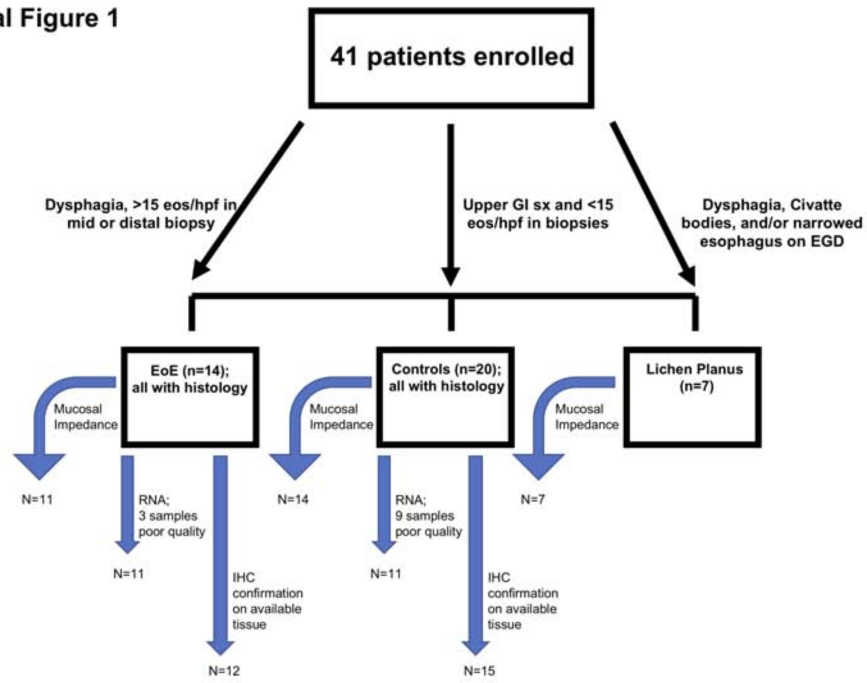
Supplemental Figure 1



Supplemental Table 1

	Sub-UES Eosinophils (n=8)	No Sub-UES Eosinophils (n=6)	p-value*
Eosinophil Number (median, range)	6, 2-60	0, 0-0	0.003
Age	29.6 ± 7.8	39.2 ± 4.8	0.02
Male Gender	75% (6/8)	67% (4/6)	0.99
Years Followed	1.0 ± 1.0	1.0 ± 0.7	0.77
Food impactions	37.5% (3/8)	0% 0/6	0.20
Endoscopy Number	1.6 ± 0.9	0.5 ± 0.5	0.03
Therapy Escalations	2.0 ± 0.5	1.0 ± 0.6	0.02
PPI at time of endoscopy	4/8 (50%)	1/6 (17%)	0.31

*Mann-Whitney

Supplementary Methods:

<u>Study Design and Patient Selection:</u>

Please see Supplemental Figure 1 for a summary of study design and patient selection. All samples were prospectively collected, and histologic evaluation was done for all patients. Our initial aim was only to do histologic analysis and extract RNA from tissue samples. However, to assess epithelial integrity of the sub-UES versus the mid/distal esophagus, mucosal impedance (MI) was added as part of the study after tissue had already been collected for the first three EoE patients and 6 controls. Thus, MI data is only available for 11 EoE patients and 14 controls.

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Moreover, data was not available for all patients for qPCR or IHC. For qPCR analysis, we were unable to extract high quality RNA from three of the EoE patient samples and 9 of the controls and thus we were unable to include these in our analysis. Since there were differences in gene expression between the sub-UES and mid/distal esophagus in patients with EoE, we decided to confirm our findings at the mRNA level with immunohistochemistry (IHC) to see if the same changes were present at the protein level. Unfortunately, we did not have enough remaining tissue in order to cut slides for 2 EoE patients and 5 controls. Since there was no difference in histology between the mid and distal esophagus, we only performed IHC on distal and sub-UES sections.

Lichen Planus Patients:

Initially, Lichen Planus (LP) patients were not part of the study design as the primary objective was to characterize the sub-UES in patients with active EoE. However, since patients with LP can be difficult to distinguish from EoE, we enrolled these patients to determine if sub-UES mucosal impedance could aid in the diagnosis of these patients. LP patients were not part of

the control group and were selected separately. The LP patients were all known to have lichen planus before the time of endoscopy when mucosal impedance was measured.

Mucosal Impedance:

A special sensor array composed of 360 degree circumferential rings (Sandhill Scientific, Inc, Highlands Ranch, CO) was engineered and mounted on a 2 mm–diameter catheter with the following specifications: (1) ring length of 3 mm, (2) ring separation of 2 mm, (3) end of distal ring mounted 1 mm away from the tip of the catheter, and (4) a soft catheter easily traversable through the working channel of an upper endoscope. The electrodes were connected to an impedance voltage transducer at the bedside via thin wires, which run the length of the catheter. The voltage generated by the transducer was limited to produce at most 2.5 mA root mean square of current. The frequency for the measuring circuit was set at 2 kHz. Impedance measurements of the esophageal mucosa were expressed in ohms as the ratio of voltage to the current, according to Ohm's law. Data were acquired with a stationary impedance data acquisition system (InSight; Sandhill Scientific, Inc) and were viewed and analyzed on BioView Analysis software (Sandhill Scientific, Inc).

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Histologic analysis:

All specimens were interpreted using a Nikon E600 microscope (Nikon Instruments Inc, Melville, NY). Eosinophils were counted using a 40x objective, a field diameter of 0.625 mm, and a field area of 0.307 mm^2.

qPCR analysis:

Mucosal biopsies were placed in RNAlater (Invitrogen) immediately after collection and then stored at -80°C. Mid and distal tissue was combined from both EoE patients and controls to increase our chances of isolating high-quality RNA. Samples were homogenized in TRIzol reagent. Total RNA was purified with RNeasy Mini Kit with on-column DNase treatment (QIAGEN) according to the manufacturer's instructions. cDNA was synthesized using the qScript XLT cDNA Supermix (Quanta) from 0.5-2 µg of total RNA. 1 µL of cDNA was used as a template in each subsequent PCR reaction. Real-time PCR was performed on Bio-Rad CFX96 real-time cyclers using LuminoCT SYBR Green Master Mix (Sigma-Aldrich). After preincubation at 95°C, 2-step cycling was performed from 95°C (10 seconds) to 60°C (30 seconds) for 40 cycles. *GAPDH* was used to calculate normalized fold change. Melting curves of the PCR products were analyzed using CFX Manager software (Bio-Rad) to exclude amplification of nonspecific products.

Immunohistochemistry:

In order to confirm changes in mRNA expression of EoE candidate genes, we performed immunohistochemistry on the EoE and control patients with remaining tissue available (n=12-15).

5µm sections were cut, dewaxed, hydrated, and endogenous peroxidase activity quenched with 0.03% hydrogen peroxide in sodium azide. Antigen retrieval was done with pH 6.0 citrate buffer (Filaggrin and Periostin) or pH 9.0 EDTA (Desmoglein-1). The following antibodies were used: anti-Filaggrin (1:75 dilution, Abcam ab218397, mouse monoclonal antibody), anti-Periostin (1:800 dilution, Abcam ab79946, rabbit polyclonal antibody), and anti-Desmoglein-1 (1:800 dilution, Proteintech 24587-1-AP, rabbit polyclonal antibody). Quantification was done according to epithelial depth and staining intensity on a severity scale (0-3) by an experienced GI

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pathologist (M.K.W.) who was blinded to the location of the biopsy and the diagnosis of the patient.

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