Supplementary Materials and Methods

Bioinformatics Analysis of RNA Sequencing Datasets

For the raw fastq files, adapters were trimmed and low-quality reads (quality cutoff 20) were filtered using cutadapt.[1] Reads were aligned to the human genome (GRCh38) using hisat2.[2,3] Aligned SAM files were converted to BAM format using samtools,[4] and featureCounts[5] was used to quantify the total number of counts for each gene. Genes with low expressions were filtered (total sum of counts <5) and differential expression analysis comparing EoE with healthy controls was performed using DESeq2[6] for each individual dataset. Genes that were consistently differentially expressed among the 3 datasets were identified by a weighted meta-analysis approach using Stouffer's P value combination method[7] in which the weights were proportional to the square root of the sample sizes. For each gene, average log2 fold-change was calculated to measure the overall expression change between EoE and controls across the 3 datasets. Among the significantly differentially expressed genes (false discovery rate <0.05 and absolute fold-change >2), enrichment of calcium signaling pathway from Kyoto Encyclopedia of Genes and Genomes[8,9] (downloaded from MSigDB[10,11]) was tested using Fisher exact test.

H&E Staining of Air–Liquid Interface Cultures

Cells were fixed in 4% paraformaldehyde added directly to the top and bottom of the Transwell. Cells on the support membranes were dehydrated with 70% ethanol followed by paraffin embedding. Serial 5-μm sections were cut and mounted on glass slides. Slides were stained with H&E for histologic evaluation. The original TIFF images of H&E-stained ALI cultures were modified in Photoshop to enhance clarity. Any adjustments in contrast, color balance, brightness, or sharpness were applied to the entire image.

Enzyme-Linked Immunosorbent Assay for Eotaxin-3

Conditioned media from esophageal cells were collected at each time point and centrifuged to remove cellular debris. Eotaxin-3 concentrations were determined using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) per manufacturer's instructions. The absorbance of each well was read at 450 nm and 540 nm using a DTX 880 Multimode plate reader (Beckman Coulter, Indianapolis, IN). All enzyme-linked immunosorbent assays were performed in at least 2 independent experiments.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated by RNeasy Mini Kit for cultured cells (Qiagen, Redwood City, CA). Reverse transcription was performed using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Grand Island, NY) per manufacturer's instructions. Primer sequences (Supplementary Table 2) were designed using Primer Express (Applied BioSystems, Foster City, CA) and manufactured by Integrated DNA Technologies (Coralville, IA). Quantitative real-time polymerase chain reaction for mRNAs was carried out with the QuantStudio 6 Flex Real-Time PCR System and SYBR Green mix (Applied Biosystems, Foster City, CA); glyceraldehyde-3-phosphate dehydrogenase was used as a reference gene. The relative quantity of mRNA with respect to reference gene was calculated. All quantitative real-time polymerase chain reaction assays were performed in triplicate in 3 independent experiments.

Protein Extraction and Immunoblot

Total protein was extracted using 1X cell lysis buffer supplemented with 1 mM phenylmethylsulfonyl fluoride according to manufacturer's instructions (Cell Signaling Technology, Danvers, MA). Protein concentrations were determined using Nanodrop 2000C (IMPLEN, Munich, Germany). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes and incubated with primary rabbit polyclonal anti-human ATP12A (Invitrogen, Waltham, MA; cat# PA5-49624) at 1:1000 dilution or rabbit polyclonal anti-human HRH2 (Origene, Rockville, MD; cat# TA308415) at 1:1000 dilution overnight at 4°C. Secondary antibody was goat anti-rabbit IgG conjugated with horseradish peroxidase (Cell Signaling Technology) at 1:2000 dilutions, and chemiluminescence was determined using ImageQuant Las4000mini (GE Healthcare, Uppsala, Sweden). Membranes were stripped and re-probed with anti–β-tubulin (Sigma, St Louis, MO) at 1:2000 dilution as loading control for total protein. All blots were repeated in at least 2 independent experiments.

Statistical Analyses

Quantitative data are expressed as mean ± SEM. Statistical analyses were performed using a paired or unpaired Student t test with Instat for Windows or Prism statistical software package (GraphPad Software, San Diego, CA). For multiple comparisons, a 1-way analysis of variance or repeated measures analysis of variance with post-hoc Student-Newman-Keuls multiple-comparisons test was performed with Instat for Windows statistical software package (GraphPad). P values $\leq.05$ were considered significant for all analyses.

Supplementary References

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10. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545–15550.
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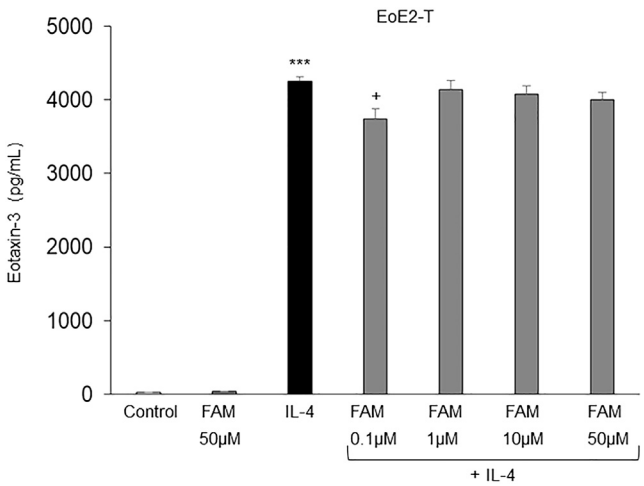
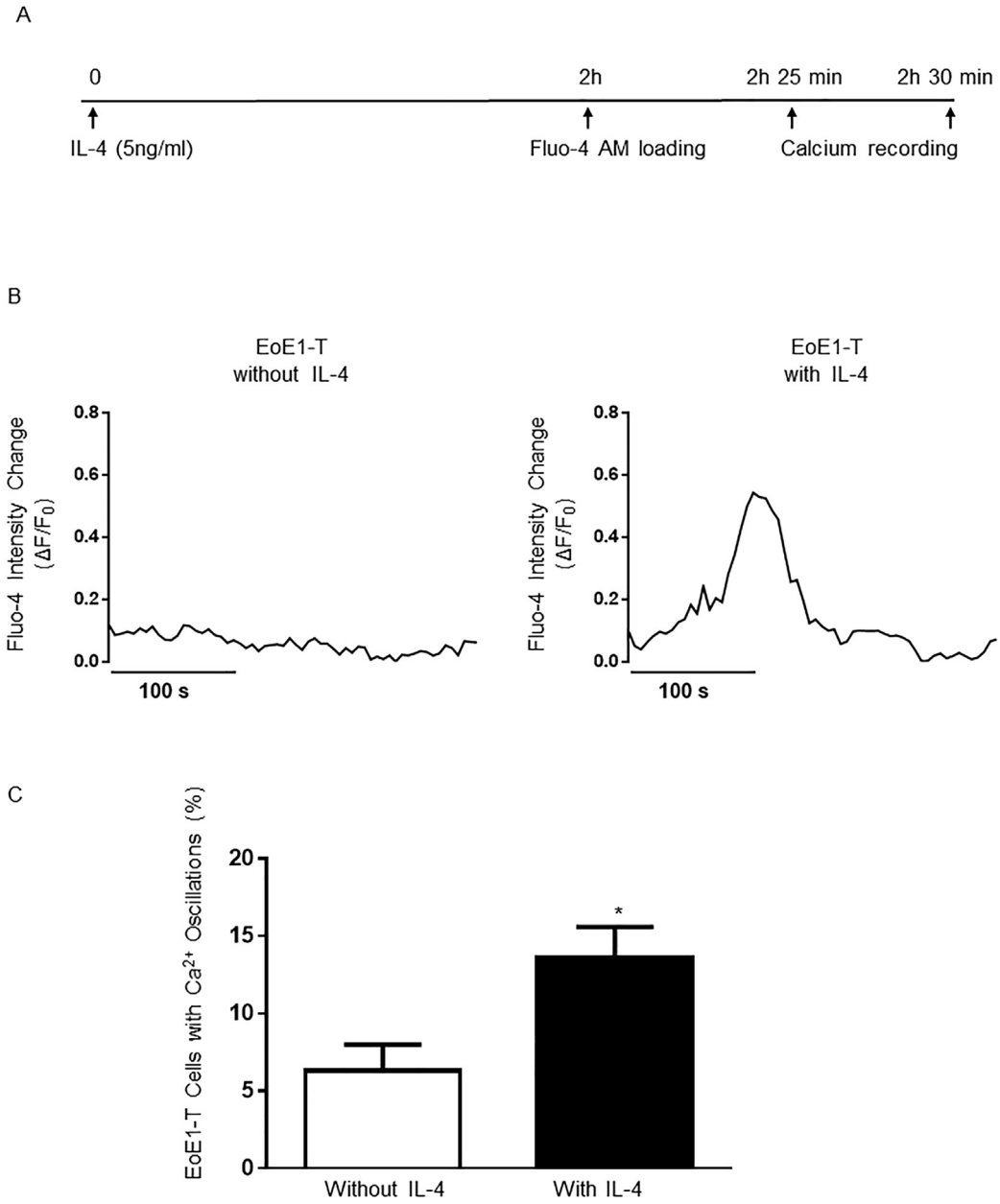
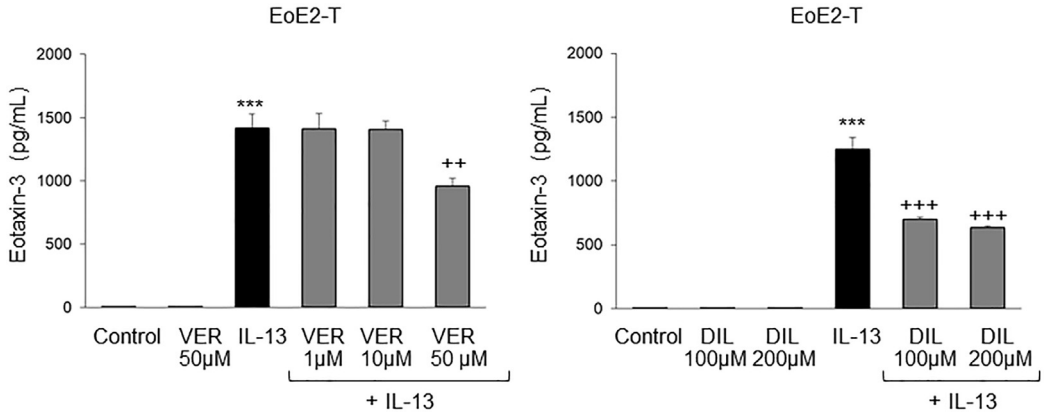


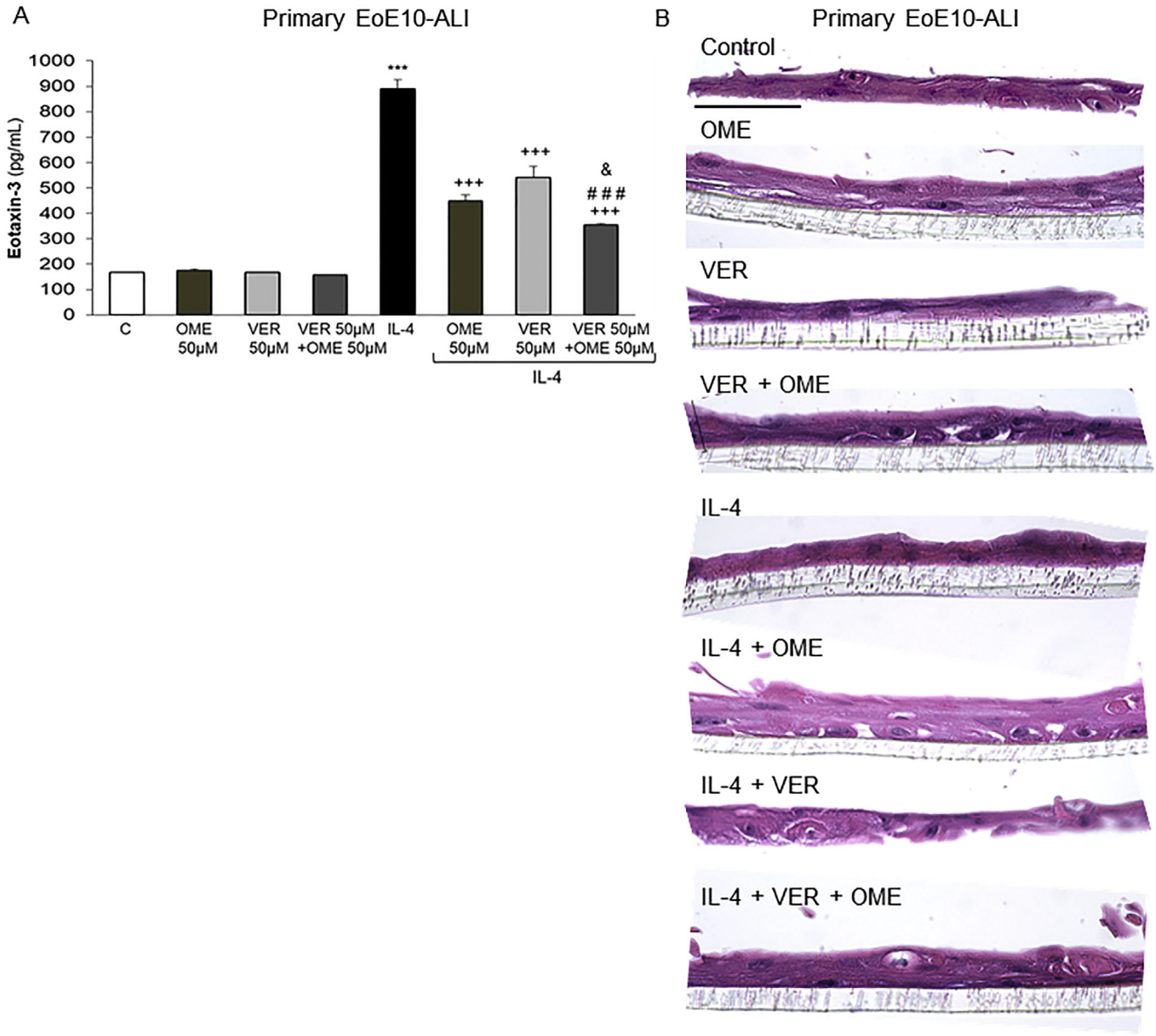
Supplementary Figure 1. Famotidine (FAM) does not decrease IL-4–stimulated eotaxin-3 secretion in EoE2-T cells. Treatment with the 0.1 μM dose of FAM caused a minimal, but statistically significant, reduction in IL-4–stimulated eotaxin-3 secretion; eotaxin-3 secretion was not decreased by FAM at doses $\geq$1 μM. *Bar graphs* show results of representative enzyme-linked immunosorbent assays for eotaxin-3 at 48 hours after IL-4 treatment with and without FAM. *Bar graphs* depict the mean $\pm$ SEM from 3 separate samples. ***$P \leq .001$ compared with control; $^{+}P \leq .05$ compared with IL-4; 1-way analysis of variance.



Supplementary Figure 2. IL-4 induces intracellular calcium oscillations in EoE1-T cells. (*A*) *Diagram* of the experimental protocol. (*B*) Representative *curves* of intracellular calcium oscillations (measured as changes in Fluo-4 fluorescence [ΔF/F0]) in single EoE1-T cells without and with IL-4 treatment. *Time scale bar:* 100 seconds. (*C*) Percentage of EoE1-T cells with calcium (Ca^{2+}) oscillations without and with IL-4 treatment. *Bar graphs* depict the mean ± SEM from 2 independent experiments with 5 views. $^*P < .05$ compared with non-IL-4–treated cells; unpaired Student *t* test.



Supplementary Figure 3. Verapamil and diltiazem block IL-13–stimulated increases in eotaxin-3 secretion in EoE2-T cells. Representative eotaxin-3 enzyme-linked immunosorbent assays at 24 hours in EoE2-T after IL-13 treatment with and without verapamil or diltiazem. *Bar graphs* depict the mean ± SEM from 3 separate samples. $^{***}P \leq .001$ compared with control; $^{++}P \leq .01$; $^{+++}P \leq .001$ compared with IL-13; 1-way analysis of variance.



Supplementary Figure 4. Omeprazole (OME) in combination with verapamil (VER) has a significantly greater suppressive effect on IL-4–stimulated eotaxin-3 secretion than either agent alone in primary EoE10-ALI cells. (*A*) Representative eotaxin-3 enzyme-linked immunosorbent assays 48 hours after IL-4 treatment in primary EoE10-ALI cells with and without OME, VER, or the combination of both. (*B*) H&E stains of primary EoE10-ALI cells under the specified conditions. *Bar graphs* depict the mean ± SEM from ALI cultures performed at least in triplicate from 1 female patient with EoE. $^{***}P \leq .001$ compared with control; $^{+++}P \leq .001$ compared with IL-4; $^{\#\#\#}P \leq .001$ compared with IL-4 + VER 50 μM; $^{\&}P \leq .05$ compared with IL-4 + OME 50 μM; 1-way analysis of variance.

Supplementary Table 2. Oligonucleotide Primers

Primer	Sequence (5′ to 3′)	Location	Use
ATP4A-F	TGCCTACACATTGACCAAGAA	Sense	qPCR
ATP4A-R	CAGAGTTCGATGAAGAGGATGG	Antisense	qPCR
ATP12A-F	GAGTGCAGGGATCAAGGTTATT	Sense	qPCR
ATP12A-R	GTGCAATGTCTTCCACTGTTTC	Antisense	qPCR
HRH2-F	CGTGTCCTTGGCTATCACTGA	Sense	qPCR
HRH2-R	GGCTGGTGTAGATATTGCAGAAG	Antisense	qPCR
GAPDH-F	TGACGCTGGGGCTGGCATTG	Sense	qPCR
GAPDH-R	GGCTGGTGGTCCAGGGGTCT	Antisense	qPCR

qPCR, quantitative real-time polymerase chain reaction.