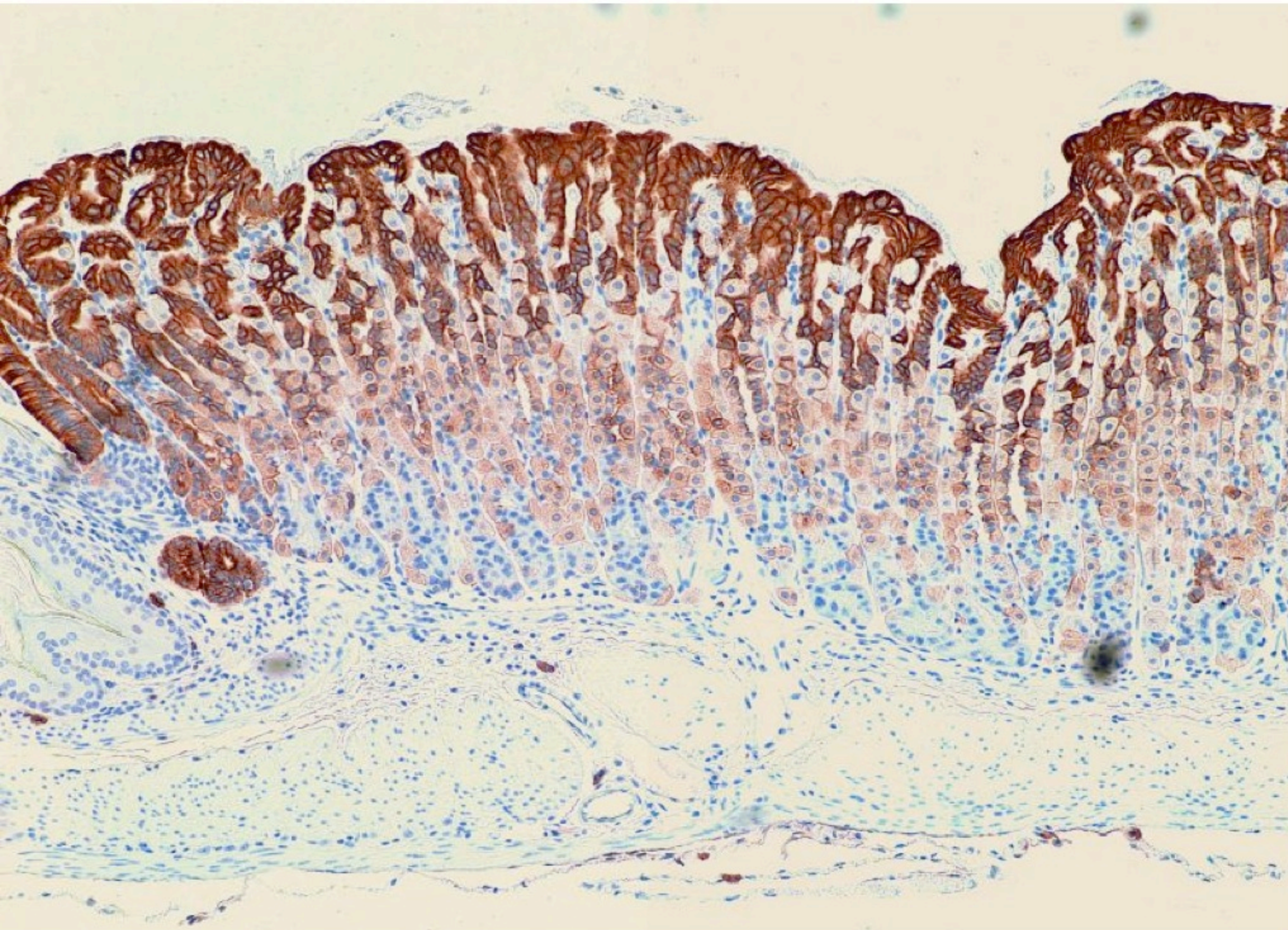
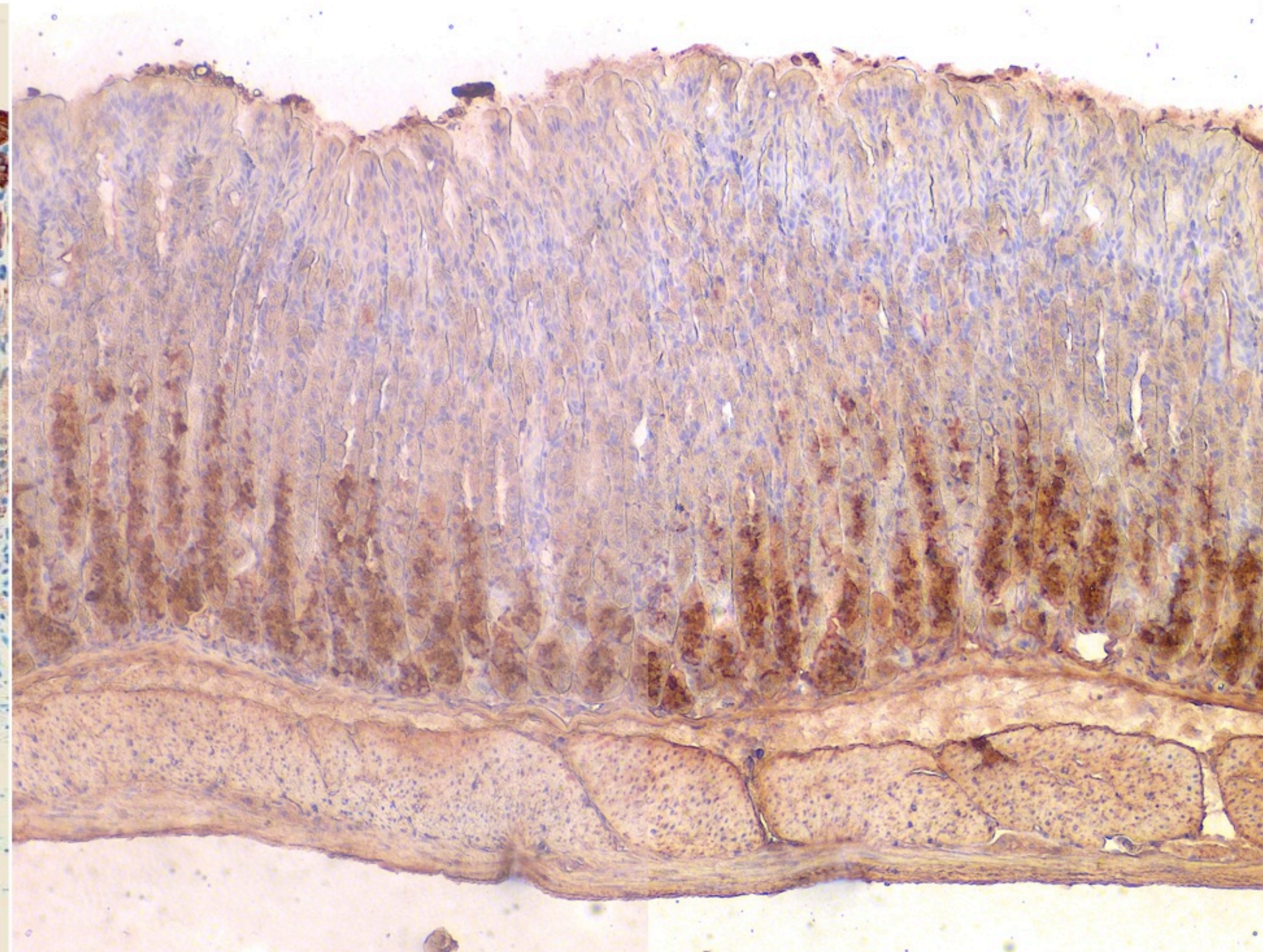


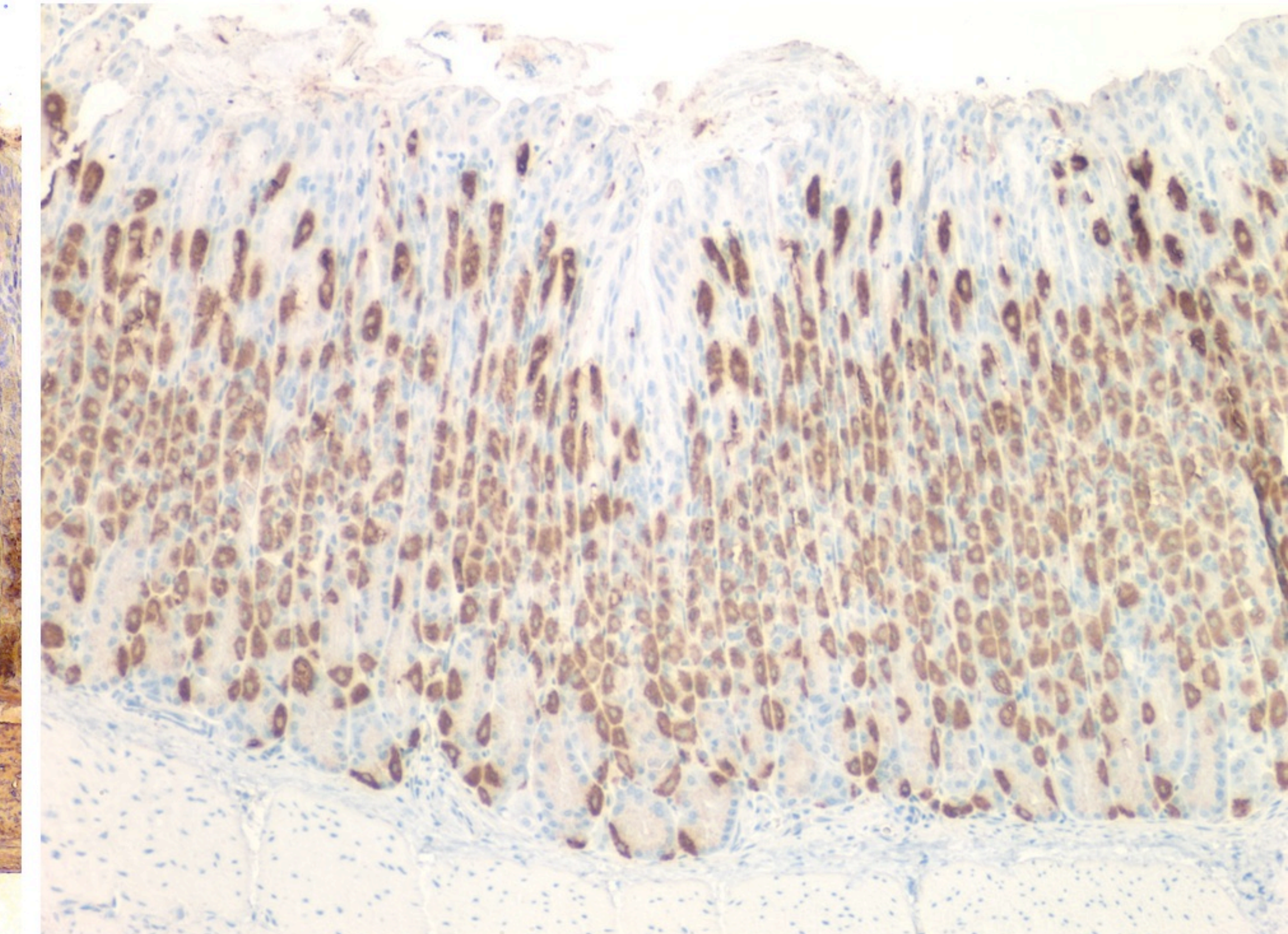
K19 DAB



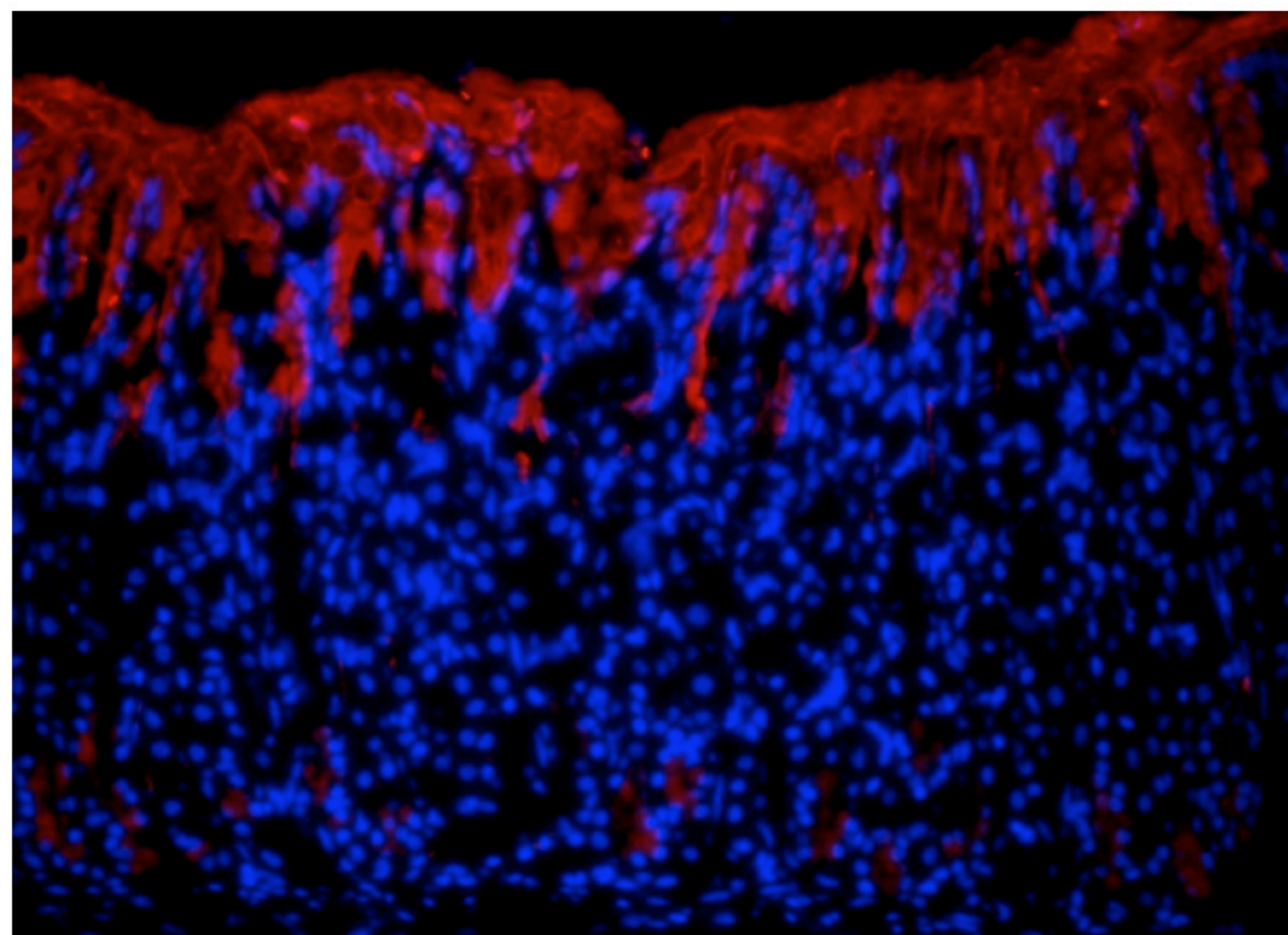
Intrinsic Factor DAB



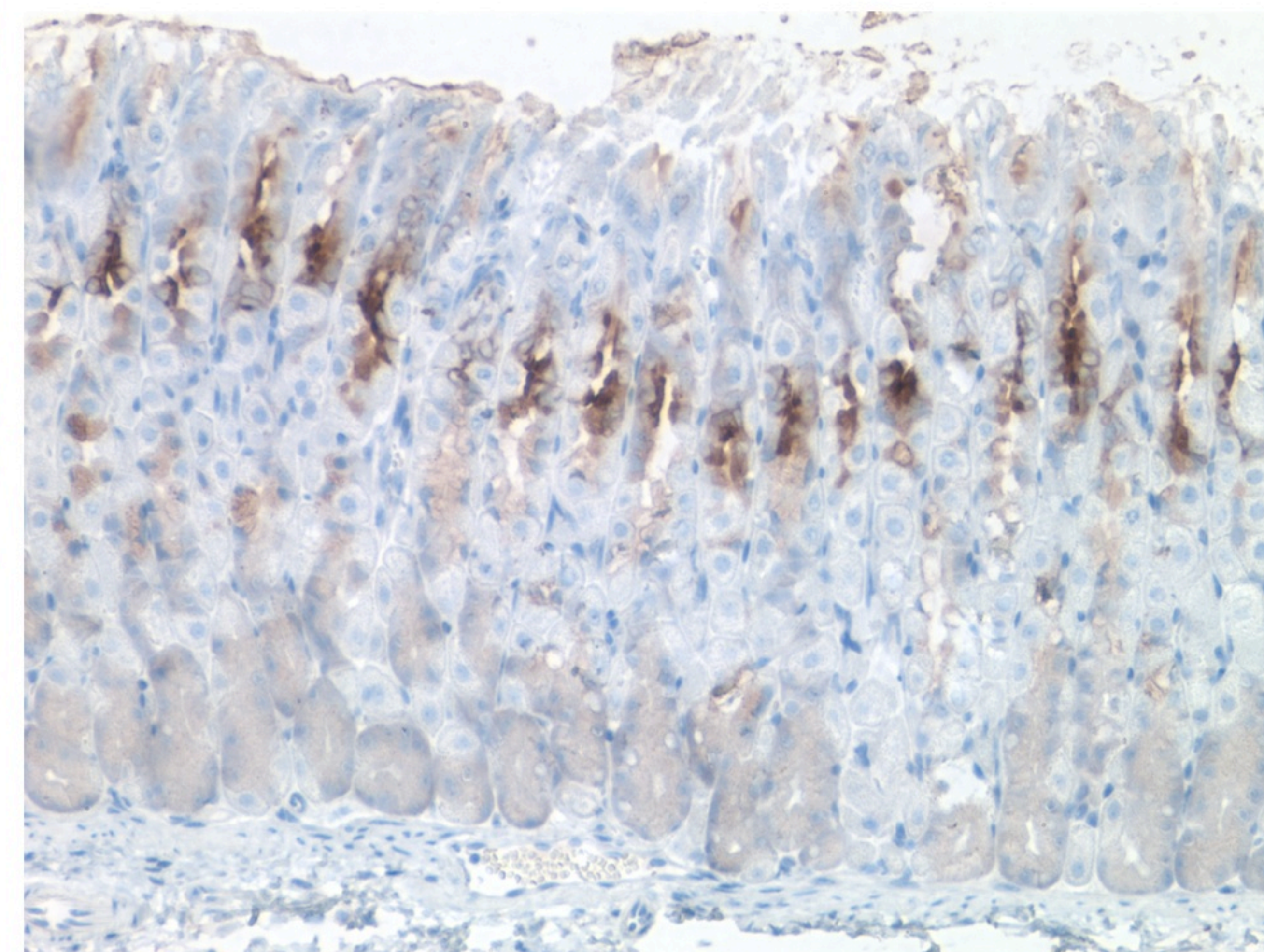
H⁺/K⁺ ATPase DAB



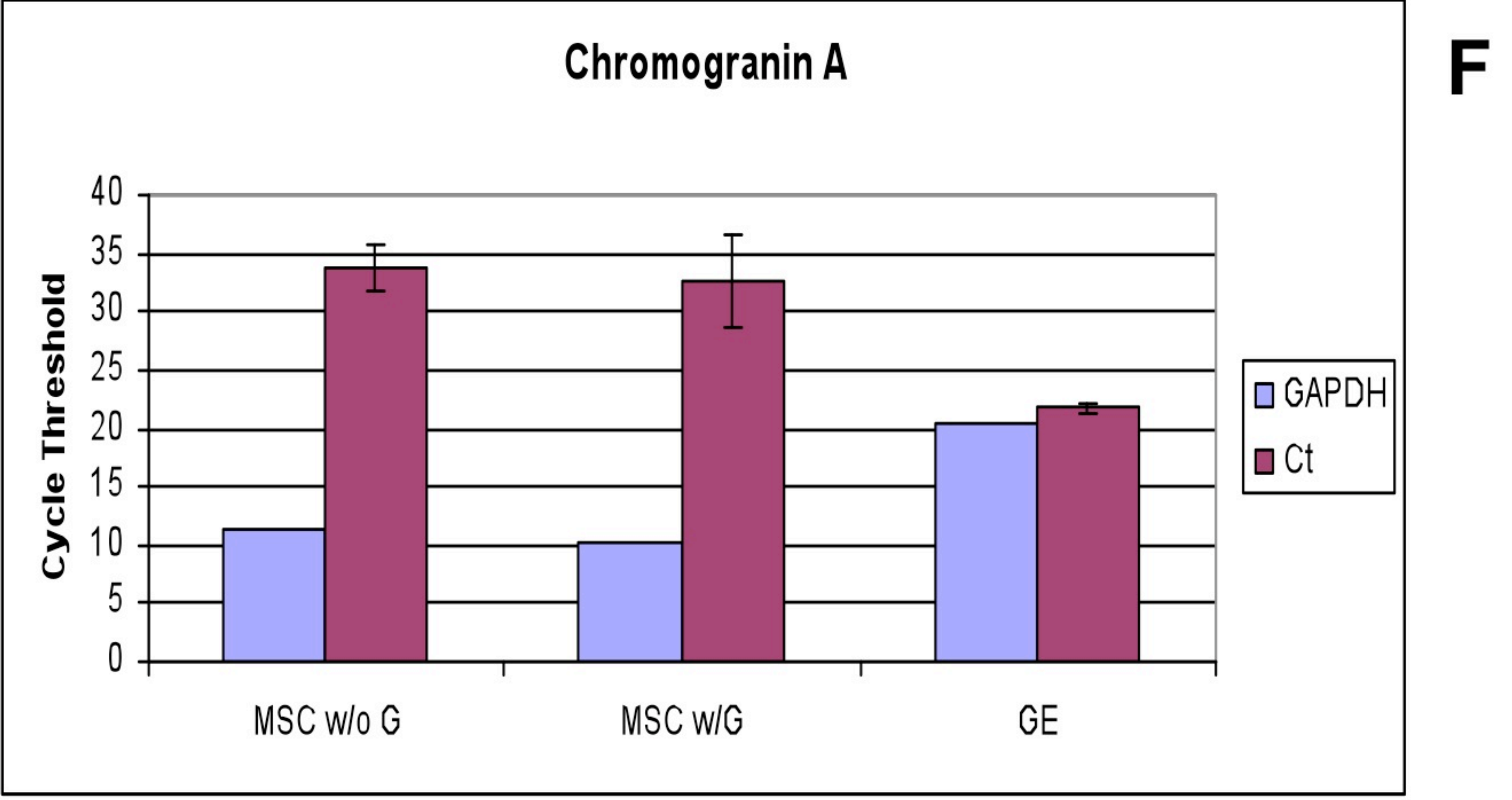
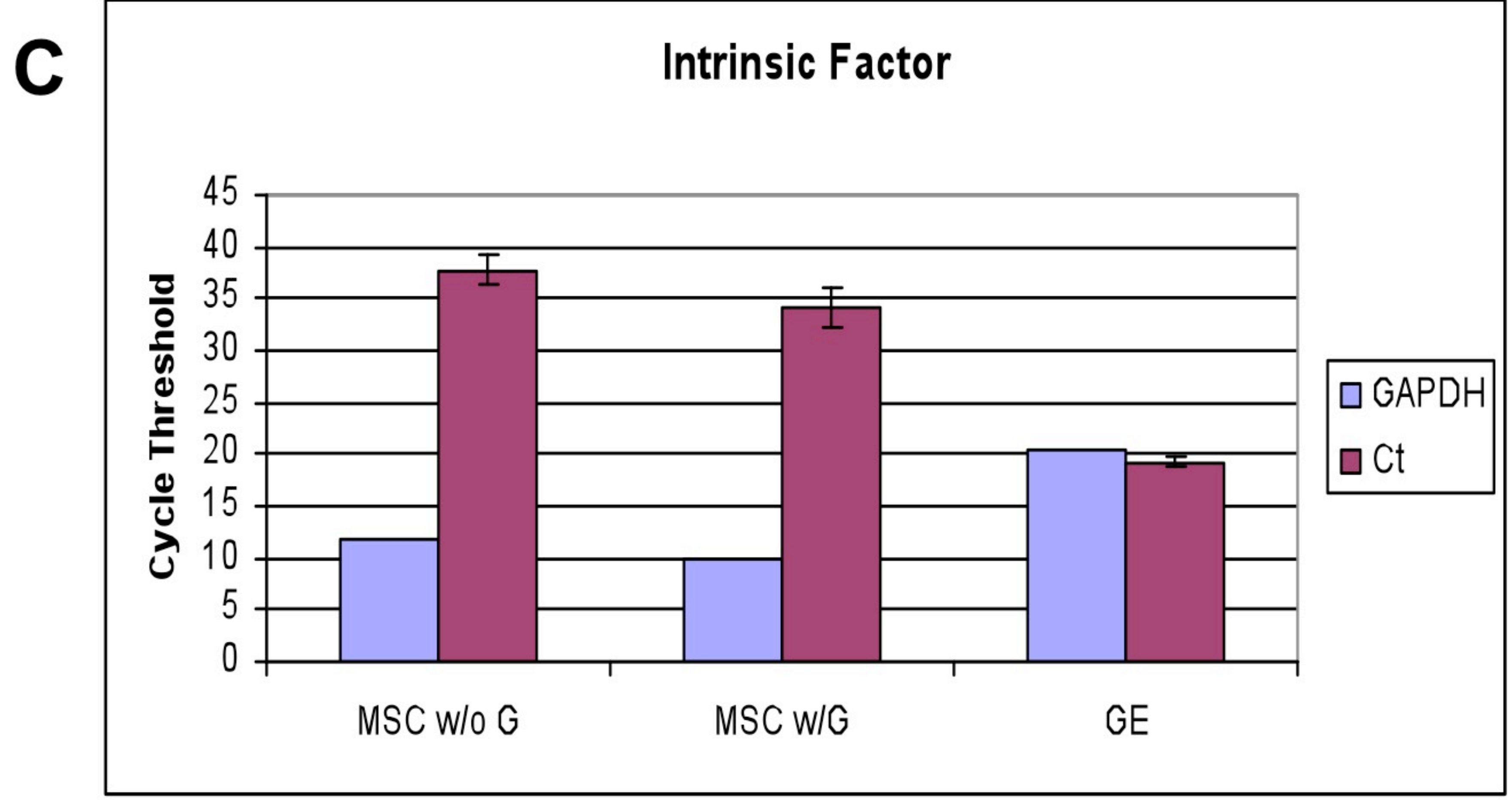
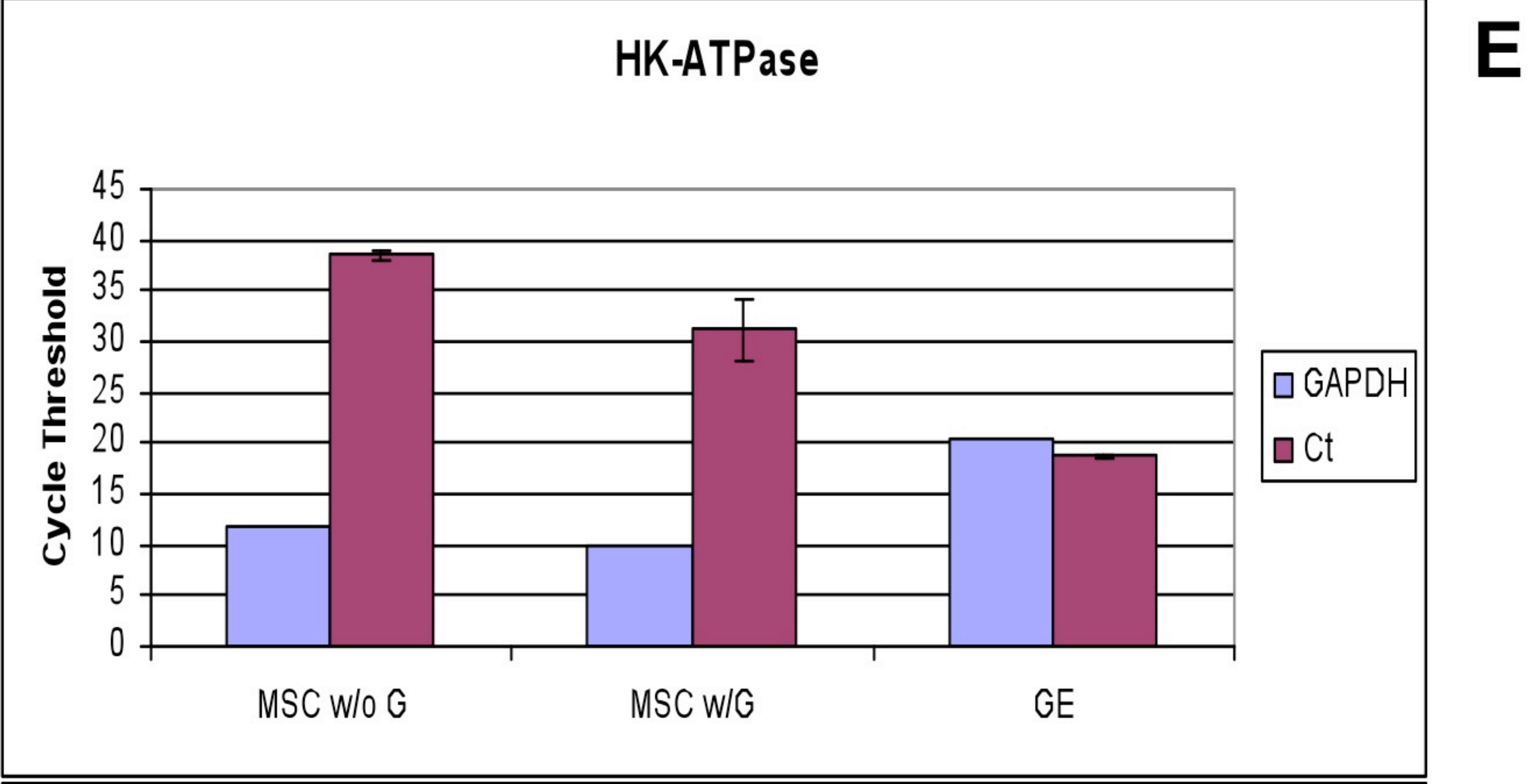
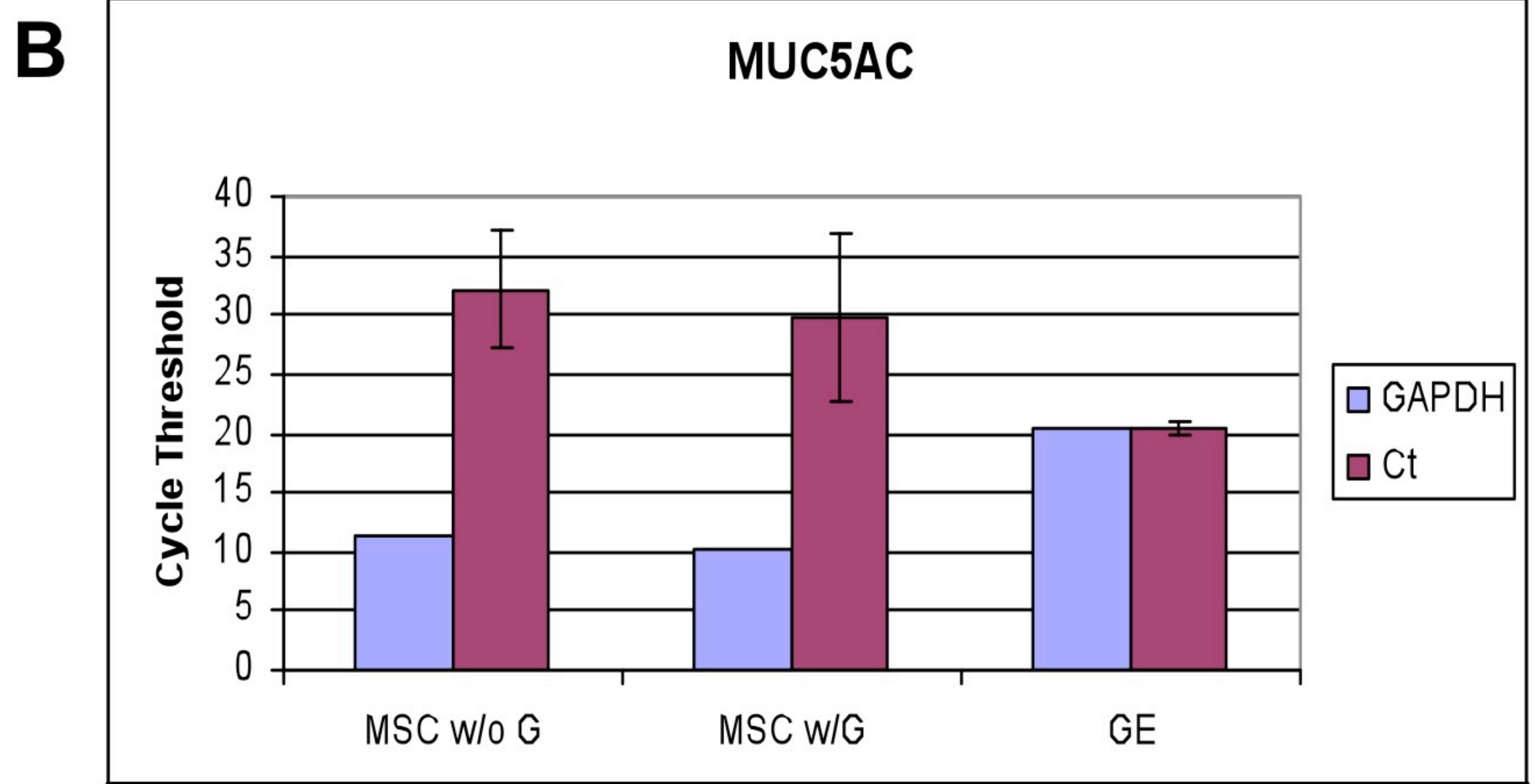
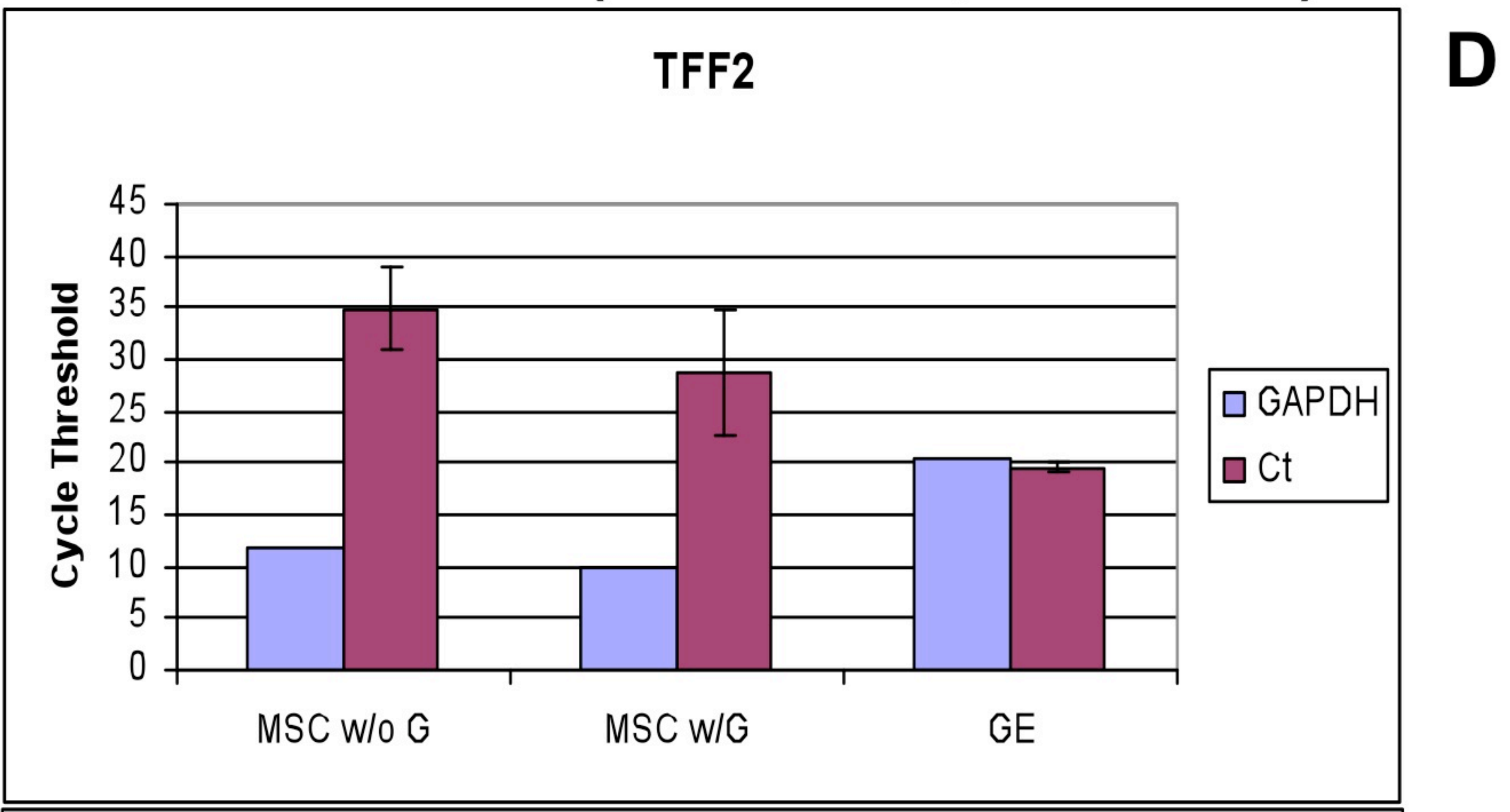
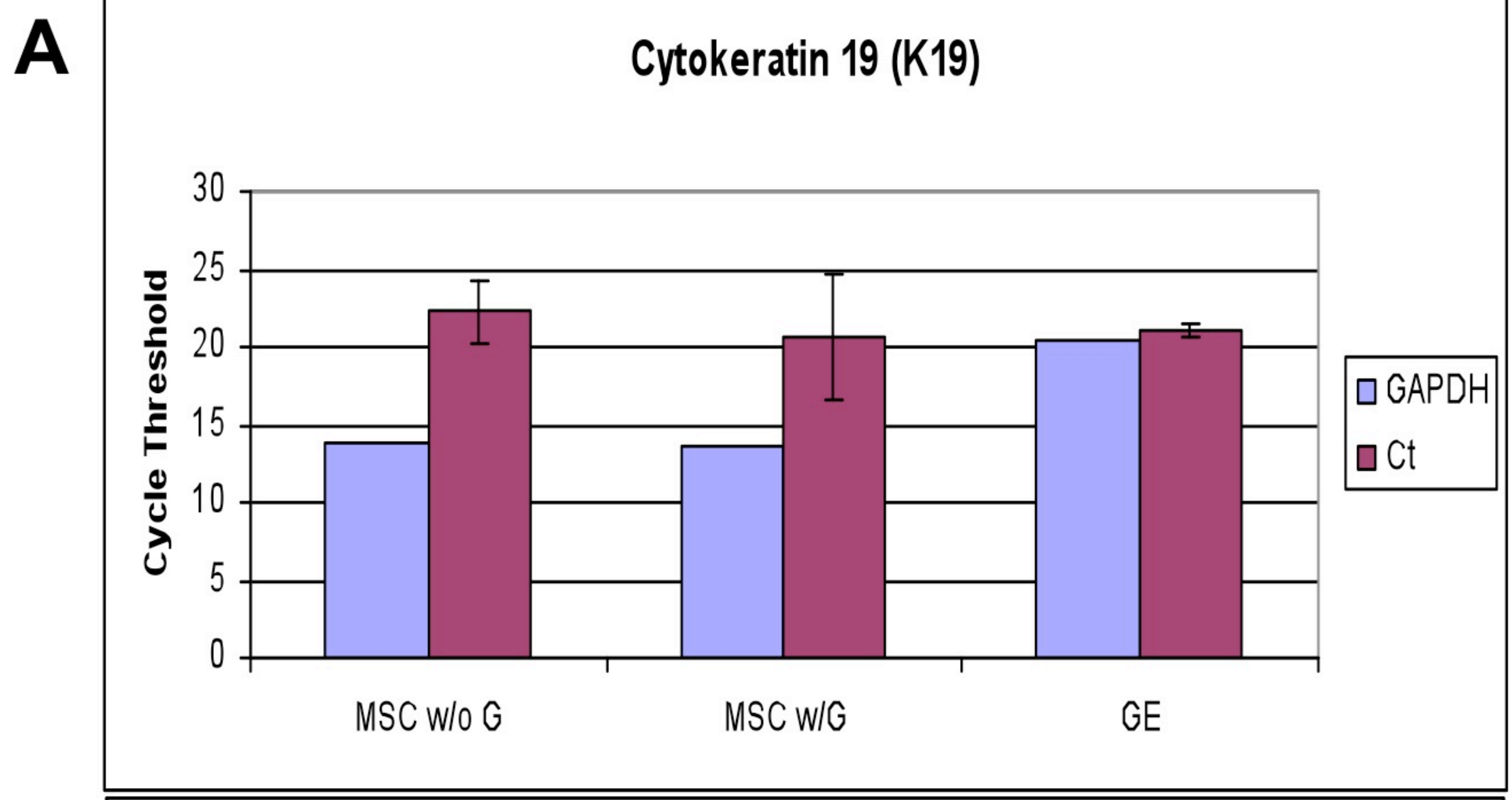
MUC 5AC Texas Red

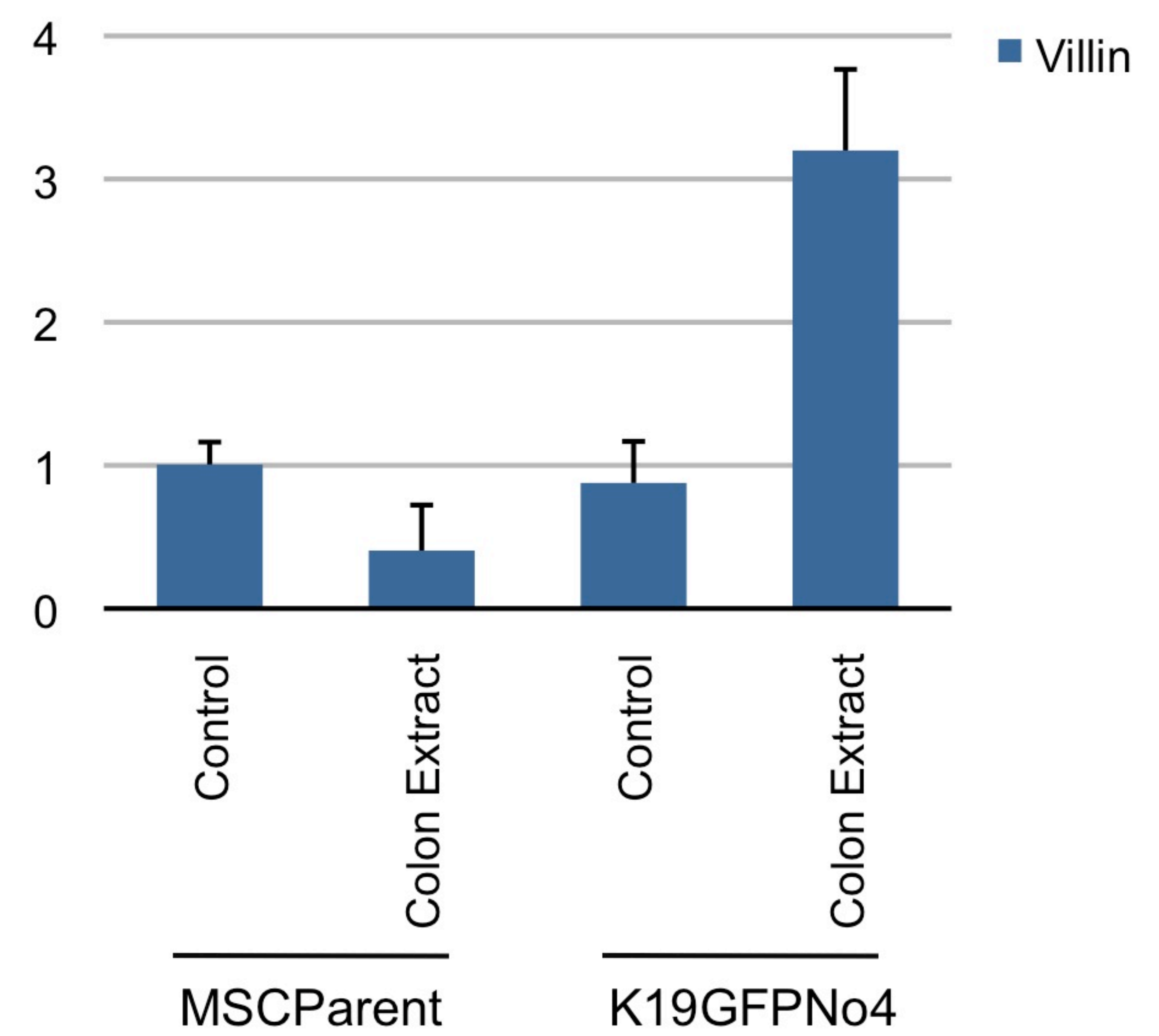
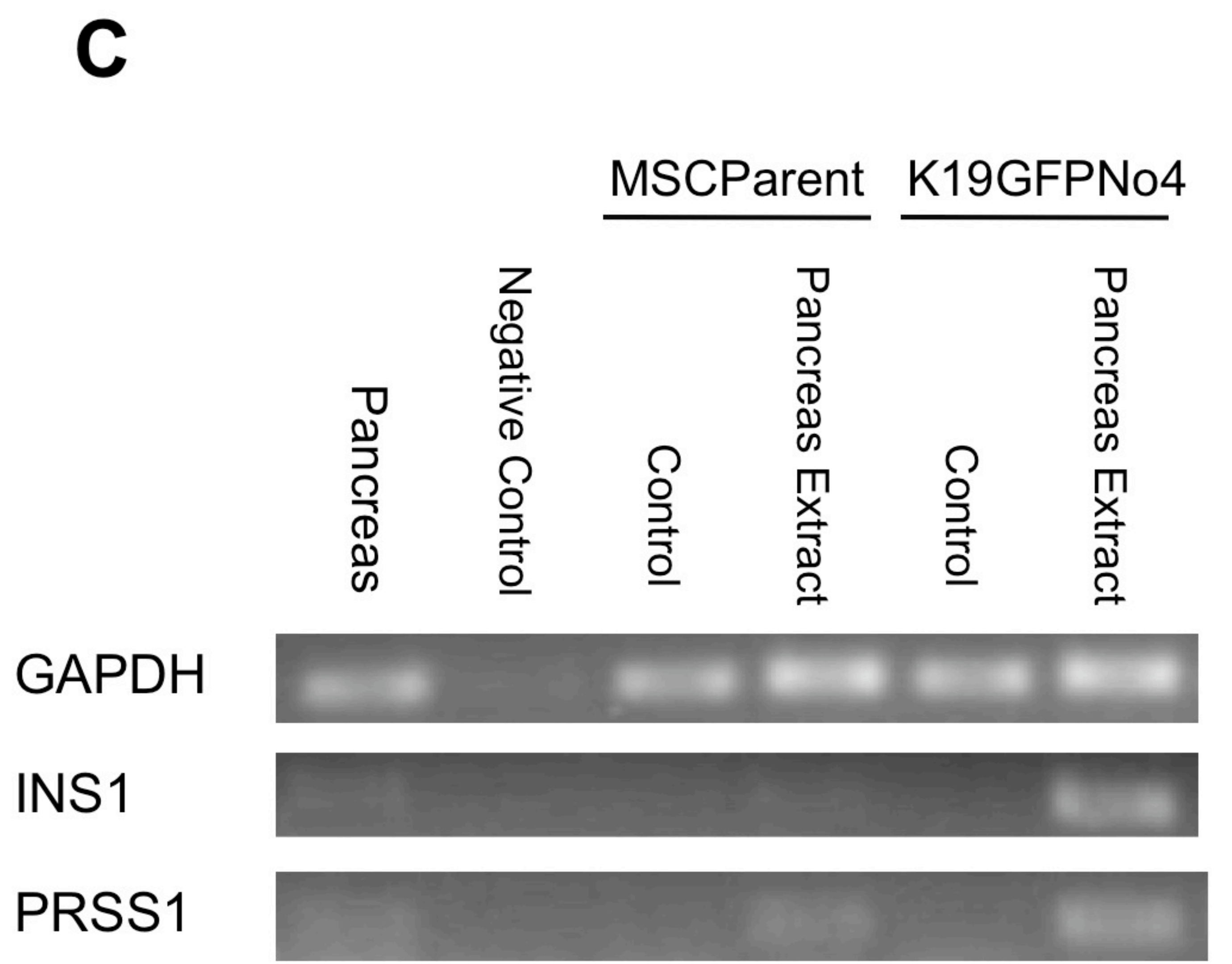
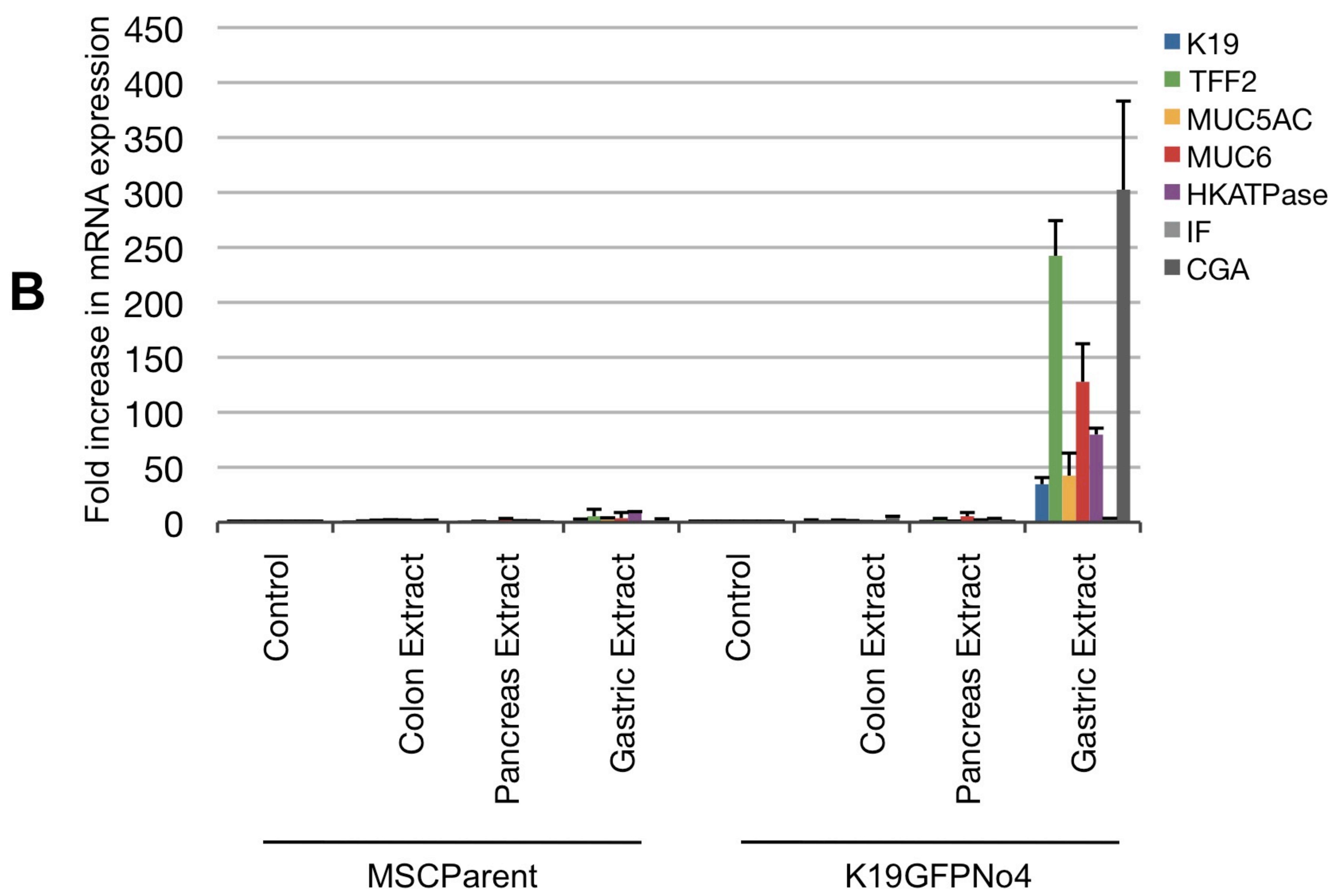
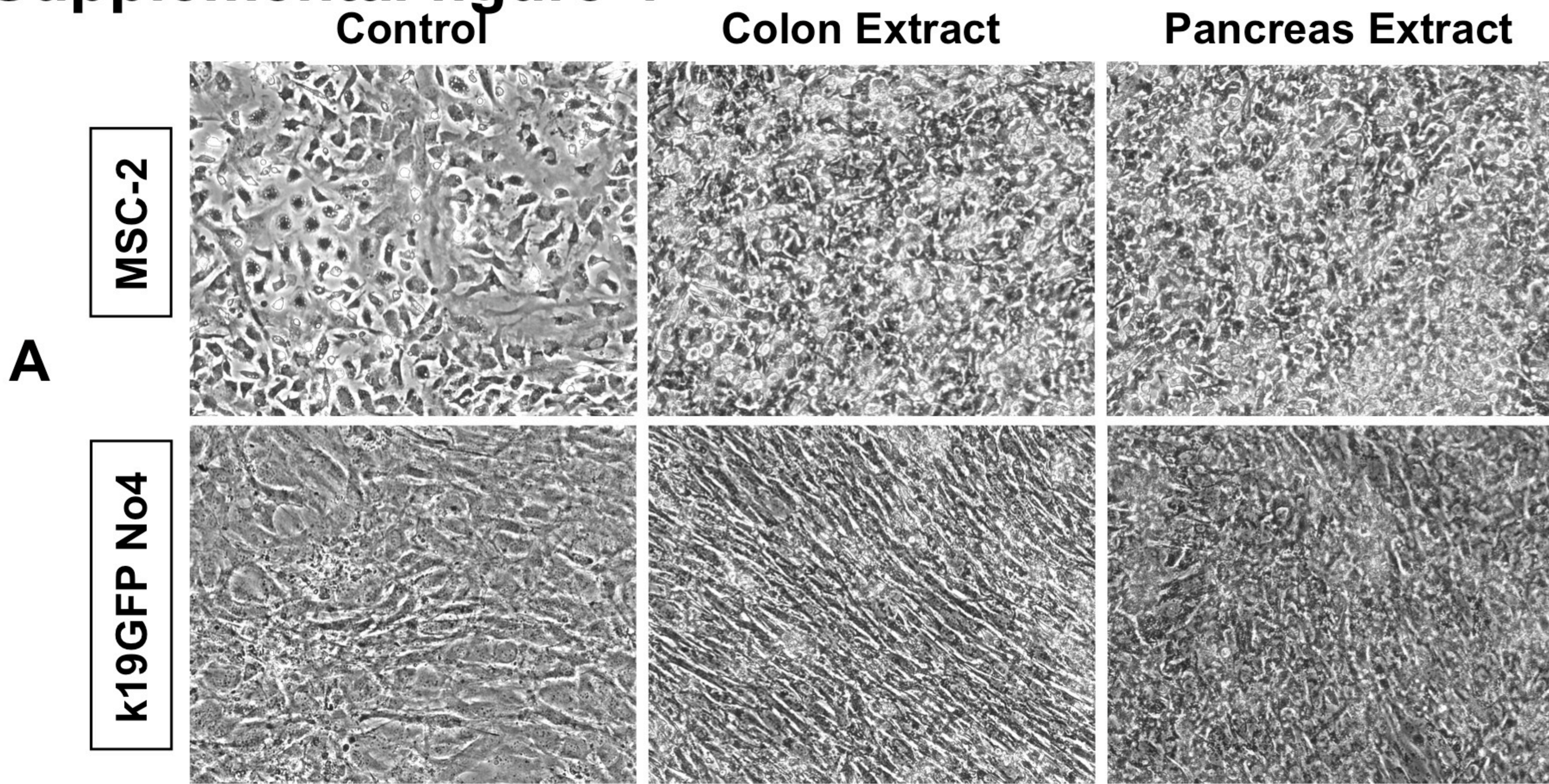


TFF2 DAB



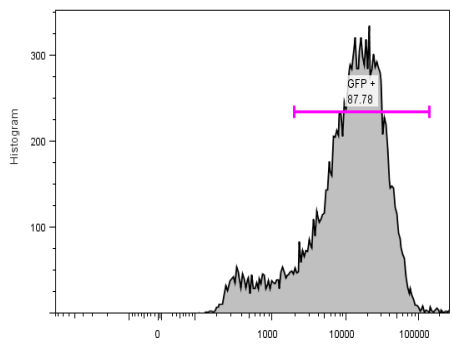
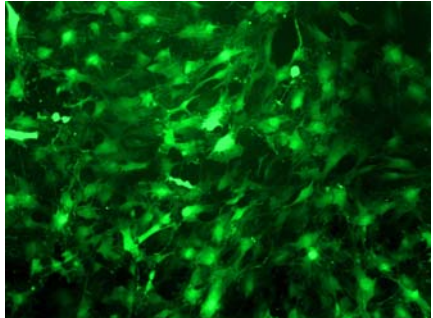
Supplemental figure 3 Gastric phenotypic gene expression in gastric tissue (GE) and K19GFP MSC +/- treatment of gastric tissue extract (MSC w/o G, MSC w/ G)





Clonal MSCs established from beta actin GFP transgenic mouse

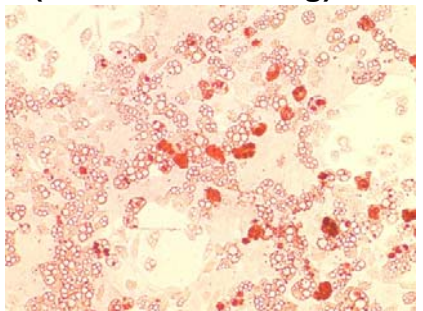
A



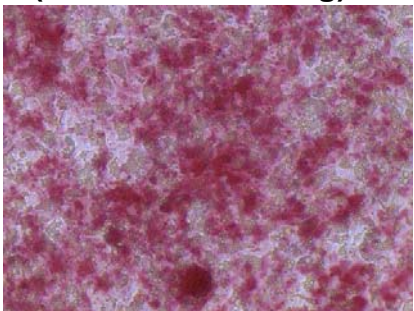
GFP

B

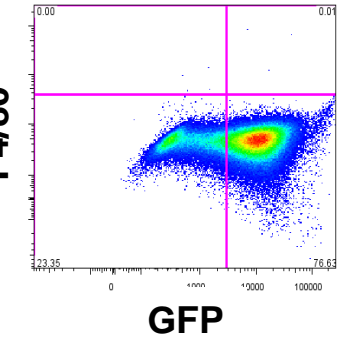
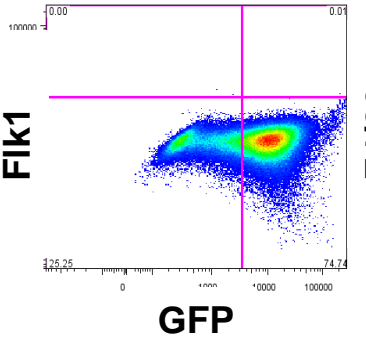
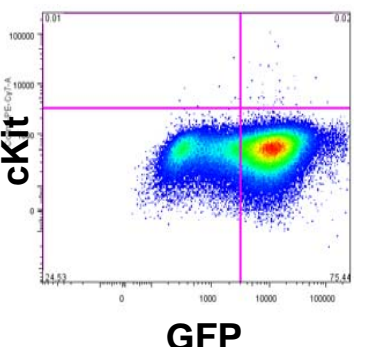
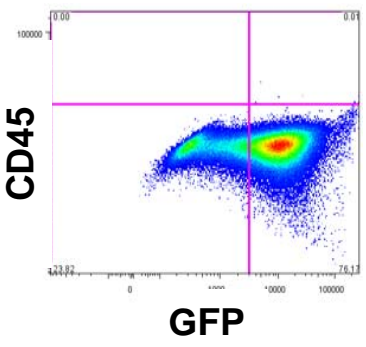
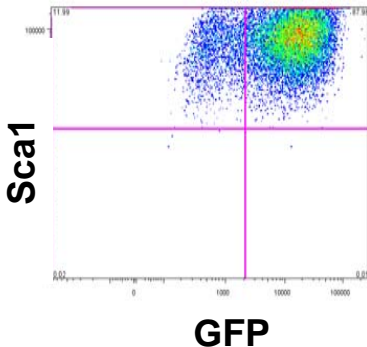
Adipocyte differentiation
(Oil Red-O staining)

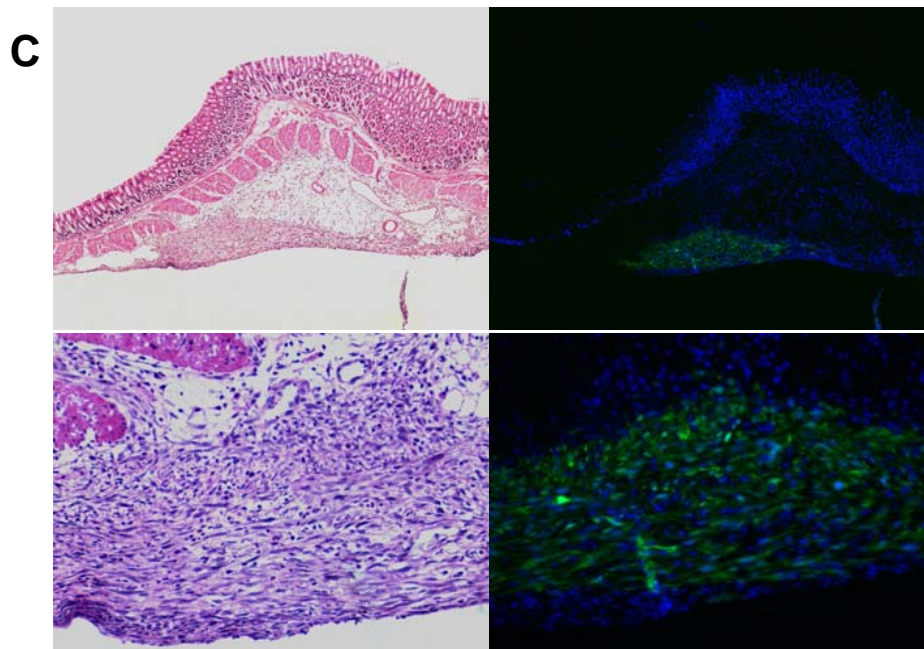
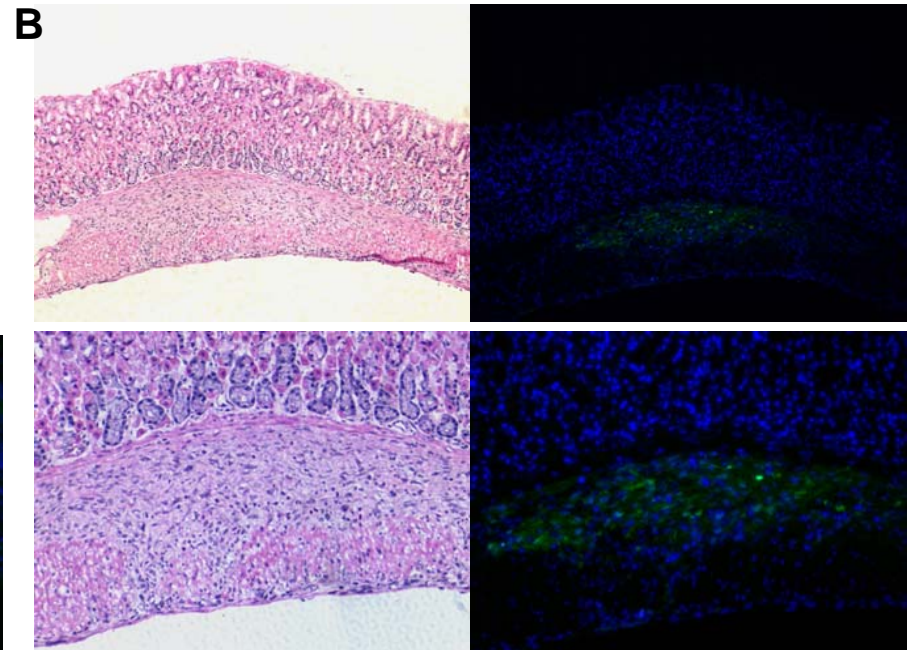
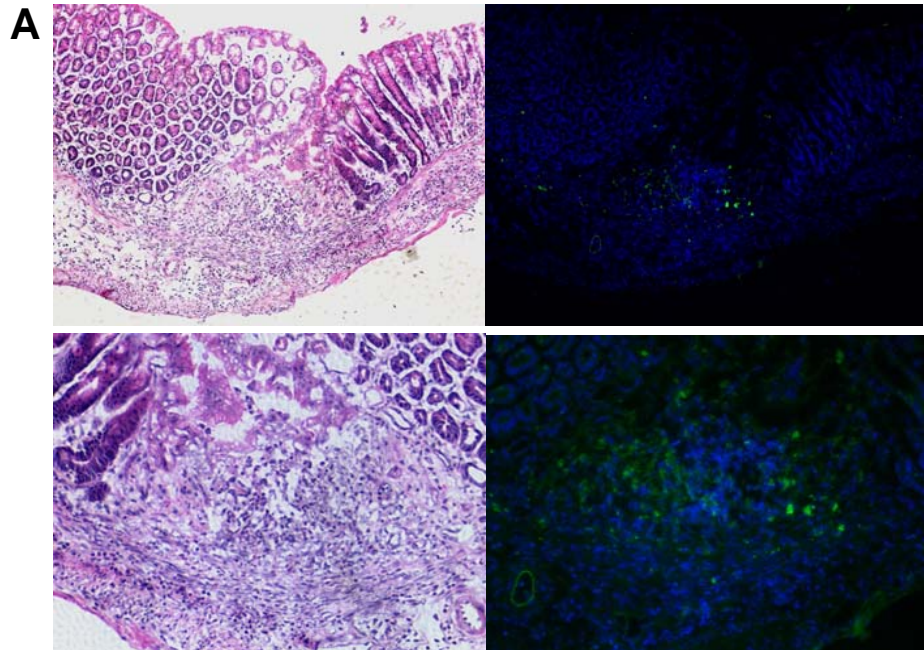


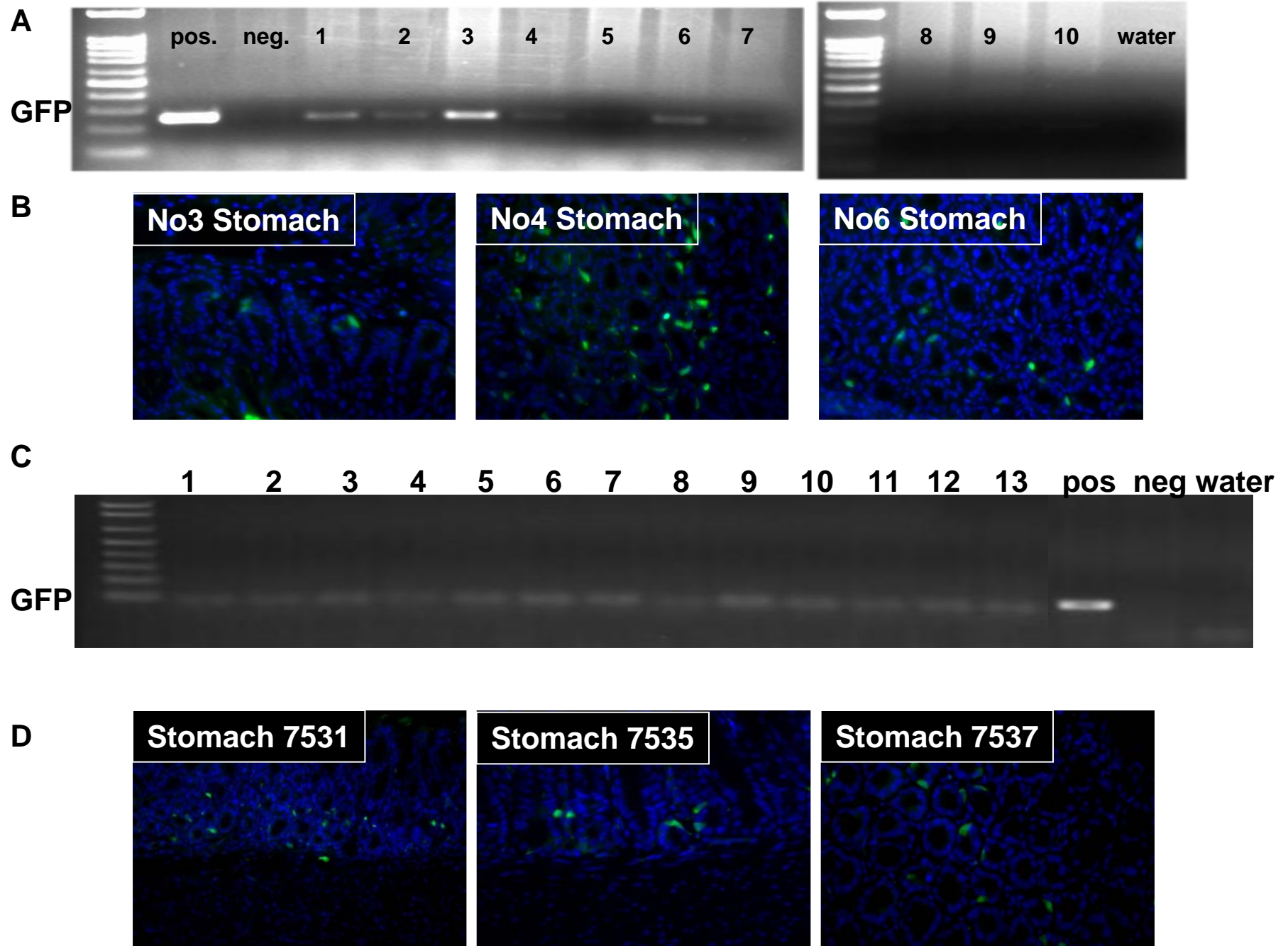
Osteocyte differentiation
(Alizaline-S staining)



C



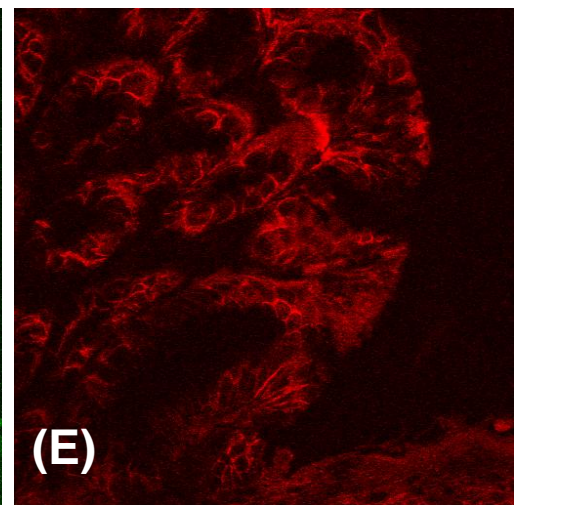
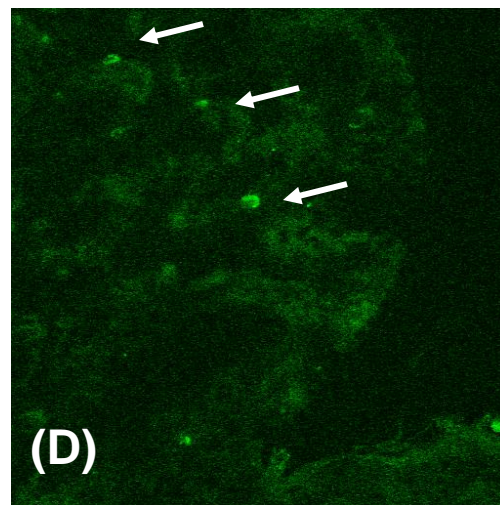
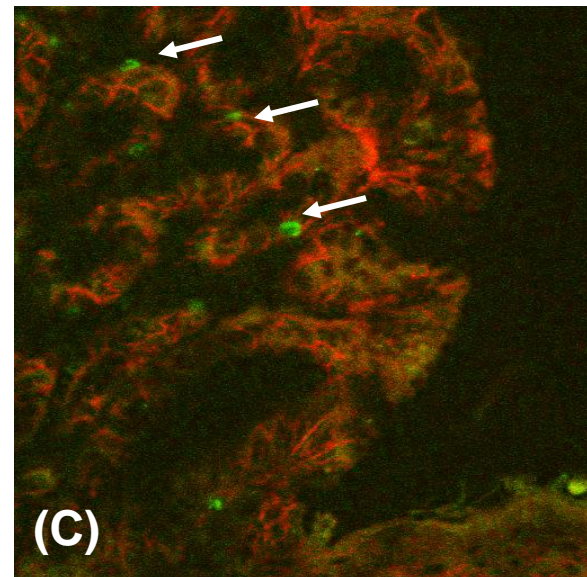
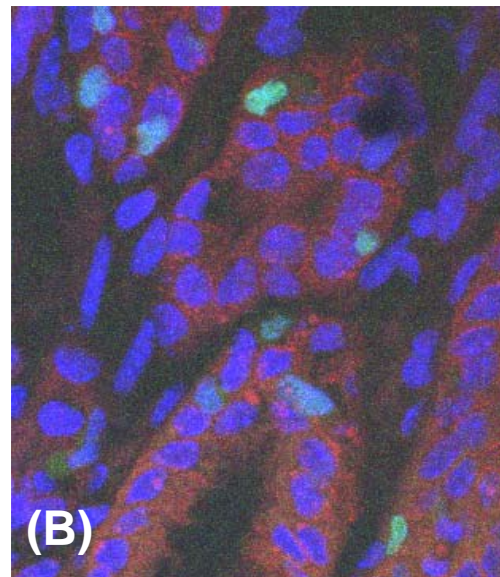
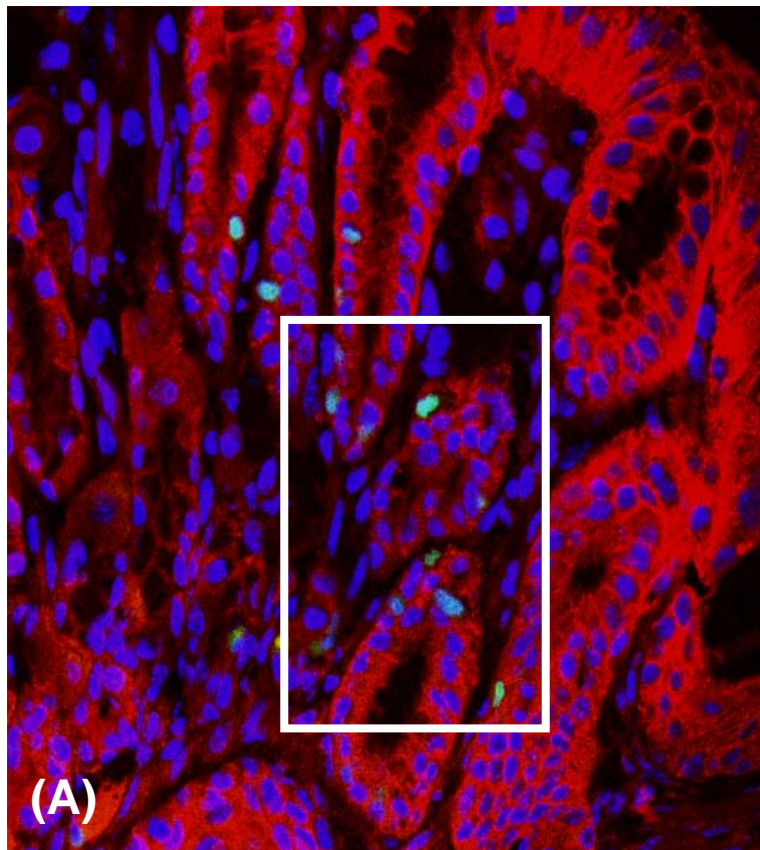




GFP positive cells detected under confocal microscope

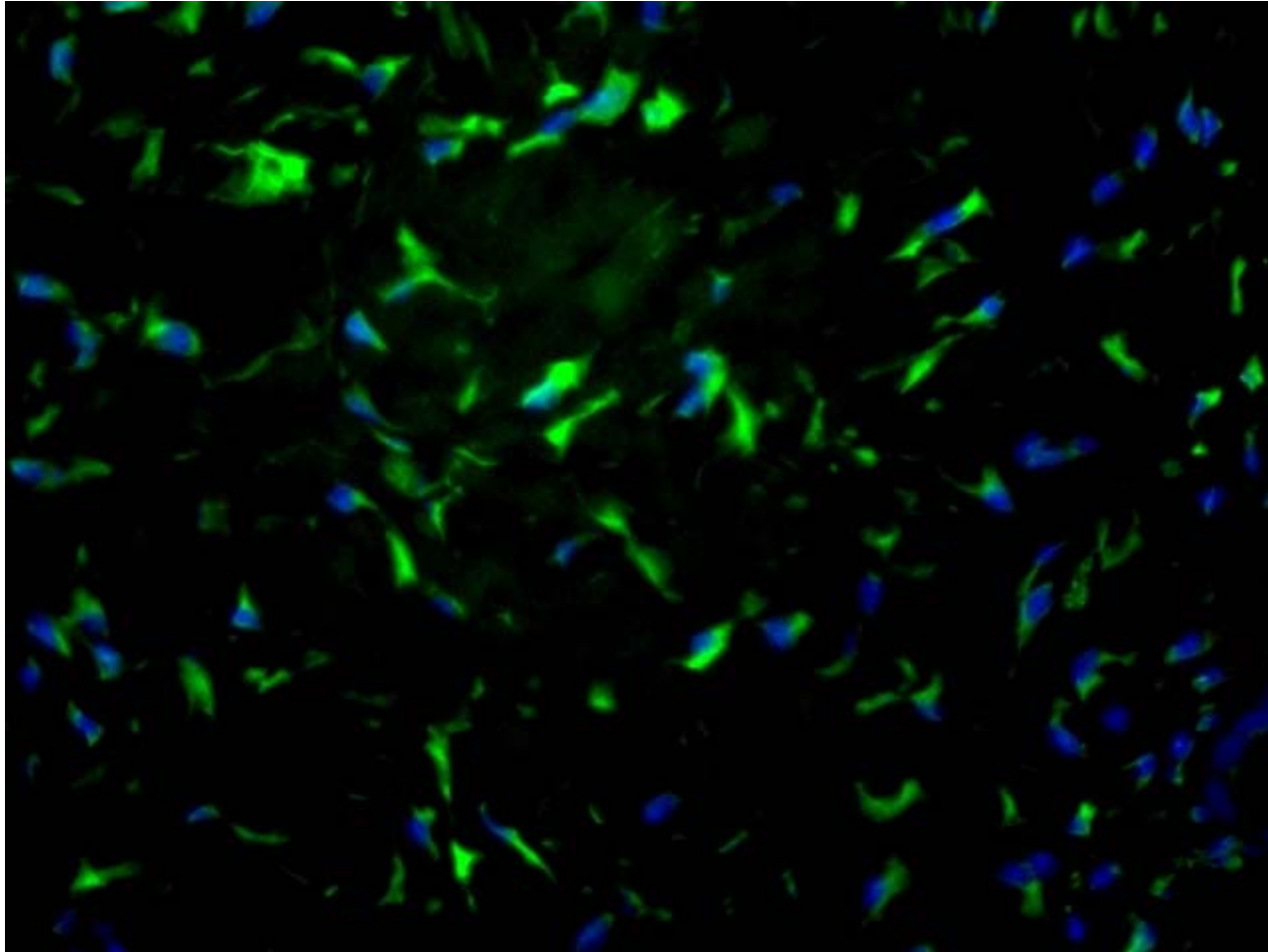
No4 Stomach of mouse from blastocyst injection of GFP MSC (A, B)

GFP MSC injection into gastric wall (C, D, E)



Green: GFP
Red: anti E-cadherin Texas Red
Blue: DAPI for nuclei

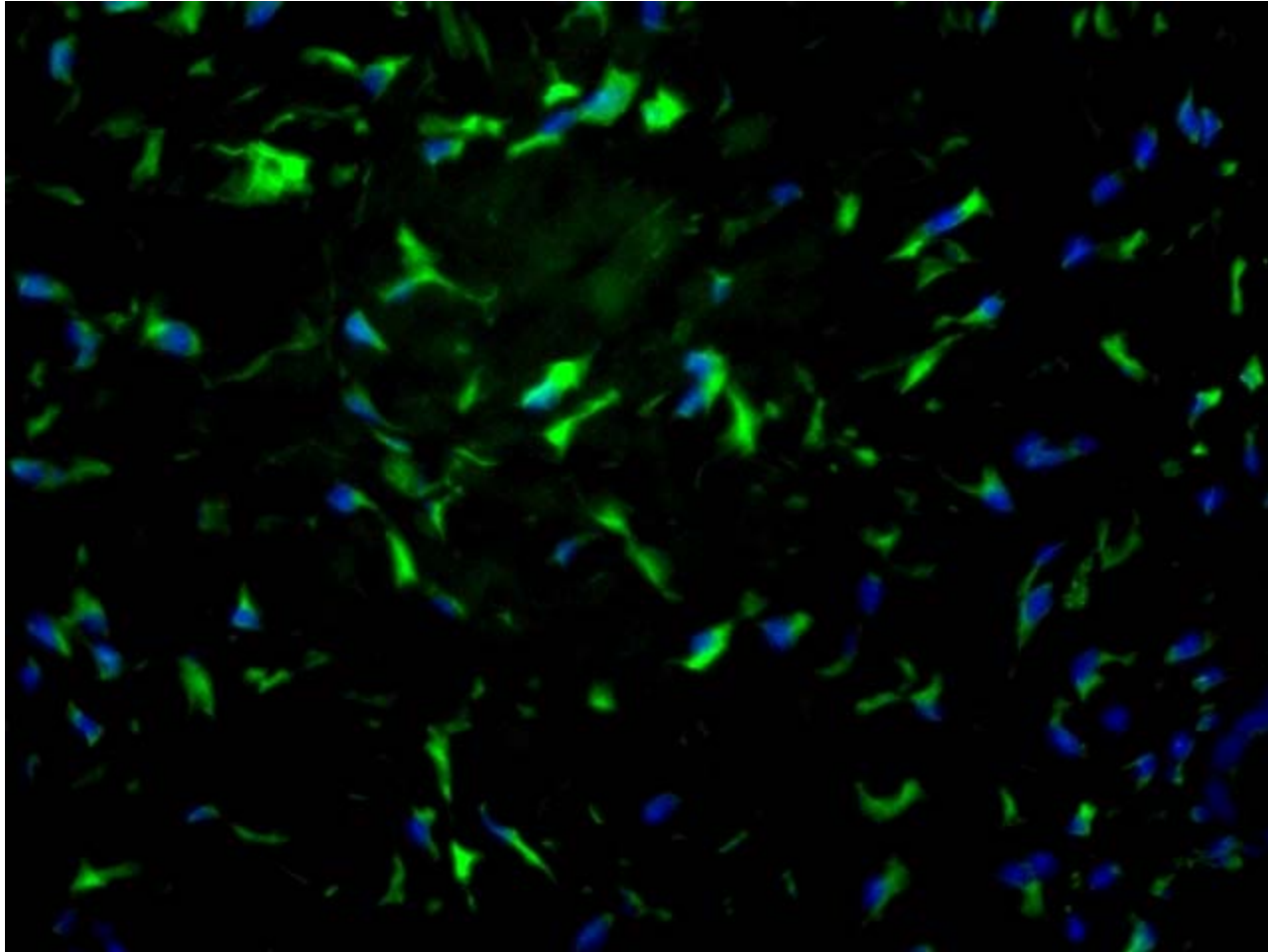
GFP positive stromal cells in epidermis of a mouse derived from blastocyst injection of k19GFP MSC No4



Green: GFP

Blue: DAPI for nuclei

GFP positive stromal cells in epidermis of a mouse derived from blastocyst injection of k19GFP MSC No4



Green: GFP

Blue: DAPI for nuclei

1 **Supplemental Methods**

2

3 **Flow cytometry**

4 Adherent cells were detached by 0.25% trypsin and 0.02% EDTA at 37°C for 2
5 min, washed with blocking buffer (PBS w/ 1% fetal bovine serum, FBS), and suspended in
6 the same buffer. Then cells were incubated with phycoerythrin (PE)-conjugated anti mouse
7 Sca-1 (eBioscience, San Diego, CA), CD45 (BD Pharmingen, San Diego, CA), ckit
8 (eBioscience), Flk1 (BD Pharmingen), or F4/80 (eBioscience) antibody at 1 micro g/1 000
9 000 cells for 30 minutes at 4°C. PE-conjugated rat IgG2a antibody (Jackson
10 ImmunoResearch, West Grove, PA) served as isotype controls. The cells were analyzed by
11 using BD LSRII (Becton, Dickinson). 4',6-diamidino-2-phenylindole (DAPI) was added to
12 exclude dead cells.

13

14 **Cell Proliferation BrdU ELISA**

15 MSC progeny from K19GFP MSC No.3 GFP(+) and GFP(-), were plated in 96-
16 well plate at a concentration of 200 000 cells/well and maintained for 24 hrs at 37°C in the
17 humid air containing 5% CO₂. Cells were then labeled with 10 microM BrdU (Cell
18 proliferation ELISA, BrdU, Colorimetri, Roche, Indianapolis, IN) for 2 hrs, fixed and
19 denatured as per manufacturer's suggestion for 30 min at room temperature, then labeled
20 with detecting antibodies for 90 min. After three washes with 1X PBS, substrate solution
21 was added for 30 min, followed by 1M H₂SO₄, and optical density was read at 450nm.

22

23 **Induction of expression of pancreatic/colonic phenotype markers in vitro**

1 A total of 2 colon or pancreas specimens from wild type C57BL/6 mice were used to make
2 tissue extracts. The paste from one pancreas or colon, made by mortar and pestle, was
3 mixed with 10 mL of MesenCult Stem Cell Medium and incubated at 4°C for 24 hours.
4 The mixture was centrifuged by 6000rpm for 20 minutes and the supernatant was obtained
5 and filtered using 0.45 micrometer membrane. MSCs were cultured with the medium
6 containing gastric extract for 5 days at 37°C in humid air containing 5% CO₂.

7

8 **Quantitative real-time PCR analysis**

9 Total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA), as recommended
10 by the manufacturer. First-strand cDNA was synthesized using the SuperScript First-
11 Strand Synthesis System with SuperScript III reverse transcriptase according to the
12 protocols of the manufacturer (Invitrogen). The cDNA generated was used as a template in
13 real-time PCR reactions with the QuantiTect™ SYBR® green PCR kit (QIAGEN,
14 Maryland, MA) and were run on an ABI Prism 7300 Sequence Detection System (Applied
15 Biosystems, Branchburg, NJ). Primer sequences are described in Supplemental Table 2.
16 Each PCR run included a 15-min activation time at 95°C as required by the instrument.
17 The three-step cycle included denaturing (94°C, 15 seconds), annealing at 55°C and
18 extension at 72°C. mRNA quantities were analyzed in duplicate, normalized against
19 GAPDH as an internal control gene. Results are expressed as relative gene expression
20 using the delta delta Ct (ddCt) method.

21

22 **Immunofluorescence staining of the cells**

23 Cells were grown in wells of Lab-Tek 8-chamber culture slides. Fixed with 4%
24 paraformaldehyde in PBS for 15 min at room temperature and digested with pepsin

1 (Abcam Inc., MA) for 10 min in 37°C. After treatment with 5% FBS in PBS for 30 min at
2 room temperature, cells were incubated with Rabbit anti Cytokeratin 19 antibody (Abcam
3 Inc.) in PBS containing 5% FBS at room temperature for 60 min. After three washes with
4 PBS, cells were incubated with Texas Red conjugated goat anti-Rabbit IgG (Jackson
5 ImmunoResearch) at room temperature for 60 min. Cells were counter stained with DAPI,
6 washed with PBS three times, and mounted using Vectashield (Vector Laboratories, Inc.
7 CA) for microscopy.

8 **PCR for GFP detection**

9 Genomic DNA from mice was extracted using a Genomic DNA isolation kit
10 (Lamda Biotech, St. Louis). Primers used for detection of GFP gene were shown in
11 Supplemental Table 2. Primers for GAPDH were used to confirm the presence of template
12 DNA in the reactions. PCR reactions were performed in 50 µL with 50 ng of DNA, each
13 with 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, and 200 µM dNTPs each, 0.4 µM
14 of each forward and reverse primer, and 1.25 U Taq DNA polymerase (Roche Diagnostics,
15 Indianapolis, IN). The PCR reactions were performed as follows: loading at 95°C for 2
16 minutes, denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and
17 elongation at 72°C for 1 minute for 30 cycles. For positive controls, DNA templates from
18 stomachs of chicken beta actin EGFP transgenic mice were used. A negative control with
19 only water was performed. All of the PCR reactions were analyzed on 1.5% agarose gels.

20 **Tissue processing and immunofluorescence staining**

21 Mice were deeply anesthetized with inhalation of isoflurane and infused through
22 the heart with PBS and then 4% paraformaldehyde. The stomachs were removed, further

1 fixed with 4% paraformaldehyde for 6 hours at 4°C, then equilibrated in 30% sucrose,
2 embedded in OCT, frozen. Four micron sections were prepared by the Research Histology
3 Service at Columbia University Medical Center. Slides were rinsed with PBS, non-specific
4 staining was blocked with 1% FBS in PBS for 1 hour at room temperature, then Rat anti E-
5 cadherin antibody (Zymed, South San Francisco, CA; 1:100 dilution), or anti mouse
6 MUC5AC (Abcam; 1:100 dilution), diluted in PBS supplemented with 1% FBS were
7 applied. Non-transplanted tissues served as additional negative controls. Following
8 overnight incubation at 4°C, slides were washed three times in PBS, and Texas Red
9 conjugated anti Rat IgG antibody (Jackson ImmunoResearch) or Texas Red conjugated
10 anti mouse IgG antibody (Jackson ImmunoResearch) were applied, respectively, with 1%
11 FBS in PBS (1: 300 dilution) and incubated for 1 hour at room temperature. Then slides
12 were stained with DAPI, washed with PBS for three times, and mounted using Vectashield
13 (Vector Laboratories) for microscopy.

14

15 **Immunohistochemistry**

16 Immunohistochemical studies were performed with avidin biotin-peroxidase
17 complex kits (Vector Laboratories, Burlingame, CA) according to the manufacturer's
18 instructions. For primary antibody, rabbit anti GFP antibody (Invitrogen), rabbit anti
19 Cytokeratin 19 antibody (Abcam; 1:100), anti TFF2 antibody (established in our
20 laboratory; 1:100), anti Intrinsic Factor (gift of Dr. David Alpers, Washington University,
21 St. Louis, MO; 1:2000), or mouse anti Hydrogen/Potassium ATPase (H/K-ATPase) beta
22 antibody (Affinity BioReagents, Golden, CO; 1:2000 dilution) was diluted in PBS
23 supplemented with 1% FBS (1:100 dilution) was applied. Diaminobenzidine (Vector

1 Laboratories) was used as the chromogen, and slides were counterstained with Mayer's
2 hematoxylin.

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1 **Supplemental Figure Legends**

2 **Figure S1.**

3 Expression of ES cell markers in cultured MSCs

4 Expression of ES cell markers, such as Nanog and Oct3/4, were investigated by RT-PCR.

5 Relative expression level of Nanog mRNA was assessed by real-time PCR. Fold increase
6 in mRNA expression, as compared to ES cells is shown.

7

8 **Figure S2.**

9 Expression of gastric phenotype markers in mouse gastric epithelium

10 Expression of k19, H/K-ATPase (parietal cells), Intrinsic Factor (chief cells), TFF2 (neck
11 cells) was detected by immunohistochemistry with DAB visualization. Slides were
12 counterstained with Mayer's hematoxylin. (original magnification x100)

13 Expression of Muc5AC (pit cells) was detected by immunofluorescent staining with Texas
14 Red conjugated secondary antibody. Nucleus was stained with DAPI.
15 (original magnification x100)

16

17 **Figure S3.**

18 Real-time PCR cycle threshold number of each gastric phenotype gene in gastric tissue and
19 K19GFPMSC with gastric tissue extract treatment. The data are presented as cycle
20 threshold. Forty (40) cycles were chosen in these experiments. Data are presented as mean
21 and standard deviation from 3 different samples.

22

23 **Figure S4.**

1 Treatment of MSCs with gastric tissue extract induces significant gastric phenotype marker
2 expression. Treatment with pancreatic or colonic tissue extract does not induce expression
3 of gastric phenotype markers, although modestly induce expression of markers for their
4 respective tissues, particularly in K19-expressing MSCs.

5 A. Morphological changes of MSCs after treatment with colonic or pancreatic tissue
6 extracts.

7 B. Expression of gastric epithelial phenotype markers in parent MSC and K19GFP
8 MSC No4 5days after treatment with gastric, colonic, or pancreatic tissue extract as
9 assessed by quantitative real-time PCR.

10 C. Expression of pancreatic phenotype markers insulin (INS1) and trypsin (PRSS1) or
11 the colonic phenotype marker villin in parent MSC and K19GFP MSC No4 5days
12 after treatment with pancreatic or colonic tissue extract, as assessed by standard or
13 quantitative real-time PCR, respectively.

14

15 **Figure S5.**

16 Establishment of MSCs from chicken beta actin EGFP transgenic mouse as a GFP labeled
17 control cells. MSC culture was established from bone marrow of chicken beta actin EGFP
18 transgenic mouse (GFP MSC).

19 A. GFP expression in GFP MSC was assessed by fluorescent microscopy and flow
20 cytometry.

21 B. Adipocyte and osteocyte differentiation of GFP MSC. MSC cultures were
22 incubated with adipocyte or osteocyte differentiation medium for 14 days and cells
23 were stained with Oil red-O and Alizarin Red, respectively.

1 C. Expression of cell surface markers (Sca1, c-kit, CD45, Flk1, and F4/80) were
2 analyzed by flow cytometry. Quadrant markers were set according to the profile of
3 corresponding control IgG staining. Representative example of three experiments.
4

5 **Figure S6.**

6 Direct injection of MSCs into the murine stomach wall. Gastric tissue sections were
7 prepared 24 hours after injection.

8 A. GFP positive cells were detected in mucosa.

9 B. GFP positive cells were detected in submucosal area.

10 C. GFP positive cells were detected in subserosal area.

11 Original magnification, 100X (upper panel). High power view is presented in lower panel.
12

13 **Figure S7.**

14 Blastocyst injection of GFP labeled MSC clones.

15 A. GFP sequence was detected by PCR in tail DNA of mice derived from blastocyst
16 injection of GFP MSC.

17 B. GFP positive cells were detected in stomach tissue sections of 3 of 10 mice derived
18 from blastocyst injection of GFP MSC. GFP positive cells were detected in
19 subserosal area.

20 C. GFP sequence was detected by PCR in tail DNA of mice derived from blastocyst
21 injection of K19GFP MSC No4.

22 D. GFP positive cells were detected in stomach tissue sections of all 13 mice derived
23 from blastocyst injection of K19GFP MSC No4.

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Figure S8.

Co-localization of GFP and E-cadherin expression in gastric glandular cells detected by confocal microscopy.

A. GFP MSCs were injected into 3.5 day-old mouse blastocysts to establish chimeric mice and gastric tissue sections were prepared at 8 weeks of age. Four micrometer thick sections were stained with anti E-cadherin antibody in combination with Texas Red conjugated secondary antibody. Nuclei were stained with DAPI.

B. 3D picture made from the same section in A.

C. GFP MSCs (200 000 cells in 10 micro L of PBS) were injected into gastric wall of C57BL/6 mice and gastric tissue sections were prepared 2 weeks after injection. Four micrometer thick sections were stained with anti E-cadherin antibody and Texas Red conjugated secondary antibody. Original magnification, 400X.

D. GFP single color photo.

E. E-cadherin in Texas-red single color photo.

Figure S9.

GFP positive stromal cells in epidermis of a mouse derived from blastocyst injection of k19GFP MSC No4

GFP positive cells were detected in tissue sections of epidermis in a mouse derived from blastocyst injection of K19GFP MSC No4. Nuclei were stained with DAPI.

Supplemental Table 1. Mice use in this study

MSC donors WT	5
MSC donors GFP	5
Gastric injection recipient--GFP MSC	10
Gastric injection recipient--K19 MSC	10
Blastocyst injection chimeric pups--GFP MSC	10
Blastocyst injection chimeric pups--K19 MSC	13
Gastric lysate	5
BMT donors	5
BMT recipients--Hf-	15
BMT recipients--Hf+	15
BMT recipients--control	5
Total	98

Supplemental Table 2. Sequence of the Primers Used for quantitative and regular RT-PCR

Gene	qRT-PCR Forward primer	qRT-PCR Reverse primer	Product size
GAPDH	5'- gac atc aag aag gtg gtg aag cag -3'	5'- ata cca gga aat gag ctt gac aaa -3'	174 bp
Keratin 19	5'- gga ccc gga ccc tcc cga gat t-3'	5'- ggc gca ggc cgt tga tgt cg-3'	205 bp
TFF2	5'- gca gtg ctt tga tct tgg atg c -3'	5'- tca ggt tgg aaa agc agc agt t -3'	185 bp
IF	5'- ccc ggt ccc cac ttc agt atc t-3'	5'- caa taa ggc ccc agg atg tca t-3'	200 bp
CgA	5'- gca gca tcc agt tcc cac ttc c-3'	5'- tcc cca tct tcc tcc tgc tga g-3'	146 bp
H/KATPase-beta	5'- gca gac cat tga ccc cta cac c-3'	5'- agg cca gcc cag gaa ctg ttt t-3'	138 bp
Mucin5ac	5'- agg gcc cag tga gca tct cct a-3'	5'- cat cat cgc agc gca gag tca -3'	150 bp
Mucin6	5'- ctc acc ttc tac ccc agt atc a-3'	5'- ggc aac gag tta gag tca cat t -3'	146 bp
Nanog	5'- gca agc ggt ggc aga aaa acc -3'	5'- cca agt ctg gct gcc cca cat -3'	158 bp
Villin	5' - gac gtt ttc act gcc aat acc a -3'	5' - ccc aag gcc cta gtg aag tct t -3'	158 bp

Gene	Regular RT-PCR Forward primer	Regular RT-PCR Reverse primer	Product size
GAPDH	5'- gaa gac tgt gga tgg ccc ct -3'	5'- gtc cac cac cct gtt gct gt -3'	424 bp
Nanog	5'- agg gcc ctg agg agg agg ag -3'	5'- tgg ccg ttc cag gac tga gc -3'	475 bp
Oct3/4	5'- gtt ctg cgg agg gat ggc ata c -3'	5'- aag gcc tcg aag cga cag atg -3'	360 bp
GFP	5'- gag ctg aag ggc atc gac ttc aag -3'	5'- gga ctg ggt gct cag gta gtg g -3'	246 bp
Insulin 1	5' - ccc agc cct tag tga cca gct ata at -3'	5' - ggg gac cac aaa gat gct gtt tga -3'	160 bp
PRSS1 (Trypsin 1)	5' - tgc tgt tgc ttt ccc tgt gga t -3'	5' - ttg gat gcg ggt ctt gta gca atg -3'	175 bp