Supplemental Data

Supplemental Methods

Enterochromaffin cell analysis

Colon tissues were isolated from *Tph1*^{CFP} mice and were opened longitudinally. The intestinal epithelial cells were harvested after the tissues were incubated at 37 °C for 20 min in PBS media containing 5 mM EDTA and 1 mM DTT. The isolated epithelial cells were stained with anti-EpCam, anti-ST2 (Biolegend) and viability dye PI (BD) and analyzed by flow cytometry.

IL-33 treatment on platelets

IL-33 was added in the purified platelets at final concentration of 100ng/ml and incubated at room temperature for 10 mins. Then 0.05U/ml thrombin (Sigma) was added together with the antibodies and incubated at room temperature for 15 mins. PBS was added to stop the reaction and the platelets were analyzed by FACS within 30 mins. All flow cytometry data were acquired on a BD X-20 cell analyzer and analyzed with FlowJo software.

5-HTP supplement

The Il33–/– mice were given 1.5mg/ml 5-HTP (Sigma) in drinking water for 2 weeks. Then the mice were tested for bleeding assay or platelet analysis.

Wound histology

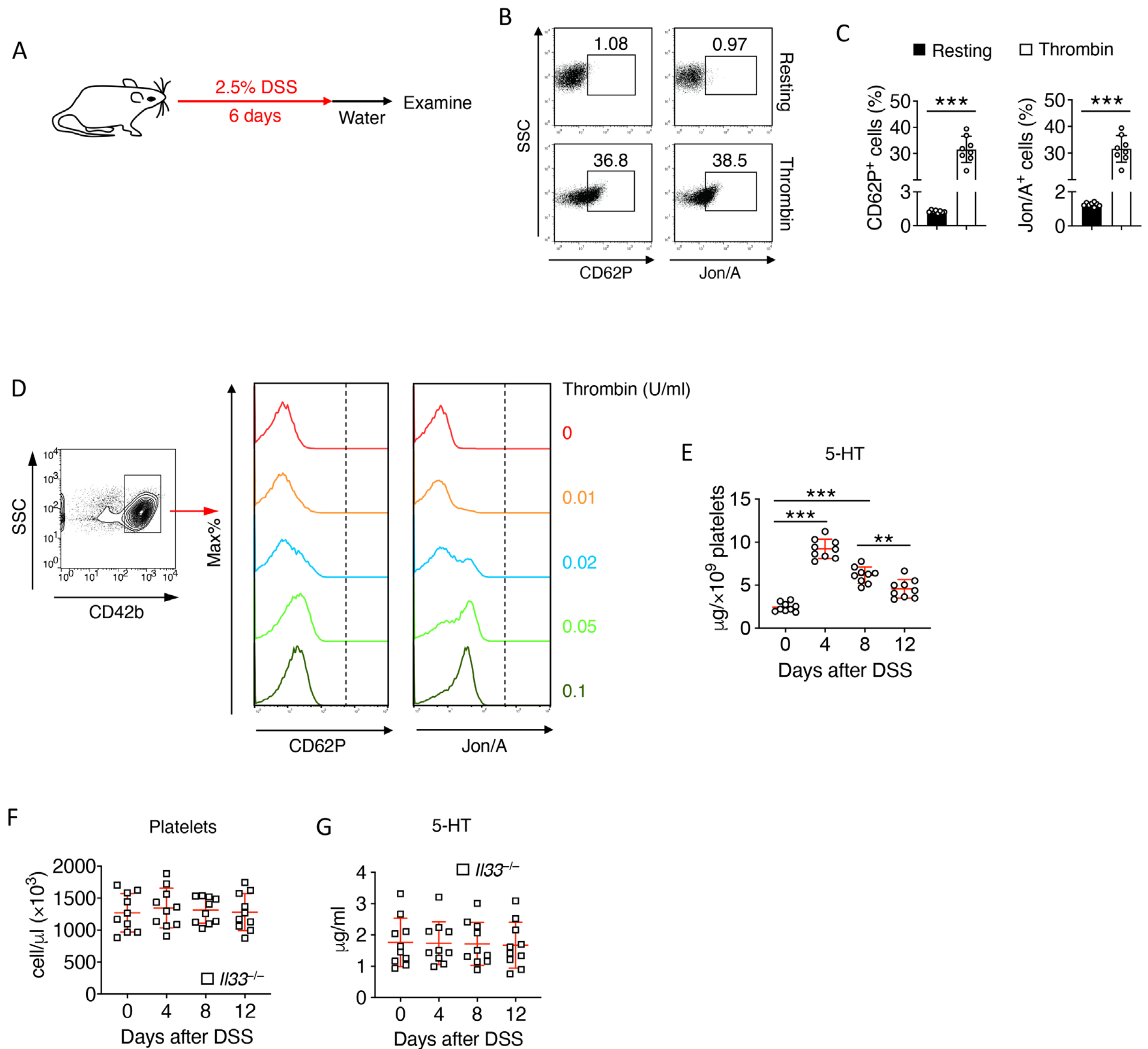
Wounded skin samples were collected 4 hours after injury. The skin was fixed in 10% neutral formalin and stored in 70% ethanol. The samples were then paraffin-embedded and cut into 10 um sections performed

by Histoserv Inc. (Germantown, MD). For immunofluorescence staining, slides were deparaffinized by xylene and antigen retrieval was conducted for 20 min in a 95℃-water bath in 10 mM sodium citrate, pH 6.0 followed by a 15 min incubation at room temperature. Slides were washed, blocked in 5% bovine serum albumin, and stained with PE conjugated anti-CD61 (Biolegend). Slides were mounted in Fluoromount-G (Thermo Fisher), and 3-15 images were taken per slide (Zeiss).

In vivo platelet quantification

Mouse platelets were labeled in vivo using 0.1 μg/g of DyLight649-anti-CD42b (Emfret) injection 30 mins before sample harvest. Wounded skin samples were homogenized in PBS. The fluorescence in the supernatant was measured and calculated according to the weight of the skin sample.

Sup Fig. 1



Sup Fig. 1 IL-33 determines enhanced blood coagulation during DSS colitis.

(A) Schematic illustration of DSS treatment on WT mice.

(B) Representative dot plots and (C) Quantification of percentile of $CD62P^{+}$ cells for WT platelets under the steady state.

(D) Representative histograms of fluorescence intensity of CD62P and Jon/A for platelets treated with thrombin at indicated concentrations.

(E) 5-HT levels in platelets were assessed by ELISA during DSS colitis.

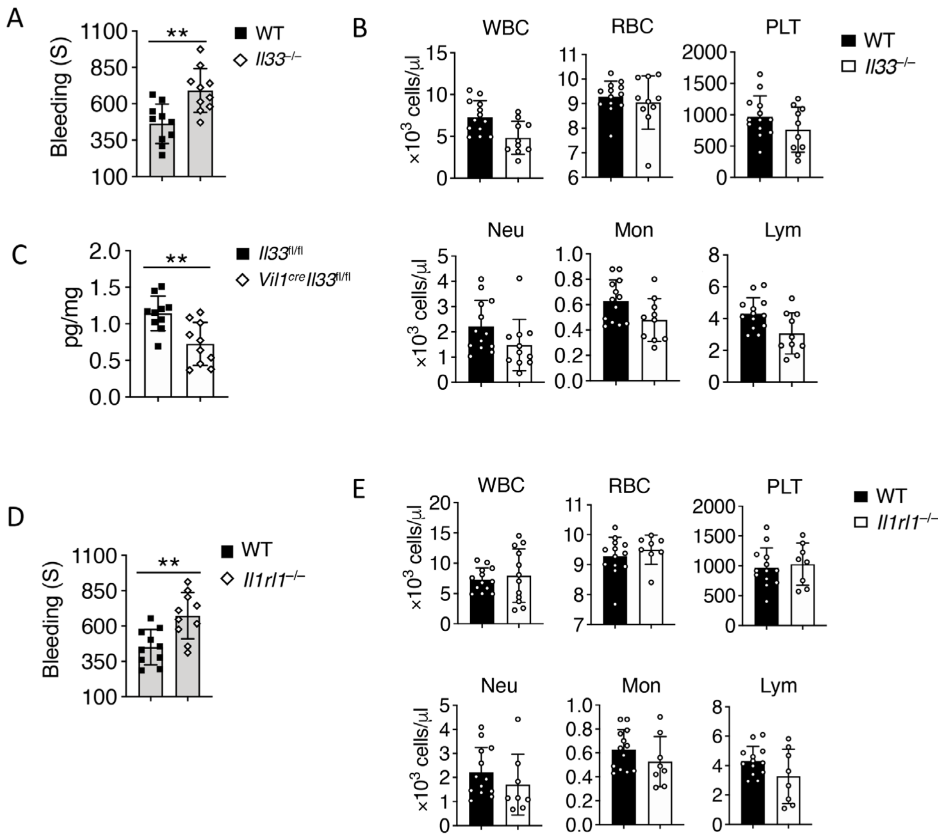
(F-G) *Il33*$^{-/-}$ mice were induced with DSS colitis and examined at indicated time points.

(F) Platelets counts during DSS colitis.

(G) 5-HT levels in serum were assessed by ELISA.

Data are representative of two independent experiments (B, D) or are pooled from two independent experiments (C, E-G). **$p<0.01$, ***$p<0.001$ (Student's t-test, error bars represent SD).

Sup Fig. 2



Sup Fig 2. IL-33-ST2 signaling regulates hemostasis.

(A) Time to cessation of bleeding in response to tail injury between WT and *Il33*$^{-/-}$ mice.

(B) Whole blood counts of white blood cells (WBC), red blood cells (RBC), platelets (PLT), neutrophils (Neu), monocytes (Mon), and lymphocytes (Lym) between WT and *Il33*$^{-/-}$ mice.

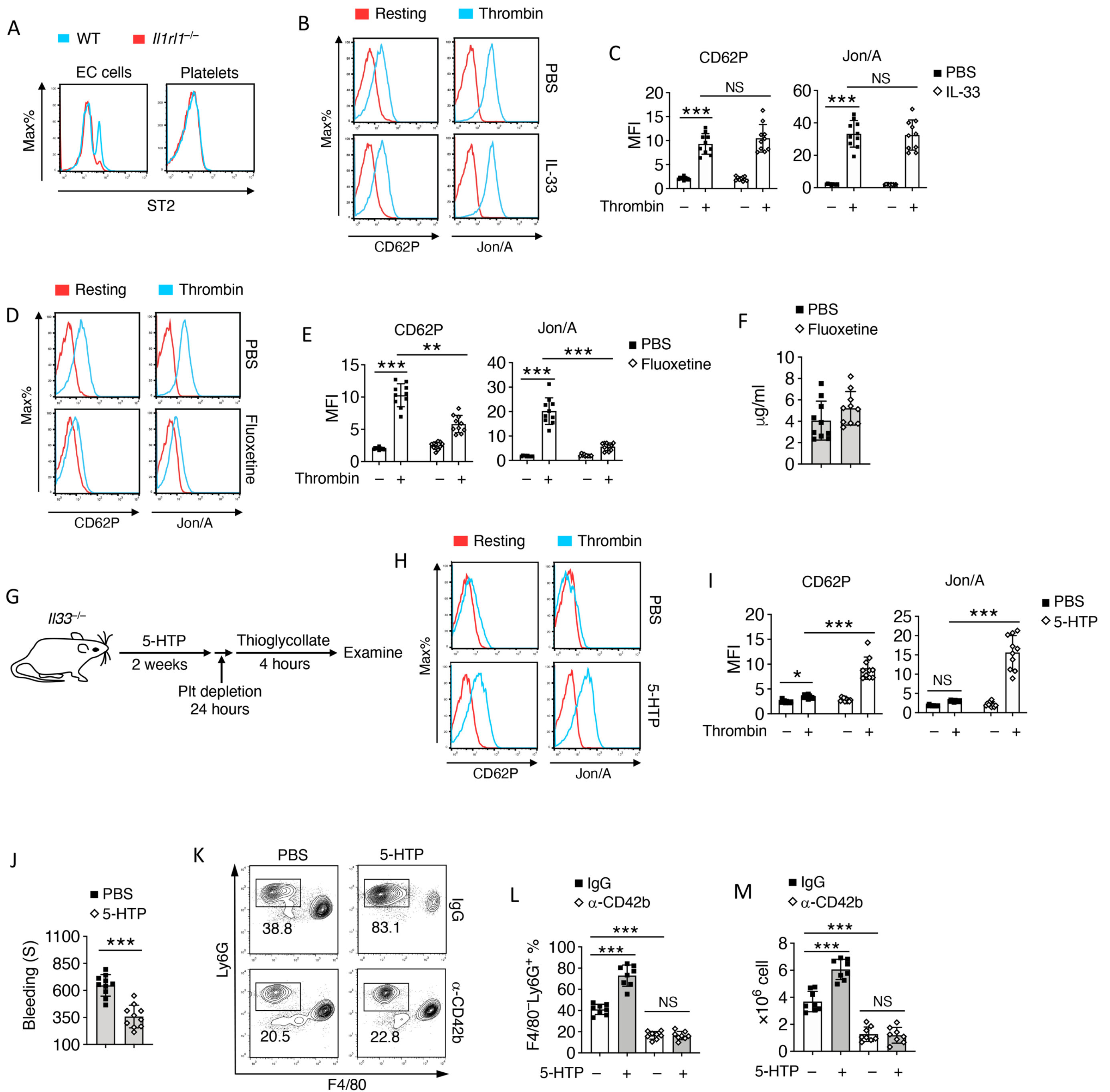
(C) IL-33 levels in colonic tissue were assessed by ELISA between *Il33*$^{fl/fl}$ and *Vil1*^{cre}*Il33*$^{fl/fl}$ mice.

(D) Time to cessation of bleeding in response to tail injury between WT and *Il1rl1*$^{-/-}$ mice.

(E) Whole blood counts of white blood cells (WBC), red blood cells (RBC), platelets (PLT), neutrophils (Neu), monocytes (Mon), and lymphocytes (Lym) between WT and *Il1rl1*$^{-/-}$ mice.

Data are representative of two independent experiments (A, C, D) or are pooled from at least two independent experiments (B, E). $^{**}p<0.01$ (Student's *t*-test, error bars represent SD).

Sup Fig. 3



Sup Fig. 3 Intestinal IL-33-ST2 signaling promotes neutrophils recruitment in acute peritonitis via platelet-derived 5-HT.

(A) Flow cytometry analysis of ST2 expression from EpCam^{+}CFP^{+} ECs and platelets isolated from

Tph1^{CFP} and *Il1rl1*$^{-/-}$*Tph1*^{CFP} mice.

(B) Platelets were isolated from WT mice and pretreated with or without IL-33 for 10 min. Representative histograms of fluorescence intensity of CD62P and Jon/A for WT platelets.

(C) Quantification of geometric mean fluorescence intensity in (B).

(D) Representative histograms of fluorescence intensity of CD62P and Jon/A for platelets from WT mice 2 hours after Fluoxetine treatment.

(E) Quantification of geometric mean fluorescence intensity in (D).

(F) Relative 5-HT levels in serum were assessed by ELISA from WT mice with or without Fluoxetine treatment.

(G-M) (G) Schematic illustration of *Il33*$^{-/-}$ mice treated with 5-HTP in the drink water for 2 weeks, followed with platelet depletion and peritonitis induction.

(H) Representative of histograms of fluorescence intensity of CD62P and Jon/A for platelets in *Il33*$^{-/-}$ mice.

(I) Quantification of histograms of fluorescence intensity in (H).

(J) Time to cessation of bleeding in response to tail injury between PBS and 5-HTP treated *Il33*$^{-/-}$ mice.

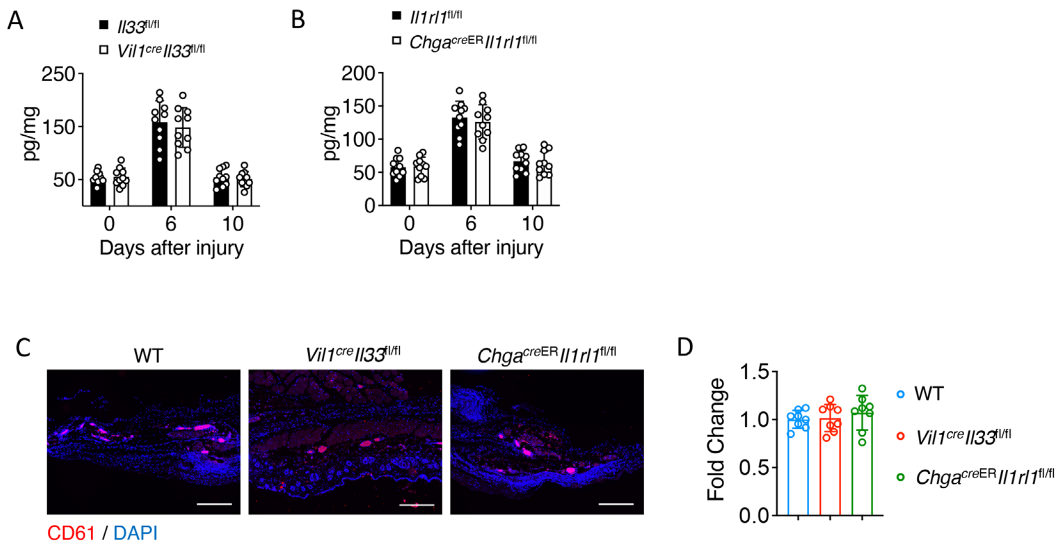
(K) Flow cytometry analysis of lymphocytes isolated from peritoneum in (G).

(L) Quantification of percentile of gated F4/80^{-}Ly6G^{+} neutrophils in (K).

(M) Quantification of F4/80^{-}Ly6G^{+} neutrophils isolated from peritoneum 4 hours after 4 hours after peritonitis induction.

Data are representative of two independent experiments (A, B, D, H, K) or are pooled from two independent experiments (C, E, F, I-M). *$p<0.05$, **$p<0.01$, ***$p<0.001$, NS: not statistically significant (Student's *t*-test, error bars represent SD).

Sup Fig. 4



Sup Fig. 4 Intestinal IL-33-ST2 signaling does not interfere with IL-33 levels in the skin during acute skin injury.

(A) IL-33 levels in the skin were assessed by ELISA at the indicated time points after wounding between *Il33*$^{fl/fl}$ and *Vil1*^{cre}*Il33*$^{fl/fl}$ mice.

(B) IL-33 levels in the skin were assessed by ELISA at the indicated time points after wounding between *Il1rl1*$^{fl/fl}$ and *Chga*^{creER}*Il1rl1*$^{fl/fl}$ mice.

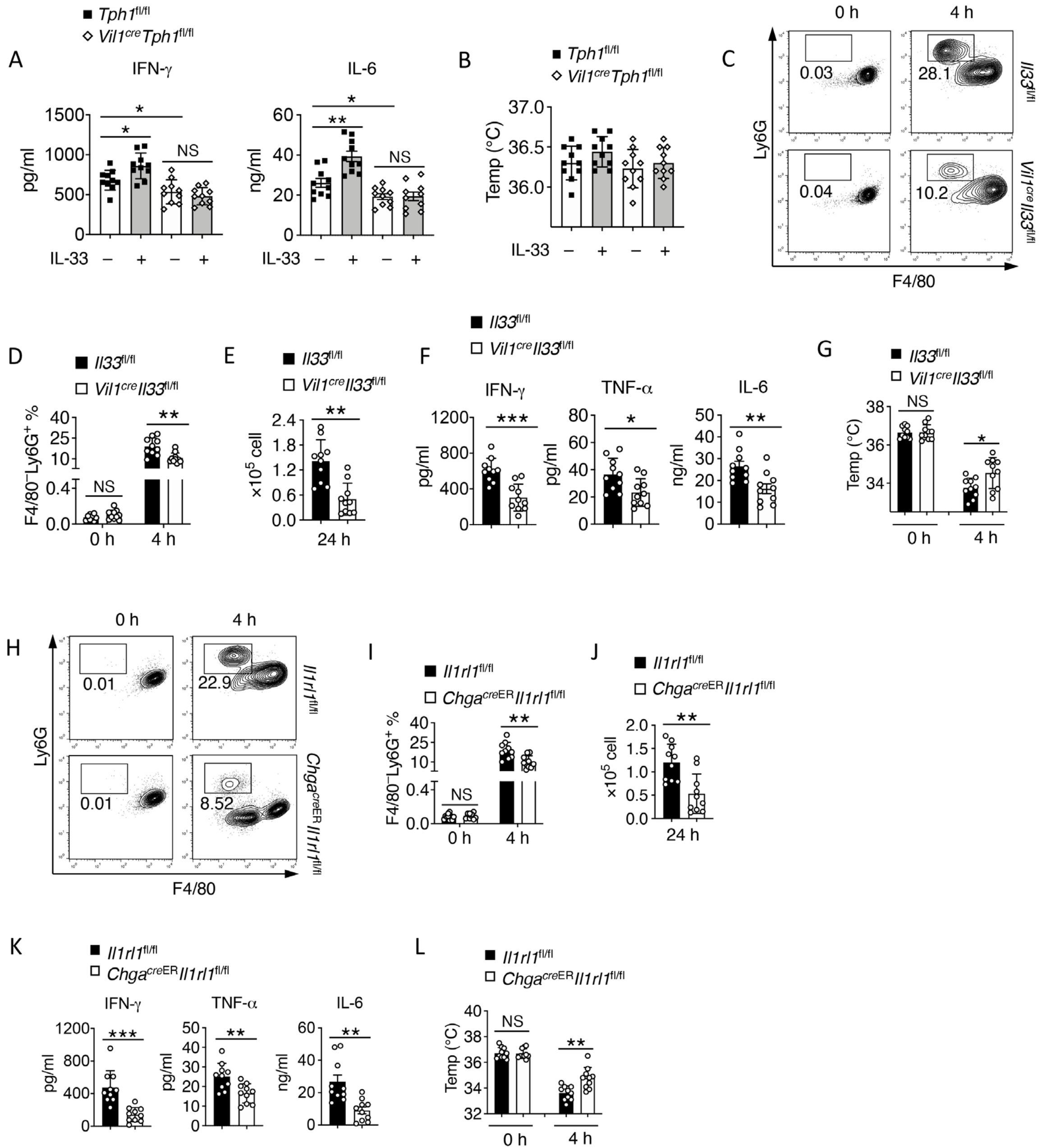
(C-D) Aseptic skin wounds were punched into the dorsal skin on indicated mouse strain.

(C) Wounded skin samples were collected 4 hours after injury. Immunofluorescence staining of CD61 (platelet). Scale bar, 40 µm.

(D) Mouse platelets from whole blood were labeled *in vivo* before skin injury. Wounded skin samples were collected 4 hours after injury and fluorescence was measured.

Data are representative of two independent experiments (C) or are pooled from two independent experiments (A, B, D).

Sup Fig. 5



Sup Fig. 5 IL-33 improves survival of LPS endotoxic shock via peripheral 5-HT.

(A) Indicated cytokines levels in the serum at 24 h after LPS injection with or without IL-33 treatment.

(B) Body temperature was measured at 0 h with or without IL-33 treatment.

(C-L) Intraperitoneal injection of *E. coli* serotype 055:B5 LPS into (C-G) *Il33*$^{fl/fl}$ and *Vil1*^{cre}*Il33*$^{fl/fl}$

mice or (H-L) *Il1rl1*$^{fl/fl}$ and *Chga*^{creER}*Il1rl1*$^{fl/fl}$ mice.

(C, H) Flow cytometry analysis of lymphocytes isolated from peritoneum 4 hours after LPS injection.

(D, I) Quantification of percentile of gated F4/80^{-}Ly6G^{+} neutrophils in (C) or (H).

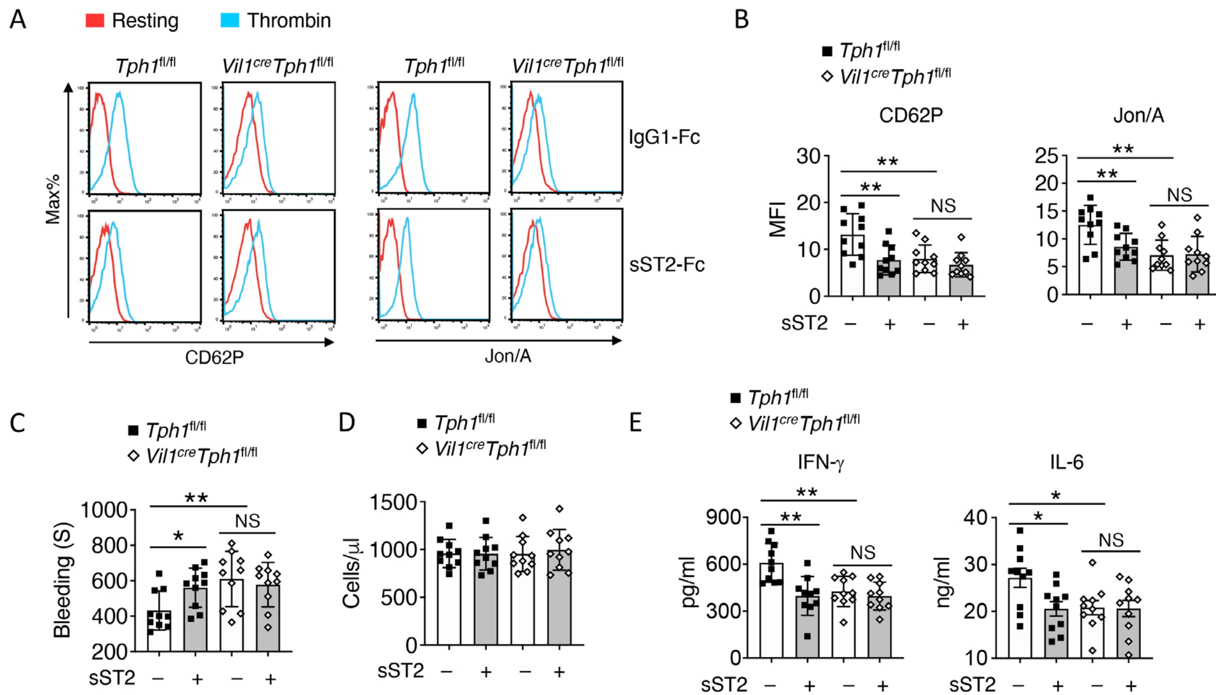
(E, J) Quantification of F4/80^{-}Ly6G^{+} neutrophils isolated from peritoneum 4 hours after LPS injection.

(F, K) Indicated cytokines levels in the serum at 24 h after LPS injection.

(G, L) Body temperature was measured at 0 and 4 h after LPS injection.

Data are representative of two independent experiments (C, H) or are pooled from two independent experiments (A, B, D-G, I-L). *$p<0.05$, **$p<0.01$, NS: not statistically significant (Student's *t*-test, error bars represent SD).

Sup Fig. 6



Sup Fig 6. Neutralization of IL-33 represses platelet activity and hemostasis.

(A-D) Control IgG-Fc or sST2-Fc-expressing plasmid was i.v. injected into *Tph1*$^{fl/fl}$ and *Vil1*^{cre}*Tph1*$^{fl/fl}$ mice.

(A) Representative histograms of fluorescence intensity of CD62P and Jon/A for platelets treated with or without thrombin.

(B) Quantification of geometric mean fluorescence intensity in (A).

(C) Time to cessation of bleeding in response to tail injury.

(D) Platelets counts between IgG and sST2-Fc treated mice.

(E) Intraperitoneal injection of *E. coli* serotype 055:B5 LPS into *Tph1*$^{fl/fl}$ and *Vil1*^{cre}*Tph1*$^{fl/fl}$ mice with 1 day pretreatment of IgG-Fc or sST2-Fc-expressing plasmid. Indicated cytokines levels in the serum at 24 h after LPS injection with or without sST2-Fc-expressing plasmid treatment.

Data are representative of two independent experiments (A) or are pooled from two independent experiments (B-E). *p<0.05, **p<0.01, ***p<0.001, NS: not statistically significant (Student's *t*-test, error bars represent SD).