

Supplementary file

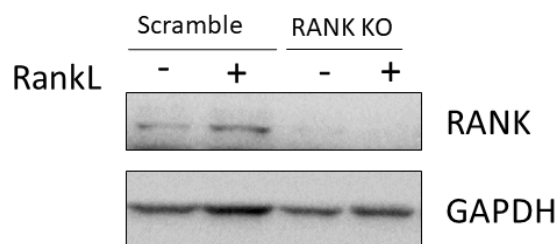


Figure S1. Immunoblot analysis of CRISPR generated RANK KO. RANK KO validation by immunoblot analysis of RANK KO and Scrambled intestinal organoids generated by CRISPR-Cas9 genome editing in C57BL/6j intestinal organoids. These organoids were grown with and without RankL 100ng for 4 days. Data from Rank KO organoids also published in 10.1016/j.jcmgh.2021.05.014 .

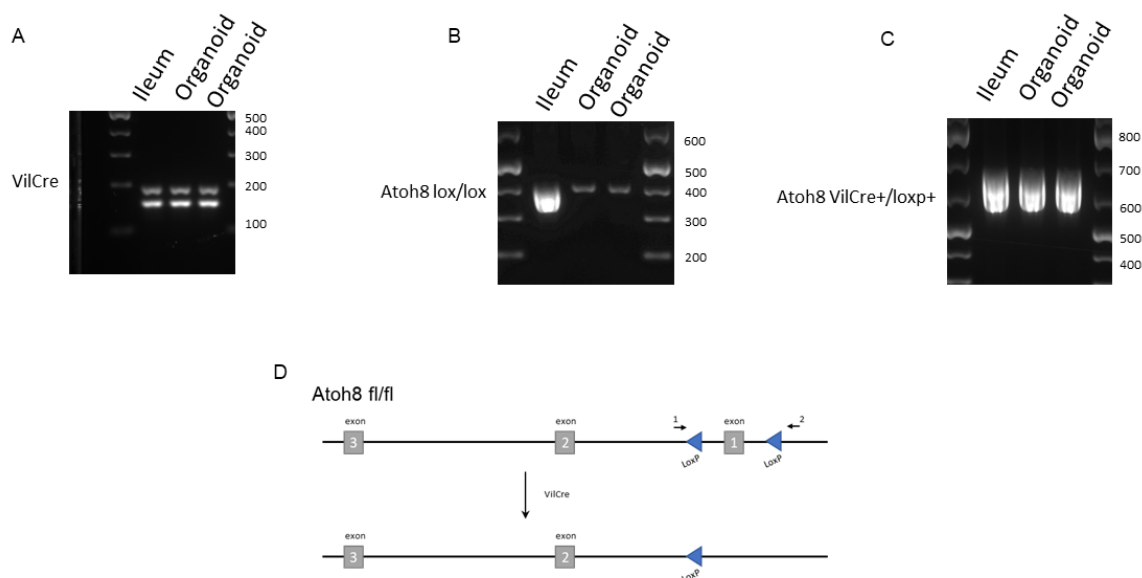


Figure S2. Representative genotyping PCR amplification of intestinal *VilCre*, *Atoh8 flox/flox* wild-type and *Atoh8 VilCre/loxp*. **A)** PCR for detection of *VilCre* recombinase is shown. Upper band: internal control (182 bp). Lower band: *VilCre* transgene (150 bp). 100 bp molecular marker brightest line corresponds to 500 bp. **B)** Floxed allele 400 bp. **C)** Genotyping of *Atoh8 lox/VilCre* 600bp. **D)** Scheme and representative PCR amplification verifying Cre-mediated recombination of the *Atoh8* floxed allele in the intestine.

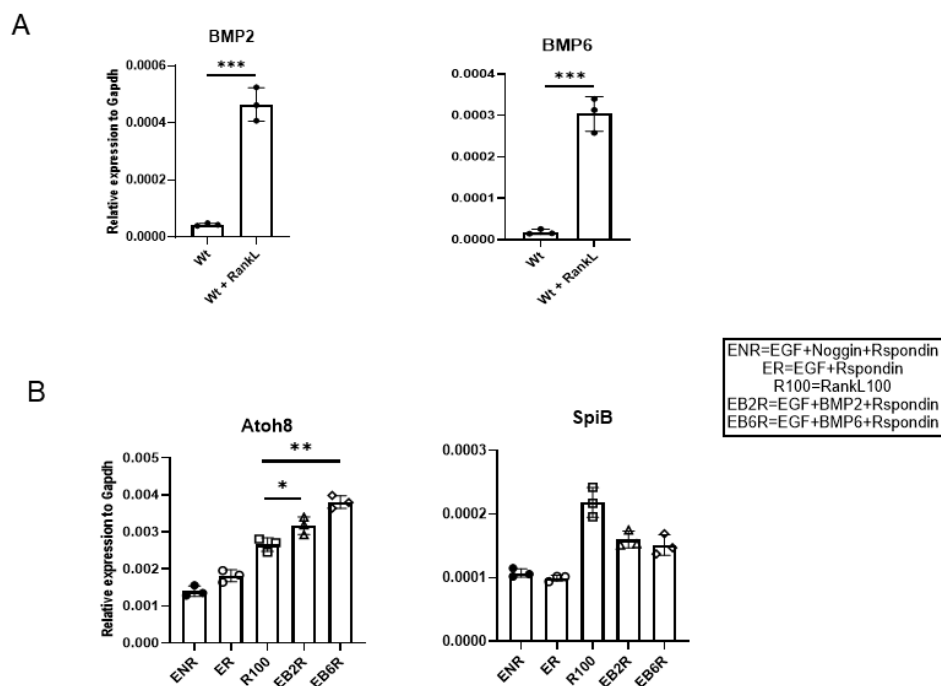


Figure S3. Atoh8 signaling is mediated by RankL/BMP2-BMP6 signaling. **(A)** Organoids from small intestinal crypts of wildtype mice were cultured with or without RANKL for 3 d. **(A)** qPCR analysis of M cell-associated genes expressed in the organoid cultures. Values are presented as the mean \pm SD; *** $p < 0.005$; unpaired two-tailed Student's t test, $n = 3$. Data are representative of two independent experiments. **(B)** Organoids isolated Wt mice were grown for 3 days in EGF, Noggin, Rspondin media, EGF, Rspondin media, 100ug of Rankl media, Egf, BMP2 Rspondin media and Egf, BMP6, Rspondin media. Values are presented as the mean \pm SD; *, $p < 0.05$, **, $p < 0.001$; unpaired two-tailed Student's t test, $n = 3$. Data are representative of two independent experiments.

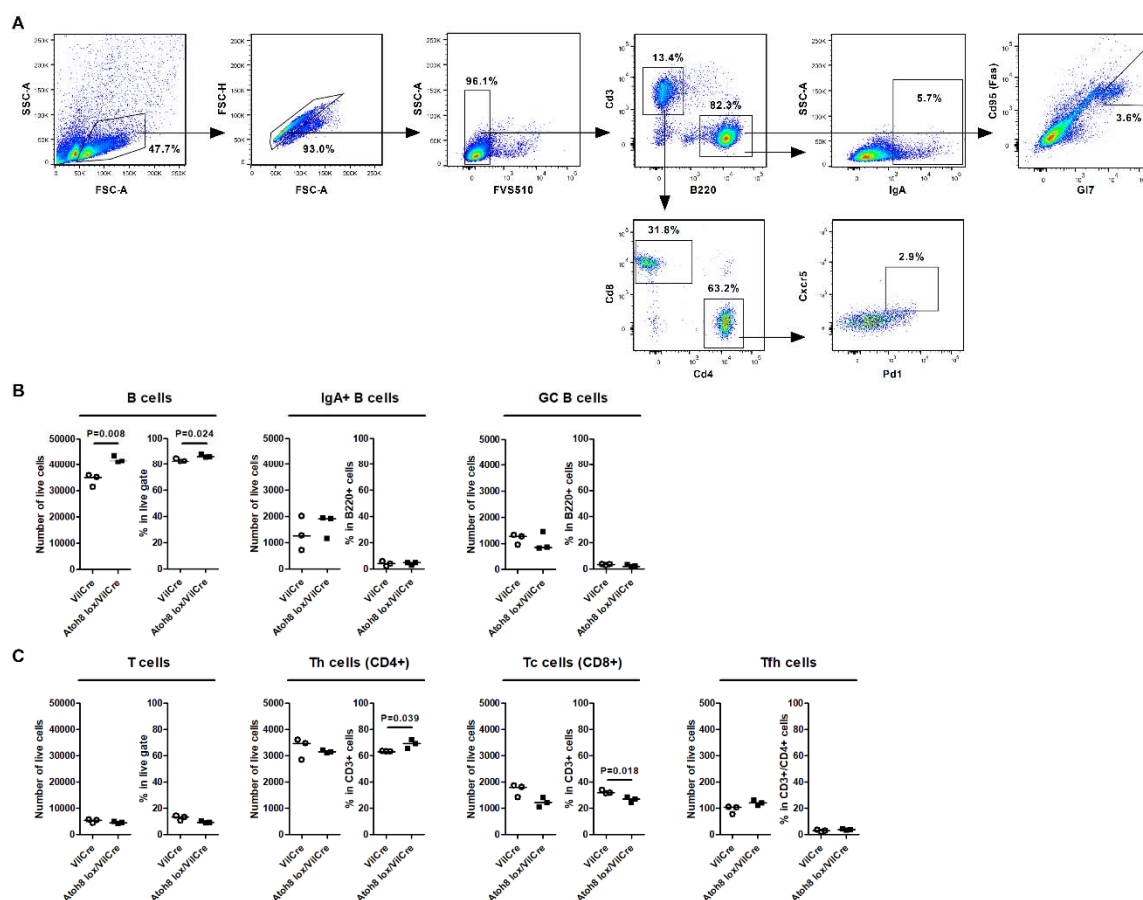


Figure S4. The loss of *Atoh8* did not change the composition of lymphoid cells in 10-week-old mice. **(A)** Gating scheme for analysis of B and T cell populations in *VilCre* and *Atoh8 lox/VilCre* ileal PPs. **(B)** (CD3 ϵ -B220+) cells were analyzed for IgA+ B and CD95+GL7+ GC B cells. T (CD3 ϵ +B220-) cells were analyzed for total CD4+ Th, CD8 α + cytotoxic T (Tc), and CD4+CD8 α -CXCR5+PD-1+ Tfh cells. **(B and C)** Flow cytometry analysis of indicated immune cells in ileal PPs. **(C)** Number of total B cells, GC B cells, and IgA+ B cells. **(D)** Number of total T cells, Tc cells, Th cells, and Tfh cells; n.s., not significant; Student's *t* test, *n* = 3 per group.

Table S1. List of primers.

Oligonucleotide	Sequence (5' to 3')
Gapdh_fwd Gapdh_rev	TGTGTCCGTCGTGGATCTGA CCTGCTTCACCACCTTCTTGA
Spi-B_fwd Spi-B_rev	GGAGTCTTCTACGACCTGGACAG GCAGGATCGAAGGCTTCATAGG
Sox8_fwd Sox8_rev	GGACCAGTACCCGCATCTCC TTCTTGTGCTGCACACGGAGC
GP2_fwd GP2_rev	GTGTACAAGTTACAGGGTACCCC GACAAGTAATCTCACAATTCTTGG

CCL9_fwd CCL9_rev	GCCCAGATCACACATGCAAC AGGACAGGCAGCAATCTGAA
MarcksL1_fwd MarcksL1_rev	CCCGTGAACGGAACAGATGA CCCACCCTCCTTCCGATTTC
Esrrg_fwd Esrrg_rev	GTGTCTCAAAGTGGGCATGC GCTGTTCTCAGCATCTATTCTGC
Aif1_fwd Aif1_rev	GGATTTGCAGGGAGGAAAA TGGGATCATCGAGGAATTG
CCL20_fwd CCL20_rev	TGTACGAGAGGCAACAGTCG TCTGCTCTTCCTTGCTTTGG
TNFAIP2_fwd TNFAIP2_rev	GTGCAGAACCTCTACCCCAATG TGGAGAATGTCGATGGCCA
18s rRNA_fwd 18s rRNA_rev	GTAACCCGTTGAACCCCAT CCATCCAATCGGTAGTAGCG
Atoh8_fwd Atoh8_rev	CGGGGGAAAGTTCCTACTCGTC CGGAAGAATCCGGGTGGTTATT
OPG_fwd OPG_rev	ACCCAGAAACTGGTCATCAGC CTGCAATACACACTCATCACT