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Supplementary Materials for

Functional role of kallikrein 5 and proteinase-activated receptor 2 in eosinophilic esophagitis

Nurit P. Azouz, Andrea M. Klingler, Purnima Pathre, John A. Besse, Netali Ben Baruch-Morgenstern, Adina Y. Ballaban, Garrett A. Osswald, Michael Brusilovsky, Jeff E. Habel, Julie M. Caldwell, Mario A. Ynga-Durand, Pablo J. Abonia, Yueh-Chiang Hu, Ting Wen, Marc E. Rothenberg*

*Corresponding author. Email: rothenberg@cchmc.org

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Other Supplementary Material for this manuscript includes the following:

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Materials and Methods

Experimental eosinophilic esophagitis (EoE) model

A mouse model of allergic EoE was established using methods described previously (*67, 68*) with some changes. In brief, BALB/c mice were sensitized by intraperitoneal injection of 100 μg of ovalbumin (OVA) and 1 mg alum in 0.9% sterile saline on two occasions separated by 14 days. On day 28, mice were subsequently anesthetized lightly with isoflurane (IsoFlo, Abbott Laboratories) and intranasally exposed to 100 μg of OVA or saline alone (50 μl) on 4-5 occasions over 8-10 days using a micropipette with the mouse held in the supine position until alert.

Generation of kallikrein 5 (*Klk5)*-deficient mice

BALB/c kallikrein 5 (*Klk5*)-deficient mice were generated by the Cincinnati Children's Hospital Medical Center (CCHMC) Transgenic core facility. Two single guide RNAs (sgRNAs) were selected to target *Klk5 gene* according to the on- and off-target scores from the web tool CRISPOR (*69, 70*). The sgRNAs were synthesized in vitro using the MEGAshorscript T7 kit (ThermoFisher) and purified by the MEGAclear Kit (ThermoFisher), as previously described (*71*). sgRNAs (50 ng/μl of each) were mixed with 200 ng/μl Cas9 protein (ThermoFisher) and incubated at 37°C for 15 min to form a ribonucleoprotein complex. The mixture was injected into the cytoplasm of one cell–stage embryos of the BALB/c genetic background using a piezo-driven microinjection technique as described previously (*71*). Injected embryos were immediately transferred into the oviducal ampulla of pseudopregnant CD-1 females. Live born pups were

genotyped by PCR, and the edited alleles were further confirmed by Sanger sequencing.

Histologic analysis of eosinophils in the esophagus

Distal esophagi were fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into 5 μm sections, fixed to positively charge slides, and immunostained with antiserum against mouse eosinophil major basic protein (anti-MBP) (Mayo Clinic) as described previously (*72*). In brief, endogenous peroxidase in the tissues was quenched with 0.3% hydrogen peroxide in methanol followed by nonspecific protein blocking with normal goat serum. Tissue sections were then incubated with rabbit anti-MBP (1:16,000) overnight at 4°C, followed by 1:200 dilution of biotinylated goat anti-rabbit IgG secondary antibody and avidin-peroxidase complex (Vector Laboratories) for 30 min each. These slides were further developed with nickel diaminobenzidine–cobalt chloride solution to form a black precipitate and counterstained with nuclear fast red. Replacing the primary antibody with normal rabbit serum, as a control, ablated the immunostaining. Quantification of immunoreactive cells was achieved by counting the positively stained cells of longitudinal sections under high-power magnification using a 10 × 10 μm ocular micrometer (Nikon Microscopes Inc.). Eosinophil number was expressed as the number of eosinophils per millimeter of whole section of the esophagus.

Analysis of epithelial thickness in the esophagus

The epithelium was immunostained with anti–desmoglein-1 (DSG1) antibody and 4’,6-diamidino-2-phenylindole (DAPI) (see Immunofluorescence staining and analysis section) in order to observe the cell boundaries and analyze the cell shape. The thickness of the epithelium was calculated using the NIKON analysis software.

Bronchoalveolar lavage fluid (BALF) collection and staining

The mice were euthanized by CO_2 inhalation. Immediately thereafter, a midline neck incision was made, and the trachea was cannulated. The lungs were lavaged twice with 1.0 ml phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS). The recovered bronchoalveolar lavage fluid (BALF) was centrifuged at 1200 rpm for 5 min at 4°C and resuspended in 200 µl PBS containing 2% FBS. Lysis of red blood cells (RBCs) was performed using RBC lysis buffer. Total cell numbers were counted with a hemocytometer. The percent of BALF eosinophil was calculated by flow cytometry as indicated below as an indication of lung airway eosinophilia.

Flow cytometry

Blood and BALF samples were processed with RBC lysis buffer and then stained with phycoerythrin (PE)–conjugated, anti–mouse Siglec-F clone E50-2440 and PE-Cy7–conjugated, anti–mouse CD11b clone M1/70 (BD Pharmingen). Staining was performed on ice for 20 min in staining buffer ((0.5% bovine serum albumin (BSA), 0.01% NaN_3 in Hank's Balanced Salt Solution (HBSS)) with manufacturer-suggested titers. Stained cells were suspended and subjected to analysis with the BD FACS Canto III flow

cytometer (BD Biosciences). The percent of eosinophils ($CD11b^+$, $SiglecF^+$) from total white blood cells were analyzed (Tree Star LLC).

Esophageal tissue processing

Esophageal sections or biopsies from mouse or human, respectively, were immediately frozen on dry ice after collection. After being thawed and incised with scissors, the samples were sonicated for 4 min in M-PER buffer (Thermo Fisher Scientific) using a S220 focused ultrasonicator (Covaris). Protein concentration was determined by bicinchoninic acid (BCA) assay (Thermo Fisher Scientific), and proteins were further analyzed for proteolytic activity and by western blot or enzyme-linked immunosorbent assay (ELISA).

ELISA

Human thymic stromal lymphopoietin (TSLP) and human IL-8 expression was measured from the supernatants of differentiated air-liquid interface (ALI) cultures by IL-8 and TSLP ELISA kits (Biolegend). Twenty µg of protein lysates from esophageal sections were subjected to human A1AT ELISA (R&D systems), mouse IgE ELISA (Biolegend), mouse CCL24 ELISA, a mouse CCL11 ELISA (R&D systems) and mouse MCPT1 ELISA (Invitrogen).

EPC2 cells, primary esophageal epithelial cells, and air-liquid interface (ALI) culture system

The esophageal epithelial cell line (human telomerase reverse transcriptase–immortalized EPC2 cell line) was a kind gift from Dr. Anil Rustgi (University of Pennsylvania). Primary esophageal epithelial cells and CRISPR/Cas9 knockout (KO) of serine peptidase inhibitor, kazal type 7 (SPINK7) in EPC2 cells were prepared as previously described (*6*). For the ALI culture system, EPC2 cells or primary esophageal epithelial cells were seeded (day 0) and grown to confluence while fully submerged in low-calcium ($CaCl_2$ 0.09 mM) keratinocyte serum-free media (KSFM) on 0.4-μm pore-size permeable supports (Corning Incorporated). Confluent monolayers (day 2) were then switched to high-calcium ($CaCl_2$ 1.8 mM) KSFM for an additional 5 days. To induce epithelial stratification and differentiation, the culture medium was removed from the inner chamber of the permeable support to expose the cell monolayer to the air interface (day 7). Differentiated esophageal epithelial equivalents were analyzed at day 11-14.

***KLK5* overexpression**

A PLX304 plasmid containing human *KLK5* open reading frame from the ORFeome Collaboration (Dana-Farber Cancer Institute, Broad Institute of Harvard and Massachusetts Institute of Technology [HsCD00442378]) was purchased from DNASU Plasmid Repository, and a control PLX304 vector was purchased from Addgene. Lentiviral particles were produced by the Cincinnati Children's Hospital Medical Center (CCHMC) Viral Vector Core. EPC2 cells grown in KSFM were transduced. Twenty-four

hours after transduction, cells were selected for stable integration using the antibiotic blasticidin.

mRNA extraction and quantitative RT-PCR (qRT-PCR)

Total RNA was isolated from cells with Quick RNA MicroPrep (Qiagen) according to the manufacturer's protocol. For RNA sequencing experiments, RNA was treated with On-Column DNase Digestion kit (ZymoResearch) according to the manufacturer's protocol. cDNA was synthesized with the Protoscript synthesis kit (New England Biolabs). Quantitative PCR (qPCR) was performed using a 7900HT Fast Real-Time PCR system from Applied Biosystems (Life Technologies) with FastStart Universal SYBR Green Master mix (Roche Diagnostics Corporation) by using the following primer sets: human glyceraldehyde 3-phosphate dehydrogenase (h*GAPDH*) (forward 5′-TGGAAATCCCATCACCATCT, reverse 5'- GTCTTCTGGGTGGCAGTGAT), h*KLK5* (forward 5'-AGTCAGAAAAGGTGCGAGGA, reverse 5'-TAATCTCCCCAGGACACGAG), h*IL8* (forward 5′- GCCTTCCTGATTTCTGCAGC, reverse 5'- CCAGTTTTCCTTGGGGTCCA), m*Hprt* (forward 5′-CCTCCTCAGACCGCTTTTT, reverse 5'- AACCTGGTTCATCATCGCTAA), m*Klk5* (forward 5′- CAATGGCTACCCTGATCACA, reverse 5'- GTGCATCCTTTTGGCAGTCT), m*Tslp* (forward 5′- CAGCTTGTCTCCTGAAAATCG, reverse 5'-AAATGTTTTGTCGGGGAGTG), h*IVL* (forward 5′-TCTGCCTCAGCCTTACTGTG), reverse 5'- TGCTCCTGATGGGTATTGAC), and h*F2RL1* (forward 5′-TGCTAGCAGCCTCTCTCTCC reverse 5'- CCAGTGAGGACAGATGCAGA).

Generation of a 3D structure of SPINK7 and KLK5

Rigid body docking analysis of SPINK7 (PDBID:2LEO chain A) and KLK5 (PDBID:2PSX chain A) was predicted by PYDOCK (*73*), GRAMM-X (*74*), and ClusPro (*75*) following each program's recommended parameters**.** Docking results were visualized using PYMOL (Schrödinger, Inc).

Single cell RNA sequencing

A single patient's biopsy from the distal esophagus was collected into RPMI medium supplemented with 10% FBS. The biopsy was then transferred into EDTA buffer (5 mM EDTA, 10% FBS, 1 mM HEPES in PBS) for 15 minutes in a 37ºC water bath, washed once with PBS, minced, and then subjected to collagenase A digestion (Roche, derived from Clostridium histolyticum, 2 mg/mL) in 10% FBS-RPMI at 37ºC for 30 minutes. The resulting suspension was diluted with 10 mL ice-cold PBS, passed through a 19-gauge needle 5 times, filtered through 2 layers of gauze, washed twice with ice-cold PBS (10 mL each), and then centrifuged at 450 g for 5 minutes, resulting in a visible, clear-edged, translucent pellet. Bulk population cells were directly subjected to the 10X mass genomics chip (10X genomics, Inc.) targeting 10,000 simultaneously captured live events per chip for next-generation sequencing. Each cell is uniquely barcoded during the cDNA library generation; libraries were subsequently sequenced on an Illumina HiSeq 2500 at CCHMC's DNA sequencing core, which allocated to a total read of ~320M (2 lanes / flow cell). Sparse data matrices, provided by 10X genomics were used as input into Seurat for further analysis, as previously described (*76*). For analysis of all sequenced esophageal samples, cells were filtered on the basis of unique feature

counts over 4,800 or less than 200. Cells with less than >20% mitochondrial counts were filtered. Only genes that expressed in at least 3 cells were retained. The total number of cells passing the filters and captured across all patients was 47,141 cells, and 18,363 genes passed quality check. Of these cells, 30,938 cells were classified as epithelial cells. Principal component analysis (PCA) was performed over the list of the variable genes. Data was subjected to Uniform Manifold Approximation and Projection (UMAP) and shared nearest neighbor (SNN) modularity optimization-based clustering. Using PCA and SNN modularity optimization-based clustering algorithm with a resolution of 0.5 and *UMAP*, we identified 14 epithelial clusters. Top markers genes with high specificity were used to classify cell markers into cell types on the basis of existing biological knowledge. Gene ontology enrichment analysis, which uses statistical methods to determine functional pathways and cellular processes associated with a given set of genes, was performed with the ToppGene suite (*77*).

Human subject recruitment (scRNA Seq)

With the overall goal of targeting normal controls, remission and active EoE samples for scRNA Seq, biopsy samples were acquired at the Allergy and Gastroenterology outpatient clinics (Cincinnati Children's Hospital Medical Center) systemically from patients who were having an endoscopy for EoE or related symptoms. EoE was defined by a histological finding of ≥ 15 eosinophils/HPF with clinical symptoms and a failed proton pump inhibitors trial. Remission was defined as any patient (with EoE history) whose tissue eosinophil count was < 15 eosinophils/HPF. Normal controls were defined as patients without history of EoE and a negative endoscopy with 0 eosinophils/HPF.

This approach resulted in successful recruitment of 2 control, 3 remission and 5 EoE patients. Samples were obtained following informed consent, under the approval of the Institutional Review Board (IRB) of the CCHMC (#2008-0090).

DNA extraction and 16S analysis

After 2 weeks of co-housing *Klk5*$^{+/+}$ mice and *Klk5*$^{-/-}$ mice, esophageal samples were frozen in liquid nitrogen and crushed with a glass pipet. Bacterial DNA was extracted using the QuickDNA miniprep kit (Zymo research). 16S rRNA gene amplicon sequencing was performed on an Illumina MiSeq at the Environmental Sample Preparation and Sequencing Facility at Argonne National Laboratory as described previously (*78*). One hundred and fifty one base paired-end reads were generated from esophageal DNA samples with 12 bp barcodes. The V4 region of the 16S rRNA gene (515F-806R) was PCR amplified with region-specific primers that include sequencer adapter sequences used in the Illumina flowcell. Analysis of commensal bacteria was carried by CLC Microbial Genomics Module (Qiagen).

Immunofluorescent staining and analysis

Formalin-fixed and paraffin-embedded (FFPE) distal esophageal biopsies or FFPE cells grown in ALI cultures were serially sectioned and de-paraffinized using xylene and then subjected to graded ethanol washes. Heat-induced epitope retrieval in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) was used. Slides were incubated with rabbit anti-human KLK5 (cat ABC470; Millipore) and mouse anti-human

DSG1 clone B-11 (Santa Cruz). Following a 1-h incubation with goat anti-rabbit Alexa Fluor 594 and goat anti-mouse Alexa Fluor 488 (From Life Technologies) and mounting with 4',6-diamidino-2phenylindole (DAPI) Fluoromount-G (Novus Biological), images of KLK5, DSG1, and fluorescent conjugates of A1AT were obtained using the Nikon A1RSi confocal microscope (Nikon). Quantifications of fluorescent intensity were done by Imaris software (Bitplane).

Transepithelial electrical resistance (TEER) and fluorescein isothiocyanate (FITC)-dextran measurements

Using differentiated cells, TEER and 3-5–kDa FITC-dextran (Sigma-Aldrich) paracellular flux were measured at day 7 using a fluorescence plate reader (BioTek) and day 14 using an EVOM (World Precision Instruments), as previously described (*79*).

Serine protease activity measurements

Supernatants from differentiated ALI cultures or 5 μg of protein lysates from processed esophagi were incubated were mixed with BOC-VPR-AMC fluorogenic peptide substrate (R&D Systems) and incubated for overnight at 37°C. The plate was read with excitation at 380 nm and emission at 460 nm using a fluorescence plate reader (BioTek) every hour for a total time of 20 h.

SPINK7, SPINK5, and A1AT in vitro inhibitory assay

Recombinant KLKs were purchased from R&D Biosystems. Recombinant KLK5, KLK7, KLK11, KLK12, or KLK13 (20 nM) were activated according to the manufacturer's protocol (R&D Biosystems) and incubated with the indicated concentrations of

recombinant SPINK7 (Abcam), SPINK5 (R&D Biosystems), or A1AT (Grifols) for 15 min. Then, substrates were added (Boc-VPR-AMC, Mca-RPKPVE-Nval-WRK(Dnp)-NH2 or Val-Leu-Lys-SBzl). Fluorescence or absorbance were measured every minute for a total time of 30 min according to the manufacturer's protocol. Inhibitory constants (Ki) were determined after serial dilutions of the substrate and were calculated according to Morrison equation (*65*).

Protease array

Supernatants from differentiated EPC2 cells were collected, and total protein was quantified using the BCA assay (Life Technologies). The Proteome Profiler Human Protease Array Kit (R&D Systems) was used to quantify protease expression, which was quantified using the Image Studio Software (LI-COR) and normalized to total protein.

Gel electrophoresis western blotting

Esophageal protein lysates were extracted with M-PER buffer (Thermo Fisher Scientific) and protease inhibitor cocktail (Roche, catalog 04 693 159 001) unless mentioned otherwise. Protein concentrations were determined by the BCA assay kit (Thermo Scientific). Loading buffer (Life Technologies) was added, and samples were heated to 95°C for 5 min and subjected to electrophoresis on 12% NuPAGE Bis-Tris gels (Life Technologies). A1AT fluorescent conjugates were immediately visualized from the NuPAGE Bis-Tris gels using the Odyssey CLx system (LI-COR Biosciences). For mucin 4 (MUC4), DSG1, and GAPDH analysis, 12% NuPAGE Bis-Tris gels were transferred to nitrocellulose membranes (Life Technologies) and visualized and analyzed using the

Odyssey CLx system (LI-COR Biosciences) with IRDye 800RD goat anti-rabbit and IRDye 680RD goat anti-mouse (LI-COR Biosciences) secondary antibodies. The primary antibodies were mouse anti-human MUC4 clone 5B12 (Abcam) and rabbit anti-human GAPDH (Catalog AB9485; Abcam) and rabbit anti-human DSG1 clone H-290 (Santa Cruz).

A1AT conjugation

A1AT (ProlastinC) was conjugated to HiLyte Fluor 647 using the AnaTag protein labeling kit according to the manufacturer's protocol (Anaspect). Briefly, 10 mg/mL of A1AT was incubated with DMSO and HiLyte Fluor 647 for 1 hour at room temp and dye-protein was purified using a desalting column. Gel electrophoresis of A1AT was performed as described above.

Analysis of A1AD and EoE prevalence rate

The patients used in this analysis were identified via the Informatics for Integrating Biology and the Bedside (i2b2) data warehouse at CCHMC. The i2b2 warehouse is a deidentified database of all patients derived from our local Epic electronic medical record with records from March 2007 through December 2019 (*80, 81*). Diagnosis codes used for eosinophilic esophagitis include: 530.19, 530.13, and K20.0 and codes for alpha-1 antitrypsin (A1AT) deficiency include 273.4 and E88.01. P value was calculated by Prism using Chi-square and Fisher's exact test.

Supplementary Figures

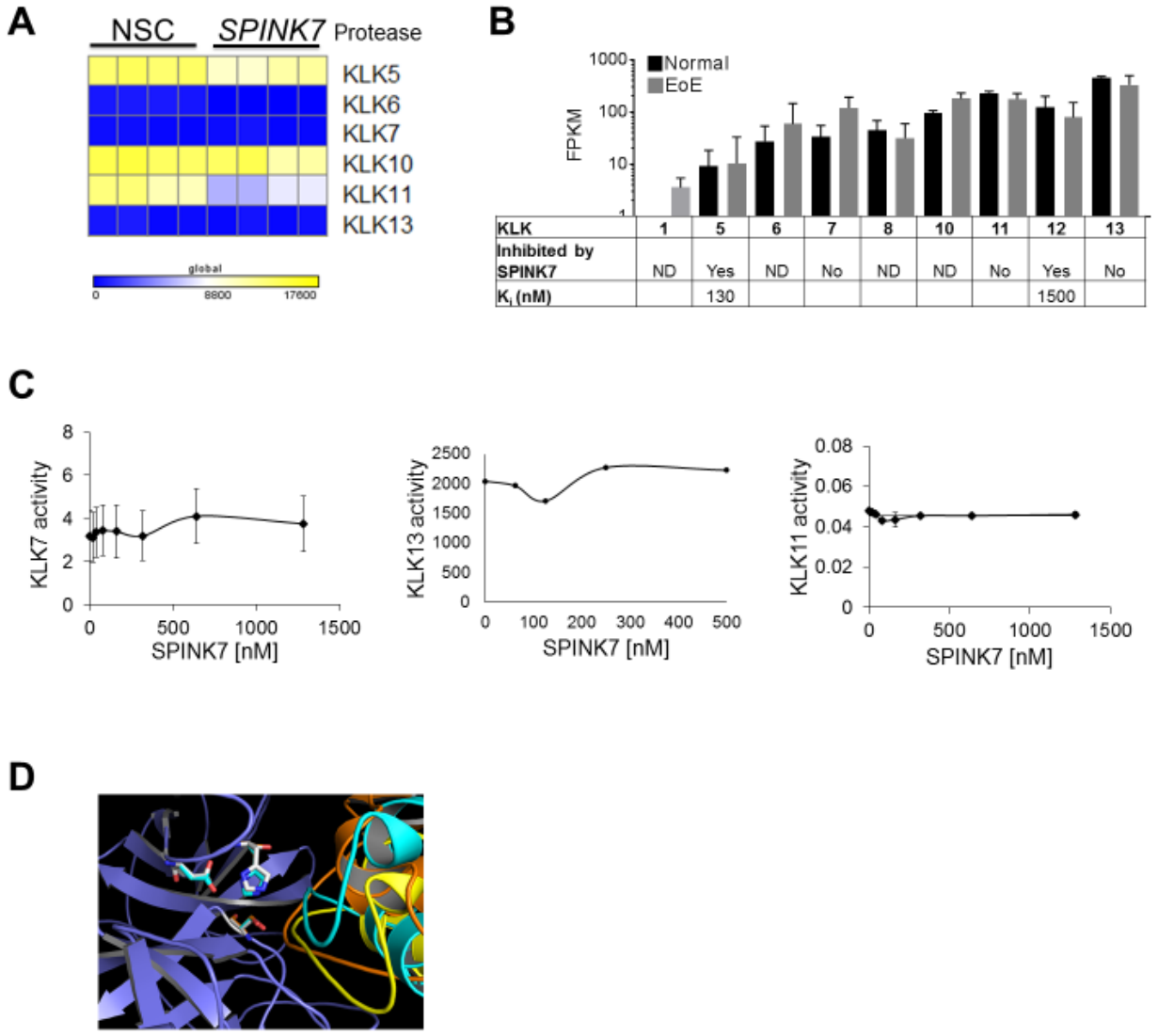
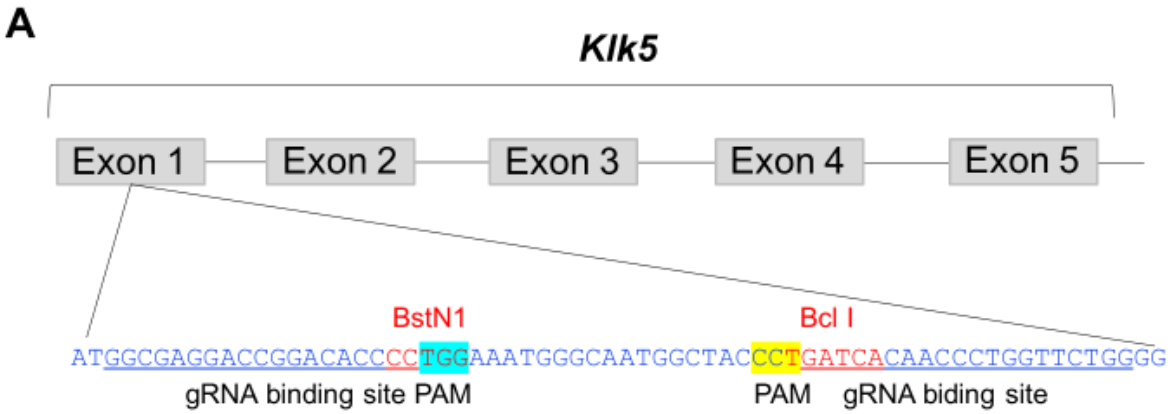
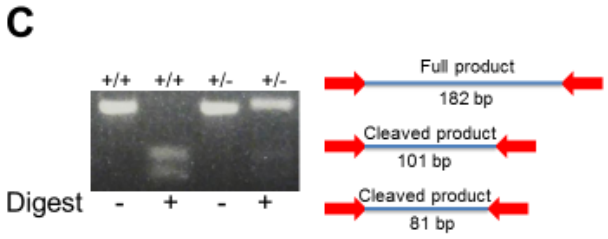
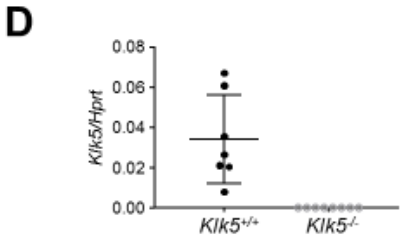


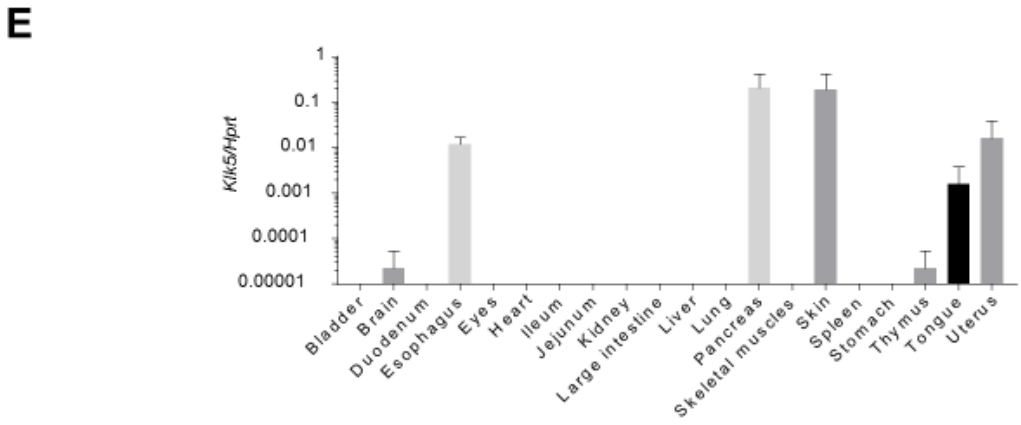
Fig. S1. SPINK7 inhibits KLK5 and KLK12. A. KLK protein expression in supernatants derived from differentiated non-silencing control (NSC)-treated or SPINK7-depleted EPC2 cells from 2 independent experiments performed in duplicates. **B.** FPKM values for *KLK* family members determined from RNA sequencing of esophageal biopsies (n = 6 control patients [Control] and n = 10 patients with active EoE [EoE]). The indicated KLKs were incubated with their substrates and a wide range of doses of SPINK7. The

proteolytic rate for each dose of SPINK7 was calculated according to serial measurements. Proteolytic activity is presented as mean values +/- SD from 3 independent experiments performed in duplicates. Values of the inhibitory constant (Ki) were calculated according to Morrison equation. ND, not determined. **C.** The indicated KLKs were incubated with their substrates and a wide range of doses of SPINK7. The proteolytic rate for each dose of SPINK7 was calculated. Results are the mean +/- SD. Values of inhibitory constants (K_i) were calculated according to the Morrison equation. **D.** Docking results of KLK5 (blue) and SPINK7 colored according to the docking program (yellow, ClusPro [as presented in Fig. 1C]; orange, GRAMM-X docking program; cyan, PYDOCK docking program). Gray lines represent the catalytic triad of KLK5.









Supplementary Figure 2. Azouz et al.

Fig. S2. Generation of *Klk5*-deficient mice. A. A schematic representation of the dual single-guide RNA (sgRNA) targeting strategy. sgRNAs were selected according to the on- and off-target scores from the web tool CRISPOR (*69, 70*) to target locations of the first exon of the *Klk5* gene. The target sites overlap with two restriction enzyme sites, BstN1 and Bcl I, respectively. **B.** Genomic sequence of the *Klk5* gene indicating the deletion site. **C.** DNA electrophoresis gel of genomic PCR products of the regions harboring the deletion site in the *Klk5* gene before and after BstN1 enzymatic digestion from $Klk5^{+/+}$ (+/+) and heterozygous $Klk5^{+/-}$ (+/-) mice. **D.** *Klk5* expression in the esophagus of $Klk5^{-/-}$ and control mice. **E.** *Klk5* expression was determined in the indicated organs from 3 BALB/c mice by qPCR. Data are the mean ± SD.

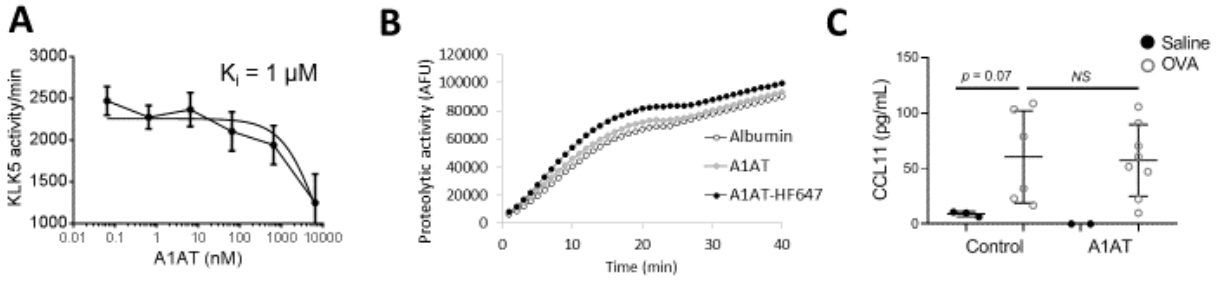


Fig. S3. A1AT inhibits KLK5 in vitro and trypsin-like proteolytic activity and CCL11 production in vivo. A. KLK5 was incubated with substrate and the indicated doses of A1AT. The proteolytic rate for each dose of A1AT was calculated. Values of inhibitory constant (Ki) were calculated according to the Morrison equation (*65*). Proteolytic activity is presented as mean values +/- SD from 3 independent experiments performed in duplicates. **B.** Proteolytic activity measurement of serum samples following 2 intraperitoneal injections of albumin, A1AT, or AIAT-HF647 (1 mg each) using the substrate BOC-VPR-AMC and 5 nM of recombinant human KLK5. Circles are fluorescence reads that represent the proteolytic activity. **C.** CCL11 expression in the esophagus of mice following induction of allergic inflammation. Data derived from 3 independent experiments with at least 2 mice in each group. Each circle represents data point from one mouse.

NS- non-significant. P value was calculated by t test (unpaired, two-tailed).

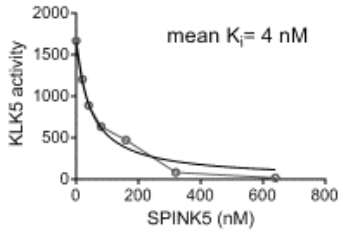
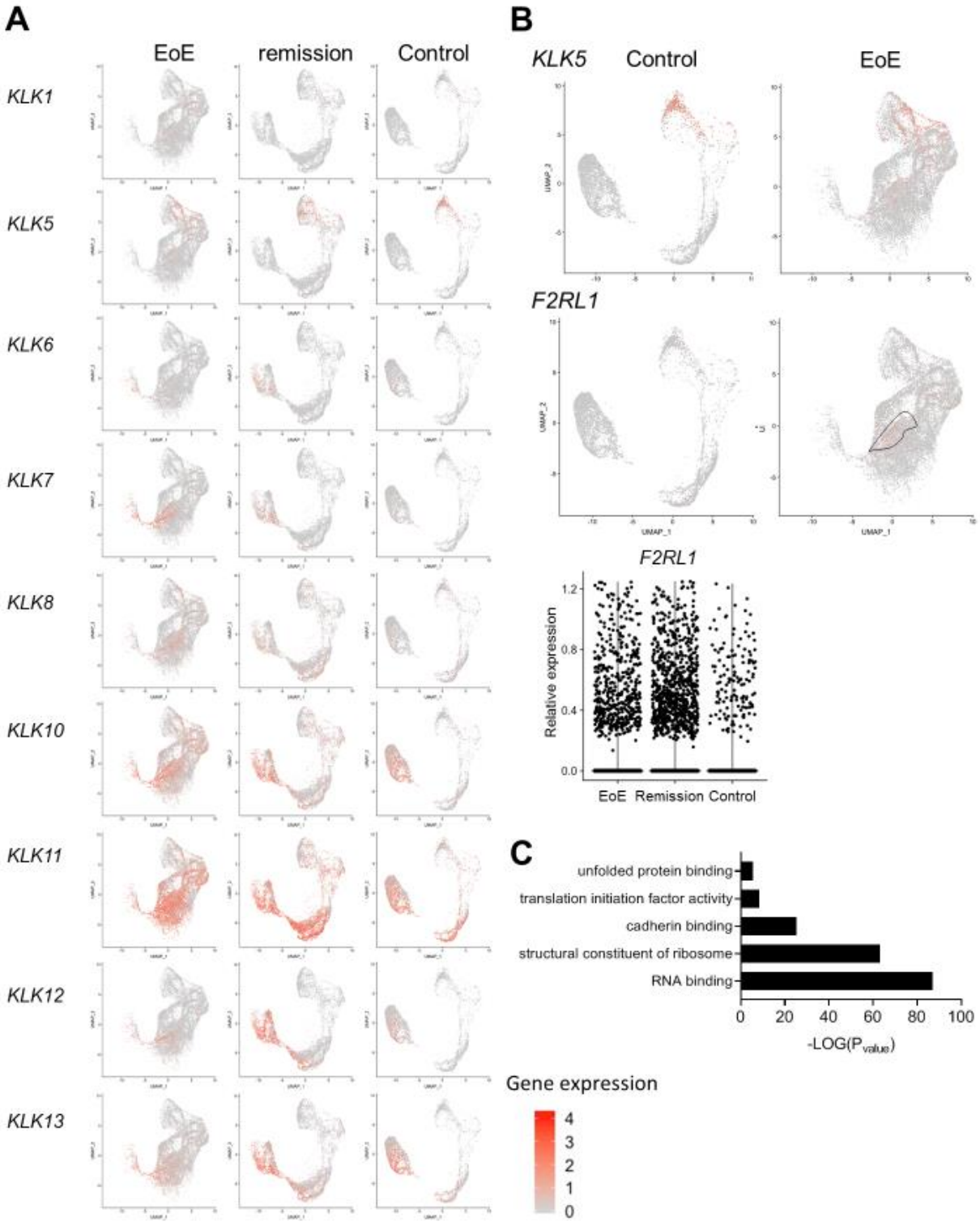


Fig. S4. SPINK5 inhibits KLK5 in vitro. KLK5 was incubated with substrate and the indicated doses of SPINK5. The proteolytic rate for each dose of SPINK5 was calculated. Values of the inhibitory constant (Ki) were calculated according to the Morrison equation (*65*). Proteolytic activity is presented as mean values +/- SD from 3 independent experiments performed in duplicates.



Supplementary Figure 5. Azouz et al.

Fig. S5. Esophageal KLK and *F2RL1* expression profile. A. UMAP plot of single-cell RNA sequencing analysis of dispersed cells from esophageal biopsies. The expression of *KLK1, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK12,* and *KLK13* in individual cells (each dot) is shown. Data derived from 2 control and 5 EoE biopsies and total of 47,141 cells. **B.** UMAP plot of single-cell RNA sequencing analysis of dispersed cells from esophageal biopsies. The expression of *KLK5* and *F2LR1* (gene product, PAR2) in individual cells (each dot) is shown in EoE and control biopsies. *F2LR1* positive cell population is surrounded by a black line. The graph in the bottom shows the relative expression of *F2LR1* in individual cells (each dot) from control, remission and EoE biopsies. **C.** Gene ontology (GO) analyses of the genes that are most enriched in epithelial clusters 1, 2 and 3.

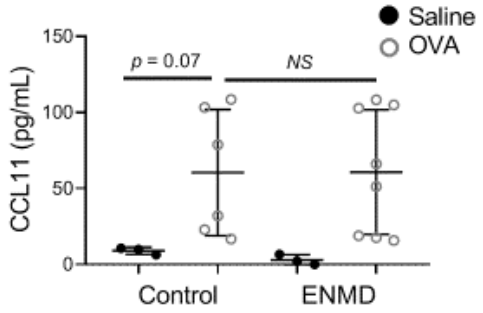

Saline
OVA
150
100
50
0
CCL11 (pg/mL)
p = 0.07
NS
Control
ENMD

Fig. S6. CCL11 production in the esophagus. CCL11 expression in the esophagus of mice following induction of allergic inflammation. Data derived from 3 independent experiments with at least 2 mice in each group. Each circle represents data point from one mouse. Control mice here are also reported in fig. S3C. NS- non-significant. (unpaired, two-tailed t-test).

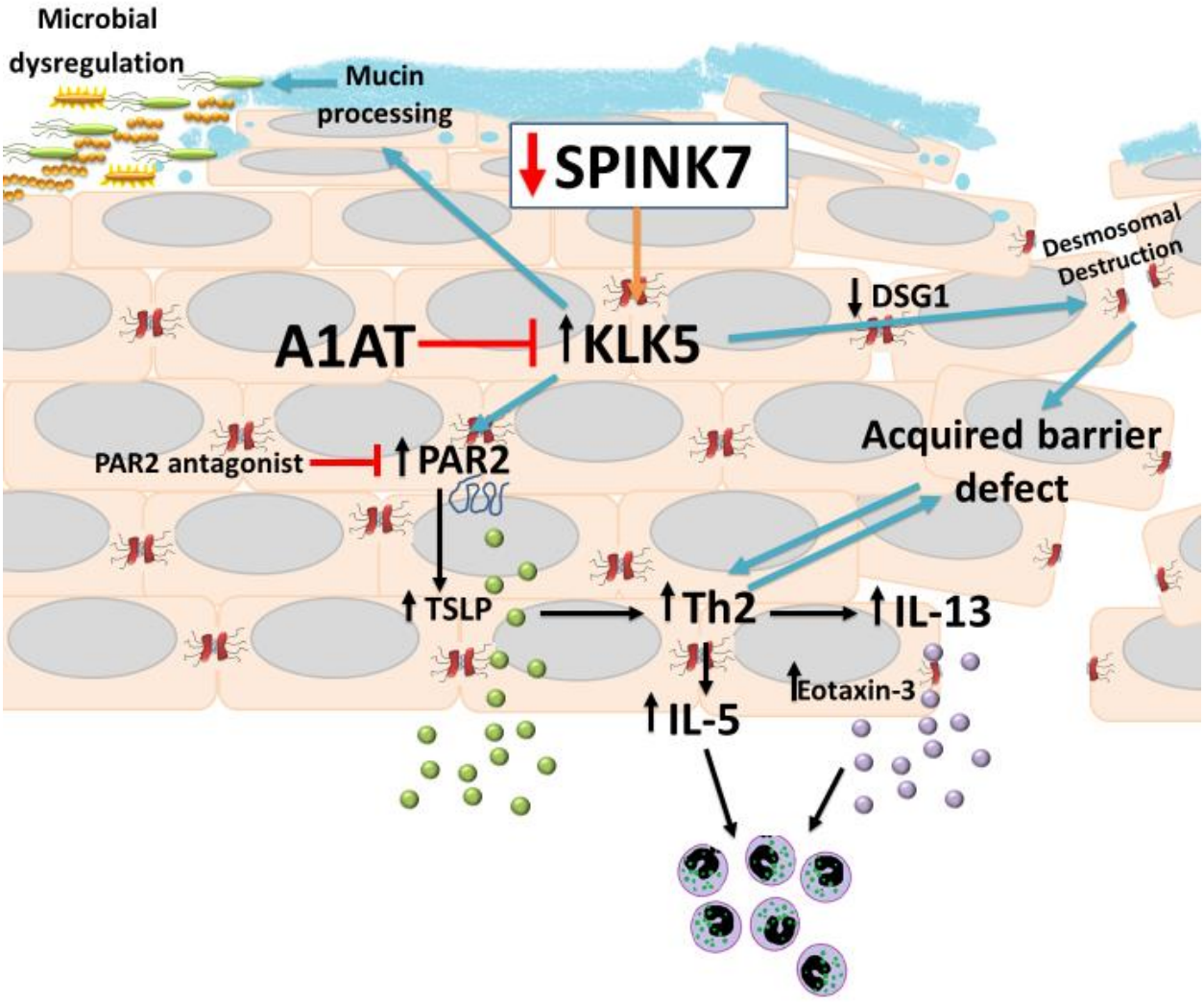


Fig. S7. A model of the SPINK7, KLK5, and PAR2 pathway in EoE. Decreased esophageal expression of the protease inhibitor SPINK7 occurs in eosinophilic esophagitis (EoE). Loss of SPINK7 unleashes uncontrolled proteolytic activity of the

serine protease KLK5. KLK5 cleaves DSG1 in epithelial desmosomes, leading to epithelial barrier impairment. As a result, luminal antigens encounter dendritic cells, which then promote type 2 immune responses. Type 2 immune responses are characterized by IL-13 production, which stimulates epithelial cells to produce eotaxin-3; eotaxin-3 recruits eosinophils and IL-5, which is indispensable in eosinophil development (*82, 83*). KLK5 cleaves mucins, which disrupts the host-microbiome homeostasis. KLK5 activates PAR2, which in turn promotes signaling pathways in epithelial cells leading to cytokine production, including TSLP that promotes type 2 immune responses. KLK5 activity can be blocked by esophageal delivery of A1AT, and PAR2 activation is blocked by PAR2-antagonized molecules.

Supplementary Tables

Table S1. Prevalence rate of A1AD in patients with EoE compared to the general population. Patients with A1AD, EoE, and concomitant A1AD / EoE were enumerated, and the proportions of patients with and without EoE and A1AD utilizing a 2 by 2 contingency table.

	EoE	A1AD
EoE	4724	8
Total	1300000	351
%	0.36	2.8
Odds ratio	**7.8-fold**	$p < 0.0001$

Table S2. List of bacteria at the family level that are differentially abundant in the esophagus of *Klk5*-deficient mice. Analysis of bacteria in the family level from the phylum *Firmicutes* that were differentially abundant in the esophagus of *Klk5*$^{-/-}$ mice compared with *Klk5*$^{+/+}$ mice.

Name	Fold change	P-value	FDR p-value
Oxalobacteraceae, 1105574	-1,316	2.34E-07	5.65E-05
Sinobacteraceae, 155989	-960	8.82E-07	8.20E-05
Lachnospiraceae, 355771	-329	1.92E-06	8.20E-05
Ruminococcaceae, 361811	-425	2.15E-06	8.20E-05
Sphingomonadaceae, 1110227	-443	2.66E-06	8.20E-05
Acetobacteraceae, 348570	-593	3.06E-06	8.20E-05
Intrasporangiaceae, 689528	-316	3.21E-06	8.20E-05
Methylocystaceae, 417366	-563	3.37E-06	8.20E-05
Koribacteraceae, 550247	-316	3.48E-06	8.20E-05

Table S3. List of the most enriched genes in epithelial clusters 1, 2, and 3.

Provided as a separate Excel file.

Data file S1. Primary data.

Provided as a separate Excel file.