Supplemental Methods

In vitro analysis of eosinophil secondary granule proteins and CLC/Gal-10 using the Oesophageal String Test

To determine whether the Enterotest™ nylon string could capture eosinophil proteins, Enterotest™ nylon string (2 cm) were incubated with eosinophil sonicates or intact IL-5-activated (25ng/ml) eosinophils (1×10^4 - 1×10^6 cells, >98% purity and viability) for various time periods in RPMI1640 tissue culture media containing 8% fetal bovine serum as previously described.[20, 21] Strings were harvested, blotted to remove excess media, and briefly incubated in trypsin/EDTA to remove adherent intact eosinophils. Some strings were boiled in SDS-PAGE sample buffer and analyzed by SDS-PAGE/Western blotting for eosinophil peroxidase (EPX), major basic protein-1 (MBP1) and CLC/Gal-10. Some strings were transferred to 0.5% NP-40 elution buffer (pH 6.0) for 30 minutes to elute ESGPs, and secreted EDN quantified by ELISA (MBL International, Woburn, MA).

Processing Oesophageal String Test samples and biopsies for ESGPs and CLC/-10 biomarker assays

String sections were placed in 2.0 ml cryotubes on ice containing 200µl PPF-E elution buffer (0.5% BSA, 10mM EDTA, 10mM EGTA, 1% protamine sulfate, 0.5M $NaPO_4$, 1% Triton-X100, pH 6.0) optimized for eluting the ESGPs, and vortexed. Before eluting string segments for biomarker assays, a protease inhibitor cocktail (complete Mini Protease Inhibitor Cocktail Tablets; Roche, Indianapolis, IN) was added, followed by vortexing and centrifugation at 14,000g for 10 minutes to obtain the EST supernatants, which were aliquoted, snap frozen and stored at -80°C for batch analysis.

Mucosal biopsies were stored at -80°C in protease inhibitor-containing tissue extraction buffer (50 mM Tris-pH6.5, 650 mM NaCl, 5% Triton-X100, 50mM NaF, 50 mM NaPi (Sodium Phosphate, pH 6.5), 50 mM NaPPi (Sodium Pyrophosphate, pH6.5), 0.2 mg/ml RNAse, 0.2 mg/ml DNAse, 1 mM PMSF, 1X Protease Inhibitor Cocktail [Roche]) until batch processed. Biopsies were extracted twice using Biomasher™ single use centrifugal homogenizers (Omni International, Kennesaw, GA) that were optimized for oesophageal biopsy extraction for this study based on manufacturer's suggestions. Biopsy extracts were harvested, centrifuged at 14,000g and total protein concentration was determined in supernatants by Pierce BCA Protein Assay (Pierce Biotechnology, Thermo Scientific, Rockford, IL). Samples were aliquoted and stored at -80°C until batch assayed by ELISA. Levels of ESGPs and CLC/Gal-10 measured in the biopsy extracts were normalized to total extracted protein prior to data analysis to account for differences in biopsy size and efficiency of extraction.

Supplemental Figure Legends

Figure S1 – Performance of the Oesophageal String Test. (A) The extended Enterotest™ nylon string is shown with the proximal end (upper right arrow) that will be taped to the subject's cheek and the distal end that is attached to the gelatin capsule (bottom left arrow). **(B)** and **(C)** The end of the string protruding from the capsule is pulled gently to expose a length of the string equal to the length of the plastic bag (~ 10cm). **(D)** The exposed string is wrapped around the index finger and held with the thumb while the capsule is swallowed by drinking a glass of water. **(E)** The string that was wrapped around the index finger is taped with Tegaderm™ to the subject's cheek. **(F)** Identification of the oesophageal and gastric sections of the Enterotest™ string is accomplished using the pH indicator stick (red arrow) with the oesophageal (pH-neutral) section in green and the stomach (acidic pH) in yellow/orange. Only the colored portions of the string were touched with the indicator stick to identify a change in pH.

Figure S2 - Capture of eosinophil granule major basic protein-1 (MBP-1) by Enterotest™ nylon string **incubated with acid extracts of eosinophils.** Acidic extracts of purified normal blood eosinophils were prepared by sonicating 1 x 10^5 or 1 x 10^6 cells in either 25mM sodium acetate/acetic acid (NaOAc) buffer (pH4.3) or 0.1N HCl (pH 3). The Enterotest™ nylon string (2 centimeter

lengths) were incubated with 0.5 ml of the eosinophil sonicate for 1 hour at 37˚C, the Enterotest™ nylon string removed, blotted to remove excess fluid, boiled in SDS-PAGE sample buffer, and analyzed by SDS-PAGE and western blotting for the presence of MBP-1 adsorbed to the Enterotest™ nylon string. Purified MBP-1 was used as the positive control for western blotting.

Figure S3 – Time-course for detection of granule major basic protein-1 (MBP-1) and eosinophil peroxidase (EPX) adsorption to Enterotests™ **incubated with extracts of blood eosinophils.** Extracts of purified normal blood eosinophils were prepared by sonication of 1 x 10^6 eosinophils in either 25mM sodium acetate/acetic acid (NaOAc) buffer (pH4.3) or in phosphate-buffered saline (PBS; pH 7.3). Enterotest™ nylon strings (2cm lengths) were incubated with 0.5ml of eosinophil extract for the indicated time points (1-16 hours at 37˚C), the strings harvested, blotted to removed excess fluid, boiled in SDS-PAGE sample buffer, and analyzed by SDS-PAGE/western blotting for the presence of MBP-1 and EPX adsorbed to Enterotest™ nylon strings. The upper panels from the same gel show both the high (52kD) and low (14kD) molecular weight EPX subunits; the lower panel, MBP-1. EPX and MBP-1 were detected at 1 hour, with somewhat more protein using the neutral pH PBS than the acidic NaOAc extract. Incubation times longer than 1 hour did not increase the amount of MBP-1 or EPX adsorbed protein detected by western blotting.

Figure S4 - IL-5 activation of blood eosinophils results in increased detection of eosinophil secondary granule proteins (ESGPs) adsorbed to Enterotest™

nylon strings. Purified blood eosinophils (1 x 10^6 cells) were incubated with 2 cm lengths of Enterotest™ nylon strings for 1 hour in complete culture media containing 8% FBS with or without the addition of IL-5 (25ng/ml) to activate eosinophil secretion of the ESGPs. Strings were removed from the culture media, blotted to remove excess media, and some strings were briefly incubated first in trypsin/EDTA to remove adherent intact eosinophils. Enterotest™ nylon strings were then boiled in SDS-PAGE sample buffer and analyzed by SDS-PAGE/western blotting for eosinophil peroxidase (EPX) and granule major basic protein-1 (MBP-1) (left). (A) Both MBP-1 and the high (~52kD) and low (14kD) molecular weight subunits of EPX were captured by Enterotest™ nylon strings. (B) As determined by scanning and quantitation of the western blot activation of eosinophils with IL-5, increased amounts of MBP-1 and EPX were detected on the Enterotest™ nylon strings, some of which was due to adherent intact eosinophils that were removed by the trypsin/EDTA treatment.

Figure S5 - EST detection of eosinophil-derived neurotoxin (EDN) secretion by IL-5-activated intact eosinophils: measurement by ELISA. Blood eosinophils (1 x 10^4 - 1 x 10^6 cells) purified from a normal subject were cultured with (+ IL-5; 25 ng/ml) or without IL-5 (- IL-5) in the presence of Enterotest™ nylon strings (2 cm) for 1 hour at 37˚C in RPMI 1640 tissue culture media containing 8% FBS. The Enterotest™ nylon strings were transferred to 0.5% NP-40 elution buffer (pH 6.0) for 30 minutes to elute secreted eosinophil granule proteins,

and the amount of secreted EDN in the supernatant analyzed by ELISA (MBL Co., Ltd).

Figure S6 - Receiver Operating Characteristic (ROC) curves for the diagnosis of EoE with biopsies and EST sampling of eosinophil-derived proteins. ROC sensitivity vs. specificity curves are shown for biopsies (left panels) and ESTs (right panels) for measurements of Eosinophil-Derived Neurotoxin (EDN) (A and D), Eosinophils Peroxidase (EPX) (B and E) and Eosinophil Cationic Protein (ECP) (C and F) in biopsy extracts and EST supernatants. C-statistics (the area under the ROC curve) are indicated as a measure of the discriminating ability of each biomarker.