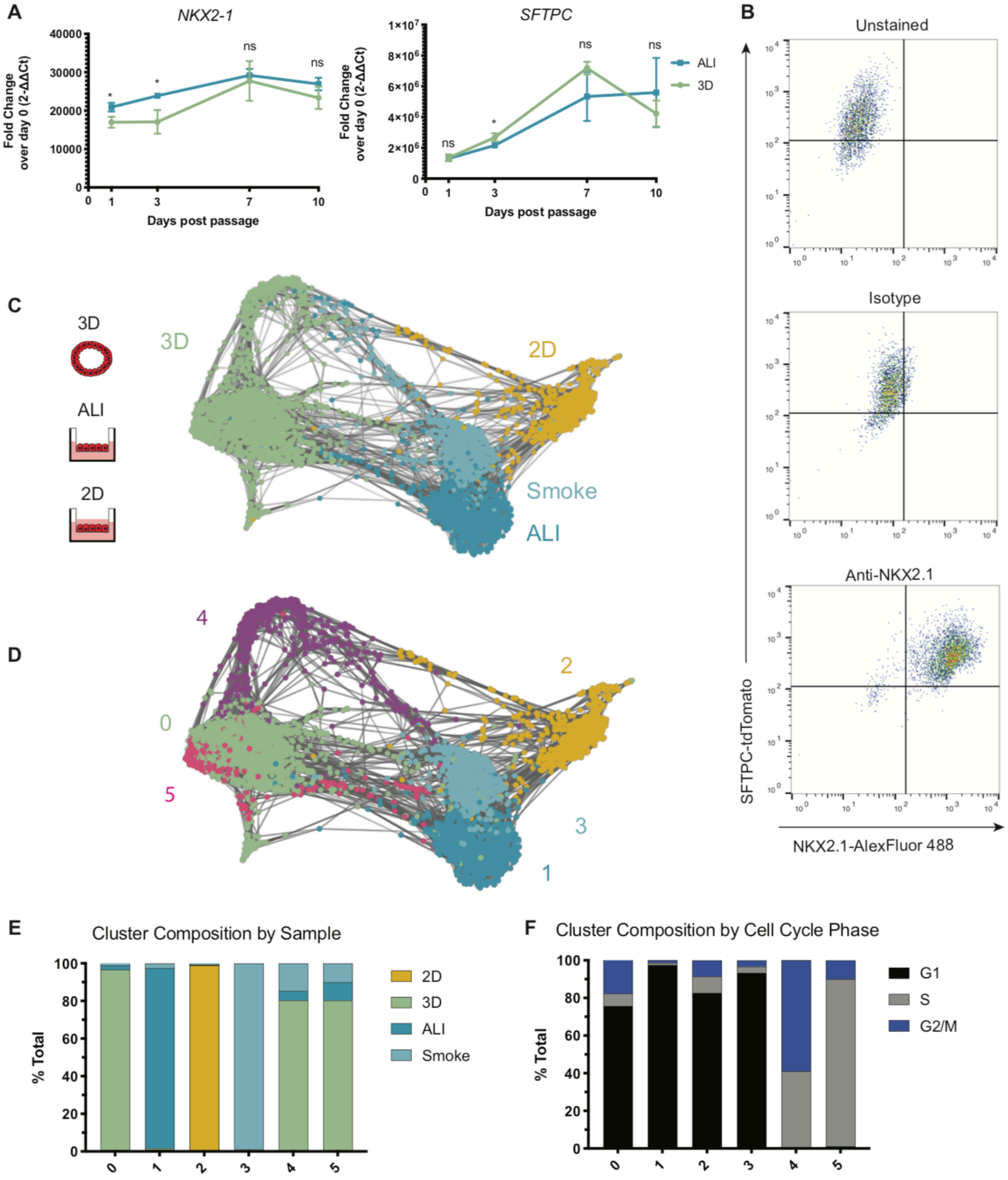


Supplemental Figure S1.

(A) RT-qPCR of *NKX2-1* and *SFTPC* expression at 1, 3, 7, and 10 days post passage in iAT2s cultured at 3D or ALI (mean ± standard deviation, unpaired two-tailed Student's t test, n=3)

(B) Representative flow cytometry plots of iAT2s with SFTPC^{tdTomato} reporter cultured in ALI and stained for NKX2.1, table of percent NKX2.1+, tdTomato+, and NKX2.1/tdTomato double + cells per replicate (n=3).

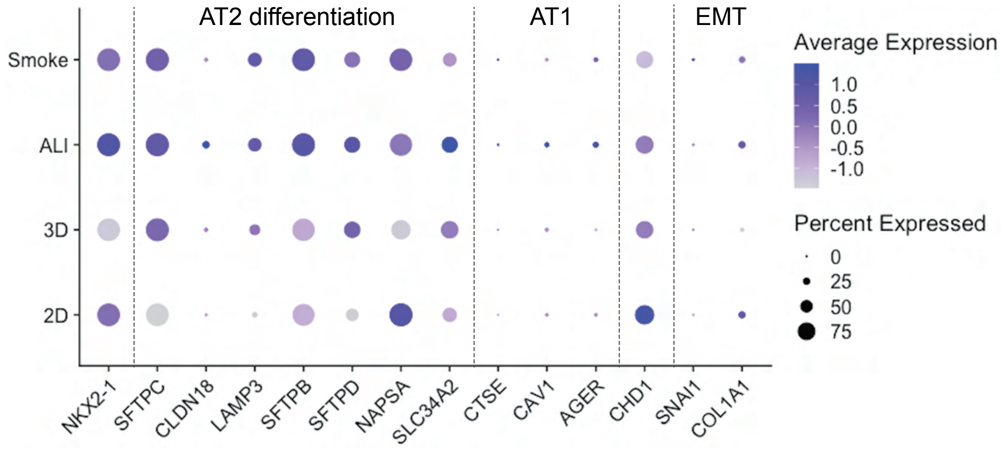
(C) SPRING plot of iAT2s cultured in 3D, 2D, or ALI and profiled by scRNA-Seq 12 hours after cigarette smoke exposure (Smoke) or air exposure (ALI), colored by condition.

(D) SPRING plot colored by Louvain cluster (res=0.25).

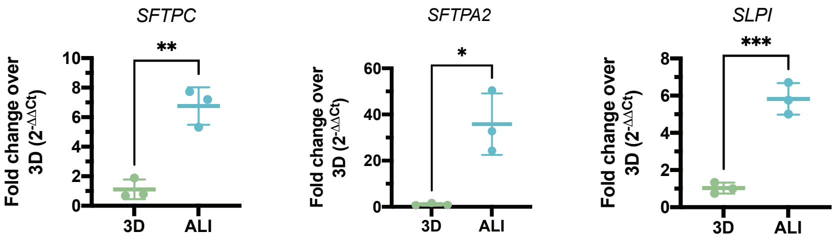
(E) Sample contribution to each Louvain cluster (res=0.25).

(F) Transcriptomically predicted cell cycle phase of each Louvain cluster (res=0.25).

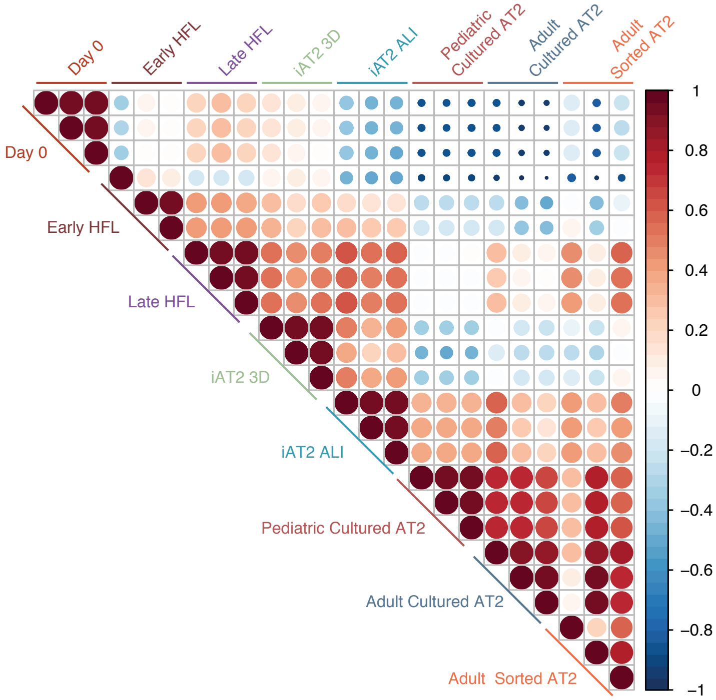
*Mean ± standard deviation. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.*



Supplemental Figure S2. Dot plot of gene expression in iAT2 cultured in 3D, ALI, and 2D profiled on day 10 by scRNA-Seq.



Supplemental Figure S3. RT-qPCR of AT2 gene expression *SFTPC, SFTPA2, SLPI* in iAT2s generated from the iPSC line BU3 cultured in 3D versus ALI. (mean ± standard deviation, unpaired two-tailed Student's t test, n=3)
Mean ± standard deviation. $*p\leq0.05$, $**p\leq0.01$, $***p\leq0.001$.



Supplemental Figure S4. Heatmap of Pearson correlation coefficients calculated between each sample based on normalized expression of human lung epithelial genes from an independent dataset.

TABLES IN SUPPLEMENTAL DATA:

Table 1. CIBERSORT signature matrix containing relative expression values of each gene (rows) in fibroblasts or AT2s (columns).

Table 2. Table of proportional contribution in each sample attributable to fibroblast or AT transcripts (CIBERSORT weights).

Table 3. Pearson correlation coefficients, individual comparisons, visualized as heat map in Supplemental Figure S3 and as group averages in Figure 3C.

Table 4. Hallmark gene sets enriched in Adult Sorted AT2 vs iAT2 ALI (FDR<0.05, ranked by direction of differential expression and P value).