SUPPLEMENTAL DATA

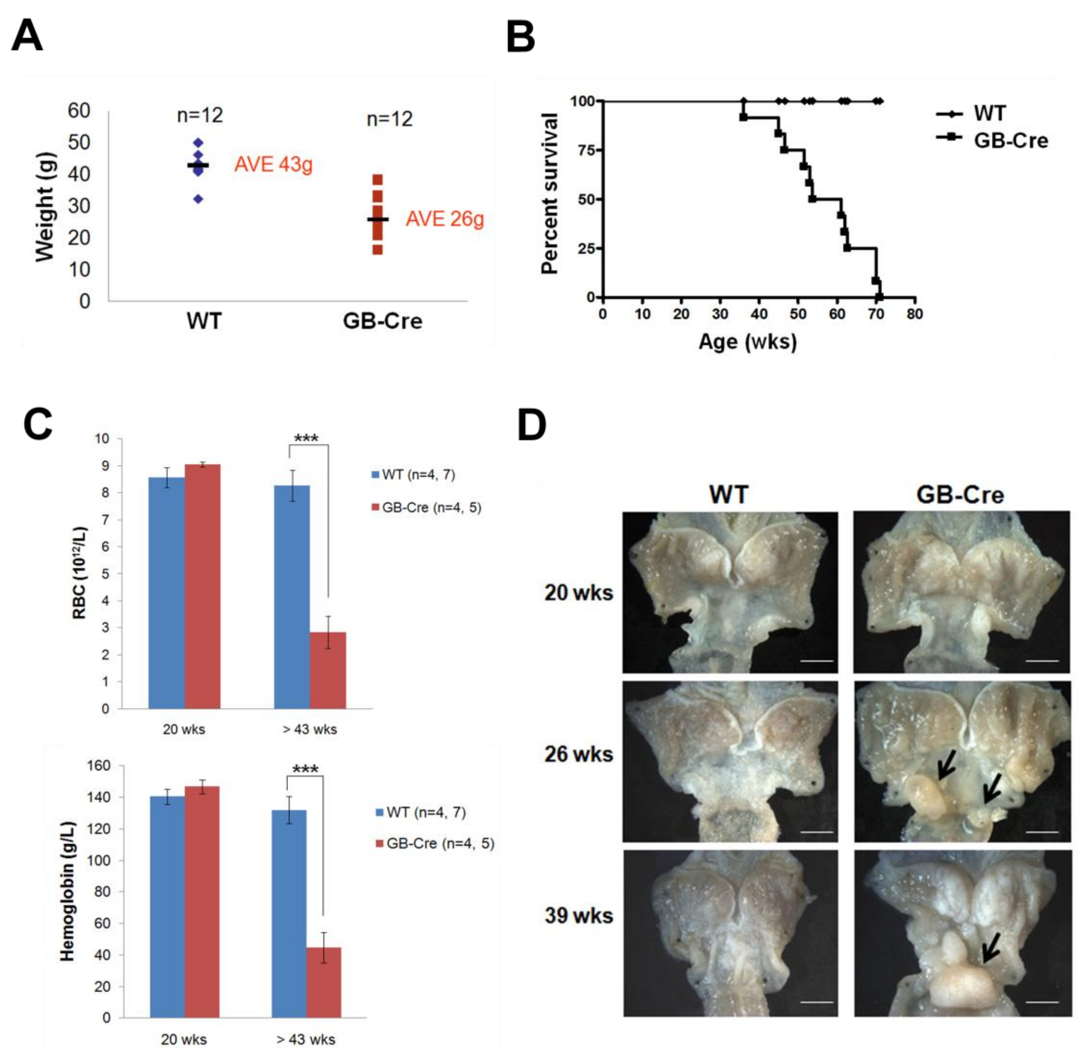

Figure S1. ***Gastric polyp-bearing GB-Cre;Smad4$^{fl/fl}$ mice exhibited weight loss and anemia***

(**A**) Weight loss in 12-18 mo old GB-Cre;*Smad4$^{fl/fl}$* mice as compared to age-matched controls (n=12 mice/group). (**B**) Survival curve for GB-Cre;*Smad4$^{fl/fl}$* mice (n = 12 mice/group). (**C**) Flow cytometric analysis for red blood cell (RBC) counts and hemoglobin concentrations from either 20 wk old, or >43 wk old control (WT) and moribund polyp-bearing animals. Data are shown as the mean +/- SEM (n >4 mice/group) from four independent experiments. Statistical analyses employed the 2-tailed unpaired T test: *$p < 0.05$, **$p < 0.01$, ***$p<0.001$. (**D**) Gastric dissections of control and experimental mice performed at the indicated time points (20, 26, or 39 weeks). Arrows indicate sites of early polyp development. Scale bar = 0.25 cm. Images are representative of 4 mice per group (WT = *Smad4$^{fl/fl}$*; GB-Cre = GB-Cre;*Smad4$^{fl/fl}$*).

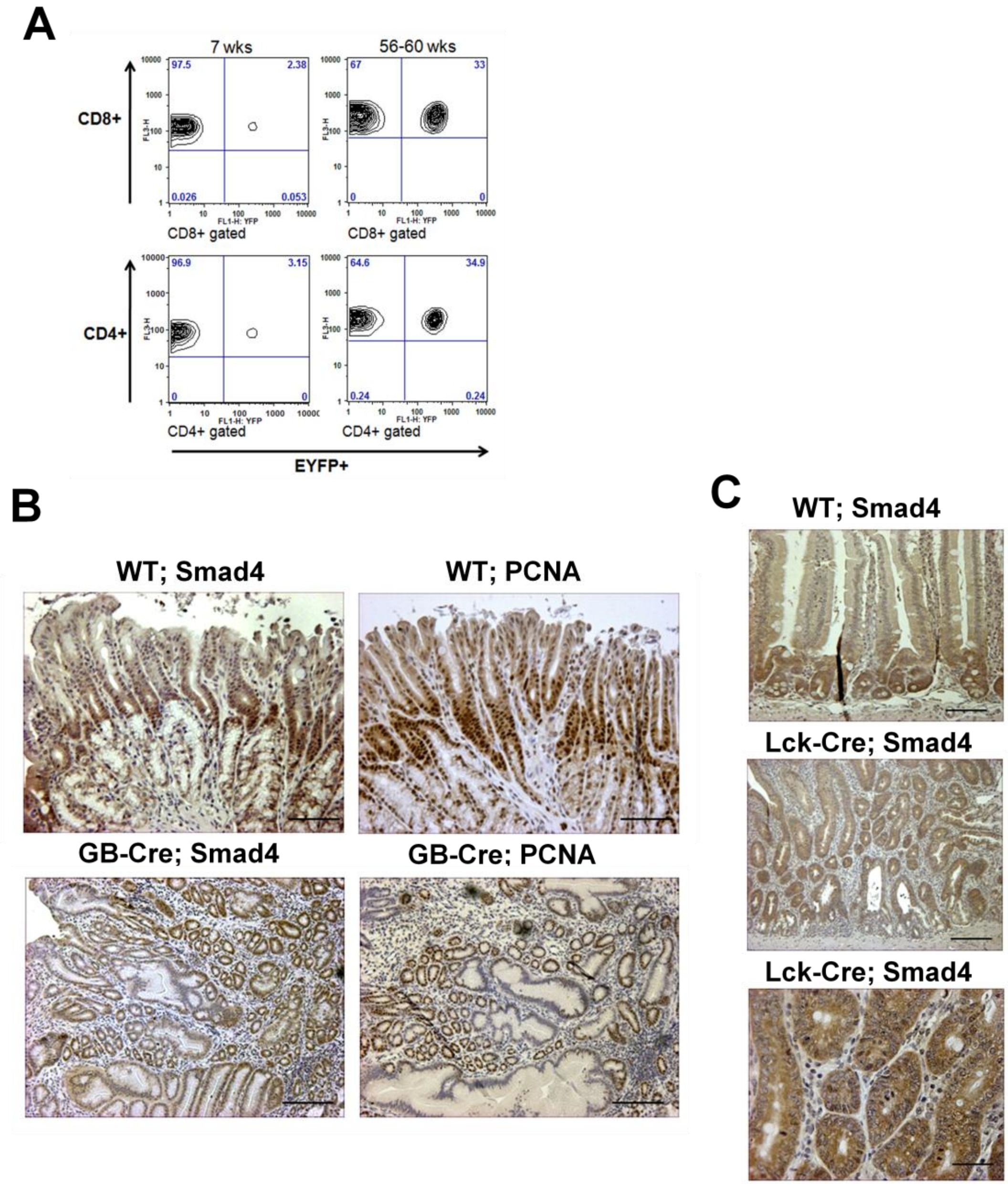

Figure S2. ***Gene excisions in GB-Cre T cells increase with age and demonstration that Smad4 protein expression is preserved in both gastric and duodenal polyp epithelium***

(**A**) A representative flow cytometry plot of EYFP expression in 7 wk, and 56-60 wk old GB-Cre;R26R-EYFP splenocytes stained with anti-CD4 and anti-CD8 antibodies (**Figure 3B** shows the results in graph form). (**B**) Antro-pyloric region histology of a *Smad4*$^{fl/fl}$ (WT) and GB-Cre;*Smad4*$^{fl/fl}$ (GB-Cre) mouse, stained for either anti-Smad4 antibody (Smad4) or anti-PCNA antibody (PCNA) (scale bars = 125 µm in top two panels; 250 µm in bottom two panels). Smad4 protein expression levels correlate with PCNA-positive (proliferative) areas in the WT antro-pyloric region (top two panels) and in the GB-Cre polyp regions (bottom two panels) (also see **Figure 1J**). Proliferative buds stained well with the anti-Smad4 antibody, whereas elongated glands stained for Smad4 at a lower level (bottom two panels). Images are a representative of 3-4 mice per group. (**C**) Duodenal histology of a *Smad4*$^{fl/fl}$ (WT) and Lck-Cre;*Smad4*$^{fl/fl}$ (Lck-Cre) mouse, stained for anti-Smad4 antibody (scale bars = 125 µm (top), 250 µm (middle), and 40 µm (bottom)). In comparison to **Figure 2C** (WT, PCNA) and **Figure 2F** (Lck-Cre, PCNA), Smad4 protein levels also correlate with proliferative regions. The bottom panel represents a high power view of Smad4-expressing dysplastic tubular epithelium. Images are a representative of four mice per group.

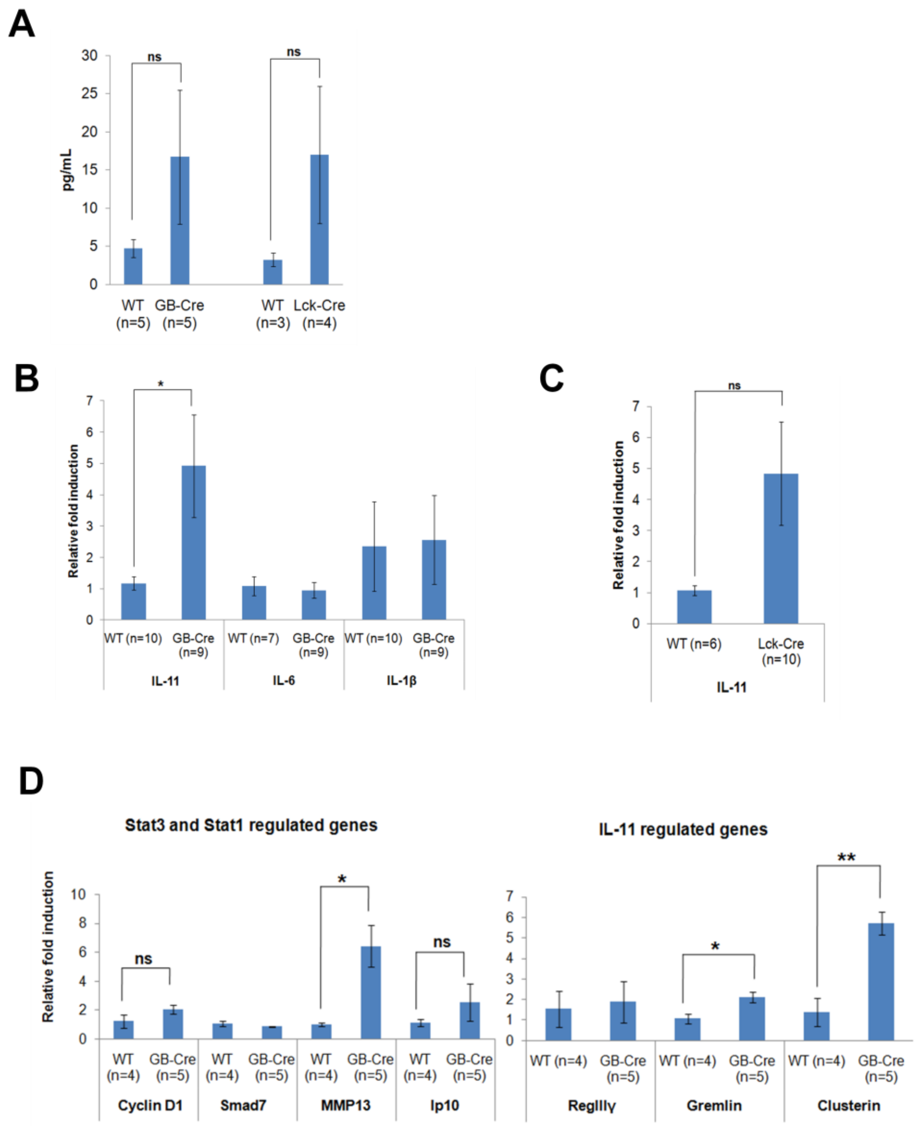

Figure S3. GB-Cre;*Smad4$^{fl/fl}$* and Lck-Cre;*Smad4$^{fl/fl}$ IL-6 serum concentrations and transcript levels of Stat3-, Stat1- and IL-11-regulated genes in GB-Cre;Smad4$^{fl/fl}$ gastric polyp RNA samples*

(**A**) IL-6 serum levels from 12-18 mo old control (WT), GB-Cre;*Smad4$^{fl/fl}$* (GB-Cre) and Lck-Cre;*Smad4$^{fl/fl}$* (Lck-Cre) mice. Data shown are the mean +/- SEM (n = 3-5 mice/group). (**B**) qRT-PCR real-time analysis of the indicated transcripts within the RNA samples isolated from the antro-pyloric regions of the indicated mice (this included 7 GB-Cre samples lacking polyps and 2 samples containing polyp nodules < 2 mm in diameter), and proximal duodenal region (Lck-Cre samples included 6 samples with no polyps, 3 samples with < 2 mm diameter polyps, and 1 sample containing a ~3 mm diameter duodenal nodule) (**C**) of 20-26 wk old mice. Data shown are the mean +/- SEM (n = 6-10 mice/group). (**D**) qRT-PCR real-time analysis of the indicated transcripts within the RNA isolated from the antro-pyloric regions of 12-18 mo old polyp-containing GB-Cre;*Smad4$^{fl/fl}$* and age matched control (WT) mice. Data are the mean +/- SEM (n = 4-5 mice/group) from two independent experiments. Statistical analyses employed the 2-tailed unpaired T test: *p < 0.05, **p < 0.01, ns = not significant. WT = *Smad4$^{fl/fl}$*; GB-Cre = GB-Cre;*Smad4$^{fl/fl}$*; Lck-Cre = Lck-Cre;*Smad4$^{fl/fl}$*

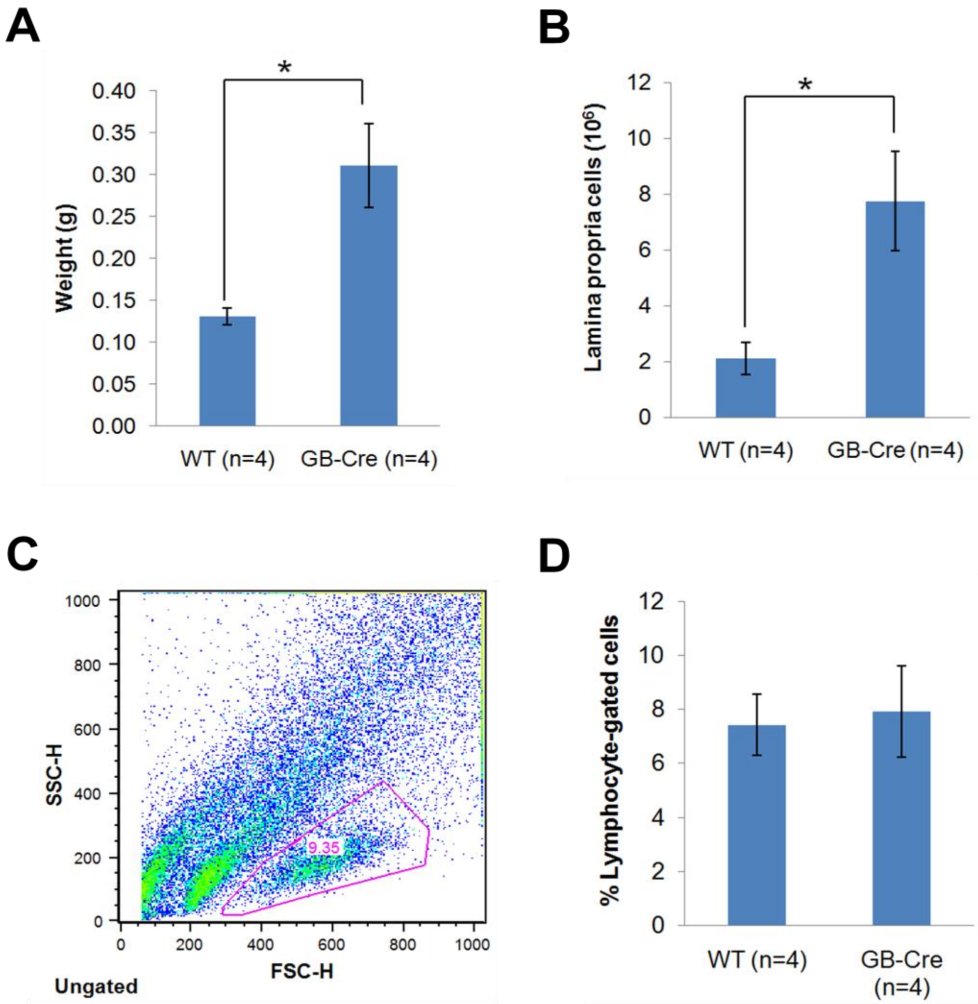

Figure S4. ***Flow cytometry of antro-pyloric region lamina propria cell populations***

Antro-pyloric regions of polyp-containing GB-Cre;*Smad4*$^{fl/fl}$ and age-matched controls (WT) were weighed (**A**), and lamina propria (LP) cells isolated and quantified (**B**). Flow cytometry was used to gate the lymphocyte-enriched cell population (**C**). Percentage of the lymphocyte-gated cells of the total number of cells within experimental and control LP isolations is shown (**D**). Data are shown as the mean +/- SEM (n = 4 mice/group) from four independent experiments. Statistical analyses used the 2-tailed unpaired T test, $*p < 0.05$. WT = *Smad4*$^{fl/fl}$; GB-Cre = GB-Cre;*Smad4*$^{fl/fl}$

Supplemental Experimental Procedures

Table 1: Mouse Genotyping Primer Sets

Gene	Primer names	Primer sequences	Ta	Band size
GB-Cre	G3F G3R	5' – AGC CTG CTC CAC CTC TTT CCT -3' 5' – GCT CGA CCA GTT TAG TTA CCC - 3'	57.5°C	800 bp
Lck-Cre	CreF CreR	5'-GCC TGC ATT ACC GGT CGA TGC-3' 5'- CAG GGT GTT ATA AGC AAT CCC-3'	59°C	490 bp
EYFP	JCC745 JCC746 JCC747	5' – GCG AAG AGT TTG TCC TCA ACC -3' 5' – GGA GCG GGA GAA ATG GAT ATG -3' 5' – AAA GTC GCT CTG AGT TGT TAT – 3'	67°C	Tg = 300bp Wt = 550bp
Smad4 fl/fl	Smad4-9 Smad4-10	5' – GGG CAG CGT AGC ATA TAA GA -3' 5' – GAC CCA AAC GTC ACC TTC AC -3'	56°C	Flox = 450bp Wt = 390bp
Smad4 excision	Smad4-11 Smad4-12	5' – ACA GGT TTC AGT TCA GGT GC -3' 5' – CAT TTG CAG TCA GGA AGC AG -3'	57°C	200 bp

Table 2: Real time PCR Primer Sets

mRNA	Primer names	Primer sequences	Ta	Reference
β-actin	β-actin for β-actin rev	5'- AGA GGG AAA TCG TGC GTG AC -3' 5'- CAA TAG TGA TGA CCT GGC CGT -3'	59°C	(1)
Activin βA	ActβA for ActβA rev	5'-GGA GAA CGG GTA TGT GGA GA-3' 5'-TGG TCC TGG TTC TGT TAG CC-3'	60°C	(2)
Activin βB	ActβB for ActβB rev	5'-CCT GAG TGA ATG CAC ACC AC-3' 5'-CGA GTC CAG TTT CGC CTA GT-3'	60°C	(2)
BMP2	Bmp2 for Bmp2 rev	5'-TGG AAG TGG CCC ATT TAG AG-3' 5'-TGA CGC TTT TCT CGT TTG TG-3'	58°C	(3)
BMP4	Bmp2 for Bmp2 rev	5'-ACG TAG TCC CAA GCA TCA CC-3' 5'-TCA GTT CAG TGG GGA CAC AA-3'	59°C	(3)
BMP7	Bmp7 for Bmp7 rev	5'-TAC GTC AGC TTC CGA GAC CT-3' 5'-GGT GGC GTT CAT GTA GGA GT-3'	60°C	(3)
Clusterin	Clusterin for Clusterin rev	5'- GAA GGG CCA GTG TGA AAA GTG -3' 5 - TTA GCC TGG GCA GGA TTG TT -3'	58°C	(4)
Cyclin D1	Cyclin D1 for Cyclin D1 rev	5'- CAC AAC GCA CTT TCT TTC CA -3' 5'- GAC CAG CCT CTT CCT CCA C -3'	57°C	(5)
FoxP3	FoxP3 for FoxP3 rev	5'-GGC CCT TCT CCA GGA CAG A-3 5'-GCT GAT CAT GGC TGG GTT GT-3'	60°C	(6)
Gastrin	Gastrin for Gastrin rev	5'-ACA CAA CAG CCA ACT ATT C-3' 5-CAA AGT CCA TCC ATC CGT AG-3'	52°C	(7)
Gremlin	Gremlin for Gremlin rev	5'- TGA AGC AGT GCC GTT GCA TA -3' 5'-TGA ATG TGC CCG CTT TGA -3'	53°C	(4)
IFNγ	IFNγ for IFNγ rev	5'- TCA AGT GGC ATA GAT GTG GAA GAA -3' 5'- TGG CTC TGC AGG ATT TTC ATG -3'	60°C	(1)
IL-1β	IL-1β for IL-1β rev	5'- CAA CCA ACA AGT GAT ATT CTC CAT G -3' 5'- GAT CCA CAC TCT CCA GCT GCA -3'	60°C	(1)
IL-4	IL-4 for IL-4 rev	5'- ACA GGA GAA GGG ACG CCA T -3' 5'- GAA GCC CTA CAG ACG AGC TCA -3'	58°C	(1)
IL-6	IL-6 for IL-6 rev	5'- TCC AGT TGC CTT CTT GGG AC -3' 5'- GTG TAA TTA AGC CTC CGA CTT G -3'	60°C	(5)
IL-10	IL-10 for IL-10 rev	5'- GGT TGC CAA GCC TTA TCG GA -3' 5'- ACC TGC TCC ACT GCC TTG CT -3'	60°C	(1)
IL-11	IL-11 for IL-11 rev	5'- CTG CAC AGA TGA GAG ACA AAT TCC-3' 5'- GAA GCT GCA AAG ATC CCA ATG-3'	60°C	(5)
IL-17	IL-17 for IL-17 rev	5′- GAA GCT CAG TGC CGC CA -3′ 5′- TTC ATG TGG TGG TCC AGC TTT -3′	58°C	(8)
IL-23p19	IL-23p19 for IL-23p19 rev	5'- TGC TGG ATT GCA GAG CAG TAA -3' 5'- ATG CAG AGA TTC CGA GAG A -3'	58°C	(9)
Ip10	Ip10 for Ip10 rev	5'- GCC GTC ATT TTC TGC CTC AT -3' 5'- GCT TCC CTA TGG CCC TCA TT -3'	58°C	(5)
MMP13	MMP13 for MMP13 rev	5'- CTG GTC TGA TGT GAC ACC ACT -3' 5'- CCA GAA GAC CAG AAG GTC CAT -3'	60°C	(5)
RegIIIγ	RegIIIγ for RegIIIγ rev	5'- GAA CCC ATC TAC TGC CTT AGA CC -3' 5'- CTT CAC ATT TTG TCC CTT GTC -3'	60°C	(5)
Runx3	Runx3 for Runx3 rev	5'-GGT TCA ACG ACC TTC GAT TC-3' 5'-AGG CCT TGG TCT GGT CTT CT-3'	58°C	(10)
TFF1	TFF1 for TFF1 rev	5' – AAT TGT GGC TTC CCC GGT GTC A - 3' 5' – ATT CAT CTC TTT TAA TTC TCA GGC C - 3'	54°C	(11)
TGFβ1	TGFβ1 for TGFβ1 rev	5' - TGA CGT CAC TGG AGT TGT ACG G - 3' 5' - GGT TCA TGT CAT GGA TGG TGC - 3'	60°C	(1)
TNFα	TNFα for TNFα rev	5'- CAT CTT CTC AAA ATT CGA GTG ACA A -3' 5'- TGG GAG TAG ACA AGG TAC AAC CC -3'	63°C	(1)

Supplemental References

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