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Supplementary Materials for

p53 terminates the regenerative fetal-like state after colitis-associated injury

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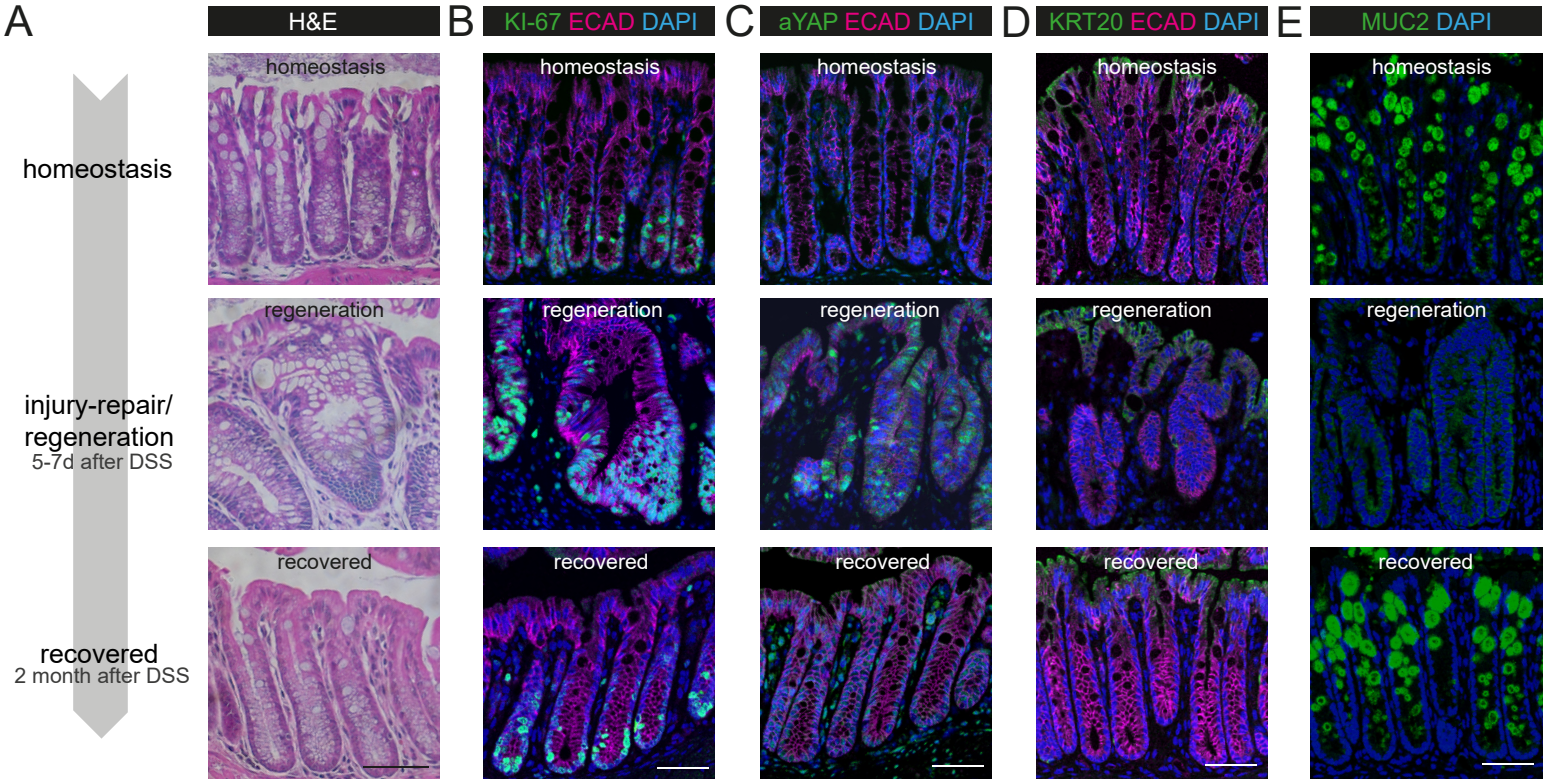
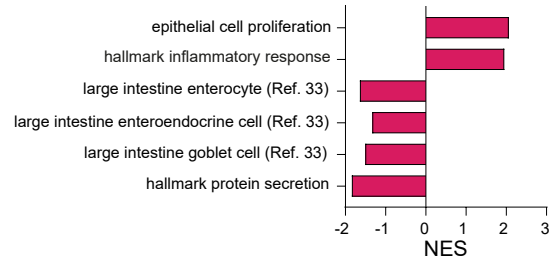
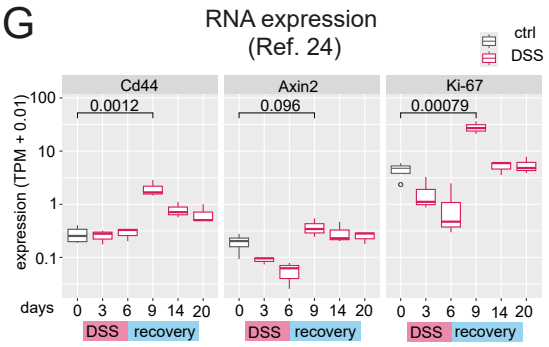
The PDF file includes:

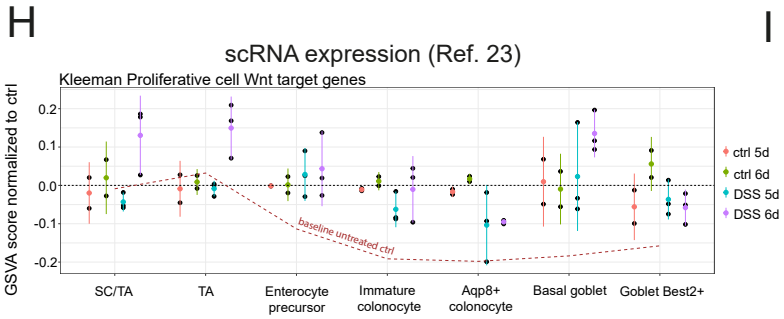
Other Supplementary Material for this manuscript includes the following:

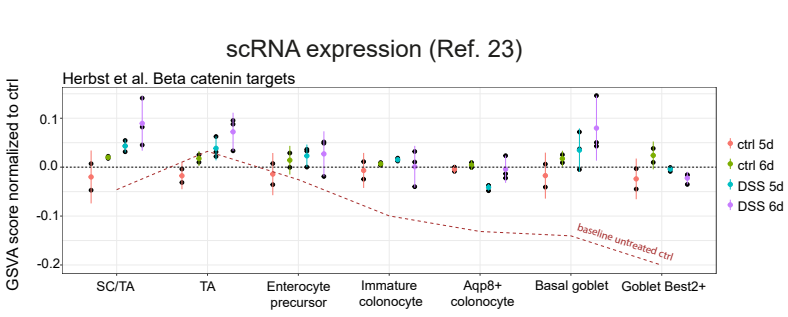
Fig. S1: Epithelial injury transiently induces Wnt-boost, regenerative signaling and loss of differentiation

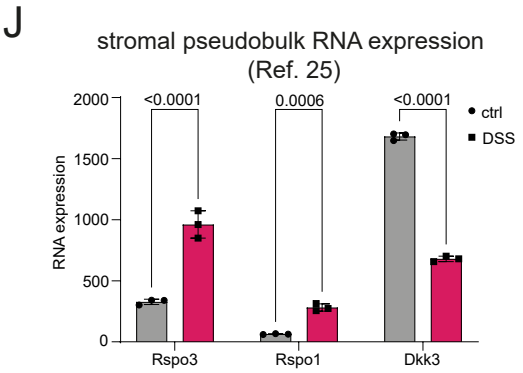


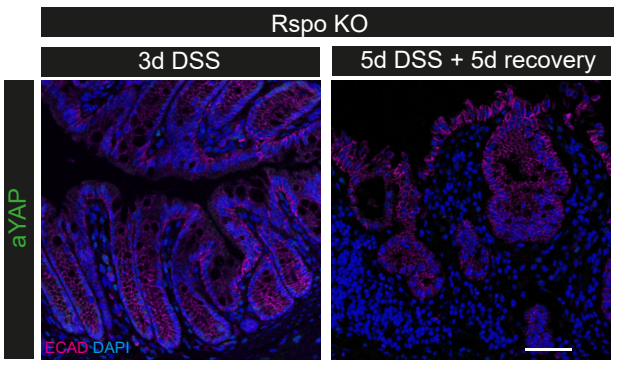








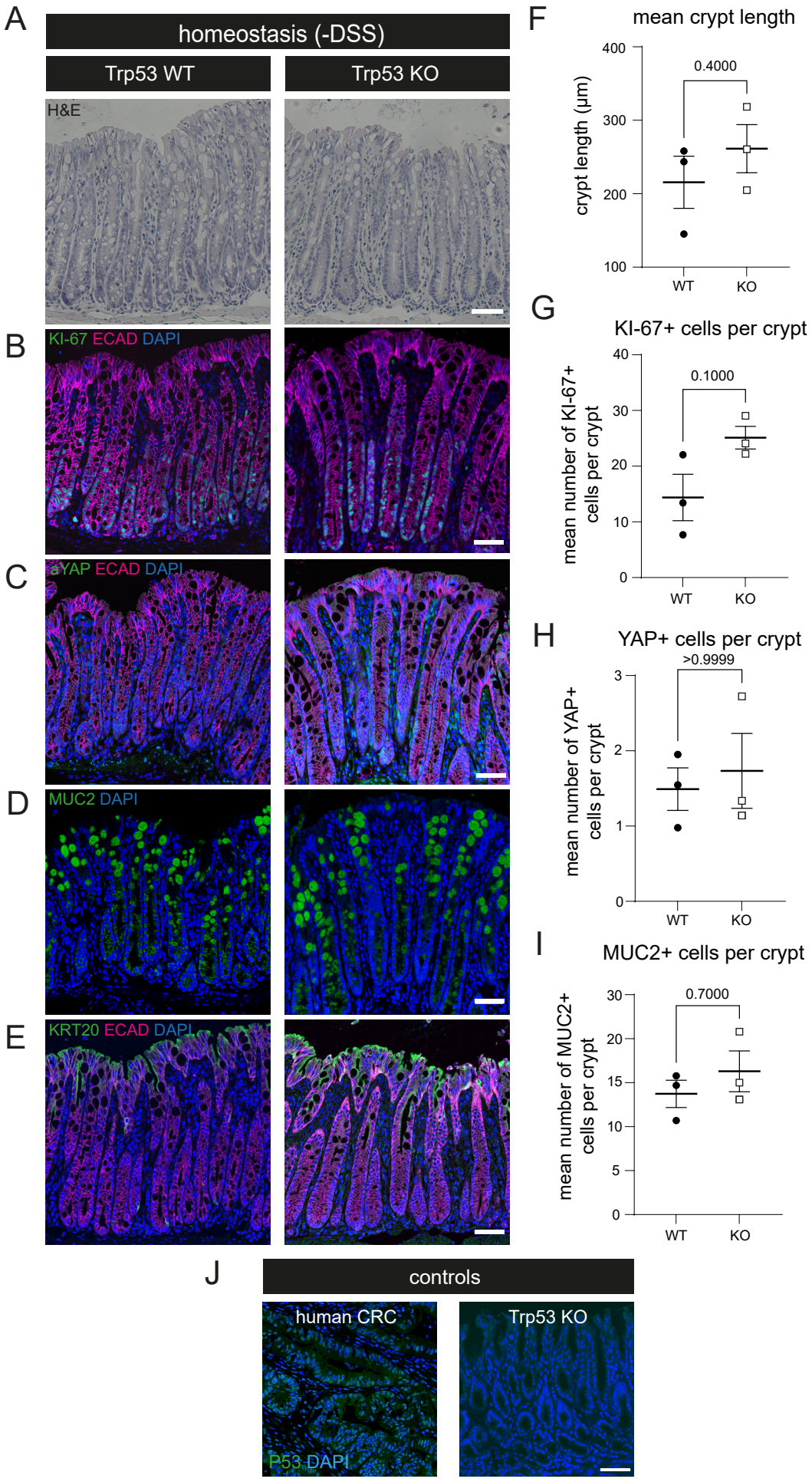




Supplementary Figure 1: Epithelial injury transiently induces Wnt boost, regenerative signaling and loss of differentiation

A Schematic of DSS-induced colitis and H&E staining for homeostasis, regeneration and recovered colonic epithelium. n = 5 mice for homeostasis, n = 6 mice for regeneration, n = 3 mice for 2-month recovery. **B-E** IF staining for KI-67, active YAP (aYAP), KRT20 or MUC2 (green) counterstained for E-cadherin (magenta) and DAPI (blue). n = 5 mice for homeostasis, n = 6 mice for regeneration, n = 3 mice for 2-month recovery. **F** Normalized enrichment scores (NES) from gene set enrichment analysis (GSEA) for MSigDB gene sets for DSS-colitis data from (*4*). **G** RNA expression for Cd44, Axin2 and Ki-67 of a DSS time-course experiment (*24*). **H+I** scRNA Seq expression data for Wnt and β-catenin target gene sets from epithelial cells at different time points in DSS colitis compared to untreated controls derived from published data (*23*). Brown dashed lines: Baseline of untreated controls. Scores are normalized by subtracting the average level in untreated condition samples. **J** Pseudobulk RNA expression of Rspo3, Rspo1 and Dkk3 in stromal cells from published data (*25*). **K** IF staining for aYAP (green) in *Myh11Cre*^{ERT2} x *Rspo* flox/flox mice treated with DSS for 3 days (n = 3 mice) or 5 days with 5 days of recovery (n = 6 mice). Scale bars: 50µm.

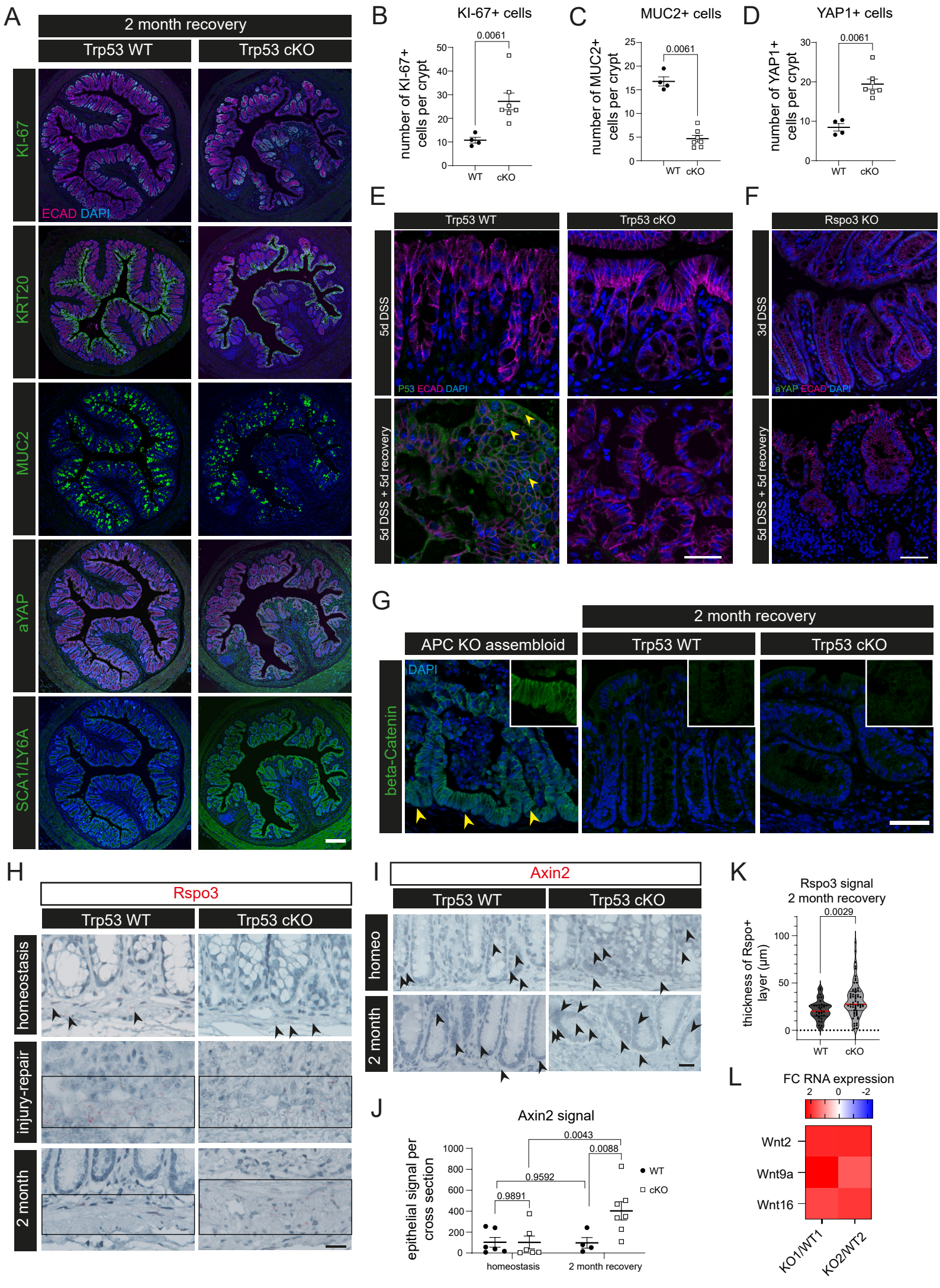
Fig. S2: Loss of *Trp53* does not alter gross tissue morphology in homeostasis



Supplementary Figure 2: Loss of *Trp53* does not alter gross tissue morphology in homeostasis

A Representative H&E image of colon of *Trp53* WT and KO mice. n = 3 mice. **B-E** Representative IF staining of *Trp53* WT and KO colon for either KI-67, aYAP, MUC2 or KRT20 (all green) counterstained for E-cadherin (magenta) and DAPI (blue). n = 3 mice. **F** Mean of crypt length measurement of *Trp53* WT and KO mice. n = 3 mice. **G** Quantification of B. n = 3 mice. **H** Quantification of C. n = 3 mice. **I** Quantification of D. n = 3 mice. **J** IF control staining for p53 on human colorectal carcinoma (CRC) and *Trp53* KO murine colon. Scale bars: 50 µm.

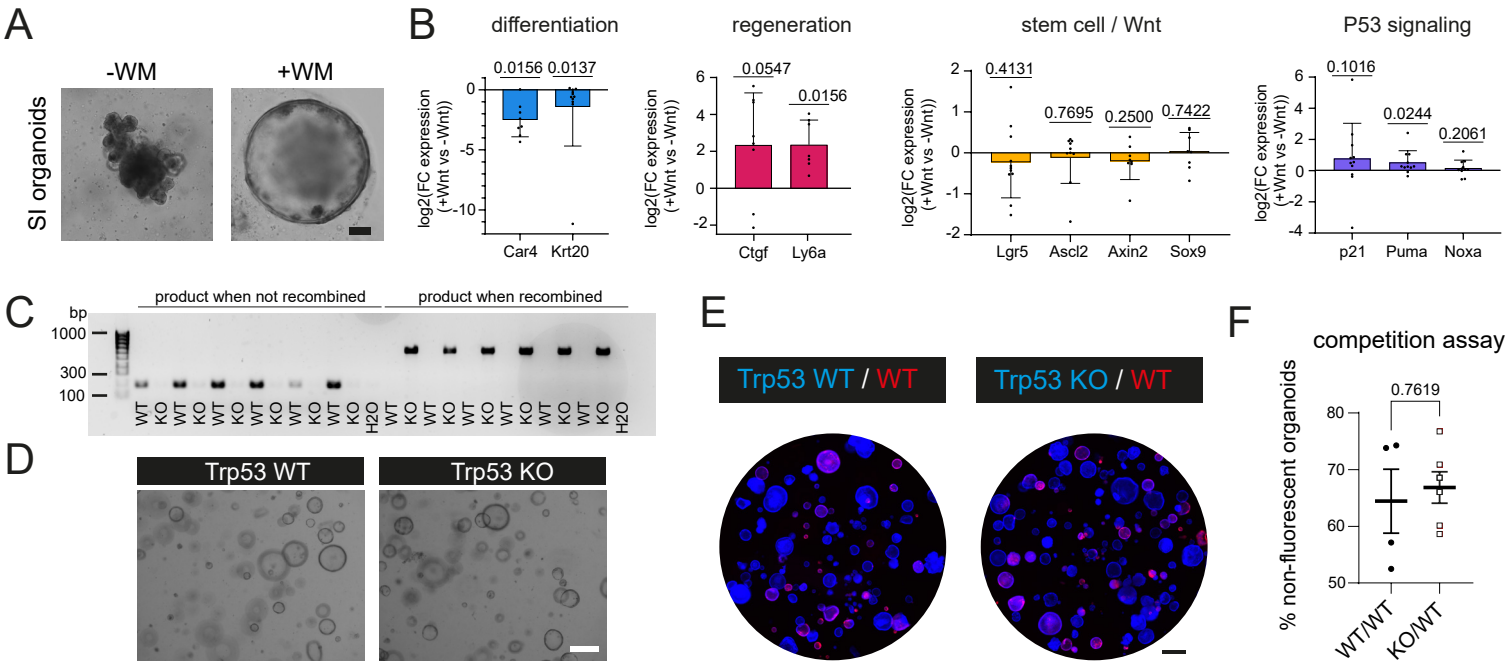
Fig. S3: Loss of *Trp53* locks the colonic epithelium in a regenerative, inflammatory, high Wnt state



Supplementary Figure 3: Loss of *Trp53* locks the colonic epithelium in a regenerative, high Wnt state

A Overview images of colonic cross sections IF-stained for KI-67, KRT20, MUC2, aYAP or SCA1/LY6A (all green), E-Cadherin (magenta) and DAPI (blue) of *Trp53* WT and cKO mice 2 months after DSS treatment. Scale bar: 200 µm. WT n = 2 mice, cKO n = 3 mice. **B-D** Quantification for Fig. 2G (n = 2 mice for WT, n = 3 mice for cKO; data points represent quantification from different colon cross-sections). **E** Single molecule RNA in-situ hybridization images for Rspo3 in *Trp53* WT and cKO mice during homeostasis, injury repair and 2 months recovery. WT n = 2 mice, cKO n = 2-3 mice**.** Scale bar: 20µm. **F** Quantification of E for 2 months recovery time point. Dots represent measurements at different sites per colon cross section. **G** Single molecule RNA in-situ hybridization images for Axin2 in *Trp53* WT and cKO mice during homeostasis and 2 months recovery. WT n = 2 mice, cKO n = 2-3 mice. Scale bar: 20µm. **H** Quantification of H for homeostasis and 2 months recovery time point. Data points represent quantification of different colon cross sections **I** IF stainings for β-catenin (green) in APC KO assembloids, *Trp53* WT and cKO mice at 2 months recovery time points counterstained for DAPI (blue). APC KO assembloids n = 2, WT n = 2 mice, cKO n = 3 mice. Scale bar: 50µm. **J** IF stainings for P53 (green) in *Trp53* WT and cKO mice at 5d DSS and 5d DSS with 5d recovery counterstained for E-cadherin (magenta) and DAPI (blue). WT n = 2 mice, cKO n = 2 mice. Scale bar: 50µm.

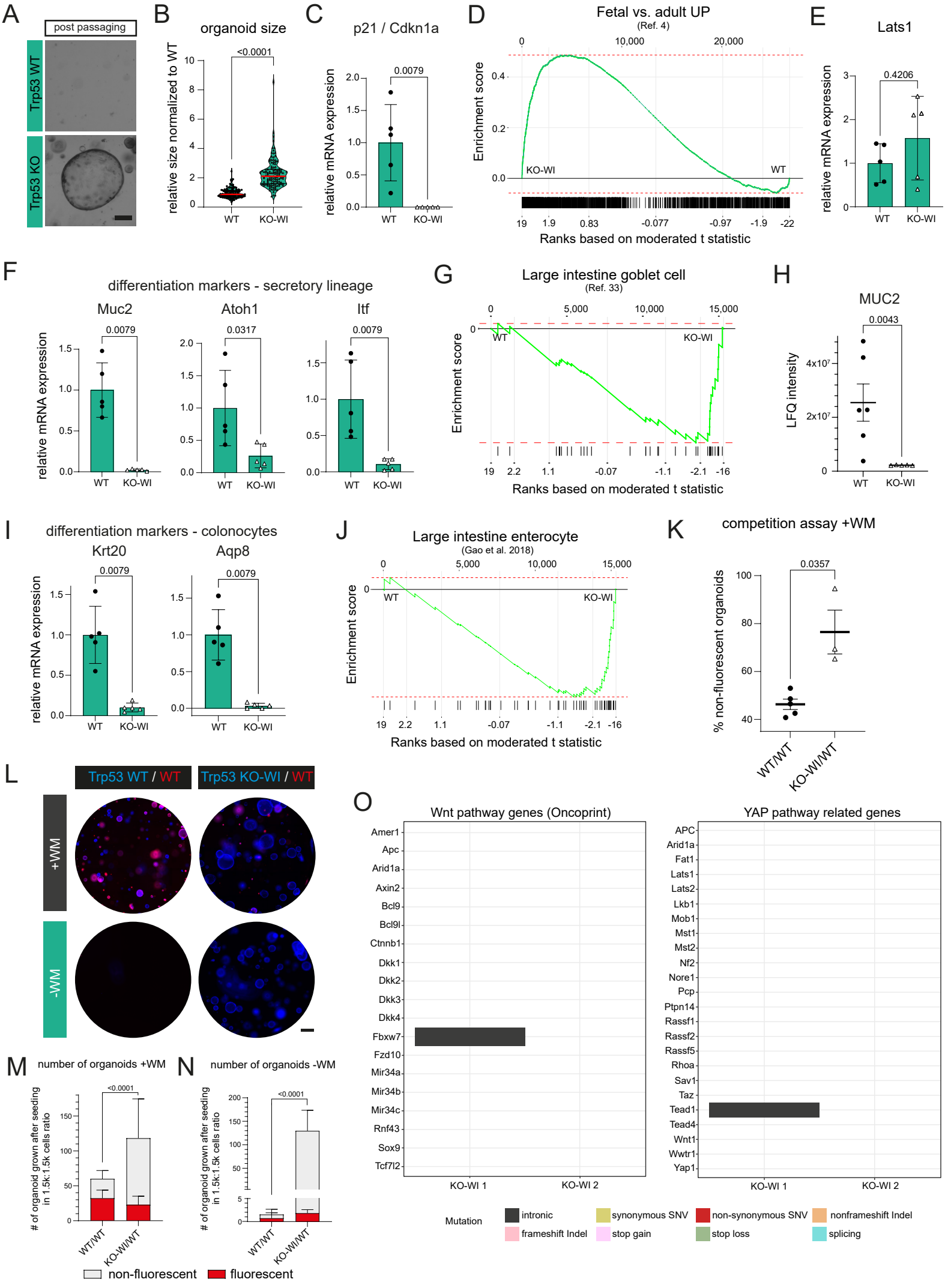
Fig. S4: sWnt induces regeneration and p53 signaling, but WT and *Trp53* KO organoids grow at the same rate in regeneration medium



Supplementary Figure 4: sWnt induces regeneration and WT and *Trp53* KO organoids grow at the same rate in regeneration medium

A Representative BF images of small intestinal (SI) organoids grown in medium without (-WM) or with (+WM) supplementation of Wnt-activating growth factors. Scale bar: 100 µm. n = 11 experimental replicates from 6 biological replicates. **B** logFC of mRNA expression of SI organoids in +WM vs -WM culture condition for differentiation, regeneration and stem cell/Wnt signaling markers and p53 signaling. n = 8-11 experimental replicates from 6 biological replicates. **C** DNA gel of genotyping PCR for WT and KO colon organoids. **D** Representative BF overview images of *Trp53* WT and KO organoid culture from n> 10 biological replicates. Scale bar: 300 µm. **E** Representative IF whole-mount images of *Trp53* WT and KO organoids grown in co-culture with fluorescent (red) WT organoids (n = 2 biological replicates for WT, n = 3 biological replicates for KO). All organoids were counterstained with DAPI. Scale bar: 500 µm. **F** Quantification of E (n = 2 biological replicates for WT, n = 3 biological replicates for KO). Plotted as mean +/- s.e.m., data points represent technical replicates.

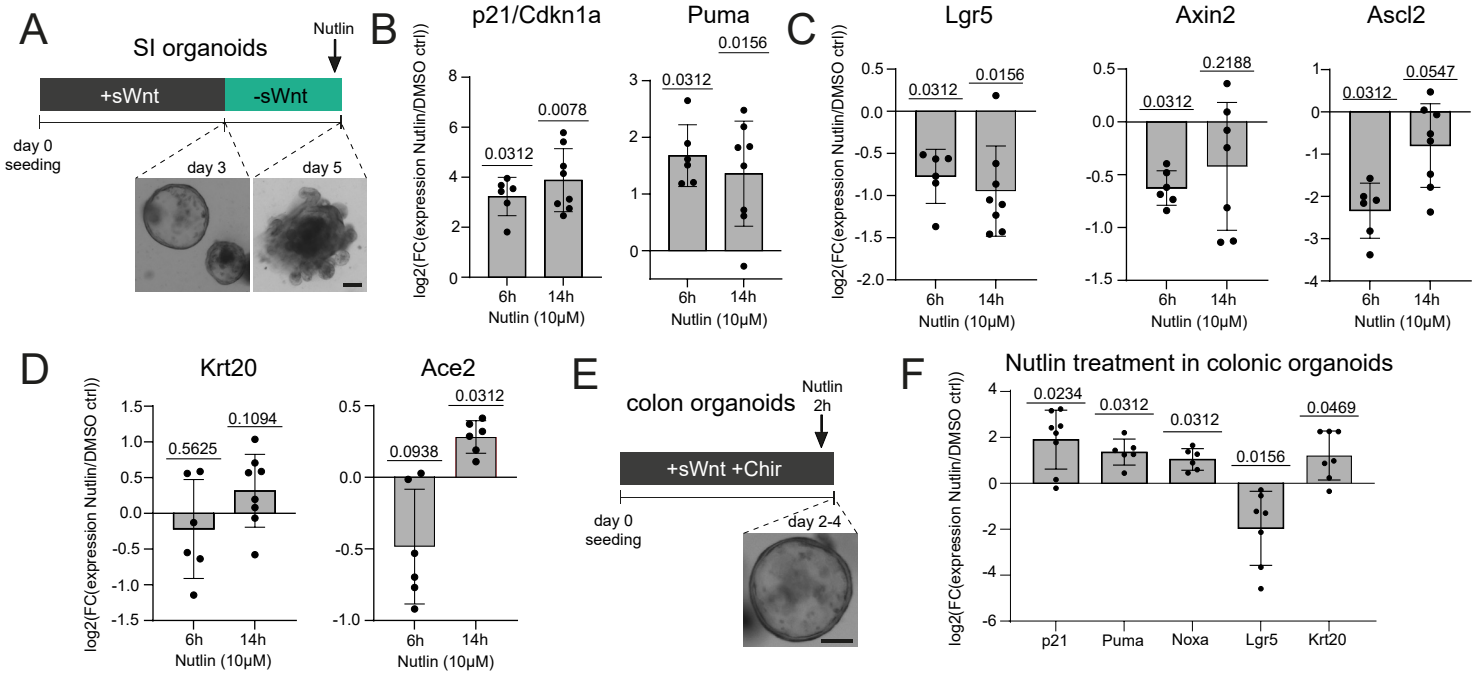
Fig. S5: Regenerative *Trp53* KO organoids exhibit accelerated growth kinetics and lack differentiation



Supplementary Figure 5: Regenerative *Trp53* KO organoids exhibit accelerated growth kinetics and lack differentiation

A Representative BF images of *Trp53* WT and KO organoids cultured in -WM medium. n > 6 biological replicates. Scale bar: 200 µm. **B** Relative size of organoids grown in -WM medium normalized to WT organoids. Median (red line) and quartiles (black lines). n = 6 biological replicates for WT, n = 4 biological replicates for KO-WI. **C** mRNA expression of p21 of *Trp53* WT and KO-WI grown in -WM condition. n = 5 biological replicates. **D** Enrichment plot for KO-WI vs WT organoids in -WM condition for the fetal signature (FDR= 0.022, p= 0.00054, ES= 0.48, NES= 2.3). n = 2 biological replicates for WT vs n = 2 biological replicates for KO-WI. **E** mRNA expression of Lats1 in *Trp53* WT and KO-WI grown in -WM condition. n = 5 biological replicates. **F** mRNA expression of differentiation markers of the secretory lineage of *Trp53* WT and KO-WI grown in -WM condition. n = 4-5 biological replicates. **G** Enrichment plot for KO-WI vs WT organoids in -WM condition for the large intestinal goblet cell signature (FDR= 0.035, p= 0.0012, ES= −0.58, NES= −1.9). n = 2 biological replicates for WT vs n = 2 biological replicates for KO-WI. **H** Protein expression for MUC2 in WT and KO-WI organoids in -WM condition. n = 3 biological replicates. **I** mRNA expression of differentiation markers of the colonocyte lineage of *Trp53* WT and KO-WI grown in -WM condition. n = 5 biological replicates. **J** Enrichment plot for KO-WI vs WT organoids in -WM condition for the large intestine enterocyte signature (FDR= 0.018, p= 0.00039, ES= −0.6, NES= −2.2). n = 2 biological replicates for WT vs n = 2 biological replicates for KO-WI. **K** Representative IF whole-mount images of *Trp53* WT and KO-WI organoids grown in co-culture with fluorescent (red) WT organoids in +WM or -WM medium condition (n = 5 biological replicates for WT, n = 3 biological replicates for KO-WI). All organoids were counterstained with DAPI. Scale bar: 500 µm. **L** Quantification of K in +WM condition (n = 5 biological replicates for WT, n = 3 biological replicates for KO-WI). Plotted as mean +/- s.e.m. **M** Absolute numbers of organoids grown in +WM condition. Grey: non-fluorescent, red: fluorescent organoids. n = 5 biological replicates for WT, n = 3 biological replicates for KO-WI. **N** Absolute numbers of organoids grown in -WM condition. Grey: non-fluorescent, red: fluorescent organoids. n = 5 biological replicates for WT, n = 3 biological replicates for KO-WI. **O** Mutation data from two KO-WI clones sequenced by whole genome sequencing (WGS) for Wnt pathway- and YAP pathway-related genes.

Fig. S6: Activation of *Trp53* signaling suppresses Wnt targets followed by induction of differentiation



Supplementary Figure 6: Activation of *Trp53* signaling suppresses Wnt targets followed by induction of differentiation

A Schematic and representative BF images of SI organoids with sWnt supplementation and 10 µM Nutlin-3a treatment. **B** mRNA expression of p53 targets 6 h and 14 h after Nutlin induction. n = 6 biological replicates (6h) or n = 9 (14h) experimental replicates from 6 biological replicates. **C** mRNA expression of Wnt targets 6 h and 14 h after Nutlin induction. n = 6 biological replicates (6 h) or n = 9 (14 h) experimental replicates from 6 biological replicates. **D** mRNA expression of differentiation markers 6 h and 14 h after Nutlin induction. n = 7 (6 h) or n = 7-8 (14 h) experimental replicates from 6 biological replicates. **E** Schematic and BF picture of colonic organoids in +WM condition treated with 10 µM Nutlin-3a. **F** mRNA expression of p53 targets, stem cell marker Lgr5 and differentiation marker Krt20 2 h after Nutlin induction. n = 6-8 biological replicates. Scale bars: 100 µm.

Supplementary Table 1: Primer sequences

ID	Sequence (5' to 3')
Genotyping Primer 1 fw	AGTCACAGCACATGACGGAG
Genotyping Primer 1 rev	CACCCGGATAAGATGCTGGG
Genotyping Primer 2 fw	CACAAAAACAGGTTAAACCCAG
Genotyping Primer 2 rev	GAAGACAGAAAAGGGGAGGG
Sanger sequencing Primer betaCat 1 fw	TTCAGGTAGCATTTTCAGTTCAC
Sanger sequencing Primer betaCat 1 rev	TAGCTTCCAAACACAAATGC
Sanger sequencing Primer betaCat 2 fw	TGGGTCATTGTCGTAGATGGC
Sanger sequencing Primer betaCat 2 rev	GCTAGCTTCCAAACACAAATGC
Gapdh fw	ACCCTTAAGAGGGATGCTGC
Gapdh rev	CCCAATACGGCCAAATCCGT
bMgl fw	ACGTAACACAGTTCCACCCG
bMgl rev	GATCACATGTCTCGATCCCAGT
Hprt1 fw	TCCCAGCGTCGTGATTAGC
Hprt1 rev	TGATGGCCTCCCATCTCCTT
Lgr5 fw	CAGTGTTGTGCATTTGGGGG
Lgr5 rev	CAAGGTCCCGCTCATCTTGA
Ascl2 fw	TTGGTCCGGTTCTTCATCCG
Ascl2 rev	CGTACCAGTCAAGGTGTGCT
Axin2 fw	CCTGACCAAACAGACGACGA
Axin2 rev	CACCTCTGCTGCCACAAAAC
Sox9 fw	CGTGCAGCACAAGAAAGACC
Sox9 rev	GGACCCTGAGATTGCCCAGA
Cd44 fw	GCACGCCATGGACAAGTTTT
Cd44 rev	TCTTCTTCAGGAGGGGCTGA
Krt20 fw	GTCCCACCTCAGCATGAAAGA
Krt20 rev	TCTGGCGTTCTGTGTCACTC
Ace2 fw	CCTGCTAAGAAACGGAGCCA
Ace2 rev	CCAACGATCTCCCGCTTCAT
Aqp8 fw	TACCTTGGGGAACATCAGCG
Aqp8 rev	TCTGGACTCACCACTTTAGCC
Car4 fw	CTGGGCAGCGTCTTTCC
Car 4 rev	ATCTCCACTGTGTGTTGATTGTT
Muc2 fw	TGTCCTGACCAAGAGCGAAC
Muc2 rev	CTTGATTGGGCCTTCCAGGT
Atoh1/Math1 fw	GTTGCGCTCACTCACAAATAAGGG
Atoh1/Math1 rev	TGGCAGTTGAGTTTCTTCAAGGCG
Itf fw	AGATTACGTTGGCCTGTCTCC
Itf rev	ATGTGACAGAGGGGTAGCCA
Ctgf fw	AGAACTGTGTACGGAGCGTG
Ctgf rev	GTGCACCATCTTTGGCAGTG
Ly6a fw	ACCCCTCCCTCTTCAGGATG
Ly6a rev	GCTGCACAGATAAAACCTAGCA
Anxa1 fw	GGAGAAAGGGGACAGACGTG
Anxa1 rev	CTGGTGGCACACTTCACGAT
Ankrd1 fw	CAATGCCAAGGACAGAGAAGG
Ankrd1 rev	TCAGTTTTCCTTGGCCCCAT
Igfbp3 fw	CCAGTGCGCGCCTCC
Igfbp3 rev	CTCACTGATGTTTCCTGGAGCA

Cyr61 fw	AAGAGGCTTCCTGTCTTTGGC
Cyr61 rev	AACTCGTGTGGAGATGCCAG
Cdkn1a/p21 fw	GGTGGAGACCTGATGATACCC
Cdkn1a/p21 rev	CGAAGAGACAACGGCACACT
Puma fw	ACGACCTCAACGCGCAGTACG
Puma rev	GAGGAGTCCCATG AAGAGATTG
Noxa fw	GTGGAGTGCACCGGACATAA
Noxa rev	CACTCGTCCTTCAAGTCTGCT
Lats1 fw	AGCAGCACGTAGAGAACGTCC
Lats1 rev	AATCCAACCCGCATCATTTC
Lats2 fw	GTACTACCAGAAAGGGAACCAC
Lats2 rev	ATCACACCGACGCTCCAC
Pkm1/2 fw	GCGACTCGTCTTCACTTGACT
Pkm1/2 rev	GCATGGTTCCTGAAGTCCTCG

Supplementary Table 2: Primary Antibodies

Antibody	**Manufacturer, details**	**Dilution**
Rabbit anti-KI67	Cell Signaling Technology, 9129S, clone D3B5	1:200
Rabbit anti-keratin 20	Cell Signaling Technology, 13063S, clone D9Z1Z	1:200
Rabbit anti-MUC2	a gift from Prof. Gunnar C. Hansson	1:1000
Rabbit anti-active YAP1	Abcam, ab205270, clone EPR19812	1:150
Mouse anti-E-cadherin	BD, 610181, clone 36	1:300
Mouse anti-P53	Santa Cruz Biotechnology, DO-1, sc-126	1:50
Rabbit anti-P21	Abcam, ab205270, clone EPR19812	1:400
Rat Anti-SCA1/LY6A	R&D, MAB1226, clone 177228	1:50
Rabbit Anti-Iba1	Fujifilm Wako Chemicals, 019-19741, batch LEE6425	1:200
Rabbit Anti-PKM2	Cell Signaling Technology, 4053, clone D78A4	1:200

Supplementary Table 3: Secondary Antibodies

Antibody	Manufacturer, details	Dilution
AlexaFluor 488 donkey anti-mouse IgG	Jackson Immunoresearch, 715-546-150	1:300
AlexaFluor 647 donkey anti-rabbit IgG	Jackson Immunoresearch, 711-605-152	1:300
AlexaFluor 488 goat anti-rat IgG	Jackson Immunoresearch, 112-546-062	1:300
Cy3 goat anti-rabbit IgG	Jackson Immunoresearch, 111-165-144	1:300
AlexaFluor 647 goat anti-mouse IgG	Jackson Immunoresearch, 115-605-008	1:300

Supplementary Data 1: Sequence data from Sanger sequencing of KO-WI clones.