

A smooth muscle-derived, BRAF-driven mouse model of gastrointestinal stromal tumor (GIST): evidence for an alternative GIST cell-of-origin

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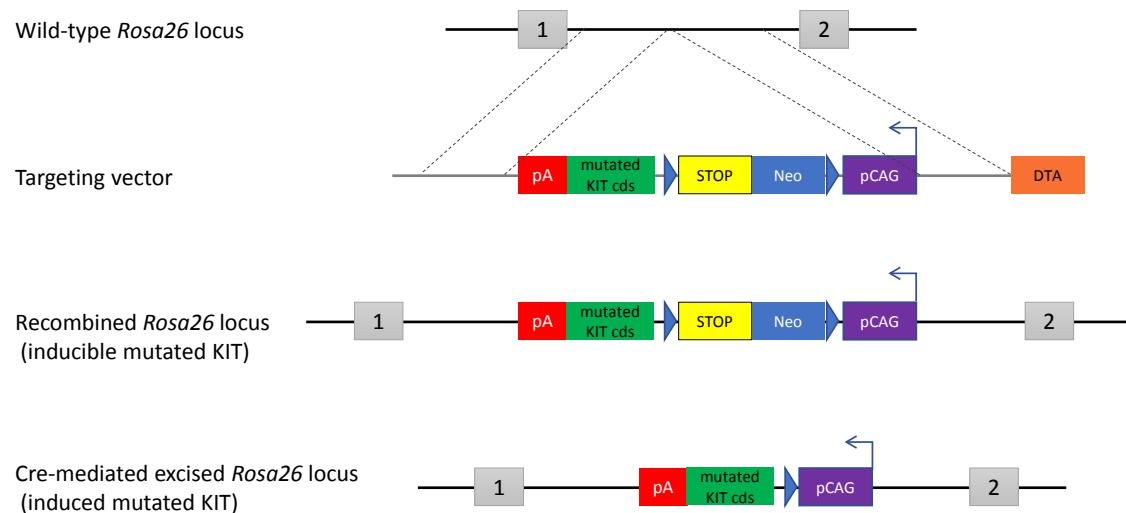


Figure S1. Schematic representation of the *Rosa26* targeting strategy. The scheme is not depicted to scale. Endogenous *Rosa26* exons are indicated as gray boxes. loxP sites are shown as blue triangles. The mutated human KIT cDNA is depicted as a green box, and the hGH polyA as a red box. The combined STOP-neomycin selection cassettes are indicated as yellow and blue boxes, respectively. The DTA negative selection cassette is represented by an orange box and the CAG promoter by a purple box.

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$Lrig1^{CreERT2/+}; Brafl^{SL-V600E/+}$

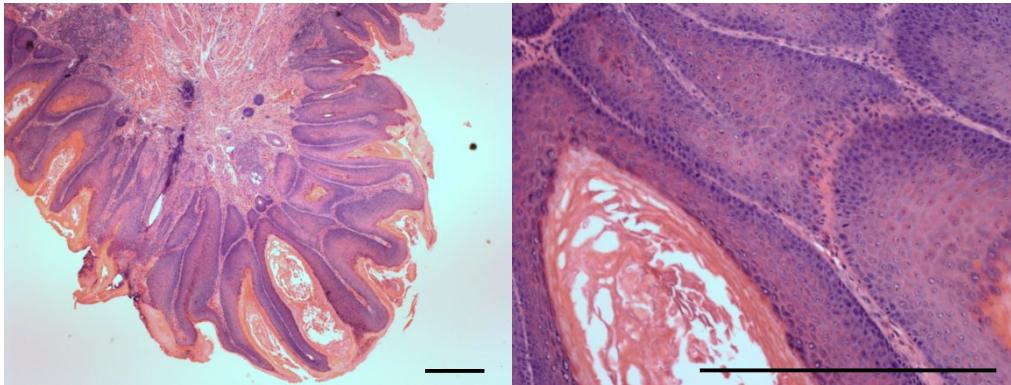


Figure S2. Oral tumor formed in an $Lrig1^{CreERT2/+}; Brafl^{SL-V600E/+}$ mouse. The $Lrig1^{CreERT2/+}; Brafl^{SL-V600E/+}$ mouse was given a single intraperitoneal injection of tamoxifen (2 mg per mouse) at six weeks of age. Two weeks later the mouse was euthanized and the oral lesion harvested and processed for microscopic examination. H&E staining of an oral tumor is shown. Scale bars; 500 μ m.

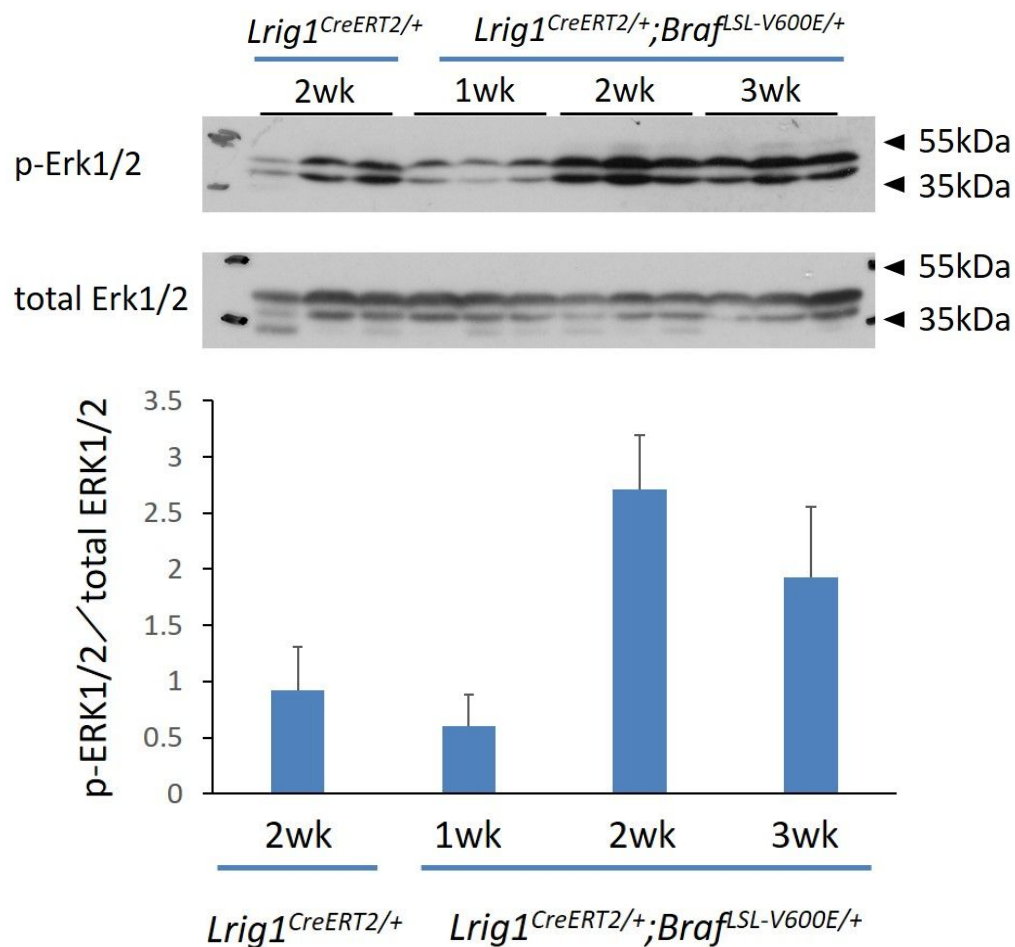


Figure S3. Verification of ERK phosphorylation after induction of BRAF^{V600E}. Western blotting analysis of the jejunal walls from *Lrig1^{CreERT2/+};Braf^{LSL-V600E/+}* and *Lrig1^{CreERT2/+}* mouse given a single intraperitoneal injection of tamoxifen (2 mg per mouse) (upper panel). Tissues were collected after indicated time after tamoxifen injection (N=3). Densitometric analysis was performed for the blots (lower panel).

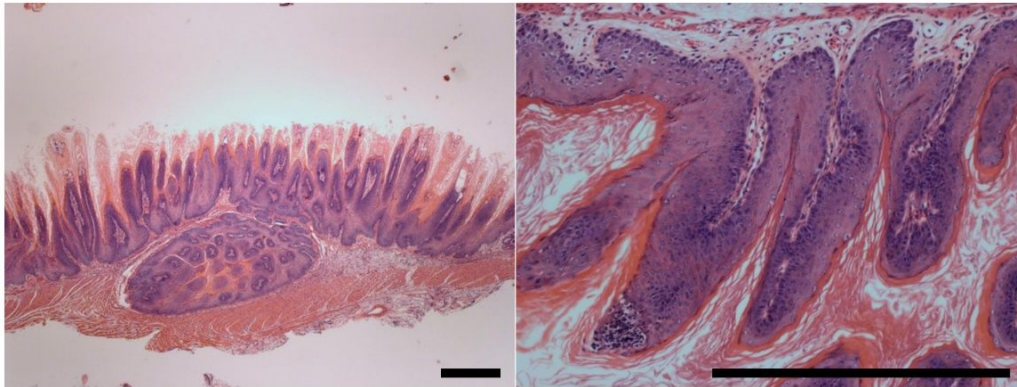


Figure S4. Forestomach hyperplasia of an *Lrig1*^{CreERT2/+}; *Braf*^{SL-V600E/+} mouse. An *Lrig1*^{CreERT2/+}; *Braf*^{SL-V600E/+} mouse was given a single intraperitoneal injection of tamoxifen (2 mg per mouse) at six weeks of age. Two weeks later the mouse was euthanized and stomach harvested and processed for microscopic examination. H&E staining shows hyperplasia of forestomach. Scale bars; 500 μ m.

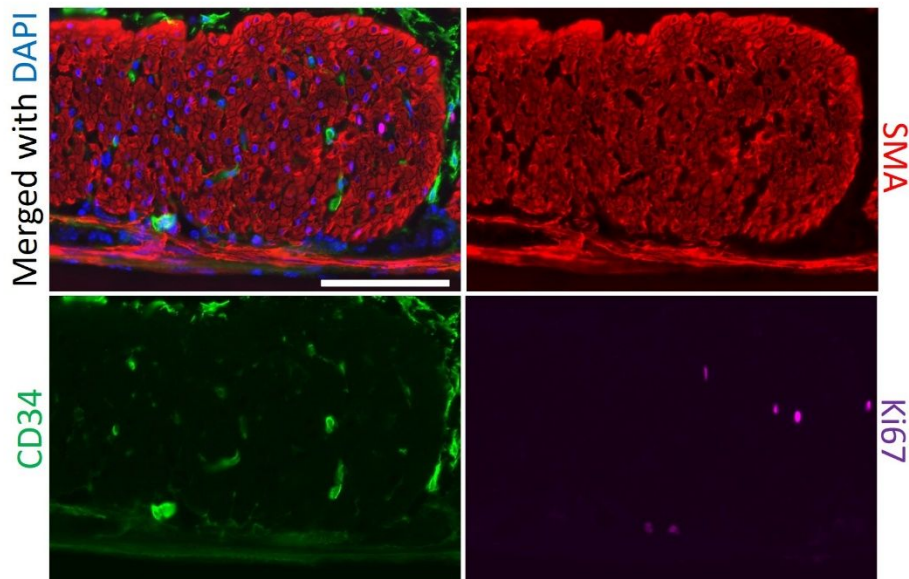


Figure S5. Low levels of proliferation in normal gastric smooth muscle cells.

Immunofluorescence images of the gastric muscularis propria of an *Lrig1^{CreERT2/+}; Braf^{LSL-V600E/+}* mouse after control corn oil injection stained for CD34 (green), SMA (red) and Ki67 (magenta). Images of the greater curvature of the gastric body were captured. Nuclei were stained with DAPI. Scale bar, 100 μ m.

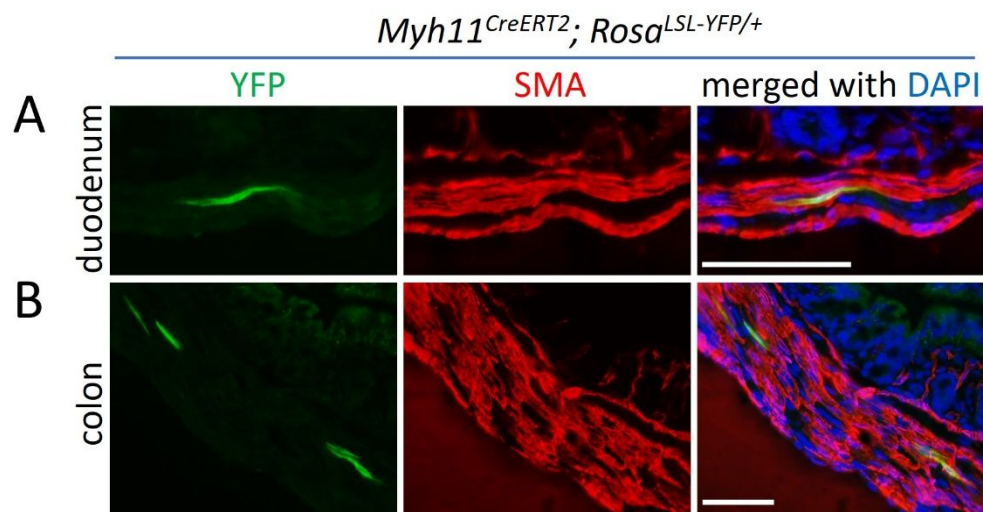


Figure S6. Immunofluorescence images of tissue sections from a *Myh11^{CreERT2}; Rosa^{LSL-YFP/+}* mouse that did not receive tamoxifen. (A) duodenal and (B) colonic sections from a two-week-old mouse stained for YFP (green) and SMA (red). Nuclei were stained with DAPI. Scale bars, 100 μ m.

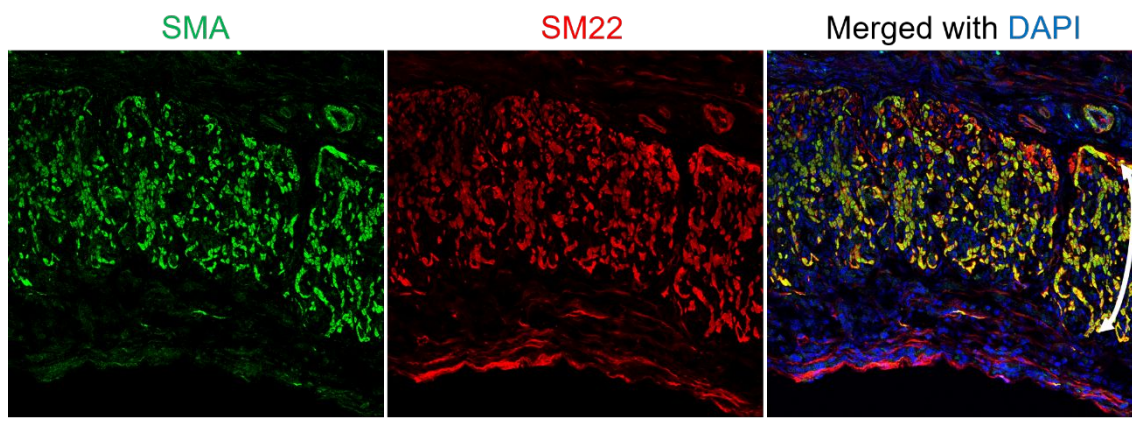


Figure S7. Decreased expression of smooth muscle markers in *Myh11^{CreERT2}; Braf^{SL-V600E/+} mouse.* Immunofluorescence images of gastric a tissue section from a *Myh11^{CreERT2}; Braf^{SL-V600E/+} mouse* two weeks after tamoxifen injection (0.1 mg per mouse) stained for SMA (green) and SM22 (red). Double-headed arrow indicates circular muscular layer. Nuclei were stained with DAPI. Scale bar, 100 μ m.

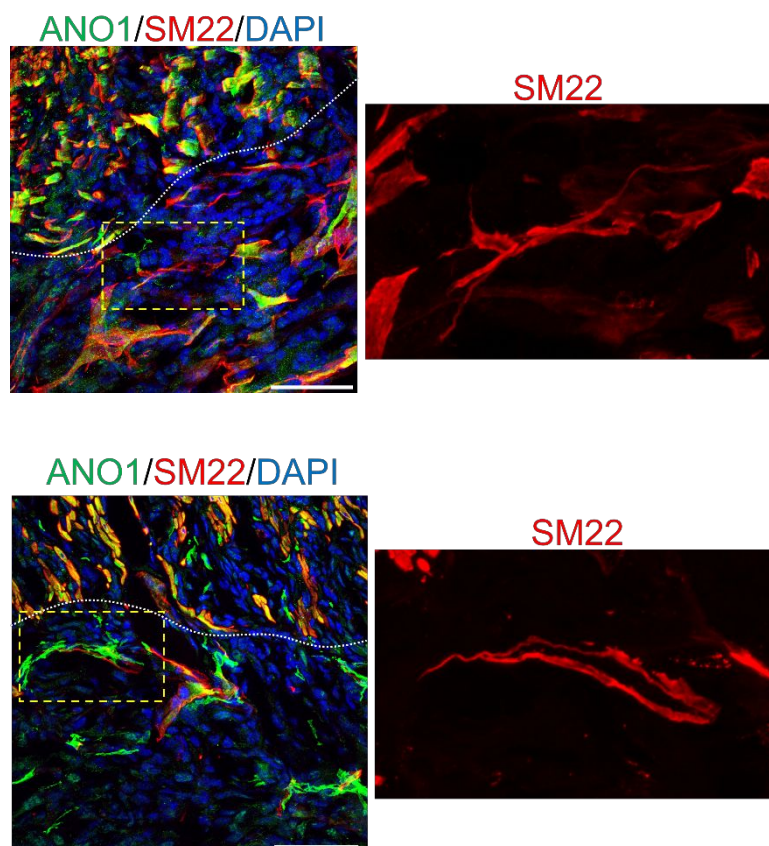
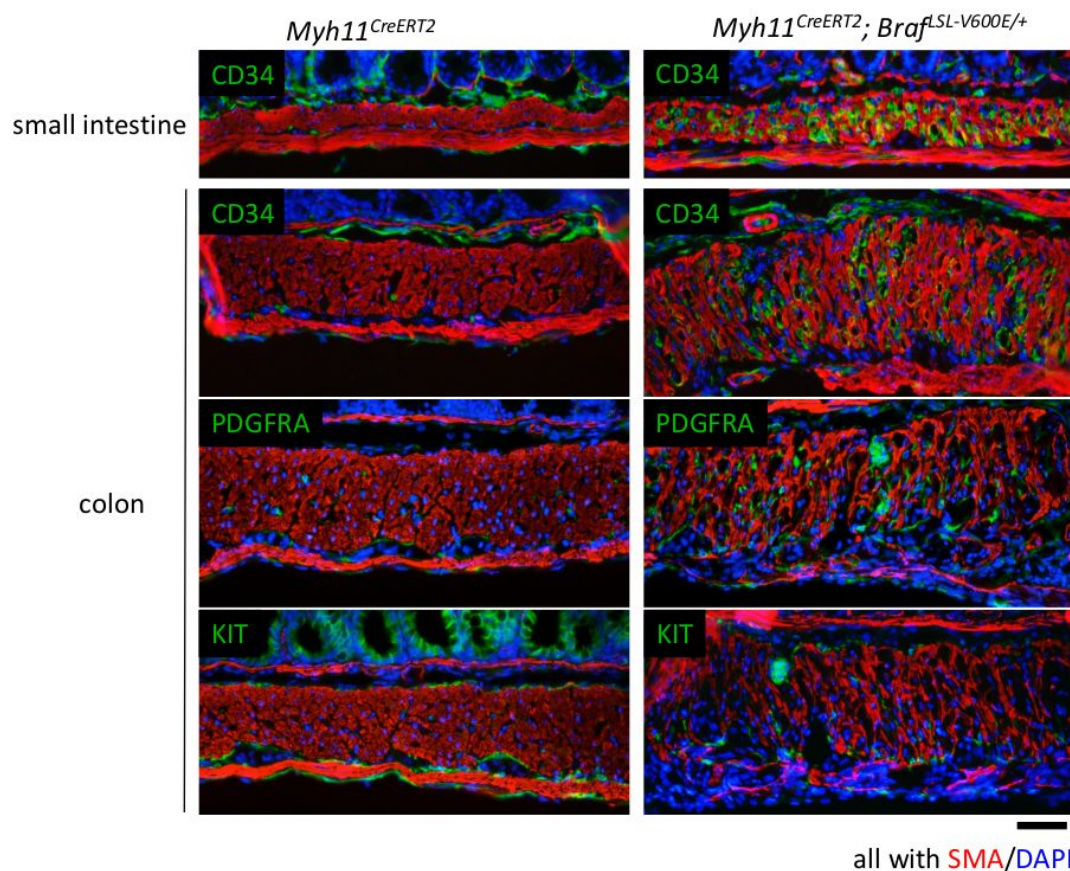


Figure S8. Gastric smooth muscle cells with ICC-like morphology in a *Myh11*^{CreERT2}; *Braf*^{SL-V600E/+} mouse. Z-stacked immunofluorescence confocal images of gastric tissue sections from a *Myh11*^{CreERT2}; *Braf*^{SL-V600E/+} mouse two weeks after tamoxifen injection (0.1 mg per mouse) stained for ANO1 (green) and SM22 (red). White dotted lines indicate the boundary of the inner circular (upper side) and the outer longitudinal (lower side) smooth muscular regions. Areas enclosed in yellow dotted boxes of the left panels are enlarged in the right panels. Nuclei were stained with DAPI. Scale bar, 50 μ m



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Figure S9. Small intestinal and colonic lesions in *Myh11^{CreERT2}* and *Myh11^{CreERT2}; Brafl^{SL-V600E/+}* mouse. Immunofluorescence images of small intestinal (ileum) and colonic (proximal colon) tissue sections from *Myh11^{CreERT2}* and *Myh11^{CreERT2}; Brafl^{SL-V600E/+}* mice each given 0.1 mg tamoxifen at six weeks of age and sacrificed two weeks later. Tissue sections were stained for CD34 (green), PDGFRA (green), KIT (green) and SMA (red). Nuclei were stained with DAPI. Scale bars, 100 μ m.