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Supplementary Materials for

m^6A mRNA modification maintains colonic epithelial cell homeostasis via NF-κB–mediated antiapoptotic pathway

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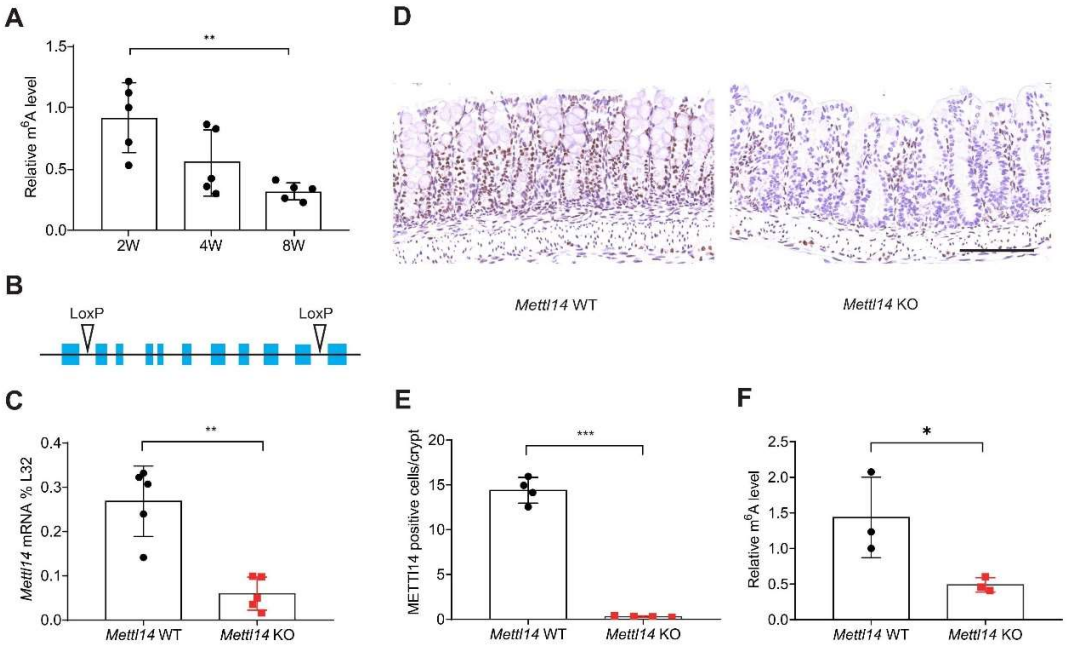

Fig. S1. Generation and verification of *Mettl14* KO mice in intestinal epithelial cells

A, m^{6}A dot plot quantitative analysis of colonic epithelial cells from mice of two weeks, four weeks and eight weeks (n = 5 mice per group). **B**, Two loxP sites were inserted into the first and last introns of *Mettl14* by CRISPR technology. **C**-**F**, Verification of METTL14 depletion in colonic epithelial cells by qRT-PCR (**C**, n = 5 mice per group), representative immunohistochemistry staining (**D**, Scale bars = 100 μm) and quantitative analysis (**E**, n = 4 mice per group), overall m^{6}A mRNA level (**F**, n = 3 mice per group). Data are presented as mean ± s.d. One-way ANOVA analysis (**A**) and two-sided Student's t-test (**C**, **E**, and **F**) were performed (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

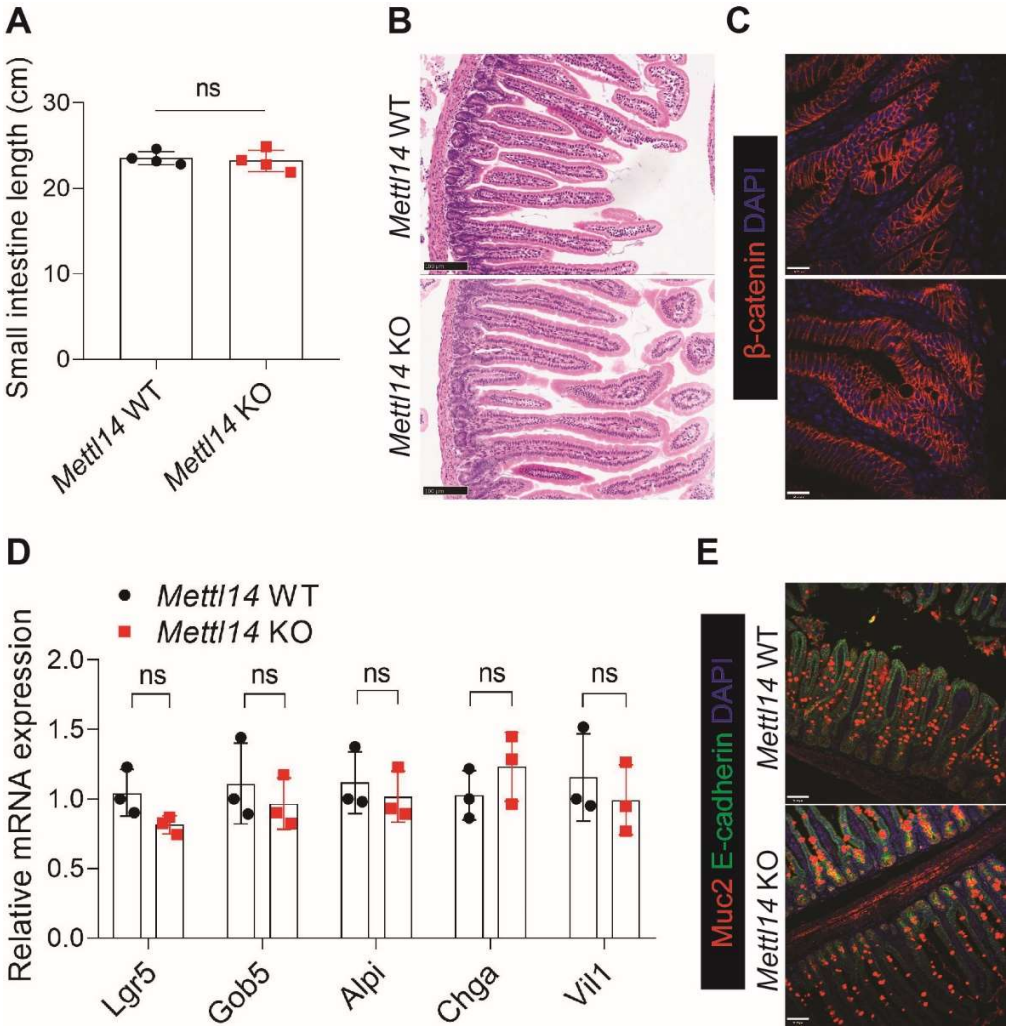

Fig. S2. *Mettl14* KO in intestinal tract does not impair the normal development and crypt integrity of small intestine.

A, quantitative analysis of the length of small intestine from *Mettl14* WT and *Mettl14* KO mice of two weeks (n = 4 per group). **B**, Representative H&E staining of small intestine sections from *Mettl14* WT and *Mettl14* KO mice at 2-week old (Scale bars = 100 μm). **C**, Representative β-catenin staining of small intestine sections from *Mettl14* WT and *Mettl14* KO mice at 2-week old (Scale bars = 18 μm). **D**, qRT-PCR analysis of different IECs specific marker genes in small intestine from *Mettl14* WT and *Mettl14* KO mice of two weeks (n = 3 per group). **E**, Representative Muc2 staining of small intestine sections from *Mettl14* WT and *Mettl14* KO mice at 2-week old (Scale bars = 70 μm). ns, no significance.

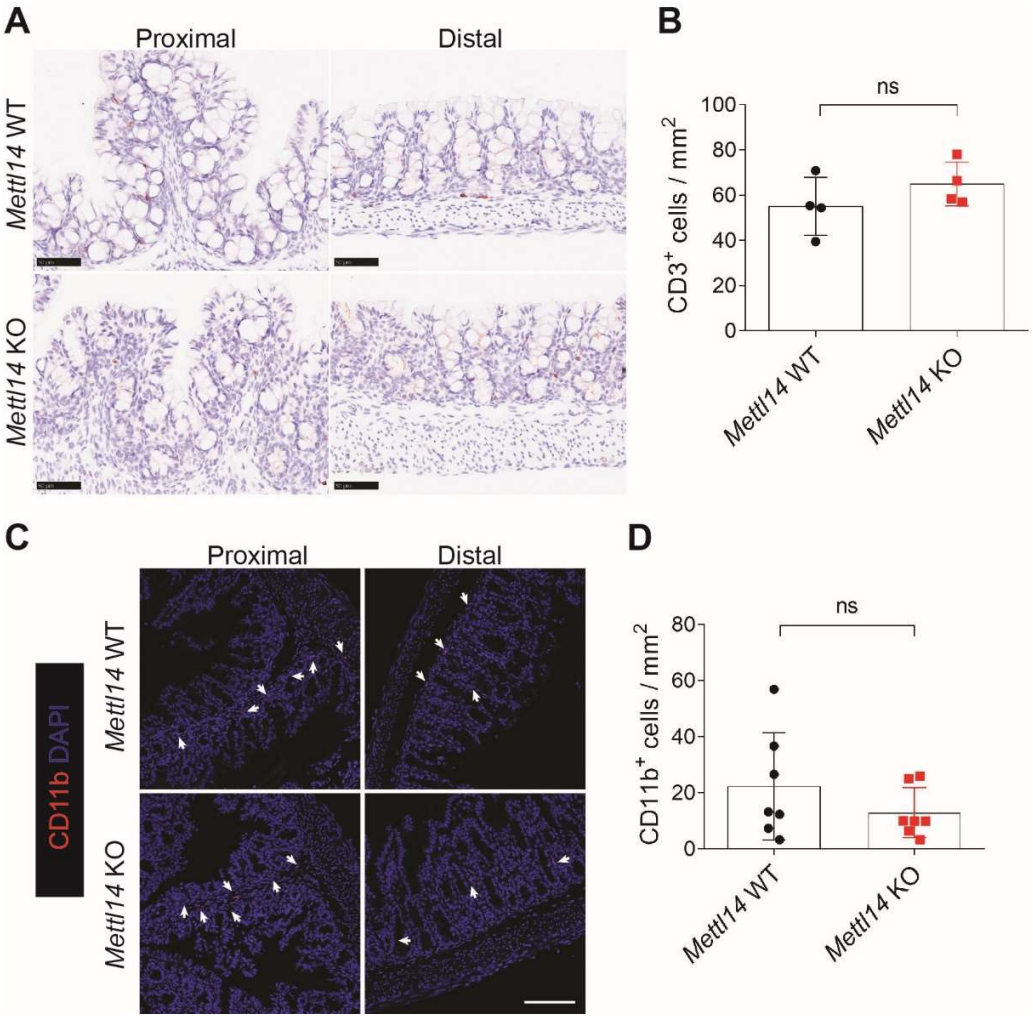

Fig. S3. Inflammatory cells infiltrated in colon showed no difference between *Mettl14* WT and *Mettl14* KO mice

A and **B**, CD3 stained proximal and distal colon sections of *Mettl14* WT and *Mettl14* KO mice of two weeks (**A**, Scale bar = 50 μm) and quantitative analysis (**B**, $n = 4$ per group). **C** and **D**, CD11b stained proximal and distal colon sections of *Mettl14* WT and *Mettl14* KO mice of two weeks (**C**, Scale bar = 100 μm) and quantitative analysis (**D**, $n = 7$ per group), CD11b positive cell (arrow). Data are presented as mean ± s.d. Two-sided Student's *t*-test (**B** and **D**) was performed. ns, no significance.

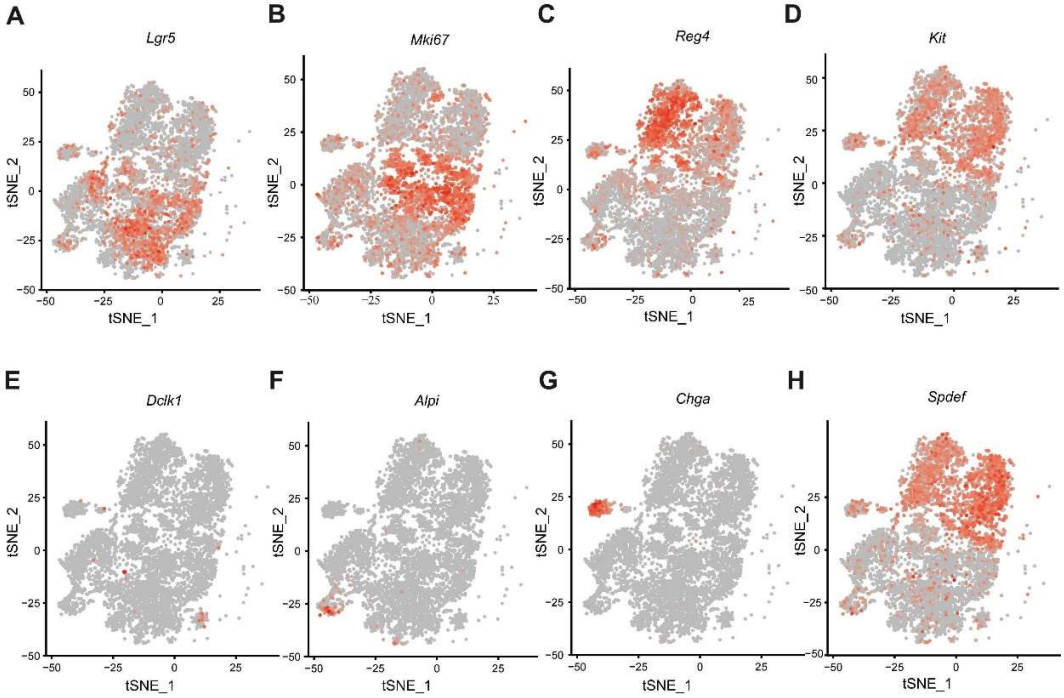

Fig. S4. Expression levels of signature genes in each cell cluster

A-H, t-SNE plot of expression levels of signature genes in different clusters. Stem cell marker gene, *Lgr5* (**A**), TA cell marker gene, *Mki67* (**B**), DCS cell marker gene, *Reg4* (**C**), DCS cell marker gene, *Kit* (**D**), Tuft cell marker gene, *Dclk1* (**E**), Colonocyte marker gene, *Alpi* (**F**), Enteroendocrine cell marker gene, *Chga* (**G**), Goblet cell marker gene, *Spdef* (**H**).

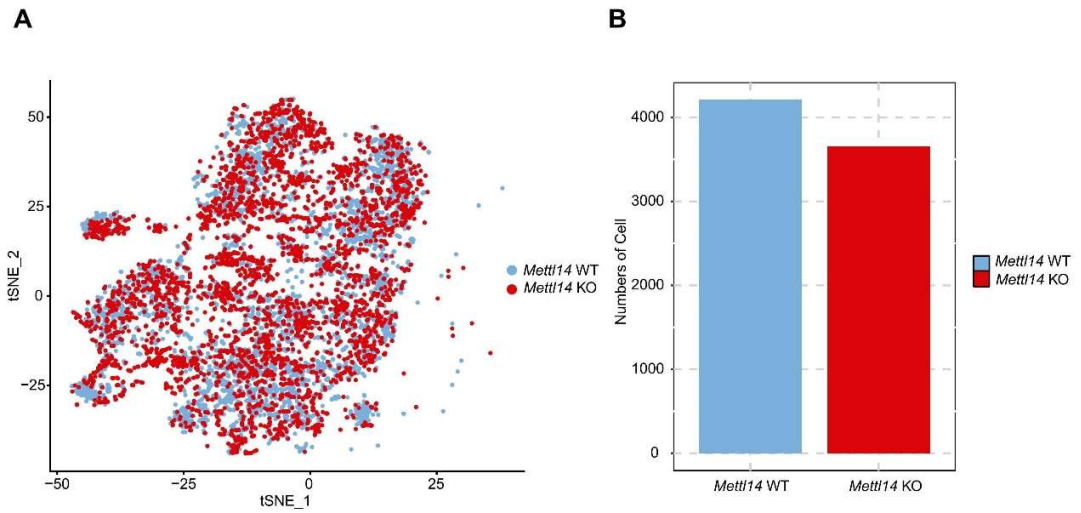

Fig. S5. Separated *t*-SNE plot of colonic epithelial cells from *Mettl14* WT and *Mettl14* KO mice

A and **B**, 7863 epithelial cells from *Mettl14* WT and *Mettl14* KO mouse colon of two weeks were separated and *t*-SNE plot was defined.

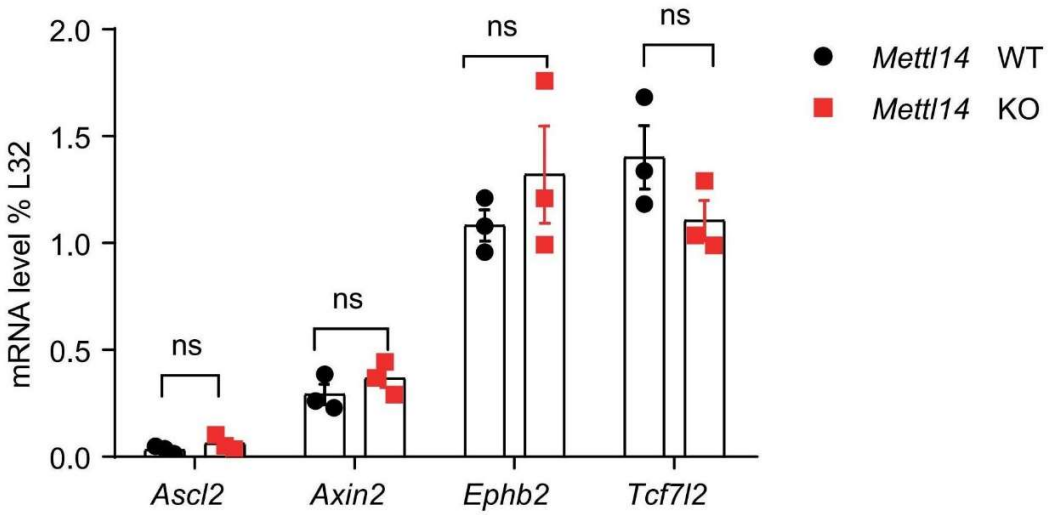

Fig. S6. Wnt signaling is not changed between *Mettl14* WT and *Mettl14* KO mice

qRT-PCR analysis of Wnt downstream target genes in *Mettl14* WT and *Mettl14* KO mice of two weeks (n = 3 per group). ns, no significance.

Table S1. Antibodies used in immunohistochemical and immunofluorescence analysis

Antibodies	Supplier	Conjugated	Catalog number
β-catenin	Biolegend	Unconjugated	844602
CD3	Abcam	Unconjugated	ab237721
CD11b	Abcam	Unconjugated	ab133357
Cleaved caspase-3	Cell Signaling Technology	Unconjugated	9579
E-cadherin	BD Transduction Laboratories	FITC	612130
Mettl14	Sigma	Unconjugated	HPA038002
Muc2	Novus Biologicals	Unconjugated	NBP1-31231
Ki67	Abcam	Unconjugated	ab16667
Donkey anti-rabbit IgG	Biolegend	Alexa Fluor 647	406414
Donkey anti-rabbit IgG	Biolegend	Alexa Fluor 594	406418
Goat anti-mouse IgG	Biolegend	Alexa Fluor 594	405326