Genetic, inflammatory, and tissue-specific factors control expression of human calpain-14

Daniel E. Miller[*,†]; Carmy Forney[*,†]; Mark Rochman[‡]; Stacey Cranert [†]; Jeffery Habel[‡]; Jeffrey Rymer[‡]; Arthur Lynch[†]; Connor Schroeder[†]; Josh Lee[†]; Amber Sauder[†]; Quinton Smith[†]; Mehak Chawla[†]; Michael P. Trimarchi[‡]; Xiaoming Lu[†]; Ellen Fjellman[†]; Michael Brusilovsky[‡]; Stephen Waggoner[†,§]; Matthew T. Weirauch[†,§,**]; Marc E. Rothenberg[‡,§]; Leah C. Kottyan[†,‡,§]

[†]Center for Autoimmune Genomics and Etiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229.

[‡]Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229.

[§]Department of Pediatrics, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45229.

[**]Divisions of Biomedical Informatics and Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229.

*Contributed jointly

RNAseq data: GSE113341

IRB approval: CCHMC 2008-0090

19 Running title: Regulation of *CAPN14* expression

20

21 Keywords: CAPN14, Eosinophilic Esophagitis, IL-13, IL-4, STAT6

22

23 Correspondence should be directed towards Leah Kottyan: Center for Autoimmune Genomics and

24 Etiology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, ML 15012,

25 Cincinnati, OH 45229; Leah.Kottyan@cchmc.org; 513-636-1316 (phone), 513-803-5246 (fax).

26

ABSTRACT

Eosinophilic esophagitis (EoE) is a chronic, food-driven allergic disease resulting in eosinophilic esophageal inflammation. We recently found that EoE susceptibility is associated with genetic variants in the promoter of *CAPN14*, a gene with esophagus-specific expression. *CAPN14* is dynamically up-regulated as a function of EoE disease activity and after exposure of epithelial cells to interleukin-13 (IL-13). Herein, we aimed to explore molecular modulation of *CAPN14* expression. We identified three putative binding sites for the IL-13-activated transcription factor STAT6 in the promoter and first intron of *CAPN14*. Luciferase reporter assays revealed that the two most distal STAT6 elements were required for the ~10-fold increase in promoter activity subsequent to stimulation with IL-13 or IL-4, and also for the genotype-dependent reduction in IL-13-induced promoter activity. One of the STAT6 elements in the promoter was necessary for IL-13-mediated induction of *CAPN14* promoter activity while the other STAT6 promoter element was necessary for full induction. Chromatin immunoprecipitation in IL-13 stimulated esophageal epithelial cells was used to further support STAT6 binding to the promoter of *CAPN14* at these STAT6 binding sites. The highest *CAPN14* and calpain-14 expression occurred with IL-13 or IL-4 stimulation of esophageal epithelial cells under culture conditions that allow the cells to differentiate into a stratified epithelium. This work establishes a candidate molecular mechanism for EoE disease etiology in which the risk variant at 2p23 dampens mediated *CAPN14* expression in differentiated esophageal epithelial cells following IL-13/STAT6 induction of *CAPN14* promoter activity.

47 **INTRODUCTION**

48 Eosinophilic esophagitis (EoE) is a chronic, allergic disease associated with marked mucosal
49 eosinophil accumulation (Liacouras et al. 2011). EoE remits after removal of specific food types, and
50 food re-introduction causes disease reoccurrence, including dysregulation of esophageal transcripts.
51 The etiology of EoE includes environmental, immunological, and genetic components (Alexander et
52 al. 2014; Kottyan et al. 2014; Kottyan et al. 2015). One of the central questions in the EoE field, and
53 allergy in general, is to understand why individuals develop certain manifestations of disease.

54

55 Until recently, studies have identified EoE-genetic risk loci that were broadly linked to other allergic
56 diseases (Sherrill and Rothenberg 2014). For example, genetic variants at 5q22, encoding *TSLP*
57 and *WDR36,* and 11q13, encoding *LRRC32* and *EMSY*, have been associated with allergic
58 sensitization, asthma, allergic rhinitis, atopic dermatitis, food allergy and/or EoE (Greisenegger et al.
59 2013; Tang et al. 2012; Ferreira et al. 2011; Li et al. 2015; Ferreira et al. 2014; Weidinger et al. 2013;
60 Ramasamy et al. 2011; Hinds et al. 2013; Sleiman et al. 2014). The shared association of risk
61 variants among these phenotypes suggests that these loci contain variants that participate in the
62 allele-dependent regulation of a molecular pathway that is central to the etiology of allergic disease.

63

64 We recently found that, in addition to genetic risk loci for allergic sensitization, EoE susceptibility is
65 linked to one or more tissue-specific genetic factors at 2p23, encoding the *CAPN14* gene (Kottyan
66 et al. 2014). This genetic linkage has been replicated at genome-wide significance in multiple
67 cohorts, as well as in a recent independent study (Sleiman et al. 2014), adding credence to the
68 importance of the 2p23 genetic linkage. *CAPN14* (encoding calpain-14) belongs to the classical
69 calpain sub-family, a set of calcium activated intracellular regulatory proteases (Sorimachi et al.
70 2011). In our initial studies, we identified *CAPN14* as dynamically up-regulated as a function of EoE

71 disease activity as well as after exposure of epithelial cells to IL-13 (Konikoff et al. 2006; Davis et al.
72 2016). Expression quantitative trait loci (eQTL) analysis revealed that patients with active EoE
73 expressed *CAPN14* in a genotype-dependent manner (Konikoff et al. 2006). While *CAPN14* was
74 expressed at a higher level in individuals with EoE compared to those without EoE, patients with the
75 risk genotype had decreased expression of *CAPN14* compared to patients with the non-risk
76 genotype (Konikoff et al. 2006). Consistent with these findings, overexpression of the calpain-14
77 protein in esophageal epithelial cells leads to morphological changes and barrier defects
78 independently of IL-13-mediated inflammation, while *CAPN14* gene silencing in these cells leads to
79 defects in barrier repair after IL-13 stimulation (Davis et al. 2016), suggesting that calpain-14 might
80 contribute to EoE via a regulatory loop (Litosh et al. 2017).

81

82 In this study, we aimed to identify regulatory factors that control the expression of *CAPN14*. We used
83 data from the GTEx project (Carithers and Moore 2015; Bahcall 2015) to identify the esophageal
84 mucosa as the region of the esophagus that expressed *CAPN14* and to show that *CAPN14* is
85 expressed in an EoE-risk genotype-dependent manner in individuals without EoE. Using luciferase
86 reporter constructs, we determined that IL-13 is sufficient to induce *CAPN14* promoter activity and
87 identified one putative STAT6 binding sites as necessary for IL-13-induced *CAPN14* promoter
88 activity with a second putative STAT6 binding site in the promoter necessary for full induction of
89 *CAPN14* promoter activity. Using chromatin immunoprecipitation, we identified STAT6 binding to the
90 promoter of *CAPN14* at the site of the two putative STAT6 binding sites in EPC2 esophageal
91 epithelial cells. One single nucleotide polymorphism (SNP) that is highly associated with EoE risk
92 and located in the promoter of *CAPN14* was sufficient to decrease *CAPN14* promoter activity in a
93 manner that is consistent with the eQTL seen in individuals with and without EoE. By measuring
94 mRNA and protein expression of *CAPN14*/calpain-14 over a variety of *in vitro* culture conditions, we

95 further found that *CAPN14* expression is highest in differentiated esophageal epithelial cells after IL-
96 13 exposure. IL-4 also signals in esophageal epithelial cells in a STAT6-dependent manner, and we
97 obtained consistent results for IL-4 and IL-13 promoter activity and gene expression. Altogether, this
98 study identified immunological, genetic, and tissue-specific mechanisms that regulate the expression
99 of *CAPN14*.

100

101 **METHODS**

102 **Esophageal epithelial cell cultures.** EPC2 esophageal epithelial cells were grown in keratinocyte
103 serum-free media (K-SFM) (Life Technologies, Grand Island, NY) to various levels of confluence
104 (80% or 100%) with relatively low (0.09 mM) or high (1.8 mM) Ca^{2+} and with or without 100 ng/mL
105 IL-13 or IL-4 for 24 hours, as a monolayer submerged culture or in an air-liquid interface (ALI) culture
106 (Davis et al. 2016; Kottyan et al. 2014). For the ALI culture system, the esophageal epithelial EPC2
107 cell line was grown to confluence on 0.4 μm pore–size polyester permeable supports (Corning
108 Incorporated, Corning, NY) in K-SFM supplemented with 1.8 mM calcium. Epithelial differentiation
109 was then induced by removing culture media from the inner chamber of the permeable support and
110 maintaining the esophageal epithelial cells for 8 days in the ALI in the presence or absence of IL-13
111 or IL-4 (100 ng/mL). TE7 esophageal epithelial cells were grown in RPMI1640 supplemented with
112 10% FBS (HyClone, Pittsburgh, PA).

113 **Luciferase reporter assays.** The *CCL26* nanoluciferase reporter construct was engineered using
114 the firefly reporter construct reported previously (Lim et al. 2011). The first 964 bp before the *CCL26*
115 start codon were subcloned into the pNL1.1 nanoluciferase reporter vector (Promega, Madison, WI).
116 For the *CAPN14* promoter reporter construct, PCR primers (listed in **Table S1**) were used to amplify
117 the 1988 base pair region around rs76562819 and subsequently clone it into the pNL1.1
118 nanoluciferase reporter vector. Site-directed mutagenesis was performed to generate the construct

119 with scrambled STAT6 binding sites and the EoE non-risk allele of rs76562819 using the GeneArt
120 Site-Directed Mutagenesis System (Invitrogen, Darmstadt, Germany). Sequences were confirmed
121 by Sanger sequencing. All mutagenesis primers are provided in **Table S1**.

122 250 ng of nanoluciferase experimental constructs were transiently co-transfected into esophageal
123 epithelial cells with 250 ng of pGL3-control firefly luciferase reporter plasmid. After 24 hours, cells
124 were treated with 100 ng/mL of IL-13 or IL-4 (Promega, Madison, WI). 24 hours after cytokine
125 administration, cells were lysed and nano and firefly luciferase activity was assayed using the
126 Promega Nano-Glo Luciferase Assay System. The nanoluciferase measurement for each well was
127 divided by the firefly luciferase measurement in order to account for cytotoxicity, small differences in
128 cell number, and transfection efficiency. Each well was further normalized to the promoterless
129 pNL1.1 vector to control for baseline activity of the vector.

130 **Chromatin immunoprecipitation analysis.** EPC2 cells were treated with IL-13 at 100 ng/mL for 45
131 min and fixed with 1% formaldehyde for ChIP. ChIP was performed as reported previously (Rochman
132 et al. 2015) with the exception that the sonication was for 4 min and ChIP was performed manually
133 for the anti-STAT6 experiments. Anti-STAT6 antibody S-20 (D3H4 Rabbit mAb #5397, Cell
134 Signaling) anti-H3K4me3 antibody ab8580 (Abcam), and anti-H3K27Ac antibody ab4279 (Abcam).
135 We fixed 10–20×10^6 cells with 0.8% formaldehyde by adding 1 ml of 10X fixation buffer (50 mM
136 Hepes-KOH, pH 7.5; 100 mM NaCl; 1 mM EDTA; 0.5 mM EGTA; 8% formaldehyde) to 9 ml of growth
137 medium for 8 minutes at room temperature with shaking. The reaction was stopped by adding glycine
138 to a final concentration of 125 mM for an additional 5 minutes. After washing with PBS, pellets were
139 frozen at −80°C for at least overnight. Nuclei were prepared by re-suspending pellets in 1 ml of L1
140 buffer (50 mM Hepes-KOH, pH 7.5; 140 mM NaCl; 1 mM EDTA; 10% glycerol; 0.5% NP-40; 0.25%
141 Triton X-100) and incubated at 4°C for 10 min. Nuclei were pelleted and re-suspended in 1 ml of L2
142 buffer (10 mM Tris-HCl, pH 8.0; 200 mM NaCl; 1 mM EDTA, pH 8.0; 0.5 mM EGTA, pH 8.0) and

143 rotated for 10 min at room temperature. Nuclei were briefly washed with sonication buffer (Tris-EDTA
144 [TE] buffer + 0.1% SDS) and re-suspended in 1 ml of sonication buffer. All buffers were
145 supplemented with complete EDTA-free protease inhibitors (Roche Diagnostics Corporation
146 Indianapolis, IN). Sonication was performed by a Covaris S220 focused ultrasonicator (Covaris, Inc.
147 Woburn, MA) at 175W Peak Incident Power, 10% output, 200 bursts for 10 min in 12×12-mm, round-
148 bottom glass tubes. Efficient DNA fragmentation was verified by agarose gel electrophoresis. Anti-
149 histone mark ChIP was performed by SX-8G IP-Star® Automated System (Diagenode Inc., Denville,
150 NJ) in RIPA buffer (TE + 0.1% SDS, 1% Triton X-100, 150 mM NaCl, 0.1% sodium deoxycholate)
151 following the protocol of the manufacturer with 2–4 μg of indicated antibodies. Libraries were
152 sequenced on the Illumina HiSeq 2500, and ChIP-seq data were visualized and analyzed in
153 Biowardrobe (Rochman et al. 2015).

154 **RNA purification and expression analyses.** For the real-time PCR analysis of CAPN14
155 expression, total RNA was purified from esophageal epithelial cells using the MirVana miRNA
156 Isolation kit (ThermoFisher, Waltham, MA). RNA was reverse-transcribed with the High-Capacity
157 RNA-to-cDNA kit (Applied Biosystems, Grand Island, NY). Gene expression was determined by real-
158 time PCR using a 7500 Real-time PCR system (Applied Biosystems, Foster City, CA) using Taqman
159 Gene Expression Assays CAPN14- Hs00871882_m1, CCL26- Hs00171146_m1 and GAPDH-
160 Hs02786624_g1. Relative quantification was calculated by the comparative CT method (Livak and
161 Schmittgen 2001). Briefly, expression levels for *CAPN14* or *CCL26* were normalized to levels of
162 *GAPDH*. All samples were then normalized to cells grown at 80% confluence without high calcium
163 or IL-13 and the data were exponentially transformed.

164 For the RNA-sequencing study performed to identify the isoforms of CAPN14 expressed in the
165 esophageal biopsies of people with and without EoE (**Figure S2**), RNA was isolated from distal
166 esophageal biopsy RNA from EoE patients (5 male and 5 female) with active disease and from

167 unaffected controls (5 male and 5 female) as previously described (Kottyan et al. 2014; Blanchard
168 et al. 2006; Namjou et al. 2014). EoE biopsies showed active disease pathology at the time when
169 they were taken, and all patients reported no glucocorticoid treatment at the time of biopsy.
170 RNA sequencing acquiring 50 million mappable 125 base-pair reads from paired-end libraries was
171 performed at the Genetic Variation and Gene Discovery Core Facility at CCHMC. Data were aligned
172 to the GrCh37 build of the human genome using the Ensembl (Flicek et al. 2013) annotations as a
173 guide for TopHat (Trapnell et al. 2009). Expression analysis was performed using Kallisto's quant
174 function (Bray et al. 2016). Data has been deposited in NCBI Geo database as GSE113341.
175 **Protein isolation and analysis.** Whole cell lysate was extracted from EPC2 cells lysed with RIPA
176 Buffer (Millipore, Burlington, MA) and Halt™ phosphatase inhibitor cocktail. Samples were exposed
177 to electrophoresis in NuPAGE Novex 4-12% Bis-Tris Gels and transferred to nitrocellulose using the
178 iBlot system (Invitrogen, Carlsbad, CA). Primary antibodies: anti-CAPN14 (HPA035721, Sigma
179 Aldrich, St. Louis, MO) and anti-β-actin (4967L, Cell Signaling Technologies, Danvers, MA).
180 Secondary antibody: IRDye 800CW donkey anti-rabbit (926-32213, LI-COR, Lincoln, NE).
181 Nitrocellulose membranes were imaged using the Odyssey CLx system (LI-COR) and analyzed with
182 Image Studio (LI-COR). EPC2 cells that constitutively express *CAPN14* behind a CMV promoter
183 were used as a positive control.

184 Statistical analysis and data visualization were done with GraphPad Prism (GraphPad Software, La
185 Jolla, CA).

186 **Data and reagent avaliblitiy.** Constructs are avalible upon request. For the *CAPN14* promoter
187 reporter construct, PCR primers (listed in **Table S1**) were used to amplify the 1988 base pair region
188 around rs76562819 and subsequently clone it into the pNL1.1 nanoluciferase reporter vector. All
189 mutagenesis primers are provided in **Table S1**. RNA sequencing data has been deposited in NCBI
190 Geo database as GSE113341.

191 **RESULTS**

192 IL-13 administration to esophageal epithelial cells is sufficient to induce *CAPN14* expression similar
193 to *CCL26*, a chemokine whose promoter activity is dependent on the STAT6 transcription factor (Lim
194 et al. 2011). Based upon these previous results, we hypothesized that IL-13 acted through the
195 transcription factor STAT6 to directly promote *CAPN14* expression. Thus, we first constructed
196 promoter reporters by placing the promoters of either *CAPN14* or *CCL26* in front of the
197 nanoluciferase gene (**Figure 1 A**). After treatment with IL-13 or IL-4, esophageal epithelial cells
198 transfected with the *CAPN14* reporter construct exhibited a similar upregulation of nanoluciferase
199 expression to the cells transfected with a *CCL26* reporter construct (**Figure 1 B and Figure S1 A**).
200 Based upon the canonical 5'-TTC...GAA-3' core motif of STAT6, we identified two putative STAT6
201 binding sequences in the promoter located within 90 base pairs of the transcription start site of
202 *CAPN14* and one putative STAT6 binding site in the first intron of *CAPN14*. We tested the necessity
203 of each individual predicted STAT6 binding site for IL-13-induced promoter activity by creating
204 luciferase reporter constructs with mutated putative STAT6 sites (**Figure 1 A**). Mutation of the first
205 STAT6 site attenuated IL-13-mediated induction of CAPN14 promoter activity, while mutation of the
206 second STAT6 site completely blocked CAPN14 promoter induction (**Figure 1 C**). Mutation of the
207 third putative STAT6 site in the first intron of CAPN14 did not affect the promoter activity of CAPN14
208 following treatment with IL-13 (**Figure 1 C**). From these experiments, we concluded that the two
209 sites in the promoter are necessary for full IL-13-induced promoter activity.

210

211 Chromatin immunoprecipitation of STAT6 in esophageal epithelial cells treated with IL-13 was used
212 to further establish STAT6 binding to the promoter of *CAPN14* at the two binding sites that were
213 necessary for IL-13-inducible *CAPN14* promoter activity. We identified ChIP-seq peaks in the
214 promoter of *CAPN14* spanning these two STAT6 sites from chromatin extracted from IL-13

215 stimulated esophageal epithelial cells (**Figure 2**). There was not evidence of STAT6 binding at the
216 third putative STAT6 site in the first intron of *CAPN14*. These STAT6 ChIP-seq peaks overlap ChIP-
217 seq peaks from active histone marks H3K4me3 and H3K27Ac that appear after induction by IL-13
218 (**Figure 2**). Altogether, these functional genomic data support our initial hypothesis that IL-13 and
219 IL-4 activate *CAPN14* expression through the binding of STAT6 to two sites in the promoter of
220 *CAPN14*.

221

222 Patients with EoE and the genetic-risk haplotype at 2p23, marked by single nucleotide polymorphism
223 (SNP) rs76562819**,** have 50% less *CAPN14* in their esophageal biopsies compared to patients with
224 the non-risk haplotype (Kottyan et al. 2014). Data from GTEx (Carithers and Moore 2015; Bahcall
225 2015) indicates that this genotype-dependent expression of *CAPN14* is also seen in a larger cohort
226 of subjects without EoE ($p=5.9x10^{-12}$, effect size=0.36, **Figure S2 A**). These data also highlighted
227 the tissue-specificity of *CAPN14* to the esophageal mucosa (**Figure S2 B**). An analysis of differential
228 isoform usage of *CAPN14* identified two major isoforms of CAPN14 that did not change with EoE
229 disease status or sex, consistent with the isoform usage reported from GTEx (**Figure S2 C**). Of the
230 two isoforms of *CAPN14* that are expressed, the major isoform (ENST00000444918) includes exon
231 7, while the less highly expressed isoform of *CAPN14* (ENST00000398824) does not. Furthermore,
232 the less highly expressed isoform of *CAPN14* (ENST00000398824) is predicted to undergo
233 nonsense mediated decay based upon manual annotation from the VEGA/Havana projects (Wilming
234 et al. 2008). The EoE-risk haplotype at 2p23 includes 12 variants in linkage disequilibrium (r^2>0.8),
235 but functional genomic and biochemical evidence supported a specific role for rs76562819 (Davis et
236 al. 2016; Kottyan et al. 2014). We tested the hypothesis that rs76562819 was sufficient to result in
237 genotype-dependent promoter activity of *CAPN14* using two identical luciferase constructs
238 containing the promoter and a portion of the first intron of *CAPN14* with either the risk or the non-

239 risk allele of rs76562819 (**Figure 3 A**). We found that the EoE risk allele at rs76562819 resulted in
240 a 40.0% reduction of IL-13 and IL-4-induced *CAPN14* promoter activity compared to the EoE non-
241 risk allele (**Figure 3 B**). Thus, the single base change from non-risk to risk at rs76562819 is both
242 necessary and sufficient to explain most of the reduced *CAPN14* expression observed in EoE patient
243 biopsies.

244

245 We next sought to determine the factors leading to tissue specificity of *CAPN14*. Unlike *CCL26*,
246 *CAPN14* expression is highly dependent upon the cell culture conditions. Esophageal epithelial
247 differentiation is a physiological process that increases mechanical strength and barrier function to
248 squamous epithelia (Macara et al. 2014). Factors that are necessary for differentiation *in vitro* are
249 calcium concentration, cellular confluence, and the culture system (Macara et al. 2014; Kc et al.
250 2015). Without esophageal epithelial differentiation by confluence, calcium, and growth in an air-
251 liquid interface (ALI), *CAPN14* mRNA expression is low (**Figure 4 A**). Following differentiation,
252 *CAPN14* is induced 10,000-fold in the ALI, $p<0.0001$ (**Figure 4 A**). At a protein level, calpain-14 was
253 detected only in the most differentiated system (ALI) following treatment with IL-13 (**Figure 4 B**). As
254 seen in the promoter reporter experiments, IL-4 and IL-13 are equally capable of induction of
255 *CAPN14* mRNA and protein (**Figure S3**).

256

257 **DISCUSSION**

258 Altogether, we identified immunological (IL-13/IL-4/STAT6), genetic (rs76562819), and tissue-
259 specific (esophageal epithelia differentiation) factors that regulate the expression of *CAPN14*. These
260 results are important because calpain-14 represents a candidate for treatment and prevention of
261 EoE. Previous studies supported a model in which IL-13 induction of *CAPN14* occurs in a STAT6-
262 dependent manner. We identified the STAT6 binding sites, the necessity of these sites for *CAPN14*

263 promoter activity, and the binding of STAT6 to the promoter of *CAPN14* in esophageal cells
264 stimulated with IL-13. We demonstrated genotype-dependent expression of *CAPN14* and identified
265 rs76562819 as the genetic variant that is sufficient to produce genotype-dependent promoter activity.
266 Indeed, the 40.0% reduction of *CAPN14* promoter activity in the genotype-dependent reporter
267 assays mirrors the effect size of the eQTLs measured in subjects with and without EoE (Kottyan et
268 al. 2014). The reporter experiments did not demonstrate statistically significant genotype-dependent
269 promoter activity in cells that were not stimulated with IL-13; however, GTEx data demonstrated
270 genotype-dependent *CAPN14* expression in the esophageal mucosa of subjects without EoE. This
271 could be due to several factors, including the sensitivity of the reporter system, the non-differentiated
272 context of the reporter experiments, the superior statistical power of the GTEx study, or genotype-
273 dependent differences in chromatin accessibility. The fact that relatively undifferentiated esophageal
274 epithelial cell culture systems are sufficient for luciferase reporter systems could be due to how
275 differentiation affects chromatin availability – a factor that is not assessed in the cytoplasmic
276 luciferase reporter assay. *CAPN14* mRNA and protein expression levels strongly support the
277 conclusion that both differentiation and stimulation with IL-13 or IL-4 is critical for the highest
278 expression of calpain-14 in esophageal epithelial cells. The requirement for esophageal epithelial
279 cell differentiation before *CAPN14* expression might be the primary mechanism through which
280 *CAPN14*'s expression is specifically induced in the esophageal mucosa (**Figure S2 B**).

281 IL-13 is a critical cytokine in the pathoetiology of EoE (Blanchard et al. 2011; O'Shea et al. 2017;
282 Davis et al. 2016; D'Mello et al. 2016; Cianferoni and Spergel 2016; Rothenberg et al. 2015; Sherrill
283 et al. 2014b; Sherrill et al. 2014a; Zuo et al. 2010; Blanchard et al. 2007; Mishra and Rothenberg
284 2003). Indeed, treating esophageal epithelial cells with IL-13 is an effective way to model
285 approximately 25% of the transcriptome and cytokine secretion profiles found in the esophageal
286 biopsies of patients with EoE (Sherrill et al. 2014b; Lu et al. 2012; Blanchard et al. 2007). IL-13

287 directly controls the expression of chemokines that recruit eosinophils to the esophagus (*CCL11*,
288 *CCL24*, *CCL26*) through STAT6-dependent mechanisms (Lim et al. 2011). IL-13 treatment can also
289 activate other cellular and transcriptional regulators including the protein kinase B (Akt) and mitogen-
290 activated protein kinase (MAPK) pathways (Demoulin and Renauld 1998; Harris et al. 2007), and
291 transcriptional regulation downstream of IL-13 signaling can also be indirect (e.g. genes transcribed
292 in response to IL-13 can regulate the expression of other genes). It was therefore important to
293 establish that two STAT6 sites in the *CAPN14* promoter control IL-13-stimulated expression *of*
294 *CAPN14*. IL-13 shares many functional properties with IL-4, stemming from the fact that they share
295 a common receptor subunit, the alpha subunit of the IL-4 receptor (Hershey 2003). Both IL-13 and
296 IL-4 signaling lead to STAT6 dimerization, phosphorylation, and nuclear translocation (Kuperman
297 and Schleimer 2008), so it is therefore not surprising that IL-4 might also regulate *CAPN14* mRNA
298 expression and promoter activity (**Figures S1 and S3**).
299

300 Very little is known about the factors regulating the expression, function, and tissue specificity of
301 *CAPN14* (Litosh et al. 2017; Davis et al. 2016; Sleiman et al. 2014; Ueta et al. 2010; Kottyan et al.
302 2014; Dear and Boehm 2001). This study focused on the regulation of *CAPN14* transcription. Future
303 studies will assess the post-translational regulation and activity of calpain-14. The structure of
304 calpain-14 strongly suggests that it might dimerize with calpain-S1 (Litosh et al. 2017). Other
305 members of the calpain family are known to undergo autolysis (Ono et al. 2016a; Ermolova et al.
306 2011; Taveau et al. 2003; Badugu et al. 2008), and proteomic analysis of calpain-14 expression
307 found evidence consistent with cleavage of calpain-14, especially in non-differentiated conditions
308 (**Figure 4 B**). We used an overexpression construct of calpain-14 to identify the specific band bound
309 by the anti-calpain-14 antibody in the Western blots (**Figure 4 B**). Calpains cleave substrates in a
310 calcium-dependent manner based upon the conformation, rather than the amino acid sequence, of

311 their targets (Ono et al. 2016b; Sorimachi et al. 2012). Full biochemical analyses of the substrates
312 of calpain-14 will be critical as the functions of calpain-14 in health and EoE are discovered.

313

314 The current study identifies critical factors that regulate *CAPN14* expression. Altogether, this study
315 makes an important advance towards identification of the factors that control the genotype-
316 dependent transcriptional regulation of *CAPN14* in the esophagus.

317

ACKNOLEDGMENTS

This work was supported by National Institutes of Health [R37 AI045898, R01 AI124355, R01 DK107502, R01 NS099068, R21 HG008186, P30 AR070549, U01 HG008666]; the Campaign Urging Research for Eosinophilic Disease (CURED); the Buckeye Foundation; the Sunshine Charitable Foundation and its supporters, Denise A. Bunning and David G. Bunning; the American Partnership of Eosinophilic Disorders (APFED); Cincinnati Children's Hospital CpG Pilot Study and Endowed Scholar awards to M.T.W.

This project was supported in part by PHS Grant P30 DK078392 DNA sequencing core of the Digestive Disease Research Core Center in Cincinnati. N.W. and S.A.C. were supported by NIH Grant DA038017 and funds from CCRF, including an Arnold Strauss Fellowship to S.A.C.

CONFLICT OF INTERST STATEMENT

M.E.R. is a consultant for Pulm One, Spoon Guru, ClostraBio, Celgene, Shire, Astra Zeneca, GlaxoSmithKline, Allakos, Adare, Regeneron and Novartis and has an equity interest in the first four listed and Immune Pharmaceuticals, and royalties from reslizumab (Teva Pharmaceuticals). M.E.R. is an inventor of patents, owned by Cincinnati Children's.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

339 **Figure 1. STAT6 binding sites in the promoter region of the *CAPN14* gene affect IL-13-induced**
340 ***CAPN14* promoter activity.** *A*, schematic representation of the nucleotide sequence of the promoter
341 of the human *CAPN14* gene. Putative binding sites for STAT6 are defined by grey boxes. *B*, Site-
342 directed mutagenesis elimination of putative STAT6-binding sites is denoted by crossed boxes. The
343 (*B*) CCL26 and CAPN14 constructs or the (*C*) mutagenized CAPN14 constructs were transfected
344 into esophageal epithelial cells followed by treatment with or without IL-13 (100 ng/mL) for 24 h. For
345 each sample, nanoluciferase activity was normalized to firefly luciferase activity. Data are shown as
346 mean ± S.E.M. (***, $p < 0.001$; $n = 3$ per group; data representative of three independent
347 experiments).

348

349 **Figure 2. Epigenetic signature and STAT6 binding to the promoter region of *CAPN14* in**
350 **response to IL-13 stimulation.** An image capture from the UC Santa Cruz genome browser
351 shows the tag density profile of aligned ChIP-seq reads in untreated and IL-13-treated EPC2 cells.
352 The read depths of the aligned sequences are of the same scale. Arrow represents direction of
353 transcription, black boxes represent exons. In the IL-13-treated cells, elevated levels of H3K4me3
354 and H3K27Ac are detected in the promoter region. The scale is the same for all track pairs
355 (untreated and IL-13).Three putative STAT6 binding sites around the TSS are indicated by blue
356 lines. TSS – transcription start site.

357

358 **Figure 3. The risk variant of rs76562819 results in a genotype-dependent decrease in IL-13**
359 **induced promoter activity of *CAPN14*.** *A,* Diagrams of constructs containing the *CAPN14* promoter
360 are shown. *B,* Reporter constructs were transfected into esophageal epithelial cells followed by
361 treatment with or without IL-13 (100 ng/mL) for 24 h. For each sample, nanoluciferase activity was
362 normalized to firefly luciferase activity. Data are shown as mean ± S.E.M. (***, t-test p-value<0.001;

363 2-way ANOVA: $p<0.05$, genotype $p<0.0001$ accounting for 12.6% of total variation; $n = 3$ per group;
364 data representative of three independent experiments).
365
366 **Figure 4. The expression of *CAPN14* in esophageal epithelial cells is dependent upon**
367 **calcium, confluency, and IL-13.** EPC2 esophageal epithelial cells were grown to various levels of
368 confluence (80%- indicated by -, or 100%-indicated by +) with relatively low (0.09 mM) or high (1.8
369 mM) Ca^{2+} and with or without 100 ng/mL IL-13 for 24 hours. Cultures were grown either as a
370 monolayer submerged culture or in an air-liquid interface (ALI) setup. *A,* RNA was isolated and
371 *CAPN14* was measured relative to the expression of the house keeping gene *GAPDH*. All RNA
372 expression values are normalized to cells at 80% confluence without high calcium or IL-13. *B,*
373 Calpain-14 protein expression levels. EPC2 cells that constitutively express *CAPN14* behind a CMV
374 promoter were used as a positive control for calpain-14 protein expression. RNA and protein level
375 expression: ***, t-test p-value<0.001, n=3-5 per group, data representative of 4 independent
376 experiments.
377
378
379

380 **REFERENCES**

381 Alexander, E.S., L.J. Martin, M.H. Collins, L.C. Kottyan, H. Sucharew *et al.*, 2014 Twin and family
382 studies reveal strong environmental and weaker genetic cues explaining heritability of
383 eosinophilic esophagitis. *J Allergy Clin Immunol* 134 (5):1084-1092 e1081.

384 Badugu, R., M. Garcia, V. Bondada, A. Joshi, and J.W. Geddes, 2008 N terminus of calpain 1 is a
385 mitochondrial targeting sequence. *J Biol Chem* 283 (6):3409-3417.

386 Bahcall, O.G., 2015 Human genetics: GTEx pilot quantifies eQTL variation across tissues and
387 individuals. *Nat Rev Genet* 16 (7):375.

388 Blanchard, C., M.K. Mingler, M. Vicario, J.P. Abonia, Y.Y. Wu *et al.*, 2007 IL-13 involvement in
389 eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J*
390 *Allergy Clin Immunol* 120 (6):1292-1300.

391 Blanchard, C., E.M. Stucke, B. Rodriguez-Jimenez, K. Burwinkel, M.H. Collins *et al.*, 2011 A striking
392 local esophageal cytokine expression profile in eosinophilic esophagitis. *J Allergy Clin*
393 *Immunol* 127 (1):208-217, 217 e201-207.

394 Blanchard, C., N. Wang, K.F. Stringer, A. Mishra, P.C. Fulkerson *et al.*, 2006 Eotaxin-3 and a
395 uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest* 116
396 (2):536-547.

397 Bray, N.L., H. Pimentel, P. Melsted, and L. Pachter, 2016 Near-optimal probabilistic RNA-seq
398 quantification. *Nat Biotechnol* 34 (5):525-527.

399 Carithers, L.J., and H.M. Moore, 2015 The Genotype-Tissue Expression (GTEx) Project. *Biopreserv*
400 *Biobank* 13 (5):307-308.

401 Cianferoni, A., and J. Spergel, 2016 Eosinophilic Esophagitis: A Comprehensive Review. *Clin Rev*
402 *Allergy Immunol* 50 (2):159-174.

403 D'Mello, R.J., J.M. Caldwell, N.P. Azouz, T. Wen, J.D. Sherrill *et al.*, 2016 LRRC31 is induced by IL-
404 13 and regulates kallikrein expression and barrier function in the esophageal epithelium.
405 *Mucosal Immunol* 9 (3):744-756.

406 Davis, B.P., E.M. Stucke, M.E. Khorki, V.A. Litosh, J.K. Rymer *et al.*, 2016 Eosinophilic esophagitis-
407 linked calpain 14 is an IL-13-induced protease that mediates esophageal epithelial barrier
408 impairment. *JCI Insight* 1 (4):e86355.

409 Dear, T.N., and T. Boehm, 2001 Identification and characterization of two novel calpain large subunit
410 genes. *Gene* 274 (1-2):245-252.

411 Demoulin, J.B., and J.C. Renauld, 1998 Signalling by cytokines interacting with the interleukin-2
412 receptor gamma chain. *Cytokines Cell Mol Ther* 4 (4):243-256.

413 Ermolova, N., E. Kudryashova, M. DiFranco, J. Vergara, I. Kramerova *et al.*, 2011 Pathogenity of
414 some limb girdle muscular dystrophy mutations can result from reduced anchorage to
415 myofibrils and altered stability of calpain 3. *Hum Mol Genet* 20 (17):3331-3345.

416 Ferreira, M.A., M.C. Matheson, D.L. Duffy, G.B. Marks, J. Hui *et al.*, 2011 Identification of IL6R and
417 chromosome 11q13.5 as risk loci for asthma. *Lancet* 378 (9795):1006-1014.

418 Ferreira, M.A., M.C. Matheson, C.S. Tang, R. Granell, W. Ang *et al.*, 2014 Genome-wide association
419 analysis identifies 11 risk variants associated with the asthma with hay fever phenotype. *J*
420 *Allergy Clin Immunol* 133 (6):1564-1571.

421 Flicek, P., I. Ahmed, M.R. Amode, D. Barrell, K. Beal *et al.*, 2013 Ensembl 2013. *Nucleic Acids Res*
422 41 (Database issue):D48-55.

423 Greisenegger, E.K., F. Zimprich, A. Zimprich, A. Gleiss, and T. Kopp, 2013 Association of the
424 chromosome 11q13.5 variant with atopic dermatitis in Austrian patients. *Eur J Dermatol* 23
425 (2):142-145.

426 Harris, J., S.A. De Haro, S.S. Master, J. Keane, E.A. Roberts *et al.*, 2007 T helper 2 cytokines inhibit
427 autophagic control of intracellular Mycobacterium tuberculosis. *Immunity* 27 (3):505-517.

428 Hershey, G.K., 2003 IL-13 receptors and signaling pathways: an evolving web. *J Allergy Clin*
429 *Immunol* 111 (4):677-690; quiz 691.

430 Hinds, D.A., G. McMahon, A.K. Kiefer, C.B. Do, N. Eriksson *et al.*, 2013 A genome-wide association
431 meta-analysis of self-reported allergy identifies shared and allergy-specific susceptibility loci.
432 *Nat Genet* 45 (8):907-911.

433 Kc, K., M.E. Rothenberg, and J.D. Sherrill, 2015 In vitro model for studying esophageal epithelial
434 differentiation and allergic inflammatory responses identifies keratin involvement in
435 eosinophilic esophagitis. *PLoS One* 10 (6):e0127755.

436 Konikoff, M.R., R.J. Noel, C. Blanchard, C. Kirby, S.C. Jameson *et al.*, 2006 A randomized double-
437 blind-placebo controlled trial of fluticasone proprionate for pediatric eosinophilic esophagitis.
438 *Gastroenterology* 131:1381-1391.

439 Kottyan, L.C., B.P. Davis, J.D. Sherrill, K. Liu, M. Rochman *et al.*, 2014 Genome-wide association
440 analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic
441 disease. *Nat Genet* 46 (8):895-900.

442 Kottyan, L.C., M.T. Weirauch, and M.E. Rothenberg, 2015 Making it big in allergy. *J Allergy Clin*
443 *Immunol* 135 (1):43-45.

444 Kuperman, D.A., and R.P. Schleimer, 2008 Interleukin-4, interleukin-13, signal transducer and
445 activator of transcription factor 6, and allergic asthma. *Curr Mol Med* 8 (5):384-392.

446 Li, J., Y. Zhang, and L. Zhang, 2015 Discovering susceptibility genes for allergic rhinitis and allergy
447 using a genome-wide association study strategy. *Curr Opin Allergy Clin Immunol* 15 (1):33-
448 40.

449 Liacouras, C.A., G.T. Furuta, I. Hirano, D. Atkins, S.E. Attwood *et al.*, 2011 Eosinophilic esophagitis:
450 updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 128
451 (1):3-20 e26; quiz 21-22.

452 Lim, E.J., T.X. Lu, C. Blanchard, and M.E. Rothenberg, 2011 Epigenetic regulation of the IL-13-
453 induced human eotaxin-3 gene by CREB-binding protein-mediated histone 3 acetylation. *J*
454 *Biol Chem* 286 (15):13193-13204.

455 Litosh, V.A., M. Rochman, J.K. Rymer, A. Porollo, L.C. Kottyan *et al.*, 2017 Calpain-14 and its
456 association with eosinophilic esophagitis. *J Allergy Clin Immunol* 139 (6):1762-1771 e1767.

457 Livak, K.J., and T.D. Schmittgen, 2001 Analysis of relative gene expression data using real-time
458 quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25 (4):402-408.

459 Lu, T.X., E.J. Lim, T. Wen, A.J. Plassard, S.P. Hogan *et al.*, 2012 MiR-375 is downregulated in
460 epithelial cells after IL-13 stimulation and regulates an IL-13-induced epithelial transcriptome.
461 *Mucosal Immunol* 5 (4):388-396.

462 Macara, I.G., R. Guyer, G. Richardson, Y. Huo, and S.M. Ahmed, 2014 Epithelial homeostasis. *Curr*
463 *Biol* 24 (17):R815-825.

464 Mishra, A., and M.E. Rothenberg, 2003 Intratracheal IL-13 induces eosinophilic esophagitis by an
465 IL-5, eotaxin-1, and STAT6-dependent mechanism. *Gastroenterology* 125 (5):1419-1427.

466 Namjou, B., K. Marsolo, R.J. Caroll, J.C. Denny, M.D. Ritchie *et al.*, 2014 Phenome-wide association
467 study (PheWAS) in EMR-linked pediatric cohorts, genetically links PLCL1 to speech language
468 development and IL5-IL13 to Eosinophilic Esophagitis. *Front Genet* 5:401.

469 O'Shea, K.M., S.S. Aceves, E.S. Dellon, S.K. Gupta, J.M. Spergel *et al.*, 2017 Pathophysiology of
470 Eosinophilic Esophagitis. *Gastroenterology*.

471 Ono, Y., K. Ojima, F. Shinkai-Ouchi, S. Hata, and H. Sorimachi, 2016a An eccentric calpain,
472 CAPN3/p94/calpain-3. *Biochimie* 122:169-187.

473 Ono, Y., T.C. Saido, and H. Sorimachi, 2016b Calpain research for drug discovery: challenges and
474 potential. *Nat Rev Drug Discov* 15 (12):854-876.

475 Ramasamy, A., I. Curjuric, L.J. Coin, A. Kumar, W.L. McArdle *et al.*, 2011 A genome-wide meta-
476 analysis of genetic variants associated with allergic rhinitis and grass sensitization and their
477 interaction with birth order. *J Allergy Clin Immunol* 128 (5):996-1005.

478 Rochman, M., A.V. Kartashov, J.M. Caldwell, M.H. Collins, E.M. Stucke *et al.*, 2015 Neurotrophic
479 tyrosine kinase receptor 1 is a direct transcriptional and epigenetic target of IL-13 involved in
480 allergic inflammation. *Mucosal Immunol* 8 (4):785-798.

481 Rothenberg, M.E., T. Wen, A. Greenberg, O. Alpan, B. Enav *et al.*, 2015 Intravenous anti-IL-13 mAb
482 QAX576 for the treatment of eosinophilic esophagitis. *J Allergy Clin Immunol* 135 (2):500-507.

483 Sherrill, J.D., K. Kc, D. Wu, Z. Djukic, J.M. Caldwell *et al.*, 2014a Desmoglein-1 regulates esophageal
484 epithelial barrier function and immune responses in eosinophilic esophagitis. *Mucosal*
485 *Immunol* 7 (3):718-729.

486 Sherrill, J.D., K.C. Kiran, C. Blanchard, E.M. Stucke, K.A. Kemme *et al.*, 2014b Analysis and
487 expansion of the eosinophilic esophagitis transcriptome by RNA sequencing. *Genes Immun*
488 15 (6):361-369.

489 Sherrill, J.D., and M.E. Rothenberg, 2014 Genetic and epigenetic underpinnings of eosinophilic
490 esophagitis. *Gastroenterol Clin North Am* 43 (2):269-280.

491 Sleiman, P.M., M.L. Wang, A. Cianferoni, S. Aceves, N. Gonsalves *et al.*, 2014 GWAS identifies four
492 novel eosinophilic esophagitis loci. *Nat Commun* 5:5593.

493 Sorimachi, H., S. Hata, and Y. Ono, 2011 Impact of genetic insights into calpain biology. *J Biochem*
494 150 (1):23-37.

495 Sorimachi, H., H. Mamitsuka, and Y. Ono, 2012 Understanding the substrate specificity of
496 conventional calpains. *Biol Chem* 393 (9):853-871.

497 Tang, X.F., H.Y. Tang, L.D. Sun, F.L. Xiao, Z. Zhang *et al.*, 2012 Genetic variant rs4982958 at
498 14q11.2 is associated with allergic rhinitis in a Chinese Han population running title: 14q11.2
499 is a susceptibility locus for allergic rhinitis. *J Investig Allergol Clin Immunol* 22 (1):55-62.

500 Taveau, M., N. Bourg, G. Sillon, C. Roudaut, M. Bartoli *et al.*, 2003 Calpain 3 is activated through
501 autolysis within the active site and lyses sarcomeric and sarcolemmal components. *Mol Cell*
502 *Biol* 23 (24):9127-9135.

503 Trapnell, C., L. Pachter, and S.L. Salzberg, 2009 TopHat: discovering splice junctions with RNA-
504 Seq. *Bioinformatics* 25 (9):1105-1111.

505 Ueta, M., K. Mizushima, N. Yokoi, Y. Naito, and S. Kinoshita, 2010 Expression of the interleukin-4
506 receptor alpha in human conjunctival epithelial cells. *Br J Ophthalmol* 94 (9):1239-1243.

507 Weidinger, S., S.A. Willis-Owen, Y. Kamatani, H. Baurecht, N. Morar *et al.*, 2013 A genome-wide
508 association study of atopic dermatitis identifies loci with overlapping effects on asthma and
509 psoriasis. *Hum Mol Genet* 22 (23):4841-4856.

510 Wilming, L.G., J.G. Gilbert, K. Howe, S. Trevanion, T. Hubbard *et al.*, 2008 The vertebrate genome
511 annotation (Vega) database. *Nucleic Acids Res* 36 (Database issue):D753-760.

512 Zuo, L., P.C. Fulkerson, F.D. Finkelman, M. Mingler, C.A. Fischetti *et al.*, 2010 IL-13 induces
513 esophageal remodeling and gene expression by an eosinophil-independent, IL-13R alpha 2-
514 inhibited pathway. *J Immunol* 185 (1):660-669.

515

Figure 1. Three STAT6 binding sites in promoter region of the *CAPN14* gene affects IL-13-induced *CAPN14* promoter activity.

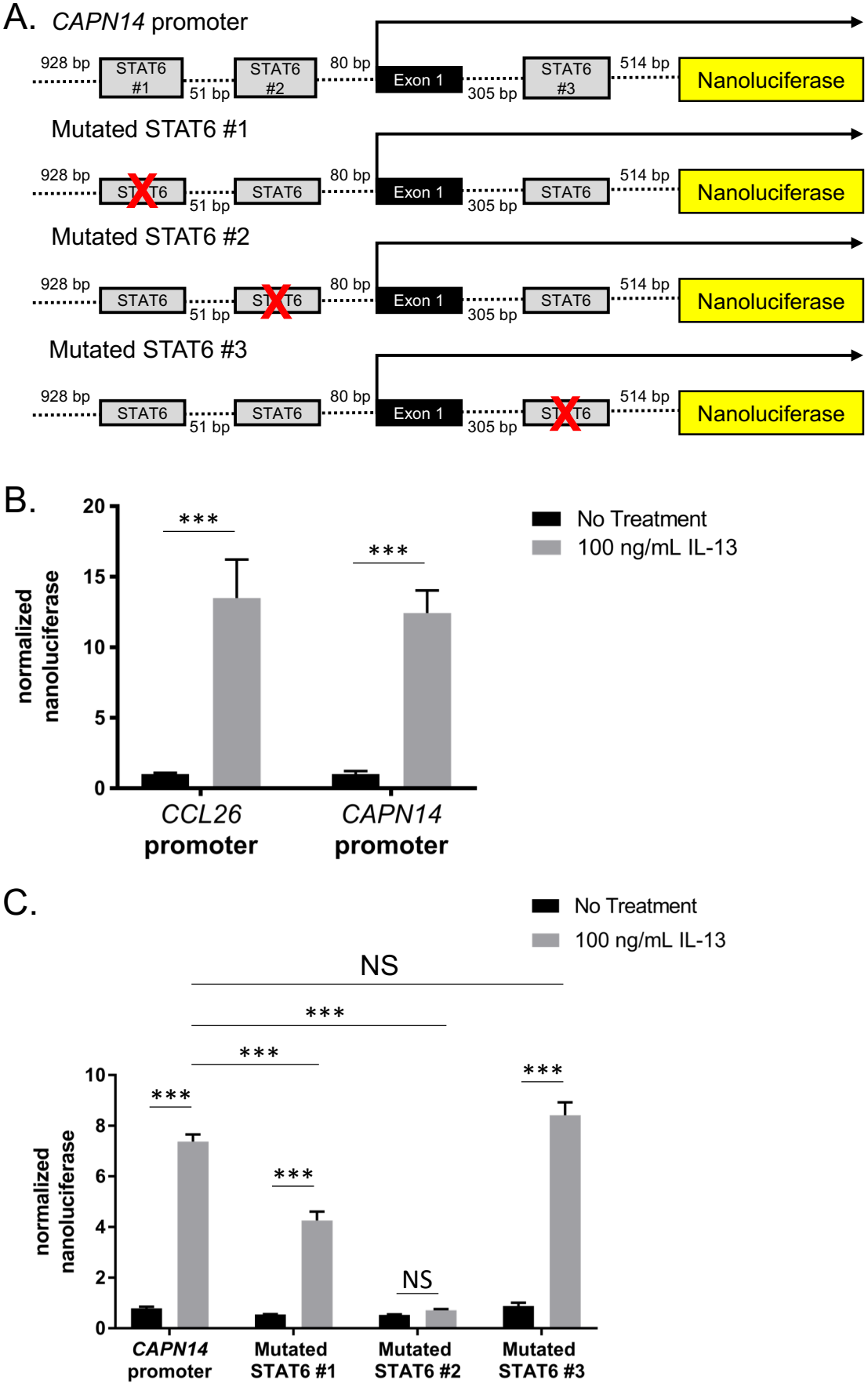


Figure 2. Chromatin immunoprecipitation of STAT6 confirms STAT6 binding at the the promoter of *CAPN14*.

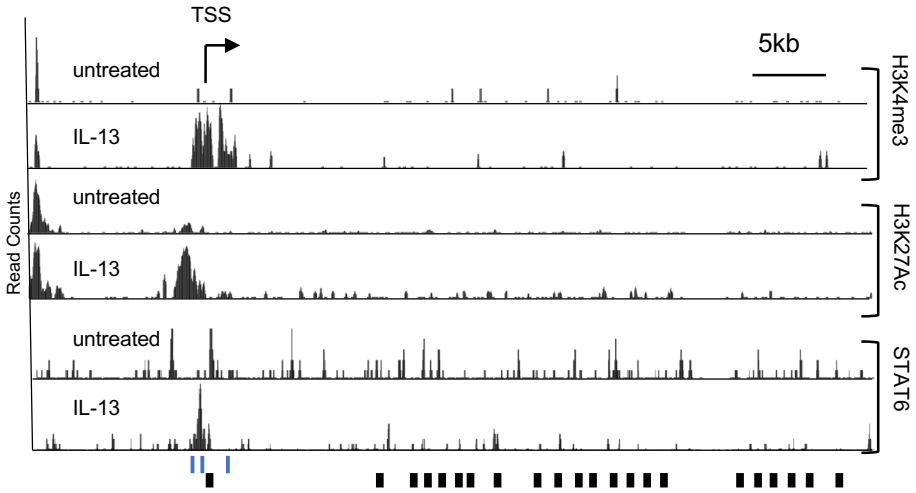


Figure 3. The risk variant of rs76562819 results in genotype-dependent decrease in IL-13 induced promoter activity of *CAPN14*

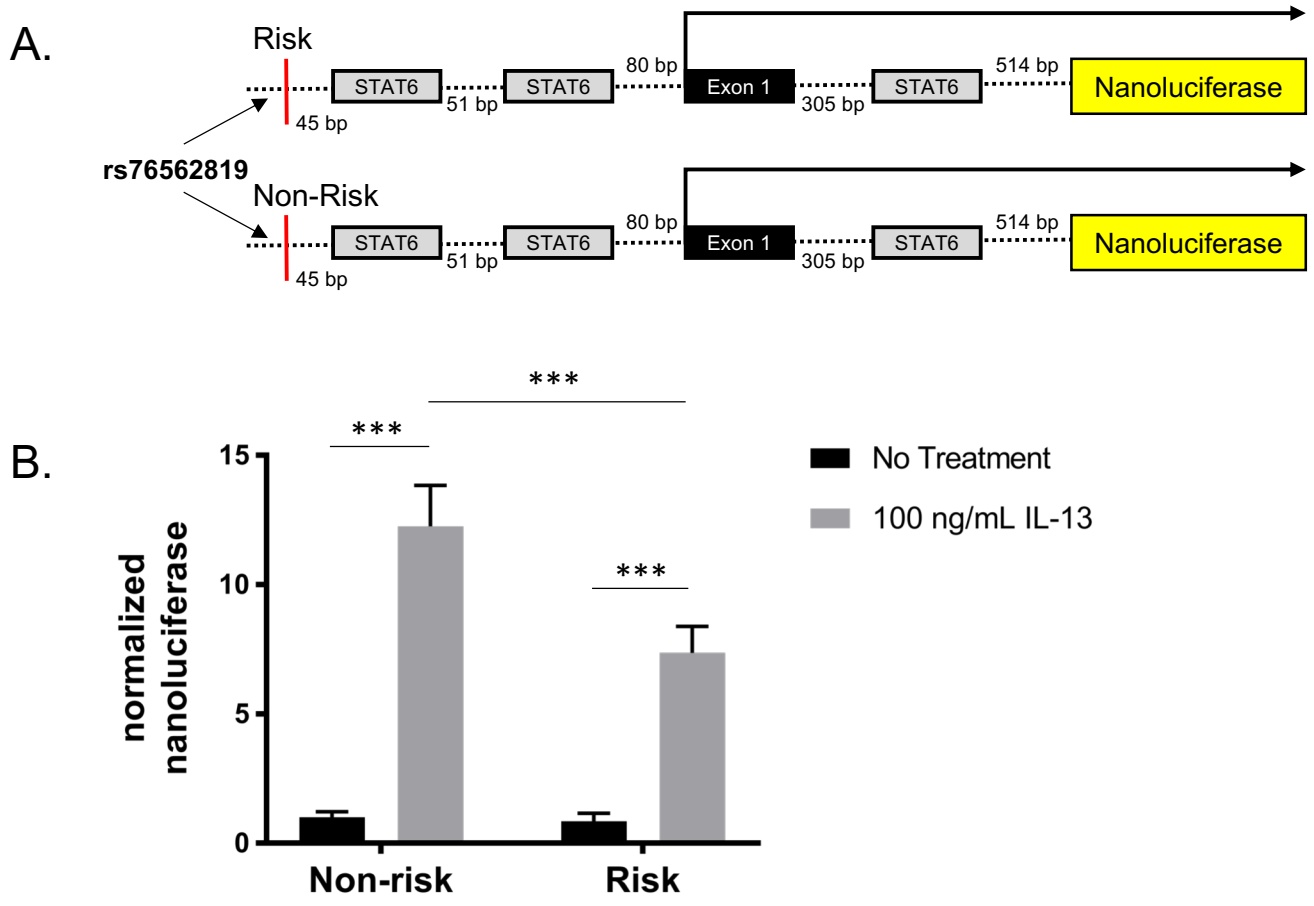
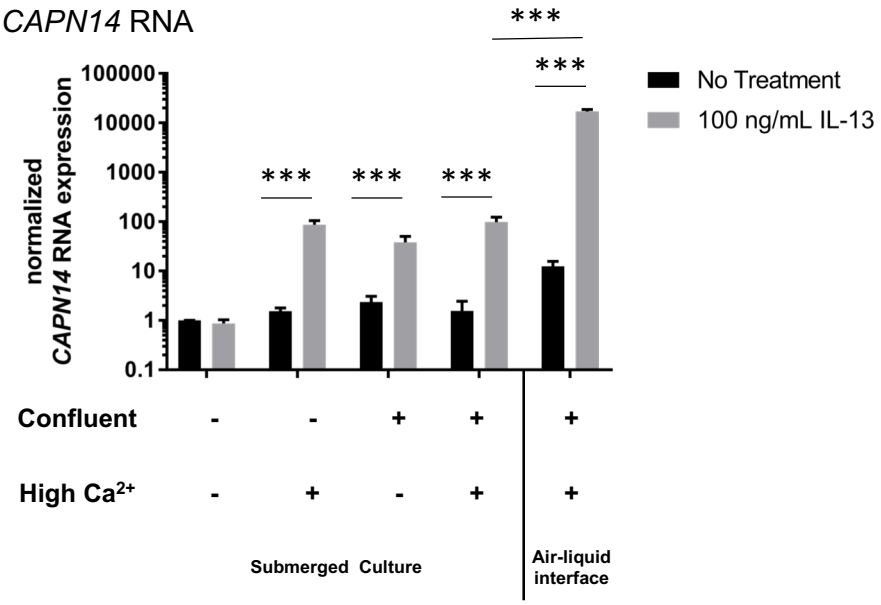
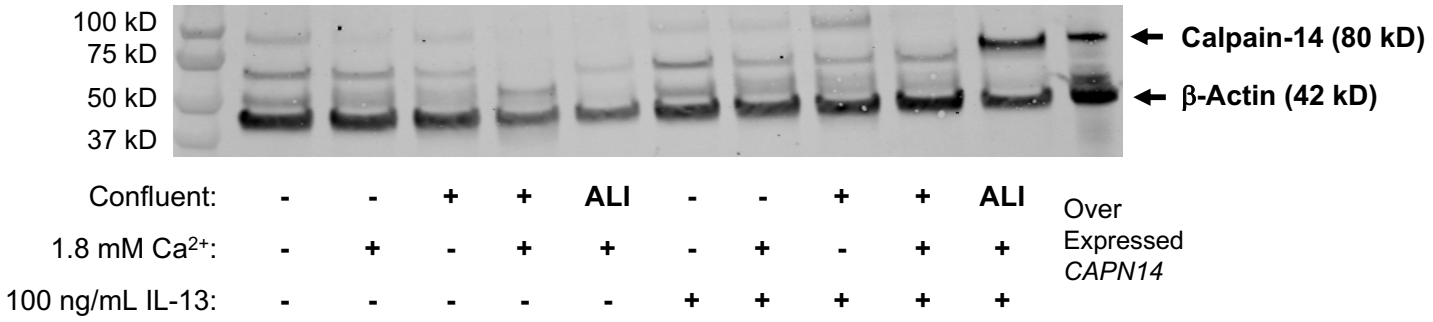


Figure 4. The expression of *CAPN14* in esophageal epithelial cells is dependent upon calcium, confluency, and IL-13.

A. *CAPN14* RNA



B. Calpain-14 Protein



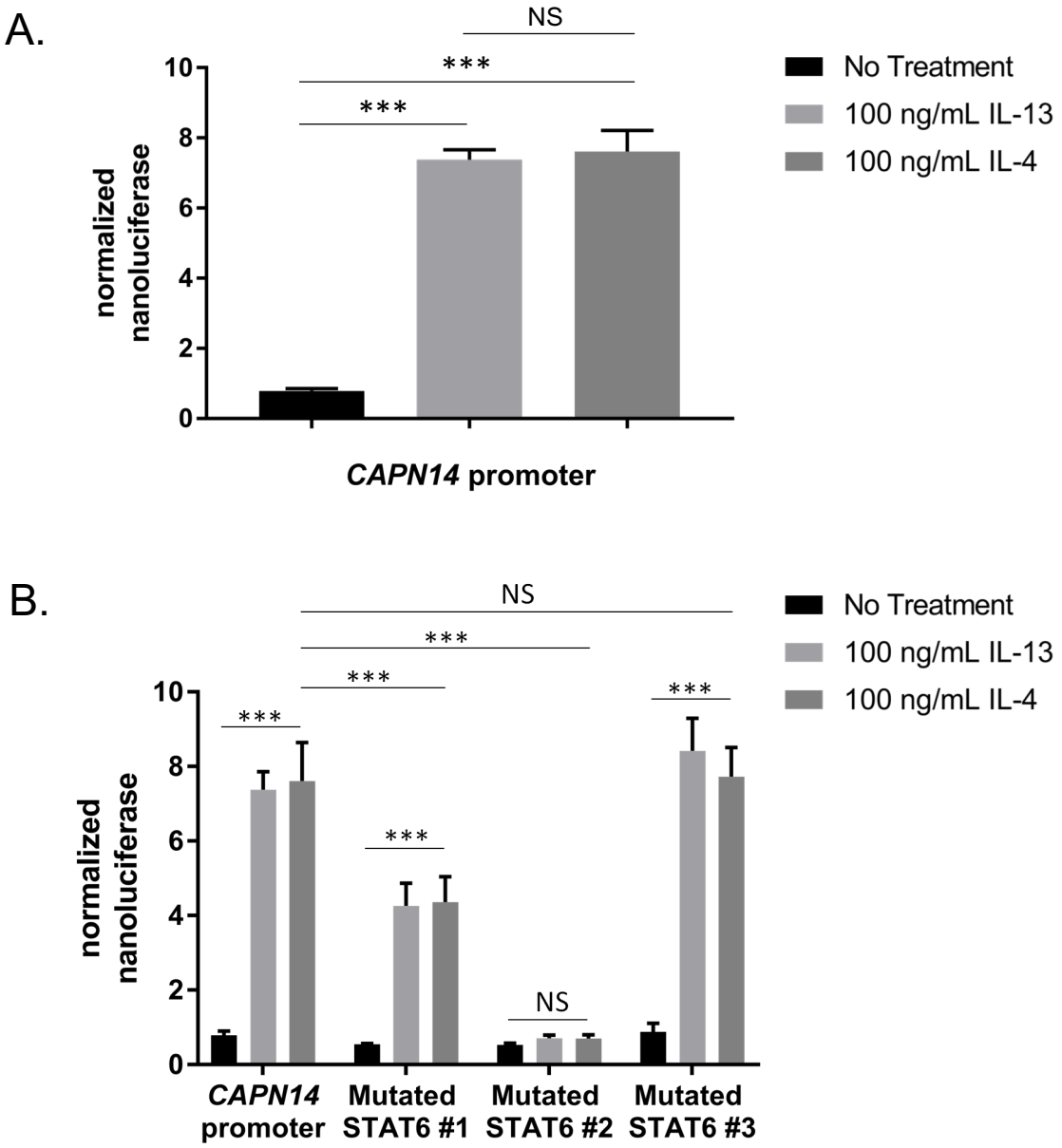


Figure S1. Two STAT6 binding sites in the promoter region of the *CAPN14* gene affect IL-4-induced *CAPN14* promoter activity. *A, B, CAPN14* promoter reporter constructs with or without mutated STAT6 sites (See **Figure 1 A**) were transfected into esophageal epithelial cells followed by treatment with or without IL-4 or IL-13 (100 ng/mL) for 24 h. For each sample, nanoluciferase activity was normalized to firefly luciferase activity. Data are shown as mean ± S.E.M (***, $p < 0.001$; $n = 3$ per group; data representative of three independent experiments).

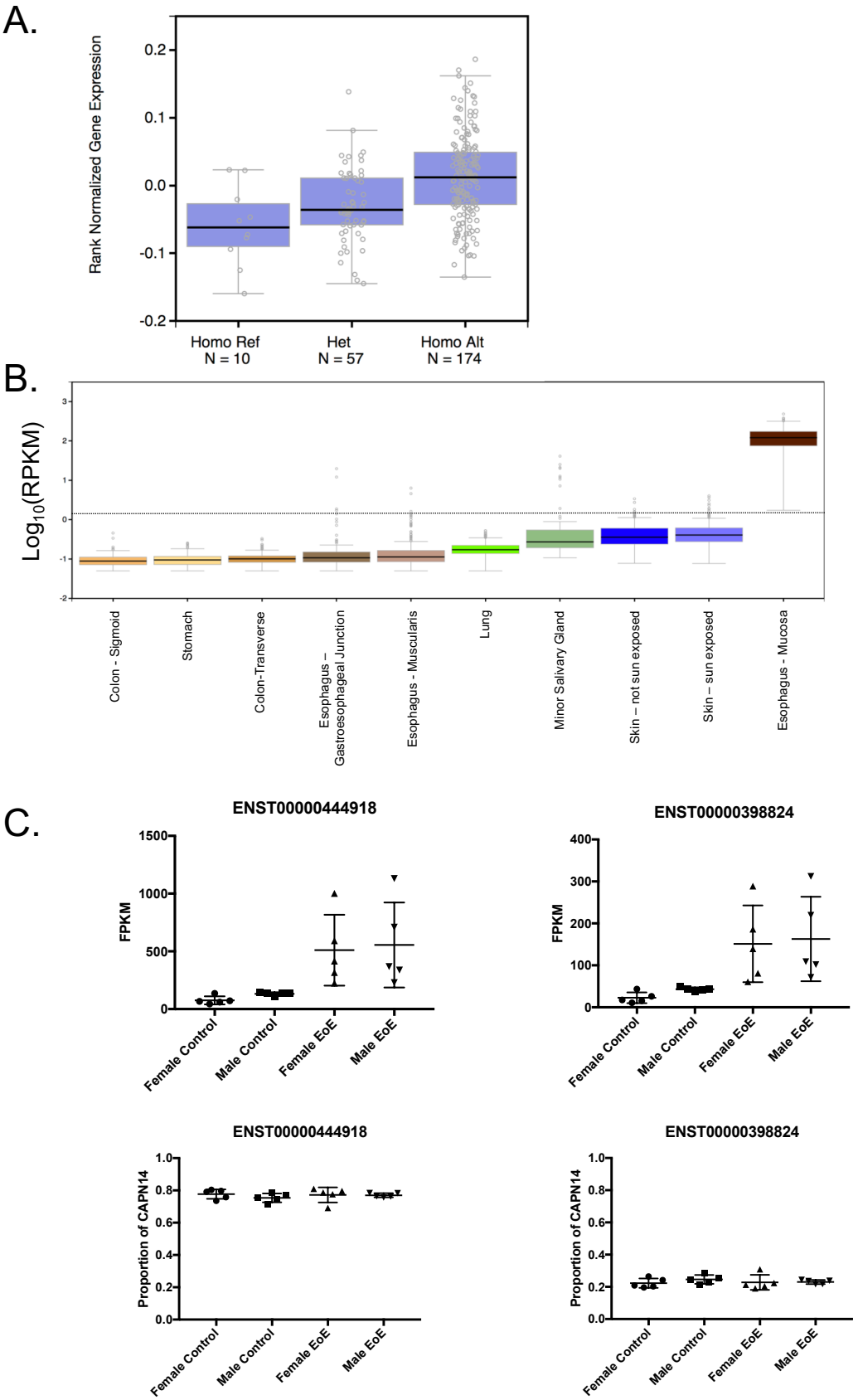
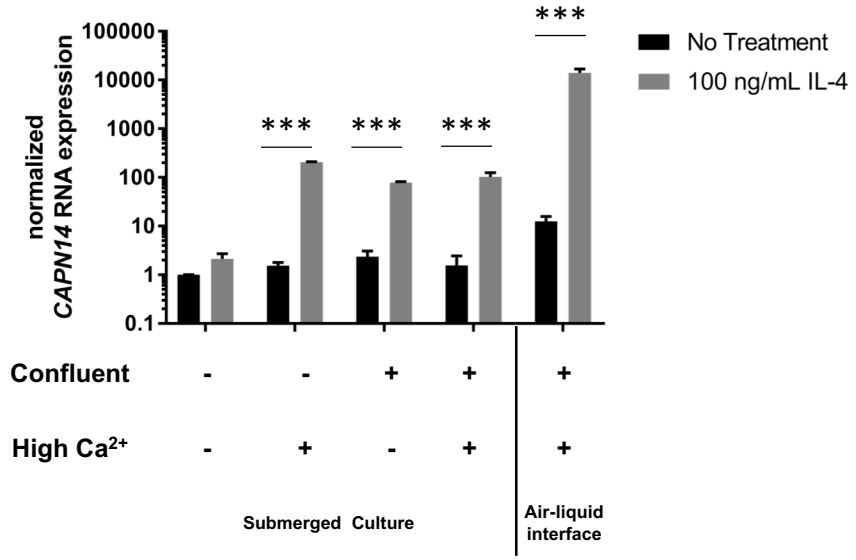


Figure S2. *CAPN14* is expressed in a genotype-dependent manner in the esophageal mucosa of individuals without EoE. *A*, An expression versus genotype box plot shows the association between *CAPN14* expression and the SNP rs207415 in esophageal mucosa tissues. Expression values are z-standardized after preprocessing as described (19, 20). The reference (Ref) genotype is the minor and EoE-risk allele. The alternative (Alt) allele is the more common EoE-non-risk allele. rs207415 is in perfect LD (r^2=1) with rs76562819. *B*, Expression of *CAPN14* in various tissues assayed in the GTEx project. A gene is "expressed" when the transcript has at least 1 read per kilobase per million mapped reads (RPKM) as indicated by the dashed line. Boxes are shown as the median with 25-75% ranges. *C*, Expression of four isoforms of *CAPN14* from an RNAseq study of subjects with (n=10) and without (n=10) EoE.

A. *CAPN14* RNA



B. Calpain-14 Protein

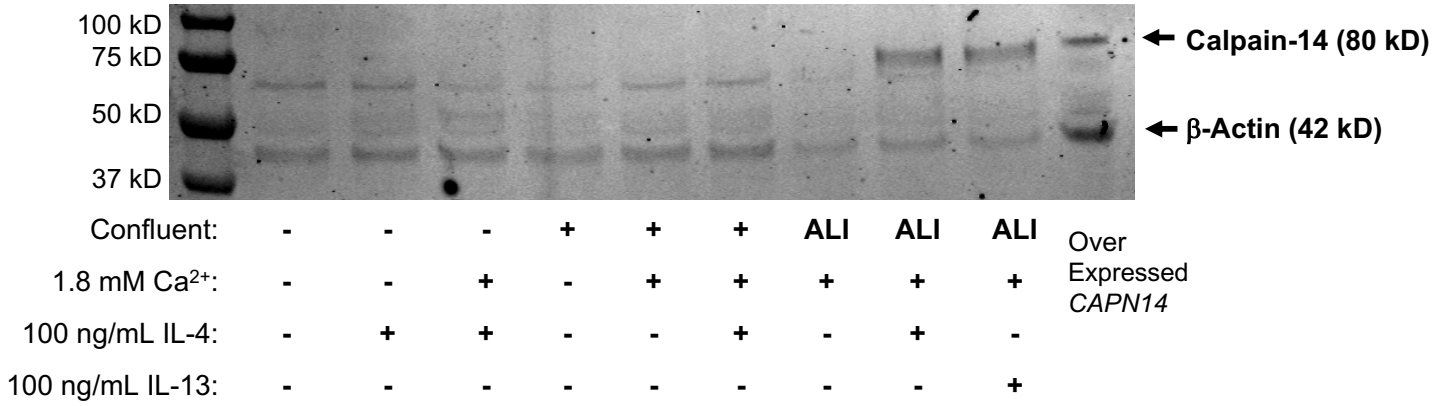


Figure S3. The expression of *CAPN14* in esophageal epithelial cells is also regulated by IL-4. EPC2 esophageal epithelial cells were grown to various levels of confluence (80% or 100%, a + indicates 100% confluence) with relatively low (0.09 mM) or high (1.8 mM) Ca^{2+} and with or without 100 ng/mL IL-4 for 24 h. Cultures were grown either as a monolayer submerged culture or in an air-liquid interface (ALI) setup. *A,* RNA was isolated and *CAPN14* was measured relative to the expression of the housekeeping gene *GAPDH*. All RNA expression values are normalized to cells at 80% confluence without high calcium or IL-4. *B,* Calpain-14 protein expression levels were assessed by immunoblot. EPC2 cells that constitutively express *CAPN14* behind a CMV promoter were used as a positive control for calpain-14 protein expression. RNA and protein level expression: ***, t-test p-value<0.001, n=3-5 per group, data representative of 4 independent experiments.

Table S1. Table of primers and probes

Luciferase Reporter Construct Primers	5' to 3'
CAPN14 PCR Primer Forward	CTActcgagGATTCTGCATGCAATGTGCT
CAPN14 PCR Primer Reverse	GGAagatctGGAAGATGTACCCAGGCTGA
rs76562819 to Non-Reference SDM Primer Forward	GCCTTAATGAGAGAGGGTTAGTCATCTTCTC
rs76562819 to Non-Reference SDM Primer Reverse	GAGAAGATGACTAACCCTCTCTCATTAAGGC
STAT6 Site 1 Scramble SDM Primer Forward	TTTCTCTGTATGGTGCTATGGCATCATAATTGGATTTGTG
STAT6 Site 1 Scramble SDM Primer Reverse	CACAAATCCAATTATGATGCCATAGCACCATACAGAGAAA
STAT6 Site 2 Scramble SDM Primer Forward	GGGTACTCCACTCCCTCCGAACTCACACACAGTGGGGAGG
STAT6 Site 2 Scramble SDM Primer Reverse	CCTCCCCACTGTGTGTGAGTTCGGAGGGAGTGGAGTACCC
STAT6 Site 3 Scramble SDM Primer Forward	ACTCTACCAAATGACACTCAGCATCACCAGAGCTGGAGGG
STAT6 Site 3 Scramble SDM Primer Reverse	CCCTCCAGCTCTGGTGATGCTGAGTGTCATTTGGTAGAGT