IL-20 subfamily cytokines impair the esophageal epithelial barrier by diminishing filaggrins in eosinophilic esophagitis

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SUPPLEMENTAL MATERIAL AND METHODS

Mouse lines

C57BL/6 (WT), *Il19-TdTomato (Il19^{tdT}),*[1] *Il20R2$^{-/-}$* mice kindly provided by Franz Oswald, Internal Medicine I, University Hospital Ulm, Ulm, Germany,[2] *Stat3$^{flox/flox}$* mice kindly provided by Radek Skoda, Department of Biomedicine, University of Basel, Basel, Switzerland,[3] *Cx3cr1^{CreER}* (B6.129P2(Cg)-*Cx3cr1$^{tm2.1(cre/ERT2)Litt}$*/WganJ) and *Krt5-CreERT2* (B6N.129S6(Cg)-*Krt5$^{tm1.1(cre/ERT2)Blh}$*/J) mice were bred and maintained under specific pathogen-free (SPF) conditions in the animal facility of Department of Biomedicine, University of Basel, Switzerland. *Il19$^{flox/flox}$* have been generated as described in detail below. *Stat3$^{flox/flox}$* have been crossed with *Krt5-CreERT2* to obtain *Stat3$^{\Delta Krt5}$* mice, and *Il19$^{flox/flox}$* have been breed with *Cx3cr1^{CreER}* mice to generate *Il19$^{\Delta CX3CR1}$* mice. In *Stat3$^{\Delta Krt5}$* mice, the tamoxifen-inducible, Cre-mediated recombination will lead to the excision of *Stat3* in Keratin5^{+} squamous epithelial cells, and in *Il19$^{\Delta CX3CR1}$* the tamoxifen-inducible, Cre-mediated recombination will excise *Il19* in CX3CR1^{+} cells. We have randomly assigned the selected animals between 6-12 weeks of age to the experimental groups. At least three animals per group were used for the respective *in vitro* and *in vivo* experiments. Mouse experiments have been carried out following the Swiss Federal and Cantonal regulations (animal protocol number 2938 (canton Basel Stadt)).

Generation of *Il19*-flox mice

The *Il19* conditional knockout mice were generated by Biocytogen Pharmaceuticals (Wakefiled, USA). In brief, homology regions covering 4.5 kb upstream of *Il19* exon 4 and 4.0 kb downstream of 3'UTR were subcloned from a BAC clone (RP23-190F9; Invitrogen) from a *C57BL/6J* mouse genomic BAC library. FRT-flanked Neo resistance positive selection cassette was inserted downstream of 3'UTR, and two loxP sites were introduced

upstream of exon 4 and downstream of 3xStop, respectively. After linearisation, the targeting vector was transfected into *C57BL/6* embryonic stem (ES) cells by electroporation. Seven positive clones were identified by Southern blotting with 5'probe, 3'probe, and Neo probe (3'). Three positive clones were injected into Balb/c blastocysts and implanted into pseudopregnant females. Chimeric mice were crossed with Flp mice to obtain F1 mice carrying the recombined allele containing the floxed *Il9* and E2 SA IRES-TdTomato-WPRE-pA allele (**supplemental figure 5C**). The *Il19*-flox mice were genotyped by PCR by denaturation at 95^0C for 3 min, amplifying 40 cycles at 95^0C 30 sec, 62^0C 30 sec, 72^0C 25 sec, and elongating at 72^0C for 10 min. Primers are listed in **supplemental table 3**.

Esophageal organoids

Esophageal organoids have been generated from biopsies obtained from control and EoE subjects adapted to a protocol reported by Kasagi et al..[4] Briefly, after the preservation of biopsies, taken during upper endoscopy, in keratinocyte serum-free medium (KSFM; Keratinocyte-SFM (Gibco) containing 100 U/ml Penicillin, 100 µg/ml Streptomycin (Gibco), 50 µg/ml bovine pituitary extract (BPE; Gibco), 1 ng/ml epidermal growth factor (EGF; Gibco), and 0.09 mM Calcium chloride; (Sigma-Aldrich) on ice, the KSFM medium was replaced with 10 U/mL Dispase I (Corning) and incubated for 10 min at RT. Afterward, the biopsies were rinsed with PBS and digested in 0.05% Trypsin-EDTA (Sigma-Aldrich) using a ThermoMixer C (Eppendorf) at 37°C and 800rpm for 10 min. The cells were further dissociated with the plunger of a tuberculin syringe, filtered through a 70 µm cell strainer, and then filtered through a 35 µm cell strainer (BD Bioscience) in a round bottom polystyrene tube using 250 ug/ml soybean trypsin inhibitor (Sigma-Aldrich). After transfer in 15 ml falcon tubes, the single-cell suspension was spun down at 300xg, 4°C for 5 min, and counted after resuspending in KSFM using an EVE automated cell counter (NanoEntek).

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25000-30000 cells were seeded in a 40uL droplet Cultrex Basement Membrane Extract (BME), Type 2, Pathclear (R&D Systems). BME droplets were polymerised for 25min at 37°C before KSFM-C (Ca^{2+} concentration titrated to 0.6 mM using calcium chloride; Sigma-Aldrich) supplemented with 10 μM Y27632 small molecule ROCK inhibitor (Tocris) was added. The medium was changed every other day (without the Y27632 small molecule ROCK inhibitor). Organoids were cultured for 5-11 days.

Esophageal air-liquid interface cultures (ALIs)

Esophageal biopsies from control subjects obtained during upper endoscopy were collected in KSFM medium (Keratinocyte-SFM medium supplemented with 50ug/ml BPE, 100 U/ml Penicillin, 100 μg/ml Streptomycin and 1ng/ml EGF). After the dispersion of biopsies with 10 U/mL Dispase I (Corning) and 0.05% Trypsin/EDTA (Sigma-Aldrich), the primary keratinocytes were expanded in KSFM (Ca^{2+} 0.09mM) medium in T25 Primaria culture flasks (Corning; #353808) and passaged using TrypLE Express Enzyme (Gibco). The expanded keratinocytes (≥P2) were then seeded on inserts of 12-well or 24-well Transwell plates with 0.4 μm polyester membrane inserts (Corning; #3460 or #3470) at a density of 200'000 keratinocytes per 0.6 cm^2 for 12-well plates and 155'000 keratinocytes per 0.5 cm^2 for 24-well plates. ALIs were cultured with KSFM medium (Ca^{2+} 0.09mM) for 2 days until a confluent monolayer was formed. KSFM medium was replaced with a Calcium-rich KSFM medium (Ca^{2+} 1.8mM) on day 2 and replaced every other day until day 7. On day 7 the medium was removed from the upper chamber to expose the epithelium to air. Calcium-rich KSFM was supplemented with 10ng/ml KGF/FGF7 (R&D Systems) and 75ug/ml L-Ascorbic acid (Sigma-Aldrich) from day 7. ALIs were cultured for 14 days. Where indicated, ALIs were stimulated with100 ng /ml IL-19, IL-20, IL-24, and 50 μM ERK1/2 inhibitor PD98059 (Cell Signaling Technology) or 1 μM Cucurtabicin I (Tocris) from day 7 until day 14.

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Cell lines

The esophagus squamous cell carcinoma cell line KYSE-180 (ACC 379) was obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. The KYSE-180 cell line was cultured in 90% RPMI-1640 / 10% FBS supplemented with 100 U/ml Penicillin, 100 µg/ml Streptomycin. The cells were cultured at 37^0C, 5% CO_2, medium replaced every 2-3 days, and were passaged with 0.25% Trypsin-EDTA (SAFC Biosciences) upon 60-80% confluency.

Cytokine stimulation

Esophageal organoids, ALIs, and KYSE-180 cells were stimulated with 100ng/ml IL-19 (R&D Systems), IL-20 (R&D Systems), and IL-24 (R&D Systems) for 6-24h (KYSE-180), 24h (organoids) or 8d (ALIs). Where indicated, the STAT3 inhibitor Cucurtabicin I (Tocris) or ERK1/2 inhibitor PD98059 (Cell Signaling Technology) at a final concentration of 1-10 µM or 50 µM was added 2 h or 1 h before cytokine stimulation.

Measurement of transepithelial electrical resistance (TEER) and fluorescein isothiocyanate (FITC)-dextran flux

Transepithelial electrical resistance (TEER) was measured to monitor the epithelial integrity in ALI cultures using an EVOM 3 (World Precision Instruments) on days 0, 2, 4, 7, 9, 11, 12, 13, and 14 of culture. Paracellular flux was measured using FITC-dextran 3-5 kDa (Sigma-Alrdrich) at day 14 of culture in 15 to 30 min intervals for a total of 180 min using a fluorescence plate reader (BioTek).

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OVA-induced EoE mouse model

The protocol for an EoE mouse model has been adapted to a protocol reported by Noti et al..[5] In brief, mice were sensitised by treating both ears with 1 nmol MC903 (calcipotriol; Tocris) in 20 µl absolute ethanol followed by 10 µl of 5 mg/mL OVA Grade 5 (Sigma-Aldrich) dissolved in PBS. Treatment with the same volume of ethanol and OVA alone served as vehicle control. Sensitisation treatment is repeated daily for 14 days. On day 14 the drinking water is replaced with autoclaved water containing 1.5 g OVA / L and given for four days. On days 15 and 17, the mice are additionally challenged by gavage with 50 mg OVA in 100 µl water. Mice are sacrificed for organ collection on day 18 (**supplemental figure 4A**). *Stat3*$^{\Delta Krt5}$ and *Il19*$^{\Delta CX3CR1}$ mice and their respective *Stat3*$^{flox/flox}$ and *Il19*$^{flox/flox}$ littermates were administered 100 µL (75mg/kg body weight) tamoxifen (MedChemExpress) dissolved in corn oil (Sigma-Aldrich) via intraperitoneal injection (i.p.) daily from day 10 of sensitisation until the end of the experiment. Where indicated *WT* mice were treated with PD98059 (10mg/kg; MedChemExpress) diluted in 90% saline and 10% dimethyl sulfoxide (DMSO) v/v i.p. daily from day 14 until the end of the experiment. Experimental groups are defined as following: ctrl: non-sensitised+non-challenged, non-sens: non-sensitised+challenged, non-chal: sensitised+non-challenged, sens+chal: sensitised+challenged.

Mouse esophagus cell isolation

After preparing the esophagus by separating the esophagus from the trachea, cutting the distal and proximal ends of the esophagus, and pulling the esophagus out from the mediastinum, the esophagus is washed in PBS. Remnant connective tissue is removed, the esophagus is opened longitudinally, washed again with PBS, and minced in small pieces. The tissue is then digested in Roswell Park Memorial Institute (RPMI) 1640 (Sigma-Aldrich) containing 0.5mg/ml collagenase IV (Sigma-Aldrich) and 10U/ml DNase I (Roche) for 30mins in a

shaking (200rpm) water bath at 37°. The samples are vortexed every 10 min. Digested tissue was filtered through a 70 μM cell strainer (Sarstedt). The single-cell suspension was centrifuged 5 mins at 600xg (4°C) and transferred to conical 96-well plates (Sarstedt) for further analysis by flow cytometry.

Flow cytometry, cell Staining, and antibodies

Esophageal cells were stained for 30 mins at 4°C with fixable viability dye eFluor455UV (Invitrogen) and anti-CD16/CD32 (Fc receptor) clone 93 (BioLegend) for live/dead cell exclusion and to prevent non-specific binding. Cells were resuspended in PBS containing 2% Fetal Bovine Serum (FBS), 0.1% sodium azide, and 10 mM EDTA (FACS buffer) and stained for surface antigen markers for 20min at 4 ˚C. Stained cells were resuspended in FACS buffer and acquired on a Fortessa flow cytometer (BD Biosciences). Data were analysed using FlowJo software version 10.4.0 (TreeStar). Doublet discrimination on forward scatter (FSC-H) versus FSC-A plot was done in all analyses. The following antibodies have been used: Superbright 645-conjugated anti-mouse CD45 clone 30-F11 (Invitrogen), APC-conjugated anti-mouse CD49b (Integrin α2) clone DX5 (Invitrogen), APC/Fire 750-conjugated anti-mouse CD117 (c-kit) clone 2B8 (BioLegend), BV711-conjugated anti-mouse Siglec-F clone E50-2440 (BD Biosciences), FITC-conjugated anti-mouse IgE clone 23G3 (Invitrogen), PE/Cy7-conjugated anti-mouse CD64 (FcγR1) clone X54-5/7.1 (BioLegend) and FITC-conjugated anti-mouse CD11b clone M1/70 (BioLegend). Biotin-conjugated anti-mouse CD11c clone N418 (BioLegend), anti-mouse CD3e clone 145-2C11 (BioLegend), Biotin-conjugated anti-mouse CD19 clone 6D5 (BioLegend), Biotin-conjugated anti-mouse NK1.1 clone PK136 (BioLegend) and Biotin-conjugated anti-mouse TER-119/Erythroid cells clone TER-119 (BioLegend) followed by either eFluor450/Pacific Blue-conjugated Streptavidin (Invitrogen) or PE-Cy5-conjugated Streptavidin (BioLegend) was added for

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lineage exclusion. Eosinophils were identified as live, lin^-, $CD45^+$, Siglec-F^+ cells (**supplemental figure 7**). IL-19 producing macrophages were identified as live, lin^-, $CD45^+$, $CD11b^+$, $CD64^+$, $tdTomato^+$ cells.

RNA extraction and quantitative PCR

RNA was extracted from human esophageal biopsies, mouse esophagus, bone marrow-derived macrophages, and the KYSE-180 cell line using TRI Reagent (Sigma-Aldrich) or Direct-zol RNA MiniPrep (Zymo Research) according to the manufacturer's instructions. After removing DNA contaminants with DNase Max Kit (Qiagen), RNA samples were reverse transcribed using High Capacity cDNA Reverse Transcription (Applied Biosystems) according to the manufacturer's instructions. The SYBR Green PCR (Qiagen) or TakyonLow Rox SYBR MasterMix blue (Eurogentec) kits were used for the quantitative PCR, and PCR was run on an ABI ViiA 7 cycler. After normalisation of Ct to *GAPDH* or *ACTB*/*Actb,* the relative expression was calculated with the 2^(-ΔCt) formula. Used primers are listed in **supplemental table 2**.

Esophageal organoid embedding in histogel

After BME digestion with 1.5 U/mL Dispase II (Sigma-Aldrich), organoids are washed with PBS and fixated with 1 ml 4 % paraformaldehyde (PFA) for 30-60mins at RT. After fixation, organoids are centrifuged with 300xg for 5mins at 4°C and washed with PBS supplemented with 1% BSA. After washing, organoids are centrifuged (300xg for 5mins at 4°C) again and resuspended in 70 µL Histogel (Epredia). After polymerisation of the Histogel (60 min), organoids are stored in 50% EtOH until Paraffin embedding using the TPC 15 Tissue Processor (Medite Medizintechnik) and TES Valida embedding station (Medite Medizintechnik).

Hematoxylin-Eosin (H&E) staining

Esophageal tissues were fixed in 4 % paraformaldehyde and embedded in paraffin blocks. After sectioning, four-micrometer sections were stained with H&E and analysed with a Nikon Eclipse Ti2 microscope using the Nikon DS-Ri2 (RGB CMOS) camera. Eosinophil numbers were quantified using Fiji (ImageJ, Version 2.0.0-rc-68/1.52h).[6]

Immunohistochemistry

Tissue and organoid sections were deparaffinised in xylene and rehydrated in graded EtOH. Antigen retrieval was performed by incubation in citrate buffer solution (pH=6) for 20mins at 95°C in a microwave tissue processor (KOS). Endogenous peroxidases were blocked with 3% hydrogen peroxide (Roth) for 10 min at room temperature followed by 1 h blocking with PBS containing 0.1% Tween20 and 5-10% goat serum (all Sigma-Aldrich) before overnight incubation at 4°C with a rabbit anti-human IL-20Rα monoclonal antibody (Sino Biological Inc.), a rabbit anti-human IL-20Rβ polyclonal antibody (Abcam), a rabbit anti-Ki67 monoclonal antibody SP6 (Abcam; kindly provided by Rishika Agarwal, Skin biology group, Department of Biomedicine, University of Basel, Basel, Switzerland) or a rabbit anti-human FLG2 polyclonal (Novus; 1:200). On the next day, the sections were washed with PBS and incubated with a goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch; 1:500) for 2h at room temperature. 3,3’ Diaminobenzidine substrate (BD Pharmingen) was used to reveal staining. Sections were counterstained with hematoxylin, dehydrated with graded EtOH and xylene, and mounted with a xylene-based mounting medium.

Immunohistochemistry for FLG in human paraffin sections was performed by Ventana Discovery Ultra (Roche Diagnostics Suisse SA) automated stainer. In brief, tissue sections were deparaffinised and rehydrated. Antigen retrieval was performed by heat in Cell Conditioning buffer 1 (CC1, Ventana; #950-124) at 95°C for 40 minutes. Primary rabbit anti-

human FLG polyclonal (Abcam, 1:100) antibody was manually applied and incubated for 1 hour at 37°C. After washing, sections were incubated with an HRP-Polymer secondary antibody for 1 hour at 37°C. Alternatively, an amplification kit (Ventana; #760-080) was used to further amplify the signal before the detection by an HRP-Polymer antibody. Detection was performed using the Ventana DISCOVERY ChromoMap DAB (Ventana; #760-159) detection kit. Tissues were then counterstained with hematoxylin II, followed by the bluing reagent (Ventana; respectively #790-2208 and #760-2037). Sections were then dehydrated, cleared and mounted with permanent mounting and coverslips. Imaging was performed using a Nikon Eclipse Ti2 microscope using the Nikon DS-Ri2 (RGB CMOS) camera and analysed with Fiji (ImageJ, Version 2.0.0-rc-68/1.52h)[6] or QuPath[7] software.

Immunofluorescence staining

Mouse tissues were fixed with 4% PFA and embedded in paraffin. All tissues were sectioned at 4 μM. Blocking and permeabilisation were done using PBS containing 0.1% Tween20 and 5-10% Goat serum (all Sigma-Aldrich). Tissue sections were stained with Chicken anti-Keratin 5 polyclonal clone Poly9059 (BioLegend) and Alexa Fluor 488 AffiniPure goat anti-chicken IgY (IgG) (H+L) secondary antibody (Jackson ImmunoResearch) together with rabbit anti-Stat3 monoclonal clone D3Z2G (Cell Signaling Technology) and Alexa Fluor 647 goat anti-rabbit IgG secondary antibody (Invitrogen). For all samples, nuclear staining was performed using Nuc Blue™ Live Cell Stain (Invitrogen), and imaging was done using a Nikon A1R confocal microscope. Brightness and contrast settings were maintained between images using NIS software.

Esophageal organoid RNA isolation

BME is digested by incubation with 1.5 U/mL Dispase II (Sigma-Aldrich) for 20-25 min at 37°C. After digestion Dispase II (Sigma-Aldrich) is removed, the organoids are washed with PBS supplemented with 1% BSA and centrifuged at 300xg for 5mins at 4°C. PBS is removed, and 1 ml RNA lysis buffer (Qiagen) is added. The organoids are vortexed and resuspended with a pipette until all organoids are lysed and transferred to 1.5 ml Eppendorf Tube (Sarstedt) and frozen at -80°C until usage. RNA was isolated using RNeasy Plus Mini Kit (Qiagen).

RNA sequencing

Organoids derived from esophagus biopsies of five control donors were subjected to a cytokine treatment (IL19, IL20 and IL24) for 24h after 11 days of culture. Unstimulated organoids from the same donors were kept in culture in parallel. Total RNA was extracted using RNeasy Plus Mini Kit (Qiagen) and quality-checked on the Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA, USA) using the RNA 6000 Nano Chip (Agilent Technologies, Santa Clara, CA, USA) and then quantified by Spectrophotometry using the NanoDrop ND-1000 Instrument (NanoDrop Technologies, Wilmington, DE, USA). RNA-seq library preparation was performed at the Genomics Facility Basel of the ETH Zurich, Basel: 220ng total RNA of each sample was aliquoted for library preparation with the TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA, USA). After quality-check of the libraries on the Fragment Analyzer (Advanced Analytical, Ames, IA, USA) with the Standard Sensitivity NGS Fragment Analysis Kit (Advanced Analytical) (average concentration was 126±13 nmol/L and average library size was 345±7 base pairs), samples were pooled to equal molarity. Each pool was quantified by PicoGreen Fluorometric measurement to be adjusted to 1.5 pM and used for clustering on the NextSeq 500 instrument

(Illumina). Paired-end 38nt reads were produced using the NextSeq 500 High Output Kit 75-cycles (Illumina). Raw fastq files were generated using the Illumina RTA version 2.4.11 and bcl2fastq-2.18.0.12.

Data analysis was performed by the Bioinformatics Core Facility, Department of Biomedicine, University of Basel. Read quality was assessed with the FastQC tool (version 0.11.5). Reads were mapped to the human genome hg38 "analysis set" with STAR (version 2.7.0c)[8] with default parameters, except filtering out multimapping reads with more than 10 alignment locations (*outFilterMultimapNmax=10*) and filtering reads without evidence in the spliced junction table (*outFilterType="BySJout"*). All subsequent analyses were performed using the R software (version 4.0.3) and Bioconductor 3.12 packages. The *featureCounts* function from the Bioconductor Rsubread package (version 2.0.1)[9] was used to count the number of read (5' ends) overlapping with the exons of each gene (Ensembl release 96) assuming an exon union model.[10, 11]

The Bioconductor package edgeR (version 3.30.3)[12] was used for differential gene expression analysis. Between samples normalisation was performed using the TMM method.[13] Genes with CPM values above 1 in at least 4 samples were retained for the differential expression analysis. To test for differences between the stimulated and unstimulated organoids a model accounting for the condition and donor effects was fitted to the read counts using a quasi-likelihood testing framework (edgeR functions *glmQLFit* and *glmQLFTest*).[14] P-values were adjusted by controlling the false discovery rate (FDR; Benjamini-Hochberg method) and genes with an FDR lower than 1% were considered significant. Gene set enrichment analysis was performed with the function camera[15] from the edgeR package (using the default parameter value of 0.01 for the correlations of genes within gene sets) using gene sets from the c5 collection (Gene Ontology categories) of the

MSigDB Molecular Signature Database (version 7.0).[16] We filtered out sets containing less than 10 genes, and gene sets with an FDR lower than 5% were considered significant.

The processed read count table of the RNA-seq dataset is available on GEO under accession GSE181261 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE181261).[17]

Reanalysis of public RNA-seq datasets

Raw data for the accessions GSE58640,[18] GSE65335,[19] and GSE103356[20] were retrieved from the GEO database and processed similarly to described above, to extract genes and gene sets differentially regulated between respectively (i) esophageal tissues from patients with eosinophilic esophagitis vs. control donors, (ii) IL-13 stimulated vs. unstimulated differentiated EPC2 immortalised esophageal epithelial cells and (iii) SPINK7-silenced vs. control differentiated EPC2 cells.

For unknown reasons, in GSE58640, all samples but one included paired-end reads. The sample GSM1415921 sequenced with single-end reads was excluded, leaving us with a total of 6 control donors and 9 EoE patients. In both groups donors of different sex were present, a factor which was associated to the second component of a principal component analysis. We thus included sex as a covariate in the model for differential expression analysis.

Global Proteomics Analysis using Tandem Mass Tags (TMT)

After the digestion of the BME with 1.5 U/mL Dispase II (Sigma-Aldrich), 100 µL protein lysis buffer (5% SDS, 10 mM TCEP, 100 mM Triethyloammonium bicarbonate (TEAB), pH = 8.5) was added, and organoids were lysed by tissue lyser (Qiagen) in the 4°C cold room at 25/s. Then three freeze/thaw-cycles with dry ice were performed, and the samples were centrifuged with maximum speed at 4°C for 20min to transfer the protein lysate without debris into a new 1.5 ml Eppendorf tube. Protein concentration was determined by BCA assay (Thermo Fisher Scientific) using a small sample aliquot. Sample aliquots containing 50

µg of total proteins were reduced for 10 min at 95 °C and alkylated at 15 mM iodoacetamide for 30 min at 25 °C in the dark. Proteins were purified and digested using S-traps micro columns (Protifi, NY, US) according to the manufacturer's instructions. Samples were dried under vacuum and stored at -80 °C until further use.

Sample aliquots comprising 5 µg of peptides were labeled with isobaric tandem mass tags (TMT 10-plex, Thermo Fisher Scientific) as described previously[21] using a Freedom Evo 100 liquid handling platform (Tecan Group Ltd., Männedorf, Switzerland). Shortly, peptides were resuspended in a 10 µl labeling buffer (2 M urea, 0.2 M HEPES, pH 8.3), and 2.5 µl of each TMT reagent was added to the individual peptide samples followed by a 1 h incubation at 25°C, shaking at 500 rpm. The labeling reaction was quenched by adding 0.75 µl aqueous 1.5 M hydroxylamine solution and incubation for 10 min at 25°C. After pooling, the pH of the obtained sample was increased to 11.9 by adding 1 M phosphate buffer (pH 12) and incubated for 20 min at 25°C to remove TMT labels linked to peptide hydroxyl groups. Subsequently, the reaction was stopped by adding 2 M hydrochloric acid until a pH < 2 was reached. Finally, the peptide sample was further acidified using 5 % TFA, desalted using Sep-Pak Vac 1cc (50 mg) C18 cartridges (Waters) according to the manufacturer's instructions, and dried under vacuum.

TMT-labeled peptides were fractionated by high-pH reversed-phase separation using an XBridge Peptide BEH C18 column (3,5 µm, 130 Å, 1 mm x 150 mm, Waters) on an Agilent 1260 Infinity HPLC system. Peptides were loaded on a column in buffer A (20 mM ammonium formate in water, pH 10) and eluted using a two-step linear gradient from 2% to 10% in 5 minutes and then to 50% buffer B (20 mM ammonium formate in 90% acetonitrile, pH 10) over 55 minutes at a flow rate of 42 µl/min. The elution of peptides was monitored with a UV detector (215 nm, 254 nm). Thirty-six fractions were collected and pooled into 12

fractions using a post-concatenation strategy as previously described[22] and dried under vacuum.

Dried peptides were resuspended in 0.1% aqueous formic acid and subjected to LC-MS/MS analysis using an Exploris 480 Mass Spectrometer fitted with an Ultimate 3000 nano-LC (both Thermo Fisher Scientific) and a custom-made column heater set to 60°C. Peptides were resolved using a RP-HPLC column (75µm × 30cm) packed in-house with C18 resin (ReproSil-Pur C18–AQ, 1.9 µm resin; Dr. Maisch GmbH) at a flow rate of 0.3 µLmin-1. The following gradient was used for peptide separation: from 2% B to 10% B over 5 min to 30% B over 70 min to 50 % B over 15 min to 95% B over 2 min followed by 18 min at 95% B. Buffer A was 0.1% formic acid in water, and buffer B was 80% acetonitrile, 0.1% formic acid in water.

The mass spectrometer was operated in DDA mode with a FAIMS device attached. FAIMS was run in standard resolution mode with 2 alternating CV voltages of -45 and -60V. Total cycle time of approximately 3 s (1.5s per CV voltage). Each MS1 scan was followed by high-collision-dissociation (HCD) of the most abundant precursor ions with dynamic exclusion set to 30 seconds. For MS1, AGC target was set to 300% with a fill time of 25 ms using a resolution of 120,000 FWHM (at 200 m/z). MS2 scans were acquired at a target setting of 200%, a maximum accumulation time of 100 ms, and a resolution of 30,000 FWHM (at 200 m/z) with the enabled TurboTMT option. Singly charged ions and ions with unassigned charge state were excluded from triggering MS2 events. The normalised collision energy was set to 38%, the mass isolation window was set to 0.7 m/z, and one microscan was acquired for each spectrum. The precursor fit threshold was set to 70 % at a fit mass window size of 0.7 m/z.

The acquired raw files were searched against a protein database containing sequences of the

predicted SwissProt entries of *homo sapiens* (www.ebi.ac.uk, release date 2020/04/17) and commonly observed contaminants (in total 20,742 sequences) using the SpectroMine software (Biognosys, version 1.0.20235.13.16424). Standard Pulsar search settings for TMT ("TMT_Quantification") were used. Missing intensities were replaced by the global minimum value, and in a given sample the intensities of all peptides belonging to the same protein were summed up.

The differences in protein levels between the stimulated and unstimulated organoids were then tested using the package limma (version 3.44.3)[23] by fitting a linear model accounting for the condition and donor effects to the TMM-normalised logCPM (counts per million) values with the *eBayes* function options *trend=TRUE* and *robust=TRUE*. Given the low number of differentially expressed proteins, we used an FDR cutoff of 20% for significance. The gene set enrichment analysis was performed similarly to the RNA-seq dataset. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[24] partner repository with the dataset identifier PXD031509 and 10.6019/PXD031509.[25]

Bone marrow cell isolation and mouse bone marrow-derived macrophages

After the preparation of femurs and tibias and removal of connective tissues and muscles, the bones were cut at the epiphysis, and the bone marrow was flushed out using RPMI 1640 medium (Sigma-Aldrich). After the passage of collected cells through a 70 µm cell strainer, the bone marrow cells were further differentiated into macrophages. In brief, macrophages were generated by culturing in in RPMI 1640 medium containing 10% FCS and supplemented with 0.05 mM 2-ME, 100 U/ml penicillin, 100 µg/ml streptomycin, and 20 ng/ml M-CSF (BioLegend). After 7 days, macrophages were either stimulated with 100 ng/ml Lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 (Sigma-Aldrich) and 10

ng/ml recombinant mouse IFN-γ (rmIFN-γ; BioLegend) or with 10 ng/ml recombinant mouse IL-4 (rmIL-4; BioLegend) and 10 ng/ml recombinant mouse IL-13 (rmIL-13; BioLegend) for 6 hours before cell analysis.

scRNA sequencing

Esophageal samples from *WT* and *Il20R2*$^{-/-}$ mice with induced experimental EoE were digested as indicated above and TotalSeq-B antibodies (BioLegend) were used to barcode the individual single-cell suspensions. Hash tagging antibodies TotalSeq-B0301 to B0305 (BioLegend) were used for samples from 5 *WT* animals and TotalSeq-B0306 to B0310 for samples from 5 *Il20R2*$^{-/-}$ animals. The pooled cell suspension was sorted for viable CD45.2^{+} (BioLegend) cells, and volumes aiming at a targeted recovery of 10,000 cells were loaded on 2 wells of a 10x Genomics Chromium Single Cell Controller to generate 3'end libraries using v3 chemistry. Single-cell libraries were sequenced on an SP flow-cell of an Illumina NovaSeq 6000 sequencer at the Genomics Facility Basel of the ETH Zurich (with 90nt-long R2 reads).

Library and cell barcode demultiplexing, read alignment to the mouse transcriptome (Ensembl release 98)[26] and generation of the table of UMI counts were performed using Cellranger (version 6.0.1). Further processing of the UMI counts table was performed by using R 4.0.5 and Bioconductor 3.12 packages, notably DropletUtils (version 1.10.3),[27, 28] scran (version 1.18.7),[29] and scater (version 1.18.6),[30] following mostly the steps illustrated in the Bioconductor OSCA book (https://bioconductor.org/books/release/OSCA/).[10, 29].

Cells were demultiplexed into their samples of origin using the function *hashedDrops* from the DropletUtils package (with default parameters except *min.prop=0.01*, and providing as input the relative abundances of HTOs in the ambient solution). Based on the observed

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distributions, cells with 0% or more than 10% of UMI counts attributed to the mitochondrial genes,[31] with less than 1,000 UMI counts, or with less than 500 detected genes were excluded. Doublets identified with the *hashedDrops* function or with the scDblFinder package (version 1.4.0) were excluded. A total of 2,633 KO cells (ranging from 500 to 897 cells per sample) and 3,421 WT cells (ranging from 271 to 877 cells per sample) were retained for the next steps of the analysis.

UMI counts were normalised with size factors estimated from pools of cells created with the scran package *quickCluster* function.[29, 32] To distinguish between genuine biological variability and technical noise we modeled the variance of the log-expression across genes using a Poisson-based mean-variance trend. The scran package *denoisePCA* function was used denoise log-expression data by removing principal components corresponding to technical noise (20 PCs retained). The scRNA-seq dataset is available on GEO under accession GSE190482 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190482).[33]

Enzyme-linked immunosorbent assay (ELISA)

The human IL-19 Quantikine ELISA Kit (R&D Systems), the human IL-20 Quantikine ELISA Kit (R&D Systems), and the human IL-24 DuoSet ELISA (R&D Systems) were used to determine the IL-19, IL-20, and IL-24 concentrations in sera from control and EoE subjects.

Mouse IgE was measured with the ELISA MAX™ Deluxe Set Mouse IgE kit (BioLegend), mouse OVA-specific IgE was detected with the LEGEND MAX™ Mouse OVA-specific IgE ELISA kit (BioLegend) in the serum of *WT*, *Il19*^{tdT} and *Il20R2*$^{-/-}$ mice. Absorbance was measured at 450 nm and 570 nm on a microplate-ELISA reader (BioTek) and the absorbance at 570 nm was subtracted from absorbance at 450 nm.

Western blotting

Following stimulation with IL-19, IL-20, and IL-24, an ice-cold RIPA buffer containing sodium orthovanadate, PMSF, and protease inhibitor cocktail (Santa Cruz) was used to lyse the cells. After quantifying the protein concentrations with the Bicinchoninic Acid (BCA) method, 10-20 μg of each protein sample was transferred onto a nitrocellulose membrane by electrophoretic separation. After blocking the membranes with 5% dry milk in Tris Buffered Saline + 0.1% Tween20 (TBS-T) buffer for 1 h, the membrane was incubated with the following primary antibodies overnight at 4°C: phospho- NF-κB (p65), NF-κB, phospho-ERK1/2, ERK1/2, phospho- STAT3, STAT3 (all from Cell Signaling Technology) and β-actin (BD Biosciences) at 1:1000 or 1:2000 dilution. After washing in TBS-T for 5 min (3x), the membrane was incubated with horseradish peroxidase-conjugated secondary antibodies anti-rabbit IgG (H+L) and anti-mouse IgG (H+L) (both Jackson ImmunoResearch) at 1:30000 dilution for 1h at room temperature before SuperSignal™ West Femto Maximum Sensitivity Substrate or SuperSignal™ West Pico PLUS Chemiluminescent Substrate (both Thermo Scientific) were used to develop the blots.

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SUPPLEMENTAL TABLES

Supplemental Table 1. Patient characteristics

	Controls (n=30)	Active EoE (n=22)	EoE steroids/ remission (n=12)
Age, mean ± SD	37.74 ± 11.09	38.77 ± 8.55	37.33 ± 11.52
Male sex n (%)	21 (67.74)	16 (72.73)	9 (75)
Body weight, kg, mean ± SD (n)	76.7 ± 17.8 (31)	80.97 ± 18.11 (18)	68.57 ± 9.45 (12)
Time since first EoE symptoms, y. mean ± SD	-	8.09 ± 4.92	7.67 ± 8.02
Time since first EoE diagnosis, y. mean ± SD	-	3.14 ± 3.82	3.25 ± 2.64
History of esophageal dilatations, n (%)	0 (0)	0 (0)	2 (16.67)
History of atopic diseases, n (%)	7 (22.58)	16 (72.73)	9 (75)
Having experienced, n (%)			
– **Nausea/Vomiting**	11 (36.67)	0 (0)	1 (8.33)
– **Heartburn**	11 (36.67)	3 (13.64)	4 (33.33)
– **Epigastric pain**	9 (30)	1 (4.55)	1 (8.33)
– **Regurgitation**	13 (43.33)	3 (13.64)	3 (25)
– **Bloating**	2 (6.67)	0 (0)	0 (0)
– **Dysphagia**	4 (13.33)	22 (100)	12 (100)
– **Odynophagia**	0 (0)	3 (13.64)	3 (25)
– **Food impaction**	0 (0)	21 (95.45)	9 (75)
PPI treatment, n (%)	12 (38.71)	7 (31.82)	4 (33.33)
Steroids treatment, n (%)	0 (0)	0 (0)	9 (75)
Peak eos/hpf, mean ± SD	-	83.05 ± 34.96	35.17 ± 59.06
– **≥15 eos/hpf n (%)**	-	22 (100)	5 (41.67)
Endoscopist assessment, n (%)			
No signs of EoE	28 (90.32)	1 (4.55)	1 (8.33)
Moderate to severe signs of EoE	3 (9.68)	21 (95.45)	11 (91.67)
– **edema**	1 (4.76)	10 (45.45)	5 (41.67)
– **exsudates**	0 (0)	14 (63.64)	6 (50)
– **furrows**	3 (9.68)	19 (86.36)	7 (58.34)
– **rings**	0 (0)	14 (63.64)	6 (50)
– **stricture**	0 (0)	3 (13.64)	2 (16.67)

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Supplemental Table 2. qPCR primers

Target gene	ENSEMBL gene code	Forward sequence	Reverese sequence
Human IL20RA	ENSG00000016402	AGA GGT GGC ACT GAC TAC AGA	ACT GGG ACC ACG TTC TGT TTG
Human IL20RB	ENSG00000174564	AAC TCA ACC ATC CTT ACC CGA C	TTC ACA CAG TAT GCA GCC CC
Human IL22RA 1	ENSG00000142677	CTC TGC AGC ACA CTA CCC TCA	CGT GGG GGT AGG ATG AAC AAT C
Human IL19	ENSG00000142224	*Qiagen QT00022015*	
Human IL20	ENSG00000162891	*Qiagen QT00044905*	
Human IL24	ENSG00000162892	*Qiagen QT00059059*	
Human FLG	ENSG00000143631	TCG GCA AAT CCT GAA GAA TCC A	TGC TTT CTG TGC TTG TGT CCT
Human FLG2	ENSG00000143520	ACC CAG ATG ATC CAG ACA CAG	TTG CTG AGG ACC TTG TTG CAG
Human SPINK7	ENSG00000145879	TCC CAG GCT CTG ACT GAG TTT	TGG GTT TGT AGG GGTAGC ACA
Human CCL5	ENSG00000271503	CTG CTG CTT TGC CTA CAT TGC C	CAC ACA CTT GGC GGT TCT TTC G
Human CCL11	ENSG00000172156	AGA GGC TGA GAC CAA CCC AGA	ACT TCT TCT TGG GGT CGG CA
Human CCL24	ENSG00000106178	GGA GTG GGT CCA GAG GTA CA	TTA GCA GGT GGT TTG GTT GC
Human CCL26	ENSG00000006606	TGG AAT TGA GGC TGA GCC AAA G	CTC CCA CGT GTG GCA GTT
Human ACTB	ENSG00000075624	AGC CTC GCC TTT GCC GA	CTG GTG CCT GGG GCG
Mouse Il20ra	ENSMUSG00000020007	AAG TCG AGA AGA ACG TGG TC	GGG TGT TTT TCC TTG CCA AC
Mouse Il20rb	ENSMUSG00000044244	AAT GCT CAC CGA CCA AAA GT	AGG ACA GTT GCA TTT CGG TT
Mouse Il22ra1	ENSMUSG00000037157	GTT CTG CAA CCT GAC TAT GGA G	GTA CAG GTG GCT TGG TGA TG
Mouse Il19	ENSMUSG00000016524	CTG GGC ATG ACG TTG ATT CT	TCT CCA GGC TTA ATG CTC CT
Mouse Il20	ENSMUSG00000026416	Qiagen; Cat No. QT00126735	
Mouse Il24	ENSMUSG00000026420	Qiagen; Cat No. QT01054634	
Mouse Il5	ENSMUSG00000036117	GAT GAG GCT TCC TGT CCC TAC T	TGA CAG GTT TTG GAA TAG CAT TTC C
Mouse Il13	ENSMUSG00000020383	AAC GGC AGC ATG GTA TGG AGT G	TGG GTC CTG TAG ATG GCA TTG C

Mouse Flg	ENSMUSG00000102439	TTC TCA GAA GGC CAG GCA GTA	CCT CGC TGT GTT CTT GCT CAT
Mouse Flg2	ENSMUSG00000049133	ACA TTC TGG ATC CGG TCA CG	GGG CAC TTC TGG TCT GAC TG
Mouse Spink7	ENSMUSG00000060201	Bio-Rad; 10025636	
Mouse Actb	ENSMUSG00000029580	TTC TTT GCA GCT CCT TCG TT	ATG GAG GGG AAT ACA GCC C

Supplemental Table 3. Genotyping primers

Il20R2$^{-/-}$	**Stat3-flox**
CAG TCC CAT AGA GTA CAC TGA G	CAC CAA CAC ATG CTA TTT GTA GG
GGG AGA GAA AAT GCC CCA AAC C	CCT GTC TCT GAC AGG CCA TC
	GCA GCA GAA TAC TCT ACA GCT C
Il19^{tdT}	**Cx3cr1-CreER**
TGC TGC ATG ACC AAC AAC CT	AAG ACT CAC GTG GAC CTG CT
GAA TGA CAA TGT CCT GAC TCT GCA	CGG TTA TTC AAC TTG CAC CA
CAC GAC ATT CAA CAG ACC TTG CAT	AGG ATG TTG ACT TCC GAG TTG
Il19-flox	**Krt5-CreER**
CAA ACT GCA AGG GAA CTC AGT AGT G	GGA GGA AGT CAG AAC CAG GAC
CAC AGA CAA GGT TTG TTC CAC AGC	GCA AGA CCC TGG TCC TCA C
	ACC GGC CTT ATT CCA AGC

Supplemental Table 4. Key resources

Reagent or Material	**Supplier**	**Catalog No.**
Cell culture Media / Supplements		
Ascorbic Acid	Sigma-Aldrich (Merck)	A4544
Bovine pituitary extract	Gibco (Thermo Fischer Scientific)	3700015
Calcium chloride	Sigma-Aldrich (Merck)	21115
Cucurtabicin I	Tocris (Bio-Techne)	1571/1
Dimethyl sulfoxide (DMSO), anhydrous >99,9%	Sigma-Aldrich (Merck)	276855
Epidermal growth factor	Gibco (Thermo Fischer Scientific)	3700015
Fetal Bovine Serum (FBS)	Gibco (ThermoFischer Scientific)	10500064

Keratinocyte-SFM	Gibco (Thermo Fischer Scientific)	17005042
Lipopolysaccharides from Escherichia coli O111:B4	Sigma-Aldrich (Merck)	L2630
PD98059	Cell Signaling Technology	9900
Penicillin-Streptomycin	Gibco (Thermo Fischer Scientific)	15140122
Recombinant Human IL-19 Protein	R&D Systems (Bio-Techne)	1035-IL-025
Recombinant Human IL-20 Protein	R&D Systems (Bio-Techne)	1102-IL-025
Recombinant Human IL-24 Protein	R&D Systems (Bio-Techne)	1965-IL-025
Recombinant Human KGF/FGF-7 Protein	R&D Systems (Bio-Techne)	251-KG-010/CF
Recombinant Mouse IFN-γ (carrier-free)	BioLegend	575306
Recombinant Mouse IL-13 (carrier-free)	BioLegend	575904
Recombinant Mouse IL-4 (carrier-free)	BioLegend	574304
Recombinant Mouse M-CSF (carrier-free)	BioLegend	576406
Rosell Park Memorial Institute (RPMI) 1640 Medium	Sigma-Aldrich (Merck)	R8758
Y-27632 dihydrochloride	Tocris (Bio-Techne)	1254
Reagents		
Bovine Serum Albumin (BSA)	Sigma-Aldrich (Merck)	A2153
Buffer RLT	Qiagen	79216
Calcipotriol (MC903)	Tocris (Bio-Techne)	2700

Collagenase from *Clostridium histolyticum* Type IV	Sigma-Aldrich (Merck)	C5138
Corn oil	Sigma-Aldrich (Merck)	C8267
Cultrex Basement Membrane Extract (BME), Type 2, Pathclear	R&D Systems (Bio-Techne)	3532-010-02
DAB Substrate Kit	BD Biosciences	550880
Dimethyl sulfoxide (DMSO), >99,5% BioScience Grade	Carl Roth	A994
Dispase I	Corning	354235
Dispase II	Sigma-Aldrich (Merck)	D4693
DNase I recombinant	Roche	04536282001
Dulbeccos Phosphate Buffered Saline (DPBS)	Sigma-Aldrich (Merck)	D8537
eBioscience™ Fixable Viability Dye eFluor™ 455UV	Invitrogen (Thermo Fischer Scientific)	65-0868-18
eBioscience™ Streptavidin eFluor™ 450 Conjugate	Invitrogen (Thermo Fischer Scientific)	48-4317-82
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich (Merck)	EDS
Fluorescin isothiocyanate (FITC)-dextran ; average mol wt 3000-5000	Sigma-Aldrich (Merck)	FD4
Goat serum	Sigma-Aldrich (Merck)	G9023
HistoGel	Epredia	HG-4000-012
Hydrogen peroxide	Carl Roth	9681
Nuc Blue™ Live Ready Probes™ Reagent (Hoechst 33342)	Invitrogen (Thermo Fischer Scientific)	R37605
Ovalbumin Grade 5	Sigma-Aldrich (Merck)	A5503

Formaldehyde solution	Sigma-Aldrich (Merck)	F8775
PD98059	MedChemExpress	HY-12028
RIPA Lysis Buffer System	Santa Cruz	sc-24948
Sodium azide	Sigma-Aldrich (Merck)	71289
Streptavidin PE/Cyanine5	BioLegend	405205
SuperSignal™ West Femto Maximum Sensitivity Substrate	Thermo Scientific (Thermo Fischer Scientific)	34095
SuperSignal™ West Pico PLUS Chemiluminescent Substrate	Thermo Scientific (Thermo Fischer Scientific)	34580
Takyon™ Low ROX SYBR 2X MasterMix blue dTTP	Eurogentec	UF-LSMT-B0701
Tamoxifen	MedChemExpress	HY-13757A
TRI Reagent®	Sigma-Aldrich (Merck)	T9424
TrypLE Express Enzyme (1X), phenol red	Gibco (Thermo Fischer Scientific)	12605010
Trypsin inhibitor from *Glycine max* (soybean)	Sigma-Aldrich (Merck)	T9128
Trypsin-EDTA	SAFC Biosciences (Merck)	59418C
TWEEN® 20	Sigma-Aldrich (Merck)	P1379
Critical Commercial Assays		
Direct-zol RNA MiniPrep	Zymo Research	R2052
DNase Max Kit (discontinued)	Qiagen	15200
High-Capacity cDNA Reverse Transcription Kit	Applied Biosystems (Thermo Fischer Scientific)	4368813
QuantiNova SYBR Green PCR Kit	Qiagen	208056
RNeasy Plus Mini Kit	Qiagen	74136
Human IL-19 Quantikine ELISA Kit	R&D Systems (Bio-Techne)	D1900

Human IL-20 Quantikine ELISA Kit	R&D Systems (Bio-Techne)	DL200
Human IL-24 DuoSet ELISA	R&D Systems (Bio-Techne)	DY1965
ELISA MAX™ Deluxe Set Mouse IgE	BioLegend	432404
LEGEND MAX™ Mouse OVA Specific IgE ELISA Kit	BioLegend	439807

Supplemental Table 5. Cell lines

Name	**Description**	**Source**	**Reference**
KYSE-180	Esophageal squamous cell carcinoma cell line established from a well differentiated esophageal squamous cell carcinoma prior to treatment	Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (ACC379)	(PMID: 1728357)

Supplemental Table 6. Antibodies

Antibody	**Clone**	**Probe/Fluorophore**	**Supplier**	**Cat No.**
Antibodies for Immunohistochemistry staining				
Filaggrin	polyclonal		Abcam	Ab81468
Filaggrin family member 2	polyclonal		Novus Biologicals (Bio-Techne)	NBP1-91901
IL-20Ra	024		Sino Biological	10397-R024
IL-20Rb	polyclonal		Abcam	ab124332
Ki67	SP6		Abcam	ab16667
Peroxidase AffiniPure Goat	polyclonal	Horseradish Peroxidase (HRP)	Jackson ImmunoResearch	111-035-003

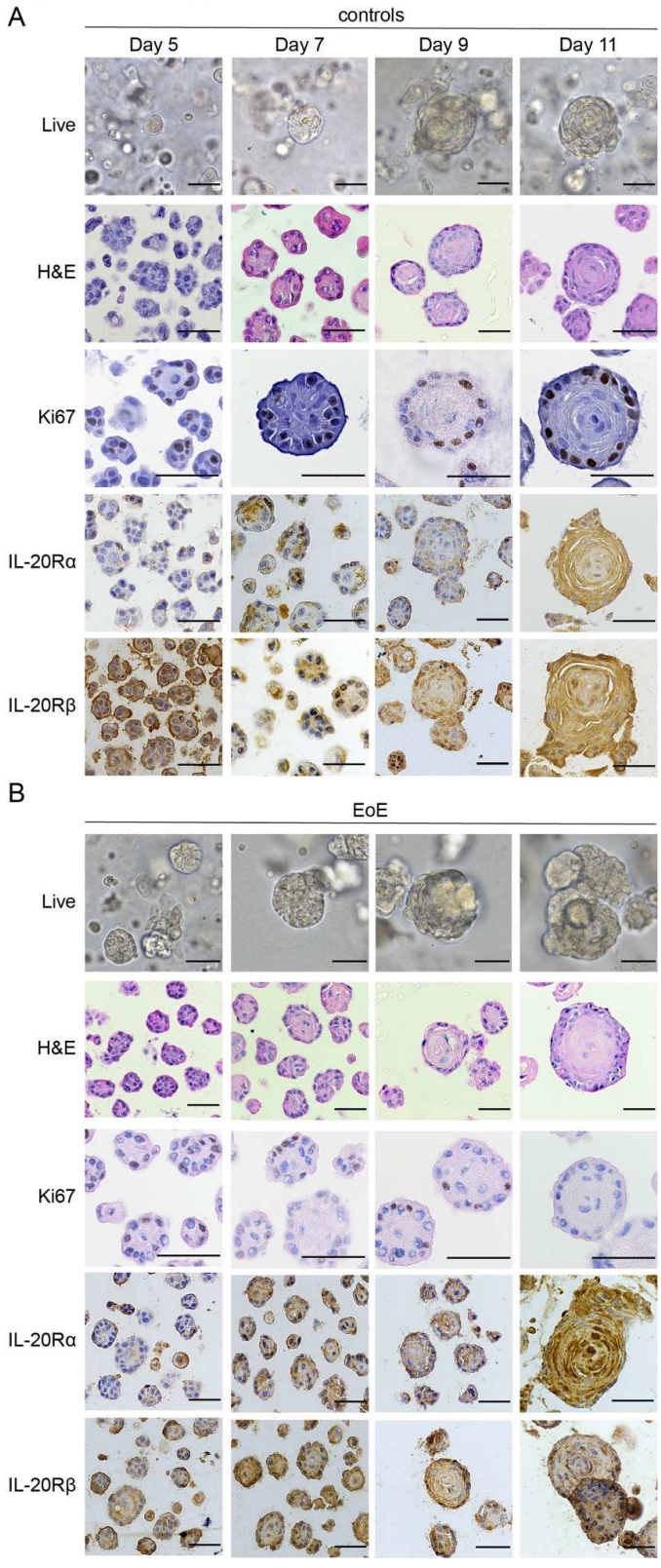
anti-rabbit IgG (H+L)				
Antibodies used for flow cytometry surface staining (Fortessa)				
CD11c	N418	Biotin	BioLegend	117304
CD19	6D56	Biotin	BioLegend	115504
CD3e	145-2C11	Biotin	BioLegend	100304
NK1.1	PK136	Biotin	BioLegend	108704
TER-119/Erythroid cells	TER-119	Biotin	BioLegend	116204
CD117 (c-kit)	2B8	APC/Fire 750	BioLegend	105838
CD11b	M1/70	Pacific Blue	BioLegend	101206
CD16/32	93		BioLegend	101302
CD45	30-F11	Superbright 645	Invitrogen (Thermo Fischer Scientific)	64-0451-82
CD49b (Integrin alpha2)	DX5	APC	Invitrogen (Thermo Fischer Scientific)	17-5971-82
CD64 (FcgR1)	X54-5/7.1	PE/Cy7	BioLegend	139*314*
IgE	23G3	FITC	Invitrogen (Thermo Fischer Scientific)	11-5992-81
Siglec-F	E50-2440	BV711	BD Biosciences	740764
Antibodies used for Immunofluorescence staining				
Keratin 5	Poly9059		BioLegend	905903
Stat3	D3Z2G		Cell Signaling Technology	12640S
AffiniPure Goat Anti-Chicken IgY (IgG) (H+L)	polyclonal	Alexa Fluor 488	Jackson ImmunoResearch	103-545-155

Goat Anti-Rabbit IgG (H+L)		Alexa Fluor® 647	Invitrogen (Thermo Fischer Scientific)	A21244
Antibodies used for Cell Sorting				
CD45.2	104	BV650	BioLegend	109836
Antibodies used for Cell Hashing				
TotalSeq-B0301	M1/42; 30-F11		BioLegend	155831
TotalSeq-B0302	M1/42; 30-F11		BioLegend	155833
TotalSeq-B0303	M1/42; 30-F11		BioLegend	155835
TotalSeq-B0304	M1/42; 30-F11		BioLegend	155837
TotalSeq-B0305	M1/42; 30-F11		BioLegend	155839
TotalSeq-B0306	M1/42; 30-F11		BioLegend	155841
TotalSeq-B0307	M1/42; 30-F11		BioLegend	155843
TotalSeq-B0308	M1/42; 30-F11		BioLegend	155845
TotalSeq-B0309	M1/42; 30-F11		BioLegend	155847
TotalSeq-B0310	M1/42; 30-F11		BioLegend	155849
Antibodies used for Western Blotting				
Phospho-Stat3 (Tyr705)	D3A7		Cell Signaling Technology	9145
Stat3	124H6		Cell Signaling Technology	9139
Phospho-p44/42 MAPK	D13.14.4E		Cell Signaling Technology	4370

(ERK1/2) (Thr202/Tyr204)			
p44/42 MAPK (ERK1/2)	137F5	Cell Signaling Technology	4695
Phospho-NF-κB p65 (Ser536)	93H1	Cell Signaling Technology	3033
NF-κB p65	D14E12	Cell Signaling Technology	8242
Actin Ab-5	C4	BD Biosciences	612656
Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L)	polyclonal	Jackson ImmunoResearch	111-035-144
Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L)	polyclonal	Jackson ImmunoResearch	111-035-146

SUPPLEMENTAL FIGURES

Supplemental Figure 1



Supplemental Figure 1. Characterisation of patient-derived esophageal organoids

In culture brightfield images, H&E, Ki67, IL-20Rα and IL-20Rβ staining of esophageal organoids from (A) control individuals and (B) EoE patients at indicated timepoints of culture. Scale bars, 50 μM

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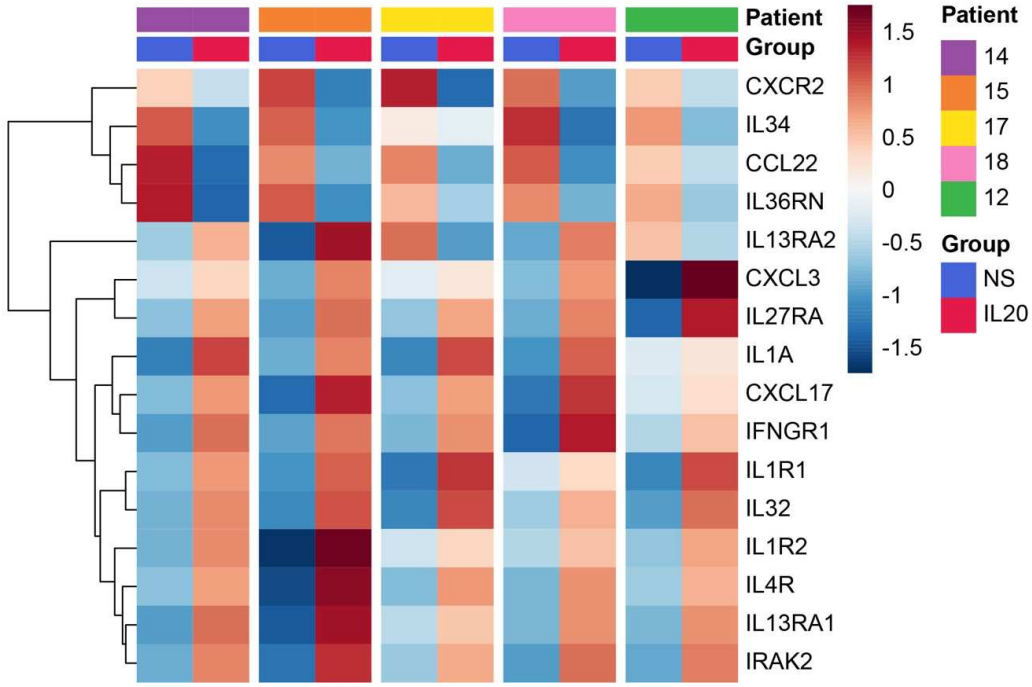
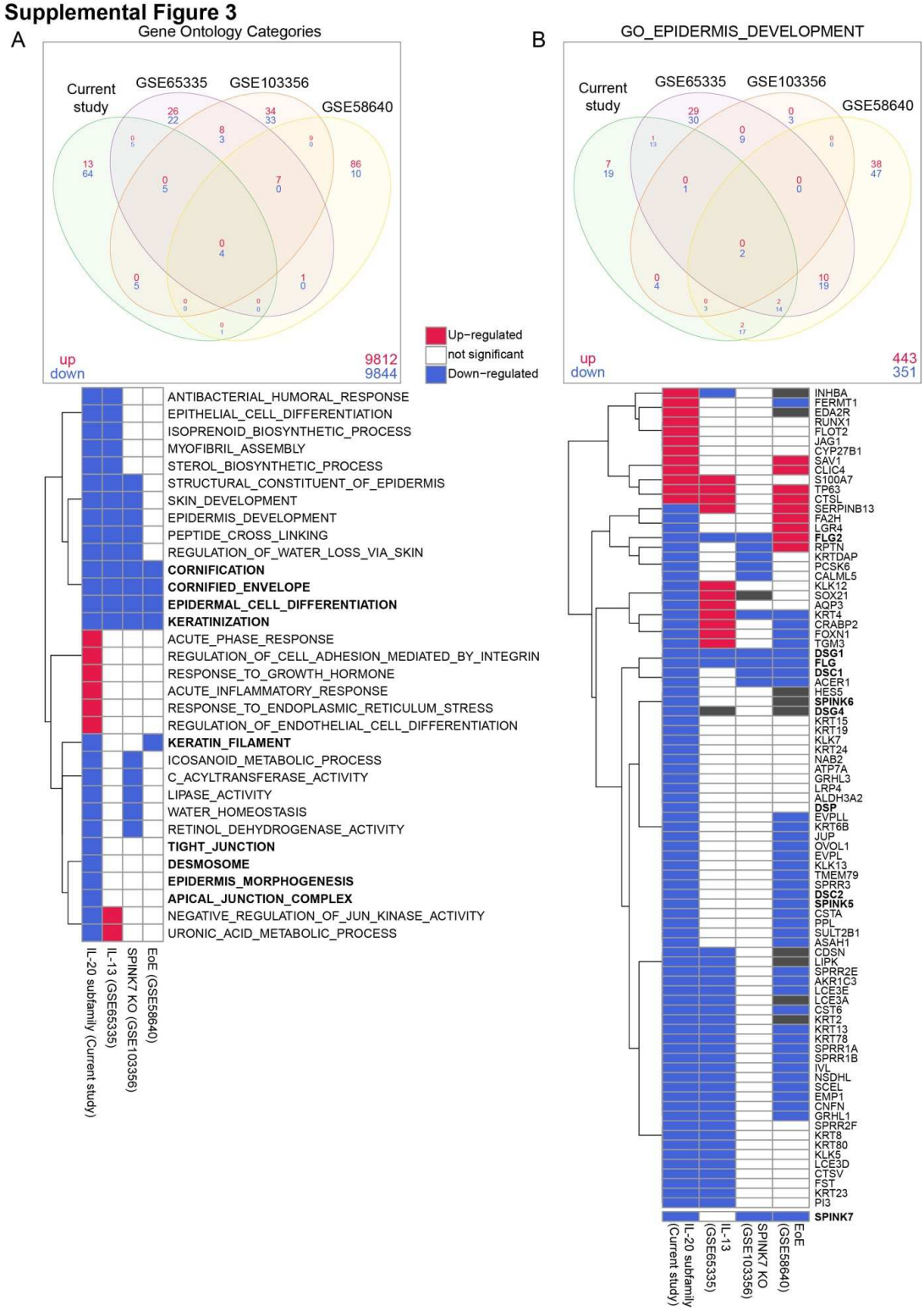
Supplemental Figure 2



Supplemental Figure 2. Heatmap showing the centered and scaled expression levels of selected cytokines and cytokine receptors in the RNA-seq dataset

Supplemental Figure 3



Supplemental Figure 3. Comparison of published RNA-seq transcriptomes to the IL-20 subfamily stimulated esophageal organoid transcriptome

(A) Comparison of GSEA results from our dataset to those from reanalysed published datasets: keratinocytes stimulated with IL-13 (GSE65335), SPINK7-deficient keratinocytes (GSE103356), and the EoE transcriptome (GSE58640). In the heatmap, a selected subset of categories significant in our dataset are shown.

(B) Comparison of differently expressed genes annotated to the Gene Ontology category "Epidermis development" in the different datasets. Only genes significant in our dataset are shown. *SPINK7* was added at the bottom of the heatmap.

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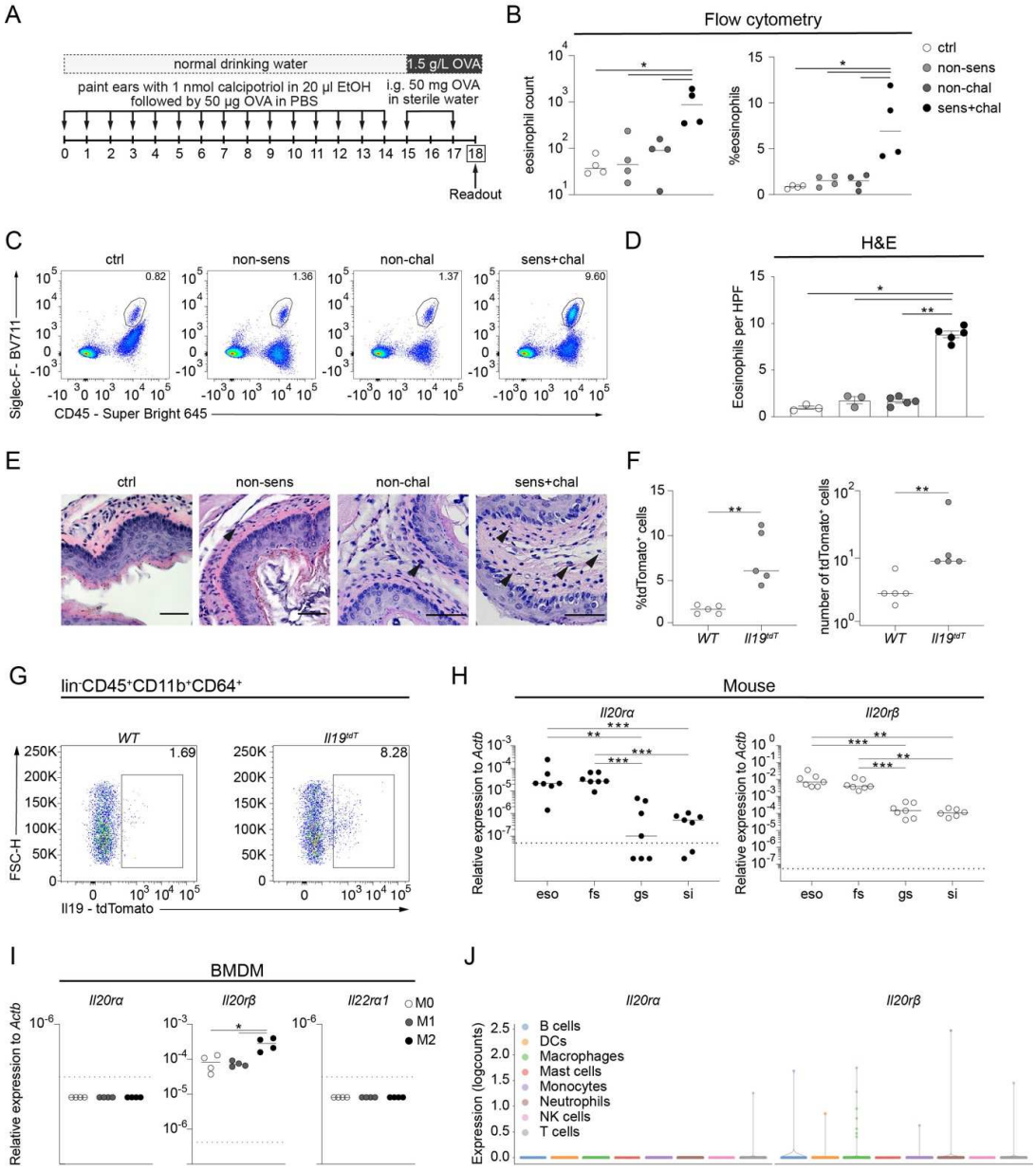
Supplemental Figure 4



Supplemental Figure 4. OVA-induced EoE mouse model, *Il20* subfamily expression by murine macrophages and murine *Il20r* expression

(A) Schematic illustration of experimental EoE model.

(B) Quantification of eosinophil infiltration from flow cytometry data in absolute cell counts and in percentages.

(C) Dot blots indicate the esophageal eosinophil infiltration by flow cytometry. Numbers indicate the percentage of eosinophils.

(D) Counted eosinophils (per HPF) from (E).

(E) H&E staining of esophageal sections obtained from *WT* mice; ctrl: non-sensitised+non-challenged, non-sens: non-sensitised+challenged, non-chal: sensitised+non-challenged, sens+chal: sensitised+challenged. Arrows indicate eosinophils. Scale bars, 50 μm.

(F) Quantification of Il19-producing macrophage infiltration in percentages and absolute cell counts from flow cytometry data in (G).

(G) Dot blots indicate the esophageal infiltration of Il19-producing macrophages ($CD45^+CD11b^+CD64^+tdTomato^+$) by flow cytometry. Numbers indicate the percentage of Il19-producing macrophages.

(H) *Il20rα* and *Il20rβ* mRNA expression in murine esophagus (eso), forestomach (fs), glandular stomach (gs) and small intestine (si) by qPCR.

(I) *Il20rα, Il20rβ and Il22rα1* mRNA expression in murine M0, M1 and M2 polarised BMDMs by qPCR.

(J) Normalised expression levels of *Il20rα* and *Il20rβ* in annotated cell types from the scRNA-seq dataset.

Data are presented as individual values with medians, with each dot representing one biological replicate. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, by Mann-Whitney U test

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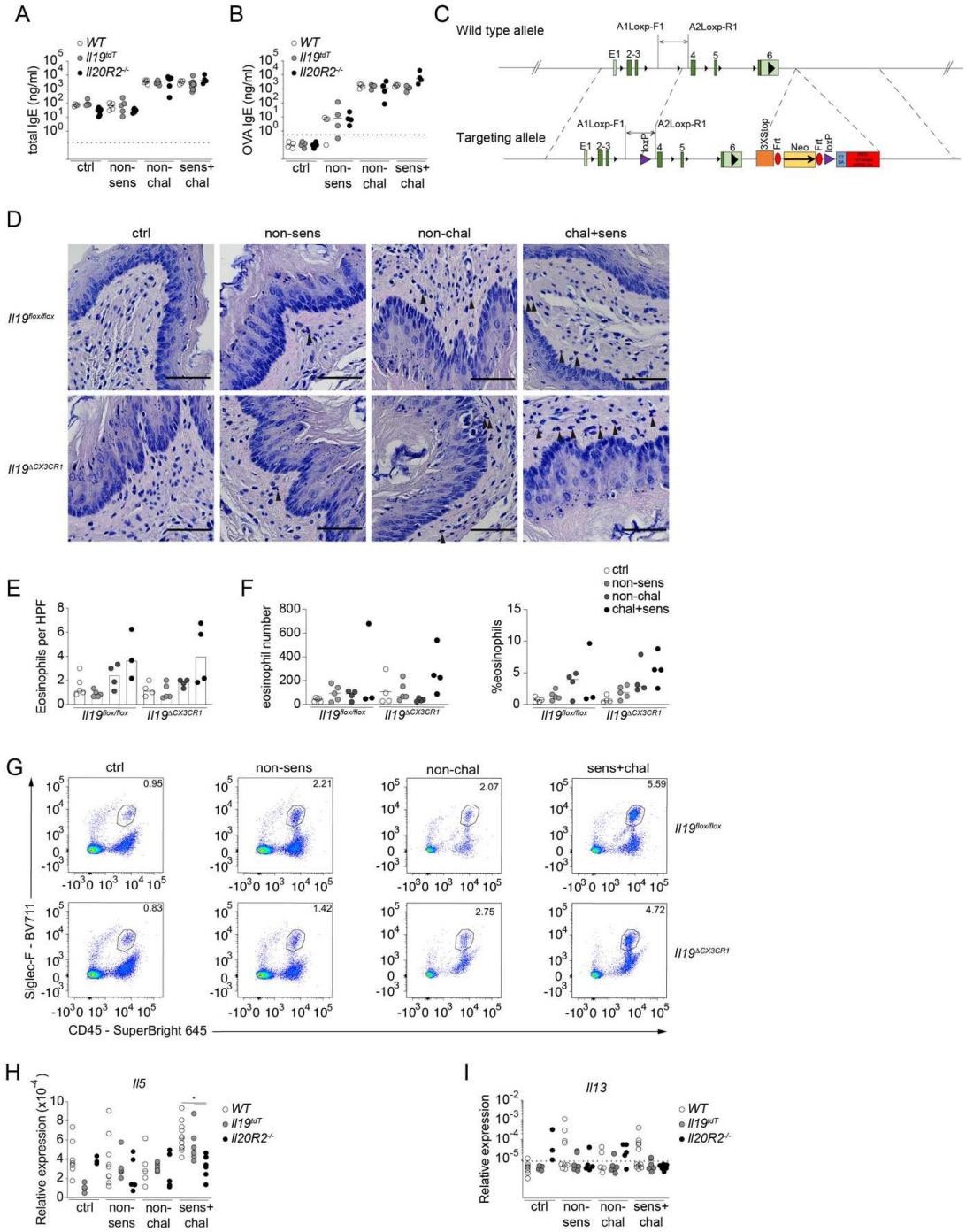
Supplemental Figure 5



Supplemental Figure 5. Total and OVA-specific IgE levels and Th2-cytokine expression in *WT*, *Il19^{tdT}* and *Il20R2$^{-/-}$* mice and OVA-induced EoE model in *Il19$^{\Delta CX3CR1}$* mice

(A) Total and (B) OVA-specific IgE levels in *WT*, *Il19^{tdT}* and *Il20R2$^{-/-}$* mice. ctrl: non-sensitised+non-challenged, non-sens: non-sensitised+challenged, non-chal: sensitised+non-challenged, sens+chal: sensitised+challenged

(C) Construct design of *Il19$^{flox/flox}$* mice.

(D) H&E staining of esophageal sections obtained from *Il19$^{flox/flox}$* and *Il19$^{\Delta CX3CR1}$* mice; ctrl: non-sensitised+non-challenged, non-sens: non-sensitised+challenged, non-chal: sensitised+non-challenged, sens+chal: sensitised+challenged. Arrows indicate eosinophils. Scale bars, 50 μm.

(E) Counted eosinophils (per HPF) from (D).

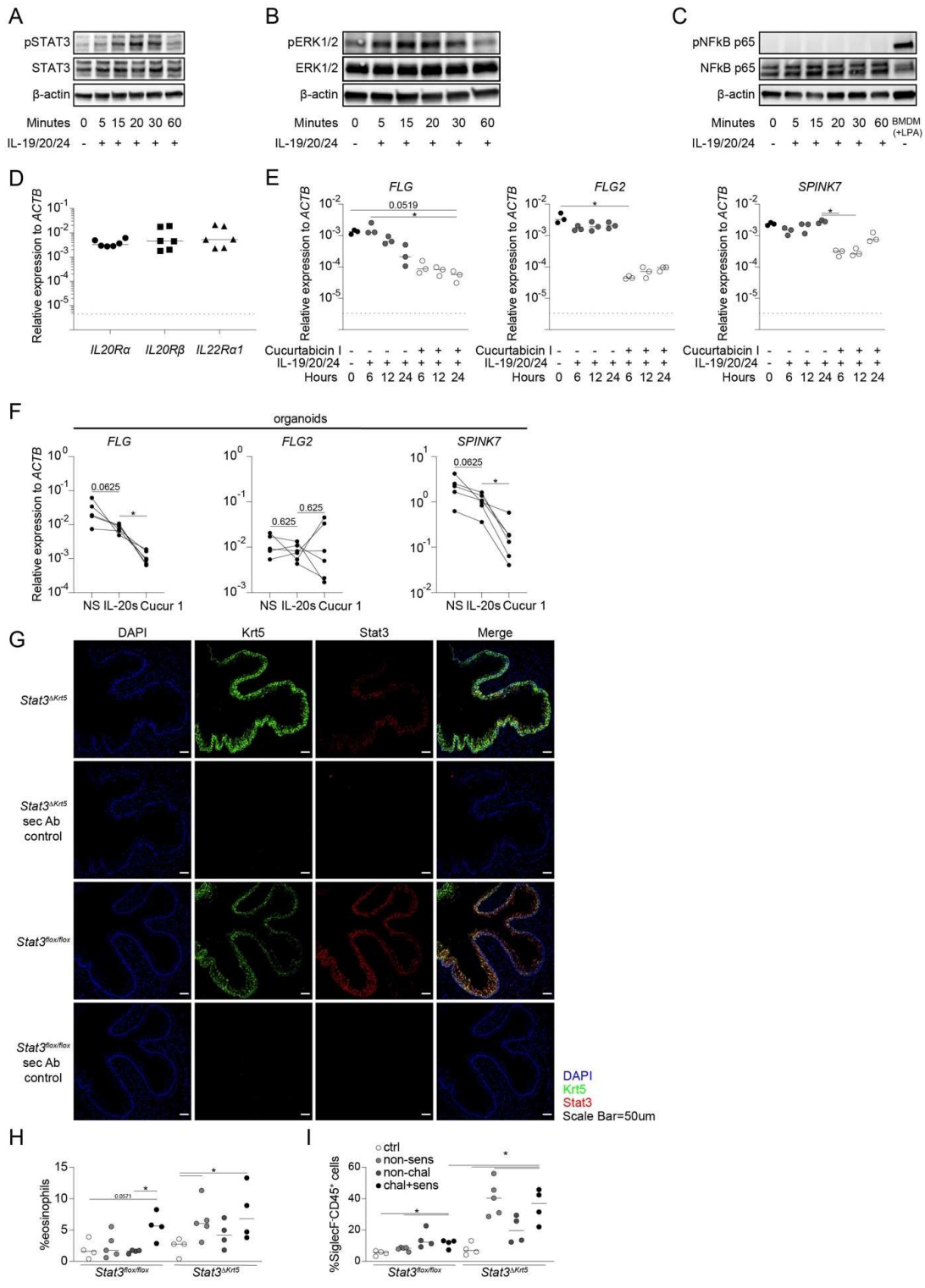
(F) Quantification of eosinophil infiltration from flow cytometry data in absolute cell counts and in percentages.

(G) Dot blots indicate esophageal eosinophil infiltration by flow cytometry. Numbers indicate the percentage of eosinophils.

(H) *Il5* and (I) *Il13* mRNA expression relative to *Actb* in *WT*, *Il19^{tdT}* and *Il20R2$^{-/-}$* mice by qPCR. ctrl: non-sensitised+non-challenged, non-sens: non-sensitised+challenged, non-chal: sensitised+non-challenged, sens+chal: sensitised+challenged

Data are presented as individual values with medians, with each dot representing one biological replicate. * $p<0.05$, by Mann-Whitney U test

Supplemental Figure 6



Supplemental Figure 6. STAT3, ERK1/2 and NFκB phosphorylation and expression of *FLG*, *FLG2* and *SPINK7* upon stimulation with IL-20 subfamily in KYSE-180 cell line and esophageal organoids, and immunofluorescence of tamoxifen induced Stat3 deletion in *Stat3*$^{\Delta Krt5cre}$ mice.

(A-C) Western blot for activation of (A) STAT3, (B) ERK1/2 and (C) NFκB in keratinocyte cell line KYSE-180 upon stimulation with IL-20 subfamily cytokines. Protein lysate from BMDMs stimulated with lysophosphatidic acid (LPA) was used as a positive control for pNFκB p65 in (C).

(D) *IL20Rα, IL20Rβ* and *IL22Rα1* mRNA expression in KYSE-180 cell line by qPCR

(E) *FLG, FLG2, SPINK7* mRNA expression in KYSE-180 cell line after stimulation with IL-20 subfamily cytokines over indicated timespan by qPCR.

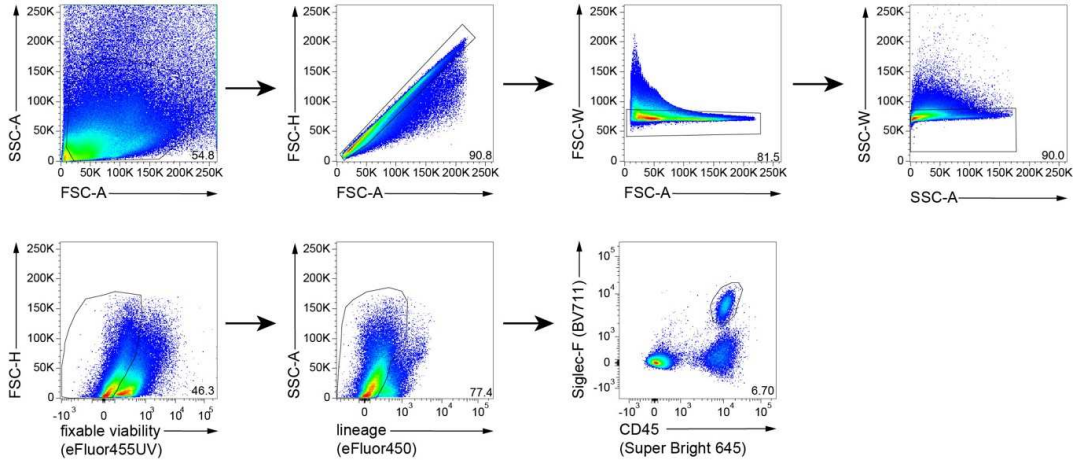
(F) *FLG, FLG2 and SPINK7* mRNA expression in patient-derived esophageal organoids stimulated with IL-20 subfamily cytokines (IL-20s) and IL-20s stimulated organoids pretreated with Cucurtabicin 1 (Cucur 1) by qPCR.

(G) Immunofluoresence staining for Krt5 and Stat3 in tamoxifen-treated *Stat3*$^{flox/flox}$ and *Stat3*$^{\Delta Krt5cre}$ mice.

(H+I) Percentages of (H) eosinophils and (I) Siglec-F^{-}CD45^{+} cells in flow cytometry analysis of *Stat3*$^{flox/flox}$ and *Stat3*$^{\Delta Krt5}$ *mice.* ctrl: non-sensitised+non-challenged, non-sens: non-sensitised+challenged, non-chal: sensitised+non-challenged, sens+chal: sensitised+challenged

Data are presented as individual values with medians, with each dot representing one biological replicate. * $p<0.05$, by Mann-Whitney U, Wilcoxon or Kruskal-Wallis test

Supplemental Figure 7



Supplemental Figure 7. Flow cytometry gating strategy for murine eosinophils

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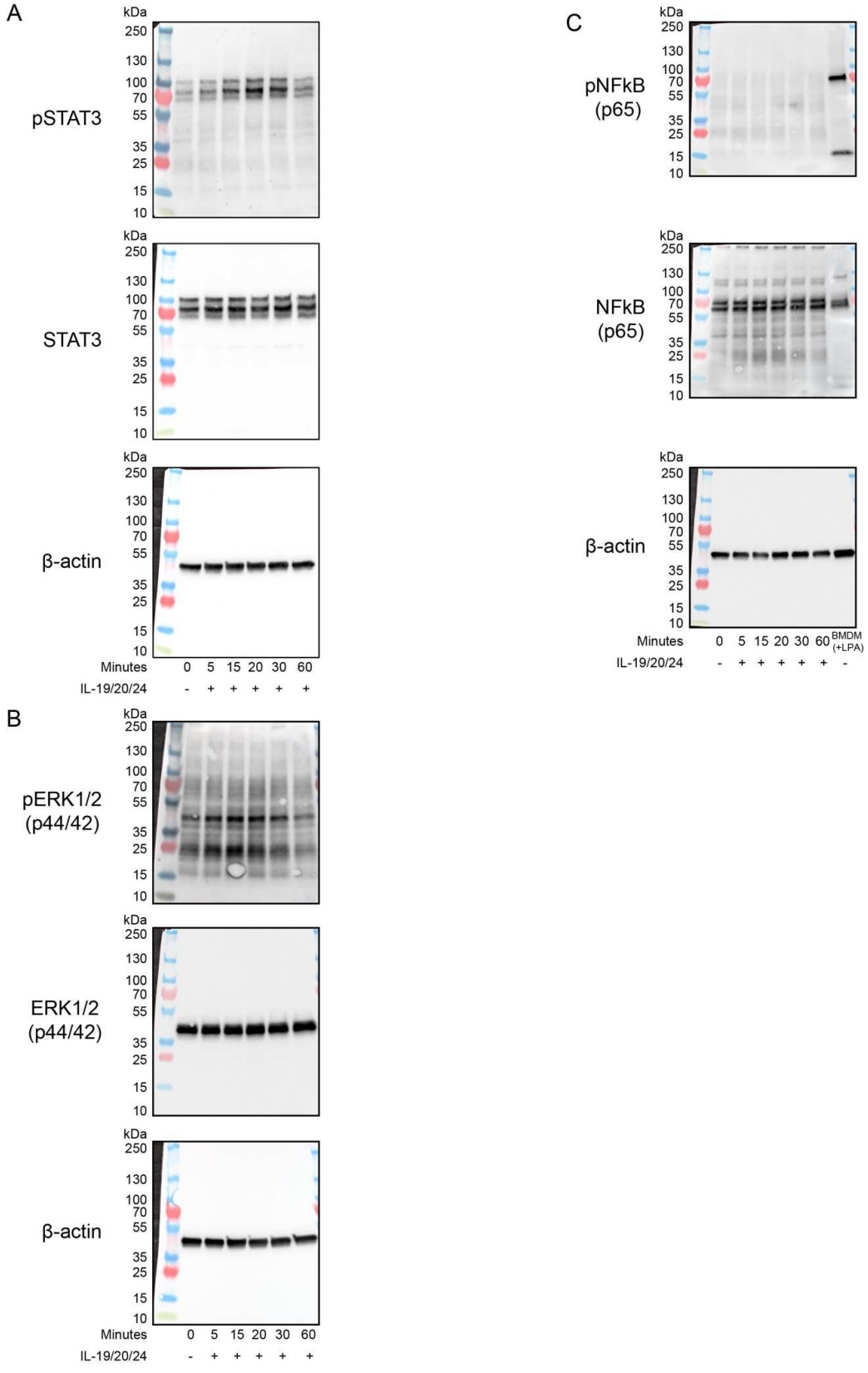
Supplemental Figure 8



Supplemental Figure 8. Activation of STAT3, ERK1/2 and NFκB upon stimulation with IL-20 subfamily cytokines

Whole membrane images of western blots represented in supplemental figure 6A-C.

Kaymak T, *et al. Gut* 2023; 72:821–833. doi: 10.1136/gutjnl-2022-327166

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