UPMC TRAUMA
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Advances in understanding hemostasis after trauma: microparticles and vWF

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- External Scientific Advisory Board CSL Behring
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- Laboratory funding: NIH, DoD, UPMC
- **US Patent** 9,072,760 *TLR4 inhibitors for the treatment of human infectious and inflammatory disorders*

Impaired Hemostasis

The Journal of TRAUMA® Injury, Infection, and Critical Care

Acute Traumatic Coagulopathy

Karim Brohi, BSc, FRCS, FRCA, Jasmin Singh, MB, BS, BSc, Mischa Heron, MRCP, FFAEM, and Timothy Coats, MD, FRCS, FFAEM

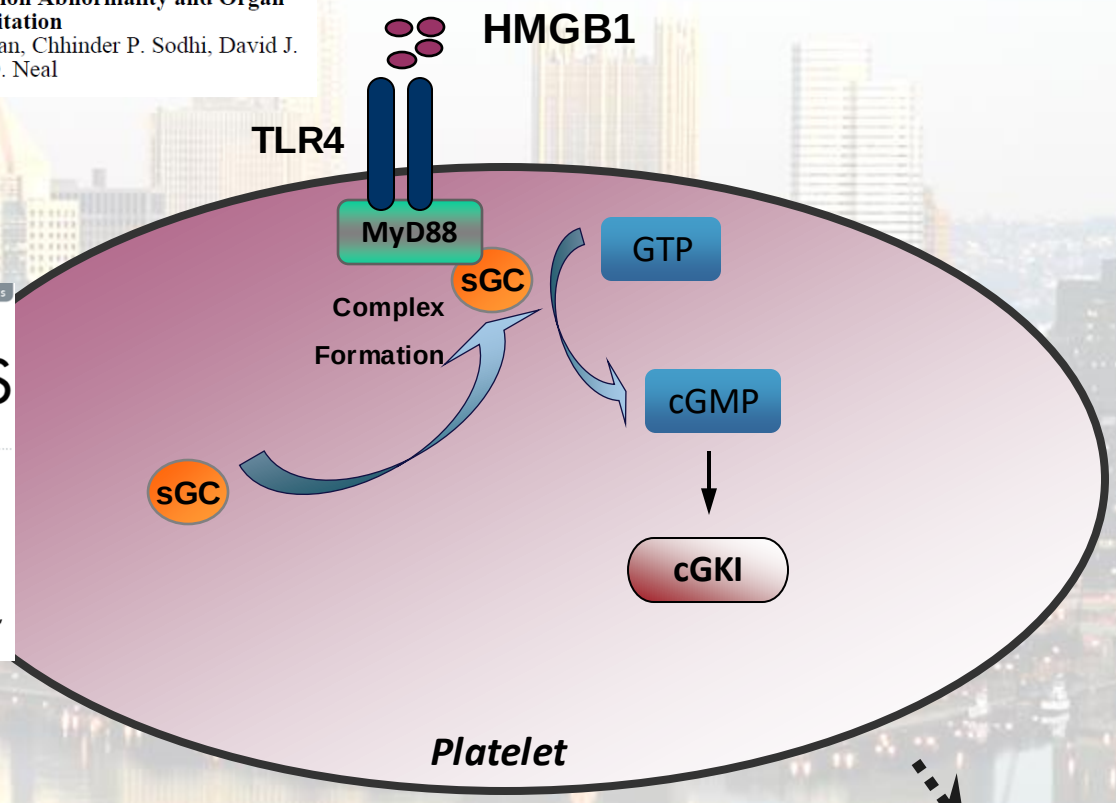
Inappropriate thrombosis

Circulation
Cardiovascular Genetics

American Heart Association.

TLR4 Regulates Platelet Function and Contributes to Coagulation Abnormality and Organ Injury in Hemorrhagic Shock and Resuscitation

Ning Ding, Guoqiang Chen, Rosemary Hoffman, Patricia A. Loughran, Chhinder P. Sodhi, David J. Hackam, Timothy R. Billiar and Matthew D. Neal



www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Deep vein thrombosis in mice is regulated by platelet HMGB1 through release of neutrophil-extracellular traps and DNA

Received: 27 July 2017
Accepted: 6 December 2017
Published online: 01 February 2018

Mitchell R. Dyer[1], Qiwei Chen[1], Shannon Haldeman[1], Hamza Yazdani[1], Rosemary Hoffman[1], Patricia Loughran[1,2], Allan Tsung[1], Brian S. Zuckerbraun[1], Richard L. Simmons[1] & Matthew D. Neal[1]

The Journal of Clinical Investigation *J Clin Invest* 2015;125(12):4638-4654 RESEARCH ARTICLE

Platelet-derived HMGB1 is a critical mediator of thrombosis

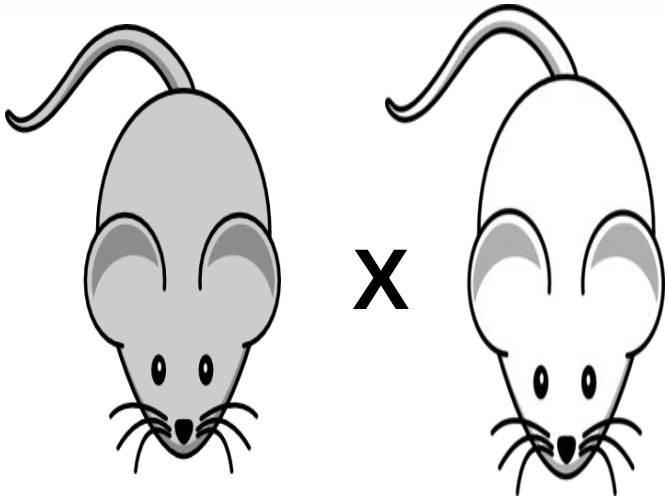
Sebastian Vogel,[1] Rebecca Bodenstein,[1] Qiwei Chen,[2] Susanne Feil,[3] Robert Feil,[3] Johannes Rheinlaender,[4] Tilman E. Schäffer,[4] Erwin Bohn,[5] Julia-Stefanie Frick,[5] Oliver Borst,[1] Patrick Münzer,[1] Britta Walker,[1] Justin Markel,[2] Gabor Csanyi,[6] Patrick J. Pagano,[6] Patricia Loughran,[2,7] Morgan E. Jessup,[7] Simon C. Watkins,[7] Grant C. Bullock,[8] Jason L. Sperry,[2] Brian S. Zuckerbraun,[2] Timothy R. Billiar,[2] Michael T. Lotze,[2] Meinrad Gawaz,[1] and Matthew D. Neal[2]

Platelet aggregation

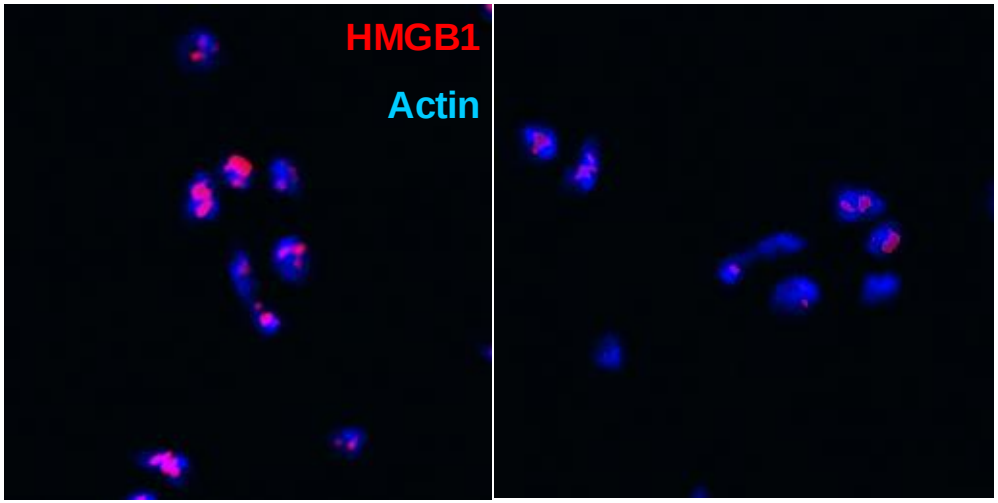
Organ injury

DVT

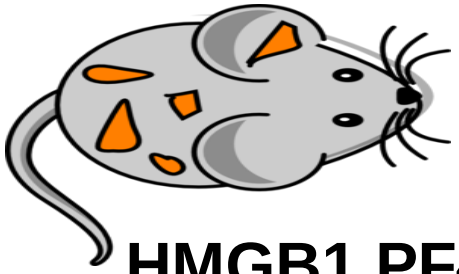
Selective deletion of HMGB1 from platelets in mice



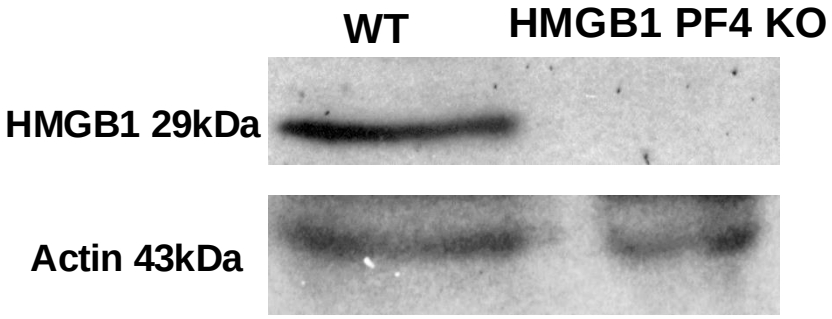
HMGB1 flox (WT) PF4 cre



WT HMGB1 PF4 KO

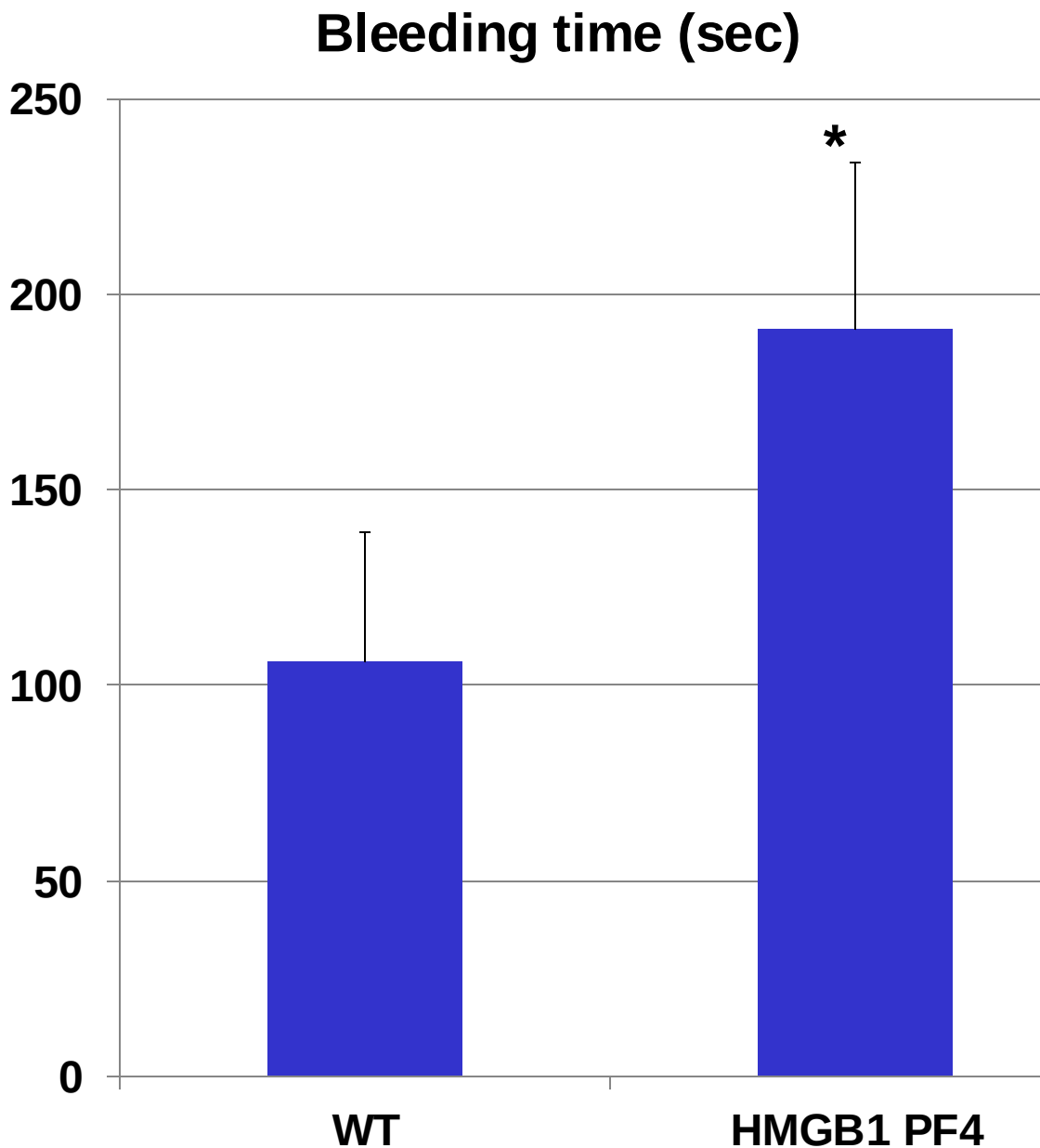


HMGB1 PF4



HMGB1 Platelet KO mice have increased bleeding time

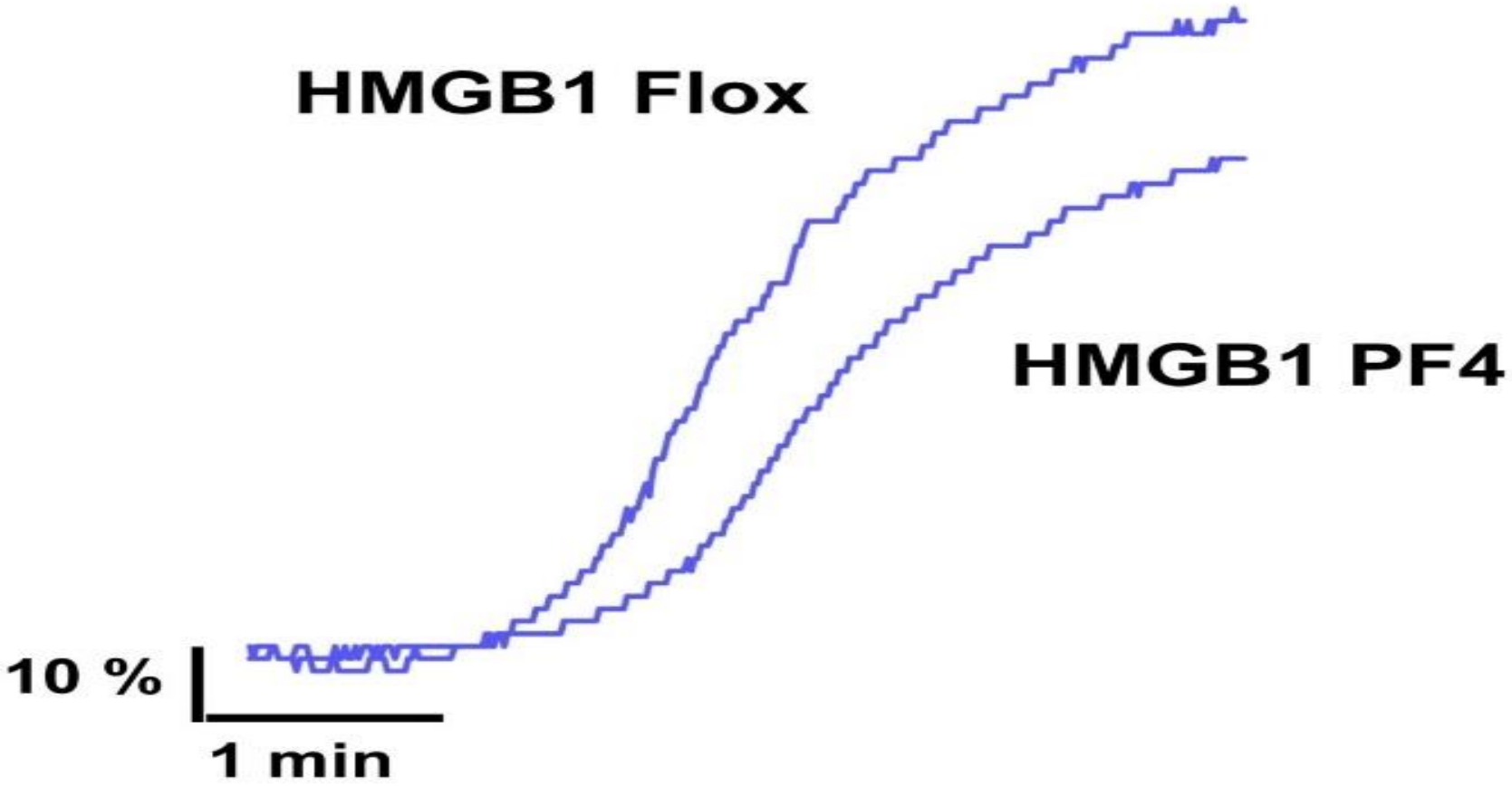
- Tail vein bleed assay
- N=18 mice per group



* p<0.01

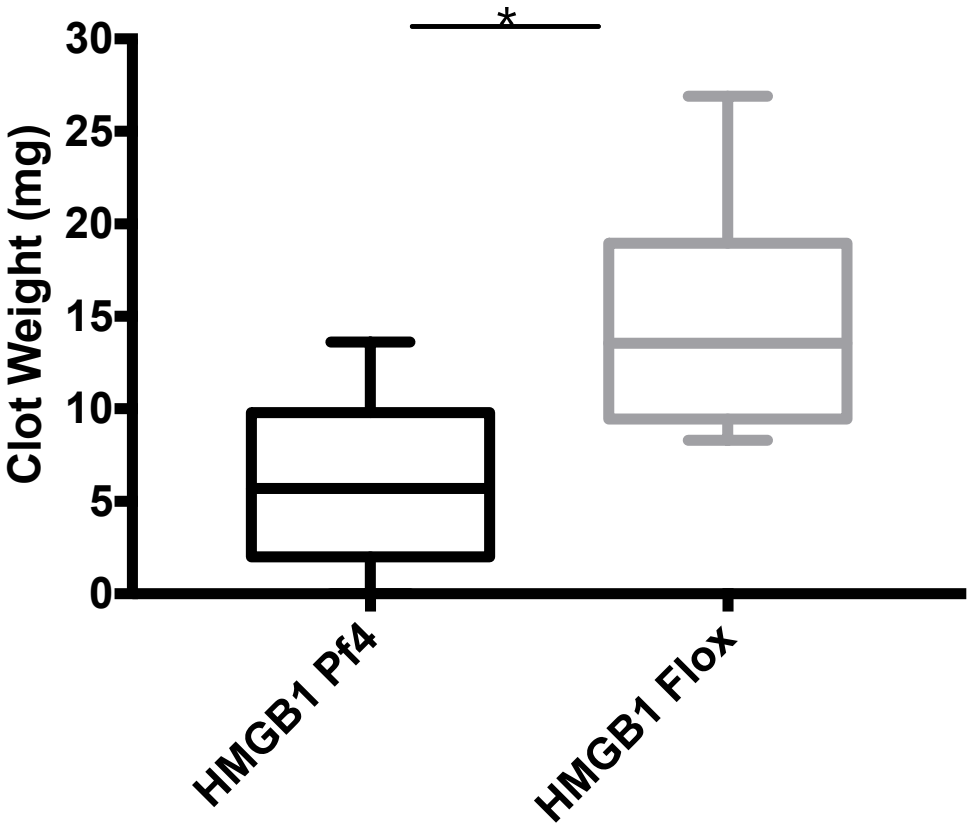
Vogel, JCI, 2016

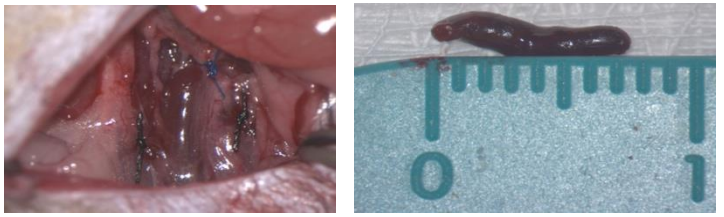
HMGB1 PF4 mice have decreased aggregation in response to collagen

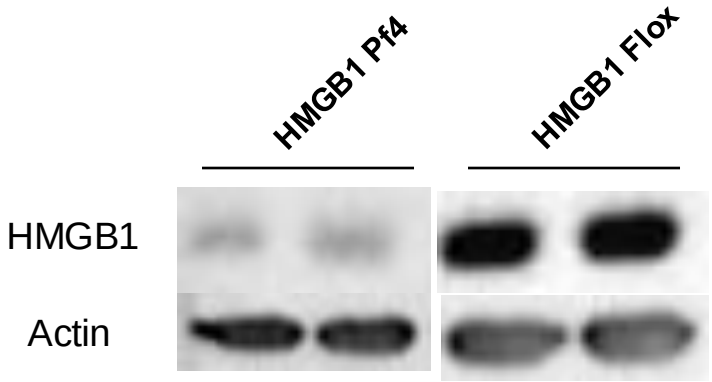


Vogel, JCI, 2016

Platelet HMGB1 promotes thrombus formation and is the major source of HMGB1 in developing thrombi







Dyer, Sci Rep, 2018

What is the mechanism of release of HMGB1 from platelets?

EAST 2015 PLENARY PAPER

Thrombin generation and procoagulant microparticle profiles after acute trauma: A prospective cohort study

Myung S. Park, MD, Ailing Xue, MD, Grant M. Spears, Timothy M. Halling, Michael J. Ferrara, MA, Melissa M. Kuntz, Sabtir K. Dhillon, MD, Donald H. Jenkins, MD, William S. Harmsen, MA, Karla V. Ballman, PhD, Paul Harrison, PhD, *and* John A. Heit, MD, *Rochester, Minnesota*

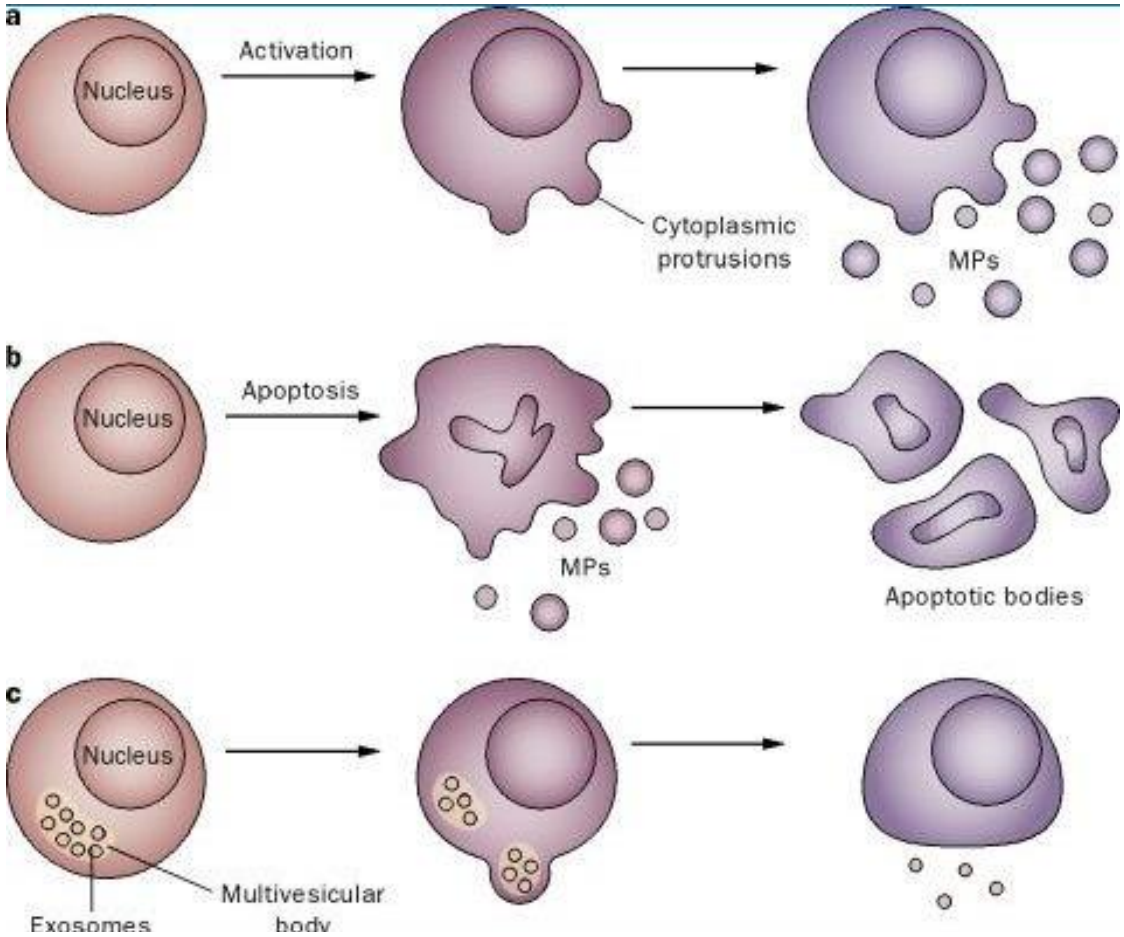
Journal of Trauma and Acute Care Surgery. Publish Ahead of Print():, FEB 2019
DOI: 10.1097/TA.0000000000002230, PMID: 30768560
Issn Print: Model.IssnPrint
Publication Date: 2019/02/01

Print

Regulation of Endothelial Cell Permeability by Platelet-Derived Extracellular Vesicles

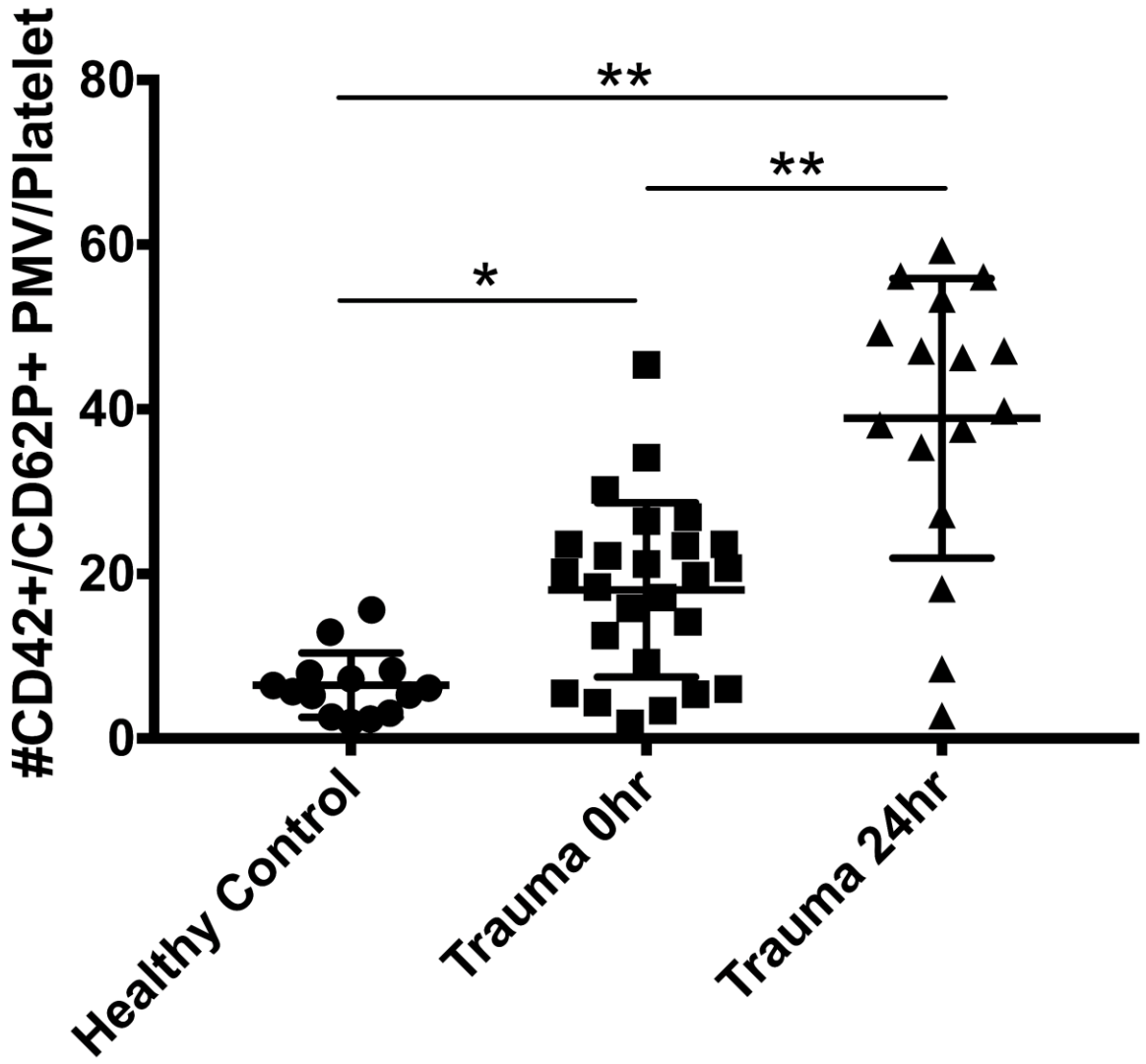
Byron Miyazawa;Alpa Trivedi;Padma Togarrati;Daniel Potter;Gyulnar Baimukanova;Lindsay Vivona;Maximillian Lin;Ernesto Lopez;Rachael Callcut;Amit Srivastava;Lucy Kornblith;Alexander Fields;Martin Schreiber;Charles Wade;John Holcomb;Shibani Pati;

Extracellular vesicles (EVs)

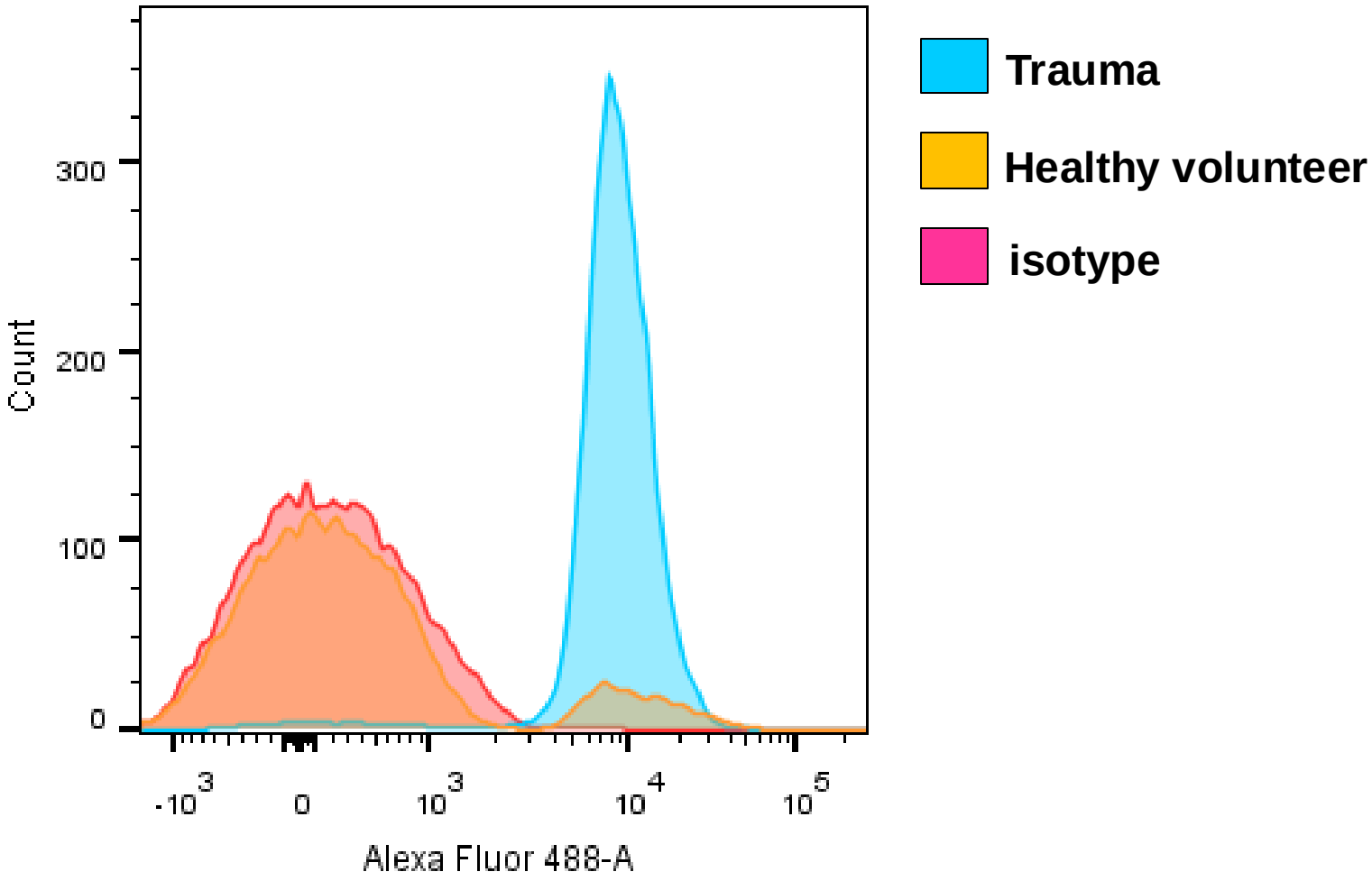


- vesicles with a diameter of less than 1000 nm
- released by budding of the plasma membrane
- express antigens specific from their parental cells
- Size exclusion chromatography, FACS, Nanoparticle tracking analysis

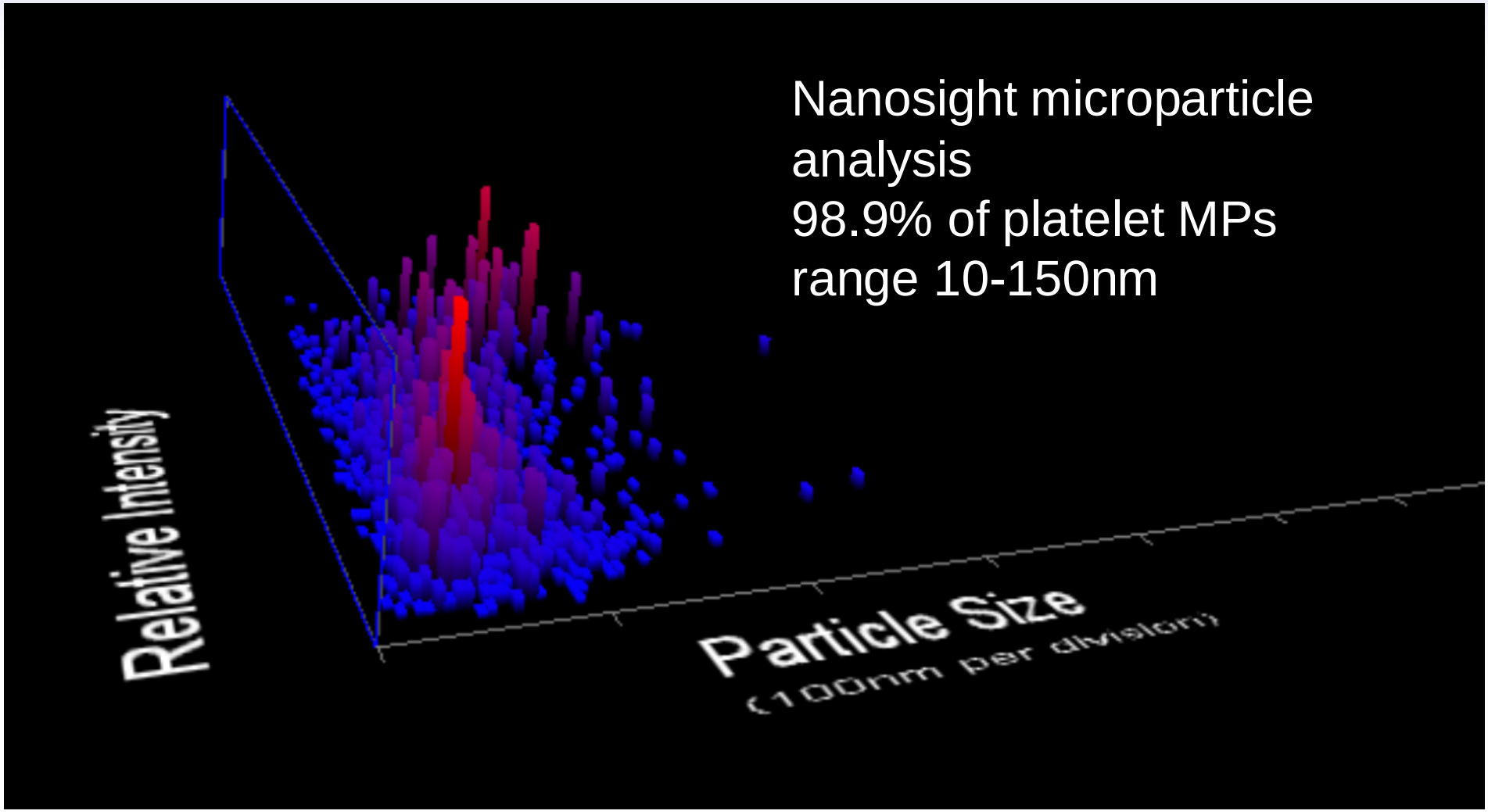
Trauma patients have increased platelet extracellular vesicles



Platelet Extracellular Vesicles are Rich in HMGB1



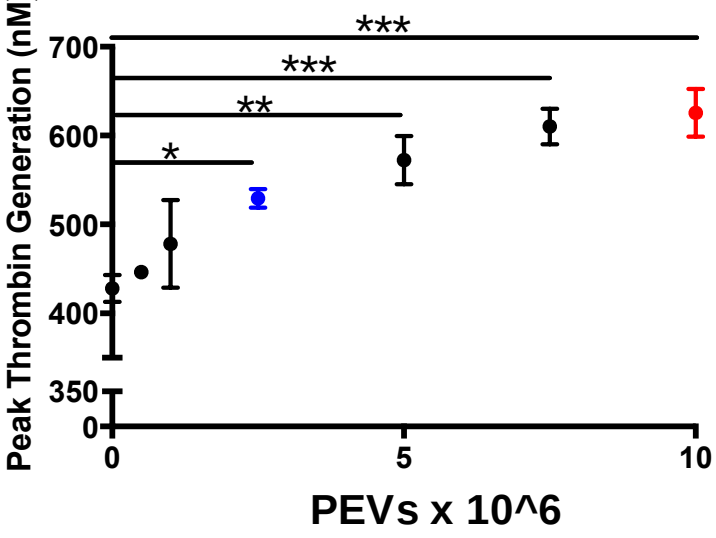
HMGB1 is presents on platelet exosomes

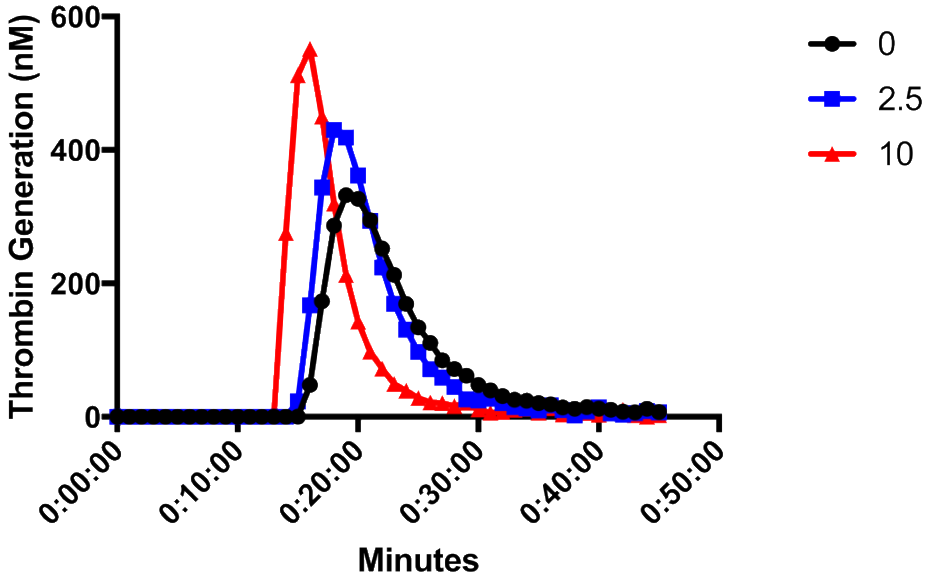


Particle Size / Relative Intensity 3D plot

Yingjie Liu
Yoel Sadovsky, MD

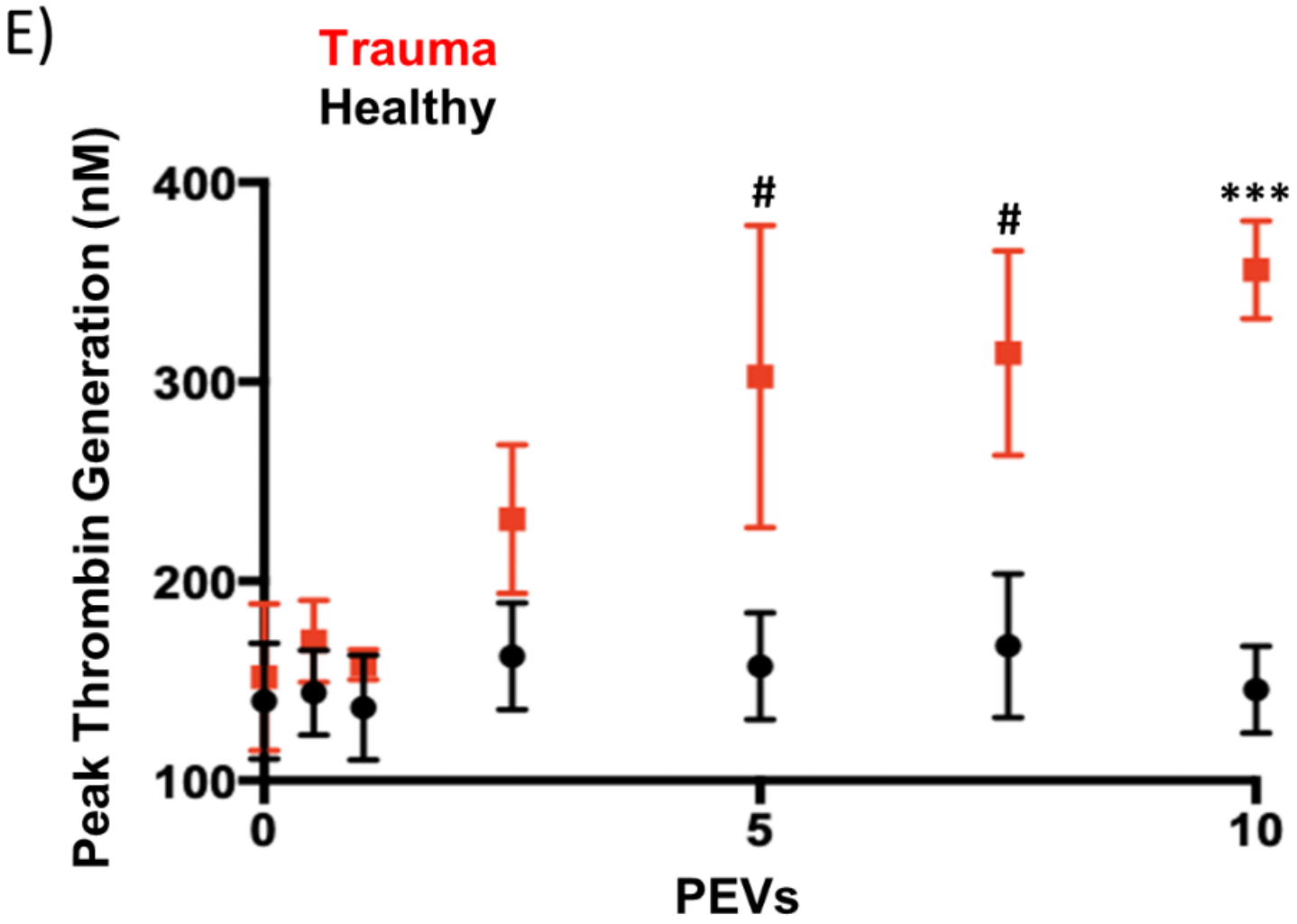
Trauma derived platelet EVs drive thrombin generation



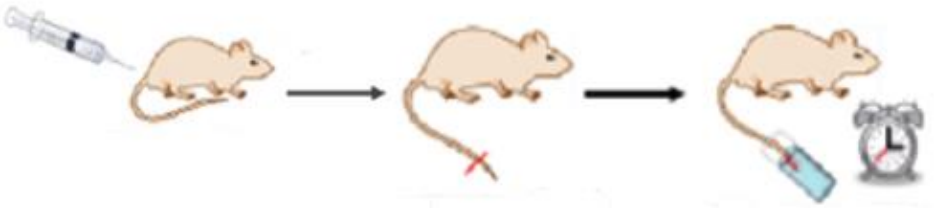


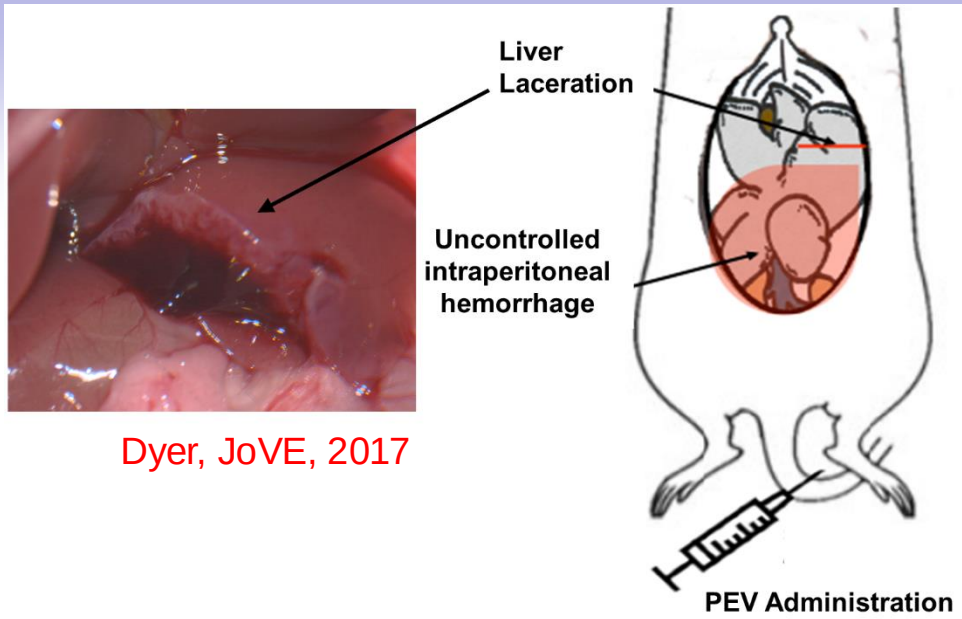
Dyer, et al, in revision (*again….*)

Trauma derived platelet EVs have increased thrombin generation over stored platelet EVs

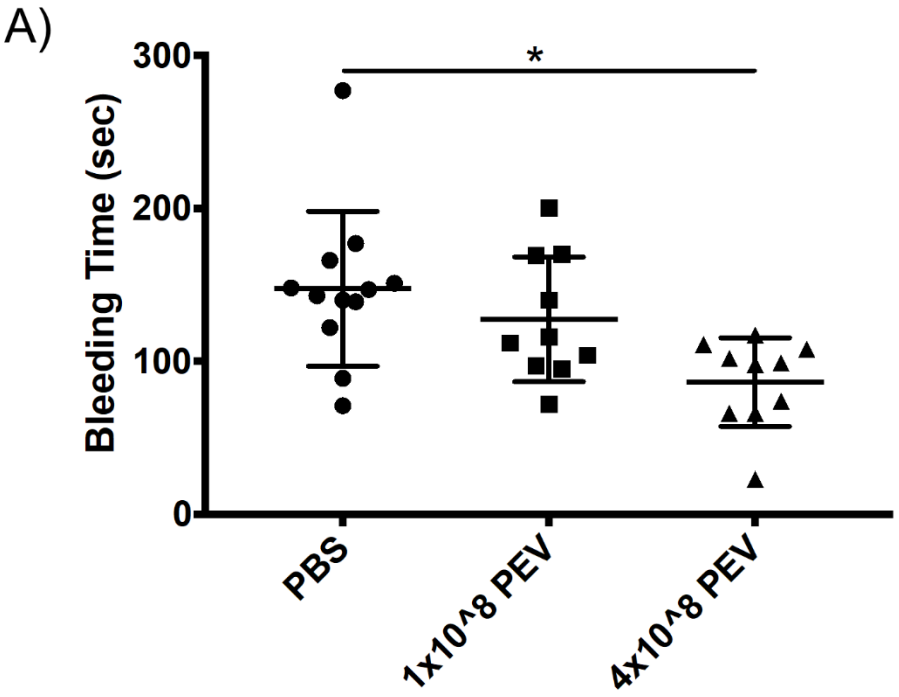


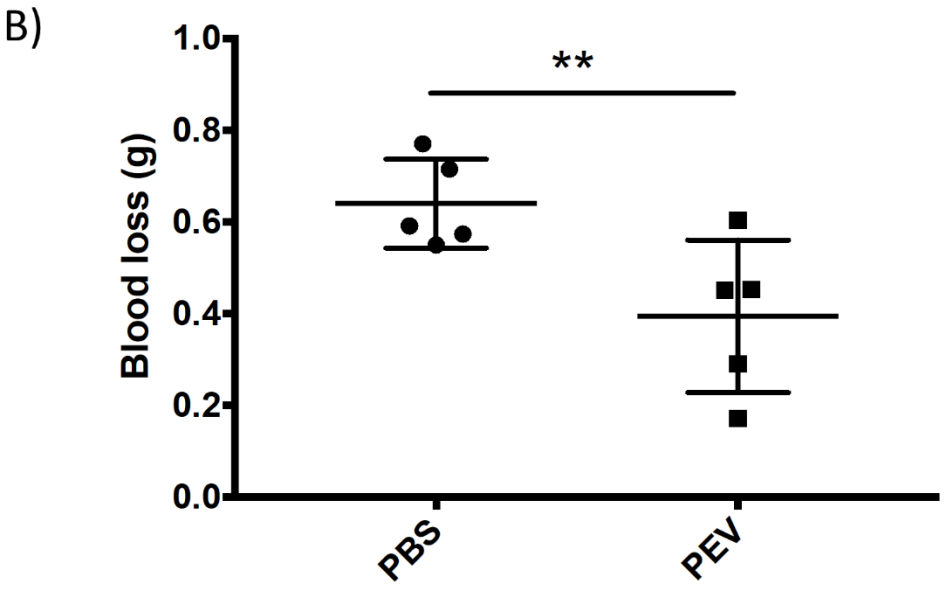
Trauma derived platelet Evs promote hemostasis



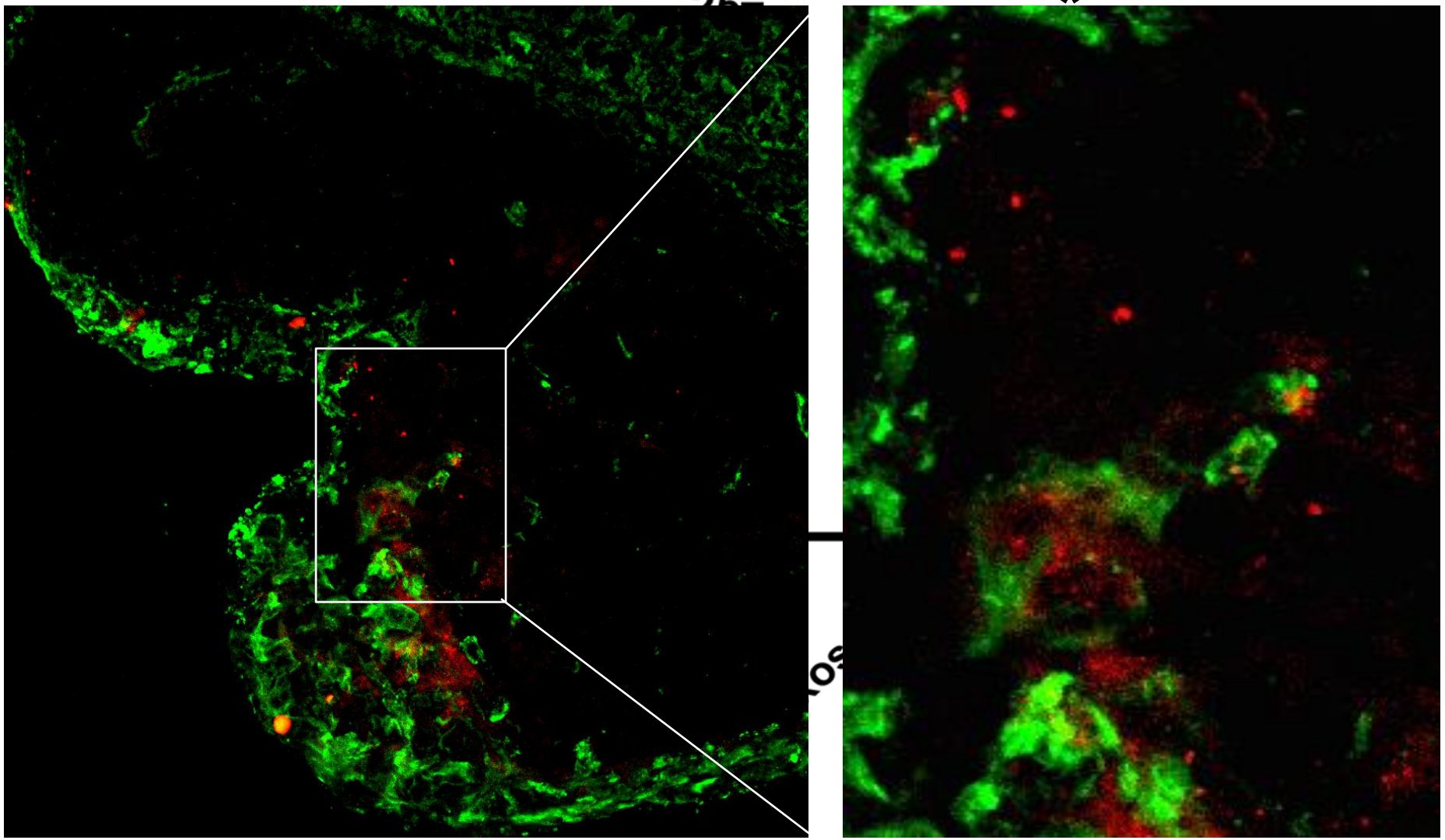


Dyer, JoVE, 2017





Adoptive transfusion of trauma-derived platelet exosomes promotes development of venous thrombosis



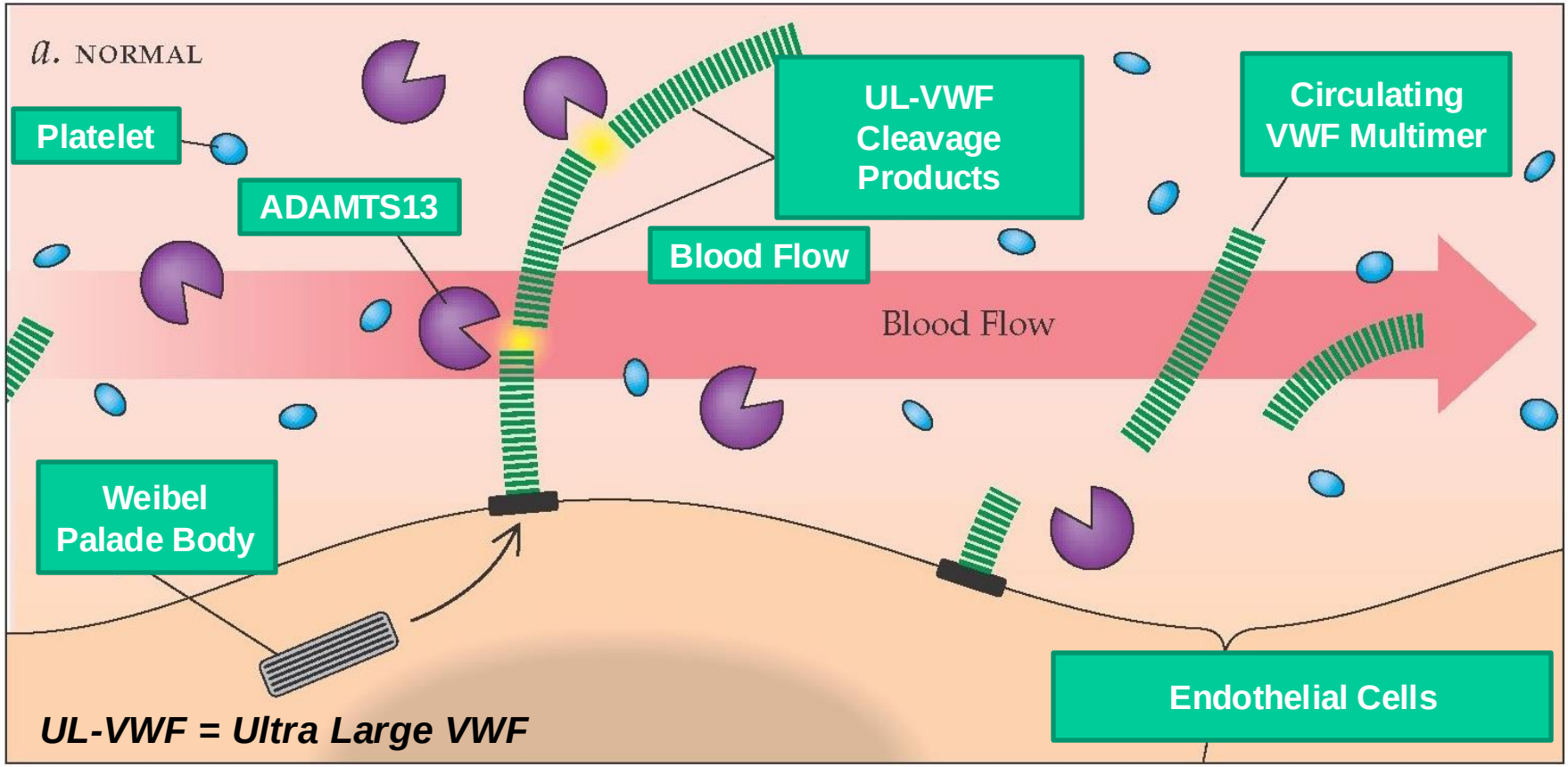
Dyer et al, in revision

*p = 0.01

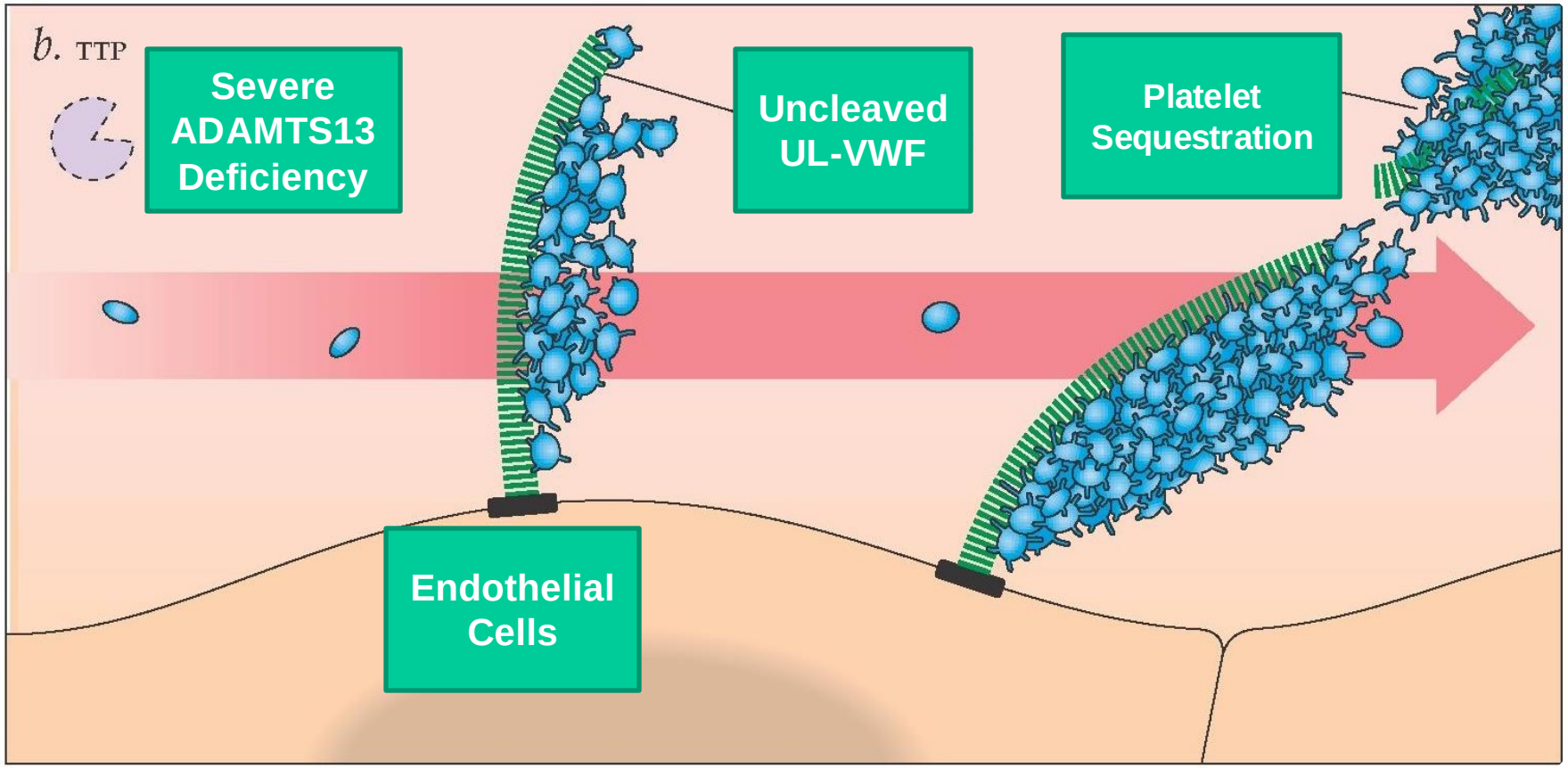
Interim summary (DVT)

- Platelet HMGB1 is a critical regulator of hemostasis and also contributes to thrombosis
- Platelets are the major source of circulating HMGB1 in murine thrombus
- Platelets release HMGB1 via extracellular vesicles following trauma
- Trauma derived platelet EVs drive thrombin generation with important consequences to hemostasis and thrombosis

ADAMTS13 and vWF: A critical balance in hemostasis



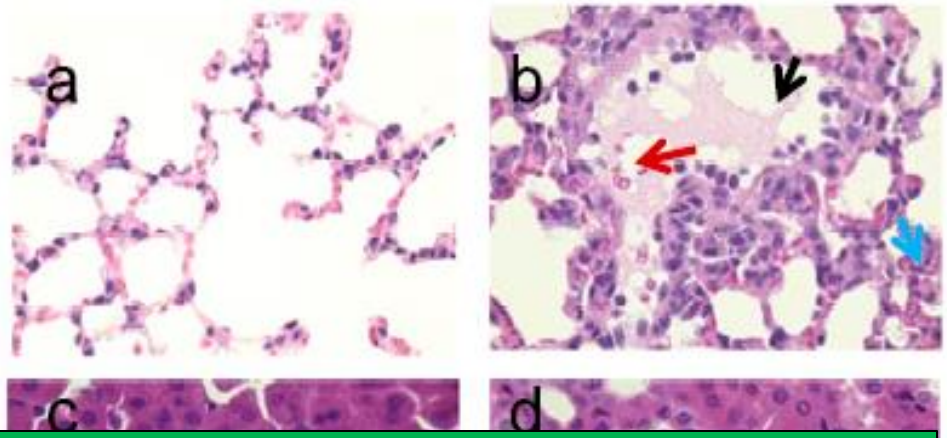
Loss of ADAMTS13 Pathology = microvascular injury/angiopathy



Dong JF, Moake JL, Nolasco L, et al. *Blood.* 2002;100(12):4033-4039
Crawley JT, de Groot R, Luken BM. *J Thromb Haemost.* 2009;7(12):2085-2087

Trauma is characterized by microvascular thrombosis

- Early reports of ‘microangiopathy’
- Autopsy and tissue studies reveal microvascular



bel of thrombogenic substances into the blood stream.[13] The fact that capillary thrombi can be found both following production of hemorrhagic shock and injection of thrombogenic materials suggested that the mechanism of the late coagulation disorder in hemorrhagic shock involved con-

Simmons, et al, Annals of Surgery, 1969

Hypotheses

Following severe trauma, a burst of ultra-large vWF is released into the bloodstream by activated, damaged endothelium to promote hemostasis

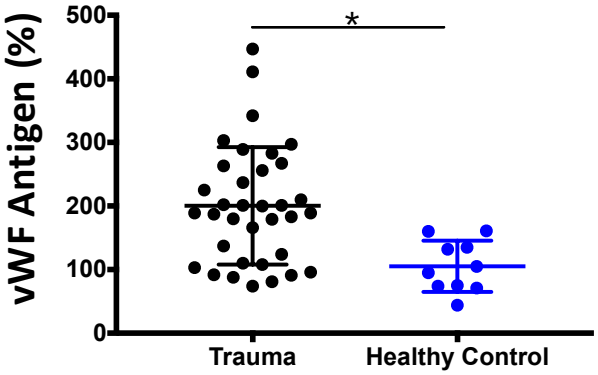
ADAMTS13 activity is impaired and insufficient to cleave the burst of ultra-large vWF, leading to organ injury

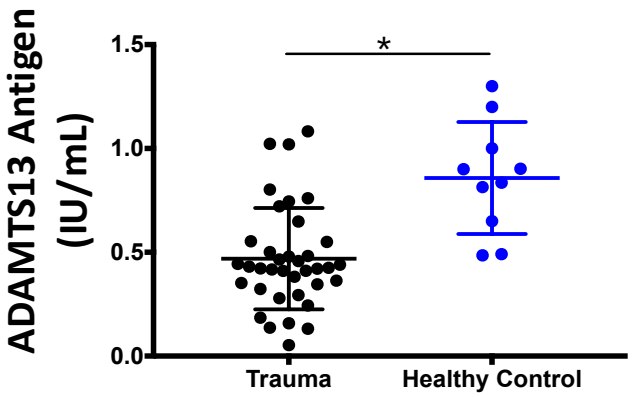
Analysis of severely injured patient samples for vWF and ADAMTS13

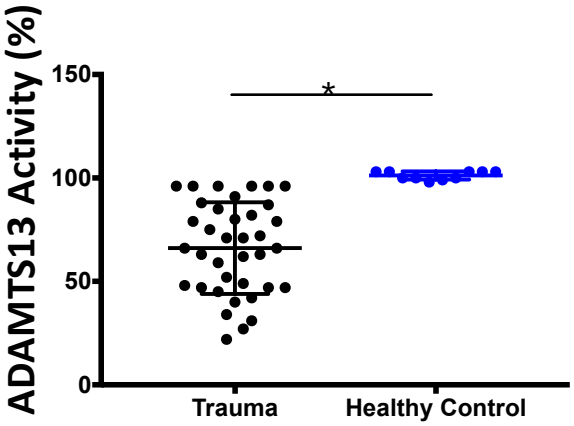
Table 1. Baseline Characteristics/Demographics	
Characteristic	N=37
Median age (IQR), yr	46 (26-63)
Male Sex (%)	81
Race (%)	
White	81
African-American	16
Blunt Trauma (%)	81
Anticoagulant (%)	11
Aspirin (%)	14
Median ISS (IQR)	18 (10-25)
Median prehospital SBP (IQR), mmHg	77 (66-86)
Coagulopathic (%)	43
30-day mortality (%)	22

vWF and ADAMTS13 Levels at ED evaluation (time 0) and 24-hrs Following Severe Trauma

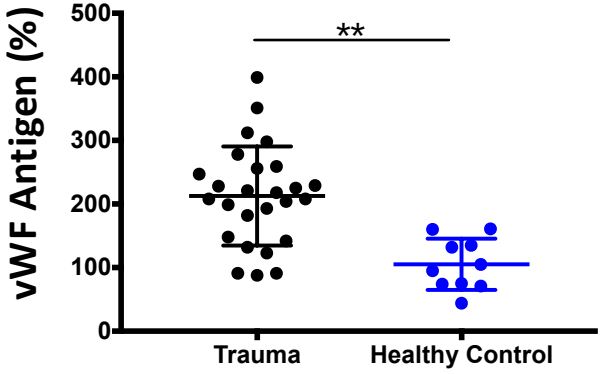
0-Hour Values

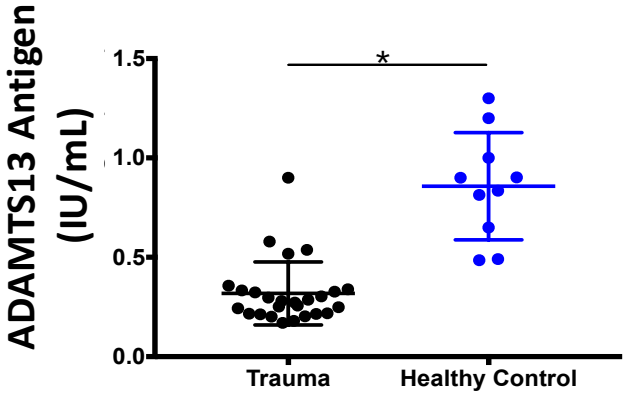


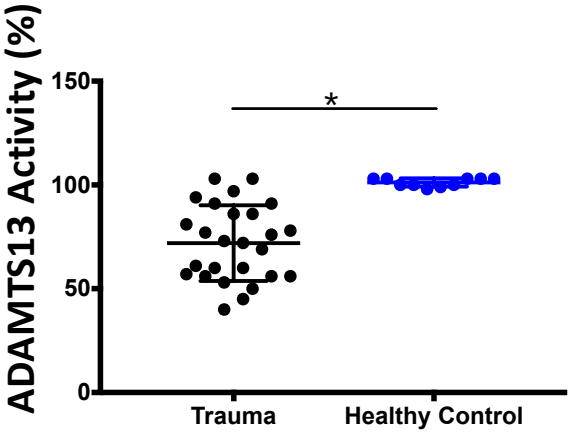




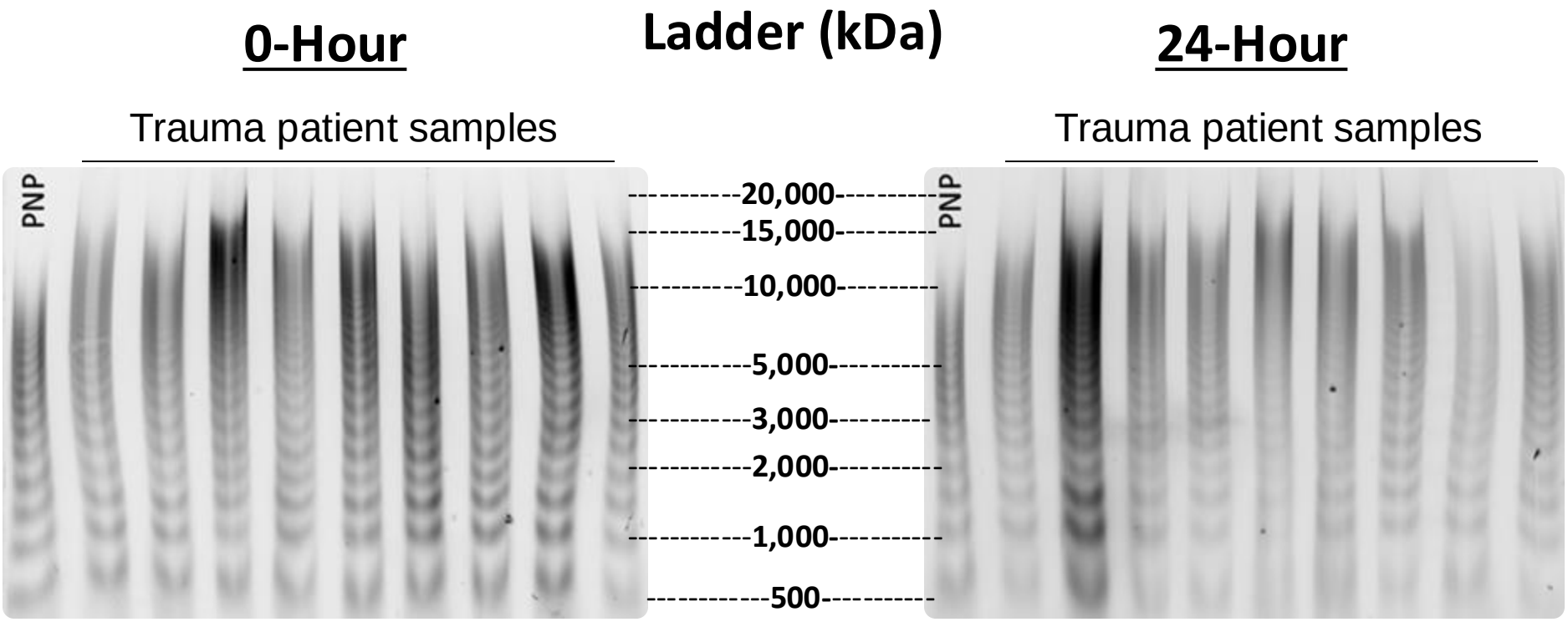
24-Hour Values







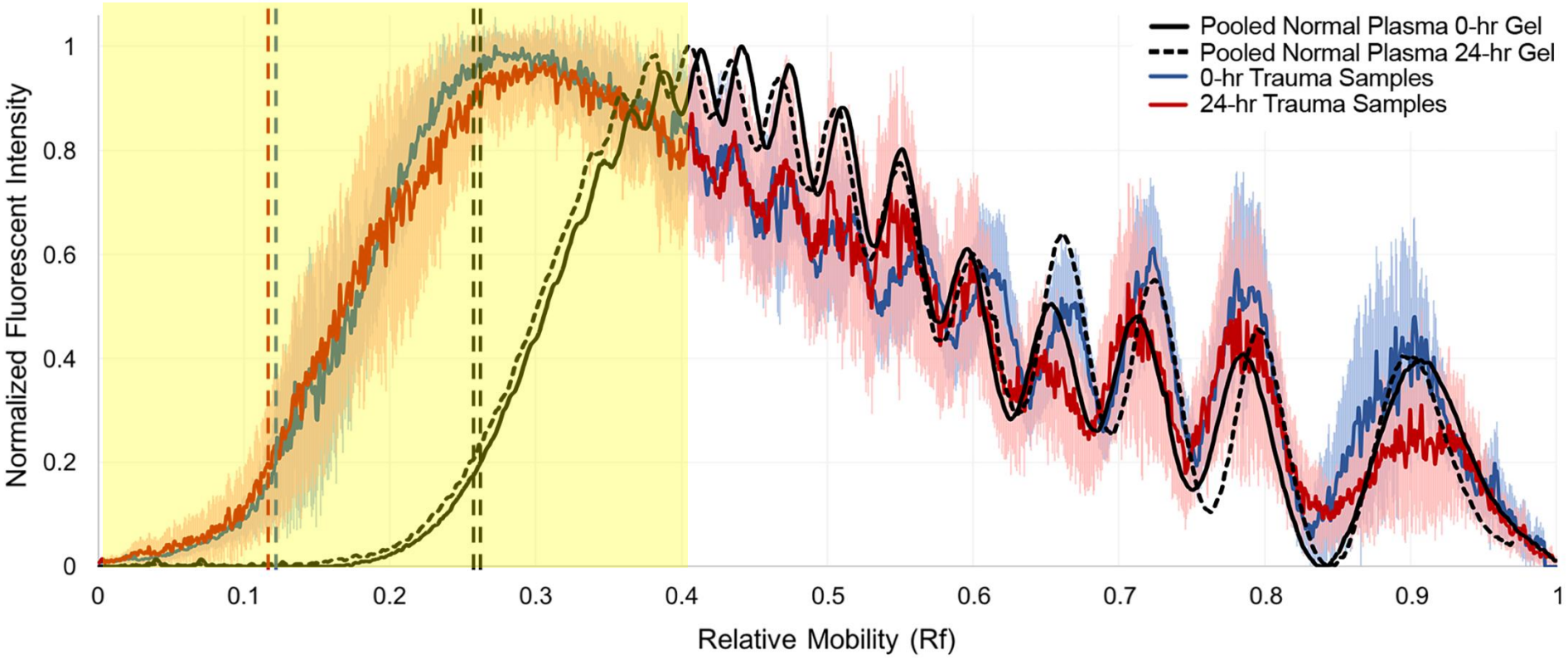
vWF present after injury is in ultra-large multimer form



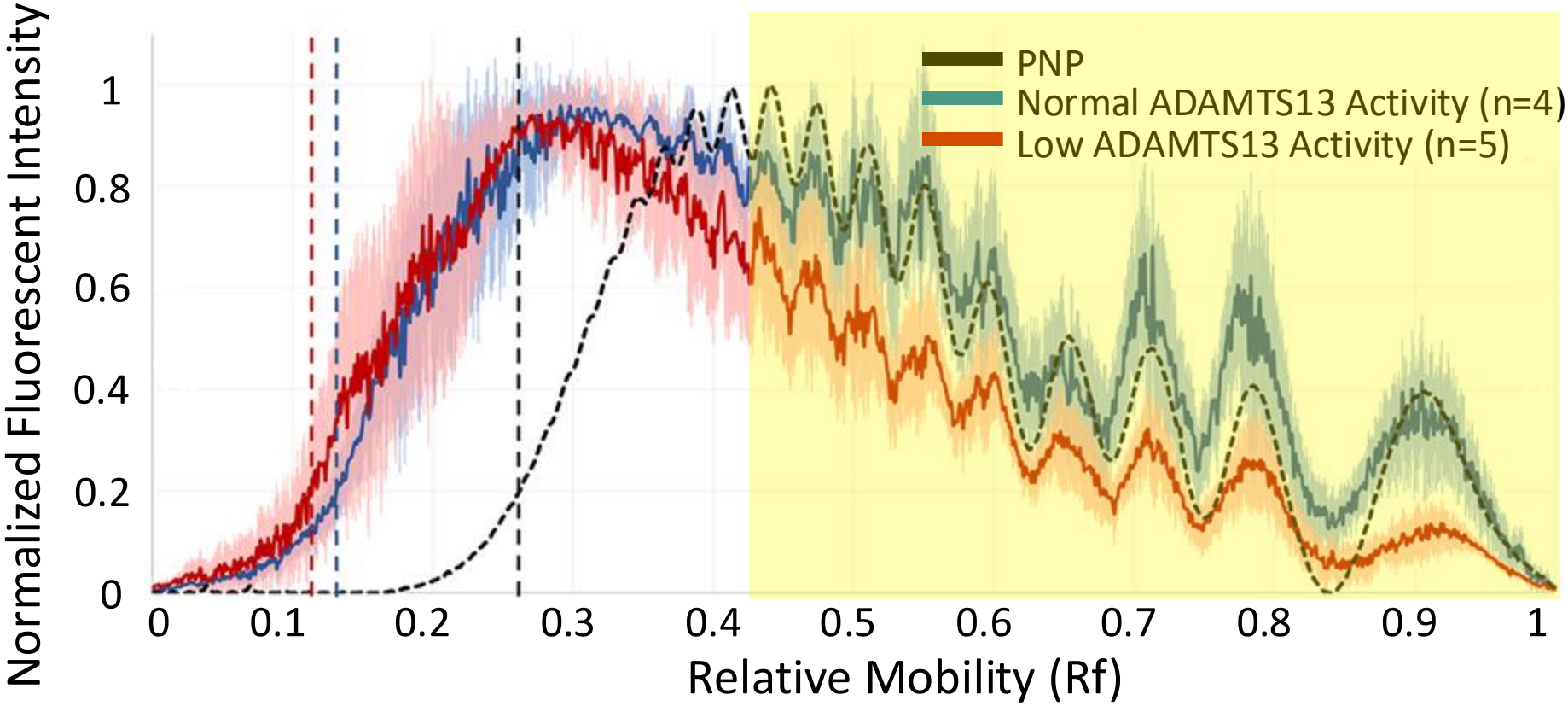
Vertical Agarose vWF Multimer Gel

--To achieve this resolving power on an 8% SDS-PAGE gel, it would have to be >0.4 meters in height

vWF after severe injury is released and persists in ultra-large multimer form



ADAMTS13 Activity regulates return to vWF homeostasis



Following Severe Traumatic Injury, we have:

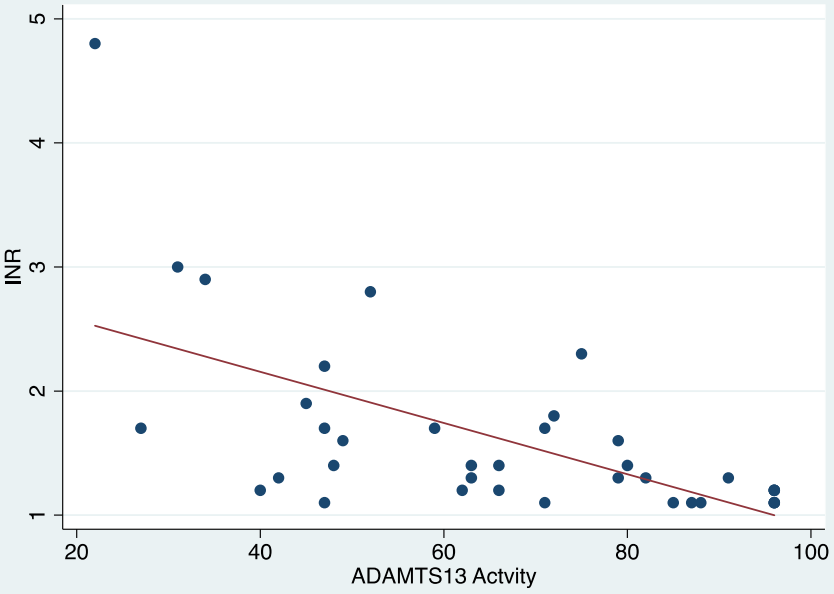
1) a near instantaneous *increase in UL-vWF* with a concomitant decrease in ADAMTS13 within the patient circulation

2) Extending 24-hours after admission

3) Demonstrating an ADAMTS13 dependent return to vWF homeostasis at 24-hours

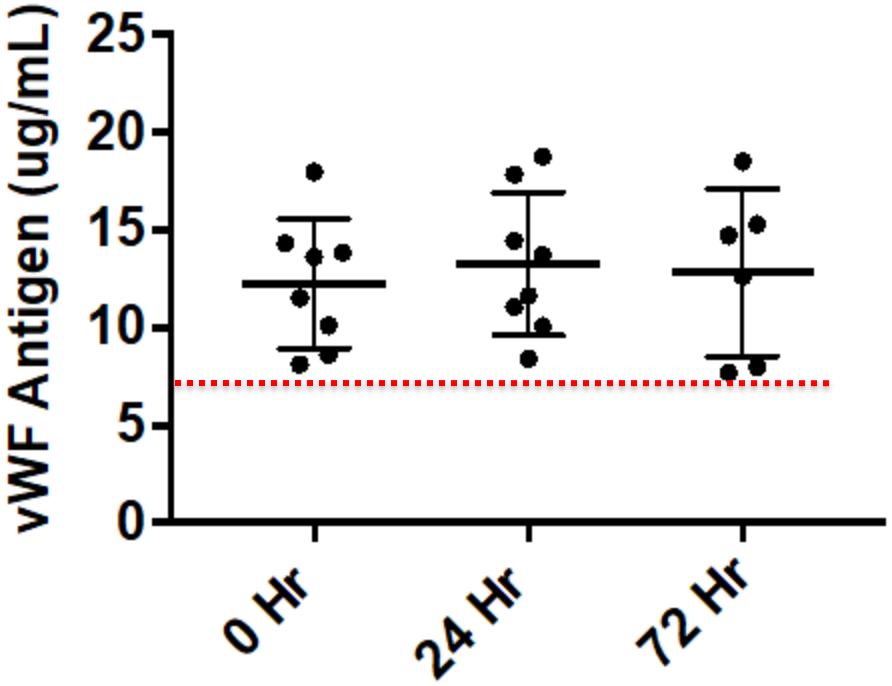
ADAMTS13 levels correlate with coagulopathy and clinical injury signs

- **ADAMTS13 activity on admission correlated with:**
  - Lab Markers of Coagulopathy (INR and TEG MA)
    ρ=-0.63, p<0.0001
  - Clinical Markers of Coagulopathy (Transfusion requirements)
    --Overall blood product transfusion (first 24 hours) (ρ=0.45, p=0.008)
  - Injury Severity Score
    --(ρ=-0.34, p=0.049)

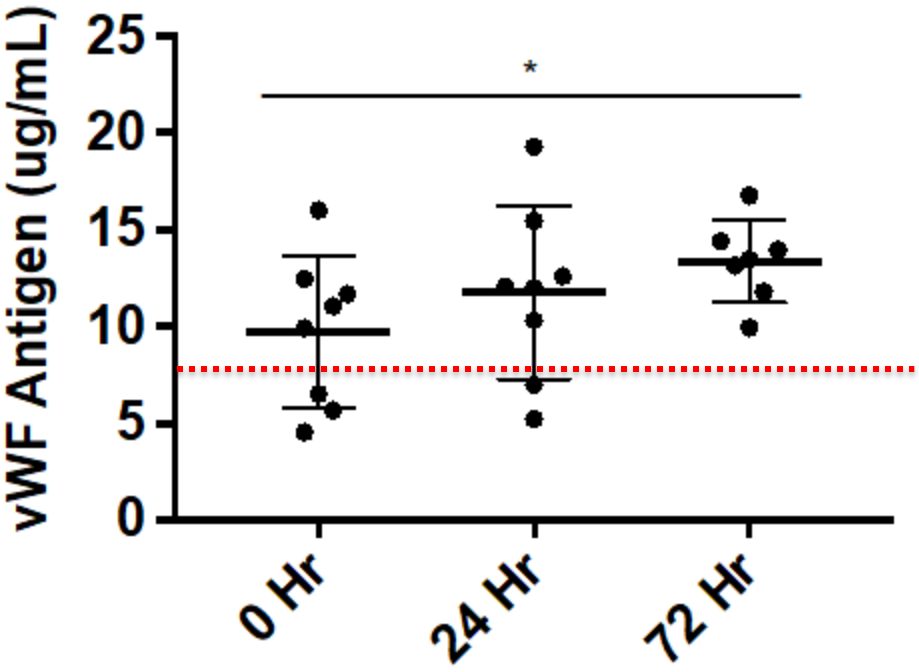


Plasma vWF Antigen Levels Correlate With AKI after Trauma

Control Trauma Patients (No AKI)



Trauma Patients that Developed AKI



Red Dotted Line = Normal Pooled Plasma (>= 20 Subjects) vWF Antigen Level

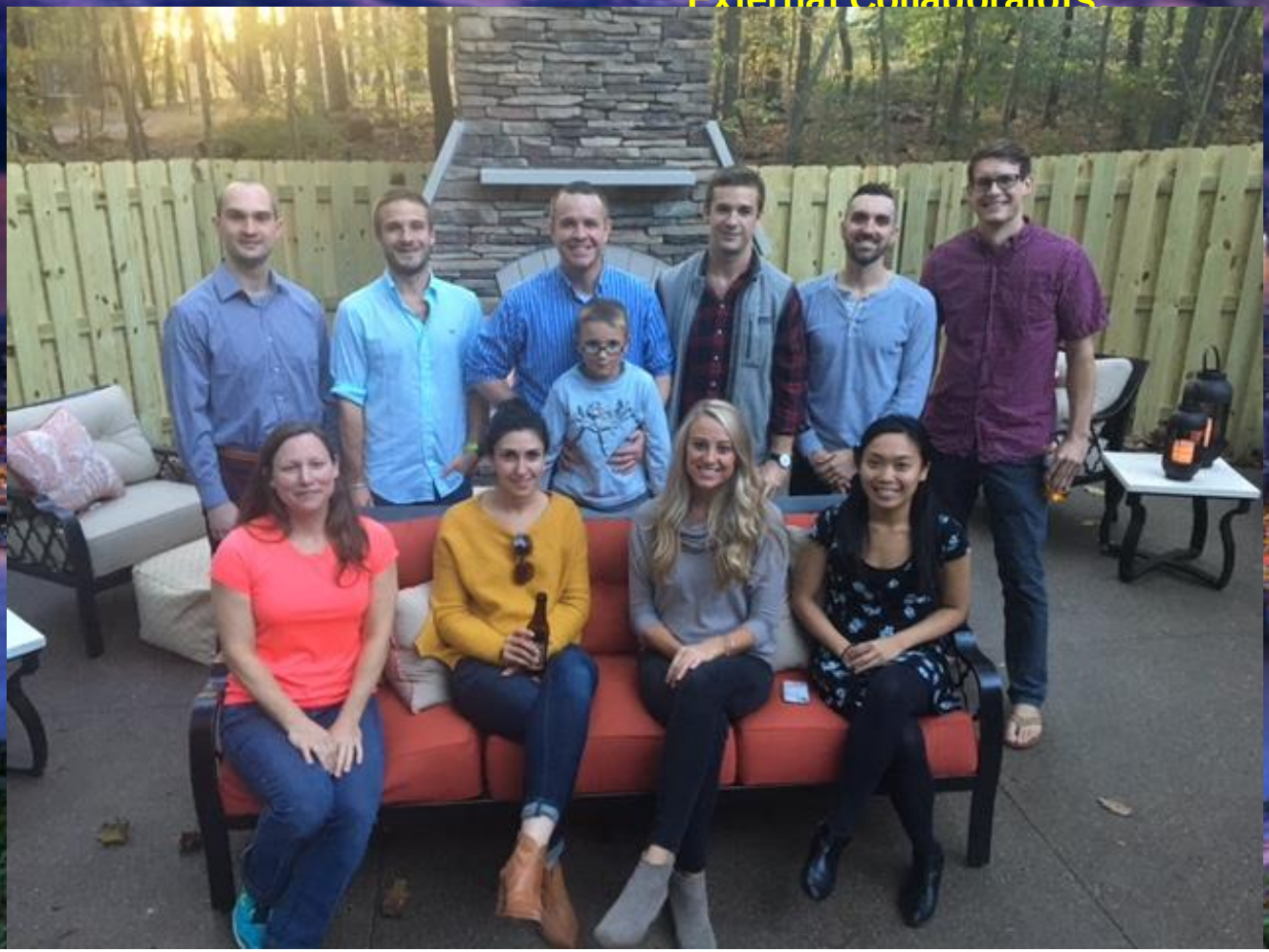
Summary/conclusions

- Platelet HMGB1 is a critical mediator of hemostasis and thrombosis after trauma
- Trauma derived platelet extracellular vesicles are potent thrombin generators
- An acute surge of vWF in ultra-large form occurs immediately after injury and persists
- ADAMTS13 level and activity are reduced after injury
- vWF antigen may be correlated with organ injury

Acknowledgements

The TEAM:

External Collaborators:



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- NIH 1 R34 HL135224 01A1 (Co-I)
- NIH 1R21HL133891-01 (Co-I)
- NIH UM1HL120877-01 (Co-I)
- BARDA #HHS01002015000081 (Co-PI)
- UPMC ITTC
- Vascular Medicine Institute Pilot Project Program in Hemostasis and Vascular Biology (P3HVB)
- Janssen Pharmaceuticals
- AAST Research Fellowship

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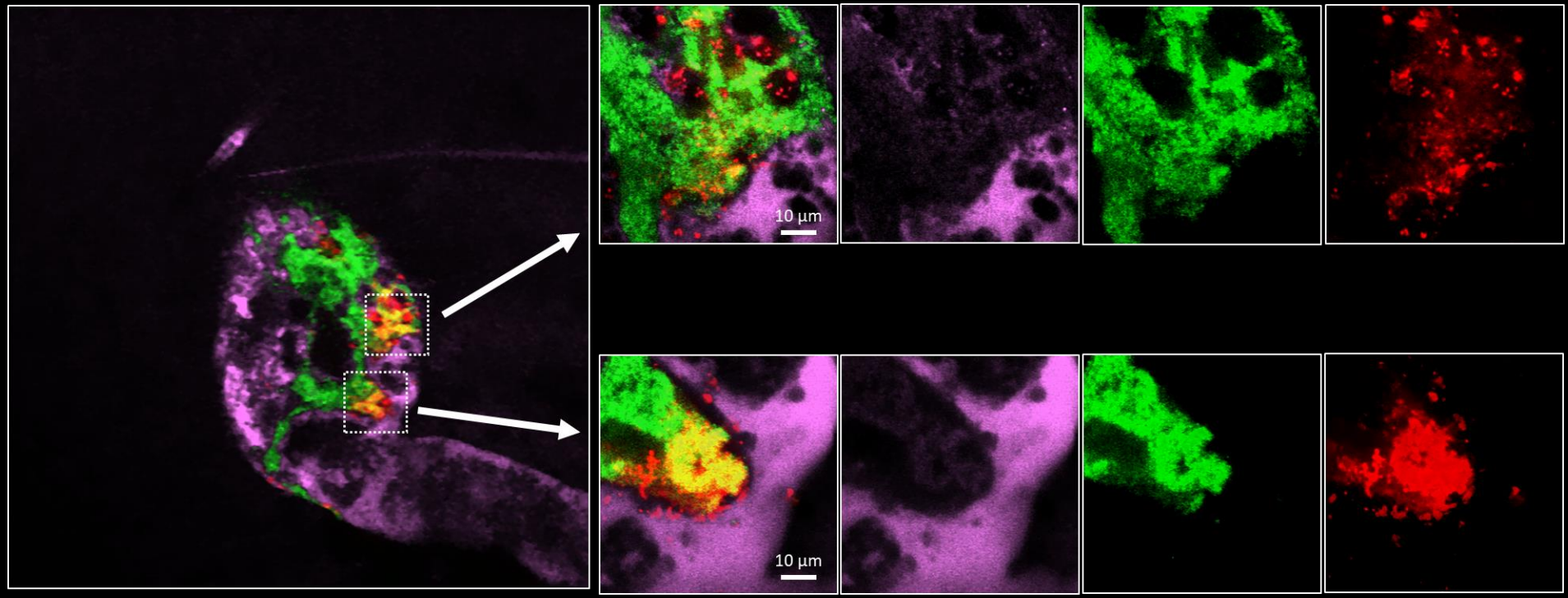
Intravital multi-photon confocal microscopy showing targeted binding to activated platelets using platelet inspired nanotechnology



vasculature: magenta (FITC dextran)
platelets: green (V450-CD 49b Ab)
Platelet-inspired nanoparticles: red (RhB)