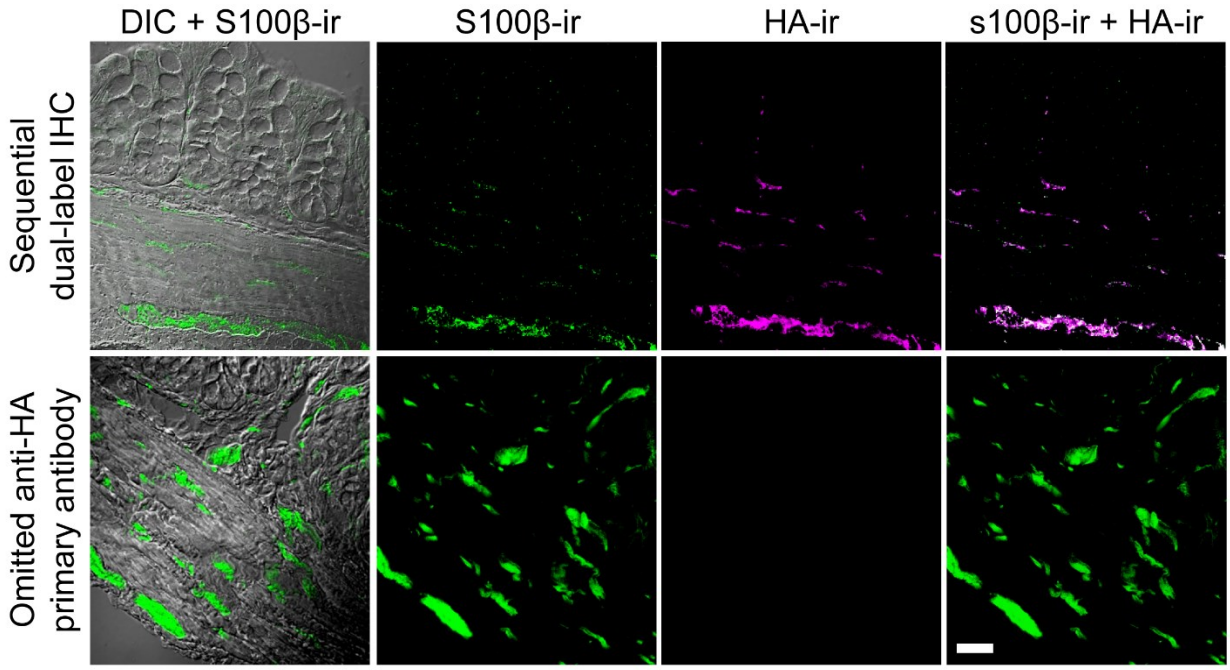
Supplementary information for:

Enteric glial activity regulates secretomotor function in the mouse colon but does not acutely affect gut permeability

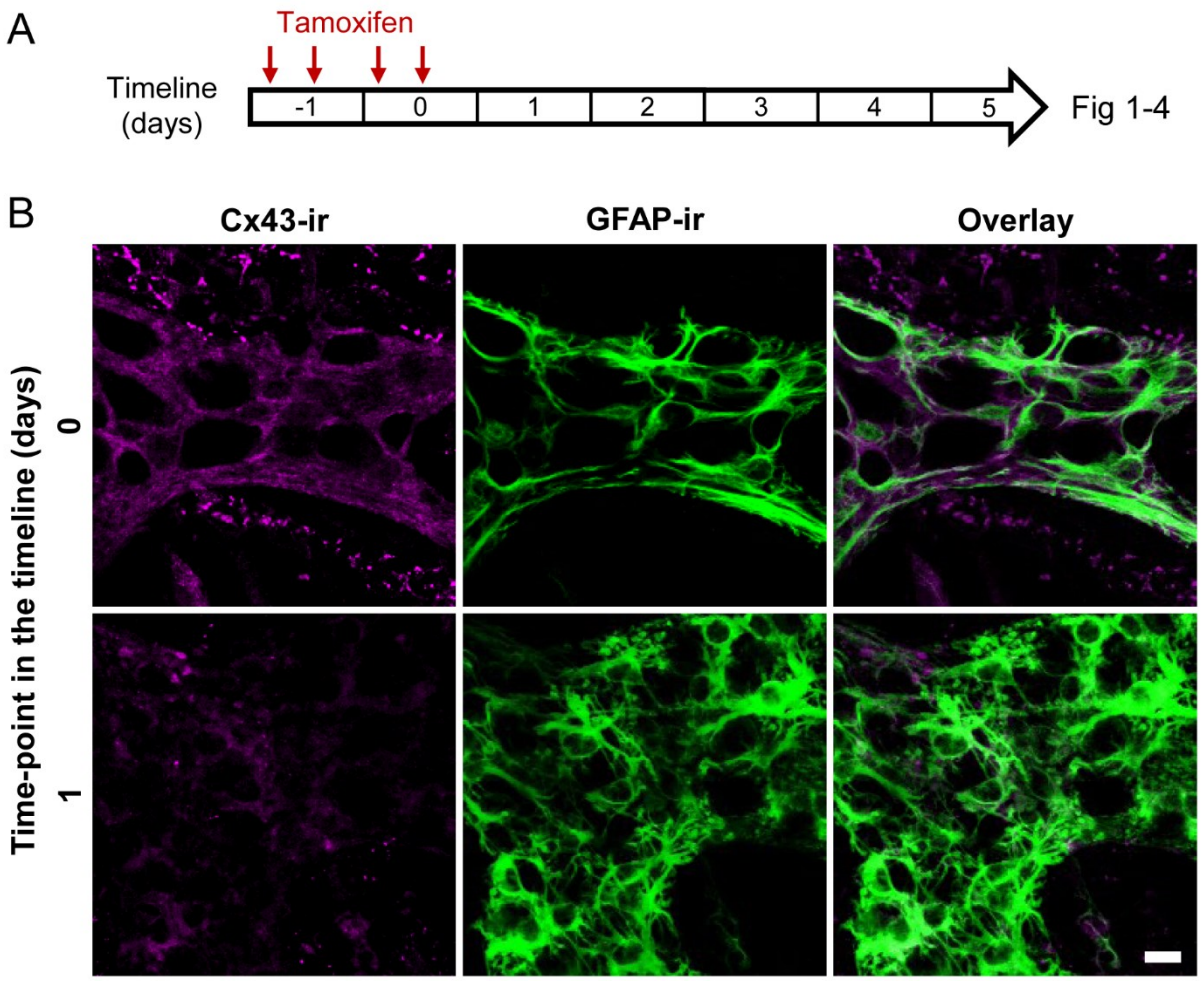
Vladimir Grubišić and Brian D. Gulbransen

This file includes:

Supplementary Figures 1 – 3 and their legends

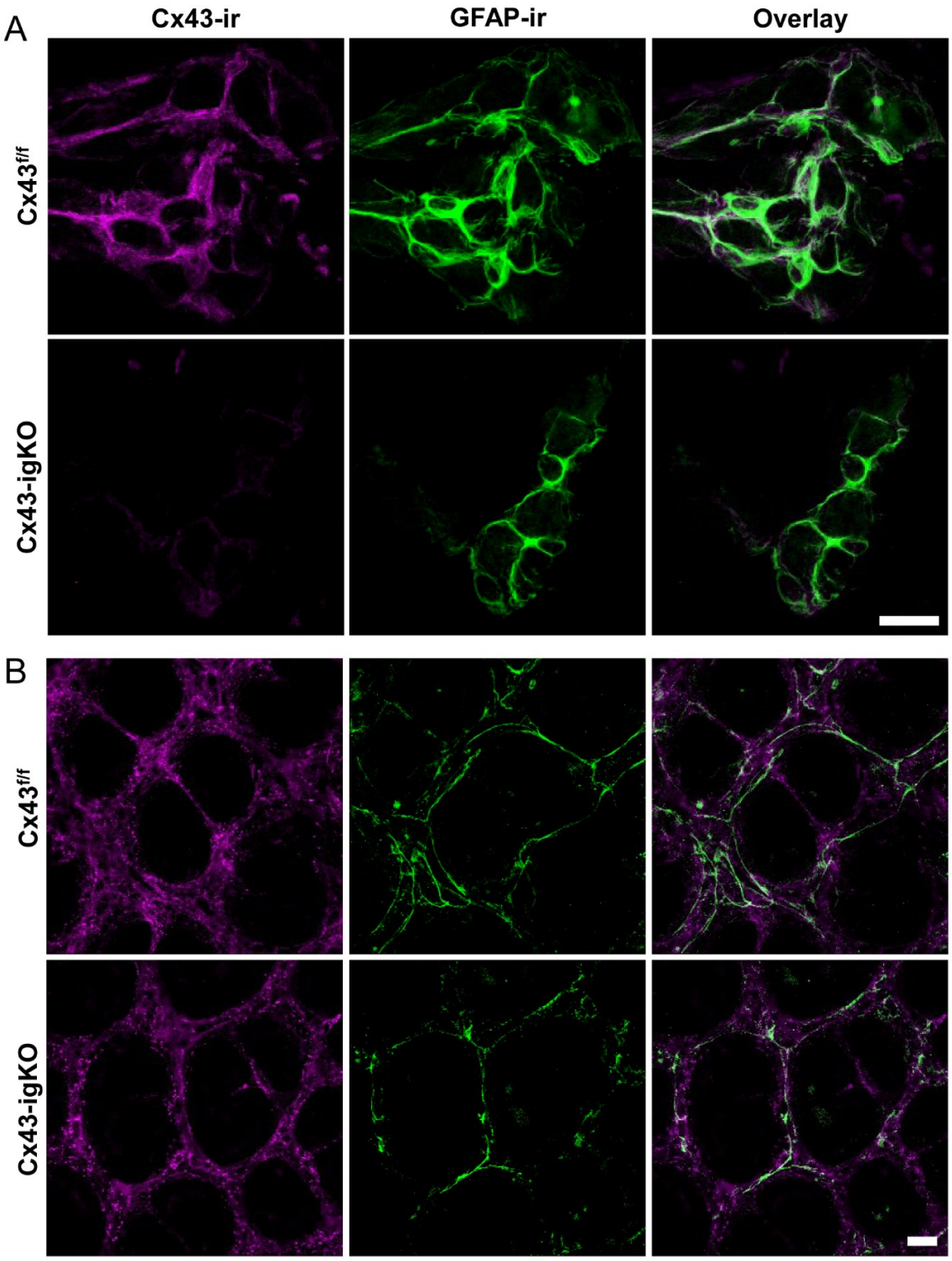


Supplementary Figure 1. Control for immunohistochemistry protocol using primary antibodies raised in the same host (Fig 5D). Confocal images of transversal “ring” sections of colons from *GFAP*::hM3Dq transgenic mice after processing with sequential dual-label immunohistochemistry (IHC) using anti-S100β (green) and anti-HA (magenta) primary antibodies (**top row**) or after the IHC procedure where only the primary anti-HA antibody incubation step was omitted (**bottom row**). HA-ir images are presented using the same fluorescence linear dynamic range for the paired images. Differential interference contrast (DIC) images at left are provided for orientation within the gut wall. Overlays of the anti-S100β and anti-HA immunoreactivities (ir) are merged in the last column. Scale bar, 25 μm.



Supplementary Figure 2. Dynamic expression of the connexin 43 (Cx43) in the Cx43 inducible and glia-specific knock out (Cx43-igKO) animal model. **A.** Timeline of the tamoxifen induction protocol. Cre recombinase activity was induced by 2 intraperitoneal injections (red arrows) of tamoxifen per day for 2 days and experiments were performed 5 days after the last injection. For details about genetics of the animal model and Cx43 expression 5 day post-induction see main text (Fig 1). **B.** Dynamic reduction of the glial Cx43 expression following Cre-lox recombination. Confocal images of dual-label immunohistochemistry showing immunoreactivity (ir) for Cx43 (Cx43-ir, magenta, left) and glial fibrillary acidic protein (GFAP-ir, green, center) in whole-mount preparations of myenteric plexus and the adherent longitudinal muscle from the

colons of tamoxifen treated Cx43-igKO (*Sox10::*CreERT2$^{+/-}$ / Cx43$^{f/f}$) mice immediately following the completion of the two-day tamoxifen induction procedure (top row) or one day following the final injection (bottom row). Cx43-ir images (left) are presented using the same fluorescence linear dynamic range. Overlays of Cx43- and GFAP-ir are shown in panels at right. Scale bar, 10 μm.



Supplementary Figure 3. Expression of glial Cx43 in the submucosal plexus and mucosa. Confocal images of dual-label immunohistochemistry showing Cx43-ir (magenta, left) and

GFAP-ir (green, center) in whole-mount preparations of the submucosal plexus (**A**) and mucosa (**B**) from the colons of tamoxifen treated $Cx43^{f/f}$ transgenic mice (top rows) and their Cx43-igKO (*Sox10::*$CreERT2^{+/-}$ / $Cx43^{f/f}$) littermates (bottom rows). Cx43-ir images (left) are presented using the same fluorescence linear dynamic range for the paired images. Overlays of Cx43- and GFAP-ir are shown in panels at right. Note that tamoxifen treated Cx43-igKO animals exhibited a loss of Cx43-ir in submucosal glial cells (A) that was comparable to that observed in the myenteric glia (Fig. 1C) while the loss of glial Cx43-ir was less apparent in mucosal glia (B) due to the abundance of Cx43-ir in the surrounding epithelial cells and subepithelial fibroblasts. Scale bars, 20 μm.