Airway secretory cell fate conversion via YAP-mTORC1-dependent essential amino acid metabolism

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Abstract

Tissue homeostasis requires lineage fidelity of stem cells. Dysregulation of cell fate specification and differentiation leads to various diseases, yet the cellular and molecular mechanisms governing these processes remain elusive. We demonstrate that YAP/TAZ activation reprograms airway secretory cells to lose their cellular identity and acquire squamous alveolar type 1 (AT1) fate in the lung. Significantly, this cell fate conversion is mediated via distinctive transitional cell states of Damage-Associated Transient Progenitors (DATPs), recently known to emerge during injury repair, in mouse and human lungs. Further, we describe a YAP/TAZ signalling cascade to be integral for the fate conversion of secretory cells into AT1 fate, by modulating mTORC1-ATF4-mediated amino acids metabolism. Critically, we observed aberrant activation of the YAP/TAZ-mTORC1-ATF4 axis in the altered airway epithelium of bronchiolitis obliterans (BO) syndrome showing the substantial emergence of DATPs and AT1 cells with severe pulmonary fibrosis. Genetic and pharmacologic inhibition of mTORC1 activity restores lineage alteration and subepithelial fibrosis driven by YAP/TAZ activation, proposing a potential therapeutic target for human fibrotic lung diseases.

Keywords

Hippo-YAP signalling; Damage-Associated Transient Progenitors (DATPs); mTORC1-ATF4 axis; Essential amino acid metabolism; Pulmonary fibrosis and bronchiolitis obliterans

Introduction

Epithelial integrity and homeostasis are of central importance to tissue maintenance and function. The alterations in epithelial identity and cellular state can induce lineage infidelity and eventually develop various diseases. However, the cellular and molecular mechanisms that reprogram the lineage identity and fate specification are little known.

The proliferation and differentiation of distinct stem and progenitor cells that reside alongside the pulmonary axis are vital to maintain the lung epithelium (Hogan *et al*, 2014). In the airways, secretory cells marked by the expression of *Scgb1a1*, which encodes the club cell secretory protein (CCSP, CC10), are key stem/progenitor cells that are highly prevalent in both human and mouse. They maintain the airway epithelium by self-renewal and differentiation into ciliated cells during homeostasis and regeneration (Rawlins *et al*, 2009). Secretory cells in the proximal airways can also de-differentiate into basal stem cells after genetic depletion of resident basal cells, whereas secretory cells in the distal airways can contribute to alveolar regeneration following alveolar injury, indicating the plasticity of airway secretory cells in context-dependent manner (Rock *et al*, 2011b; Barkauskas *et al*, 2013; Zhao *et al*, 2014). Besides the functional role of secretory cells as stem and progenitor cells in the lung, they are critical for maintaining barrier integrity by involving in host defence and xenobiotic metabolism (Jones *et al*, 1983; Wang *et al*, 2003; Gamez *et al*, 2015). Importantly, several lines of evidence reveal that dysregulation of secretory cell maintenance is highly relevant to lung diseases such as bronchiolitis obliterans (BO) syndrome (Yanagi *et al*, 2015; Liu *et al*, 2019). Even though the regulation of secretory cells during homeostasis and injury repair has been established, the molecular program that influences the fate behaviour of secretory cells remains unknown. In particular, how the reprograming of secretory cell identity impairs tissue integrity and causes lung dysfunction is poorly understood.

The Hippo signalling pathway, an evolutionally conserved kinase cascade, plays a key role in maintaining tissue homeostasis and function by regulating stem cell behaviours (Zhao *et al*, 2010). In the canonical Hippo pathway, upstream kinases MST1/2 phosphorylate LATS1/2 kinases, which then phosphorylate transcriptional co-activators of YAP/TAZ, causing their cytoplasmic localization and degradation, and inhibit their transcriptional activities (Zhao *et al*, 2010). Proper regulation of Hippo/YAP signalling is critical for proliferation and differentiation of stem and progenitor cells during lung development. Disruption of dynamic YAP/TAZ activity causes defects in airway morphogenesis, cell proliferation, and lineage differentiation in developing lungs (Mahoney *et al*, 2014; Lange *et al*, 2015; Lin *et al*, 2015; Nantie *et al*, 2018; van Soldt *et al*, 2019). In particular, dysregulation

of Hippo/YAP signalling is suggested to impair lung maintenance and function, which is implicated in lung diseases such as pulmonary fibrosis (Gokey *et al*, 2018; LaCanna *et al*, 2019). Nevertheless, the molecular mechanisms of how YAP/TAZ signalling controls the fate behaviours of airway stem and progenitor cells in adult lungs and its implication in lung diseases remain largely unknown.

Here we show that YAP/TAZ signalling modulates the fate behaviours of airway secretory cells, which is implicated in human lung diseases. Single cell RNA-sequencing (scRNA-seq) analysis reveals that sustained activation of YAP/TAZ by LATS1/2 deletion reprograms secretory cells to lose their cellular identity and convert into squamous alveolar type 1 (AT1) cells, via the distinct transitional cell state of Damage-Associated Transient Progenitors (DATPs) (Choi *et al*, 2020; Kobayashi *et al*, 2020; Strunz *et al*, 2020). We further demonstrate that mTORC1-ATF4 activity mediated by YAP/TAZ activation realigns amino acid metabolism, integral to the transition of secretory cells into DATP-AT1 fate. Importantly, aberrant activation of the YAP/TAZ-mTORC1-ATF4 axis is detected in the airways of human BO lungs concomitant with the loss of secretory cell identity and the emergence of DATP and AT1-like cells. Overall, our study identifies a core molecular programme regulating the fate behaviours of secretory cells in lungs and provides new insights into how dysregulation of stem and progenitor cell fate impairs epithelium integrity and causes human lung diseases.

Results

Sustained activation of YAP/TAZ drives the differentiation of airway secretory cells into AT1 cells

To investigate the regulatory role of YAP/TAZ signalling in the cellular identity of airway stem and progenitor cells, we established *Lats1*$^{fl/fl}$;*Lats2*$^{fl/fl}$;*Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$ mice (hereafter Lats1/2 dKO) to delete both LATS1 and LATS2 genes in secretory cells (Fig. 1A). Two days after tamoxifen induction, *Scgb1a1*$^{+}$ lineage-labeled secretory cells of the control lungs (*Lats1*$^{+/+}$;*Lats2*$^{+/+}$;*Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$) barely expressed YAP and TAZ (Appendix Fig. S1A, B). By contrast, *Lats1/2*-deficient secretory cells showed a significant increase of nuclear expression of YAP and TAZ (Appendix Fig. S1A, B). Consistent with YAP/TAZ nuclear localization, *Lats1/2*-deficient secretory cells significantly upregulated the expression levels of genes known to be regulated by YAP and TAZ (Appendix Fig. S1C). Histological analysis revealed that, at day 5 following YAP/TAZ activation, the pseudostratified airway epithelium was disrupted and begun to be stratified and flattened in Lats1/2 dKO lungs (Fig. 1B). Remarkably, the expression of secretory cell marker CC10 was substantially decreased in *Lats1/2*-deficient secretory cells, suggesting the loss of cellular identity by YAP/TAZ activation (Fig. 1B, C). Furthermore, we also observed the subepithelial fibrosis with an increase in collagen deposition and proliferating mesenchymal cells following the alteration of airway epithelium in Lats1/2 dKO lungs, which is consistent with previous reports (Lee *et al*., 2016; McNeill and Reginensi, 2017) (Appendix Fig. S1D).

Of interest, *Lats1/2*-deficient secretory cells significantly lost their identity, but still maintained epithelial identity based on the expression of E-cadherin with no sign of apoptosis (Fig. EV1A). Thus, we next sought to further investigate the changes in cellular property of secretory cells driven by YAP/TAZ activation. Given the differentiation potential of secretory cells into basal or alveolar type 2 (AT2) cells (Barkauskas *et al*, 2013; Rock *et al*, 2011b; Zhao *et al*, 2014), we first checked the lineage conversion of *Scgb1a1*$^{+}$ lineage-labeled secretory cells into these cell types in Lats1/2 dKO lungs. Immunofluorescent (IF) staining showed that neither p63^{+} basal nor SPC^{+} AT2 cells arose from *Lats1/2*-deficient secretory cells (Fig. EV1B, C). Lineage-labeled airway epithelium in Lats1/2 dKO lungs displayed the squamous morphology (Figs. 1B and EV1A-C). Therefore, we then examined the acquisition of AT1 cell fate. Surprisingly, the expression of AQP5, a mature marker for AT1 cells, emerged in *Scgb1a1*$^{+}$ lineage-labeled cells at day 14 post tamoxifen treatment in Lats1/2 dKO airways (Fig. 1D, E). By additional IF staining for other canonical AT1 markers, such as T1α and AGER,

we further verified that lineage-labeled cells in Lats1/2 dKO airways displayed positive for T1α and AGER (Fig. EV1D-F). We also observed the microvascular structure expressing endothelial marker VECAM adjacent to lineage-labeled AT1 cells, suggesting that the secretory cells are converted into bona-fide AT1 cells retaining the potential for gas-exchange with capillary endothelial cells (Fig. EV1F). Significantly, deletion of *Yap* and *Taz* in Lats1/2 dKO lungs, by establishing *Yap*$^{fl/fl}$;*Taz*$^{fl/fl}$;*Lats1*$^{fl/fl}$;*Lats2*$^{fl/fl}$;*Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$ mice (hereafter quadruple knockout mice, or qKO mice), completely rescued the alterations of airway epithelial lineage fate including the restoration of secretory cell identity with CC10 expression (Fig. 1F-H). Taken together, we conclude that sustained activation of YAP/TAZ by *Lats1/2* deletion drives the lineage conversion of secretory cells into squamous AT1 cell fate.

Sustained activation of YAP/TAZ reprograms airway secretory cells to enter DATP cell states leading to AT1 cell fate conversion

We next attempted to define differentiation programmes mediating the transition of secretory cells to AT1 cell fate by YAP/TAZ activation. To do this, we performed scRNA-seq analysis of freshly isolated *Scgb1a1*$^{+}$ lineage-labeled cells (CD45^{-}CD31^{-}EpCAM^{+}Tomato^{+}) from control and Lats1/2 dKO lungs post tamoxifen treatment (Figs. 2A and Appendix Fig. S2A). To focus on the trajectory and molecular identity of secretory to AT1 fate conversion, we further analyzed lineage-labeled epithelial cells of control and Lats1/2 dKO lungs across different time points after excluding non-epithelial populations and ciliated cells (Appendix Fig. S2B, C; see Method section). Based on the expression of canonical markers for secretory and AT1 cells, we identified three distinct cell populations from the scRNA-seq analysis (Fig. 2B, C). Distribution of each cluster across the time points allowed us to assess how YAP/TAZ activation modulates the loss of secretory cell identity and further differentiation into AT1 cells (Fig. 2D). As expected, most lineage-labeled cells in control lungs were secretory cells based on the expression of secretory cell markers such as *Scgb1a1* and *Scgb3a2* (Fig. 2D, E). However, *Lats1/2*-deficient secretory cells showed much lower expression levels of most secretory cell markers such as *Cyp2f2* and *Scgb3a2* (Appendix Fig. S2D). Yet, they still express a comparable level of *Scgb1a1*, which assigns them as 'secretory cell' at day 5 post tamoxifen treatment (Appendix Fig. S2D). Consistent with our IF staining results (Figs. 1D and EV1D, E), scRNA-seq analysis revealed that AT1 cells emerged with a substantial reduction of secretory cells at day 14 post tamoxifen treatment (Fig. 2B-E). Significantly, we also identified a population showing distinct transcriptional signatures that emerged at day 5 and persisted until day 14 (Fig. 2B-E). This population expressed neither secretory nor AT1 cell markers.

Instead, they were marked by enriched expressions of *Cldn4*, *Krt8*, or *Ndrg1*, which are known as the markers for the transitional cell state of DATPs emerged during alveolar regeneration (Choi *et al*, 2020; Kobayashi *et al*, 2020; Strunz *et al*, 2020) (Fig. 2E). Pseudotime analysis suggested that AT1 cell differentiation from secretory cells is mediated by DATPs (Fig. 2F). To further validate the emergence of DATPs from lineage-labeled secretory cells upon YAP/TAZ activation, we performed IF staining for a DATP marker, CLDN4, in Lats1/2 dKO airways. Consistent with our scRNA-seq analysis, $CLDN4^+$ DATPs were detected in lineage-labeled cells at day 5 following tamoxifen treatment (Fig. 2G-I). The frequency of DATPs was reduced with the differentiation of AT1 cells at day 14 (Fig. 2H, I). We observed no detectable levels of CLDN4 expression in control airways (Fig. 2G-I). Collectively, these data suggest that activation of YAP/TAZ reprograms secretory cells to lose their cellular identity and acquire AT1 cell fate through DATP cell state that is known to be induced during lung regeneration.

Uptake of essential amino acids mediated by YAP/TAZ activation is integral to the transition of secretory cells to DATP-AT1 fate

We next sought to determine the molecular programmes, driven by YAP/TAZ activation, responsible for modulating the fate conversion of secretory cells. We found that transcriptional signatures of DATPs showed marked increases in the genes regulating metabolic process, especially in the amino acid synthesis (Fig. EV2A, B). Importantly, DATPs showed much higher expression of solute carrier transporters such as *Slc7a5/Lat1* and *Slc3a2*, which form a heterodimeric complex and uptake essential amino acid (EAA) from the extracellular region (Yanagida *et al*, 2001; Dejure *et al*, 2020) (Fig. 3A). Previous studies revealed that subsets of EAA transporters, such as *Slc7a5/Lat1*, are directly regulated by YAP/TAZ (Hansen *et al*, 2015; Park *et al*, 2016). Therefore, we aimed to evaluate the functional role of EAAs in directing the fate conversion of secretory cells into DATP-AT1 cells using an *ex vivo* three-dimensional (3D) organoid culture system. We recently identified KDR/FLK1 as the surface marker of airway secretory cells (Choi *et al*, 2021, Jiang *et al*, 2021). Using this marker, we isolated secretory cells (KDR^+EpCAM^+) from Lats1/2 dKO lungs, followed by organoid co-culture with stromal cells (Fig. 3B). At day 9 after cell seeding, we replaced culture medium with medium supplemented with AA-limited basal medium (BME) or EAA-enriched medium (BME+EAA) (Fig. 3C). Ethanol (EtOH) or 4-hydroxytamoxifen (4-OHT) was added for control or *Lats1/2* deletion, respectively, at the following day post medium replacement (Fig. 3C). As expected, control secretory cells formed normal cystic organoids (Fig. 3D). By contrast, organoids

derived from 4-OHT-treated secretory cells showed marked morphological changes with dense and folded structures (Fig. 3D). Significantly, these organoids contained CLDN4$^+$ DATPs and AGER$^+$ AT1 cells, whereas control cystic organoids mainly retained CC10$^+$ secretory cells (Fig. 3D-G). Thus, we named folded organoids as "transitioned organoids". Notably, amino acid depletion in the 3D medium dramatically restored these alterations, including the maintenance of secretory cell identity and reduction of DATP and AT1 cell conversion, driven by YAP/TAZ activation (Fig. 3D-G). However, the addition of EAA in organoids derived from *Lats1/2*-deficient secretory cells enhanced the generation of transitioned organoids retaining DATPs and AT1 cells (Fig. 3D-G). We also verified increased nuclear expression of YAP and TAZ by 4-OHT treatment in organoids with 3D and BME medium (Fig. 3H). We then further asked whether a solute carrier transporter SLC7A5/LAT1, modulated by YAP/TAZ signalling, is a crucial regulator for secretory cell conversion by mediating EAA uptake. To do this, we treated JPH-203, a potent inhibitor for SLC7A5/LAT1, in secretory organoids derived from control and Lats1/2 dKO lungs (Oda *et al*, 2010) (Fig. EV2C). Notably, pharmacological inhibition of SLC7A5/LAT1 substantially rescued the loss of secretory cell identity and their conversion into AT1 cell fate mediated by YAP/TAZ activation (Fig. EV2D-F). Taken together, these results indicate that fate conversion of secretory cells into DATPs and AT1 cells by YAP/TAZ activation requires SLC7A5/LAT1-mediated EAA uptake.

mTORC1-ATF4 activity mediated by YAP/TAZ activation regulates amino acid uptake

The Gene Ontology (GO) enrichment analysis of our scRNA-seq data showed that transcriptional signatures of DATPs retained not only 'AA transporter' genes but also a wide range of mTORC1-related targets, including 'AA synthesis', '1C metabolism' and 'tRNA charging' genes (Fig. 4A). Interestingly, we also found that DATPs showed upregulated expression levels of the ATF4 transcript and its downstream target genes (Fig. 4A). Recent studies have suggested a functional interaction between mTORC1 and ATF4 signalling, and Slc7a5/Lat1 was identified as its potent target gene (Adams *et al*, 2007; Ben-Sahra *et al*, 2016; Torrence *et al*, 2021). Therefore, we hypothesized that upregulation of an mTORC1-ATF4 response in DATPs, induced by YAP/TAZ activation, controls EAA uptake, which is crucial for fate conversion of secretory cells into AT1 cells. To test our hypothesis, we first checked whether mTORC1 signalling is activated in Lats1/2 dKO airways by assessing phosphorylated S6 ribosomal protein (p-S6), the best-characterized marker for mTORC1 signalling activation. We detected elevated expression of p-S6 in *Scgb1a1*$^+$ lineage-labeled cells at day 5 post tamoxifen treatment, which coincides with lineage labeled dKO secretory cells converting to

CLDN4^{+} DATPs (Fig. 4B). Elevated levels of p-S6 expression reduced by day 21 when secretory cells were completely converted into AT1 fate, and DATPs were no longer present (Fig. 4B). This suggests that YAP/TAZ-mediated mTORC1 activation is transient with the emergence of DATPs during cell fate conversion. Furthermore, consistent with the p-S6 expression pattern and scRNA-seq analysis, we also observed increased expression of ATF4 in *Scgb1a1*$^{+}$ lineage-labeled cells at day 5 following *Lats1/2* deletion, suggesting the activation of an mTORC1-ATF4 axis in DATPs induced by YAP/TAZ activation (Fig. 4C).

We next sought to validate whether the activation of mTORC1 signalling causes fate conversion of secretory cells, mediated by YAP/TAZ activation. To address this question, we ablated Raptor, a core regulator for mTORC1 kinase in Lats1/2 dKO lungs by establishing *Raptor*$^{fl/fl}$;*Lats1*$^{fl/fl}$;*Lats2*$^{fl/fl}$;*Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$ mice (hereafter triple knockout mice, or tKO mice) (Fig. 4D). Deletion of *Raptor* significantly impaired mTORC1 activity based on the expression of p-S6 in lineage-labeled cells (Fig. EV3A, B). We also observed a significant reduction of ATF4 expression in the cells from tKO mice, despite maintenance of strong nuclear expression of YAP (Fig. EV3C). Importantly, ablation of mTORC1 activity by deletion of *Raptor* substantially rescued the loss of secretory cell identity driven by YAP/TAZ activation (Fig. 4E, F). Further, *Raptor* deletion also blocked the fate conversion of secretory cells into AT1 cells (Fig. 4E, F). We also verified that the expression of EAA transporters, such as *Slc7a5 and Slc3a2*, was augmented by YAP/TAZ activation in secretory cells (Fig. 4G). However, disruption of mTORC1 activity significantly decreased their expressions in tKO secretory cells (Fig. 4G). Consistently, we confirmed the enhanced uptake of EAA in Lats1/2 deficient secretory organoids where secretory cells extensively transited into DATPs and AT1 fate (Fig. 4H). Further, increased EAA uptake by YAP/TAZ activation was inhibited in tKO secretory organoids, suggesting the direct effect of mTORC1 activity on EAA uptake (Fig. 4H). Pharmacological inhibition of mTOR signalling by treatment of AZD8055, a potent mTOR inhibitor, also suppressed the alterations of secretory cell fate by maintaining columnar airway epithelium retaining CC10 expression but lacking AGER expression (Fig. EV3D-F). Notably, we also found that AZD8055 treatment alleviated the accumulation of peribronchiolar collagen and mesenchymal cells (Fig. EV3G).

We further assessed the increased mTORC1-ATF4 activity in *Lats1/2*-deficient secretory organoids. The elevation of p-S6 level and ATF4 expression coincided with the activation of YAP/TAZ in organoids treated with 4-OHT in 3D medium (Fig. 4I). Significantly, depletion of EAA retained the activity of mTORC1-ATF4, yet blocked the transition of secretory cells into DATP-AT1 cell fate in organoids with AA-limited BME media (Fig. 4I).

These results indicate that EAA uptake via mTORC1-ATF4 activity is determinant for the transition of secretory cells into DATPs and AT1 cells.

Finally, we validated whether ATF4 activity regulated by YAP/TAZ activation is essential for fate conversion of secretory cells. To do this, we generated the airway cell line overexpressing YAP 5SA mutant in which all five Lats1/2 target sites are mutated (Kim *et al*, 2015). Consistent with Lats1/2 dKO lungs, constitutive activation of YAP signalling enhanced the EAA uptake with increased expression of ATF4 and its target genes, including Slc7a5 (Fig. 5A-C). However, knockdown (KD) of ATF4 caused the defects in EAA uptake with reduced expression of Slc7a5 (Fig. 5A, C). Furthermore, ATF4 KD in organoids derived from *Lats1/2*-deficient secretory cells blocked the fate conversion of secretory cells into DATPs and AT1 cells (Fig. 5D-F). We also confirmed that Slc7a5 KD in Lats1/2 dKO secretory organoids inhibited the transition into DATPs and AT1 cells (Fig. 5D-F). In contrast, sustained overexpression of ATF4 in secretory organoids significantly promoted the fate conversion of secretory cells into DATPs and AT1 cells (Fig. 5D, G, H). These results strongly support that ATF4 activity induced by YAP/TAZ signalling is crucial for fate conversion of secretory cells into DATP and AT1 cells via regulation of Slc7a5/Lat1 expression, allowing EAA uptake.

Aberrant activation of YAP/TAZ and mTORC1 signalling in human lungs of bronchiolitis obliterans syndrome

Dysregulation of airway secretory cell maintenance is implicated in human lung diseases such as bronchiolitis obliterans (BO); characterized by severe fibrosis with collagen deposition and loss of epithelial integrity (Liu *et al*, 2019). Our data demonstrated that persistent activation of YAP/TAZ leads to fate changes of secretory cells, coincided with peribronchiolar fibrosis (Appendix Fig. S1D). We therefore asked if YAP/TAZ and mTORC1-ATF4 signalling are activated in the lungs of patients with pulmonary fibrosis (PF), including BO. In the distal airways of normal regions, the expression of nuclear YAP was barely detected (Fig. 6A, B). However, we found a marked increase in nuclear YAP protein in the airways of BO where there were flattened epithelial layers and evident subepithelial fibrosis (Fig. 6A, B). Significantly, DATP-like cells expressing CLDN4 and AT1-like cells expressing AQP5 were readily detected in this region, whereas they were barely seen in the airways of normal regions (Fig. 6A, B). Moreover, we found increased levels of p-S6 in the airway epithelium of BO lungs accompanied by the loss of secretory cells (Fig. 6C). Nuclear ATF4 expression was also correlated with the nuclear expression of YAP in this altered epithelium of BO lungs (Fig. 6D). Notably, we readily detected an early transitioning region with partial loss of CC10 expression

in secretory cells showing higher expression of p-S6, implying that acquisition of mTORC1 activity may trigger the loss of secretory cell identity in the airways of BO (Fig. 6E).

Airway epithelium alterations and subepithelial fibrosis are also evident in other types of PF. Therefore, we sought to gain further insights into the role of YAP/TAZ in PF, where we also observed persistent activation of YAP/TAZ-mTORC1 signalling. We re-analyzed scRNA-seq data of PF (GSE135893) and identified the presence of DATP-like cells marked by *CLDN4* and *KRT8* (Habermann *et al*, 2020) (Fig. EV4A-D). Notably, this population has been reported as basaloid cells that are aberrantly expanded in human IPF lungs (Kobayashi *et al*, 2020; Strunz *et al*, 2020; Habermann *et al*, 2020). Furthermore, in human PF we identified elevated transcript levels of YAP/TAZ target genes (*CYR61, AMOTL2, CTGF,* and *GAS6)*, *ATF4* and its targets (*ATF3*, *PPPR15A* and *SLC3A2*) in DATP-like/basaloid and AT1 cells (Fig. EV4E). Finally, we evaluated the potential direct transition of airway secretory cells into DATP-like and AT1-like cells by performing IF staining for these lineage markers in IPF lung tissues. The airways of IPF lungs exhibited flattened epithelial morphology, accompanied with subepithelial fibrosis (Fig. EV4F). Surprisingly, consistent with BO lungs, nuclear YAP protein was readily observed in the airway epithelium where $CLDN4^+$ DATP-like/basaloid and $AQP5^+$ AT1-like cells were prominent but not $CC10^+$ secretory cells. (Fig. EV4G). We also verified increased mTORC1-ATF4 signalling in this altered airway epithelium of IPF lungs (Fig. EV4G). Taken together, these results indicate that the aberrant activation of YAP/TAZ in the airway epithelium may modulate the fate conversion of secretory cells into DATP states and AT1 cells via regulating mTORC1-ATF4 activity, leading to development of peribronchiolar fibrosis in human lung diseases.

Discussion

The loss of stem cell lineage fidelity leads to disruption and failure in maintaining epithelial barriers, resulting in severe pathological diseases. Here, we demonstrated that sustained activation of YAP/TAZ reprograms airway secretory cells to lose their cellular identity and acquire squamous AT1 cell fate via DATP transitional cell state. This transitional cell state derived from airway secretory cells shared very similar transcriptional signatures of DATPs derived from AT2 cells during alveolar regeneration upon damage signals(Choi *et al*, 2020; Kobayashi *et al*, 2020; Strunz *et al*, 2020). Notably, the fate transition of secretory cells into DATP and AT1 cells, driven by YAP/TAZ activation, coincided with severe subepithelial fibrosis, strongly suggesting that dysregulation of lineage fidelity within the airway epithelium causes pulmonary fibrosis. Further, we discovered a core molecular programme directing this process. Activation of YAP/TAZ induces mTORC1-ATF4 activity, enabling amino acid uptake which is required for the fate conversion of secretory cells. Remarkably, elevated YAP and mTORC1-ATF4 activities were observed in the airway epithelium of human PF lungs, including BO, where the loss of secretory cells and aberrant emergence of DATP-like and AT1-like cells within the airways are evident. These results indicate that YAP/TAZ-mediated mTOR-ATF4 signalling is a key molecular programme regulating the fate behaviour of secretory cells in human and mouse lungs, and its dysregulation leads to fibrotic human lung diseases.

By combining lineage-tracing and scRNA-seq analysis, we identified the trajectory of secretory cell conversion into AT1 cell fate, driven by YAP/TAZ activation. Airway secretory cells have been reported to give rise to AT2 cells during alveolar regeneration after injury (Rock *et al*, 2011a; Barkauskas *et al*, 2013). However, in the context of sustained YAP/TAZ activation, we found no evidence of the role for AT2 cells mediating the differentiation of secretory cells into AT1 cells. Instead, we discovered $CLDN4^{+}$ DATPs mediated this process, similar to a previous study which suggested a new route for secretory to AT1 fate transition via an alveolar differentiation intermediate (ADI) during alveolar regeneration after injury (Strunz *et al*, 2020). Importantly, together with previous studies showing AT1 cell differentiation mediated by YAP/Hippo signalling during lung development and regeneration (Mahoney *et al*, 2014; Zhao *et al*, 2014; Lange *et al*, 2015; Lin *et al*, 2015; Nantie *et al*, 2018; LaCanna *et al*, 2019; van Soldt *et al*, 2019; Penkala *et al*, 2021), our study indicates that YAP/TAZ is a key regulator in directing AT1 cell fate in the lung. Secretory cell-derived AT1 cells by YAP/TAZ activation showed similar transcriptional signatures compared to bona-fide AT1 cells in our scRNA-seq analysis. However, whether the proper functional features of AT1

cells converted from secretory cells in the airways are comparable to those in the alveoli remains unanswered.

The loss of secretory cells and/or their squamous alterations have been suggested to cause a severe fibrotic eruption in the distal airways. Previous studies reported that the transient activation of YAP/TAZ promotes regeneration of the airway epithelium (Lange *et al*, 2015; Sun *et al*, 2019). Further, it is noted that YAP is activated in transitional cell states during lung injury repair or in disease cell states (Kobayashi *et al*, 2020; Choi *et al*, 2020; Gokey *et al*, 2018; Strunz *et al*, 2020). Based on these reports and our observations, we propose that YAP/TAZ signalling is a core programme regulating the differentiation of DATP cell states, which arise from either AT2 or airway secretory cells in a spatiotemporal manner during lung regeneration. Interestingly, we observed transient upregulation of mTORC1-ATF4 activity with enhanced nuclear YAP/TAZ localization during airway injury repair post naphthalene treatment (Fig. EV5A, B). In accordance with YAP/TAZ activation, secretory cells lost their cellular identity and transiently acquired CLDN4^{+} DATP cell states prior to repopulating secretory cells for injury resolution. However, repetitive naphthalene injuries caused impaired injury repair and the loss of epithelial integrity. DATPs were persisted, and, of note, we observed the fate conversion of lineage-labeled secretory cells into AT1 cells in the airway epithelium, which is similar to the phenotype of Lats1/2 dKO lungs (Fig. EV5C, D). These results suggest that persistent YAP/TAZ activation induced by chronic lung damage promotes fate conversion of secretory cells into DATP/AT1 cells, which could be directly implicated in lung diseases. Indeed, we observed increased expression of nuclear YAP/TAZ in the airway epithelium of human PF, where the loss of cellular integrity coinciding with the accumulation of DATP-like and AT1-like cells was evident. Further, the emergence of DATP cell states after YAP/TAZ activation coincided with the observation of severe subepithelial fibrosis in Lats1/2 dKO mouse lungs. As previously suggested, it is likely that prolonged presence of DATPs by sustained YAP activation establishes fibrotic microenvironments by secreting pro-fibrotic factors, such as *Tgfb1*, *Ctgf*, and *Edn1*, which may cause the subepithelial fibrotic lesions (Choi *et al*, 2020; Habermann *et al*, 2020; Kobayashi *et al*, 2020; Strunz *et al*, 2020). Thus, a better understanding of the cellular events and molecular programmes governing the fate decisions of secretory cells by YAP/TAZ activation, and its impacts on inducing remodelling of surrounding microenvironments, could potentially enhance our insight into human lung diseases.

Our scRNA-seq analysis revealed that the regulation of secretory cell fate driven by YAP/TAZ activation requires dynamic regulation of metabolism; the synthesis and uptake of

amino acids were highly elevated in DATP cell states. Our *ex vivo* organoid results strongly suggested the crucial role of extracellular EAAs in directing the YAP/TAZ-mediated fate transition of secretory cells into DATPs and AT1 cells. Notably, the expressions of amino acid transporters, such as *Slc7a5*/*Lat1,* are highly enriched in DATPs driven by YAP/TAZ activation. YAP has been reported to directly regulate the expression of *Slc7a5/Lat1* (Hansen *et al*, 2015; Park *et al*, 2016; Edwards *et al*, 2017). However, our study suggests that mTORC1 signalling is essential for YAP/TAZ-mediated upregulation of *Slc7a5/Lat1,* enabling the promotion of EAA uptake in airway secretory cells. Notably, both amino acid withdrawals and pharmacological inhibition of SLC7A5/LAT1 significantly alleviated YAP/TAZ-induced fate conversion of secretory cells into AT1 cells.

We further provide an *in vivo* demonstration that activation of mTOR-dependent ATF4 signalling, mediated by YAP/TAZ activation, is essential for the fate conversion of secretory cells into DATPs and AT1 cells. YAP/TAZ is known to modulate mTORC1 activity, which acts as gatekeepers in cellular differentiation and growth (Tumaneng *et al*, 2012; Hansen *et al*, 2015; Hu *et al*, 2017). We found that inactivation of mTORC1 signalling, by ablating *Raptor* in YAP/TAZ-activated lungs, suppressed upregulation of ATF4, the loss of secretory cell identity, and the emergence of DATPs and AT1 cells. Remarkably, genetic and pharmacologic inhibition of mTORC1 activity substantially suppressed aberrant fibrotic lesions by YAP/TAZ activation in secretory cells. These results suggest the direct interconnection between epithelial lineage alterations and the development of subepithelial fibrosis in the lungs. Significantly, despite the activation of an mTORC1-ATF4 axis in *Lats1/2*-deficient secretory cells, they failed to convert into DATPs and AT1 fate in the absence of EAAs. These results strongly suggest that mTOR-ATF4 activity mediated by YAP/TAZ is required for fate conversion of secretory cells into DATPs and AT1 fate by modulating the expression of amino acid transporters such as *Slc7a5*/*Lat1,* which enables EAA uptake.

BO syndrome is a poorly understood airway disease characterized by severe fibrotic bronchiolar occlusions (Liu *et al*, 2019). The lack of understanding of the molecular mechanisms for its initiation and progression limits the therapeutic interventions possible for BO patients. Previous studies suggested that damage to secretory cells, or decrease of CC10 expression in secretory cells, are related to BO (Nord *et al*, 2002; Bourdin *et al*, 2012; Kelly *et al*, 2012; Liu *et al*, 2019). Importantly, we discovered aberrant YAP and mTORC1-ATF4 signalling in the airway epithelium of human BO lungs with the loss of secretory cells and emergence of $CLDN4^{+}$ DATP-like and $AQP5^{+}$ AT1-like cells in the airways. These were strikingly similar observations to those made in our LATS1/2 dKO mouse model. Our findings

suggest the YAP/TAZ-mTORC1-ATF4 axis as a potential therapeutic target for human fibrotic lung diseases such as BO by modulating the differentiation programme of secretory or DATP cells in PF lungs.

In summary, our results identify the molecular and cellular mechanisms governing the fate and identity of airway secretory cells, which are critical for maintaining epithelial integrity and functions. The regulatory axis between YAP/TAZ and mTORC1-ATF4 signalling determines the fate behaviours of secretory cells in the lungs. Hyperactivation of this axis causes lineage infidelity of secretory cells, coupled with severe subepithelial fibrosis. Our study provides clues for potential therapeutic targets of mTORC1 signalling in the lung diseases of severe fibrosis, including BO lungs.

Materials and Methods

Human Tissues. For histological analysis, human distal lung parenchymal tissues with BO and IPF were retrieved from surgical pathology archives, with the approval of the institutional review board of Yonsei University Severance Hospital, Seoul, Korea. The fibrotic lung diseases were classified according to the American Thoracic Society and European Respiratory Society criteria. After histologic review, paraffin-embedded lung tissues from 5 patients with IPF and 5 patients with BO were collected and analysed. We defined regions with normal morphology as "normal".

Animals. *Lats1*$^{fl/fl}$ mice (Heallen *et al*, 2011) were generated and kindly provided by Dr. Randy L. Johnson. *Yap*$^{fl/fl}$ (Xin *et al*, 2011) and *Taz*$^{fl/fl}$ mice (Xin *et al*, 2013) were generated and generously donated by Dr. Eric N. Olson. *Lats2*$^{fl/fl}$ mice were generated in our laboratory (Kim *et al*, 2013). *Scgb1a1-CreER*^{TM} mice (Rawlins *et al*, 2009), *R26-LSL-tdTomato* mice (Madisen *et al*, 2010) and *Raptor*$^{fl/fl}$ mice (Sengupta *et al*, 2010) were purchased from Jackson Laboratory. For analysis, C57BL/6J male mice were used throughout the study at the age of 4-5 weeks. Mice were bred and maintained under specific-pathogen-free conditions. Experiments were performed in accordance with the procedures approved by Korea Advanced Institute of Science and Technology-Animal Care and Use Committee, approved by local ethical review committees, and conducted according to UK Home Office project license PC7F8AE82.

Chemical treatment in mice. Tamoxifen (CAY-13258-2, Cayman) was dissolved in sterile corn oil at a 25 mg/ml dosage and administered into mice through intraperitoneal injection at a 0.25 mg/g body weight. AZD8055 mTORC1/2 inhibitor (HY-10422, MedChemExpress) was injected into mice via intraperitoneal injection 24 hours prior to tamoxifen treatment at a 10 mg/kg dose. After tamoxifen injection, AZD8055 was re-injected 6 hours later. Then, AZD8055 inhibitor was continuously injected every 12 hours until the indicated day of tissue collection. AZD8055 was dissolved in the solution of 5% Tween80, 10% DMSO, 40% PEG300, and 45% Saline. Naphthalene (176044, Sigma) was dissolved in corn oil and administered to mice through intraperitoneal injection at a 250mg/kg body weight. Single dose of naphthalene was injected and analyzed at day 5 and 14 post treatment. For repetitive injuries, naphthalene was injected once a week for 5 weeks, and tissues were analyzed at 5 days after a final dose.

Tissue sample preparation for histology. Lung tissues were exsanguinated using filtered PBS and inflated with freshly prepared 4% Paraformaldehyde (PFA). After incubation for 2-4 hrs at 4°C, lung tissues were washed with PBS at 4°C for 16 hours. Lung lobes were cut in half, to process half of the tissue for paraffin blocks and the half for frozen blocks. For formalin-fixed paraffin (FFPE) blocks, tissue was dehydrated in ethanol, cleared with xylene then embedded in paraffin. For frozen tissue blocks, tissues were submerged in 30% sucrose for 2-3 days at 4°C. Tissues were embedded in the Optimal Cutting Temperature (OCT) compound and stored at -80°C. Frozen tissue blocks were sectioned using a Leica cryostat into 10 µm thick sections and attached to Superfrost Plus™ Adhesion Microscope slides (ThermoFisher Scientific, J1800AMNT). Paraffin blocks were sectioned into 4 µm thick sections.

Hematoxylin and Eosin staining. Hematoxylin and Eosin staining was performed on 4 µm thick formalin-fixed paraffin-embedded tissue sections. Briefly, slides were de-paraffinized and hydrated. The sections were then placed in Mayers Hematoxylin (Sigma HHS32) for 30 sec. Slides were placed in HCl and rinsed excessively in tap water. The intensity of the hematoxylin was checked under a bright microscope. Sections were then advanced to Ammonium Chloride, dehydrated, and mounted in DPX mountant (44581, Sigma Aldrich).

Sirius Red staining. Collagen fibers were observed in lung tissues, as previously reported (Gomer Ronkainen *et al*, 2021). Briefly, tissue samples were de-paraffinized and incubated overnight at room temperature with a saturated picric acid (Sigma 80456) with 0.1% Fast Green (Sigma F7252) and 0.1% Direct Red (Sigma 365548). Sections were repeatedly washed with distilled water, then dehydrated and mounted with DPX Mounting.

Immunofluorescent staining. FFPE sections were de-paraffinized, hydrated, and emerged in PBS. OCT embedded frozen sections were directly submerged in PBS for 5 min. After antigen retrieval with citrate acid (0.01M, pH6.0), blocking was performed with 10% donkey serum in 0.2% Triton-X/PBS (PBST) at room temperature (RT) for 1 hr. Primary antibodies were diluted in 0.2% PBST at appropriate dilution (**Refer to Table1**) and incubated overnight at 4°C. For immunofluorescence, Alexa-Fluor-488, -594, or -647 were used as 2'ab and incubated for 1 hr at RT or 25 min at 37°C. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma D9542). Slides were then mounted in ProLong Gold antifade reagent (Invitrogen P36930) and sealed with cover glass (Marienfeld, 0101172 or 0101192).

Image analysis and Cell counting. Microscope slides were imaged on Leica TCS SP5, Zeiss LSM800, and Zeiss LSM880 confocal imaging system. For tissue images, at least three different sections, including at least two different bronchial regions from three individual mice per group, were used. For 3D organoid co-culture images, qualitative images were taken at a 20X magnification without zoom and were processed using ImageJ. Quantifications were performed in a blinded fashion using ImageJ and the cell counter plugin. Statistical significance was determined by student's unpaired t-test and set to $p<0.05$ or 0.1. Raw data was recorded, and graphs were generated in GraphPad Prism 5.

Quantitative Real-Time PCR. A quantitative PCR reaction mix was prepared using cDNA from lineage-labeled cells, SYBR qPCR mixture, and primers listed below. Comparative C_T ($\Delta\Delta C_T$) experiment was run on a Bio-Rad Connect machine, using Gapdh as endogenous control and cDNA samples from lineage-labeled cells as reference samples. Data were analysed using the $\Delta\Delta C_T$ quantitation method. ImageJ was used for quantifying the relative gene expression from gel images. The primers used for qRT-PCR are as follows:

Target	Forward (5' – 3')	Reverse (5' – 3')
Ctgf	AGCTGACCTGGAGGAAAACA	GACAGGCTTGGCGATTTTAG
Cyr61	CTGCGCTAAACAACTCAACGA	GCAGATCCCTTTCAGAGCGG
Thbs1	GGTAGCTGGAAATGTGGTGCGT	GCACCGATGTTCTCCGTTGTGA
Tgfb2	CTCCGACGTGACAGACGCT	GCAGGGGCAGTGTAAACTTATT
Atf4	ATGGCCGGCTATGGATGAT	CGAAGTCAAACTCTTTCAGATCCATT
Trib3	CTGCGTCGCTTTGTCTTCAGCA	CTGAGTATCTCTGGTCCCACGT
Slc7a1	GTCTATGTCCTAGCCGGTGC	GAGCCTAGGAGACTGGTGGA
Slc7a3	ATTTGCTTTCTCCGAGGGCA	ATACCCAGCTCCAACACACG
Slc7a5	GGTCTCTGTTCACGTCCTCAAG	GAACACCAGTGATGGCACAGGT
Slc3a2	GAGCGTACTGAATCCCTAGTCAC	GCTGGTAGAGTCGGAGAAGATG
Slc7a11	CTTTGTTGCCCTCTCCTGCTTC	CAGAGGAGTGTGCTTGTGGACA
Gapdh	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG
L32	GGCCTCTGGTGAAGCCCAAGATCG	CCTCTGGGTTTCCGCCAGTTTCGC

Mouse lung tissue dissociation and flow cytometry. Lung tissues were dissociated with a collagenase/dispase solution as previously described (Lee *et al*, 2014). Briefly, after lungs were exsanguinated using filtered PBS, 2 ml of warm dispase (Corning 354235, 50 U/ml) was injected into lung tissues through the trachea. Each lobe was dissected and chopped excessively with a scissor in a 50 ml falcon tube. Per lung tissue, 3 ml PBS with 60 µl collagenase (Roche 10 269 638 001, diluted to 100 mg/ml in cold PBS) was added and incubated for 35 min in 37°C shaking incubator. 25 µl DNase I (Sigma D4527, made to 1% solution in sterile water) were added and further incubated in 37°C shaking incubator for 10 min. Dissociated cells were filtered through series of 100 µm and 40 µm filter strainer, and centrifuged at 1000 rpm for 5 min. The supernatant was aspirated, and the remaining red blood cell (RBC) in the pellet was lysed in 1 ml 1xACK buffer (Lonza 10-548E) for 90 sec at RT. To stop the lysis, 6 ml FBS-free DMEM was first added, then 500 µl FBS was gently layered to the bottom of the tube. Cells were centrifuged at 1000 rpm for 5 min, and the cell pellet was resuspended in 10% FBS in PBS (PF10) for further staining. Cell sorting was performed with FACS Aria II (Beckton Dickinson).

The antibodies used for Flow cytometry and sorting are as follows:

Antibody	Fluorophore	Company	Cat. No.
CD45	APC	BD Pharmingen	559864
CD31	APC	BD Pharmingen	551262
EpCAM	PE-Cy7	Biolegend	118216
KDR	APC	Biolegend	136405

***Ex vivo* 3D lung organoid co-culture.** Freshly sorted secretory cells were resuspended in culture medium (3D basic medium (DMEM/F12, Gibco) supplemented with 10% FBS (Gibco) and ITS (Insulin-Transferrin-Selenium, Corning). These cells were mixed with cultured lung stromal cells negatively isolated by microbeads of CD326/EpCAM, CD45, and CD31 via MACS (Miltenyi Biotech), followed by resuspension in growth factor-reduced Matrigel (BD Biosciences) at a ratio of 1:5. A 100 µl mixture was placed in a 24-well Transwell insert with a 0.4 µm pore (Corning) (Lee *et al*, 2014). Approximately 5×10^3 epithelial cells were seeded in each insert. 500 µl of culture medium was placed in the lower chamber, and the medium was changed every other day. For amino acid media change experiment, 500 µl of 3D basic media, 10% dialyzed FBS in BME media (Thermofisher, 21010046), or 10% dialysed FBS in BME

media with EAA (1mM of L-Histidine, L-Isoleucine, L-Leucine, L-Methionine, L-Threonine, L-Valine, L-Glutamine, L-Arginine, L-Cysteine) was replaced every day.

5'-Single cell RNA library preparation and scRNA-sequencing analysis. Four mice from each group of day 5 (D5) *Scgb1a1-CreER$^{TM/+}$;R26R$^{tdTomato/+}$*, D5 *Lats1$^{fl/fl}$;Lats2$^{fl/fl}$;Scgb1a1-CreER$^{TM/+}$;R26R$^{tdTomato/+}$*, and day 14 (D14) *Lats1$^{fl/fl}$;Lats2$^{fl/fl}$*; *Scgb1a1-CreER$^{TM/+}$*; *R26R$^{tdTomato/+}$* were used. Isolated primary lung epithelial cells from the same group were pooled and sorted for DAPI$^-$CD31$^-$CD45$^-$EpCAM$^+$tdTom$^+$ cells using BD AriaII FACS sorter. The purified tdTom$^+$ cells were then processed using the droplet-based 10X Genomics Chromium approach. In brief, Gel beads in Emulsion (GEMs) were prepared using barcoded Chromium Single Cell 3'Library & Gel Bead Kit v3 (PN1000075) and Chromium B Chip Kit (PN1000154). To recover 2x10^3 cells for control and 4x10^3 cells each for D5 and D14 samples, approximately 5-8x10^3 cells were loaded onto the Chromium controller. Immediately after GEMs formation, they were reverse transcribed, and the resulting cDNAs were tagged with Unique Molecular Index (UMI) and cell barcodes. Next, GEMs were broken, and cDNA was amplified and quantified using Agilent 2100 Bioanalyzer. The final 3' Gene Expression Library was constructed by enzymatic fragmentation and size selection. The samples were then sequenced on the Illumina HiSeq X by Macrogen with a target of 2.5-3.0x10^4 reads/cells (2x100 paired-end reads).

Alignment, quantification, and quality control of scRNA-seq data. Droplet-based sequencing data was aligned and quantified using the Cell Ranger Single-Cell Software Suite (version 3.0.2, 10x Genomics Inc) using the *Mus musculus* genome (GRCm38) (official Cell Ranger reference, version 1.2.0). Cells were filtered by custom cutoff (more than 500 and less than 7000 detected genes, more than 2000 UMI count) to remove potential empty droplets and doublets. The downstream analysis included data normalisation, highly variable gene detection, log transformation, principal component analysis, neighbourhood graph generation, and Louvain graph-based clustering, which was done by python package scanpy (version 1.3.6) (Wolf *et al*, 2018) using default parameters.

Doublet Exclusion. To exclude doublets from scRNA-seq data, we applied scrublet algorithm per sample to calculate scrublet-predicted doublet score per cell with following parameters: sim_doublet_ratio = 2; n_neighbors=30; expected_doublet_rate= 0.1. Any cell with a scrublet score > 0.7 was flagged as doublet. To propagate the doublet detection into potential false-

negatives from scrublet analysis, we over-clustered the dataset (*sc.tl.louvain* function from scanpy package version 1.3.4; resolution = 20), and calculated the average doublet score within each cluster. Any cluster with averaged scrublet score > 0.6 was flagged as a doublet cluster. All remaining cell clusters were further examined to detect potential false-negatives from scrublet analysis according to the following criteria: (1) Expression of marker genes from two distinct cell types which are unlikely according to prior knowledge, (2) higher number of UMI counts.

Pseudotime Analysis. All data contained within our processed Seurat object for the wildtype data set was converted to the AnnaData format for pseudotime analysis in Scanpy (version 1.3.6). We recalculated *k*-nearest neighbours at k = 15. Pseudotime was calculated using Scanpy's partitioned-based graph abstraction function, PAGA. Diffusion pseudotime was performed using Scanpy's DPT function with default parameters.

Plasmid construction

For conditional shRNA expression in organoid culture, pSico plasmid was used as cloned as previously reported (Ventura *et al*, 2004). The shRNA sequences used are as follows: shscramble forward: 5'-TGACACGCGACTTGTACCACTTCAAGAGAGTGGTACAAGT CGCGTGTCTTTTTTC-3', shscramble reverse: 5'-TCGAGAAAAAAGACACGCGACTTG TACCACTCTCTTGAAGTGGTACAAGTCGCGTGTCA-3', shAtf4 forward: 5'-TGGAGT TAGTTTGACAGCTATTCAAGAGATAGCTGTCAAACTAACTCCTTTTTTC-3', shAtf4 reverse: 5'- TCGAGAAAAAAGGAGTTAGTTTGACAGCTATCTCTTGAATAGCTGTCA AACTAACTCCA-3', shSlc7a5 forward: 5'- TGCAGCCTGCAATCCTAATATTCAAGAG ATATTAGGATTGCAGGCTGCTTTTTTC-3', shSlc7a5 reverse: 5'- TCGAGAAAAAAGC AGCCTGCAATCCTAATATCTCTTGAATATTAGGATTGCAGGCTGCA-3'.

For Atf4 overexpression, pMSCV-ERT2-Atf4-IRES-EGFP was constructed in-house. Mouse Atf4 coding sequence (CDS) was PCR amplified from mouse cDNA using the following primer set: 5'-GGTACCGAGCTCGGATCCAACATGACCGAGATGAGCTTCC-3' and 5'-GCTGGATATCTGCAGAATTCTTACGGAACTCTCTTCTTCCC-3'. The Atf4 CDS, ERT2, and IRES-EGFP DNA fragments were assembled and cloned into *EcoR*I/*BamH*I digested pMSCV plasmid using the In-Fusion Cloning kit (Takara), and confirmed by DNA sequencing.

Amino acid uptake assay

L-amino acid assay kit (Sigma, ab65347, for total L-amino acid) and Branched chain amino acid (BCAA) assay kit (Sigma, ab83374, for EAA) were used to measure amino acid content in 3D organoids or C22 murine airway cell lines. For 3D organoids, Matrigel was first removed by dispase (Corning 354235, 50 U/ml) incubation for 40 min. tdTomato-expressing organoids were picked with a pipette under a fluorescent microscope to exclude co-cultured endothelial cells. The selected organoids or C22 cells were further dissociated into single cells using TrypLE (Gibco 2604021) and the number of cells was counted using Countess II automated cell counter. $1x10^6$ cells were each used to measure total L-amino acids or BCAAs contents according to the manufacturer's protocols.

Statistical Analysis

Values are presented as mean ± SEM. All experiments used at least 2-5 mice per group due to the individual variation between mice. For JPH203 or AZD8055 chemical treatment, male mice of 4~6 weeks were randomly assigned to treatment or control groups. Statistical significance was determined by student's unpaired t-test and set to $p<0.05$ or 0.1. All graphs were generated with GraphPad Prism 5.

Data Availability

The Gene Expression Omnibus accession number for raw expression data for scRNA-seq analysis of *Lats1*$^{fl/fl}$;*Lats2*$^{fl/fl}$;*Scgb1a1-CreER*$^{TM/+}$;*R26R*$^{tdTomato/+}$ mice reported in the manuscript is GSE178829 (https://www-ncbi-nlm-nih-gov.ezproxy.u-pec.fr/geo/query/acc.cgi?acc=GSE178829). The Rds files for control and idiopathic pulmonary fibrosis (IPF) lungs were obtained from GEO (GSE135893) (Habermann *et al*, 2020). Cell clusters of 'Club', 'CLDN4^{+}/KRT8^{+}' and 'AT1' cells were extracted and analysed.

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Author Contributions

H.-Y.J., J.C., D.-S.L., J.-H.L., conceived and designed the experiments, interpreted the data, and wrote the manuscript; H.-Y.J. performed most of the experiments and data analysis; J.C. analysed murine scRNA-seq data; D.E. re-analysed human scRNA-seq data; L.K. designed the experiments, performed IF analysis, and interpreted IF image data; Y.H.K. helped with genotyping and staining of qKO mice and qPCR of C22 cell lines; K.Y. aligned raw scRNA-seq data; J.-G.K constructed Atf4 overexpressing plasmid; H.S.S. provided and analysed pathology of human IPF tissue; J.W.K shared Raptor$^{fl/fl}$ mice.

Disclosure and Competing Interests Statement

The authors declare that they have no conflict of interest.

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Figure Legends

Figure 1. *Lats1/2* deletion induces the differentiation of secretory cells into AT1 cells in the airways

A Experimental design for lineage-tracing analysis of secretory cells following genetic depletion of *Lats1* and *Lats2*. Specific time points for tamoxifen induction and tissue analysis are indicated.

B Representative H&E (top) and immunofluorescent (IF) images (bottom) of control and Lats1/2 dKO airways at indicated time points post tamoxifen treatment. Tomato (for Scgb1a1 lineage, red), CC10 (for secretory cell, white), Acetylated tubulin (Act-Tub, for ciliated cell, green), and DAPI (blue). Scale bar, 100μm.

C Statistical quantification of *Scgb1a1* lineage-labeled tdTomato$^+$CC10$^+$ secretory cells in (B). Data are presented as mean ± SEM (n= 5 mice for each group,). ***p<0.001 (Student's t-test).

D Representative IF images of control and Lats1/2 dKO airways at indicated time points post tamoxifen treatment. Tomato (for Scgb1a1 lineage, red), CC10 (green), AQP5 (for AT1 cell, white), and DAPI (blue). Scale bar, 100μm.

E Quantification of *Scgb1a1* lineage-labeled tdTomato$^+$AQP5$^+$ AT1 cells in (D). Data are presented as mean ± SEM (n=5 mice for each group). **p<0.01, ***p<0.001 (Student's t-test).

F Experimental design for lineage-tracing analysis of secretory cells following genetic depletion of *Lats1/2* (double knockout, dKO) and *Lats1/2*;*Yap*;*Taz* (quadruple knockout, qKO). Specific time points for tamoxifen induction and tissue analysis are indicated.

G Representative H&E (top) and IF images (bottom) of control, dKO, and qKO airways at day 21 post tamoxifen treatment. Tomato (for Scgb1a1 lineage, red), CC10 (green), AQP5 (white), and DAPI (blue). Scale bar, 100μm.

H Quantification of *Scgb1a1* lineage-labeled tdTomato$^+$CC10$^+$ secretory cells in (G). Data are presented as mean ± SEM (n=3 mice for each genotype). ***p<0.001, NS=not significant (Student's t-test).

Figure 2. Sustained activation of YAP/TAZ drives the fate conversion of secretory cells into AT1 cells via DATP cell state

A Experimental design for isolating *Scgb1a1* lineage-labeled cells from control and Lats1/2 dKO lungs at indicated time points post tamoxifen treatment. Isolated cells were subjected to scRNA-seq analysis.

B Clusters of *Scgb1a1* lineage-labeled cells from 10XGenomics 3' single-cell RNA-sequencing (scRNA-seq) analysis visualized by Uniform Manifold Approximation and Projection (UMAP), assigned by specific colors at day 5 and 14 post tamoxifen treatment. The number of cells in the individual cluster is depicted in the figure.

C Distribution of each cluster across indicated time points after tamoxifen treatment.

D Bar graph showing the percentage of secretory, DATP, and AT1 cell clusters in control and Lats1/2 dKO lungs at day 5 and 14 post tamoxifen treatment.

E Blob plot depicting selected marker genes in cell clusters. Dot size encodes the percentage of cells expressing the gene, and color encodes the average per cell gene expression level.

F Pseudotime ordering of *Scgb1a1* lineage-labeled cells colored by cluster assignment (right) according to diffusion pseudotime (DPT, left) order.

G, H Representative IF images of control and Lats1/2 dKO airways at indicated time points post tamoxifen treatment. Tomato (for Scgb1a1 lineage, red), CLDN4 (for DATPs, white), CC10 (G, green), AGER (H, for AT1 cell, green), and DAPI (blue). Scale bar, 100μm.

I Quantification of *Scgb1a1* lineage-labeled tdTomato$^+$CLDN4$^+$ DATPs in (G and H). Data are presented as mean ± SEM (n=5 mice for each genotype). ***p<0.001 (Student's t-test).

Figure 3. The uptake of essential amino acids is required for cell fate conversion of secretory cells mediated by YAP/TAZ activation

A Violin plots of DATP cell markers (*Cldn4, Ndrg1*) and essential amino acid (EAA) transporters (*Slc7a5, Slc3a2, Slc6a9*) in control and Lats1/2 dKO lung cells at day 5 post tamoxifen treatment.

B, C Flow cytometry sorting scheme for isolation of secretory cells (EpCAM$^+$KDR$^+$) (B) and experimental designs for organoid cultures (C). Secretory cells were isolated from unlabeled Lats1/2 dKO lungs, followed by 3D organoid co-cultures with stromal cells. 3D normal media was changed to experimental media 1 day prior to 4-hydroxytamoxifen (4-OHT) or ethanol (EtOH) treatment. Organoids were analyzed at day 8 post 4-OHT or EtOH treatment.

D Representative brightfield and H&E images of 3D organoids. Arrow indicates transitioned 3D organoids retaining DATP and AT1 cells. Scale bars, 1mm for brightfield images, 200μm for H&E images.

E, F Representative IF images of secretory, DATP, or AT1 cells in secretory organoids in indicated culture conditions. Tomato (for Scgb1a1 lineage, red), CC10 (E, green), CLDN4 (E, white), AGER (F, green), and DAPI (blue). Scale bars, 100μm.

G Quantification of transitioned 3D organoids in 3D normal media (3D), amino acid-limited Basal MEM media (BME), or affluent repletion of EAA in BME media (BME+EAA) with 4-OHT or EtOH treatment. Data are presented as mean ± SEM (n= 3 technical replicates per group). *p<0.05, NS= not significant (Student's t-test).

H Representative IF images of YAP and TAZ expression of secretory organoids in indicated culture conditions. Tomato (for Scgb1a1 lineage, red), YAP (green), TAZ (white), and DAPI (blue). Scale bars, 100μm.

Figure 4. mTORC1-ATF4 activity regulates the amino acid uptake for fate conversion of secretory cells

A Heatmap of ATF4-dependent mTORC1 targets (AA synthesis, 1C metabolism, AA transport, tRNA charging), ATF4 targets, and ATF4-independent mTORC1 targets (HIF1a, mt tRNA).

B Representative IF images showing the expression of secretory cell marker CC10, mTORC1 activity p-S6, and DATP marker CLDN4 in *Scgb1a1* lineage-labeled toTomato^{+} cells from serial sections of control and Lats1/2 dKO lung tissues. Tomato (for Scgb1a1 lineage, red), CC10 (green), p-S6 (white), CLDN4 (magenta), and DAPI (blue). Scale bars, 100μm.

C Representative IF images showing the nuclear expression of YAP and ATF4 in *Scgb1a1* lineage-labeled tdTomato^{+} cells from serial sections of control and Lats1/2 dKO lung tissues. Of note, CLDN4 expression in lineage-labeled cells having the nuclear YAP and TAZ. Tomato (for Scgb1a1 lineage, red), YAP (green), ATF4 (white), CLDN4 (magenta) and DAPI (blue). Scale bars, 100μm.

D Experimental designs for lineage-tracing of control, *Lats1/2* double knockout (dKO) and *Raptor*;*Lats1/2* homozygous triple-knockout (tKO) lungs. Specific time points for tamoxifen treatment and analysis are indicated.

E Representative H&E and IF images of control, Lats1/2 dKO, and Raptor;Lats1/2 tKO airways at day 21 post tamoxifen treatment. Tomato (for Scgb1a1 lineage, red), CC10 (green, upper), AGER (green, lower), and DAPI (blue). Scale bar, 100μm.

F Quantification of *Scgb1a1* lineage-labeled tdTomato$^+$CC10$^+$ secretory cells (left) and tdTomato$^+$AGER$^+$ AT1 cells (right) in (E). Data are presented as mean ± SEM (n=2-3 mice for each genotype). ***p<0.001 (Student's t-test).

G Quantitative PCR (qPCR) analysis of ATF4-dependent amino acid transporters (*Slc7a1, Slc7a3, Slc7a5, Slc34a2, Slc7a11*), on freshly isolated *Scgb1a1* lineage-labeled cells from control and Lats1/2 dKO lungs at day 2 post tamoxifen treatment. Data are presented as mean ± SEM (n=2 mice for each group). *p<0.05, ***p<0.0001 (Student's t-test).

H Measurement of L-amino acid or essential amino acids (leucine, isoleucine, valine) contents in *Scgb1a1* lineage-labeled tdTomato$^+$ organoids derived from control, Lats1/2 dKO or Raptor;Lats1/2 tKO mice. Data are presented as mean ± SEM (n=3 technical replicates for each genotype). *p<0.05, **p<0.001 (Student's t-test).

I Representative IF images showing the expression of YAP, p-S6, and ATF4 in serial sections of 3D organoids derived from secretory cells in indicated culture conditions. Tomato (for Scgb1a1 lineage, red), YAP (green), p-S6 (white), ATF4 (magenta), and DAPI (blue). Scale bars, 100μm.

Figure 5. ATF4-dependent essential amino acid uptake is crucial for the cell fate decision of secretory cells

A Western blot images for the expression of YAP, ATF4, and LAT1/Slc7a5 in control or YAP5SA overexpressed C22 airway epithelial cell line. Knockdown of ATF4 was induced by using lentiviral-mediated inducible scramble shRNA (shScramble, control) or shATF4.

B Quantitative PCR (qPCR) analysis of YAP/TAZ targets (*Cyr61*, *Thbs1*, *Amotl2*), *ATF4* and the targets of ATF4 (*Trib3*, *Slc7a5*, *Slc7a11*).

C Concentration of L-amino acids (L-AA, left) or EAA (right; leucine, isoleucine, valine) in cell lines in (A). Data are presented as mean ± SEM (n=3 biological replicates for each group). *p<0.05, NS=not significant (Student's t-test).

D Experimental designs for culture and viral transduction for knockdown constructs (shRNA vectors) targeting Atf4 or Slc7a5 and ectopic expression of Atf4 in 3D secretory organoids.

E Representative H&E images and IF images showing the expression of secretory cell marker CC10, DATP cell marker CLDN4, and AT1 cell marker CAV1 in organoids derived from Lats1/2 dKO secretory cells with shscramble, shAtf4, or shSlc7a5. CC10 (green), CLDN4 (white), CAV1 (white) and DAPI (blue). Scale bars, 100μm.

F Quantification of *Scgb1a1*$^+$ lineage-labeled tdTomato$^+$CC10$^+$ secretory cells (red), tdTomato$^+$CLDN4$^+$ DATP cells (green), and tdTomato$^+$CAV1$^+$ AT1 cells (blue) in (E).

Data are presented as mean ± SEM (n=3 technical replicates for each group). ***p<0.0001, NS=not significant (Student's t-test).

G Representative H&E images and IF images showing the expression of secretory cell marker CC10, DATP cell marker CLDN4, or AT1 cell marker CAV1 in organoids derived from *Scgb1a1* lineage-labeled secretory cells with or without hydroxytamoxifen-induced ATF4 overexpression. CC10 (green), CLDN4 (white), CAV1 (white), and DAPI (blue). Scale bars, 100μm.

H Quantification of *Scgb1a1* lineage-labeled tdTomato$^+$CC10$^+$ secretory cells (red), tdTomato$^+$CLDN4$^+$ DATP cells (green), and tdTomato$^+$CAV1$^+$ AT1 cells (blue) in (G). Data are presented as mean ± SEM (n=3 technical replicates for each group). ***p<0.0001 (Student's t-test).

Figure 6. Aberrant activation of YAP/TAZ-mTORC1 signalling in the human lungs of bronchiolitis obliterans syndrome (BO)

A Representative H&E images of human lung tissues from normal background and bronchiolitis obliterans (BO) with progressive fibrosis.

B Representative IF images showing the expressions of nuclear YAP, DATP cell marker CLDN4, and AT1 cell marker AQP5 in the airways of normal background (top) and BO (bottom) human lungs. CLDN4 (white), AQP5 (red), YAP (green), and DAPI (blue). Of note, a flattened airway layer in BO lungs. Scale bars, 100μm.

C Representative IF images showing the expressions of secretory cell marker CC10 and mTORC1 activation marker p-S6 in the airways of normal background (top) and BO (bottom) human lungs. CC10 (green), p-S6 (white), and DAPI (blue). Scale bars, 100μm.

D Representative IF images showing the expression of nuclear YAP, ATF4, and AQP5 in the airways of normal background (top) and BO (bottom) human lungs. YAP (green), ATF4 (white), AQP5 (red), and DAPI (blue). Of note, co-expressions of nuclear YAP, nuclear ATF4, and AQP5 in the airways of BO lungs. Scale bars, 100μm.

E Representative IF images showing the expression of p-S6 (white) in the airway cells losing CC10 (green) expression of BO human lungs. Scale bars, 100μm.

Figure Expanded View 1. YAP/TAZ activation in secretory cells enforces fate conversion into AT1 cells, but bypasses basal or AT2 cells.

A Representative IF images showing the expression of epithelial cell marker E-cadherin and apoptosis marker TUNEL in *Scgb1a1* lineage-labeled tdTomato$^+$ cells of control and

Lats1/2 dKO lungs. Tomato (for secretory lineage, red), E-cadherin (for epithelial cell, white), TUNEL (for apoptotic cells, green), and DAPI (blue). Scale bars, 100μm.

B Representative IF images showing the expression of basal cell marker P63 in *Scgb1a1* lineage-labeled tdTomato$^+$ cells in control and Lats1/2 dKO lungs. Tomato (for secretory lineage, red), P63 (green), and DAPI (blue). Scale bars, 100μm.

C Representative IF images showing the expression of AT2 cell marker SPC in *Scgb1a1* lineage-labeled tdTomato$^+$ cells in control and Lats1/2 dKO lungs. Tomato (for secretory lineage, red), SPC (green), and DAPI (blue). Scale bars, 100μm.

D, E Representative IF images showing the expression of AT1 cell markers T1α (D), AGER (E), and endothelial marker VECAM (E) in *Scgb1a1* lineage-labeled tdTomato$^+$ cells in control and Lats1/2 dKO lungs. Tomato (for secretory lineage, red), T1α (D, green), AGER (E, green), VECAM (E, white), and DAPI (blue). Scale bars, 100μm.

F Quantification of *Scgb1a1* lineage-labeled tdTomato$^+$AGER$^+$ AT1 cells in (E). Data are presented as mean ± SEM (n=5 mice for each group). *p<0.05, ***p<0.001 (Student's t-test).

Figure Expanded View 2. YAP/TAZ signalling modulates the uptake of essential amino acids that is crucial for cell fate conversion of secretory cells

A Analysis of Gene Ontology (GO) terms in DATP cell states driven by YAP/TAZ activation.

B Heatmap of secretory, DATP, and AT1 cells for metabolism-related genes including hypoxia response, one-carbon metabolism, and amino acid synthesis/serine/glycine metabolism.

C Experimental designs for organoid cultures derived from secretory cells isolated from unlabeled control and Lats1/2 dKO lungs. Isolated secretory cells were seeded in 3D organoid co-cultures with stromal cells. At day 7 post-seeding, 4-hydroxytamoxifen (4-OHT) was treated, followed by the addition of SLC7A5/LAT1 inhibitor (JPH203) twice every day for 7 days. Organoids were analyzed at day 7 post 4-OHT treatment.

D Representative H&E and IF images of 3D organoids in (C). Tomato (red), CC10 (green), CAV1 (for AT1 cells, white), and DAPI (blue). Scale bars, 100μm.

E, F Quantification of *Scgb1a1* lineage-labeled tdTomato$^+$CC10$^+$ secretory (E) and CAV1$^+$ AT1 (F) cells in 3D organoids in (D). Data are presented as mean ± SEM (n=at least 2 technical replicates per group). *p<0.05, **p<0.01, NS=not significant (Student's t-test).

Figure Expanded View 3. Ablation of *Raptor* suppresses YAP/TAZ-induced mTORC1 and ATF4 activation.

A, B Representative H&E and IF images (A), and quantification (B) of secretory cells and mTORC1 activity in control, Lats1/2 dKO (dKO), and Raptor;Lats1/2 triple knockout (tKO) airways at day 5 post tamoxifen treatment. Tomato (A, for Scgb1a1 lineage, red), CC10 (A, green), pS6 (A, white), and DAPI (blue). Scale bars, 100μm. Data are presented as mean ± SEM (n=3 mice for each genotype). **p<0.01, ***p<0.0001 for $pS6^{+}CC10^{-}$ cells (Student's t-test).

C Representative IF images showing the expression of nuclear YAP and ATF4 in *Scgb1a1*$^{+}$ lineage-labeled cells of control, Lats1/2 dKO, and Raptor;Lats1/2 tKO airways at day 5 post tamoxifen treatment. Tomato (for Scgb1a1 lineage, red), YAP (green), ATF4 (white), and DAPI (blue). Scale bars, 100μm.

D Experimental designs for treating mTOR inhibitor (AZD8055) or vehicle to control and Lats1/2 dKO mice twice a day at indicated time points post tamoxifen treatment.

E Representative H&E and IF images of control and Lats1/2 dKO lungs with vehicle or AZD8055 treatment at day 14 post tamoxifen treatment. Tomato (for secretory lineage, red), CC10 (middle, green), AGER (bottom, green), and DAPI (blue). Scale bars, 100μm.

F Quantification of *Scgb1a1* lineage-labeled $tdTomato^{+}CC10^{+}$ secretory (top) and $AGER^{+}$ AT1 cells (bottom) in (E). Data are presented as mean ± SEM (n=3 mice for each group). **p<0.005, ***p<0.001 (Student's t-test).

G Representative Sirius red staining for collagen (top) and IF images showing the expression of mesenchymal marker Vimentin (bottom) in control and Lats1/2 dKO lungs with vehicle or AZD8055 treatment. Tomato (for secretory lineage, red), Vimentin (VIM, green), and DAPI (blue). Scale bars, 100μm.

Figure Expanded View 4. Aberrant activation of YAP/TAZ-mTORC1 signaling in the human lungs of idiopathic pulmonary fibrosis (IPF)

A Representative IF images showing the expressions of AT1 cell marker AQP5 and CAV1 in the airways of normal background (top), IPF (middle), and BO (bottom) human lungs. AQP5 (red), CAV1 (white), and DAPI (blue). Scale bars, 100μm.

B UMAP plot showing single cells from normal background (control, 5552) and pulmonary fibrosis (PF, 12477) of human lung tissues (GSE135893).

C UMAP plot representing 5 colour-coded cell clusters identified in merged single-cell transcriptomes from control and PF lungs.

D Blob plot depicting selected marker genes in each cell cluster. Dot size encodes the percentage of cells expressing the genes, and color encodes the average per cell gene expression level.

E Violin plots showing normalized expression levels of YAP/TAZ targets (*CYR61, AMOTL2, CTGF, GAS6*) and mTORC1-ATF4 targets (*ATF4, ATF3, PPPR15A, SLC3A2*).

F Representative H&E images of human airway from normal background or idiopathic pulmonary fibrosis (IPF). Scale bars, 100μm.

G Representative IF images showing the expression of YAP, ATF4, DATP marker CLDN4, AT1 cell marker AQP5, secretory cell marker CC10, and mTORC1 activity marker p-S6 in human lungs of IPF. YAP (green, top and bottom), ATF4 (white, bottom), CLDN4 (white, top), AQP5 (red, top and bottom), CC10 (green, middle), p-S6 (white, middle), and DAPI (blue). Scale bars, 100μm.

Figure Expanded View 5. Sustained YAP/TAZ-mTORC1-ATF4 signalling by chronic injury caused fate conversion of secretory cells into AT1 cells in the airways.

A Experimental schematic diagram of naphthalene-induced acute injury in *Scgb1a1-CreER$^{TM/+}$;R26R$^{tdTomato/+}$* mice post tamoxifen treatment.

B Representative IF images showing the expression of YAP and TAZ, secretory cell marker CC10, DATP cell marker CLDN4, mTORC1 activity marker p-S6 and ATF4 in *Scgb1a1* lineage-labeled tdTomato$^+$ cells in control and naphthalene treated lungs. YAP/TAZ (green, 1st panel), CC10 (green, 2nd and 4th panels), p-S6 (white, 2nd panel), CLDN4 (white, 3rd panel), ATF4 (white, 4th and 5th panels). Scale bars, 100μm.

C Experimental schematic diagram of repetitive naphthalene-induced chronic injury in *Scgb1a1-CreER$^{TM/+}$;R26R$^{tdTomato/+}$* mice post tamoxifen treatment.

D Representative IF images showing the expression of AT1 cell marker AGER and DATP cell marker CLDN4 in *Scgb1a1* lineage-labeled tdTomato$^+$ cells in control and naphthalene treated lungs. AGER (green), CLDN4 (white), and DAPI (blue). Scale bars, 100μm.

Table 1. The antibodies used for immunostaining are:

Antibody	Dilution	Sample prep	Company	Cat. No.
CC10	1:200	Frozen/FFPE	SantaCruz	sc-9772
CC10	1:100	Frozen/FFPE	SantaCruz	sc-365992
Acetylated-tubulin	1:1000	Frozen/FFPE	Sigma-Aldrich	T7451
p63	1:100	Frozen	Abcam	AB735
SPC	1:200	Frozen/FFPE	Millipore	AB3786
AGER	1:100	Frozen/FFPE	R&D Systems	MAB1179
CAVEOLIN-1	1:200	FFPE	Cell Signaling	3238
AQUAPORIN5	1:400	FFPE	Abcam	78486
T1α	1:300	Frozen	DSHB	8-1-1
CLAUDIN4	1:100	Frozen	Invitrogen	36-4800
tdTomato	1:500	Frozen/FFPE	Sicgen	AB8181-200
YAP	1:100	Frozen/FFPE	Novus	H00010413
TAZ	1:300	Frozen/FFPE	Sigma-Aldrich	HPA007415
Phospho-S6 (S235/236)	1:100	FFPE	Cell Signaling	2211S
ATF4	1:100	FFPE	Novus	NBP2-67766