**Cell Reports, Volume *30***

## Supplemental Information

# Platform Effects on Regeneration by Pulmonary Basal Cells as Evaluated by Single-Cell RNA Sequencing

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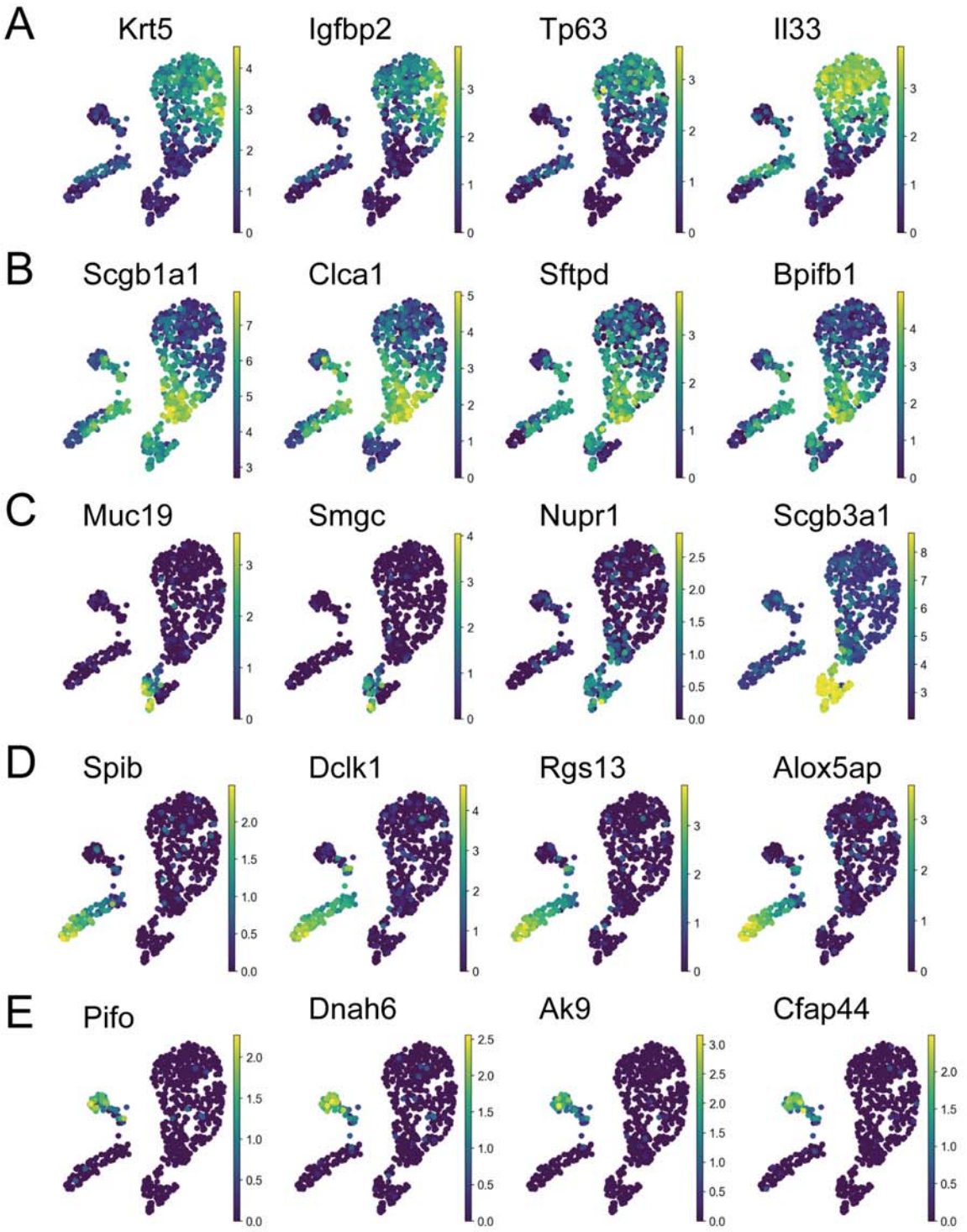


Figure S1. Population distribution of differentially expressed genes in native rat tracheal epithelium by scRNAseq, related to Figure 1. umaps of A) BC markers Krt5, Igfbp2, Tp63, Il33, B) secretory cell markers Scgb1a1, Clca1, Sftpd, Bpifb1, C) sub-mucosal gland secretory cell markers Muc19, Smgc, Nupr1, Scgb3a1, D) tuft cell markers Spib, Dclk1, Rgs13, Alox5ap, and E) ciliated cell markers Pifo, Dnah6, Ak9, Cfap44.

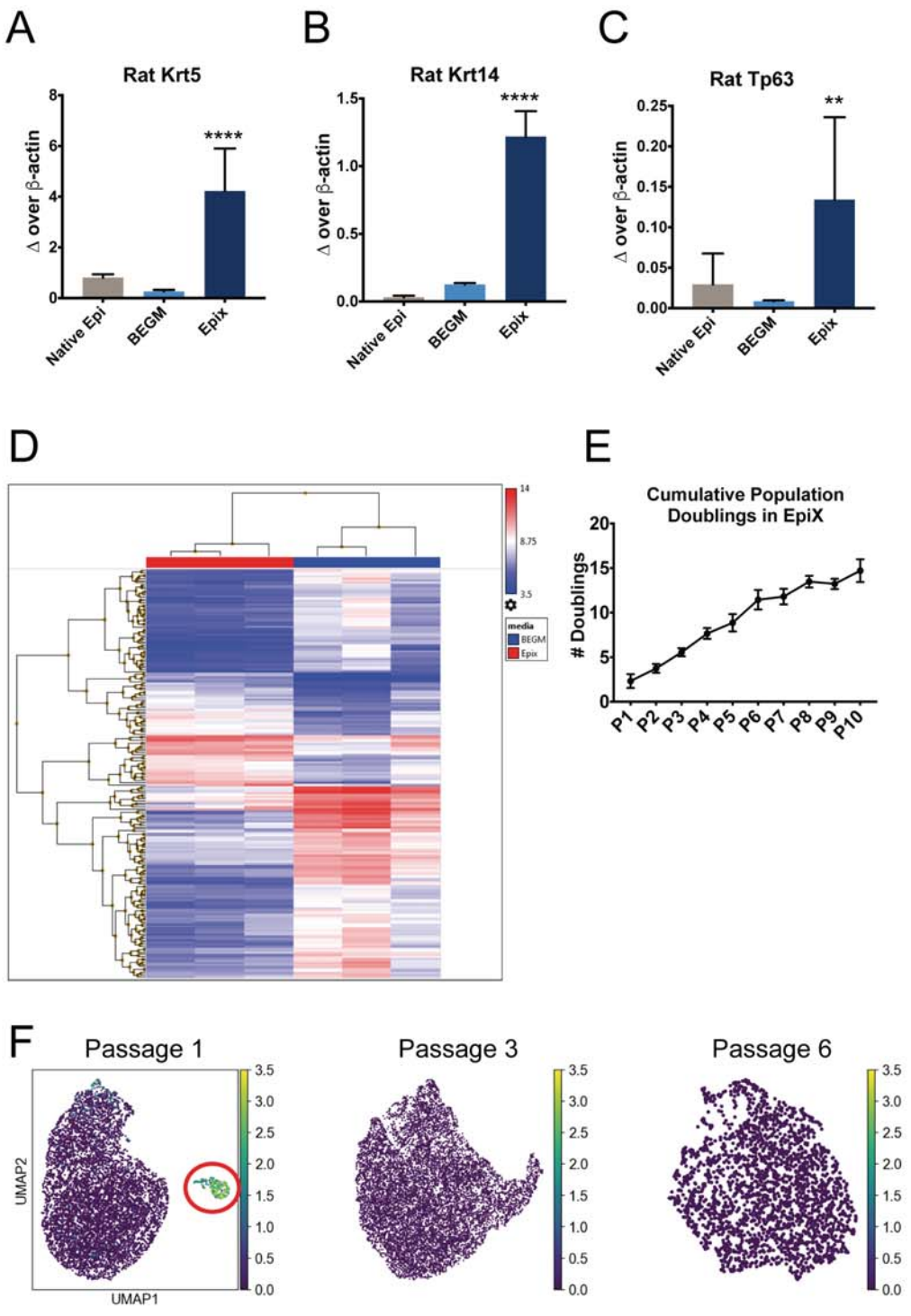


Figure S2. Media comparison between BEGM and EpiX, related to Figure 2. PCR gene expression for cells cultured in BEGM or EpiX for 9 days at P1, relative to bulk native tracheal epithelium for A) Krt5, B) Krt14, and C) Tp63 (****p<0.0001, **p<0.01). D) Microarray gene analysis showing bulk differential gene patterning for three lines of cells grown for 8 days at P1 in either BEGM (blue column label) or EpiX (red column label), showing distinctive transcriptomic variation. E) Growth curve of three lines of peBC in EpiX showing 15 population doublings over 10 passages. F) scRNAseq umaps of peBC clustering at P1, P3, and P6, colored by expression of Vimentin; red circle indicating Vim+ population in P1.

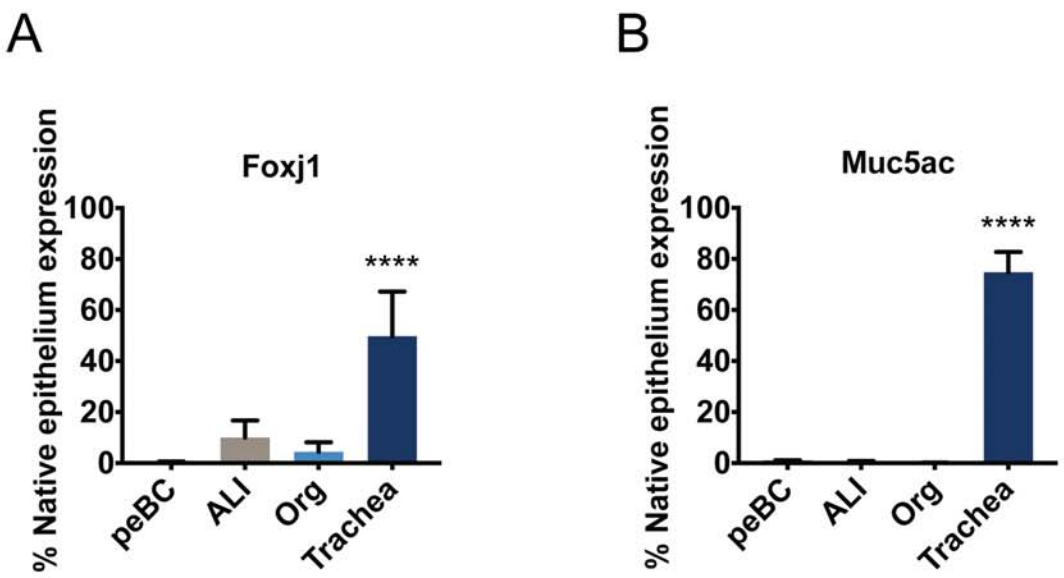


Figure S3. Relative mucociliary maturity in engineered samples, related to Figure 3. qRT-PCR gene expression of A) ciliated marker Foxj1 and B) secretory marker Muc5ac, in proximal engineered conditions ALI, organoid, and engineered trachea, including starting peBC at P3 (n=3 each), all expressed as a percentage of expression in bulk native tracheal epithelium (****$p<0.0001$).

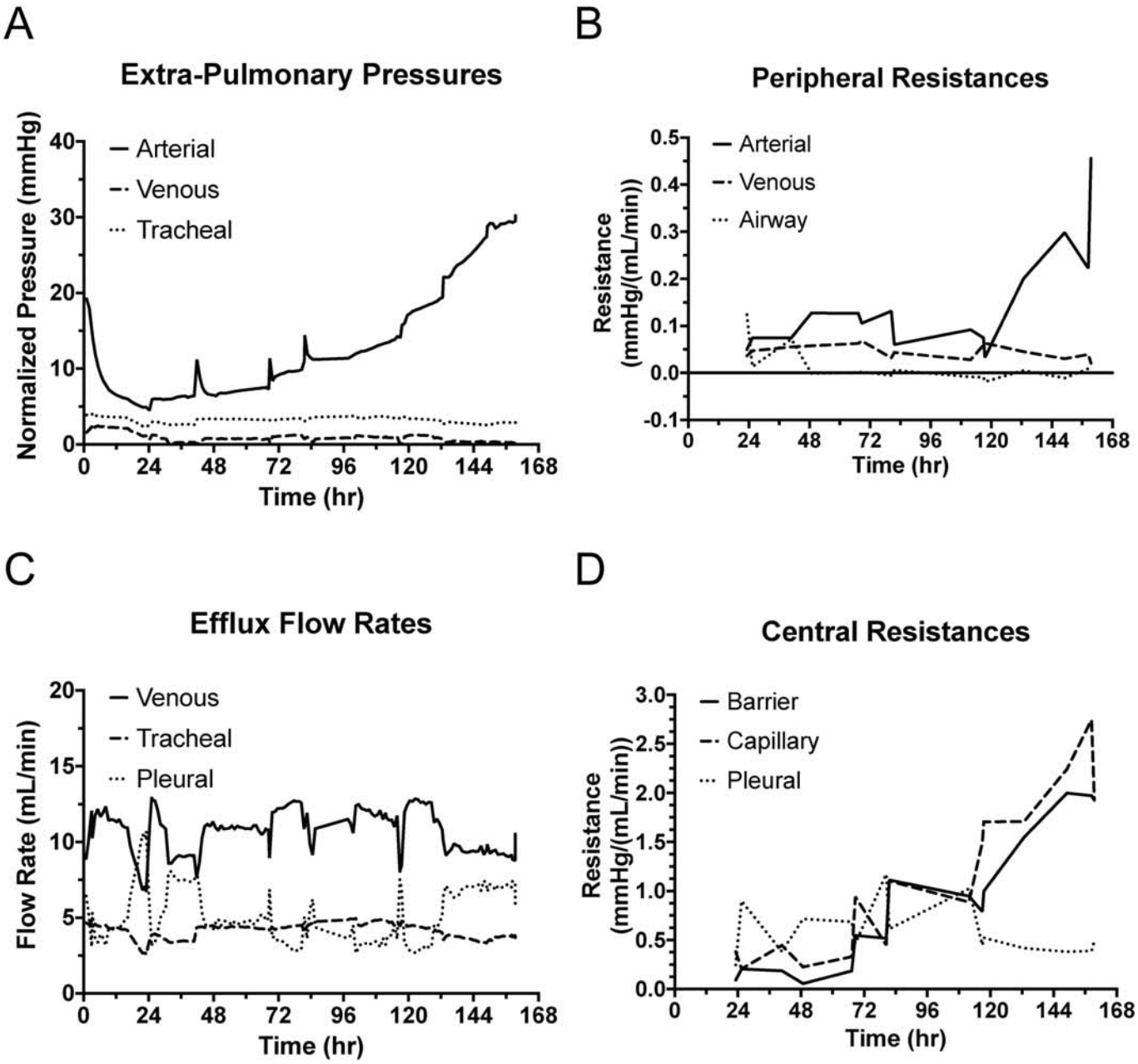


Figure S4. Mechanics of peBC culture in an engineered lung, related to Figure 3. A) Extra-pulmonary pressures measured continuously for the duration of culture (1 week) at the inlet (pulmonary artery (PA), “Arterial”) and outlets (pulmonary vein (PV), “Venous” and trachea, “Tracheal”) of the lung, normalized to measured bioreactor pressure. B) Peripheral resistances of arterial and venous vasculature, and airway tree calculated from data recorded during manual barrier measurements. C) Efflux flow rates extrapolated from these pressures and resistances for all paths through which medium introduced through the PA could exit the organ. D) Central resistances extrapolated from these values representing resistance of the organ to fluid crossover from vasculature to alveoli (“Barrier”), resistance through the capillaries (“Capillary”), and resistance to fluid across the pleura (“Pleural”).

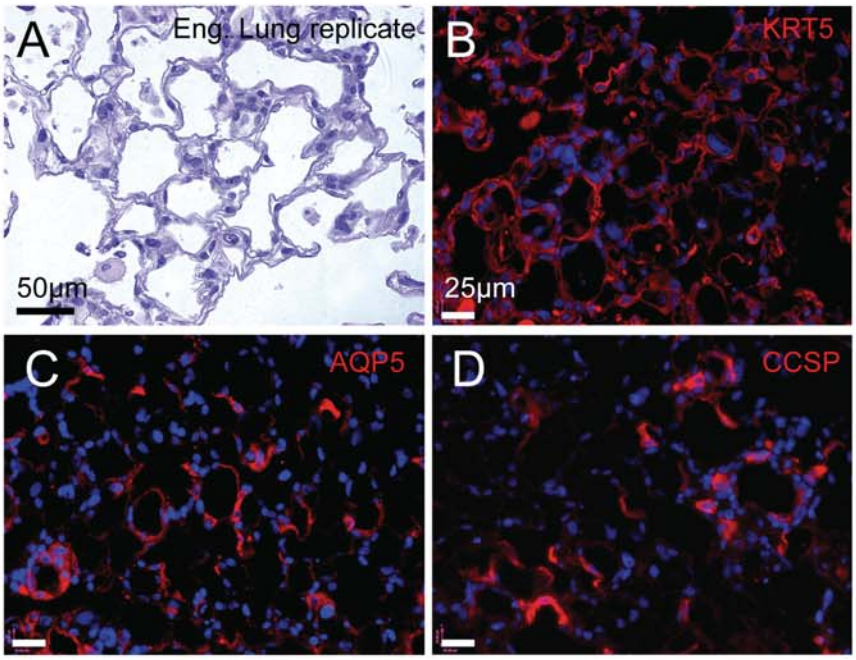


Figure S5. Repeatability of engineered lung culture with peBC, related to Figure 3. Histology of an engineered lung replicate: A) H&E (scale bar = 50µm) , IF staining for B) KRT5, C) AQP5, D) CCSP (scale bars = 25µm).

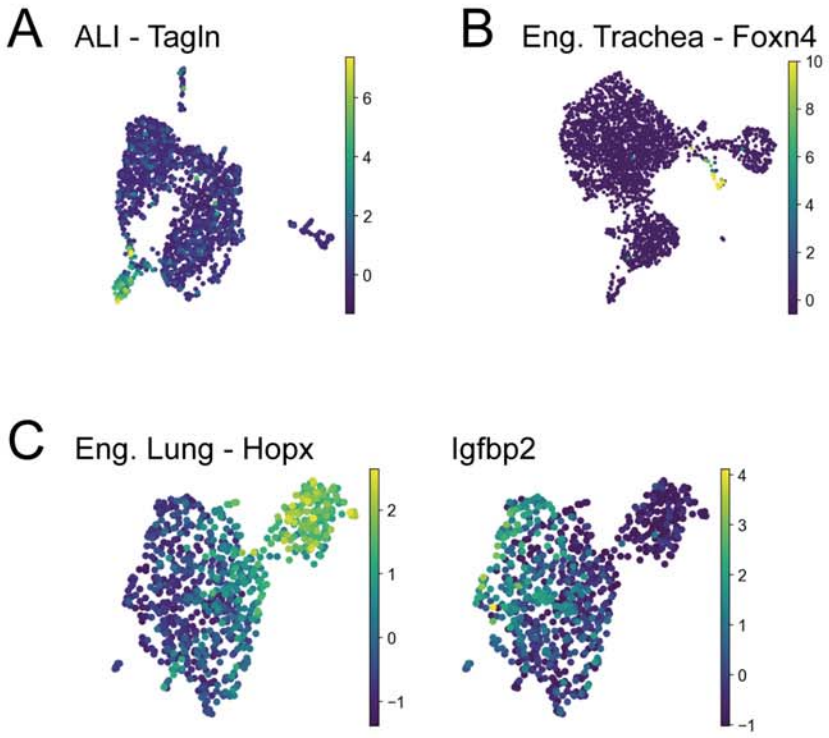


Figure S6. Unique populations in engineered samples, related to Figure 4. umaps of A) Tagln in ALI, B) Foxn4 in engineered trachea, and C) Hopx and Igfbp2 in engineered lung.

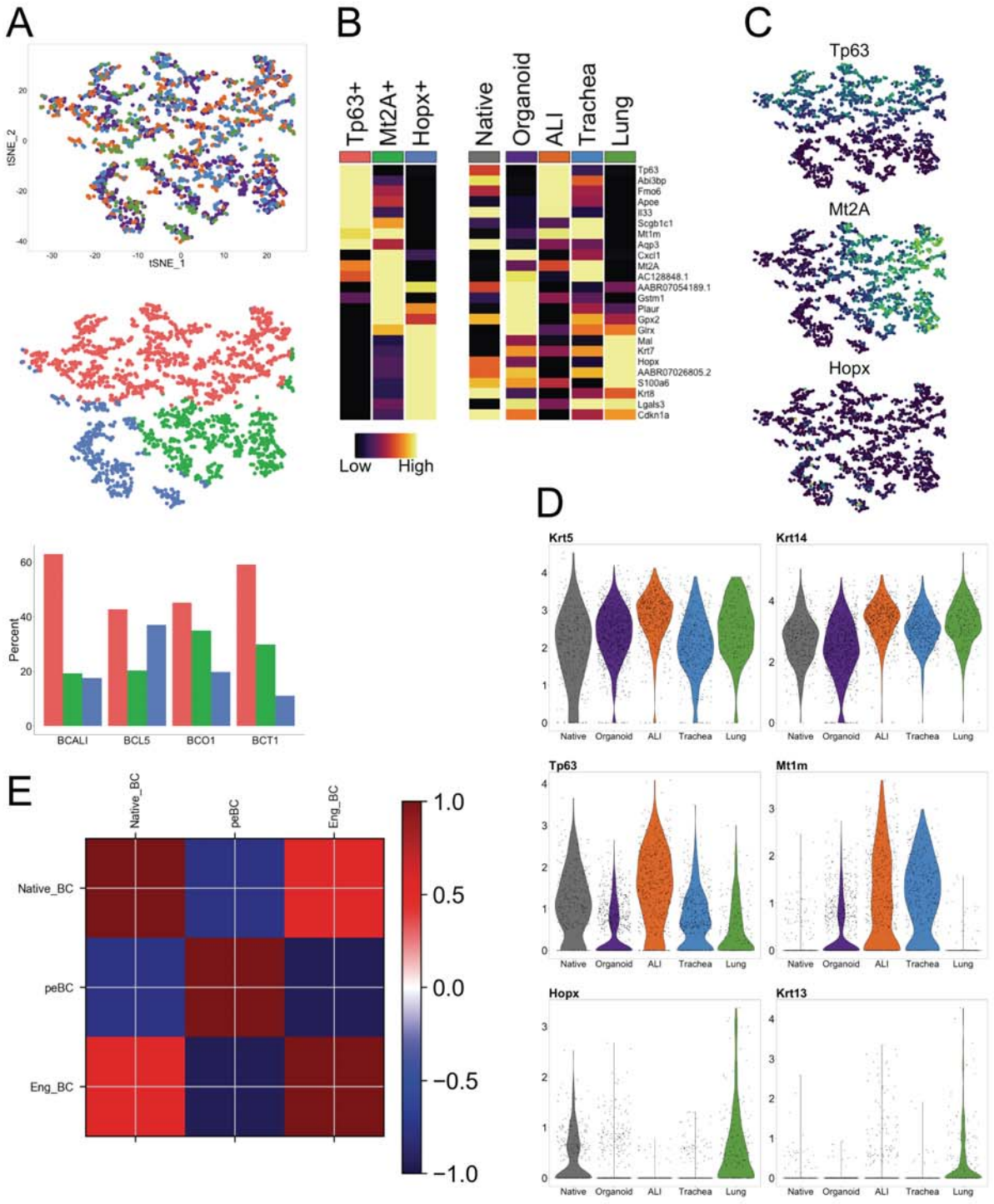


Figure S7. Heterogeneity of engineered BC compared to native BC and starting peBC, related to Figure 7. A) TSNE plots showing alignment of engineered BC from organoid, ALI, engineered trachea, and engineered lung (top), subclustering of this aligned sample (middle), and the proportions of each subcluster comprising engineered samples (bottom). B) Heatmap of top differentially expressed genes (greatest average log fold-change, see Method Details) for each subcluster of the engineered BC alignment with comparison to native BC and each individual engineered sample; heatmap columns show average gene expression for each cluster; rows represent unity normalized expression of the top defining genes across all clusters. C) Featureplots of engineered BC alignment showing heterogeneous expression of Tp63, Mt2A, and Hopx. D) Violin plots showing relative gene expression of native BC and engineered BC from organoid, ALI, engineered trachea, and engineered lung (all scaled together) of Krt5, Krt14, Tp63, Mt1m, Hopx, and Krt13. E) Heatmap of pearson correlation of native BC, merged peBC across passage (Fig 2), and merged engineered BC based on the variable genes used to cluster the native sample (2,424 genes).

Table S1. Rat primer sequences for qRT-PCR, related to Figures 2, S2, & S3, and STAR Methods.

| Gene | Forward | Reverse |
|---|---|---|
| β-actin | GCAGGAGTACGATGAGTCCG | ACGCAGCTCAGTAACAGTCC |
| Foxj1 | ATCGTCGTGCACATCTCGAA | TGTGGGTTGGTGGCATAGTC |
| Krt14 | CATCGAGGACCTGCCGAGCA | GTAGGCCATTGATGTCAGACTC |
| Krt5 | ACCTGCAAATCGATCCCACC | ATGACCCCATCCAGCTGTCTA |
| Muc5ac | AAGGGGTTAGTCAGGACTGC | TGGGGCGGTAGATGTGGATA |
| Tp63 | GGAAAGCAATGCCCAGACTC | AGGGGCTGGTAGATGAGGAG |