Supplemental Material and methods

Quantitation of mouse and human eosinophils

Mouse lungs were flushed with 10mL of PBS to remove blood from the lung. Lungs were macerated and mixed with 3mL of digestion buffer consisting of HBSS with Ca and Mg (ThermoFisher, 24020117), 5% FBS, 1mg/mL collagenase A (Roche, 10103578001), and 0.05mg/mL DNase I (Roche, 4716728001) and incubated at 37°C for 1h with shaking. Mouse lung cell suspension was passed through a cell strainer (70μm) and washed twice in ice-cold PBS prior to the staining with conjugated surface markers. Mouse spleen was homogenised and passed through a cell strainer (70μM). The recovered spleen cell suspension was lysed with lysis buffer, consisting of of UltraPure distilled water (Invitrogen, 15657708) supplemented with 150mM NH_4Cl, 10mM $KHCO_3$, 0.1mM EDTA, at room temperature for 5 minutes and washed with ice-cold PBS twice prior to the staining with conjugated surface markers. Mouse blood was collected from the eye sinus with a capillary under terminal anesthesia. The recovered mouse blood was lysed with lysis buffer at room temperature for 5 minutes and washed with ice-cold PBS twice prior to the staining with conjugated surface markers. All recovered lung, spleen, and blood single-cell suspensions were stained with anti-CCR3-APC (BioLegend, J073E5), anti-Siglec-F-PE (BD Biosciences, E50-2440), anti-CD3-FITC (BD Biosciences, 55306), anti-CD19-APC-eFluor 780 (eBioscience, 47-0193-80), anti-Ly-6G-PE-Cy7 (BD Biosciences, 560601), anti-CD45-V500 (BD Biosciences, 562129), anti-CD11b-BV421 (BD Biosciences, 562605), and green nucleic acid stain (BD Biosciences, 565799). Staining reactions were performed at 4°C following a 5-minute incubation with Fc receptor antibodies to reduce non-specific binding (BD Biosciences, 553141). Mouse eosinophils were defined as $CCR3^+$Siglec-F^+ cells (Figure E1). Absolute mouse eosinophil counts were quantitated using CountBright absolute counting beads for flow cytometry (ThermoFisher, C36950). Human whole blood eosinophils were quantitated using an automated hematology analyzer (Cell-Dyn, Abbott Laboratories).

Isolation of eosinophils from mouse bone marrow

Single-cell mouse bone marrow suspensions were recovered from hind leg bones (pelvis, tibia, femur). Bones were flushed with 10mL of ice-cold PBS containing 10mM EDTA and passed through a cell strainer (70μM). The recovered single-cell suspension was lysed in lysis buffer at room temperature for 5 minutes and washed with ice-cold PBS twice. Mouse bone marrow cells were stained with anti-CCR3-APC (BioLegend, J073E5), anti-Siglec-F-

PE (BD, E50-2440), and green nucleic acid stain (BD Biosciences, 565799). Absolute eosinophil counts were quantitated using CountBright absolute counting beads for flow cytometry (ThermoFisher, C36950). Bone marrow eosinophils ($CCR3^+$ $Siglec\text{-}F^+$ cells) were sorted into TRIzol® (ThermoFisher, 15596026) using a BD FACSAria III (BD Biosciences) at a purity of 90 per cent or higher and stored at -80°C for downstream RNA applications.

Isolation of eosinophils from human blood

From every donor patient, we sampled 30mL of venous blood in BD Vacutainer EDTA tubes (BD biosciences, 367525). Polymorphonuclear cells were isolated by double-layer density gradient centrifugation. Recovered cells were purified by negative selection immunomagnetic purification (Stemcell™ Technologies, 17956) following the manufacturer's instructions. Briefly, cells were washed twice with ice-cold PBS supplemented with 2% heat inactivated fetal bovine serum (FBS, Sigma-Aldrich) and 1mM EDTA. Cell suspension was sedimented at 300xg for 5 minutes and adjusted to $5x10^7$ cells per mL. For every mL of sample, 50µL of antibody cocktail was added, and cells were incubated at room temperature for 5 minutes. An equal volume of RapidSpheres™ was added and samples were immediately incubated in an EasySep™ magnet at room temperature for 3 minutes. Pre-sort eosinophil viability was 95 per cent or superior as assessed by trypan blue exclusion. Human blood eosinophils were stained with anti-CCR3-FITC (REA574, Miltenyi Biotec), anti-CD3-PE (REA613, Miltenyi Biotec), anti-CD19-PE (REA675, Milenyi Biotec), anti-Siglec-8-APC (REA1045, Milentyi Biotec), and anti-CD16-APC-H7 (3G8, BD Biosciences). Staining reactions were performed at 4°C following a 5-minute incubation with Fc receptor antibodies to reduce non-specific binding (BD Biosciences, 564219). Eosinophils were sorted into TRIzol® (ThermoFisher, 15596026) using a BD FACSAria III (BD Biosciences) at a purity of 95% or higher and stored at -80°C for downstream RNA applications.

Bright-field microscopy

Sorted human blood- and mouse bone marrow eosinophils were sedimented at 300xg for 5 minutes and resuspended in Freeflex Geloplasma 3% (Fresenius Kabi, RVG 20107), loaded into a cytofunnel and spun at 800xg for 4 minutes. Slides were left to dry overnight and cells and stained using Hemacolor Rapid staining kit (Sigma-Aldrich, 1116610001). Bright-field cell images were taken at 1500x total magnification using an oil immersion objective.

Ex vivo activation of eosinophils

Culture medium consisted of sterile filtered IMDM supplemented with 20% heat inactivated fetal bovine serum (Sigma-Aldrich), 1% non-essential amino acids (Sigma-Aldrich), 1mM sodium pyruvate (Sigma-Aldrich), 2mM l-glutamine (Sigma-Aldrich), 25mM HEPES (Sigma-Aldrich), 50μM β-mercaptoethanol (Sigma-Aldrich), 100U/mL penicillin (Millipore). Human blood eosinophils (purity≥95%) were cultured for 6 hours at 37°C and 5% CO_2 in culture medium with additional 100ng/mL purified IL33 (PeproTech, 200-33). Stimulated human blood eosinophils were sedimented at 300xg for 5 minutes, resuspended in TRIzol® (ThermoFisher) and stored at -80°C for downstream RNA extraction. Mouse bone marrow eosinophils (purity≥90%) were cultured for 4 hours at 37°C and 5% CO_2 in culture medium with additional 100ng/mL purified IL33 (BioLegend, 580504), and 10ng/mL purified IL5 (Peprotech, 215-15). Stimulated mouse bone marrow eosinophils were sedimented at 300xg for 5 minutes, resuspended in TRIzol® (ThermoFisher) and stored at -80°C for downstream RNA extraction.

RNA extraction

Human blood and mouse bone marrow eosinophils were sorted as described above directly into TRIzol (ThermoFisher). For every mL of TRIzol, 200μL of chloroform was added, and the samples were vigorously mixed and incubated for 2 minutes at room temperature. Samples were centrifuged at 10,000xg for 15 minutes at 4°C to separate the phases. The RNA-containing upper aqueous phase was transferred to a new microcentrifuge tube containing 475μL of isopropanol and 2μL of glycoblue (ThermoFisher, AM9515). Samples were centrifuged at 10,000xg for 15 minutes and supernatant was discarded. One volume of 75% ethanol was added and samples were centrifuged at 10,000xg for 1 minute to precipitate the RNA and discard the supernatant. RNA pellet was resuspended in 40μL of DNase/RNase-free water for a 15-minute DNase treatment (Zymo Research, E1010). DNase treatment was followed by column-based RNA purification with the RNA Clean & concentrator-5 kit (Zymo Research, R1016). Briefly, 100μL of RNA binding buffer was added to every 50μL sample and mixed thoroughly. One volume of 100% ethanol was added and the sample was transferred into a Zymo-Spin™ IC column in a collection tube. Columns were centrifuged at 10,000xg for 30 seconds and flow-through was discarded. The column was washed once with RNA prep buffer and twice with RNA wash buffer, following the manufacturer's instructions. RNA was eluted in 10μL of DNase/RNase-free water and stored

at -80°C. Purified RNA integrity and quantity was assessed using the RNA 6000 Pico kit (Agilent) for the presence of 18s and 28s rRNA peaks. All human samples had RNA integrity number (RIN) >7.9.

RNA sequencing & data processing

Full length cDNA was prepared from isolated RNA using SMART-Seq v4 Ultra Low Input RNA kit (Takara Bio, 634889) following the manufacturer's instructions, with 17 cycles of cDNA amplification. Final cDNA quality was assessed using Agilent High Sensitivity DNA kit (Agilent, 5067-4626). cDNA libraries were prepared for sequencing using Nextera XT DNA library preparation kit (Illumina, FC-131-1024) using the manufacturer's instructions and samples were sequenced on a NovaSeq™ 6000 sequencing system (Illumina). If samples were sequenced in different batches, groups were kept equal within every batch.

Differential gene expression analyses

Sequenced reads were aligned to the mouse genome (UCSC mm10) or the human genome (HG19) with RNA-seq Alignment (v2.0.2) using STAR aligner (version 2.6.1a) on BaseSpace (https://basespace.illumina.com). Uniquely mapped reads were used to calculate gene expression. Differential gene expression was calculated using DESeq2 (1.26.0) in R (3.6.3 and 4.0.3) (19). If samples were sequenced in different batches, sample batch was taken into account in the DESeq2 design. GSEAR analyses on differentially expressed genes were performed on preranked list of significantly differentially expressed genes with baseMean>50 ordered according to their log2 fold change. Online GSEAR v7.2.1 (https://genepattern.broadinstitute.org/gp/pages/index.jsf) was used with the "h.all.v7.2.symbols" (Hallmarks) gene sets and default parameters, except for a "classic" scoring scheme and minimal gene set size of 20.

Statistical analyses

All statistical analyses were performed in R (3.5.0). Patient demographic and functional characteristics were expressed as mean ± SD. Comparisons between patient groups were performed using unpaired t tests. Chi-square test was applied for categorical variables. All mouse experiments followed a randomized design. Sample sizes were determined by power analysis. Respect of tests assumptions and model fit were evaluated using diagnostic plots. Raw data were transformed when needed and back-transformed for graphical presentation.

Error bars in all graphs represent mean +/- 95% confidence interval. In Figure 1A, data was pooled from 2 to 3 independent experiments and was analyzed by one-way ANOVA followed by Tukey honestly significant difference tests. In Figure 3A, data was analyzed by one-way ANOVA followed by Tukey honestly significant difference tests. A p-value lower than 0.05 was considered significant. For clarity of presentation, only results of intergroup comparisons of interest are displayed in figures.

Data deposition

RNA-sequencing data have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession numbers E-MTAB-10188, E-MTAB-10189 and E-MTAB-10190