



## ORIGINAL ARTICLE

# Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88

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## ABSTRACT

**Background** The gut microbiota has profound effects on host physiology but local host–microbial interactions in the gut are only poorly characterised and are likely to vary from the sparsely colonised duodenum to the densely colonised colon. Microorganisms are recognised by pattern recognition receptors such as Toll-like receptors, which signal through the adaptor molecule MyD88.

**Methods** To identify host responses induced by gut microbiota along the length of the gut and whether these required MyD88, transcriptional profiles of duodenum, jejunum, ileum and colon were compared from germ-free and conventionally raised wild-type and *Myd88*–/– mice. The gut microbial ecology was assessed by 454-based pyrosequencing and viruses were analysed by PCR.

**Results** The gut microbiota modulated the expression of a large set of genes in the small intestine and fewer genes in the colon but surprisingly few microbiota-regulated genes required MyD88 signalling. However, MyD88 was essential for microbiota-induced colonic expression of the antimicrobial genes *Reg3β* and *Reg3γ* in the epithelium, and *Myd88* deficiency was associated with both a shift in bacterial diversity and a greater proportion of segmented filamentous bacteria in the small intestine. In addition, conventionally raised *Myd88*–/– mice had increased expression of antiviral genes in the colon, which correlated with norovirus infection in the colonic epithelium.

**Conclusion** This study provides a detailed description of tissue-specific host transcriptional responses to the normal gut microbiota along the length of the gut and demonstrates that the absence of MyD88 alters gut microbial ecology.

The human gut is home to trillions of bacteria (gut microbiota) that have co-evolved with us and established a finely tuned symbiosis.<sup>1</sup> Their combined genomes (metagenome), which contain 150-fold more genes compared with our own genome, provide us with functions that we did not have to evolve ourselves.<sup>2–3</sup> Recent data suggest that if this symbiosis is disrupted we are exposed to the increased risk of developing common diseases such as inflammatory bowel disease and obesity.<sup>4</sup> Germ-free mice provide a powerful tool to gain mechanistic insights into host–microbial interactions and their effect on host physiology. Colonisation of germ-free mice is associated with profound morphological changes in the small intestine such as shortening and widening of the

## Significance of this study

### What is already known about the subject?

- The gut harbours a vast ensemble of bacteria, the gut microbiota, encoding 150-fold more genes than our own genome.
- The gut microbiota is altered in inflammatory bowel disease and obesity.
- Germ-free mice are protected against inflammatory bowel disease and diet-induced obesity.
- Toll-like receptors recognise microorganisms and signal via MyD88.

### What are the new findings?

- Provides an extensive survey of host responses to the normal gut microbiota along the length of the gut.
- Analysis of the gut microbial ecology along the length of the gut.
- Demonstrates that *Myd88*-deficient mice harbour norovirus in the colonic epithelium.

### How might it impact on clinical practice in the foreseeable future?

- Understanding the fundamental factors underlying host–microbial interactions in the mammalian gut is essential for future studies directed at targeting the gut microbiota in order to improve health. We also provide a web-accessible database, <http://microbiota.wall.gu.se>, to investigate whether specific genes are regulated by the gut microbiota and/or MyD88. This resource will facilitate the identification of microbially regulated genes for researchers interested in all aspects of gastroenterology.

villus,<sup>5</sup> increased vascularisation,<sup>6</sup> recruitment of lymphocytes<sup>7</sup> and activation of the innate and adaptive immune systems.<sup>8</sup>

The innate immune system recognises bacteria and other infectious agents by pattern recognition receptors such as intracellular nucleotide-binding oligomerization domain-like receptors and Toll-like receptors (TLR).<sup>9</sup> All TLR (except TLR3) as well as interleukin (IL) 1 and IL-18 signal via MyD88-dependent pathways, which activate nuclear factor kappa B-driven pro-inflammatory signalling. Accordingly, *MYD88* deficiency in humans is associated with increased susceptibility to pyogenic bacterial



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infections and *Myd88*-deficient mice have enhanced susceptibility and morbidity to most viral and bacterial experimental infections.<sup>10</sup>

The microbial ecology of the gut is governed by several factors, such as age, diet and host genotype, and recent findings suggest an important role for the innate immune system.<sup>11</sup> In particular, TLR5 has been shown to have a significant impact in shaping the colonic gut microbiota, whereas TLR2 and TLR4 appear to play a minor role.<sup>12–13</sup> Furthermore, in a mouse model of non-obese diabetes, *Myd88* deficiency is associated with altered microbial community composition that confers protection against developing the disease.<sup>14</sup>

The function and architecture of the gut differs along its length: for example, nutrient absorption is most prevalent in the duodenum and jejunum, whereas the ileum is more immunologically active.<sup>15</sup> The colon is more of a fermentative reactor producing short chain fatty acids and is the major site of water reabsorption.<sup>15</sup> However, little is known about how the host responds to gut microbiota along the length of the gut. The concentration of bacteria increases along the length of the gut, from 10<sup>4</sup> cells/g in the duodenum to 10<sup>12</sup> cells/g in the colon,<sup>15</sup> and most published studies of gut microbial ecology have focused on the colon and feces, with less emphasis on the less populated and more inaccessible small intestine. The aim of our study was to identify the influence of innate immunity on microbiota-induced host responses and microbial composition along the length of the gut; we used *Myd88* deficiency as a model for the loss of innate immune signalling.

## MATERIALS AND METHODS

### Mice

Germ-free C57Bl6/J and Swiss Webster male mice (three to five per cage) were maintained in flexible film isolators under a 12-h light cycle and freely fed an autoclaved chow diet (Labdiet, St Louis, Missouri, USA). The *Myd88*<sup>−/−</sup> were backcrossed at least eight generations to C57Bl6/J and the last two crossings were performed using mice from our colony. These two lines were thereafter separated by a maximum of two generations. Germ-free and conventionally raised mice were separated by a maximum of three generations. Conventionally raised mice were fed the same autoclaved diet upon weaning and all mice were used at 12 weeks of age.

Mice were killed by cervical dislocation and the small intestines, cecum and colon were removed. The small intestine was divided into eight equal-sized segments and the colon into three. For RNA analyses, we used the first (duodenum), fifth (jejunum), eighth (ileum) segments and the proximal piece of the colon. For analysis of the gut ecology, all segments were analysed. Epithelial cells were isolated as described previously.<sup>16</sup> Animal protocols were approved by the Research Animal Ethics Committee in Gothenburg.

### RNA isolation

RNA was isolated from the gut tissues and epithelial cells immediately after cell harvest using the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA concentration and quality were evaluated by spectrophotometric analysis (ND-1000; NanoDrop Technologies, Wilmington, DE, USA) and capillary electrophoresis on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

### Microarray processing and statistical analysis

RNA labelling, microarray hybridisation and scanning were performed at the Uppsala array platform core facility at Uppsala University using MoGene 1.0 ST chips (Affymetrix, Santa Clara, CA, USA), according to the manufacturer's instructions. Normalisation and probe set summarisation were performed on

each tissue separately using the expression console software (Affymetrix). CEL files and normalised data were deposited into the NCBI GEO repository, accession number GSE17438. Downstream analyses were performed in Matlab using proprietary scripts and functions from the Bioinformatics toolbox. Genes were annotated against the ENSEMBL<sup>17</sup> set of genes using the MoGene 1.0 ST probe set mapping provided using BioMart.<sup>18</sup> Probe sets that were mapped to more than one gene were excluded while redundant probe sets were averaged. This resulted in a final dataset of 22 073 unique genes, each associated with one or more unambiguously mapped probe sets. Principal components analysis and hierarchical clustering (average linkage with Pearson's correlation coefficient) was performed on the processed dataset. Differential expression was evaluated in each tissue by calculating two-way analysis of variance (ANOVA) *p* values, including a test for interaction, using colonisation status (germ-free or conventionally raised) and genotype (*Myd88*<sup>−/−</sup> or wild type) as independent variables. This test was applied to each individual gene, and was followed by adjustment for multiple comparisons using the *q* value method proposed by Benjamini and Hochberg.<sup>19</sup> Gene ontology (GO) statistics, based on ENSEMBL annotated terms and their ancestors in the GO hierarchy, were calculated using Fisher's exact test. To account for multiple testing, we used permutation simulations to determine that an enrichment *p* value threshold of 10<sup>−4</sup> resulted in a low fraction of false positives (<5%). Genes without annotated GO terms were excluded from the analysis.

### cDNA synthesis and quantitative PCR

An aliquot of 0.5 µg of total RNA was reverse transcribed (high capacity cDNA reverse transcription kit; Applied Biosystems, Foster City, California, USA) and SYBR green-based quantitative reverse transcription (qRT)–PCR was performed as described previously.<sup>16</sup> Primers are listed in supplementary table S1, available online only.

### Bacterial community composition analysis

Intestines were removed, flash frozen in liquid nitrogen and divided into 12 segments. The small intestine was divided equally into eight segments, the ninth segment corresponded to the cecum, and the large intestine was sectioned into three equal pieces. Whole community DNA was isolated from the segments using the tissue and cells DNA isolation kit (MoBio, Carlsbad, CA, USA). DNA concentration and quality was evaluated by Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA, USA) and a plate reader.

PCR amplification of 16S ribosomal RNA genes was carried out using barcoded primers 27F–338R for the V1–V3 region of the 16S rRNA gene.<sup>20</sup> Three independent 50 µl PCR reactions were performed for each sample, each consisting of 2.5 U EasyA high fidelity enzyme, 1× buffer, 2 µM of each primer and 10–100 ng of the DNA template. Reaction conditions consisted of one cycle of 2 min at 95°C, 30 cycles of 30 s at 95°C, 45 s at 57°C, 60 s at 72°C and a final cycle of 2 min at 75°C on a thermocycler (Eppendorf, Hamburg, Germany). The PCR amplification products from each reaction were pooled and purified using Ampure magnetic purification beads (Agencourt Bioscience, Brea, CA, USA) and sequenced at the Cornell University Life Sciences Core Laboratories Centre (using Roche/454 FLX genome sequencer; Roche, Basel, Switzerland).

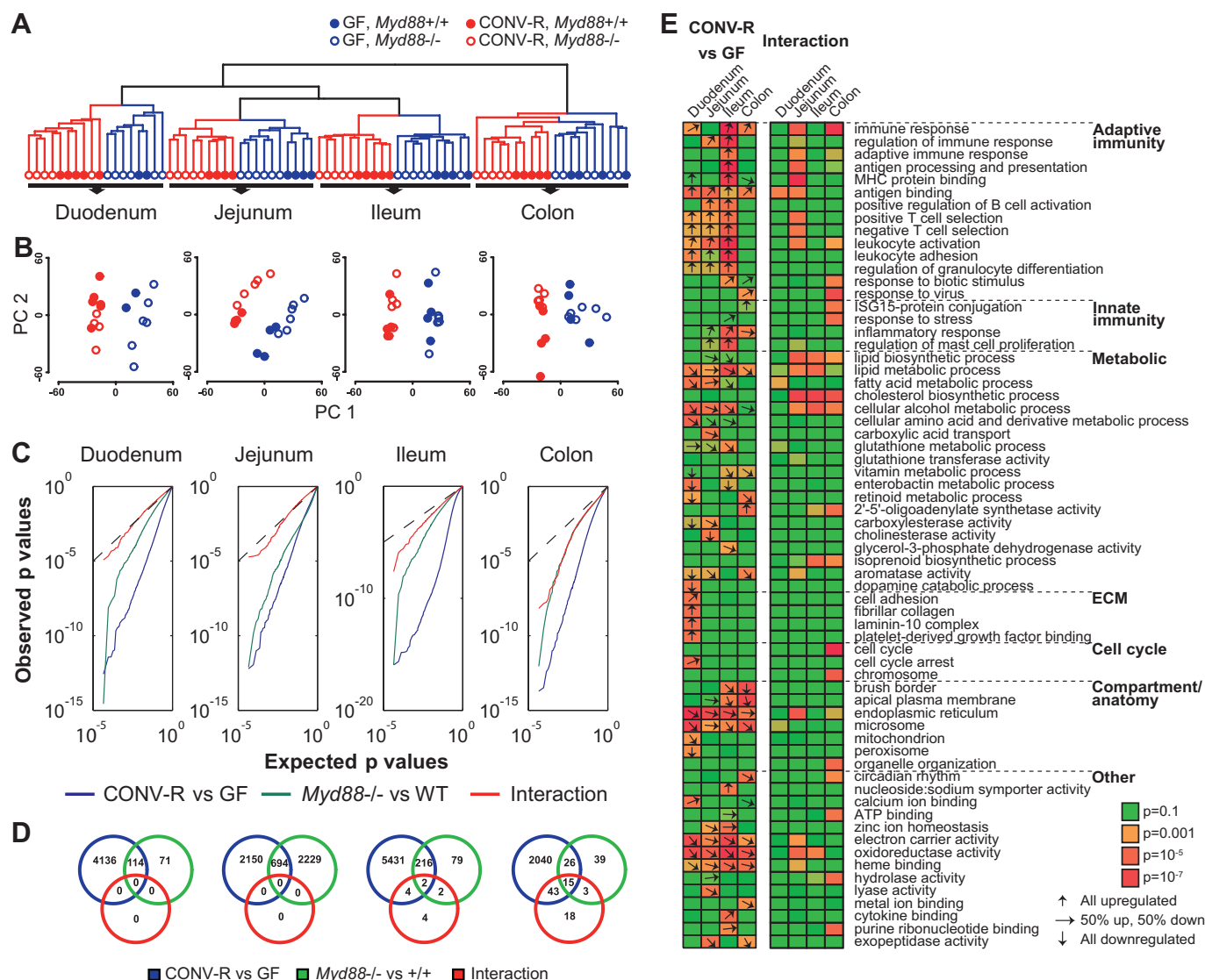
Sequences were analysed using the quantitative insights into microbial ecology (QIIME) software package.<sup>21</sup> Sequences were removed from further analysis if their length was outside the range of 200–1000 nt, or if they contained ambiguous bases, primer mismatches, homopolymer runs greater than six nucleotides, or

uncorrectable barcodes. The remaining sequences were denoised<sup>22</sup> and assigned to operational taxonomic units using UCLUST,<sup>23</sup> with a 97% pairwise identity threshold, and classified taxonomically using Greengenes.<sup>24</sup> Changes in bacterial abundance were compared using a two-tailed Student's *t* test with a Bonferroni correction for multiple comparisons. For tree-based analyses, single representative sequences for each operational taxonomic unit were aligned using PyNAST, and the phylogenetic tree used in the UniFrac<sup>25</sup> analysis was built using FastTree.<sup>26</sup> Sequences were deposited into MG-RAST, accession number MGP151.

## RESULTS AND DISCUSSION

### Gene expression profiling of germ-free and conventionally raised wild-type and *Myd88*<sup>-/-</sup> mice

We performed 77 microarray hybridisations to obtain global transcriptional profiles from duodenum, jejunum, ileum and colon from germ-free and conventionally raised wild-type and *Myd88*<sup>-/-</sup> mice. Hierarchical clustering revealed distinct clusters representing each of the four tissues and further subclustering based on colonisation status and genotype (wild type or *Myd88*<sup>-/-</sup>; figure 1A). Clustering by genotype was most clearly





seen for colonised animals. A similar pattern was revealed by principal component analysis (figure 1B). We next applied a two-way ANOVA test to score for differential expression depending on colonisation status or genotype, as well as interaction between these factors to identify cases in which MyD88 modulates the microbial response. We observed dramatic transcriptional responses to the microbiota in all segments of the gut (figure 1C). In the small intestine, between 2844 and 5653 genes were regulated by the microbiota (5% false discovery rate; blue circles in figure 1D; a list of all microbiota-regulated genes is provided in supplementary table S2, available online only). Fewer genes were regulated by the microbiota in the colon compared with other segments (2124 genes; figure 1D). The limited microbial regulation in the colon may be explained by the presence of a thick continuous mucosal layer, which shields the epithelium from direct bacterial exposure.<sup>27</sup>

Given the importance of TLR as pattern recognition receptors for bacteria, we anticipated a major role for MyD88 in regulating the transcriptional responses to the gut microbiota. We observed a significant impact of MyD88 on host transcriptional profiles in the jejunum but not in other segments of the gut (figure 1D, green circle); however, these responses were independent of the gut microbiota (figure 1D, red circle). Although few microbiota-induced changes in gene expression required MyD88, such interaction effects became increasingly larger in the distal segments; 79 genes were significant in the interaction test in the colon at 5% false discovery rate (red circles in figure 1D).

### Genes involved in immune responses and metabolism are predominantly regulated by the gut microbiota in the small intestine

To identify major regulated pathways and processes, we evaluated the 500 most significant microbiota-regulated genes in each tissue for enrichment of functional gene categories. Several GO categories were statistically linked to microbial status in all tissues (figure 1E, see supplementary table S3, available online only). Similar to previous studies<sup>28–30</sup> we observed significant microbial induction of genes related to immunity. The majority of these genes were associated with adaptive immunity, and are probably the result of lymphocyte migration to the mucosa and differentiation in response to the gut microbiota.<sup>7 28 31</sup> In addition, we evaluated transcriptional responses to the gut microbiota that depended on MyD88 (500 most significant genes in the interaction test). Immune categories were only weakly enriched in this set, although a stronger association was observed in the jejunum (figure 1E). Interestingly, these categories were also identified to be affected by MyD88 under germ-free conditions in the jejunum and ileum.

Metabolism-related genes including lipid and fatty acid metabolism-related categories, as well as genes related to cellular compartments involved in nutrient absorption and metabolism, were regulated in response to the microbiota throughout the gut (figure 1E). Interestingly, genes related to cholesterol biosynthesis were enriched among the top genes that responded differentially to the microbiota depending on genotype in the jejunum, ileum and colon. Furthermore, genes associated with energy-yielding compartments, mitochondrion and peroxisome, were found to be among those downregulated in the duodenum of conventionally raised compared with germ-free mice.

### Most microbially regulated genes involved in immune responses and barrier function are not dependent on Myd88

Expression levels of genes encoding immunoglobulins were altered in all segments of the gut, and this regulation was

independent of MyD88 (see supplementary table S2, available online only). In agreement, we observed increased chemokine gene expression in gut tissues from conventionally raised compared with germ-free mice (table 1). By isolating primary epithelial cells from the ileum in a separate set of animals, we found that these genes were regulated by the gut microbiota in the epithelium (see supplementary figure S1A, available online only). An increase in chemokine levels would be expected to result in lymphocyte infiltration to the mucosa.<sup>7</sup>

We also observed microbiota-induced upregulation of genes associated with barrier function (table 1). Genes encoding small proline-rich protein 1A and 1B, which play important roles as components of the cell envelope, and are cross-bridging proteins,<sup>32</sup> were significantly upregulated in the colon of conventionally raised mice (table 1). Expression of MUC2, the major mucin component of the colonic mucus layer, and expression of MUC4 and MUC13 were significantly elevated in all segments of the small intestine and colon (table 1). These responses were evident in epithelial cells (see supplementary figure S1B, available online only), but were unaffected by *Myd88* deficiency (table 1).

### The inability to signal through MyD88 is associated with increased susceptibility to viral infections in conventionally raised mice

Antiviral genes were induced by the microbiota in the colons of *Myd88*<sup>−/−</sup> mice but not in wild-type counterparts (figure 2). These results were corroborated by qRT-PCR (see supplementary figure S2, available online only) and could be localised to the epithelial layer (see supplementary figure S3, available online only). In contrast, we did not observe any increases in antiviral gene expression in the small intestine of *Myd88*<sup>−/−</sup> mice (see supplementary table S2, available online only).

At first these results may be counterintuitive as MyD88-independent pathways are mainly invoked in the regulation of antiviral response genes. However, *Myd88*-deficient mice exhibit increased mortality in several models of experimental viral infections.<sup>10</sup> To gauge whether the elevated expression of antiviral genes in the colonic epithelium of conventionally raised *Myd88*-deficient mice could be attributed to a viral infection, we performed a PCR-based screen of 15 selected viruses. This analysis revealed that our *Myd88*-deficient mice were infected by murine norovirus (table 2). Murine norovirus is common in animal facilities around the world and does not cause symptoms in immune-competent mice.<sup>33</sup>

Our data thus suggest that *Myd88*-deficient mice are susceptible to naturally occurring viral infections and that the presence of norovirus in the colonic epithelium of *Myd88*-deficient mice is the most likely cause of antiviral gene expression in these mice.

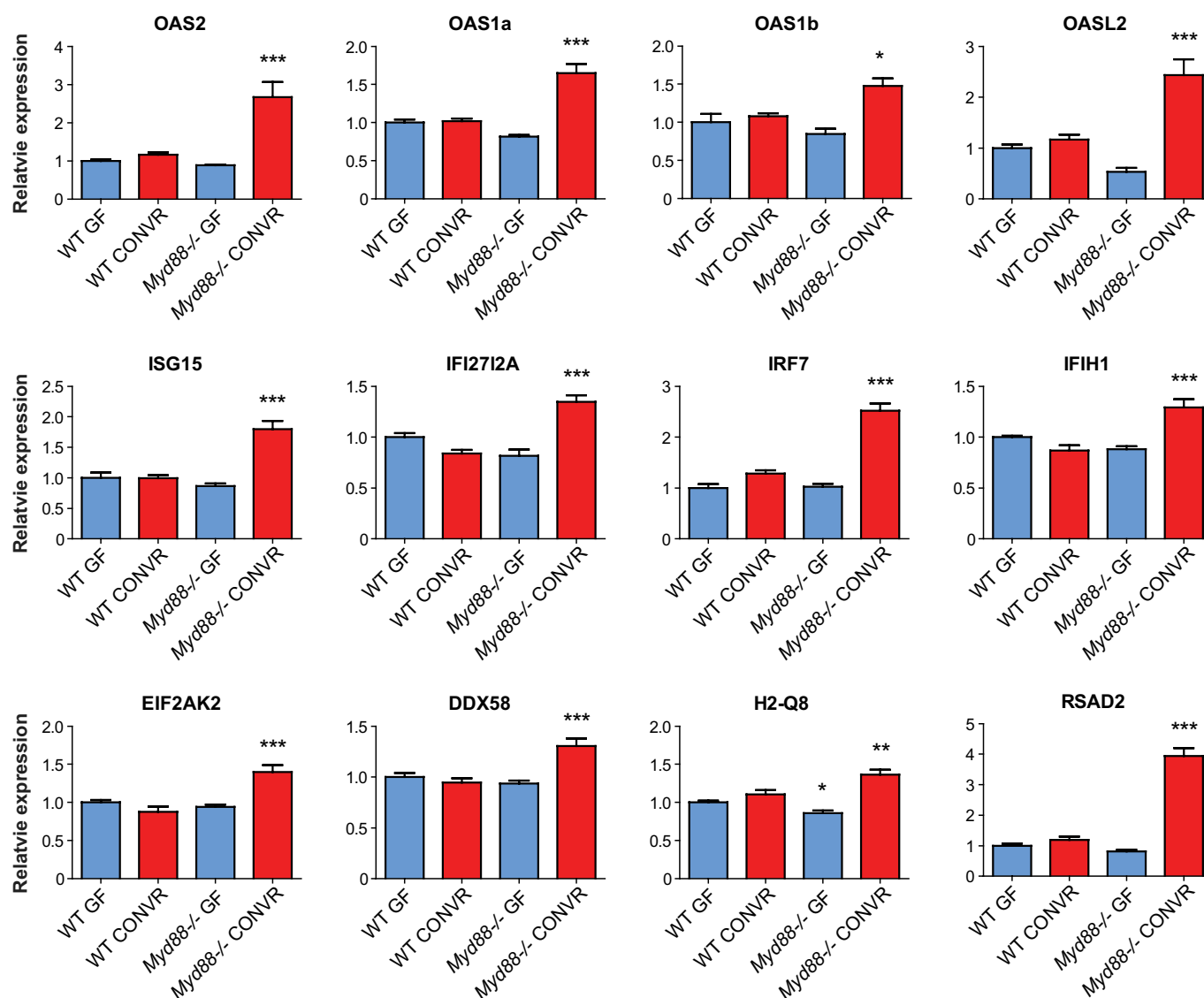
### Microbial regulation of REG3β and REG3γ requires MyD88 in colon but not ileum

Two recently identified antimicrobial peptides, REG3β and REG3γ, which are mainly produced by the Paneth cells but also by the absorptive epithelium,<sup>34 35</sup> were among the most significantly upregulated genes by the presence of a microbiota in both the small intestine and the colon (table 1). Interestingly, microbially induced expression of REG3β and REG3γ was dependent on MyD88 in the colon but not in the small intestine, (table 1), demonstrating distinct signalling pathways in these tissues. We also observed microbiota-induced expression of genes involved in the production of reactive oxygen (ROS) and nitrogen species (NOX1, NOS2 and DUOX2 and the maturation factor DUOX2A2), which are also potent

**Table 1** Selected microbially regulated genes

Gene	Duodenum				Jejunum				Ileum				Colon			
	<i>Myd88</i> +/+		<i>Myd88</i> -/-		<i>Myd88</i> +/+		<i>Myd88</i> -/-		<i>Myd88</i> +/+		<i>Myd88</i> -/-		<i>Myd88</i> +/+		<i>Myd88</i> -/-	
	GF	CONV-R	GF	CONV-R	GF	CONV-R	GF	CONV-R	GF	CONV-R	GF	CONV-R	GF	CONV-R	GF	CONV-R
<b>Chemokines</b>																
CCL5 (RANTES)	1.0±0.02	1.3±0.1	0.9±0.05	1.5±0.1*	1.0±0.03	1.0±0.1	0.4±0.06***	0.9±0.1	1.0±0.05	3.8±0.3***	0.4±0.05	4.6±0.6***	1.0±0.05	1.4±0.1	1.0±0.06	2.5±0.1***
CXCL9 (MIG)	1.0±0.04	2.3±0.3**	0.8±0.04	1.3±0.09	1.0±0.09	1.6±0.2**	0.5±0.04**	0.8±0.06	1.0±0.04	3.7±0.5***	0.7±0.04	2.3±0.07*	1.0±0.05	1.1±0.05	0.9±0.03	1.4±0.1**
CXCL10 (IP-10)	1.0±0.1	1.4±0.2	0.7±0.06	1.1±0.05	1.0±0.08	1.3±0.09	0.5±0.01***	0.8±0.09	1.0±0.1	2.7±0.3***	0.6±0.04	2.1±0.1**	1.0±0.1	1.1±0.02	0.7±0.02*	1.9±0.1***
<b>Barrier function</b>																
SPRR1A	1.0±0.2	1.4±0.3	1.4±0.2	2.2±1.2	1.0±0.04	1.4±0.3	1.2±0.06	1.3±0.04	1.0±0.5	0.7±0.1	0.7±0.09	0.6±0.01	1.0±0.09	3.4±0.3***	0.9±0.09	3.2±0.5***
SPRR1B	1.0±0.06	1.0±0.07	1.0±0.05	1.1±0.1	1.0±0.03	1.0±0.05	1.0±0.04	1.0±0.02	1.0±0.1	1.0±0.05	1.0±0.07	1.0±0.03	1.0±0.06	1.6±0.07***	1.0±0.05	1.4±0.1*
MUC2	1.0±0.01	1.5±0.06*	1.0±0.04	1.5±0.1*	1.0±0.07	1.4±0.05**	1.2±0.07	1.6±0.04***	1.0±0.01	1.0±0.02	1.0±0.02	1.2±0.05***	1.0±0.05	1.2±0.03**	1.0±0.03	1.1±0.01*
MUC4	1.0±0.06	3.4±0.2***	0.9±0.09	2.5±0.1***	1.0±0.03	2.2±0.1**	0.8±0.06	1.9±0.2***	1.0±0.02	2.0±0.05***	1.0±0.02	2.2±0.2***	1.0±0.08	1.5±0.05*	0.9±0.07	1.6±0.2**
MUC13	1.0±0.06	1.3±0.01**	0.9±0.06	1.3±0.03**	1.0±0.02	1.2±0.03**	0.9±0.03	1.1±0.03	1.0±0.01	1.3±0.02***	1.0±0.02	1.3±0.03***	1.0±0.01	1.1±0.01	1.0±0.01	1.0±0.02
<b>Antibacterial genes</b>																
REG3γ	1.0±0.2	6.5±0.3***	0.6±0.02	3.3±0.3***	1.0±0.04	4.2±0.2***	1.0±0.07	3.6±0.3***	1.0±0.03	1.6±0.05***	1.0±0.03	1.5±0.03***	1.0±0.3	5.2±1.5**	0.7±0.06	1.6±0.2
REG3β	1.0±0.9	2.4±0.2*	0.1±0.01	0.6±0.09	1.0±0.5	7.1±0.4***	0.7±0.07	5.1±0.7***	1.0±0.09	1.6±0.04***	1.0±0.02	1.4±0.05***	1.0±0.4	5.2±1.5*	0.7±0.1	0.7±0.1
NOX1	1.0±0.4	1.7±0.1	0.8±0.06	2.7±0.5*	1.0±0.2	2.0±0.5	1.0±0.04	2.7±0.4**	1.0±0.2	2.4±0.4**	1.1±0.09	4.5±0.2***	1.0±0.06	1.2±0.06	0.7±0.06	1.5±0.2
NOS2	1.0±0.03	1.4±0.09	1.0±0.05	1.8±0.4	1.0±0.08	5.2±1.9*	1.2±0.06	3.8±0.6	1.0±0.05	9.2±0.6***	1.0±0.08	8.2±0.7***	1.0±0.05	1.3±0.2	1.0±0.05	1.0±0.02
DUOX2	1.0±0.06	2.7±0.2***	1.1±0.04	2.0±0.2*	1.0±0.09	5.0±1.2	1.0±0.09	7.4±1.6**	1.0±0.04	6.0±0.4***	1.1±0.07	7.2±0.8***	1.0±0.081	2.1±0.1***	1.0±0.05	1.6±0.2*
DUOX2A2	1.0±0.05	1.4±0.1	0.9±0.05	1.4±0.2	1.0±0.05	3.6±1.2	1.0±0.09	6.7±1.7**	1.0±0.02	10.3±0.8***	1.2±0.09	14.4±2.3***	1.0±0.02	4.0±0.6***	1.0±0.06	2.6±0.5

CONV-R, conventionally raised; GF, germ free. \*p&lt;0.05; \*\*p&lt;0.01; \*\*\*p&lt;0.001.



**Figure 2** Relative expression levels of antiviral genes in the colon of germ-free (GF) and conventionally raised (CONV-R) wild-type (WT) and *Myd88*<sup>-/-</sup> mice. Data are from the microarray experiment and were corroborated by quantitated reverse transcription PCR in supplementary figure S2 available online only. n=4–6 per group. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001; analysis of variance.

antimicrobial agents,<sup>36, 37</sup> with the most pronounced effect in the ileum (table 1; validated by qRT-PCR in supplementary table S4, available online only). However, in contrast to REG3 $\beta$  and REG3 $\gamma$ , regulation of genes related to ROS production was independent of *Myd88*. qRT-PCR analysis of isolated epithelial cells from ilea and colons of germ-free and conventionally raised mice revealed that most of these genes were microbially regulated in the epithelium (see supplementary figure S4, available online only). Interestingly, we also noted a similar gene expression profile in Swiss Webster mice (see supplementary table S5, available online only), which are considered to have a T<sub>H</sub>2-biased immune system.

#### **Myd88 deficiency is associated with altered bacterial community composition**

We also examined bacterial diversity and community structure along the length of the gut in conventionally raised wild-type (n=7) and *Myd88*-deficient (n=4) mice using 454-based pyrosequencing of 16S rRNA gene (see supplementary figure S5, available online only). *Myd88*-deficient mice had a significant

increase in the relative abundance of segmented filamentous bacteria (SFB), especially in the jejunum and ileum (figure 3A), indicating that SFB are particularly affected by the loss of *Myd88*. SFB are tightly associated with the epithelium, especially in the small intestine,<sup>38</sup> and play a fundamental role in the maturation of intestinal T-cell responses and in the induction of gut inflammation, antimicrobial defence as well as mucosal IgA production.<sup>28, 39, 40</sup> Therefore, it is possible to speculate that localisation of SFB close to the epithelium may make them more sensitive to antimicrobial molecules that are expressed by the epithelium in a *Myd88*-dependent fashion compared with other bacteria located further away from the surface of the epithelium.

To assess how the overall diversity of the microbiota was affected by *Myd88* deficiency irrespective of the relative abundances of taxa, we compared samples using the unweighted UniFrac distance metric, which assumes that similar communities have similar evolutionary histories that can be represented as shared branches in a joint phylogenetic tree.<sup>25</sup> This analysis revealed that the two genotypes harbour different bacterial communities (evidenced as clustering of samples by genotype in

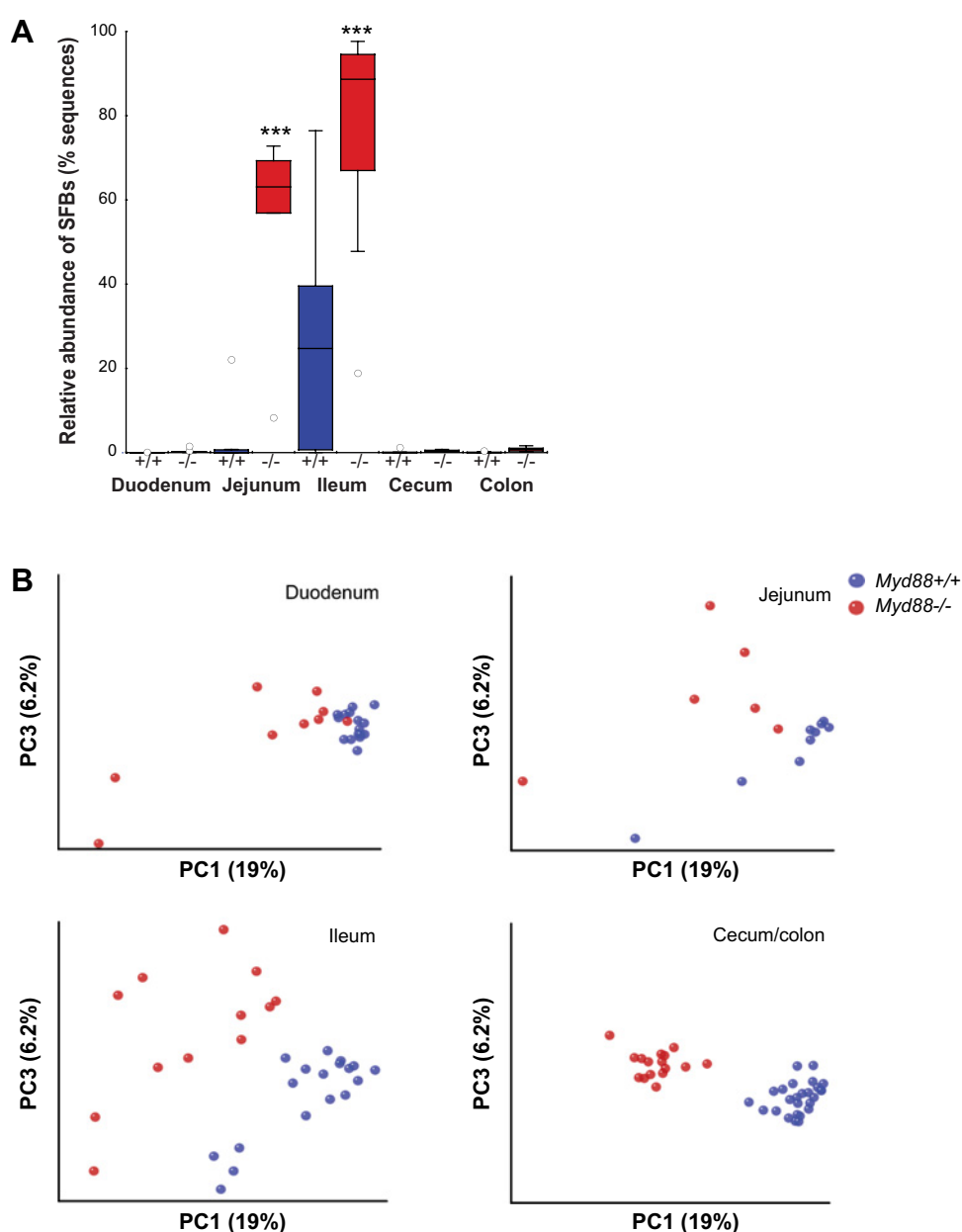
**Table 2** PCR-based screening for mouse viruses in isolated colonic intestinal epithelial cells from conventionally raised mice

Viral agent	Genotype	
	Myd88 +/+	Myd88 -/-
Sendai virus	-(0/3)	-(0/3)
Mouse hepatitis virus	-(0/3)	-(0/3)
Pneumonia virus of mice	-(0/3)	-(0/3)
Minute virus of mice	-(0/3)	-(0/3)
Mouse parvovirus (MPV1, MPV2, MPV3)	-(0/3)	-(0/3)
Theiler's murine encephalomyelitis virus	-(0/3)	-(0/3)
Murine norovirus	-(0/3)	+(3/3)
Reovirus 3	-(0/3)	-(0/3)
Mouse rotavirus (EDIM)	-(0/3)	-(0/3)
Ectromelia virus	-(0/3)	-(0/3)
Lymphocytic choriomeningitis virus	-(0/3)	-(0/3)
Polyoma virus	-(0/3)	-(0/3)
Lactate dehydrogenase-elevating virus	-(0/3)	-(0/3)
Mouse adenovirus (MAD1, MAD2)	-(0/3)	-(0/3)
Mouse cytomegalovirus	-(0/3)	-(0/3)

each of the different segments of the intestine, figure 3B). In addition, duodenum, jejunum and ileum-associated microbial communities show significantly higher levels of inter-mouse variation in bacterial diversity in *Myd88*-deficient mice compared with wild-type mice (see supplementary figure S6, available online only). In contrast, the bacterial communities in the cecum and colon clustered tightly (low mouse–mouse variation in microbial diversity, supplementary figure S6, available online only) and very clearly segregated by host genotype (figure 3B). Generally, difference in mouse to mouse variation along the length of the gut may reflect the biomass difference between gut segments. However, the greater mouse to mouse variation in *Myd88*-deficient animals compared with wild-type mice may signify a loss of control over microbial diversity.

Earlier studies have shown that the host genotype can affect the microbial ecology of the gut: for instance,  $\alpha$ -defensin deficiency has recently been shown to modify the composition of gut microbiota significantly in mice.<sup>41</sup> We therefore propose that the MyD88-dependent increases in REG3 $\beta$  and REG3 $\gamma$  gene

**Figure 3** Effect of host genotype on the microbiota composition along the length of the gut from conventionally raised wild-type and *Myd88*<sup>-/-</sup> mice. (A) Relative abundance of segmented filamentous bacteria (SFB) in the two genotypes (*Myd88*<sup>+/+</sup> or <sup>-/-</sup>) along the length of the gut. \*\*\**p* < 0.001; two-tailed Student's *t* test with Bonferroni correction for multiple comparisons. (B) Principal coordinates analysis of unweighted UniFrac distances between bacterial communities determined from 16S rRNA genes. Percentage variation explained by each principal coordinate (PC) is indicated on the axis. Symbols represent individual intestinal segments obtained from replicate mice: duodenum, first three segments; jejunum, segments 4–5; ileum, segments 6–8; cecum, 9; large intestine, 10–12. *n* = 4–7 mice per group. Low sequence counts were obtained for some samples, which were removed from further analysis.



expression in the colon could contribute to the genotype-specific clustering of bacterial communities in the colon, but further experiments are required to prove this hypothesis.

## CONCLUSION AND PROSPECTUS

In summary, we have extensively analysed the influence of the microbiota on gene expression and microbial ecology along the length of the gut, in both wild-type and Myd88-deficient animals. Furthermore, we identified limited but selective roles of Myd88 in increasing susceptibility to norovirus infections and modifying microbial ecology. Despite relatively small effects on mediating microbial-induced host gene expression changes, Myd88 affects host microbial ecology in the gut.

To make our gene expression data easily available to the scientific community, we constructed a searchable database (<http://microbiota.wall.gu.se>). The database enables interactive queries on gene name and detailed gene views with graphical illustrations of measured levels in different tissues and conditions, as well as statistical results and additional information about tissue expression profiles.<sup>42</sup> Data on host microbial responses in tissues (liver, epididymal and subcutaneous fat), that were not analysed in this report, are provided through the web interface.

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**Competing interests** None.

**Contributors** EL performed research, analysed data, wrote the manuscript; VT performed research, analysed data, commented on the manuscript; YL performed research, analysed data, commented on the manuscript; OK analysed data, commented on the manuscript; IN analysed data, commented on the manuscript; AF performed research; JN analysed data, commented on the manuscript; REL analysed data, assisting in writing the manuscript; FB conceived the study, analysed data, wrote the manuscript.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** CEL files and normalised array data are available at NCBI GEO repository, accession number GSE17438. The expression data are also freely available through <http://microbiota.wall.gu.se/>. The 454 data set will be available at MGRAST—accession number MGP151.

## REFERENCES

- Ley RE, Hamady M, Lozupone C, et al. Evolution of mammals and their gut microbes. *Science* 2008;**320**:1647–51.
- Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010;**464**:59–65.
- Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature* 2009;**457**:480–4.
- Ott SJ, Musfeldt M, Wenderoth DF, et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 2004;**53**:685–93.
- Westmann BS. The germfree animal in nutritional studies. *Annu Rev Nutr* 1981;**1**:257–79.
- Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc Natl Acad Sci U S A* 2002;**99**:15451–5.
- Umesaki Y, Setoyama H, Matsumoto S, et al. Expansion of alpha beta T-cell receptor-bearing intestinal intraepithelial lymphocytes after microbial colonization in germ-free mice and its independence from thymus. *Immunology* 1993;**79**:32–7.
- Slack E, Hapfelmeier S, Stecher B, et al. Innate and adaptive immunity cooperate flexibly to maintain host–microbiota mutualism. *Science* 2009;**325**:617–20.
- Fukata M, Vamadevan AS, Abreu MT. Toll-like receptors (TLRs) and Nod-like receptors (NLRs) in inflammatory disorders. *Semin Immunol* 2009;**21**:242–53.
- von Bernuth H, Picard C, Jin Z, et al. Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* 2008;**321**:691–6.
- Walter J, Ley RE. The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol* 2011;**65**:411–29.
- Loh G, Brodzia F, Blaut M. The Toll-like receptors TLR2 and TLR4 do not affect the intestinal microbiota composition in mice. *Environ Microbiol* 2008;**10**:709–15.
- Vijay-Kumar M, Aitken JD, Carvalho FA, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* 2010;**328**:228–31.
- Wen L, Ley RE, Volchok PY, et al. Innate immunity and intestinal microbiota in the development of type 1 diabetes. *Nature* 2008;**455**:1109–13.
- Kleerebezem M, Vaughan EE. Probiotic and gut lactobacilli and bifidobacteria: molecular approaches to study diversity and activity. *Annu Rev Microbiol* 2009;**63**:269–90.
- Reigstad CS, Lunden GO, Felin J, et al. Regulation of serum amyloid A3 (SAA3) in mouse colonic epithelium and adipose tissue by the intestinal microbiota. *PLoS One* 2009;**4**:e5842.
- Hubbard T, Barker D, Birney E, et al. The Ensembl genome database project. *Nucleic Acids Res* 2002;**30**:38–41.
- Smedley D, Haider S, Ballester B, et al. BioMart — biological queries made easy. *BMC Genomics* 2009;**10**:22.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Statist Soc* 1995;**57**:289–300.
- Koren O, Spor A, Felin J, et al. Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proc Natl Acad Sci U S A* 2010;**108**:4592–8.
- Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;**7**:335–6.
- Reeder J, Knight R. Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. *Nat Methods* 2010;**7**:668–9.
- Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**:2460–1.
- DeSantis TZ, Hugenholtz P, Larsen N, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 2006;**72**:5069–72.
- Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol* 2005;**71**:8228–35.
- Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol Biol Evol* 2009;**26**:1641–50.
- Johansson ME, Phillipson M, Petersson J, et al. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci U S A* 2008;**105**:15064–9.
- Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 2009;**31**:677–89.
- Hooper LV, Wong MH, Thelin A, et al. Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 2001;**291**:881–4.
- Rawls JF, Mahowald MA, Ley RE, et al. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. *Cell* 2006;**127**:423–33.
- Macpherson AJ, Hunziker L, McCoy K, et al. IgA responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. *Microbes Infect* 2001;**3**:1021–35.
- Steinert PM, Marekov LN. Initiation of assembly of the cell envelope barrier structure of stratified squamous epithelia. *Mol Biol Cell* 1999;**10**:4247–61.
- Pritchett-Corning KR, Cosentino J, Clifford CB. Contemporary prevalence of infectious agents in laboratory mice and rats. *Lab Anim* 2009;**43**:165–73.
- Mukherjee S, Partch CL, Lehotzky RE, et al. Regulation of C-type lectin antimicrobial activity by a flexible N-terminal prosegment. *J Biol Chem* 2009;**284**:4881–8.
- Cash HL, Whitham CV, Behrendt CL, et al. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 2006;**313**:1126–30.
- Nathan C. Inducible nitric oxide synthase: what difference does it make? *J Clin Invest* 1997;**100**:2417–23.
- Leto TL, Geiszt M. Role of Nox family NADPH oxidases in host defense. *Antioxid Redox Signal* 2006;**8**:1549–61.
- Davis CP, Savage DC. Habitat, succession, attachment, and morphology of segmented, filamentous microbes indigenous to the murine gastrointestinal tract. *Infect Immun* 1974;**10**:948–56.
- Klaasen HL, Van der Heijden PJ, Stok W, et al. Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. *Infect Immun* 1993;**61**:303–6.
- Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009;**139**:485–98.
- Salzman NH, Hung K, Haribhai D, et al. Enteric defensins are essential regulators of intestinal microbial ecology. *Nat Immunol* 2010;**11**:76–83.
- Su AI, Wiltshire T, Batalov S, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci U S A* 2004;**101**:6062–7.



## **Supplementary material to:**

**Analysis of gut microbial regulation of host gene expression along the length of the gut  
and regulation of gut microbial ecology through MyD88**

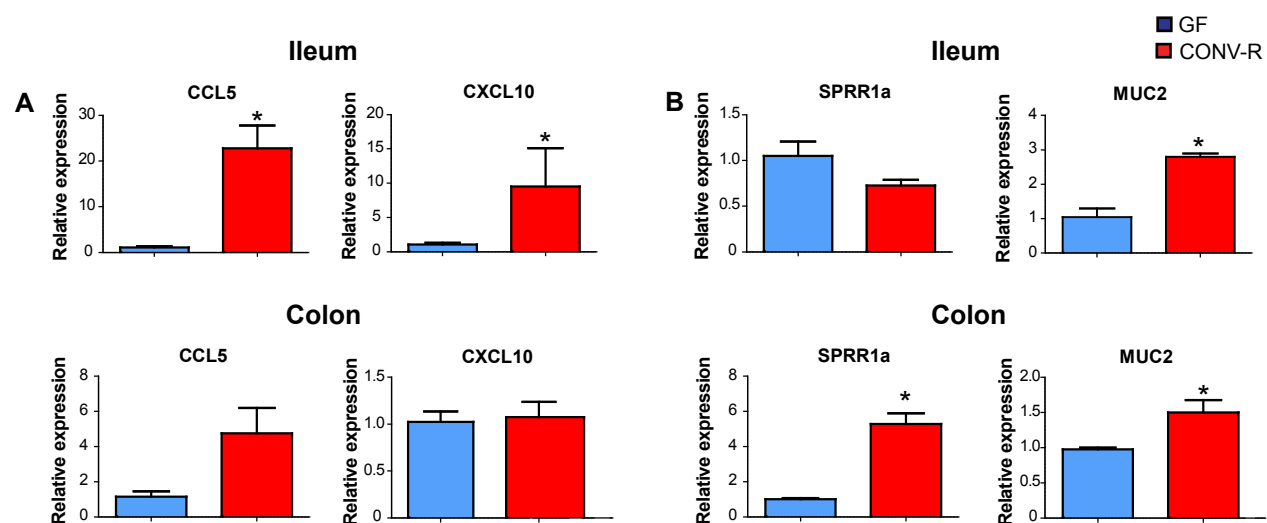
Erik Larsson, Valentina Tremaroli, Ying Shiuan Lee, Omry Koren, Intawat Nookaew,  
Ashwana Fricker, Jens Nielsen, Ruth E. Ley, Fredrik Bäckhed

Contents:

Figures S1-6

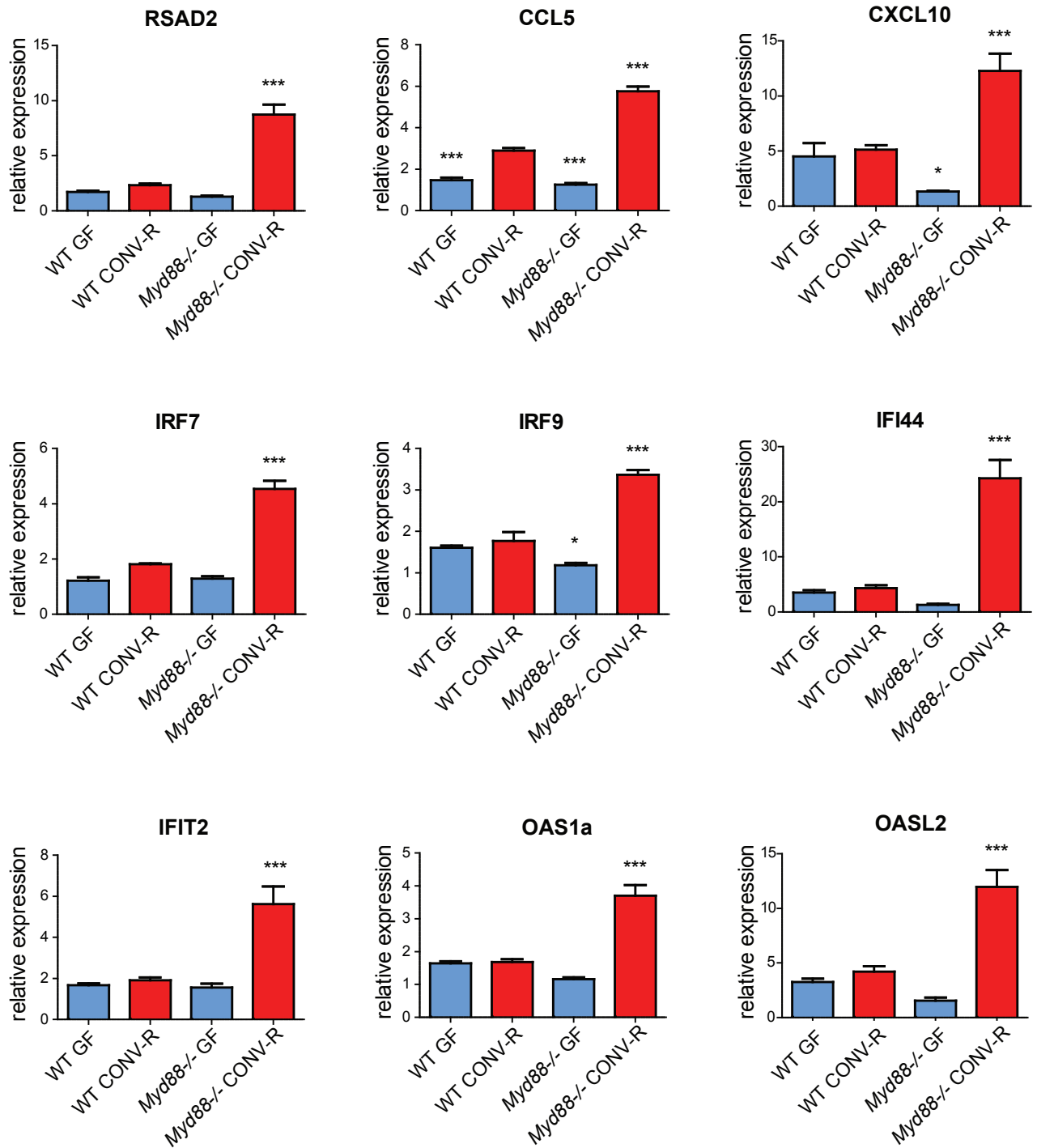
Tables S1,4,5

**Figure S1**



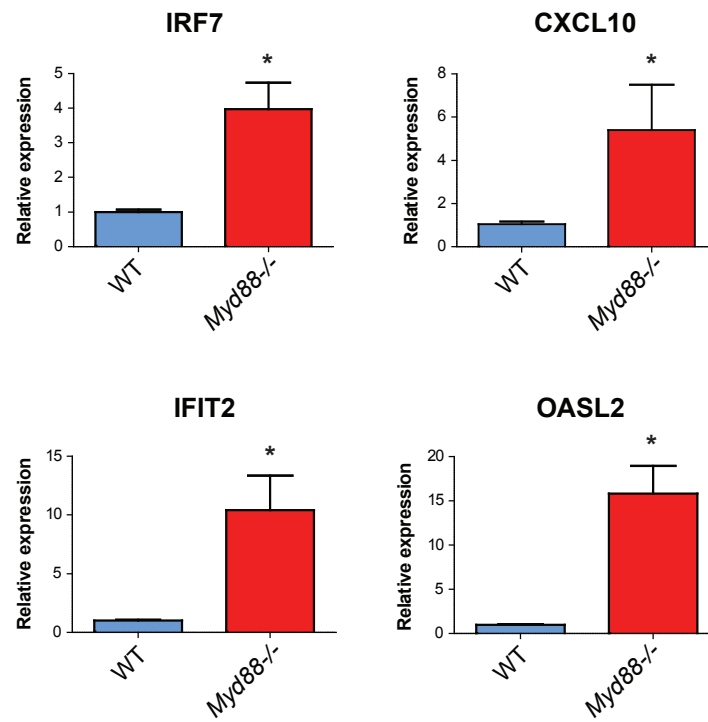
**Figure S1.** qRT-PCR analysis of (A) chemokines and (B) genes involved in mucosa fortification in epithelial cells isolated from the ileum and colon of GF and CONV-R mice. n = 4 mice/group. \* P < 0.05; Mann Whitney U-test.

**Figure S2**



**Figure S2.** qRT-PCR validation of antiviral gene expression in the proximal colon of CONV-R wildtype and *Myd88*<sup>-/-</sup> mice. n = 4-6/group. \* P < 0.05; \*\*\* P < 0.0001; ANOVA.

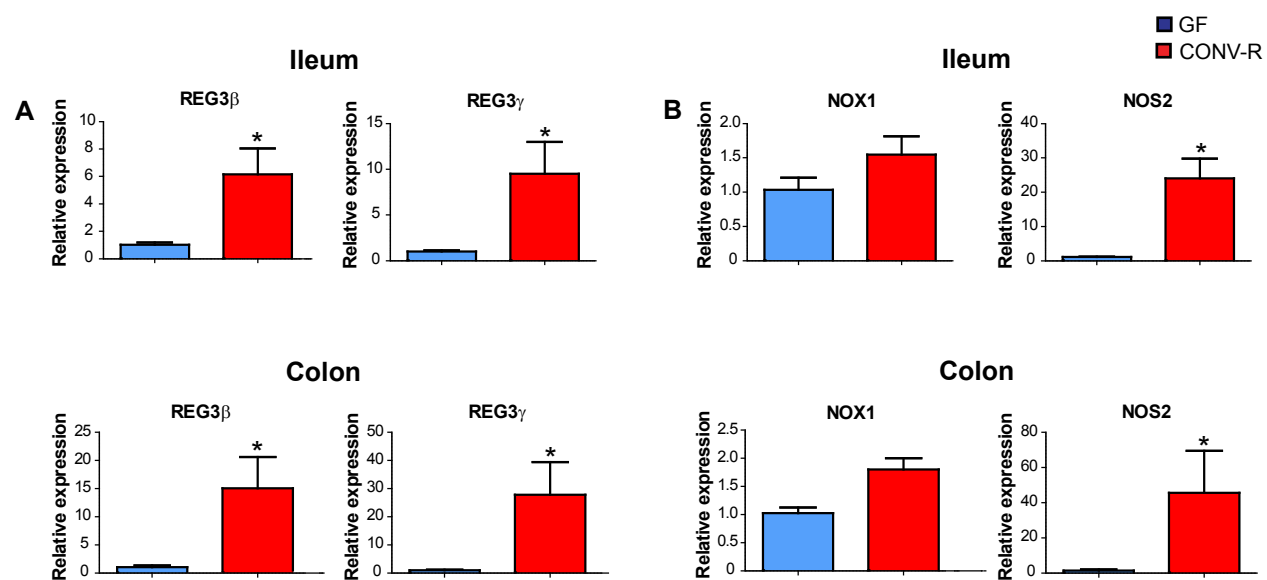
**Figure S3**



**Figure S3.** qPCR analysis of antiviral gene expression in epithelial cells isolated from the colon of CONV-R wildtype and Myd88<sup>-/-</sup> mice. n = 4/group. \* P < 0.05; Mann-Whitney U-test.

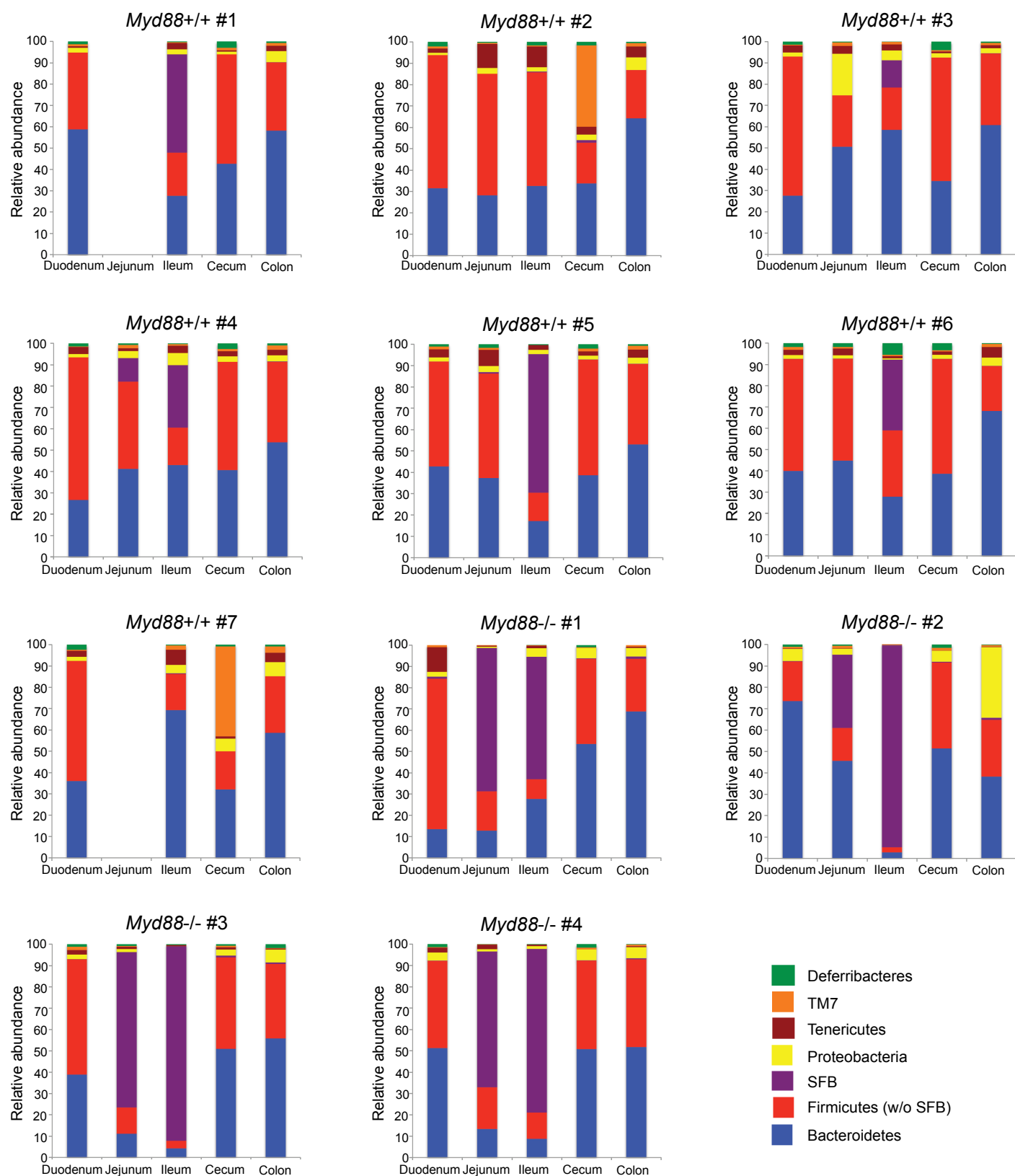


**Figure S4**



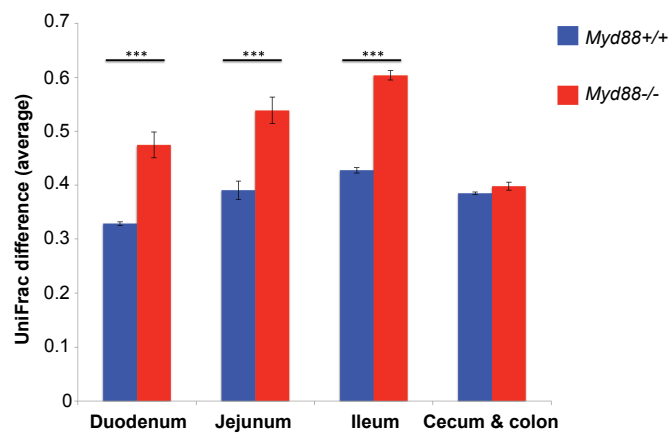
**Figure S4.** qRT-PCR analysis of antimicrobial genes in epithelial cells isolated from ileum and colon of GF and CONV-R mice. n = 4 mice/group. \* P < 0.05; Mann Whitney U-test.

**Figure S5**



**Figure S5.** Relative abundance of the different phyla in individual mice (*Myd88*  $+/+$  or  $-/-$ ) along the length of the gut.

**Figure S6**



**Figure S6.** Mean unweighted UniFrac distances derived from the different sections of the gut. P-values were corrected for multiple comparisons using the Bonferroni correction; \*\*\*  $p < 0.0005$ .  $n = 4-7$  mice/group.

**Table S1. Primer sequences used for validation of DNA microarray data by qRT-PCR.**

Gene		Primer Sequence (5'-3')	Reference
Rsad2	F	TGCTGGCTGAGAATAGCATTAGG	Primer bank <sup>1*</sup> (ID: 31543946a1)
	R	GCTGAGTGCTGTTCCCATCT	
Irf7	F	GAGACTGGCTATTGGGGGAG	Primer bank <sup>1*</sup> (ID: 8567364a1)
	R	GACCGAAATGCTTCCAGGG	
Cxcl10	F	CCAAGTGCTGCCGTCATTTTC	Primer bank <sup>1*</sup> (ID: 10946576a1)
	R	GGCTCGCAGGGATGATTTCAA	
Ccl5	F	GCTGCTTTGCCTACCTCTCC	Primer bank <sup>1*</sup> (ID: 7305461a1)
	R	TCGAGTGACAAACACGACTGC	
Ifi44	F	AACTGACTGCTCGCAATAATGT	Primer bank <sup>1*</sup> (ID: 19527086a1)
	R	GTAACACAGCAATGCCTCTTGT	
Irf9	F	GCCGAGTGGTGGGTAAGAC	Primer bank <sup>1*</sup> (ID: 6680474a1)
	R	GCAAAGGCGCTGAACAAAGAG	
Ifit2	F	AGTACAACGAGTAAGGAGTCACT	Primer bank <sup>1*</sup> (ID: 6680363a1)
	R	AGGCCAGTATGTTGCACATGG	
Oas1a	F	ATGGAGCACGGACTCAGGA	Primer bank <sup>1*</sup> (ID: 21630283a1)
	R	TCACACACGACATTGACGGC	
Oas12	F	AGGGGACAACCCTGAACCA	Primer bank <sup>1*</sup> (ID: 16924024a1)
	R	TAGGCCAGGCTTCTGCTACA	
Sprr1a	F	TTGTGCCCCCAAACCAAG	Primer bank <sup>1*</sup> (ID: 6678115a1)
	R	GGCTCTGGTGCCTTAGGTTG	
Muc2	F	CAAGGGCTCGGAACCTCCAG	Primer bank <sup>1*</sup> (ID: 294610642b1)
	R	CCAGGGAATCGGTAGACATCG	
Reg3β	F	TACTGCCTTAGACCGTGCTTTCTG	Vaishnava (2008) PNAS 105:20858-20863
	R	GACATAGGGCAACTTCACCTCACA	
Reg3γ	F	TTCCTGTCCTCCATGATCAAAA	Vaishnava (2008) PNAS 105:20858-20863
	R	CATCCACCTCTGTTGGGTTCA	
Nos2	F	AGCCTTGCATCCTCATTGG	Huang (2009) Mol Cell 35:48-57
	R	CACTCTCTTGCGGACCATCT	
Nox1	F	CTGACAAGTACTATTACACGAGAG	Ibi (2008) J Neurosci 28:9486-9494
	R	CATATATGCCACCAGCTTATGGAAG	

<sup>1</sup> Spandidos (2010) Nucl Acids Res 38:D792-9.\* available at: <http://pga.mgh.harvard.edu/primerbank/index.html>



**Table S4. qRT-PCR validation of selected microbially regulated genes in whole tissues from C57BL/6 (one-way ANOVA)**

<b>Ileum</b>					
Gene	<i>Myd88</i> <sup>+/+</sup>		<i>Myd88</i> <sup>-/-</sup>		<b>Confirmed results</b>
<i>Antibacterial</i>	GF	CONV-R	GF	CONV-R	
Nox1	1.08±0.4	3.5±1.2 <sup>**</sup>	1.3±0.3	7.6±1.1 <sup>***</sup>	Yes
Nos2	1.0±0.1	18.7±3.3 <sup>***</sup>	1.1±0.3	18.1±3.4 <sup>***</sup>	Yes
Duox2	1.0±0.08	11.0±2.0 <sup>***</sup>	1.2±0.05	16.8±5.2 <sup>***</sup>	Yes

Yes, qRT-PCR confirmed microarray data; No, qRT-PCR did not confirm microarray data.

**Table S5. qRT-PCR validation of selected microbially regulated genes in ileal tissue from Swiss Webster mice (one-way ANOVA)**

Gene	Ileum		Confirmed results (GF vs CONVR)
	GF	CONV-R	
<i>Chemokines, mucosa fortification, antibacterial</i>			
Ccl5	1.1±0.4	5.8±1.9 <sup>*</sup>	Yes
Cxcl10	1.1±0.5	3.4±1.4 <sup>*</sup>	Yes
Sprr1a	1.0±0.3	2.2±2.5	Yes
Muc2	1.0±0.2	1.5±0.1 <sup>*</sup>	No <sup>a</sup>
Muc4	1.0±0.3	2.8±3.1	No <sup>b</sup>
Muc13	1.0±0.2	1.8±0.2 <sup>*</sup>	Yes
Reg3γ	1.0±0.2	2.5±0.6 <sup>*</sup>	Yes
Reg3β	1.0±0.3	3.2±0.6 <sup>*</sup>	Yes
Nox1	1.4±1.6	39.7±72.3	No <sup>b</sup>
Nos2	1.0±0.4	9.2±4.9 <sup>*</sup>	Yes
Duox2	1.0±0.1	2.2±0.9 <sup>*</sup>	Yes

Yes, qRT-PCR confirmed microarray data; No, qRT-PCR did not confirm microarray data.

<sup>a</sup>Small difference in expression levels between GF and CONVR detected by microarray.

<sup>b</sup>High deviation in qRT-PCR data of CONVR mice.

**Table S3.** GO terms that are overrepresented among top regulated**Duodenum: overrepresented terms among top 500 genes regul**

<b>GO Term</b>	<b>P-val</b>	<b>Frequency</b>	<b>Genome frequency</b>
GO:0016020	2.42E-12	240/500 (48.0%)	5797/17360 (33.4%)
GO:0005783	8.11E-11	56/500 (11.2%)	760/17360 (4.4%)
GO:0019752	1.16E-08	39/500 (7.8%)	492/17360 (2.8%)
GO:0006082	1.23E-08	39/500 (7.8%)	493/17360 (2.8%)
GO:0005792	1.34E-08	21/500 (4.2%)	166/17360 (1.0%)
GO:0042598	2.52E-08	21/500 (4.2%)	172/17360 (1.0%)
GO:0016491	1.65E-07	49/500 (9.8%)	769/17360 (4.4%)
GO:0009055	1.99E-07	24/500 (4.8%)	246/17360 (1.4%)
GO:0055114	3.27E-07	42/500 (8.4%)	625/17360 (3.6%)
GO:0044420	1.18E-06	13/500 (2.6%)	87/17360 (0.5%)
GO:0032787	1.73E-06	23/500 (4.6%)	258/17360 (1.5%)
GO:0018958	2.21E-06	12/500 (2.4%)	78/17360 (0.4%)
GO:0006066	2.28E-06	28/500 (5.6%)	362/17360 (2.1%)
GO:0044444	2.58E-06	149/500 (29.8%)	3675/17360 (21.2%)
GO:0044432	2.91E-06	34/500 (6.8%)	496/17360 (2.9%)
GO:0006519	8.09E-06	24/500 (4.8%)	303/17360 (1.7%)
GO:0003824	8.53E-06	202/500 (40.4%)	5439/17360 (31.3%)
GO:0003823	1.09E-05	6/500 (1.2%)	19/17360 (0.1%)
GO:0006807	1.29E-05	27/500 (5.4%)	375/17360 (2.2%)
GO:0005789	1.36E-05	30/500 (6.0%)	442/17360 (2.5%)
GO:0051186	1.89E-05	19/500 (3.8%)	218/17360 (1.3%)
GO:0009308	2.09E-05	25/500 (5.0%)	342/17360 (2.0%)
GO:0007050	2.13E-05	9/500 (1.8%)	54/17360 (0.3%)
GO:0005583	2.22E-05	4/500 (0.8%)	7/17360 (0.0%)
GO:0043259	2.38E-05	3/500 (0.6%)	3/17360 (0.0%)
GO:0044255	2.72E-05	36/500 (7.2%)	599/17360 (3.5%)
GO:0007155	3.41E-05	38/500 (7.6%)	654/17360 (3.8%)
GO:0022610	3.41E-05	38/500 (7.6%)	654/17360 (3.8%)
GO:0034641	3.53E-05	25/500 (5.0%)	353/17360 (2.0%)
GO:0005201	3.78E-05	8/500 (1.6%)	45/17360 (0.3%)
GO:0048407	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0005509	4.81E-05	48/500 (9.6%)	918/17360 (5.3%)
GO:0008667	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0009237	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0009238	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0009239	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0009712	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0019184	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0019290	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0019540	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0030155	5.41E-05	11/500 (2.2%)	90/17360 (0.5%)
GO:0006631	5.46E-05	17/500 (3.4%)	196/17360 (1.1%)
GO:0006518	5.81E-05	9/500 (1.8%)	61/17360 (0.4%)
GO:0006725	6.60E-05	15/500 (3.0%)	161/17360 (0.9%)
GO:0043043	7.14E-05	8/500 (1.6%)	49/17360 (0.3%)
GO:0016627	7.34E-05	11/500 (2.2%)	93/17360 (0.5%)
GO:0016712	7.54E-05	9/500 (1.8%)	63/17360 (0.4%)
GO:0031012	8.27E-05	22/500 (4.4%)	306/17360 (1.8%)
GO:0042420	9.30E-05	3/500 (0.6%)	4/17360 (0.0%)

**Duodenum: overrepresented terms among top 500 genes regul**

<b>GO Term</b>	<b>P-val</b>	<b>Frequency</b>	<b>Genome frequency</b>
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GO:0055114	4.52E-25	73/500 (14.6%)	625/17360 (3.6%)
GO:0016491	7.49E-23	78/500 (15.6%)	769/17360 (4.4%)
GO:0003824	2.41E-19	251/500 (50.2%)	5439/17360 (31.3%)
GO:0008152	4.00E-12	279/500 (55.8%)	7112/17360 (41.0%)
GO:0004091	8.78E-12	20/500 (4.0%)	102/17360 (0.6%)
GO:0006629	1.19E-11	55/500 (11.0%)	705/17360 (4.1%)
GO:0016616	4.14E-11	21/500 (4.2%)	123/17360 (0.7%)
GO:0016614	7.58E-11	22/500 (4.4%)	140/17360 (0.8%)
GO:0044255	1.10E-10	48/500 (9.6%)	599/17360 (3.5%)
GO:0004364	2.84E-10	10/500 (2.0%)	23/17360 (0.1%)
GO:0051186	8.81E-10	26/500 (5.2%)	218/17360 (1.3%)
GO:0032787	1.80E-09	28/500 (5.6%)	258/17360 (1.5%)
GO:0009055	2.73E-09	27/500 (5.4%)	246/17360 (1.4%)
GO:0019752	3.63E-09	40/500 (8.0%)	492/17360 (2.8%)
GO:0020037	3.79E-09	21/500 (4.2%)	155/17360 (0.9%)
GO:0046906	3.79E-09	21/500 (4.2%)	155/17360 (0.9%)
GO:0006082	3.83E-09	40/500 (8.0%)	493/17360 (2.8%)
GO:0006066	5.53E-09	33/500 (6.6%)	362/17360 (2.1%)
GO:0006631	1.17E-08	23/500 (4.6%)	196/17360 (1.1%)
GO:0005792	1.34E-08	21/500 (4.2%)	166/17360 (1.0%)
GO:0004104	1.51E-08	8/500 (1.6%)	18/17360 (0.1%)
GO:0016712	2.25E-08	13/500 (2.6%)	63/17360 (0.4%)
GO:0042598	2.52E-08	21/500 (4.2%)	172/17360 (1.0%)
GO:0044444	3.78E-08	157/500 (31.4%)	3675/17360 (21.2%)
GO:0006725	4.05E-08	20/500 (4.0%)	161/17360 (0.9%)
GO:0004497	6.45E-08	19/500 (3.8%)	150/17360 (0.9%)
GO:0042287	8.30E-08	7/500 (1.4%)	15/17360 (0.1%)
GO:0018958	3.21E-07	13/500 (2.6%)	78/17360 (0.4%)
GO:0016705	3.26E-07	19/500 (3.8%)	166/17360 (1.0%)
GO:0005506	7.01E-07	28/500 (5.6%)	341/17360 (2.0%)
GO:0042288	8.00E-07	6/500 (1.2%)	13/17360 (0.1%)
GO:0016765	1.31E-06	10/500 (2.0%)	50/17360 (0.3%)
GO:0051188	1.57E-06	16/500 (3.2%)	134/17360 (0.8%)
GO:0005783	1.68E-06	46/500 (9.2%)	760/17360 (4.4%)
GO:0002478	1.85E-06	7/500 (1.4%)	22/17360 (0.1%)
GO:0002495	2.22E-06	6/500 (1.2%)	15/17360 (0.1%)
GO:0019886	2.22E-06	6/500 (1.2%)	15/17360 (0.1%)
GO:0042613	2.22E-06	5/500 (1.0%)	9/17360 (0.1%)
GO:0006766	2.53E-06	12/500 (2.4%)	79/17360 (0.5%)
GO:0016627	2.55E-06	13/500 (2.6%)	93/17360 (0.5%)
GO:0006749	2.59E-06	7/500 (1.4%)	23/17360 (0.1%)
GO:0044432	2.91E-06	34/500 (6.8%)	496/17360 (2.9%)
GO:0006732	3.10E-06	16/500 (3.2%)	141/17360 (0.8%)
GO:0005777	4.63E-06	13/500 (2.6%)	98/17360 (0.6%)
GO:0042579	5.19E-06	13/500 (2.6%)	99/17360 (0.6%)
GO:0002504	5.23E-06	6/500 (1.2%)	17/17360 (0.1%)
GO:0008667	6.53E-06	9/500 (1.8%)	47/17360 (0.3%)
GO:0009237	6.53E-06	9/500 (1.8%)	47/17360 (0.3%)
GO:0009238	6.53E-06	9/500 (1.8%)	47/17360 (0.3%)
GO:0009239	6.53E-06	9/500 (1.8%)	47/17360 (0.3%)
GO:0009712	6.53E-06	9/500 (1.8%)	47/17360 (0.3%)
GO:0019184	6.53E-06	9/500 (1.8%)	47/17360 (0.3%)
GO:0019290	6.53E-06	9/500 (1.8%)	47/17360 (0.3%)
GO:0019540	6.53E-06	9/500 (1.8%)	47/17360 (0.3%)
GO:0042825	7.78E-06	5/500 (1.0%)	11/17360 (0.1%)
GO:0019884	8.49E-06	7/500 (1.4%)	27/17360 (0.2%)



GO:0043043	9.35E-06	9/500 (1.8%)	49/17360 (0.3%)
GO:0008202	1.46E-05	16/500 (3.2%)	159/17360 (0.9%)
GO:0006790	1.55E-05	10/500 (2.0%)	65/17360 (0.4%)
GO:0006955	2.15E-05	28/500 (5.6%)	408/17360 (2.4%)
GO:0070330	2.27E-05	7/500 (1.4%)	31/17360 (0.2%)
GO:0034045	2.38E-05	3/500 (0.6%)	3/17360 (0.0%)
GO:0048002	2.84E-05	7/500 (1.4%)	32/17360 (0.2%)
GO:0005771	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0016628	4.43E-05	9/500 (1.8%)	59/17360 (0.3%)
GO:0006776	4.79E-05	6/500 (1.2%)	24/17360 (0.1%)
GO:0006518	5.81E-05	9/500 (1.8%)	61/17360 (0.4%)
GO:0016125	6.23E-05	10/500 (2.0%)	76/17360 (0.4%)

**Duodenum: overrepresented terms among top 500 genes where**

GO Term	P-val	Frequency	Genome frequency
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**Jejunum: overrepresented terms among top 500 genes regulating**

GO Term	P-val	Frequency	Genome frequency
GO:0003824	9.99E-09	216/500 (43.2%)	5439/17360 (31.3%)
GO:0005783	1.16E-07	49/500 (9.8%)	760/17360 (4.4%)
GO:0055114	1.23E-07	43/500 (8.6%)	625/17360 (3.6%)
GO:0002682	6.84E-07	25/500 (5.0%)	282/17360 (1.6%)
GO:0002684	1.35E-06	19/500 (3.8%)	182/17360 (1.0%)
GO:0045582	1.50E-06	8/500 (1.6%)	30/17360 (0.2%)
GO:0016491	2.31E-06	46/500 (9.2%)	769/17360 (4.4%)
GO:0045621	2.56E-06	8/500 (1.6%)	32/17360 (0.2%)
GO:0003823	1.09E-05	6/500 (1.2%)	19/17360 (0.1%)
GO:0045321	1.19E-05	19/500 (3.8%)	211/17360 (1.2%)
GO:0050870	1.35E-05	10/500 (2.0%)	64/17360 (0.4%)
GO:0006082	1.72E-05	32/500 (6.4%)	493/17360 (2.8%)
GO:0001775	1.84E-05	20/500 (4.0%)	237/17360 (1.4%)
GO:0045597	2.32E-05	16/500 (3.2%)	165/17360 (1.0%)
GO:0045580	2.67E-05	8/500 (1.6%)	43/17360 (0.2%)
GO:0051251	2.82E-05	11/500 (2.2%)	84/17360 (0.5%)
GO:0002376	3.45E-05	37/500 (7.4%)	630/17360 (3.6%)
GO:0005789	3.51E-05	29/500 (5.8%)	442/17360 (2.5%)
GO:0050778	3.55E-05	13/500 (2.6%)	118/17360 (0.7%)
GO:0008238	3.94E-05	11/500 (2.2%)	87/17360 (0.5%)
GO:0019752	4.11E-05	31/500 (6.2%)	492/17360 (2.8%)
GO:0002696	4.38E-05	11/500 (2.2%)	88/17360 (0.5%)
GO:0044432	4.78E-05	31/500 (6.2%)	496/17360 (2.9%)
GO:0016614	5.32E-05	14/500 (2.8%)	140/17360 (0.8%)
GO:0006066	5.33E-05	25/500 (5.0%)	362/17360 (2.1%)
GO:0016616	5.50E-05	13/500 (2.6%)	123/17360 (0.7%)
GO:0050867	6.00E-05	11/500 (2.2%)	91/17360 (0.5%)
GO:0002253	6.23E-05	10/500 (2.0%)	76/17360 (0.4%)
GO:0051249	9.02E-05	13/500 (2.6%)	129/17360 (0.7%)
GO:0045619	9.59E-05	8/500 (1.6%)	51/17360 (0.3%)
GO:0016829	9.75E-05	14/500 (2.8%)	148/17360 (0.9%)
GO:0046942	9.75E-05	14/500 (2.8%)	148/17360 (0.9%)

**Jejunum: overrepresented terms among top 500 genes regulating**

GO Term	P-val	Frequency	Genome frequency
GO:0006955	2.83E-18	50/500 (10.0%)	408/17360 (2.4%)
GO:0002376	2.00E-16	60/500 (12.0%)	630/17360 (3.6%)
GO:0042287	5.36E-11	9/500 (1.8%)	15/17360 (0.1%)

GO:0042825	6.79E-11	8/500 (1.6%)	11/17360 (0.1%)
GO:0042288	5.00E-10	8/500 (1.6%)	13/17360 (0.1%)
GO:0048002	7.19E-10	11/500 (2.2%)	32/17360 (0.2%)
GO:0009615	1.82E-09	13/500 (2.6%)	52/17360 (0.3%)
GO:0019882	1.45E-08	14/500 (2.8%)	72/17360 (0.4%)
GO:0002478	1.00E-07	8/500 (1.6%)	22/17360 (0.1%)
GO:0032553	1.34E-07	94/500 (18.8%)	1915/17360 (11.0%)
GO:0032555	1.34E-07	94/500 (18.8%)	1915/17360 (11.0%)
GO:0007049	1.64E-07	36/500 (7.2%)	479/17360 (2.8%)
GO:0000280	1.82E-07	19/500 (3.8%)	160/17360 (0.9%)
GO:0007067	1.82E-07	19/500 (3.8%)	160/17360 (0.9%)
GO:0050896	2.05E-07	111/500 (22.2%)	2409/17360 (13.9%)
GO:0017076	2.66E-07	96/500 (19.2%)	1999/17360 (11.5%)
GO:0002449	2.93E-07	10/500 (2.0%)	43/17360 (0.2%)
GO:0042611	2.93E-07	10/500 (2.0%)	43/17360 (0.2%)
GO:0048285	3.26E-07	19/500 (3.8%)	166/17360 (1.0%)
GO:0019884	6.14E-07	8/500 (1.6%)	27/17360 (0.2%)
GO:0002443	7.14E-07	10/500 (2.0%)	47/17360 (0.3%)
GO:0002250	1.08E-06	10/500 (2.0%)	49/17360 (0.3%)
GO:0002460	1.08E-06	10/500 (2.0%)	49/17360 (0.3%)
GO:0051301	1.52E-06	23/500 (4.6%)	256/17360 (1.5%)
GO:0002252	1.67E-06	14/500 (2.8%)	104/17360 (0.6%)
GO:0000166	1.78E-06	102/500 (20.4%)	2256/17360 (13.0%)
GO:0002495	2.22E-06	6/500 (1.2%)	15/17360 (0.1%)
GO:0019886	2.22E-06	6/500 (1.2%)	15/17360 (0.1%)
GO:0042613	2.22E-06	5/500 (1.0%)	9/17360 (0.1%)
GO:0003824	3.59E-06	204/500 (40.8%)	5439/17360 (31.3%)
GO:0002504	5.23E-06	6/500 (1.2%)	17/17360 (0.1%)
GO:0005524	5.48E-06	76/500 (15.2%)	1579/17360 (9.1%)
GO:0009607	5.63E-06	22/500 (4.4%)	257/17360 (1.5%)
GO:0051707	7.57E-06	16/500 (3.2%)	151/17360 (0.9%)
GO:0032559	8.80E-06	76/500 (15.2%)	1600/17360 (9.2%)
GO:0022403	1.08E-05	21/500 (4.2%)	248/17360 (1.4%)
GO:0006952	1.39E-05	31/500 (6.2%)	465/17360 (2.7%)
GO:0030554	1.62E-05	78/500 (15.6%)	1684/17360 (9.7%)
GO:0005771	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0043368	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0045059	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0045061	4.59E-05	5/500 (1.0%)	15/17360 (0.1%)
GO:0044432	4.78E-05	31/500 (6.2%)	496/17360 (2.9%)
GO:0016064	4.79E-05	6/500 (1.2%)	24/17360 (0.1%)
GO:0043383	7.64E-05	4/500 (0.8%)	9/17360 (0.1%)
GO:0045060	7.64E-05	4/500 (0.8%)	9/17360 (0.1%)
GO:0016817	7.74E-05	44/500 (8.8%)	832/17360 (4.8%)
GO:0022402	8.00E-05	24/500 (4.8%)	349/17360 (2.0%)
GO:0019724	9.79E-05	6/500 (1.2%)	27/17360 (0.2%)

**Jejunum: overrepresented terms among top 500 genes where  $\lambda$**

GO Term	P-val	Frequency	Genome frequency
GO:0042287	5.36E-11	9/500 (1.8%)	15/17360 (0.1%)
GO:0044432	4.55E-09	40/500 (8.0%)	496/17360 (2.9%)
GO:0042288	2.32E-08	7/500 (1.4%)	13/17360 (0.1%)
GO:0006695	4.16E-08	8/500 (1.6%)	20/17360 (0.1%)
GO:0048002	1.97E-07	9/500 (1.8%)	32/17360 (0.2%)
GO:0042825	2.26E-07	6/500 (1.2%)	11/17360 (0.1%)
GO:0002376	4.05E-07	42/500 (8.4%)	630/17360 (3.6%)

GO:0008203	5.66E-07	12/500 (2.4%)	69/17360 (0.4%)
GO:0016126	6.14E-07	8/500 (1.6%)	27/17360 (0.2%)
GO:0050863	1.32E-06	14/500 (2.8%)	102/17360 (0.6%)
GO:0016125	1.66E-06	12/500 (2.4%)	76/17360 (0.4%)
GO:0005783	1.68E-06	46/500 (9.2%)	760/17360 (4.4%)
GO:0002478	1.85E-06	7/500 (1.4%)	22/17360 (0.1%)
GO:0002495	2.22E-06	6/500 (1.2%)	15/17360 (0.1%)
GO:0019886	2.22E-06	6/500 (1.2%)	15/17360 (0.1%)
GO:0042613	2.22E-06	5/500 (1.0%)	9/17360 (0.1%)
GO:0006955	2.80E-06	30/500 (6.0%)	408/17360 (2.4%)
GO:0008610	4.80E-06	23/500 (4.6%)	274/17360 (1.6%)
GO:0005789	5.05E-06	31/500 (6.2%)	442/17360 (2.5%)
GO:0002504	5.23E-06	6/500 (1.2%)	17/17360 (0.1%)
GO:0019882	6.26E-06	11/500 (2.2%)	72/17360 (0.4%)
GO:0019884	8.49E-06	7/500 (1.4%)	27/17360 (0.2%)
GO:0002252	9.00E-06	13/500 (2.6%)	104/17360 (0.6%)
GO:0044255	1.15E-05	37/500 (7.4%)	599/17360 (3.5%)
GO:0016491	1.19E-05	44/500 (8.8%)	769/17360 (4.4%)
GO:0008202	1.46E-05	16/500 (3.2%)	159/17360 (0.9%)
GO:0051249	2.13E-05	14/500 (2.8%)	129/17360 (0.7%)
GO:0055114	2.91E-05	37/500 (7.4%)	625/17360 (3.6%)
GO:0044444	4.11E-05	143/500 (28.6%)	3675/17360 (21.2%)
GO:0002694	4.18E-05	14/500 (2.8%)	137/17360 (0.8%)
GO:0016020	4.18E-05	209/500 (41.8%)	5797/17360 (33.4%)
GO:0005771	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0043368	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0045059	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0045061	4.59E-05	5/500 (1.0%)	15/17360 (0.1%)
GO:0031090	4.67E-05	66/500 (13.2%)	1400/17360 (8.1%)
GO:0050865	5.32E-05	14/500 (2.8%)	140/17360 (0.8%)
GO:0006694	6.23E-05	10/500 (2.0%)	76/17360 (0.4%)
GO:0043383	7.64E-05	4/500 (0.8%)	9/17360 (0.1%)
GO:0045060	7.64E-05	4/500 (0.8%)	9/17360 (0.1%)
GO:0050868	9.17E-05	7/500 (1.4%)	38/17360 (0.2%)
GO:0016705	9.36E-05	15/500 (3.0%)	166/17360 (1.0%)

#### Ileum: overrepresented terms among top 500 genes regulated k

GO Term	P-val	Frequency	Genome frequency
GO:0002376	7.36E-25	73/500 (14.6%)	630/17360 (3.6%)
GO:0016020	3.52E-17	256/500 (51.2%)	5797/17360 (33.4%)
GO:0009897	4.42E-17	30/500 (6.0%)	152/17360 (0.9%)
GO:0006955	7.17E-17	48/500 (9.6%)	408/17360 (2.4%)
GO:0002682	2.59E-13	35/500 (7.0%)	282/17360 (1.6%)
GO:0002684	3.39E-13	28/500 (5.6%)	182/17360 (1.0%)
GO:0019882	1.24E-12	18/500 (3.6%)	72/17360 (0.4%)
GO:0050865	1.11E-11	23/500 (4.6%)	140/17360 (0.8%)
GO:0051249	1.46E-11	22/500 (4.4%)	129/17360 (0.7%)
GO:0048002	3.65E-11	12/500 (2.4%)	32/17360 (0.2%)
GO:0055114	4.43E-11	50/500 (10.0%)	625/17360 (3.6%)
GO:0002694	4.92E-11	22/500 (4.4%)	137/17360 (0.8%)
GO:0045321	6.98E-11	27/500 (5.4%)	211/17360 (1.2%)
GO:0050863	7.38E-11	19/500 (3.8%)	102/17360 (0.6%)
GO:0050867	8.27E-11	18/500 (3.6%)	91/17360 (0.5%)
GO:0019884	8.38E-11	11/500 (2.2%)	27/17360 (0.2%)
GO:0051251	1.90E-10	17/500 (3.4%)	84/17360 (0.5%)
GO:0007159	1.98E-10	8/500 (1.6%)	12/17360 (0.1%)

GO:0050870	2.51E-10	15/500 (3.0%)	64/17360 (0.4%)
GO:0002696	4.06E-10	17/500 (3.4%)	88/17360 (0.5%)
GO:0050776	4.07E-10	23/500 (4.6%)	166/17360 (1.0%)
GO:0016491	4.22E-10	55/500 (11.0%)	769/17360 (4.4%)
GO:0001775	1.17E-09	27/500 (5.4%)	237/17360 (1.4%)
GO:0003824	1.88E-09	219/500 (43.8%)	5439/17360 (31.3%)
GO:0042287	2.39E-09	8/500 (1.6%)	15/17360 (0.1%)
GO:0002478	4.49E-09	9/500 (1.8%)	22/17360 (0.1%)
GO:0042825	4.68E-09	7/500 (1.4%)	11/17360 (0.1%)
GO:0050778	7.51E-09	18/500 (3.6%)	118/17360 (0.7%)
GO:0044459	1.23E-08	75/500 (15.0%)	1328/17360 (7.6%)
GO:0045621	1.31E-08	10/500 (2.0%)	32/17360 (0.2%)
GO:0050896	1.63E-08	115/500 (23.0%)	2409/17360 (13.9%)
GO:0042288	2.32E-08	7/500 (1.4%)	13/17360 (0.1%)
GO:0006952	2.53E-08	37/500 (7.4%)	465/17360 (2.7%)
GO:0046649	2.56E-08	22/500 (4.4%)	188/17360 (1.1%)
GO:0005886	3.45E-08	95/500 (19.0%)	1888/17360 (10.9%)
GO:0009055	4.98E-08	25/500 (5.0%)	246/17360 (1.4%)
GO:0042221	5.73E-08	52/500 (10.4%)	812/17360 (4.7%)
GO:0002495	8.30E-08	7/500 (1.4%)	15/17360 (0.1%)
GO:0019886	8.30E-08	7/500 (1.4%)	15/17360 (0.1%)
GO:0044255	1.03E-07	42/500 (8.4%)	599/17360 (3.5%)
GO:0045582	1.06E-07	9/500 (1.8%)	30/17360 (0.2%)
GO:0006629	2.06E-07	46/500 (9.2%)	705/17360 (4.1%)
GO:0002504	2.39E-07	7/500 (1.4%)	17/17360 (0.1%)
GO:0005783	2.89E-07	48/500 (9.6%)	760/17360 (4.4%)
GO:0042110	3.10E-07	16/500 (3.2%)	119/17360 (0.7%)
GO:0045058	3.81E-07	7/500 (1.4%)	18/17360 (0.1%)
GO:0070666	5.66E-07	12/500 (2.4%)	69/17360 (0.4%)
GO:0044444	5.68E-07	152/500 (30.4%)	3675/17360 (21.2%)
GO:0032944	1.06E-06	12/500 (2.4%)	73/17360 (0.4%)
GO:0050670	1.06E-06	12/500 (2.4%)	73/17360 (0.4%)
GO:0070663	1.06E-06	12/500 (2.4%)	73/17360 (0.4%)
GO:0044432	1.07E-06	35/500 (7.0%)	496/17360 (2.9%)
GO:0048584	1.35E-06	18/500 (3.6%)	165/17360 (1.0%)
GO:0045619	1.59E-06	10/500 (2.0%)	51/17360 (0.3%)
GO:0006954	1.64E-06	20/500 (4.0%)	202/17360 (1.2%)
GO:0045061	2.22E-06	6/500 (1.2%)	15/17360 (0.1%)
GO:0042613	2.22E-06	5/500 (1.0%)	9/17360 (0.1%)
GO:0043383	2.22E-06	5/500 (1.0%)	9/17360 (0.1%)
GO:0045060	2.22E-06	5/500 (1.0%)	9/17360 (0.1%)
GO:0048518	2.78E-06	68/500 (13.6%)	1335/17360 (7.7%)
GO:0016614	2.82E-06	16/500 (3.2%)	140/17360 (0.8%)
GO:0042611	2.99E-06	9/500 (1.8%)	43/17360 (0.2%)
GO:0045580	2.99E-06	9/500 (1.8%)	43/17360 (0.2%)
GO:0009605	4.25E-06	37/500 (7.4%)	573/17360 (3.3%)
GO:0001772	4.34E-06	5/500 (1.0%)	10/17360 (0.1%)
GO:0048583	4.84E-06	24/500 (4.8%)	294/17360 (1.7%)
GO:0032787	5.99E-06	22/500 (4.4%)	258/17360 (1.5%)
GO:0046635	6.44E-06	7/500 (1.4%)	26/17360 (0.1%)
GO:0019752	6.45E-06	33/500 (6.6%)	492/17360 (2.8%)
GO:0006082	6.73E-06	33/500 (6.6%)	493/17360 (2.8%)
GO:0002252	9.00E-06	13/500 (2.6%)	104/17360 (0.6%)
GO:0020037	1.06E-05	16/500 (3.2%)	155/17360 (0.9%)
GO:0046906	1.06E-05	16/500 (3.2%)	155/17360 (0.9%)
GO:0009611	1.08E-05	21/500 (4.2%)	248/17360 (1.4%)



GO:0016616	1.23E-05	14/500 (2.8%)	123/17360 (0.7%)
GO:0006725	1.71E-05	16/500 (3.2%)	161/17360 (0.9%)
GO:0006066	1.94E-05	26/500 (5.2%)	362/17360 (2.1%)
GO:0019955	1.95E-05	14/500 (2.8%)	128/17360 (0.7%)
GO:0050853	2.06E-05	5/500 (1.0%)	13/17360 (0.1%)
GO:0043235	2.24E-05	11/500 (2.2%)	82/17360 (0.5%)
GO:0046983	2.33E-05	24/500 (4.8%)	323/17360 (1.9%)
GO:0030852	2.38E-05	3/500 (0.6%)	3/17360 (0.0%)
GO:0000267	2.68E-05	34/500 (6.8%)	551/17360 (3.2%)
GO:0042129	2.88E-05	9/500 (1.8%)	56/17360 (0.3%)
GO:0002757	3.18E-05	8/500 (1.6%)	44/17360 (0.3%)
GO:0008305	3.51E-05	7/500 (1.4%)	33/17360 (0.2%)
GO:0050851	3.51E-05	7/500 (1.4%)	33/17360 (0.2%)
GO:0050871	3.51E-05	7/500 (1.4%)	33/17360 (0.2%)
GO:0032946	3.78E-05	8/500 (1.6%)	45/17360 (0.3%)
GO:0050671	3.78E-05	8/500 (1.6%)	45/17360 (0.3%)
GO:0070665	3.78E-05	8/500 (1.6%)	45/17360 (0.3%)
GO:0070668	3.78E-05	8/500 (1.6%)	45/17360 (0.3%)
GO:0005626	3.80E-05	31/500 (6.2%)	490/17360 (2.8%)
GO:0042102	4.32E-05	7/500 (1.4%)	34/17360 (0.2%)
GO:0005771	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0043368	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0045059	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0050798	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0055069	4.34E-05	4/500 (0.8%)	8/17360 (0.0%)
GO:0005624	5.03E-05	30/500 (6.0%)	474/17360 (2.7%)
GO:0017076	5.06E-05	87/500 (17.4%)	1999/17360 (11.5%)
GO:0002443	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0046634	5.26E-05	7/500 (1.4%)	35/17360 (0.2%)
GO:0005506	5.57E-05	24/500 (4.8%)	341/17360 (2.0%)
GO:0002526	6.13E-05	8/500 (1.6%)	48/17360 (0.3%)
GO:0002764	6.13E-05	8/500 (1.6%)	48/17360 (0.3%)
GO:0002819	6.13E-05	8/500 (1.6%)	48/17360 (0.3%)
GO:0002822	6.13E-05	8/500 (1.6%)	48/17360 (0.3%)
GO:0050864	6.13E-05	8/500 (1.6%)	48/17360 (0.3%)
GO:0002253	6.23E-05	10/500 (2.0%)	76/17360 (0.4%)
GO:0002250	7.14E-05	8/500 (1.6%)	49/17360 (0.3%)
GO:0002460	7.14E-05	8/500 (1.6%)	49/17360 (0.3%)
GO:0005887	7.62E-05	20/500 (4.0%)	262/17360 (1.5%)
GO:0002429	9.17E-05	7/500 (1.4%)	38/17360 (0.2%)
GO:0005415	9.30E-05	3/500 (0.6%)	4/17360 (0.0%)
GO:0002821	9.79E-05	6/500 (1.2%)	27/17360 (0.2%)
GO:0002824	9.79E-05	6/500 (1.2%)	27/17360 (0.2%)

#### Ileum: overrepresented terms among top 500 genes regulated b

GO Term	P-val	Frequency	Genome frequency
GO:0006955	7.17E-17	48/500 (9.6%)	408/17360 (2.4%)
GO:0002376	2.95E-15	58/500 (11.6%)	630/17360 (3.6%)
GO:0002252	6.68E-09	17/500 (3.4%)	104/17360 (0.6%)
GO:0006952	7.84E-08	36/500 (7.2%)	465/17360 (2.7%)
GO:0045087	1.28E-07	15/500 (3.0%)	98/17360 (0.6%)
GO:0003824	1.34E-07	211/500 (42.2%)	5439/17360 (31.3%)
GO:0032020	6.80E-07	4/500 (0.8%)	4/17360 (0.0%)
GO:0050776	1.47E-06	18/500 (3.6%)	166/17360 (1.0%)
GO:0050778	1.50E-06	15/500 (3.0%)	118/17360 (0.7%)
GO:0004364	2.59E-06	7/500 (1.4%)	23/17360 (0.1%)

GO:0009611	3.15E-06	22/500 (4.4%)	248/17360 (1.4%)
GO:0003924	4.96E-06	20/500 (4.0%)	217/17360 (1.3%)
GO:0009607	5.63E-06	22/500 (4.4%)	257/17360 (1.5%)
GO:0019882	6.26E-06	11/500 (2.2%)	72/17360 (0.4%)
GO:0006954	6.35E-06	19/500 (3.8%)	202/17360 (1.2%)
GO:0016787	8.14E-06	105/500 (21.0%)	2422/17360 (14.0%)
GO:0016020	8.30E-06	213/500 (42.6%)	5797/17360 (33.4%)
GO:0005525	9.16E-06	27/500 (5.4%)	368/17360 (2.1%)
GO:0002253	1.07E-05	11/500 (2.2%)	76/17360 (0.4%)
GO:0032561	1.42E-05	27/500 (5.4%)	377/17360 (2.2%)
GO:0019001	1.49E-05	27/500 (5.4%)	378/17360 (2.2%)
GO:0050896	1.84E-05	103/500 (20.6%)	2409/17360 (13.9%)
GO:0048584	2.32E-05	16/500 (3.2%)	165/17360 (1.0%)
GO:0002682	2.43E-05	22/500 (4.4%)	282/17360 (1.6%)
GO:0016817	3.76E-05	45/500 (9.0%)	832/17360 (4.8%)
GO:0042221	4.40E-05	44/500 (8.8%)	812/17360 (4.7%)
GO:0006959	4.46E-05	8/500 (1.6%)	46/17360 (0.3%)
GO:0002443	5.24E-05	8/500 (1.6%)	47/17360 (0.3%)
GO:0002684	7.66E-05	16/500 (3.2%)	182/17360 (1.0%)

#### Ileum: overrepresented terms among top 500 genes where Myd

GO Term	P-val	Frequency	Genome frequency
GO:0006695	4.16E-08	8/500 (1.6%)	20/17360 (0.1%)
GO:0016126	6.14E-07	8/500 (1.6%)	27/17360 (0.2%)
GO:0006066	2.28E-06	28/500 (5.6%)	362/17360 (2.1%)
GO:0008202	3.49E-06	17/500 (3.4%)	159/17360 (0.9%)
GO:0008203	4.09E-06	11/500 (2.2%)	69/17360 (0.4%)
GO:0006694	1.07E-05	11/500 (2.2%)	76/17360 (0.4%)
GO:0016125	1.07E-05	11/500 (2.2%)	76/17360 (0.4%)
GO:0044255	1.15E-05	37/500 (7.4%)	599/17360 (3.5%)
GO:0006629	1.63E-05	41/500 (8.2%)	705/17360 (4.1%)
GO:0008299	2.06E-05	5/500 (1.0%)	13/17360 (0.1%)
GO:0008152	3.18E-05	249/500 (49.8%)	7112/17360 (41.0%)
GO:0006720	3.51E-05	7/500 (1.4%)	33/17360 (0.2%)
GO:0008610	4.79E-05	21/500 (4.2%)	274/17360 (1.6%)
GO:0055114	6.56E-05	36/500 (7.2%)	625/17360 (3.6%)

#### Proximal colon: overrepresented terms among top 500 genes r

GO Term	P-val	Frequency	Genome frequency
GO:0003824	2.90E-08	214/500 (42.8%)	5439/17360 (31.3%)
GO:0016324	5.34E-08	17/500 (3.4%)	119/17360 (0.7%)
GO:0005903	3.81E-07	7/500 (1.4%)	18/17360 (0.1%)
GO:0042598	5.67E-07	19/500 (3.8%)	172/17360 (1.0%)
GO:0016491	9.82E-07	47/500 (9.4%)	769/17360 (4.4%)
GO:0005792	1.47E-06	18/500 (3.6%)	166/17360 (1.0%)
GO:0016712	1.61E-06	11/500 (2.2%)	63/17360 (0.4%)
GO:0004497	6.95E-06	16/500 (3.2%)	150/17360 (0.9%)
GO:0000267	1.11E-05	35/500 (7.0%)	551/17360 (3.2%)
GO:0055114	1.25E-05	38/500 (7.6%)	625/17360 (3.6%)
GO:0016020	1.26E-05	212/500 (42.4%)	5797/17360 (33.4%)
GO:0005626	1.53E-05	32/500 (6.4%)	490/17360 (2.8%)
GO:0044432	1.94E-05	32/500 (6.4%)	496/17360 (2.9%)
GO:0032787	1.97E-05	21/500 (4.2%)	258/17360 (1.5%)
GO:0001730	2.38E-05	3/500 (0.6%)	3/17360 (0.0%)
GO:0020037	4.26E-05	15/500 (3.0%)	155/17360 (0.9%)
GO:0046906	4.26E-05	15/500 (3.0%)	155/17360 (0.9%)

GO:0005624	5.03E-05	30/500 (6.0%)	474/17360 (2.7%)
GO:0007623	5.26E-05	7/500 (1.4%)	35/17360 (0.2%)
GO:0005783	8.87E-05	41/500 (8.2%)	760/17360 (4.4%)
GO:0001523	9.02E-05	5/500 (1.0%)	17/17360 (0.1%)
GO:0006721	9.02E-05	5/500 (1.0%)	17/17360 (0.1%)
GO:0016101	9.02E-05	5/500 (1.0%)	17/17360 (0.1%)

**Proximal colon: overrepresented terms among top 500 genes r**

GO Term	P-val	Frequency	Genome frequency
GO:0046777	1.78E-05	10/500 (2.0%)	66/17360 (0.4%)
GO:0003824	2.94E-05	199/500 (39.8%)	5439/17360 (31.3%)
GO:0044237	4.12E-05	214/500 (42.8%)	5961/17360 (34.3%)
GO:0005488	9.34E-05	376/500 (75.2%)	11727/17360 (67.6%)
GO:0008152	9.72E-05	246/500 (49.2%)	7112/17360 (41.0%)

**Proximal colon: overrepresented terms among top 500 genes w**

GO Term	P-val	Frequency	Genome frequency
GO:0000280	9.17E-14	27/500 (5.4%)	160/17360 (0.9%)
GO:0007067	9.17E-14	27/500 (5.4%)	160/17360 (0.9%)
GO:0048285	2.29E-13	27/500 (5.4%)	166/17360 (1.0%)
GO:0022403	9.62E-13	32/500 (6.4%)	248/17360 (1.4%)
GO:0051301	1.15E-11	31/500 (6.2%)	256/17360 (1.5%)
GO:0003824	6.77E-11	224/500 (44.8%)	5439/17360 (31.3%)
GO:0007049	1.04E-10	42/500 (8.4%)	479/17360 (2.8%)
GO:0022402	1.10E-10	35/500 (7.0%)	349/17360 (2.0%)
GO:0002376	2.41E-09	47/500 (9.4%)	630/17360 (3.6%)
GO:0006955	2.62E-09	36/500 (7.2%)	408/17360 (2.4%)
GO:0000776	2.53E-08	11/500 (2.2%)	43/17360 (0.2%)
GO:0016126	3.75E-08	9/500 (1.8%)	27/17360 (0.2%)
GO:0000775	6.91E-08	11/500 (2.2%)	47/17360 (0.3%)
GO:0009615	2.10E-07	11/500 (2.2%)	52/17360 (0.3%)
GO:0006695	8.83E-07	7/500 (1.4%)	20/17360 (0.1%)
GO:0044427	1.06E-06	27/500 (5.4%)	328/17360 (1.9%)
GO:0016125	1.66E-06	12/500 (2.4%)	76/17360 (0.4%)
GO:0017076	1.72E-06	93/500 (18.6%)	1999/17360 (11.5%)
GO:0005694	3.16E-06	18/500 (3.6%)	175/17360 (1.0%)
GO:0050896	3.72E-06	106/500 (21.2%)	2409/17360 (13.9%)
GO:0000166	5.53E-06	100/500 (20.0%)	2256/17360 (13.0%)
GO:0032553	1.02E-05	87/500 (17.4%)	1915/17360 (11.0%)
GO:0032555	1.02E-05	87/500 (17.4%)	1915/17360 (11.0%)
GO:0006694	1.07E-05	11/500 (2.2%)	76/17360 (0.4%)
GO:0006996	1.51E-05	53/500 (10.6%)	1005/17360 (5.8%)
GO:0030554	1.62E-05	78/500 (15.6%)	1684/17360 (9.7%)
GO:0000777	1.80E-05	7/500 (1.4%)	30/17360 (0.2%)
GO:0016787	2.30E-05	103/500 (20.6%)	2422/17360 (14.0%)
GO:0006950	2.32E-05	63/500 (12.6%)	1287/17360 (7.4%)
GO:0001730	2.38E-05	3/500 (0.6%)	3/17360 (0.0%)
GO:0033554	2.46E-05	26/500 (5.2%)	367/17360 (2.1%)
GO:0008203	2.66E-05	10/500 (2.0%)	69/17360 (0.4%)
GO:0005524	5.96E-05	72/500 (14.4%)	1579/17360 (9.1%)
GO:0006323	6.15E-05	6/500 (1.2%)	25/17360 (0.1%)
GO:0016779	6.49E-05	12/500 (2.4%)	108/17360 (0.6%)
GO:0016817	7.74E-05	44/500 (8.8%)	832/17360 (4.8%)
GO:0032559	9.02E-05	72/500 (14.4%)	1600/17360 (9.2%)
GO:0032020	9.30E-05	3/500 (0.6%)	4/17360 (0.0%)

genes from 2-way ANOVA tests.

#### **ated by microbiota**

##### **Description**

membrane  
endoplasmic reticulum  
carboxylic acid metabolic process  
organic acid metabolic process  
microsome  
vesicular fraction  
oxidoreductase activity  
electron carrier activity  
oxidation reduction  
extracellular matrix part  
monocarboxylic acid metabolic process  
phenol metabolic process  
cellular alcohol metabolic process  
cytoplasmic part  
endoplasmic reticulum part  
cellular amino acid and derivative metabolic process  
catalytic activity  
antigen binding  
nitrogen compound metabolic process  
endoplasmic reticulum membrane  
cofactor metabolic process  
cellular amine metabolic process  
cell cycle arrest  
fibrillar collagen  
laminin-10 complex  
cellular lipid metabolic process  
cell adhesion  
biological adhesion  
cellular nitrogen compound metabolic process  
extracellular matrix structural constituent  
platelet-derived growth factor binding  
calcium ion binding  
2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase activity  
siderophore metabolic process  
enterobactin metabolic process  
enterobactin biosynthetic process  
catechol metabolic process  
nonribosomal peptide biosynthetic process  
siderophore biosynthetic process  
siderophore biosynthetic process from catechol  
regulation of cell adhesion  
fatty acid metabolic process  
peptide metabolic process  
cellular aromatic compound metabolic process  
peptide biosynthetic process  
oxidoreductase activity, acting on the CH-CH group of donors  
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen...  
extracellular matrix  
dopamine catabolic process

#### **ated by Myd88 genotype**

##### **Description**

oxidation reduction  
oxidoreductase activity  
catalytic activity  
metabolic process  
carboxylesterase activity  
lipid metabolic process  
oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor  
oxidoreductase activity, acting on CH-OH group of donors  
cellular lipid metabolic process  
glutathione transferase activity  
cofactor metabolic process  
monocarboxylic acid metabolic process  
electron carrier activity  
carboxylic acid metabolic process  
heme binding  
tetrapyrrole binding  
organic acid metabolic process  
cellular alcohol metabolic process  
fatty acid metabolic process  
microsome  
cholinesterase activity  
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen...  
vesicular fraction  
cytoplasmic part  
cellular aromatic compound metabolic process  
monooxygenase activity  
MHC protein binding  
phenol metabolic process  
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen  
iron ion binding  
MHC class I protein binding  
transferase activity, transferring alkyl or aryl (other than methyl) groups  
cofactor biosynthetic process  
endoplasmic reticulum  
antigen processing and presentation of exogenous peptide antigen  
antigen processing and presentation of peptide antigen via MHC class II  
antigen processing and presentation of exogenous peptide antigen via MHC class II  
MHC class II protein complex  
vitamin metabolic process  
oxidoreductase activity, acting on the CH-CH group of donors  
glutathione metabolic process  
endoplasmic reticulum part  
coenzyme metabolic process  
peroxisome  
microbody  
antigen processing and presentation of peptide or polysaccharide antigen via MHC class II  
2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase activity  
siderophore metabolic process  
enterobactin metabolic process  
enterobactin biosynthetic process  
catechol metabolic process  
nonribosomal peptide biosynthetic process  
siderophore biosynthetic process  
siderophore biosynthetic process from catechol  
TAP complex  
antigen processing and presentation of exogenous antigen

- peptide biosynthetic process
- steroid metabolic process
- sulfur metabolic process
- immune response
- aromatase activity
- pre-autophagosomal structure membrane
- antigen processing and presentation of peptide antigen
- multivesicular body
- oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor
- vitamin A metabolic process
- peptide metabolic process
- sterol metabolic process

## **e Myd88 genotype modulates response to microbiota**

### **Description**

#### **enriched by microbiota**

##### **Description**

- catalytic activity
- endoplasmic reticulum
- oxidation reduction
- regulation of immune system process
- positive regulation of immune system process
- positive regulation of T cell differentiation
- oxidoreductase activity
- positive regulation of lymphocyte differentiation
- antigen binding
- leukocyte activation
- positive regulation of T cell activation
- organic acid metabolic process
- cell activation
- positive regulation of cell differentiation
- regulation of T cell differentiation
- positive regulation of lymphocyte activation
- immune system process
- endoplasmic reticulum membrane
- positive regulation of immune response
- exopeptidase activity
- carboxylic acid metabolic process
- positive regulation of leukocyte activation
- endoplasmic reticulum part
- oxidoreductase activity, acting on CH-OH group of donors
- cellular alcohol metabolic process
- oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor
- positive regulation of cell activation
- activation of immune response
- regulation of lymphocyte activation
- regulation of lymphocyte differentiation
- lyase activity
- carboxylic acid transport

#### **enriched by Myd88 genotype**

##### **Description**

- immune response
- immune system process
- MHC protein binding

TAP complex  
 MHC class I protein binding  
 antigen processing and presentation of peptide antigen  
 response to virus  
 antigen processing and presentation  
 antigen processing and presentation of exogenous peptide antigen  
 ribonucleotide binding  
 purine ribonucleotide binding  
 cell cycle  
 nuclear division  
 mitosis  
 response to stimulus  
 purine nucleotide binding  
 lymphocyte mediated immunity  
 MHC protein complex  
 organelle fission  
 antigen processing and presentation of exogenous antigen  
 leukocyte mediated immunity  
 adaptive immune response  
 adaptive immune response based on somatic recombination of immune receptors built from...  
 cell division  
 immune effector process  
 nucleotide binding  
 antigen processing and presentation of peptide antigen via MHC class II  
 antigen processing and presentation of exogenous peptide antigen via MHC class II  
 MHC class II protein complex  
 catalytic activity  
 antigen processing and presentation of peptide or polysaccharide antigen via MHC class II  
 ATP binding  
 response to biotic stimulus  
 response to other organism  
 adenylyl ribonucleotide binding  
 cell cycle phase  
 defense response  
 adenylyl nucleotide binding  
 multivesicular body  
 positive T cell selection  
 positive thymic T cell selection  
 thymic T cell selection  
 endoplasmic reticulum part  
 immunoglobulin mediated immune response  
 negative T cell selection  
 negative thymic T cell selection  
 hydrolase activity, acting on acid anhydrides  
 cell cycle process  
 B cell mediated immunity

### **lyd88 genotype modulates response to microbiota**

#### **Description**

MHC protein binding  
 endoplasmic reticulum part  
 MHC class I protein binding  
 cholesterol biosynthetic process  
 antigen processing and presentation of peptide antigen  
 TAP complex  
 immune system process

cholesterol metabolic process  
 sterol biosynthetic process  
 regulation of T cell activation  
 sterol metabolic process  
 endoplasmic reticulum  
 antigen processing and presentation of exogenous peptide antigen  
 antigen processing and presentation of peptide antigen via MHC class II  
 antigen processing and presentation of exogenous peptide antigen via MHC class II  
 MHC class II protein complex  
 immune response  
 lipid biosynthetic process  
 endoplasmic reticulum membrane  
 antigen processing and presentation of peptide or polysaccharide antigen via MHC class II  
 antigen processing and presentation  
 antigen processing and presentation of exogenous antigen  
 immune effector process  
 cellular lipid metabolic process  
 oxidoreductase activity  
 steroid metabolic process  
 regulation of lymphocyte activation  
 oxidation reduction  
 cytoplasmic part  
 regulation of leukocyte activation  
 membrane  
 multivesicular body  
 positive T cell selection  
 positive thymic T cell selection  
 thymic T cell selection  
 organelle membrane  
 regulation of cell activation  
 steroid biosynthetic process  
 negative T cell selection  
 negative thymic T cell selection  
 negative regulation of T cell activation  
 oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen

## by microbiota

### Description

immune system process  
 membrane  
 external side of plasma membrane  
 immune response  
 regulation of immune system process  
 positive regulation of immune system process  
 antigen processing and presentation  
 regulation of cell activation  
 regulation of lymphocyte activation  
 antigen processing and presentation of peptide antigen  
 oxidation reduction  
 regulation of leukocyte activation  
 leukocyte activation  
 regulation of T cell activation  
 positive regulation of cell activation  
 antigen processing and presentation of exogenous antigen  
 positive regulation of lymphocyte activation  
 leukocyte adhesion



positive regulation of T cell activation  
positive regulation of leukocyte activation  
regulation of immune response  
oxidoreductase activity  
cell activation  
catalytic activity  
MHC protein binding  
antigen processing and presentation of exogenous peptide antigen  
TAP complex  
positive regulation of immune response  
plasma membrane part  
positive regulation of lymphocyte differentiation  
response to stimulus  
MHC class I protein binding  
defense response  
lymphocyte activation  
plasma membrane  
electron carrier activity  
response to chemical stimulus  
antigen processing and presentation of peptide antigen via MHC class II  
antigen processing and presentation of exogenous peptide antigen via MHC class II  
cellular lipid metabolic process  
positive regulation of T cell differentiation  
lipid metabolic process  
antigen processing and presentation of peptide or polysaccharide antigen via MHC class II  
endoplasmic reticulum  
T cell activation  
T cell selection  
regulation of mast cell proliferation  
cytoplasmic part  
regulation of mononuclear cell proliferation  
regulation of lymphocyte proliferation  
regulation of leukocyte proliferation  
endoplasmic reticulum part  
positive regulation of response to stimulus  
regulation of lymphocyte differentiation  
inflammatory response  
thymic T cell selection  
MHC class II protein complex  
negative T cell selection  
negative thymic T cell selection  
positive regulation of biological process  
oxidoreductase activity, acting on CH-OH group of donors  
MHC protein complex  
regulation of T cell differentiation  
response to external stimulus  
immunological synapse  
regulation of response to stimulus  
monocarboxylic acid metabolic process  
positive regulation of alpha-beta T cell activation  
carboxylic acid metabolic process  
organic acid metabolic process  
immune effector process  
heme binding  
tetrapyrrole binding  
response to wounding

oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor  
 cellular aromatic compound metabolic process  
 cellular alcohol metabolic process  
 cytokine binding  
 B cell receptor signaling pathway  
 receptor complex  
 protein dimerization activity  
 regulation of granulocyte differentiation  
 cell fraction  
 regulation of T cell proliferation  
 immune response-activating signal transduction  
 integrin complex  
 antigen receptor-mediated signaling pathway  
 positive regulation of B cell activation  
 positive regulation of mononuclear cell proliferation  
 positive regulation of lymphocyte proliferation  
 positive regulation of leukocyte proliferation  
 positive regulation of mast cell proliferation  
 insoluble fraction  
 positive regulation of T cell proliferation  
 multivesicular body  
 positive T cell selection  
 positive thymic T cell selection  
 activated T cell proliferation  
 zinc ion homeostasis  
 membrane fraction  
 purine nucleotide binding  
 leukocyte mediated immunity  
 regulation of alpha-beta T cell activation  
 iron ion binding  
 acute inflammatory response  
 immune response-regulating signal transduction  
 regulation of adaptive immune response  
 regulation of adaptive immune response based on somatic recombination of immune receptors...  
 regulation of B cell activation  
 activation of immune response  
 adaptive immune response  
 adaptive immune response based on somatic recombination of immune receptors built from...  
 integral to plasma membrane  
 immune response-activating cell surface receptor signaling pathway  
 nucleoside:sodium symporter activity  
 positive regulation of adaptive immune response  
 positive regulation of adaptive immune response based on somatic recombination of immune...

## Myd88 genotype

### Description

immune response  
 immune system process  
 immune effector process  
 defense response  
 innate immune response  
 catalytic activity  
 ISG15-protein conjugation  
 regulation of immune response  
 positive regulation of immune response  
 glutathione transferase activity

response to wounding  
GTPase activity  
response to biotic stimulus  
antigen processing and presentation  
inflammatory response  
hydrolase activity  
membrane  
GTP binding  
activation of immune response  
guanyl ribonucleotide binding  
guanyl nucleotide binding  
response to stimulus  
positive regulation of response to stimulus  
regulation of immune system process  
hydrolase activity, acting on acid anhydrides  
response to chemical stimulus  
humoral immune response  
leukocyte mediated immunity  
positive regulation of immune system process

### **188 genotype modulates response to microbiota**

#### **Description**

cholesterol biosynthetic process  
sterol biosynthetic process  
cellular alcohol metabolic process  
steroid metabolic process  
cholesterol metabolic process  
steroid biosynthetic process  
sterol metabolic process  
cellular lipid metabolic process  
lipid metabolic process  
isoprenoid biosynthetic process  
metabolic process  
isoprenoid metabolic process  
lipid biosynthetic process  
oxidation reduction

### **regulated by microbiota**

#### **Description**

catalytic activity  
apical plasma membrane  
brush border  
vesicular fraction  
oxidoreductase activity  
microsome  
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen...  
monooxygenase activity  
cell fraction  
oxidation reduction  
membrane  
insoluble fraction  
endoplasmic reticulum part  
monocarboxylic acid metabolic process  
2'-5'-oligoadenylate synthetase activity  
heme binding  
tetrapyrrole binding

membrane fraction  
circadian rhythm  
endoplasmic reticulum  
retinoid metabolic process  
terpenoid metabolic process  
diterpenoid metabolic process

#### **regulated by Myd88 genotype**

##### **Description**

protein amino acid autophosphorylation  
catalytic activity  
cellular metabolic process  
binding  
metabolic process

#### **where Myd88 genotype modulates response to microbiota**

##### **Description**

nuclear division  
mitosis  
organelle fission  
cell cycle phase  
cell division  
catalytic activity  
cell cycle  
cell cycle process  
immune system process  
immune response  
kinetochore  
sterol biosynthetic process  
chromosome, centromeric region  
response to virus  
cholesterol biosynthetic process  
chromosomal part  
sterol metabolic process  
purine nucleotide binding  
chromosome  
response to stimulus  
nucleotide binding  
ribonucleotide binding  
purine ribonucleotide binding  
steroid biosynthetic process  
organelle organization  
adenyl nucleotide binding  
condensed chromosome kinetochore  
hydrolase activity  
response to stress  
2'-5'-oligoadenylate synthetase activity  
cellular response to stress  
cholesterol metabolic process  
ATP binding  
DNA packaging  
nucleotidyltransferase activity  
hydrolase activity, acting on acid anhydrides  
adenyl ribonucleotide binding  
ISG15-protein conjugation