**SUPPLEMENTAL MATERIAL**

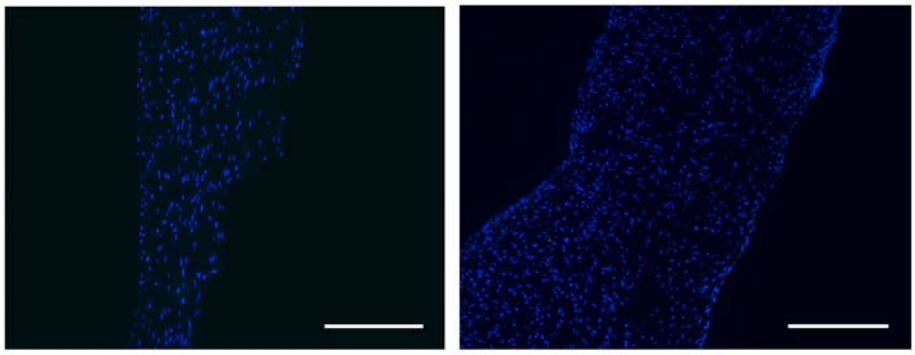

**Figure S1. VICs are quiescent in freshly explanted porcine aortic valve leaflets.** Immunofluorescent staining for αSMA demonstrated the absence of activated VICs in healthy aortic valves. αSMA - green, DAPI – blue. Scale bar represents 200 μm.

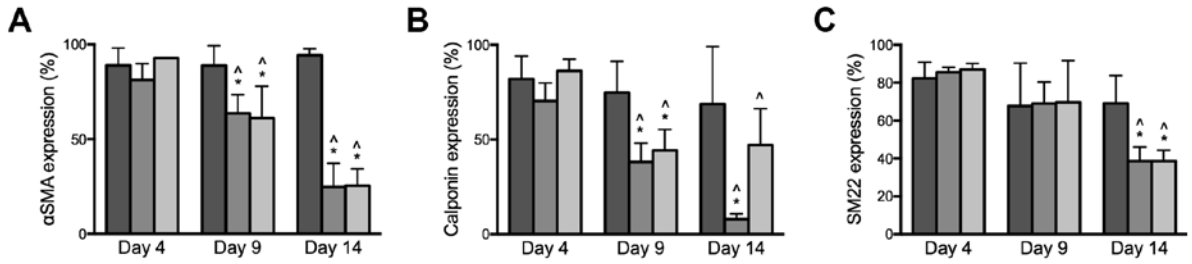


**Figure S2. The percentage of VICs positive for myofibroblastic markers decreases after culture in FIB or FIB-Coll.** Quantification of A) αSMA, B) calponin, and C) SM22 protein expression via flow cytometry. *$p < 0.05$ compared to control VICs at the same timepoint. ^$p < 0.05$ compared to day 4 VICs cultured in the same condition.

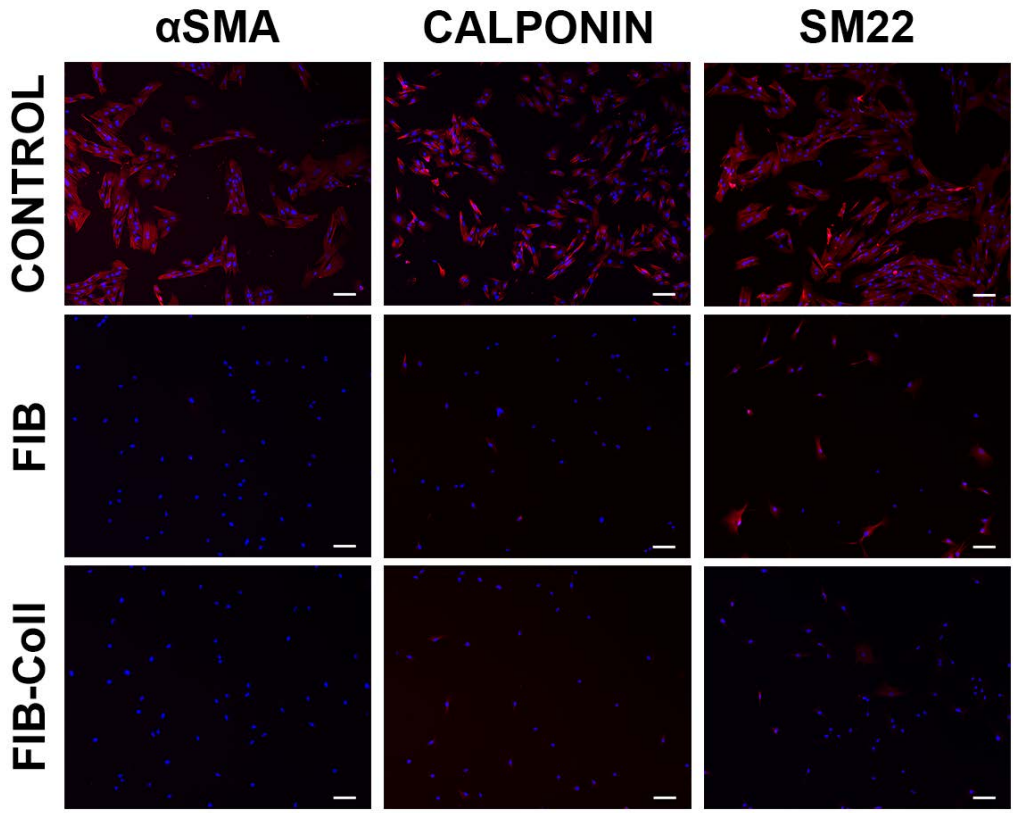


**Figure S3. Myofibroblastic marker expression after 14 days of culture in control, FIB, or FIB-Coll.** Myofibroblastic markers are stained red; nuclei are stained blue with DAPI. Scale bar represents 100 μm.

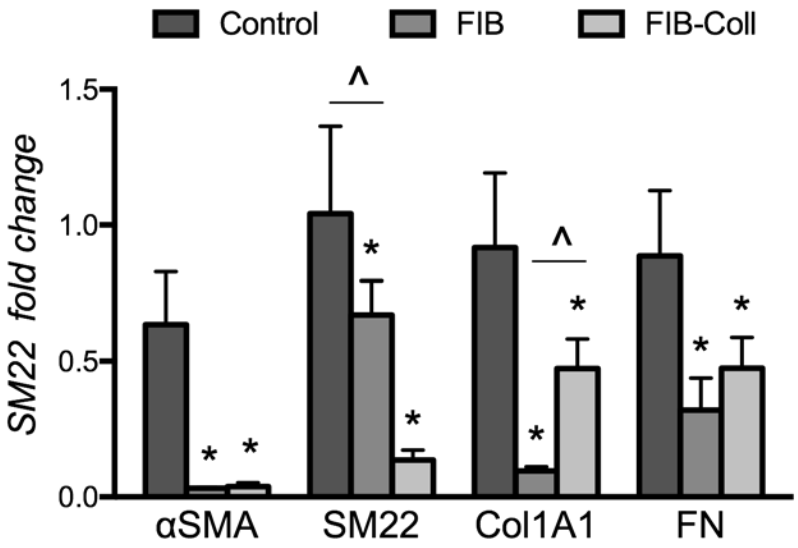


**Figure S4. VICs generated via FIB or FIB-Coll culture remained quiescent for 5 days following removal of FIB or FIB-Coll conditions.** Quantification of myofibroblastic (αSMA and SM22) and ECM (Col1A1 and FN) gene expression. *p <0.05 compared to control, ^p <0.05 for indicated comparison.

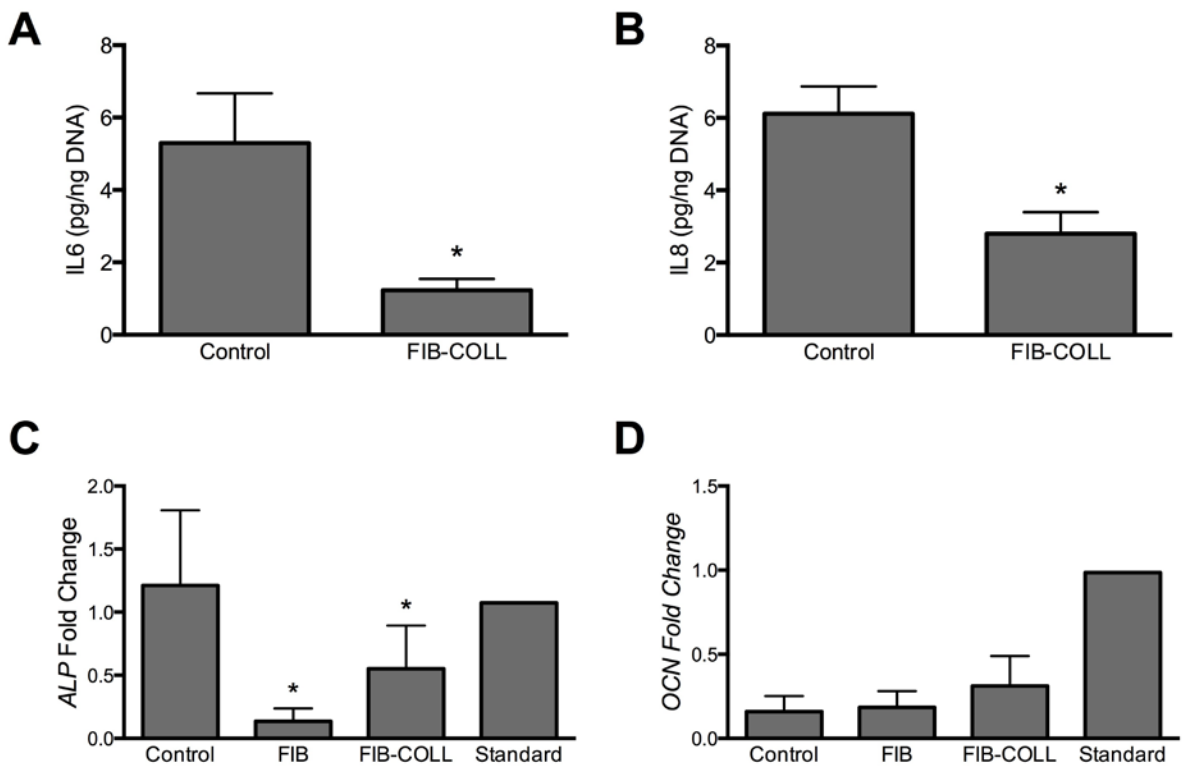


**Figure S5. VICs cultured in FIB-Coll conditions exhibited decreased inflammatory cytokine production and osteogenic activity relative to control.** The inflammatory activity of VICs was assessed using ELISAs for A) IL-6 and B) IL-8. The expression of C) alkaline phosphatase (*ALP*) and D) osteocalcin (*OCN*) genes by VICs in different culture environments was evaluated as a measure of osteogenic activity. The "Standard" is a positive control generated by culture of VICs on uncoated tissue culture polystyrene and treatment with osteogenic medium (DMEM with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, 10 mM β-glycerophosphate, and 50 μg/ml ascorbic acid). * $P < 0.05$ compared to control VICs.

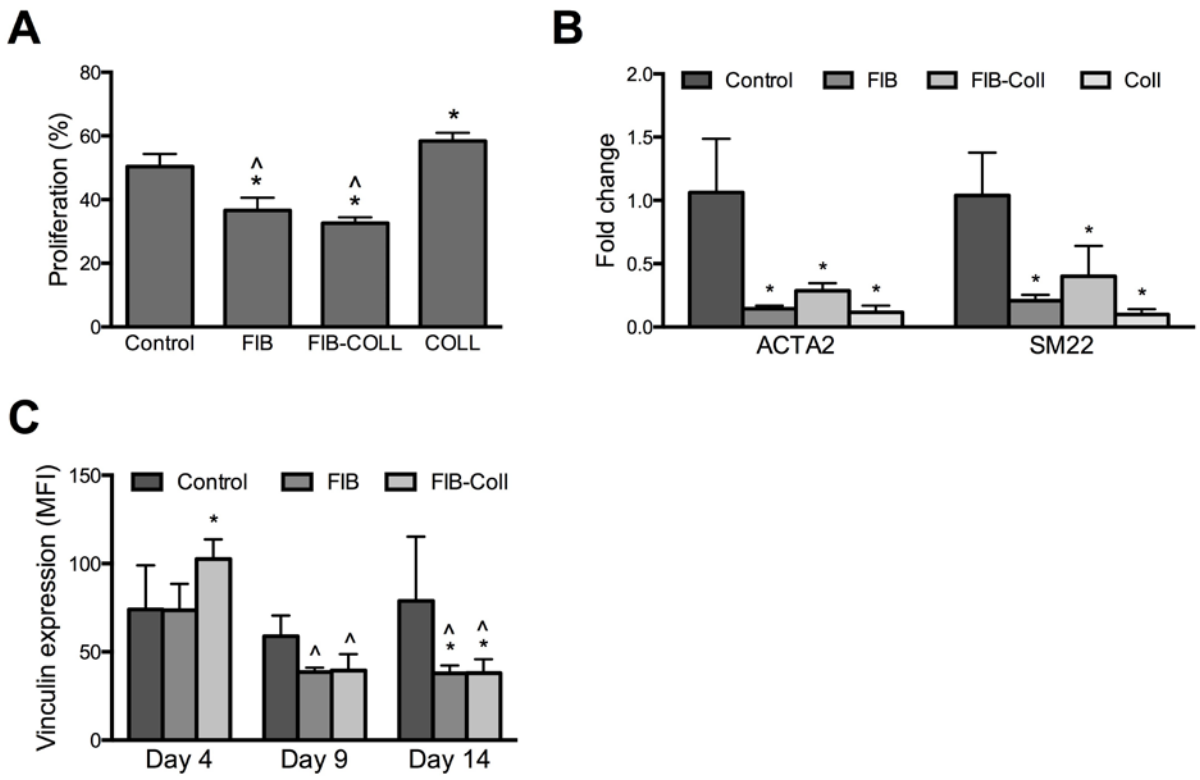


**Figure S6. Culture on collagen alone (Coll) was insufficient to yield a quiescent phenotype.** A) VIC proliferation on Coll was significantly increased relative to the control and conditions containing FIB media, although B) VICs on Coll exhibited decreased expression of contractile proteins relative to the control. * $P < 0.05$ compared to control VICs. ^ $P < 0.05$ compared to VICs cultured in COLL. C) An initial increase in vinculin expression was noted in FIB-COLL conditions at Day 4, but then significantly decreased at later time points. *$P < 0.05$ compared to control VICs at the same time point. ^ $P < 0.05$ compared to day 4 VICs cultured in the same condition.