

DR. RONALD CRYSTAL (Orcid ID : 0000-0001-6177-6839)

DR. ODELYA PAGOVIKH (Orcid ID : 0000-0002-9888-198X)

Article type : Original article

Manuscript Acceptance Date: 15-Jan-2021

Gene Therapy for a Murine Model of Eosinophilic Esophagitis

Anna E. Camilleri, Sagar Nag, Anthony R. Russo, Katie M. Stiles

Ronald G. Crystal and Odelya E. Pagovich

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ALL.14822](#)

This article is protected by copyright. All rights reserved

Department of Genetic Medicine
Weill Cornell Medical College
New York, NY

Conflict:

The authors declare no conflict of interest

Funding:

These studies were supported, in part, by the Department of Genetic Medicine,
Weill Cornell Medicine.

Running head:

Gene Therapy for Eosinophilic Esophagitis

Correspondence:

Department of Genetic Medicine
Weill Cornell Medical College
1300 York Avenue, Box 164
New York, New York 10065
Phone: (646) 962-4363
Fax: (646) 962-0220
E-mail: geneticmedicine1@med.cornell.edu

Abstract

Background: Eosinophils are specialized granulocytic effector cells that store and release highly active mediators used in immune defense. Eosinophils are also implicated in the pathogenesis of allergic disorders, including eosinophilic esophagitis (EoE), a chronic disorder characterized by infiltration of eosinophils into the esophagus and release of mediators that damage tissue, resulting in gastrointestinal morbidity, food impaction and dysphagia. Treatment with elimination diets and/or topical corticosteroid therapy slow disease progression, but are complicated by adverse effects, limited compliance and loss of response to therapy. We hypothesized that a single administration of an adeno-associated virus (AAV) coding for an anti-eosinophil monoclonal antibody that induces eosinophil clearance (anti-Siglec-F) would treat on a persistent basis a murine model of EoE.

Methods: A mouse model of peanut-induced EoE that mimics the human disease was established by sensitization and challenge with peanut extract. After challenge, these mice exhibited an EoE phenotype demonstrated by elevated levels of blood eosinophils, infiltration of eosinophils in the esophagus with associated esophageal remodeling and food impaction.

Results. The mice were treated with a single intravenous administration (10^{11} genome copies) of AAVrh.10mAnti-Eos, a serotype rh.10 AAV vector coding for an anti-Siglec-F monoclonal antibody. Vector administration resulted in persistent, high levels of anti-Siglec-F antibody expression. Administration of AAVrh.10mAnti-Eos to the mouse model of EoE reduced blood ($p < 0.02$) and esophageal eosinophil numbers ($p < 0.002$), protected from esophageal tissue remodeling, and minimized food impaction.

Conclusion. These results suggest that a single treatment with AAVrh.10mAnti-Eos has the potential to provide persistent therapeutic benefit to patients with EoE.

Introduction

Eosinophilic esophagitis (EoE) is a cause of chronic upper gastrointestinal morbidity characterized by esophageal dysfunction and histologically by an eosinophilic infiltrate in the esophageal mucosa in the absence of other causes of eosinophilia¹⁻³. Clinically, EoE presents with dysphagia, food impaction and heartburn¹. Other symptoms include nausea, vomiting, weight loss, abdominal pain, poor appetite, and malnutrition^{1,4}. Adult EoE patients primarily suffer from dysphagia, often culminating in food impaction necessitating endoscopic bolus removal^{1,4}. The diagnosis of EoE requires three criteria: clinical symptoms of esophageal dysfunction; an esophageal biopsy with a minimum eosinophil count of at least 15 eosinophils per high-power microscopy field; and exclusion of other possible causes of esophagus eosinophil accumulation⁵.

The prevalence of EoE is approximately 1 in 1000, with the incidence increasing in both the pediatric and adult populations⁵. The goals of EoE treatment include symptom reduction/resolution and histological remission to avoid long-term complications such as esophageal stricture formation, furrowing, trachealization, and food impaction^{2,6}. Current therapies are complicated by high costs, limited compliance due to drug side effects, loss of or lack of response to therapy and difficulty with lifestyle modification. Many treated individuals have disease relapse after ceasing therapy⁷⁻¹⁰. No therapy for EoE has been approved by regulatory authorities in the United States¹¹.

Based on this background, we hypothesized that gene therapy delivering an anti-eosinophil monoclonal antibody directed against Siglecs on the eosinophil surface would provide persistent effective therapy for EoE with a single treatment. Siglecs are sialic acid-binding, immunoglobulin-like lectins found predominantly on leukocytes¹². The extracellular domain binds to glycans while the intracellular signaling domain of most Siglecs contains two tyrosine-based inhibitory motifs. Siglec-8 is predominantly expressed on human eosinophils and mast cells and its functional murine paralogue, Siglec-F is predominantly expressed on mouse eosinophils¹². Engagement of these receptors by the anti-Siglec-8 or anti-Siglec-F monoclonal antibodies activates a caspase dependent apoptotic pathway specifically in eosinophils resulting in programmed cell death¹²⁻¹⁵. Recent clinical studies using an anti-Siglec-8 antibody, particularly in eosinophilic gastrointestinal disorders have shown promising results in reducing tissue eosinophils and clinical symptoms^{9,16}. The use of gene therapy in the treatment of animal models of allergic disorders, including food allergies, complement mediated

disorders and hypereosinophilic disorders have demonstrated that gene transfer either with delivery of a monoclonal antibody or with gene augmentation can result in molecular and clinical phenotype improvement and may be curative¹⁷⁻²². In this context, a gene therapy delivering an anti-eosinophil monoclonal antibody should provide persistent effective therapy for EoE with a single administration.

To test this hypothesis, we developed a mouse model of peanut-induced esophageal eosinophilia associated with esophageal remodeling, which allowed us to investigate the effectiveness of a gene therapy approach targeting Siglec-F. We used an adeno-associated virus serotype rh.10 (AAVrh.10) to genetically modify the liver to express and secrete an anti-Siglec-F anti-eosinophil monoclonal antibody to generate AAVrh.10mAnti-Eos, a gene therapy-based treatment to provide single dose, long-lasting reduction of eosinophilia and associated manifestations for proof-of-principle in a mouse model of peanut-induced EoE disease.

Methods

AAVrh.10mAnti-Eos Vector

The nucleotide sequence for heavy and light chain variable regions of the anti-Siglec-F monoclonal antibody (clone 9C7, rat IgG2b) was obtained from S. Duan and J. Paulson, Scripps Research Institute (La Jolla, CA)²³. The rat IgG2b heavy chain constant region amino acid sequence was obtained from UniProtKB (P20761.1) and the rat Ig Kappa constant amino acid sequence from Genbank (AAB03702.1) and back-translated using EMBOSS Backtranseq (EMBL-EBI, Cambridge, UK) to obtain the nucleotide sequence. The heavy and light chain sequences were separated by a furin 2A protease sequence²³. The AAVrh.10mAnti-Eos vector is comprised of the nonhuman primate-derived rh.10 capsid pseudotyped with AAV2 inverted terminal repeats surrounding the expression cassette. The expression cassette consists of the cytomegalovirus (CMV) enhancer, chicken- β -actin promoter and intron, rabbit β -globin splice acceptor (CAG promoter), the anti-Siglec-F cDNA sequence, and rabbit β -globin polyadenylation signal (Supplemental Figure 1).

The optimized full-length anti-Siglec-F cDNA sequence was synthesized (Genscript, Piscataway, NJ) and cloned into the pAAV plasmid under control of the CAG promoter. The AAVrh.10mAnti-Eos vector was produced by co-transfection into human embryonic kidney 293T cells (HEK 293T; American Type Culture Collection) of the pAAV2-CAG-anti-SiglecF plasmid together with a plasmid carrying the AAV Rep proteins derived from AAV2 needed for vector

134 replication, the AAVrh.10 viral structural (Cap) proteins VP1, 2 and 3 and the adenovirus helper
135 functions of E2, E4 and VA RNA. The AAVrh.10mAnti-Eos vector was purified by iodixanol
136 gradient and QHP anion exchange chromatography as previously described²⁴. Vector genome titers
137 were determined by quantitative TaqMan real-time PCR^{25,26}. A vector coding for anti-anthrax
138 protective antigen antibody (anti-PA; AAVrh.10IgGcontrol) modified with a Rat IgG2b Fc to match
139 anti-Siglec-F was used as control for the *in vivo* studies.

140 **Flow Cytometry Apoptosis Assay**

141 Leukocytes were isolated from a hypereosinophilic mouse generated by overexpressing mouse
142 interleukin-5 with an AAV vector (AAVrh.10mIL5); leukocytes were >90% eosinophils based on
143 both complete blood counts (ADVIA hematology analyzer) and flow cytometry with anti-CD45 and
144 anti-Siglec-F antibodies (BD Biosciences, San Jose, CA) (Supplemental Figure4). The leukocytes
145 were incubated with serum equivalent to 50 µg of antibody by ELISA from mice administered
146 AAVrh.10mAnti-Eos (10¹¹ genome copies [gc]) 10 wk prior or controls for 24 hr at 37°C. The
147 leukocytes were incubated with lactadherin-FITC, which binds exposed phosphatidylserine to identify
148 cells in the early stages of apoptosis, and Sytox blue (Thermo Fisher Scientific, Waltham, MA) to stain
149 and exclude dead cells, and analyzed by flow cytometry (Beckman-Coulter Cytoflex LX).

150 ***In Vitro* Expression of AAVrh.10mAnti-Eos and AAVrh.10IgGcontrol by Western Analysis**

151 HEK 293T cells were transfected with anti-Siglec-F or anti-PA plasmid and the cell
152 media collected after 72 hours. The supernatant was concentrated, reduced and denatured, and run on
153 an SDS-Page gel (4-12% Tris-Glycine, Thermo Fisher Scientific, Waltham, MA) and blotted on a
154 polyvinylidene difluoride membrane (Thermo Fisher Scientific). The blot was stained using a goat
155 anti-rat HRP conjugated antibody (Santa Cruz Biotechnology, Dallas, TX) and bands detected using
156 ECL Plus (Thermo Fisher Scientific) and the C-Digit Blot Scanner (LI-COR Biosciences, Lincoln,
157 NE).

158 ***In Vivo* Expression of AAVrh.10mAnti-Eos and AAVrh.10IgGcontrol**

159 All animal studies were conducted under protocols approved by the Weill Cornell Medicine
160 Institutional Animal Care and Use Committee. To assess AAVrh.10mAnti-Eos directed expression of
161 anti-Siglec-F or anti-PA *in vivo*, male Balb/c mice (n=5 mice/group, age 6 to 8 wk; Taconic Farms),
162 were administered intravenously in 100 µl a one-time dose of AAVrh.10mAnti-Eos (10¹¹ gc),

AAVrh.10IgGcontrol (10^{11} gc), orphosphate buffered saline, pH 7.4 (PBS) control. Blood (100 μ l) was collected from the tail vein and allowed to clot at 23°C, followed by centrifugation at 5,000 rpm for 10 min to collect serum. Anti-Siglec-F was measured at 0 wk, and at time points over the course of 32 wk by ELISA, and anti-PA (IgGcontrol) over the course of 8 wk(Alpha Diagnostic, San Antonio, TX), conducted per the manufacturer's protocol.

Characterization of the EoE Murine Model

Balb/c male mice were obtained from Taconic Farms (6-8 wk) (NY, catalog # Balb/cAnN) and housed under pathogen-free conditions according to standard guidelines and maintained on peanut-free chow. Only male mice were used in experiments, as EoE is a male dominant disease². Mice were sensitized with 200 μ g peanut extract (*Arachis hypogaea*; Stallergenes Greer, Lenoir, NC) plus 1 mg alum adjuvant (Thermo Fisher Scientific, Waltham, MA) by intraperitoneal injection (wk 0, 2; liquid diluent, in PBS) and challenged with 100 μ g peanut extract (liquid diluent, in PBS) by intranasal administration at set intervals from 3 to 11 wk. Mice were rested for 3 wk (wk 8 to 10) to avoid tolerance to the peanut antigen. Animals were fasted for 30 min to 2 hr before sacrifice, 24 hr after final peanut challenge (wk 4 and 11). Naive mice were sensitized and challenged with PBS. The esophagus was harvested and assessed for the presence of impacted food, defined as food particles affixed in the esophageal tissue after fasting. Esophageal tissue was fixed in Carnoy's solution (60% ethanol, 30% chloroform, and 10% acetic acid) for 2 hr, followed by 100% ethanol for 1 hr and stored in 70% ethanol. Esophagi were embedded in paraffin and sectioned longitudinally.

Therapeutic Intervention with AAVrh.10mAnti-Eos

Groups of mice with EoE were treated with either AAVrh.10mAnti-Eos vector (10^{11} gc), AAVrh.10IgGcontrol vector (10^{11} gc), or PBS (100 μ l) 4 wk after the first sensitization (n=15 mice/group) via intravenous tail vein injection 24 hr following intranasal peanut extract challenge (wk 4). Mice were injected with PBS (100 μ l) via intravenous tail vein injection (wk 4) following intranasal challenge with PBS (50 μ l).

Peanut-Specific IgE and IgG1 Levels in Serumby ELISA

Peanut-specific IgE and IgG1 levels in sera were assessed by ELISA as previously described²⁷. Plates were coated with 20 μ g/ml peanut extract (*Arachis hypogaea*; Stallergenes Greer, Lenoir, NC) for sample wells and 20 μ g/ml DNP-HSA (Alpha Diagnostic, San Antonio, TX) for

192 standard wells overnight at 4°C. Plates were blocked for 2hr with PBS 0.05% tween and 2% BSA.
193 Serum from EoE and naive mice at wk 0, 4, and 11, and all groups after therapy at wk 11 (n=4
194 mice/group, randomly selected from the n=15mice/group) were diluted and incubated in the wells for
195 1hr, 37°C. A standard curve was created using anti-DNP mouse IgE (Sigma-Aldrich, Burlington,
196 MA) or anti-DNP mouse IgG1 (Acro Biosystems, Newark, DE). For detection of peanut-specific IgE,
197 plates were incubated with 0.5µg/ml goat anti-mouse IgE (Thermo Fisher Scientific) for 1hr, 37°C,
198 followed by 10ng/ml donkey anti-goat-HRP (Abcam, Cambridge, MA) for 1hr, 37°C. For peanut-
199 specific IgG1, plates were incubated with 10ng/ml goat anti-mouse IgG1-HRP (Thermo Fisher
200 Scientific) for 1hr, 37°C. The signal was detected by adding TMB peroxidase EIA substrate (Bio-Rad,
201 Hercules, CA) for 10 min and stopping with 1N H₂SO₄. Plates were read at 450 nm detection
202 wavelength.

203 **Blood and Esophageal Eosinophil Quantification**

204 Blood eosinophil counts were monitored at wk 0, 4, and 11 using the ProCyté DX Hematology
205 Analyzer (IDEXX veterinary, Westbrook, ME). For analysis of eosinophil infiltration of the esophagus
206 by immunohistochemistry, 5 esophageal sections were chosen randomly per group (from the
207 n=15mice/group), deparaffinized and stained with a primary rat anti-mouse major basic protein
208 (MBP) antibody (Mayo Clinic, Scottsdale, AZ), followed by a secondary biotinylated anti-rat IgG
209 (Vector Laboratories, Burlingame, CA), avidin-biotin complex (Vector Laboratories), DAB substrate
210 kit with nickel (Vector Laboratories) and hematoxylin counterstain. The esophagus was imaged at
211 20x, resulting in 5 to 6 images per esophageal tissue section, total MBP-positive cells counted and the
212 total area of the esophageal lamina propria outlined and quantified (mm²) using ImageJ (ImageJ/Fiji
213 software version 1.52i - National Institutes of Health, Bethesda, MD). Eosinophil quantification is
214 expressed as total MBP-positive cells per mm² lamina propria and was calculated by dividing the total
215 number of MBP-positive cells by the total area of the lamina propria. Anti-major basic protein (MBP)
216 immunohistochemistry stained esophageal sections were quantified by 3 blinded observers.
217 Eosinophils in the stomach and intestine were stained in the same manner. Eosinophil quantification in
218 the stomach was expressed as total MBP-positive cells per mm² lamina propria (n=4/group from the
219 n=15mice/group), and quantification in the duodenum was expressed as total MBP-positive cells per

mm² villus/crypt unit (n=4/group from the n=15mice/group). Villi for quantification were selected by measuring 5 mm from the pylorus in each tissue sample, and the first 20 villi were quantified.

Quantification of the Extent of the Epithelial Basal Layer

Esophageal sections were stained with hematoxylin and eosin for analysis of the proportion of area of the represented basal layer. One longitudinal esophageal tissue section was selected at random from each of four mice per group for analysis (from the n=15mice/group). The percent basal layer area of the esophageal epithelium for each mouse was determined by imaging the entire area of the 10-12 mm hematoxylin and eosinstained esophageal tissue at 20x, resulting in 5-6 images per esophageal tissue section. The area of the basal layer and the area of the epithelium were outlined and measured using ImageJ. The percent basal layer area of the epithelium was calculated by dividing the area of the basal layer by the total area of the epithelium for each esophageal section.

Quantification of Collagen Deposition

Matrix collagen deposition was assessed using Masson's trichrome stain (Abcam, Cambridge, MA) on esophageal sections according to the manufacturer's instruction. One longitudinal esophageal tissue section was selected at random from each of four mice per group (EoE model + AAVrh.10mAnti-Eos, EoE model + AAVrh.10IgGcontrol, EoE model + PBS, and naive; from the n=15 mice/group) for analysis. The percent collagen of the esophageal wall was determined by imaging the entire area of the 10-12 mm trichrome stained esophageal tissue at 20x, resulting in 5-6 images per esophageal tissue section. The area of trichrome stain in each image was selected by adjusting the color threshold to select for blue trichrome stain (hue: 130-200, saturation: 35-255, brightness: 175-250) using ImageJ. The selected area of collagen was measured, and the area of the esophageal wall outlined and measured. The area of collagen deposition was calculated by dividing the total area of trichrome stain by the total area of the esophageal wall for each esophageal section.

Quantification of Fibronectin Deposition

Deposition of matrix fibronectin for each mouse was evaluated using an anti-fibronectin antibody (Abcam), followed by a secondary donkey-anti-mouse IgG – Alexa Fluor555 and DAPI nuclear stain. One longitudinal esophageal tissue section was selected at random from each of four mice per group for analysis (from the n=15mice/group). The percent fibronectin area of the esophageal wall was determined by imaging the entire area of the 10-12 mm anti-fibronectin stained

249 esophageal tissue at 20x, resulting in 5-6 images per esophageal tissue section. The area of fibronectin
250 staining in each image was determined using ImageJ by adjusting the color threshold to adjust for red
251 fibronectin stain (hue: 215-255, saturation: 24-255, brightness: 47-255) and the entire area of the
252 esophageal wall per image outlined and measured. The percent fibronectin area of the esophageal wall
253 was calculated by dividing the total area of fibronectin by the total area of the esophageal wall for
254 each esophageal section.
255

256 **Statistics**

257 All data are presented as means $\pm$ standard error of the mean (SEM) unless otherwise stated;
258 the “n” value for each group is stated in the figures or figure legends. Differences between groups
259 were analyzed using an unpaired two-tailed Student's t test unless otherwise stated. For histological
260 quantification of esophageal, stomach and intestinal eosinophils, basal layer area and fibronectin
261 deposition, and peanut-specific IgE and IgG1 by ELISA at wk11, a one-way ANOVA analysis was
262 performed using Tukey's multiple comparisons for pairwise analyses, reported as adjusted p-values
263 (GraphPad Prism; San Diego, CA). For analysis of peripheral eosinophilia including
264 AAVrh.10mAnti-Eos and AAVrh.10IgGcontrol vector groups, all time points and groups were
265 evaluated collectively using a mixed-effects model with Uncorrected Fisher's LSD test for multiple
266 comparisons (GraphPad Prism; San Diego, CA); p values of less than 0.05 were considered
267 statistically significant for all comparisons.

268 **Results**

269 **Eosinophilic Esophagitis Murine Model**

270 Balb/c male mice were challenged and sensitized with peanut extract to induce EoE-like
271 disease over an 11 wk period (Figure 1A). Mice receiving peanut exhibited increased esophageal
272 tissue eosinophils at wk 4 ($p<0.003$; Supplemental Figure 2). Peanut challenged mice also
273 demonstrated increased blood eosinophil numbers at wk 4 and 11 ($p<0.0005$, $p<0.004$ respectively;
274 Figure 1B) elevated serum peanut-specific IgG1 (wk 4, 11; $p<0.0001$; Supplemental Figure 3A) and
275 peanut-specific IgE (wk 4, 11; $p<0.0001$; Supplemental Figure 3B) compared to control mice. At 11
276 wk, all mice were sacrificed after fasting and the esophagus evaluated for infiltration of eosinophils
277 and food impaction (Figure 1C-E). Peanut sensitized and challenged mice exhibited increased
278 eosinophil infiltration ($p<0.001$; Figure 1C, D) and food impaction (26.7%, Figure 1E) in the
279 esophagus compared with control mice. Similar to the human disease, EoE mice showed a peanut-
280 induced EoE phenotype at wk 4 and 11, defined by eosinophil infiltration in the esophagus and food
281 particle impaction. Food impaction was not associated with vomiting, refusal to eat or drink, or weight
282 loss. Of note, during the final peanut challenge, mice did not display any systemic signs or symptoms
283 suggestive of anaphylaxis. Mice administered peanut antigen did display local erythema of the nose
284 (the site of antigen administration) compared with PBS administered mice.

285 **AAVrh.10mAnti-Eos-Mediated Therapy**

286 *In vivo* expression of anti-Siglec-F after AAVrh.10mAnti-Eos gene transfer was demonstrated
287 in mice after a single intravenous administration of the vector (10^{11} gc; Figure 2A) for 32 wk. No
288 ratIgG2b expression was detected in PBS injected mice. Similarly, AAVrh.10IgGcontrol gene transfer
289 was demonstrated in mice for 8 wk (10^{11} gc; Figure 2B). Expression of anti-Siglec-F and anti-PA
290 were validated by Western analysis (Figure 2C). Livers fromAAVrh.10mAnti-Eos and
291 AAVrh.10IgGcontrol injected micewere evaluated histologicallyafter sacrifice (wk 11, wk 7 after
292 vector injection). No abnormal pathology relating to antibody expression was observed.An increase in
293 apoptotic cells was observed when serum collected from AAVrh.10mAnti-Eos administered mice was
294 incubated with eosinophils *in vitro* compared with eosinophils incubated with serum from control
295 mice (Figure 2D, Supplemental Figure 4).

296 To determine the timing for therapeutic treatment with AAVrh.10mAnti-Eos in our EoEmouse
297 model, we examined eosinophils in the esophagus and blood after the third peanut challenge at wk 4.
298 Peanut-sensitized and -challenged mice had significantly increased eosinophils in the esophagus and
299 blood at wk 4 compared with control mice ($p<0.003$; Supplemental Figure 2; Figure 1B; $p<$
300 0.0005).To assess the ability of AAVrh.10mAnti-Eos to alleviate the phenotypic and histological
301 parameters of the disease, EoE model mice were treated with AAVrh.10mAnti-Eos (10^{11} gc),
302 AAVrh.10IgGcontrol (10^{11} gc) or PBS intravenously at wk 4 (Figure 3A). Blood eosinophils of peanut
303 sensitized and challenged mice were decreased after administration of AAVrh.10mAnti-Eos to levels
304 comparable to naive mice ($p>0.9$) as compared to AAVrh.10IgGcontrol and PBS controls in which
305 eosinophil levels remained elevated ($p<0.002$ and $p<0.02$, respectively); at wk 11(Figure 3B).Other
306 core blood values remained mostly unchanged over time (Supplemental Table I).EoE mice
307 administered AAVrh.10mAnti-Eos demonstrated significantly lowered eosinophil infiltration per
308 mm^2 of the esophageal lamina propria compared to control mice administered AAVrh.10IgGcontrol
309 and PBS ($p<0.0002$ and $p<0.001$, respectively; Figure 3C, D) at wk 11. Esophageal eosinophil
310 numbers were reduced to the same levels asPBS-challenged mice ($p>0.3$; Figure 3D). There was no
311 evidence of food impaction in treated miceafter fasting before sacrifice, but food impaction was
312 observed in AAVrh.10IgGcontrol and PBS controls (20%and 26.7% respectively; Figure 3E).

313 Peanut-specific IgG1 and IgE levels in sera were assessed at wk 11 after AAVrh.10mAnti-Eos

314 therapy. Levels of peanut-specific IgG1 remained unchanged after therapy compared to control mice
315 administered AAVrh.10IgGcontrol and PBS ($p>0.9$, Supplemental Figure 5A) as expected. Similarly,
316 levels of peanut-specific IgE were unchanged after therapy compared to AAVrh.10IgGcontrol and
317 PBS controls ($p>0.9$ and $p>0.8$, respectively; Supplemental Figure 5B).

318 Peanut challenged mice treated with AAVrh.10mAnti-Eos showed significantly decreased
319 epithelial basal layer area of the epithelium compared to EoE mice treated with AAVrh.10IgGcontrol
320 or PBS ($p<0.008$, $p<0.003$; Figure 4A-B) at wk 11. The basal layer area of AAVrh.10mAnti-Eos
321 treated mice appeared similar to that of PBS-challenged mice ($p>0.1$, Figure 4A-B).

322 The area of extracellular matrix fibronectin deposition was significantly decreased in the
323 lamina propria of mice expressing AAVrh.10mAnti-Eos compared to AAVrh.10IgGcontrol or PBS at
324 wk 11 ($p<0.05$, $p<0.02$; Figure 4C-D). The percent area fibronectin of AAVrh.10mAnti-Eos treated
325 mice was similar to that of PBS-challenged mice ($p>0.5$, Figure 4C-D). Fibrosis, as assessed by
326 Masson's trichrome staining to visualize and quantify matrix collagen deposition, showed a trend
327 toward reduction in mice administered AAVrh.10mAnti-Eos compared to EoE mice
328 administered AAVrh.10IgGcontrol or PBS ($p>0.1$, $p>0.07$; Supplemental Figure 6A, B).

329 Eosinophil numbers in the other areas of the gastrointestinal tract were also evaluated
330 (Supplemental Figure 7A-D). Eosinophils in the lamina propria of the stomach of peanut-challenged
331 mice were higher in the AAVrh.10IgGcontrol and PBS groups compared to saline challenged mice,
332 though not significantly ($p>0.3$, Supplemental Figure 7B). Mice administered AAVrh.10mAnti-Eos
333 demonstrated a significant decrease in eosinophils in the lamina propria of the stomach compared to
334 AAVrh.10IgGcontrol and PBS controls ($p<0.04$, Supplemental Figure 7B). Eosinophils in the
335 villi/crypts of the duodenum were not significantly increased in AAVrh.10IgGcontrol and PBS
336 control groups compared to naive mice ($p>0.8$ and $p>0.3$, respectively; Supplemental Figure 7D).
337 AAVrh.10mAnti-Eos therapy decreased eosinophil numbers in the duodenum compared to
338 AAVrh.10IgGcontrol and PBS control groups ($p>0.1$ and $p<0.05$, respectively; Supplemental Figure
339 7D).

340 Discussion

341 Eosinophilic esophagitis (EoE) is a chronic antigen-mediated condition that if left untreated can
342 lead to esophageal dysfunction and ultimately fibrosis^{1,2}. To date, no therapies have been approved by

the Food and Drug Administration for the treatment of EoE, and the pharmacologic agents that are used are prescribed off label¹¹. The mainstay of therapy includes chronic swallowed or dissolving topical corticosteroids to maintain histologic and clinical remission, dietary restriction to address the inflammatory response and endoscopic dilation to ameliorate fibrosis^{6,28,29}. As a possible new experimental approach to treating EoE, experimental animal and clinical studies demonstrate that antibodies to eosinophil Siglec receptors induce eosinophil apoptosis and reduce the numbers of blood, bone marrow, and tissue eosinophils³⁰⁻³³. However, a key challenge to the use of monoclonal antibodies is that protection provided by a single administration of antibody is short and repeated administration is required to maintain persistent efficacy.

To circumvent the requirement for frequent administration of anti-eosinophil monoclonal antibodies, we developed a novel AAV-mediated gene therapy strategy to induce persistent clearance of eosinophils with a single administration of a gene transfer vector coding for anti-Siglec-F, a monoclonal antibody that induces depletion of eosinophils. We established a murine model of peanut-induced EoE that is characterized by esophageal eosinophil accumulation, food impaction and histological changes consistent with esophageal damage, including increased basal cell area and deposition of fibronectin and collagen, all hallmarks of human clinical EoE. Similar to other murine models of EoE, our peanut induced EoE mouse model, was characterized by increased peanut-specific IgE and IgG levels³⁴⁻³⁸.

Therapy with AAVrh.10mAnti-Eos

The data demonstrates that therapy with AAVrh.10mAnti-Eos mediates suppression of esophageal eosinophil accumulation, eosinophil-mediated esophageal remodeling, and structural changes in the esophagus in EoE that may be the result of persistent inflammation and/or aberrant tissue repair mechanisms^{39,40}. Previous studies have demonstrated that antibody-mediated cross-linking of Siglec-F induces eosinophil apoptosis¹³⁻¹⁵ and that administration of an anti-Siglec-F antibody to mice reduces levels of eosinophilic inflammation in the lung³⁰, blood³⁰⁻³², and jejunum^{31,32}. In an ovalbumin-induced EoE mouse model, administration of anti-Siglec-F mAb induced eosinophil apoptosis, a reduction in blood and tissue eosinophilia³⁰⁻³³ and was associated with a significant decrease in deposition of fibronectin, and basal zone hyperplasia³³, suggesting that

371 Siglec-F and human paralog Siglec-8 may be therapeutic targets to reduce eosinophilic inflammation
372 in EoE³³.

373 Basal cell hyperplasia of the esophageal epithelium is a characteristic finding of tissue
374 remodeling in EoE, and eosinophil numbers correlate with the degree of hyperplasia in biopsies of
375 patients with EoE⁴¹. A variety of pro-inflammatory cytokines, chemokines, and mediators are
376 produced by the esophageal epithelium, and therefore, reducing the thickness of this layer may
377 contribute to reduced inflammation and remodeling in EoE^{42,43}. The decrease in basal layer area in
378 EoE mice treated with AAVrh.10mAnti-Eos was likely secondary to the decreased eosinophil
379 accumulation in the esophagus, as the basal epithelium does not express Siglec-F receptors³³.
380 Esophageal eosinophils in EoE produce the profibrotic factor, transforming growth factor beta-1
381 (TGF β 1), which can increase the production of collagen, fibronectin and other extracellular matrix
382 proteins^{44,45} and contribute to the pro-inflammatory effects of eosinophils in EoE⁴⁶. The reduction in
383 fibronectin levels in EoE mice administered AAVrh.10mAnti-Eos may contribute to decreased
384 eosinophil viability and activation and may provide a therapy for long-term control of esophageal
385 eosinophilic inflammation. Collagen deposition was not significantly reduced after AAVrh.10mAnti-
386 Eos therapy in our study, despite the significant reduction in other markers of esophageal remodeling.
387 These results are similar to those obtained in a clinical study of EoE patients treated with
388 corticosteroids for 12 months, despite a marked reduction in eosinophil density in both the epithelium
389 and the lamina propria⁴⁷. Additionally, in patients with bronchial asthma treated with inhaled topical
390 steroids, collagen deposits persisted in spite of treatment^{48,49}. It is possible that a longer duration of
391 therapy is needed to detect a significant reduction of collagen deposition in the esophagus as well as
392 further mechanistic studies of the complex interplay of tissue damage and repair caused by the
393 eosinophil-mediated inflammatory response.

394 **Future Directions**

395 There are limitations to our study that will require further assessment as AAV-mediated gene
396 therapy is translated to the clinic. First, one highly unlikely theoretical risk with the proposed therapy
397 would be complete, permanent suppression of eosinophils. Eosinophils have important innate and
398 adaptive immunological roles in the defense against parasites and pathogens⁵⁰. Animal studies (mice
399 and nonhuman primate) using anti-interleukin-5 monoclonal antibodies report no adverse effects on

400 health and longevity and eosinophil deficient mice failed to suggest any defects in animal host
401 defense^{51,52}. Overall, clinical studies suggest that a reduction of eosinophils has been associated with
402 bacterial and viral infections, autoimmune and allergic disorders and as a potential marker for sepsis
403 and predictor of mortality in critical illness⁵³⁻⁵⁵. The data shows that we have not been able to drive
404 eosinophils to zero, nor suppress eosinophils below normal baseline levels observed in wild-type
405 mice. Thus, the data supports the hypothesis that persistent expression of antibody would likely be
406 efficacious to control eosinophil numbers. Persistent expression from AAVrh.10mAnti-Eos is similar
407 to monthly infusions of a monoclonal antibody and a gene therapy approach offers the advantage of
408 consistent steady-state levels of anti-Siglec-F.

409 Second, as with any animal model of human disease, this murine model of EoE may not
410 reproduce all aspects of the disease in humans. A number of allergen and ovalbumin-induced EoE
411 murine models with both high and low magnitudes of esophageal eosinophilia and the presence or
412 absence of a number of human EoE characteristics have been described³⁴. We chose a peanut induced
413 model as although the pathophysiology of EoE is not well understood, current evidence from clinical
414 studies implicates dietary antigens and aeroallergens as triggers and/or exacerbating factors of EoE^{2,4}.
415 Furthermore, symptoms and esophageal eosinophilic infiltration in patients with EoE can be
416 ameliorated by a food allergen elimination diet (i.e., dairy, wheat, soy, eggs, nuts, and
417 seafood/shellfish)^{2,4} which suggests that sensitized individuals may develop eosinophilic inflammation
418 in response to food allergens. The role of IgE in the pathogenesis of EoE remains unclear and recent
419 clinical trials targeting IgE have failed to ameliorate symptoms of disease⁵⁶⁻⁶⁰, suggesting that IgE
420 may not be critical for EoE pathogenesis. Thus, our mouse model of EoE may recapitulate the
421 immunologic state of affected individuals with EoE, but further studies, including evaluating
422 chemokine and cytokine expression profiles and alveolar macrophages will be required to investigate
423 the immunological factors associated with the eosinophilia in this EoE model. Of note, although blood
424 eosinophil levels are not useful in diagnosing or monitoring EoE, in part because they are elevated in
425 other allergic disorders, blood eosinophilia has been reported in up to 50% of patients with EoE⁶¹.
426 Similar to our findings, blood eosinophil levels correlate with the degree of esophageal eosinophilia
427 and decrease after histological remission of the disease⁶². Future studies using Siglec-F null mice
428 would be useful in identifying the direct effects of AAVrh.10mAnti-Eos on eosinophils and

translation of this therapy to humans.

The murine model of EoE is limited to 7 wk after AAVrh.10mAnti-Eos administration, as mice develop tolerance to the peanut antigen after repeated exposure and therefore, long-term effects of the vector cannot be fully assessed in this time-frame. Despite this limitation, AAV-based gene therapies in our laboratory and others have demonstrated long-term expression measured in years and limited only by the longest time point evaluated^{63,64}.

Third, while Siglec-F is highly specific to eosinophils, it is also expressed to a lesser extent on a number of murine cell-types including alveolar macrophages, T cells and neutrophils, but not on mast cells^{12,65,66}. Siglec-8, the functional convergent paralog of Siglec-F in humans, is expressed on the surface of eosinophils, mast cells and weakly on basophils^{12,67,68}. Studies of Siglec-F *in vivo* showed that administration of the antibody to mice selectively reduced blood and tissue eosinophils in an interleukin-5 transgenic mouse model without adverse effects³². In another transgenic mouse study of eosinophilic gastritis, administration of a monoclonal antibody targeting Siglec-8, resulted in selective depletion of tissue and blood eosinophils and reduction of mast cells⁶⁹. Furthermore, studies using antibodies targeting Siglec-8 have shown cross-linking of mast cells does not induce apoptosis as with eosinophils or affect mast cell viability⁶⁷, but may inhibit activation or degranulation^{49,70}. This is supported by *ex vivo* patient data showing that an anti-Siglec-8 antibody depleted sputum eosinophils and inhibited mast cells from asthmatic patients⁷¹. A recent clinical study evaluated a novel humanized mAb to Siglec-8 in healthy subjects that depleted blood eosinophils after a single dose with an acceptable safety profile^{9,72}. Thus, although there is a low possibility of off-target effects from the use of anti-Siglec antibodies, the anticipated detrimental effects are minimal.

Overall, the data demonstrates potent, consistent efficacy for treating EoE using AAVrh.10mAnti-Eos, but translation to the clinic will require formal safety and toxicology studies that will inform the viability of this gene therapy approach, including testing of a gene therapy vector expressing an equivalent antibody against Siglec-8 (AAVrh.10hAnti-Eos). AAV therapies have been generally shown to be safe in the short-term but long-term follow up continues to be necessary to understand any toxicities or alterations that may develop over time, such as malignancy⁷³. In a clinical setting, AAVrh.10hAnti-Eos gene therapy could potentially provide long-term protection to individuals affected by EoE following a single administration of the vector, a therapeutic strategy that

458 could be applied to other eosinophilic allergic and inflammatory disorders with unmet treatment
459 needs.

Acknowledgments. We thank S. Duan and J. Paulson (Scripps Research Institute) for providing us the sequence of the anti-Siglec-F antibody, E. Frenk, for technical assistance and N. Mohamed for editorial assistance.

Conflict of interest: None

Author contributions: AC: conceived experimental design of the study, performed experimental work and data analysis/interpretation, prepared manuscript; ARR, SN: performed experimental work and data analysis/interpretation, reviewed manuscript for content; KMS: performed vector design, experimental work and data analysis/interpretation, reviewed manuscript for content; RGC, OEP: conceived study, performed data analysis/interpretation, prepared manuscript.

References

1. Dellon ES, Liacouras CA. Advances in clinical management of eosinophilic esophagitis. *Gastroenterology*. 2014;147(6):1238-1254.
2. Liacouras CA, Furuta GT, Hirano I, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol*. 2011;128(1):3-20 e26; quiz 21-22.
3. Jensen ET, Kappelman MD, Martin CF, Dellon ES. Health-care utilization, costs, and the burden of disease related to eosinophilic esophagitis in the United States. *Am J Gastroenterol*. 2015;110(5):626-632.
4. Gonsalves N. Clinical Presentation and Approach to Dietary Management of Eosinophilic Esophagitis. *Gastroenterol Hepatol (N Y)*. 2018;14(12):706-712.
5. Dellon ES, Liacouras CA, Molina-Infante J, et al. Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference. *Gastroenterology*. 2018;155(4):1022-1033 e1010.
6. Straumann A, Katzka DA. Diagnosis and Treatment of Eosinophilic Esophagitis. *Gastroenterology*. 2018;154(2):346-359.
7. Cianferoni A, Spergel JM. Immunotherapeutic approaches for the treatment of eosinophilic esophagitis. *Immunotherapy*. 2014;6(3):321-331.
8. Schroeder S, Atkins D, Furuta GT. Recent advances in the treatment of eosinophilic esophagitis. *Expert Rev Clin Immunol*. 2010;6(6):929-937.
9. Dellon ES, Woosley JT, Arrington A, et al. Rapid Recurrence of Eosinophilic Esophagitis Activity After Successful Treatment in the Observation Phase of a Randomized, Double-Blind, Double-Dummy Trial. *Clin Gastroenterol Hepatol*. 2020;18(7):1483-1492 e1482.
10. Eluri S, Runge TM, Hansen J, et al. Diminishing Effectiveness of Long-Term Maintenance Topical Steroid Therapy in PPI Non-Responsive Eosinophilic Esophagitis. *Clin Transl Gastroenterol*. 2017;8(6):e97.
11. Gonsalves NP, Aceves SS. Diagnosis and treatment of eosinophilic esophagitis. *J Allergy Clin Immunol*. 2020;145(1):1-7.

12. Bochner BS. Siglec-8 on human eosinophils and mast cells, and Siglec-F on murine eosinophils, are functionally related inhibitory receptors. *Clin Exp Allergy*. 2009;39(3):317-324.
13. Nutku E, Hudson SA, Bochner BS. Mechanism of Siglec-8-induced human eosinophil apoptosis: role of caspases and mitochondrial injury. *Biochem Biophys Res Commun*. 2005;336(3):918-924.
14. Nutku-Bilir E, Hudson SA, Bochner BS. Interleukin-5 priming of human eosinophils alters siglec-8 mediated apoptosis pathways. *Am J Respir Cell Mol Biol*. 2008;38(1):121-124.
15. Mao H, Kano G, Hudson SA, et al. Mechanisms of Siglec-F-induced eosinophil apoptosis: a role for caspases but not for SHP-1, Src kinases, NADPH oxidase or reactive oxygen. *PLoS One*. 2013;8(6):e68143.
16. Hirano I, Peterson K, Murray J, et al. AK002, an Anti-Siglec-8 Antibody, Depletes Tissue Eosinophils and Improves Dysphagia Symptoms in Patients with Eosinophilic Esophagitis. *Journal of Allergy and Clinical Immunology*. 2020;145(2):AB167.
17. Crystal RG, Pagovich OE. The Jeremiah Metzger Lecture Novel Therapeutic Strategies of Allergic and Immunologic Disorders. *Trans Am Clin Climatol Assoc*. 2018;129:250-265.
18. Pagovich OE, Wang B, Chiuchiolo MJ, et al. Anti-hIgE gene therapy of peanut-induced anaphylaxis in a humanized murine model of peanut allergy. *J Allergy Clin Immunol*. 2016;138(6):1652-1662 e1657.
19. Qiu T, Chiuchiolo MJ, Whaley AS, et al. Gene therapy for C1 esterase inhibitor deficiency in a Murine Model of Hereditary angioedema. *Allergy*. 2019;74(6):1081-1089.
20. Pagovich OE, Camilleri AE, Nag S, Stiles KM, Crystal RG. AAV-mediated anti-eosinophil gene therapy for eosinophilic esophagitis. *Mol Ther*. 2020;28:90.
21. Pagovich OE, Nag S, Camilleri AE, Stiles KM, Crystal RG. Gene therapy for chronic eosinophilic leukemia. *Mol Ther*. 2019;27.
22. Pagovich OE, Russo AR, A.S. W, et al. Gene Therapy for chronic eosinophilic esophagitis. *Mol Ther* 2018;26:161
23. Gicheva N, Macauley MS, Arlian BM, Paulson JC, Kawasaki N. Siglec-F is a novel intestinal M cell marker. *Biochem Biophys Res Commun*. 2016;479(1):1-4.

24. Shi J, Shi Y, Waehrens LN, Rasmussen JT, Heegaard CW, Gilbert GE. Lactadherin detects early phosphatidylserine exposure on immortalized leukemia cells undergoing programmed cell death. *Cytometry A*. 2006;69(12):1193-1201.
25. Mayginnes JP, Reed SE, Berg HG, Staley EM, Pintel DJ, Tullis GE. Quantitation of encapsidated recombinant adeno-associated virus DNA in crude cell lysates and tissue culture medium by quantitative, real-time PCR. *J Virol Methods*. 2006;137(2):193-204.
26. Wang F, Cui X, Wang M, Xiao W, Xu R. A reliable and feasible qPCR strategy for titrating AAV vectors. *Med Sci Monit Basic Res*. 2013;19:187-193.
27. Orgel K, Kulis M. A Mouse Model of Peanut Allergy Induced by Sensitization Through the Gastrointestinal Tract. *Methods in molecular biology (Clifton, NJ)*. 2018;1799:39-47.
28. Greuter T, Safroneeva E, Bussmann C, et al. Maintenance Treatment Of Eosinophilic Esophagitis With Swallowed Topical Steroids Alters Disease Course Over A 5-Year Follow-up Period In Adult Patients. *Clin Gastroenterol Hepatol*. 2019;17(3):419-428.e416.
29. Lucendo AJ, Miehlke S, Schlag C, et al. Efficacy of Budesonide Orodispersible Tablets as Induction Therapy for Eosinophilic Esophagitis in a Randomized Placebo-Controlled Trial. *Gastroenterology*. 2019;157(1):74-86.e15.
30. Song DJ, Cho JY, Lee SY, et al. Anti-Siglec-F antibody reduces allergen-induced eosinophilic inflammation and airway remodeling. *J Immunol*. 2009;183(8):5333-5341.
31. Song DJ, Cho JY, Miller M, et al. Anti-Siglec-F antibody inhibits oral egg allergen induced intestinal eosinophilic inflammation in a mouse model. *Clin Immunol*. 2009;131(1):157-169.
32. Zimmermann N, McBride ML, Yamada Y, et al. Siglec-F antibody administration to mice selectively reduces blood and tissue eosinophils. *Allergy*. 2008;63(9):1156-1163.
33. Rubinstein E, Cho JY, Rosenthal P, et al. Siglec-F inhibition reduces esophageal eosinophilia and angiogenesis in a mouse model of eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr*. 2011;53(4):409-416.
34. Mishra A. Significance of Mouse Models in Dissecting the Mechanism of Human Eosinophilic Gastrointestinal Diseases (EGID). *J Gastroenterol Hepatol Res*. 2013;2(11):845-853.

35. Hogan SP, Mishra A, Brandt EB, Foster PS, Rothenberg ME. A critical role for eotaxin in experimental oral antigen-induced eosinophilic gastrointestinal allergy. *Proc Natl Acad Sci U S A*. 2000;97(12):6681-6686.
36. Hogan SP, Mishra A, Brandt EB, et al. A pathological function for eotaxin and eosinophils in eosinophilic gastrointestinal inflammation. *Nature immunology*. 2001;2(4):353-360.
37. Zaidi AK, Mussarat A, Mishra A. Diagnostic and therapeutic strategies for eosinophilic esophagitis. *Clinical practice (London, England)*. 2014;11(3):351-367.
38. Rajavelu P, Rayapudi M, Moffitt M, Mishra A, Mishra A. Significance of para-esophageal lymph nodes in food or aeroallergen-induced iNKT cell-mediated experimental eosinophilic esophagitis. *Am J Physiol Gastrointest Liver Physiol*. 2012;302(7):G645-654.
39. Rothenberg ME, Mishra A, Collins MH, Putnam PE. Pathogenesis and clinical features of eosinophilic esophagitis. *J Allergy Clin Immunol*. 2001;108(6):891-894.
40. Mishra A, Wang M, Pemmaraju VR, et al. Esophageal remodeling develops as a consequence of tissue specific IL-5-induced eosinophilia. *Gastroenterology*. 2008;134(1):204-214.
41. Blanchard C, Wang N, Stringer KF, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest*. 2006;116(2):536-547.
42. Aceves SS. Remodeling and fibrosis in chronic eosinophil inflammation. *Dig Dis*. 2014;32(1-2):15-21.
43. Hill DA, Spergel JM. The Immunologic Mechanisms of Eosinophilic Esophagitis. *Curr Allergy Asthma Rep*. 2016;16(2):9.
44. Rieder F, Nonevski I, Ma J, et al. T-helper 2 cytokines, transforming growth factor beta1, and eosinophil products induce fibrogenesis and alter muscle motility in patients with eosinophilic esophagitis. *Gastroenterology*. 2014;146(5):1266-1277 e1261-1269.
45. Mishra A. Mechanism of eosinophilic esophagitis. *Immunol Allergy Clin North Am*. 2009;29(1):29-40, viii.
46. Anwar AR, Walsh GM, Cromwell O, Kay AB, Wardlaw AJ. Adhesion to fibronectin primes eosinophils via alpha 4 beta 1 (VLA-4). *Immunology*. 1994;82(2):222-228.
47. Lucendo AJ, Arias A, De Rezende LC, et al. Subepithelial collagen deposition, profibrogenic cytokine gene expression, and changes after prolonged fluticasone propionate treatment in

- adult eosinophilic esophagitis: a prospective study. *J Allergy Clin Immunol*. 2011;128(5):1037-1046.
48. Ward C, De Soyza A, Fisher AJ, Pritchard G, Forrest IA, Corris PA. Reticular basement membrane thickening in airways of lung transplant recipients is not affected by inhaled corticosteroids. *Clin Exp Allergy*. 2004;34(12):1905-1909.
49. Chakir J, Shannon J, Molet S, et al. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol*. 2003;111(6):1293-1298.
50. Rosenberg HF, Dyer KD, Foster PS. Eosinophils: changing perspectives in health and disease. *Nat Rev Immunol*. 2013;13(1):9-22.
51. Hassani M, Koenderman L. Immunological and hematological effects of IL-5(Ralpha)-targeted therapy: An overview. *Allergy*. 2018;73(10):1979-1988.
52. Roufosse F. Targeting the Interleukin-5 Pathway for Treatment of Eosinophilic Conditions Other than Asthma. *Front Med (Lausanne)*. 2018;5:49.
53. Abidi K, Belayachi J, Derras Y, et al. Eosinopenia, an early marker of increased mortality in critically ill medical patients. *Intensive Care Med*. 2011;37(7):1136-1142.
54. Gleich GJ, Klion AD, Lee JJ, Weller PF. The consequences of not having eosinophils. *Allergy*. 2013;68(7):829-835.
55. Terradas R, Grau S, Blanch J, et al. Eosinophil count and neutrophil-lymphocyte count ratio as prognostic markers in patients with bacteremia: a retrospective cohort study. *PLoS One*. 2012;7(8):e42860.
56. Foroughi S, Foster B, Kim N, et al. Anti-IgE treatment of eosinophil-associated gastrointestinal disorders. *J Allergy Clin Immunol*. 2007;120(3):594-601.
57. Rocha R, Vitor AB, Trindade E, et al. Omalizumab in the treatment of eosinophilic esophagitis and food allergy. *European journal of pediatrics*. 2011;170(11):1471-1474.
58. Clayton F, Fang JC, Gleich GJ, et al. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology*. 2014;147(3):602-609.
59. Loizou D, Enav B, Komlodi-Pasztor E, et al. A pilot study of omalizumab in eosinophilic esophagitis. *PLoS One*. 2015;10(3):e0113483.

60. Goyal A, Cheng E. Recent discoveries and emerging therapeutics in eosinophilic esophagitis. *World journal of gastrointestinal pharmacology and therapeutics*. 2016;7(1):21-32.
61. Teitelbaum JE, Fox VL, Twarog FJ, et al. Eosinophilic esophagitis in children: immunopathological analysis and response to fluticasone propionate. *Gastroenterology*. 2002;122(5):1216-1225.
62. Hines BT, Rank MA, Wright BL, et al. Minimally invasive biomarker studies in eosinophilic esophagitis: A systematic review. *Ann Allergy Asthma Immunol*. 2018;121(2):218-228.
63. Hastie E, Samulski RJ. Recombinant adeno-associated virus vectors in the treatment of rare diseases. *Expert Opin Orphan Drugs*. 2015;3(6):675-689.
64. Mingozzi F, High KA. Therapeutic in vivo gene transfer for genetic disease using AAV: progress and challenges. *Nat Rev Genet*. 2011;12(5):341-355.
65. Zhang JQ, Biedermann B, Nitschke L, Crocker PR. The murine inhibitory receptor mSiglec-E is expressed broadly on cells of the innate immune system whereas mSiglec-F is restricted to eosinophils. *Eur J Immunol*. 2004;34(4):1175-1184.
66. Yokoi H, Choi OH, Hubbard W, et al. Inhibition of FcepsilonRI-dependent mediator release and calcium flux from human mast cells by sialic acid-binding immunoglobulin-like lectin 8 engagement. *J Allergy Clin Immunol*. 2008;121(2):499-505.e491.
67. Nutku E, Aizawa H, Hudson SA, Bochner BS. Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis. *Blood*. 2003;101(12):5014-5020.
68. Varki A, Angata T. Siglecs--the major subfamily of I-type lectins. *Glycobiology*. 2006;16(1):1R-27R.
69. Youngblood BA, Brock EC, Leung J, et al. Siglec-8 antibody reduces eosinophils and mast cells in a transgenic mouse model of eosinophilic gastroenteritis. *JCI Insight*. 2019;4(19).
70. Schanin J, Gebremeskel S, Korver W, et al. A monoclonal antibody to Siglec-8 suppresses non-allergic airway inflammation and inhibits IgE-independent mast cell activation. *Mucosal Immunology*. 2020.
71. Kerr SC, Gonzalez JR, Schanin J, et al. An anti-siglec-8 antibody depletes sputum eosinophils from asthmatic subjects and inhibits lung mast cells. *Clin Exp Allergy*. 2020.

72. Rasmussen HS, Chang AT, Tomasevic N, Bebbington C. A Randomized, Double-Blind, Placebo-Controlled, Ascending Dose Phase 1 Study of AK002, a Novel Siglec-8 Selective Monoclonal Antibody, in Healthy Subjects. *Journal of Allergy and Clinical Immunology*. 2018;141(2):AB403.
73. Batty P, Lillicrap D. Advances and challenges for hemophilia gene therapy. *Human molecular genetics*. 2019;28(R1):R95-r101.

Figure Legends

Figure 1. Mouse model of eosinophilic esophagitis. **A.** Timeline of 11 wk model based on intraperitoneal sensitization and intranasal challenge with peanut extract. Wild-type Balb/c (6-8 wk) male mice were sensitized with 200 μ g peanut extract plus 1 mg Alum intraperitoneally (wk 0, 2) then challenged intranasally with 100 μ g peanut extract (wk 3-11) for 18 doses (2-3 days apart; 3x/wk) and sacrificed wk 11, 24 hr after final challenge. As a control, naive mice were sensitized and challenged with PBS. (n=15 mice/group). **B.** Eosinophil numbers in blood before and after peanut sensitization and challenge in EoE mice. (n=3-8 mice/group). **C.** Immunohistochemistry major basic protein staining of esophageal tissue in naive and EoE mice (wk 11). Arrowheads identify major basic protein positive eosinophils. Bar = 20 μ m. **D.** Quantification of eosinophils in naive and EoE mice (wk 11; n=5 mice/ group). **E.** Food impaction in esophagus of naive and EoE mice (wk 11), arrowhead identifies impacted food (n=15 mice/ group). Percentages represent esophagi with detectable food impaction. Results are shown as means $\pm$ SEM.

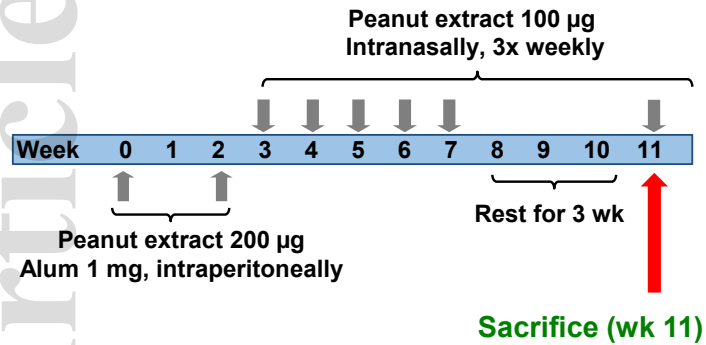
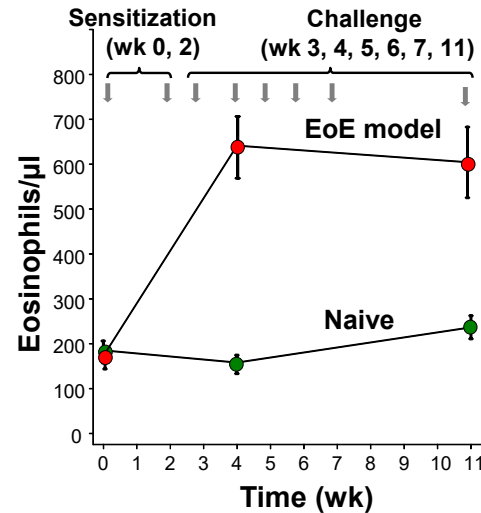
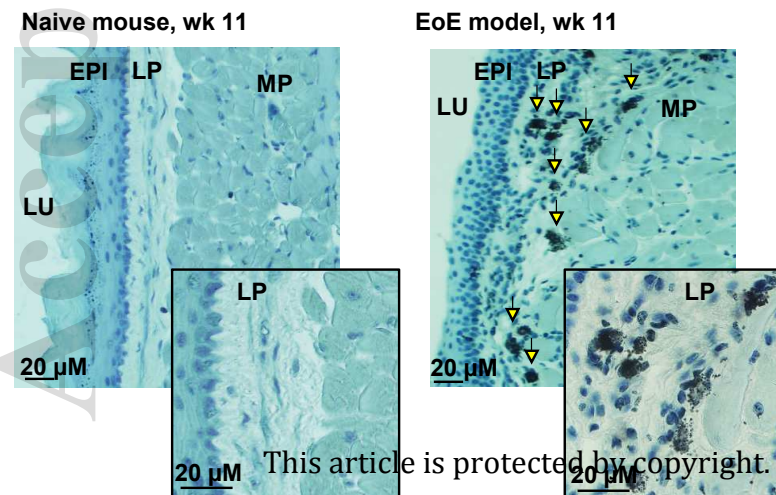
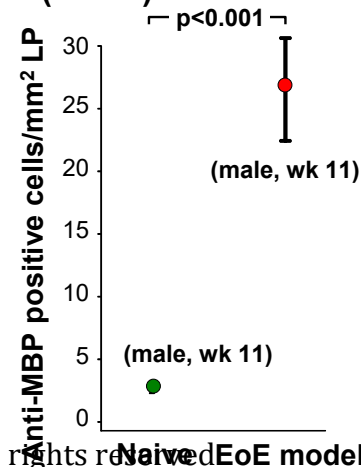
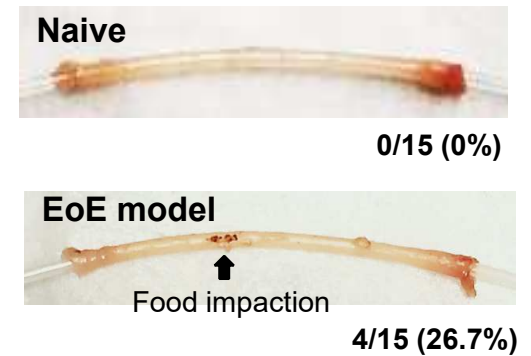
Figure 2. Characterization of the AAVrh.10mAnti-Eos vector coding for an anti-eosinophil monoclonal antibody to induce eosinophil clearance. **A.** Murine anti-Siglec-F monoclonal antibody levels over time following intravenous administration of AAVrh.10mAnti-Eos (10^{11} genome copies). Data shown for male Balb/c mice (n=5 mice/ group). **B.** Murine anti-PA ratIgG2b expression levels over time following intravenous administration of AAVrh.10IgGcontrol (10^{11} genome copies). Data shown for male Balb/c mice (n=4 mice/ group). **C.** Expression of anti-Siglec-F and anti-PA *in vitro* by Western analysis. **D.** Induction of apoptosis of murine eosinophils by the product of the vector. Flow cytometry assessment of lactadherin (apoptosis marker) staining of mouse eosinophils incubated *in vitro* with serum from AAVrh.10mAnti-Eos or control administered mice.

Figure 3. Murine model of EoE treated with AAVrh.10mAnti-Eos. **A.** Timeline of 11 wk model based on intraperitoneal sensitization and intranasal challenge with peanut extract. Wild-type Balb/c (6-8 wk) male mice were sensitized with 200 μ g peanut extract plus 1 mg Alum intraperitoneally (wk 0, 2) then challenged intranasally with 100 μ g peanut extract (wk 3-11) for 18 doses (2-3 days apart; 3x/wk) and sacrificed wk 11, 24 hr after final challenge. Mice were treated with AAVrh.10mAnti-Eos (10^{11} gc), AAVrh.10IgGcontrol (10^{11} gc) or PBS intravenously at wk 4. (n=15 mice/ group). **B.**

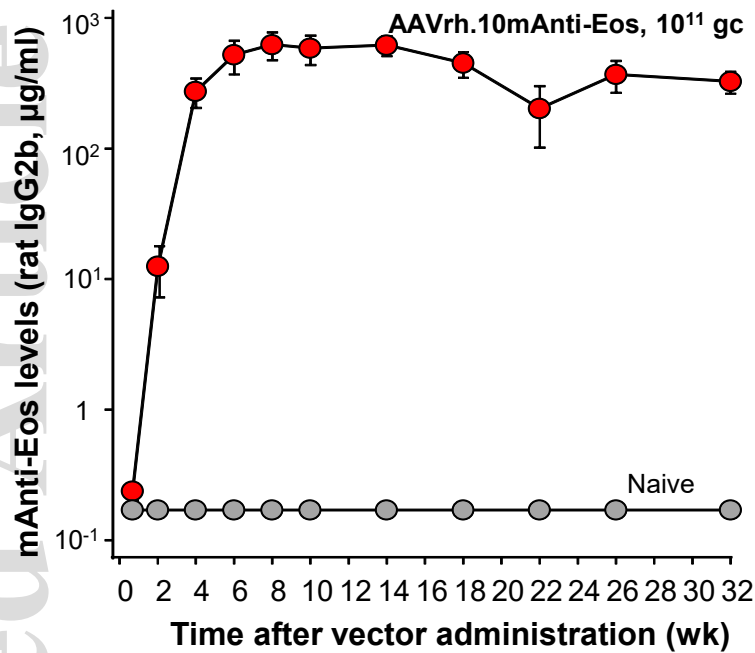
Eosinophil numbers in blood of EoE mice treated with AAVrh.10mAnti-Eos and controls. (n=3-8 mice/group). **C.** Immunohistochemistry: major basic protein staining of esophageal tissue in naive and EoE mice following AAVrh.10mAnti-Eos therapy. EPI, epithelium; LP, lamina propria; MP, muscularis propria. Arrowheads identify major basic protein (MBP+) eosinophils. Bar = 25 μ m. **D.** Quantification of eosinophils in naive and EoE mice following AAVrh.10mAnti-Eos therapy (n=5 mice/group). **E.** Food impaction in esophagus of control and EoE mice (wk 11) following AAVrh.10mAnti-Eos therapy; arrowhead identifies impacted food (n=15 mice/group). Results are shown as means $\pm$ SEM.

Figure 4. Tissue remodeling in a murine model of EoE treated with AAVrh.10mAnti-Eos.

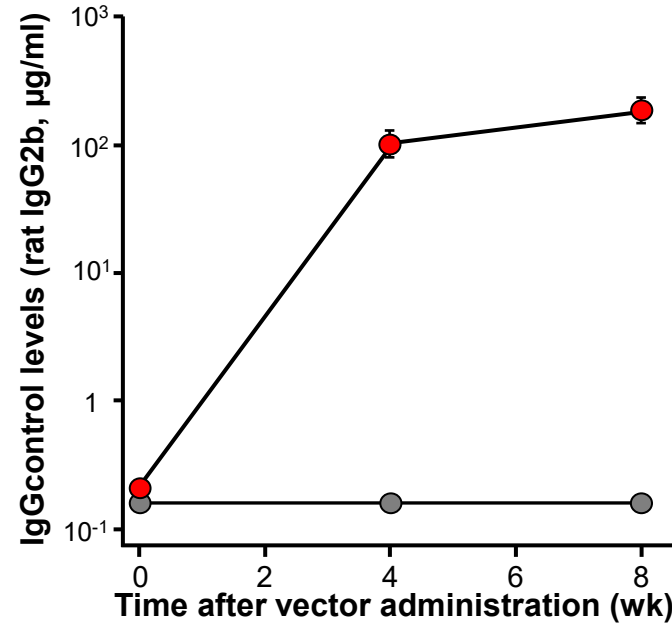
Esophageal lamina propria basal layer area in EoE and naive mice. **A.** Hematoxylin and eosin stained tissue sections of EoE mice showing the basal layer following peanut challenges. Images taken 5 mm above the gastroesophageal junction. Bar = 20 μ m. EPI, epithelium; BL, basal layer; LP, lamina propria; MP, muscularis propria. White bars denote the basal layer. **B.** Quantification of average basal layer area of the esophageal epithelium (n=4 mice/group). **C.** Immunofluorescence analysis of fibronectin deposition in the lamina propria after AAVrh.10mAnti-Eos therapy. Red- fibronectin, cyan – nuclei. Bar = 50 μ m. **D.** Quantification of matrix fibronectin deposition area (n=4 mice/group).

A. Timeline**B. Blood eosinophils****C. Eosinophils in esophagus****D. Eosinophils in esophagus (wk 11)****E. Food impaction (wk 11)**

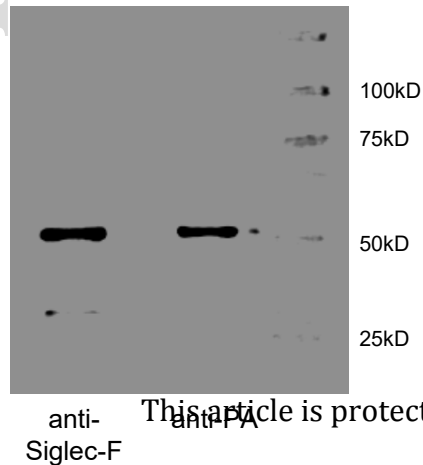
A. *In vivo* expression of AAVrh.10mAnti-Eos in Balb/c males



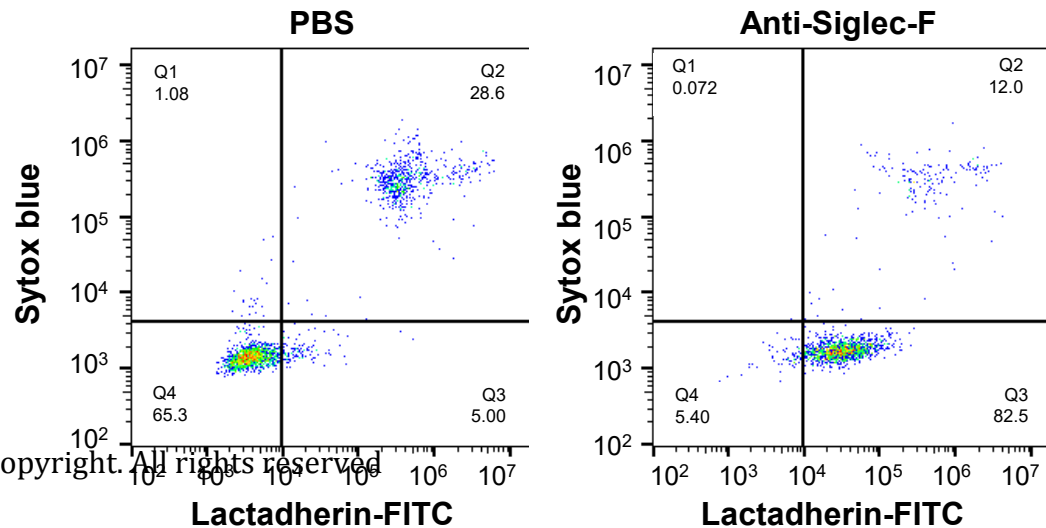
B. *In vivo* expression of AAVrh.10IgGcontrol in Balb/c males



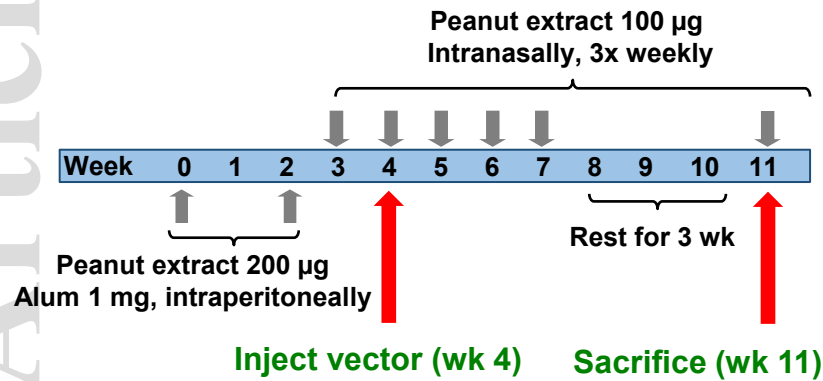
C. Expression of anti-Siglec-F and ratIgG2b control



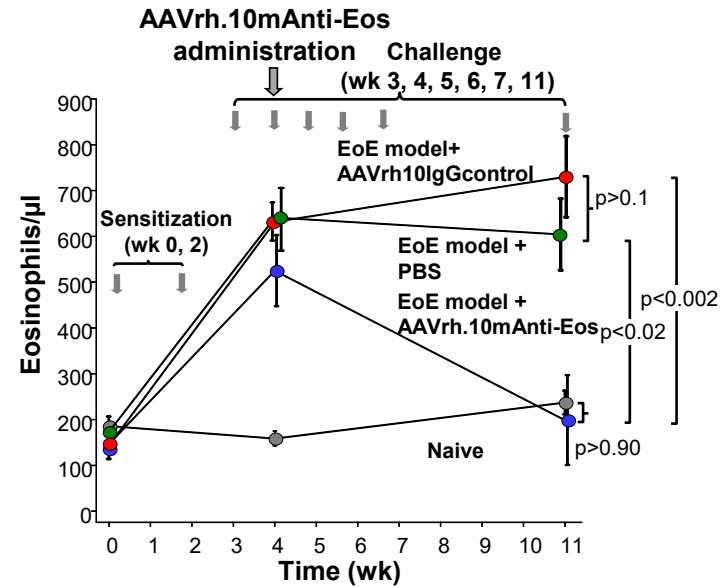
D. Induction of apoptosis of eosinophils, 10 wk



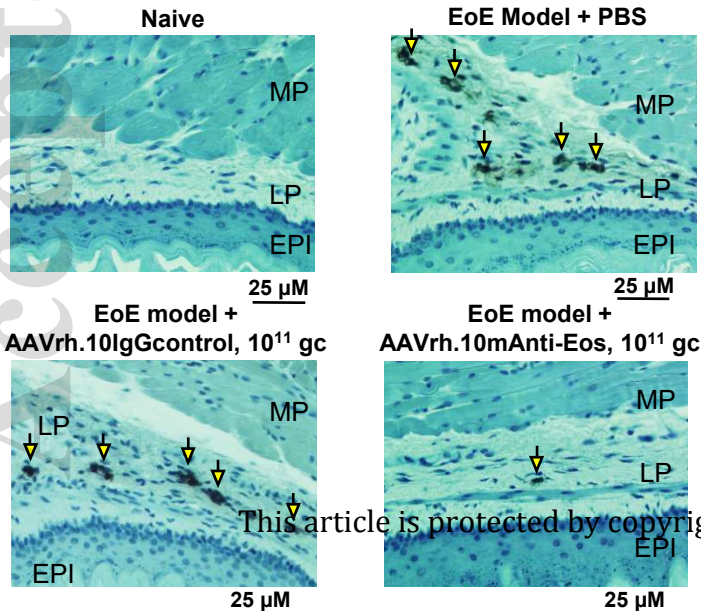
A. Timeline



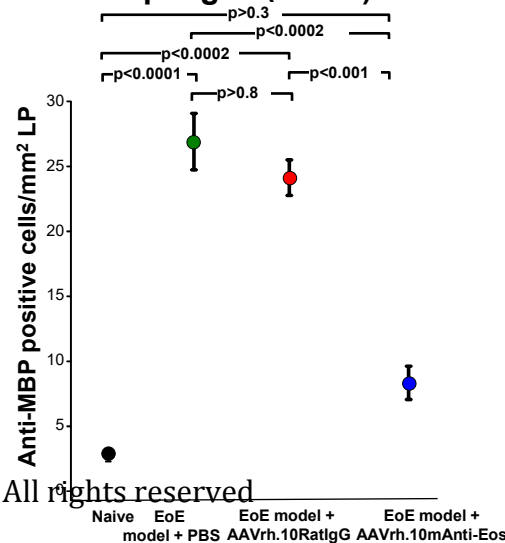
B. Blood eosinophils



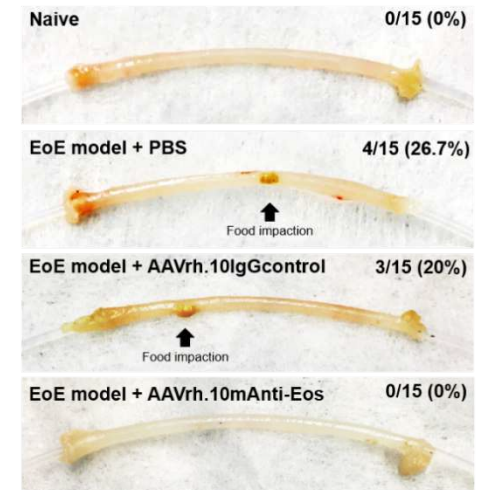
C. Eosinophils in esophagus



D. Eosinophil quantification in esophagus (wk 11)



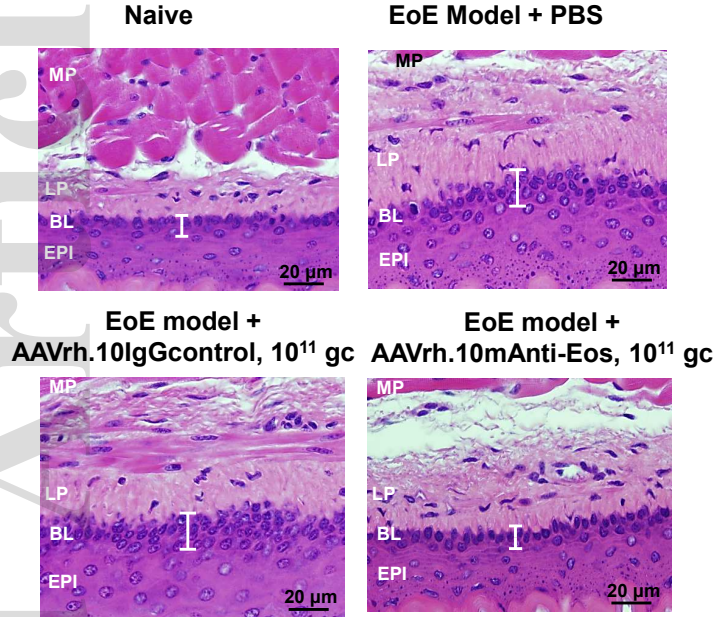
E. Food impaction (wk 11)



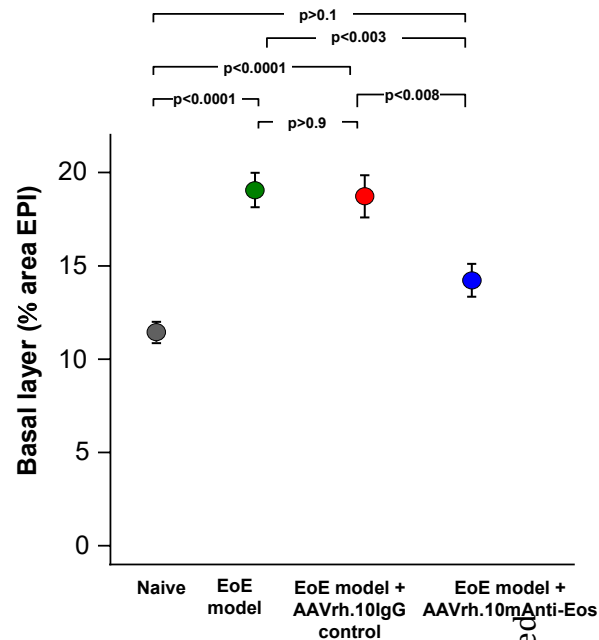
This article is protected by copyright. All rights reserved

Figure 4

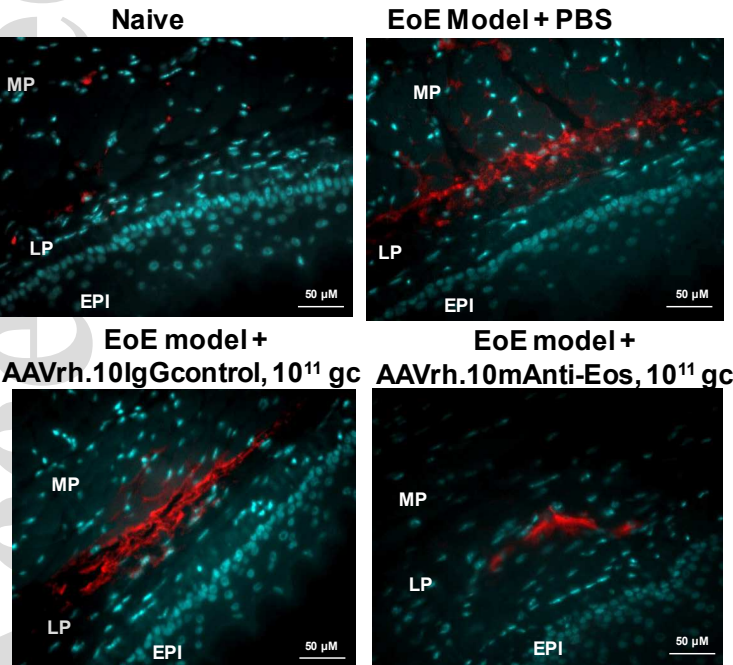
A. Basal layer (wk11)



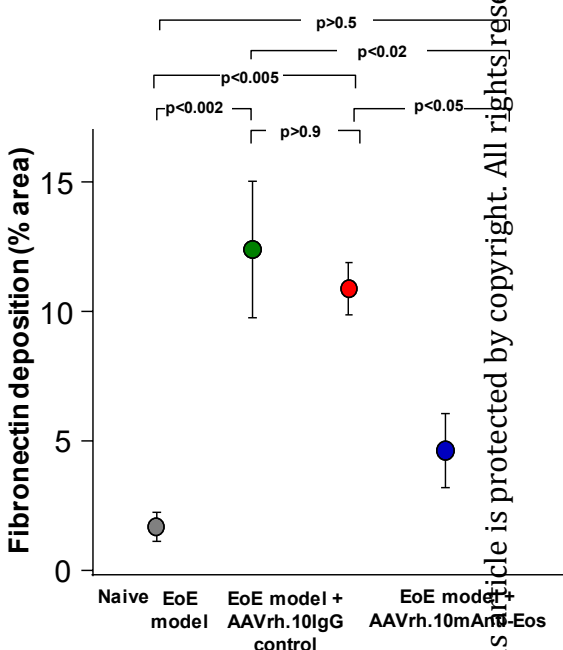
B. Basal layer (% area epithelium)



C. Fibronectin deposition (wk11)



D. Fibronectin deposition (% area)



This article is protected by copyright. All rights reserved.

Supplemental Table I. Complete Blood Counts in EoE¹ Balb/c Male Mice

Mouse treatment group	RBC and Platelet Indices, mean (SEM)					Automated Differentials, mean (SEM)					
	RBC ⁴ (x10 ⁶ cells/ μ L) (6.93-12.24)	Hemoglobin (g/dL) (12.4-20.5)	Hematocrit (%) (42.1-68.3)	MCV ⁵ (fL) (50.7-64.4)	PLT ⁶ (x10 ³ cells/ μ L) (420-1698)	WBC ⁷ (x10 ³ cells/ μ L) (3.48-14.84)	Neutrophil (%) (9.86-39.11)	Lymphocyte (%) (48.81-83.82)	Monocyte (%) (3.29-14.33)	Eosinophil (%) (0.07-4.91)	Basophil (%) (0.00-1.84)
Wk 0											
Naive	10.53 ($\pm$ 0.17)	16.40 ($\pm$ 0.27)	49.48 ($\pm$ 0.99)	46.98 ($\pm$ 0.23)	974.17 ($\pm$ 35.27)	5.37 ($\pm$ 0.42)	23.63 ($\pm$ 1.01)	72.22 ($\pm$ 0.91)	1.18 ($\pm$ 0.05)	2.90 ($\pm$ 0.21)	0.07 ($\pm$ 0.21)
Peanut + PBS	10.47 ($\pm$ 0.12)	16.33 ($\pm$ 0.18)	49.16 ($\pm$ 0.56)	46.93 ($\pm$ 0.16)	1006.71 ($\pm$ 32.91)	5.38 ($\pm$ 0.38)	20.71 ($\pm$ 0.83)	74.91 ($\pm$ 0.83)	1.51 ($\pm$ 0.13)	2.71 ($\pm$ 0.19)	0.14 ($\pm$ 0.04)
Peanut + AAVrh.10IgGcontrol ²	10.76 ($\pm$ 0.36)	16.70 ($\pm$ 0.47)	50.83 ($\pm$ 1.44)	47.27 ($\pm$ 0.27)	883.33 ($\pm$ 75.22)	6.07 ($\pm$ 1.28)	19.90 ($\pm$ 3.15)	76.00 ($\pm$ 3.02)	1.47 ($\pm$ 0.12)	2.57 ($\pm$ 0.47)	0.07 ($\pm$ 0.07)
Peanut + AAVrh.10mAnti-Eos ³	10.67 ($\pm$ 0.04)	16.53 ($\pm$ 0.12)	50.20 ($\pm$ 0.36)	47.03 ($\pm$ 0.24)	1024.33 ($\pm$ 50.68)	7.03 ($\pm$ 0.91)	25.63 ($\pm$ 3.47)	70.83 ($\pm$ 2.92)	1.27 ($\pm$ 0.12)	2.20 ($\pm$ 0.55)	0.07 ($\pm$ 0.03)
Wk 4											
Naive	10.73 ($\pm$ 0.12)	16.67 ($\pm$ 0.14)	51.97 ($\pm$ 0.67)	48.43 ($\pm$ 0.23)	970.17 ($\pm$ 40.97)	9.62 ($\pm$ 1.02)	19.00 ($\pm$ 0.79)	77.25 ($\pm$ 0.87)	2.07 ($\pm$ 0.24)	1.52 ($\pm$ 0.17)	0.17 ($\pm$ 0.06)
Peanut + PBS	10.96 ($\pm$ 0.19)	16.04 ($\pm$ 0.22)	50.17 ($\pm$ 0.68)	45.80 ($\pm$ 0.33)	931.29 ($\pm$ 32.67)	6.65 ($\pm$ 0.53)	26.00 ($\pm$ 1.52)	58.96 ($\pm$ 1.14)	5.90 ($\pm$ 0.38)	9.33 ($\pm$ 0.88)	0.23 ($\pm$ 0.06)
Peanut + AAVrh.10IgGcontrol	11.44 ($\pm$ 0.27)	16.64 ($\pm$ 0.36)	52.24 ($\pm$ 1.30)	45.66 ($\pm$ 0.30)	936.00 ($\pm$ 29.25)	6.85 ($\pm$ 0.61)	28.62 ($\pm$ 1.18)	55.62 ($\pm$ 1.21)	6.08 ($\pm$ 0.53)	9.02 ($\pm$ 0.81)	0.26 ($\pm$ 0.08)
Peanut + AAVrh.10mAnti-Eos	11.06 ($\pm$ 0.11)	16.20 ($\pm$ 0.13)	50.88 ($\pm$ 0.45)	46.00 ($\pm$ 0.39)	983.20 ($\pm$ 41.80)	6.28 ($\pm$ 0.47)	29.82 ($\pm$ 0.20)	55.50 ($\pm$ 1.09)	6.02 ($\pm$ 0.84)	8.60 ($\pm$ 0.62)	0.16 ($\pm$ 0.09)
Wk 11											
Naive	10.81 ($\pm$ 0.11)	16.08 ($\pm$ 0.24)	50.13 ($\pm$ 0.73)	46.42 ($\pm$ 0.68)	1123.17 ($\pm$ 87.85)	8.71 ($\pm$ 0.72)	24.37 ($\pm$ 1.72)	71.92 ($\pm$ 1.72)	1.38 ($\pm$ 0.09)	2.20 ($\pm$ 0.17)	0.13 ($\pm$ 0.04)
Peanut + PBS	10.82 ($\pm$ 0.09)	16.27 ($\pm$ 0.16)	52.20 ($\pm$ 0.52)	48.24 ($\pm$ 0.27)	807.71 ($\pm$ 63.45)	7.50 ($\pm$ 0.44)	30.90 ($\pm$ 0.99)	58.03 ($\pm$ 1.77)	3.86 ($\pm$ 0.17)	7.11 ($\pm$ 0.82)	0.10 ($\pm$ 0.02)
Peanut + AAVrh.10IgGcontrol	11.12 ($\pm$ 0.09)	16.59 ($\pm$ 0.19)	52.54 ($\pm$ 0.54)	47.24 ($\pm$ 0.32)	829.14 ($\pm$ 53.41)	9.95 ($\pm$ 1.02)	26.27 ($\pm$ 0.97)	61.69 ($\pm$ 1.57)	4.40 ($\pm$ 0.22)	7.54 ($\pm$ 0.92)	0.10 ($\pm$ 0.00)
Peanut + AAVrh.10mAnti-Eos	10.94 ($\pm$ 0.12)	16.52 ($\pm$ 0.26)	52.23 ($\pm$ 0.70)	47.77 ($\pm$ 0.26)	965.50 ($\pm$ 101.73)	7.08 ($\pm$ 0.56)	25.37 ($\pm$ 1.19)	68.02 ($\pm$ 1.59)	3.95 ($\pm$ 0.31)	2.55 ($\pm$ 0.97)	0.12 ($\pm$ 0.04)

¹ EoE: Eosinophilic esophagitis

² AAVrh.10IgGcontrol: adeno-associated virus (AAV) vector coding for anti-anthrax protective antigen antibody modified with a Rat IgG Fc to match anti-Siglec-F

³ AAVrh.10mAnti-Eos: adeno-associated virus (AAV) expressing an anti-Siglec-F monoclonal antibody

⁴ RBC: red blood cell

⁵ MCV: mean corpuscular volume

⁶ PLT: platelets

⁷ WBC: white blood cell

Supplemental Figure Legends

Supplemental Figure 1. Schematic of expression cassette. Anti-Siglec-F heavy and light chain cDNA sequence (clone 9C7, rat IgG2b)²³ separated by furin 2A protease site inserted between AAV2 ITRs following CAG promoter (CMV enhancer, CBA promoter, rabbit β -globin splice acceptor site) and before rabbit β -globin polyadenylation signal.

Supplemental Figure 2. Mouse model of eosinophilic esophagitis at wk 4 (prior to AAVrh.1-mAnti-Eos treatment). **A.** Immunohistochemistry major basic protein staining of esophageal tissue in naive and EoE mice (wk 4). Arrowheads identify major basic protein positive eosinophils. Bar = 50 μ m **B.** Quantification of eosinophils in naive and EoE mice (wk 4; n=5 mice/group) .

Supplemental Figure 3. Assessment of peanut-specific IgG1 and IgE in the EoE model mice. **A.** Peanut-specific IgG1 levels in serum before and after peanut sensitization and challenge in naive and EoE model mice (n=4 mice/ group). **B.** Peanut-specific IgE levels in serum before and after peanut sensitization and challenge in naive and EoE model mice (n=4 mice/group).

Supplemental Figure 4. Anti-CD45 and anti-Siglec-F staining by flow cytometry. Leukocytes isolated from AAVrh.10mIL5 injected mice show >90% eosinophils in the sample. The same population of leukocytes was used in the apoptosis assay in Figure 2D.

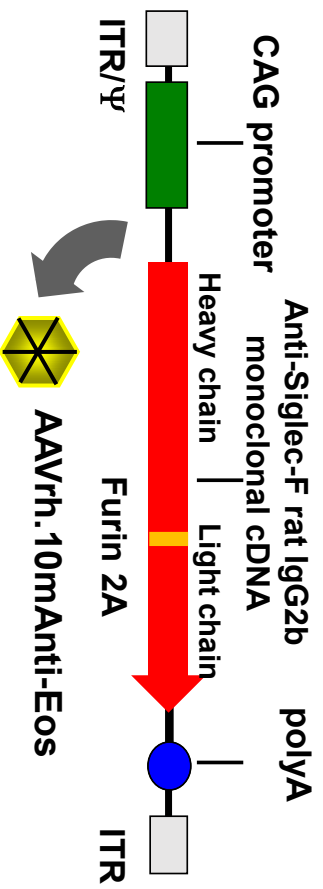
Supplemental Figure 5. Assessment of peanut-specific IgG1 and IgE in EoE mice after AAVrh.10mAnti-Eos therapy. **A.** Peanut-specific IgG1 levels in serum of AAVrh.10mAnti-Eos treated mice and controls at wk 11 (n=4 mice/group). **B.** Peanut-specific IgE levels in serum of AAVrh.10mAnti-Eos treated mice and controls at wk 11 (n=4 mice/group).

Supplemental Figure 6. Collagen deposition in a murine model of EoE treated with AAVrh.10mAnti-Eos. **A.** Masson's trichrome-stained analysis of esophageal tissue after AAVrh.10mAnti-Eos therapy. Bar = 100 μ m **B.** Quantification of collagen deposition (wk 11;

n=4 mice/group). Collagen – blue, muscle fibers – red, nuclei, red/dark red. EPI, epithelium; LP, lamina propria; MP, muscularis propria; LU, lumen.

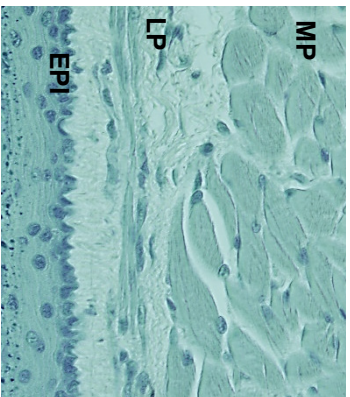
Supplemental Figure 7. Eosinophil quantification in the GI tract of peanut challenged mice treated with AAVrh.10mAnti-Eos. **A.** Immunohistochemistry major basic protein staining of stomach tissue in naive and EoE mice following AAVrh.10mAnti-Eos therapy. Bar = 200 μ m. LU, lumen; LP, lamina propria. Arrowheads identify major basic protein (MBP⁺) eosinophils. **B.** Quantification of eosinophils in the gastric lamina propria of naive and EoE mice following AAVrh.10mAnti-Eos therapy (n=4 mice/group). **C.** Immunohistochemistry major basic protein staining of duodenal tissue in naive and EoE mice following AAVrh.10mAnti-Eos therapy. Bar = 100 μ m. Arrowheads identify major basic protein (MBP⁺) eosinophils in the villi/crypts. **D.** Quantification of eosinophils in the duodenal villi and crypts of naive and EoE mice following AAVrh.10mAnti-Eos therapy (n=4 mice/group).

AAVrh.10mAnti-Eos vector

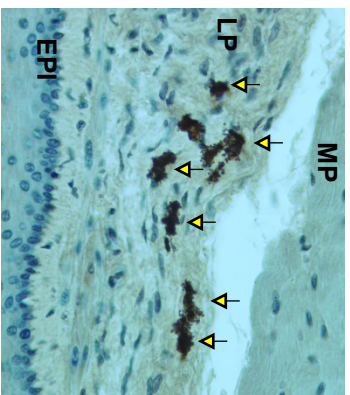


A. Eosinophils in esophagus

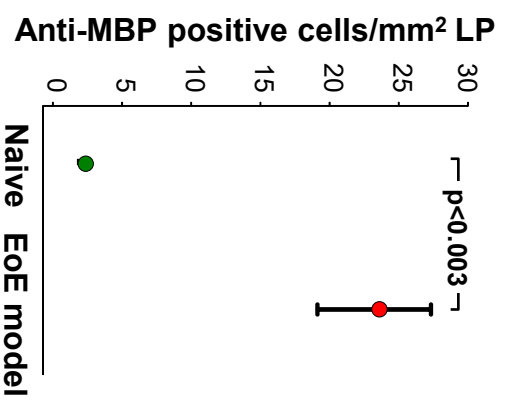
Naive mouse, wk 4



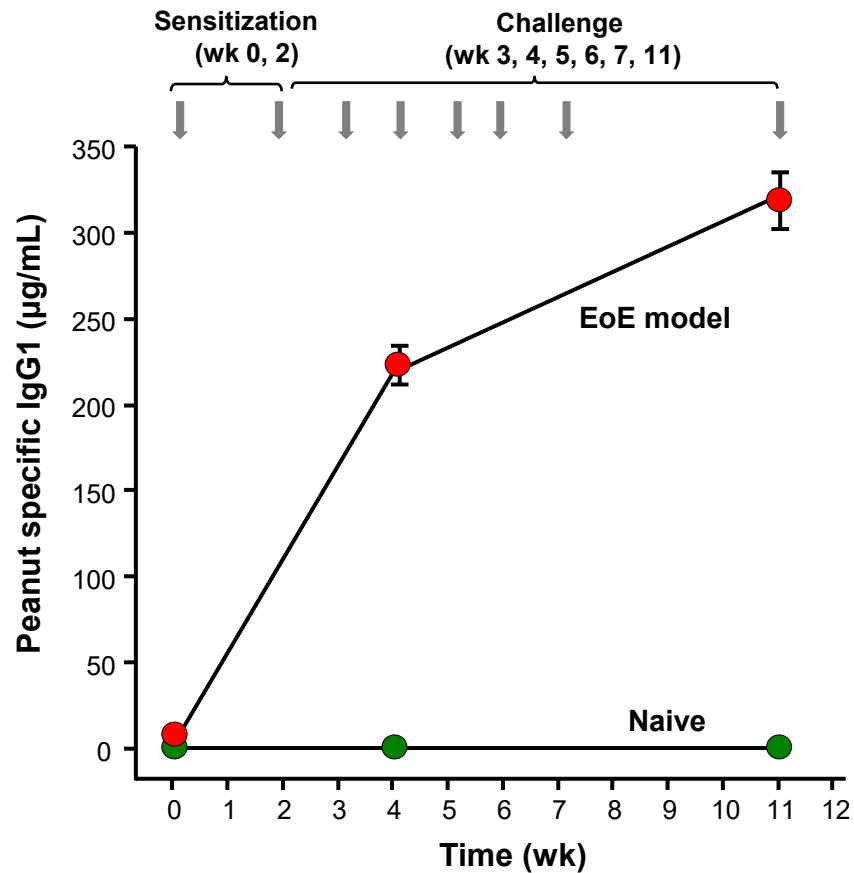
EoE model, wk 4



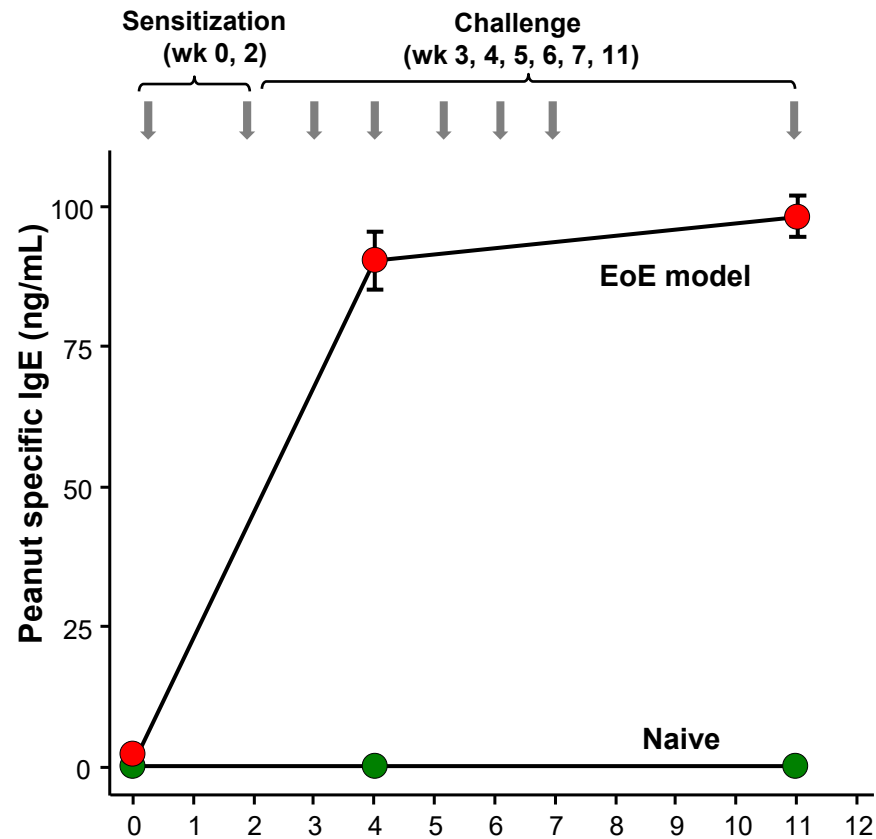
B. Eosinophils in esophagus (wk 4)



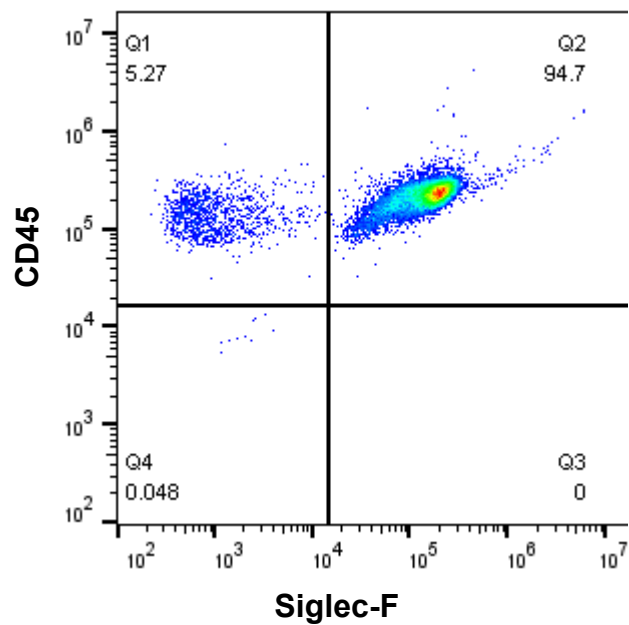
A. Serum Peanut-Specific IgG1



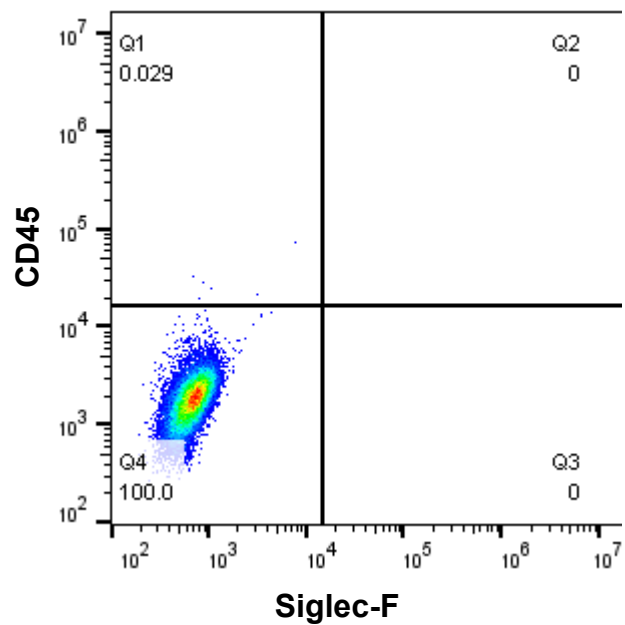
B. Serum Peanut-Specific IgE

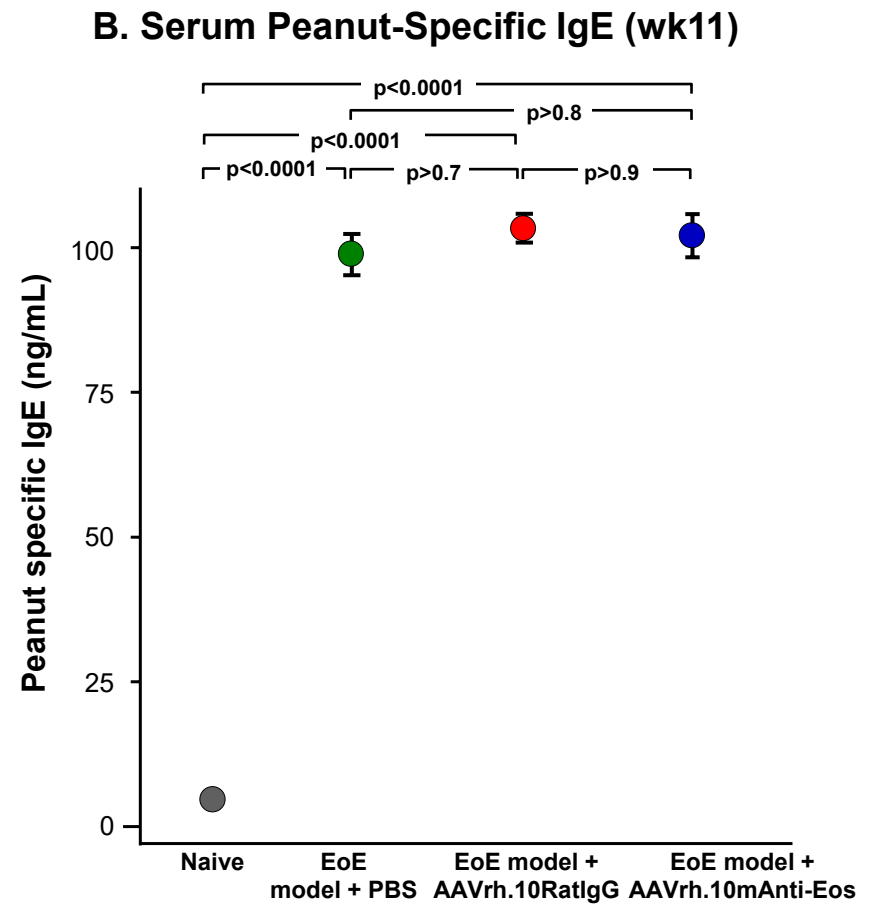
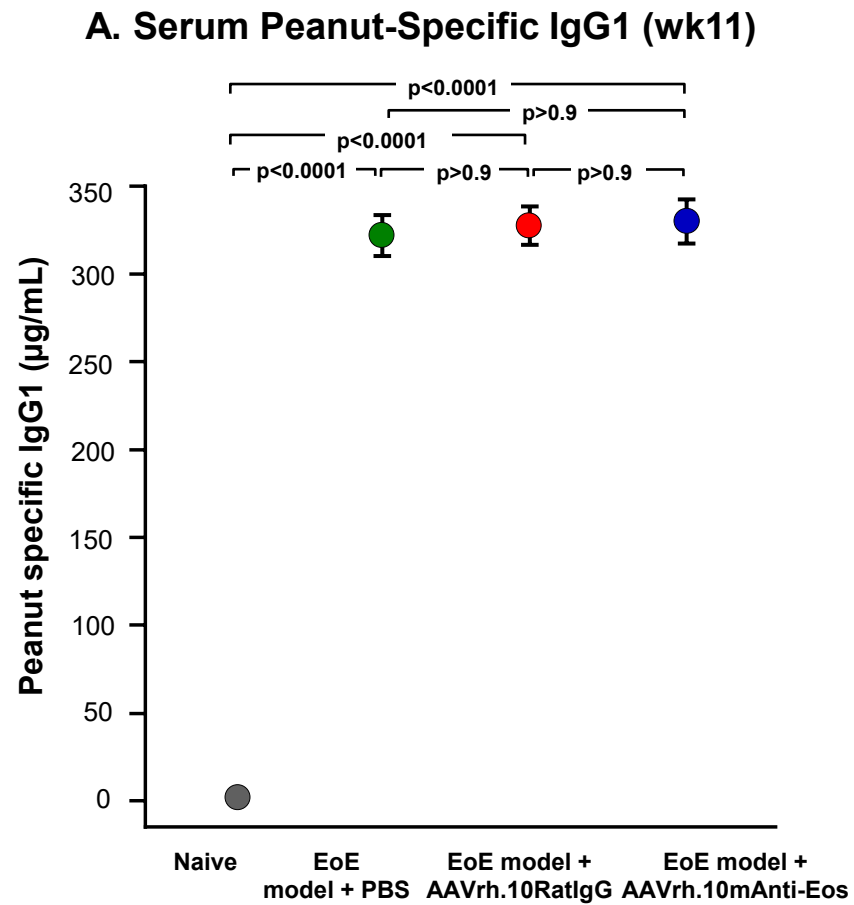


Anti-CD45 + anti-Siglec-F



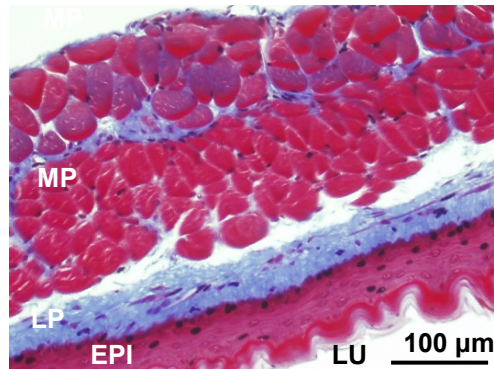
Isotype controls



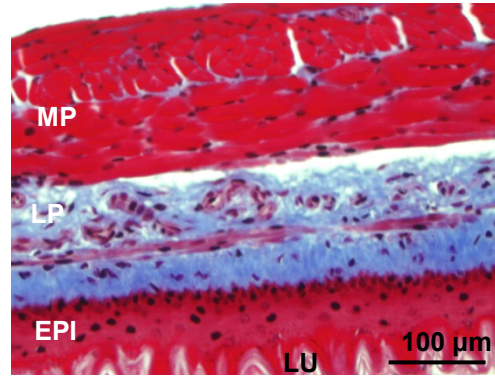


A. Collagen deposition (wk11)

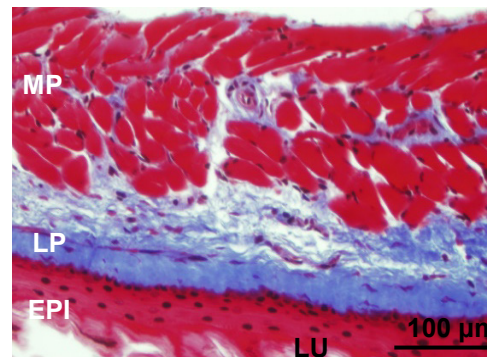
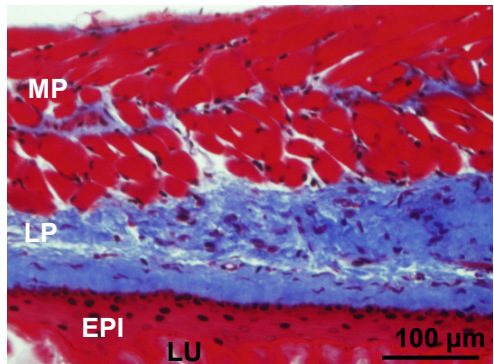
Naive



EoE Model + PBS

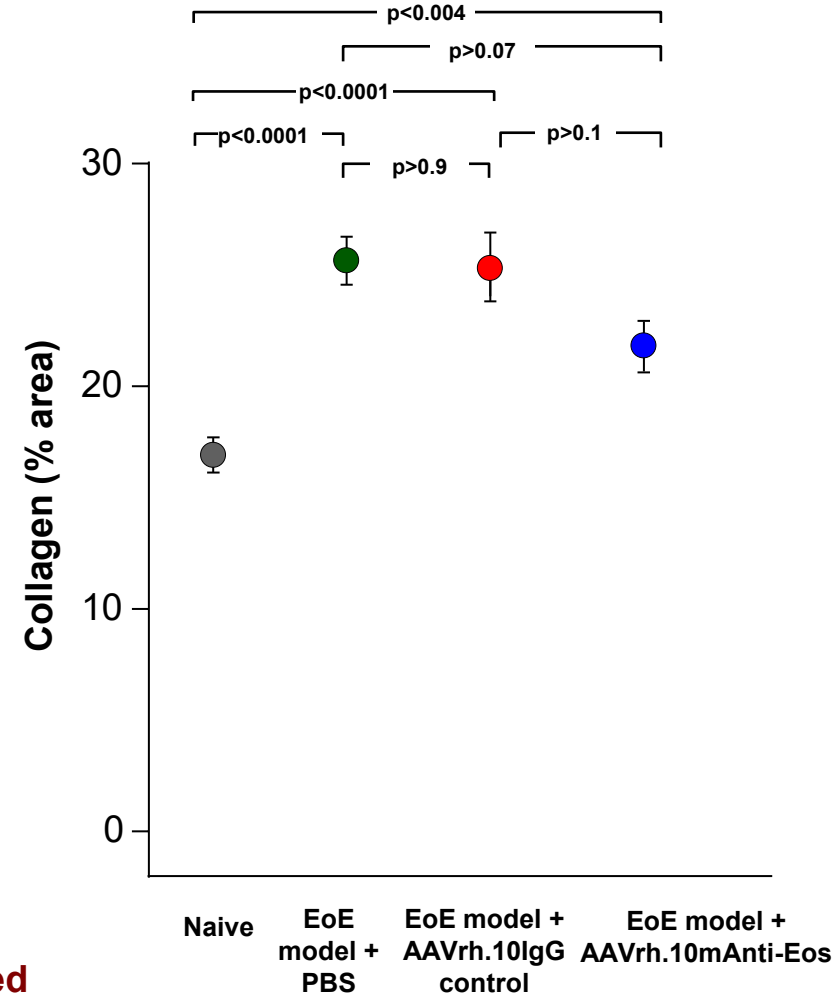


AAVrh.10IgGcontrol, 10^{11} gc AAVrh.10mAnti-Eos, 10^{11} gc

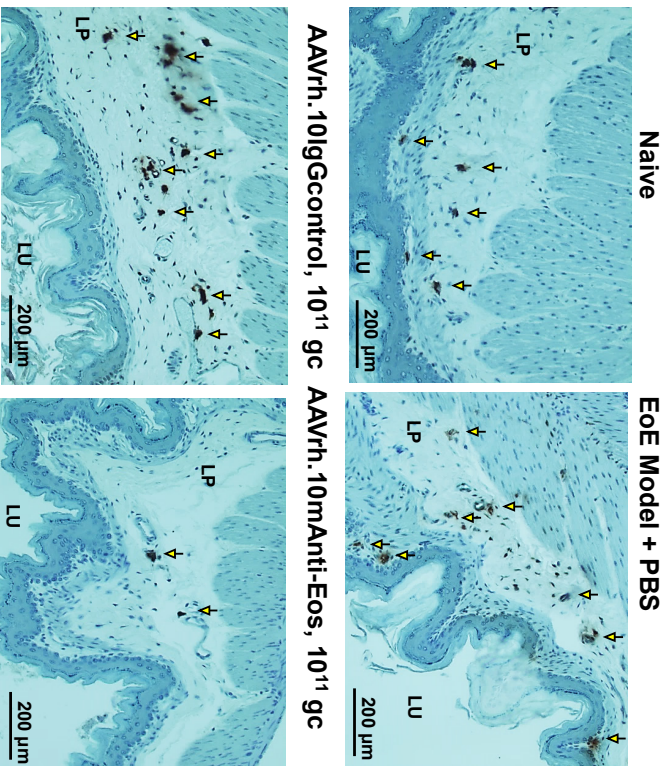


Collagen – blue Muscle fibers – red Nuclei - red/dark red

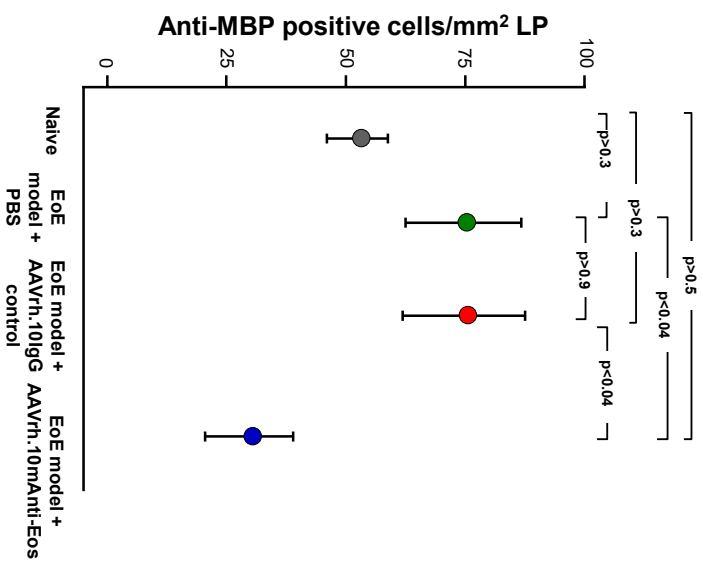
B. Collagen thickness (% area)



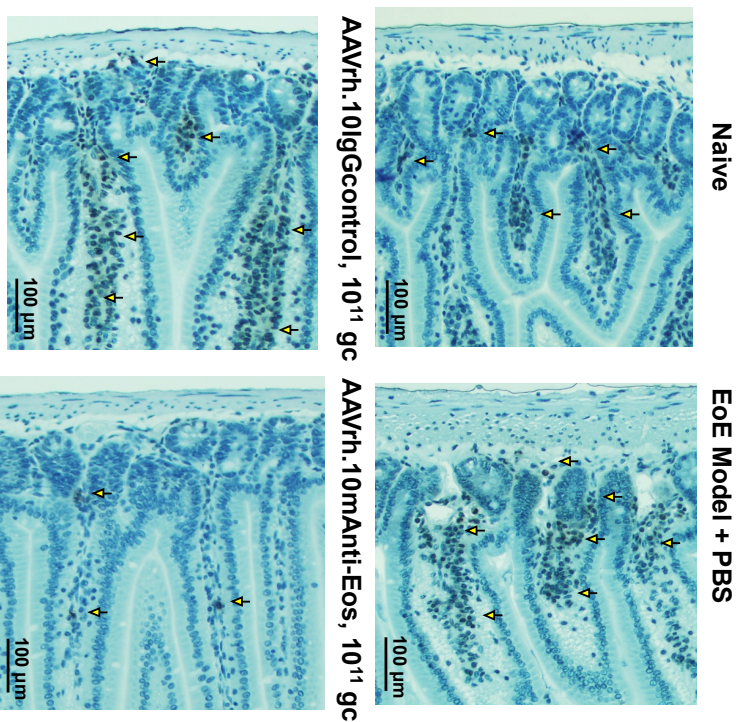
A. Eosinophils in stomach



B. Eosinophil quantification in stomach



C. Eosinophils in intestine



D. Eosinophil quantification in intestine

