Supplemental Methods

Reagents

Recombinant mouse P-selectin-IgM, E-selectin-IgM, and control CD45-IgM Fc chimeras were described previously[1]. Recombinant mouse ICAM-1-IgG Fc, mouse CXCL1, rat anti-mouse CXCL1 mAb, and mouse TNF-α were from R&D Systems. Rabbit antibodies to SLP-76, SFK, phospho-SFK (Y416), Syk, phospho-Syk, PLCγ2, phospho-PLCγ2, p38 MAPK, and phospho-p38 MAPK, and goat HRP-conjugated anti-rat IgG and HRP-conjugated anti-rabbit IgG were from Cell Signaling Technology. Rabbit antibodies to phospho-Vav (Y174), β2 integrin, and β-actin were from Santa Cruz Biotechnology. Hybridomas producing rat anti-mouse PSGL-1 mAb 4RA10 (blocking) and 4RB12 (non-blocking)[2,3] and rat anti-mouse P-selectin mAb RB40.34 (blocking)[4] were from Dietmar Vestweber. Blocking rat anti-E-selectin mAb was described previously[5]. Rat anti-mouse β2 integrin mAb (GAME-46), FITC-conjugated rat mAbs to mouse L-selectin (MEL-14), PSGL-1 (4RA10), CD44, β2 integrin, Ly6G, CD3, M-CSFR (CD115), and isotype control rat IgG; PE-conjugated rat mAbs to Ly6G, M-CSFR (CD115), and isotype control rat IgG; PE-conjugated mouse mAb to human SLP-76 (cross-reacts with mouse SLP-76) and isotype control mouse IgG2a; and hamster anti-mouse ICAM-1 mAb (3E2) were from BD Biosciences. Rabbit antibody to mouse Ly6G was from Biorbyt. Rabbit antibody to human fibrin (cross-reacts with mouse fibrin) was from Dako. Rabbit antibody to citrullinated histone and to mouse tissue factor were from Abcam. Alexa 488-conjugated goat anti-rabbit IgG, SYTOX Orange, and rat antibodies to mouse M-CSFR (CD115) and TER-119 were from Invitrogen. $F(ab')_2$ fragments of control rat IgG, GAME-46, 4RA10, and MEL-14 were generated with an

$F(ab')_2$ purification kit (ThermoFisher), following the manufacturer's protocol. CXCR2 antagonist (SB225002) was from Calbiochem.

Mice

All mice were backcrossed with C57BL/6J mice more than 10 times. C57BL/6J, *Cxcr2*$^{-/-}$, and *Itgb2*$^{-/-}$ mice were from The Jackson Laboratory. *Selplg*$^{-/-}$ mice were generated as described previously[1]. *Sell*$^{-/-}$ mice were made as described[6], with retention of the loxP-flanked *neostop* cassette to prevent expression of *Sell*. *Lcp2*$^{f/f}$LysMCre^{-}, *Lcp2*$^{f/f}$ LysMCre^{+}, *Lcp2*$^{Y112/128F}$, and *Lcp2*^{Y145F} mice[7,8] were gifts from Gary Koretzky. The mutations in SLP-76 were confirmed by PCR of tail DNA and DNA sequencing. PCR primer pairs were: F112: sense, 5'-ccactgtgttgacaggaa gatgactt-3', antisence: 5'-ttaacctagagaggcagtcttcagc-3'; F128, sense: 5'-ccagatgggga ggacgatggtgactt-3', antisense: 5'-ttaacctagagaggcagtcttcagc-3'; F145, sense: 5'-tctgtctcaaagcatgggtttccaatc-3', antisense: 5'-ctcgttgttggagggtggaggctcaa-3'. For DNA sequencing, the tail DNA was amplified by PCR using the primer pair, sense: 5'-tctgtctcaaagcatgggtttccaatc-3', antisense: 5'-attaacctagagaggcagtcttcagc-3', and the amplified PCR product was purified by QIAquick PCR Purification Kit (QIAGEN). Purified DNA (8 ng/μl) was sequenced using the sequence primer: 5'-caattctgtctc aaagcatgggtttc-3'.

All mouse protocols were approved by the Institutional Animal Care and Use Committee of the Oklahoma Medical Research Foundation.

Cells

Mouse bone marrow leukocytes were isolated as described previously[9]. Briefly, cells were isolated by gently flushing femurs and tibias with 10 ml Hanks' balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+}. After lysing red blood cells in 150 mM NH_4Cl, 10 mM $NaHCO_3$, and 1 mM EDTA, the cells were washed with HBSS and resuspended at 2 x 10^6/ml in HBSS

containing 1.26 mM Ca^{2+}, 0.81 mM Mg^{2+}, and 0.5% human serum albumin. Bone marrow neutrophils were isolated from bone marrow leukocyte by a density gradient method[10]. Briefly, the cells were resuspended in 3 ml of 45% Percoll (GE Healthcare) solution in 150 mM NaCl. The cells were loaded on top of a Percoll density gradient prepared by layering 81, 62, 55, and 50% Percoll solution. After centrifugation at 1200 g for 30 min, the cell band between the 81 and 62% layer was harvested and resuspended in HBSS containing Ca^{2+} and Mg^{2+} with 0.5% human serum albumin.

Mouse peripheral blood platelets were isolated as described[11].

Flow cytometry

Mouse bone marrow leukocytes were pre-incubated with 5 μg/ml Fc blocking antibody (BD Biosciences) for 20 min at 4˚C and then incubated with 5 μg/ml FITC-conjugated anti-L-selectin, PSGL-1, CD44, or β2 integrin, plus 5 μg/ml PE-conjugated anti-Ly6G for 20 min at 4˚C. Data were acquired on a FACSCalibur and analyzed with Cell Quest.

In some experiments, heparinized peripheral blood was obtained by heart puncture, and red blood cells were lysed. After lysis, the cells were washed in HBSS with 0.5% human serum albumin. The cells were pre-incubated with 5 μg/ml Fc blocking antibody (BD Biosciences) for 20 min at 4˚C. After washing in HBSS with 0.5% human serum albumin, they were fixed and permeabilized with a BD Cytofix/Cytoperm fixation and permeabilization kit (BD Biosciences). The cells were then washed with BD Perm/Wash buffer (BD Biosciences) and stained with μg/ml FITC-conjugated anti-Ly6G, anti-M-CSFR (CD115), or anti-CD3, plus PE-conjugated anti-SLP-76.

In other experiments, thrombi collected from the IVC 24 h after stenosis were minced into small pieces and digested in HBSS with Ca^{2+} and Mg^{2+} containing 1 mg/ml collagenase type IV

(Worthington) and 100 μg/ml DNAse I (Roche) for 15 min at 37˚C, with occasional vortex. After digestion, the single-cell suspension was passed through a 100-μm cell strainer and washed in HBSS without Ca^{2+} and Mg^{2+}, containing 5 mM EDTA and 1% human serum albumin. The cells were pre-incubated with 5 μg/ml Fc blocking antibody (BD Biosciences) for 20 min at 4˚C, and then incubated with 5 μg/ml PE-conjugated anti-Ly6G or anti-M-CSFR (CD115) for 20 min at 4˚C. Six-μm polystyrene beads (1 $x10^5$) (PolySciences) were added to 500 μl of cell suspension. The neutrophil or monocyte population was identified by its scatter properties and by Ly6G or M-CSFR (CD115) staining. The number of neutrophils or monocytes per thrombus was quantified.

Blood counts

Peripheral blood counts were measured using a veterinary hematology analyzer (Hemavet 950, Drew Scientific Inc.).

Flow chamber assay

Goat anti-human IgM Fc antibody (10 μg/ml) was adsorbed in 35-mm polystyrene dishes. In some experiments, 20 μg/ml mouse ICAM-1-Fc was co-adsorbed with or without 0.1 μg/ml or 1 μg/ml mouse CXCL1. After incubation at 4˚C overnight, the dishes were blocked with 1% human serum albumin, and then P-selectin-IgM or E-selectin-IgM was captured on the dishes. Bone marrow leukocytes were perfused over dishes mounted in a parallel-plate flow chamber at a wall shear stress of 1.0 dyne/cm^2. After 5-10 min, rolling and arrested cells were analyzed using a video microscopy system coupled to Element digital image-analysis software (Nikon). Arrested cells were scored as “round” (round and bright) or “spread” (irregular and dark)[12]. In this assay, virtually all rolling and adherent leukocytes are mature neutrophils[13].

Western blot and measurement of fibrin in thrombus

Bone marrow leukocytes (1 x 10^7) were incubated in six-well plates with P-selectin-IgM, E-selectin-IgM, or control CD45-IgM captured on immobilized anti-human IgM Fc Ab on a rotary shaker at 65 rpm for 10 min at room temperature. Alternatively, the plates were incubated with 50 μg/ml F(ab')$_2$ fragments of control rat IgG, GAME-46, 4RA10, or MEL-14 for 10 min. Some cells were pre-incubated with 10 mM methyl-β-cyclodextrin (Sigma-Aldrich) or its inactive analog α-cyclodextrin for 30 min at 37˚C. Isolated bone marrow neutrophils (5 x 10^6) were then incubated in plates with the immobilized F(ab')$_2$ fragments for 10 min at room temperature[14]. After incubation the cells were lysed in 1% Triton X-100, 125 mM NaCl, 50 mM Tris, pH 7.4, 10 mM EDTA, 2 mM PMSF, 0.1% SDS, with a protease inhibitor cocktail (1:50; Thermo Fisher Scientific), and analyzed by Western blotting using rabbit Abs to SFK, phospho-SFK, Syk, phospho-Syk, PLCγ2, phospho-PLCγ2, p38, phospho-p38, phospho-Vav, and β-actin, followed by HRP-conjugated goat anti-rabbit IgG.

In some experiments, bone marrow neutrophils and platelets were lysed and analyzed by Western blotting using rabbit antibodies to SLP-76 and β-actin, followed by HRP-conjugated goat anti-rabbit IgG.

Western blot analysis of thrombi was performed as described previously[6]. Briefly, thrombi collected 24 h after stenosis of the IVC were minced and lysed in 1% Triton X-100, 125 mM NaCl, 50 mM Tris pH 8.0, 0.1% SDS, 10 mM EDTA, and 1:50 dilution of protease inhibitor cocktail. Aliquots of supernatant, each containing 150-200 μg of protein, were resolved by SDS-PAGE, transferred to PVDF membrane, and probed with rabbit anti-Ly6G (Biorbyt, #orb13552, 1:1000), anti-human fibrin IgG (cross-reacts with mouse fibrin; DAKO, #A0080, 1:2000), rabbit anti-citrullinated histone IgG (Abcam, #5201, 1:1000), or rabbit anti-tissue factor IgG followed by goat horseradish peroxidase-conjugated anti-rabbit IgG (Cell Signaling Technology), or rat

anti-mouse mouse M-CSFR (CD115) IgG (ThermoFisher, #14-1152-82, 1:1000), or rat anti-mouse TER-119 (Invitrogen, #14-5921-81, 1:500) followed by goat horseradish peroxidase-conjugated anti-rat IgG (Cell Signaling Technology). The data were presented as the densitometric ratio of fibrin Aα chain or citrullinated histone to Ly6G, or the ratio of fibrin Aα chain or tissue factor to M-CSFR (CD115).

Fibrin in thrombus lysate was measured with a mouse fibrin ELISA kit (Biomatik).

Measurement of neutrophil extracellular traps (NETs)

NETs were measured as described previously[15]. Isolated bone marrow neutrophils in DMEM-Ham F12 medium containing 15 mM HEPES were incubated in chamber slides with 100 nM PMA, P-selectin-IgM, CXCL, or P-selectin-IgM and CXCL1 for 4 hours at 37˚C/5% CO_2. After 4 h, cells were fixed and permeabilized with a kit (BD Biosciences). The cells were incubated with 0.5 μg/ml rabbit anti-mouse citrullinated histone IgG or control IgG for 1 h at room temperature, followed by Alexa 488-conjugated anti-rabbit IgG. DNA was stained with 2 μM Sytox Orange for 5 min. Fluorescent images were visualized and digitalized by Hamamatsu ORCA-FLASH 2.8, and captured using Element digital image-analysis software (Nikon). NET-forming cells were identified as cells positive for both extracellular citrullinated histone and DNA. Six fields were randomly chosen per well for image acquisition. The percentage of neutrophils positive for citrullinated histone and/or DNA per field was quantified.

Deep vein thrombosis

DVT induced by flow restriction of the IVC was performed as described[6,16], with minor modifications. During the surgical procedure, mice were anaesthetized by continuous inhalation of 1-2% isoflurane in 100% oxygen using a veterinary vaporizer and placed on a heating pad in a supine position. After laparotomy, intestines were exteriorized and covered with saline-moistened

gauze to prevent drying. The IVC was gently separated from the aorta just below the left renal veins. Permanent ligation of the IVC over a 30-gauge needle (outside diameter of 0.31 mm serving as a spacer) placed on the vessel was achieved with a 7.0 nylon, non-absorbable suture (Braintree Scientific, Inc.). The needle spacer was then removed to prevent complete vein occlusion. Side branches were not ligated. Control mice underwent sham surgery without occlusion of the IVC. The peritoneum and skin were then closed. The entire procedure was completed within 10-15 min.

At various times after surgery, ultrasonography was performed with a Vevo 2100 system with 40 MHz mouse scan head (VisualSonics) to confirm flow restriction and to monitor thrombus progression. Mice placed on a heating pad were anesthetized as described above. Heart rates were monitored and kept at 450-500 beats per minute during observation. Sagittal orientation view under B-mode was used to visualize the IVC and the stenosis, and to analyze the frequency and size of the thrombus. In B-mode view, color Doppler mode was used to monitor flow direction. Turbulent flow was visualized as a color code with flow toward or away from the transducer indicated as blue or red, respectively. Pulse-wave Doppler mode was used to measure the flow velocity.

In some mice, spinning-disk intravital microscopy of the IVC was performed as described previously[17]. The IVC was observed within 15 min after occlusion. Alternatively, the vein was re-exposed 3 h after occlusion. The exposed vein was superfused with thermocontrolled (35˚C) bicarbonate-buffered saline. Fluoresbrite Green microspheres (0.5-μm diameter, PolySciences, Inc.) were coated with control or anti-CXCL1 mAb[10]. One hour before microscopy, 10^{10} microspheres and 4 μg PE-conjugated anti-Ly6G mAb, or 4 μg PE-conjugated anti-mouse M-CSFR (CD115) mAb in 200 μl saline were injected through the retro-orbital venous plexus. The

Fluoresbrite Green microspheres and PE-labeled Ly6G-positive neutrophils or M-CSFR-positive monocytes in the IVC were observed 1 mm below the ligation in the experimental group or 1-2 mm below the left renal vein in sham-surgery control mice. Rolling cells were defined as cells moving in the direction of the blood flow at slower velocity in the focal plane than free-flowing cells (100-150 m/s). Adherent cells were defined as cells that remained stationary for at least 30 s.

In other experiments, 50 μg of RB40.34, 4RB12, 4RA10, 9A9, RB40.34 and 9A9, or GAME-46 per mouse was injected intravenously through the retroorbital plexus 1 h before and 12 h after the ligation. 1 mg/kg of SB225002, dissolved in DMSO and diluted in saline, was injected intraperitoneally 1 h before and 12 h after the ligation, following a published protocol[18].

The incidence and size of thrombi were monitored by ultrasonography. After 24 h, mice were killed, and the IVC was excised below the ligation and proximal to the confluence of the common iliac vein. Thrombus formed in the IVC was removed for measurements of weight and length, flow cytometry, and Western blots.

Statistical analysis

Statistical differences were analyzed using unpaired and two-sided tail Student's *t*-test between two groups, or a one-way ANOVA with post hoc multiple-comparison test between more than two groups. Values were considered significant at $P < 0.05$.

References

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Table S1

Genotype	Total leukocytes	Neutrophils	Lymphocytes	Monocytes	Red blood cells	Hemoglobin	Platelets
	$x10^3/\mu l$	$x10^3/\mu l$	$x10^3/\mu l$	$x10^3/\mu l$	$x10^6/\mu l$	g/dl	$x10^6/\mu l$
WT	4.3 ± 0.3	0.8 ± 0.1	3.4 ± 0.5	0.1 ± 0.0	9.8 ± 1.0	11 ± 2.0	0.9 ± 0.1
Selplg$^{-/-}$	9.1 ± 0.4*	4.5 ± 0.6*	4.4 ± 0.4	0.1 ± 0.0	10.0 ± 1.0	13 ± 2.0	1.2 ± 0.5
Cxcr2$^{-/-}$	7.2 ± 1.0*	3.8 ± 0.4*	3.3 ± 0.6	0.1 ± 0.1	9.7 ± 1.4	13 ± 1.0	0.9 ± 0.1
Itgb2$^{-/-}$	10.0 ± 1.0*	4.5 ± 0.8*	5.1 ± 0.3*	0.2 ± 0.1	10.5 ± 1.2	12 ± 1.0	0.9 ± 0.2
Sell$^{-/-}$	4.0 ± 0.5	0.9 ± 0.1	3.2 ± 0.4	0.1 ± 0.0	9.5 ± 1.0	13 ± 1.5	1.0 ± 0.1
Lcp2$^{f/f}$*LysMCre*$^{-}$	3.8 ± 0.8	0.9 ± 0.3	3.5 ± 0.6	0.1 ± 0.1	9.7 ± 1.3	11 ± 2.0	1.3 ± 0.4
Lcp2$^{f/f}$*LysMCre*$^{+}$	4.1 ± 0.6	1.1 ± 0.2	3.2 ± 0.3	0.1 ± 0.0	9.0 ± 1.6	12 ± 1.5	1.0 ± 0.3
Lcp2^{Y145F}	3.9 ± 0.7	1.0 ± 0.2	2.9 ± 0.5	0.1 ± 0.0	9.9 ± 1.5	14 ± 2.0	1.0 ± 0.2
Lcp2$^{Y112/128F}$	3.7 ± 0.5	1.0 ± 0.4	3.3 ± 0.7	0.1 ± 0.0	10.1 ± 1.2	10 ± 1.5	1.1 ± 0.1

The data represent the mean ± SEM from 10-15 mice in each genotype. * $p < 0.05$ vs. WT. The data for WT and *Cxcr2*$^{-/-}$ mice are from Yago et al. *Blood Adv* 2:731-744, 2018.

Fig. S1. **Confirmation of SLP-76 deletion in neutrophils from *Lcp2*$^{f/f}$LysMCre^{+} mice and confirmation of tyrosine mutations in SLP-76 from *Lcp2*$^{Y112/128F}$ and *Lcp2*^{Y145F} mice.** (A) Isolated bone marrow neutrophils or peripheral blood platelets from WT or *Lcp2*$^{f/f}$LysMCre^{+} mice were lysed and analyzed by immunoblotting with antibody against SLP-76 or β-actin (loading control). (B) Flow cytometric analysis of intracellular expression of SLP-76 in Ly6G positive neutrophils, M-CSFR positive monocytes, or CD3-positive lymphocytes. (C) PCR of tail DNA with *Lcp2* primers confirms homozygosity for alleles encoding WT SLP-76 or SLP-76 with substitutions of the indicated tyrosines. (D) PCR of tail DNA with primers specific for Y112F, Y128F, or Y145F substitutions on SLP-76. (E) DNA sequence of purified tail DNA of the indicated genotype, confirming each mutation.

Fig. S2. **E-selectin and integrin signaling pathways in neutrophils require distinct tyrosine residues on SLP-76.** (A and B) Bone marrow leukocytes of the indicated genotype were incubated on immobilized control CD45-IgM or E-selectin-IgM. Lysates were analyzed by immunoblotting with the indicated antibodies. The data are representative of three experiments. (C and D) Rolling velocities of neutrophils of the indicated genotype on E-selectin with or without coimmobilized ICAM-1 in the presence or absence of anti-ICAM-1 mAb. (E and G) Rolling velocities of neutrophils of the indicated genotype on E-selectin coimmobilized with ICAM-1 and low-dose CXCL1 (0.1 μg/ml) in the presence or absence of anti-ICAM-1 mAb. (F and H) Percentages of neutrophils of the indicated genotype rolling, arrested and round, or arrested and spread on coimmobilized E-selectin, ICAM-1, and low-dose CXCL1. The data represent the mean ± SEM from five experiments, with five mice in each experimental group. *, $P < 0.05$ for rolling velocity; #, $P < 0.05$ for number of rolling cells.

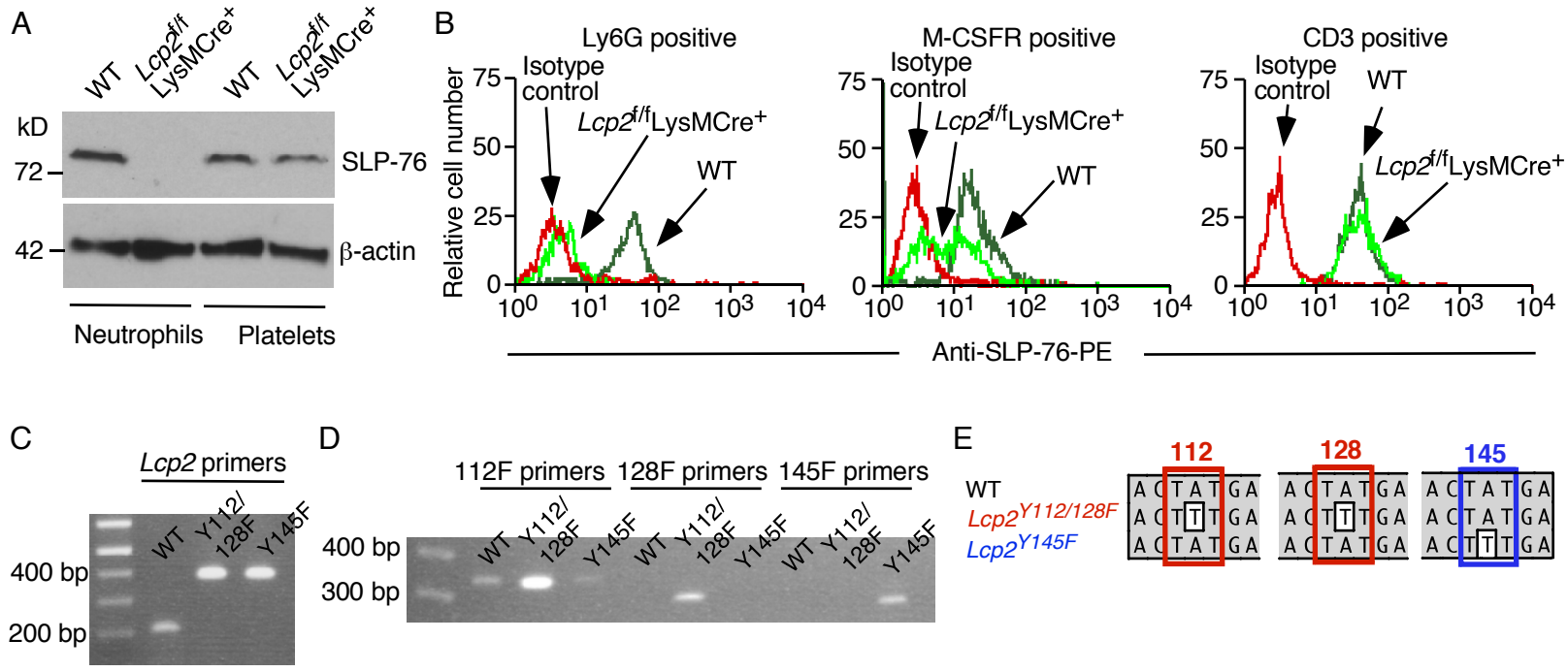
Fig. S3. **E-selectin-triggered signaling through PSGL-1 does not require L-selectin.** (A) Rolling velocities of WT or *Sell*$^{-/-}$ neutrophils on E-selectin with or without coimmobilized ICAM-1 in the presence or absence of anti-ICAM-1 mAb. (B) Rolling velocities of WT or *Sell*$^{-/-}$ neutrophils on E-selectin coimmobilized with ICAM-1 and low-dose CXCL1 (0.1 μg/ml) in the presence or absence of anti-ICAM-1 mAb. (C) Percentages of WT or *Sell*$^{-/-}$ neutrophils rolling, arrested and round, or arrested and spread on coimmobilized E-selectin, ICAM-1, and low-dose CXCL1. (D-G) Bone marrow leukocytes from WT or *Sell*$^{-/-}$ mice were incubated on immobilized control CD45-IgM or E-selectin-IgM in the presence or absence of EDTA. Lysates were analyzed by immunoblotting with the indicated antibodies. The data in A-C represent the mean ± SEM from five experiments, with five mice in each experimental group. The data in D-G are representative of three experiments. *, $P < 0.05$.

Fig. S4. **In all genotypes, thrombus weight is proportional to the total amount of fibrin.** After weighing, the thrombus from each mouse was lysed, and the total amount of fibrin was determined by ELISA. Thrombus weight was plotted vs. fibrin amount. Each panel represents data for the indicated genotype. Each symbol represents an individual thrombus. In each panel, the data are fitted by a straight line.

Fig. S5. **In all genotypes, the ratio of the red blood cell marker TER-119 to fibrin is constant.** (A) Western blot of thrombus lysates probed with antibodies to fibrin and TER-119. The Aα, Bβ, and γ chains of fibrin are marked. The data are representative of three experiments. (B)

Normalized densitometric ratio of TER-119 to fibrin Aα chain. The data represent the mean ± SD from three mice in each experimental group.

Figure S1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WT
Lcp2f/f LysMCre+
kD
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42
SLP-76
β-actin
Neutrophils
Platelets
B
Ly6G positive
M-CSFR positive
CD3 positive
Isotype control
Lcp2f/fLysMCre+
WT
Relative cell number
Anti-SLP-76-PE

C
Lcp2 primers
WT
Y112/128F
Y145F
400 bp
200 bp

D
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E
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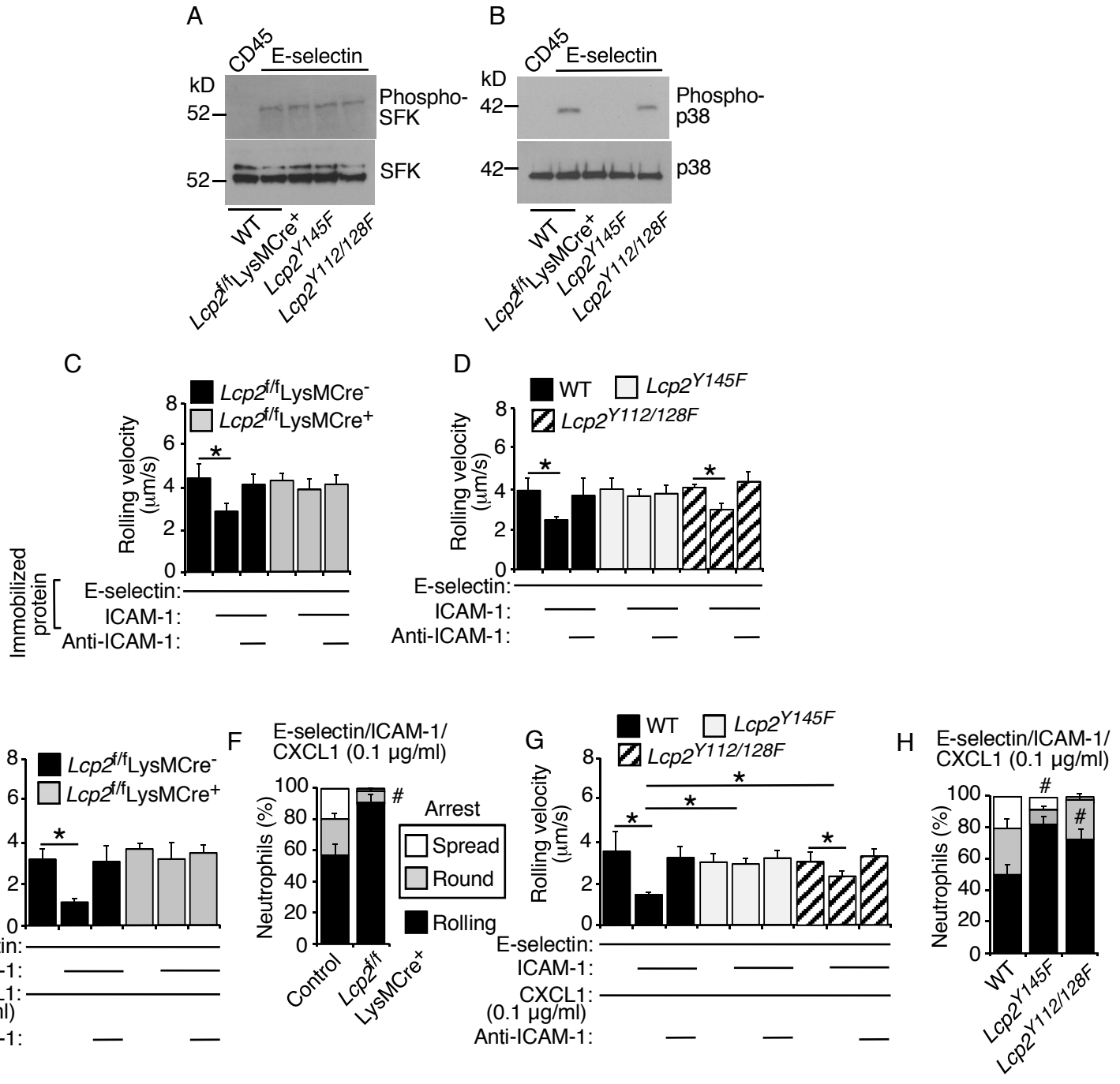
Figure S2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B
CD45
E-selectin
kD
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Phospho-p38
42
p38
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Lcp2Y112/128F
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Rolling velocity (μm/s)
Immobilized protein
E-selectin:
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Rolling velocity (μm/s)
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Anti-ICAM-1:
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Lcp2f/fLysMCre-
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Rolling velocity (μm/s)
Immobilized protein
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ICAM-1:
CXCL1: (0.1 μg/ml)
Anti-ICAM-1:
F
E-selectin/ICAM-1/ CXCL1 (0.1 μg/ml)
Neutrophils (%)
Arrest
Spread
Round
Rolling
Control
Lcp2f/f LysMCre+
G
WT
Lcp2Y145F
Lcp2Y112/128F
Rolling velocity (μm/s)
E-selectin:
ICAM-1:
CXCL1: (0.1 μg/ml)
Anti-ICAM-1:
H
E-selectin/ICAM-1/ CXCL1 (0.1 μg/ml)
Neutrophils (%)
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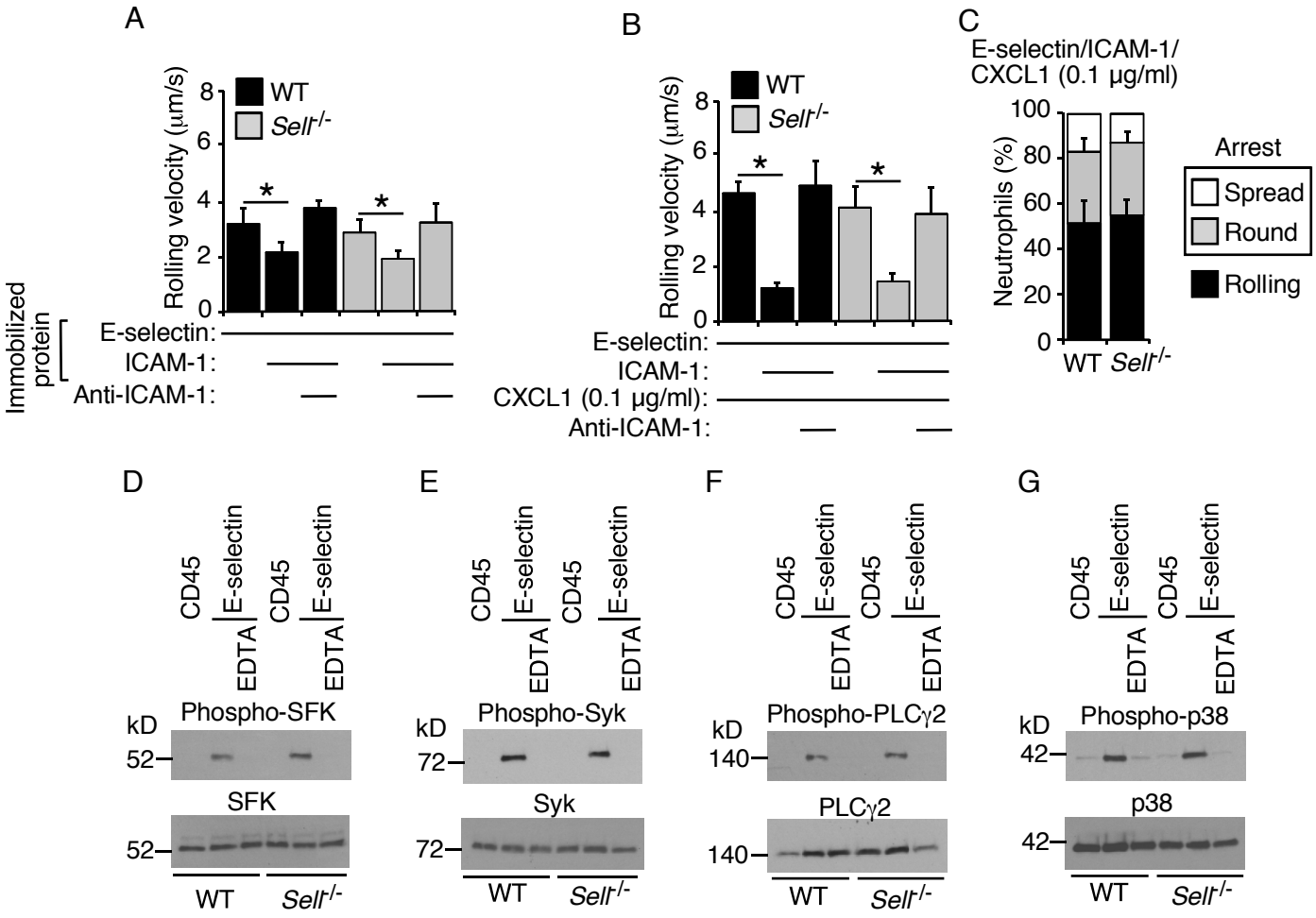
Lcp2Y145F
Lcp2Y112/128F

Figure S3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E-selectin/ICAM-1/
CXCL1 (0.1 μg/ml)
Neutrophils (%)
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SFK
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Syk
F
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140
PLCγ2
G
Phospho-p38
42
p38

Figure S4

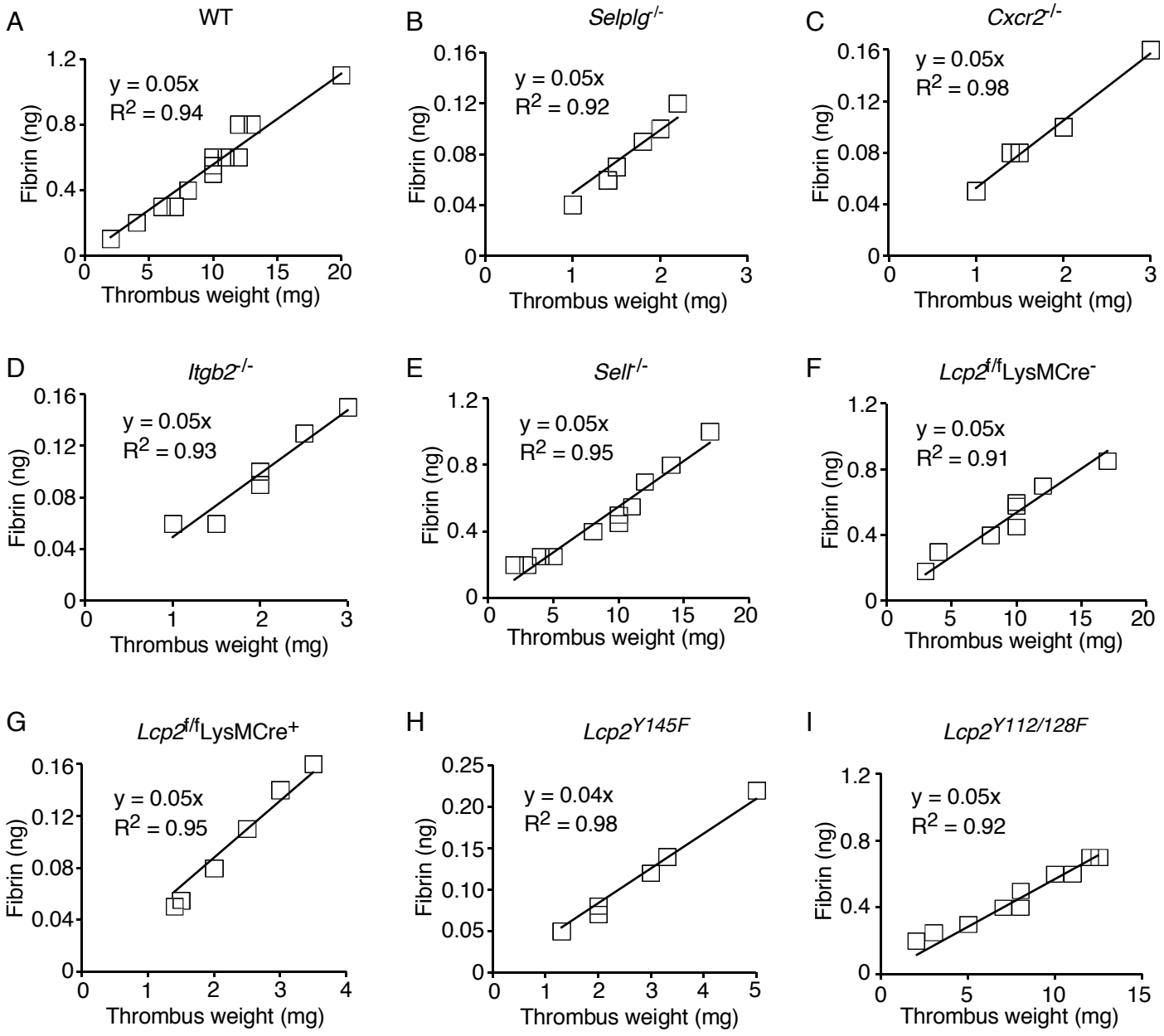
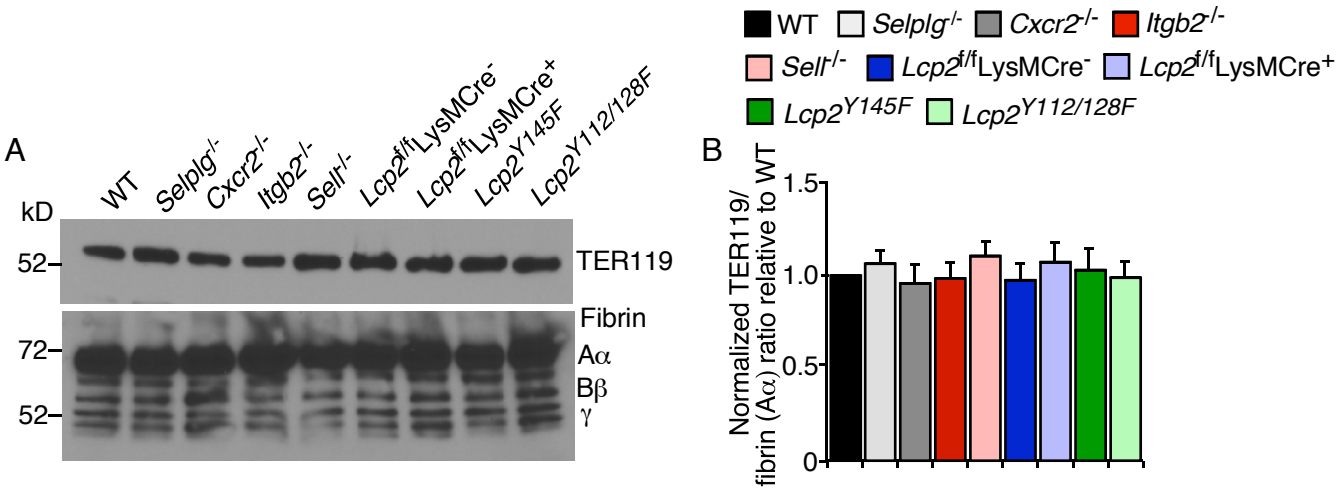

A
WT
y = 0.05x
R2 = 0.94
B
Selplg-/-
y = 0.05x
R2 = 0.92
C
Cxcr2-/-
y = 0.05x
R2 = 0.98
D
Itgb2-/-
y = 0.05x
R2 = 0.93
E
Selt-/-
y = 0.05x
R2 = 0.95
F
Lcp2f/fLysMCre-
y = 0.05x
R2 = 0.91
G
Lcp2f/fLysMCre+
y = 0.05x
R2 = 0.95
H
Lcp2Y145F
y = 0.04x
R2 = 0.98
I
Lcp2Y112/128F
y = 0.05x
R2 = 0.92
Fibrin (ng)
Thrombus weight (mg)

Figure S5


A
WT
Selplg-/-
Cxcr2-/-
Itgb2-/-
Sell-/-
Lcp2f/fLysMCre-
Lcp2f/fLysMCre+
Lcp2Y145F
Lcp2Y112/128F
kD
52
72
52
TER119
Fibrin
Aα
Bβ
γ
B
WT
Selplg-/-
Cxcr2-/-
Itgb2-/-
Sell-/-
Lcp2f/fLysMCre-
Lcp2f/fLysMCre+
Lcp2Y145F
Lcp2Y112/128F
Normalized TER119/ fibrin (Aα) ratio relative to WT
1.5
1.0
0.5
0