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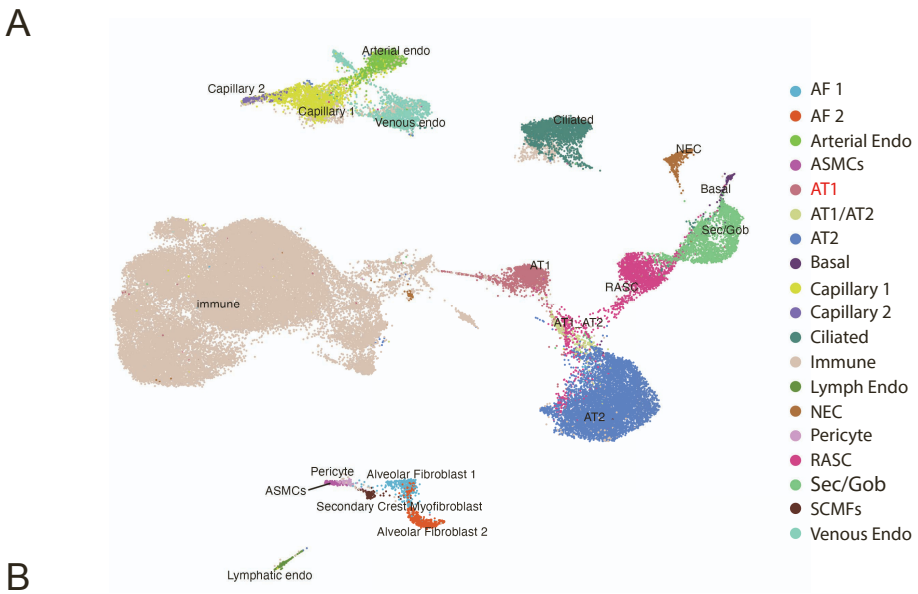
Supplemental Information

Generation of human alveolar epithelial type I cells from pluripotent stem cells

Claire L. Burgess, Jessie Huang, Pushpinder S. Bawa, Konstantinos-Dionysios Alysandratos, Kasey Minakin, Lauren J. Ayers, Michael P. Morley, Apoorva Babu, Carlos Villacorta-Martin, Maria Yampolskaya, Anne Hinds, Bibek R. Thapa, Feiya Wang, Adeline Matschulat, Pankaj Mehta, Edward E. Morrisey, Xaralabos Varelas, and Darrell N. Kotton

Supplemental Figure 1



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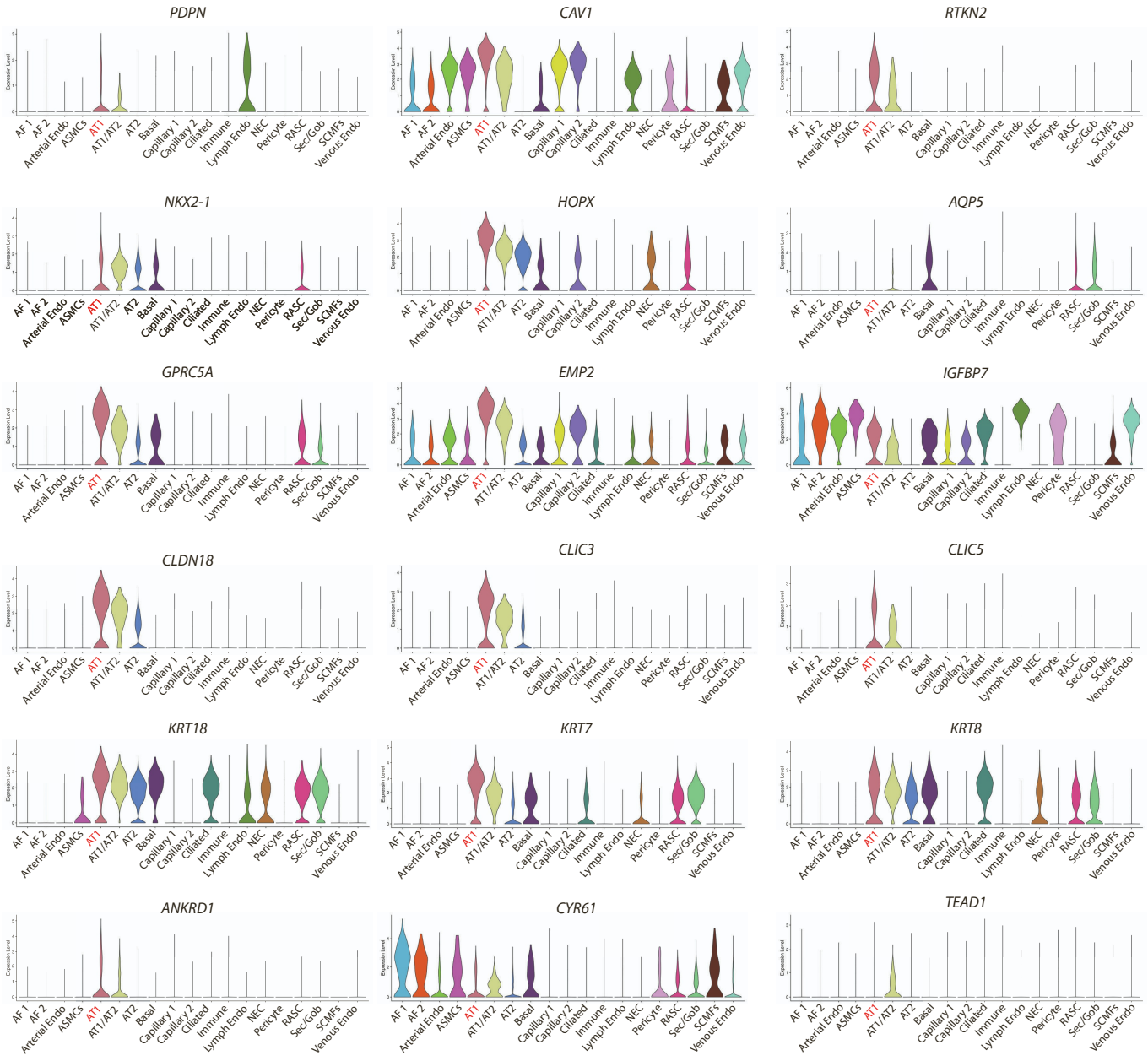
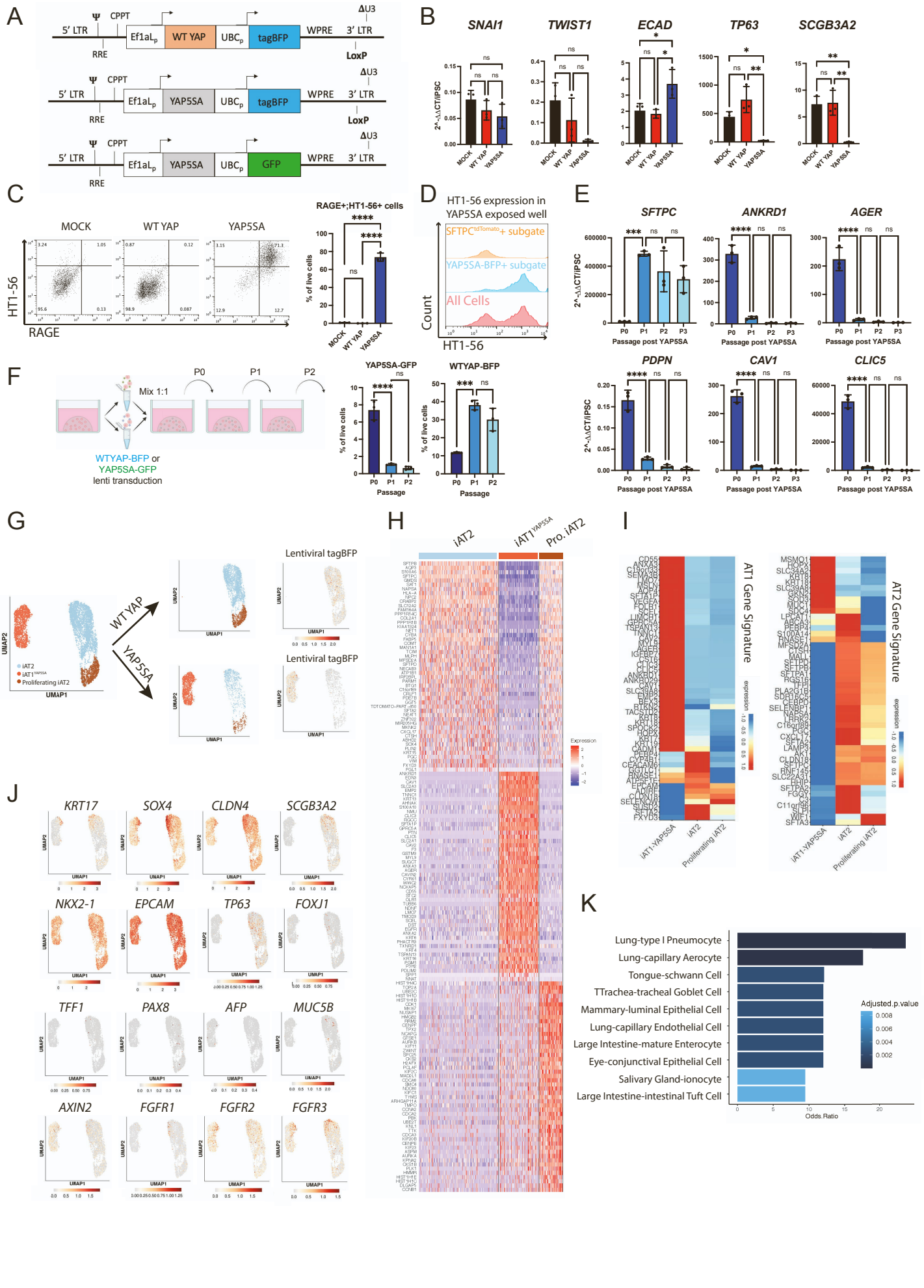


Supplemental Figure 1: Human AT1 marker gene expression across all cell types in the lung, related to Figure 1.

A) UMAP projection of all lung cells in previously published dataset by Basil et al.[37] (N=5, 58567 total cells, 1401 AT1s). B) Violin plots showing gene expression of selected AT1 marker genes across all lung cell types represented in the dataset.

Supplemental Figure 2

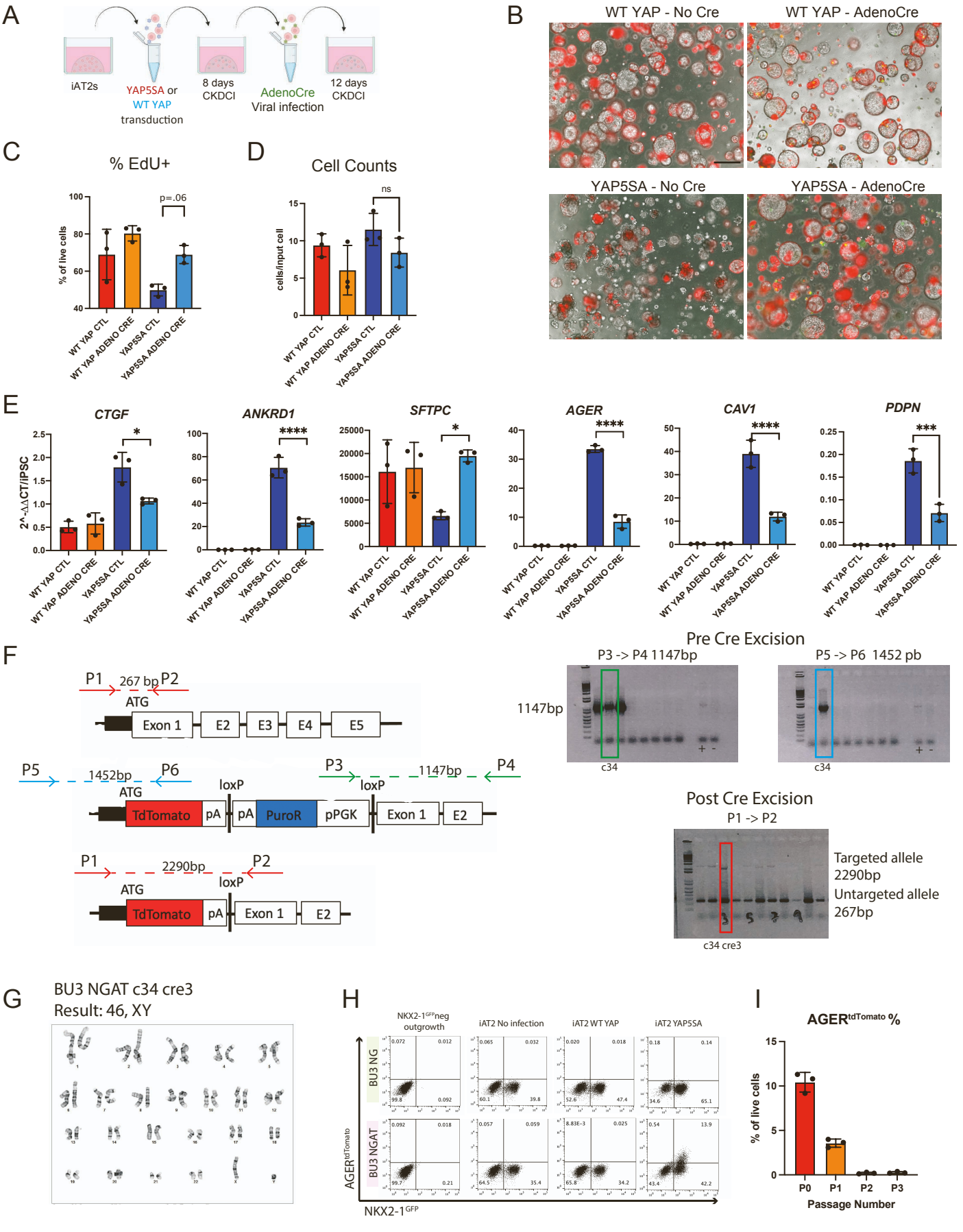


Supplemental Figure 2: YAP5SA transduction of SPC2B2 iAT2s, related to Figures 2 and 3.

A) Diagram of lentiviruses showing dual promoter system with either: WT YAP or YAP5SA in the first gene position, tagBFP or GFP in the second gene position, and LoxP site in the 3'LTR. B) Whole well gene expression by RT-qPCR of EMT markers, airway lung lineage markers and non-lung endoderm markers 14 days post YAP5SA, WT YAP, or Mock lentiviral transduction of SPC2-ST-B2 iAT2s, relative to Day 0 iPSCs. (N=3 wells per condition, one way ANOVA) C) Live cell flow cytometry analysis of staining for RAGE protein and AT1 marker HT1-56 in wells exposed to either WT YAP, YAP5SA lentivirus or Mock control (N=3 per condition, 1 way ANOVA). D) Representative FACS histograms of HT1-56 cell surface protein staining in cells exposed to YAP5SA lentivirus, showing HT1-56 expression levels in sub-gates of either lentiviral tagBFP+ cells, $SFTPC^{tdTomato}$+ cells, or all cells. E) Whole well gene expression by RT-qPCR of AT2 and AT1 markers over 4 passages of cells transduced with YAP5SA lentivirus. (Passaging every 14 days for a total of 56 days post lentiviral transduction). (N=3 wells per condition, one way ANOVA). F) Competition assay – SPC2-ST-B2 iAT2s were transduced with either a WT YAP–tagBFP or YAP5SA-GFP lentivirus and mixed 1:1 before 3D plating. Cells were assessed by FACS with percentage of positive cells calculated based on mock-transduced negative control. Whole wells were passaged normally every 14 days for 3 passages (1-way ANOVA). G) Sub plots of UMAP projections of WT YAP and YAP5SA wells to show lentiviral tagBFP expression in each condition (SPC2-ST-B2). H) Full heatmap of top 50 differentially upregulated genes in the iAT2, $iAT1^{YAP5SA}$, and Proliferating iAT2 populations. I) Heatmap of genes in the 50 AT1 gene signature or 50 AT2 gene signature across each population. J) Gene expression overlays of aberrant basaloid transitional markers *KRT17, SOX4*, and *CLDN4*; Non-lung endoderm markers *TFF1, PAX8*, and *AFP*; other lung markers *TP63, MCU5B, FOXJ1, SCGB3A2*, *NKX2-1; WNT target gene AXIN2;* and FGF receptors. K) Enrichr analysis of top 92 (FDR < 0.05, logFC > 1) differentially upregulated genes in $iAT1^{YAP5SA}$ population using Tabula Sapiens dataset.

*p<0.05, **p<0.01, ***p<0.001, and ****p<0.001, bars represent mean +/- SD for all panels.

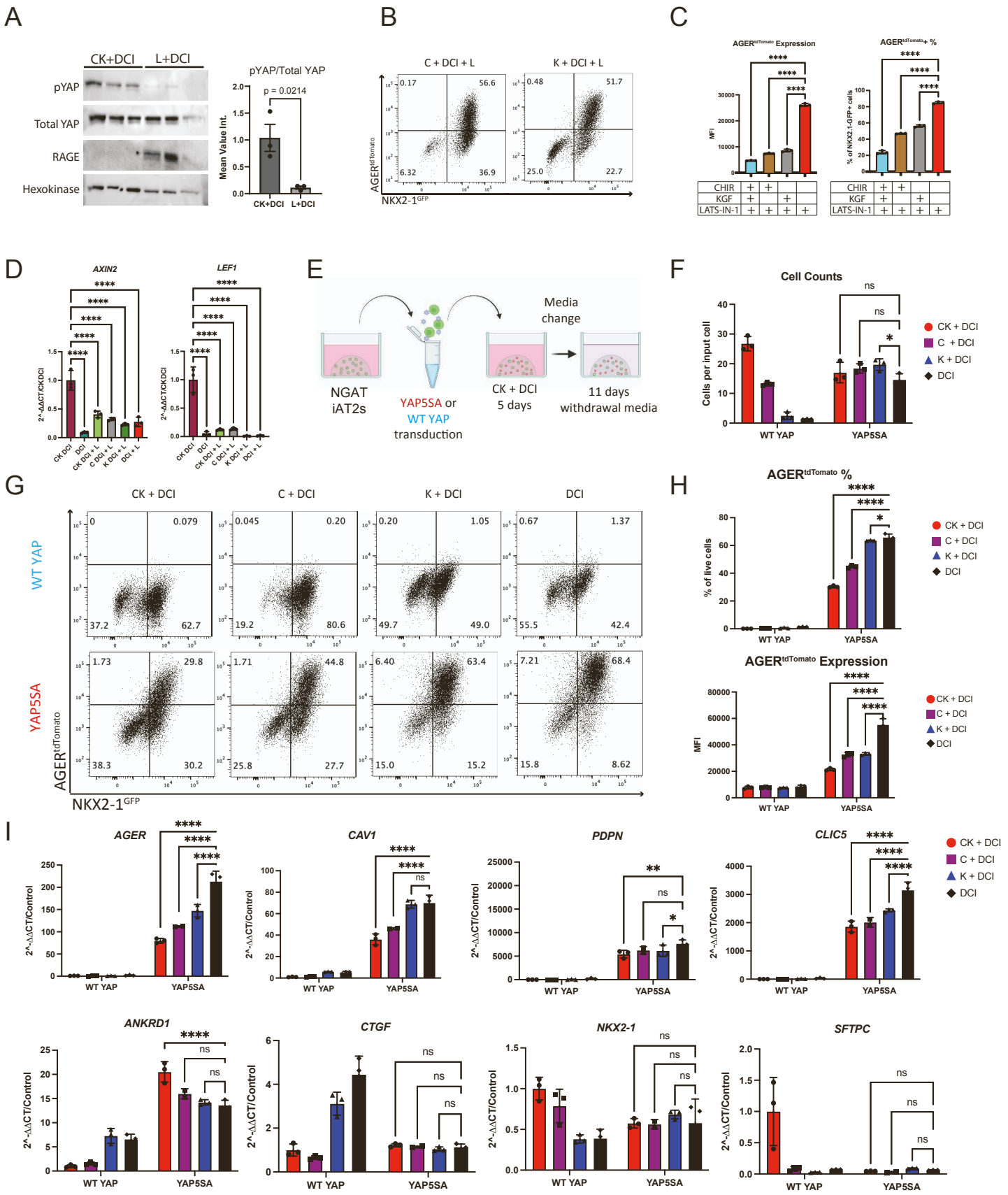
Supplemental Figure 3



Supplemental Figure 3: Cre excision of YAP5SA lentivirus leads to reversion to iAT2-like phenotype and Gene editing and characterization of a NKX2-1^{GFP};AGER^{tdTomato} dual reporter iPSC line, related to Figures 2 and 4.

A) SPC2-ST-B2 iAT2s were transduced with either WT YAP or YAP5SA lentivirus and then plated in 3D Matrigel in CK+DCI for 8 days. They were then infected in single cell suspensions with either Adeno-Cre to excise lentivirus or mock and replated into 3D Matrigel in CK+DCI for 12 days. B) Representative live cell imaging of SPC2-ST-B2 iAT2s following AdenoCre infection (SFTPC^{tdTomato}/Phase Contrast overlay, scale bar = 500um). C) Quantification of flow cytometry analysis showing proliferation by 24hr EdU percentage. (N=3 wells per condition, 1-way ANOVA). D) Cell counts per input cell. E) Gene expression of YAP downstream targets, AT2 and AT1 markers by whole-well RT-qPCR (N=3, 1-way ANOVA). F) CRISPR targeting of tdTomato reporter to *AGER* locus and Cre excision of puromycin resistance cassette. PCR primer binding sites (P1-P6) and resulting PCR amplicons are schematized. Final clone does not include puromycin cassette by lack of P3 -> P4 band and has both long and short bands from P1 -> P2 showing one copy of unedited *AGER* and one copy of tdTomato reporter. G) Karyotype of BU3 NGAT iPSC line. H) Representative flow cytometry of NKX2-1^{GFP} negative outgrowth, iAT2s, WT YAP transduced and YAP5SA transduced cells in both BU3 NGAT and parent BU3 NG lines. I) Quantification of AGER^{tdTomato} over 4 passages of YAP5SA transduced cells. (Passaged every 14 days for a total of 56 days post lentiviral transduction). (N=3 wells per condition).
*p<0.05, **p<0.01, ***p<0.001, and ****p<0.001, bars represent mean +/- SD for all panels.

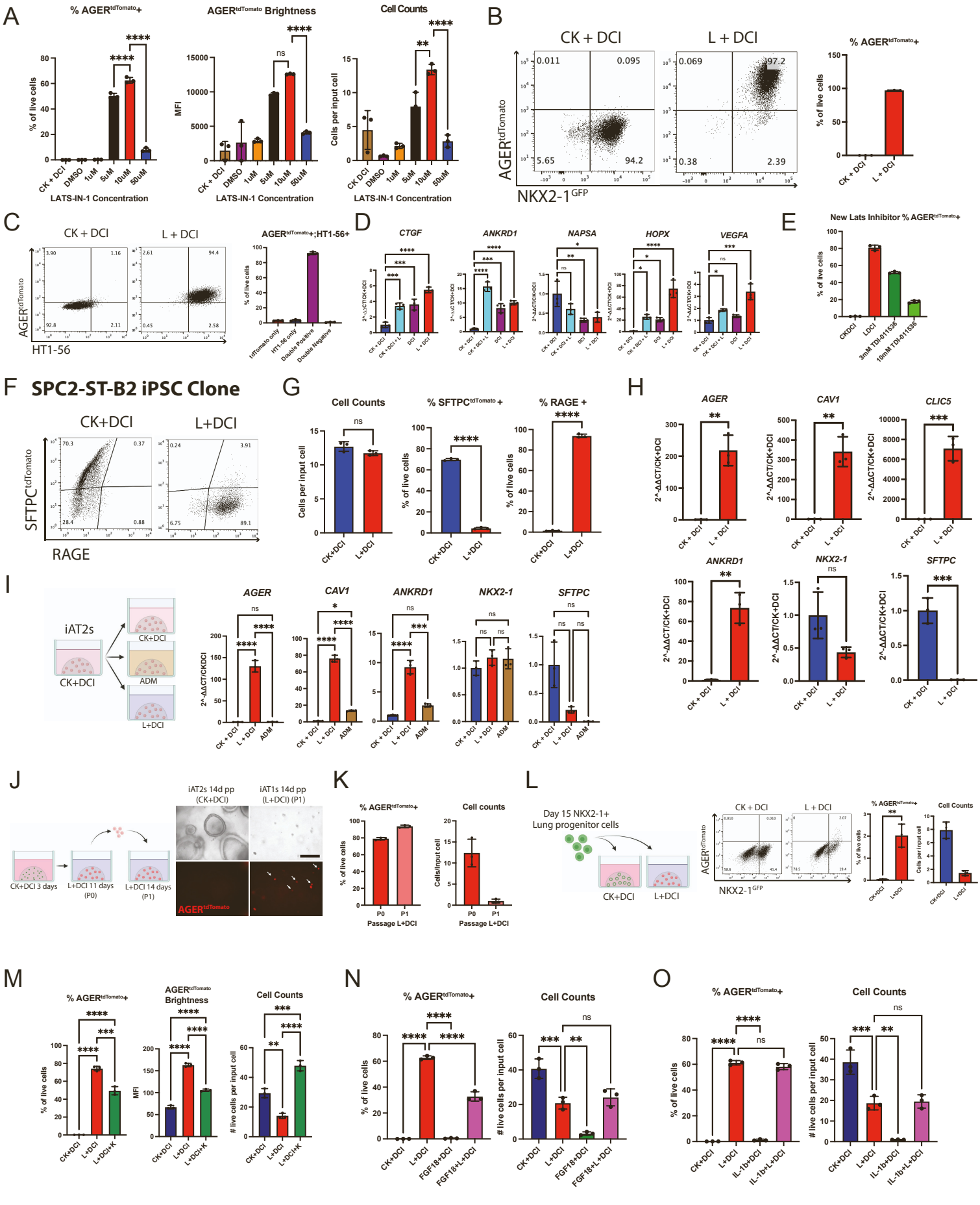
Supplemental Figure 4



Supplemental Figure 4: Both Chir and KGF are inhibitory towards AT1 program induced by both serum-free media and YAP5SA lentivirus, related to Figure 5.

A) SPC2-ST-B2 iAT2s were grown in either CK+DCI for 14 days or CK+DCI for 3 days followed by 11 days of L+DCI. Western blots for total YAP protein, Phosphorylated YAP (pYAP) and RAGE protein shown, with quantification of ratio of pYAP to Total YAP. (N=3 wells, student's t test). B) BU3 NGAT iAT2s were passaged into 3D Matrigel in CK+DCI for 3 days, and then switched to medium containing LATS-IN-1 and either Chir or KGF and grown for a further 11 days. Representative flow cytometry of $NKX2\text{-}1^{GFP}$ and $AGER^{tdTomato}$. C) Quantification of $AGER^{tdTomato}$ compared to CK+DCI + L and DCI + L quantification in Figure 5. (N=3 wells per condition, 1-way ANOVA). D) Whole well relative gene expression by RT-qPCR of Wnt target genes *AXIN2* and *LEF1* across medias containing CHIR (C), KGF (K), and LATS-IN-1 (L). E) BU3 NGAT iAT2s were transduced with either WT YAP or YAP5SA lentivirus in suspension for 4 hours and then replated into 3D Matrigel in CK+DCI. After 5 days, one or both growth factors were withdrawn from the media. Cells were analyzed 11 days post factor withdrawal. F) Cell counts of WT YAP and YAP5SA transduced cells in different medias (2-way ANOVA). G) Representative flow cytometry of $NKX2\text{-}1^{GFP}$ and $AGER^{tdTomato}$. H) Quantification of $AGER^{tdTomato}$ percentage and Mean Fluorescence Intensity following YAP5SA transduction. (2-way ANOVA). I) Gene expression of AT1 markers, YAP downstream targets, and AT2 markers by whole well RT-qPCR. (N=3 wells, 2-way ANOVA). $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.001$, bars represent mean +/- SD for all panels.

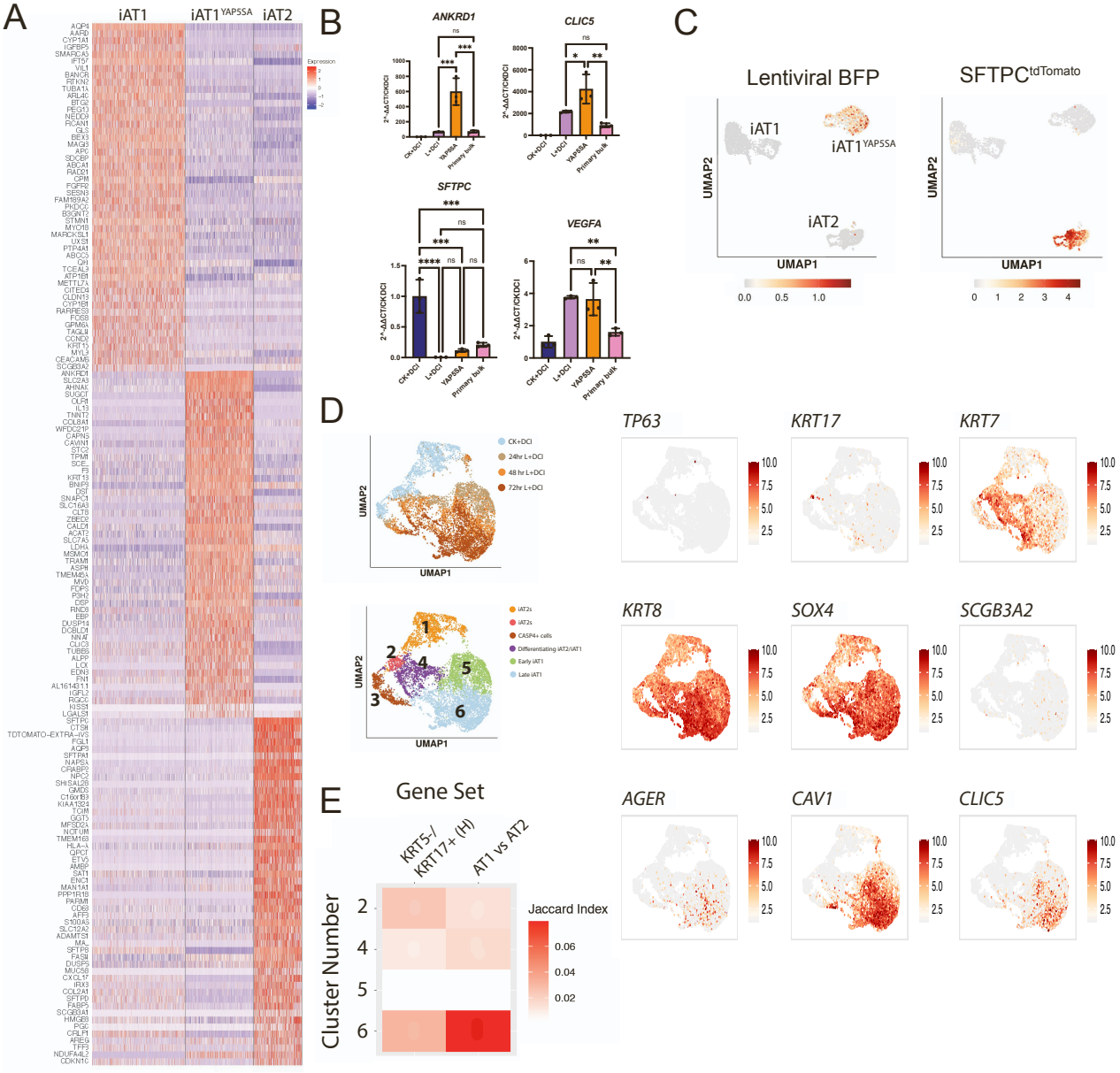
Supplemental Figure 5



Supplemental Figure 5: Optimization of LATS inhibitor-containing medium for iAT1 induction, related to Figure 5.

A) Quantification of AGER^{tdTomato} expression by flow cytometry and cell counts of different concentrations of LATS-IN-1 vs carrier vehicle only control (DMSO) added to base DCI medium 3 days post passage, analyzed 9 days post medium change. (N=3 wells per condition, 1-way ANOVA). Continued AT2 condition (CK+DCI) shown for comparison. B) Flow cytometry analysis and quantification of AGER^{tdTomato} in BU3 NGAT cells that were sorted on NKX2-1^{GFP} on day 201 and replated into CK+DCI before medium was switched to L+DCI 3 days later. Cells were analyzed 11 days post medium change. (N=3 wells per condition). C) Representative flow cytometry and quantification of AGER^{tdTomato} reporter expression and staining for human AT1 extracellular marker HT1-56. D) Gene expression of YAP downstream targets, and AT2 and AT1 markers in LATS inhibitor media by whole well RT-qPCR. (1-way ANOVA). E) Quantification of AGER^{tdTomato} reporter expression of LATS-IN-1 medium (L +DCI) compared to another LATS inhibitor (TDI-011536). F) SPC2-ST-B2 iAT2s were passaged into CK+DCI and then medium was switched to L+DCI 3 days post passage. Representative flow cytometry of SFTPC^{tdTomato} reporter expression and RAGE-AF488 staining on live cells 11 days post media change. G) Quantification of SFTPC^{tdTomato}, RAGE staining, and cell counts of SPC2-ST-B2 cells in CK+DCI and L+DCI (N=3 wells per condition, students t test). H) Gene expression by bulk RT-qPCR at 8 days post media change. (N=3 wells per condition) I) BU3 NGAT iAT2s were cultured in CK+DCI for three days post passage before changing to Alveolar Differentiation Medium ("ADM"; Katsura et al. 2020)[31] or L+DCI and whole well RNA was taken for RT-qPCR 7 days post media change. (N=3 wells per condition, One-way ANOVA). J) Differentiated BU3 NGAT iAT1s were passaged at single cell into 3D Matrigel and grown for 14 days in L+DCI. Live cell imaging at 14 days post passage compared to sister iAT2s. Scale bar = 200um K) Quantification of AGER^{tdTomato} reporter and cell counts of iAT1s 14 days post passage. L) Day 15 NKX2-1+ lung progenitor cells were passaged in to CK+DCI or L +DCI and grown for 14 days. Representative flow cytometry and quantification of NKX2-1^{GFP} and AGER^{tdTomato} reporters. M) BU3 NGAT iAT1s were differentiated from iAT2s and then KGF was added 7 days post passage. Quantification of AGER^{tdTomato} and cell counts at 14 days post passage. N) Quantification of AGER^{tdTomato} and cell counts 14 days post passage where FGF18 was added alone or in combination with LATS-IN-1 (L) during iAT1 differentiation from iAT2s. O) Quantification of AGER^{tdTomato} and cell counts 14 days post passage where IL1β was added alone or in combination with LATS-IN-1 (L) during iAT1 differentiation from iAT2s. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.001, bars represent mean +/- SD for all panels.

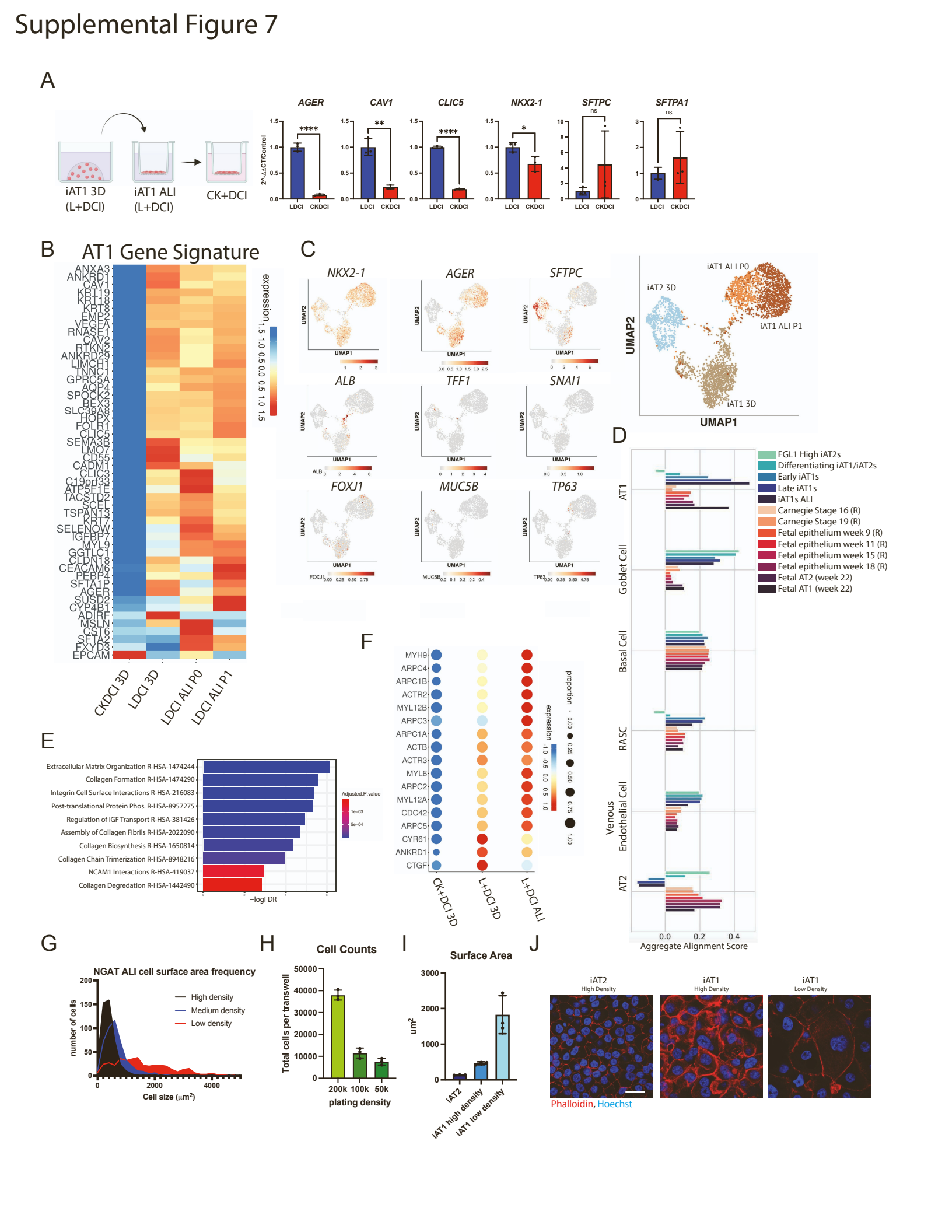
Supplemental Figure 6



Supplemental Figure 6: Single Cell RNA sequencing analysis of medium-based and lentiviral induction of iAT1s, related to Figure 6.

A) Heatmap showing top 50 differentially upregulated genes between iAT2, iAT1^{YAP5SA}, and iAT1 populations. (SPC-ST-B2) B) Comparison of expression levels of indicated transcripts in whole well RNA extracts from iAT2, iAT1^{YAP5SA}, and iAT1 populations compared to bulk primary human distal lung (RT-qPCR 2^-DDCt fold change compared to iAT2s in CK+DCI; N=3 wells per condition, 1-way ANOVA). C) Gene expression overlays of lentiviral tagBFP and SFTPC^{tdTomato} reporter expression. D) Gene expression overlays of "transitional" markers in time series single cell dataset. (BU3 NGAT) E) Jaccard index of differentiating iAT2 to iAT1 clusters compared to KRT5-/KRT17+ gene set from Habermann et al.[2] (H) and AT1 gene set from primary dataset.[2] *p<0.05, **p<0.01, ***p<0.001, and ****p<0.001, bars represent mean +/- SD for all panels.

Supplemental Figure 7



Supplemental Figure 7: Profiling of iAT1s after Air Liquid Interface (ALI) Culture, related to Figure 7.

A) iAT1s were plated at ALI for 7 days and then media was switched back to iAT2 medium (CK+DCI) for 7 days. Gene expression of key AT1 and AT2 markers from whole well RT-qPCR.(N=3 wells per condition, student's t test) (SPC2-ST-B2) B) Heatmap showing average expression of genes in 50 gene primary adult human AT1 gene signature across populations iAT2 3D, iAT1 3D, and iAT1 ALI conditions. (BU3 NGAT) C) UMAP expression overlays of lung epithelial markers and Non-lung markers. D) scTOP scores of iPSC-derived iAT2 and iAT1s compared to UPenn LungMap reference dataset. of E) Enrichr analysis of top 100 (FDR < 0.05, logFC > 1) differentially upregulated genes in iAT1 ALI vs 3D population using Reactome dataset. F) Expression of transcripts encoding Actin and non-muscle myosin and Arp2/3 complex genes as associated with AT1 cells by Shiraishi et al.[47] G) Frequency distribution of cell surface areas from high, medium, and low plating densities of iAT1s after ALI culture. Calculated using ImageJ on ZO-1 staining from ALIs fixed at day 10 post plating. N=457, 455, and 399 respectively across 3 different transwell inserts. H) Cell counts of iAT1s at ALI from different plating densities after 14 days in culture. I) Surface area iAT2 vs iAT1 SPC2-ST-B2 N=3, averaged from ~30-50 cells per image. J) Immunofluorescence images of SPC2B2 iAT2s and iAT1s at high and low density. (F-actin: Phalloidin Red, Hoechst: blue) Scale bar = 20um.

*p<0.05, **p<0.01, ***p<0.001, and ****p<0.001, bars represent mean +/- SD for all panels.