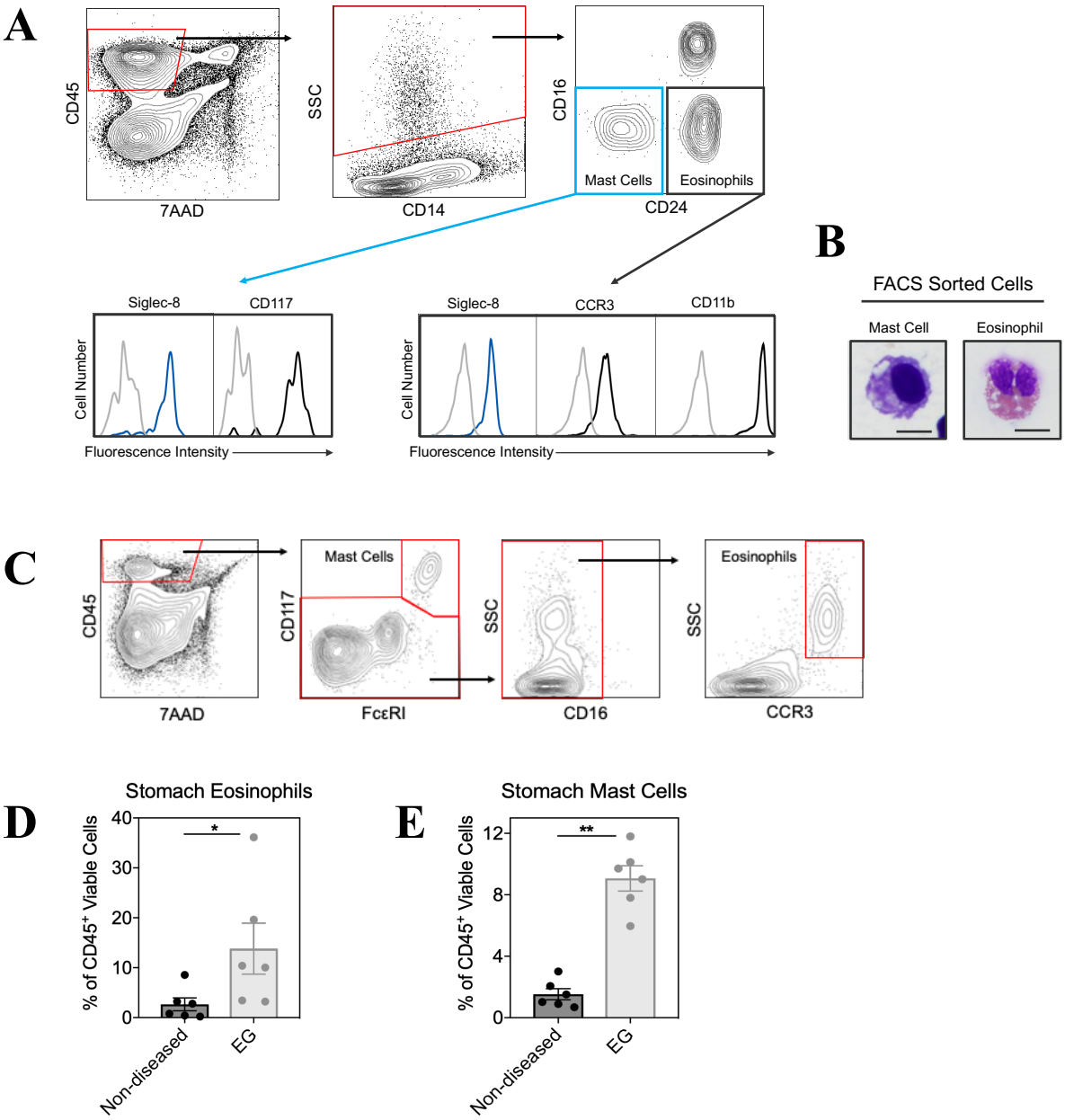
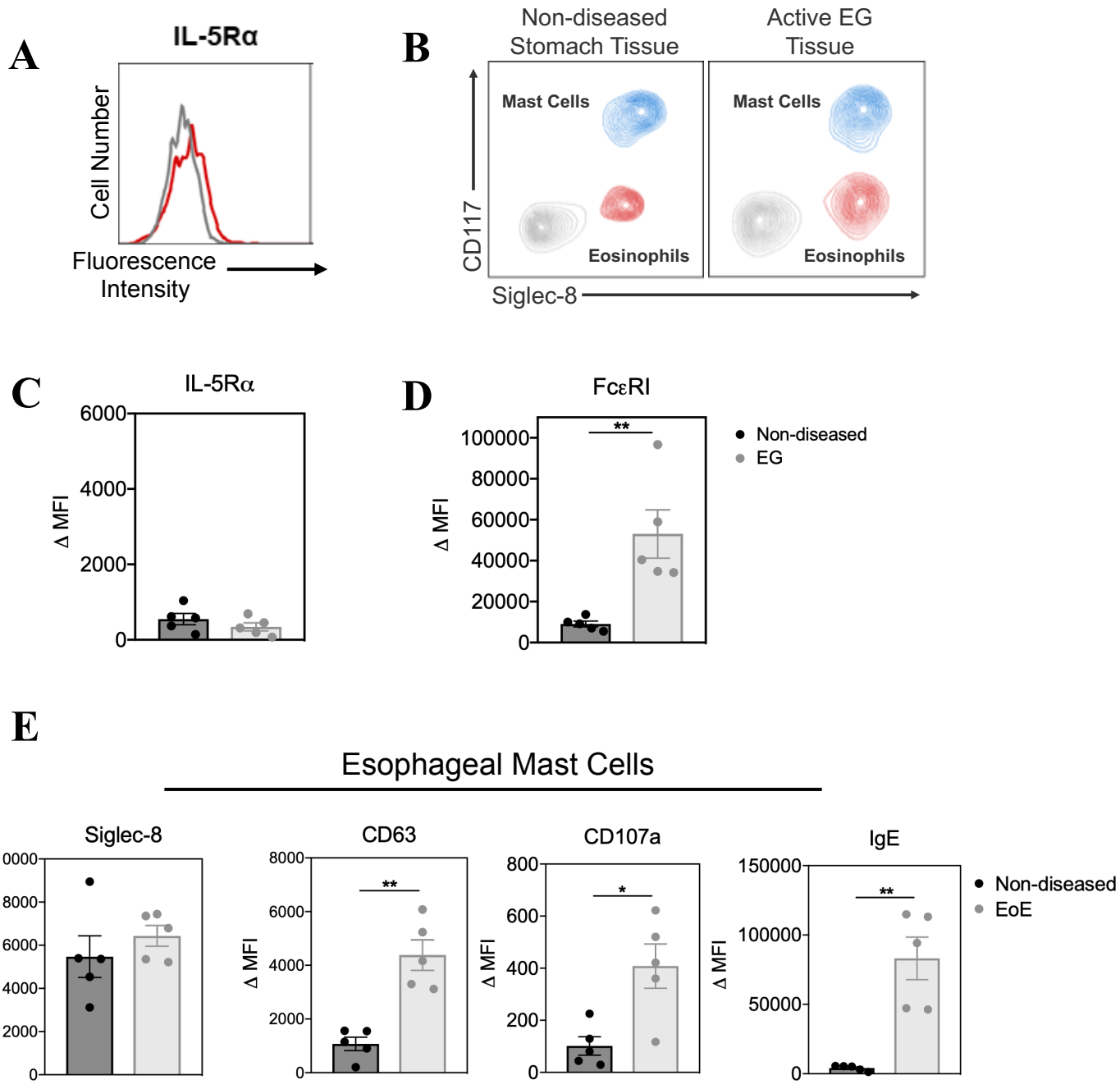
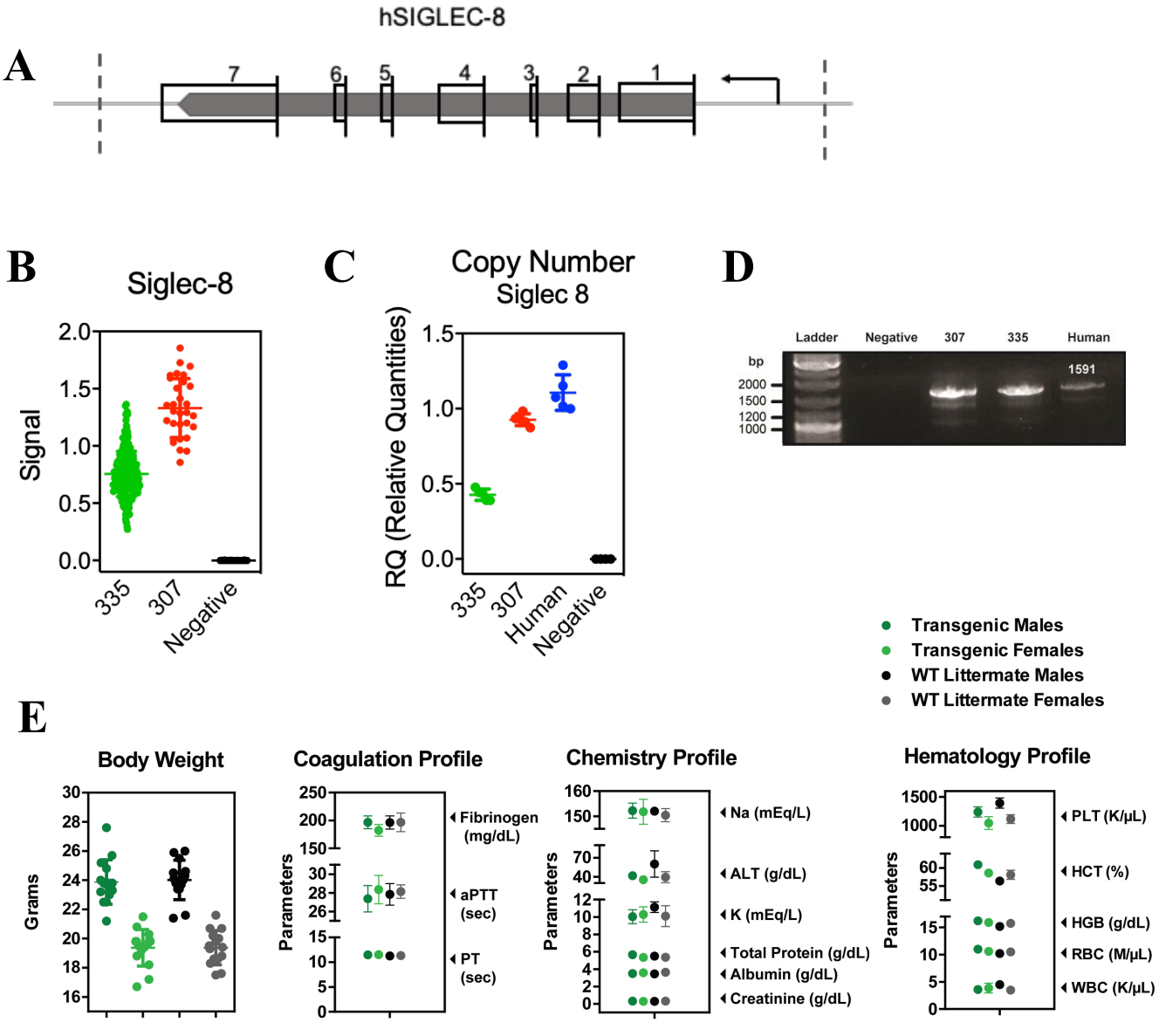


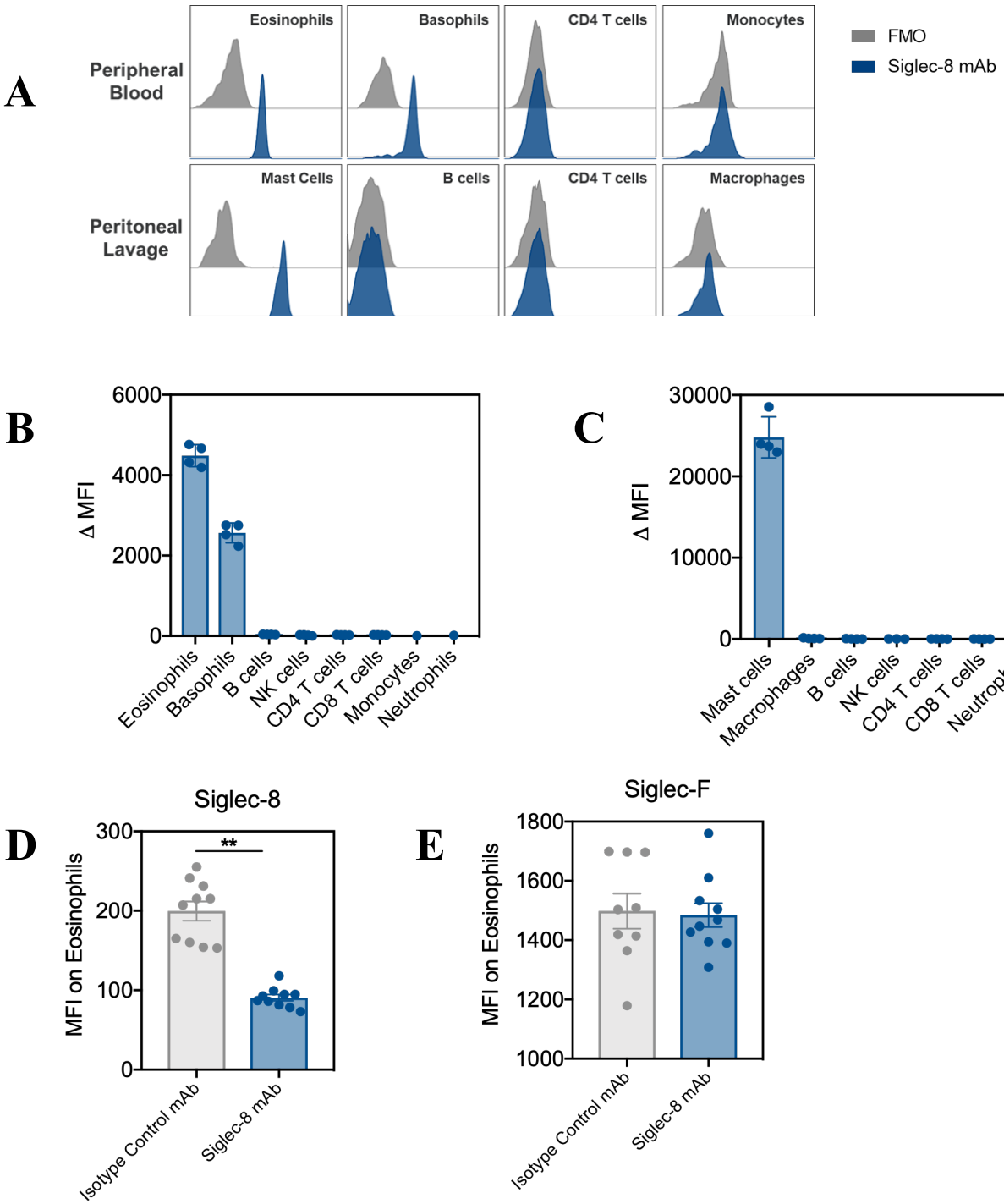
Supplemental Figure 1: EG patient tissue has significantly increased eosinophils and mast cells compared to non-diseased control tissue. (A) Flow cytometry gating strategy used in Figure 1A to identify mast cells ($CD45^+$ $7AAD^-$ SSC^{Hi} $CD16^-$ $CD24^-$) and eosinophils ($CD45^+$ $7AAD^-$ SSC^{Hi} $CD16^-$ $CD24^+$) in human GI tissue. Histogram of gated mast cells (blue) labeled for analysis of surface expression of Siglec-8 and CD117 or an FMO negative control (gray). Histogram of gated eosinophils (black) labeled for analysis of surface expression of Siglec-8, CCR3, and CD11b or an FMO negative control (gray). (B) Representative images of FACS-sorted mast cells stained with May-Grunwald Giemsa and eosinophils stained with H&E from their respective windows (40x magnification; scale bar is 10μM). (C) Alternative flow cytometry gating strategy used to identify mast cells ($CD45^+$ $7AAD^-$ $CD117^+$ $FcεRI^+$) and eosinophils ($CD45^+$ $7AAD^-$ $CD16^-$ SSC^{Hi} $CCR3^+$) in human GI tissue. Percentage of (D) eosinophils or (E) mast cells present in non-diseased (black) or EG (gray) tissue identified using the gating strategy in panel C. Data are plotted as mean ± SEM for n=7 non-diseased stomach tissue; n=2 EG, n=4 EG+EoE. *P<0.05, ** P<0.01 by Mann Whitney U test.



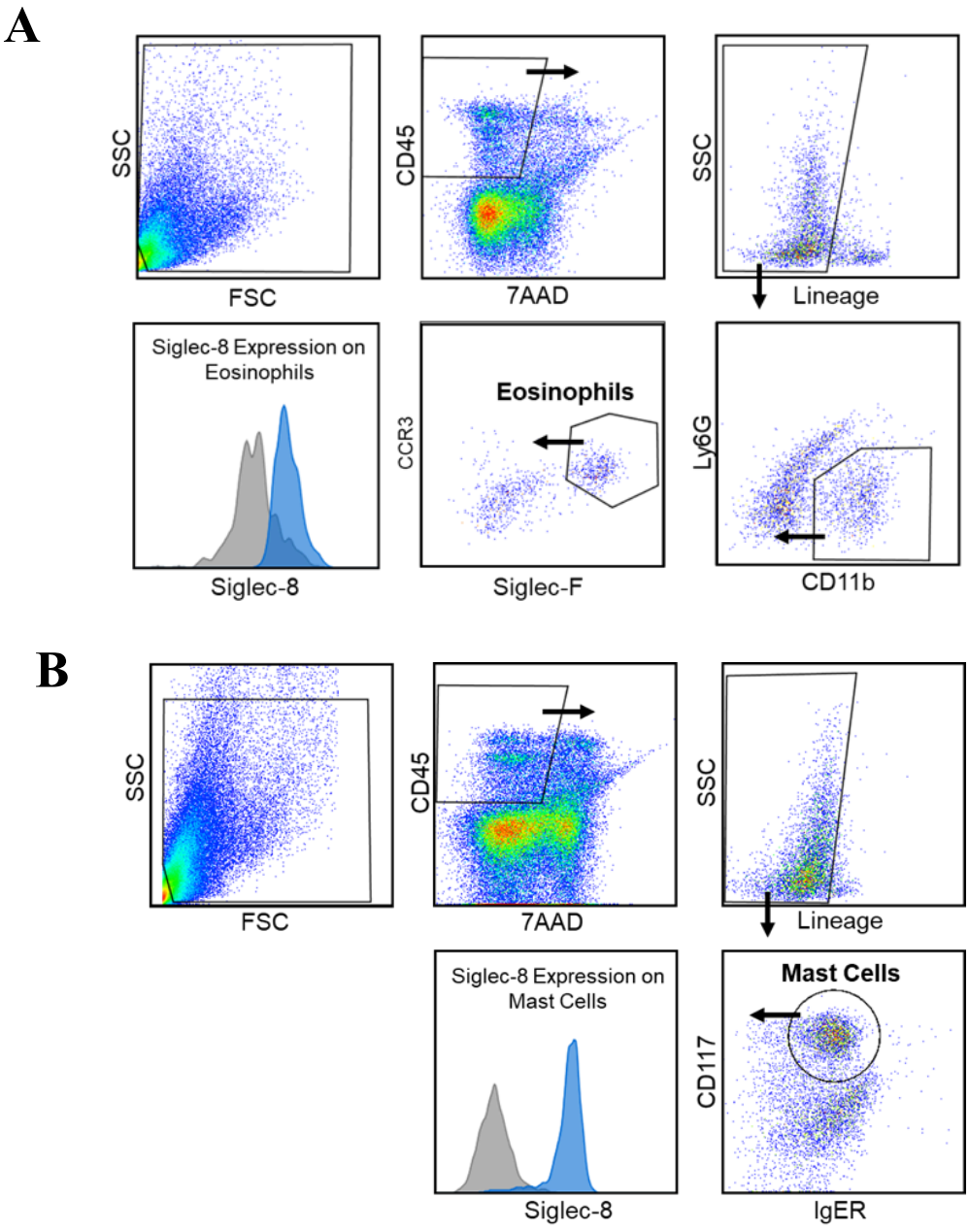
Supplemental Figure 2: Siglec-8 is selectively expressed on mast cells and eosinophils in human stomach tissue and mast cells from EoE patient tissue are highly activated. (A) Histogram of EG mast cells stained with either IL-5Rα or an FMO negative control (gray). (B) Flow cytometry dot plots of stomach eosinophils (red) and mast cells (blue) from (left) non-diseased control or (right) EG patient tissue stained with a Siglec-8 mAb. Mast cells and eosinophils were identified by flow cytometry as described in Supplemental Figure 1, C. (C and D) Expression as shown by ΔMFI of IL-5Rα and FcεRI on stomach mast cells from non-diseased controls (black) or EG patients (gray). (E) Expression as shown by ΔMFI of Siglec-8, CD63, CD107a, and IgE on esophageal mast cells from non-diseased controls (black) or EoE patients (gray). Data are plotted as mean ± SEM for n=7 non-diseased stomach tissue and n=4 non-diseased esophageal tissue; n=2 EG, n=3 EG+EoE, and n=2 EoE patients. *P<0.05, ** P<0.01 by Mann Whitney U test.



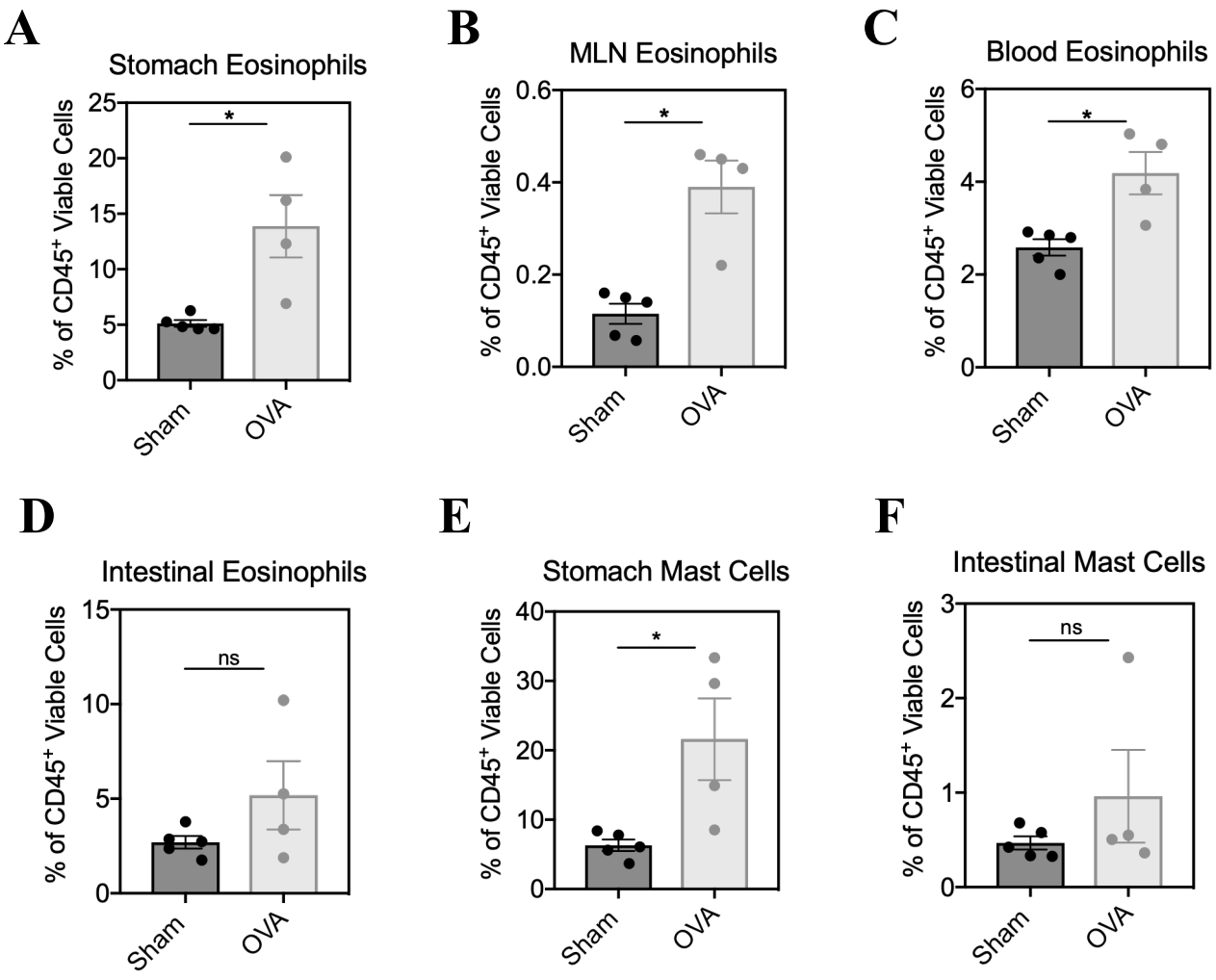
Supplemental Figure 3: Genotyping and Phenotyping of Siglec-8 Transgenic Mice. (A) Schematic of the human Siglec-8 DNA fragment used to generate Siglec-8 transgenic mice. Arrow represents the native putative promoter. (B) Tail biopsy genotyping of Siglec-8 transgenic mice or non-transgenic WT littermates by real-time PCR with specific probes designed to detect the hSiglec-8 gene. Siglec-8 raw signal data for each of the progeny derived from Siglec-8 transgenic lines 335 (n=263), 307 (n=30), and non-transgenic WT littermates (negative, n=228) are plotted. (C) Siglec-8 gDNA copy number evaluation in tail biopsies using qPCR. The presence of Siglec-8 DNA was quantified by qPCR and human values were defined as a relative quantity of 1. Values of 0.5 are consistent with 1 copy per genome. (D) cDNA from two Siglec-8 transgenic murine lines, littermate controls (negative), or a normal human donor were generated from RNA extracted from mouse or human blood. The expected 1591 bp mRNA product supports the presence of the full-length Siglec-8 coding sequence. (E) Body weights of Siglec-8 transgenic mice or WT littermates at 11 weeks of age from each sex were evaluated (n=15/group). Age-matched Siglec-8 transgenic mice or WT littermates were evaluated for fibrinogen, PTT, or PT in plasma (n=5/group). Age matched Siglec-8 transgenic mice or WT littermates were evaluated for serum chemistry for Na, ALT, K, total protein, albumin, and creatinine in serum (n=5/group). Anticoagulated blood from age-matched Siglec-8 transgenic mice or WT littermates were evaluated for PLT count, HCT, HGB, RBC count, and WBC count.



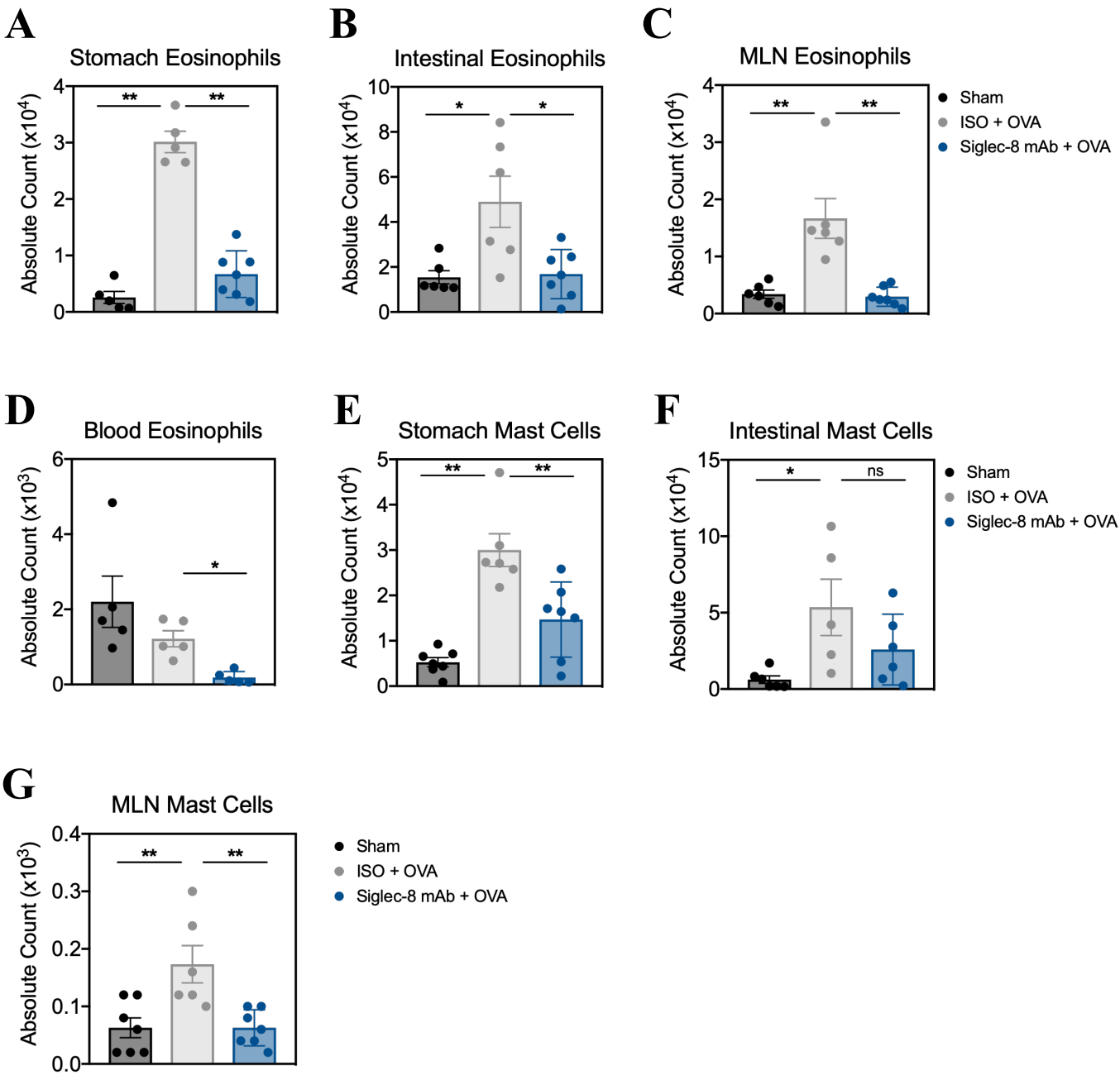
Supplemental Figure 4: Siglec-8 expression is restricted to eosinophils, mast cells, and basophils in Siglec-8 transgenic mice. (A) Immune cells in PBL and PL were identified by flow cytometry. Representative histograms for the immune cells stained with either an anti-Siglec-8 mAb (blue) or isotype control mAb (gray). Siglec-8 expression plotted as Δ MFI on (B) PBL or (C) PL from panel A (mean ± SD n=4 mice). (D) Siglec-8 and (E) Siglec-F MFI on blood eosinophils in Siglec-8 transgenic mice dosed with either an isotype control mAb (gray) or anti-Siglec-8 mAb (blue). Graphs are plotted as mean ± SEM (n= 9-10 mice/group). *P<0.05, ** P<0.01 by Mann Whitney U test.



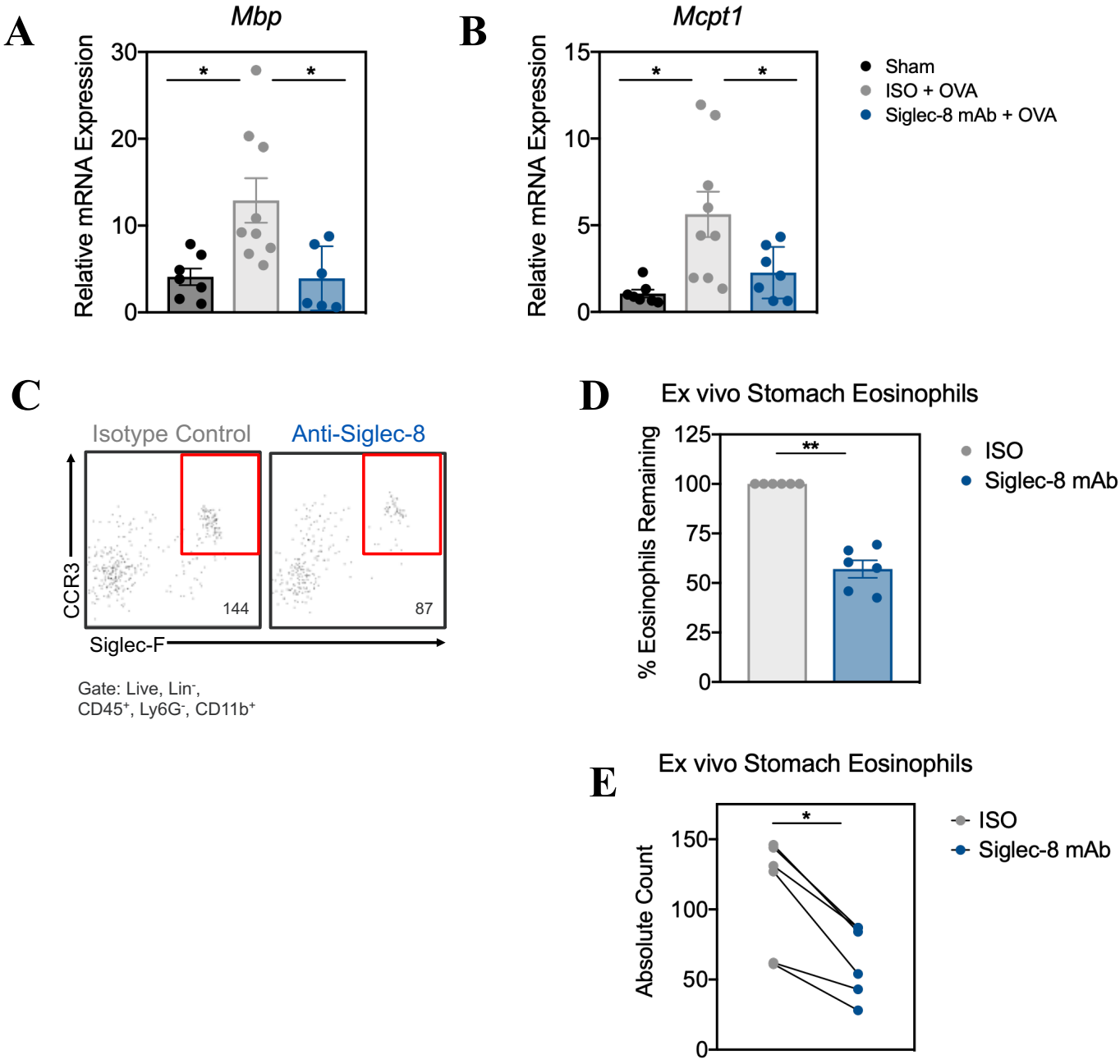
Supplemental Figure 5. Gating strategy for murine eosinophils and mast cells in stomach tissue. (A) Eosinophils were identified as $CD45^+$ $7AAD^-$ $Lineage^-$ $CD11b^+$ $Siglec\text{-}F^+$ $CCR3^+$. Histogram of Siglec-8 (blue) or FMO control (gray) MFI on eosinophils (B) Mast cells were identified as $CD45^+$ $7AAD^-$ Lineage- $IgER^+$ $CD117^+$. Histogram of Siglec-8 (blue) or FMO control (gray) MFI on mast cells. Lineage markers: CD3, CD4, CD8, CD19, TER119, CD5.



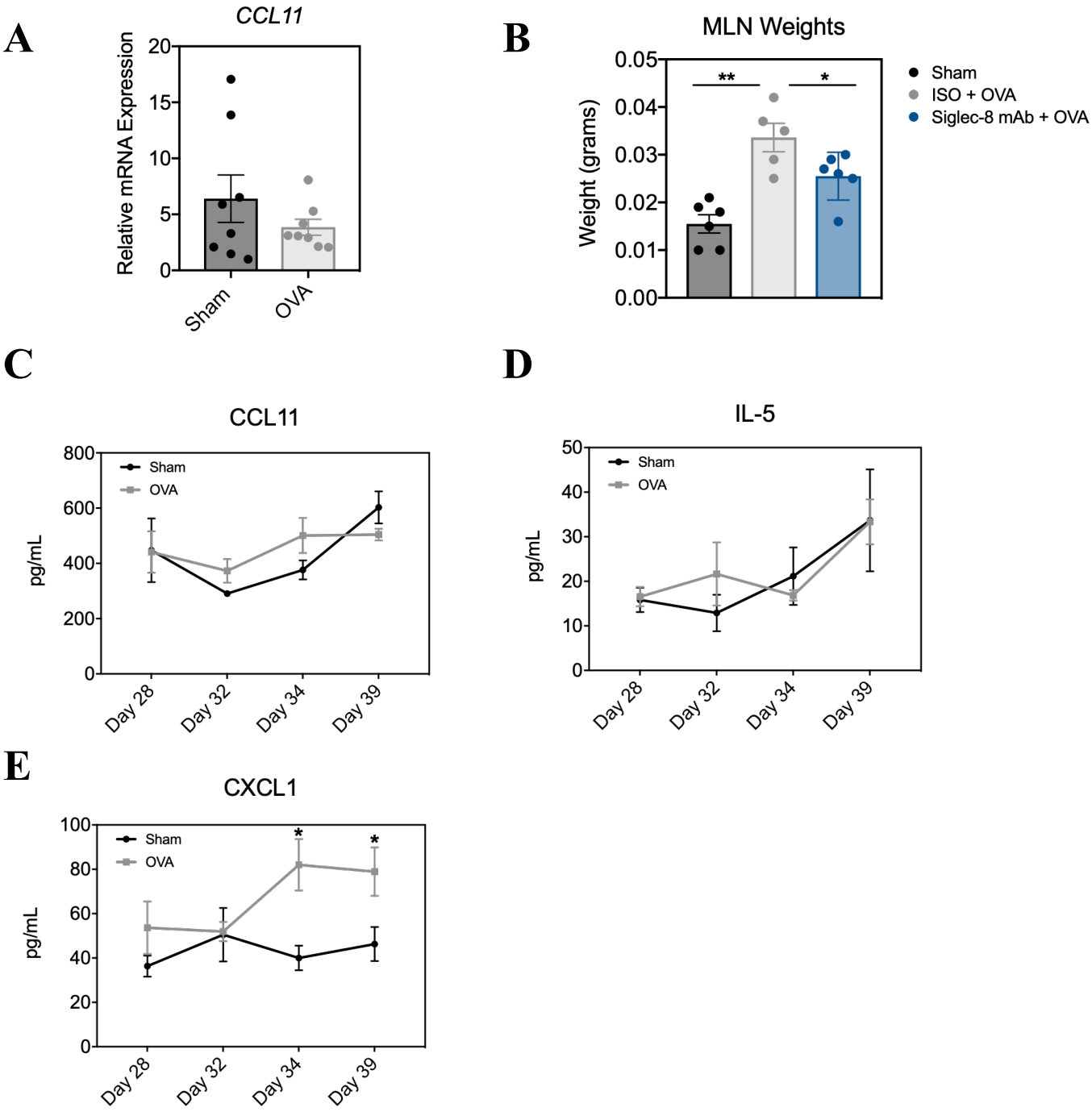
Supplemental Figure 6. Siglec-8 transgenic mice sensitized and intragastrically challenged with OVA have elevated stomach eosinophils and mast cells on day 32. Percentage of eosinophils on day 32 in the (A) stomach, (B) MLN, (C) peripheral blood and (D) duodenum in sham-treated mice (black) or mice sensitized and challenged with OVA (gray). Percentage of mast cells on day 32 in the (E) stomach and (F) duodenum in sham mice (black) or mice sensitized and challenged with OVA (gray). The percentage of mast cells and eosinophils are derived from the $CD45^+$, viable cell population. Data are plotted as mean ± SEM (n=4-5mice/ group). *P<0.05, ** P<0.01 by Mann Whitney U test.



Supplemental Figure 7. Administration of an anti-Siglec-8 mAb reduces eosinophil and mast cell numbers in gastrointestinal tissues in mice with EG and EGE. The absolute counts of eosinophils per tissue on day 39 in the (A) stomach, (B) small intestine, (C) MLN and (D) peripheral blood quantified by flow cytometry in sham mice (black) or mice sensitized and challenged with OVA and dosed with either an isotype control mAb (gray) or anti-Siglec-8 mAb (blue). The absolute counts of mast cells per tissue on day 39 in the (E) stomach, (F) duodenum, and (G) MLN quantified by flow cytometry in sham mice (black) or mice sensitized and challenged with OVA and dosed with either an isotype control mAb (gray) or anti-Siglec-8 mAb (blue). Data are plotted as mean ± SEM (n= 6-7 mice/group) and are representative of 3 experiments. *P<0.05; **P<0.01 by one-way ANOVA with Tukey's multiple comparisons test.



Supplemental Figure 8. Siglec-8 mAb treated mice display reduced expression of mast cell and eosinophil specific genes in intestinal tissue and Siglec-8 mAb directly decreased stomach eosinophils ex vivo. qPCR based gene expression of (A) *Mbp* and (B) *Mcpt1* in the duodenum on day 39 of in sham mice (black) or mice sensitized and challenged with OVA and dosed with either an isotype control mAb (gray) or anti-Siglec-8 mAb (blue) (mean ± SEM; n= 6-8 mice/group). (C) Representative dot plots of murine stomach eosinophils after overnight treatment with an isotype control-mAb (gray) or anti-Siglec-8 mAb (blue). (D) The percentage of stomach eosinophils remaining or (E) absolute eosinophil counts after overnight treatment with either 1µg/mL isotype control mAb (gray) or anti-Siglec-8 mAb (blue) in ex vivo stomach tissue (mean ± SEM; n= 6 mice/group). **P*<0.05; ***P*<0.01 by one-way ANOVA with Tukey's multiple comparisons test (A and B) or by Mann Whitney U test (D and E). The percentage of eosinophils remaining was calculated by normalizing the number of eosinophils in the isotype control-treated group to 100 percent.



Supplemental Figure 9. Siglec-8 mAb treated mice display reduced expression of OVA-induced type 2 immune-associated inflammatory cytokines and chemokines in serum. (A) qPCR-based gene expression of *CCL11* in the duodenum on day 39 of in sham mice (black) or mice sensitized and challenged with OVA (gray). (B) MLN weights on day 39. (C, D, and E) CCL11, IL-5, and CXCL1 levels in serum in sham (black) or OVA (gray) treated mice on day 28 (before first OVA challenge) and days 32, 34, and 39. (n=6-7 mice/group). Graphs are plotted as mean ± SEM (n= 6-8 mice/group) *P<0.05; **P<0.01 by one-way ANOVA with Tukey's multiple comparisons test (B) or two-tailed t test with Sidak's post-test (C-E).

Subject/Disease	Tissue	GI Disease
EG 1	Stomach	EGID
EG 2	Stomach	EGID
EG 3	Stomach	EGID
EoE/EG 4	Eso/Stomach	EGID
EoE/EG 5	Eso/Stomach	EGID
EoE/EG 6	Eso/Stomach	EGID
EoE 7	Esophagus	EGID
EoE 8	Esophagus	EGID
EoE 9	Esophagus	EGID
Control 1	Stomach	Normal
Control 2	Stomach	Normal
Control 3	Stomach	Normal
Control 4	Stomach	Normal
Control 5	Stomach	Normal
Control 6	Stomach	Normal
Control 7	Stomach	Normal
Control 8	Esophagus	Normal
Control 9	Esophagus	Normal
Control 10	Esophagus	Normal
Control 11	Esophagus	Normal

Supplemental Table 1. EGID patients and non-diseased, normal tissue subjects.

EG, eosinophilic gastritis

EoE, eosinophilic esophagitis

EGID, eosinophilic gastrointestinal disease