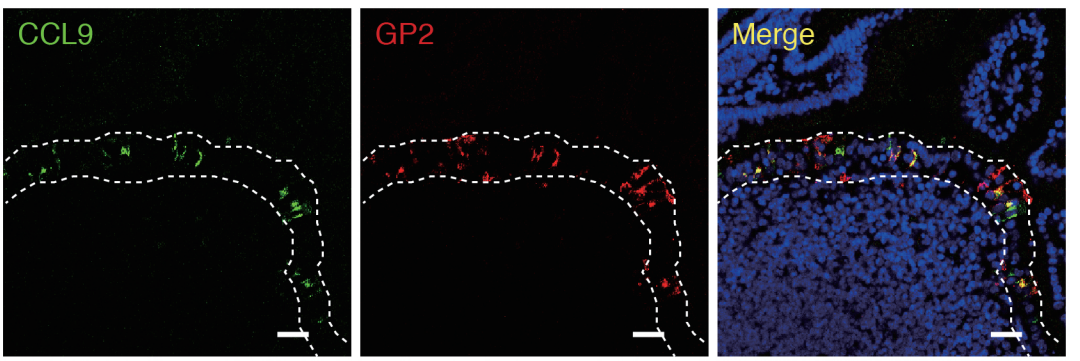
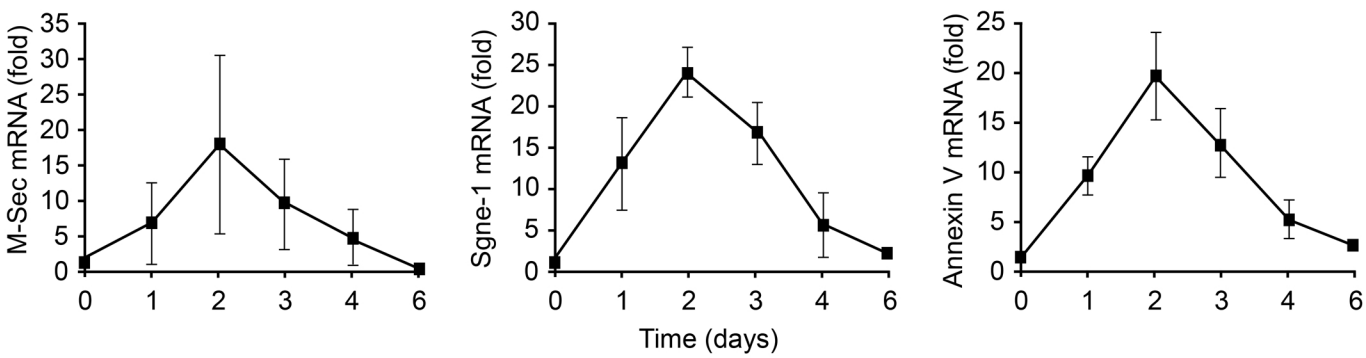
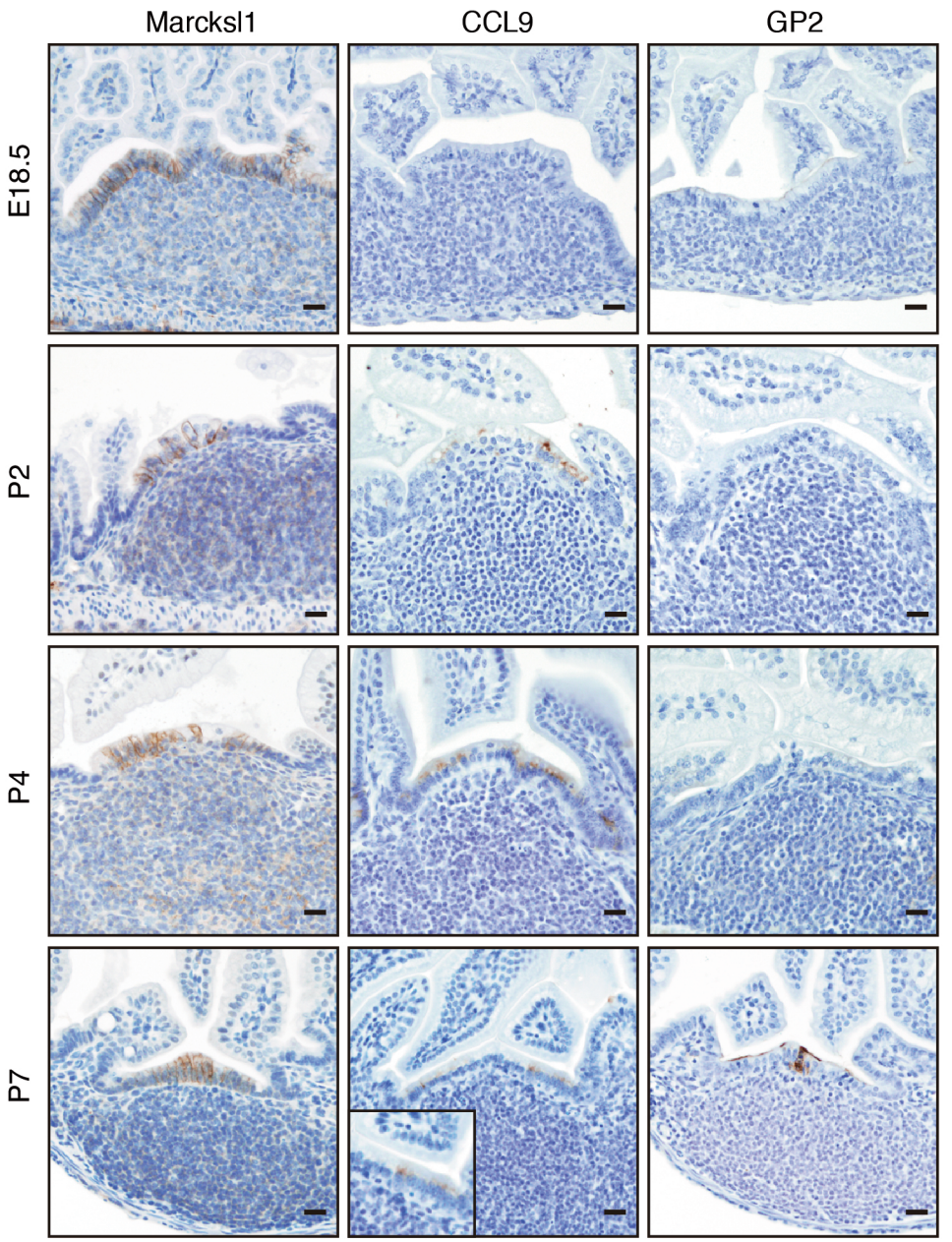


Supplementary Figure 1. Coexpression of CCL9 and GP2 in adult PP M cells. The preferential expression of CCL9 in PP M cells of adult mice was demonstrated by dual staining with anti-CCL9 antibody (green) and anti-GP2 antibody (red). Counterstaining was performed with DAPI (blue). Dotted lines depict the position of FAE. Scale bar: 20 μm. Data are representative of three independent experiments.



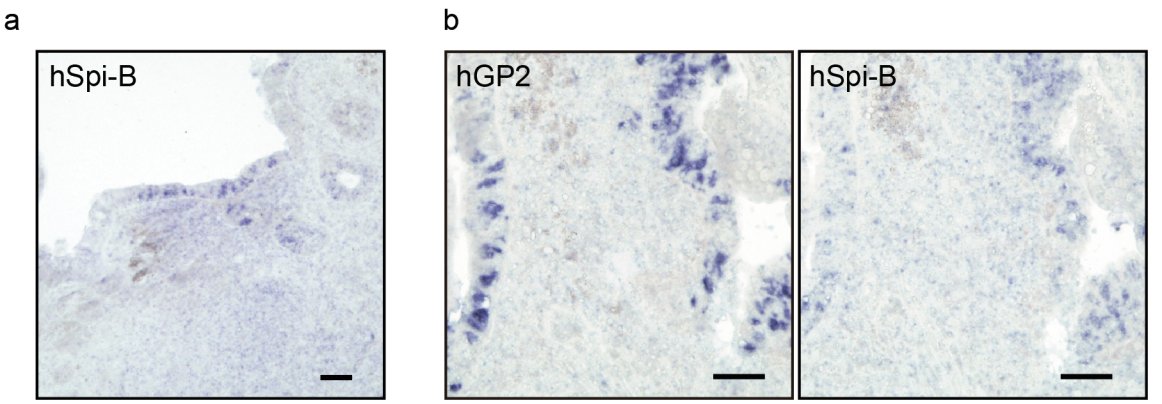
Supplementary Figure 2. Kinetics of expression of additional M-cell markers in RANKL-induced M-cell differentiation.

The kinetics of expression of M-Sec, Sgne-1 and Annexin V in the mouse intestinal epithelial cells upon GST-RANKL treatment was assessed by qPCR. Data represent fold change compared to the normalized value of expression of each transcript in epithelial cells from untreated mice. All samples were normalized to the expression level of GAPDH (n = 3). Error bars indicate s.d. Data are representative of three independent experiments.



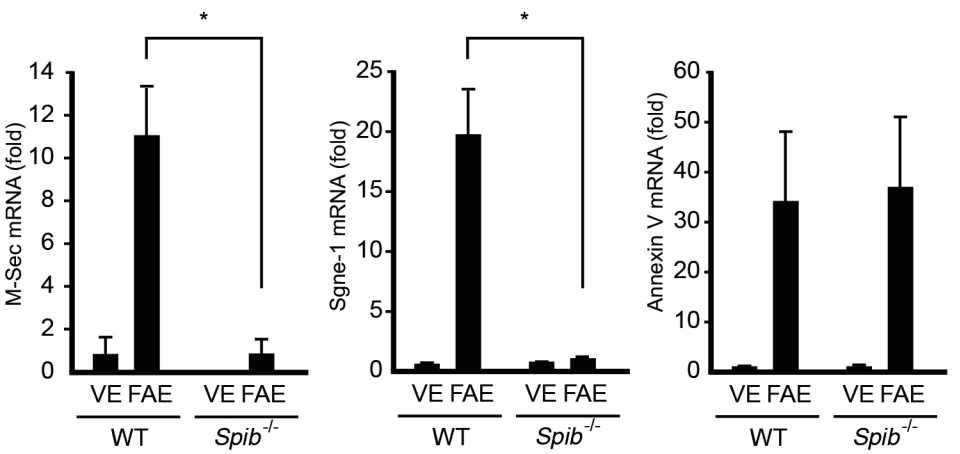
Supplementary Figure 3. Sequence of expression of M-cell markers during perinatal PP development.

PPs were isolated from wild-type mice at embryonic day 18.5 (E18.5), 2 and 4 days postpartum, and 1 week postpartum (P2, P4 and P7). Sections of PPs were immunostained with anti-Marcksl1, CCL9, and GP2 antibodies. Consequently the sections were incubated with biotinylated secondary antibodies and HRP-conjugated streptavidin. Finally the distribution of proteins were visualized DAB substrate was used for the visualization. Scale bar: 20 µm. Data are representative of four independent experiments



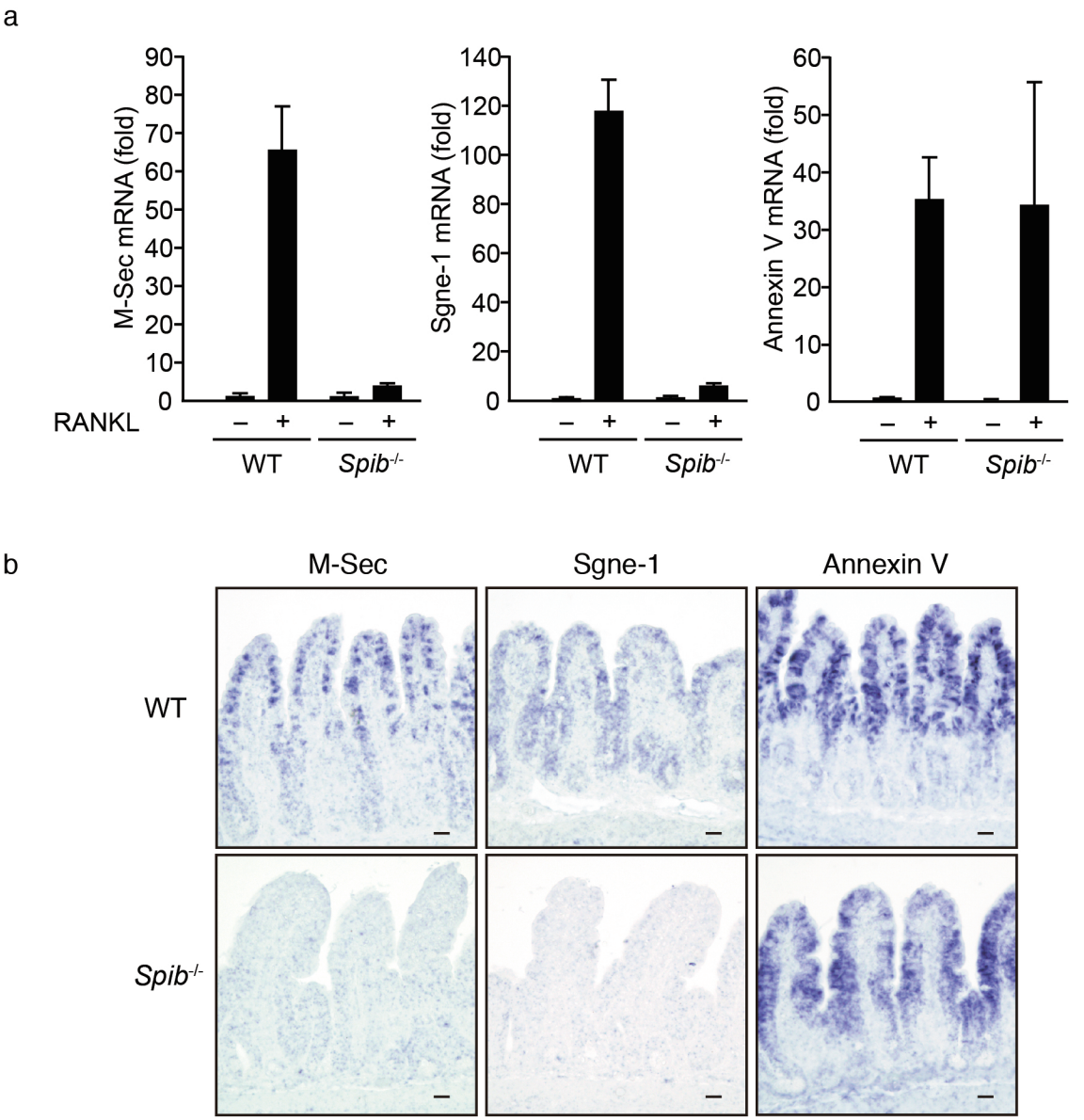
Supplementary Figure 4. The expression of Spi-B in human M cells

(a) An ISH probe for human Spi-B mRNA exhibited M-cell like distribution in the human FAE of PPs. The signal of mRNA was visualized by NBT-BCIP substrate (blue). Scale bar = 50 μm. **(b)** Serial sections of human PPs demonstrated that Spi-B mRNA was associated with GP2 positive human M cells. Bars = 50 μm. Data are representative of two independent experiments.

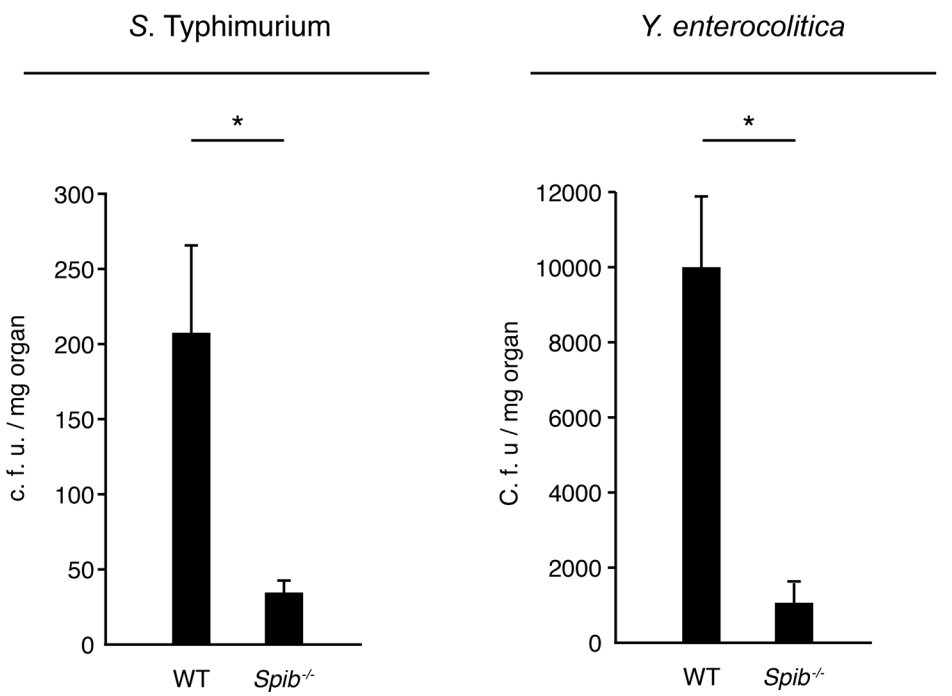


Supplementary Figure 5. The expression of additional M-cell markers in *Spib*$^{-/-}$ and wild-type mice.

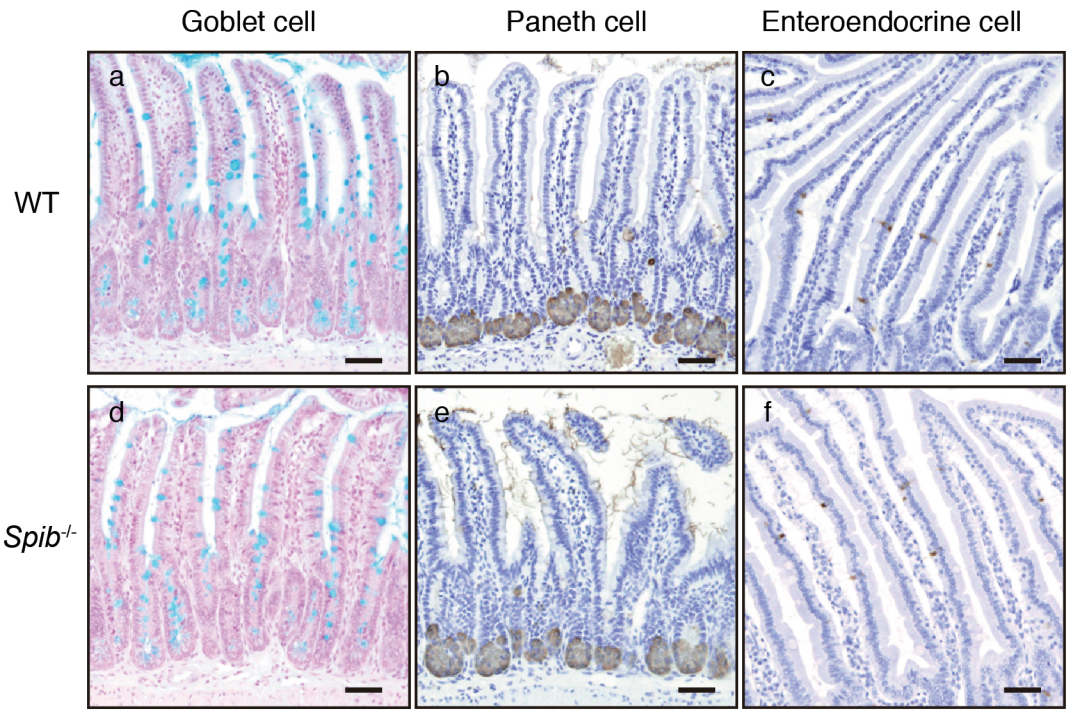
Comparison of M-cell marker expression in FAE and villous epithelium (VE) between wild-type and *Spib*$^{-/-}$ mice by qPCR analysis. Data represent fold change compared to the normalized value of expression of each transcript in VE from wild-type mice. Error bars indicate s.d. (One-way ANOVA with Bonferroni post hoc test, $n = 3$). *, $P<0.01$. Data are representative of two independent experiments.



Supplementary Figure 6. Expression analysis of additional M-cell markers during RANKL-induced M-cell differentiation in *Spib*$^{-/-}$ and wild-type mice. **(a)** Comparative analysis of 3 additional RANKL-induced M-cell markers not presented in the main figures in wild-type and *Spib*$^{-/-}$ and wild-type mice. Data represent fold change compared to the normalized value of expression of each transcript in villous epithelial cells from untreated wild-type mice. All samples were normalized to the expression level of GAPDH. Error bars indicate s.d. (n = 3). **(b)** ISH of M-Sec, Sgne-1 and Annexin V mRNA in villous epithelium (VE) of *Spib*$^{-/-}$ and wild-type mice treated for 3 days with RANKL. Scale bar: 20 μm. Data are representative of two independent experiments.

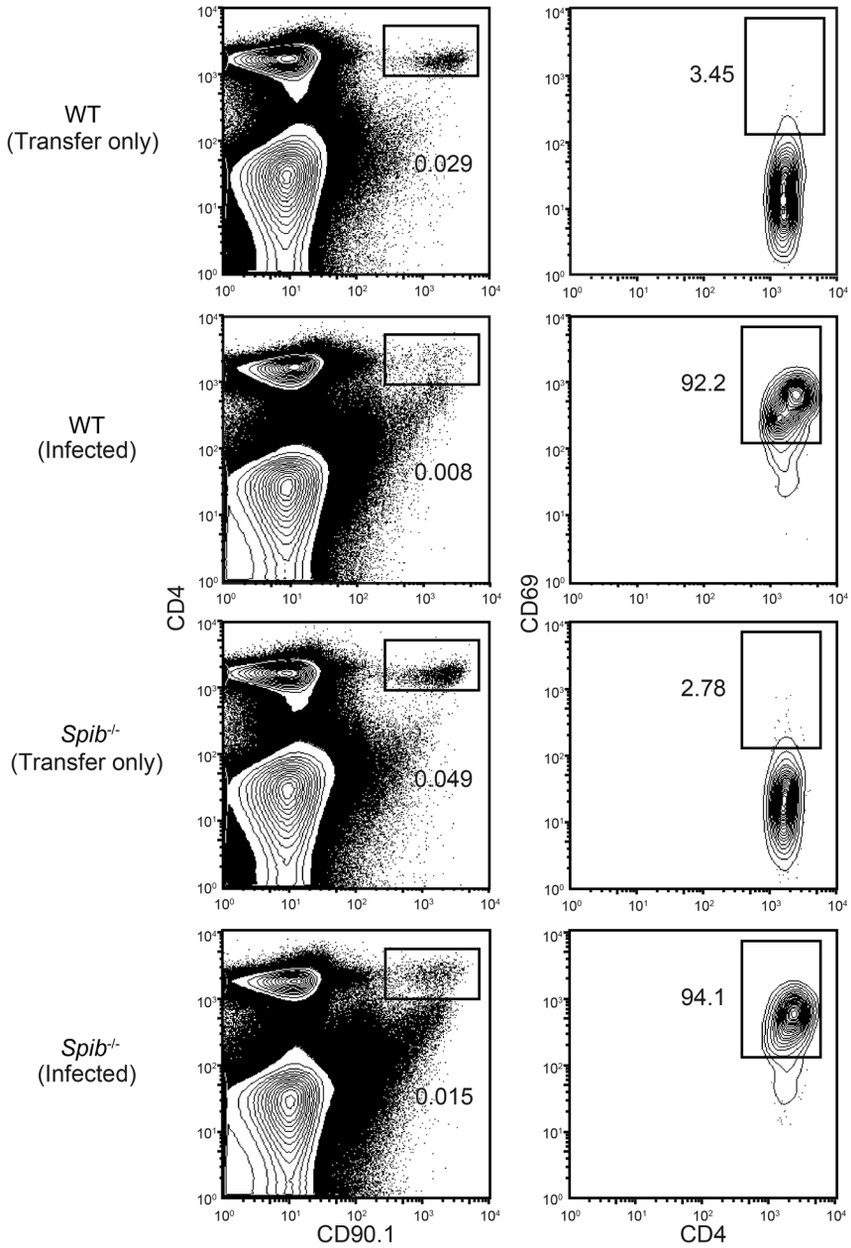


Supplementary Figure 7. Impairment of bacterial translocation in PPs of *Spib*$^{-/-}$ mice
Wild-type or *Spib*$^{-/-}$ mice were orally infected with 5 x 10^7 c.f.u. of *S.* Typhimurium or 2 x 10^8 c.f.u. of *Y. enterocolitica*. PPs were collected 24 h after the infection to prepare tissue homogenates. The colonies of culturable bacteria in the tissue homogenates were counted and normalized to the weight of tissue samples, and are shown as colony-forming units. Error bars indicates s.e. Mann–Whitney U-test were used for calculating a significant difference in c. f. u. of *S.* Typhimurium (wild-type: $n = 10$, *Spib*$^{-/-}$: $n = 8$), and Student' s *t*-test were used for that of *Y. enterocolitica* (wild-type: $n = 10$, *Spib*$^{-/-}$: $n = 9$). *, $P<0.01$. Data are representative of two independent experiments.



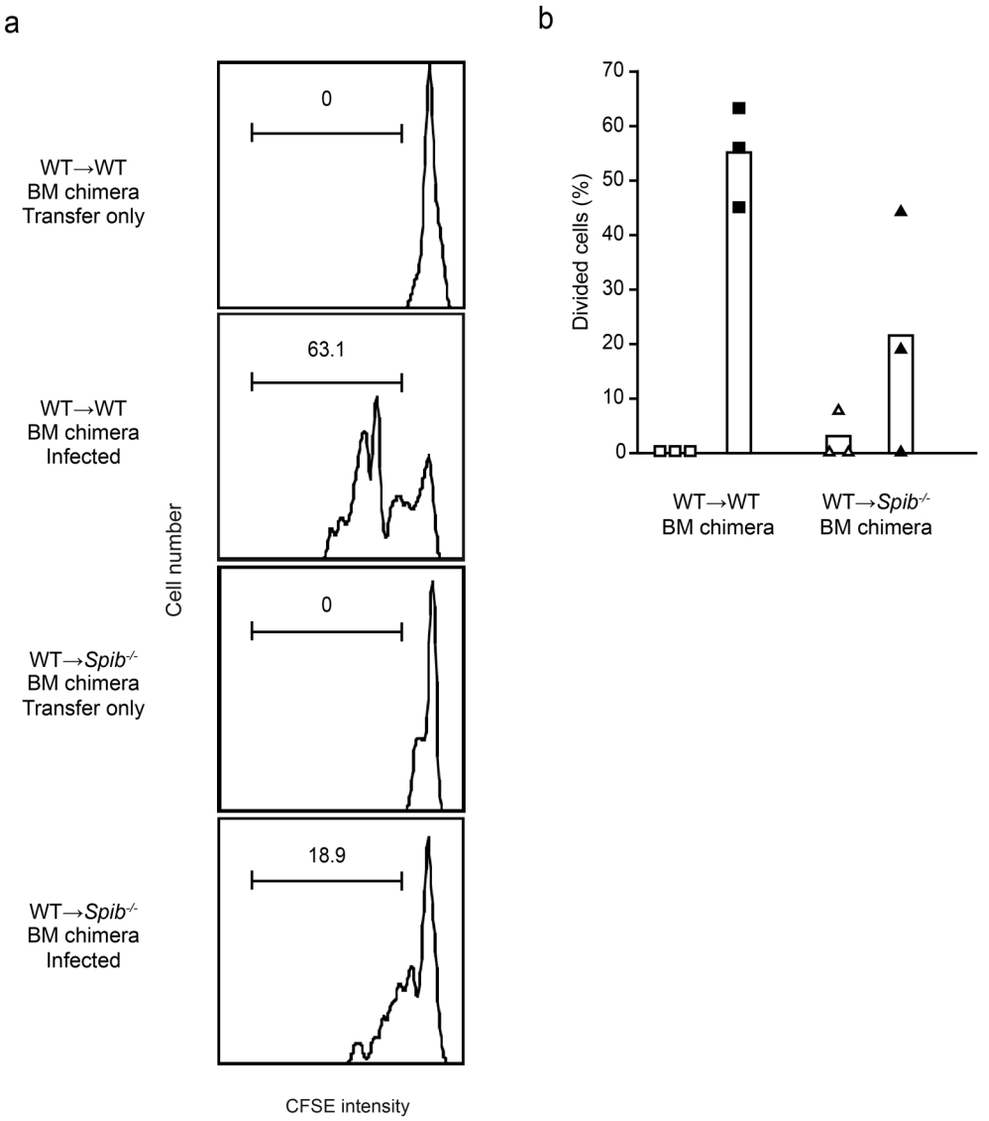
Supplementary Figure 8. Intestinal epithelial cells of the secretory lineage develop normally in *Spib*$^{-/-}$ mice

To observe the distribution of goblet cells, Alcian blue staining was performed (cyan in **a** and **d**). Counterstaining was performed with nuclear fast red (pink). For the detection of Paneth or enteroendocrine cells, small intestines were immunostained with anti-lysozyme antibody (brown in **b** and **e**) or anti-chromogranin A antibody (brown in **c** and **f**), respectively. Scale bar: 50 μm. Data are representative of two independent experiments.



Supplementary Figure 9. Normal activation status of transferred SM1 T cells in systemic immune response to *S.* Typhimurium in *Spib*$^{-/-}$ mice

Wild-type (WT) or *Spib*$^{-/-}$ mice were adoptively transferred with SM1 T cells, and intraperitoneally inoculated with *S.* Typhimurium at 24 h after transfer. Left, dot plots of CD90.1 and CD4 expressions on splenocytes from uninfected (Transfer only) and infected (Infected) mice at 24 h after infection. Rectangles in the left panels indicate the gate for CD90.1^{+} SM1 cells. The percentage of the gated cells in the total cells is indicated below. Right, contour plots of CD4 and CD69 expressions on the cells in the gate of the left panels. Rectangles indicate the CD69^{+} activated cells among the gated SM1 cells. The percentage of the gated cells among all SM1 cells is indicated. Data are representative of three independent experiments



Supplementary Figure 10. The defect of Spi-B in M cells but not in immune cells impaired immune response to *S.* Typhimurium

(a) Bone marrow (BM) cells from wild-type (WT) CD45.1 congenic mice were transferred into irradiated WT or *Spib*$^{-/-}$ mice. After the reconstitution of hematopoietic cells, CFSE-labeled SM1 T cells were transferred into these BM chimera mice followed by oral inoculation with *S.* Typhimurium (5 x 10^9 c.f.u.) at 24 h after transfer. Control mice received SM1 T cells, but were not inoculated with *S.* Typhimurium (transfer only). Three days after infection, PP cells were collected and the dilution of the CFSE label in gated SM1 T cells was examined. Representative histograms from mice in each group are shown. The numbers in histograms represent the percentage of divided CD90.1$^+$ CD4$^+$ SM1 T cells. **(b)** Bar graph shows the mean percentage of divided SM1 T cells in each group (n = 3). Open boxes and triangles indicate uninfected (transfer only) WT (WT→WT) and *Spib*$^{-/-}$ (WT→*Spib*$^{-/-}$) BM chimeras, respectively. Filled boxes and triangles indicate those mice orally inoculated with *S.* Typhimurium. Data are representative of two independent experiments.

Supplementary Table 1. List of antibodies used in this study.

Primary antibodies for immunohistochemistry

Antibody	Host	Company	Clone	Specificity	Dilution
Marcksl1*	Rabbit	Proteintech	Polyclonal	Mouse M cell	1: 200
Mouse CCL9	Goat	R&D Systems	Polyclonal	Mouse M cell	1: 100
Mouse GP2	Rat	Prepared at our lab.	2F11-C3	Mouse M cell	1: 100
Chromogranin A*	Rabbit	ImmunoStar	Polyclonal	Enteroendocrine cell	1: 500
Lysozyme	Rabbit	DakoCytomation	Polyclonal	Paneth cell	1: 1000
Mouse Spi-B*	Sheep	R&D Systems	Polyclonal	Mouse M cells	1: 500

*For the staining of Marcksl1, Chromogranin A, and Spi-B, antigen retrieval was performed with citrate buffer (pH7.0) (121ºC, 5 min).

Secondary antibodies for immunohistochemistry and whole mount staining

Antibody	Host	Label	Company	Dilution
Anti-rabbit IgG	Donkey	Biotin	Jackson ImmunoResearch	1: 400
Anti-rabbit IgG	Donkey	DyLight 549	Jackson ImmunoResearch	1: 400
Anti-goat IgG	Donkey	Biotin	Jackson ImmunoResearch	1: 400
Anti-goat IgG	Donkey	Alexa 555	Invitrogen	1: 400
Anti-rat IgG	Donkey	Biotin	Jackson ImmunoResearch	1: 400
Anti-rat IgG	Donkey	DyLight 549	Jackson ImmunoResearch	1: 400
Anti-rat IgG	Donkey	DyLight 649	Jackson ImmunoResearch	1: 400
Anti-rat IgG	Goat	Alexa 488	Invitrogen	1: 400
Anti-sheep IgG	Donkey	Alexa 555	Invitrogen	1: 400

Antibodies for flow cytometry

Antibody	Label	Clone	Company	Dilution
CD4	PE-Cy7	GK1.5	eBioscience	1: 400
CD90.1	FITC	HIS51	eBioscience	1: 100
CD69	PE	H1. 2F3	eBioscience	1: 200

Supplementary Table 2. Primer sequences for realtime PCR

Mouse GP2 forward 5′-GATACTGCACAGACCCCTCCA-3′ (ref. 6)
Mouse GP2 reverse 5′-GCAGTTCCGGTCATTGAGGTA-3′ (ref. 6)

Mouse Marcksl1 forward 5′-TTTTGCCCTCCTGTGGATTCT-3′
Mouse Marcksl1 reverse 5′-CCACTAGGCACAGCACAAGAGA-3′

Mouse Annexin V forward 5′-TTTCCGTTGCACGGAGTTGT-3′
Mouse Annexin V reverse 5′-TTTCCTGGCGCTGAGCATT-3′

Mouse Sgne-1 forward 5′-ACGGTTAAAAATGGCCTCAAGG-3′
Mouse Sgne-1 reverse 5′-AAGGACCCAGATGCTGAAGACC-3′

Mouse CCL9 forward 5′-TACTGCCCTCTCCTTCCTCA-3′
Mouse CCL9 reverse 5′-TTGAAAGCCCATGTGAAACA-3′

Mouse Spi-B forward 5′-AGCGCATGACGTATCAGAAGC-3′
Mouse Spi-B reverse 5′-GGAATCCTATACACGGCACAGG-3′

Mouse M-Sec forward 5′-GTGCAGAACCTCTACCCCAATG-3′
Mouse M-Sec reverse 5′-TGGAGAATGTCGATGGCCA-3′

Mouse GAPDH forward 5′-TGTGTCCGTCGTGGATCTGA-3′
Mouse GAPDH reverse 5′-TTGCTGTTGAAGTCGCAGGAG-3′

Supplementary Table 3. Primer sequences for ISH probe

Mouse Spi-B (EcoRV) forward 5′-GTGTGAATCCCACCATGCTTGCTCTGG-3′
Mouse Spi-B (NotI) reverse 5′-GAGAGCGGCCGCGTGCTCAGACATGCCG-3′

Mouse Sgne-1 (BamHI) forward 5′-ATTGGATCCAGCATTCGCTTATAGTCC-3′ (ref. 8)
Mouse Sgne-1 (XhoI) forward 5′-TATCTCGAGAAGTGGGGGACAGATTTC-3′ (ref. 8)

Mouse M-Sec (BamHI) forward 5′-ATAGGATCCATGTCTGAGGCGTCCTCT-3′
Mouse M-Sec (SalI) reverse 5′-TAAGTCGACAGTAACCTTTATAAGGGAGAACAG-3′

Mouse Annexin V (EcoRV) forward 5′-GATATCAACCTGTTGACATCCCGAAG-3′
Mouse Annexin V (NotI) reverse 5′-GCGGCCGCTCTCTGCAAGGTAGGCAGGT-3′

Human Spi-B (EcoRI) forward 5′-CTGAATTCCCACACTTCAGCTGTCTGTA-3′
Human Spi-B (NotI) reverse 5′-TTGCGGCCGCTCGAACTGGTAGGTGAGCTT-3′

Human GP2 (BamHI) forward 5′-CGCGGATCCGCGATAAAAACATGAGCGGC-3′ (ref. 6)
Human GP2 (XhoI) reverse 5′-CCGCTCGAGCTCTCCAGAATGTTCCTGCAG-3′ (ref. 6)