

Supplemental Material**IKK β in intestinal epithelial cells regulates allergen-specific IgA and allergic inflammation
at distant mucosal sites**

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Supplemental Materials and Methods

Analysis of gut microbiota

The bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) (Roche Titanium 454 FLX pyrosequencing platform) was used for detection and identification of the primary populations of microbes in fecal pellet samples. Briefly, freshly emitted fecal pellets samples were collected, snap frozen and stored at -80°C . Samples were collected twice a day from each individual mouse and pooled to minimize potential daily variation of the microbiota. Bacterial DNA was extracted by conventional methods (Qiagen, Valencia, CA), and 16S rRNA genes were amplified with the modified 16S Eubacterial primers 28F, 5'-GAG TTT GAT CNT GGC TCA G-3' and 519R, 5'-GTN TTA CNG CGG CKG CTG-3' for amplifying the 500 bp region of 16S rRNA genes. The primer sets used for FLX-Titanium amplicon pyrosequencing were designed with adding linker A and 8 base pair barcode sequence at the 5' end of forward primers as follow: 28F-A, 5'-CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG-barcode-GAG TTT GAT CNT GGC TCA G-3'. The biotin and linker B sequence at the 5' end of reverse primer 519R-B: 5'-Biotin-CCT ATC CCC TGT GTG CCT TGG CAG TCT CAG GTN TTA CNG CGG CKG CTG-3'. HotStarTaq Plus Master Mix Kit (QIAGEN, CA, USA) was used for PCR under the following conditions: 95°C for 5 minutes followed by 35 cycles of 95°C for 30 second; 54°C for 40 second and 72°C for 1 minute, a final elongation step at 72°C for 10 minutes was also included. The PCR products were cleaned by using Diffinity Rapid Tip (Diffinity Genomics, Inc, West Henrietta, NY), and the small fragments were removed by using Agencourt Ampure Beads (Beckman Coulter, CA, USA).

Bacterial tag-encoded FLX-Titanium amplicon pyrosequencing (bTEFAP) was performed as described previously ¹. In preparation for FLX-Titanium sequencing (Roche, Nutley, New

Jersey), DNA fragment sizes and concentration were accurately measured using DNA chips under a Bio-Rad Experion Automated Electrophoresis Station (Bio-Rad Laboratories, CA, USA) and a TBS-380 Fluorometer (Turner Biosystems, CA, USA). A sample of double-stranded DNA, 9.6 million molecules/ml, with an average size of 625 bp were combined with 9.6 million DNA capture beads, and then amplified by emulsion PCR. After bead recovery and bead enrichment, the bead attached DNAs were denatured with NaOH, and sequencing primers (Roche) were annealed. A four-region 454 sequencing run was performed on a GS PicoTiterPlate (PTP) using the Genome Sequencer FLX System (Roche). Forty tags were used on each quarter region of the PTP. All FLX procedures were performed using Genome Sequencer FLX System manufacturer's instructions (Roche). After denoising (USEARCH application) and chimera removal (UCHIIME in *de novo* mode), the sequences were clustered into *operational taxonomic units (OTU)* clusters with 96.5% identity (3.5% divergence) using USEARCH and the seed sequence put into a FASTA formatted sequence file. The FASTA files were then queried against a database of high quality sequences derived from NCBI using a distributed .NET algorithm that utilizes BLASTN+ (KrakenBLAST www.krakenblast.com). For identification of segmented filamentous bacteria, blast search was performed with the fasta sequences against *Candidatus Arthromitus* (taxid:49082) genome sequences (*Candidatus Arthromitus* sp. SFB-mouse-Yit and *Candidatus Arthromitus* sp. SFB-mouse-Japan), using Megablast (optimized for highly similar sequences; $\geq 95\%$) and E-values below $1e-15$.

Reference

1. Dowd SE, Wolcott RD, Sun Y, McKeethan T, Smith E, Rhoads D. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). PLoS One 2008;3:e3326.

Supplemental Figure Legends

Figure S1. STAT3 responses in gut tissues of IKK $\beta^{\Delta\text{IEC}}$ after oral administration of cholera toxin. Control IKK β -competent C57BL/6 mice and IKK $\beta^{\Delta\text{IEC}}$ mice were orally administered cholera toxin (10 μg) by intragastric gavage. After 16 h, mice were euthanized and small intestines were collected. Tissue sections were labeled with anti-pSTAT3 Ab and counter-stained with hematoxylin and eosin (Original magnification: x40). The figure is representative of at least three independent experiments.

Figure S2. Cholera toxin does not induce fluid accumulation in the gut of IKK $\beta^{\Delta\text{IEC}}$ mice. Control IKK β -competent C57BL/6 mice and IKK $\beta^{\Delta\text{IEC}}$ mice were orally administered cholera toxin (10 μg) by intragastric gavage. Mice were euthanized 10 or 16 hours later and intestinal fluid accumulation was evaluated by measuring the weight of intestine. The results are expressed as mean percentage of intestine weight over whole mouse body weight. (*, $p < 0.05$ compared to control C57BL/6 mice).

Figure S3. Oral cholera toxin treatment alters the distribution of bacteria families in the gut of IKK $\beta^{\Delta\text{IEC}}$ mice. Control IKK β -competent C57BL/6 mice and IKK $\beta^{\Delta\text{IEC}}$ mice were orally administered cholera toxin (10 μg) by intragastric gavage and fecal pellets were collected before (day 0) and 2 and 4 days later. The composition of the bacterial community was analyzed by pyrosequencing and heatmap of percentage of main bacteria family (at least 1 %) was generated.

The results are expressed as percentage of bacteria in individual samples with 3-4 mice per groups.

Figure S4. Oral cholera toxin treatment alters the distribution of bacteria genus in the gut of IKK β ^{ΔIEC} mice. Control IKK β -competent C57BL/6 mice and IKK β ^{ΔIEC} mice were orally administered cholera toxin (10 μ g) by intragastric gavage and fecal pellets were collected before (day 0) and 2 and 4 days later. The composition of the bacterial community was analyzed by pyrosequencing and heatmap of percentage of main bacteria genus (at least 1 %) was generated. The results are expressed as percentage of bacteria in individual samples with 3-4 mice per groups.

Figure S5. Oral cholera toxin treatment alters the distribution of bacteria species in the gut of IKK β ^{ΔIEC} mice. Control IKK β -competent C57BL/6 mice and IKK β ^{ΔIEC} mice were orally administered cholera toxin (10 μ g) by intragastric gavage and fecal pellets were collected before (day 0) and 2 and 4 days later. The composition of the bacterial community was analyzed by pyrosequencing and heatmap of percentage of the main bacteria species (at least 1 %) was generated. The results are expressed as percentage of bacteria in individual samples with 3-4 mice per groups.

Figure S6. IKK β -deficiency in myeloid cells also alters innate and adaptive immune responses to ingested allergen. (A) STAT3 responses in gut tissues after oral administration of cholera toxin. Control IKK β -competent C57BL/6 mice, IKK β ^{ΔMye} and IKK β ^{ΔIEC} mice were

orally administered cholera toxin (10 μg) by intragastric gavage. After 16 h, mice were euthanized and small intestines were collected, labeled with anti-pSTAT3 Ab and counter-stained with hematoxylin and eosin (Original magnification: x40). The figure is representative of at least three independent experiments. (B-C) Serum antibody responses. Control C57BL/6 (open bars), $\text{IKK}\beta^{\Delta\text{Mye}}$ (grey bars) and $\text{IKK}\beta^{\Delta\text{IEC}}$ (solid bars) mice were orally sensitized on days 0 and 7 with of OVA (1 mg) and cholera toxin (10 μg). Blood was collected on day 14 and OVA-specific Ab responses were analyzed by ELISA. The results are expressed as the mean \log_2 titers \pm one SD and are from three separate experiments and four mice / group. (*, $p < 0.05$).

Figure S7. Frequency of IgA secreting cells (ASC) in the lung and mesenteric lymph nodes of $\text{IKK}\beta^{\Delta\text{IEC}}$ mice. Mice were sensitized on days 0 and 7 by oral administration of ovalbumin (OVA, 1 mg) and cholera toxin (10 μg). Nasal challenges were performed on days 15, 16, and 19 and lungs and mesenteric lymph nodes (MLN) were collected on day 20 and subjected to an IgA-specific ELISPOT assay. The results are expressed as mean number of ASC/ 10^6 cells \pm one SD and are from three experiments and four mice/group. (*, $p < 0.05$ compared to control C57BL/6 mice).

Figure S8. $\text{IKK}\beta$ -deficiency in intestinal epithelial cells alters serum and lung responses in mice sensitized by parenteral injection. Control C57BL/6 (open bars), and $\text{IKK}\beta^{\Delta\text{IEC}}$ (solid bars) mice were sensitized on days 0 and 7 by intraperitoneal injection of OVA (100 μg) and cholera toxin (1 μg). (A) Serum Ab responses. Blood was collected on day 14 and OVA-

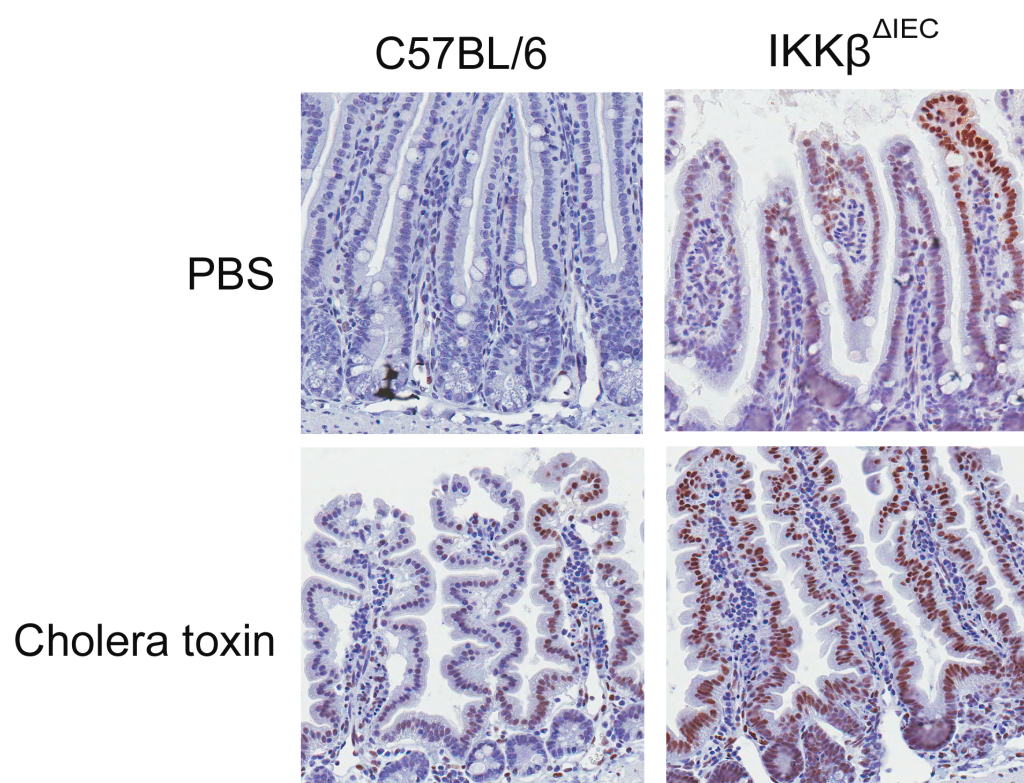
specific Ab responses were analyzed by ELISA. The results are expressed as the mean \log_2 titers \pm one SD and are from three separate experiments and four mice / group. (*, $p < 0.05$). (B) Mucus secretion in the lungs after antigen challenge. Nasal challenges were performed on days 15, 16, and 19 and animals were euthanized on day 20. Lung sections were stained with PAS and counter stained with hematoxylin and eosin. Pictures are representative of three separate experiments and four mice / group. Original magnifications are indicated.

Figure S9. IKK β -deficiency in intestinal epithelial cells alters immune cell recruitment and cytokine responses in the lungs of orally sensitized mice. Mice were sensitized on days 0 and 7 by oral administration of ovalbumin (OVA, 1 mg) and cholera toxin (10 μ g). Nasal challenges were performed on days 15, 16, and 19. Flow cytometry analysis of the frequency of (A) F4/80⁺CD11c⁺ alveolar macrophages, F4/80⁺CD11c⁻ interstitial macrophages and F4/80⁻CD11⁺ dendritic cells and (B) CD103⁺ dendritic cells in the lungs after nasal antigen challenge of orally sensitized mice (C) Flow cytometry analysis of T cell subsets. Results are expressed as mean \pm SD of three separate experiments, with 4 mice per group. (*, $p < 0.05$ compared to control C57BL/6 mice).

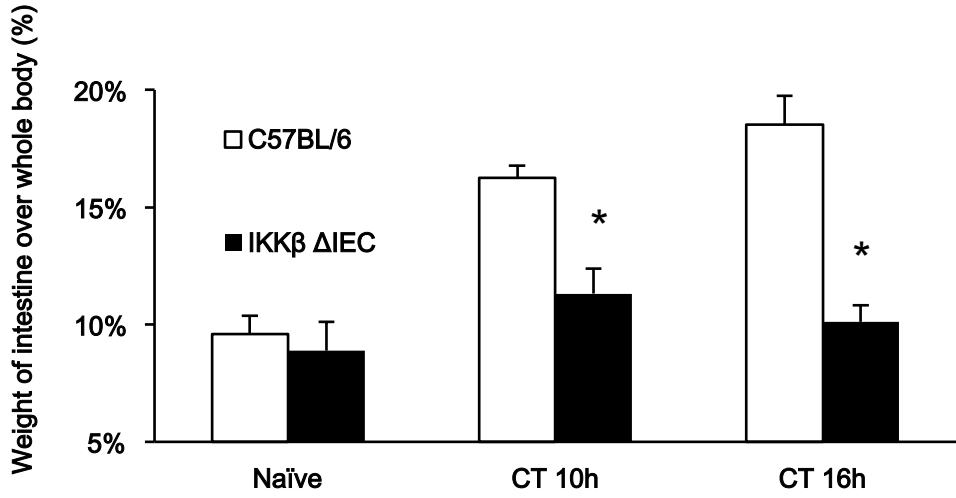
Figure S10. IKK β -deficiency in intestinal epithelial cells alters airway cytokine responses to nasal antigen challenge of orally sensitized mice. Mice were sensitized on days 0 and 7 by oral administration of ovalbumin (OVA, 1 mg) and cholera toxin (10 μ g). Nasal challenges were performed on days 15, 16, and 19. Bronchoalveolar lavage fluids (BAL) were collected on day 20 cytokine/chemokine responses were analyzed by conventional ELISA. Results are expressed

as mean \pm SD of three separate experiments, with 4 mice per group. (*, $p < 0.05$ compared to control C57BL/6 mice).

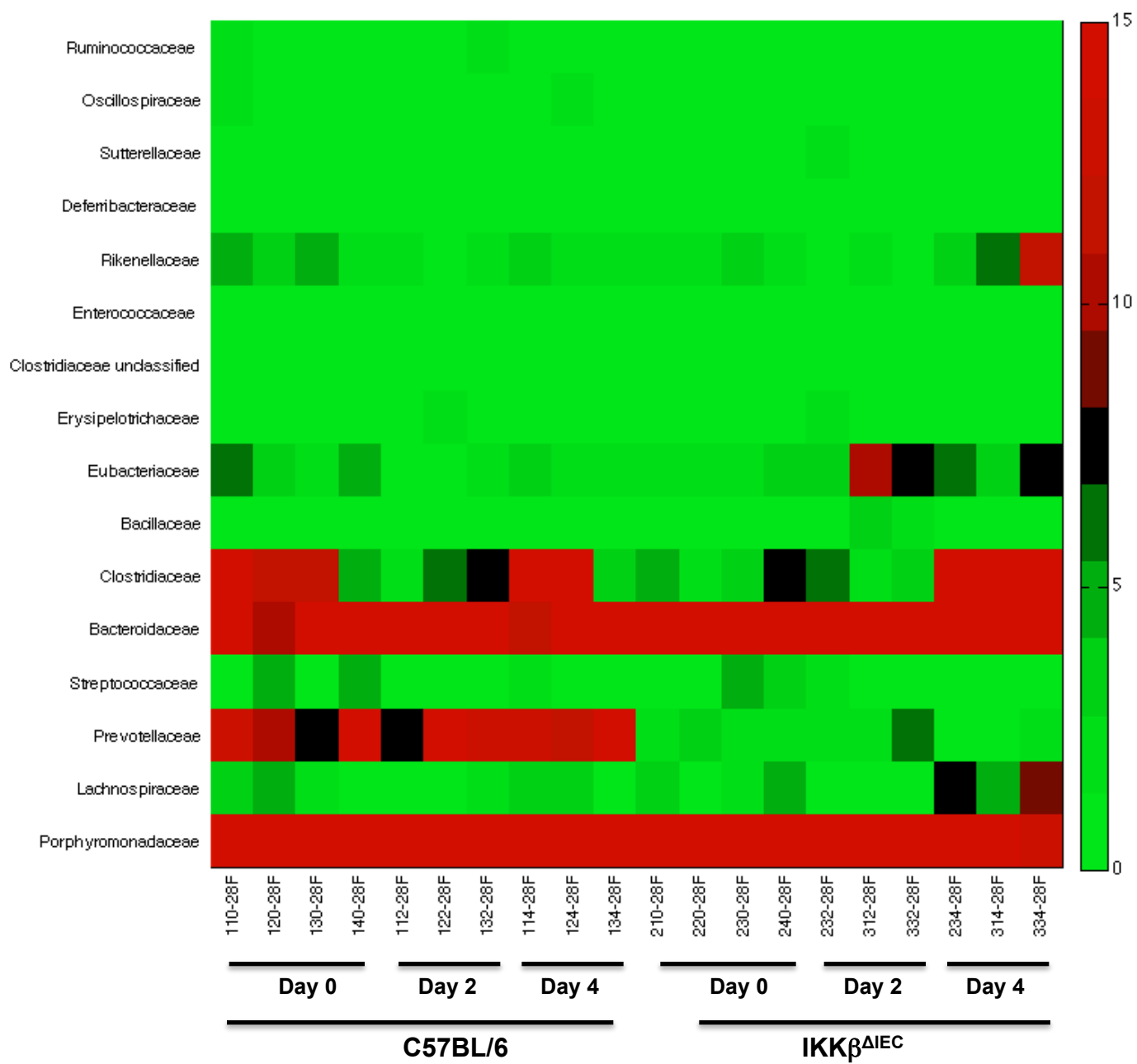
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Figure S1.



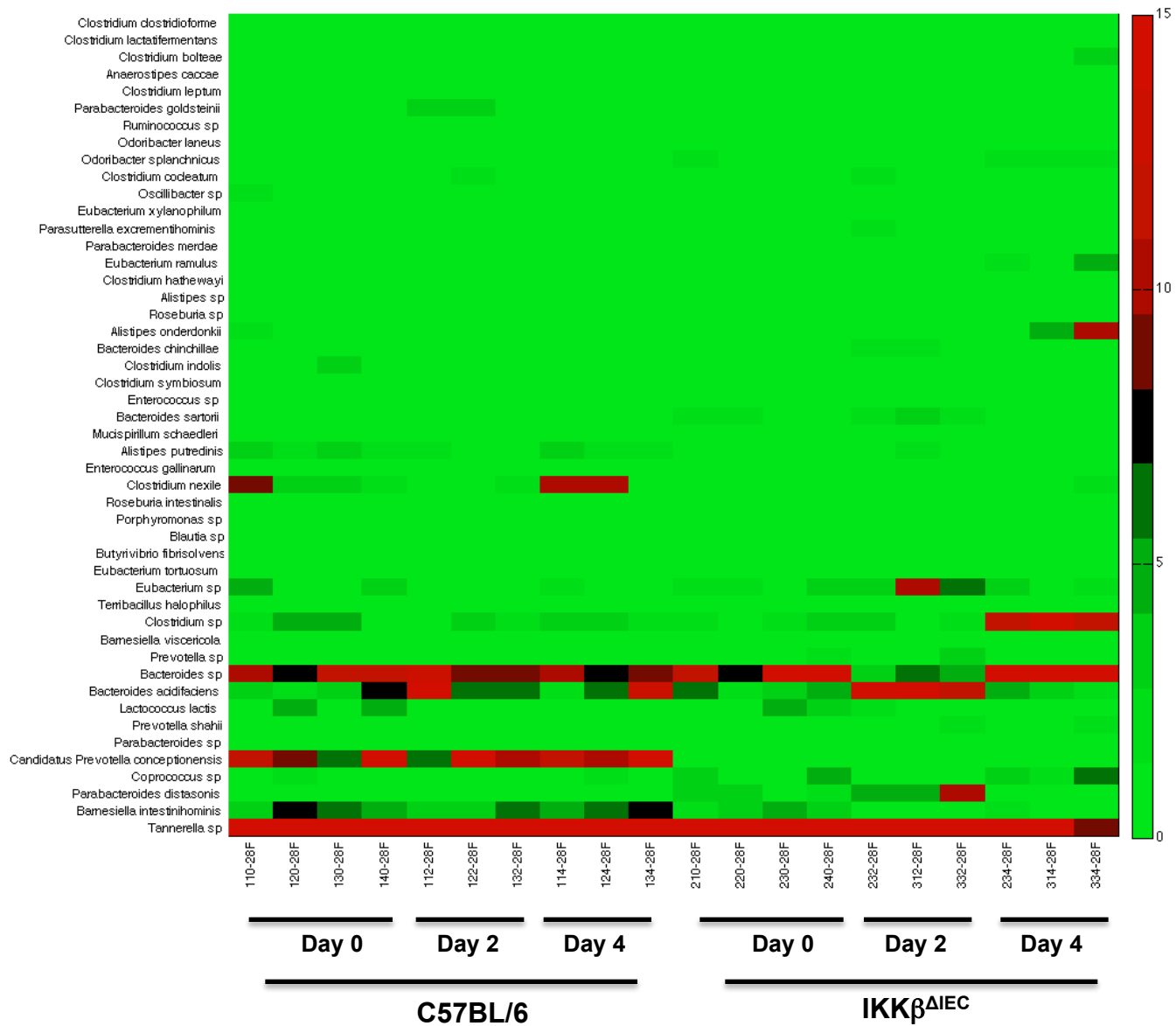
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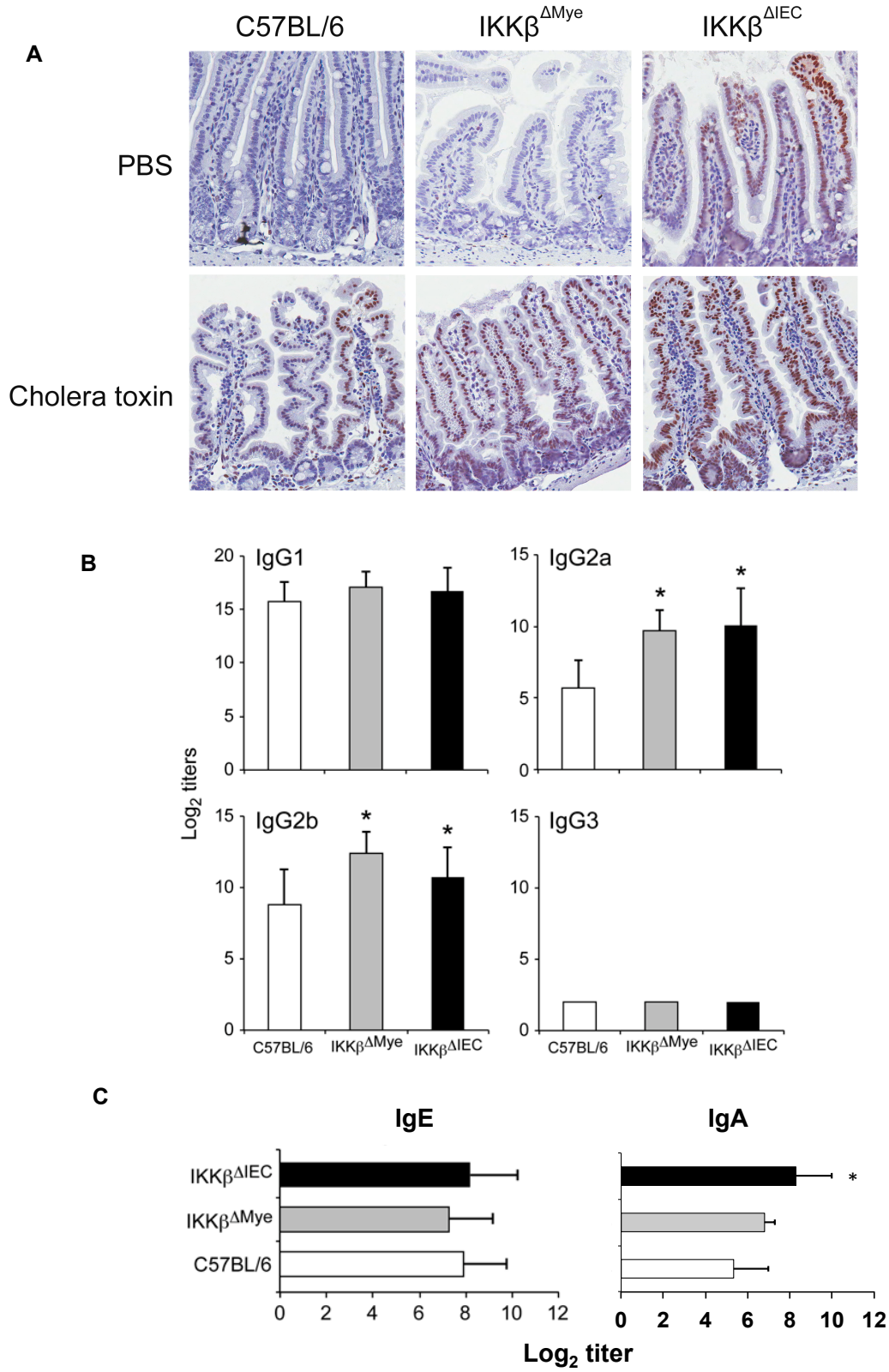


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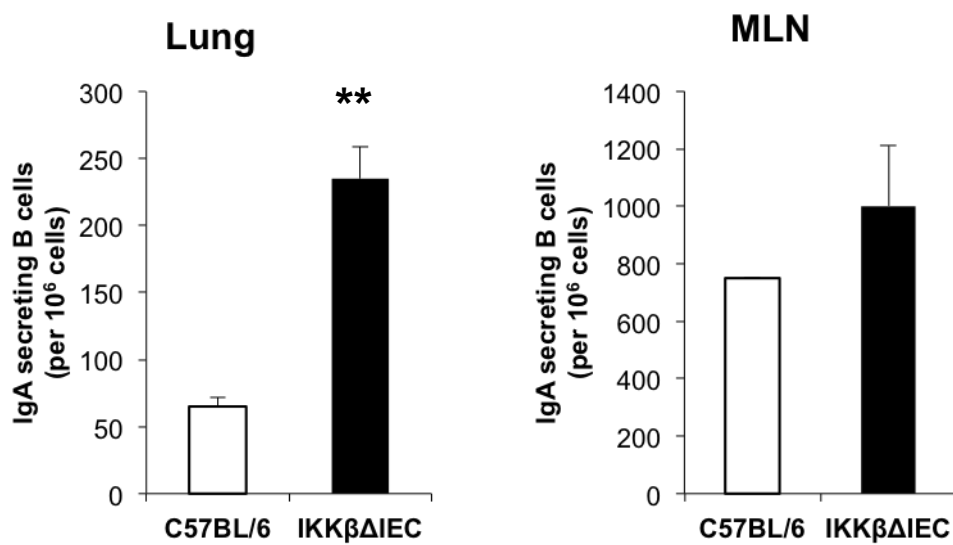


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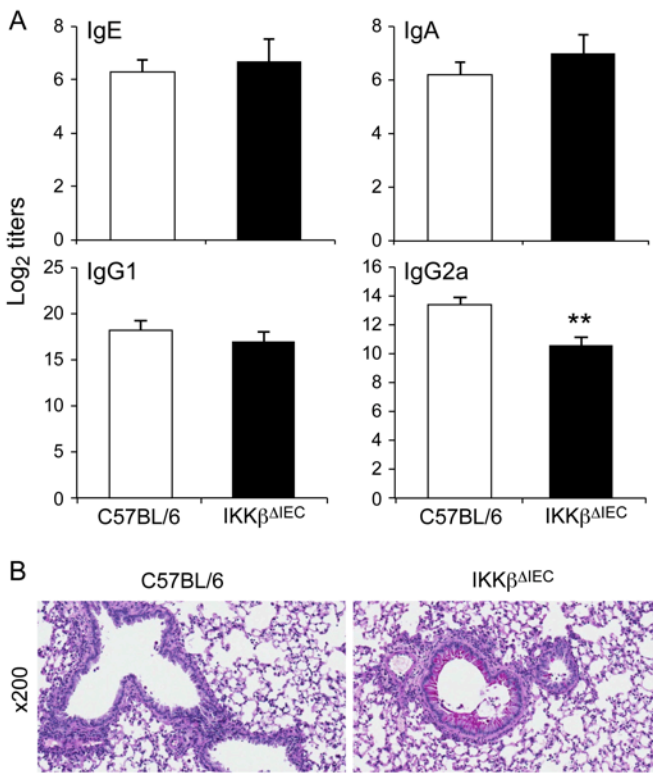




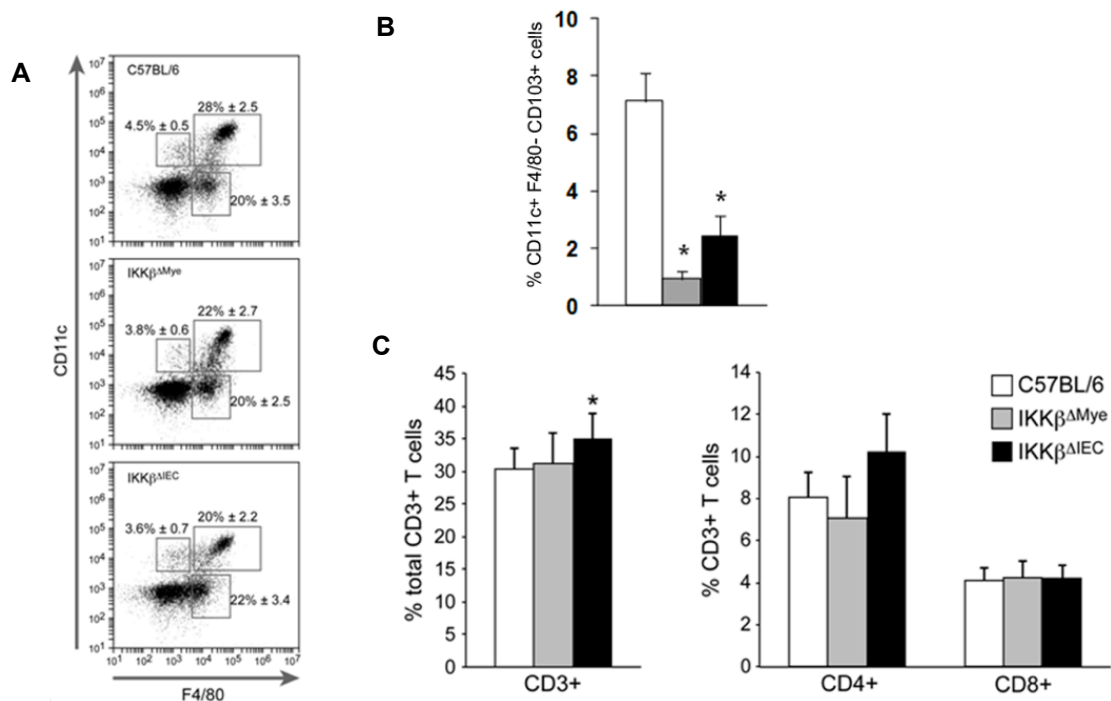
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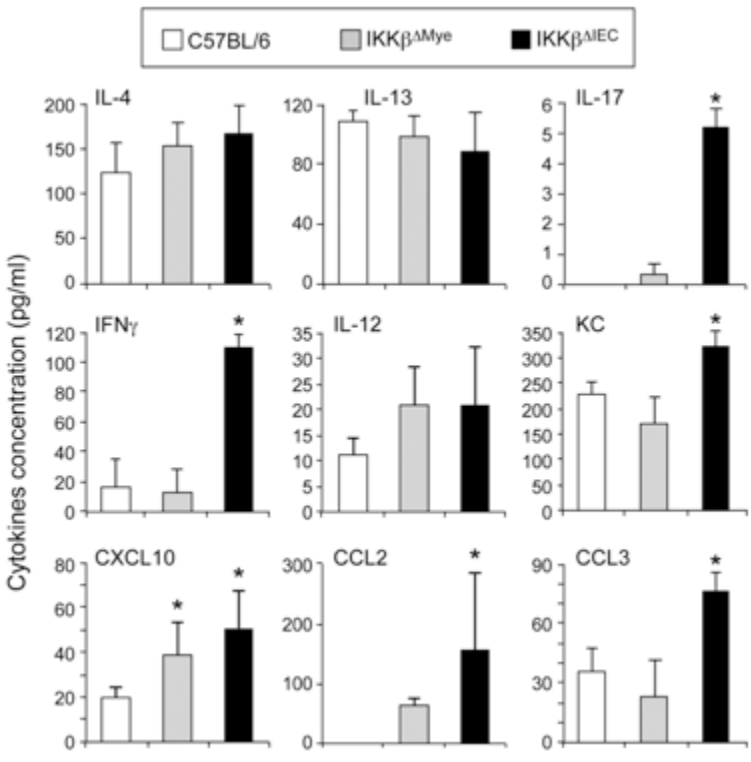


Table S1. Relative percentage of each Phylum

Phylum	C57BL/6						IKK β Δ IEC					
	Day 0		Day 2		Day 4		Day 0		Day 2		Day 4	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
Bacteroidetes	76.78	7.17	82.32	3.55	75.80	7.62	82.68	6.12	79.24	3.82	53.05	1.42
Firmicutes	22.13	6.75	9.87	6.18	20.63	11.28	13.74	5.75	15.75	2.19	40.99	1.29
Deferribacteres	0.30	0.25	0.05	0.02	0.30	0.27	0.03	0.02	0.06	0.10	1.02	0.29
Proteobacteria	0.30	0.11	6.40	7.70	2.51	3.93	2.38	1.14	1.78	0.88	4.24	0.65
Tenericutes	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.44	0.13	0.18	0.20	0.10
Actinobacteria	0.10	0.04	0.08	0.08	0.04	0.05	0.10	0.07	0.42	0.37	0.04	0.04
Verrucomicrobia	0.08	0.10	1.09	1.54	0.15	0.13	0.00	0.00	0.00	0.00	0.00	0.00
Cyanobacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.26	2.07	2.74	0.22	0.22
TM7	0.32	0.17	0.18	0.30	0.57	0.13	0.06	0.01	0.00	0.00	0.24	0.15
Spirochaetes	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.49	0.54	0.00	0.00
Acidobacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.07	0.05	0.09	0.01	0.01
Chlorobi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
Chloroflexi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00

Blue: IKK β ^{AIEC} mice different than control C57B/L 6 group at day 0.

Red: Decreased percentage in IKK β ^{AIEC} mice compared to same group at day 0.

Green: Increased percentage in IKK β ^{AIEC} mice compared to same group at day 0.

Table S2. Relative percentage of bacteria classes

Class	C57BL/6						IKK β Δ IEC					
	Day 0		Day 2		Day 4		Day 0		Day 2		Day 4	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
Bacteroidia	76.39	6.15	81.76	3.61	75.38	7.88	82.32	4.67	78.59	3.41	52.91	1.48
Clostridia	19.06	5.77	8.28	5.34	19.17	10.36	10.86	2.47	12.30	0.50	39.40	1.76
Bacilli	3.04	2.19	0.75	0.54	1.10	1.12	2.68	3.18	2.69	0.93	1.49	0.40
Erysipelotrichi	0.03	0.06	0.85	1.03	0.37	0.21	0.19	0.14	0.76	1.10	0.10	0.12
Deferribacteres (class)	0.30	0.21	0.05	0.02	0.30	0.27	0.03	0.03	0.06	0.10	1.02	0.29
Betaproteobacteria	0.04	0.02	0.19	0.16	0.06	0.06	0.59	0.42	0.63	0.87	0.32	0.13
Alphaproteobacteria	0.23	0.15	6.20	7.72	2.33	3.93	0.17	0.02	0.69	0.88	0.26	0.31
Mollicutes	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.05	0.13	0.18	0.20	0.10
Deltaproteobacteria	0.02	0.02	0.01	0.01	0.12	0.06	0.15	0.34	0.01	0.01	0.13	0.15
Actinobacteria (class)	0.10	0.04	0.08	0.08	0.04	0.05	0.10	0.09	0.42	0.37	0.04	0.04
Verrucomicrobiae	0.08	0.13	1.09	1.54	0.15	0.13	0.00	0.00	0.00	0.00	0.00	0.00
Cyanobacteria (class)	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.18	0.26	0.18	0.20
Chroococcales	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.22	1.88	2.48	0.04	0.04
Epsilonproteobacteria	0.00	0.00	0.00	0.00	0.00	0.00	1.30	1.36	0.32	0.30	3.47	0.76
TM7 (class)	0.32	0.16	0.18	0.30	0.57	0.13	0.06	0.01	0.00	0.00	0.24	0.15
Spirochaetia	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.04	0.49	0.54	0.00	0.00
Flavobacteria	0.34	0.20	0.55	0.25	0.42	0.31	0.35	0.15	0.65	0.41	0.12	0.11
Gammaproteobacteria	0.00	0.00	0.00	0.00	0.01	0.01	0.16	0.11	0.13	0.13	0.06	0.05
Sphingobacteria	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cytophagia	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02
Acidobacteria (class)	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.07	0.05	0.09	0.01	0.01
Chlorobia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
Anaerolineae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00

Blue: IKK β ^{Δ IEC} mice different than control C57B/L 6 group at day 0.

Red: Decreased percentage in IKK β ^{Δ IEC} mice compared to same group at day 0.

Green: Increased percentage in IKK β ^{Δ IEC} mice compared to same group at day 0.

Order	C57BL/6						IKK β Δ IEC					
	Day 0		Day 2		Day 4		Day 0		Day 2		Day 4	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
Bacteroidales	76.16	7.09	81.59	3.69	75.27	7.84	82.31	6.10	78.54	3.44	52.90	1.47
Clostridiales	19.06	7.46	8.28	5.34	19.17	10.36	10.86	4.44	12.30	0.50	39.40	1.76
Lactobacillales	3.00	2.05	0.65	0.53	1.07	1.14	2.56	2.75	1.01	1.63	1.08	0.44
Bacillales	0.04	0.04	0.10	0.09	0.03	0.04	0.13	0.09	1.68	1.33	0.41	0.41
Erysipelotrichales	0.03	0.05	0.85	1.03	0.37	0.21	0.19	0.14	0.76	1.10	0.10	0.12
Deferribacterales	0.30	0.25	0.05	0.02	0.30	0.27	0.03	0.02	0.06	0.10	1.02	0.29
Burkholderiales	0.04	0.02	0.19	0.16	0.06	0.06	0.59	0.56	0.63	0.87	0.32	0.13
Rhodospirillales	0.23	0.12	6.15	7.70	2.31	3.90	0.01	0.03	0.01	0.02	0.00	0.00
Mycoplasmatales	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.12	0.10	0.18	0.12	0.11
Desulfovibrionales	0.01	0.02	0.00	0.00	0.05	0.08	0.15	0.30	0.00	0.00	0.11	0.16
Coriobacteriales	0.07	0.04	0.05	0.03	0.02	0.02	0.04	0.02	0.02	0.02	0.04	0.04
Anaeroplasmatales	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.32	0.02	0.01	0.06	0.07
Verrucomicrobiales	0.08	0.10	1.09	1.54	0.15	0.13	0.00	0.00	0.00	0.00	0.00	0.00
Rickettsiales	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.04	0.06	0.24	0.29
Actinomycetales	0.03	0.01	0.03	0.05	0.02	0.03	0.06	0.05	0.40	0.39	0.00	0.00
Chroococcales	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.04	0.18	0.26	0.00	0.00
Cyanobium (order)	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.26	1.88	2.48	0.04	0.04
Caulobacterales	0.00	0.00	0.01	0.01	0.00	0.00	0.12	0.04	0.41	0.55	0.02	0.02
Kiloniellales	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.02	0.13	0.20	0.00	0.00
Rhizobiales	0.00	0.00	0.04	0.03	0.02	0.03	0.00	0.00	0.01	0.01	0.00	0.00
Campylobacteriales	0.00	0.00	0.00	0.00	0.00	0.00	1.30	1.22	0.32	0.30	3.47	0.76
TM7 (order)	0.32	0.17	0.18	0.30	0.57	0.13	0.06	0.01	0.00	0.00	0.24	0.15
Bacteroidia (order)	0.23	0.12	0.17	0.09	0.11	0.05	0.02	0.04	0.04	0.05	0.01	0.02
Spirochaetales	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.49	0.54	0.00	0.00
Flavobacteriales	0.34	0.17	0.55	0.25	0.42	0.31	0.35	0.14	0.65	0.41	0.12	0.11
Pasteurellales	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Bdellovibrionales	0.01	0.02	0.00	0.00	0.05	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonadales	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.01	0.01
Acholeplasmatales	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
Enterobacteriales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.04
Sphingobacteriales	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cytophagales	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02
Desulfuromonadales	0.00	0.00	0.01	0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.01	0.02
Mollicutes (order)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
Nostocales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.18	0.20
Kopriimonadales	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.09	0.08	0.00	0.00
Methylococcales	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.13	0.08	0.09	0.00	0.00
Acidobacteriales	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.07	0.05	0.09	0.01	0.01
Thiotrichales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Chlorobiales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
Anaerolineales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Entomoplasmatales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.02

Blue: IKK β Δ IEC mice different than control C57B/L 6 group at day 0.

Red: Decreased percentage in IKK β Δ IEC mice compared to same group at day 0.

Green: Increased percentage in IKK β Δ IEC mice compared to same group at day 0.

Page 5 of 84. **Table S4. Relative percentage of bacteria families**

Family	C57BL/6						IKK β Δ IEC					
	Day 0		Day 2		Day 4		Day 0		Day 2		Day 4	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
Porphyromonadaceae	45.65	7.34	44.05	6.79	39.38	4.92	59.03	8.60	49.04	5.05	17.67	4.64
Lachnospiraceae	2.41	1.56	0.88	0.83	2.50	1.45	2.82	1.37	0.49	0.11	6.84	2.02
Prevotellaceae	11.51	3.83	15.41	9.68	16.49	7.65	2.42	0.36	3.18	2.20	0.92	0.52
Streptococcaceae	2.99	2.07	0.46	0.43	0.71	0.63	2.30	2.51	0.77	1.30	0.41	0.28
Bacteroidaceae	14.96	3.88	20.39	7.87	16.65	5.79	18.65	2.82	25.08	3.81	27.60	3.95
Clostridiaceae	10.56	4.56	5.03	2.81	12.16	7.19	4.81	2.46	3.90	2.45	25.34	6.23
Bacillaceae	0.01	0.02	0.07	0.07	0.00	0.00	0.09	0.07	1.64	1.35	0.18	0.06
Eubacteriaceae	3.81	1.78	0.75	0.64	2.59	1.36	2.54	0.50	6.61	3.21	5.35	2.48
Erysipelotrichaceae	0.03	0.05	0.85	1.03	0.37	0.21	0.19	0.14	0.76	1.10	0.10	0.12
Clostridiaceae unclassified	0.25	0.14	0.07	0.10	0.05	0.03	0.14	0.11	0.31	0.23	0.20	0.13
Enterococcaceae	0.00	0.00	0.06	0.05	0.01	0.02	0.01	0.02	0.03	0.04	0.00	0.00
Rikenellaceae	4.03	1.42	1.72	0.55	2.75	0.81	2.15	0.44	1.24	0.51	6.68	4.33
Deferribacteraceae	0.30	0.25	0.05	0.02	0.30	0.27	0.03	0.02	0.06	0.10	1.02	0.29
Sutterellaceae	0.04	0.02	0.19	0.16	0.05	0.05	0.59	0.56	0.63	0.87	0.32	0.13
Oscillospiraceae	1.10	0.72	0.59	0.50	0.98	0.46	0.16	0.15	0.36	0.23	0.48	0.07
Ruminococcaceae	0.78	0.50	0.87	0.71	0.82	0.41	0.32	0.13	0.37	0.21	1.07	0.24
Rhodospirillaceae	0.23	0.12	6.13	7.68	2.31	3.89	0.01	0.03	0.01	0.02	0.00	0.00
Mycoplasmataceae	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.12	0.10	0.18	0.12	0.11
Desulfovibrionaceae	0.01	0.02	0.00	0.00	0.05	0.08	0.15	0.30	0.00	0.00	0.11	0.16
Paenibacillaceae	0.01	0.01	0.03	0.05	0.03	0.04	0.01	0.02	0.03	0.03	0.02	0.00
Planococcaceae	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.32
Marinilabiaceae	0.02	0.02	0.01	0.01	0.00	0.00	0.05	0.03	0.01	0.02	0.03	0.03
Lactobacillaceae	0.02	0.03	0.14	0.14	0.34	0.53	0.25	0.25	0.22	0.35	0.68	0.16
Coriobacteriaceae	0.07	0.04	0.05	0.03	0.02	0.02	0.04	0.02	0.02	0.02	0.04	0.04
Anaeroplasmataceae	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.32	0.02	0.01	0.06	0.07
Verrucomicrobiaceae	0.08	0.10	1.09	1.54	0.15	0.13	0.00	0.00	0.00	0.00	0.00	0.00
Rickettsiaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.04	0.06	0.24	0.29
Nocardiaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.20	0.19	0.00	0.00
Chroococcaceae unclassified	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.04	0.18	0.26	0.00	0.00
Cyanobium (family)	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.26	1.88	2.48	0.04	0.04
Caulobacteraceae	0.00	0.00	0.01	0.01	0.00	0.00	0.12	0.04	0.41	0.55	0.02	0.02
Kiloniellaceae	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.02	0.13	0.20	0.00	0.00
Hypomicrobiaceae	0.00	0.00	0.03	0.03	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Helicobacteraceae	0.00	0.00	0.00	0.00	0.00	0.00	1.30	1.22	0.32	0.30	3.47	0.76
TM7 (family)	0.32	0.17	0.18	0.30	0.57	0.13	0.06	0.01	0.00	0.00	0.24	0.15
Peptococcaceae	0.14	0.06	0.05	0.05	0.08	0.05	0.06	0.05	0.26	0.40	0.12	0.08
Bacteroidia (family)	0.23	0.12	0.17	0.09	0.11	0.05	0.02	0.04	0.04	0.05	0.01	0.02
Spirochaetaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.49	0.54	0.00	0.00
Bacillaceae unclassified	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.02	0.04
Flavobacteriaceae	0.34	0.17	0.55	0.25	0.42	0.31	0.35	0.14	0.65	0.41	0.12	0.11
Pasteurellaceae	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Bacteriovoracaceae	0.01	0.02	0.00	0.00	0.05	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonadaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.01	0.01
Acholeplasmataceae	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
Enterobacteriaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.04
Streptomycetaceae	0.03	0.01	0.03	0.05	0.02	0.03	0.00	0.01	0.00	0.00	0.00	0.00
Chitinophagaceae	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cytophagaceae	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02
Desulfuromonadaceae	0.00	0.00	0.01	0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.00	0.00
Sphingobacteriaceae	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acetobacteraceae	0.00	0.00	0.01	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Comamonadaceae	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Mollicutes (family)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
Nostocaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.18	0.20
Micromonosporaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.20	0.31	0.00	0.00
Kopriimonadaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.09	0.08	0.00	0.00
Methylococcaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.13	0.08	0.09	0.00	0.00
Acidobacteriaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.07	0.05	0.09	0.01	0.01
Staphylococcaceae	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Catabacteriaceae	0.01	0.01	0.03	0.06	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
Rhodobiaceae	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Micrococcaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
Geobacteraceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02
Francisellaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Chlorobiaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00
Anaerolineaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Spiroplasmataceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.02
Bradyrhizobiaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00

Blue: IKK β Δ IEC mice different than control C57BL/6 group at day 0.

Red: Decreased percentage in IKK β Δ IEC mice compared to same group at day 0.

Green: Increased percentage in IKK β Δ IEC mice compared to same group at day 0.

Table S6. Relative percentage of bacteria species

Species	C57BL/6						IKK β AIEC						Species	C57BL/6						IKK β AIEC							
	Day 0		Day 2		Day 4		Day 0		Day 2		Day 4			Day 0		Day 2		Day 4		Day 0		Day 2		Day 4			
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD		Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD		
Tamarella sp	38.27	5.13	34.92	6.99	31.84	5.43	49.79	38.59	2.33	33.27	4.22	Sporobacter sp	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
Bacterella intestinihominis	5.59	1.79	4.72	1.63	5.98	0.87	3.12	2.25	1.20	0.20	0.27	Prevotella detrita	0.15	0.07	0.09	0.15	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
Parabacteroides distasonis	0.08	0.07	0.03	0.03	0.03	0.05	2.39	1.03	6.31	2.93	0.75	Blautia putredinis	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.03	0.01	
Coprococcus sp	0.64	0.82	0.49	0.42	1.18	1.27	2.20	1.64	0.00	0.00	3.80	1.90	Prevotella dentans	0.14	0.12	0.84	0.81	0.28	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Candidatus Prevotella conceptinensis	10.01	3.49	13.06	9.09	14.58	7.27	0.18	0.14	0.36	0.04	0.03	Clostridium proteolyticum	0.05	0.04	0.01	0.02	0.02	0.02	0.02	0.03	0.01	0.04	0.01	0.00	0.00	0.00	
Parabacteroides sp	0.15	0.11	0.11	0.17	0.11	0.07	0.66	0.09	0.33	0.24	0.16	Tannerella forsythia	0.00	0.04	0.03	0.06	0.03	0.04	0.04	0.03	0.04	0.04	0.01	0.01	0.01	0.01	
Parabacteroides sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Lactobacillus flavifaciens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Lactococcus lactis	2.90	2.03	4.43	6.40	0.59	0.54	2.18	2.56	0.77	1.30	0.31	Clostridium populi	0.07	0.07	0.00	0.00	0.10	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Bacteroides acidifaciens	4.09	3.20	9.64	6.17	7.20	5.86	4.02	1.69	15.60	3.79	3.38	1.93	Clostridium xyloferum	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03
Prevotella sp	9.98	1.87	9.97	2.14	8.88	1.38	12.35	3.20	4.40	1.03	22.17	3.64	Acetivibrio sp	0.10	0.02	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteroides sp	0.70	0.24	1.05	0.30	1.05	0.41	1.16	0.65	1.58	1.40	0.12	0.64	Desulfotuberculum sp	0.12	0.03	0.05	0.05	0.06	0.04	0.06	0.05	0.24	0.37	0.12	0.08	0.00	0.00
Blautia obeum	0.34	0.23	0.24	0.37	0.02	0.04	1.18	0.15	0.14	0.26	0.46	0.13	Ruminococcus sp	0.04	0.05	0.00	0.00	0.02	0.03	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.00
Clostridium sp	3.03	1.74	2.21	1.11	2.42	0.72	2.34	0.87	2.26	1.31	14.37	5.31	Ruminococcus sp	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00
Terribacillus halophilus	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	Phaeosporillum sp	0.01	0.01	0.13	0.14	0.05	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eubacterium sp	2.35	1.52	0.45	0.22	1.42	0.99	1.54	0.97	6.55	2.34	2.04	0.89	Bacteroides bacterium	0.23	0.12	0.17	0.09	0.11	0.05	0.02	0.04	0.04	0.05	0.01	0.00	0.00	0.00
Eubacterium tortuosum	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Catella sp	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.08	0.00	0.00	0.00	0.00	0.00	0.00
Butyrivibrio fibrosolvens	0.21	0.25	0.06	0.03	0.07	0.04	0.06	0.11	0.01	0.03	0.14	0.07	Tropea sp	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.49	0.54	0.00	0.00	0.00	0.00
Blautia sp	0.01	0.01	0.00	0.00	0.03	0.04	0.08	0.10	0.13	0.23	0.05	0.06	Genella morbillorum	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.02	0.04	0.00	0.00
Porphyromonadaceae	0.44	0.25	0.63	0.24	0.44	0.14	0.30	0.24	0.18	0.15	0.27	0.05	Ruminococcus sp	0.04	0.03	0.10	0.13	0.03	0.04	0.02	0.04	0.01	0.01	0.00	0.00	0.00	0.00
Roseburia sp	0.06	0.05	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.03	0.04	Streptococcus constellatus	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.08	0.00	0.00	0.00	0.07	0.12	0.00
Clostridium nexile	4.63	3.28	0.80	1.01	7.23	5.14	0.27	0.15	0.11	0.08	1.63	0.52	Alistipes finegoldii	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.01	0.02	0.00	0.00
Enterococcus gallinarum	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Lactobacillus sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02
Enterococcus sp	2.51	1.17	1.14	0.54	1.71	0.79	1.01	0.15	0.04	0.11	0.91	0.15	Dysgonomus sp	0.00	0.00	0.01	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mucinipillum schaedleri	0.30	0.25	0.05	0.02	0.30	0.27	0.03	0.02	0.06	0.10	1.02	0.29	Lactobacillus murinus	0.00	0.00	0.02	0.02	0.04	0.04	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Bacteroides sartorii	0.00	0.00	0.00	0.00	0.00	0.00	1.32	0.91	2.93	0.86	0.50	0.36	Prevotella albensis	0.03	0.02	0.02	0.02	0.04	0.04	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Enterococcus sp	0.00	0.00	0.04	0.04	0.01	0.02	0.00	0.00	0.03	0.04	0.00	0.00	Gelidibacter sp	0.00	0.00	0.02	0.02	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Clostridium xylanolyticum	0.04	0.05	0.02	0.02	0.05	0.04	0.06	0.09	0.01	0.01	0.03	0.03	Helicobacter mastotyrinus	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.12	0.00	0.00	0.00	0.01	0.02	0.00
Clostridium indolis	1.18	1.22	0.44	0.63	0.86	0.50	1.18	0.15	0.14	0.24	0.64	0.23	Haemophilus sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteroides chinchibae	0.00	0.00	0.00	0.00	0.00	0.00	0.70	0.36	1.70	0.38	0.34	0.31	Anaerophaga thermophilophila	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alistipes onderdonkii	0.93	0.45	0.01	0.01	0.27	0.22	0.80	0.38	0.18	0.17	5.37	4.50	Clostridium ramosum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Roseburia sp	0.51	0.39	0.01	0.01	0.44	0.14	0.04	0.03	0.05	0.04	0.26	0.14	Butyrivibrio cacaecolentis	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.06	0.00	0.00	0.00	0.01	0.01	0.00
Alistipes sp	0.10	0.07	0.26	0.12	0.11	0.08	0.00	0.00	0.01	0.02	0.02	0.02	Butyrivibrio synergeticus	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Streptococcus sp	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.05	0.03	Bacteriostreptococcus	0.01	0.02	0.00	0.00	0.05	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eubacterium ramulus	0.24	0.46	0.00	0.00	0.16	0.15	0.64	0.33	0.01	0.03	2.77	2.06	Pseudomonas sp	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.01	0.01	0.00
Parabacteroides merdae	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.11	0.32	0.15	0.16	0.17	Acetivibrio granulum	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Parasuterella excrementihominis	0.04	0.02	0.19	0.16	0.05	0.05	0.56	0.56	0.63	0.87	0.32	0.13	Eumecostium coli	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Eubacterium xylanophilum	0.24	0.19	0.12	0.21	0.26	0.14	0.04	0.08	0.00	0.11	0.38	0.31	Faecalibacterium sp	0.00	0.00	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.02	0.00	0.00	0.00	0.00
Oscillibacter sp	0.74	0.54	0.38	0.39	0.63	0.24	0.10	0.11	0.07	0.11	0.28	0.03	Streptomyces sp	0.03	0.01	0.03	0.05	0.02	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Streptococcus lacteus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.69	0.12	0.46	0.80	0.02	Prevotella loescheii	0.07	0.05	0.05	0.03	0.01	0.01	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
Odoribacter lanicus	0.01	0.02	0.03	0.05	0.02	0.03	0.01	0.03	0.00	0.00	0.94	0.16	Zhangella mubiis	0.00	0.00	0.02	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ruminococcus sp	0.65	0.33	0.50	0.15	0.51	0.19	0.01	0.03	0.00	0.00	0.01	0.01	Streptococcus pleomorphaus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00
Ruminococcus sp	0.15	0.04	0.33	0.37	0.22	0.15	0.05	0.03	0.01	0.01	0.22	0.22	Shackia piriformis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Parabacteroides goldsteini	0.01	0.01	2.77	1.42	0.09	0.10	0.00	0.01	0.36	0.34	0.01	0.01	Nitastella sp	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eubacterium leptum	0.05	0.03	0.01	0.01	0.01	0.01	0.00	0.01	0.04	0.04	0.02	0.04	Flexibacter sp	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Anaerostipes cacaecae	0.50	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Prevotella buccae	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Parabacteroides merdae	0.07	0.06	0.02	0.03	0.10	0.15	0.06	0.13	0.29	0.51	1.15	1.87	Desulfuromonas sp	0.00	0.00	0.01	0.01	0.02	0.00	0.00	0.01	0.01	0.01	0			



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