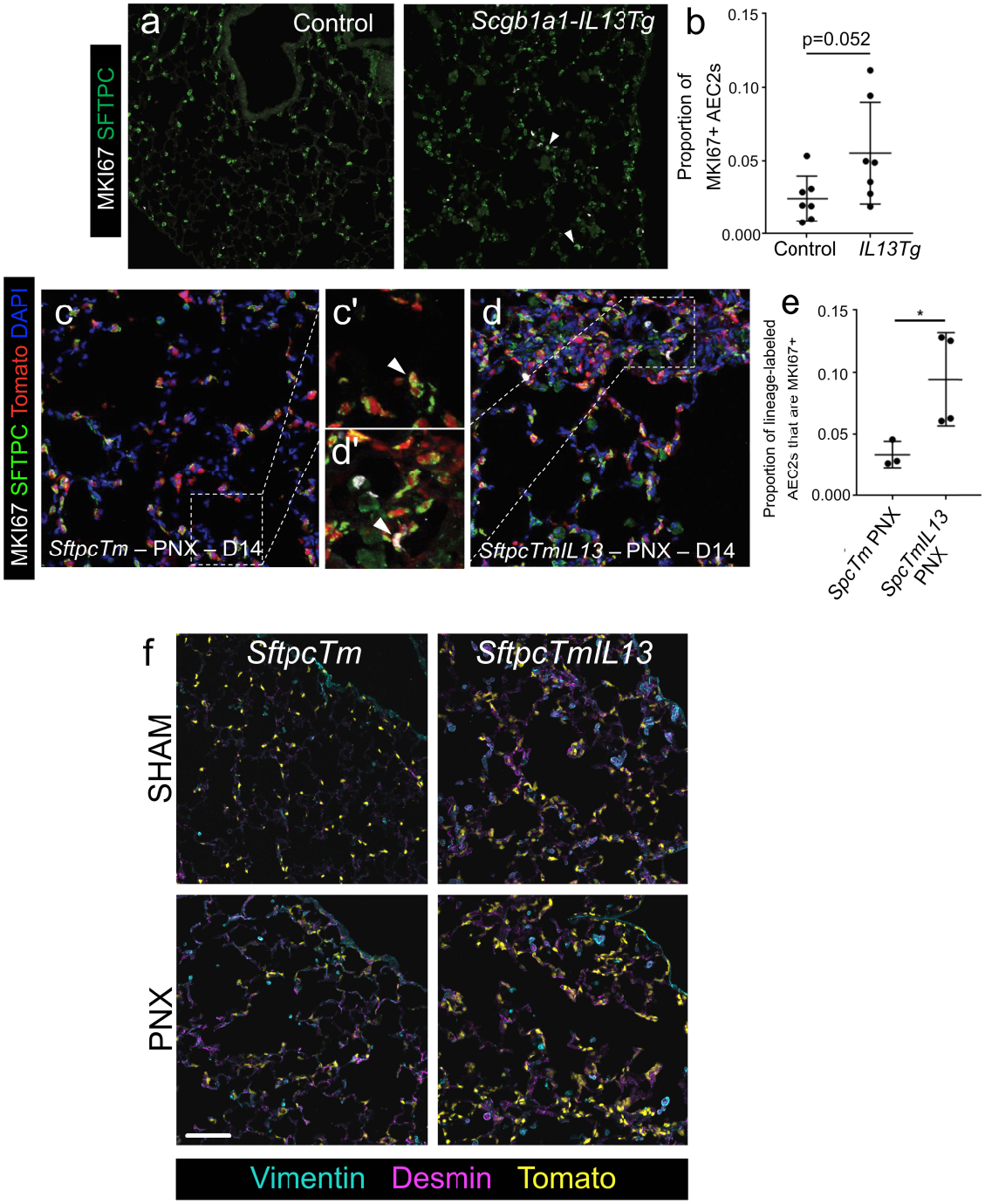
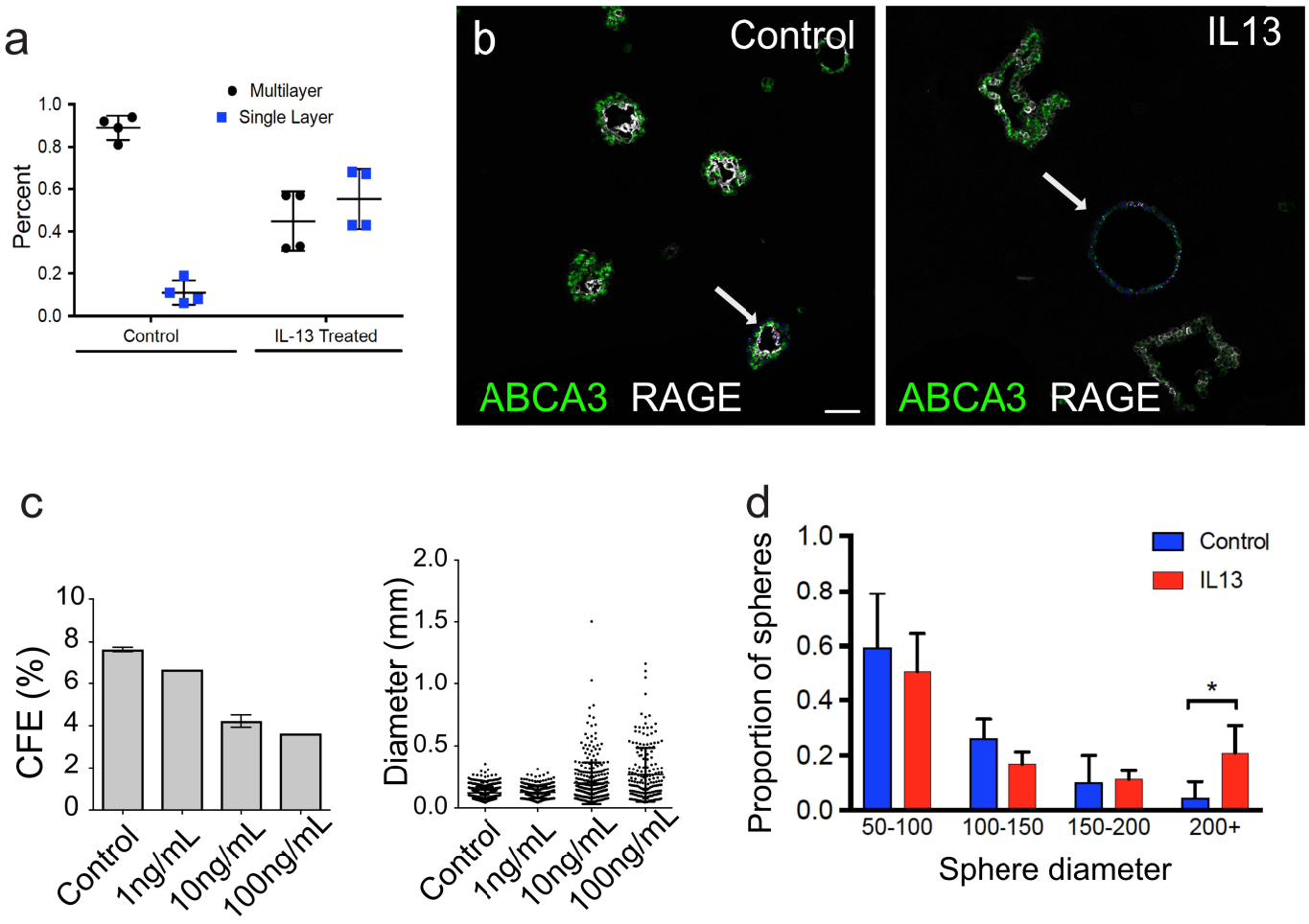
Supplemental Figure 1



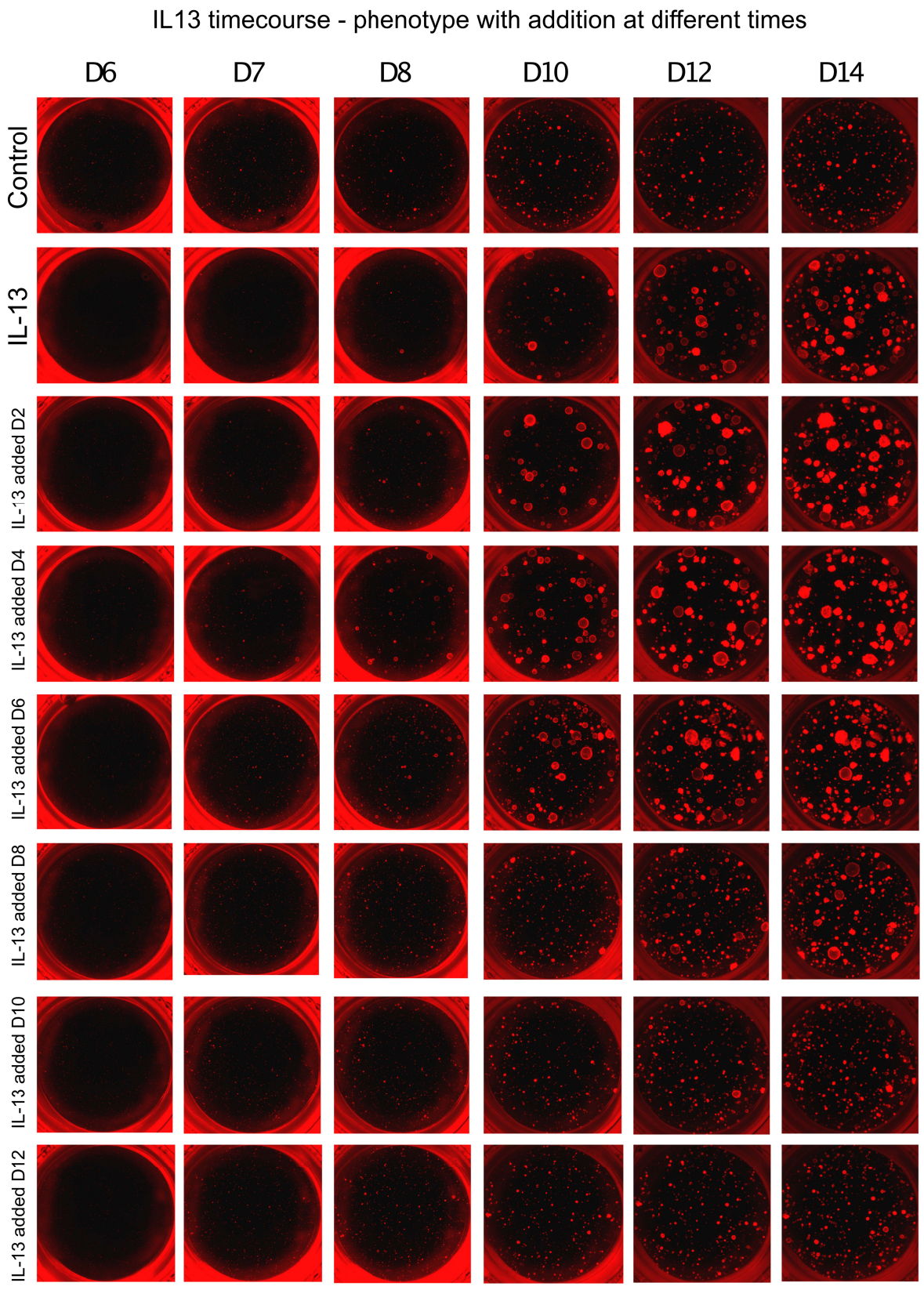
Supplemental Figure 1. There is increased proliferation of AEC2s in lungs with overexpression of IL13. (a) Proliferation of AEC2s was assessed at steady-state in control vs. *Scgb1a1-IL13Tg* mice. Proliferating AEC2s were defined as being dual positive for Ki67 and SFTPC. AEC2 proliferation was assessed as a proportion of Ki67+ AEC2s out of all AEC2s. (b) There is a trend towards increased proliferation of AEC2s in *Scgb1a1-IL13Tg* lungs compared to controls at steady-state. (c) Mice of genotypes *SftpcTm* (control; Sup Fig c and c') and *SftpcTmIL13* (IL13-overexpressing; Sup Fig d and d') underwent L lobe pneumonectomy (PNX) at least two weeks after receiving tamoxifen (TMX) to lineage-label AEC2s. (e) The proportion of proliferating (MKI67+), lineage-labeled (Tomato+) AEC2s (SFTPC+) out of all lineage-labeled AEC2s was assessed 14 days after PNX. (f) There is no evidence of notable septal expansion in *SftpcTmIL13* mice compared to *SftpcTm* mice following PNX nor are there obvious differences in vimentin or desmin expression following PNX. * = $p<0.05$. Each dot represents an individual biological replicate. Unpaired t-test, two-tailed. Error bars represent SD.

Supplemental Figure 2



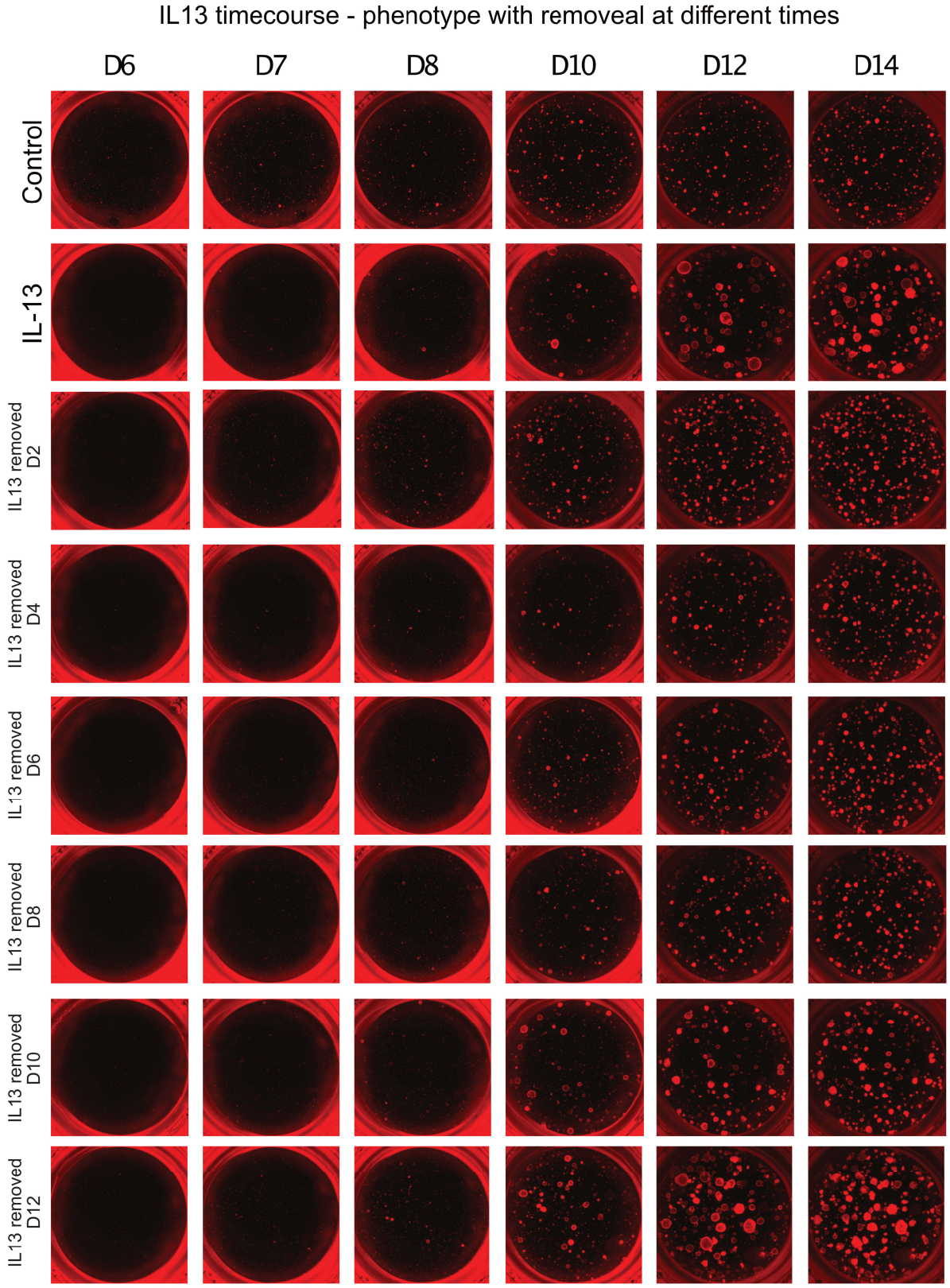
Supplemental Figure 2. Effect of adding IL13 to AEC2 organoid culture. (a) There is an increase in the number of "single layer" organoids grown in the presence of IL13 compared to control. (b) Representative images of the multilayer spheres in control conditions and the multilayer and single layer spheres in IL13-treated conditions. AEC2 marker = ABCA3; AEC1 marker = RAGE. Arrows highlight spheres annotated for cell type counting (summary data presented in Fig 3b). (c) CFE drops and average sphere diameter increases with increasing concentrations of IL13. Data are from one cell culture experiment. Data represent averages from technical replicates (2 technical replicates for 1 ng/mL and 100 ng/mL conditions; 3 technical replicates for control and 10 ng/mL conditions). Error bars reflect standard deviation when calculated for n=3 technical replicates. Sphere diameter data from the same experiment. Each dot represents the diameter of an individual organoid. (d) Distribution of diameters for control and IL13-treated organoids. Note the distributions are quite similar with the exception of the largest size range. There is a statistically significant increase in proportion of organoids >200um in diameter in the IL13-treated conditions. One-way ANOVA; error bars represent SD. Scale bar (b) 50μm.

Supplemental Figure 3



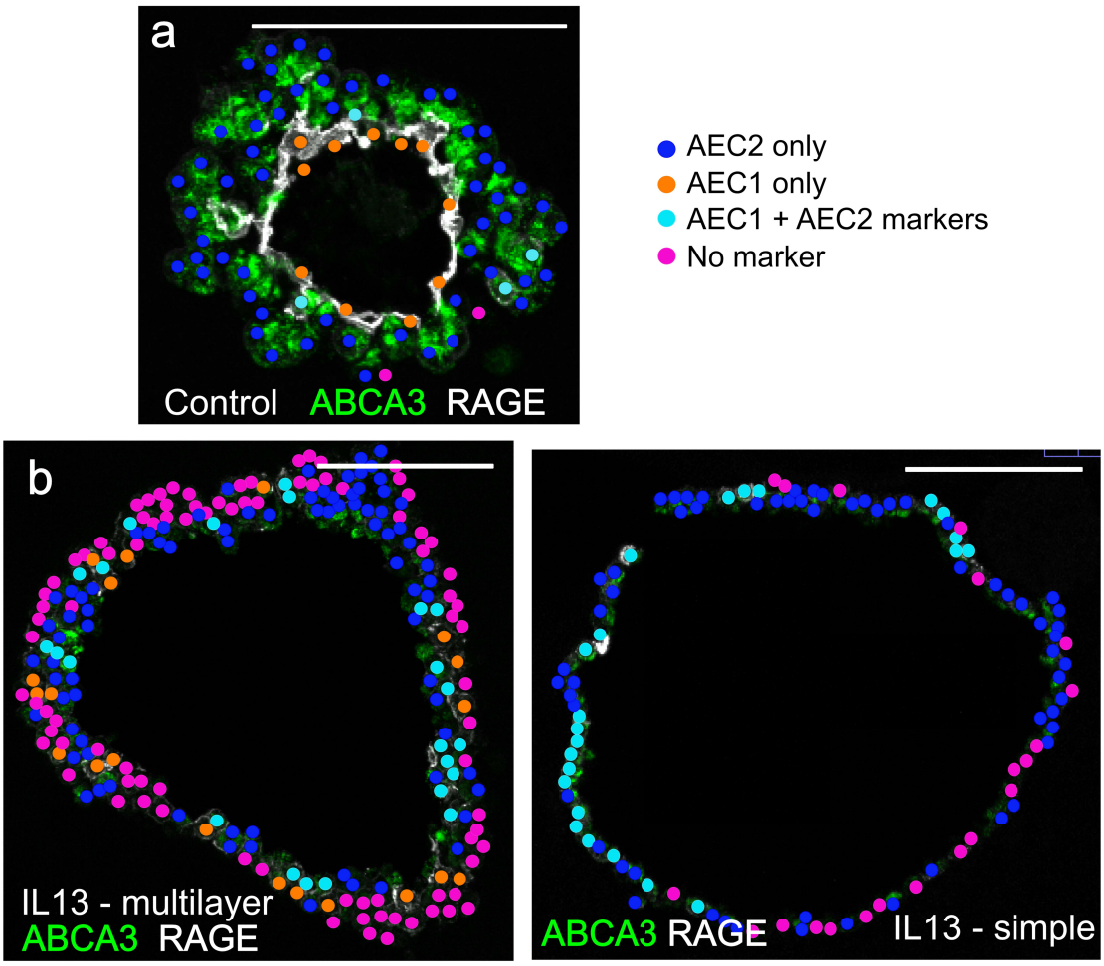
Supplemental Figure 3. Effect of adding IL13 at different time points. The IL13 phenotype is attenuated if added to the culture system beyond day 6-8.

Supplemental Figure 4



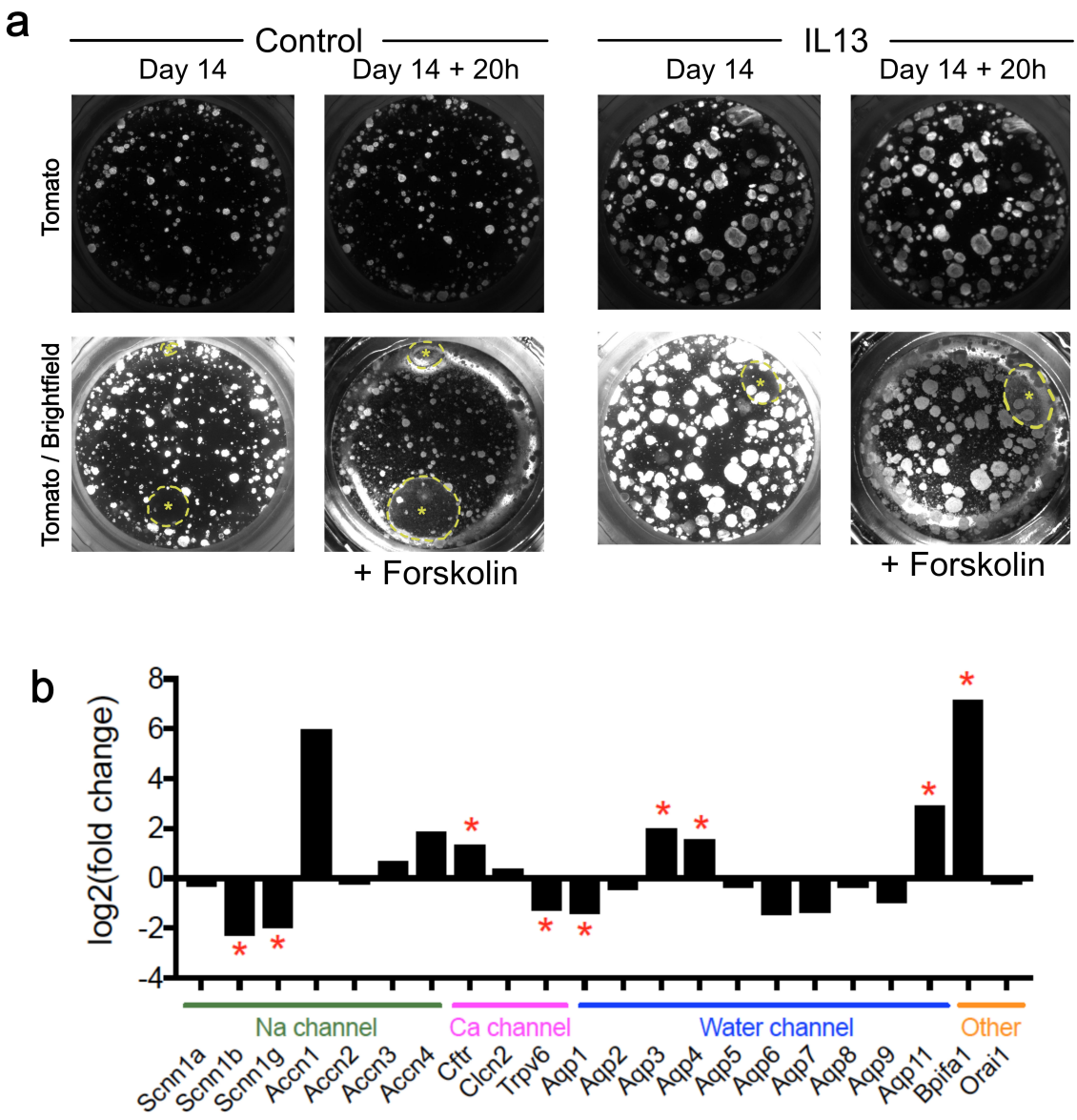
Supplemental Figure 4. Effect of removing IL13 at different time points. If IL13 is removed any time before day 10, the IL13 phenotype at day 14 is attenuated.

Supplemental Figure 5



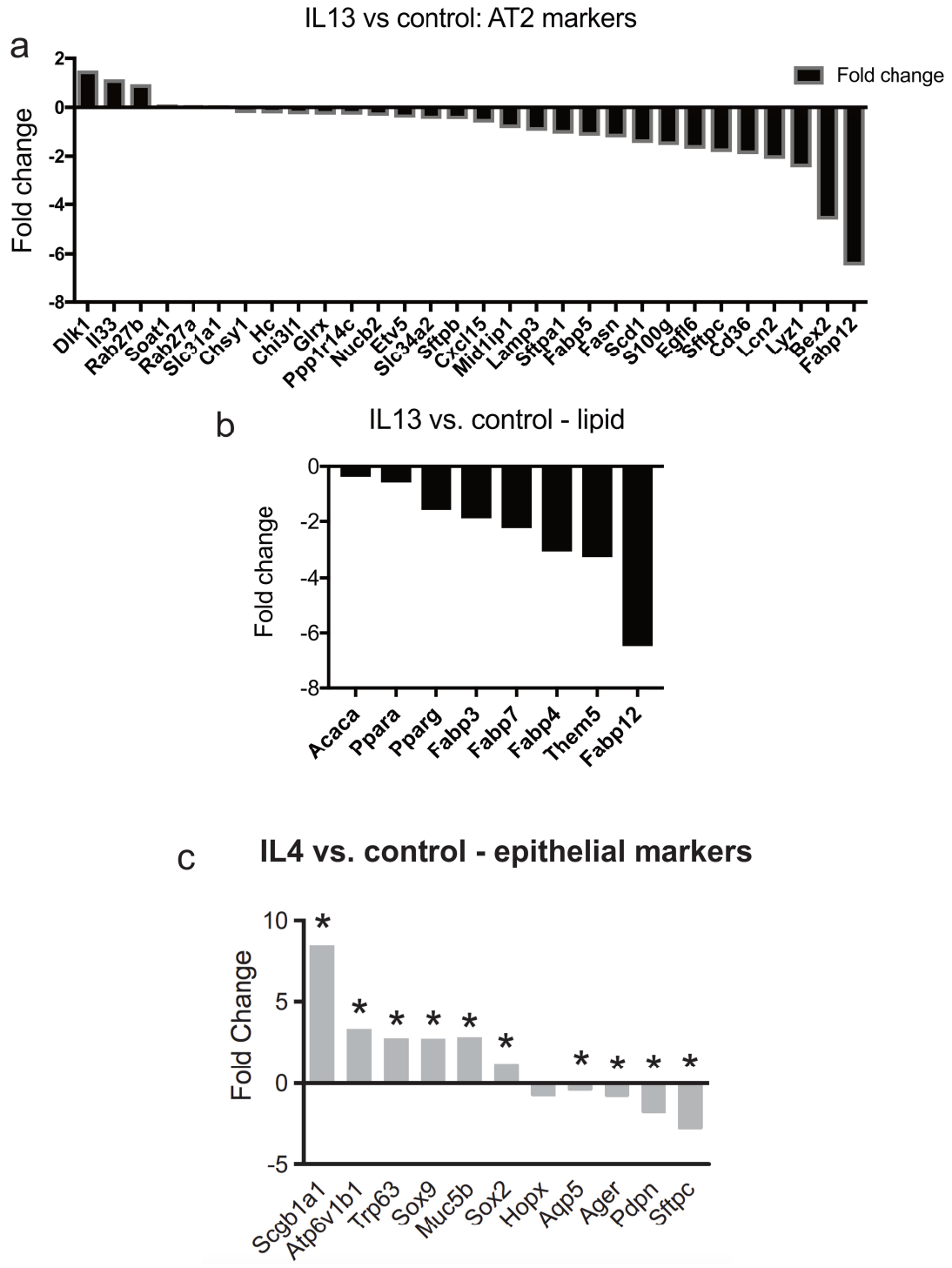
Supplemental Figure 5. Scoring system use to assess polarity of organoids. (a) Control sphere with obvious polarity: the vast majority of AEC2s (ABCA3 positive) are on the outer surface of the sphere while the majority of AEC1s (RAGE positive) are on the inner (luminal) surface. There are few cells that lack AEC2 or AEC1 makers in control conditions. (b) Representative multilayer and single layer organoids grown in the presence of IL13. Note the increase in negative (no marker) cells in both spheres (supported by quantitative data in Fig 3b). Also note the lack of luminal polarity of AEC1s in the IL13-treated spheres. The organoids lack the predictable architecture seen in the control organoids. Scale bars: (a,b) 100μm.

Supplemental Figure 6



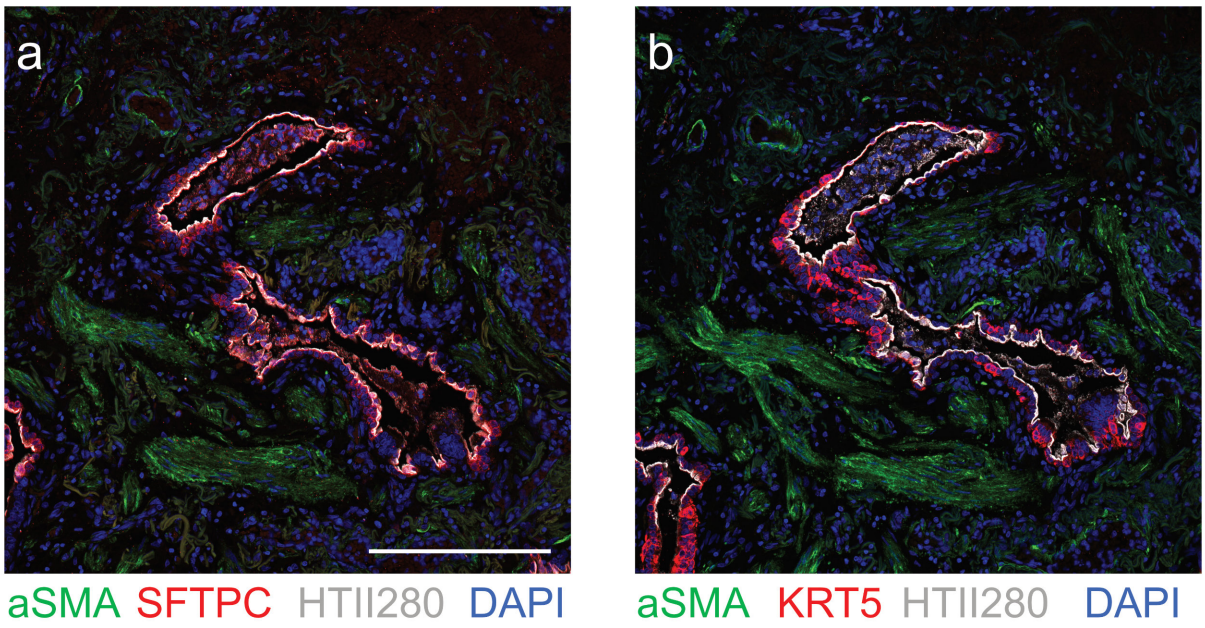
Supplemental Figure 6. Forskolin treatment of AEC2-derived organoid cultures. (a) Organoids grown in the presence and absence of IL13 were treated with forskolin to assess organoid swelling that occurs through modulation of ion channels via activation of adenylate cyclase and an increase in cAMP levels. In both control and IL13-treated conditions, there was no increase in the diameters of Tomato+ (AEC2-derived) organoids after 20 hours of forskolin treatment (top panels, Tomato+), when forskolin was applied starting at culture Day 14. Conversely, rare non-lineage labeled organoids (likely derived from tracheal basal cells and a product of contamination at time of FACS; outlined by yellow dashed line and marked with asterisk) demonstrate significant increase in sphere diameter 20 hours after treatment. This suggests that AEC2-derived sphere diameter is not mediated by cAMP activated ion channels, but that tracheal basal cell-derived sphere diameter can be modulated by cAMP activated ion channels. This data is representative of one biological replicate for each condition. (b) RNAseq data from Day 16 organoids demonstrating changes in the expression of a variety of ion and water channels when organoids are grown in the presence of IL13 compared to controls. * Indicates genes that are significantly differentially expressed. Data represent analysis of 3 biological replicates for each condition.

Supplemental Figure 7



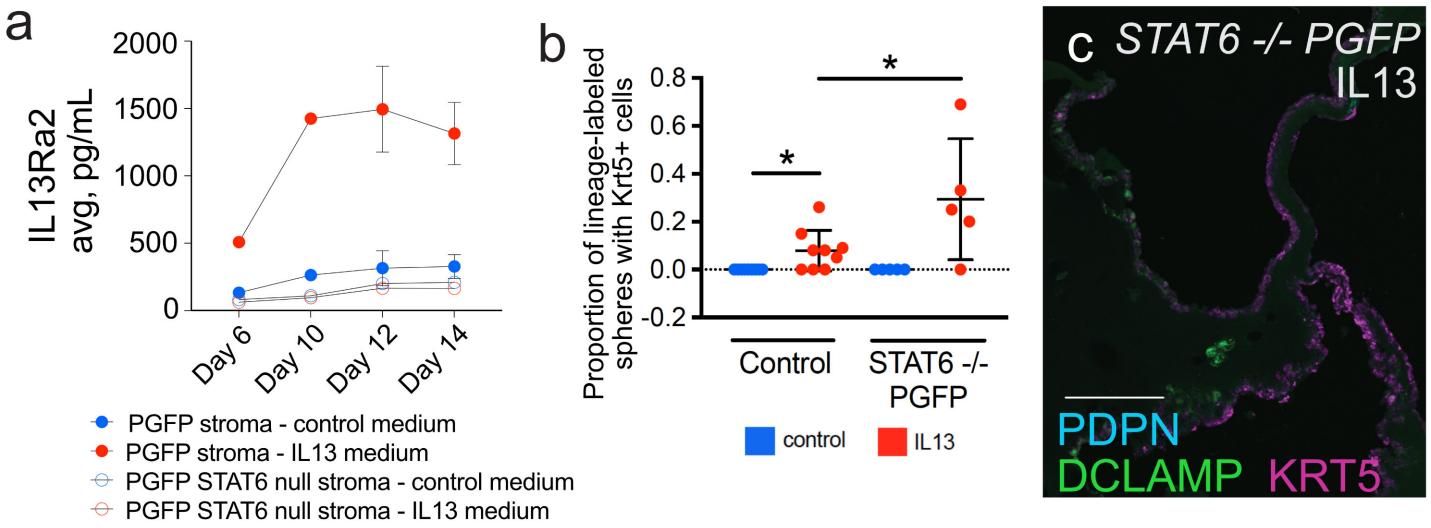
Supplemental Figure 7. RNAseq data demonstrating changes in the expression of (a) AEC2 epithelial markers (previously validated in single cell transcriptomics(26)) and (b) lipid-related genes in AEC2s grown in the presence of IL13 compared to controls. (c) Bulk RNAseq data from Day 16 organoids treated with and without IL4. IL4 induces a change in AEC2s similar to that induced by IL13. * Indicates genes that are significantly differentially expressed. Data represent 3 biological replicates for each condition.

Supplemental Figure 8



Supplemental Figure 8. Serial sections from explanted lungs from a patient with IPF. (a) "Hyperplastic" AEC2 epithelium in a region of fibrotic lung tissue. This epithelium contains AEC2 markers (SFTPC and HTII280) and a paucity of morphologic AEC1s. (b) A serial section reveals that these "hyperplastic" AEC2s are also positive for KRT5. Scale bar: 200μm.

Supplemental Figure 9



Supplemental Figure 9. IL3RA2 levels assessed with ELISA in conditioned medium (CM) from organoids grown with and without intact STAT6 signaling. (a) IL13RA2 levels go up when organoids are grown in the presence of IL13 and are supported by WT fibroblasts; there is no increase in IL13RA2 levels when organoids are grown in the presence of IL13 but are supported by Stat6 -/- fibroblasts. (b,c) A higher proportion of organoids supported by Stat6 -/- fibroblasts in the presence of IL13 contain KRT5+ cells, suggesting that IL13RA2 serves to attenuate the effect of IL13. Scale bar: (c) 150µm.

Supplemental Table 1 – Proteins and genes significantly altered by IL13[a]

Major pathways/proteins/genes increased with IL13	
<u>Th2 signature</u> Acidic mammalian chitinase (AmCase/CHIA; *Chia*) Chitinase-like 3 (CHIL3; *Chi3l3*) Chitinase-like 4 (CHIL4; *Chi3l4*) Chitinase-3-like protein 1 (CH3L1) Resistin-like alpha/FIZZ1 (RETNA; *Retnla*) *Resistin-like beta (Retnlb)* Alpha-aminodipic semialdehyde synthase (AASS; *Aass*) Adseverin (ADSV; *Scin*) Pendrin (S26A4, *Slc26a4*) Sodium-glucose transporter 1 (SC5A1; *Slc5a1*) Polymeric Ig receptor (PIGR; *Pigr*) <u>Proliferation/differentiation</u> Keratin type I cytoskeletal 18 (K1C18) Proliferation marker Ki-67 (KI67; *Mki67*) Structural maintenance of chromosomes 2 (SMC2; *Smc2*) Structural maintenance of chromosomes 2 (SMC4; *Smc4*) *Transformation-related protein 63 (Trp63)*	<u>Bronchiolar / Club cell markers</u> *Uteroglobin/CCSP* (*Scgb1a1*) Cytochrome P450 2F2 (CP2F2; *Cyp2f2*) Cytochrome P450 2S1 (CP2S1, *Cyp2s1*) <u>Remodeling of extracellular matrix</u> Collagen alpha-1(XVIII) chain (COIA1; *Col18a1*) Collagen alpha-1(XIV) chain (COEA1; *Col14a1*) Pro-collagen-lysine,2-oxoglutarate 5-dioxygenase 2 (PLOD2, *Plod2*) Protein-glutamine gamma-glutamyltransferase K (TGM1; *Tgm1*) Protein-glutamine gamma-glutamyltransferase 2 (TGM2)
Major pathways/proteins/genes decreased with IL13	
<u>Pulmonary surfactant: lipid synthesis and function</u> Acetyl-CoA carboxylase 1 (ACACA) Choline-phosphate cytidylyltransferase A (PCY1A; *Pcyt1a*) Fructose 1,6-bisphosphatase 1 (F16P11; *Fbp1*) Fructose 1,6-biophosphatase 2 (F16P2; *Fbp2*) Fatty acid synthase (FAS) Fatty acid binding protein 5 (FABP5; *Fabp5*) *Fatty acid binding protein 12 (Fabp12)* Lysozyme C-2 (LYZ2, *Lyz2*)	

[a] Descriptions in italics indicate differentially expressed genes without corresponding identified/quantified protein. If proteins and genes were both significantly affected, the protein and gene name are listed in parentheses.