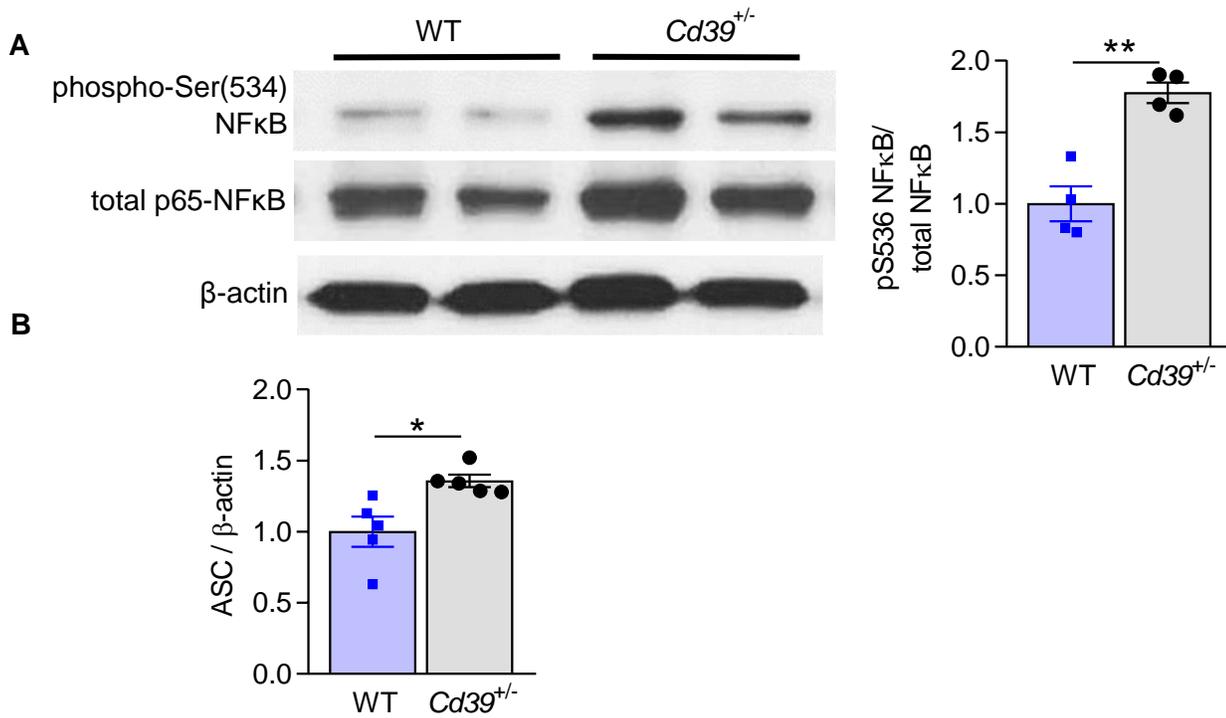


Supplemental Figure 1. CD39 tempers neutrophil extracellular trap formation during venous thrombosis. (A) Flow cytometric analysis of cell surface CD39 expression on neutrophils from WT and *Cd39*^{+/-} mice (n=3, each). (B) Immunoblot quantification for Ly6G in venous thrombi from WT and *Cd39*^{+/-} mice (n=5, each). Data represent mean \pm SEM. * P <0.05; **<0.01; ***<0.01 using two-tailed Student's t test



Supplemental Figure 2. CD39 regulates inflammasome activation in mice with flow-restricted venous thrombosis. (A) Increased Ser(534) phosphorylation of the NFκB p65 subunit in thrombus lysates of *Cd39^{+/-}* compared with WT mice (n= 4, each). **(B)** Quantification of ASC in thrombus lysates (n=5, each). Data represent mean ± SEM. * $P < 0.05$; ** $P < 0.01$ using two-tailed Student's *t* test.

Supplemental Methods:

Animal housing, surgery, and treatments. All experiments were approved by, and carried out in accordance with the University of Michigan Institutional Animal Care and Use Committee guidelines. Mice were housed in a specific pathogen-free barrier facility, and fed standard chow *ad libitum*. *Cd39^{+/-}* mice and genotype controls were generated as previously described.(1) Male mice, aged 8-12 weeks were used for all experiments, and decreased CD39 expression verified by flow cytometry (Accuri C6 plus, BD) using FlowJo v10 analysis software. Flow-restriction (“stenosis”) of the IVC was performed as previously described.(2, 3) Briefly, following anesthesia with ketamine/xylazine, a laparotomy was performed and a ligature (7-0 proline) was fastened around the infra-renal IVC over a blunted 30-gauge needle (which served as a spacer). The spacer was removed, abdomen closed with 5-0 vicryl suture and the mouse allowed to recover. At the given time points, mice were humanely anesthetized and thrombus formation was assessed. IL-1 β neutralizing antibody (BE122) or IgG isotype controls (BioXCell) were administered to mice via IP injection (200 μ g) 24 hours prior to surgery. In some experiments, the IL-1 receptor inhibitor anakinra (Amgen, 100 mg/kg/day, IP) or saline 100 μ L were administered to mice.(4) Bleeding time was assessed as previously described.(5)

Immunohistochemistry and immunofluorescence. Formalin-fixed thrombi were sectioned, and stained with hematoxylin/eosin (H&E), or with rat anti-mouse Ly6G (1A8 clone, Abcam) and appropriate secondary antibody (Thermo) and chromogenic development using 3-amino-9-ethylcarbazole (Vector Labs). Images were acquired with a Nikon Eclipse Ti microscope, and when necessary, stitched together using Nikon Elements software.

Western blotting. Thrombi were homogenized in RIPA buffer with Roche protease inhibitor cocktail pellet and 1% SDS. Protein quantified using BCA protein assay kit (Pierce), and equal quantities were resolved by SDS-PAGE, and transferred to a PVDF membrane. Non-specific

binding was blocked with 4% non-fat milk, followed by incubation with primary antibodies directed against fibrin (59D8, generated by Dr. Marshall Runge,(6) generously maintained and provided by Dr. Charles Esmon), H3cit (Abcam, #5103), total p65 NFkB (Cell Signaling Technologies, CST #8242S), phospho-Ser⁵³⁴ (p65) NFkB (CST, #3033S), NLRP3 (Abcam, #210491), ASC (Santa Cruz, #sc514414), caspase-1 p20 (Adipogen, #AG20B0042c100), interleukin-1 beta (Abcam, #9722), Ly6G (1A8 clone, BD pharm). Appropriate HRP-labeled secondary antibodies (Sigma) and standard enhanced chemiluminescence were used for detection. Band densities were quantified using ImageJ software.

Neutrophil purification, NETosis assay, and adoptive transfer. Neutrophils from bone marrow were isolated as previously described.(2, 7) Briefly, extracted bone marrow cells were separated using a discontinuous Percoll gradient (52%, 69%, 78%) at 1500xg for 30 minutes. Cells were collected from the 69-78% interface. Cells isolated from this layer were verified to have typical nuclear morphology by microscopy, and were >95% Ly-6G-positive by flow cytometry. NETosis was triggered by stimulation with phorbol-12-myristate-13-acetate (PMA, 100 nM, Sigma) or vehicle control for 4 hours at 37°C in RPMI supplemented with 2% bovine serum albumin and 10 mM HEPES buffer. Cells were fixed with 4% paraformaldehyde (PFA). DNA was stained with Hoechst 33342 (Invitrogen). Primary antibody staining with citrullinated histone H3 (Abcam) or IgG control was followed by an appropriate secondary antibody (Thermo). Images were acquired with a Nikon Eclipse Ti microscope as above. NETs were quantified as previously described.(2, 7) Adoptive transfer was performed by retro-orbital injection of 2.5×10^6 neutrophils in 150 μ L saline, isolated using an EasySep mouse neutrophil enrichment kit (StemCell Tech).(8)

IL-1 β ELISA. Quantitative measurement of IL-1 β in plasma following venous thrombosis was performed using a mouse IL-1 β sandwich ELISA (R&D, DY401) per manufacturer instructions.

Statistics. Data analysis was performed using GraphPad Prism 8 software. Normally distributed data were analyzed by unpaired two-tailed Student's *t* test and skewed data were analyzed with Welch's correction. Thrombus frequency was analyzed by Chi squared analysis. Statistical significance was defined as $P < 0.05$.

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