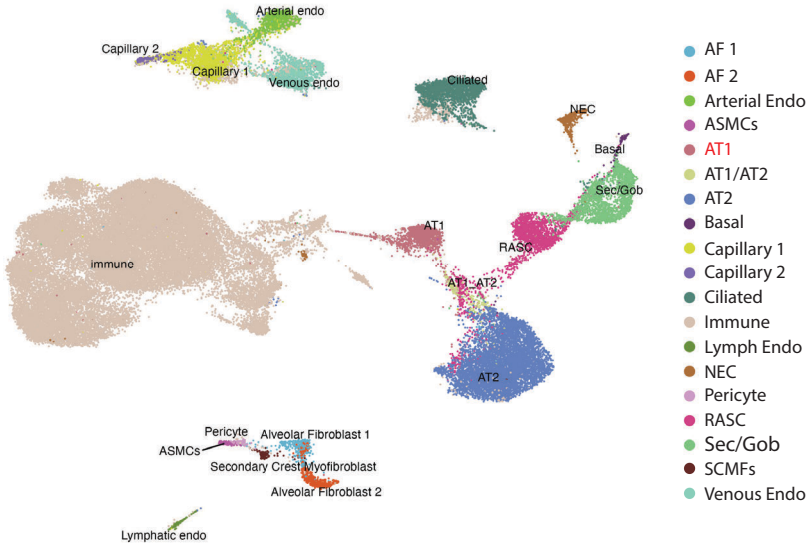
Supplemental Figure 1

A



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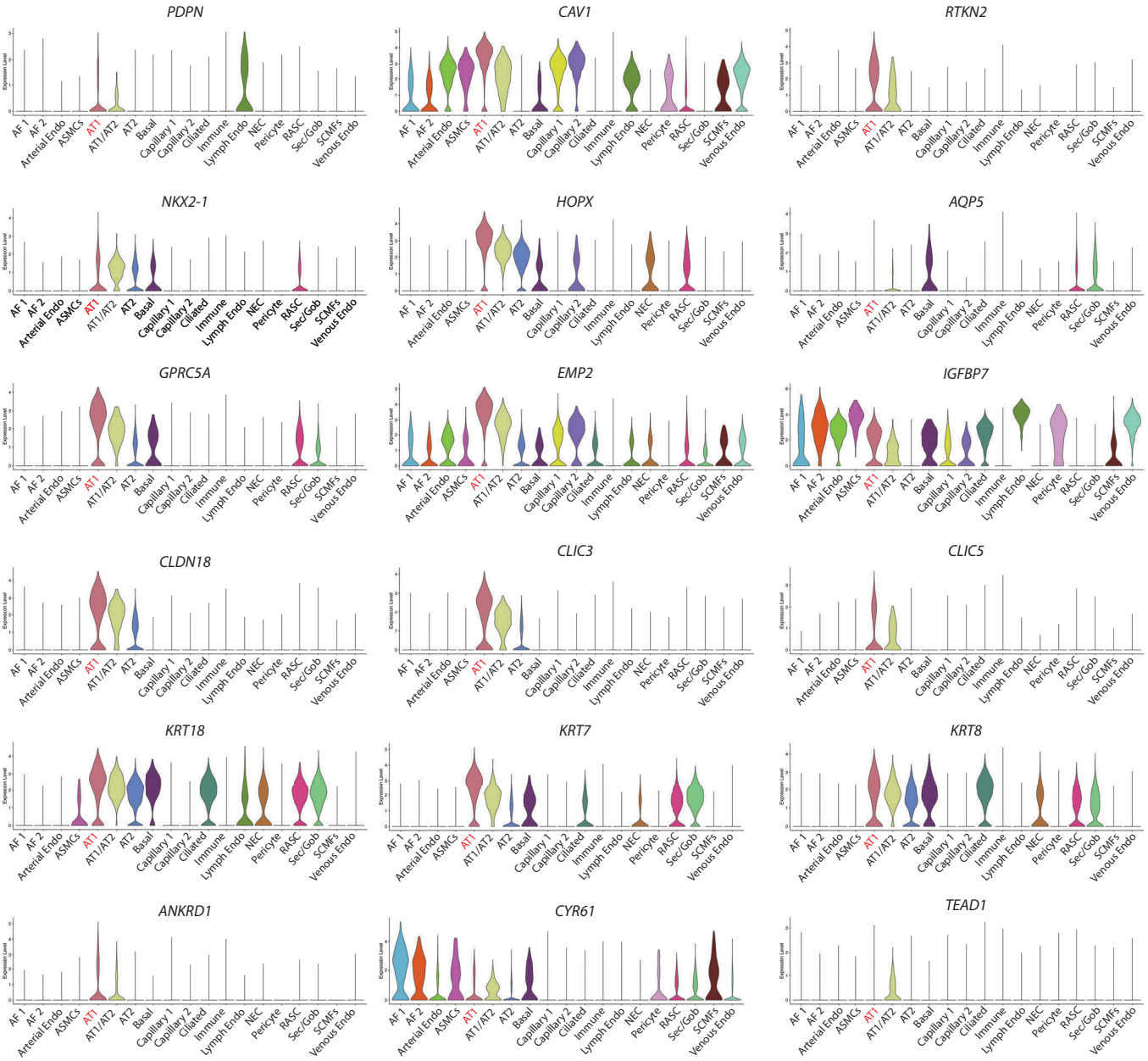
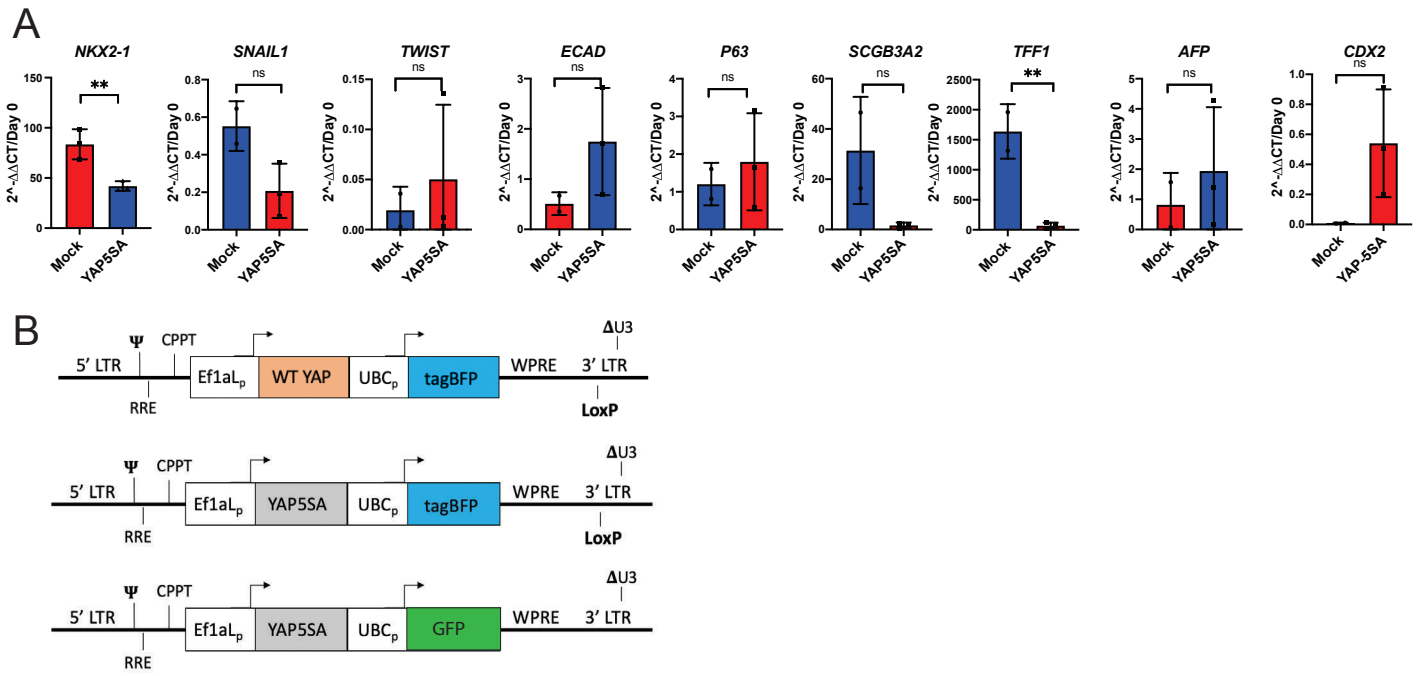


Supplemental Figure 1: Human AT1 marker gene expression across all cell types in the lung.

A) UMAP projection of all lung cells in previously published dataset by Basil et al.[41] (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.

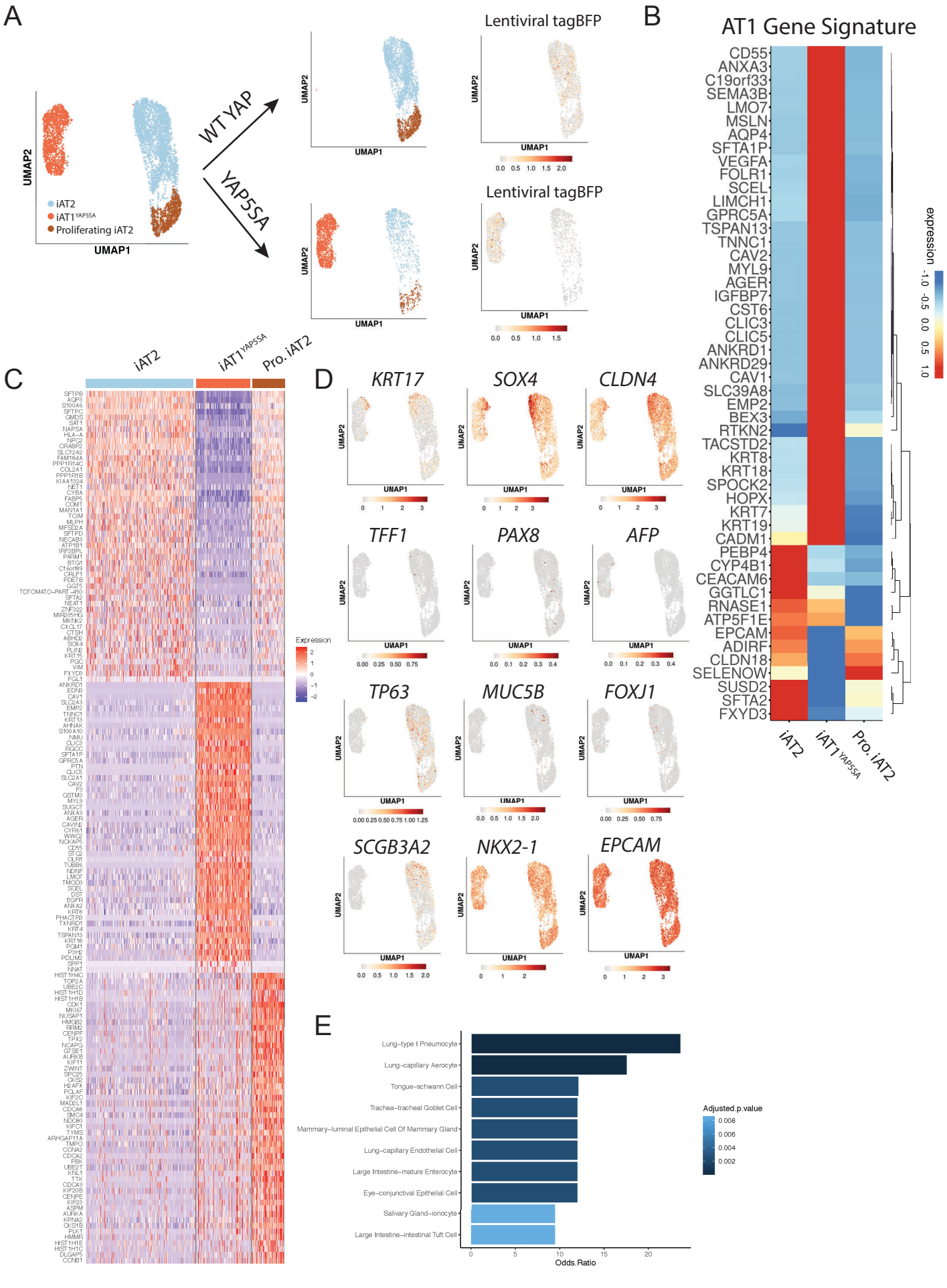
Supplemental Figure 2



Supplemental Figure 2: YAP5SA transduction of SPC2B2 iAT2s.

A) Whole well gene expression by RT-qPCR of EMT markers, airway lung lineage markers and non-lung endoderm markers 14 days post YAP5SA or mock lentiviral transduction of SPC2B2 iAT2s, relative to Day 0 iPSCs. (N=3 per condition, student's t test) B) Diagram of WT YAP and YAP5SA lentiviruses showing dual promoter system with tagBFP and LoxP site. YAP5SA-GFP lentivirus used for competition assay. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.001 for all panels.

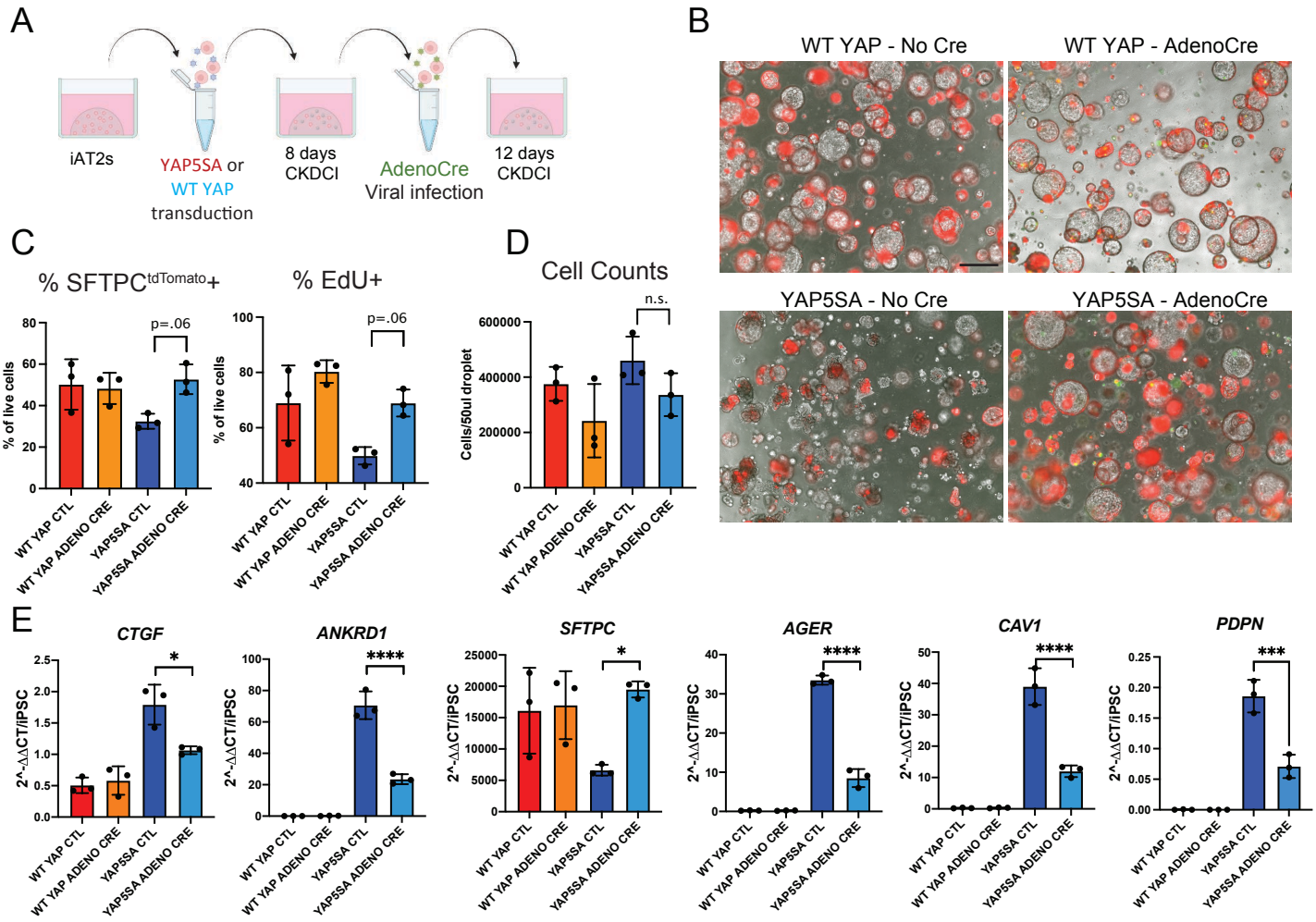
Supplemental Figure 3



Supplemental Figure 3: Single Cell RNA sequencing of YAP5SA transduced iAT2s.

A) Sub plots of UMAP projections of WT YAP and YAP5SA wells to show lentiviral tagBFP expression in each condition. B) Heatmap of genes in the 50 AT1 gene signature across each population. C) Full heatmap of top 50 differentially upregulated genes in the iAT2, iAT1^{YAP5SA}, and Proliferating iAT2 populations. D) Gene expression overlays of aberrant basaloid transitional markers *KRT17, SOX4*, and *CLDN4*; Non-lung endoderm markers *TFF1, PAX8*, and *AFP*; and other lung markers *TP63, MCU5B, FOXJ1, SCGB3A2*, and *NKX2-1*. E) Enrichr analysis of top 92 (FDR > 0.05, logFC > 1) differentially upregulated genes in iAT1^{YAP5SA} population using Tabula Sapiens dataset.

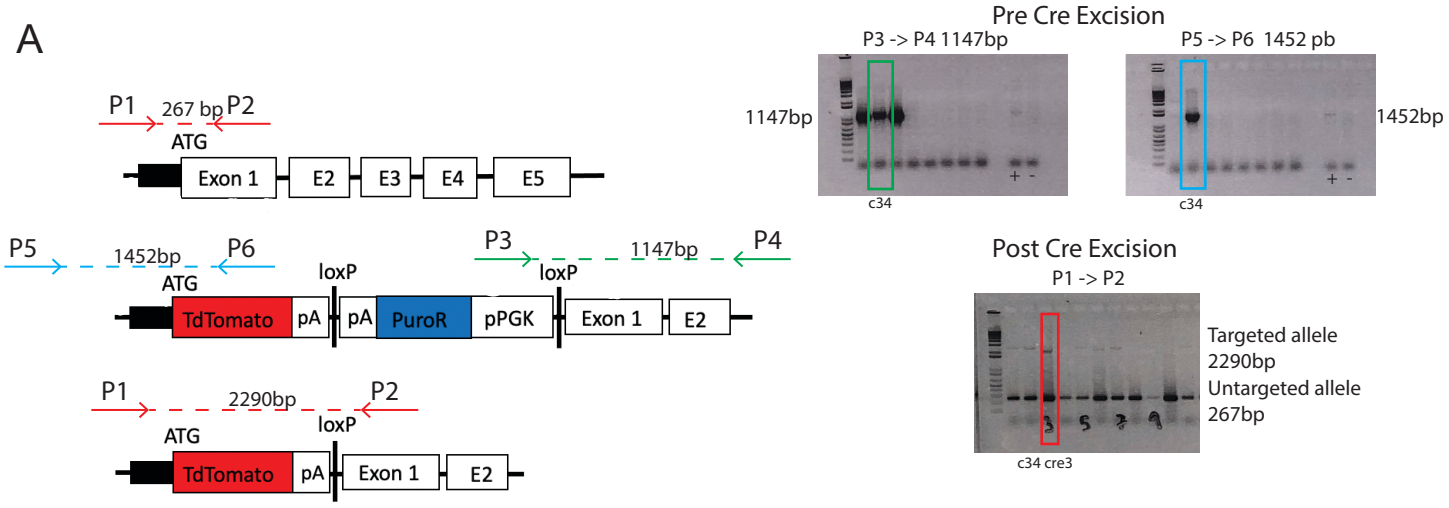
Supplemental Figure 4



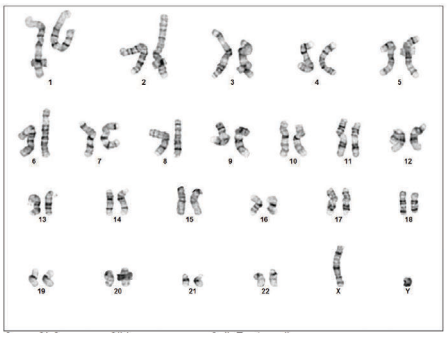
Supplemental Figure 4: Cre excision of YAP5SA lentivirus leads to reversion to iAT2-like phenotype.

A) 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 and $SFTPC^{tdTomato}$ percentage. (N=3, 1-way ANOVA). D) Cell counts per 50uL droplet. E) Gene expression of YAP downstream targets, AT2 and AT1 markers by bulk RT-qPCR (N=3, 1-way ANOVA). *p<0.05, **p<0.01, ***p<0.001, and ****p<0.001 for all panels.

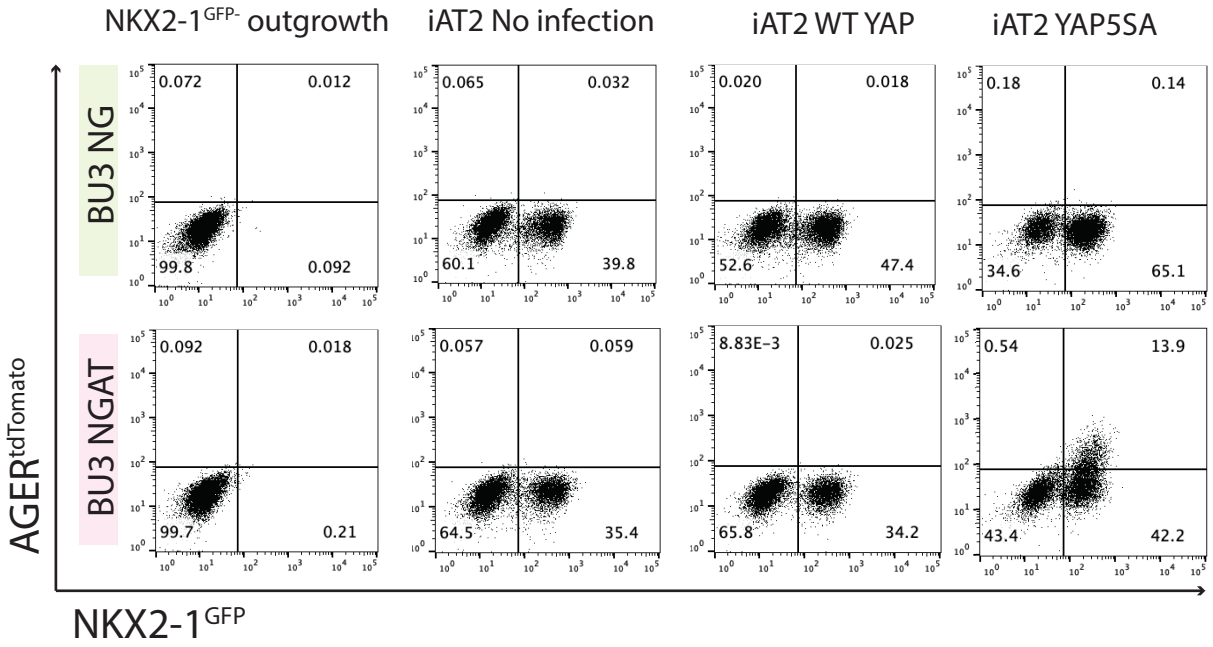
Supplemental Figure 5



B BU3 NGAT c34 cre3
Result: 46, XY



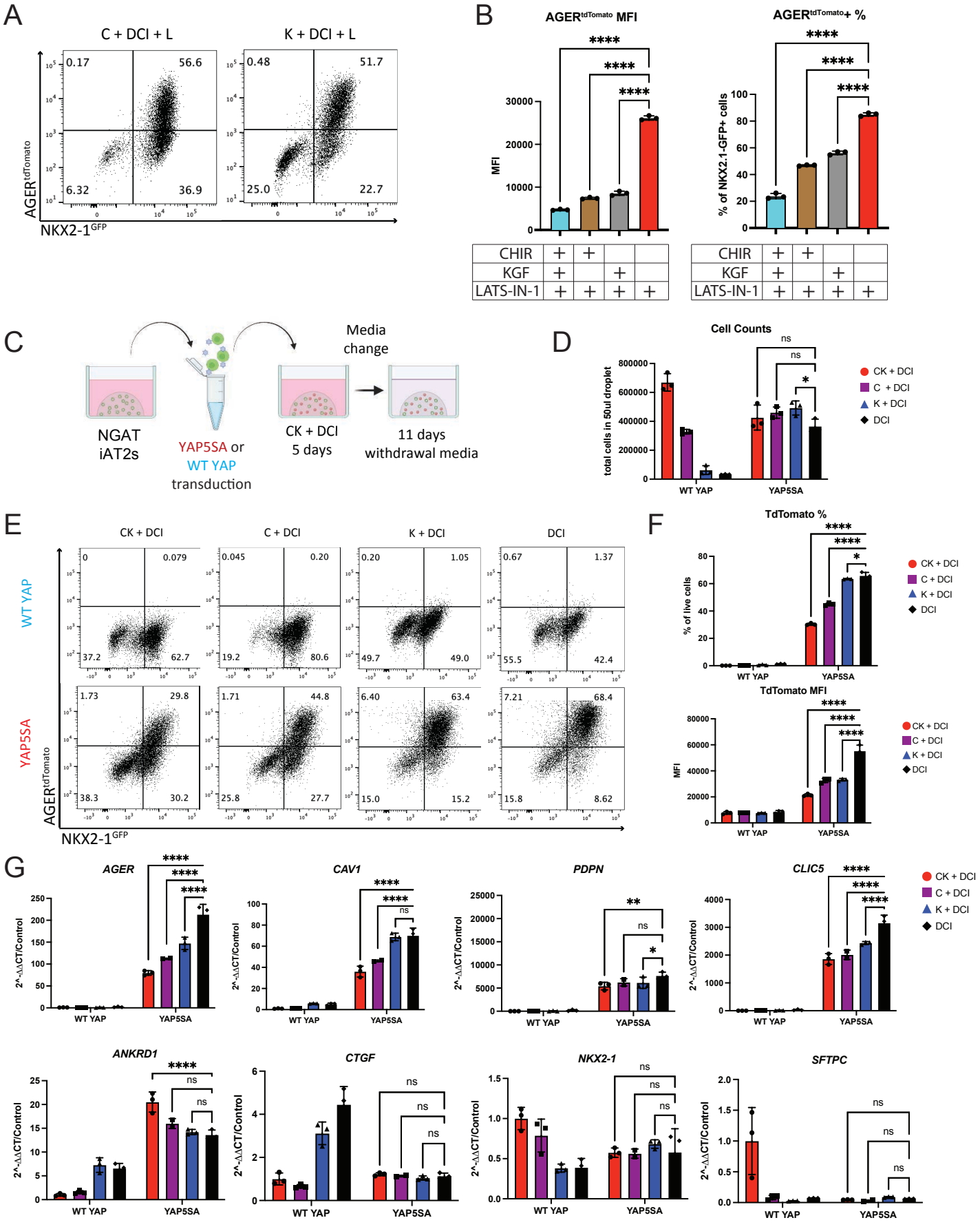
C



Supplemental Figure 5: Gene editing and characterization of a NKX2-1^{GFP}/AGER^{tdTomato} dual reporter iPSC line.

A) CRISPR targeting of tdTomato reporter to *AGER* locus and Cre excision of puromycin resistance cassette. Final clone does not include puromycin cassette by lack of Fp2 -> Rp2 band and has both long and short bands from Fp1 -> Fp2 showing one copy of unedited *AGER* and one copy of tdTomato reporter. B) Karyotype of BU3 NGAT iPSC line. C) 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.

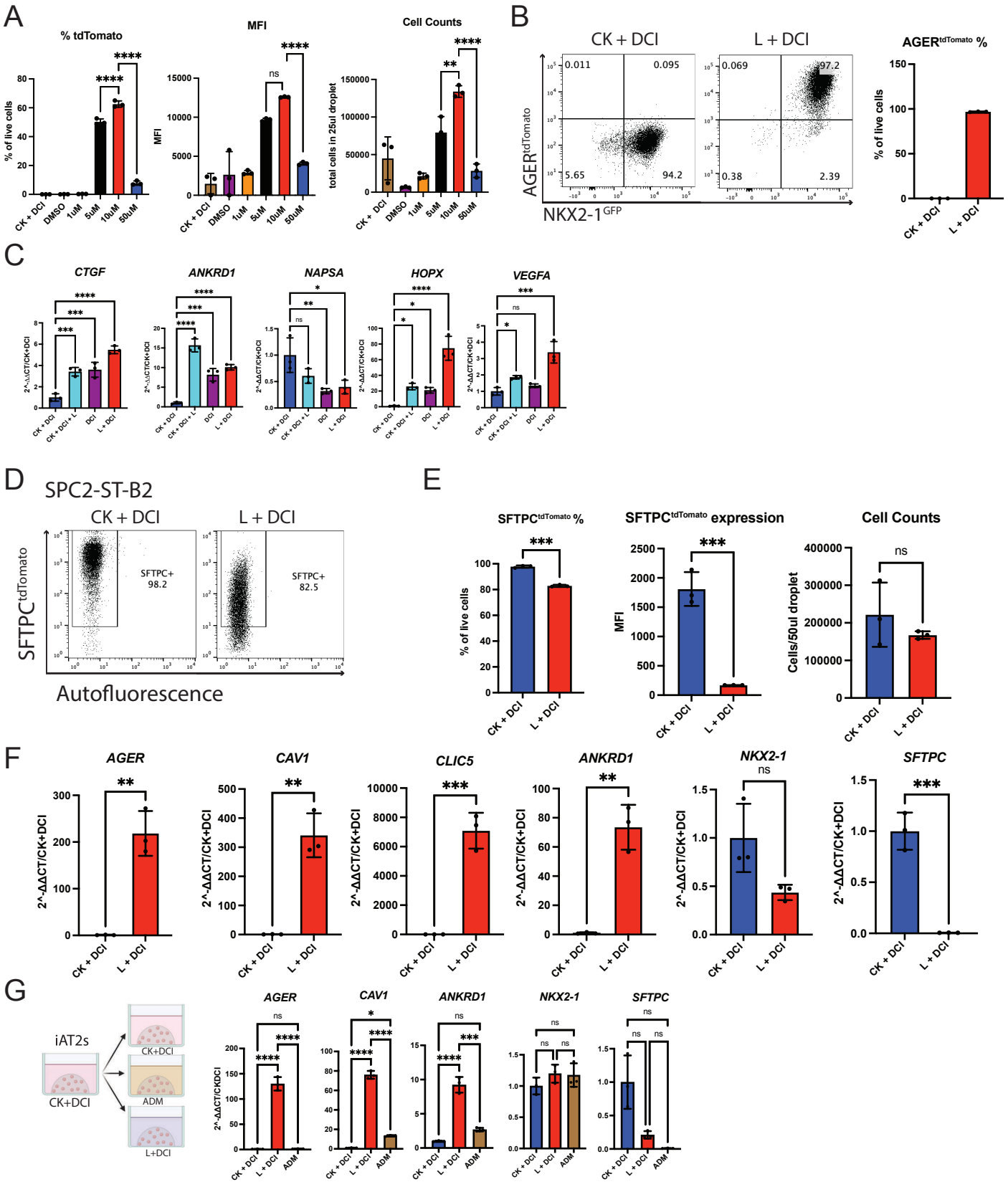
Supplemental Figure 6



Supplemental Figure 6: Both Chir and KGF are inhibitory towards AT1 program induced by both serum-free media and YAP5SA lentivirus.

A) BU3 NGAT iAT2s were passaged into 3D Matrigel in CK+DCI for 3 days, and then media was switched to media containing LATS-IN-1 and either Chir or KGF and grown for a further 11 days. Representative flow cytometry of NKX2-1^{GFP} and AGER^{tdTomato}. B) Quantification of AGER^{tdTomato} compared to CK DCI + L and DCI + L quantification in Figure 5. (N=3 per condition, 1-way ANOVA). C) 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, media was changed to withdraw one or both growth factors from the media. Cells were analyzed 11 days post media change. D) Cell counts of WT YAP and YAP5SA transduced cells in different medias (2-way ANOVA). E) Representative flow cytometry of NKX2-1^{GFP} and AGER^{tdTomato}. F) Quantification of AGER^{tdTomato} percentage and Mean Fluorescence Intensity following YAP5SA transduction. (2-way ANOVA). G) Gene expression of AT1 markers, YAP downstream targets, and AT2 markers by whole well RT-qPCR. (N=3, 2-way ANOVA). $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.001$ for all panels.

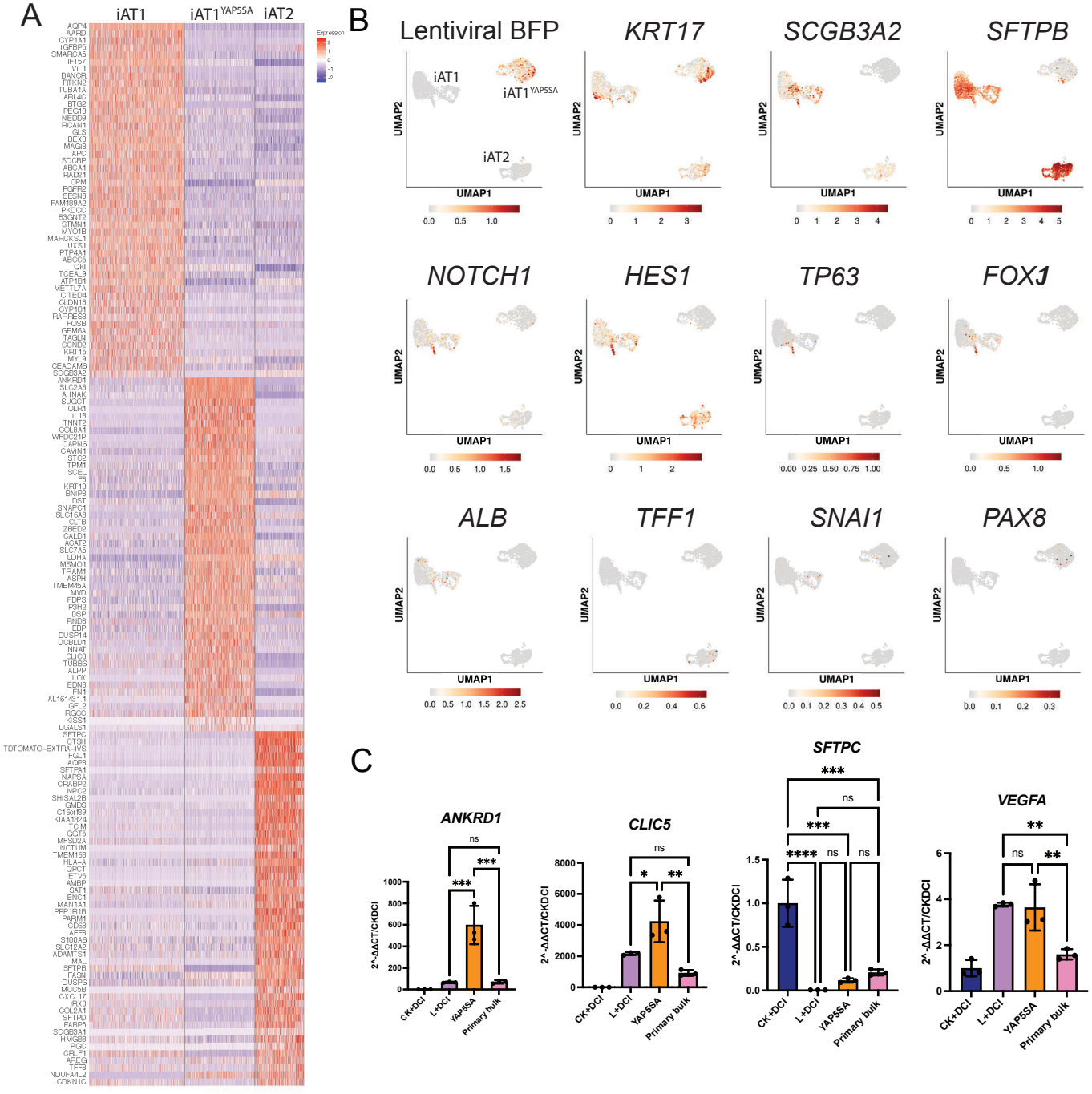
Supplemental Figure 7



Supplemental Figure 7: Optimization of LATS inhibitor-containing media for iAT1 induction.

A) Quantification of AGER^{tdTomato} expression by flow cytometry and cell counts of different concentrations of LATS-IN-1 added 3 days post passage, analyzed 9 days post media change. (N=3 per condition, 1-way ANOVA). 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+RI before media was switched to L+DCI 3 days later. Cells were analyzed 11 days post media change. (N=3). C) Gene expression of YAP downstream targets, and AT2 and AT1 markers in LATS inhibitor media by whole well RT-qPCR. (1-way ANOVA). D) SPC2-ST-B2 iAT2s (>95% SFTPC^{tdTomato}+) 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 8 days post media change. E) Quantification of SFTPC^{tdTomato} and cell counts of SPC2-ST-B2 cells in CK+DCI and L+DCI (N=3 per condition, students t test). F) Gene expression by bulk RT-qPCR at 8 days post media change. (N=3) G) BU3 NGAT iAT2s were cultured in CK+DCI for three days post passage before changing to Alveolar Differentiation Medium ("ADM"; Katsura et al. 2020)[32] or L+DCI and whole well RNA was taken for RT-qPCR 7 days post media change. (N=3, One-way ANOVA). $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.001$ for all panels.

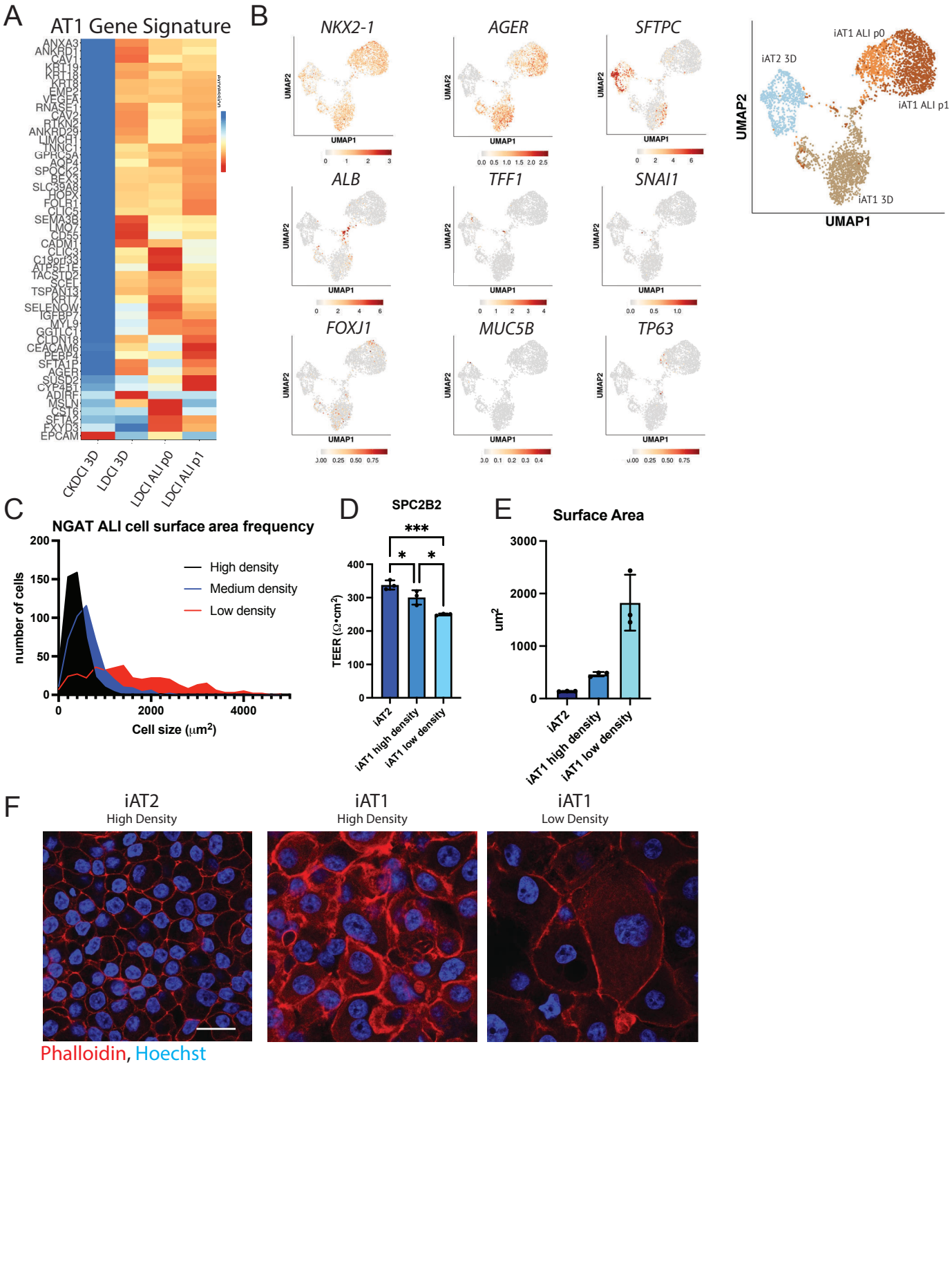
Supplemental Figure 8



Supplemental Figure 8: Single Cell RNA sequencing analysis of media and lentiviral induction of iAT1s.

A) Heatmap showing top 50 differentially upregulated genes between iAT2, iAT1^{YAP5SA}, and iAT1 populations. B) Gene expression overlays of lentiviral tagBFP, as well as transitional state markers, airway markers, and non-lung endoderm markers. C) 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, 1-way ANOVA). p<0.05, **p<0.01, ***p<0.001, and ****p<0.001 for all panels.

Supplemental Figure 9



Supplemental Figure 9: Profiling of iAT1s after Air Liquid Interface (ALI) Culture

A) 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. B) UMAP expression overlays of lung epithelial markers and Non-lung markers. C) 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 transwells. D) TEER of SPC2-ST-B2 iAT2s plated in CK+DCI after ALI culturing as published[68] compared to iAT1s at high (200k) and low (50k) density plating in L+DCI and cultured at ALI. E) Surface area iAT2 vs iAT1 SPC2-ST-B2 N=3, averaged from ~30-50 cells per image. F) 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 for all panels.