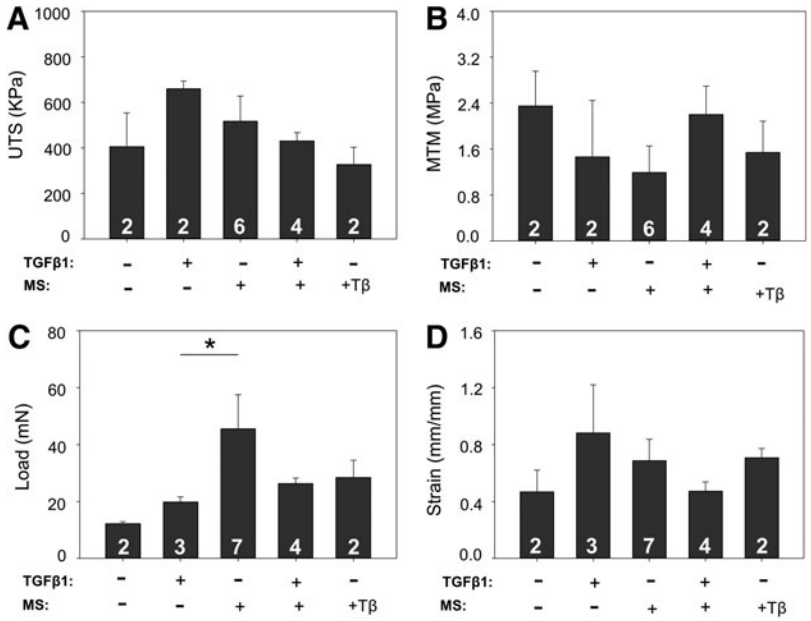
Supplementary Data

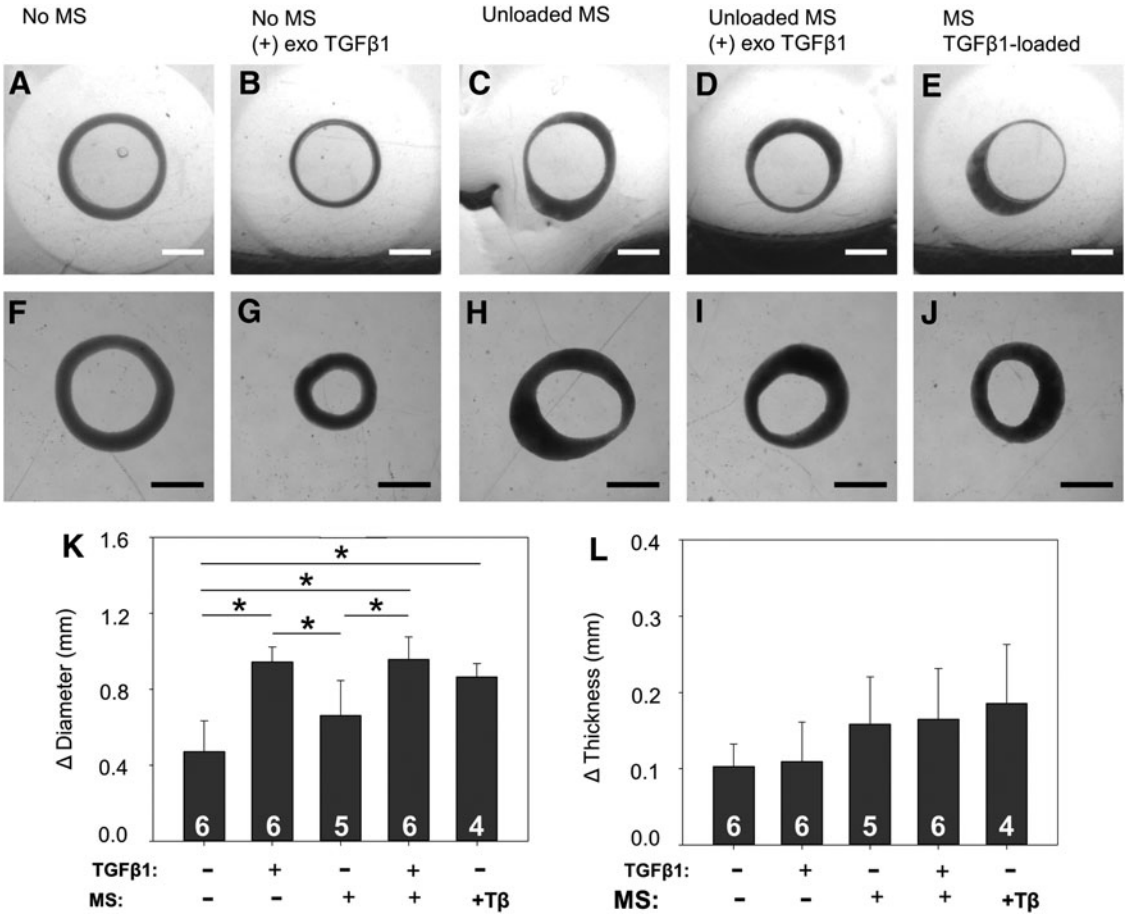


SUPPLEMENTARY FIG. S1. Mechanical properties of rings treated with TGF-β1. Sample groups included untreated rings with no microspheres, rings treated with 10 ng/mL exogenous TGF-β1, rings with unloaded gelatin microspheres untreated or treated with exogenous TGF-β1, and rings with TGF-β1-loaded microsphere incorporation, but no exogenous TGF-β1. Mean values for **(A)** UTS, **(B)** MTM, **(C)** failure load, and **(D)** failure strain were calculated from stress–strain curves for each sample. $*p < 0.05$. Values are mean ± SD, sample size for each group shown on bars. TGF-β1, transforming growth factor beta 1; UTS, ultimate tensile stress; MTM, maximum tangent modulus; SD, standard deviation.

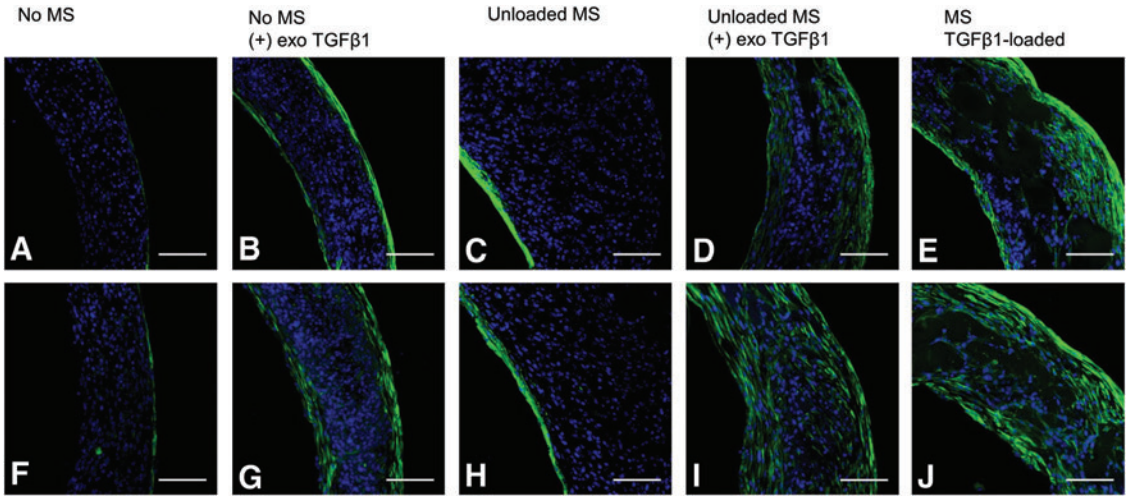
Supplementary Methods

Cell culture

For supplementary experiments, testing was repeated with human coronary artery cells from a different manufacturer (Lonza). These cells were cultured in SmGM-2 complete medium (Lonza), containing 5% fetal bovine serum, 0.1% epidermal growth factor, 0.2% fibroblast growth factor-B, 0.1% insulin, 0.1% gentamicin sulfate, amphotericin-B, and was also supplemented with 1% penicillin–streptomycin (Mediatech).



SUPPLEMENTARY FIG. S2. Effects of TGF-β1 treatment in SMC rings sourced from a different donor. Rings were seeded in growth medium, switched to differentiation medium at day 1, and cultured for a total of 14 days. Rings were photographed **(A–E)** before and **(F–J)** after removal from the agarose posts to measure changes in ring inner diameter and wall thickness. **(A**, **F)** Untreated control ring with no microspheres. **(B**, **G)** Tissue rings treated with 10 ng/mL soluble exogenous (exo) TGF-β1 in the culture medium. Tissue rings with unloaded gelatin microspheres (0.6 mg/million cells) **(C**, **H)** untreated or **(D**, **I)** treated with 10 ng/mL exogenous TGF-β1. **(E**, **J)** Tissue rings with microspheres loaded with TGF-β1, but no exogenous TGF-β1 in the medium. Tissue rings contracted after they were removed from agarose posts, resulting in changes in **(K)** inner diameter and **(L)** thickness. Initial images and thicknesses were measured using the DVT imaging system **(A–E)**, while secondary measurements were taken with the stereoscope **(F–J)**. Scale = 1 mm. $*p < 0.05$. Values are mean ± standard error of mean, sample size for each group shown on bars. SMC, smooth muscle cell.



SUPPLEMENTARY FIG. S3. Effects of TGF-β1 treatment on SMC protein expression in rings self-assembled from human SMCs from a different donor. Rings were seeded in growth medium, switched to differentiation medium at day 1, and cultured for a total of 14 days. **(A**, **F)** Control (untreated) rings. **(B**, **G)** Rings cultured with exogenous TGF-β1 (10 ng/mL) added to the medium. Rings with unloaded microspheres (0.6 mg per million cells) **(C**, **H)** untreated or **(D**, **I)** treated with 10 ng/mL exogenous TGF-β1. **(E**, **J)**, Rings with TGF-β1-loaded microspheres (0.6 mg microspheres per million cells) but without exogenous TGF-β1. Rings were stained for **(A**–**E)** smooth muscle alpha actin and **(F**–**J)** calponin (*green* fluorescence). Nuclei are shown in *blue* (Hoechst). Scale = 100 μm.