

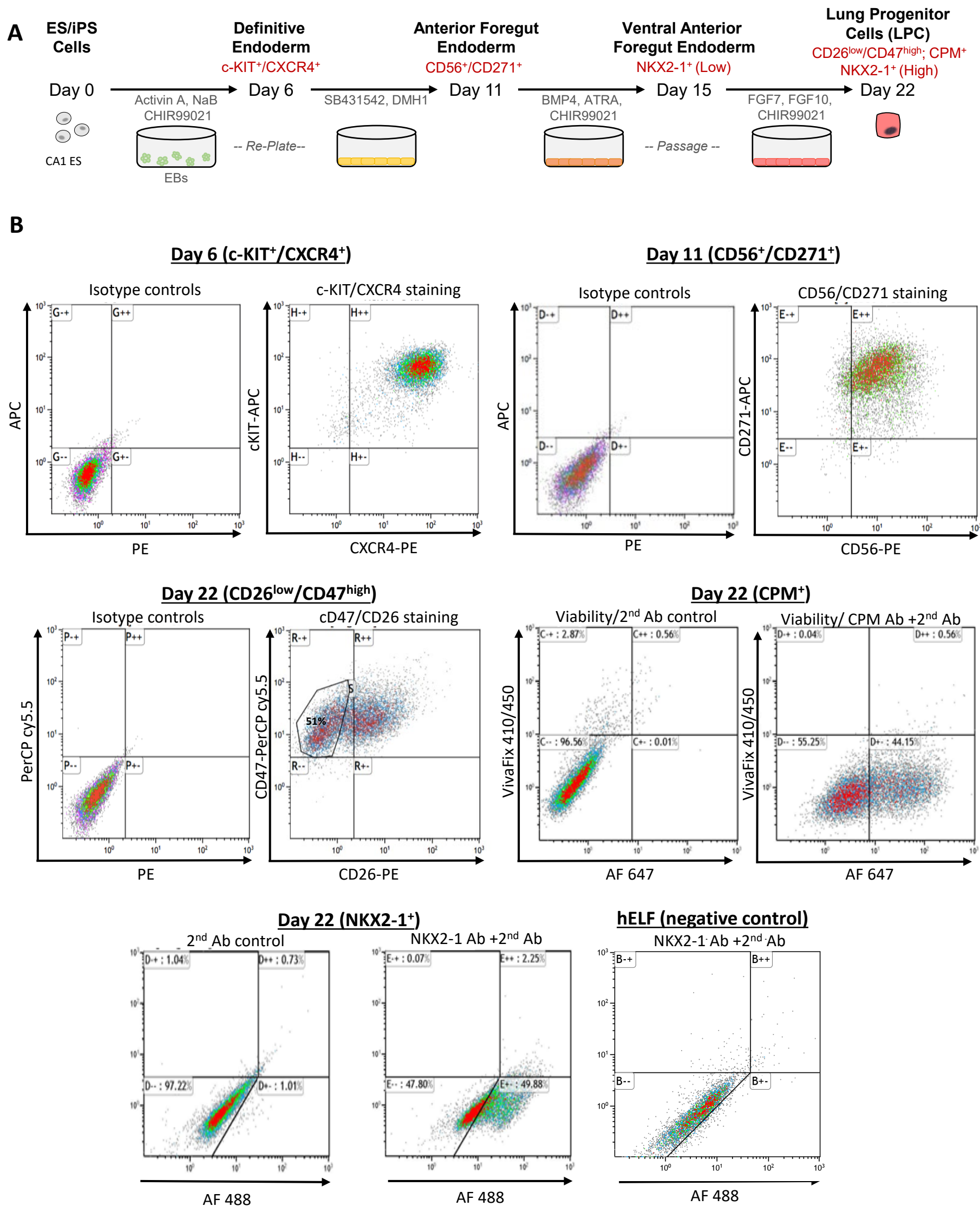
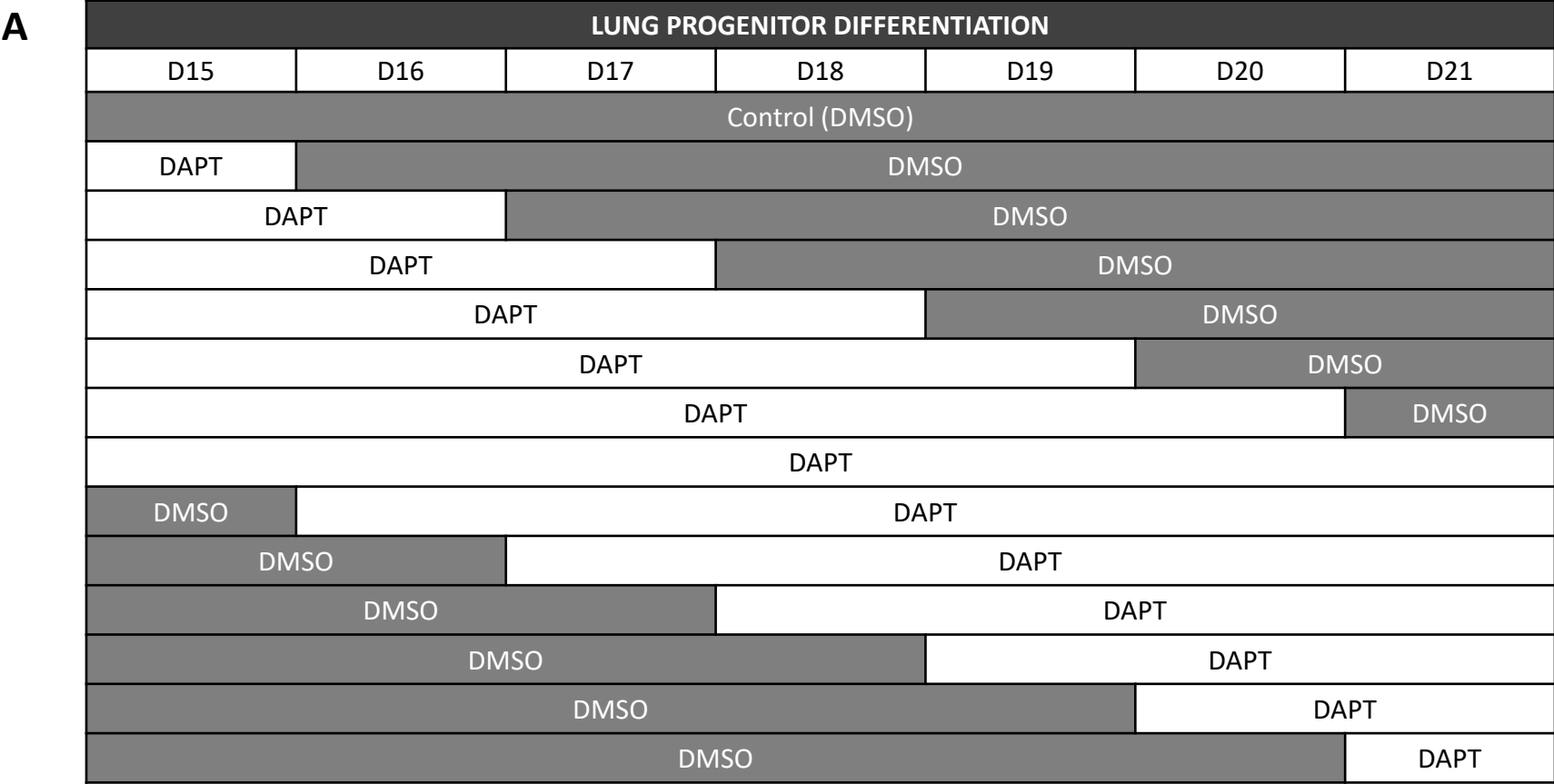
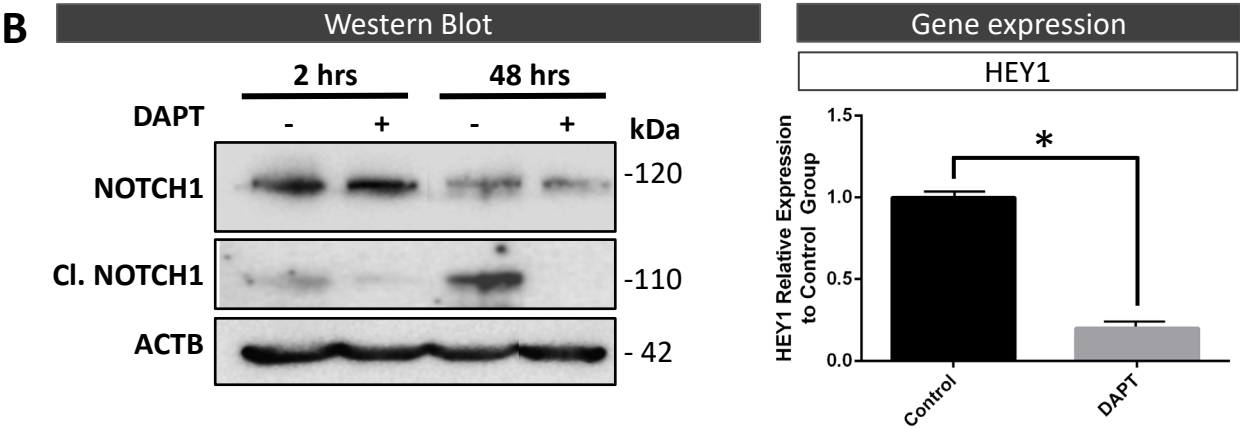
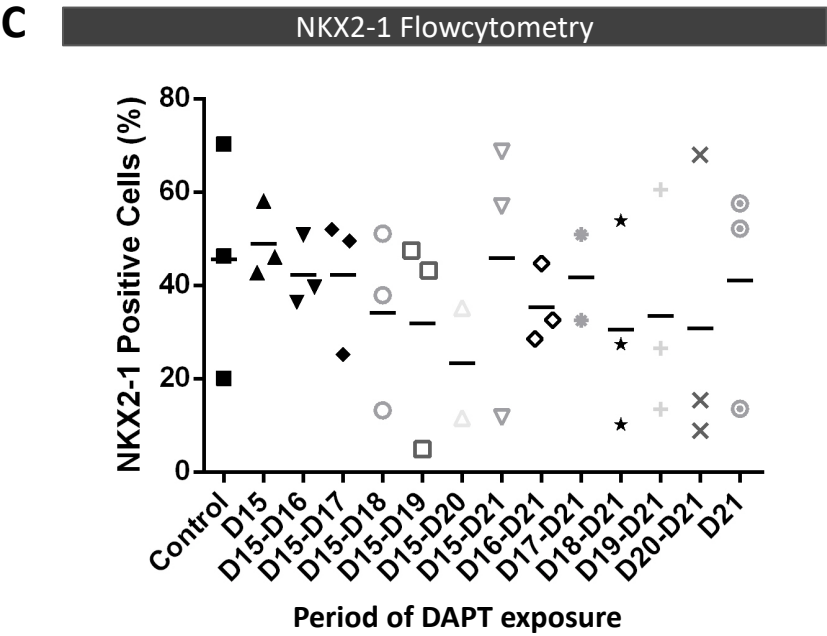
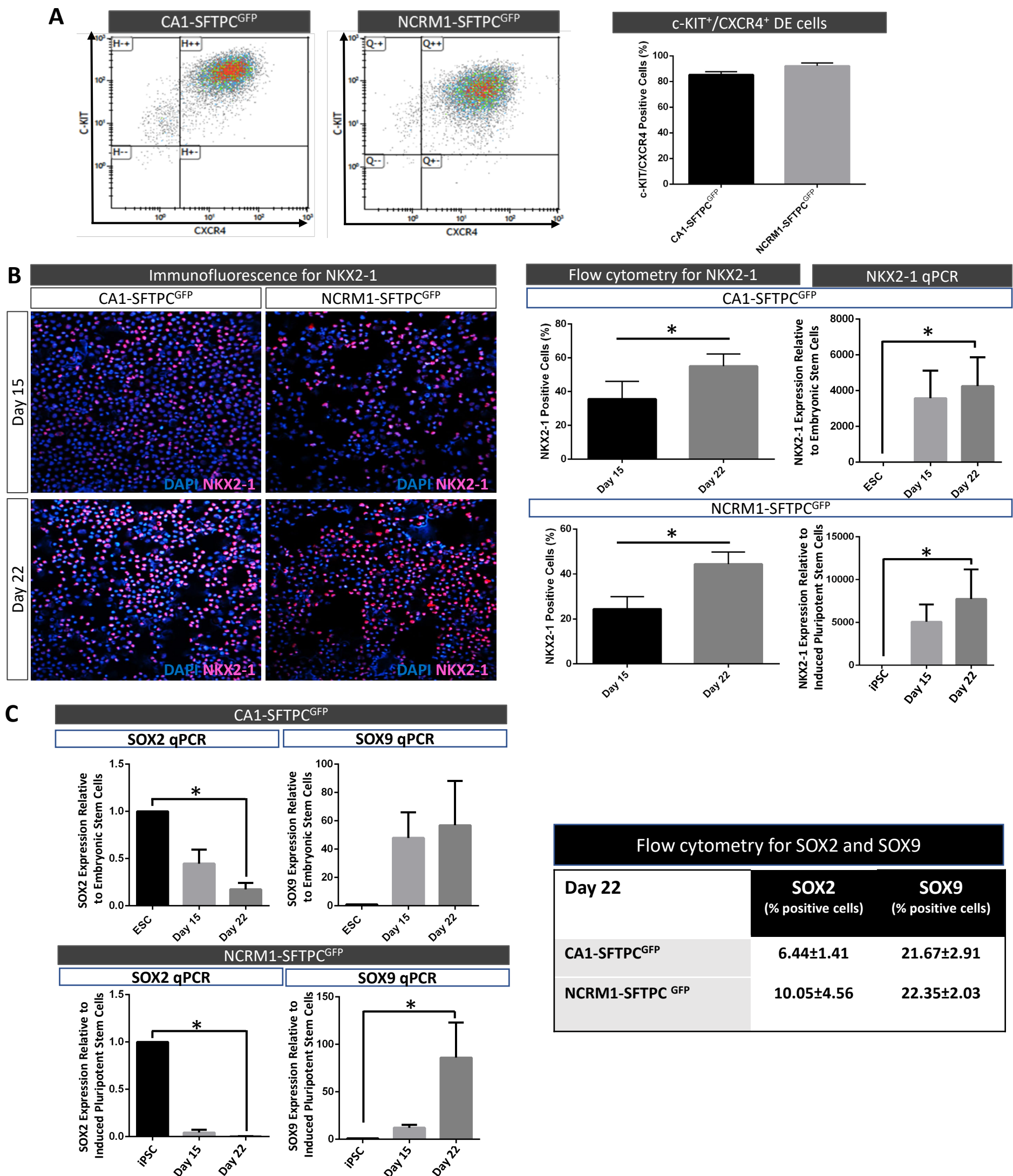


Figure S1: Directed differentiation of pluripotent stem (ES/iPS) cells into lung progenitor (LPC) cells. (A) Stepwise differentiation protocol with stage specific markers in red. **(B)** representative isotype, secondary and primary plus secondary antibody flow scatter plots for established definitive endoderm ($c\text{-KIT}/\text{CXCR4}$), anterior foregut endoderm ($\text{CD56}/\text{CD271}$) and lung progenitor ($\text{CD26}^{\text{low}}/\text{CD47}^{\text{high}}$, CPM , NKX2-1) markers corroborate robustness of protocol. Human embryonic lung fibroblasts (hELF) were used as negative cell control for intracellular NKX-2-1 staining. Viability was assessed with VivaFix401/450 stain.





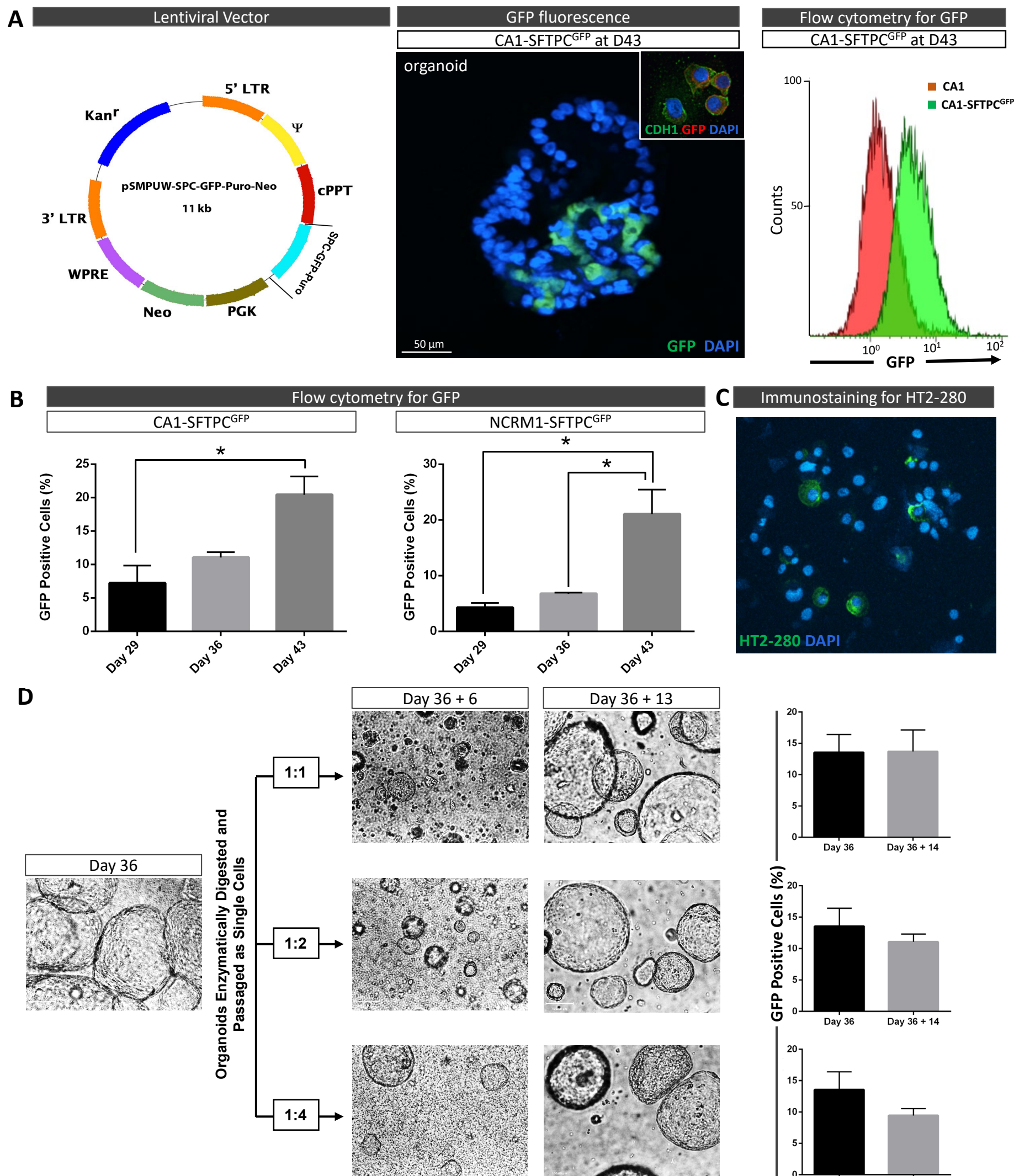


Figure S4. Differentiation of lung progenitor cells derived from pluripotent stem cells stably transduced with a hSFTPC^{GFP} reporter into AEC2 cells. (A-left) A schematic of the lentiviral vector containing a 5 kb hSFTPC promoter driving GFP-IRES-puromycin (hSFTPC^{GFP}) for selection and readout. (A-right panels) Representative GFP confocal microscopy and GFP flow histogram of CA1-hSFTPC^{GFP} organoids at D43 of differentiation. Non-transduced CA1 cells served as negative flow control. *Inlet*: cytopsin of D43 CA1-hSFTPC^{GFP} organoid cells stained with anti-GFP and anti-CDH1. (B) GFP expression over time in CA1-hSFTPC^{GFP} or NCRM1-hSFTPC^{GFP} organoid cultures measured by flow cytometry. Mean $\pm$ SEM, $n \geq 4$ biological replicates, * $p < 0.05$ by one-way ANOVA). (C) Representative immunostaining for HT2-280 of cells isolated from CA1-hSFTPC^{GFP} organoids at D43 of differentiation. (D-left panels) Representative organoid formation after passaging at different densities. Organoids started with D22 CA1-hSFTPC^{GFP} LPCs were harvested at day 14 of initial organoid formation (day 36 of differentiation), dispersed into single cells, reseeded in fresh 50% Matrigel and cultured for another 2 weeks. (D-right panels) Flow cytometry analysis of GFP expressing cells after passaging. Mean $\pm$ SEM, $n \geq 3$ biological replicates.

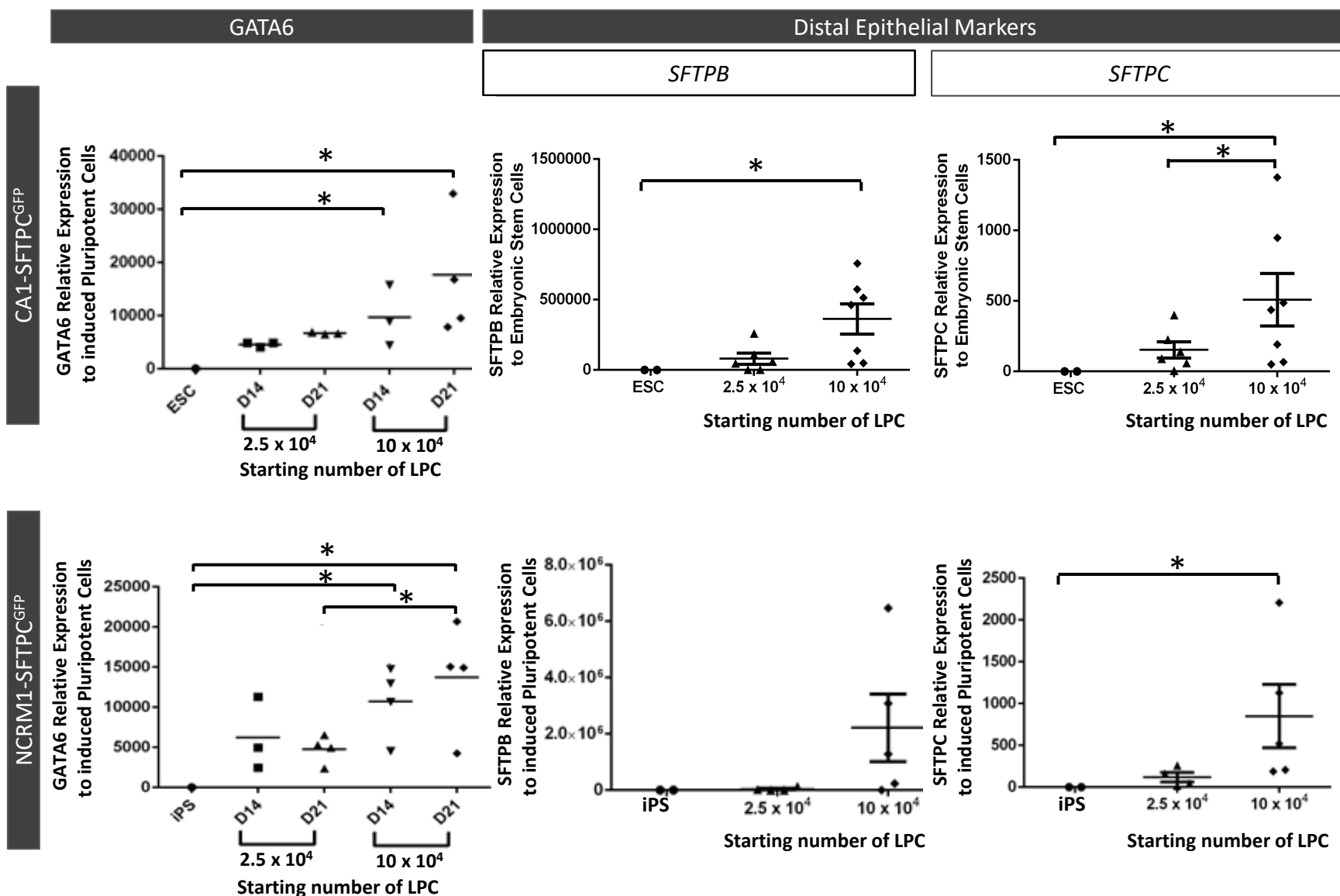
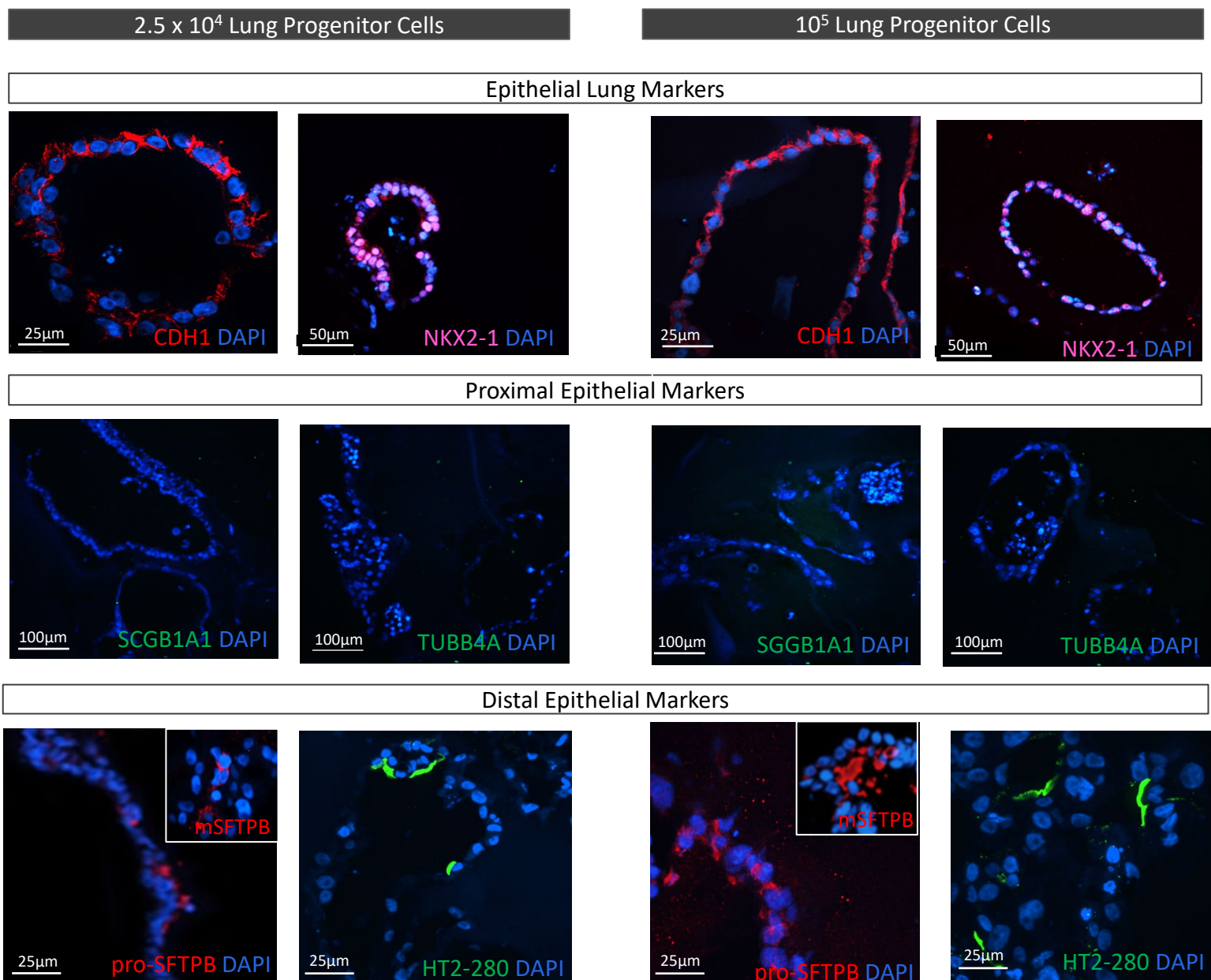


Figure S5. Increased gene expression of distal lung epithelial markers in high-density seeded organoids. Real-time PCR analysis of *GATA6*, *SFTPB* and *SFTPC* in D43 organoid cultures started with either 2.5×10^4 or 10^5 D22 LPCs derived from either CA1-hSFTPC^{GFP} ES or NCRM1-hSFTPC^{GFP} iPS cells (mean $\pm$ SEM, $n \geq 3$ differentiations, * $p < 0.05$ by one-way ANOVA). LPC: lung progenitor cell.

A



B

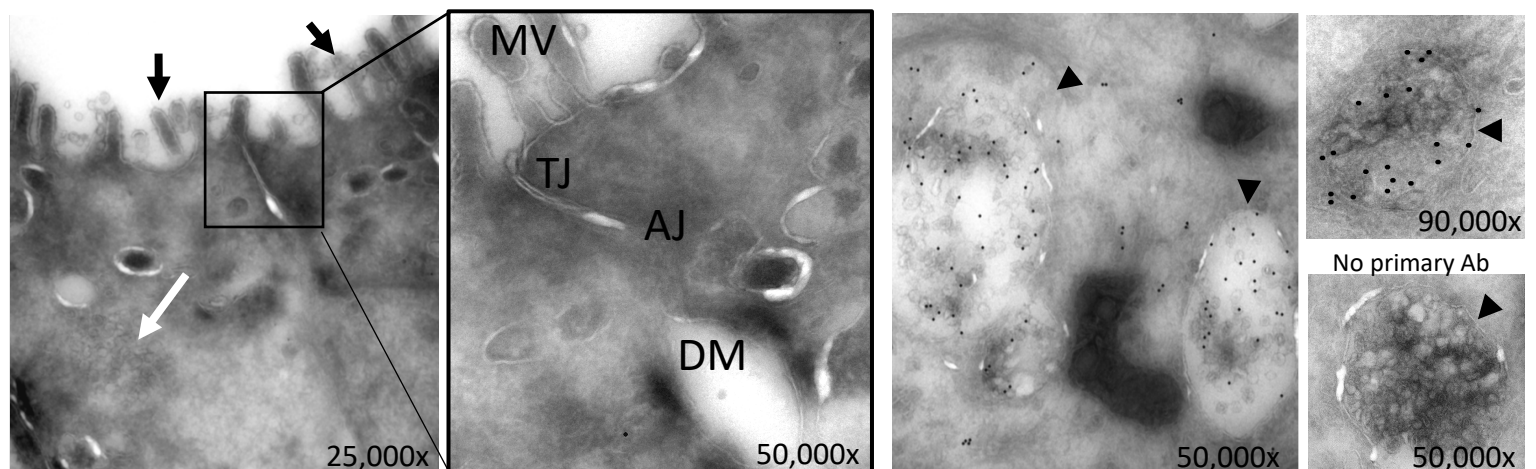


Figure S6. Visualisation of the diversity of cells found within organoids. (A) Representative immunofluorescence images of epithelial (CDH1), lung (NKX-2-1), proximal (TUBB4, SCGB1A1) and distal (pro-SFTPb, mature SFTPb (mSFTPb, insert), and HT2-280) markers. **(B)** Representative transmission (left panels) and mSFTPb immunogold EM (right panels) images of day 42 lung organoids. MV; microvilli; TJ: tight junction; AJ: adherens junction; DM: desmosomes. Black arrows indicate microvilli while white arrows indicate pre-lamellar multivesicular structures that are surrounded by a limiting membrane (black arrow heads) and contain mSFTPb (immunogold dots = 10 nm).

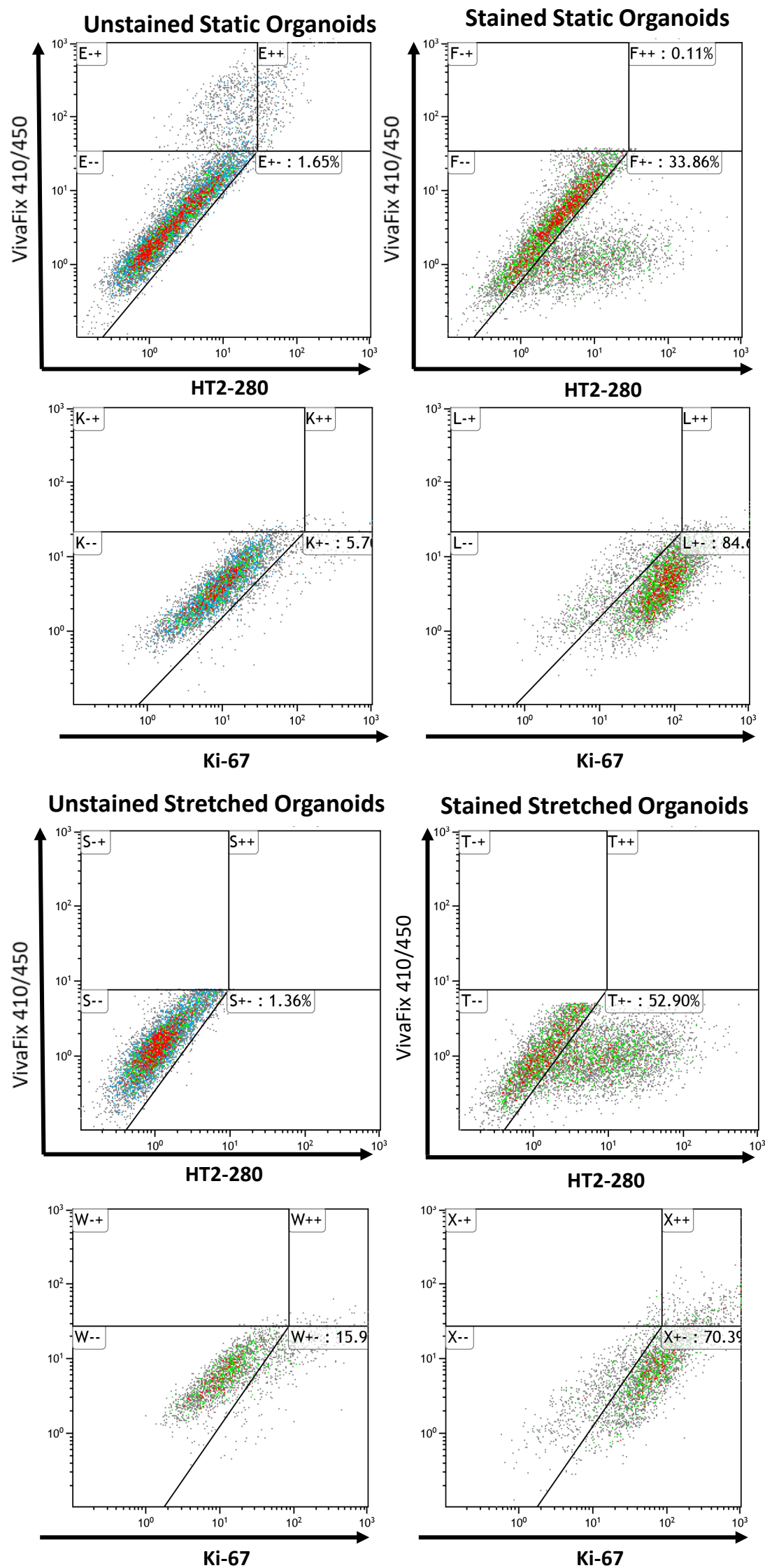


Figure S7: Representative flow scatter plots for HT2-280, Ki-67 and VivaFix of static and episodically stretched organoid cultures. Flow cytometry analysis was used to measure the viability (VivaFix 410/450) and expression of HT2-280 (AEC2 marker) and Ki-67 (proliferation marker) in D43 static and stretched organoid cultures. Both static and stretched cultures were started at the same time from the same D21 lung progenitor cells.