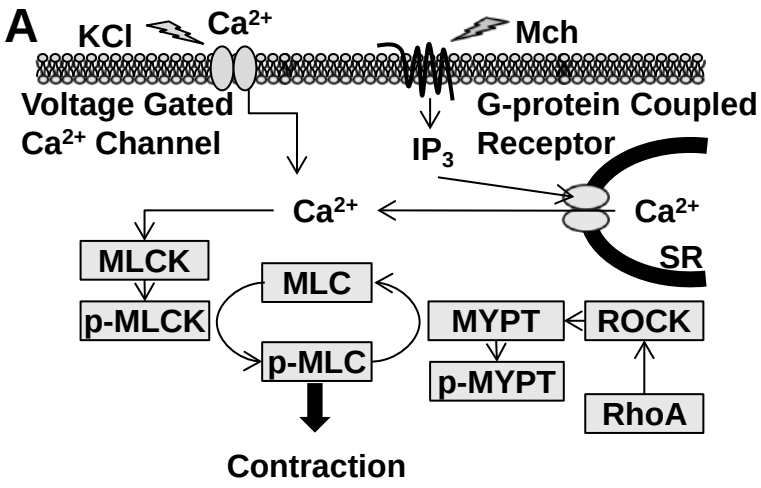
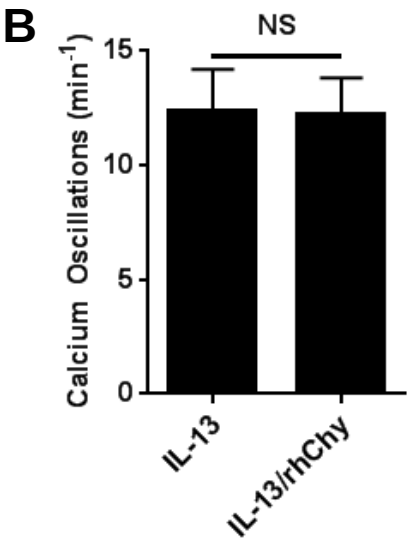
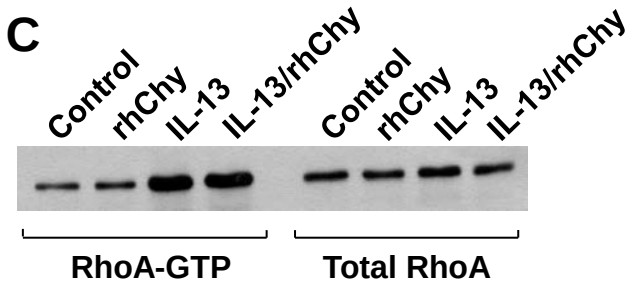
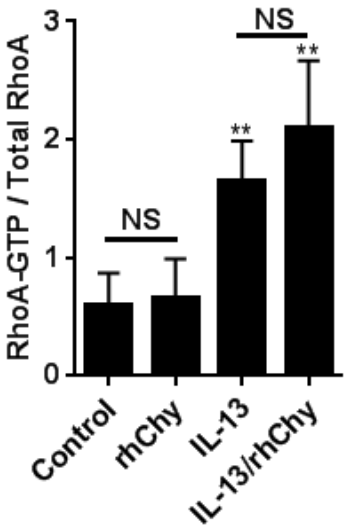
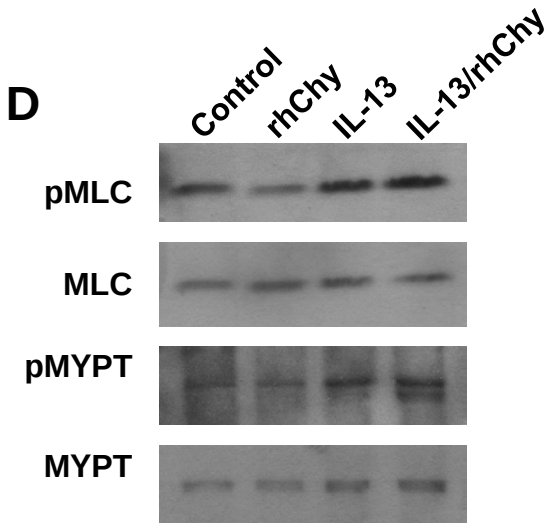
Supplemental Figure 1

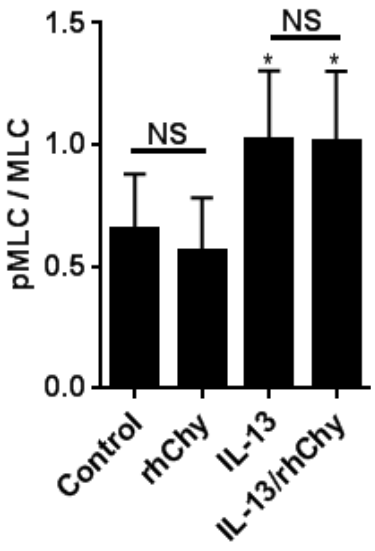


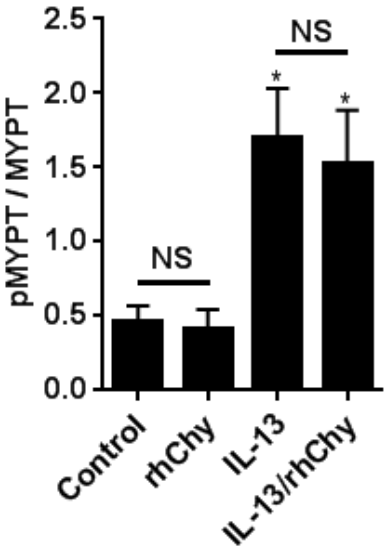








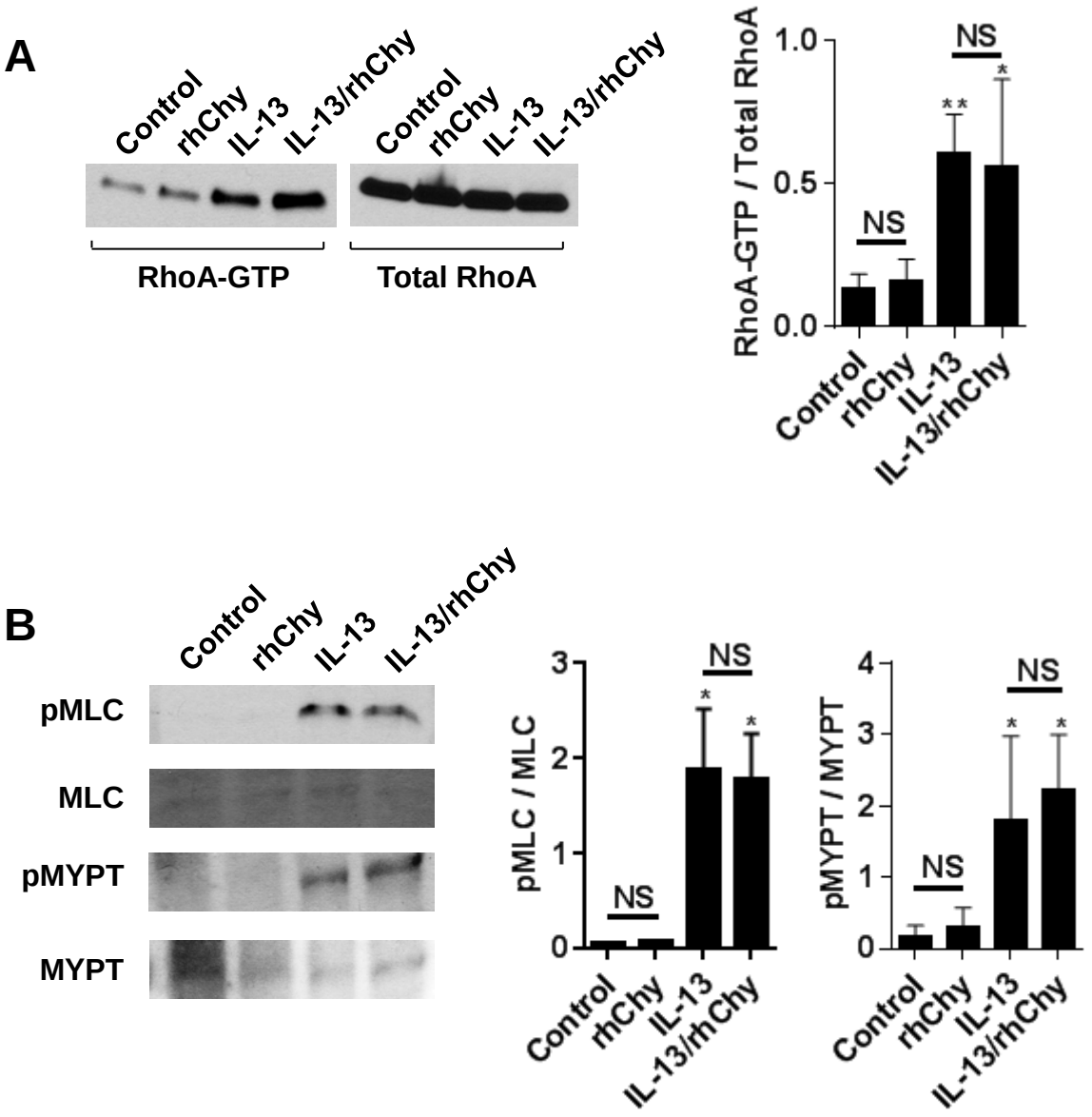




Supplemental Figure 1. Chymase does not alter the classical actin-myosin pathway. (A) Schematic of classical pathways of force generation in airway smooth muscle. Mch = methacholine, IP_3 = inositol triphosphate, SR = sarcoplasmic reticulum. (B) Frequency of calcium

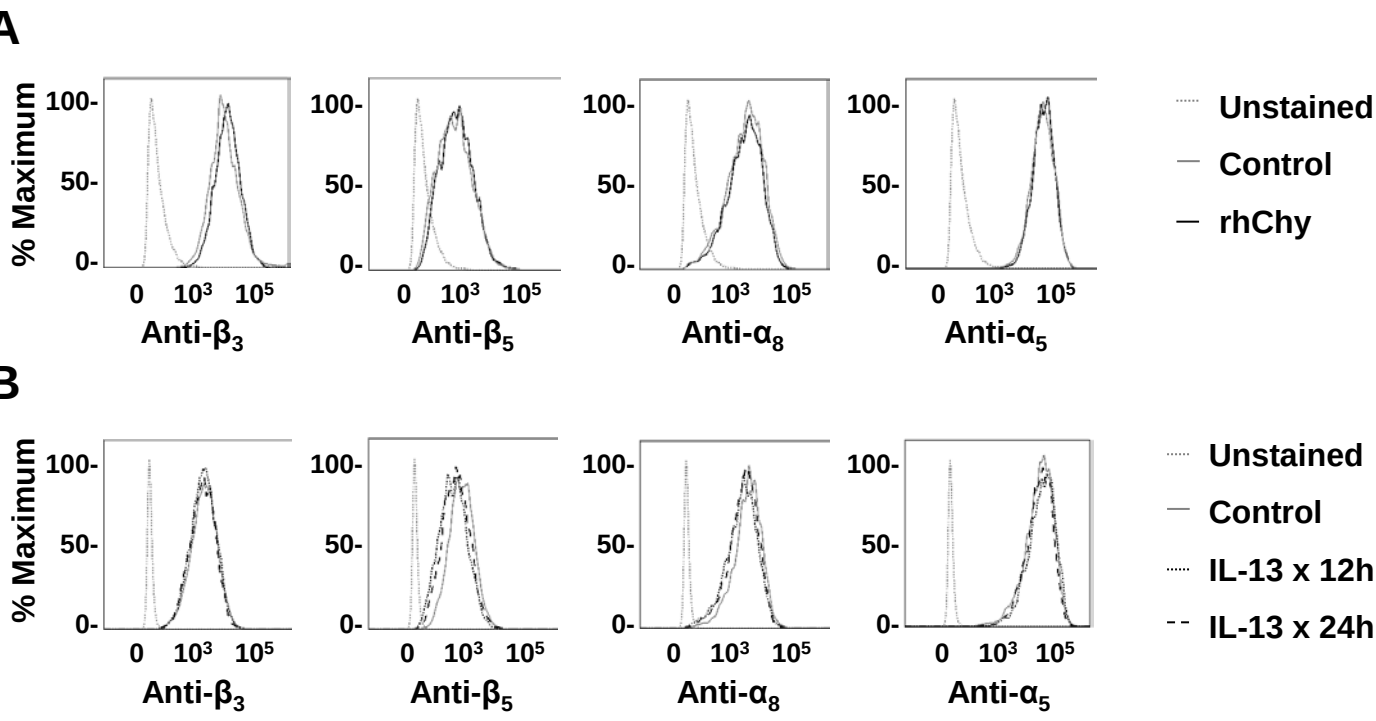
Supplemental Figure 1 (continued)
oscillations in mouse lung slices after incubation for 12 h in DMEM with IL-13 (100 ng/mL), then for 20 min with rhChy (30 nM) or vehicle with a single dose of 10^{-4} M methacholine. n=4 lung slices per group, 2-tailed Student's t-test. (C) Representative western blot and quantitative densitometry of active (RhoA-GTP) and total RhoA in mouse tracheal strips after incubation for 12 h in DMEM with IL-13 (100 ng/mL) or saline, then for 20 min with rhChy (30 nM) or vehicle with a single dose of 10^{-4} M methacholine. (D) Representative western blot and quantitative densitometry for phospho- and total- myosin light chain (MLC) and myosin light chain phosphatase (MYPT1) in mouse posterior tracheal strips after incubation for 12 h in DMEM with IL-13 (100 ng/mL) or saline, then for 20 min with rhChy (30 nM) or vehicle with a single dose of 10^{-4} M methacholine. n=3-5 distinct experiments for C-D. *P<0.05, **P<0.01 vs Control, NS = not significant, 2-way ANOVA for C-D. Data are mean ± SEM for B-D.

Supplemental Figure 2



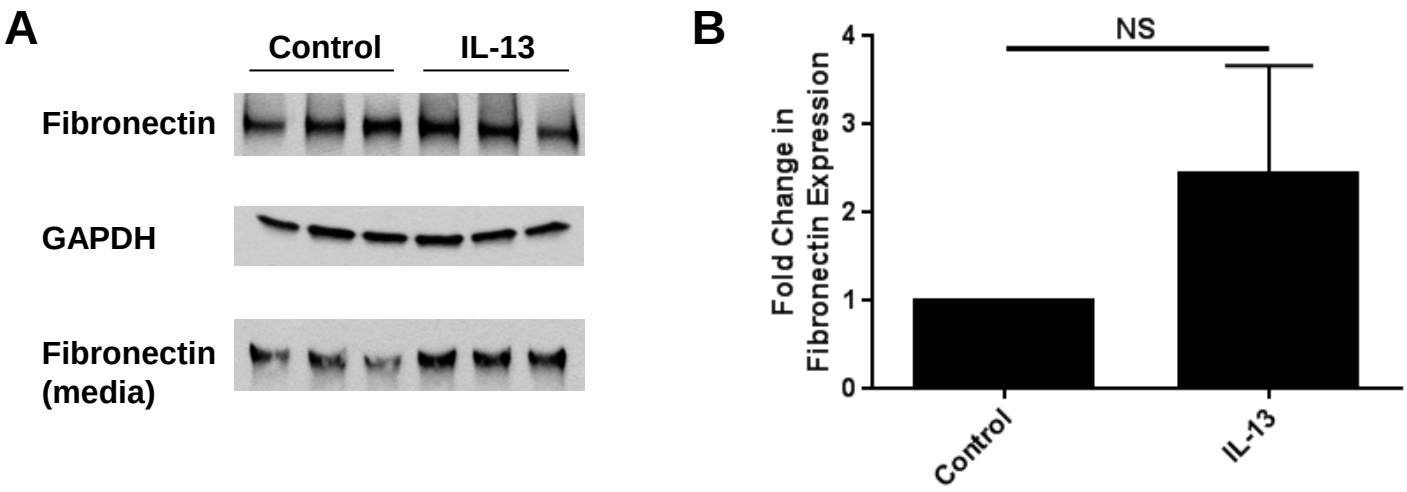
Supplemental Figure 2. Chymase does not alter the classical actin-myosin pathway in human airway smooth muscle cells. (A) Representative western blot and quantitative densitometry of active (RhoA-GTP) and total RhoA in human airway smooth muscle cells after incubation for 12 h in DMEM with IL-13 (100 ng/mL) or saline, then for 20 min with rhChy (30 nM) or vehicle with a single dose of 10^{-4} M methacholine. (B) Representative western blot and quantitative densitometry for phospho- and total- myosin light chain (MLC) and myosin light chain phosphatase (MYPT1) in human airway smooth muscle cells after incubation for 12 h in DMEM with IL-13 (100 ng/mL) or saline, then for 20 min with rhChy (30 nM) or vehicle with a single dose of 10^{-4} M methacholine. n=3-4 distinct experiments for all panels. *P<0.05, **P<0.01 vs Control, NS = not significant, 2-way ANOVA for all panels. Data are mean ± SEM for all panels.

Supplemental Figure 3



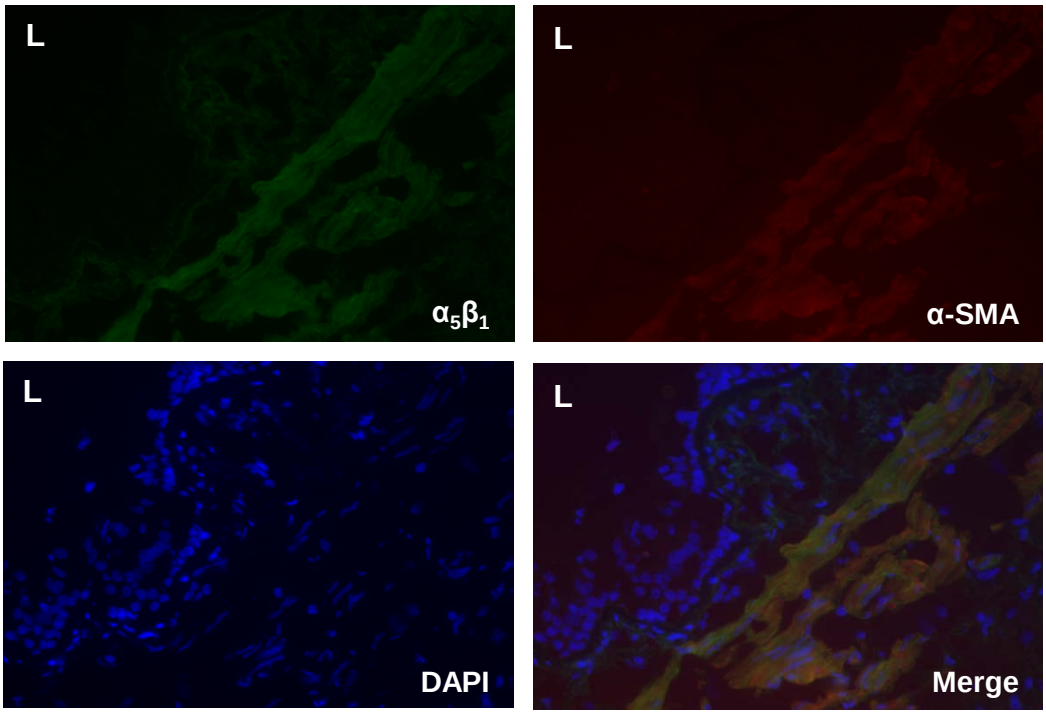
Supplemental Figure 3. Cell surface expression of integrins is not modulated by treatment with chymase or IL-13. Human airway smooth muscle cells were labeled with primary antibodies specific for cell surface integrins β_3, β_5, α_8, α_5 and a secondary antibody conjugated to allophycocyanin (APC). The cells were analyzed by flow cytometry and gated for live cells. The resultant population was analyzed for APC expression. Cells labeled with secondary antibody alone served as a control (Unstained=grey dashed line). Representative histograms of APC expression versus cell count are shown. x axis = APC expression (mean fluorescence intensity), y axis = cell count (percent of maximum). (A) Human airway smooth muscle cells were treated for 20 min with recombinant human chymase (rhChy, 30 nM) or vehicle followed by chymostatin (10 ug/mL). Control vehicle=grey solid line, rhChy=black solid line. (B) Human airway smooth muscle cells were treated with IL-13 (100 ng/mL) for 12 and 24 h or saline. Control saline=grey solid line, IL-13 x12h=black short dashed line, IL-13 x24h=black long dashed line.

Supplemental Figure 4



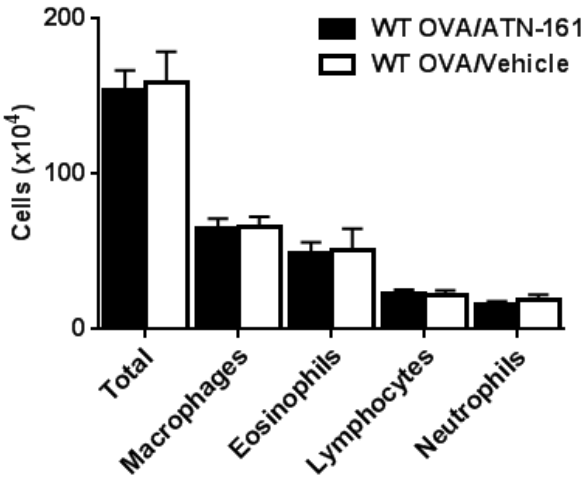
Supplemental Figure 4. Fibronectin expression is not significantly modulated by treatment with IL-13. (A) Western blot of fibronectin in lysates and media of human airway smooth muscle cells that were serum-starved and treated with IL-13 (100 ng/mL) or saline for 12 h. (B) qRT-PCR of fibronectin transcript identified in human airway smooth muscle cells treated with IL-13 (100 ng/mL) or saline for 12 h. RNA was harvested, reverse transcribed, and amplified by qRT-PCR. Values for each mRNA were normalized to GAPDH, and relative quantity was calculated in comparison to expression in saline-treated cells. NS = not significant, 2-tailed Student's t-test. Data are mean ± SEM of three distinct experiments.

Supplemental Figure 5



Supplemental Figure 5. Integrin $\alpha_5\beta_1$ is highly expressed in human airway smooth muscle. Human bronchial rings were embedded in OCT, sectioned into 5 μm sections, and fixed in 4% paraformaldehyde. Immunostaining was performed with anti-integrin $\alpha_5\beta_1$ (green), anti-α-SMA (red), and DAPI (blue). L = airway lumen. Original magnification, x40.

Supplemental Figure 6



Supplemental Figure 6. Treatment with a small molecule inhibitor of $\alpha_5\beta_1$ did not affect allergen induced inflammation. Bronchoalveolar lavage cell counts of total cells, macrophages, eosinophils, lymphocytes, and neutrophils in WT C57Bl/6 mice following immunization and intranasal challenge with OVA, with intranasal administration of ATN-161 (12.5 mg/kg) or vehicle (5% DMSO, 0.9% saline) 1 hour prior to measurements. Data are mean ± SEM, n=8 animals per group.