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IL1A promotes inflammation while safeguarding epithelial basal cell identity and barrier function

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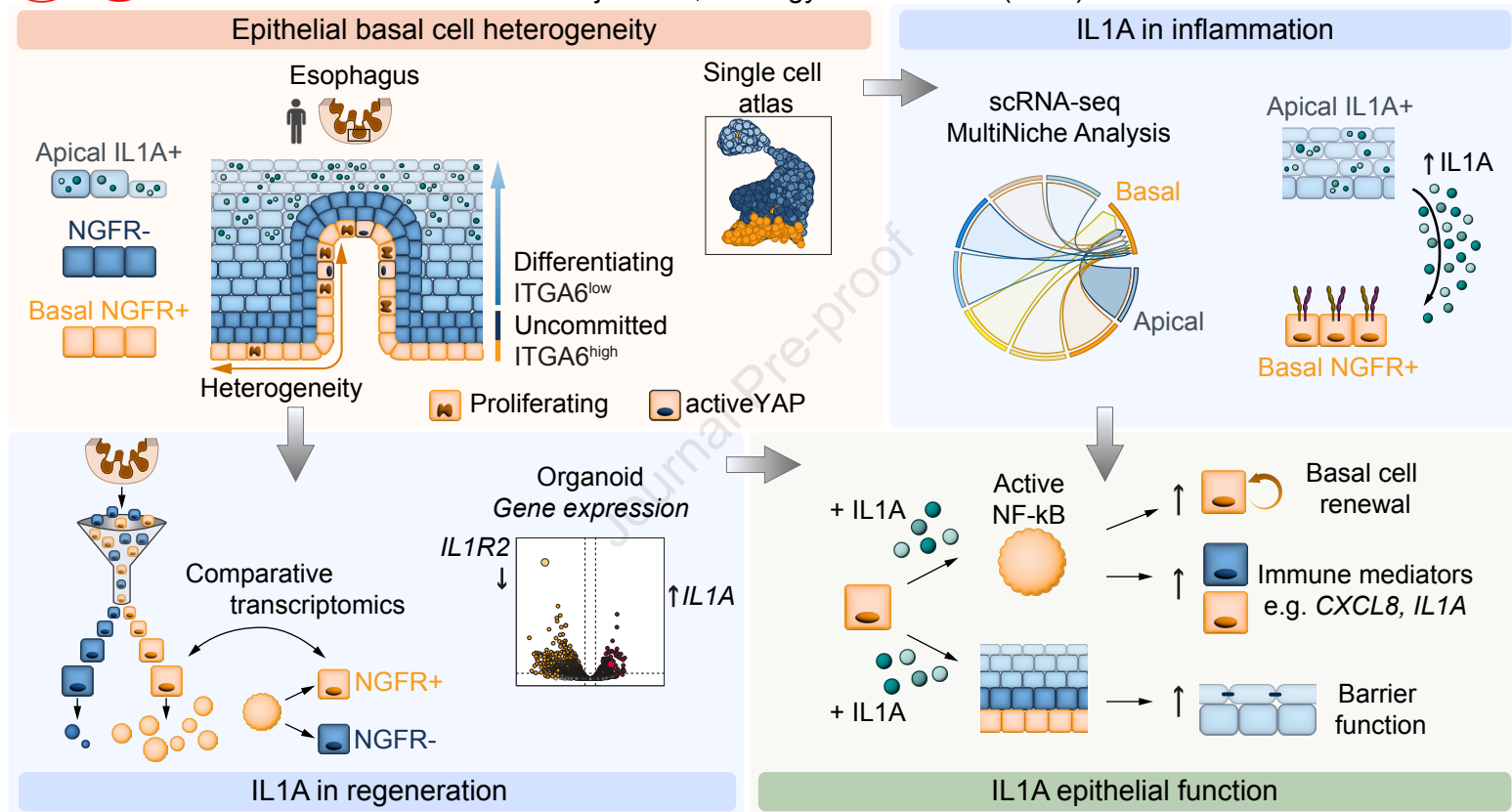
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**Abbreviations:** ITGA6: Integrin alpha 6, YAP: Yes-associated protein 1, IL1A: Interleukin 1 Alpha, IL1R2: Interleukin 1 Receptor 2, NGFR: Nerve Growth Factor Receptor, CXCL8: Chemokine (C-X-C motif) ligand 8, NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B cells



**IL1A promotes inflammation  
while safeguarding epithelial basal cell identity and barrier function**

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**Conflict of Interest Statement**

The authors declare that they have no conflict of interest.

**Abstract****Background**

The ability of barrier epithelia to sense environmental perturbations and initiate local inflammation is essential for progenitor-mediated epithelial repair and restoration of tissue integrity.

**Objective**

To characterize human esophageal epithelial progenitor cell heterogeneity and identify molecular mechanisms regulating epithelial regeneration in eosinophilic esophagitis (EoE).

**Methods**

Single-cell RNA sequencing of the adult human esophagus was integrated with spatial characterization of epithelial cell populations and functional analyses using primary human esophageal organoids and air-liquid interface cultures.

**Results**

Using NGFR and P73, we defined a layer of human basal progenitor cells spanning both papillary and interpapillary regions of the human esophageal epithelium. Proliferating basal progenitor cells were enriched within the papillary epithelium, and a subset of papillary basal progenitor cells displayed active YAP signaling, revealing spatially organized heterogeneity within the progenitor compartment. NGFR-positive basal cells exhibited enhanced organoid-forming capacity and suppressed NOTCH signaling to maintain basal identity. During epithelial regeneration, IL1A was induced in primary human organoids, and an intraepithelial apical-to-basal IL1A signaling axis was identified in patients with EoE. IL1A activated NF- $\kappa$ B-dependent transcription, promoted basal progenitor expansion, and enhanced epithelial barrier function. Although IL13 selectively rewired IL1A-dependent transcriptional and regenerative programs, IL1A signaling remained active under inflammatory conditions.

**Conclusion**

These findings demonstrate that IL1A coordinates epithelial inflammatory and regenerative responses in the human esophagus. They further show that the outcome of IL1A signaling is determined by the surrounding inflammatory milieu, providing a framework for understanding epithelial remodeling in eosinophilic esophagitis.

### Key Messages

- Spatially distinct basal progenitor niches define functional heterogeneity within the human esophageal epithelium.
- An intraepithelial IL1A signaling axis links epithelial injury to progenitor expansion and barrier maintenance.
- IL13 selectively modifies IL1A-dependent epithelial programs in eosinophilic esophagitis.

### Capsule Summary

Spatially organized human esophageal basal progenitors respond to IL1A by renewing and supporting epithelial barrier function. These IL1A-dependent epithelial responses are selectively rewired by IL13 in eosinophilic esophagitis.

**Keywords:** Basal cell heterogeneity, Esophagus, Epithelium, Inflammation, IL1A

### Abbreviations

DEGs (Differentially Expressed Genes)

EoE (Eosinophilic Esophagitis)

EPC2 (Esophageal Primary Cell, clone 2)

GSEA (Gene Set Enrichment Analysis)

IL1A (Interleukin 1A)

NGFR (Nerve Growth Factor Receptor)

NF- $\kappa$ B (Nuclear Factor kappa-light-chain-enhancer of activated B cells)

P73 (Tumor protein 73)

PDPN (Podoplanin)

UMAP (Uniform Manifold Approximation and Projection)

## Introduction

Barrier epithelia provide the primary interface between the body and the external environment and must continuously balance host defense with preservation of tissue integrity. Following injury, epithelial and immune cells coordinate inflammatory responses to eliminate harmful stimuli and activate progenitor cells to restore tissue architecture and barrier function (1). The mechanisms that integrate inflammatory signaling with epithelial repair remain incompletely understood.

The human esophageal epithelium is architecturally distinct from that of commonly used experimental models such as the mouse (2), limiting our understanding of the mechanisms governing human epithelial homeostasis. The stratified epithelium is organized into papillary and interpapillary regions that create specialized epithelial-stromal interfaces (2). Although papillae are a defining feature of the human esophagus, whether they establish spatially distinct progenitor niches with unique functional properties remains unknown. Understanding how epithelial architecture influences progenitor behavior is therefore fundamental to defining mechanisms of tissue maintenance and repair.

Chronic inflammation disrupts the balance between tissue repair and inflammatory activation. In the esophagus, food antigen-driven eosinophilic esophagitis (EoE) is characterized by epithelial remodeling, basal cell hyperplasia, fibrosis, and barrier dysfunction (3, 4). While inflammatory mediators are produced by infiltrating immune cells, epithelial cells also orchestrate inflammatory responses through cytokines, including members of the IL1 family (5). Although IL1 cytokines are classically regarded as epithelial alarmins that are rapidly released following tissue damage to activate innate immunity (6), they also regulate epithelial proliferation, differentiation, and tissue repair in multiple barrier epithelia (6-10). Whether epithelial IL1 signaling regulates progenitor cell behavior in the human esophagus, and how these responses are influenced by the type 2 inflammatory environment of EoE, remains unknown.

## Materials and Methods

### Human ethical statement and considerations

Human esophageal tissue was collected at the Karolinska University Hospital (Stockholm, Sweden) under the ethical approvals 2018/1711-3 and 2021-01574 (Regionala Etikprövningsnämnden Stockholm). Esophagi used for organoid culture and immunofluorescence were obtained from patients undergoing esophagectomy for esophageal cancer. Tissue was collected at least 5cm proximal to the tumor and considered healthy, cancer-free mucosa. EoE biopsies were obtained from patients undergoing diagnostic endoscopy. Samples were blinded with respect to age and sex; esophagectomy samples were additionally blinded with respect to previous treatments. Sex was not considered a biological variable. Sample use and available treatment information are provided in Supplemental Table 8.

### Esophageal tissue processing

The epithelium was separated from the stroma macroscopically and a small part of epithelium was fixed overnight at 4°C in 4% paraformaldehyde solution (V/V, PFA, Sigma, #252549), embedded in OCT Embedding Matrix (VWR, #361603E) and sectioned at 10µm to be used for immunofluorescence analysis.

The residual tissue was cut into smaller pieces, immersed in 0.25mg/ml Liberase<sup>TM</sup> (Sigma-Aldrich, #5401127001), 0.25mg/mL DNase I (Sigma-Aldrich, #11284932001) and 2U/mL Dispase (Corning<sup>TM</sup>, #354235) in Hank's Balanced Salt Solution (HBSS, Gibco, #14175-129), incubated on a shaker for 30 minutes at 37°C, while resuspending the solution every 10 minutes. The cell suspension was spun down at 400g for 10 minutes and resuspended in 0.25% Trypsin-EDTA (Thermo Fisher Scientific, #25200056) before being incubated on a shaker at 37°C for 20 minutes. Cells were resuspended after 10 minutes. Twice the volume of 2.5mg/ mL Soybean Trypsin Inhibitor (SBTI; Sigma Aldrich #T9128) was added and the cell suspension was filtered through a 40µm Nylon strainer (Corning, #431750). Cells were spun down at 400g for 10 minutes then either mixed with Basement Membrane Extract 2

(BME2, R&D Systems, #3533-001-02) to generate organoids, collected for 2D culture or processed directly for Fluorescence-Activated Cell Sorting (FACS).

### **Fluorescence-Activated Cell Sorting (FACS)**

Single-cell suspensions were derived from fresh human esophagi (above) or from organoids. Organoids were retrieved by resuspending the BME2 domes in ice-cold PBS (Gibco, #14190250). Organoids were then centrifuged at 400g for 5 minutes and dissociated to single cells using 0.25% Trypsin-EDTA for 20-30 minutes at 37°C, with resuspension of the mix every 10 minutes. Trypsinization was neutralized using twice the volume SBTI (Sigma Aldrich #T9128), and the cell suspension was filtered through a 40µm pipet tip strainer (FlowMi® Cell Strainer, #BAH136800040) to remove cell clumps and achieve single cell suspensions.

Cells isolated from organoids were stained with CD271-APC (NGFR, clone ME20.4, BioLegend, #345107). Cells isolated directly from human esophagi were stained with CD271-APC, CD31-FITC (clone WM59, BioLegend, #303104), CD45-FITC (clone 2D1, BioLegend, #368508), CD49f-PE (ITGA6, clone eBioGoH3, Thermo Fisher Scientific, #12-0495-82) with/without CD327-PE/Cy7 (ECAD, clone DECMA-1, BioLegend, #147310). Staining with conjugated antibodies was done in 1X PBS containing 2% Fetal Bovine Serum (FBS, V/V, Cytiva, #SH30071.03) for 20 minutes on ice. After staining, cells were washed once with 1X PBS containing 2% FBS. Prior to sorting, cells were incubated with SYTOX Blue Dead Cell stain for 3 minutes on ice (1:10,000, Invitrogen, #S34857). Cells were sorted on a FACS Aria™ Fusion (BD Biosciences) and either collected for RNA sequencing or organoid culture.

### **Primary organoid culture**

From a vial or directly from tissue, epithelial cells were counted and centrifuged for 10 minutes at 400g, after which the pellet was reconstituted in a mixture of organoid medium (see below) and BME2 (R&D Systems, #3533-001-02) at a ratio of 1:5. 10µl domes, containing between 3000-5000 cells, were plated and incubated invertedly for 20 minutes at 37°C with 5% CO<sub>2</sub>. Organoid medium supplemented with

10 $\mu$ M RhoK inhibitor (Sigma-Aldrich, #Y0503) was used to initiate organoid formation during the first 2 days. RhoK inhibitor was removed from the medium after 2 days. Organoids were cultured at 37°C, 5% CO<sub>2</sub> and the medium was refreshed every other day. Upon passaging, ice-cold PBS was used to dissolve the BME2 domes and isolate the organoids. Subsequently, organoids were dissociated into single cells using 0.25% Trypsin-EDTA for 20-30 minutes at 37°C, with resuspension of the mix every 10 minutes. Trypsinization was neutralized with SBTI, after which cells were counted and either plated at 3000-5000 cells per dome for passaging or prepared for staining for FACS. When indicated, organoids were treated with either 5 $\mu$ M DAPT (Sigma-Aldrich, #D5942), 10ng/mL IL1A (Preprotech, #200-01A), 10ng/mL IL1B (Preprotech, #200-01B) or 50 $\mu$ g/ml Anakinra (MedChemExpress, #HY1088415).

#### **Primary esophageal organoid medium**

Organoids were cultured in Advanced DMEM/F12 (Thermo Fisher Scientific, 12634-010) supplemented with 2mM L-glutamine (Thermo Fisher Scientific, A2916801), 10mM HEPES (Thermo Fisher Scientific, 15630080), and penicillin/streptomycin (100U/mL and 100 $\mu$ g/mL, respectively; Thermo Fisher Scientific, 15140122). The medium was further supplemented with 5mM nicotinamide (Sigma-Aldrich, N0636), 1mM N-acetyl cysteine (Sigma-Aldrich, A9165), and 1 $\times$  B27 minus vitamin A (Thermo Fisher Scientific, 12587010). Growth factors included 100ng/mL Noggin (PeproTech, 120-10C), 250ng/mL R-spondin (PeproTech, 120-38), 100ng/mL FGF10 (PeproTech, 100-26), 25ng/mL FGF7 (PeproTech, 100-19), and 100ng/mL IGF-1 (PeproTech, 100-11). Additional supplements comprised 10mM forskolin (R&D Systems, 1099-10), 500 $\mu$ M A83-01 (Sigma-Aldrich, SML0788), 1 $\mu$ M SB202190 (R&D Systems, 1264/10), 4ng/mL hydrocortisone (Millipore, 386698), and 100 $\mu$ g/mL Primocin (InvivoGen, Ant-pm-05).

#### **Primary esophageal cell 2D culture**

Cell suspensions derived from esophageal epithelia, were spun down and resuspended in Keratinocyte Serum Free Medium (KSFM) containing 0.09mM Ca<sup>2+</sup> (Gibco, 17005042), supplemented with recombinant EGF (rEGF, 1ng/mL), bovine pituitary extract (BPE, 50 $\mu$ g/mL), and penicillin-

streptomycin (100U/mL, 100µg/mL, ThermoFisher Scientific, 15140122) for 2D expansion. Culture plates were coated with 10µg/mL Collagen (Gibco, A1048301) and 1µg/mL Laminin (LN511-0202, BioLamina) for 2 hours at 37°C prior to plating the cells. Primary cells were cultured at 37°C, 5% CO<sub>2</sub> and media was refreshed every other day. For freezing or passaging of the primary epithelial cells, cells were detached from the plate using 0.25% Trypsin-EDTA for 5-10 minutes at 37°C and neutralized with SBTI.

### **EPC2-hTERT organoids and 2D cultures**

EPC2-hTERT cells were maintained 2D in keratinocyte serum-free medium (KSFM; 0.09mM Ca<sup>2+</sup>) supplemented with recombinant EGF (1ng/mL), bovine pituitary extract (BPE; 50µg/mL), and penicillin-streptomycin (100U/mL and 100µg/mL; Thermo Fisher Scientific, #15140122) at 37°C with 5% CO<sub>2</sub>. Medium was changed every other day. For routine passaging of 2D cultures, cells were dissociated using Gibco TrypLE Express Enzyme (1X, Thermo Fisher Scientific/Gibco, #12605010), phenol red, and digestion was stopped by adding an equal volume of soybean trypsin inhibitor (SBTI; 0.25mg/mL in DPBS).

For 3D organoid culture, EPC2-hTERT cells were harvested, counted, and centrifuged for 3 minutes at 1400rpm. Cell pellets were resuspended in the same complete organoid medium used for primary esophageal organoid cultures and mixed with growth factor-reduced Matrigel matrix (Corning) at a 1:4 ratio. Each 8µL dome contained 2µL of cell suspension, corresponding to 2000 cells, and 6µL of Matrigel. Domes were allowed to polymerize by incubating the plate upside down for 20 minutes at 37°C with 5% CO<sub>2</sub>, after which complete organoid medium was added. During the first 24 hours, the medium was supplemented with 10µM ROCK inhibitor Y-27632 (Sigma-Aldrich, #Y0503) to support organoid establishment. Thereafter, organoids were maintained in the same complete organoid medium with regular medium changes.

For cytokine stimulation experiments, EPC2 organoids were treated with IL13 (100ng/mL), IL1A (10ng/mL), IL33 (10 or 100ng/mL) or TSLP (10 or 100ng/mL) for different times as indicated in the



corresponding figure legends. For 7-day treatments, recombinant proteins were refreshed every other day.

To assess dynamics of IL1A protein expression and secretion, EPC2-hTERT cells were expanded in a 6-well plate (2D) and treated with LPS (1 $\mu$ g/mL) or Poly I:C (10 $\mu$ g/mL) for 16 hours when 80% confluent. EPC2 cells were collected for IL1A western blot and media for IL1A ELISA at the end of experiment.

To assess proliferation, EPC2-hTERT cells were expanded on glass coverslips in the absence or presence of IL1A (10ng/mL) and incubated with 10 $\mu$ M EdU (Thermo Fisher Scientific, #E10187) in KSFM medium for 2 hours prior to fixation in 4% PFA.

Growth of EPC2-hTERT cells was determined by plating 15000 cells in a well of a 6-well plate in the presence or absence of IL1A (10ng/mL). Cells were trypsinized when the wells reached 50-70% confluency, collected using 0.25% Trypsin-EDTA for 5-10 minutes at 37°C, neutralized with SBTI and counted again.

### **IL1A ELISA**

IL1A secretion was measured using a commercial human IL1A ELISA kit (BMS243-2, ThermoFisher Scientific) according to manufacturer's instructions, using 50 $\mu$ l of cell culture media per reaction. Cultures treated with recombinant IL1A were washed to remove residual recombinant protein and then incubated for 3 hours in fresh media without IL1A before ELISA samples were collected.

### **Air-Liquid Interface (ALI) Culture of EPC2-hTERT and primary epithelial cells**

EPC2-hTERT or primary human epithelial cells were cultured at a density of 250000 cells per 0.4 $\mu$ m transwell insert in a 12-well plate (Corning, 3460). Transwell inserts were coated with 10 $\mu$ g/mL Collagen and 1 $\mu$ g/mL Laminin for 2 hours at 37°C prior to the plating of cells. Cells were grown submerged in PneumaCult<sup>TM</sup>-Expansion Medium (Stemcell Technologies, #05008) until confluent, and

then maintained in PneumaCult™-ALI Medium (Stemcell Technologies, #05001) for 2 days before exposing to air. To induce differentiation and stratification, PneumaCult™-ALI Medium in the upper chamber was removed and cells were exposed to air for 14 days. PneumaCult™-ALI Medium was kept in the basal chamber and refreshed every other day. Primary human epithelial cells were passaged no more than 2 times before used for ALI cultures. Recombinant IL1A (10ng/mL) or IL13 (100ng/mL) was refreshed every other day.

### **FITC-Dextran Permeability Assay**

Air-liquid interface cultures were exposed to air for 14 days to obtain a stratified epithelial layer with basal and differentiated cells. 500µl of FITC-conjugated Dextran beads (3-5kDa, 1mg/ml, Sigma-Aldrich, FD4) in PneumaCult™-ALI Medium was added to the apical chamber of the insert. The lower chamber was refreshed with 1.5mL PneumaCult™-ALI Medium. 100µl of medium was collected at different time points (15, 30, 60, 90, 120, 150, 180 and 240 minutes) and transferred into a 96-well transparent flat bottom plate. Medium of the different time points was stored in the dark at 4°C. The fluorescence intensity was measured on SpectraMax iD3 with an excitation wavelength at 490nm and an emission wavelength at 520nm using the Softmax Pro software.

### **TEER assay**

Barrier integrity was assessed by measuring transepithelial electrical resistance at day 6 of EPC2 ALI and day 8 of primary cell ALI culture, using a Millicell® ERS 3.0 Digital Voltohmmeter (Millipore, MERS03000) according to standard protocols.

### **Immunofluorescence Staining**

Organoids were fixed for 30 minutes in 4% PFA solution at room temperature and fresh esophageal tissue was fixed overnight at 4°C in 4% PFA solution. After fixation, the tissue or organoids were left overnight at 4°C in 20% sucrose (Sigma, #84100) in 1X PBS, embedded in OCT embedding matrix and sectioned at 10µm slices for immunofluorescence analysis.

FFPE sections from EoE patient biopsies were deparaffinized and rehydrated by immersing the FFPE tissue sections twice for 10 minutes in Xylene (VWR chemicals, #28975.291), followed by 2 minutes in decreasing Ethanol solutions (2x100%, 75% and 50%) and deionized water. After, antigen retrieval was performed by boiling the sections for 20 minutes in 0.1mM EDTA pH8.

OCT sections that were stained for P63 required antigen retrieval in 0.1M citrate buffer for 15 minutes prior to blocking for nonspecific binding of the antibodies. All sections (OCT and FFPE) were washed in 1x PBS and incubated for 30 minutes at RT in blocking buffer containing 1% BSA (W/V, Sigma-Aldrich, #A4503), 5% Normal Donkey Serum (V/V, Abcam, # ab138579), and 0.3% Triton X-100 (0V/V, Sigma-Aldrich, #93443) in 1X PBS. Sections were incubated with primary antibody in blocking buffer at 4°C overnight, followed by three times washing with PBS containing 0.05% Tween (PBST, Bio-Rad, #1706531). Subsequently, the sections were incubated for 2 hours at room temperature with secondary antibodies (1:500, Jackson ImmunoResearch) and DAPI (0.25µg/mL, Thermo Fisher Scientific, #D1306) in blocking buffer. Finally, the sections were washed three times with PBST, followed by a single wash with 1xPBS, and then mounted using Mowiol 4-88 (Sigma, #81381). Immunofluorescent images were obtained using the Zeiss Axio Imager Z2 microscope and the ZEN 2.3 software.

#### *Air-Liquid Interface cultures*

The ALI cultures were washed with PBS and fixed in 4% PFA for 30 minutes. Inserts were washed 3 times for 5 minutes in 0.3% Triton X-100 in PBS and blocked for 1 hour at room temperature in blocking buffer containing 1% (W/V), 5% (V/V) Normal Donkey Serum, and 0.3% (V/V) Triton X-100 in 1XPBS. Inserts were incubated with primary antibody in blocking buffer at 4°C overnight, followed by three times washing with PBS containing 0.03% (V/V) Triton X-100. Subsequently, the inserts were incubated for 2 hours at room temperature with secondary antibodies (1:500, Jackson ImmunoResearch) and DAPI (0.25 µg/mL) in blocking buffer. Finally, the inserts were washed three times with PBS containing 0.03% (V/V) TritonX, followed by a single wash with 1x PBS, and then

mounted using Mowiol 4-88. Immunofluorescent images were obtained using the Leica Stellaris confocal microscope.

### **Cell proliferation and DNA content assay for flow cytometry**

Organoids were incubated with 10 $\mu$ M EdU (ThermoFisher Scientific, # E10187) in organoid medium for 2 hours. Organoids were then trypsinized to generate single cells and collected. Cell solutions were filtered through a 40 $\mu$ m Flowmi cell strainer for 1000 $\mu$ l pipette tips (VWR, #734-5950). EdU detection and DNA content staining was done using the Click-it EdU Alexa Fluor 647 Flow Cytometry Assay Kit (Invitrogen, #C-10424) and FxCycle Violet stain (Invitrogen, #F10347) according to manufacturer's instructions. Flow cytometry was performed using the LSRIII (BD) and data was analyzed using the Flowjo 10 software.

### **Western Blot Analysis**

Cells or patient biopsies were collected in RIPA lysis buffer (ThermoFisher Scientific, #89901) containing protease inhibitors (MerckSigma, #4693116001) and lysed for 1 hour on ice. Samples were centrifuged at 13000g for 15 minutes at 4°C. Protein concentration per sample was measured using Pierce<sup>TM</sup> BCA protein assay kit (ThermoFisher Scientific, #23225) according to manufacturing's instructions. A total of 10 $\mu$ g of protein per sample was prepared with 6X Laemmli SDS buffer (VWR, #J61337) and boiled at 95°C for 5 minutes. Samples were run on SDS/PAGE gels (Biorad, #4561093) and transferred onto polyvinylidene difluoride (PVDF, Merck, #IPVH00010) membranes. Membranes were blocked with 5% BSA in TRIS-buffered saline (TBS, Fisher Scientific, #J62662) containing 0.1% Tween-20 (TBS-T) for 1 hour at room temperature and incubated with primary antibody overnight at 4°C. The next day, membranes were washed 3 times in TBS-T, followed by 1 hour incubation with HRP-conjugated secondary antibody at RT. Membranes were washed again for 3 times with TBS-T and were subsequent developed using SuperSignal West Femto Maximum Sensitivity Substrate (ThermoFisher Scientific, #34095) and imaged with ChemiDoc (Bio-Rad).

### **RNA isolation, cDNA synthesis and RT-qPCR**

Ice-cold PBS was used to isolate organoids from the BME2 domes, and the organoids were spun down at 400g for 5 minutes. Cells from 2D cultures were washed with PBS and directly lysed in the plate. Lysis and RNA isolation was according to manufacturer's instructions of the RNeasy Mini Kit (QIAGEN, #74106). RNA was eluted in 30 µl DNase/RNase free water and quantified using Nanodrop. Equal amounts of RNA (500ng – 2µg) per experiment was used for cDNA synthesis using SuperScript™ VILO™ cDNA Synthesis Kit (Invitrogen, #11754250) following manufacturer's instructions.

Diluted cDNA was used with selected primers together with Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific, #4368702) on a ViiA7 device (Applied biosystems). Relative gene expression was calculated with the use of both *GAPDH* and *ACTIN* as housekeeping genes.

### Primary antibodies

The following primary antibodies were used for IF: COL17A1 (1:500, rabbit, ab28440, Abcam), KI67 (1:250, rat, 14-5698, eBioscience), KRT4 (1:500, mouse, ab9004, Abcam), KRT14 (1:200, guinea pig, BP5009, Acris Antibodies), P63 (1:100, mouse, ab735, Abcam), P73 (1:500, rabbit, ab40658, Abcam), NGFR (1:200, rabbit, ab52987, Abcam; and 1:400, mouse, 345102, BioLegend), KLF4 (1:200, rabbit, ab214666, Abcam), IL1A (1:100, rabbit, ab300501, Abcam), IL1R2 (1:100, goat, AF-263, R&D Systems), aYAP (1:100, rabbit, ab205270, Abcam), TGM1 (1:1000, rabbit, ab103814, Abcam), K5 (1:100, chicken, 905904, Biolegend) and ZO-1 (1:100, rabbit, 61-7300, ThermoFisher).

In addition, we used P65 (1:800 for IF and 1:500 for WB, rabbit, 8242, Cell Signaling Technology), phospho-P65 (1:500 WB, rabbit, 3033, Cell Signaling Technology), GAPDH (1:1000 WB, mouse, sc47724, Santa Cruz Biotechnology), IL1A (1:1000 WB, rabbit, 16765-1-AP, Proteintech), ECAD (1:1000 WB, rabbit, 3194, Cell Signaling), alpha-tubulin (1:1000 WB, mouse, ab7291, Abcam), GAPDH (1:1000 WB, mouse, ab8245, Abcam).

### Secondary antibodies

The following secondary antibodies were used: Alexa Fluor 488–conjugated donkey anti–guinea pig (706-545-148, Jackson ImmunoResearch), donkey anti–mouse (715-545-150, Jackson ImmunoResearch), and donkey anti–rat (712-545-153, Jackson ImmunoResearch). Alexa Fluor 647–conjugated donkey anti–mouse (715-605-151, Jackson ImmunoResearch) and donkey anti–rabbit (711-605-152, Jackson ImmunoResearch) were also used. For Cy3 detection, Alexa Fluor–Cy3 donkey anti–rabbit (711-165-152), donkey anti–guinea pig (706-165-148), and donkey anti–mouse (715-165-150) antibodies were obtained from Jackson ImmunoResearch. For Western blot detection, HRP-conjugated donkey anti–rabbit (A16023, Thermo Fisher Scientific) and donkey anti–mouse (A16011, Thermo Fisher Scientific) secondary antibodies were used.

### **Bulk RNA sequencing library preparation**

Libraries for bulk RNA-sequencing were prepared according to the prime-seq protocol as previously described (11). In short, fresh tissue as well as corresponding organoids from 5 different donors were used to isolate 10000 NGFR-negative and 10000 NGFR-positive cells. Cell populations were lysed in TRI Reagent (ZymoResearch, #R2050) and stored at -80°C until RNA isolation. RNA was isolated using the Direct-zol RNA Microprep kit (ZymoResearch, #R2060) according to manufacturing's instructions. From each sample, 16ng of RNA was used for cDNA synthesis. cDNA quantity was determined using the Qubit dsDNA HS assay kit (Thermo Fisher, #Q32851). The sequencing library was constructed from 20ng of cDNA using the NEBNext Ultra II FS Kit (New England #E6177S). Size selection was performed using SPRIselect beads (Beckman Coulter, #B23318), and the quantity and quality of the library was assessed using the Bioanalyzer High Sensitivity DNA kit (Agilent # 5067-4626). The samples were sequenced on Illumina NovaSeq 6000 at an average depth of 10 million reads per sample.

### **Bulk RNA sequencing library data analysis**

Bulk Prime-seq data was processed following the Deng Lab Prime-seq analysis pipeline (see Data and Code Availability). After standard quality control and trimming, sequencing reads were processed with zUMIs using a customized YAML configuration that specified sample barcodes, read structure and

STAR genome indices. All downstream analyses were conducted in R (4.3.1) using the resulting count matrices.

To remove lowly and sparsely expressed genes prior to downstream analysis, genes were retained only if their total counts across all samples exceeded 100 and they were detected (count > 0) in at least 50% of samples. Differential expression analysis was performed using DESeq2 (12) v1.40.2. For paired comparisons, patient identity was included as a blocking factor and condition was modelled as a fixed effect (design = ~ patient + condition). Statistical inference was performed on the condition coefficient, corresponding to the log<sub>2</sub> fold change (logFC) after adjusting for patient-specific effects. Gene set enrichment analysis (GSEA) was performed using clusterProfiler v4.10.1 (13), and enrichment was assessed against v2024.1 human gene sets (14) using permutation-based scoring. Pathways with a normalized enrichment score (|NES|) > 1 and an adjusted p-value (FDR) < 0.05 were considered significantly impacted.

To bridge bulk and single-cell datasets, the top 50 differentially expressed genes ranked by logFC from bulk RNA-seq were used to compute transcriptional module scores across single cells. Gene signature was evaluated using Seurat's AddModuleScore (Seurat v4.4.0) (15), and scores were visualized on UMAP embeddings to map bulk-derived transcriptional programs onto individual epithelial cell states.

### **Single-cell RNA sequencing data analysis**

Processed single-cell RNA-sequencing data were downloaded from the Broad Institute Single Cell Portal (SCP1242) (16). Low-quality cells with fewer than 50 detected genes (nFeature\_RNA > 50) or more than 45% mitochondrial RNA content (percent.mt < 45) were removed. Sample-level QC summaries were assessed across all donors, and two samples (E1178 and E1611) showed markedly reduced cell numbers and contained very few epithelial cells; these samples behaved as outliers during downstream clustering and were therefore excluded from further analysis. Doublet identification and

removal were performed using DoubletFinder v2.0.4 (17). To correct for inter-individual batch effects, we applied Harmony v1.2.0 (18), using patient identity as the integration variable.

Following batch correction, we performed stratified random downsampling across all annotated cell types using the same strategy as described in the original study (16). A total target of 100,000 cells was allocated proportionally to each cell type, with rare populations fully retained and abundant populations randomly downsampled according to group-specific quotas. After subsampling, the final dataset were subsequently used for normalization, feature selection, and downstream analyses. Principal component analysis (PCA) was performed for dimensionality reduction, and the top 25 principal components were retained for construction of a shared nearest neighbor graph. Cell-type annotation was not re-derived; instead, we retained the curated annotations provided in the original dataset (16), which demonstrated robust separation across epithelial, immune, and stromal compartments.

Cell–cell communication was inferred using the multinichenetr (19, 20) (nichenetr v2.0.6). Only cell types represented by at least 10 cells per group were retained for analysis. Genes were required to be expressed in at least 50% of samples and to exhibit non-zero expression in a minimum of 5% of cells within each sample. For visualization purposes, inferred ligand activities were subsequently filtered to interactions targeting basal cells, and the top 20 incoming ligand–receiver interactions were selected for display.

## Image Analysis

### *Organoid size and organoid-forming efficiency analysis*

Organoid size was measured seven days after plating. BME2 domes containing live organoids were imaged using the Leica DMI6000 (10x magnification; brightfield) and the LAS X software. Image analysis was done using ImageJ (v2.14.0/1.54f). Organoids bigger than  $2500\mu\text{m}^2$  were included in the analysis.

### *Immunofluorescence analysis on organoid or tissue sections*



QuPath (v0.5.1) was used for the analysis of NGFR-positive, P73-positive, P63-positive, KI67-positive, KLF4-positive and EdU-positive cells. Thresholds were manually altered to accurately count cells positive for specific markers or to determine the percentage of NGFR-positive area in each organoid. Thresholds were kept consistent for each esophagus and organoid passage.

### Statistical analysis

GraphPad Prism (v10.6.0) was used for statistical analyses. Statistical tests included two-tailed paired Student's t-test, two-tailed unpaired Student's t-test, One-Way ANOVA with a Tukey's multiple comparisons test or Two-Way ANOVA with a Tukey's multiple comparisons test. The statistical tests used per experiment are indicated in the figure legends. Error bars represent the mean and the standard deviation (SD).

## Results

### Basal progenitor cell heterogeneity is spatially regulated

Single-cell RNA-sequencing has enabled comprehensive characterization of human tissue cell states. A recently generated atlas of the adult human esophagus (16) encompasses all major epithelial and stromal cell populations (Figure S1A–C; Supplemental Table 1). We subclustered epithelial cells into basal (*COL17A1*, *PDPN*), suprabasal (*KRT6B*, *TGM1*), and apical (*FLG*, *KRT78*) cell states (21, 22), representing successive stages of epithelial differentiation (Figure 1A–B and S1D). In addition, we identified a proliferative cluster marked by *MKI67* and *CDKI*, likely comprising cells derived from both the basal and suprabasal compartments (23) (Figure 1A–B and S1E–F).

Within the basal compartment, *TP73* and the transmembrane receptor *NGFR* (23) were exclusively expressed in basal cells (Figure 1B) spanning both papillary and interpapillary regions (Figure 1C and S1D). In contrast, the progenitor marker P63 (24, 25) overlapped with NGFR/P73-positive basal cells but also extended into several suprabasal layers, whereas the differentiation markers KLF4 (26) and TGM1 were confined to more differentiated suprabasal cells (Figure 1C and S1G–H). These findings identify spatially distinct basal and suprabasal progenitor compartments that differ in marker expression.

To assess functional heterogeneity within the basal compartment, we quantified KI67-positive cells in esophageal tissue sections. Consistent with previous reports (21), the NGFR-negative suprabasal compartment contained more cycling cells than the NGFR-positive basal layer (Figure 1D). Within the basal layer, however, nearly 80% of cycling cells localized to papillary regions (Figure 1E), demonstrating spatial heterogeneity among basal progenitors.

Although NGFR marked a single basal cell layer throughout the epithelium (Figure 1D), the basal marker KRT14 extended into papillary suprabasal cells, where it overlapped with the differentiation marker KRT4 (white arrow) (Figure 1F and S1I). Similarly, active YAP, a marker for cells committing to differentiation (26), was detected in differentiating suprabasal cells as well as NGFR-positive basal cells at papillary tips (Figure 1G). Together, these findings demonstrate that the human esophageal epithelium follows a stereotypic basal-to-suprabasal differentiation trajectory while exhibiting pronounced spatial heterogeneity correlating to the location of basal cells along the papillary axis.

### **NGFR defines basal progenitor cells capable of renewal**

Basal progenitor cells exhibit high clonogenic capacity and are commonly isolated using ITGA6 or PDPN (22, 27). However, we found that ITGA6<sup>high</sup> epithelial cells extended beyond the NGFR-positive basal layer (Figure 2A) and comprised both NGFR-positive basal and NGFR-negative suprabasal cells (Figure S2A–B), rendering ITGA6 alone suboptimal for basal progenitor isolation. Within the ITGA6<sup>high</sup> population, PDPN and NGFR were highly co-expressed (Figure S2C–D), confirming NGFR as a specific marker of human basal progenitor cells.

We next FACS-isolated ITGA6<sup>high</sup> NGFR-positive and ITGA6<sup>high</sup> NGFR-negative epithelial cells from seven esophagectomy donors and assessed their organoid-forming capacity (Figure 2B–C). NGFR-positive basal cells generated significantly more organoids than NGFR-negative suprabasal cells (Figure 2D), indicating that clonogenic potential is largely restricted to the NGFR-positive basal compartment.

Organoids derived from NGFR-positive basal cells recapitulated the architecture and differentiation trajectory of the human esophageal epithelium (Figure 2E). KI67-positive proliferating

and P73-positive cells were confined to the outer basal cell layer, whereas P63 extended into suprabasal layers (Figure 2E–F). Consistent with the native epithelium, NGFR expression was restricted to P73-positive basal cells (Figure 2G).

### Repression of NOTCH signaling restricts basal cell differentiation

To further characterize basal and suprabasal epithelial cell states, we transcriptionally profiled ITGA6<sup>high</sup>, NGFR-positive and NGFR-negative cells from five individual donors. Employing pair-wise statistical comparisons, we identified differentially expressed genes (DEGs) associated with each cell state (Figure 2H and S2E–F) (Supplemental Table 2). Projecting the top 50 NGFR-positive and NGFR-negative DEGs onto the single-cell RNA sequencing UMAP confirmed that that NGFR-positive signatures mapped to basal progenitor clusters, whereas NGFR-negative profiles aligned with suprabasal and apical cells (Figure 2I). Gene set enrichment analysis (GSEA) revealed that NGFR-positive basal cells expressed genes associated with known mitogenic pathways, such as WNT, FGF, IGF and HH signaling (2), as well as signatures linked to integrin and laminin signaling, indicative of basal cell interactions with the basement membrane or extracellular matrix (Figure 2J) (Supplemental Table 3). In contrast, genes upregulated in the NGFR-negative population were associated with cell-cell junctions and retinoic acid signaling, previously linked to epithelial differentiation (25, 28). Additionally, NGFR-positive and NGFR-negative cell states were predicted to differentially regulate NOTCH signaling (Figure 2J). We confirmed differential expression of NOTCH ligands *DLL1* and *JAG2* in NGFR-positive basal cells, whereas in contrast, *NOTCH2* and *NOTCH3* receptors were enriched in the NGFR-negative population (Figure 2K and S2G) and correlated to HES1 (a proxy for active NOTCH signaling) immunoreactivity (Figure 2L).

Pharmacologic NOTCH inhibition (using DAPT) in human organoids reduced *HES1* transcription (Figure S2H) and organoid size (Figure 2M–N). The percentage of KLF4-positive differentiated cells was decreased (Figure 2N) while the proportion of the P73-, but not P63-positive, basal progenitor cell population increased (Figure 2O). These data indicate that NOTCH signaling facilitates epithelial differentiation (22, 29, 30).

### **The basal layer is maintained through renewal of NGFR-positive progenitor cells**

A large proportion of NGFR-expressing basal cells co-expressed the proliferation marker KI67 in organoids (Figure 3A). To determine whether basal progenitor cell states change during organoid expansion, NGFR-positive and NGFR-negative cells were isolated from human esophageal organoids and challenged to generate secondary organoids (Figure 3B and S3A). In contrast to NGFR-negative cells isolated directly from the esophageal epithelium, which failed to establish organoids (Figure 2D), NGFR-negative organoid-derived cells readily formed secondary organoids with efficiency and size comparable to NGFR-positive cells (Figure 3B). These findings indicate that NGFR-negative suprabasal cells acquire renewal capacity when specified in culture.

Organoids established from NGFR-positive and NGFR-negative cells (Figure 3B, schematics) exhibited similar proliferation kinetics (Figure 3C), proportions of P63-positive cells (Figure S3B), and differentiation as assessed by KLF4 expression (Figure 3D). However, organoids derived from NGFR-positive cells contained significantly higher proportions of P73- and NGFR-positive basal cells, as determined by immunofluorescence (Figure 3E) and flow cytometry (Figure 3F and S3C). Thus, although NGFR-negative suprabasal cells acquire organoid-forming capacity and regenerate NGFR-positive basal cells during culture, NGFR-positive cells retain a greater capacity to generate P73/NGFR-positive basal progenitors.

### **Characterization of culture-induced cell state changes**

To gain some mechanistic insights into how culture affects NGFR-positive and NGFR-negative cell states, we isolated both populations from organoids derived from the same five donors previously used to sequence primary NGFR-positive and NGFR-negative cell populations (Figure 2) and performed bulk RNA-sequencing (Figure 3G and S3D) (Supplemental Table 2).

We confirmed that *NGFR*, together with *COL17A1* and *KRT15*, was enriched in NGFR-positive basal organoid cells, whereas differentiation markers were low, validating the isolation strategy (Figure 3H). Projection of the top 50 NGFR-positive and NGFR-negative signature genes onto the human

single-cell sequencing UMAP demonstrated that NGFR-negative cells retained similarity to *in vivo* suprabasal/apical populations (Figure 3I) despite acquiring organoid-forming capacity (Figure 3B). In contrast, NGFR-positive cells projected towards the proliferative cluster (Figure 3I), consistent with loss of basal quiescence in organoids (Figure 3A).

Probing deeper, we performed GSEA analysis to understand differences in cell identities induced *in vitro* (Supplemental Table 3). NGFR-positive cells upregulated programs linked to cell cycle progression, NF- $\kappa$ B, and Interleukin 1 (IL1) signaling (Figure 3J) - pathways associated with inflammation and regeneration - while downregulating basal markers such as *KRT15*, *TP73*, and *NGFR* (Figure 3K and S3E). Focusing on transcriptional changes in the NGFR-negative cell population, we recorded silencing of transcripts associated with epithelial differentiation (*KRT13*, *KRT4* and *KRT78*) and cell-cell adhesion, whereas upregulation of genes associated with IL1 signaling (*IL1A* and *IL1B*) reoccurred (Figure 3L-M). Together, these data suggest that organoid culture induces a regenerative, inflammation-associated transcriptional state in both populations.

Intrigued by the finding that both NGFR-positive and NGFR-negative cells isolated from tissue alter the IL1 pathway in culture (Figure 3J-N), we examined this pathway in more detail. Interestingly, both NGFR-positive and NGFR-negative cells upregulated the transcription of several IL1 ligands, most notable *IL1A* and *IL1B*, while NGFR-positive cells silenced the expression of the IL1 decoy receptor *IL1R2*, facilitating active IL1 signaling in basal progenitor cells (Figure 3N).

We further observed that cultured NGFR-positive and NGFR-negative cell states upregulate transcription of EGF-like growth factors (*AREG*, *EREG* and *TGFA*) (Figure 3K and M, S3F). Given the established role of EGF signaling in epithelial proliferation and repair (7, 31-33), autocrine EGF signaling may contribute to the acquisition of organoid-forming capacity by NGFR-negative cells.

### **Intraepithelial IL1 signaling is accentuated during esophageal inflammation**

To determine whether the epithelial injury-response programs identified in organoids are also associated with eosinophilic esophagitis (EoE), a food antigen-driven type 2 allergic inflammation (3), we revisited the published single-cell atlas (Figure 1) (16). Epithelial cells from both healthy and EoE patients with active disease were subclustered (n=5 and n=8, respectively) (Figure 4A and S4A). In

agreement with previous publications, we detected a subtle expansion of suprabasal and apical cell clusters (Figure S4B) (22, 34-36), reflecting an altered epithelial architecture.

Cell cycle inference (23) failed to identify differences in cycling behavior when comparing epithelial subclusters from healthy and EoE donors (Figure S4C), indicating epithelial hyperplasia results from impaired terminal differentiation rather than increased cell division (22, 37). Differential expression analysis identified relatively few transcriptional changes between healthy and EoE epithelial cell states (Figure 4B-D) (Supplemental Table 4) (16), indicating preservation of overall epithelial identity despite shifts in cell proportions. Suprabasal and apical cells downregulated genes required for epithelial barrier integrity (*SPRR2A*, *SPRR2E*, *SPRR1B* and *KRT6A*) (22, 35), while upregulating enzymes (*HPGD*) and inhibitors (*SERPINB4*) involved in modulating inflammatory responses. In addition, known proinflammatory cytokines such as *CCL26*, *IL18*, *IL36A* and *IL1A* were upregulated in differentiated cell clusters derived from EoE donors.

To understand if signaling directed towards basal cells, the cell population predicted to mediate epithelial regeneration, was altered in EoE, we implemented Multiniche, which uses receptor-ligand expression to predict cell-cell communication patterns (19, 20, 38). Employing the entire single-cell atlas (encompassing all profiled cell clusters) (Figure S4A) (Supplemental Table 5) we identified the 20 strongest receptor-ligand interactions directed towards basal cells and mapped the sending cell populations (Figure 4E-F). Multiple cell types were predicted to direct signaling towards basal cells normally. Interestingly, this profile changed in EoE, and additional communication pathways directed towards basal cells was predicted to originate from T-cells as well as apical epithelial cells (Figure 4E-F).

Identifying the most significantly altered ligand-receptor interactions in EoE compared to healthy esophagus, we linked T-cell-to-basal cell communication to IL13-IL13RA1 signaling, confirming previous work establishing IL13 as a key driver of esophageal inflammation in EoE (Figure S4D) (39-42). In addition, signaling emanating from the IL1 ligands was activated in EoE compared to healthy patients (Figure S4D-E). We recorded a significant upregulation of *IL36B*- and *IL1A*-mediated apical-to-basal intraepithelial communication and additionally demonstrated that increased *IL33* produced by endothelial cells was predicted to impact basal epithelial cells via binding to the *IL1RAP*

receptor (Figure 4G and S4D-E) (40, 43). *IL1B*, while not significantly altered in our top 20 cell-cell communication analysis, was upregulated in both myeloid and mast cells (Figure S4E). Taken together, this data indicates that the cell-cell communication landscape is significantly altered during inflammation, pinpointing the involvement of the IL1 family.

We realized that *IL1A*, in contrast to the other IL1 ligands, was predominantly produced by epithelial cells (Figure S4E) (44). In line with the Multiniche prediction, *IL1A* was expressed by apical cells, and *IL1A* transcription was increased in patients diagnosed with EoE, when compared to healthy donors or remission patients (Figure 4H and S4F). Comparing the abundance of IL1A receptors in healthy and EoE patients demonstrated that expression of both the decoy *IL1R2* and the functional *IL1RAP* receptors were maintained by basal cells, suggesting that basal progenitors are equipped to respond to the apical IL1A source (Figure S4G). Probing a potential autocrine IL1A loop, we recorded expression of both the *IL1R1* receptor and IL1A antagonist (*IL1RN*) in apical cells derived from EoE patients, indicating that also apical cells could sense IL1A (Figure S4G).

Localizing *IL1R2* and *IL1A* on esophageal tissue sections, we confirmed the compartmentalized expression profile indicated by the transcriptional data. Whereas *IL1R2* exclusively marked basal progenitor cells, *IL1A* was detected in the apical cell layers of the healthy human esophageal epithelium (Figure S4H and 4I). We noted that while basal cells in interpapillary regions were shielded from apical IL1A by intermediate, IL1A-negative, suprabasal cell layers, basal cells in papillary tip regions neighbored IL1A-expressing cells. These differences in IL1A-basal cell proximity suggest that cycling basal cells located at papillary tips (Figure 1D and E) could be exposed to relatively high IL1A levels, even during epithelial homeostasis. Turning to epithelium from EoE patients diagnosed as either in active or remission phase (Supplemental Table 8), we confirmed high IL1A in patients with active EoE, both in epithelial as well as stromal cells (Figure 4J and S4I), indicating that IL1A signaling is dynamically regulated during EoE.

### **IL1A induces epithelial expression of proinflammatory cytokines**

Since our data indicated that intraepithelial IL1A signaling could impact both basal and suprabasal cell states, we revisited our human primary organoids. We recorded IL1A transcript and

protein preferentially in the inner, most differentiated, cell layers but also in the basal layer recapitulating IL1A expression in the esophageal epithelium (Figure 4K and S4J). Interestingly, the percentage of basal cells expressing IL1A correlated to increasing organoid passaging, suggesting that basal cells could respond to the regenerative stress associated with forming organoids by inducing endogenous IL1A (Figure 4J-K).

We then established organoids from five donors in the presence or absence of IL1A and isolated NGFR-positive and NGFR-negative cell populations for transcriptional profiling (Figure 5A and S5A). Continuous IL1A treatment resulted in a low number of differentially expressed genes (53 in NGFR-negative and 31 in NGFR-positive cells), where most deregulated genes were enriched, rather than silenced (Figure 5B) (Supplemental Table 6). We realized that many of the genes found upregulated after IL1A treatment were shared between the NGFR-positive basal and NGFR-negative suprabasal populations (Figure 5C). This shared transcriptional signature was reflected in the GSEA analysis, which highlighted enrichment of pathways associated with immune cell recruitment and activation (neutrophil chemotaxis and IL6 signaling) or cytokine signaling (Figure S5B) (Supplemental Table 7). Commonly regulated genes included several proinflammatory cytokines (*CCL20*, *CXCL1*, *CXCL2*, *CXCL3*, *CXCL8* and *CSF3*) as well as secreted calcium-binding proteins (*S100A8* and *S100A9*) known to be produced by epithelial cells in response to inflammation (45). In addition, both NGFR-positive and NGFR-negative cells responded to IL1A by upregulating *IL36G* and *IL1A* itself, indicating a feed-forward IL1-transcriptional program. Consistent with this, IL1A treatment increased the proportion of IL1A-positive basal cells when compared to control organoids, although this did not reach statistical significance ( $p=0.0514$ ) (Figure S5C). Upregulated signatures unique for suprabasal, NGFR-negative, cells included genes associated with epithelial differentiation and barrier function (*CNFN*, *LCE3D*, *SPRR1B*, *SPRR2A*, *SPRR2D*, *SPRR2E* and *TGMI*) (Figure 5C). These data suggest that IL1A can impact, directly or indirectly, both basal and suprabasal epithelial cell populations.

### **IL1A activates NF- $\kappa$ B signaling in both basal and suprabasal cells**

GSEA analysis pointed towards NF- $\kappa$ B signaling as a downstream mediator of the transcriptional changes we detect when exposing human primary organoids to IL1A (Figure S5B). To



understand the relationship between IL1A and NF- $\kappa$ B transcriptional activity in progenitor cells, we treated EPC2-hTERT (EPC2) human esophageal basal cells with IL1A and determined the subcellular location of P65 (RELA), the key NF- $\kappa$ B transcriptional subunit, in a defined time course (Figure 5D and S5D-F). While the immortalized nature of EPC2-hTERT cells limits their ability to fully recapitulate basal progenitor behavior *in vivo*, they are a useful model for studying epithelial signaling, expressing basal markers and IL1-receptors comparable to primary cells. (Figure S5D-E). When administrating IL1A, P65 rapidly, but transiently, translocated to the nuclei of EPC2 cells (Figure S5E). Increased P65 phosphorylation confirmed activation of NF- $\kappa$ B activity in response to IL1A treatment (Figure S5G). We then explored the expression of NF- $\kappa$ B target genes *NFKB1A*, *TNF* and *CXCL8* (46-48) and demonstrated a time-dependent increase in transcription, correlating with the nuclear localization dynamics of P65 (Figure 5E).

Providing a 1-hour pulse of IL1A to organoids, resulted in prominent P65 nuclear translocation in both basal and suprabasal cells (Figure 5F), indicating that IL1A can target both cell states. Expanding organoids in the presence of IL1A significantly upregulated NF- $\kappa$ B target genes *NFKB1A*, *TNF* and *CXCL8*, as well as *IL1A* itself (Figure S5H). To verify the direct relationship between IL1A-IL1R1 and NF- $\kappa$ B signaling, we made use of Anakinra, an IL1-receptor antagonist (Figure 5G). Whereas IL1A alone induced expression of NF- $\kappa$ B target genes, this effect was ablated when IL1A was combined with Anakinra (Figure 5H). These data indicate that IL1A, through activation of NF- $\kappa$ B signaling, mediates gene expression changes, which includes upregulation of *IL1A* itself, thereby bolstering the IL1A-mediated signaling loop in both basal progenitor and suprabasal cells.

### **IL1A promotes expansion of the basal progenitor cell pool**

To determine how IL1A affects epithelial cell behaviors, we treated primary human organoids with IL1A. While organoid-forming efficiency was unchanged (Figure 6A), IL1A significantly increased organoid size at days 4 and 7 (Figure 6A and S6A), an effect reversed by the IL1 receptor antagonist Anakinra (Figure S6B), confirming IL1A-dependent growth. Analysis of mature organoids revealed an increased proportion of P73-positive basal cells following IL1A treatment (Figure 6B, left graph), whereas P63- and KLF4-positive populations were unchanged (Figure 6B-C), suggesting

selective expansion of basal progenitors. However, EdU incorporation and KI67 staining at day 7 showed no increase in proliferation at this stage (Figure 6D and S6C). To address if IL1A can affect proliferation of basal progenitor cells, we administered IL1A to expanding EPC2 basal cells and recorded a small but significant increase in the number of basal cells in culture (Figure S6D) as well as the percentage of cells incorporating EdU (Figure S6E), indicating that IL1A positively regulates renewal of esophageal epithelial basal cells.

IL1A and IL1B activates the same receptors. IL1B is upregulated in myeloid cells during EoE (Figure S4E), and we therefore asked if IL1B affected basal cells analogous to IL1A. Administering IL1B to primary organoid cultures phenocopied the effect of IL1A; increasing organoid size but not organoid forming efficiency (Figure S6F). Together, these findings indicate that IL1A affects basal cell renewal, an effect which may be further enhanced *in vivo* by complementary IL1B activity.

### **IL1A positively affects epithelial barrier function**

To assess if IL1A can impact epithelial barrier integrity, we turned to 3D air-liquid interface cultures. Epithelial sheaths from both control and IL1A treated air-liquid interface cultures recapitulated normal epithelial organization, with layered, largely non-overlapping, expression of basal P63 and suprabasal KLF4 expression (Figure 6E and S6G). Basal cells retained COL17A1 expression and proliferated (Figure 6F), both normally and after exposure to IL1A.

Tight junctions are essential for epithelial barrier integrity, and ZO-1 is a central scaffolding protein that organizes tight junction complexes. IL1A-treated epithelial sheaths exhibited improved ZO-1 organization (Figure 6G and S6H), accompanied by reduced passage of apically applied fluorescent dextran (3–5 kDa) to the basal compartment, indicating enhanced epithelial barrier function (Figure 6H and S6I). Together, these findings demonstrate that IL1A promotes the establishment of a functional epithelial barrier.

### **Innate immune agonists, but not epithelial alarmins, alter IL1A production and secretion**

To determine whether IL1A production and secretion are dynamically regulated by epithelial stress, EPC2 cells were treated with LPS or Poly I:C for 16 hours to mimic bacterial or viral challenge,

respectively. Poly I:C, but not LPS, increased IL1A protein levels (detected at 31 kDa), corresponding to the uncleaved but biologically active IL1A precursor (49) (Figure 7A). The same IL1A species was confirmed to be expressed in EPC2 organoids and EoE patient biopsies (Figure S7A). Consistent with Poly I:C-induced IL1A protein, ELISA demonstrated significantly increased IL1A secretion following Poly I:C, but not LPS, treatment (Figure 7A). Together, these results demonstrate that epithelial IL1A production and secretion are dynamically regulated in response to environmental stress.

We then asked if IL1A expression is dependent on the presence of TSLP or IL33, alarmins previously associated with initiation of EoE (50). Administering TSLP or IL33 for 3 hours to EPC2 organoids was able to induce CXCL8, but not IL1A, transcription, indicating that IL1A is not directly regulated by these alarmins (Figure S7B). Conversely, treating primary organoids with IL1A did not alter TSLP or IL33 expression (Figure S7C-D). Collectively, these findings suggest that crosstalk between IL1A and TSLP/IL33 is limited.

### **Inflammation reshapes IL1A-dependent changes in epithelial cell state programs**

To investigate dependencies between the Th2 cytokine IL13 and IL1A signaling, EPC2 organoids were pre-treated with IL13 for 1 day before IL1A was pulsed for 3 hours. Quantification of the IL1A target genes *NFKBIA*, *TNF*, *CXCL8*, and *IL1A* (Figure 5H) revealed that IL13 selectively modulates the epithelial transcriptional response to IL1A. While IL13 suppressed IL1A-induced expression of *NFKBIA* and *CXCL8*, induction of *TNF* and *IL1A* was preserved (Figure 7B), indicating that IL13 reshapes rather than uniformly inhibits IL1A signaling.

Since IL13 may influence the effects of IL1A on epithelial barrier formation (Figure 6H and S6I), EPC2 organoids were treated with IL13, IL1A, or the combination for 7 days (Figure 7C–G). We then assessed genes associated with epithelial differentiation (Figure 7C), epithelial repair (Figure 7D), and tight junctions (Figure 7E). As expected (51), expression of the differentiation-associated genes *KRTDAP*, *IVL*, *CNFN*, and *CALML3* was reduced following IL13 treatment. In contrast, IL1A maintained expression of *KRTDAP*, *IVL*, and *CALML3*, while significantly increasing *CNFN*. Combined IL13 and IL1A treatment largely recapitulated the IL13 phenotype (Figure 7C).

We next examined genes associated with epithelial repair (Figure 7D). *SPRR1A*, *SPRR2D*, and *SPRR2E*, encoding proteins that reinforce the structural integrity of the differentiated epithelium during tissue repair, together with *SLPI*, a secreted protease inhibitor that protects epithelial tissues from protease-mediated damage, were significantly induced by IL1A but not IL13. Co-treatment with IL13 prevented IL1A-mediated induction of these genes (Figure 7D).

Finally, we quantified expression of the tight junction component *TJPI* (ZO-1). IL1A, but not IL13, increased *TJPI* expression, and this effect was maintained in the presence of IL13 (Figure 7E). Consistent with these findings, western blot analysis demonstrated that IL13 reduced both ZO-1 and E-cadherin protein levels, whereas the effect on ZO-1 was attenuated by co-treatment with IL1A (Figure 7F). Collectively, these findings indicate that IL1A promotes a coordinated epithelial repair program by maintaining epithelial differentiation, inducing epithelial repair-associated genes, and increasing expression of a key tight junction component involved in barrier formation.

Since IL1A induces its own transcription (Figure 5H), we next examined whether this feed-forward loop was maintained under inflammatory conditions by quantifying IL1A secretion from EPC2 organoids treated with IL13, IL1A, or the combination for 7 days. As expected, IL1A treatment increased IL1A secretion, and this effect was maintained in the presence of IL13 (Figure 7G), indicating that an IL1A feed-forward loop remains active despite inflammatory signaling.

Finally, we assessed the combined effects of IL13 and IL1A on epithelial barrier integrity. Transepithelial electrical resistance (TEER) measurements after 6 days of differentiation in EPC2 ALI cultures confirmed that high (100ng/mL) IL13 impaired barrier formation (51). Co-treatment with IL1A did not significantly restore barrier integrity (Figure S7E). Similarly, IL1A did not rescue the effect of low (10ng/mL) IL13 at day 8 of ALI cultures using primary cells (Figure 7H). Together, these findings indicate that although IL1A is unable to overcome the detrimental effects of sustained IL13 on epithelial barrier function, IL1A signaling remains active, supporting a model in which IL13 selectively reshapes rather than abolishes epithelial IL1A responses.

## Discussion

Effective tissue repair requires epithelial cells to rapidly detect environmental insults and coordinate inflammatory responses with regeneration to restore barrier integrity. Here, we identify previously unrecognized spatial heterogeneity within the human esophageal basal progenitor compartment and demonstrate that the pro-inflammatory cytokine IL1A promotes basal progenitor renewal while enhancing epithelial barrier function.

A central finding of this study is that the basal compartment of the human esophageal epithelium exhibits marked spatial and functional heterogeneity. While NGFR is expressed broadly across basal cells, proliferation is concentrated to basal cells at papillary tips, which exhibit active YAP signaling and reside in close proximity to the luminal surface (Figure 1). These observations suggest that papillary architecture creates specialized progenitor niches with local microenvironmental cues, consistent with emerging concepts that epithelial stem cell function is spatially regulated by niche-derived signals and tissue architecture (52). The proximity of papillary basal cells to differentiated IL1A-expressing epithelial cells further raises the possibility that local cytokine gradients contribute to regional differences in progenitor cell states (Figure 4).

We further identified an intraepithelial apical-to-basal IL1A signaling cascade that is enhanced in patients with eosinophilic esophagitis (Figure 4) and demonstrate that IL1A signaling promotes basal progenitor expansion while simultaneously improving epithelial barrier function (Figure 6). Although eosinophilic esophagitis is classically viewed as a type 2 cytokine-driven disease, increasing evidence suggests that epithelial-derived stress signals are important mediators of disease initiation (37, 53, 54). Alongside the established epithelial alarmins IL-33 and TSLP, we identify IL1A as an additional epithelial-derived injury-response cytokine that couple inflammatory signaling to esophageal epithelial regeneration, in line with the role of IL1A in other barrier epithelia (55). Our data extend previous observations by demonstrating that epithelial-derived IL1A directly promotes esophageal basal progenitor cell renewal and barrier integrity while simultaneously activating NF- $\kappa$ B-dependent inflammatory transcriptional programs.

The dual functions of IL1A may provide insight into EoE pathophysiology. We propose that transient IL1A signaling promotes epithelial regeneration following tissue damage, whereas persistent activation during chronic inflammation could contribute to pathological tissue remodeling. Active IL13

signaling dampens and rewires epithelial transcriptional and functional responses to IL1A (Figure 7), suggesting that local immune-cell driven cytokine environments influence how epithelial cells interpret injury-associated alarmins. Consequently, the biological outcome of IL1A signaling could promote distinct epithelial programs depending on the local inflammatory environment.

Together, our findings support a model in which IL1 signaling constitutes an integral component of the epithelial injury-response program in the human esophagus. Rather than functioning solely as a classical pro-inflammatory pathway, IL1A appears capable of coordinating inflammatory activation with epithelial regeneration and maintenance of barrier integrity. Future studies defining how IL1 signaling integrates with type 2 inflammatory pathways within distinct basal progenitor niches may provide opportunities to selectively enhance epithelial repair while limiting chronic inflammatory remodeling in eosinophilic esophagitis.

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### **Author contributions**

EE and MG conceptualized the project. EE, FK, MN, KT and MG designed the experiments. EE, MLT, LL, AK, MTM and DG performed the experiments. FK, MN and KT identified donors and collected human samples. EE, LL, AK and MLT analyzed and curated the data. EE, MLT, LL and MG drafted the manuscript. EE and MG supervised the project. MG was responsible for resource and funding acquisition. All authors have proof-read and approved the final version of the manuscript.

## Data and Code Availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Single cell-sequencing EoE atlas was downloaded from the Broad Institute Single Cell Portal (SCP1242) and the bulk RNA Prime-sequencing data is available at GEO (GSE314119, reviewer token glszqyaspoxfkj). All code supporting single-cell and bulk transcriptional data processing, analysis, and visualization is or will be publicly available before publication at [github.com/paulojannig/Prime-seq\\_analysis](https://github.com/paulojannig/Prime-seq_analysis).

## References

1. Niec RE, Rudensky AY, Fuchs E. Inflammatory adaptation in barrier tissues. *Cell*. 2021;184(13):3361-75.
2. Grommisch D, Eenjes E, Troost ML, Genander M. Epithelial architecture and signaling activity in the adult human esophagus. *Front Cell Dev Biol*. 2025;13:1632255.
3. Merves J, Muir A, Modayur Chandramouleeswaran P, Cianferoni A, Wang ML, Spergel JM. Eosinophilic esophagitis. *Ann Allergy Asthma Immunol*. 2014;112(5):397-403.
4. Rochman M, Rothenberg ME. Immune functions of the esophagus. *J Allergy Clin Immunol*. 2026;157(2):316-28.
5. Cavalli G, Colafrancesco S, Emmi G, Imazio M, Lopalco G, Maggio MC, et al. Interleukin 1alpha: a comprehensive review on the role of IL-1alpha in the pathogenesis and treatment of autoimmune and inflammatory diseases. *Autoimmun Rev*. 2021;20(3):102763.
6. Imai Y. Interleukin-33 in atopic dermatitis. *J Dermatol Sci*. 2019;96(1):2-7.
7. Calafiore M, Fu YY, Vinci P, Arnhold V, Chang WY, Jansen SA, et al. A tissue-intrinsic IL-33/EGF circuit promotes epithelial regeneration after intestinal injury. *Nat Commun*. 2023;14(1):5411.
8. Bersudsky M, Luski L, Fishman D, White RM, Ziv-Sokolovskaya N, Dotan S, et al. Non-redundant properties of IL-1alpha and IL-1beta during acute colon inflammation in mice. *Gut*. 2014;63(4):598-609.
9. Choi J, Park JE, Tsagkogeorga G, Yanagita M, Koo BK, Han N, et al. Inflammatory Signals Induce AT2 Cell-Derived Damage-Associated Transient Progenitors that Mediate Alveolar Regeneration. *Cell Stem Cell*. 2020;27(3):366-82 e7.
10. Liu C, Xu Y, Yang G, Tao Y, Chang J, Wang S, et al. Niche inflammatory signals control oscillating mammary regeneration and protect stem cells from cytotoxic stress. *Cell Stem Cell*. 2024;31(1):89-105 e6.
11. Janjic A, Wange LE, Bagnoli JW, Geuder J, Nguyen P, Richter D, et al. Prime-seq, efficient and powerful bulk RNA sequencing. *Genome Biol*. 2022;23(1):88.
12. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
13. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Camb)*. 2021;2(3):100141.
14. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst*. 2015;1(6):417-25.
15. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, 3rd, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. *Cell*. 2021;184(13):3573-87 e29.
16. Ding J, Garber JJ, Uchida A, Lefkovith A, Carter GT, Vimalathas P, et al. An esophagus cell atlas reveals dynamic rewiring during active eosinophilic esophagitis and remission. *Nat Commun*. 2024;15(1):3344.



17. McGinnis CS, Murrow LM, Gartner ZJ. DoubletFinder: Doublet Detection in Single-Cell RNA Sequencing Data Using Artificial Nearest Neighbors. *Cell Syst.* 2019;8(4):329-37 e4.
18. Korsunsky I, Millard N, Fan J, Slowikowski K, Zhang F, Wei K, et al. Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat Methods.* 2019;16(12):1289-96.
19. Browaeys R, Gilis J, Sang-Aram C, De Bleser P, Hoste L, Tavernier S, et al. MultiNicheNet: a flexible framework for differential cell-cell communication analysis from multi-sample multi-condition single-cell transcriptomics data. *bioRxiv.* 2023:2023.06.13.544751.
20. Browaeys R, Saelens W, Saeys Y. NicheNet: modeling intercellular communication by linking ligands to target genes. *Nat Methods.* 2020;17(2):159-62.
21. Busslinger GA, Weusten BLA, Bogte A, Begthel H, Brosens LAA, Clevers H. Human gastrointestinal epithelia of the esophagus, stomach, and duodenum resolved at single-cell resolution. *Cell Rep.* 2021;34(10):108819.
22. Rochman M, Wen T, Kotliar M, Dexheimer PJ, Ben-Baruch Morgenstern N, Caldwell JM, et al. Single-cell RNA-Seq of human esophageal epithelium in homeostasis and allergic inflammation. *JCI Insight.* 2022;7(11).
23. Tirosch I, Venteicher AS, Hebert C, Escalante LE, Patel AP, Yizhak K, et al. Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. *Nature.* 2016;539(7628):309-13.
24. Zhang Y, Jiang M, Kim E, Lin S, Liu K, Lan X, et al. Development and stem cells of the esophagus. *Semin Cell Dev Biol.* 2017;66:25-35.
25. Grommisch D, Wang M, Eenjes E, Svetlicic M, Deng Q, Giselsson P, et al. Defining the contribution of Troy-positive progenitor cells to the mouse esophageal epithelium. *Dev Cell.* 2024.
26. McGinn J, Hallou A, Han S, Krizic K, Ulyanchenko S, Iglesias-Bartolome R, et al. A biomechanical switch regulates the transition towards homeostasis in oesophageal epithelium. *Nat Cell Biol.* 2021;23(5):511-25.
27. Jeong Y, Rhee H, Martin S, Klass D, Lin Y, Nguyen le XT, et al. Identification and genetic manipulation of human and mouse oesophageal stem cells. *Gut.* 2016;65(7):1077-86.
28. Doupe DP, Alcolea MP, Roshan A, Zhang G, Klein AM, Simons BD, et al. A single progenitor population switches behavior to maintain and repair esophageal epithelium. *Science.* 2012;337(6098):1091-3.
29. Kasagi Y, Chandramouleeswaran PM, Whelan KA, Tanaka K, Giroux V, Sharma M, et al. The Esophageal Organoid System Reveals Functional Interplay Between Notch and Cytokines in Reactive Epithelial Changes. *Cell Mol Gastroenterol Hepatol.* 2018;5(3):333-52.
30. Ohashi S, Natsuzaka M, Yashiro-Ohtani Y, Kalman RA, Nakagawa M, Wu L, et al. NOTCH1 and NOTCH3 coordinate esophageal squamous differentiation through a CSL-dependent transcriptional network. *Gastroenterology.* 2010;139(6):2113-23.
31. Sarosiek J, Feng T, McCallum RW. The interrelationship between salivary epidermal growth factor and the functional integrity of the esophageal mucosal barrier in the rat. *Am J Med Sci.* 1991;302(6):359-63.
32. Skrupskelyte G, Rojo Arias JE, Ajith H, Dang Y, Rossetti D, Han S, et al. Precancerous niche remodelling dictates nascent tumour persistence. *Nature.* 2026;653(8113):242-53.
33. Das S, Parigi SM, Luo X, Fransson J, Kern BC, Okhovat A, et al. Liver X receptor unlinks intestinal regeneration and tumorigenesis. *Nature.* 2025;637(8048):1198-206.
34. Clevenger MH, Karami AL, Carlson DA, Kahrilas PJ, Gonsalves N, Pandolfino JE, et al. Suprabasal cells retain progenitor cell identity programs in eosinophilic esophagitis-driven basal cell hyperplasia. *JCI Insight.* 2023;8(19).
35. Rochman M, Travers J, Miracle CE, Bedard MC, Wen T, Azouz NP, et al. Profound loss of esophageal tissue differentiation in patients with eosinophilic esophagitis. *J Allergy Clin Immunol.* 2017;140(3):738-49 e3.
36. Underwood B, Troutman TD, Schwartz JT. Breaking down the complex pathophysiology of eosinophilic esophagitis. *Ann Allergy Asthma Immunol.* 2023;130(1):28-39.
37. Rochman M, Azouz NP, Rothenberg ME. Epithelial origin of eosinophilic esophagitis. *J Allergy Clin Immunol.* 2018;142(1):10-23.
38. Crowell HL, Sonesson C, Germain PL, Calini D, Collin L, Raposo C, et al. muscat detects subpopulation-specific state transitions from multi-sample multi-condition single-cell transcriptomics data. *Nat Commun.* 2020;11(1):6077.



39. Blanchard C, Mingler MK, Vicario M, Abonia JP, Wu YY, Lu TX, et al. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J Allergy Clin Immunol*. 2007;120(6):1292-300.
40. Masuda MY, Pyon GC, Luo H, LeSuer WE, Putikova A, Dao A, et al. Epithelial overexpression of IL-33 induces eosinophilic esophagitis dependent on IL-13. *J Allergy Clin Immunol*. 2024;153(5):1355-68.
41. Mishra A, Rothenberg ME. Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6-dependent mechanism. *Gastroenterology*. 2003;125(5):1419-27.
42. Zuo L, Fulkerson PC, Finkelman FD, Mingler M, Fischetti CA, Blanchard C, et al. IL-13 induces esophageal remodeling and gene expression by an eosinophil-independent, IL-13R alpha 2-inhibited pathway. *J Immunol*. 2010;185(1):660-9.
43. Pyon GC, Masuda MY, Putikova A, Luo H, Gibson JB, Dao AD, et al. Tissue-specific inducible IL-33 expression elicits features of eosinophilic esophagitis. *J Allergy Clin Immunol*. 2024;154(6):1545-53 e2.
44. Rochman M, Kellerman K, Jankowski MP, Rothenberg ME. The oesophagus as an immune organ. *Nat Rev Gastroenterol Hepatol*. 2025;22(9):657-67.
45. Gebhardt C, Nemeth J, Angel P, Hess J. S100A8 and S100A9 in inflammation and cancer. *Biochem Pharmacol*. 2006;72(11):1622-31.
46. Haskill S, Beg AA, Tompkins SM, Morris JS, Yurochko AD, Sampson-Johannes A, et al. Characterization of an immediate-early gene induced in adherent monocytes that encodes I kappa B-like activity. *Cell*. 1991;65(7):1281-9.
47. Shakhov AN, Collart MA, Vassalli P, Nedospasov SA, Jongeneel CV. Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor alpha gene in primary macrophages. *J Exp Med*. 1990;171(1):35-47.
48. Richmond A. Nf-kappa B, chemokine gene transcription and tumour growth. *Nat Rev Immunol*. 2002;2(9):664-74.
49. Kim B, Lee Y, Kim E, Kwak A, Ryoo S, Bae SH, et al. The Interleukin-1alpha Precursor is Biologically Active and is Likely a Key Alarmin in the IL-1 Family of Cytokines. *Front Immunol*. 2013;4:391.
50. Muir A, Falk GW. Eosinophilic Esophagitis: A Review. *JAMA*. 2021;326(13):1310-8.
51. Sasaki M, Wang JX, Zhou Y, Kennedy KV, Teranishi R, Itami T, et al. FOXM1 Modulation Alleviates Epithelial Remodeling and Inflammation in Eosinophilic Esophagitis. *bioRxiv*. 2025.
52. Thompson SM, Yaple VS, Searle GH, Phan QM, Makkar J, Cheng X, et al. Rete ridges form via evolutionarily distinct mechanisms in mammalian skin. *Nature*. 2026;651(8104):135-45.
53. O'Shea KM, Aceves SS, Dellon ES, Gupta SK, Spergel JM, Furuta GT, et al. Pathophysiology of Eosinophilic Esophagitis. *Gastroenterology*. 2018;154(2):333-45.
54. Travers J, Rochman M, Caldwell JM, Besse JA, Miracle CE, Rothenberg ME. IL-33 is induced in undifferentiated, non-dividing esophageal epithelial cells in eosinophilic esophagitis. *Sci Rep*. 2017;7(1):17563.
55. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev*. 2018;281(1):8-27.

## Main Figure Legends

### Figure 1. Spatial heterogeneity within the esophageal epithelium

(A) Single cell UMAP of human adult esophageal epithelium (16). (B) Heatmap displaying scaled expression of epithelial subcluster signature markers. (C) Protein localization of basal markers NGFR and P73 (left panel), or NGFR with P63 (right panel) in adult human esophageal tissue sections. Scale bar=25µm. (D) Immunofluorescent localization and quantification of the percentage of proliferating (KI67-positive) cells in either basal (NGFR-positive, 1<sup>st</sup> layer) or suprabasal (NGFR-negative, 2<sup>nd</sup> layer) cells. Closed arrows mark NGFR/KI67 double positive cells, whereas open arrows mark NGFR-negative, KI67-positive cells. Scale bar=25µm. n=4 donors, \*\* p<0.01, paired t-test. (E) Quantification of percentage of basal, KI67-positive cells, separated into papillary or interpapillary basal layer (IBL). Scale bar=25µm. n=3 donors, \*\* p<0.01, paired t-test. (F) Immunofluorescent staining of basal marker

NGFR and KRT4, marking differentiated cells (left panel) or basal marker KRT14 and KRT4 (right panel). Closed arrows indicate cells that co-express KRT14 and KRT4. Scale bar=25 $\mu$ m. (G) Basal NGFR-positive cells co-express nuclear, active, YAP (aYAP) in papillary tip regions. Closed and open arrows indicate NGFR-positive cells which are either positive or negative for aYAP, respectively. Scale bar=25 $\mu$ m.

### Figure 2. Notch signaling represses differentiation of NGFR-positive basal cells

(A) Immunofluorescent staining of ITGA6 and NGFR on human esophageal tissue. (B) Schematics of epithelial cell isolation strategy. (C) FACS graph confirming separation of ITGA6<sup>high</sup> epithelial cells into NGFR-negative (blue) and NGFR-positive (orange). (D) Representative images and quantification of organoid forming efficiency (OFE) comparing organoids established from basal (ITGA6<sup>high</sup>,NGFR<sup>pos</sup>) or suprabasal (ITGA6<sup>high</sup>,NGFR<sup>neg</sup>) cells. n=7 donors, \*\*\* p<0.001, paired t-test. (E-G) Organoids derived from NGFR-positive basal cells. (E) Organoids express KRT14 in the basal layer, whereas KLF4 and KRT4 mark differentiated layers. KI67 is restricted to the basal layer. (F) Organoids retain restricted P73 expression compared with P63. Closed arrows: P73/P63 double-positive basal cells. Open arrows: P63-positive suprabasal cells. (G) Basal cells co-express NGFR and P73. (H) Volcano plot showing DEGs between NGFR-negative and NGFR-positive primary human esophageal epithelial cells. (I) Projection of the top 50 NGFR-negative and NGFR-positive gene signatures onto the human esophageal epithelial single-cell UMAP (16). (J) GSEA analysis of DEGs identified when comparing NGFR-positive to NGFR-negative cells. (K) Dot plot displaying the relative expression of NOTCH receptors and ligands. (L) HES1 localization in human esophageal epithelium. (M) Bright-field images and quantification of organoid size in control and DAPT-treated organoids. n=7 independent experiments, 5 donors. \*\* p<0.01, paired t-test. (N-O) Characterization of control or DAPT-treated organoids. (N) DAPT treatment does not alter the percentage of KI67-positive cells (upper panel), the percentage of KLF4-positive cells is reduced (lower panel). n=4 (4 independent experiments, 3 donors), \*\* p<0.01, paired t-test. (O) DAPT administration does not alter the percentage of P63-positive cells (upper panel), the percentage of P73-positive cells per organoid is increased (lower panel). 4 independent experiments, 3 donors, \* p<0.05, paired t-test. (A,E,F,G and K) Scale bar=25 $\mu$ m. (D and M) Scale bar=200 $\mu$ m.

### Figure 3. Characterization of culture-induced cell state changes

(A) NGFR-positive basal cells are positive for proliferation marker KI67 in primary human organoids. Scale bar=25 $\mu$ m. (B) Schematic illustration relevant for (B-F). Bright-field images of organoids established from NGFR-negative and NGFR-positive organoid cells. Quantification of organoid-forming efficiency (OFE, left) and organoid size (right). n=5 independent experiments (3 donors), paired t-test. Scale bar=200 $\mu$ m. (C-E) Characterization of organoids derived from NGFR-negative or NGFR-positive organoid cells. Scale bar=25 $\mu$ m. (C) Quantification of KI67 in organoids derived from NGFR-negative or NGFR-positive cells. n=39 (39 organoids, 2 donors), unpaired t-test. (D) Localization and quantification of differentiation marker KLF4. n=25 (25 organoids, 2 donors), unpaired t-test. (E) Distribution and quantification of basal markers P73 (left, percentage) and NGFR (right, area). n=39 (39 organoids, 2 donors), unpaired t-test, \*\*\*\* p<0.0001. (F) FACS analysis of NGFR comparing organoids derived from NGFR-negative or NGFR-positive organoid cells. n=5 (5 independent experiments from 3 donors), \*\* p<0.01, paired t-test. (G) Schematic illustrating isolation and RNA sequencing of NGFR-negative and NGFR-positive cells from human esophagi and primary organoids. (H) Volcano plot comparing NGFR-negative and NGFR-positive organoid cell DEGs. P-value < 0.05 and Log2FoldChange > 0.58. (I) Signature of top 50 DEGs identified comparing NGFR-negative to NGFR-positive organoid cells projected onto the esophageal epithelial UMAP (16). (J)

GSEA analysis of DEGs identified when comparing organoid- to epithelial-derived NGFR-positive cells. **(K)** Volcano plot highlighting DEGs identified by comparing organoid- to epithelial-derived NGFR-positive cells. P-value <0.05 and Log2FoldChange>0.58. **(L)** GSEA analysis of DEGs identified when comparing organoid- to epithelial-derived NGFR-negative cells. **(M)** Volcano plot highlighting DEGs identified by comparing organoid- to epithelial-derived NGFR-negative cells. P-value <0.05 and Log2FoldChange>0.58. **(N)** Expression of IL1 signaling ligands and receptors in NGFR-positive (upper) and NGFR-negative (lower) epithelial- and organoid-derived cell populations. Data are shown as normalized RNA-sequencing counts.

#### Figure 4. Intraepithelial IL1 signaling is enhanced in EoE

**(A)** UMAP of epithelial cells from the human esophageal single-cell dataset (16), including healthy donors (n=5) and patients with active eosinophilic esophagitis (EoE; n=8). **(B-D)** Volcano plots showing differentially expressed genes in basal **(B)**, suprabasal **(C)**, and apical **(D)** cell populations comparing healthy and EoE samples (P-value < 0.05 and Log2FoldChange >0.58). **(E-F)** MultiNiche analysis (19, 20) identifying predicted cell-cell communication networks in healthy **(E)** or EoE **(F)** esophagi (16). Chord diagram showing the top 20 strongest ligand-receptor pairs towards basal cells. **(G)** Bubble plot showing the 20 most increased ligand-receptor interactions in healthy versus EoE. Color indicates scaled differences in ligand-receptor expression across donors. (\*) Indicates interactions ranked among the top 50. **(H)** Violin plot comparing *IL1A* expression in healthy, active EoE and remission patients. 0.22, 0.41 and 0.32 indicate the average expression levels of *IL1A*. **(I)** Immunofluorescence of IL1A and NGFR in human esophageal epithelium. Scale bar=25µm. **(J)** IL1A and KRT5 in active and remission EoE patient biopsies. Dotted line delineates the basal membrane. Scale bar=25µm. **(K)** IL1A immunoreactivity and quantification in primary organoids. Closed triangles: IL1A-positive basal cells. Open triangles: IL1A-negative basal cells. Arrows: IL1A-negative suprabasal cells. n=5 independent experiments (3 patients). Light grey dots represent P0. Scale bar=25µm. \*\*\*\*p<0.0001, unpaired t-test.

#### Figure 5. IL1A activates NF-κB signaling in both basal and suprabasal cells

**(A)** Schematic illustration for IL1A treatment and isolation of NGFR-negative and -positive organoid cells sent for RNA-sequencing. Sequenced cell populations were compared pairwise between organoids derived from the same donor. **(B)** DEGs in NGFR-negative (left) or NGFR-positive (right) cells treated with IL1A. P-value <0.05 and Log2FoldChange>0.58. **(C)** Venn Diagram displaying the overlap in significantly up- and downregulated genes in NGFR-negative and NGFR-positive cells after IL1A treatment. **(D)** Images of EPC2 cells treated with IL1A for 0 (Control), 20, 40 or 60 minutes (min). Scale bar=25µm. **(E)** EPC2 cells treated with IL1A for 20, 40, 60 or 360 minutes followed by qPCR analysis of IL1A target genes *NFKBIA*, *TNF* and *CXCL8*. Expression is relative to housekeeping genes *GAPDH* and *ACTIN*. n=3, \*p<0.05, \*\* p<0.01, \*\*\*p<0.001, One-way ANOVA. **(F)** Representative images of organoids treated with IL1A for 1 hour and stained for P65. Closed and open arrows indicate nuclear P65 in basal and suprabasal cells, respectively. Scale bar=25µm. **(G)** Illustration of IL1A signaling. **(H)** Organoids treated for 3 hours with Ana, IL1A or IL1A and Ana together followed by qPCR analysis target genes. n=4 (4 independent experiments from 2 donors), \*p>0.05, \*\* p<0.01, \*\*\*p<0.001, paired t-test.

#### Figure 6. IL1A couples expansion of the basal progenitor cell pool with barrier integrity

**(A)** Quantification of organoid forming efficiency (OFE) (left) or organoid size (right) comparing control to IL1A treatment at day 7. n=6 (6 independent experiments from 2 donors), \* p<0.01, paired t-test. Scale bar=200µm. **(B)** Immunofluorescence images and quantification of P63- (left) and P73-

positive (right) cells in control and IL1A-treated organoids. P73: n=10 (7 donors), \*\*\*\* p<0.0001; paired t-test. P63: n=7 (4 donors). Scale bar=25µm. (C) Quantification of percentage of cells expressing the differentiation marker KLF4 in control and IL1A-treated organoids. n=6 (6 independent experiments from 3 donors), paired t-test. Scale bar=25µm. (D) Quantification of KI67 in control and IL1A-treated organoids. n=10 (10 independent experiments from 5 donors), paired t-test. Scale bar=25µm. (E–G) EPC2 cells were cultured on inserts until confluence and differentiated at air-liquid interface (ALI) for 14 days in the absence or presence of IL1A. (E) Immunofluorescence for P63 (basal) and KLF4 (differentiation). (F) COL17A1 and KI67 localize to the basal layer. (G) ZO-1 staining is more pronounced in suprabasal cells following IL1A treatment. Scale bars = 25µm. (H) Barrier integrity assay using ALI cultures. IL1A reduced transepithelial passage of apically applied fluorescent dextran (3–5 kDa). \*\* p<0.01, \*\*\*\* p<0.0001, Two-way ANOVA with Bonferroni's multiple comparison test (left graph), paired t-test (right graph).

**Figure 7. Inflammation reshapes IL1A-dependent changes in epithelial cell state programs**

(A) Western blot and ELISA for IL1A after 16 hours of stimulation with LPS or Poly I:C in EPC2 cells grown as 2D. Tubulin is used as loading control in western blot. \*\*p<0.01, One-way ANOVA. (B) Relative gene expression of IL1A target genes in EPC2 organoids after priming organoids with IL13 for 1 day, then supplementing with IL1A for 3 hours. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001\*\*\*\*, p<0.0001, One-way ANOVA. (C–E) Relative expression of genes linked to epithelial differentiation (C), barrier repair (D) and tight junctions (E). \* p<0.05, \*\*\* p<0.001\*\*\*\*, p<0.0001, One-way ANOVA. (F) Western blot for ZO-1 and ECAD from EPC2 organoids treated with IL13, IL1A or IL13+IL1A for 7 days. GAPDH and Tubulin are used as loading controls. (G) IL1A protein secretion in EPC2 organoids after 7days of IL13, IL1A or IL13+IL1A treatment. \* p<0.05, \*\*\* p<0.001\*\*\*\*, p<0.0001, One-way ANOVA. (H) Transepithelial resistance (TEER) measurement at day 8 of primary cells ALI comparing the effect of IL13, IL1A or IL13+IL1A.