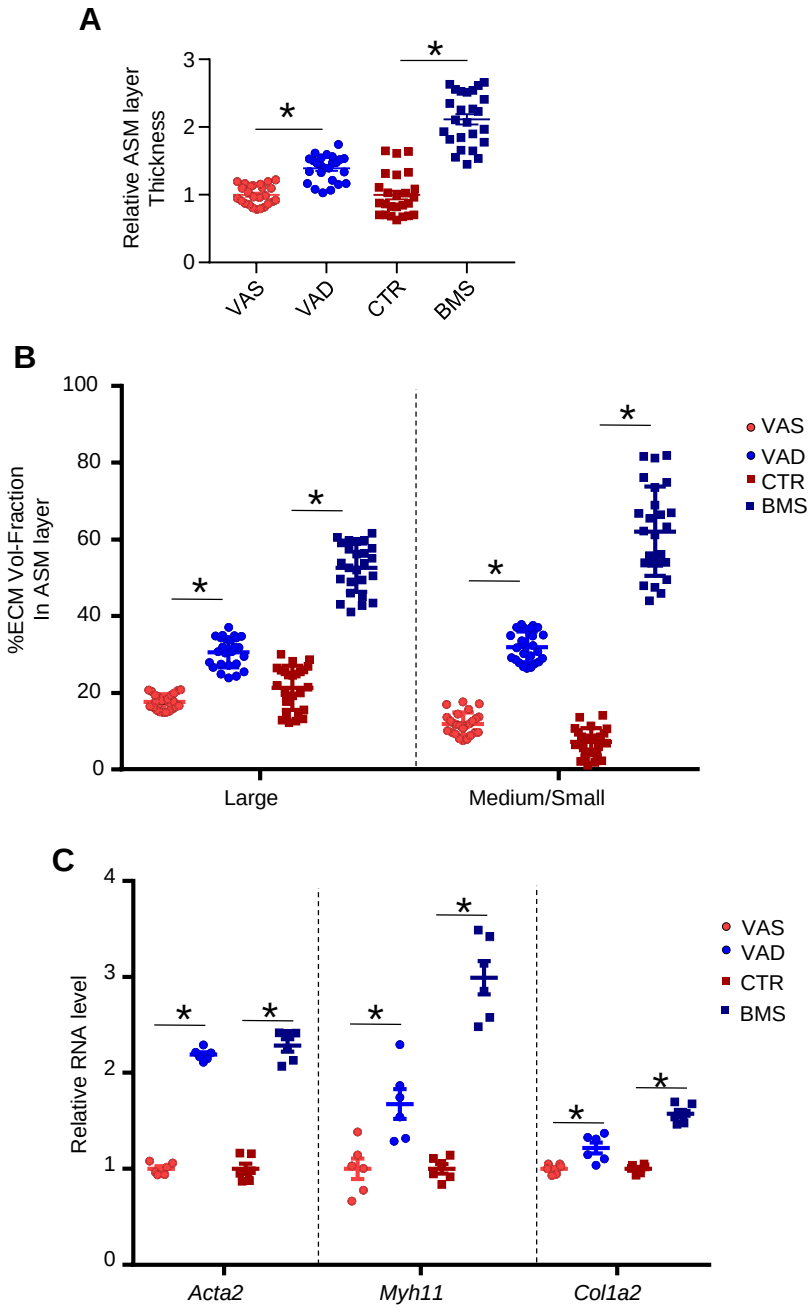
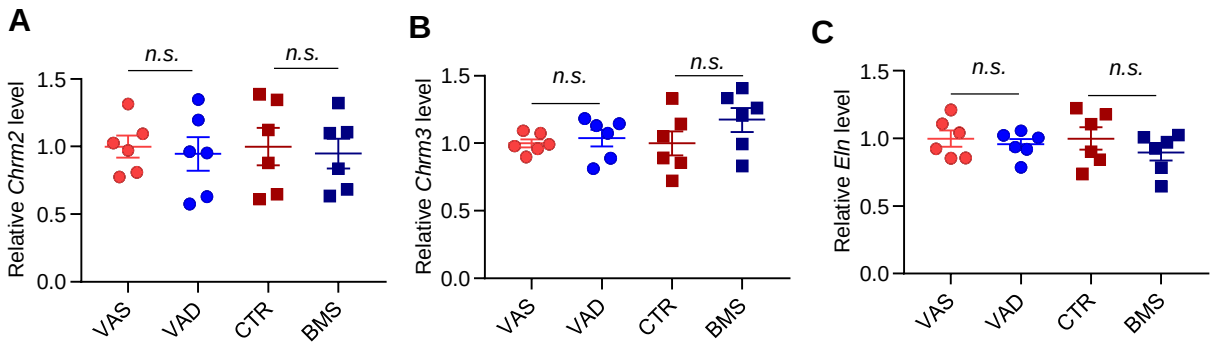
# Supplemental Figure 1



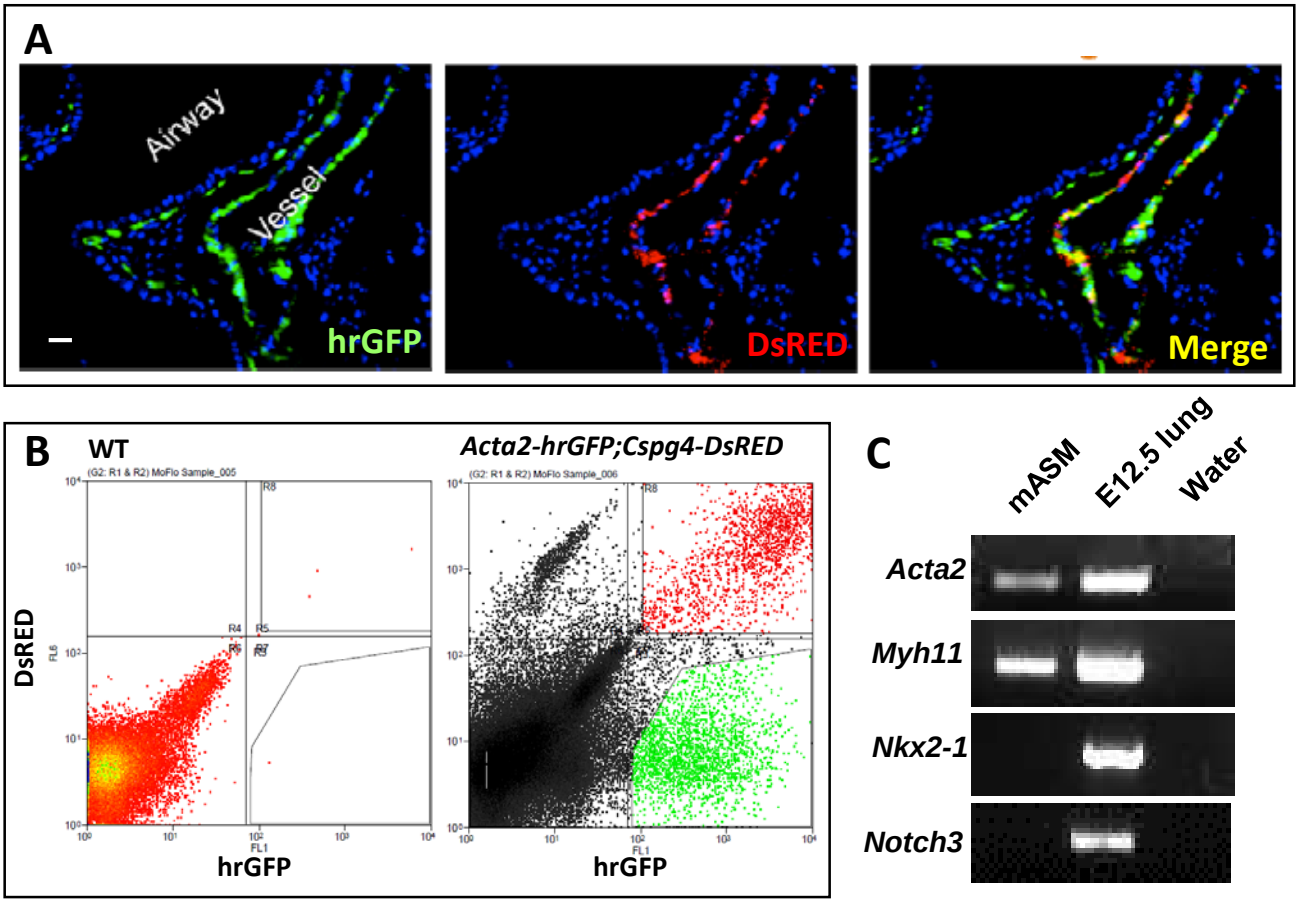

**Supplemental Figure 1. Disruption of RA signaling in vivo for five days results in the increase in ASM layer thickness, airway collagen deposition, and expression of smooth muscle-specific genes and collagen.** (A) ASM layer is significantly thicker in airways of mice fed with VADthan the airways of mice fed with VAS diet. Similarly, ASM layer in BMS mice is also significantly thicker than the CTR mice (n = 3 mice/group; 8-9 airways measured per mouse). (B) The amount of extracellular matrix (ECM) deposition within the large and medium/small airways, quantified as % ECM volume (vol)-fraction, is increased in VAD (compared to VAS) and BMS (compared to CTR) airways (n = 3 mice/group; 8-9 airways measured per mouse). (C) Expressions of smooth muscle-specific markers (*Acta2* and *Myh11*) and collagen (*Col1a2*) are increased in VAD lung (compared to VAS lung) and BMS lung (compared to CTR lung) (n = 6 mice/group). Data represent mean ± SEM. *$p < 0.05$, (Student's *t*-test).

# Supplemental Figure 2



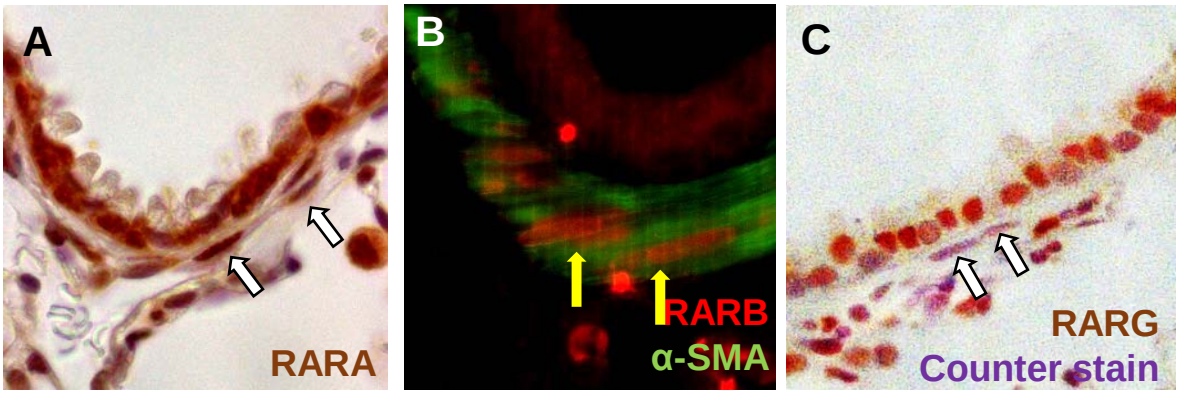

**Supplemental Figure 2. Disruption of RA signaling in vivo for five days does not change expression level of cholinergic receptors and elastin in the lung.** (A-C) Expression levels of cholinergic receptor, muscarinic 2 (*Chrm2*) (A), cholinergic receptor, muscarinic 3 (*Chrm3*) (B), and elastin *(Eln*) (C) are not changed between VAS and VAD lungs, nor are they different between CTR and BMS lungs. (n = 6 mice/group). Data represent mean ± SEM . *n.s.* indicate $p \geq 0.05$ by Student's *t*-test.

# Supplemental Figure 3



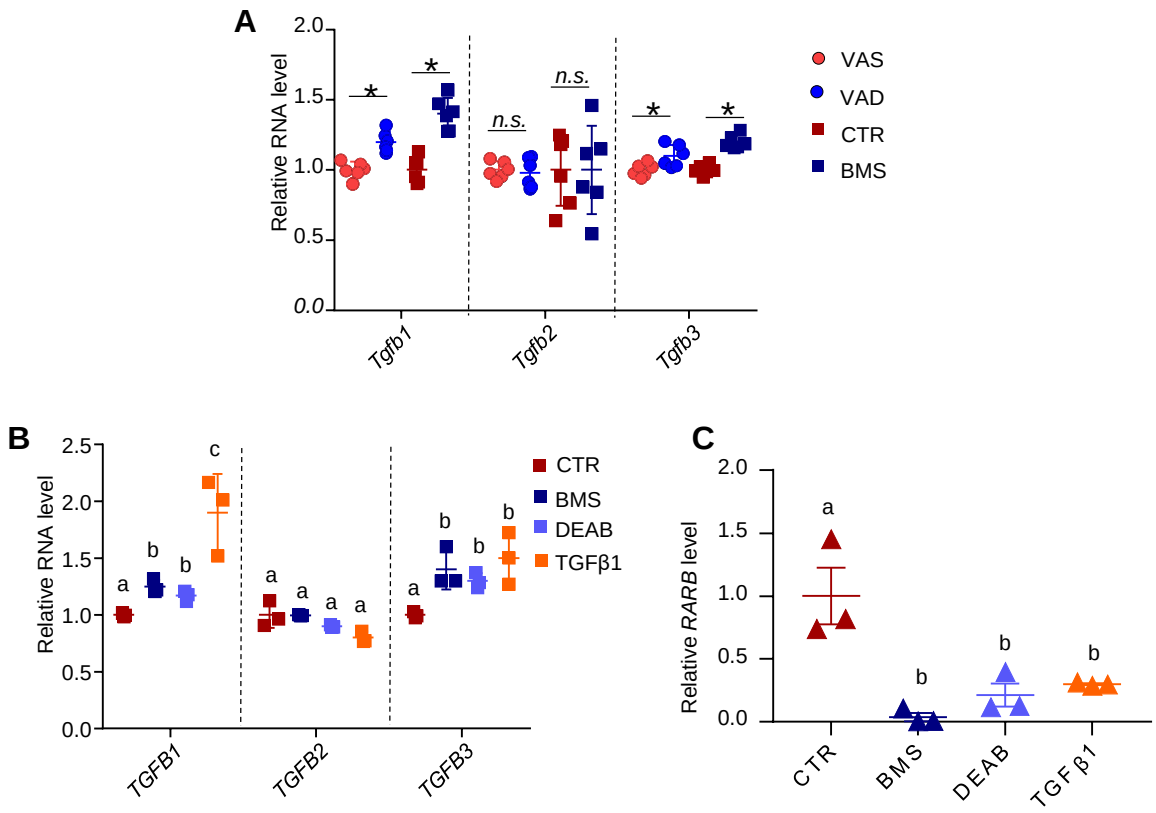

**Supplemental Figure 3. Isolation of mouse ASM (mASM) from *Acta2-hrGFP*;*Cspg4-DsRED* lung.** (A) Representative images from *Acta2-hrGFP*;*Cspg-DsRED* lung. hrGFP localizes to both ASM and vascular smooth muscle (left) whereas DsRED localizes only to vascular smooth muscle (middle) in *Acta2-hrGFP*;*Cspg-DsRED* lung. Merged image shows that ASM was hrGFP$^{+}$DsRED$^{-}$ (right) (n = 3). (B) Flow cytometry of WT (left) and *Acta2-hrGFP*;*Cspg4-DsRED* (right) lung cell preparations. The box at lower right quadrant indicates hrGFP$^{+}$DsRED$^{-}$ cells (mASM) (n = 6 per group). (C) PCR analysis of isolated mASM reveals expression of smooth muscle markers (*Acta2*, *Myh11*), but not the expression of lung epithelial marker *Nkx2-1* or the vascular smooth muscle marker *Notch3*. cDNA from E12.5 lung is used as a positive control (n = 3). Bar in A = 10 µm.

# Supplemental Figure 4




**Supplemental Figure 4. RA receptors are expressed in mouse ASM**. (A-C) Immunostaining of RA receptors in WT mouse lung reveals expression of retinoic acid receptor-alpha (RARA) (A) and beta (RARB) (B) but not gamma (RARG) (C) in the ASM (arrows), suggesting that mouse ASM is capable of turning on RA receptor-mediated signaling (n = 3). Lung in (B) is also immunostained with the smooth muscle specific marker α –SMA, showing colocalization with RARB signals.

# Supplemental Figure 5




**Supplemental Figure 5. TGF-β ligands are induced in RA-deficient and TGF-β activated ASM**. (A) Expression of TGF-β ligands *Tgfb1* and *Tgfb3*, but not *Tgfb2* are upregulated in RA-deficient mASM (VAD compared to VAS; BMS compared to CTR) (n = 6 per group). (B) Expression of *TGFB1* and *TGFB3*, but not *TGFB2* are induced in RA-deficient hASM (BMS and DEAB compared to CTR). Both *TGFB1* and *TGFB3* are also induced in TGFβ1-treated hASM compared to CTR-treated hASM (n = 3). (C) Expression of the RA transcriptional target *RARB* in hASM is down-regulated by TGFβ1 treatment compared to CTR, suggesting suppression of RA signaling when TGF-β pathway is activated. Data represent mean ± SEM. Student's *t*-test is used to calculate *p*-values in A (*$p < 0.05$). Two-way ANOVA is used for statistical analysis in B and C where *p*-values are adjusted with Bonferroni correction (means with different letters are statistically different with corrected $p < 0.05$).

**Supplemental Table 1**: Primers used for reverse transcription-PCR (RT-PCR)

| **Gene** | Forward sequence | Reverse sequence |
|---|---|---|
| *Acta2* | CCAGCACCATGAAGATCAAG | AGGGGGCCACCCTATAATAA |
| *Myh11* | GGCTTCATTTGTTCCTTCCA | GTCAGGGAAAGGTTGGGAGT |
| *Nkx2-1* | TCCGTTCTCAGTGTCTGACA | GTTGCTTGAAACGTCGCTCGA |
| *Notch3* | GATGACACATCAGCCAGCAT | GGCTCCATTTTTCAGCAGAG |
| *Rara* | CTGAACGGGTGATCACATTG | GTTTGCTGGTGATGAAGACG |
| *Rarb* | TGCTGCAGTGAGACATTTCC | CTCCACAACCTCGGTGTCTT |
| *Rarg* | GAGAACCCGGAGATGTTTGA | TGGCAGAGGAAAAGGCTATG |
| *Aldh1a1* | GCCCTCAGATTGACAAGGAA | TCATTGCGACTGTCTTGAGC |
| *Aldh1a2* | CAAGCTGGGACAGTTTGGAT | ATTTGGCAGCTCAGGAGAGA |
| *Aldh1a3* | ATGGAGCCTCACATTTGACC | TGCTCAGCCCAACTTTATCC |