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

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Figure S1

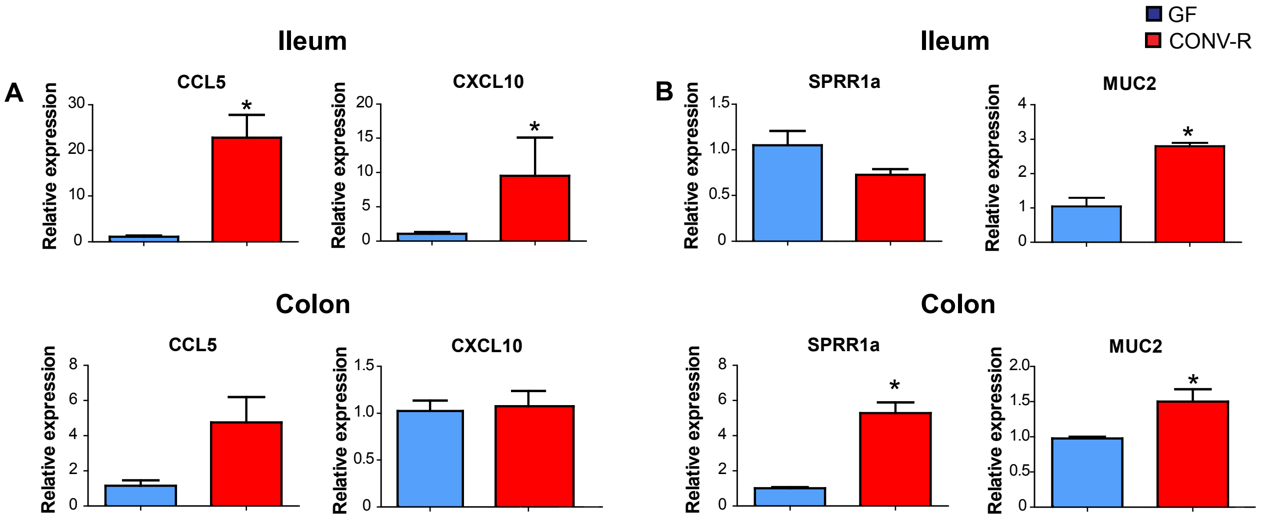

Figure S1. qRT-PCR analysis of (A) chemokines and (B) genes involved in mucosa fortification in epithelial cells isolated from the ileum and colon of GF and CONV-R mice. n = 4 mice/group. * $P < 0.05$; Mann Whitney U-test.

Figure S2

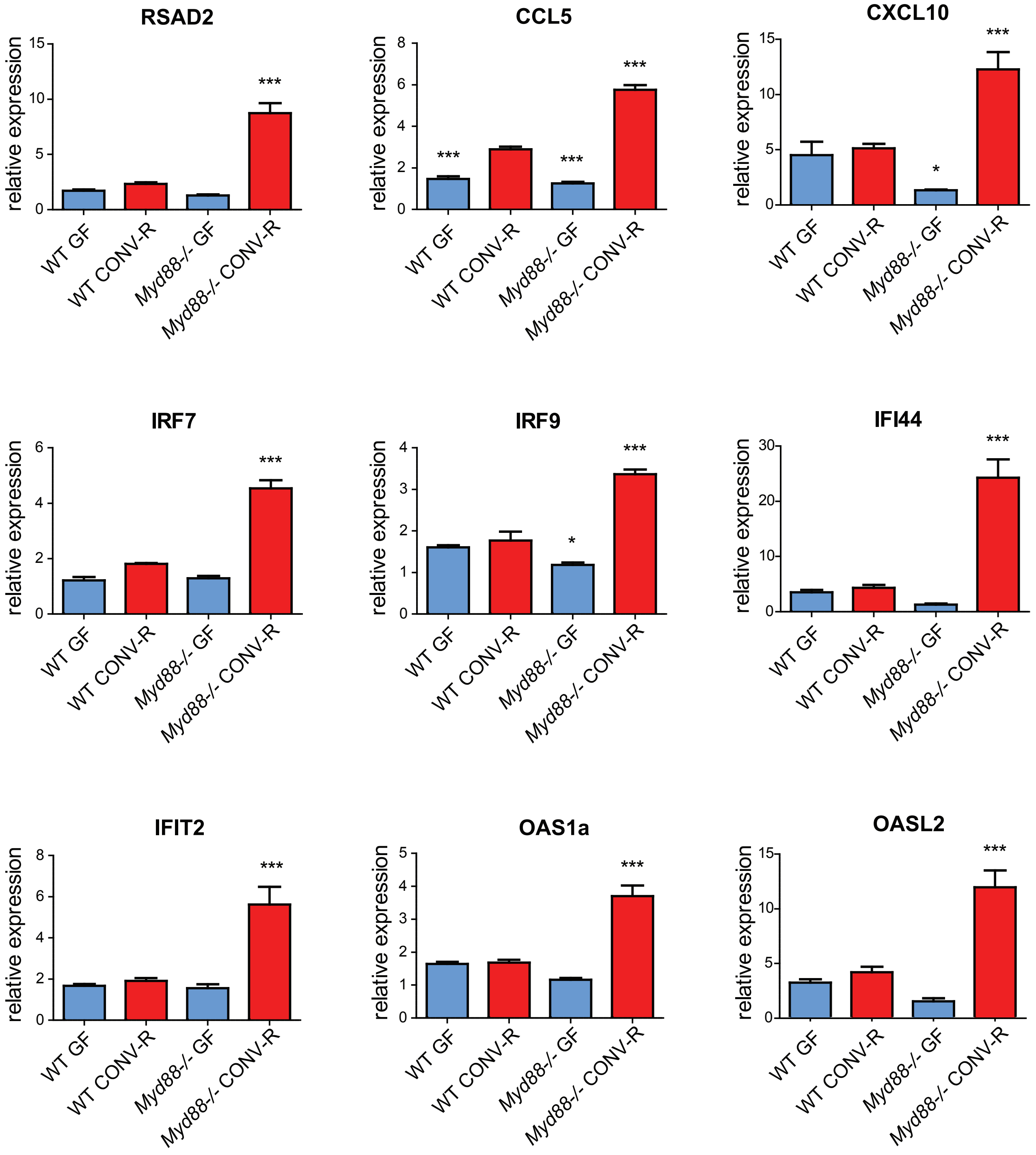

Figure S2. qRT-PCR validation of antiviral gene expression in the proximal colon of CONV-R wildtype and *Myd88-/-* mice. n = 4-6/group. * P < 0.05; *** P < 0.0001; ANOVA.

Figure S3

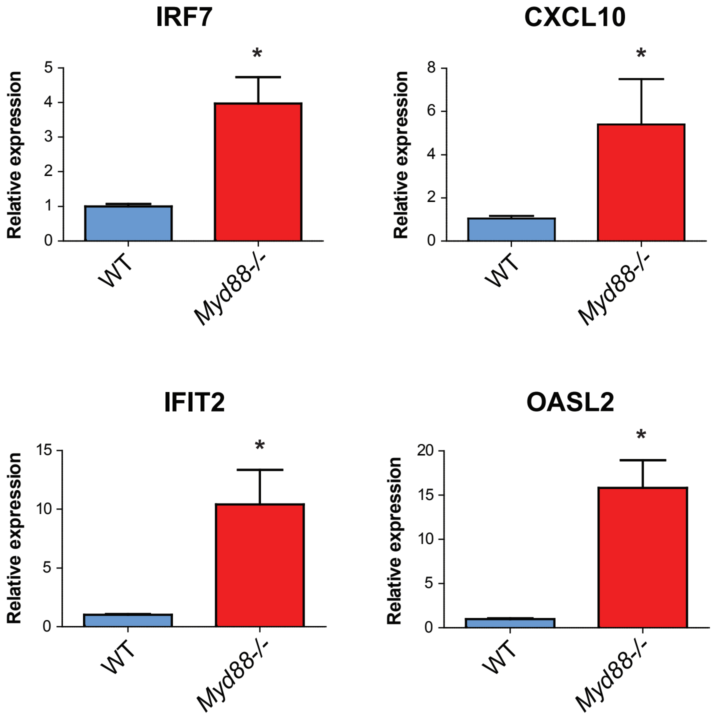

Figure S3. qPCR analysis of antiviral gene expression in epithelial cells isolated from the colon of CONV-R wildtype and Myd88-/- mice. n = 4/group. * P < 0.05; Mann-Whitney U-test.

Figure S4

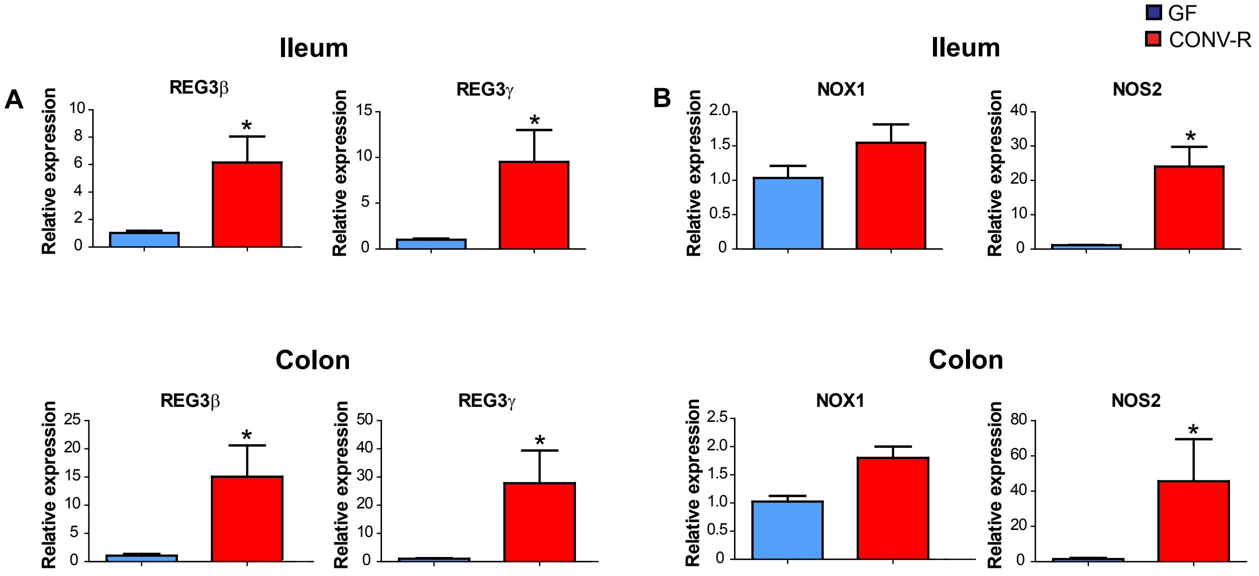

Figure S4. qRT-PCR analysis of antimicrobial genes in epithelial cells isolated from ileum and colon of GF and CONV-R mice. n = 4 mice/group. * $P < 0.05$; Mann Whitney U-test.

Figure S5

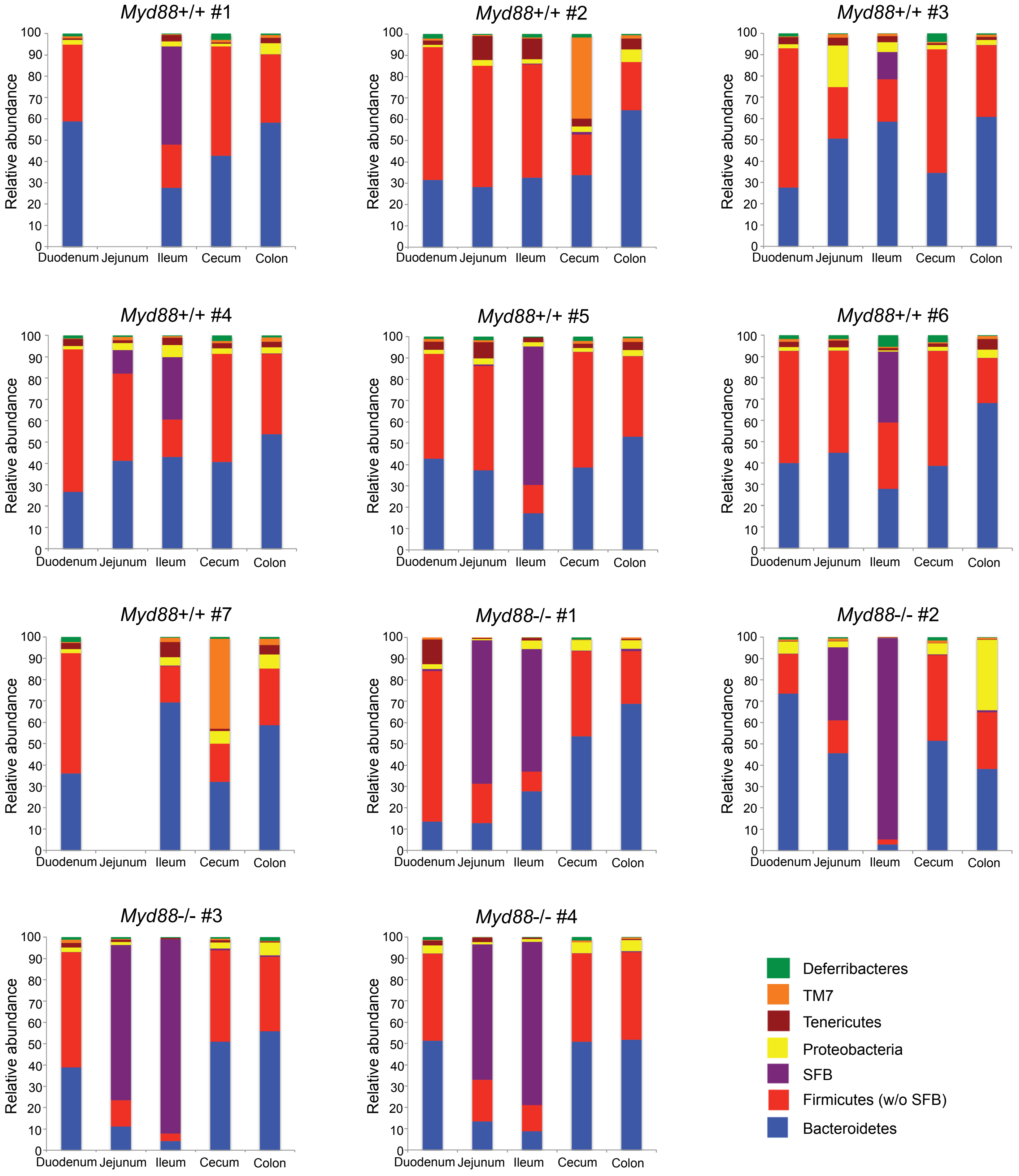

Figure S5. Relative abundance of the different phyla in individual mice (*Myd88* +/+ or -/-) along the length of the gut.

Figure S6

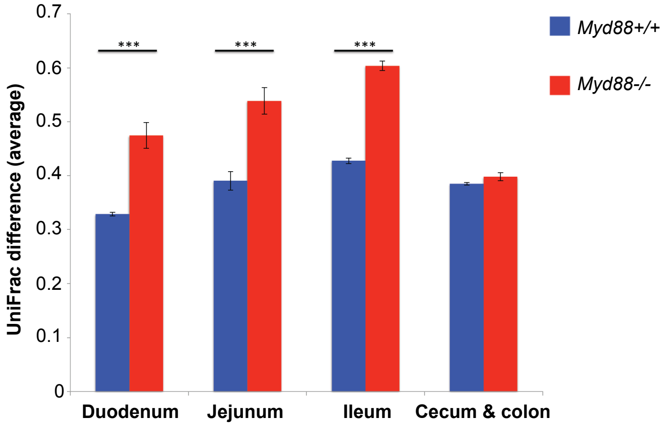

Figure S6. Mean unweighted UniFrac distances derived from the different sections of the gut. P-values were corrected for multiple comparisons using the Bonferroni correction; *** $p<0.0005$. n = 4-7 mice/group.

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[1*] (ID: 31543946a1)
	R	GCTGAGTGCTGTTCCCATCT	
Irf7	F	GAGACTGGCTATTGGGGGAG	Primer bank[1*] (ID: 8567364a1)
	R	GACCGAAATGCTTCCAGGG	
Cxcl10	F	CCAAGTGCTGCCGTCATTTTC	Primer bank[1*] (ID: 10946576a1)
	R	GGCTCGCAGGGATGATTTCAA	
Ccl5	F	GCTGCTTTGCCTACCTCTCC	Primer bank[1*] (ID: 7305461a1)
	R	TCGAGTGACAAACACGACTGC	
Ifi44	F	AACTGACTGCTCGCAATAATGT	Primer bank[1*] (ID: 19527086a1)
	R	GTAACACAGCAATGCCTCTTGT	
Irf9	F	GCCGAGTGGTGGGTAAGAC	Primer bank[1*] (ID: 6680474a1)
	R	GCAAAGGCGCTGAACAAAGAG	
Ifit2	F	AGTACAACGAGTAAGGAGTCACT	Primer bank[1*] (ID: 6680363a1)
	R	AGGCCAGTATGTTGCACATGG	
Oas1a	F	ATGGAGCACGGACTCAGGA	Primer bank[1*] (ID: 21630283a1)
	R	TCACACACGACATTGACGGC	
Oasl2	F	AGGGGACAACCCTGAACCA	Primer bank[1*] (ID: 16924024a1)
	R	TAGGCCAGGCTTCTGCTACA	
Sprr1a	F	TTGTGCCCCCAAAACCAAG	Primer bank[1*] (ID: 6678115a1)
	R	GGCTCTGGTGCCTTAGGTTG	
Muc2	F	CAAGGGCTCGGAACTCCAG	Primer bank[1*] (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	

[1] 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)

Ileum					
Gene	*Myd88*+/+		*Myd88*-/-		**Confirmed results**
Antibacterial	GF	CONV-R	GF	CONV-R	
Nox1	1.08±0.4	3.5±1.2**	1.3±0.3	7.6±1.1***	Yes
Nos2	1.0±0.1	18.7±3.3***	1.1±0.3	18.1±3.4***	Yes
Duox2	1.0±0.08	11.0±2.0***	1.2±0.05	16.8±5.2***	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)**
Chemokines, mucosa fortification, antibacterial	GF	CONV-R	
Ccl5	1.1±0.4	5.8±1.9*	Yes
Cxcl10	1.1±0.5	3.4±1.4*	Yes
Sprr1a	1.0±0.3	2.2±2.5	Yes
Muc2	1.0±0.2	1.5±0.1*	No [a]
Muc4	1.0±0.3	2.8±3.1	No [b]
Muc13	1.0±0.2	1.8±0.2*	Yes
Reg3γ	1.0±0.2	2.5±0.6*	Yes
Reg3β	1.0±0.3	3.2±0.6*	Yes
Nox1	1.4±1.6	39.7±72.3	No [b]
Nos2	1.0±0.4	9.2±4.9*	Yes
Duox2	1.0±0.1	2.2±0.9*	Yes

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

[a] Small difference in expression levels between GF and CONVR detected by microarray.

[b] High deviation in qRT-PCR data of CONVR mice.