Peptides and Probiotics as Alternative to Antibiotics: Reports from *in-vitro* and *in-vivo* Studies

By

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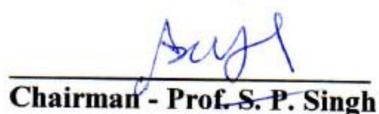

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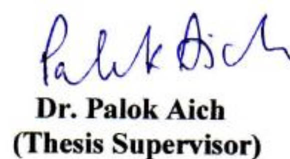

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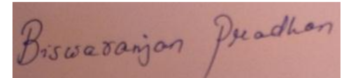

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DECLARATION

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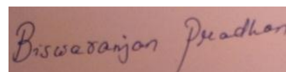

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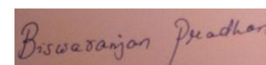

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Biswaranjan Pradhan

DEDICATION

I dedicate this thesis to my family for nursing me with love and affections and their dedicated partnership for success in my life

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I joined as a PhD student with lots of passion, curiosity and spirit for exploring new things in science for the service of mankind. However I did not have any clue with what I will work on. During course work I have gone through many literatures suggested by my Ph.D. supervisor Dr. Palok Aich, with the hope of finding a challenging problem; and I found that "antibiotic resistance" is the biggest problem of the current human society. We experimented on many things, but boiled down to concentrate on using probiotics and host defense peptides as an alternative to antibiotics. Thankfully, now I know how far I have come in this area. This journey was not smooth but would not have been possible, without the company of many individuals who helped me scientifically, and inspired me in many ways.

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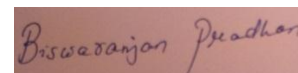

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Homi Bhabha National Institute

Ph. D. PROGRAMME
Synopsis of Ph.D. Thesis

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SYNOPSIS

The ability to treat bacterial infections with antibiotics has been a cornerstone of human medicine for almost ninety years. The selection pressure caused by the use of millions of tons of antibiotics over the past 80 years since antibiotics were introduced has made almost all disease-causing bacteria resistant to antibiotics [1]. Nearly 1000 resistance-related β-lactamases, that inactivate β-lactamase class of antibiotics, have been identified. A ten times increase in the number since discovered until 1990 [2]. Bacteria also become resistance to synthetic quinolone antibiotics 30 years after its induction in health sector [3]. Recent studies suggest that quinolone resistance was a crucial factor in the evolution of hospital Methicillin-resistant Staphylococcus aureus or MRSA [4]. Carbapenem resistance among common Enterobacteriaceae has increased

sharply over the past decade [5]. In India, *E coli* isolated from urine cultures of pregnant women in their first trimesters in the community (n=1815) showed the highest overall resistance to ampicillin, nalidixic acid, and co-trimoxazole, as 75%, 73%, and 59%, respectively between 2004 and 2007 [1]. 30% showed resistance to injectable antibiotics, such as aminoglycosides (represented by gentamicin). In a study of bloodstream infections, the proportion of *E coli* producing extended-spectrum β-lactamases increased from 40% in 2002 to 61% in 2009, and the proportion of *Klebsiella pneumoniae* with carbapenem resistance increased from 2·4% to 52% [1]. These trends are globally consistent as per the WHO report 1990 to 2012.

There is a growing sense of urgency for the establishment of novel antimicrobials and treatment strategies. Modulation of host immunity is an important and emerging paradigm to treat bacterial infections [6]. In this approach of host immunity modulaton, instrinsic defense mechanisms, of the host, are targeted to derive therapeutic or prophylactic outcome. Immune modulators under investigation include host defense regulator peptides (HDPs) and agonists of the innate immune system *e.g*, Toll-like receptors and NOD-like receptors. Innate defense regulator peptides are a particularly important subset of these modulators. These peptides are often cationic (10–50 amino acids) with a high proportion of hydrophobic residues that impart amphiphilic physical properties. Cationic peptides are generally membrane-active, and have antimicrobial activity *in-vitro* [6]. Nevertheless, these peptides often have only weak antimicrobial activity under host physiological conditions and have convincing effects in modulating the host immune response. The clinical development of such molecules to treat bacterial infections has commonly focused on the antimicrobial activities with subsequent recognition during development of the immuno modulatory properties. However the high cost of these peptides makes it difficult to afford for the general public in developing countries. So

there is a need to enhance the efficacy of these peptides. We planned to enhance efficacy of select cationic peptides by conjugating with nano materials [7].

Another category of immune modulating biological agents are probiotics. Probiotics are live microorganisms which when conferred in adequate amounts imparts health benefit to the host [8]. Probiotics maintains the gut microbial homeostasis there by imparting the host metabolism and immune system [9].Treatments with probiotics have intuitive appeal to patients and are growing in popularity. The science of probiotics, indeed, remains a mysterious one at present, but the potential is clearly there for this approach and for immune modulation treatments in general. Since, ninety percent of infection takes place through mucosal surface it is therefore, a good strategy to prime innate mucosal immunity to control infection. From the perspective of priming innate mucosal immunity the focus is on a group of cationic peptides of eukaryotic origin and certain probiotics.

This study was designed with the following objectives;

1. *In-vitro* screening of selected HDPs and Probiotics for their efficacy of immune modulation
   - For this study we have selected 4 HDPs which are established for their antimicrobial activity from bovine origin (such as Indolicidin and Bactenecin) and Human origin (Such as LL-37 and Beta-defensin-1).
   - Four probiotics strains, *Lactobacillus acidophilus* MTCC-10307 (LA), *Bacillus clausii* MTCC-8326 (BC), *Saccharomyces boulardi* (ECONORM™, Dr Reddy's) (SB) and *Bifidobacterium bifidum* (Atra pharmaceuticals) (BF) were selected for screening in this study.

2. The role of probiotics in modulating host innate immune system *in-vivo* in mice models (Both BALB/c Th2 biased as well as C57BL/6 Th1 biased mice) to understand its efficacy to contain salmonella infection and its role in restoring gut microbiome homeostasis.

This thesis is divided into six chapters:

1. Introduction
2. Materials and methods

The result section is divided into three chapters:

3. *In vitro* screening of peptides with respect to their efficacy of immune modulation in macrophage cell lines.
4. *In vitro* screening of probiotics with respect to their efficacy of immune modulation in macrophage cell lines.
5. *In vivo* study of the screened probiotics in BALB/c and C57BL/6 mice for their efficacy in modulating host immunity in gut wall tissue as well as gut microbial homeostasis in containing salmonella infection

The last chapters of the thesis contains

6. Discussions and conclusion
7. Bibliography

Chapter1: Introduction

1.1 Emergence of multidrug resistance clinical strains globally and the need for alternative treatment strategy

1.2 HDPs as the potential immune modulators

1.3 Increasing efficacy of peptides by conjugation method

1.4 Probiotics as potential immune modulators and gut microbial symbiotic agent

Chapter2: Materials and methods

The materials used in this work and methods adopted, modified or developed, comprises this chapter

Chapter3: *In vitro* screening of host defense peptides

3.1 Introduction

For this study we have selected 4 HDPs which are established for their antimicrobial activity from bovine origin (such as Indolicidin and Bactenecin) [10,11] and Human origin (Such as LL37 and Beta-defensin-1) [12,13]. They are effective against bacteria in the higher concentrations, such as 20µg/ml to 50µg/ml [12]. However the host cell proliferation was decresed in this high dose [12]. Therefore we tried to enhance its efficacy by conjugating it with carbon nano tubes (CNT) and gold nano particles (GNP), so that it can be delivered into the cell efficiently. A preliminary study with respect to select innate immune gene expression in human macrophage cell line Thp1 revealed that bactenecin is not potent enough even at the concentration of 20µg/ml but the other 3 peptides activated these innate immune genes effectively. We choose 2 peptides (Indolicidin and LL-37) out of these 3 for further study, one bovine origin and other human origin.

3.2 Indolicidin Results

Indolicidin was not toxic to Thp1 (transformed human leukemia monocyte cell line) cells up to concentrations of 20µg/ml. Expression of select innate immune genes such as interleukin 6,

interleukin 10, interleukin 12, NFkB, interleukin 1 beta, interleukin 1 alpha and interferon alpha and beta increased with increasing concentration of indolicidin with optimal fold changes at 10-20µg/ml. We conjugated Indolicidin to CNT and GNP using EDC-NHS method and characterized the conjugate through various chemical and physical techniques. Conjugates were incubated with Thp1 cells as concentrations of 2, 0.2 and 0.02µg/ml of cell culture media. We found that at 1000 fold less concentrations of free indolicidin, conjugated indolicidin was activating the select innate immune genes up to 2 fold with respect to time matched untreated controls. We have compared genome wide gene expression in Thp1 cells following 6h treatment with conjugate at 0.02µg/ml and free indolicidin at 20µg/ml along with CNT and spiked peptide (a mixture of CNT and 0.02µg/ml of peptide). We found that proinflammatory cytokine expression in Thp1 was comparable or better in conjugate than free indolicidin at 1000 fold higher concentration. Thp1 cells primed with conjugate at 0.02µg/ml and free indolicidin at 20µg/ml was protected against *Salmonella typhimurium* induced cytotoxicity up to 18 hours whereas unprimed cells died within 6 hours. Similar results were also observed with murine monocytic macrophage cell line RAW 264.7.

3.3 LL-37 Results

LL-37 was not toxic to Thp1 cells up to concentrations of 20µg/ml. Expression of select innate immune genes such as interleukin 6, interleukin 10, interleukin 12, *NFkB*, interleukin 1 beta, interleukin 1 alpha and interferon alpha and beta increased with increasing concentration of LL-37 with optimal fold changes at 10-20µg/ml. We conjugated LL-37 to CNT and GNP using the EDC-NHS method and characterized the conjugate through various chemical and physical techniques. Conjugates were incubated with Thp1 cells as concentrations of 2, 0.2 and 0.02µg/ml of cell culture media. We found that at 1000 fold less concentrations of free LL-37,

conjugated LL-37 was activating the select innate immune genes up to 2 fold or more with respect to time matched untreated controls. We compared genome wide gene expression in Thp1 cells following 6h treatment with conjugate at 0.02µg/ml and free LL37 at 20µg/ml along with CNT and spiked peptide (a mixture of CNT and 0.02µg/ml of peptide). We found that proinflammatory cytokine expression in Thp1 was comparable or better with the conjugate than free LL37 at 1000 fold higher concentration. Further the primed Thp1 cells with the conjugate at 0.02µg/ml and free LL37 at 20µg/ml were protected against *Salmonella typhimurium* induced cytotoxicity up to 12 hours whereas unprimed cells depleted in 6 hours. Similar results were also observed with a murine monocytic macrophage cell line RAW 264.7.

3.4 Conclusion

Both Indolicidin and LL-37 primed Thp1 cells are effectively protected against *Salmonella typhimurium* induced cytotoxicity. Our genome wide gene expression study revealed that pro-inflammatory as well anti-apoptotic signaling in Thp1 cells upon treatment with indolicidin was mediated through the *TNFRSF1A-TRADD-TRAF2-NFkB* route whereas in the case of LL-37 treatment it was mediated through the *IL1R-TAB1-TAK1-NFkB* route. The cell proliferation signaling in indolicidin treatment went through the *TNFRSF1A-TRADD-TRAF2-MAP3K14-MAP2K7-MAPK10-cJUN* pathway where as in LL-37 treatment, cell proliferation was signaled through calcium influx followed by PPP3CA mediated de-phosphorylation and activation of *NFAT2* transcription factor followed by expression of cell proliferating genes.

Though immune modulation by indolicidin and LL37 was partly known before, our data established the complete gene expression and signaling mechanism. Our conjugation strategy enhanced the immune modulating efficacy of these two peptides by 1000 folds. The conjugation

strategy may reduce the cost of these peptides for antimicrobial treatment there by reaching a wider population of developing countries who are the most sufferers of infectious diseases.

Chapter4: *In vitro* probiotics screening

4.1 Introduction

Probiotics are the live micro-organisms which when taken in adequate amount confers health benefit to the host[14]. Probiotic and potential probiotic bacterial strains are routinely prescribed and used as supplementary therapy for a variety infectious diseases, including enteric disorders among a wide range of individuals[8]. While there are an increasing number of studies defining the possible mechanisms of probiotic activity, a great deal remains unknown regarding the diverse modes of action attributed to these therapeutic agents[8]. More precise information is required to support the appropriate application of probiotics. We selected four probiotics strains, *Lactobacillus acidophilus* MTCC-10307 (LA), *Bacillus clausii* MTCC-8326 (BC), *Saccharomyces boulardi* (SB) and *Bifidobacterium bifidum* (BF) to screen in-vitro in RAW 264.7 murine macrophage cells.

4.2 Results

LA, BC and SB are not toxic to Raw264.7 up to a multiplicity of infection (MOI) of 100 for 6 hours. However BF causes around 40% cell death on MOI of 100 in 6 hours. So we studied the BF at the MOI of 10 instead of 100. Macrophage survivability was compared with *Salmonella typhimurium* MTCC 3232 (ST) on MOI of 10. On MOI of 10, ST was cytotoxic to Raw264.7 and kills more than 50% of the cells within 2 hours. LA, BC, and SB were tested for their immune modulatory property with a few select innate immune genes such as interleukin 6, interleukin 10, interleukin 12, *NFkB*, interleukin 1 beta, interleukin 1 alpha and interferon alpha

and beta. With increasing MOI, expression of these genes increased and was optimized on MOI of 100. However SB failed to modulate most of these genes and was discarded from further studies. Whole genome gene expression kinetics was assayed in Raw264.7 cell lines following treatment with LA and BC for 1, 2, 4 and 6 hours. Our data revealed that both LA and BC induces controlled pro-inflammation in macrophage through Warburg metabolism. While Glycolytic and tricarboxylic acid metabolism enzymes are up-regulated, the electron transport chain enzymes are down-regulated in Raw264.7 cells following treatment with LA and BC. This data suggests that there was controlled inflammation in macrophage where the NADPH produced in glycolysis and TCA cycle was being used to neutralize the reactive oxygen species (ROS) produced in mitochondria to kill the invaded pathogens. This result was supported by the protection of the probiotics primed macrophage from ST induced cytotoxicity.

4.3 Conclusion

The use of Lactobacillus and Bacillus species as probiotic dietary supplements is increasing. Each year new strains of these two genera are studied and added to the probiotics list based on their immune modulatory and antimicrobial activities. In this study, we reported two new strains of LA and BC, which displayed significant effects on modulating innate immune responses in murine macrophage and protected cells from ST-induced cytotoxicity. These two strains adhere to the surface of macrophages and have moderate cytotoxic activity. Our comparative analysis of transcriptome dynamics in macrophages following treatment with these two bacterial strains revealed that *L. acidophilus* MTCC-10307 and *B. clausii* MTCC-8326 could be considered as probiotics for further usage. However, their efficacy as probiotics needs be established *in vivo*. So LA and BC were selected for further study in *in-vivo* both in BALB/c Th2 and C57BL/6 Th1 immune biased mice models.

Chapter5: *In-vivo* probiotics study

5.1 Introduction

Based on in vitro results, we selected LA and BC for further validation in vivo. We checked the effect of LA and BC on BALB/c and C57BL/6 mice with respect to their ability to modulate intestinal mucosal immunity and maintaining gut microbial homoeostasis. BALB/c mice are Th2 biased [15] whereas C57BL/6 mice are Th1 biased [15]. Apart from various proposed mechanisms of action of probiotics, maintenance of gut microbial homoeostasis was an important mechanism through which it indirectly maintains the host physiological homoeostasis in gut [16]. As the genetic backgrounds of both the mice are different we thought that microbiome composition of the gut in different mouse strain might be different and therefore probiotics action on them will be different. Thus we checked the background microbiome composition in both of these mice varieties through V3 sequencing of 16s rRNA gene. Our data revealed that BALB/c has more firmicutes and less bacteroidetes, whereas C57BL/6 has similar amounts of firmicutes and bacteroidetes. So we propose that both of these mice might behave differently to LA and BC treatment as well as to salmonella infection.

BALB/c and C57BL/6 mice were divided into 6 groups and treated/challenged with various bacterial combinations such as LA, BC, ST, LA + ST, BC + ST along with time matched untreated control (NT). Samples were collected at 3, 5 and 10 days post treatment/challenge. The samples collected were fecal and gut content (ingesta) for microbial genomic DNA collection for 16S rRNA based microbial profiling through V3 next generation sequencing; duodenum, jejunum, ileum and colon tissue for histopathology as well as for host gene expression in gut wall tissue. Probiotics site of colonization and duration of stay in gut was

evaluated through polymerase chain reaction with LA and BC specific primer. Infection by ST also evaluated through PCR assay.

5.2 Results from BALB/c study

Both LA and BC colonized the colon and distal ileum of mice after orally gavaged. The effective dose of LA titrated to be 2 x 10^9 per mouse whereas for BC it was 2 x 10^8. The infective dose of ST was titrated to be 5 x 10^8 per mouse through the oral gavage route of administration. Our gene expression data suggests that ST and STBC treatment induces severe inflammation in BALB/c mice through the expression of *TNFa* , interleukin 17, interleukin 6 , interleukin 1a and interleukin 1b and several type I interferons. Microbial data revealed the increase in gamma proteobacteria level in the group of mice treated with ST and ST + BC which are gram negative opportunistic pathogens. LA induced controlled inflammation in the gut through the moderate expression of interleukin 6, interleukin 17, interleukin 18, type I interferons and several chemokines. There were increased firmicutes and decreased gamma proteobacteria levels in the group of mice treated with LA + ST, supporting the restoration of gut microbial homoeostasis by LA after ST induced microbial dysbiosis. The species level analysis of the microbiota data revealed a 50 fold increase in *Butyricoccus pullicaecorum* and *Faecalibacterium prausnitzii,* which are bacteria known to produce butyrate and aryl hydrocarbon, which are potent inflammation suppressors in LA + ST treated group. These data suggest that LA modulates the gut microbiome in a way to neutralize the inflammation caused by ST infection in mice. Our histopathological study supports the above mentioned data where there was increased immune infiltration and villi atrophy in ST and ST + BC treated mice but normal gut structure in LA + ST treated mice. There was 100% mortality in the ST treated group whereas 100% survivability in the LA + ST treated group. The ST + BC group had 90%

survivability with gut inflammation and microbial dysbiosys. These data together suggest that LA combats the ST infection by neutralizing inflammation and restoring microbial homoeostasis whereas BC failed to do so.

5.3 Results from C57BL/6 study

Both LA and BC colonized the colon and distal ileum of mice after oral gavage. The effective dose of LA was found to be 2 x 10^9 per mouse whereas for BC it was 2 x 10^8. The infective dose of ST was found to be 5 x 10^8 per mice through the oral gavage route of administration. Our gene expression data suggest that ST and STBC treatment induces severe inflammation in C57BL/6 mice through the expression of *TNFa* , interleukin 17, interleukin 6 , interleukin 1a and interleukin 1b, several type I interferons and secretion of heavy amounts of β- defensins. Microbial data revealed the increase in gamma proteobacteria levels in the group of mice treated with ST and ST + BC which are gram negative opportunistic pathogens. LA induced controlled inflammation in the gut through the moderate expression of interleukin 6, interleukin 17, interleukin 23, type I interferon. There was no expression of chemokine which suggest that C57BL/6 mice have localized gut inflammation through ST infection and no involvement of nearby lymph node for pathogen clearance. There was increased bacteroides and decreased gamma proteobacteria levels in the group of mice treated with LA + ST supporting the restoration of gut microbial homoeostasis by LA after ST induced microbial dysbiosis. Gene expression data suggest that ST and STBC groups of mice were having severe inflammation and microbial dysbiosis in the gut whereas STLA mice have the least inflammation. Our histopathological study supports the above mentioned data where there was increased immune infiltration, villi atrophy and transmural tissue damage in ST and ST + BC treated mice but normal gut structure in LA + ST treated mice. There was 100% mortality with ST and ST + BC

treated group whereas 100% survivability with LA + ST treated group. Together these data suggests that LA combats the ST infection by neutralizing inflammation and restoring microbial homoeostasis whereas BC increased ST infection in C57BL/6 mice.

5.4 Conclusion

Among all the treatment groups STBC induced the highest inflammation in the gut for both strains of mice. The data revealed that C57BL/6 failed to induce the chemokines required for attracting leucocytes from nearby lymph nodes to the site of infection; rather it tries to fight the pathogen by inducing large amounts of β-defensins locally. Tlr4 and tlr6 induction in C57BL/6 mice was higher than in BALB/c mice, for which inflammation might be severe in C57BL/6.

Chapter 6: Summary and Conclusions

We summarize our overall observations below:

Indolicidin and LL37 at 20µg/ml primed the macrophage immunity and protect it from ST induced cytotoxicity. We increased efficacy of Indolicidin and LL37 with respect to immune modulation by 1000 fold upon conjugation with CNT. Macrophages primed with conjugates of both the peptides are protected against salmonella induced cytotoxicity. We have shown that LA and BC primed macrophage were protected against ST induced cytotoxicity. LA modulates BALB/c and C57BL/6 microbiome and gut gene expression in a way which cured ST infection but BC failed to do so.

Our study of HDPs and probiotics for modulating the host mucosal immune system and combating salmonella infection supported the alternative strategy to antibiotic treatment to combat infection. We established the mechanism of action of indolicidin and LL37 as well as

their conjugate in modulating macrophages. We established a new strain of LA as probiotics *in-vitro* and *in-vivo* and established its mode of action in modulating mucosal immunity as well as maintaining host microbial homoeostasis. Together this study contributed significantly to the dream of an alternative to antibiotics to combat infectious diseases to protect future human society from infection in case all pathogens develop resistance to all the existing antibiotics.

Publications:

1. Sur A*, **Pradhan B***, Banerjee A, Aich P* (2015) Immune activation efficacy of indolicidin is enhanced upon conjugation with carbon nanotubes and gold nanoparticles. PLoS ONE 10(4):e0123905
2. **Pradhan B**, Guha D, Ray P, Das D, Aich P* (2016) Comparative Analysis of the Effects of Two Probiotic Bacterial Strains on Metabolism and Innate immunity in the Raw264.7 Murine Macrophage Cell Line. Probiotics and Antimicrobial Proteins 8(25): PMID: 27038159
3. S. Priyadarshini, **B. Pradhan** & P. Aich*. Cortisol mediated regulation of immune and metabolic processes in murine adipocytes 3T3-L1 and macrophages RAW 264.7 via serotonin receptors HTR2a and HTR5c. (Communicated)
4. S. Priyadarshini, **B. Pradhan** & P. Aich*. Effects of Cortisol and Serotonin on Pre-adipocytes and Macrophages of Mouse Origin: A Shift in Immune Response kinetics. (communicated)
5. **Pradhan B**, Datzkiw D, Aich P. Gut microbiota composition is in unison with human health; a review with focus on metabolic and immunological disorders. (Communicated)
6. **Pradhan B**, Guha D, Banerjee A, Sur A, Murmu K, Ray P, Das D, Hancock REW, Aich P. Immune modulatory efficacy of Indolicidin and LL37 is enhanced by 1000 folds upon conjugation with CNT and GNP.(In preparation)
7. **Pradhan B**, Guha D, Naik A, Banerjee A, Tambat S, Chawla S, Senapati S, Aich P. Probiotics Lactobacillus acidophilus and Bacillus clausii modulate mice gut microbiota and ameliorate Salmonella induced dysbiosis and inflammation.(In preparation)
8. Guha D*, Banerjee A*, **Pradhan B**, Peneva M, Aleksandrov G and Aich P. Macrophage polarization is influenced following treatment with *Lactobacillus bulgaricus.*(Communicated)

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List of Abbreviations

ABI1	Abl Interactor 1
ACTN1	Actinin Alpha 1
ACVR1	Activin A Receptor Type 1
ADCY1	Adenylate Cyclase 1
ADCYAP1R1	ADCYAP Receptor Type I
AKT1	V-Akt Murine Thymoma Viral Oncogene Homolog 1
AKT1S1	AKT1 Substrate 1
AKT2	V-Akt Murine Thymoma Viral Oncogene Homolog 2
AKT3	V-Akt Murine Thymoma Viral Oncogene Homolog 3
ALOX5	Arachidonate 5-Lipoxygenase
ANAPC1	Anaphase Promoting Complex Subunit 1
APAF1	Apoptotic Peptidase Activating Factor 1
ARIH1	Ariadne RBR E3 Ubiquitin Protein Ligase 1
ARPC2	Actin Related Protein 2/3 Complex Subunit 2
ATF6B	Activating Transcription Factor 6 Beta
ATG12	Autophagy Related 12
ATP6V0A1	ATPase H+ Transporting V0 Subunit A1
ATP6V1C1	ATPase H+ Transporting V1 Subunit C1
BAD	BCL2 Associated Agonist Of Cell Death
BAX	BCL2 Associated X Protein
BC	Bacillus clausii
BCL10	B-Cell CLL/Lymphoma 10
BF	Bifidobacterium bifidum
BIRC5	Baculoviral IAP Repeat Containing 5
BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase
BRF1	BRF1, RNA Polymerase III Transcription Initiation Factor 90 KDa Subunit
C1QA	Complement Component 1, Q Subcomponent, A Chain
C1QB	Complement Component 1, Q Subcomponent, B Chain
C1QC	Complement Component 1, Q Subcomponent, C Chain
C1S	Complement Component 1, S Subcomponent
C2	Complement Component 2
C4A	Complement Component 4A
C5AR1	Complement Component 5a Receptor 1
C8A	Complement Component 8 Alpha Subunit
C9	Complement Component 9
CAB39L	Calcium Binding Protein 39 Like
CACNA1I	Calcium Voltage-Gated Channel Subunit Alpha1 I
CACNA2D3	Calcium Voltage-Gated Channel Auxiliary Subunit Alpha2delta 3
CACNB1	Calcium Voltage-Gated Channel Auxiliary Subunit Beta 1
CALM1	Calmodulin 1 (Phosphorylase Kinase, Delta)

CAMK1	Calcium/Calmodulin Dependent Protein Kinase I
CAMK1G	Calcium/Calmodulin Dependent Protein Kinase IG
CAMK2A	Calcium/Calmodulin Dependent Protein Kinase II Alpha
CAMK2B	Calcium/Calmodulin Dependent Protein Kinase II Beta
CAMK2D	Calcium/Calmodulin Dependent Protein Kinase II Delta
CAMK2G	Calcium/Calmodulin Dependent Protein Kinase II Gamma
CAMK4	Calcium/Calmodulin-Dependent Protein Kinase IV
CAMP	Cathelicidin Antimicrobial Peptide
CARD9	Caspase Recruitment Domain Family Member 9
CASP1	Caspase 1
CASP10	Caspase 10
CASP12	Caspase 12
CASP2	Caspase 2
CASP3	Caspase 3
CASP4	Caspase 4
CASP5	Caspase 5
CASP6	Caspase 6
CASP7	Caspase 7
CASP8	Caspase 8
CCK	Cholecystokinin
CCL1	C-C Motif Chemokine Ligand 1
CCL11	C-C Motif Chemokine Ligand 11
CCL14	C-C Motif Chemokine Ligand 14
CCL17	C-C Motif Chemokine Ligand 17
CCL19	C-C Motif Chemokine Ligand 19
CCL2	C-C Motif Chemokine Ligand 2
CCL20	C-C Motif Chemokine Ligand 20
CCL21	C-C Motif Chemokine Ligand 21
CCL22	C-C Motif Chemokine Ligand 22
CCL23	C-C Motif Chemokine Ligand 23
CCL24	C-C Motif Chemokine Ligand 24
CCL25	C-C Motif Chemokine Ligand 25
CCL27	C-C Motif Chemokine Ligand 27
CCL28	C-C Motif Chemokine Ligand 28
CCL3	C-C Motif Chemokine Ligand 3
CCL4	C-C Motif Chemokine Ligand 4
CCL5	C-C Motif Chemokine Ligand 5
CCL7	C-C Motif Chemokine Ligand 7
CCL8	C-C Motif Chemokine Ligand 8
CCNE2	Cyclin E2
CCR1	C-C Motif Chemokine Receptor 1
CCR10	C-C Motif Chemokine Receptor 10
CCR2	C-C Motif Chemokine Receptor 2

CCR3	C-C Motif Chemokine Receptor 3
CCR4	C-C Motif Chemokine Receptor 4
CCR5	C-C Motif Chemokine Receptor 5
CCR6	C-C Motif Chemokine Receptor 6
CCR7	C-C Motif Chemokine Receptor 7
CCR8	C-C Motif Chemokine Receptor 8
CCR9	C-C Motif Chemokine Receptor 9
CDC14B	Cell Division Cycle 14B
CDC25C	Cell Division Cycle 25C
CDH10	Cadherin 10
CDK1	Cyclin-Dependent Kinase 1
CDKN2A	Cyclin-Dependent Kinase Inhibitor 2A
CDKN2C	Cyclin-Dependent Kinase Inhibitor 2C
CFLAR	CASP8 And FADD Like Apoptosis Regulator
CHEK1	Checkpoint Kinase 1
CHEK2	Checkpoint Kinase 2
CNT	Carbon Nano Tube
CNTN2	Contactin 2
CREB1	CAMP Responsive Element Binding Protein 1
CREB3L1	CAMP Responsive Element Binding Protein 3-Like 1
CREB3L2	CAMP Responsive Element Binding Protein 3-Like 2
CREB3L3	CAMP Responsive Element Binding Protein 3-Like 3
CREB3L4	CAMP Responsive Element Binding Protein 3-Like 4
CREBBP	CREB Binding Protein
CSF1	Colony Stimulating Factor 1
CSF1R	Colony Stimulating Factor 1 Receptor
CSF2	Colony Stimulating Factor 2
CSF2RB	Colony Stimulating Factor 2 Receptor Beta Common Subunit
CSF3	Colony Stimulating Factor 3
CSF3R	Colony Stimulating Factor 3 Receptor
CTLA4	Cytotoxic T-Lymphocyte Associated Protein 4
CTNNA2	Catenin Alpha 2
CTNNB1	Catenin Beta 1
CTSE	Cathepsin E
CTSF	Cathepsin F
CTSH	Cathepsin H
CTSK	Cathepsin K
CTSL	Cathepsin L
CTSO	Cathepsin O
CXCL1	C-X-C Motif Chemokine Ligand 1
CXCL10	C-X-C Motif Chemokine Ligand 10
CXCL11	C-X-C Motif Chemokine Ligand 11
CXCL12	C-X-C Motif Chemokine Ligand 12

CXCL13	C-X-C Motif Chemokine Ligand 13
CXCL14	C-X-C Motif Chemokine Ligand 14
CXCL16	C-X-C Motif Chemokine Ligand 16
CXCL2	C-X-C Motif Chemokine Ligand 2
CXCL3	C-X-C Motif Chemokine Ligand 3
CXCL5	C-X-C Motif Chemokine Ligand 5
CXCL9	C-X-C Motif Chemokine Ligand 9
CXCR1	C-X-C Motif Chemokine Receptor 1
CXCR2	C-X-C Motif Chemokine Receptor 2
CXCR3	C-X-C Motif Chemokine Receptor 3
CXCR4	C-X-C Motif Chemokine Receptor 4
CXCR5	C-X-C Motif Chemokine Receptor 5
CXCR6	C-X-C Motif Chemokine Receptor 6
CYP2E1	Cytochrome P450 Family 2 Subfamily E Member 1
DAGLA	Diacylglycerol Lipase Alpha
DAP	Death-Associated Protein
DEFA1	Defensin Alpha 1
DEFA3	Defensin Alpha 3
DEFA4	Defensin Alpha 4
DEFA5	Defensin Alpha 5
DEFA6	Defensin Alpha 6
DEFB1	Defensin Beta 1
DEFB103B	Defensin Beta 103B
DEFB105B	Defensin Beta 105B
DEFB106B	Defensin Beta 106B
DEFB119	Defensin Beta 119
DEFB123	Defensin Beta 123
DEFB129	Defensin Beta 129
DUSP10	Dual Specificity Phosphatase 10
DUSP14	Dual Specificity Phosphatase 14
DUSP16	Dual Specificity Phosphatase 16
DUSP2	Dual Specificity Phosphatase 2
DUSP3	Dual Specificity Phosphatase 3
DUSP4	Dual Specificity Phosphatase 4
DUSP9	Dual Specificity Phosphatase 9
EGR1	Early Growth Response 1
FADD	Fas Associated Via Death Domain
FAS	Fas Cell Surface Death Receptor
FASLG	Fas Ligand
FCER1G	Fc Fragment Of IgE Receptor Ig
FGF13	Fibroblast Growth Factor 13
FGF14	Fibroblast Growth Factor 14
FGF17	Fibroblast Growth Factor 17

FGF3	Fibroblast Growth Factor 3
FGF5	Fibroblast Growth Factor 5
FGF8	Fibroblast Growth Factor 8
FGF9	Fibroblast Growth Factor 9
FGFBP1	Fibroblast Growth Factor Binding Protein 1
FGFR4	Fibroblast Growth Factor Receptor 4
FIGF	C-Fos Induced Growth Factor
FOS	FBJ Murine Osteosarcoma Viral Oncogene Homolog
FOXO4	Forkhead Box O4
FPR2	Formyl Peptide Receptor 2
FRS3	Fibroblast Growth Factor Receptor Substrate 3
GABRA4	Gamma-Aminobutyric Acid Type A Receptor Alpha4 Subunit
GADD45A	Growth Arrest And DNA Damage Inducible Alpha
GADD45B	Growth Arrest And DNA Damage Inducible Beta
GADD45G	Growth Arrest And DNA Damage Inducible Gamma
GAS1	Growth Arrest Specific 1
GAS2	Growth Arrest Specific 2
GATA4	GATA Binding Protein 4
GNG4	G Protein Subunit Gamma 4
GNP	Gold Nano Particle
GPR17	G Protein-Coupled Receptor 17
GPR4	G Protein-Coupled Receptor 4
GPR65	G Protein-Coupled Receptor 65
GRB2	Growth Factor Receptor Bound Protein 2
HDAC1	Histone Deacetylase 1
HDAC3	Histone Deacetylase 3
HIF1A	Hypoxia Inducible Factor 1 Alpha Subunit
HNF1A	HNF1 Homeobox A
HSPA1A	Heat Shock Protein Family A (Hsp70) Member 1A
HSPA2	Heat Shock Protein Family A (Hsp70) Member 2
HSPA6	Heat Shock Protein Family A (Hsp70) Member 6
HSPA8	Heat Shock Protein Family A (Hsp70) Member 8
HTR2A	5-Hydroxytryptamine Receptor 2A
HTR2B	5-Hydroxytryptamine Receptor 2B
HTR2C	5-Hydroxytryptamine Receptor 2C
ICAM1	Intercellular Adhesion Molecule 1
ICAM2	Intercellular Adhesion Molecule 2
ICAM4	Intercellular Adhesion Molecule 4
ICAM5	Intercellular Adhesion Molecule 5
IER3	Immediate Early Response 3
IFNA13	Interferon, Alpha 13
IFNA14	Interferon Alpha 14
IFNA2	Interferon, Alpha 2

IFNA4 Interferon, Alpha 4
IFNA5 Interferon Alpha 5
IFNA7 Interferon, Alpha 7
IFNAR1 Interferon Alpha And Beta Receptor Subunit 1
IFNAR2 Interferon Alpha And Beta Receptor Subunit 2
IFNB1 Interferon Beta 1
IFNG Interferon, Gamma
IFNGR1 Interferon Gamma Receptor 1
IFNGR2 Interferon Gamma Receptor 2
IFNLR1 Interferon Lambda Receptor 1
IGF1 Insulin Like Growth Factor 1
IGF2R Insulin Like Growth Factor 2 Receptor
IKBKB Inhibitor Of Kappa Light Polypeptide Gene Enhancer In B-Cells, Kinase Beta
IKBKE Inhibitor Of Kappa Light Polypeptide Gene Enhancer In B-Cells, Kinase Epsilon
IKBKG Inhibitor Of Kappa Light Polypeptide Gene Enhancer In B-Cells, Kinase Gamma
IL10 Interleukin 10
IL10RA Interleukin 10 Receptor Subunit Alpha
IL10RB Interleukin 10 Receptor Subunit Beta
IL11 Interleukin 11
IL11RA Interleukin 11 Receptor Subunit Alpha
IL12A Interleukin 12A
IL12B Interleukin 12B
IL12RB1 Interleukin 12 Receptor Subunit Beta 1
IL12RB2 Interleukin 12 Receptor Subunit Beta 2
IL13RA1 Interleukin 13 Receptor Subunit Alpha 1
IL13RA2 Interleukin 13 Receptor Subunit Alpha 2
IL15 Interleukin 15
IL15RA Interleukin 15 Receptor Subunit Alpha
IL16 Interleukin 16
IL17A Interleukin 17A
IL17B Interleukin 17B
IL17C Interleukin 17C
IL17D Interleukin 17D
IL17F Interleukin 17F
IL17RA Interleukin 17 Receptor A
IL17RB Interleukin 17 Receptor B
IL17RC Interleukin 17 Receptor C
IL17RD Interleukin 17 Receptor D
IL17RE Interleukin 17 Receptor E
IL18 Interleukin 18
IL18BP Interleukin 18 Binding Protein
IL18R1 Interleukin 18 Receptor 1
IL18RAP Interleukin 18 Receptor Accessory Protein

IL19	Interleukin 19
IL1A	Interleukin 1 Alpha
IL1B	Interleukin 1 Beta
IL1R1	Interleukin 1 Receptor Type 1
IL1R2	Interleukin 1 Receptor Type 2
IL1RAP	Interleukin 1 Receptor Accessory Protein
IL1RL1	Interleukin 1 Receptor Like 1
IL1RL2	Interleukin 1 Receptor Like 2
IL1RN	Interleukin 1 Receptor Antagonist
IL2	Interleukin 2
IL20RA	Interleukin 20 Receptor Subunit Alpha
IL20RB	Interleukin 20 Receptor Subunit Beta
IL21	Interleukin 21
IL21R	Interleukin 21 Receptor
IL22	Interleukin 22
IL22RA1	Interleukin 22 Receptor Subunit Alpha 1
IL22RA2	Interleukin 22 Receptor Subunit Alpha 2
IL23A	Interleukin 23 Subunit Alpha
IL23R	Interleukin 23 Receptor
IL24	Interleukin 24
IL27	Interleukin 27
IL27RA	Interleukin 27 Receptor Subunit Alpha
IL2RA	Interleukin 2 Receptor Subunit Alpha
IL2RB	Interleukin 2 Receptor Subunit Beta
IL2RG	Interleukin 2 Receptor Subunit Gamma
IL3	Interleukin 3
IL31	Interleukin 31
IL31RA	Interleukin 31 Receptor A
IL33	Interleukin 33
IL34	Interleukin 34
IL36B	Interleukin 36, Beta
IL36G	Interleukin 36, Gamma
IL3RA	Interleukin 3 Receptor Subunit Alpha
IL4	Interleukin 4
IL5	Interleukin 5
IL5RA	Interleukin 5 Receptor Subunit Alpha
IL6	Interleukin 6
IL6R	Interleukin 6 Receptor
IL6ST	Interleukin 6 Signal Transducer
IL7	Interleukin 7
IL7R	Interleukin 7 Receptor
IL9	Interleukin 9
IL9R	Interleukin 9 Receptor

IRAK1	Interleukin 1 Receptor Associated Kinase 1
IRAK3	Interleukin 1 Receptor Associated Kinase 3
IRF1	Interferon Regulatory Factor 1
IRF2	Interferon Regulatory Factor 2
IRF3	Interferon Regulatory Factor 3
IRF4	Interferon Regulatory Factor 4
IRF5	Interferon Regulatory Factor 5
IRF6	Interferon Regulatory Factor 6
IRF9	Interferon Regulatory Factor 9
IRS1	Insulin Receptor Substrate 1
ITGA1	Integrin Subunit Alpha 1
ITGA10	Integrin Subunit Alpha 10
ITGA11	Integrin Subunit Alpha 11
ITGA2	Integrin Subunit Alpha 2
ITGA2B	Integrin Subunit Alpha 2b
ITGA3	Integrin Subunit Alpha 3
ITGA4	Integrin Subunit Alpha 4
ITGA5	Integrin Subunit Alpha 5
ITGA6	Integrin Subunit Alpha 6
ITGA7	Integrin Subunit Alpha 7
ITGA8	Integrin Subunit Alpha 8
ITGA9	Integrin Subunit Alpha 9
ITGB1	Integrin Subunit Beta 1
ITGB2	Integrin Subunit Beta 2
ITGB3	Integrin Subunit Beta 3
ITGB5	Integrin Subunit Beta 5
JUN	Jun Proto-Oncogene
JUNB	Jun B Proto-Oncogene
JUND	Jun D Proto-Oncogene
LA	Lactobacillus acidophilus
LIF	Leukemia Inhibitory Factor
LRR1	Leucine Rich Repeat Protein 1
MAP2K1	Mitogen-Activated Protein Kinase Kinase 1
MAP2K2	Mitogen-Activated Protein Kinase Kinase 2
MAP2K3	Mitogen-Activated Protein Kinase Kinase 3
MAP2K4	Mitogen-Activated Protein Kinase Kinase 4
MAP2K5	Mitogen-Activated Protein Kinase Kinase 5
MAP2K6	Mitogen-Activated Protein Kinase Kinase 6
MAP2K7	Mitogen-Activated Protein Kinase Kinase 7
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1
MAP3K10	Mitogen-Activated Protein Kinase Kinase Kinase 10
MAP3K11	Mitogen-Activated Protein Kinase Kinase Kinase 11
MAP3K12	Mitogen-Activated Protein Kinase Kinase Kinase 12

MAP3K13	Mitogen-Activated Protein Kinase Kinase Kinase 13
MAP3K14	Mitogen-Activated Protein Kinase Kinase Kinase 14
MAP3K2	Mitogen-Activated Protein Kinase Kinase Kinase 2
MAP3K3	Mitogen-Activated Protein Kinase Kinase Kinase 3
MAP3K4	Mitogen-Activated Protein Kinase Kinase Kinase 4
MAP3K6	Mitogen-Activated Protein Kinase Kinase Kinase 6
MAP3K7	Mitogen-Activated Protein Kinase Kinase Kinase 7
MAP3K8	Mitogen-Activated Protein Kinase Kinase Kinase 8
MAP3K9	Mitogen-Activated Protein Kinase Kinase Kinase 9
MAP4K1	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 1
MAP4K3	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 3
MAP4K4	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4
MAP4K5	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 5
MAPK10	Mitogen-Activated Protein Kinase 10
MAPK11	Mitogen-Activated Protein Kinase 11
MAPK12	Mitogen-Activated Protein Kinase 12
MAPK14	Mitogen-Activated Protein Kinase 14
MAPK3	Mitogen-Activated Protein Kinase 3
MAPK4	Mitogen-Activated Protein Kinase 4
MAPK6	Mitogen-Activated Protein Kinase 6
MAPK8	Mitogen-Activated Protein Kinase 8
MAPK8IP2	Mitogen-Activated Protein Kinase 8 Interacting Protein 2
MAPK8IP3	Mitogen-Activated Protein Kinase 8 Interacting Protein 3
MAPK9	Mitogen-Activated Protein Kinase 9
MAPKAP1	Mitogen-Activated Protein Kinase Associated Protein 1
MASP2	Mannan Binding Lectin Serine Peptidase 2
MMP10	Matrix Metallopeptidase 10
MMP11	Matrix Metallopeptidase 11
MMP12	Matrix Metallopeptidase 12
MMP13	Matrix Metallopeptidase 13
MMP14	Matrix Metallopeptidase 14
MMP19	Matrix Metallopeptidase 19
MMP2	Matrix Metallopeptidase 2
MMP25	Matrix Metallopeptidase 25
MMP3	Matrix Metallopeptidase 3
MMP7	Matrix Metallopeptidase 7
MMP8	Matrix Metallopeptidase 8
MMP9	Matrix Metallopeptidase 9
MOI	Multiplicity of Infection
MUC1	Mucin 1, Cell Surface Associated
MX1	MX Dynamin Like GTPase 1
MYD88	Myeloid Differentiation Primary Response 88
NFATC1	Nuclear Factor Of Activated T-Cells 1

NFATC2	Nuclear Factor Of Activated T-Cells 2
NFATC4	Nuclear Factor Of Activated T-Cells 4
NFKB1	Nuclear Factor Kappa B Subunit 1
NFKB2	Nuclear Factor Kappa B Subunit 2
NFKBIA	NFKB Inhibitor Alpha
NFKBIB	NFKB Inhibitor Beta
NFKBIE	NFKB Inhibitor Epsilon
NLRC4	NLR Family, CARD Domain Containing 4
NLRC5	NLR Family, CARD Domain Containing 5
NLRP4	NLR Family, Pyrin Domain Containing 4
NT	No Treatment
PDGFA	Platelet Derived Growth Factor Subunit A
PDGFB	Platelet Derived Growth Factor Subunit B
PFKFB1	6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 1
PIK3C2G	Phosphatidylinositol-4-Phosphate 3-Kinase Catalytic Subunit Type 2 Gamma
PIK3C3	Phosphatidylinositol 3-Kinase Catalytic Subunit Type 3
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PIK3R1	Phosphoinositide-3-Kinase Regulatory Subunit 1
PIK3R2	Phosphoinositide-3-Kinase Regulatory Subunit 2
PIK3R3	Phosphoinositide-3-Kinase Regulatory Subunit 3
PIK3R4	Phosphoinositide-3-Kinase Regulatory Subunit 4
PIK3R5	Phosphoinositide-3-Kinase Regulatory Subunit 5
PIKFYVE	Phosphoinositide Kinase, FYVE-Type Zinc Finger Containing
PLA2G10	Phospholipase A2 Group X
PLA2G2A	Phospholipase A2 Group IIA
PLA2G3	Phospholipase A2 Group III
PLA2G4A	Phospholipase A2 Group IVA
PLCB1	Phospholipase C Beta 1
PLCB2	Phospholipase C Beta 2
PLCD3	Phospholipase C Delta 3
PLCG1	Phospholipase C Gamma 1
PLD1	Phospholipase D1
PLD3	Phospholipase D Family Member 3
PPP1R11	Protein Phosphatase 1 Regulatory Inhibitor Subunit 11
PPP1R12A	Protein Phosphatase 1 Regulatory Subunit 12A
PPP1R12B	Protein Phosphatase 1 Regulatory Subunit 12B
PPP1R13B	Protein Phosphatase 1 Regulatory Subunit 13B
PPP1R14A	Protein Phosphatase 1 Regulatory Inhibitor Subunit 14A
PPP1R15A	Protein Phosphatase 1 Regulatory Subunit 15A
PPP1R2	Protein Phosphatase 1 Regulatory Inhibitor Subunit 2
PPP1R3C	Protein Phosphatase 1 Regulatory Subunit 3C
PPP1R3D	Protein Phosphatase 1 Regulatory Subunit 3D
PPP1R9A	Protein Phosphatase 1 Regulatory Subunit 9A

PPP2CA	Protein Phosphatase 2 Catalytic Subunit Alpha
PPP2R1A	Protein Phosphatase 2 Regulatory Subunit A, Alpha
PPP2R2A	Protein Phosphatase 2 Regulatory Subunit B, Alpha
PPP2R2B	Protein Phosphatase 2 Regulatory Subunit B, Beta
PPP2R2C	Protein Phosphatase 2 Regulatory Subunit B, Gamma
PPP2R2D	Protein Phosphatase 2 Regulatory Subunit B, Delta
PPP2R5A	Protein Phosphatase 2 Regulatory Subunit B', Alpha
PPP5C	Protein Phosphatase 5 Catalytic Subunit
PRKACB	Protein Kinase CAMP-Activated Catalytic Subunit Beta
PRKAR1A	Protein Kinase CAMP-Dependent Type I Regulatory Subunit Alpha
PRKAR1B	Protein Kinase CAMP-Dependent Type I Regulatory Subunit Beta
PRKAR2A	Protein Kinase CAMP-Dependent Type II Regulatory Subunit Alpha
PRKAR2B	Protein Kinase CAMP-Dependent Type II Regulatory Subunit Beta
PRKCA	Protein Kinase C Alpha
PRKCB	Protein Kinase C Beta
PRKCG	Protein Kinase C Gamma
PTGER1	Prostaglandin E Receptor 1
PTGFR	Prostaglandin F Receptor
PTGS1	Prostaglandin-Endoperoxide Synthase 1
PTGS2	Prostaglandin-Endoperoxide Synthase 2
PTPRK	Protein Tyrosine Phosphatase, Receptor Type K
RAC1	Ras-Related C3 Botulinum Toxin Substrate 1
RAC2	Ras-Related C3 Botulinum Toxin Substrate 2
RAC3	Ras-Related C3 Botulinum Toxin Substrate 3
RALBP1	RalA Binding Protein 1
RAP2A	RAP2A, Member Of RAS Oncogene Family
RASA1	RAS P21 Protein Activator 1
RASA2	RAS P21 Protein Activator 2
RASA3	RAS P21 Protein Activator 3
RASGRP3	RAS Guanyl Releasing Protein 3
RASGRP4	RAS Guanyl Releasing Protein 4
RB1	Retinoblastoma 1
RBCK1	RANBP2-Type And C3HC4-Type Zinc Finger Containing 1
RBL1	Retinoblastoma-Like 1
RELA	RELA Proto-Oncogene, NF-KB Subunit
RELB	RELB Proto-Oncogene, NF-KB Subunit
RGS2	Regulator Of G-Protein Signaling 2
RIPK2	Receptor Interacting Serine/Threonine Kinase 2
SB	Saccharomyces boulardii
SIGLEC8	Sialic Acid Binding Ig Like Lectin 8
SKP2	S-Phase Kinase-Associated Protein 2, E3 Ubiquitin Protein Ligase
SLC11A1	Solute Carrier Family 11 Member 1
SMAD2	SMAD Family Member 2

SMAD3	SMAD Family Member 3
SMAD4	SMAD Family Member 4
SMAD6	SMAD Family Member 6
SMAD7	SMAD Family Member 7
SMAD9	SMAD Family Member 9
SOCS1	Suppressor Of Cytokine Signaling 1
SOCS3	Suppressor Of Cytokine Signaling 3
SOCS5	Suppressor Of Cytokine Signaling 5
SOS1	SOS Ras/Rac Guanine Nucleotide Exchange Factor 1
SPP1	Secreted Phosphoprotein 1
ST	Salmonella typhimurium
STAT1	Signal Transducer And Activator Of Transcription 1
STAT2	Signal Transducer And Activator Of Transcription 2
STAT3	Signal Transducer And Activator Of Transcription 3
STAT4	Signal Transducer And Activator Of Transcription 4
TAB1	TGF-Beta Activated Kinase 1/MAP3K7 Binding Protein 1
TANK	TRAF Family Member Associated NFKB Activator
TCF12	Transcription Factor 12
TCF4	Transcription Factor 4
TGFA	Transforming Growth Factor Alpha
TGFB1	Transforming Growth Factor Beta 1
TGFB1I1	Transforming Growth Factor Beta 1 Induced Transcript 1
TGFB2	Transforming Growth Factor Beta 2
TGFB3	Transforming Growth Factor Beta 3
TGFBR1	Transforming Growth Factor Beta Receptor 1
TGFBR3	Transforming Growth Factor Beta Receptor 3
TLR1	Toll Like Receptor 1
TLR2	Toll Like Receptor 2
TLR3	Toll Like Receptor 3
TLR4	Toll Like Receptor 4
TLR5	Toll Like Receptor 5
TLR6	Toll Like Receptor 6
TLR7	Toll Like Receptor 7
TLR8	Toll Like Receptor 8
TLR9	Toll Like Receptor 9
TMEM173	Transmembrane Protein 173
TNF	Tumor Necrosis Factor
TNFAIP3	TNF Alpha Induced Protein 3
TNFAIP6	TNF Alpha Induced Protein 6
TNFRSF10B	Tumor Necrosis Factor Receptor Superfamily Member 10b
TNFRSF10C	Tumor Necrosis Factor Receptor Superfamily Member 10c
TNFRSF11A	Tumor Necrosis Factor Receptor Superfamily Member 11a
TNFRSF11B	Tumor Necrosis Factor Receptor Superfamily Member 11b

TNFRSF12A	Tumor Necrosis Factor Receptor Superfamily Member 12A
TNFRSF13B	Tumor Necrosis Factor Receptor Superfamily Member 13B
TNFRSF13C	Tumor Necrosis Factor Receptor Superfamily Member 13C
TNFRSF14	Tumor Necrosis Factor Receptor Superfamily Member 14
TNFRSF17	Tumor Necrosis Factor Receptor Superfamily Member 17
TNFRSF18	Tumor Necrosis Factor Receptor Superfamily Member 18
TNFRSF19	Tumor Necrosis Factor Receptor Superfamily Member 19
TNFRSF1A	Tumor Necrosis Factor Receptor Superfamily Member 1A
TNFRSF1B	Tumor Necrosis Factor Receptor Superfamily Member 1B
TNFRSF21	Tumor Necrosis Factor Receptor Superfamily Member 21
TNFRSF25	Tumor Necrosis Factor Receptor Superfamily Member 25
TNFRSF4	Tumor Necrosis Factor Receptor Superfamily Member 4
TNFRSF9	Tumor Necrosis Factor Receptor Superfamily Member 9
TNFSF10	Tumor Necrosis Factor Superfamily Member 10
TNFSF11	Tumor Necrosis Factor Superfamily Member 11
TNFSF12	Tumor Necrosis Factor Superfamily Member 12
TNFSF13B	Tumor Necrosis Factor Superfamily Member 13b
TNFSF14	Tumor Necrosis Factor Superfamily Member 14
TNFSF15	Tumor Necrosis Factor Superfamily Member 15
TNFSF18	Tumor Necrosis Factor Superfamily Member 18
TNFSF4	Tumor Necrosis Factor Superfamily Member 4
TNFSF8	Tumor Necrosis Factor Superfamily Member 8
TNFSF9	Tumor Necrosis Factor Superfamily Member 9
TNIP1	TNFAIP3 Interacting Protein 1
TRADD	TNFRSF1A Associated Via Death Domain
TRAF1	TNF Receptor Associated Factor 1
TRIM6	Tripartite Motif Containing 6
TSG101	Tumor Susceptibility 101
UBR4	Ubiquitin Protein Ligase E3 Component N-Recognin 4
VEGFB	Vascular Endothelial Growth Factor B

CHAPTER 1

Introduction
&
Review of Literature

1.0 Introduction

1.1 Innate Mucosal Immunity

A promising new paradigm for the treatment of infectious disease is through the activation of the innate immune system, rather than through direct interaction with the microbe. This strategy harnesses the natural power of the immune responses and may minimize the likelihood of bacterial resistance as the attack is indirect, multi-faceted, and evolutionarily successful. While the roles and processes within the innate immune system are still in the early stages of characterization, it is clear that this branch of immunity has a central role in the defense against microbial challenges. Indeed, for many lower organisms the innate immune system is the sole mechanism of immunological defense [1,2]. Innate immunity is mediated through a complex network of cellular and molecular systems that mediate a spectrum of biological activities which include: up-regulation of chemokines/cytokines and their receptors, recruitment of leukocytes to sites of infection, phagocytic cell activation, activation of extracellular killing mechanisms, stimulation of histamine release, angiogenesis, dendritic cell maturation and wound healing [3,4,5,6]. Through either physiological or therapeutic activation, these responses can be rapidly induced and amplified to mediate potent and non-specific defense against microbial challenge. The ability to treat bacterial infections with antibiotics has been a cornerstone of human medicine for over sixty years. However, the extensive application of antibiotics has served as a selection pressure to drive the evolution of bacterial pathogens resistant to these treatments. There is a growing sense of urgency for the establishment of novel antimicrobials and treatment strategies. A promising new paradigm is through molecules which modulate the innate immune system rather than exert direct antimicrobial activity. These immune-modulatory molecules are

often of biological, but not necessarily of host origin. In this capacity, host defense peptides (HDPs) and probiotics are among the leading candidates to serve as templates for the creation of novel antibiotics or therapeutic regimes via induction of the innate immune system. Based on these premises, we propose an overarching hypothesis for the following study.

Hypothesis: Probiotics and HDPs can sufficiently prime the host innate immunity so that it can combat infectious agents without giving it a chance to evolve.

The ability to identify novel alternatives to antibiotics, which function through the modulation of innate immune responses, from diverse libraries of prokaryotic and eukaryotic antimicrobial peptides and prescribed probiotics, is dependent upon implementation of the appropriate assays, with defined biomarkers, as well as testing in appropriate disease and medically relevant animal models. The therapeutic administration of these molecules will require comparable efforts towards optimization of biological activity, efficient delivery and formulation.

While HDPs are traditionally noted for their antimicrobial activity, our interest is restricted to their immune-modulatory capabilities. It has been demonstrated that different individual peptides have unique capabilities with regards to influencing specific subsets of the immune response; it is therefore important to characterize the different effects of various peptides on the immune system. We anticipate that different types of infections may require different types of peptide treatment, or a combination treatment of peptides, which stimulate appropriate aspects of the innate immune system. A single peptide may not be universally effective against all infections, requiring a degree of specialization between peptides and infections. Due to the drawbacks laid out with regards to treating with a single peptide, we have opted to extensively analyze two highly potent peptides in this study, LL-37 and indolicidin. Additionally, we

screened 4 different probiotics that are most often prescribed by physicians as a therapy or supplement for their immune-modulatory potential. Of the four strains tested through *in-vitro* screening methods, we selected two probiotics - *Bacillus clausii* MTCC8326 (BC) and *Lactobacillus acidophilus* MTCC10307 (LA) for further studies *in vivo*. Our goal was to determine the efficacy of these probiotic agents followed by testing their ability to prevent infection. We have used *Salmonella typhimurium serovar enterica* MTCC3232 (ST) as the infectious agent in this study. We have reviewed the issue of antibiotic resistant super bugs worldwide, with emphasis on India, a country which is especially threatened by this problem. Additionally, we have examined some of the major recent findings in the area of HDPs and probiotics with respect to their involvement in immune modulation before designing the experiments for this project. The details of the review follow.

1.2 Antibiotic resistance by microbes and the need for an alternative treatment strategy

Antibiotic / Antimicrobial resistance is the ability for microbes to resist drugs that would otherwise have growth inhibiting or bactericidal effects. Infections with resistant pathogens are difficult to treat, requiring costly and sometimes toxic alternatives. The decreasing effectiveness of antibiotics in their ability to treat common infections has accelerated in recent years (Table 1.1); with the arrival of untreatable strains of carbapenem resistant *Enterobacteriaceae*, we are at the dawn of a post-antibiotic era [7]. In high-income countries, a continued high rate of antibiotic use in hospitals, the community, and agriculture have contributed to selection pressure that has sustained resistant strains [8], forcing a shift to more expensive and more broad-spectrum antibiotics. In low income and middle-income countries, antibiotic use is increasing

with rising incomes, high rates of hospitalization, and high prevalence of hospital infections. Resistance arises as a consequence of mutations in microbes and the selection pressure from antibiotic use provides a competitive advantage for mutated strains. Suboptimum antibiotic doses help stepwise selection of resistance. The spread of antibiotic resistance is facilitated by inter species gene transmission, poor sanitation and hygiene in communities and hospitals, and the increasing frequency of global, travel, trade, and disease transmission. Most of major disease causing bacteria in India such as *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter aerogenes* are resistant to advanced antibiotics [9] (Table 1.2). Bacteria will inevitably find ways of resisting the antibiotics developed by humans, which is why aggressive action is now needed to keep new resistance from developing and proliferating, and to prevent the resistance that already exists from spreading [7]. Thus, it is essential to explore new paradigms in anti-microbial therapy. One promising approach involves host-directed immune-modulatory therapies, whereby natural defense mechanisms in the host are exploited to enhance the therapeutic efficacy of anti-microbial agents. The objective is to initiate or enhance the protective antimicrobial immunity while limiting inflammation-induced tissue injury [10]. A range of potential immune modulators have been proposed, including host defense peptides (HDPs) and Probiotics which are used in this study as immune modulators.

Table 1. 1 Timeline for antibiotics introduction and unset of bacterial resistance (Data collected from Center for Disease Control and Prevention [7], U.S. department of health and human services)

Antibiotic Name	Year of Introduction	Year of Resistance	Resisting Organism	Disease caused
Penicillin	1943	1940 1965	*Staphylococcus* *Pneumococcus*	Abscesses, sinusitis, Food poisoning

Tetracycline	1950	1959	*Shigella*	Dysentery
Erythromycin	1953	1968	*Streptococcus*	Pharyngitis, Pneumonia
Methicillin	1960	1962	*Staphylococcus*	Abscesses, sinusitis, food poisoning
Gentamicin	1967	1979	*Enterococcus*	Bacteremia, Meningitis
Vancomycin	1972	1988	*Enterococcus*	Bacteremia, Meningitis
Imipenem	1985	1998	*Enterobacteriaceae*	Sepsis, Bacteremia
Ceftazidime	1985	1987	*Enterobacteriaceae*	Sepsis, Bacteremia
Levofloxacin	1996	1996	*Pneumococcus*	Pneumonia, Meningitis
Linezolid	2000	2001	*Staphylococcus*	Abscesses, Sinusitis
Daptomycin	2003	-	-	-
Ceftaroline	2010	2011	*Staphylococcus*	Abscesses, Sinusitis

Table 1.2 Major pathogens and their antibiotics resistance status in India as of 2012 (Data collected from The Center for Disease Dynamics, Economics and Policy[9], New Delhi, India)

Antibiotics	**Measure pathogens and their antibiotics resistance status in India**									
	Staphylococcus aureus	*Salmonella Typhi*	*Salmonella Paratyphi*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Escherichia coli*	*Enterococcus faecalis*	*Acinetobacter baumannii*	*Enterococcus faecium*	*Enterobacter aerogenes/cloacae*
Amikacin				+				+		
Aminoglycosides				+	+	+		+		+
Aminopenicillins		+	+			+	+		+	
Amoxicillin-clavulanate										+
Carbapenems		+	+	+	+	+		+		+
Ceftazidime				+						
Cephalosporins (3rd gen)		+	+		+	+				+
Fluoroquinolones		+	+	+	+	+		+		+
Gentamicin							+		+	
Glycylcyclines										+
Linezolid	+									
Macrolides		+	+							
Oxacillin	+									

Piperacillin-tazobactam				+						+
Polymyxins				+	+	+		+		+
Trimethoprim-sulfamethoxazole		+	+							
Vancomycin	+						+		+	

1.3 Cationic host defense peptides (HDPs) have multifaceted role in immune-modulation and inflammation

HDPs are innate immune effector molecules found in diverse range of species. HDPs exhibit a wide range of functions ranging from direct antimicrobial properties to immune-modulatory effects. Research in the last decade has demonstrated that HDPs are critical effectors of both innate and adaptive immunity [11]. Various studies in the last decade, have demonstrated that several cationic antimicrobial peptides have multifunctional roles as immune effector molecules, provide a link between innate and adaptive immunity, contribute to the resolution of inflammation, maintain homeostasis and aid in wound healing (Table 1.3) [12,13,14]. Various studies have demonstrated that HDPs and their synthetic derivatives, IDR peptides, have multifaceted roles in immunity (summarized in table 1.3). A primary function associated with certain HDPs is in the facilitation of chemotaxis of immune cells. HDPs, e.g. human cathelicidin LL-37 and defensins human neutrophil peptide (HNP)-1, HNP-2, hBD-1 and hBD-2, can either directly or indirectly promote recruitment of different immune cells such as neutrophils, monocytes, immature dendritic cells (iDCs), T lymphocytes, eosinophils and neutrophils to the site of infection. Human cathelicidin LL-37, human α-defensins HNP-1 and HNP-2, murine β-defensins and porcine cathelicidin PR-39 are direct chemo-attractants for cell types such as iDCs, neutrophils and T lymphocytes (Table 1.3). Moreover, at low to modest physiological

concentrations, HDPs such as LL-37, hBD-2 and hBD-3 can promote chemotaxis of immune cells indirectly by inducing the production of chemokines such as MCP-1/CCL2, MIP-1β /CCL4, RANTES/CCL5, MIP-3α/CCL20, Gro-α/CXCL1 and IL-8/CXCL8 from both immune cells and structural cells such as epithelial cells and gingival fibroblasts (Table 1.3). In addition, HDPs such as LL-37 can up-regulate the expression of chemokine receptors such as IL-8RB, CXCR4 and CCR2 in macrophages [15]. Human defensins hBD-1 and hBD-2 chemo-attract dendritic cells (DCs) and T lymphocytes via the chemokine receptor CCR6, which is preferentially expressed on iDCs and memory T cells [16]. Thus, it can be summarized that a critical innate immune function of certain HDPs and IDR peptides is the promotion of immune cell recruitment to the site of infection, which directly contributes to the clearance of infections. Another innate immune mechanism by which HDPs can protect against bacterial invasion is by prolonging the life span of neutrophils. It has been demonstrated that cathelicidin LL-37 and human defensin hBD-3 suppress neutrophil apoptosis (Table 1.3). LL-37 induces the expression of the anti-apoptotic protein BcL-XL and inhibits caspase-3 activity to suppress neutrophil apoptosis [17]. As neutrophils phagocytose and destroy infectious agents, suppressing apoptosis of neutrophils would aid in host defence mechanisms for resolution of bacterial infections. The paradox associated with HDP-mediated immune functions is that even though these peptides promote innate immune effector mechanisms which include certain 'classical' inflammatory responses required for resolution of infections, they also contribute to the resolution of inflammation, thus protecting against the detrimental effect of excessive inflammation. Several in vivo models of infections and sepsis have shown that HDPs such as cathelicidins LL-37 and BMAP-28 and defensin hBD-2, as well as the synthetic IDR peptides IDR-1 and IDR-1002, can modulate host immune responses for the resolution of pathogen-induced inflammation

[18,19,20,21,22]. The anti-inflammatory activity of these cationic peptides appears to be targeted and selective. HDPs such as LL-37 and hBD-3 have been demonstrated to target inflammatory pathways such as Toll-like receptor to NF-κB in the presence of exogenous inflammatory stimuli, resulting in selective suppression of pro-inflammatory responses, while maintaining or enhancing critical immune responses such as cell recruitment and movement and crucial anti-inflammatory mechanisms [23,24].

Even though HDPs have a multifaceted role in modulating immunity, homeostasis, and pathogen clearance it is not very popular because of its high cost [25,26]. Genetically engineering prokaryotic systems to produce recombinant fusion peptides is a potentially cheaper alternative. HDPs have shown to act synergistically with other cationic peptides (i.e., when combined, bovine indolicidin and LL-37 produced a greater-than-additive effect in the reduction of LPS-induced production of TNF-a in the human monocyte-like cell line THP-1) [27]. Because less peptide is required to get the same desired effect, synergistic formulas may help to reduce the overall cost of therapy. Another potential method to increase efficacy is by conjugating it with nanoparticles such as carbon nanotubes (CNTs) and gold nanoparticles (GNPs) for better delivery into the target cell [28]. As part of my Ph.D. work I used LL-37 and indolicidin as immune modulators in a mouse macrophage cell line Raw264.7 and a human monocytic cell line THP-1. I conjugated these peptides in carboxylated CNTs and GNPs through the EDC-NHS conjugation method and characterized the conjugates using various biophysical methods. By this method we have enhanced efficacy of indolicidin by 1000 fold with respect to immune modulation and macrophage protection against *Salmonella typhimurium* induced cytotoxicity [28] (Details in Chapter 3).

Table 1.3 List of HDPs and IDRs and their immune modulatory activity

Peptides	Biological functions	References
LL-37, PR-39, HNP-1, HNP-2, hBD-1 and hBD-2	Direct chemotaxis of cell types such as neutrophils, monocytes, DCs, T cells and eosinophils	[29,30,31,32,33]
LL-37, hBD-2, hBD-3, IDR-1 and IDR-1002	Induction of chemokine expression, e.g. MIP1/CCL4, IL-8/CXCL8, MCP-1/CCL2 and MIP-3/CCL20	[34,35,36,37] [15,19,23,38]
LL-37	Regulation of chemokine receptor expression, e.g. IL-8RB and CXCR4	[15]
LL-37 and hBD-3	Suppression of neutrophil apoptosis	[17,39]
LL-37, IDR-1 and IDR-1002	Induction of anti-inflammatory cytokines, e.g. IL-10 and IL-1RA	[15,38,40,41]
LL-37, IDR-1 and IDR-1002	Suppression of pro-inflammatory mediators, e.g. TNF-α, IL-1, IL-6, MIP-1α and nitric oxide	[15,23,38,40]
LL-37 and hBD-2	Activation of ERK1/2 and p38 MAPK signalling pathways	[37,42,43,44]
LL-37 and HNP-1–3	Modification of DC differentiation, endocytic capacity, phagocytic receptor expression and cytokine secretion	[38,43,45,46]
HNP-1–3	Enhancement of phagocytosis	[47]
LL-37	Induction of autophagy	[48]
LL-37 and hBD-2–4	Activation and degranulation of mast cells	[42,49]
BMAP-28, indolicidin, bactenecin 2A and IDR HH2	Adjuvant-like functions	[50,51,52]
IDR-1002	Protection against immune-mediated inflammation in synovial fibroblasts	[40]
LL-37	Protection against inflammatory shock or sepsis in vivo	[15,53]

1.4 Microbes and physiology

It has long been established that microbes co-exist with humans, inhabiting various bodily surfaces derived from endodermal and ectodermal tissue layers [54,55]. Microbes are present in regions such as the surface of the skin, and within the oral cavity and large intestine, the later hosting the largest population of microbes [56,57,58]. The different species within a given microbial population play various physiological roles, ranging from secretion of metabolites responsible for underarm odor [59], to producing vitamin K which in turn aids in the human blood clotting process [60]. We have co-evolved with our resident microbes, reaping numerous mutual benefits to the extent that it has been suggested that these populations serve as a sort of “microbial organ”. The microbes may be smaller in size at a cellular level, however, they outnumber the total number of cells in our body by at least 1 log in mucosal surfaces [61]. However, it is difficult to count the exact number of microbes present in our body as many of them are non-culturable. Recently developed techniques such as meta-genomics (a high throughput DNA sequencing methodology) have overcome this hurdle, giving us the ability to identify most of the microbes present in our body from mixed samples [62,63].

It has been a common goal of researchers to study and understand the microbial ecosystem that exists within humans and its respective microbiome as they play a pivotal role in our health and well-being. It is believed that perturbation of the normal state of the microbiota present in our system can compromise general health by disturbing homeostasis [64,65]. Immediately following birth, the intestinal tract is inundated with commensal gut microflora, the composition of which aids in maintenance of homeostasis in the gut. There are over 1,000 species of commensals, composing approximately 10^{12} organisms per milliliter of the content within the

lumen of the large intestine [66]; of these, 90 % are obligate anaerobes, comprised of *Eubacterium, Bifidobacterium, Bacteroides, Lactobacillus,* among others [67]. The intestinal environment provides nutrients to the commensals, and in turn the commensals benefit the host by aiding in the absorption of otherwise indigestible nutrients and preventing the overgrowth of potential pathogens within the intestinal lumen [68]. This can occur through several different mechanisms such as secreting antimicrobial substances, providing competition at adhesion sites on the intestinal epithelial cells, or enhancing the mucosal immune system [68].

Of the known commensal bacteria, there are two major species that have been established as probiotics, *Bifidobacterium* and *Lactobacillus*. Probiotics are bacteria that can be consumed or found naturally in the intestine, in an active form that confer beneficial properties to the host [69]. Recently it has been established that there are four different mechanisms by which probiotics benefit the host. The first mechanism of probiotic protection is the ability of the probiotic to normalize or alter gut permeability, which decreases pathogen translocation into the intestinal epithelial cells and thus creates a defense barrier within the gut [70,71]. Secondly, the probiotic may reduce the ability of the virus or other pathogen to survive, and as such, an infection is reduced or eliminated. Thirdly, the probiotic can modulate signal transduction in the gut wall tissue, which can regulate gut homeostasis. Lastly, innate and adaptive immune modulation through altered gene expression can occur due to the probiotics presence in the gut [71,72].

Given that the epithelial lining of the intestine is the first line of defense for intestinal infections, exploration of enhanced innate immunity in the gut through prophylactic probiotic presence is of major interest [73]. Upon pathogen entry into the epithelial cell, the host’s innate immune

response is activated via germ line encoded pattern recognition receptors (PRR) present on and within organelles in the epithelial cell [74]. PRRs recognize pathogen associated molecular patterns (PAMPs) on the virus and bacteria. This recognition of PAMPs by PRRs leads to a change in the gene expression of cytokines and chemokines [71]. These chemokines and cytokines are pro-inflammatory or anti-inflammatory, ultimately regulating the non-specific killing of bacteria and epithelial cells infected with virus, and aiding in the recruitment of immune response cells and host defense peptides [74]. Over a period of days following the induction of certain chemokines and cytokines, the host's adaptive immune response is activated, resulting in the stimulation of T and B cell responses [75]. The activation and response of T and B lymphocytes results in the antigen-specific killing of a new pathogen, efficiently clearing the pathogen with the subsequent induction of memory cells. Memory cells are capable of recognizing future infections that present similar antigens to the initial infection, with the ability to mount an immediate immune response following antigen detection.

Inducing a sufficient adaptive response is not possible without induction of the innate immunity and certain chemokines and cytokines [76]. However, it has been shown that over-stimulation of chemokines and cytokines due to PRR signaling can lead to significant intestinal inflammation, which can lead to colitis [66]. Commensal bacteria are known to stimulate a continuous activation of the host epithelial cells, which leads to continual basal production of factors involved in repairing tissue - thus maintaining homeostasis [66]. This maintenance of homeostasis or increase in the expression of certain genes involved in innate immunity is one mechanism of alleviating the clinical symptoms of pathogen infection [77]. *Lactobacillus plantarum* 299v (Lp299v) is a probiotic that has been shown to aid in passive immunity and mediation of the effects due to viral infection in humans [78]. Prophylactic therapy in humans

has been successfully accomplished using many lactobacillus strains, especially with regards to the alleviation of several diarrhea genic infections [79]. Probiotics are known to be potent modulators of mucosal immunity by reshuffling microbiota. In the next section, we have reviewed known probiotic effects on mucosal immunity and microbiota.

1.5 Modulation of immunity and microbiota in the gut by Probiotics

Probiotics are generally recognized as live microorganisms that confer a health benefit to the host when administered in adequate amounts [80], although, dead bacteria and bacterial molecular components may also exhibit probiotic properties [81]. Strains belonging to *Bifidobacterium* and *Lactobacillus* are the most widely used probiotic bacteria [82], exerting health-promoting properties, including but not limited to, the maintenance of the gut barrier function and the local and systemic modulation of the host immune system [83,84]. Clinical studies have demonstrated the clinical potential of probiotics against many diseases [82] such as allergic pathologies (including atopic eczema [85] and rhinitis [86]), diarrhea [87], inflammatory bowel disease (IBD) [88] and viral infection [84]. However, generalizations concerning the potential health benefits of probiotics should not be made because probiotic effects tend to be strain-specific [84,89]. Several important mechanisms underlying the beneficial effects of probiotics include the modification of the gut microbiota, the competitive adherence to the mucosa and epithelium, the strengthening of the gut epithelial barrier and the regulation of the immune system and inflammation [82,84]. Most of these mechanisms involve regulation of gene expression in specific tissues, particularly the intestine and liver (Table 1.4). Additionally, the expression of mucin genes (MUC) can be affected by probiotics. Likewise, toll-like receptor (TLR) and nucleotide-binding oligomerization domain (NOD) receptor genes,

as well as pro-inflammatory transcription factors, cytokines, and apoptosis related enzyme gene expression can also be affected by commensal bacteria [90] (Table 1.5).

The intestinal epithelium is constantly exposed to high levels of organic material and bacterial antigens. Under normal physiological conditions, the intestinal epithelial monolayer facilitates a controlled and selective flux of components between the lumen and the underlining mucosa [91]. The intestine and the gut-associated lymphoid tissue (GALT) are essential components of the immune defense, protecting the body from foreign antigens and pathogens while tolerating commensal bacteria and dietary antigens. The balance between tolerance and immunity in the intestine is, in part, dictated by antigen-presenting cell (APCs) populations in the gut. The dysregulation of this balance can contribute to the pathogenesis of numerous inflammatory conditions [92]. The inflammatory response in the intestinal tract is abrogated or avoided by the complex and well-regulated tolerance-inducing mechanisms in the GALT. Several cells that are capable of antigen presentation exist in the GALT, including enterocytes and other intestinal epithelial cells (IEC), such as M cells, dendritic cells (DCs), macrophages, and T and B cells [93]. Microbes activate DCs directly via the DCs' pattern recognition receptors (PRR) or indirectly by capturing the apoptotic/necrotic products of other cells that are dying in response to microbial exposure [93]. PRRs are comprised of TLRs, NOD-like receptors (NLRs), adhesion molecules and lectins [84]. Commensal bacteria and probiotics can interact with these cells, thereby exerting immune-modulatory effects. Mammalian epithelial surfaces are colonized by large numbers of microorganisms collectively known as the microbiota. Bacteria typically dominate this microbial ecosystem (>99%), and live as mutualists in close contact with mucosal surfaces [94,95]. The largest density of bacteria is found in the gastrointestinal tract and therefore this interface is widely studied in microbial research. Especially in the large intestine,

bacteria have various functions, including the production of essential nutrients and co-metabolization of food. In addition, they prevent bacterial overgrowth and infection through the formation of an ecological barrier which buffers against microbial colonization and by inducing the host's production of IgA and anti-microbial proteins. Finally, intestinal bacteria influence central physiological functions such as the development of lymphatic tissue, the induction of mucosal tolerance, angiogenesis and fat storage. Defects in host genes controlling bacterial homeostasis or alterations of the gut microbiota composition have been associated with complex diseases, including inflammatory bowel disease (IBD), diabetes mellitus [96] and asthma [97]. Although in some cases complex diseases have been linked with the presence of specific bacteria (e.g. Prevotella in reactive arthritis) [98], it seems likely that bacterial communities, and not specific bacteria, determine susceptibility towards complex diseases, a concept that has been introduced as the 'the pathobiome' [99]. The identification and manipulation of such disease-associated communities will undoubtedly be the topic of intensive research. Interestingly, the composition of gut bacterial communities is genetically determined [100,101]. Although a direct link between the microbiome and disease susceptibility is difficult to establish, proof of concept studies argue that manipulation of the microbiome represents an appealing treatment strategy. Gastrointestinal abnormalities can be improved by probiotic treatment for patients within the autism spectrum [102], and fecal microbiota transplantation is highly effective for the treatment of recurrent *Clostridium difficile* infection [103]. Likewise, disease phenotypes can be transferred by microbiota transplantation. For example, obesity can be transferred successfully using feces from obese humans to lean mice, which was associated with decreased fermentation of short-chain fatty acids, increased metabolism of branched amino acids and decreased transformation of bile acid species as compared to the lean phenotypes [104]. In addition,

simply cohousing lean and obese animals prevented the obese phenotype, an effect that was associated with the transfer of *Bacteroidales*, suggesting a phenotypic impact via horizontal microbial transfer [104].The commercial probiotics and their immune modulatory properties are summarized in the table 1.4, whereas the effect of probiotics on microbial homeostasis is summarized in table 1.5.

Table 1.4 Regulation of immunity and inflammatory gene expression by probiotics

Probiotic strain	Genes involved	References
E. coli Nissle 1917 and VSL#3	Mucins genes	[105]
L. plantarum 299v and *L. rhamnosus GG*	MUC2 and MUC3	[106]
L. plantarum MB452	Tight junction-related genes	[107]
Lactobacilli and *Bifidobacteria* strains	MAPK signaling pathway	[108]
Bifidobacteria strains	NF-κB activation, IL-8, TNF-α , COX-2, and ICAM-1	[109]
B. lactis BB12	NF-κB, MAPK signaling, and IL-6	[110]
B. lactis HN019	IL-8	[111]
Bifidobacteria	IL12p40, IL-1β, TNF-α , and SOCS1	[112]
B. breve strain Yakult and *B. bifidum* strain Yakult	IL-8 and IκB-zeta	[113]
B. breve strains M-16V, NR246 and UCC2003	CASP7, IRF3, A4, APBA1, NOX5, and LIFR	[114]
L. acidophilus	NF-κB signaling	[115]
Bifidobacteria, *lactobacilli*, and *P. freudenreichii*	FCER1A, FCER1G, IL-8, TNF-α , and IL-10	[116]
Saccharomyces cerevisiae CNCM I-3856	PPAR-γ	[117]
E. coli Nissle 1917	MCP-1, MIP-2alpha and MIP-2beta	[118]
L. casei Zhang	TLR2, TLR3, TLR4, and TLR9	[119]
L. plantarum DSMZ 12028	TLR2 and TLR4	[120]
C. butyricum	IL-8, IL-6, and TNF-α	[121]
L. paracasei CNCM I-4034	TLR9, CASP8, and TOLLIP	[84]
L. acidophilus	Genes encoding IFN, TLR-3, and IL-12	[122]
S. boulardii and *B. subtillis B10*	MyD88, NF-κ B, TLR-1, 2, 4, and 15	[123]
Bifidobacteria, *lactobacilli*, and *S.*	TNF-α , IL-1β , IL-6, IL-10, IL-12, and IFN-	[124]

thermophilus THS	γ	
L. rhamnosus 35	IL12, TNF-α, IL1B, IL6, TGFB1, IL-23, and IL-8	[125]
Bifidobacteria and *lactobacilli*	MUC2, MUC3, NAIP, HIAP1/cIAP2, and HIAP2/cIAP1	[126]
L. plantarum, *L. lactis*, and *L. mesenteroides*	IL-1β , IL-8, IL-10, TNF-α , IL-8, TLR5, and IgT	[127]
VSL#3	IL-1β , IL-6, IFN-γ, TNF-α, IL-12, IL-10, TGF-β , and IL-8	[128]
L. rhamnosus GG	TNF-α and IL-1	[129]

Table 1.5 Probiotics and their effects on gut microbiota

Probiotics	Pre-existing Disrupting factor	Claims stated in the paper	Evidence based claim	References
Escherichia coli Nissle	Liver cirrhosis	Restores	More Bifido and Lactobacillus	[130]
Saccharomyces boulardii lyo	Active diarrhoea	Improves	More 'habitual microbiota'	[131]
Lactobacillus plantarum 8PA3 + Bifidobacterium bifidum	Colon cancer	Restores	More E. coli and enterococci	[132]
L. brevis CD2+Lactobacillus salivaris FV2+L. plantarum FV9 L. paracasei Lpc37+L. acidophilus 74-2+Bifido animalis DGCC420	IBS	Restores	More clostridia and Ruminococcus	[133,134]
L. rhamnosus GG+L. rhamnosus Lc705+Propionibacterium freudenreichii shermanii JS+B. breve Bb99	IBS	Restores	More clostridia	[135,136,137]
L. acidophilus 4356+L.	Liver disease	Improves	Less	[137]

plantarum *14917+L. rhamnosus 7469*			firmicutes, more bacteriodetes	
L. acidophilus+L. paracasei +Lactobacillus delbrueckii spp bulgaricus+L. plantarum+B. longum+B. infantis+B. breve	Pouchitis	Altered	More anaerobes	[138]
L. acidophilus+L. paracasei +Lactobacillus delbrueckii spp bulgaricus+L. plantarum+B. longum+B. infantis+B. breve	IBS	Altered	Less bacteroides	[139]

1.6 Plans for this project

As antibiotic resistant bugs are growing in number and we have limited number of antibiotics; there is a growing need for the development of evolution proof mechanism to combat infectious diseases. Host immune modulation is an indirect mechanism through which infectious pathogens can be neutralized. In this project we used HDPs and probiotics as the immune modulators. Preliminary screening for immune modulation was done *in-vitro* with mice and human monocyte/macrophage cell lines. The best immune modulators were further studied *in-vivo* in mice models.

We selected two well-known HDPs, LL37 and indolicidin, to study their immune-modulatory properties *in-vitro* with mice and human macrophage cell lines. Optimal dose and time point of

treatment was selected based on the host cell viability and level of select innate immune gene expressions. With the optimal dose and time, we tried to determine the mechanism of HDP mediated immune modulation by whole genome expression microarray. LL-37 and indolicidin modulate immune genes in macrophage at 20 µg/ml concentration. This is too high a concentration which can not be afforded by the general public to use it as a prophylactic against infectious micro-organisms. In an attempt to increase their efficacy, we conjugated both of these peptides with carboxylated short multi-walled carbon nano tube (SMW-CNT) and gold nano-particles (GNP) for better delivery into the cell. Immune modulatory mechanism of the conjugates was studies through whole genome expression array and was compared with that of free peptides. At last we compared the immune priming efficiency of free peptides as well as conjugates with respect to the protectability of the primed macrophages from *Salmonella* induced cytotoxicity. Our results suggested that immune modulatory efficacy of LL37 and indolicidin was increased by 1000-folds upon conjugation with CNT and GNP.

We selected four commonly prescribed probiotic strains, *Lactobacillus acidophilus* MTCC-10307 (LA), *Bacillus clausii* MTCC-8326 (BC), *Saccharomyces boulardii* (SB) and *Bifidobacterium bifidum* (BF) for this project to study their immune modulatory efficacy in macrophages *in-vitro*. These four probiotic strains were screened based on survivability and select innate immune gene expression in macrophage cell lines. Whole gene expression array was done to study the mechanism of priming of macrophage by probiotics. At last we studied the efficacy of probiotics induced macrophage priming based on macrophage survival against *Salmonella* induced cytotoxicity.

Following the conclusions from our *in vitro* results of HDPs and probiotics, we decided to study the mechanism of action of two probiotics LA and BC further *in vivo* in mouse models. Our goal is to see the effects of LA and BC on Th1 biased C57BL/6 strain and Th2 biased BALB/c strain. Probiotics induced modulation of gut microbiota is one of the major reasons of probiotics action on host health. As the genetic background of BALB/c and C57BL/6 mice strains are different, microbiota of the gut in both of these mice strains might be different, which may trigger dissimilar probiotics action. LA and BC were orally gavaged to the mice with or without *Salmonalla typhimurium*. Gut microbiota purterbation and restoration was determined through the sequencing of gut microbial V3-V4 region of 16S rRNA gene. Host response was determined through whole genenome expression array in gut wall tissue. Gene expression kinetics in both the mice strains were compared to find out the difference in response to the probiotics. The over all health of mice was accessed through histopathology of gut, liver and spleen. Next generation sequencing data of 16S rRNA from both the mice is compared to find out the difference in microbiota purterbation by ST and restoration efficiency of probiotics LA and BC.

Chapter 2

Materials

&

Methods

2.0 Materials and Methods

In this chapter we described the methods we have been used through out the project. The manufacturing company and country of origin of the materials being used in the project is given in round brackets, within the methods. The descriptions of methods were succinct but good enough to convey detailed procedure. Few of the methods were very long and described in annexure 7 and annexure 8.

2.1 Cell culture

The murine macrophage cell line, RAW 264.7 (National Centre for Cell Science, Pune, India were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Himedia, India) supplemented with 10% heat inactivated fetal bovine serum (FBS) (Himedia, India), 2 mM L-glutamine (Himedia, India) and 1X Antibiotic/Antimycotic solution (Himedia, India). Cells were grown in 75 cm2 tissue culture flasks (BD Biosciences) at $37^{o}C$ in 5% CO2 under optimal humidity. The cells were sub-cultured twice a week to maintain them in an actively growing state. When cells were 70% confluent, they were washed with Dulbecco's phosphate buffered saline (PBS) and trypsinized with 0.25% trypsin solution containing 0.001% EDTA (Himedia, India) to produce a single cell suspension.

2.2 Bacteria culture and quantification

Lactobacillus acidophilus (LA) (MTCC-10307, India) was cultured in MRS broth (Himedia, India) whereas Bacillus clausii (BC) (MTCC-8326, India) and Salmonella typhimurium (ST) (MTCC-3232, India) were cultured in nutrient broth (Himedia, India). We determined bacterial

growth curves by measuring colony forming units (CFU) per ml. Bacteria were quantified per ml per specific optical density (OD) at 600 nm. Briefly, 100 µl of bacterial glycerol stock was inoculated into 5 ml broth and placed on a shaker incubator (Innova 42, New Brunswick Scientific, Germany) overnight to establish the primary culture. The next day, 500 µl of primary culture was inoculated into 20 ml of broth to establish a secondary culture. OD of the culture was measured every 30 min at 600 nm to quantify bacterial growth. At each time point, an aliquot of the culture was plated on nutrient agar (Himedia, India) to quantify CFU. OD versus time was plotted to determine the bacterial growth curve and CFU versus OD was plotted to establish a standard curve for bacterial quantification.

2.3 Determining macrophage viability

One million RAW 264.7 cells/well were grown in DMEM supplemented with 10% FBS in six well plates. After 16 h of incubation, cells were treated with either LA or BC at a multiplicity of infection (MOI) of 1, 10, 100 and 500. Cells were trypsinized after 2, 4, 6 and 12 h of bacterial treatment, stained with 0.2% trypan blue (Himedia, India) and counted with a hemocytometer. Time-matched untreated cells and cells treated with ST at corresponding MOIs were used as negative and positive controls.

2.4 Bacterial uptake assay by macrophage; Cell Lysis method

One million RAW 264.7 cells were treated with either LA or BC at MOIs of 1, 5, 10, 100 and 500 for 2 h in antibiotic free DMEM supplemented with 10% FBS. Media was aspirated and cells were washed thrice with PBS to remove free bacteria. Cells were then lysed with 350 µl of

lysis buffer containing 0.025% SDS (Himedia, India) dissolved in PBS. Lysate was adjusted to a final volume of one ml with PBS and a 100 µl aliquot was then plated in nutrient agar and incubated overnight at 37oC. The following day the number of CFU was counted and plotted against the respective MOI to quantify internalized bacteria.

2.5 Bacterial uptake assay by macrophage; Flow-cytometric method

Ten billion viable probiotic bacteria were re-suspended in 1 ml Nutrient broth before adding one 1 µl (25 mg/ml) 5-(and-6)-Carboxyfluorescein Diacetate, Succinimidyl Ester (CFDA/SE) (Invitrogen, USA). The bacterial suspension was placed on a shaker incubator at 37 oC for 2 to 4 h to achieve complete labeling. Bacteria retain CFDA/SE stain for 8 successive divisions [140]. Bacteria were then harvested by spinning at 1500 g for 12 min at 4 ^{o}C and washed thrice with PBS to remove free CFDA/SE. One million RAW 264.7 cells were incubated in suspension for 2 h with CFDA/SE labeled bacteria at MOIs of 1, 5, 10, 100 and 500 for 2 h. Following co-culture, medium was aspirated and cells washed thrice with PBS to remove unattached bacteria. Cells were then trypsinized and fixed with 4% paraformaldehyde (Himedia, India) for 30 min. Fixed cells were again washed 3X with PBS twice before re-suspending in 500 µl FACS buffer supplemented with bovine serum albumin (Himedia, India). Control samples included 1x106 RAW 264.7 cells incubated with unlabeled bacteria at an MOI of 10. Experiment was done using BD FACSCaliburTM (BD Biosciences, USA) with total number of events collected for each experiment is 10,000.

2.6 Bacterial uptake assay by macrophage; microscopic method

Ten thousand RAW 264.7 cells were seeded on a glass cover-slip placed in a 6 well plate for confocal microscopy. Cells were then cultured in a CO2 incubator at 37 oC and 5% CO2 under optimal humidity for 24 h. Following this period of adherence, cells were incubated for 2h with CFDA/SE labeled bacteria at MOIs of 1, 5, 10 and 100. Non-adherent bacteria were removed at the end of 2 h by washing 3X with PBS. Cells were then fixed with PFA for 30 min. Fixed cells were stained by submerging the coverslip in a solution of 300 nM DAPI (Himedia, India) and 1X Cellmask Red (Invitrogen, USA) at pH 7.2 for 15 min. The coverslip was then washed 5X with PBS and mounted on a glass slide with fluormountG (Sigma, USA) under moist condition. The glass slide was kept at room temperature and in the dark for 6 to 12 h to ensure proper mounting. Microscopy was done using Carl Zeiss LSM 780 confocal fluorescence microscope (Germany).

2.7 RNA isolation from mammalian cell and quality control

Following each indicated treatment, cells were harvested and washed twice with PBS. RNA was extracted using the RNeasy mini kit (Qiagen, Germany). Cells were gently lysed with 350 µl RLT buffer, and then homogenized using QIAshredder (Qiagen, Germany). An equal volume of 70% ethanol was added to the homogenate, mixed by pipetting and the solution passed through the RNeasy mini column. The column was then washed once with 750 µl RW1 buffer and twice with 500 µl RPE buffer to remove lipids, protein and DNA from the matrix. RNA was then eluted from the matrix with 50 µl nuclease free water and kept in ice. RNA was quantified spectrophotometrically by Nanodrop 2000 (Thermo Scientific, USA). RNA integrity was checked with a Bioanalyzer2100 (Agilent, USA) using RNA 6000 Nano kits (Agilent, USA). Only samples with an RNA integrity number (RIN) equal to or greater than 8.5 were used for

quantitative real time polymerase chain reactions (qRT-PCR) and microarray analysis of gene expression.

2.8 Reverse transcription and qRT-PCR

c-DNA was synthesized from RNA using AffinityScript One-Step RT-PCR Kit (Agilent, USA). Briefly, 5 μg total RNA was mixed with the buffer containing AffinityScript reverse transcriptase and polyT primer. The mixture was kept in the thermo cycler at 45 ^{o}C for 30 min to synthesize c-DNA. The temperature was then increased to 92 ^{o}C for 1 min to deactivate the enzyme.

qRT-PCR was done using the protocol described earlier [28]. Cycle threshold (Ct) values were noted and fold changes of the desired genes were calculated with respect to control after normalizing with internal control genes such as β-actin. The experiment was done with 3 technical replicates along with no-template-control and no-primer-control. Expression kinetics of selected genes was also checked through qRT-PCR.P Primer Specificity of designed primers were checked from melting curves and PCR products were checked for single products and validated by sequencing.

2.9 Microarray experiments and data analysis

RNA samples for gene expression analysis were labeled using Agilent Quick-Amp labeling Kit (p/n5190-0444 Agilent, USA). Time matched control and treated samples of RNA (500 ng each) containing RNA spike in mixture (p/n5188-5279 Agilent, USA) were incubated with reverse transcription mix primed by oligodT with a T7 polymerase promoter site at 40 °C for 2 h

to synthesize c-DNA. c-RNA was generated by in-vitro transcription by incorporating Cy3 labeled CTP in time matched untreated control samples and by incorporating Cy5 labeled CTP in LA- and BC- treated samples. Labeled c-RNA samples were cleaned and aliquots of the samples were assessed for yield and specific activity. 825 ng of each of Cy3- and Cy5- labeled c-RNA sample was mixed, fragmented and hybridized onto 4x44k microarray slides. Hybridization was carried out in Agilent's Surehyb Chambers at 65°C for 17 h. The hybridized slides were washed using Agilent Gene Expression wash buffers (Part Number 5188–5327, Agilent, USA). Slides were scanned to obtain images. Scanned images were analyzed to extract data using Feature Extraction software Version 10.7 (Agilent, USA) to obtain normalized intensity for each spot corresponding to a gene on the slide. We further obtained normalized fold change values for all genes present on the microarray slides using Arraypipe (v2.0) [141]. Differentially regulated genes at various conditions were functionally clustered using WEB-based GEne SeT AnaLysis Toolkit [142,143]. Pathway coloration and visualization of pathways was done using bioconductor in R [144].

2.10 Salmonella Challenge Study *in-vitro*

RAW 264.7 cells were seeded in 12 well plates with DMEM supplemented with 10% FBS. Each experiment was done in triplicate and included time matched untreated control, unprimed cells challenged with ST at an MOI of 10, and cells primed with probiotic bacteria for 6 h prior to challenge with ST. Cells from each treatment group were harvested at 4 h intervals until 16 h post-challenge, suspended in culture medium and viable cell number determined by trypan blue dye exclusion method.

2.11 Statistical analysis

Student's t-test and 2-way ANOVA were used to calculate the level of significance of different treatment groups with respect to control groups as well as among various groups. All graphs were prepared and statistical analysis was done using GraphPad Prism (V5.04, CA, USA).

2.12 UV-Vis Spectroscopy

UV-Vis spectrophotometry was conducted using NanoDrop2000 (Thermo-Scientific, USA) in the wavelength range of 200 – 550 nm.

2.13 Steady State Fluorescence Spectroscopy

Fluorimetric measurements were obtained using a PerkinElmer (LS55) instrument by exciting the samples at 280 nm and recording the emission spectra in the wavelength range of 290 nm to 600 nm using an excitation and emission slit width of 10 nm and a 1% attenuation of the emission signal in case of the samples corresponding to the CNT-peptide conjugation and at a 7 nm slit width for the GNP-peptide combinations for proper resolution of the spectra.

2.14 Time resolved florescence spectroscopy

Time-resolved fluorescence measurements were carried out using a time-correlated single-photon counting (TCSPC) spectrometer (Edinburgh, OB920). The samples were excited at 280 nm using picoseconds laser diode (EPL), and the signals were collected at magic angle (54.7°)

using a Hamamatsu micro channel plate photomultiplier tube (R3809U-50). The lamp profile was recorded by scatterer (dilute Ludox solution in water) in place of the sample. The instrument response functions (FWHM) of our setup is 840 picoseconds for 280 nm. Decay is analyzed at 358 nm. Decay curves were analyzed by nonlinear least-squares iteration procedure using F900 decay analysis software. The qualities of the fit were judged by the chi square ($\chi 2$) values, and weighted deviations were obtained by curve fitting.

2.15 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR was conducted using PerkinElmer Spectrum RX1 instrument. FTIR spectra measurements were obtained on purified lyophilized samples in the absorbance range from 4000 to 400 cm^{-1} by accumulating 20 scans with a spectral resolution of 1 cm^{-1}. Absorbance spectra were corrected versus a spectrum of potassium bromide, obtained in the same instrumental conditions.

2.16 Binding Isotherm

Free nanoparticles after activation of their carboxyl groups, titrated against increasing concentrations of the peptide yielded a gradual increase in the absorbance and fluorescence intensities till it reached a saturation point. From the saturation binding curve, a corresponding binding isotherm for CNT-Indolicidin and GNP-Indolicidin conjugation was generated in order to calculate the affinity constants of the peptide for the two different nanomaterials and the stoichiometry of binding in each case. In addition, free non-activated nanoparticles were also titrated against increasing concentrations of indolicidin in order to understand the presence of

any non-specific interactions between the free peptide and the functionalized nanoparticles. All analyses were done using GraphPad Prism 5.01 software, CA, USA.

2.17 Isothermal Calorimetry

Investigation of the peptides' potential to interact with free activated nanoparticles was conducted utilizing the GE Healthcare MicroCalTMiTC200 Isothermal Calorimetry apparatus. The settings were with few exceptions, 40 injections of 1µl with a spacing of 300 seconds and an injection time of 5 seconds and baseline setting at 10µcal. Furthermore, a 2000 second delay was applied to get a steady baseline before injection of ligand. From the signal obtained, the thermodynamic parameters related to the binding of the peptide to the carboxyl groups on the nanomaterials were calculated.

2.18 Scanning Electron Microscopy

50 µl of aqueous dispersion of the conjugates and the free nanoparticles were spotted on a silicon chip and spin coated for 3 minutes at 1000 rpm under vacuum. The spin coated samples were baked on a dry bath for 1 minute before they were inserted into the instrument for image analysis. The samples on the chips were observed in electron microscopy with an accelerating voltage of 5.0–6.0 kV.

2.19 Nano treatment of the cell

RAW264.7 murine macrophage cells and THP-1 human monocyte cell lines were obtained from ATCC (Manassas, VA). Both cell lines were maintained in RPMI-1640 (Himedia #AL008)

supplemented with 4.5 gL^{-1} D-glucose, 25mM HEPES, 0.11 gL^{-1} sodium pyruvate, 1.5 gL^{-1} sodium bicarbonate, 2mM L-glutamine and 10% FBS along with 100 units ml^{-1} Gentamycin and 100 pg ml^{-1} Amphotericin-B. For proper comparison, THP1 primary monocytes were differentiated into adherent macrophages by treating them with phorbol-12-myristate-13-acetate or PMA. Following PMA treatment, THP-1 monocytes were incubated for 48 h before treatment with all the different conditions. Around 2 million RAW264.7 cells and differentiated THP-1 cells were seeded into each well in a 6 well plate and were incubated at 37°C and 5% CO2 for 24 h to allow the cells to recover. The medium from each well was aspirated and 3 ml of fresh growth medium was added per each well. To the confluent cell layer, a standardized dose of free indolicidin (20 μg/ml) and a lower dose of indolicidin (0.02 μg/ml) conjugated to CNTs and GNPs were added, and further incubated for 6 h at 37°C. In addition, the cells were also treated with a lower dose of free peptide (0.02 μg/ml) for a relative comparison against the conjugates. The medium was then aspirated from each well and the cell layer was rinsed 3 times with PBS to remove any traces of unwanted treatment samples. About 0.3 ml of 0.1 M Trypsin-EDTA solution was added to each well to detach the cell layer from the plastic. Detached cells were dispersed in 1 ml of complete growth medium and gently pipetted out of the well. The cell suspension was transferred into a centrifuge tube and centrifuged at approximately 300 x g for 5 minutes. RNA was extracted from the cells using the Qiagen RNeasy Mini Kit as per the protocol described in the manual.

2.20 Bacterial protection assay of the nano primed cells

Around 0.5 million differentiated THP-1 cells were seeded into each well of a clean and sterile 12-well plate and incubated at 37°C for 24 h. Confluent cell layer was treated in triplicates with

a standardized dose of free indolicidin (20 µg/ml) and a lower dose of indolicidin (0.02 µg/ml) conjugated to CNTs and not conjugated to CNT. In addition, the cells were also treated with a lower dose of free peptide (0.02 µg/ml) and free CNTs for proper comparison against the conjugates. After treatment, the plates were further incubated for 6 h at 37°C. At a 6 h time point after treatment the THP-1 cells were infected with Salmonella typhimurium at a multiplicity of infection (MOI) of 10. The bacteria were grown to late log phase to ensure the expression of SP-1 genes so that both the invasive and phagocytic mechanisms can occur. After infection, THP-1 cell counts were recorded at 6 h, 12 h and 18 h time-points following trypan blue staining. At each 6 h interval, the infected cells were collected by detaching them from the plastic using 0.1M trypsin-EDTA solution and dispersing them in 0.5 ml of growth medium. The cellular suspension was further diluted and to the diluted suspension, 100 µL of trypan blue stain (1% solution) was added. After vigorous mixing, 10 µL of the cell suspension was loaded onto a haemocytometer and the cell counts at that particular time point were recorded.

2.21 Bacterial Dose Titration for Mice

Mice of 7 weeks of age were divided into 4 groups (n=3) for each bacterial dose titration. Lactobacillus acidophilus MTCC-10307 (LA), Bacillus clausii MTCC-8326 (BC) and Salmonella typhimurium MTCC-3232 (ST) were grown to log phage and orally gavaged with 250 µl of saline water at the dose of 2 x 10^6, 20 x 10^6, 200 x 10^6 and 2000 x 10^6 to their respective group of mice on day zero. Fresh fecal sample was collected once per day from each mouse and checked for the presence of treated bacteria through plating as well as PCR method for 3 days. Health of mice then followed twice every day till any abnormal symptoms observed. If mice was having disease symptoms and about to die, it was sacrificed by cervical dislocation

and tissues as well as gut contents from the duodenum, jejunum, ileum and colon were collected to see the site of colonization of bacteria and for histopahological analysis of the gut sections. Based on the symptoms and histopathology the dose and time points for sample collection were selected. Mice with the ST dose of 2000 x 10^6 have severe disease symptoms and died within 5 days but mice with 200 x 10^6 ST had disease symptoms and died within 11 days. To bring the mouse mortality to a week we increased the ST dose to 500 x 10^6.

2.22 Mice Survivability

The doses selected for probiotics treatment were 2 x 10^9 and 2 x 10^8 for LA and BC respectively. Similarly for ST the dose selected for infection was 5 x 10^8. Mice were divided into 6 groups (n=6 to 9) and orally gavaged with the bacteria described in the table 2.1. The symptoms and mortality is noted and plotted to get the survivability plot.

Table 2. 1 Mice treatment and sample collection plan

Sl. No.	Group Name	Group description	Treatment formulation
1	NT	No Treatment	250 µl saline
2	ST	ST infected	5 x 10^8 ST in 250 µl saline
3	STLA	ST infected followed by LA treated	5 x 10^8 ST and 2 x 10^9 LA in 250 µl saline
4	STBC	ST infected followed by BC treated	5 x 10^8 ST and 2 x 10^7 BC in 250 µl saline

5	LA	LA treated	2 x 10^9 LA in 250 µl saline
6	BC	BC treated	2 x 10^7 BC in 250 µl saline

2.23 Mice treatment and sample collection plan

From the dose titration data we have seen that the ST infected symptoms appear in mice from 4th day onwards. As we are planning to check the host microbiome dynamics as well as host gene expression kinetics in gut, we planned to sacrifice mice and collecting samples one day prior to the disease onset (3rd day) and one day post to the disease onset (5th day). We also kept another longer day (10th day) for observation if any group of mice was being protected. The details of the treatment plan and sample collection is depicted in figure 2.1.

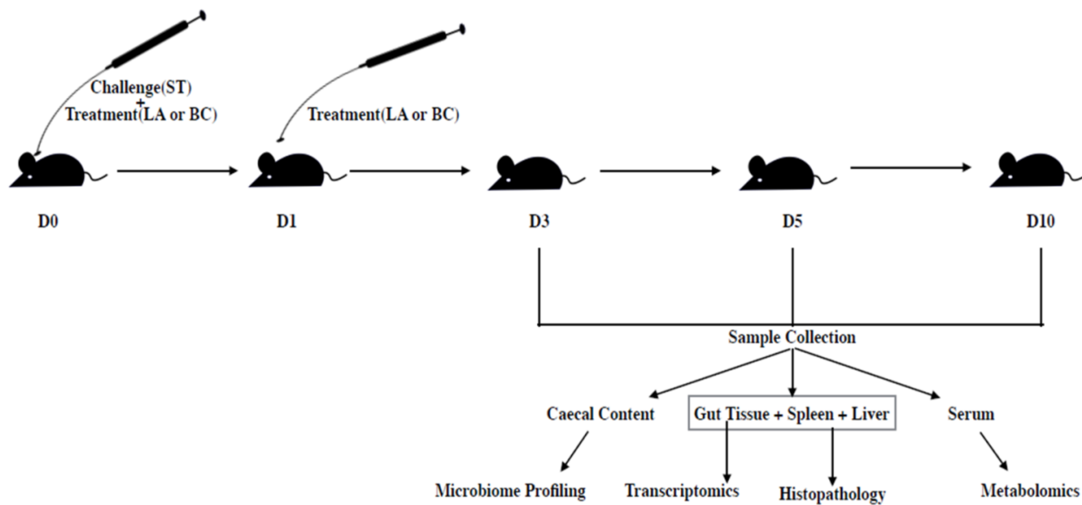


Figure 2. 1 Mice treatment plans and samples collected

2.24 Genomic DNA isolation from the gut content

Genomic DNA was isolated from the gut contents using the protocol standardized in our lab. In brief 200 mg of fresh gut content from -80°C was taken in a 2 ml sterile microfuge tube and 1 ml of sterile PBS is added to it. The sample was mixed properly by vortexing and pipetting. It is then centrifuged at 1500 g for 5 minutes. To the pellet 600 µl of lysis buffer (Tris-HCl 0.1M, EDTA 20mM, NaCl 100mM, 4% SDS) was added and mixed properly by pipetting. The mixture was kept in 70°C for 30 minutes with intermittent mixing by inverting the microfuge tube several times. The lysate was centrifuged at 15700 g in 4°C for 15 minutes. The supernatant was collected in a new 2 ml microfuge tube. To the clear lysate 1 ml of Phenol-Chloroform-isoamyl alcohol (24:24:1) was added and mixed properly by inverting several times. The mixture was centrifuged at 15700 g in 4°C for 15 minutes. The upper aqueous layer is collected in a new microfuge tube. The aqueous layer should be colorless. If there is still some color remains, repeat the above step once more. Add 3 volumes of chilled absolute ethanol to the lysate and gently mix by inverting and rotating simultaneously. Keep the solution in -80°C for 1 hour so that nucleic acid will precipitate. The precipitate was separated out by centrifuging it at 15700 g in 4°C for 15 minutes. The pellet was washed with 70% ethanol by centrifuging it at 15700 g at 4°C for 5 minutes. The pellet was dried in room temperature and solubilized with 100 µl of nuclease free water by tapping it gently. RNAse treatment was given to the nucleic acid and the ethanol precipitation step was repeated to recover the microbial gDNA. The quality and quantity of the gDNA was accessed with Nanodrop2000 and agarose gel electrophoresis.

2.25 Species specific primer designing

We designed species specific primers against 16s rRNA gene to confirm the next generation sequencing data. There is no online software to design it directly, so we followed the following

steps. We collected the sequence of 16s rRNA of the species of interest from NCBI nucleotide [145], align it with the 16s rRNA database of bacteria and archaea using BLAST [146], took the top 25 hits and align it using multiple sequence alignment software MUSCLE [147]. We downloaded the HTML output format of the MUSCLE and searched for the unique bases in our sequence of interest. We fixed the unique base G or C in our sequence of interest as the 3'-OH base and extended towards the 5' end till the melting temperature (Tm) reaches 55oC. We did not fix the length of the primer constant, as done by many of the online primer designing software. The primers are then optimized for their efficiency and specificity through qRTPCR taking different concentrations of template in different reactions (See Chapter 5 for detailed data).

2.26 Histopathological Study

Tissue samples were collected and preserved in 4% para formaldehyde solution at room temperature for 72 hours. For histopathological analysis, tissues were processed for paraffin embedding, and multiple 5-micron sections were prepared. For haematoxylin and eosin staining, slides were deparaffinized and hydrated with deionized water followed by haematoxylin (Sigma) staining for three minutes and eosin for two minutes. Slides were thoroughly washed in H_2O and dehydrated through sequential alcohol grading, then cleared in xylene and mounted with permanent mounting media (Vector Lab). Stained slides were observed under a Leica DM500 light microscope, and representative images were taken at 4x, 10x and 40x magnifications.

2.27 Immuno-histochemistry

Tissue sections and slides were prepared as explained in the previous method. Slides were then deparaffinized and hydrated with deionized water. Antigen retrieval was performed in acidic citrate buffer (Vector Lab) by incubating slides in a steam cooker for 20 minutes. Slides were then washed twice with PBS for five minutes, followed by endogenous peroxidase quenching in 3% H_2O_2 (SRL) for 15 minutes. Nonspecific binding was blocked by incubating the slides with horse serum for 30 minutes, followed by overnight incubation with primary antibody at 4^oC in a humidified chamber. Slides were then washed twice with PBS for five minutes and incubated with biotinylated anti-rabbit/mouse IgG secondary antibody for 45 minutes. Diaminobenzidine (Vector Lab) was used to detect the immunoreactivity. Slides were subsequently counterstained with haematoxylin (Sigma), dehydrated through sequential alcohol grading, cleared in xylene, and mounted with permanent mounting media (Vector Lab). Stained slides were observed under Leica DM500 light microscope, and images were taken.

2.28 RNA isolation from gut wall tissue

RNA isolation was done using Rneasy mini kit (Qiagen, Germany) with little modification to their protocol. Briefly, 20 mg of tissue was gently homogenized with 600 µl of buffer RLT in a homogenizer (Himedia, India). The homogenate was centrifuged for 5 minutes at 2300 g in 4^oC to remove the debris. The supernatant was passed through the Qiashredder (Qiagen, Germany) at 9300 g for 2 minutes. 600 µl of 70% ethanol was added to the clear lysate and mixed well by pipetting. The solution was again centrifuged at 2300 g for 5 minutes to remove the pellets formed. The clear lysate-alcohol solution was passed through RNeasy mini column (Qiagen, Germany) twice at 9300 g for 2 minutes, for RNA binding to the silica column. The column was then washed once with 750 µl RW1 buffer and twice with 500 µl RPE buffer to remove lipids,

protein and DNA from the matrix. RNA was then eluted from the matrix with 50 µl nuclease free water and kept in ice. RNA was quantified spectrophotometrically by Nanodrop 2000 (Thermo Scientific, USA). RNA integrity was checked with a Bioanalyzer2100 (Agilent, USA) using RNA 6000 Nano kits (Agilent, USA). If the concentration of RNA is low, multiple RNA samples from the same tissue were pooled together. Only samples with an RNA integrity number (RIN) equal to or greater than 8.5 were used for quantitative real time polymerase chain reactions (qRT-PCR) and microarray analysis of gene expression.

2.29 16S rRNA gene profiling through V3-V4 sequencing by NGS

2.29.1 Sequence Processing and Bioinformatics Analysis

Stringent quality control of the Illumina reads were performed using NGS QC toolkit to estimate the base call errors. Further, high quality reads from all the samples were merged and subjected to de-replication and de-noising. The de-replicated reads were processed for clustering of OTU's followed by chimera filtering. Hence obtained non-redundant and representative OTUs were annotated up to species level followed by individual sample quantitation. All the above analysis was performed using UPARSE tool [148]. Sequences which could not be classified to at least a kingdom level were excluded from subsequent analysis. Operational taxonomic units (OTUs) were determined at a threshold of phylum (80%), class (90%), order (92%), genus (95%), and species (97%) level.

2.29.2 Imputation of metagenome using different tools

To overcome the challenges in metagenomic data analysis, several standalone software, web servers, and R packages have been developed and are available in the public domain. There are many standalone tools, which may be used for the analysis of 16S rRNA marker gene sequencing. Here, we focus on the popular software, which can be used in studying caecum microbiota. For our samples we had perform the analysis with different tools such as QIIME [149] and UPARSE [148]. QIIME needs some expertise in Linux and Windows systems, and it lacks parallel processing at the OTU picking step. UPARSE investigates microbial diversity using 16S rRNA data. It provides the users with taxonomy assignments along with de-replication and quality filtering of the raw reads generated from Illumina or other platforms. As UPARSE is giving bacterial taxonomy up to genus level we subjected our samples further to get species level classification with the MG-RAST [150] tool.

2.29.3 Statistical Analysis for Biodiversity

OTUs along with their abundance in each sample were parametrically annotated with day parameters such as zero, three, five and ten and condition parameters such as NT, ST, STLA, STBC, LA and BC with each of the parameter being representing at least 3 samples as criteria for statistical analysis. OTUs abundance along with parameters was subjected to statistical and multi-variate analysis using METAGENEassist [151]. Unsupervised hierarchical clustering of OTUs based on parameters was done by applying Pearson un-centered algorithm with average linkage rule using cluster 3.0 software [152] and visualized using Java Tree View software [153]. OTUs enrichment analysis was performed independently by plotting rarefaction curves for all the samples using the MEGAN [154] tool. Parameter specific bacterial diversity was identified by calculating alpha diversity index using the EstimateS [155] tool.

Chapter 3

Immune modulatory efficacy of anti-microbial peptides, LL-37 and indolicidin is enhanced upon conjugation with CNT and GNP; an *in-vitro* study with human and mice monocyte/macrophage cell lines

3.0 HDPs modulate innate immunity; immune modulatory efficacy of HDPs increased upon conjugation with CNT and GNP

3.1 INTRODUCTION

The antimicrobial activity of cationic peptides is mostly elicited via direct interaction with microbes [28,156]. However, direct anti-microbial approaches are often hindered by the tendency for microbes to develop resistance against antimicrobial peptides (AMP) over time. An alternative paradigm of prophylactics or therapeutics would involve activating the innate immune system of the host mucosa through treatment with a sub optimal dosage of AMPs, rather than a direct attack on the microbes. This methodology could alleviate the possibility of microbes developing resistance against AMPs. Keeping this logic in mind, we have compared the antimicrobial activities of two therapeutically potential AMPs with potential medicinal properties, LL-37 and indolicidin, *in vitro*.

Indolicidin belongs from the cathelicidin class of AMPs. Indolicidin is a potent and structurally novel antimicrobial peptide, purified from the cytoplasmic granules of bovine neutrophils. Indolicidin sterilizes suspensions of *Staphylococcus aureus* and *Escherichia coli* at a concentration of 10 µg/ml [157]. Indolicidin is capable of killing gram-negative bacteria by crossing the outer membrane and causing disruption of the cytoplasmic membrane by channel formation [158]. Indolicidin is active against gram-positive bacteria, fungi, protozoa and enveloped viruses such as HIV-1 [159,160]. Apart from direct neutralization of microbes, another important function of indolicidin is its ability to modulate the host innate immune system against infectious agents [11,161]. Indolicidin exerts many immunomodulatory roles, including - but not limited to - chemotaxis, modulation of cytokine and chemokine expression, and leukocyte activation [162,163,164]. Instead of utilizing the direct antimicrobial effects of

indolicidin, its immunomodulatory properties could be exploited to facilitate pathogen clearance in the host. Interestingly, the concentration of indolicidin required to stimulate the innate immune system is comparable to its antimicrobial concentration of 10-20 μg/ml.

Another potent AMP is LL-37, which is of human origin [165]. LL-37 was first detected in leukocytes and the testis [166], thereafter it was found inside a large variety of cells, tissues and body fluids. LL-37 was initially recognized for its antimicrobial properties [167,168,169] as it can exert antimicrobial activity against bacteria, fungi and viral pathogens [170,171]. The level of LL-37 decreases during enteric infection [172] [173]. LL-37 has a strong affinity towards lipopolysaccharides [169,174]; however, LL-37 can neutralize the biological activity of lipopolysaccharides by binding to them with higher affinity [169]. LL-37 shows chemotactic activity towards T-cell leukocytes [175], as well as mononuclear cells and neutrophils [176]. Interestingly, the presence of serum does not affect the chemotactic activity of LL-37 [177] at the particular serum concentration previously shown to inhibit it's *in vitro* antimicrobial effects [167]. LL-37 also plays a significant role in wound healing, angiogenesis and apoptosis [178]. Most importantly, recent studies suggest that it is also involved in the regulation of cancer [179].

Both natural and synthetic AMPs have shown promise as 'next generation antibiotics' due to their unique mode of membranolytic action, which minimizes the development of microbial resistance. However, bacteria have evolved the following mechanisms to counteract AMPs: (i) by a transient induction of bacterial signaling systems that help the bacteria to cope with AMPs, and (ii) constitutive resistance as a result of genetic changes. Currently, there are several putative mechanisms known for bacterial resistance to AMPs [156,180,181,182,183]. When

AMPs are present at higher concentrations, bacteria modulate their cell surface by making it less negatively charged and less permeable [184,185,186].

Despite the apparent medical potential of AMPs, their activity is not clinically practical because of weak activity, nonspecific cytotoxicity and proteolytic effect on some host membrane proteins [187]. For example, indolicidin is cytotoxic for rat and human T-lymphocytes [188]. Also, *in vivo* studies have confirmed that indolicidin is toxic to erythrocytes [159] at a high concentration (10 µg/ml). Indolicidin's immune modulatory efficacy with respect to concentration needs to be increased in order to avoid damage to the host and development of indolicidin resistance in bacteria. Previous studies have demonstrated that immune modulatory efficacy as well as delivery of CpG is enhanced when conjugated with nanoparticles [34,189]. Additionally, we have recently reported that conjugation of indolicidin with small multiwalled carbon nano tubes (SM-CNT) enhanced the efficacy of indolicidin by increasing its ability to protect host cells from *Salmonella typhimurium serovar enterica* (ST) MTCC 3232 [28].

In this present study we have demonstrated the comparative efficacy and *in vitro* functioning of LL-37 and indolicidin conjugated with CNTs. We have studied the effects of free and nano-conjugated indolicidin treatment on the human monocyte cell line THP-1 through transcriptomics. We have also selected LL-37 for our current study as it has already been tested for various immune modulatory effects [15]. Our current results revealed that following conjugation of LL-37 and indolicidin with CNTs, the efficacy of LL-37 and indolicidin was significantly increased *in vitro*. Our results revealed that an effective level of activity for the

peptides is maintained following CNT-conjugation; however, effective treatment required a significantly lower (1000-fold less than free peptide) dosage.

3.2 RESULTS

3.2.1 Characterization of Nano-peptide conjugates

Indolicidin (I) and LL-37 was conjugated with CNT and/or GNP covalently using EDC-NHS chemistry as described before [190]. Conjugated CNT with I (CNT-I), GNP with I (GNP-I) and CNT with LL-37 (CNT-LL37) were characterized by various optical spectroscopy [UV-vis, fluorescence & Fourier transformed infra-red (FT-IR) spectroscopy], scanning electron microscopy (SEM) and isothermal calorimetry (ITC). Results from UV-Vis and fluorescence spectroscopy are shown in figure 3.1 a to c. Comparative FT-IR (figure 3.1 d and e) spectral analysis confirmed conjugation of indolicidin to CNT and GNP followed by validation of the conjugation by thermodynamic analysis (Table 3.1) by ITC. We also compared SEM images (figure 3.1 f to j) for further confirmation and characterization. We observed approximately 2-fold and 5-fold increase in absorbance upon covalent conjugation of indolicidin with CNT and GNP, respectively. Covalent conjugation quenched fluorescence of indolicidin in both cases but it was quenched significantly more for CNT than GNP (figure 3.1 b and c). We observed a blue shift of 18 nm in the fluorescence emission spectrum of GNP-conjugated indolicidin, however this phenomenon was not observed in case of CNT conjugation of the peptide. Fourier transformed infrared spectroscopy confirmed that indolicidin and LL-37 are covalently conjugated with CNT and GNP. Figure 3.1 d and e reveal that the C-O stretch at 1085 cm^{-1} of the COOH groups of indolicidin split into multiple bands at 1051 cm^{-1} and 974 cm^{-1} suggesting amide C-N stretch for CNT-I conjugate. Two other new spectral bands appear at 1120 cm^{-1} and

1226 cm^{-1} representing the C-O stretch. In addition, presence of new bands at 1524 cm^{-1} and 1330 cm^{-1}, corresponding to N-H in-plane and C-N bond stretching respectively, further confirms the presence of a amide linkage. The characteristic C-O stretching peak at 1085 cm^{-1} is fragmented into bands at, 1040 cm^{-1} and 980 cm^{-1} for GNP-I conjugate. The split peaks at 1040 cm^{-1} and 980 cm^{-1} represent C-N stretch present in an amide bond. Conclusive characterization of CNT-LL37 was achieved using FTIR spectra. Appearance of carbonyl (C=O) stretching at 1680 cm^{-1} and amide (C-N) bending at 1645 cm^{-1} in CNT-LL37 conjugate implied that there was formation of a peptide bond between SMW-CNT and LL-37. Within the SMW-CNT and LL-37 spike, a broader peak in the region of 1680 cm^{-1} indicated that there was no disturbance to the carboxyl group of SMW-CNT and the primary amine group of LL-37.The characteristic spectra of the free nanomaterials and the non-conjugated nanomaterials and peptide dispersions were compared with those of the conjugates where the conjugates showed remarkable band shifts and occurrence of newer bands indicating a successful conjugation reaction. SEM images of free CNT, GNP and indolicidin conjugated CNT and GNP were further collected to image the difference between free nanomaterials (CNT and GNP) against the peptide conjugated CNT and GNP (figure 3.1 g to l). Upon conjugation with indolicidin, CNT tends to form clusters (figure 3.1 g to i). Indolicidin was visible as small bulges on the surface of GNP upon conjugation (figure 3.1 j to l). Thermodynamic characterization of the conjugation as well as the spiking processes of SMW-CNT with LL-37 and indolicidin was observed using isothermal calorimetry. The SMW-CNT and LL-37 conjugation process was exothermic but spontaneous, having enthalpy (ΔH) -290.4 ± 2.2 kcal/mole and free energy change (ΔG) -9.98 ± 2.1 kcal/mole. Similarly, the spiking process of the two aforementioned reactants was also exothermic and spontaneous, having enthalpy (ΔH) -62.58 ± 2.6 kcal/mole and free energy

change (ΔG) -5.36 ± 2.63 kcal/mole. Conjugation process of SMW-CNT and indolicidin was exothermic but not spontaneous having enthalpy (ΔH) -3200 ± 483 kcal/mole and free energy change (ΔG) 78 ± 11.7 kcal/mole. Spiking process of SMW-CNT and indolicidin is exothermic but non-spontaneous having enthalpy (ΔH) -100 ± 15.6 kcal/mole and free energy change (ΔG) 4.3 ± 0.65 kcal/mole. Conjugation process of GNP and indolicidin is exothermic and spontaneous having enthalpy (ΔH) -4300 ± 638 kcal/mole and free energy change (ΔG) -128 ± 21.3 kcal/mole. Spiking process of GNP and indolicidin is exothermic and spontaneous having enthalpy (ΔH) -2800 ± 378 kcal/mole and free energy change (ΔG) -28 ± 4.43 kcal/mole. Differential thermodynamics parameters indicated that conjugation and spiking have different reaction processes. Conjugated LL-37 and indolicidin were characterized using various biochemical methods. Conclusive characterization was achieved using FTIR spectra. Appearance of carbonyl (C=O) stretching at 1680 cm^{-1} and amide (C-N) bending at 1645 cm^{-1} in CNT-LL-37 conjugate implied that there was formation of a peptide bond between SM-CNT and LL-37 (Figure 3.1 f). Within the SM-CNT and LL-37 spike, a broader peak in the region of 1680 cm^{-1} indicated that there was no disturbance to the carboxyl group of SM-CNT and the primary amine group of LL-37 (Figure 3.1 f). Similar characterization of indolicidin and CNT conjugate has been reported previously by our group [28].

Thermodynamic characterization of the conjugation as well as the spiking processes of SM-CNT with LL-37 and indolicidin was determined using isothermal calorimetry. The SM-CNT and LL-37 conjugation process was exothermic, having enthalpy (ΔH) -290.4 ± 2.2 kcal/mole and free energy change (ΔG) -9.98 ± 2.1 kcal/mole. Similarly, the spiking process of the two

aforementioned reactants was also exothermic, having enthalpy (ΔH) -62.58 ± 2.6 kcal/mole and free energy change (ΔG) -5.36 ± 2.63 kcal/mole.

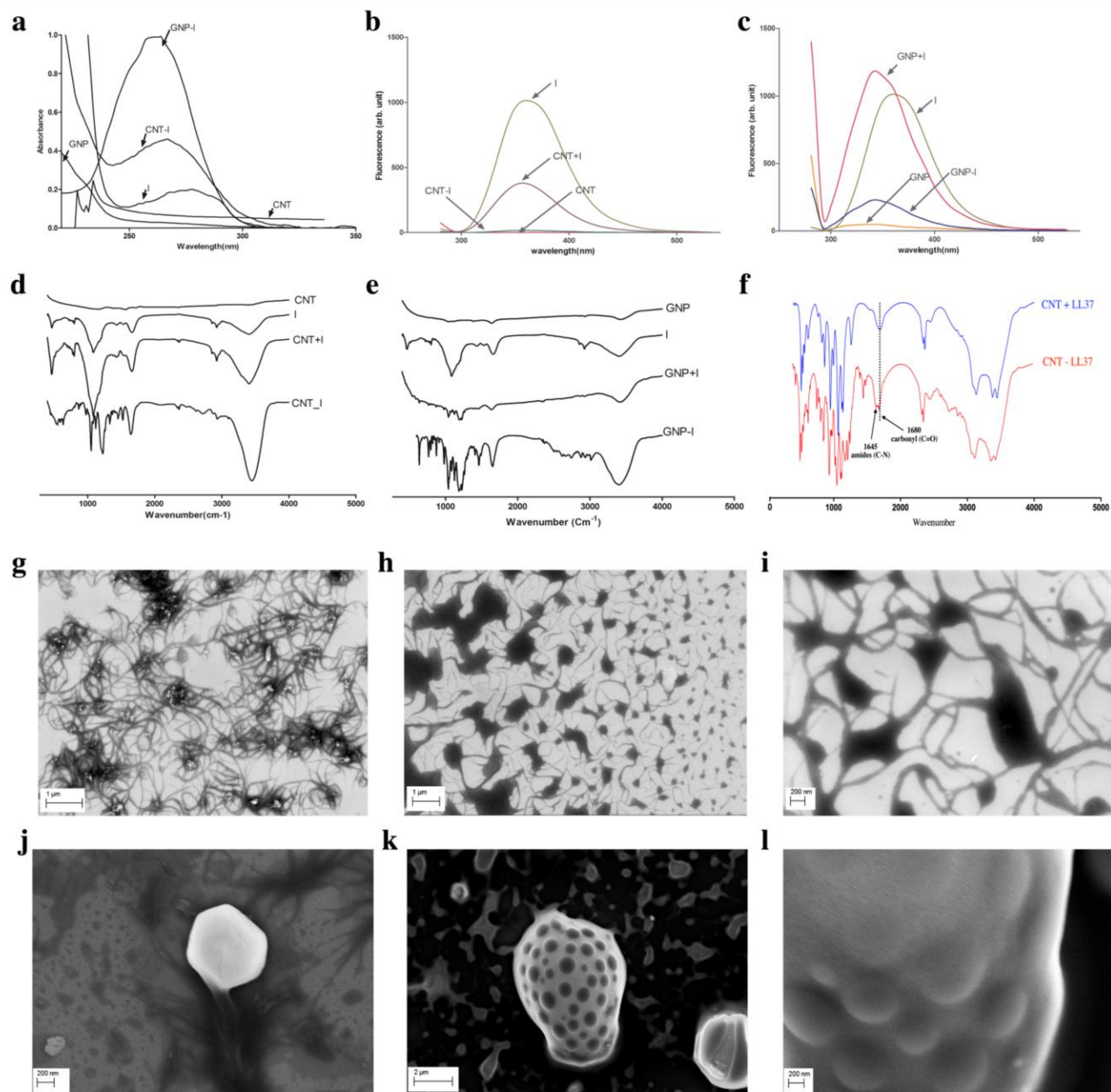


Figure 3. 1 Nano-Peptide conjugate characterization

a.UV-vis absorbance spectrum of the indolicidin conjugate, free peptide and free nano particles, **b.** Florescence emission spectra of CNT-indolicidin conjugate, **c.** Florescence emission spectra of GNP-indolicidin conjugate, **d.** FTIR absorption spectra of CNT-indolicidin, **e.** FTIR absorption spectra of GNP-indolicidin, **f.** FTIR absorption spectra of CNT-LL37 conjugate, **g.** SMW-CNT FE-SEM image, **h, i.** CNT-indolicidin conjugate FE-SEM image (note the clumping property of CNT after conjugated with indolicidin), **j.** Free GNP FE-SEM image, **k, l.** GNP-indolicidin FE-SEM image (note the bulging buds of indolicidin on the surface of GNP)

3.2.2 Cell viability of THP-1 cells treated with free and conjugated LL-37 and indolicidin

Viability of the human macrophage cell line THP-1 and mice macrophage cell line Raw264.7 were determined following treatment with free LL-37 and indolicidin for 6 h. Concentrations up to 50 µg/ml for both AMPs was found to be non-toxic for both the abovementioned cell lines until 6 h post treatment, as determined from cell viability assay (Figure 3.2 a and e).

The viability of macrophage cells after treating them with free SM-CNT, SM-CNT spiked with LL-37 and/or indolicidin and SM-CNT conjugated with LL-37 and/or indolicidin up to 2 µg/ml for 6h were also determined in order to find the toxicity. Our data revealed that none of the above treatments were toxic to the macrophage cell lines THP-1 and Raw 264.7 (Figure 3.2 b and f).

Table 3. 1 Thermodynamic parameters of the nanoparticle and HDP conjugate

Reaction Conditions	ΔH ± SD (Kcal/mole)	ΔS ± SD (Kcal/mole)	ΔG ± SD (in Room Temp.) (Kcal/mole)
CNT-LL37 (Conjugate)	-290.4 ± 2.2	-0.94 ± 0.08	-9.98 ± 2.1
CNT + LL37 (Spike)	-62.58 ± 2.60	-0.19 ± 0.03	-5.36 ± 2.63
CNT-indolicidin (Conjugate)	-3200 ± 283	-11 ± 0.82	78 ± 6.35
CNT + indolicidin (Spike)	-100 ± 12.32	-0.35 ± 0.04	4.3 ± 0.38
GNP-indolicidin (Conjugate)	-4300 ± 39.53	-14 ± 1.25	-128 ± 9.36
GNP + indolicidin (Spike)	-2800 ± 26.35	-9.3 ±1.15	-28.6 ± 3.12

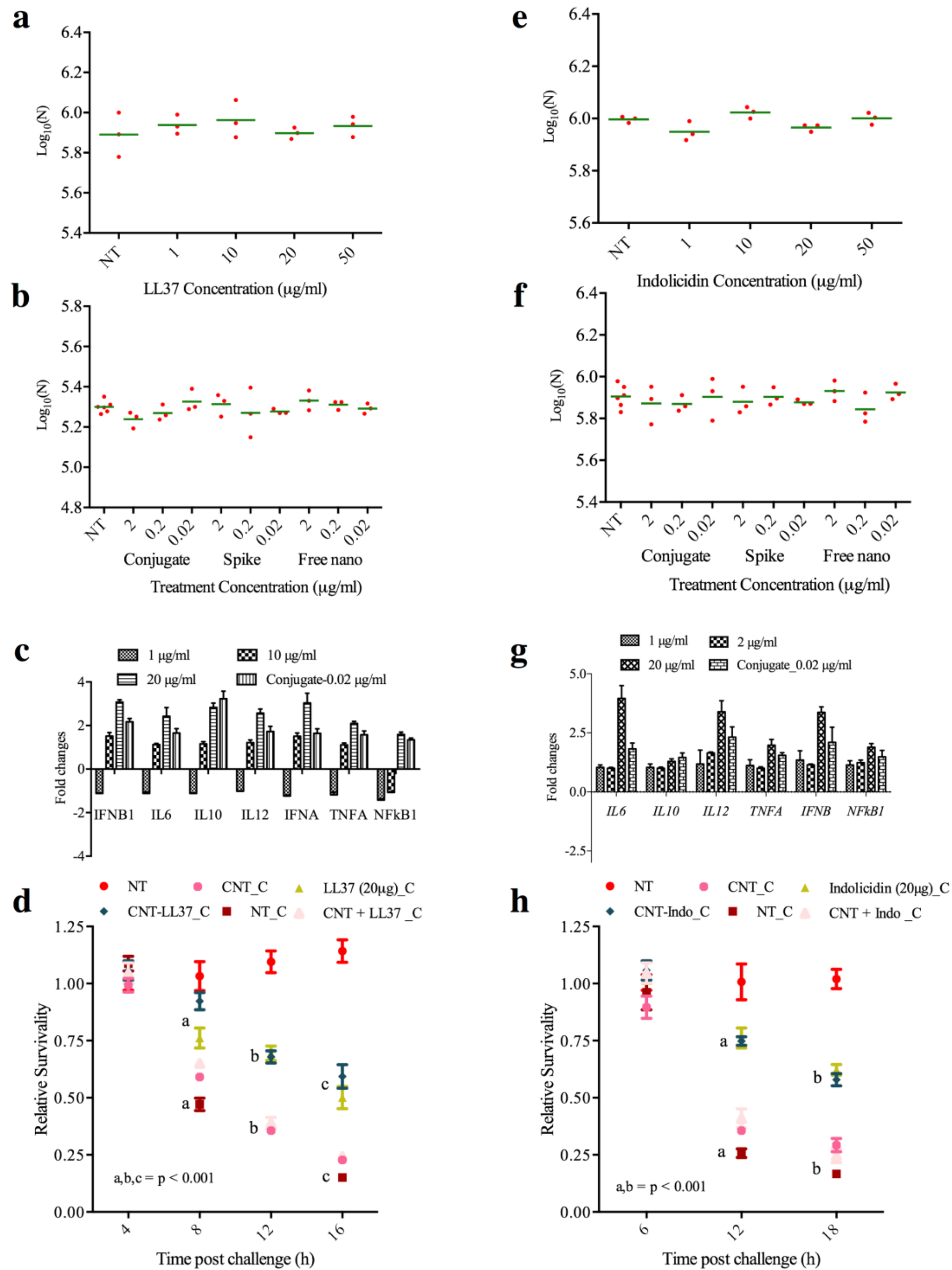


Figure 3. 2 Nanoconjugated HDPs are not toxic to THP-1 cells, but induce immune genes expression and protects the cells from ST induced cytotoxicity

a.LL-37 dose titration for survivability assay, **b**. CNT, CNT conjugated LL-37 and LL-37 spiked CNT is not toxic to Thp1 cells, **c.** Select innate immune gene modulation in Thp1 cells by Free LL-37 and CNT conjugated LL-37, **d**. Free LL-37 at 20 µg/ml and LL-37 conjugate at 0.02 µg/ml Primed Thp1 cells are protected against Salmonella induced cytotoxicity up to 16

hours ($p \leq 0.001$), **e.** Indolicidin dose titration for survivability assay, **f.** CNT, CNT conjugated Indolicidin and Indolicidin spiked CNT is not toxic to Thp1 cells, **g.** Select innate immune gene modulation in Thp1 cells by Free Indolicidin and CNT conjugated Indolicidin, **h.** Free Indolicidin at 20 μg/ml and Indolicidin conjugate at 0.02 μg/ml Primed Thp1 cells are protected against Salmonella induced cytotoxicity up to 18 hours ($p \leq 0.001$).

3.2.3 Expression of a few select innate immune genes in THP-1 following HDP treatment

Following the establishment of AMP toxicity to cell lines, we quickly decided to evaluate the effects of AMP treatment on select innate immune genes to understand AMPs potential as an immune stimulant that facilitates antimicrobial activity. Expression of a few innate immune genes at the transcriptional level in THP-1 cells following 6 h treatment with free LL-37 at concentrations of 1, 10 and 20 μg/ml was determined by qRT-PCR. Experimental data revealed that with increasing concentration of LL-37, the expression at a transcriptional level for the genes *IFNB1*, *IFNA*, *IL6*, *IL10*, *IL12*, *TNFA* and *NFkB1* increased with respect to time matched untreated control (Figure 3.2 c). When the expression of the above-mentioned genes were calculated in THP-1 cells after 6 h treatment with 0.02 μg/ml of conjugated LL-37, it was observed that the expression of these genes was similar (1-way ANOVA, $p \geq 0.05$, $n = 3$) in comparison with free LL-37 which was treated with a higher dose of 20 μg/ml (Figure 3.2 c). Similarly, with increasing dose of free indolicidin, the above innate immune gene expressions increased with respect to time matched untreated controls (Figure 3.2 g). Statistical analysis using 1-way ANOVA ($p \geq 0.05$, $n = 3$) confirms that the gene expression pattern was almost similar in 20 μg/ml indolicidin treated cells with respect to 0.02 μg/ml indolicidin conjugated cells (Figure 3.2 g). It's clear, that both of the conjugated peptides can induce controlled up-regulation to the select innate immune genes at a lower dosage (1000 fold less) than that of the free peptides.

3.2.4 Protection against ST challenge

When innate immune genes are moderately (fold changes up to 5) up-regulated in macrophages, we define the macrophages as primed. Primed macrophages can have the ability to combat bacterial pathogens more efficiently than naive macrophages. To prove this hypothesis, free LL-37 and indolicidin, as well as conjugated AMPs primed THP-1 cells, were exposed to ST with MOI of 10. Result shows that cells primed with free LL-37 at 20 µg/ml and CNT-LL-37 at 0.02 µg/ml were significantly (2-way ANOVA, $p \leq 0.001$, $n = 12$) protected against ST challenge with respect to the unprimed cell population (figure 3.2 d). Primed cell survivability was found to be 80%, 65% and 55%, whereas, unprimed cell survival was 48%, 36% and 14% after 8 h, 12 h and 16 h post ST challenge respectively. Similarly, THP-1 cells primed with free indolicidin at 20 µg/ml and CNT-indolicidin at 0.02 µg/ml were significantly (2-way ANOVA, $p \leq 0.001$, $n = 12$) protected against ST infection (figure 3.2 h). The survivability of primed cells was found to be 75% and 55%, whereas, the survival of unprimed cells was 28% and 17% at 12 h and 18 h post ST challenge respectively (figure 3.2 h). The results from our pathogenic challenge study revealed that conjugating LL-37 and indolicidin with SM-CNT increased their immune modulatory efficacy by 1000 folds. However, the exact mechanism through which priming occurs needs to be elucidated. Therefore, we conducted a genome wide gene expression microarray experiment to find out the detailed mechanism of immune priming.

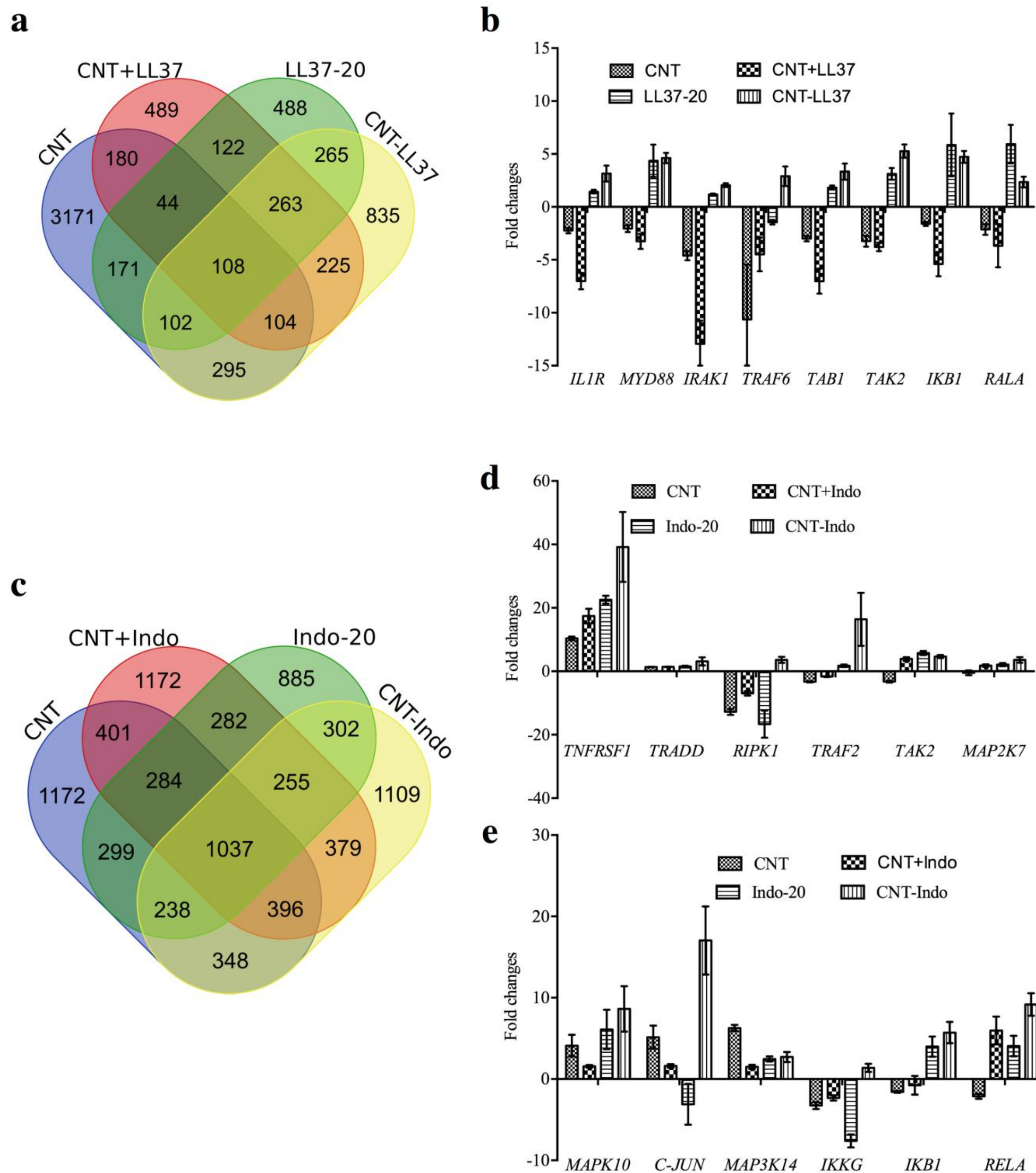


Figure 3. 3 Genome wide gene expression in Thp1 cells following 6 hours treatment with free peptides as well as their conjugates

a. Differentially regulated genes in Thp1 following treatment with LL37 and CNT conjugated LL37, **b.** Validation of few important genes of IL1 signaling pathway through qRT PCR, **c.** Differentially regulated genes in Thp1 following treatment with Indolicidin and CNT conjugated Indolicidin, **d & e**. Validation of genes of TNF signaling pathway through qRT PCR.

3.2.5 Genome wide gene expression and pathway validation

We attempted to understand genome wide transcriptomic profiling by studying gene expression changes in THP-1 cells following treatment with LL-37 and indolicidin at 20 µg/ml and their respective CNT conjugate at 0.02 µg/ml. Cells were also treated with CNT, free peptides and peptides spiked with CNT at their respective conjugate concentrations considered as negative controls. The genes were considered to be differentially expressed if their fold changes were $\geq$ 1.5 with $p \leq 0.05$. There were 3784, 1535, 2197, 1563 differentially expressed and 3171, 489, 488, 835 uniquely expressed genes in THP-1 cells following 6 h treatment with CNT, CNT + LL37, CNT-LL37 and LL37-20 respectively (figure 3.3 a). Similarly there were 3784, 2446, 2221 and 2015 differentially expressed genes were detected in THP-1 cells following 6 h treatment with CNT, CNT + indolicidin, CNT-indolicidin and indolicidin-20 respectively (figure 3.3 c); among them, 1172, 1172, 885 and 1109 were uniquely expressed following treatment with CNT, indolicidin spiked with CNT, CNT-indolicidin and indolicidin-20 respectively.

Table 3. 2 Top 5 enriched pathways in THP-1 following LL-37 and indolicidin treatment

LL-37		**Indolicidin**	
Pathways	**Enriched gene number**	**Pathways**	**Enriched gene number**
AKT signaling	63	Innate immune system	90
Infectious diseases	46	MAPK signaling	54
IGF1R signaling	20	AKT signaling	55
TGFB signaling	20	TNF signaling	21
Cell Cycle	22	Chemokine signaling	26

Table 3. 3 Important genes differentially expressed in THP-1 following 6 h treatments with CNT-LL37

Gene Symbol	Fold changes WRT NT				Entrez gene ID	Gene Function
	CNT	LL37 -20	CNT-LL37	CNT + LL37		
TSG101	2.1	1.0	6.8	1.0	7251	Acts as a negative growth regulator
GRB2	1.5	1.0	5.2	1.0	2885	Links cell surface GFRs and the Ras signaling pathway.
IL9R	1.0	2.6	2.6	2.5	3581	Interleukin-3, 5 and GM-CSF signaling
MAP4K3	1.0	1.7	6.2	1.0	8491	MAPK signaling pathway and TNF signaling
CFLAR	1.0	1.0	6.3	1.0	8837	Acts as an inhibitor of TNFRSF6 mediated apoptosis
ENPP1	3.0	1.0	7.0	1.0	5167	Appears to modulate insulin sensitivity and function
RALBP1	1.0	3.1	4.5	-2.6	10928	Can catalyze transport of glutathione and xenobiotics
SUCLA2	-1.8	4.0	5.6	4.8	8803	Catalyzes succinyl-CoA production
ALOX5	1.0	1.0	6.0	1.0	240	Catalyzes leukotriene biosynthesis and inflammation
MAOB	1.0	4.6	4.3	1.0	4129	oxidative deamination of biogenic and xenobiotic amines
CCL20	5.5	9.2	10.1	1.0	6364	Chemotactic to lymphocytes and neutrophils. Possesses antibacterial activity E.coli and S.aureus
IL33	1.0	1.0	3.5	1.0	90865	Activates NF-kappa-B and MAPK signaling pathways
IL36G	1.0	2.9	3.8	2.7	56300	Activates NF-kappa-B and MAPK

						signaling pathways
SLC2A14	1.0	1.0	4.1	3.3	144195	Facilitative glucose transporter
DEFB105B	1.0	1.0	2.8	1.0	504180	Has antibacterial activity.
DEFA5	1.0	1.0	5.0	1.0	1670	Antimicrobial activity against broad spectrum bacteria.
EFR3A	-25.4	1.0	7.3	1.0	23167	Signaling through PIP3K and G protein couples receptors
PLA1A	1.0	6.2	5.2	1.0	51365	Stimulate histamine production
INO80B	1.0	1.0	2.8	1.0	83444	Cell cycle arrests at the G1 phase of the cell cycle
TLE1	1.0	3.8	5.8	1.0	7088	Inhibits NF-kappa-B-regulated gene expression
SKP2	1.0	2.3	7.1	1.0	6502	involved in regulation of G1/S transition
NLRC4	1.0	1.0	2.9	1.0	58484	Senses specific proteins from pathogenic bacteria and fungi and responds by assembling an inflammasome complex
BTG3	1.0	5.7	6.1	1.0	10950	Blocks cell cycle at G0/G1 to S phase
SLC22A15	5.6	1.0	9.5	9.0	55356	Probably transports organic cations
IL11RA	1.0	3.6	3.2	1.0	3590	Involved in macrophage proliferation and differentiation
OGFOD2	1.0	4.9	6.9	5.4	79676	Iron ion binding and oxidoreductase activity
IL17RE	1.0	1.0	3.9	3.8	132014	crucial regulator in innate immunity to bacterial pathogens
CCNE2	1.0	4.7	4.5	3.2	9134	Blocks cell cycle at the G1-S phase
IRF1	1.0	1.0	4.7	1.0	3659	Regulation of IFNs against viral and

						bacterial infections

Table 3. 4 Important genes differentially expressed in THP-1 after treatment with GNP-LL37

Gene Symbol	Entrez Gene ID	Fold changes wrt NT			
		GNP	LL37-20	GNP-LL37	GNP+LL37
SCCPDH	51097	1.0	1.0	17.5	1.0
ABCB7	22	-1.7	1.0	14.2	1.0
TMCO2	127391	3.7	1.0	10.3	4.6
FAM55D	54827	1.0	8.3	9.8	1.0
PLEKHM1	9842	4.9	7.4	9.5	6.8
CALU	813	3.8	8.6	9.0	9.3
MAP6D1	79929	3.2	4.5	8.2	4.4
PIK3C3	5289	1.0	6.7	8.1	1.0
PDK4	5166	1.0	8.0	7.8	2.6
CNTN2	6900	1.0	6.0	7.7	3.1
ULK1	8408	1.0	1.0	7.3	1.0
GOLGA7	51125	-1.9	1.0	7.2	1.0
SGPP2	130367	1.0	1.0	7.2	1.0
PTGFR	5737	1.0	5.8	7.2	1.0
CLDN16	10686	1.0	5.4	7.0	1.0
TRIP13	9319	1.0	5.1	6.9	1.0
CCDC6	8030	1.0	3.6	6.8	1.0
PABPC5	140886	1.0	4.6	6.7	1.0
MS4A12	54860	1.0	5.6	6.4	1.0

ZFYVE28	57732	1.0	1.0	6.3	1.0
SLC22A10	387775	1.0	4.8	6.2	1.0
ITGA6	3655	1.0	4.9	6.2	3.9
HFE2	148738	1.0	1.0	6.1	1.0
CCL4	6351	2.6	1.0	5.8	1.0
PPP1R17	10842	1.0	1.0	5.7	1.0
RASA3	22821	1.9	1.0	5.7	1.0
LRRC37A4	55073	1.0	5.0	5.7	1.0
HTRA2	27429	1.0	1.0	5.5	1.0
IL36G	56300	1.0	2.9	5.4	2.8
TNFRSF10C	8794	1.0	1.0	5.3	1.0
PRKAR1A	5573	1.0	1.0	4.8	1.0
PTPRK	5796	1.0	6.5	4.7	1.0
SMAD7	4092	1.0	1.0	4.7	1.0
SLC35E3	55508	1.0	5.0	4.6	1.0
MUC5B	727897	1.0	1.0	4.4	1.0
MAOB	4129	1.0	4.6	4.4	1.0
SLC13A5	284111	1.0	5.6	4.3	1.0
IL17RE	132014	1.0	1.0	4.1	1.0
DEFA5	1670	1.0	1.0	3.9	1.0
IL28B	282617	1.0	1.0	3.4	1.0
IL11RA	3590	1.0	3.6	3.0	1.0
TLR3	7098	1.0	1.0	2.8	1.0
JUN	3725	-1.6	1.0	2.4	1.0

IL1RAPL2	26280	1.0	1.8	2.3	1.0

Table 3. 5 Important genes differentially expressed in THP-1 following GNP-Indolicidin treatment

Gene Symbol	Entrez Gene ID	Fold changes wrt NT			
		GNP	GNP + Indo	GNP-Indo	Indo-20
TNFRSF1A	7132	20.9	29.4	62.8	33.2
SOS1	6654	5.2	-1.4	21.2	1.1
NBPF3	84224	2.0	-1.9	19.3	-1.1
CATSPERG	57828	5.8	2.5	11.6	5.4
CALU	813	3.8	9.1	10.8	16.4
STAT2	6773	-2.0	4.7	10.2	6.1
SYNCRIP	10492	3.4	1.1	10.2	1.3
PFKFB1	5207	2.8	1.9	9.1	4.7
CHST9	83539	1.2	2.1	7.4	-1.2
LIF	3976	1.5	-1.5	6.7	3.1
RAB28	9364	1.7	1.3	6.5	2.7
TMEM8A	58986	1.5	1.8	6.2	1.4
PLA2G10	8399	-1.0	-2.1	5.8	-1.4
CREB3L3	84699	1.5	3.2	5.3	4.9
KTN1	3895	-1.2	2.7	5.3	2.8
GPR12	2835	2.6	3.2	5.3	1.9
ALAD	210	-1.1	1.9	5.1	2.5
MASP2	10747	-1.2	1.7	5.1	-1.0

HIF1AN	55662	-1.0	-2.6	5.0	-1.0
ARPC2	10109	2.3	3.4	4.9	4.8
CPT1A	1374	-2.4	3.2	4.6	1.4
ATP13A5	344905	1.1	1.0	4.4	-1.0
GPR173	54328	2.3	1.6	4.3	2.7
LRFN5	145581	-1.3	1.2	4.2	1.1
SIGLEC8	27181	-1.0	1.0	4.1	1.8
VEGFB	7423	-1.4	1.2	4.0	1.5
SLC30A8	169026	-4.3	-1.6	4.0	1.1
TMEM231	79583	1.1	-1.2	3.8	1.7
MC1R	4157	-2.2	-2.6	3.7	1.8
SLC16A1	6566	-1.0	1.8	3.4	-1.0
PPP2R2A	5520	1.8	2.7	3.3	3.3
SLC9A10	285335	1.8	-1.4	3.3	1.5
NFATC2	4773	2.0	1.9	3.2	1.9
MAP4K1	11184	1.4	1.6	3.2	1.3
LRRC32	2615	1.5	2.4	3.2	2.6
CSF2	1437	-2.2	3.8	3.1	2.2
CALML4	91860	-1.2	1.2	3.1	2.8
IL18BP	10068	1.1	-1.0	3.0	-2.4
MUC4	4585	1.4	-1.4	2.9	-1.3
SMAD9	4093	2.0	1.4	2.8	2.6
GPRC5D	55507	-3.0	-1.8	2.8	-1.3
AQP5	362	1.2	1.9	2.8	2.1

CXCR5	643	-1.0	-1.0	2.8	-1.0
DEFB123	245936	1.1	1.0	2.7	-1.8
JUNB	3726	-1.1	-2.4	2.5	1.4
SAMD7	344658	-1.4	1.1	2.5	-3.2
IL17C	27189	2.3	2.7	2.4	1.3
SLC16A13	201232	1.1	1.3	2.3	-1.0
SLC39A11	201266	-1.1	-2.6	2.1	-1.6
SMAD3	4088	-1.1	-1.1	2.0	1.2
SLC13A2	9058	1.0	-3.7	1.9	-3.3

The genes were clustered using GeneAnalytics based on the pathways they represent. The top 5 pathways related to innate immune signaling and cell cycle are listed in table 3.2. Innate immune signaling in THP-1 cells was activated with 90 genes enriched from the list of differentially expressed genes in the indolicidin treatment groups. The LL-37 activated pathway can combat infectious diseases; in the infectious disease signaling pathway, 46 genes were enriched from the differentially expressed gene list. LL-37 also activates TGFb signaling, which indicates feedback suppression of inflammatory pathways. The expression levels of these enriched genes are comparable with CNT conjugated peptide treatment, but at 1000 fold less concentration (Annexure 1 and 2). Important genes with their expression level and function are listed in table 3.3 (for CNT-LL-37) and in table 3.4 (for GNP-LL37). The gene expression profile revealed that conjugated AMPs show similar effects as free AMPs, but at a 1000 fold lower concentration (table 3.3 and table 3.4). It was also observed that when indolicidin spiked with CNT, the gene expression profile was similar to the CNT-indolicidin conjugate. This may be due to indolicidin stacking over the CNT surface via π electron cloud overlap of both the

substances, increasing the effectiveness of indolicidin delivery into the cell through the added hydrophobicity from CNT. Similarly conjugation of indolicidin with nanoparticles enhanced its immune modulatory property by 1000 folds. Important immunological genes differentially expressed in THP-1 following treatment with indolicidin and its conjugates with CNT and GNP were listed in table 3.5 (for GNP-indolicidin) and table 3.6 (for CNT-indolicidin). The expression values of these genes were similar with the free peptide treatment conditions at 20μg/ml and conjugated peptide treatment conditions at 0.02μg/ml.

We tried to populate the pathways with important innate immune genes which were differentially expressed following treatment with both of the conjugates. From the list of differential gene expression, we searched for receptors, adaptors, kinases and transcription factors which are related to immune signaling and match them with the KEGG pathways. It was observed that LL-37 signaling might be going through the interleukin 1 receptor (IL1R) followed by nuclear factor kappa beta 1 (NFkB1) translocation to nucleus with subsequent transcription of proinflammatory cytokines, chemokines and defensins.

Table 3. 6 Important genes differentially expressed in THP-1 following 6 h treatments with CNT-indolicidin

Gene Symbol	Fold changes WRT NT				Entrez Gene ID	Gene Function
	CNT	CNT + Indo	CNT-Indo	Indo-20		
TNFRSF1A	6.5	42.3	29.0	33.2	7132	Activate NFkB, mediated regulator of inflammation
RBCK1	3.4	10.9	6.9	1.0	10616	Activation of canonical NFkB and the JNK signaling
SLC11A1 &	4.4	6.1	6.9	4.3	6556	Transport of glucose and other

SLC5A5	2.2	5.0	8.7	1.8	6528	sugars, bile salts and organic acids, metal ions and amine compounds
ENPP7	5.1	12.1	16.3	8.5	339221	Converts sphingomyelin to ceramide
SOSTDC1	2.8	2.5	5.0	2.5	25928	Enhances Wnt and inhibits TGF-beta signaling
XPR1	1.0	1.6	47.6	-1.9	9213	G-protein coupled receptor activity
S100A5	69.2	67.4	150.4	113.5	6276	Helps in cell cycle progression and differentiation
NAPEPLD	8.0	25.7	22.6	17.7	222236	Responsible for the generation of anandamide, the ligand of cannabinoid and vanilloid receptors
RGS11	2.1	3.0	5.9	1.9	8786	Inhibits signal transduction by G Protein
RGS6	1.0	-1.6	5.9	1.5	9628	Inhibits signal transduction by G protein
RUSC1	1.1	-1.0	5.7	1.6	23623	Activation of the NFkB pathway
IBA57	2.5	4.0	4.9	1.0	200205	Activates iron-sulfur cluster assembly pathway
MYO5B	1.0	-1.3	13.7	-1.0	4645	Vesicular trafficking with the CART complex
AOX1	2.8	2.7	4.7	2.6	316	Regulation of reactive oxygen species homeostasis
RIPK4	3.7	7.3	6.9	1.4	54101	Plays a role in NF-kappa-B activation
FILIP1L	2.0	10.6	14.0	2.2	11259	leads to inhibition of cell proliferation and migration
NCOA4	2.4	5.9	10.6	5.8	8031	Co-activator of the PPARG
CREB3L3	1.7	7.0	4.5	4.9	84699	linked to acute inflammatory

						response
RASSF2	6.5	6.7	12.2	7.3	9770	May promote apoptosis and cell cycle arrest
DAP	2.8	1.7	7.0	2.7	1611	Negative regulator of autophagy
RELA	-1.2	1.7	8.4	-1.2	5970	NFkB pleiotropic transcription factor
CCL20	5.5	1.5	10.9	11.0	6364	Antibacterial activity against E.coli and S.aureus
LYRM4	3.3	3.4	6.2	2.2	57128	Nuclear and mitochondrial FE-S protein biosynthesis
ZDHHC22	17.0	41.5	44.6	44.4	283576	Feedback regulator of Calcium mediated signaling
CYP3A5	3.7	3.6	6.9	3.7	1577	Oxidizes steroids, fatty acids, and xenobiotics

Also, calcium channel (CACNA1B) activation leads to calcium release into the cytoplasm, followed by activation of protein phosphatase 3 catalytic subunit alpha (PPP3CA). Activated PPP3CA dephosphorylates the transcription factor NFAT2 which is subsequently translocated to the nucleus to transcribe genes related to cell proliferation and differentiation. The above pathway is represented in figure 3.4 a. The genes involved in this pathway were also validated through qRT-PCR and represented with their fold changes with respect to untreated time matched controls in figure 3.3 b. It was observed that indolicidin induces a proinflammatory signal, which is transmitted through TNFRSF1A and followed by activation of MAP3K11 and MAP3K14; this signaling cascade activates the transcription factors NFkB1 and c-JUN. This pathway was represented schematically in figure 3.4 b and the genes shown were validated through qRT-PCR and shown with their fold changes with respect to untreated time matched controls in figure 3.3 d and 3.3 e.

3.3 DISCUSSION

Cationic AMPs are non-toxic to cells up to a concentration of 50 μg/ml [23]. Our findings corroborate with this pre-established level of AMP toxicity. Our results revealed that upon LL-37 and indolicidin treatment up to 50μg/ml, human and mice macrophage cells show no toxic effects (Figure 3.2 a and e). The immune modulatory properties of AMPs include modulating pro- and anti-inflammatory responses through [191] various signaling pathways [23] directly [30] or indirectly [192] recruiting effector cells including phagocytes to the site of infection, enhancing intracellular [193] and extracellular [194] bactericidal activity. AMPs also mediate macrophage differentiation [195] which is required for effective clearance of pathogens from the host. AMPs also induce apoptosis [196] and pyroptosis [172] in the infected cells as a means of clearing pathogens. Despite their effectiveness in pathogen clearance, host defense peptides (HDPs) were not popular for clinical usage because of their high synthesis cost [26]. There is a need to improve the efficacy of AMPs and we tried to accomplish this by conjugating LL-37 and indolicidin to carboxylated CNT and GNP. We compared immune modulatory properties of these two peptides in treated macrophages, both in free and nano conjugated states.

3.3.1 Role of LL-37, indolicidin and their CNT conjugates in modulating pro- and anti-inflammation in the THP-1 human macrophage cell line

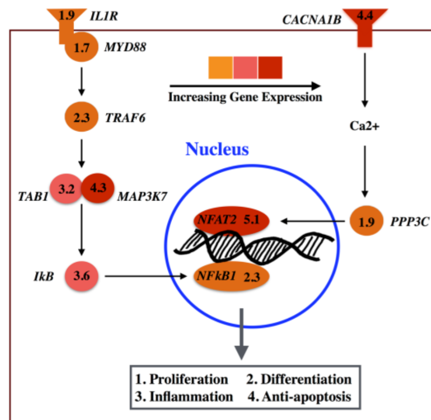
LL-37 activates the canonical NF-kB pathway responsible for modulating the expression of various genes involved in the innate immune system [162]. Our findings revealed that the expression of pro-inflammatory genes that play a critical role in regulating the NF-kB pathway (RELA, TNFRSF1A, TRAF6, ATM and BTRC) are up-regulated upon CNT-LL-37 treatment

to THP-1; notably, LL-37 conjugate treated cells also show almost the same expression pattern, but at 1000 fold less concentration than free LL-37. The expression profile of TNFRSF1A, LELA, RIPK4, RUSC1 and RBCK1 genes in CNT-indolicidin as well as free indolicidin treated cells confirms pro-inflammation mediated by the NF-kB pathway. LL-37 induced signaling through the P^{38} MAPK pathway, followed by activation of genes responsible for macrophage differentiation, pro-inflammation and proliferation [41]. Activation of 36 genes (Annexure 1) related to the P^{38} MAPK pathway upon LL-37 treatment, and up-regulation of many genes (Annexure 2) upon indolicidin treatment confirms that the conjugates stimulate signaling similar to that of the free peptide, but at 1000 fold lower dose. Through the interaction of phosphoinositide 3-kinase (PI3K), NF-kB and MAPK pathways; LL-37 induces IL-1B, followed by pro-inflammation in monocytes and macrophages [197]. Expression of IL1B (Annexure 1) in THP-1 cells following LL-37 and indolicidin conjugate treatment indicates that conjugated AMPs can induce pro-inflammation in macrophages at the same levels stimulated by free peptides, but at a 1000 fold lower concentration. AMPs also induce the production of IL17 and reactive oxygen species (ROS), enhancing the phagocytic activity of macrophages and capacity for clearing pathogens within the phagosome [198]. Expression of IL17RE (table 3.3) in THP-1 cells following treatment with LL-37 as well CNT conjugated LL-37 indicates a similar effect of the conjugate on macrophages. This is a controlled inflammation, indicated by the moderate expression of anti-inflammatory cytokine IL10 along with pro-inflammatory cytokines IL6, IL12, IL1a, IFNa and IFNb (Figure 3.2 c and g) in the conjugate treatments as well as free LL-37 and indolicidin treatments at 20 µg/ml. The primers used to validate few key microarray gene expression values and few innate immune genes were listed in table 3.7.

Table 3.7 list of primers used to validate the gene expression

GENE NAME	FORWARD PRIMER	REVERSE PRIMER	NCBI SEQUENCE ACCESSION NUMBER
C-JUN	TTGGACTGGGTTGCGTCCTG	CGAAAAGTCCAACGTTCCGTTC	NM_002228.3
IKB1	GCTGCTGCTTCAGGCAATTCAGAG	GTGCTTCAGCCACCAGTTCTTCAC	NM_001556.2
IKBKG	ACCGTGCAGTCTGCGCTTTC	GGCCGCATCTACCCCAAAAG	NM_003639.4
IL1R	ATGCCACCGATTGCAGGACA	CCCAGGAGAAAGCAGGTGGAA	M27492.1
IRAK1	GAAGCCCCTGGAAGGCAGAA	CGTCACCAATGCCCAGCTTC	NM_001569.3
MAP2K7	ACTGGGAAGGACCGGGTGAG	TTCTGCGCCTTTGGTGTTGG	NM_001297555.1
MAP3K14	CCCTGGCCAGAGGGTACTGC	AGGCTAAGCTGGGGCAATGG	NM_003954.4
MAPK10	CATCCCAACTTTTCCGGTAGGC	CCAACTGCCCCAAAGGAAGC	NM_138980.3
MYD88	TCTGTCTGCCTGTCCATGTACTTC	CCCAGAGCTATGCTTCACCATTTC	NM_001172567.1
RELA	ATGCAGTTGCGGAGACCTTCTGAC	GGTGCCATTGAGGCATGATGTGAC	NM_002908.2
RIPK2	GATATACCTCACCGAGCACGTATG	GGTGCTATCCCAACTGTGATTTCC	NM_003821.5
TAB1	AGCCTCTGGGGTGCTTGGTC	CGAGGACCCTGGGCTGAGAC	U49928.1
TAK1	AAGCTAGGATCGCCGCAACC	AGGGGTCCATGGATGACTTCG	AF218074.1
TNFRSF1	TCATGCCCGTTTTGGGTGTC	GCTGAAGGCCCCATTGTTCC	NM_001065.3
TRADD	TGGTGGAGGCACTCGAGGAG	CGTGGATGGACAGGGGTTCA	NM_003789.3
TRAF2	TCTGGCCCCTGGAGAGAAGG	TTACCCGCAGGCTGTGCTGT	NM_021138.3
TRAF6	ACCATCAAATCCGGGAGCTGACTG	CCAAGGGAGGTGGCTGTCATATTC	NM_145803.1
IL6	GAACAAGCCAGAGCTGTGCAGATG	TAAGTTCTGTGCCCAGTGGACAGG	NM_000600.3
IL10	GGCATCTACAAAGCCATGAGTGAG	TCAACAGCTAGAAAGCGTGGTCAG	NM_000572.2
IL12	TCTTGGAGCGAATGGGCATCTGTG	TGAAGGCCCATGGCAACTTGAGAG	NM_002187.2
IFNA	CGTGCTGGTACTCAGCTACAAATC	GGCACAAGGGCTGTATTTCTTCTC	NM_021057.2
IFNB1	TAGTAGGCGACACTGTTCGTGTTG	TGGCCTTCAGGTAATGCAGAATCC	NM_002176.2
TNFA	CTGTAGCCCATGTTGTAGCAAACC	CAGGGCAATGATCCCAAAGTAGAC	NM_000594.2
NFKB1	TGGAGGCGGAGGCATGTTTGGTAG	TTCACGTCTCCTGTCACCGAGTAG	NM_003998.3

a



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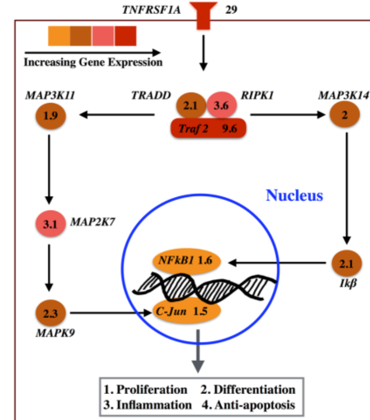

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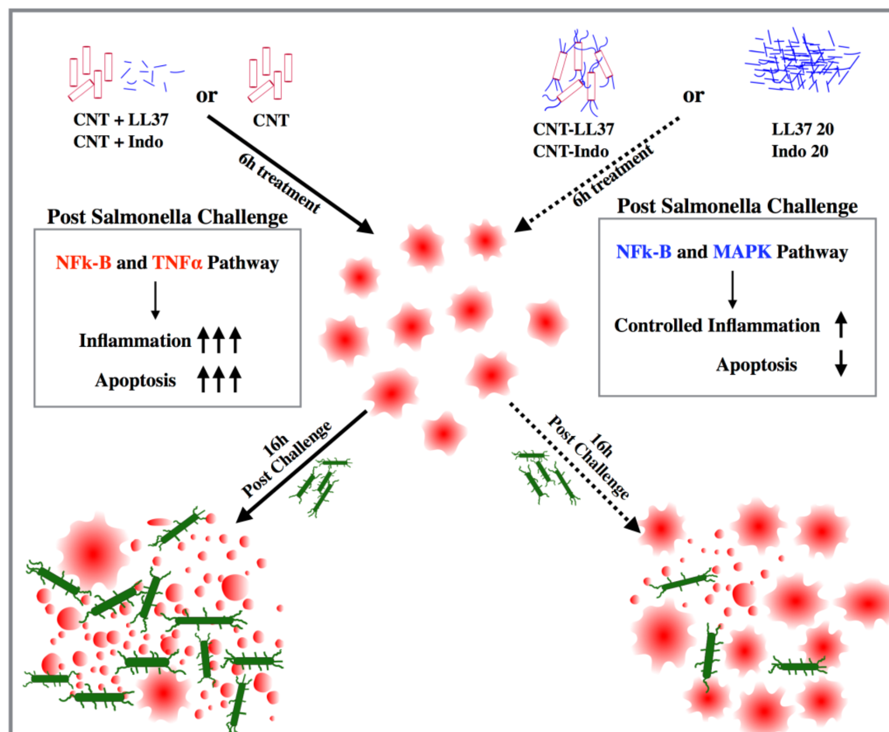

Figure 3. 4 LL37 and Indolicidin signals through NFkB pathway to modulate immunity in Thp1 cells for which it is protected against Salmonella induced cytotoxicity

a. LL37 signals through IL1R followed by activation of NFkB and NFAT to induce proinflammation and survival signal in Thp1 cells, **b.** Indolicidin signals through TNFRSF1A followed by activation of NFkB and c-JUN to induce proinflammation and survival in Thp1 cells, **c.** Graphical representation of the process through which LL37 and Indolicidin primes Thp1 cells through which it is being protected against Salmonella infection

Table 3. 8 LL-37 and indolicidin modulates immune genes and pro-apoptotic genes differently

Gene Symbol	CNT	CNT + Indo	CNT-Indo	Indo-20	CNT + LL37	CNT-LL37	LL37-20
ANAPC11	-2.4	1.2	1.7	-1.4	1	1	1
CCL20	5.5	1.5	10.9	11	1	10.1	9.2
DEFA3	1.1	1	1.6	1.1	1	1	1
IL31RA	1.4	-6.7	1.6	-1	1	4.4	1
NFATC2	3.1	2.1	4.3	1.9	1.7	4.9	12.4
NFATC2IP	1.3	-1.8	2	1.1	1	1	1
PKMYT1	-1	-1.4	1.6	-1.3	1	1	1
RB1	-3.3	-4.1	1.6	1.6	1	1	1
SMAD3	-1.3	-2.3	2.5	1.2	1	2.5	1
TGFBR1	1	-1	1.5	-1.1	1	2.5	1.7
CHEK1	-3	-1.1	-6.5	1.6	1	-3.9	1
CDKN2A	1.5	-1.2	-4.2	-2	1	4.6	4.3
TNFSF4	1.1	1	-3.3	5.5	1	-3.2	-2.9
CDC25C	-1	-1	-2.9	1	-3.6	1	1
CDC14B	-1	-1.3	-2.4	-1.7	-3.8	-7.4	1
GADD45B	1.8	-1.8	-2.1	-2	1	2.2	1.6
TLR1	-4.4	-1.2	-1.4	-1.8	1	1	1
TLR3	1	1	2.3	1	1	2.8	1
TNFAIP3	-2.5	-7	-9	1.6	-4.4	1	1
CCL14	4.3	-1.3	3.9	-1.2	1	1	1

BCL2L2	1.4	2.7	2.5	1.1	1.1	3.2	1.3
Apaf1	4.3	1.1	1.1	1.0	1.2	1.1	1.1

3.3.2 Role of LL-37, indolicidin and their CNT conjugates in modulating chemokine expression in the THP-1 human macrophage cell line

AMPs are chemo attractants for monocytes, neutrophils, macrophages and T-cells. These molecules can induce various chemokines and chemokine receptors in macrophages to attract these immune cells to the site of infection [192,199]. Expression of CCL20, CCL4 and CCL19 (table 3.3) in CNT-LL-37 and CCL20, CCL19, CCL7, CCL4 (table 3.6) in indolicidin conjugate treated THP-1 cells indicates that both conjugates are able to stimulate similar signaling pathways involved in macrophage chemotaxis.

3.3.3 Additional functions of free and conjugated AMPs in THP-1 cells

AMPs induce autophagy in infected macrophages to facilitate the clearance of intercellular debris, which is controlled through ATG5 gene [200]. Expression of ATG5 in LL-37 and its CNT conjugate treated cells results in similar autophagic activity. However, CNT itself is a very efficient autophagic inducer, as CNT treatment shows 16 fold up-regulation of ATG5 in THP-1 cells. Apart from enhancing the efficacy of AMPs, CNT itself has this added effect, which was absent in the case of free AMP treatments. LL-37 and beta-defensin, induce epidermal growth factor receptor (EGFR) signaling, followed by activation of the PI3K-AKT and MAPK pathways responsible for cell proliferation during wound healing [201]. THP-1 cells treated with LL-37 and its conjugate also show activation of the PI3K-AKT and MAPK signaling pathways (table 3.2), as well as the PDGFRA gene (annexure 1).

It's reported that LL-37 delays apoptosis in monocytes and neutrophils by activating G protein coupled receptor (GPCR) mediated signaling [196]. THP-1 cells treated with LL-37 or its conjugate also appear to exhibit active GPCR signaling as we have recorded the expression of GPR180, GPRC5C, GPR174 and GPR3 (annexure 1); similarly, THP-1 cells treated with indolicidin or its conjugate resulted in GPCR signaling as shown by the expression of GPR135, GPR176, GPR112, GPR110, GPR173 and GPR3 (annexure 2). Compared to LL-37, indolicidin imparts more pro-inflammatory effect in THP-1 cells; however, the regulation of inflammation through TGFB and IL10 pathway was stronger in LL-37 treated cells, indicated by the genes listed in table 3.8. The overall mechanism through which LL-37, indolicidin and their CNT conjugates protect macrophages from salmonella induced cytotoxicity was graphically summarized in figure 3.4 c.

Experimental data revealed that, LL-37 or indolicidin primed THP-1 cells can efficiently protect themselves against ST induced cytotoxicity for 16 h post challenge. The genome wide gene expression study shows that pro-inflammatory and anti-apoptotic signaling in THP-1 cells treated with indolicidin was mediated through *TNFRSF1A,* followed by activation of *NFkB* and *c-JUN*. However, LL-37 treatment was mediated through *IL1R,* followed by activation *of NFkB* and *NFAT2*. Though immune modulation by LL-37 and indolicidin was partly known before, our data established the complete gene expression and signaling mechanism. The conjugation strategy enhanced the immune modulating efficacy of these two peptides by 1000 fold, which will reduce the cost of these peptides for antimicrobial treatment, thereby increasing treatment access to a wider population of developing countries. Although LL-37 and indolicidin

conjugation with CNT shows promise with regards to resisting ST infection *in-vitro*, further trials need to be conducted *in-vivo* for better understanding of its working mechanisms.

Chapter 4

Probiotics can prime innate immunity in macrophages and protect it from *Salmonella* induced cytotoxicity; an *in-vitro* study with mouse and human monocyte/macrophage cell lines

4.0 Probiotics primed innate immunity; and protect macrophage from *Salmonella*

4.1 Introduction

Microbiota, a diverse group of microorganisms, resides on all body surfaces, including skin, oral mucosa, conjunctiva and the reproductive and gastrointestinal tracts. They include bacteria, fungi and archaea. The composition and abundance of microbes varies with the site they inhabit [202]. The number of microorganisms that reside in the human gastroenterological tract is around 10^{14}, including over 10^{4} bacterial species [203]. Host physiology and homoeostasis are influenced by the composition of this microbial community [204] which can be perturbed by both internal and external factors. For example, during infection pathogens can perturb the microbiota and alter host physiology [204]. It has been reported that probiotics, a microbial food supplement, can restore this perturbed physiological state of the host [205]. Probiotic bacteria have been shown to interact with a variety of immune cells and intestinal mucosal epithelium to alter host immunity and metabolism. The mechanisms mediating these interactions between probiotics bacteria and host cells remain, however, to be fully defined.

There has been much research on bacteria that cause disease, but the human body contains many bacteria which have beneficial effects on metabolism and multiple organ systems. Probiotic bacteria are defined as those strains with beneficial effects on health, especially through the digestive system [206]. Probiotics are delivered through food and food supplements [207] with doctors prescribing probiotics for digestive problems, to restore antibiotic mediated depletion of microbiome, and to provide nutrients and vitamins [208]. In many countries, taking probiotic

supplemented food is a common practice. Thus, the search to better understand probiotics and their health benefits has intensified since the 1990s.

Two common types of probiotic bacteria include *Lactobacillus* spp and *Bifidobacterium* spp [209]. These bacteria may have beneficial effects through reducing gut permeability, which decreases pathogen translocation across the intestinal epithelial barrier. Some probiotic bacteria may adhere to intestinal epithelial cells and subsequently block pathogen attachment [210]. Probiotic bacteria may also act by reducing pathogen survival in the gastrointestinal tract. For example, some Lactobacilli strains produce acetic acid, lactic acid and propionic acid, which lower the local pH. This lower pH can inhibit the growth of a wide range of Gram negative pathogenic bacteria [211]. Probiotic bacteria also modulate cell signal transduction pathways, which regulate gut homeostasis. Microbial-associated molecular pattern (MAMPS) on probiotic bacteria can interact with pattern recognition receptors (PRRs) on host cells. These PRRs play an important role in maintenance of immune homeostasis [212,213]. Furthermore, there is growing evidence that this interaction between probiotic bacteria and the innate immune system can have effects at both a systemic and mucosal level. Treatment of lipopolysaccharide (LPS) activated Caco-2 epithelial cells with specific strains of Lactobacilli reduced IL-23 secretion and modulated IL-17 production [214]. Similar results were also observed when peripheral blood mononuclear cells (PBMCs) were co-cultured with intestinal epithelial cells that had been treated with probiotics [214].

Intestinal macrophages are key players in mucosal immune responses, leading to either activation of inflammatory responses following infection with a pathogen or tolerance to luminal content such as food antigens and commensal bacteria [215]. Probiotic bacterial strains have been reported to modulate macrophage functioning by activating pro-inflammatory cytokines in macrophages, such as the human Thp1 monocyte cell line and the murine RAW 264.7 macrophage cell line [216]. Activated or primed macrophages more efficiently kill pathogenic bacteria [217] but pathogens can compromise effector functions in unprimed macrophages to promote their own survival [218]. It has been reported that probiotics can prime macrophages to protect against pathogenic infection [219] but the effect of probiotics may be strain specific. Thus each probiotic candidate must be tested to identify specific biological activities. In the current investigation, we evaluated the macrophage activation properties of two probiotic strains, *Lactobacillus acidophilus* MTCC-10307 (LA) and *Bacillus clausii* MTCC-8326 (BC) using a murine macrophage cell line, RAW 264.7. We compared genome wide transcriptomic expression in macrophages following treatment with LA and BC. *Salmonella typhimurium* serovar enterica MTCC-3232 (ST) was used as a control non-probiotic or pathogenic bacteria to further distinguish LA and BC probiotic induced gene expression. LA and BC primed macrophages were then challenged with ST to determine the protection efficacy of these potential probiotic strains against a pathogen. Our analysis confirms that LA and BC can be considered as probiotic strains that modulate both metabolic and innate immune functions in macrophages.

4.2 RESULTS

4.2.1 Effect of LA, BC and BF on mice and human macrophage at MOI1

We treated human monocytic cell line THP-1 and mice monocytic/macrophage cell line Raw264.7 with the probiotics strains LA, BC and BF at multiplicity of infection 1 for 6 hours and followed the differentially expressed genes against time matched untreated control through expression microarray. In case of THP-1 there are 221, 159 and 129 genes expressed differentially in LA, BC and BF treatments respectively. 5 genes expressed in common whereas 185, 102 and 86 genes are expressed uniquely in LA, BC and BF treatment respectively (figure 4.1 a). Similarly in Raw264.7 cell there are 734, 3915 and 265 genes expressed differentially in LA, BC and BF treatments. 35 genes expressed in common whereas 505, 3604 and 105 genes are expressed uniquely in LA, BC and BF treatment respectively (figure 4.1 b). We validated a few of the immune genes through quantitative real time polymerase chain reaction (figure 4.1 c). The genes are clustered based on the pathways they constitute for each treatment conditions using geneanalytics from genecards.

The major pathways enriched by the differentially expressed genes in the case of LA treatment in mice are integrin signaling pathway, G protein coupled receptor signaling pathway, interleukin 3, 5 and GM-CSF signaling pathway and ERK signaling pathway. The significant immune genes expressed in Raw264.7 following LA treatment are chemokine ligand CCL1 (11.4 fold), chemokine receptor CCR6 (1.7 fold) which indicate the immune priming of the macrophage. However down-regulation of interleukins IL7 (-2.1 fold) and IL23A (-1.5 fold) indicate the anti-inflammatory status in macrophage.

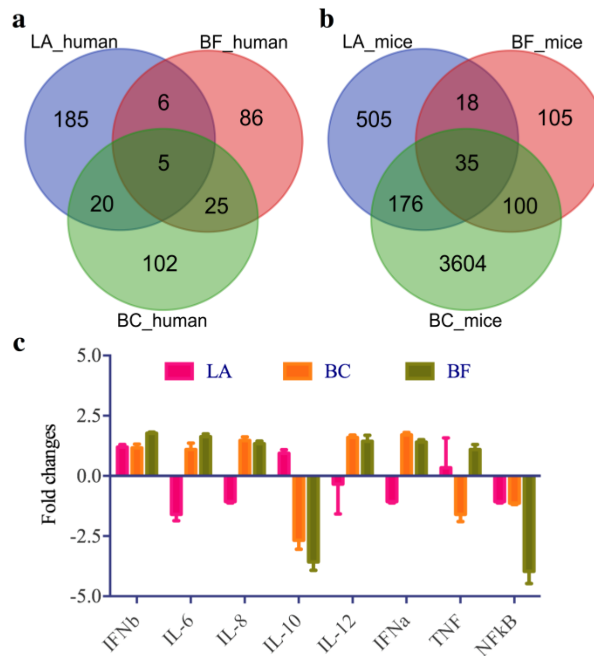

Figure 4. 1 Differential expression of genes in THP-1 and Raw264.7 following probiotics treatment for 6 hours

a. Genes differentially expressed in THP-1, **b.** Genes differentially expressed in Raw264.7, **c.** Expression of few select innate immune genes in Raw264.7 following probiotics treatment determined through qRTPCR.

Interestingly the expression of ATPase H^+ transporting V_0 subunit A1 ($ATP6V_0A1$) (1.5 fold) which is a known acidifier of the lysosomal vacuoles during pathogen clearance indicates the state of priming of mice macrophage in LA treatment. The important differentially expressed genes and their corresponding fold changes are listed in the table 4.1.

Table 4. 1 Important genes expressed differentially in Raw264.7 following LA treatment for 6h at an MOI1.

Gene Symbol	Gene Name	Fold changes
ATP6V0A1	ATPase H+ Transporting V0 Subunit A1	1.5
BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase	2
CAB39L	Calcium Binding Protein 39 Like	1.7

CAMK2D	Calcium/Calmodulin Dependent Protein Kinase II Delta	1.8
CASP7	Caspase 7	-1.6
CCL1	C-C Motif Chemokine Ligand 1	11.4
CCR6	C-C Motif Chemokine Receptor 6	1.7
CHGA	Chromogranin A	1.6
CTTN	Cortactin	1.5
FGF13	Fibroblast Growth Factor 13	1.6
GNG4	G Protein Subunit Gamma 4	29.8
HNF1A	HNF1 Homeobox A	1.6
IL23A	Interleukin 23 Subunit Alpha	-1.5
IL7	Interleukin 7	-2.1
PPP1R12B	Protein Phosphatase 1 Regulatory Subunit 12B	12.5
RAP2A	RAP2A, Member Of RAS Oncogene Family	1.6
TCF12	Transcription Factor 12	2.2
TCF4	Transcription Factor 4	1.6
TNS1	Tensin 1	1.6

The important enriched pathways in THP-1 following LA treatment are type II interferon signaling, epidermal growth factor 1 signaling, IL2 signaling and TGF-b signaling. Expression of catenin beta 1 (CTNNB1) (1.5 fold), and N-meristoyltransferase 2 (NMT2) (1.5 fold) indicated the activation of THP-1 monocytic cells to macrophage. Expression of C-X-C motive chemokine ligand 10 (CXCL10) (1.6 fold), defensing beta 129 (DEFB129) (1.7 fold), and interferon regulatory factor 9 (IRF9) (1.5 fold) indicated the immune priming status of human

macrophage. The important differentially expressed genes and their corresponding fold changes are listed in the table 4.2.

Table 4. 2 Important genes expressed differentially in THP-1 following LA treatment for 6h at an MOI1.

Gene Symbol	Gene Name	Fold change
ANAPC7	Anaphase Promoting Complex Subunit 7	1.5
CTNNB1	Catenin Beta 1	1.5
FOXO4	Forkhead Box O4	2.4
CREB1	CAMP Responsive Element Binding Protein 1	1.6
IRF9	Interferon Regulatory Factor 9	1.5
ABI1	Abl Interactor 1	1.5
PLD1	Phospholipase D1	2
CXCL10	C-X-C Motif Chemokine Ligand 10	1.6
ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1	2.1
DEFB129	defensin, beta 129	1.7
NLRP5	NLR family, pyrin domain containing 5	1.5
NMT2	N-myristoyltransferase 2	1.5
BTN3A2	butyrophilin, subfamily 3, member A2	1.5

Major pathways populated by the differential genes expressed in mouse macrophage cell line Raw264.7 following BF treatment are chromatin organization, cell cycle, packaging of telomere ends, immune response through integrins and regulation of actin cytoskeleton. Expression of chemokine ligand 2 (CCL2) (1.5 fold), interleukin enhancer binding factor 3 (ILF3) (1.5 fold), and inhibitor of kappa beta kinase gamma (IkBKG) (1.5 fold) indicated the immune priming of

macrophage through the NFkB pathway. Down-regulation of check point kinase 2 (CHEK2) (-2 fold) indicated that BF does not interfere with the cell cycle progression of mice macrophage. The important differentially expressed genes and their corresponding fold changes are listed in the table 4.3.

Table 4. 3 Important genes expressed differentially in Raw264.7 following BF treatment for 6h at an MOI1.

Gene Symbol	Gene Name	Fold Changes
Irf6	interferon regulatory factor 6	3.3
Gpx8	glutathione peroxidase 8	1.8
Nqo1	NAD(P)H dehydrogenase, quinone 1	1.8
Cox17	Cytochrome C oxidase 17	2
Rgs2	regulator of G-protein signaling 2	1.6
Ikbkg	inhibitor of kappaB kinase gamma	1.5
Ubr4	ubiquitin protein ligase E3 component n-recognin 4	1.5
Ccl2	chemokine (C-C motif) ligand 2	1.5
Ilf3	interleukin enhancer binding factor 3	1.5
Chek2	checkpoint kinase 2	-2

Important pathways enriched by the differentially expressed genes in THP-1 following BF treatment are G- protein coupled receptor signaling, TGFb signaling, IL5 signaling, IFNa and IFNb signaling and immune cell transmigration signaling. The major genes related to immunity and metabolisms are listed in table 4.4. Expression of CTNNB1 (1.7 fold) indicated the activation of monocyte to macrophage for transmigration to the site of infection. However down-regulation of CCL23 (-1.5 fold), IL18 (-1.5 fold) and TNFRSF1B (-2.1 fold) indicated the

induction of anti-inflammatory status in THP-1 following BF treatment. The important differentially expressed genes and their corresponding fold changes are listed in the table 4.4.

Table 4. 4 Important genes expressed differentially in THP-1 following BF treatment for 6h at an MOI1

Gene Symbol	Gene Name	Fold Changes
CTNNB1	catenin (cadherin-associated protein), beta 1	1.7
SLC12A2	solute carrier family 12 (sodium/potassium/chloride transporters), member 2	1.6
ACTN1	actinin, alpha 1	1.6
ITGB1	integrin, beta 1	1.5
GPR172B	G protein-coupled receptor 172B	1.5
CCL23	chemokine (C-C motif) ligand 23	-1.5
IL18	interleukin 18	-1.5
TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B	-2.1

The major pathways enriched by the differentially expressed genes in THP-1 cells following BC treatment are CXCR3 mediated signaling, innate immune signaling, type II interferon signaling, blood brain barrier and immune cell migration signaling and TGFb signaling. Important genes and the corresponding fold changes are listed in the table 4.5. Expression of CTNNB1 (1.7 fold), early growth response 1 (EGR1) (3.3 fold) indicated the activation of THP-1 monocyte into macrophages. Similarly expression of immune genes like CXCL10 (1.6 fold) and TNFSF15 (2.1 fold) indicated the induction of pro-inflammatory status in THP-1. Expression of several G protein coupled receptors, such as GPR31 (1.7 fold), GPR150 (1.5 fold) and GPR153 (1.6 fold)

indicated the signaling through GPCR by BC in THP-1 cells. The important differentially expressed genes and their corresponding fold changes are listed in the table 4.5.

Table 4. 5 Important genes expressed differentially in THP-1 following BC treatment for 6h at an MOI1.

Gene Symbol	Gene Name	Fold Changes
CTNNB1	Catenin Beta 1	1.7
SPP1	Secreted Phosphoprotein 1	1.9
ACTN1	Actinin Alpha 1	1.5
ITGB2	Integrin Subunit Beta 2	1.9
ARIH1	Ariadne RBR E3 Ubiquitin Protein Ligase 1	1.5
CARD9	Caspase Recruitment Domain Family Member 9	1.6
CXCL10	C-X-C Motif Chemokine Ligand 10	1.6
EGR1	Early Growth Response 1	3.3
PDGFA	platelet-derived growth factor alpha polypeptide	3.5
TNFSF15	tumor necrosis factor (ligand) superfamily, member 15	2.1
GPR31	G protein-coupled receptor 31	1.7
SLC12A2	solute carrier family 12	1.6
GPR153	G protein-coupled receptor 153	1.6
MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78	1.5
GPR150	G protein-coupled receptor 150	1.5

Bacillus clausii induced several genes in a mouse macrophage cell line Raw264.7 even at MOI1 indicated the robustness of BC in macrophage activation. The major pathways enriched by the

differentially expressed genes in Raw264.7 following BC treatment are innate immune signaling pathways, GM-CSF signaling, toll like receptor signaling, TNF signaling and cell cycle regulation. The important genes and their corresponding fold changes are listed in table 4.6. Expression of the pro-inflammatory cytokines CXCL2, CXCL10, CXCL12, DEFA3, IFNb1, IL1F10 and TNFAIP3 indicated the robust immune priming status of Raw264.7 following BC treatment. The activation of cholesterol synthesis pathway represented by the expression of squalene epoxydase (SQLE) and 3-hydroxy-3-methylglutaryl-coenzymeA reductase (HMGCR) indicated the monocyte differentiation into macrophage induced by BC. Expression of several growth factors such as fibroblast growth factor 17 (FGF17), c-FOS induced growth factor (FIGF), platelet derived growth factor subunit A (PDGFA) and PDGFB indicated the induction of differentiation and proliferation of Raw264.7. The important differentially expressed genes and their corresponding fold changes are listed in the table 4.6. Except for BC in Raw264.7, no other probiotics strains induce that many genes at an MOI1. Even BC at an MOI1 induces many genes in Raw264.7 cells with moderate fold changes. So we increased the MOI of probiotics strains for better priming of the macrophages. We titrated the macrophage survivability and select immune gene expression with different doses of probiotics.

Table 4. 6 Important genes expressed differentially in Raw264.7 following BC treatment for 6 h at an MOI1.

Gene Symbol	Gene Name	Fold Changes
AKT1	V-Akt Murine Thymoma Viral Oncogene Homolog 1	1.6
C2	Complement Component 2	1.5
CAMK4	Calcium/Calmodulin-Dependent Protein Kinase IV	1.6
Camkk2	calcium/calmodulin-dependent protein kinase kinase 2, beta	2.4
CASP1	Caspase 1	1.6

CREB1	CAMP Responsive Element Binding Protein 1	1.8
CXCL10	C-X-C Motif Chemokine Ligand 10	3.3
Cxcl12	chemokine (C-X-C motif) ligand 12	2.4
Cxcl2	chemokine (C-X-C motif) ligand 2	2.1
DEFA3	Defensin Alpha 3	1.7
FGF17	Fibroblast Growth Factor 17	2.0
Figf	c-fos induced growth factor	3.0
FRS3	Fibroblast Growth Factor Receptor Substrate 3	1.5
Gabra4	gamma-aminobutyric acid	2.1
Gpr139	G protein-coupled receptor 139	2.6
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	2.1
IER3	Immediate Early Response 3	2.5
IFNB1	Interferon Beta 1	3.5
Ifnb1	interferon beta 1, fibroblast	3.5
Il1f10	interleukin 1 family, member 10	3.8
IL3RA	Interleukin 3 Receptor Subunit Alpha	1.5
JUN	Jun Proto-Oncogene	2.3
NFKB2	Nuclear Factor Kappa B Subunit 2	1.5
NFKBIB	NFKB Inhibitor Beta	1.6
PDGFA	Platelet Derived Growth Factor Subunit A	1.5
PDGFB	Platelet Derived Growth Factor Subunit B	2.4
Ptgs2	prostaglandin-endoperoxide synthase 2	2.1
RAC1	Ras-Related C3 Botulinum Toxin Substrate 1	1.8
Rgs1	regulator of G-protein signaling 1	3.1

Slc13a2	solute carrier family 13	2.7
Slc2a1	solute carrier family 2	2.3
Slc6a4	solute carrier family 6	2.3
SOCS1	Suppressor Of Cytokine Signaling 1	1.7
Sqle	squalene epoxidase	2.5
Tank	TRAF family member-associated Nf-kappa B activator	7.2
Tgfb1i1	transforming growth factor beta 1 induced transcript 1	4.2
TNFAIP3	TNF Alpha Induced Protein 3	2.1

4.2.2 Effect of LA and BC on RAW 264.7 viability

The murine RAW264.7 macrophage cell line was treated with LA, BC and ST. ST was used as a positive control for cell death and non-treated (NT) cells were used as a negative control. Figure 4.2 shows percent survival of RAW 264.7 cells at 2, 4, 6 and 12 h post-treatment with BC, LA and ST at MOIs of 1, 10, 100 and 500. There was significant cell death with ST, even at an MOI of 1 and at 4 h post-treatment. LA and BC were not cytotoxic for RAW264.7 cells, even at an MOI of 100 and with a 6 h treatment; ST displayed significant ($p \leq 0.001$) cytotoxic (Figure 4.2) with 100% macrophages dead 1 h post-treatment with an MOI of 500. In contrast, neither of the two probiotics strains had any cytotoxic effects.

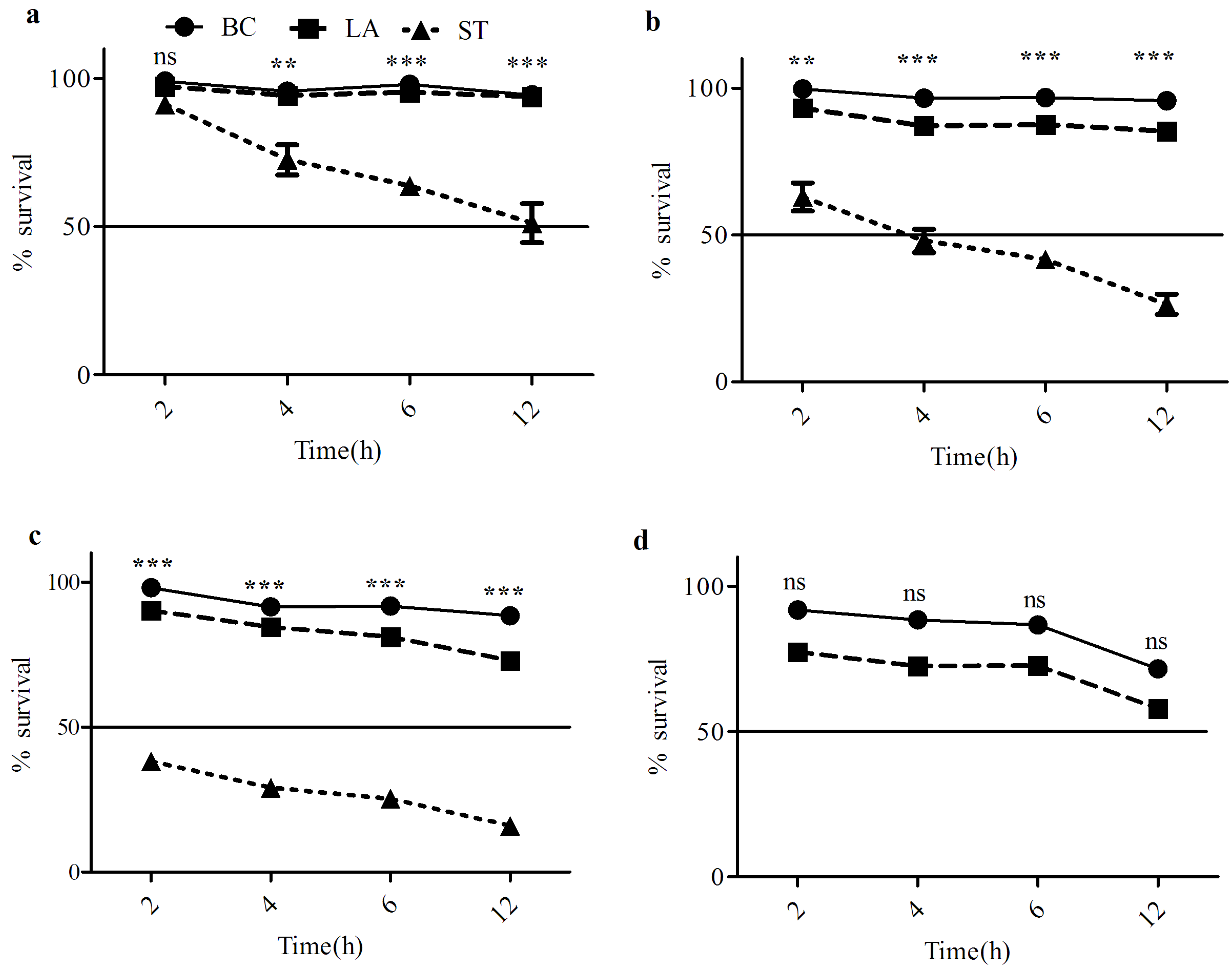

Figure 4. 2 Macrophage survival assay following LA, BC and ST treatment

Survival curve of RAW 264.7 cells following treatment with different probiotics along with ST are depicted. Treatment was done at MOI of **a.** 1, **b.** 10, **c.** 100 and **d.** 500 at different time points (Statistical significance was calculated using 2 way ANOVA, based on the statistical analysis, '**' corresponds to p B 0.01, '***' corresponds to p B 0.001 and 'ns' corresponds to not significant, error bars shown are standard deviations determined from average values of 3 replicates).

4.2.3 LA and BC modulates host innate immune genes in dose-dependent manner

We first evaluated the expression of selected pro- and anti-inflammatory innate immune genes; interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon α (IFNα) and

tumor necrosis factor (TNF), following treatment of RAW 264.7 with LA and BC at various MOI and for a duration of 6 h. qRT-PCR analysis revealed all target genes were up-regulated approximately 2-fold in cells treated with LA (MOI =100) relative to NT (figure 4.3 a).

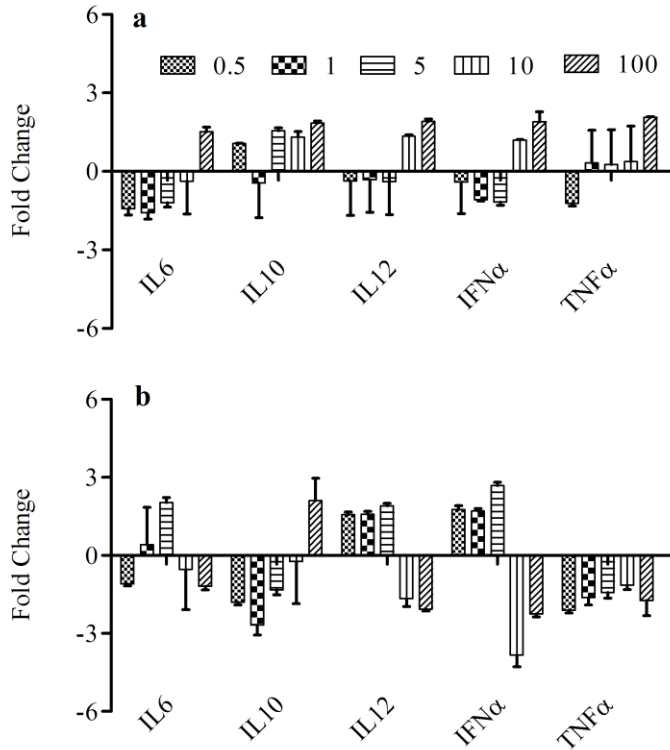

Figure 4. 3 LA and BC modulates select innate immune genes in Raw264.7 and it is dose dependent

Fold change values of the select innate immune genes in macrophage at 6 h following treatment with LA (**a**) and BC (**b**)

Furthermore, IL-10 gene was up-regulated with LA at an MOI (as low as) 5 following treatment for 6 h. BC treatment, at MOIs of 5 and 100, up-regulated IL-6 and IL-10 gene expression approximately 2-fold relative to untreated controls (figure 4.3 b). Furthermore, with treatment the IL-12 and IFNα genes were up-regulated at an MOI of 0.5 but repressed at MOIs equal to

greater than 10. TNFα was down-regulated at all MOIs of BC. Both LA and BC activated IL10 gene coincidentally with the pro-inflammatory cytokine genes, including IL6, IL12 and TNFα. Cytokine expression with MOIs below 100 was significantly lower for both probiotic strains. Therefore, an MOI of 100 was selected for subsequent analysis of whole transcriptomic expression kinetics.

4.2.4 Cytokine gene expression kinetics in Raw264.7 cells following treatment with ST, LA, and BC

qRT-PCR analysis revealed that following a 6h co-culture of Raw 264.7 with ST (MOI = 10) there was a 2 to 8-fold increase in the expression of genes such as IL6, IL8, IL10, IFNα, IFNβ and PI3KCα with respect to the time-matched untreated control (Figure 4.4). Expression of these genes prior to 6h was, however, significantly ($P < 0.05$) reduced. Robust expression of pro-inflammatory cytokines in ST treated macrophages may trigger apoptosis but we hypothesized that moderate expression (2-8 fold increase) of pro-inflammatory cytokines may prime macrophages against pathogenic bacteria. To test this hypothesis we assayed bacterial uptake using several methodologies such as FACS, Confocal fluorescence microscopy and cell lysis assay as described in the materials and methods sections.

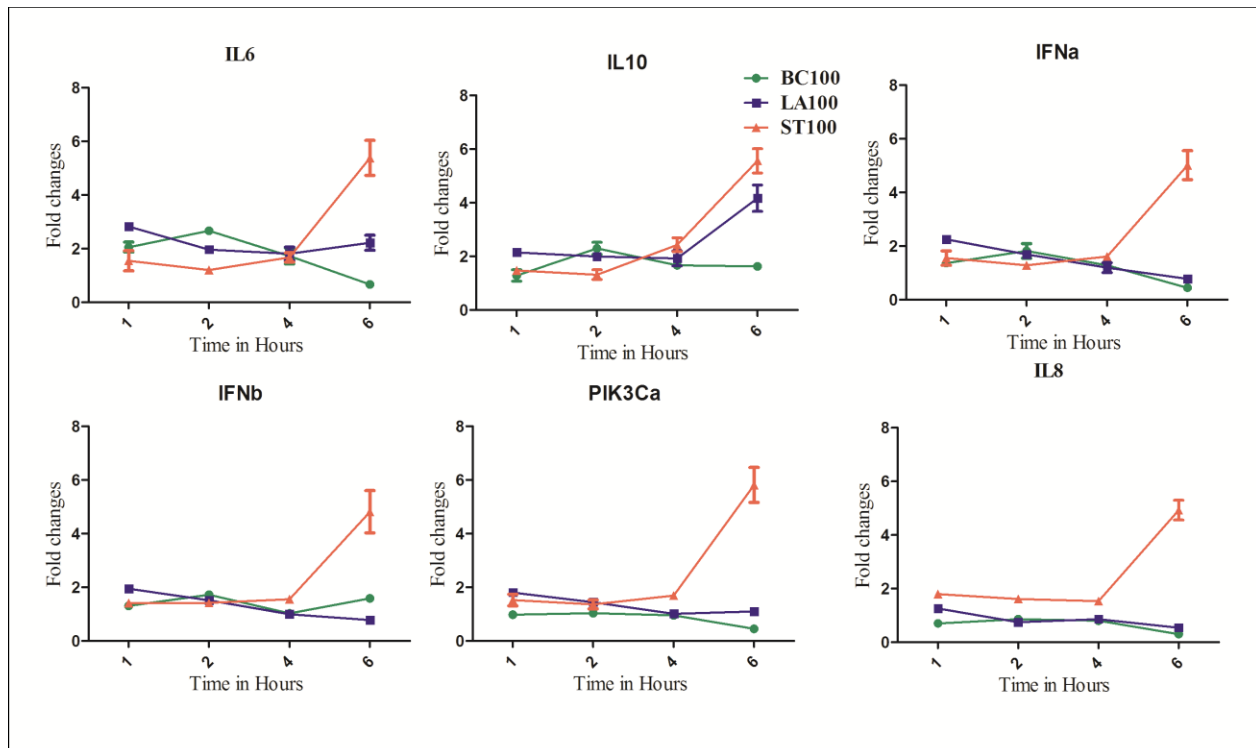

Figure 4. 4 Cytokine expression kinetics in Raw264.7 following treatment with LA, BC and ST

Cytokine expression kinetics in Raw264.7 is different with *Salmonella typhimurium* MTCC-3232 than *Lactobacillus acidophilus* MTCC-10307 and *Bacillus clausii* MTCC-8326 treatment. Expression kinetics of Il6 (a), Il10 (b), Ifna (c), Ifnb (d), PIK3Ca (e) and Il8 (f) in Raw264.7 with ST (red), LA (blue) and BC (green) treatment

4.2.5 Macrophages ingest and capture probiotic bacteria on their surface

Fluorescently labeled bacteria were used to determine the amount of bacteria ingested or adhered to the surface of RAW 264.7 cells. FACS analysis revealed a shift in fluorescence for RAW 264.7 cells incubated with labelled bacteria (figure 4.5 a). This shift in fluorescence was confirmed to be due to bacterial ingestion at different MOIs (figure 4.5 b). The percent labeled bacteria ingested by RAW 264.7 cells at MOIs 1, 5, 10, 100 and 500 is shown in figures 4.5 c to

g. FACS data was further validated by high resolution confocal microscopy. Figure 4.5 h shows both uptake and adherence of labeled (green) bacteria at an MOI 100.

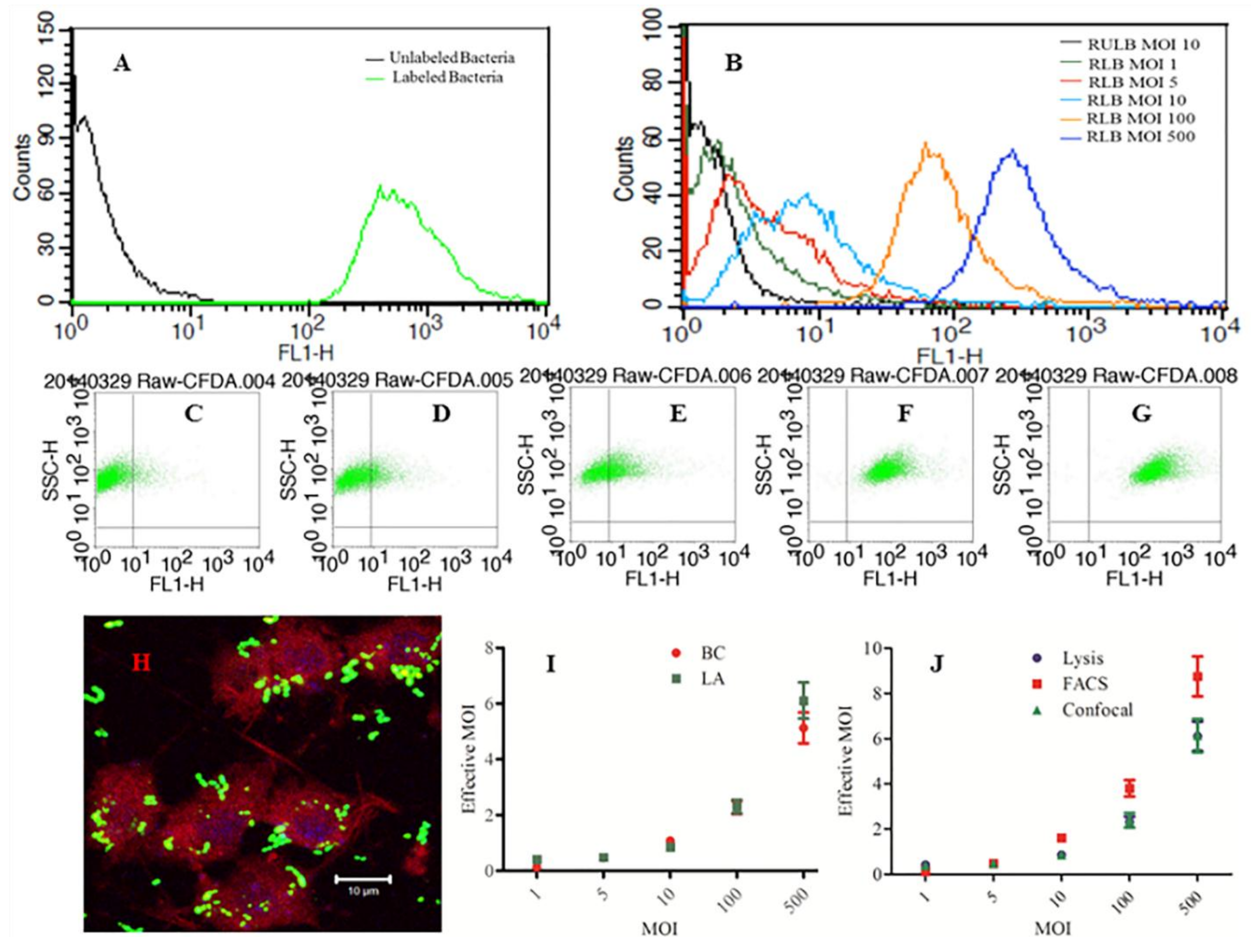

Figure 4. 5 Adhesion and integration of probiotics with macrophages

a. Mean fluorescence intensity (MFI) counts of the CFDA/SE-labeled LA. The shift in X axis through Filter 1 (FL1) is because of CFDA/SE green fluorescence. **b.** MFI counts of the macrophages associated with CFDA/SE-labeled LA. Note the shift in X axis with increasing MOI of LA treatment. Dot plot of the macrophage treated with CFDA/SE labeled LA at MOI of 1 (**c**), MOI of 5 (**d**), MOI of 10 (**e**), MOI of 100 (**f**), and at MOI of 500 (**g**). Representative visualization of the macrophage treated with CFDA/SE-labeled LA (green) at MOI of 100 where cell membrane is labeled with cell mask Red (red) and nucleus is labeled with Hoechst (blue) (**h**). **i.** Effective MOI of LA and BC determined through cell lysis method. **J.** Effective MOI of LA determined through 3 different methods.

We also quantified number of bacteria taken up by RAW 264.7 cells by using a cell lysis assay method as described in materials and methods. Figure 4.5 i reveals the effective MOI, calculated by dividing the number of bacteria recovered from RAW cells by the number of total host RAW cells present for treatment with either LA or BC. Similarly, values for effective MOI were also determined for FACS and confocal methodologies. Figure 4.5 j compares effective MOIs determined by three methodologies at each MOI. Results revealed a correlation among all three methodologies. The number of probiotic bacteria recovered per macrophage (Effective MOI) increased with exposure to an increasing MOI.

4.2.6 Macrophage transcriptome kinetics following LA and BC treatment

4.2.6.1 Overall transcriptional responses

Microarray analysis probed 29116 coding transcripts and 3316 non-coding transcripts. Genes were identified as significantly altered in expression if fold change was at least 1.5 with p value ≤ 0.05. Figures 4.6a and 4.6b show the number of genes differentially regulated at each time point following BC and LA treatment. Our analysis revealed that 4544, 3847, 4258 and 4128 genes were significantly and differentially regulated following LA treatment for 1, 2, 4 and 6 h, respectively. Similarly, 5164, 4781, 4625 and 4576 genes were significantly and differentially regulated following BC treatment for 1, 2, 4 and 6 h, respectively. There were 1455, 941, 795 and 1130 genes that were uniquely expressed following 1, 2, 4 and 6h of BC treatment, respectively. Similarly, 1100, 707, 903 and 1200 unique genes were uniquely expressed following LA treatment for 1, 2 4 and 6 h, respectively. At all-time points, following BC and LA treatment, there were 842 and 747 genes conserved in their expression respectively. Significantly expressed genes for all treatment conditions are listed in annexure 3.

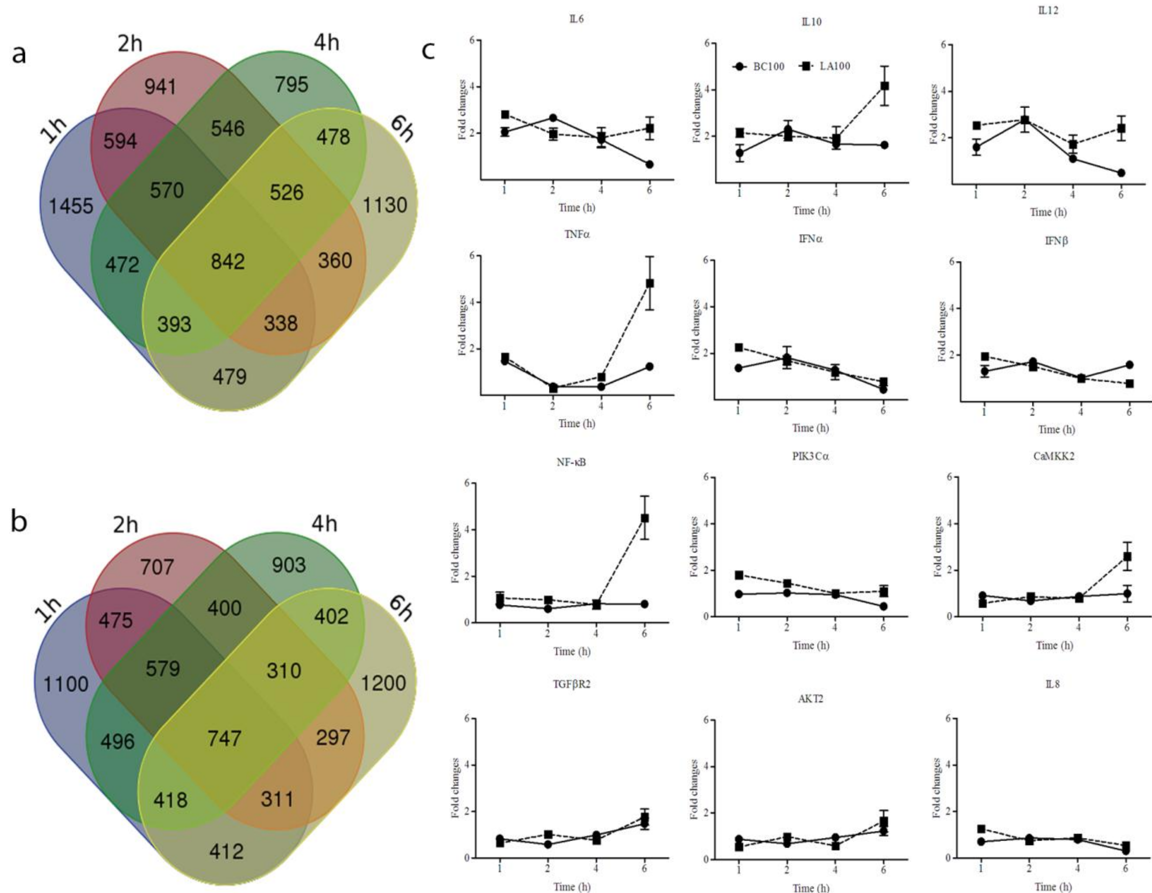

Figure 4. 6 Expression kinetics of the differentially expressed genes in Raw264.7 following treatment with LA and BC

Genes that were differentially expressed following treatment with BC (**a**), and LA (**b**). qRTPCR validation of a few genes and cytokines (**c**)

4.2.6.2 Comparison of transcriptomic responses at pathway level

Significantly altered gene expression was further analyzed for pathway enrichment. The top 10 pathways identified, based on their gene enrichment, are listed in table 4.7. A complete list of differentially regulated genes to differentiate treatment following LA and BC treatment are listed in annexure 3.

Table 4. 7 Pathway kinetics in RAW264.7 with LA and BC treatment

Pathway Name	KEGG genes (Total)	Number of enriched genes in							
		LA treatment				BC treatment			
		1h	2h	4h	6h	1h	2h	4h	6h
Pathways in cancer	325	97	–	–	83	104	105	–	73
Metabolic pathways	1184	213	182	185	207	236	208	208	235
Neuroactive ligand–receptor interaction	277	81	74	79	–	88	97	80	75
Focal adhesion	200	67	51	62	52	67	63	57	53
Calcium signaling pathway	178	57	45	49	47	62	67	54	56
Regulation of actin cytoskeleton	216	63	–	–	52	–	–	–	–
MAPK signaling pathway	268	–	–	–	73	84	74	–	62
Endocytosis	220	–	48	–	–	74	64	61	67
Cell adhesion molecules (CAMs)	149	–	39	47	–	57	54	54	–
Cytokine–cytokine receptor interaction	245	–	62	63	57	68	–	69	–
ECM–receptor interaction	86	–	32	–	31	–	–	–	–
Jac-STAT signaling pathway	153	–	–	–	–	–	–	49	–

4.2.6.3 Comparison of transcriptomic responses at gene level

All innate immune genes on the microarray with their respective fold changes are listed in annexure 3. Lists of genes we consider important for the present investigation are listed in table

4.8. Among the metabolic genes, genes regulating carbohydrate metabolism were mostly up-regulated. The list for carbohydrate metabolism includes genes from the GO pathways of Glycolysis, Tri carboxylic acid cycle, electron transport and oxidative phosphorylation. The nucleotide metabolism pathway was also activated as active transcription was occurring in the macrophages. Fatty acid beta oxidation was down-regulated whereas cholesterol biosynthesis was active for the first 2 h (table 4.9) and subsequently down-regulated at 4 and 6 h following treatment with both LA and BC. Both LA and BC induced similar changes in metabolic pathways. The primers used to determine the expression levels of genes through qRTPCR were listed in table 4.10.

Table 4. 8 Expression kinetics of important innate immune genes in Raw264.7

Genes	LA treatment				BC treatment			
	1h	2h	4h	6h	1h	2h	4h	6h
ccl2	2.6	3.3	2.3	2.5	2.8	2.7	1.4	–1.05
Ccl3	1.9	1.9	1.0	–1.24	2.1	1.5	–1.31	–1.38
Ccl4	2.6	2.1	–1.74	–1.60	3.1	2.2	–1.58	–1.54
Ccl7	1.8	3.0	2.6	3.5	2.6	2.4	1.5	–1.15
Ccl19	1.5	1.3	1.9	–1.03	1.2	1.1	–1.48	–1.50
Cxcl10	1.3	1.5	1.6	1.3	1.4	1.7	1.7	1.3
Cxcl12	1.0	2.4	–1.23	2.1	2.7	3.0	2.3	1.0
Cxcl2	4.9	4.1	–1.13	1.8	6.5	2.3	–2.20	–1.36
Cxcr4	2.0	1.6	–1.22	3.0	1.4	2.0	2.8	–2.04
Cxcr7	1.0	1.7	3.4	1.9	1.1	1.9	1.1	1.3

Csf1	–1.23	–1.05	2.3	–1.02	–1.20	1.2	2.4	–1.06
Csf1r	1.2	1.2	–1.29	–1.14	1.4	1.4	1.4	1.5
Csf3	–2.40	2.1	–1.18	1.4	1.5	2.4	1.6	1.8
Defa-rs12	1.0	–1.31	1.9	1.4	1.4	–1.14	2.7	1.6
Defb10	–1.11	2.8	–4.89	1.1	1.0	1.2	1.2	3.9
Defb15	1.0	2.3	3.2	2.1	1.5	1.5	3.1	2.2
Defb34	1.1	1.2	1.2	1.8	1.4	1.4	2.9	2.0
Defb35	1.0	–1.17	1.6	1.1	1.1	4.2	7.3	2.8
Defb38	–1.07	1.1	–1.03	–1.21	1.1	1.9	1.5	1.4
Defb39	1.6	1.9	–1.08	1.3	1.4	2.1	1.2	1.1
Il10ra	1.4	1.1	–1.04	1.3	1.7	1.7	1.6	1.4
Il12rb2	1.0	1.8	1.6	1.6	1.5	1.1	1.9	1.1
Il15ra	–1.48	–1.63	3.2	–2.15	–2.76	–2.09	2.8	–1.63
Il16	2.8	2.5	1.7	1.3	1.7	1.2	2.7	1.3
Il17b	–1.04	1.6	2.7	–1.01	1.1	–1.01	2.0	1.1
Il17d	1.6	1.6	1.2	1.9	1.7	–1.56	1.1	1.4
Il17rd	1.8	1.6	1.3	–5.67	1.3	3.9	1.1	1.3
Il5	–1.69	–1.94	–2.94	–1.22	–1.66	–5.06	–2.49	–2.34
Ifna12	1.8	1.6	1.6	1.6	–1.04	1.5	1.9	1.4
Ifna2	1.5	1.1	1.5	1.8	6.8	2.0	1.2	1.0
Ifnlr1	2.9	3.0	2.8	1.7	1.6	1.1	4.3	2.4
Ptgds	1.3	1.5	–1.10	1.2	–1.06	1.3	–1.10	1.5
Ptger1	1.5	1.1	2.0	2.6	–1.19	–1.40	–1.47	1.4

Tgfa	2.2	1.9	1.4	1.2	1.3	1.7	1.4	1.4
Tgfbr1	1.4	1.0	1.6	1.8	1.8	–1.22	1.8	1.8
Tlr5	1.2	–1.08	1.4	–1.02	–1.16	1.5	1.5	1.9
Tlr9	1.2	1.2	1.2	1.3	1.5	1.5	1.3	1.3
Tnf	1.2	–1.13	1.5	1.4	1.6	–1.15	1.6	1.8
Tnfrsf1b	1.2	1.6	2.8	1.1	1.4	1.9	2.6	1.1
Tnfsf13b	1.7	1.7	1.4	3.1	1.7	1.6	1.6	1.4

Significantly regulated innate immune genes during this study included chemokines (Ccl and Cxcl), macrophage colony stimulating factors (Csf), defensins (Def), interleukins (Il), type I interferons (Ifn), eicosanoids synthesizing enzymes (Ptgs), transforming growth factors (Tgf) and tumor necrosis factors (Tnf). These genes and their expression values at different time points post-treatment are listed in annexure 3. Although most innate immune genes were similarly regulated with both LA and BC there were some exceptions. These exceptions included Ccl17, Ccl19, Cxcl1, Cxcl15. Cxcr5, Csf1r, Defa6, IL10ra, IL17a, IL17b, Tgfb3, Tlr5 and Tnfsf8.

Table 4. 9 Expression kinetics of cholesterol biosynthesis pathway genes in Raw264.7

Gene name	Fold changes							
	LA treatment				BC treatment			
	1h	2h	4h	6h	1h	2h	4h	6h
Sqle	1.5	1.5	1.1	–1.41	1.7	1.5	1.2	–1.04
Hmgcs1	3.1	1.9	–1.16	–1.93	1.2	1.1	–1.43	–3.58

Hmgcr	1.8	1.4	1.1	–1.71	2.0	1.4	1.2	–1.17
Fdps	1.8	1.2	–1.10	–1.34	2.4	1.8	1.1	1.3
Fdft1	1.2	1.3	2.7	–1.37	1.2	1.2	1.1	3.6
Sc4mol	1.3	1.2	–1.25	–1.88	1.3	1.1	–1.28	–1.83
Cyp51	1.3	1.3	–1.25	–1.99	1.4	1.2	–1.50	–2.03
SC5d	1.2	1.2	–1.20	1.3	1.6	1.2	–1.19	–1.43
IDI1	1.3	1.2	1.0	–1.34	1.2	1.1	–1.06	–1.73
Dhcr7	1.3	1.2	–1.07	–1.51	1.4	1.2	–1.05	–1.58
Lss	1.2	1.1	–1.39	–1.52	1.4	1.3	–1.20	–1.44

Table 4. 10 Primers used in qRTPCR validation of gene expression

Gene name	Forward Primer	Reverse Primer	NCBI Sequence Accession number
IL6	AGACAAAGCCAGAGTCCTTCAGAG	CCACAGTGAGGAATGTCCACAAAC	NM_031168.1
IL10	AGGCAGTGGAGCAGGTGAAGAGTG	GCTCTCAAGTGTGGCCAGCCTTAG	NM_010548.2
IL12	CGCCCAAGAACTTGCAGATGAAGC	CGCCTTTGCATTGGACTTCGGTAG	NM_001303244.1
TNFα	CCCACGTCGTAGCAAACCACCAAG	TGCCCGGACTCCGCAAAGTCTAAG	NM_013693.2
IFNα	GAGCTGACCCAGCAGATCCTGAAC	TGAGGAAGACAGGGCTCTCCAGAC	NM_008334.3
IFNβ	AACTCCACCAGCAGACAGTGTTTC	TCCGCCTCTGATGCTTAAAGGTTG	NM_010510.1
NFkβ1	CAGGGTATGGCTACTCGAACTACG	CCAGATGTGACTTCCAGCAGATCC	NM_008689.2
PIK3CA	GTGGTGATGATGGTGGATTTC	TGAAGCACGGATTCTTGGAG	NM_008839.2
CAMKK2	TAGGCGCAGGATAACATCTG	AATCTTCCCTAGCACCTTGG	NM_001199676.1
TGFβR2	CTTCGAACACCATGGAAACC	ATGATGACCCGGAAGTGAAG	NM_029575.3
AKT2	TTCTCCTGGTTTTGCTCTCC	AACAGATGACCCCCATAACC	NM_001110208.1

IL8Rβ	GCTGACCTGTTCTTTGCCCTGACC	GCAGTACGACCCTCAAACGGGATG	NM_009909.3
β-Actin	CTGACGGCCAGGTCATCACTATTG	GACAGCACTGTGTTGGCATAGAGG	NM_007393.3
GAPDH	CCTACATGGCCTCCAAGGAGTAAG	TGTGATGTGGAGCACTGACCTCTG	NM_008084.3
αTubulin	TGCCATTGCCACCATCAAGAC	GGCACGCTTGGCATACATCAG	NM_011653.2

4.2.6.4 Probiotic primed cells were more resistant to ST induced cytotoxicity

Results from microarray data analysis revealed that immune pathways were activated in RAW 264.7 cells following co-culture with both LA and BC. We hypothesized that macrophages activated by probiotic bacteria may be more resistant to pathogenic bacteria, such as ST. Therefore, we analyzed the effect of ST infection at an MOI of 100 following treatment with LA or BC, either individually or in combination of LA and BC. Results in figure 4.7a revealed that LA:BC ratio of 50:50 and 70:30 showed promise among other conditions.

4.3 DISCUSSION

Probiotics are expected to be non- cytotoxic for host cells and modulate host immunity and metabolism. Both LA and BC satisfied these properties with cultured Raw264.7 cells and were significantly different when compared to ST. The probiotic bacterial uptake assay, established by three different methodologies (Figure 4), demonstrated their cellular adherence property. Probiotic bacterial uptake increased as MOI increased, which indicated that macrophage activation also increased with higher MOIs. The transcriptomic profile in macrophages following exposure to LA and BC further supported the probiotic action of these two strains at a molecular label. Although the significantly regulated genes correspond to several pathways, we focused on metabolism, immune modulation, cell cycle progression and apoptosis in this paper.

4.3.1 Metabolism

Probiotic bacteria treated RAW 264.7 cells increased in size, had higher cell density, and displayed an increased number of filopodia than non-treated cells. Gene expression studies revealed that both cholesterol biosynthetic and fatty acid biosynthetic pathway genes were significantly up-regulated (table 4.9). These results support the conclusion that RAW 264.7 cells were differentiating into activated macrophages [220]. The increased phagocytosis of probiotics with longer co-culture intervals (figure 4.5) also supports that RAW 264.7 cell differentiated into activated macrophages.

Glycolytic pathway genes as well as pentose phosphate pathway (PPP) genes are up-regulated while oxidative phosphorylation (OXPHOS) genes are down regulated (annexure 3). This suggests increased metabolism in probiotic treated macrophages with decreased mitochondrial respiration to conserve NADPH produced by glycolytic as well as PPP pathway for reactive oxygen species (ROS) neutralization [221].

Over-expression of retinoic acid induced gene-1 (RIG-1) and type-I interferon indicates macrophages are primed against virus infection [222,223]. Enhanced expression of these genes in LA and BC treated macrophage is consistent with cells being primed against viral and bacterial infections. It has also been reported that *Lactobacillus plantarum* 299v primed bovine primary intestinal epithelial cells against rotavirus infection [65].

In normoxic condition when OXPHOS is active, regeneration of NAD+ in the cytoplasm and NADH in the mitochondrial matrix occurs through the malate aspartate shuttle. However, when OXPHOS is inhibited the conversion of pyruvate to lactate becomes an essential means of regenerating NAD+ in the cytoplasm [224]. Hypoxia inducible factor 3 alpha (Hif3a) is generally expressed in hypoxic conditions but is also expressed by the TNF-α and Il-1β induced pathway during Warburg metabolism. Hif-1α, in contrast, induces production of lactate dehydrogenase (Ldhd and Ldhl6b) which produces lactate from pyruvate, and the induction of pyruvate dehydrogenase kinase (Pdk2, Pdk4) which phosphorylates and there by inhibits pyruvate dehydrogenase (Pdha2 and Pdhx), an enzyme which converts pyruvate into acetyl CoA [225]. These genes are expressed similarly in LA and BC treated macrophages further supporting induction of Warburg metabolism in probiotic treated RAW 264.7 cells. Warburg metabolism in macrophages results in increased production of reactive oxygen species (ROS) to enhance clearance of pathogens.

The arachidonic acid metabolism pathway was also increased in LA and BC treated macrophages. This pathway produces inflammatory leukotrienes and prostanoids which increase the pro-inflammatory response in macrophages [226]. Suppression of 24-dehydrocholesterol reductase (Dhcr24) catalyzes the synthesis of desmosterol which induces expression of inflammatory genes in activated macrophages [227]. Peroxisome proliferator activator receptor delta (PPARδ) was down regulated in LA treated macrophages but up regulated in BC treated macrophages. PPARδ promotes expression of genes involved in substrate oxidation and OXPHOS [228]. This is consistent with truncated Warburg metabolism in BC treated macrophages, resulting in less priming against pathogens. We did not observe, however, a

significant difference in macrophage protection against ST challenge with combined LA and BC treatment (figure 4.7 a). PPARδ expressing macrophage tolerate parasitic helminthes [229], is down regulated in LA and up-regulated in BC treatment. So whether LA primed macrophage can clear pathogens better than BC primed macrophage will need to be tested *in-vivo*.

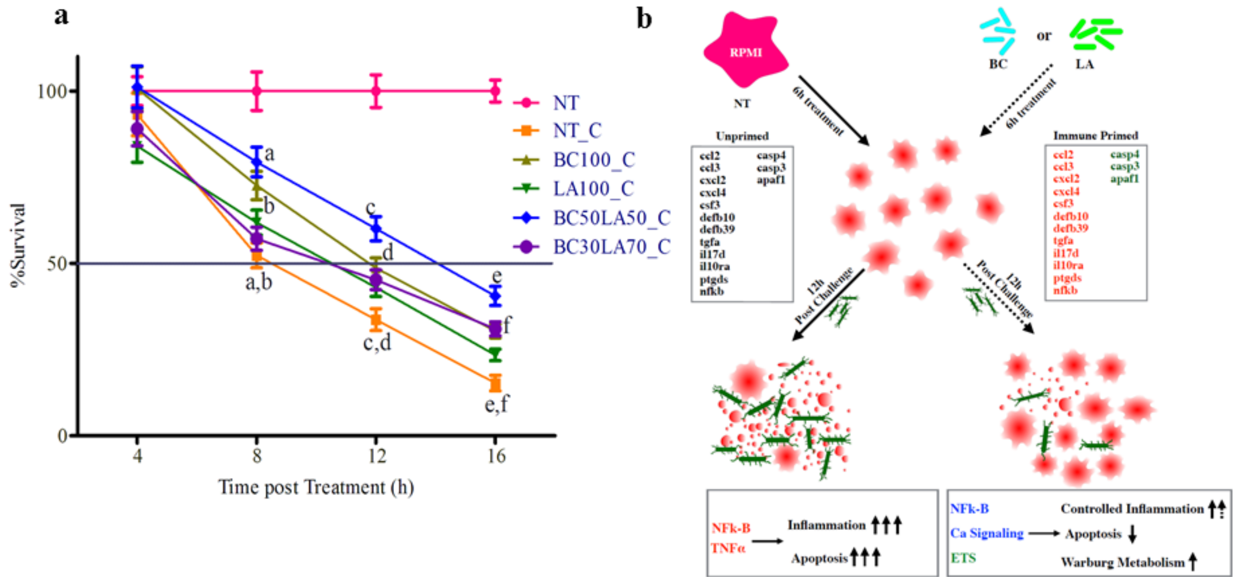

Figure 4. 7 Probiotics primed cells are protected against ST induced cytotoxicity

a. Percentage survival of RAW 264.7 cells post ST challenge with respect to time-matched untreated control. (a, c, e correspond to $p \leq 0.001$, b correspond to $p \leq 0.01$, d, f corresponds to $p \leq 0.05$,Two-way ANOVA at n = 4) **b.** graphical summary of the mechanism of protection of macrophage from ST through probiotics induction of innate immune pathways.

4.3.2 Immunity

The expression of several chemokine-chemokine receptors was significantly altered in Raw 264.7 cells incubated with LA and BC. Expression of chemokines such as Cxcl1, Cxcl2, cxcl5 which are ligands for the IL8rb; Cxcl12 which is ligand for Cxcr4; Tnfsf11 which is ligand for Tnfrsf11b; Tnf which is ligand for Tnfrsf1b; Il17β which is ligand for Il17rβ, indicated the

activation of various immunological signaling in Raw 264.7 cells [230]. These ligand-receptor interactions and subsequent signaling induces a pro-inflammatory condition in macrophage. Signaling by the Il10 family occurs through an interaction between Il10 and Il10rα/β; Il19 and Il20rα/β; Il24 and Il20rβ; Il22 and Il22rα1; Tgfβ2 and Tgfβr2; Tgfβ3 and Tgfβr1 [230]. Expression of these genes in Raw464.7 following LA and BC treatment indicated an anti-inflammatory state in macrophage. Induction of a pro-inflammation response at 2 hours and an anti-inflammatory response at 6 hours indicated the induction of controlled inflammatory response in macrophages by LA and BC. This indicates that LA and BC are ideal candidates for macrophage immune priming as they induce controlled inflammation. Expression of several defensins and interferons in macrophages following LA and BC treatment (table 4.7 and annexure 3) indicated antibacterial and antiviral priming of the macrophage [231]. Expression of several Il17 family genes (annexure 3) at 2 hours but downregulated at 6 hours in Raw264.7 cells following LA and BC treatment also supported the controlled inflammatory status in macrophages [232].

4.3.3 Cell Cycle

The cell survival assay (figure 2) indicated that macrophage proliferation was arrested following LA and BC treatment. Cyclin E1 and cyclin-dependent kinase 2 (CDK2) forms a complex that blocks the cell cycle at the G1/S transition phase [233]. The down regulation of cyclin E1 in Raw264.7 cells suggests that cell cycle progression was blocked at the G1/S phase. Similar to cyclin E1, cyclin D2/3 forms a complex with cdk6 whose activity is required for cell cycle progression at the G1/S phase. The net effect of cyclin E1 and cyclin D2/3, growth arrest and DNA-damage-inducible 45 alpha (Gadd45a) and cell division cycle 25A (Cdc25a) appears to

inhibit cell cycle progression at G1/S [234] phase in RAW 264.7 cells after treatment with LA and BC.

4.3.4 Apoptosis

IL1 receptor signals through Map3k14 (NIK) and NF-kB1 which in turn transcribes the survival genes BIRC3, BIRC7, Bcl2l1 and Bcl2 [235]. Down-regulation of Apaf1 complex, Caspase3, Caspase4, and Caspase7 indicates blockage of apoptosis through mitochondrial signaling [235]. The down regulation of apoptosis-inducing factor mitochondrion-associated 1 (Aifm1) and Aifm3 is consistent with an intrinsic blockade of apoptosis [236]. This result may explain why LA and BC primed macrophages survived better following ST challenge. The mechanism of protection of probiotics primed macrophage from ST induced cytotoxicity is graphically summarized in figure 4.7b.

4.4 Conclusion

The use of *Lactobacillus* and *Bacillus* species as probiotic dietary supplements is increasing. Each year new strains of these two genera are studied and added to the probiotics list based on their immune modulatory and antimicrobial activities. In this study, we reported two new strains of LA and BC, which displayed significant effects on modulating innate immune responses in murine macrophage and protected cells from ST induced cytotoxicity. These two strains adhere to the surface of macrophages and have moderate cytotoxic activity. Our comparative analysis of transcriptome dynamics in macrophage following treatment with these two bacterial strains revealed that *L. acidophilus* MTCC-10307 and *B. clausii* MTCC-8326 could be considered as probiotics for further usage. However, their efficacy as probiotics needs be established *in-vivo*.

Chapter 5

Probiotics *Lactobacillus acidophilus* modulate mice gut microbiota and ameliorate *Salmonella* induced dysbiosis and inflammation

5.0 Probiotics LA and BC can modulate mice gut microbiota and ameliorate Salmonella induced dysbiosis and inflammation

5.1 Introduction

The gut is a vital organ in the body, responsible for transporting and digesting foodstuffs, absorbing nutrients and expelling waste [237,238,239]. The gut is also a highly dynamic and multifunctional organ, unique in the fact that it is closely tied to the microbiota which interacts with both nutrients and host cells. Early scientists studied microorganisms and host interactions from a primarily pathogenic point of view, as some bacteria were known to produce toxins [169,240,241,242], and moreover, some of these same bacteria were observed to invade the gut mucosa and cause systemic infections [241,242]. However, modern day science has significantly changed the way we approach the gut microbiota by thoroughly demonstrating the symbiotic interactions that exist between commensal microbiota and the host; these new revelations reveal that the microbiota are not only beneficial, but a vital component of the host [243]. The mutualistic partnership between microbiota and the host begins at birth and stabilizes in humans at around the age of 3 [58,244].

Gut bacterial destabilization (recognized as "dysbiosis") is primarily caused through pathogenic bacterial colonization [245] and antibiotic administration [246]. *Salmonella enterica serovar typhimurium* and *Helicobacter pylori* are two common pathogens which can efficiently induce destabilization of gut microbiota populations [247,248]. It has been observed that *Salmonella enterica* subspecies 1 serovar Typhimurium cannot colonize inside the intestine of susceptible *Nramp1*−/− (*Slc11α1*−/−) mice, but can cause typhoid-like infection [249]; pretreatment with streptomycin causes a certain degree of dysbiosis within the gut, thus helping the same strain of

Salmonella to avoid competitive exclusion, allowing it to establish and induce colitis in C57BL/6 and BALB/c mice more efficiently [250].

Dysbiosis of the gut microbiota can result in long lasting and potentially fatal diseases, marked by chronic inflammation and diarrhea. Effective administration of probiotics with proper regimen times can cure gut bacterial destabilization. Lilly and Stillwell first introduced the word "probiotic" in 1965, serving as an antonym to the word "antibiotic" [251]. *Bifidobacteria* and *Lactobacilli* are highly reputable and effective probiotics [252] which can be administrated to the host through fermented vegetable and dairy products. Probiotic bacteria can decrease the permeability of the gut, helping prevent pathogenic attacks [75]. Adhesion of probiotic bacteria to the intestinal epithelial cells helps block and resist the adhesion of pathogenic agents [210]. Through competitive exclusion, the presence of a well-established population of probiotic bacteria can reduce the potential for a pathogen to thrive and survive within the gut [211]. Additionally, probiotic bacteria can modulate several host signaling pathways which regulate gut homeostasis [212]. Recently, our group has shown that probiotics have the ability to prime host immune cells, and in doing so, increase the effectiveness of the host immune response when fighting future pathogenic infections [64].

In this report we have evaluated the efficacy of *Lactobacillus acidophilus* MTCC-10307 (LA) and *Bacillus clausii* MTCC-8326 (BC) with respect to their potent probiotic effects in C57Bl/6 mice, which are Th1 immune biased and in Balb/C mice, which are Th2 biased. We used *Salmonella typhimurium serovar enterica* MTCC-3232 (ST) as pathogenic bacteria and reported the mechanism of pathogenic clearance facilitated by LA and BC. Genome-wide transcriptomic

expression data has shed light on how LA and BC behave and interact during ST clearance in Th1 and Th2 biased mice. Overall, the concept of gut microbiota restoration was demonstrated using probiotic administration and this was reflected in our Next-Generation Sequencing (NGS) analysis. Histology of gut and liver tissues show the extent of damage rendered by the pathogenic bacteria ST; and most importantly, the degree of disease prevention incurred through probiotic treatment.

5.2 Results

5.2.1 Microbial constitution of the BALB/c and C57BL/6 gut differs

The genetic makeup and the immunological milieu of the gut of BALB/c and C57BL/6 mice are different. It is expected that the microbiota of these two mice strains might differ, thus they may respond differently to *Salmonella typhimurium serovar enterica* MTCC3232 (ST) mediated perturbation, as well as following treatment with probiotics *Lactobacilllus acidophilus* MTCC10307 (LA) and *Bacillus clausii* MTCC8326 (BC). We performed 16s rRNA V3 sequencing to profile the gut microbiota in both of these mice of 7 weeks of age. The major phyla that constitute BALB/c gut microbiota are 83.7 ± 10.6 % *Firmicutes*, 11 ± 1.4 % *Bacteroides*, and 3.3 ± 0.4 % *Proteobacteria*. Whereas C57BL/6 microbiota consists of 49.5 ± 6.2 % *Firmicutes*, 46.2 ± 5.8 % *Bacteroides*, and 3.4 ± 0.4 % *Spirochaetes* (figure 5.1). At the genus level, the BALB/c microbiota is more diverse, consisting of 39.7 ± 5.1 % *moryella*, 14.4 ± 1.8 % *lachnospiracea*, 8.7 ± 1.1 % *flavonifractor*, 6.3 ± 0.8 % *anaerostipes*, 3.2 ± 0.4 % *alistipes*, 2.8 ± 0.4 % *desulfovibrio*, and several other genera. C57BL/6 microbiota is less diverse, consisting of 54.9 ± 7.1 % *prevotella*, 16.7 ± 2.1 % *oscillospira*, 12.2 ± 1.6 % *bacteroides*, 3.9 ± 0.5 % *ruminococcus*, 3.4 ± 0.5 % *odoribacter* and several other genera in

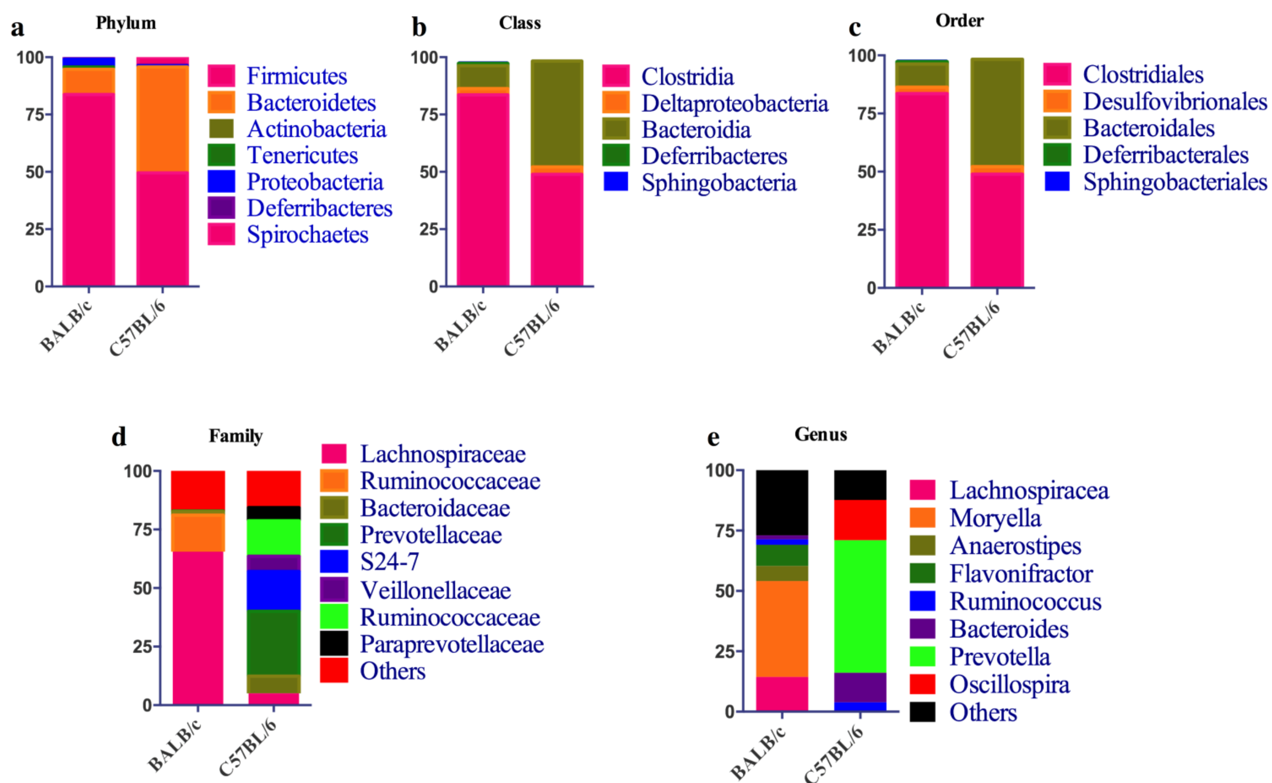

Figure 5. 1 Gut microbial composition of BALB/c and C57BL/6

Gut microbial composition of BALB/c and C57BL/6 in the level of Phylum (**a**), Class (**b**), Order (**c**), Family (**d**) and Genus (**e**).

Table 5. 1 BALB/c gut microbiota is more diverse than C57BL/6

Microbial Genus	Percentage Abundance	
	BALB/c	C57BL/6
Lachnospiracea	14.4	0.0
Moryella	39.7	0.0
Anaerostipes	6.3	0.0
Flavonifractor	8.7	0.0
Desulfovibrio	2.8	1.9
Ruminococcus	2.3	3.9
Marvinbryantia	2.6	0.0
Butyricimonas	1.1	0.0
Anaerotruncus	1.7	0.0

Oscillibacter	1.4	0.0
Alistipes	3.2	0.0
Bacteroides	1.6	12.2
Parabacteroides	1.6	1.4
Lactobacillus	0.0	1.3
Prevotella	0.0	54.9
Oscillospira	0.0	16.7
Coprococcus	0.0	1.1
Odoribacter	0.0	3.4

small percentages (Table 5.1). Diversity of BALB/c microbiota was correlated with its ability to secrete diverse polyreactive IgA into the gut [253], which is reflected by our data also.

5.2.2 ST, LA and BC were able to colonize in distal ileum and proximal colon of mice gut

The strains of probiotics (LA and BC) as well as pathogen ST were tested *in-vitro* for their immune-modulatory and cytotoxic ability respectively [64]; respectively, the strains were also evaluated for their colonization efficacy as well as infectivity *in-vivo*. BALB/c mice were infected with ST through oral gavage with doses ranging from 20-500 million bacteria. Bacterial presence in fecal samples was determined through qRT-PCR using ST specific primer against 16S rRNA. We also tracked the health of mice with respect to their feeding, drinking, grooming and playful activities. Histopathology of the gut, spleen and liver of the dead mice was performed to quantitate the level of infection. Our data revealed that ST was able to colonize the mice ileum for at least 3 days (Figure 5.2e). The optimal ST infective dose per mouse was determined to be 500 million bacteria based on mice survivability and histopathology. Symptoms including semi-liquid greenish brown stools and drowsiness in mice were observed after ST infection from the 3rd day onwards. BALB/c mice were also treated with LA and BC

through oral gavage with doses ranging from 20- 2000 million bacteria per mouse. Bacterial presence in the fecal samples of mice as well as health and behavior of the mice was tracked for

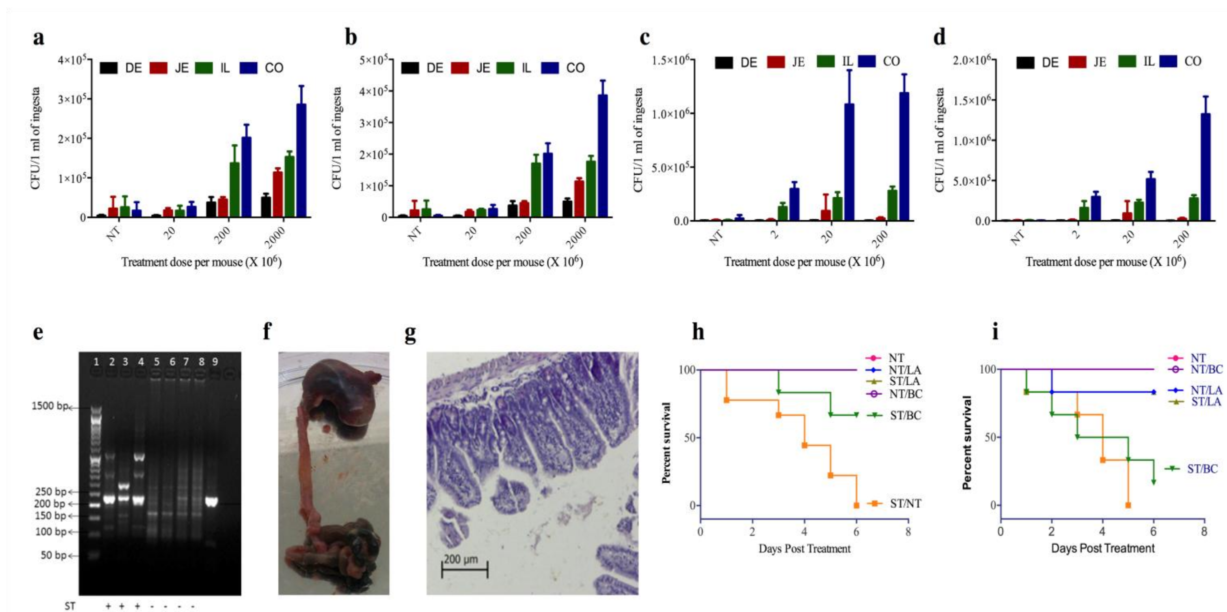

Figure 5. 2 LA, BC and ST could colonize in mice ileum and colon. LA protected mice from ST

BALB/c and C57BL/6 are being protected by LA from ST, whereas BALB/c (not C57BL/6) is being protected by BC from ST. Number of colony forming units of LA per ml of ingesta after 24 hour treatment of LA in different doses through oral gavage in BALB/c (**a**) and C57BL/6 (**b**). Number of colony forming units of BC per ml of ingesta after 24 hour treatment of BC in different doses through oral gavage in BALB/c (**c**) and C57BL/6 (**d**). **e.** ST detection in fecal sample of BALB/c for 24hours through PCR method. Lane 2, 3 and 4 corresponds to the presence of ST in fecal sample in 24h, 48h and 72h respectively. Lane 5, 6, 7 and 8 are the negative control of non treated mice. Lane 9 is the PCR positive from the pure ST culture. **f.** Bloating in BALB/c after 3 days, treated with 200 million of BC **g.** immune infiltration and villi atrophy in BALB/c ileum after 3 days, treated with 200 million of BC, **h.** Percentage survivability of BALB/c with different treatment conditions, **i.** Percentage survivability of C57BL/6 with different treatment conditions.

several days to find the optimal dose of LA and BC. Both LA and BC were able to colonize in the distal ileum and proximal colon (Figure 5.2 a to d). However, when LA was being tolerated by mice up to the dose of 2 billion, BC induced bloating (figure 5.2 f) and inflammation (figure 5.2 g) in mice when administered beyond the dose of 20 million. Therefore, the optimized doses were 2 billion LA and 20 million BC per mouse.

5.2.3 LA and BC protects BALB/c mice from ST infection, but only LA (not BC) was able to protect C57BL/6 mice from ST infection

Mice were divided into 6 groups as described in methods (chapter 2) and treated with ST, LA and BC separately; LA or BC was administered to mice recently infected with ST. A booster dose of LA or BC was given 24h after the first dose to the group of mice infected with ST, as well as to the groups that were treated with only LA or BC. Our data revealed that BALB/c mice treated with ST died within 6 days, but the mice that were challenged with ST in the presence of LA, followed by a booster dose of LA on the next day post challenge, had a 100% rate of survival (figure 5.2 h) as observed up to 10 days. However, mice that were challenged by ST along with BC, followed by a booster dose the next day, only had a survival rate of 67 %. All of the unchallenged BALB/c mice treated with either LA or BC survived (figure 5.2 h). Our data for C57BL/6 mice revealed that 100 % mice challenged with ST died within 5 days, but the mice that were challenged with ST in the presence of LA, followed by a booster dose of LA on the next day, had a survival rate of 100% (figure 5.2 i) as observed up to 10 days. The mice that were challenged by ST along with BC, with a booster dose the next day, all died within the duration of the experiment. All of the unchallenged C57BL/6 mice treated with either LA or BC survived.

5.2.4 Mice treated with ST and STBC had severe dysbiosys

LA treatment did not perturb the gut microbiota of either mouse strain (BALB/c or C57BL/6) while treatment with BC caused significant perturbation, resulting in decreased *Bacteroides* and increased *Anaerostipes*. Both LA and BC treated mice gut microbiota was comparable with time matched untreated control mice gut microbiota. However, ST reduced the number of short chain

fatty acid (SCFA) producing bacteria drastically within the gut microbiota, such as *lachnospiraceae* and *Morella* in BALB/c (figure 5.3 a and b); *prevotella* and *ruminococcus* in C57BL/6 mice (figure 5.3 c and d). SCFA producing bacteria maintain gut homoeostasis via signaling through anti-inflammatory pathways in IBD patients. Reduced SCFA producers in ST and STBC treated mice indicated the inflammatory condition in their gut. The overall diversity of the gut microbiota was decreased significantly in ST and STBC treated mice (figure 5.3). Mice treated with ST or STBC had increased numbers of inflammation inducing bacteria present in the gut, such as *proteobacteria*. Surprisingly, the mice treated with STLA had a balanced microbiota profile similar to the untreated control mice (figure 5.3). Species level information in the case of BALB/c revealed that *Butyricicoccus pullicaecorum* (6.3-fold), *Clostridium scidens* (41.6-fold), *Candidatus arthromitus* (16.7-fold), *Clostridium tertium* (20.6-fold), *Clostridium xylanolyticum* (11.2-fold) and *Faecalibacterium prausnitzii* (143.2-fold) levels increased by the fold changes indicated in brackets with respect to time matched untreated control mice (table 5.2). All of these bacteria are known complex carbohydrate fermenters, and producers of anti-inflammatory SCFAs such as butyrate, propionate, acetate, isobutyrate, isovalerate, and valerate. The presence of all these species in fecal samples was confirmed through qRTPCR using species specific primers designed against 16s rRNA (annexure 8). The excessive increase of these SCFA producing species in STLA treated mice might explain the non-inflammed status of the BALB/c mice; however, more experiments would be required to confirm it.

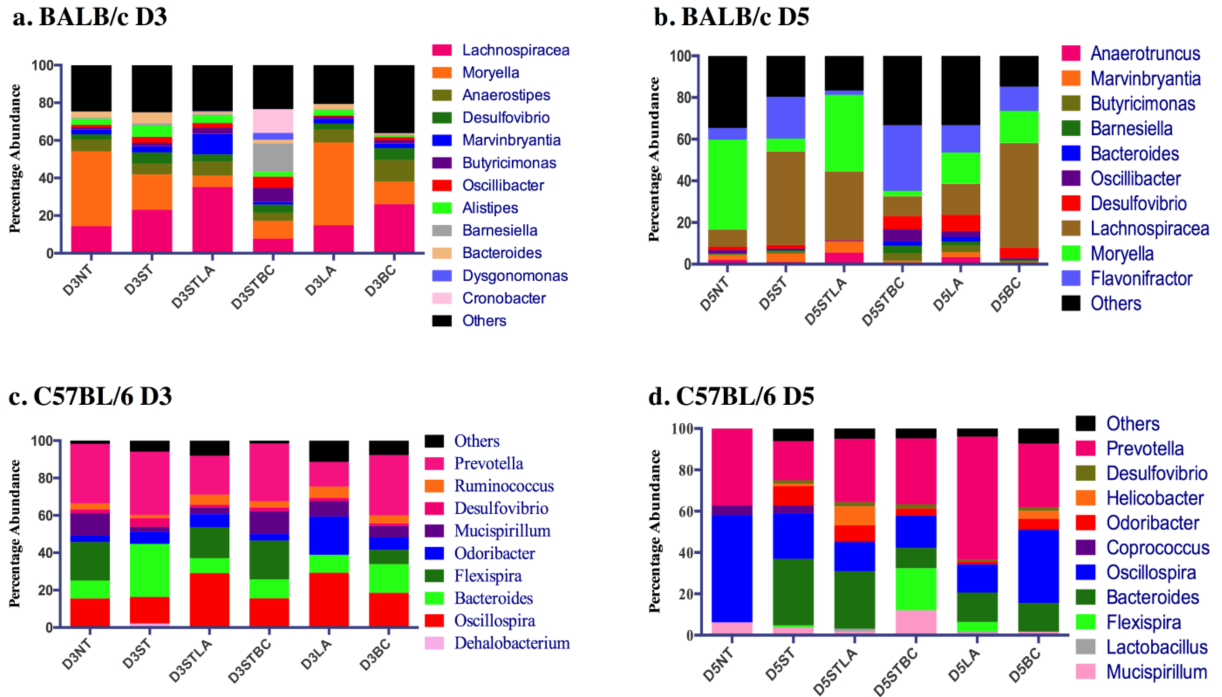

Figure 5. 3 Gut microbial composition of mice treated with different treatment conditions in genus level

Gut microbial composition of BALB/c mice treated with different probiotics and ST after 3 days (**a**) and 5 days (**b**). Gut microbial composition of C57BL/6 mice treated with different probiotics and ST after 3 days (**c**) and 5 days (**d**).

Results in the case of C57BL/6 mice revealed the increased number of SCFA producers in STLA treated mice, such as *Clostridium lepum* (13.7-fold), *Clostridium polysaccharolyticum* (2-fold), *Alistipes putredinis* (1.6-fold), *Prevotella dentalis* (2.8-fold), *Herbinix hemicellulosilytica* (17.8-fold) (table 5.3). C57BL/6 mice treated with STBC have increased numbers of inflammatory-inducing sulphate reducing bacteria, such as *Desulfovibrio intestinalis* (2.6-fold), and *Helicobacter typhlonius* (17.4-fold) (table 5.3). Together these data suggest that LA relieves inflammation caused by ST in Th2 biased BALB/c mice and Th1 biased C57BL/6 mice by stimulating SCFA producers in gut microbiota, whereas BC failed to do so. However, BC

Table 5. 2 BALB/c gut microbiota changes in species level folloing probiotics treatments

OTU_ID	Species Name	Fold changes wrt time matched NT mice			
		D3STLA	D3STBC	D5STLA	D5STBC
gi_343205971	*Butyricicoccus pullicaecorum*	2.6	-3.0	6.3	3.4
gi_444304121	*Candidatus arthromitus*	-2.0	-1.2	41.6	1.2
gi_265678482	*Clostridium scindens*	1.7	-1.4	16.7	-1.8
gi_636558671	*Clostridium tertium*	-4.2	-10.4	20.6	-2.6
gi_310975204	*Clostridium xylanolyticum*	1.0	-2.0	11.2	-2.0
gi_265678656	*Faecalibacterium prausnitzii*	-4.6	-3.4	143.2	5.8

Table 5. 3 C57BL/6 gut microbiota changes in species level following probiotics treatments

OTU_ID	Species Name	Fold changes wrt time matched NT mice			
		D3STLA	D3STBC	D5STLA	D5STBC
denovo1416	*Clostridium polysaccharolyticum*	2	1.3	-5	-2.4
denovo3052	*Desulfovibrio intestinalis*	2.1	2.6	1.6	-6.4
denovo4952	*Oscillibacter ruminantium*	2	-1.7	-1.1	-1.5
denovo9209	*Acetatifactor muris*	1	1	6846	6
denovo1769	*Ruminococcus gnavus*	-2.8	-1.6	8.8	36.6
denovo8429	*Herbinix hemicellulosilytica*	17.8	1.5	1	2.2
denovo3701	*Mucispirillum schaedleri*	1	6	-2.1	18
denovo2541	*Helicobacter typhlonius*	6.3	17.4	-3	3.3
denovo13416	*Prevotella dentalis*	-4.1	-12.5	2.8	-251.6
denovo5601	*Alistipes putredinis*	1.6	-3.3	1.1	-4.1
denovo465	*Clostridium lepum*	-1.6	30.3	13.7	-44.5

treatment of C57BL/6 mice infected with ST aids in destabilizing the microbiota by stimulating the growth of a few inflammation-inducing sulphate reducers. Altogether, we have revealed that LA induced anti-inflammatory microbiota, whereas BC induces inflammatory microbiota in the gut of ST infected mice. Gene expression in the gut wall tissue needs to be assessed to confirm the predicted inflammatory status.

5.2.5 Gene expression in the gut wall tissue of treated mice

Gut wall tissue comprised of a mixed population of cells including intestinal epithelial cells, goblet cells, M cells, enterochromafin cells, and immune cells such as macrophages, dendritic cells, B cells, T cells and NKT cells. Gut microbiota and the gut tissues are in continuous crosstalk through the interaction of various secretory molecules with gut tissue receptors, and the respective activation of various metabolic and immunological signaling pathways required for gut homoeostasis. Therefore, it is necessary to check gene expression in the gut wall tissue following its response to microbial perturbation, rather than analyzing intestinal epithelial cells alone. We extracted RNA from mouse gut wall tissue to check host gene expression. The genes were considered to be differentially expressed if their fold changes were more than 1.5 with respect to time matched untreated controls, and if the log ration of the p-value was less than 0.05. Our whole gene expression data revealed that at day 3, BALB/c gut wall tissue expressed 4850, 4813, 6548, 3980 and 8354 genes with ST, STLA, STBC, LA and BC treatments respectively. Similarly, in day 5 there are 5496, 5032, 8479, 5795 and 8421 genes expressed in ST, STLA, STBC, LA and BC treatments respectively in BALB/c mice gut. We followed the STLA treated BALB/c mice group up to day 10, and our gene expression data revealed the expression of 4919 genes (figure 5.4 a to d). A selection of immunological genes involved in the TLR pathway was validated through qRTPCR (figure 5.4 i and j).

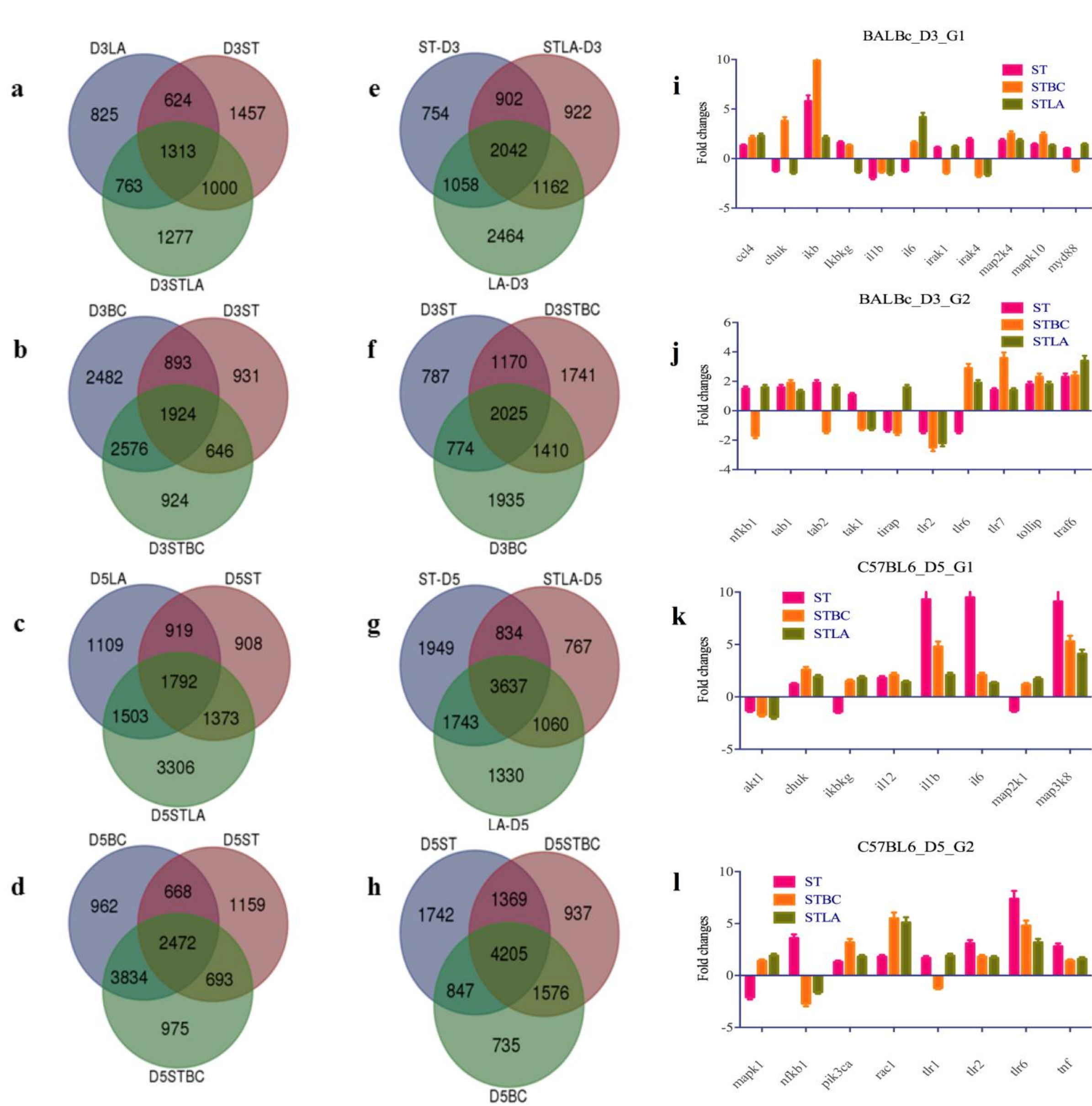

Figure 5. 4 Genes differentially expressed in mice with different treatment conditions and validation of few select immune genes through qRTPCR

a. Genes differentially expressed in BALB/c following treatment with LA, ST and STLA after 3 days, **b.** Genes differentially expressed in BALB/c following treatment with BC, ST and STBC after 3 days, **c.** Genes differentially expressed in BALB/c following treatment with LA, ST and STLA after 5 days, **d.** Genes differentially expressed in BALB/c following treatment with BC, ST and STBC after 5 days, **e.** Genes differentially expressed in C57BL/6 following treatment with LA, ST and STLA after 3 days, **f.** Genes differentially expressed in C57BL/6 following treatment with BC, ST and STBC after 3 days, **g.** Genes differentially expressed in C57BL/6 following treatment with LA, ST and STLA after 5 days, **h**. Genes differentially expressed in C57BL/6 following treatment with BC, ST and STBC after 5 days, **i,j.** Validation of innate

immune genes expressed in BALB/c following different treatments after 3 days, **k,l.** Validation of innate immune genes expressed in C57BL/6 following different treatments after 5 days.

For C57BL/6 mice in the day 3 group, there are 4756, 5028, 6346, 6726, 6144 differentially expressed genes in ST, STLA, STBC, LA and BC treatments, respectively. Similarly, for the day 5 C57BL/6 mice group there are 8163, 6298, 8087, 7770 and 7363 genes expressed differentially in ST, STLA, STBC, LA and BC treatments respectively. The day 10 STLA treated C57BL/6 mice had 7575 differentially expressed genes in their gut wall tissue (figure 5.4 e to h). Genes were clustered together based on their function and the pathways they represent. Several pathways had gene hits from our analysis; however, we concentrated mostly on immunologic, metabolic and gut barrier maintenance pathways in this study. A selection of the immunological genes involved in the TLR pathway was validated through qRTPCR (figure 5.4 k and l).

Table 5.4 lists some of the important genes and their fold changes with respect to time matched untreated controls in the day 3 group of BALB/c mice. The data suggests the expression of mucin genes such as MUC2, glucose transporters - such as Slc2a2 and Slc2a7, monocarboxylic acid transporters like Slc27a5 in mice treated with STLA. These data suggest that gene expression in STLA mice is shifting in order to promote maintenance of the gut mucous as well as epithelial tissue barrier function. Also, the STLA treated mice express the toxin-clearing cytochrome p450 gene Cyp26b1, which suggests the neutralization of toxins, is being facilitated by the gut epithelium. Tumor necrosis factor (TNF) and interleukin 1 beta (IL1b) was not expressed in STLA treated mice, but was expressed in STBC treated mice, suggesting an anti-

inflammatory condition and pro-inflammatory condition in STLA and STBC treated mice respectively (see annexure 4 for the entire list of differentially expressed genes in BALB/c).

Table 5. 4 Important genes differentially expressed in BALB/c after 3 days of probiotics treatment

Gene Symbol	D3BC	D3LA	D3ST	D3STBC	D3STLA
Adh4	11.6±1.5	1.5±0.2	4.2±0.5	4.7±0.6	7±0.9
Maob	2±0.3	-1.1±0.1	1.9±0.2	3.1±0.4	2.9±0.4
Ugt2a3	9.6±1.2	1.2±0.1	5.4±0.7	7.9±1.0	6.3±0.8
Ugt1a6a	-2.8±0.3	-1.2±0.1	-1.2±0.1	-3±0.4	3.4±0.4
Gzmg	1.3±0.1	1.7±0.2	1.9±0.2	1.1±0.1	140.8±17.8
Slc27a5	12.5±1.6	4.1±0.5	1.8±0.2	5.6±0.7	5.6±0.5
Nme6	1.2±0.1	1.2±0.1	1.3±0.1	-1±0.1	124.7±15.9
Oas1h	-1.1±0.1	1±0.1	1.2±0.1	1±0.1	31±3.9
Ppp1r12b	-5.4±0.6	-1.3±0.1	-1.3±0.1	1±0.1	29.1±3.7
Ptger4	-2.6±0.3	1.4±0.1	1.4±0.1	-1.5±0.1	22.6±2.9
Slc2a2	202.2±25.4	-1.2±0.1	1.8±0.2	40.2±5.3	22.1±2.8
Slc2a7	7±0.9	6.4±0.8	2.4±0.3	3.3±0.4	15.9±2.5
Mpo	1.2±0.1	1.2±0.1	9.7±1.2	-1.1±0.1	1.3±0.1
Lect2	5±0.6	-1.5±0.1	7.4±1.2	25.6±3.4	5.6±0.9
Hdc	2.6±0.3	1.9±0.2	1.1±0.1	7.4±1.2	3.1±0.4
Trim6	5.7±0.7	7.6±1.1	4.3±0.6	3.2±0.4	5.6±0.7
Cyp26b1	4.3±0.6	1.2±0.1	7±1.2	8.4±1.1	1.8±0.2

Table 5. 5 Important genes differentially expressed in C57BL/6 after 3 days of probiotics treatment

Gene Symbol	D3BC	D3LA	D3ST	D3STBC	D3STLA
DEFA4	-2.1±0.3	-1.9±0.2	-1.1±0.1	42.4±5.3	7.7±1
DEFA1	2.3±0.3	1±0.1	1.3±0.1	25.1±3.2	7.6±1
TNFSF4	-1.8±0.2	4.5±0.6	7.7±1.2	3.1±0.5	7.2±0.8
ITGA5	-5.5±0.6	-1.3±0.1	3.7±0.5	7±0.6	6.4±0.9
IL17F	2.4±0.4	4±0.5	1.2±0.1	3.1±0.3	4.5±0.6
IL19	2.2±0.3	-1.3±0.1	1.2±0.1	-1.6±0.2	4.1±0.5

TLR6	13.2±1.7	5.1±0.6	2.5±0.3	8.9±1.1	3.6±0.5
IL17A	2.3±0.3	1.5±0.2	-1.3±0.1	1.9±0.3	3.5±0.4
TGFB3	-1±0.1	1.3±0.1	1.3±0.1	1.4±0.1	2.4±0.3
CCR4	3.3±0.4	4.1±0.5	4.5±0.6	2±0.3	2.1±0.3
CXCL1	1.2±0.1	2.2±0.2	1.8±0.2	-1.8±0.1	2±0.3
IL12B	-1.1±0.1	1.2±0.1	-1.1±0.1	1.4±0.1	1.9±0.2
CTSE	1.7±0.2	10.8±1.5	5.3±0.6	11.6±1.6	1.9±0.2
CTLA4	2.3±0.3	1.9±0.2	-1.1±0.1	2.5±0.4	1.7±0.2
CSF3	3.9±0.5	2.5±0.3	3.5±0.4	1.7±0.2	1.7±0.2
C3	1.3±0.1	1.4±0.1	1±0.1	1.7±0.2	1.4±0.1
IL6	1.7±0.2	1.6±0.2	-1.4±0.1	1.2±0.1	1.3±0.1
CCL2	-1.1±0.1	1.9±0.2	-1.1±0.1	1.1±0.1	1.1±0.1
FCER1G	-1.3±0.1	-1.4±0.1	-1.2±0.1	1.5±0.1	-1.1±0.1
CAMP	1.4±0.1	2.2±0.2	-1.1±0.1	1.8±0.1	-1.1±0.1
IL1B	1.9±0.3	2±0.2	1.2±0.1	1.3±0.1	-1.1±0.1
TNF	1.4±0.1	1.4±0.1	1±0.1	1.9±0.2	-1.1±0.1
FPR2	1±0.1	-2.1±0.3	1.2±0.1	2.1±0.2	-1.2±0.1
CCL7	1.4±0.1	2.6±0.3	1.4±0.2	4.2±0.6	-1.3±0.1

Table 5.5 lists some of the important genes and their fold changes with respect to time matched untreated control in day 3 group of C57BL/6 mice. Heavy expression of the defensins, DEFA4 and DEFA1, in STBC treated mice suggests that tissue damage occurred in the gut. Interleukin 12 beta (IL12b) and Il17A expression in STLA treated mice suggest upregulation of pro-inflammatory signals for pathogen clearance. Down-regulation of IL1b and TNF in STLA treated mice, but up-regulation in STBC treated mice suggested the presence of severe inflammation in the STBC group, whereas no inflammation was present in the STLA group of mice (see annexure 5 for the entire list of differentially expressed genes in C57BL/6). Together, these data suggest that LA induce anti-inflammation and BC induce inflammation in ST infected mice, findings which are in accordance with the previous data on the microbiota. However, the

inflammatory damage to the gut by ST and STBC needs to be further explored for confirmation and mechanism.

5.2.6 BALB/c and C57BL/6 mice treated with ST and STBC had intensive damage to ileum, colon, spleen and Liver

Histo-pathological scoring of the eosin and haematoxylin (H&E) stained transverse slices of BALB/c and C57BL/6 mice ileum, colon, spleen and liver were performed using the method explained in the materials and methods section. Extensive damage, immune infiltration into the tissue, tissue abscises, and immune infiltrations into the red pulp area were observed in the BALB/c mice treated with ST and STBC. This was further supported by a higher pathological score of 3.4 ± 0.2, 1.9 ± 0.2, 2.6 ± 0.2, 1.9 ± 0.3 for the ileum, colon, spleen and liver respectively in BALB/c mice treated with ST. Similarly pathological score of 3.6 ± 0.1, 2.6 ± 0.2, 2.4 ± 0.4 and 2.1 ± 0.5 for the ileum, colon, spleen and liver respectively in BALB/c mice treated with STBC suggested an inflammatory state in this group of mice. Tissues of the above mentioned organs have lower pathological scores compared to untreated mice in the groups of mice treated with LA, BC and STLA (figure 5.5).

Extensive damage, immune infiltration into the tissue, tissue abscises, and immune infiltrations into the red pulp area were also observed in the C57BL/6 mice treated with ST and STBC. This was further supported by a higher pathological score of 3.2 ± 0.2, 2.3 ± 0.3, 2.7 ± 0.2, 3.2 ± 0.3 for the ileum, colon, spleen and liver respectively in C57BL/6 mice treated with ST. Similarly, a pathological score of 3.6 ± 0.2, 3.6 ± 0.1, 2.7 ± 0.4 and 2.6 ± 0.6 for the ileum, colon, spleen and liver respectively in C57BL/6 mice treated with STBC suggests an inflammatory state in this group of mice. Tissues of the above mentioned organs had lower pathological scores and

comparable to untreated mice in the group of mice treated with LA, BC and STLA (figure 5.6). Microbial dysbiosys and inflammatory gene expression in ST and STBC treated BALB/c and C57BL/6 mice were further supported with significantly higher pathological scores in these groups of mice with respect to time matched untreated control groups of mice.

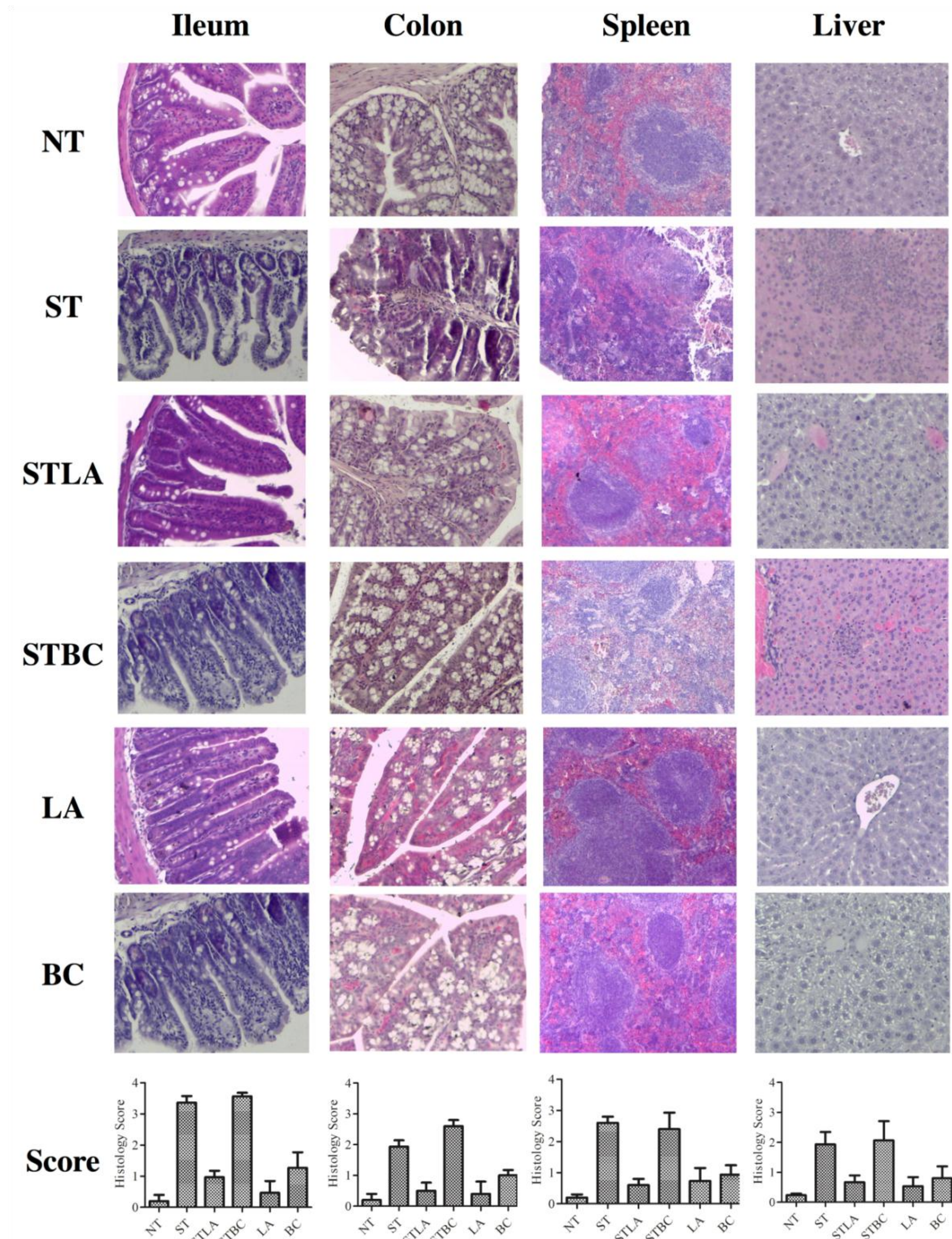

Figure 5. 5 Histopathology of BALB/c treated with ST, LA, BC and their combinations

(Note the highest score of the group treated with ST and STBC. ST and STBC treated mice have high inflammation.)

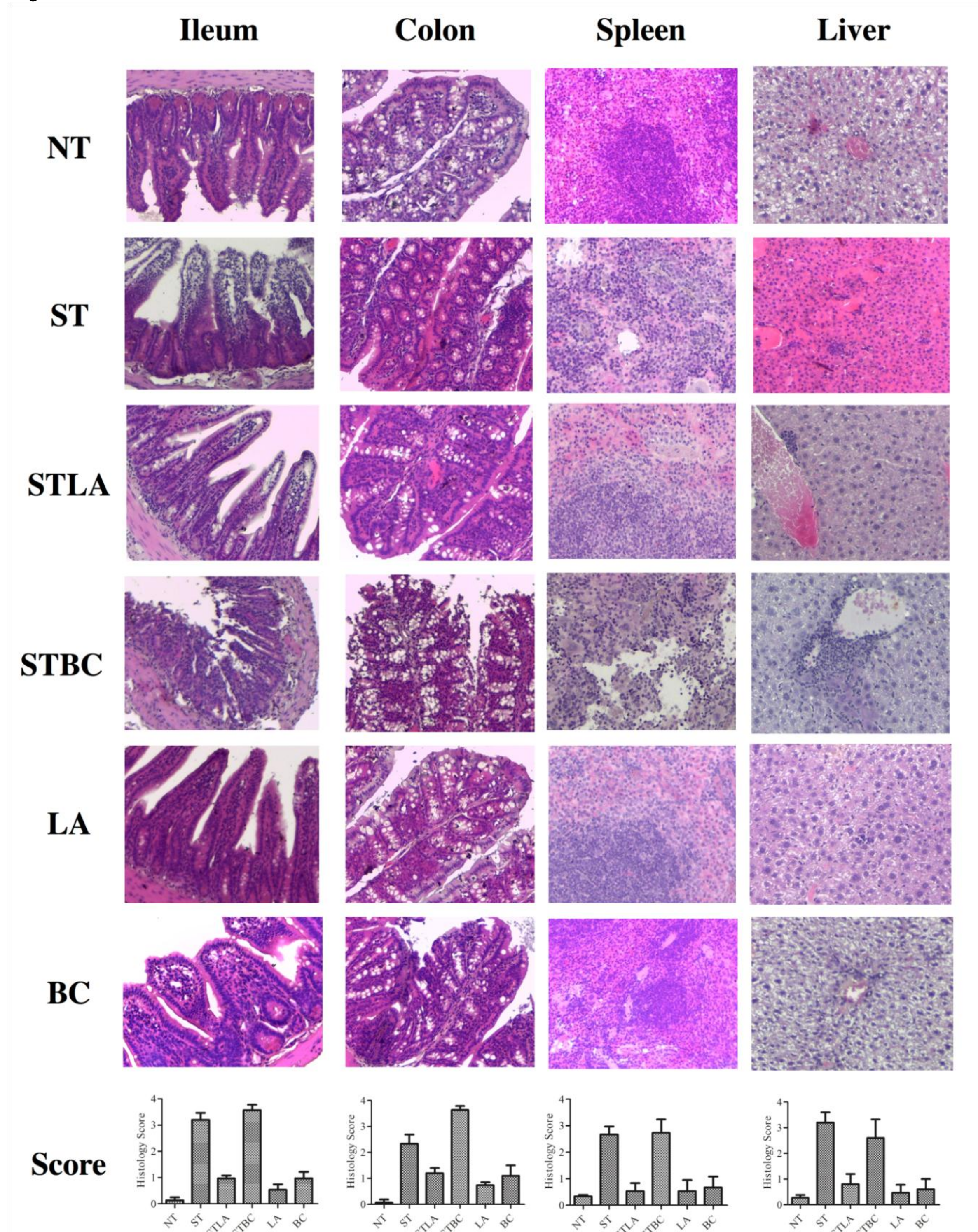

Figure 5. 6 Histopathology of C57BL/6 treated with ST, LA, BC and their combinations

(Note the highest score of the group treated with ST and STBC. ST and STBC treated mice have high inflammation.)

5.3 Discussion

5.3.1 Microbial diversity and its resilience to perturbation

Microbial diversity has been seen to be reduced in the gut from the patients suffering from Crohn's disease [254], ulcerative colitis [255], irritable bowel syndrome [256], Clostridium difficile associated diarrhea [257] and antibiotic associated diarrhea [258]. Fransen et al have shown that BALB/c gut microbiota was more diverse than that of C57BL/6 mice [253], and our study also reproduced these findings. Species-rich microbial communities are less susceptible to invasion because they use limiting resources more efficiently, with different species specialized to each potentially limiting resource. Therefore, we suspect that BALB/c mice might be less vulnerable to perturbation by ST than C57BL/6 mice. Our 16s rRNA profiling data from the mice gut microbiota has suggested that ST perturbed the microbiota of C57BL/6 mice more than that of the BALB/c mice. Surprisingly, the microbial perturbation was more pronounced in STBC treated mice than in ST treated mice. There was little perturbation of microbiota in STLA, LA and BC treated mice, which implied that while LA and BC by itself did not disturb the microbiota, LA was restoring ST perturbed microbiota to normalcy. Our species level information revealed an increase in number of SCFA producing firmicutes, such as *Butyricoccus pullicaecorum*, *Clostridium scindens*, *Candidatus arthromitus*, *Clostridium tertium*, *Clostridium xylanolyticum* and *Faecalibacterium prausnitzii,* in STLA treated BALB/c mice. In the case of STLA treated C57BL/6 mice, there was an increase in SCFA producing species such as *Clostridium lepum*, *Clostridium polysaccharolyticum*, *Prevotella dentalis* and *Herbinix hemicellulosilytica*. SCFAs induce anti-inflammatory responses in the intestinal

epithelial cells of mice by inhibiting HDACs, followed by NF-kB suppression and down regulation of TNF and

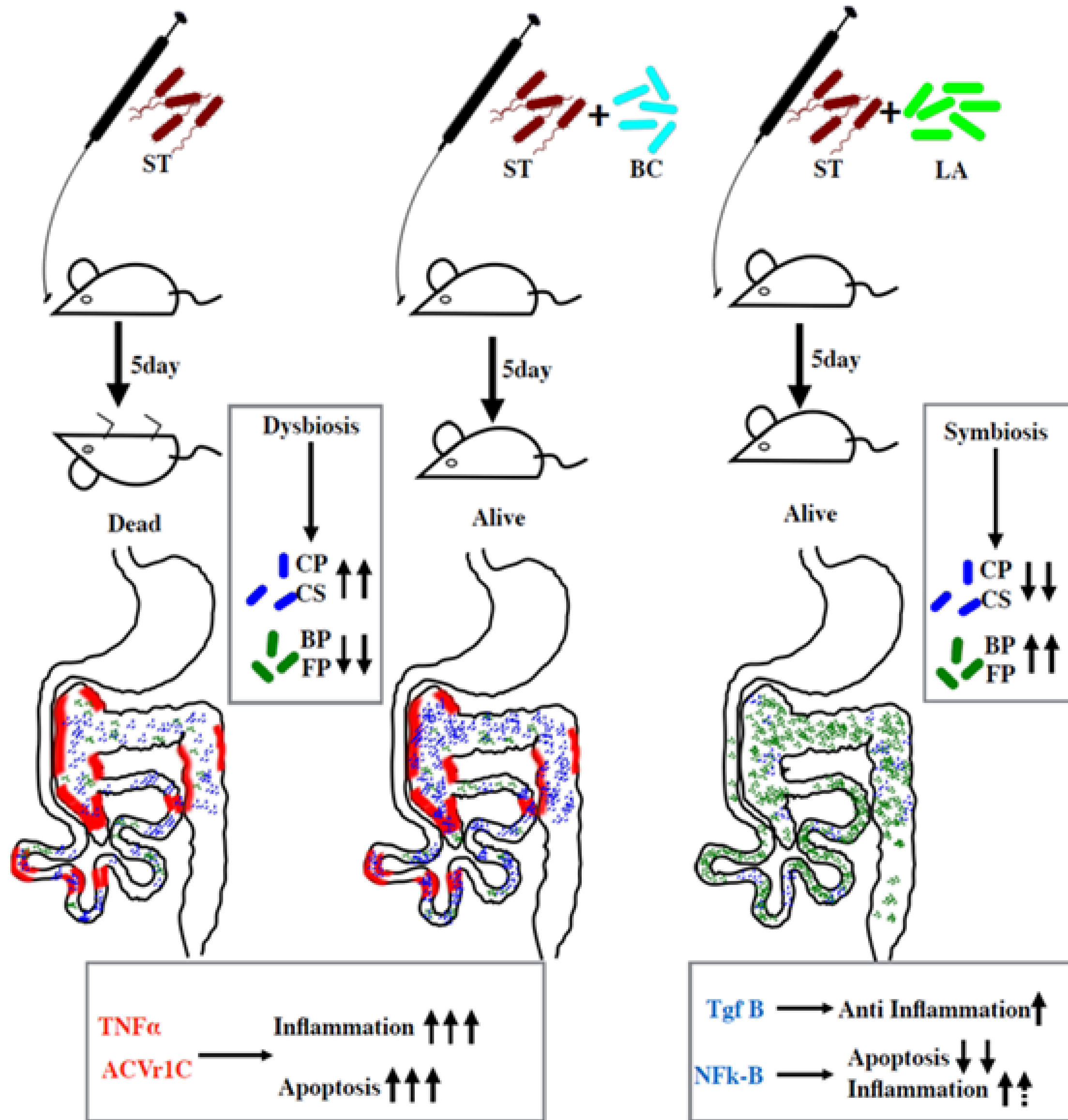

Figure 5. 7 Mechanism of protection of BALB/c against ST infection by LA and BC

LA modulates SCFA producing microbiota in gut; thereby reducing ST induced inflammation through activation of Tgfb pathway and deactivation of NFkb pathway. However BC could not reduce inflammation induced by ST, but STBC treated mice survive with inflammation. ST treated mice died within 6 days; however LA and BC treated mice do not have any inflammation and fatality.

IL1B [259]. The bloom of SCFA producing bacteria in STLA treated mice suggested that LA was trying to bring down the ST induced inflammation in the gut. However, STBC treated C57BL/6 mice had an increased number of sulphate reducing bacteria, such as *Desulphovibrio intestinalis* and *Helicobacter typhlonius*, which are known inflammation inducers in the gut. Dysbiosys and inflammation in the gut co-occurs in patients suffering from CD, UC, IBD, IBS, CDAD and AAD. Dysbiosys was evident in ST and STBC treated mice, whereas there was little perturbation in STLA, LA and BC treated mice. Therefore, inflammatory and physiological status in the gut needs to be evaluated, which we have accomplished through gene expression analysis using an expression microarray. The inflammatory status of the gut was also visualized through H & E stained transverse sections of the gut, as well as spleen and liver.

5.3.2 ST and STBC treated mice exhibit severe inflammation in gut compared to STLA treated mice

SCFAs are inhibitors of histone deacetylases (HDACs) and ligands for G protein-couples receptors (GPCRs), and thereby act as signaling molecules that influence the expansion and function of hematopoietic and non-hematopoietic cell lineages. SCFA-driven inhibition of HDACs tends to promote a tolerogenic, anti-inflammatory cell phenotype that is crucial for maintaining immune homoeostasis [259]. Exposure of peripheral blood mononuclear cells and neutrophils to SCFAs inhibits HDAC and inactivated nuclear factor–kB (NF-kB), and down regulated production of the pro-inflammatory cytokine, tumor necrosis factor (TNF) [260,261]. Activation of HDAC1, HDAC3, HDAC6, HDAC7, HDAC8, and HDAC9 by BC and STBC in BALB/c mice was followed by the activation of TNFSF10, TNFSF13B and TNFSF14 through NF-kB pathway; this corroborates the high level of inflammation and immune cell infiltration observed in STBC treated groups of mice. However, LA treated BALB/c mice had down

regulated HDACs and TNFs which are corroborated with low inflammation and low immune cell infiltration in STLA treated mice. Up-regulated HDACs and TNFs by BC and ST might explain the synergistic activity of ST and BC with respect to inflammation in BALB/c mice, whereas HDAC down-regulation by LA antagonized the ST induced inflammation. However, moderate activation (low fold changes) of chemokines like CCL17, CCL24, CCRL2, CXCL12 and interleukins such as IL2, IL6, IL22, IL22RA1, and IL24 in STLA treated BALB/c mice indicated the immune activation for ST clearance without inducing inflammation. Robust activation (High fold changes) of the aforementioned chemokines and cytokines, along with TNFs and IL1B in STBC treated BALB/c mice, indicates immune activation for ST clearance, but over inflammation might have caused the severe immune infiltration and damage to the gut mucosa. Over expression of formyl peptide receptor 1 (FPR1) in STBC treated BALB/c mice, which are known to be aberrantly expressed during inflammation[262], corroborated with the inflammatory condition observed in these groups of mice. SCFAs are also essential for the maintenance of mucosal immunity through fortifying the intestinal epithelial cell barrier function. In response to SCFAs, intestinal epithelial goblet cells increased their transcription of mucin genes [263,264]. In our transcriptome study we found that ST down regulates mucins, including MUC5Ac and MUC5B, whereas LA restored the expression of these genes in STLA treated BALB/c mice. Expression of free fatty acid receptor 4 (FFAR4) in STLA treated mice might be acting as the receptor for the SCFAs produced in the gut by the microbiota, which would induce the restoration of mucin genes. Apart from mucins, other proteins contributing to IEC barrier function, are intercellular junction proteins such as occludins, zonula occludens 1 (known as TJP1) and E-cadherins [265,266].Down regulation of tight junction protein 2 (TJP2)

in STBC and ST treated mice implicated the compromised intestinal barrier in these groups of mice.

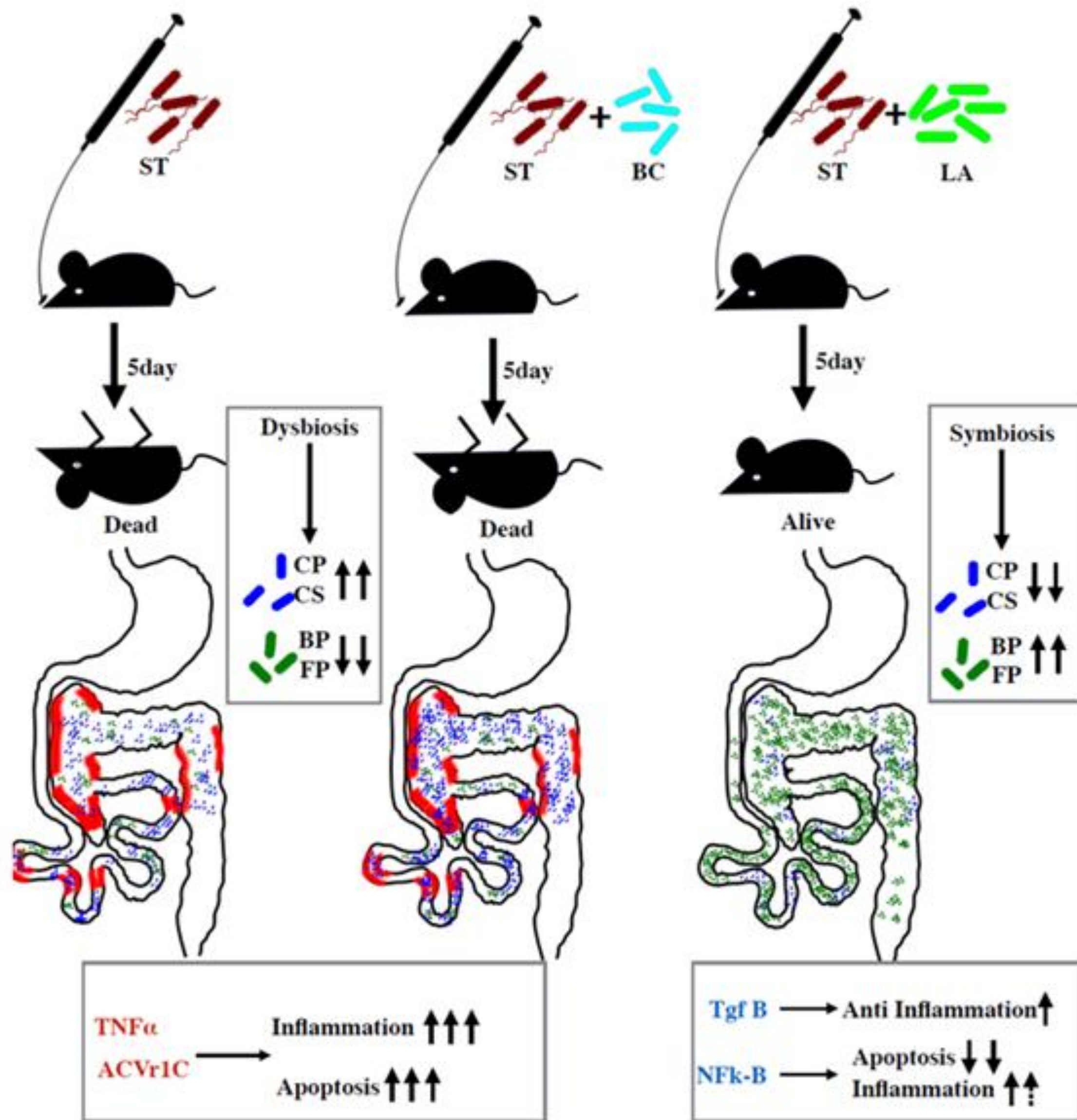

Figure 5. 8 Mechanism of protection of C57BL/6 against ST infection by LA

LA modulates SCFA producing microbiota in gut; thereby reducing ST induced inflammation through activation of Tgfb pathway and deactivation of NFkb pathway. However BC could not reduce inflammation induced by ST, thereby STBC treated mice died because of inflammation. ST treated mice died within 5 days; however LA and BC treated mice do not have any inflammation and fatality.

However, TJP2 is over expressed in STLA and LA treated mice, indicating high integrity of the intestinal barrier in these groups of mice. Down regulation of E-cadherins and its receptors, such as CDH10, CDH17, CDH22, CDHR1, CDHR2 and CDHR5 in ST and STBC treated mice, indicated a compromised gut barrier in these group of mice.

Activation of HDAC3 and HDAC6 by BC and STBC in C57BL/6 mice followed by activation of TNF, TNFSF4, IL1B, and TNFRSF4 through NF-kB pathway corroborates with the high inflammation and immune cell infiltration observed in STBC treated groups of mice. However, LA treated C57BL/6 mice had down regulated HDACs and TNFs, which are corroborated with low inflammation and low immune cell infiltration in STLA treated mice. Up-regulated HDACs and TNFs by BC and ST might explain the synergistic activity of ST and BC with respect to inflammation in C57Bl/6 mice, whereas HDAC down-regulation by LA antagonized the ST induced inflammation. However, moderate activation (low fold changes) of chemokines, such as CCL2, CCL7, CCR4, CXCL1, CXCL13, and interleukins, like IL12B, IL6, IL17A, IL17F, IL19, and IL22 in STLA treated C57BL/6 mice, indicated the immune activation for ST clearance without inducing inflammation. Robust activation (High fold changes) of the aforementioned chemokines and cytokines along with TNFs and IL1B in STBC treated C57BL/6 mice indicated immune activation for ST clearance, but over inflammation might have caused the observed casualties. Aberrant expression of formyl peptide receptors (FPRs) has been well established in states of inflammation, autoimmune diseases, neurodegenerative disorders, and cancer [262]. Over expression of FPR1 and FPR2 in ST treated mice corroborated the inflammatory status of ST treated mice after 5 days. Expression of free fatty acid receptor 2 (FFAR2), followed by expression of mucin genes such as MUC2, MUC3 and

MUC15 in LA and STLA treated mice, indicates an intact IEC barrier; however, downregulation of these genes in ST and STBC treated mice indicates compromising of the IEC barrier. TJP1 was moderately expressed in LA and STLA treated mice, but was down regulated in ST and STBC treated mice, indicating the compromising of IEC barrier integrity in ST and STBC treated mice. E-cadherines, and their receptors (CDH2, CDH7, CDH12, CDH20, CDHR2 and CDHR4), were downregulated in ST and STBC treated mice, indicating compromised gut barrier integrity in these groups of mice.

5.4 Conclusion

Together, the microbiota, host transcriptome and host histo-pathological data suggest that BALB/c mice micobiota was less perturbed than that of C57BL/6 mice following ST infection. LA antagonizes the ST infection completely by inducing SCFA producing microbial species like, *B. pullicaecorum*, *C. scindens*, *C. arthromitus*, *C. tertium*, *C. xylanolyticum* and *F. prausnitzii* in BALB/c mice. However, BC could not induce these SCFA producing bacteria significantly, therefore STBC treated BALB/c mice survived, but with severe inflammation of the gut. Downregulated TNF and IL1B in STLA treated mice, but up-regulated TNF and IL1B in STBC treated mice, in addition to evidence from histological data, was in accordance with the previously mentioned observations. Further expression of several mucins, zonula occludens 1, and several E-cadherins in STLA treated mice indicated the intact IEC barrier function, which was supported by gut histological data (See figure 5.7 for the graphical summary).

The microbiota of C57BL/6 mice was readily perturbed by the ST-induced growth of sulphate reducing proteobacteria *D. intestinalis* and inflammation inducing proteobacteria *H. typhlonius.*

However, LA induced the anti-inflammatory SCFA producing bacteria such as *C. lepum*, *C. polysaccharolyticum*, *P. dentalis* and *H. hemicellulosilytica* which consequently neutralized the ST induced inflammation in the mouse gut. The over activation of HDACs by BC and ST in C57BL/6 mice induces NF-kB mediated production of inflammation inducing TNF and IL1B. The inflammation is again accompanied with compromised IEC barrier function through downregulation of mucins, TJP1 and several E-cadherins in STBC treated C57BL/6 mice, which is evident from the histology of the mouse gut (See figure 5.8 for the graphical summary).

Microbial diversity has been reported to be depleted in CD, UC, IBD, IBS, CDAD and AAD. In this study we reported that the gut microbiota is also depleted in ST induced diarrhea accompanied by severe gut inflammation. Therefore, we propose that depletion of microbial diversity can be regarded as a marker for several gut related diseases. While probiotics were supplemented successfully offset the dysbiosis in gut microbiota caused by antibiotics, it should be kept in mind that a few probiotics species exist, such as BC, which may exacerbate inflammation during ST infection. We conclude that each species and each strain of probiotics should be pre-screened for their compatibility with a particular type of infection or disease; further study in this area is highly needed and future insights may serve as promising prospects for probiotic use in clinical settings.

Chapter 6

Summary
&
Conclusions

6.0 Summary and Conclusions

6.1 Summary

Despite our efforts to curb the increase and spread of antimicrobial resistance, bacteria continue to become less susceptible to antimicrobial drugs over time, and rates of discovery for new antibiotics are declining. Thus, it is essential to explore new paradigms for anti-infective therapy. One promising approach involves host-directed immune-modulatory therapies, whereby natural mechanisms in the host are exploited to enhance therapeutic benefit. In the current study, our objective is to initiate or enhance protective antimicrobial immunity while limiting inflammation-induced tissue injury.

We have tested the immune-modulatory effects of select HDPs, such as indolicidin and LL37. Our whole genome expression data following indolicidin and LL37 treatment of macrophages are in accordance with the previously published partial data sets. We further tried to enhance the immune modulatory efficacy of HDPs by covalently conjugating them to CNT and GNP. Because of conjugation with nanomaterials, efficacy of indolicidin and LL37 was increased by 1000 fold. The HDP primed mice macrophage cell line Raw264.7 and human monocytic cell line THP-1 were protected from *Salmonella typhimurium* induced cytotoxicity for 16 hours post challenge. Activation of cytokines like IL6, IL8, IL12 and several type I and type II interferons, but no expression of TNFs in macrophages indicated the inflammation proof priming of the innate immune system through indolicidin and LL37. Our systematic study on HDP induced immune priming of macrophages and its protection against ST, strengthened the concept of

modulating innate mucosal immunity as an alternative to antibiotics to combat infectious diseases in an evolution proof manner.

Probiotics are the living micro-organisms which were consumed through food unknowingly by human civilization from Neolithic era itself in the form of fermented foods. It was only after the discovery of modern fermentation technology in the 19th century; people industrialized the fermentation process and consumed fermented foods containing probiotics and prebiotics. It was observed that people consuming yoghurt habitually have longer life span and better combating capacity to infectious diseases. It was only recently in the 21st century when the menace of antibiotics resistance bugs went out of hand, people began to think about probiotics as an alternative to antibiotics. However the mechanisms through which probiotics work remained unknown until recently when people started looking at their immune modulatory properties and its ability to modify the gut mucosal microbiota. We have screened *in vitro* 4 highly prescribed probiotics species, but of different strains; with murine and human macrophage cell lines with respect to their immune-modulatory property and protection against ST induced cytotoxicity. *Saccharomyces boulardii*, and *Bifidobacterium bifidum* could not induce expression of select innate immune genes in macrophages which in turn were sensitive to ST induced cytotoxicity. However *Lactobacillus acidophilus* MTCC10307 and *Bacillus clausii* MTCC8326 could activate the monocytes into macrophages and modulate several cytokines, chemokines and defensins. Macrophages primed by LA and BC were found to be through NFkB and p38 MAPK pathways. The primed macrophages were protected against ST induced cytotoxicity for 16 hours post challenge. We orally gavaged these two probiotics to Th1 biased C57BL/6 mice and Th2 biased BALB/c mice with or without ST and gave a booster dose of probiotics 24 hours after

first gavage. We found that there was severe inflammation and colitis in the group of mice treated with ST after 3 days of infection and they eventually died after 6 days in the case of BALB/c and 5 days in the case of C57BL/6. We have seen that mice treated with BC and ST also have severe inflammation and colitis. However, LA and ST treated mice have no inflammation and colitis. Although both these mice strains are inbred and have little genetic difference; their response to ST, STBC and STLA were different. This differential response might be because of the different composition and abundance of gut microbiota. We profiled the gut microbiota using 16s rRNA V3 sequencing. While BALB/c microbiota was mainly dominated by firmicutes, C57BL/6 microbiota was composed of firmicutes and bacteroides in equal percentage. While major SCFA producers in BALB/c are lachnospira, that of C57BL/6 are morella. We confirmed the differential species through qRT-PCR and found that *Butyricoccus pullicaecorum* and *Faecalibacter prausnitzii* numbers increased drastically in STLA treated BALB/c mice. These are the two key bacteria found to be depleted in IBD, IBS and CD patients because of severe colitis occur. These are the two key bacteria which produce butyrate and propionate from complex carbohydrates which has anti-inflammatory property. In the case of C57BL/6 mice *Clostridium lepum*, *Clostridium polysaccharolyticum*, *Prevotella dentalis* and *Herbinix hemicellulosilytica* are the SCFA producers which increased with STLA treatment. LA might have reversed the inflammation caused by ST in this group of mice by inducing the growth of SCFA producers. But BC could not modulate the microbiome of the mouse gut in a way which could induce anti-inflammation. The inflammatory conditions in the ST and STBC treated mice are further confirmed through inflammatory gene expression in gut wall tissue and lesions in the gut evaluated through histopathological studies.

6.2 Conclusions

LL-37 or indolicidin primed THP-1 cells can efficiently protect themselves against ST induced cytotoxicity for 16 h post challenge. The genome wide gene expression study shows that pro-inflammatory and anti-apoptotic signaling in THP-1 cells treated with indolicidin was mediated through *TNFRSF1A,* followed by activation of *NFkB* and *c-JUN*. However, LL-37 treatment was mediated through *IL1R,* followed by activation *of NFkB* and *NFAT2*. Though immune modulation by LL-37 and indolicidin was partly known before, our data established the complete gene expression and signaling mechanism. The conjugation strategy enhanced the immune modulating efficacy of these two peptides by 1000 fold, which will reduce the cost of these peptides for antimicrobial treatment, thereby increasing treatment access to a wider population of developing countries.

The use of Lactobacillus and Bacillus species as probiotic dietary supplements is increasing. Each year new strains of these two genera are studied and added to the probiotics list based on their immune modulatory and antimicrobial activities. We screened two new strains of LA and BC, which displayed significant effects on modulating innate immune responses in murine macrophages and protected their cells from ST induced cytotoxicity. These two strains adhere to the surface of macrophages and have moderate cytotoxic activity. We tested LA and BC in mice as probiotics with respect to their ability to modify gut microbiota and inducing innate mucosal immunity. Microbial diversity was reported to be depleted in CD, UC, IBD, IBS, CDAD and AAD. Here we report the microbiota is also depleted in ST induced diarrhea accompanied with severe gut inflammation. However the LA induces several SCFA producing bacteria in the gut

which maintained the gut homoeostasis. But BC failed to induce such bacteria, rather induced proteobacteria which enhanced inflammation even further. So we propose that depletion of microbial diversity can be regarded as a marker for several gut related diseases. While probiotics was supplemented with many antibiotic medications to maintain the gut microbiota, it should be kept in mind that few probiotics species like BC may trigger more inflammation during ST infection. Thus each species and each strain of probiotics should be checked for their compatibility with a particular type of infection. Our comparative analysis of gene expression dynamics in macrophages following treatment with LA and further confirmed *in-vivo* in mice in maintaining gut microbial homoeostasis confirms the potential of LA MTCC10307 as a probiotic. Immune modulation by LL-37, indolicidin, and LA MTCC10307 followed by protection against ST infection, have shown the promise of the idea to combat infection by modulating natural mechanisms in the host through evolution proof manner.

6.3 Key Findings from the study

- ❖ Efficacy of Indolicidin and LL-37 was increased by 1000 fold by conjugation with CNT and GNP.
- ❖ Immune-modulation by Indolicidin was signaled through TNFRSF1A followed by activation of NFkB and c-JUN.
- ❖ Immune-modulation by LL-37 was mediated through IL1R, followed by activation of NFkB and NFAT2.
- ❖ LA (MTCC-10307) and BC (MTCC-8326) modulated immune genes in macrophages (Raw264.7 and THP-1) without inducing inflammation and protected them from ST (MTCC-3232) induced cytotoxicity.

- BALB/c microbiota is composed of 83.7% firmicutes, 11% bacteroides, and 3.3% proteobacteria. The major genera in BALB/c microbiota were moryella (39.7%) and lachnospiracea (14.4%).
- C57BL/6 microbiota consists of 49.5% firmicutes, 46.2% bacteroides, and 3.4% spirochaetes. The major genera in C57BL/6 microbiota were 54.9% prevotella, 16.7% oscillospira.
- BALB/c microbiota is more diverse than C57BL/6 microbiota. This might be the cause why BALB/c microbiota is less perturbed by ST than that of C57BL/6.
- *Butyricoccus pullicaecorum, Clostridium scindens, Candidatus arthromitus, Clostridium tertium, Clostridium xylanolyticum* and *Faecalibacterium prausnitzii* are the SCFA producing species induced in STLA treated BALB/c mice for countering inflammation.
- *Clostridium lepum, Clostridium polysaccharolyticum, Prevotella dentalis* and *Herbinix hemicellulosilytic* are the SCFA producing species induced in STLA treated C57BL/6 mice for countering inflammation.
- BC prescribed with antibiotics to maintain gut microbiota in typhoid cases may induce severe dysbiosis and inflammation in gut.

6.4 Methods developed during the course of this project

- Extraction of gDNA from gut contents (Details in Methods section).
- Designing species specific primers against 16s rRNA gene (Details in Methods section and Annexure 8).
- Profiling microbial composition through qRTPCR, qualitatively and quantitatively. (Details in Annexure 8).

6.5 Future Prospective

- CNT and GNP conjugated indolicidin and LL-37 has 1000 fold better efficacy *in-vitro,* than the free peptides with respect to immune modulation in macrophage. However their efficacy needs to be proved *in-vivo*.
- High microbial diversity can be considered as a marker of good gut health. We have seen that ST and STBC decreased microbial diversity in BALB/c and C57BL/6 mice, which in turn increased inflammation. Also it was reported that microbial diversity was reduced drastically in CD, IBD and IBS patients who have severe inflammation in the gastrointestinal tract.
- Probiotics and prebiotics which can induce higher microbial diversity in the host can have significant implications in clearing the infectious microbes out of the gut, by depleting the substrates which sustain infectious microbes in gut.
- Morella, lachnospira, and prevetolla are the genera which contains many species producing SCFA from complex carbohydrates. Probiotics which can induce these genera can be used to treat the gut inflammatory disorders like CD, IBD and IBS.

Chapter 7

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Annexures

Annexures

Annexure 1: Important genes expressed differentially in THP-1 following 6 hour treatment with Nano-LL37

Gene Symbol	Entrez Gene ID	CNT	GNP	LL37-20	LL37-0.02	GNP-LL37	GNP + LL37	CNT-LL37	CNT + LL37	CNT + GNP + LL37	CNT_LL37+ GNP_LL37
AKT2	208	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
ALPK2	115701	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
ANAPC11	51529	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4
APOPT1	84334	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7
BCL2L12	83596	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
BCL7C	9274	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
BRF1	2972	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
CALU	813	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
CAMKMT	79823	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
CCL19	6363	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
CCL20	6364	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
CCL28	56477	-2.3	-2.3	-2.3	-2.3	-2.3	-2.3	-2.3	-2.3	-2.3	-2.3
CCL4	6351	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
CDK10	8558	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
CDK17	5128	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
CDK4	1019	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1

CDK5RAP2	55755	-5.6	-5.6	-5.6	-5.6	-5.6	-5.6	-5.6	-5.6	-5.6	-5.6
CDKL3	51265	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
CDKN2C	1031	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
COX1	4512	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
COX7B2	170712	-10.9	-10.9	-10.9	-10.9	-10.9	-10.9	-10.9	-10.9	-10.9	-10.9
COX7C	1350	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3
CTNNB1	1499	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
CXCR1	3577	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0
CYP11B1	1584	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
CYP2A7	1549	-7.0	-7.0	-7.0	-7.0	-7.0	-7.0	-7.0	-7.0	-7.0	-7.0
CYP2F1	1572	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
CYP2J2	1573	-21.4	-21.4	-21.4	-21.4	-21.4	-21.4	-21.4	-21.4	-21.4	-21.4
CYP3A43	64816	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0
CYP3A5	1577	-2.1	-2.1	-2.1	-2.1	-2.1	-2.1	-2.1	-2.1	-2.1	-2.1
CYP4A11	1579	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
DEFB123	245936	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
DEFB129	140881	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4
GAD2	2572	-1.8	-1.8	-1.8	-1.8	-1.8	-1.8	-1.8	-1.8	-1.8	-1.8
GPR114	221188	-5.7	-5.7	-5.7	-5.7	-5.7	-5.7	-5.7	-5.7	-5.7	-5.7
GPR115	221393	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
GPR135	64582	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1

GPR153	38750 9	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
GPR176	11245	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
GPR35	2859	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
GPR77	27202	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
HK3	3101	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
IFNAR1	3454	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
IL17C	27189	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
IL18BP	10068	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
IL1F10	84639	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0
IL1R1	3554	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7
IL27RA	9466	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
IL6ST	3572	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
MAP2K2	5605	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
MAP3K4	4216	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5	-3.5
MAP3K6	9064	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
MAP3K9	4293	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
MAP6D1	79929	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
MAP7D2	25671 4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4
MAPK15	22568 9	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
MUC1	4582	-3.9	-3.9	-3.9	-3.9	-3.9	-3.9	-3.9	-3.9	-3.9	-3.9
MUC12	10071	-1.6	-1.6	-1.6	-1.6	-1.6	-1.6	-1.6	-1.6	-1.6	-1.6
MUC4	4585	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
NFATC2	4773	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
NFATC4	4776	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9
PIK3C2A	5286	-3.1	-3.1	-3.1	-3.1	-3.1	-3.1	-3.1	-3.1	-3.1	-3.1

PIK3R5	23533	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
PPP1CA	5499	-4.7	-4.7	-4.7	-4.7	-4.7	-4.7	-4.7	-4.7	-4.7	-4.7
PPP1R11	6992	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
PPP1R13B	23368	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
PPP1R15A	23645	-6.2	-6.2	-6.2	-6.2	-6.2	-6.2	-6.2	-6.2	-6.2	-6.2
PPP1R16B	26051	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4
PPP1R21	129285	-3.6	-3.6	-3.6	-3.6	-3.6	-3.6	-3.6	-3.6	-3.6	-3.6
PPP2R1A	5518	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
PPP2R2A	5520	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4	-3.4
PPP2R2D	55844	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
PPP2R5A	5525	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
PPP6R2	9701	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0
RASA3	22821	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
RASGRP3	25780	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
RASGRP4	115727	-4.0	-4.0	-4.0	-4.0	-4.0	-4.0	-4.0	-4.0	-4.0	-4.0
RB1	5925	-3.3	-3.3	-3.3	-3.3	-3.3	-3.3	-3.3	-3.3	-3.3	-3.3
RIPK4	54101	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
SAMD4A	23034	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
SAMD4B	55095	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
SERPINB1	1992	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
SERPINB5	5268	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
SLC12A9	56996	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
SLC13A2	9058	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7	-2.7

SLC16A7	9194	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9
SLC22A15	55356	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
SLC25A32	81034	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
SLC28A3	64078	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7
SLC29A3	55315	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
SLC29A4	222962	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
SLC35A1	10559	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
SLC35A3	23443	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7	-1.7
SLC35E4	339665	-1.5	-1.5	-1.5	-1.5	-1.5	-1.5	-1.5	-1.5	-1.5	-1.5
SLC41A3	54946	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
SLC44A1	23446	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
SLC4A3	6508	-8.4	-8.4	-8.4	-8.4	-8.4	-8.4	-8.4	-8.4	-8.4	-8.4
SLC50A1	55974	-2.2	-2.2	-2.2	-2.2	-2.2	-2.2	-2.2	-2.2	-2.2	-2.2
SLC5A12	159963	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
SLC6A13	6540	-5.2	-5.2	-5.2	-5.2	-5.2	-5.2	-5.2	-5.2	-5.2	-5.2
SLC6A15	55117	-2.8	-2.8	-2.8	-2.8	-2.8	-2.8	-2.8	-2.8	-2.8	-2.8
SLC6A4	6532	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
SLC6A6	6533	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
SLC8A1	6546	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
SLC9A3R2	9351	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9	-1.9
SMAD9	4093	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
TNFAIP8	25816	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
TNFRSF1A	7132	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5

TNFSF15	9966	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0	-3.0
TNFSF18	8995	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
TRAF3IP3	80342	-8.9	-8.9	-8.9	-8.9	-8.9	-8.9	-8.9	-8.9	-8.9	-8.9
TRAF6	7189	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6

Annexure 2: Differential expression of the genes in THP-1 following 6 h treatments with indolicidin and nano particles

Gene Symbol	Entrez Gene ID	Fold changes wrt NT										
		CNT	CNT + GNP	CNT + GNP + Indo	CNT + Indo	CNT-Indo	CNT-Indo + GNP-Indo	GNP	GNP + Indo	GNP-Indo	Indo-0.02	Indo -20
ACVR1	90	-2.1	-2.9	-1.3	-2.1	1.4	1.3	-1.5	1.5	1.2	2.0	1.4
ACVR1B	91	1.3	1.2	1.3	1.6	1.5	1.5	1.9	1.3	1.4	-1.1	-7.7
ACVR1C	130399	2.6	2.3	4.0	-1.1	4.0	3.8	2.9	3.0	2.2	1.2	2.8
ADCY3	109	1.2	1.3	1.0	1.8	-1.3	1.3	1.7	2.0	-3.4	-1.0	1.6
AKT1	207	1.7	1.2	-1.3	1.1	1.4	-1.0	1.5	1.4	1.2	1.5	1.4
AKT1S1	84335	1.8	-4.0	1.9	2.4	1.7	3.0	1.8	1.8	1.7	1.7	1.6
AKT2	208	2.2	1.0	-1.3	2.3	1.6	1.8	1.6	2.1	1.9	-1.2	1.4
AKT3	10000	1.5	1.0	-1.2	1.4	-2.1	1.1	-3.6	1.0	1.3	-3.8	-1.0
ANAPC1	64682	1.4	1.4	-1.8	1.4	-1.4	-5.3	-1.5	1.4	-1.8	-1.8	1.2
ANAPC10	10393	1.2	-1.0	1.5	1.1	-1.3	-1.1	-1.3	-1.0	-1.4	1.7	-1.1
ANAP	51529	-2.4	-3.8	-2.6	1.2	1.7	2.3	-1.4	1.1	2.1	-2.0	-1.4

C11												
ATF6B	1388	1.8	3.3	3.0	2.1	2.1	-1.1	3.8	3.3	3.1	1.6	3.4
ATF7I P	55729	5.4	5.5	3.3	6.9	4.4	2.8	6.6	5.2	3.5	3.6	2.5
BAX	581	1.2	1.1	1.2	1.1	1.5	1.3	1.5	1.2	1.2	1.1	1.1
BCL11 A	53335	-1.1	-1.2	-1.1	-1.1	-1.2	-1.2	-1.1	-1.6	-1.2	-12.9	2.3
BCL2	596	1.0	1.2	-1.0	1.1	1.2	1.6	-1.1	-1.2	1.3	1.9	1.1
BCL2L 1	598	1.2	1.3	1.1	1.1	1.5	1.4	1.4	1.2	1.4	-1.2	-1.0
BCL2L 11	10018	-1.9	-1.4	-1.4	-1.1	-1.9	-1.4	-1.0	-1.5	-2.1	-1.4	-1.7
BCL2L 12	83596	1.9	-1.3	1.5	1.6	2.0	1.2	-1.1	1.1	1.6	-4.6	1.3
BCL2L 13	23786	1.0	-7.2	1.0	-1.0	-1.1	-1.1	-1.0	-1.0	-1.1	1.0	-1.1
BCL2L 14	79370	1.1	1.0	-1.4	1.1	1.0	-1.4	-6.0	1.1	1.1	-1.3	1.0
BCL6	604	1.1	1.4	1.3	1.5	1.4	1.4	1.2	1.5	1.1	-1.0	1.1
BCL6B	255877	1.1	1.0	1.5	1.2	-1.1	1.1	1.1	-8.1	-1.9	-2.4	-1.5
BCL7A	605	2.5	1.6	-1.2	2.2	2.0	-3.2	2.0	-1.4	1.1	-2.6	1.8
BCL7C	9274	1.7	1.9	1.2	2.1	1.4	-1.4	2.0	1.8	1.5	2.0	-2.4
CALM L4	91860	2.6	-8.4	1.9	1.9	2.0	1.7	-1.2	1.2	3.1	1.0	2.8
CALN 1	83698	2.8	-5.1	1.3	1.3	1.0	1.8	1.8	1.3	3.1	1.6	1.7
CALR3	125972	-3.7	-2.3	1.3	1.5	-5.7	-1.2	-2.3	1.6	-1.8	1.1	-2.1
CALU	813	6.9	12.2	10.4	1.5	10.1	6.7	3.8	9.1	10.8	6.1	16.4
CALY	50632	2.4	2.8	2.6	-1.0	1.3	2.1	2.1	-2.6	-1.6	-1.5	2.1
CAMK 4	814	1.1	1.1	1.1	-9.0	-1.4	-1.0	2.6	2.1	-1.1	1.1	1.3

CAMK K1	84254	1.0	-2.5	-1.0	2.3	-1.2	1.6	-8.9	-1.1	1.5	1.0	1.3
CAMK K2	10645	-1.0	1.5	1.8	1.3	1.2	1.2	2.0	1.9	1.2	1.5	-2.0
CASP1 0	843	-4.2	-12.7	-1.7	-6.2	-1.0	1.0	-1.4	1.0	1.0	-1.2	1.1
CASP1 4L	197350	1.4	-1.8	1.4	1.4	1.4	1.4	1.4	1.4	1.2	3.3	1.4
CASP2	835	-1.0	1.0	1.1	-1.0	-5.7	-1.0	1.0	1.0	-1.0	1.3	-1.0
CASP4	837	1.5	-1.6	1.3	1.8	1.3	1.5	2.0	1.6	1.8	-1.4	3.6
CASP5	838	1.9	4.0	-10.9	1.1	1.8	2.4	1.7	-1.2	2.6	1.9	1.7
CASP8	841	1.4	1.2	1.3	1.5	1.6	1.1	1.8	1.4	1.4	1.0	-1.1
CCL1	6346	1.0	-1.0	1.0	-1.0	-1.1	-14.1	-1.0	1.1	-1.1	1.0	-1.1
CCL11	6356	-8.8	-1.0	1.7	1.0	1.0	1.0	1.1	-2.9	1.0	1.5	-1.3
CCL19	6363	3.2	4.7	3.0	2.7	1.0	2.9	3.3	3.2	-1.7	-1.2	3.2
CCL20	6364	5.5	1.5	12.7	1.5	10.9	1.7	12.2	11.8	10.9	12.3	11.0
CCL21	6366	4.7	1.6	2.7	7.2	1.2	5.6	2.4	1.4	1.9	1.7	1.4
CCL23	6368	1.1	1.0	1.1	1.1	1.1	1.1	-10	1.1	1.1	-1.1	1.1
CCL24	6369	-1.2	2.0	4.6	1.8	-1.1	2.0	-1.6	-1.8	1.0	2.4	1.7
CCL27	10850	1.7	-1.0	1.8	1.7	1.7	1.6	3.4	3.5	-11.7	1.6	1.0
CCL28	56477	-2.3	-4.3	-2.9	-3.0	-2.9	-1.3	-1.0	-4.8	-1.1	1.1	-2.9
CCL3	6348	1.0	2.8	-1.2	-1.0	-6.3	-1.0	1.5	-1.0	-1.0	-1.2	-1.1
CCL4	6351	4.3	2.1	1.6	-1.3	3.9	4.1	2.6	1.9	1.7	1.8	-1.2
CCR1	1230	-1.0	-1.1	-1.0	-41.0	-1.1	-1.1	-1.1	-1.0	-1.1	-1.2	-1.1
CCR10	2826	1.1	1.3	1.1	-1.9	-4.3	-1.8	-1.1	-2.5	-3.3	-4.1	-1.4
CCR5	1234	1.0	1.2	-1.1	1.4	1.0	1.3	1.0	-1.0	1.6	-1.3	-1.1
CCR7	1236	-1.0	-1.0	1.1	4.0	1.3	-1.0	-1.0	-1.0	-1.1	-1.2	1.0
CCR8	1237	-5.5	-1.0	1.0	-1.0	-1.0	-3.7	-1.0	1.0	1.4	-1.1	1.0
CDH3	1001	2.1	-5.3	1.3	1.3	1.9	3.3	1.6	1.3	3.6	-1.0	1.3

CDH5	1003	1.0	-1.0	1.0	1.0	-1.0	-1.6	1.5	-13.1	2.3	-1.1	1.8
CDH6	1004	-3.3	1.3	1.4	1.4	2.5	1.3	1.4	1.4	-4.4	1.5	1.0
CDK1	983	1.4	1.5	1.2	1.5	1.6	1.4	1.5	1.9	1.3	1.1	1.0
CDK3	1018	1.3	1.4	1.3	1.4	1.1	-1.1	1.5	1.5	1.4	-1.4	-1.5
CDK4	1019	3.1	2.8	4.8	3.0	1.1	6.1	2.4	5.9	6.0	3.3	8.4
CDK5	1020	1.5	1.3	-1.3	1.6	1.3	-1.1	1.2	1.3	1.3	-1.0	1.8
CDK6	1021	-1.6	-1.0	1.0	1.0	1.0	1.0	1.0	-47.8	1.0	-1.1	1.0
CDK8	1024	-1.0	1.4	-1.7	1.4	1.1	1.2	1.3	1.2	1.3	1.2	1.3
CHEK 1	1111	-3.0	-1.1	-8.9	-1.1	-6.5	-1.1	-2.1	-3.9	-1.1	-2.1	1.6
CREB3 L1	90993	-1.9	1.2	-3.1	-1.7	-4.4	-2.5	-2.0	-3.5	-3.0	-1.9	-1.8
CREB3 L3	84699	1.7	8.8	3.2	7.0	4.5	4.5	1.5	3.2	5.3	2.4	4.9
CREB BP	1387	1.5	1.4	1.5	1.4	1.4	1.3	1.4	-3.0	-1.3	1.4	1.3
CREB L2	1389	1.0	-1.0	1.0	1.0	1.0	1.0	-7.4	1.0	1.0	-1.4	1.0
CSF1	1435	1.8	1.1	1.7	-4.6	1.1	-4.9	1.9	1.2	1.2	2.6	-1.3
CSF2	1437	3.8	3.1	1.9	4.8	3.2	4.1	-2.2	3.8	3.1	4.9	2.2
CSF2R B	1439	1.1	1.5	1.3	1.6	-1.2	1.4	1.7	1.0	-2.1	1.2	-1.1
CSF3R	1441	1.1	1.2	-4.6	1.3	-6.6	-1.9	1.0	-4.9	-2.5	-4.3	-2.2
CTLA4	1493	1.4	-4.3	-1.4	2.9	1.6	1.2	1.6	1.7	1.1	-1.2	-1.2
CXCL1	2919	-1.2	-1.2	1.2	-3.2	-1.2	-1.2	-1.2	-1.1	-1.2	-4.7	-1.2
CXCL1 2	6387	-1.0	-1.3	-1.1	1.9	2.6	3.1	1.5	-1.3	-1.4	-1.5	1.5
CXCL1 6	58191	-1.1	-1.6	-2.0	1.8	-1.4	-1.0	2.9	1.1	-4.2	1.2	-3.4
CXCL3	2921	-1.0	-1.1	1.0	-1.1	-1.1	-1.1	-1.1	-1.0	-6.0	-19.2	-1.1

CXCL9	4283	-1.4	-5.5	-1.4	-1.4	-2.0	-1.6	-2.9	1.2	-1.6	-1.4	-1.7
CXCR1	3577	-2.0	-4.9	-2.0	-1.6	-1.1	-1.8	-4.9	-2.6	-1.3	-1.6	-1.5
CXCR2	3579	1.0	-1.0	1.0	-1.0	-1.1	-1.0	-1.0	-1.0	-1.0	5.9	-1.1
CXCR3	2833	-1.5	-1.3	-1.1	-1.0	-1.5	-1.5	-2.0	-1.2	-1.5	1.0	-1.4
CXCR5	643	-1.0	-1.0	1.0	-1.0	-25.4	-1.1	-1.0	-1.0	2.8	-1.3	-1.0
CXCR6	10663	1.0	-1.0	1.0	-1.0	-1.1	-1.0	10.8	-1.0	-1.0	-1.0	-1.1
DEF6	50619	1.3	1.4	1.1	1.4	1.3	1.6	1.5	1.2	-1.2	1.1	1.8
DEF8	54849	5.3	5.4	3.6	8.2	6.3	3.5	9.4	5.9	5.2	5.7	2.7
DEFA3	1668	1.1	-1.0	2.2	1.0	1.6	1.2	1.0	1.9	1.0	-1.0	1.1
DEFA4	1669	1.1	1.0	-1.1	1.1	1.1	1.1	-1.2	-1.0	-4.8	-2.7	-1.7
DEFA5	1670	1.0	1.1	1.1	1.5	1.1	-3.7	1.1	-1.0	-1.0	1.0	-1.0
DEFB103B	55894	-4.8	-1.0	1.0	-1.0	1.3	-1.0	1.0	1.0	-1.0	-1.1	1.5
DEFB106B	503841	1.0	-4.6	-2.9	-2.0	-1.3	-2.6	-1.0	-1.3	-2.7	-4.6	-1.0
DEFB119	245932	-72	1.0	1.1	-2.1	1.0	1.2	-1.1	1.1	1.0	-1.1	1.9
DEFB123	245936	1.8	-1.4	-1.4	3.3	1.7	4.0	1.1	1.0	2.7	1.1	-1.8
DEFB129	140881	8.4	10.8	5.1	7.9	8.3	12.9	10.9	3.4	7.9	7.9	8.0
DICER1	23405	1.7	1.5	1.6	1.7	1.2	3.7	1.9	1.3	1.9	5.7	1.1
DUSP16	80824	1.3	1.1	-2.5	-1.6	1.0	1.1	-1.2	2.9	1.3	2.3	-3.8
DUSP3	1845	1.0	1.4	-1.8	1.8	-1.5	1.2	2.0	1.5	-1.2	1.0	1.4
DUSP4	1846	1.3	1.5	-1.6	-1.1	-1.2	1.0	1.9	1.3	1.4	-3.3	1.1

FADD	8772	-1.3	-2.8	-2.3	-1.4	-2.3	-2.7	-2.4	-2.9	-1.2	-1.7	-6.8
FASLG	356	1.4	1.5	1.3	1.8	1.6	1.5	-6.0	-1.0	1.4	-1.4	-1.1
FGF14	2259	6.6	6.9	7.1	6.5	6.5	6.7	4.8	6.5	6.3	6.7	7.3
FGF3	2248	1.8	1.1	1.1	1.3	-1.3	-1.5	1.4	1.1	-1.1	2.1	1.0
FGF5	2250	2.3	2.2	-1.1	2.3	-1.2	2.0	2.3	-2.8	-1.0	2.2	-1.4
FGFR4	2264	-1.2	1.5	-1.2	-1.8	1.2	1.8	-11	1.7	1.4	-2.0	-1.6
G6PD	2539	1.5	1.8	-1.0	1.8	-1.5	1.6	-1.1	2.0	1.0	2.0	1.4
GADD45A	1647	-2.0	-2.9	-3.9	-2.8	-2.5	-2.2	-2.3	-3.4	-1.9	-1.0	-1.2
GADD45B	4616	1.8	-3.4	-1.0	-1.8	-2.1	-2.3	-1.8	-1.1	-2.0	-2.3	-2.0
GALNT1	2589	4.2	4.1	4.2	4.7	1.0	1.7	4.5	4.7	2.4	4.8	3.4
GATA3	2625	2.1	-1.9	2.0	2.1	1.1	-2.4	-4.3	2.1	2.0	2.2	1.9
GP6	51206	4.0	5.6	2.4	4.0	4.4	3.6	4.7	3.9	-1.8	4.7	2.4
GPR114	221188	-5.7	1.2	2.7	1.0	1.4	1.6	2.4	2.1	1.9	-1.0	1.8
GPR115	221393	3.3	3.1	3.3	3.3	-2.4	3.1	3.3	3.3	3.2	3.2	2.9
GPR12	2835	3.3	4.1	1.2	5.5	3.8	2.3	2.6	3.2	5.3	7.3	1.9
GPR124	25960	5.4	1.7	3.4	3.4	3.8	4.5	9.8	3.7	3.4	3.4	3.8
GPR135	64582	9.1	4.6	7.7	5.2	4.8	2.5	6.4	4.3	6.4	4.3	5.7
GPR137	56834	3.4	1.6	3.6	-1.4	2.5	-1.4	-1.1	4.5	-2.2	-2.2	2.1
GPR157	80045	1.6	1.5	1.5	1.5	1.5	-1.5	1.5	1.6	-1.2	1.7	-6.1
GPR161	23432	3.1	3.5	2.2	2.0	2.1	1.5	3.3	1.4	2.8	1.6	1.7
GPR17	54328	1.3	4.8	2.7	1.8	1.7	2.6	2.3	1.6	4.3	2.7	2.7

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GPR176	11245	3.3	5.1	3.3	4.8	3.2	4.0	6.1	5.6	3.2	5.2	5.7
GPR20	2843	-1.5	-1.4	3.3	1.8	2.4	1.5	1.1	2.4	1.7	3.3	-1.1
GPR35	2859	1.6	-4.2	1.0	2.2	-1.0	1.0	3.4	2.4	-1.5	1.5	1.4
GPR61	83873	-2.6	-1.6	-1.2	-1.5	3.2	1.7	1.0	1.1	-1.5	-1.1	1.2
GPR97	222487	-1.0	-1.0	1.3	2.1	-1.0	-1.2	2.0	2.7	-1.3	1.2	-2.5
HDAC1	3065	1.7	1.3	1.5	1.8	1.4	1.4	1.8	1.6	1.3	1.0	1.4
HDAC3	8841	-1.2	1.0	-1.8	-1.2	-1.4	4.8	-1.3	-1.1	-1.2	-1.6	1.1
HDAC4	9759	-1.2	-1.1	1.2	1.4	1.8	1.2	2.1	1.7	1.6	1.2	-1.1
HIF1AN	55662	-1.0	-2.0	-1.3	-1.0	-1.0	1.1	-1.0	-2.6	5.0	-1.4	-1.0
HSP90AB3P	3327	1.4	1.2	-2.8	2.6	1.3	1.5	1.3	1.4	1.2	-2.2	-1.0
HSP90AB6P	541611	1.7	2.8	-1.1	4.6	-1.0	2.3	-1.4	-1.7	2.2	2.8	-1.1
HSPA12A	259217	1.9	1.8	-1.1	1.2	1.1	1.8	1.0	-1.1	-1.4	-3.0	1.0
HSPA6	3310	8.6	14.9	9.0	6.0	8.4	9.0	6.9	10.4	11.2	13.9	3.9
HSPA9	3313	30.0	28.4	29.8	26.1	26.6	13.4	12.9	20.2	2.1	29.2	27.6
ICAM5	7087	-1.0	1.3	1.4	2.2	2.3	1.9	1.9	1.4	-1.4	-1.8	1.0
IFNAR1	3454	1.6	1.4	1.5	1.6	1.5	1.4	-7.5	1.5	-1.7	-1.1	1.1
IFNB1	3456	1.1	-1.0	-11.2	-1.8	-1.0	1.0	1.1	1.2	1.0	1.0	1.0
IFNG	3458	1.1	1.1	-1.5	-1.0	-1.3	1.1	1.0	1.1	-1.3	-1.1	1.0
IFNGR2	3460	-4.8	1.3	1.3	1.1	1.3	1.3	1.4	1.4	-1.1	2.6	-1.2
IL10RA	3587	1.4	1.1	2.0	1.9	1.4	1.5	2.4	2.2	2.7	1.2	1.1

IL12B	3593	1.1	-1.1	-2.6	-5.9	1.0	-2.2	-1.1	-1.0	-1.1	-1.3	-1.1
IL12RB1	3594	-3.2	-2.0	1.8	1.9	1.1	1.2	1.1	1.4	-1.1	2.2	-1.4
IL13RA1	3597	1.0	1.1	1.1	-16.4	1.1	1.1	1.1	1.1	1.0	1.1	1.0
IL13RA2	3598	3.2	-1.1	-1.0	-1.4	-1.1	-1.1	-1.3	-1.0	-1.1	-1.3	-1.1
IL15RA	3601	-1.8	1.8	-3.4	1.3	1.1	-1.0	1.7	-1.0	-11.9	-1.2	-2.8
IL16	3603	1.0	-1.4	1.1	1.0	-2.7	1.0	1.0	1.0	1.0	-2.8	-3.8
IL17C	27189	1.8	2.5	1.5	2.4	1.3	2.5	2.3	2.7	2.4	1.3	1.3
IL17F	112744	-1.2	-1.3	-1.2	-3.9	-1.3	1.1	1.1	-1.2	-5.5	-1.4	-1.2
IL17RC	84818	2.6	1.5	-1.0	3.0	1.8	2.3	3.3	2.6	1.9	-1.2	2.0
IL18BP	10068	1.7	-1.4	-2.6	1.1	1.7	3.4	1.1	-1.0	3.0	2.4	-2.4
IL18R1	8809	1.0	1.3	1.1	-2.5	1.0	1.0	1.6	1.0	1.0	-2.0	-7.7
IL18RAP	8807	1.3	1.3	-2.6	1.5	1.3	1.3	1.3	1.3	1.3	-1.6	1.8
IL1F10	84639	-2.0	1.7	-4.4	1.0	-1.4	1.4	-5.2	-1.7	-2.0	-1.4	-2.2
IL1R1	3554	-1.7	1.2	-3.3	-2.2	-3.9	1.5	-1.6	-6.5	-2.4	-2.0	-1.9
IL1RAP	3556	-1.0	-1.0	1.0	-1.0	-3.0	-1.0	-1.0	-8.0	1.4	-1.6	-1.0
IL1RN	3557	-1.0	-1.0	1.0	-4.5	-1.1	-1.0	-1.0	-1.0	-8.6	-1.2	-1.1
IL2	3558	-1.0	-1.5	-1.4	1.0	1.1	-1.1	-1.1	-1.6	-1.2	-1.7	-1.3
IL21	59067	1.1	1.0	1.5	2.7	1.7	1.1	1.9	-2.5	-1.0	2.0	3.4
IL21R	50615	1.5	1.4	3.0	-1.0	1.5	2.1	1.7	-1.2	1.5	-2.6	1.9
IL22	50616	1.1	-1.1	1.1	1.1	1.1	1.1	1.1	1.2	-6.8	-1.1	1.4
IL24	11009	-1.1	-4.4	-1.1	2.9	-1.1	-1.1	-1.5	1.5	1.2	1.3	1.1
IL27	246778	1.3	-2.0	-1.2	-1.2	1.2	-1.0	1.9	1.2	1.6	1.6	1.3
IL27R	9466	2.0	3.8	2.3	4.9	3.9	-1.4	3.9	-1.1	3.3	1.5	3.1

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IL28A	282616	1.3	-1.1	1.4	2.2	1.2	-1.6	1.2	1.8	1.2	-4.7	-1.2
IL29	282618	-5.8	-1.2	-1.8	-1.4	-1.3	1.9	-8.0	1.5	1.2	1.8	-1.3
IL2RG	3561	2.3	1.2	2.1	1.2	1.9	1.7	2.3	1.4	1.3	-1.5	-10.5
IL31RA	133396	1.4	1.3	1.4	-6.7	1.6	-1.1	-3.2	1.3	1.3	1.1	-1.0
IL34	146433	-1.6	-1.7	-1.6	-13.6	-1.6	-1.2	-4.1	1.2	1.0	-1.9	-1.6
IL36B	27177	1.1	1.3	1.0	-5.0	-1.1	-5.5	1.4	1.0	-1.0	1.0	1.0
IL6R	3570	1.2	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	-1.8	-3.9
IL6ST	3572	3.0	2.2	2.3	4.4	2.0	4.7	4.4	1.3	1.5	1.3	2.8
IRF3	3661	-1.1	-3.4	-1.7	1.6	1.8	1.6	1.5	1.2	1.1	-1.2	1.4
IRF4	3662	-1.6	1.3	1.4	1.2	-3.2	1.4	-1.3	1.9	-1.4	2.0	1.5
IRF5	3663	3.1	1.8	4.6	2.3	1.3	4.4	4.1	4.0	2.0	1.4	2.4
ITGA11	22801	-1.8	2.5	1.9	3.2	3.5	2.2	4.0	3.1	2.0	4.3	1.0
ITGA2	3673	-3.1	2.3	1.3	1.7	1.1	1.6	1.8	1.7	1.6	1.7	1.1
ITGA2B	3674	-1.1	1.4	1.7	-4.8	1.7	-6.1	-1.3	-1.1	1.6	-1.1	-1.1
ITGA5	3678	-1.0	1.1	1.4	-11.2	-1.7	1.6	-1.4	1.3	1.4	-2.7	-2.4
ITGA7	3679	2.4	2.5	1.5	3.9	-1.1	1.9	2.1	2.2	1.7	1.4	11.7
ITGB5	3693	-1.1	1.1	1.6	2.0	1.4	2.3	1.4	2.7	1.8	2.3	-1.4
JUNB	3726	1.8	-1.3	-1.8	-1.2	-1.3	2.6	-1.1	-2.4	2.5	2.0	1.4
LIF	3976	6.2	3.1	1.0	4.6	1.9	4.1	1.5	-1.5	6.7	1.3	3.1
LRRC15	131578	5.1	5.0	4.9	4.7	4.5	4.7	5.0	4.7	4.5	-1.4	5.0
LRRC18	474354	2.2	2.4	1.8	2.2	-2.1	2.1	2.9	2.7	1.8	1.6	-2.9
LRRC28	123355	-2.2	1.6	-4.5	-1.8	-1.1	-2.3	1.6	-1.3	-1.5	-1.1	1.4

LRRC32	2615	1.8	-8.5	-1.3	3.5	-1.5	1.6	1.5	2.4	3.2	-1.0	2.6
MAP2K2	5605	7.6	5.7	2.0	10.9	-1.1	3.0	3.1	4.6	3.6	3.6	2.9
MAP2K4	6416	1.0	1.1	1.3	1.3	1.1	1.2	-1.0	1.1	-1.0	2.0	-1.2
MAP2K6	5608	1.2	1.9	1.3	-5.4	1.1	1.0	2.1	1.1	1.2	-7.3	1.1
MAP2K7	5609	-1.4	-2.1	-2.0	-1.2	-1.6	-1.6	2.1	-1.9	-1.1	-1.9	-3.1
MAP3K1	4214	1.2	-1.6	-1.2	1.1	-2.7	1.1	1.1	1.7	-5.2	-1.2	1.1
MAP3K11	4296	3.1	1.8	1.3	2.1	1.9	1.2	3.2	1.4	1.8	2.4	1.7
MAP3K12	7786	1.1	1.0	-1.0	-3.2	-1.2	-1.1	1.1	2.6	1.0	-1.5	-1.6
MAP3K13	9175	1.1	1.9	1.2	1.9	1.3	-3.9	1.6	1.7	1.8	2.0	1.5
MAP3K14	9020	2.9	-1.9	1.8	1.8	3.1	2.5	2.5	1.3	2.9	-1.0	1.8
MAP3K3	4215	2.8	3.0	2.1	2.7	2.3	1.7	4.4	2.5	2.5	1.4	-1.2
MAP3K6	9064	2.8	-1.1	1.8	5.6	2.1	2.5	2.5	1.5	-10.8	-1.6	1.0
MAP4K1	11184	1.6	1.2	1.3	1.3	1.2	1.3	1.4	1.6	3.2	-8.6	1.3
MAPK11	5600	1.5	1.6	-1.0	-1.0	1.2	-1.1	1.4	1.1	1.2	-1.3	1.4
MAPK14	1432	1.6	-11.4	-1.1	2.0	2.1	1.5	2.2	1.7	-5.9	2.0	1.5
MAPK15	225689	1.7	-2.2	-1.3	2.1	1.3	1.2	-1.7	-1.5	2.0	-1.2	-5.8
MAPK3	5595	1.1	-1.1	-2.4	1.6	-1.2	1.2	1.6	-1.2	1.1	-1.2	-1.1

MAPK6	5597	1.4	2.6	1.3	3.1	1.8	1.8	2.0	2.5	1.4	1.3	1.6
MAPK8	5599	1.2	1.2	2.0	1.3	-1.2	-1.0	-1.0	1.2	-1.2	-1.1	-1.5
MMP15	4324	1.5	1.1	1.2	1.5	-1.1	-1.2	1.9	1.3	-1.3	1.6	-1.3
MMP17	4326	-2.1	1.1	1.1	1.8	-1.2	1.3	3.6	1.3	-3.3	-1.7	1.9
MMP19	4327	3.4	2.2	1.8	2.0	4.3	3.9	2.9	1.2	2.8	2.8	1.7
MMP24	10893	-1.1	1.2	1.9	1.6	1.8	1.4	-32	-5.6	1.5	-2.0	1.9
MMP25	64386	1.9	-2.0	1.8	1.4	1.4	1.8	3.4	2.2	-1.7	-1.4	-3.7
MMP8	4317	1.5	1.3	-1.6	1.8	1.4	1.3	1.4	1.9	-8.2	-2.1	-1.2
MUC4	4585	2.0	-1.0	1.4	2.2	1.9	1.2	1.4	-1.4	2.9	1.8	-1.3
MUC5AC	4586	-2.5	2.6	-1.0	1.7	-3.3	1.7	1.8	2.0	3.0	-1.1	1.7
NFATC1	4772	1.5	-12.5	-1.3	-1.0	1.2	1.1	1.9	1.0	2.2	-1.1	1.0
NFATC2	4773	3.1	1.6	4.5	2.1	4.3	1.2	2.0	1.9	3.2	4.0	1.9
NFATC4	4776	3.9	2.4	1.3	5.9	-1.9	4.2	3.7	6.3	2.1	1.8	-4.0
NFKB2	4791	1.2	1.3	1.4	2.3	-1.1	1.7	1.3	2.0	1.1	1.3	1.6
NFKBIB	4793	-1.2	-2.3	-1.9	-3.0	-2.3	-1.5	1.6	-2.4	-1.5	1.3	3.0
NFRKB	4798	1.6	5.6	1.3	4.3	1.1	8.0	1.3	3.3	2.4	2.2	3.5
NLRC3	197358	1.3	-1.6	-2.2	1.5	-4.2	1.3	-2.0	1.2	1.7	1.7	1.1
NLRC5	84166	1.3	1.4	-1.0	1.3	1.2	1.2	1.9	-1.0	1.0	-2.1	1.3
NLRP4	147945	-3.7	1.9	2.0	2.1	1.9	-1.3	1.1	1.3	1.6	-1.9	2.0
PIK3R	8503	1.0	1.4	1.7	3.8	1.0	1.6	4.3	-1.3	-3.2	-1.5	1.6

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PIK3R4	30849	3.2	1.6	1.7	1.7	-3.3	3.3	-1.7	-1.1	-1.6	1.4	1.5
PLCB1	23236	1.7	1.3	1.5	1.6	1.8	1.4	2.2	1.5	1.7	-1.1	1.1
PLCB2	5330	5.6	5.3	5.6	6.9	5.4	8.5	5.6	5.6	5.4	3.6	5.9
PLCD3	113026	3.0	3.8	-1.4	2.6	2.1	1.8	2.3	-1.9	2.5	5.5	3.9
PRKACB	5567	-2.0	-1.2	-1.2	1.9	2.5	1.6	1.4	1.8	2.8	-1.2	3.0
PRKCA	5578	1.3	1.2	-1.0	1.9	1.4	1.5	1.2	1.8	1.4	1.0	1.2
PRKCG	5582	3.8	-1.0	-1.3	2.8	1.3	1.6	3.4	1.7	2.6	1.2	2.4
RAC2	5880	2.8	1.2	-1.4	3.0	-1.1	3.7	3.8	2.2	-1.0	2.2	4.6
RAC3	5881	-2.3	1.3	-1.2	1.5	1.0	2.1	-2.0	-1.1	-4.1	1.1	1.8
RB1	5925	-3.3	-1.6	1.7	-4.1	1.6	-1.7	-4.5	1.6	1.2	-1.3	1.6
RELA	5970	-1.2	-3.2	-1.2	1.7	8.4	-1.2	-1.2	-1.2	-1.0	-3.1	-1.2
RIPK4	54101	3.7	5.6	2.8	7.3	6.9	6.4	7.4	6.6	6.0	3.9	1.4
SAMD14	201191	1.1	4.9	1.0	1.0	1.3	1.0	-1.0	-2.3	1.5	1.1	1.1
SAMD4A	23034	1.9	1.6	1.4	-1.5	2.2	1.9	2.8	-1.4	1.6	-2.1	-4.7
SAMD4B	55095	1.9	2.7	1.1	3.1	1.7	2.1	2.9	-1.2	1.4	-3.1	3.2
SAMD7	344658	2.4	-1.1	1.2	2.4	1.0	3.3	-1.4	1.1	2.5	-2.0	-3.2
SLC11A1	6556	1.4	1.4	1.0	1.9	1.3	1.4	1.1	1.3	1.4	1.1	1.0
SLC12A1	6557	1.3	1.2	1.2	1.3	1.2	-3.2	1.3	3.0	1.2	1.2	1.0
SLC12A9	56996	2.1	1.6	1.2	1.7	1.6	1.4	1.3	1.2	1.9	1.4	1.5
SLC14	8170	22.3	21.4	35.3	13.9	22.6	22.4	22.2	22.4	14.7	16.8	22.1

A2												
SLC16A1	6566	1.4	1.7	1.0	3.2	1.3	1.2	-1.0	1.8	3.4	1.8	-1.0
SLC1A1	6505	-3.3	-1.4	-1.7	-1.1	-1.0	-2.9	4.5	-1.0	1.1	-1.5	1.4
SLC1A4	6509	1.0	-1.0	1.0	-1.0	9.8	-1.0	-1.0	-1.0	-1.0	-1.0	-1.1
SLC22A1	6580	1.4	1.0	2.0	1.2	1.3	-1.2	-6.0	1.1	1.0	-1.1	1.7
SLC22A14	9389	2.2	1.5	-3.1	-2.6	2.4	2.0	1.4	2.5	1.5	-1.1	-2.3
SLC22A16	85413	2.5	1.8	3.9	6.9	1.9	1.8	6.2	1.4	3.4	2.7	7.4
SLC22A7	10864	1.9	1.9	1.8	-1.9	2.5	2.0	2.6	2.0	2.1	1.9	-1.2
SLC25A43	203427	3.3	3.1	3.3	3.1	3.5	3.1	27.7	3.2	3.1	1.8	3.0
SLC26A1	10861	5.2	4.4	5.1	4.2	3.0	3.9	4.3	6.8	4.8	1.3	1.1
SLC2A8	29988	1.0	-1.0	-1.1	-1.0	1.4	-1.1	1.8	1.1	1.4	-1.1	-1.3
SLC30A2	7780	2.9	2.7	2.5	4.3	2.7	2.7	6.6	4.1	2.8	5.2	2.4
SLC34A3	142680	3.1	1.6	1.8	2.5	1.8	1.6	2.0	1.7	1.4	1.9	1.8
SLC38A2	54407	1.3	2.6	1.6	2.9	3.9	1.8	2.2	3.6	1.4	3.3	2.9
SLC39A14	23516	-1.2	1.4	1.4	1.5	1.4	1.4	1.5	2.7	1.4	-1.1	1.4
SLC39A3	29985	1.7	-1.3	1.5	2.1	-2.7	1.7	1.5	1.7	2.0	-1.1	1.2
SLC41A3	54946	1.6	1.1	1.2	1.1	1.9	1.7	1.7	1.2	1.7	-1.3	-1.2
SLC43	29015	1.9	1.2	-1.8	3.4	2.2	2.4	-1.2	1.5	2.3	-1.0	-1.5

A3												
SLC44A1	23446	2.9	2.8	2.4	4.5	3.7	2.8	3.2	3.8	2.4	-1.1	1.8
SLC46A1	113235	2.1	-2.4	-2.0	1.8	-1.6	1.1	2.4	-1.6	1.3	-1.8	1.3
SLC47A1	55244	9.6	2.3	1.8	6.0	10.1	6.7	2.8	10.7	2.8	7.5	1.1
SLC5A5	6528	2.2	2.1	3.0	5.0	8.7	4.7	2.2	2.0	3.2	2.9	1.8
SLC6A18	348932	2.7	3.9	2.2	3.2	4.4	3.7	4.2	3.1	3.6	-1.1	2.5
SMAD3	4088	-1.3	-1.6	-3.7	-2.3	2.5	-1.4	-1.1	-1.1	2.0	-4.1	1.2
SMAD9	4093	5.7	4.4	1.2	4.7	3.1	3.6	2.0	1.4	2.8	1.6	2.6
SP5	389058	1.9	1.2	-1.0	2.1	-1.7	1.6	2.8	1.2	1.4	1.9	-1.9
SP7	121340	-5.2	1.1	1.1	1.1	-1.0	1.0	2.3	1.0	-1.0	1.1	-1.0
STAT2	6773	10.7	7.2	4.6	3.9	9.6	-1.8	-2.0	4.7	10.2	2.3	6.1
STAT3	6774	1.6	-1.1	-1.1	1.6	1.1	1.3	1.6	1.4	1.4	1.2	1.1
TGFB1	7040	1.2	1.4	-1.2	1.6	1.3	1.3	1.4	1.4	1.3	1.2	1.3
TMEM173	340061	1.5	2.9	4.8	1.3	1.4	-1.2	3.4	1.4	3.7	2.0	3.3
TMEM176A	55365	1.8	1.2	2.1	3.5	2.2	1.7	1.6	2.3	1.8	2.7	3.6
TNFAIP8	25816	1.9	-3.8	1.5	1.6	-1.1	-1.3	4.1	1.8	2.6	-1.0	-3.0
TNFRSF13C	115650	21.5	17.4	12.2	13.4	5.9	15.2	21.3	23.7	14.4	25.2	15.4
TNFRSF1A	7132	6.5	47.2	49.7	42.3	29.0	46.2	20.9	29.4	62.8	48.7	33.2
TNFRSF1B	7133	-1.1	-2.6	1.1	1.5	1.0	2.1	1.2	1.0	1.4	1.1	1.3
TNFRS	27242	1.6	3.5	-1.1	-2.6	1.1	-1.0	1.3	-1.5	-1.2	2.4	1.9

F21											
TNFSF 9	8744	1.3	-1.1	1.0	1.8	1.8	1.0	-3.9	1.4	1.7	1.2	1.1
TRAF1	7185	1.4	2.5	-1.1	1.8	-1.6	1.3	1.1	1.3	1.5	1.5	-1.4
TRAF2	7186	1.1	1.3	1.2	1.1	-1.0	-3.6	1.5	1.8	1.0	1.0	1.4

Annexure 3: Important genes differentially expressed in Raw264.7 following LA and BC treatment

Gene Symbol	Entrez gene ID	LA treatment				BC treatment			
		1h	2h	4h	6h	1h	2h	4h	6h
Acvr1	11477							1.7	1.6
Acvr1b	11479	2.2	1.9	1.8	3.4	2.0	2.6	3.6	1.8
Acvr1c	269275	3.8	2.6	4.2		2.6	2.4	2.3	
Acvr2a	11480	-2.6	-1.6	-1.7		1.6	-2.7	-2.2	-2.0
Acvr2b	11481					1.6			
Acvrl1	11482				-1.7				-1.6
Adcy1	432530		4.1			-1.8	1.7	2.0	2.1
Adcy10	271639					1.6			
Adcy2	210044								1.5
Adcy3	104111		-3.2			1.7	-3.9		-2.4
Adcy4	104110			-1.5	1.6		-2.5		-2.4
Adcy5	224129	2.1	-2.0		1.7		-1.6		1.6
Adcy7	11513					1.5			
Adcy8	11514	-5.0	-4.8	-2.1	-3.2		-2.5	-2.2	1.5
Adcy9	11515	1.6				1.9	1.6		
Akt1	11651	-2.2				-1.9	-1.9		

Anapc1	17222	1.6			-1.6				
Anapc11	66156		1.5		-3.1	-1.9			
Anapc15	75430			-1.8	-1.8	2.2	2.2	-2.3	1.7
Anapc16	52717				1.9	1.6			
Anapc2	99152				-1.6				
Bcl11b	58208	2.1		2.2		2.3	3.5	1.7	1.5
Bcl2	1.01E+08	1.6					1.6		
Bcl2a1c	12046								-1.7
Bcl2l1	12048	-2.0		-1.9		7.4			
Bcl2l10	12049	-2.2	-2.7		1.7			2.1	5.7
Bcl2l11	12125	-1.5		1.6	1.8	2.0	1.8	1.8	1.7
Bcl2l14	66813		-2.3		-1.5	1.9			2.4
Bcl6b	320414						-2.4	-1.5	
Bcl7a	77045								1.6
Bcl7b	12054					1.6			
Bcl7c	12055				-1.7				2.3
Bcl9	77578	2.3		1.8	2.1	2.4	2.5		4.3
Bcl9l	80288	2.0			1.7				
C3	64918			-1.6			-2.4	-1.7	
C4a	171543	-6.7	-3.0	-3.7	-3.1		-1.5	-1.8	-2.1
C7	109828			-1.7		1.5			
C8a	329581						1.7		
C8g	69379	1.7	-1.7						
C9	12279				1.8				
Calca	12310		2.5					2.6	

Calcb	116903					1.6		-1.7	
Casp3	12367	-1.6				-2.1			-1.5
Casp4	12363	-1.5				-1.5			-1.7
Casp7	12369			-2.4	-1.7				-2.2
Cck	12424	1.6	-2.0		1.9	-1.7			2.4
Ccl1	20290							1.5	
Ccl17	20295		1.5	1.8					-1.5
Ccl19	24047	1.5		1.9					-1.5
Ccl2	20296	2.6	3.3	2.3	2.5	2.8	2.7		
Ccl20	12322	1.8					2.1		
Ccl21a	18829					-1.6	-2.6		
Ccl24	56221		1.6	2.4				1.6	
Ccl27a	20301	-3.0	-2.0						
Ccl3	20302	1.9	1.9			2.1	1.5		
Ccl4	20303	2.6	2.1	-1.7	-1.6	3.0	2.2	-1.6	-1.5
Ccl6	20305					1.6			
Ccl7	20306	1.8	2.9	2.6	3.5	2.6	2.4		
Ccl8	12326		-3.8	-2.0			-5.7	-1.8	-1.6
Ccr1	12768					7.7			
Ccr10	12777							-1.8	
Ccr1l1	12770		3.5	3.0		2.3			
Ccr2	12335	2.0	-3.3	-2.6	-2.8		-1.9	-2.8	
Ccr3	12771		2.0			2.6	-1.5	-2.4	
Ccr4	12773					1.5			
Ccr5	12774	2.2							

Ccr6	12458	1.6		1.9		2.0			1.8
Ccr8	12776					3.9			
Cdh15	12555	2.3		1.8		2.4	2.1	2.7	
Cdh16	12556	1.8	5.0		3.0	2.6		2.0	1.6
Cdh5	12562	2.6	1.6	2.6	2.2	5.3		1.9	3.0
Cdk12	69131	1.9		1.7	2.6	1.6			2.1
Cdk13	330188	-1.7	-1.6		1.7		-1.8		-1.7
Cdk14	18647	3.9		2.4	-3.2	1.6	2.5	2.4	-2.0
Cdk16	18555	-1.7				-1.6			
Cdk17	237459	1.9		2.1	1.7	3.1	2.1	-2.5	
Cdk2	12566				-1.9	1.5			
Cdk20	105278			-1.6				-1.6	
Cxcl1	66200						2.8		
Cxcl10	70349			1.6			1.7	1.7	
Cxcl12	20315		2.4		2.1	2.7	3.0	2.3	
Cxcl15	20309		-2.0			-4.6	7.7		
Cxcl16	66102		-2.1	-1.7	-2.2	-2.4		-1.6	-1.9
Cxcl17	232983	-2.6	-2.4	-2.1	-2.0	-1.9	-1.7	-1.9	
Cxcl2	20310	4.9			1.8	6.5		-2.2	
Cxcl3	330122			-4.7					
Cxcl5	20311	-2.6				-1.9			
Cxcr2	26754	-1.5					-1.9	-2.2	
Cxcr4	56358	2.0					1.9	2.8	-2.0
Cxcr5	67876						1.6		
Cxcr7	107684		1.7	3.4	1.9		1.9		

Cyp26a1	13082	3.6	4.0						1.6
Cyp26b1	232174		2.2	2.1		1.6		1.5	
Cyp2a22	235415		1.6				3.3	1.5	1.7
Def6	23853							1.9	1.6
Defa1	13216					-1.8			
Defa21	66298								-2.9
Defa22	382059							1.9	
Defa4	13238				-1.7				
Defa6	13240		1.9						
Defa-rs12	13221			1.9				2.7	1.6
Defb1	13214	3.6							
Defb10	246085		2.8	-4.9					3.9
Defb11	246081		-2.7			-1.7		-1.6	
Defb12	77674					1.6			
Defb13	246083					1.5			
Defb15	246082		2.3	3.2	2.1	1.5		3.1	2.2
Defb19	246700				-1.8	1.9			
Defb22	442835					-1.9			
Defb29	75400		-1.7	-1.8		2.2			-1.8
Defb3	27358	1.6							
Defb34	360211				1.8			2.9	2.0
Defb35	232983			1.6			4.2	7.3	2.8
Defb36	266620							-2.7	1.8
Defb37	353320	-1.5				1.5			-1.8
Defb38	20310						1.8	1.5	

Defb39	12765	1.6	1.8				2.1		
Defb40	12765						1.6		
Defb41	77673		-2.5	-1.7		1.5	-1.6		
Defb42	619548				-2.2				
Defb50	387334					1.5			
Defb6	116746	-1.7			-4.0				
Defb7	246080		-2.7			-1.6	-2.7	-2.4	-1.6
Dusp23	110052						1.8		1.6
Dusp27	329260	-1.8	1.7	-1.5			2.5	2.1	
Fgf8	14179				2.3	1.9	1.7	2.1	2.2
Fgf9	14180		1.6	2.7	1.5	1.5	1.7		
Fgfbp1	14181	2.3	2.0	1.8	2.0	2.6	8.6	2.3	9.9
Gpr137	107173	2.2	2.2	2.0				1.6	-2.1
Gpr137c	70713			1.7	1.6			2.3	-1.7
Gpr139	209776	3.0	2.2	1.7		-0.4	-3.4		
Gpr141	14365		2.5	2.1	2.5		2.8	2.1	
Gpr152	269053	3.1	2.3			1.9	1.5	-2.3	
Hif3a	53417	2.3	2.3	3.2	2.5	1.6	2.3	2.0	
Hk1	1E+08	-3.3	-2.8	-1.9			-2.4		
Hk2	15277	2.2	1.7			1.9	1.7		
Hkdc1	1E+08	1.9	2.0	1.7	1.8		3.5	1.8	
Htr2a	15558	2.1	-2.8					-2.4	
Htr2b	15559					1.9	1.6		
Htr2c	15560	1.8		1.6		-1.7	1.7		
Htr3a	14729		2.6		2.0		-6.2		

Icam5	15898				1.5				1.8
Ifna12	242519	1.8	1.6	1.6	1.6			1.9	
Ifna2	15965			1.5	1.8	6.8	2.0		
Ifna4	574402		1.6				-2.7	-1.9	
Ifna5	15968		-2.2						1.6
Ifna9	15972				1.7				1.8
Ifnb1	15977								1.7
Igf2r	16004	1.6	1.6	1.6		2.0	1.8	1.8	
Igfals	319197	2.0	1.8	3.1			-1.7	2.9	
Igfbp1	16006		-1.7	2.0					-2.2
Il10ra	16154					1.6	1.7	1.6	
Il10rb	16155							-1.8	-1.6
Il12a	53623		-3.6		-1.5		-2.1	-2.1	-1.5
Il12rb2	16162		1.8	1.6	1.6			1.9	
Il13ra2	16165	-2.3				1.8			
Il15ra	16169		-1.6	3.2	-2.1	-2.8	-2.1	2.8	-1.6
Il16	16170	2.8	2.5	1.7		1.7		2.7	
Il17a	16171	4.2							
Il17b	56069		1.6	2.7				2.0	
Il17c	234836			1.9		4.2			
Il17d	239114	1.6	1.5		1.9	1.7	-1.6		
Il17f	257630				2.1			1.8	1.5
Il17rb	50905		-3.0					-2.0	
Il17rc	171095					1.6			
Il17rd	14804	1.8			-5.7		3.9		

Il17re	57890	-1.8	-4.6		1.7	-1.9		1.7	2.1
Il18bp	14806		1.7				1.7	1.8	1.5
Il18rap	16174	-1.5						-1.5	1.5
Il19	14809	-2.1		-3.1			-2.1	-2.7	
Il1b	16176			-1.7	1.7				
Il1f10	14811	-2.0		-1.7			2.2		
Il1f5	54450	-2.0	-3.7	-3.1		1.5			
Il1f6	54448	-1.9		-2.1		-2.2	-2.6	-1.7	-1.8
Il1f8	69677	1.8	1.6			-1.7		1.8	
Il1f9	14813	-1.8		-1.5	-1.7		-1.6	-1.9	-2.7
Il1rap	16180	-1.6	2.0	-1.8	1.8				
Il1rapl2	60367	-2.1			-1.9	-1.6	-1.6	-1.6	
Il1rl1	17082	1.5	2.5	-2.6	3.3	1.5	2.6	-1.9	
Il1rl2	107527	1.6			1.8	-1.8	-2.1	-1.9	-1.6
Il1rn	16181				1.6			-2.8	-1.6
Il2	16183				-1.5				
Il20ra	237313		2.1			1.5		1.5	
Il20rb	213208	2.6		2.3	1.7	2.6	2.7	2.4	2.3
Il21	60505								1.6
Il21r	60504					1.8		1.6	
Il22	50929	-1.7	-1.7					-1.8	
Il22ra1	230828	1.6	1.6		2.5				
Il22ra2	237310			-2.5	-3.2				
Il23a	83430			-1.7					
Il23r	209590		-2.5		-1.6	-1.7			-1.5

Il24	93672							-1.6	
Il27ra	50931	3.5	2.0	2.2	1.8				2.2
Il2ra	16184	1.6	3.0	2.2					
Il2rb	16185		2.7	4.1	4.2	10.2		2.4	
Il31	76399					-1.7			
Il31ra	218624		1.5						-1.6
Il34	76527					1.7			
Il3ra	16188				2.8				1.5
Il4i1	14204					-1.9			
Il4ra	16190							1.9	
Il5	16191	-1.7	-1.9	-2.9		-1.7	-5.1	-2.5	-2.3
Il5ra	16192					1.7			
Il6ra	16194	-1.6			-1.8	-2.2			-1.6
Il7	16196	-3.1		-1.8					
Il7r	16197	-3.8	-5.4	-2.2	-2.2	-2.2	-2.4	-3.4	
Il9	16198	-2.8	-2.7	-4.1		-2.6	-2.7	-1.9	
Il9r	16199		2.3	2.1	2.7	2.6		2.6	2.7
Ins2	16334				1.7	4.4	2.4		
Irf1	16362	2.8	-2.3	2.4	1.9	5.0	1.8	2.8	
Irf6	54139	3.0		5.3	4.0			4.6	
Itga3	381091		-1.7	1.7			1.8		
Itga5	16402				1.6			1.8	2.4
Itga9	104099	3.2	2.5	1.7	1.7	2.2		3.6	2.4
Itgb2	16414					1.6	1.6	1.6	
Itgb3	16416	1.8	2.1	3.3	2.9	2.9	3.2	2.5	3.5

Jun	16476					1.6			
Junb	16477					1.5			
Map2k6	16579	1.9	1.9	1.7			1.8	-3.0	
Map2k7	26400			1.9	1.6	-2.3		-2.5	1.6
Map3k1	26401	-2.2	-4.4	-5.0	-2.8	-1.7	-2.9	-2.7	-2.9
Map3k12	67703	-2.3	-2.3	-3.0	-1.8		-1.7		
Map6	17760	1.7		2.7				3.0	2.9
Mapk15	332110		1.5	1.6	2.9				1.6
Mapk6	50772	2.1	4.1	1.8	2.0	2.9	4.3	1.9	
Mapk8	26419	1.8			-3.5	-1.7	-1.7	-5.0	-1.6
Mmp10	17384	1.7	2.0	1.5	1.5				-1.7
Mmp23	26561	3.5	1.7	2.4	2.2			1.9	
Mmp24	66180		2.1	-2.2			2.0		
Muc5ac	17833	1.7	1.7	1.6	1.6	1.5	1.6	1.6	1.7
Nlrc4	98682	1.9	-1.7	1.5			1.9	-2.3	
Nlrp12	378425		2.7	1.8	4.3	10.2			
Nlrp9c	330490			1.5		1.9	-2.2	1.5	-1.5
Pik3c2a	18704	-2.2		-2.4		2.0	-4.9	-2.9	-2.5
Pik3c2g	18705	2.1	1.5	2.0	1.7	2.1	3.1		3.0
Pik3r1	18708		-1.5	1.6		3.7	-1.9	1.7	1.6
Pla2r1	18779			3.0		1.5		3.1	-1.9
Ppp1r9a	243725	1.9	1.8	2.4	2.1	1.6	1.8	-1.6	1.9
Ppp2ca	19052	3.2	3.0	2.0	2.4	1.7			
Ppp2r2b	72930	1.9	-1.7	-1.6	-1.6	1.5		2.0	
Ppp2r2c	107975	2.3	1.8		1.6		1.5		

Ppp4r1l-ps	1E+08	1.7	-0.5	1.6	1.8			2.9	2.0
Ptgdr	19214					1.6			
Ptgds	19215		1.5						
Ptger1	19216			2.0	2.6				
Ptger2	19217				1.6				
Ptk6	20459	1.6	1.9			5.7	10.4	5.0	3.6
Rab14	68365	2.0	2.1	1.9	2.6	2.8	2.0	2.2	3.2
Rab39	270160	2.1	2.9	1.7	2.3	8.8	3.2		1.9
Rasa2	114713	3.5			2.3	6.6	7.0		2.9
Rela	19697	-1.7				-2.0			
Slc10a1	57262	2.3	1.9	1.9			3.7		1.6
Slc10a2	20494				2.8	1.6			
Slc12a5	57138	1.7				1.7	1.6		
Slc13a5	237831	1.6	1.9	2.0		1.8			
Slc14a1	108052		1.8	1.9		7.1	1.6		
Slc16a10	72472				1.7	1.5			2.5
Slc16a5	217316	1.7		1.9					4.5
Slc17a6	140919	3.7	1.8	0.7	-2.3			-3.7	-1.9
Slc18a2	215160			1.8	1.6		2.1	1.9	2.7
Slc1a2	20511	2.4	-3.0	3.6				1.7	
Slc1a3	20512					1.5	-1.6		
Slc1a4	55963				1.8				3.6
Slc1a6	20513	1.7		3.4				2.0	
Slc22a1	11848	1.5	1.6	1.6			2.0	1.7	2.2
Slc24a2	76376	2.7	1.7	2.3	-2.6	1.5	3.7		

Slc30a2	230810		3.0	2.7	3.5	11.8		2.4	1.6
Slc43a2	215113	1.6	1.8	3.2	3.6	2.2		3.1	
Slc45a2	22293	1.6	1.6			2.0	1.9	2.0	2.1
Smad7	67860	-2.9	1.9	2.3	1.7		1.6		
Smad9	55994		1.6	2.2					1.5
Tgfa	71726	2.2	1.9				1.7		
Tgfb1i1	21804		-1.6	-2.2		-1.6	-2.9	-4.5	1.7
Tgfbr1	21812			1.6	1.8	1.8		1.8	1.8
Tlr5	20679						1.5	1.5	1.9
Tlr8	20679						1.7		
Tlr9	78912						1.5		
Tmem145	330485		1.9	2.1	2.3			2.1	1.9
Tmem163	72160		2.1	3.9	6.1	3.0	8.8	6.0	11.4
Tmem191c	224019	1.8		1.6	1.6	22.7	2.8	2.3	3.4
Tmem205	235043	2.1		1.6	5.1	1.7		2.1	4.0
Tnf	21926					1.6		1.6	1.8
Tnfaip3	21929	1.6				1.9			
Tnfrsf10b	26938	2.0	1.9	2.0			2.4	2.1	1.8
Tnfrsf1b	20843		1.6	2.8			1.9	2.6	
Tnfrsf21	94185	-2.1		2.1	-1.6	-1.7	-2.1		
Tnfrsf25	85030	2.2	3.7	2.4	2.2	-1.7	2.8	2.7	
Tnfsf13b	24099	1.7	1.7		3.1	1.7	1.6	1.6	
Tnfsf14	20850	1.7					1.7	1.7	

Annexure 4: Differentially expressed genes in BALB/c following probiotics treatment

Gene Symbol	Entrez Gene ID	D10 STLA	D3 BC	D3 LA	D3 ST	D3 STBC	D3 STLA	D5 BC	D5 LA	D5 ST	D5 STBC	D5 STLA
Acvr1c	269275	-1.4	3.8	1.2	1.0	1.3	-1.3	1.1	-1.1	-1.2	1.5	1.0
Acvr2b	11481	1.3	2.6	1.4	1.3	1.8	1.2	2.1	1.2	1.6	2.2	1.9
Acvrl1	11482	1.2	1.5	1.4	1.2	1.3	1.0	2.1	1.2	1.2	2.2	1.0
Adcy5	224129	-4.3	3.1	1.4	-1.2	2.2	-2.1	4.0	-1.2	1.0	4.1	-1.2
Adcy6	11512	-1.5	-1.2	-1.1	-1.3	-1.4	1.0	1.0	-1.0	-1.1	1.1	1.1
Adcy7	11513	-1.0	1.3	-1.5	-1.1	1.3	-1.2	1.2	1.1	-1.2	1.8	-1.3
Adcy8	11514	1.2	1.7	2.3	1.2	4.0	1.3	2.1	-1.4	2.2	-1.0	1.1
Adcy9	11515	-1.6	1.0	-1.3	-1.4	-1.2	-1.0	1.1	-1.0	1.4	-1.3	1.2
Afap1	70292	-1.3	-1.8	-1.6	-1.1	-1.0	-1.2	-1.4	-1.8	-1.6	-1.4	-1.9
Afap1l1	106877	1.2	1.8	1.2	1.8	1.2	1.3	1.4	-2.1	-3.2	1.6	-1.3
Afap1l2	226250	-2.6	6.6	-1.5	-1.9	5.3	-1.3	1.5	-1.7	-2.7	5.3	-2.2
Akt1	11651	-1.3	-2.9	-1.2	-1.7	-1.3	-1.1	-2.7	-1.6	-1.1	-2.7	-1.4
Akt1s1	67605	-1.5	-2.9	1.0	-1.4	-2.2	-1.0	-4.0	1.2	1.9	-4.6	1.5
Akt2	11652	1.2	1.7	1.2	1.1	2.0	1.1	1.7	-1.3	1.3	1.7	-1.0
Akt3	23797	1.3	1.4	1.4	1.4	1.4	1.3	1.3	-1.0	-1.0	1.9	1.1
Anapc1	17222	1.2	3.8	1.2	-1.1	2.2	1.0	4.1	-1.1	1.4	3.6	1.1
Anapc11	66156	-1.1	-1.6	-1.1	-1.1	-1.2	-1.3	-1.3	-1.3	-1.1	-1.5	-1.3
Anapc13	69010	-1.3	1.1	-1.2	-1.1	1.0	-1.3	1.1	-1.5	1.3	1.2	-1.6
Anapc15	75430	1.1	-1.9	-1.1	-1.1	-1.1	1.1	-1.8	1.1	-1.3	-1.7	-1.1
Anapc2	99152	1.2	-1.8	-1.2	1.1	-1.0	-1.0	-1.5	-1.0	-1.1	-1.9	1.1
Anapc4	52206	-1.3	1.6	-1.4	-1.4	-1.1	-1.3	1.4	-2.3	-1.1	1.5	-1.6
Anapc5	59008	1.0	-1.9	-1.2	-1.9	-1.4	-1.0	-1.8	-1.0	-1.1	-1.9	1.4

Anapc7	56317	1.0	1.6	1.1	-1.2	1.4	1.1	1.5	1.1	1.2	1.5	1.0
Atg10	66795	1.4	1.5	1.3	-3.8	6.3	-1.3	1.1	1.1	-1.9	1.1	1.4
Atg12	67526	1.0	1.7	1.0	1.2	-1.0	1.1	1.6	-1.1	-1.1	1.4	-1.1
Atg14	1.01E+08	-1.1	1.2	-1.4	-1.1	1.3	-1.1	1.5	-1.0	-1.1	1.2	1.2
Atg16l1	77040	2.7	1.5	1.9	2.4	2.3	2.0	1.6	2.7	2.0	1.8	1.8
Atg16l2	73683	2.2	1.8	1.6	2.0	1.4	1.5	1.8	-1.1	1.2	3.4	1.4
Atg2a	329015	-1.4	-2.3	-1.0	-1.9	-1.4	-1.1	-1.9	1.1	1.2	-2.6	1.1
Atg2b	76559	-1.5	2.3	-1.5	-1.4	1.6	1.1	1.8	-1.0	-1.6	1.6	1.2
Atg4b	66615	1.1	-1.6	1.0	1.5	-1.3	1.3	-1.7	-1.1	1.2	-2.0	1.3
Atg4d	235040	-1.8	-1.7	-1.5	-1.1	-1.6	-1.6	-1.3	-1.6	-1.6	-1.6	-1.9
Atg5	11793	-1.2	1.8	1.1	-1.1	1.4	-1.1	1.8	-1.2	1.3	1.6	-1.1
Atg7	74244	-1.7	-2.5	1.4	-1.2	-3.6	-2.4	-1.9	-1.9	-3.9	-5.3	-2.6
Atg9a	245860	-1.3	-1.9	-1.4	1.2	1.4	-1.2	-2.5	-1.0	1.4	-2.0	1.3
Bad	12015	1.7	-2.2	1.5	-1.2	-1.7	1.3	-2.8	2.0	1.4	-2.7	1.5
Bcl10	12042	-1.6	1.7	-1.4	-1.5	1.0	-1.7	1.6	-2.3	-1.2	1.7	-2.1
Bcl11a	14025	-1.3	1.4	-1.7	-1.2	1.9	1.0	-2.1	-1.1	4.0	2.7	1.6
Bcl11b	58208	1.1	1.8	1.3	2.5	1.6	1.2	2.2	1.7	2.0	2.5	1.7
Bcl2	12043	1.3	-1.5	1.0	1.4	-1.1	1.1	-1.4	-1.1	1.5	-1.3	1.3
Bcl2a1c	12046	2.7	2.3	1.9	1.9	2.1	1.3	2.2	1.6	1.6	4.8	1.4
Bcl2a1d	12047	1.6	1.5	-1.4	-1.3	1.2	-1.6	1.2	-1.0	-1.0	2.4	-1.1
Bcl2l1	12048	1.2	1.1	1.1	1.6	1.5	1.3	1.2	1.4	1.4	1.0	1.1
Bcl2l11	12125	-1.7	1.6	2.7	-1.0	2.6	-1.0	2.3	2.1	3.3	-3.3	1.9
Bcl2l13	94044	-1.4	5.2	-1.0	-1.0	1.2	-1.4	5.2	-1.4	-1.1	2.4	-1.1
Bcl2l14	66813	-1.2	1.1	-1.1	-1.3	1.1	-1.2	-1.1	-1.7	-1.4	1.2	-1.3
Bcl2l15	229672	1.5	-1.2	1.1	-1.5	1.2	-1.1	-1.0	1.3	1.5	-1.1	1.1
Bcl2l2	12050	1.1	1.5	1.1	1.1	19.2	1.6	2.7	1.2	1.5	1.4	1.4
Bcl3	12051	-1.2	-1.3	-1.1	1.0	1.1	1.2	1.3	1.7	1.6	-1.0	-1.0

Bcl6	12053	1.1	2.2	-1.2	1.2	1.9	1.0	2.1	-1.0	-1.0	2.7	-1.0
Bcl7a	77045	1.4	-1.0	-1.4	1.1	2.3	1.3	1.3	1.5	-1.5	2.5	-5.1
Bcl7b	12054	1.8	-1.1	1.6	1.9	1.3	1.9	-1.3	1.5	1.7	-1.2	1.6
Bcl7c	12055	1.0	-2.0	-1.0	1.4	-2.0	-1.0	-2.7	1.0	1.1	-2.7	1.1
Bcl9	77578	1.8	1.1	1.5	1.2	-1.8	1.5	-1.0	-1.6	-1.1	-5.5	2.0
Bcl9l	80288	-3.2	-3.7	-1.8	-1.0	-2.3	1.4	-1.8	-5.1	-1.2	-1.7	1.4
C1galt1	94192	-1.3	-2.9	-1.1	-1.0	-1.6	-1.0	-2.9	-1.8	-3.1	-3.2	-1.5
C1galt1c1	59048	-1.1	1.0	1.0	1.1	-1.1	-1.5	-1.0	-1.6	-1.4	1.0	-1.3
C1qa	12259	-1.2	-1.6	1.1	-1.2	-1.6	-1.5	-1.8	-1.1	1.0	-1.5	1.1
C1qb	12260	-1.2	-2.6	-1.4	-2.0	-1.8	-2.1	-2.5	-1.8	-1.7	-1.8	-1.1
C1qc	12262	-1.1	-2.1	-1.2	-1.1	-1.1	-1.5	-2.1	-1.2	-1.2	-1.7	-1.1
C1qtnf1	56745	-1.4	1.1	4.5	-1.1	4.3	-1.2	1.7	-1.2	1.8	1.9	-2.3
C1qtnf2	69183	-2.1	-1.3	-3.2	-5.0	-1.1	9.2	-1.6	-1.8	-3.4	-2.1	-2.7
C1qtnf3	81799	-1.3	1.0	-1.5	-1.6	-1.3	-1.4	-1.1	-2.1	-2.6	-1.4	-1.7
C1qtnf4	67445	2.4	1.2	1.2	1.9	1.8	1.7	1.4	1.1	-1.6	1.1	1.4
C1qtnf5	235312	1.4	-1.4	1.1	-1.4	-1.1	1.1	-1.5	1.3	-1.3	-1.5	-1.3
C1qtnf7	109323	-1.4	-1.3	-1.3	-2.0	-1.4	1.2	-1.2	-6.0	-3.0	-1.7	-2.8
C1qtnf9	239126	-1.1	-1.3	1.3	1.4	-1.6	1.2	-1.3	-1.1	5.5	-1.2	-1.5
C1ra	50909	1.2	1.9	1.2	-1.7	1.5	1.2	2.2	1.3	1.1	3.1	1.2
C1rb	667277	-2.2	-5.0	-3.9	-5.7	-2.5	-1.4	-9.7	-2.6	-4.1	-4.2	-2.0
C1rl	232371	-1.1	1.6	1.1	-1.1	-1.1	-1.6	-1.2	-1.2	-2.1	2.4	-1.2
C1s	50908	1.4	1.2	1.3	-1.5	1.3	-1.0	1.1	-1.9	-1.1	1.8	-1.4
C2	12263	1.3	1.3	-1.0	1.4	1.1	1.4	1.2	2.2	2.1	1.8	1.8
C3	12266	-1.2	-1.6	-1.7	-1.8	-1.4	-2.0	-1.6	-3.6	-2.3	-1.0	-3.3
Calm1	12313	-1.1	-1.7	-1.0	1.6	1.5	1.2	-1.5	1.8	1.6	-1.4	1.4
Calm2	12314	-1.6	1.6	-1.3	-1.3	1.1	-2.1	1.5	-1.8	1.2	1.8	-1.9
Calm3	12315	-1.1	1.2	1.4	1.1	2.2	1.5	1.3	1.0	2.3	1.3	-1.1

Calml3	70405	1.2	1.3	1.3	1.6	-26.5	1.4	1.2	1.1	2.5	1.2	1.4
Calml4	75600	-1.2	1.5	1.1	1.2	1.1	-1.2	1.3	1.2	1.9	1.3	-1.1
Camk1	52163	-1.3	1.7	-1.1	-1.6	-1.9	-1.7	1.9	-1.5	-1.8	1.0	-1.1
Camk1d	227541	1.2	1.8	1.2	-1.1	1.4	-1.1	1.4	1.0	1.0	1.6	1.2
Camk1g	215303	1.6	1.3	-1.4	-1.2	5.6	1.1	3.1	1.9	-1.9	1.5	-1.0
Camk2a	12322	-2.3	-2.7	1.4	-1.6	-1.7	2.5	-2.9	-2.6	-2.2	-1.1	-1.7
Camk2d	108058	1.0	-1.5	1.1	-1.7	1.2	-1.1	-1.9	-1.2	1.3	-1.7	1.2
Camk2g	12325	-1.0	2.8	-1.1	-1.0	1.6	1.1	2.6	-1.5	1.4	2.7	-1.2
Camk2n1	66259	1.3	4.4	2.6	4.7	3.4	3.6	2.1	1.2	2.7	1.9	3.1
Camk2n2	73047	-1.8	-1.1	-1.2	-1.1	1.4	-1.0	-1.1	-1.3	-1.0	-1.5	-1.1
Camk4	12326	1.2	-1.2	1.1	245.2	-1.4	1.2	1.0	-1.0	1.9	-1.1	1.4
Camkk1	55984	-1.4	2.9	-1.3	1.9	1.4	1.1	2.4	1.0	1.1	1.6	1.4
Camkk2	207565	-1.0	1.2	1.1	1.2	1.5	1.3	1.2	-1.5	-1.1	1.2	-1.3
Casp1	12362	-1.4	1.4	-1.3	-1.0	1.4	-1.5	1.3	-1.6	1.2	1.6	-1.6
Casp14	12365	2.3	-1.1	-2.6	-2.6	-3.2	-1.2	-1.6	-1.3	-1.5	6.4	1.3
Casp2	12366	-1.4	1.6	-1.4	-1.1	1.3	-1.4	2.2	-1.7	-1.1	1.9	-1.5
Casp3	12367	-2.9	-4.9	1.7	-1.8	-1.9	-1.5	-3.7	-3.7	1.0	-3.0	-1.8
Casp4	12363	-1.9	-1.3	-1.4	-1.8	-1.5	-1.4	-2.8	-1.8	-1.6	-1.4	-1.5
Casp6	12368	-1.1	2.0	1.2	-1.1	1.4	1.0	1.4	1.1	-1.0	1.2	-1.0
Casp7	12369	-1.2	-1.1	1.1	1.4	1.4	1.4	-1.3	1.6	1.4	1.3	1.4
Casp8	12370	-1.8	-3.8	-2.2	-3.0	-1.9	-2.2	-3.2	-1.6	-1.4	-3.2	-2.0
Casp8ap2	26885	1.2	1.4	-1.1	1.4	1.2	-1.0	1.2	-1.0	-1.6	1.3	-1.3
Ccl1	20290	2.0	-9.4	3.2	1.0	2.0	1.0	1.2	1.3	1.9	-1.2	1.5
Ccl11	20292	1.5	1.7	1.2	-1.8	1.3	1.3	1.5	-1.0	-1.4	4.7	-1.2
Ccl12	20293	1.4	1.0	1.1	-2.2	1.0	-1.7	1.4	-1.1	-1.8	2.3	1.1
Ccl17	20295	2.4	3.2	1.6	1.7	4.2	2.1	-1.1	6.1	3.8	1.0	2.0
Ccl19	24047	1.9	1.4	-1.3	-1.2	-1.1	-2.1	1.2	-1.7	-1.8	1.7	-1.3

Ccl2	20296	2.5	1.1	1.4	-2.0	-1.2	-1.0	1.5	-1.2	-1.6	3.1	1.1
Ccl24	56221	-1.4	5.2	1.4	5.2	10.1	3.3	2.1	12.5	5.5	3.8	7.9
Ccl25	20300	-1.4	1.4	-1.3	1.3	1.2	1.1	1.2	1.2	2.5	-1.0	-1.1
Ccl27a	20301	-1.7	1.2	-1.4	2.1	1.0	-1.3	-1.5	1.4	-2.9	1.1	-1.2
Ccl28	56838	1.0	5.2	-1.0	1.4	2.0	1.2	2.9	-1.3	1.6	6.2	-1.0
Ccl4	20303	1.2	1.5	1.8	1.3	2.1	1.5	-1.1	1.5	-1.8	3.3	1.3
Ccl5	20304	-1.2	1.2	-1.5	1.5	1.5	1.0	1.1	1.1	2.0	2.0	-1.4
Ccl6	20305	-1.9	-1.6	-1.6	1.0	-1.2	-2.4	-1.3	-1.5	-1.2	-1.7	-1.6
Ccl7	20306	1.7	-1.4	1.6	-1.9	-1.7	1.0	1.2	-1.2	1.1	4.7	1.9
Ccl8	20307	-1.4	-1.6	-1.4	-2.0	-2.8	-4.3	-1.8	-11	-2.8	1.1	-3.0
Ccl9	20308	1.2	-2.0	1.1	1.3	-1.0	-1.2	-1.5	1.4	-1.2	-2.3	-1.2
Ccr10	12777	-2.1	1.4	-1.1	-1.1	1.0	1.1	2.4	-1.2	1.4	2.3	-1.1
Ccr5	12774	1.2	5.1	-1.4	-1.5	1.5	-1.1	1.5	-1.2	-1.4	5.8	1.1
Ccr6	12458	-1.3	-1.6	-2.0	-1.1	-1.6	-2.5	-1.3	-3.3	-1.7	1.5	-5.6
Ccr7	12775	2.5	-1.6	-1.3	-1.3	-1.2	-2.2	-1.1	-1.5	-1.1	1.8	-6.0
Ccr8	12776	4.1	1.1	-1.3	-1.3	6.6	1.0	2.4	1.1	3.4	1.5	-1.6
Ccr9	12769	-1.6	-2.1	-1.6	1.7	-1.1	2.2	-4.7	2.6	1.6	-2.3	1.6
Cdh10	320873	-2.0	-1.3	-1.0	1.0	-3.4	-2.5	-1.3	-1.1	-5.1	-1.2	-1.7
Cdh13	12554	-1.5	-1.2	1.2	1.5	-1.0	1.5	2.0	-1.3	1.0	1.6	-1.1
Cdh17	12557	-1.1	-1.9	1.3	-1.0	-1.0	-1.1	-1.9	1.4	1.3	-2.3	1.0
Cdh2	12558	1.3	3.3	-1.0	1.2	2.6	-1.1	3.8	-1.5	-1.1	3.5	-1.2
Cdh20	23836	-5.5	-3.4	-1.8	-1.9	-3.5	-2.7	-2.8	-1.5	-1.9	-4.7	-3.7
Cdh22	104010	-1.9	1.0	1.0	-3.9	1.8	-1.8	-2.3	-2.5	-1.9	-1.2	-1.5
Cdh26	381409	3.1	1.7	3.4	4.6	1.8	1.2	1.7	1.4	1.3	1.4	1.6
Cdh5	12562	-1.8	-1.3	-1.5	-1.6	-1.2	1.4	1.1	1.0	1.2	-1.0	-1.8
Cdh8	12564	1.1	-2.5	-1.2	-1.4	-1.1	-1.6	-2.0	-1.3	-1.7	-1.9	-4.0
Cdhr1	170677	-2.2	1.3	-3.9	-2.6	-1.3	-15.7	1.2	-5.3	-3.4	1.4	-2.5

Cdhr2	268663	-1.9	-1.4	-1.2	1.0	1.3	-1.1	-1.1	-1.3	1.7	-1.6	-1.2
Cdhr4	69398	2.5	-1.5	-1.4	-2.1	1.3	-1.4	-1.1	1.3	2.4	1.0	-1.4
Cdhr5	72040	-1.8	-2.6	-1.2	1.1	-1.6	-1.1	-2.5	-1.3	1.4	-3.4	-1.1
Cdk7	12572	-1.4	2.5	1.4	1.1	-1.3	-1.3	2.0	-1.5	1.6	2.8	1.1
Cflar	12633	-2.5	-4.8	1.4	1.4	-1.7	1.2	-5.3	1.1	-1.6	-3.6	-1.6
Creb1	12912	-1.1	4.8	-1.9	1.4	3.8	1.2	3.4	1.8	-1.1	2.5	-1.3
Creb3l2	208647	-3.0	-1.1	1.3	-1.2	1.8	1.8	1.3	1.7	-1.8	-1.4	-1.3
Crebzf	233490	-1.3	1.8	1.9	-1.1	4.4	-1.3	1.6	-1.1	2.5	1.6	-1.7
Csf1	12977	1.3	6.1	1.4	1.2	1.4	1.0	6.2	-1.2	-1.5	7.3	-1.3
Csf1r	12978	-1.1	-3.0	-1.3	-2.6	-1.9	-4.8	-4.3	-2.9	-3.1	-4.0	-1.0
Csf2	12981	1.3	1.5	1.8	1.3	-1.1	1.3	1.7	1.5	1.2	1.6	1.4
Csf2rb	12983	1.1	-2.2	-1.1	-1.9	-1.3	-1.6	-1.4	-1.7	-1.8	-1.1	-1.4
Csf2rb2	12984	1.5	3.2	1.7	-1.8	1.5	-1.2	3.0	-1.5	-1.9	1.6	-1.2
Csf3r	12986	-1.2	-5.0	-2.0	-2.1	-2.7	-2.0	-3.2	-1.8	-1.2	-2.6	-1.8
Ctnna2	12386	-1.7	2.4	-1.4	-2.8	2.6	-1.6	2.6	1.2	-2.3	-1.0	1.0
Ctnnb1	12387	-1.1	1.3	-1.1	-1.2	1.4	-1.0	1.4	-1.1	1.6	1.3	1.0
Ctsf	56464	2.0	1.7	1.8	2.5	1.1	1.3	2.3	1.4	1.4	1.8	1.5
Ctsg	13035	1.1	-1.3	4.7	-4.7	-3.8	-2.1	3.0	-3.3	-7.3	1.2	-9.1
Ctsh	13036	1.8	1.7	1.1	1.2	1.6	-1.0	1.6	1.0	1.2	2.3	1.1
Ctsk	13038	1.5	2.7	-1.0	1.1	1.4	1.1	2.9	-1.1	1.3	2.7	1.0
Ctsl	13039	-1.3	2.1	-1.3	-1.5	-1.4	-1.3	1.8	-2.5	-1.2	2.0	-1.8
Ctso	229445	-1.8	1.1	-1.2	1.3	-1.0	1.3	1.5	3.2	-1.0	-1.7	1.8
Cxcl1	14825	5.1	1.4	3.1	-1.6	-4.2	4.1	1.6	1.1	-1.8	5.2	-1.1
Cxcl10	15945	3.0	-1.4	1.7	-1.6	-1.7	-1.6	2.1	1.0	-2.9	2.6	-1.2
Cxcl11	56066	-1.1	-6.0	-1.8	-2.2	-4.0	-2.8	-3.9	-2.6	1.5	-4.0	-1.5
Cxcl12	20315	1.4	2.7	1.4	1.6	2.9	2.2	2.6	1.2	1.6	2.3	1.9
Cxcl13	55985	1.4	1.3	-2.3	-1.4	1.5	-2.6	-1.0	-1.8	-2.6	1.7	-1.8

Cxcl14	57266	-1.3	1.3	1.2	3.5	2.4	2.6	-1.2	2.1	1.3	1.3	2.8
Cxcl15	20309	1.1	-1.1	-1.0	1.2	-1.4	1.2	-1.1	-1.2	1.3	-11.0	-1.0
Cxcl16	66102	1.2	-1.2	-1.3	-1.4	-1.2	-1.3	1.1	-1.8	-1.5	1.0	-1.6
Cxcl5	20311	3.9	7.0	1.6	-1.1	3.0	2.1	3.9	1.7	1.1	27.2	1.7
Cxcl9	17329	2.8	-1.2	1.4	-2.9	-1.2	-2.0	1.8	-1.7	-3.4	1.7	-2.4
Cxcr3	12766	1.5	2.5	1.1	-1.0	1.6	1.6	1.8	-1.4	-1.1	4.3	1.2
Cxcr4	12767	-1.1	-3.8	-2.2	-3.9	-1.7	-1.7	-1.0	-2.8	-2.8	1.2	-4.4
Cxcr5	12145	1.2	-2.0	-3.6	-1.2	-1.4	-2.6	-1.0	-1.6	-1.3	1.9	-3.6
Cxcr6	80901	-1.2	1.2	1.3	-1.3	-1.2	1.1	-1.1	-1.5	-2.3	1.7	-1.2
Cxcr7	12778	1.1	2.2	-1.1	-1.1	1.2	1.1	2.5	-1.2	-1.2	3.1	1.2
Cyp19a1	13075	1.3	1.2	1.3	1.3	1.6	6.2	1.1	-1.0	1.9	1.2	1.3
Cyp1a1	13076	1.3	2.1	1.0	2.9	-1.3	2.7	2.7	4.3	2.4	2.2	-1.2
Cyp1b1	13078	2.2	1.6	1.1	1.2	2.4	1.7	1.3	2.1	1.6	2.7	1.5
Cyp20a1	77951	1.2	2.9	1.1	-2.1	1.2	1.0	2.2	1.1	1.0	2.0	1.2
Cyp26b1	232174	1.8	4.3	1.2	7.0	8.4	1.8	1.5	2.9	1.2	1.3	10.6
Cyp26c1	546726	1.3	1.0	-4.1	1.2	-1.2	1.3	-1.0	1.0	1.7	1.0	1.3
Cyp27a1	104086	-1.3	2.1	-1.1	1.2	2.4	1.6	1.1	3.3	1.3	-1.1	2.6
Cyp2ab1	224044	1.3	-1.1	1.2	-1.2	2.6	-3.4	1.3	-1.0	1.3	1.1	1.2
Cyp2b10	13088	-1.8	2.8	-1.2	1.6	4.1	6.8	3.4	10.8	-1.1	1.0	1.0
Cyp2c44	226143	1.2	10.3	6.3	2.3	6.3	2.5	4.1	1.5	2.3	9.2	2.6
Cyp2d26	76279	-2.0	1.7	-1.9	1.3	1.3	1.7	2.3	1.3	-1.5	-1.4	-1.8
Cyp2d40	71754	-1.0	2.1	-1.7	1.4	1.3	-1.3	6.4	1.6	2.3	2.0	-1.4
Cyp3a16	13114	-1.5	12.2	-1.7	-1.8	14.3	4.3	5.7	10.4	1.5	2.2	1.2
Cyp3a25	56388	-1.6	3.7	-1.2	1.9	4.3	3.5	2.9	3.1	2.0	1.0	2.3
Cyp3a44	337924	-2.9	13.6	1.9	2.8	12.2	4.2	13.0	2.0	4.6	2.8	4.4
Def6	23853	-1.0	-1.2	-1.4	-1.8	-1.5	-1.4	-1.5	-1.7	-1.2	1.1	-1.8
Def8	23854	-1.3	1.4	-1.3	-1.1	1.2	1.1	1.7	-1.4	-1.2	1.1	-1.3

Defa1	13216	-1.5	-1.2	-1.5	-1.9	1.2	-2.3	-1.8	-1.0	1.5	-2.1	-1.6
Defa21	66298	1.3	-1.4	1.1	1.2	-6.3	1.3	-6.0	-1.6	1.3	-7.0	1.2
Defa23	497114	-1.5	1.2	-1.3	1.1	1.2	-1.2	-1.3	1.1	1.7	-1.7	-1.5
Defa25	13236	1.5	1.1	1.3	1.2	1.5	-1.0	-1.7	2.1	1.8	-1.9	2.2
Defa26	626708	-1.5	-3.1	-1.9	-1.6	-1.3	-1.4	-5.0	-1.3	1.0	-6.0	-1.2
Defa3	13237	-1.8	-1.8	-1.6	-1.2	-1.4	-1.6	-2.3	-1.2	1.3	-2.8	-1.7
Defa4	13238	1.2	-1.1	-1.2	1.1	1.4	-1.4	-1.6	1.8	2.2	-1.8	1.1
Defa-ps1	727720	1.0	-2.3	-1.1	-1.1	1.2	-1.3	-3.4	1.9	1.6	-4.1	1.3
Defa-rs1	13218	-1.4	2.4	-1.3	1.1	2.1	-1.0	1.0	2.0	1.8	-1.1	-1.2
Defa-rs10	13219	-2.1	-3.5	-2.2	-1.5	-1.1	-3.2	-2.9	-2.0	-1.3	-3.4	-2.6
Defa-rs12	13221	-1.7	-3.5	-2.2	-1.4	-1.6	-3.0	-2.9	-1.5	1.0	-4.1	1.2
Defa-rs2	13222	-1.7	-1.4	-1.2	1.0	1.3	-2.0	-1.3	-1.3	1.4	-1.8	-1.9
Defa-rs4	13223	-2.1	-3.4	-2.7	-1.4	-1.8	-2.4	-3.2	-1.2	-1.2	-4.6	-2.4
Defa-rs7	13226	-2.3	-2.0	-1.5	-1.3	-1.4	-3.4	-2.0	-1.3	-1.2	-2.7	-2.2
Defb1	13214	2.2	-1.4	-1.0	-1.3	-1.4	-1.9	-1.4	-1.0	1.1	-1.1	-1.4
Defb14	244332	1.1	-1.0	-1.0	1.6	14.4	1.4	-1.1	-1.1	1.2	1.0	1.2
Defb25	654459	-1.4	4.4	2.7	4.5	4.4	2.1	8.5	6.5	1.3	3.2	2.8
Defb30	73670	-1.2	-1.6	-1.1	-1.2	-1.9	1.0	-1.5	-2.9	-1.3	-1.6	1.3
Defb36	266620	1.1	1.1	1.1	1.4	-1.1	1.3	1.0	-1.1	1.7	-8.2	1.1
Defb41	77673	1.4	1.3	1.3	-3.4	-3.1	-1.2	-2.9	1.1	1.9	1.2	1.5
Defb42	619548	3.0	1.5	-2.0	-1.1	2.7	-1.0	2.1	1.1	1.7	2.1	-2.4
Defb45	433490	-1.2	3.8	2.7	1.7	2.3	-1.1	2.9	1.0	1.0	5.9	-1.1
Defb48	432867	-3.7	-2.1	1.2	-2.2	-4.3	-1.8	-1.6	-1.1	-1.4	-2.1	-2.9
Defb7	246080	1.0	-1.4	-2.7	-1.3	-3.9	1.4	-2.8	1.4	1.1	-7.6	-1.3
Dusp19	68082	-1.0	-1.3	-1.1	-1.3	-1.7	283.4	-1.3	1.5	-1.7	1.8	-1.1
Dusp22	105352	-1.1	-1.2	-1.4	1.4	1.9	1.8	4.1	1.3	-1.7	2.0	-2.4
Dusp23	68440	1.2	3.4	2.0	-1.1	3.6	1.2	2.7	-1.7	-2.4	3.8	-1.3

Dusp26	66959	1.1	1.4	-1.2	-1.0	1.2	-1.3	2.3	-1.5	-1.4	1.1	-1.1
Dusp4	319520	1.4	2.7	-1.2	1.4	2.7	4.4	-1.2	3.5	1.6	1.1	-1.1
Fas	14102	2.1	4.9	1.4	1.4	2.9	1.9	3.5	1.4	2.0	3.7	1.4
Fasl	14103	-1.2	2.1	-2.7	1.1	1.3	-2.2	5.2	-1.5	1.4	1.9	-1.2
Fos	14281	-1.3	-1.1	1.0	1.6	2.0	1.8	1.3	2.0	1.0	-1.4	-1.1
Gadd45a	13197	1.0	2.0	1.1	1.7	1.1	-1.0	1.9	1.5	1.9	2.6	1.4
Gadd45b	17873	1.1	-2.6	1.1	1.1	-1.0	1.7	-2.7	1.2	-1.1	-2.3	1.0
Gadd45gip1	102060	1.0	7.8	1.2	2.2	-1.1	1.7	1.4	1.2	-1.1	1.2	1.5
Gas1	14451	1.1	1.2	1.1	1.3	1.1	2.2	-1.3	1.2	1.6	1.3	-1.8
Gas2	14453	1.1	2.8	-3.1	-1.6	2.9	-1.4	1.5	1.2	-1.2	1.0	1.6
Gata2	14461	-1.0	1.3	1.2	-1.2	1.0	1.2	1.2	-1.2	2.1	1.7	-3.2
Gata4	14463	-1.7	-1.8	-1.4	-1.2	1.3	1.2	-1.9	2.2	-1.6	-3.1	-1.4
Gata5	14464	1.1	1.1	1.2	1.5	1.6	1.4	1.4	1.9	2.3	1.1	1.4
Gata6	14465	-1.4	1.1	-1.1	-1.1	1.3	-1.1	-1.1	-1.2	2.5	-1.1	-1.0
Gpr110	77596	-1.3	5.6	-2.4	2.0	1.0	1.7	1.2	2.6	2.1	7.4	1.2
Gpr111	435529	2.2	3.2	-1.7	-1.2	1.7	1.8	2.0	1.2	3.1	2.8	3.7
Gpr112	236798	-1.4	3.2	-1.9	1.1	5.1	-1.1	2.4	-1.0	-1.8	-1.0	-2.3
Gpr114	382045	1.2	1.9	-1.1	1.7	1.2	1.2	1.6	1.3	1.4	2.1	1.5
Gpr116	224792	-1.5	10.4	1.7	1.5	4.2	2.9	13.8	1.2	1.7	9.4	1.5
Gpr126	215798	1.5	3.3	1.1	3.5	3.6	1.1	1.4	1.8	1.4	4.6	1.1
Gpr133	243277	-1.1	1.3	1.1	1.8	2.5	3.2	4.0	1.7	3.1	1.4	1.7
Gpr137b	83924	1.5	5.5	1.6	1.1	-1.0	1.4	5.4	-1.6	-1.5	4.7	1.4
Gpr137b-ps	664862	1.7	2.6	1.5	-1.7	1.4	1.5	1.3	-1.7	-5.6	3.4	-1.0
Gpr153	100129	-1.6	1.3	1.1	2.1	-1.5	1.4	1.5	-1.0	-1.8	1.6	-1.3
Gpr17	574402	-1.2	1.4	1.1	1.2	1.1	1.8	2.9	9.5	3.3	1.9	3.9
Gpr171	229323	2.4	1.6	1.7	1.2	1.7	1.5	2.1	1.4	1.6	3.7	1.3
Gpr182	11536	-1.2	1.6	-1.2	-1.3	-1.1	1.5	1.4	-1.1	-1.6	3.0	1.2

Gpr183	321019	-1.2	-1.3	-1.8	-1.6	-1.2	-5.2	1.2	-1.7	-1.7	1.8	-2.5
Gpr20	239530	1.4	1.5	1.7	1.9	1.6	1.4	2.4	-1.7	-1.1	2.2	1.4
Gpr27	14761	1.1	-1.4	-1.3	-1.3	1.0	-1.5	1.4	1.9	1.4	1.2	1.2
Gpr3	14748	3.8	-1.2	1.4	1.2	1.5	1.6	6.5	1.9	2.0	1.0	-1.0
Gpr31b	436440	2.7	2.1	1.3	1.7	1.8	-1.1	-1.3	3.1	1.9	2.2	2.4
Gpr82	319200	-2.3	2.7	1.2	-2.3	-1.1	-1.4	3.1	-2.4	-1.9	4.1	-1.1
Gpr84	80910	7.0	2.8	1.2	2.6	1.7	2.0	3.4	-1.1	2.2	4.5	3.3
Gpr85	64450	-3.9	-1.1	-1.3	-5.0	1.1	-1.3	2.0	-3.4	-2.7	2.0	-2.8
Gpr97	54672	1.1	1.8	1.0	1.2	1.5	1.6	2.1	1.6	1.3	1.5	1.8
Gprasp1	67298	-1.7	2.3	-2.0	-1.1	2.1	-1.5	2.9	-1.6	-1.5	3.1	-2.0
Gprasp2	245607	-1.9	2.8	-1.3	1.1	1.4	-1.2	2.4	-1.7	-1.4	2.0	-2.4
Gprc5b	64297	1.1	3.1	-1.3	-1.3	1.6	1.1	2.9	-1.2	-1.3	2.1	-1.1
Hif1a	15251	-2.5	-3.8	-2.3	1.1	-2.6	1.1	-1.4	-3.5	-1.4	-2.6	-4.4
Hif1an	319594	1.0	2.3	1.9	2.3	1.4	1.5	1.9	1.4	1.7	1.9	2.0
Hif3a	53417	1.8	1.4	1.2	1.2	3.3	1.4	1.5	-1.3	-1.2	1.3	1.9
Hk2	15277	1.1	3.4	1.0	-1.5	-1.1	-1.1	2.0	-1.2	-1.1	3.9	-1.2
Hk3	212032	3.7	6.0	4.9	1.1	6.4	2.4	1.6	6.2	1.3	10.8	6.5
Hkdc1	216019	1.2	1.6	1.0	1.6	1.5	1.1	1.8	1.6	2.5	1.5	1.5
Hspa12b	72630	1.1	2.2	2.4	1.8	2.4	2.6	4.2	2.1	1.3	2.9	1.2
Hspa1a	193740	1.5	1.3	1.4	5.4	1.5	1.6	8.2	4.4	1.6	1.0	1.4
Hspa2	15512	2.9	1.9	1.2	1.9	2.7	2.3	1.4	1.7	3.4	2.0	1.5
Hspa8	15481	1.3	-2.7	-1.0	2.5	-1.6	-1.1	-2.0	2.3	1.7	-2.6	1.8
Hspb11	72938	1.0	2.2	1.2	1.1	1.3	-1.0	2.7	-1.3	1.3	2.6	1.0
Icam1	15894	-6.2	-8.6	-4.9	-5.1	-8.7	-4.9	-7.1	-11	-10	-6.3	-5.7
Icam2	15896	1.6	1.8	1.0	1.4	1.3	1.1	1.7	-1.1	-1.1	2.5	-1.0
Ifna13	230396	1.9	-1.1	-1.1	1.5	-1.4	1.3	1.5	-9.2	1.2	1.3	-1.0
Ifna14	404549	-2.5	-2.0	-3.4	-3.3	-2.5	-7.2	-1.5	-2.1	-1.3	-2.0	-3.0

Ifna2	15965	4.4	-4.2	1.2	3.0	-1.0	1.3	1.2	1.0	5.6	1.1	1.3
Ifna5	15968	-1.3	1.2	1.2	1.7	-1.4	1.1	-1.1	3.2	-1.6	1.8	-1.3
Ifnab	15974	-1.2	-1.3	-4.9	-1.9	-1.3	-4.3	-1.2	-1.5	-1.1	1.1	-2.3
Ifnar1	15975	-1.0	2.6	1.3	-1.3	1.8	1.3	2.2	1.4	1.3	1.7	1.9
Ifnar2	15976	-1.0	-1.2	-1.0	-1.2	-1.3	-1.0	-1.3	-1.3	-1.8	-1.2	-1.3
Ifng	15978	2.8	1.0	1.1	-1.7	5.8	-1.1	1.5	-1.8	-2.9	5.0	-1.7
Ifngr1	15979	-1.5	-1.2	-1.2	-1.5	-1.0	-1.7	-1.2	-3.0	-1.5	1.2	-2.3
Ifngr2	15980	-1.3	-1.9	-1.2	-1.2	-1.3	1.0	-1.7	-1.2	1.3	-2.3	-1.3
Igf1	16000	1.0	1.5	4.1	-1.8	1.5	1.9	4.9	-1.2	-1.4	3.9	-1.9
Igf2as	111975	-3.5	1.0	1.5	1.0	2.6	1.9	2.9	5.2	2.7	-1.0	-1.2
Igf2r	16004	1.1	1.7	-1.0	1.1	1.4	-1.5	1.9	-1.3	1.3	1.9	-1.1
Igfbp4	16010	-1.2	2.1	2.0	2.6	-1.2	2.7	-1.1	2.5	-2.4	1.0	-2.2
Igfbp5	16011	-1.4	-1.9	1.2	1.1	1.1	1.7	-1.7	-1.5	-1.2	-1.4	-1.3
Igfbp7	29817	-1.3	1.7	-1.1	-1.5	-1.5	1.5	1.3	1.1	2.2	-1.5	-1.0
Ikbip	67454	1.2	2.1	-1.2	-1.4	1.2	-1.7	1.7	-1.5	-1.1	2.2	-1.4
Ikbkb	16150	-1.2	-1.2	-1.6	1.0	-1.0	-1.3	-1.9	-1.0	-1.0	-1.7	-1.2
Ikbke	56489	-1.4	-1.8	1.1	-1.1	1.3	1.3	-1.1	1.2	1.5	-1.4	1.7
Ikbkg	16151	-1.0	1.0	-1.3	1.6	1.3	-1.1	1.1	1.5	-1.3	1.2	-1.4
Il10	16153	-1.6	1.2	-2.1	-1.6	-1.7	-1.5	-1.2	-1.3	-1.7	1.6	-2.1
Il10ra	16154	1.2	1.2	-1.4	-1.3	-1.0	-1.8	1.2	-1.7	-1.0	1.4	-1.6
Il10rb	16155	1.4	1.7	1.3	1.6	1.3	1.5	1.7	1.7	1.8	1.5	1.5
Il11	16156	-1.8	-1.4	-3.6	-2.6	-2.0	2.1	-1.2	-1.5	-1.7	-1.0	1.9
Il11ra1	16157	1.1	2.5	1.0	1.4	1.6	-1.1	3.1	-1.4	-1.2	2.7	-1.0
Il12b	16160	-1.4	-2.2	-1.2	-2.6	-5.0	-2.5	-2.5	-2.2	-2.7	-1.7	-3.7
Il12rb1	16161	2.1	-1.1	-1.2	1.1	1.2	1.4	-1.7	-1.7	-2.2	-1.1	-2.1
Il13ra1	16164	1.2	-1.2	1.0	1.3	-1.6	-1.1	-1.8	1.3	-1.2	-1.5	1.3
Il13ra2	16165	-1.4	4.4	1.4	-1.9	1.2	-2.7	-1.1	1.9	-2.5	2.3	-3.9

Il15	16168	-1.9	4.7	-1.1	-1.6	1.3	-1.5	1.7	1.5	2.0	1.2	-2.8
Il15ra	16169	1.7	-2.2	-1.3	-1.2	-2.6	-1.5	-1.4	1.3	1.4	-1.0	1.0
Il16	16170	-1.2	-1.4	-1.9	-1.1	-1.3	-1.5	-1.6	-1.3	-1.2	-1.0	-2.3
Il17a	16171	-1.5	3.0	-1.4	3.9	-1.2	1.1	-1.0	-1.3	1.9	4.5	1.9
Il17d	239114	-1.1	-3.3	2.5	-1.5	-3.0	-1.2	1.3	-1.1	-1.4	-2.1	1.6
Il17rb	50905	1.4	2.5	-1.1	-2.3	-1.1	1.5	2.5	1.7	-1.9	3.5	1.5
Il17rc	171095	1.1	-1.2	1.4	1.2	1.0	1.0	-1.0	1.2	1.9	-1.0	1.2
Il17rd	171463	-1.0	5.9	1.1	-1.1	16.9	1.3	10.4	-1.0	1.6	3.6	1.3
Il17re	57890	-1.0	-1.3	1.3	1.2	-2.2	-1.0	1.0	-2.2	-1.3	1.1	1.1
Il18	16173	1.2	-1.2	1.1	-1.7	1.7	-1.1	1.3	1.2	-1.4	1.6	1.3
Il18bp	16068	1.2	-1.8	1.3	-1.3	-1.2	-1.0	-2.2	1.4	1.8	-1.5	1.6
Il18r1	16182	-1.4	2.0	-1.8	-2.0	1.2	-1.0	2.6	-1.6	-1.7	1.6	-1.8
Il1b	16176	2.6	1.7	1.0	-1.9	-1.3	-1.2	2.7	-1.8	-2.2	3.9	1.3
Il1f5	54450	-1.0	-1.3	-1.1	1.3	-1.7	1.1	-12	-1.1	-1.1	-1.2	-1.1
Il1r1	16177	1.3	1.9	1.0	1.1	1.2	1.2	1.9	-1.4	-1.2	2.1	-1.3
Il1r2	16178	2.0	-4.1	1.6	-2.1	-1.1	-1.9	1.0	-1.3	-4.0	1.5	1.2
Il1rap	16180	-1.9	-1.2	2.2	3.0	2.7	1.1	1.4	-1.4	-2.1	2.9	-1.7
Il1rapl2	60367	-1.6	-7.1	-2.3	2.4	1.1	-1.5	-1.6	-1.4	1.2	-1.3	1.2
Il1rl1	17082	-1.2	4.6	1.5	1.4	1.9	2.6	6.8	-1.3	-1.8	7.5	-1.3
Il1rl2	107527	1.3	-1.0	-1.4	-1.5	-9.6	1.2	-1.1	-1.1	-1.0	-1.0	1.1
Il1rn	16181	2.4	7.5	1.5	-1.0	2.2	2.1	4.3	-1.1	-1.6	5.5	1.0
Il2	16183	-1.2	-1.9	1.1	3.1	-1.5	1.5	-1.5	1.6	1.8	3.6	-1.4
Il20rb	213208	2.7	1.8	1.1	-1.5	5.2	1.1	2.2	1.3	1.1	3.5	1.6
Il21r	60504	1.1	-1.1	-1.0	1.6	1.2	-1.5	-1.5	-1.2	2.8	1.3	1.5
Il22	50929	1.6	-2.2	1.8	-1.6	1.8	3.0	1.6	8.3	-1.5	3.8	-1.0
Il22ra1	230828	-1.1	-1.4	1.1	1.3	1.4	1.5	-1.5	2.4	1.7	-1.7	1.9
Il22ra2	237310	-2.6	2.0	-2.6	1.4	1.2	-2.9	2.4	-1.2	-1.1	2.7	1.1

Il24	93672	1.7	-1.1	-1.5	-1.1	1.7	1.6	1.4	1.1	-1.1	-4.0	-1.9
Il27	246779	1.7	-1.2	-1.4	-1.0	-1.5	-2.3	-1.2	-1.0	1.7	1.1	1.1
Il27ra	50931	-2.3	-3.4	-2.5	-15.3	-1.7	-5.1	-5.6	-1.4	-2.5	-1.4	-1.7
Il2ra	16184	-1.1	1.3	-12	-2.3	-1.3	-2.4	1.2	-2.6	-4.1	2.2	-4.1
Il2rb	16185	1.5	1.9	1.3	1.7	1.7	1.3	1.9	1.5	1.6	3.0	1.1
Il2rg	16186	1.1	-3.6	-1.5	-1.6	-1.7	-1.5	-3.9	-1.5	-1.5	-2.0	-1.8
Il3	16187	1.0	-1.2	1.0	1.2	-1.5	-1.1	-1.2	-1.2	-1.3	-14.9	-2.1
Il33	77125	1.8	4.2	1.1	-1.4	2.6	1.3	5.0	1.2	-2.1	10.1	1.5
Il3ra	16188	1.4	1.2	1.1	1.4	1.1	1.5	-1.1	1.3	1.1	1.3	1.4
Il4	16189	1.1	1.3	1.3	-1.2	1.2	-1.6	1.2	-1.1	-1.4	2.1	-1.2
Il4i1	14204	1.1	-1.3	-1.4	-1.5	-1.1	-2.3	-1.0	-2.1	-1.8	1.4	-4.0
Il4ra	16190	-4.0	-1.1	-3.0	-1.9	-1.4	-3.0	-1.2	-1.7	-1.7	-2.3	-3.6
Il5ra	16192	1.0	6.5	1.2	1.2	8.6	-1.0	1.6	2.8	1.7	1.1	-1.5
Il6	16193	2.2	1.6	3.7	-1.2	1.6	3.2	2.3	-1.1	-1.6	10.7	3.4
Il6ra	16194	1.5	-1.4	-1.2	1.7	1.4	1.4	-1.5	1.2	1.4	-1.7	1.7
Il6st	16195	-1.0	1.2	-1.2	1.1	1.1	-1.1	1.4	-1.9	-1.6	1.3	-1.4
Il7	16196	1.4	2.3	1.3	2.0	6.3	-1.1	2.9	-1.4	1.3	3.8	2.1
Il7r	16197	1.1	1.9	1.5	-1.3	2.2	1.1	2.8	-1.9	1.4	4.6	1.1
Il9r	16199	-1.0	1.6	-1.6	-1.2	1.8	1.0	1.8	-1.1	-1.0	2.7	-1.1
Ing2	69260	-1.2	7.9	1.0	1.5	6.4	1.2	5.4	3.1	1.3	7.1	-1.6
Ing3	71777	2.3	5.9	2.0	1.8	4.2	1.6	4.2	2.0	1.6	12.3	1.6
Irf1	16362	1.0	2.1	1.2	-1.0	1.4	-1.5	1.7	-1.3	1.6	2.8	1.1
Irs1	16367	1.2	2.8	1.3	1.7	5.1	1.5	3.7	-1.1	1.3	4.2	-1.0
Itga1	109700	-1.6	-3.8	-1.0	1.0	-1.3	-1.1	-1.9	-1.3	1.0	-2.5	-1.5
Itga10	213119	2.2	5.4	1.6	3.1	3.5	3.7	7.6	5.8	1.7	5.5	1.8
Itga11	319480	-1.3	1.9	-1.5	-1.0	1.4	-1.1	1.7	1.1	1.2	1.7	1.4
Itga3	16400	1.1	-1.1	1.4	2.0	-2.0	2.1	-1.3	1.5	1.8	-1.8	1.9

Itga4	16401	-1.4	2.6	-2.0	-1.7	1.2	-1.0	1.1	-1.2	-1.3	1.9	-1.2
Itga5	16402	-1.1	1.1	2.0	-1.5	-2.0	1.5	2.0	-2.0	-1.2	1.3	1.2
Itga6	16403	1.4	1.9	1.0	-1.2	1.5	-1.1	1.4	-1.1	-1.0	1.6	-1.2
Itga7	16404	1.0	-1.2	-1.1	1.1	-1.4	-1.4	1.3	-2.2	-1.4	-1.4	-1.7
Itga8	241226	1.1	4.7	1.5	1.1	2.3	1.5	4.6	1.8	-1.0	4.4	1.0
Junb	16477	1.3	-2.2	2.3	4.1	2.5	6.3	-1.8	4.0	2.5	-2.0	2.9
Jund	16478	-1.2	-3.0	-1.0	1.6	1.1	-1.1	-1.9	1.0	1.7	-2.6	-1.1
Lrr1	69706	1.4	1.8	1.0	1.3	1.5	1.2	2.3	1.5	1.2	2.2	1.2
Lrrc17	74511	1.3	8.2	1.1	1.7	2.8	1.6	4.9	1.1	1.3	8.7	1.1
Lrrc24	378937	1.0	-1.1	2.5	1.6	1.5	1.7	-1.1	2.2	3.2	-1.7	1.5
Lrrc26	227618	1.4	-2.2	1.4	1.9	-1.0	1.2	-1.8	1.2	1.5	-2.1	1.4
Lrrc32	434215	1.7	2.8	-1.0	1.5	3.2	2.3	2.6	2.9	2.1	2.2	3.4
Lrrc38	242735	-2.2	1.4	1.1	4.3	5.6	1.3	1.8	2.8	1.8	1.0	2.5
Map2k3	26397	1.3	-1.3	1.5	1.6	-1.0	1.3	-1.6	1.5	1.8	-1.6	1.4
Map2k4	26398	-1.7	1.7	1.3	1.8	5.4	1.4	-1.0	1.6	1.6	-1.3	1.3
Map2k5	23938	1.8	2.5	1.5	-1.2	1.6	-1.7	-1.6	-4.3	-1.7	1.0	1.5
Map3k1	26401	1.0	1.4	-1.2	1.0	1.9	1.1	1.3	-1.1	1.2	1.4	-1.0
Map3k10	269881	4.0	1.3	1.2	-1.4	-2.5	3.4	3.3	2.9	-1.7	1.7	-1.0
Map3k11	26403	1.2	-1.8	1.3	1.6	1.1	1.6	-2.1	1.5	-1.0	-2.4	1.4
Map3k15	270672	1.6	-1.4	1.0	-1.4	-4.1	1.0	1.3	2.1	1.0	-1.4	-1.5
Map3k2	26405	1.2	4.8	-1.2	1.0	1.8	-1.1	3.8	1.3	1.3	5.7	1.5
Map3k4	26407	-1.1	2.6	-1.0	1.3	1.7	-1.2	2.5	-1.4	1.5	2.5	1.1
Map3k6	53608	1.1	-1.3	1.5	1.3	1.8	1.5	-1.0	2.7	2.2	-1.3	2.6
Map3k7	26409	-1.1	-2.3	-1.2	1.9	9.1	1.6	-1.4	1.5	-1.3	-2.0	1.4
Map4k5	399510	1.1	4.3	1.1	1.3	2.0	-1.1	5.3	-1.6	-1.0	3.9	1.0
Mapk10	26414	1.1	2.3	1.3	1.4	2.1	1.0	4.6	1.1	1.1	4.2	-1.0
Mapk12	29857	1.0	1.2	1.5	-2.3	1.4	1.5	1.3	-1.3	-2.9	1.2	1.5

Mapk14	26416	1.3	2.5	1.3	1.7	1.8	1.3	3.3	1.2	1.6	2.5	1.6
Mapk1ip1	69546	-6.0	3.2	-1.9	-1.0	2.5	-1.8	-5.1	-1.2	-1.6	-1.2	-1.1
Mapk8	26419	-1.2	6.9	-1.5	-1.2	-1.9	1.2	5.3	1.1	1.9	6.7	1.4
Mapk9	26420	3.7	1.0	3.3	2.7	2.1	-1.0	-1.0	-1.2	1.6	1.1	4.3
Mapkap1	227743	1.5	1.5	1.7	2.2	-1.3	2.6	1.1	2.3	3.7	-1.0	1.6
Mapkbp1	26390	1.6	-1.3	-3.1	-1.2	1.9	1.1	-2.4	2.0	1.9	-1.1	2.5
Mapre2	212307	-1.0	1.9	-1.1	1.3	1.1	-1.1	1.9	-1.0	-1.0	2.5	-1.0
Mmp11	17385	-1.3	1.5	-1.2	-2.1	1.7	-1.0	2.2	1.6	-1.2	1.3	1.6
Mmp12	17381	1.2	-1.0	4.6	1.9	-1.7	1.3	2.0	1.2	1.1	1.1	1.0
Mmp13	17386	-1.2	4.3	1.2	-7.0	1.9	-1.5	4.1	-1.6	-1.1	7.8	-1.2
Mmp14	17387	1.5	-2.1	-1.4	1.2	-2.0	3.3	-1.2	1.3	6.1	-2.4	1.8
Mmp23	26561	-1.4	-3.2	-1.4	-2.4	-4.3	-1.7	-5.3	-1.7	6.2	-3.1	-2.0
Mmp3	17392	1.9	1.9	2.5	-1.9	-1.8	2.3	1.9	-3.0	-2.3	7.4	1.7
Mmp7	17393	1.5	1.4	1.0	1.5	1.8	-1.3	1.0	1.7	1.7	1.0	1.3
Mmp8	17394	1.1	1.1	1.0	1.6	-1.2	-1.3	1.0	-1.1	1.1	3.2	4.8
Muc16	73732	3.0	5.1	1.6	1.7	2.7	2.7	3.3	1.5	-1.2	3.0	1.2
Muc20	224116	-1.2	1.3	-1.4	-1.7	-1.5	-1.1	1.3	-2.3	-2.0	1.4	1.4
Muc5b	74180	1.1	-1.3	-1.1	6.2	-1.2	1.5	-1.3	1.0	1.2	-1.2	-1.2
Nfkb1	18033	1.9	-2.9	2.6	1.3	-1.7	1.3	-2.6	3.8	1.8	-2.5	2.7
Nfkb2	18034	1.0	-2.2	1.1	1.1	-1.3	1.3	-1.5	-1.1	1.0	-1.8	1.1
Nfkbib	18036	1.2	-1.8	1.0	1.2	1.4	1.3	-1.6	1.5	1.5	-1.8	1.2
Nfkbid	243910	1.2	-2.1	-1.3	1.1	1.5	1.0	-1.7	1.3	1.2	-1.5	-1.1
Nfkbie	18037	1.3	-1.1	1.0	-1.4	-1.6	-1.1	1.2	1.2	-1.1	1.1	-1.2
Nfkbiz	80859	-3.8	-4.0	-1.3	-1.6	-2.2	-1.1	-3.9	-1.3	-2.0	-3.6	1.2
Nfrkb	235134	-1.1	1.1	-1.3	-2.0	-1.2	-1.7	-1.2	-1.3	-1.4	-1.6	1.0
Nlrc3	268857	1.2	-1.7	-2.4	-1.8	-1.3	-2.0	-1.3	1.3	-1.2	-1.2	-1.3
Nlrc4	268973	-1.5	-2.2	1.1	1.3	-1.7	1.2	-2.8	1.1	1.0	-2.5	1.1

Nlrc5	434341	1.1	-2.7	1.1	-5.2	2.8	-1.2	-1.2	1.3	-5.7	1.7	1.1
Pi4k2a	84095	1.2	-1.9	-1.2	-1.1	-1.3	-1.2	-1.6	1.1	1.5	-1.7	1.2
Pi4k2b	67073	1.0	2.6	-1.1	1.3	1.8	1.2	1.7	1.5	1.9	1.7	1.4
Pi4ka	224020	-1.3	1.9	-1.2	-1.3	1.2	-1.3	1.7	-1.5	1.4	1.5	-1.3
Pi4kb	107650	-1.1	-4.7	-1.1	-1.0	-1.3	1.3	-3.7	1.6	1.2	-3.7	1.1
Pik3c2b	240752	-2.1	1.1	-1.5	-1.9	2.0	-1.0	-1.5	-1.1	-1.1	-5.8	-1.4
Pik3c2g	18705	-1.1	2.3	1.7	2.7	4.7	3.1	-1.6	-1.9	-1.7	1.5	2.2
Pik3ca	18706	-1.1	1.2	1.9	2.6	-1.6	1.3	2.2	-1.1	1.2	3.6	1.7
Pik3ip1	216505	2.0	-1.0	1.3	1.3	-1.1	1.8	-1.5	-1.1	1.9	-1.0	1.3
Pik3r1	18708	1.5	-2.0	1.1	1.2	1.1	1.5	-2.8	1.5	1.5	-1.9	1.6
Pik3r2	18709	1.8	-1.2	1.6	1.9	1.3	2.1	-1.3	1.9	1.7	-1.6	1.7
Pik3r3	18710	1.3	1.9	1.3	1.3	1.1	-1.1	2.8	-1.7	-1.1	2.3	-1.1
Pilra	231805	4.2	3.1	2.2	5.0	7.5	1.2	4.4	4.5	3.3	3.0	4.0
Pilrb1	170741	2.9	2.4	3.1	-1.7	8.0	-1.2	1.9	1.3	-1.6	1.2	1.9
Plcg1	18803	1.1	1.1	1.2	-1.2	10.8	1.1	1.6	-1.2	1.3	1.2	1.3
Plcl1	227120	1.3	1.5	-1.5	-1.4	3.2	-2.4	1.8	1.0	2.0	2.2	1.4
Plcl2	224860	1.4	2.4	1.4	1.2	1.9	1.3	2.1	1.0	1.6	2.6	1.4
Plcxd2	433022	-1.2	1.4	-1.2	1.6	1.8	1.0	1.0	1.6	1.1	-1.1	1.4
Pld3	18807	1.0	1.3	-1.0	-1.1	1.2	1.1	2.3	-1.2	-1.2	1.5	-1.0
Pld6	194908	10.2	1.7	2.1	-1.3	1.3	1.2	3.0	1.1	1.2	-1.2	1.3
Ppp1cb	19046	-1.3	3.9	-1.2	1.0	2.0	-1.5	4.2	-1.9	1.3	3.5	-1.5
Ppp1r12a	17931	-1.1	1.3	1.5	2.0	1.7	1.3	-1.1	1.5	1.7	1.9	1.2
Ppp1r12b	329251	-1.3	-5.4	-1.3	-1.3	1.0	29.1	-3.1	-1.7	-1.0	-1.6	-1.5
Ppp1r13l	333654	1.2	1.6	1.8	1.7	1.5	1.7	1.9	1.5	-1.1	2.1	1.5
Ppp1r2	66849	-1.0	1.4	1.4	-1.2	2.4	1.1	9.5	1.9	1.6	2.0	1.5
Ppp1r21	73825	1.7	7.1	2.0	-1.0	9.4	3.0	5.6	2.8	1.5	6.9	1.9
Ppp1r26	241289	-2.1	6.5	1.3	2.0	4.9	2.4	1.6	1.6	-1.5	7.2	2.0

Ppp1r3c	53412	-2.1	2.9	-1.3	-1.3	-1.3	-1.4	3.7	-2.8	-1.5	1.1	-2.8
Ppp1r3d	228966	1.3	2.5	1.5	1.5	1.9	-1.6	5.0	-1.4	-1.5	5.5	-1.0
Ppp5c	19060	3.2	-1.3	-1.4	4.4	1.4	1.6	13.0	2.8	-1.3	1.2	1.1
Prkar1b	19085	1.0	1.7	-1.0	1.4	1.9	1.5	2.5	-1.1	-1.1	1.4	1.0
Prkar2b	19088	-1.1	2.3	1.8	1.2	-1.1	1.3	2.9	-1.7	-1.7	1.3	-1.2
Prkcb	18751	1.1	1.1	-1.6	-1.7	1.5	-1.6	4.0	-1.9	-1.3	6.4	-2.4
Ptger1	19216	-1.2	-1.3	-1.2	-2.1	4.8	-1.3	1.0	-1.6	-3.3	-1.7	-1.6
Ptger3	19218	11.0	3.6	2.3	1.8	2.4	2.2	4.0	1.4	1.4	2.6	1.5
Ptger4	19219	-1.1	-2.6	1.4	1.4	-1.5	22.6	-2.2	1.8	1.5	-2.3	-1.1
Ptgfr	19220	4.6	1.9	-1.3	1.2	-1.4	-1.6	1.3	1.4	1.4	-1.1	-1.5
Ptgir	19222	1.1	5.7	1.2	1.3	3.0	1.2	4.5	-1.4	-1.9	6.3	1.1
Ptgis	19223	1.7	2.0	1.2	1.4	1.1	1.0	1.7	1.3	1.5	2.0	-1.0
Ptgr1	67103	1.2	4.6	1.2	1.3	2.0	1.8	2.9	2.3	2.6	2.8	1.4
Ptgr2	77219	-3.1	1.4	-1.0	-1.5	2.9	-1.1	2.0	2.5	-3.6	2.2	-1.5
Ptgs1	19224	1.2	1.6	1.1	1.3	2.0	1.5	1.5	1.1	1.3	1.3	1.3
Ptgs2	19225	1.1	2.5	1.3	1.3	2.7	2.6	2.8	1.3	-1.3	2.1	-1.1
Rasa1	218397	3.1	1.6	2.2	4.0	1.5	7.4	1.6	-1.3	1.6	1.6	2.0
Rasa2	114713	-1.9	1.5	-1.2	1.0	1.1	1.1	1.8	-1.5	-1.0	19.2	-1.3
Rbl1	19650	-1.8	6.6	1.5	-1.4	2.3	1.6	2.6	1.1	1.6	5.6	-1.3
Rela	19697	-1.5	1.1	-1.7	-1.6	-1.3	-1.3	-1.0	-2.2	-1.2	1.0	-1.6
Relb	19698	-1.0	-1.2	1.1	1.6	1.3	-1.0	-1.0	1.6	1.7	-1.3	1.7
Slc10a2	20494	1.5	-2.6	-1.1	2.0	-2.0	-1.7	-3.6	1.2	4.3	-1.2	1.8
Slc10a6	75750	1.6	5.7	1.8	1.6	8.2	3.5	2.7	-1.5	-1.6	7.7	1.6
Slc10a7	76775	-2.1	-1.2	2.1	-1.1	1.9	-2.2	-3.1	-1.9	2.0	1.1	-3.2
Slc11a1	18173	1.2	1.3	-1.0	-1.1	1.8	1.6	-1.1	2.5	1.6	1.4	1.8
Slc13a1	55961	1.1	1.0	1.1	1.4	-1.2	1.1	-1.9	2.6	3.6	1.0	2.3
Slc13a2	20500	1.2	1.7	1.5	1.4	1.6	1.0	1.7	1.5	2.8	2.1	1.3

Slc13a3	114644	1.3	1.6	3.9	1.4	-1.3	1.2	-1.1	1.1	1.6	2.1	1.3
Slc14a1	108052	-1.1	2.6	-1.2	-1.5	1.4	-1.5	3.9	2.1	-1.2	3.9	-2.0
Slc15a3	65221	2.9	-2.9	2.2	1.0	-1.7	-1.5	-4.7	2.0	1.4	-1.8	1.4
Slc15a4	100561	1.2	1.8	-1.1	1.4	1.5	-1.2	2.6	-1.3	1.3	2.0	-1.1
Slc16a1	20501	1.4	2.9	-1.1	1.6	5.3	2.1	3.3	4.0	-2.1	1.7	-1.5
Slc16a10	72472	-1.1	1.8	1.2	-1.7	1.9	1.4	1.7	2.3	1.5	-1.2	-1.0
Slc16a11	216867	1.6	1.9	1.6	1.7	2.4	2.0	2.2	1.6	2.6	1.4	1.5
Slc16a12	240638	1.1	5.0	1.9	-1.9	6.2	1.8	5.7	1.7	-2.2	1.8	1.4
Slc18a1	110877	2.2	6.4	2.4	2.1	4.2	2.4	4.3	2.7	2.6	6.6	2.2
Slc18a3	20508	1.7	1.7	-1.1	1.2	2.5	1.0	2.4	1.2	1.2	2.4	1.5
Slc19a2	116914	1.4	5.2	1.5	1.5	2.3	1.3	4.4	1.9	1.5	2.8	1.3
Slc1a1	20510	1.2	-2.4	1.1	1.4	-1.0	-1.1	-2.0	1.8	1.6	-2.2	1.6
Slc1a3	20512	2.7	3.7	3.1	2.6	4.4	-1.2	7.1	1.4	-1.2	8.1	1.5
Slc1a4	55963	1.9	-3.7	1.0	-1.3	-3.1	-1.8	-1.7	1.1	1.4	-2.2	-1.2
Slc1a6	20513	1.6	3.9	2.6	-1.7	-1.2	1.4	3.8	1.1	2.5	4.8	3.1
Slc1a7	242607	1.2	6.2	-1.0	1.6	3.9	3.8	11.0	2.7	2.6	7.4	1.7
Slc22a13b	109280	-1.3	7.2	1.1	-1.3	2.1	4.7	4.7	3.1	-1.0	1.7	1.8
Slc23a1	20522	-1.7	6.3	2.9	1.9	9.4	4.5	1.3	10.8	-1.2	1.7	2.3
Slc23a2	54338	1.1	3.1	1.5	1.9	2.8	1.8	4.3	1.5	1.4	3.1	1.4
Slc23a3	22626	1.8	2.8	1.5	1.2	1.2	1.3	1.3	-1.1	1.1	2.1	1.2
Slc24a2	76376	1.4	1.3	1.3	2.0	1.1	1.4	-3.8	1.7	3.1	1.3	1.6
Slc25a16	73132	1.5	1.2	1.3	1.1	2.1	1.5	5.7	1.1	1.7	4.4	1.5
Slc25a17	20524	-1.1	2.2	-1.1	-1.5	1.5	-1.6	2.3	-1.7	-1.2	2.3	-1.6
Slc25a22	68267	-1.4	3.9	3.0	3.1	3.4	2.0	6.1	1.2	2.2	6.2	1.6
Slc25a27	74011	1.8	5.0	3.1	2.2	10.0	2.2	9.3	-1.2	1.7	4.3	4.3
Slc28a1	434203	1.2	3.5	1.2	1.4	1.7	2.7	4.0	2.5	-1.6	1.0	-1.1
Slc29a2	13340	1.5	6.4	3.0	5.8	5.2	4.6	10.9	5.3	1.3	1.5	3.4

Slc2a2	20526	1.2	202.2	-1.2	1.8	40.2	22.1	11.2	18.3	5.1	10.7	5.1
Slc2a5	56485	6.7	10.7	2.0	2.7	6.1	4.2	2.6	13.4	8.1	3.2	5.6
Slc34a3	142681	2.2	18.3	2.6	5.6	7.3	12.9	11.5	11.5	3.2	4.2	6.8
Slc35d2	70484	1.1	12.4	2.0	1.5	5.6	1.4	7.6	2.2	1.8	8.0	1.6
Slc35g2	245020	-2.1	1.3	-1.2	-2.9	5.3	-2.5	3.7	-2.8	-1.5	1.4	-1.9
Slc39a10	227059	-1.2	1.5	1.5	1.6	7.0	1.4	1.5	-1.0	1.1	2.1	1.1
Slc39a13	68427	1.1	1.9	1.0	1.0	1.4	-1.2	2.7	-1.2	-1.0	2.7	-1.0
Slc43a1	72401	1.1	3.6	1.1	-1.1	1.9	1.3	3.3	1.1	-1.4	2.6	1.1
Slc43a2	215113	1.1	-1.2	1.4	1.2	1.7	2.1	-1.8	2.0	2.0	-1.7	1.9
Slc46a1	52466	1.1	1.3	1.7	1.4	1.8	1.9	1.1	2.3	1.9	1.2	1.9
Slc46a3	71706	1.1	1.9	1.3	1.9	1.4	1.7	2.0	2.3	3.6	2.0	2.2
Slc47a1	67473	1.2	3.7	-1.3	1.3	2.2	2.3	2.1	4.0	2.7	2.0	2.4
Slc4a10	94229	1.1	1.5	2.1	2.2	1.3	-1.1	1.7	2.4	1.6	1.5	2.2
Slc4a11	269356	2.3	1.2	3.4	1.0	-1.4	4.8	5.5	1.1	-2.5	9.3	1.5
Slc5a4a	64452	1.2	7.4	1.8	2.4	2.4	6.9	5.9	22.8	7.9	3.0	2.9
Slc6a18	22598	3.3	7.0	-1.1	1.5	4.8	2.1	3.4	3.8	5.3	3.5	3.9
Slc6a19	74338	1.7	3.3	1.4	1.3	3.0	1.6	1.8	3.8	4.9	1.7	2.2
Slc6a20a	102680	1.0	2.2	1.3	2.9	1.4	2.4	1.8	3.8	3.9	1.3	3.4
Slc6a3	13162	1.8	32.5	1.0	2.5	13.6	2.1	2.4	2.8	1.5	1.9	-1.5
Smad2	17126	-1.2	2.3	1.2	1.2	1.5	1.2	1.7	-1.2	-1.1	1.7	1.2
Smad3	17127	1.1	1.8	1.1	1.2	1.7	1.2	1.6	1.1	1.7	1.5	1.4
Smad6	17130	1.4	1.9	1.4	2.5	2.8	2.1	1.8	2.7	2.2	2.5	1.4
Smad7	17131	-2.0	2.8	-1.5	-1.0	4.6	2.2	2.6	1.4	3.6	2.7	1.0
Smad9	55994	-2.2	6.1	1.2	-1.3	2.3	2.2	3.0	-1.0	1.2	2.1	-1.1
Smarca1	93761	-2.8	4.2	-3.2	-1.0	1.2	1.1	1.6	-1.3	-2.7	2.4	1.0
Smarca2	67155	-1.4	1.7	-1.4	-1.3	1.3	-1.1	2.4	-1.6	-1.1	2.1	-1.1
Smarca4	20586	-1.4	-5.7	1.9	1.7	-3.8	-1.2	-7.6	1.5	1.8	-6.3	1.2

Stat1	20846	1.4	4.0	-1.1	1.8	3.2	1.3	4.1	2.5	-2.1	2.4	2.5
Stat2	20847	-1.2	1.5	-1.1	-1.0	1.5	-1.3	2.3	1.0	1.0	2.4	1.0
Stat4	20849	1.3	1.4	1.3	1.3	2.9	2.4	2.9	1.8	1.6	2.9	1.3
Tgfa	21802	-1.2	1.2	1.5	-1.4	1.2	-1.3	1.8	1.6	-1.4	1.5	1.2
Tgfb1	21803	1.0	-1.8	-1.0	-1.1	-2.2	-1.1	-1.6	1.3	-1.7	-1.5	1.1
Tgfb1i1	21804	-1.1	-2.6	1.1	1.1	-1.1	1.3	-2.3	-1.0	1.3	-2.9	-1.2
Tgfb2	21808	-2.9	2.5	-1.7	-7.8	1.8	-1.5	2.1	-3.4	-1.5	1.4	-1.8
Tgfbr1	21812	-1.2	1.7	1.4	-1.4	2.0	1.7	1.2	1.4	-2.3	1.1	1.0
Tlr12	384059	1.0	2.0	-1.3	2.2	2.5	1.5	1.7	1.7	1.8	1.8	1.5
Tlr13	279572	1.5	2.2	1.1	1.4	2.0	1.4	1.8	1.0	1.4	3.3	1.8
Tlr4	21898	-1.2	1.1	-1.1	-1.0	-1.7	-2.7	1.4	-2.2	-2.3	1.6	-1.3
Tlr6	21899	-1.0	4.5	-1.6	-1.4	2.1	1.5	2.1	-1.1	1.0	1.5	1.3
Tlr7	170743	2.2	7.1	1.4	1.4	2.9	1.3	3.7	-1.0	1.3	3.1	1.6
Tlr9	81897	1.9	-1.5	-1.6	-1.2	-1.6	-2.4	1.1	-1.1	1.4	-1.1	-1.3
Tmem106a	217203	2.2	1.2	4.5	2.6	3.0	3.3	1.4	4.7	4.1	1.1	3.3
Tmem108	81907	1.7	1.3	-1.6	-1.5	4.1	-1.6	1.4	1.3	2.3	-1.5	-1.3
Tmem116	77462	2.8	4.3	3.3	1.5	9.5	2.7	3.1	6.3	2.3	2.1	3.6
Tmem117	320709	-1.3	2.6	-1.0	1.7	2.6	-1.0	2.2	-1.1	1.7	1.7	1.1
Tmem121	69195	1.6	3.0	1.1	-1.1	2.1	1.6	5.4	2.0	1.3	9.8	-2.1
Tnfaip3	21929	-1.0	1.8	1.3	1.1	1.1	1.1	2.0	-1.0	-1.1	2.4	-1.1
Tnfaip6	21930	1.9	5.4	-2.4	2.4	4.9	-1.1	6.3	1.8	-2.0	3.2	1.5
Tnfaip8l3	244882	-1.4	1.0	2.0	-2.5	1.1	1.2	-1.8	2.0	1.8	2.3	1.0
Tnfrsf10b	21933	-1.1	-1.1	-1.0	-1.0	1.3	1.3	1.7	1.1	-1.1	-1.0	-1.1
Tnfrsf13b	57916	1.1	1.5	-1.6	-1.4	1.2	1.0	1.2	-1.3	1.0	2.5	-1.4
Tnfrsf13c	72049	1.5	-1.4	-5.2	-1.3	-1.6	-1.8	-1.0	-1.7	-1.9	2.3	-5.1
Tnfrsf17	21935	1.1	2.3	1.5	1.3	-1.1	1.9	1.7	1.2	1.4	2.9	-1.1
Tnfrsf18	21936	1.4	-1.0	-1.5	-1.1	-1.1	-1.1	1.0	-1.6	-1.7	2.0	-1.3

Tnfrsf19	29820	-2.7	2.6	-1.4	1.0	1.9	-1.2	2.4	-1.0	-1.1	1.5	-1.4
Tnfrsf1b	21938	-1.1	1.8	-1.3	-1.6	-1.0	-1.5	1.9	-2.1	-1.4	2.1	-1.5
Tnfrsf23	79201	1.1	1.5	1.2	-1.0	1.4	-1.4	1.7	-2.3	-1.9	1.5	1.1
Tnfrsf25	85030	1.2	3.1	1.0	1.2	1.2	-1.4	3.0	1.1	-1.2	3.1	1.1
Tnfrsf9	21942	1.8	1.4	-1.5	-5.1	-1.2	1.0	2.6	-1.3	-2.5	2.5	-1.4
Tnfsf10	22035	1.3	2.9	-1.1	-1.1	2.0	-1.1	1.5	1.1	1.1	2.4	1.2
Tnfsf13b	24099	-1.1	2.1	-1.0	1.3	1.3	1.6	1.5	1.4	1.4	2.0	1.4
Tnfsf14	50930	-1.3	2.7	1.2	-1.9	-2.1	-1.1	-1.7	-1.0	-4.0	3.7	-1.3
Tnfsf9	21950	-1.7	4.3	-1.3	-1.7	1.7	1.3	4.5	1.8	-1.2	1.9	1.8
Tnik	665113	1.2	2.2	1.2	1.7	2.1	1.2	2.0	1.8	1.9	1.8	1.8
Tnip1	57783	-1.6	1.8	-1.1	-1.5	1.3	-1.2	1.7	-1.5	1.8	1.9	-1.5
Traf4	22032	1.3	1.8	1.1	1.6	1.3	-1.0	1.5	1.3	1.5	1.8	1.5
Traf5	22033	1.0	-1.0	-1.5	-1.2	-1.1	-1.3	1.7	-1.3	-1.2	1.7	-1.7
Traf6	22034	1.3	1.4	-1.0	2.3	1.6	2.1	1.9	-1.3	2.1	-2.7	1.0

Annexure 5: Differentially expressed genes in C57BL/6 following probiotics treatment

Gene Symbol	Entrez Gene ID	D10 STLA	D3 BC	D3 LA	D3 ST	D3 STBC	D3 STLA	D5 BC	D5 LA	D5 ST	D5 STBC	D5 STLA
Acvr1c	269275	2.9	-1.1	-1.7	-1.1	-1.3	1.6	6.2	4.1	-1.6	1.8	-10.9
Acvr2b	11481	1.0	1.2	1.0	-1.0	1.1	1.0	1.6	1.3	1.0	1.1	1.1
Acvrl1	11482	1.2	1.3	-1.1	-1.1	1.4	1.2	1.2	1.6	-1.2	1.3	1.1
Acyp2	75572	2.2	-1.6	-1.1	-2.1	-1.3	-1.7	-2.5	-2.5	-1.6	2.3	1.0
Adcy1	432530	1.5	-1.2	2.8	-1.4	-1.3	2.2	1.5	2.6	-2.2	1.8	2.8
Adcy2	210044	1.3	1.7	2.3	1.8	-1.4	1.7	2.4	2.4	3.8	2.6	1.6
Adcy4	104110	1.7	1.7	1.5	-1.2	1.4	1.1	-1.6	-1.1	1.3	-1.1	3.3
Adcy5	224129	3.1	1.4	-2.0	1.8	3.0	2.2	5.8	2.4	-1.0	2.4	1.6
Adcy6	11512	-1.1	1.0	-1.1	1.4	1.8	1.3	-1.0	-1.1	-1.0	-1.3	-1.1
Adcy8	11514	1.3	4.3	2.1	1.8	-2.5	1.6	2.0	-1.9	1.5	2.4	1.9
Adcyap1r1	11517	2.7	-1.2	2.8	1.3	6.1	2.1	3.1	5.0	3.8	5.6	2.5
Akt2	11652	2.0	1.6	-1.2	1.4	2.4	1.4	1.5	1.4	2.9	2.0	1.1
Akt3	23797	1.0	-2.4	-1.2	1.9	1.6	2.0	-1.8	-1.4	1.8	-2.4	-1.5
Aldh1l2	216188	11.4	6.8	56.5	6.0	4.7	3.4	18.4	18.5	17.7	32.3	15.6
Anapc1	17222	1.4	1.2	1.2	-1.0	-1.1	-1.1	1.3	1.5	1.4	1.5	1.2
Anapc15	75430	1.1	-1.0	1.1	1.2	1.0	1.0	1.1	1.2	1.7	1.4	1.0
Anapc16	52717	1.2	-1.0	-1.0	1.1	1.2	1.1	1.3	1.2	2.0	1.3	-1.0
Apaf1	11783	2.5	1.9	1.4	1.3	-1.0	-1.0	1.7	1.4	1.2	3.5	1.1
Bad	12015	-1.0	-1.0	-1.1	-1.1	-1.1	-1.0	-1.2	-1.2	-2.6	-1.4	-1.1
Bcl10	12042	1.4	1.3	1.5	1.2	1.1	1.2	1.8	1.4	1.7	1.7	1.2
Bcl11a	14025	1.6	1.7	2.7	3.0	1.9	1.3	-1.5	5.8	2.7	1.7	-1.9
Bcl11b	58208	1.5	1.3	1.1	-1.1	-1.5	1.1	1.8	1.9	-2.4	1.2	1.1

Bcl2	12043	-1.7	1.5	1.0	-1.0	1.6	1.4	1.4	-1.1	1.4	-1.0	-1.3
Bcl2a1c	12046	1.3	-1.2	-1.3	-2.0	-1.2	-1.2	-1.2	-1.4	2.5	-1.5	1.0
Bcl2l1	12048	-4.2	-2.9	-3.7	2.0	1.2	-1.2	-5.9	-10.7	-2.0	-3.5	-3.6
Bcl2l11	12125	4.5	3.1	4.9	1.9	2.3	3.7	3.1	2.9	9.1	3.6	1.6
Bcl2l12	75736	2.1	1.9	2.0	1.1	1.0	-1.0	1.8	2.1	1.7	2.3	1.7
Bcl2l15	229672	1.2	-1.0	-1.5	2.0	-1.0	1.2	-1.8	-1.8	1.9	1.0	1.1
Bcl7a	77045	1.7	1.4	-1.6	-1.7	-2.2	-1.7	1.3	2.1	-3.9	1.4	-1.1
Birc5	11799	-1.3	2.5	2.4	1.8	1.3	3.7	2.7	-1.5	2.3	-1.3	2.3
Birc6	12211	1.9	1.0	1.1	-1.4	-2.0	-1.7	1.7	2.1	1.8	1.6	1.5
C1galt1	94192	1.1	-1.1	1.6	1.2	1.0	-1.1	1.1	1.0	2.1	1.2	1.1
C1galt1c1	59048	1.8	1.1	1.3	1.2	-1.3	1.0	1.6	1.5	1.2	1.6	1.2
C1qa	12259	1.2	-1.0	-1.0	1.2	2.0	1.3	-1.1	1.1	1.9	-1.1	-1.2
C1qb	12260	-1.8	-1.4	-1.4	1.1	2.3	1.3	-1.7	-1.1	1.7	-1.8	-1.5
C1qbp	12261	1.4	1.2	1.0	1.2	1.1	-1.0	1.4	1.4	1.6	1.9	-1.1
C1ql2	226359	1.2	-2.4	-1.2	2.4	-1.3	-3.5	1.5	-1.1	-1.8	1.2	2.8
C1qtnf1	56745	2.0	2.3	2.0	-1.0	2.1	1.6	4.5	1.7	1.5	2.3	4.2
C1qtnf2	69183	2.7	2.4	1.7	1.7	2.7	3.6	2.0	2.7	2.4	2.0	1.5
C1qtnf4	67445	-1.1	-1.1	-1.0	1.1	1.5	-1.2	-1.0	-1.6	1.8	-1.3	-1.3
C1qtnf5	235312	1.2	1.1	1.0	1.9	3.2	2.3	1.2	-1.1	1.1	1.2	-1.1
C1qtnf6	72709	-2.4	-1.2	1.5	3.1	2.9	3.7	1.1	1.1	-1.4	-2.2	3.2
C1qtnf7	109323	1.0	-1.0	1.3	1.1	1.6	1.4	1.4	1.1	1.7	-1.1	-1.2
C1ra	50909	1.9	1.3	1.4	1.4	-1.1	1.8	1.2	1.8	6.9	1.6	1.9
C1rb	667277	-2.9	-3.4	-2.9	1.8	2.3	1.8	-3.9	-2.7	1.6	-3.6	-4.1
C1rl	232371	3.0	1.8	2.3	-1.4	-1.1	-1.1	4.5	4.3	2.1	5.7	1.7
C1s	50908	1.5	1.2	1.2	1.5	2.1	2.3	1.2	1.5	4.5	1.3	1.3
C2cd2	207781	1.0	1.5	1.3	1.8	1.5	1.4	1.0	1.1	1.2	1.3	1.3
C2cd4a	244911	-1.3	1.1	2.9	3.6	2.9	2.9	-1.3	1.2	4.2	2.2	1.5

C2cd4b	75697	-1.7	-1.7	2.0	3.1	2.5	2.1	-1.8	1.1	5.4	2.5	-1.2
C3	12266	1.4	1.3	1.4	1.0	1.7	1.4	-1.5	1.2	8.8	-1.2	2.0
C4b	12268	1.8	1.4	1.3	1.1	1.5	1.3	-1.2	1.9	3.0	1.4	1.9
C4bp	12269	3.2	2.7	3.5	2.2	2.5	1.9	2.8	1.9	-1.3	1.9	2.8
C5ar1	12273	2.2	7.2	4.1	-2.1	3.4	-1.8	3.4	11.9	5.0	3.3	2.2
C6	12274	3.1	1.8	1.3	1.5	2.3	1.5	1.8	2.6	1.9	2.5	1.4
C7	109828	-1.3	-2.8	1.2	1.3	1.6	-1.4	-1.6	1.3	1.6	-1.2	1.7
Cacna1i	239556	2.1	2.3	-1.7	5.9	1.7	1.3	1.6	1.7	3.0	1.3	2.7
Cacna2d3	12294	3.1	1.3	1.3	-1.6	1.3	2.5	1.2	2.2	1.3	3.1	-1.1
Cacnb1	12295	3.3	1.7	1.9	1.7	4.4	7.2	1.9	3.3	2.4	1.8	4.2
Cacnb3	12297	-1.5	1.1	1.1	1.6	1.8	1.8	-1.2	-1.3	1.1	-1.3	-1.2
Cacnb4	12298	1.4	2.2	1.9	2.1	-1.1	-1.3	1.4	1.3	2.9	1.1	2.1
Calr3	73316	3.7	1.2	3.3	1.6	-1.4	1.4	1.4	3.7	1.7	3.6	1.1
Calu	12321	2.1	1.8	1.9	1.3	-1.0	1.1	1.8	1.9	2.3	2.0	2.0
Camk1d	227541	2.6	7.1	5.0	1.9	2.2	2.3	5.4	1.8	1.0	1.8	3.4
Camk1g	215303	2.0	1.1	1.1	1.3	-1.1	1.2	2.4	1.6	1.3	-1.1	1.2
Camk2b	12323	2.1	-1.2	1.4	1.1	1.9	1.1	1.2	1.4	1.3	2.2	1.4
Camk2d	108058	1.1	1.1	1.1	1.2	1.2	1.1	1.1	1.0	2.7	-1.0	1.3
Camk4	12326	3.9	3.7	3.5	3.0	4.8	5.2	5.0	1.9	4.5	3.5	8.8
Casp1	12362	1.3	1.0	1.1	-1.1	-1.3	-1.0	-1.0	1.2	-1.6	-1.1	1.1
Casp12	12364	3.1	1.4	1.4	-1.2	-1.7	-1.2	5.6	1.7	1.1	2.2	1.7
Casp14	12365	6.2	3.5	1.4	6.9	82.0	1.9	2.4	8.2	1.3	1.5	5.6
Casp2	12366	-1.0	-1.1	-1.1	-1.5	-1.4	-1.4	-1.1	1.1	-1.6	-1.1	-1.2
Casp3	12367	-1.8	-6.1	-1.7	-1.7	-3.4	-2.4	-5.1	-2.4	-4.8	-4.1	-1.1
Casp6	12368	-1.8	-1.6	-2.9	-1.7	-2.9	-2.5	-2.4	-2.3	-1.9	-2.9	-1.1
Casp7	12369	2.2	1.2	1.4	-1.1	-1.9	-1.5	1.4	1.4	-1.1	1.5	1.9
Casp8	12370	1.8	1.1	1.3	1.5	1.2	1.3	1.0	-1.1	-1.2	1.3	1.2

Casp8ap2	26885	2.5	1.8	1.9	-1.1	-1.6	-1.1	2.0	2.3	1.6	2.7	1.8
Ccl1	20290	2.1	1.4	2.8	-2.7	1.6	1.1	1.9	2.5	3.4	-3.2	1.8
Ccl11	20292	1.6	1.8	1.0	1.4	-1.5	1.5	2.0	1.1	1.5	1.8	-1.5
Ccl12	20293	1.5	-1.0	1.5	-2.2	-1.0	-1.5	-3.3	1.7	4.5	-1.5	-1.0
Ccl17	20295	-1.1	-1.4	-1.2	-1.3	1.3	1.2	1.4	-1.4	3.0	1.6	-1.3
Ccl19	24047	-3.0	1.6	-2.9	-1.8	-2.3	-1.5	-2.7	-3.9	2.3	-2.6	-1.5
Ccl2	20296	1.1	-1.1	1.9	-1.1	1.1	1.1	-1.3	1.6	6.1	-1.4	1.5
Ccl22	20299	-14.8	-1.3	-1.4	-1.1	-1.0	1.0	-1.0	-1.1	-1.1	-1.4	1.0
Ccl24	56221	1.1	-1.4	-2.4	1.2	-6.8	-2.2	-1.2	-3.0	-1.6	1.7	1.7
Ccl25	20300	-1.0	-1.0	-1.7	-1.7	-2.3	-1.2	-1.0	-1.3	-2.6	-1.3	-1.3
Ccl27a	20301	4.0	1.0	1.7	-1.1	-1.1	-1.1	1.1	1.4	2.2	1.4	1.1
Ccl28	56838	1.6	1.4	1.9	1.0	1.5	1.3	1.6	2.4	1.7	2.2	1.2
Ccl3	20302	2.6	-2.4	-2.7	-1.1	-1.2	-1.8	-1.7	-3.7	-1.2	-1.7	3.1
Ccl4	20303	-1.1	-4.2	-1.1	-4.1	-2.7	-3.9	-1.7	-1.3	-1.5	-2.3	1.2
Ccl5	20304	-1.0	-2.7	-1.2	-2.5	-1.7	-2.4	-1.5	-1.1	-2.1	-1.7	1.0
Ccl6	20305	1.4	1.3	1.1	1.1	1.3	1.2	1.2	1.1	-2.1	1.0	-1.1
Ccl7	20306	1.9	1.4	2.6	1.4	4.2	-1.3	-1.1	4.2	13.6	1.8	2.6
Ccl8	20307	1.3	-2.3	2.8	1.6	2.3	-1.5	-2.7	5.6	4.3	-2.5	1.6
Ccl9	20308	-1.1	1.1	1.0	1.1	-1.5	-1.3	-1.8	-1.2	-1.4	1.1	-1.1
Ccr10	12777	1.7	1.7	1.2	-1.5	1.0	-1.0	1.0	1.8	-1.9	-1.2	-1.0
Ccr4	12773	3.1	3.3	4.1	4.5	2.0	2.1	5.7	3.3	38.1	2.4	5.9
Ccr5	12774	2.4	1.3	2.0	-2.3	1.6	-1.8	1.8	4.0	2.4	1.7	2.0
Ccr6	12458	-4.4	1.3	-3.2	-3.6	-2.9	-1.8	-4.3	-3.1	-1.3	-3.8	-1.1
Ccr7	12775	-4.2	1.7	-2.7	-3.5	-2.2	-2.7	-5.6	-3.5	1.6	-3.6	-2.2
Ccr9	12769	-1.4	-1.4	-1.7	-2.0	-1.1	-1.3	-1.6	-1.2	1.0	-2.0	-1.3
Cdh11	12552	2.4	6.2	1.2	-1.1	1.5	-1.6	3.0	1.1	1.3	2.8	3.1
Cdh12	215654	-1.3	-1.3	-1.3	4.1	2.2	2.2	1.2	-1.1	-1.8	-1.3	-1.0

Cdh13	12554	2.0	1.7	2.0	1.3	2.0	2.0	1.5	1.7	1.5	1.8	1.9
Cdh2	12558	1.3	1.1	1.9	-1.2	-2.2	-1.3	1.2	2.4	1.1	1.5	1.8
Cdh20	23836	1.2	-1.4	-1.4	-1.6	-1.8	-1.3	-2.8	1.6	-1.3	-1.1	-1.0
Cdh26	381409	1.7	-1.1	1.9	1.3	-1.1	2.6	1.7	1.8	1.2	1.2	-1.3
Cdh3	12560	1.8	1.4	3.3	1.7	2.4	2.1	4.7	1.7	1.9	1.5	1.1
Cdh5	12562	6.0	1.5	-1.2	1.1	1.4	1.8	1.7	2.0	2.1	2.4	1.4
Cdhr1	170677	4.3	6.8	14.0	4.4	15.3	-1.3	9.1	15.3	17.8	4.5	11.3
Cdk1	12534	2.5	2.5	3.2	-1.0	4.2	1.2	3.7	8.1	2.3	7.8	1.9
Cdk13	69562	4.0	1.7	1.5	1.1	-1.0	1.0	4.5	2.6	2.0	5.2	1.4
Cdk17	237459	4.5	2.1	1.8	1.4	1.8	1.8	4.7	3.1	3.0	4.8	2.2
Cdk18	18557	2.4	2.3	1.1	1.7	1.2	1.6	1.8	1.4	1.2	2.0	2.1
Cdk2	12566	2.0	1.6	1.5	1.2	-1.0	1.0	1.6	2.3	1.4	1.1	1.4
Creb1	12912	3.9	2.1	1.3	2.3	-1.4	-1.5	1.3	1.5	3.8	3.2	1.5
Creb3l1	26427	1.0	1.1	2.2	1.3	1.6	-1.2	1.2	1.8	1.5	1.3	2.1
Creb3l4	78284	1.1	1.2	1.6	1.3	1.4	1.3	1.2	1.5	-1.2	1.3	1.1
Cxcl1	14825	1.2	1.2	2.2	1.8	-1.8	2.0	1.6	1.4	13.4	1.2	1.6
Cxcl10	15945	1.5	-1.6	1.6	-1.8	-1.2	-1.6	-1.6	1.3	2.6	-1.5	1.9
Cxcl11	56066	-2.7	-2.0	-2.0	-1.5	-1.8	-1.4	-1.5	-2.0	-2.4	-2.3	-1.8
Cxcl12	20315	1.6	1.7	1.1	1.4	2.3	2.0	2.4	2.3	2.0	3.0	1.6
Cxcl13	55985	-1.0	1.7	-1.5	-2.1	1.5	-2.2	-1.9	-1.1	13.2	1.2	1.2
Cxcl14	57266	1.3	1.3	1.1	-1.4	-1.0	1.4	1.5	1.7	-1.8	1.7	1.1
Cxcl15	20309	-1.2	-1.3	-1.4	-1.9	1.0	-2.2	1.0	-1.1	-1.2	4.3	1.1
Cxcl16	66102	1.3	1.4	1.2	-1.2	1.6	1.1	1.6	1.2	2.3	1.4	1.4
Cxcl2	20310	-4.4	-4.5	-4.4	-3.1	-3.7	-3.4	-3.5	-3.9	1.9	-4.3	-3.4
Cxcl3	330122	1.9	1.8	1.7	-1.2	2.4	1.1	2.4	2.1	31.7	1.7	2.5
Cxcl5	20311	19.9	4.4	11.2	13.1	2.0	2.5	4.4	9.6	172.5	6.4	5.2
Cxcl9	17329	2.8	1.1	3.0	-1.4	-1.4	-2.1	-1.9	3.3	6.3	1.3	2.8

Cxcr1	227288	-2.3	-6.4	-2.4	-1.5	-1.9	-1.8	-1.8	-2.0	-2.0	-2.2	-1.8
Cxcr3	12766	1.5	1.1	-1.1	-1.7	1.2	-1.2	-1.2	-1.0	1.2	-1.2	1.2
Cxcr4	12767	-2.6	-1.2	-3.4	-20	-1.4	-24.3	-7.7	-3.7	-1.4	-4.4	-4.5
Cxcr5	12145	-6.5	1.4	-3.0	-4.6	-4.8	-2.2	-8.4	-4.3	-1.9	-9.2	-1.1
Cxcr6	80901	2.4	1.2	-2.1	-1.3	-1.2	-1.1	1.4	-1.2	-1.7	1.7	1.2
Cxcr7	12778	1.9	1.3	1.2	1.3	2.1	2.0	1.6	2.5	3.4	2.2	1.5
Cyp1b1	13078	3.1	1.1	-1.3	1.1	1.2	2.6	2.3	2.0	6.4	2.3	1.2
Cyp20a1	77951	3.9	3.0	3.4	2.5	3.1	2.7	3.1	3.3	4.4	4.1	2.2
Cyp21a1	13079	2.0	-1.2	-1.3	2.1	-1.1	1.8	2.2	-1.1	1.5	-2.0	1.7
Cyp27b1	13115	-1.7	-2.5	-1.7	2.0	1.7	1.2	1.5	-1.5	1.3	-9.6	-1.4
Cyp2d10	13101	-1.5	-1.9	8.5	7.8	29.1	-1.1	-2.0	3.9	2.2	-1.4	5.6
Cyp2d13	68444	2.7	1.4	8.1	5.4	8.0	-1.1	1.5	3.1	1.5	2.7	2.0
Cyp2d34	223706	-2.0	-2.4	4.5	3.4	7.1	-1.7	-2.1	2.4	1.0	-2.4	2.5
Cyp2d37-ps	627860	1.4	1.4	31.8	10.2	9.4	1.6	1.4	12.1	8.4	2.1	14.3
Cyp2d40	71754	2.3	3.3	1.5	-1.6	1.1	2.8	5.0	1.1	2.6	5.9	2.5
Cyp2d9	13105	3.0	-1.5	14.4	21.6	67.2	1.0	-1.7	6.8	7.0	1.0	6.3
Cyp2e1	13106	-2.4	-13	-1.7	-8.5	4.0	-2.5	-10	2.2	-1.3	-1.7	-1.4
Cyp2f2	13107	1.1	-1.1	1.5	6.8	35.2	1.2	1.2	1.4	-1.2	1.3	5.7
Dagla	269060	2.1	1.5	4.8	3.2	2.6	1.6	2.6	3.7	-3.2	3.5	2.7
Def6	23853	-1.4	-1.3	-1.6	-1.8	-1.5	-1.5	-1.8	-1.4	-1.2	-1.9	-1.2
Def8	23854	-1.3	1.2	-1.3	-1.4	-1.3	-1.2	1.0	1.0	-1.5	-1.1	-1.1
Defa-ps1	727720	1.5	1.3	-2.9	2.4	1.3	2.8	1.7	-3.6	-1.7	1.6	-2.1
Defa-ps13	654456	-1.2	-1.8	-1.3	1.1	1.0	1.0	1.0	-4.6	-1.0	-1.2	1.1
Defa-rs1	13218	3.4	2.4	-1.4	2.1	-1.5	1.9	2.1	-3.5	-2.2	3.0	-1.2
Defa-rs10	13219	-2.0	-2.5	-2.2	1.0	22.9	7.4	2.1	28.0	11.5	-3.2	-2.0
Defa-rs12	13221	-2.0	-2.3	-2.0	1.0	24.5	6.8	1.4	18.8	12.1	-2.7	-1.8
Defa-rs2	13222	-1.9	-2.4	-1.4	-1.2	119.8	24.8	7.7	137.8	63.5	-2.5	-1.7

Defa-rs4	13223	-2.0	-2.5	-2.0	-1.1	17.1	4.3	1.7	16.3	8.0	-2.4	-2.1
Defa-rs7	13226	-2.1	-2.2	-1.9	-1.2	23.0	5.0	1.7	17.8	10.4	-2.7	-1.9
Defa1	13216	2.1	2.3	1.0	1.3	25.1	7.6	2.8	14.3	15.5	2.8	-1.2
Defa21	66298	4.1	1.9	-1.9	1.9	-1.9	2.4	3.0	-4.2	-2.5	2.9	-1.3
Defa23	497114	3.2	2.4	-1.8	2.1	1.1	2.3	2.1	-1.4	-1.2	3.0	-1.4
Defa25	13236	1.7	1.8	-1.9	2.3	-1.0	2.7	1.5	-5.2	-3.0	2.1	-1.6
Defa26	626708	1.4	1.3	-2.7	2.5	1.1	2.7	1.5	-4.0	-2.0	1.4	-2.1
Defa3	13237	2.6	2.2	-1.9	2.0	1.4	2.7	2.9	-1.3	-1.3	2.9	-1.4
Defa4	13238	-2.3	-2.1	-1.9	-1.1	42.4	7.7	1.3	29.3	31.5	-2.5	-1.7
Defb12	77674	-4.9	1.2	-1.2	1.5	1.6	1.5	1.6	1.3	1.4	1.1	1.6
Defb14	244332	-1.6	-3.5	21.1	-2.9	-1.2	-1.1	-1.2	-1.4	-1.4	-3.9	-1.2
Defb2	13215	1.5	1.4	1.4	1.9	1.8	-4.8	1.8	-1.0	1.7	-1.1	1.3
Defb21	403172	-1.2	-1.2	8.2	1.1	-1.0	1.1	1.1	-1.0	-1.0	-1.2	1.1
Defb22	442835	1.5	1.5	1.5	1.1	2.2	1.9	1.9	1.8	1.8	-3.5	2.0
Defb26	654457	1.2	1.1	-2.9	-1.2	-2.0	-1.5	-3.3	1.0	1.4	1.1	-1.3
Defb28	545475	-1.1	-1.4	-1.9	-1.4	-1.2	1.1	-1.7	-1.3	-1.2	-9.3	1.0
Defb3	27358	-1.2	-3.7	-4.5	-2.9	-2.0	-5.6	-3.1	-3.9	-2.6	-1.7	-3.0
Defb39	360214	-1.5	-1.5	-1.7	-1.1	-1.5	6.1	-1.2	1.3	-1.3	-1.9	-1.2
Defb40	360217	-2.4	-1.7	-1.9	-4.0	-2.0	1.7	-1.8	1.5	-3.6	-2.5	-1.9
Defb41	77673	1.3	-1.3	-3.8	-4.6	-1.3	-1.9	-1.1	-4.4	-1.1	-1.2	1.0
Defb42	619548	-2.9	-1.5	-5.3	-1.7	-1.1	1.3	-1.3	-1.3	1.3	-1.8	1.5
Defb45	433490	-1.4	-1.5	-1.9	-1.1	6.5	-1.4	1.7	-1.3	1.4	-1.5	-1.1
Defb6	116746	-1.7	-1.1	1.5	-2.8	-3.8	-1.5	-1.4	-1.0	1.8	-1.1	-1.8
Defb7	246080	-2.5	-1.5	-1.4	-1.0	1.2	-1.5	-2.1	-1.4	-1.9	1.1	1.2
Dusp10	63953	3.0	2.4	4.1	-1.2	-1.1	1.4	1.5	3.5	5.0	2.6	2.3
Dusp13	27389	1.2	-3.7	1.1	3.1	3.1	-1.0	-2.4	-3.0	1.3	1.6	-1.3
Dusp14	56405	2.8	2.0	1.7	1.2	1.5	2.1	1.2	1.4	13.6	2.2	2.3

Dusp18	75219	-1.1	1.3	1.7	1.3	2.1	1.8	1.5	1.3	-1.1	2.3	1.2
Dusp2	13537	-2.2	1.2	-2.1	-3.3	-1.3	-1.8	-2.3	-1.5	2.2	-2.1	-1.2
Dusp26	66959	2.2	1.5	2.2	1.7	3.0	1.9	4.4	2.1	4.7	2.2	2.1
Dusp3	72349	1.1	-1.0	1.4	1.0	-1.1	-1.2	-1.3	1.4	-1.1	-1.1	1.6
Dusp9	75590	1.6	4.1	-1.2	1.8	4.7	1.4	1.2	1.1	11.1	2.8	1.3
Gadd45a	13197	1.1	1.9	3.0	-1.0	-1.4	-1.0	1.3	1.1	1.0	1.5	2.1
Gadd45g	23882	1.0	1.3	1.8	1.3	1.6	1.8	-1.2	-1.1	1.7	2.0	1.5
Gadd45gip1	102060	1.3	1.9	1.5	1.0	-1.4	-1.1	3.2	3.4	1.1	1.6	1.5
Gata1	14460	2.4	2.2	-1.3	6.6	1.2	3.4	3.0	2.6	2.7	2.3	2.6
Gata2	14461	2.4	2.2	1.4	-1.6	2.4	1.7	1.6	3.2	4.6	6.9	2.4
Gata3	14462	-1.9	2.5	-1.1	-1.0	1.3	-1.1	-2.0	-1.0	1.3	3.3	1.6
Gpr116	224792	2.3	3.1	1.3	-1.8	1.4	1.8	2.6	3.7	7.5	5.6	1.9
Gpr124	78560	2.2	2.5	1.2	1.1	1.8	1.4	2.0	-1.0	-1.1	2.3	1.9
Gpr126	215798	2.8	2.9	3.7	-1.3	1.1	2.0	5.5	4.3	2.9	1.9	2.2
Gpr137b	83924	1.6	-1.1	5.6	2.2	2.0	-1.5	-1.8	1.1	2.7	-1.2	2.5
Gpr137b-ps	664862	-1.1	1.2	3.4	2.6	4.2	1.0	1.1	1.3	2.7	1.1	2.9
Gpr146	80290	1.0	1.0	-1.5	-1.3	1.3	-1.0	1.3	1.4	4.2	1.8	1.2
Gpr153	100129	2.7	1.6	1.4	1.3	1.2	2.0	1.7	2.0	1.2	2.7	1.7
Gpr155	68526	4.8	1.4	-1.3	1.1	-1.3	-1.6	1.5	2.5	-1.8	4.0	1.3
Gpr182	11536	2.4	2.6	1.9	2.0	3.2	2.3	3.5	3.3	4.7	3.5	2.6
Gpr4	319197	2.7	5.5	2.1	1.5	2.9	2.3	1.3	1.5	3.3	5.1	2.0
Gpr6	140741	2.0	2.3	1.9	1.3	1.8	-1.1	1.7	1.5	5.5	2.1	1.2
Gpr65	14744	1.7	-1.0	-1.0	-1.6	1.1	-1.2	1.1	1.6	2.0	1.1	1.1
Gpr82	319200	1.1	1.5	-1.0	-3.7	-1.7	-1.3	1.4	3.8	2.8	1.9	2.4
Gpr83	14608	-2.4	1.5	1.8	2.3	1.8	2.0	1.1	1.6	2.6	1.6	2.0
Gpr84	80910	1.8	2.4	1.7	1.8	1.0	-1.2	1.6	1.3	12.6	2.8	1.3
Gpr85	64450	1.2	2.1	3.3	-1.1	1.7	1.2	5.7	4.9	6.5	2.4	6.9

Gpr98	110789	5.9	2.1	2.5	2.6	4.1	4.3	3.3	14.0	11.8	3.1	8.2
Hif1a	15251	-2.4	-3.6	-1.6	1.4	2.3	-1.9	-1.5	-1.4	-1.2	-1.6	1.3
Hif1an	319594	1.1	1.2	1.2	1.6	1.4	1.5	1.1	-1.2	1.0	1.2	-1.0
Hif3a	53417	-1.2	1.3	1.4	1.4	3.5	1.7	1.3	1.4	6.6	2.9	1.7
Hk1	15275	1.0	-1.2	4.0	2.1	3.6	-1.3	-1.1	2.1	2.1	-1.1	2.9
Hk2	15277	1.5	1.1	5.0	1.5	2.3	1.4	-1.0	2.5	6.2	1.9	3.9
Hk3	212032	-1.0	1.1	-1.0	1.1	-1.0	1.0	-1.5	1.2	2.3	1.2	1.1
Hspa12a	73442	-1.4	-2.0	2.0	1.5	1.6	1.6	2.8	-1.1	1.3	2.0	-1.1
Hspa12b	72630	2.3	3.2	1.7	1.4	3.5	3.2	3.6	2.8	1.9	3.1	2.3
Hspa1a	193740	-1.2	1.2	2.0	1.4	1.6	2.3	-1.6	-1.4	-1.2	-1.9	2.7
Htr2b	15559	1.5	-1.6	1.1	1.3	1.9	-1.1	-1.0	1.7	-1.1	-1.1	-1.1
Htr3a	15561	3.7	1.6	4.7	1.5	3.8	2.0	4.5	3.1	3.8	3.2	1.9
Htr4	15562	1.2	-1.4	3.7	1.3	1.9	-3.4	-1.0	2.9	-2.0	2.7	3.6
Htr6	15565	-1.4	3.1	2.1	1.1	1.9	1.6	3.7	2.5	2.2	1.8	-5.8
Htr7	15566	10.0	6.0	6.4	4.0	2.4	1.5	3.4	9.6	14.1	20.2	4.4
Icam1	15894	3.0	-1.1	2.1	3.0	1.5	4.9	1.1	1.2	4.4	-1.2	2.5
Icam2	15896	1.0	1.3	-1.5	-1.3	1.1	1.2	-1.2	1.1	-1.5	-1.1	-1.0
Icam4	78369	1.4	3.6	2.9	3.3	1.3	2.7	5.6	3.7	5.9	2.2	3.2
Ifna11	15964	1.0	-1.6	-1.0	1.4	1.6	1.3	1.3	-10.8	1.2	2.0	2.7
Ifna12	242519	-4.4	-1.5	-1.5	1.1	-1.1	-1.0	-1.1	-1.3	-1.2	-1.6	-2.1
Ifna14	404549	-12.0	-10	-9.2	-2.8	-10.1	-4.1	-6.6	-10.5	-7.2	-7.5	-7.3
Ifna5	15968	-1.3	2.6	-1.4	2.0	-4.1	1.3	1.1	1.2	-1.1	-1.3	1.1
Ifna7	15970	-3.3	1.0	1.0	3.1	2.0	1.1	2.0	1.8	3.6	1.6	2.1
Ifnab	15974	-2.2	-1.2	-1.2	-4.9	-2.4	2.0	-1.2	-1.2	-1.1	-1.9	1.1
Ifnar1	15975	-2.6	-1.5	-2.9	-1.9	-1.7	-1.5	-7.4	-1.9	-4.5	-2.7	-1.1
Ifnar2	15976	2.5	1.4	-1.8	1.9	-1.4	2.0	1.9	-1.2	1.2	1.2	-1.0
Ifng	15978	1.7	-3.5	-1.2	-2.2	-2.5	-3.0	-1.5	1.1	1.1	1.0	1.3

Ifngr2	15980	1.0	1.1	1.1	-1.2	-1.2	1.0	1.1	-1.3	-1.6	-1.1	1.1
Igf2bp2	319765	2.5	1.6	2.2	1.0	-1.8	-1.2	1.2	-1.2	1.6	1.4	1.9
Igfbp2	16008	1.6	-2.1	3.3	2.1	1.1	1.3	1.0	2.4	2.3	-1.5	2.1
Igfbp3	16009	1.5	1.1	-1.3	1.1	3.5	2.0	1.6	1.6	2.9	2.1	-1.3
Igfbp4	16010	2.7	-1.1	1.7	1.7	1.5	2.7	-1.1	1.5	2.6	-1.2	-1.0
Igfbp5	16011	1.2	-1.2	-1.1	1.6	1.7	1.5	1.6	1.4	1.3	1.2	1.5
Igfbp6	16012	1.5	-1.1	1.4	1.4	1.7	2.1	1.1	1.1	1.8	1.2	1.6
Il10	16153	1.3	1.2	3.2	-2.2	-1.5	-2.4	1.2	2.5	7.7	1.5	1.6
Il10ra	16154	1.8	2.1	1.8	1.3	2.2	1.4	1.5	1.4	2.5	1.4	1.4
Il11	16156	1.4	1.5	-1.1	1.6	-1.3	1.0	1.3	-2.1	2.5	3.3	-1.3
Il11ra1	16157	-1.5	-1.1	1.6	-1.4	2.2	-1.5	-1.1	1.4	1.2	-1.4	1.0
Il12b	16160	1.2	-1.1	1.2	-1.1	1.4	1.9	2.7	-1.1	1.6	1.8	1.1
Il12rb1	16161	1.2	1.7	1.6	-1.0	-1.2	-1.1	-1.0	1.2	1.1	-6.4	-1.0
Il12rb2	16162	-1.4	1.4	-3.1	1.0	1.3	1.1	-1.5	-1.0	-1.5	-1.0	-1.6
Il13ra1	16164	1.3	-1.7	-1.4	1.2	-1.7	-1.2	-1.4	-1.5	-1.6	-1.3	-1.1
Il13ra2	16165	2.5	2.7	3.9	2.5	4.3	1.1	1.0	3.5	4.4	2.7	3.9
Il15	16168	3.9	2.1	1.0	-1.1	1.1	1.2	1.6	1.3	1.6	2.0	1.6
Il15ra	16169	-2.4	-1.4	-2.0	-1.2	-1.7	-1.4	-1.3	-2.2	-1.4	-1.7	-1.7
Il16	16170	-1.1	-1.2	-1.6	-1.9	-1.5	-1.8	-2.4	-1.9	-3.4	-1.7	-1.1
Il17a	16171	-6.4	2.3	1.5	-1.3	1.9	3.5	2.0	1.8	10.5	1.6	2.0
Il17d	239114	1.5	-1.3	-1.2	1.2	2.2	1.2	1.2	1.8	1.3	1.5	2.7
Il17f	257630	1.6	2.4	4.0	1.2	3.1	4.5	-1.2	2.1	4.4	-2.9	2.1
Il17ra	16172	-1.7	-2.1	-2.2	1.6	1.4	1.3	-2.3	-2.5	-1.4	-2.3	-1.8
Il17rb	50905	3.5	2.1	1.1	1.7	1.6	2.0	1.1	1.2	-1.3	-1.8	1.7
Il17rc	171095	1.4	1.3	1.1	-1.0	-1.2	-1.0	-1.0	1.2	-1.6	-1.1	1.4
Il17rd	171463	1.5	1.2	-1.1	-1.0	1.1	1.5	1.5	-1.0	2.1	1.8	-1.1
Il17re	57890	1.0	1.7	3.6	2.7	7.4	1.6	1.4	2.1	1.4	-1.2	2.1

Il18	16173	1.7	2.0	1.3	3.4	3.4	2.0	1.1	-1.8	5.5	3.9	1.1
Il18bp	16068	2.0	-2.3	1.5	2.1	1.0	-1.1	-2.3	-1.1	1.8	-1.1	1.7
Il18r1	16182	3.0	1.4	1.2	-1.4	-1.4	-1.0	2.7	2.9	1.4	-1.0	1.4
Il18rap	16174	-1.2	-1.8	1.3	-1.6	-5.0	-2.5	-1.4	1.1	2.0	1.8	-2.4
Il19	329244	-1.2	2.2	-1.3	1.2	-1.6	4.1	1.1	-1.1	2.1	-1.3	1.2
Il1a	16175	-1.2	-1.2	-1.3	1.1	1.0	1.1	1.1	-1.0	4.4	-1.2	1.1
Il1b	16176	2.5	1.9	2.0	1.2	1.3	-1.1	2.1	2.7	14.8	2.0	1.9
Il1r1	16177	1.2	-1.0	-1.1	1.1	1.2	1.5	1.2	1.5	1.8	1.4	-1.1
Il1r2	16178	1.8	1.9	1.1	1.4	1.8	1.5	3.0	1.3	20.4	4.0	1.2
Il1rap	16180	-1.5	1.3	1.1	-2.1	-6.0	-1.8	1.3	-1.4	-2.2	-1.2	-1.2
Il1rapl1	331461	-1.2	1.5	2.9	1.3	1.2	-2.3	1.9	-1.1	1.8	-1.2	2.0
Il1rapl2	60367	1.6	1.3	1.7	2.3	1.5	2.1	1.5	-1.1	1.3	-1.9	-2.1
Il1rl1	17082	12.2	1.2	1.9	-1.5	-4.5	-1.1	2.9	5.8	-1.1	3.8	7.6
Il1rn	16181	-4.1	-1.9	1.4	4.3	9.9	-2.0	-3.0	-1.2	1.7	-4.8	1.1
Il20rb	213208	-1.2	-1.1	-1.2	1.0	-1.1	-1.6	1.0	-1.1	2.1	2.2	-1.2
Il21	60505	-1.2	1.0	1.8	-2.6	1.6	-4.2	-1.0	1.4	2.0	1.5	1.6
Il21r	60504	-1.5	-2.0	-1.6	-3.1	-3.3	-2.4	-2.8	-1.3	-1.2	-2.7	1.1
Il22	50929	1.4	-1.8	-1.1	1.3	-1.9	-1.9	1.1	-2.8	12.5	2.3	-1.2
Il22ra1	230828	-1.7	1.1	-2.0	1.7	1.1	-1.2	-1.8	-3.3	-2.8	-2.7	1.1
Il22ra2	237310	1.0	1.3	-1.5	-3.0	-4.2	-2.0	-1.0	-1.1	-14.2	-2.2	-1.6
Il27	246779	-1.6	-1.4	-1.4	-1.1	-1.4	-1.4	-1.8	-2.0	-1.4	-1.8	-1.9
Il27ra	50931	1.9	1.4	1.0	-1.8	-1.6	-1.6	1.1	1.1	-2.2	-1.3	2.0
Il2ra	16184	-1.0	1.6	-2.9	-1.6	-1.0	-1.1	1.3	3.9	1.2	4.3	-2.5
Il2rb	16185	-1.2	-1.3	-1.4	-2.0	-1.1	-1.5	-1.5	-1.1	1.1	-1.1	1.1
Il2rg	16186	-1.8	-1.2	-1.8	-1.4	-1.0	-1.2	-2.4	-1.9	-1.1	-2.4	-1.2
Il33	77125	2.4	2.6	1.0	1.6	-2.3	-1.3	1.0	-1.4	1.9	1.3	2.9
Il34	76527	1.4	1.6	1.2	1.4	2.4	2.0	1.0	1.5	2.0	1.8	1.4

Il3ra	16188	1.4	1.2	1.3	-1.4	-1.2	-1.2	1.0	1.4	2.9	1.0	1.3
Il4	16189	1.2	1.9	1.1	-1.5	-1.7	-1.3	-1.0	1.4	-1.2	-1.2	1.3
Il4i1	14204	-2.2	1.6	-2.1	-2.7	-2.3	-1.9	-2.6	-1.9	-1.1	-2.7	1.0
Il4ra	16190	-1.1	-1.4	-1.1	1.1	-1.8	-1.3	-1.8	-1.5	1.4	-1.1	1.0
Il5ra	16192	-1.2	-1.6	2.3	-1.1	1.0	1.3	-1.2	-1.2	-3.5	-1.8	-1.5
Il6	16193	1.8	1.7	1.6	-1.4	1.2	1.3	2.3	2.0	9.2	1.8	-1.3
Il6st	16195	1.4	1.4	1.5	1.5	2.5	2.0	1.3	1.3	2.4	1.6	1.4
Il7	16196	2.6	1.4	1.2	-1.3	1.4	1.1	1.4	1.2	1.9	1.4	4.7
Il7r	16197	2.2	1.2	-1.3	-2.3	-1.1	-1.6	-1.0	2.6	1.5	3.7	1.3
Il9	16198	-2.2	-2.3	-1.1	-1.8	1.7	-1.0	-1.5	1.2	-1.8	1.1	-1.6
Il9r	16199	-4.8	-2.5	-5.4	-1.0	-3.8	-1.6	-3.8	-4.3	-3.2	-5.0	-2.1
Irak3	73914	-1.1	1.2	-1.0	1.2	1.3	1.6	1.5	1.2	2.8	1.1	1.1
Irf1	16362	1.3	-1.5	1.1	-1.3	-1.9	-1.4	1.0	1.1	-2.2	-1.2	1.2
Irf2	16363	2.3	1.6	1.6	-1.2	-1.3	1.1	2.4	1.8	1.1	2.0	1.9
Irf4	16364	1.2	1.5	-1.2	-1.3	1.4	1.4	1.5	1.3	1.8	-1.0	1.2
Irf5	27056	-1.1	1.4	-1.3	-1.6	1.2	1.1	-1.3	-1.1	1.7	1.0	-1.2
Irs1	16367	2.5	1.1	1.2	1.1	2.5	1.7	1.8	3.4	1.1	2.0	2.3
Irs3	16369	-1.9	-1.2	2.6	-1.4	1.2	-1.3	-1.2	1.9	-1.2	1.8	1.0
Irx2	16372	1.6	1.8	1.0	-2.0	-2.9	-2.6	1.3	1.7	1.2	-1.3	1.7
Irx4	50916	2.8	-1.1	-1.4	-1.1	-1.2	-1.3	-1.1	1.7	1.9	1.1	-2.7
Irx5	54352	1.3	-1.0	1.2	-2.5	1.9	1.0	-1.4	-1.3	1.3	1.2	-1.1
Isg20l2	229504	2.3	2.2	1.8	1.1	1.2	1.1	2.9	2.6	2.8	3.5	1.8
Isl1	16392	2.2	3.6	2.3	-1.4	1.7	-1.0	3.0	4.2	3.1	3.4	2.6
Isl2	104360	4.5	1.9	4.2	1.6	3.4	1.1	1.3	2.1	2.6	-1.4	2.0
Itga1	109700	-1.2	-1.3	1.1	-1.1	1.3	1.5	-1.5	1.2	1.2	1.0	1.1
Itga11	319480	2.1	1.5	-1.3	1.4	1.9	2.6	2.0	1.9	-1.4	1.5	1.3
Itga2b	16399	1.1	2.0	1.0	2.6	1.4	3.6	2.1	1.9	1.3	1.5	5.3

Itga5	16402	-3.3	-5.5	-1.3	3.7	7.0	6.4	-9.9	-8.1	-1.9	-12.2	-2.7
Itga6	16403	1.1	1.6	1.5	-1.0	-1.3	-1.1	1.7	1.4	1.3	1.4	1.5
Itga7	16404	-1.2	-1.5	2.0	-1.0	1.3	-1.1	1.1	1.8	1.0	-1.1	1.6
Itga8	241226	1.9	1.3	1.3	1.0	-1.1	1.6	1.6	2.1	-1.8	1.1	-1.1
Itga9	104099	1.2	-1.1	-1.4	1.1	1.0	1.8	1.1	-1.1	-1.5	1.0	-1.0
Itgb1	16412	1.3	1.0	1.2	1.2	-1.1	1.3	1.5	1.3	1.8	1.4	1.6
Itgb2l	16415	2.2	2.1	4.1	1.6	2.7	2.6	2.8	2.5	2.8	-1.0	5.7
Itgb3	16416	1.7	-1.0	-1.0	1.1	1.4	1.8	1.4	1.6	1.4	1.2	1.2
Jund	16478	1.1	1.0	1.3	1.1	-1.0	1.0	1.5	1.4	-1.4	-1.0	1.2
Lrrc10	237560	2.8	2.4	2.4	2.7	2.2	2.0	-1.2	3.1	-1.1	2.3	3.8
Lrrc15	74488	-1.3	-1.3	-1.4	1.2	-1.0	11.0	1.1	-1.1	-1.1	-1.4	1.0
Lrrc17	74511	3.5	2.5	1.6	1.5	1.8	1.8	2.6	3.1	-1.8	2.6	1.8
Lrrc18	67580	2.8	3.1	2.7	6.8	3.3	1.4	3.5	11.1	4.4	2.9	3.5
Lrrc2	74249	3.2	1.9	5.4	1.2	2.6	1.2	1.3	1.2	2.5	5.8	2.7
Lrrc27	76612	2.2	5.0	-2.1	1.9	3.0	1.4	1.7	1.0	2.7	4.9	2.3
Lrrc32	434215	2.2	1.9	1.5	1.0	1.9	1.6	1.8	2.3	2.1	2.2	1.9
Lrrc38	242735	-3.6	1.6	3.3	-1.1	1.8	-1.3	1.7	1.4	1.6	1.2	-1.2
Map2k1	26395	1.2	1.1	1.6	1.2	1.2	1.1	1.2	-1.1	-1.1	1.1	1.5
Map2k3	26397	-1.1	1.1	1.2	2.0	1.4	1.6	1.0	-1.6	1.1	1.0	1.1
Map2k5	23938	-1.1	2.0	1.8	2.2	1.0	1.8	1.1	1.5	1.9	1.8	1.3
Map2k7	26400	-1.8	1.3	1.0	1.9	3.0	1.9	-1.1	-2.5	-1.8	1.4	-1.8
Map3k13	71751	-1.0	4.5	3.4	-1.5	3.2	1.0	4.8	1.9	3.6	1.7	1.8
Map3k14	53859	-1.1	1.2	-1.1	-1.1	1.0	-1.0	-1.1	1.6	-1.0	-1.0	-1.1
Map3k15	270672	8.9	9.8	7.2	2.3	10.6	5.9	1.8	6.7	5.1	8.2	7.7
Map3k2	26405	1.5	1.2	1.3	-1.1	-1.6	-1.6	1.2	1.0	-1.7	1.4	2.6
Map3k8	26410	-1.6	2.7	2.5	2.1	3.8	2.0	4.1	6.5	9.0	4.5	3.1
Map4k3	225028	2.2	1.6	1.6	-1.0	-1.0	1.2	2.3	2.2	1.3	2.0	1.5

Map4k4	26921	-1.1	1.1	-1.4	1.1	-1.1	1.1	-1.1	-1.4	1.6	-1.1	1.1
Map4k5	399510	2.1	1.4	1.5	1.1	1.1	1.3	1.5	1.6	1.9	1.8	1.6
Mapk10	26414	1.4	1.0	1.5	1.3	1.8	1.6	1.6	1.2	1.6	-1.0	1.3
Mapk12	29857	1.5	2.6	1.8	1.0	2.1	1.8	1.7	1.6	2.4	2.1	1.3
Mapk15	332110	1.2	1.3	1.9	2.9	5.5	5.8	1.8	1.4	1.4	1.2	3.2
Mapk4	225724	-1.4	-1.3	-1.3	-2.0	2.8	-1.1	1.2	1.5	-1.4	-3.3	-1.3
Mapk8ip2	60597	1.4	1.6	5.0	1.3	2.9	1.6	2.8	1.8	2.7	2.6	2.4
Mapk8ip3	30957	-4.1	-3.6	-3.9	1.8	1.7	-1.4	-5.8	-6.5	-3.7	-2.4	-2.9
Mmp10	17384	1.3	1.4	-1.9	1.4	-1.3	1.8	1.2	-1.1	1.2	1.2	-1.2
Mmp11	17385	1.7	1.2	1.2	1.2	2.5	2.4	1.7	1.6	2.1	1.3	-1.2
Mmp13	17386	-1.0	2.0	1.6	1.2	1.3	1.5	2.0	4.1	3.0	3.2	1.4
Mmp17	23948	1.3	-1.3	1.7	1.3	1.9	2.4	1.6	1.4	-1.3	-1.1	1.6
Mmp19	58223	3.3	10.4	4.7	5.5	6.9	9.2	5.6	4.6	21.4	1.4	11.9
Mmp2	17390	1.4	1.4	1.1	1.7	2.9	2.4	1.3	1.5	2.3	1.3	1.5
Mmp23	26561	-1.5	-1.0	-1.1	1.3	1.1	2.0	-1.4	-1.4	-1.1	-1.5	-1.2
Mmp24	17391	-1.2	1.8	1.8	1.3	2.6	2.1	1.2	1.3	1.0	1.1	-2.4
Mmp3	17392	1.3	-1.0	2.4	3.7	1.3	2.0	2.8	3.0	107.4	3.7	2.6
Mmp7	17393	1.5	2.2	1.1	1.8	2.9	1.8	1.4	1.3	4.1	2.1	1.1
Mmp8	17394	2.2	2.0	2.1	1.4	-1.7	1.2	1.7	2.5	23.4	2.2	2.8
Mmp9	17395	3.3	3.0	-1.2	5.3	-1.9	3.3	2.2	-2.0	2.1	4.7	1.1
Muc13	17063	2.0	1.2	1.3	1.4	1.2	1.4	1.2	1.2	1.1	2.3	1.3
Muc16	73732	5.9	1.6	4.0	1.9	1.8	2.2	2.5	1.8	8.0	1.9	2.2
Muc20	224116	3.8	1.2	-1.2	1.0	-3.6	-1.2	2.2	-1.1	1.0	1.9	-1.5
Nfkb1	18033	-1.7	-1.8	-1.9	1.2	-1.5	-1.1	-2.0	-2.3	-1.9	-2.1	-1.4
Nfkb2	18034	-2.9	-1.7	-1.9	-1.1	1.1	-1.2	-2.9	-3.0	-2.0	-3.1	-1.7
Nfkbia	18035	-1.3	1.1	1.1	-1.2	1.4	1.3	-1.0	-1.1	3.8	1.3	-1.1
Nfkbib	18036	-1.4	-1.2	-1.3	1.1	-1.2	1.1	-1.4	-1.8	-1.5	-1.3	-1.3

Nfkbid	243910	-2.0	-1.2	-1.8	-1.5	-1.7	-1.5	-2.3	-1.7	-2.3	-1.8	-1.4
Nfkbie	18037	-2.0	-1.1	-2.6	-2.5	-2.4	-1.8	-2.6	-1.6	-1.7	-1.7	-2.3
Nfkbil1	18038	1.4	1.4	1.3	-1.1	1.1	1.1	1.6	1.5	1.7	1.4	1.4
Nfkbiz	80859	-2.7	-1.4	1.1	1.2	1.6	1.2	-1.9	-1.6	-1.5	-1.5	-1.9
Nfrkb	235134	1.1	1.3	1.1	1.2	1.3	1.2	1.3	1.0	2.0	1.6	1.2
Nlrc3	268857	-1.9	-2.2	-2.1	1.1	1.5	-1.1	-2.3	-2.5	-1.9	-2.8	-1.3
Nlrc4	268973	-1.5	-1.2	-1.7	-1.0	-1.2	1.2	-1.4	-1.9	-1.4	-1.2	-1.7
Nlrc5	434341	-2.1	-3.2	-1.4	-2.9	-1.4	-2.3	-2.5	1.0	-1.2	-1.4	-1.0
Nlrp10	244202	1.3	2.7	11.3	6.5	13.2	2.1	4.1	2.8	5.3	6.1	8.2
Nox4	50490	3.1	6.3	3.0	-1.1	-1.2	2.7	4.0	3.6	3.6	3.7	3.9
Noxa1	241275	-1.0	-1.1	1.4	1.0	1.3	1.2	-1.2	1.7	-1.2	-1.0	-1.2
Pcdh20	219257	8.7	6.5	5.0	-1.1	2.1	2.4	7.5	13.4	4.1	10.8	11.1
Pcdh7	54216	3.7	2.6	2.1	3.1	8.7	3.2	2.4	2.5	1.0	1.6	3.4
Pcdh8	18530	7.9	4.4	4.0	1.3	1.4	5.4	21.3	10.9	1.5	8.0	1.1
Pcdhac2	353237	-2.4	4.6	4.7	1.4	2.4	1.7	4.2	9.3	3.6	5.6	3.6
Pcdhb14	93885	7.8	5.3	4.1	3.6	7.6	5.9	12.0	7.2	1.6	11.2	2.1
Pcdhb17	93888	2.1	2.7	2.2	-1.1	-2.3	3.6	4.8	4.0	1.8	2.4	1.6
Pcdhb20	93891	3.8	5.6	2.0	1.3	1.3	1.7	3.3	7.5	3.2	4.3	-2.0
Pcdhb21	93892	2.9	-1.3	-1.4	-1.2	-1.1	1.1	2.8	2.0	1.9	2.8	-1.4
Pik3r3	18710	1.7	1.2	3.7	1.3	1.8	-1.7	1.1	2.1	-1.3	1.1	3.1
Pik3r5	320207	1.4	1.4	1.4	1.4	2.0	1.4	1.7	1.2	2.3	1.7	1.3
Pik3r6	104709	1.5	1.3	-1.2	-1.1	1.1	1.2	1.1	1.5	1.4	1.3	1.3
Pikfyve	18711	1.4	-1.2	5.5	2.6	1.1	1.5	5.3	1.7	1.9	1.1	1.0
Pilra	231805	1.8	1.7	1.2	1.1	1.0	1.5	-1.1	1.1	1.7	1.0	1.1
Pilrb1	170741	5.2	1.9	3.5	1.5	1.2	2.3	2.4	1.5	4.4	2.7	2.2
Pla2g2a	18780	1.7	1.5	-1.2	3.0	2.5	2.8	1.5	-1.3	3.8	3.1	-1.2
Pla2g2c	18781	2.1	1.7	10.3	4.7	5.3	1.2	3.5	3.3	3.8	3.5	11.4

Pla2g3	237625	-1.0	-1.7	5.7	1.0	1.6	-1.4	-1.3	3.4	1.2	-1.0	3.4
Pla2g4a	18783	1.6	1.1	1.6	1.1	1.0	-1.1	1.3	2.3	-1.1	1.4	1.3
Ppp1cb	19046	1.8	1.3	1.9	1.2	1.0	1.1	1.9	1.9	1.5	1.9	1.6
Ppp1cc	19047	1.4	1.4	1.1	1.1	1.2	1.1	1.7	1.5	1.8	1.9	1.1
Ppp1r12a	17931	1.2	1.6	1.2	1.4	-1.3	1.3	1.3	-1.0	2.5	1.4	1.7
Ppp1r13l	333654	1.7	1.8	1.8	1.4	1.4	1.2	1.3	1.2	1.4	1.5	1.5
Ppp1r14a	68458	1.1	-1.2	1.6	1.7	3.3	2.1	1.0	-1.1	1.1	-1.2	1.4
Ppp1r15a	17872	-1.2	1.1	1.2	-1.1	1.4	1.2	-1.0	-1.1	1.9	1.0	-1.0
Ppp1r2	66849	2.0	1.5	2.0	1.4	1.8	1.1	1.9	2.2	2.2	2.5	2.2
Ppp1r21	73825	1.5	1.8	-1.1	-1.2	1.0	-1.3	1.2	1.4	1.5	1.3	2.0
Ppp1r26	241289	3.7	3.2	3.8	-1.0	1.4	1.6	4.7	5.3	1.6	4.9	-1.5
Ppp1r27	68701	7.0	2.0	1.3	1.3	1.5	2.0	2.2	1.0	1.1	1.6	2.3
Ppp1r32	67752	1.8	1.9	1.5	1.2	1.3	1.2	2.3	1.2	2.4	1.7	1.6
Prkar1a	19084	3.1	1.4	-1.0	2.0	-1.2	2.9	2.9	1.7	1.3	3.4	1.6
Prkar1b	19085	-2.0	-3.8	-2.4	-1.3	2.3	-1.0	-1.8	-1.3	-1.4	-1.1	-2.0
Prkar2a	19087	-3.0	-3.0	-2.5	1.1	1.1	-1.0	-3.6	-3.1	-2.9	-3.9	-2.5
Prkar2b	19088	1.3	1.1	-1.2	-1.2	1.5	1.8	1.8	3.0	1.2	1.8	1.0
Ptger1	19216	3.0	2.3	2.4	2.7	-1.0	1.6	1.2	2.4	3.0	1.1	1.7
Ptger2	19217	1.9	1.2	1.4	1.6	1.2	1.4	4.0	2.1	2.1	2.4	-3.3
Ptger3	19218	-1.9	-6.7	-1.4	-1.1	1.7	1.5	-1.4	1.5	-1.3	1.3	-2.1
Ptgir	19222	1.7	1.4	2.6	-1.9	2.0	1.6	1.5	3.3	-2.3	5.6	2.4
Ptgis	19223	2.4	1.4	1.8	1.4	2.4	2.1	1.2	1.6	3.7	1.8	2.0
Ptgs1	19224	1.3	1.2	1.2	-1.3	1.4	1.4	1.6	1.8	1.7	1.7	1.1
Ptgs2	19225	1.9	1.8	2.7	-1.5	-1.0	1.2	2.0	3.2	1.3	3.6	1.4
Rb1	19645	2.3	1.7	1.4	1.0	-1.8	1.1	2.1	1.7	-1.0	1.7	2.0
Samd12	320679	3.7	5.3	63.4	5.7	4.3	1.3	2.1	5.8	5.1	8.7	6.8
Samd14	217125	1.2	1.6	1.4	1.6	2.4	1.9	1.3	1.3	1.3	1.4	1.5

Samd4	74480	2.3	1.5	2.7	1.0	21.8	-1.0	1.7	7.2	14.2	2.3	1.8
Samd4b	233033	1.7	1.8	1.8	1.3	1.2	1.3	1.5	1.6	2.6	2.2	2.0
Samd5	320825	4.2	2.3	-1.6	1.6	1.1	2.2	4.8	2.3	1.8	2.9	1.4
Slc10a2	20494	2.6	5.6	2.5	-1.0	1.5	4.9	1.6	4.2	10.9	2.3	3.2
Slc10a7	76775	-2.0	1.1	-1.3	1.0	1.4	4.3	-1.3	2.1	1.4	1.9	1.2
Slc11a1	18173	1.3	1.4	1.1	1.3	2.5	1.4	1.3	1.5	3.6	1.6	-1.0
Slc12a1	20495	6.7	8.5	7.6	9.8	10.8	5.8	11.0	9.8	10.1	8.2	11.2
Slc12a2	20496	1.8	1.1	1.1	1.1	-1.2	1.2	1.9	2.0	1.1	1.7	1.0
Slc13a1	55961	2.2	1.3	1.9	1.1	-1.5	2.8	1.9	2.4	-1.4	1.8	1.9
Slc13a3	114644	1.6	1.6	1.5	1.2	2.5	2.1	2.1	1.0	10.4	1.5	1.7
Slc14a1	108052	5.4	5.3	1.4	1.2	-1.1	1.2	1.8	2.1	-2.9	1.9	3.8
Slc15a1	56643	1.9	2.5	1.3	-2.5	2.3	-3.2	2.1	1.2	1.7	-1.7	2.4
Slc15a2	57738	-1.4	2.6	1.6	-5.7	-6.7	-13.1	2.3	2.5	1.4	-1.5	1.8
Slc15a3	65221	-1.2	1.0	-1.1	-1.0	1.1	1.0	-1.2	-1.0	2.7	1.0	-1.0
Slc15a5	277898	1.1	1.1	1.1	1.5	1.3	1.4	-1.3	3.8	1.3	1.2	1.4
Slc16a10	72472	2.5	4.2	6.1	2.6	3.0	1.8	3.3	4.5	6.1	-3.0	3.3
Slc16a12	240638	2.1	1.7	-1.7	1.3	1.9	1.7	3.3	10.5	-1.8	3.7	1.5
Slc16a13	69309	-4.1	7.8	3.4	1.1	-1.3	1.1	1.6	3.2	1.7	-2.8	1.5
Slc16a2	20502	1.8	5.1	2.8	2.4	3.7	6.0	2.8	4.8	12.9	4.0	2.3
Slc16a8	57274	-1.5	-1.3	2.0	-1.2	-1.6	-1.2	-1.6	1.8	2.0	1.1	-4.7
Slc16a9	66859	1.4	1.9	1.5	-1.3	1.3	-1.2	1.3	1.6	3.7	2.2	1.9
Slc17a8	216227	1.6	3.0	2.7	1.2	1.3	3.6	2.8	3.4	6.9	4.2	3.3
Slc17a9	228993	-1.2	-1.1	4.1	2.1	1.8	-1.4	-1.1	2.5	1.8	1.3	3.1
Slc18a1	110877	1.1	1.5	-1.0	-1.1	-1.5	1.1	1.6	-1.0	1.2	1.9	1.4
Slc18a3	20508	1.9	-1.1	2.2	-1.0	2.9	1.6	2.1	1.5	2.5	-1.0	1.0
Slc19a1	20509	1.6	-1.2	-1.0	2.9	2.3	2.8	1.4	1.4	4.0	1.4	2.3
Slc1a5	20514	-1.3	1.1	1.1	1.0	1.8	1.0	1.0	1.3	1.5	1.1	-1.1

Slc1a6	20513	9.4	8.7	5.0	9.0	12.9	6.7	13.1	6.5	6.6	4.6	13.5
Slc20a1	20515	1.4	1.8	2.8	2.0	4.2	-1.1	1.7	2.1	1.8	1.7	3.9
Slc20a2	20516	-1.6	-2.1	-1.6	2.0	1.9	1.5	-1.5	-2.0	1.4	-1.7	-2.1
Slc22a13	102570	1.0	3.0	1.5	2.0	1.1	1.2	4.8	2.5	2.4	3.2	2.7
Slc22a15	242126	2.5	1.7	-1.0	1.6	-1.1	1.3	2.9	1.7	1.8	3.3	1.2
Slc22a16	70840	1.5	1.4	1.3	-1.2	1.9	1.9	1.9	1.7	1.7	1.4	2.0
Slc22a17	59049	1.1	1.1	1.6	-1.1	1.3	1.3	1.6	1.9	1.6	1.3	1.0
Slc25a16	73132	4.4	2.2	1.7	1.1	-1.0	1.5	6.4	3.8	2.4	4.5	2.1
Slc25a32	69906	2.7	1.8	1.6	1.1	-1.1	1.0	2.6	2.2	4.4	3.5	1.7
Slc25a33	70556	1.7	3.0	3.7	2.0	2.1	1.4	1.8	2.2	13.3	5.2	2.6
Slc27a5	26459	2.3	11.2	-2.2	1.7	3.8	1.4	4.2	4.4	1.0	4.6	1.8
Slc29a2	13340	2.2	3.0	2.5	1.4	3.4	1.5	3.6	3.2	2.2	6.6	4.1
Slc29a3	71279	-1.1	-1.5	-1.2	2.1	2.9	4.8	-1.1	-1.5	-1.0	1.1	-1.7
Slc2a1	20525	-1.1	-1.1	5.1	2.8	3.6	1.0	1.0	2.6	3.0	1.2	2.9
Slc34a1	20505	2.1	2.9	1.1	-1.3	2.5	3.6	1.6	1.9	15.0	3.7	2.3
Slc35d2	70484	-1.6	2.7	2.0	1.8	-2.0	1.2	8.3	6.1	2.6	15.2	5.2
Slc35d3	76157	2.2	1.7	4.0	-1.6	2.7	3.3	2.9	2.2	1.4	2.1	2.9
Slc35f4	75288	8.0	5.2	6.2	3.4	3.7	2.7	2.0	3.4	3.9	8.0	5.4
Slc35g1	240660	1.3	1.4	-1.0	-1.0	1.0	1.0	1.8	1.1	2.1	1.6	1.1
Slc35g2	245020	1.6	1.4	1.7	1.3	1.1	1.3	1.8	1.6	1.1	1.8	1.2
Slc37a2	56857	1.5	1.2	-1.7	3.4	30.3	-1.1	1.1	1.3	1.0	1.2	1.9
Slc41a1	98396	5.2	2.4	1.6	2.7	2.6	2.9	1.7	1.6	2.9	2.8	-1.3
Slc43a1	72401	2.7	2.3	2.1	1.3	2.2	1.7	1.7	2.8	3.8	1.7	2.2
Slc4a10	94229	3.8	-1.2	-1.3	1.1	-2.2	1.1	1.1	-1.6	-1.0	-1.2	-1.6
Slc4a11	269356	2.4	4.1	2.8	3.0	1.4	1.7	2.5	2.0	2.4	5.2	8.4
Smad2	17126	1.5	1.9	1.5	1.1	1.1	1.0	1.6	1.5	1.4	1.6	1.4
Smad3	17127	1.9	2.0	1.4	1.3	2.2	1.5	2.5	2.7	2.1	1.6	3.4

Smad4	17128	1.0	-1.3	-1.5	1.5	1.6	1.5	-1.1	-1.5	2.2	1.2	-1.4
Smad6	17130	3.8	3.0	1.4	1.5	1.0	1.4	3.5	1.7	-1.1	1.6	2.2
Smad7	17131	-2.2	1.3	2.0	-1.1	-2.1	-1.3	1.9	-1.1	1.0	2.4	1.4
Smad9	55994	2.2	3.3	-1.0	-1.2	1.2	1.2	2.5	3.4	1.8	3.4	2.4
Socs1	12703	1.2	-1.7	1.8	-1.4	-1.6	-1.2	-1.4	1.4	2.4	-1.1	2.0
Socs3	12702	-1.2	-1.0	-1.3	1.6	1.2	1.3	1.1	-1.3	3.2	2.2	-1.1
Socs5	56468	3.9	2.8	4.2	1.4	2.1	2.2	4.2	6.3	1.8	3.5	5.7
Stat1	20846	-1.3	-1.1	3.9	-1.5	-1.9	-1.6	-1.6	2.6	-1.6	1.4	2.1
Stat2	20847	1.4	-1.0	2.4	-1.4	-1.4	-1.2	-1.2	1.5	-1.3	-1.3	2.1
Stat3	20848	-2.0	-2.6	-2.1	2.7	1.5	1.3	-2.9	-3.9	-1.8	-1.9	-2.9
Tgfa	21802	2.3	1.8	2.2	1.8	1.3	1.4	2.5	1.7	2.2	3.0	2.0
Tgfb1	21803	-2.3	-1.7	-2.2	1.3	2.0	1.5	-3.4	-3.5	-1.3	-3.3	-1.8
Tgfb1i1	21804	-1.7	-2.4	-1.9	-1.4	3.7	1.6	-2.5	1.4	-1.6	-1.7	-5.1
Tgfb2	21808	3.3	4.1	4.2	1.9	2.0	2.3	4.7	5.1	3.8	5.4	2.0
Tgfb3	21809	1.6	-1.0	1.3	1.3	1.4	2.4	1.5	1.6	1.0	1.2	1.5
Tgfbr1	21812	-1.0	-3.0	1.0	-2.2	1.2	1.1	1.8	3.1	-1.1	1.2	-1.3
Tgfbr3	21814	2.5	1.5	1.7	1.5	3.2	2.9	3.5	2.2	4.2	2.9	1.8
Tgm1	21816	1.6	3.3	2.8	-1.7	-1.0	1.3	-1.4	2.0	4.6	3.2	2.1
Tlr2	24088	2.2	1.1	2.3	1.0	1.0	1.2	1.3	2.6	2.6	1.4	1.4
Tlr4	21898	1.1	-1.6	4.5	1.9	3.6	1.3	-1.3	2.6	1.9	-1.0	2.3
Tlr6	21899	2.8	13.2	5.1	2.5	8.9	3.6	2.4	5.8	12.7	3.9	2.3
Tlr7	170743	-1.8	1.3	1.3	1.1	1.1	-1.3	1.3	1.2	2.2	1.1	-2.1
Tlr8	170744	2.8	-1.9	1.6	1.0	1.1	-1.9	-1.1	-1.6	1.2	-2.1	-1.6
Tlr9	81897	-1.5	-1.5	-1.6	-1.1	-1.8	-1.3	-1.0	-1.7	-1.4	-1.3	-1.4
Tmem132e	270893	2.1	1.8	1.4	1.1	1.0	1.3	2.1	1.9	1.5	1.3	1.7
Tmem136	235300	1.8	3.2	2.9	-1.2	2.8	1.3	4.6	5.1	4.5	3.6	1.3
Tmem150a	232086	-1.4	1.0	-1.6	1.4	2.7	1.7	-1.1	-1.7	1.1	-1.1	-1.2

Tmem158	72309	1.6	1.4	-1.4	1.6	-1.3	1.9	1.8	-1.4	-1.4	1.7	-1.1
Tmem159	233806	1.3	1.6	1.2	1.4	1.7	1.7	1.7	1.3	1.8	1.5	1.1
Tmem161b	72745	3.9	1.9	1.5	-1.2	-1.8	-1.5	2.5	3.1	3.4	3.9	1.7
Tmem165	21982	1.6	1.3	1.1	1.1	-1.2	-1.0	1.8	1.4	1.4	1.6	1.4
Tmem167	66074	1.9	1.0	-1.1	1.1	-1.6	1.0	1.7	-1.0	-1.6	1.5	-1.0
Tmem167b	67495	1.9	2.0	1.9	1.1	1.0	1.1	2.0	2.0	1.7	1.9	2.2
Tnf	21926	1.3	1.4	1.4	1.0	1.9	-1.1	-1.1	-1.3	2.2	1.1	1.5
Tnfaip2	21928	1.4	1.2	1.0	1.1	1.1	1.4	1.2	1.3	2.6	1.5	1.3
Tnfaip3	21929	1.2	1.2	1.4	-1.2	1.0	-1.7	-1.1	1.1	2.0	1.1	1.6
Tnfaip6	21930	-1.3	-1.3	-1.3	1.1	-1.1	1.0	1.0	-1.1	-5.0	1.1	1.0
Tnfaip8	106869	-1.6	-1.5	-1.3	-1.0	-1.8	1.5	-1.1	-2.0	-4.5	-2.0	-1.2
Tnfaip8l1	66443	1.2	1.1	1.3	1.1	1.3	1.5	1.3	1.5	2.6	1.5	-1.2
Tnfaip8l3	244882	1.9	-1.5	-1.3	1.2	-2.8	-1.0	1.2	1.1	-1.2	1.1	-1.2
Tnfrsf10b	21933	-1.1	1.2	1.0	1.0	-1.9	-1.3	1.4	-1.1	-2.5	-1.1	1.2
Tnfrsf11a	21934	-1.3	-1.1	1.2	1.2	-1.1	-1.3	1.1	1.2	-1.7	-1.2	1.1
Tnfrsf11b	18383	2.9	-1.2	1.2	-1.1	-1.3	1.6	2.7	1.6	1.2	1.7	1.2
Tnfrsf12a	27279	-1.1	1.1	2.3	1.4	1.2	1.5	-1.2	-1.2	1.4	-1.1	1.4
Tnfrsf13b	57916	-1.1	1.3	-1.4	-1.8	-1.1	1.0	1.1	1.3	1.4	-1.6	1.0
Tnfrsf13c	72049	-3.2	-1.0	-3.9	-6.7	-5.1	-2.1	-4.8	-3.5	-1.2	-5.2	1.0
Tnfrsf14	230979	-1.4	-1.7	-2.1	1.4	-1.5	1.2	-1.3	-2.0	-1.6	-1.3	-1.5
Tnfrsf17	21935	1.5	1.9	1.3	1.1	2.6	1.8	2.2	2.3	1.3	1.2	1.4
Tnfrsf18	21936	1.1	1.6	1.2	-1.5	1.1	-1.4	-1.3	1.1	1.2	1.1	1.0
Tnfrsf19	29820	1.6	1.5	-1.1	-1.2	1.3	1.2	1.9	1.8	-1.8	1.8	-1.3
Tnfrsf1a	21937	1.4	1.4	1.2	1.1	1.0	1.1	1.3	-1.1	2.1	1.7	1.4
Tnfrsf1b	21938	2.0	1.4	2.0	1.1	1.7	-1.1	-1.0	4.6	2.6	2.6	2.7
Tnfrsf21	94185	-3.2	-2.6	1.1	1.6	2.0	1.5	-2.9	-3.2	1.3	-2.3	-1.1
Tnfrsf22	79202	2.1	-1.1	1.1	1.1	-1.3	-1.1	-2.7	1.3	3.1	2.7	1.7

Tnfrsf23	79201	-1.5	-1.1	1.9	1.0	-1.4	-1.6	-3.1	1.3	1.8	-1.3	2.5
Tnfrsf25	85030	-1.5	-1.1	-1.3	-2.0	-2.0	-1.3	-1.7	-1.4	-1.0	-1.5	-1.3
Tnfrsf4	22163	-1.1	1.2	1.0	-1.9	-1.2	-1.8	-1.5	-1.0	2.5	1.1	1.1
Tnfrsf9	21942	1.3	-1.5	-1.1	-2.3	-2.0	-2.1	-2.1	2.0	1.2	1.1	-1.2
Tnfsf10	22035	3.1	-1.5	-1.5	-2.3	-3.4	-2.1	1.1	1.3	-2.4	-1.1	-1.0
Tnfsf11	21943	-1.5	-1.6	1.5	-1.7	-1.6	-1.3	1.3	1.8	-1.0	1.5	2.1
Tnfsf12	21944	-1.4	-1.4	-2.2	-1.2	-1.3	-1.0	-1.9	-2.4	-2.5	-2.7	-2.0
Tnfsf13b	24099	1.1	-1.2	1.1	-2.3	-2.0	-1.5	-1.5	-1.1	-2.2	-1.3	1.2
Tnfsf14	50930	-1.4	-1.2	-1.9	-1.5	-1.5	-1.2	-1.0	1.8	-2.1	1.9	-1.4
Tnfsf15	326623	1.1	1.7	-1.4	-1.4	-1.8	-1.2	1.5	1.0	-1.6	1.5	-1.3
Tnfsf4	22164	5.1	-1.8	4.5	7.7	3.1	7.2	6.8	5.6	5.9	4.3	7.2
Tnfsf8	21949	-3.3	1.2	-1.1	-6.2	-2.6	-1.1	-6.2	-1.1	-1.9	-1.1	1.3
Tnfsf9	21950	1.1	1.8	1.9	-1.5	-1.5	1.2	1.1	1.8	4.1	1.6	1.1

Annexure 6: Gut histopathology scoring methodology

Category	Criterion	Definition	Score value
Inflammatory cell infiltrate	Severity	Leukocyte density of lamina propria area infiltrated in evaluated hpf.	
		Minimal: <10%	1
		Mild: 10-25%;scattered neutrophils	2
		Moderate: 26-50%	3
		Marked: >51%; dense infiltrate	4
	Extent	Expansion of leukocyte infiltration:	
		Mucosal	1
		Mucosal and Submucosal	2
		Mucosal, Submucosal and transmural	3
Epithelial changes	Hyperplasia	Increase in epithelial cell numbers in longitudinal crypts relative to baseline epithelial cell numbers per crypt; visible as crypt elongation	
		Minimal: <25%	1
		Mild: 21-35%	2 or 3
		Moderate: 36-50%	3 or 4

		Marked: >50%	4 or 5
	Goblet cell loss	Reduction of Goblet cell numbers relative to besaline goblet cell numbers per crypt:	
		Minimal: <20%	1 or 2
		Mild: 21-35%	2 or 3
		Moderate: 36-50%	3 or 4
		Marked: >50%	4
	Cryptitis	Neutrophils between crypt epithelial cells	2 or 3
	Crypt abscesses	Neutrophils in crypt lumen	3 to 5
	Erosion	Loss of surface epithelium	1 to 4
Mucosal architecture	Ulceration	Epithelial defect reaching beyond muscularis mucosae	3 to 5
	Granulation tissue	Connective tissue repair with new capilaries, surrounded by spindle-shaped fibroblasts, myofibroblasts, macrophages, neutrophils and mononuclear cells as well as cell debris; pserdopolyps: villiformous,hypertrophied areas projecting into the lumen	4 or 5
	Irregular crypts	Non-parallel crypts, variable crypt diameters, bifurcation and branched crypts	4 or 5
	Crypt loss	Mucosa devoid of crypts	4 or 5
	Villous blunting	Mild: villous-to-crypt-length ratio of 2:1 to 3:1	1 to 3
		Moderate: villous-to-crypt-length ratio of 1:1 to 2:1	2 to 4
		Villous atrophy	3 to 5

Annexure 7: Histopathology Scores

Treatment conditions	Histopathology Scores (Score ± SD)							
	5th Day BALB/c				5th Day C57BL/6			
	Ileum	Colon	Spleen	Liver	Ileum	Colon	Spleen	Liver
NT	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.8	2.3 ± 0.5	1.3 ± 0.9	0.7 ± 0.9	3.3 ± 0.5	2.7 ± 0.9
ST	32.3 ± 0.5	19.3 ± 1.7	26.0 ± 1.6	19.3 ± 3.4	32.0 ± 2.2	23.3 ± 2.9	17.7 ± 6.4	32.0 ± 3.3
STLA	8.3 ± 0.5	5.0 ± 2.2	6.0 ± 1.6	6.7 ± 1.9	9.7 ± 0.9	12.0 ± 1.6	5.3 ± 2.5	8.0 ± 3.3
STBC	35.7 ± 0.9	26.0 ± 1.6	24.0 ± 4.3	20.7 ± 5.2	39.7 ± 0.5	38.7 ± 2.6	27.3 ± 4.1	26.0 ± 5.9
LA	4.7 ± 3.1	4.0 ± 3.3	7.3 ± 3.4	5.3 ± 2.5	5.3 ± 1.7	7.3 ± 0.9	5.3 ± 3.4	4.7 ± 2.5
BC	12.7 ± 4.1	10.0 ± 1.4	9.3 ± 2.5	8.0 ± 3.3	9.7 ± 2.1	11.0 ± 3.3	6.7 ± 3.4	6.0 ± 3.3

Annexure 8: Species specific primer designing and primer optimization protocol against 16S rRNA

8.1 Primer designing method

1. Collect nucleotide sequence of 16s rRNA of the species of interest from NCBI nucleotide database. Align it with the 16s rRNA database of bacteria and archaea using NCBI-BLAST.
2. Take top 25 hits and align it using multiple sequence alignment software MUSCLE. Download aligned sequenceses in HTML output format of the MUSCLE.
3. Search for unique bases in the sequence of interest from the 5'end, fix the unique base G or C as the 3'-OH base for the forward primer and extend towards the 5' end till the melting temperature (Tm) reaches 55°C (we generally keep Tm of the primer at 55°C).
4. Similarly searxh for unique bases in the sequence of interest from the 3' end, fix the unique base G or C as the 3'-OH base for the reverse primer and extend towards the 3' end till Tm reaches 55°C. While extending the sequence, write the complementary sequence. This will give you the reverse primer.
5. Don't fix length of the primer and product constant, as done by many existing online primer designing softwares. This will reduce the chance of finding a pair of unique primers from the whole 16s rRNA sequence.
6. Check primer efficiency and specificity through qRTPCR as described below.

8.2 Primer optimization methodology

1. In every reaction use 300 nM primers (Recommended, but can be varied from 100 to 400 nM) each of forward and reverse primer.

2. Serially dilute the template mixture (From Faecal sample) by 10 folds and use 1 ul of the 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions as the template in each reaction.
3. Perform the qRTPCR reaction for 40 cycles along with melting curve in triplicates along with no template control (NTC).
4. Do a semi-log Plot **Ct Vs dilution** and find out the slope of the curve.
5. Calculate the efficiency of the primer by using the formula **e= 10^(-1/slope)**
6. From the melting curve make sure that, you are getting only single type of product and there is no primer dimer in the NTC reaction.
7. If the efficiency of primer is 100%, the slope of the semi-log plot will be -3.321928.

8.3 Species specific primers designed and optimized in this project

Listed below are the bacterial species and their respective primers against 16S rRNA designed by the method described above.

Name of Bacteria	NCBI Assn.No	Primer name	Primer Sequence from 5' to 3'	Nt. Length	Tm in °C
Clostridium Xylanolyticum	NR_037068.1	Clxy61F	ATT TTA AAG GAA GTT TTC GGA TGG AAT TTA G	31	54.9
		Clxy217R	AGTTTTTCACACTGTACTATGCAGC	25	55.7
Clostridium Perfinges	NR_121697.1	Clpe184F	GTTGAAAGATGGCATCATCATTCAA C	26	55.1
		Clpe449R	CCCCAAAGACAGAGCTTTACG	21	55.8
Clostridium subterminale	NR_113027.1	Clsu186F	GCATGGTAGAAACATCAAAGGAGC	24	56.4
		Clsu639R	CCTGCACTCTAGATATCCAGTTTG	24	54.9
Butyricicoccus pullicaecorum	NR_044490.1	Bupu72F	GGAAATCCTTCGGGATGGAATC	22	55.6
		Bupu648R	ACTCAAGACTCGCAGTTTTGAAAG	24	55.7
Aerococcus sanguinicola	AY837833.1	Aesa166F	GCATAGTAATTTGTCAGGCATCTGA C	26	56
		Aesa498R	TGGTAAGATACCGTCAAGACTGTAG	25	55.6
Clostridium scindens	NR_028785.1	Clsc79F	CTTCTTCGGAACGAGGAGC	19	55.4
		Clsc647R	TTCGACACTCCAGCCACG	18	56.1
Lachnospira multipara	FR733699.1	Lamu76F	TTCCTTCGGGATGACGATTTG	21	55.1
		Lamu209R	TCACACTGCATCATGCGATC	20	55.8
Candidatus	D86302.1	Caar61F	GAGGTAGATGGAGCTTGCTC	20	54.8

arthromitus		Caar177R	TGTATGCGGTATTAATCCAACTTTC ATC	28	55.5
Clostridium tertium	NR_113325.1	Clte63F	GAGGAGTTCCTTCGGGAAC	19	55
		Clte225R	GCCCATCTTGTAGCGGATTG	20	56.2
Faecalibacterium prausnitzii	AJ413954.1	Fapr53F	GAGCGAGAGAGAGCTTGC	18	55.5
		Fapr244R	GCCATCTCAAAGCGGATTG	19	54.3
Finegoldia magna	NR_113383.1	Fima80F	GCTTCGGTGGAAGATTACTAATGAG	25	55.3
		Fima222R	CCGTTAAAACATGTGTTTCTACGAT TTTATG	31	55.5

Efficiency and specificity of each of these primers are tested through qRTPCR using the method described above. Few of those results are given below.

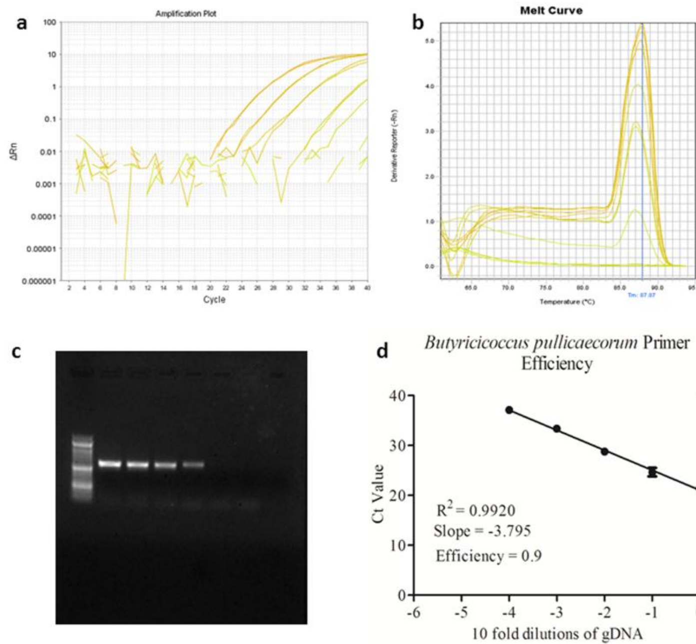


Figure Annex1: Validation of Primer efficiency for *Butyricicoccus pullicaecorum*.
a. Amplification plot **b.** Melting curve showing the single peak **c.** Agarose gel electrophoresis of the PCR products showing the single product **d.** Ct vs Dilution curve has the slope of -3.795 and primer efficiency of 90%.

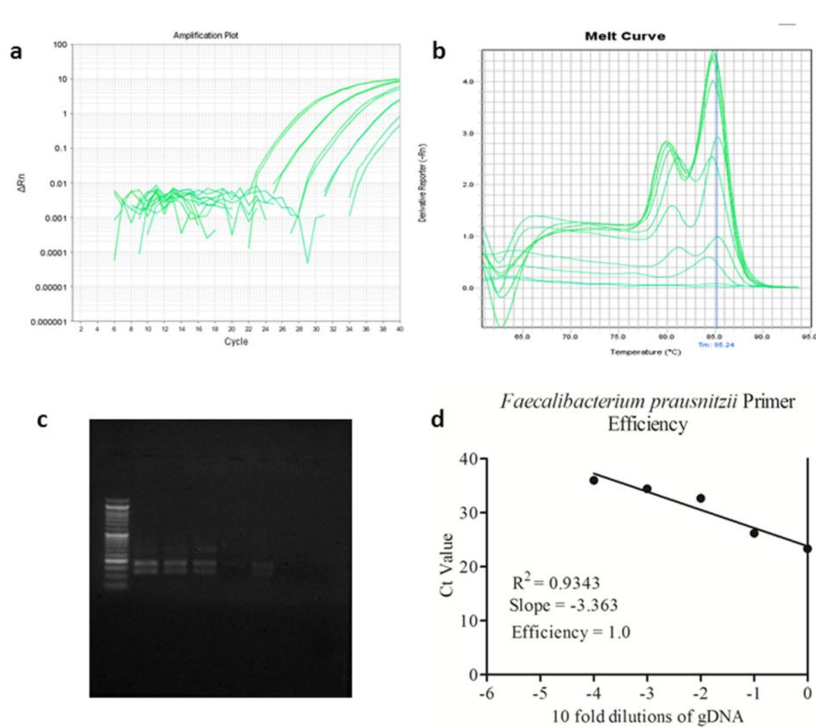


Figure Annex2: Validation of Primer efficiency for *Faecalibacterium prausnitzii*.
a. Amplification plot **b.** Melting curve showing the single peak **c.** Agarose gel electrophoresis of the PCR products showing the single product **d.** Ct vs Dilution curve has the slope of -3.363 and primer efficiency of 100%.

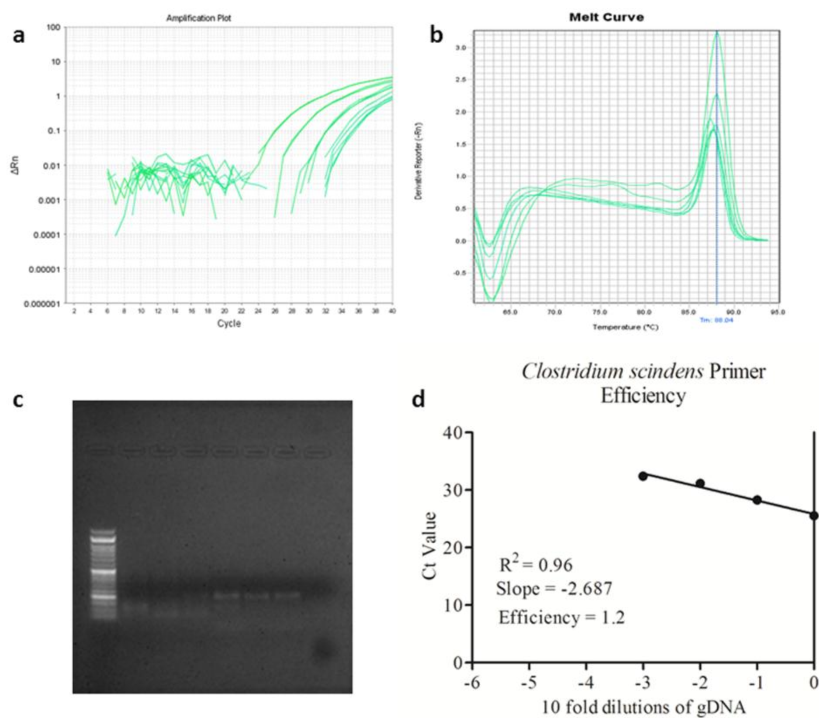


Figure Annex3: Validation of primer efficiency for *Clostridium scindens*.
a. Amplification plot **b.** Melting curve showing the single peak **c.** Agarose gel electrophoresis of the PCR products showing the single product **d.** Ct vs Dilution curve has the slope of -2.687 and primer efficiency of 120%.

8.4: Determination of microbial composition through qRTPCR in phylum level

Below are the list of primers against 16S rRNA reported for major phyla present in mice and human gut.

Phylum Name	Primer Name	Nucleotide Sequence	Nt. Length	Tm in ^{o}C
Universal Bacterial primer	U926F	AAACTCAAAKGAATTGACGG	20	61.5
	U1062R	CTCACRRCACGAGCTGAC	18	61.5
α-Proteobacteria	α682F	CIAGTGTAGAGGTGAAATT	19	61.5
	α908R	CCCCGTCAATTCCTTTGAGTT	21	61.5
γ-Proteeobacteria	γ1080F	TCGTCAGCTCGTGTYGTGA	19	61.5
	γ1202R	CGTAAGGGCCATGATG	16	61.5
Bacteroidetes	Bact798F	CRAACAGGATTAGATACCCT	20	61.5
	Bact967R	GGTAAGGTTCCTCGCGTAT	19	61.5
Firmicutes	Firm928F	TGAAACTYAAAGGAATTGACG	21	61.5
	Firm1040R	ACCATGCACCACCTGTC	17	61.5
Actinobacteria	Act920F	TACGGCCGCAAGGCTA	16	61.5
	Act1200R	TCRTCCCCACCTTCCTCCG	19	61.5

We reverified the primer efficiency and specificity using the method described above. We found that all these primers amplify single product and their efficiency is close to 100%.

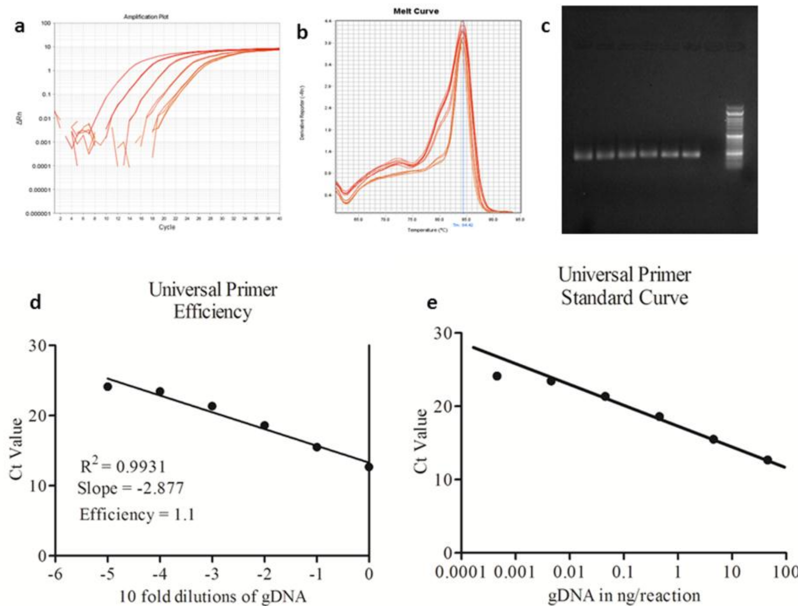
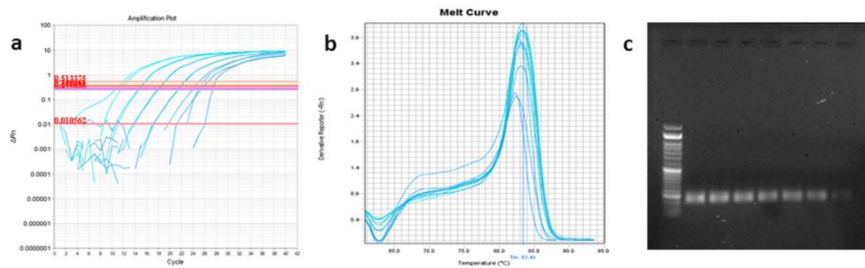


Figure Annex4: Primer efficiency and specificity for Universal bacterial primer. **a.** Amplification plot, **b.** Melting curve showing the single peak, **c.** Agarose gel electrophoresis of the PCR products showing the single product, **d.** Ct vs Dilution curve has the slope of -2.877 and primer efficiency of 110%, **e.** Ct vs Template amount (standard) curve is a straight line



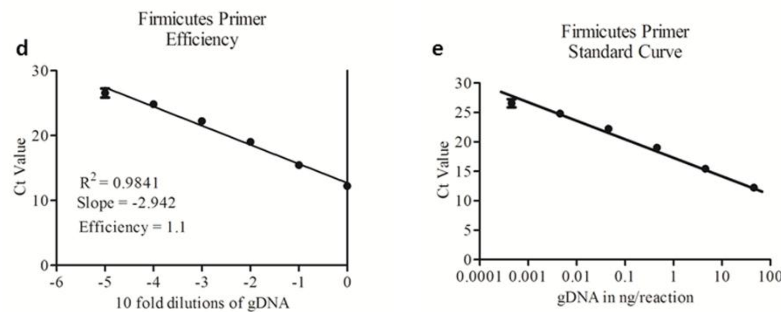
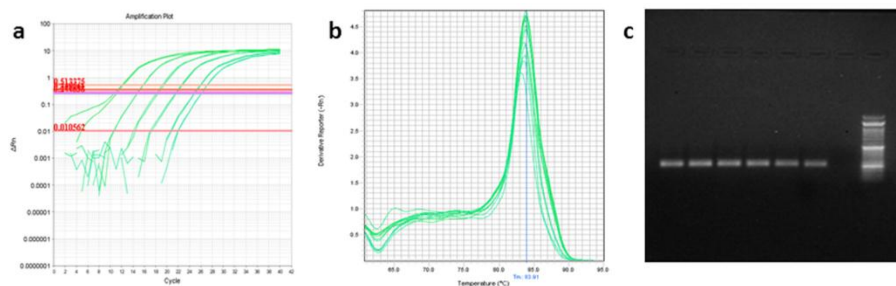


Figure Annex5: Primer efficiency and specificity for Firmicutes.
a. Amplification plot, **b.** Melting curve showing the single peak, **c.** Agarose gel electrophoresis of the PCR products showing the single product, **d.** Ct vs Dilution curve has the slope of -2.942 and primer efficiency of 110%, **e.** Ct vs Template amount (standard) curve is a straight line



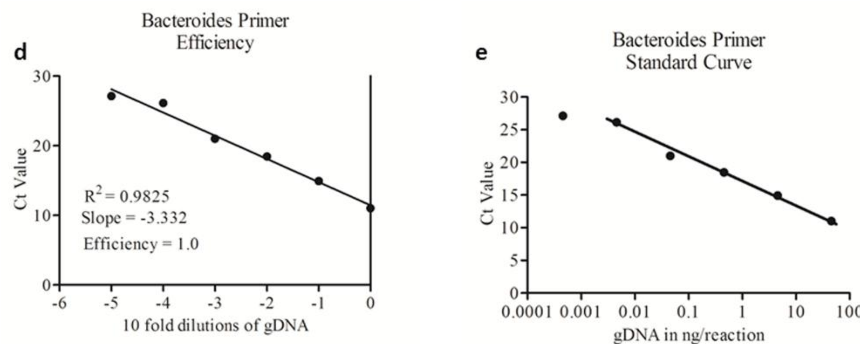


Figure Annex6: Primer efficiency and specificity for Bacteroides.
a. Amplification plot, **b.** Melting curve showing the single peak, **c.** Agarose gel electrophoresis of the PCR products showing the single product, **d.** Ct vs Dilution curve has the slope of -3.332 and primer efficiency of 100%, **e.** Ct vs Template amount (standard) curve is a straight line

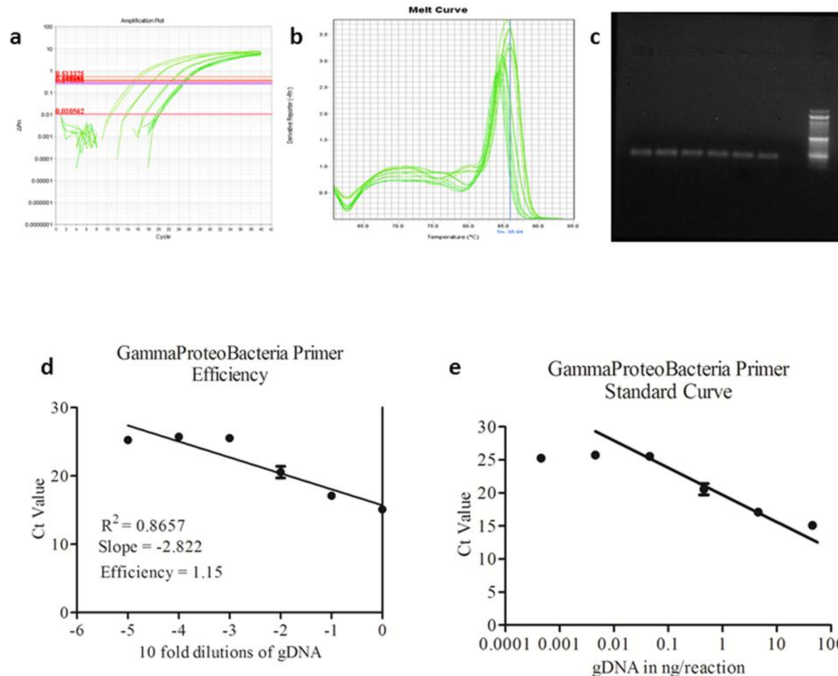


Figure Annex7: Primer efficiency and specificity for GammaProteoBacteria. **a.** Amplification plot, **b.** Melting curve showing the single peak, **c.** Agarose gel electrophoresis of the PCR products showing the single product, **d.** Ct vs Dilution curve has the slope of -2.822 and primer efficiency of 115%, **e.** Ct vs Template amount (standard) curve is a straight line

The average size of the genome of microbiota is about 2 Mb, corresponding to the molecular weight of 1.32 x 10^9 amu. 100 ng of gDNA is equivalent to 7.6 x 10^{-17} moles. Assuming a single copy of 16S rRNA gene per genome, number of copies of 16S rRNA per 100 ng of gDNA will be 4.6 x 10^7. Accordingly copy numbers in other concentrations of DNA can be calculated, and Ct vs Copy number of 16S rRNA can be plotted as a standard curve for universal primer. Ct values for different phyla can be compared with the standard curve for universal primer and corresponding copy numbers can be determined. From the copy numbers of all phyla, percentage abundance of phyla in a particular gDNA can be calculated. This is an easy, economical and fast procedure to profile the microbial composition in a sample without going for next generation genome sequencing.

Thesis Evaluation Reports

Homi Bhabha National Institute

Ph.D. Thesis Evaluation Report

1. **Name of the Constituent Institution:** National Institute of Science Education and Research
2. **Name of the Student:** Biswaranjan Pradhan
3. **Enrolment No.:** LIFE07201004014
4. **Title of the Thesis:**
5. **Name of the Board of Studies:** Life Sciences

Recommendations

Tick one of the following:

1. The thesis in its present form is commended for the award of the Ph.D. Degree. ☐
2. The thesis is commended for the award of the Ph.D. degree. However, my suggestions for improving the thesis may be considered at the time of the viva voce examination and if the viva voce board deems it appropriate, the same may be incorporated in the thesis based on the discussions during the viva voce examination. The revised thesis need not be sent to me. ☒
3. The thesis should be revised as per the suggestions enclosed. I would like to see the revised thesis incorporating my suggestions before I give further recommendations. ☐
4. The thesis is not acceptable for the award of the Ph.D. degree. ☐

Date: Jan 2 - 2017

(Signature): Lorne A Babiuk

Name of Examiner:

And affiliation

Please give your detailed report in the attached sheet. You may use additional sheets, if required.

Version approved during the meeting of Deans held during 29-30 Nov 2013

1. **Name of the Student:**

2. **Title of the Thesis:**

DETAILED REPORT

The current thesis is a well-focused series of experiments designed to help reduce (eventually) the use of antibiotics. Currently, there is a great concern regarding the overuse of antibiotics in society, and a push to treat individuals in a more natural way to control infectious diseases. The thesis focus is on stimulating innate immune responses – as a first defense against infection. Initially they used host defense peptides to stimulate innate immune responses. The interest in society to use probiotics as health food additives and in host defense is the focus of the second aspect of the study. More specifically, to determine how and if specific probiotics can stimulate innate immune pathways that can protect individuals against other pathogenic bacteria. The analysis of protection was dissected at the gene level as well as at the level of pathology. A very important aspect of the study was the use of various mouse strains that are known to have different immune biases. Regardless of the mouse strain, probiotics chosen reduced inflammation and modulated the microbial flora. This study provides scientific evidence for the merits and mechanism of probiotic use.

The writing was not as crisp as would be needed for final approval but I have provided extensive editorial comments, which I am told have been incorporated into the final thesis edition. Thus, if they are incorporated into the final copy, I would be happy to recommend acceptance and congratulate the student on a well-designed and carefully executed thesis.

I do not know if it is customary at your University to have the extended reviewer ask the student questions. If it is allowed, please ask the student what the next steps are for a follow-up thesis? The answer will clearly demonstrate the maturity of the student in designing future studies and show how solid his training has been. Secondly, tease him with what public policies might be forthcoming from his work, and how might he design approaches to ensure these policies are well understood.

Name of Examiner:

Signature and Date:

Homi Bhabha National Institute

Ph.D. Thesis Evaluation Report

1. **Name of the Constituent Institution:** NISER
2. **Name of the Student:** MR BISWARANJAN PRADHAN
3. **Enrolment No.:** LIFE11201004014
4. **Title of the Thesis:** Peptides and Probiotics as Alternative to Antibiotics: Results from in-vitro and in-vivo Studies.
5. **Name of the Board of Studies:** LIFE SCIENCES

Recommendations

Tick one of the following:

1. The thesis in its present form is commended for the award of the Ph.D. Degree. ☑
2. The thesis is commended for the award of the Ph.D. degree. However, my suggestions for improving the thesis may be considered at the time of the viva voce examination and if the viva voce board deems it appropriate, the same may be incorporated in the thesis based on the discussions during the viva voce examination. The revised thesis need not be sent to me. ☐
3. The thesis should be revised as per the suggestions enclosed. I would like to see the revised thesis incorporating my suggestions before I give further recommendations. ☐
4. The thesis is not acceptable for the award of the Ph.D. degree. ☐

Date: 03.01.2017

(Signature): [signature]

Name of Examiner: PROF. BHABATOSH DAS

And affiliation THSTI, FARIDABAD

Please give your detailed report in the attached sheet. You may use additional sheets, if required.

Version approved during the meeting of Deans held during 29-30 Nov 2013

1. **Name of the Student:** MR. BISWARANJAN PRADHAN

2. **Title of the Thesis:** Peptides and Probiotics as alternative to antibiotics: Reports from in-vitro and in-vivo studies

DETAILED REPORT

Emergence of antimicrobial resistant Gram-negative bacteria have created a serious global health crisis and threaten the effectiveness of most, if not all, antibiotics commonly used to prevent and treat bacterial infections. Several laboratories in the world have turned their research activities for developing non-antibiotic promising alternatives like phage therapy, vaccination, identification of bacteriocins, boosting innate immunity by developing complementary medicine, and introducing prebiotics and probiotics to tackle resistant pathogens and health disorders.

Among several non-antibiotic promising alternatives, focus on host derived peptides and microbiota living in the human gastro-intestinal track are appeared as most encouraging candidates. Although, several small cyclic and non-cyclic peptides synthesized by the neutrophils, macrophages and other immune cells exhibiting antimicrobial effects are potential candidates to tackle resistant pathogens, most of the peptides are not very stable and have some limitations to eliminate infectious bacterial species in *in vivo* condition. There is a dearth to develop effective peptide compounds for clinical use. Similarly, several human and non-human derived microbiota have been introduced in several food products to promote human health, prevent colonization of exogenous microbiota in human body sites and restore native microbial consortia in gut like complex ecosystem. Still now, little is known about the biology of these probiotic species and their interactions with the host immune system and impact on host physiology.

In the first part of the present report, Mr. Biswaranjan Pradhan, has investigated antimicrobial activity of four human and bovine origin Host Defense Peptides (HDPs) conjugated with Carbon Nanotubes (CNTs) and Gold Nanoparticles (GNPs). Mr. Pradhan reported that CNT and GNT conjugated HDPs boost host immune system and help experimental animal (mice) to subsist when challenge with specific pathogen (*Salmonella* Typhimurium). In my opinion, the finding is very interesting and will be useful to develop research programme to tackle resistant pathogens.

In the second part of his thesis work, Mr. Pradhan, has studied the modulation of different host inflammatory and anti-inflammatory molecules in the presence of different probiotic species including *Lactobacillus acidophilus, Bacillus clausii, Saccharomyces boulardi* and *Bifidobacterium bifidum*. Outcome of the present study indicated that *L. acidophilus* and *B. clausii* could be used as potential probiotic candidates, but additional animal and human originated experimental supports are needed.

The findings of the present thesis work have great scientific value in the understanding of HDPs antimicrobial activity and fundamental of host immune modulation in the presence of probiotic strains. In my opinion, the work performed by Mr. Biswaranjan's is of outstanding quality and I strongly recommend acceptance of the thesis in its present format without any further modifications.

Name of Examiner: BHABATOSH DAS

Signature and Date:
03.01.2017

Homi Bhabha National Institute

Ph.D. Thesis Evaluation Report

1. **Name of the Constituent Institution:** National Institute of Science Education and Research Bhubaneswar
2. **Name of the Student:** Biswaranjan Pradhan
3. **Enrolment No.:** LIFE11201004014
4. **Title of the Thesis:** Peptides and Probiotics as alternative to Antibiotics; Reports from in-vitro and in-vivo studies
5. **Name of the Board of Studies:** Life Sciences

Recommendations

Tick one of the following:

1. The thesis in its present form is commended for the award of the Ph.D. Degree. [x]
2. The thesis is commended for the award of the Ph.D. degree. However, my suggestions for improving the thesis may be considered at the time of the viva voce examination and if the viva voce board deems it appropriate, the same may be incorporated in the thesis based on the discussions during the viva voce examination. The revised thesis need not be sent to me. [ ]
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4. The thesis is not acceptable for the award of the Ph.D. degree. [ ]

Date: 07/01/2017

(Signature): Palok Aich

Name of Examiner: PALOK AICH
And affiliation Associate Prof.

Please give your detailed report in the attached sheet. You may use additional sheets, if required.

Version approved during the meeting of Deans held during 29-30 Nov 2013

1. **Name of the Student:** Biswaranjan Pradhan

2. **Title of the Thesis:** Peptides and Probiotics as alternative to Antibiotics; Reports from in-vitro and in-vivo studies

DETAILED REPORT

The present thesis carried out a novel work to understand how a novel paradigm to develop alternative to antibiotics can be developed. The work hypothesized that by priming host innate immune system using immune stimulants such as host defense peptides (HDPs) and probiotics, one can combat infection and alleviate the possibility of developing antibiotic resistance. Since the concentration of the HDPs and probiotics are much less than the concentrations required to kill the pathogen microbes, therefore these agents are never seen as a threat to the microbes and chance is that the pathogens will not be threatened to develop resistance.
The thesis has two parts, in the first part he has screened various HDPs and probiotics and established the mode of action using high-throughput omics, histopathology and biochemical and molecular biology techniques. Second part deals with two shortlisted probiotics Lactobacillus acidophilus (LA) and Bacillus clausii (BC) in mouse model. Two differently immune biased mice strains BALB/c (Th2-biased) and C57BL/6 (Th1 biased) were compared before and after treatment with the probiotics. It was observed that LA could protect both mouse strains against Salmonella challenge better than BC. It was also shown that microbiota difference could be the major reasons of providing protection. Detailed microbiome profiling, comparison and correlation with host response in terms of innate mucosal immunity and metabolism were made.
The work is one of first kind in this area.
I strongly recommend that not only the degree of PhD be awarded to the candidate but if possible to nominate the work for further appreciation, if possible.

Palok Aich

Name of Examiner:

Palok Aich

Signature and Date: 07/01/17

List of Publications

A. Published

1. # Sur A*, **Pradhan B***, Banerjee A, Aich P. Immune activation efficacy of indolicidin is enhanced upon conjugation with carbon nanotubes and gold nanoparticles. PloS one. 2015 Apr 15;10(4):e0123905.
2. # **Pradhan B**, Guha D, Ray P, Das D, Aich P. Comparative Analysis of the Effects of Two Probiotic Bacterial Strains on Metabolism and Innate Immunity in the RAW 264.7 Murine Macrophage Cell Line. Probiotics and antimicrobial proteins. 2016 Jun 1;8(2):73-84.
3. # **Pradhan B,** Datzkiw D, Aich P. Gut microbiota and health: a review with focus on metabolic and immunological disorders and microbial remediation. Biomedical Reviews 2016; 27: 1-17

B. Communicated

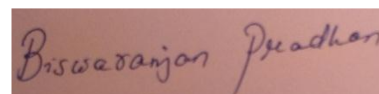
4. # **Pradhan B**, Guha D, Sur A, Murmu KC, Ray P, Das D, and Aich P. Comparative efficacy analysis of anti-microbial peptides, LL-37 and indolicidin upon conjugation with CNT in human monocytes.
5. Guha D, Banerjee A, **Pradhan B**, Peneva M, Aleksandrov G and Aich P. Macrophage polarization is influenced following treatment with *Lactobacillus bulgaricus*

C. Manuscripts

6. # **Pradhan B**, Guha D, Naik A, Banerjee A, Tambat S, Chawla S, Senapati S, and Aich P. Probiotics *Lactobacillus acidophilus* modulate mice gut microbiota and ameliorate Salmonella induced dysbiosis and inflammation.
7. Priyadarshini S, **Pradhan B**, Aich P. Cortisol mediated serotonergic regulation of immune and metabolic processes in adipocytes and macrophages. Part -1
8. Priyadarshini S, **Pradhan B**, Aich P. Cortisol mediated serotonergic regulation of immune and metabolic processes in adipocytes and macrophages. Part -2
9. # **Pradhan B**, Guha D, Banerjee A, Naik A, Datzkiw D and Aich P. A new approach in making bacterial species specific primers for qRT-PCR against 16S rRNA

*Authors equally contributed

Pertaining to Thesis



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