**Luciferase reporter assays** HeLa cells were transiently transfected using FuGENE 6 (Roche Diagnostics) with NFκB luciferase reporter plasmid following the manufacturer's protocol. Empty vector was used to ensure all wells received equal amounts of DNA. 24 h after transfection, cells were stimulated with 1ng/mL IL-1β for 8 hours. Cells were lysed and luciferase activity was assessed using Reporter lysis buffer and Luciferase Assay Reagent (Promega). All results reported are technical triplicates representing at least three independent experiments.

**Plasmid** DNA encoding N-terminal FLAG-tagged SIGIRR was cloned into the vector pCDAN3.1 (+) purchased from Invitrogen. Site-directed mutagenesis was performed on N-terminal FLAG-tagged SIGIRR on pcDNA3.1(+) using QuickChange kit from Agilent Technologies according to the manufacturer's instruction. In all cases, the asparagine was mutated to serine on the plasmid. 5'RACE was performed using a kit from Invitrogen (18374-058) according to the manufacturer's instruction. HA and FLAG-tagged Full-length SIGIRR and SIGIRR$^{\Delta E8}$ expression plasmids were constructed by subcloning from the 5'RACE product into a pcDNA3.1(+) vector.

**Construction of SIGIRR-Transgenic Mouse** Transgenic construct was generated as described before. DNA encoding mouse SIGIRR was placed under the control of transcriptional regulatory elements derived from a fatty acid-binding protein gene followed by the human growth hormone reporter gene (hGH). A Flag tag was included at the N terminus of SIGIRR to distinguish the transgene from the endogenous gene. Site-directed mutagenesis was performed on the construct carrying wildtype SIGIRR sequence to generate mutant transgene. Both constructs were sent to the Transgenic and Targeting Facility at Case Western Reserve University. Mice carrying transgenes were genotyped with polymerase chain reaction to detect presence of hGH sequence. Trasngenes were bred with SIGIRR knockout mice to generate SIGIRR-/-, WT-SIGIRR and SIGIRR-/-, MT-SIGIRR strains.

**Tumorigenesis** Procedure 8-week-old mice (SIGIRR-/-, Fabpl-SIGIRR/Fabpl-SIGIRR$^{N85/101S}$ and their SIGIRR-/- littermates were on mixed C57BL/6×129/SvJ background) were injected with AOM (Sigma) dissolved in 0.9% NaCl intraperitoneally at a dose of 12.5 mg/kg body weight. 5 days after injection, mice were treated with 2.5% DSS in drinking water, then followed by regular water for 16 days. This cycle was repeated twice. 2 weeks after the last DSS treatment, mice were sacrificed and murine colon was removed and flushed carefully with PBS buffer. Colon tumors were counted and measured under a stereomicroscope. Representative tumors were paraffin embedded and sectioned at 5 μm. Histology analysis was carried out on H&E-stained tumor sections.

***In situ* Biotinylation, Immunoprecipitaion** Biotinylation was performed by rinsing transfected cells with cold PBS followed by incubation with freshly prepared 10mM sulfo-NHS-biotin dissolved in cold PBS for 2 hours. The labeling process was stopped by siphoning away the labeling reagent and quenching the with 100mM glycine dissolved in PBS. The cells were then harvested and lysed for lysates. The supernatant was collected for western blot or ELISA analysis. Co-immunoprecipitation was performed by incubating cell lysates with antibodies and protein A beads, or avidin conjugated beads at 4 ℃ overnight. Precipitated protein-beads complex was washed with lysis buffer followed by elution with 2X SDS-PAGE loading buffer.

**Transfection, kifunensine, PNGase F treatment and western blot**. Transfection was performed using Fugene 6 according to the manufacturer's protocol. For kifunensine treatment, the inhibitor was added 8 hours after transfection and the cells were harvested 48 hours after

transfection. PNGase F was purchased from New England Biolabs and used according to the manufacturer's instruction. Cells were lysed in lysis buffer (0.5% Triton X-100, 20 mM HEPES (pH 7.4), 150 mM NaCl, 12.5 mM β-glycerophosphate, 1.5 mM $MgCl_2$, 10 mM NaF, 2 mM dithiothreitol (DTT), 1 mM sodium orthovanadate, 2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride and complete protease inhibitor cocktail from Roche). Western blots were performed after the SDS-PAGE following standard procedure. For analysis of immunoprecipitation samples, anti-light chain secondary antibody (Jackson Immuno Research) was used.

**Quantitative real-time PCR** In all experiments, RNA was extracted with TRIZol (Invitrogen) followed by reverse transcription with SuperScript II Reverse Transcriptase (Life Technologies) according to the manufacturer's instruction. Real-time PCR analysis was performed use SYBR Green master mixes (Agilent Technologies). Primer sequences are as follow: Mmp8 forward -5' CCAGCACCTATTCACTACCTC 3' reverse-5' AGCATCAAATCTCAGGTGGG3' ; Duox2 forward-5' CTTCCACATCTACTTCCTGGTC 3' reverse-5' AATGTCTTGGGTCTCTGGAAC 3'; SIGIRR exon4 forward -5' ACTACAGCCTCCACGAGTAC 3' reverse-5' CCATAGACTTCAGTGCTGGTC 3' ; SIGIRR exon8 forward -5' CTCTTGGTGAACCTGAGCC 3' reverse-5' CCCTCGAAGGTGATGAAGATG 3'. The standard curve for quantification of $SIGIRR^{\Delta E8}$ was established by amplifying cDNA of full-length SIGIRR and $SIGIRR^{\Delta E8}$ mixed at indicated ratio (**Supple. Fig 1A** total input 10ng plasmids) and calculating the Ct difference.

**Immunofluorescence** Formalin-fixed and paraffin-embedded colon sections or tumor samples were deparaffined, rehydrated, and pretreated with 3% hydrogen peroxidase in PBS buffer for 20 minutes. Antigen retrieval in DAKO's antigen retrieval buffer was conducted in a steam cooker for 20 minutes at 96℃, followed by slowly c ooling down at room temperature. After blocking with 10% normal goat serum, sections were incubated with primary antibody overnight at 4℃. Then, the sections are washed with PBST and stained with corresponding secondary antibodies and DAPI. The stained slides are subjected to confocal microscopy for analysis.

**Colon culture** Colon tissue from mice on the day 15 of the AOM-DSS protocol was washed in cold PBS supplemented with penicillin and streptomycin. The tissue was then cut into small pieces and cultured in 12-well flat bottom culture plates (Falcon) in serum-free RPMI medium supplemented with penicillin and streptomycin. After incubation at 37℃ for 24 hr, medium was collected and subjected to ELISA using kit purchased from R&D. Colon orgagnoids were isolated following previously described protocol. Briefly, the normal colon mucosa was isolated and minced. Minced tissue was subjected to collagenase I (Sigma) incubation for 30 minutes at 37 degree. Digested tissue was then filtered through a 70μM cell strainer and washed with DMEM/F12 medium. Isolated crypts were precipitated and embedded in matrigel and cultured in the presence of mouse wnt3a, human R-spoind1, human EGF and mouse Norggin (+WNR) or in the presence of human EGF only.

**Cell sorting** Normal human mucosa and cancer tissue was washed with cold PBS and minced. Minced tissue was then digested with collagenase I at 37 degree for 30 minutes. Isolated crypts were further digested with Trypsin LE (Life Technologies) for 10 minutes to create single cell suspension. Cells were then washed and stained with fluorophore conjugated antibodies. anti-human LGR5 antibody and anti-human EpCam antibody were purchased from Miltenyi Biotec .

**Statistical Analysis** Normality of data was not formally tested. Therefore non-parametric statistics was applied in all data analysis. Mann–Whitney U was used to determine the p value of mean difference in two-group comparison.

**Legends for Supplemental Figures and Table**

**Supplemental Figure 1. A.** Standard curve for the quantification of the percentage of SIGIRR$^{\Delta E8}$ over total SIGIRR. **B.** Real-time PCR analysis of total SIGIRR expression in indicated samples using amplicon targeting the exon4. Error bar represents standard error of mean (S.E.M) of biological replicates. **C.** Increasing amounts of SIGIRR$^{\Delta E8}$ were co-transfected with full-length SIGIRR into HeLa cells. Cell lysates were subjected to western blot using anti-

SIGIRR antibody. Note the similar band pattern between co-expression of SIGIRR$^{\Delta E8}$ with full-length SIGIRR and the endogenous SIGIRR in Vaco400 cells.

**Supplemental Figure 2. A.** $Log_2(RPKM^{Exon8}/RPKM^{Reference\ Exon})$ values computed using RPKM values of other coding region exons as reference were plotted for each sample. **B.** $Log_2(RPKM^{Exon8}/RPKM^{Reference\ Exon})$ Values computed using RPKM values of other coding region exons as reference were plotted as bar graph showing the significant reduction in the tumor samples. * P value <0.01 **P value<0.001

**Supplemental Figure 3. A.** Normal colon mucosa and colorectal cancer tissue was enzymatically dissociated to generate single cell suspension. Cells were stained with FITC conjugated anti-Epcam and PE congjugated LGR5. Epcam+LGR5+ cells were sorted and subjected to real-time PCR analysis. **B.** Normal colon crypts were cultured under previously described condition to derive organoids. Established organoids were then cultured under stem cell condition (+WRN) or differentiating condition (-WRN) followed by real-time PCR analysis. **C.** Normal human colon paraffin section were stained with anti-SIGIRR (green),anti Na+-K+ ATPase antibody (red) and DAPI. **D.** Normal human colon paraffin section were stained with anti-SIGIRR (green),anti β-Catenin antibody (red) and DAPI.

**Supplemental Figure 4.** Colon cancer tissue array of 110 cases was stained with anti-SIGIRR (green), anti-$Na^+$-$K^+$ATPase(red) antibodies and DAPI (blue). Representative images from normal colon tissues **(A)** and adenoma **(B)**, grade I~II colorectal cancer tissues**(C and D)** and grade III colorectal cancer tissues **(E and F)** are shown. Scale bar= 25□m

**Supplemental Figure 5. A.** Co-localization signal of SIGIRR and $Na^+$-$K^+$ATPase was quantified for each sample on the tissue array described in supplemental figure 4. The signal intensity was correlated with the tumor grade. **B.** Immunostaining of a stage II colon cancer for SIGIRR showing the progressive changes from normal to cancer. **C.** RNA from adenoma tissue (7 case in total) and normal tissue was analyzed with real-time PCR.

**Supplemental Figure 6.** Mice of indicated genotypes were subjected to AOM-DSS induced colon tumorigenesis. **A.**Tumor numbers were recorded and plotted. (N=8 for SIGIRR+/-, N=7 for SIGIRR-/-, N=15 for WT-SIGIRR, N=12 for MT-SIGIRR.) **B.** Tumor size distribution in mice underwent experiment described for A. **C.** Representative macroscopic view of colons from mice of indicated genotypes after the AOM-DSS treatment and H&E staining of tumors from mice of indicated genotypes. **D.** 15 days after the initiation of the tumorigenic protocol, the colons were taken for *ex vivo* culture for 12 hours. Supernatant from the organ culture were subjected to ELISA. (N=5, error bar represents S.E.M) **E.** Colons from experiments described for panel D were lysed and the lysates were subjected to western blot. Each lane represents one mouse. **F.** Gene expression analysis by real-time PCR of tumors from mice underwent AOM-DSS. Error bar represents S.E.M * indicates p<0.05, ** indicates p<0.0001

**Supplemental Table 1** Top 100 up-regulated genes in SIGIRR $^{\Delta E8}$ high colorectal cancers

Supplemental Table 1

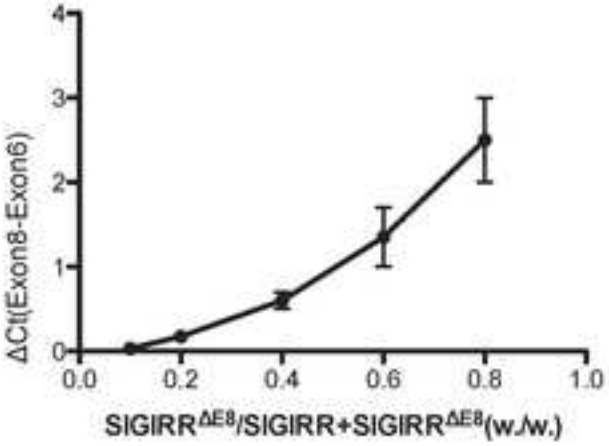
| Gene Description | Fold Change | Adjusted p value |
|---|---|---|
| Immunoglobulin heavy variable 1-3 | 10.79100639 | 1.25E-08 |
| Immunoglobulin heavy variable 1/OR15-3 | 6.885605208 | 7.03E-06 |
| Aldehyde dehydrogenase 1 family, member L1 | 6.084714683 | 2.41E-05 |
| Immunoglobulin kappa variable 6D-21 | 6.082007054 | 8.45E-05 |
| Cathepsin E | 6.068421107 | 5.08E-05 |
| RAD17 homolog | 5.671697711 | 2.86E-05 |
| Defensin, beta 4A | 5.661011918 | 9.59E-05 |
| Tripartite motif containing 72, E3 ubiquitin protein ligase | 5.193330706 | 0.00035181 |
| WAP four-disulfide core domain 12 | 4.940887474 | 0.00044488 |
| Family with sequence similarity 83, member C | 4.897490171 | 0.000705923 |
| Mucin 4, cell surface associated | 4.804269863 | 2.35E-05 |
| Membrane-spanning 4-domains, subfamily A, member 12 | 4.773043002 | 0.000770139 |
| Microsomal triglyceride transfer protein | 4.750526007 | 0.000393668 |
| Keratin 6B | 4.737665939 | 0.000953002 |
| Transient receptor potential cation channel, subfamily V | 4.565926869 | 0.000767522 |
| D-amino-acid oxidase [ | 4.502168041 | 0.000577826 |
| Deleted in malignant brain tumors 1 | 4.499204033 | 0.001384927 |
| Transient receptor potential cation channel, subfamily V | 4.49755197 | 0.000704776 |
| SLC7A11 antisense RNA 1 | 4.496734603 | 0.000152557 |
| TMLHE antisense RNA 1 | 4.48738292 | 0.001209422 |
| Protease, serine, 1 (trypsin 1) | 4.486833221 | 0.001317118 |
| Cystatin S | 4.485949581 | 0.000244128 |
| MicroRNA 5587 | 4.463470593 | 0.001340198 |
| Myosin binding protein C, slow type | 4.450216641 | 0.001263645 |
| Matrix metallopeptidase 8 (neutrophil collagenase) | 4.421040045 | 0.001009067 |
| Neuropeptide Y receptor Y6 | 4.337598106 | 0.001181456 |
| Hydroxycarboxylic acid receptor 3 | 4.296538597 | 6.88E-05 |
| Immunoglobulin kappa joining 5 | 4.255872519 | 0.001498937 |
| Chloride channel accessory 4 | 4.229012122 | 0.001313344 |
| mmunoglobulin kappa variable 1D-27 | 4.185603042 | 0.001232669 |
| Cholinergic receptor, nicotinic, alpha 7 | 4.178820533 | 0.000571709 |
| V-set and immunoglobulin domain containing 2 | 4.127279852 | 0.001359981 |
| Dual oxidase 2 | 4.087002751 | 0.001836333 |
| Immunoglobulin heavy variable 6-1 | 4.063108143 | 0.000511038 |
| Phosphatase and actin regulator 2 pseudogene 1 [ | 4.04135711 | 0.001686791 |
| Chymotrypsin-like elastase family, member 3B | 4.035446872 | 0.001626975 |
| Protease, serine, 33 | 4.034198651 | 0.001232605 |
| FERM and PDZ domain containing 3 | 4.02858679 | 0.001024602 |
| Xanthine dehydrogenase | 4.010987274 | 0.001104457 |
| Interleukin 13 receptor, alpha 2 | 4.002680226 | 4.42E-05 |
| T cell receptor alpha variable 18 | 3.998687428 | 0.000725179 |
| Matrix metallopeptidase 10 (stromelysin 2) | 3.992558873 | 0.001271347 |
| Immunoglobulin kappa variable 2-26 | 3.951600401 | 0.001279844 |
| Keratin 32 | 3.94905481 | 0.003522221 |

| | | |
|---|---|---|
| Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2 | 3.9438296 | 0.003061587 |
| ATP-binding cassette, sub-family A (ABC1), member 12 | 3.935535025 | 0.001448535 |
| Nuclear receptor subfamily 1, group H, member 4 | 3.923146338 | 0.003684918 |
| Immunoglobulin kappa variable 1-16 | 3.909612378 | 0.002932452 |
| Immunoglobulin lambda variable 2-18 | 3.897372209 | 0.003857212 |
| Immunoglobulin heavy variable 3-48 | 3.895695358 | 0.001156257 |
| Hydroxycarboxylic acid receptor 2 | 3.881397594 | 0.00388553 |
| Immunoglobulin heavy variable 3-15 | 3.867017991 | 0.000457937 |
| Regenerating islet-derived 1 beta | 3.863446162 | 0.000914453 |
| Matrix metallopeptidase 20 | 3.844007957 | 0.000254919 |
| Proprotein convertase subtilisin/kexin type 1 | 3.841079319 | 5.30E-06 |
| Acyl-CoA oxidase-like | 3.834592062 | 0.004072552 |
| Keratin 37 | 3.830986264 | 0.002483995 |
| Transmembrane protease, serine 11E | 3.789640306 | 0.001257516 |
| Surfactant associated 2 | 3.787418565 | 3.46E-07 |
| Maestro heat-like repeat family member 2A | 3.768030838 | 0.001586669 |
| CD300 molecule-like family member d | 3.738782812 | 0.004875004 |
| Gliomedin | 3.738144225 | 0.004709175 |
| Solute carrier organic anion transporter family, member 4C1 | 3.698445541 | 0.004974919 |
| Glucosaminyl (N-acetyl) transferase 3, mucin type | 3.696547887 | 0.004475873 |
| Long intergenic non-protein coding RNA 1511 | 3.680602174 | 0.000427588 |
| Fructose-1,6-bisphosphatase 2 | 3.671008112 | 0.005737687 |
| POM121 transmembrane nucleoporin B (pseudogene) | 3.6694481 | 0.003659366 |
| V-set domain containing T cell activation inhibitor 1 | 3.665879761 | 0.003414012 |
| Hepatitis A virus cellular receptor 1 | 3.641632281 | 0.004996819 |
| ADP-ribosyltransferase 5 | 3.641173813 | 0.001865793 |
| Arachidonate 15-lipoxygenase | 3.636888903 | 0.005733402 |
| Immunoglobulin kappa variable 2D-24 | 3.634753674 | 0.002443329 |
| TAR DNA binding protein pseudogene 2 | 3.628337692 | 0.000589396 |
| Immunoglobulin lambda variable 2-23 | 3.62810549 | 0.006184943 |
| Immunoglobulin lambda constant 6 | 3.625289388 | 0.001810775 |
| Small proline-rich protein 2D | 3.614528068 | 0.004130302 |
| Transient receptor potential cation channel, subfamily A, member 1 | 3.611342872 | 0.006382652 |
| Hepsin | 3.589679497 | 0.000777594 |
| Pleckstrin homology domain containing, family G | 3.563481725 | 0.006934812 |
| Immunoglobulin lambda variable 6-57 | 3.560848072 | 0.002290553 |
| Keratin 6A | 3.560529202 | 0.005949099 |
| Insulin-like growth factor binding protein 1 | 3.554819224 | 0.003979318 |
| Carbonic anhydrase II | 3.554701171 | 0.004198011 |
| Mucin 5B, oligomeric mucus/gel-forming | 3.549785303 | 0.001247391 |
| Immunoglobulin heavy variable 3-23 | 3.539717146 | 0.007236282 |
| Solute carrier family 4 (sodium bicarbonate cotransporter), member 4 | 3.538834282 | 0.001099452 |
| Immunoglobulin heavy variable 3/OR16-6 | 3.530049121 | 0.002957845 |
| Mucin-like 1 | 3.525221616 | 0.000948065 |

| | | |
|---|---|---|
| Immunoglobulin lambda variable 8-61 | 3.519610177 | 0.006039831 |
| Immunoglobulin kappa variable 1-27 | 3.516856642 | 0.005259029 |
| Keratin 3 | 3.51663443 | 0.005218816 |
| Sperm associated antigen 17 | 3.513435344 | 0.007608932 |
| EYA transcriptional coactivator and phosphatase 4 | 3.49913034 | 0.006622943 |
| Ubiquitin-conjugating enzyme E2 variant 2 | 3.485678047 | 0.000557363 |
| Dehydrogenase/reductase (SDR family) member 9 | 3.478656468 | 0.008038889 |
| Androgen-dependent TFPI-regulating protein | 3.472637185 | 0.007586122 |
| Butyrophilin-like 8 | 3.471366973 | 0.000919719 |
| Immunoglobulin heavy variable 2-5 | 3.446423183 | 0.008586999 |
| Immunoglobulin J polypeptide, linker protein | 3.442054932 | 0.008562378 |
| Protease, serine, 41 | 3.423634323 | 0.007638857 |

## Supplemental Figure 1.

A.

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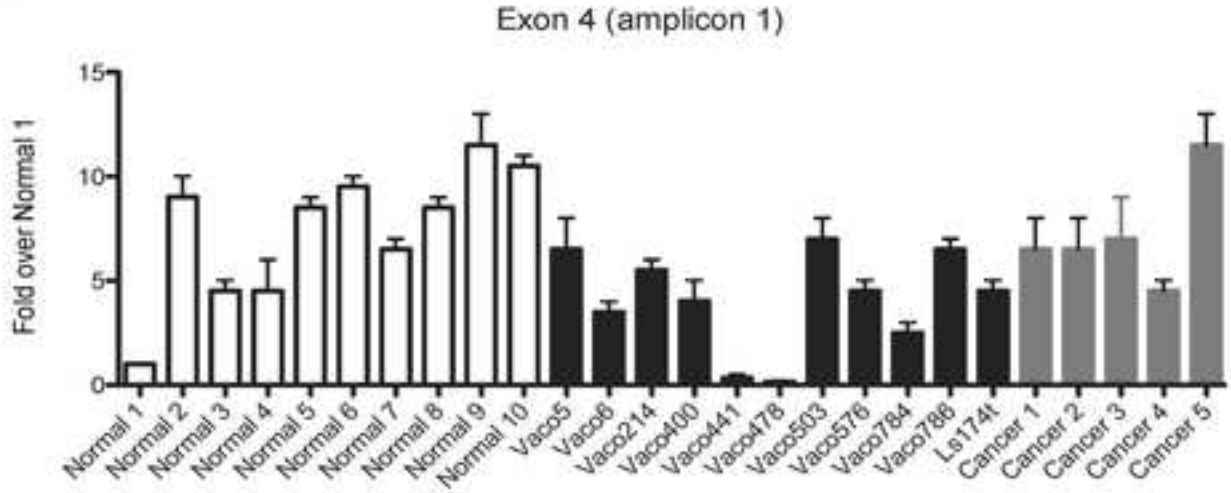


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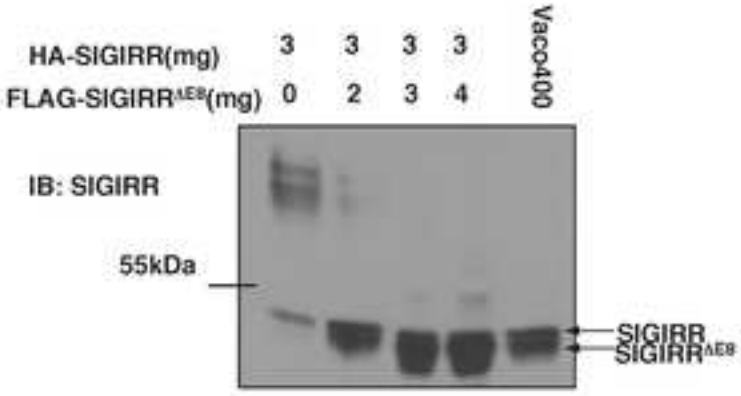

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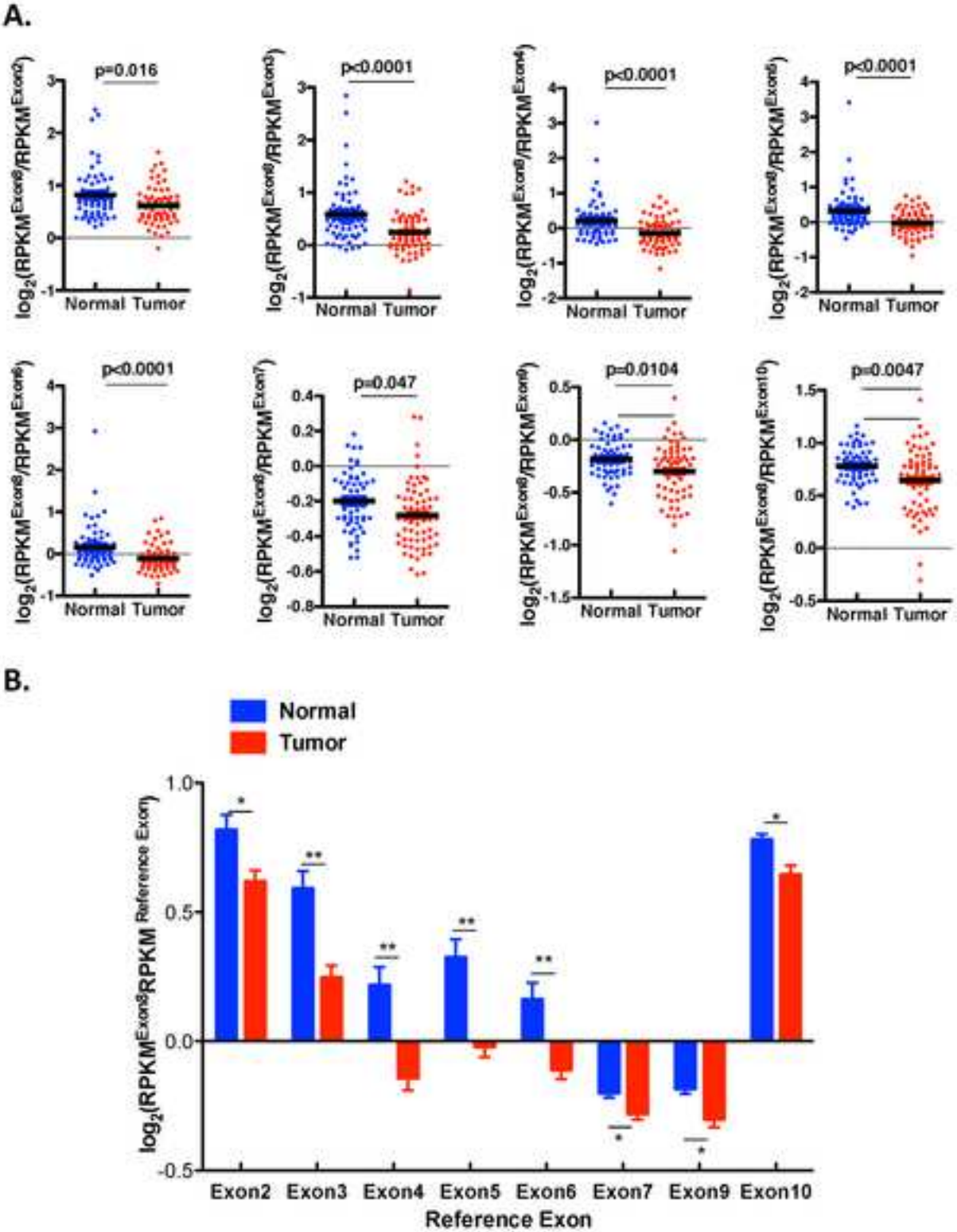

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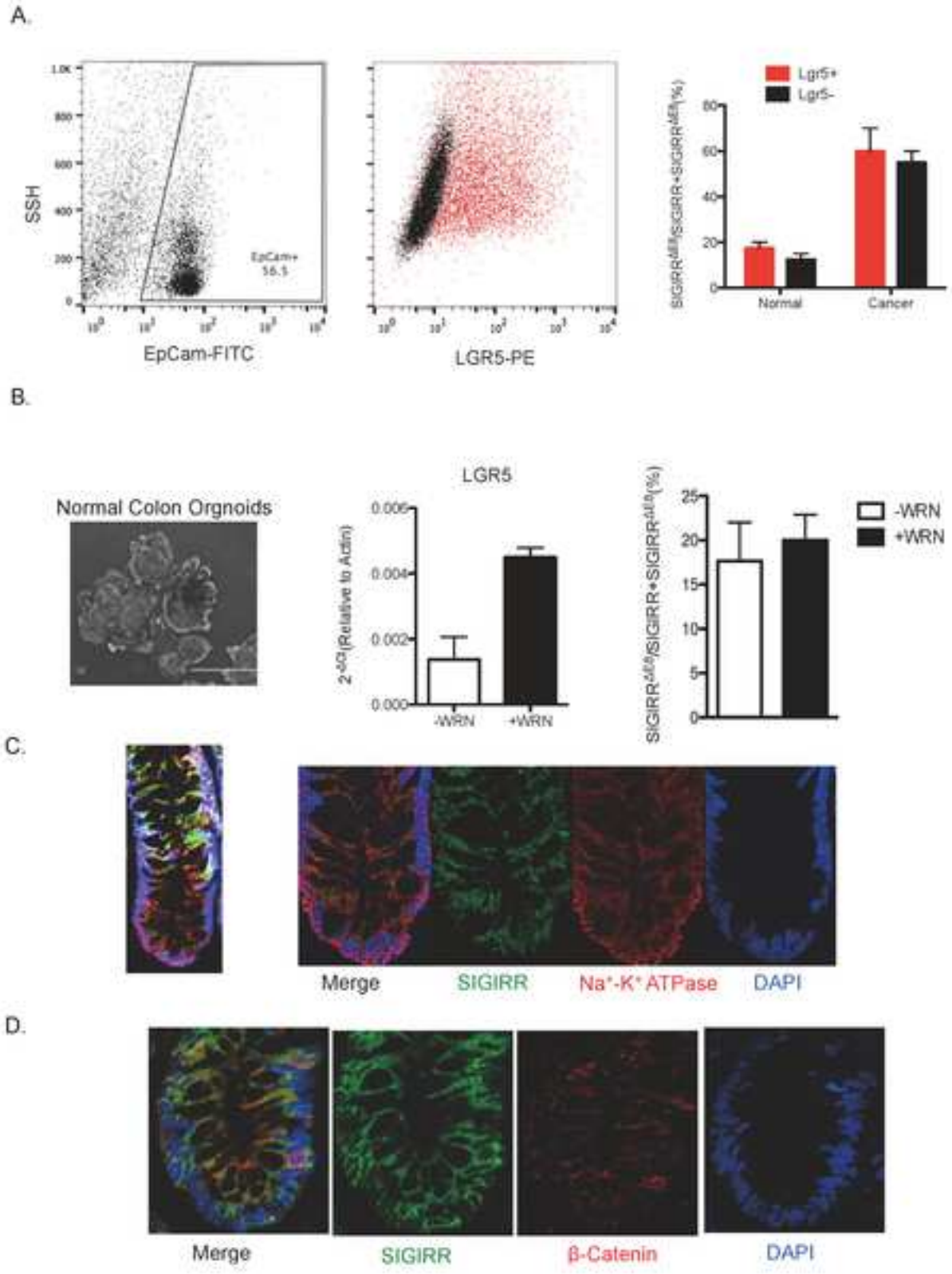
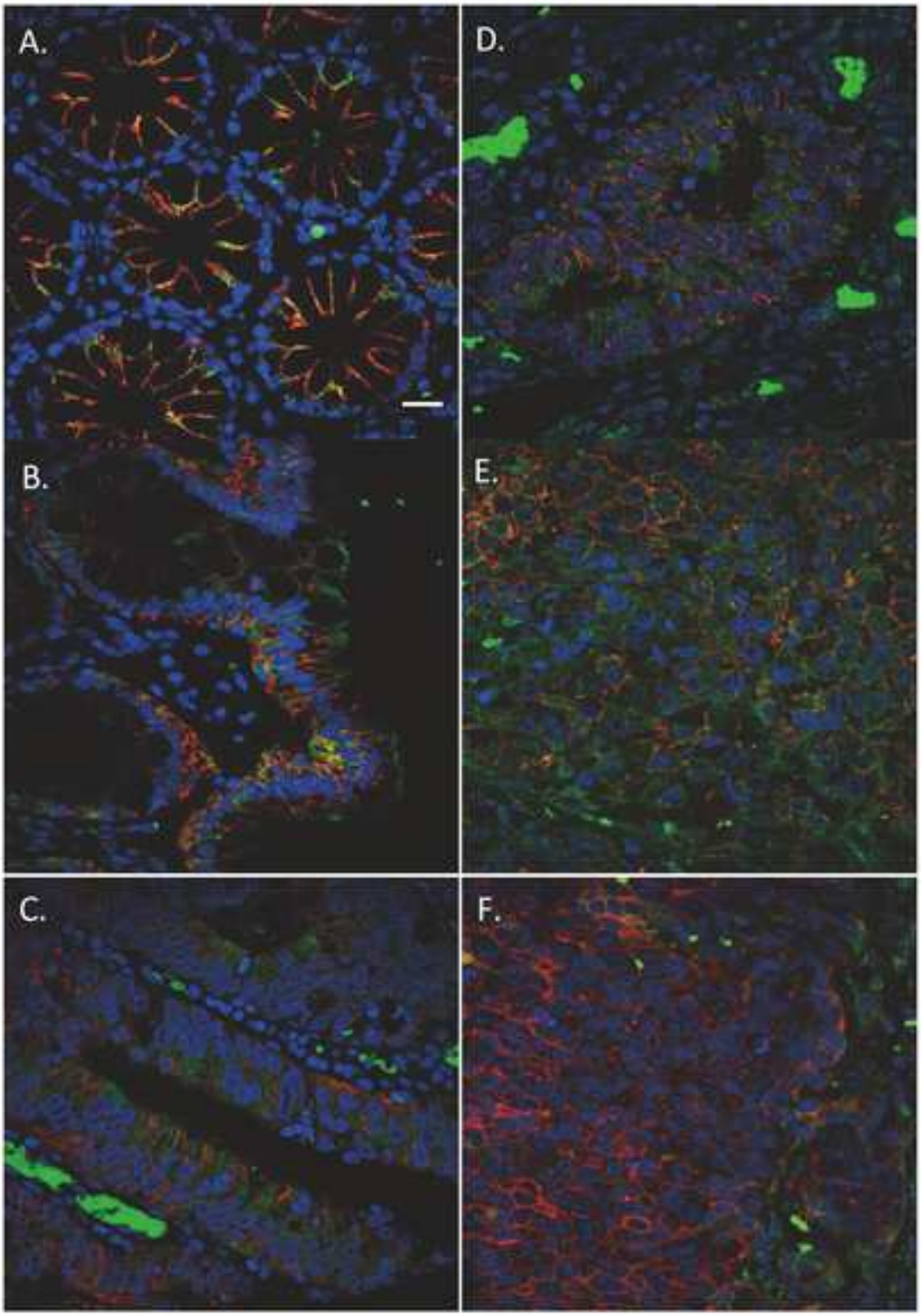
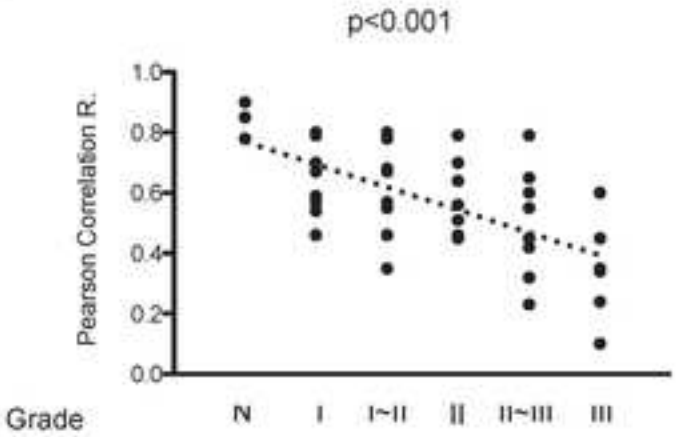


**Supplemental Figure 4.**



## Supplemental Figure 5.

A.

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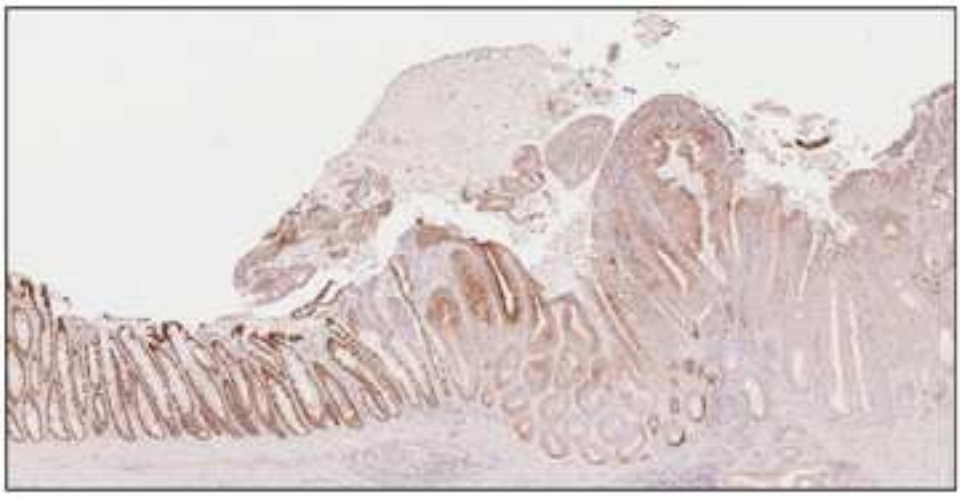

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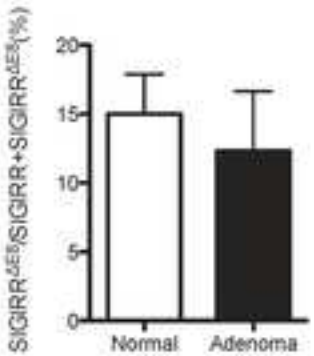

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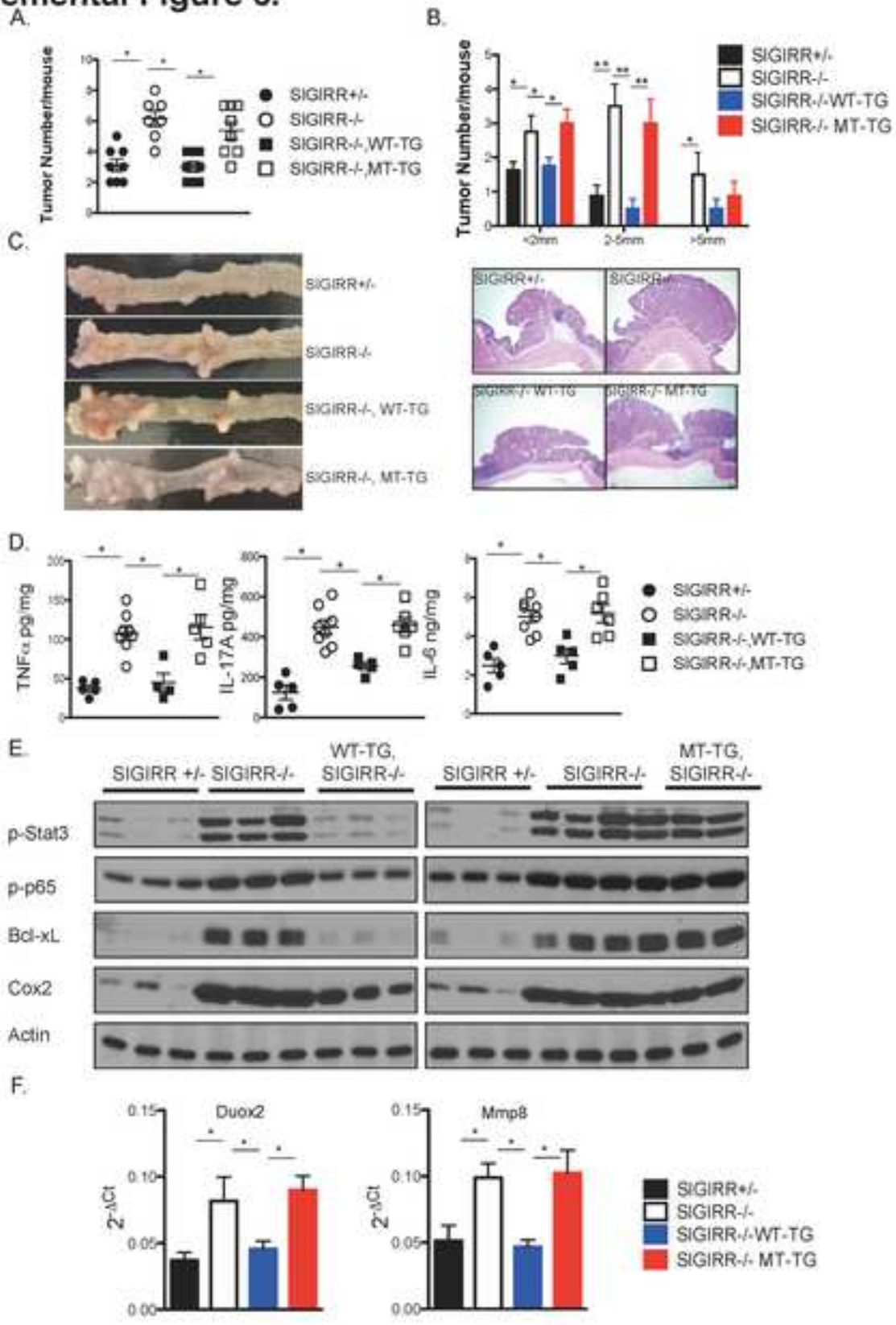

**Supplemental Figure 7.**