Supplemental Figure Legends

Figure S1. Generation of miR^{flox} and miR^{-} alleles, related to Figure 1

(A) Schematic representation of the generation of miR^{flox} and miR^{-} alleles. Green arrowheads, LoxP sites; blue arrowheads, FRT sites; gray arrows, locations of genotyping primers.

(B) Northern blot analysis of bladder miRNA expression in mice of indicated genotypes.

Figure S2. Normal epithelial turnover in miR$^{-/-}$ mice, related to Figure 1

BrdU immunohistochemistry showing small intestinal epithelial cell turnover 2, 24, 48, and 72 hours after BrdU injection.

Figure S3. Equivalent DSS injury in miR$^{+/+}$ and miR$^{-/-}$ mice, related to Figure 1

(A) Normalized body weight of miR$^{+/+}$ and miR$^{-/-}$ mice after DSS treatment. n=10 mice per genotype.

(B) Representative colon sections of miR$^{+/+}$ and miR$^{-/-}$ mice dissected after 5 days of DSS, documenting completely de-epithelialized ulcers (upper panels, H&E) and apoptosis in residual crypts (lower panels, cleaved caspase 3).

Figure S4. miR-143/145 are exclusively expressed in the intestinal mesenchyme, related to Figure 2

(A) Quantitative RT-PCR analysis of miR-143/145 expression in full-thickness colon specimens and purified epithelium (crypts) of wild-type mice treated with DSS and

dissected at the indicated time points. Measurements from 2 independent animals are shown for each condition.

(B) Quantitative RT-PCR analysis of miR-143/145 expression in wild-type small intestine, adenomas from *Apc*$^{min/+}$ mice, and purified epithelium from normal intestine or adenomas. n=3 animals per condition.

Figure S5. Cre-mediated recombination in *Villin-Cre* and *Twist2*^{Cre} animals, related to Figure 3

(A) PCR detection of miR^{WT}, miR^{flox} and miR^{-} alleles in the liver, small intestine (total SI), large intestine (total LI), and purified intestinal epithelia from 2 mice for each indicated genotype.

(B) Quantitative PCR detection of miR^{flox} recombination in bone marrow (BM), hematopoietic stem cells (HSC), and various hematopoietic lineages in *Twist2*$^{Cre/+}$*; miR*$^{flox/flox}$ animals. Black and gray bars represent measurements from 2 independent animals.

(C) Quantitative RT-PCR analysis of mRNA and miRNA expression in full-thickness colon specimens and purified epithelium of mice of indicated genotypes. All values were normalized to expression in wild-type colon. n ≥ 3 samples from independent animals per condition.

Figure S6. Colons and myofibroblasts after DSS treatment, related to Figures 4-5

(A) Representative colons from animals of the indicated genotypes, dissected 2 days after completion of DSS treatment.

(B) SMA immunofluorescence showing muscularis mucosa and myofibroblasts in ulcerated regions of colon from mice of the indicated genotypes 2 days after completion of DSS treatment. n=3 mice per genotype examined.

(C) A subset of SMA+ cells in the lamina propria of ulcerated regions of DSS treated *Myh11-Cre/GFP; mTmG* mice also express GFP. White arrowheads, SMA+GFP+ cells; open arrowheads, SMA+GFP– cells.

Figure S7. Differential expression of *Igfbp4* and *Bmp4* in cultured ISEMFs but not DSS-treated colons, related to Figure 6

(A) Quantitative RT-PCR analysis of *Igfbp4* and *Bmp4* expression in cultured ISEMFs in the presence or absence of LPS. n=3 independently-derived WT or KO ISEMF cell lines per condition.

(B) Quantitative RT-PCR analysis of *Igfbp4* and *Bmp4* expression in untreated and DSS-treated mouse colons from WT and KO mice. n=3 animals per condition.

Supplemental Experimental Procedures

Mouse strains

Lgr5$^{+/eGFP}$ (Barker et al., 2007), *Villin-Cre* (Madison et al., 2002), *Twist2*$^{+/Cre}$ (Sosic et al., 2003), *Myh11-Cre/eGFP* (Xin et al., 2002), *Apc*$^{min/+}$ (Su et al., 1992), *Rosa26*$^{+/LacZ}$ (Soriano, 1999), and *Rosa26*$^{+/mTmG}$ (Muzumdar et al., 2007) mice were obtained from the Jackson Laboratory and maintained on a C57BL6/J background. Standard breeding schemes were used to generate most compound mutant mice. The *Myh11-Cre/eGFP* transgene was always carried by *miR*$^{+/–}$ males, due to its expression in the male and female germline and its ability to recombine the parental allele when transmitted through the female germline.

Histology, immunofluorescence, and immunohistochemistry

The following primary antibodies and dilutions were used: anti-cleaved caspase 3 (Cell Signaling Technology #9661, 1:5000), anti-phosphorylated histone 3 (Millipore #06-570, 1:100), anti-Ki67 (Leica Microsystems #KI67-MM1-CE, 1:100), anti-smooth muscle actin Cy3 conjugate (Sigma #C6198, 1:500), and anti-phosphorylated Igf1r (abcam #39398, 1:50). Signal detection was achieved using fluorophore-conjugated secondary antibodies (Invitrogen, 1:200) or VECTASTAIN Elite ABC Reagent with 3, 3′-diaminobenzidine substrate (Vector Labs). LacZ staining was performed as described (Barker and Clevers, 2010). Images were acquired using a Zeiss Axio Observer Z1 microscope and analyzed using AxioVision software.

For visualizing GFP expression, intestinal tissue was fixed overnight at 4°C in 4% paraformaldehyde and incubated with 30% sucrose (dissolved in PBS) at 4°C for 6

hours. Intestine segments were then wrapped into “Swiss rolls”, embedded in O.C.T. freezing medium (Tissue-Tek) and sectioned on a cryostat (Leica) at -20°C.

Isolation of intestinal crypts

Mouse intestines were dissected, rinsed with ice-cold PBS, and (for mouse small intestine) depleted of villi using a coverslip. Tissue was minced into ~2 mm pieces and washed in PBS containing 5 mM EDTA before a 30-minute incubation in the same buffer. After incubation, vigorous shaking liberated intact crypts which were then filtered through a 100 μm cell strainer (BD) and collected by centrifugation at 200*g* for 3 minutes. For isolation of Lgr5+ ISCs, the same procedure was followed except purified crypts were dissociated in TrypLE (Invitrogen) supplemented with 1 mg/mL DNase I (Roche) for 30 minutes at 37°C, passed through a 50 μm filter (BD), washed in ice-cold PBS, and sorted. For purification of crypts from *Apc*$^{min/+}$ adenomas, ~10 adenomas per mouse were pooled to obtain adequate material for RNA isolation. Paired human colorectal tumors and normal colon specimens were obtained through the University of Texas Southwestern Medical Center Tissue Resource. Fresh full-thickness colon and tumor biopsies were immediately washed and incubated with a modified buffer containing 3% fetal bovine serum, 1 mM DTT, and 1 mM EDTA to release crypts.

RNA extraction and analysis

Total RNA was isolated from cells and tissues with the miRNeasy kit (Qiagen) according to the manufacturer’s protocol. Northern blots were performed as described previously (Hwang et al., 2007) using Ultrahyb-oligo buffer (Ambion) and oligonucleotide probes

perfectly complementary to the mature miRNA sequences. TaqMan microRNA assays (Applied Biosystems) were performed per manufacturer's instructions and normalized to U6 snRNA. For measurements of mRNAs, RNA was DNase digested (Qiagen), reverse transcribed using the Superscript III First-Strand Synthesis Supermix (Invitrogen), and quantitative PCR was performed using Power SYBR Green PCR Master Mix (Invitrogen) with primers listed in Table S2 or using pre-designed Taqman assays (Invitrogen). All reactions were performed in triplicate. Transcriptome profiling was performed using the MouseWG-6 V2 BeadChip (illumina) at the UTSW microarray core facility and analyzed using the GeneSpring software (Agilent). Microarray data were deposited in the GEO repository under accession number GSE55651.

In situ hybridization

In situ hybridization detection of miRNAs was performed using the miRCURY LNA miRNA ISH Kit (Exiqon), with slight modifications to the manufacturer's protocol. Paraffin slides were prepared from intestine tissues as described above except with the use of RNAse-free reagents. Slides were deparaffinized in xylenes and digested with 20 μg/ml Proteinase K at 37°C for 8 minutes. miRNA LNA probes were used at 50 nM final concentration. Hybridization was carried out at 55°C overnight in a hybridization incubator. After hybridization, slides were washed and stained using the DIG detection kit (Roche). Stained slides were counterstained in nuclear fast red (Vector Labs), dehydrated, and mounted using VectaMount permanent mounting medium.

Luciferase assay

The predicted miR-143 binding sites in the 3′ UTRs of human and mouse *IGFBP5* plus ~250 bp of flanking sequence were amplified and ligated into the XbaI site of pGL3-control (Promega). Mutagenesis was performed by re-amplifying each fragment with primers that contained the mutations (primer sequences provided in Table S2). Mutated fragments were then re-introduced into the pGL3 3′ UTR reporter plasmid using the In-Fusion HD cloning kit (Clontech). HEK293T cells were cultured in high glucose DMEM media supplemented with 10% fetal bovine serum, penicillin, and streptomycin (Invitrogen). $1.25x10^5$ HEK293T cells were plated in each well of a 24-well plate and transfected 16 hours later with 50 ng of the indicated pGL3 3' UTR reporter construct and 2 ng of phRL-SV40 (Promega) using Lipofectamine 2000 (Invitrogen) per manufacturer's instructions. Where indicated, control, miR-143, or miR-145 mimics (Dharmacon) were co-transfected at 25 nM final concentration. 24 hours after transfection, cells were lysed and assayed for firefly and renilla luciferase activity using the Dual-Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized to renilla luciferase activity to obtain relative luciferase activity. All transfections were performed in triplicate and multiple experimental trials were performed.

BrdU labeling

BrdU pulse chase experiments were performed with BrdU labeling reagent and staining kit (Invitrogen) according to manufacturer's instructions.

Cell isolation from the bone marrow

Mouse bone marrow cells were obtained by flushing the femur and tibia with PBS and then filtering through a 30 μm mesh. Hematopoietic stem cells were isolated using a Lineage Cell Depletion Kit (Miltenyi Biotec) followed by CD117 MicroBead (Miltenyi Biotec) selection. Alternatively, mouse bone marrow cells were incubated with mouse Hematopoietic Lineage Antibody (Biotin) Panel (eBioscience) and each lineage was separated using Anti-Biotin MicroBeads (Miltenyi Biotec) according to manufacturer's instructions.

Supplemental References

Barker, N., and Clevers, H. (2010). Lineage Tracing in the Intestinal Epithelium. In Current Protocols in Stem Cell Biology (John Wiley & Sons, Inc.).

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., *et al.* (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature *449*, 1003-1007.

Hwang, H.-W., Wentzel, E.A., and Mendell, J.T. (2007). A Hexanucleotide Element Directs MicroRNA Nuclear Import. Science *315*, 97-100.

Madison, B.B., Dunbar, L., Qiao, X.T., Braunstein, K., Braunstein, E., and Gumucio, D.L. (2002). cis Elements of the Villin Gene Control Expression in Restricted Domains of the Vertical (Crypt) and Horizontal (Duodenum, Cecum) Axes of the Intestine. Journal of Biological Chemistry *277*, 33275-33283.

Muzumdar, M.D., Tasic, B., Miyamichi, K., Li, L., and Luo, L. (2007). A global double-fluorescent Cre reporter mouse. genesis *45*, 593-605.

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet *21*, 70-71.

Sosic, D., Richardson, J.A., Yu, K., Ornitz, D.M., and Olson, E.N. (2003). Twist Regulates Cytokine Gene Expression through a Negative Feedback Loop that Represses NF-[kappa]B Activity. Cell *112*, 169-180.

Su, L., Kinzler, K., Vogelstein, B., Preisinger, A., Moser, A., Luongo, C., Gould, K., and Dove, W. (1992). Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. Science *256*, 668-670.

Xin, H.-B., Deng, K.-Y., Rishniw, M., Ji, G., and Kotlikoff, M.I. (2002). Smooth muscle expression of Cre recombinase and eGFP in transgenic mice. Physiological Genomics *10*, 211-215.

Table S1. Microarray profiling of WT and KO ISEMFs

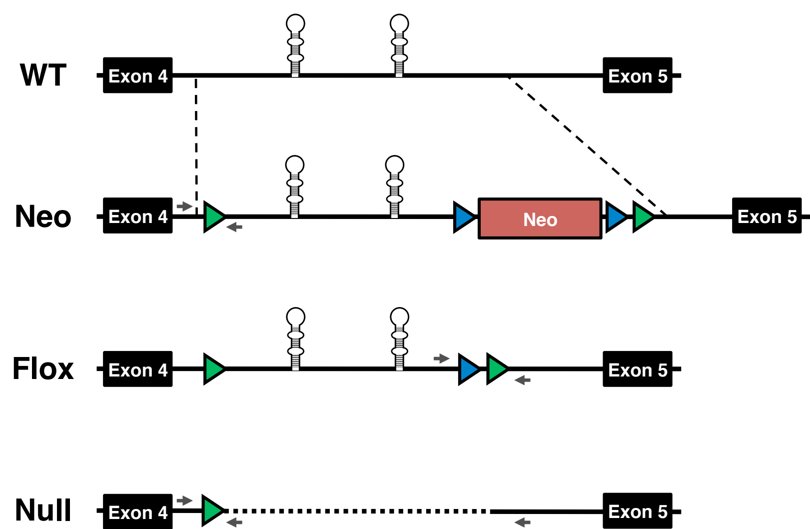
Symbol	Regulation (KO vs WT)	Fold change (KO vs WT)	Regulation (KO+LPS vs WT+LPS)	Fold change (KO+LPS vs WT+LPS)
Ifitm1	Up	2.13	Up	3.87
Igfbp5	Up	2.12	Up	3.03
Tcrb-V13	Up	2.51	Up	2.71
Ptn	Up	2.75	Up	2.62
Il11	Up	1.42	Up	2.60
Spon2	Up	1.85	Up	2.53
Bmp4	Up	2.12	Up	2.38
Serping1	Up	1.94	Up	2.30
1810009M01Rik	Up	2.45	Up	2.25
Tcrb-V8.2	Up	2.13	Up	2.17
Igfbp4	Up	1.60	Up	2.04
Tgfbi	Up	2.41	Up	2.00
Tmem176a	Up	2.11	Up	1.97
Gpc3	Up	2.33	Up	1.80
Ly6a	Up	2.13	Up	1.77
Ccdc3	Up	2.01	Up	1.45
Dcn	Up	2.00	Up	1.44
2310002L13Rik	Up	2.28	Up	1.41
Usp18	Up	2.15	Down	-1.24
Slc5a7	Down	-2.01	Down	-1.70
3110018A08Rik	Down	-2.38	Down	-1.90
Serpinb2	Down	-1.53	Down	-2.14
Pmaip1	Down	-2.04	Down	-2.24

Differentially expressed genes in KO *versus* WT ISEMFs that show at least 2-fold change with or without LPS treatment. 3 independently-derived WT or KO ISEMF cell lines were analyzed per condition (+/- LPS).

Table S2 List of primers

Amplicon	Forward Primer Sequence	Reverse Primer Sequence
Genotyping		
miR$^{flox/+}$.	CGCCCTGTCCATATTTCCTA	CAACGGAGATGAATGGCTTT
miR$^{+/–}$	CCAGCAGACTCCCTTTCCTT	CCAACTGACCAGAGATGCAG and GAAAACTCGTTTCCCTGTGG
qRT-PCR		
mouse 18S	TGTCTCAAAGATTAAGCCATGC	GCGACCAAAGGAACCATAAC
mouse SMA	GTCCCAGACATCAGGGAGTAA	GCCACACGAAGCTCGTTATAG
mouse Vimentin	AGGAGATGCTCCAGAGAGAGG	CTCCTGGATCTCTTCATCGTG
mouse E-cadherin	AAGCTGGAGACCAGTTTCCTC	CTTCTGAGACCTGGGTACACG
mouse Villin	ACTCCCGGGATACAGATATGG	AGTCCTGGCCAATCCAGTAGT
human 18S	CAGTAAGTGCGGGTCATAAGC	CAAGTTCGACCGTCTTCTCAG
human SMA	ACTGCCTTGGTGTGTGACAAT	CTCTTTTGCTCTGTGCTTCGT
human Vimentin	GACCAGCTAACCAACGACAAA	ACTTTGCGTTCAAGGTCAAGA
human E-cadherin	GTCAAAAGGCCTCTACGGTTT	AATGTGAGCAATTCTGCTTGG
human Villin	CTGCAGATATGGAGGATCGAG	AGTCCTGGCCAATCCAGTAGT
Luciferase construct		
mouse Igfbp5 3'UTR WT	AGTCTAGATTCGACAGCAGTAACGTTGAG	AGTCTAGAGTCCGTTCAACTTGCTTCAAA
human IGFBP5 3'UTR WT	AGTCTAGAACACCTTCGACAGCAGCAA	AGTCTAGAGGGGGTGTCTTTTTAGCTTTT
mouse Igfbp5 3'UTR mutant left	GCCGTGTAATTCTAGATTCGAC	TAAATCACAAGTAATGAGTGACGTCCTGGG
mouse Igfbp5 3'UTR mutant right	ATTACTTGTGATTTAGGGGAAATATATATACATA	CCGCCCCGACTCTAGAG
human Igfbp5 3'UTR mutant left	GCCGTGTAATTCTAGACCCTCT	AAATCACAAGTAATGAGTGGCGTCCTGG
human Igfbp5 3'UTR mutant right	CATTACTTGTGATTTAAGGGAAAAATATATATCTATCTATTTG	CCGCCCCGACTCTAGAG
In situ probe		
mouse Igfbp5 probe	GTTACCCCGCCTCTCTTCC	TGTCTGAACGTAACACTATAGAGAGC

Figure S1



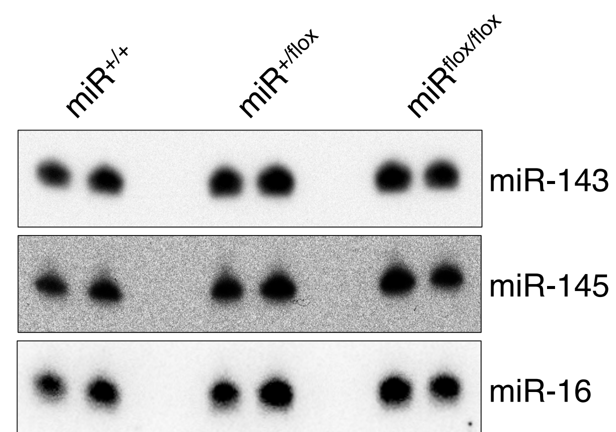


Figure S2

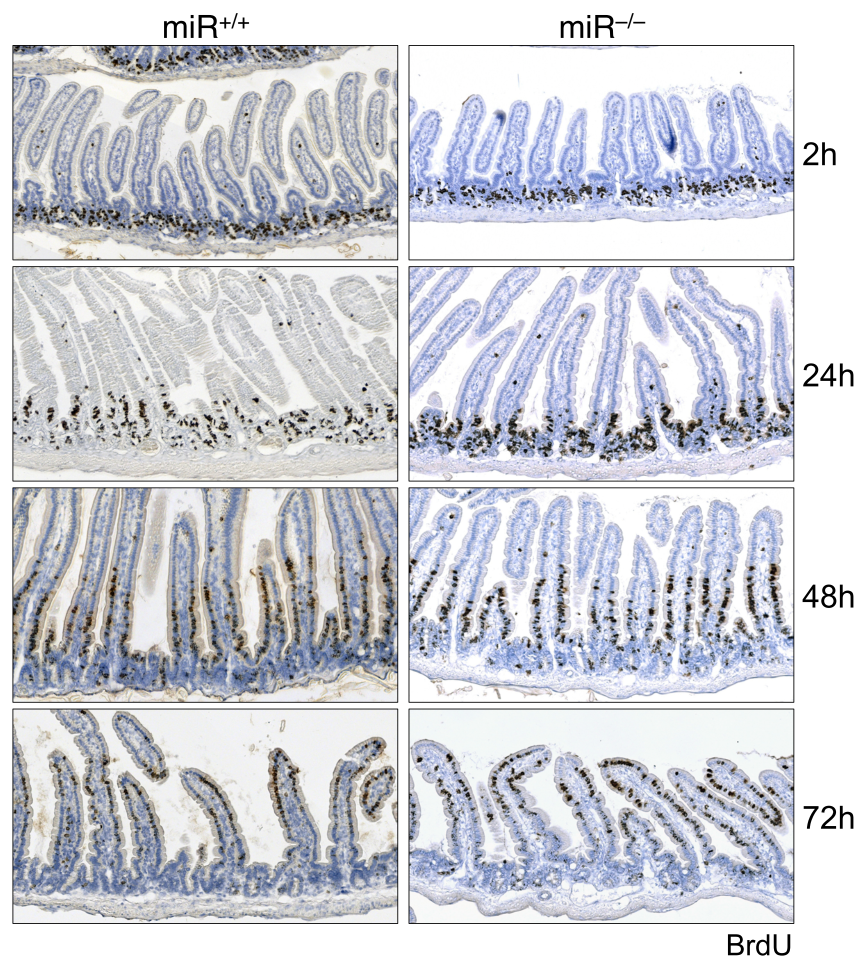
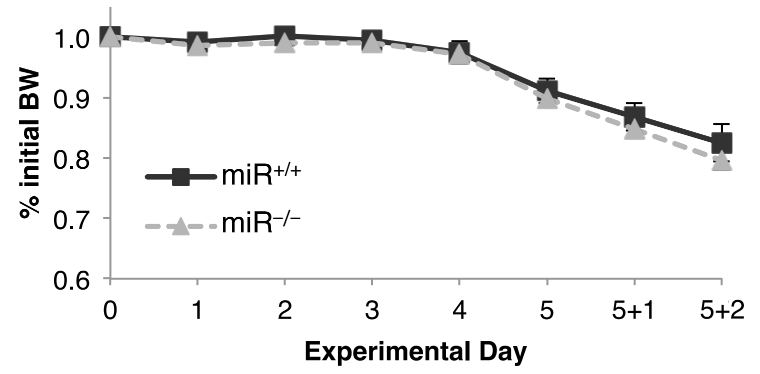


Figure S3



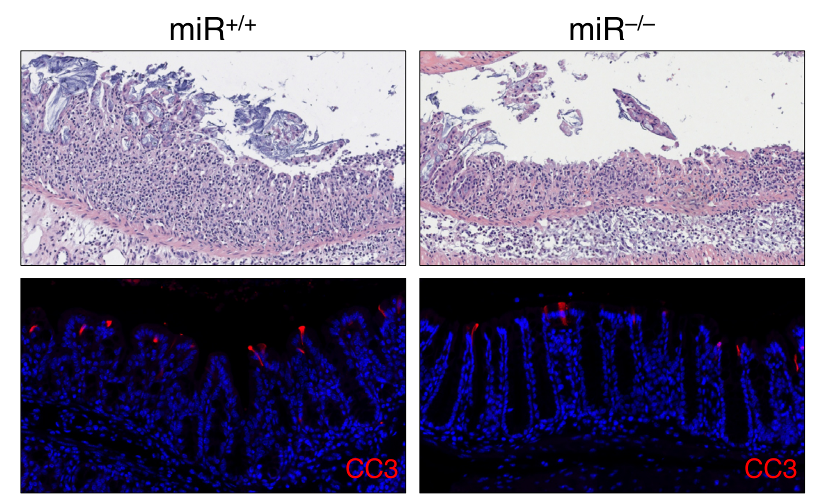
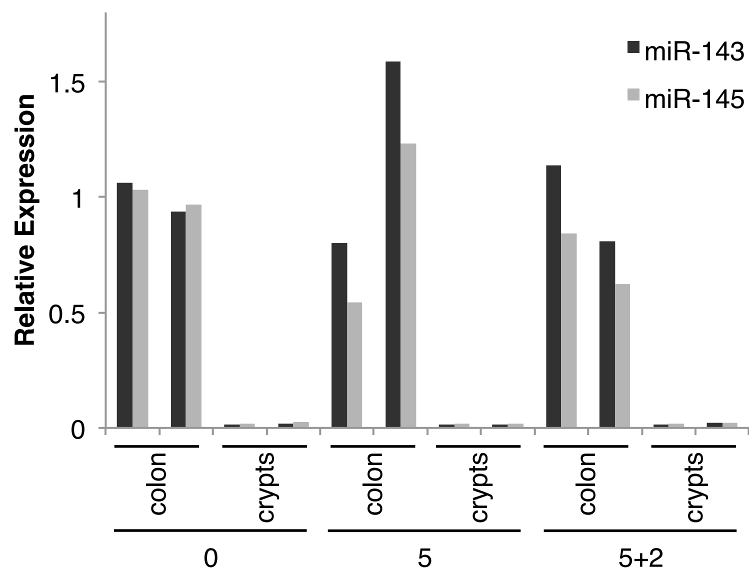


Figure S4



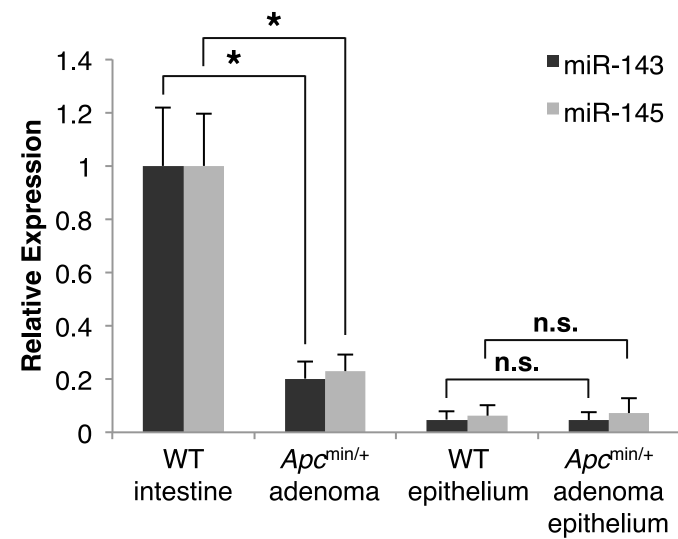


Figure S5

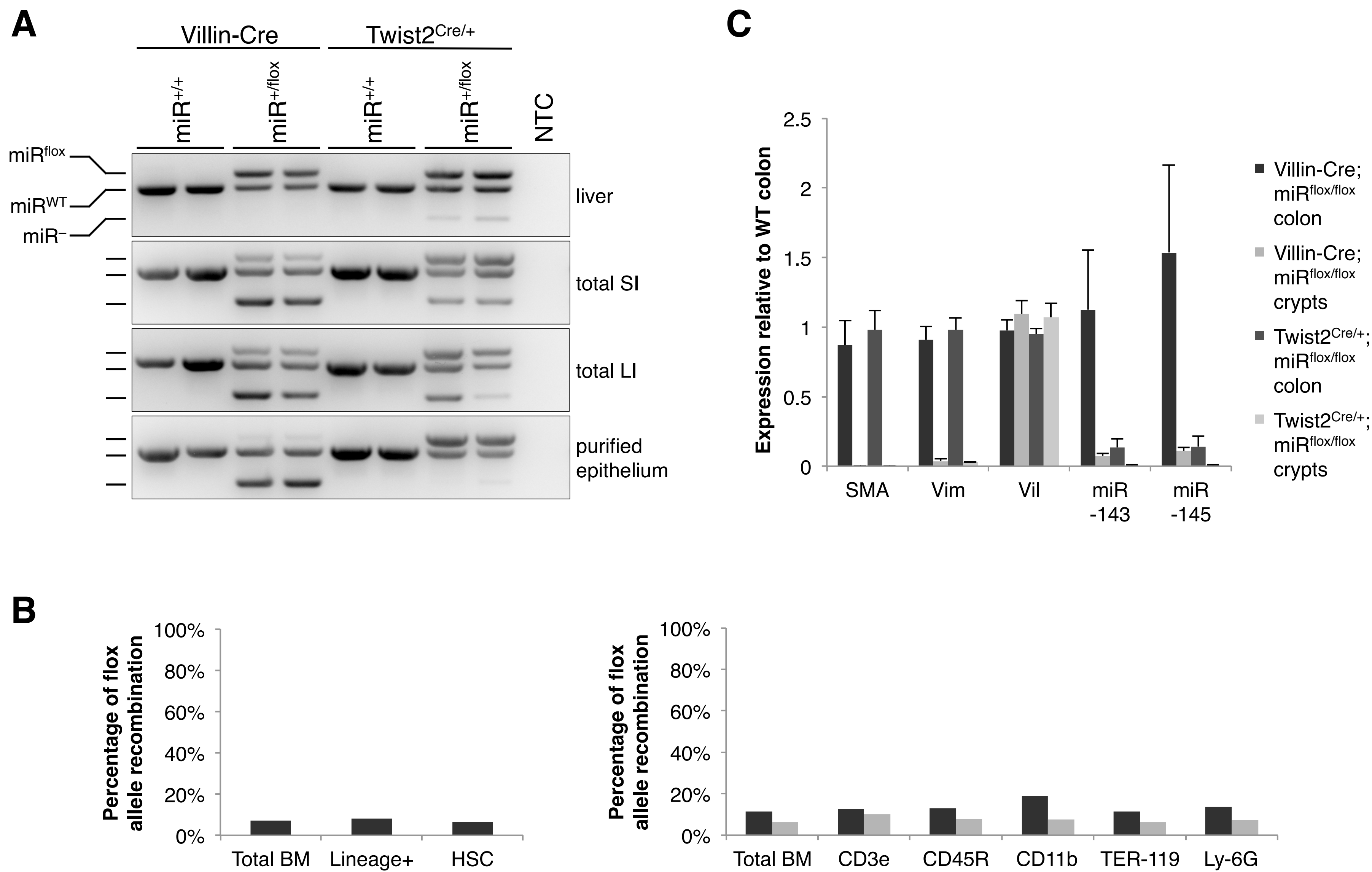


Figure S6

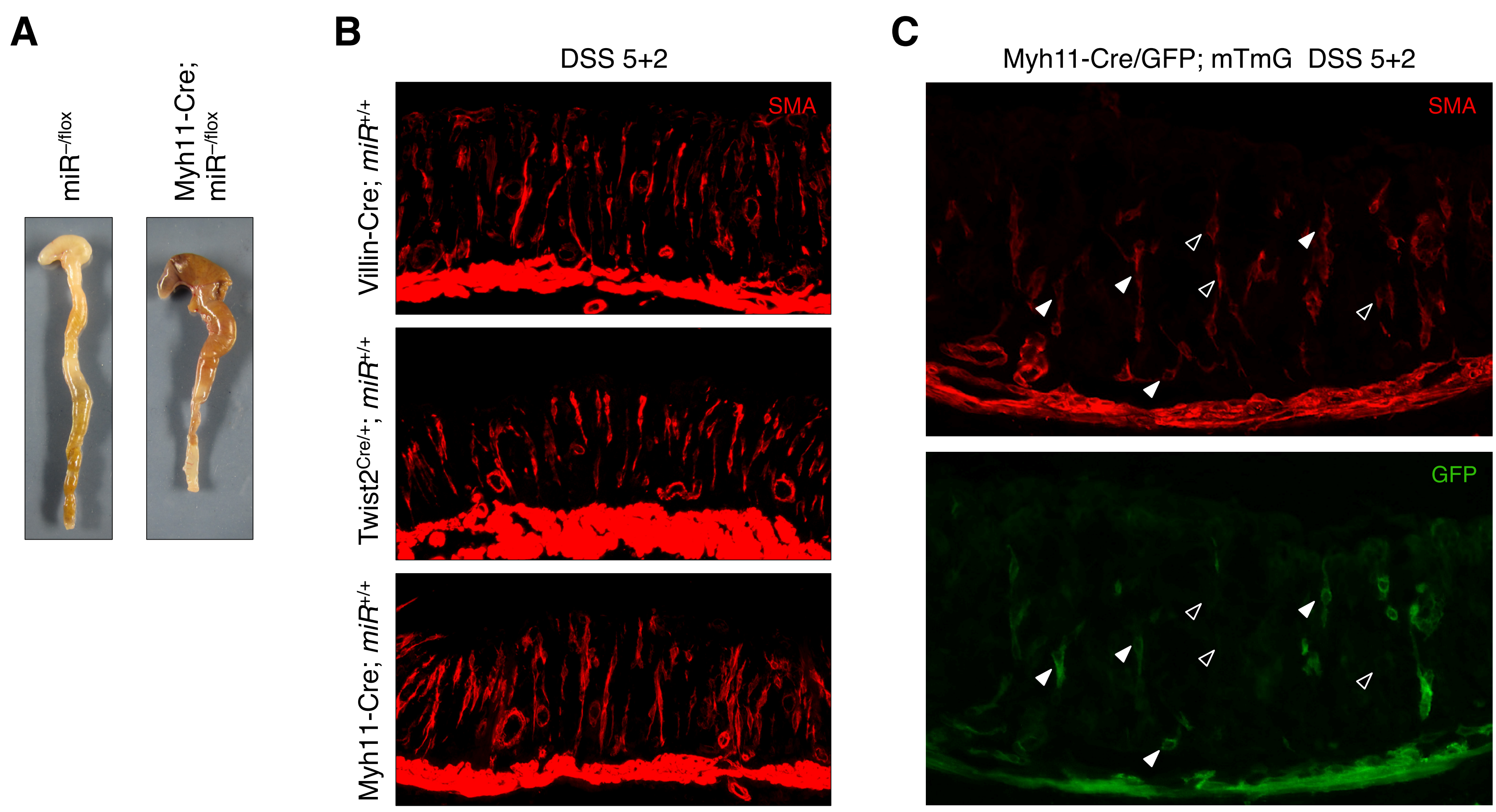


Figure S7