Supplementary Materials and Methods

Primary Myofibroblast Immunostaining

Colonic stromal cells were extracted from α-SMA–RFP transgenic mice (a gift from Dr David Brenner).[1] This transgenic line is well validated and has been backcrossed to C57BL/6 for more than 20 generations. Colonic stromal cells were harvested and cultured as previously described in Materials and Methods. After 2 weeks, colonic stromal cells were plated onto glass chamber slides, cultured overnight, and fixed using 4% paraformaldehyde. Cells were immunostained for vimentin using 1:300 anti-vimentin (catalog no. 2707-1; Epitomics, Burlingame, CA) and an anti-rabbit Alexa Fluor 488 secondary (catalog no. A11008; Life Technologies, Grand Island, NY) using a standard immunocytofluorescence approach. Cell nuclei were stained using DAPI. In addition, a no-primary antibody control was performed to confirm the specificity of the anti-vimentin immunostaining. One hundred cells were counted to determine the relative expression of α-SMA and vimentin.

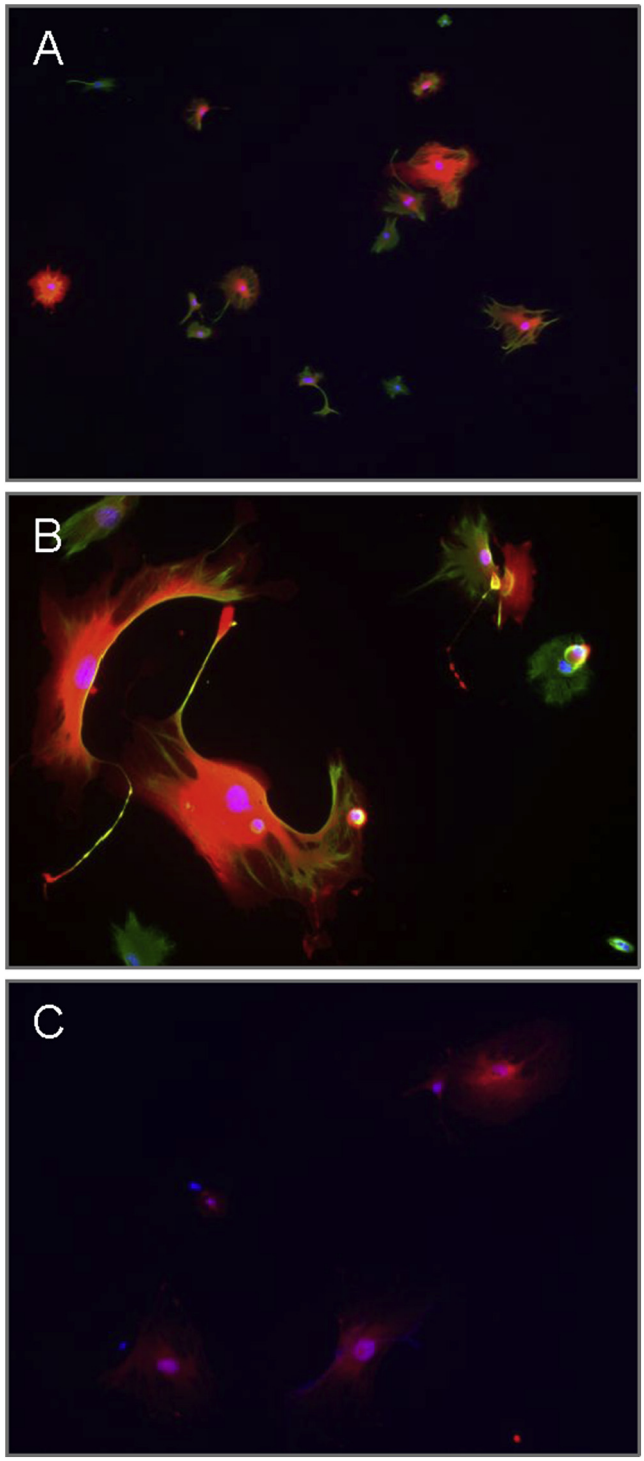
Supplementary Results

Primary Stromal Cell Cultures Are Predominantly Myofibroblasts Under Subculture Conditions

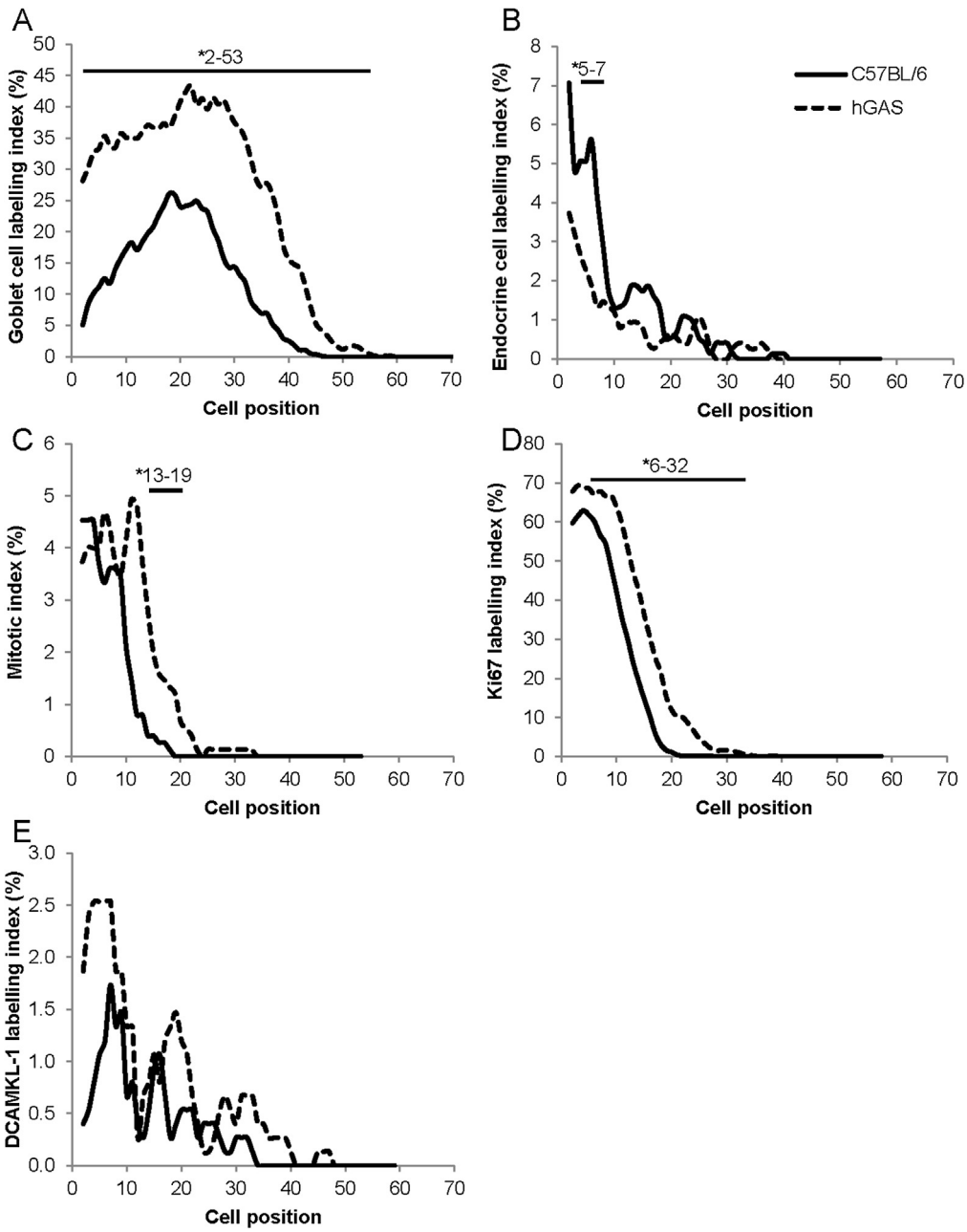
The primary stromal culture method we have used in this study also results in the extraction of mesenchymal stem cells. After subculture, we observed an increase in α-SMA expression over time (a well-accepted arbiter of myofibroblastic differentiation). The distinction between mesenchymal stem cells and differentiated mesenchymal cells on the basis of α-SMA expression, however, is less well established, and α-SMA is believed by some to be a marker of mesenchymal stem cells.[2] We therefore assessed the coexpression of α-SMA with vimentin to determine the percentage of cells that are myofibroblasts under the subculture conditions used in this study. We determined that 100% of the cells were positive for vimentin and ~70% were also positive for expression of α-SMA, suggesting that the majority of the cells are myofibroblasts (Supplementary Figure 1).

Supplementary References

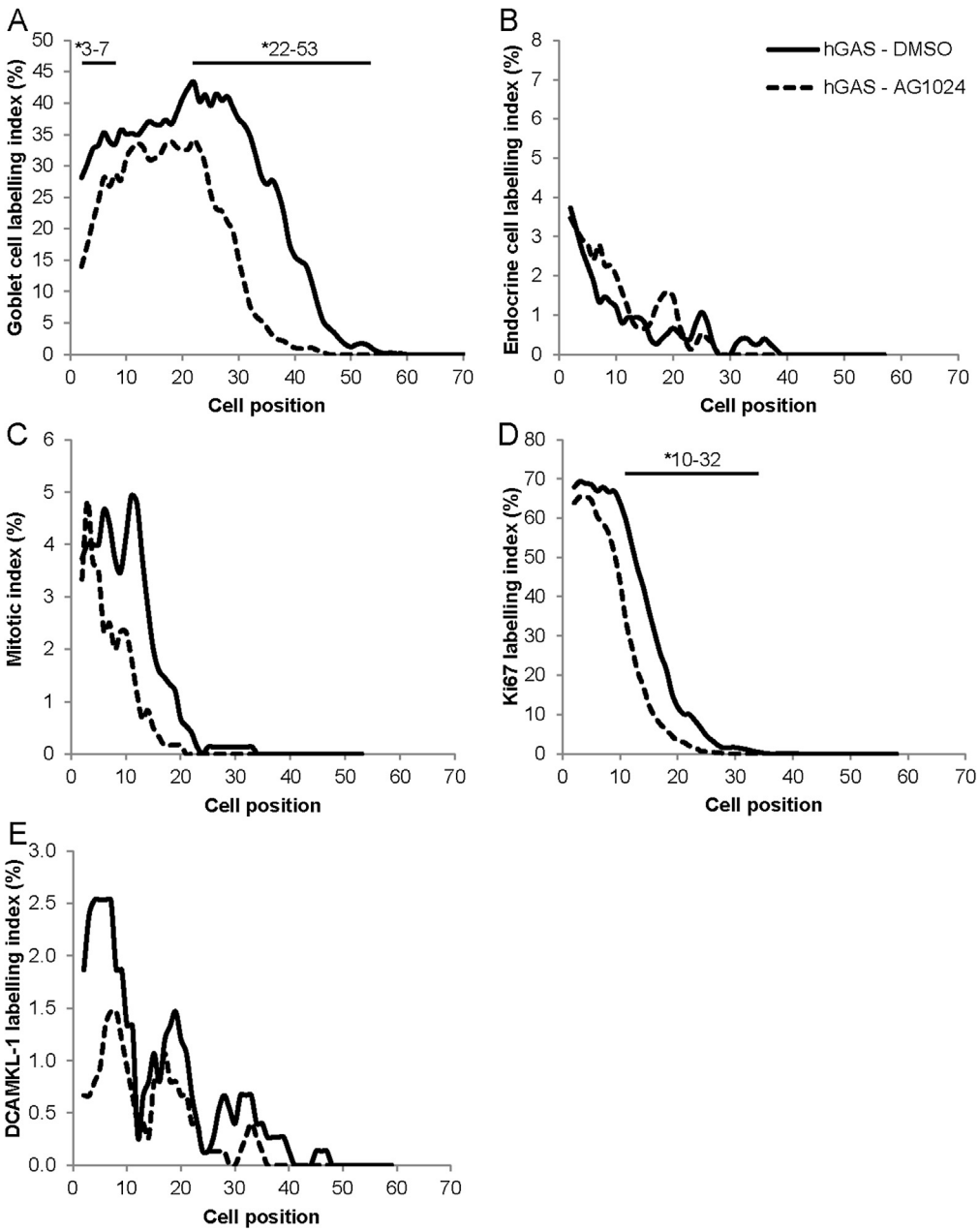
1. Magness ST, Bataller R, Yang L, et al. A dual reporter gene transgenic mouse demonstrates heterogeneity in hepatic fibrogenic cell populations. Hepatology 2004;40:1151–1159.
2. Grcevic D, Pejda S, Matthews BG, et al. In vivo fate mapping identifies mesenchymal progenitor cells. Stem Cells 2012;30:187–196.



Supplementary Figure 1. Colonic stromal cultures showing that the majority of colonic cells cultured were myofibroblasts as defined by immunostaining (coexpression of vimentin and α-SMA). α-SMA–RFP expression (*red*), vimentin (*green*), and nuclei (*blue*) are shown at (*A*) 100× and (*B*) 200× original magnification. (*C*) No primary antibody control confirming the specificity of anti-vimentin immunostaining.



Supplementary Figure 2. Distal colonic cell positional labeling indices of (*A*) goblet cells, (*B*) CgA-positive cells, (*C*) mitotic figures, (*D*) Ki67-positive cells, and (*E*) DCAMKL-1–positive cells in DMSO-treated C57BL/6 (*solid line*) and hGAS (*dotted line*) mice. n = 5 mice/group. The *asterisk* indicates significantly different cell positions by modified median test.



Supplementary Figure 3. Distal colonic cell positional labeling indices of (*A*) goblet cells, (*B*) CgA-positive cells, (*C*) mitotic figures, (*D*) Ki67-positive cells, and (*E*) DCAMKL-1–positive cells in hGAS mice treated with DMSO (*solid line*) or AG1024 (*dotted line*). n = 5 mice/group. The *asterisk* indicates significantly different cell positions by modified median test.