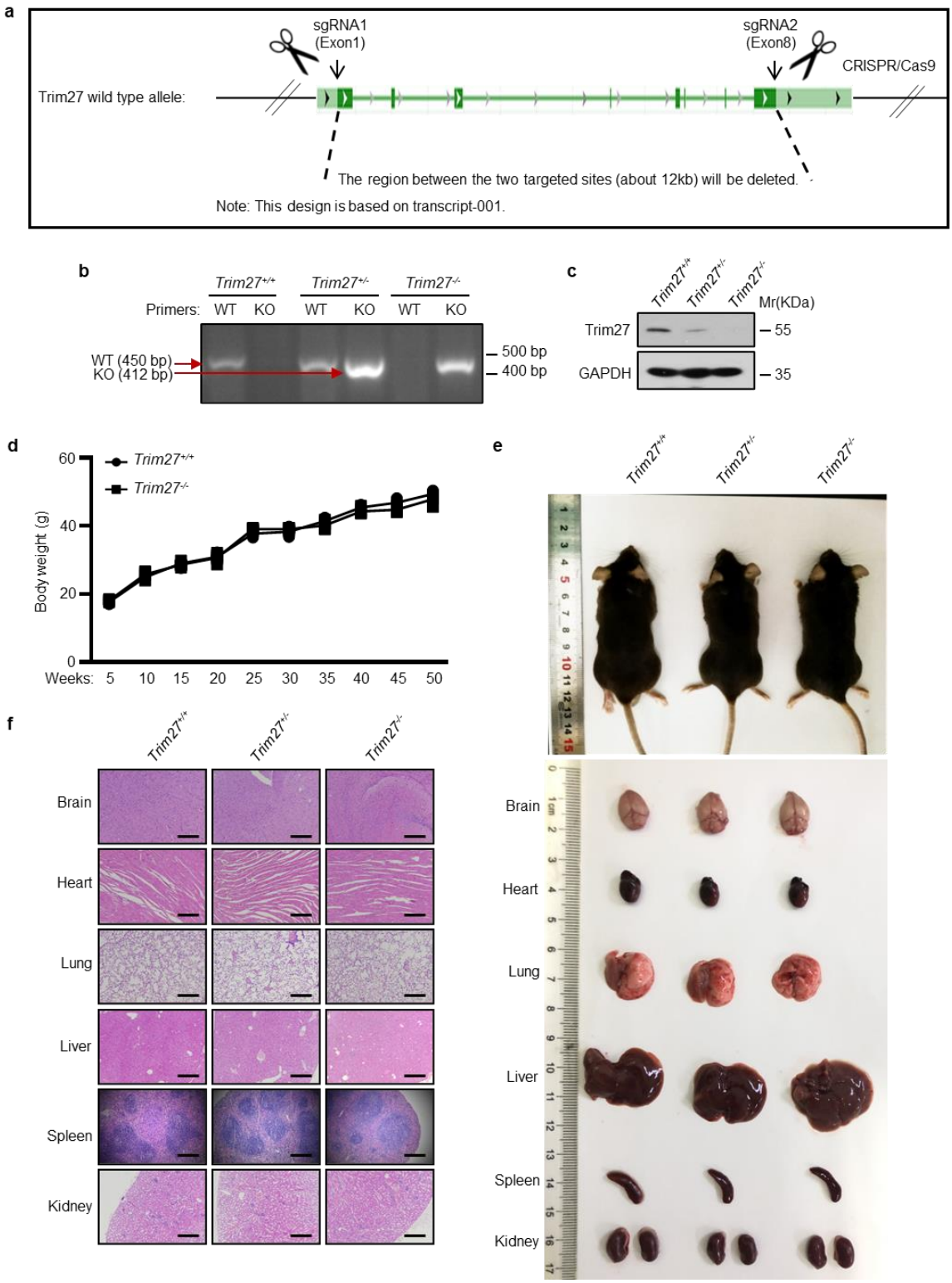
Supplementary Information

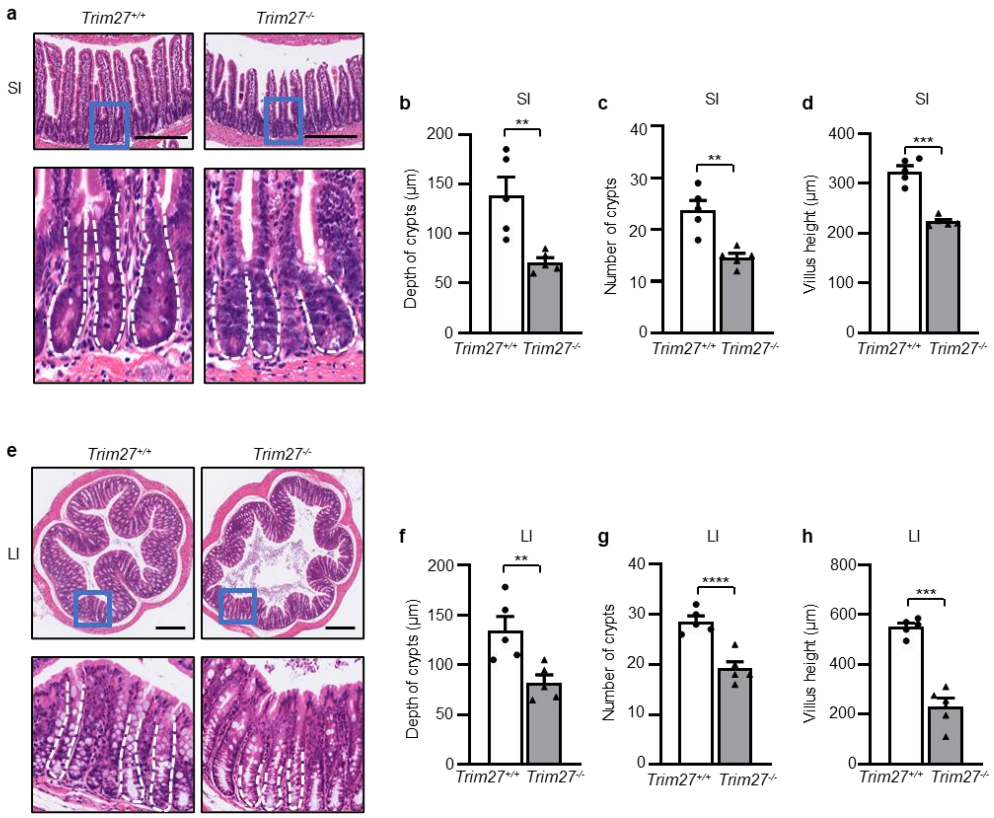
TRIM27 maintains gut homeostasis by promoting intestinal stem cell self-renewal

Wang *et al*.



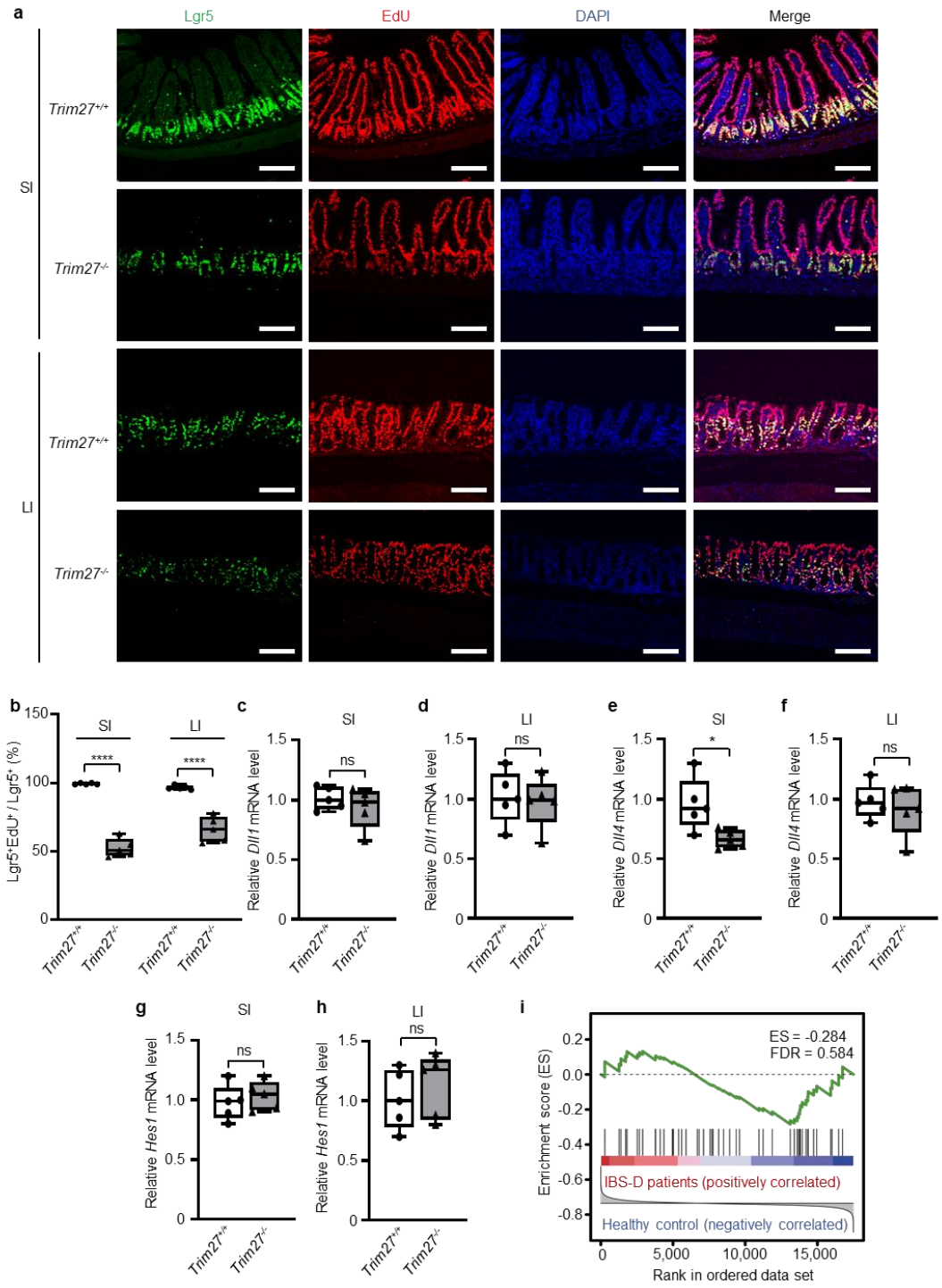
Supplementary Fig. 1 The constructive strategy and analysis of *Trim27*-knockout mice. a Strategy for the generation of *Trim27*-knockout (*Trim27*$^{-/-}$) mice. **b** PCR analysis of the mice. The predicted sizes of PCR products were 450 bp (*Trim27*$^{+/+}$) and 412 bp (*Trim27*$^{-/-}$). WT, primers for identification of *Trim27*$^{+/+}$ mice; KO, primers for identification of *Trim27*$^{-/-}$ mice.

c Immunoblots of Trim27 in BMDMs from *Trim27*$^{+/+}$, heterozygous (*Trim27*$^{+/-}$) and *Trim27*$^{-/-}$ mice. **d** Time course of the body weight of *Trim27*$^{+/+}$ and *Trim27*$^{-/-}$ mice. **e** The morphology of 16-week-old *Trim27*$^{+/+}$, *Trim27*$^{+/-}$ and *Trim27*$^{-/-}$ mice and their organs. **f** Hematoxylin and Eosin (H&E) staining of mouse organs from mice as in **e**. Scale bars, 200 μm.



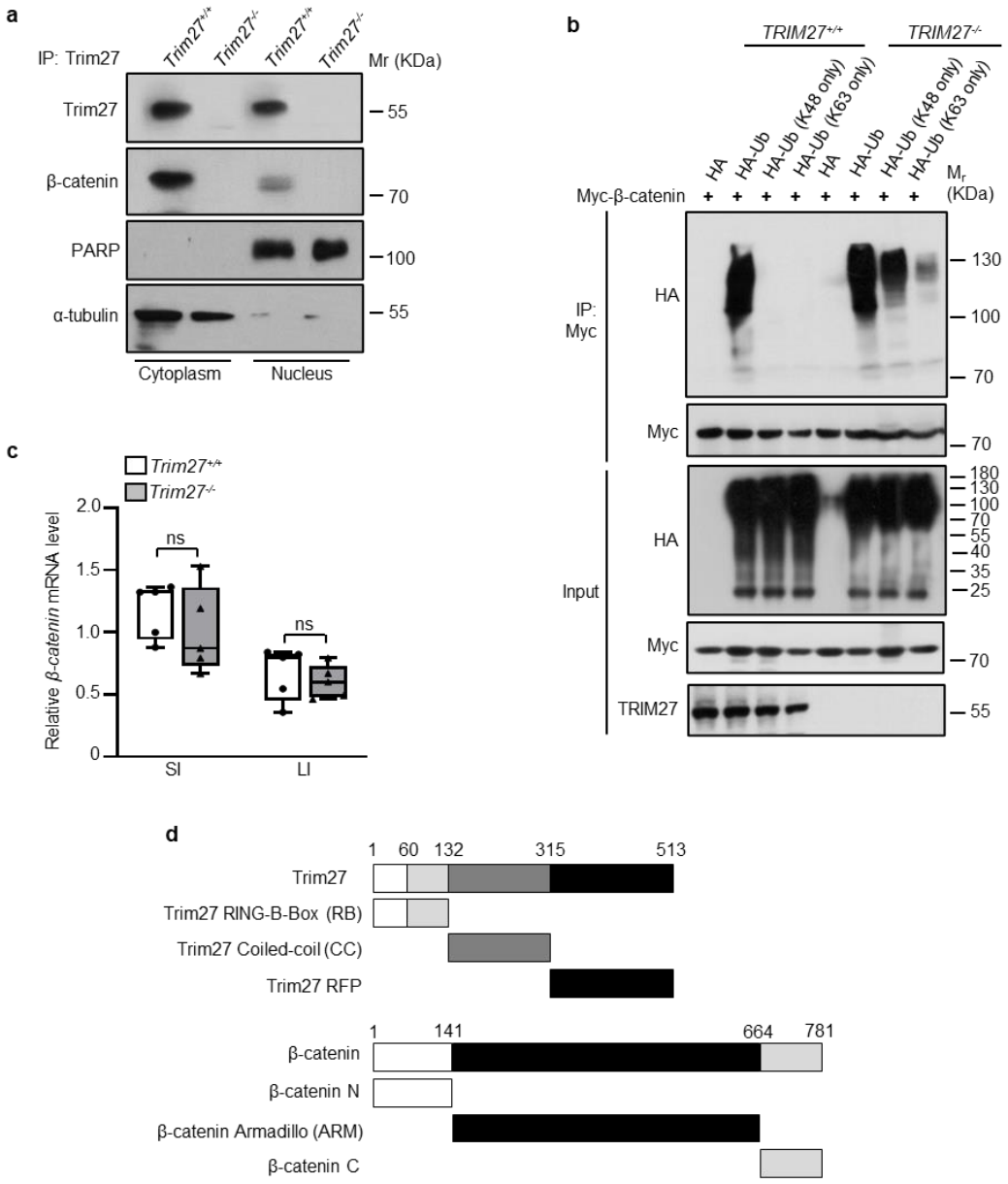
Supplementary Fig. 2 TRIM27 promotes the development of intestines. a H&E staining of the small intestine (SI) from 16-week-old *Trim27*$^{+/+}$ and *Trim27*$^{-/-}$ mice. Bottom, enlarged images of regions outlined in upper panels. Scale bars, 300 μm. White dashed lines mark crypt borders. **b–d** Crypt depth (**b**), crypt numbers per mm (**c**) and villus height (**d**) of the SI from mice as in **a**. At least 20 randomly selected crypts (**b**, **d**) or randomly selected fields (**c**) from the SI of each mouse were analyzed, and the average was calculated. Five mice per group were analyzed. **e** H&E staining of the large intestine (LI) from 16-week-old *Trim27*$^{+/+}$ and *Trim27*$^{-/-}$ mice. Bottom, enlarged images of regions outlined in upper panels. Scale bars, 300 μm. White dashed lines mark crypt borders. **f–h** Crypt depth (**f**), crypt numbers per mm (**g**) and villus height (**h**) of the LI from mice as in **e**. At least 20 randomly selected crypts (**f**, **h**) or randomly selected fields (**g**) from the LI of each mouse were analyzed, and the average was calculated. Five mice per group were analyzed. For **b–d and f–h**, statistical analyses were performed using unpaired two-tailed Student's *t*-test. Data are mean ± SEM (n=5). $^{**}P < 0.01$;

***$P < 0.001$; ****$P < 0.0001$.



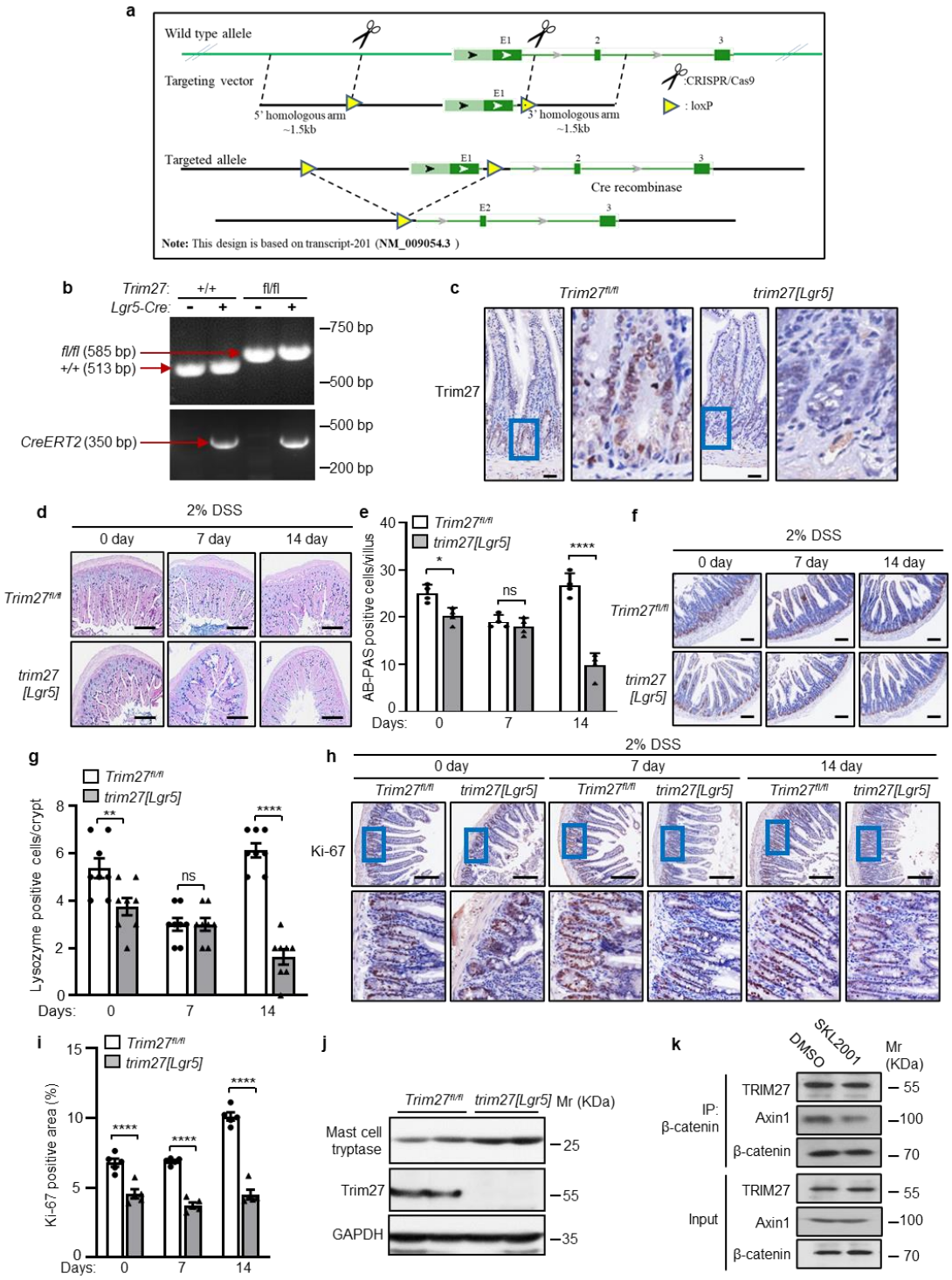
Supplementary Fig. 3 TRIM27 promotes the proliferation of intestinal stem cells (ISCs) in a Notch signaling pathway-independent manner. a Representative image of Lgr5 (green) and EdU signal (red) in the SI and LI from 16-week-old *Trim27*$^{+/+}$ and *Trim27*$^{-/-}$ mice on day 0.5 after a 4-day EdU administration. Scale bars, 100 μm. **b** The proportion of Lgr5^{+}EdU^{+} cells in the SI and LI from mice as in **a**. At least 20 randomly

selected crypts from the SI and LI of each mouse were analyzed, and the average was calculated. Five mice per group were analyzed. **c, d** Quantitative PCR (qPCR) analysis of Notch target gene *Dll1* mRNA of crypts freshly isolated from the SI (**c**) and LI (**d**) from 16-week-old *Trim27*$^{+/+}$ and *Trim27*$^{-/-}$ mice. **e, f** qPCR analysis of Notch target gene *Dll4* mRNA of crypts freshly isolated from the SI (**e**) and LI (**f**) from mice as in **c**. **g, h** qPCR analysis of Notch target gene *Hes1* mRNA of crypts freshly isolated from the SI (**g**) and LI (**h**) from mice as in **c**. **i** Gene Set Enrichment Analysis (GSEA) results for Notch signaling pathway in IBS-D patients. FDR, false discovery rate. For **b**, **c**–**h**, statistical analyses were performed using unpaired two-tailed Student's t-test. Data are box-and-whisker plots displaying median, interquartile range (boxes) and minima and maxima (whiskers) (**b**, **c**–**h**, n=5). $p > 0.05$, not significant (ns); *$p < 0.05$; ****$P < 0.0001$.



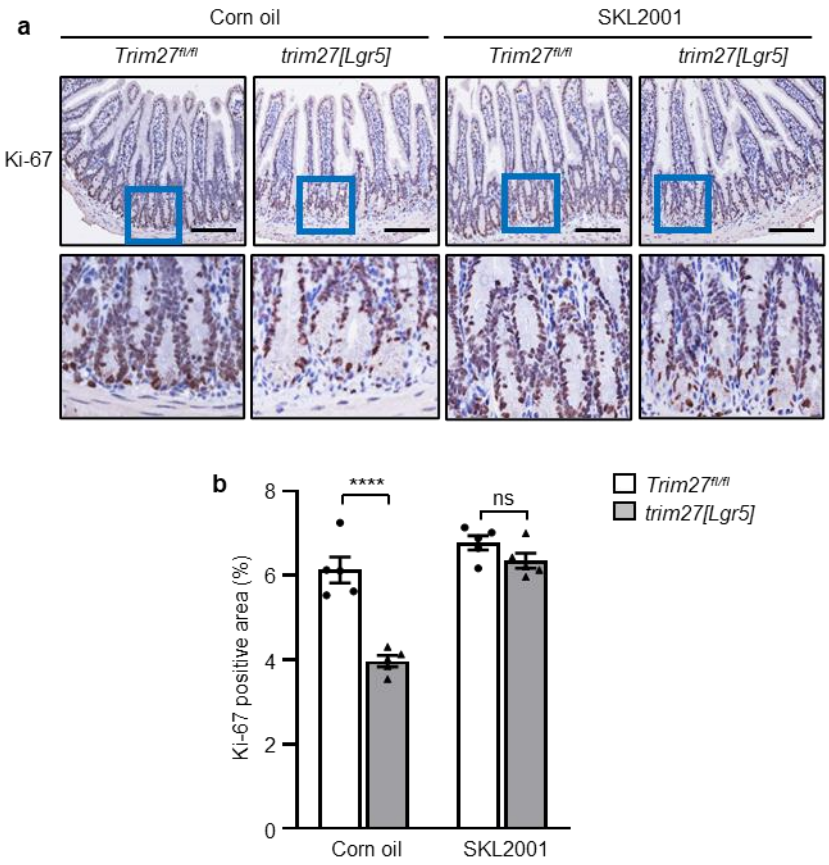
Supplementary Fig. 4 TRIM27 interacts with β-catenin and mainly inhibits its K48-linked polyubiquitination. a Immunoprecipitation (IP) of β-catenin by Trim27 in cytoplasm and nucleus of crypts from 16-week-old *Trim27*$^{+/+}$ and *Trim27*$^{-/-}$ mice. Crypts were lysed and immunoprecipitated with anti-Trim27 antibody. IP products were immunoblotted with the indicated antibodies. **b** *In vivo* ubiquitination assay of Myc-β-catenin in *TRIM27*$^{+/+}$ and *TRIM27*$^{-/-}$ HEK293T cells transfected with the indicated plasmids for 24 h. Cells were lysed and immunoprecipitated with the antibody against Myc. The immunoprecipitated proteins were immunoblotted with the antibody against

HA. **c** qPCR analysis of *β-catenin* mRNA of crypts freshly isolated from the SI and LI from 16-week-old *Trim27*$^{+/+}$ and *Trim27*$^{-/-}$ mice. **d** Schematic diagram of Trim27 and β-catenin domains. For **c**, statistical analyses were performed using unpaired two-tailed Student's t-test. Data are box-and-whisker plots displaying median, interquartile range (boxes) and minima and maxima (whiskers) (**c**, n=5). $p > 0.05$, not significant (ns).



Supplementary Fig. 5 Establishment and analysis of *Trim27*$^{fl/fl}$ and *trim27[Lgr5]* mice. a Strategy for the generation of *trim27[Lgr5]* mice. **b** PCR analysis of the mice. The predicted sizes of PCR products were 585 bp (*Trim27*$^{fl/fl}$), 513 bp (*Trim27*$^{+/+}$) and 350 bp (*creERT2*). **c** Immunohistochemical analysis of Trim27 in the SI from 16-week-old *Trim27*$^{fl/fl}$ and *trim27[Lgr5]* mice. Scale bars, 50 μm. **d** AB-PAS staining of the SI

from 16-week-old *Trim27$^{fl/fl}$* and *trim27[Lgr5]* mice after 2% DSS treatment. Scale bars, 200 μm. **e** The number of AB-PAS-positive goblet cells in intestinal tissue sections from mice as in **d**. At least 20 randomly selected villi from the SI of each mouse were analyzed, and the average was calculated. Five mice per group were analyzed. **f** Immunohistochemical analysis of lysozyme in the SI from 16-week-old *Trim27$^{fl/fl}$* and *trim27[Lgr5]* mice after 2% DSS treatment. Scale bars, 200 μm. **g** The number of lysozyme-positive Paneth cells per crypt in intestinal tissue sections from mice as in **f**. At least 20 randomly selected crypts from the SI of each mouse were analyzed, and the average was calculated. Five mice per group were analyzed. **h** Ki-67 staining of the SI from 16-week-old *Trim27$^{fl/fl}$* and *trim27[Lgr5]* mice after 2% DSS treatment. Scale bars, 100 μm. **i** Ki-67 positive area in intestinal tissue sections from mice as in **h**. Five mice per group were analyzed. **j** Immunoblots of mast cell tryptase, Trim27 and GAPDH in intestinal tissues from 16-week-old *Trim27$^{fl/fl}$* and *trim27[Lgr5]* mice. **k** IP of TRIM27 and Axin1 by β-catenin in HEK293T cells. Cells were lysed and immunoprecipitated with anti-β-catenin antibody in the presence or absence of SKL2001 (20 μM). IP products were immunoblotted with the indicated antibodies. For **e**, **g and i**, statistical analyses were performed using two-way ANOVA and Sidak's multiple comparisons test. Data are mean ± SEM (**e** and **i**, n=5; **g**, n=8). $p > 0.05$, not significant (ns); *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$.



Supplementary Fig. 6 The Wnt/β-catenin signaling activator SKL2001 promotes ISC self-renewal in *trim27[Lgr5]* mice. a Ki-67 staining of the SI from 12–16-week-old *Trim27*$^{fl/fl}$ and *trim27[Lgr5]* mice fed with corn oil or SKL2001. Scale bars, 300 μm. **b** Ki-67 positive area in the SI from mice as in **a**. Five mice per group were analyzed. statistical analyses were performed using two-way ANOVA and Sidak's multiple comparisons test. Data are mean ± SEM (b, n=5). $p > 0.05$, not significant (ns); ****$p < 0.0001$.

Supplementary Table 1. Oligonucleotides used for qPCR analysis or mouse genotyping PCR.

Name	Oligonucleotides (5'-3')
Gapdh-F	GGAGCGAGATCCCTCCAAAAT
Gapdh-R	GGCTGTTGTCATACTTCTCATGG
Tnf-F	CCTCTCTCTAATCAGCCCTCTG
Tnf-R	GAGGACCTGGGAGTAGATGAG
Il1b-F	GCAACTGTTCCTGAACTCAACT
Il1b-R	ATCTTTTGGGGTCCGTCAACT
Gper-F	ATGGATGCGACTACTCCAGC
Gper-R	AAGAGGGCAATCACGTACTGC
Axin2-F	GTCCACCAAACCTATGCCCG
Axin2-R	GAGTGTAAAGACTTGGTCCA
Myc-F	CAACGTCTTGGAACGTCAGA
Myc-R	TCGTCTGCTTGAATGGACAG
Ctnnb1-F	GATTCTGGAATCCATTCTGG
Ctnnb1-R	GGAAAAGCCTTGCTCCCATT
Wnt3a-F	CCTCGGAGATGGTGGTAGA
Wnt3a-R	GTTAGGTTCGCAGAAGTTGG
Dll1-F	CGGGGGCTCTGGCCCGCCTT
Dll1-R	AGCACTGGCGTGACAGCACT
Dll4-F	ACGCCGGTATTGGGCACCAA
Dll4-R	TTGGATGTTGAGTGAGAAGG
Hes1-F	AATGCCGGGAGCTATCTTTCT
Hes1-R	CCAGCCAGTGTCAACACGA
β-catenin-F	CCCAGTCCTTCACGCAAGAG
β-catenin-R	CATCTAGCGTCTCAGGGAACA
Trim27$^{+/+}$-F	CTCCGGAGCCGAGAAGCGGAGCGAG
Trim27$^{+/+}$-R	TCCTGCTCGCAGTACAGCTTCAGAG
Trim27$^{-/-}$-F	GAGCAGCTGTGCGGGGACGGGTCTG
Trim27$^{-/-}$-R	TGGAAAGTGGGTCTCCAAATCCAAATC
Trim27$^{fl/fl}$-F	GCACGTGCTCTCTCACTAAGCTGT
Trim27$^{fl/fl}$-R	GTGGCGCACTCCATTAATCCTCTTGT
CreERT2-F	CGATGCAACGAGTGATGAGG
CreERT2-R	CGCATAACCAGTGAAACAGC