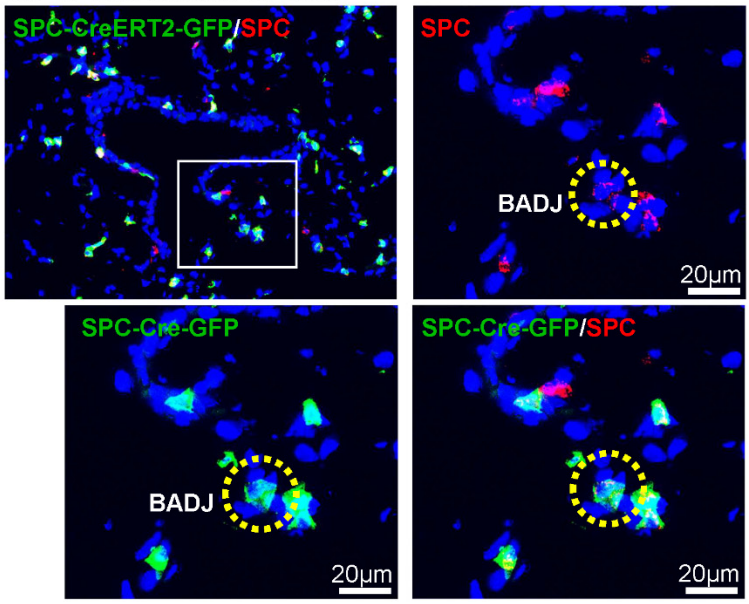
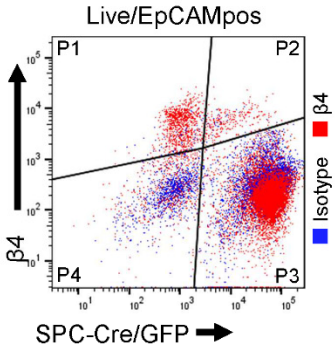
Supplementary Figure S1

A SPC-Cre labels BASCs



B Isolation of non-BASC Airway Cells



P1: β4+ Airway cells (without BASCs)

P2 + P3: AEC2s + BASC

C P1 Colony (β4+/SPC-Cre-neg)

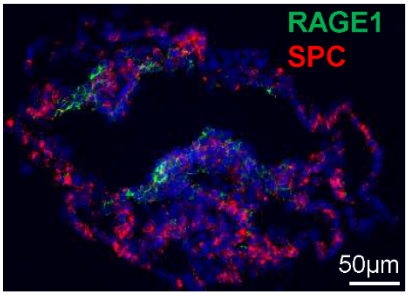


Figure S1, Related to Figure 1: Identification of non-BASC airway epithelial cells as major source of regenerative progenitor cells. (A) BASCs are labeled with SPC-CreERT2-lineage label and display low levels of SPC protein expression, which indicates SPC-CreERT2+ cell population as mature AEC2s + BASCs.

(B) Representative flow plot of isolation of three distinct populations from a tamoxifen labeled SPC-CreERT2/mTmG mouse. P1 represents non-BASC airway cells (β4+/SPC-CreERT2neg), P2 and P3 represent all mature AEC2s and BASCs.

(C) β4+/SPC-CreERT2neg cells cultured in 3D organoid assay with mesenchymal support display differentiation into AGER+/SPC+ alveolar colonies. Image represents a composite of image of multiple images captured at 20X and stitched together (see STAR Methods). Scale bar = 50 μm.

Supplementary Figure S2

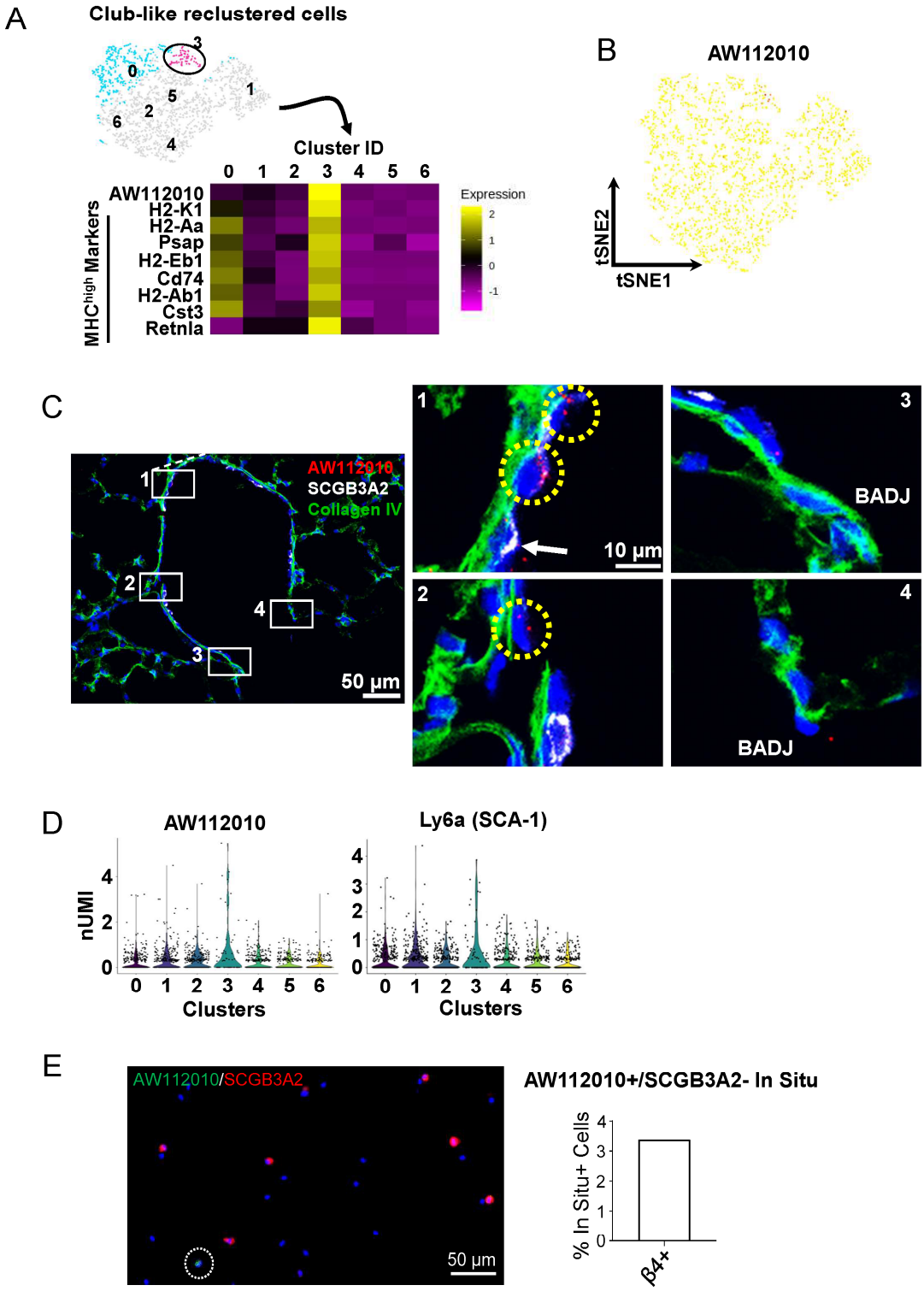


Figure S2, Related to Figure 2: Elevated expression of MHC I and II genes and a long noncoding RNA marks H2-K1^{high} and SCA-1^{high} cluster of cells. (A) MHC class I and class II gene expression is elevated in cluster 3 (H2-K1^{high} progenitor cells). Average expression of genes in every cluster was used to generate Heatmap. These genes are selected from Strunz et al., which identify a club-like cell subset, defined by elevated MHC class II signature (Strunz et al., 2019), that expands during bleomycin injury, ultimately differentiating into RAGE1+ AEC1 cells.

(B) A feature plot of AW112010 transcript shows presence of AW112010^{high} cells in the H2-K1^{high} cluster of cells.

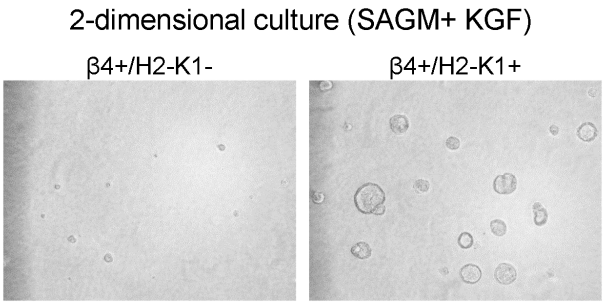
(C) In situ staining for AW112010 lncRNA localizes the progenitor cells in the airway. AW112010 positive cells (red) localize away from the broncheoalveolar duct junctions (BADJ) and have low or no detectable expression of club cell marker Scgb3a2 (white).

(D) These cells are also positive for Sca-1, a marker attributed to BASCs, which suggests that cell sorting via flow cytometry using Sca1 as a surface marker will also isolate H2-K1^{high} cells, which are not BASCs.

(E) Cytospin analysis of in situ hybridized airway cells identifies ~3% of β4pos cells are marked by AW112010^{high}/SCGB3A2^{low} expression, which correlates with ~3% H2-K1^{high} cells isolated via flow cytometry and 2.2% H2-K1^{high} cells identified via single cell mRNA sequencing (70 of ~3100 cells sequenced cells). Image represents a composite of image of multiple images captured at 20X and stitched together (see STAR Methods).

Supplementary Figure S3

A

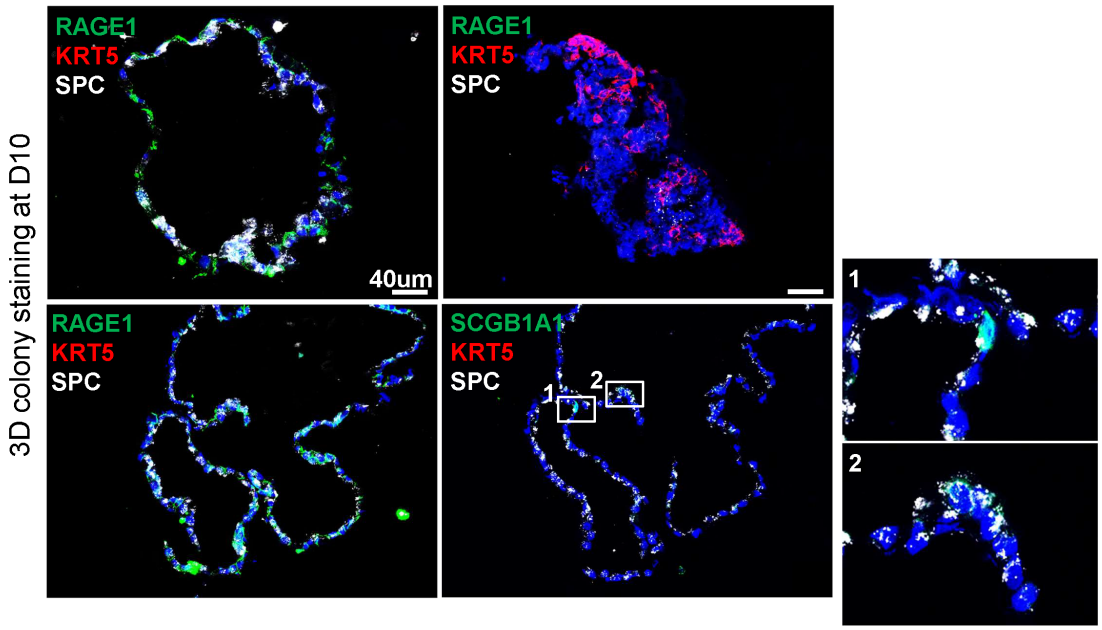


B

Lung Progenitor Media (LPM)

Additive	Concentration	Additive	Concentration
FGF10	50 ng/mL	p38 inhibitor	1 μM
FGF9	50 ng/mL	Rock inhibitor	10 μM
EGF	50 ng/mL	Heparin	5 μg/mL
GSK-3β inhibitor	3 μM	Insulin	10 μg/mL
TGF-β inhibitor	1 μM	Transferrin	15 μg/mL

C



D

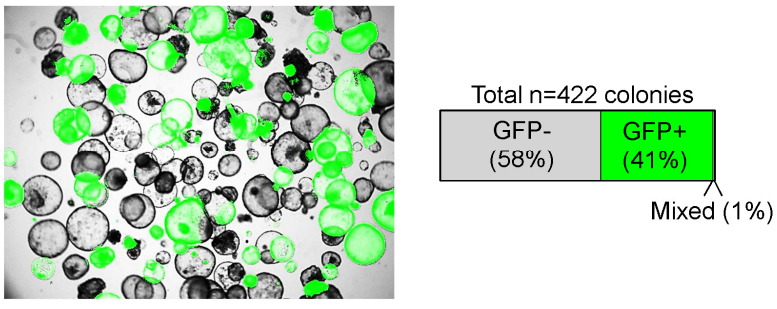


Figure S3, Related to Figure 3: H2-K1^{high} progenitor cells can give rise to alveolar (SPC+/RAGE1+) and airway (Krt5+) colonies.

(A) β4+/H2-K1^{high} cells represented the primary colony forming cells when cultured on 2D matrigel in small airway growth media supplemented with KGF.

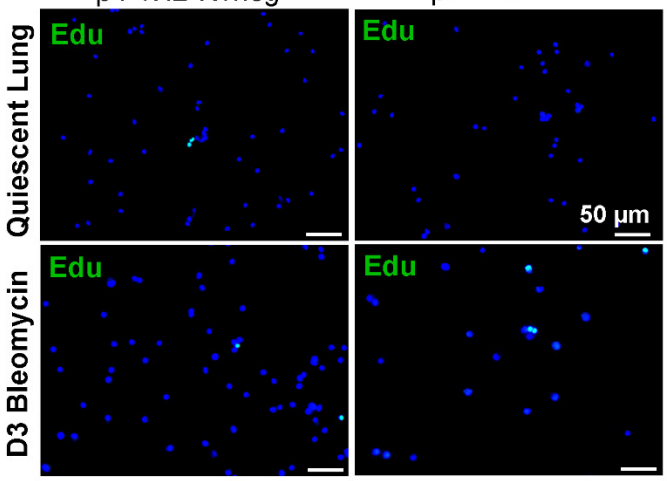
(B) Lung progenitor media (LPM) was used to culture airway progenitors without mesenchyme support. The constituents of the LPM included growth factors that are thought to be released from the mesenchymal cells in a 3D organoid assay.

(C) β4+/H2-K1^{high} cells were cultured in 3D matrigel droplet without mesenchyme in lung progenitor media, which gave rise to either SPC+/RAGE1+ alveolar or KRT5+ airway colonies. Infrequent Scgb1a1+ cells were also observed in colonies that were otherwise SPC+/RAGE1+.

(D) Colony mixing experiments was performed by mixing equal number of progenitor cells from either a C57Bl/6 or a UBC-GFP mouse and colonies that were either GFP- or GFP+ or both were counted.

Supplementary Figure S4

A

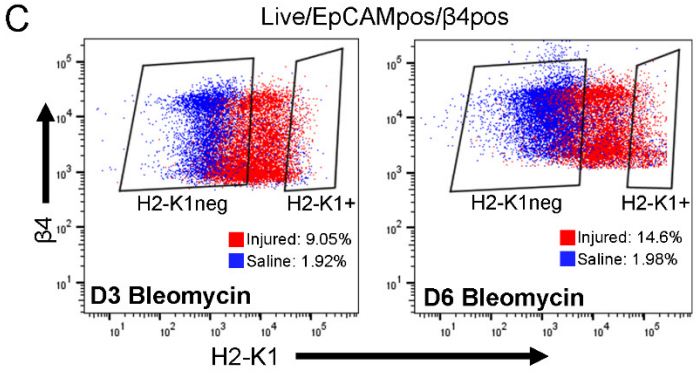


B

Quantification

Cell Type	D0 Edu (Quiescent)	D3 Edu
β4+/H2-K1neg	11/635 (1.7%)	10/490 (2.04%)
β4+/H2-K1+	0/585 (0%)	62/564 (10.99%)

C



D

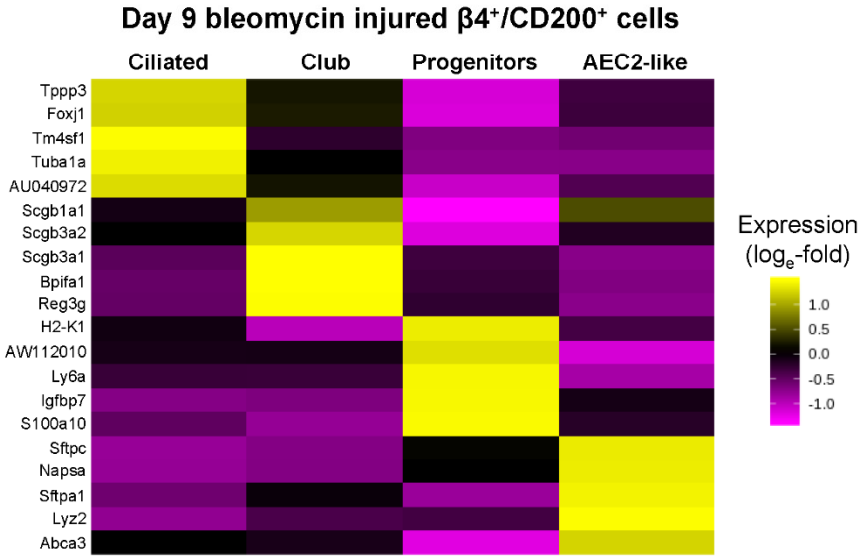


Figure S4, Related to Figure 4: Quiescent H2-K1^{high} airway cells proliferate and expand during early stages of bleomycin injury.

(A-B) β4+/H2-K1^{high} cells were isolated and cytospins were prepared from Edu labeled (once every 24 hours for a total of 72 hours) uninjured or bleomycin injured. Quiescent H2-K1^{high} cells are non-proliferative (0% Edu+ cells). ~11% of β4+/H2-K1^{high} cells proliferate after injury, while only 2% of remaining airway cells proliferate after injury – a rate consistent with an uninjured lung.

(C) Representative flow plots of bleomycin injured lungs show that ~2% β4+/H2-K1^{high} cells (at Day 0) expand to ~9% (Day 3) and ~15% (Day 6) after bleomycin injury.

(D) Heatmap displays average expression of top five markers from each of the cell type identified from injured β4+/CD200+ cells.

Supplementary Figure S5

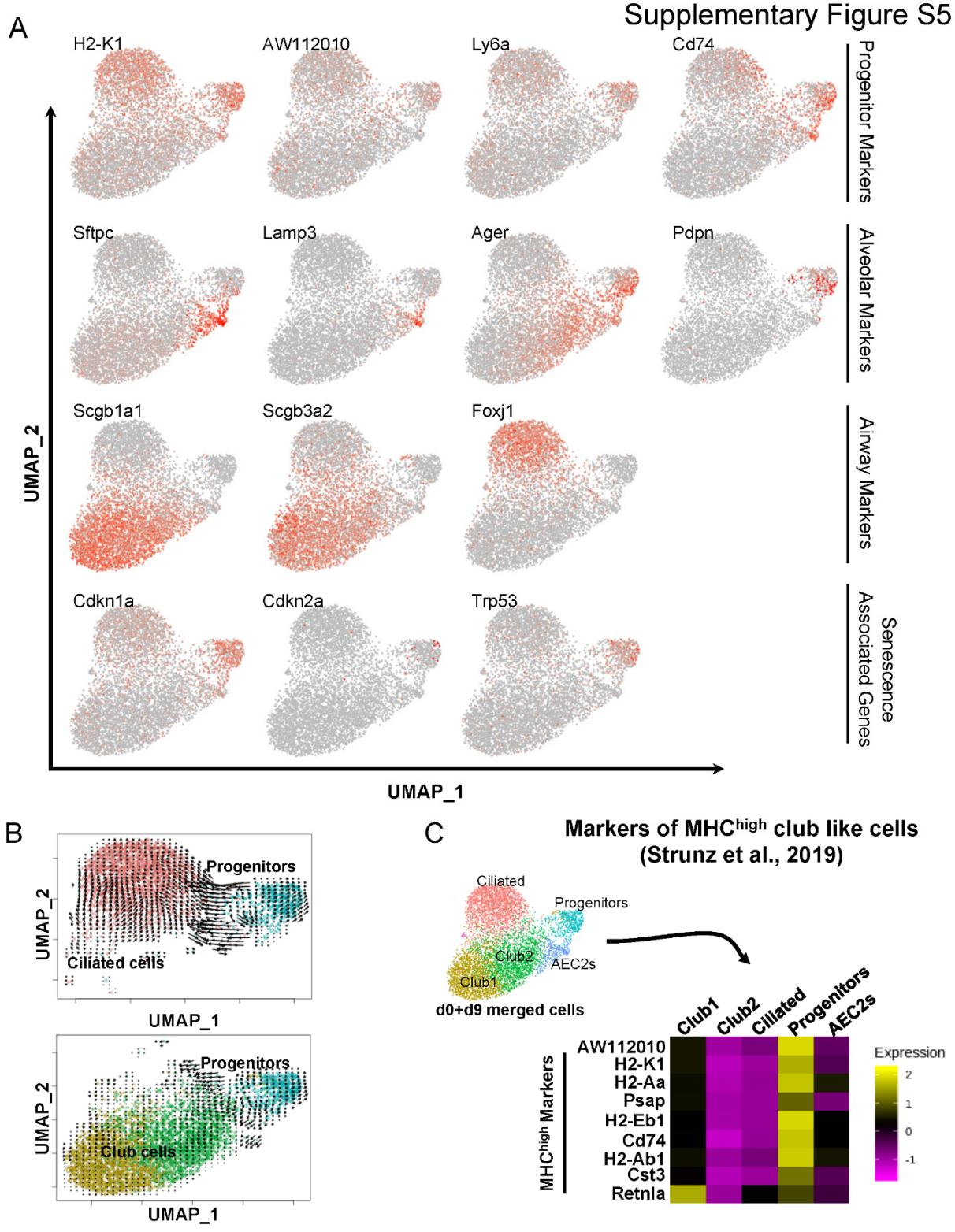


Figure S5, Related to Figure 5: Cluster identification of injured and uninjured cells.

(A) Featureplots of progenitor markers, alveolar markers, airway markers, and senescence-associated markers are plotted, highlighting a H2-K1^{high} progenitor cluster, club cell clusters, and ciliated cells.

(B) RNA velocity plotted on top of the UMAP embedding of either progenitors and ciliated clusters or progenitors and club cell clusters.

(C) Injured H2-K1^{high} cluster is marked by elevated expression of the MHC class I and class II genes, identified as features of specialized club-like cells (Strunz et al., 2019). Expression of these same set of genes were also elevated in uninjured H2-K1^{high} cells (Figure S2).

D14 post transplant

Supplementary Figure S6

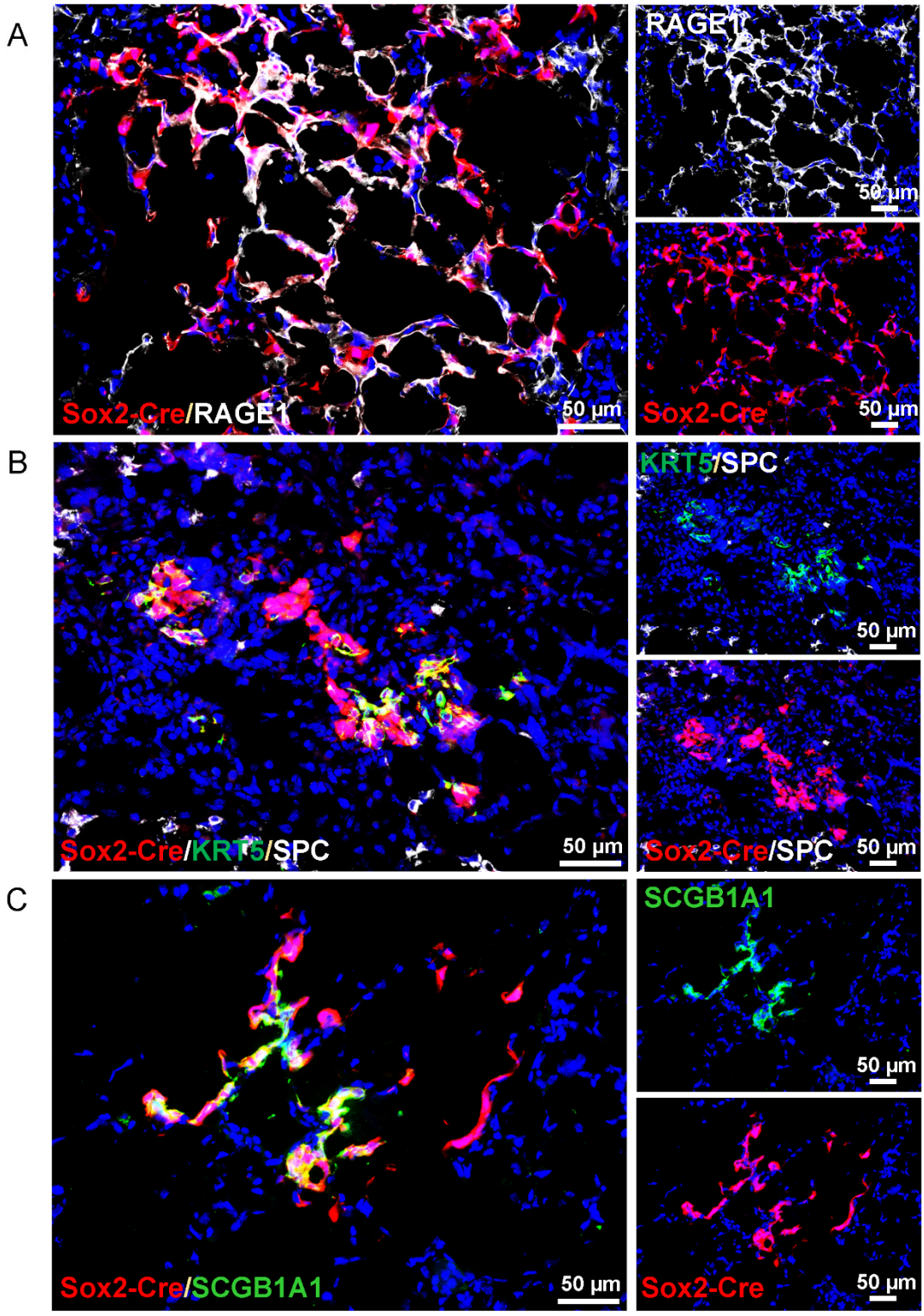


Figure S6, Related to Figure 5: H2-K1^{high} progenitors engraft and differentiate in injured lungs. Freshly sorted Sox2-CreERT2+/TdTomato+ (referred to as Sox2-Cre) cells were transplanted in bleomycin injured lungs (d10 post injury), which engrafted and differentiated into **(A)** RAGE1+ AEC1s, **(B)** KRT5+ basal cells and **(C)** Scgb1a1+ club cells in injured lungs.

Supplementary Figure S7

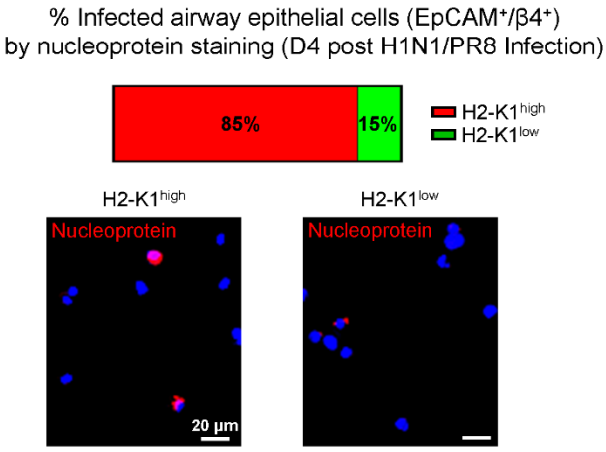


Figure S7, Related to Figure 7: Influenza virus preferentially targets β4+/H2-K1^{high} cells.

Analysis of all airway epithelial cells from mice injured with H1N1 PR8 influenza virus revealed that 85% of the infected airway cells were β4+/H2-K1^{high} as determined by staining for viral nucleoprotein on day 4 post influenza injury (n=2 mice).