Stem Cell Reports, Volume *7*

Supplemental Information

Myofibroblasts Derived from Hepatic Progenitor Cells Create the Tumor Microenvironment

Sayaka Sekiya, Shizuka Miura, Kanae Matsuda-Ito, and Atsushi Suzuki

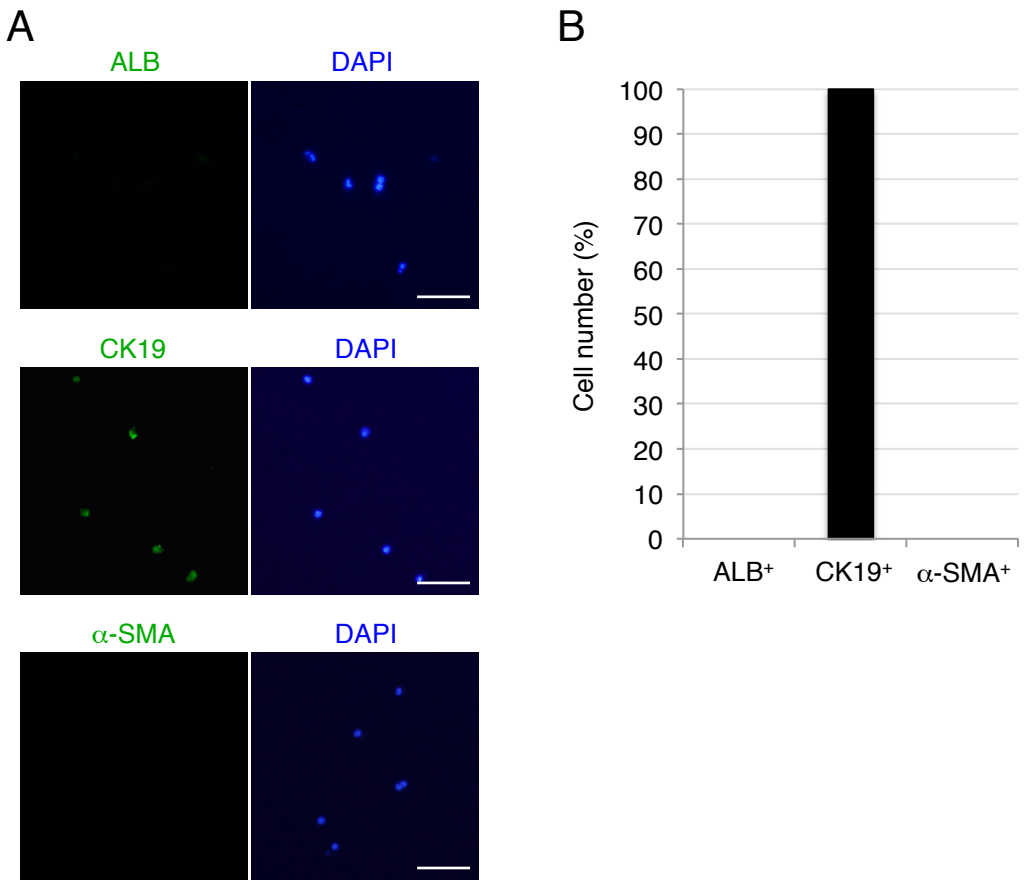

Figure S1 (related to Figure 1). α-SMA$^+$ cells are not contained in the CD133$^+$ CD45$^-$ TER119$^-$ cells isolated from the chronically injured adult mouse liver. (A) Immunofluorescence staining of ALB, CK19, and α-SMA was conducted for CD133$^+$ CD45$^-$ TER119$^-$ cells soon after their isolation from the livers of DDC-treated mice by flow cytometry. DNA was stained with DAPI. Scale bars: 50 μm. (B) The percentages of ALB$^+$, CK19$^+$, and α-SMA$^+$ cells in the isolated CD133$^+$ CD45$^-$ TER119$^-$ cells are shown. CK19 was expressed, while ALB and α-SMA were not expressed, in all CD133$^+$ CD45$^-$ TER119$^-$ cells. The data represent means ± SD of three independent experiments (n = 3).

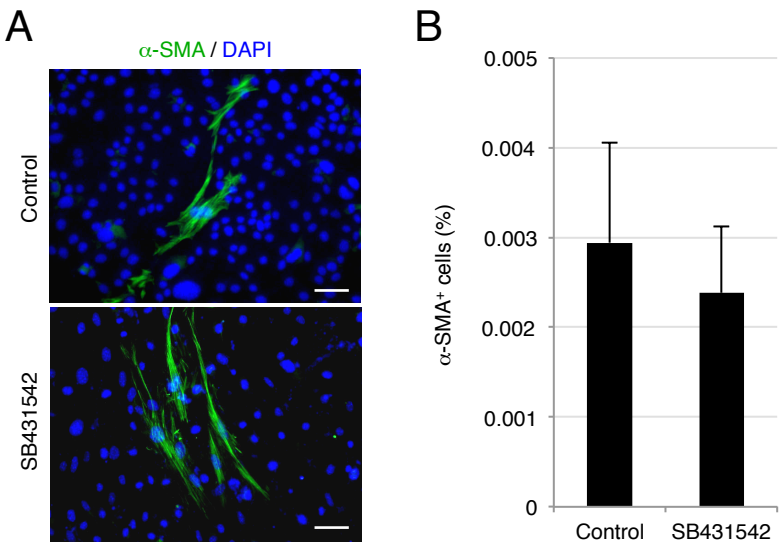

Figure S2 (related to Figure 1). HPCs produce myofibroblasts even in culture with an inhibitor of TGF-β signaling. (A) Immunofluorescence staining of α-SMA was conducted for cells in a clonal culture of HPCs in the presence or absence (control) of 2 μM SB431542. DNA was stained with DAPI. Scale bars: 50 μm. (B) The percentages of cells immunoreactive for α-SMA in a HPC clone cultured with or without SB431542 were calculated after counting ~7 × 10^5 cells in individual culture dishes. The data represent means ± SD of three technical replicates (*n* = 3).

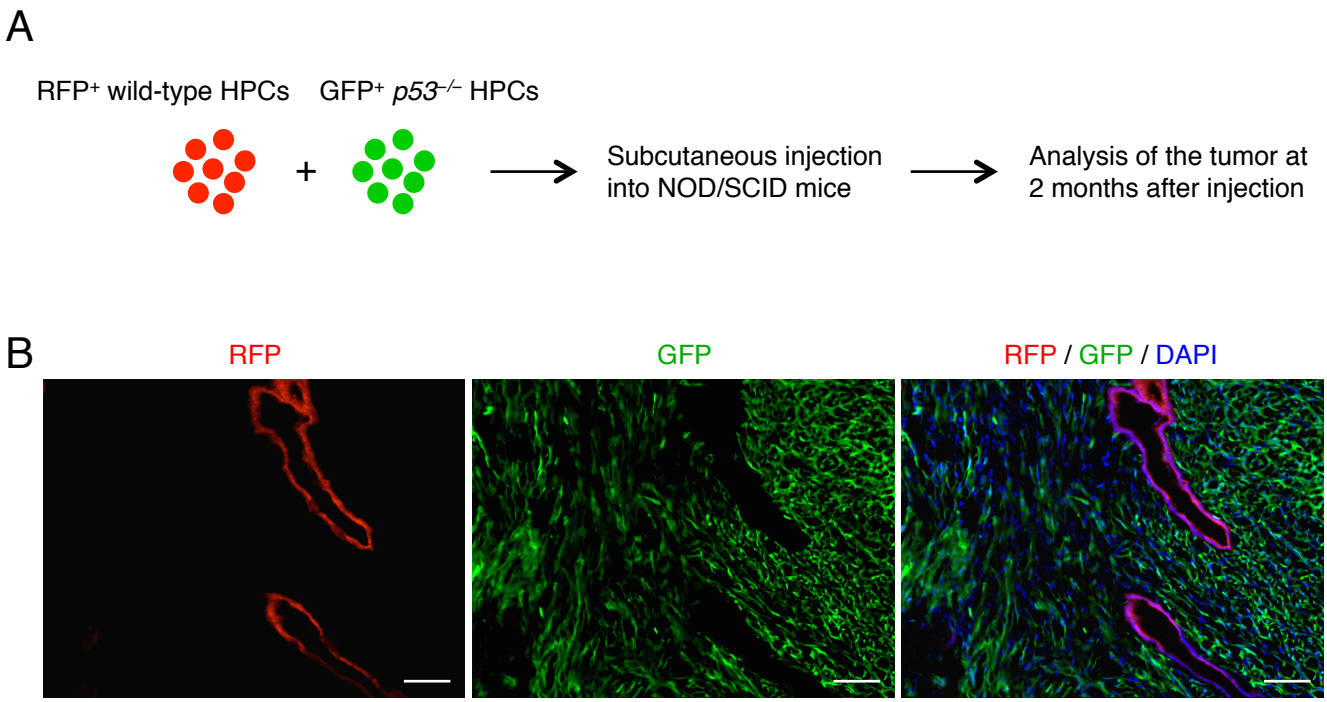

Figure S3 (related to Figure 5). Wild-type HPCs generate only a small number of ductal structures surrounded by *p53*$^{-/-}$ HPC-derived mesenchymal tissues in the tumor. (A) Experimental procedure to examine the behavior of wild-type HPCs in the tumors formed by *p53*$^{-/-}$ HPCs. HPC clones derived from the chronically injured livers of wild-type and *p53*$^{-/-}$ mice were mixed and injected into NOD/SCID recipient mice, after marking the cells by expression of red fluorescent protein (RFP) and GFP, respectively. The mixture of donor cells consisted of 1.5×10^7 RFP$^+$ wild-type HPCs and 1.5×10^7 GFP$^+$ *p53*$^{-/-}$ HPCs. (B) Representative fluorescence images of RFP$^+$ and GFP$^+$ tissues in tumors are shown. DNA was stained with DAPI. Scale bars: 100 μm.

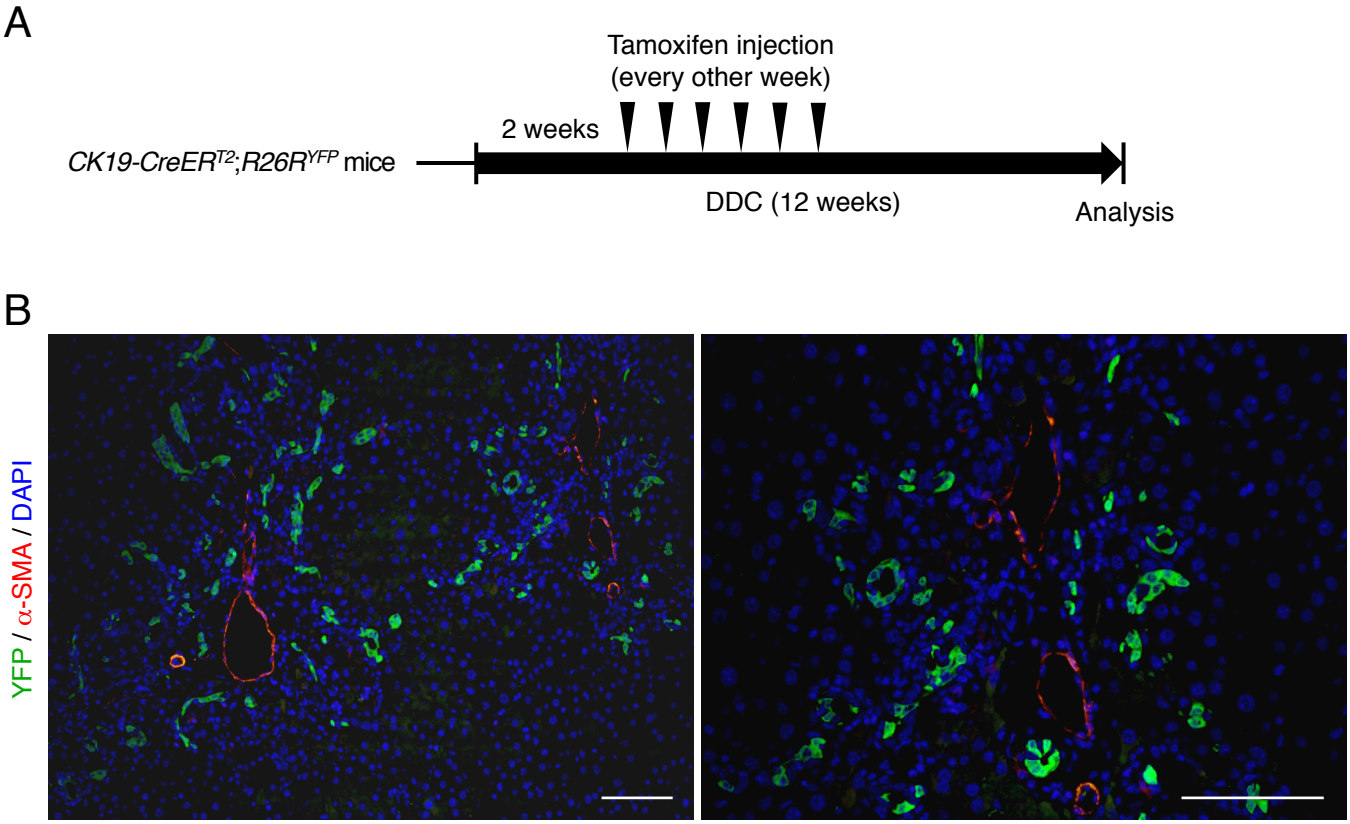

Figure S4 (related to Figure 5). Absence of HPC-derived myofibroblasts in the chronically injured adult mouse liver. (A) Experimental procedure to follow the fate of HPCs in the chronically injured liver. Two weeks after initiation of DDC administration, CK19^{+} HPCs and cholangiocytes were marked with the expression of yellow fluorescent protein (YFP) by intraperitoneal injection of Tamoxifen into *CK19-CreER^{T2};R26R^{YFP}* mice. DDC was continuously administered to mice for 12 weeks until the liver was analyzed. (B) Co-immunofluorescence staining of YFP with α-SMA was conducted for the chronically injured liver of Tamoxifen-injected *CK19-CreER^{T2};R26R^{YFP}* mice after 12 weeks of DDC treatment. Representative images are shown. DNA was stained with DAPI. Scale bars: 100 μm.