

Platelet Genomics *In Injury*

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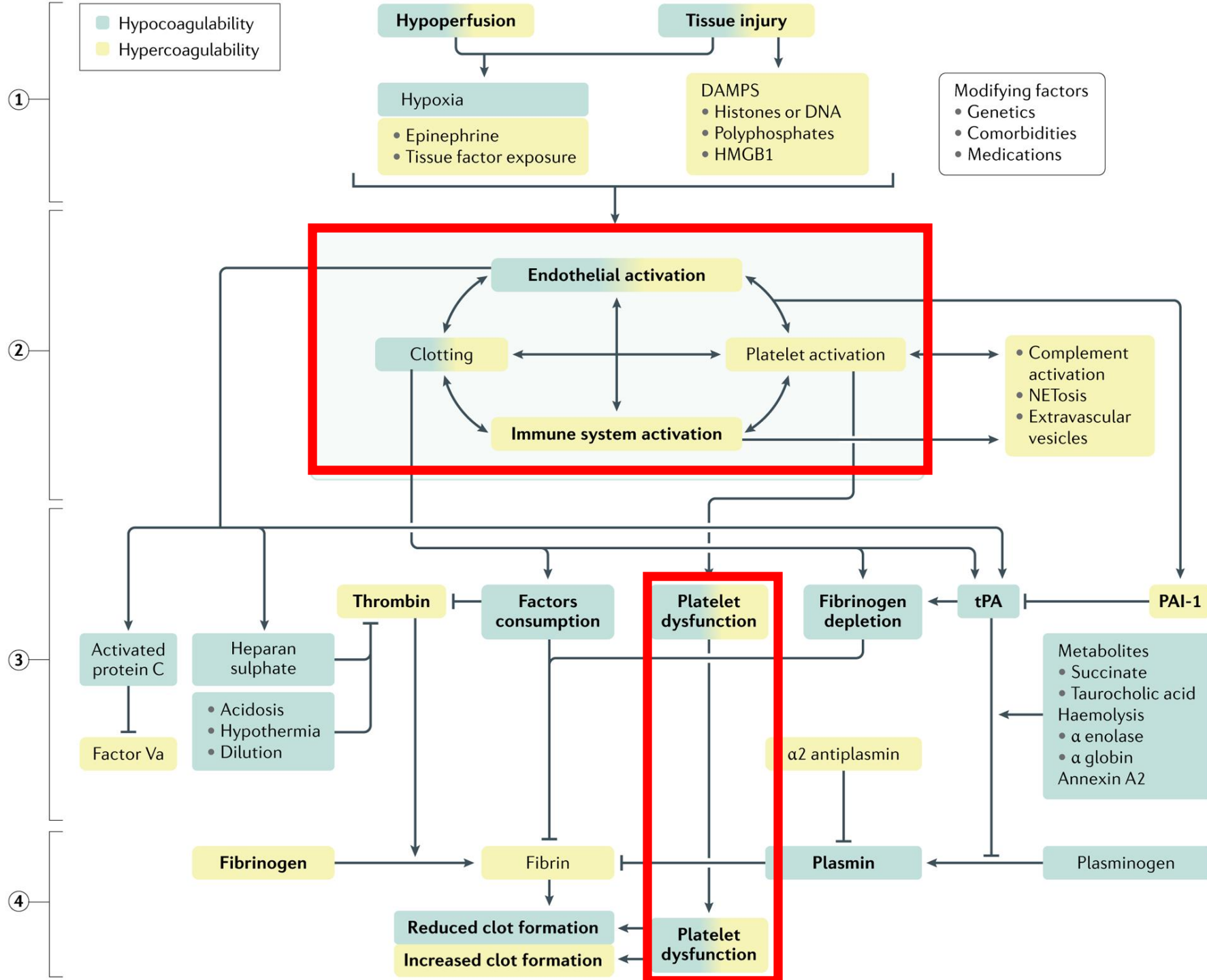


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 - Coagulant Therapeutics
- Family
 - Husband, founder of CaptureDx

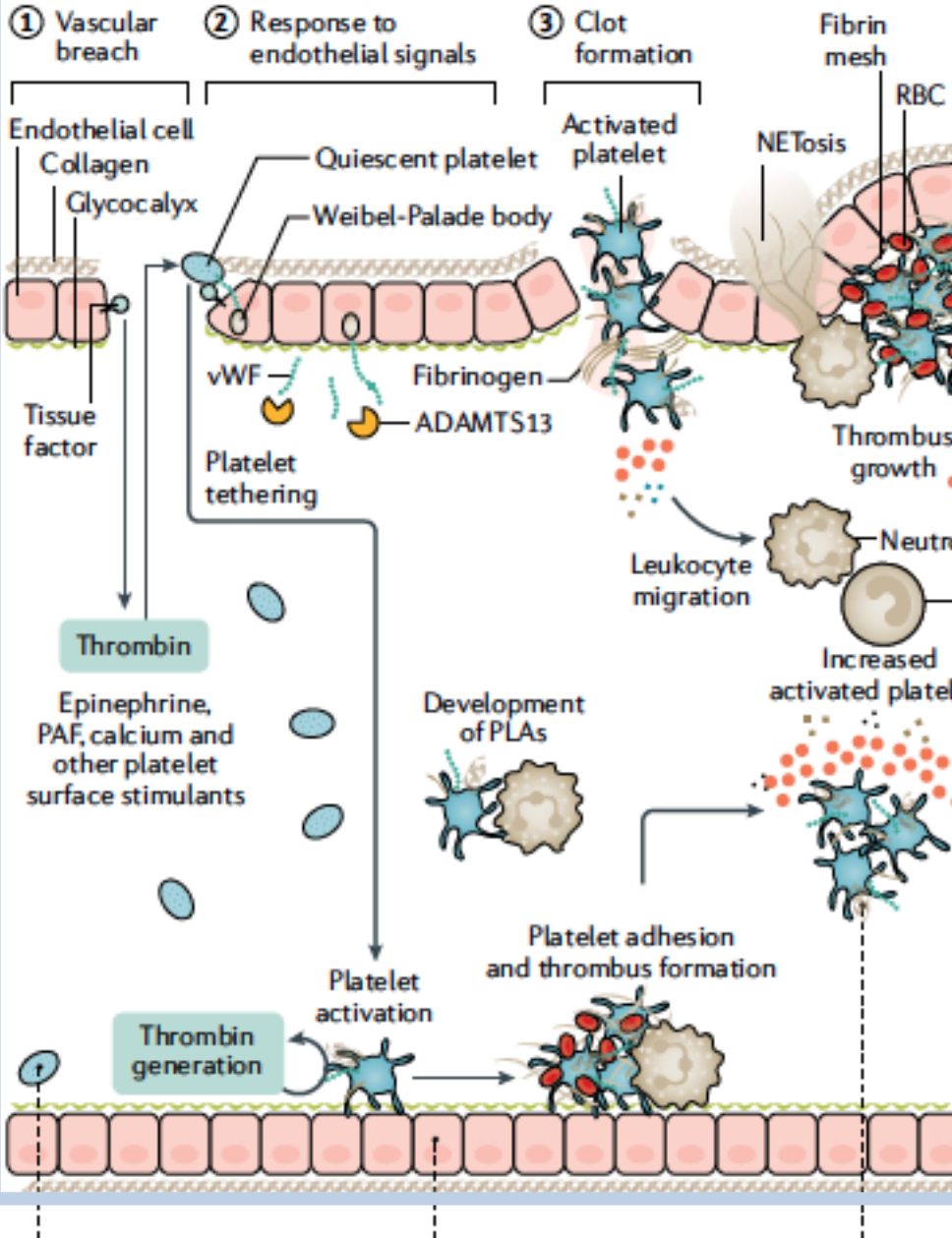


Platelets

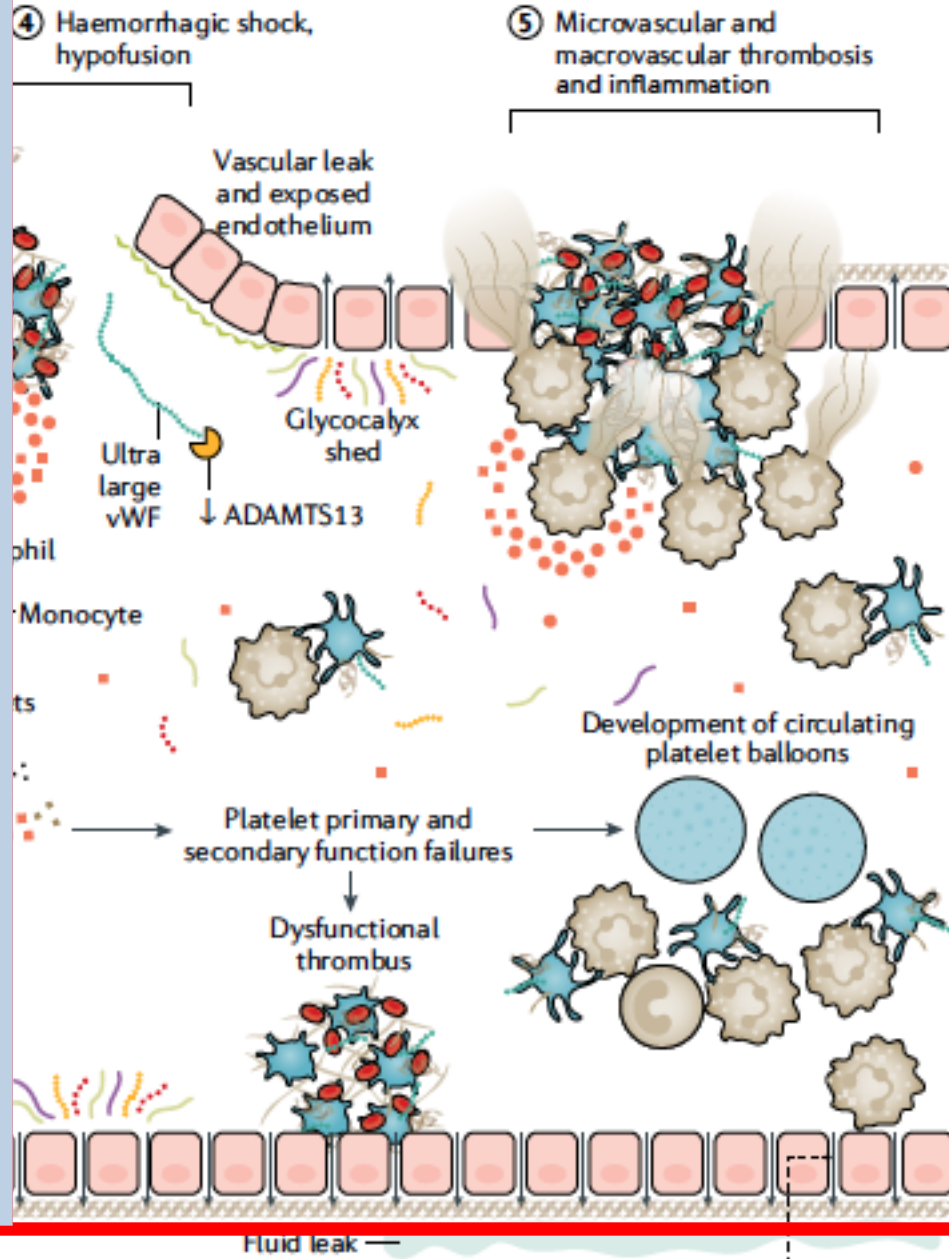


Moore EE, Moore HB, Kornblith LZ, Neal MD, Hoffman M, Mutch NJ, Schöchl H, Hunt BJ, Sauaia A. Trauma-induced coagulopathy. Nat Rev Dis Primers. 2021 Apr 29;7(1):30.(2021).

Adaptive



Maladaptive



Moore EE, Moore HB, Kornblith LZ, Neal MD, Hoffman M, Mutch NJ, Schöchl H, Hunt BJ, Sauaia A. Trauma-induced coagulopathy. *Nat Rev Dis Primers.* 2021 Apr 29;7(1):30. doi: 10.1038/s41572-021-00264-3. Erratum in: *Nat Rev Dis Primers.* 2022 Apr 22;8(1):25.

Post-injury platelet biology: *what we know*

- Platelet counts are normal
- Platelets are activated

BUT

- Measured ex-vivo platelet aggregatory function is impaired
- AND.... clinical associations are variable
- AND... platelet impairments are not reversed with platelet transfusion

- Multiple pathways potentially contribute

Post-injury platelet biology: *what we don't know*

- Adaptive or maladaptive?
- Defined biology explained by ex-vivo assays?
- Requires treatment?
- What is the treatment?

Identification of injury and shock driven effects on ex vivo platelet aggregometry: A cautionary tale of phenotyping

Nichole R. Rachael¹
¹A. Matthay, MD, Alexander T. Fields, PhD, Brenda Nunez-Garcia, BA, et al J. Cohen, MD, and Lucy Z. Kornblith, MD, San Francisco, California

2018 WTA PODIUM PAPER

Perhaps it's not the platelet: Ristocetin uncovers the potential role of von Willebrand factor in impaired platelet aggregation following traumatic brain injury

Lucy Z. Kornblith, MD, Anamaria J. Robles, MD, Amanda S. Conroy, Carolyn M. Hendrickson, MD, MPH, Carolyn S. Calfee, MD, MAS, Alexander T. Fields, PhD, Rachael A. Callcut, MD, MSPH, and Mitchell J. Cohen, MD, San Francisco, California

SCIENCE ADVANCES | RESEARCH ARTICLE CORONAVIRUS Platelets amplify endotheliopathy in COVID-19

Tessa J. Barrett^{1,2}, Macintosh Cornwell^{1,2}, Khrystyna Mymnazar¹, Christina C. Rolling¹, Yuhua Xia¹, Kamella Drenkova¹, Antoine Babuyck¹, Alexander T. Fields³, Michael Tawil¹, Elliot Luttrell-Williams¹, Eugene Yuriditsky¹, Grace Smith⁴, Paola Cotsis^{3,5}, Matthew D. Neal⁶, Lucy Z. Kornblith², Stefana Pittaluga¹, Amy V. Raskiewicz², Hannah M. Burgess⁷, Ian Mohr⁸, Kenneth A. Stapleford⁹, Deepak Voora¹, Kelly Reggles¹, Judith Hochman¹, Jeffrey S. Berger^{1,11}

Postinjury platelet aggregation and venous thromboembolism

Zachary A. Matthay, MD, Zane J. Hellmann, MD, Brenda Nunez-Garcia, BS, Alexander T. Fields, PhD, Joseph Cuschieri, MD, Matthew D. Neal, MD, Jeffrey S. Berger, MD, Elliot Luttrell-Williams, BA, M. Margaret Knudson, MD, Mitchell J. Cohen, MD, Rachael A. Callcut, MD, MSPH, and Lucy Z. Kornblith, MD, San Francisco, California

2021 AAST PODIUM PAPER

Effects of the circulating environment of COVID-19 platelet and neutrophil behavior

Alexander T. Fields¹, Elizabeth A. Andrasika², Christof Kallenmieser³, Zachary A. Matthay¹, Kimberly Herrera¹, Brenda Nunez-Garcia¹, Chayse M. Jones², Katherine D. Wick^{1,4}, Silvia Liu¹, Juan-Yua Luo¹, Yan-Ping Yu¹, Michael A. Matthay^{1,5}, Carolyn M. Hendrickson¹, Roland J. Bainton¹, Tessa J. Barrett^{1,6}, Jeffrey S. Berger^{1,7,8}, Matthew D. Neal⁹, Lucy Z. Kornblith¹⁰, and the COVID-19 Associated Coagulopathy, Inflammation and Thrombosis (Co-ACT) Study Group

OPEN

1786 Open Access Research Article
Published: 14 May 2021
doi:10.3389/fimmu.2021.612509

AAST 2019 PODIUM PAPER

A journey upstream: Fluctuating platelet-specific genes in cell-free plasma as proof-of-concept for using ribonucleic acid sequencing to improve understanding of postinjury platelet biology

Lucy Z. Kornblith, MD, Cedric M.V. Bainton, Alexander T. Fields, PhD, Zachary A. Matthay, MD, Nina T. Magid, Brenda Nunez-Garcia, BA, Arun Prakash, MD, PhD, Philip A. Kurien, MD, Rachael A. Callcut, MD, MSPH, Mitchell J. Cohen, MD, and Roland J. Bainton, MD, PhD, San Francisco, California

2019 WTA PODIUM PAPER

It's About Time: Transfusion effects on postinjury platelet aggregation over time

Lucy Z. Kornblith, MD, Anna Decker, PhD, Amanda S. Conroy, RN, Carolyn M. Hendrickson, MD, MPH, Alexander T. Fields, PhD, Anamaria J. Robles, MD, Rachael A. Callcut, MD, MSPH, and Mitchell J. Cohen, MD, San Francisco, California

PRIMER

Trauma-induced coagulopathy

Ernest E. Moore^{1,2,3,4}, Hunter B. Moore², Lucy Z. Kornblith⁵, Matthew D. Neal⁶, Maureane Hoffman⁶, Nicola J. Mutch⁶, Herbert Schöchl⁷, Beverley J. Hunt⁸, and Angela Savaia^{9,10}

Published in final edited form as:
J Thromb Haemost. 2019 June ; 17(6): 852–862. doi:10.1111/jth.14450.

Trauma-Induced Coagulopathy: The Past, Present, and Future

Lucy Z. Kornblith¹, Hunter B. Moore², and Mitchell J. Cohen³
¹Department of Surgery, Zuckerberg San Francisco General Hospital and the University of California, San Francisco, San Francisco, California, 1001 Potrero Avenue, Building 1, Suite 210, San Francisco, CA 94110
²Department of Surgery, Denver Health Medical Center and the University of Colorado, Denver, Colorado, 777 Bannock Street. Mail Code 0206, Denver, CO 80203

WTA PODIUM - 2020

Dynamic effects of calcium on in vivo and ex vivo platelet behavior after trauma

Zachary A. Matthay, MD, Alexander T. Fields, PhD, Brenda Nunez-Garcia, BA, Maya H. Patel, HSD, Mitchell J. Cohen, MD, Rachael A. Callcut, MD, MSPH, and Lucy Z. Kornblith, MD, San Francisco, California

SHOCK, Vol. 55, No. 2, pp. 189–197, 2021

GOOD PLATELETS GONE BAD: THE EFFECTS OF TRAUMA PATIENT PLASMA ON HEALTHY PLATELET AGGREGATION

Alexander T. Fields¹, Zachary A. Matthay¹, Brenda Nunez-Garcia¹, Ellicott C. Matthay¹, Roland J. Bainton¹, Rachael A. Callcut¹, and Lucy Z. Kornblith¹
¹Department of Surgery, University of California, San Francisco; ²Department of Epidemiology and Biostatistics, University of California, San Francisco; and ³Department of Anesthesia and Perioperative Care, University of California, San Francisco

ORIGINAL ARTICLE

Importance of catecholamine signaling in the development of platelet exhaustion after traumatic injury

Zachary A. Matthay¹, Alexander T. Fields¹, Chayse Jones², Aleksandra Leligdowicz², Brenda Nunez-Garcia¹, John J. Park³, Rachael A. Callcut³, Michael A. Matthay², and Lucy Z. Kornblith¹



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PubMed: 2021, 1532, 202-204
https://doi.org/10.1080/09537104.2020.171863



Brohl¹ & Matthew D. Neal⁶

AAST 2013 PLENARY PAPER

Fibrinogen and platelet contributions to clot formation: Implications for trauma resuscitation and thromboprophylaxis

Lucy Z. Kornblith, MD, Matthew E. Kutcher, MD, Brittney J. Redick, BA, Carolyn S. Calfee, MD, Ryan F. Vilardi, BS, and Mitchell Jay Cohen, MD, San Francisco, California

The problems with studying platelet function

- Highly sensitive
- Highly sticky
- Markedly diminutive
- Subpopulations likely have different biology
- Anucleate
- Model systems are imperfect

A guide to molecular and functional investigations of platelets to bridge basic and clinical sciences

Tarun Tyagi^{1,6}, Kanika Jain^{1,6}, Sean X. Gu^{1,2}, Miaoyun Qiu³, Vivian W. Gu¹, Hannah Melchinger¹, Henry Rinder², Kathleen A. Martin¹, Elizabeth E. Gardiner⁴, Alfred I. Lee⁵, Wai Ho Tang³ and John Hwa¹✉


NATURE CARDIOVASCULAR RESEARCH

REVIEW ARTICLE

Table 4 | A concise guide to research approaches used to assess platelet properties

Platelet state	Immature/young	Resting	Activated	Apoptotic	Defective	Cell conjugates
Technique	Flow cytometry (reticulated platelets) Hematology analyzer (Sysmex IPF, Abbott retPLT)	Flow cytometry Western blotting Imaging (SEM, TEM) Phase-contrast/confocal microscopy Proteomics RNA sequencing	Flow cytometry Western blotting Aggregometry Imaging (SEM, TEM) ELISA PFA-100 Proteomics RNA sequencing Mass cytometry Intravital imaging	Flow cytometry Western blotting Spectroscopy Confocal microscopy	Flow cytometry Lumiaggregometry ROTEM platelet PFA-100 Imaging (TEM) Genomic sequencing Immunofluorescence	Flow cytometry Confocal microscopy Single-cell morphometry Mass cytometry Real-time microfluidic assays Intravital imaging

SCIENTIFIC REPORTS



OPEN

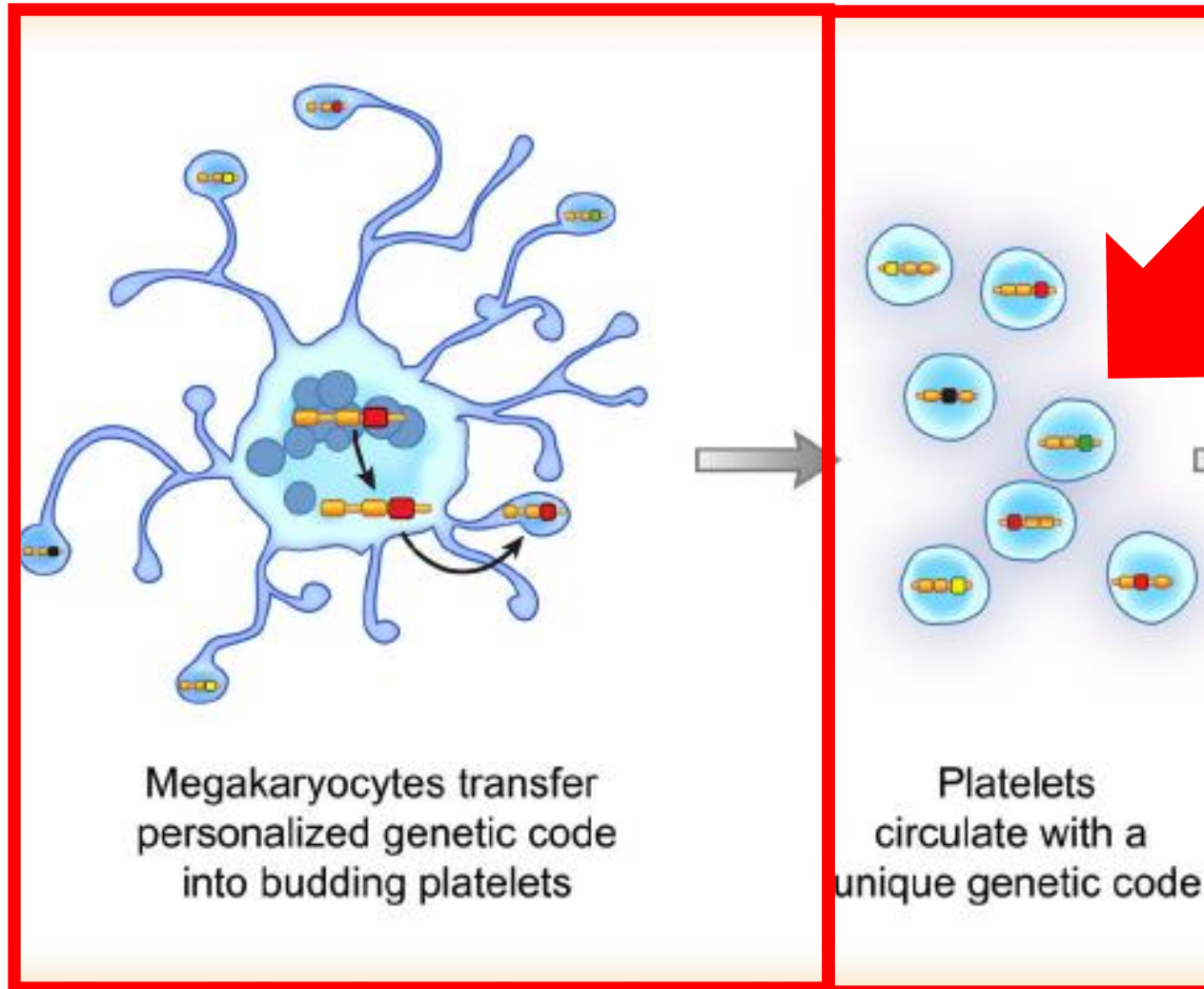
Splicing of platelet resident pre-mRNAs upon activation by physiological stimuli results in functionally relevant proteome modifications

Received: 1 November 2017

Accepted: 19 December 2017

Published online: 11 January 2018

Giovanni Nassa ¹, Giorgio Giurato^{1,2}, Giovanni Cimmino³, Francesca Rizzo¹, Maria Ravo^{1,2}, Annamaria Salvati¹, Tuula A. Nyman ⁴, Yafeng Zhu⁵, Mattias Vesterlund ⁵, Janne Lehtiö ⁵, Paolo Golino³, Alessandro Weisz¹ & Roberta Tarallo ¹



Megakaryocytes transfer personalized genetic code into budding platelets

Platelets circulate with a unique genetic code

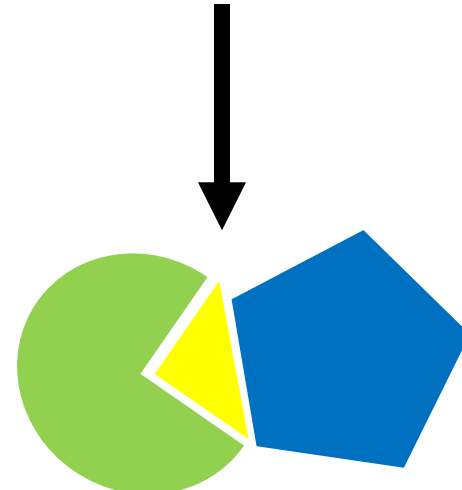
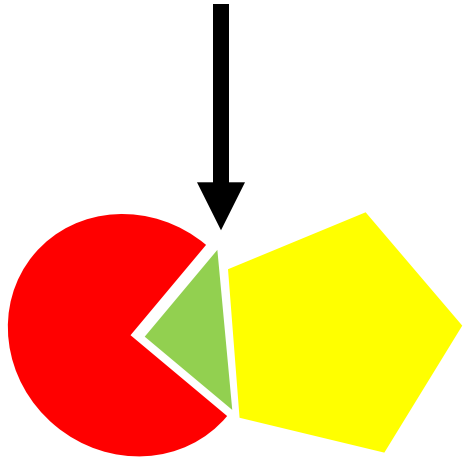
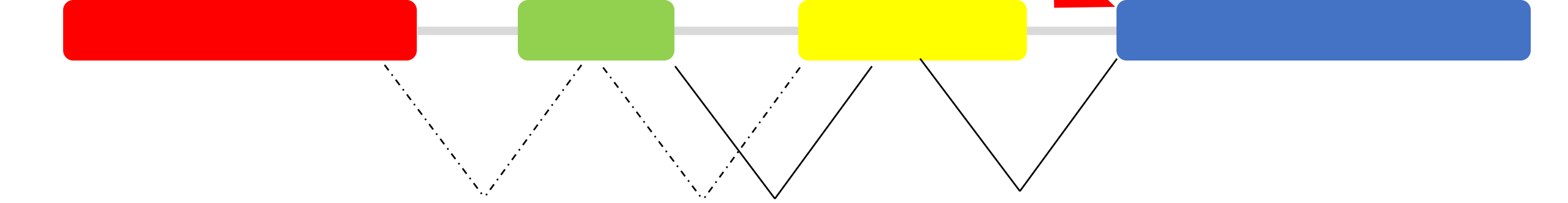
PHYSIOLOGIC SIGNAL

Selected
Schwartz et al (2006). *J Exp Med*
Weyrich et al. (2004) *Semin Thromb Hemost*
Nassa et al. (2018). *Sci Re*
Bray et al (2013). *BMC Genomics*
Rondina et al (2015). *J Thromb Haemost*

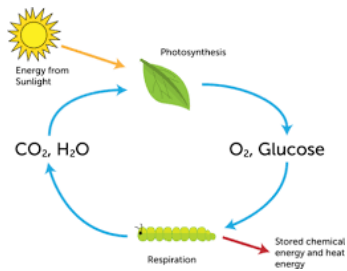
**PHYSIOLOGIC
SIGNAL 2**



**PHYSIOLOGIC
SIGNAL 1**



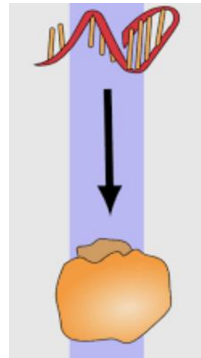
Speed of biologic transitions



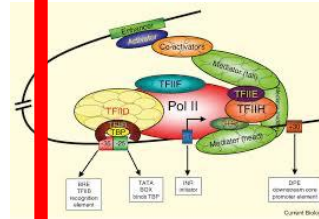
**Biochemistry
Enzymology**



Neurophysiology



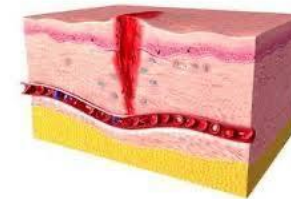
RNA Splicing
↓
Translation



**Novel
Transcription
Initiation**



**Circadian
Rhythms**



**Healing and
Repair**



Aging

Microseconds

Milliseconds

**Seconds
to
Minutes**

**Minutes
to
Hours**

**Fractions
of
a Day**

**Fractions
of
a Year**

Years

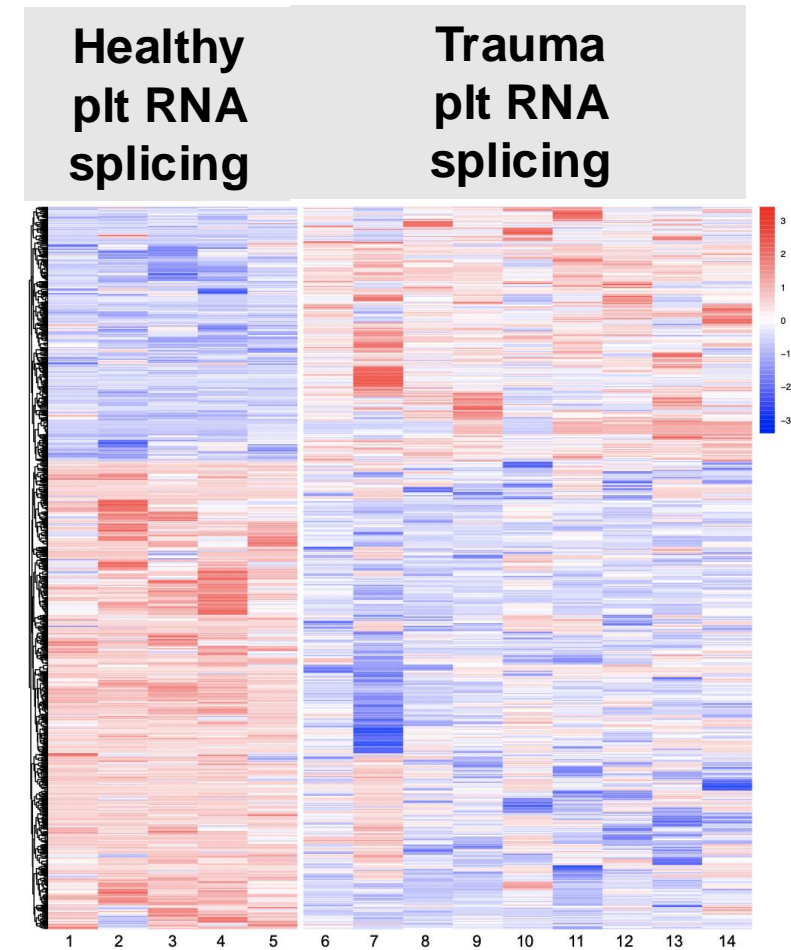
Methods

- Whole blood prospective collected
- Platelet aggregometry and rotational thromboelastometry
- Platelets isolated and stored in trizol

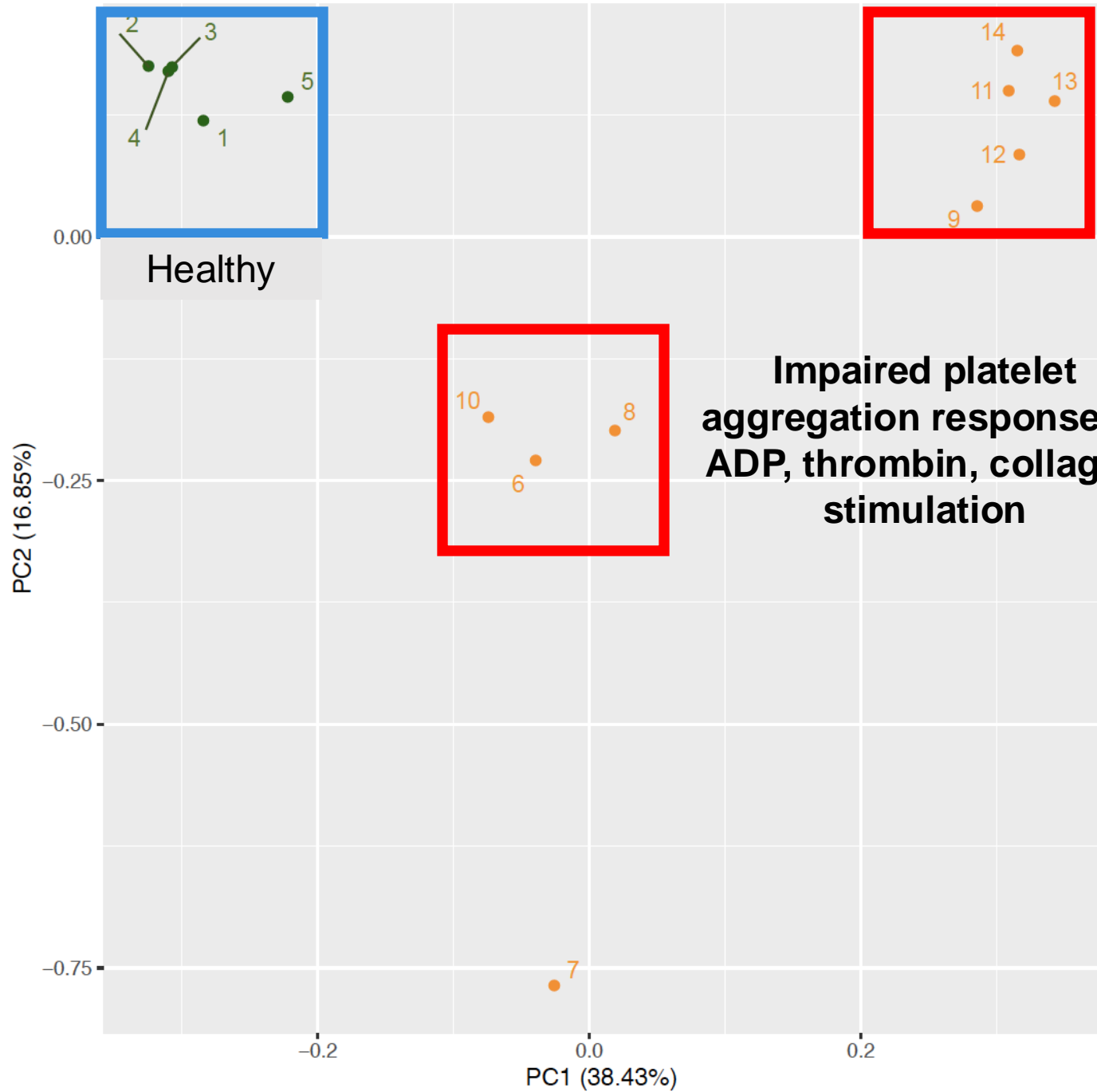
- RNA Sequencing of platelet isolates
 - RNA isolated (QIAamp RNA Blood Mini Kit)
 - 5 nanograms of RNA per sample, random primer based complementary DNA amplification
 - Sequenced at high-read depth (100-400 mil reads/sample)

Platelet RNA: trauma v. healthy

- 49 platelet genes with differing abundance
 - Primarily mitochondrial
- 1188 splicing events across 691 platelet genes (FDR <0.001)
 - Coagulation, platelet activation, wound healing
 - Immune responses, post-transcriptional gene regulation, other signaling and cell physiology



Fields AT, Lee MC, Mayer F, Santos YA, Bainton CMV, Matthay ZA, Callcut RA, Mayer N, Cuschieri J, Kober KM, Bainton RJ, Kornblith LZ. A new trauma frontier: Exploratory pilot study of platelet transcriptomics in trauma patients. *J Trauma Acute Care Surg.* 2022 Feb 1;92(2):313-322. PMID: 34738997



Normal platelet aggregation responses BUT prolonged clot formation, decreased fibrin crosslinking, and weaker clot strength

Impaired platelet aggregation response to ADP, thrombin, collagen stimulation

Healthy

Unique platelet transcriptome of trauma

- Unique platelet transcriptome in trauma patients vs. healthy donors
- Trauma platelet transcriptome
 - Physiologic driven finetuning of platelet RNA
 - Clusters trauma patients
 - Clusters differ in platelet hemostatic responses

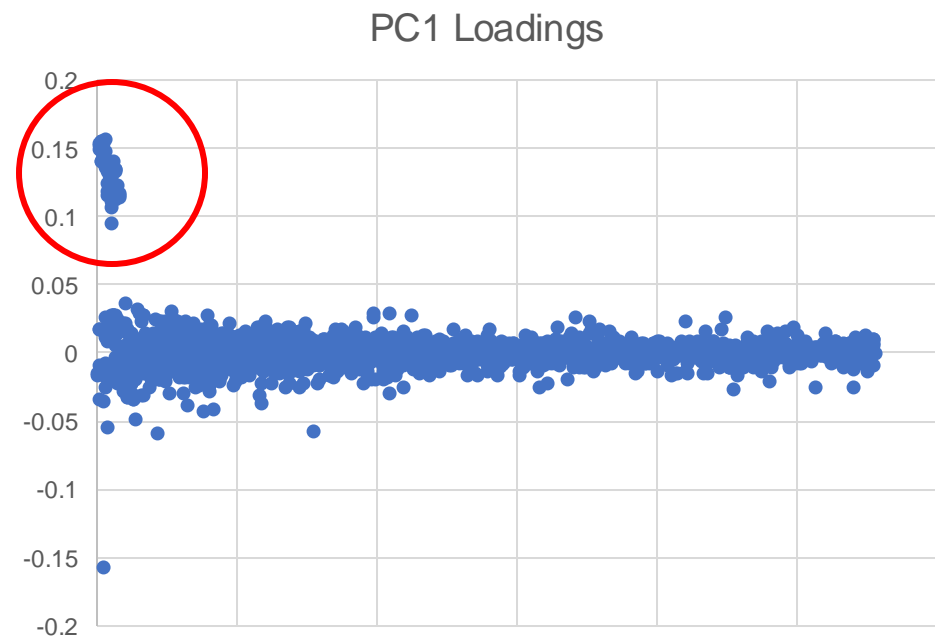
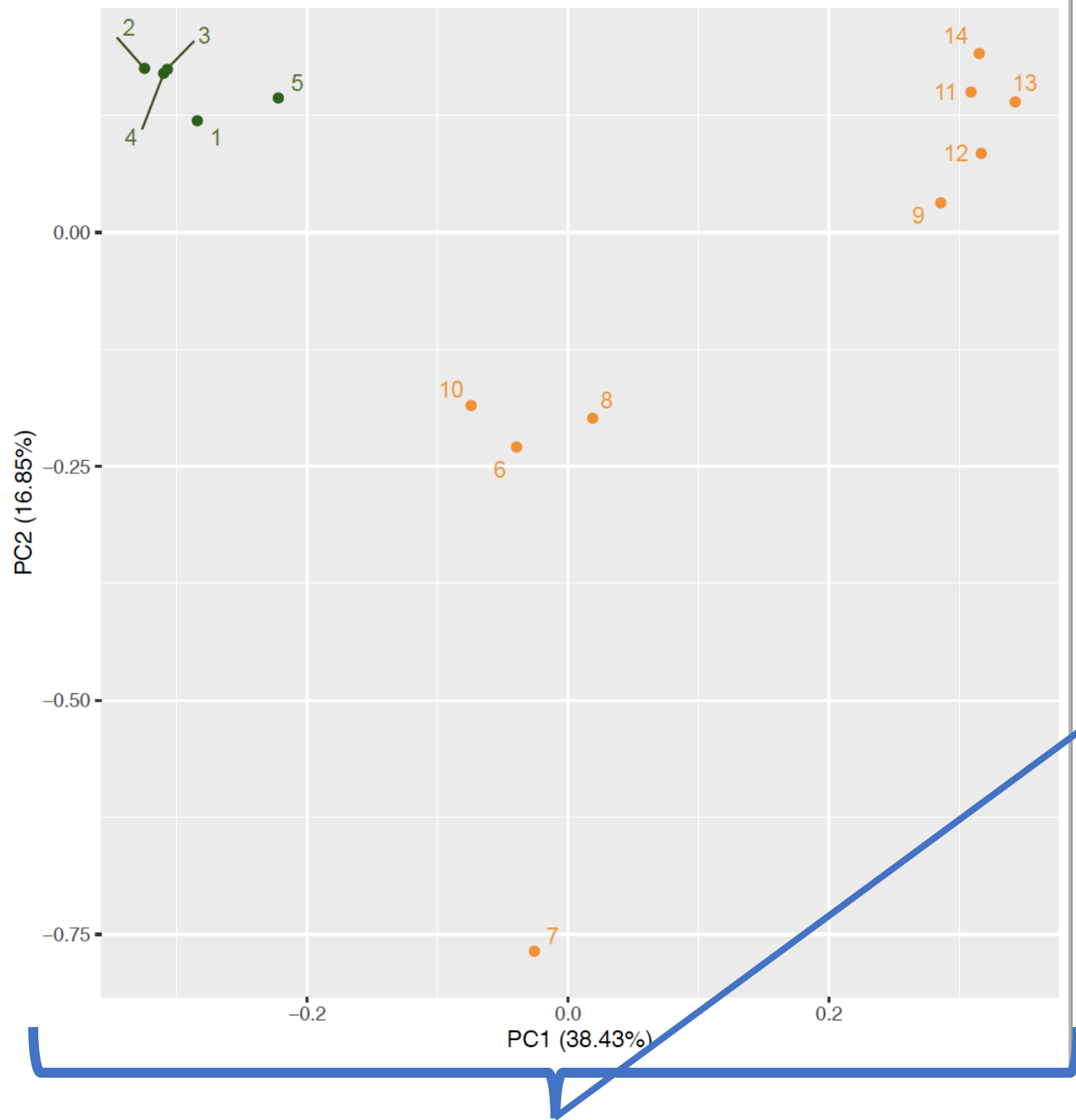
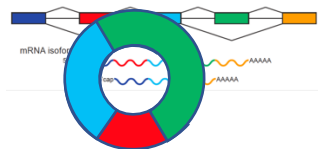


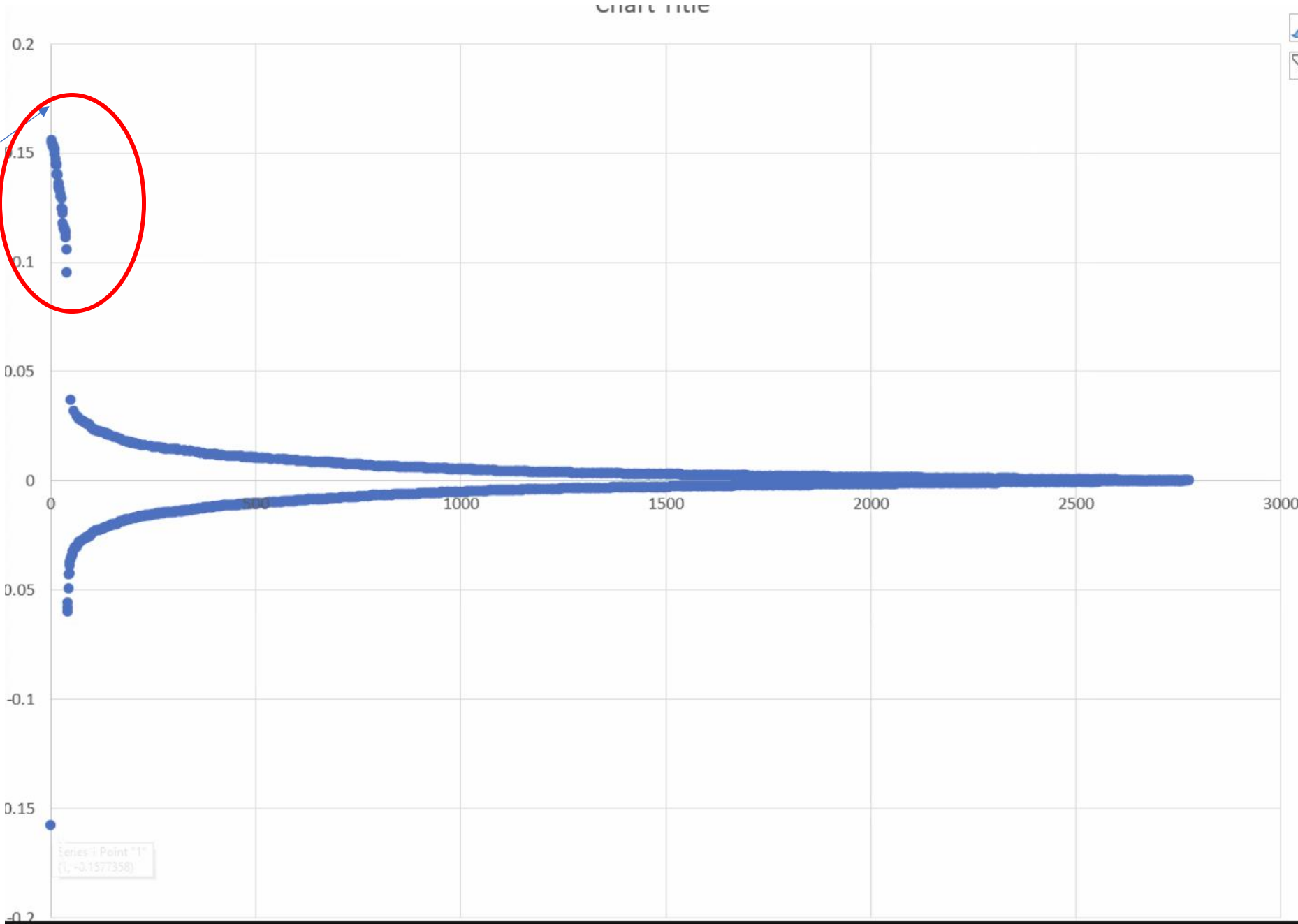
Chart Title



Splice Variants of One Gene

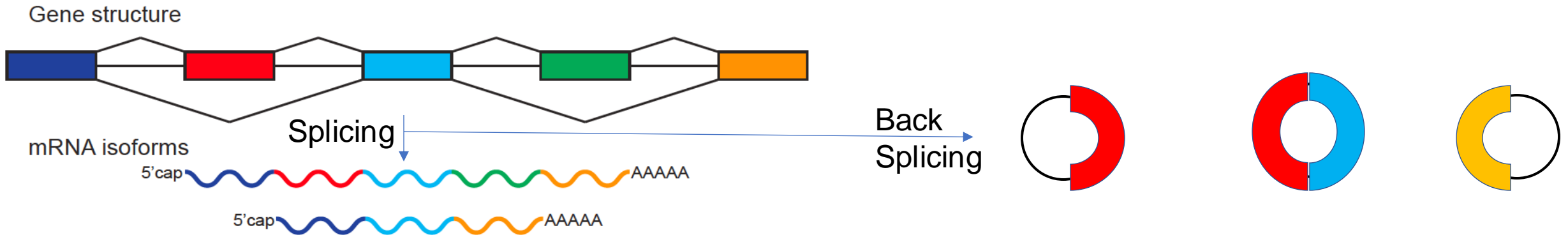


PC1 Loadings



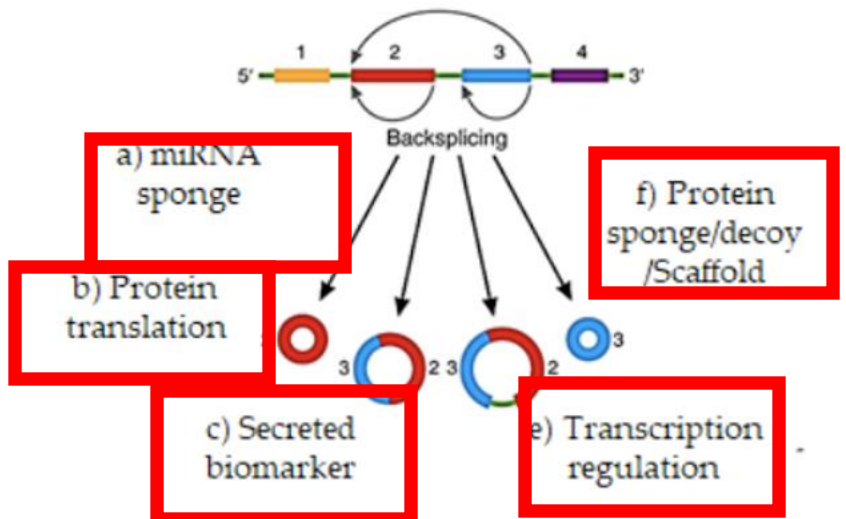
Genes

Circular RNA



RNA Circles

7) CircRNA



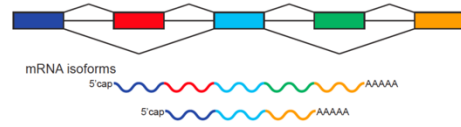
Giovanni Cimmino , Stefano Conte, Domenico Palumbo, Simona Sperlongano, Michele Torella, Alessandro Della Corte and Paolo Golino. "The Novel Role of Noncoding RNAs in Modulating Platelet Function: Implications in Activation and Aggregation" Int. J. Mol. Sci. 2023

Trauma patients v. healthy donors

RNA Expression
FDR < .05

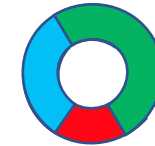
49

RNA Splicing
FDR < .001

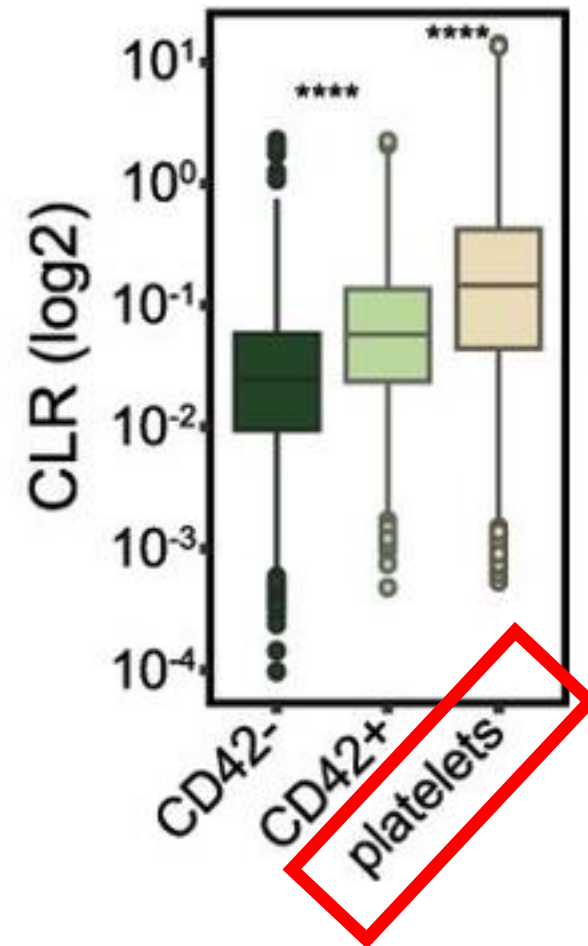
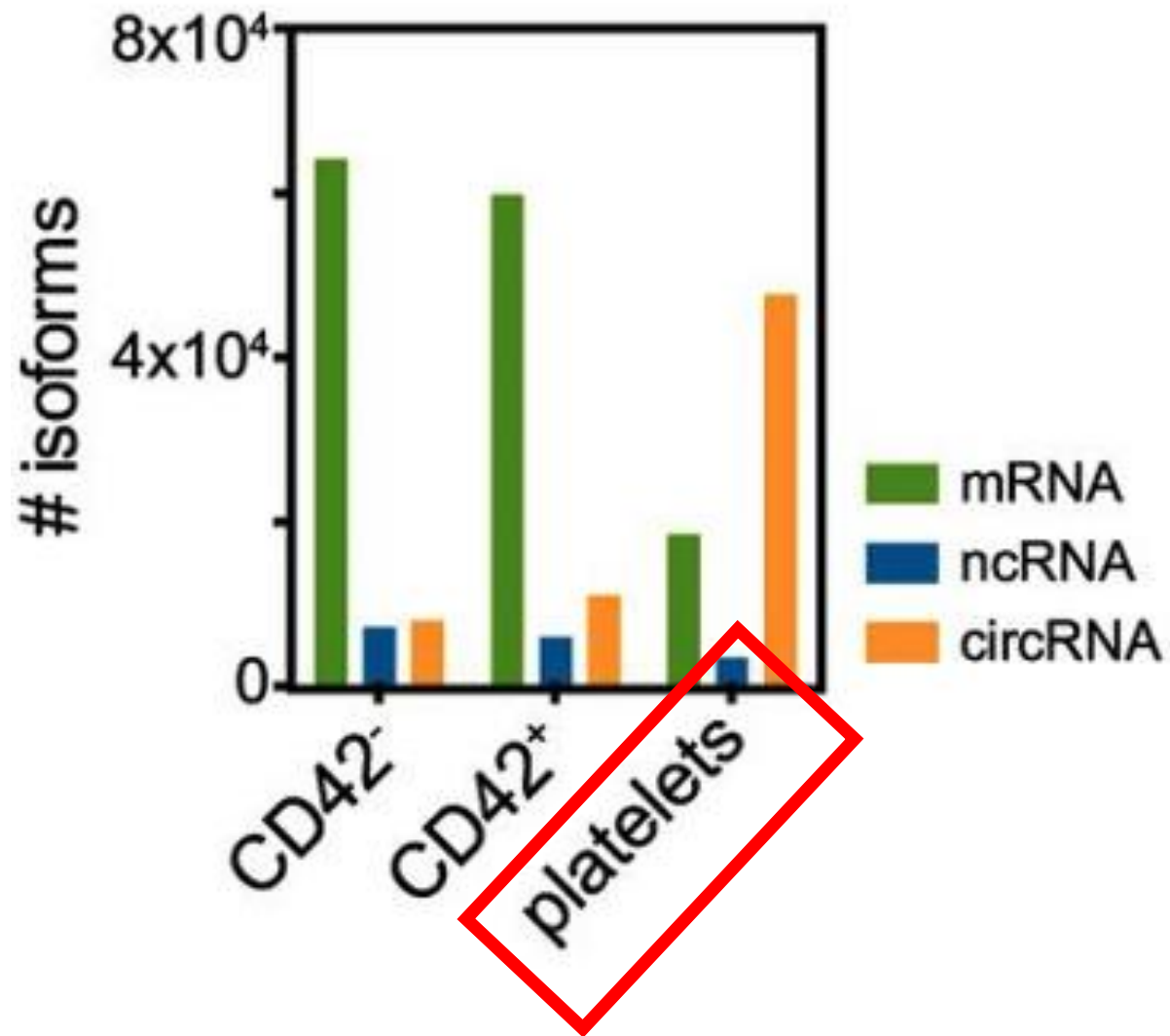


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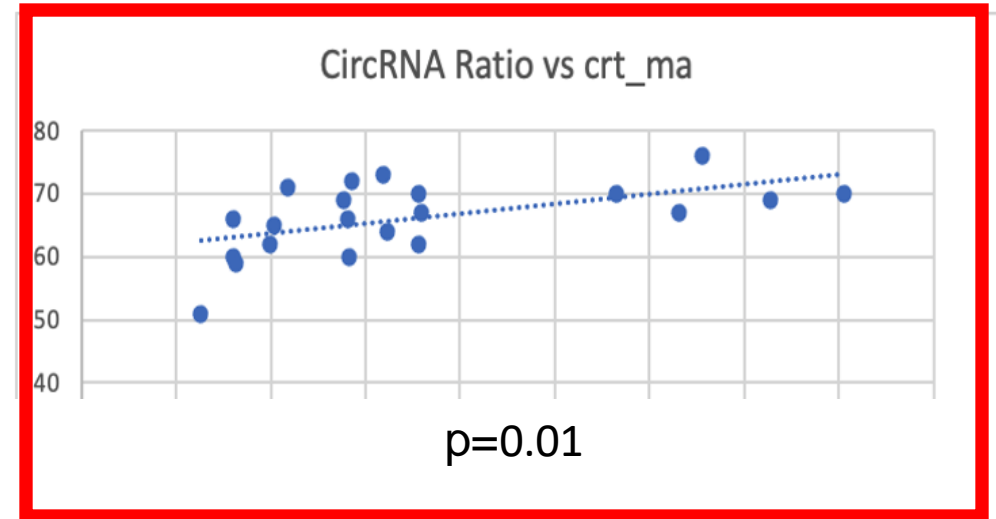
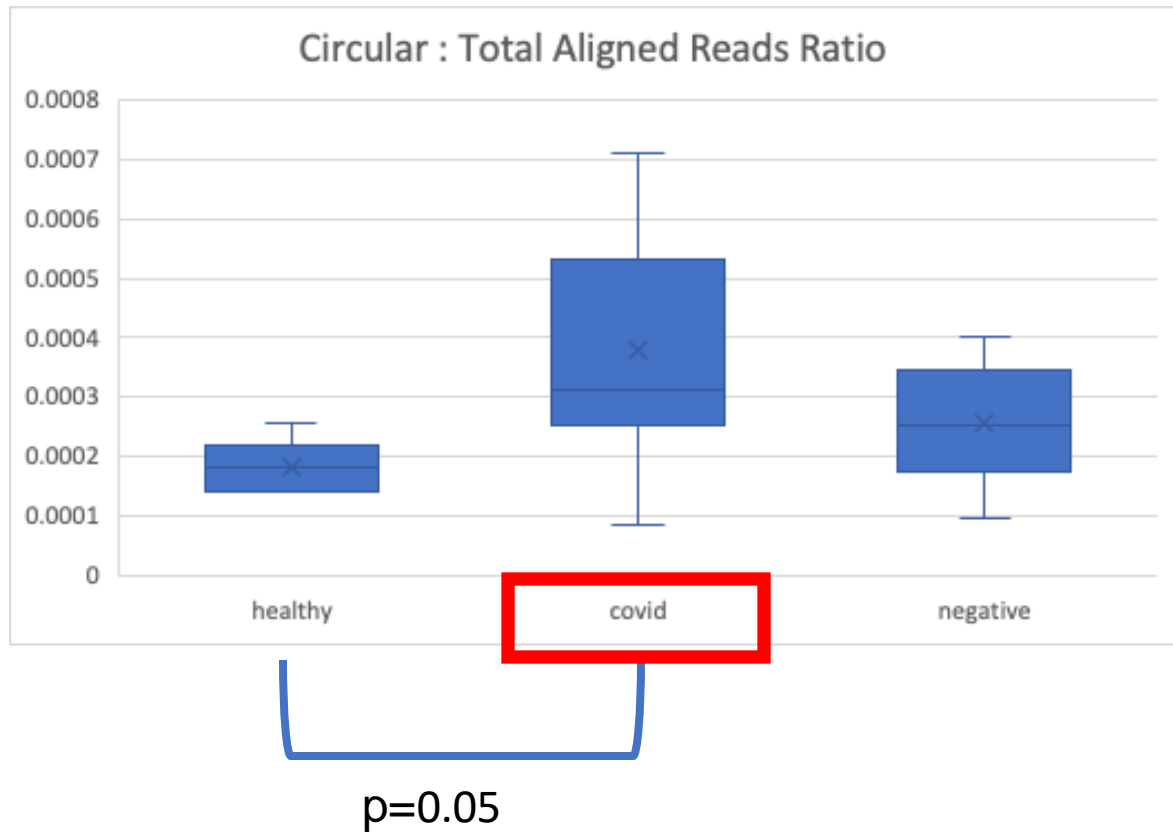
RNA Circles
FDR < .05

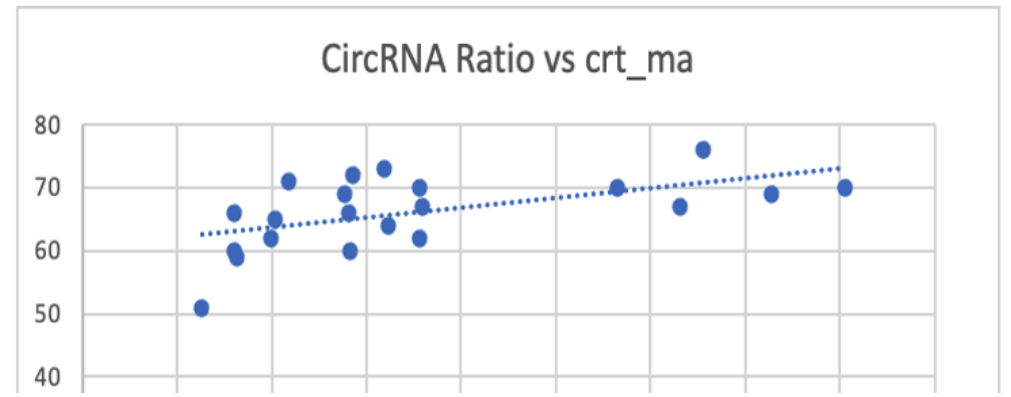
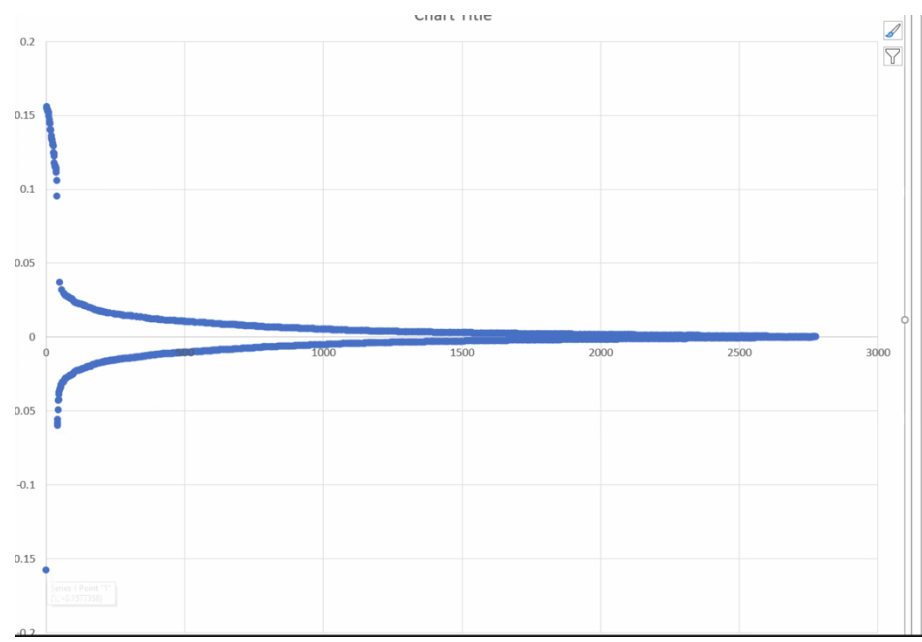
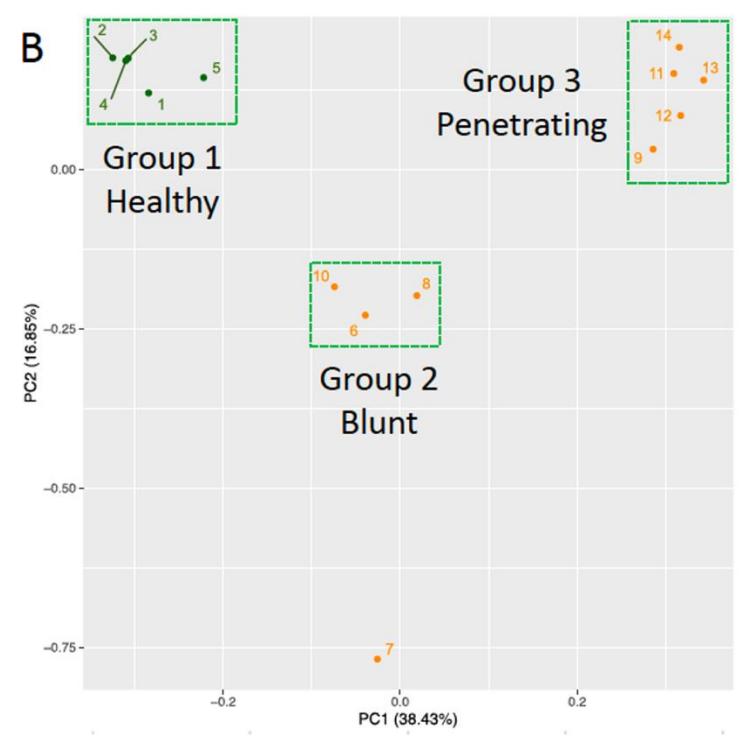
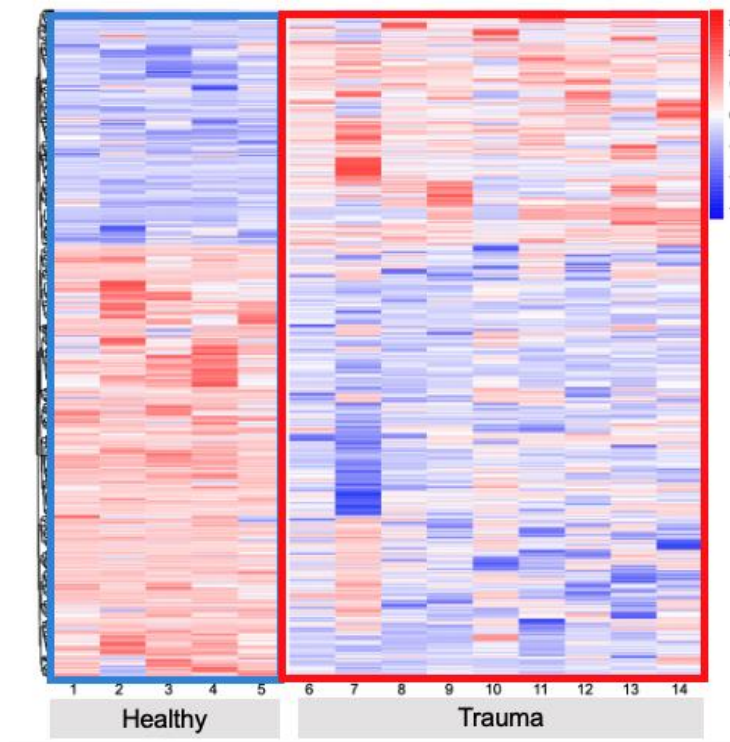


650



Total circular RNA increases in COVID-19





Future

- Extensive methods of studying post-injury platelet biology...
 - In-vivo systems
 - Microfluidics
 - Cell culture
 - Mitochondrial respiration
 - Ultrastructure microscopy
 - ***Platelet genomics***
- What should a native platelet be doing?
- What should a transfused platelet be doing?

WHAT NOW?

Bulk sequencing larger number of platelet

PCR of candidate RNAs

Ribosome footprint profiling

Westerns of protein products

Inducing trauma genomic signatures ex-vivo with plasma treatments

Model systems: ex-vivo CD34+ cell culture models, KO and humanized platelet murine models

THE KORNBLOTH LAB

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Pictured (left to right):

Alex Fields, PhD-Senior staff scientist
Kim Herrera Rodriguez-Research assoc.
Christopher Lee-CRC

Lucy Kornblith-Lab Director

Jake Corvera-Undergraduate student
Aubrey Fife-Undergraduate student
Marcela Matheus-CRC
Carolyn Hendrickson-Collaborator
Brenda Nunez-Garcia-Program Manager
Rocco-Resident canine

Not pictured:

Yale Santos-Data Scientist
Nasima Mayer-PhD candidate
Deanna Lee-CRC
Celine Chou-CRC
Suzanna Chak-CRC
Seif Elmankabadi-CRC
Saigeetha Bhaskar-Graduate student
Nikoo Marageh-Undergraduate student
Jordyn Pinochi-Volunteer

Bainton lab: not pictured

