Online Supplement

Supplemental Results

Generation of kallikrein through the contact factor pathway. The data in Figure 1 shows that addition of kaolin to whole blood initiates release of α-defs by neutrophils, indicating that activation of the intrinsic pathway of coagulation is responsible. To help validate this conclusion, we monitored activation of the contact factor pathway by measuring the generation of kallikrein under the same conditions. Addition of kaolin to whole blood caused a significant increase in kallikrein activity compared with addition of thrombin (145.6 ± 11.7 mU/ml vs. 11.8 ± 3.3 mU/ml, n=7 donors, $p<0.0063$) (Figure S1A). The increase in kallikrein generated by kaolin (but not by thrombin) was associated with an increase in the concentration of α-defs in the serum (406.6 ± 36.4 nM vs 14.9 ± 2.3 nM, n=7, $p<0.0044$). Thus, the conditions that generate kallikrein correspond to those leading to the release of α-defs.

Reinforcement of α-def release by the extrinsic pathway of coagulation. Rapid activation of the extrinsic pathway by adding a high concentrations of tissue factor (5 pM)[7,8] to citrated blood had minimal impact on the generation of kallikrein and subsequent release of α-defs (Figure S1B). In contrast, when a lower concentration of tissue factor was added (0.2 pM), kallikrein activity increased from 9.7 ± 1.5 to 23.5 ± 1.9 mU/ml (n=6, $P<0.05$) in parallel with an increase in the concentration of α-defs in the serum from 12.6 ± 1.5 to 34.5 ± 2.4 (n=6, $p<0.05$) (Figure S1B). At this low concentration of tissue factor, addition of CTI decreased kallikrein generation from 22.6 ± 1.9 to 13.9 ± 1.2 mU/ml (n=6, $p<0.05$) and the level of α-defs from 32.4 ± 2.8 to 11.4 ± 1.6 nM (n=6, $p<0.05$) (Figure S1B). Together, these results indicate that low levels of tissue factor and slow activation of the extrinsic pathway augments the release of α-defs by generating additional kallikrein.

Kallikrein induces the release of endogenous α-defs from neutrophils in whole blood. Activation of the intrinsic, but not the extrinsic, pathway of coagulation in whole blood initiates release of α-defs from neutrophils (Figures 1 A-F). As an independent approach, kaolin, purified FXIIa, FXIa, FVIIa, FXa or thrombin was added to citrated whole blood, followed by addition of calcium. Only the addition of kaolin and FXIIa to whole blood induced the release of α-defs, an effect that was inhibited by CTI (50 µg/ml), which blocks FXIIa, or by aprotinin, which blocks

kallikrein (Figure S2). These results support the finding that the combination of FXIIa and prekallikrein induces the release of α-defs from isolated human neutrophils (Figure 1).

Effect of endogenous α-defs from neutrophils on clot formation *in vitro*. Exogenous α-Def-1 increases the rate and extent of fibrin polymerization (Figures 2A and B). To see whether the same effect occurs in a more physiologic environment, supernatants from isolated human neutrophils incubated with phorbol myristate acetate (PMA) or with FXIIa and prekallikrein were added to fibrinogen followed by adding thrombin. Supernatants from activated, but not unactivated, neutrophils increased the rate of fibrin polymerization (Figure S3A) and the maximal clot turbidity (Figure S3B), replicating the effect of purified α-Def-1. Fibrin formation was prevented when isolated neutrophils were incubated with FXIIa and prekallikrein in the presence of CTI or aprotinin (Figures S3A-B). No change in clot dynamics was seen following addition of supernatants from neutrophils incubated with FXIIa alone (Figures S3A and B). These findings demonstrate that release of α-defs from activated neutrophils accelerates fibrin clot formation.

Effect of endogenous α-defs from neutrophils on tPA-mediated clot lysis. Synthetic α-Def-1 prolongs the time required to cause 50% lysis of preformed fibrin clots by tPA in a dose-dependent manner (Figures 4A and 4B). To see whether the same effect is seen in a more physiologic environment, supernatants from isolated human neutrophils incubated with PMA or with FXIIa and prekallikrein were studied in lieu of synthetic α-Def-1. Supernatants from activated, but not unactivated, neutrophils inhibited fibrinolysis and the effect was abrogated by CTI and by aprotinin (Figure S4A). Again, no effect on clot lysis was seen when supernatants from neutrophils incubated with FXIIa alone were studied (Figure S4A).

Effect of α-Def-1 on uPA mediated clot lysis. α-defs inhibit tPA- but not uPA-mediated lysis of preformed fibrin clots *in vitro*[1]. In addition, α-defs directly affect fibrin structure (Figures 3A, 3B, 5E, 6A and 6B). Therefore, we next compared the effect of tPA and uPA on the lysis of clots formed from fibrinogen in the presence of α-defs. To address this question, α-Def-1 was either added to fibrinogen prior to clotting or after its formation together with plasminogen and either tPA or uPA. Addition of α-Def-1 and plasminogen prior to clot formation inhibited uPA-mediated lysis (Figure S4B), whereas once fibrin was formed addition of α-Def-1 together with plasminogen had no effect on uPA-mediated fibrinolysis (Figure S4B). The same outcome was observed using supernatants from isolated human neutrophils activated by FXIIa and prekallikrein as the source of α-defs (data not shown). The absence of an inhibitory effect of α-defs on plasminogen or

urokinase added to fibrinogen supports the concept that the predominant site of anti-fibrinolytic activity is on fibrin itself. This is consistent with the fibrin specificity of tPA but not uPA[2]

Incorporation of endogenous α-defs from neutrophils into fibrin clots *in vivo*. Exogenous α-defs added to purified human fibrinogen bind to fibrin following addition of thrombin (Figure 1F) and when clotting of human blood is initiated by kaolin (Figure 1G). Similarly, over 90% of the α-defs released when blood from Def^{++} mice was activated by addition of kaolin were incorporated into fibrin (Figure S5). The release of α-defs from mouse neutrophils was inhibited by colchicine, which inhibits degranulation, as well as by inhibitors of FXIIa (CTI) and kallikrein (aprotinin) (Figure S5).

Prothrombotic effects of endogenous α-defs from neutrophils on venous thrombosis *in vivo*. Clots formed in the IVC of Def^{++} mice are dramatically larger than those formed in WT mice (Figures 5C-E). To examine the contribution of neutrophil-derived α-defs to this phenotype, neutrophils were depleted from $Def^{+/+}$ mice using the anti-mouse mAb Ly6G[3]. Clotting of neutrophil depleted blood was initiated by adding kaolin *in vitro* and clot formation in response to IVC occlusion was examined *in vivo*. Neutrophil depletion inhibited release of α-Def-1 into serum (Figure S6A) and abolished the difference in clot formation between $Def^{+/+}$ and WT mice (Figure S6B), similar to the effect of colchicine (Figure 6B). Neither neutrophil depletion nor colchicine had a comparable effect on clot size in WT mice (Figure S6B), nor did either affect the prothrombin time or partial thromboplastin time in $Def^{+/+}$ or WT mice (data not shown).

Activation of the intrinsic pathway of coagulation, release of α-Def-1 release, and clot size *in vivo*. Activation of the intrinsic pathway of coagulation triggers the release of α-defs from neutrophils *in vitro* (Figures 1B-E). To determine if this sequence of effects mediates enhanced formation *in* vivo, Def^{++} mice were given CTI (4 μg/g body weight[4]) intravenously immediately before IVC stenosis was initiated and every 12 hours thereafter until thrombi were extracted. CTI inhibited release of α-Def-1 in the plasma (Figure S6A) and reduced clot size *in vivo* (Figure S6B).

Supplemental Methods

α-Def-1. α-Def-1 provided by Dr. Wuyan Lu (Univ. MD School of Medicine) was produced by solid-phase peptide synthesis using 3 different pairs of protection groups to ensure correct disulfide coupling[5]. The final protein product was purified using reverse-phase HPLC,

characterized by acid urea gels, Western blots and LC-MS/MS. The antimicrobial activity was confirmed using radial diffusion[6] and its coagulant function measured by inhibition of ADAMTS13 activity[7]. α-Def-1 was radiolabeled with I^{125} as previously reported[8].

Binding of ^{125}I-α-def to fibrin(ogen). Binding of α-Def-1 to fibrinogen and fibrin was measured as described[8]. Briefly, ^{125}I-α-def (28 µM) was incubated with fibrinogen (100 µg/ml) or soluble fibrin[9] (100 µg/ml) in 200 µL of phosphate buffered saline (PBS) or in PBS alone for 60 min at 24°C. The mixture was loaded onto a Sephacryl S-100 gel filtration column. Radioactivity in successive 0.5 mL fractions was measured and the protein concentration estimated by measuring the optical density (OD) at 280 nm.

Dynamic clot turbidity. Clotting of purified fibrinogen (2.5 mg/mL in 50 µL; HYPHEN-BioMed, Neuville-Sur-Oise, France) was initiated by adding an "activation mix" (50 µl) containing 0.07 U/ml human α-thrombin (Sigma) in 50 mM Tris-HCl/140mM NaCl, pH=7.4, plus 0-10 µM α-Def-1 and 10 mM calcium chloride using low volume 1 x 8 Stripwell 96-well plates (Corning, Corning, NY). Fibrin formation was evaluated by monitoring the change in turbidity (A_{405}) over 2 hours at 37°C using a Synergy 2 multi-mode microplate reader and Gene5 software (BioTeK, Winooski, VT). A turbidimetry curve was processed using SigmaPlot (Systat Software, San Jose, CA) with the following parameters of fibrin formation extracted: 1) the lag phase (Lag), i.e. the time from activation until an increase in the optical density of 0.01, which measures the time needed for protofibril formation; 2) the slope of the curve or the rate of polymerization (V) taken between the end of the lag phase through the linear part of the curve, which measures the velocity of lateral aggregation of protofibrils and fiber formation; 3) the maximum optical density (A_{max}) at the plateau, which reflects the average cross-sectional area of fibrin fibers.

Release of α-defs through activation of the extrinsic pathway of coagulation. Tissue factor (0.2 or 5 pM[7,8]), kaolin or thrombin (0.33 U/mL) was added to citrated blood from healthy donors for 30 min at 37°C. Clots were separated by centrifugation at 1500 x g for 10 min. Kallikrein activity in serum was measured using the Bio Vision kit (Milpitas CA) and the concentration of α-defs by ELISA.

Quantification of neutrophils in blood clots formed in-vivo. We followed the method described by Wang et al.[14] Clot sections were stained with H&E. Clot images were captured at 40X magnification using a Nikon Ti motorized inverted microscope with a NIKON DS-Fi1 color

CCD camera. On each slide, the number of neutrophils in 12 non-overlapping high power fields was counted. Each slide was examined by two observers blinded to the study protocol. The mean number of neutrophils counted was reported.

Dynamics of α-defs release during the clot formation. Isolated PMNs were resuspended in autologous plasma. In some samples, coagulation was activated with kaolin and the time to clot formation was measured by TEG. In a second set of samples, clotting was initiated by adding kaolin after the first set; the reaction was stopped by adding HCl at different times before clotting was first detected as assessed by TEG followed by centrifugation and the release of α-defs was measured by ELISA.

Change in the internal structure and composition of thrombi induced by α-defs. We quantified differences in the internal structure and composition of thrombi formed in Def^{++} mice in the absence and presence of colchicine by analyzing 3-5 randomly selected scanning electron microscopy images of each type. To quantify the density (or porosity) of the thrombi, the relative area occupied by intercellular space was calculated manually by applying a grid with 2-μm squares on the scanning micrographs and counting the number of squares (n=3285) containing individual RBCs and tightly packed RBC arrays (or other components) versus the number of squares (n=1875) that were unoccupied, in other words the empty space between cells or other thrombus components. The results show that in thrombi from Def^{++} mice without colchicine, the average unoccupied area between cells (i.e. the porous area) comprised only 15% of the total, while in thrombi from Def^{++} mice given colchicine, the unoccupied porous area was 65% of the total area (p=4.7E-289, chi-square test). To quantify the fraction of RBCs compressed during intravital contraction of thrombi, between 300 and 1200 RBCs were analyzed on the scanning micrographs for percentage of biconcave (n = 301) and polyhedral or polyhedral-like cells (n = 1272). In thrombi from Def^{++} mice without colchicine, the percentage of deformed (polyhedral or polyhedral-like) RBCs comprised 96% of the total, while in thrombi from Def^{++} mice given colchicine, the fraction of deformed RBCs comprised only 30% of the total (2.9E-172, chi-square test).

Rate of fibrinolysis by tPA. Fibrin clots were formed as described above but the A_{405} was monitored for up to 4.5 hours. Plasminogen (200 nM) was added to the fibrinogen before clotting, followed by thrombin (0.33 U/ml final) and tPA (50 pM final) in the presence or absence of α-Def-1. Because the turbidities were far higher in the presence of α-Def-1, data were normalized based

on maximal turbidity. We measured the 50% lysis time, defined as the time elapsed from the maximal to the half-maximal A_{405} value ($Lys50_{MA}$), and the time from initiation of clotting needed to reduce the maximum turbidity of the clot to the half-maximal value ($Lys50_{t0}$).

Immunofluorescent staining and confocal microscopy of fibrin clots. To study the structure of hydrated clots, fibrin was allowed to form over 30 min at room temperature after mixing the components as described above, in the presence or absence of α-Def-1 (5 μM final concentration, concertation within the lower range found in fibrin clots (Figure 1G)) and then incubated with 5 μg/ml Alexa Fluor 546-conjugated anti-human fibrin mouse monoclonal antibody (clone 59D8[10]; provided by M. Poncz, Childrens Hospital of Philadelphia) at in 50 mM Tris-HCl/140mM NaCl, pH=7.4 for 18 hours at 4°C. Immunolabeled fibrin networks were imaged using a Zeiss LSM 710 laser scanning confocal microscope equipped with a Plan Apo 40x water immersion objective lens (NA 1.2). The z-stack distance between the slices was set as 0.3 μm with a 1024x1024 pixels resolution for each slice. Three-dimensional (3D) reconstruction and maximal projection was performed using the Volocity 6.3 software (Perkin Elmer).

Scanning electron microscopy. Fibrin clots formed in the absence or presence of α-Def-1 were washed 4 times in 0.05 M sodium cacodylate supplemented with 150 mM NaCl (cacodylate buffer hereafter), pH 7.4, fixed overnight in the cacodylate buffer containing 2% glutaraldehyde, dehydrated in ethanol, dried with hexamethyldisilazane, and sputter coated with gold-palladium. Triplicate samples formed in the presence of 0, 1, 2.5 or 5 μM α-Def-1 were examined using a FEI Quanta FEG250 scanning electron microscope (FEI, Hillsboro, OR).

Release of α-defs *ex vivo*. To prepare serum or plasma, blood was collected from healthy volunteers into a glass vacutainer tube in the absence of an anticoagulant or in citrate (0.32% final concentration). Thrombin, tissue factor or kaolin plus calcium chloride, were added to citrated blood where indicated to generate serum. The concentrations of α-defs 1-3 in plasma and serum were measured by ELISA[11]. Clot formation and lysis were monitored by thromboelastography using a TEG 5000 (Haemonetics, Braintree, MA)[2].

Effect of α–Def-1 on urokinase (uPA)-mediated clot lysis. Fibrin clots were generated using purified fibrinogen (2.5 mg/mL in PBS) and thrombin (0.07 U/ml). Plasminogen and uPA (50 pM final), alone or with α-Def-1 (1 μM), were added either to fibrinogen prior to clot formation induced by thrombin or on the surface of preformed clots[1]. Fibrinolysis was determined by measuring

fibrin degradation products released into the supernatant (OD 280) until total fibrinolysis was achieved and the time to 50% lysis was determined.

Isolation of neutrophils. Neutrophils were isolated from the blood of healthy volunteers using dextran sedimentation, hypotonic lysis, and separation over Ficoll-Hypaque, as previously reported[1,12]**.** The neutrophils (7×10^7 cells) were suspended in 1 ml of HBSS supplemented with 1 mg/ml gelatin and kept on ice until use[13].

α-defs in blood clots ex vivo. Clots were formed from whole blood either by activating the contact pathway on glass or by adding thrombin (0.33 U/mL) and calcium chloride (10 mM) to citrated blood. Clots were separated by centrifugation at 1500 x g for 10 min. PBS with or without tPA (2 μM) was added to the isolated blood clot and incubated for one hour at 37ºC to initiate lysis. Residual pieces of fibrin were removed by centrifugation (1500 x g for 10 min) and incubated in PBS containing tPA (2 μM) and plasminogen (5 μM) for additional 6 hours at 37ºC. Residual fibrin was re-centrifuged (1500 x g for 10 minutes), the supernatants were separated, chilled to 4ºC, and the concentration of α-defs released into the supernatants as a result of lysis was measured by ELISA.

Effect of α-defensins on venous clot formation in vivo: Inferior vena cava venous thrombosis model. To study venous thrombosis, partial IVC occlusion was performed as described[14] using a model that combines external compression with a reduction in blood flow. Briefly, mice were anesthetized by intraperitoneal injection of ketamine and xylazine. A midline incision was performed and the small bowel was exteriorized with care not to damage the endothelium. No branches were ligated. A 5-0 proline suture was placed longitudinally along the ventral surface of the IVC. A 4-0 mersilk suture was tied around the IVC and a proline suture was immediately placed beneath the branch of the left renal vein. The suture was then removed, allowing blood flow to resume. Animals were sacrificed 48 hours later and the thrombi were extracted and weighed. In some experiments colchicine (1 mg/liter) or a corresponding volume of saline was added to the drinking water twice weekly for two weeks prior to IVC ligation[11]. Plasma levels of α-defs were measured and clot weights were compared.

Bone marrow transplantation (BMT). BMT was performed as previously reported[11]**.** Briefly, bone marrow (BM) from male Def^{++} mice or WT mice was transplanted into 6-8 week-old irradiated syngeneic male WT mice or BM from WT mice was similarly transplanted into $Def^{+/+}$ mice. All

mice were on a C57BL/6 background. Briefly, bone marrow was collected from femurs and tibias of donor WT and $Def^{+/+}$ mice[15,16]. On the day of BMT, 6- to 8-week-old recipient WT and $Def^{+/+}$ mice received 1000 cGy total body irradiation in split doses. Three hours post-irradiation, 2-5 × 10^6 BM cells in a volume of 150-200 µL of PBS were injected via the tail vein. Blood was obtained by retro-orbital puncture and white blood cell recovery and plasma levels of α-defs were measured. Upon recovery, typically four weeks post-BMT, partial IVC stenosis was induced and clot weight was measured.

Neutrophil depletion. In some experiments, mice were depleted of neutrophils by four intraperitoneal injections (100 µg/mouse) of rat anti-mouse Ly6G mAb over a span of one week as reported by others[3] Depletion was confirmed by flow cytometric analysis 5 days post-administration.

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Legends

Figure S1A. Activation of the contact pathway. Citrated blood collected from healthy donors was activated by adding thrombin (0.33 U/mL) or kaolin plus calcium chloride for 30 min. Sera were separated by centrifugation and kallikrein activity was measured in the sera. The results shown are the mean ± SD kallikrein activity in samples from 7 healthy volunteers in each group (*p <0.05).

Legend S1B. Reinforcement of α-def release by the extrinsic pathway of coagulation. Isolated human PMNs were suspended in autologous normal plasma. Coagulation was initiated by adding 5pM tissue factor (TF) (white bars) or 0.2 pM TF (0.2 pM) alone (black bars) or in presence (grey bars) of CTI (50 µg/ml) for 30 min. The clots were centrifuged, sera were removed and the amout of of kallikrein generated and the amount of α-defs released from the neutrophils were measured. The Y axis shows kallikrein activity (mU/ml) and α-def (nM). The results shown are the mean ± SD outcomes in samples from 7 healthy volunteers (*p <0.05).

Figure S2. Release of endogenous α-defs from neutrophils by kallikrein. Citrated plasma from healthy volunteers was prepared. Coagulation was initiated by adding calcium chloride (1 mM) plus purified kaolin, FXIIa (8 nM), FXIa (0.5 nM), FVIIa (5 nM), FXa (0.5 nM) or thrombin

(0.33 U/mL) alone or with CTI (50 µg/ml), colchicine (10 nM), or aprotinin (400 KIU/mL) (final concentrations) for 60 min at 37°C. The blood was centrifuged at 1500 x g for 10 min and the concentration of α-defs in the supernatants was measured by ELISA[11]. The results shown are the mean ± SD from 9 human healthy volunteers (*p<0.05) respectively.

Figure S3A. Effect of endogenous α-defs released by neutrophils on the rate of fibrin clot formation *in vitro*. Isolated human neutrophils were incubated in the presence of calcium chloride (10 mM) together with PMA (100 ng/ml) or with FXIIa (8 nM) alone or with prekallikrein (20 µg/ml), with CTI (50 µg/ml) or with aprotinin (400 KIU/mL) at 37°C for 30 minutes. The blood was centrifuged at 1500 x g for 10 min and the supernatants were added to purified fibrinogen (2.5 mg/ml) (1/10 v/v). Clotting was initiated by adding an "activation mix" containing 0.07 U/ml human α-thrombin and 10 mM calcium chloride. The lag time to fibrin formation was evaluated by monitoring the change in turbidity (A_{405}) as in Figure 2. In some experiments, synthetic α-def-1 (1 µM) was used as a positive control. The mean ± SD of 3 experiments is shown (*p<0.05).

Figure S3B. Effect of endogenous α-defs from neutrophils on the size of fibrin clots *in vitro*. Supernatants from isolated human neutrophils were prepared as in Figure S2. The experiment was performed as in the previous figure and Figures 2A and B. The maximum absorbance (Amax) was used to determine clot size as described in Figure S2. The mean ± SD of 3 experiments is shown.

Figure S4A. Effect of endogenous α-defs from neutrophils on the rate of tPA-mediated fibrinolysis. Supernatants from isolated human neutrophils were prepared as in Figure S2. Fibrin clots were prepared as in Figure S2 from fibrinogen supplemented with plasminogen (200 nM) and tPA (50 pM final). Neutrophil supernatants were added for 60 min at 37°C. Dynamic

clot turbidity (A_{405nm}) was measured as in Figure 4A. The time to attain 50% lysis of preformed fibrin clots was determined as in Figure 4B. In some experiments synthetic α-Def-1 (1 µM) was used as the positive control. The mean ± SD of 3 experiments is shown (*p<0.05).

Figure S4B. Effect of α-Def-1 and endogenous α-defs from neutrophils on the rate of uPA-mediated fibrinolysis. Fibrin clots were generated using purified fibrinogen (2.5 mg/mL in PBS) and thrombin (0.07 U/ml). Plasminogen and either tPA or uPA (50 pM final) alone or with α-Def-1 were added either to fibrinogen prior to clot formation or added onto the surface of preformed fibrin clots (1 µM). Fibrinolysis was determined by repetitively measuring fibrin degradation products released into the supernatant (OD 280) until total fibrinolysis was achieved and the time to 50% lysis was determined. Similar results were found using supernatants from isolated neutrophils stimulated with FXIIa and prekallikrein (data not shown). The mean ± SD of 3 experiments is shown (*p<0.05).

Figure S5. Incorporation of endogenous α-defs from neutrophils into fibrin clots. Citrated whole blood from $Def^{+/+}$ mice was clotted by adding calcium chloride (10 mM) together with kaolin alone or together with either CTI (50 µg/ml), colchicine (10 nM) or aprotinin (400 KIU/mL) at 37°C for 30 min. Clots were separated by centrifugation at 1500 x g for 10 min, lysed by addition tPA (1 µM final), re-centrifuged and the concentration of α-defs in the supernatants was measured by ELISA[11]. Unclotted citrated plasma from $Def^{+/+}$ mice was used as the negative control. The mean ± SD of 3 experiments is shown.

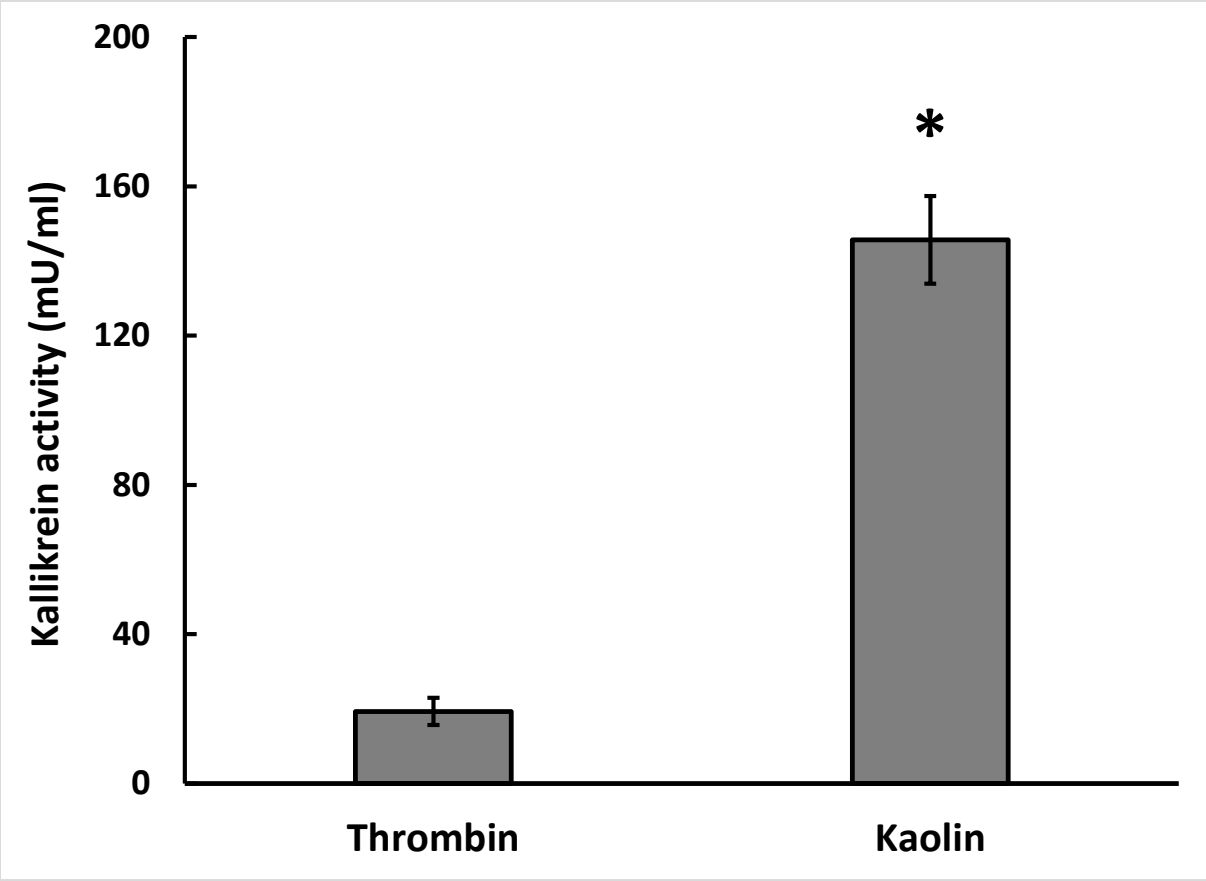
Figure S6A. Prothrombotic effects of endogenous α-defs from neutrophils on venous thrombosis *in vivo*. Neutrophils were depleted from $Def^{+/+}$ mice using mAb Ly6G[3]. In some experiments, mice were given CTI (4 µg/g bodyweight)[4] intravenously immediately before IVC

stenosis was initiated and every 12 hours thereafter until thrombi were extracted. In other experiments, colchicine was given intravenously (0.5 mg/kg) as in Figure 7B. Thrombus weight was measured as in Figure 4. The mean ± SD in 8 mice is shown (* and #, $p<0.05$).

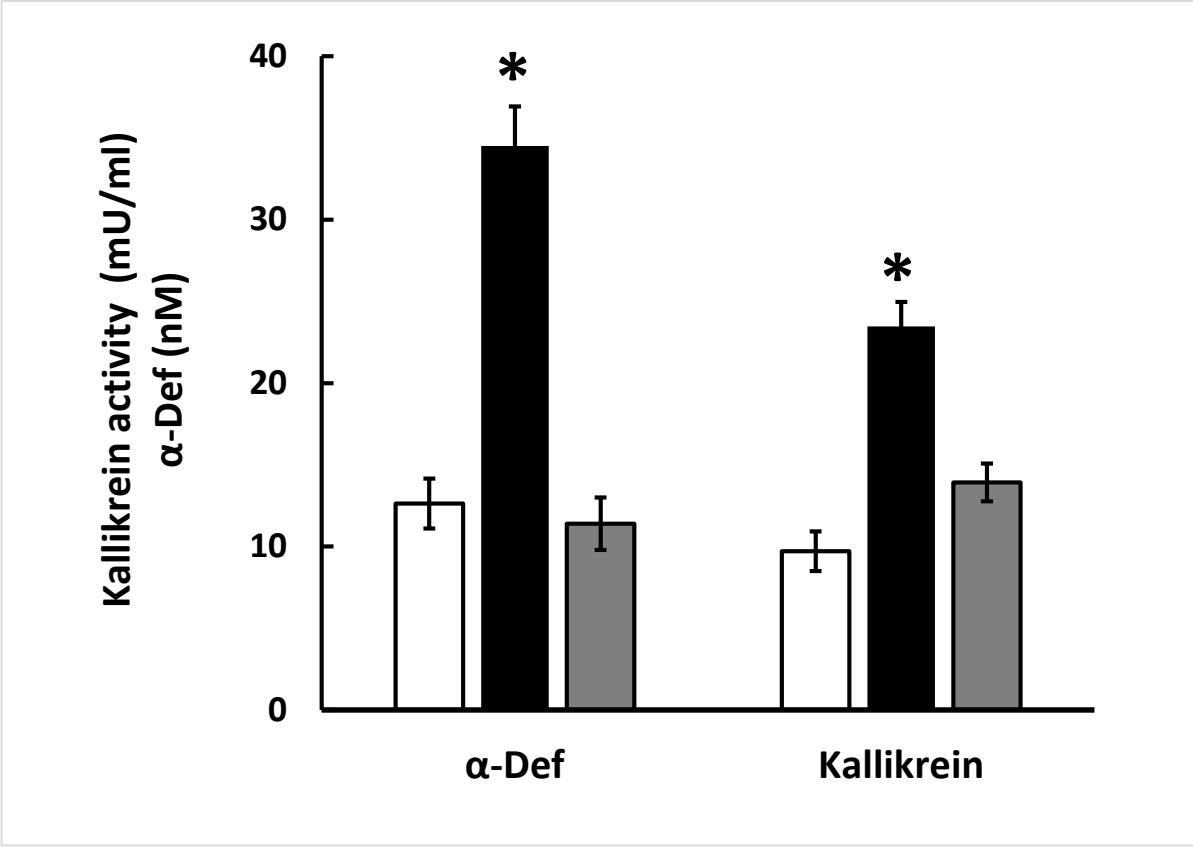
Figure S6B. Effect of neutrophil activation on the concentration of α-defs *in vivo*. Def^{++} and WT mice were given CTI (4 µg/g bodyweight)[4] intravenously immediately before IVC stenosis or neutrophils were depleted as in Figure S7. Two hours later, blood was taken into citrate, centrifuged as in Figures S1 and 1E, and the concentration of α-defs in the plasma was measured by ELISA[11]. The mean ± SD in 7 mice is shown (* and #, $p<0.05$).

Figure S7. Effect of α-defs on resistance to heparin *in vitro*. Coagulation was initiated by adding kaolin and calcium chloride to FX deficient plasma (FX^- plasma) supplemented with 1-12.7 µg/ml FX. Coagulation was monitored using TEG. The R value was measured as in Figures 5A and B. Increasing concentrations of FX were added to replicate samples of FX^- alone (open circles) or together with 0.1 U/ml heparin (closed circles), heparin and 1 µM α-defs (triangles) or α-defs alone (squares). The effect of heparin alone on the FX concentration necessary to generate a clot at an R value of 40 is shown by the dotted vertical line on the right and the effect of heparin in the presence of α-defs by the dashed vertical line on the left. The shift in FX concentration needed to result in the same R value is depicted by the double-sided arrow horizontal line.

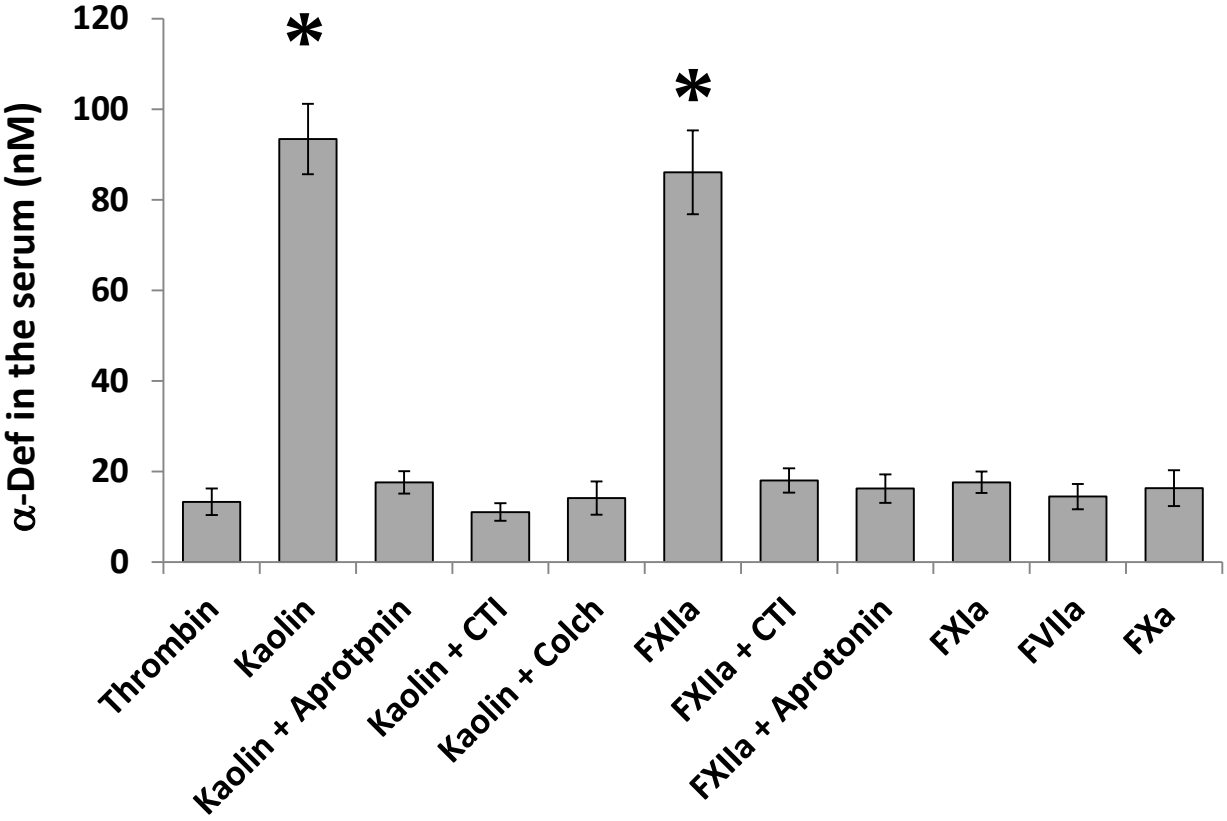
Supplement Figure S1A



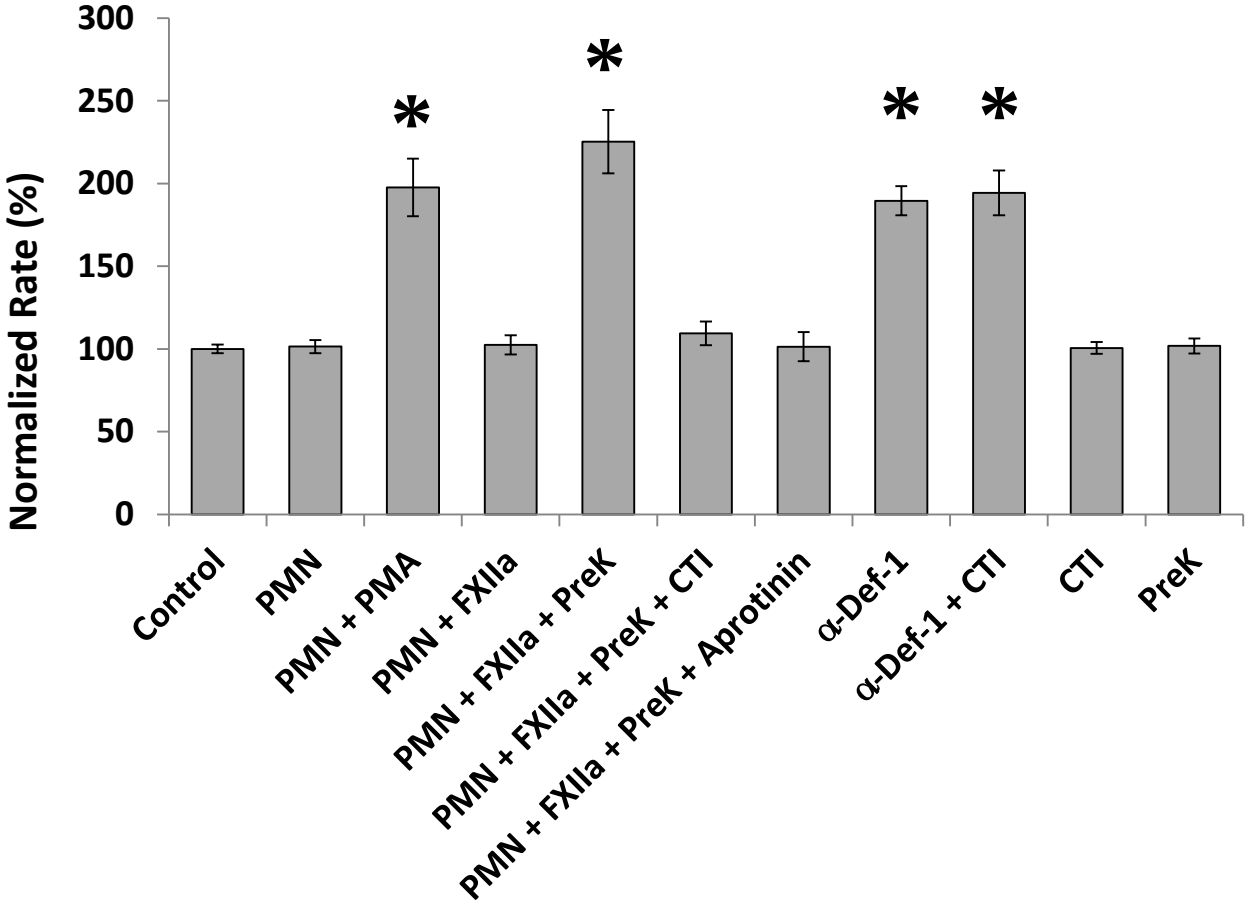
Supplement Figure S1B



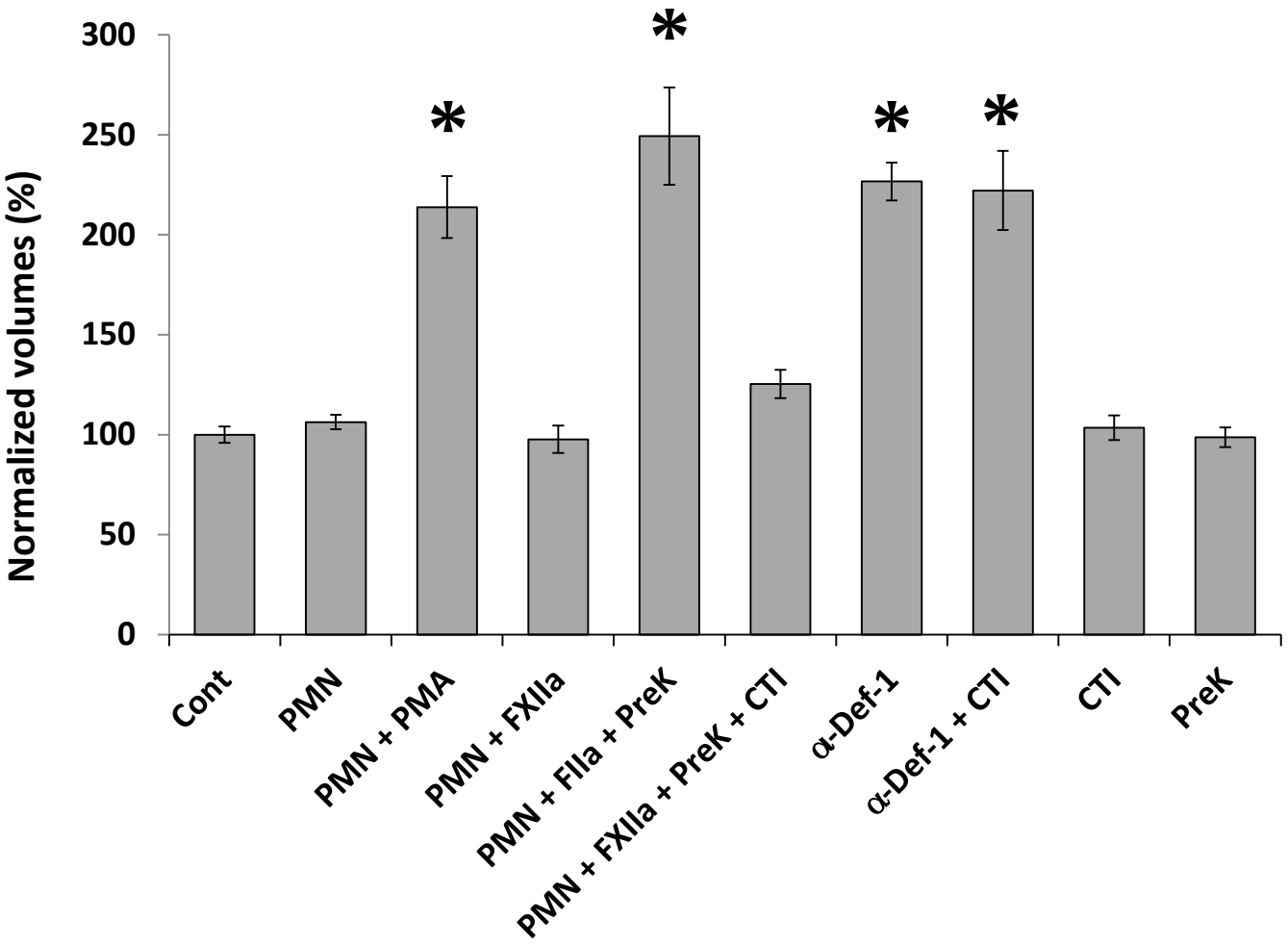
Supplement Figure S2



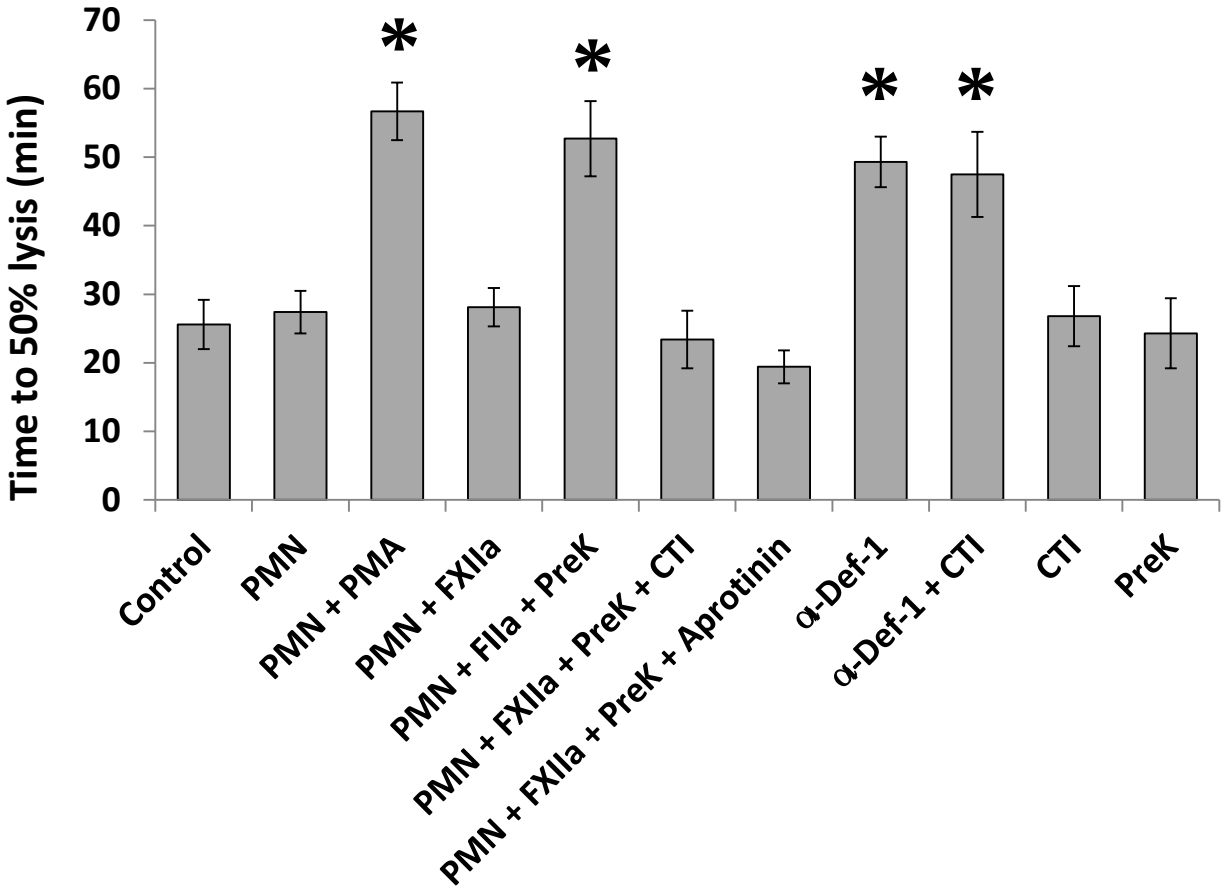
Supplement Figure S3A



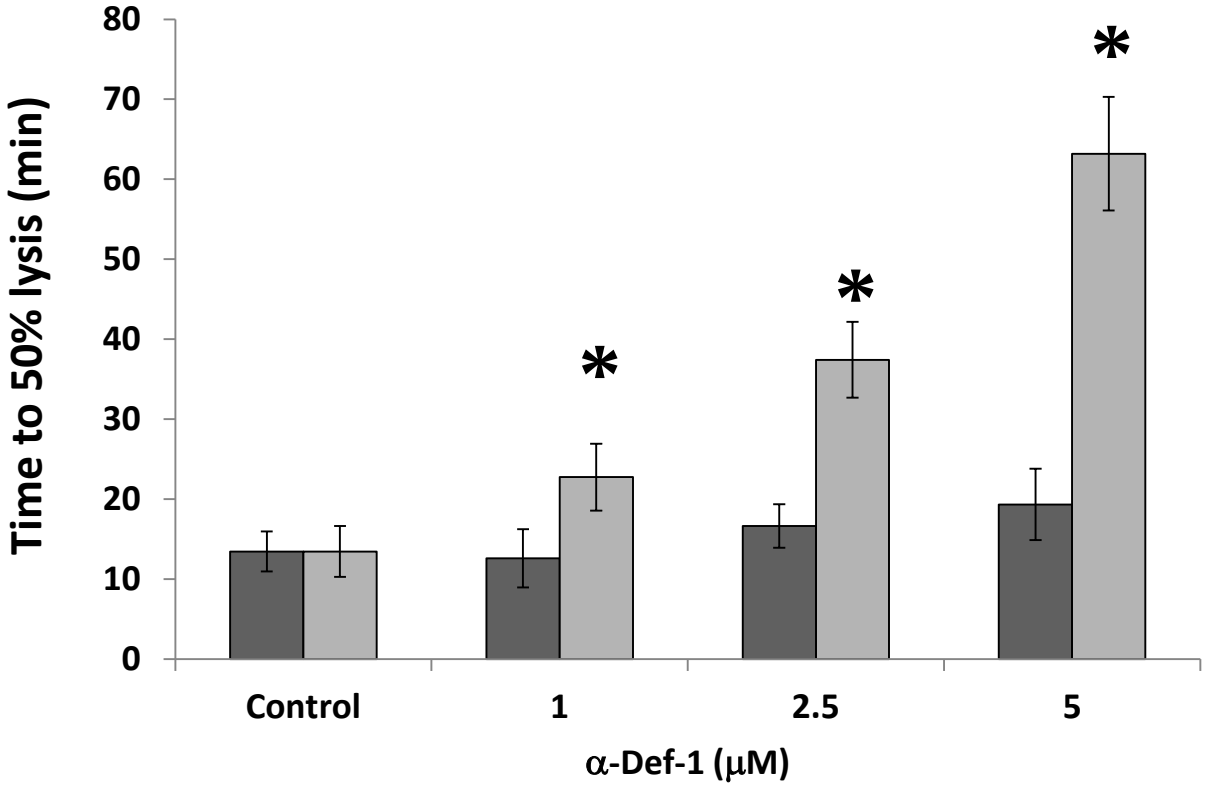
Supplement Figure S3B



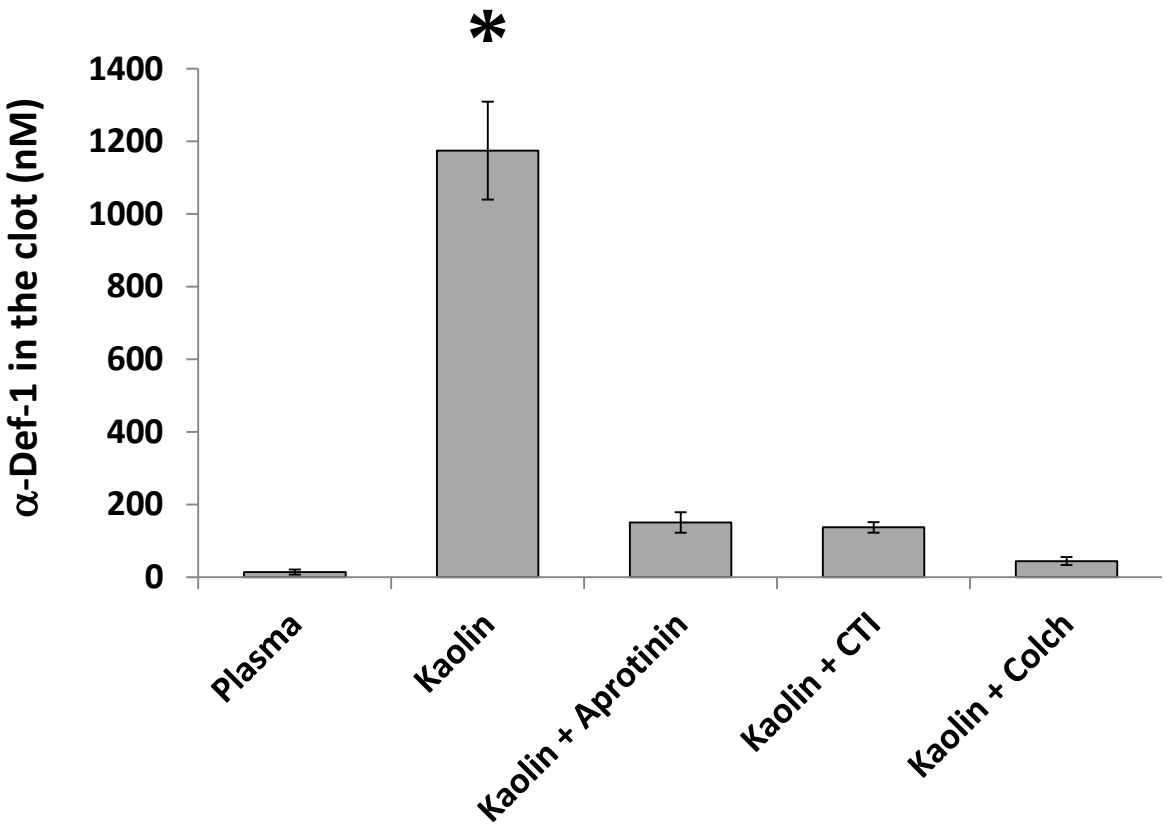
Supplement Figure S4A



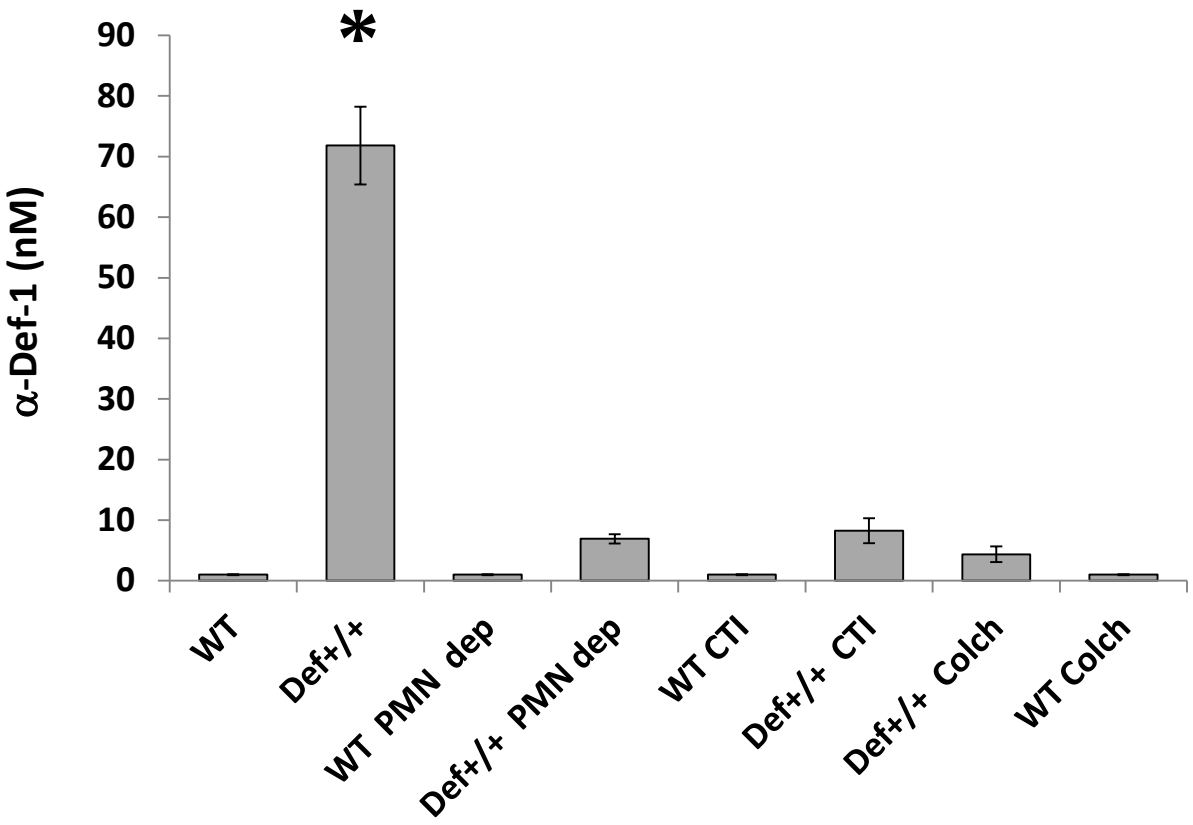
Supplement Figure S4B



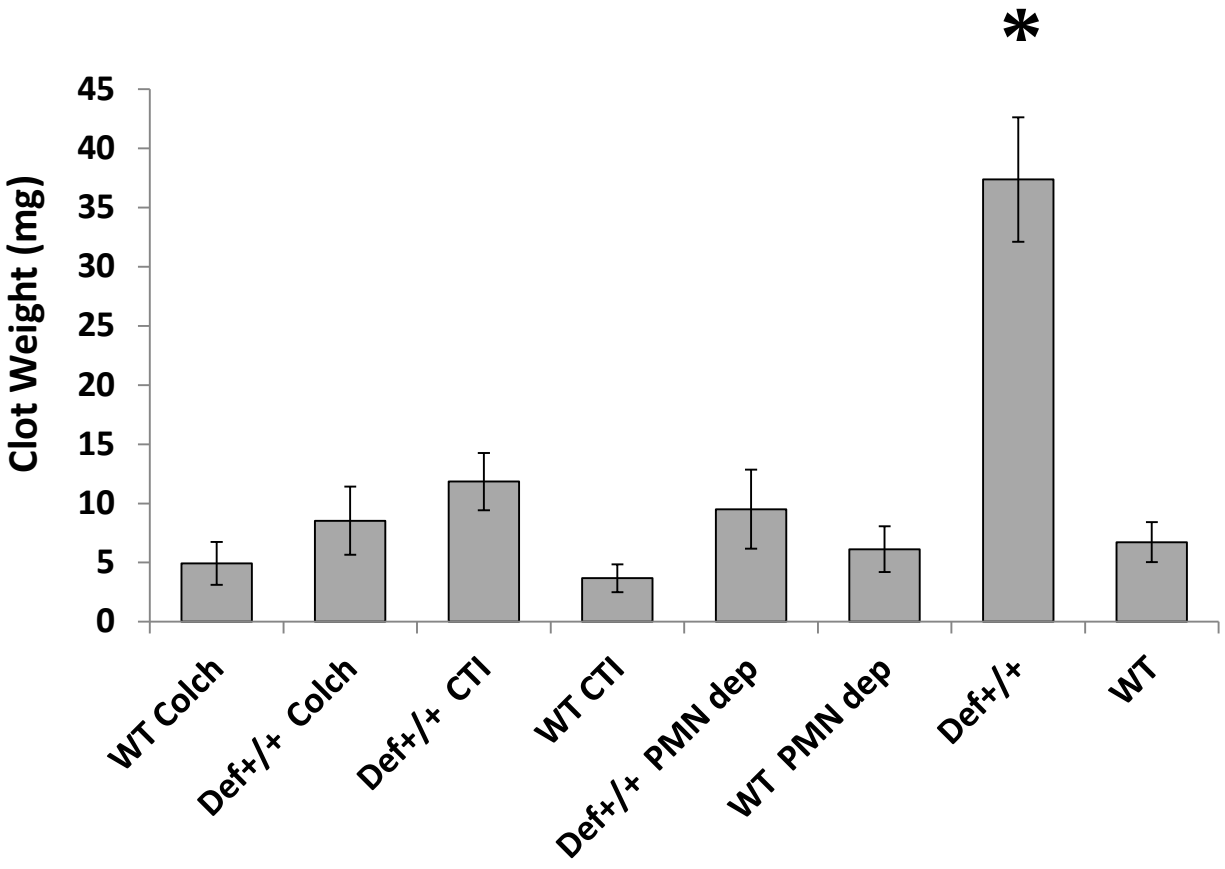
Supplement Figure S5



Supplement Figure S6A



Supplement Figure S6B



Supplement Figure S7