**SUPPLEMENTAL DATA**

**Generation of PDI CKO mice.**

Heterozygous PDI-floxed ($PDI^{flox/+}$) mice in which exons 1 and 2 containing the first thioredoxin domain are flanked by loxP sites were generated from GenOway (Lyon, France) and bred with CMV-Cre mice to generate heterozygous $PDI^{+/-}$ mice. To generate myeloid-specific PDI CKO mice, $PDI^{+/-}$ mice were crossed with Lys-Cre mice. The resulting mice ($PDI^{+/-}$;Lys-Cre) were further bred with $PDI^{flox/flox}$ mice to generate littermate WT control ($PDI^{flox/+}$) and PDI CKO ($PDI^{flox/-}$;Lys-Cre) mice. Homologous recombination in embryonic stem cells was confirmed by Southern blot analysis of the genomic DNA isolated from mouse tails. The expected targeted alleles of 13.3-, 10.0-, and 7.0-kb KpnI/BamHI fragments for WT, LoxP, and KO, respectively, were identified by the external 3' probe. Following PCR primers were used for genotyping: 5'-GGCTGAGTTATCTGGTGATTGACCAATG-3' and 5'-TCCA GGCTCCACAAAAATTCCTTTAGC-3' for PDI WT (305 bp) and flox (422 bp); and 5'-CCCAGAAATGCCAGATTACG-3', 5'-CTTGGGCTGCCAGAATTTCTC-3', and 5'-TT ACAGTCGGCCAGGCTGAC-3' for Lys-Cre (700 bp). The University of Illinois Institutional Animal Care and Use Committee approved all animal care and experimental procedures.

**Reagents.** N-formyl-methionyl-leucyl-phenylalanine (fMLF), PGE1, rabbit polyclonal anti-PDI antibodies, and scrambled and PDI siRNA oligonucleotides were from Sigma (St. Louis, MO). Recombinant human and mouse TNF-$\alpha$ and ICAM-1/Fc, and polyclonal anti-human $\beta$2 antibodies were obtained from R&D systems (Minneapolis, MN). Rabbit polyclonal anti-ERp57 and anti-ERp72 antibodies, a blocking mouse

monoclonal antibody against PDI (RL90), SSB (cell-impermeable sulfo-NHS-biotin), avidin agarose beads, and protein G agarose beads were from Thermo Scientific (Rockford, IL). Mouse monoclonal antibodies against PDI (BD34) and mouse P-selectin glycoprotein ligand-1 were purchased from BD Biosciences (San Jose, CA). The blocking anti-PDI antibodies (RL90 and BD34) were further purified by protein A/G affinity chromatography and used as pure IgG. Isotype control IgGs, mouse monoclonal blocking antibodies against human αLβ2 (HI111), αMβ2 (ICRF44 and CBRM1/5), αXβ2 (clone 3.9), or β2 (TS1/18), rat monoclonal blocking antibodies against mouse αLβ2 (M17/4) or αMβ2 (M1/70), PE-conjugated rat IgG2a or antibodies against mouse L-selectin (MEL-14), αXβ2, P-selectin glycoprotein ligand-1, or CD44, and Alexa Fluor 647- or 488-conjugated antibodies against mouse Gr-1 or F4/80 were from BioLegend (San Diego, CA). Anti-mouse CD42b and Dylight 488-conjugated anti-mouse CD42c antibodies were obtained from Emfret Analytics (Eibelstadt, Germany). Mouse monoclonal blocking anti-human αLβ2 (TS1/22) and anti-human αVβ3 (LM609) antibodies were from Developmental Studies Hybridoma Bank (Iowa City, IA) and Millipore, respectively. FITC-conjugated mouse IgG1 or anti-human L-selectin (DREG56), and PE-conjugated mouse IgG1 or anti-human activated αMβ2 (CBRM1/5) antibodies were obtained from eBioscience (San Diego, CA). Alexa Fluor 488-labeled F(ab)'$_2$ rabbit anti-mouse IgG, MPB (cell-impermeable N-alpha-[3-maleimidylpropionyl]biocytin), Alexa Fluor 488-conjugated human fibrinogen (FG), and the Alexa Fluor labeling kit were purchased from Invitrogen (Eugene, OR). Mouse anti-human αM and rabbit anti-human αL antibodies were from Santa Cruz (Santa Cruz, CA) and Abcam (Cambridge, MA), respectively. The extracellular domain of recombinant human αMβ2 integrin (Met1-Asn1104 for αM with a flag tag at the C-terminus and Met1-Asn700 for β2 with a poly His tag at the

C-terminus) expressed in HEK293 cells were obtained from Sino Biological (Beijing, China). Purified human FG was kindly provided by Deane F. Mosher (University of Wisconsin-Madison, WI).

**Expression and purification of recombinant PDI.** cDNA for His-tagged rat wtPDI and double mutant PDI (dmPDI) was provided by Dr. Takashi Uehara (Hokkaido University, Sapporo, Japan), cloned into pET-15b vector, expressed in BL21 cells and purified using Ni-affinity beads. The two CGHC residues were mutated into SGHS residues in dmPDI to abolish its isomerase activity. Endotoxin was removed with a level of <0.1 EU/μg of the protein using the endotoxin removal kit.

**Isolation of mouse blood platelets, splenic lymphocytes, and bone marrow monocytes.** Sodium citrate-treated mouse blood (0.9 ml) was drawn from WT or PDI CKO mice and centrifuged at 200g for 20 minutes. The plasma and buffy coat were transferred to a separate tube and re-centrifuged at 200g for 3 minutes. The platelet-rich plasma was collected and centrifuged at 800g for 3 minutes in the presence of 0.5 μM PGE1. The pellet was suspended in HEPES-Tyrode buffer, pH 7.3, containing 10% sodium citrate solution and 0.15 μM PGE1, and centrifuged at 800g for 3 minutes. Platelets were counted and lysed at a concentration of $1 \times 10^{10}$ cells/ml in ice-cold lysis buffer (TBS, pH 7.4, containing 1% Triton X-100, 0.1% SDS, 2 mM EDTA, protease inhibitor cocktail, and 1 mM phenylmethylsulfonyl fluoride). Splenic lymphocytes were isolated by ficoll gradient. After lysis of RBCs, lymphocytes were lysed at a concentration of $3 \times 10^7$ cells/ml. Bone marrow monocytes were also isolated by ficoll gradient. The cell layer at the interface between ficoll and HBSS were collected and resuspended with RPMI1640 medium.

After 3 hour incubation on a tissue culture plate at 37ºC, non-adherent cells were removed by harsh washing with PBS. Adherent monocytes were extracted and analyzed by the forward and side scatter as well as F4/80 expression. Cell lysates were electrophoresed and immunoblotted.

**Flow chamber assay under venous shear.** Confluent HUVECs on FG-coated glass coverslips were stimulated with TNF-$\alpha$ (20 ng/mL) for 6 hours and placed into a parallel plate flow chamber (Bioptech). Neutrophils, 3 x $10^6$, were incubated with or without blocking antibodies (20 minutes at RT), followed by stimulation with fMLF. After washing, neutrophils were perfused for 10 minutes over activated HUVECs under venous shear of 1 dyne/$cm^2$. Then, the medium was perfused for 5 minutes to wash out weakly bound cells. Images were obtained using a Nikon microscope (ECLIPSE Ti, Melville, NY) equipped with 10 x/0.25 NA objective lens and recorded with a camera (CoolSNAP $ES^2$). The data were analyzed using NIS Elements (AR 3.2). Adherent neutrophils were monitored in a field of 0.15 $mm^2$ and counted in 5-7 separate fields.

**Static adhesion assay.** Human or mouse ICAM-1/Fc chimera, 10 $\mu$g/ml, was spotted (25 $\mu$l) onto Petri dishes for 2 hours. Human or mouse neutrophils, 1 x $10^6$, in HBSS containing 10 mM HEPES, pH 7.4 and 1 mM MgCl2, were incubated with or without inhibitors or recombinant PDI in the presence of fMLF. After 10-minute incubation at 37°C, cells were incubated onto the dishes for 4 minutes at RT. After washing with HBSS, the numbers of round versus spread adherent neutrophils were counted in 5-7 separate fields.

**Isolation of lipid rafts.** Neutrophil pellet (5 x $10^7$ cells/sample) were suspended with 2 ml of 0.5 M sodium carbonate, pH 11, containing protease inhibitor cocktail and PMSF and lysed by 10 strokes in a homogenizer and by sonication. The homogenate (1.8 ml) was mixed with an equal volume of MES-buffered saline (MBS, 25 mM MES, pH 6.5, 150 mM NaCl) containing 90% sucrose and placed in the bottom of a tube. MBS containing 35% sucrose (3.6 ml) was layered on top, followed by 3.6 ml of MBS containing 5% sucrose. Gradients were centrifuged for 16 hours at 35,000 rpm. Each fraction (1 ml) was collected from the top, stained with GelCode Blue (Coomassie Blue, Thermo Scientific) or immunoblotted.

**Pull-down and immunoprecipitation assay.** Human or mouse neutrophils, 2 x $10^7$ cells/ml, were pretreated with an anti-PDI antibody, followed by stimulation with fMLF. Cells were further incubated for 10 minutes at 37°C with 100 μM cell-impermeable probes, either the primary amine-reactive biotin (SSB) or the thiol-reactive maleimide (MPB). The reaction was quenched with 20 mM Tris-HCl, pH 7.4 for SSB and 100 μM reduced glutathione for MPB. Cell lysates were pulled down with avidin agarose beads. For immunoprecipitation assays, lysates of SSB-labeled neutrophils were precleared with protein G beads coupled with control IgG, followed by incubation with protein G beads coupled with anti-PDI antibodies. The bound fractions were analyzed by SDS-PAGE under reduced conditions and immunoblotted. The blots were stripped and re-probed with peroxidase-conjugated avidin or other antibodies. The band density was measured by densitometry using Scion Image (v4.0). To calculate the exposure of sulfhydryl groups on surface αM, β2, and PDI, the surface free thiol levels of each protein (band density of MPB labeling) were normalized to its surface expression (band density of SSB labeling). Then, the fold

change of free thiol levels during cell activation was obtained by dividing the normalized value in stimulated cells by that in unstimulated cells in each group.

**Surface plasmon resonance.** Direct binding of PDI to αMβ2 was determined by surface plasmon resonance with Biacore T100. The extracellular domain of recombinant αMβ2 (25 μg/ml in 10 mM acetate buffer, pH 5.0) was immobilized onto a CM5 chip (Series S, Sensor Chip CM5, GE Healthcare) according to the manufacturer's instructions. Various concentrations of recombinant PDI (0.03 to 21 μM) or purified fibrinogen (0.02 to 5 μM) in running buffer (10 mM HEPES, pH 7.5, 150 mM NaCl, 0.005% P20 with 2 mM MnCl2) were infused over the reference and integrin-immobilized surfaces at a flow rate of 5 μl/minute for 240 seconds, followed by a dissociation phase of 300 seconds. Specific binding between two proteins was calculated by subtracting the reference sensorgram from the sample. Data were analyzed with Biacore T100 evaluation software.

**Confocal microscopy.** Human neutrophils were incubated with fMLF in the presence or absence of 10 μg/ml control IgG or a blocking anti-PDI antibody on glass coverslips coated with human ICAM-1/Fc. Adherent cells were stained with PE-conjugated mouse IgG1 or anti-activated αM (CBRM1/5) or αL (TS1/22) antibodies, followed by incubation of rabbit IgG or rabbit anti-PDI antibodies equivalently conjugated with Alexa Fluor 488. After mounting, images were obtained using a laser scanning confocal microscope (LSM 510 META, Zeiss) equipped with a 63 x/1.2 NA water immersion objective lens and analyzed using LSM Image Browser (v.4). Since inhibition of PDI affected neutrophil adhesion to immobilized ICAM-1, images were taken at the region with a similar number of adherent neutrophils in the

groups treated with control IgG or an anti-PDI antibody. Co-localization histograms were generated with Image J (v.1.45). To calculate the size and number of punctates of activated αMβ2 integrin as a clustering indicator, images were further processed with a threshold (9 pixels) in Image J and the fluorescence signal of an anti-activated αMβ2 integrin antibody was quantified as particles (n = 25-30 cells in 4 independent experiments). The medium and large punctates were defined as 10-100 and 100-5000 pixels, respectively.

**Supplemental Figure Legend**

**Figure S1. Surface interaction of PDI with αMβ2 and αLβ2 on human neutrophils.** (A-B) Human neutrophils were incubated without or with fMLF. After SSB labeling, lysates were immunoprecipitated with anti-αM (A) or anti-αL (B) antibodies and immunoblotted (total αM, αL, or PDI). The blots were re-probed with peroxidase-conjugated avidin (surface αM, αL, or PDI). (C) Confocal microscopy was performed as described in Methods. Adherent cells were stained with control IgG or anti-αL (TS1/22) and then rhodamine-conjugated anti-mouse IgG (red), followed by incubation with rabbit IgG or anti-PDI antibodies equivalently conjugated with Alexa Fluor 488 (green). Representative blots and images were obtained from three independent experiments. Bar = 10 μm.

**Figure S2. Binding of ICAM-1 and fibrinogen to the extracellular domain of human αMβ2 integrin.** (A-B) The extracellular domain of recombinant human αMβ2 integrin was analyzed by SDS-PAGE under reduced conditions, followed by Coomassie Blue staining (A) and immunoblotting (B). αMβ2; 1 (protein staining) and 0.1 μg (immunoblotting) of the protein, and lysate: 2.5 x $10^5$ neutrophils per lane. (C) Binding of ICAM-1 and FG to immobilized αMβ2 was determined by enzyme-linked immunosorbent assay. The wells of a 96-well plate were coated with the extracellular domain of αMβ2 and incubated with Alexa 488-conjugated ICAM-1 and FG, 10 μg/ml, in the presence of 2 mM Mn2+. After washing, the wells were added with rabbit IgG or rabbit anti-Alexa 488 antibody, and followed by incubation with HRP-conjugated anti-rabbit IgG antibodies. O-phenylenediamine (Sigma) solution and 0.03% H2O2 were added. The absorbance was measured at 490 nm. Specific binding of an antibody was calculated by subtracting the signal by nonspecific binding of rabbit IgG

from the signal by binding of rabbit anti-Alexa 488 antibodies to αMβ2. Data represent mean ± SD (n = 3).

**Figure S3. Protein amounts in lipid raft and non-raft fractions.** Fractions containing lipid rafts and non-rafts were analyzed by SDS-PAGE under reduced conditions, and the gel was stained with Coomassie Blue (A). (B) The protein amount in each fraction was determined by a BCA assay (B, mean ± SD, n = 3).

**Figure S4. Effect of a blocking anti-PDI antibody on the translocation of PDI and αMβ2 to lipid rafts during neutrophil activation.** Human neutrophils were incubated with fMLF in the presence of control IgG or a blocking anti-PDI antibody. Fractions containing lipid rafts and non-rafts were collected and immunoblotted. Representative blots and densitometric analysis are shown in A and B, respectively (n = 3).

**Figure S5. Visualization of neutrophils, monocytes, and platelets during vascular inflammation.** Intravital microscopy was performed as described in Methods. Neutrophils, monocytes, and platelets were visualized by infusion of Alexa Fluor 647-conjugated anti-Gr-1, Alexa Fluor 488-conjugated anti-F4/80 (A-B), and Dylight 488-conjugated anti-CD42c antibodies (C-D), respectively, in WT mice. (C-D) WT mice were infused with rat IgG (control) or anti-CD42b antibodies (thrombocytopenic), 0.5 μg/g BW, to deplete circulating platelets. (B and D) The number of adherent neutrophils (B and D) and monocytes (B) is shown. Small (gray) and large arrows show adherent monocytes and blood flow, respectively. Data represent mean ± SEM (n = 20-22 venules in 3 mice per group). ***:P<0.001 vs the

number of adherent neutrophils after Student *t*-test.

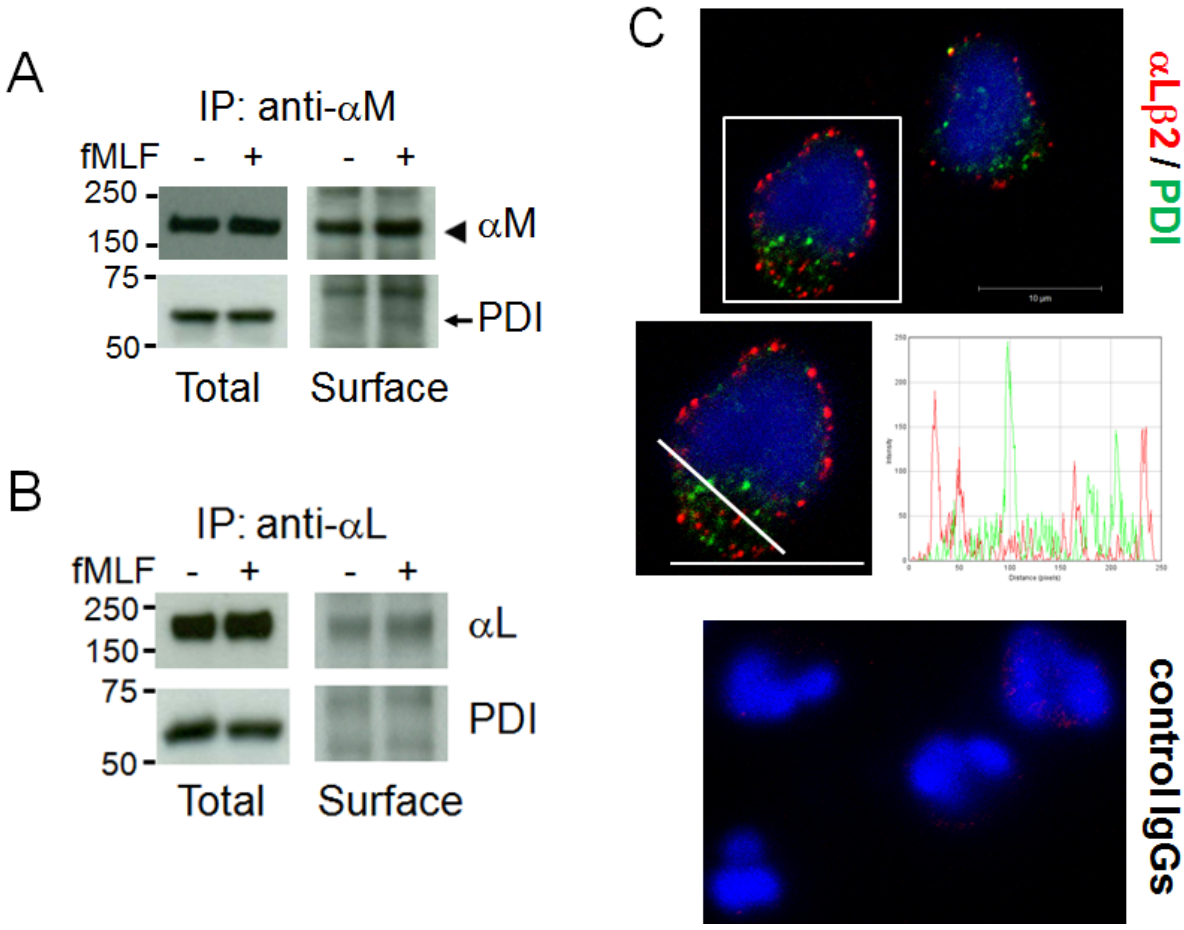
**Supplemental Videos 1-2.** Neutrophil recruitment during TNF-α-induced cremaster venular inflammation in littermate WT control (Video 1) or PDI CKO (Video 2) mice. Neutrophils were monitored by Alexa Fluor 647-conjugated anti-mouse Gr-1 antibody.

**Supplemental Videos 3-4.** Recombinant wtPDI (Video 3) or dmPDI (Video 4) was infused into PDI CKO mice as described in Methods. Neutrophils were monitored by Alexa Fluor 647-conjugated anti-mouse Gr-1 antibody.

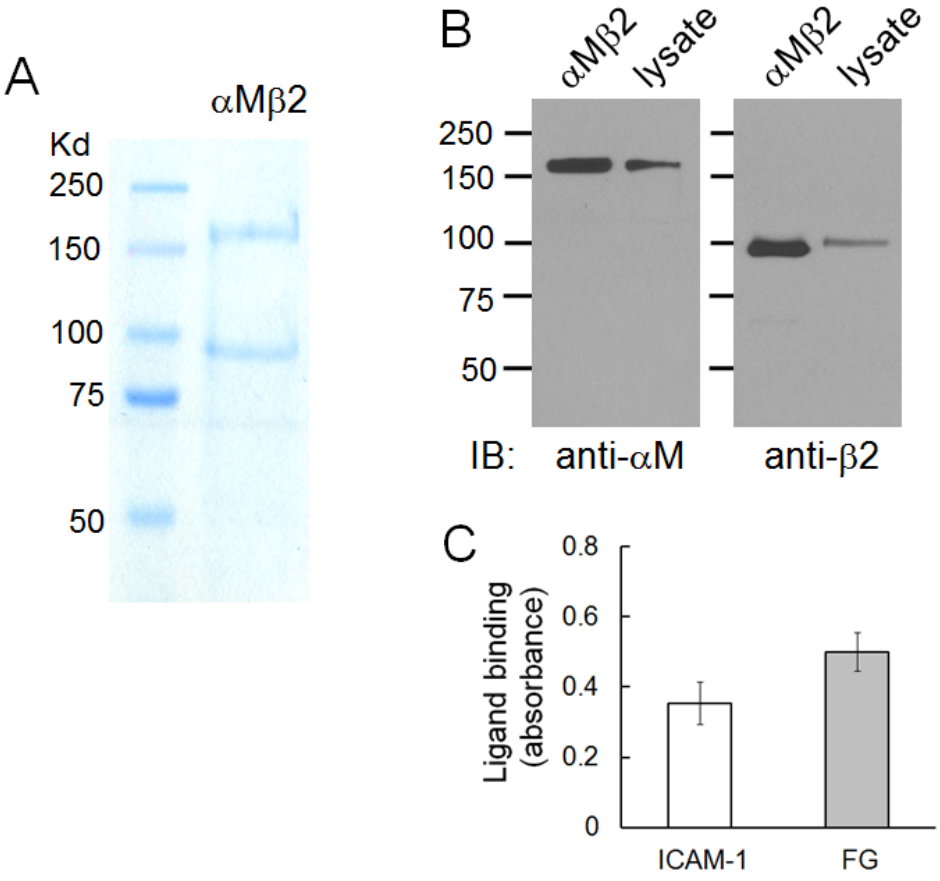
**Supplemental Videos 5-6.** Binding of recombinant wtPDI (Video 5) or dmPDI (Video 6) to intravascular cells was visualized by infusion of Alexa Fluor 647-conjugated anti-mouse Gr-1, Alexa Fluor 488-conjugated anti-β2, and PE-conjugated anti-poly His antibodies.

**Supplemental Videos 7-8.** Isotype control IgG (Video 7) or a function-blocking anti-PDI antibody (RL90, Video 8), 3 μg/g BW, was infused into WT mice as described in Methods. Neutrophils were visualized by infusion of an Alexa Fluor 647-conjugated anti-mouse Gr-1 antibody.

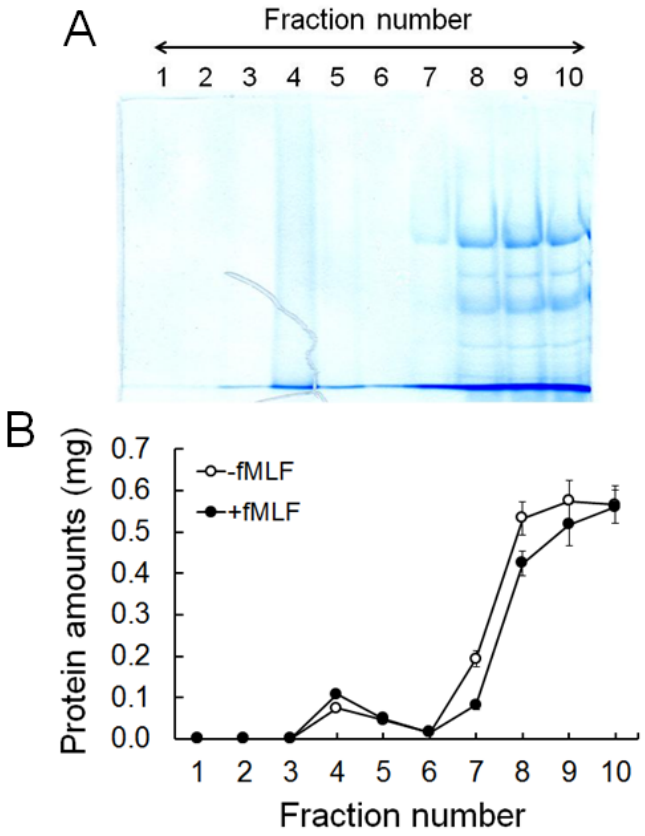
# Figure S1


A
IP: anti-αM
fMLF - + - +
250
150
75
50
αM
PDI
Total
Surface
B
IP: anti-αL
fMLF - + - +
250
150
75
50
αL
PDI
Total
Surface
C
αLβ2 / PDI
control IgGs

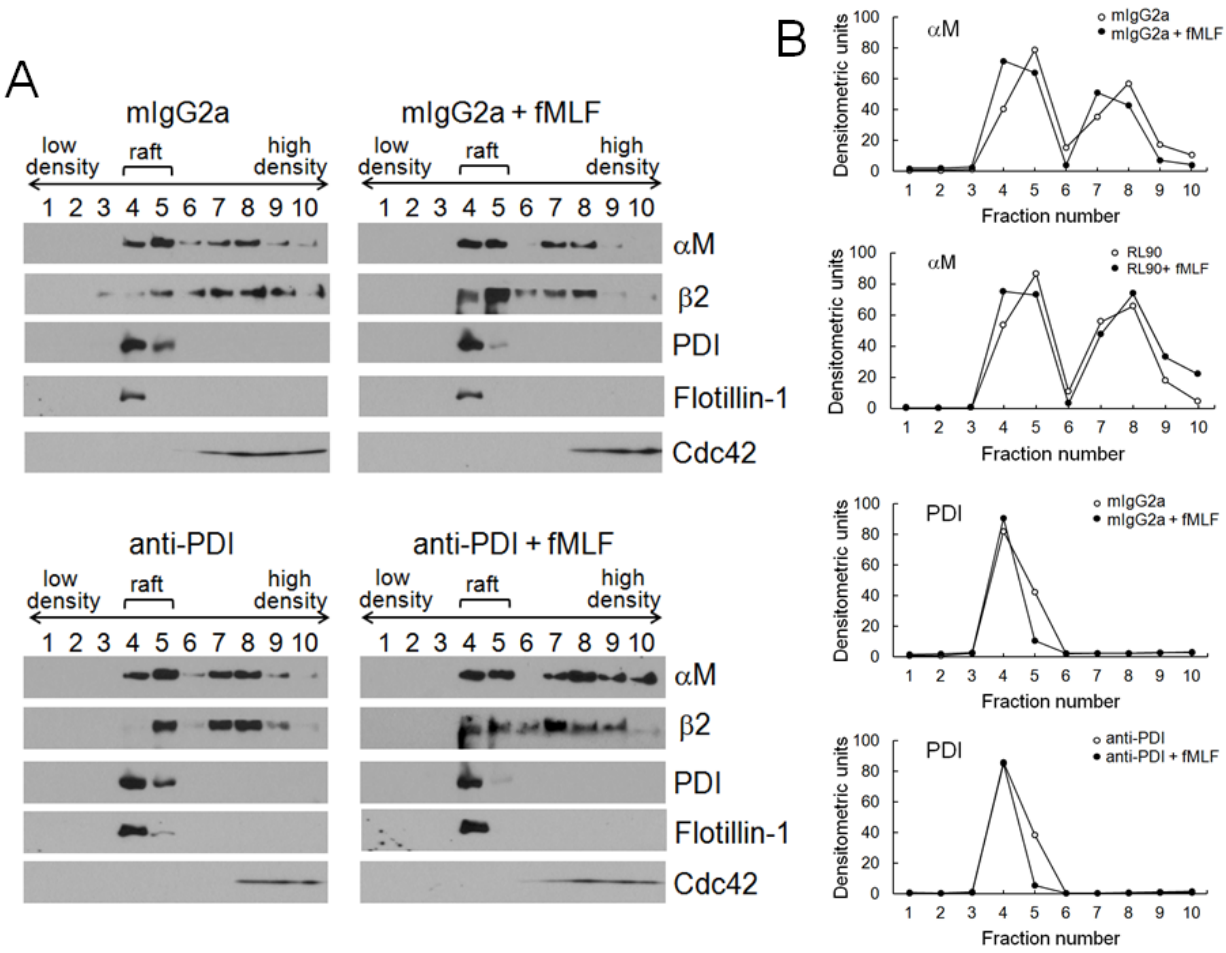
# Figure S2


A
αMβ2
Kd
250
150
100
75
50
B
αMβ2
lysate
αMβ2
lysate
250
150
100
75
50
IB:
anti-αM
anti-β2
C
Ligand binding (absorbance)
0.8
0.6
0.4
0.2
0
ICAM-1
FG

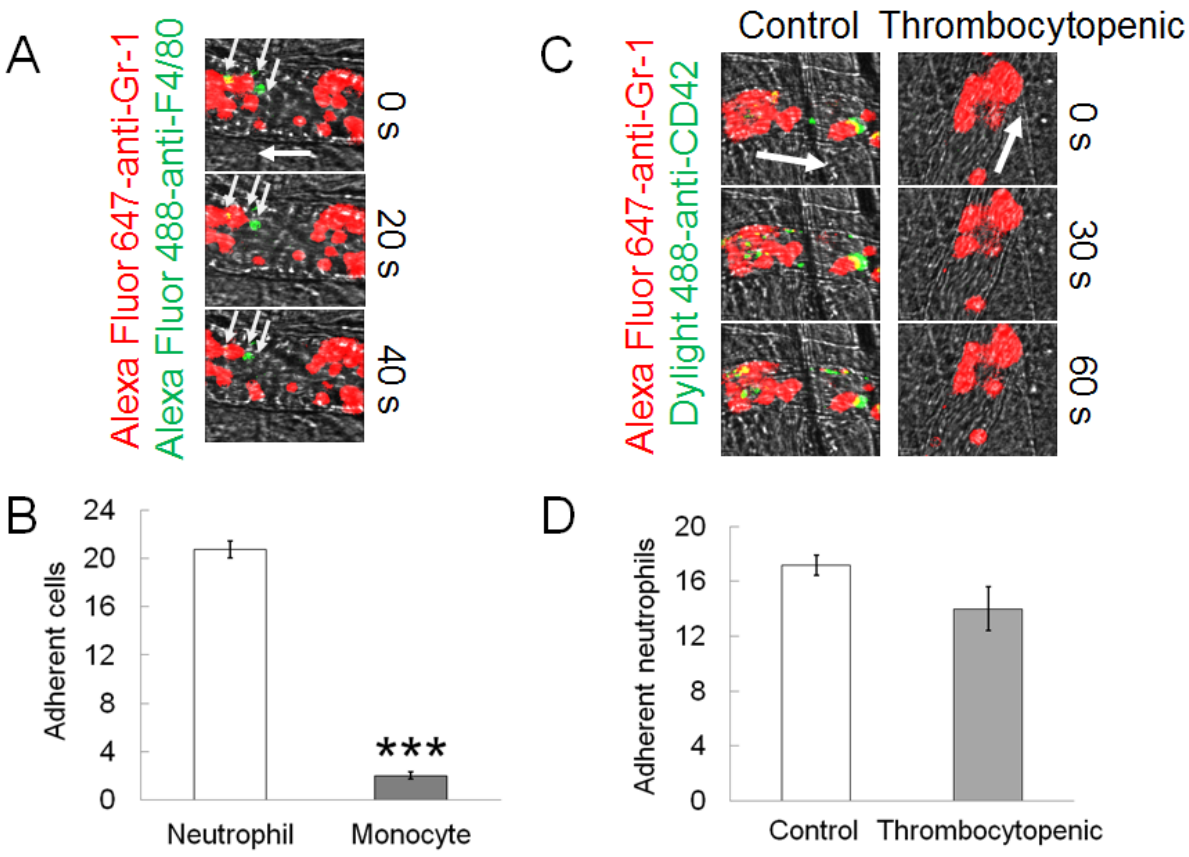
# Figure S3


A
Fraction number
1 2 3 4 5 6 7 8 9 10
B
Protein amounts (mg)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-fMLF
+fMLF
1 2 3 4 5 6 7 8 9 10
Fraction number

# Figure S4


A
mIgG2a
mIgG2a + fMLF
low density
raft
high density
1 2 3 4 5 6 7 8 9 10
αM
β2
PDI
Flotillin-1
Cdc42
anti-PDI
anti-PDI + fMLF
B
αM
mIgG2a
mIgG2a + fMLF
RL90
RL90+ fMLF
PDI
anti-PDI
anti-PDI + fMLF
Densitometric units
Fraction number

# Figure S5


A
Alexa Fluor 647-anti-Gr-1
Alexa Fluor 488-anti-F4/80
0 s
20 s
40 s
C
Control
Thrombocytopenic
Alexa Fluor 647-anti-Gr-1
Dylight 488-anti-CD42
0 s
30 s
60 s
B
Adherent cells
24
20
16
12
8
4
0
***
Neutrophil
Monocyte
D
Adherent neutrophils
20
16
12
8
4
0
Control
Thrombocytopenic