Supplemental methods

Detailed description of ELISAs

Human VWF propeptide ELISA

Microtiter plates (96 well) were coated in a solution of 5µg/mL monoclonal CLB-pro35 immunoglobulin G (IgG) antibodies dissolved in 50 mM sodium carbonate buffer (pH=9.8). Plates were stored overnight at 4°C and were subsequently blocked and incubated for 2 hours at 37 °C using a 0.2% Tween-20, 0.2% gelatin solution in phosphate buffered saline (PBS). Plasma samples and a reference standard were added in duplicates and incubated at 37°C for 2 hours. CLB-pro14-3 was added followed by another incubation at 37°C for 2 hours. A coloring solution of tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) was used for visualization. After 5 minutes, the reaction was stopped using 1M H_2SO_4. The optical density of the samples was measured using a spectrophotometer set to 450 nm.

Human and rat VWF antigen ELISAs

Human VWF antigen was measured by coating 96-well microtiter plates with an equimolar mixture of 4 monoclonal immunoglobulin G antibodies: CLB-RAG 20, CLB-RAG 35, CLB-RAG 42 and CLB-RAG 50. Plates were stored at 4°C. Plates were blocked and incubated for 2 hours at 37 °C using a solution of 2% bovine serum albumin (BSA) in PBS. Human plasma samples and a reference standard were added in duplicates and incubated at 37°C for 2 hours. The reference standard consisted of pooled plasma calibrated to the most recent World Health Organization (WHO) standard for VWF antigen in plasma.[29] Horseradish peroxidase (HRP) labeled polyclonal anti-human VWF antibodies (DAKO, Glostrup, Denmark) were added followed by another incubation at 37°C for 2 hours. Coloring reaction was started and stopped as described in the VWF propeptide ELISA.

Rat VWF antigen levels were determined using a murine VWF antigen ELISA as previously described [49]. Microtiter plates were coated with polyclonal anti-human VWF antibodies overnight at 4°C (1/1000 in carbonate/bicarbonate buffer) and subsequently blocked with a solution of 3% dried milk powder in PBS for 2 hours at room temperature (RT). Next, a serial dilution of the rat plasma samples (starting dilution of 1/20) was added and samples were incubated for 1 hour at 37°C. Captured rat VWF was detected using a mixture of two biotinylated monoclonal anti-murine VWF antibodies (15H2 and 2C12 each at 1 µg/mL, incubation for 1 hour at RT), followed by addition of HRP-labeled streptavidin (1/10,000; incubation for 1 hour at RT). The colorimetric reaction was initiated by addition of o-phenylenediamine (OPD) and H_2O_2, stopped with 4M sulfuric acid, and the absorbance was measured

at 490 nm. A reference curve was used to calculate the values. The mean of baseline VWF antigen concentration was used as reference value, from which the rat VWF antigen levels were calculated.

Human and rat ADAMTS13 antigen ELISAs

Human ADAMTS13 antigen was determined, as previously described.[30] Microtiter plates were coated with the monoclonal anti-human ADAMTS13 antibody 3H9 [30,31] overnight at 4°C (5 µg/mL in carbonate/bicarbonate buffer) and subsequently blocked with a solution of 3% dried milk powder in PBS for 2 hours at RT. Next, human plasma samples (starting dilution of 1/100) were added in a 1.5 over 2.5 dilution series and incubated for 1.5 hours at 37°C. Captured ADAMTS13 was detected using a mixture of biotinylated anti-human ADAMTS13 antibodies 17G2 [30,32] and 19H4 [30,32] (1.5 µg/mL each, incubation for 1 hour at RT, followed by HRP-labeled streptavidin (1/10,000; Roche Diagnostics, Mannheim, Germany) (incubation for 1 hour at RT). The colorimetric reaction was performed as described in the rat VWF antigen ELISA. A dilution series of a normal human plasma pool (NHP, plasma from ≥ 20 healthy donors, set at 100%) was used as a reference curve, from which the ADAMTS13 antigen levels were interpolated.

To determine rat ADAMTS13, an ELISA that measures murine as well as rat ADAMTS13 antigen was used as previously described.[36] Microtiter plates were coated with the monoclonal anti-mouse ADAMTS13 antibody 9F2 overnight at 4°C (5 µg/mL in carbonate/bicarbonate buffer) and subsequently blocked with a solution of 3% dried milk powder in PBS for 2 hours at RT. Next, a serial dilution of the rat plasma samples (starting dilution of 1/20) was added and samples were incubated for 1 hour at 37°C. Captured rat ADAMTS13 was detected using polyclonal rabbit anti-mouse ADAMTS13 antibodies (5 µg/mL, incubation for 1 hour at RT) followed by addition of HRP-labeled goat anti-rabbit antibodies (1/10,000; Jackson Immunoresearch Laboratories Inc., West Grove, PA, USA; incubation for 1 hour at RT). The colorimetric reaction was performed as described above in the rat VWF antigen ELISA. The mean of baseline ADAMTS13 was used as reference value, from which the rat ADAMTS13 antigen levels were calculated.

Human ADAMTS13 activity

ADAMTS13 activity levels were measured using the FRETS-VWF71 assay, with minor modifications.[33] ADAMTS13 activity levels in patient plasma samples were assayed by adding 7.5% v/v patient plasma to 2.23 µM of the FRETS-VWF71 substrate in HEPES-buffered saline (pH 7.4) containing 1 mg/mL BSA. A reference curve was obtained using different dilutions of NHP (2.5%, 5%, 7.5%, 10% and 12.5%, v/v, NHP was set at 100%) and the inhibitory anti-human ADAMTS13 antibody 3H9 (10 µg/mL) was used as a negative control. Proteolysis of FRETS-VWF71 by plasma ADAMTS13 was determined by

measuring the change in fluorescence intensities (FLUOstar OPTIMA reader, BMG Labtech GmbH, Offenburg, Germany), during 30 cycles of 120 seconds using excitation at 620 nm and emission at 660 nm. The proteolytic activity was determined by plotting the fluorescence intensities in function of time and slopes of the linear part of the resulting curves were used to calculate ADAMTS13 activity levels according to the reference curve.

Human and rat VWF multimers

VWF multimers were separated on a sodium dodecyl sulphate (SDS) isoelectric focusing (IEF) 1.2% agarose gel. After electrophoresis, the gel was fixed on a Gelbond (Cambrex Bio Science Rockland Inc., Rockland, ME) and the VWF multimer pattern was visualized with anti-human VWF antibodies labelled with alkaline phosphatase (AP) and the AP-conjugate-substrate kit (BioRad, Hercules, CA). Densitometric analysis was performed using ImageJ software (version 1.47, NIH, MD) to determine the percentage of HMW VWF multimers. The low molecular weight VWF multimers (protein band 1-5), the medium molecular weight VWF multimers (protein band 6-10), and HMW VWF multimers (protein bands >10) were selected. The density of the HMW VWF multimers relative to the total multimer density was calculated as a percentage.

In vitro human microvascular lung endothelial cell transwell system

Human lung microvascular endothelial cells (HMVEC-L, Lonza, Basel, Switzerland) were seeded onto matrigel coated transwells (12 mm insert, 12-well plate, 0.4µm pores, Corning Incorporated). After 48 hours, cells formed a confluent monolayer. The experiment started 24 hours after the second seeding. Cells from passage 6 were used in these experiments. On the day of the experiment, medium was replenished, in positive controls with thrombin (10 IU/ml), to the upper and lower chamber, respectively 500 µL and 1500 µL. Dose-response was tested in thrombin stimulated endothelial cells for rhADAMTS13 (1 nM, 5nM, 10nM, 40nM). FITC-Dextran (70kDa, 5 mg/ml) was used as tracer molecule and added to the upper chamber after an equilibration period of 5 minutes. At 1, 3 and 6 hours after adding the tracer molecule to the upper compartment, 20 µL sample from the lower compartment was taken without disturbing the transwell. These samples were diluted in 180µL MilliQ and analyzed using a spectrophotometer (Spectramax M2e plate reader, Molecular devices LLC, St. José, USA). Fluorescence was measured in 96-wells non-binding plate, with excitation at 485 nm and emission 535 nm, using SoftMaxPro 6.5.1. software. A standard curve was used to determine the concentration of FITC-dextran leakage. The medium was used for determination of syndecan-1 as described for human syndecan-1 and lactate dehydrogenase. For lactate dehydrogenase activity 200

µl (0.5M kPi buffer, 10% Triton, 0.12 mMNADH and milliQ, pH 7.5), 2.5 µl medium and 2.5 µl 0.5mM pyruvate was added to a clear 96-well plate. The optical density at 340 nm was determined every second for 10 minutes (Spectramax M2e plate reader, Molecular devices LLC, St. José, USA). The decrease of optical density reflects the conversion of NADH to NAD+ by LDH. The LDH activity was calculated using the slope of the curve, the extinction coefficient for NADH and the dilution factor. Total VE-cadherin was determined using western blotting and corrected for GAPDH. Furthermore, immunostaining for F-actin with rhodamin phalloidin (Invitrogen, USA), mouse-anti-human VE-cadherin/goat-anti mouse IgG alexa fluor 488 (Thermo Fisher Scientific, USA) and fluorescent microscopy (LEICA DM6 B, Wetzlar, Germany, 10x magnification) was performed to obtain an overview of the cells with different concentrations of rhADAMTS13.

Supplemental Tables

Supplemental table 1: In-/exclusion criteria ACIT-III

Inclusion criteria
Adult trauma patients were enrolled if they sustained a blunt or penetrating trauma and for whom the trauma team was activated, with at least one of the following clinical parameters: • Respiratory rate <10 or >25 times per minute • Heart rate ≥120 per minute • Systolic blood pressure < 90 mmHg • Oxygen saturation <90% • Estimated blood loss ≥500 mL • Glasgow Coma Score ≤ 13 or abnormal pupil size and/or reaction Or clinical signs of at least one of the following diagnoses: • 1 femur fracture • Signs of flail thorax/pneumothorax/hematothorax or multiple rib fractures • Signs of significant abdominal injury • Pelvic fracture • Spine injury
Exclusion criteria
• Age <18 • Patients transferred from other hospitals • Patients presenting more than 120 minutes after time of injury • Patients who have received more than 2000 mL of intravenous fluids prior to emergency department arrival • Patients with burns >5% of their body surface area • Patients taking anticoagulant medication other than aspirin (<650mg/day) • Patients with a known bleeding diathesis • Patients with moderate to severe liver disease (Child's classification B or C3)

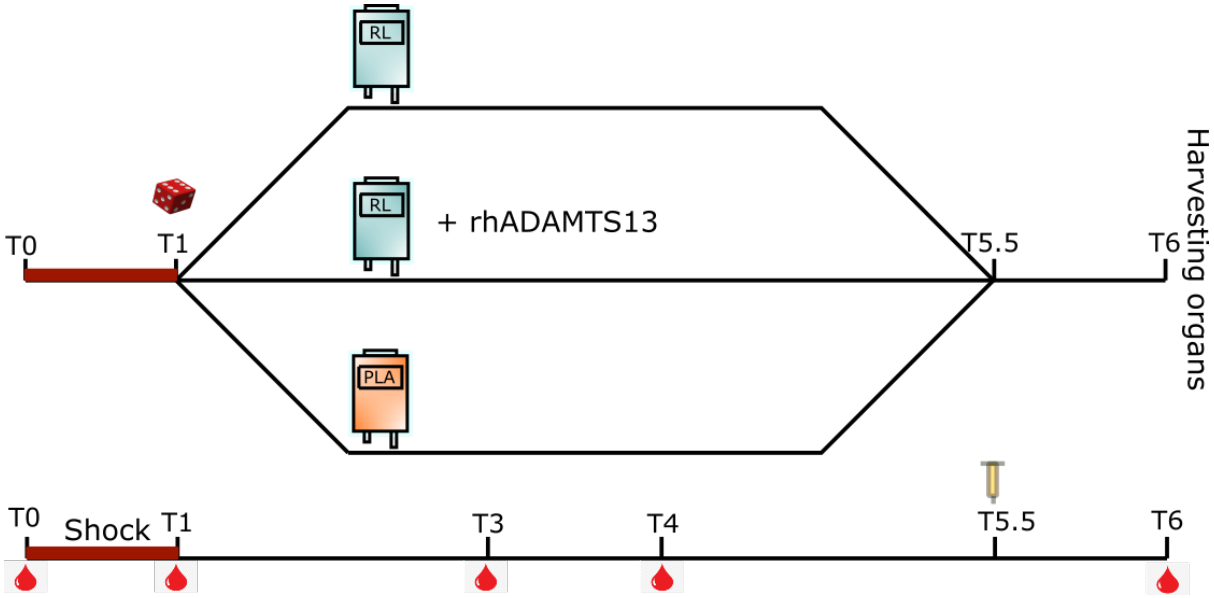
Supplemental table 2: Animal organ failure assessments scoring list

Lung injury was scored as follow:
• Lung edema • Interstitial inflammatory cell infiltration • Endothelial inflammation • Hemorrhage
The severity of **kidney injury** was scored as follow:
• Tubules in the cortex or the outer medulla that showed epithelial necrosis or had luminal necrotic debris • Tubular dilation • Neutrophil extravasation • Interstitial changes • Hemorrhage
The severity of **liver injury** observed in the tissue sections was scored as follows:
• Loss of intercellular borders • Necrosis • Hemorrhage • Portal inflammation • Neutrophil infiltration
The severity of **spleen injury** was scored as follows:
• Neutrophil infiltration • Necrosis • Hemorrhage
The severity of **small intestine injury** was scored from 0 to 3 as follows:
• Focal epithelial edema • Diffuse swelling of the villi • Neutrophil infiltration in the submucosa • Necrosis • Hemorrhage

All evaluations were made on five fields per section and five sections per organ. Severity was assessed based on a 0 to 3 scale: 0, absent; 1, mild; 2, moderate; 3, severe.

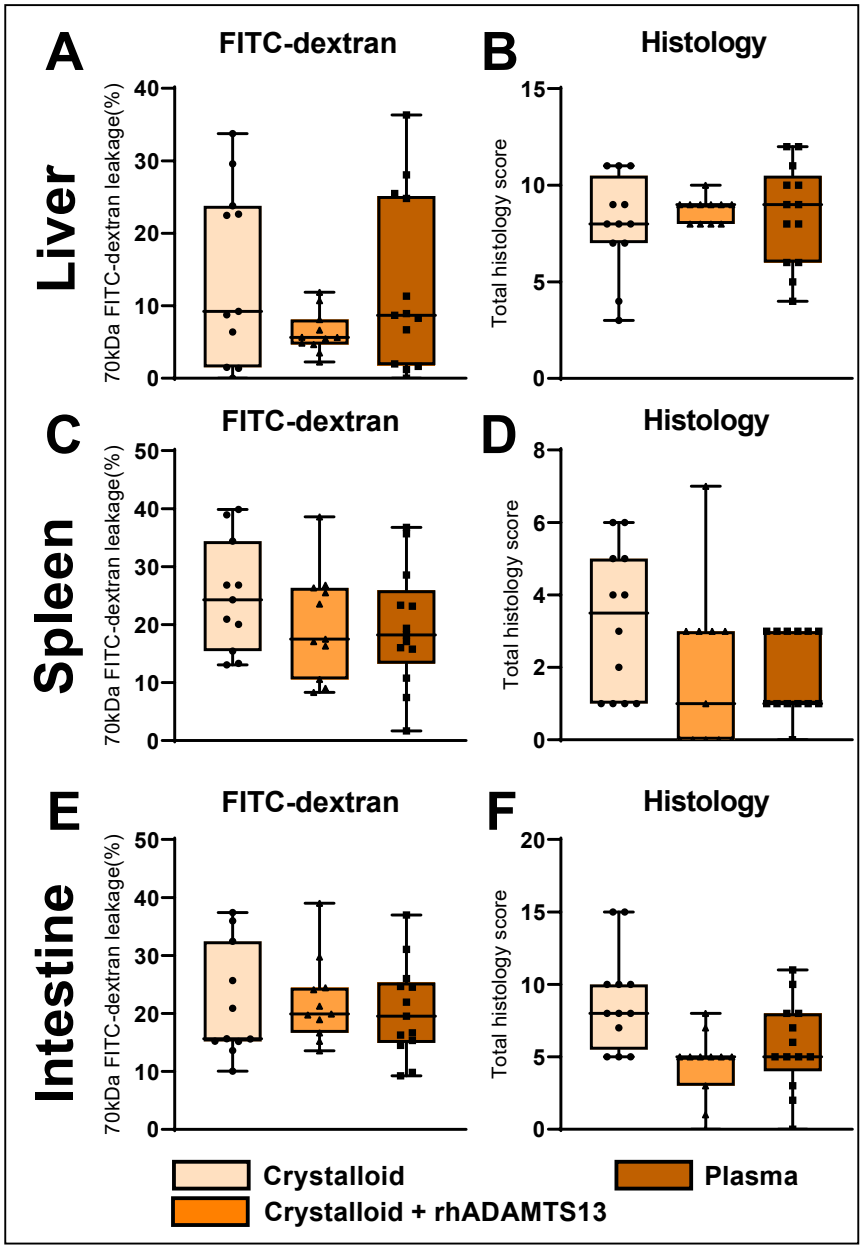
Supplemental figures

Figure 1: Schematic overview of experimental model



Description of trauma transfusion model. Baseline sample before trauma was taken (T0), after which multiple trauma and controlled hemorrhaged was inflicted. After 1 hour of shock with mean arterial pressures below 40mmHg, a blood sample was taken (T1) and rats were randomized to crystalloid Ringer's lactate (RL), RL + rhADAMTS13 or plasma transfusion (PLA). Resuscitation goal was to achieve a mean arterial pressure of 60 mmHg for 5 minutes. Thereafter, resuscitation was stopped. Additional post-resuscitation samples were taken 3 hours and 4 hours after trauma (T3, T4). At 5.5 hours post-trauma a FITC-labelled 70kDa dextran was infused. This label circulated for 30 minutes. Then, last samples were taken, quickly followed by a bolus of heparin, tying-off of the hilum of left lung and left kidney (for later W/D ratio assessments), and flushing of the circulation with 50ml of 0.9% NaCl against gravitational force. Organs were harvested for endothelial leakage assessments and organ failure scores.

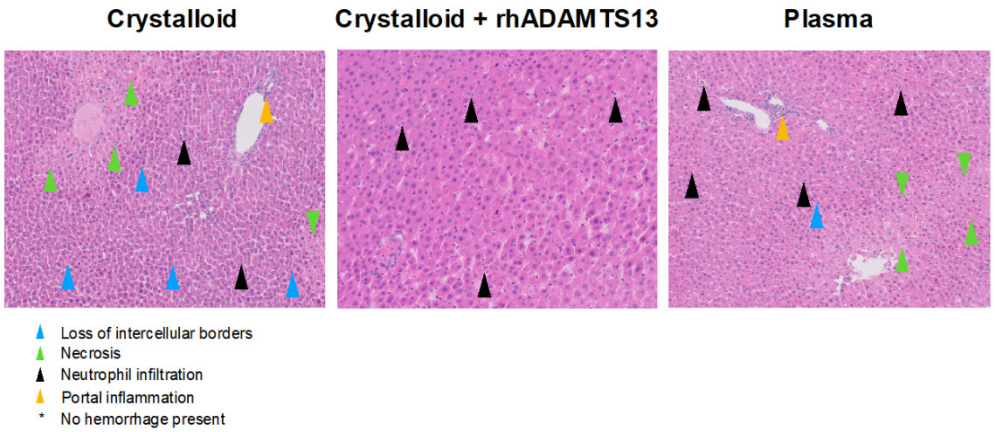
Supplemental figure 2: Splanchnic endothelial leakage and organ failure



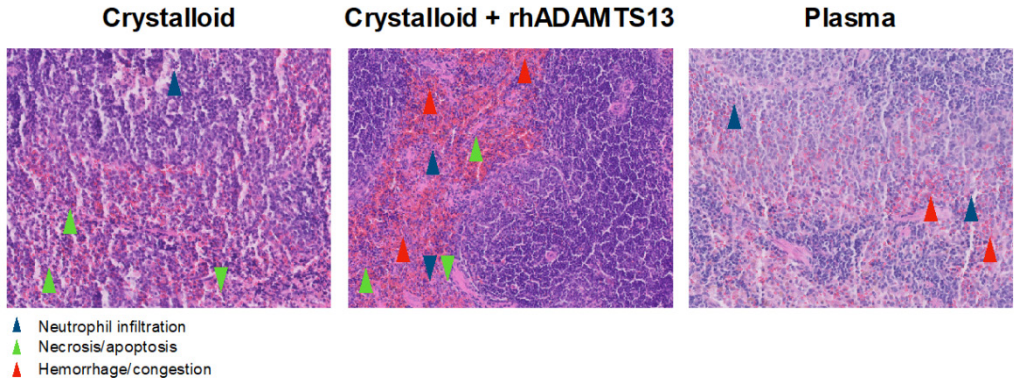
Data are boxplots with range. A – B Liver area of 70kDa FITC-dextran leakage and liver histology scores (**Supplemental Table 2**). C-D Spleen FITC-dextran leakage and spleen histology score. E – F Small intestine FITC-dextran leakage and intestine histology score.

Supplemental figure 3: Histology of the liver, spleen and small intestine

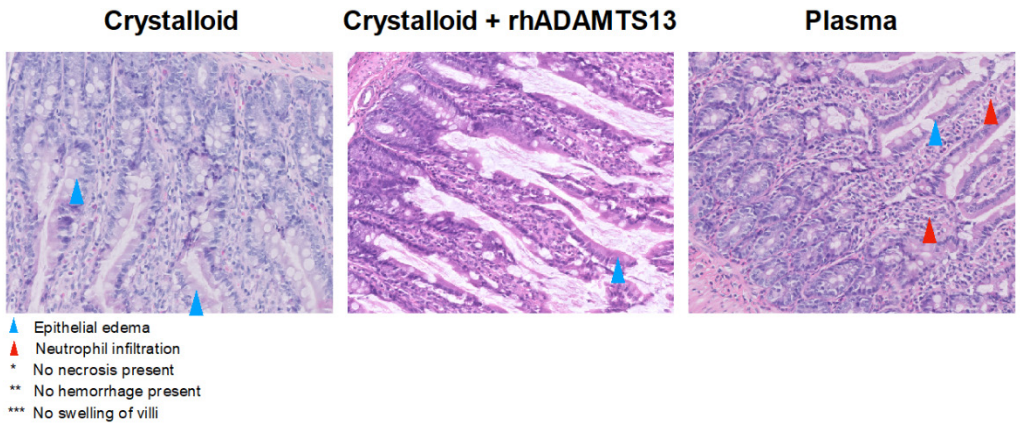
A. Liver



B. Spleen

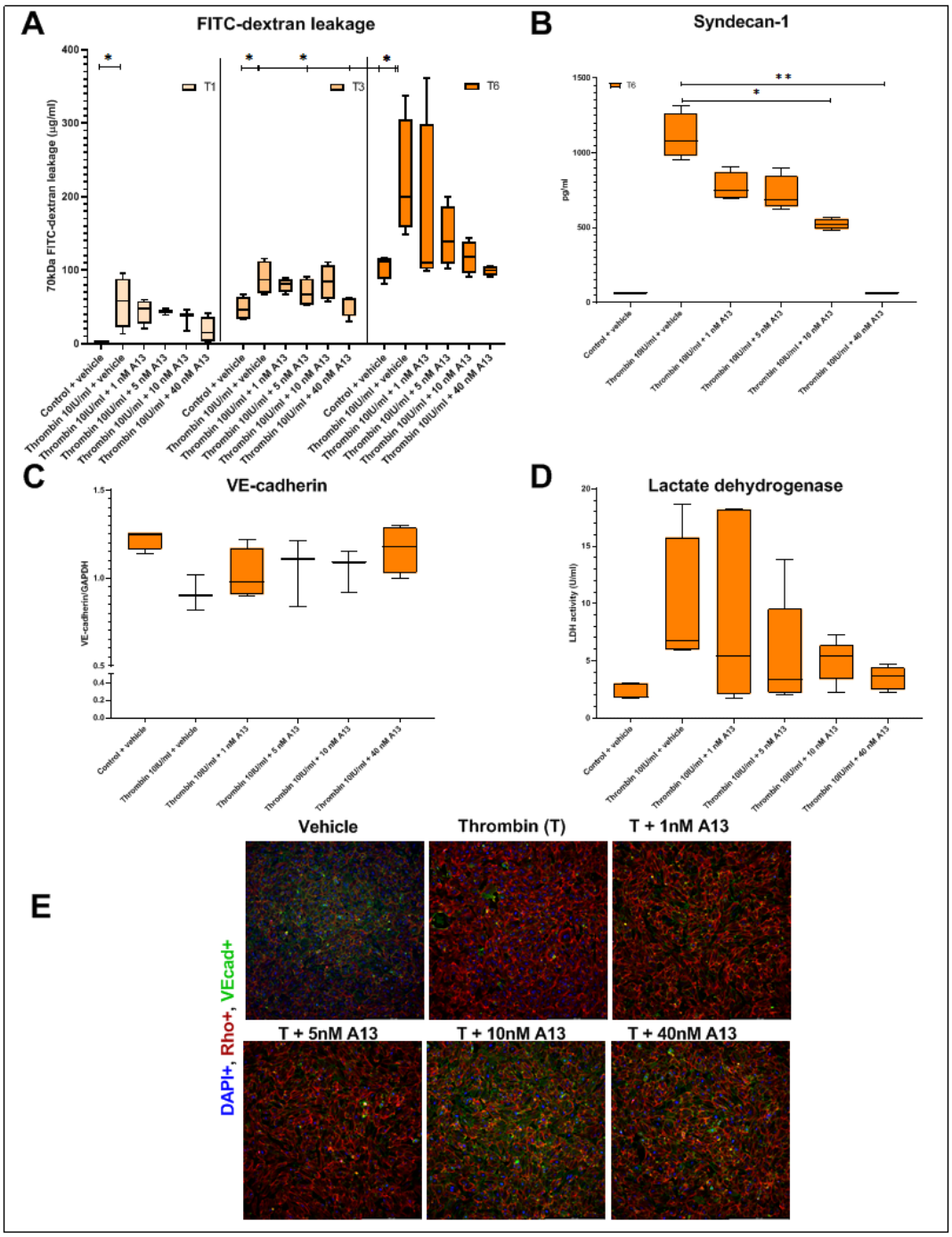


C. Small intestine



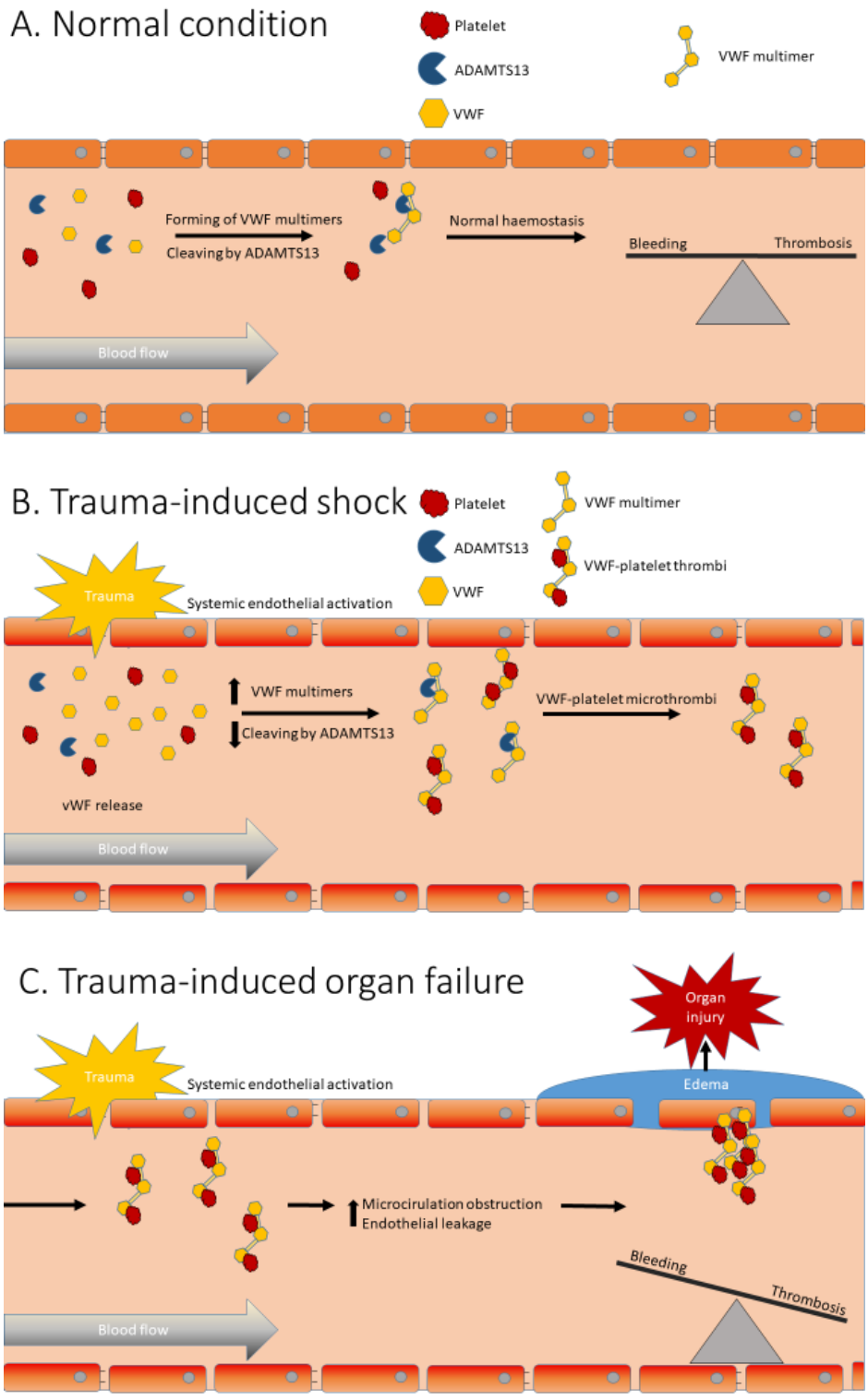
Hematoxylin and eosin staining of the A. Liver, B. Spleen, C. Small intestine. Arrows represent presence of damage to the specific organ which was used for the scoring of organ failure (**Supplemental Table 2**).

Supplemental figure 4: ADAMTS13 reduces endothelial leakage in a dose-dependent manner in vitro, associated with less syndecan-1 release.



Transwell system with thrombin stimulated human microvascular lung endothelial cells treated with different doses of rhADAMTS13 (1nM, 5nM, 10nM and 40nM). Values represent boxplots of n=4 per condition. A. Amount of 70kDa FITC-dextran leakage. B. Syndecan-1 release in the medium. For the control and thrombin + 40nM rhADAMTS13 the values were below the detection range of 62.5 pg/ml. This lowest value was used as imputation. C. Total VE-cadherin/GAPDH. D. Amount of cell death as determined by release of lactate dehydrogenase in the medium. E. Immunostainings (10x magnification) of the cell nucleus (blue), actin filaments (red) and VE-cadherin (green).

Supplemental figure 5: Proposed mechanism on endothelial VWF-ADAMTS13 imbalance in trauma-induced organ failure



In normal conditions (A) there is a balance between VWF antigen molecules and ADAMTS13 resulting in adequate cleavage of VWF multimers, preventing formation of microthrombi. In traumatic shock (B), increased release of multiple VWF molecules into the circulation stimulates the formation of VWF multimers, while a relative shortage of ADAMTS13 and possible inhibiting factors contribute to formation of highly sticky microthrombi which adhere to the vessels in the microcirculation further amplifying endothelial damage resulting in endothelial leakage and eventually (C) organ damage.