Supplemental Fig. S1 Purity assessment and collagen content of cells isolated from explants and collagenase digests. (A) Cells isolated from explants and collagenase digests onto collagen-coated silicone substrates of different stiffness were assessed by Western blotting for markers of endothelial (VE-cadherin), smooth muscle (desmin), and epithelial cell (E-cadherin) contamination. (B) Samples from epithelium, smooth muscle and aorta served as antibody controls. Fibroblast cultures were positive for collagen and the mesenchymal protein vimentin but not for any other marker. Cells retrieved by tissue explanation had a higher level of collagen production than isolated by collagen digestion. Due to ease of isolation and increased capacity for collagen production, all further experiments were performed using fibroblasts sequestered from tissue explants.
Supplemental Fig. S2 Myofibroblast potential of fibroblasts exclusively cultured on soft substrates and mechanical priming effects of TCP. In order to assess the effect of mechanical priming using plastic as a fibrotic model (as opposed to silicone), rat lung fibroblasts were seeded at a concentration of 2,500 cells/cm\(^2\) onto collagen-coated silicone substrates and cultured for four passages to model: healthy lung tissue (5 kPa), mature fibrotic tissue (TCP), the migration of cells from soft to fibrotic-stiff zones (5T TCP), and the migration of cells out of stiff fibrotic-zones to soft tissue (TCP T5). Fourteen days (two passages) after the divergence, lung fibroblasts were assessed for myofibroblast presence as by assaying α-SMA expression via Western blot analysis. As seen previously, cells primed on stiff surfaces (TCP T5) maintained the pro-fibrotic myofibroblast phenotype and cells primed on soft surfaces (5T TCP) maintained a reduced level of fibrotic activity even 14 days after the removal of the stiffness stimulus.
Supplemental Fig. S3 General schematic view on mechanical priming of fibroblasts. (A) Fibroblasts cultured on soft surfaces and stiff surfaces result in priming of fibroblasts. Expression levels of α-SMA are low and saturate after 2 passages on soft substrates, while α-SMA levels are higher and continued to increase over time on the stiff substrates. After the substrate switch, cells preconditioned on these surfaces will maintain similar levels of fibrotic activity as compared with previous culture conditions. (B) Hours after the mechanical switch of surfaces, lung fibroblasts initially respond to changes on substrate stiffness that correspond with literature. After approximately 24 hours however, fibroblasts will demonstrate effects of mechanical priming.