SUPPLLIMENTAL MATERIAL

METHODS

RNA sequencing and data analysis

Neutrophil cell-pellets were lysed, and RNA was isolated using the RNeasy Mini Kit (Qiagen #74106) as per manufacturer's instructions. Libraries were prepared with the Stranded mRNA Prep, Ligation Kit (Illumina). One µg of RNA was processed for each sample and mRNA was purified and fragmented. cDNA was synthesized, and 3' ends were adenylated. Anchor sequences were ligated to each sample and a limited-cycle PCR was performed to amplify and index the libraries. The average library size was determined using an Agilent TapeStation D1000 assay (Agilent Technologies) and libraries were quantitated with qPCR (Bio-rad CFX96 Touch Real-Time PCR, NEB Library Quant Kit for Illumina). Libraries were normalized to 0.5 nM and pooled. The library pool was denatured and diluted to approximately 100pM. A 1% library of 2.5pM PhiX was spiked in as an internal control. Paired end 76 x 76 base pair sequencing was performed on an Illumina NovaSeq 6000. Primary analysis, including base calling and quality scoring, was performed onboard the Illumina NovaSeq 6000 (NovaSeq Control Software v1.8.0; RTA v3). Samples were de-multiplexed, the adapter sequences were removed (the first 9 cycles of sequencing were trimmed), and FASTQ files were generated. Data analysis was performed as we previously described. The quality of the raw FASTQ files was checked through FastQC v0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Trimmomatic v.0.35 was used to trim the low-quality reads with the parameters: SLIDINGWINDOW:4:20 MINLEN:25. The resulted high-quality reads were then mapped to the mouse reference genome (GRCm38.90) using STAR 2.5.1b11 and quantified by RSEM 1.2.3112 following the ENCODE-DCC/RNA-seq pipeline. Differentially expression analysis was performed using Bioconductor limma + Voom (Version: 3.48.3) and EdgeR (Version: 3.34.1) packages.13,14 Benjamini-Hochberg correction was used to obtain adjusted p-values. Statistically significant differentially expressed genes were filtered based on adjusted p< 0.01. Genes with adjusted P value < 0.01 were considered as significant DEGs. The upregulated and downregulated DEGs were analyzed for significantly enriched Gene Ontology pathways using the clusterProfiler package (version 4.8.3). The significance of the enrichment was determined by right-tailed Fisher's exact test followed by Benjamini-Hochberg multiple testing adjustment. The heatmap of all shared DEG between

stimulated vs unstimulated and KO vs control (Figure 4H) was generated by ComplexHeatmap R package (version 2.16.0). Hierarchical clustering of top 100 differentially expressed genes (supplementary figure 6 A and B) was visualized as a heatmap using R package gplots (version 3.1.3).

Peptide and protein identification

Protein identification was evaluated by commercial service from Cell Signaling Technology (CST®), TMT10plex™ Total Proteome profiling service, that provides accurate global profiling of protein abundance in cells and uses multiplexed sample labeling with Tandem Mass Tags™ (TMT™) and liquid chromatography tandem mass spectrometry (LC-MS/MS). Mass spectra were evaluated by CST® using SEQUEST and the GFY-Core platform (Harvard University). Searches were performed against the 20180718 update of the Uniprot Homo sapiens database with a mass accuracy of ± 50 ppm for precursor ions and 0.02 Da for product ions. Total proteome data were filtered to a 1% peptide-level false discovery rate (FDR) with mass accuracy ± 5 ppm on precursor ions and presence ions; IMAC data were filtered to samples with a phosphorylated residue prior to filtering to a 1% protein-level FDR. All IMAC quantitative results were generated using Skyline 16 to extract the integrated peak area of the corresponding peptide assignments. Accuracy of quantitative data was ensured by manual review in Skyline or in the ion chromatogram files. TMT quantitative results were generated using the GFY-Core platform (Harvard University).

Immunofluorescence staining

IVC thrombi were harvested 48-hour post-stenosis, detached from the vessel wall, dried and weighed in a microbalance followed by embedded in optimal cutting temperature compound, frozen at −80°C, and was cut with a cryotome (CryoStar NX70 Cryostat; ThermoFisher Scientific) into 10-µm sections. 10 µm cryosections were then blocked in a ready-to-use protein block (#ab64226, Abcam) for 30 min at room temperature followed by three times washing with 1X PBS-Tween (PBST). Sections were incubated overnight with primary antibodies for anti-rabbit Cit-Histone H3 (Arg2, Arg8, Arg17) (Thermo Fisher Scientific, #630-180ABBOMAX, 1:300) and FITC-Ly6G (Thermo Fisher Scientific, 11-9668-82, 1:250) at 4°C overnight. Next day, for anti-rabbit Cit-Histone H3 staining only, sections were washed with 1X PBST thrice and incubated with Alexa Fluor 647 goat anti-mouse (Thermo Fisher Scientific, #A21244, 1:500) secondary antibody for 1 h in the dark at room temperature. Slides were then washed

three times with 1X PBST. All slides were then blotted dry, sealed with mounting medium containing DAPI (Thermo Fisher Scientific, #P36983) and allowed to dry overnight before imaging. Images were acquired using a fluorescence microscope EVOS™ M5000 Imaging System (Invitrogen) at 20 x magnification. Quantification was carried out using ImageJ software (NIH Image J, USA) and assessed with GraphPad Prism 8.0.0 to identify statistically significant differences among groups.

Antibody treatment

Male WT mice were randomly assigned to receive either 4C1 (BD Pharmingen, purified NA/LE Rat Anti-Mouse CD14 # 557896) or isotype control antibody (BD Pharmingen Purified Rat IgG2b, κ Isotype Control # 553986) at the dose of 4 mg/kg intravenously, 30 mins before the surgery and 24 hours post-surgery.

ELISA assay

Plasma G-CSF (R&D systems # MELA20), plasma IL-10 (R&D systems # M1000B-1), plasma elastase (R&D systems # MELA20) and plasma MPO (R&D systems # DY3667) were analyzed using commercially available kits.

FIGURE LEGENDS

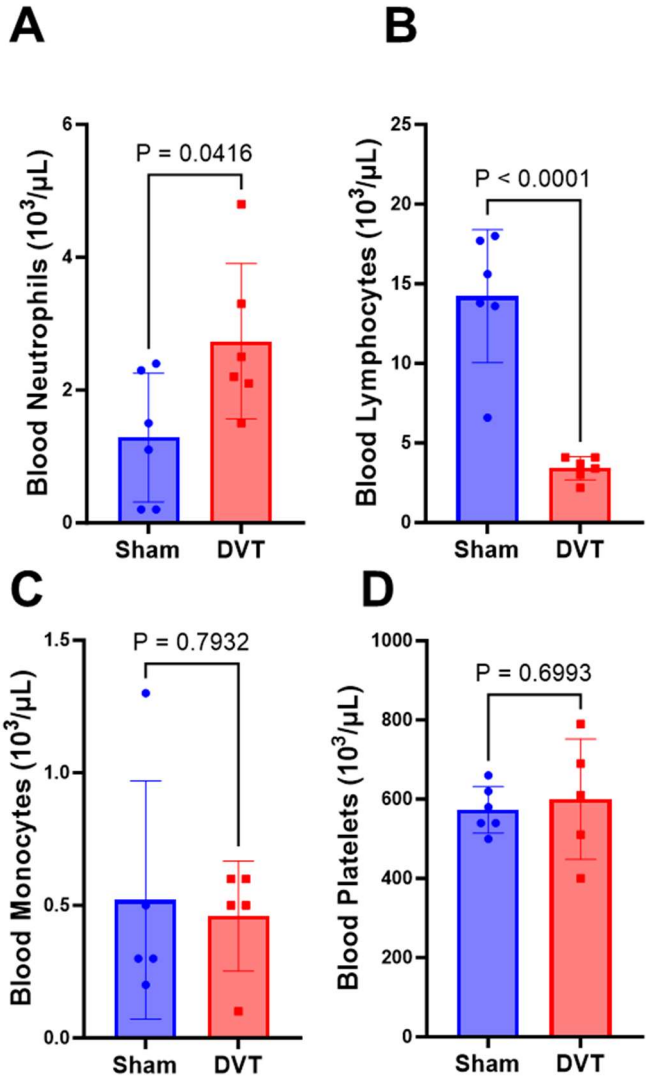
Supplemental Figure 1: Blood was collected at 3-hr post-surgery from mice with sham-surgery and mice with DVT for analysis of blood neutrophil (A), blood lymphocytes (B), blood monocytes (C), platelets (D). All data are from male mice and are mean ± SEM and by an unpaired Student t test; n = 5 (A-D).

Supplemental Figure 2: Hierarchical clustering of gene expression from RNA-seq data (3-hour post-surgery) of neutrophils isolated from mice with DVT and mice with sham-surgery.

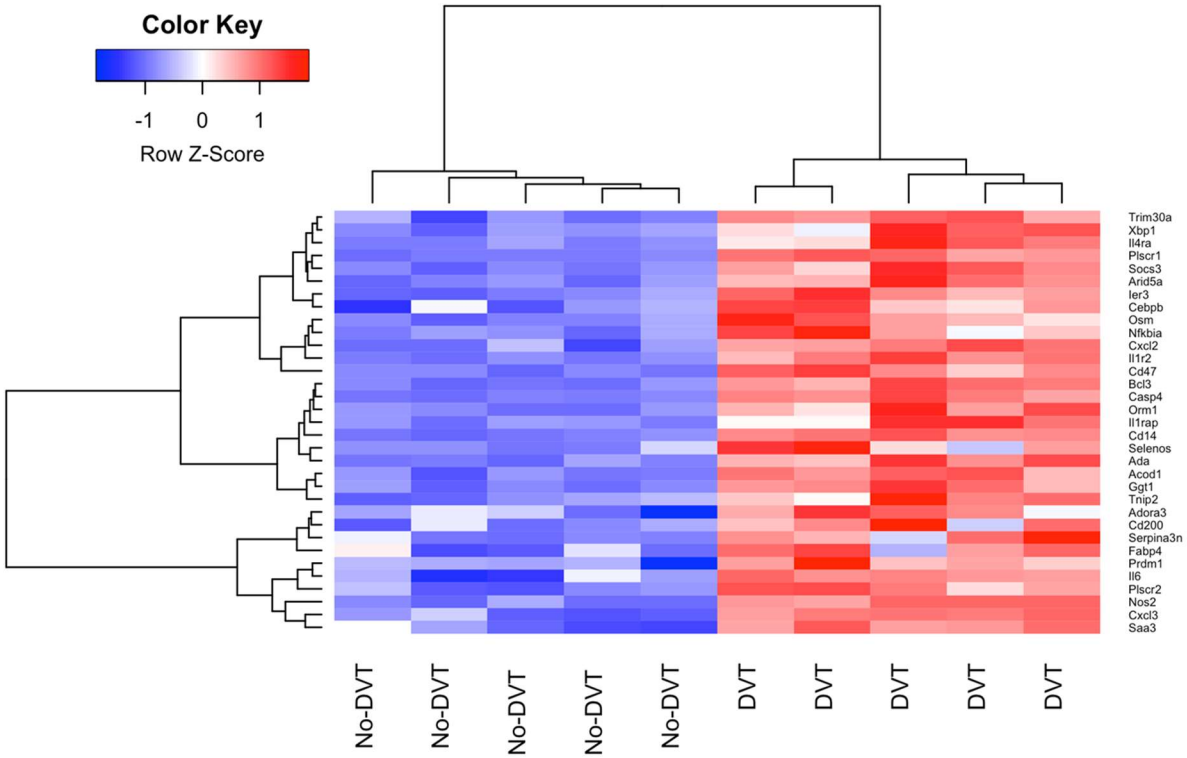
Supplemental Figure 3: List of top 10 downregulated protein from neutrophils isolated from mice with DVT and mice with sham-surgery.

Supplemental Figure 4: Left, representative image of flow-cytometric analysis of CD14 positive neutrophils. Right, quantification of CD14 expression in peripheral monocytes and neutrophils following IVC stenosis or sham-surgery in mice. Data are mean ± SEM, analyzed by two-way ANOVA followed by Holm-Šídák's multiple comparisons test. n = 6.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

Sr No	Protein Name	Normalized Sum Signal : Noise (Mean ± SEM)		Raw ratio
		Sham	DVT	
1	SEC11L3	223 ± 21.4	118 ± 32.9	0.53
2	DDX27	30 ± 3.7	16 ± 1.5	0.52
3	BAI3	496 ± 27.3	248 ± 42.9	0.5
4	OAS1	396 ± 24.6	197 ± 18.3	0.5
5	GRN	707 ± 4.5	346 ± 77.4	0.49
6	SLPI	1882 ± 285.5	884 ± 85.8	0.47
7	OSTC	596 ± 109.6	262 ± 34.3	0.44
8	ABITRAM	753 ± 97.7	316 ± 22.8	0.42
9	MPC2	899 ± 105.3	325 ± 30.0	0.36
10	UGT1A1	246 ± 11.9	75 ± 10.0	0.31

Supplemental Figure 4