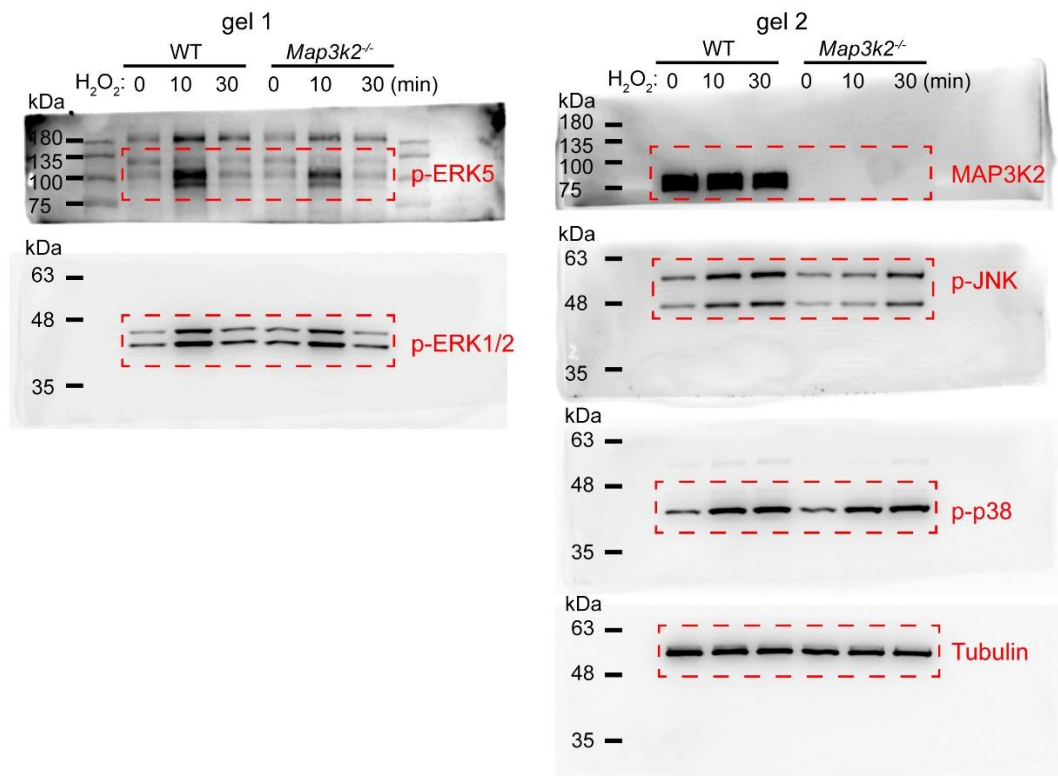
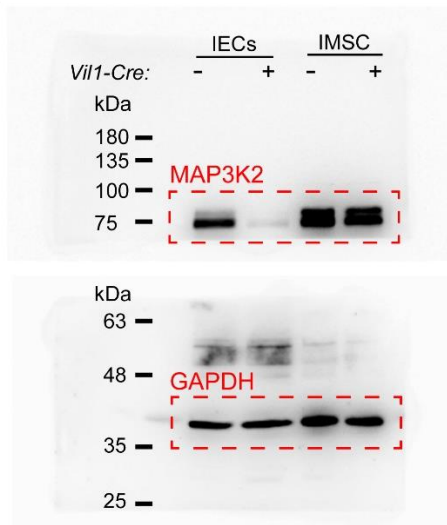
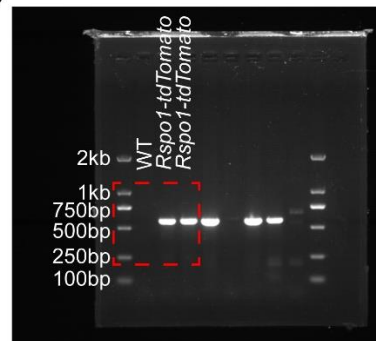

Supplementary information

MAP3K2-regulated intestinal stromal cells define a distinct stem cell niche

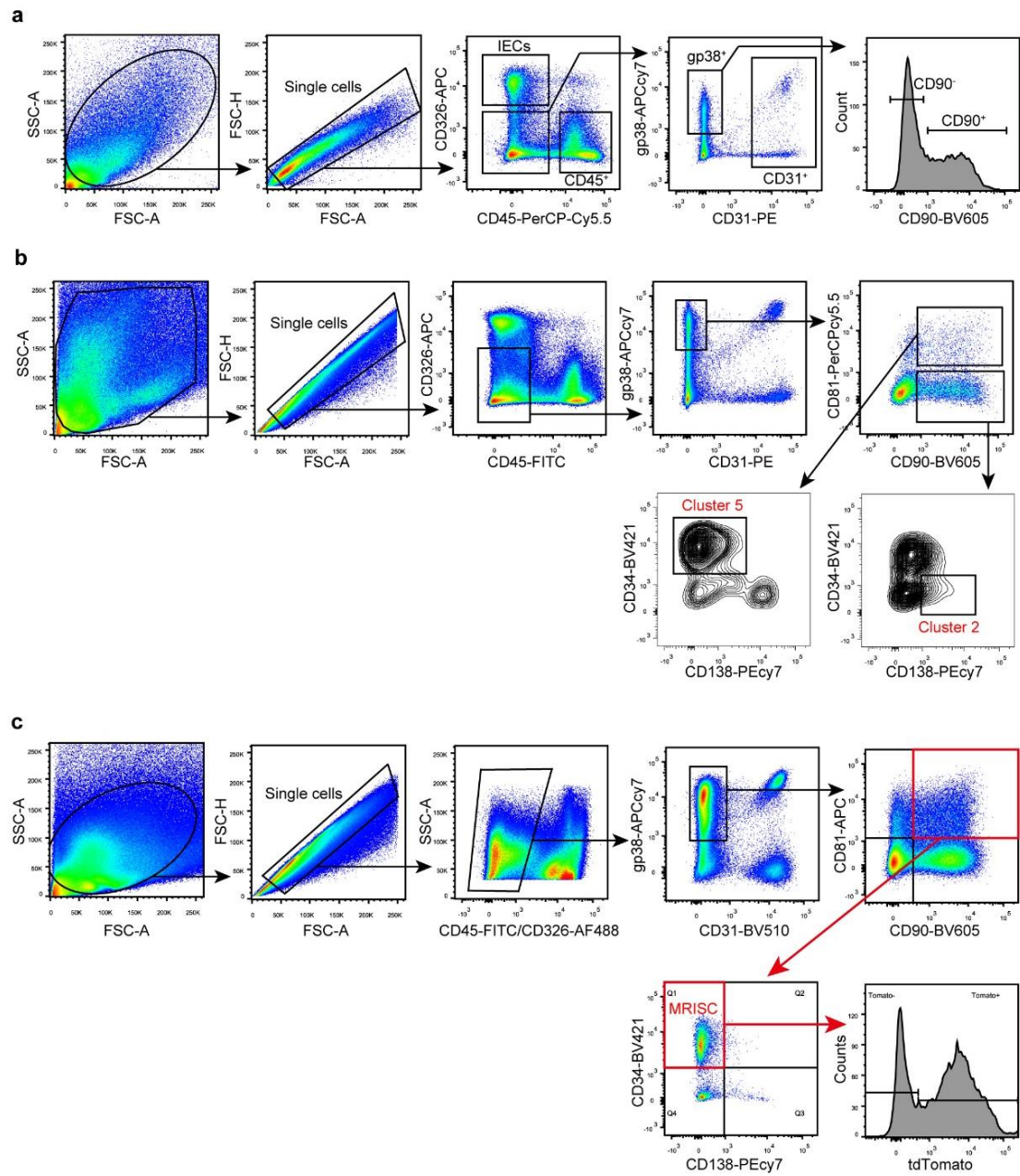
In the format provided by the
authors and unedited

a**b****c**

Supplementary Fig. 1. (see next page for caption)

Supplementary Fig. 1. The uncropped scans of the blots and gel.

a. The uncropped scans of the blots used in **Fig. 4j**. The dotted red boxes indicate cropped areas used in the figure. The same samples were used for gel 1 and gel 2. For gel 1, membrane was cut into two blots (upper blot: 75-180 kDa, lower blot: 35-63 kDa as indicated). The upper blot was blotted for p-ERK5 (upper) and the lower blot for p-ERK1/2. For gel 2, membrane was cut into two blots (upper blot: 75-180 kDa, lower blot: 35-63 kDa as indicated). The upper blot was blotted for MAP3K2 and the lower blot for p-JNK, p-p38 and Tubulin as indicated. **b.** The uncropped scans of the blots used in **Extended Data Fig. 2k**. The dotted red boxes indicate cropped areas used in the figure. Membrane was cut into two blots (upper blot: 75-180 kDa, lower blot: 35-63 kDa as indicated). The upper blot was blotted for MAP3K2 and the lower blot for GAPDH. **c.** The uncropped scans of the gel used in **Extended Data Fig. 7d**. The dotted red box indicates cropped area used in the figure.



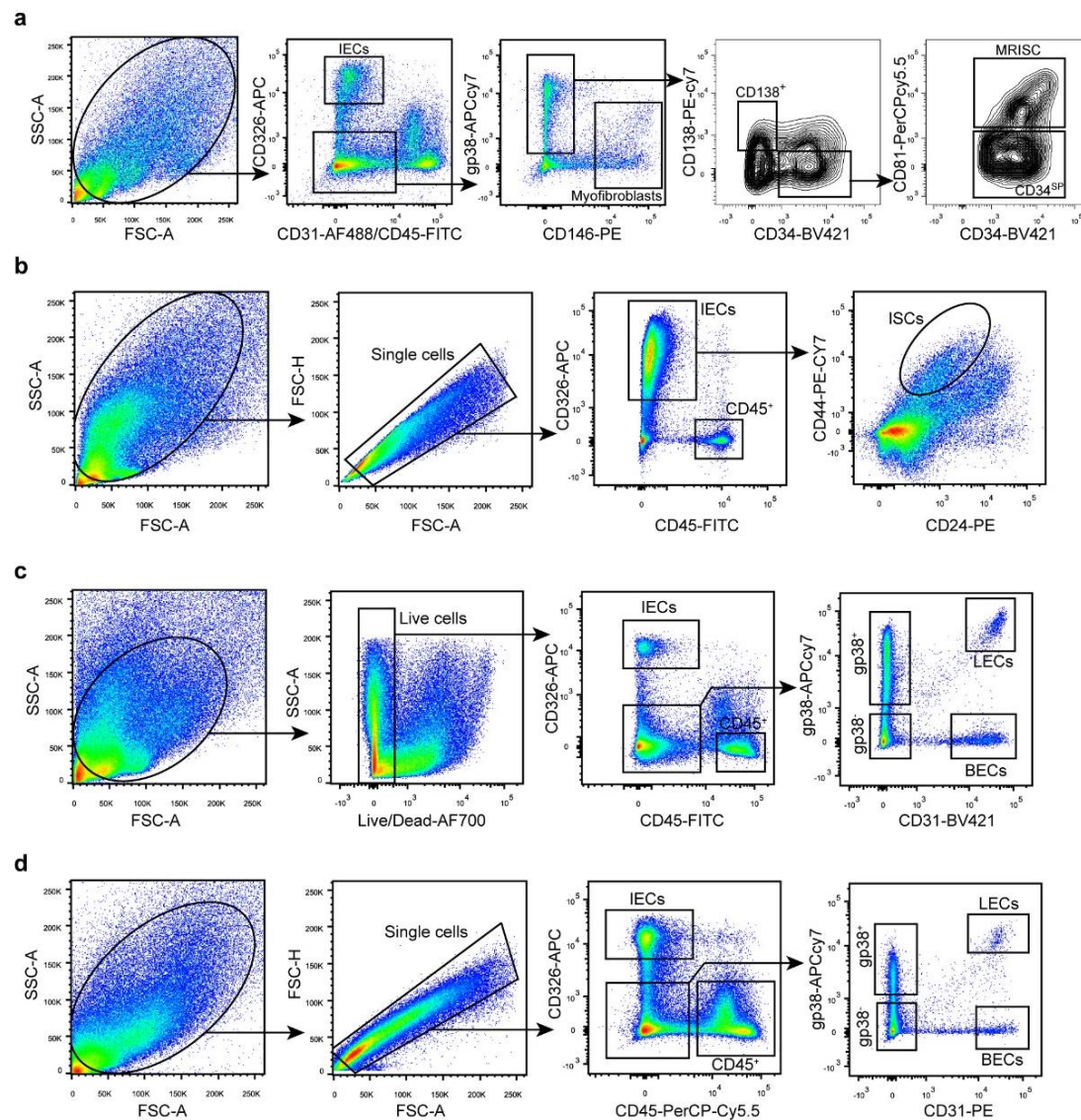
Supplementary Fig. 2. (see next page for caption)

Supplementary Fig. 2. The gating strategies for FACS analysis and sorting for Figures 3a, 3d, 3h, 3j; 4a, 4c, 4f, 4l; and Extended Data Figures 4a-c, 4e; 6d-f; 8a, 8c; and 10e, 10f.

a. Gating strategy for FACS sorting of IECs, CD45⁺, gp38⁺ stroma, CD31⁺ cells, CD90⁻ and CD90⁺ stromal cells from the colons of mice for qRT-PCR and RNA-Seq presented in Figures 3a, 3d; Extended Data Figures 4a-c, 4e.

b. Gating strategy for FACS sorting and analysis of Cluster 2 and Cluster 5 (MRISC) stromal cells from the colon of mice for qRT-PCR, organoid culture, and orthotopic surgical delivery presented in Figures 3h; 4a, 4c, 4f, 4l; Extended Data Figures 6d-f; 8a, 8c; 10e, 10f.

c. Gating strategy for FACS analysis of tdTomato⁺ cells in colon MRISC from *Rspo1*-tdTomato reporter mice presented in Figure 3j.



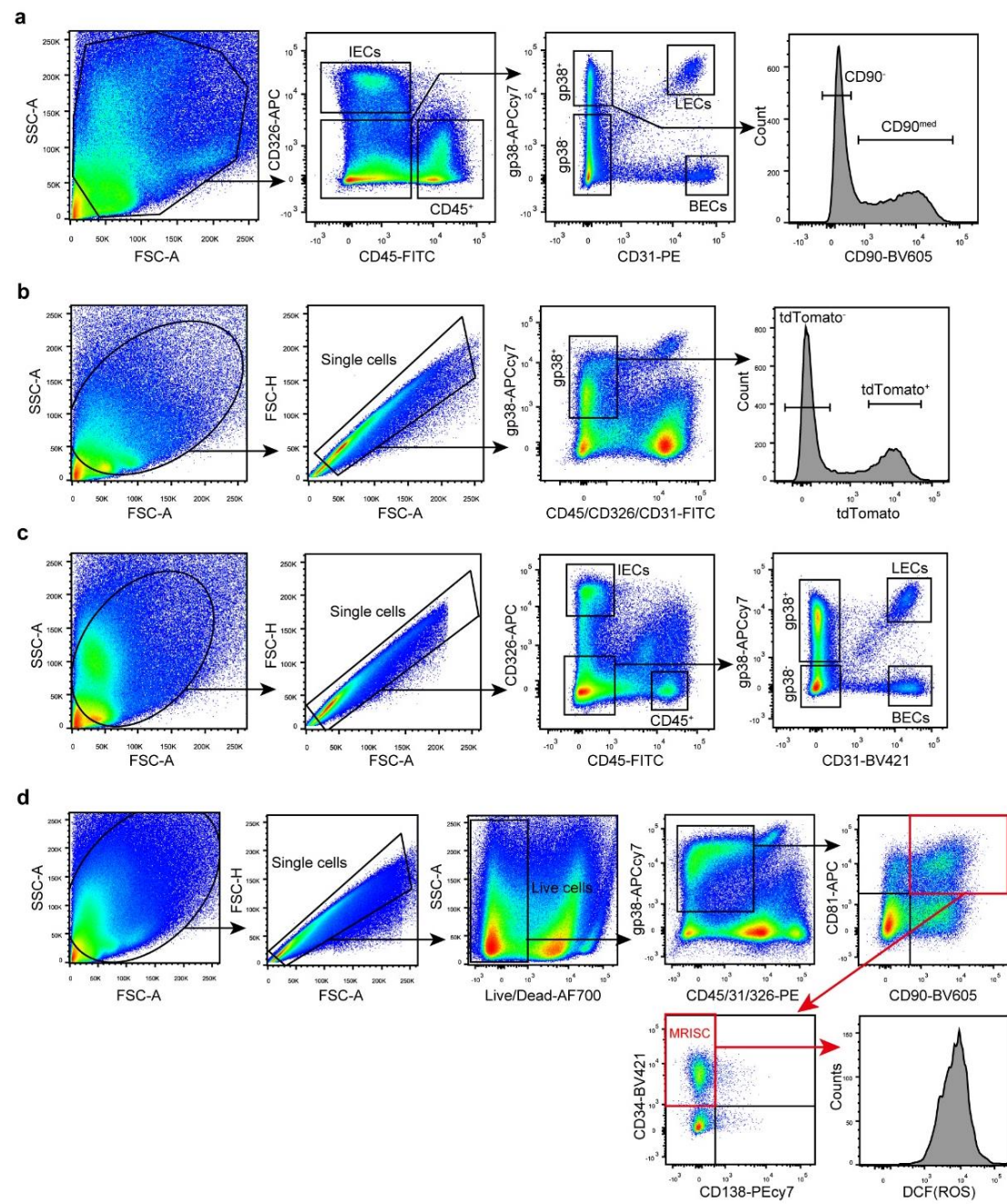
Supplementary Fig. 3. The gating strategies for FACS analysis and sorting for Fig. 4d and Extended Data Figure 1h; 4d; 5d; and 9a.

a. Gating strategy for FACS sorting of IECs, Myofibroblasts, CD138⁺, CD34^{SP} and MRISC cells from the colon of mice for ATAC-seq presented in Figure 4d, Extended Data Figure 9a.

b. Gating strategy for FACS analysis of intestinal stem cells in the colon of mice presented in Extended Data Figure 1h.

c. Gating strategy for FACS analysis of tdTomato⁺ cells in the colon of *Col1a2-Cre^{ERT2}:R26-loxP-stop-loxP-tdTomato* mice presented in Extended Data Figure 4d.

d. Gating strategy for FACS analysis of CD90 expression in the colon of mice presented in Extended Data Figure 5d.



Supplementary Fig. 4. (see next page for caption)

Supplementary Fig. 4. The gating strategies for FACS analysis and sorting for Extended Data Fig. 7a, 7e, 7g; and 10i.

- a.** Gating strategy for FACS analysis of CD34⁺CD81⁺ cells in the colon of mice presented in Extended Data Figure 7a.
- b.** Gating strategy for FACS sorting of tdTomato⁺ intestinal stromal cells in *Rspo1*-tdTomato reporter mice presented in Extended Data Figure 7e.
- c.** Gating strategy for FACS analysis of tdTomato⁺ cell in the colon of *Rspo1*-tdTomato reporter mice presented in Extended Data Figure 7g.
- d.** Gating strategy for FACS analysis of the ROS level of MRISC in the colon of mice presented in Extended Data Figure 10i.