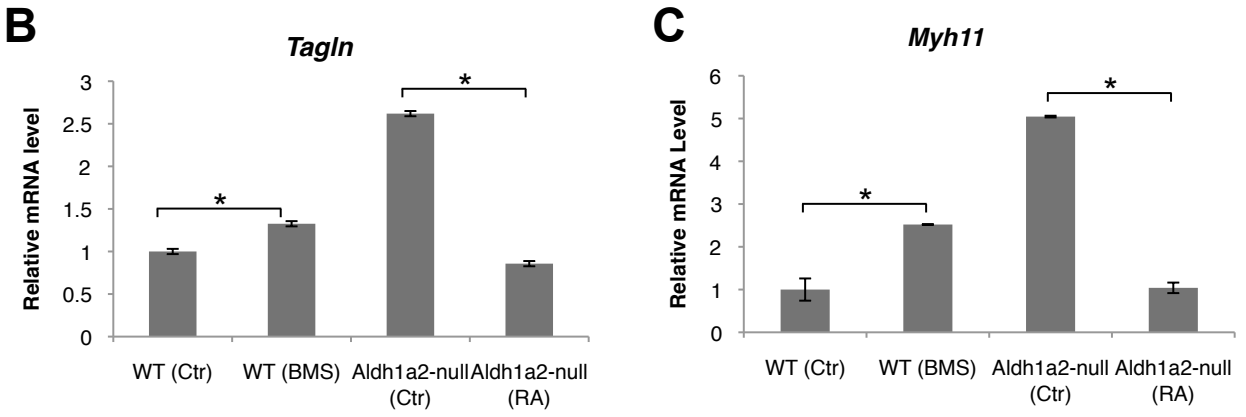
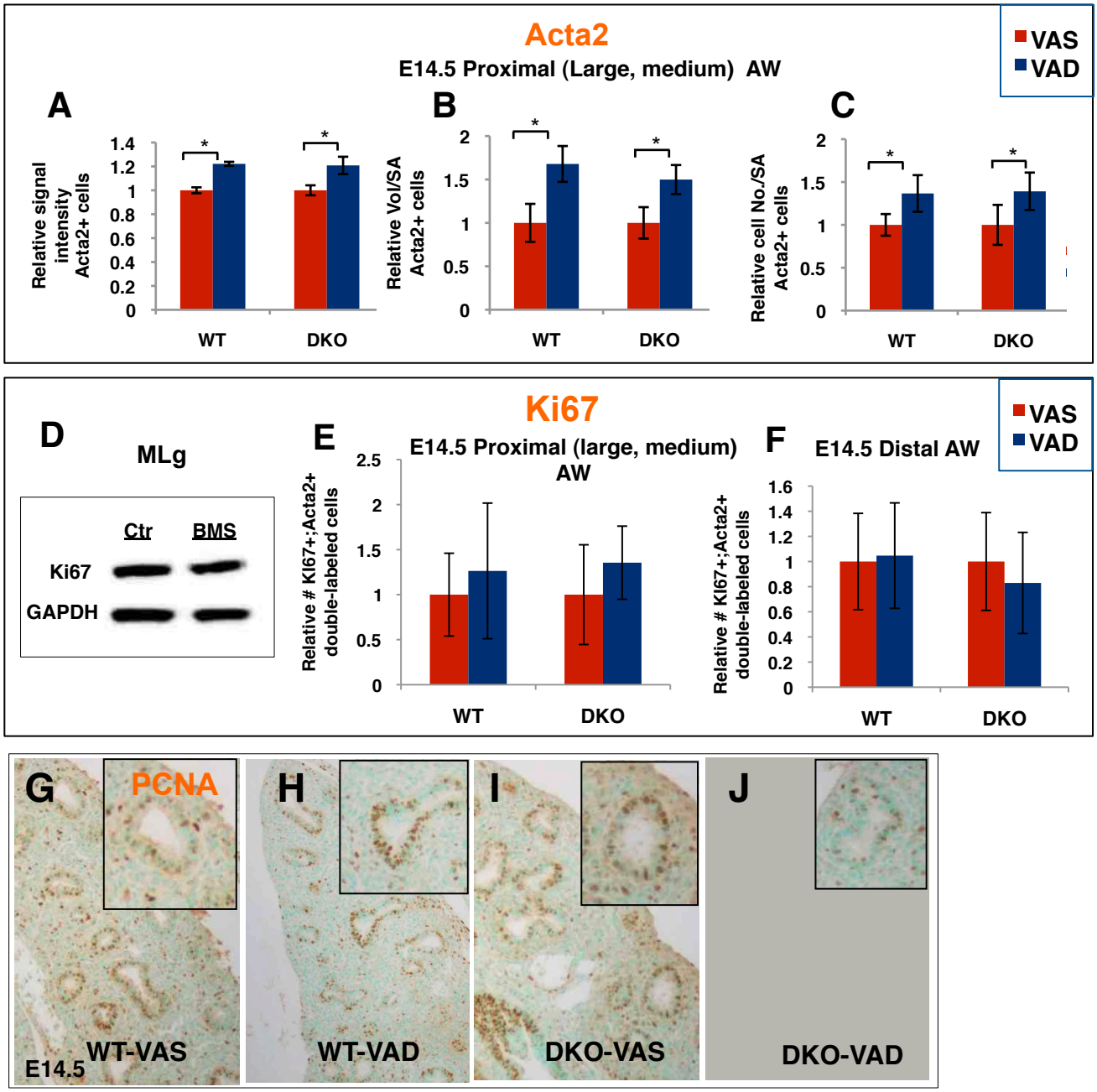
A

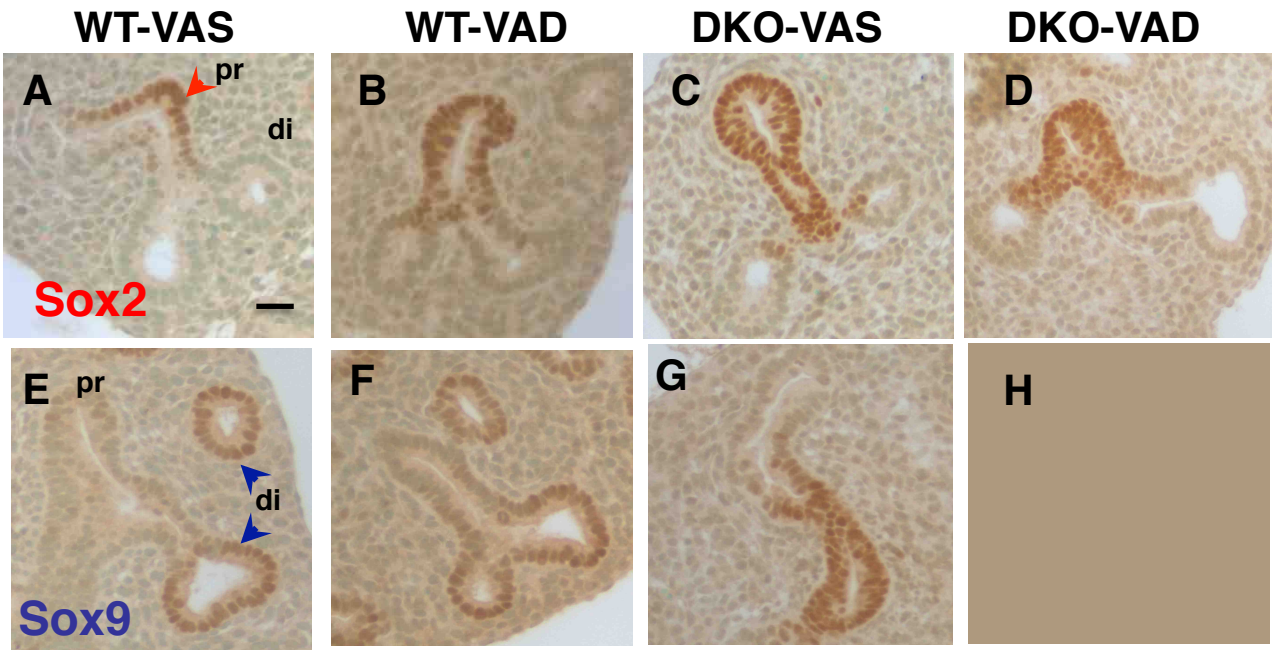
Gene Name	Gene Symbol	p (RA)	FC (RA)	p (BMS)	FC (BMS)
Cysteine and glycine-rich protein 1	*Csrp1*	5.92×10^{-7}	-1.7	9.62×10^{-7}	1.9
Cysteine and glycine-rich protein 2	*Csrp2*	1.12×10^{-2}	-1.2	7.65×10^{-3}	1.4
Actin, alpha 2, smooth muscle, aorta	*Acta2*	1.43×10^{-1}	-2.4	1.88×10^{-2}	2.9
Actin, gamma 2, smooth muscle, enteric	*Actg2*	1.42×10^{-1}	-1.5	6.08×10^{-4}	2.1
Transgelin	*Tagln*	8.40×10^{-1}	-1.0	6.42×10^{-9}	2.8
Transgelin 2	*Tagln2*	2.21×10^{-1}	-1.1	2.52×10^{-4}	1.5
Myosin heavy chain 11, smooth muscle	*Myh11*	3.10×10^{-1}	-1.8	7.84×10^{-3}	1.2
Calponin 2	*Cnn2*	6.71×10^{-1}	-1.0	6.29×10^{-4}	1.4



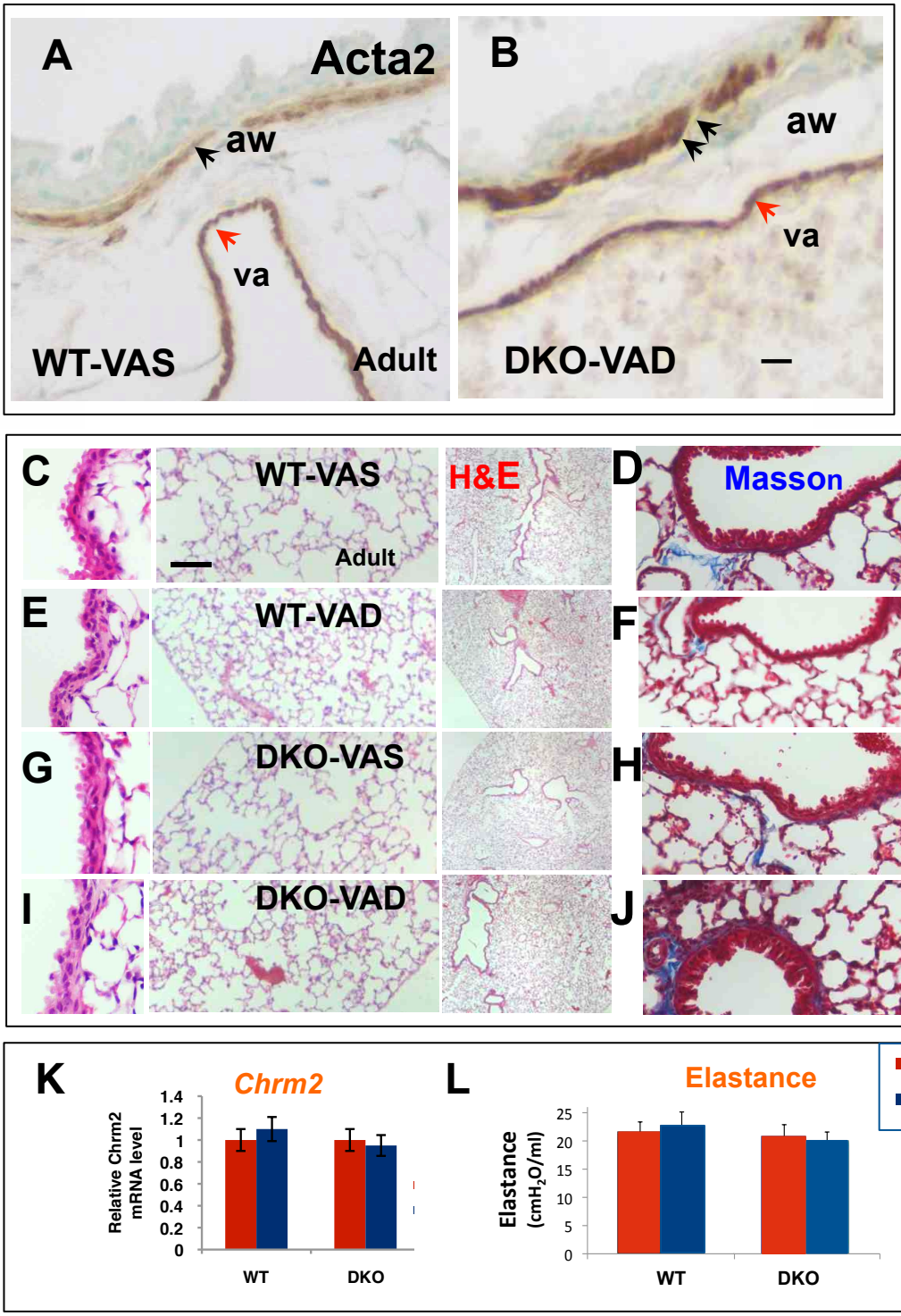
Supplemental Figure 1. Disruption of RA signaling results in upregulation of SM markers in the foregut at the onset of lung development. (A) Gene array analysis of a pharmacological (BMS: RAR antagonist) and a genetic (*Aldh1a2*-null mice) model of RA deficiency using mouse embryonic foregut explants cultured for 24h (32). p value and fold change (FC) of RA (mutant non-rescued vs. rescued by RA) and BMS (WT control vs RAR antagonist). In both models, RA deficiency is associated with up-regulation of SM markers. (B-C) qPCR confirms increased expression of SM markers (*Tagln* and *Myh11*) by disruption of RA signaling (BMS or *Aldh1a2-/-*) and down-regulation by supplementation with RA. Asterisks: significance at $p<0.05$ (mean, $\pm$ se; n=3 explants per condition).



Supplemental Figure 2. The abnormal SM phenotype by deficient RA signaling is not associated with changes in cell proliferation. (A-C) Quantitative assessment of Acta2 IHC in proximal airways (AW) of WT and *DKO* E14.5 mouse lungs: increase in the relative signal intensity, relative volume per surface area (SA), and relative number per SA of Acta2-positive cells in VAD mice compared to VAS. (D-F) No significant changes in cell proliferation as seen by Ki67 Western blot in MLg cells control and BMS (D) and IHC-morphometric assessment of the relative number of Ki67;Acta2 double-labeled cells (E-F) in proximal and distal airways of E14.5 WT and *DKO* lungs (VAS or VAD). (G-J) PCNA IHC in E14.5 lungs further shows no obvious changes in proliferation between groups. Asterisks: significance at $p<0.05$ (mean, $\pm$ se; n=3-4 explants per condition).



Supplemental Figure 3. The SM phenotype of RA-deficient lungs is not associated with changes in lung epithelial patterning. IHC of Sox2 (A-D) and Sox9 (E-H) showing labeling of the proximal (pr) and distal (di) epithelium, respectively in E14.5 lungs from WT (VAS, VAD) and DKO (VAS, VAD) mice. No difference in intensity or proximal-distal distribution of these markers among the groups (n=3 per condition). Scale bar in A: 80 µm.



Supplemental Figure. 4. Effects of pre-natal Vitamin A deficiency in the adult lung. (A, B) Acta2 IHC: major increase in expression in airway SM but not in vascular (va) SM in VAD-DKO mice compared to WT-VAS (representative section). (C, E, G, I) H&E, no obvious morphological changes in the alveolar spaces between groups. (D, F, H, J) Masson staining: no evidence of aberrant extra-cellular matrix deposition among groups. (K-L) No significant changes in muscarinic receptor (Chrm2) expression by qRT-PCR or changes in lung elastance when VAS and VAD groups are compared in WT and DKO mice. Scale bars in B, C: 24 μm and 40 μm