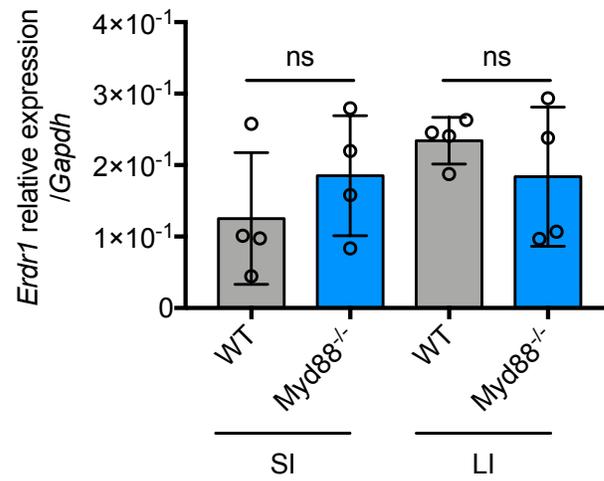


## **Supplementary Information**

### **Erythroid differentiation regulator-1 induced by microbiota in early-life drives intestinal stem cell proliferation and regeneration**

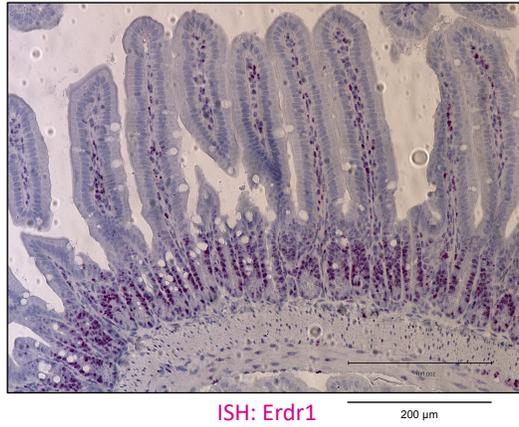
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This PDF file includes supplementary figure 1 to 16



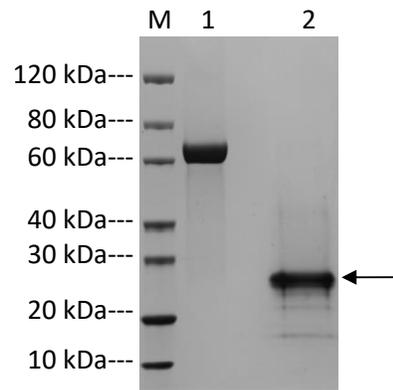
**Supplementary Figure 1 | Erdr1 expression is independent of Myd88 in the small and large intestine.**

Expression of Erdr1 in total intestinal tissue from the indicated mice. n=4; Data are presented as mean  $\pm$  SD; ns, no significance by unpaired, two-tailed *t*-tests.



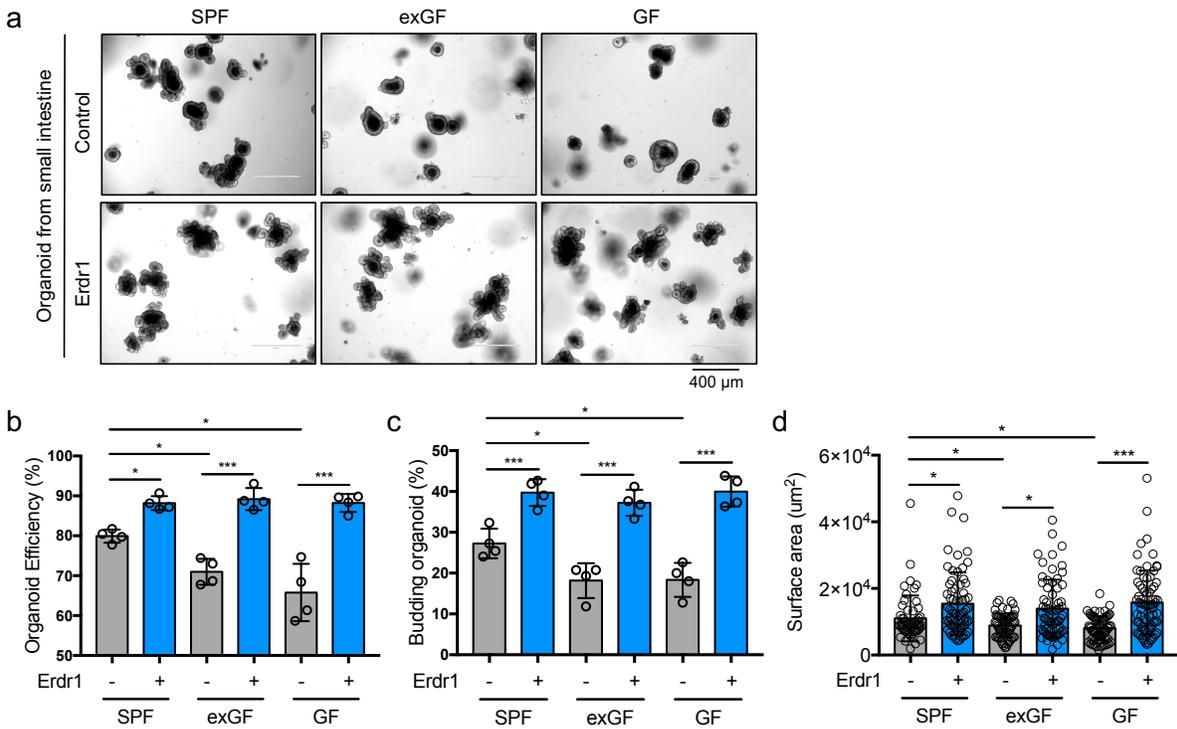
**Supplementary Figure 2 | RNAscope for *Erdr1* in mouse small intestine.**

RNAscope staining for *Erdr1* within small intestine from SPF mice. Purple dots indicate *Erdr1* RNA.



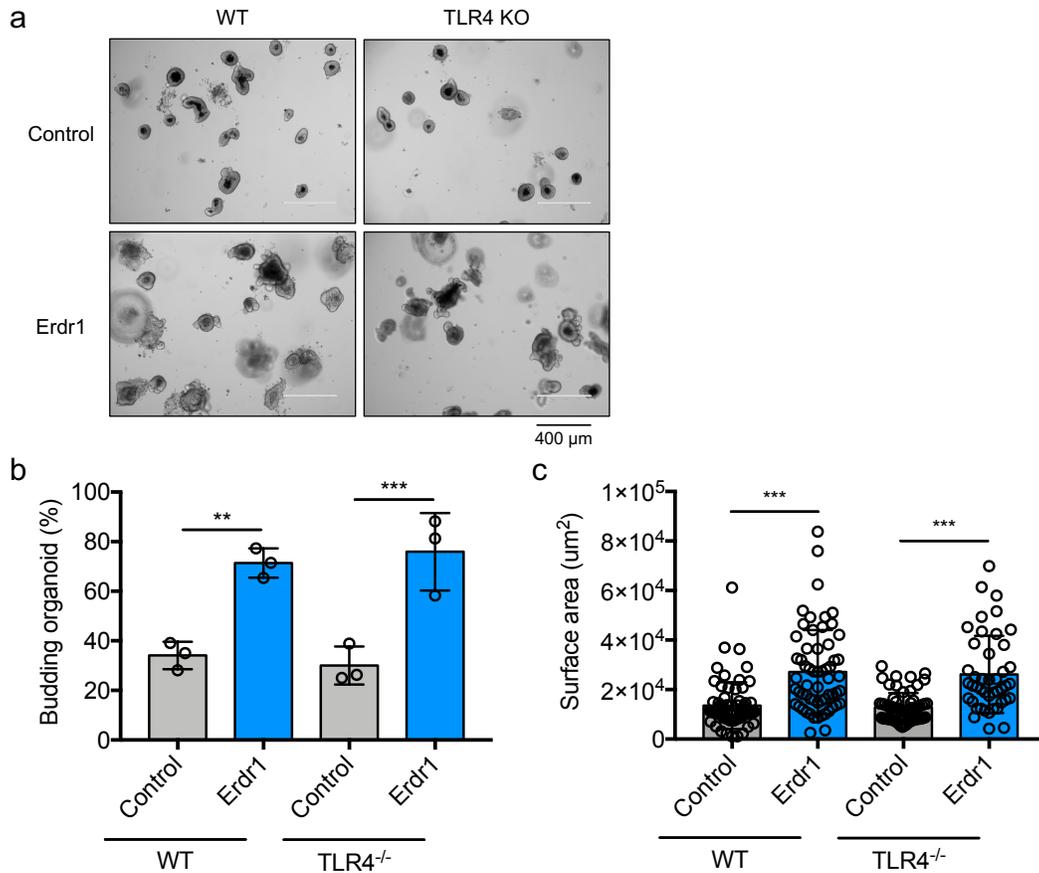
**Supplementary Figure 3 | SDS-PAGE gel of recombinant Erdr1 stained by coomassie blue.**

Lane M;Protein marker, Lane 1;BSA, Lane;2 Erdr1.



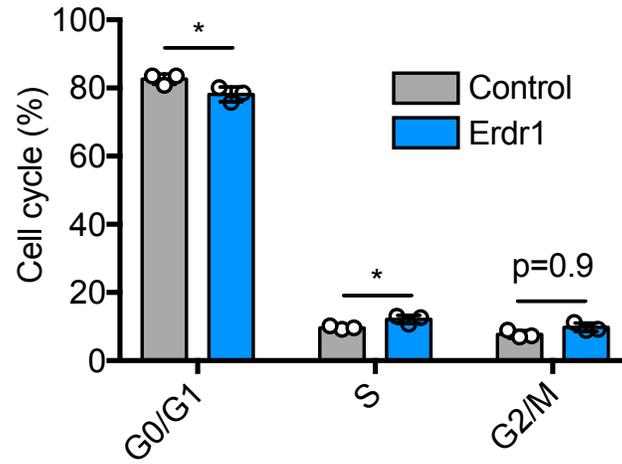
**Supplementary Figure 4 | Erdr1 increases growth of small intestine organoids.**

**a**, Representative images of small intestine (SI) organoids derived from SPF, exGF and GF mice and cultured +/- Erdr1 (1  $\mu\text{g}$ ) for 6 days. **b**, Organoid efficiency of SI organoids cultured +/- Erdr1.  $n=4$ ; representative of three experiments. **c**, Frequency of budding organoids cultured +/- Erdr1 for 6 days.  $n=4$ ; representative of three experiments. **d**, Surface area of SI organoids cultured +/- Erdr1.  $n=55$  (SPF),  $n=67$  (exGF),  $n=63$  (GF); representative of three experiments. All data are presented as mean  $\pm$  SD; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  by unpaired, two-tailed  $t$ -tests.



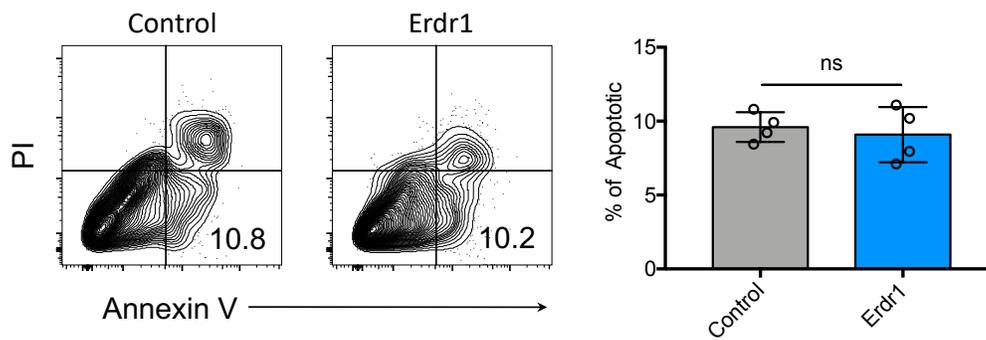
**Supplementary Figure 5 | TLR4 signaling does not influence organoid growth.**

**a**, Representative images of LI organoids derived from WT and TLR4 KO mice and cultured with or without Erdr1 for 6 days. **b**, Frequency of budding organoids with or without Erdr1.  $n=3$ ; representative of two experiments. **c**, Surface area of LI organoids cultured with or without Erdr1.  $n=67$  (WT/control),  $n=62$  (WT/Erdr1),  $n=58$  (TLR4<sup>-/-</sup>/control),  $n=63$  (TLR4<sup>-/-</sup>/Erdr1); representative of two experiments. All data are presented as mean  $\pm$  SD; \*\* $P < 0.01$ , \*\*\* $P < 0.001$  by one-way ANOVA with Tukey's multiple comparison test.



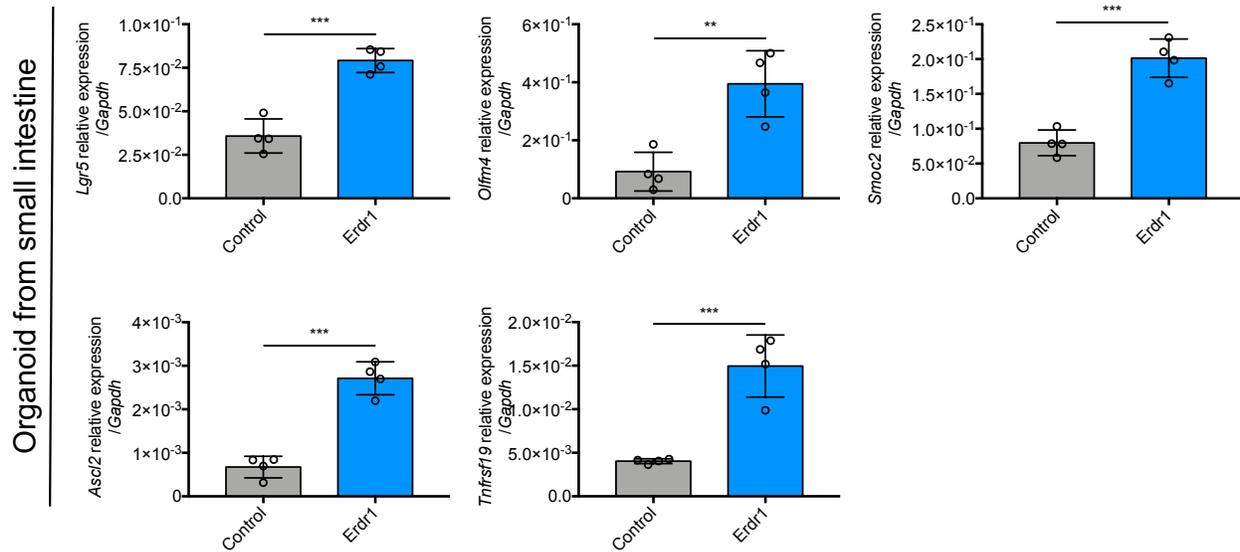
**Supplementary Figure 6 | Cell cycle analysis of small intestine organoids cultured +/- Erdr1.**

Cell cycle analysis of small intestine (SI) organoids cultured +/- Erdr1 (1  $\mu\text{g/mL}$ ). n=3; representative of two experiments. All data are presented as mean  $\pm$  SD; \* $P < 0.05$ , two-tailed  $t$ -tests.



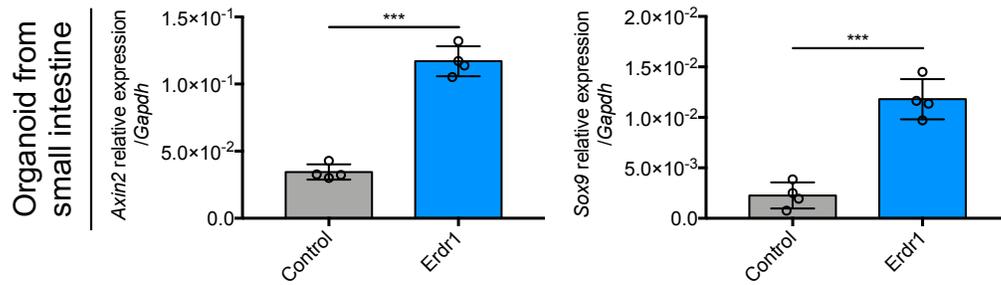
**Supplementary Figure 7 | Erdr1 does not influence apoptosis in organoid cultures.**

Frequency of pre-apoptotic cells in organoids derived from large intestine cultures with or without Erdr1. n=4; representative of two experiments. Data are presented as mean  $\pm$  SD; ns, no significant by unpaired, two-tailed *t*-tests.



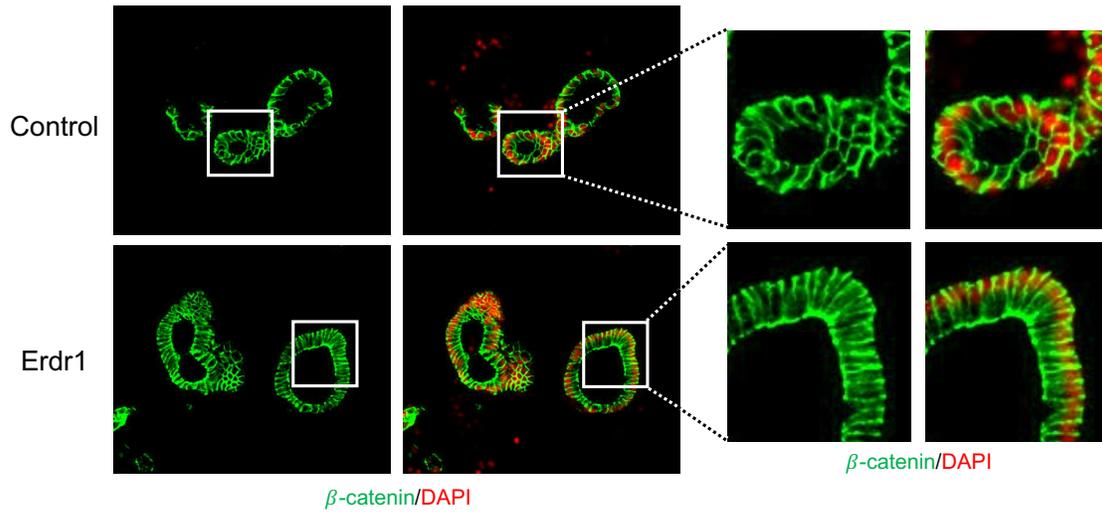
**Supplementary Figure 8 | Erdr1 induces ISC signature gene expression.**

**a**, Expression of stem cell signature genes (*Lgr5*, *Olmf4*, *Smoc2*, *Ascl2*, *Tnfrsf19*) in SI organoids cultured +/- Erdr1 for 6 days. n=4; representative of three experiments. All data are presented as mean  $\pm$  SD; \*\* $P < 0.01$ , \*\*\* $P < 0.001$  by unpaired, two-tailed *t*-tests.



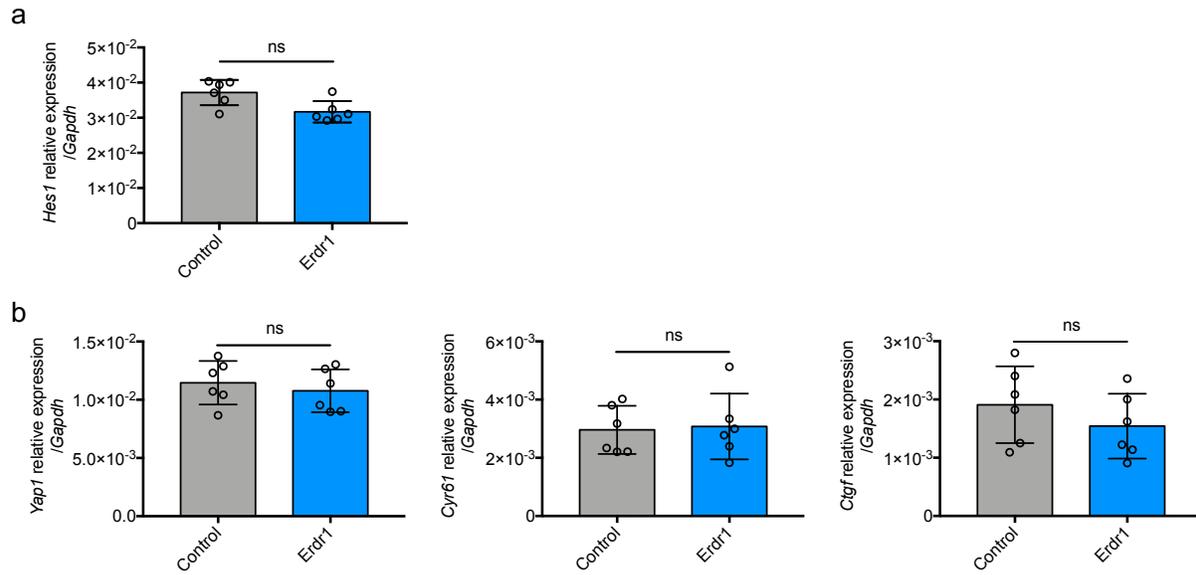
### Supplementary Figure 9 | Erdr1 enhances Wnt signaling in small intestine organoids.

Expression of Wnt signaling pathway target genes (*Axin2* and *Sox9*) in SI organoid cultures +/- Erdr1 (1  $\mu\text{g}/\text{mL}$ ).  $n=4$ ; representative of three experiments. All data are presented as mean  $\pm$  SD; \*\*\* $P < 0.001$  by unpaired, two-tailed  $t$ -tests.



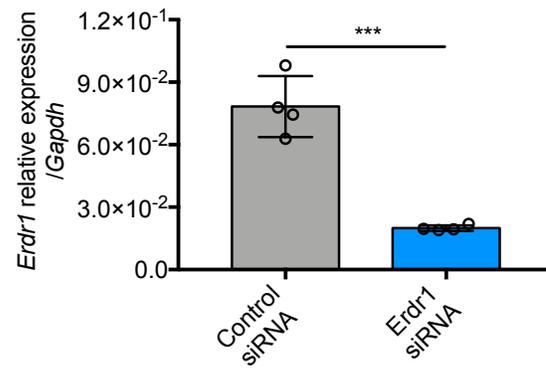
**Supplementary Figure 10 | Erdr1 induces nuclear translocation of  $\beta$ -catenin.**

Immunofluorescent staining of  $\beta$ -catenin in LI organoid cultures with or without Erdr1 (green,  $\beta$ -catenin; blue, DAPI). Representative of two experiments.



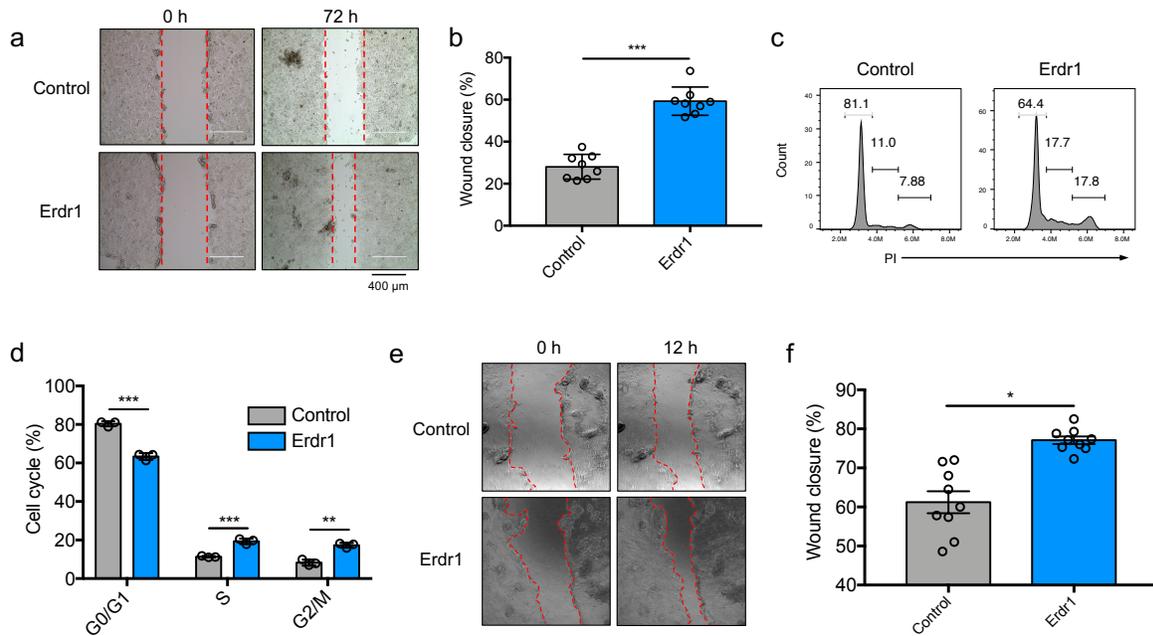
**Supplementary Figure 11 | Erdr1 increases organoid growth without inducing Notch or Yap/Taz mRNA expression.**

**a**, Expression of *Hes1* in organoids derived from the large intestine of SPF mice. n=6; two independent experiments. **b**, Expression of *Yap1* and its target genes (*Cyr61*, *Ctgf*) in organoids derived from the large intestine of SPF mice. n=6; representative of two experiments. Data are presented as mean ± SD; ns, no significant difference ( $p > 0.5$ ) by unpaired, two-tailed *t*-tests.



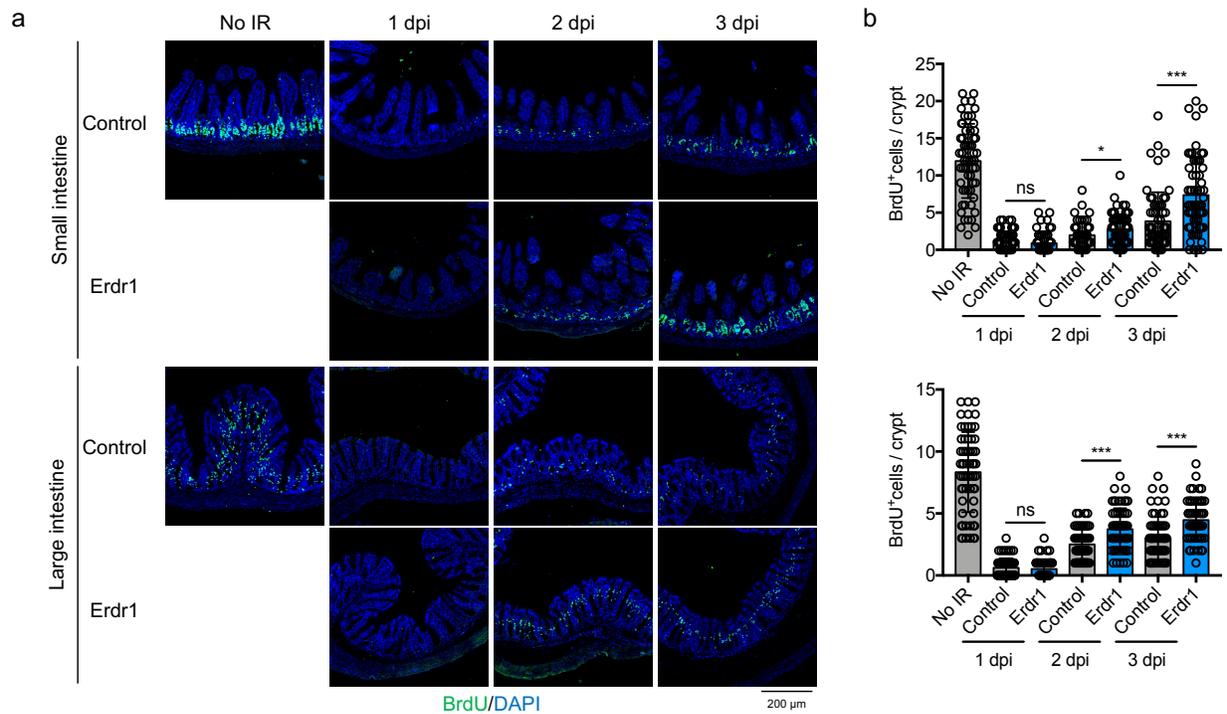
**Supplementary Figure 12 | siRNA knockdown of Erdr1.**

**a**, *Erdr1* expression after treatment of Mode-K cells with control siRNA or Erdr1 siRNA for 24 hours. n=4; representative of two experiments. All data are presented as mean  $\pm$  SD; \*\*\* $P < 0.001$  by unpaired, two-tailed *t*-tests.



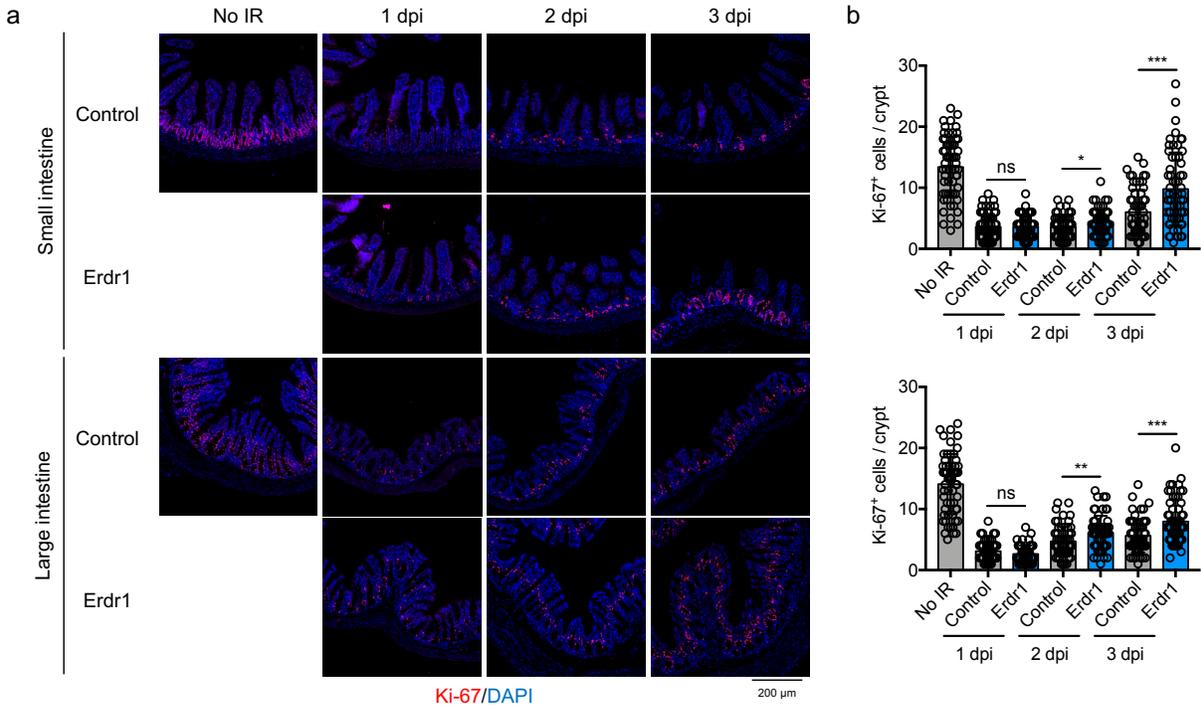
### Supplementary Figure 13 | Erdr1 accelerates wound closure in human intestinal epithelial cell.

**a-b**, Wound closure of HT-29 cells cultured +/- Erdr1 (1  $\mu$ g/mL) for 72 hours (**a**, picture; **b**, % of closure).  $n=8$ ; representative of three experiments. **c-d**, Cell cycle analysis of HT-29 cells cultured +/- Erdr1.  $n=3$ ; representative of three experiments. **e-f**, Wound closure of human 2D organoid monolayers +/- Erdr1 (1  $\mu$ g/mL) for 12 hours post scratching (**e**, picture; **f**, % of closure).  $n=9$ ; Data are cumulative of three experiments. All data are presented as mean  $\pm$  SD; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  by unpaired, two-tailed  $t$ -tests.



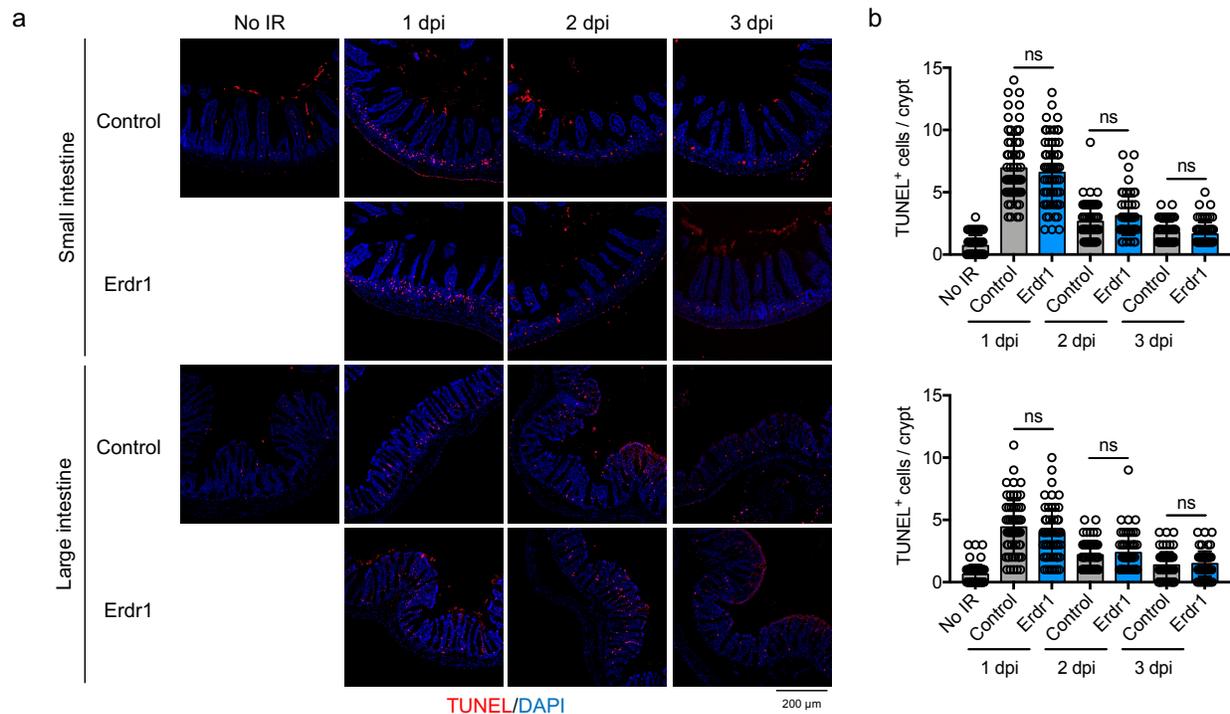
**Supplementary Figure 14 | Erdr1 promotes regeneration following radiation-induced injury.**

**a**, Representative images of BrdU staining in ileum and colon sections (green, BrdU; blue, DAPI). Mice were treated with 10 Gy of radiation, and euthanized at the indicated time points. **b**, Quantification of BrdU positive cells per crypt from the SI (top) and LI (bottom). SI, n=60; LI, n=60; All data are presented as mean  $\pm$  SD; \* $P$  < 0.01, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001 by unpaired, two-tailed  $t$ -tests.



**Supplementary Figure 15 | Erdr1 promotes the regeneration following radiation-induced injury.**

**a**, Representative images of Ki-67 staining in ileum and colon sections (pink, Ki-67; blue, DAPI). Mice were treated with 10 Gy of radiation, and euthanized at the indicated time points. **b**, Quantification of Ki-67 positive cells per crypt from the SI (top) and LI (bottom). SI, n=60; LI, n=60; All data are presented as mean  $\pm$  SD; \* $P$  < 0.01, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001 by unpaired, two-tailed  $t$ -tests.



**Supplementary Figure 16 | Erdr1 does not influence apoptosis following radiation-induced injury.**

**a**, Representative images of TUNEL staining in ileum and colon sections (pink, TUNEL; blue, DAPI). Mice were treated with 10 Gy of radiation, and euthanized at the indicated time points. **b**, Quantification of TUNEL positive cells per crypt from the SI (top) and LI (bottom). SI, n=60; LI, n=60; All data are presented as mean ± SD; \* $P < 0.01$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  by unpaired, two-tailed  $t$ -tests.