SUPPLEMENTAL INFORMATION

EXTENDED EXPERIMENTAL PROCEDURES

Mice. C57BL/6 (CD45.2^{+}) and congenic CD45.1^{+} C57BL/6 were purchased from the Jackson Laboratory or Charles River Laboratories. *Cx$_3$cr1*$^{GFP/GFP}$ C57BL/6 knock in mice (D. Littman, NYU) were bred with WT C57BL/6 mice to obtain heterozygote CX$_3$XR1$^{GFP/WT}$ mice. *Csf1r*$^{-/-}$ C57BL/6 fetal liver cells were used to obtain mouse chimeras and *Csf1r*$^{-/-}$ FVB/NJ and *Csf1*$^{op/op}$ FVB/NJ mice were analyzed within 4 weeks of age because of their early lethality.

Immunofluorescence. Separated small- or large-intestinal muscularis externa or cultured enteric neurons were fixed for 5-10 minutes in PBS with 4% PFA / 4% sucrose and stained as described (Bogunovic et al., 2009). For soluble factor staining removed intestines were placed in the medium with 1xBrefeldin A (Biolegend) and separated muscularis was fixed in 2.5% PFA / 20% sucrose. Immunostaining was performed and analyzed as described(Bogunovic et al., 2009). Antibodies: MHC Class II (clone M5/144.15.2) and CD3 (clone 145-2C11) from eBioscience, BMPRII (clone 73805) and CSF1 (clone 131614) from R&D, BMP2 (clone 1A11) from Sigma, pSMAD1/5/8 (rabbit polyclonal IgG) from Cell Signaling, βIII-Tubulin (rabbit polyclonal IgG) and GFAP (goat polyclonal IgG) from Abcam, Serotonin from Novocastra (rabbit polyclonal IgG). TUNEL staining was performed using TUNEL Apoptosis detection kit (Millipore) according to the manufacturer's protocol. DNase I (3 μg/ml) was used for a positive control prior applying dUTP antibody.

Real-time PCR was performed as described (Bogunovic et al., 2009) with some modifications. Total RNA from sorted cells or muscularis tissue was extracted using Trizol reagent (Life Technologies) following the manufacturer's protocol. RT-PCR was performed using RNA to cDNA Ecodry kit (Clontech Laboratories). For real time PCR Primetime qPCR primers (Integrated DNA Technologies) were used.

Colonic peristalsis *ex vivo*. Mice were cervically dislocated and the entire colon was surgically removed and placed into room temperature Krebs-Ringer bicarbonate buffer (pH=7.4). One cm of proximal colon was removed and three successive colonic rings of 3 mm each were cut. In all experiments the third ring was used. Rings were then allowed to equilibrate for one hour with solution changes every 15 min in separate 8 ml organ baths filled with Krebs-Ringer buffer and constantly perfused with 95% O_2 and 5% CO_2. Colonic rings were then suspended on wires in a four-bath wire myograph system (Danish Myo Technology) and distended in increments of 0.25 mm followed by 10 minutes of rest at each distension length. The data were acquired using the PowerLab acquisition system and analyzed with LabChart Software (AD Instruments).

***In vitro* culture of enteric neuron.** Embryonic intestines from E17 were digested with 0.05% trypsin (Life Technologies) and 0.05% DNAse I (Roche) in HBSS (Life Technologies) at 37^{o} C for 18 min. The digested tissue was homogenized, washed and plated on ultra low binding 24-well plates in DMEM/F12 containing 1:100 N2 (Life Technologies), 20 ng/ml bFGF (eBioscience), 20 ng/ml EGF (Life Technologies) and 1:100 Penicillin/Streptomycin (Sigma) to grow neurospheres. In 7 days neuropsheres were collected, dissociated with accutase (Global Cell Solutions) for 15 min at 37^{o} C, washed and plated for differentiation on round glass cover slips coated with 1μg/ml poly-

D-lysine (MP Biomedicals) and 20 μg/ml Laminin (Life Technologies) in Neurobasal Media containing 1:100 of N2 and 1:50 of B27 (Life Technologies). At day 12 the mature differentiated neurons were used for experiments. For immunofluorescence staining cells were grown on sterile glass cover slips.

Bacterial 16S rDNA qPCR. Fecal DNA Isolation was done using the ZR Fecal DNA MINIPrep (Zymo Research Corporation). Fecal pellets were weighed and total fecal DNA was resuspended in 100 μl of elution buffer. Samples were further diluted 1:100 in RT-PCR grade water (Ambion). Universal 16S primers were purchased from IDT for the V2 and V6 region of 16S rDNA. PCR product was isolated from control feces and serial dilutions (1:10) were made from 1 ng to 10^{-6} ng. A standard curve was created from these dilutions, which was used to calculate the corresponding ng concentration from Ct values of 16S rDNA present per mg of fecal matter.

Fecal transfer. Intact cecum with adjacent 2 cm pieces of ileum and colon from WT B6 mice were homogenized in 2 ml of cold sterile PBS. Obtained suspensions were filtered through 70 μm cell strainers to remove larger debris, spun down at 6000x g and washed twice with cold PBS. Obtained pellets were resuspended in PBS and gavaged to donor mice. Intestinal contents from one donor mouse were divided between three recipients.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1.

FACS analysis of MMs: gating strategy. Related to Figure 1. (A) $CD11c^{lo}MHCII^{hi}$ MMs (oval gate, solid line) in a single cell suspension of SB muscularis separated from the mucosa and submucosa (top) or whole SB (bottom) and $CD11c^{hi}MHCII^{hi}$ LP phagocytes (oval gate, dashed line) in a single cell suspension of whole SB (bottom).

CSF1R expression in the gut is restricted to macrophages. **Related to Figure 2.** (B) FACS plots of SB muscularis single cell suspension from MAFIA mice that express GFP under control of *Csf1r* promoter compared to WT mice. (C) FACS histograms of GFP expression by $CD45^{+}CD11c^{lo}CD11b^{+}MHCII^{hi}$ MMs under control of *Csf1r* promoter in SB and LB muscularis from MAFIA mice compared to WT mice. (D) FACS plots of LB mucosa and submucosa single cell suspension from MAFIA mice compared to WT mice.

Figure S2.

Model of MM depletion. Related to Figure 2. (A) FACS plots of separated LB muscularis single cell suspensions from WT mice 2 days after i.p. injection of isotype IgG or αCSF1R mAb show % of $CD11c^{lo}MHCII^{hi}$ MMs (gated on total viable cells). (B) Absolute numbers of MMs (Muscularis) and mucosal macrophages (LP $CD103^{-}$ $CD11b^{+}$), quantified by FACS, in LB of WT mice 2 days after i.p. injection with isotype IgG or αCSF1R mAb. (C) Absolute numbers of $CD45^{+}B220^{+}$ B cells (top) and $CD45^{+}CD3^{+}$ T cells (bottom), quantified by FACS, in SB muscularis of WT mice 2 days after i.p. injection with isotype IgG or αCSF1R mAb. (D) Absolute numbers of $SSC^{lo}CD45^{+}CD11c^{-}CD11b^{-}$ lymphocytes, quantified by FACS, in the LB muscularis

(top) and mucosa (bottom) of WT mice 2 days after i.p. injection with isotype IgG or αCSF1R mAb. (E) Detection of apoptotic cells by TUNEL assay using anti-dUTP Ab in cross-sections of ileum (top) and proximal colon (bottom) from WT mice 2 days after i.p. injection of isotype IgG or αCSF1R mAb. One sample from IgG treated mouse was pre-treated with DNase I as a control to ensure Ab immunoreactivity in the assay. Scale bars – 100 nm (ileum) and 200 nm (proximal colon).

Gastrointestinal motility in mice depleted of MMs. Related to Figure 3. (F) Gastric emptying measured as percent of total fluorescence expelled from the stomach 15 minutes after oral gavage with rhodamine B conjugated dextran containing methylcellulose meal in mice 5 days after i.p. injection of isotype IgG or αCSF1R mAb. (G) Relative SB fluorescence in 10 SB segments (1 – most proximal, 10 – most distal) measured as percent of total possible fluorescence 15 min after oral gavage with rhodamine B conjugated dextran containing methylcellulose meal 5 days after i.p. injection of isotype IgG or αCSF1R mAb. (H) Gastrointestinal (GI) transit time that represents the time required to expel feces containing carmine red dye. Mice were given carmine red dye containing methylcellulose meal by oral gavage 4 days after i.p. injection of isotype IgG or αCSF1R mAb. (I) Colonic transit time measured by bead expulsion assay in WT mice that were lethally irradiated and received WT or *Csf1r*$^{-/-}$ BM 8 weeks prior to analysis.

Figure S3.

MMs control intestinal peristalsis by secreting BMP2. Related to Figure 4. (A) *Ex vivo* recordings of stretch-induced contractions of colonic rings from WT mice treated *in vivo* with Dorsomorphin or control vehicle (2.75 mm stretch distance).

Conditional MM depletion does not affect various parameters of gastrointestinal physiology. Related to Figure 5. (B) Increase of contractile force of colonic rings isolated from WT mice two days after i.p. injection of isotype IgG or αCSF1R mAb in response to step-wise distension normalized to weight of each colonic ring (mN/g). (C) Relative increase of contractile force of colonic rings isolated from WT mice two days after i.p. injection of isotype IgG or αCSF1R mAb upon increasing concentrations of KCl. (D) IF analysis of ICC network in LB muscularis from WT mice two days after i/p injection of isotype IgG or αCSF1R mAb stained with anti-cKit Ab and counterstained with DAPI. Scale bar – 100 nm. (E) Representative images of the distribution of Serotonin$^+$ enteroendocrine cells in the SB from WT mice two days after i.p. injection of isotype IgG or αCSF1R mAb. Tissues were stained with anti-Serotonin Ab and counterstained with DAPI. Scale bars – 100 nm. (F) Quantitative summary of the distribution of Serotonin$^+$ enteroendocrine cells in the SB mucosa from WT mice two days after i.p. injection of isotype IgG or αCSF1R mAb. Each data point represents numbers of Serotonin$^+$ cells in the mucosa of a complete intestinal cross-section; each column summarizes the results from 3 mice. (G) Transepithelial resistance of ileum, proximal colon and distal colon from WT mice 3-10 days after i.p. injection of a maximal dose (150 ng/g of body weight) of isotype IgG or αCSF1R mAb. (H) Flux of FITC-dextran across the intestinal tissue of ileum, proximal colon and distal colon from WT mice 3-10 days after i.p. injection of a maximal dose of isotype IgG or αCSF1R mAb.

Figure S4.

BMP receptor is not expressed by c-Kit$^+$ ICC network and GFAP$^+$ enteric glia. Related to Figure 5. (A) IF analysis of LB muscularis from WT mice stained with anti-

βIII Tubulin and anti-cKit Abs and counterstained with DAPI. (B) IF analysis of LB muscularis from WT mice stained with anti-BMPRII and anti-cKit Abs and counterstained with DAPI. C. IF analysis of LB muscularis from WT mice stained with anti-G□□□ and anti-BMPRII Abs and counterstained with DAPI. (A-C) Scale bar – 200 nm.

Cultured ENs express functional BMP receptor. Related to Figure 5. (D) *Bmp2*, *BmprIa*, *BmprIb* and *BmprII* relative gene expression levels (FI) in cultured ENs measured by qPCR as compared to *Bmp2*. (E) IF analysis of primary ENs fixed 30 min after adding 10 ng/ml of BMP2 or control vehicle and stained with anti-pSMAD1/5/8 and anti-BMPRII Abs and counterstained with DAPI. Scale bar – 5 nm.

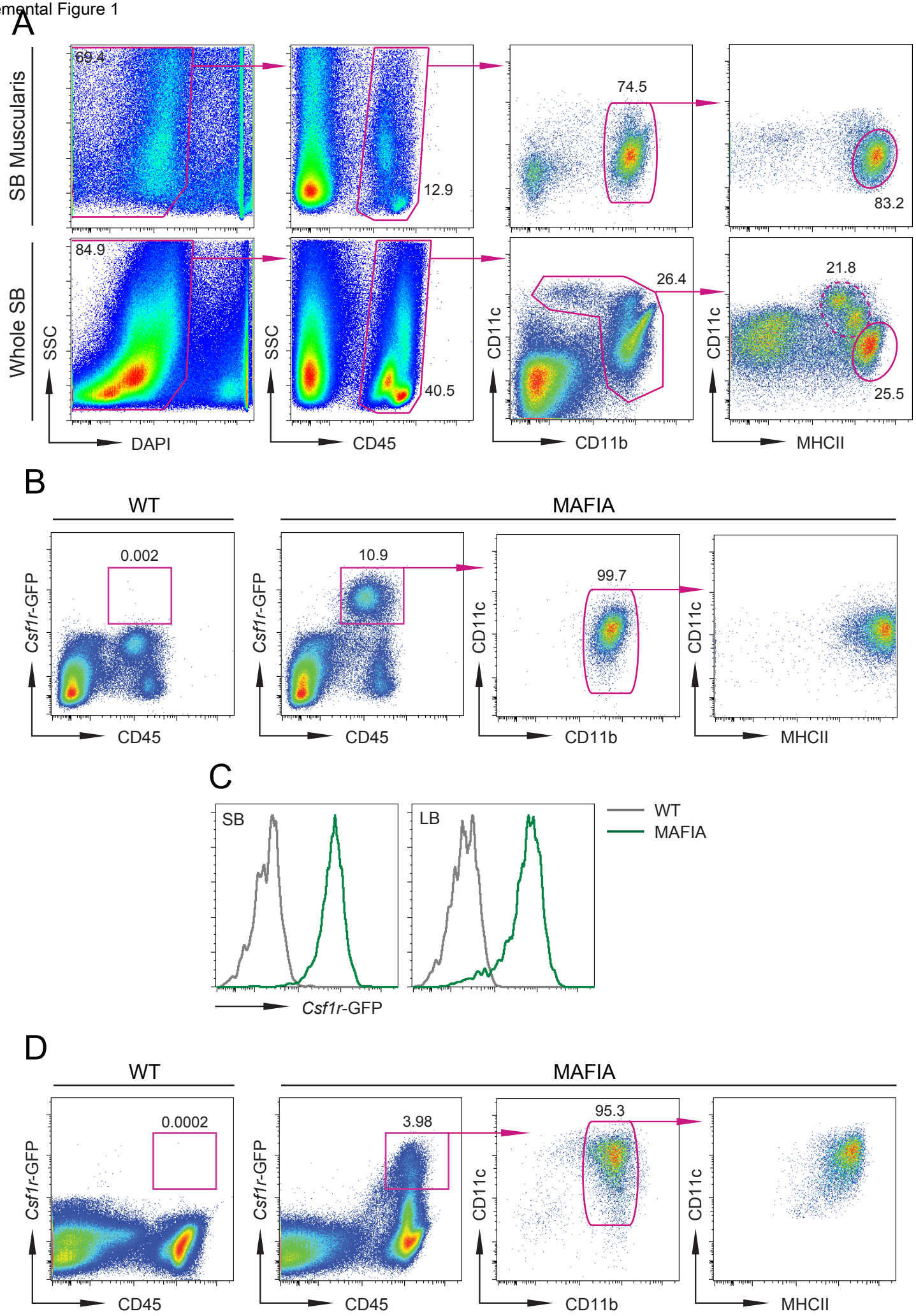
Figure S5. Characterization of *Csf1*$^{op/op}$ mice. Related to Figure 6. (A) Image of a 4-week-old *Csf1*$^{op/op}$ mouse next to its WT littermate (ruler in inches). (B) Image of LB from a 4-weeks-old *Csf1*$^{op/op}$ mouse next to the LB from a WT littermate (ruler in inches). Both pups were kept on a homogenized soft diet. (C) IF analysis of LB muscularis from 4-weeks-old WT and *Csf1*$^{op/op}$ mice stained with anti-pSMAD1/5/8 and anti-BMPRII Abs and counterstained with DAPI. Scale bars – 100 nm.

Figure S6. Intestinal dysmotility and reduced neuronal BMPR signaling in mice treated with broad-spectrum antibiotics. Related to Figure 7. WT mice received antibiotics with drinking water for 4 weeks and control littermates received only water. (A) Gastric emptying in antibiotic-treated and control mice. (B) Relative SB fluorescence in 10 SB segments (1 – most proximal, 10 – most distal) measured as percent of total possible fluorescence 15 min after oral gavage with rhodamine B conjugated dextran

containing methylcellulose meal in antibiotic-treated and control mice. (C) *Ex vivo* recordings of stretch-induced contractions of colonic rings from antibiotic-treated and control mice (2.75 mm stretch distance). (D) Representative images of the distribution of pSMAD1/5/8^{+}BMPRII^{+} neurons in the LB muscularis from antibiotic-treated and control mice. Tissues were stained with anti-pSMAD1/5/8 and anti-BMPRII Abs and counterstained with DAPI. Scale bars – 100 nm.

Figure S7. Luminal microbiota regulates intestinal motility and MM-neuronal crosstalk. Related to Figure 7. (A) *Csf1* relative gene expression levels measured by qPCR in LB muscularis from WT mice received antibiotics with drinking water for 1, 2, 3 and 4 weeks and control littermates received only water. FI – fold increase as compared to average *Csf1* levels in the control group. (B) Absolute numbers of MMs quantified by FACS in LB of WT mice received antibiotics with drinking water for 1, 2, 3 and 4 weeks and control littermates received only water. (C) Absolute numbers of MMs quantified by FACS in SB from WT mice treated with antibiotics in drinking water for 4 weeks (left), from WT mice treated with antibiotics with 50 μg/ml LPS in drinking water for 4 weeks (middle) and from WT mice 3 weeks after they received FT following a 4-week course of antibiotics (right). The data are compared to control littermates received only water. (D) Percentage (%) of SSC^{lo}CD45^{+}CD11b^{+}CD115^{+}Gr1^{hi} monocytes among blood leukocytes quantified by FACS in WT mice received antibiotics with drinking water for 4 weeks and control littermates received only water. (E) Image of LB from age matched WT mice received antibiotics for 1 or 4 weeks in drinking water, and a control littermate received only water (ruler in cm). (F) Amount of bacterial 16S rDNA (ng/mg of feces) in fecal pellets from WT mice received antibiotics with drinking water for 1, 2, 3 and 4 weeks

and control littermates received only water. (G) Image of LB from age matched WT mice received antibiotics in drinking water for 4 weeks or antibiotics with 50 μg/ml LPS in drinking water for 4 weeks, and a control littermate received only water (ruler in cm). (H) Amount of bacterial 16S rDNA (ng/mg of feces) in fecal pellets from WT mice 3 weeks after they received FT following a 4-week course of antibiotics, mice received antibiotics for 4 weeks or control littermates maintained on water. (I) Image of LB from WT mice 3 weeks after they received FT following a 4-week course of antibiotics and control littermates maintained on water (ruler in cm).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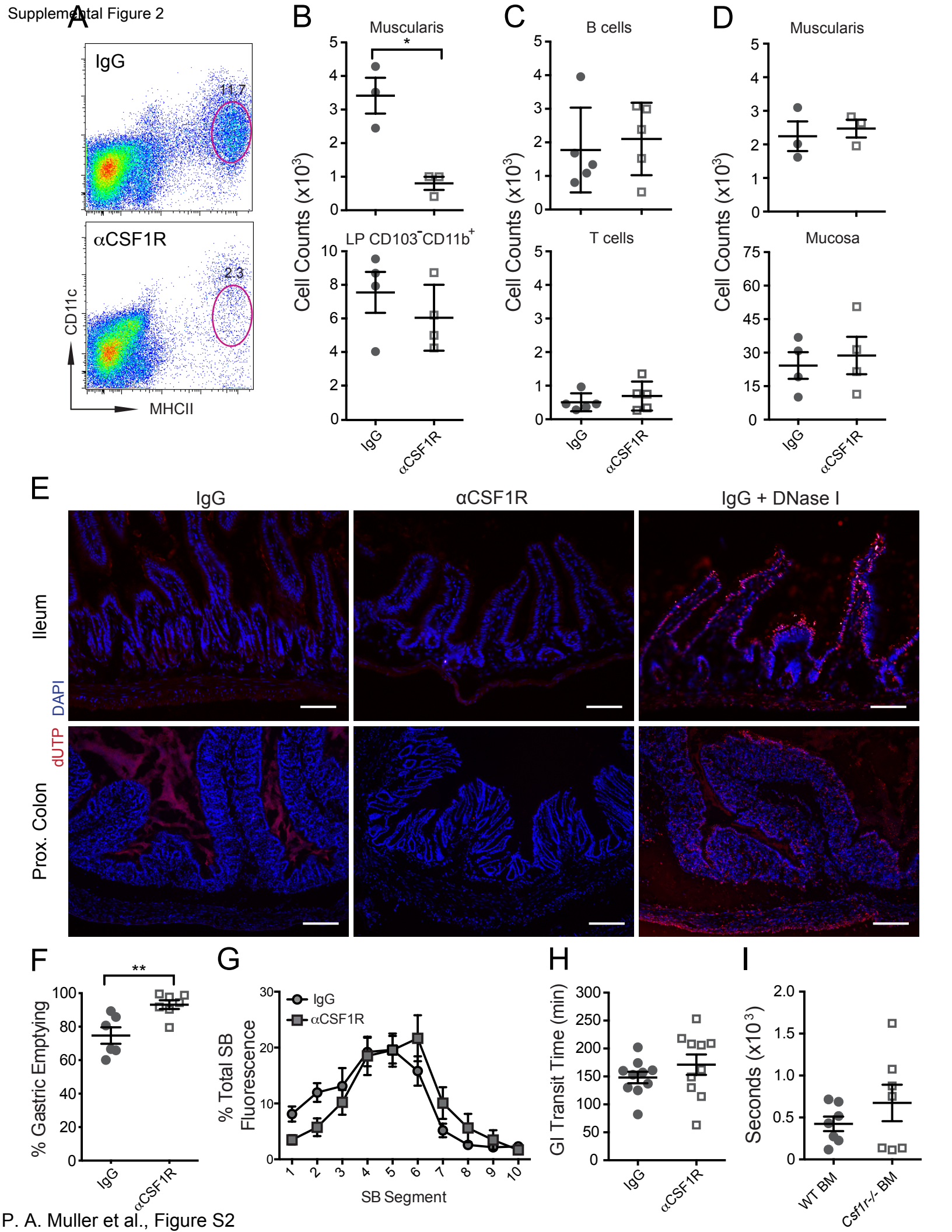
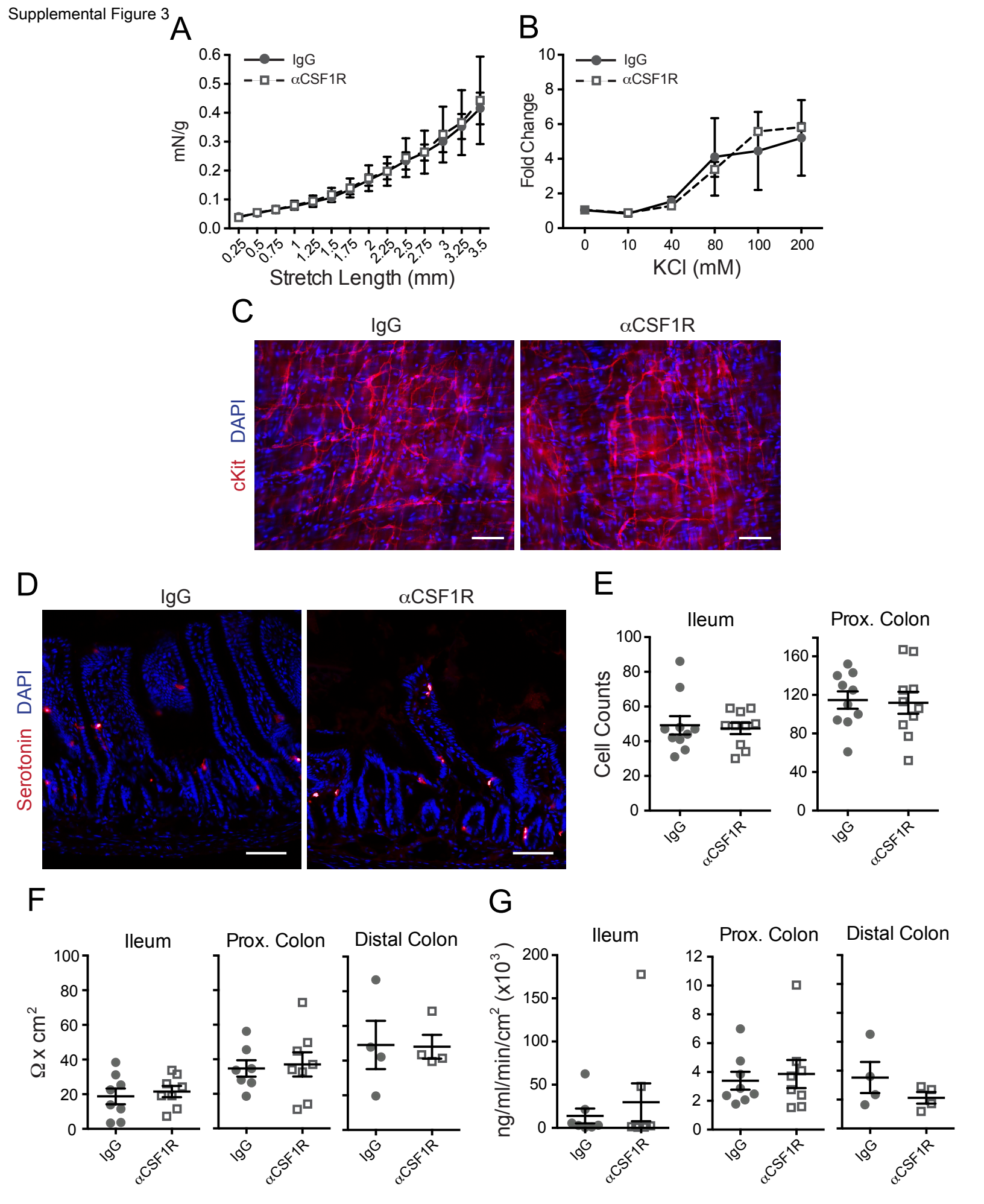
Supplemental Figure 2



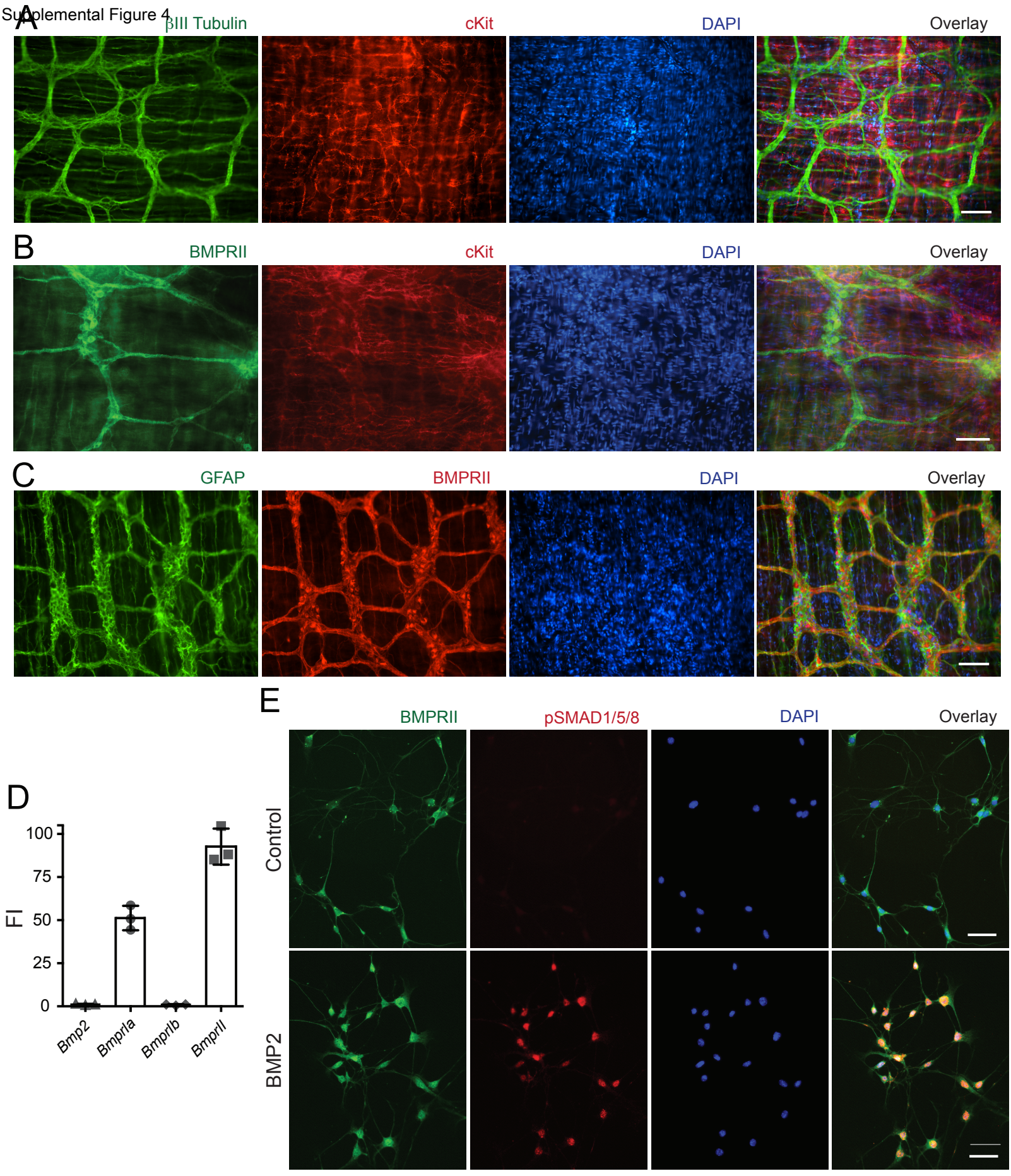
P. A. Muller et al., Figure S2

Supplemental Figure 3



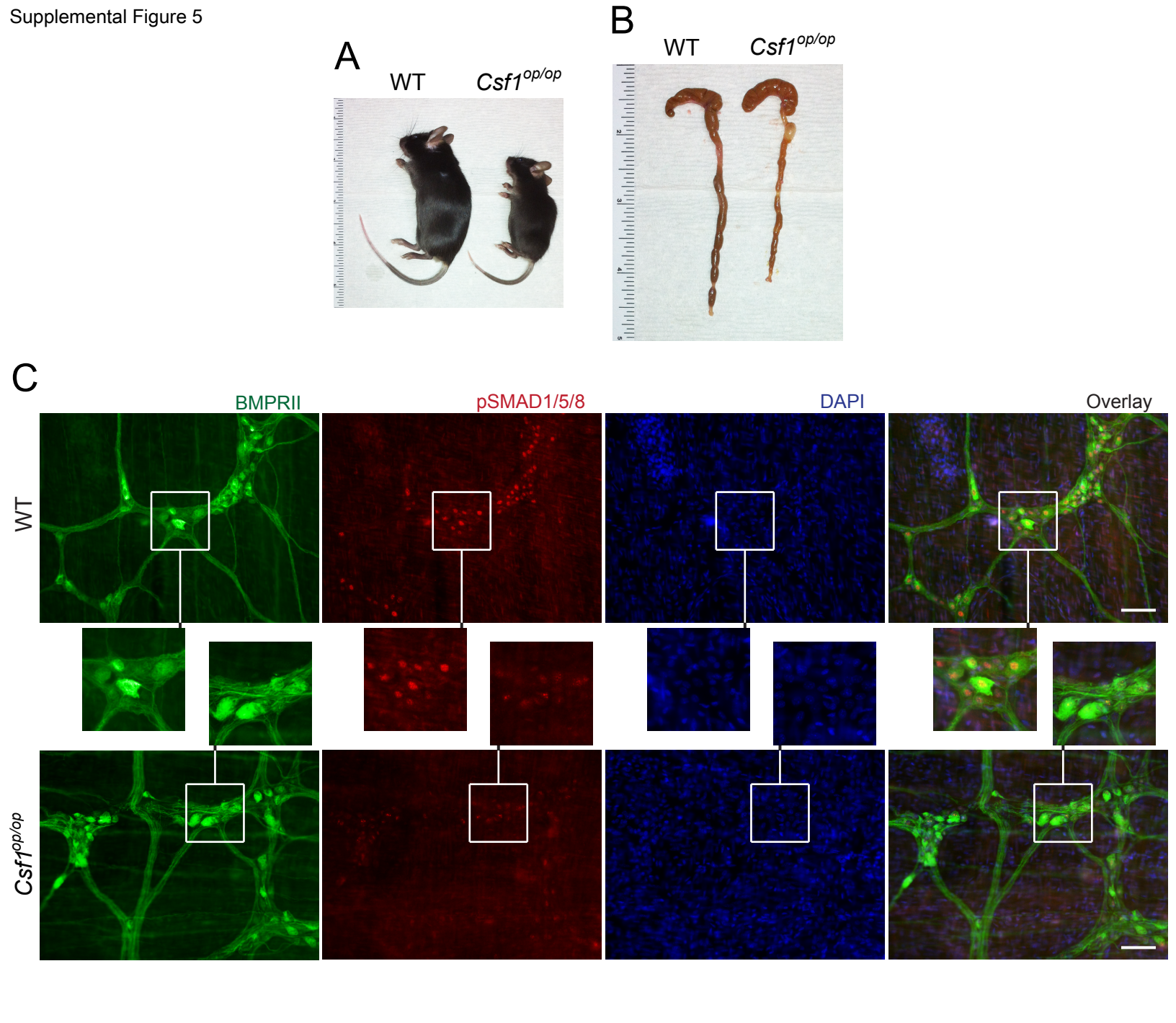
P. A. Muller et al., Figure S3

Supplemental Figure 4



P. A. Muller et al., Figure S4

Supplemental Figure 5



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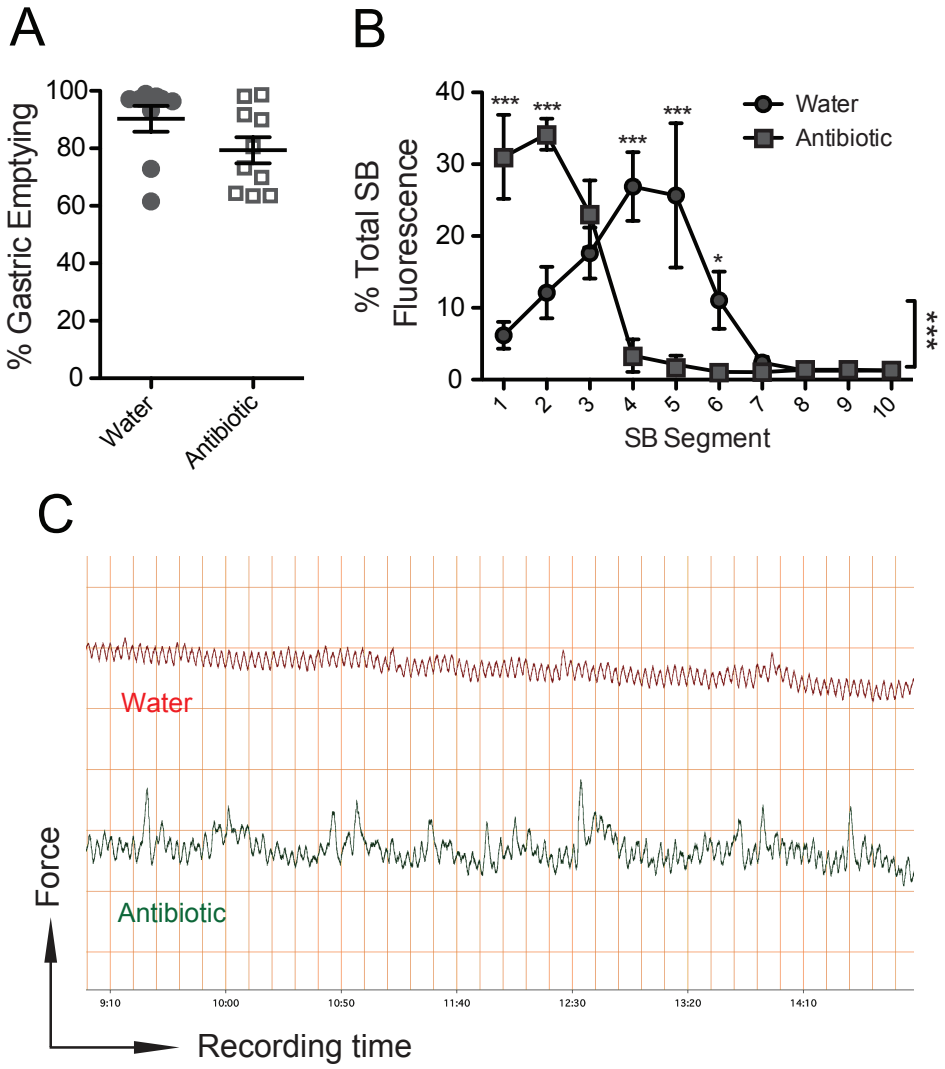
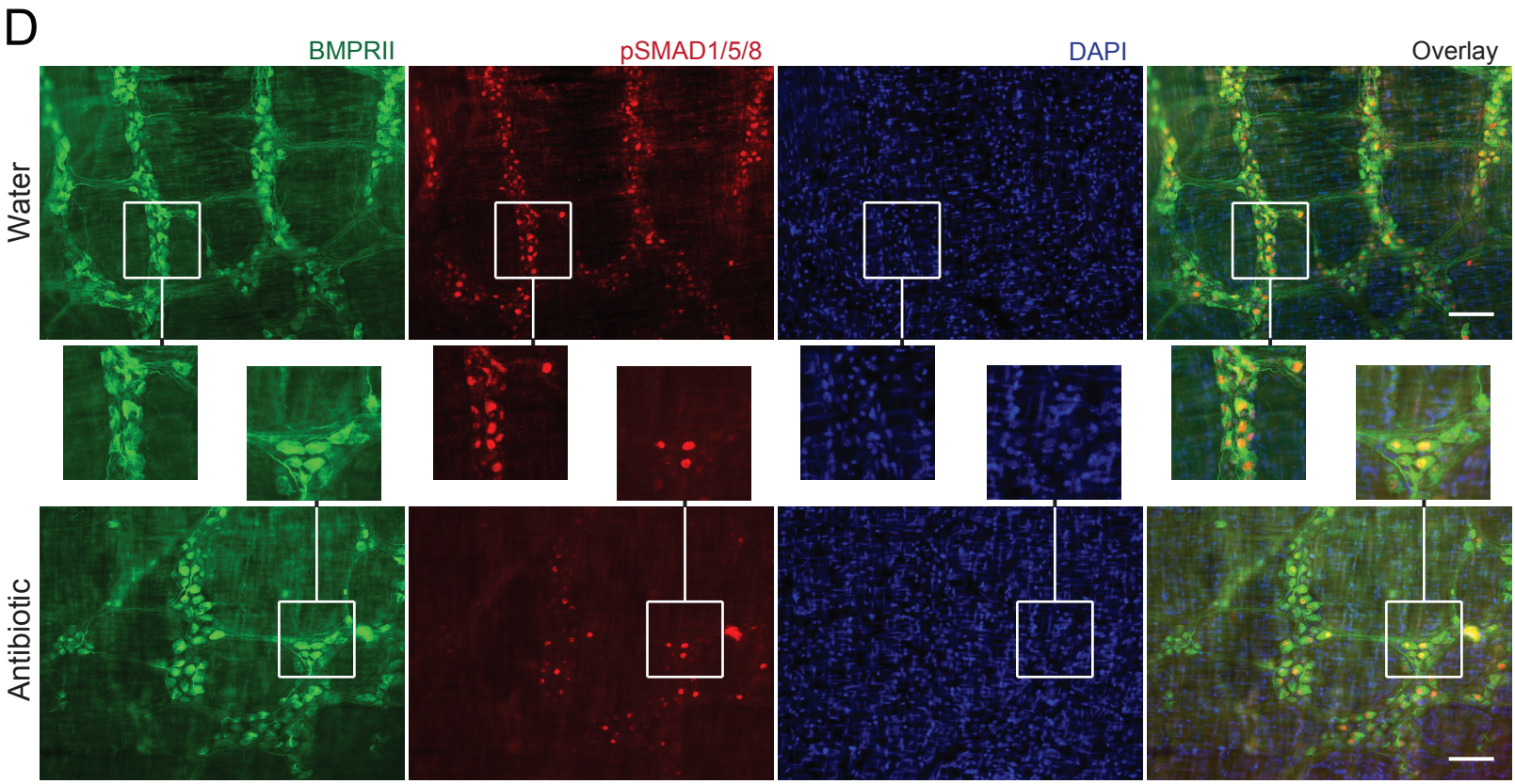
Supplemental Figure 6





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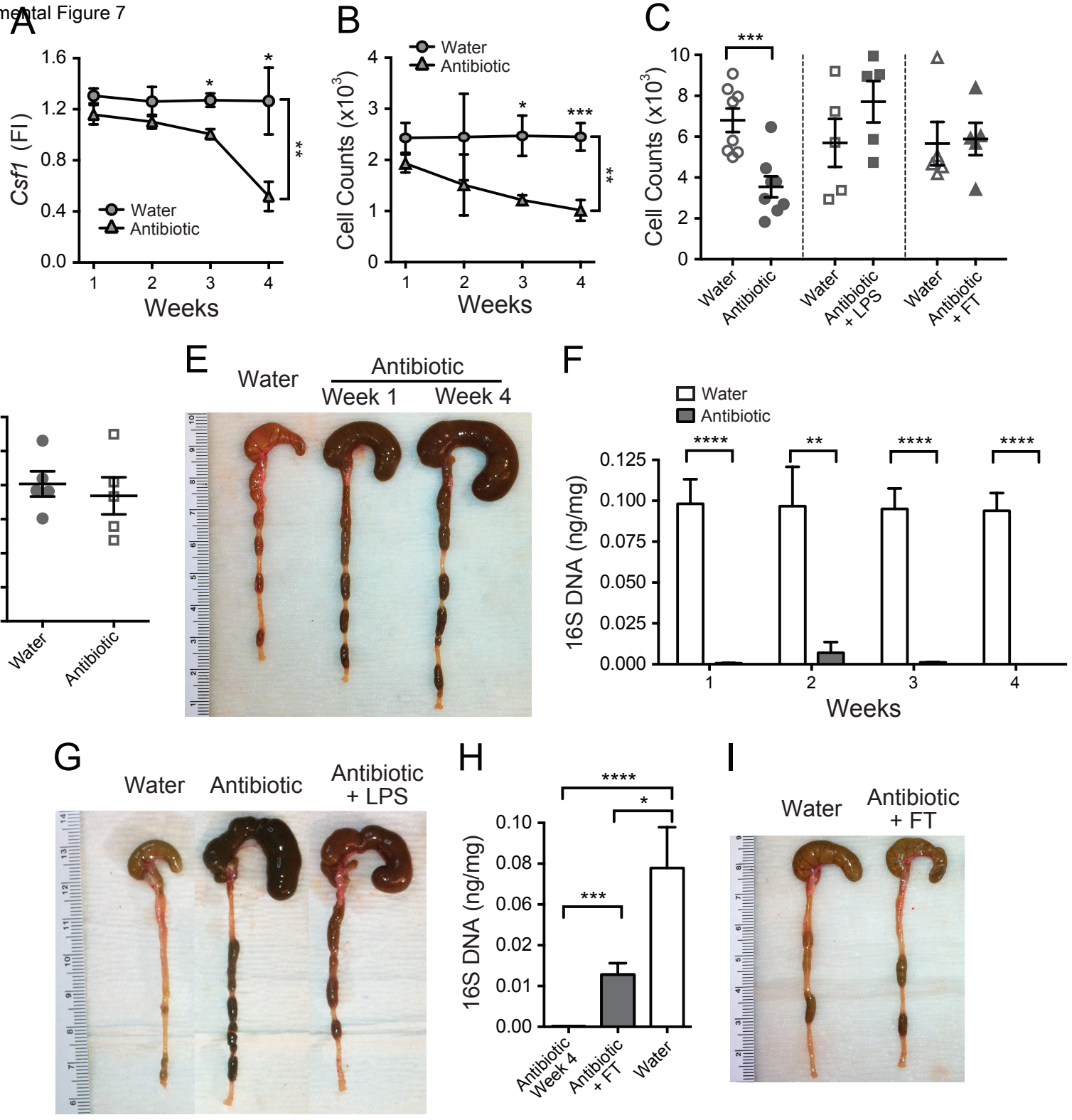
Supplemental Figure 7



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