HOKKAIDO UNIVERSITY

Title	Gut commensals suppress interleukin-2 production through microRNA-200/BCL11B and microRNA-200/ETS-1 axes in lamina propria leukocytes of murine large intestine
Author(s)	Ohsaka, Fumina; Karatsu, Yugo; Kadota, Yoshihiro et al.
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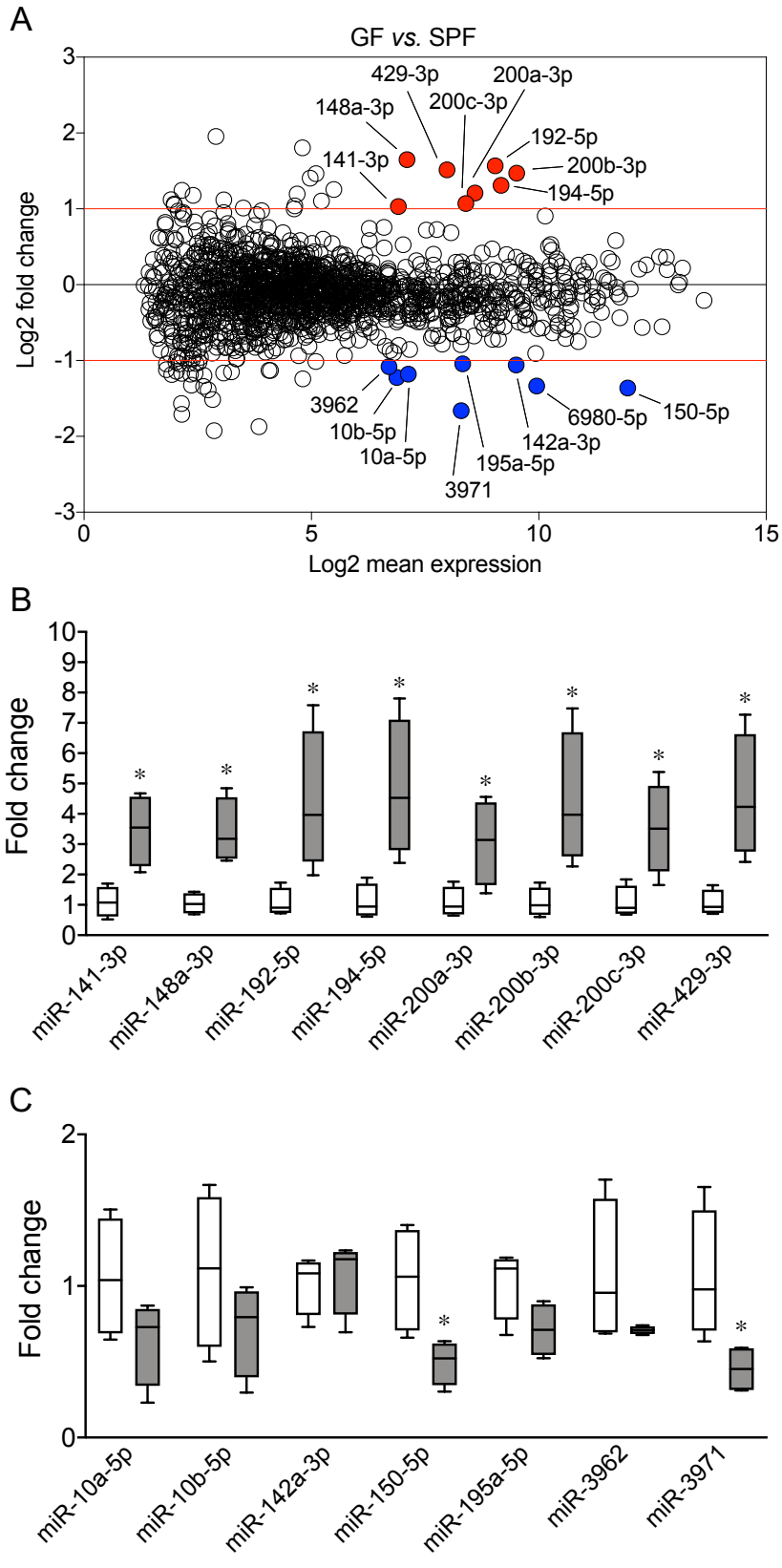


Fig. 1 Ohsaka *et al.*

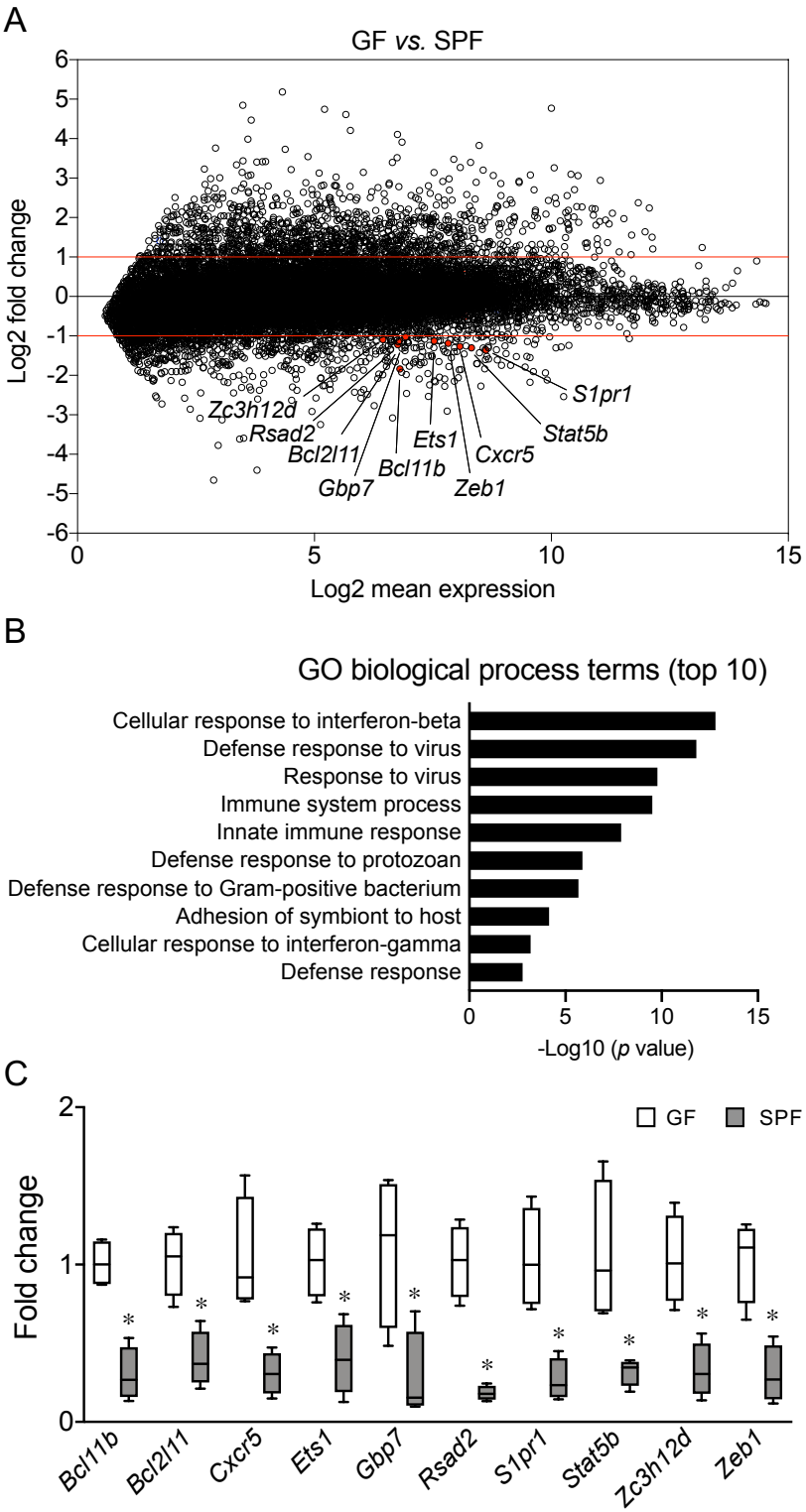


Fig. 2 Ohsaka *et al.*

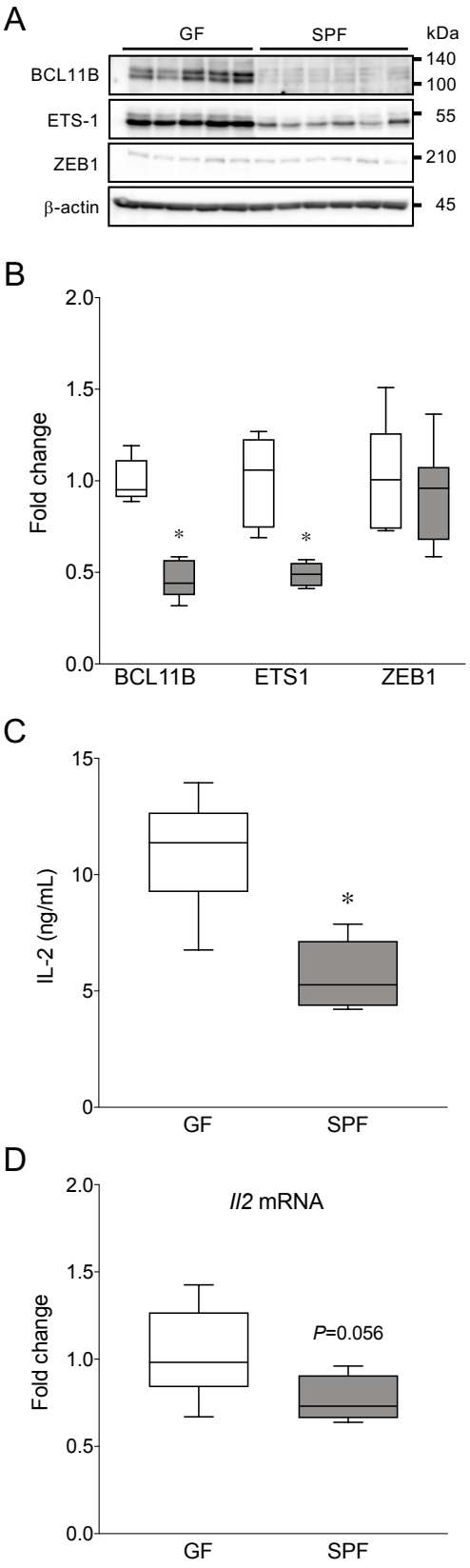


Fig. 3 Ohsaka *et al.*

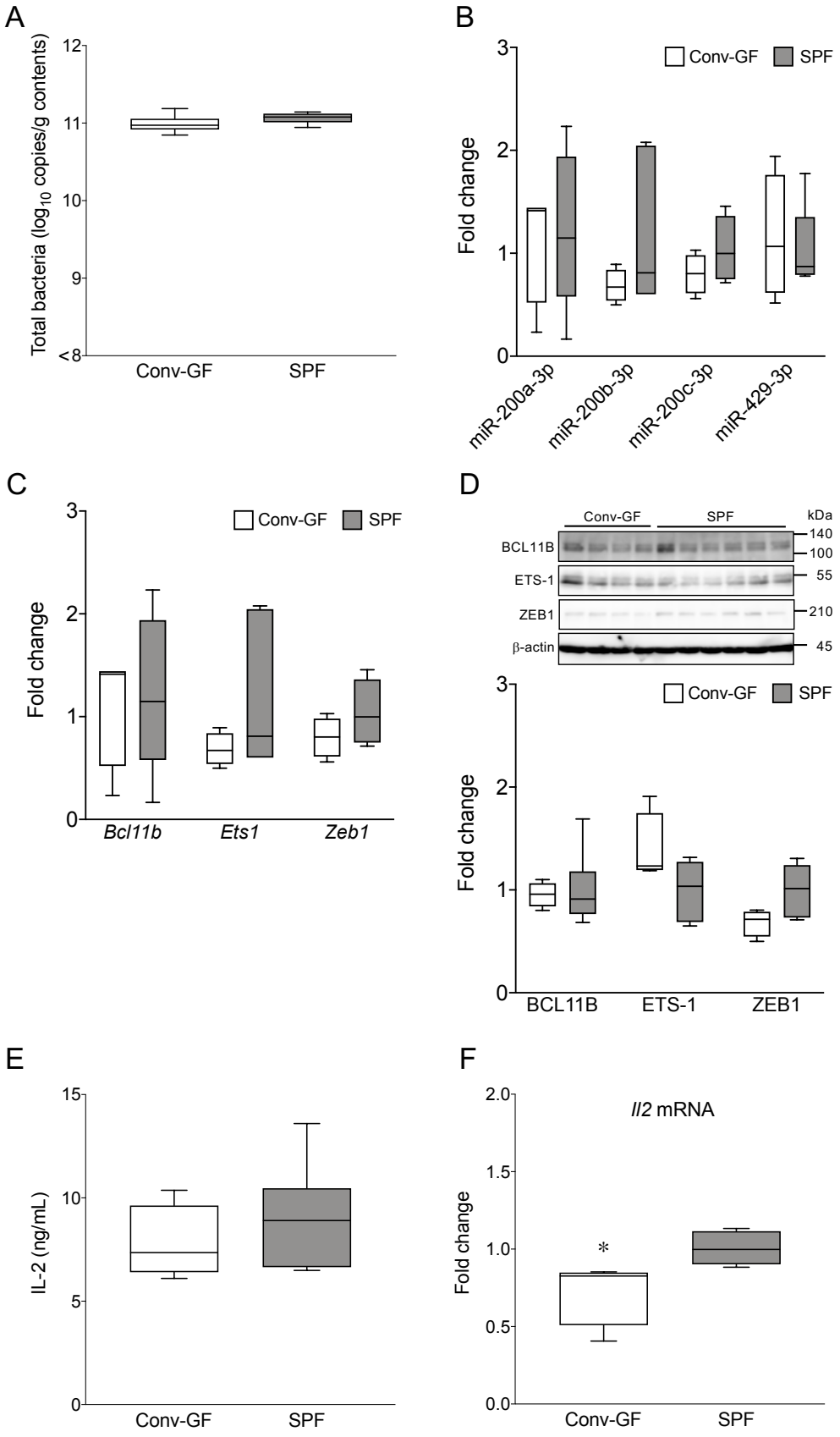


Fig. 4 Ohsaka *et al.*

Supplementary Table 1 Primer sequences for qRT-PCR of miRNA

miRNA	Forward
mmu-miR-10a-5p	TACCCTGTAGATCCGAATTTGTG
mmu-miR-10b-5p	TACCCTGTAGAACCGAATTTGTG
mmu-miR-141-3p	TAACACTGTCTGGTAAAGATGG
mmu-miR-142a-3p	CATAAAGTAGAAAGCACTACT
mmu-miR-148a-3p	TCAGTGCACTACAGAACTTTGT
mmu-miR-150-5p	TCTCCCAACCCTTGTACCAGTG
mmu-miR-192-5p	CTGACCTATGAATTGACAGCC
mmu-miR-194-5p	TGTAACAGCAACTCCATGTGGA
mmu-miR-195a-5p	TAGCAGCACAGAAATATTGGC
mmu-miR-200a-3p	TAACACTGTCTGGTAACGATGT
mmu-miR-200b-3p	TAATACTGCCTGGTAATGATGA
mmu-miR-200c-3p	TAATACTGCCGGGTAATGATGGA
mmu-miR-429-3p	TAATACTGTCTGGTAATGCCGT
mmu-miR-3962	AGGTAGTAGTTTGTACATTT
mmu-miR-3971	CTCCCCACCCCTGTACCAGTGA
cel-miR-39-3p	TCACCGGGTGTAAATCAGCTTG

Supplementary Table 2 Primer sequences for qRT-PCR of mRNA

Gene	Forward	Reverse
Actb	CTGGGACGATATGGAGAAGA	AGAGGCATACAGGGACAACA
Bcl11b	GGCGATGCCAGAATAGATGCCG	CCAGGCCACTTGGCTCCTCTATCTCCAGAC
Bcl2l11	AATGTCTGACTCTGATTCTCGGAC	TCTCAGCAGGCTGCAATTGTCCAC
Cxcr5	GACTCCTTACCACAGTGCACCTT	GGAAACGGGAGGTGAACCA
Ets1	CGATCCAGCTGTGGCAGTT	ATCTCCTGGCCACCTCATCT
Gbp7	TTGAGGAAATGCCAGAGGACCAGT	GTCTCCACTATTGATAGCATCCACG
Il2	TGATGGACCTACAGGAGCTCCTGAG	GAGTCAAATCCAGAACATGCCGCAG
Rsad2	TGCTGGCTGAGAATAGCATTAGG	GCTGAGTGCTGTTCCCATCT
S1pr1	ATGGTGTCCACTAGCATCCC	CGATGTTCAACTTGCCTGTGTAG
Stat5b	GGACTCCGTCCTTGATACCG	TCCATCGTGTCTTCCAGATCG
Zc3h12d	TGGCAGCAATGTGGCTATGA	AGCACGTGTTGCTCTCTGAT
Zeb1	GCTGGCAAGACAACGTGAAAG	GCCTCAGGATAAATGACGGC

Supplementary Table 3 Primary antibodies for western blot analysis

Target	Cat#	Source	Species raised in; clonality	Dilution	RRID*
BCL11B	55414-1-AP	Proteintech (Rosemont, IL)	Rabbit; poly	1:300	AB_11182609
ETS-1	sc-55581	Santa Cruz Biotechnology (Santa Cruz, CA)	Mouse; mono	1:300	AB_831289
ZEB1	NBP1-05987	Novus Biologicals (Littleton, CO)	Rabbit; poly	1:1,000	AB_2273178
β-actin	sc-47778	Santa Cruz Biotechnology	Rabbit; poly	1:200	AB_2714189

* RRID, Research Resource Identifier

Supplementary Table 4 Secondary antibodies for western blot analysis

Target	Cat#; label	Source	Species raised in; clonality	Dilution	RRID*
Rabbit IgG	HAF008; HRP	R&D Systems (Minneapolis, MN)	Goat; poly	1:1,000	AB_357235
Mouse IgG	HAF007; HRP	R&D Systems	Goat; poly	1:1,000	AB_357234

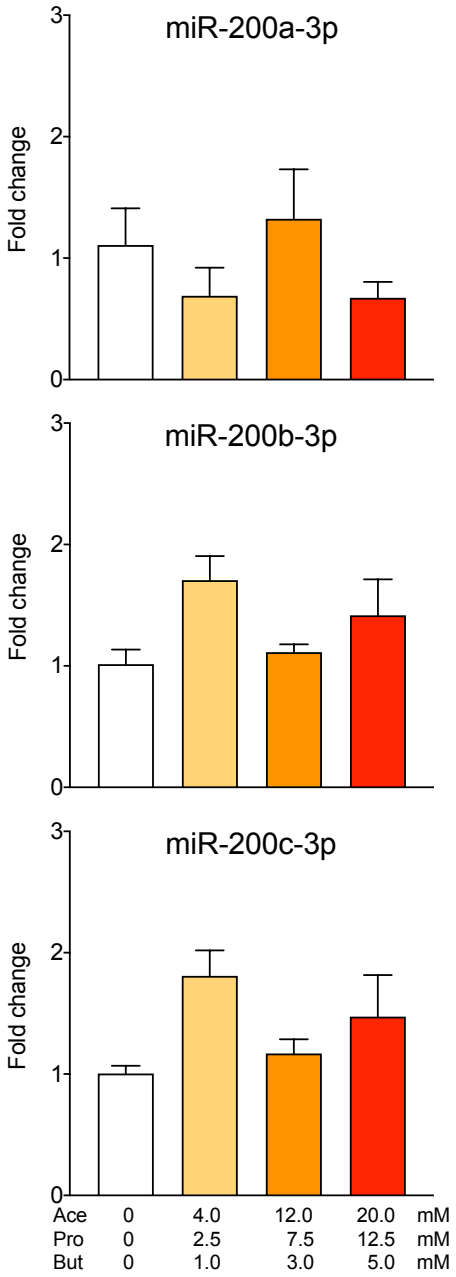
* RRID, Research Resource Identifier

Supplementary Table 5 miRNAs increased by gut microbiota and their predicted target genes

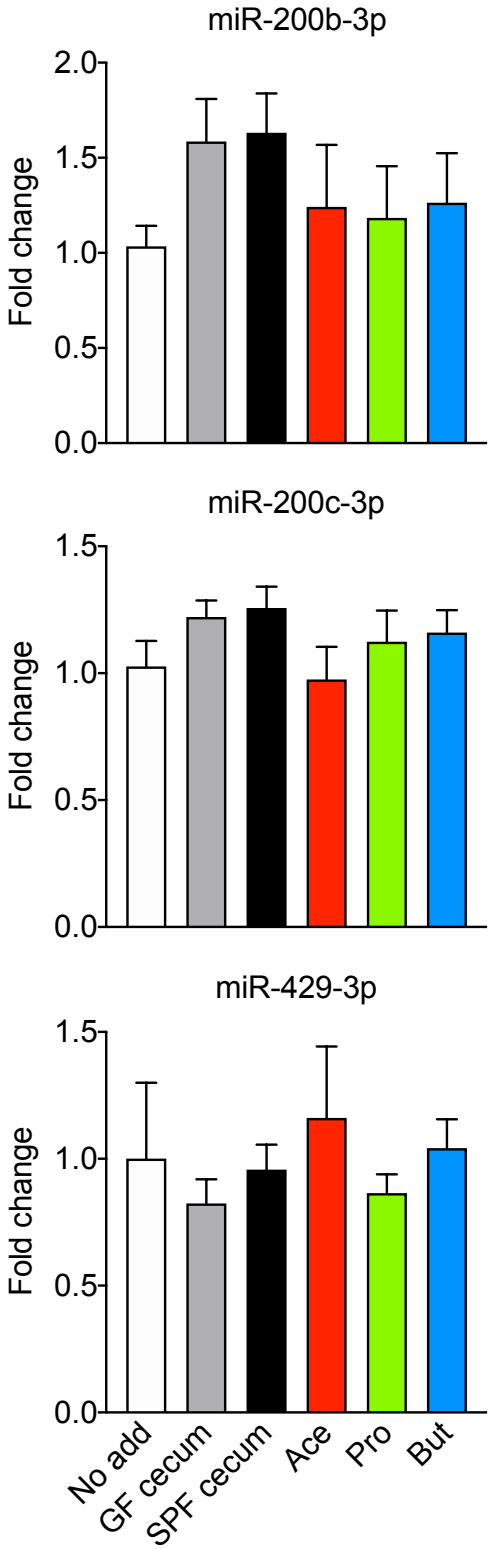
miRNA*	Target gene**			
mmu-miR-148a-3p	*Bcl2l11*	*S1pr1*		
mmu-miR-192-5p	*Cxcr5*	*Rsad2*	*S1pr1*	
mmu-miR-194-5p	*Stat5b*	*Zc3h12d*	*Zeb1*	
mmu-miR-141-3p/200a-3p	*Bcl11b*	*Gbp7*	*Stat5b*	*Zeb1*
mmu-miR-200b-3p/200c-3p/429-3p	*Bcl11b*	*Ets1*	*Zeb1*	

* These miRNAs show higher levels in SPF mice than in GF mice as determined by qRT-PCR (**Fig. 1B**).

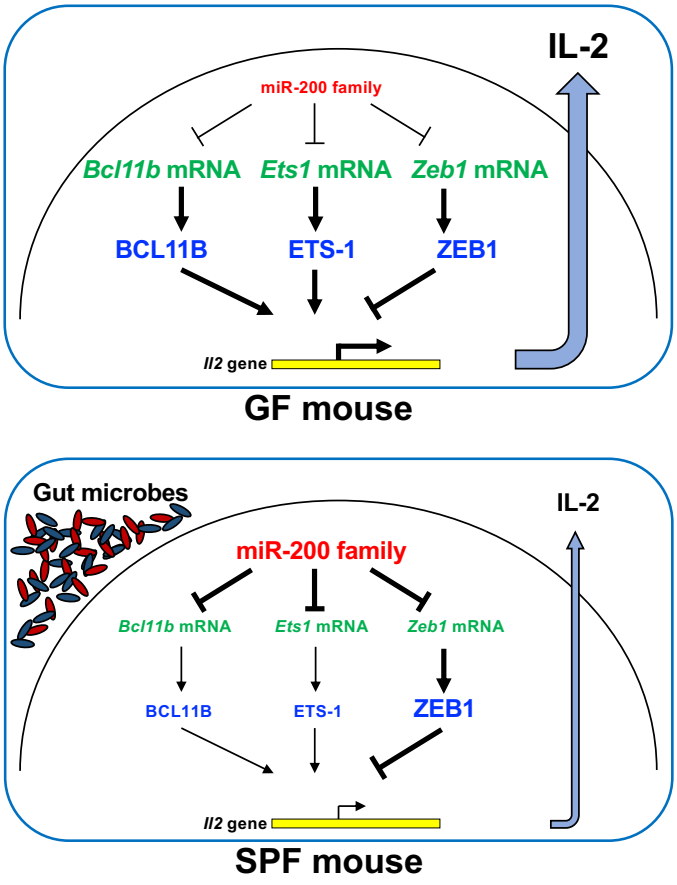
** GO terms of these genes are associated with infection defense and the immune system as shown in **Fig. 2B**, and lower levels of their mRNAs in SPF mice than in GF mice were shown by qRT-PCR (**Fig. 2C**).



Supplementary Figure 1 Expression of miRNAs in large intestinal lamina propria leukocytes cultured in the presence of short-chain fatty acids. The cells were isolated from large intestine of specific-pathogen free BALB/c mice and cultured with a mixture of different concentrations of sodium acetate, sodium propionate and sodium butyrate (Ace, Pro and But, respectively) for 24 h. Relative miRNA levels were determined by qRT-PCR. Data are expressed as means ± SEM (n = 3) and are shown relative to levels in the negative control (white bar), which were set to 1.



Supplementary Figure 2 Expression of miRNAs in large intestinal lamina propria leukocytes cultured in the presence of fecal extracts or short-chain fatty acids. The cells were isolated from large intestine of germ-free BALB/c mice and cultured with fecal extracts (200 μg protein/mL) prepared from cecal contents of germ-free (GF cecum) or specific-pathogen free (SPF cecum) mice, or 0.1 mM acetate (Ace), propionate (Pro) or butyrate (But) upon stimulation with 25 ng/mL PMA and 1 μg/mL ionomycin for 24 h. Relative miRNA levels were determined by qRT-PCR. Data are expressed as means ± SEM (n = 6) and are shown relative to levels in the negative control (No add; white bar), which were set to 1.



Graphical abstract