

Figure S1 (Related to Figure 1): Single-cell RNA sequencing analysis of the intrapulmonary airway epithelium from control and NAPH-injured mice. (A) Purification of EPCAM⁺ cells from control and NAPH-injured lungs. (B) Individual cell populations are distinguished by the corresponding protein markers. (C) Club cells are separated into three subpopulations in both controls and NAPH-injured lungs. (D) Expression of *Kdr* in club cells.

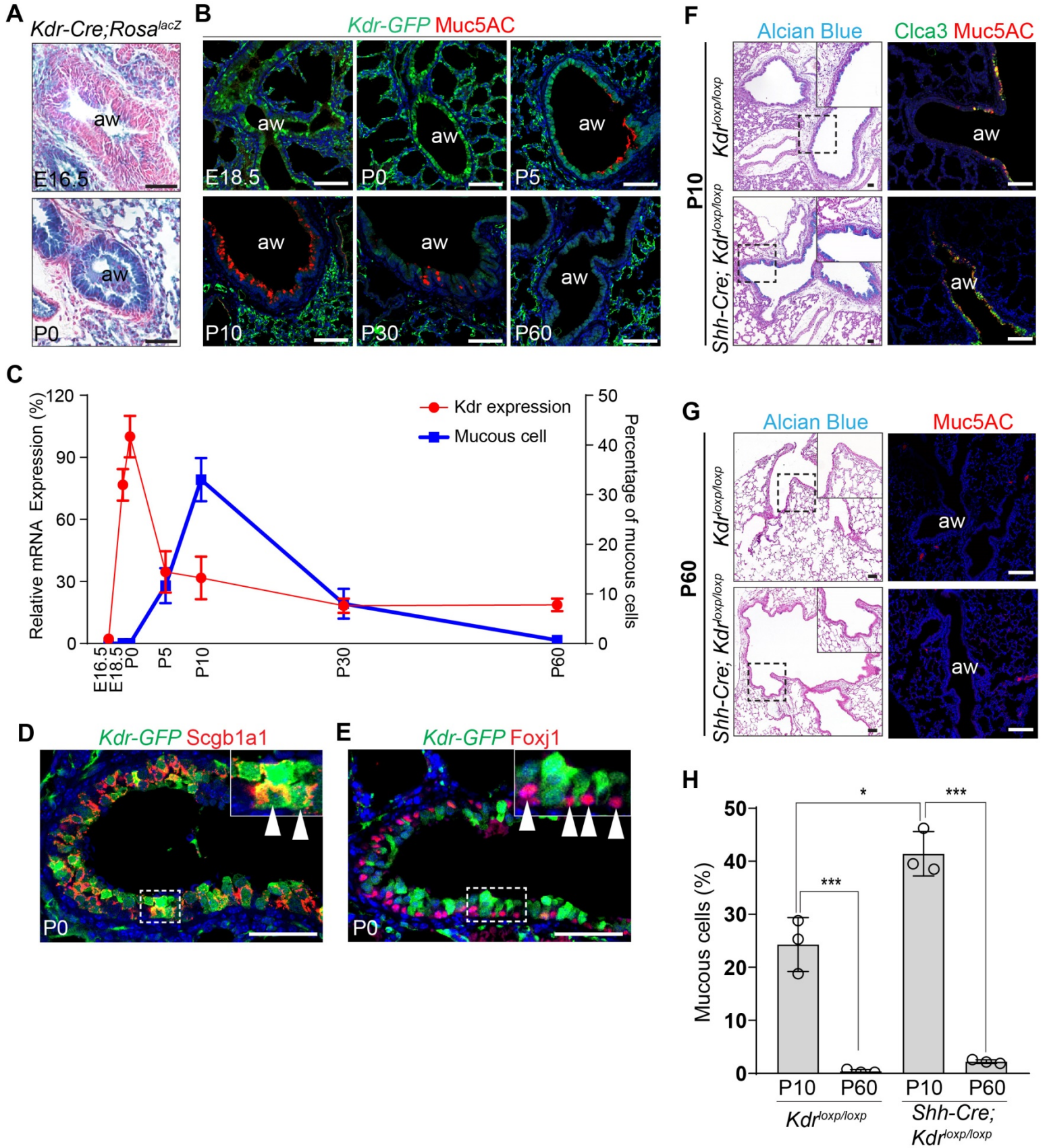


Figure S2 (Related to Figure 2): Loss of *Kdr* promotes mucous cell differentiation during the early postnatal stage. (A) *Kdr* lineage-labeled cells are barely detected in the airways (aw) at embryonic day (E)16.5 while prominent at postnatal (P)0 in *Kdr-Cre;Rosa^{lacZ}* reporter mice. Note positive signals in the blood vessels. (B and C) While *Kdr-GFP* expression increases from E16.5 to P0 and decreases from P5 to adult, the numbers of mucous cells increase from P5 to P10, and decrease towards adult. Data represent mean \pm s.e.m (n=3). (D and E) *Kdr-GFP* is expressed in club cells (D) but not ciliated cells (E) at P0. (F-H) Loss of *Kdr* increases the numbers of airway mucous cells (*Muc5AC⁺ Clca3⁺*) in *Shh-Cre;Kdr^{loxp/loxp}* mutants at P10 (F and H). The numbers of mucous cells are dramatically reduced at P60 in both controls and mutants (G and H). Note slightly increased number of mucous cells in mutants (non-significant) at P60 (n=3). Data represent mean \pm s.e.m. * $p < 0.05$, *** $p < 0.001$; statistical analysis by unpaired two-tailed Student's *t*-test. Scale bar: 50 μ m.

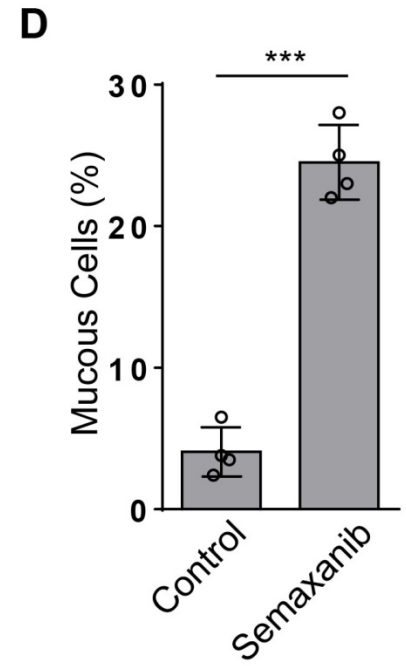
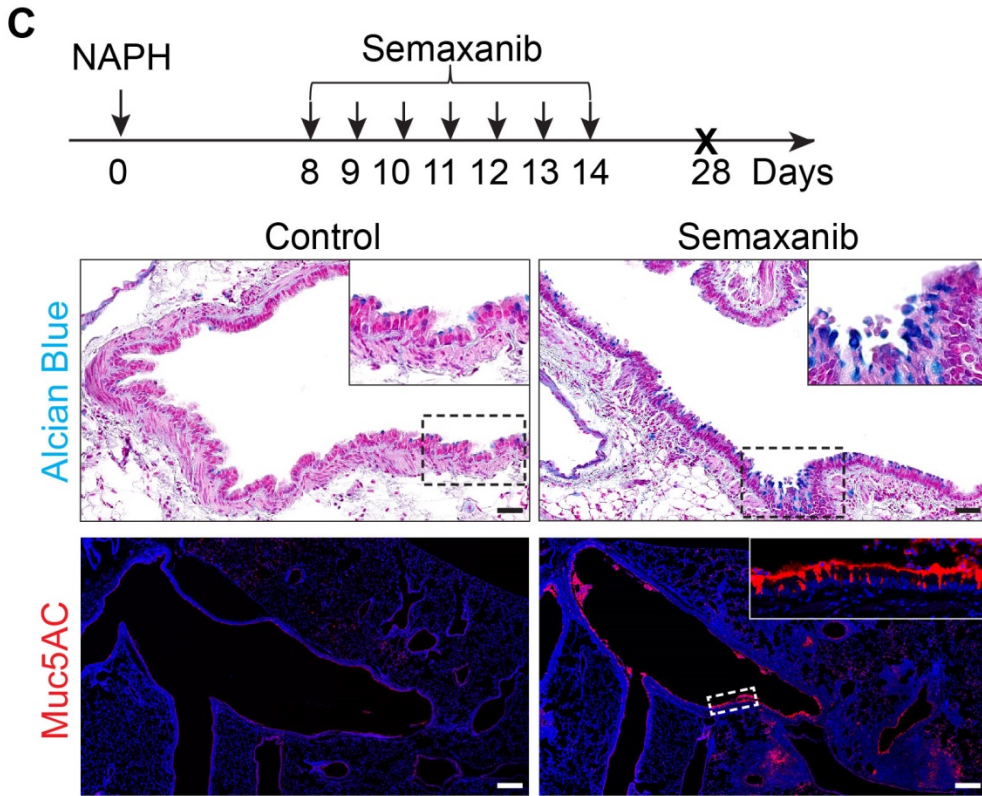
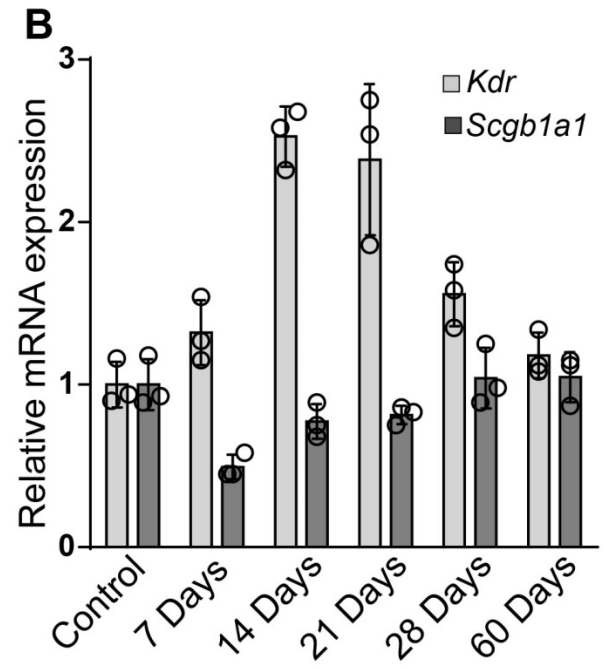
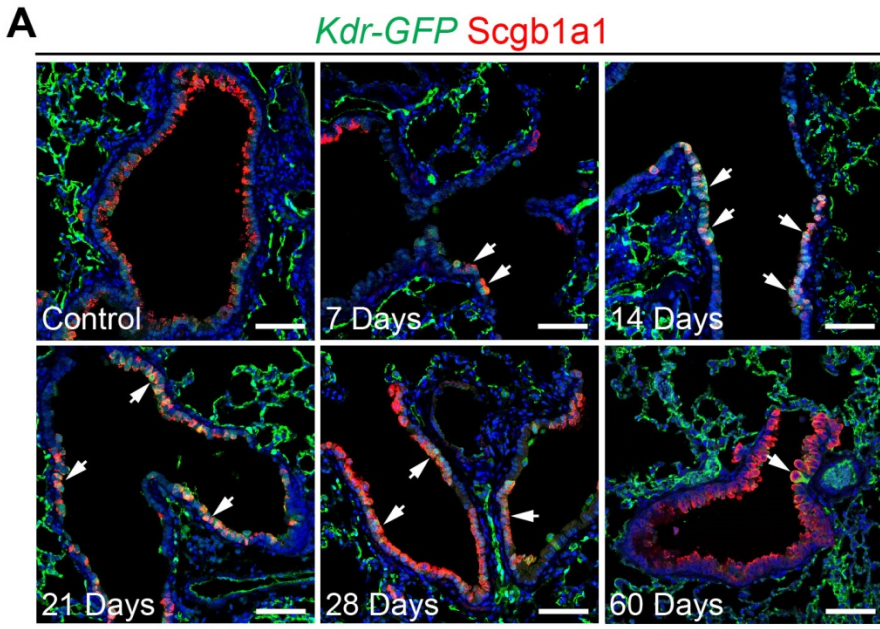


Figure S3 (Related to Figure 2): Transiently increased *Kdr* expression in the regenerated club cells while inhibition of its activity by Semaxanib promotes mucous metaplasia of the regenerated airway epithelium following NAPH challenge. (A) *Kdr-GFP* expression increases in club cells during regeneration and returns to low levels upon recovery. (B) Quantitative PCR shows a transient increase of *Kdr* transcripts during regeneration (n=3). (C and D) *Kdr* inhibition by Semaxanib induces mucous metaplasia following NAPH challenge (n=4). Data represent mean \pm s.e.m. * p <0.001; statistical analysis by unpaired two-tailed Student's *t*-test. Scale bar: 50 μ m.**

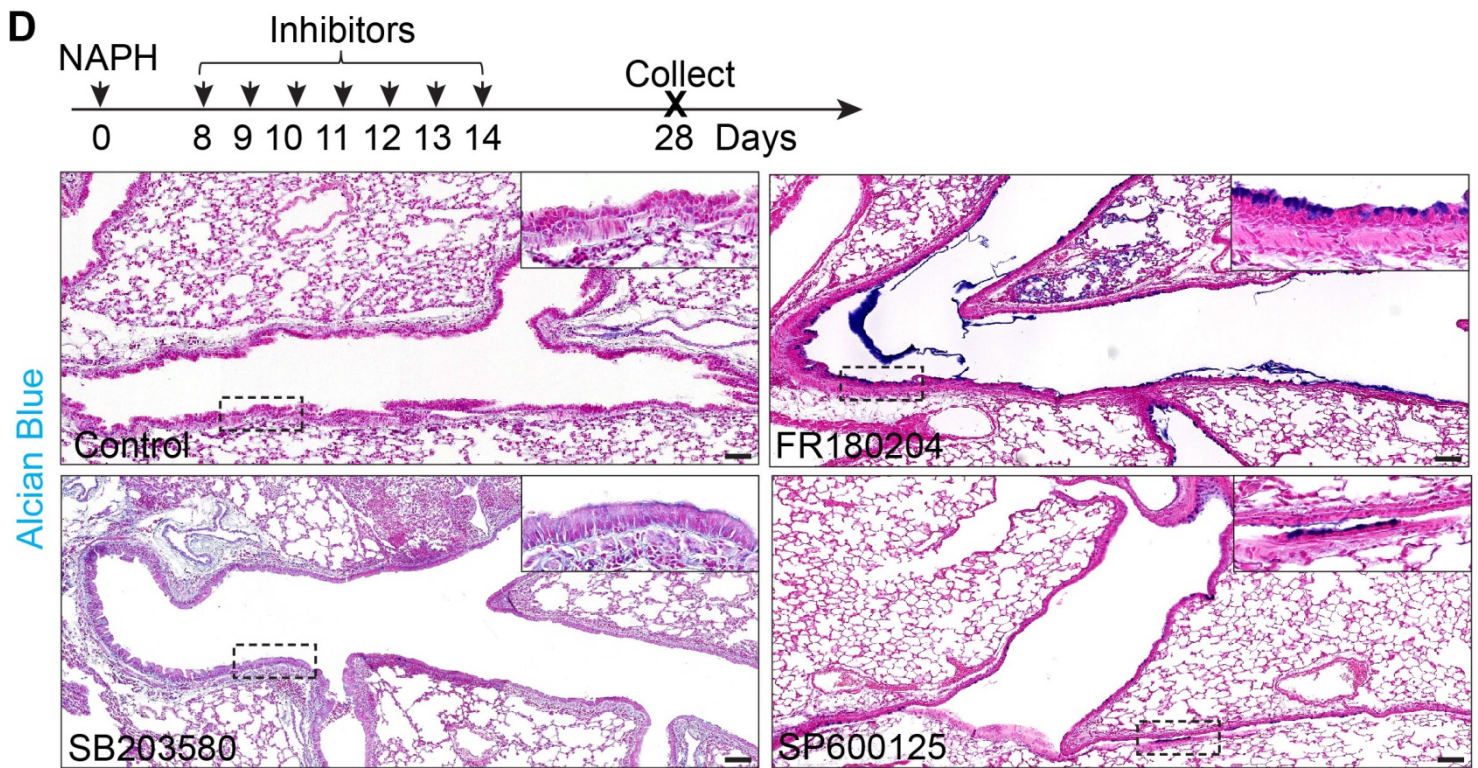
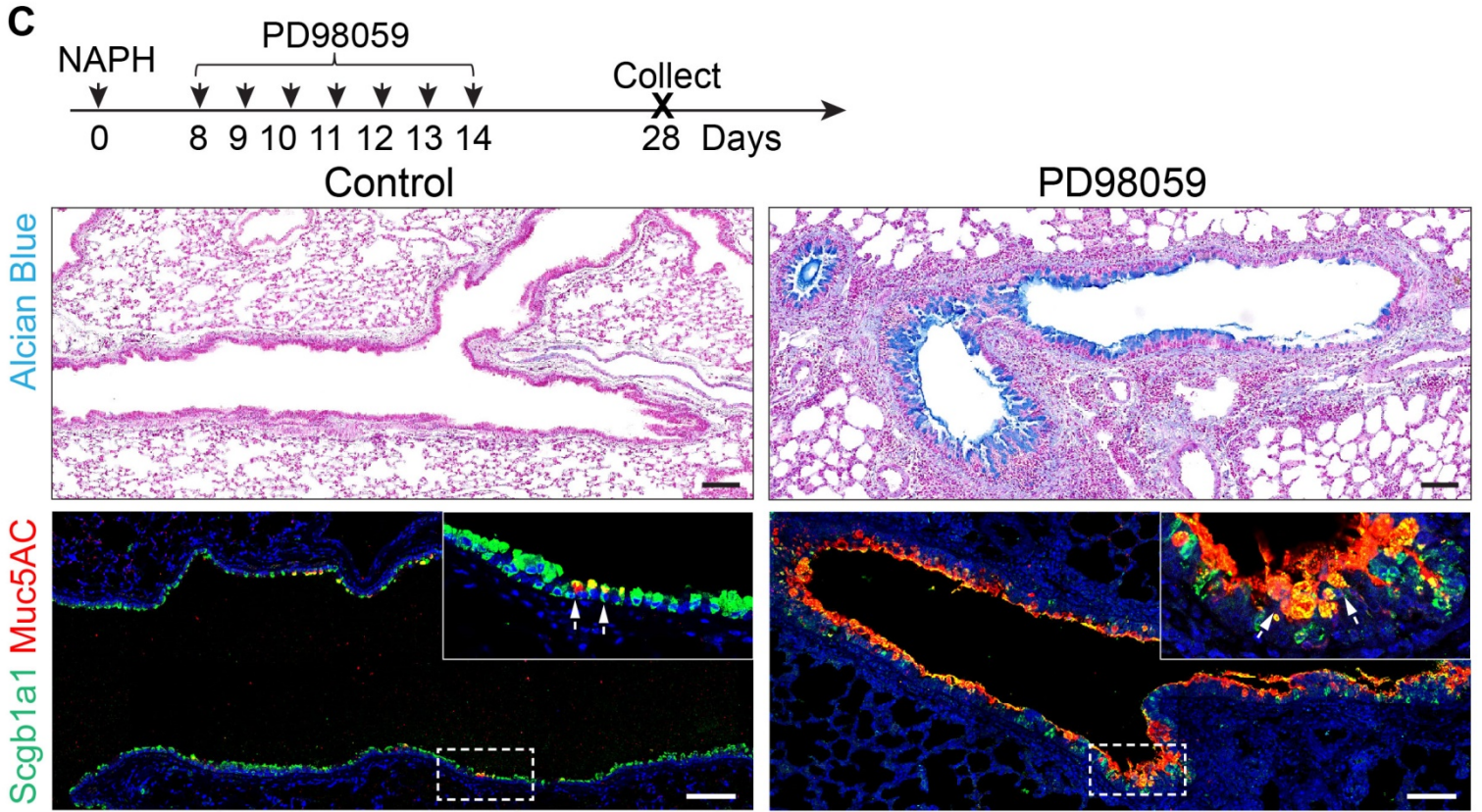
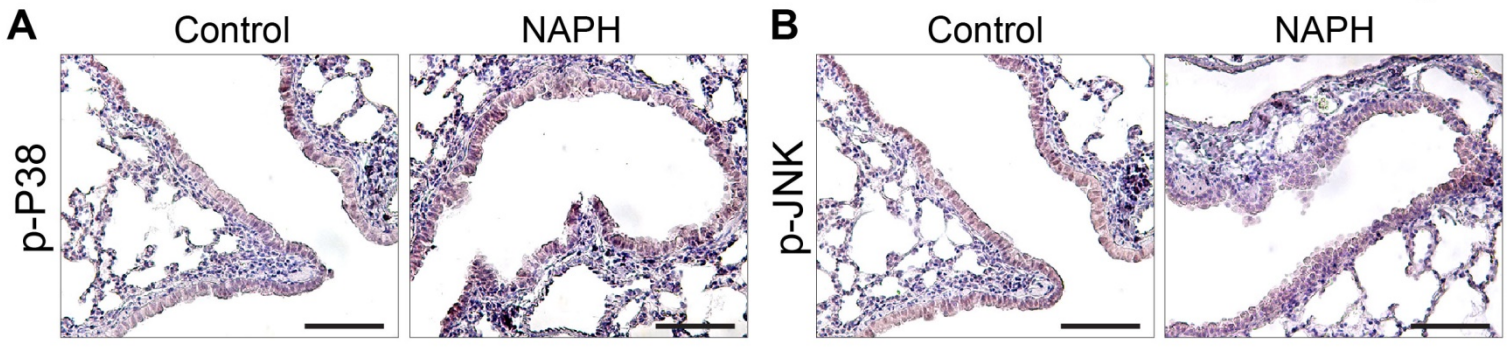


Figure S4 (Related to Figure 4): Inhibition of Mek/Erk signaling promotes mucous metaplasia of the regenerated airway epithelium. (A and B) Expression of p-P38 (A) and p-JNK (B) are not increased in the airway epithelium following NAPH challenge. (C) Mek inhibition with PD98059 promotes mucous metaplasia in the airways of wildtype mice following NAPH challenge. (D) Inhibition of Erk signaling with FR180204 but not p38 MAPK (SB203580) or JNK (SP600125) leads to severe mucous metaplasia following NAPH challenge. Scale bar: 100 μ m.

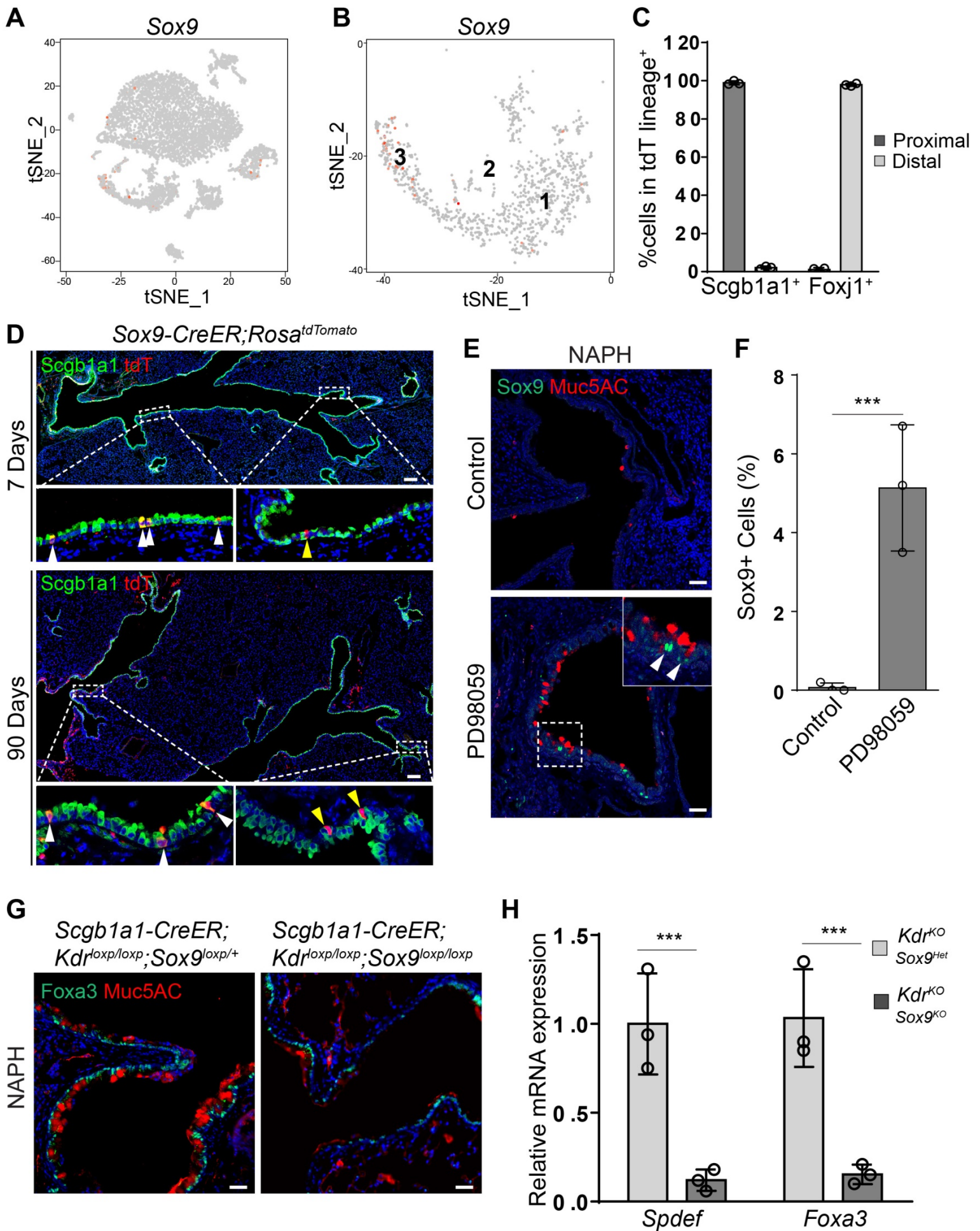


Figure S5 (Related to Figure 5): Sox9 is required for mucous metaplasia downstream of Kdr inhibition. (A and B) Sox9 transcripts are relatively enriched in Sub3. (C) Few solitary lineage-labeled epithelial cells are club cells in the proximal airways and ciliated cells in the distal airways of *Sox9-CreER;Rosa^{tdTomato}* reporter mice. (D) Sox9 lineage-labeled cells remain solitary. Note lineage-labeled mesenchymal cells close to the airway epithelium. (E and F) Inhibition of Kdr signaling by the Mek inhibitor PD98059 leads to Sox9 expression in airway mucous cells. (G and H) *Sox9* deletion attenuates mucous metaplasia of the regenerated airway epithelium accompanied with reduced expression of *Spdef* and *Foxa3* in *Scgb1a1-CreER;Kdr^{loxp/loxp};Sox9^{loxp/loxp}* mutants (n=3). Data represent mean \pm s.e.m. *** $p < 0.001$; statistical analysis by unpaired two-tailed Student's *t*-test. Scale bar: 100 μ m.

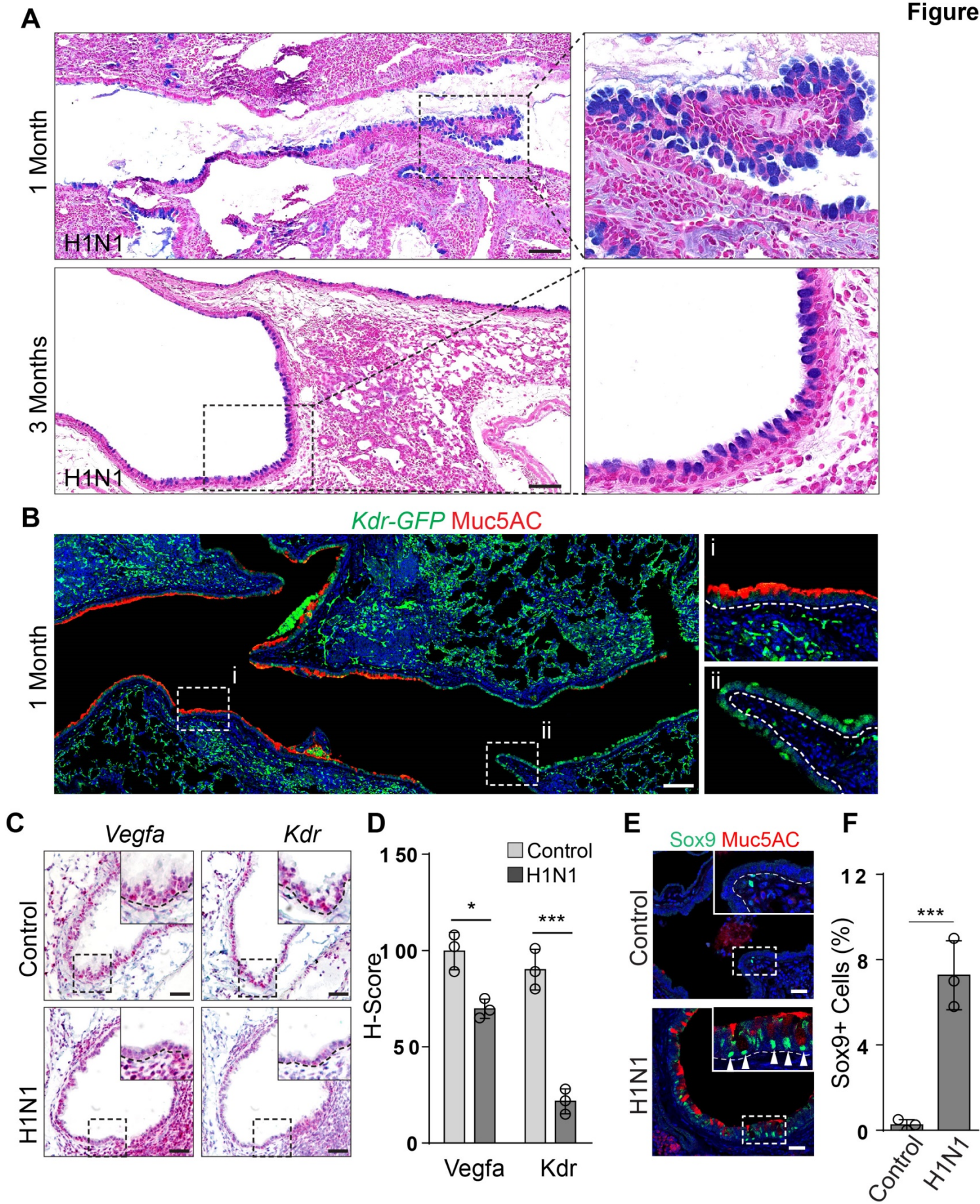


Figure S6 (Related to Figure 6): Extensive mucous metaplasia present in the airways following H1N1 PR8 viral infection accompanied by reduced *Vegfa* and *Kdr* transcripts. (A) Persistent mucous metaplasia (Alcian blue⁺) in the airways of wildtype mice treated with influenza. (B) *Kdr-GFP* expression is reduced in the airway epithelium one month after viral infection. Note the regenerated epithelium without mucous metaplasia shows *Kdr-GFP* expression (insert ii). (C and D) Reduced *Vegfa* and *Kdr* transcripts in the airway epithelium of wildtype mice following viral infection as revealed by RNAscope analysis (n=3). (E and F) Mucous metaplastic epithelial cells express Sox9 in H1N1-infected mouse lung (n=3). Data represent mean \pm s.e.m. * $p < 0.05$; *** $p < 0.001$; statistical analysis by unpaired two-tailed Student's *t*-test. Scale bar: 100 μ m.

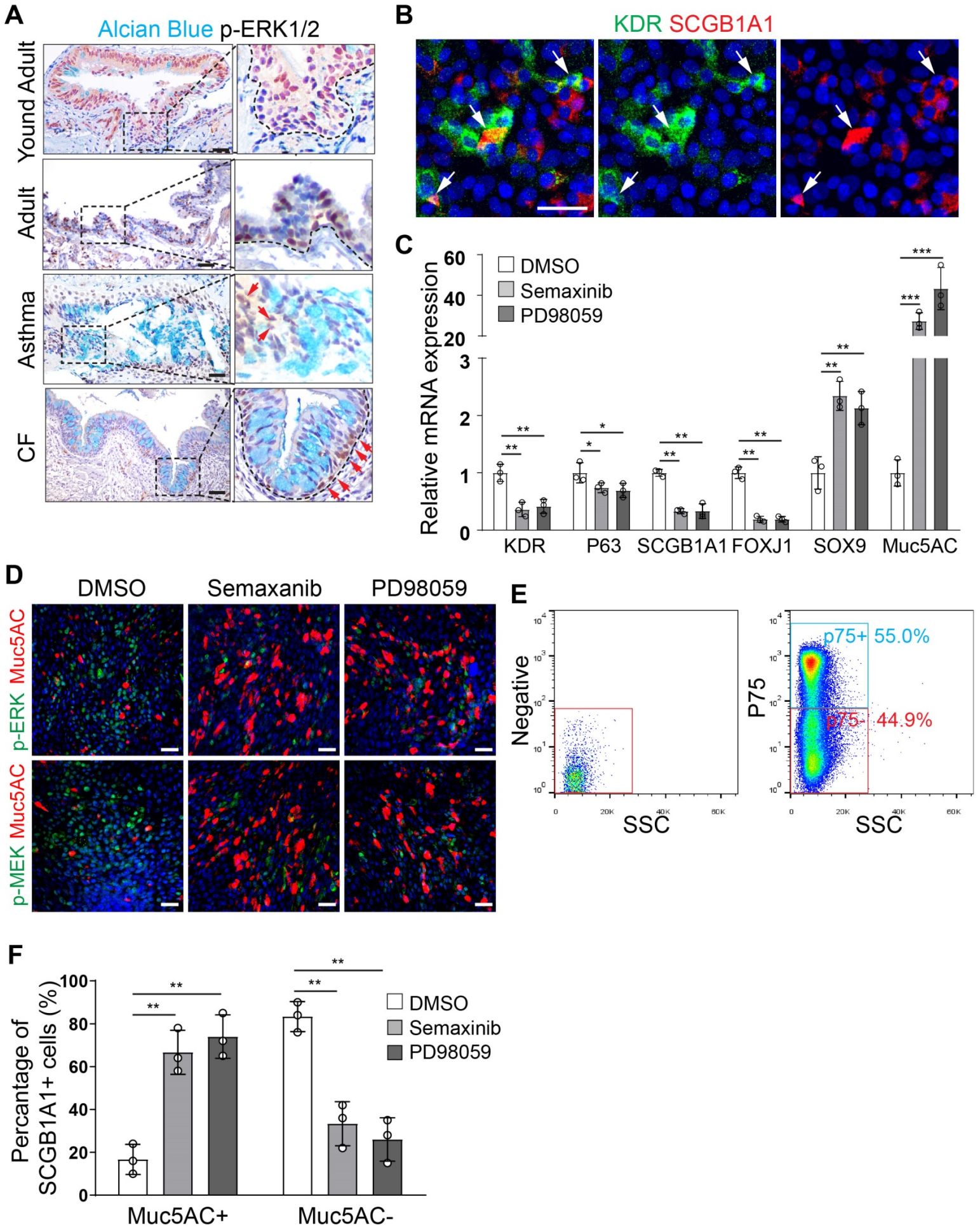


Figure S7 (Related to Figure 7): Suppressed VEGF-KDR signaling during mucous metaplasia of human airway epithelium. (A) Reduced p-ERK1/2 in the airway epithelium of asthma and CF patients. Note pERK1/2⁺ cells (arrows) are not stained by Alcian blue. (B) KDR is co-expressed with SCGB1A1 in air-liquid interface culture of human small airway epithelial cells (HSAECs). (C) Inhibition of KDR signaling with Semaxinib or PD98059 alters the transcript levels of *KDR*, *P63*, *SCGB1A1*, *FOXJ1*, *SOX9*, and *MUC5AC* (n=3). (D) Inhibition of KDR (Semaxinib) and MEK (PD98059) reduces expression of p-ERK and p-MEK. (E) The cell surface marker p75 is used to exclude basal cells. (F) Treatment of Semaxinib or PD98059 promotes mucous cell differentiation of club cells (n=3). Data represent mean ± s.e.m. **p*<0.05, ***p*<0.01, ****p*<0.001; statistical analysis by unpaired two-tailed Student's *t*-test. Scale bar: 100 μm.

Table S1 (Related to Figure 1): Differential gene expression between subpopulations of club cells.

	sub1 vs sub3(avg_logFC)	sub2 vs sub3(avg_logFC)	sub1 vs sub2(avg_logFC)
Scgb1a1	0.27365	0.10258	0.17106
Scgb13a2	-1.17434	-0.91607	-0.25826
Muc5b	-1.79667	-1.6147	-0.181972
Tff2	-2.73341	-2.25524	-0.47816
Agr2	-0.68333	-0.68478	0.00147
Cla1	-0.55428	-0.56432	-0.01004
Reg3g	-3.6599	-3.57156	-0.08834
Scgb3a1	-4.86245	-4.02362	-0.84884
Bpifb1	-3.68443	-3.55663	-0.12784
Bpifa1	-4.39985	-4.36042	-0.03985
Tmsb4x	0.363901	0.38291	-0.019021
Rps5	-0.12537	0.16304	-0.28842
Rpl13	-0.10984	0.15627	-0.25612
Rps6	-0.156872	0.13865	-0.29551
Rps14	-0.91172	0.15923	-0.2504
Rps27	-0.10895	0.14931	-0.25826
Rps37a	-0.111223	0.14524	-0.25645
Rplp0	-0.15433	0.12253	-0.27685
Rps8	-0.09088	0.16096	-0.25182
Rpl18a	-0.128266	0.145724	-0.27399
Rpl9	0.14322	0.42818	-0.28497
Kdr	0.300497	0.255597	0.041473
Scgb1c1	1.030853	1.244696	-0.215648
Igfbp6	0.558976	0.366504	0.192375
Prdx6	0.574796	0.55718	0.01762