Supplementary Discussion for “Allergic inflammatory memory in human respiratory epithelial progenitor cells” by Ordovas-Montanes et al., *Nature*, 2018

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I. Sampling the CRS spectrum through sinus tissue resections

Our single-cell RNA-seq (scRNA-seq) data spans tissue specimens ranging from mild chronic inflammation and no eosinophilia, through moderate eosinophilia (the primary histologic signature of T2I), to more severe eosinophilic rhinosinusitis, presenting an opportunity to identify cellular features that correlate with T2I inflammatory disease (chronic rhinosinusitis (CRS)) severity and gross anatomical features, such as the presence of polyps ($n_{Non\text{-}Polyp}$=6; n_{Polyp}=6; **Supplementary Table 1)**[1]. As no healthy individual had a clinical indication for sinus tissue resections during enrollment, and nasal brushings cannot fully recapitulate the nasal mucosal ecosystem given a bias towards apical cells[2], we initially focused on globally assessing the cell types/states that inform the spectrum of T2I-mediated CRS **(Fig. 1)** and its gross anatomical manifestations (e.g., polyps vs non-polyps**)**[1,3-5]. We later provide contextualization with healthy inferior turbinate and chronic rhinosinusitis CRS polyp brushings **(Fig. 2e-f)**.

II. Tissue dissociation and frequencies of cell types recovered

Identified clusters were present across all patients within the non-polyp and polyp groups **(Extended Data Fig. 1c,d; Supplementary Table 3)**. We used lists of cluster-specific genes to define epithelial, immune, and stromal cells[6] **(Fig. 1b,c; Extended Data Fig. 2a; Supplementary Table 3)**, and display lineage-defining genes to highlight the major cell types recovered: basal[7,8] (*KRT5*) and apical[7,8] (*KRT8*) to orient the major division present in pseudostratified epithelia of the respiratory tract, and further specialization present in ciliated[7,9] (*FOXJ1*) and glandular[10,11] (*LTF*) apical epithelium. We also highlight the supportive endothelial[12] (*DARC*), fibroblast[13] (*COL1A2*), plasma[14] (*CD79A*), myeloid[15,16] (*HLA-DRA*), T[17,18] (*TRBC2*), and mast cells[19] (*TPSAB1*) **(Extended Data Fig. 1e).** Despite the potential challenges associated with isolating single parenchymal and immune cells from tissues[20], we sequenced and annotated a highly reproducible distribution of cell types across patients **(Extended Data Fig. 1c,d)**. Collectively, we recovered abundant cell numbers for all expected cell types across each patient, yet note the exploratory nature of this study regarding specific disease endotypes[1,3,21,22] **(Extended Data Fig. 1d, Supplementary Table 3)**. Given the rarity of ILC2s in unfractionated tissue[23], we would not expect to detect them as a distinct cluster **(Methods)**. The frequency of eosinophils expressing *CLC* **(Extended Data Fig. 2b)** was lower than anticipated based on the literature[1] and matched clinical pathology reports. Since we measured

a robust eosinophil and T-helper 2 (Th2) signature in snap-frozen whole tissue nasal RNA-seq libraries from nasal polyps[1] **(Extended Data Fig. 2c-e)**, and they can be recovered by flow cytometry, our data suggest that eosinophil transcripts may be particularly susceptible to degradation during isolation, likely due to high levels of endogenous RNAses[24] **(Methods)**.

III. Myeloid, fibroblast, and endothelial cell type sub-clustering

For each cell type recovered, sub-clustering revealed further, potentially meaningful heterogeneity **(Extended Data Fig. 3)**. For example, within the myeloid compartment, we identify a small number of eosinophils (Myeloid-3), along with inflammatory macrophages (Myeloid-2) and two subsets of dendritic cells, expressing many of the marker genes of the recently described[25] 'Non-inflammatory DC2' (Myeloid-1) and 'Inflammatory DC3' (Myeloid-0) CD1c+ blood DCs, though the latter appear to lose expression of *CD1C* in tissue **(Extended Data Fig. 3a; Supplementary Table 3)**. Intriguingly, canonical genes associated with alternatively activated macrophages (*CD163, MRC1, MARCO, IL10, AREG*) are found distributed across macrophage and DC subsets co-expressing *TNF* and *IL1B*, highlighting the plasticity of these cells in tissues[26,27] and the importance of studying these states in their native tissue setting **(Extended Data Fig. 3a)**. Similar analyses of fibroblasts reveal a spectrum of activation ranging from preferential production of chemokines to growth factors[28,29] **(Extended Data Fig. 3b; Supplementary Table 3),** and that endothelial cells are largely divided in two distinct classes, one of which represents post-capillary venules specialized for leukocyte recruitment (Endothelial-0)[12,30] **(Extended Data Fig. 3c; Supplementary Table 3)**. While this manuscript focuses largely on epithelial cells, as well as selected interactions with immune and stromal cells, in order to comprehensively characterize basal cell hyperplasia, we expect that our data will serve as a useful reference to investigators with specific interests within each cell type, and will increase in value as other tissues are profiled across various inflammatory disease contexts. For example, it could be of interest to identify the tissue-specific nature of vascular beds specialized in the recruitment of immune cells to distinct organs[12,30].

IV. Cell-of-origin for chemokines, lipid mediators, and cytokines

Lymphoid and myeloid cells are recruited and positioned in tissues to facilitate effector functions during T2I through the action of chemokines and lipid mediators, but the cell of origin for these molecules can be obscured by bulk cellular analyses[22,31]. The eotaxins (*CCL26, CCL11,*

CCL24) are chemotactic for eosinophils, acting through CCR3[24]. In our scRNA-seq data, we detected each eotaxin in distinct sources, including basal cells (*CCL26*), fibroblasts (*CCL26* and *CCL11*), and myeloid cells (*CCL24*) **(Extended Data Fig. 4a)**. The mucosal cytokine *CXCL17*[32] was largely detected in apical and glandular cells, while *CCL28*, a chemokine involved in the positioning of IgA+ plasma cells in mucosal tissues[33], was specifically detected in glandular epithelium **(Extended Data Fig. 4a)**. This cell-type distributed (eotaxins) or restricted (*CCL28*) expression pattern of chemokines may help to clarify recurrent patterns observed in histological studies of human tissue, explaining the diffuse distribution of eosinophils or the preferential recruitment of plasma cells to glandular epithelium, respectively[5].

Lipid mediators, such as prostaglandins and leukotrienes, also play key roles in T2I[34]. We found mast cells specifically enriched for *HPGDS* and *PTGS2*, and *ALOX5,* suggesting they may be a dominant source of prostaglandin D2, implicated in activation of Th2 cells via *CRTH2* **(Extended Data Fig. 4a)**[35], and leukotriene biosynthesis within CRS, respectively. Myeloid cells were enriched for *TBXAS1*, responsible for thromboxane A2 biosynthesis, and basal, apical and ciliated epithelial cells were high expressers of *ALOX15*, the key enzyme in synthesizing a mediator found in high levels in T2I, 15-HETE[36], and recently reported to regulate ferroptotic cell death in airway epithelial cells[37] **(Extended Data Fig. 4a)**.

Notably, patients with or without polyps showed consistent cells-of-origin for the T2I-related chemokines, lipids, and cytokines, except for select mediators such as *CCL26* and *AREG*, which were more broadly detected in polyp tissue, and *PTGS2* (e.g. COX2), which was reduced in polyp epithelium **(Extended Data Fig. 4a,b)**. Intriguingly, the reduction in COX-2 from epithelial cells in polyps would help explain the high PGD2/PGE2 ratios associated with increasing disease severity, as mast cells primarily synthesize PGD2 while epithelial cells are capable of PGE2 synthesis[38].

Consistent with previous reports, we did not detect *IL25* in our system[39] **(Fig. 1d)**. *IL33* was present in basal cells, identifying their contribution to both upper and lower airway disease[40], but was also localized to apical and ciliated cells, highlighting important differences across disease states and tissues. However, the key instructive cytokine *TSLP* was uniquely restricted to basal cells, which may link increases in basal cell number to activation of effector cells associated with allergic inflammation potentially through polarization of DCs[41,42] **(Fig. 1d, Extended Data Fig. 3a, Extended Data Fig. 4b,c)**. Furthermore, amplifying cytokines (e.g. IL-1 family members)[3]

were either distributed throughout both epithelial and immune subsets (*IL18*), or limited to myeloid cells (*IL1B*) **(Fig. 1d; Extended Data Fig. 3a, Extended Data Fig. 4b)**.

V. T cell polarization in CRS

We identified a substantial T2I bias to the overall inflammatory ecosystem **(Fig. 1d; Extended Data Fig. 4c-e)**. All identified T cell sub-clusters scored evenly for a set of TCR complex genes **(Extended Data Fig. 4d; Methods),** and we could not detect robust signals for Type-1 or Type-17 inducer or effector cytokines across any cell subset **(Extended Data Fig. 4e).**

VI. Mapping GWAS risk genes

Of specific interest in the context of human diseased tissues is leveraging the literature on genome-wide association studies (GWAS) for allergic diseases[43]. While several risk genes have been identified, their links with phenotype have proven complex to unravel[44]. In many cases, the precise pattern of expression within a complex tissue has not formally been clarified due to technological limitations of bulk gene expression analyses. We noted that several risk genes (*IL13, IL33, TSLP*) mapped to a restricted set of cell types **(Fig. 1d; Extended Data Fig. 4b)**. Thus, we formally investigated the distribution of risk alleles throughout our cell types, identifying *GATA2* and *IL1RL1* (IL-33R) enriched in mast cells, and *CDHR3, KIF3A* and *TMEM232* specifically mapping to ciliated cells **(Extended Data Fig. 4f)**. While current GWAS literature describes the rhinovirus C receptor *CDHR3* as an epithelial-enriched gene, the fine-mapping of *CDHR3* to ciliated cells[45] could provide novel therapeutic angles such as supporting cilium motility in at-risk individuals[46]. Furthermore, enrichment of ubiquitously expressed genes such as *MYC* to basal cells indicates targeted investigation of MYC-related programs specifically within this cell type during chronic allergic inflammation[47] **(Extended Data Fig. 4f)**.

VII. Classification of cell types across non-polyp and polyp samples

Marker gene analysis across epithelial cell types (e.g. basal vs. differentiating/secretory vs. glandular vs. ciliated) identified conserved programs present in the three clusters with a basal phenotype (*TP63, KRT5,* and high basal cell score[48]), the three with a differentiating/secretory phenotype (*KRT8, SERPINB3, SCGB1A1,* and baseline basal cell score[48]), the two with a

glandular phenotype (*LTF, TCN1, LYZ*), and the one with a ciliated phenotype (*CAPS, OMG, FOXJ1, PIFO*; **Fig. 2a,b; Extended Data Fig. 5a-d; Supplementary Table 3)**[7,49].

Marker gene analysis within each epithelial cell type (e.g. 12 vs. 8 vs. 2 only) revealed further granularity associated with the presence or absence of polyps **(Fig. 2c; Extended Data Fig. 5e; Supplementary Table 3)**. Within basal cells and differentiating/secretory cells, we observed that canonical Type 2 cytokine induced genes (*POSTN*[50] and *ALOX15*) amongst others **(Supplementary Table 3)**, drive clustering, suggesting that the separations we observe may be mediated by differences in the sensing or impact of cytokines between disease states **(Fig. 2c; Extended Data Fig. 5e)**. Importantly, a canonical correlation analysis (CCA)[51], performed across non-polyp and polyp samples, returned similar basal and differentiating/secretory cluster groupings **(Extended Data Fig. 5c).**

VIII. Gene expression changes specific to secretory cells

In non-polyp epithelium, we detected robust expression of antimicrobials (*MSMB, SCGB1A1, STEAP4, PSCA*, and *LYPD2*) which were diminished in polyp epithelium **(Fig. 2d)**. In contrast, secretory cells recovered from polyps expressed *CST4* and *CST1,* associated with inhibition of protease activity[52], and *IGFBP3, TFF3*, and *EGLN3,* indicating attempts at curtailing tissue growth[53] and promoting tissue tolerance and repair[3,54,55] **(Fig. 2d)**. Thus, secretory cells from polyps appear to supplant antimicrobial function with tissue-repair.

IX. Goblet and glandular cell subsets, and their relationship to secretory cells

The two predominant secreted mucins of the upper respiratory tract are MUC5B and MUC5AC, which differ in their biophysical properties and potential to mediate microbe adhesion[56]. Although the production of both has been commonly ascribed to a set of goblet cells in human airway[49], we observed that expression of *MUC5B* is restricted to glandular mucus cells (cluster 13) in nasal mucosa, which do not express *MUC5AC* ($r=-0.0375$; $p=0.4952$, corrected Holm's method**; Fig. 2b; Extended Data Fig. 5f)**. Instead, *MUC5AC* is expressed in a subset of secretory cells (clusters 0 and 4) co-expressing *SCGB1A1* and *FOXA3* (*MUC5B* vs *AZGP1*: $r=0.491$, *MUC5AC* vs *AZGP1*: $r=-0.084$, *MUC5AC* vs *SCGB1A1*: $r=0.184$, *MUC5AC* vs *FOXA3*: $r=0.181$; $p<0.0001$, corrected Holm's method**; Extended Data Fig. 5f,g)**. This suggests that the

goblet cell program is layered atop a secretory cell base[57-61], and that glandular mucus cells are the predominant source of *MUC5B*[62]. Expression of *SPDEF*, a putative goblet cell transcription factor[49], was shared amongst mucin producing cells, but relatively enriched in *MUC5AC* expressing cells (SPDEF vs MUC5B: r=0.107, SPDEF vs MUC5AC: r=0.213; p<0.0001, corrected Holm's method; **Extended Data Fig. 5f,g)**. Crucially, MUC5AC and MUC5B are not functionally interchangeable—in murine models, MUC5B, but not MUC5AC, is essential for maintaining immune homeostasis and controlling infections of the upper airway, primarily through promoting mucocilliary clearance[63]—suggesting imbalances among these cell types could have profound effects on host defense.

Having identified the restricted expression of *MUC5B* to a subset of glandular cells, we more formally assessed the heterogeneity present within all glandular cells[64] (clusters 3 and 13, **Fig. 2a)**. We isolated all glandular cells within our epithelial cells **(Extended Data Fig. 6a)** and re-clustered them independently, finding five main clusters **(Extended Data Fig. 6b; Supplementary Table 3)**. Importantly, each cluster had cells from all non-polyp samples, suggesting that these are not patient-specific subsets (data not shown). All clusters shared expression of *TCN1*, and the majority expressed *LTF*, but expression of *LCN2, SERPINB3, MUC5B* and *BPIFB2*, and *PRB1* were restricted to specific subsets[65,66] **(Extended Data Fig. 6b; Supplementary Table 3)**. This expression from restricted subsets may represent a regulatory mechanism for controlling the production and release of certain antimicrobial factors and secretory proteins without inducing global release of gene products[67,68]. No comparison between polyp and non-polyp glandular and ciliated cells was possible as these clusters were nearly absent from polyp ecosystems **(Fig. 2c; Extended Data Fig. 6; Supplementary Table 3)**.

X. Epithelial and immune cells from healthy and diseased nasal scrapings

Comparisons of nasal scraping tSNE plots colored by location/diagnosis and by cell type reveal a striking separation between the epithelial cells from the inferior turbinates and from polyps, yet a remarkable similarity in the inferior turbinate of healthy controls and individuals with polyps **(Extended Data Fig. 6c,d)**, underscoring the importance of local cues in driving disease at barrier sites.

We recovered T cells, neutrophils, and myeloid cells from both anatomical locations, while mast cells and eosinophils were exclusively found in polyp epithelium **(Extended Data Fig. 6d,e; Supplementary Table 3)**. We display genes of interest enriched within each immune cell type **(Extended Data Fig. 6d,e)**, and, as myeloid cells were sampled evenly across all three sites, take the opportunity to identify genes preferentially expressed within each region sampled **(Extended Data Fig. 6f)**. From healthy inferior turbinate, cells expressed *TXNRD1* and *RALA* involved in an anti-inflammatory macrophage phenotype[69] and phagocytic function[70]. From polyp inferior turbinate cells expressed *TLR2* and *RIPK2*, involved in microbial sensing[71]. And from polyp tissue, cells expressed *C1QA* and *FGL2* indicative of the pro-inflammatory environment[72] **(Extended Data Fig. 6f)**.

XI. Secretory cells from nasal scrapings

To identify conserved gene modules in secretory cells, as well as shifts in cell states across anatomical regions in health and disease, we combined all epithelial cells recovered from surgical resections of non-polyp and polyp ethmoid sinus tissue **(Fig. 2a-c)** with scrapings from healthy inferior turbinate, polyp inferior turbinate, and accessible polyp tissue **(Fig. 2e)**. Importantly, glandular cells continued to cluster separately, and we did not recover any cells scoring for the glandular marker gene set **(Fig. 2b; Supplementary Table 3)** through scraping. This suggests that we correctly classified glandular epithelium in our original data set and in iterative clustering **(Extended Data Fig. 6)**. Using marker discovery for secretory cells across all samples led us to identify a conserved core gene set found across secretory cells from all diseases and sites sampled[73-75], including *WFDC2, VMO1, TSPAN1, AGR2, S100A6, PIGR,* and *CD55* (Core: **Fig. 2e,f, Supplementary Table 3)**. Extracting all secretory cells across the four categories and performing marker discovery, we found a set of genes (*S100A8/A9, MUC4, ANKRD36C, LCN2*, and others)[65,76,77] shared across healthy and polyp inferior turbinate (Healthy: **Fig. 2f, Supplementary Table 3)**, with only slightly elevated expression of some markers in inferior turbinate from patients with polyps. By relating non-polyp and polyp ethmoid sinus secretory cells to healthy inferior turbinate secretory cells, we find a significant decrease in *S100A8/A9, MUC4, ANKRD36C,* and *MUC1* highlighting a conserved alteration in secretory cell state in CRS tissue (CRS: **Fig. 2f;** inferior turbinate vs. ethmoid sinus $p<5.43x10^{-186}$ or less with Bonferroni correction for multiple comparisons). Nevertheless, in non-polyp secretory cells relative to all others, we find an increase in *SCGB1A1, UGT2A2, SLPI, S100A13,* and

RARRES1, suggesting that non-polyp secretory cells are altered in the typical production seen in healthy and polyp inferior turbinate tissues to yield a distinct cell state[78-80] (CRS non-polyp: **Fig. 2f)**. Additionally, there is an overall loss of antimicrobial effector programs in polyp secretory cells, whether compared to non-polyp secretory cells from the ethmoid sinus **(Fig. 2d, Fig. 2f)**, or either condition in the inferior turbinate (CRS polyp: **Fig. 2f**). Using gene lists derived from cytokine-treated air-liquid interface (ALI) cultures **(Methods; Supplementary Table 4)**, we find that IFNα- and IFNγ-induced genes[81] are enriched in healthy inferior turbinate, and there is a progressive loss of these signatures through CRS tissue accompanied by a large increase in IL-4/IL-13 induced genes[81] such as *CST1*, *ALOX15*, and *SERPINB3*, in polyp secretory cells (CRS polyp: **Fig 2f,g)**. We conclude that secretory cells from involved CRS tissue of the ethmoid sinus differ significantly from the inferior turbinate, and that secretory cells in non-polyp and polyp tissues reach distinct states whose altered functionality may be linked to disease trajectory and severity.

XII. Ecological diversity of epithelial cells and the overall tissue ecosystem

To test if our findings on reduced ecological diversity in nasal polyp epithelia **(Fig. 3b)** were driven by biases introduced in cell type curation, we calculated the number of clusters present within epithelial cells from each patient independently and calculated Simpson's index of diversity for each, obtaining consistent results **(Extended Data Fig. 7c,d)**.

Intriguingly, when calculating Simpson's index of diversity across stromal and immune cells alone (e.g. fibroblast, endothelium, immune), or the entire cellular ecosystem for each patient, we observed a reduced index of diversity in polyps for stromal and immune cells, but an overall increase across all cell types **(Extended Data Fig. 7f)**. Given the relative distribution of cell types present **(Extended Data Fig. 7a)**, and how evenness factors into this equation, we speculate that the immune cells in polyps may represent an overcorrection in attempting to restore balance to the epithelial compartment **(Extended Data Fig. 7f)**.

XIII. Bulk RNA-seq deconvolution of epithelial cell types across disease

We identified four clusters of patients (K-nearest neighbors (KNN) on a principal components analysis (PCA)**; Fig. 3f,g; Methods)**: a non-polyp cluster enriched in secretory and glandular signatures (grey) and then three increasingly polyp-enriched clusters that showed more pronounced basal and ciliated cell programs (cyan), then lost glandular ones (lilac), and eventually, in the most severe cases, lost core ciliated genes (coral, as determined by number of previous surgeries, time to polyp regrowth, and eosinophilia; **Fig. 3g; Supplementary Table 1, Supplementary Table 3, Methods)**. Within polyps, we also confirmed upregulation of the tissue reparative program observed in **Fig. 2d,f** for differentiating/secretory cells **(Fig. 3g)**.

To address if basal cell hyperplasia, in addition to loss of secretory cell function **(Fig. 2 and 3)**, characterizes deviations from healthy tissue, as well as from the CRS non-polyp disease state, we used two publicly-available RNA-seq datasets containing normal human sinus mucosal biopsies, and non-eosinophilic and eosinophilic nasal polyps[1,82,83]. Re-analyzing each sample for the fraction of basal or secretory cell markers present amongst genes representative of those two lineages, we identify a significantly increased basal and decreased secretory cell fraction in polyp tissues relative to healthy controls **(Extended Data Fig. 7k,l; Supplementary Table 3; Methods)**. This mirrors the findings from our cohorts between non-polyp and polyp tissues, highlighting that the changes in cellular composition we observe also typify the divergence between the healthy and polyp states.

XIV. Changes in basal cell gene expression in polyps

We next sought to understand what mechanisms account for decreased epithelial diversity in polyps. By comparing the transcriptomes of basal cells[7,48] from non-polyp or polyp ecosystems **(Fig. 4a)**, we identified elevated polyp expression of a set of transcripts—including *POSTN, PTHLH, ALOX15, SERPINB2, HS3ST1, CDH26, MMP10* and *CCL26*—involved in extracellular matrix remodeling and chemo-attraction of effector cells[50,84], along with a decrease in protease inhibitor expression (*SPINK5*)[85] and metabolic genes (*ALDH3A1, CLCA4, GLUL*) **(Fig. 4a).** As several of these genes are known to be IL-4/IL-13 responsive[81], and IL-4/IL-13 targets were enriched in ethmoid sinus secretory cells **(Fig. 2g)**, we looked more globally at gene sets induced by these cytokines.

XV. Fibroblast alterations that correlate with basal cell hyperplasia

To contextualize our basal cell findings related to extracellular matrix remodeling within their larger cellular ecosystems, we asked whether cells which compose the basal cell niche, such as fibroblasts[28], were altered in polyps potentially contributing to basal cell dysfunction. Thus, we looked at averaged gene-expression values in single-cell fibroblasts whose expression correlated with basal cell frequency **(Extended Data Fig. 7b, Extended Data Fig. 8c;** all genes: abs(r)>0.7651, p<0.0037**)**. Clustering over fibroblast genes that either positively or negatively correlated with basal cell frequency in our single-cell data identified a polyp-enriched gene module in fibroblasts associated with *ITGA8*, a hallmark gene of these cells during development of new airways but also fibrosis **(Extended Data Fig. 8d)**[86,87]. We also identify significant changes in gene expression within myeloid and endothelial cells **(Fig. 1b, Extended Data Fig. 8e,f)** by non-polyp and polyp disease state suggesting further alterations to the tissue microenvironment. We note that despite gene expression changes within all cell types recovered by disease state, the most striking changes we observe comparing across disease within specific cell types were those seen in epithelial cells **(Fig. 2c)**. Taken together, our data suggest that basal cells harbor an imprint of IL-4/IL-13 signaling, are found amongst an altered niche constituent, and a broadly altered microenvironment.

XVI. Genes correlated with changes in pseudotime differentiation trajectory

Ordering the cells according to this common axis, we examined our full gene expression matrix in order to identify which genes may become dysregulated in polyps during differentiation **(Extended Data Fig. 9b; Supplementary Table 3)**. We found that: 1) DLK2, DLL1, JAG2, DKK3 (mediators of Wnt and Notch signaling, **Fig. 4c**) and 2) POSTN, FN1, TNC (extracellular matrix components, FN1 and TNC are ligands for ITGA8, **Extended Data Fig. 8d)** were both significantly negatively correlated with pseudotime in non-polyps and had altered correlations in polyps (based on abs(Fisher's Z) > 3.8; **Extended Data Fig. 9b, Supplementary Table 3** for full list and statistics). This suggests important contributions of cell-extrinsic developmental pathways and matrix-interacting receptors in regulating basal cell fate.

XVII. Epigenetic profiling via Omni-ATAC-seq

As our data highlighted an impairment in differentiation of basal cells in polyp tissue, we next sorted basal cells **(Extended Data Fig. 7h)** from 4 non-polyp and 7 polyp tissues (3 non-polyp and 7 polyp retained through data quality filtering, reflecting sample size in main text) and performed Omni-assay for transposase accessible chromatin (ATAC)-seq to identify intrinsic epigenetic changes from the integration of extrinsic cellular signaling events[88,89] **(Methods)**. Chromatin can exist in several states within stem cells[90]—including a poised state[91]—providing a form of epigenetic memory[92,93] that may influence the propensity to differentiate towards particular cell fates. Among our data, we identified that polyp basal cells had an enrichment in peaks, indicative of more accessible chromatin **(Methods)**, for bZIP transcription factor target motifs[94], including various AP-1 family members[95,96] such as JUN, FOXA1[97], ATF3[98], KLF5[99] and p63[100] itself **(NB:** *TP63* and *JUNB* were significantly negatively correlated with a productive differentiation trajectory in non-polyps, with a deviation observed in polyps **(Fig. 4f; Extended Data Fig. 9b-f; Supplementary Table 5)**. These proteins have previously been associated with the maintenance of an undifferentiated state, chromatin opening, and oncogenesis[101]. Conversely, accessible motifs in non-polyp basal cells were enriched for Sox10, Sox4, and Sox2 (implicated in stemness, early progenitor cell differentiation programs, and lung branching morphogenesis[102]), as well as STAT6 involved in allergic inflammation[103], and Mef2-family transcription factors, which bias towards specialized cell types within tissues[104] **(Fig. 4f; Supplementary Table 5)**. Clustering of enriched motifs revealed Sox2/Sox4 and Sox3/Sox6/Sox10 modules in non-polyp tissue **(Extended Data Fig. 9c,d)**. Furthermore, in polyp basal cells, p63 shows reduced correlation with the Sox and AP-1 modules, suggesting that higher order changes in chromatin structure may affect the activity of this defining basal cell transcription factor in severe disease[90] **(Extended Data Fig. 9c-f)**. As p63 is essential for the proliferative potential of stem cells in stratified epithelia, yet largely dispensable for commitment and differentiation during development[105], it will be of interest to further understand the contextual activity of p63 in distinct types of tissue remodeling.

XVIII. Transcription factors in basal cell RNA-seq data and relation to Omni-ATAC-seq

Motif enrichment analysis of accessible chromatin illustrates the potential for activity of closely related transcription factors[94]. To refine which putative factors may be responsible for the

differences we observed, we confirmed and extended our Omni-ATAC-seq findings by performing low-input bulk RNA-seq on sorted basal cells from these same patients to further refine putative transcription factors. In non-polyp basal cells, *MEF2A, MEF2C, SOX10, STAT5B,* and *THRB* are significantly upregulated transcription factors corresponding with enriched motifs **(Fig. 4f,g; Supplementary Table 5).** In polyp basal cells, we find increased expression of *TP63, ATF3, KLF4, KLF5, FOSL1,* and *FOXA1* **(Figure 4f,g; Supplementary Table 5).** Intriguingly, transcription factors such as *ATF3* and *KLF5* showed increased accessibility in the promoters and regions adjacent to the genes themselves, raising the possibility that the transcription factor networks associated with basal cell hyperplasia could persist in the absence of inflammatory triggers, or be recalled more rapidly in their presence **(Extended Data Fig. 8e,f)**. Taken together, these basal cell gene signature, differentiation trajectory analyses and epigenetic studies led us to predict that during chronic T2I, basal cell differentiation is intrinsically impaired through the influence of extrinsic cues (e.g., IL-4/IL-13 and Wnt pathway), and hypothesize that the intrinsic persistence of such aberrant signaling may contribute to failed basal cell differentiation. This results in a barrier with reduced cellular and functional diversity, akin to an ecosystem with compromised biodiversity, and consequently an overall reduction in tissue health[106].

XIX. Air-liquid interface cultures to model basal cell differentiation

After 21 days of differentiation from basal cells, these air-liquid interface (ALI) cultures yield a differentiated and pseudostratified epithelium. We applied our epithelial deconvolution gene lists **(Supplementary Table 3; Methods)** to relate the cell types generated in ALI cultures to *in vivo*. We recovered four main cell types including basal, secretory, ciliated, and a hybrid cell type expressing canonical transcription factors of both secretory (*SPDEF*[107]) and ciliated (*FOXJ1*[9]) cells **(Fig. 5a; Extended Data Fig. 9g; Supplementary Table 3)**. We note a bias in ALI cultures for producing a larger fraction of ciliated cells than what we recover *in vivo* (compare **Fig. 3a)**, and the appearance of hybrid ciliated and secretory cells, suggesting a strong push towards terminal differentiation[7,61]. As we had previously noted striking differences in the cell states of secretory cells from polyps relative to other tissues **(Fig. 2d,f)**, we assessed the expression of *in vivo* secretory gene sets **(Extended Data Fig. 9h)** in these ALI cultures, noting recovery of gene expression present in healthy inferior turbinate (*S100A9, MUC4*) and non-polyp sinus tissue (*PSCA, SCGB1A1*), with a complete absence of genes highly expressed in polyp secretory cells *in vivo* (*CST1, POSTN*) **(Fig. 5b; Extended Data Fig. 9h)**. Intriguingly,

genes such as *S100A9* and *MUC4* have increased chromatin accessibility in basal cells from polyps relative to non-polyps **(Extended Data Fig. 9f)**, suggesting that if basal cells could be "released" from their trapped state, the expression of these gene products might occur *in vivo*.

As canonical IL-13 induced genes[81,108] (*CST1* and *POSTN*) were not detected in ALI cultures, we hypothesized that the addition of IL-13 might reveal a defect in basal cell differentiation. We tested this by addition of a range of IL-13 concentrations to non-polyp or polyp-derived ALI cultures, and performed flow cytometry as in **(Fig. 3c; Extended Data Fig. 7h)** to assess the relative ratio of basal to differentiated epithelial cells **(Extended Data Fig. 9i)**. While the addition of IL-13 did increase the ratio of basal to differentiated cells (*$p<0.0224$, 2-way ANOVA of IL-13 dosage; **Methods)**, it did not act to preferentially inhibit differentiation in polyp-derived cultures at any of the doses tested **(Fig. 9i)**. Taken together, these data suggest that basal cells from polyps, if provided with strong and sustained extrinsic cues, have the potential to differentiate towards a mixed-tissue secretory cell phenotype observed *in vivo* even in the presence of IL-13 **(Extended Data Fig. 9h,i)**. It will be important to formally assess whether relevant clinical features of barrier tissue diseases are maintained *ex vivo* in relevant organoid systems[109].

XX. Wnt pathway in allergic inflammatory memory and basal cell hyperplasia

We also highlight the increased expression of *CTGF* in polyp basal cells at baseline and across several doses of cytokine, as this growth factor is both a Wnt/β-catenin-regulated gene and an extracellular regulator of the pathway[21,110] **(Fig. 5d; Extended Data Fig. 9j)**. Intriguingly, a recent study suggested that exogenous Wnt provided to ALI cultures led to morphological changes in cells resembling basal cell hyperplasia[111]. Furthermore, the Wnt pathway is a key regulator of epithelial-mesenchymal transition[112] (EMT), which has been identified as a key element of barrier dysfunction in nasal polyps and other type 2 inflammatory diseases[113].

XXI. In vivo IL-4Rα blockade and effects on basal and secretory cells

While the distinct nature of the sampling methods and limited sample size preclude a formal analysis of basal to differentiated cell ratios, we leveraged the power of our scRNA-seq data to identify clusters of the same cell type in order to look within relatively homogenous populations for treatment-induced changes in gene expression **(Fig. 5e; Extended Data Fig. 10a,b**; **NB**

given the unique nature of our *in vivo* validation, we limit our reporting of data to differentially expressed genes which were previously contextualized in the manuscript through *in vivo* and/or *ex vivo* analyses, with a focus on basal and secretory cells). To compare changes in gene expression after IL-4/IL-13 receptor blockade, we identified the cluster with the highest basal cell score **(Extended Data Fig. 10a,b; Supplementary Table 3)**. This cluster contained cells from both the pre-dupilumab scraping and the post-dupilumab surgery **(Fig. 5e, Supplementary Table 3)**.

Contextualizing these findings within our previous data, we identify several key gene sets, including a conserved core set of basal cell genes unchanged by treatment (*TP63, CLDN1, ITGA2*) **(Extended Data Fig. 10d)**. Intriguingly, transcription factors upregulated in polyp basal cells identified through Omni-ATAC-seq and low-input RNA-seq (*ATF3, KLF5, FOSB*) were significantly decreased by treatment[96,98,99,114] **(Fig. 5g)**. While Wnt pathway target gene expression was globally reduced (Wnt pathway gene set, *ID2*), *CTNNB1* expression was notably retained **(Fig. 5g),** as might be predicted its persistence observed in our *in vitro* data. However, several genes upregulated at baseline in *ex vivo* polyp basal cells were significantly decreased, including *CTGF* and *SERPINE1* **(Extended Data Fig. 10d)**. Based on our previous analysis of non-polyp vs. polyp basal cells in vivo, we had identified *POSTN, NTRK2* and *HS3ST1*, amongst others, as significantly upregulated in polyps whereas here we note conserved expression of *POSTN* and *NTRK2*, with diminished expression of *HS3ST1* **(Fig. 5g)**. Lastly, we report that for genes that were upregulated both *in vitro* and *in vivo* in polyp basal cells (*TNC, SOX4, PTHLH*), none were significantly decreased by IL-4/IL-13 blockade **(Extended Data Fig. 10d)**, suggesting that some genes in this patient, and at this timepoint sampled, persist.

After identifying differentiating/secretory cells related to those found in **(Fig. 2d)** within inferior turbinate and polyp scrapings **(Extended Data Fig. 10e,f; Methods)**, we tested for candidate pathways of interest, observing in polyps a specific decrease in Wnt-target genes (Wnt pathway gene set, *CD44*), a significant decrease in IL-4/IL-13 induced genes (IL-4/IL-13 gene set, *ALOX15*), and a concomitant increase in IFNα induced genes (IFNα gene set, *IFI27*) **(Extended Data Fig. 10g)**. We utilized our healthy and diseased secretory cell gene sets **(Fig. 2f; Supplementary Table 3)** to identify a core gene set conserved with treatment (*WFDC2, S100A6, PIGR*). Pairwise differential expression tests on samples grouped by anatomical location and treatment identified sets of genes expressed in healthy inferior turbinate restored in

polyp secretory cells (*S100A9, LCN2, MUC1*) and genes restored in both inferior turbinate and polyp secretory cells (*ANKRD36C, MUC4, RARRES1*; **Extended Data Fig. 10h)**. Furthermore, we observed a significant decrease in the expression of non-polyp secretory genes (*TPT1, SCGB1A1, DUSP1*), polyp secretory cell genes (*CST1, TFF3, EGLN3*), and the transcription factors *ATF3, KLF5,* and *FOSB* **(Extended Data Fig. 10h)**.

Further investigation will be required to formally test whether these changes in gene expression of secretory cells represent the natural potential of these cells in a non-inflammatory EthSin environment **(Fig. 5a)**. Our exploration of the shifts induced by IL-4Rα blockade within the basal and secretory cells of an individual requires further examination. However, it highlights the potential of single-cell approaches to deconvolve tissues, allowing for *in vivo* validation of *in vitro* mechanisms in humans relating to shifts in specific cell states and differentiation potential of accessible barrier tissues.

XXII. References

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