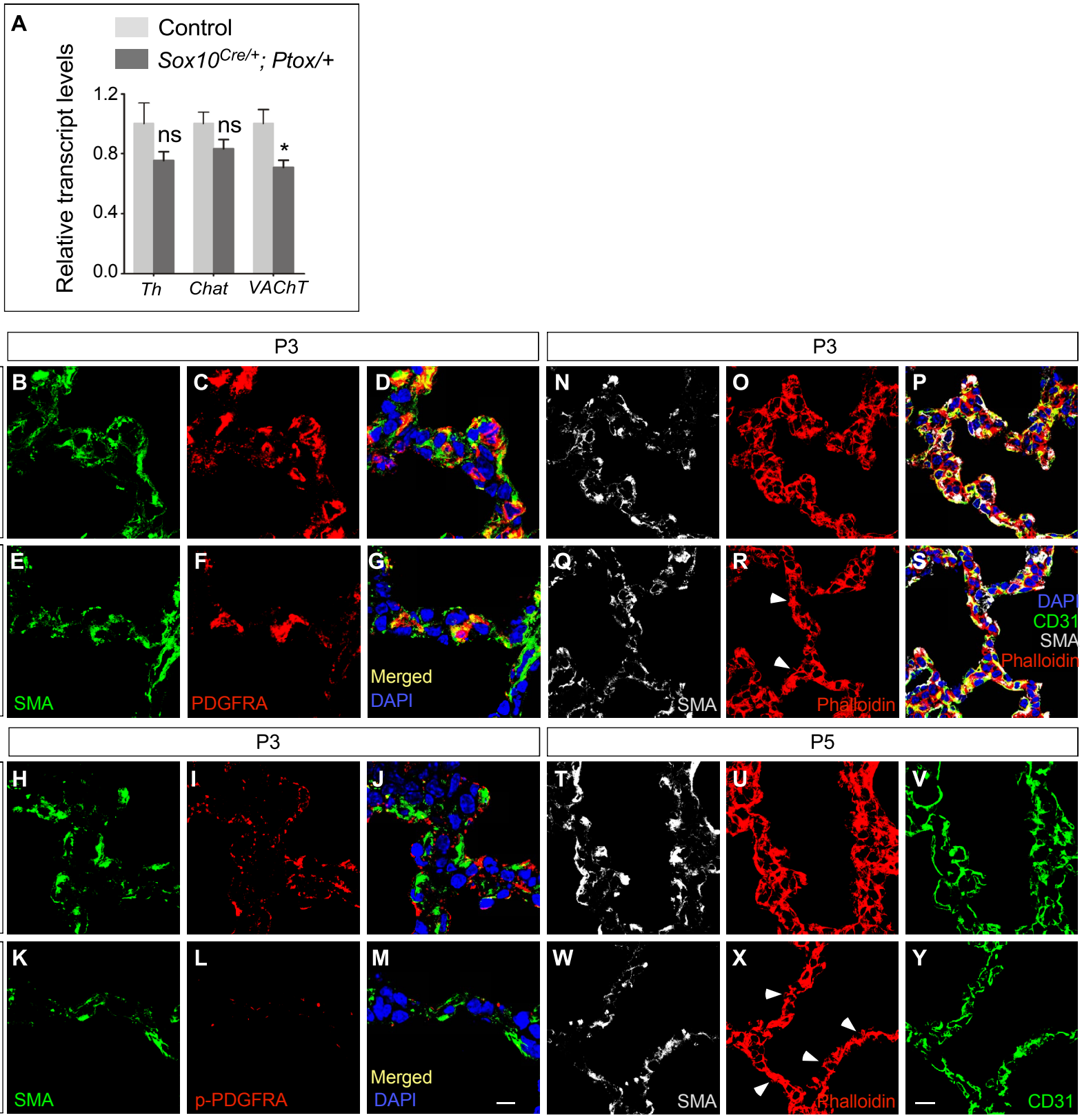

Figure S1. Analysis of control and *Sox10*$^{Cre/+}$*; Ptox/+* mouse lungs, in which autonomic nerve activity was abolished by *Ptox* expression in SOX10^{+} cells (related to Figure 3). (A) qPCR analysis of transcript levels in control and *Sox10*$^{Cre/+}$*; Ptox/+* lungs. The expression levels of *Th* and *Chat* were unaltered, while a slight reduction in *VAChT* expression in *Sox10*$^{Cre/+}$*; Ptox/+* lungs was noted. *Th* is expressed in sympathetic nerves while *Chat* and *VAChT* mark parasympathetic nerves. *Th*, *tyrosine hydroxylase*; *Chat*, *choline acetyltransferase*; *VAChT*, *vesicular acetylcholine transporter*. (B-Y) Immunohistochemical analysis of control and *Sox10*$^{Cre/+}$*; Ptox/+* mouse lungs at P3 and P5. The levels of phosphorylated (p) PDGFRA, indicative of PDGF signaling, were reduced in *Sox10*$^{Cre/+}$*; Ptox/+* lungs compared to controls while the levels of PDGFRA were unchanged. Phalloidin staining revealed disorganized cytoskeleton (arrowheads) in the mutant lungs. Scale bars, 7.5 µm (B-M),10 µm (N-Y). All values are mean ± SEM. (*) $p<0.05$; ns, not significant (unpaired Student's *t*-test).

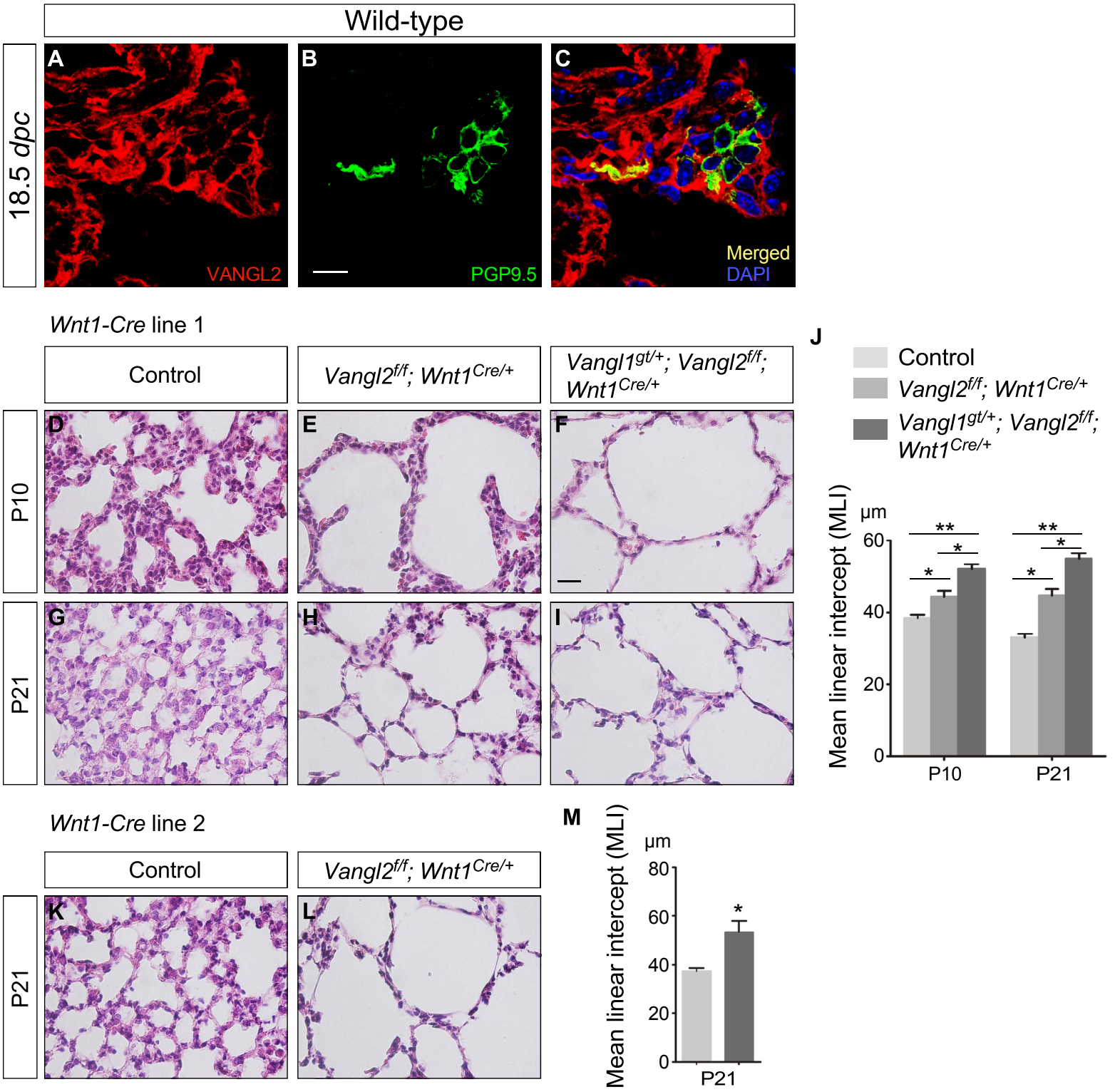

Figure S2. Analysis of control and *Vangl1/2*-deficient mouse lungs, in which planar cell polarity (PCP) signaling in autonomic nerves was abolished (related to Figure 4). (A-C) Immunohistochemical analysis of wild-type mouse lungs at 18.5 *days post coitus* (*dpc*). VANGL2 was broadly expressed in many cells including its expression in nerve cells (labeled by PGP9.5). (D-I) Hematoxylin and eosin-stained lung sections from control mice and mice deficient in *Vangl2* alone or in both *Vangl1* and *Vangl2* induced by *Wnt1-Cre* (line 1) at postnatal (P) day 10 and 21. Loss of PCP signaling led to alveolar defects. Elimination of one copy of *Vangl1* (*Vangl1^{gt}* is a null allele) exacebated the alveolar defects due to *Vangl2* removal. (**J**) Measurement of the mean linear intercept (MLI) in control, *Vangl2$^{f/f}$; Wnt1$^{Cre/+}$* and *Vangl1$^{gt/+}$; Vangl2$^{f/f}$; Wnt1$^{Cre/+}$* lungs. The MLI was increased in the absence of PCP signaling in *Wnt1-Cre^{+}* cells. (K, L) Hematoxylin and eosin-stained lung sections from control mice and mice deficient in *Vangl2* at P21. A second *Wnt1-Cre* line (line 2) was used in this experiment and similar alveolar defects were observed. (M) Measurement of the MLI in control and *Vangl2$^{f/f}$; Wnt1$^{Cre/+}$* lungs. The MLI was increased in the absence of PCP signaling in *Wnt1-Cre^{+}* cells. Scale bars, 25 μm (A-C), 25 μm (D-I, K, L). All values are mean ± SEM. (*) $p<0.05$; (**) $p<0.01$ (unpaired Student's *t*-test and one-way ANOVA).

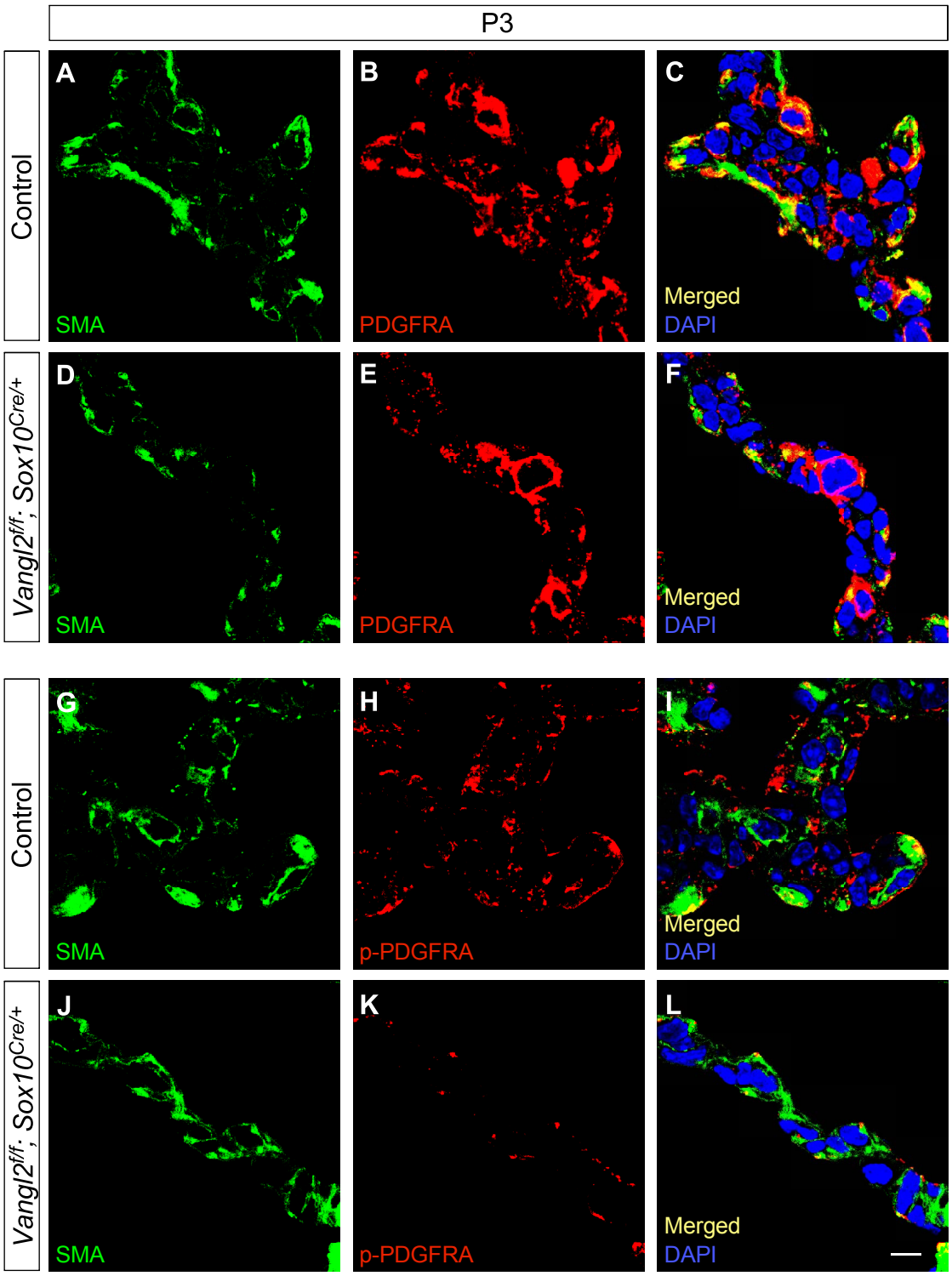

Figure S3 Analysis of control and *Vangl2$^{f/f}$; Sox10$^{Cre/+}$* mouse lungs, in which planar cell polarity (PCP) signaling in autonomic nerves was abolished (related to Figure 4). (A-L) Immunohistochemical analysis of control and *Vangl2$^{f/f}$; Sox10$^{Cre/+}$* mouse lungs at postnatal (P) day 3. The levels of phosphorylated (p) PDGFRA, indicative of PDGF signaling, were reduced in *Vangl2$^{f/f}$; Sox10$^{Cre/+}$* lungs compared to controls while the levels of PDGFRA were unchanged. Scale bars, 7.5 μm (A-L).

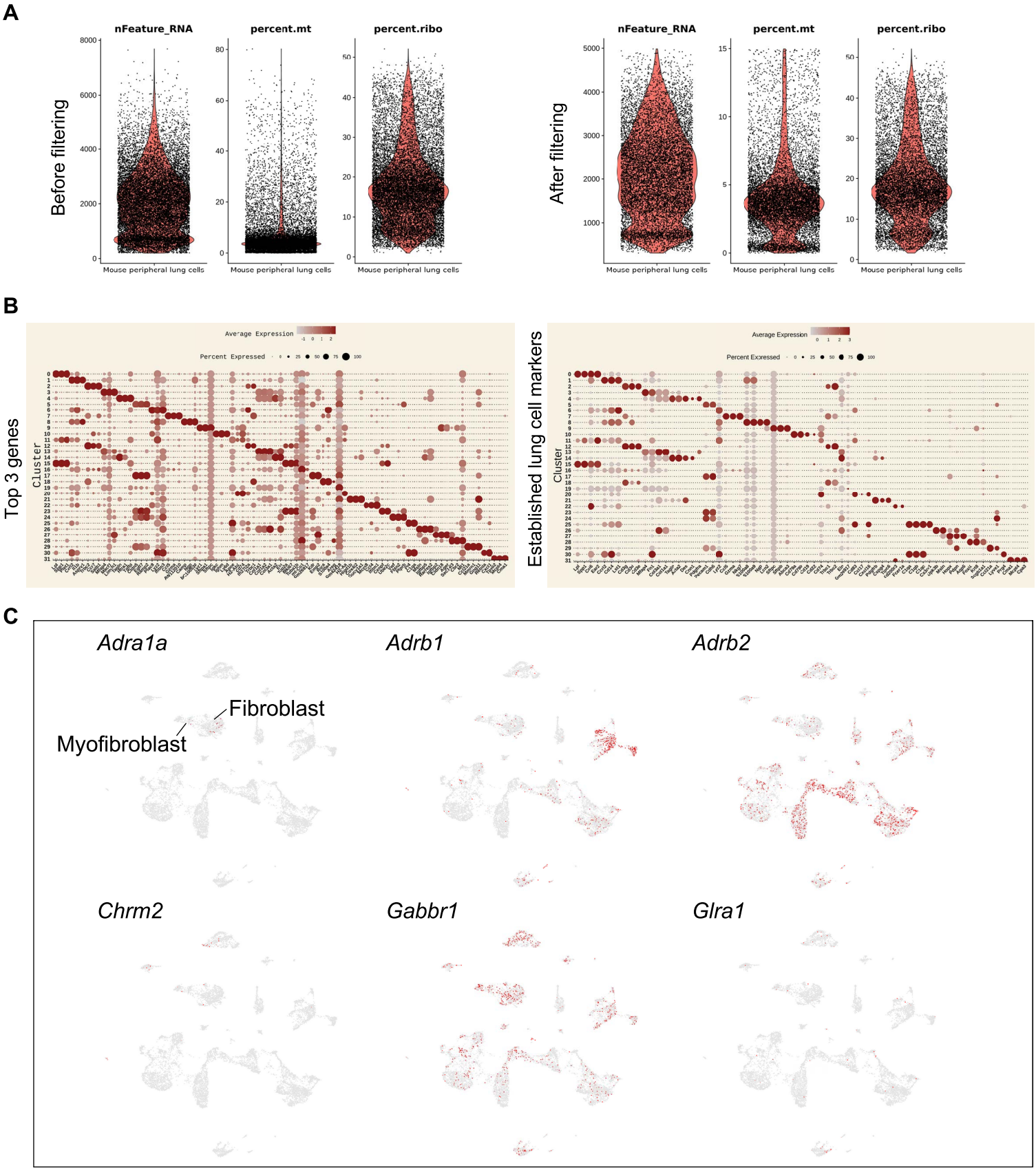

Figure S4. scRNA-seq analysis of distal mouse lung cells (related to Figure 6). (A) Violin plots before and after filtering for low-quality cells using nFeature_RNA (genes per cell), percent.mt (mitochondrial content %), and percent.ribo (ribosomal content %). (B) Dotplots showing the top 3 gene markers per cluster (left) and selected canonical lung cell markers (right) by average log fold change. (C) UMAP plots of neurotransmitter receptor expression across all cell clusters (see Fig. 6L), darker red indicating higher relative expression.

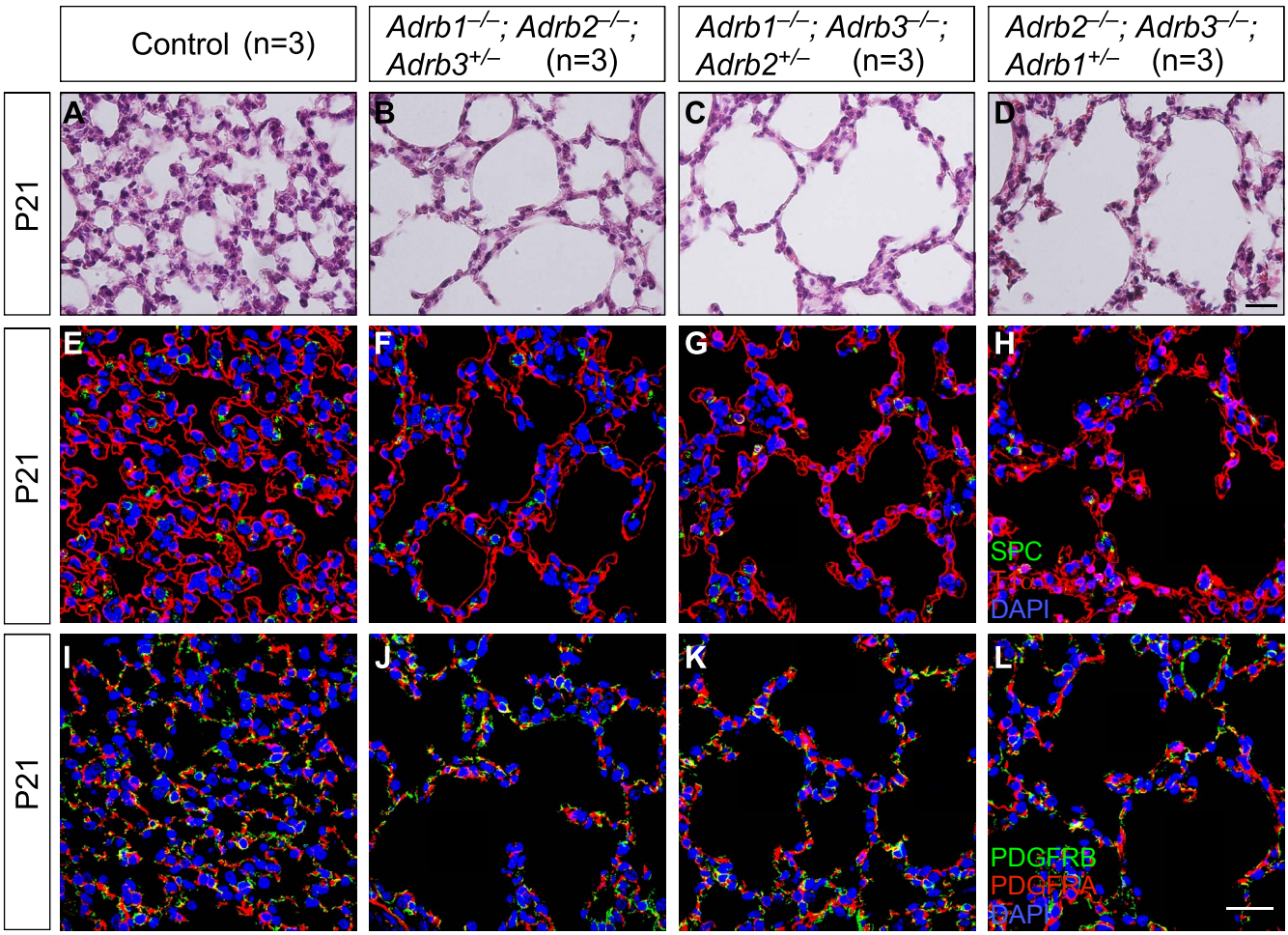

Figure S5. Phenotypic analysis of mouse lungs deficient in adrenergic receptors (related to Figures 6&7). (A-D) Hematoxylin and eosin-stained lung sections from control mice and mice deficient in two of the three adrenergic receptors (*Adrb1*, *2* and *3*) at postnatal (P) day 21. Loss of adrenergic receptors compromised alveolar formation in mice and increased air space was observed. (E-H) Immunohistochemical analysis of control mice and mice deficient in two of the three adrenergic receptors (*Adrb1*, *2* and *3*) at P21. SPC labeled alveolar type II cells while T1α marked alveolar type I cells. (I-L) Immunohistochemical analysis of control mice and mice deficient in two of the three adrenergic receptors (*Adrb1*, *2* and *3*) at P21. PDGFRA labeled fibroblasts/myofibroblasts; PDGFRB marked pericytes. Mice deficient in all three adrenergic receptors (*Adrb1*$^{-/-}$*; Adrb2*$^{-/-}$*; Adrb3*$^{-/-}$) displayed varying degrees of embryonic and postnatal lethality. Many of the survivors also exhibited alveolar defects. Scale bars, 25 µm (A-D), 25 µm (E-L).

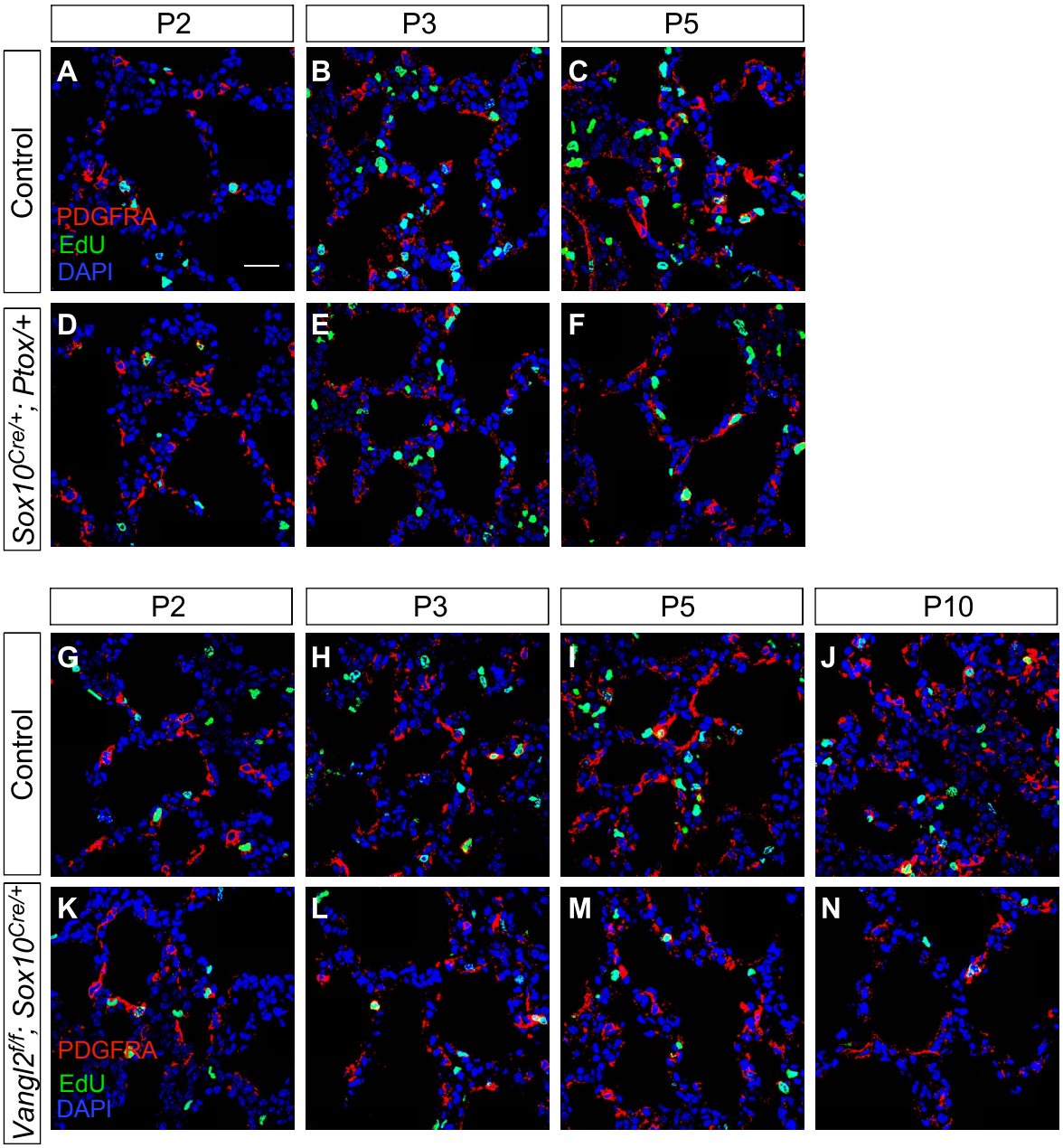

Figure S6. EdU staining to assess cell proliferation of fibroblasts/myofibroblasts (related to Figure 7). (A-F) Immunofluorescence of lung sections from control and *Sox10$^{Cre/+}$; Ptox/+* mice, which were injected with EdU. PDGFRA marked fibroblasts/myofibroblasts. (G-N) Immunofluorescence of lung sections from control and *Vangl2$^{f/f}$; Sox10$^{Cre/+}$* mice, which were injected with EdU. PDGFRA marked fibroblasts/myofibroblasts. n=3 pairs for each stage. Scale bars, 25 μm (A-N).

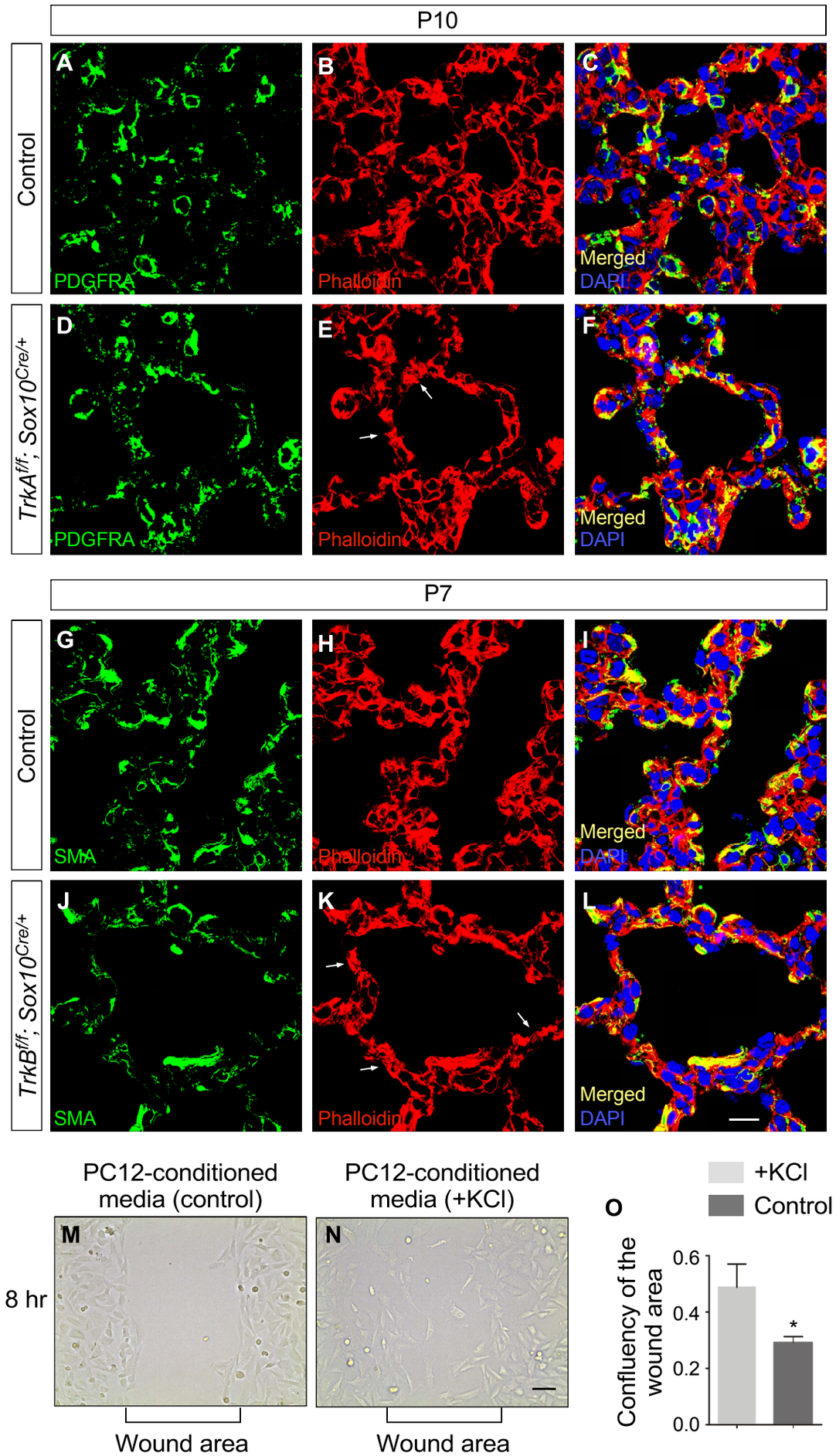

Figure S7. Analysis of control, *TrkA$^{f/f}$; Sox10$^{Cre/+}$* and *TrkB$^{f/f}$; Sox10$^{Cre/+}$* mouse lungs, in which neurotrophin signaling in autonomic nerves was abolished (related to Figure 7). (A-F) Immunohistochemical analysis of control and *TrkA$^{f/f}$; Sox10$^{Cre/+}$* mouse lungs at postnatal (P) day 10. Myofibroblasts (marked by PDGFRA) failed to migrate to the sites of secondary septation. Phalloidin labeled F-actin. (G-L) Immunohistochemical analysis of control and *TrkB$^{f/f}$; Sox10$^{Cre/+}$* mouse lungs at P7. Myofibroblasts expressing SMA failed to migrate to the sites of secondary septation. Arrows point to the disorganized actomyosin cytoskeleton, which interfered with contraction of myofibroblasts. (M, N) Migration assays of wild-type fibroblasts/myofibroblasts treated with conditioned media from PC12 cells (control) or conditioned media with KCl added to release the neurotransmitters. Fibroblasts/myofibroblasts treated with media (+KCl) covered the wound area faster than those treated with control media. (O) Quantification of the confluency of the wound area at 48 hr. KCl-treated media enhanced the confluency of the wound area. Scale bars, 10 μm (A-L), 50 μm (M, N). All values are mean ± SEM. (*) $p<0.05$ (unpaired Student's *t*-test).

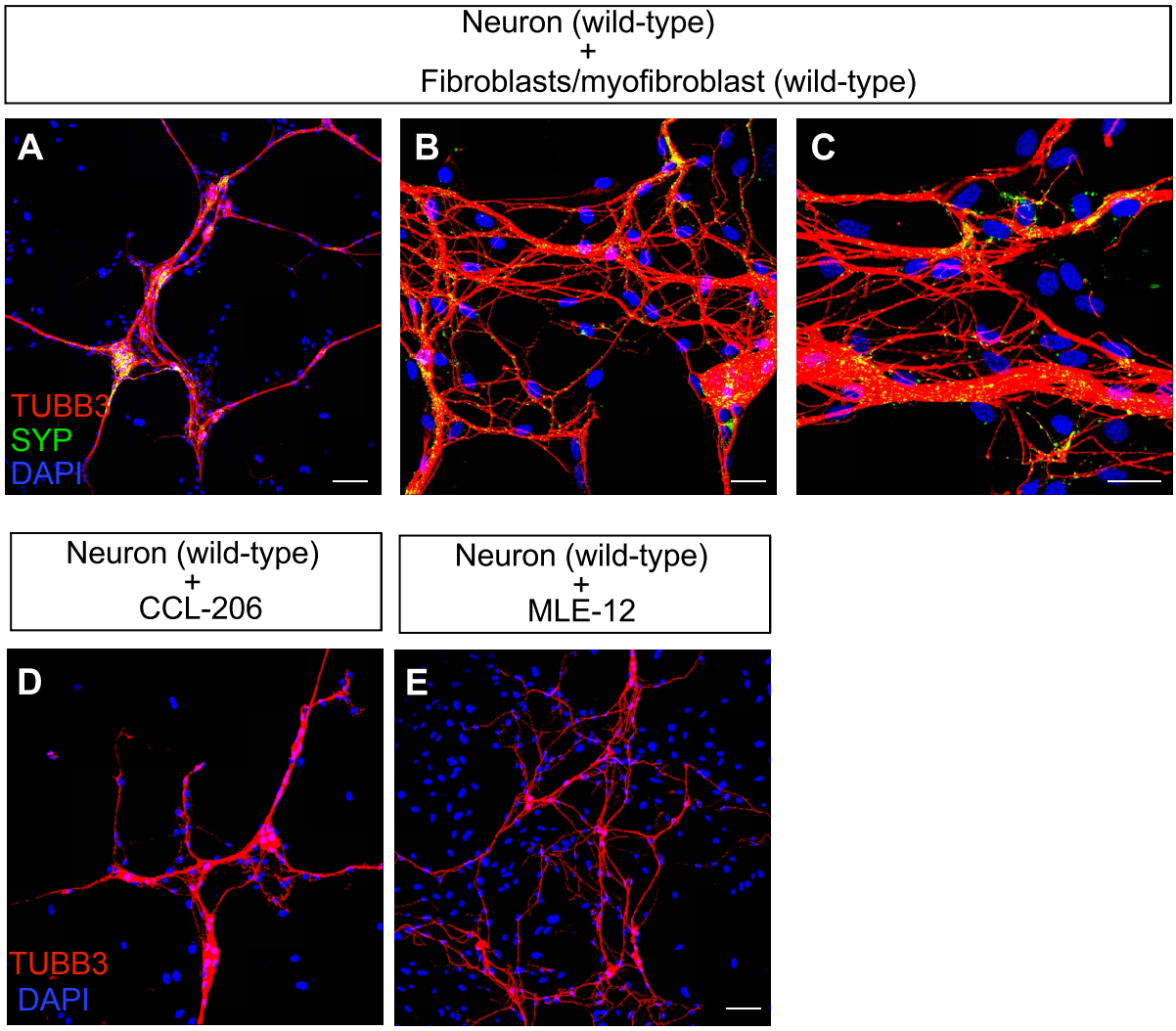

Figure S8. Fibroblasts/myofibroblasts aggregate around autonomic nerve fibers in coculture (related to Figure 7).
(A-C) Immunofluorescence of cultured neurons and fibroblasts/myofibroblasts. (D). Immunofluorescence of cultured neurons and CCL-206 fibroblasts. (E) Immunofluorescence of cultured neurons and MLE-12 cells. Scale bars, 100 μm (A, D), 25 μm (B), 25 μm (C), 50 μm (E).

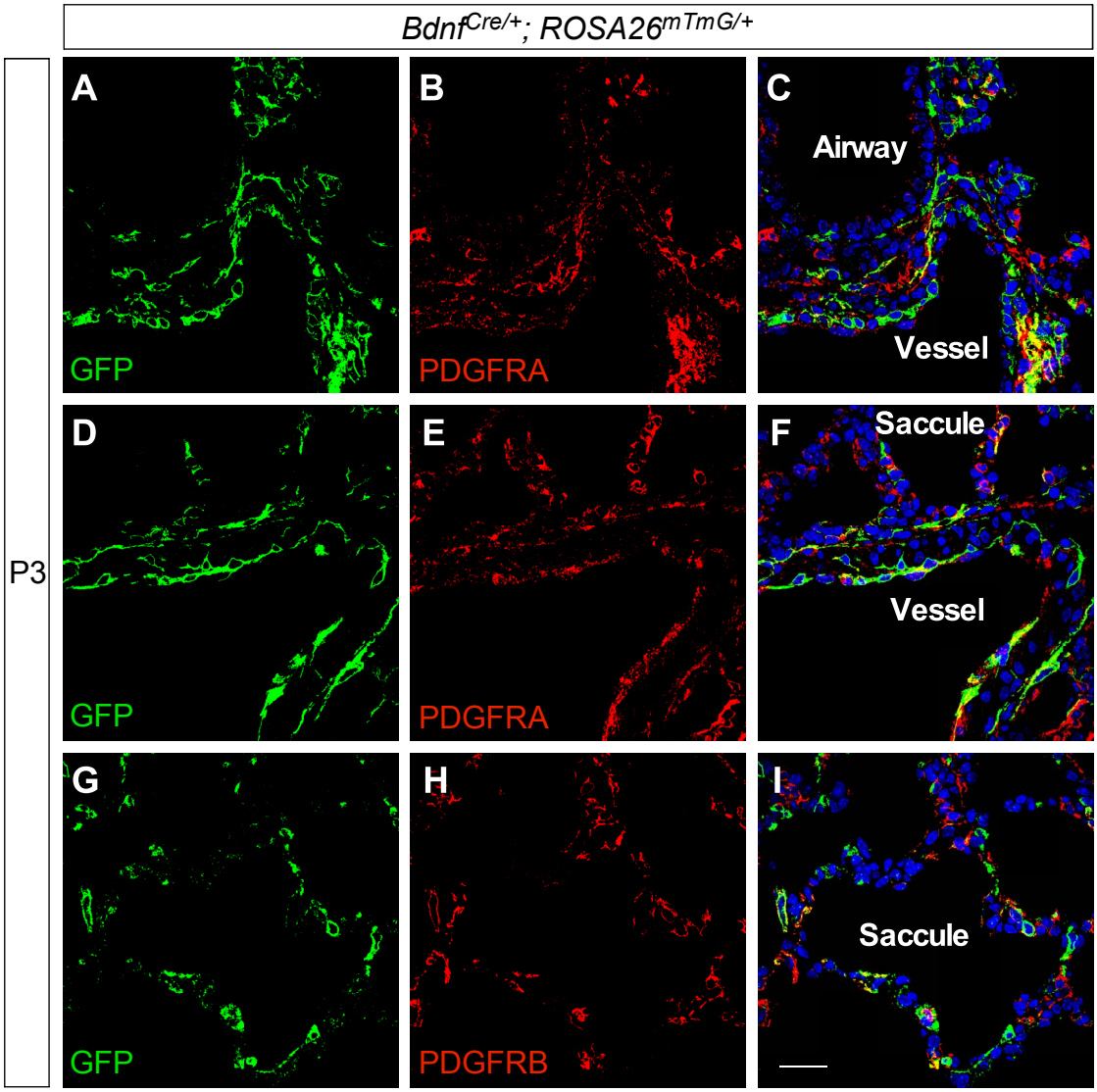

Figure S9. *Bdnf-Cre* can label multiple cell lineages (related to Figure 8).
(A-I) Immunofluorescence of lung sections derived from *Bdnf*$^{Cre/+}$; *ROSA26*$^{mTmG/+}$ mice at postnatal (P) day 3. PDGFRA marked airway smooth muscle cells and vascular smooth muscle cells while PDGFRB labeled pericytes in saccules. Scale bars, 25 μm (A-I).

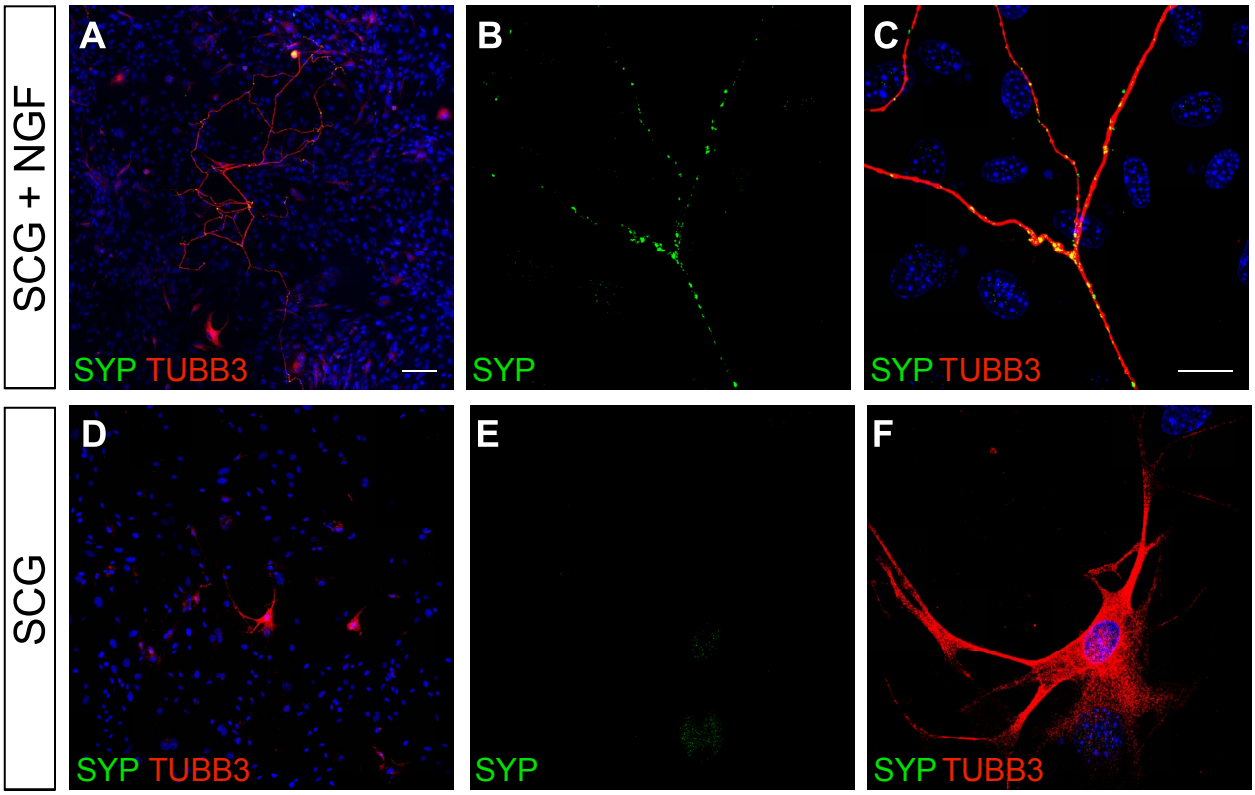

Figure S10. NGF is required for survival and function of autonomic nerve fibers in culture (related to Figure 8).
(A-F) Immunofluorescence of neurons derived from the superior cervical ganglions (SCG) in culture, which were stained with anti-SYP and anti-TUBB3. SYP marked synaptic vesicles and TUBB3 labeled nerve fibers. Scale bars, 100 μm (A, D), 25 μm (B, C, E, F).

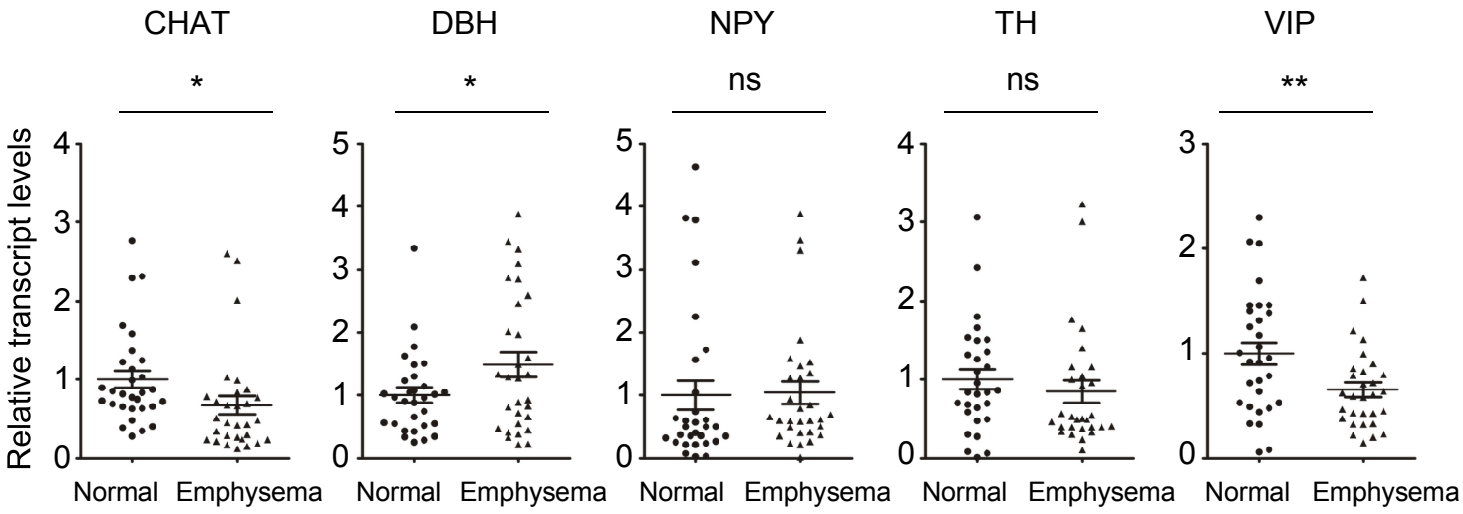

Figure S11. qPCR analysis of transcript levels of neurotransmitters or enzymes in lungs from normal and COPD/emphysema patients (related to STAR Methods). The expression levels of several neurotransmitters or enzymes involved in their synthesis were perturbed in lungs of COPD/emphysema patients (n=30 pairs for CHAT, DBH, NPY and VIP; n=29 pairs for TH). CHAT, choline acetyltransferase; DBH, dopamine beta hydroxylase; NPY, neuropeptide Y; TH, tyrosine hydroxylase; VIP, vasoactive intestinal polypeptide. All values are mean ± SEM. (*) $p<0.05$; (**) $p<0.01$; ns, not significant (unpaired Student's *t*-test).