

Supplementary Table 1. The Mmean Expression Values and the Standard Deviation of All Analyzed the Genes

Gene	Description	Mean						SD									
		HC	BL	Mesalazine	Placebo	HC	BL	Mesalazine	Placebo	HC	BL	Mesalazine	Placebo				
ALOX15B	Arachidonate 15-lipoxygenase type B	0.80	0.30	0.30	0.34	1.12	0.23	0.21	0.25	0.80	0.30	0.30	0.34	1.12	0.23	0.21	0.25
AQP3	Aquaporin 3	5.60	3.80	3.41	3.68	3.09	1.22	1.20	1.03	5.60	3.80	3.41	3.68	3.09	1.22	1.20	1.03
ATP12A	ATPase H ⁺ /K ⁺ transporting non-gastric alpha2 subunit	3.52	0.32	0.25	0.43	5.54	0.51	0.37	0.75	3.52	0.32	0.25	0.43	5.54	0.51	0.37	0.75
BDKRB2	Bradykinin receptor B2	10.37	17.06	12.12	13.46	3.35	6.39	3.45	3.21	10.37	17.06	12.12	13.46	3.35	6.39	3.45	3.21
CCL28	C-C motif chemokine ligand 28	69.19	57.51	50.86	46.83	28.72	22.44	14.54	14.86	69.19	57.51	50.86	46.83	28.72	22.44	14.54	14.86
CELA1	Chymotrypsin like elastase 1	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	0.00	0.00
CLDN2	Claudin 2	0.18	0.20	0.11	0.09	0.36	0.48	0.12	0.06	0.18	0.20	0.11	0.09	0.36	0.48	0.12	0.06
CMA1	Chymase 1	0.56	0.32	0.36	0.29	0.41	0.31	0.31	0.27	0.56	0.32	0.36	0.29	0.41	0.31	0.31	0.27
CMKLR1	Chemerin chemokine-like receptor 1	0.77	1.31	1.21	1.30	0.27	0.62	0.56	0.37	0.77	1.31	1.21	1.30	0.27	0.62	0.56	0.37
CMTM2	CKLF like MARVEL transmembrane domain containing 2	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01
CYP2J2	Cytochrome P450 family 2 subfamily j member 2	6.14	4.49	4.49	4.34	2.61	1.42	1.41	1.27	6.14	4.49	4.49	4.34	2.61	1.42	1.41	1.27
EPHX2	Epoxide hydrolase 2	15.94	17.88	16.32	15.43	4.62	5.40	4.45	4.18	15.94	17.88	16.32	15.43	4.62	5.40	4.45	4.18
F11R	F11 receptor	29.89	35.16	28.20	30.76	8.47	10.80	9.27	10.05	29.89	35.16	28.20	30.76	8.47	10.80	9.27	10.05
F2RL1	F2R like trypsin receptor 1	28.44	28.21	22.51	23.95	36.36	9.61	10.08	7.49	28.44	28.21	22.51	23.95	36.36	9.61	10.08	7.49
F2RL2	Coagulation factor II thrombin receptor like 2	0.08	0.17	0.14	0.19	0.03	0.09	0.09	0.09	0.08	0.17	0.14	0.19	0.03	0.09	0.09	0.09
F2RL3	F2R like thrombin or trypsin receptor 3	0.29	0.51	0.46	0.54	0.14	0.25	0.20	0.26	0.29	0.51	0.46	0.54	0.14	0.25	0.20	0.26
FABP2	Fatty acid binding protein 2	2.93	6.19	5.81	4.91	1.25	2.75	3.26	2.49	2.93	6.19	5.81	4.91	1.25	2.75	3.26	2.49
FCERIA	Fc fragment of IgE receptor Ia	1.45	0.97	0.98	0.94	0.79	0.72	0.59	0.49	1.45	0.97	0.98	0.94	0.79	0.72	0.59	0.49
FCERIG	Fc fragment of IgE receptor Ig	23.31	21.17	23.54	22.06	9.46	7.82	9.61	6.38	23.31	21.17	23.54	22.06	9.46	7.82	9.61	6.38
FPR2	Formyl peptide receptor 2	0.07	0.18	0.05	0.03	0.16	0.81	0.09	0.04	0.07	0.18	0.05	0.03	0.16	0.81	0.09	0.04
FST	Follistatin	0.06	0.11	0.10	0.12	0.06	0.08	0.05	0.06	0.06	0.11	0.10	0.12	0.06	0.08	0.05	0.06
GJA1	Gap junction protein alpha 1	6.76	9.09	9.52	9.78	2.82	3.53	3.52	2.62	6.76	9.09	9.52	9.78	2.82	3.53	3.52	2.62
IL10	Interleukin 10	0.22	0.16	0.12	0.13	0.21	0.08	0.05	0.06	0.22	0.16	0.12	0.13	0.21	0.08	0.05	0.06
IL12RB1	Interleukin 12 receptor subunit beta 1	1.03	0.93	0.99	0.93	0.53	0.39	0.44	0.29	1.03	0.93	0.99	0.93	0.53	0.39	0.44	0.29
IL13	Interleukin 13	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01
IL18	Interleukin 18	6.11	4.29	4.62	4.08	4.81	1.38	1.68	1.12	6.11	4.29	4.62	4.08	4.81	1.38	1.68	1.12
IL1B	Interleukin 1 beta	2.57	1.77	2.04	1.94	3.61	0.93	3.53	1.30	2.57	1.77	2.04	1.94	3.61	0.93	3.53	1.30
IL1RN	Interleukin 1 receptor antagonist	2.62	1.48	1.25	1.35	2.77	1.34	0.66	1.50	2.62	1.48	1.25	1.35	2.77	1.34	0.66	1.50
IL23A	Interleukin 23 subunit alpha	0.12	0.06	0.06	0.05	0.09	0.03	0.02	0.03	0.12	0.06	0.06	0.05	0.09	0.03	0.02	0.03
IL33	Interleukin 33	81.93	2.41	2.03	6.91	354.64	7.85	2.44	16.34	81.93	2.41	2.03	6.91	354.64	7.85	2.44	16.34
IL37	Interleukin 37	0.53	0.33	0.40	0.34	0.25	0.23	0.26	0.18	0.53	0.33	0.40	0.34	0.25	0.23	0.26	0.18
IL6	Interleukin 6	0.10	0.03	0.03	0.02	0.21	0.02	0.04	0.01	0.10	0.03	0.03	0.02	0.21	0.02	0.04	0.01
JAM2	Junctional adhesion molecule 2	1.45	1.44	1.37	1.54	1.07	0.56	0.54	0.52	1.45	1.44	1.37	1.54	1.07	0.56	0.54	0.52
JAM3	Junctional adhesion molecule 3	1.74	2.00	1.92	1.98	0.69	0.71	0.71	0.56	1.74	2.00	1.92	1.98	0.69	0.71	0.71	0.56

Supplementary Table 1. Continued

Gene	Description	Mean				SD			
		HC	BL	Mesalazine	Placebo	HC	BL	Mesalazine	Placebo
KIT	KIT proto-oncogene, receptor tyrosine kinase	4.81	3.77	4.17	3.76	2.05	1.55	1.64	1.37
LRBA	LPS responsive beige-like anchor protein	6.48	6.26	6.03	5.83	2.24	1.57	1.45	1.41
MIADCAM1	Mucosal vascular addressin cell adhesion molecule 1	1.01	1.21	1.01	0.90	0.67	1.44	0.58	0.25
MCL1	MCL1 apoptosis regulator, BCL2 family member	73.47	57.43	52.04	58.90	87.43	16.94	16.20	26.52
MMP2	Matrix metalloproteinase 2	8.31	12.90	11.26	11.30	2.99	5.14	4.31	3.03
MT2A	Metallothionein 2A	2.79	3.52	2.45	2.58	1.55	6.74	2.16	3.22
MUC1	Mucin 1, cell surface associated	27.03	18.23	18.68	16.34	12.18	5.83	9.64	5.82
MUC3B	Mucin 3B, cell surface associated	12.46	6.80	5.78	6.37	17.73	4.93	2.15	3.77
MYD88	MYD88 innate immune signal transduction adaptor	18.18	19.73	16.15	16.36	6.14	7.94	5.81	5.59
NAT1	N-acetyltransferase 1	2.00	2.43	2.25	2.01	0.70	0.94	0.86	0.48
NAT2	N-acetyltransferase 2	1.70	0.94	0.79	0.74	3.24	0.40	0.23	0.26
NOD2	Nucleotide binding oligomerization domain containing 2	0.16	0.20	0.18	0.20	0.09	0.10	0.09	0.10
NOS2	Nitric oxide synthase 2	6.54	3.81	4.07	2.53	11.73	2.85	4.52	1.16
NT5E	5'-nucleotidase ecto	9.72	14.07	11.77	12.74	2.96	6.77	5.07	4.93
OCLN	Occludin	38.84	13.15	11.63	11.55	103.77	4.30	4.10	4.09
PDE4B	Phosphodiesterase 4B	0.81	0.88	0.83	0.91	0.40	0.34	0.32	0.32
PDE7A	Phosphodiesterase 7A	2.92	2.22	2.16	2.03	2.24	0.89	0.90	0.64
PLA2G2A	Phospholipase A2 group IIA	182.07	173.14	165.37	151.98	196.23	102.00	189.65	99.29
PPARG	Peroxisome proliferator activated receptor gamma	29.71	16.16	13.10	11.88	59.25	6.11	3.53	2.99
PTGER1	Prostaglandin E receptor 1	0.10	0.13	0.09	0.13	0.06	0.08	0.06	0.09
PTGES	Prostaglandin E synthase	0.73	0.67	0.64	0.59	0.32	0.29	0.34	0.19
PTGES2	Prostaglandin E synthase 2	15.49	13.95	13.48	24.16	11.63	5.86	5.78	45.40
PTGS1	Prostaglandin-endoperoxide synthase 1	2.95	4.20	3.75	4.08	1.00	1.61	1.25	1.09
PTGS2	Prostaglandin-endoperoxide synthase 2	0.39	0.43	0.41	0.42	0.22	0.22	0.17	0.15
SE	Fucosyltransferase 2	26.33	21.07	17.46	17.16	21.69	12.92	8.85	9.95
SOD1	Superoxide dismutase 1	29.22	24.13	23.31	21.27	9.58	7.00	6.58	5.53
TBXAS1	Thromboxane A synthase 1	19.46	4.45	3.71	3.94	51.61	2.20	1.55	1.31
TGFB1	Transforming growth factor beta 1	16.04	7.96	7.42	8.34	15.30	3.18	2.74	2.21
TGFBR1	Transforming growth factor beta receptor 1	21.31	4.69	4.22	4.22	79.05	1.53	1.33	0.98
TGFBR2	Transforming growth factor beta receptor 2	18.66	18.31	17.49	17.44	16.39	4.89	4.69	4.04
TJP1	Tight junction protein 1	7.13	10.35	8.57	8.62	2.95	3.08	2.79	2.79
TJP2	Tight junction protein 2	14.84	15.18	13.24	13.47	8.59	5.12	4.77	5.47
TJP3	Tight junction protein 3	8.88	11.20	9.37	9.41	2.55	3.53	3.81	3.06
TTLR2	Toll like receptor 2	0.19	0.27	0.21	0.18	0.08	0.40	0.12	0.07
TTLR4	Toll like receptor 4	1.49	2.56	1.89	2.04	0.54	0.80	0.79	0.56
TTLR5	Toll like receptor 5	0.22	0.29	0.17	0.19	0.13	0.46	0.06	0.06

Supplementary Table 1. Continued

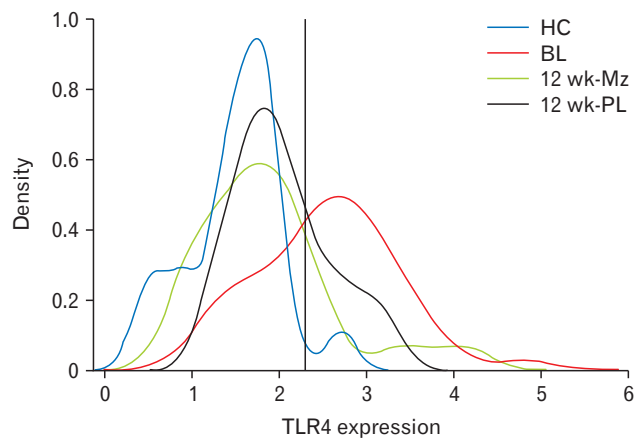
Gene	Description	Mean				SD			
		HC	BL	Mesalazine	Placebo	HC	BL	Mesalazine	Placebo
TLR7	Toll like receptor 7	1.04	0.45	0.36	0.39	3.06	0.26	0.16	0.16
TLR9	Toll like receptor 9	2.07	1.30	0.87	1.00	1.76	1.47	0.48	0.37
TNF	Tumor necrosis factor	2.07	1.30	0.87	1.00	1.76	1.47	0.48	0.37
TNFRSF10A	TNF receptor superfamily member 10a	4.33	2.66	2.48	2.70	6.88	1.04	1.14	1.08
TNFRSF25	TNF receptor superfamily member 25	8.47	6.25	6.21	5.64	3.40	2.12	1.89	2.36
TNFSF10	TNF superfamily member 10	22.81	28.95	22.66	23.13	8.77	12.51	6.12	11.82
TNFSF15	TNF superfamily member 15	1.12	1.19	0.87	0.90	0.94	0.85	0.43	0.29
TOLLIP	Toll interacting protein	8.23	8.62	7.29	7.81	2.89	2.53	1.91	2.47
TPH1	Tryptophan hydroxylase 1	24.75	7.48	6.38	7.13	44.51	3.01	3.21	2.79
TPSB2	Tryptase beta 2 (gene/pseudogene)	150.05	106.36	102.11	97.56	64.39	61.85	44.51	61.41
TRPA1	Transient receptor potential cation channel subfamily A member 1	7.12	8.12	6.50	8.78	11.02	3.84	2.07	3.16
VCAM1	Vascular cell adhesion molecule 1	2.43	2.53	3.11	2.75	1.51	1.21	1.38	1.02
VNN1	Vanin 1	8.64	2.39	1.57	1.75	23.55	3.44	2.08	2.45

SD, standard deviation; HC, healthy controls; BL, baseline.

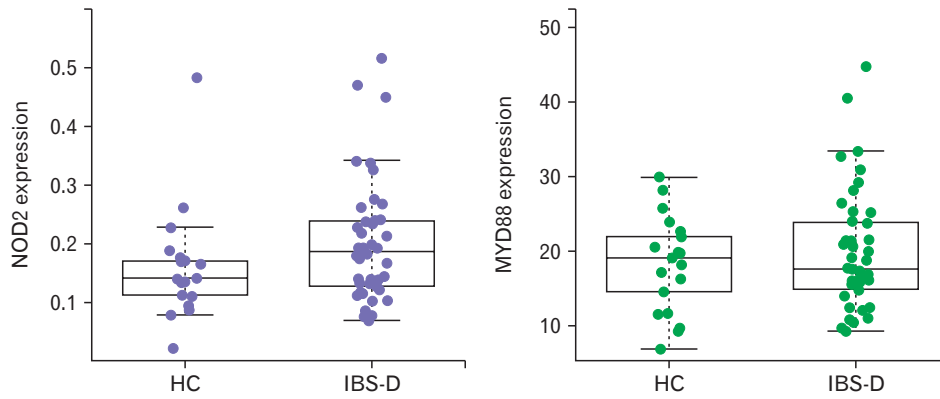
Gene expression measurements were calculated as $2^{-(Ct)}$ using the geometric mean of 4 reference genes described in Materials and Methods section and values are given as arbitrary units.

Supplementary Table 2. Genes Correlating With Toll-like 4 Expression in the Irritable Bowel Syndrome Patient Cohort

Genes	Spearman correlation	q-value
BDKRB2	0.526	0
CMKLR1	0.515	5.97E-08
F11R	0.572	2.17E-06
F2RL1	0.575	1.77E-05
FUT2	0.418	0.002366
MCL1	0.581	1.34E-05
MMP2	0.403	3.12E-06
MYD88	0.639	3.95E-08
OCLN	0.515	6.40E-05
PPARG	0.525	0.00053
TGFBR2	0.516	1.77E-07
TJP1	0.539	1.09E-05
TJP2	0.406	0.00084
TNBP2	0.368	1.06E-05
TNFSF10	0.496	4.04E-06
TNFSF15	0.388	0.000906
TOLLIP	0.426	0.00043
TRPA1	0.552	5.34E-06



Supplementary Figure 1. Estimation of the Toll-like receptor 4 (TLR4) threshold. The distribution of the TLR4 expression values was plotted as a density plot to evaluate the threshold of high and low TLR4 expression. The 1.5 times the standard deviation of the healthy controls (HC) expression values was selected. The threshold value is indicated with a straight line at 2.4 expression value. BL, baseline; MZ, mesalazine group; PL, Placebo group.



Supplementary Figure 2. Mucosal biopsy gene expression at baseline in healthy controls (HC) and patients with diarrhea-predominant irritable bowel syndrome (IBS-D). There was no statistically significant difference in the expression levels of (A) nucleotide-binding oligomerization domain-containing protein 2 (NOD2) or (B) myeloid differentiation factor 88 (MyD88) between HC and IBS-D patients at baseline. The line in the box shows the median expression value, boxes indicate the upper (75th percentile) and lower quartiles (25th percentile) of the data, significant difference between HC and IBS-D patients is indicated with asterisk.

Supplementary Method. Micro-fluidic cards were loaded and analyzed as per manufacturer's instructions. In brief, 60 μL of cDNA was created using 1 μg of RNA and Superscript III reverse transcriptase (Cat. No. 18080-093; Invitrogen, Carlsbad, CA, USA), according to manufacturer's instructions. Fifty microliter of diethyl pyrocarbonate (D5758; Sigma-Aldrich, St. Louis, MO, USA) treated high-performance liquid chromatography grade water and 110 μL of Universal Taqman master mix (P/N 4304437; Applied Biosystems, Foster City, CA, USA) was added to create a final volume of 220 μL . The micro-fluidic gene card has 8 wells, with 2 wells for each of the 4 samples. One hundred microliter of the samples were placed in their corresponding wells and the plate centrifuged twice at 1200 rpm for 1 minute using a Sorvall ST40 centrifuge (Thermoscientific, Loughborough, UK). The plate was sealed with a plate sealer (Model 4331770 Rev A.5; Applied Biosystems) and loaded into the 7900HT Fast Real-Time PCR system analyser (Applied Biosystems).