Supplemental Methods

Analysis of mRNA and protein expression

Intestinal mucosa scraping, isolation of total RNA and protein extracts from mouse tissues were performed as described (1, 2). CDK4/6 inhibitors were administered as described (3). Equal amounts of lysate or RNA were pooled from three individual mice/group before addition of sample buffer and gel electrophoresis, or cDNA synthesis. Real-time PCR was performed on CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA) with SYBR Green (Invitrogen, Carlsbad CA). Total protein extracts were subjected to NuPage gel (Invitrogen, Carlsbad CA) electrophoresis followed by Western blotting. Representative data were shown, and similar results were obtained in at least three independent experiments. Primers are listed in Table S1. The primary antibodies included p53 (sc-6243, Santa Cruz, Dallas, TX, USA), p21 (SC-397, Santa Cruz); PUMA (ab9643, Abcam, Cambridge, MA, USA); Bax (SC-493, Santa Cruz); Bak (#06-536, Upstate, Billerica, MA, USA); Bcl-2 (#M0887, Dako, Carpenteria, CA, USA); Mcl-1 (#559027, BD Biosciences, Bedford, MA, USA); p-p53 (S20, #9287, Cell Signaling, Danvers, MA, USA); p-Chk1 (S317, #2344, Cell Signaling, Danvers, MA, USA); p-BRCA1 (S1387, #NB100-225, Littleton, CO, USA); p-Rb (S780, #SC-12901, Santa Cruz, Dallas, TX, USA); p-AKT (Ser473, #4058, Cell Signaling); p-ERK (Thr202/Tyr204 , #4376, Cell Signaling); p-GSK-3α/β (Ser21/9, #8566, Cell Signaling); p-JNK (Thr183/Tyr185, #4668, Cell Signaling); p-p38 (Thy180/Tyr182, #4631, Cell Signaling,) and p-p65 (S536, #3033, Cell Signaling).

Tissue processing, Histological Analysis, TUNEL and BrdU staining

BrdU (Sigma Chemical Co., Louis, MO) was given by intraperitoneal injection to all the mice at a dose of 100 mg/kg 2 hours prior to sacrifice. For CDK4/6 inhibitor compounds administration, mice were treated as previous description (3).Tissues were harvested, fixed and embedded in paraffin as described (4, 5). 5μm sections were performed hematoxylin and eosin (H&E) staining for histological analysis.

Villus and crypts measures were made using H&E sections (5). Villus height measurements were based on 50-80 villi from different locations of the jejunum from three mice per group using ImageJ 1.46 software (National Institutes of Health, USA). Crypts numbers were based 3-5 full cross sections from three mice per group.

The crypt microcolony assay was used to quantitate stem cell survival by counting regenerated crypts in H&E-stained and BrdU-stained cross-sections 4 days after radiation. Surviving crypts were defined as containing 5 or more adjacent chromophilic non-Paneth cells, at least one Paneth cell and a lumen. The number of surviving crypts was counted in 6-8 circumferences per mouse, with each ~1 cm apart. For the definition of BrdU labeled regenerated crypts, it contained 10 or more BrdU-positive cells with a lumen.

TUNEL (Terminal deoxynucleotidyl transferase dUTP nick-end labelling) staining was conducted with ApopTag Peroxidase or ApopTag Fluorescein *In Situ* Apoptosis Detection Kit (Millipore, Billerica, MA) according to the manufacturer's instructions.

For BrdU staining, sections were treated with Proteinase K following deparaffinization. After incubation with anti-BrdU antibody (A21301MP, Invitrogen, Carlsbad, CA; 1:100 in 10% goat serum) and secondary antibody (Goat-anti-Mouse-biotin, 1:100; #31802; Pierce, Rockford, IL),

sections were performed with VectaStain ABC kit and 3,3'-Diaminobenzidine (DAB) (Vector Laboratories, Burlingame, CA).

Immunohistochemistry (IHC) and immunofluorescence (IF)

For immunostaining, the paraffin embedded sections were subjected to deparaffinization and antigen retrieval (boiling for 10 min in 0.1 M citrate buffer, pH 6.0, with 1mM EDTA). The images were acquired using an Olympus BX51 system microscope equipped with SPOT camera and SPOT Advanced 5.1 software. The confocal images were acquired using an Olympus FV2000 confocal microscope system equipped with FV10-ASW software.

Cleaved Caspase-3 IHC. Non-specific antibody binding was blocked using 20% goat serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in a humidified chamber with 1:25 diluted Rabbit-anti-Cleaved Caspase-3 antibodies (#9661; Cell Signaling Technologies, Beverly, MA). After incubating with biotinylated goat-anti-rabbit secondary antibodies (#31822; Pierce, Rockford, IL) for 1 hour at room temperature, the sections were developed with ABC kit and DAB.

γH2AX IF. Non-specific antibody binding was blocked using 20% goat serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in a humidified chamber with 1:100 diluted Mouse-anti- γH2AX (#05-636; Millipore, Billerica, MA). Then the sections were incubated with AlexaFluor-594 goat-anti-mouse secondary antibodies (1:100; A11005, Invitrogen) for 1 hour at room temperature and counterstained with VectaShield plus DAPI (Vector Laboratories).

p-β-catenin (S552) IF. Non-specific antibody binding was blocked using 20% goat serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in a humidified chamber with 1:100 diluted Rabbit-anti-p-β-catenin (gift from Linheng Li, Stowers Insitute for Medical Research, and The University of Kansas School of Medicine, Kansas City, Missouri) (6). Then the sections were incubated with AlexaFluor-594 goat-anti-Rabbit secondary antibodies (1:100; A11012, Invitrogen) for 1 hour at room temperature and counterstained with VectaShield plus DAPI (Vector Laboratories).

PCNA IF. Non-specific antibody binding was blocked using 20% goat serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in a humidified chamber with 1:100 diluted mouse-anti-PCNA (sc-56; Santa Cruz Biotechnology, Santa Cruz, CA). Then the sections were incubated with AlexaFluor-594 goat anti-mouse secondary antibodies (1:100; A11001, Invitrogen) for 1 hour at room temperature and counterstained with VectaShield plus DAPI (Vector Laboratories).

GFP IF. Non-specific antibody binding was blocked using 20% goat serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in a humidified chamber with 1:100 diluted mouse-anti-GFP (sc-9996; Santa Cruz Biotechnology, Santa Cruz, CA). Then the sections were incubated with AlexaFluor-488 goat anti-mouse secondary antibodies (1:100; A11001, Invitrogen) for 1 hour at room temperature and counterstained with VectaShield plus DAPI (Vector Laboratories).

GFP/TUNEL-double IF. GFP staining was processed as described except the secondary antibodies was AlexaFluor-594 goat anti-mouse IgG (1:100; A11005, Invitrogen). Before counterstaining, sections were washed with PBS and stained for TUNEL with the ApopTag

Fluorescein Kit (Millipore) according to the manufacturer's instructions. Sections were then counterstained as described.

53BP1 IF. Non-specific antibody binding was blocked using 20% goat serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in a humidified chamber with 1:100 diluted Mouse-anti- 53BP1 (IHC-00001; Bethyl Laboratories). Then the sections were incubated with AlexaFluor-594 goat-anti-mouse secondary antibodies (1:100; A11005, Invitrogen) for 1 hour at room temperature and counterstained with VectaShield plus DAPI (Vector Laboratories).

RAD51 IF. Non-specific antibody binding was blocked using 20% goat serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in a humidified chamber with 1:100 diluted Mouse-anti- RAD51 (ab1837; Abcam, Cambridge, MA, USA). Then the sections were incubated with AlexaFluor-594 goat-anti-mouse secondary antibodies (1:100; A11005, Invitrogen) for 1 hour at room temperature and counterstained with VectaShield plus DAPI (Vector Laboratories).

DNA-PKcs IF. Non-specific antibody binding was blocked using 20% goat serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in a humidified chamber with 1:100 diluted Mouse-anti- DNA-PKcs (#ab18356; Abcam, Cambridge, MA, USA). Then the sections were incubated with AlexaFluor-594 goat-anti-mouse secondary antibodies (1:100; A11005, Invitrogen) for 1 hour at room temperature and counterstained with VectaShield plus DAPI (Vector Laboratories).

BRCA1 IF. Non-specific antibody binding was blocked using 20% goat serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in a humidified chamber with

1:100 diluted Rabbit-anti- BRCA1 (S1387) (NB100-225; Novus, Littleton CO, USA). Then the sections were incubated with AlexaFluor-594 goat-anti-Rabbit secondary antibodies (1:100; A11005, Invitrogen) for 1 hour at room temperature and counterstained with VectaShield plus DAPI (Vector Laboratories).

γH2AX/p-H3 double-IF. γH2AX staining was processed as described except the secondary antibodies was AlexaFluor-488 goat anti-mouse secondary antibodies (1:100; A11001, Invitrogen). Before counterstaining, sections were incubated overnight at 4 °C in a humidified chamber with 1:100 diluted Rabbit-anti- p-Histone-H3 (#06-570; Millipore, Billerica, MA, USA). Then the sections were incubated with AlexaFluor-594 goat-anti-Rabbit secondary antibodies (1:100; A11012, Invitrogen) for 1 hour at room temperature and counterstained with VectaShield plus DAPI (Vector Laboratories). Sections were then counterstained as described.

X-gal staining for β-Galactosidase

β-Galactosidase tissue whole mount staining was performed as previously described (7) . In brief, 10 mg/ml tamoxifen (T5648; Sigma; 100 mg/kg) in corn oil (Cat# C8267 , Sigma) , was given through intraperitoneal injection 18 hours prior to IR in *Lgr5-EGFP/Rosa*$^{B/+}$ (LacZ reporter) mice. The top 1/3 small intestines (~10 cm) were collected and fixed at 4 °C (1% Formaldehyde, 0.2% Glutaraldehyde and 0.02% NP-40 in PBS) 96 hours after IR. After twice washing, the small intestines were stained overnight at room temperature in the dark on rolling platform (5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6 \cdot 3H_2O$, 2 mM $MgCl_2 \cdot 6H_2O$, 1 mg/ml X-Gal, 0.02% NP40 and 0.1% NaDeo in PBS). Whole-mount tissues were fixed in 4% paraformaldehyde and photographed, which was Swiss-rolled and followed by paraffin

embedding. The whole-mount pictures were taken with an Olympus SZX10 stereo microscope equipped a DP26, 5MP high-fidelity digital color camera and cellSens imaging software. Embedded tissues were cut to 5 μm and stained by Eosin as described in H&E staining and photographed. The numbers and fractions of entirely blue crypts were quantitated in 3 longitudinal 10-cm jujenal sections.

Supplemental Table

Table S1: Mouse primers used for RT-PCR analysis

Gene	Primer	Sequence
p53	Forward	5′-TGAAACGCCGACCTATCCTTA-3′
	Reverse	5′-GGCACAAACACGAACCTCAAA-3′
PUMA	Forward	5′-ATGGCGGACGACCTCAAC-3′
	Reverse	5′-AGTCCCATGAAGAGATTGTACATGAC-3′
p21	Forward	5′-ATGTCCAATCCTGGTGATGT-3′
	Reverse	5′-TGCAGCAGGGCAGAGGAAGT-3′
Bax	Forward	5′-GGGTTGTCGCCCTTTTCTACTT-3′
	Reverse	5′-AGCCCATGATGGTTCTGATCA-3′
Bak	Forward	5′-ATATTAACCGGCGCTACGAC-3′
	Reverse	5′-AGGCGATCTTGGTGAAGAGT-3′
GAPDH	Forward	5′-CTCTGGAAAGCTGTGGCGTGATG-3′
	Reverse	5′-ATGCCAGTGAGCTTCCCGTTCAG-3′

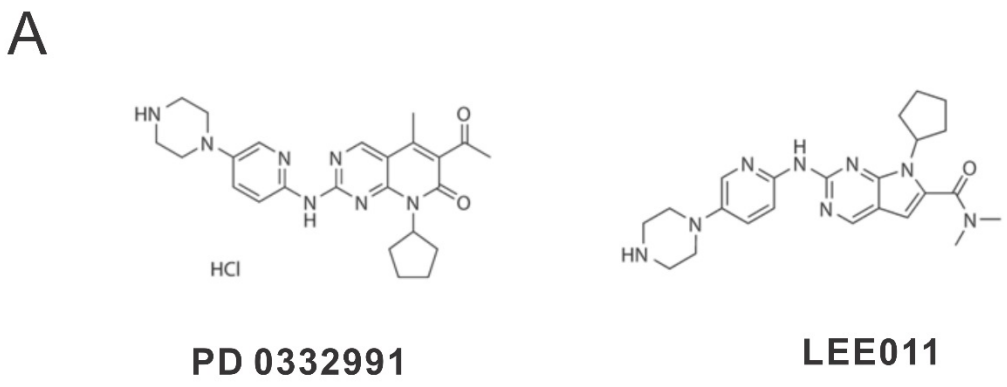
Supplemental References

1. Wu B, Qiu W, Wang P, Yu H, Cheng T, Zambetti GP, Zhang L, and Yu J. p53 independent induction of PUMA mediates intestinal apoptosis in response to ischaemia-reperfusion. *Gut.* 2007;56(5):645-54.
2. Qiu W, Carson-Walter EB, Kuan SF, Zhang L, and Yu J. PUMA suppresses intestinal tumorigenesis in mice. *Cancer Res.* 2009;69(12):4999-5006.

3. Parry JV, Farrington CP, Perry KR, Waight PA, Mortimer PP, and Miller E. Rational programme for screening travellers for antibodies to hepatitis A virus. *Lancet.* 1988;1(8600):1447-9.
4. Qiu W, Carson-Walter EB, Liu H, Epperly M, Greenberger JS, Zambetti GP, Zhang L, and Yu J. PUMA regulates intestinal progenitor cell radiosensitivity and gastrointestinal syndrome. *Cell stem cell.* 2008;2(6):576-83.
5. Leibowitz BJ, Wei L, Zhang L, Ping X, Epperly M, Greenberger J, Cheng T, and Yu J. Ionizing irradiation induces acute haematopoietic syndrome and gastrointestinal syndrome independently in mice. *Nat Commun.* 2014;5(3494.
6. He XC, Yin T, Grindley JC, Tian Q, Sato T, Tao WA, Dirisina R, Porter-Westpfahl KS, Hembree M, Johnson T, et al. PTEN-deficient intestinal stem cells initiate intestinal polyposis. *Nat Genet.* 2007;39(2):189-98.
7. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature.* 2007;449(7165):1003-7.

Supplemental Figures and legends

Figure S1, Wei et al. related to Figs. 1 and 3



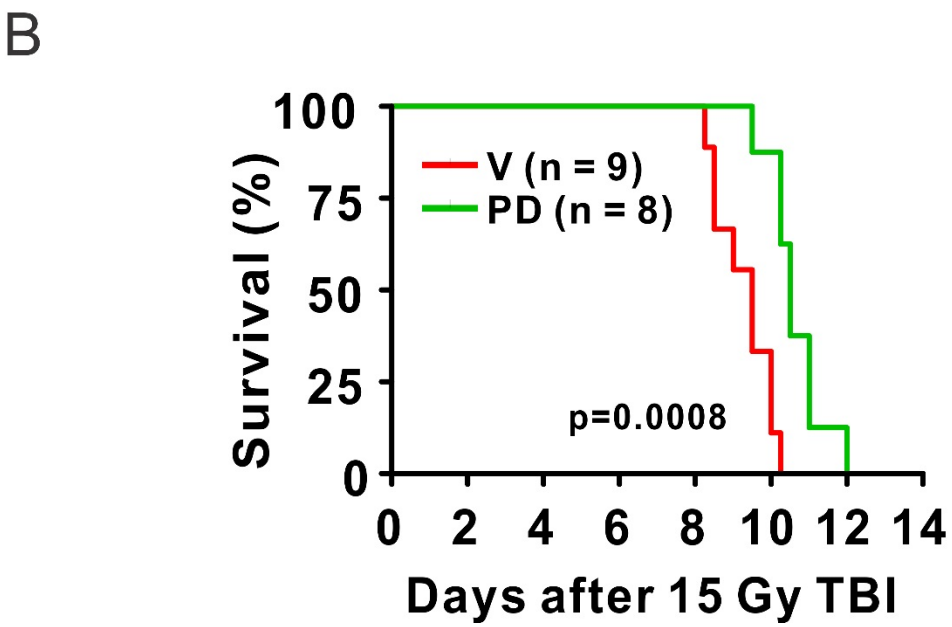


Figure S1. Radioprotection by CDK4/6 inhibitors. (A) Two structurally distinct and orally bioavailable compounds used in the study. (B) Kaplan-Meier survival curve of WT mice pretreated with three doses of vehicle (V) or PD 0332991 (PD) as depicted in Fig. 1A, and subjected to 15 Gy total body irradiation (TBI). N = 9 mice in V, and N = 8 mice in PD group. ***p = 0.0008 (Logrank Test).

Figure S2, Wei et al. related to Fig. 2

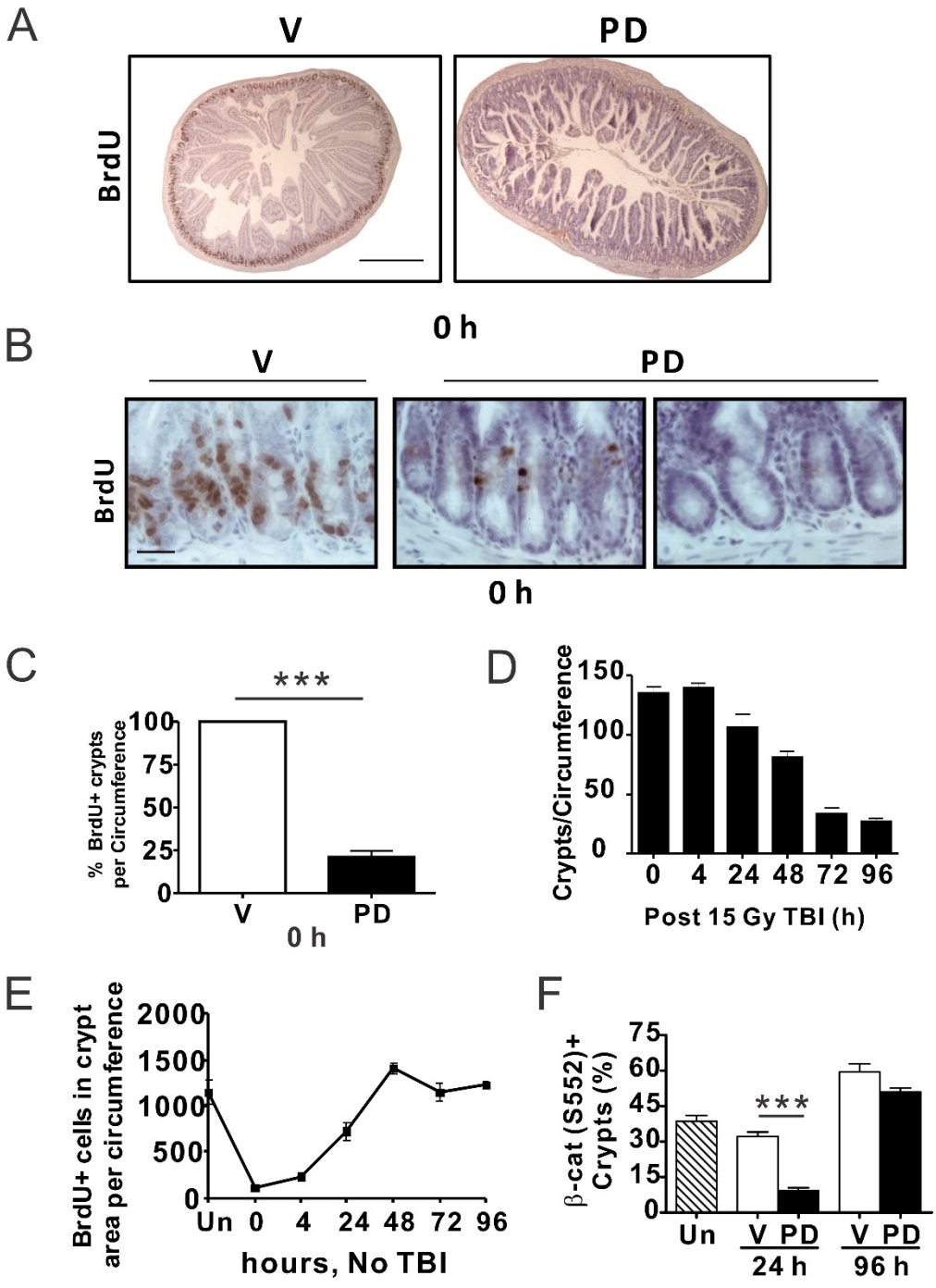


Figure S2. PD 0332991 blocks intestinal proliferation. Mice were pretreated with vehicle (V) or PD 0332991 (PD) in Figure 1A. The small intestine was analyzed at indicated times with or without 15 Gy TBI. (A) Representative pictures of BrdU IHC in intestinal cross sections. Scale bar: 500 μm. (B) Representative pictures of BrdU IHC in the intestinal crypts. Scale bar: 20 μm. (C) Quantification of BrdU+ crypt (%). (D) The number of crypts in the cross sections at

indicated times after 15 Gy TBI. (E) Quantification of BrdU+ crypt cells per cross section in mice treated with three dose of PD but no TBI from 0-96 hr, compared to unirradiated mice (Un). (F) Quantitation of p-β-catenin+ crypt fractions. Values are Mean ± SEM. N = 3 mice in each group. ***$p < 0.001$, V *vs.* PD (Student's t-test, two-tailed).

Figure S3, Wei et al. related to Fig. 3

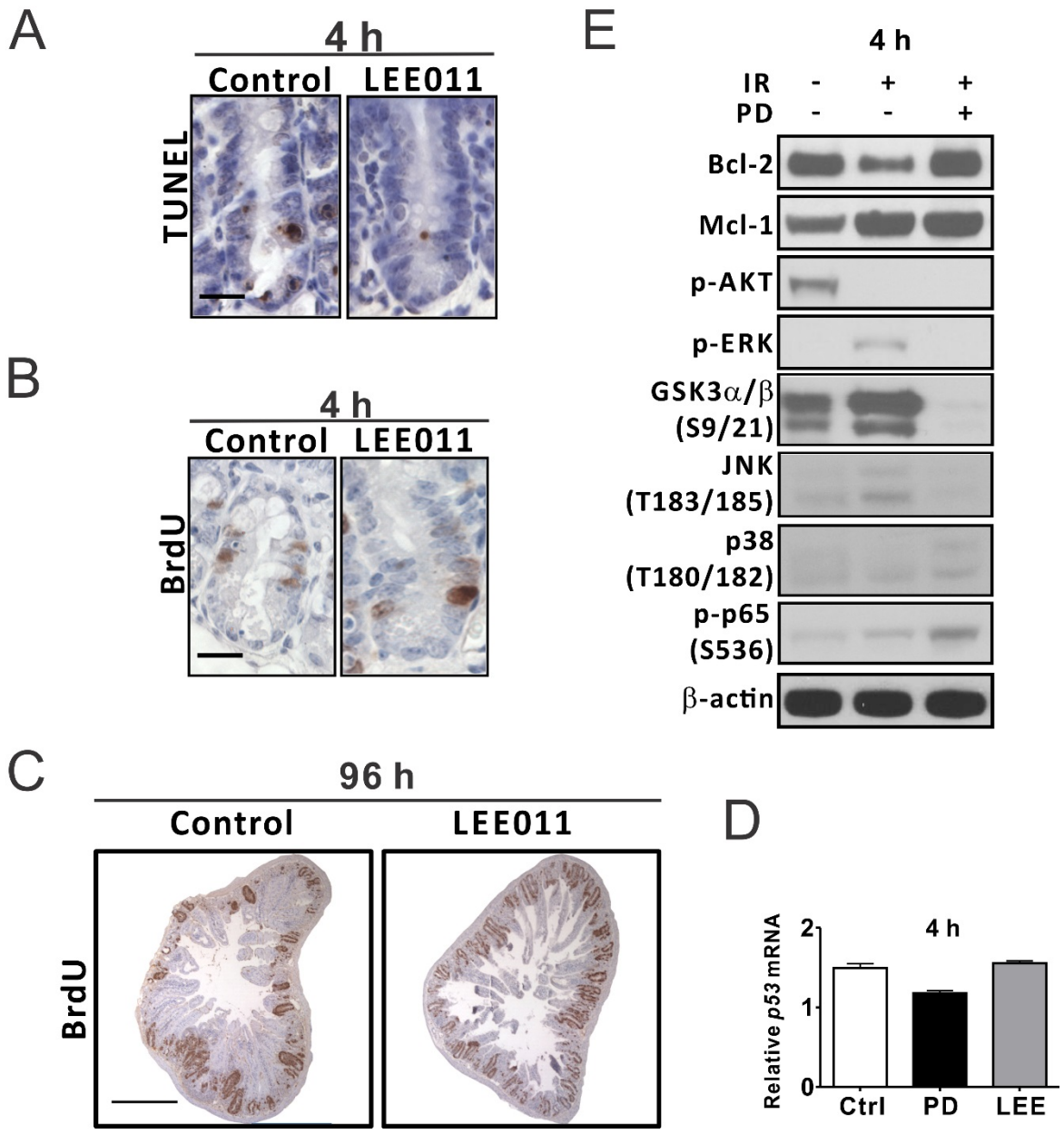


Figure S3. Pathway modulation by PD 0332991 and LEE011. Mice were pretreated with vehicle (V), PD 0332991 (PD), or LEE011 (LEE), and subjected to 15 Gy TBI. The small intestines were analyzed at indicated times. (A) Representative pictures of TUNEL staining at 4 hr. Scale bar: 20 μm. (B) Representative pictures of BrdU IHC at 4 hr. Scale bar: 20 μm. (C) Representative pictures of BrdU IHC in the crypts at 96 hr. Scale bar: 500 μm. (D) Intestinal expression of *p53* mRNAs was analyzed by real-time RT-PCR. cDNA was synthesized from RNA pooled from 3 mice. (E) Intestinal expression of indicated proteins at 4 hr was analyzed by western blotting. β-actin was used as the loading control. Lysates were pooled from 3 mice. Replicate samples run on separate gels are presented. Similar results were obtained in at least three independent experiments for western blotting and RT-PCR.

Figure S4, Wei et al. related to Fig. 4

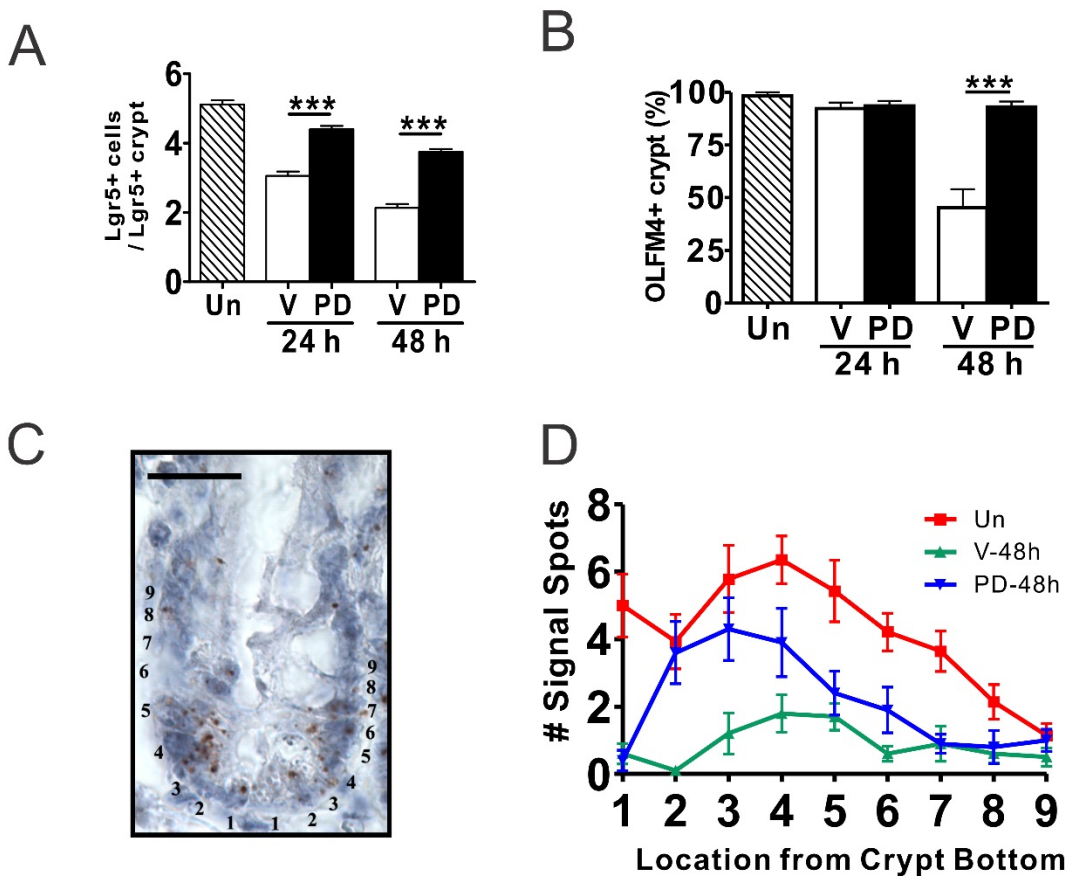


Figure S4. PD 0332991 protects intestinal stem cells. Mice were pretreated with vehicle (V) or PD 0332991 (PD), and subjected to 15 Gy TBI. The small intestines were analyzed at indicated times. (A) Quantification of number of Lgr5 cells in Lgr5+ crypts at 24 and 48 hr in *Lgr5-EGFP* mice. (B) Quantification of *OLFM4*+ crypts fractions at 24 and 48 hr. (C) Representative picture of *OLFM4* RNAscope in unirradiated mice. (D) Quantification of *OLFM4* spot signals based on cell location in unirradiated (Un) mice and those at 48 hr. (A), (B) and (D), values are Mean ± SEM. N = 3 mice in each group. ***$p < 0.001$, V *vs.* PD (Student's t-test, two-tailed).

Figure S5, Wei et al. related to Fig. 5

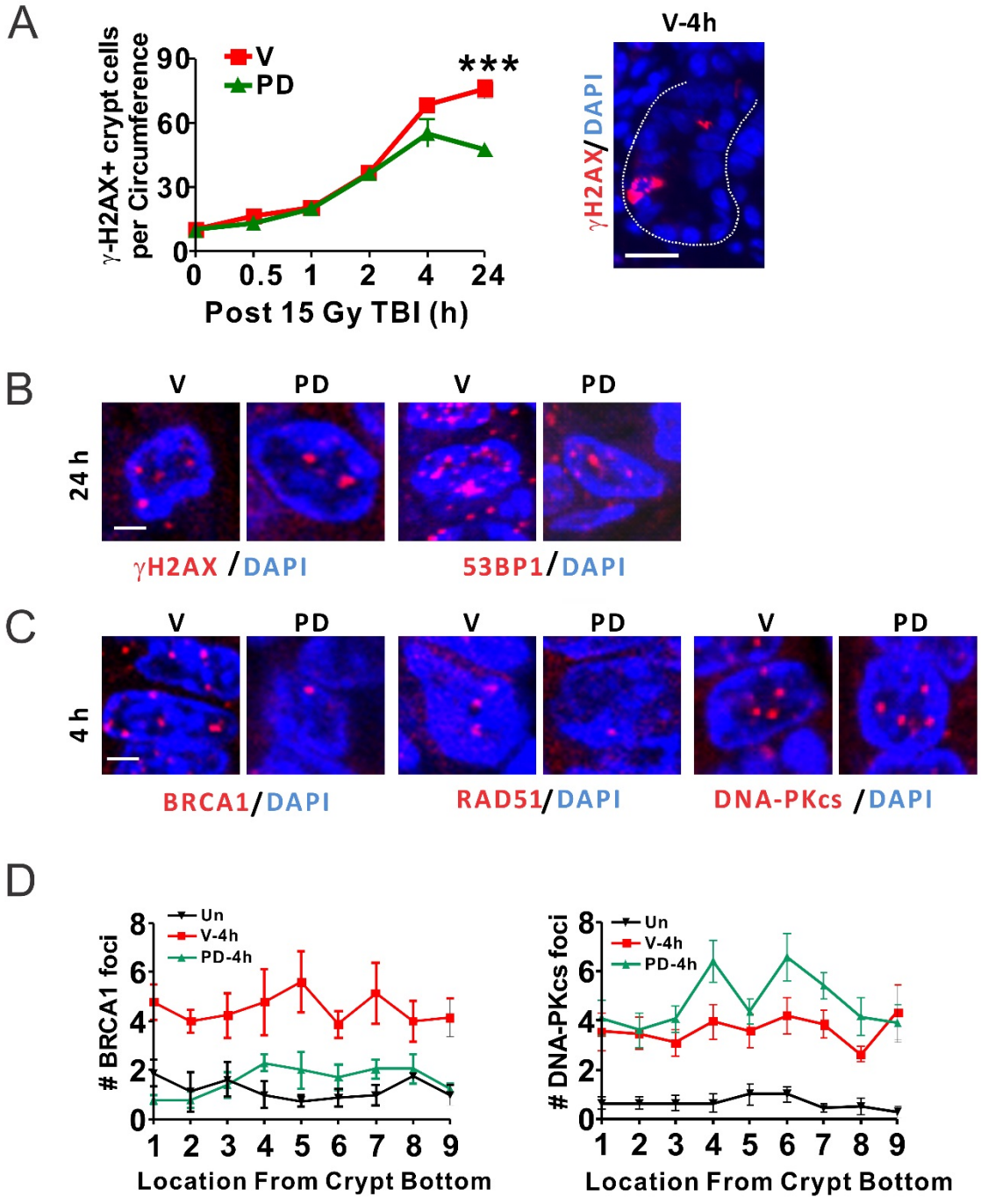


Figure S5. PD 0332991 modulates intestinal DNA damage response. Mice were pretreated with vehicle (V) or PD 0332991 (PD), and subjected to 15 Gy TBI. The small intestines were analyzed at indicated times. (A) Quantification of γH2AX+ crypt cells from 0-24 hr. Right, representative pictures of γH2AX IF at 4 hr. Red-γH2AX; Blue-DAPI. Dashed white line outlines the crypt. Scale bar: 20 μm. (B) Enlarged confocal pictures of γH2AX and 53BP1 foci

(red) in crypt cells at 24 hr. Scale bar: 2 μm. (C) Enlarged confocal pictures of BRCA1, RAD51, and DNA-PKcs foci (red) in crypt cells at 4 hr. Blue-DAPI. (D) Quantification of BRCA1 and DNA-PKcs foci in the crypts from cell position 1-9. (A) and (D), values are Mean ± SEM; N = 3 mice in each group. ***$p < 0.001$, V *vs.* PD (Student's t-test, two-tailed).

Figure S6, Wei et al. related to Fig. 6

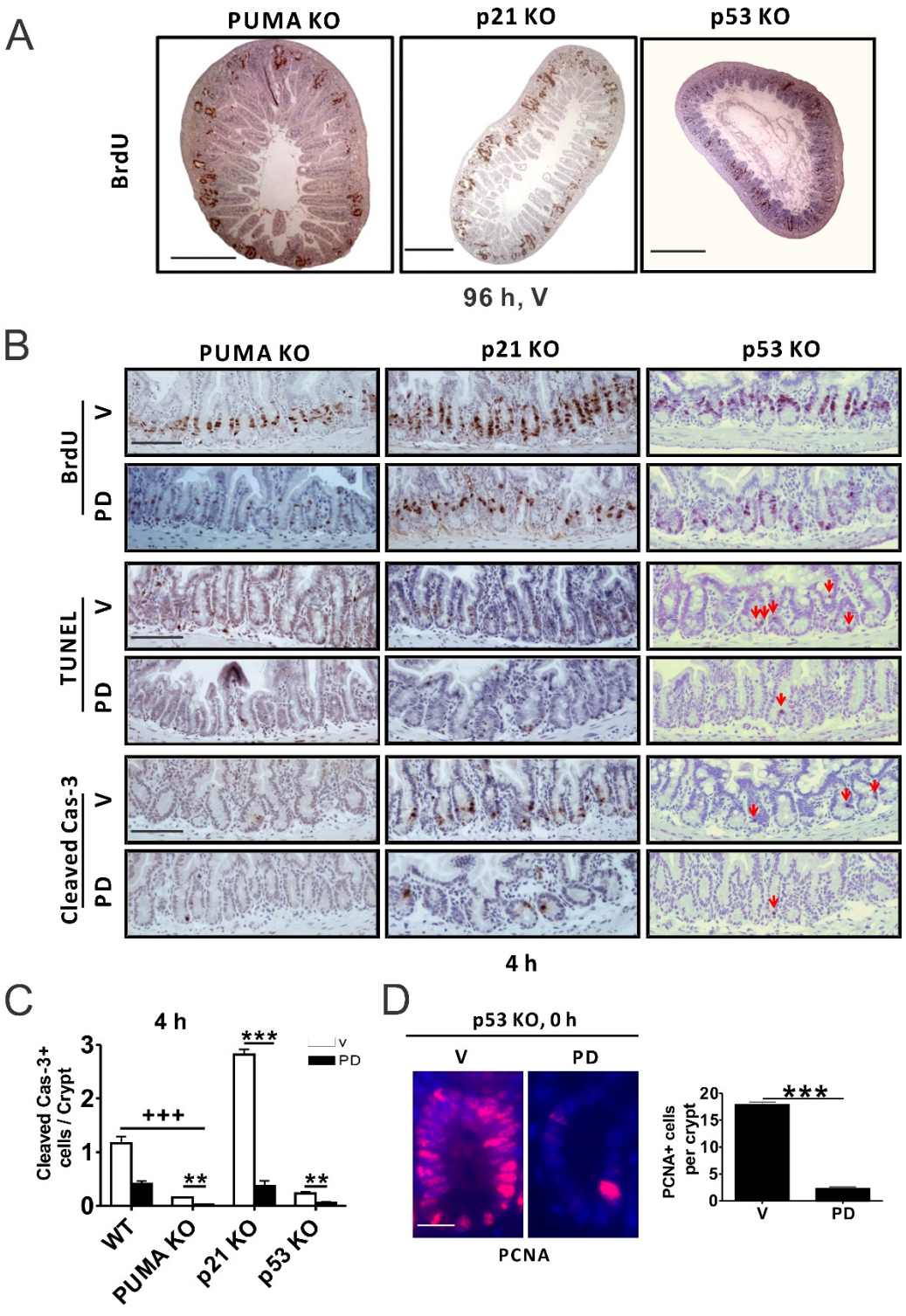


Figure S6. The effects of PD 0332991 in *PUMA* KO, *p21* KO and *p53* KO crypts after TBI. Mice with indicated genotypes were pretreated with vehicle (V) or PD 0332991 (PD) and

subjected to 15 Gy TBI. The small intestines were analyzed at indicated times. (A) Representative pictures of BrdU IHC in vehicle treated KO crypts at 96 hr. Scale bar: 500 μm. (B) Representative pictures of BrdU, TUNEL and cleaved-caspase-3 IHC in crypts at 4 hr. Scale bar: 200 μm. Arrowheads indicate positive cells. (C) Quantitation of cleaved-caspase-3 positive crypt cells with four genotypes. (D) Representative pictures of PCNA (Red) IF in *p53* KO crypts prior to TBI (0 h). Blue-DAPI. Scale bar: 20 μm. Right, quantification of PCNA+ *p53* KO crypt cells. C and D, values are Mean ± SEM. N = 3 mice in each group. $^{**}p < 0.01$, $^{***}p < 0.001$, V *vs.* PD within the same genotype (Student's t-test, two-tailed). $^{+++}p < 0.001$, WT/V *vs*. *PUMA* KO/PD (One-way ANOVA followed by Bonferroni's Multiple Comparison Test).

Figure S7, Wei et al.related to Fig. 6

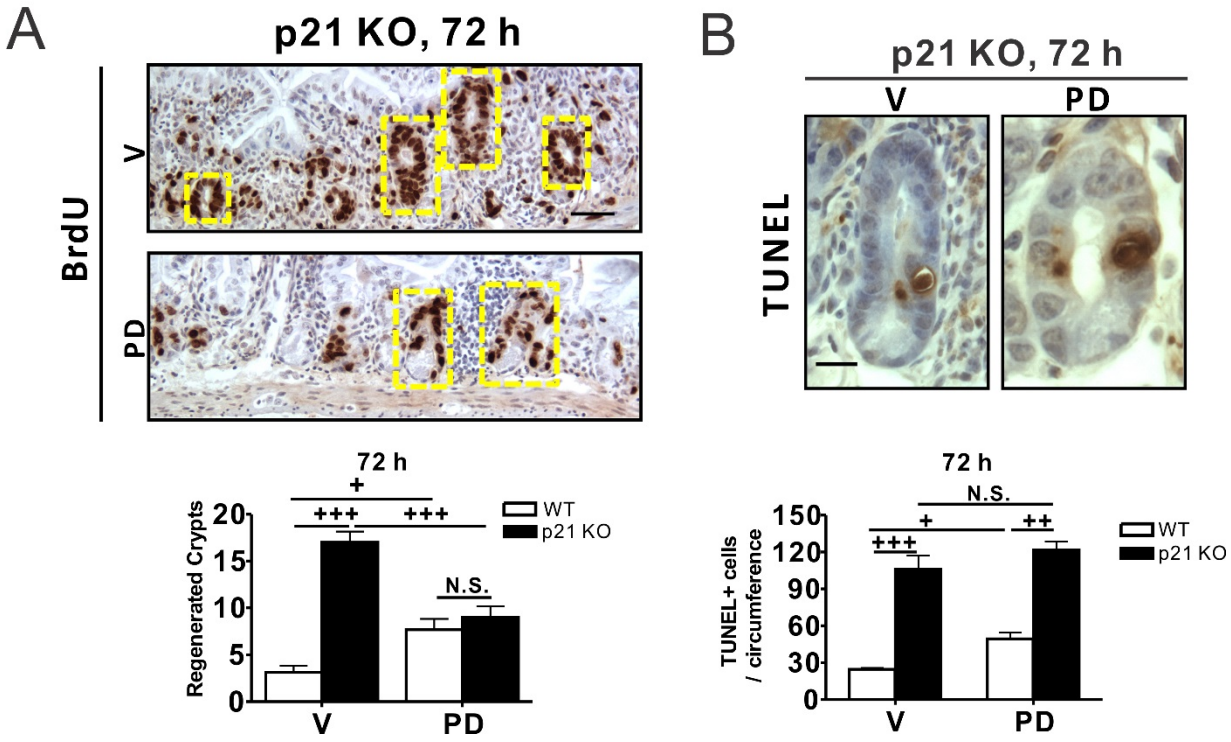


Figure S7. PD does not suppress nonapoptotic cell death in *p21* KO crypts after TBI. *p21* KO mice were pretreated with vehicle (V) or PD 0332991 (PD) and subjected to 15 Gy TBI. (A) Representative pictures of BrdU IHC in *p21* KO crypts at 72 hr. Scale bar: 50 μm. Bottom, quantification of regenerated crypts. (B) Representative pictures of TUNEL staining in *p21* KO crypts at 72 hr. Scale bar: 20 μm. Bottom, quantification of TUNEL+ cells in the crypt area. Values are Mean ± SEM. N = 3 mice in each group. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$, WT *vs.* *p21* KO and or indicated treatment (One-way ANOVA followed by Bonferroni's Multiple Comparison Test). N.S., non-significant.