Supplementary Information

Integrin α9β1 in airway smooth muscle regulates a novel brake on exaggerated murine and human airway narrowing

By

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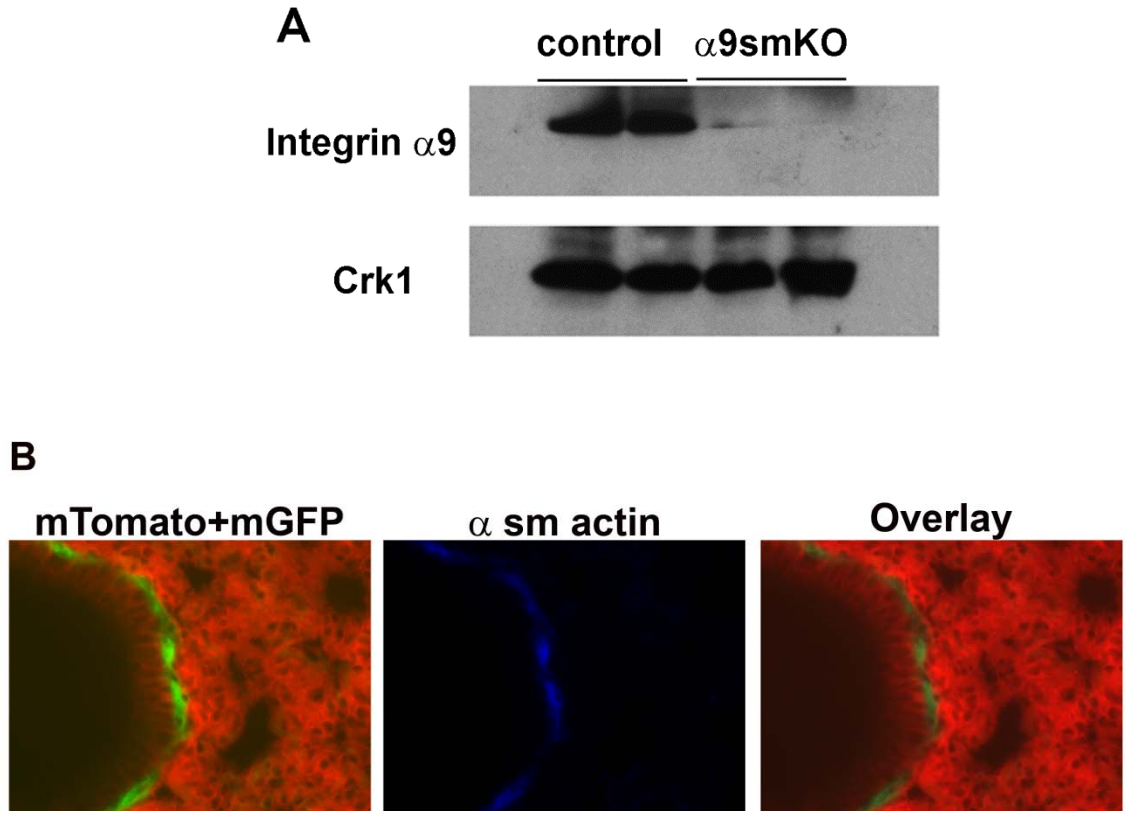
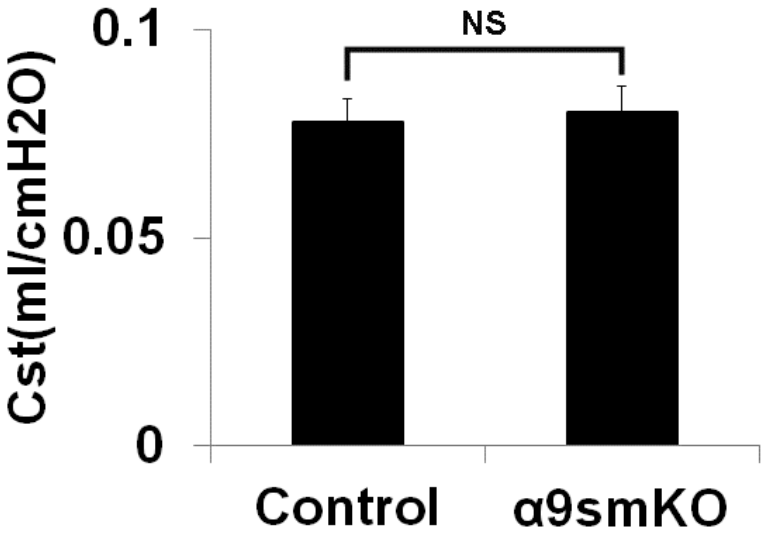
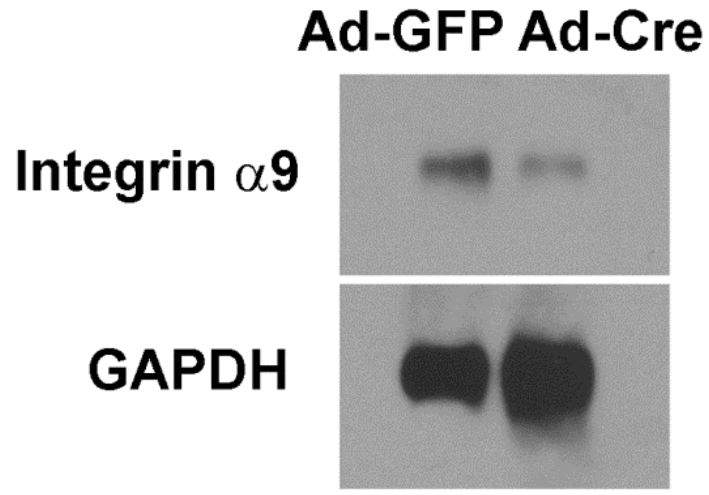


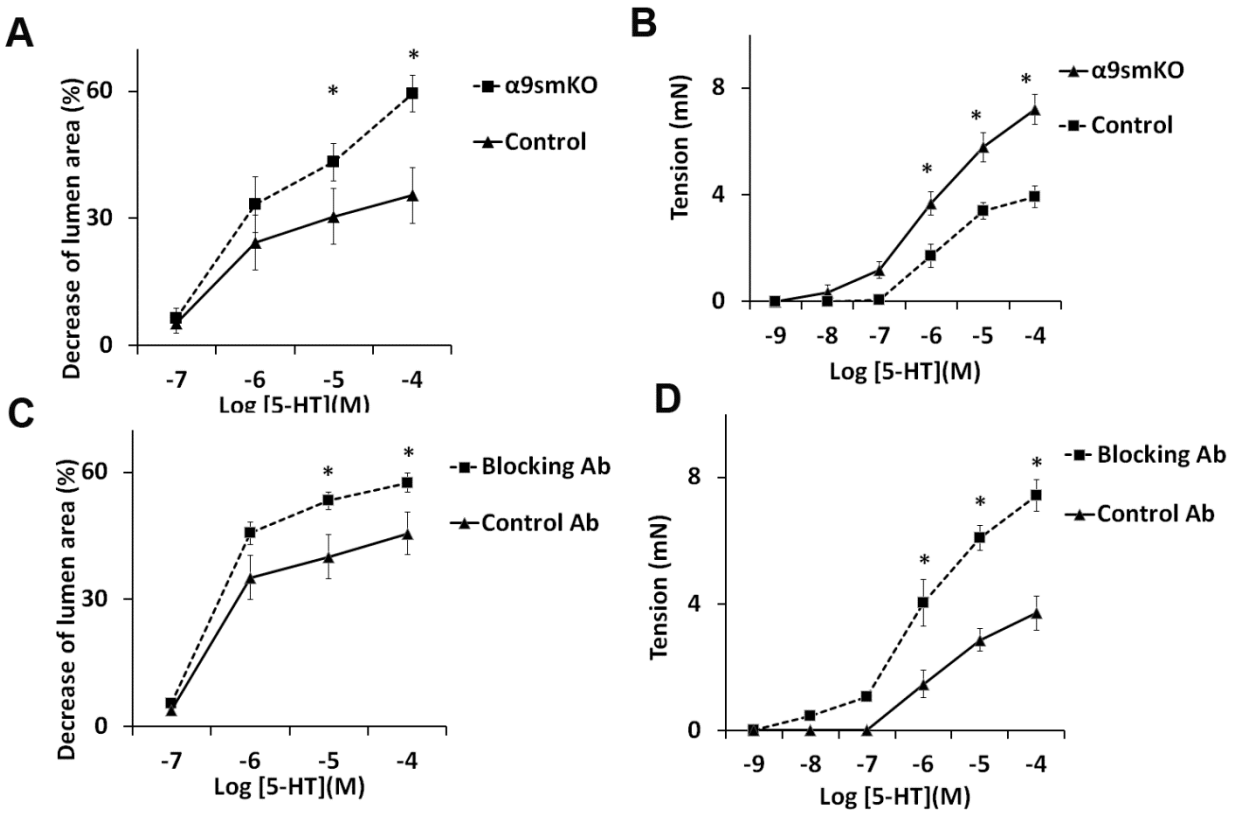
Supplementary Figure 1: (A) α9smKO mice have decreased integrin α9β1 expression. Airway smooth muscle cells were purified from mouse tracheal tissues. Integrin α9 was detected by Western blotting with rabbit polyclonal anti-α9. Western blot for Crk1 was used as a control for equal protein loading. (B) The tissue specificity of Cre recombinase. Image of fixed tissue sections of mT/mG; α-sm-rTTA; (tetO)7-Cre mouse lung demonstrating the specific expression of Cre recombinase in smooth muscle(left panel) which is confirmed by α smooth muscle actin staining(right panel, overlay of EGFP and Alexa 350 signals).



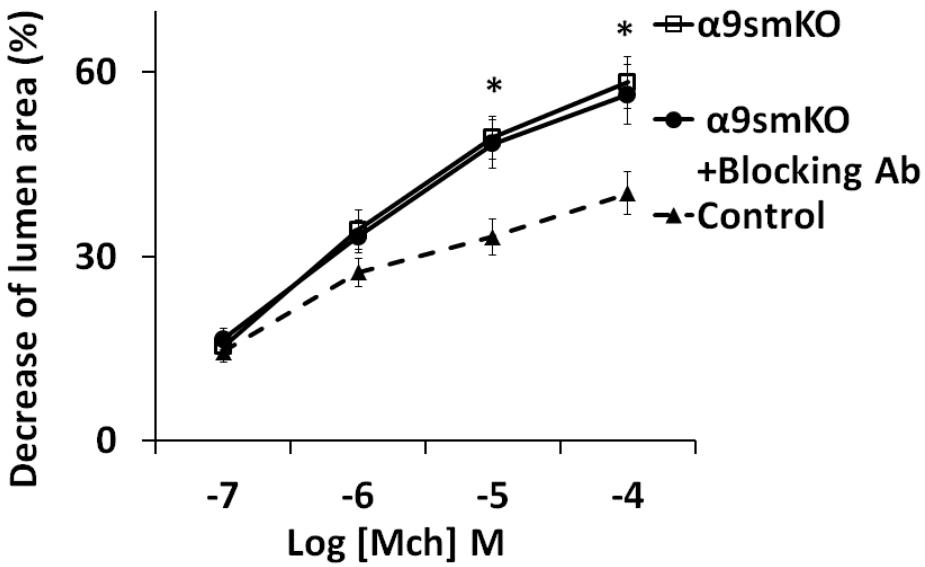
Supplementary figure 2: No different in static lung compliance between α9smKO and control mice. Cst stands for Quasi-static Compliance.



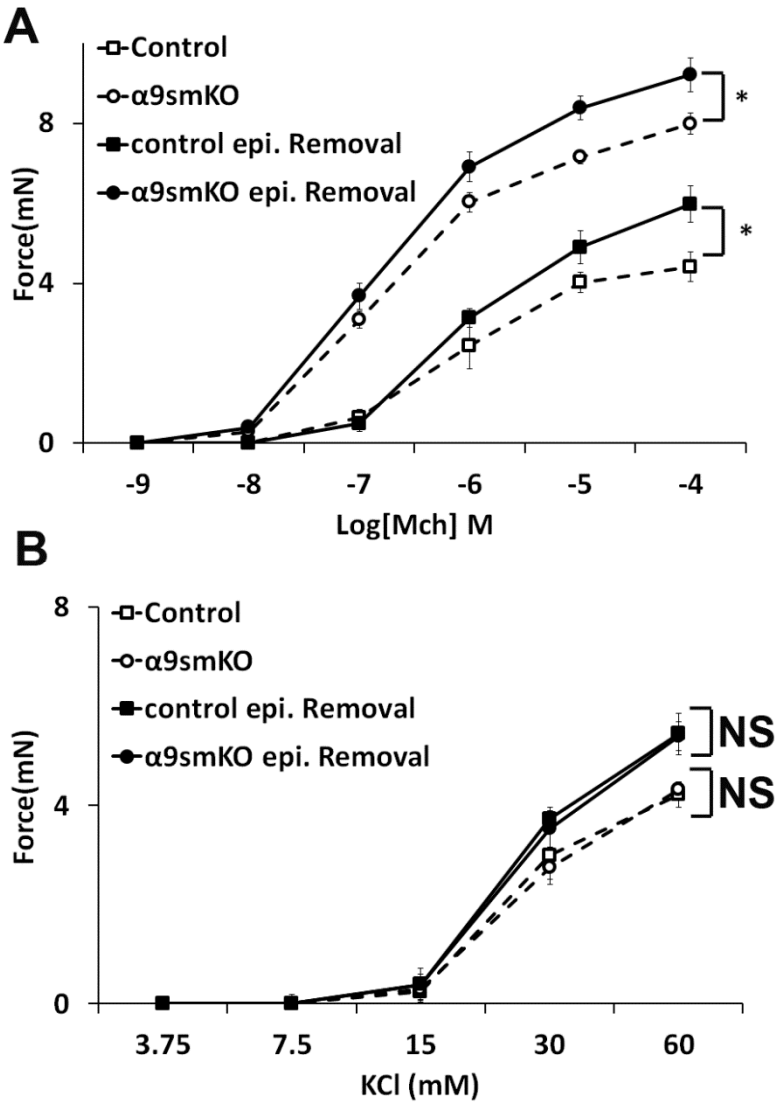
Supplementary Figure 3: Integrin α9β1 expression in mouse lung slices was reduced by adenovirus-Cre infection after 3 days. Integrin α9 was detected by Western blotting with rabbit polyclonal anti-α9. Western blot for GAPDH was used as a control for equal protein loading. Adenovirus expression GFP was used as virus infection control.



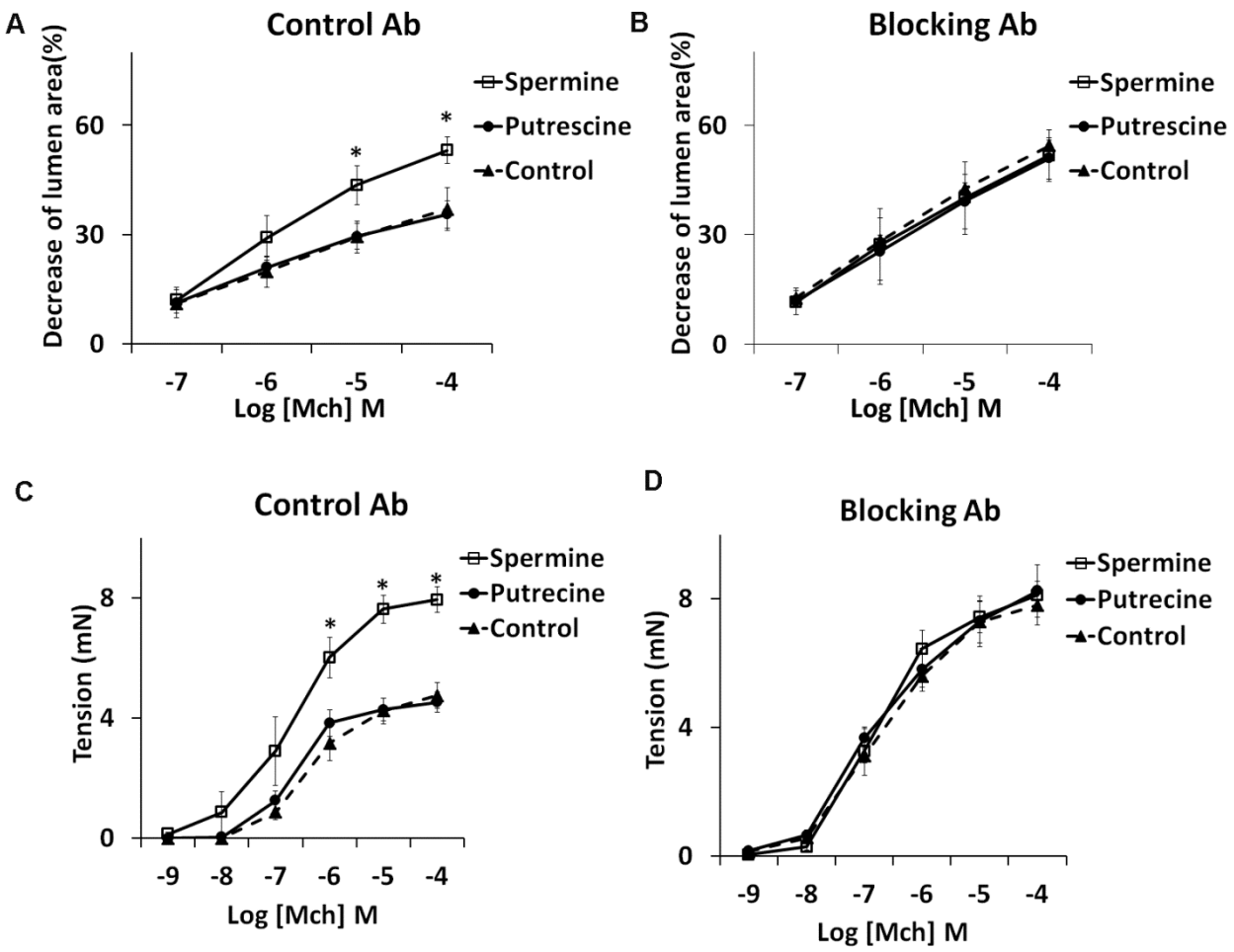
Supplementary figure 4 Airway narrowing and tension generation induced by 5-HT. Lumen area in response to 5-HT was assessed in mouse lung slices (A and C) from α9smKO or control mice (A) or wild type lung slices treated with α9-blocking antibody(C). Tension generation in response to 5-HT was measured in tracheal ring from α9smKO or control mice (B) or in rings from wild type mice treated with blocking antibody or control IgG (D). * $p<0.01$.



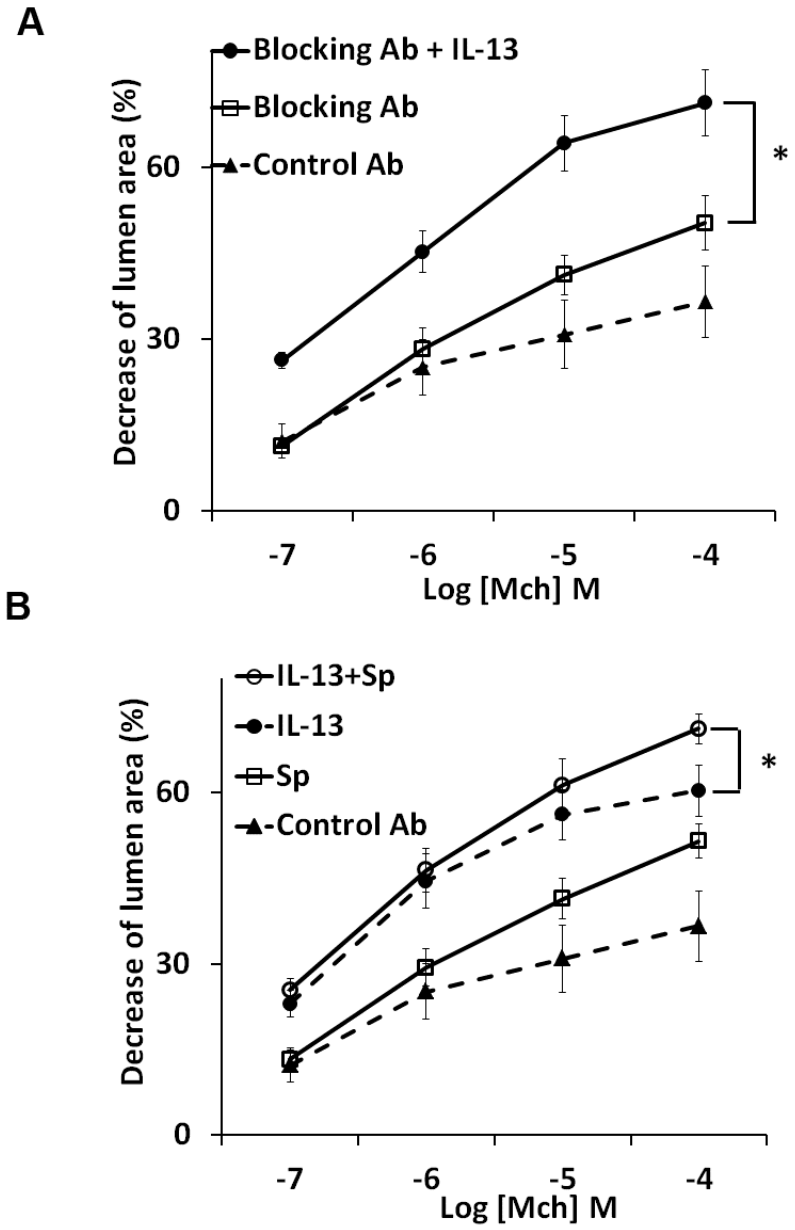
Supplementary Figure 5: Integrin α9β1 blocking antibody does not further increase airway narrowing in lung slices from α9smKO mice. Lung slices from α9smKO mice were treated with blocking hamster monoclonal antibody to murine α9β1 (or control hamster IgG) for 24 hours. Methacholine-induced airway narrowing was measured.



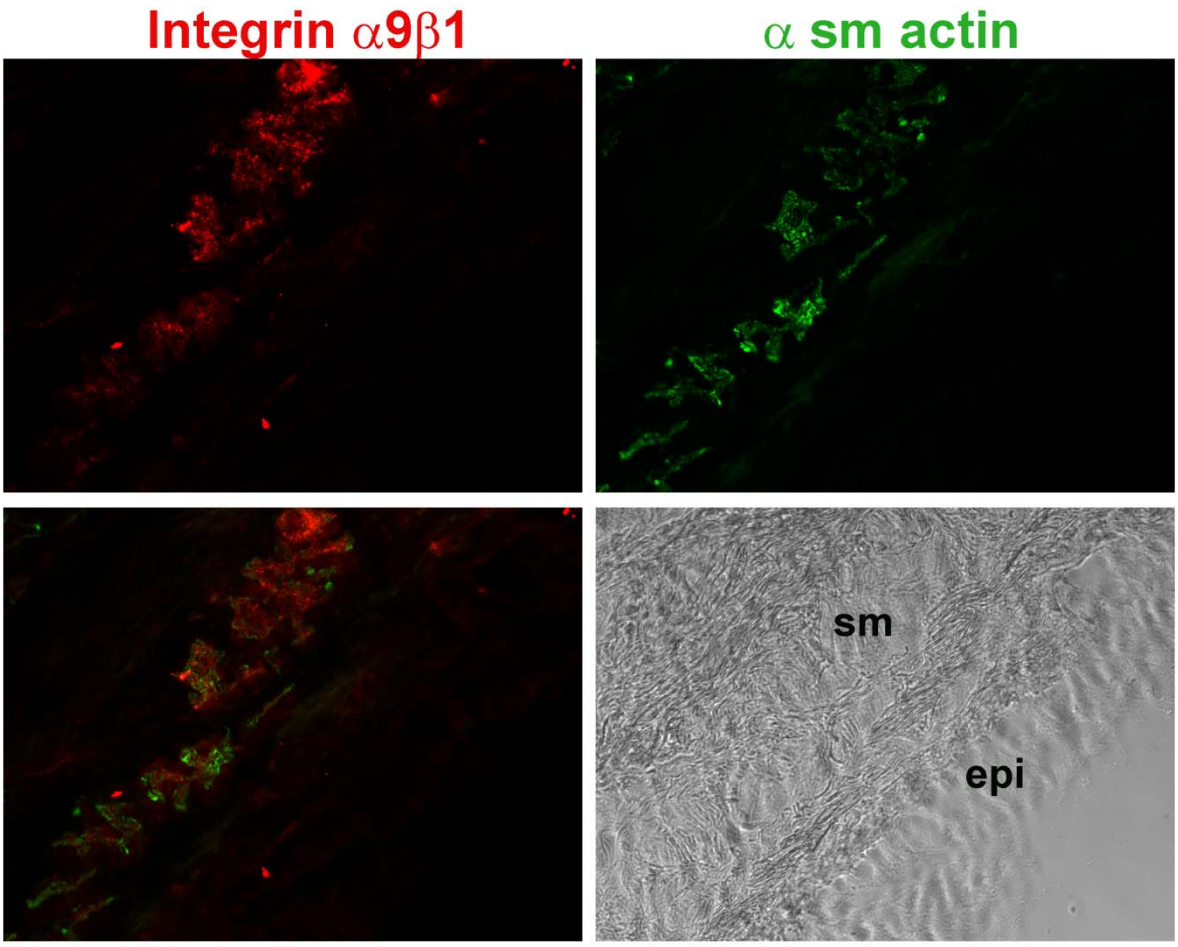
Supplementary Figure 6: Tracheal rings with epithelium intact or removed (n = 6 per group) from control or α9smKO. Contractility to methacholine (A) and KCl (B) were measured. There is significant difference between control and α9smKO after epithelium removed, but the effects of removal are similar in control and α9smKO tracheal rings. * P<0.01.



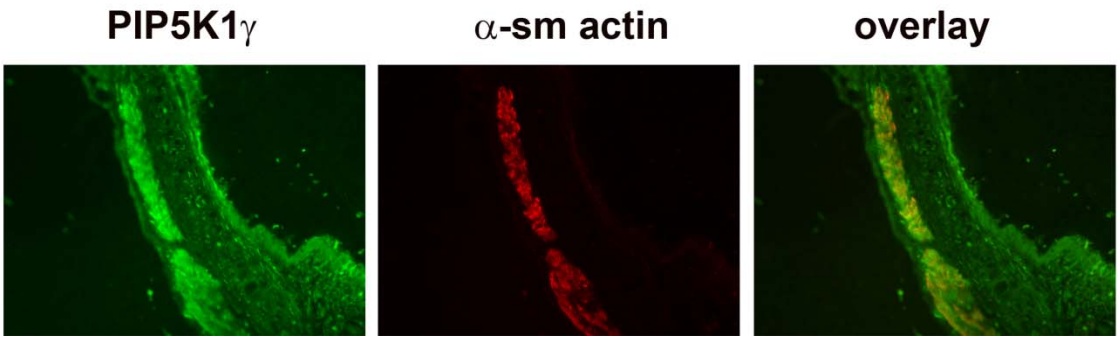
Supplementary figure 7: Dependence of effect of airway smooth muscle α9β1 on polyamine catabolism(A and B). Airway narrowing in lung slices (treated with control IgG (A) or blocking Ab (B) incubated with exogenous spermine (100 uM) or putrescine(100 uM) for 24 hours. Force generation in response to methacholine by tracheal rings treated with spermine (100 uM) or putrescine (100 uM) together with control Ab(C) or blocking Ab(D) for 24 hours. * $p<0.01$



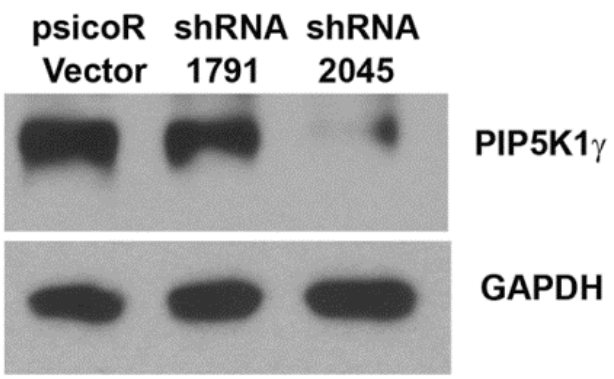
Supplementary Figure 8: Mouse lung slices treated with IL-13. (A) Mouse lung slices (n=5 per group) were treated with blocking antibody (10 µg/ml) and/or IL-13 (50ng/ml) for 24 hours before assessment of airway narrowing to methacholine. (B) Mouse lung slices (n=5 per group) were treated with IL13 with or without spermine (Sp, 100 µM) for 24 hours. * $p<0.01$.



Supplementary Figure 9: Integrin α9β1 is expressed in human bronchial smooth muscle. Immunofluorescence staining of human bronchial smooth muscle cells (arrow) with antibodies against integrin-α9 (red) and α-smooth muscle actin(green). sm : smooth muscle cells; epi: epithelial cells.



Supplementary Figure 10: Expression of PIP5K1γ in tracheal smooth muscle. Immunofluorescence staining of adult mouse trachea with rabbit anti-PIP5K1γ (Abcam,) and Cy3 labeled anti- α-smooth muscle actin (Sigma).



Supplementary figure 11: Knockdown of PIP5K1γ expression by shRNA. (A) Lysate from mouse embryonic fibroblast cells infected with empty lentivirus (pSicoR) or two shRNA lentivirus designed to target PIP5K1γ (1791 and 2045) were blotted by anti-PIPKIγ and GAPDH.