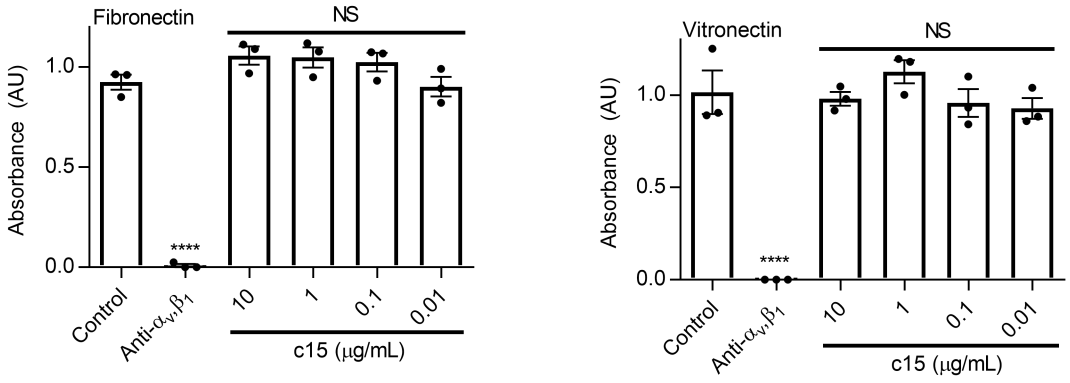
Supplemental Table 1

Gene	F Primer	R Primer
ITGA1	5’-GCTCCTCACTGTTGTTCTACG-3’	5’-CGGGCCGCTGAAAGTCATT-3’
ITGA2	5’-CCTACAATGTTGGTCTCCCAGA-3’	5’-AGTAACCAGTTGCCTTTTGGATT-3’
ITGA2B	5’-GATGAGACCCGAAATGTAGGC-3’	5’-GTCTTTTCTAGGACGTTCCAGTG-3’
ITGA3	5’-TCAACCTGGATACCCGATTCC-3’	5’-GCTCTGTCTGCCGATGGAG-3’
ITGA4	5’-CACAACACGCTGTTCGGCTA-3’	5’-CGATCCTGCATCTGTAAATCGC-3’
ITGA5	5’-GGCTTCAACTTAGACGCGGAG-3’	5’-TGGCTGGTATTAGCCTTGGGT-3’
ITGA6	5’-ATGCACGCGGATCGAGTTT-3’	5’-TTCCTGCTTCGTATTAACATGCT-3’
ITGA7	5’-CAGCGAGTGGACCAGATCC-3’	5’-CCAAAGAGGAGGTAGTGGCTATC-3’
ITGA8	5’-TCAGGCGTTCAACCTGGAC-3’	5’-GCGTCGGGTATGTGGAAGTC-3’
ITGA9	5’-TCGTTCTTCGGCTACGCAG-3’	5’-CCCCATCCACTCATCATCGC-3’
ITGA10	5’-AACATCACCCACGCCTATTCC-3’	5’-GTTGGTAGTCACCTAAGTGGC-3’
ITGA11	5’-GTGGCAATAAGTGGCTGGTC-3’	5’-GTTCCCGTGGATCACTGGAC-3’
ITGAD	5’-TCGGTGGATCTCGACTCGT-3’	5’-GCAGGAACCCTTTGAGTATGAG-3’
ITGAE	5’-TGCTGGCCGCTTTCAATGT-3’	5’-ACAGGATGGCAAAGGATTTCAT-3’
ITGAL	5’-TGCTTATCATCATCACGGATGG-3’	5’-CTCTCCTTGGTCTGAAAATGCT-3’
ITGAM	5’-GCCTTGACCTTATGTCATGGG-3’	5’-CCTGTGCTGTAGTCGCACT-3’
ITGAX	5’-AGAGCTGTGATAAGCCAGTTCC-3’	5’-AATTCCTCGAAAGTGAAGTGTGT-3’
ITGB3	5’-GTGACCTGAAGGAGAATCTGC-3’	5’-CCGGAGTGCAATCCTCTGG-3’
ITGB4	5’-GCAGCTTCCAAATCACAGAGG-3’	5’-CCAGATCATCGGACATGGAGTT-3’
ITGB5	5’-TCTCGGTGTGATCTGAGGG-3’	5’-TGGCGAACCTGTAGCTGGA-3’
ITGB6	5’-TCCATCTGGAGTTGGCGAAAG-3’	5’-TCTGTCTGCCTACACTGAGAG-3’
ITGB7	5’-AGAATGGCGGAATCCTCACCT-3’	5’-TGAAGTTCAGTTGCTTGCACC-3’
ITGB8	5’-ACCAGGAGAAGTGTCTATCCAG-3’	5’-CCAAGACGAAAGTCACGGGA-3’
GAPDH	5’-GGAGCGAGATCCCTCCAAAAT-3’	5’-GGCTGTTGTCATACTTCTCATGG-3’

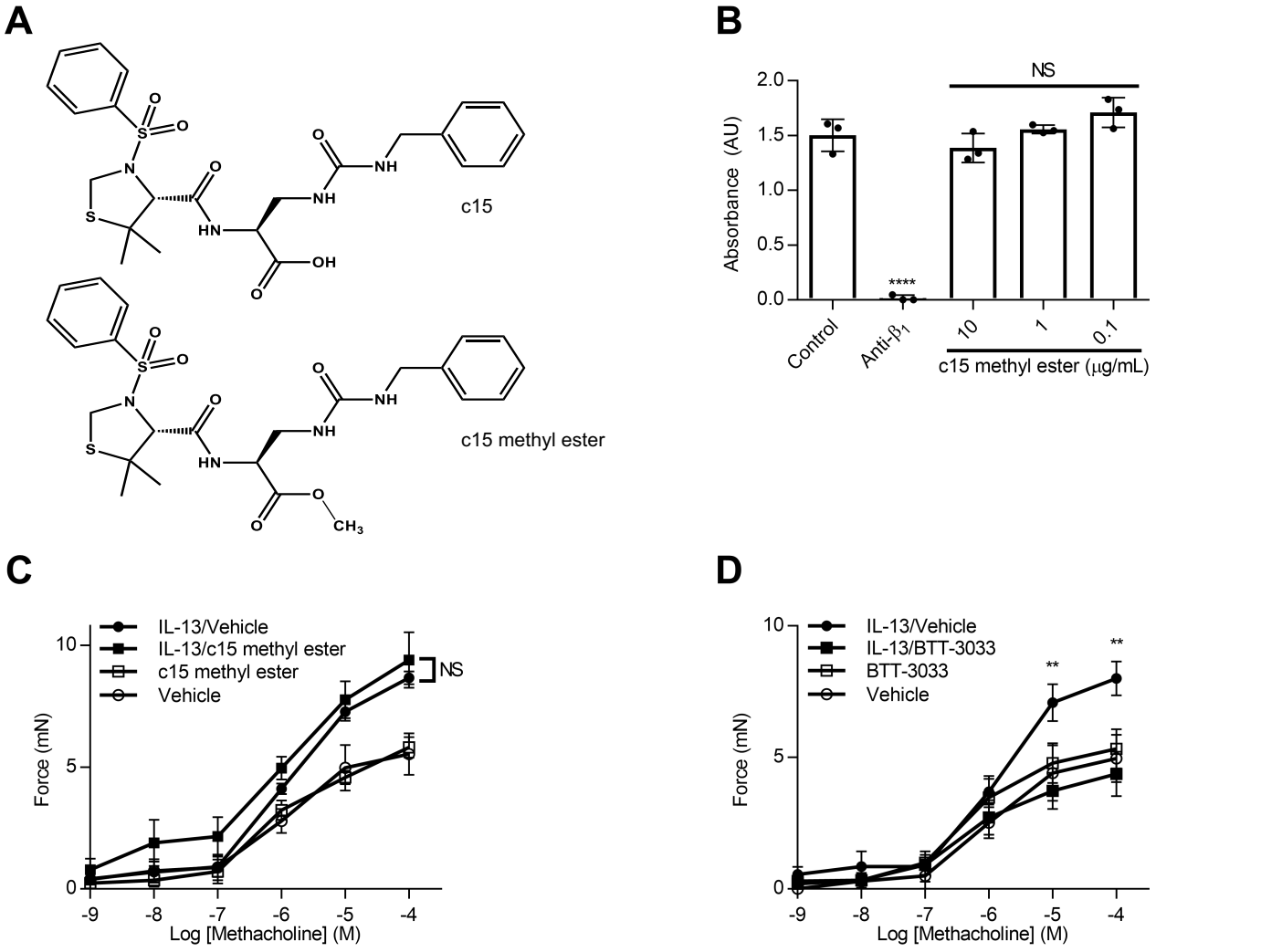
Supplemental Table 1. Primers used for qRT-PCR analysis.

Supplemental Figure 1



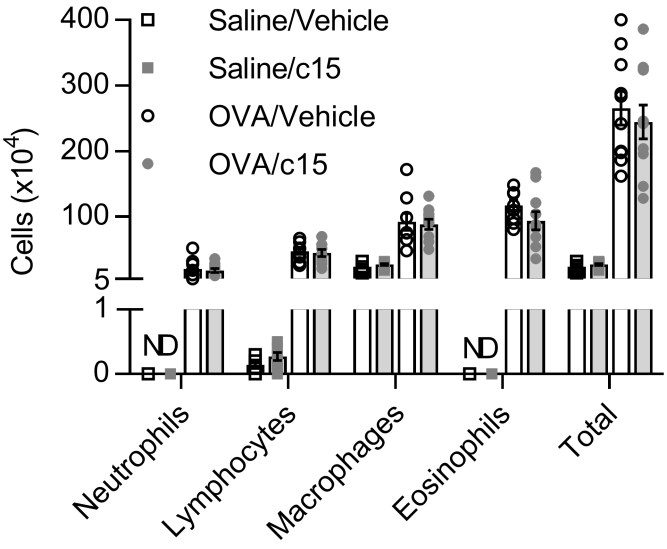
Supplemental Figure 1. c15 does not mediate adhesion to fibronectin or vitronectin. Adhesion (measured by absorbance of crystal violet at 595 nm) of human airway smooth muscle cells to fibronectin (5 μg/mL) and vitronectin (5 μg/mL) after treatment with the indicated concentrations of c15. Experiment performed in triplicate with three biologic replicates. NS=not significant, ****P<0.0001 compared to control. 1-way ANOVA with Dunnett's multiple comparisons test. Data are mean ± SEM.

Supplemental Figure 2



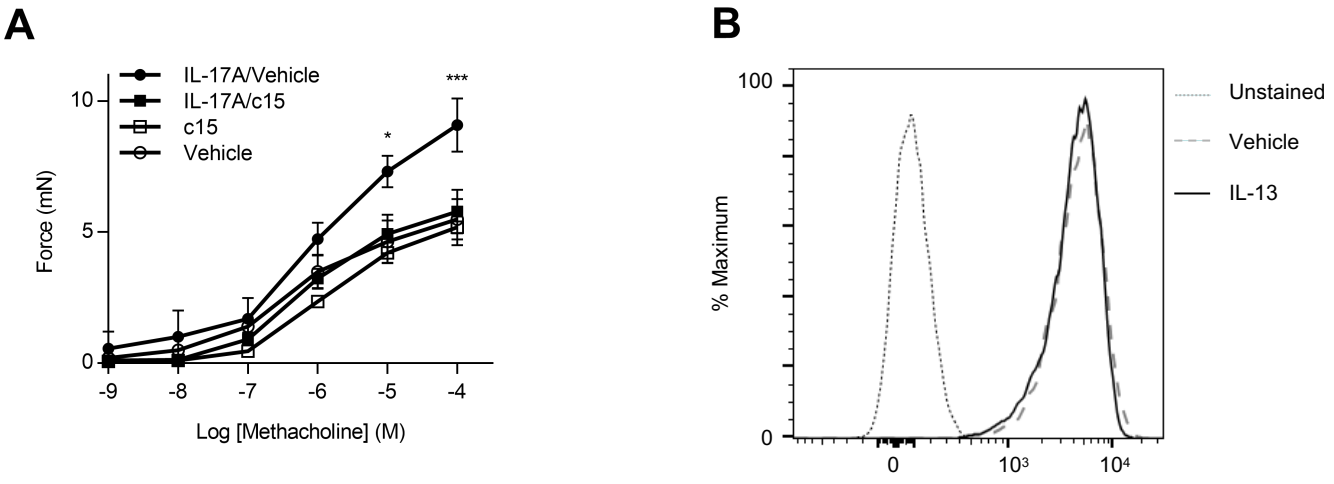
Supplemental Figure 2. The protective effect of c15 is mediated by integrin $\alpha_2\beta_1$. (A) Chemical structure of c15 and c15 methyl ester. (B) Adhesion (measured by absorbance of crystal violet at 595 nm) of human airway smooth muscle cells to collagen I (0.1 μg/mL) after treatment with the indicated concentrations of c15 methyl ester. Experiment performed in triplicate with two biologic replicates. NS=not significant, ****P<0.0001 compared to control. 1-way ANOVA with Dunnett's multiple comparisons test. (C-D) Force exerted on mouse tracheal rings measured after incubation for 12 h with IL-13 (100 ng/mL) or saline, then 1 h with (C) c15 methyl ester (10 μg/mL) or (D) BTT-3033 (10 μM), or vehicle with a range of concentrations of methacholine. n=3-5 rings per group. NS=not significant, **P<0.01 between IL-13 treated conditions. 2-way ANOVA with repeated measures, Tukey's multiple comparisons test. Data are mean ± SEM for all panels.

Supplemental Figure 3



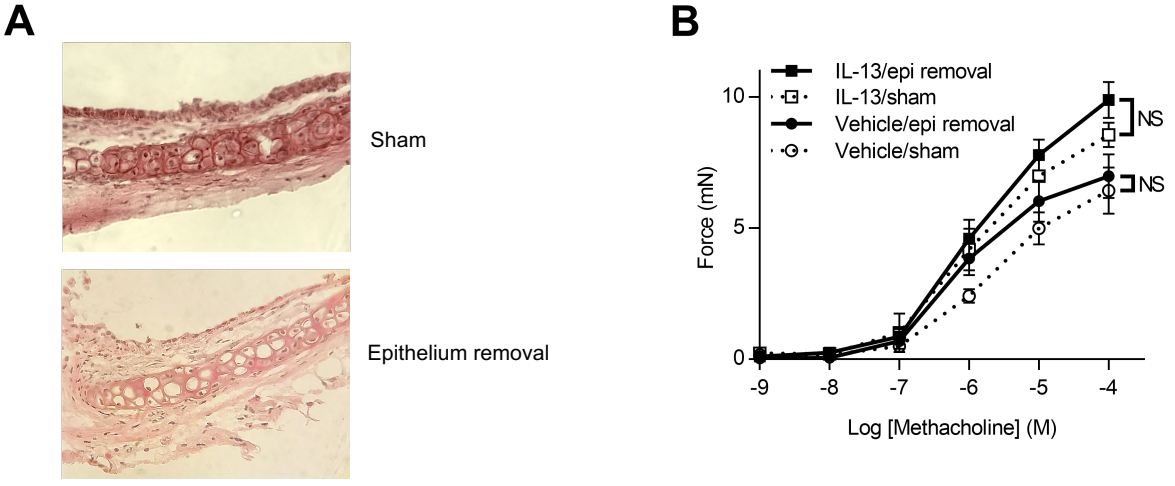
Supplemental Figure 3. Treatment with a small molecule inhibitor of $\alpha_2\beta_1$ did not affect allergen induced inflammation. Bronchoalveolar lavage cell counts in WT C57Bl/6 mice following immunization and intranasal challenge with OVA, with intraperitoneal administration of c15 (120 mg/kg) or vehicle (50% DMSO, 0.9% saline) 30 mins prior to measurements. n=9-10 animals per group. ND=not detected. Data are mean ± SEM.

Supplemental Figure 4



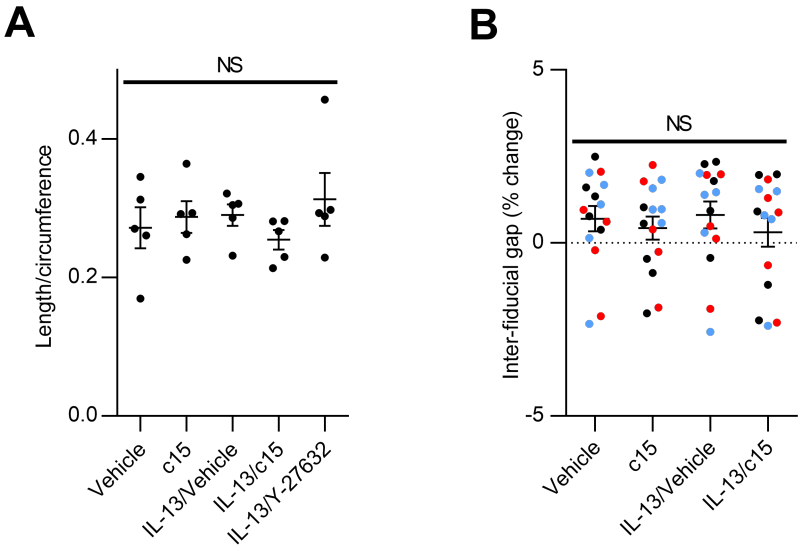
Supplemental Figure 4. c15 protects against IL-17A exaggerated contraction and IL-13 does not affect surface expression of integrin $\alpha_2\beta_1$. (A) Force exerted on mouse tracheal rings measured after incubation for 12 h with IL-17A (100 ng/mL) or saline, then 1 h with c15 (10 μg/mL) or vehicle, followed by a range of concentrations of methacholine. n=3 rings per group. ***P<0.001, *P<0.05 between IL-13 treated conditions. 2-way ANOVA with repeated measures, Tukey's multiple comparisons test. Data are mean ± SEM. (B) Human airway smooth muscle cells were incubated for 12 h with IL-13 (100 ng/mL) or saline, then suspended and labeled with a primary antibody specific for integrin α_2 and a secondary antibody conjugated to allophycocyanin (APC). Cells were then analyzed by flow cytometry and gated for live cells. The resultant population was analyzed for APC expression. Human airway smooth muscle cells labeled with secondary antibody alone served as an unstained control (grey short dashed line). Vehicle=grey long dashed line. IL-13=black solid line. Representative histogram of APC expression (mean fluorescence intensity) versus cell count scaled to mode (percent of maximum) is shown. Results verified with two biologic replicates.

Supplemental Figure 5



Supplemental Figure 5. The protective effect of c15 persists after epithelial debridement. (A) Representative examples of H&E stained sections from tracheal rings that underwent brushing to remove epithelium or a sham treatment, 40X magnification. (B) Force exerted on mouse tracheal rings with or without debridement of the epithelium, measured after incubation for 12 h with IL-13 (100 ng/mL) or saline with a range of concentrations of methacholine. NS=not significant between indicated conditions. (C) Force exerted on mouse tracheal rings debrided of the epithelium after incubation for 12 h with IL-13 (100 ng/mL) or saline, then 1 h with c15 (10 µg/mL) or vehicle, followed by a range of concentrations of methacholine. n=3-4 rings per group. NS=not significant, **P<0.01, *P<0.05 between IL-13 treated conditions. 2-way ANOVA with repeated measures, Tukey's multiple comparisons test for all panels. Data are mean ± SEM for all panels.

Supplemental Figure 6



Supplemental Figure 6. Tracheal rings are similar at baseline and c15 does not alter non-muscle portions of the trachea. (A) Ratio of smooth muscle length to tracheal ring circumference was calculated at baseline for tracheal rings used in two-photon experiments in Figure 5. n=5 rings per group. NS=not significant. 1-way ANOVA with Tukey's multiple comparisons test. (B) Quantification of change in distance between intrinsic fiducial markers after methacholine (10^{-4} M) relative to baseline after incubation for 12 h with IL-13 (100 ng/mL) or saline, then 1 h with c15 (10 µg/mL) or vehicle. n=3 rings per group, each sample labeled by color. NS=not significant. 2-way ANOVA with Tukey's multiple comparisons test. Data are mean ± SEM for all panels.

Supplemental Video 1

Supplemental Video 1. 3-Dimensional View of Trachea. 3-D rotation of tracheal ring showing α-SMA (red) and collagen fibrils and tissue autofluorescence (green) before treatment with methacholine (Scale Bar: 200 μm).

Supplemental Video 2

Supplemental Video 2. 3-Dimensional View of Trachea. 3-D rotation of tracheal ring showing α-SMA (red) and collagen fibrils and tissue autofluorescence (green) after treatment with methacholine (Scale Bar: 200 μm).