Supporting Information

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SI Materials and Methods

Mice. Mice were purchased from Taconic Farms (TAC) (BALB/cAnNTac, C57BL/6NTac, NOD/MrkTac) or the Jackson Laboratory (JAX) (BALB/cJ, C57BL/6J, NOD/ShiLtJ) or were housed at our specific pathogen-free facility at the New Research Building (NRB) at Harvard Medical School. For mice from either JAX or TAC, feces were collected within 24 h of arrival for quantification of segmented filamentous bacteria (SFB). Female nonobese diabetic (NOD) mice housed at the NRB facility were used except when noted otherwise. The NOD colony at the NRB facility is replenished occasionally from a separate breeding stock kept at the Jackson Laboratory (independent of the commercial facility there). All mouse experiments were approved by the Institutional Animal Care and Use Committee of Harvard Medical School.

Tissue Collection and DNA Isolation. Fecal pellets were collected with alcohol-cleaned forceps under a laminar flow hood, placed in sterile tubes, frozen immediately on dry ice, and stored at −80°C until processing. Intestinal tissues were dissected surgically, separated into the relevant anatomical segments (small intestine, colon), and frozen immediately on dry ice before storage at −80 °C. The distal small intestine was defined as the lower half of the complete small intestine, to avoid any sex- or age-related differences in length. Weighed tissue segments first were homogenized with an autoclaved sonicator in 0.5 mL of a buffer containing 0.2 mg/mL proteinase K, 100 mM NaCl, 10 mM Tris, and 100 mM EDTA. The sonicator was washed with sterile double-distilled water and alcohol between each sample, and sterile spleens were processed after every 5–10 tissue samples as negative controls. Two or three frozen fecal pellets in lysis buffer or 0.5 mL of each homogenate were transferred into autoclaved cryotubes containing 500 μL of 0.1-mm zirconium beads (Biospec Products) and freshly added 20% SDS (210 μL). Fecal pellets were bead beaten for 2 min and tissues were bead beaten for 4 min using a minibeater (Biospec), followed by phenol-chloroform extraction of DNA, as described elsewhere (1). SYBR Green real-time PCR was performed with 20 ng of fecal or tissue DNA using SFB-specific [SFB736 forward and SFB884 reverse (2)] and conserved eubacterial (EUB) 1114 forward and 1221 reverse (3) 16S ribosomal DNA primers. Primer sequences are as follows:

SFB736 forward: GACGCTGAGGCATGAGAGCAT
SFB844 reverse: GACGGCACGGATTGTTATTCA
EUB1114 forward: CGGCAACGAGCGCAACCC
EUB1221 reverse: CCATTGTAGCACGTGTGTAGCC

Genomic DNA from spleens and bacterial DNA from *Staphylococcus epidermidis* and *Escherichia coli* were used as SFB-negative controls.

Diabetes and Insulitis Assessments. Mice were screened weekly for diabetes development starting at 10 wk of age. Glucosuria was measured by dipstick analysis of urine (Diastix; Ames). Onset of diabetes was confirmed by blood-glucose measurements. Mice were considered diabetic if measurements of blood glucose were >250 mg/dL on three consecutive readings. Mice without glucosuria were followed until at least 30 wk of age, at which point they were considered protected.

For evaluation of insulitis, pancreata were dissected and immediately fixed in 10% formalin (Sigma Aldrich) before being embedded in paraffin, sectioned, and stained with H&E. Insulitis was assessed by light microscopy and was graded according to the following scheme: no insulitis, peri-insulitis (perivascular/periductular infiltrates with leukocytes touching islet perimeters), or insulitis (leukocytic penetration of any degree).

Preparation of Intestinal Cell Suspensions for Immunological Analyses. The small intestine and colon were dissected from 6- to 10-wk-old NOD mice, and residual mesenteric fat was removed. Peyer's patches were excised carefully before longitudinal opening of the intestinal segment, which then was thoroughly washed in ice-cold PBS and cut into 2- to 3-cm pieces. Segments from a single intestine were incubated twice in 25 mL of 5 mM EDTA and 0.145 mg/mL DL-DTT in DMEM for 30 min at 37 °C at a rotation speed of 200 rpm. Next, the epithelial cell layer containing intraepithelial lymphocytes was removed by intensive vortexing and passage through a 100-μm cell strainer. The intestinal bits were rolled over paper tissue to remove excess mucus and then were washed in PBS, cut into 1-mm^2 pieces using scissors, and placed in 25 mL digestion solution containing 1 mg/mL each of type II collagenase from *Clostridium histolyticum* (Sigma), 0.15 mg/mL DNase I (Sigma), and 200 ng/mL Liberase Cl (Roche). After the intestinal bits were incubated at 37 °C for 40 min with rotation, the cell suspension was vortexed intensely and passed through a 100-μm strainer. Supernatants then were passed through a 40-μm cell strainer, and the cells were resuspended in 10% DMEM for flow cytometric analysis.

Flow Cytometry. Cell suspensions were isolated as above from intestinal regions of female NOD mice at 6–10 wk of age or from the pancreatic lymph nodes and spleens by organ dissection and glass slide disruption (4). Cells were surface stained with fluorophore-labeled mAbs specific for CD45 (30-F11), CD4 (RM4-5), CD8 (53-6.7), T-cell receptor β (TCRβ) (H57–597), and T-cell receptor γδ (TCRγδ) (GL3), all purchased from BioLegend. For intracellular cytokine staining, cells were incubated immediately after isolation for 4 h with 50 ng/mL phorbol 12-myristate 13-acetate (Sigma), 1 μM ionomycin (Sigma), and BD GolgiPlug (1:1,000 dilution) at 37 °C. Staining was performed using Cytofix/Cytoperm (BD Pharmingen) according to the manufacturer's instructions. mAbs recognizing IL-17 (TC11-18H10.1) or IFN-γ (XMG1.2) were purchased from BioLegend and BD Pharmingen, respectively. The forkhead box P3 Staining Buffer Set (clone FJK-16S; eBioscience) was used for intracellular staining following the manufacturer's instructions. Cells were run on an LSRII machine (BD Biosciences), and data were analyzed with FloJo software (TreeStar).

Microarray Analysis. $CD4^+$ T cells were isolated from spleen and small-intestinal lamina propria (SI-LP) of 6- to 10-wk-old mice as described previously (5). Cells were sorted using a Becton Dickinson FACSAria into TRIzol reagent (Invitrogen) based on the following cell surface markers: $CD45^+$, $CD8a^-$, $CD11b^-$, $CD11c^-$, $CD19^-$, $B220^-$, $Gr\text{-}1^-$, $TCR\beta^+$, and $CD4^+$. RNA was isolated following the manufacturer's protocol. RNA was amplified for two rounds (MessageAmp aRNA; Ambion), biotin labeled (BioArray High Yield RNA Transcription Labeling; Enzo), and purified using the RNeasy Mini Kit (Qiagen). The resulting cRNAs were hybridized to Affymetrix GeneChip Mouse Gene 1.0 ST arrays (Expression Analysis). Raw data were normalized using the robust means analysis algorithm. Data were visualized using the Multiplot and Hierarchical Clustering Viewer modules from the GenePattern suite (Broad Institute) (6). All cell populations

analyzed were generated from individual mice from two to four independent experiments. Transcriptional profiling did not reveal any consistent differences between male and female NOD mice outside of sex-specific gene expression; therefore, data from different genders were pooled and a coefficient of variation filter of < 1 was used to exclude the probes with high variability. Datasets are available at the National Center for Biotechnology Information under accession no. GSE 29806.

1. Turnbaugh PJ, et al. (2009) A core gut microbiome in obese and lean twins. *Nature* 457: 480–484.
2. Barman M, et al. (2008) Enteric salmonellosis disrupts the microbial ecology of the murine gastrointestinal tract. *Infect Immun* 76:907–915.
3. Denman SE, McSweeney CS (2006) Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. *FEMS Microbiol Ecol* 58:572–582.
4. Turley SJ, Lee JW, Dutton-Swain N, Mathis D, Benoist C (2005) Endocrine self and gut non-self intersect in the pancreatic lymph nodes. *Proc Natl Acad Sci USA* 102: 17729–17733.
5. Feuerer M, et al. (2010) Genomic definition of multiple ex vivo regulatory T cell subphenotypes. *Proc Natl Acad Sci USA* 107:5919–5924.
6. Reich M, et al. (2006) GenePattern 2.0. *Nat Genet* 38:500–501.

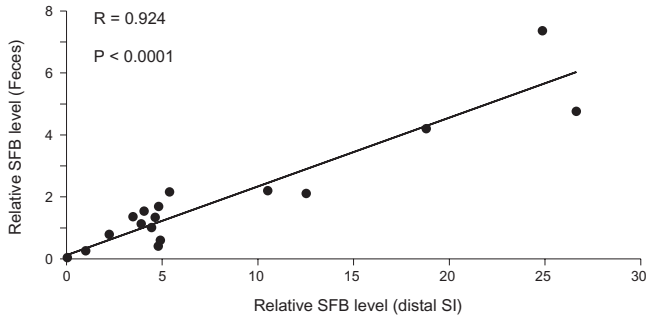

Fig. S1. Correlation of relative SFB levels from small intestine and feces in NOD mice. SFB levels in the distal small intestine and feces of 16 mice were quantified by PCR. SFB were not detectable in five additional paired small intestine and fecal samples. The *P* value was determined by Pearson correlation.

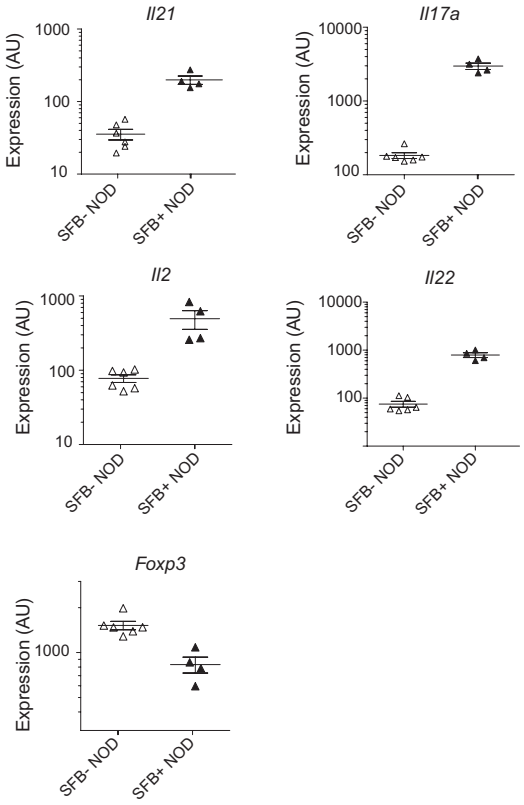

Fig. S2. Quantification in arbitrary units (AU) of selected signature genes in SI-LP CD4$^+$ T cells of SFB-negative and SFB-positive NOD mice. Affymetrix microarray analysis of SI-LP CD4$^+$ T cells from NOD mice that were 6–10 wk of age was performed as described in *SI Materials and Methods*. See Fig. 5*A* for the corresponding expression plot. Open triangles represent SFB-negative samples, closed triangles SFB-positive samples.

Table S1. Genes encoding transcripts that were up-regulated or down-regulated more than twofold in SI-LP CD4$^+$ T cells sorted from SFB-positive vs. SFB-negative NOD mice

ProbeSet ID	Gene symbol	NOD SI-LP (SFB$^+$) average (n = 4)	Coefficient of variation among SFB$^+$ mice	NOD SI-LP (SFB$^-$) average (n = 6)	Coefficient of variation among SFB$^-$ mice	SFB$^+$ vs. SFB$^-$fold-change	False discovery rate
Genes up-regulated twofold							
10345032	Il17a	2975.9	0.20	183.0	0.22	16.26	0
10353415	Il17f	2219.8	0.10	159.3	0.12	13.93	0
10366572	Il22	792.2	0.23	75.2	0.34	10.53	5.03E-11
10373695	Il22	786.4	0.24	75.1	0.34	10.47	8.66E-10
10568956	Olfr60	270.9	0.23	36.2	0.18	7.48	2.70E-09
10372730	Iltifb	114.6	0.34	15.6	0.18	7.35	0.01398307
10345762	Il1r1	455.2	0.26	67.1	0.11	6.79	1.13E-06
10497878	Il2	495.2	0.56	77.7	0.29	6.37	NS
10497886	Il21	198.8	0.26	35.5	0.41	5.60	2.28E-05
10513729	Tnfsf8	1051.7	0.14	236.9	0.23	4.44	0
10408629	1300014I06Rik	587.7	0.38	138.7	0.33	4.24	NS
10598004	Ccr1	74.4	0.49	20.9	0.18	3.56	NS
10407281	Esm1	310.2	0.22	99.8	0.32	3.11	0.000364066
10408557	Serpinb1a	274.3	0.26	92.0	0.24	2.98	0.02559328
10598013	Ccr5	731.8	0.21	248.3	0.26	2.95	8.51E-05
10514221	Plin2	1166.1	0.14	402.2	0.34	2.90	2.29E-10
10564960	Furin	3788.7	0.24	1330.3	0.18	2.85	0.002189427
10578904	Cpe	109.1	0.40	40.1	0.15	2.72	NS
10480286	Nebl	116.3	0.06	42.9	0.15	2.71	0
10545588	Hk2	569.6	0.21	211.1	0.27	2.70	0.000681032
10405727	2410127L17Rik	222.8	0.26	83.0	0.23	2.68	0.07768437
10438738	Bcl6	778.1	0.14	294.6	0.65	2.64	0.007192369
10380927	Grb7	599.5	0.24	228.2	0.17	2.63	0.01042621
10401891	Ston2	415.1	0.27	158.4	0.20	2.62	0.174866
10461909	BC016495	91.2	0.21	35.2	0.20	2.59	0.000803299
10590623	Cxcr6	2562.0	0.17	1004.3	0.50	2.55	0.004468818
10545154	Il23r	673.6	0.13	264.5	0.27	2.55	1.73E-10
10595205	2410127L17Rik	367.4	0.25	145.2	0.26	2.53	0.1580564
10461921	2410127L17Rik	364.1	0.25	144.1	0.25	2.53	0.1282091
10547054	Gm14335	145.7	0.40	57.7	0.25	2.52	NS
10392183	Ern1	2261.0	0.26	914.2	0.91	2.47	NS
10592888	Cxcr5	90.3	0.37	36.6	0.27	2.47	NS
10558655	Olfr46	166.7	0.19	68.4	0.12	2.44	2.27E-05
10568691	A130023I24Rik	162.8	0.26	69.0	0.24	2.36	NS
10495794	Pde5a	95.1	0.38	40.6	0.27	2.34	NS
10389786	Hlf	197.5	0.22	85.1	0.11	2.32	0.009723693
10354732	Hspd1	915.1	0.27	395.8	0.63	2.31	NS
10345752	Il1r2	192.5	0.15	83.8	0.14	2.30	1.92E-08
10406941	Sgtb	132.5	0.35	58.6	0.51	2.26	NS
10466835	Snora19	89.3	0.28	39.5	0.37	2.26	NS
10512030	3110043O21Rik	396.3	0.24	176.4	0.44	2.25	NS
10453715	Rab18	294.9	0.34	133.3	0.27	2.21	NS
10582997	Casp4	641.7	0.20	293.9	0.38	2.18	NS
10535904	Hsph1	2050.1	0.36	944.9	0.72	2.17	NS
10347888	Ccl20	307.2	0.28	142.2	0.25	2.16	NS
10428728	9330154K18Rik	267.8	0.26	125.7	0.23	2.13	NS
10444589	Hspa1a	1276.8	0.50	607.1	0.53	2.10	NS
10501046	Gm10673	226.4	0.31	108.7	0.20	2.08	NS
10599192	Lonrf3	118.0	0.24	57.1	0.21	2.07	NS
10458028	Gypc	576.0	0.15	279.9	0.18	2.06	2.60E-05
10406407	Arrdc3	345.3	0.36	168.1	0.27	2.05	NS
10480275	Nebl	197.6	0.09	96.2	0.11	2.05	0
10498345	Gpr171	601.9	0.11	295.6	0.16	2.04	4.47E-11
10607933	Hccs	1064.4	0.15	523.5	0.23	2.03	0.000139185
10491780	Hspa4l	507.7	0.12	251.7	0.28	2.02	2.34E-05
Genes down-regulated twofold							
10430425	Lgals2	53.5	0.40	244.6	0.76	0.22	NS
10585005	Apoa1	141.4	0.24	434.1	0.99	0.33	NS

Table S1. Cont.

ProbeSet ID	Gene symbol	NOD SI-LP (SFB^+) average (n = 4)	Coefficient of variation among SFB^+ mice	NOD SI-LP (SFB^-) average (n = 6)	Coefficient of variation among SFB^- mice	SFB^+ vs. SFB^- fold-change	False discovery rate
10438405	Igl-V1	38.2	0.06	107.5	0.63	0.36	NS
10502622	Clca3	48.2	0.13	123.0	0.64	0.39	NS
10502613	AI747448	22.9	0.24	55.5	0.62	0.41	NS
10418341	Il17rb	89.3	0.25	215.2	0.39	0.41	NS
10359689	Atp1b1	109.1	0.17	260.8	0.40	0.42	NS
10384223	Igfbp3	57.1	0.21	135.8	0.76	0.42	NS
10545569	Reg3g	30.6	0.20	72.5	0.77	0.42	NS
10503098	Lyn	88.3	0.28	209.1	0.76	0.42	NS
10558992	Muc2	58.6	0.38	134.6	0.52	0.44	NS
10447383	Epcam	135.8	0.28	310.6	0.45	0.44	NS
10460746	Naaladl1	25.6	0.10	58.1	0.57	0.44	NS
10379615	Sl	74.1	0.17	166.2	0.33	0.45	NS
10416689	Olfm4	62.8	0.44	137.6	0.44	0.46	NS
10538921	LOC100046350	28.1	0.06	59.9	0.92	0.47	NS
10506488	Ppap2b	60.6	0.88	126.7	0.81	0.48	NS
10491952	Mgst2	155.6	0.11	325.5	0.19	0.48	3.46E-06
10531931	Sparcl1	76.5	0.62	159.3	0.97	0.48	NS

Average values, coefficients of variation, and false-discovery rates are listed for each up- or down-regulated transcript. Note that these mouse-to-mouse comparisons showed more within-group variability than we routinely observe in comparisons of cells from secondary lymphoid organs, as reflected by high within-group coefficients of variation and poor false discovery rates (FDR) (Table S1), even if the shifts are fully reproducible (Fig. S2).

Table S2. Genes encoding transcripts that were up-regulated or down-regulated more than twofold in colonic lamina propria CD4$^+$ T cells sorted from SFB-positive vs. SFB-negative NOD mice

ProbeID	Gene symbol	NOD colon lamina propria (SFB$^+$) average*	Coefficient of variation among SFB$^+$ mice	NOD colon lamina propria (SFB$^-$) average†	Coefficient of variation among SFB$^-$ mice	SFB$^+$ vs. SFB$^-$fold-change	False discovery rate
Genes up-regulated more than twofold							
10545135	Il12rb2	199.9	0.04	60.3	0.36	3.32	0.00
10345791	Il1rl1	330.5	0.19	105.2	0.43	3.14	0.16
10361771	Plagl1	325.6	0.02	113.4	0.44	2.87	0.00
10502052	Alpk1	293.9	0.21	106.9	0.23	2.75	NS
10533569	Kdm2b	1234.6	0.05	458.8	0.46	2.69	0.00
10535904	Hsph1	1386.7	0.59	523.3	0.29	2.65	NS
10586252	Dennd4a	395.2	0.12	153.0	0.72	2.58	NS
10586254	Dennd4a	1103.4	0.03	431.1	0.73	2.56	NS
10586248	Dennd4a	593.8	0.03	234.0	0.67	2.54	0.15
10502050	Alpk1	240.5	0.02	95.0	0.09	2.53	0.00
10586246	Dennd4a	834.5	0.06	330.8	0.68	2.52	NS
10586250	Dennd4a	495.0	0.13	197.9	0.74	2.50	NS
10392183	Ern1	493.2	0.08	205.3	0.36	2.40	0.00
10420308	Gzmb	951.6	0.24	397.1	0.45	2.40	NS
10580382	Neto2	105.7	0.62	44.5	0.85	2.37	NS
10349081	Phlpp1	339.0	0.05	144.2	0.23	2.35	0.00
10365933	Eea1	468.9	0.23	202.0	0.17	2.32	NS
10586700	Rora	761.8	0.02	328.4	0.34	2.32	0.00
10363070	Gp49a	591.6	0.37	255.3	0.34	2.32	NS
10453867	Rbbp8	314.5	0.17	135.8	0.36	2.32	NS
10594251	Kif23	657.6	0.23	292.3	0.65	2.25	NS
10428536	Trps1	260.2	0.08	116.6	0.32	2.23	0.00
10568956	Olfr60	39.6	0.12	17.9	0.13	2.21	0.00
10586244	Dennd4a	580.2	0.04	264.4	0.60	2.19	NS
10436209	Cblb	2597.4	0.28	1188.7	0.59	2.19	NS
10392207	Tex2	802.9	0.04	372.8	0.52	2.15	0.34
10544084	Zc3hav1l	404.1	0.14	189.6	0.61	2.13	NS
10540472	Bhlhe40	948.7	0.11	447.3	0.33	2.12	0.03
10429926	Dgat1	1434.4	0.19	682.8	0.76	2.10	NS
10453678	Zeb1	824.0	0.08	395.7	0.19	2.08	0.00
10406254	Ell2	277.5	0.09	134.3	0.49	2.07	NS
10358408	Rgs1	888.8	0.48	432.6	0.22	2.05	NS
10409278	Nfil3	740.8	0.10	363.2	0.14	2.04	0.00
10548735	Dusp16	336.1	0.21	164.8	0.43	2.04	NS
10349603	Il10	731.4	0.48	360.0	0.33	2.03	NS
10531415	Cxcl10	794.9	0.15	391.4	0.30	2.03	NS
10358191	Camsap1l1	703.6	0.02	348.0	0.33	2.02	0.00
10439527	Tigit	1316.7	0.26	654.5	0.47	2.01	NS
10346651	Bmpr2	409.7	0.30	204.1	0.06	2.01	NS
10586240	Dennd4a	1869.5	0.18	931.6	0.65	2.01	NS
Genes down-regulated more than twofold							
10472235	Dapl1	106.3	0.10	375.0	0.44	0.28	NS
10530615	Ociad2	29.2	0.16	76.9	0.99	0.38	NS
10347915	Gm7609	43.8	0.03	114.9	0.12	0.38	0.00
10512640	Gne	116.2	0.05	293.1	0.84	0.40	NS
10351959	1810030J14Rik	19.2	0.19	48.5	0.96	0.40	NS
10451818	Sult1c2	18.8	0.00	45.1	0.95	0.42	NS
10429564	Ly6a	686.5	0.05	1647.9	0.23	0.42	0.01
10571984	Ddx60	53.4	0.09	125.9	0.28	0.42	NS
10345855	Slc9a2	46.0	0.12	103.3	0.95	0.45	NS
10533345	Aldh2	96.5	0.12	215.7	0.84	0.45	NS
10393573	Lgals3bp	219.5	0.27	490.9	0.07	0.45	0.00
10503508	Ggh	46.5	0.27	103.4	0.97	0.45	NS
10347925	Gm7609	54.3	0.16	119.5	0.08	0.45	0.00
10403054	LOC435333	63.3	0.01	137.8	1.00	0.46	NS
10441254	Tmprss2	57.5	0.07	125.3	0.93	0.46	NS
10389143	Slfn8	80.0	0.03	168.4	0.29	0.47	NS

Table S2. Cont.

ProbeID	Gene symbol	NOD colon lamina propria (SFB^+) average*	Coefficient of variation among SFB^+ mice	NOD colon lamina propria (SFB^-) average†	Coefficient of variation among SFB^- mice	SFB^+ vs. SFB^- fold-change	False discovery rate
10379615	Slfn5	89.0	0.11	186.7	0.33	0.48	NS
10494085	Selenbp2	67.8	0.19	141.3	0.84	0.48	NS
10514779	Prkaa2	22.6	0.08	47.0	0.93	0.48	NS
10578361	Asah1	50.7	0.02	104.0	0.42	0.49	NS
10393166	St6galnac2	280.8	0.03	575.5	0.28	0.49	NS
10458992	C330018D20Rik	29.5	0.19	60.4	0.48	0.49	NS
10406423	Mblac2	45.8	0.17	93.3	0.35	0.49	NS
10530201	Ugdh	277.6	0.07	562.8	0.96	0.49	NS
10411082	Thbs4	51.1	0.07	103.3	0.97	0.49	NS
10570516	Kbtbd11	193.5	0.19	388.2	0.61	0.50	NS

Average values, coefficients of variation, and false-discovery rates are listed for each up- or down-regulated transcript.
*For NOD colon-LP samples that were SFB-positive, $n = 2$.
†For NOD colon-LP samples that were SFB-negative, $n = 4$.