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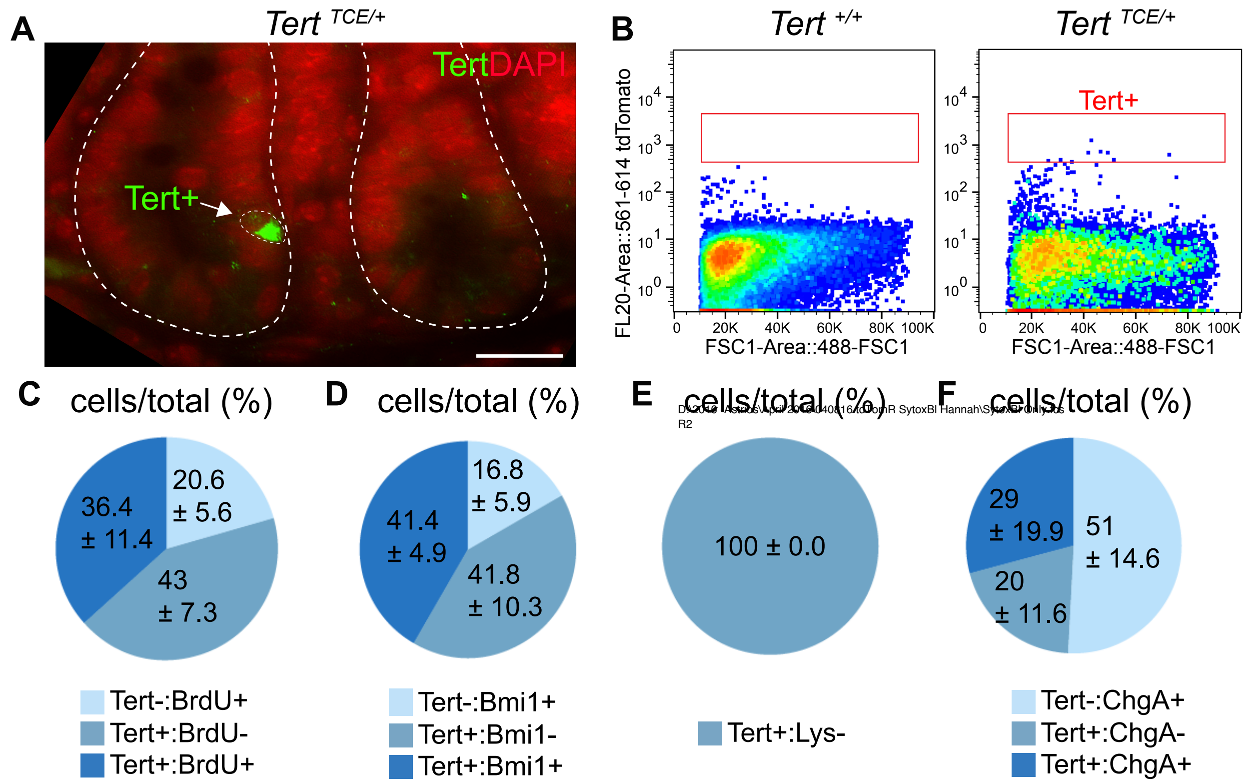
SUPPLEMENTAL FIGURES

Figure S1. Characterization of Tert+ cells in the small intestine (Related to Figure 1)



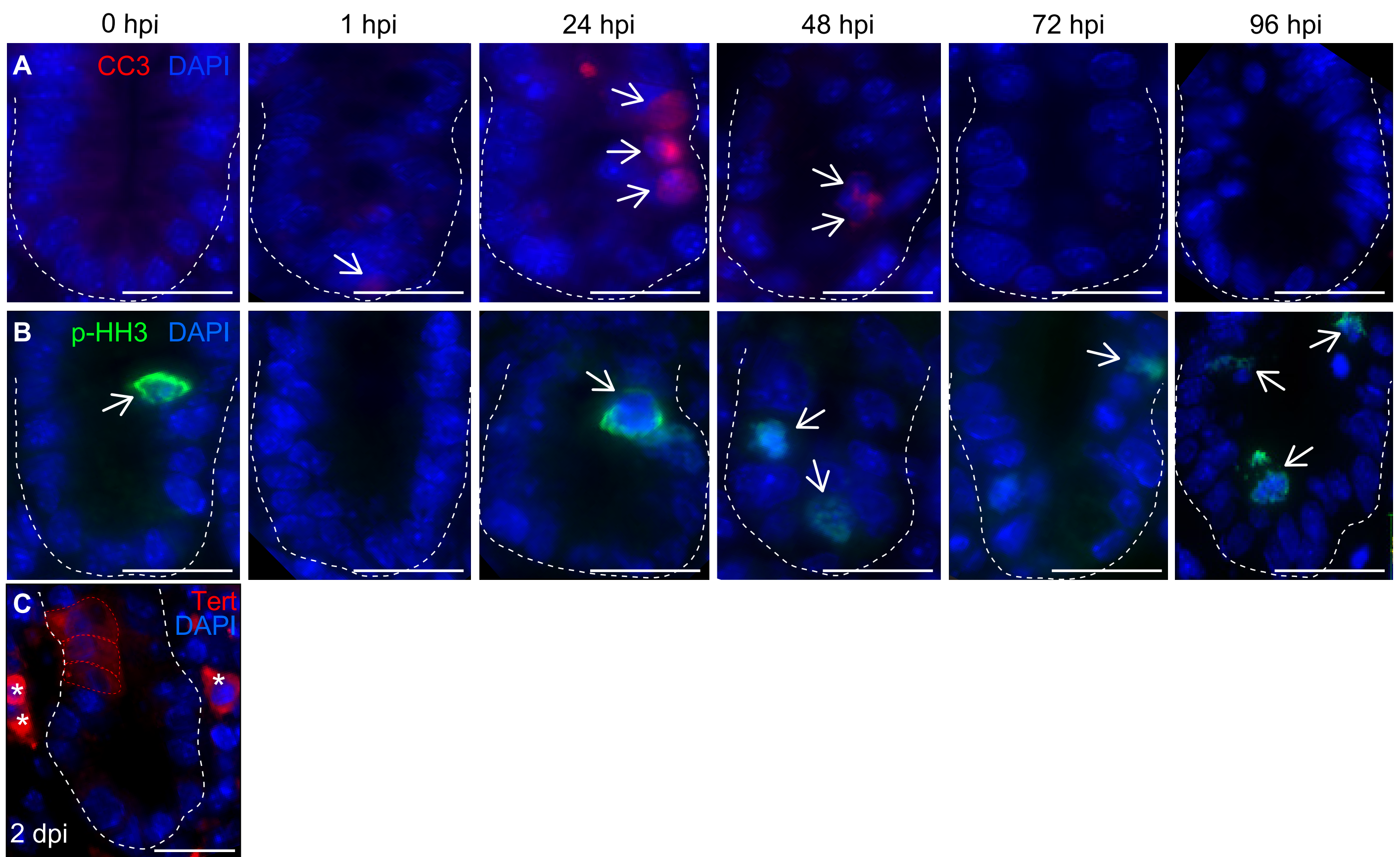
(A) Location of Tert+ cell in the crypts. Tert+ cells are rare (1 Tert+ cell per 120.5 ± 26.50 crypts).

(B) Assessment of Tert+ cell population in the small intestine.

Cells isolated from *Tert*$^{TCE/+}$ mice were quantified based on tdTomato fluorescence. Cells from *Tert*$^{+/+}$ mice were used as a negative control for gating. Quantitative analysis of Tert+ cells by FACS.

(C-F) Quantification graph of Figure 1F (**C**); Figure 1K (**D**); Figure 1L (**E**); Figure 1M (**F**).

Figure S2. IR-induced intestinal damage and regeneration (Related to Figure 2)

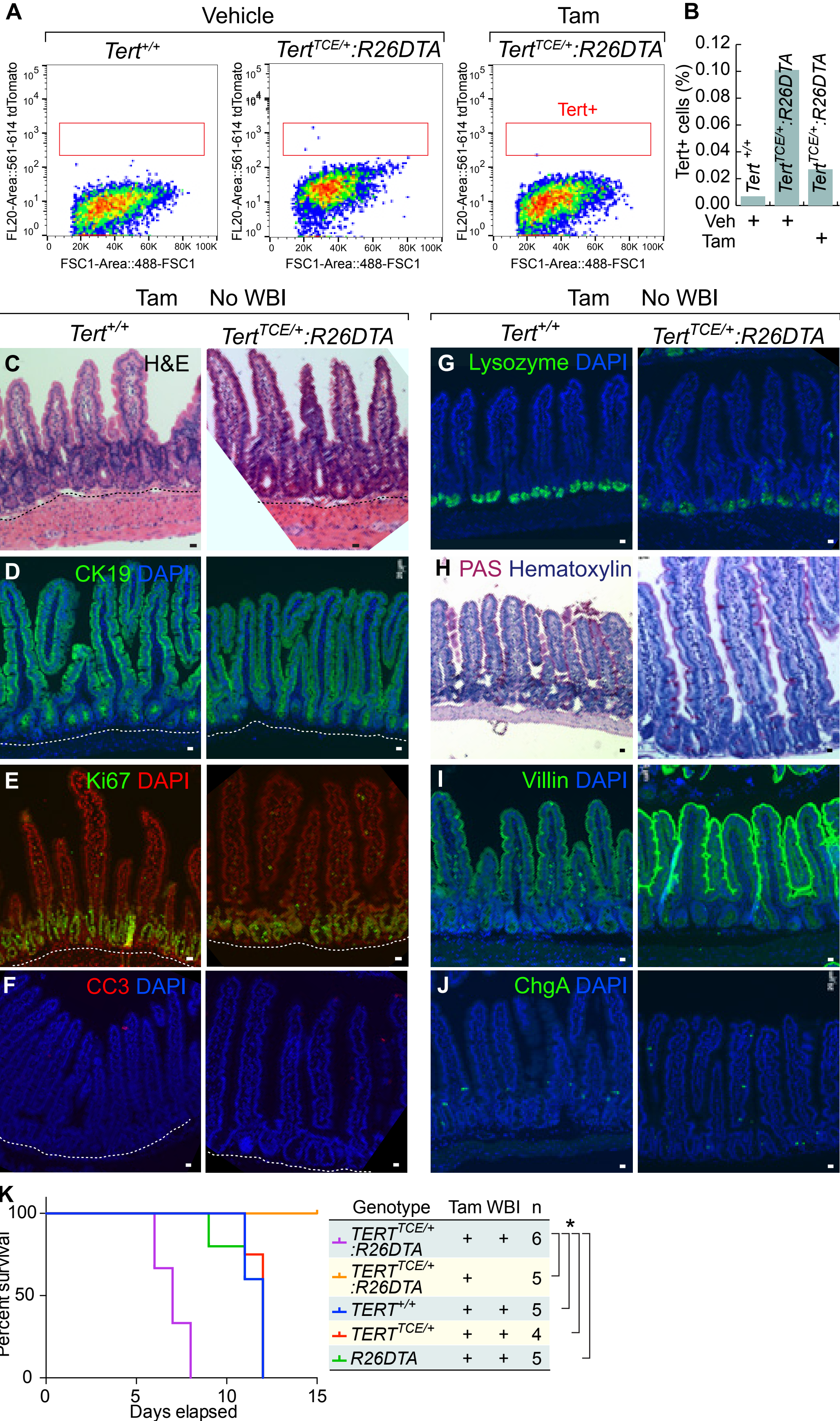


(A, B) WBI (10 Gy)-induced intestinal damage and regeneration.

Apoptosis (cleaved caspase 3) (**A**); mitosis (phospho-histone H3) (**B**). Arrows indicate the positive cells of each antibody. Hpi: hours post injury. Scale bars=20µm. The representative images are shown; N≥3.

(C) Expansion of Tert+ cells during regeneration. Tert+ cells rebuild the damaged intestinal epithelial at 2 dpi. Asterisk: non-specific signal. Scale bars=20µm.

Figure S3. Impaired intestinal regeneration by Tert+ cell ablation (Related to Figure 3)



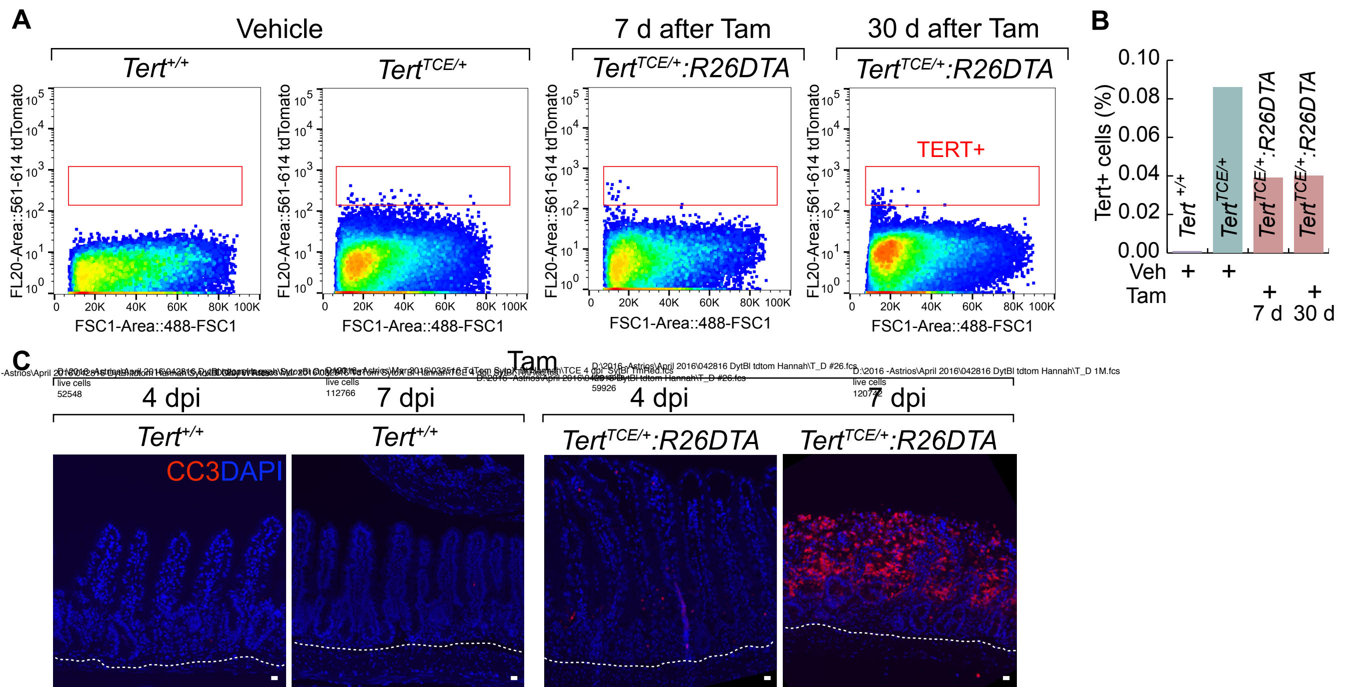
(A, B) Confirmation of Tert+ cell ablation after Tam treatment.

Tert$^{TCE/+}$:*Rosa26DTA* mice were administered by Vehicle (cone oil) or Tam (50 mg/kg, 5 times, 1 day interval). Tert+ cells were then quantified by FACS (**A**). Quantification graph (**B**). Tam treatment removed about 70% Tert+ cells in *Tert*$^{TCE/+}$:*Rosa26DTA* mice.

(C-J) No defect of intestinal epithelium after Tert+ cell ablation in homeostasis. H&E staining (**C**); epithelial structure (cytokeratin 19; CK19) (**D**); proliferation (Ki67) (**E**); apoptosis (CC3) (**F**); lysozyme (**G**); PAS (**H**); villin (**I**); chromogranin A (**J**). Scale bars=20μm; dot lines indicate the basal membranes below crypts. The representative images are shown; N≥3. Of note, in the absence of tissue injury (irradiation), Tert+ cell ablated mice (*Tert*$^{TCE/+}$*:Rosa26DTA*) were viable without any discernible phenotype.

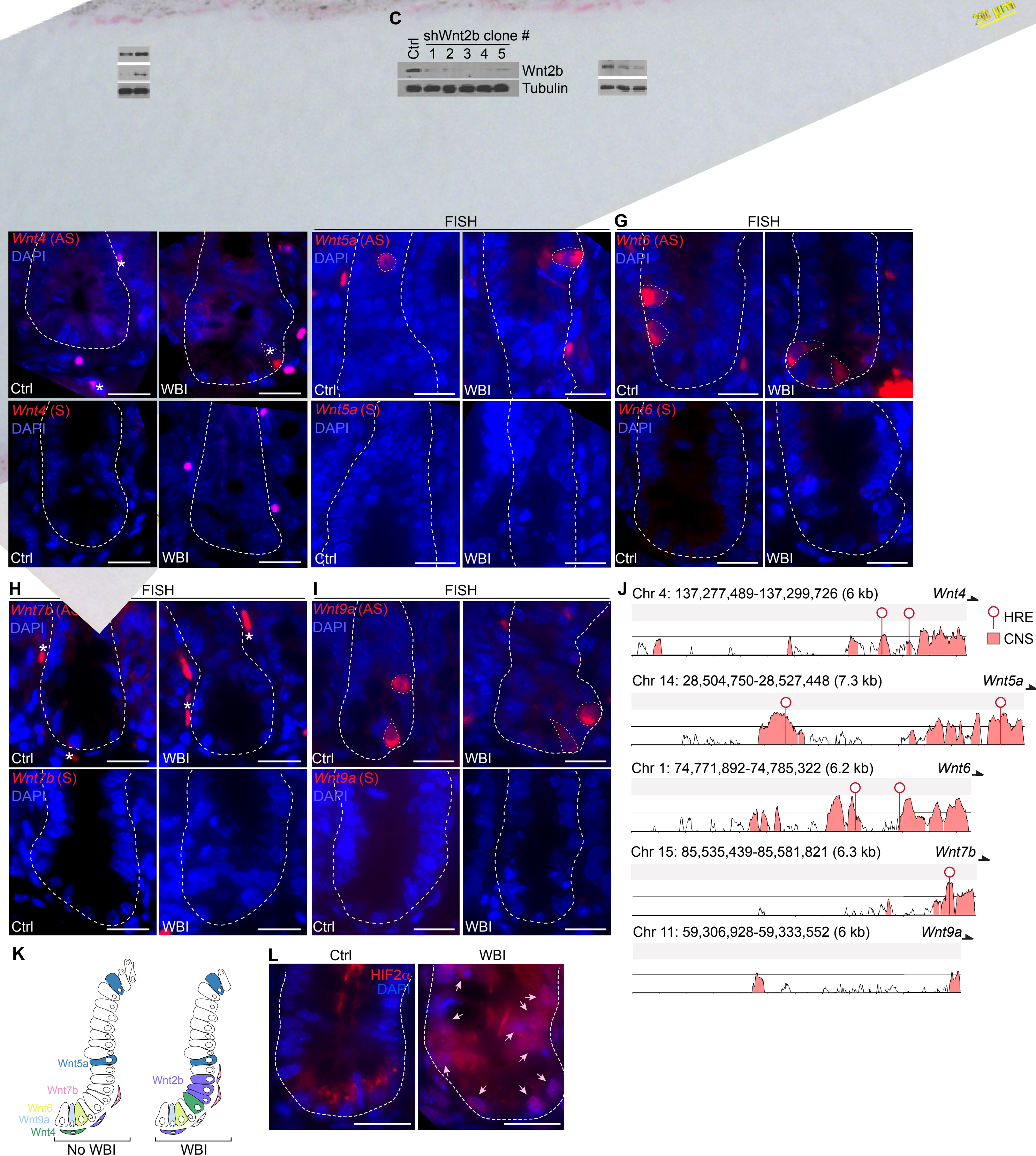
(**K**) Kaplan Meier survival graph. *Tert*$^{TCE/+}$*:Rosa26DTA* treated with Tam and WBI showed the early lethality compared to *Tert*$^{+/+}$*, Tert*$^{TCE/+}$*,* and *Rosa26DTA.* Asterisks (*)=$P<0.05$.

Figure S4. Defects in intestinal regeneration by Tert+ cell ablation (Related to Figure 3)



(A, B) No *de novo* generation of Tert+ cells after Tert+ cell ablation. $Tert^{TCE/+}$:*Rosa26DTA* mice were treated with Tam (50 mg/kg, 5 times, 1 day interval). 7 or 30 days after treatment, Tert+ cells were quantified by FACS (**A**). Quantification graph (**B**). $Tert^{TCE/+}$ mice were used as a positive control and $Tert^{+/+}$ mice were used as a negative control.

(C) Impaired intestinal regeneration by Tert+ cell ablation. Cleaved caspase-3 (CC3). Scale bars=20µm; dot lines indicate the basal membranes below crypts. The representative images are shown; N≥3.


C
shWnt2b clone #
Ctrl
1 2 3 4 5
Wnt2b
Tubulin
FISH
G
FISH
Wnt4 (AS)
DAPI
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Wnt4 (S)
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Wnt5a (AS)
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Wnt5a (S)
DAPI
Wnt6 (AS)
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Wnt6 (S)
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FISH
Wnt7b (S)
DAPI
I
FISH
Wnt9a (AS)
DAPI
Wnt9a (S)
DAPI
J
Chr 4: 137,277,489-137,299,726 (6 kb)
Wnt4
HRE
CNS
Chr 14: 28,504,750-28,527,448 (7.3 kb)
Wnt5a
Chr 1: 74,771,892-74,785,322 (6.2 kb)
Wnt6
Chr 15: 85,535,439-85,581,821 (6.3 kb)
Wnt7b
Chr 11: 59,306,928-59,333,552 (6 kb)
Wnt9a
K
Wnt5a
Wnt7b
Wnt6
Wnt9a
Wnt4
Wnt2b
No WBI
WBI
L
Ctrl
WBI
HIF2α
DAPI

(A) Expression of β-catenin in the crypt after IR(10 Gy, 24 hpi). Both total β-catenin and active β-catenin were upregulated by IR.

(B) Expression of nineteen Wnt ligands in the crypt after IR (10 Gy, 24 hpi). The expression of *Wnt2b, Wnt4, Wnt5a, Wnt6, Wnt7b*, and *Wnt9a* mRNA was upregulated by IR.

(C) Depletion of endogenous Wnt2b using shRNAs. HCT116 cells were transduced with lentiviruses encoding shRNAs against Wnt2b (five clones; #1~#5) and analyzed for Wnt2b Western blot assays.

(D) Depletion of endogenous Wnt2b using mouse shRNAs. NIH/3T3 cells were transduced with lentiviruses encoding shRNAs against Wnt2b (two clones; #1~#2) and analyzed for Wnt2b Western blot assays.

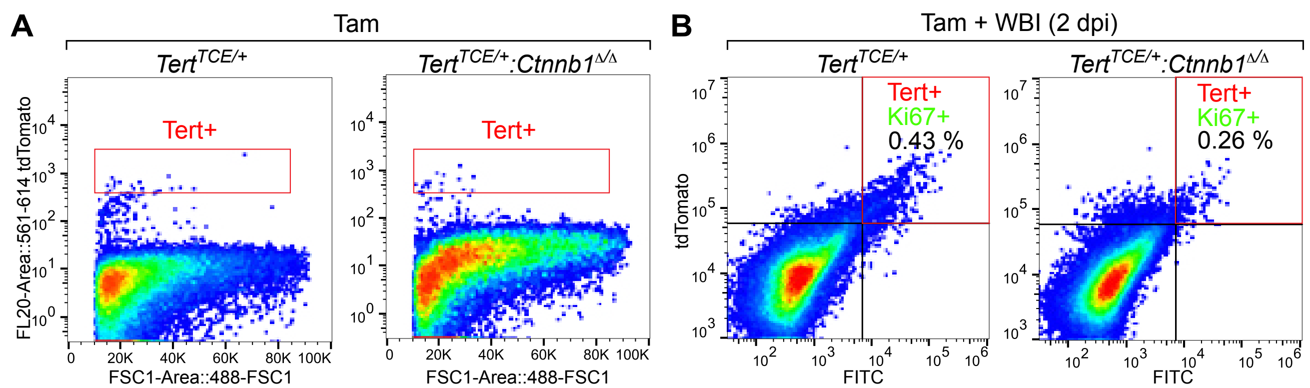
(E-I) FISH for *Wnt4* (**E**), *Wnt5a* (**F**), *Wnt6* (**G**), *Wnt7b* (**H**), and *Wnt9a* (**I**) expression by WBI (10 Gy, 24 hpi). AS: antisense; S: sense; scale bars=20μm; asterisks: Wnt4+ cells (mesenchymal or epithelial), Wnt7b+ cells (mesenchymal). Among the six Wnt ligands examined, we selected *Wnt2b* to further study how IR activates Wnt/β-catenin signaling.

(J) Wnt ligand (Wnt4, Wnt5a, Wnt6, Wnt7b, and Wnt9a) promoter analysis for hypoxia response element (HRE). Conserved non-coding sequence (CNS); arrow indicates the transcription start site.

(K) Illustration of Wnt ligands localization and expression pattern during homeostasis and regeneration.

(L) Nuclear translocation of HIF2α after IR (10 Gy, 24 hpi). In addition to HIF1α (Figure 4M), HIF2α also showed nuclear translocation by IR. Scale bars=20mm.

Figure S6. β-catenin CKO in Tert+ cells (Related to Figure 5)



(A) Representative FACS plots of Figure 5N. β-catenin CKO in Tert+ cells did not affect the quantity of Tert+ cells in the small intestine.

(B) Representative FACS plots of Figure 5O. β-catenin CKO in Tert+ cells decreased the number of proliferative Tert+ cells (Tert+:Ki67+) upon WBI.

The representative images are shown; N≥3.

SUPPLEMENTAL TABLES

Table S1. Primer sequence information (Related to Figures 1, 2, 4, and 5)

Gene	**Forward (5' to 3')**	**Reverse (5' to 3')**
Tert	TGGGTCTC CCTGTACCAAAT	GGCCTGTAACTAGCGGACACA
Bmi1	TGATTCTGGTTCGATGC	TGGCTCGCATTCATTTTATG
Dll1	TGAGCCAGTCTTTCCTTGAA	AGACCCGAAGTGCCTTTGTA
Krt19	GGGGGTTCAGTACGCATTGG	GAGGACGAGGTCACGAAGC
Lrig1	TTGAGGACTTGACGAATCTGC	CTTGTTGTGCTGCAAAAAGAGAG
Alpi	CATGGACCGCTTCCCATA	CTTGCACTGTCTGGAACCTG
Lgr5	ACCTGTGGGTAGATGACAATGC	TCCAAAGGGGTAGTCTGCTAT
Lig4	AGTCTGCAAAGGGGACATGA	CTTTCTCTTCCACCATGGCT
Xrcc5	TGTCCAACGACAGGTATTTTCG	AAGGGCATTATCAGTGCCATC
Xrcc4	CTTGCTTCTGAACCCAACGTA	TGGCCGTCAGTAAGTGTAATAAC
Blm	AGCGACACTCAGCCAGAAAAC	GCCTCAGACACGTTCACATCTT
Rpa1	GGGACACAGTCCAAAGTGGTG	GACACGGGCACAAATAGTCCA
Rad51c	CGGGAGTTGGTGGGTTATCC	CCGGCACATCTTGGTTTATTTGT
p19	TCAGGAGCTCCAAAGCAACT	TTCTTCATCGGGAGCTGGT
p107	AGGGAGAAGTTATACACTGGCT	CCCTTTCCCACAGTAGGAATGA
trp53bp2	AGTAAAGGCTCTAAAGCTCACCC	GTAAGAGGTCGGCATTGGAAG
p130	AACTTCCCCATGATTAGCCATG	GGTTAGAACACTGAAGGGCATTT
trp53ip2	GCGCCCTCCTTGATGGATG	TCCTCCAGCGGATTGCTCT
p21	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
Rb1	TGCATCTTTATCGCAGCAGTT	GTTCACACGTCCGTTCTAATTTG

Ccne1	GTGGCTCCGACCTTTCAGTC	CACAGTCTTGTCATTCTTGGCA
p16	CAAAGTGACAGATGCTCCAATCC	TTTTCCTTCTACGGCTCGTTTT
Ccnd1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
CD133	TCGTACTGGTGGCTGGGTGGC	ACCACAAGGATCATCAATATC
Axin2	TGCATCTCTCTCTGGAGGTG	TATGTCTTTGCACCAGCCAC
CD44	AGCGGCAGGTTACATTCAAA	CAAGTTTTGGTGGCACACAG
Ctgf	AGCCTCAAACTCCAAACACC	CAACAGGGATTTGACCAC
Glil1	ACCACCCTACCTCTGTCTATTC	TTCAGACCATTGCCCATCAC
Hes1	GGTATTTCCCCAACACGGT	GGCAGACATTCTGGAAATGA
Ptprq	CGGAGGTTACTGGAACCGTG	CAGGGTCCCCACATAGCCT
Nestin	CTGCAGGCCACTGAAAAGTT	GACCCTGCTTCTCCTGCTC
ChgA	GCAGAGGACCAGGAGCTAGA	CAGGGGCTGAGAACAAGAGA
ChgB	ACAGGAAGAAGGCAGACGAA	TCCTTCAGTGAAAGGCTCGT
Mmp7	CCCGGTACTGTGATGTACCC	AATGGAGGACCCAGTGAGTG
Wnt1	TCTTTGGCCGAGAGTTCGTG	AGAGAACACGGTCGTTCGC
Wnt2	ATCTCTTCAGCTGGCGTTGT	AGCCAGCATGTCCTCAGAGT
Wnt2b	CACGTCACAACAATGAGGCT	TCGGCACCTTGAAGTACGTG
Wnt3	TGGAACTGTACCACCATAGAT	ACACCAGCCGAGGCCATG
Wnt3a	ACCGTCACAACAATGACGCT	TCGGCACCTTGAAGTACGTG
Wnt4	AACGGAACCTTGAGGTGATG	GGACGTCCACAAAGGACTGT
Wnt5a	CACGCTATACCAACTCCTCTGC	AATATTCCAATGGGGTTCTTC
Wnt5b	GCCGCGGATGAGGAGTG	GCCTCAACCCATCCCAATGC
Wnt6	CGGAGACGATGTGGACTT	GGAACCCGAAAGCCCATG
Wnt7a	ATCAAGCAGAATGCCCGGAC	TAGCTCTCGGAACTGTGGCA

Wnt7b	ACTCCGAGTAGGGAGTCGAGA	GCGACGAGAAAAGTCGATGC
Wnt8a	TGGGAACGGTGGAATTGTCC	GCAGAGCGGATGGCATGAAT
Wnt8b	GTGGACTTCGAAGCGCTAAC	TTACACGTGCGTTTCATGGT
Wnt9a	TGCTTTCCTCTACGCCATCT	TATCACCTTCACACCCACGA
Wnt9b	GTGTGGTGACAATCTGAAG	GTGTGGTGACAATCTGAAG
Wnt10a	GCTTCGGAGAACGCTTCTCT	ATTTGCACTTACGCCGCATG
Wnt10b	GGAAGGGTAGTGGTGAGCAA	CACTTCCGCTTCAGGTTTTC
Wnt11	GTTCTCCGTGATTGCAGGCG	TTGCGTCTGATTCAGTGCCA
Wnt16	CTGTGACACCACCTTGCAGA	CAGGTTTTCACAGCACAGGA
18S rRNA	AAGTCCCTGCCCTTTGTACACA	GATCCGAGGGCCTCACTAAAC
Wnt2b i	CTAGTCCCAGTGTGGGGAAA	TTCTCGGTGTCTGGCTTTCT
Wnt2b ii	CTGGGGACATTTGCTCTGTT	TGGGGTTCTTGGCTTGTTAC
Wnt2b iii	GGAACACAGCCTCTTCTGG	CACAGATGCTCGGCTATTGA
Wnt2b iv	GGGCACTCTGCTCCATTTAG	CACGGGGAATGCTACAAAGT
Wnt2b v	AAAGCACCAAGGTGGACAAG	TGCGCTTCTAGGAAACTGGT

Table S2. Antibody information for IHC (Related to Figures 1-5)

Protein; Company (cat #); Dilution

BrdU and CldU; Abcam (ab6326); 1/100

Ki67; Abcam (ab1667); 1/100

IdU; Abcam (ab181664); 1/100

Bmi1; Abcam (ab14389); 1/100

p-γH2AX; Cell signaling (#9718);1/200

Cleaved Capase 3; Cell signaling (#9664s); 1/200

p-HH3; Cell signaling (#9706); 1/200

Lysozyme; Abcam (ab108508); 1/400

Villin; Thermo (PA5-22072); 1/200

ChgA; Abcam (ab15160); 1/200

CK19; Abcam (ab133496); 1/200

β-catenin; Cell signaling (#9587s); 1/250

β-galactosidase (LacZ); Abcam (ab4761); 1/200

CD44; BD Biosciences (550538); 1/100

Cyclin D1; Cell signaling (#2978S); 1/50

8-oxo-dG; Abcam (ab62623); 1/200

HIF1α; Abcam (ab1); 1/100

HIF2α; Abcam (ab199); 1/100

C-Myc; Santa Cruz (sc 764); 1/80

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Crypt and single cell organoids

For crypt organoids, intestinal crypts were isolated. For single cell isolation, crypts were digested with Accumax (Stem cell technology 07921) for 5 min and single cells were collected through 70 µm (BD 087712) and 40 µm (BD 087711) cell strainers. Crypts or single cells were then suspended in growth factor-reduced Matrigel (Corning 356231). After polymerization, organoid culture medium composed of 50% conditioned medium from L-WRN cells (ATCC® CRL-3276™) (1:1 dilution with Advanced DMDM/F12) containing 1 mM *N*-acetyl cysteine, B27 supplement, N2 supplement, 50 ng/ml mouse EGF, 10 µM Y-27632 was overlayed.

Organoid lentiviral infection

Single cells isolated from crypts of *C57BL/6* were incubated with media containing lentiviruses expressing mouse Wnt2b shRNAs (Dharmacon clone ID V3LMM_505459, V3LMM_505462), 7 µg/ml polybrene, 1 mM Jagged-1 peptide, and 10 µM Y-27632 for 2 h at 37 °C. Infected cells were then suspended with Matrigel, seeded in 24 well culture plates, and overlayed with organoid culture medium. Next day, fresh organoid culture medium with selection antibiotic (puromycin, 2 µg/ml) was added to the organoids. Survival of GFP+ cells was considered as infected cells. GFP shRNA (Sigma SHC005) was used as a negative control.

X-gal staining

Axin2-LacZ mice were treated with 10 Gy WBI and intestine was collected for 5-bromo-4-chloro-3-indolyl-β-D-galacto-pyranoside (X-gal) staining (Sigma GALS-1KT). Intestine was fixed with 2% formaldehyde containing 0.2% glutaraldehyde for 10 min at room temperature, rinsed with PBS, and incubated with Staining solution [2 mM $MgCl_2$, 4 mM $K_3Fe(CN)_6$, 4 mM $K_4Fe(CN)_6$, 1 mg/ml X-gal] for 2 h at 37°C. Stained intestine was washed, post-fixed, and analyzed. Nuclear fast red was used for nuclear counterstaining.

Cell line

CCD 841 CoN cell line was acquired from ATCC (CRL-1790™) and maintained with Eagle's Minimum Essential Medium (ATCC® 30-2003) containing 10% fetal bovine serum. CCD 841 CoN is originated from 21 weeks gestation female fetus. NIH/3T3 cell line was acquired from ATCC (CRL-1658™) and maintained with Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum.

Isolation of mesenchymal and epithelial cells

Mouse intestine was collected, cut transversely, and minced into small pieces in cold PBS. Tissue was incubated in 5 mM EDTA for 1 h at 4°C on orbital shaker. After incubation, tissues were passed through a 100 μm cell strainer. Flow thorough (epithelium) and remained pieces on the strainer (mesenchyme) were collected for further analysis.

Detection of intracellular reactive oxygen species

CCD841CoN was cultured on coverglass until 50% confluent. After 10 Gy IR, cells were incubated with cell-permeable 2',7'-dichlorodihydrofluorescein diacetate (1 μM, H_2DCFDA; Thermo) for 30 min. Cells were then washed twice with PBS, stained with Hoechst 33342 for nucleus counterstaining, and photographed using fluorescence microscope (Zeiss; AxioVision).

Immunohistochemistry

Mouse intestinal tissues were fixed in 10% neutral buffered formalin overnight, made into Swiss role, and embedded in paraffin. Tissue samples were then sectioned (5 μm), deparaffinized, processed for antigen retrieval, blocked, incubated with primary antibody, and fluorescence or peroxidase-conjugated secondary antibody. Samples were mounted and photographed using a microscope (Zeiss; AxioVision). For comparison among the experiment groups, images were captured with the same exposure time. For peroxidase-conjugated secondary antibody, 3,3'-Diaminobenzidine (DAB) substrate was used, followed by hematoxylin for nuclear counterstaining. All antibody information is listed in Supplemental Table2.

Immunofluorescence staining

Cells grown on coverglass were fixed with 4% paraformaldehyde, permeabilized with 0.01% Triton X-100 in PBS, blocked with 5% BSA, incubated with primary antibody (HIF-1α) and fluorescence-conjugated secondary antibody. Cells were stained with Hoechst 33342 for nuclear counterstaining and photographed using a fluorescence microscope (Zeiss; AxioVision). For comparison, images were captured under the same exposure time. Representative images were shown.