Endotheliopathy and TXA: An Old Dog Continues to Bark

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Disclosures

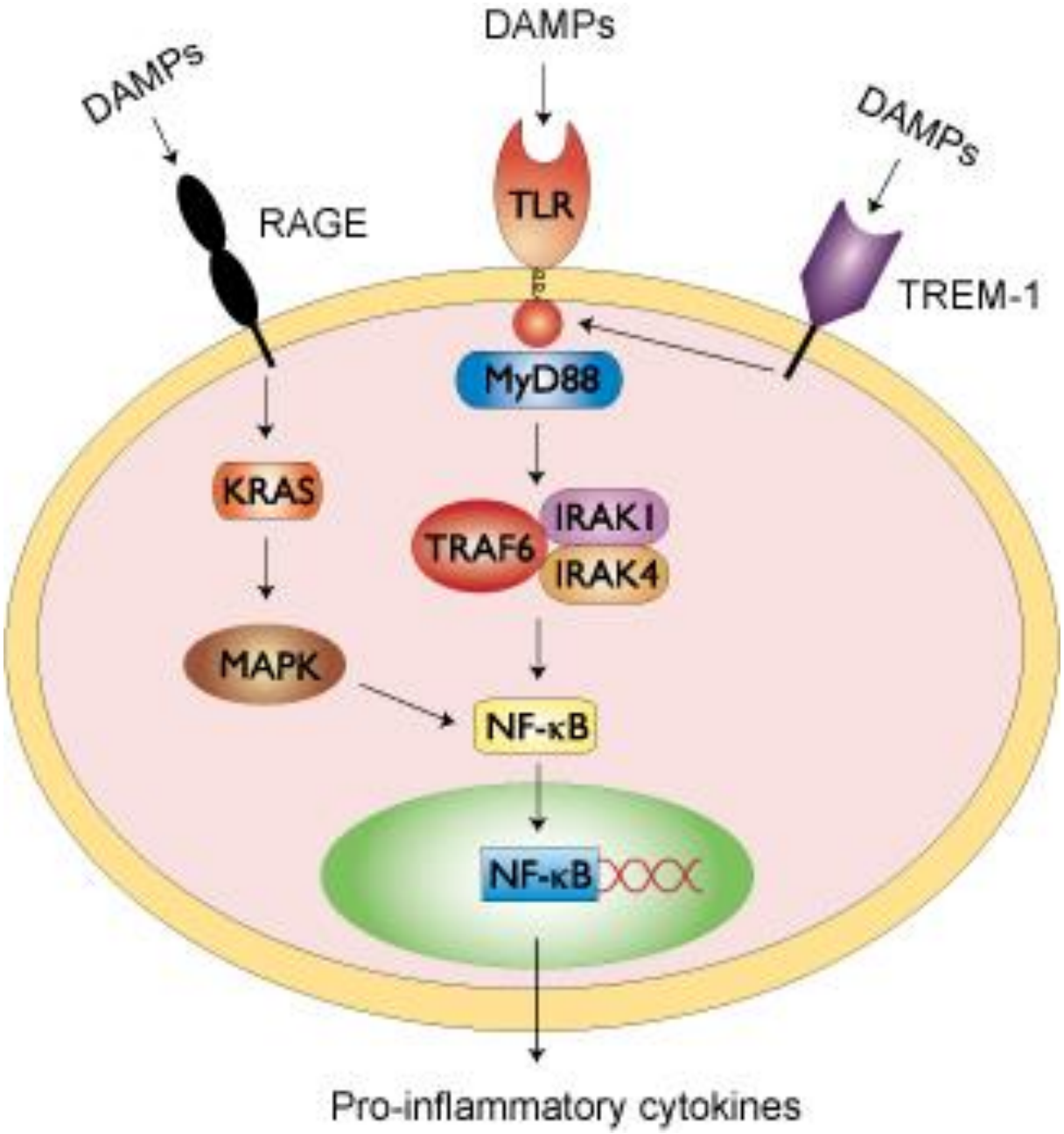
- Funding Sources: DoD and pilot grants from the Maine Medical Cardiovascular Research Institute, MDIBL COBRE and Tufts University School of Medicine CTSI
- Consultant for Cellphire Therapeutics

Events following vascular trauma

- Release of Damage Associated Molecular Patterns (DAMPs)
- Enhanced production and secretion of pro-inflammatory cytokines
- Loss of glycocalyx from endothelial cells (Kozar/Pati)
- Increased permeability of endothelial monolayer
  - Edema
- ARDS/AKI

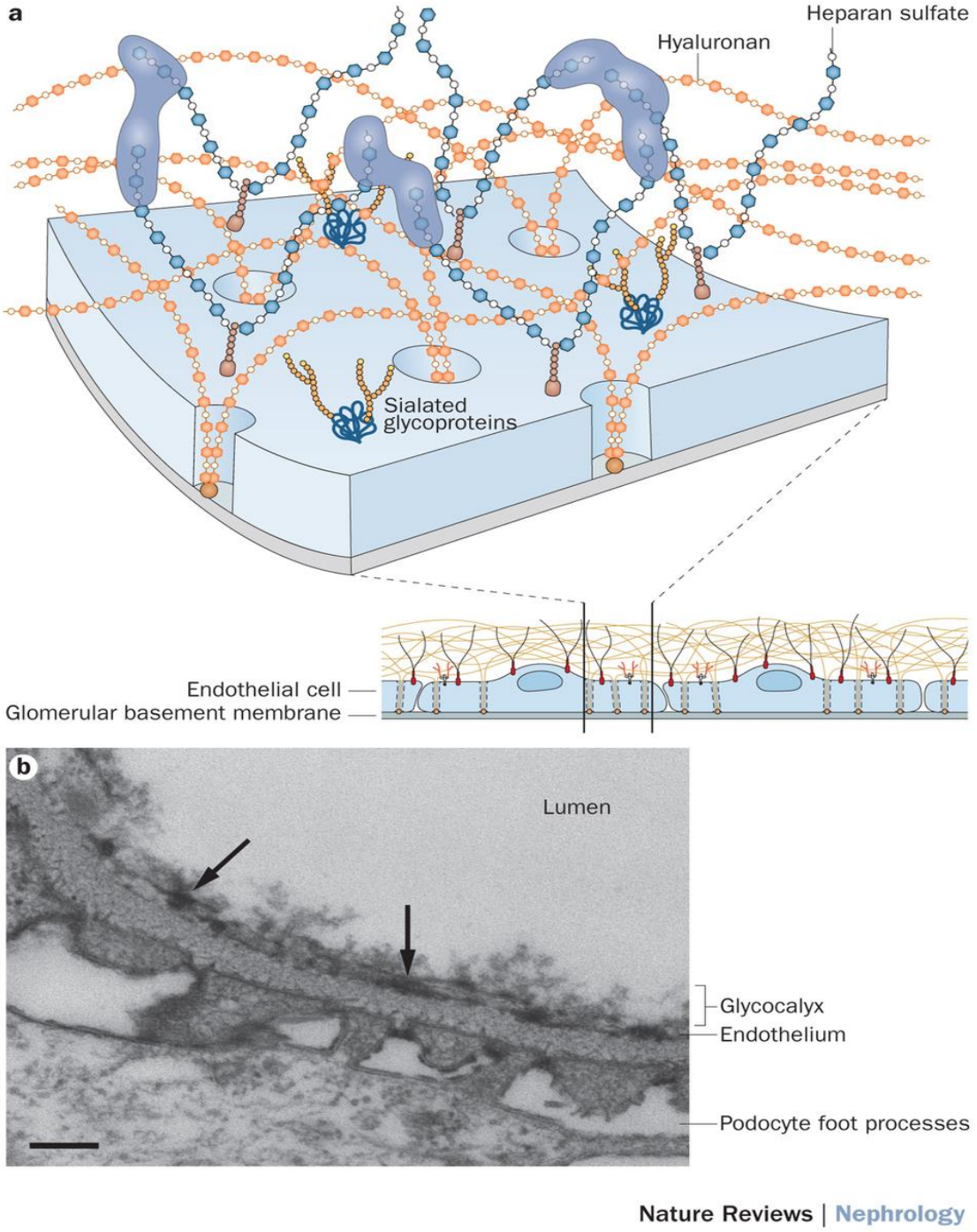
Examples of DAMPs

- HMGB1
- S100 family proteins
- Histones
- mtDNA*
- Formylated peptides
- Nuclear DNA



IL1α, Il1β, IL6, IL8, TNFα

Endothelial glycocalyx



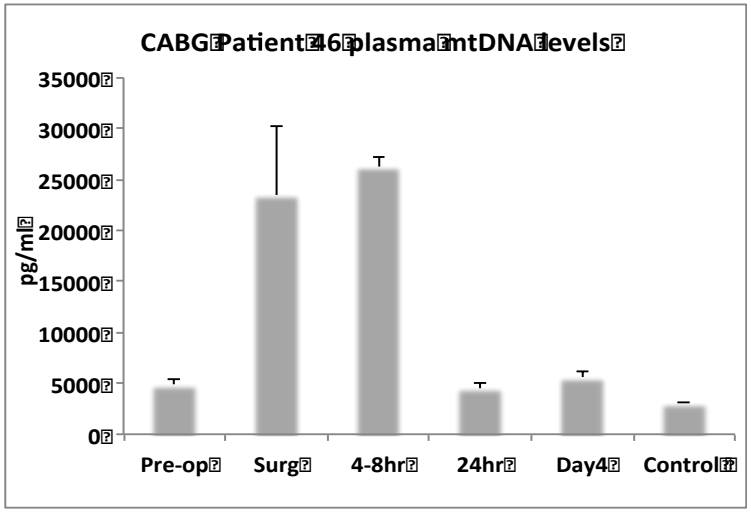
Damage to the glycocalyx

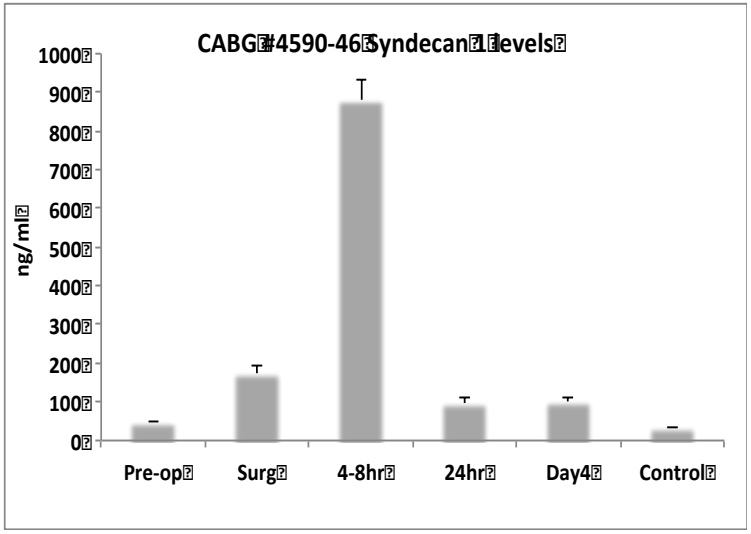
- Affects luminal surface of the endothelial cell (EC)
- Compromises the status of adherence junctions (AJ) between the lateral membranes of the capillary EC.

The release of DAMPs and shedding of the glycocalyx are provoked not only by acute hemorrhagic trauma but also by other physiological stressors such as cardiovascular surgery, burns and sepsis

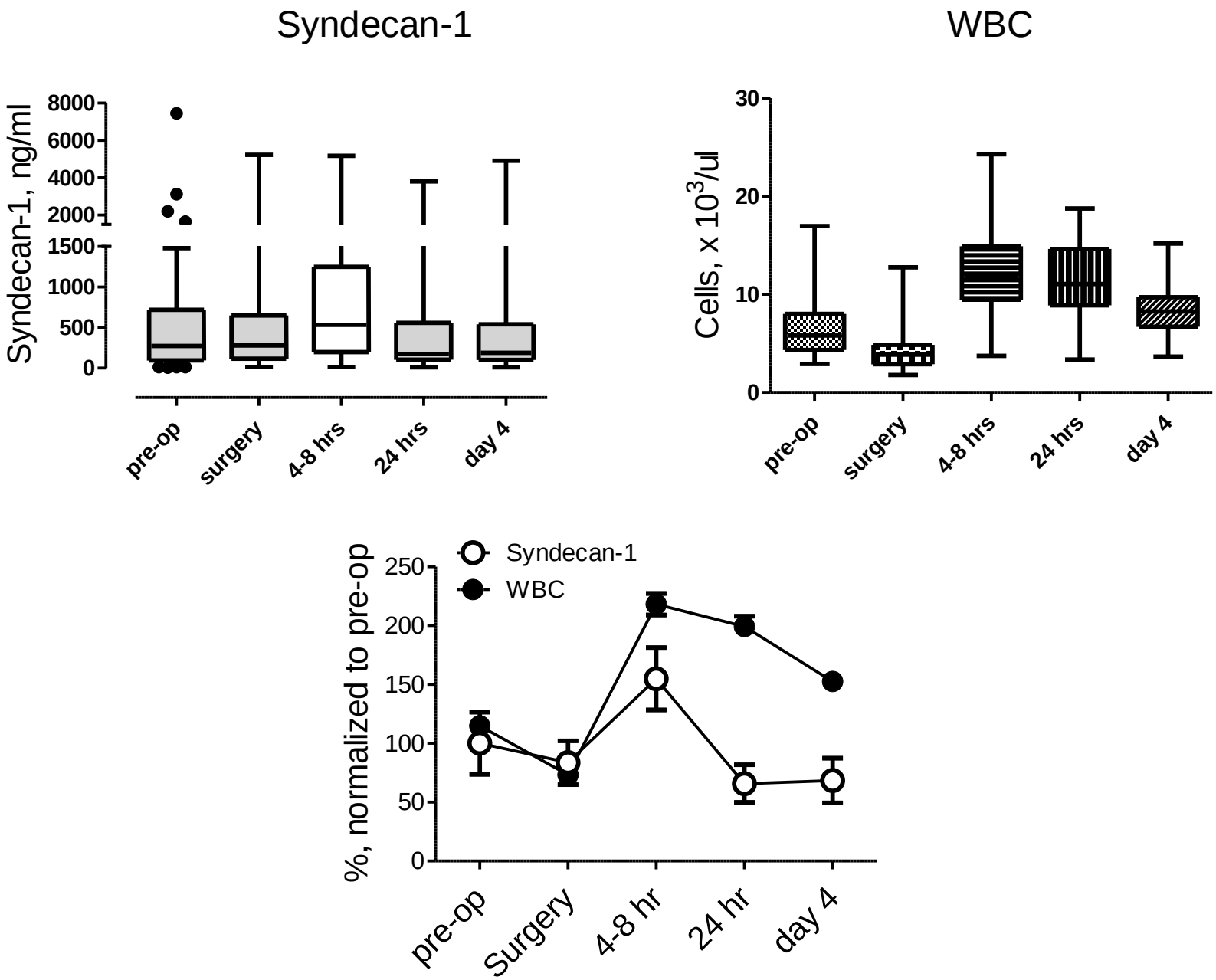
The shedding of gycocalyx follows the trauma-induced release of DAMPs

(Example: cardiac bypass)

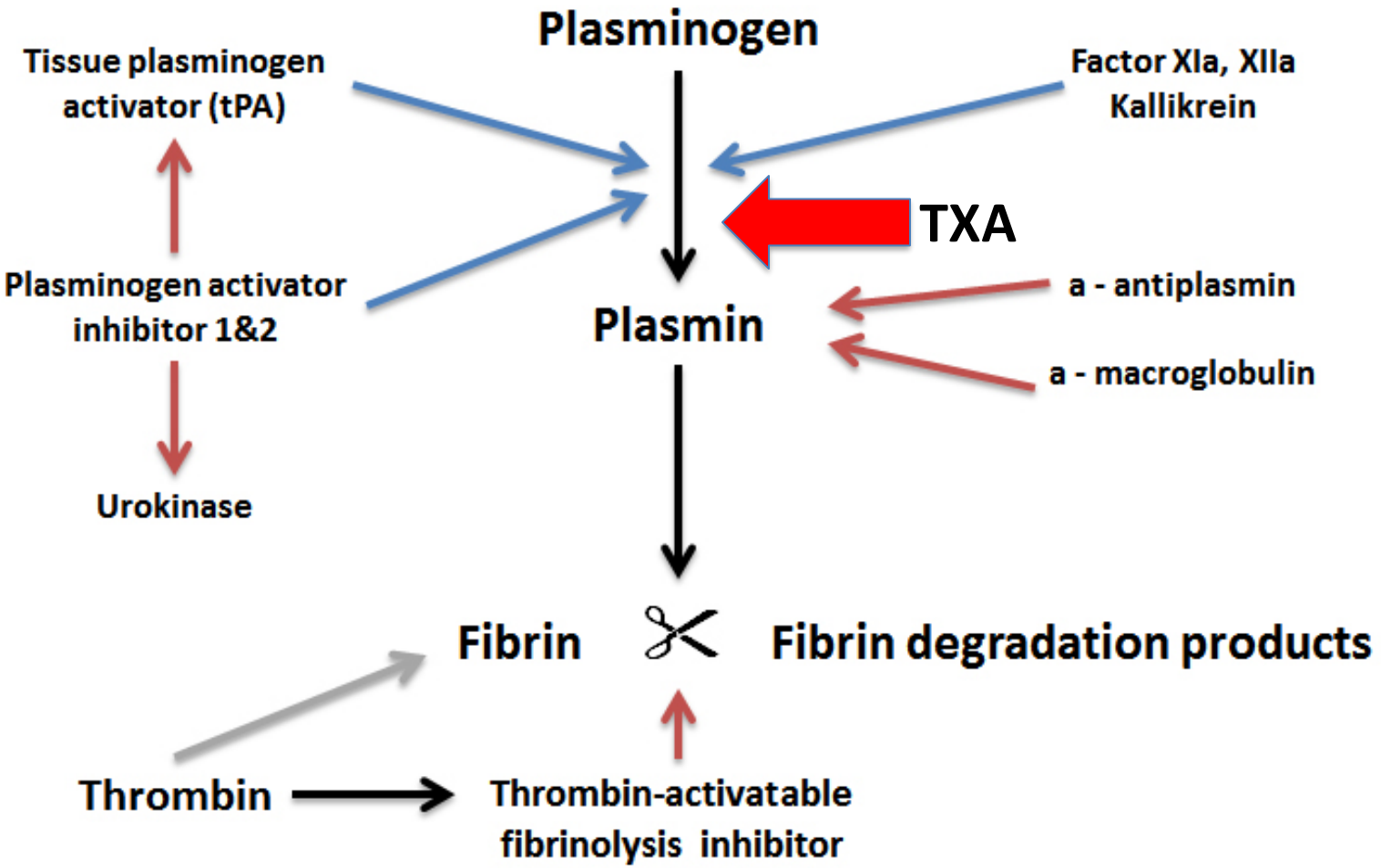




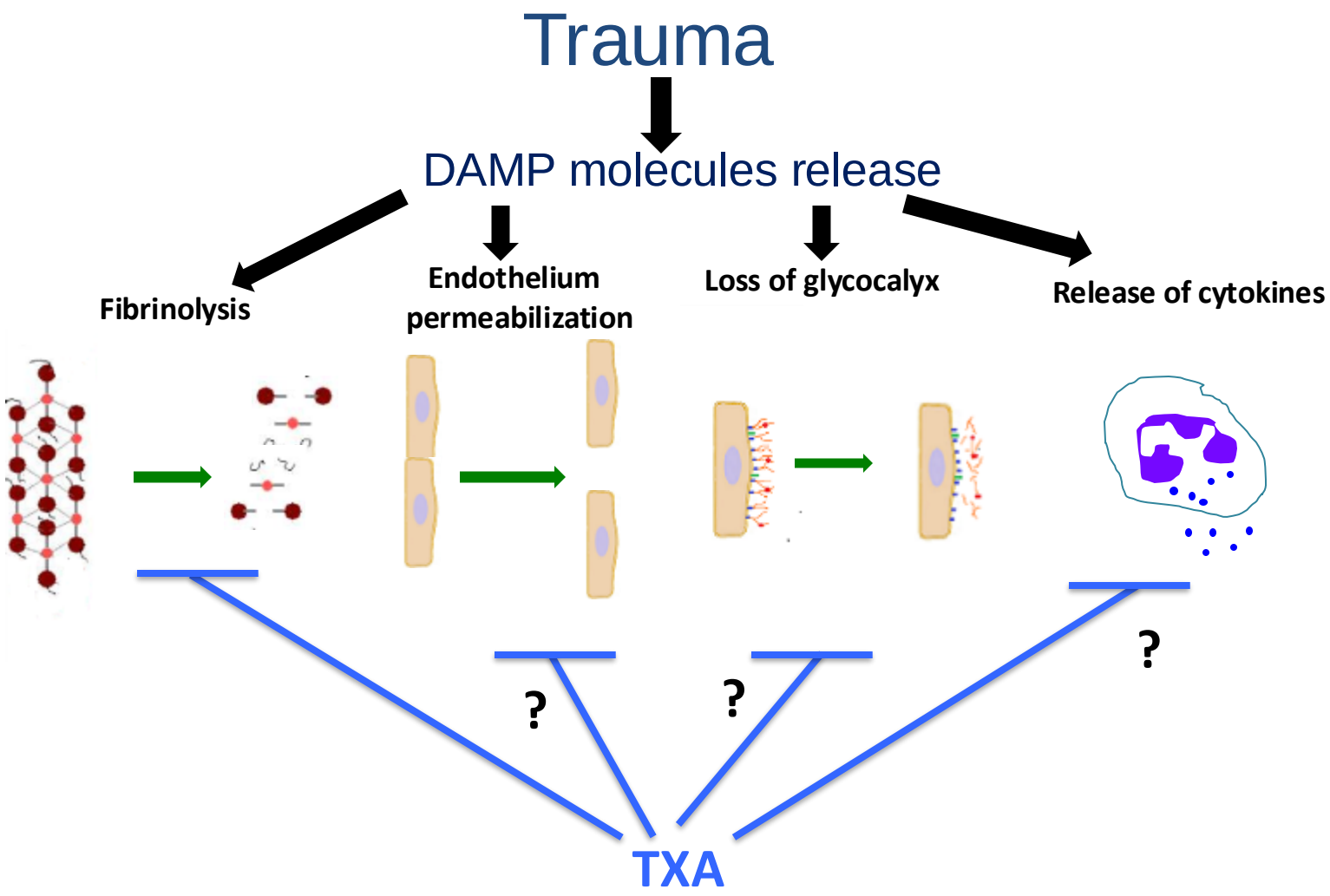
The shedding of glycocalyx takes place simultaneously with the increase of leukocyte count in circulation



- Tranexamic acid (TXA) is an anti-fibrinolyic agent that potentially improves outcome in severe hemorrhagic trauma
- Bulk of data indicates that mechanisms underlying protective effect of TXA transcend its anti-fibrinolytic activity



Fibrinolysis (simplified) - Blue arrows denote simulation, red arrows inhibition



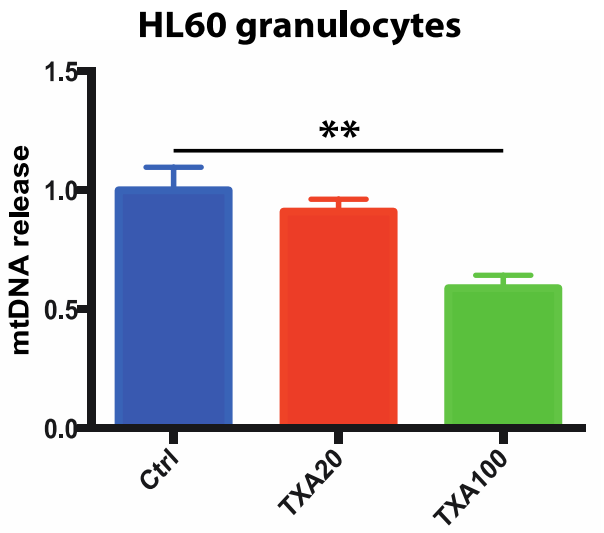
What are the cell and molecular mechanisms underlying the extensive protective effects of TXA in traumatic hemorrhage?

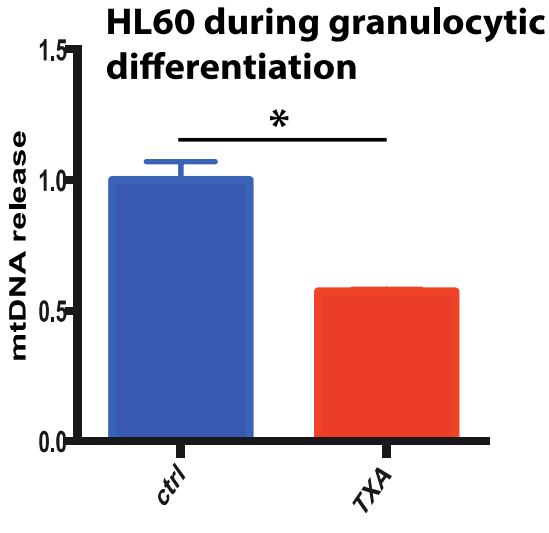
TXA inhibits lung injury and inflammation in hemorrhagic rats and suppresses the activity of enzymes shedding glycocalyx in the gut (Peng et al., J of Trauma and Acute Care Surgery, 2016)

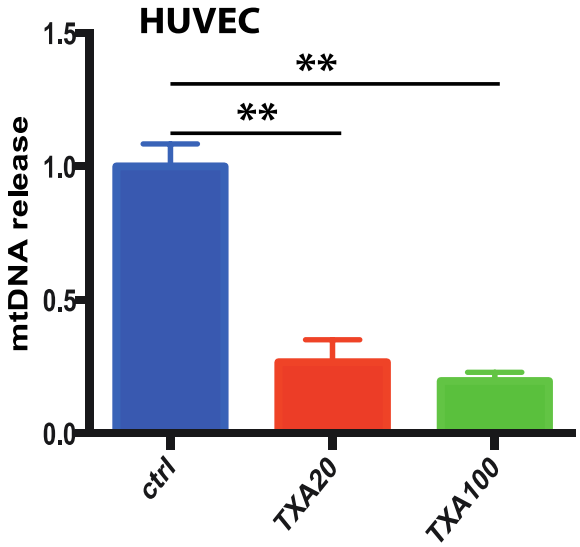
TXA decreases the blood levels of proinflammatory cytokines in hemorrhagic rats (Teng et al, Experimental Animals, 2018)

In the endothelial cell culture, TXA decreases the shedding of glycocalyx induced by hydrogen peroxide and protects the integrity of the monolayer (Diebel et al, J of Trauma and Acute Care Surgery, 2017,2018)

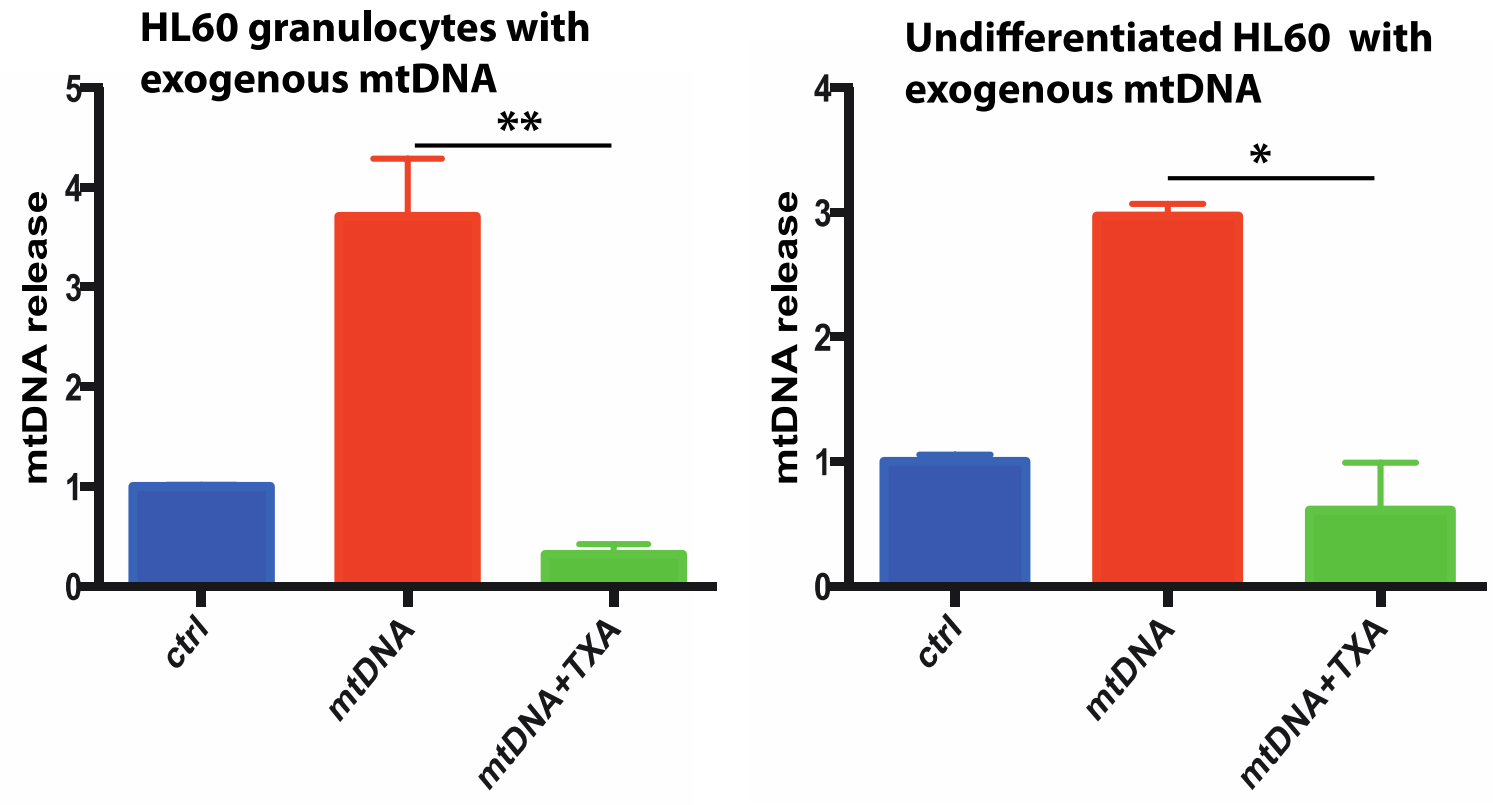
TXA suppresses the spontaneous release of mtDNA from granulocytes and endothelial cells



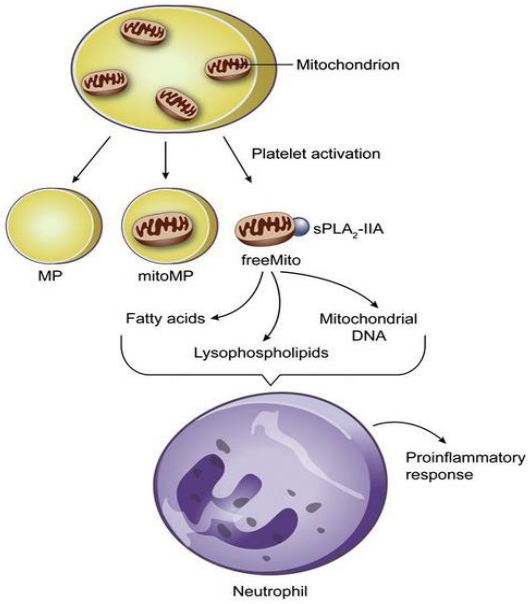


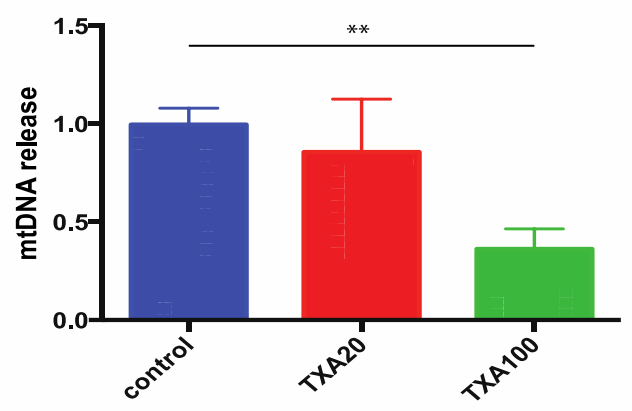


TXA suppresses the release of endogenous mtDNA induced by exogenous mtDNA

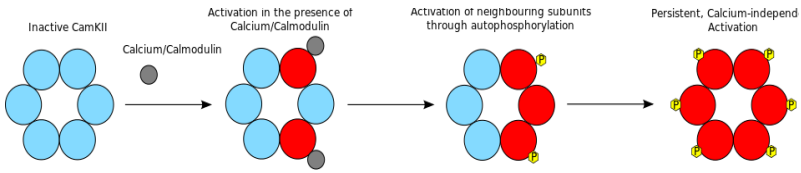


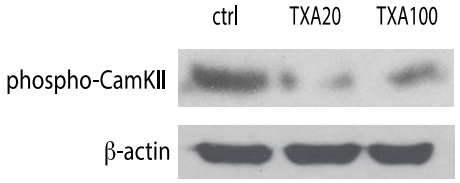
TXA suppresses the release of mtDNA from isolated platelets in vitro



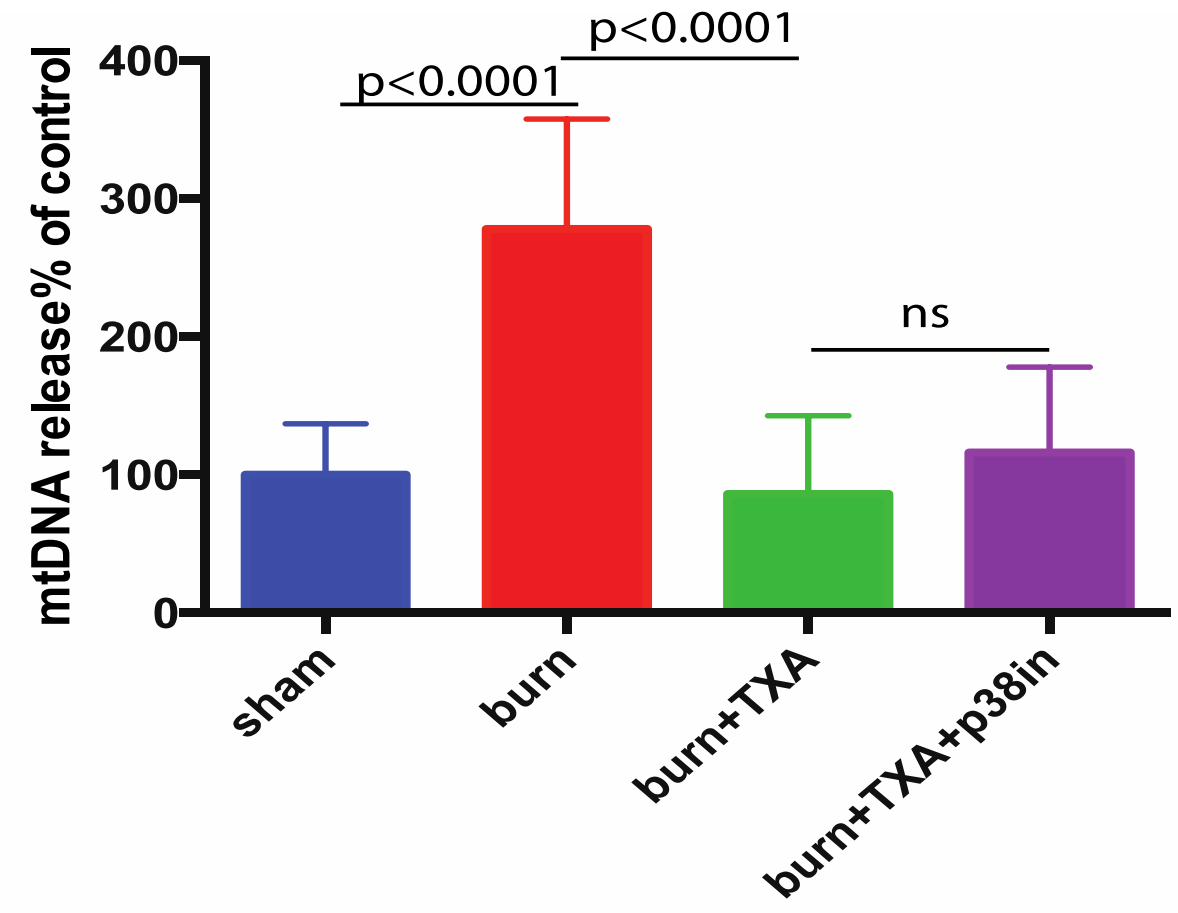


In platelets, TXA suppresses the phosphorylation of the stress response enzyme CamKII

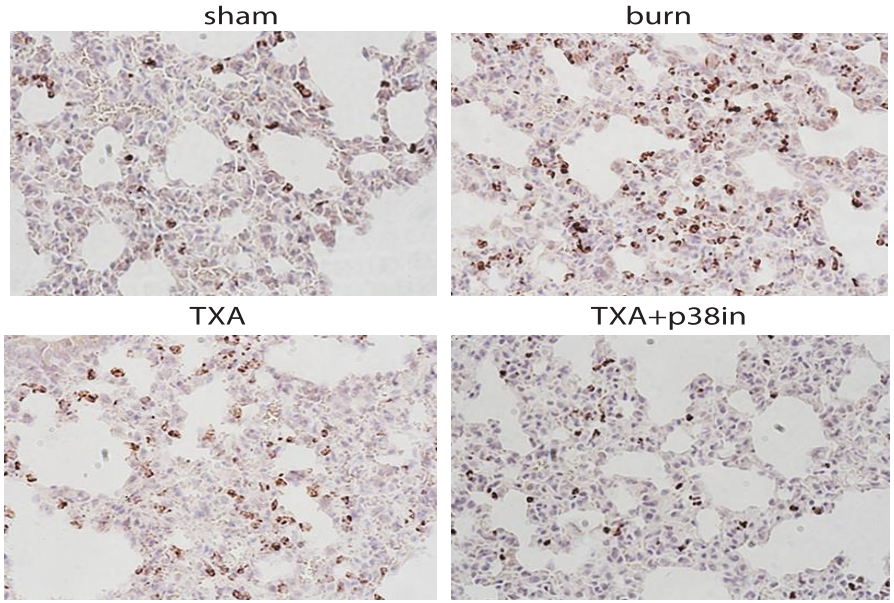


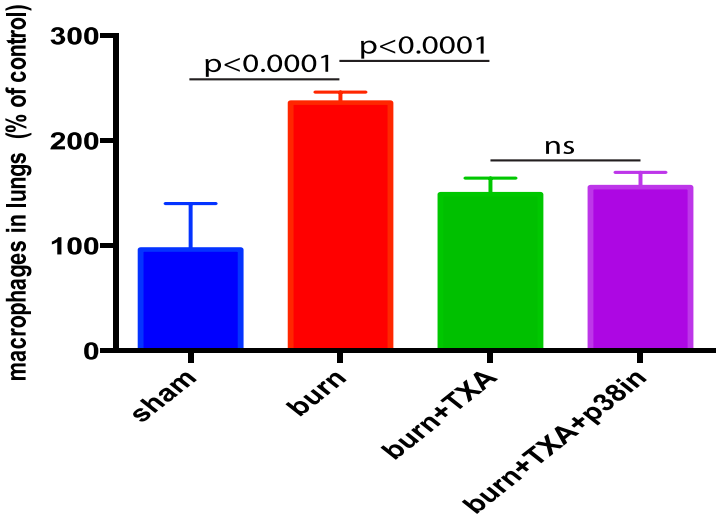


TXA inhibits the burn-induced release of mtDNA to the bloodstream of mice

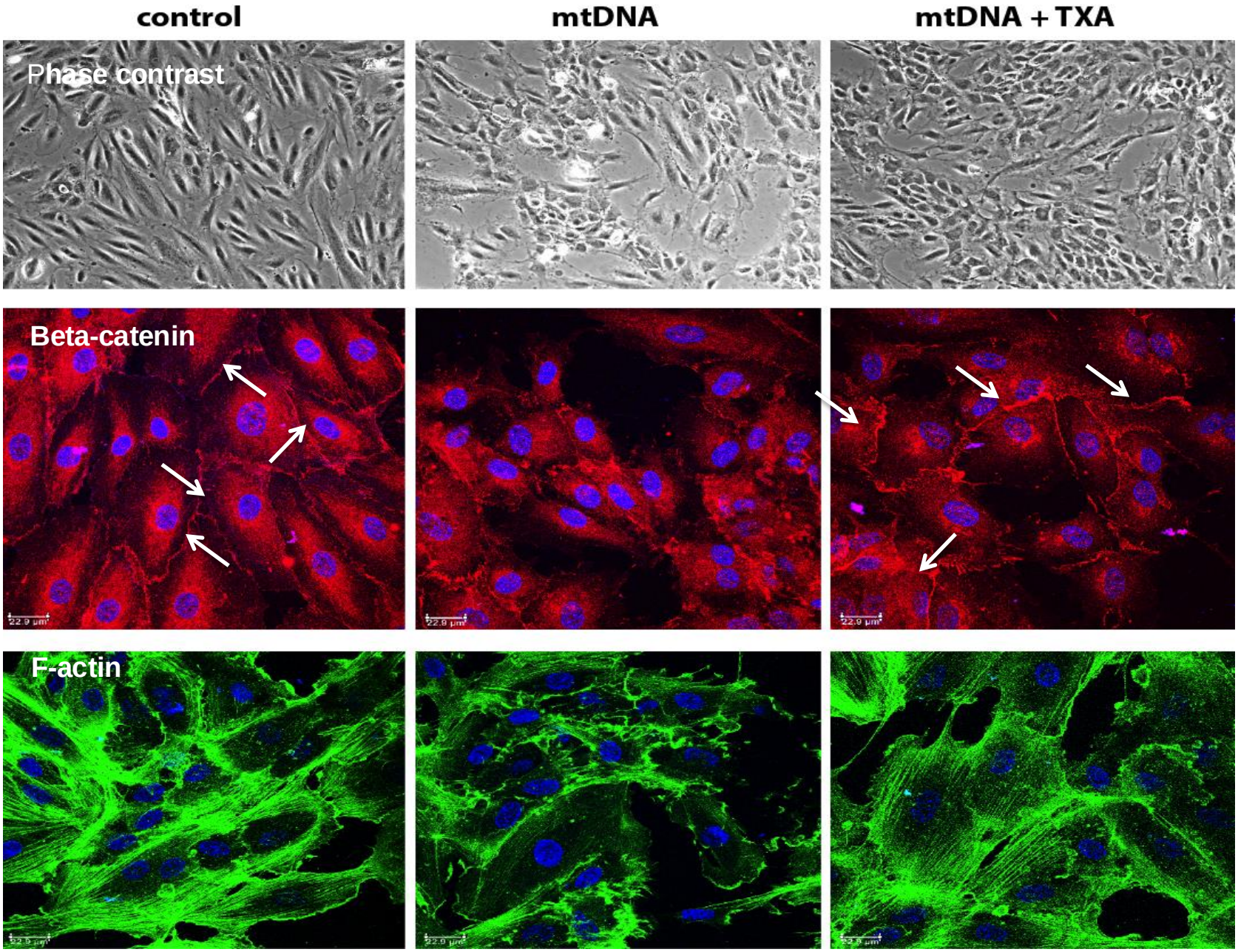


TXA suppresses the burn-induced infiltration of macrophages to the lungs of mice

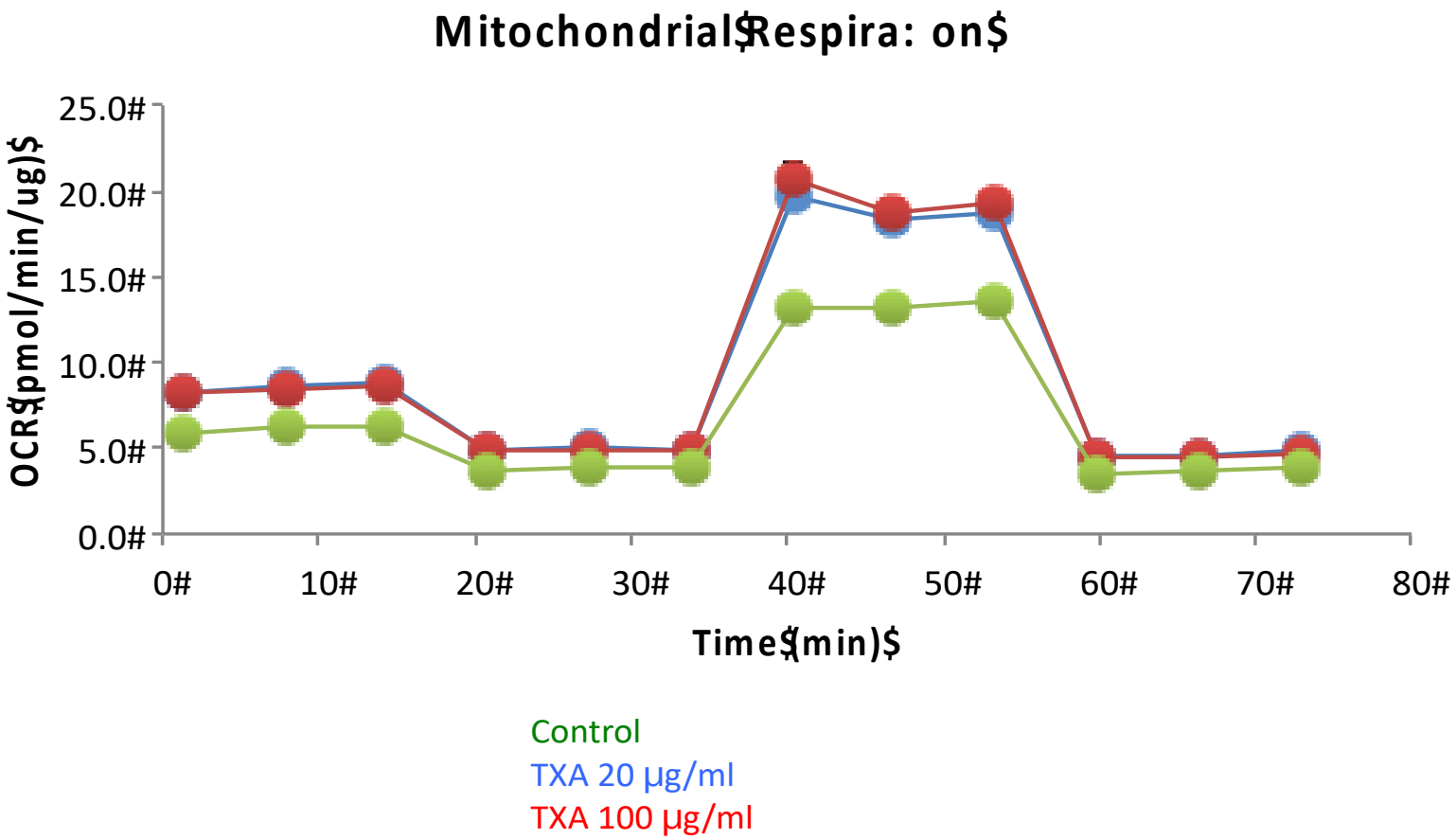




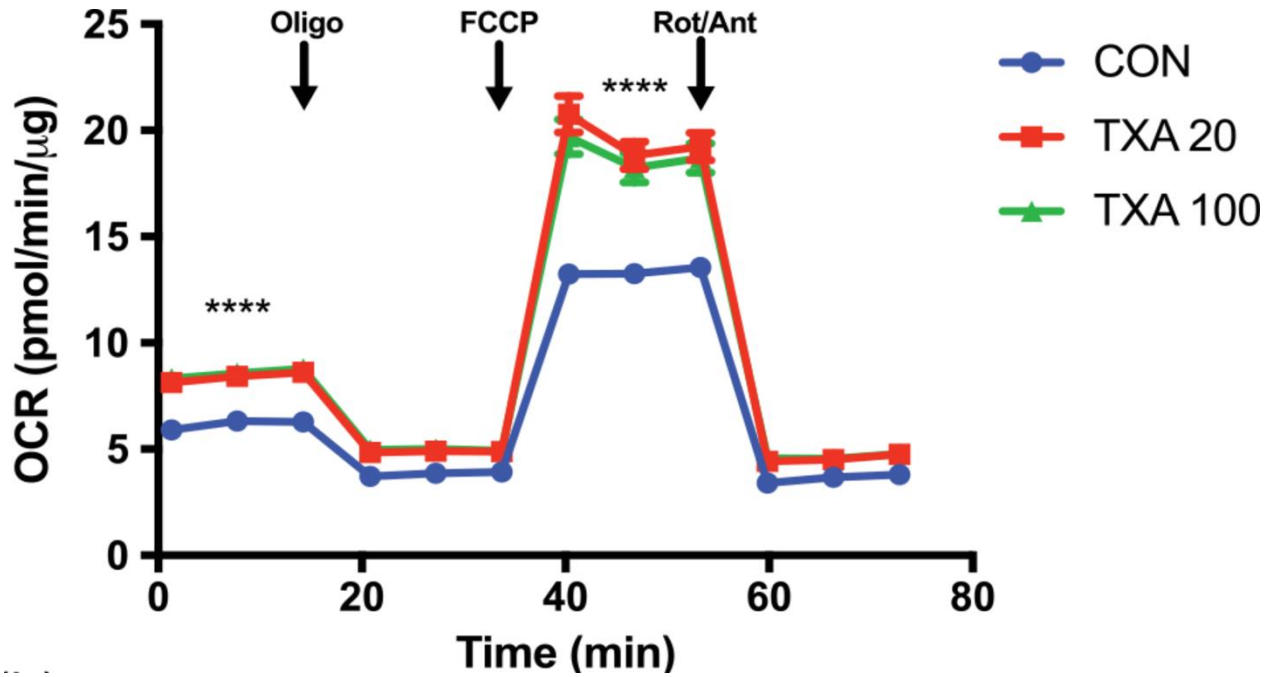
TXA protects endothelial monolayer from the damage induced by exogenous mtDNA



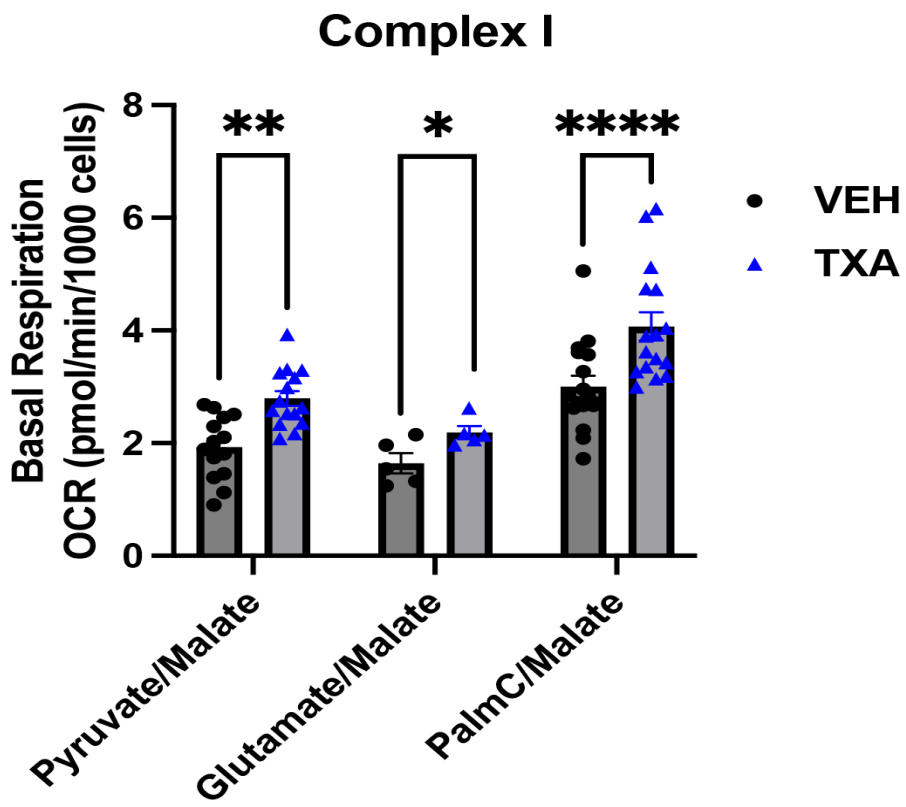
Seahorse study of metabolic activity in TXA-treated HUVEC



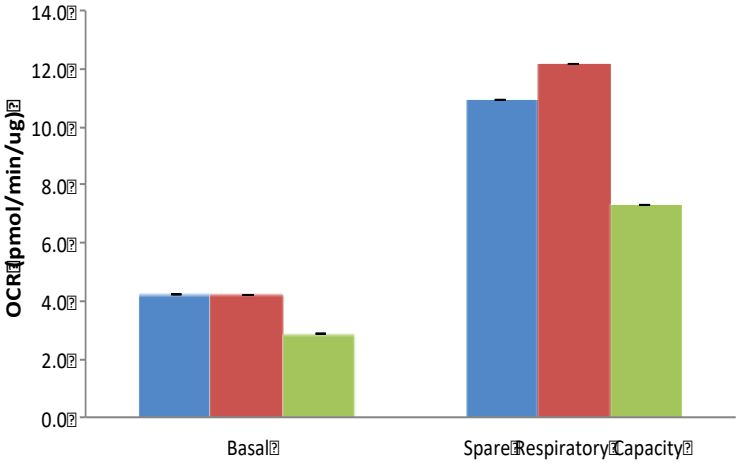
TXA enhances mitochondrial respiration in endothelial cells. Seahorse study.

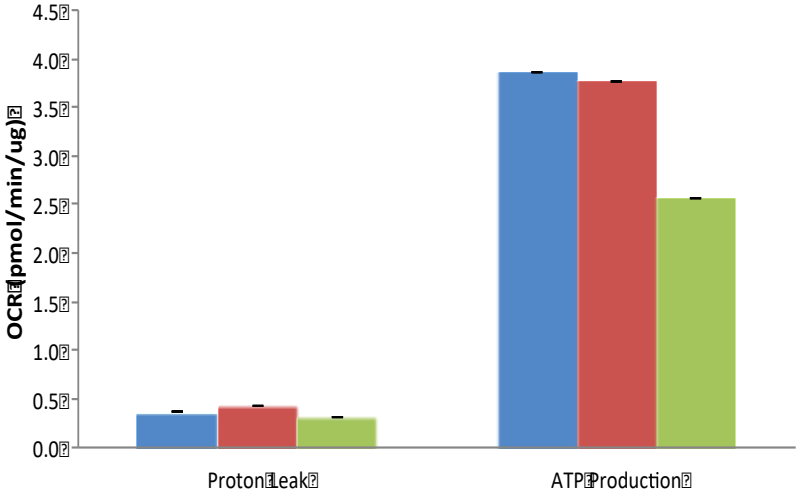


TXA stimulates the activity of mitochondrial complex I in permeabilized endothelial cells. Seahorse study.



TXA enhances the mitochondrial spare respiratory capacity and ATP production in the endothelial cells





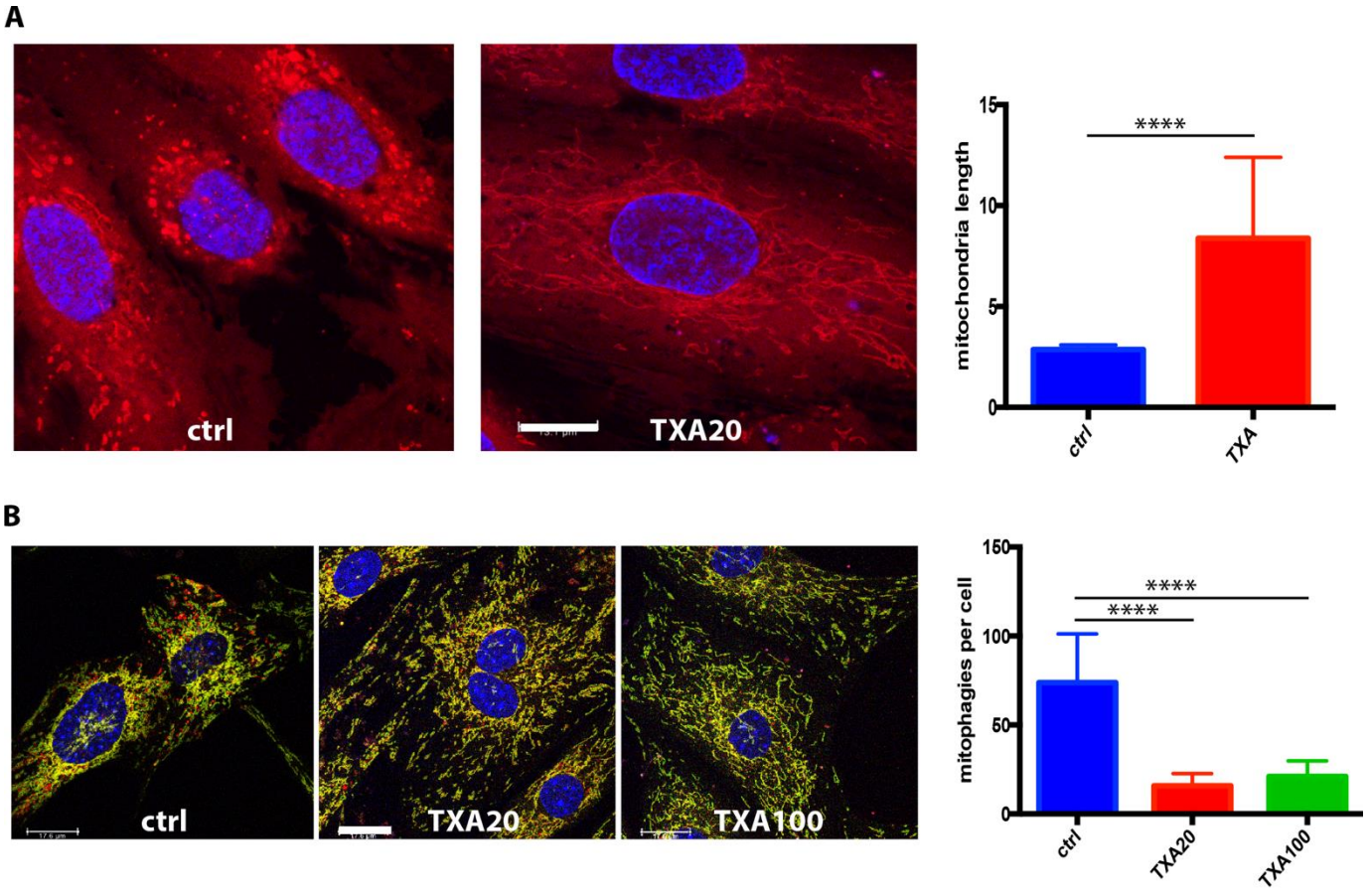
TXA 20

TXA 100

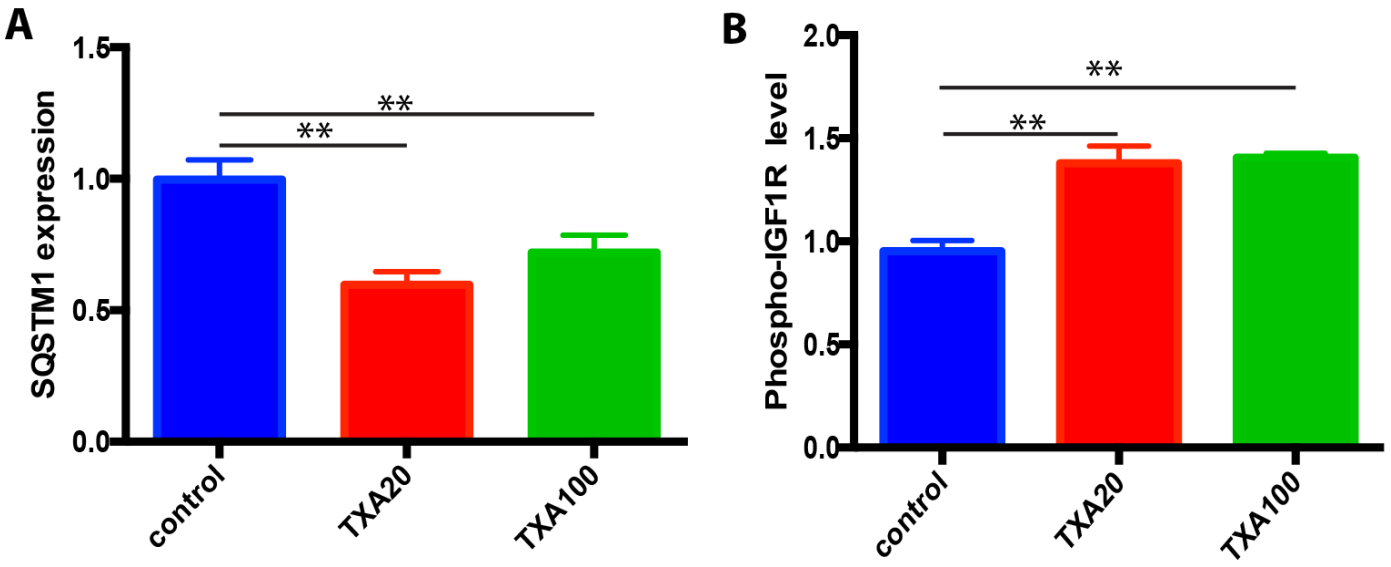
Control

TXA increases the length of mitochondria (A) and suppresses mitophagy (B) in the endothelial cells

Mitophagies: red structures in Figure B

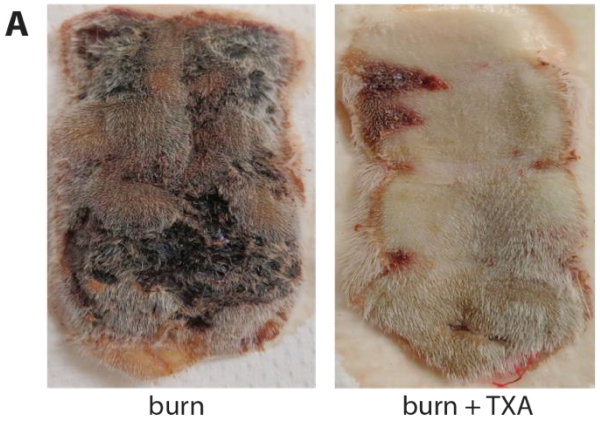


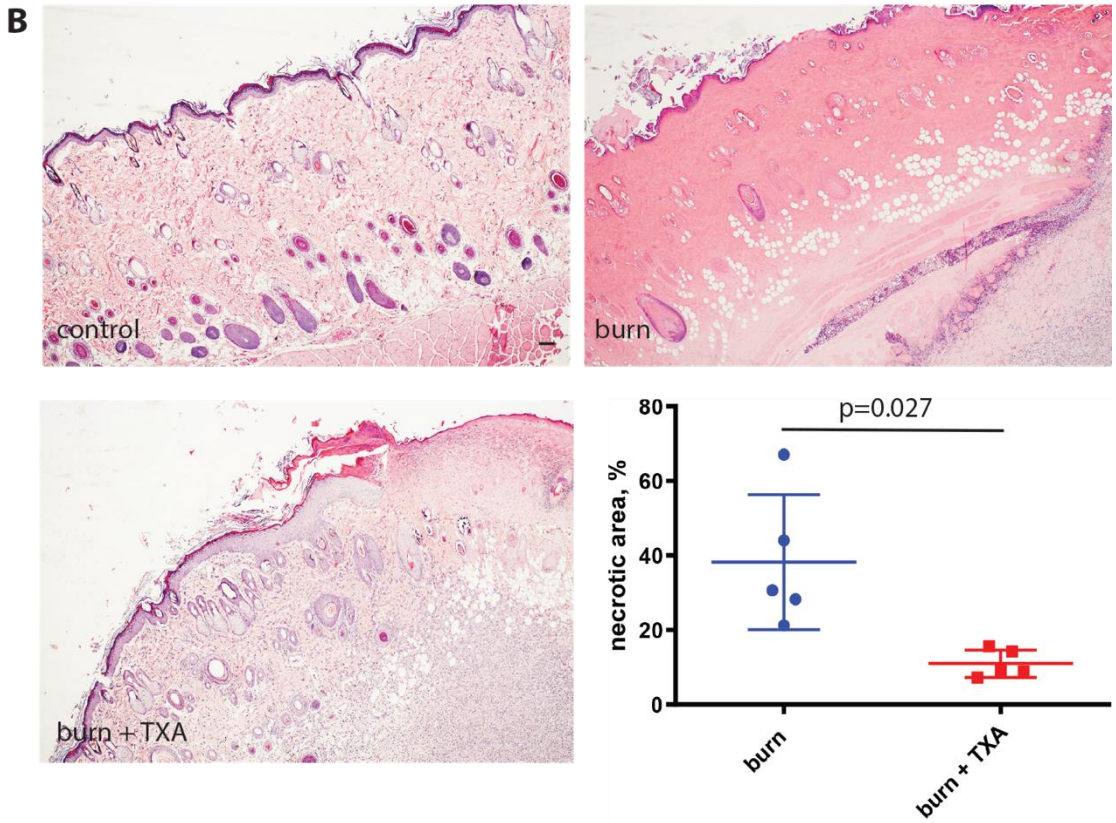
A study using the ActivSignal array demonstrates that in endothelial cells TXA suppresses the level of the mitophagy pathway component SQSTM1 and enhances the IGFR1 activity known to stimulate the mitochondrial biogenesis



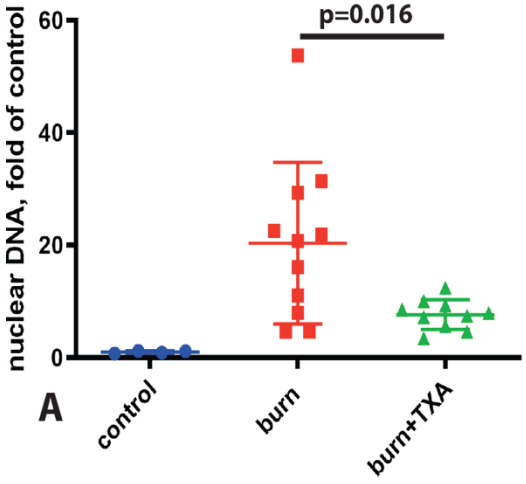
TXA effects in rat burn injury model

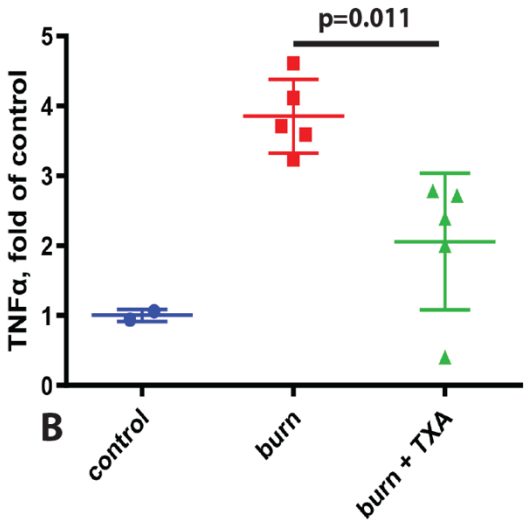
Daily injections of TXA improve burn wound healing in rats

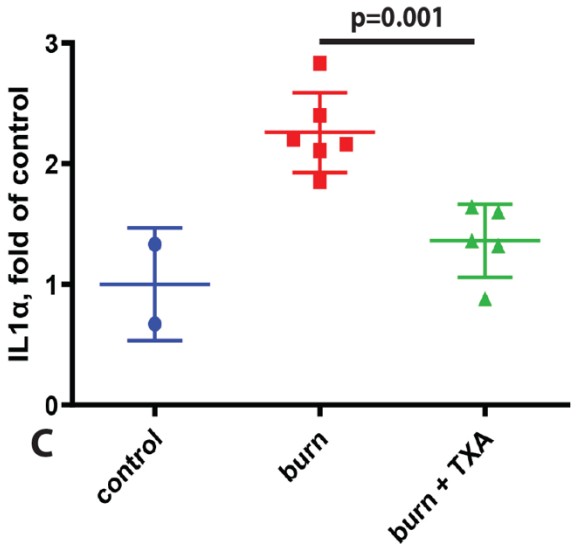




TXA injection to rats suppresses burn-induced release of nuclear DNA to bloodstream (A), and expression of proinflammatory cytokines TNFα (B) and IL1α (C) in liver.

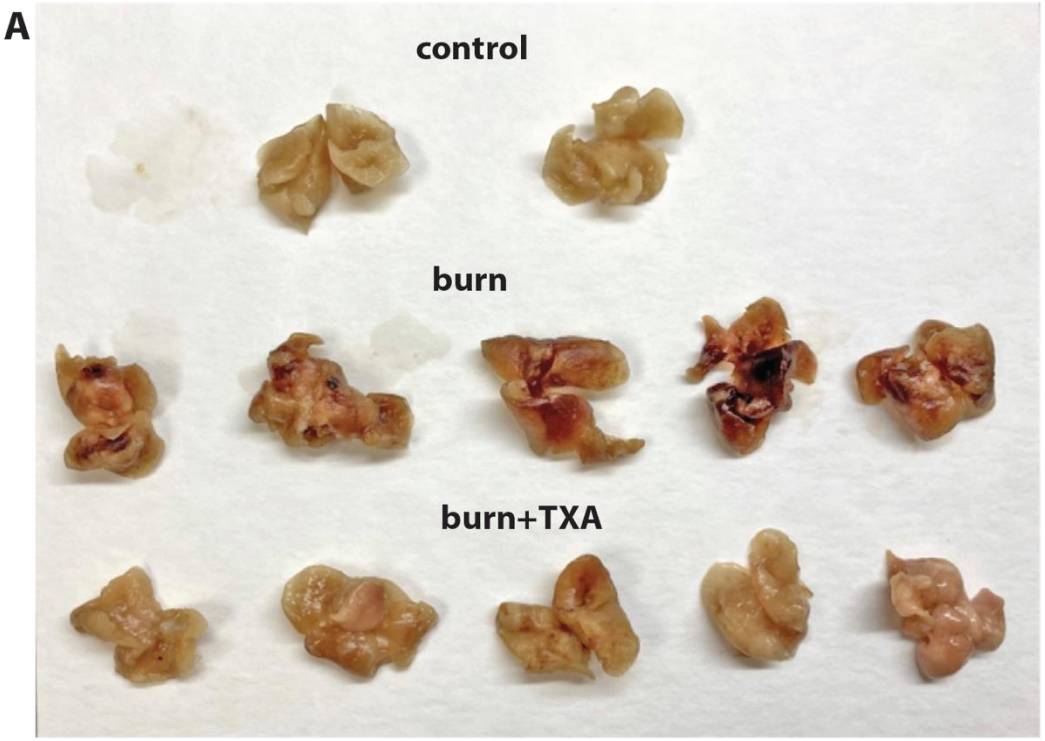




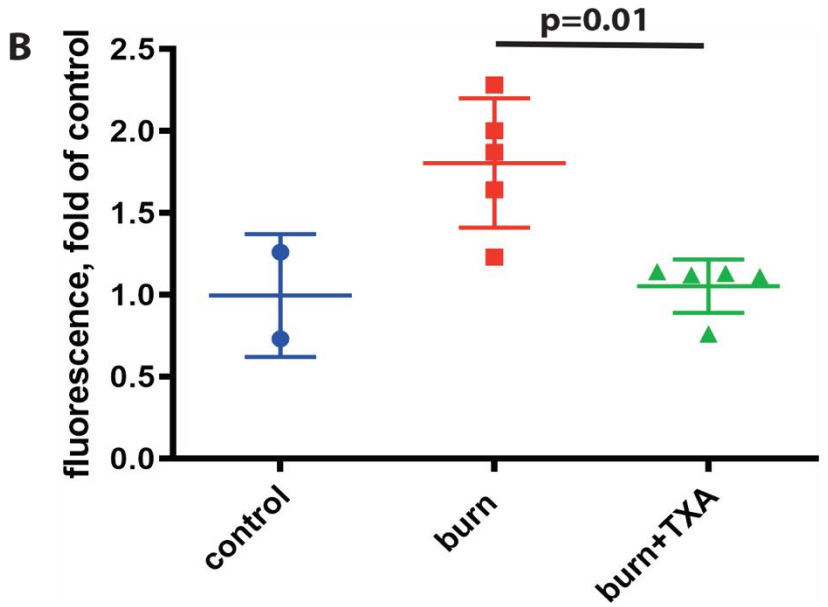


TXA suppresses burn-induced capillary leakage in rats

A. Decrease of red blood cells leakage

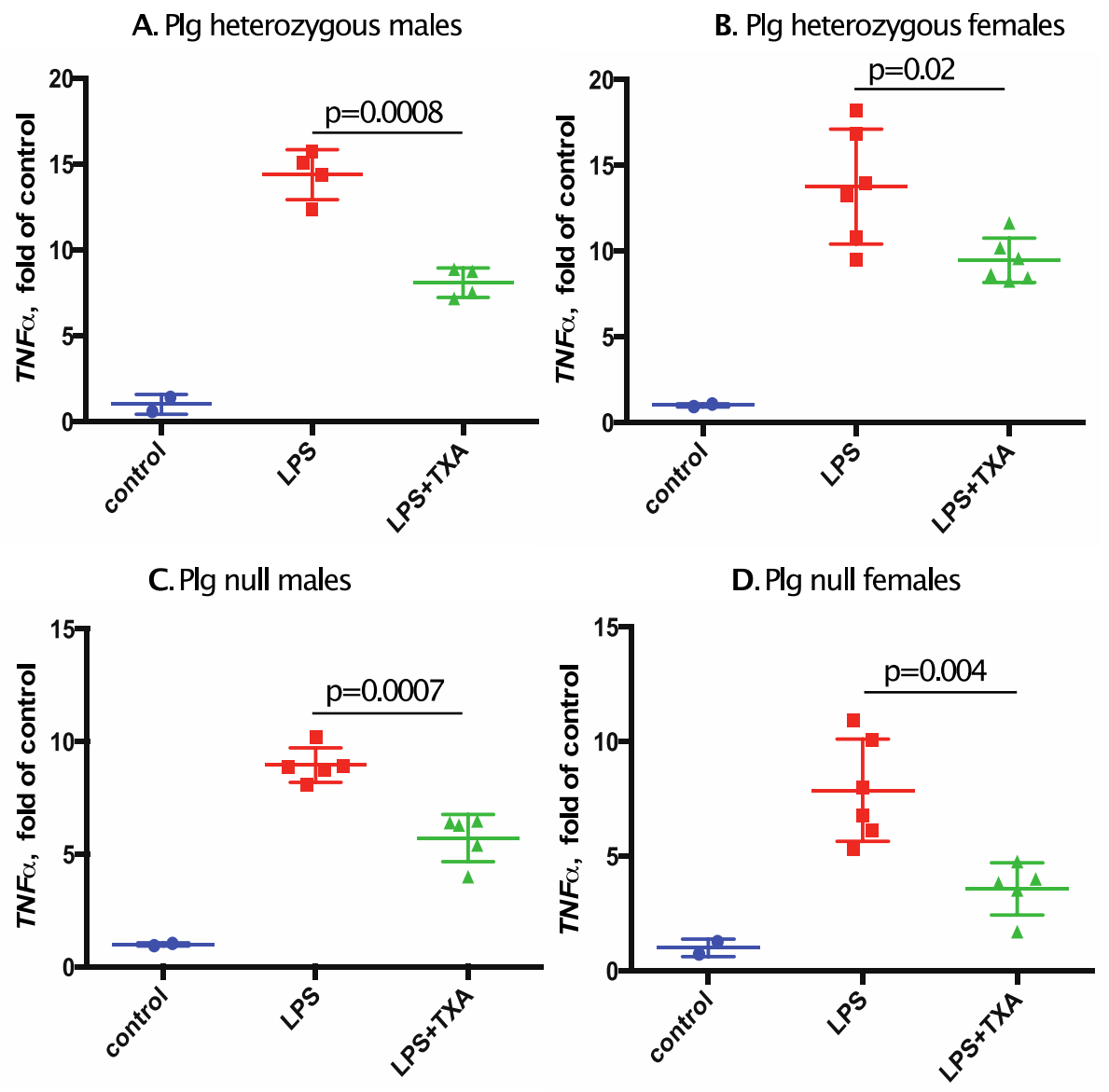


B. Decrease of Evans Blue leakage

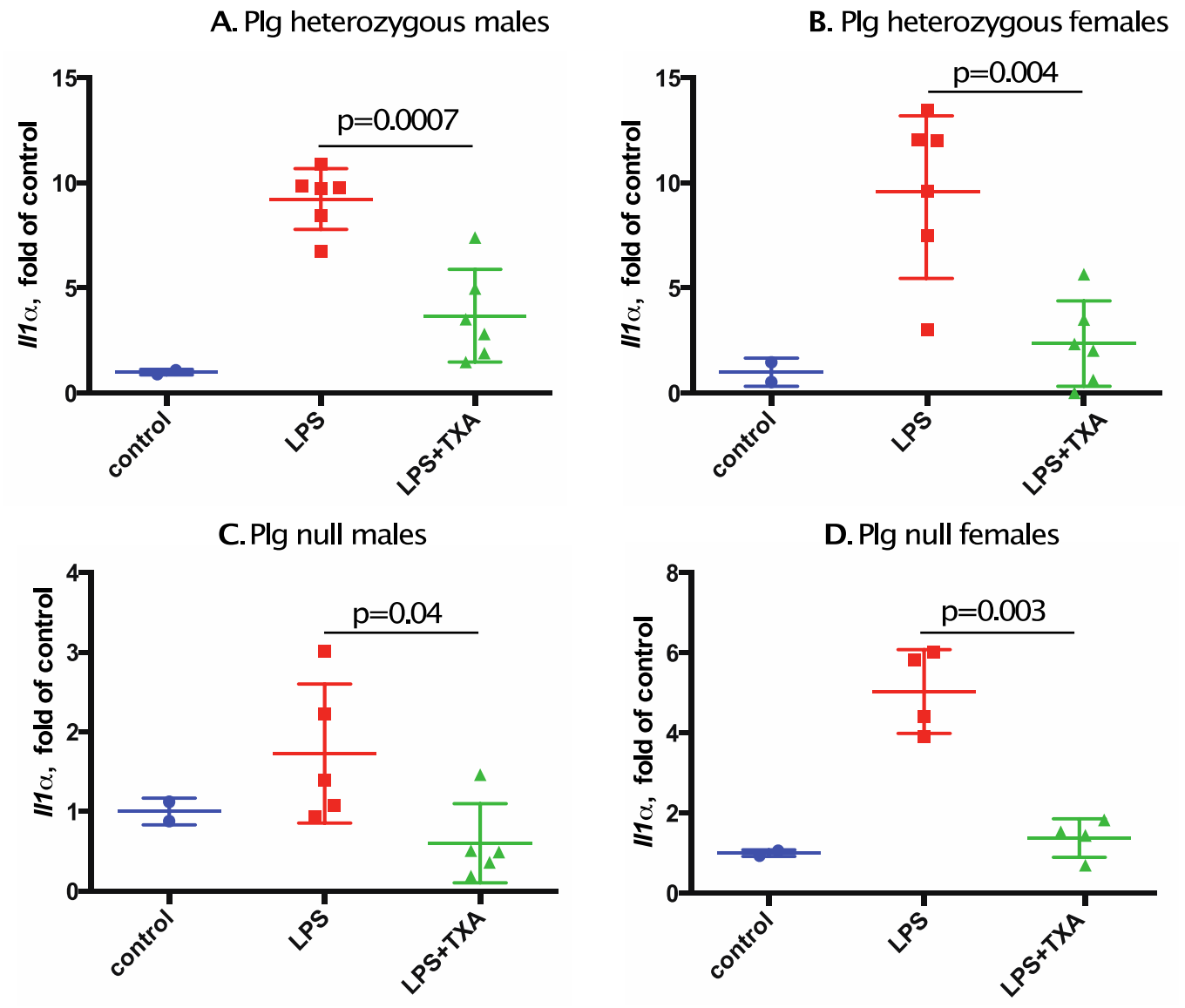


Do proinflammatory effects of TXA depend on plasmin inhibition?

TXA suppresses LPS-induced TNFα expression in the livers of Plg heterozygous and Plg null mice

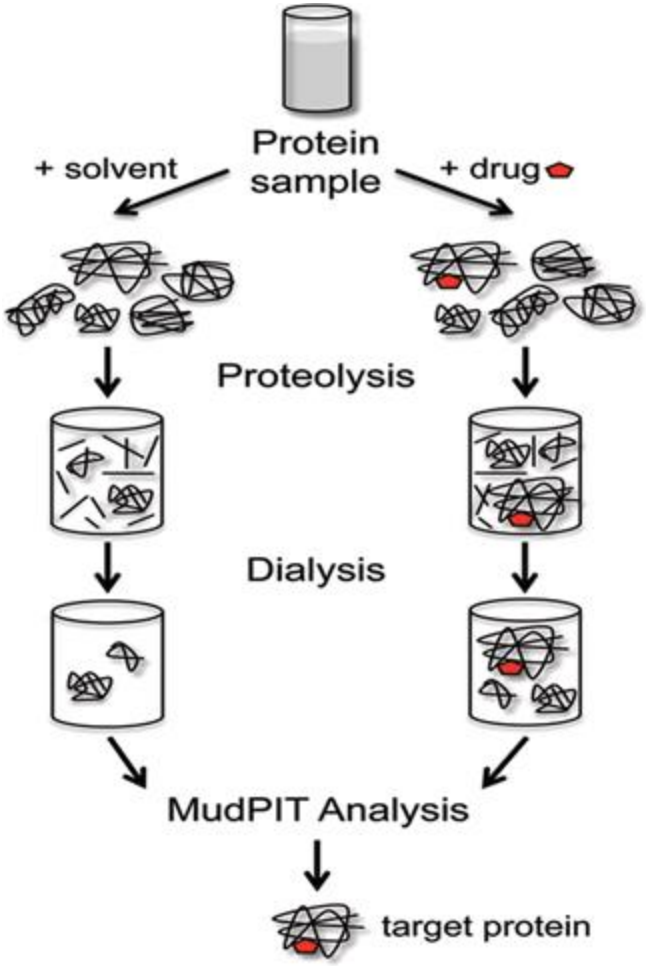


TXA suppresses LPS-induced IL1α expression in the hearts of Plg heterozygous and Plg null mice



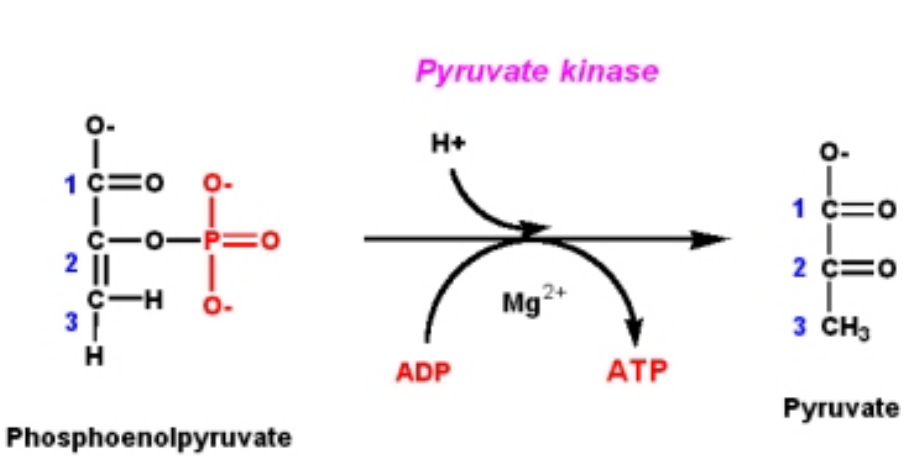
What is the protein target(s) of TXA, besides plasmin?

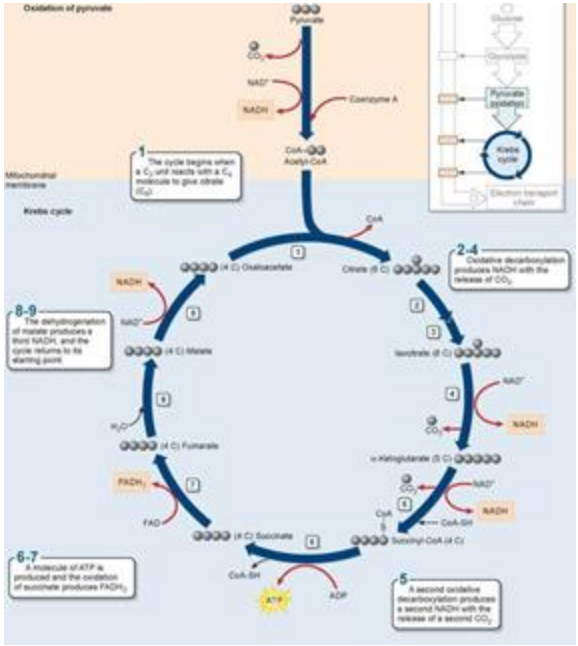
We used the DARTS method that is based on mass-spectrometry analysis of cell lysate proteins protected by a small ligand (TXA in our case) from the degradation by pronase

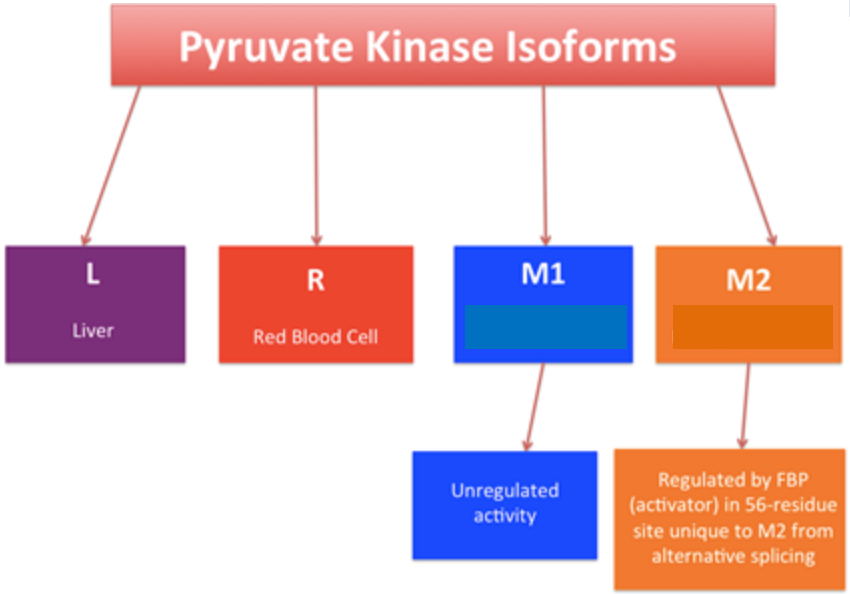


Results of DARTS analysis of TXA protein targets in endothelial cells

- Pyruvate kinase M (PKM2)
- RAB GDP dissociation inhibitor beta
- Nucleoside diphosphatase kinase B
- S-adenosylmethionine synthase isoforms type-2
- Leucine-rich repeat containing protein 59
- Nucleoside diphosphatase kinase B


Pyruvate kinase
H+
Mg2+
ADP
ATP
Phosphoenolpyruvate
Pyruvate




Pyruvate Kinase Isoforms
L
Liver
R
Red Blood Cell
M1
M2
Unregulated activity
Regulated by FBP (activator) in 56-residue site unique to M2 from alternative splicing

Neurotherapeutics (2018) 15:770–784
https://doi.org/10.1007/s13311-018-0635-2

ORIGINAL ARTICLE

Pyruvate Kinase M2 Increases Angiogenesis, Neurogenesis, and Functional Recovery Mediated by Upregulation of STAT3 and Focal Adhesion Kinase Activities After Ischemic Stroke in Adult Mice

Dongdong Chen[1] · Ling Wei[1] · Zhi-Ren Liu[2] · Jenny J. Yang[2] · Xiaohuan Gu[1] · Zheng Z. Wei[1,2] · Li-Ping Liu[4] · Shan Ping Yu[1,3]

Nat Med. 2017 June ; 23(6): 753–762. doi:10.1038/nm.4328.

Pyruvate kinase M2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction

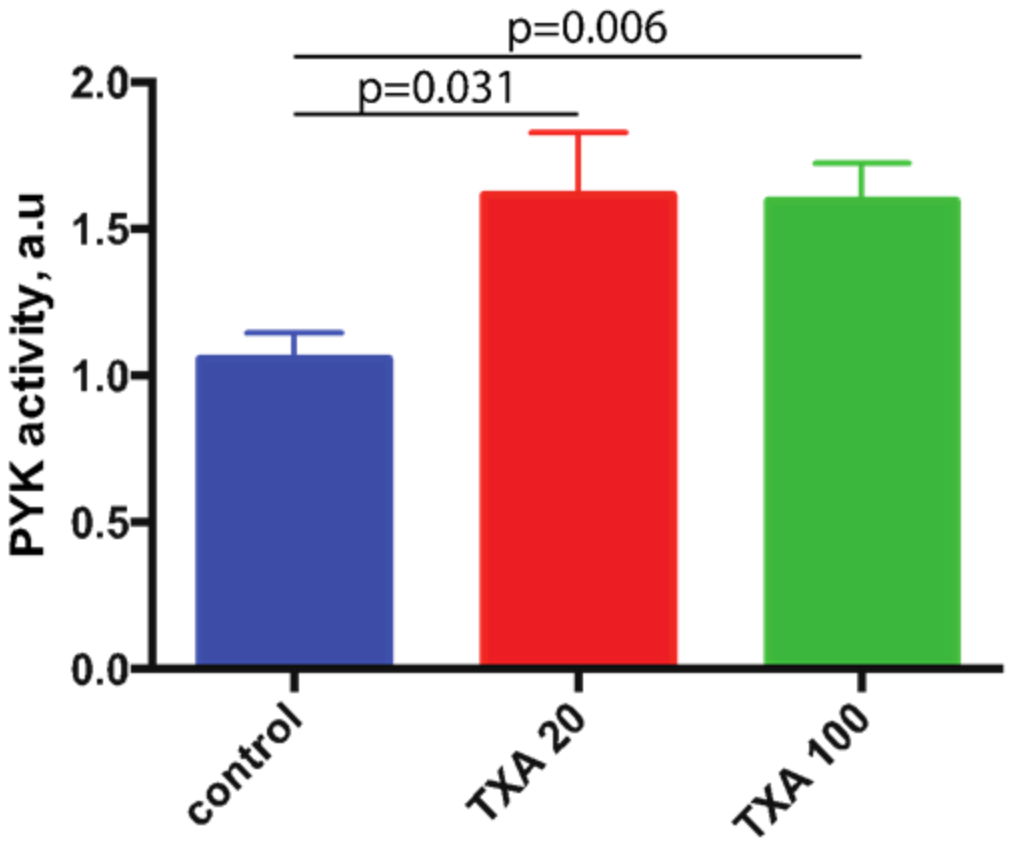
Weier Qi[1], Hillary A Keenan[1], Qian Li[1], Atsushi Ishikado[1], Aimo Kannt[2], Thorsten Sadowski[2], Mark A Yorek[3], I-Hsien Wu[1], Samuel Lockhart[4], Lawrence J Coppey[3], Anja Pfenninger[2], Chong Wee Liew[5], Guifen Qiang[5,6], Alison M Burkart[1], Stephanie Hastings[1], David Pober[1], Christopher Cahill[1], Monika A Niewczas[1], William J Israelsen[7], Liane Tinsley[1], Isaac E Stillman[8], Peter S Amenta[1], Edward P Feener[1], Matthew G Vander Heiden[9], Robert C Stanton[1], and George L King[1]

Wound Repair Regen. 2016 March ; 24(2): 328–336. doi:10.1111/wrr.12411.

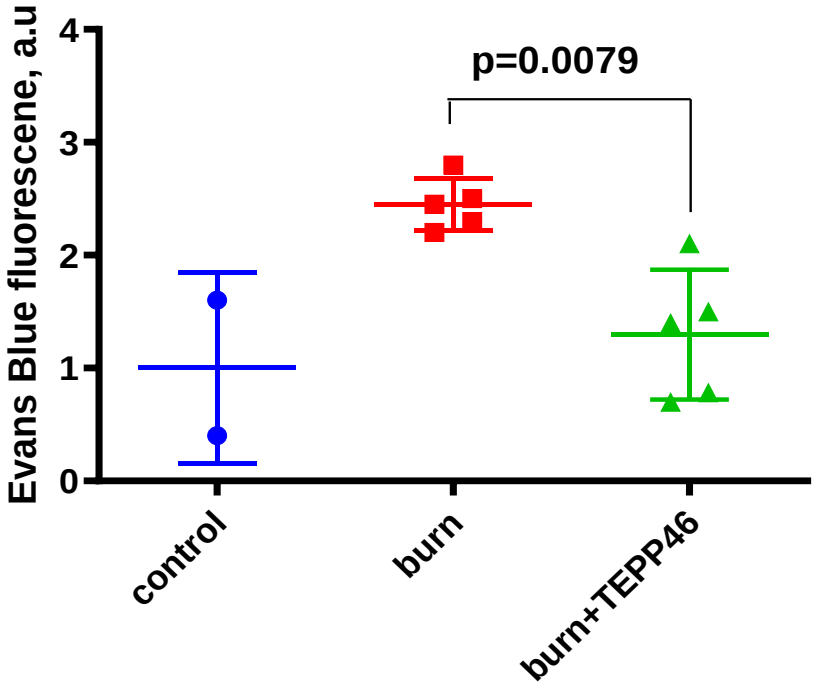
PKM2 released by neutrophils at wound site facilitates early wound healing by promoting angiogenesis

Yinwei Zhang, PhD, Liangwei Li, PhD, Yuan Liu, PhD, and **Zhi-Ren Liu, PhD**
Department of Biology, Georgia State University, Atlanta, Georgia

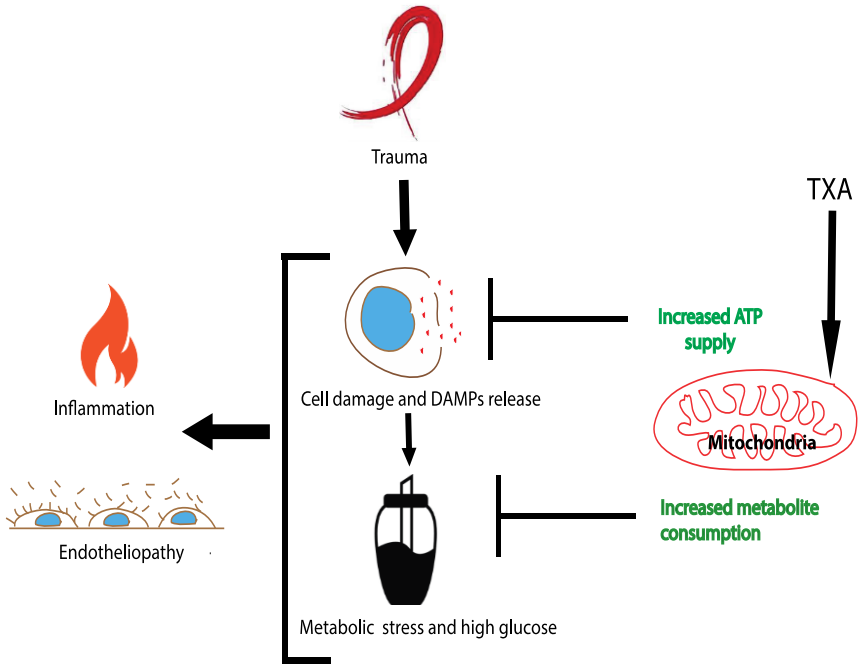
TXA enhances pyruvate kinase activity in endothelial cells



TEPP46, a potent activator of PKM2, suppresses the burn-induced vessel leakage in rat lungs



Working hypothesis: by enhancing mitochondrial respiration, TXA suppresses the trauma-induced DAMP release and metabolic stress, which results in the protection of the endothelial monolayer and decrease of inflammation



Conclusions:

1. TXA suppresses the release of such DAMPs as mtDNA and nuclear DNA in vitro and in vivo
2. TXA protects the endothelial monolayer from damage
3. TXA suppresses the burn-induced invasion of inflammatory cells to lungs
4. TXA suppresses burn-induced edema
5. TXA improves the healing of burn-induced wounds
6. TXA enhances mitochondrial respiration
7. TXA suppresses mitophagy and enhances mitochondrial biogenesis
8. TXA-induced decrease of proinflammatory cytokines expression is not mediated by plasmin inhibition

Questions to be addressed/future plans:

1. Do protective effects of TXA in vitro and in vivo depend on mitochondria?
2. Does TXA enhance mitochondrial function directly by enhancing the expression of mitochondrial proteins encoded in the nucleus?
4. What are the roles of individual TXA-binding proteins in its protective effects?
5. Can other chemical stimulators of the mitochondrial function be used in trauma patients to improve their recovery?
6. Does TXA regulate the levels of metabolic stress markers (lactate, glucose, succinate, ATP) in trauma?

Rappold/Prudosky Laboratory

- Igor Prudovsky, PhD, DSc
- Damien Carter, MD
- Elizabeth Turner, MD
- Doreen Kacer, MHS
- Matthew Siviski, PhD
- Shivangi Pande, PhD
- Ethan Griffin, BS
- Jack Vernamonti, MD
- Kathleen Pyburn
- Chloe Kumpel
- Autumm Green
- Angus Fung
- Eva Machnitzki

We are grateful to Ilya Alexandrov for ActivSignal array analysis and to Keyran Wynne for mass-spectroscopy studies