SUPPLEMENTAL MATERIAL

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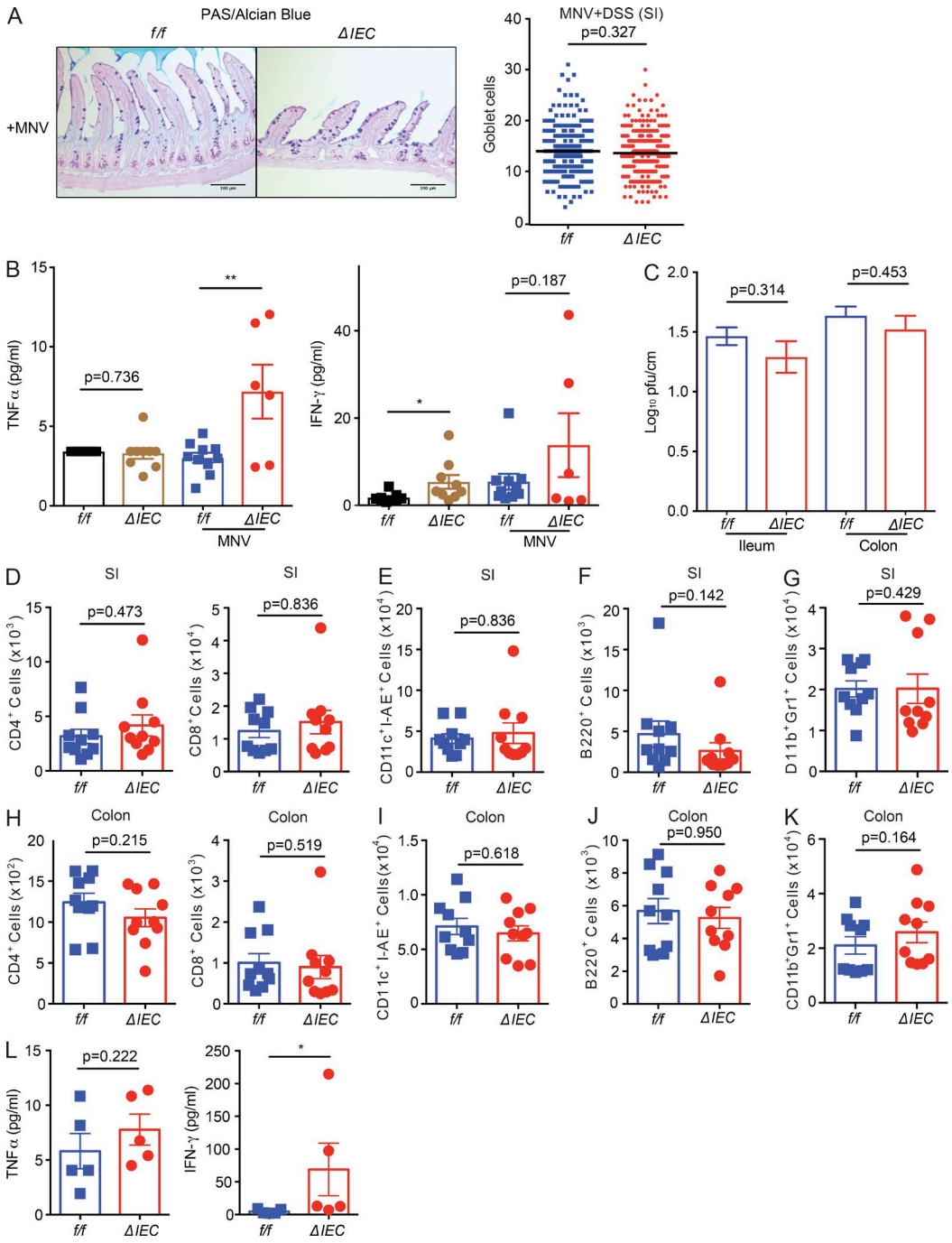


Figure S1. **Additional characterization of mice deficient in ATG16L1 in the epithelium after MNV+DSS and allo-HSCT treatment.** (A) Representative images of periodic acid–Schiff (PAS)/Alcian blue staining and quantification of goblet cells in the small intestinal samples harvested from MNV-infected *Atg16L1*$^{f/f}$ and *Atg16L1*$^{\Delta IEC}$ mice on day 15 after infection. At least 50 villi were quantified from three mice per group. Bars, 100 µm. (B) Quantification of TNFα and IFN-γ in sera harvested from mice on day 15 after infection. $n = 9$ (*f/f*), 9 (*ΔIEC*), 10 (*f/f* + MNV), and 6 (*ΔIEC* + MNV). (C) PFUs of MNV in ileum and colon harvested from *Atg16L1*$^{f/f}$ (*f/f*) and *Atg16L1*$^{\Delta IEC}$ (*ΔIEC*) mice on day 10 after infection. $n = 3$ per group. (D–K) Flow cytometric analysis of CD4$^+$ and CD8$^+$ T cells (D), CD11c$^+$I-AE$^+$ cells (E), B220$^+$ cells (F), and CD11b$^+$ cells (G) in the lamina propria of the small intestine and CD4$^+$ and CD8$^+$ T cells (H), CD11c$^+$I-AE$^+$ cells (I), B220$^+$ cells (J), and CD11b$^+$ cells (K) in the lamina propria of the colon on day 4 after transplantation. $n = 9$ (*f/f*) and 10 (*ΔIEC*). Bars represent mean ± SEM, and data points represent individual mice. (L) Quantification of TNFα and IFN-γ in sera harvested from mice on day 4 after transplantation. $n = 5$ per group. Data points in A represent individual villi, individual mice in B and D–L, and data points in C are mean of three technical replicates. Bars represent mean ± SEM, and at least two independent experiments were performed. *, $P < 0.05$; **, $P < 0.01$ by unpaired *t* test in A, one-way ANOVA and Tukey's test in B and C, and Mann-Whitney *U* test in D–L.

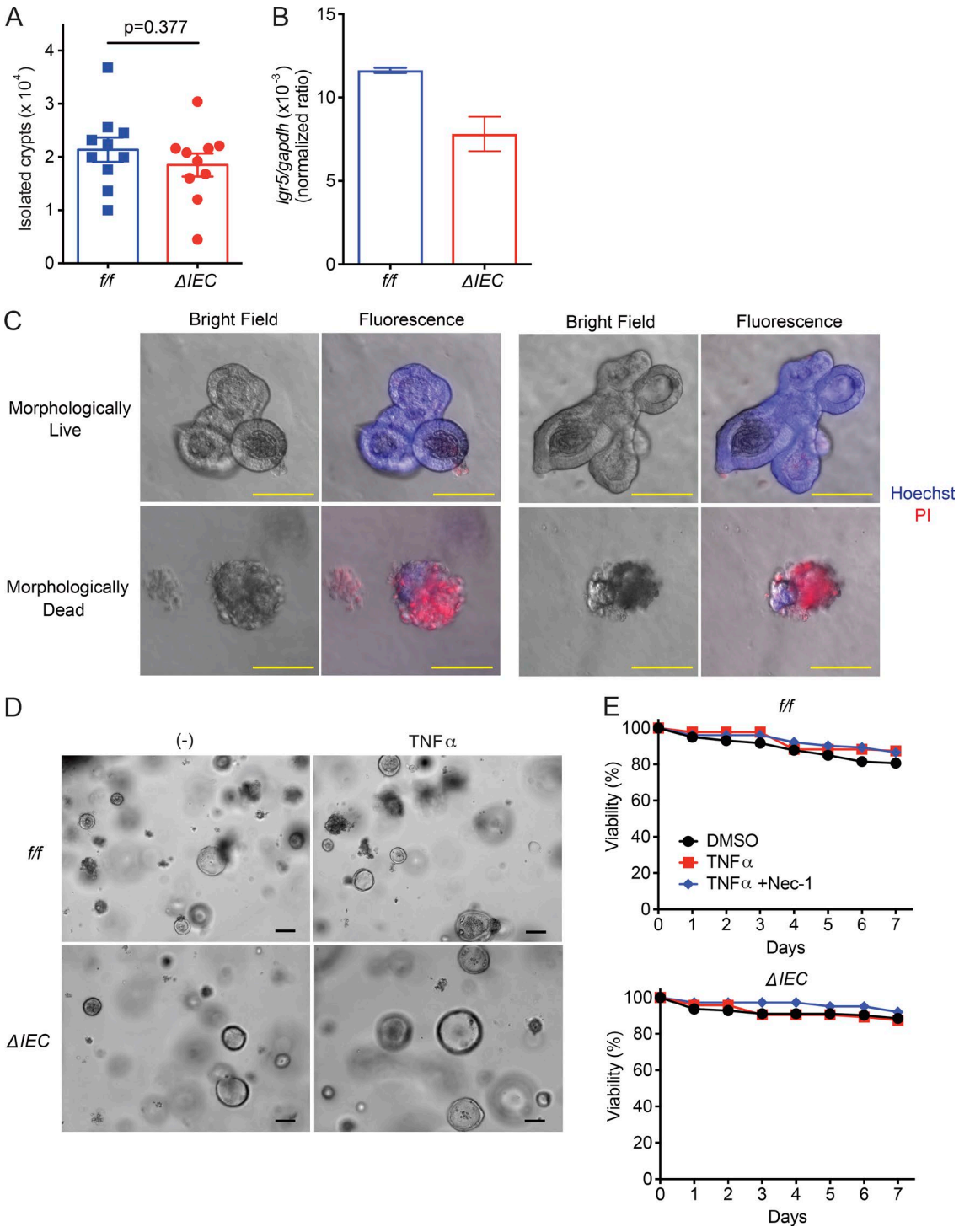


Figure S2. **Effect of ATG16L1 deletion on small intestinal stem cells and colonic organoids.** (A) Number of crypts isolated from each genotype. $n = 10$ per group. (B) Quantitative RT-PCR measurement of *Lgr5* expression normalized to *Gapdh* in *Atg16L1*$^{f/f}$ (*f/f*) and *Atg16L1*$^{\Delta IEC}$ (*ΔIEC*) organoids on day 3. $n = 3$ per group. (C) Number of viable organoids was quantified in several figures on the basis of appearance. Organoids displaying visible lumens were considered alive (representative pictures in top row), whereas organoids displaying a collapsed structure with an absence of buds or lumen, such as those in the bottom row, were considered dead. Loss of viability on the basis of these morphological criteria was confirmed by staining the organoids with PI (red) and Hoechst 33342 (blue) where uptake of PI indicated the presence of dead cells. (D and E) Representative images (D) and quantification of viability (E) of colonic organoids on day 3 ± TNFα and Nec-1. $n = 3$ mice each. (C and D) Bars: 50 µm (C); 100 µm (D). Data points in A represent individual mice, and data points in B and E are mean of three technical replicates. Bars represent ±SEM, and at least two independent experiments were performed.

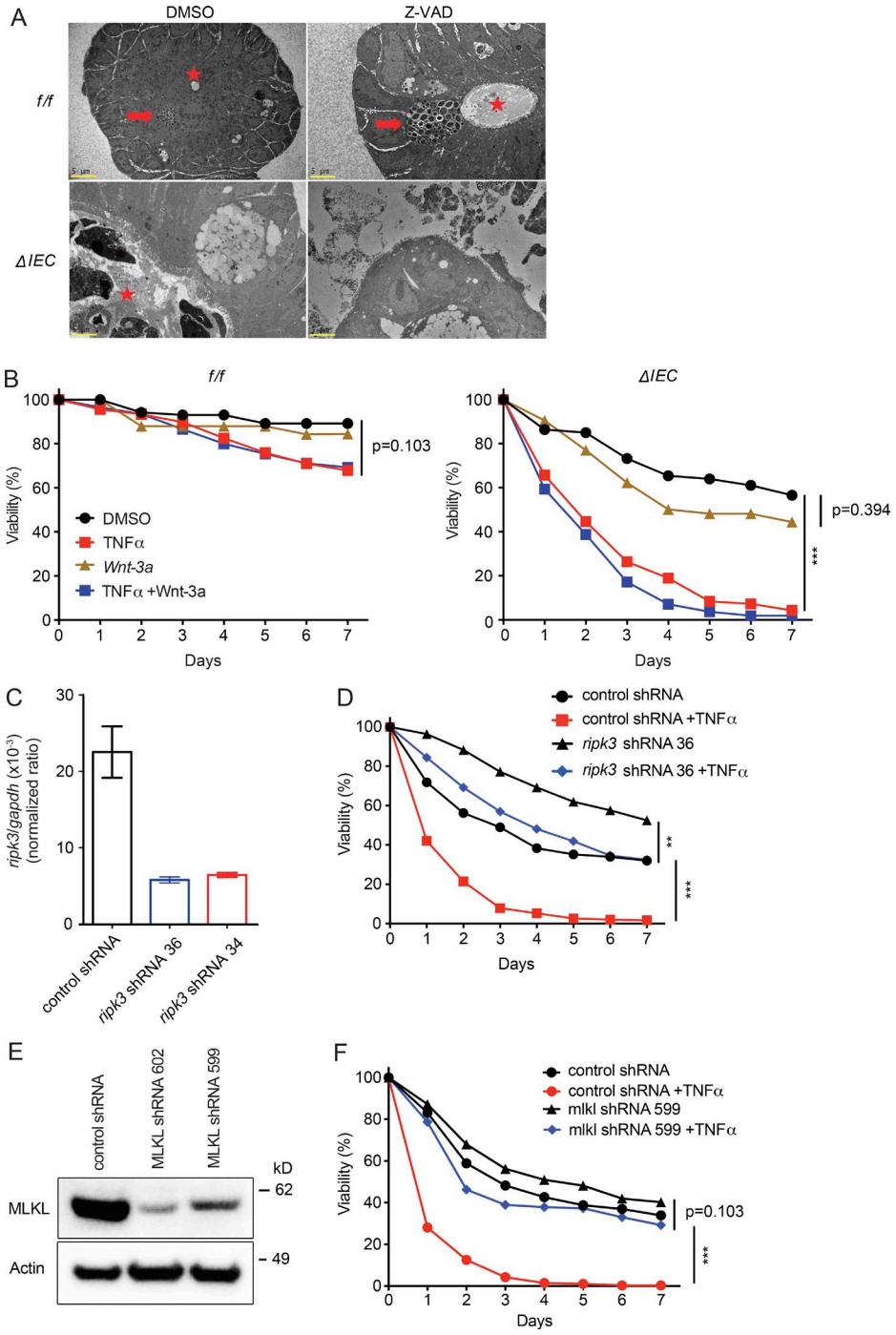


Figure S3. **Additional analyses of the effect of Z-VAD and TNFα on ATG16L1-deficient organoids.** (A) Representative lower magnification TEM images of organoids ± Z-VAD-FMK to complement the TEM images from Fig. 5 E. Stars indicate organoid lumen, and arrows indicate Paneth cells. Bars, 5 µm. *n* = 3 mice each. (B) Organoids from *Atg16L1*$^{f/f}$ (*f/f*) and *Atg16L1*$^{\Delta IEC}$ (*ΔIEC*) mice were incubated with Wnt-3a to determine whether its presence interferes with the viability assay. Quantification of viable organoids ± TNFα indicate that *Atg16L1*$^{\Delta IEC}$ organoids remain susceptible to TNFα-induced death. *n* = 3 mice each. (C) Quantitative RT-PCR measurement of *Ripk3* expression normalized to *Gapdh* in *Atg16L1*$^{\Delta IEC}$ organoids after transduction with lentiviruses encoding indicated shRNAs. *n* = 3 mice each. (D) The restoration of viability after *Ripk3* KD in *Atg16L1*$^{\Delta IEC}$ organoids (Fig. 5 H) was confirmed using a second shRNA targeting a different region of *Ripk3*. *n* = 3 mice each. (E) Representative Western blot image of MLKL and β-actin after transduction with lentiviruses encoding indicated shRNAs. (F) The restoration of viability after *Mlkl* KD in *Atg16L1*$^{\Delta IEC}$ organoids (Fig. 5 I) was confirmed using a second shRNA targeting a different region of *Mlkl*. Data points in B–D and F are mean of three technical replicates. Bars represent ±SEM, and at least two independent experiments were performed. **, P < 0.01; ***, P < 0.001 by unpaired *t* test.

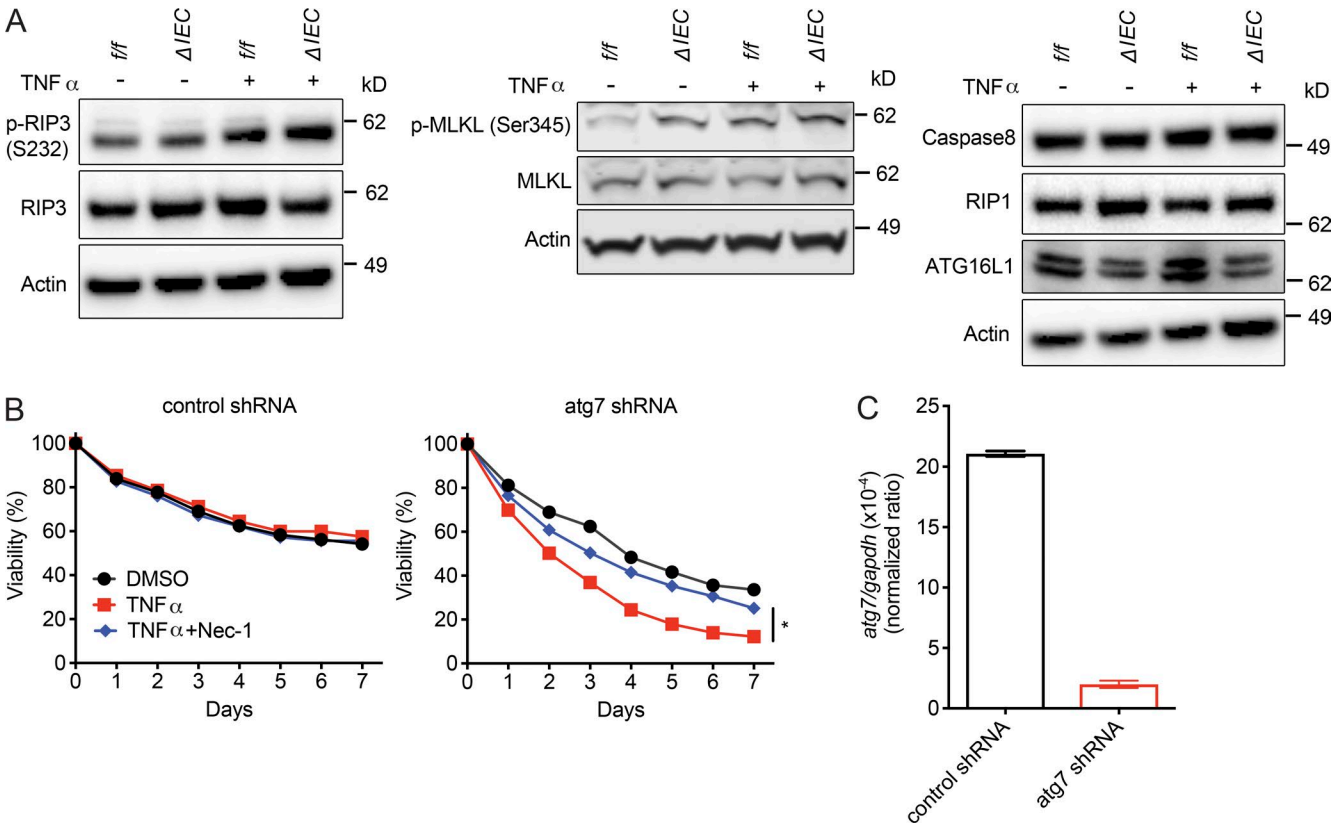


Figure S4. **ATG7-deficient organoids are susceptible to TNFα-induced death.** (A) Western blot analysis of necroptosis-related proteins in day 3 *Atg16L1*$^{f/f}$ and *Atg16L1*$^{\Delta IEC}$ organoids treated with TNFα for 2 h. Blots are representative of at least two independent repeats. (B) Viability of lentivirus-infected organoids from B6 mice treated ± TNFα and Nec-1. (C) Quantitative RT-PCR measurement of *Atg7* expression normalized to *Gapdh* in B6 organoids after transduction with lentiviruses encoding *Atg7* shRNA. Bars represent mean ± SEM, and at least two independent experiments were performed. Data points are mean of three technical replicates. *, $P < 0.05$ by unpaired *t* test.

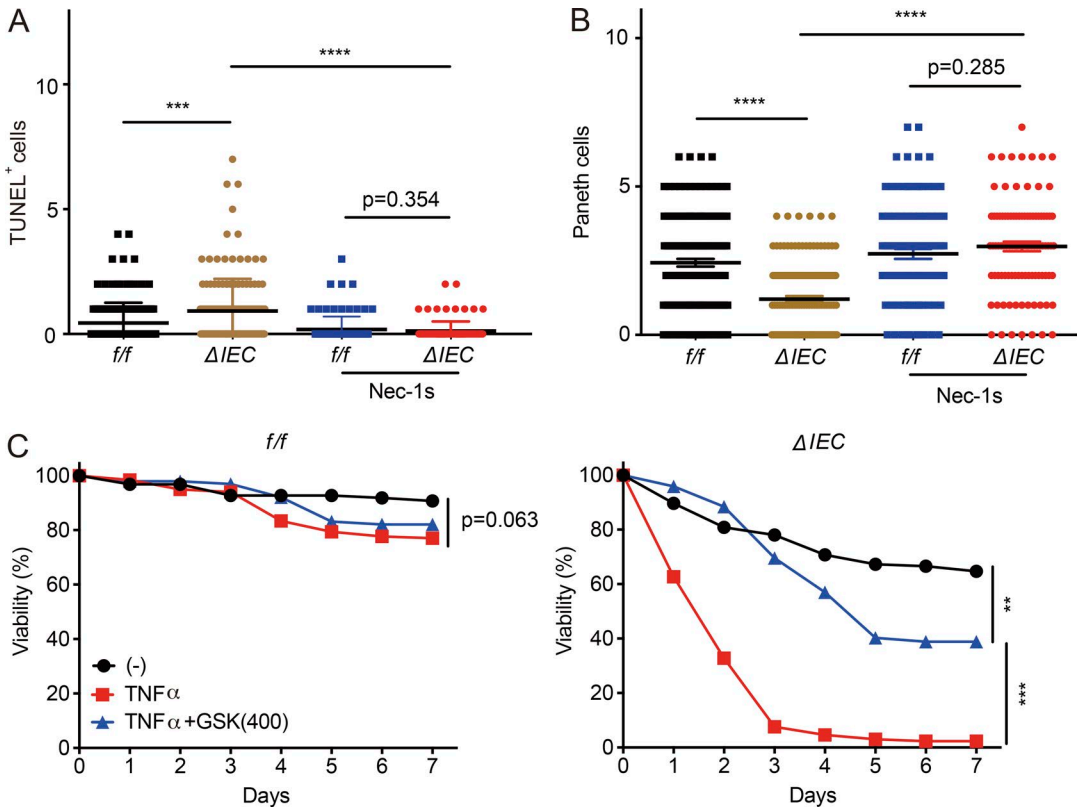
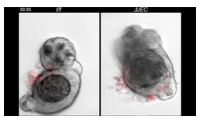


Figure S5. **Chemical inhibition of RIPK1 protects ATG16L1-deficient epithelial cells in vivo and in vitro.** (A and B) Number of TUNEL$^+$ (A) and Paneth (B) cells per crypt on day 15 in mice ± daily injection of Nec-1s from day 9 to 14. At least 50 crypts were quantified from 3 mice per group. (C) Viability of organoids from *Atg16L1*$^{f/f}$ and *Atg16L1*$^{\Delta IEC}$ mice treated ± TNFα and GSK547. Data points in A and B represent individual crypts, and data points in C are mean of three technical replicates. Bars represent mean ± SEM, and at least two independent experiments were performed. **, P < 0.01; ***, P < 0.001; ****, P < 0.0001 by one-way ANOVA and Tukey's test in A and B and unpaired *t* test in (C).



Video 1. **Live imaging analysis of TNFα-induced cell death in ATG16L1-deficient intestinal organoids.** Organoids from *Atg16L1*$^{f/f}$ and *Atg16L1*$^{\Delta IEC}$ mice on day 3 were treated with 20 ng/ml TNFα and stained with 100 µg/ml PI before imaging. Time-lapse images of multiple fields were collected at 5-min intervals over 17 h. Digits represent hours:minutes. *Atg16L1*$^{\Delta IEC}$ organoids rapidly took up PI, underscoring the dramatic increase in sensitivity to cell death. At least two independent experiments were performed.