Supplemental Methods

TGF-B1 ELISA

Supernatants were collected from AT2 cells cultured for 1, 2, 3, or 4 days and frozen at -80 degrees C until use in the ELISA. A commercially available mouse TGF-B1 Duo Set was used according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Latent TGF- β1 was calculated by subtracting active TGF-β1 from total TGF-β1.

Supplemental Table 1.

Primers used for real time RT-PCR.

	Forward 5'-3'	Reverse 5'-3'
18s	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT
Has1	GCCCTCCTCCTTCCTTCGT	GTATAGCCACTCTCGGAAGTAAGATTGG
Has2	TCATGGGTAACCAATGCAGTTTT	TTTAGTTGCATAGCCCAGACTCAA
Has3	CCTATGAATCAGTGGTCACAGGTTT	TGCGGCCACGGTAGAAAA
Hyal1	GTGCTGCCCTATGTCCAGAT	ATTTTCCCAGCTCACCCAGA
α-SMA	GCGTGGCTATTCCTTCGTTAC	GGCCATCTCATTTTCAAATC C
Spc	CAGCTCCAGGAACCTACTGC	CACAGCAAGGCCTAGGAAAG
vimentin	TTGATACCCTACAGCCCTGG	AAGAGTGGCAG GG CTGGA
E-cad	ATG GGG CAC CAC CAT CAC	CTGGGTACACG TGGGAAAC
WISP-1	GTC CTG AGG GTG GGC AAC AT	GGG CGT GTA GTC GTT TCC TCT

Supplemental Figure 1.

AT2 were 92.9 +/- 3.2% pure at isolation and 100% positive for pro-surfactant protein C in static culture after 4 days. A. Pap stain and B. anti-pro surfactant protein C staining of alveolar type II epithelium.

Supplemental Figure 2.

Size of sonicated Healon. Lane 1. High molecular weight hyaluronan ladder. Lane 2. Low molecular weight hyaluronan ladder. Lane 3. Blank. Lane 4. Sonicated Healon.

Supplemental Figure 3.

mRNA expression of Wnt/beta-catenin responsive genes. A. There was a trend for CCND1 gene expression increase with stretch, which did not reach statistical significance. B. WNT10a gene expression was not significantly changed with stretch. C. TCF4 gene expression was significantly increased with stretch compared to all other groups. D. SRFP1 gene expression was significantly increased with stretch compared to static CD44-/- cells. Data are presented as mean +/- standard error. N=3 per group, *$p<0.05$ as calculated by one way ANOVA with Tukey post-hoc analysis.

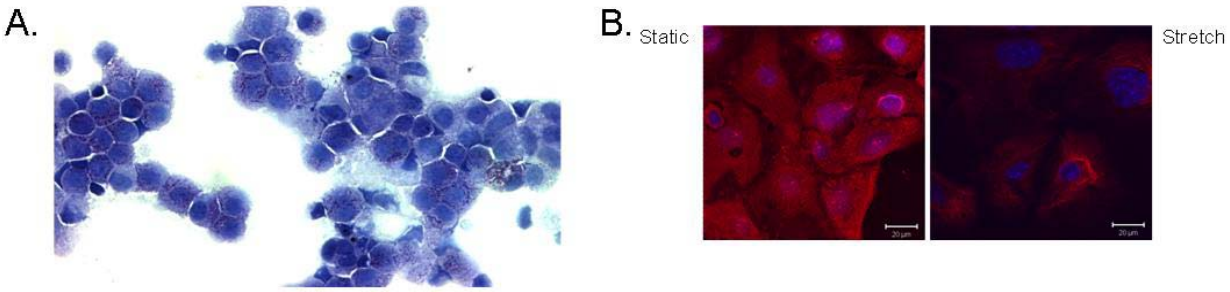
Supplemental Figure 4.

TGF-β1 induced EMT is separate from mechanical stretch. A. TGF-β1 ELISA results show that there is no significant increase in active or latent TGF-β1 in stretched cultures compared to stretch. B-D. Gene expression of AT2 CD44-/- cells exposed to TGF-β1 or stretch. B. E-cadherin gene expression is not significantly changed in CD44-/- cells treated with TGF-β1. C. Vimentin gene expression is significantly increased in CD44-/- cells treated with TGF-β1 compared to statically cultured cells and stretched cells. D. Alpha-SMA gene expression was significantly increased in CD44-/- cells treated with TGF-β1 compared to statically cultured and stretched cells. E-G. Gene expression of AT2 MyD88-/- cells exposed to TGF- β1 or stretch. E. E-cadherin gene expression was significantly reduced in MyD88-/- cells treated with TGF-β1 compared to stretched cells. F. Vimentin gene expression is significantly increased in MyD88-/- cells treated with TGF-β1 compared to statically cultured cells and stretched cells. G. Alpha-SMA gene expression was significantly increased in MyD88-/- cells treated with TGF-β1 compared to

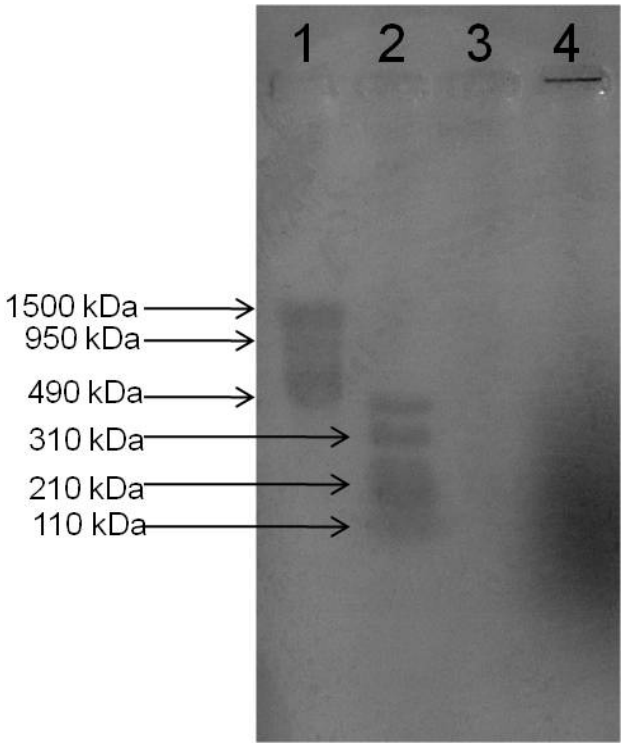
statically cultured and stretched cells. Data are presented as mean+/- standard error. N=3 per group, *p<0.05, **p<0.01, ***p<0.001 as calculated by one way ANOVA with Tukey post-hoc analysis.

Supplemental Figure 5.
TGF-β1 blockade does not affect stretch-induced or hyaluronan-induced EMT. Cells were stretched for 4 days according to experimental protocol, or exposed to short-fragment hyaluronan at 0.2 mg/ml for 4 days with either anti- TGF-β1 antibody treatment, or IgG control treatment. As evident from western blot detection of αSMA, vimentin, and E-cadherin after 4 days incubation, TGF-β1 blockade has no consistent effect on either stretch-induced or sHA-induced EMT marker expression.

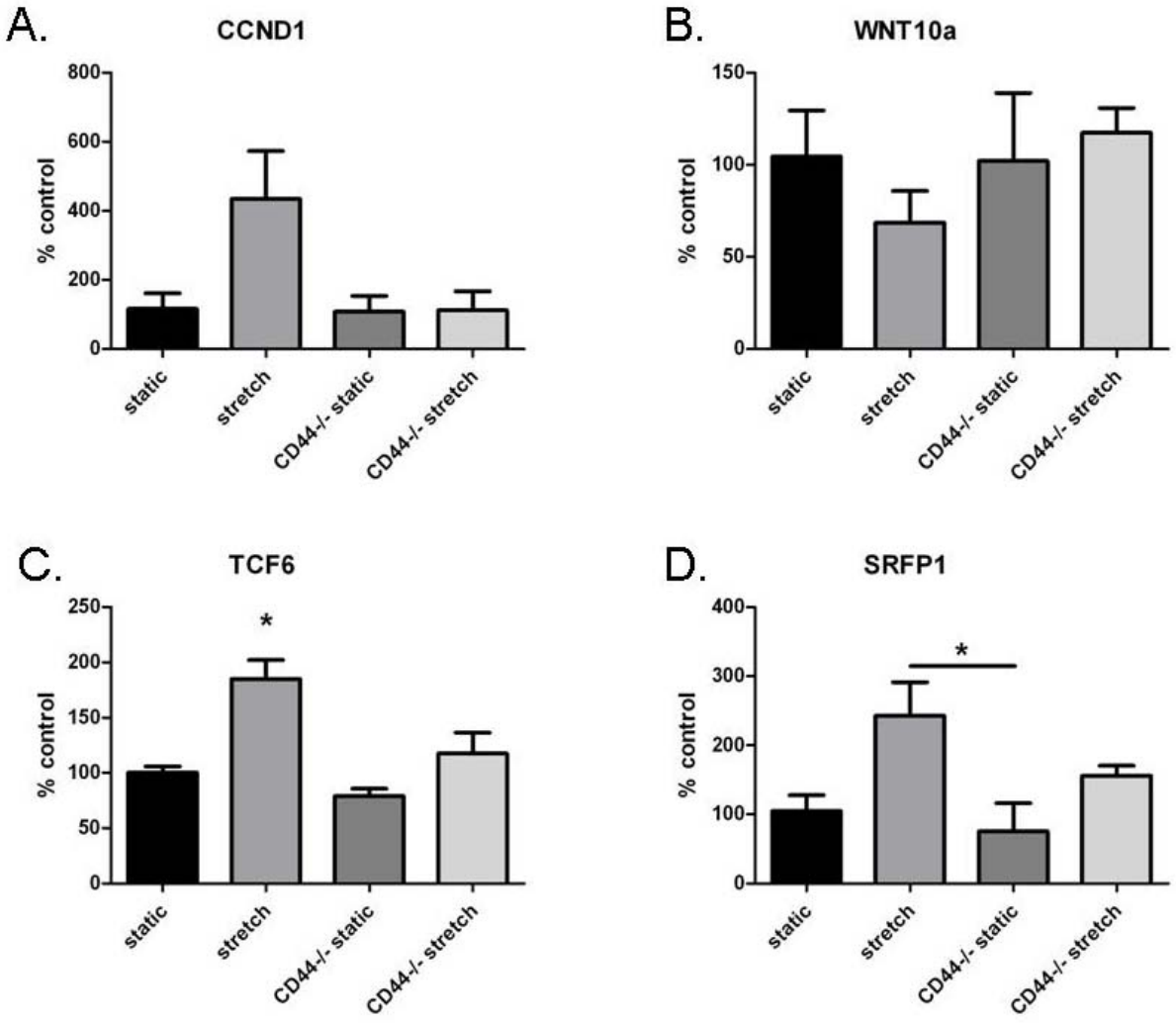
Supplemental Figure 1



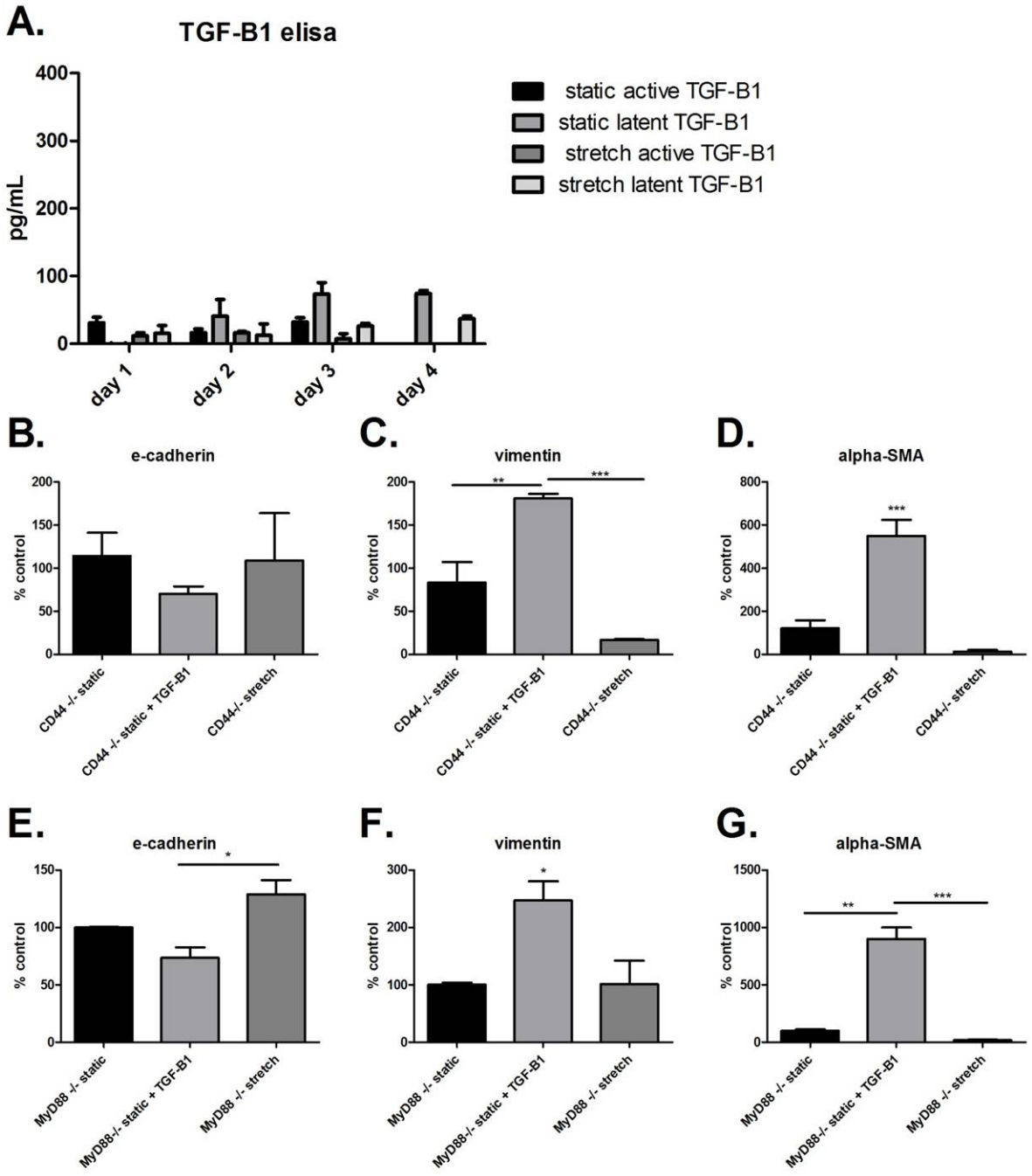
Supplemental Figure 2



Supplemental Figure 3.



Supplemental Figure 4.



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