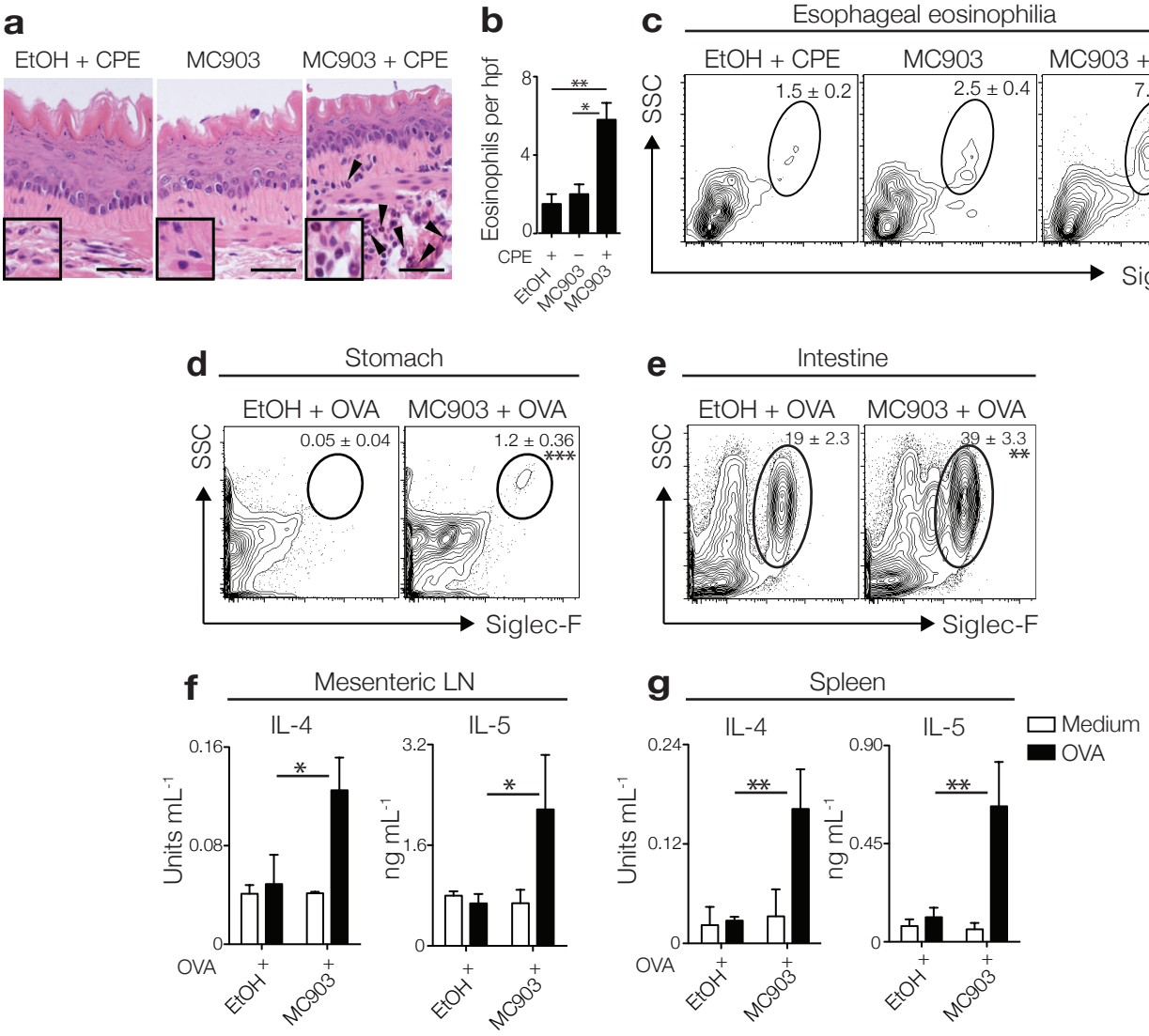
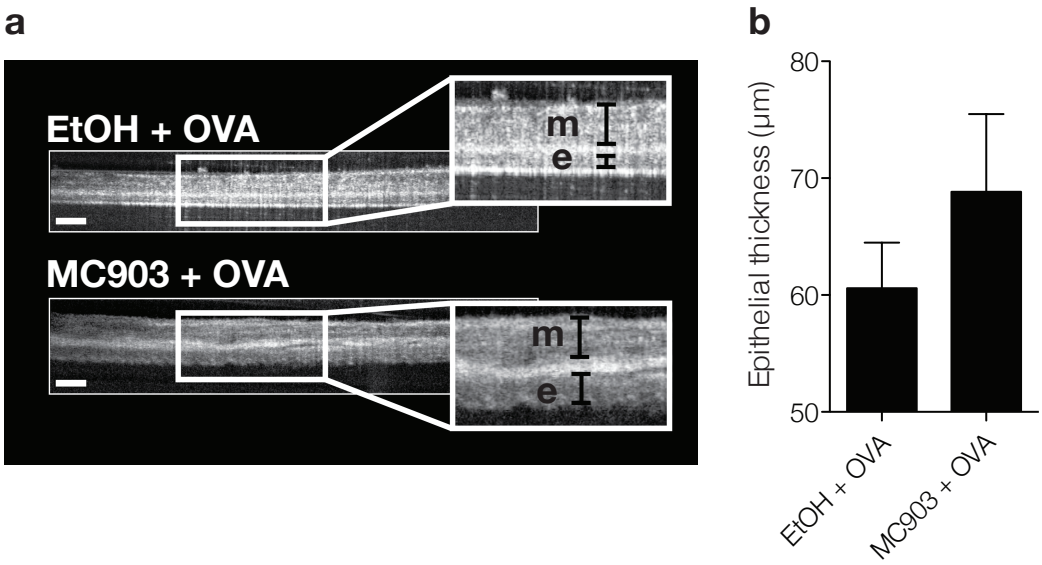
**TSLP-elicited basophil responses can mediate the pathogenesis of eosinophilic esophagitis.**

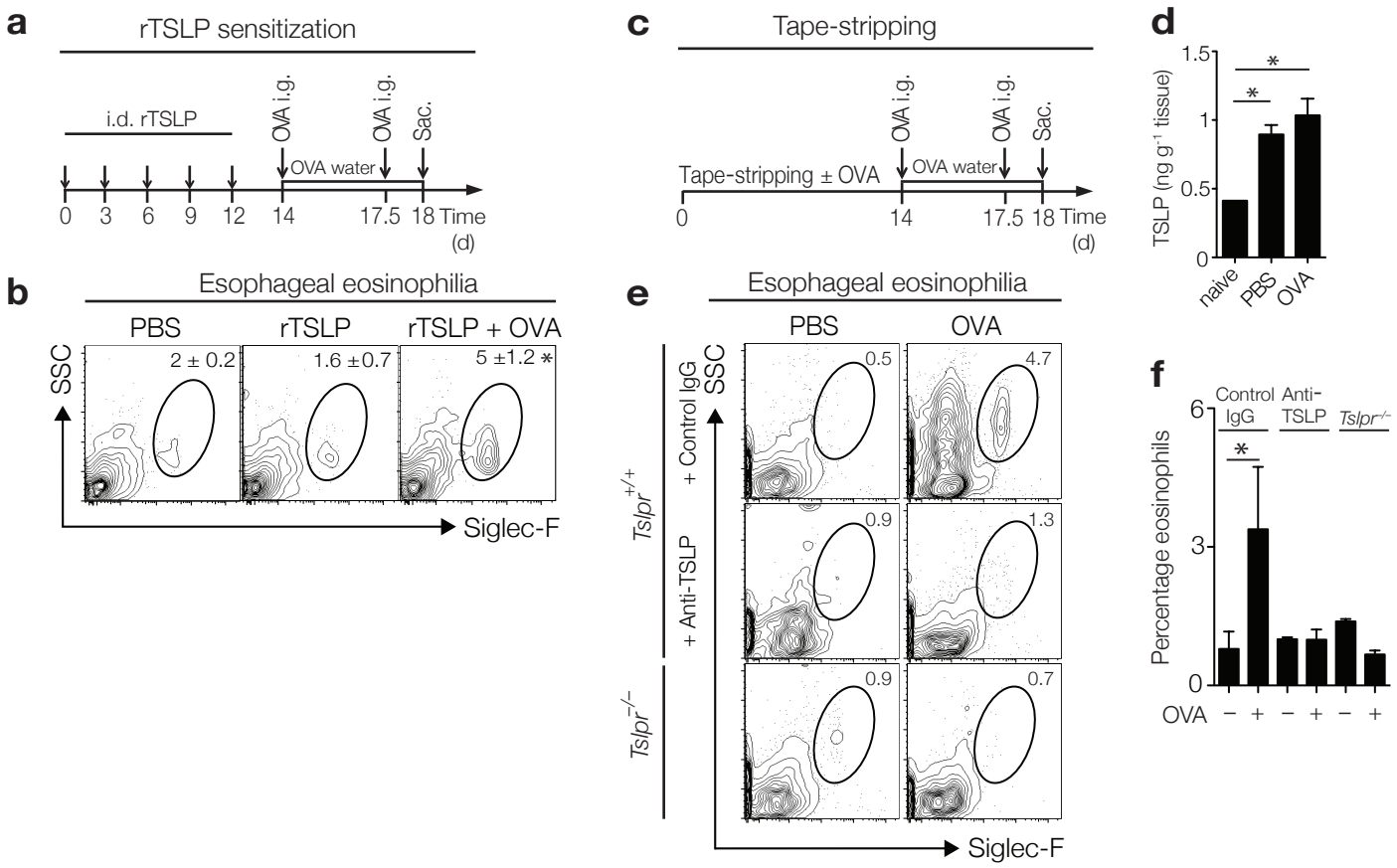
Mario Noti, Elia D. Tait Wojno, Brian S. Kim, Mark C. Siracusa, Paul R. Giacomin, Meera G. Nair, Alain J. Benitez, Kathryn R. Ruymann, Amanda B. Muir, David A. Hill, Kudakwashe R. Chikwava, Amin E. Moghaddam, Quentin J. Sattentau, Aneesh Alex, Chao Zhou, Jennifer H. Yearley, Paul Menard-Katcher, Masato Kubo, Kazushige Obata-Ninomiya, Hajime Karasuyama, Michael R. Comeau, Terri Brown-Whitehorn, Rene de Waal Malefyt, Patrick M. Sleiman, Hakon Hakonarson, Antonella Cianferoni, Gary W. Falk, Mei-Lun Wang, Jonathan M. Spergel, and David Artis



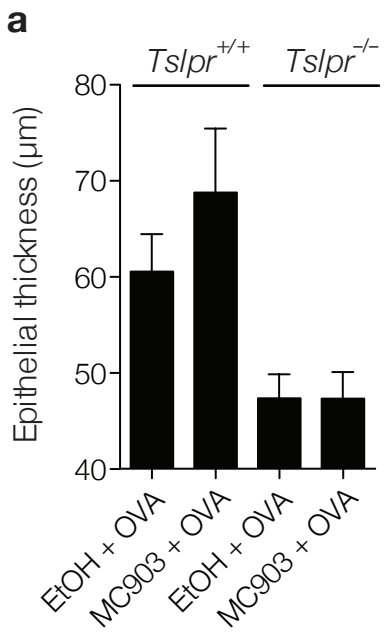

**Supplementary Figure 1** Epicutaneous sensitization with peanut antigen and antigen-induced immune responses in the GI tract. (**a**) Histological sections (H & E staining) from the esophagus of WT BALB/c mice. Arrows identify tissue-infiltrating eosinophils. Scale bar: 25 μm. (**b**) Number of eosinophils per hpf in the esophagus. (**c**) Representative flow cytometry plots showing frequencies of eosinophils in esophageal tissues. Data depicted in (**a–c**) are from one experiment (EtOH + CPE, $n = 3$; MC903, $n = 3$; MC903 + CPE, $n = 4$), and are representative of three independent experiments. Representative flow cytometry plots showing frequencies of eosinophils in (**d**) the stomach and (**e**) small intestine of control (EtOH + OVA) and MC903 + OVA treated WT BALB/c mice. $T_H2$ cytokines in cell-free supernatants of antigen re-stimulated (**f**) mesenteric lymph nodes (LN) and (**g**) splenocytes of control (EtOH + OVA) and MC903 + OVA treated mice as measured by ELISA. Data depicted in (**d–g**) are from one experiment (EtOH + OVA, $n = 3$; MC903 + OVA, $n = 4$), and are representative of three independent experiments. All parameters in (**a–g**) were assessed 12 h post-final oral antigen challenge. All data depicted in (**a–g**) are from mice challenged twice with OVA. Results are shown as mean ± sem, and a non-parametric, two-tailed Mann-Whitney *t*-test or a non-parametric, two-way ANOVA with Bonferroni post-hoc testing were used to determine significance. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.




**Supplementary Figure 2** OCT analysis reveals epithelial thickening in the esophagus of mice with EoE-like disease. (**a**) Representative OCT images of the esophagus of WT BALB/c mice. Scale bar: 200 µm. (**b**) Quantification of epithelial thickness of the esophagus as measured by OCT. Data depicted in (**a,b**) are from two pooled experiments (EtOH + OVA, $n = 7$; MC903 + OVA, $n = 9$). All parameters were assessed 12 h post-final oral antigen challenge. Data depicted are from mice challenged repeatedly with OVA to induce prolonged inflammation. Results are shown as mean ± sem.




**Supplementary Figure 3** Epicutaneous sensitization and oral challenge with a model antigen in the context of elevated TSLP levels results in EoE-like disease. (**a**) Schematic of sensitization in the presence of recombinant TSLP (rTSLP). WT BALB/c mice were injected intradermally (i.d.) on the ears with PBS as control or rTSLP in the presence or absence of OVA. (**b**) Representative flow cytometry plots showing frequencies of eosinophils in esophageal tissues. Data depicted are from one experiment (PBS, $n = 3$; rTSLP, $n = 3$; rTSLP + OVA, $n = 4$), and are representative of three independent experiments. (**c**) Schematic of sensitization on tape-stripped skin. WT BALB/c mice were shaved on the back and sensitized with PBS as control or OVA on tape-stripped skin. (**d**) TSLP expression in cell-free supernatants of overnight-cultured skin (ears) as measured by ELISA. (**e**) Representative flow cytometry plots showing frequencies of eosinophils in esophageal tissues. Data depicted in (**d, e**) are from one experiment experiment (*Tslpr*$^{+/+}$ PBS + IgG, $n = 3$; *Tslpr*$^{+/+}$ OVA + IgG, $n = 3$; *Tslpr*$^{+/+}$ PBS + anti-TSLP mAb, $n = 3$; *Tslpr*$^{+/+}$ OVA + anti-TSLP mAb, $n = 3$; *Tslpr*$^{-/-}$ PBS, $n = 3$; *Tslpr*$^{-/-}$ OVA, $n = 3$), and are representative of three independent experiments. (**f**) Frequencies of eosinophils in esophageal tissues as measured by flow cytometry. Data depicted are from two pooled experiments (*Tslpr*$^{+/+}$ PBS + IgG, $n = 5$; *Tslpr*$^{+/+}$ OVA + IgG, $n = 6$; *Tslpr*$^{+/+}$ PBS + anti-TSLP mAb, $n = 5$; *Tslpr*$^{+/+}$ OVA + anti-TSLP mAb, $n = 7$; *Tslpr*$^{-/-}$ PBS, $n = 5$; *Tslpr*$^{-/-}$ OVA, $n = 6$). All parameters were assessed 12 h post-final oral antigen challenge. Data depicted are from mice challenged twice with OVA. Results are shown as mean ± sem, and a non-parametric, one-way Kruskal-Wallis ANOVA with Dunn's post-hoc testing or a non-parametric, two-way ANOVA with Bonferroni post-hoc testing were used to determine significance. *, $P \leq 0.05$.

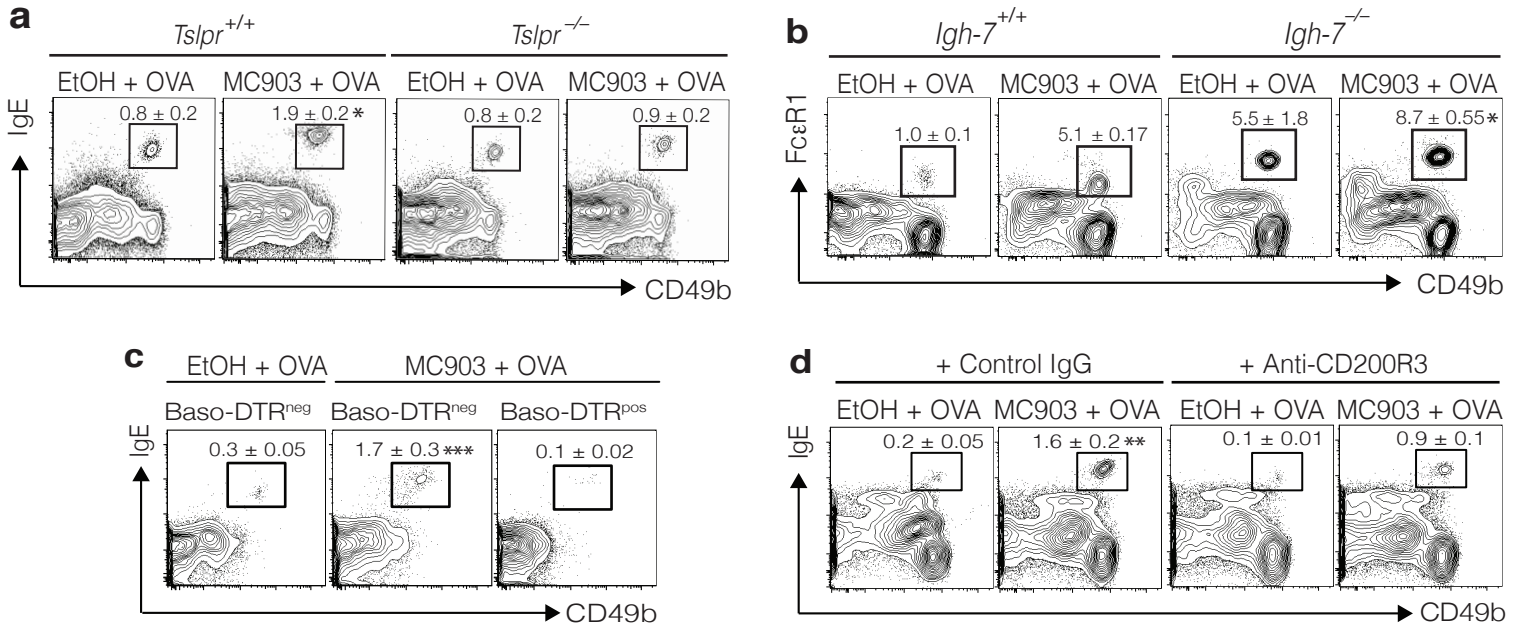



**b**

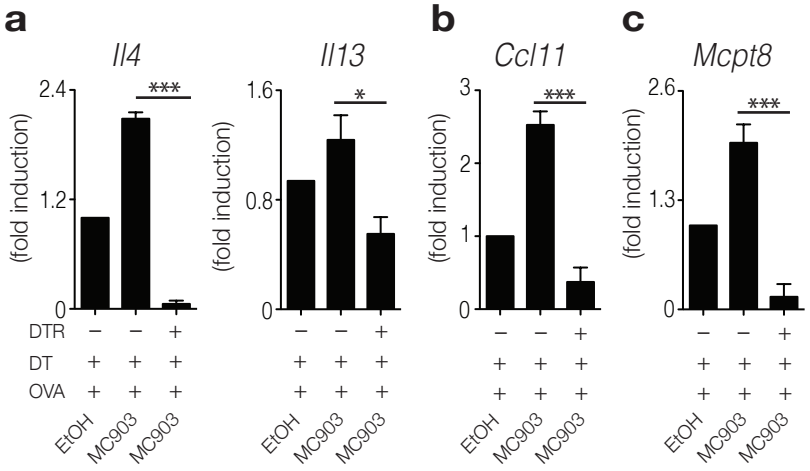
Food impaction

| | *Tslpr*$^{+/+}$ | *Tslpr*$^{-/-}$ |
|---|---|---|
| EtOH + OVA | 0/7 (0%) | 0/6 (0%) |
| MC903 + OVA | 4/14 (29%) | 0/10 (0%) |

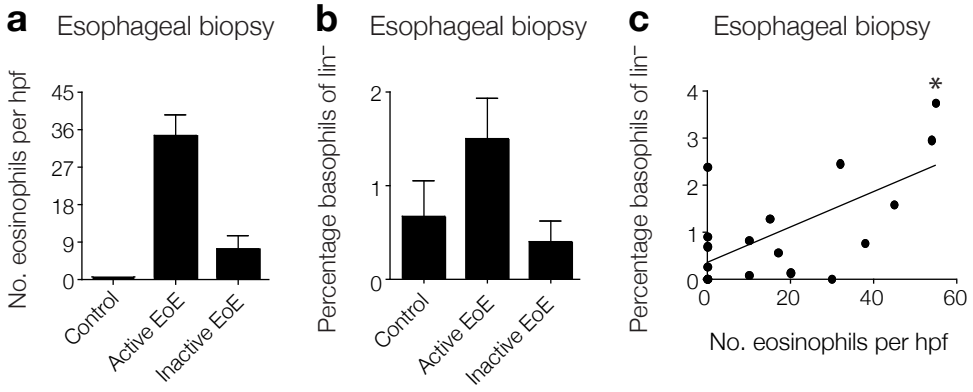
**Supplementary Figure 4** Reduced epithelial thickening of the esophagus and absence of food impaction in *Tslpr*$^{-/-}$ mice. (**a**) Quantification of epithelial thickness of the esophagus of BALB/c *Tslpr*$^{+/+}$ and BALB/c *Tslpr*$^{-/-}$ mice as measured by OCT. Data depicted are from one experiment (EtOH + OVA *Tslpr*$^{+/+}$, $n = 6$; MC903 + OVA *Tslpr*$^{+/+}$, $n = 9$; EtOH + OVA *Tslp*$^{-/-}$, $n = 4$; MC903 + OVA *Tslpr*$^{-/-}$, $n = 6$). (**b**) Table summarizing the incidence of food impaction in the esophagus. Data depicted are from two pooled experiments (EtOH + OVA *Tslpr*$^{+/+}$, $n = 7$; MC903 + OVA *Tslpr*$^{+/+}$, $n = 14$; EtOH + OVA *Tslpr*$^{-/-}$, $n = 6$ ; MC903 + OVA *Tslpr*$^{-/-}$, $n = 10$). All parameters were assessed 12 h post-final oral antigen challenge. Data depicted are from mice challenged repeatedly with OVA to induce extended inflammation. Results are shown as mean ± sem.



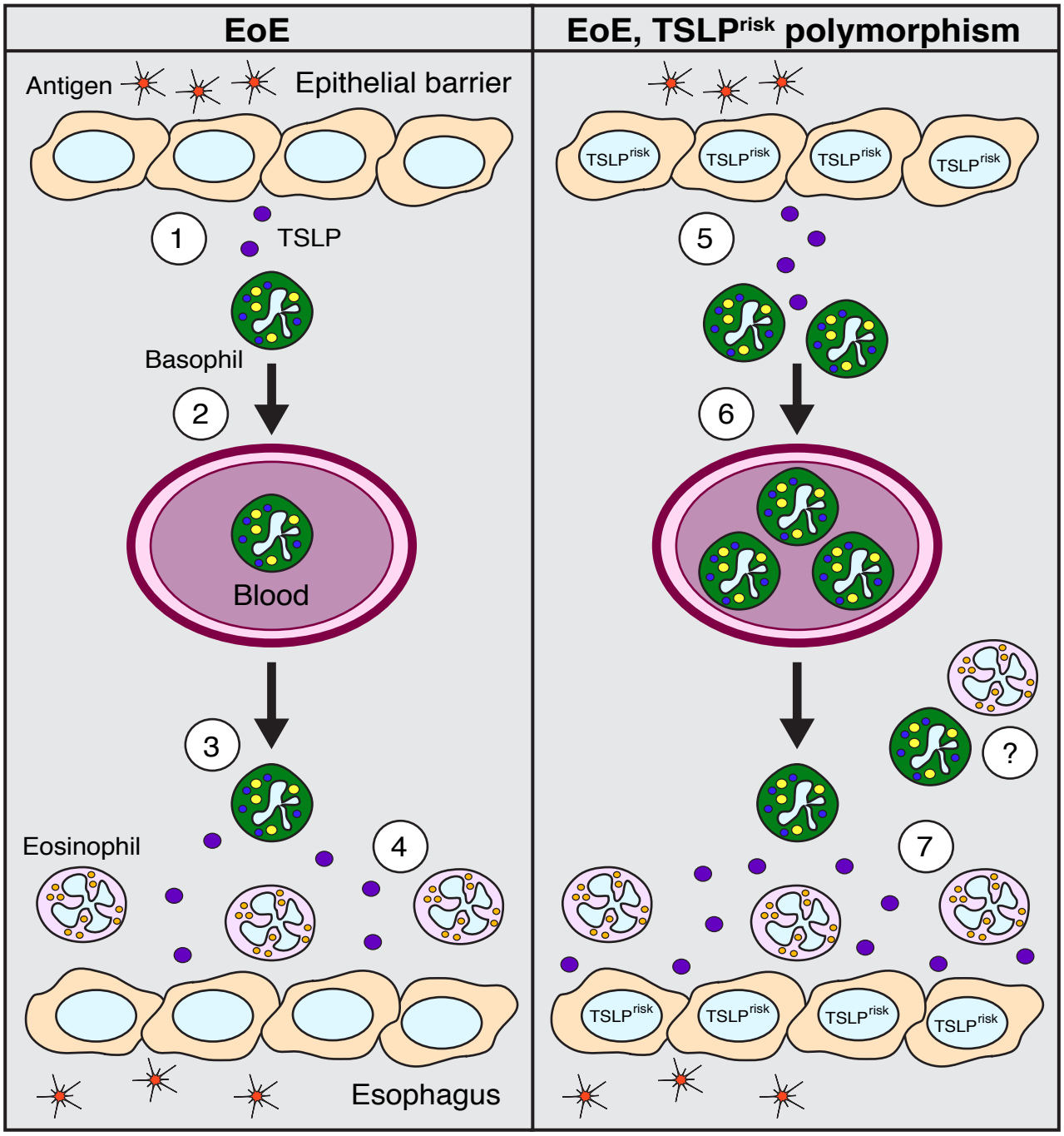

**Supplementary Figure 5** Peripheral basophil responses. (**a**) Representative flow cytometry plots showing frequencies of basophils in the periphery of BALB/c *Tslpr*$^{+/+}$ and BALB/c *Tslpr*$^{-/-}$ mice. Data depicted are from one experiment (EtOH + OVA *Tslpr*$^{+/+}$, $n = 3$; MC903 + OVA *Tslpr*$^{+/+}$, $n = 3$; EtOH + OVA *Tslpr*$^{-/-}$, $n = 3$; MC903 + OVA *Tslpr*$^{-/-}$, $n = 4$), and are representative of three or more independent experiments. For statistical analysis, MC903 + OVA *Tslpr*$^{+/+}$ and MC903 + OVA *Tslpr*$^{-/-}$ are compared. (**b**) Representative flow cytometry plots showing frequencies of basophils in the periphery of BALB/c *Igh-7*$^{+/+}$ and BALB/c *Igh-7*$^{-/-}$ mice. Data depicted are from one experiment (EtOH+OVA *Igh-7*$^{+/+}$, $n=3$; MC903+OVA *Igh-7*$^{+/+}$, $n=3$; EtOH+OVA *Igh-7*$^{-/-}$, $n = 3$; MC903 + OVA *Igh-7*$^{-/-}$, $n = 4$), and are representative of three independent experiments. For statistical analysis, MC903 + OVA *Igh-7*$^{+/+}$ and MC903 + OVA *Igh-7*$^{-/-}$ are compared. (**c**) Representative flow cytometry plots showing frequencies of basophils in the periphery of C57BL/6 Baso-DTR$^{neg}$ and C57BL/6 Baso-DTR$^{pos}$ mice. Data depicted are from one experiment (EtOH + OVA Baso-DTR$^{neg}$, $n = 3$; MC903 + OVA Baso-DTR$^{neg}$, $n = 3$; MC903 + OVA Baso-DTR$^{pos}$, $n = 4$), and are representative of three independent experiments. For statistical analysis, MC903 + OVA Baso-DTR$^{neg}$ and MC903 + OVA Baso-DTR$^{pos}$ are compared. (**d**) Representative flow cytometry plots showing frequencies of basophils in the periphery of control antibody or anti-CD200R3 mAb treated WT BALB/c mice. Data depicted are from one experiment (EtOH + OVA+ IgG, $n = 3$; MC903 + OVA + IgG, $n = 3$; EtOH + OVA + anti-CD200R3 mAb, $n = 3$; MC903 + OVA + anti-CD200R3 mAb, $n = 4$), and are representative of three independent experiments. For statistical analysis, MC903 + OVA + IgG or MC903 + OVA + anti-CD200R3 mAb are compared. All parameters were assessed 12 h post-final oral antigen challenge. All data depicted are from mice challenged twice with OVA. Results are shown as mean ± sem, and a non-parametric, two-way ANOVA with Bonferroni post-hoc testing was used to determine significance. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ .




**Supplementary Figure 6** Reduced inflammatory responses in the esophagus of mice with EoE-like disease depleted of basophils. mRNA expression levels of (**a**) $T_H2$ cytokines (*Il4*, *Il13*), (**b**) *Ccl11*, and (**c**) the basophil-specific protease *Mcpt8* in the esophagus of C57BL/6 Baso-DTR$^{neg}$ and C57BL/6 Baso-DTR$^{pos}$ mice. Data depicted in (**a–c**) are from one experiment (EtOH + OVA Baso-DTR$^{neg}$, $n = 3$; MC903 + OVA Baso-DTR$^{neg}$, $n = 4$; MC903 + OVA Baso-DTR$^{pos}$, $n = 4$), and are representative of two independent experiments. All parameters were assessed 12 h post-final oral antigen challenge. Data depicted are from mice challenged twice with OVA. Results are shown as mean ± sem, and a non-parametric, one-way Kruskal-Wallis ANOVA with Dunn's post-hoc testing was used to determine significance. *, $P \leq 0.05$; ***, $P \leq 0.001$.




**Supplementary Figure 7** Elevated basophil responses in adult subjects with EoE positively correlate with esophageal eosinophil counts. (**a**) Number of eosinophils per hpf in adult esophageal biopsy tissue sections were quantified for control subjects ($n$ = 6), subjects with active EoE ($n$ = 9), and subjects with inactive EoE ($n$ = 3). (**b**) Frequencies of basophils in the lin$^-$ compartment (see Methods) in esophageal biopsies from control subjects ($n$ = 6), active subjects with EoE ($n$ = 9), and inactive subjects with EoE ($n$ = 3). (**c**) Correlation of frequencies of basophils in the lin$^-$ compartment in adult esophageal biopsies and the number of eosinophils per hpf observed histologically ($n$ = 18) (Spearman r = 0.5282). Data are shown as mean ± sem. Correlation analysis was performed using a non-parametric Spearman correlation (sensitivity analyses were performed), and a linear regression of the data is displayed. *, $P \leq 0.05$.




**Supplementary Figure 8** Proposed model of the relationship between a gain-of-function *TSLP* polymorphism (TSLP$^{risk}$), peripheral basophil responses, and the development of EoE in humans. In humans that do not carry the TSLP$^{risk}$ polymorphism, exposure to antigens at epithelial barriers may induce TSLP expression which can result in local (**1**) and systemic (**2**) TSLP-elicited basophil responses. Encounter with the antigen in the esophagus may promote additional TSLP expression and mobilization of TSLP-elicited basophil populations from the blood to esophageal tissue (**3**). The studies presented here suggest that TSLP-elicited basophils and their products contribute to esophageal inflammation, including the accumulation of eosinophils, and other immune cells, such as T cells, B cells, and mast cells (not depicted) (**4**). In humans that carry the TSLP$^{risk}$ polymorphism, exposure to antigens at epithelial barriers is more likely to result in enhanced TSLP expression (**5**) associated with TSLP-elicited peripheral basophilia (**6**), which together increase the likelihood of, but are not required for, developing EoE in the context of antigen-induced TSLP over-expression in the esophagus (**7**). It is currently unknown whether the TSLP$^{risk}$ polymorphism and peripheral basophilia also promote increased accumulation of basophils and eosinophils in the esophagus in the context of EoE.