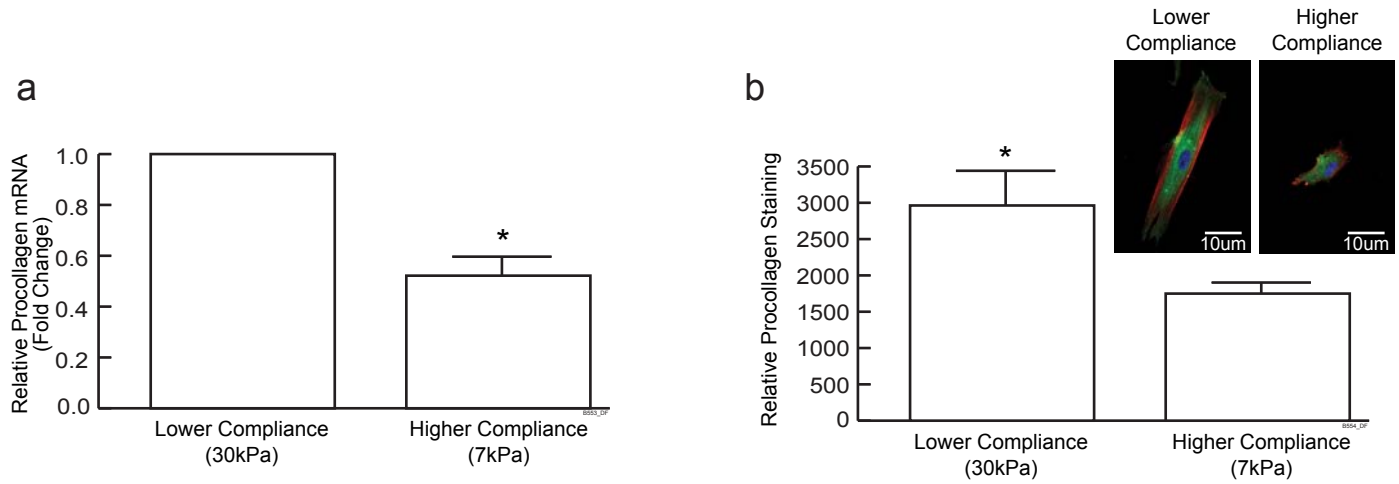
Supplemental Table S1

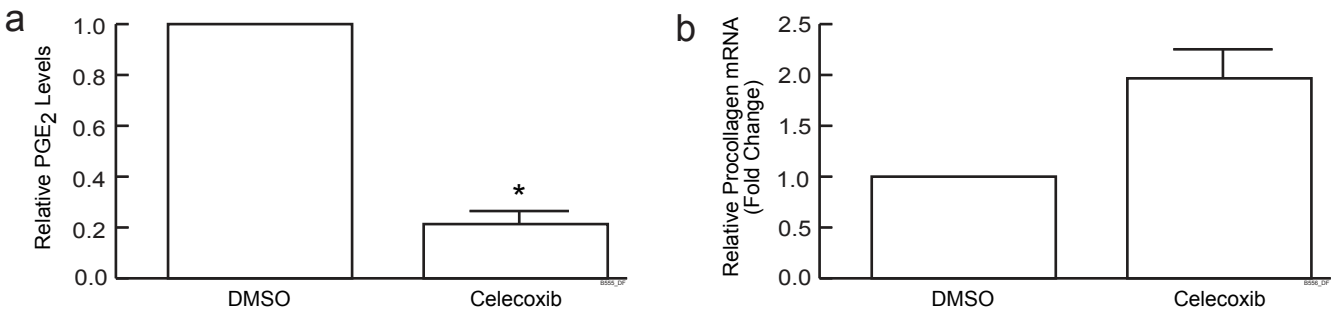
Relative mRNA expression of cell markers in fibroblast-enriched and fibroblast-depleted cells.

	Fibroblasts	Non-Fibroblasts	Fibroblasts/Non-fibroblasts
Type I Procollagen	1.2±0.27	$8.3x\ 10^{-3}$±$2.8x10^{-3}$	145
Keratin-14	$6.9x10^{-5}$±$1.9x10^{-5}$	$8.8x10^{-3}$±$2.5x10^{-3}$	0.0078
CD31	Undetectable	$6.1x10^{-5}$±$4.32x10^{-5}$	
CD45	Undetectable	$3.1x10^{-5}$±$5.76x10^{-5}$	

Supplemental Table S1: Cells were released from fresh skin samples by collagenase digestion. Released cells were fractionated by immino-affinity magnetic beads to separate fibroblasts from other cell types. Total RNA was extracted from fibroblast-enriched and fibroblast-depleted cell fractions. mRNA expression of type I procollagen (fibroblast marker), Keratin-14 (keratinocyte marker), CD31 (endothelial cell marker), and CD45 (leukocyte marker) was determined by reverse transcription and qPCR. The relative mRNA expression values normalized to 36B4 mRNA. Data are mean±SEM. (N=4). mRNA expression of CD31 and CD45 were below the limit of qPCR detection.



Supplemental Figure 1: Reduced spreading/mechanical force attenuates procollagen expression in human skin fibroblasts. Reduced spreading/mechanical force attenuates procollagen expression in human skin fibroblasts. Fibroblasts cultured from young skin were seeded on type I collagen-coated hydrogels with low (30kPa) or high (7kPa) compliance. (a) Type I procollagen mRNA levels were quantified by qPCR and normalized to 36B4 mRNA levels. Data are mean±SEM (N=3, *p<0.05). (b) Type I procollagen protein expression was revealed by immunofluorescence staining. The staining per cell was quantified by image analysis using NIH ImageJ. At least 5 cells per condition were quantified. The average procollagen staining per cell was calculated based on four independent experiments. (N=4, *p<0.05). Data are mean±SEM. Insets show representative immunostaining images. Nuclei (blue) were stained by DAPI. F-actin (red) was stained by phalloidin. Procollagen (green) was stained by anti-procollagen antibody (EMD Millipore). In addition to reduced collagen expression, fibroblasts cultured on 7kPa hydrogels displayed less prominent F-actin staining and reduced cell size compared to that of 30kPa.



Supplemental Figure 2: COX2-specific inhibition reduces PGE2 and increases type I procollagen expression in human skin organ culture. Skin samples were incubated in serum-free α-MEM media with the addition of celecoxib (2 μM) or vehicle (DMSO) for 16 hours. (a) PGE2 levels in conditioned media were quantified by PGE2 EIA and normalized to protein content of corresponding skin samples (N=3, *p<0.05). Data are mean±SEM. (b)Type I collagen mRNA levels were quantified by qPCR and normalized to 36B4 mRNA levels (N=3, *p<0.05). Data are mean±SEM.