

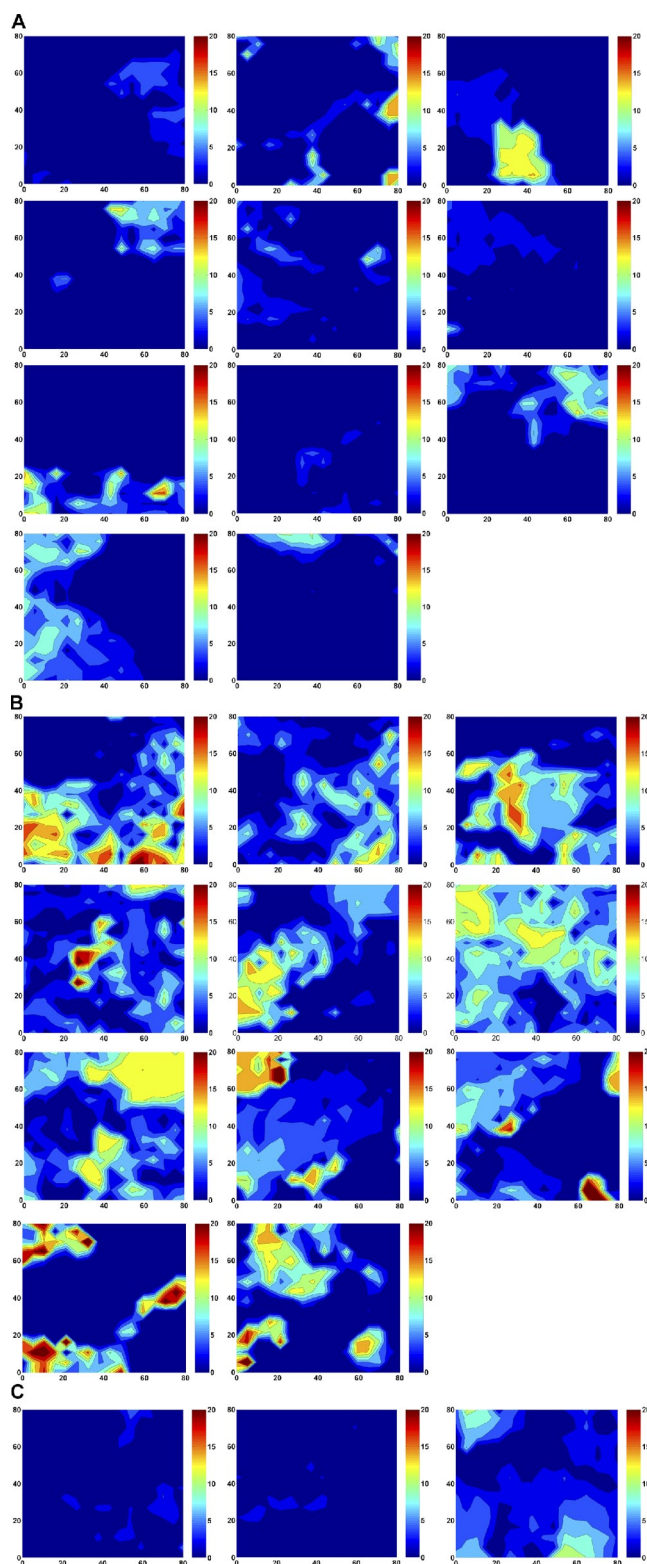
Liu et al., <http://www.jcb.org/cgi/content/full/jcb.201004082/DC1>

Figure S1. **Stiffness maps collected from five saline- and eight bleomycin-treated mouse lungs.** (A and B) Elastographs of lung parenchyma from five saline-treated mice in two injection experiments (A) and eight bleomycin-treated mice in two injection experiments (B) obtained in fibrotic lesion areas. (C) Elastographs of lung parenchyma from three bleomycin-treated mice in two injection experiments obtained outside fibrotic lesion areas. The color bars indicate shear modulus in kilopascals. Axis labels indicate spatial scale in micrometers.

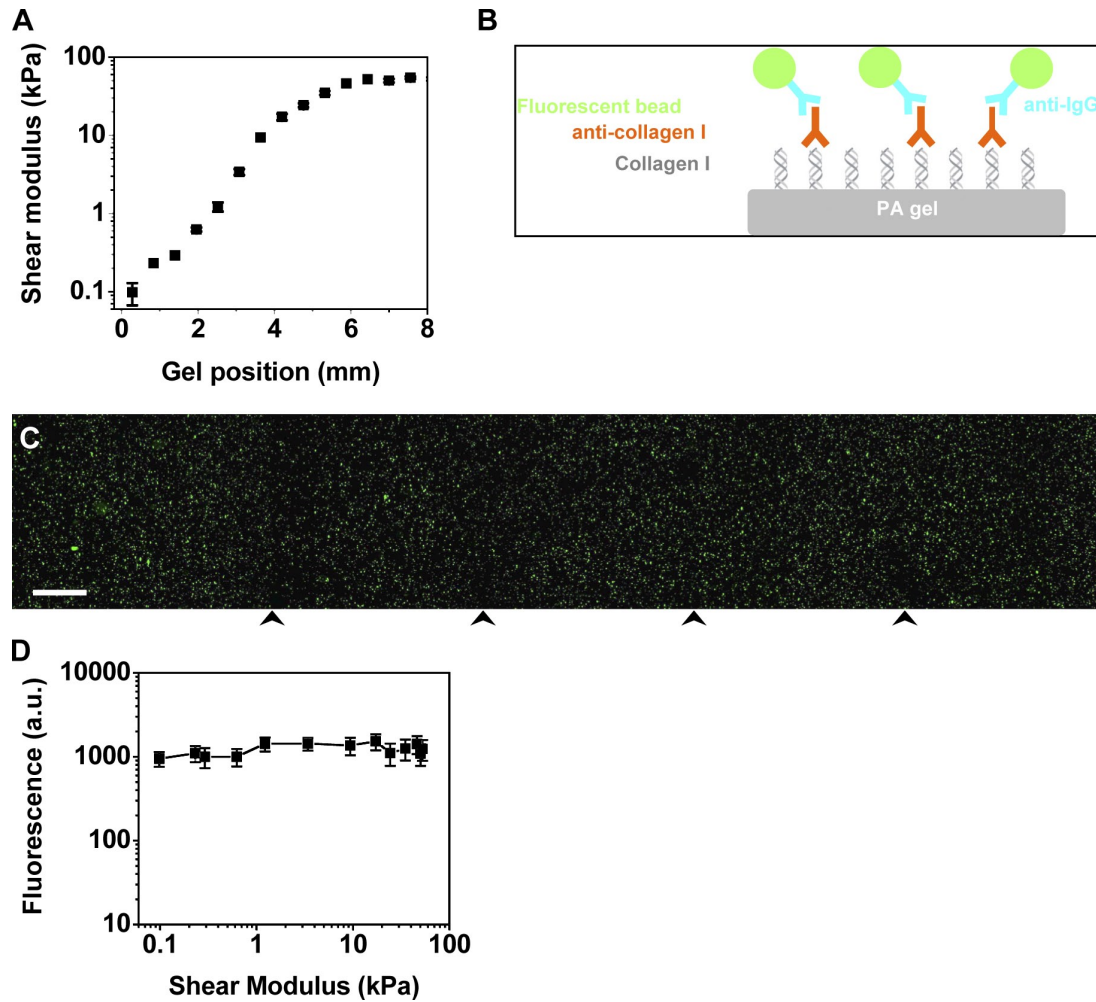


Figure S2. **Mechanical validation of the gradient stiffness system and uniform collagen density across the stiffness gradient.** (A) Shear modulus along polyacrylamide gel position measured using AFM microindentation. A stiffness gradient spanning the range of ~ 0.1 – 50 kPa was observed. Error bars indicate SD from three independent experiments. (B) The inert polyacrylamide (PA) surface was derivatized with $100\text{ }\mu\text{g/ml}$ monomeric collagen I using a heterobifunctional cross-linker. The uniformity of the collagen I surface density across the stiffness gradient was confirmed by immunostaining with collagen I primary antibody, followed by secondary antibody conjugated to fluorescently labeled $1\text{-}\mu\text{m}$ beads, which restricted the fluorescent detection to surface-available antigens. (C) Immunostaining of collagen I across stiffness gradient. Panorama image was generated by imaging the entire gel width along the stiffness gradient and then tiling five to seven adjacent pictures. Arrowheads below the image indicate stitching positions. Bar, $500\text{ }\mu\text{m}$. (D) Mean bead fluorescence is uniform across the overall stiffness gradient. Error bars indicate SD from four to six measurements obtained from one representative experiment. a.u., arbitrary unit.

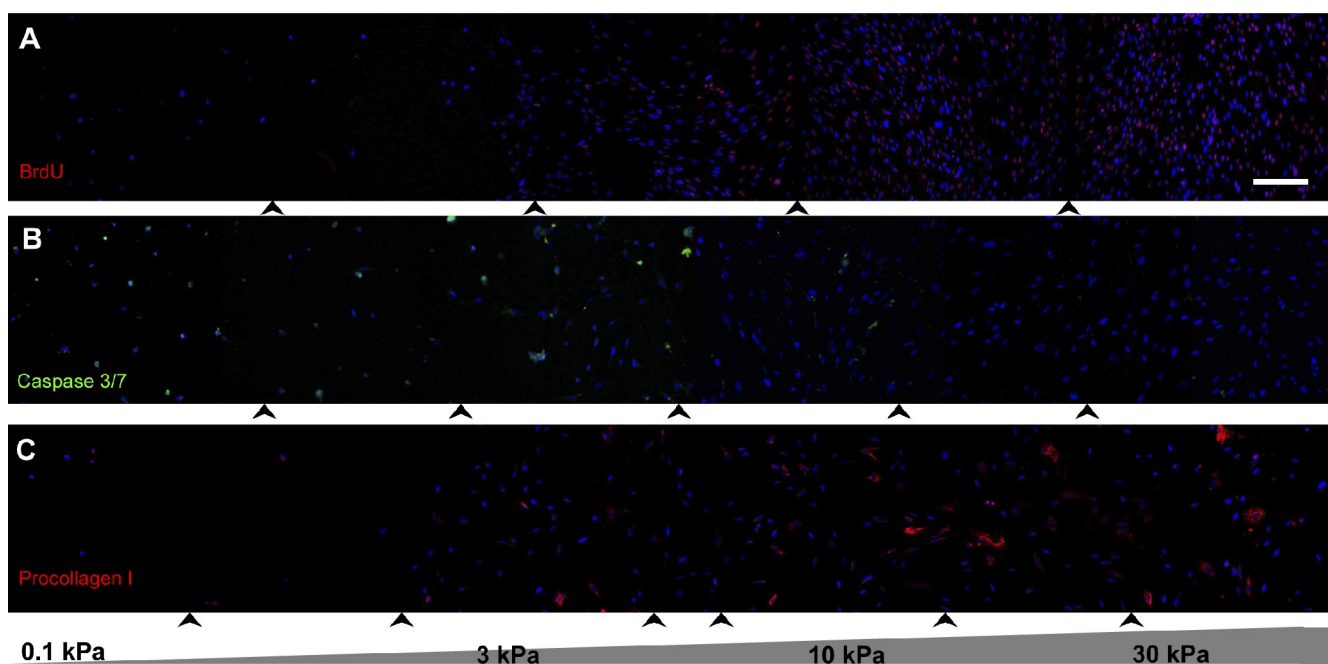


Figure S3. **Panorama fluorescence micrographs showing BrdU, caspase 3/7 activity, and procollagen staining across stiffness gradients.** (A–C) Immunostaining of BrdU (A), caspase 3/7 activity (B), and procollagen I (C) in human lung fibroblasts cultured on stiffness gradients. Each panorama image was generated by imaging the entire gel width along the stiffness gradient and then tiling five to seven adjacent pictures. Arrowheads below each image indicate stitching positions. Bar, 200 μ m.

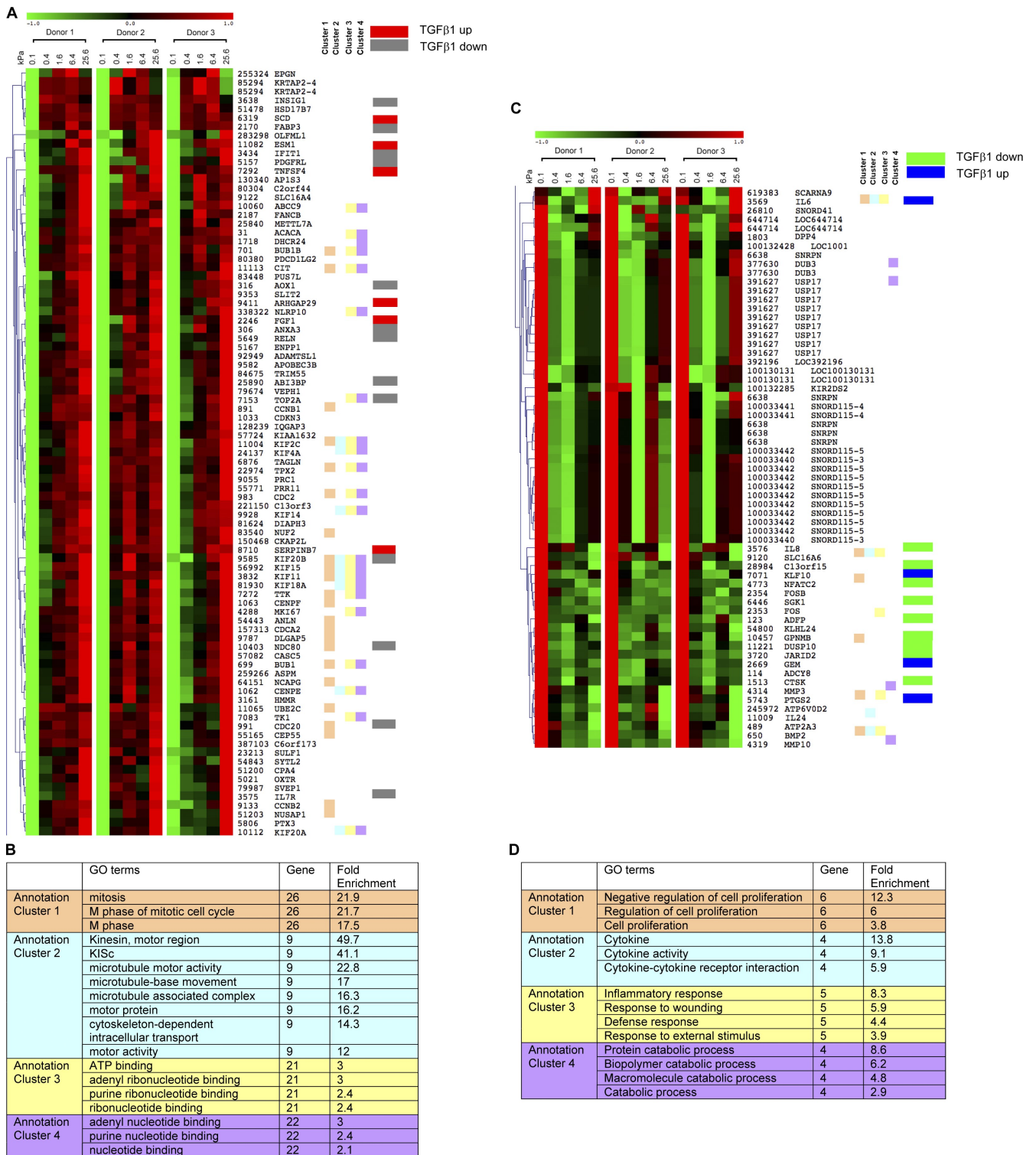


Figure S4. Gene expression profiles of NHLFs in response to substrate stiffness. (A and C) Hierarchical clustering based on linear correlation across the three donors identified two major clusters of genes, those whose expression generally increased with stiffness (A) and those that decreased with increasing stiffness (C). Each row displays expression data from three donors for each single gene that has a minimum coefficient variance of 0.2 per donor. Columns within each donor represent gene expression for stiffness effect at 0.1, 0.4, 1.6, 6.4, and 25.6 kPa. Genes are listed with their EntrezID, followed by their symbols according to the HUGO nomenclature. The subcluster of genes most prominently attenuated with increasing stiffness (linear correlation vs. stiffness <-0.26) is shown in Fig. 4 A. (B and D) Top four annotation clusters from functional annotation clustering analysis of genes in A and C. Corresponding genes within each annotation cluster are marked with the same color next to the gene name in A and C. Genes that are responsive to both stiffness and TGF- β 1, as reported in Kapoun et al. (2006), are marked at the end of the heat map.

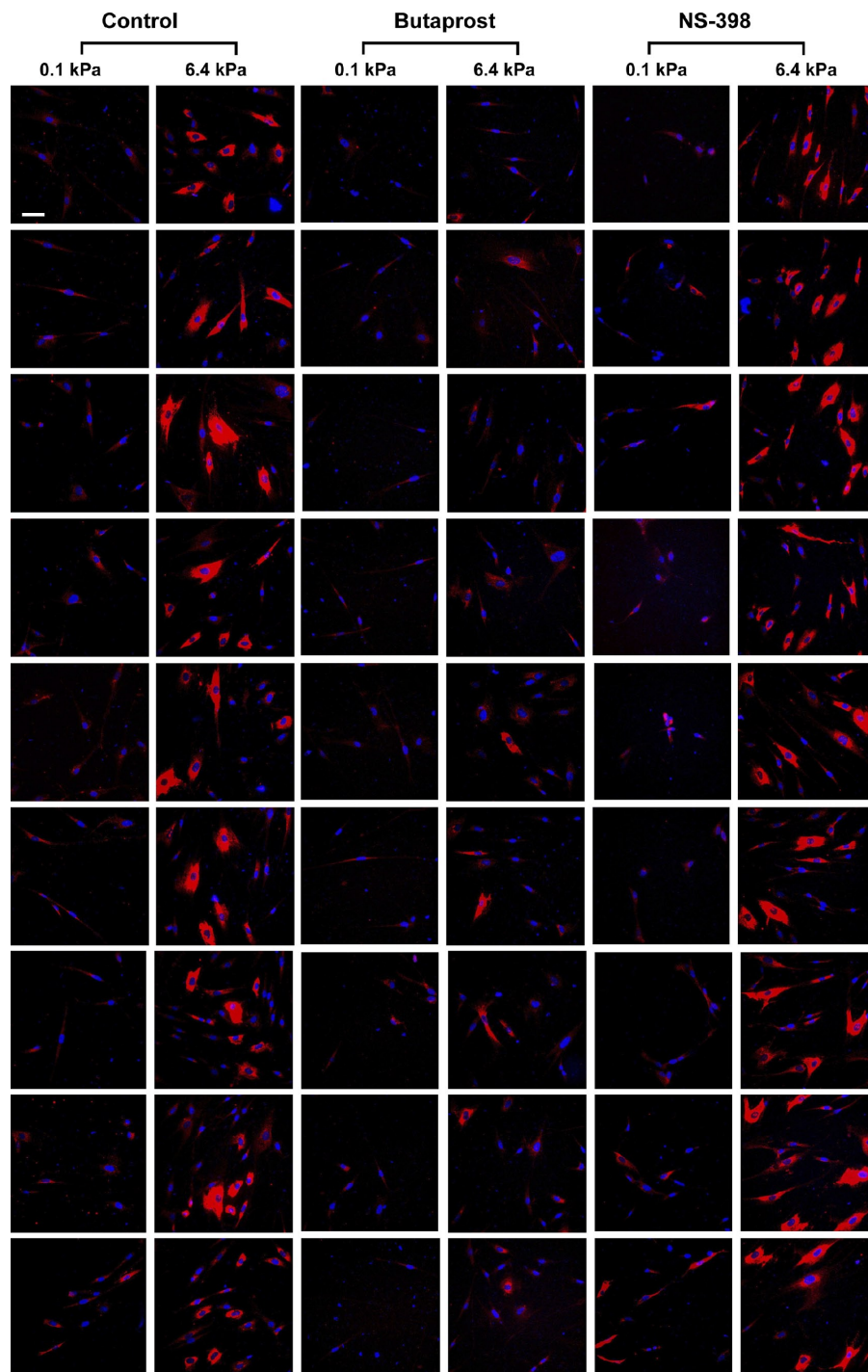


Figure S5. **Immunostaining of procollagen I in NHLFs cultured on 0.1 and 6.4 kPa substrates.** EP2-selective agonist (5 μ M butaprost) diminishes the stiffness effect on procollagen I expression, whereas COX-2-selective inhibitor (3 μ M NS-398) is unable to augment procollagen I expression at low stiffness. Bar, 50 μ m.

Table S1. PCR primers designed with qPrimerDepot

Gene	Primers (forward vs. reverse)
<i>GAPDH</i>	5'-AATGAAGGGGTCATTGATGG-3' 5'-AAGGTGAAGGTCGGAGTCAA-3'
<i>COL1A1</i>	5'-CACACGTCTCGGTCATGGTA-3' 5'-AAGAGGAAGGCCAAGTCGAG-3'
<i>COL3A1</i>	5'-AGGACTGACCAAGATGGGAA-3' 5'-AGGGGAGCTGGCTACTTCTC-3'
<i>MMP1</i>	5'-TTGTGGCCAGAAAACAGAAA-3' 5'-TTCGGGGAGAAGTGATGTTTC-3'
<i>MMP10</i>	5'-CTGATGGCCCAGAACTCATT-3' 5'-ATTTTGGCCCTCTCTTCCAT-3'
<i>MMP3</i>	5'-CAATTCATGAGCAGCAACG-3' 5'-AGGGATTAAATGGAGATGCCC-3'
<i>CTSK</i>	5'-CATTTAGCTGCCTTGCCCTGT-3' 5'-ATATGTGCAGAAGAACCGGG-3'
<i>PTGS2</i>	5'-CCGGGTACAATCGCACTTAT-3' 5'-GGCGCTCAGCCATACAG-3'

Reference

Kapoun, A.M., N.J. Gaspar, Y. Wang, D. Damm, Y.W. Liu, G. O'young, D. Quon, A. Lam, K. Munson, T.T. Tran, et al. 2006. Transforming growth factor-beta receptor type 1 (TGFbetaRI) kinase activity but not p38 activation is required for TGFbetaRI-induced myofibroblast differentiation and profibrotic gene expression. *Mol. Pharmacol.* 70:518–531. doi:10.1124/mol.105.021600