

Supplemental Figures and Figure Legends

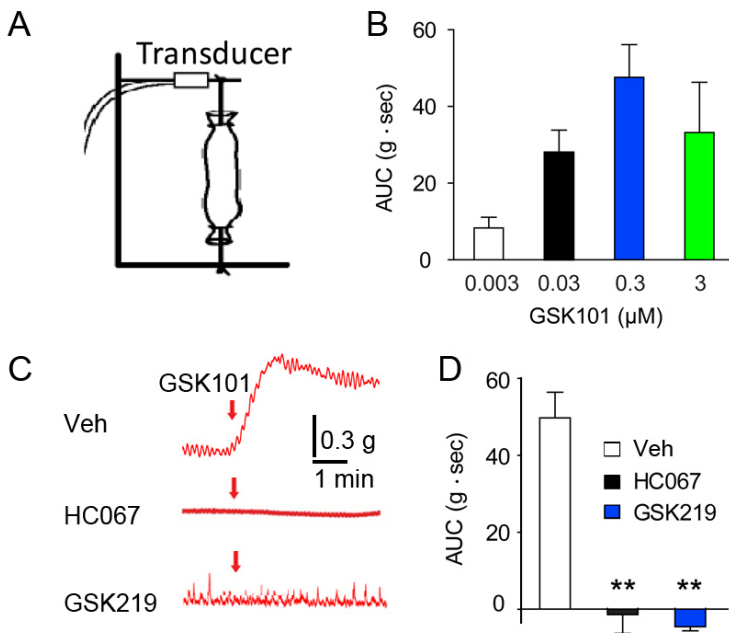


Figure S1, related to Figure 1, Selective activation of TRPV4 regulates GI motility.

(A) Schematic of the contractility recording apparatus using colon strips.

(B) Quantification of GSK101-elicited contractile responses in colon strips from *Trpv4*^{+/+} mice exposed to increasing concentrations of GSK101. n=16 segments from 4 mice per group.

(C-D) Representative traces (C) and quantification (D) of GSK101-induced colon contraction in the presence of the TRPV4 antagonists HC067 (3 μM) or GSK219 (0.3 μM). n= 16 segments from 4 mice per group.

All data are pooled from two to three independent experiments. Data are mean ± SEM. Statistical significance was determined using one-way ANOVA with Tukey's post-test (D). ** p<0.01.

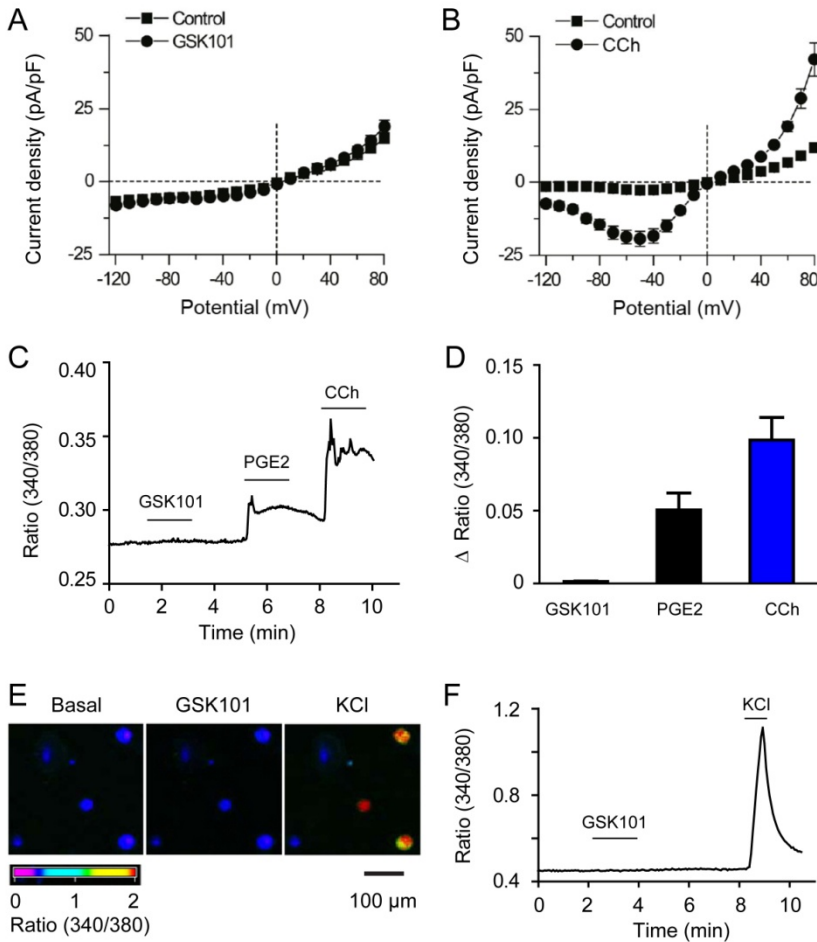


Figure S2, related to Figure 3, Enteric neurons and SMCs are unresponsive to TRPV4 stimulation.

(A-B) Averaged membrane currents at holding potentials from -120 mV to +80 mV (10 mV steps) before and after the application of GSK101 (0.3 μM, n=9 cells) (A) or CCh (50 μM, n=13 cells) (B).

(C) Averaged time lapse trace showing the [Ca²⁺]_i responses elicited by GSK101 (0.3 μM), PGE2 (30 μM), and CCh (10 μM) in freshly isolated intestinal SMCs from one cover slip.

(D) Quantification of changes in 340/380 ratio elicited by GSK101, PGE2, or CCh. n=5 coverslips.

(E) Representative images showing [Ca²⁺]_i responses in dissociated myenteric neurons elicited by GSK101 (0.2 μM) and KCl (100 mM).

(F) Averaged time-lapse trace showing [Ca²⁺]_i responses of myenteric neurons in response to GSK101 (0.2 μM) and KCl (100 mM) from one cover slip. KCl was used as a positive control.

All data are pooled from three independent experiments.

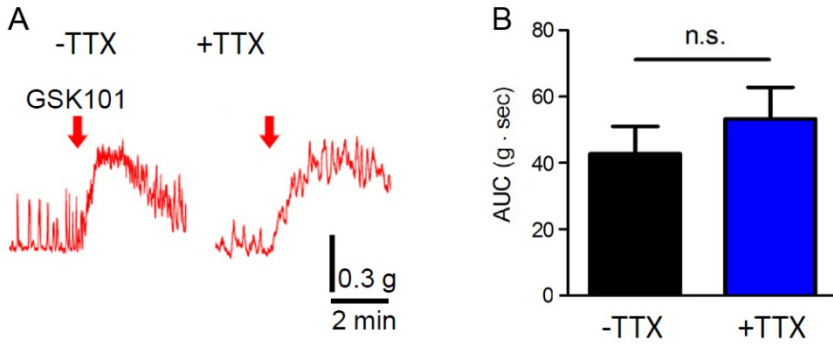


Figure S3, related to Figure 3, TRPV4-mediated colon contraction is not reduced by blocking enteric neurotransmission.

(A-B) Representative traces (A) and quantification (B) of GSK101-induced colon contractions in the absence and presence of TTX (0.3 μ M). n=12 segments from 3 mice.

All data are pooled from three independent experiments. Data are mean \pm SEM. Statistical significance was determined using Student's t-test (B). n.s., not significant.

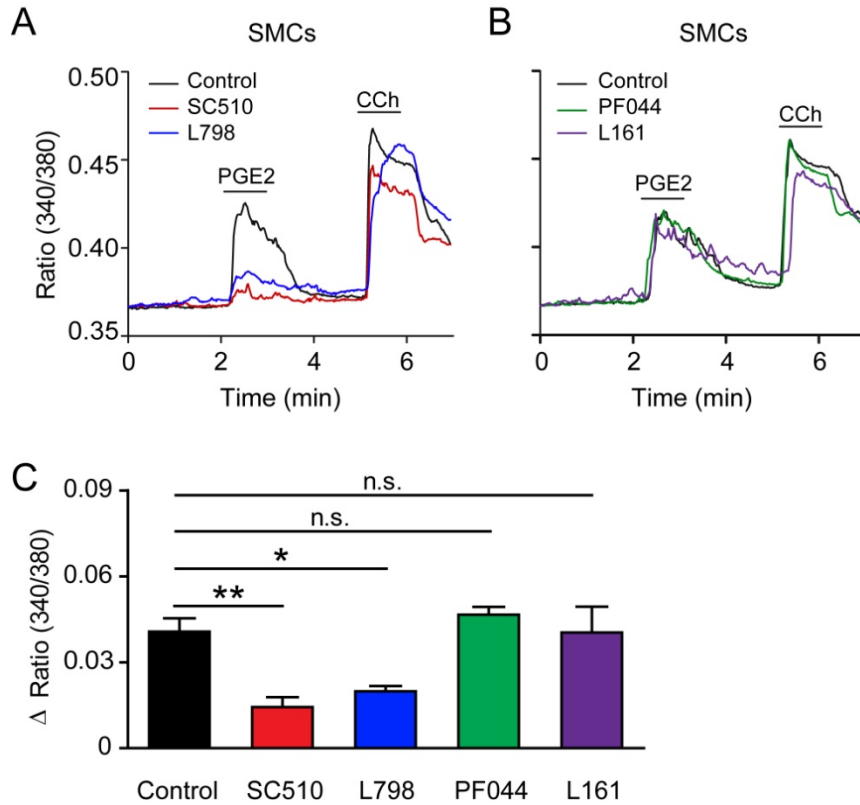


Figure S4, related to Figure 5, PGE2 evokes smooth muscle cell response via EP1/3 receptors.

(A) Averaged $[Ca^{2+}]_i$ response induced by PGE2 (30 μ M) in the absence and presence of the EP1 antagonist SC510 (red line, 5 μ M) or EP3 antagonist L798 (blue line, 3 μ M) in freshly isolated intestinal SMCs. CCh was used as a positive control.

(B) Averaged $[Ca^{2+}]_i$ response induced by PGE2 in the absence and presence of the EP2 antagonist PF044 (red line, 0.3 μ M) or EP4 antagonist L161 (blue line, 0.3 μ M) in freshly isolated intestinal SMCs. CCh was used as a positive control.

(C) Quantification of PGE2-induced $[Ca^{2+}]_i$ response in the absence or presence of EP1 and EP3 antagonists or EP2 and EP4 antagonists. $n=5$ coverslips per group.

All data are pooled from two to three independent experiments. Data are mean \pm SEM. Statistical significance was determined using one-way ANOVA with Tukey's post-test (C). * $p<0.05$, ** $p<0.01$, n.s., not significant.

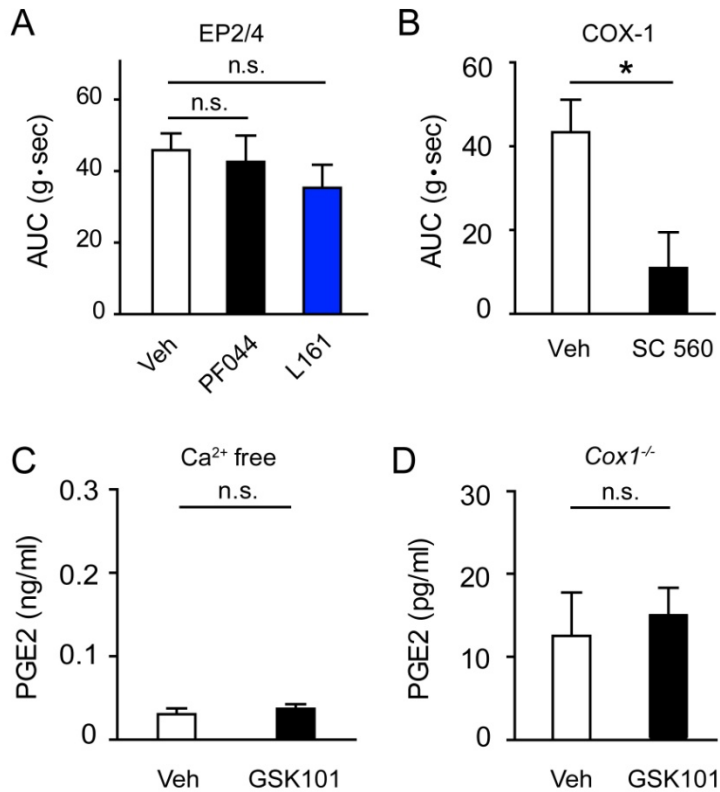


Figure S5, related to Figure 5, TRPV4-mediated colon contraction involves PGE2 signaling.

(A) GSK101 (0.3 μ M)-induced colon contraction in the presence of EP2 antagonist (PF044, 0.3 μ M) and EP4 antagonist (L161, 0.3 μ M). n=16 segments from 4 mice per group.

(B) GSK101-induced colon contraction in the presence of pharmacologic COX-1 inhibition by SC 560 (0.1 μ M) in *wt* mice. n=12 segments from 3 mice per group.

(C) GSK101-induced PGE2 release in a Ca²⁺-free extracellular buffer from MMs. n=5 mice.

(D) GSK101-induced PGE2 release in MMs from COX-1 KO mice. n=5 mice per group.

* p<0.05; ** p<0.01; n.s., not significant.

All data are pooled from two to three independent experiments. Data are mean \pm SEM. In this figure, statistical significance was determined using one-way ANOVA with Tukey's post-test (A) and Student's t-test (B, C, and D). * p<0.05, n.s., not significant.