

## Supplementary Tables for Online Repository

**Table E1:** Characteristics of donor lungs used for cell traction force measurements

| <i>Donor</i>      | <i>Age</i> | <i>Gender</i> | <i>Race and Ethnicity</i> | <i>Cause of Death</i>   |
|-------------------|------------|---------------|---------------------------|-------------------------|
| <b>Non-asthma</b> |            |               |                           |                         |
| <b>1</b>          | 25         | M             | Unknown                   | Multiple trauma, anoxia |
| <b>2</b>          | 69         | M             | Unknown                   | Intracranial bleed      |
| <b>3</b>          | 53         | F             | White, not Hispanic       | Intracranial bleed      |
| <b>4</b>          | 40         | M             | White, not Hispanic       | Drug overdose           |
| <b>5</b>          | 61         | M             | Black, not Hispanic       | Intracranial bleed      |
| <b>6</b>          | 49         | M             | White, not Hispanic       | Intracranial bleed      |
| <b>Asthma</b>     |            |               |                           |                         |
| <b>7</b>          | 42         | M             | Unknown                   | Unknown                 |
| <b>8</b>          | 44         | F             | Unknown                   | Asthma attack           |
| <b>9</b>          | 45         | M             | White, not Hispanic       | Intracranial bleed      |
| <b>10</b>         | 48         | F             | Black, not Hispanic       | Intracranial bleed      |
| <b>11</b>         | 51         | F             | Black, unknown            | Intracranial bleed      |
| <b>12</b>         | 11         | M             | White, not Hispanic       | Anoxia                  |

Notes:

Human airway smooth muscle (ASM) cells used for cell traction force measurements were obtained from the University of Chicago, through the Gift of Hope Organ and Tissue Donor Network.

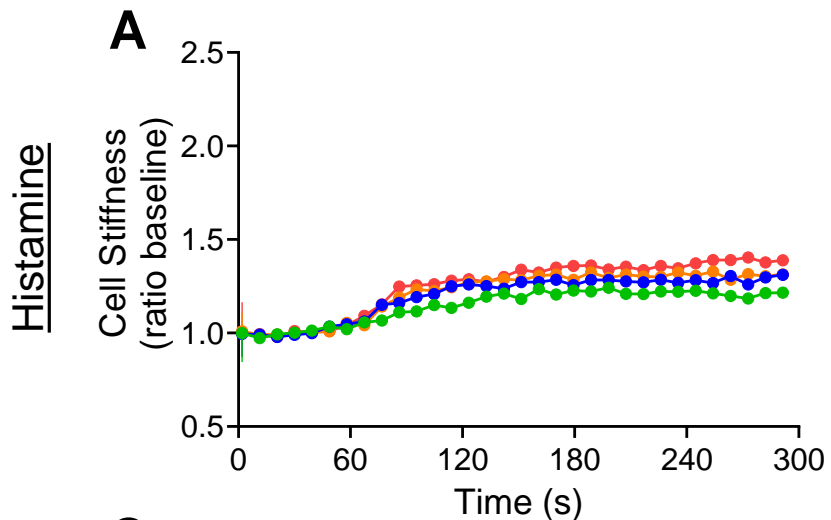
**Table E2:** Characteristics of donor lungs used for cell stiffness measurements

| <i>Donor</i>      | <i>Age</i> | <i>Gender</i> | <i>Race and Ethnicity</i> | <i>Cause of Death</i> |
|-------------------|------------|---------------|---------------------------|-----------------------|
| <b>Non-asthma</b> |            |               |                           |                       |
| <b>13</b>         | 16         | F             | White, not Hispanic       | Head trauma           |
| <b>14</b>         | 37         | M             | Black, not Hispanic       | Intracranial bleed    |
| <b>15</b>         | 19         | M             | Black, not Hispanic       | Closed head injury    |
| <b>16</b>         | 19         | F             | Black, not Hispanic       | Head trauma           |
| <b>17</b>         | 55         | F             | Hispanic                  | Hypertensive bleed    |
| <b>18</b>         | 55         | F             | White, not Hispanic       | CNS tumor             |
| <b>Asthma</b>     |            |               |                           |                       |
| <b>19</b>         | 13         | M             | White, not Hispanic       | Asthma attack, anoxia |
| <b>20</b>         | 44         | M             | Hispanic                  | Asthma attack, anoxia |
| <b>21</b>         | 15         | F             | Hispanic                  | Asthma attack, anoxia |
| <b>22</b>         | 25         | F             | White, not Hispanic       | Anoxia                |
| <b>23</b>         | 38         | M             | White, not Hispanic       | Asthma attack, anoxia |
| <b>24</b>         | 9          | M             | White, not Hispanic       | Asthma attack         |

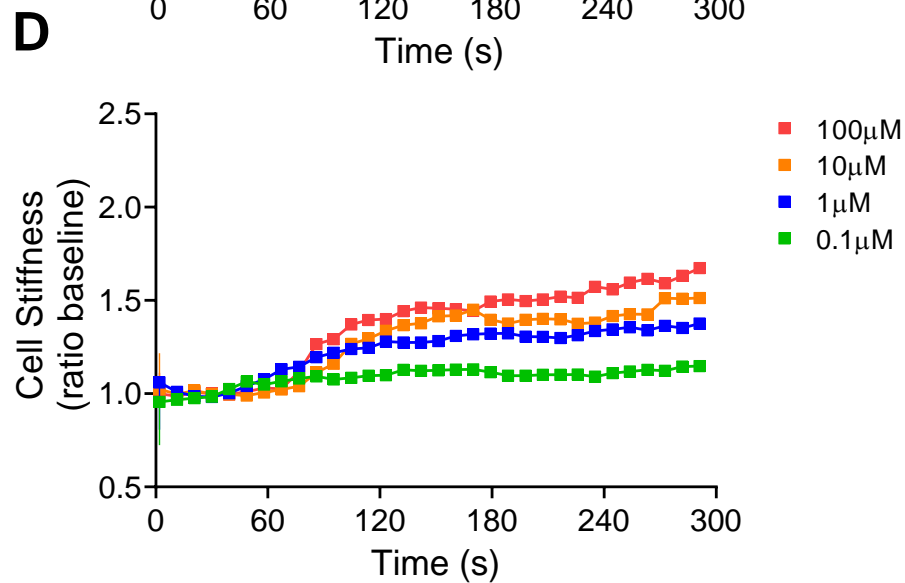
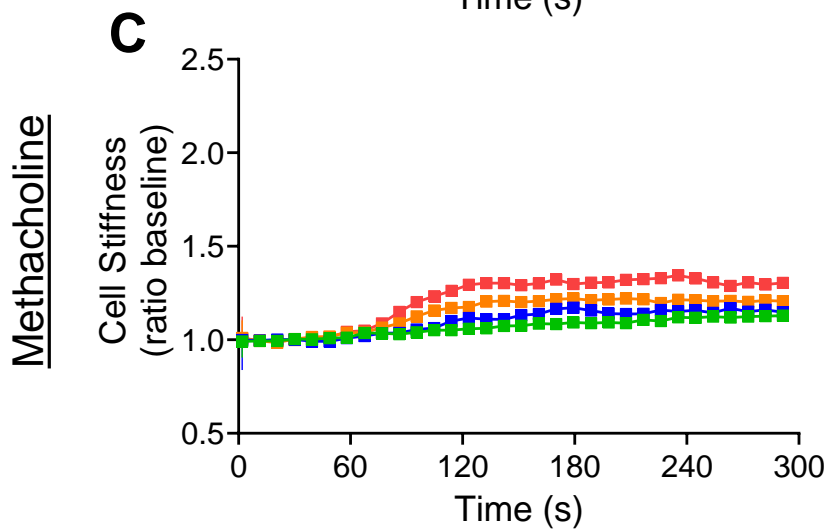
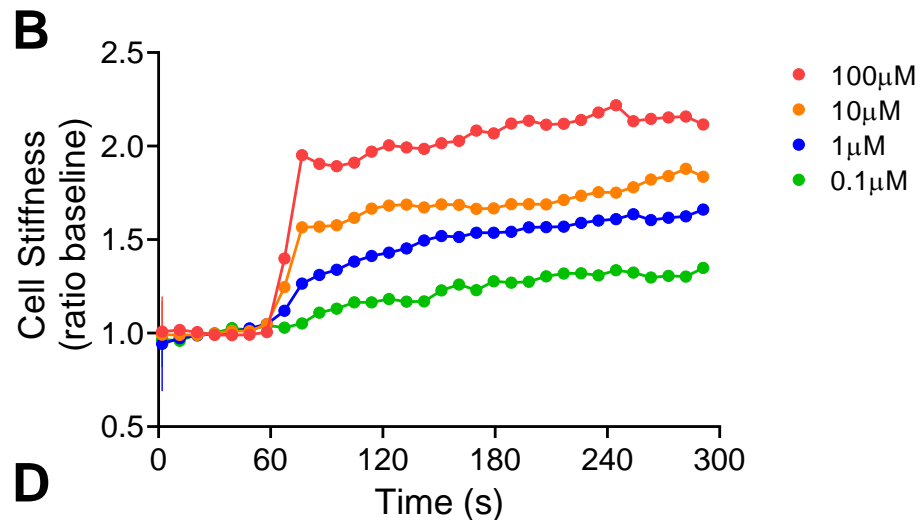
Notes:

Human ASM cells used for cell stiffness measurements were obtained from the University of Pennsylvania. Lungs were procured through the National Disease Research Interchange and the International Institute for the Advancement of Medicine.

### ASM (non-asthma)



### ASM (asthma)



## **Supplementary Material for Online Repository**

### **Materials and Methods**

#### **Materials**

Unless otherwise noted, all reagents were obtained from Sigma-Aldrich with the exception of DMEM-Ham's F-12 (1:1) which was purchased from GIBCO. The synthetic arginine-glycine-aspartic acid (RGD) containing peptide was purchased from American Peptide Company.

#### **ASM cell culture and characterization**

Human ASM cells were prepared from donor lungs unsuitable for transplantation in accordance with of the respective Institutional Review Boards at the University of Chicago and the University of Pennsylvania. Because the availability of large numbers of early passage primary ASM cells from donor lungs is a challenge, and because the propagation of cells from distal airways often necessitate greater numbers of passages in culture, here we harvested cells from the proximal airways (first through third order bronchi) as described.<sup>E1</sup> Cells were maintained in serum-free media for 24 h at 37°C in humidified air containing 5% CO<sub>2</sub> prior to study. These conditions have been optimized for seeding cultured cells on collagen matrix and for assessing their mechanical properties.<sup>E1-E5</sup>

#### **Fourier transform traction microscopy**

Briefly, cells were plated sparsely on collagen-coated elastic gel blocks precisely tuned to mimic a (patho)physiological range of airway wall rigidity (Young's modulus from 1 kPa to 8 kPa),<sup>E5</sup> and allowed to adhere and stabilize for 24 h. The contractile stress arising at the interface between each adherent cell and its substrate was measured with traction microscopy,<sup>E4</sup> and the computed traction field was used to obtain net contractile moment, which is a scalar measure of the cell's contractile amplitude. Net contractile moment is expressed in units of pico-Newton meters (pNm).

#### **Magnetic twisting cytometry**

Dynamic increases in cell stiffness to bronchoconstrictive agonists were measured as an indicator of the single-cell contraction of isolated human ASM cells as we have previously described.<sup>E1-E3</sup>

In brief, RGD-coated ferrimagnetic microbeads (4.5  $\mu\text{m}$  in diameter) bound to the cytoskeleton through cell surface integrin receptors were magnetized horizontally and then twisted in a vertically aligned homogeneous magnetic field that was varying sinusoidally in time. This sinusoidal twisting magnetic field caused both a rotation and a pivoting displacement of the bead: as the bead moves, the cell develops internal stresses which in turn resist bead motions.<sup>E3</sup> Lateral bead displacements in response to the resulting oscillatory torque were detected with a spatial resolution of  $\sim 5$  nm, and the ratio of specific torque to bead displacements was computed and expressed here as the cell stiffness in units of Pascal per nm (Pa/nm).

### Statistical Analysis

For cell traction force measurements, we used nested design analysis to control for random effects from repeated measurements of multiple cells in the same subject, and to increase the power.<sup>E6</sup> To satisfy the normal distribution assumptions associated with the Analysis of Variance (ANOVA), cell traction data were converted to log scale prior to analyses. Unless otherwise stated, we used Student's *t*-test and ANOVA with adjusting for multiple comparisons by applying Bonferroni's methods. All analyses were performed using SAS V.9.2 (SAS Institute Inc., Cary, NC), and 2-sided *P*-values less than 0.05 were considered significant.

### References

- E1. Deshpande DA, Wang WC, McIlmoyle EL, Robinett KS, Schillinger RM, An SS, Sham JS, Liggett SB. Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nat Med* 2010;16:1299-1304.
- E2. An SS, Fabry B, Trepas X, Wang N, Fredberg JJ. Do biophysical properties of the airway smooth muscle in culture predict airway hyperresponsiveness? *Am J Respir Cell Mol Biol* 2006;35:55-64.
- E3. Fabry B, Maksym GN, Butler JP, Glogauer M, Navajas D, Fredberg JJ. Scaling the microrheology of living cells. *Phys Rev Lett* 2001;87:148102.
- E4. Butler JP, Tolic-Norrelykke IM, Fabry B, Fredberg JJ. Traction fields, moments, and strain energy that cells exert on their surroundings. *Am J Physiol* 2002;282:C595-C605.
- E5. An SS, Kim J, Ahn K, Trepas X, Drake KJ, Kumar S, Ling G, Purington C, Rangasamy T, Kensler TW, Mitzner W, Fredberg JJ, Biswal S. Cell stiffness, contractile stress and the role of extracellular matrix. *Biochem Biophys Res Commun* 2009;382:697-703.
- E6. Krzywinski M, Altman N, Blainey P. Points of significance: nested designs. *Nat Methods*. 2014;11:977-978.

## Figure Legends

**Figure E1.** Cell stiffening responses to histamine (**A** and **B**) and methacholine (**C** and **D**) of ASM derived from non-asthma (*left*) and asthma (*right*) lung donors measured by magnetic twisting cytometry. For each individual ASM cell, baseline stiffness was measured for the first 60 s, and after drug addition stiffness was measured continuously for the next 240 s. For each cell, stiffness was normalized to its baseline stiffness before the agonist stimulation. Data are presented as mean  $\pm$  SE ( $n = 68-283$  individual cell measurements for each dose of the agonists).