Cell Reports, Volume *18*

Supplemental Information

Canonical Wnt Signaling Ameliorates Aging of Intestinal Stem Cells

Kodandaramireddy Nalapareddy, Kalpana J. Nattamai, Rupali S. Kumar, Rebekah Karns, Kathryn A. Wikenheiser-Brokamp, Leesa L. Sampson, Maxime M. Mahe, Nambirajan Sundaram, Mary-Beth Yacyshyn, Bruce Yacyshyn, Michael A. Helmrath, Yi Zheng, and Hartmut Geiger

Supplemental Information

Youthful levels of canonical Wnt signaling ameliorate aging of intestinal stem cells

Kodandaramireddy Nalapareddy, Kalpana J. Nattamai, Rupali S. Kumar , Rebekah Karns, Kathryn A. Wikenheiser-Brokamp [3], Leesa L. Sampson, Maxime M. Mahe, Nambirajan Sundaram , Mary-Beth Yacyshyn , Bruce Yacyshyn , Michael Helmrath , Yi Zheng and Hartmut Geiger

Figure S1

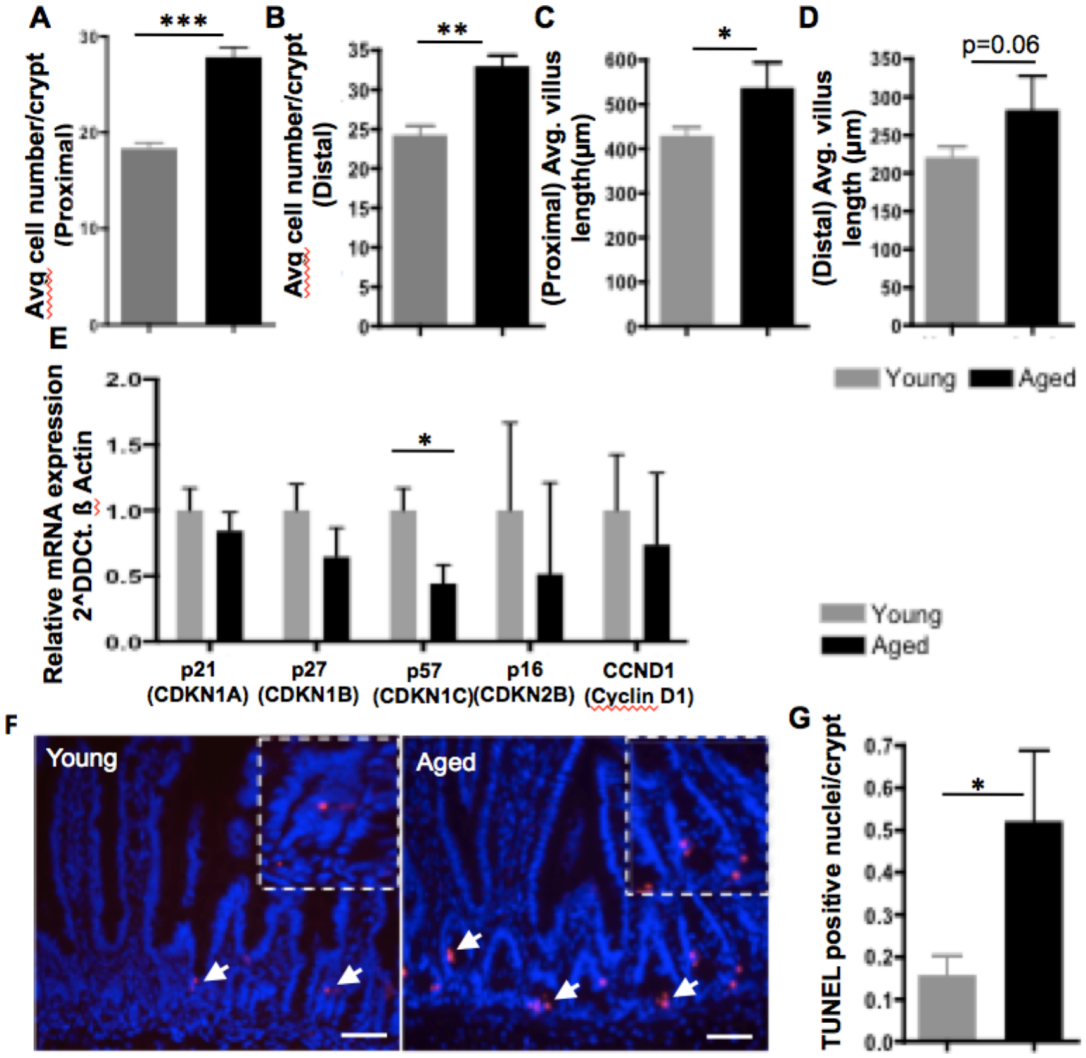


Figure S1. Changes in Cell number, villus length, proliferative response, cell cycle and apoptosis in young and aged intestine. Related to Figure 1 (A and B) Number of cells per crypt in the proximal and distal part of mouse small intestine (C and D) Histogram showing average length of the villus in µm in both the proximal and distal part of mouse small intestine. (E) Expression of *p21* (CDKN1A), *p27* (CDKN1B), *p57* (CDKN1C), *p16* (CDKN2B) and *Cyclin D1* (CCND1) normalized to ß actin transcript levels in young and aged crypts. (F) Representative picture of TUNEL staining in young and aged crypts, scale bar=50µm (G) Number of TUNEL positive nuclei per crypt in the proximal part of mouse small intestine. *=p<0.05, **=p<0.01, ***=p<0.001, error bars represent standard deviation.

Figure S2

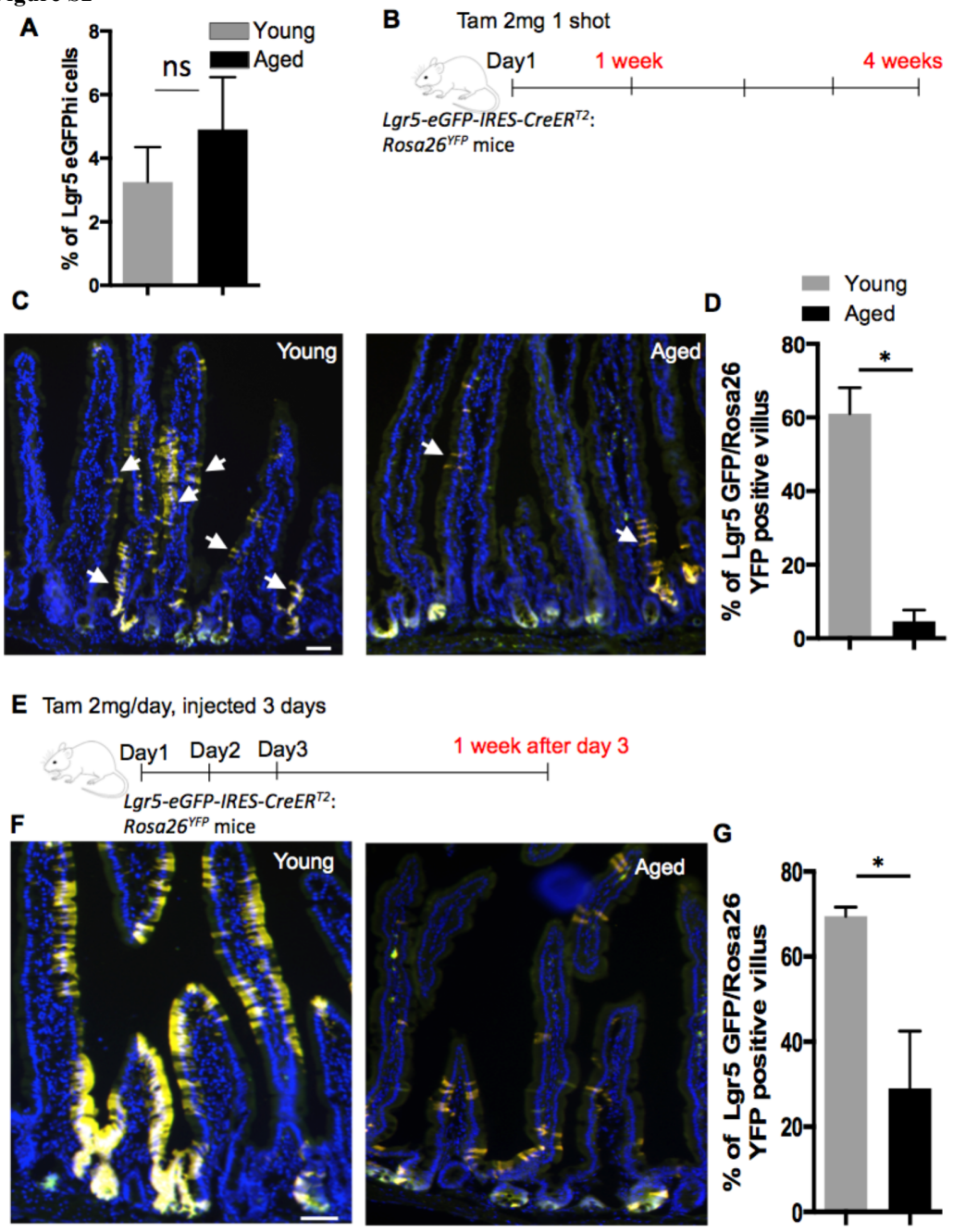


Figure S2. Number of Lgr5 eGFP hi cells and lineage tracing experiments. Related to Figure 2
(A) Percentage of Lgr5 eGFPhi cells from the proximal part of young and aged intestinal epithelium. (B) Experimental setup for lineage tracing (C) Representative picture of a longitudinal section of young and aged *Lgr5*$^{eGFPCreER(T2)}$ *Rosa26* ^{YFP} mice 1 week after 1 shot tamoxifin treatment. (D) Number of YFP positive villus/Lgr5 eGFP positive crypts in the proximal part of young and aged mouse intestine 1 week after 1 shot tamoxifen (E) Experimental setup for lineage tracing (F) Representative picture of longitudinal section of the proximal part of young and aged *Lgr5*$^{eGFPCreER(T2)}$ *Rosa26* ^{YFP} mice 1 week after 3 shots (1shot /day) of tamoxifin (G) Number of YFP positive villus/Lgr5 eGFP positive crypts in the proximal part of young and aged mouse intestine, 1 week after 3 shots (1shot/day) of tamoxifin.

Figure S3

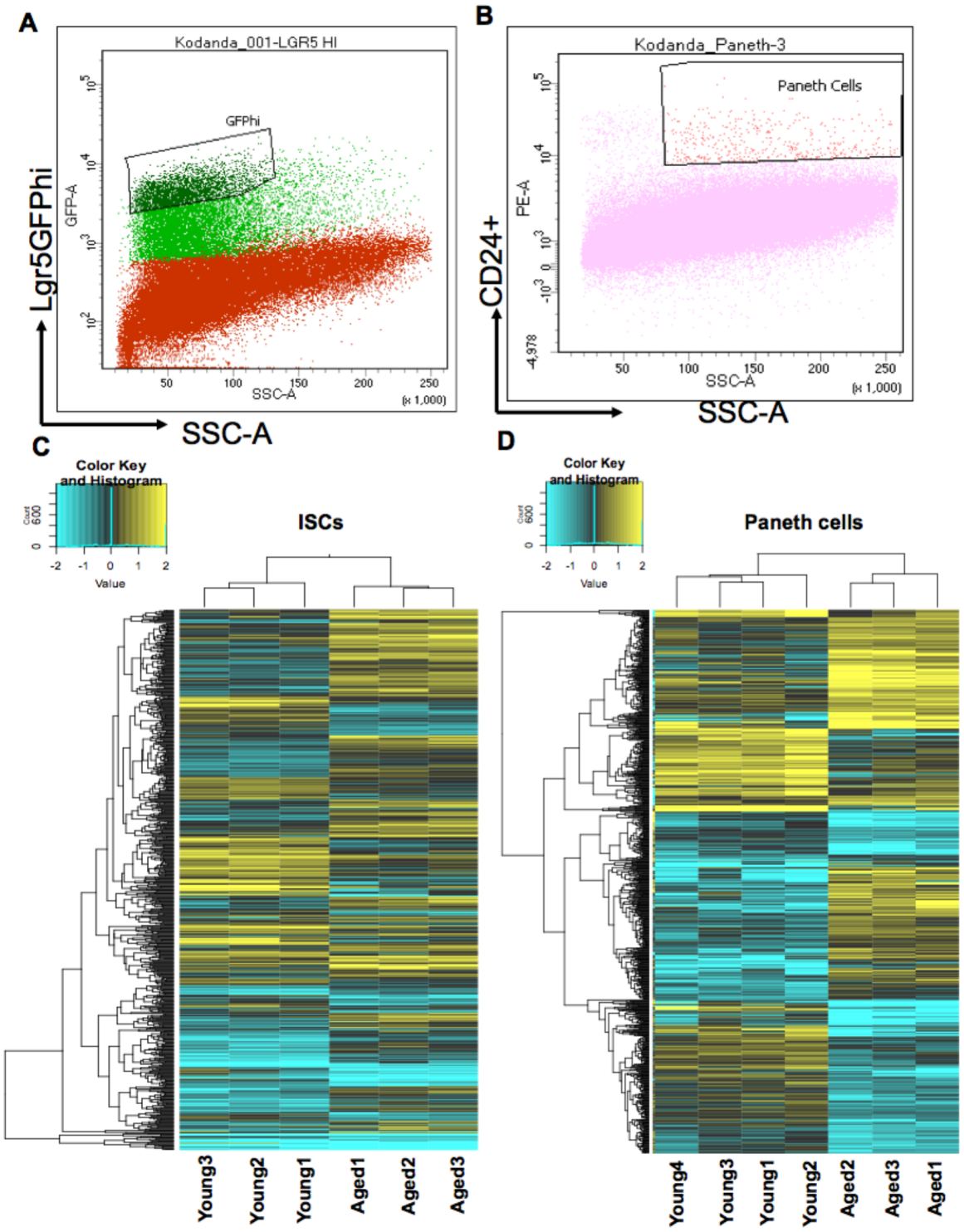
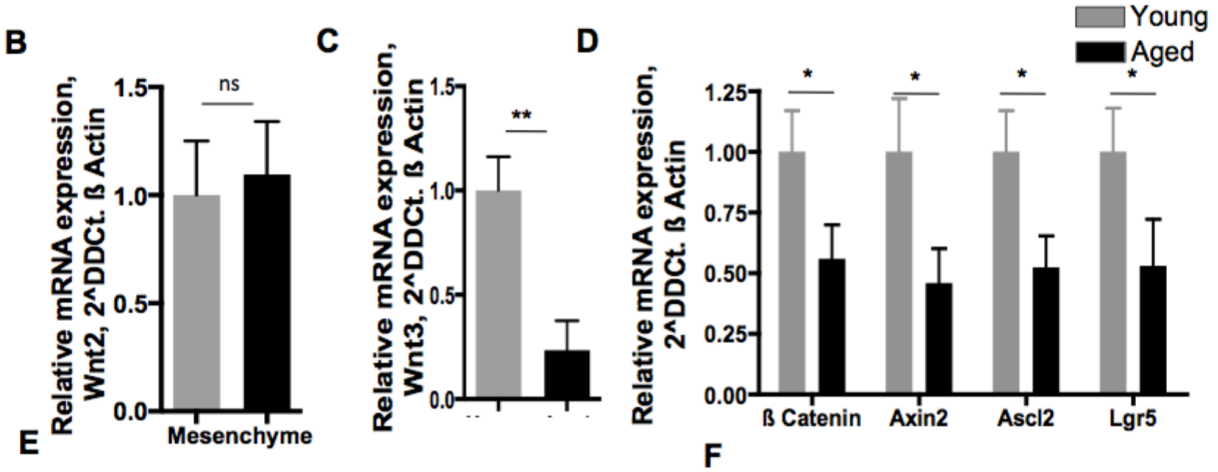


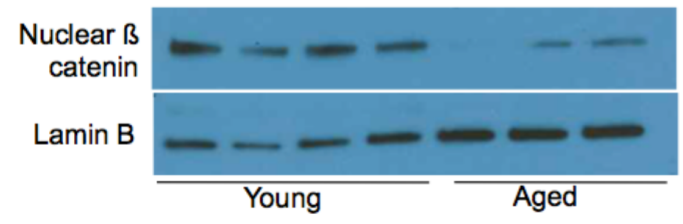
Figure S3. Isolated ISCs and Paneth cells, changes in gene expression pattern in ISCs ans Paneth cells. Related to Figure3 (A) FACs plot showing ISC (Lgr5GFPhi) population sorted and used for RNA seq. analysis (B) FACs plot showing Cd24hi (Paneth cells) population sorted and used for RNA seq. analysis (C &D) Heat maps showing differential gene expression pattern both in young and aged ISCs and Paneth cells in mouse intestine.

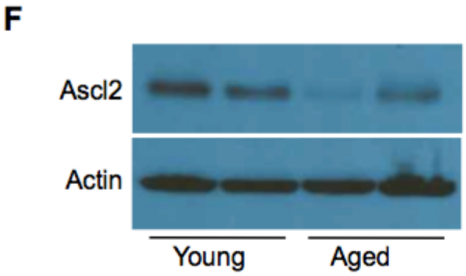
Figure S4

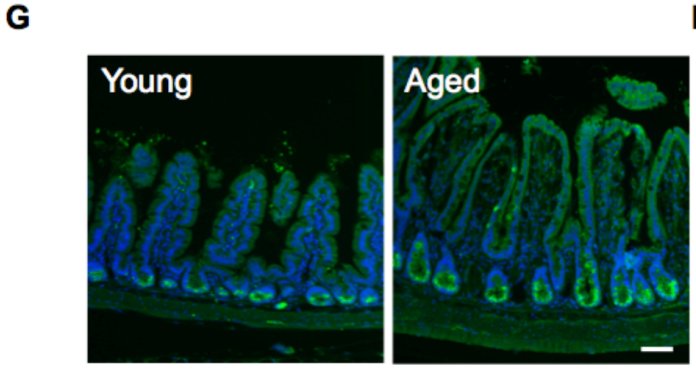
A

Downregulated Process	-log10(p-value)	Genes
Wnt signaling pathway (N=3)	1.34	Ppp3r1, Prickle1, Cacybp
SMAD binding (N=4)	1.85	Ankrd1, Col5a2, Col1a2, Eid2
PPAR signaling (N=4)	1.94	Hmgcs2, Scd1, Acadl, Me1
Cell proliferation (N=27)	3.09	F2r, Ftmt, Pdgfc, Gtpbp4, Mef2c, Fyn, Ascl1, Zbtb16, Trim35, Il2, Cyp7b1, Gja1, Cav1, Nampt, Sparc, Sash3, Wt1, Srpx, Fgf5, Chst11, Plag1, Jag2, Eid2, Pgr, Dock2, Lifr, Nkx2-9
Extracellular matrix (N=14)	4.17	Col14a1, Col1a2, Sparc, Marco, Bgn, Hmcn2, Matn2, Lamc1, Col6a3, Adamtsl1, Fn1, Mmp2, Col1a1, Col5a2









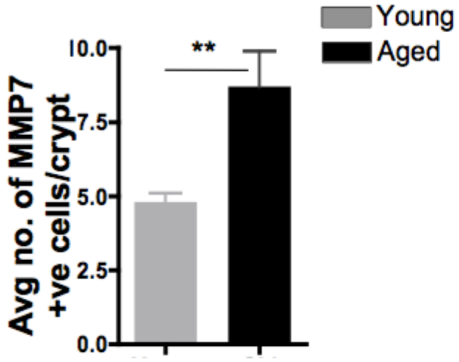


Figure S4. Down regulated process in in ISCs and quantification in ISCs, gene expression changes in crypts and MMP7 positive (Paneth) cells. Related to Figure 3 and Figure 4 (A) Table showing down regulated gene list from Figure 3A histogram respectively. (B) Expression of *Wnt2b* normalized to *ß-actin* in young and aged mesenchyme of the small intestine (C) *Wnt3* expression normalized to *ß Actin* transcript levels in young and aged crypts of mouse small intestine. (D) *ß Catenin, Axin 2, Ascl2* and *Lgr5* expression normalized to ß Actin transcript levels in young and aged crypts of mouse small intestine. (E) Ascl2 and actin protein levels in young and aged crypt epithelial cells of mouse small intestine. (F) ß catenin and lamin B nuclear protein levels (control) in young and aged duodenal crypt epithelial cells (G) Representative picture of MMP7 staining (Paneth cells) in ileum from young and aged mice. (H) Number of MMP7 positive cells per crypt (ileum) in young and aged mice, scale bar=50µm). n= 3 to 5 mice per experimental group. **=$p<0.01$, error bars represent standard deviation.

Figure S5

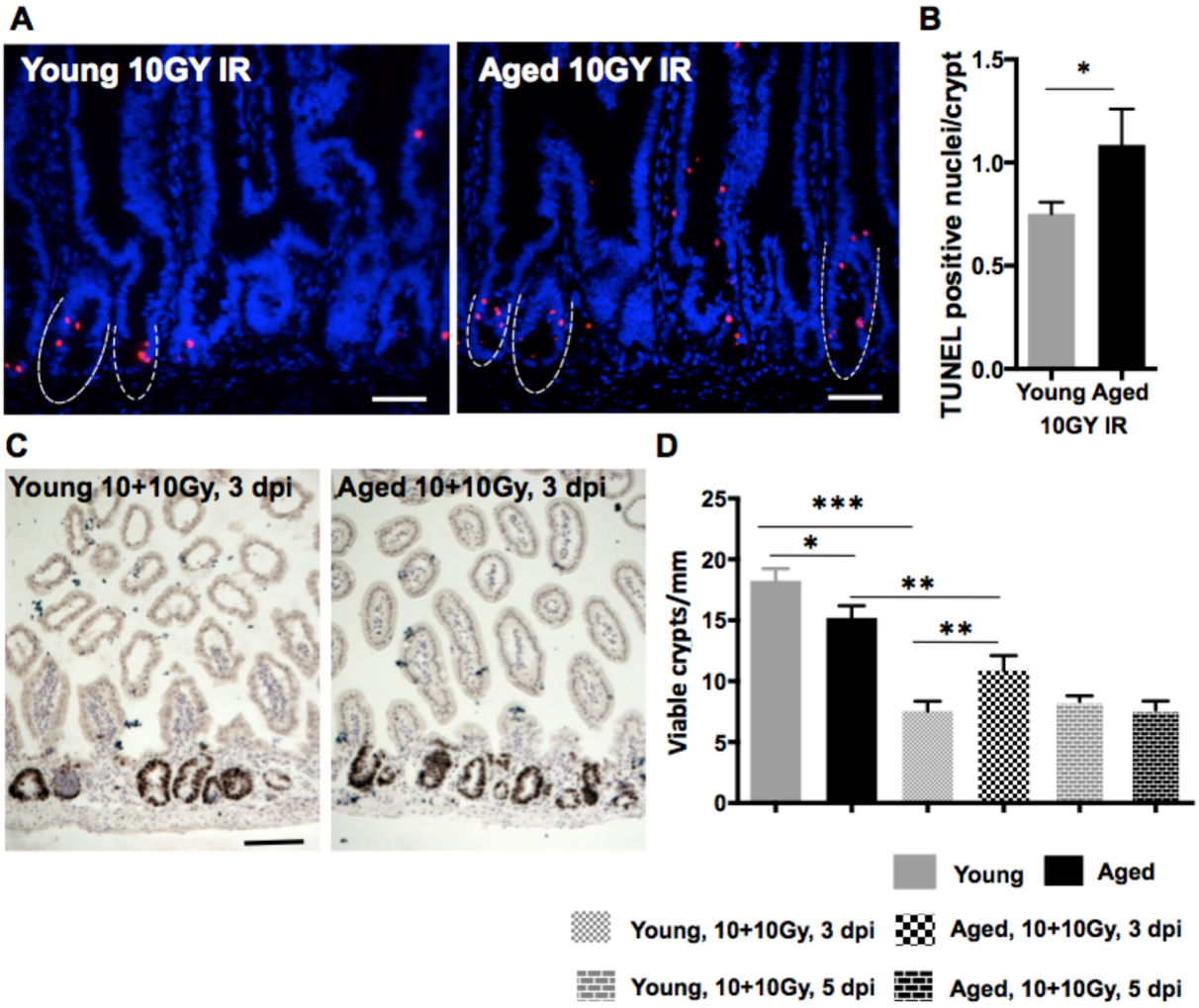


Figure S5. Apoptosis in 10Gy irradiated crypts and quantification of proliferating crypts in young and aged intestine.Related to Figure 5

(A) Representative picture of TUNEL staining in young and aged crypts 5 days after 10Gy radiation, scale=100µm. (B) Number of TUNEL positive nuclei per crypt in young and aged mice (C) Representative picture of Ki67 staining in young and aged crypts 3 days after 10+10Gy radiation, scale=100µm. (D) Number of viable crypts per mm in non irradiated (control) in young and aged mouse intestine and 3 days and 5 days after 10+10Gy radiation, n=3 to 5 mice per experimental group. *=p<0.05, **=p<0.01, ***=p<0.001, error bars represent standard deviation.

Figure S6

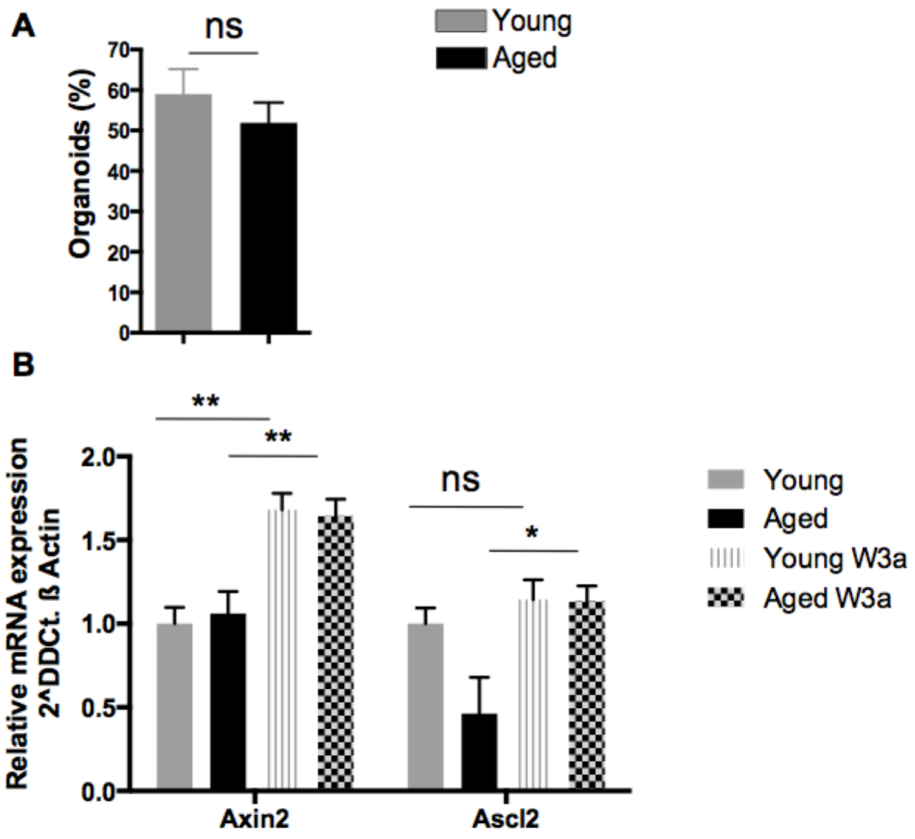


Figure S6. Organoid formation quantification and activation of Wnt targets after treating organoids with recombinant Wnt3a. Related to Figure 6

(A) Percentage of organoids growing six days after initial plating of murine crypts (first passage). (B) *Axin2* and *Ascl2* transcript levels in young, aged and aged mouse crypt organoids from the 3rd passage in the presence of Wnt3a, n= 5 to 6 mice per experimental group. *=p<0.05, **=p<0.01, error bars represent standard deviation.