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Supporting Information

Molecular Tension Probes to Investigate the Mechanopharmacology of Single Cells: A Step toward Personalized Mechanomedicine

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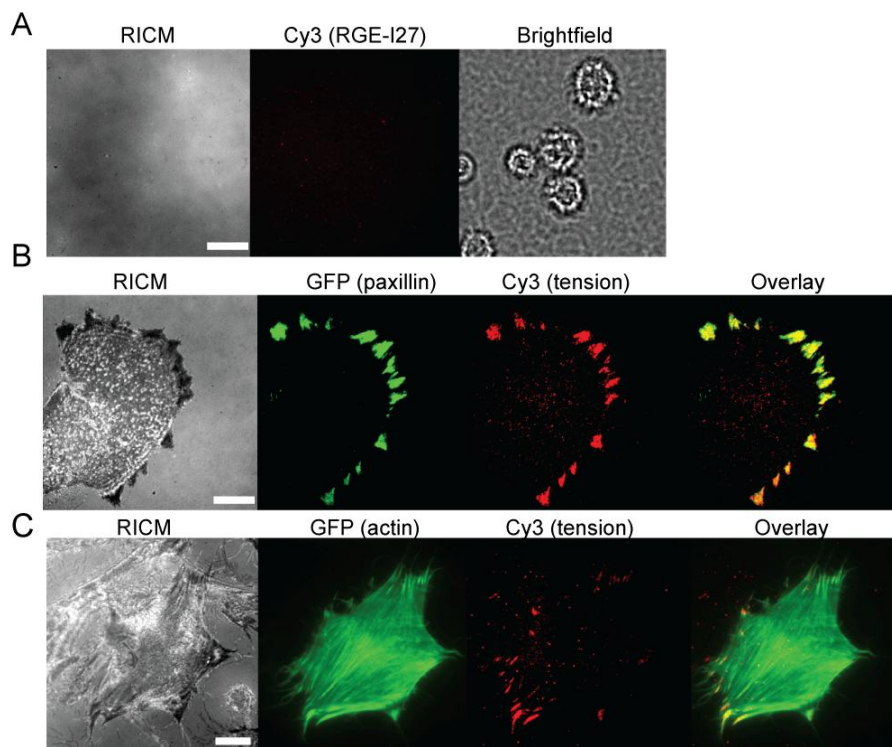
Figure S1. Tension signals are specifically generated through integrin-RGD interactions and are mediated by the cytoskeleton. A) Representative RICM, fluorescence and brightfield images of human ASM cells cells plated onto a surface coated with the RGE-Cy3-I27 nanoparticle sensors. Mutating RGD to RGE completely inhibits cell binding and spreading. Scale bar, 10 μm. **B)** Representative RICM, GFP-paxillin and Cy3 tension images of human ASM cells incubated on the RGD-Cy3-I27 functionalized on the AuNPs for 1 h. An overlay of Cy3/GFP demonstrates that tension signal was colocalized with the FAs. Scale bar, 10 μm. **C)** Representative RICM, GFP-actin, Cy3 tension, and overlay of Cy3/GFP demonstrate that tension signal was colocalized with actin at the cell periphery. Scale bar, 10 μm.
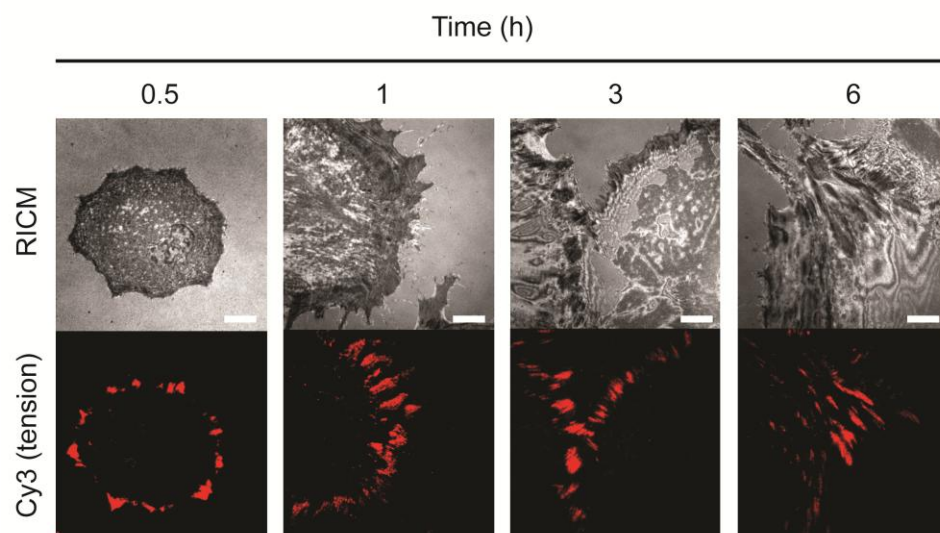
Figure S2. Representative time-lapse RICM and fluorescence images of ASM cells cultured on the tension sensor surface and imaged at the indicated time points. Note the changes in the cell morphology over time. Scale bar, 10 µm.
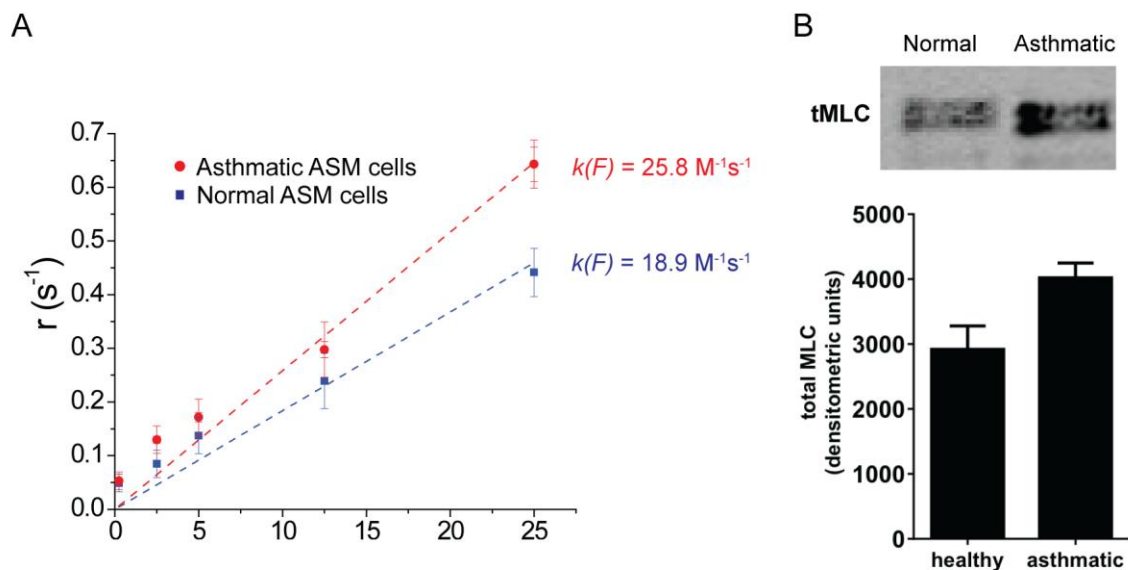
Figure S3. Asthmatic human ASM cells have higher levels of contractile proteins and higher rates of I27 unfolding compared to normal human ASM cells. A) Plot of *r* (rate of disulfide reduction) as a function of [DTT] for normal human ASM cells (blue) and asthmatic human ASM cells (red). Error bars represent mean±SEM from *n* = 5 cells from 5 different surfaces **B)** Total myosin light chain (total MLC) expression was measured for normal and asthmatic human ASM cells of a normal and an asthamic donor by using Western blot analysis and quantified by densitometric analysis. Error bars represent mean±SEM from 3 samples of normal donors and 4 samples of asthmatic donors.
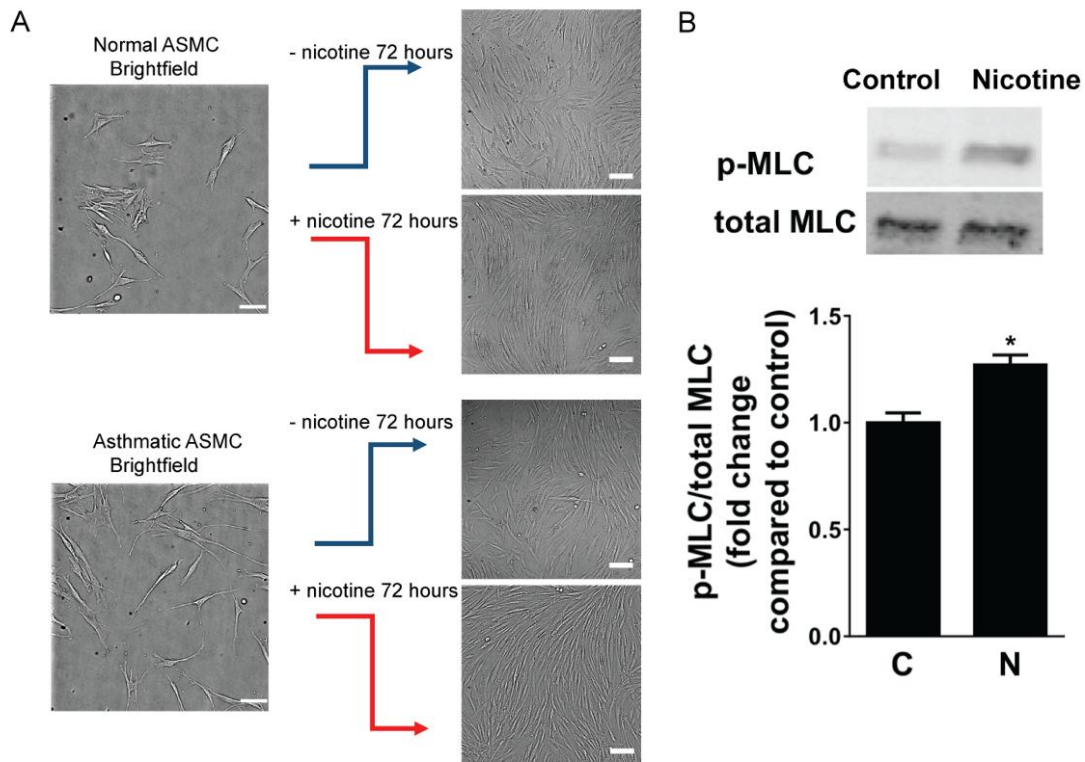
Figure S4. Changes in morphology and gene expression of human ASM cells incubated with and without nicotine over 72 h. A) Representative brightfield images of normal and asthmatic human ASM cells incubated in the presence of 50 μg/ml of nicotine. Scale bar, 100 μm. **B)** Total myosin light chain (total MLC) and phosphorylated myosin light chain (p-MLC) expression levels were measured by Western blot analysis and quantified by densitometric analysis. Error bars represent mean±SEM from 3 samples of normal donors and 4 samples of asthmatic donors. *$P<0.05$ using Student's *t*-test.
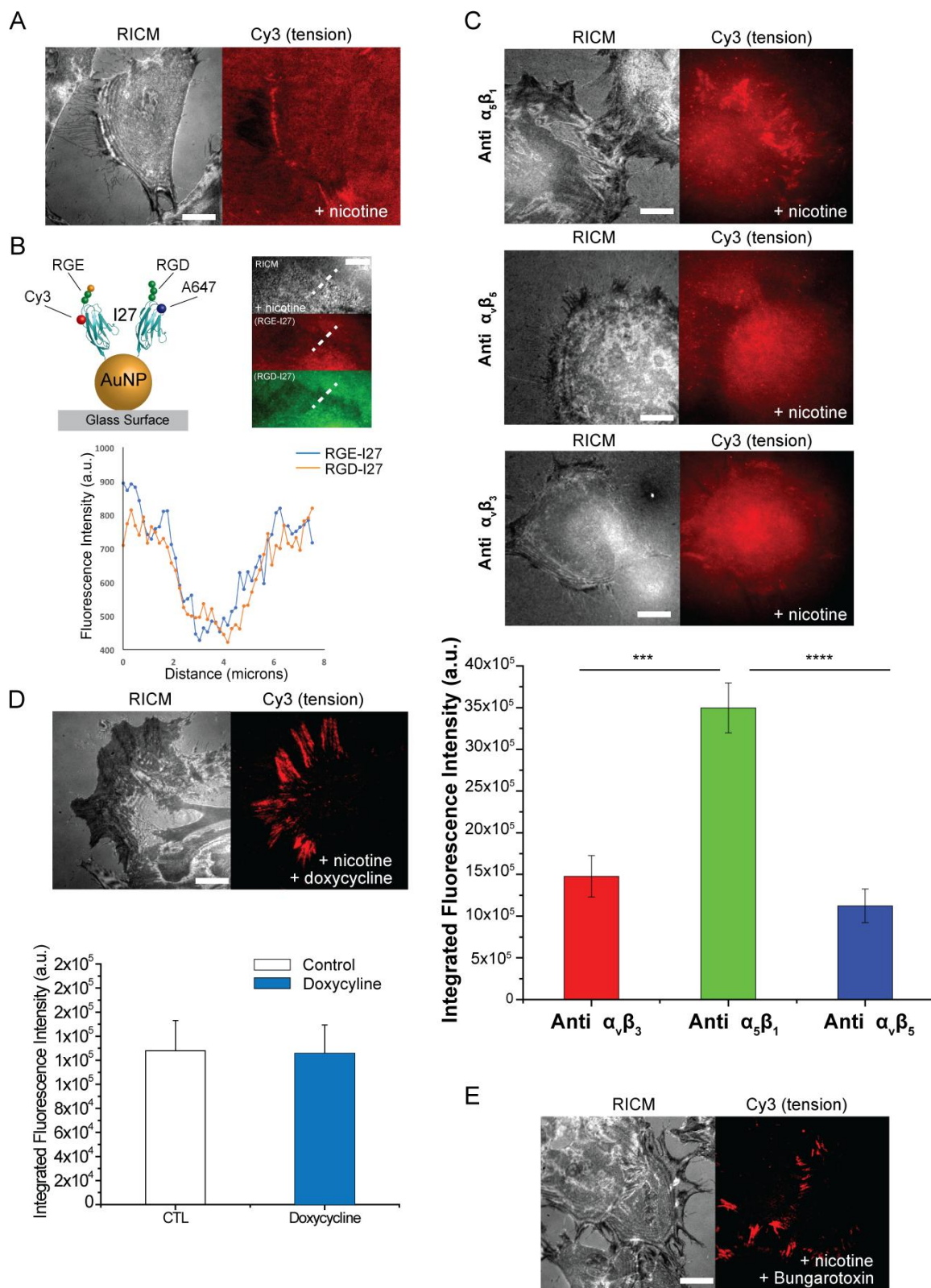
Figure S5. Chronic treatment of human ASM cells with nicotine induces the release of matrix metalloproteinases (MMPs). A) Representative RICM and fluorescence images of ASM cells treated with 50 µg/ml of nicotine for 72 h and seeded on the RGD-Cy3-I27 tension sensor for 2 h. Note the negative signals in the image. Scale bar, 10 µm. **B)** ASM cells were treated with 50 µg/ml nicotine for 72 h and seeded on surfaces co-incubated with the RGD-

A647-I27 and RGE-Cy3-I27 sensors for 2 h to visualize protease activity. Both channels showed negative signal which suggested that the proteases were cleaving the sensors from the AuNPs. Line scan (dashed white line) analysis through the adhesion site shows correlation between Cy3 (RGE-I27) and A647 (RGD-I27). Scale bar, 10 µm. **C)** Representative RICM and fluorescence images of human ASM cells that were treated with 50 µg/ml of nicotine for 72 h, blocked with monoclonal antibodies against $\alpha_v\beta_3$, $\alpha_5\beta_1$, and $\alpha_v\beta_5$ integrins for 30 min before seeding the cells on the RGD-Cy3-I27 sensor surface. Scale bar, 10 µm. Plot shows the integrated fluorescence intensity for each condition. The error bars represent SEM from $n = 20$ cells for each group collected from three surfaces, $***P<0.001$ and $****P<0.0001$ using Student's t-test. **D)** Representative RICM and integrin tension images of ASM cells treated with 50 µg/ml of nicotine for 72 h, followed by 4 µg/ml of doxycycline, an MMP inhibitor, for 24 h before adding cells on the RGD-Cy3-I27 sensor surface. Scale bar, 10 µm. Plot shows the integrated fluorescence intensity of healthy cells not treated (control) and treated with doxycycline (4 µg/ml). This result indicates doxycycline does not impact integrin tension. The error bars represent SEM from $n = 10$ cells from 3 surfaces. **E)** Representative RICM and RGD-Cy3-I27 tension images of ASM cells treated with 50 µg/ml of nicotine for 72 h and 5 nM of α-bungarotoxin (BGT), an α7-nAChR antagonist, for 72 h. Incubating cells with bungarotoxin inhibited the release of MMPs. Scale bar, 10 µm.
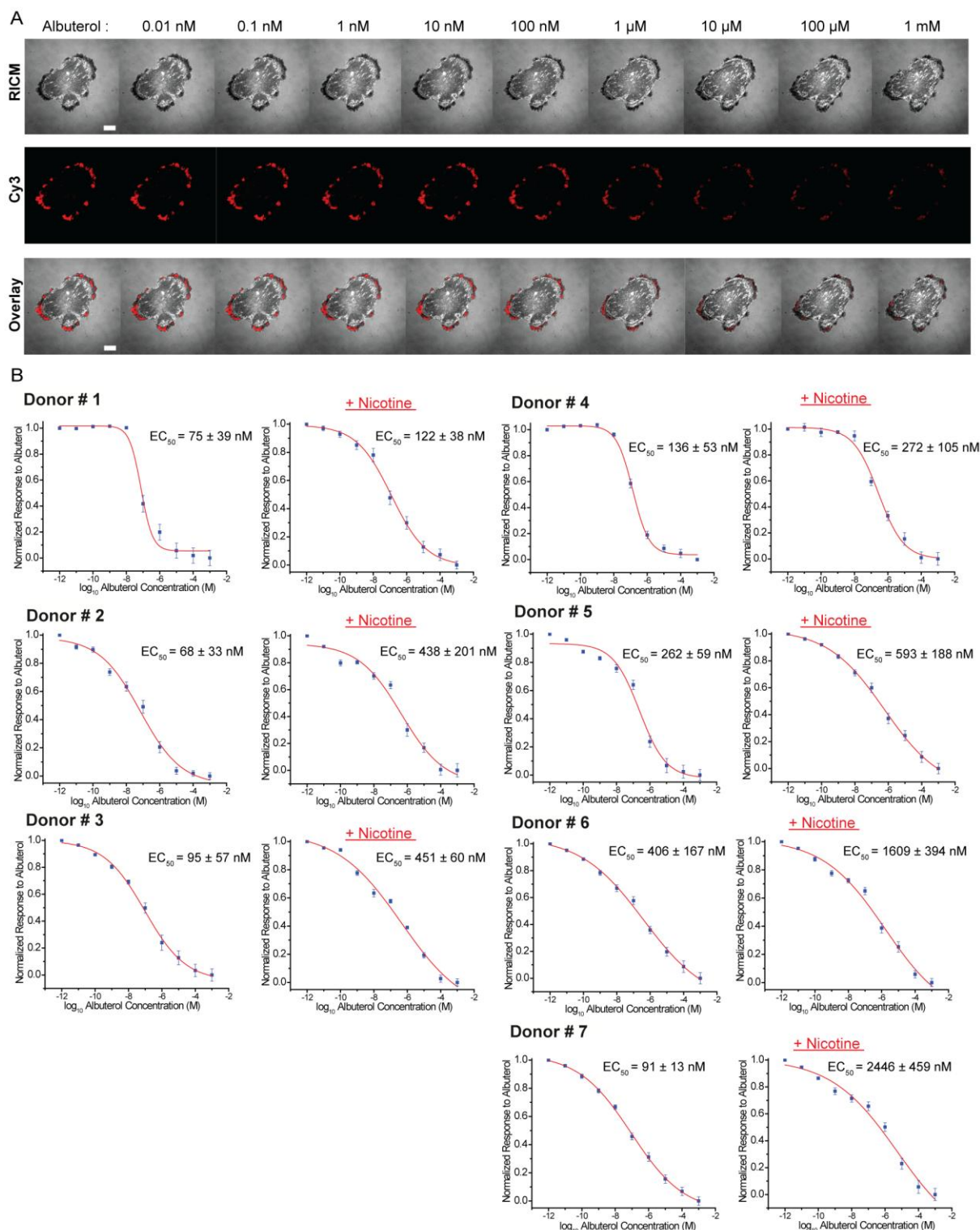
Figure S6. Dose dependent curves of integrin mediated tension with and without the chronic treatment of nicotine (50 µg/ml) for 72 h. A) Representative RICM, integrin tension, and overlay of RICM and tension images for nicotine- and doxycycline-treated ASM cell on the RGD-Cy3-I27 tension surface dosed with a stepwise addition of albuterol (0.01 nM–1 mM). Scale bar, 10 µm. **B)** EC_{50} values were calculated from dose-dependent curves from normal ASM cells (Donors #1-3) and from asthmatic ASM cells (Donors #4-7). Each pair of measurements are performed on the same day using 5−10 cells per EC_{50} measurement. Also, each EC_{50} plot is the average of cells cultured on 5−10 different substrates. All the donor samples are age and gender matched.
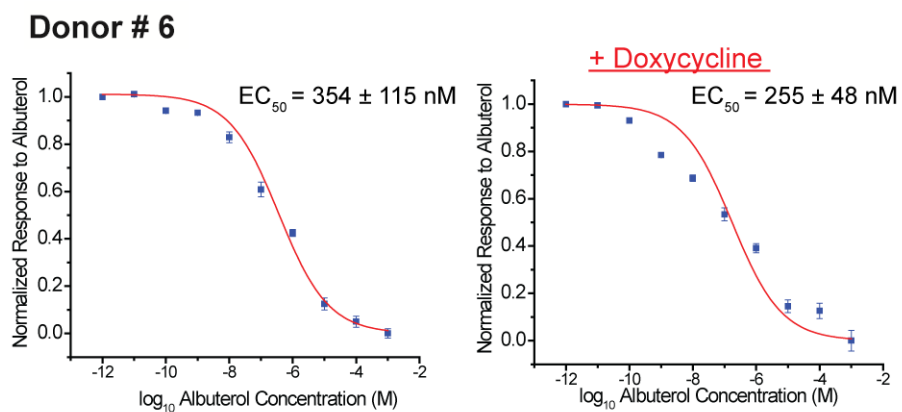
Donor # 6

Figure S7. Representative dose-dependent curves for nicotine treated ASM cells pre-incubated with or without doxycycline for 24 h, cultured on the tension sensor, and treated with the stepwise addition of albuterol.
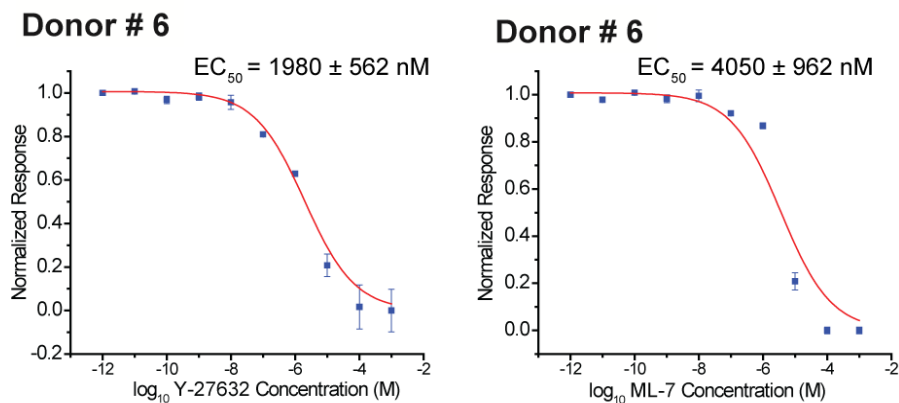
Figure S8. Representative dose-dependent curves for ASM cells cultured on the tension sensor and treated with the stepwise addition of Y-27632 and ML-7.

Table S1. Data of anonymous donors used in this study

Donor	Asthma	Age	Male/Female	Tobacco use
1	no	48	M	no
2	no	49	M	Chewing tobacco
3	no	39	F	no
4	yes	27	F	cigarettes
5	yes	46	F	no
6	yes	9	M	no
7	yes	54	M	no