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Gemeinsam. Fürsorglich. Wegweisend.

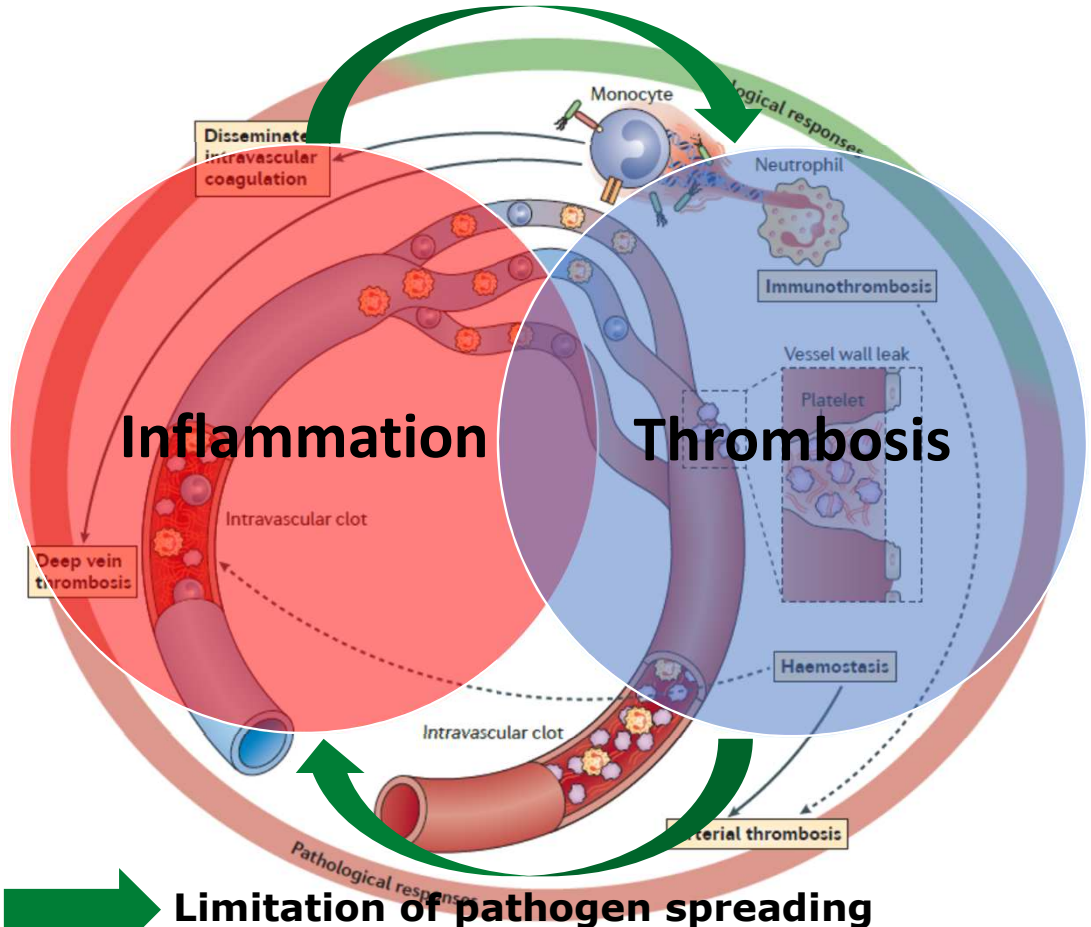
State of the art lecture: *Immuno-thrombosis: the interplay of inflammation and thrombosis*

Portal vein thrombosis meeting
Paris, 29.11.2022

Prof. Konstantin Stark
Department of Cardiology
Ludwig-Maximilians University, Munich, Germany

Immunothrombosis and thromboinflammation

Two sides of the same coin?



- **Immunothrombosis**
  - Host defense mechanism limiting the systemic spreading of pathogens
  - Protects the host against infections and pathogen invasion into the blood stream upon barrier breaches
  - Involves the activation of platelets and the coagulation system by inflammatory mechanisms

- **Thromboinflammation**
  - Pathologic process where overshooting and aberrant activation of immunothrombosis exacerbates tissue damage through tissue ischemia
  - Thrombosis feeds back on inflammation generating a *vicious circle* resulting in organ failure
  - Aberrant activation of thromboinflammation is a therapeutic target for cardiovascular, inflammatory/infectious diseases, and cancer

Limitation of pathogen spreading

Tissue ischemia and exacerbated inflammation

Inflammation and thrombosis

An evolutionary conserved host defense mechanism

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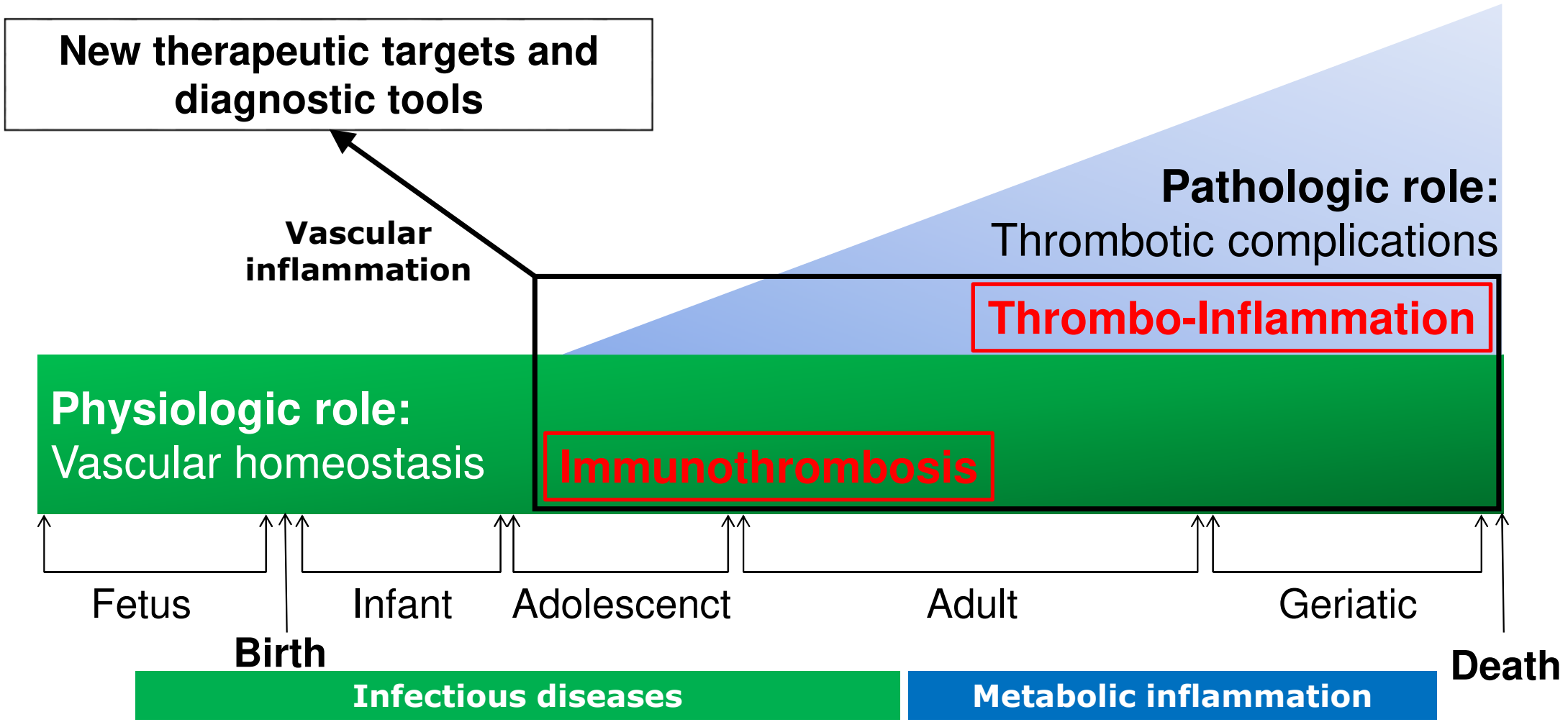


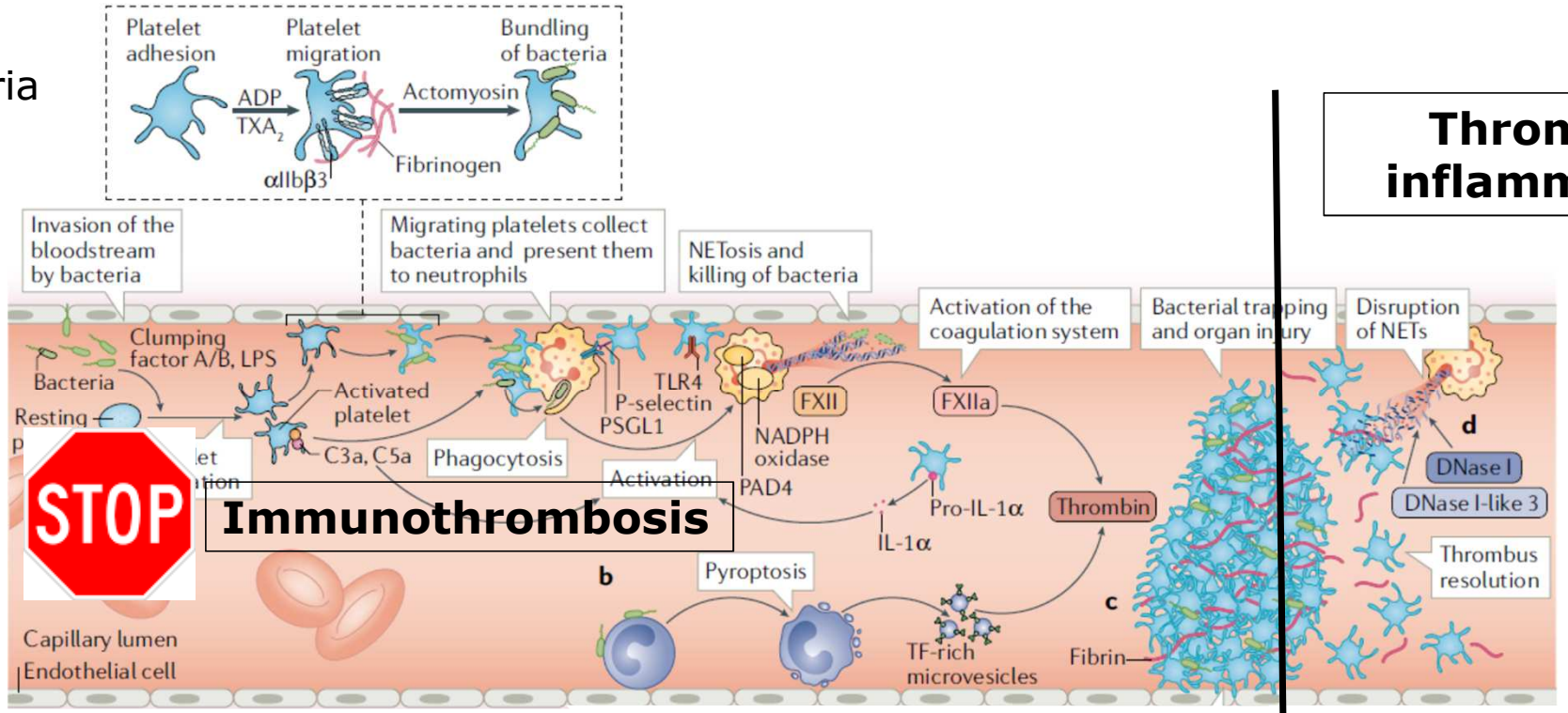
Immunothrombosis

An emergency mechanism preventing systemic pathogen dissemination

Interstitium

Circulation

Bacteria

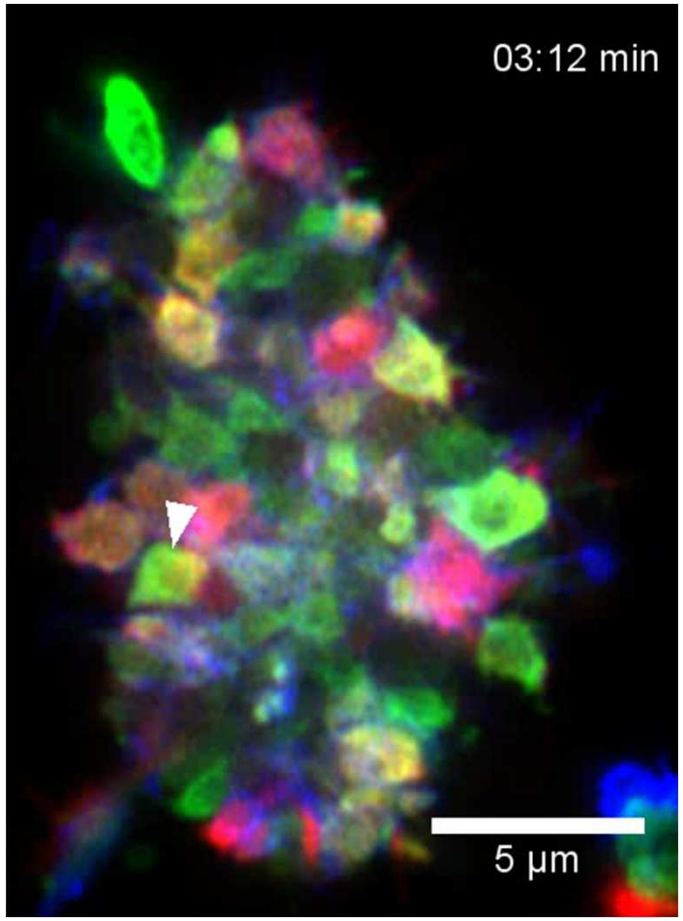


Thrombo-inflammation

Pathogen-translocation

Stark et al., *Nature Reviews Cardiology* 2021

Autonomous migration – a new asset of platelets



Gärtner et al., Cell 2017

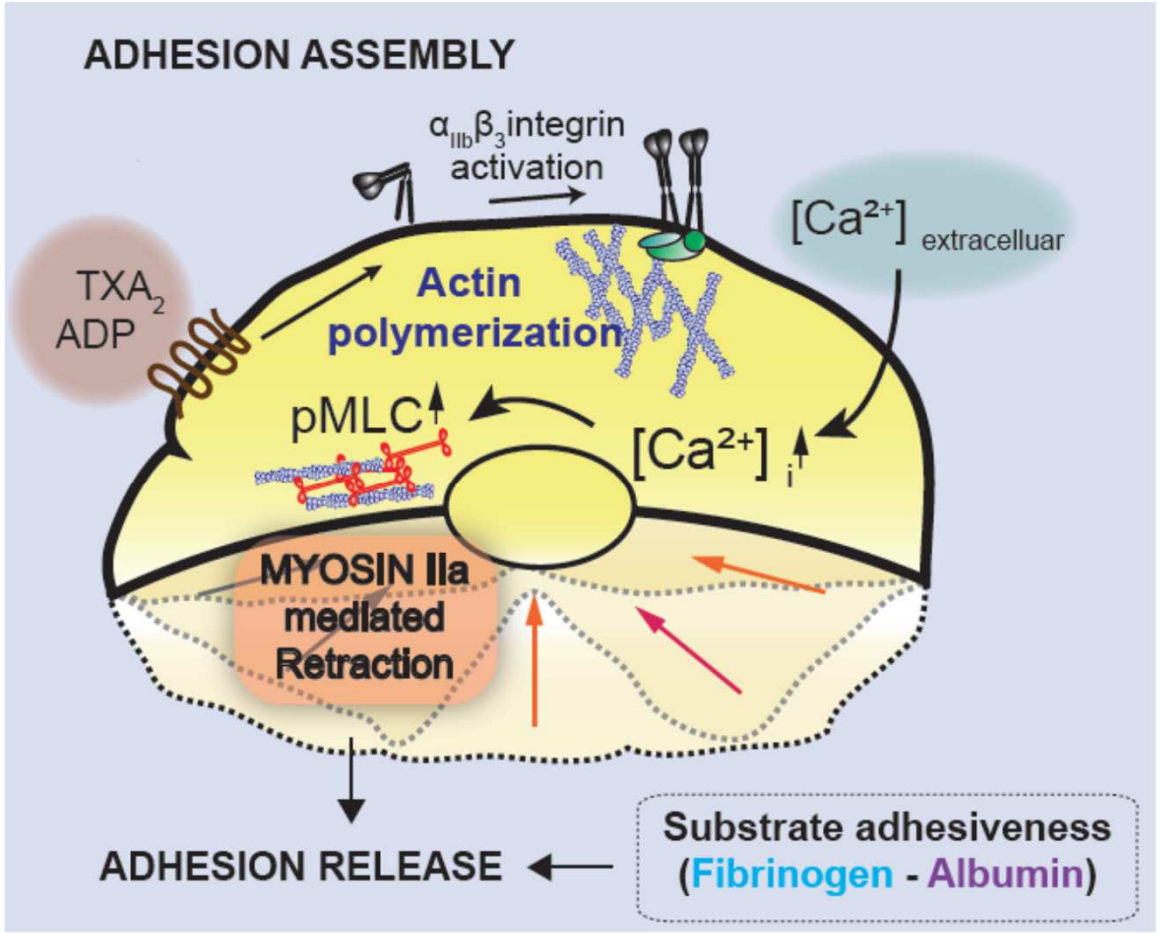
MIGRATION

FRONT

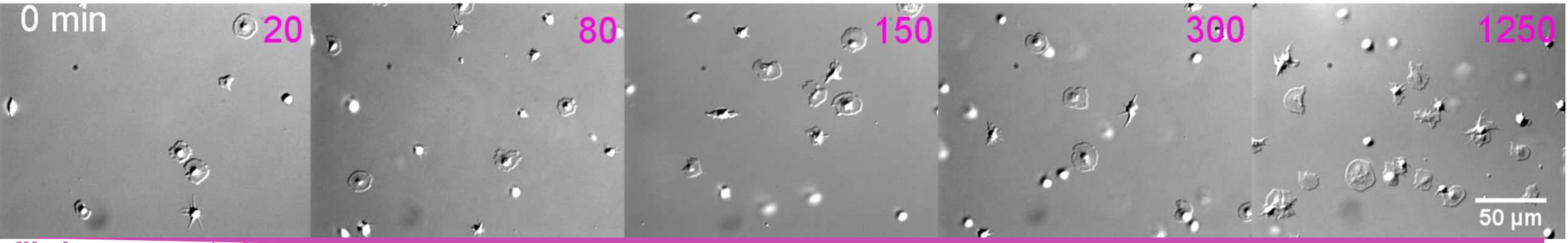
POLARIZATION

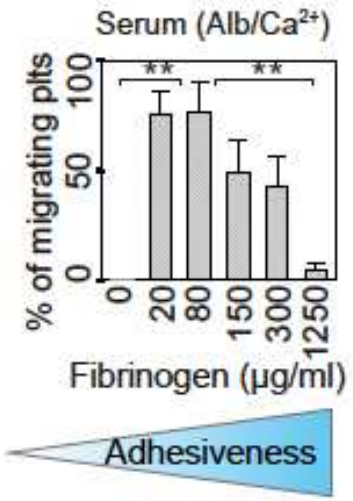
REAR

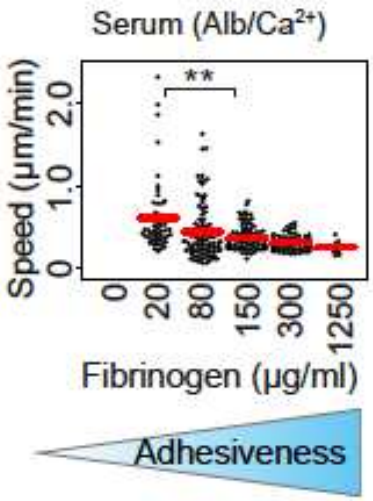
SPREADING

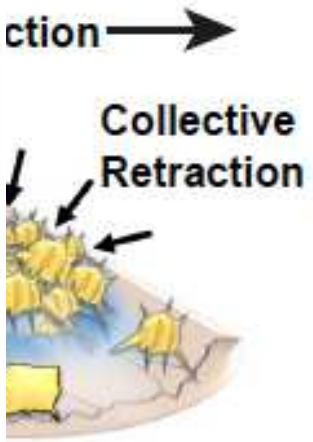


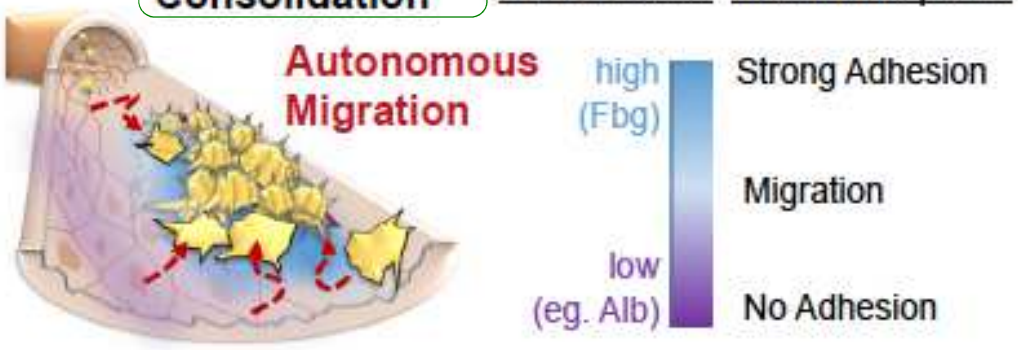
Platelet migration is haptotactic and contributes to clot organization











Migrating platelets collect fibrin(ogen) and with it all types of particles including bacteria

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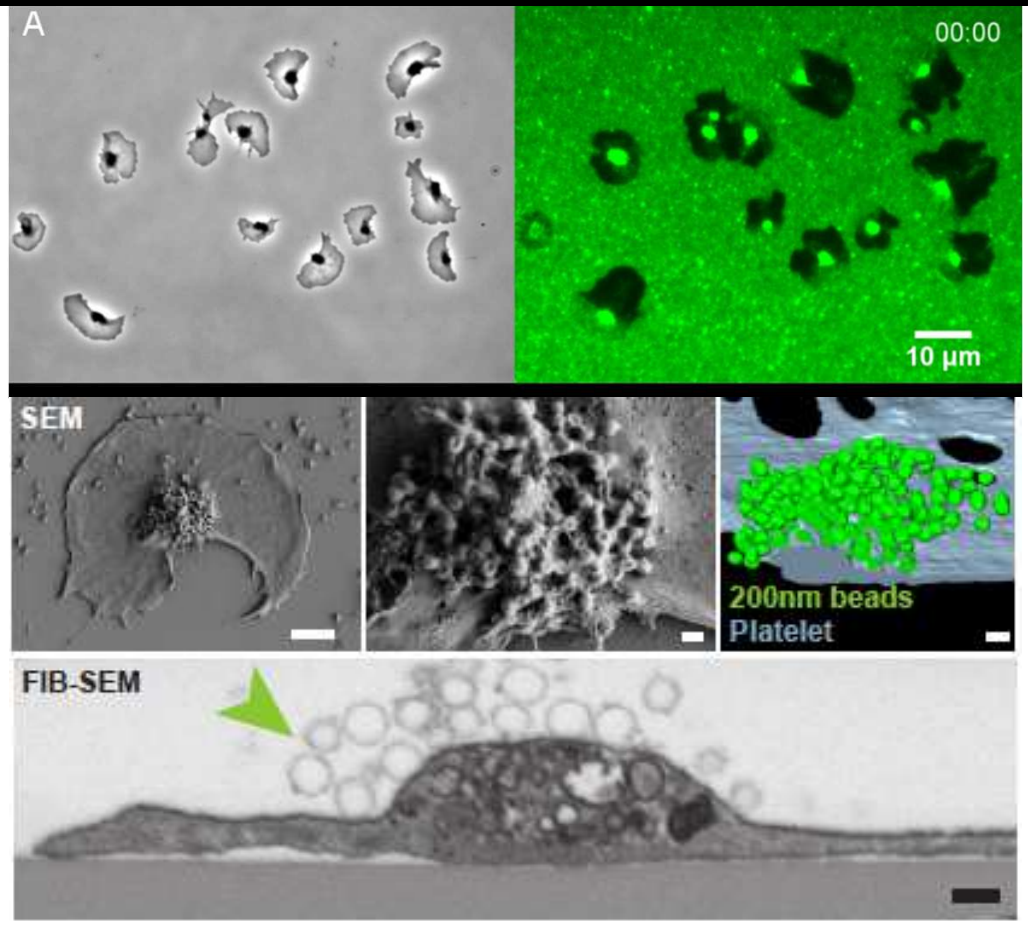
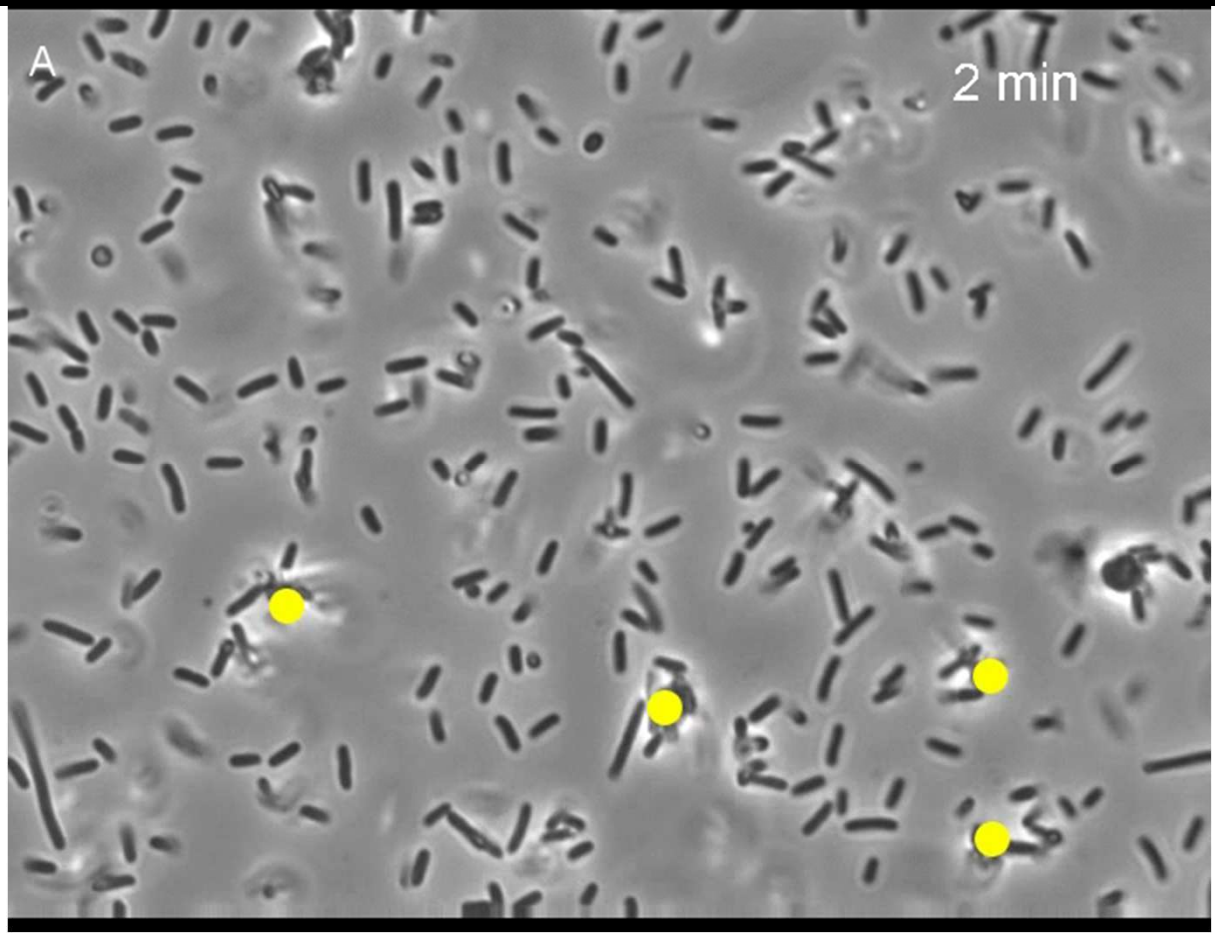
DIC

fibrinogen

bacteria

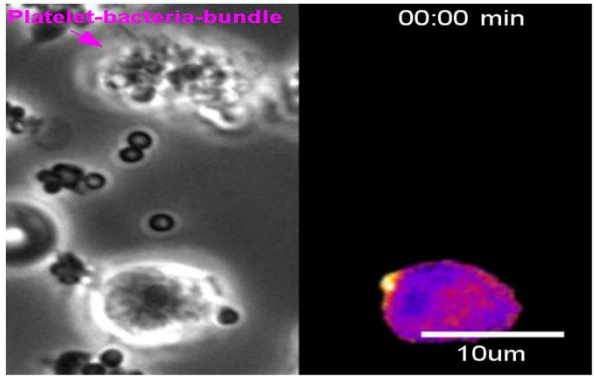
platelets

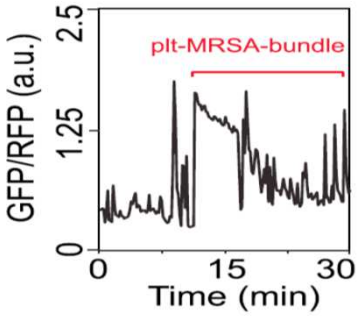




Platelet bacteria bundles boost neutrophil activation

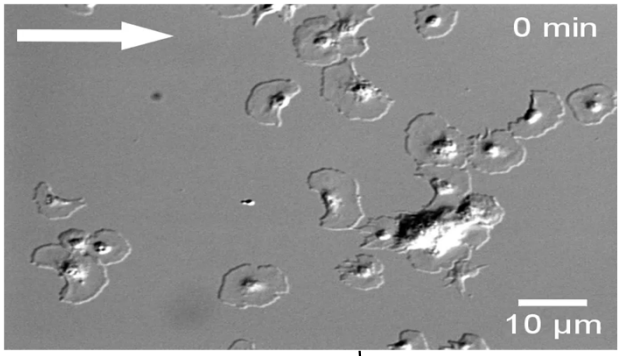
Calcium influx

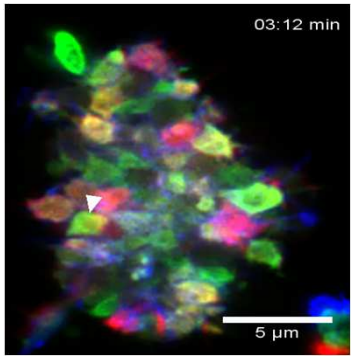


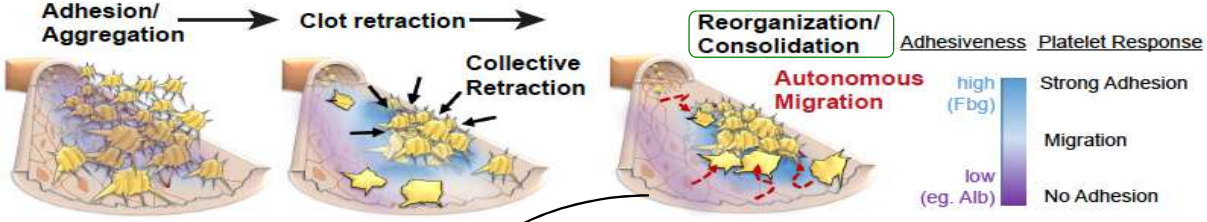


Migrating platelets drive NETosis

Platelets migrate autonomously…

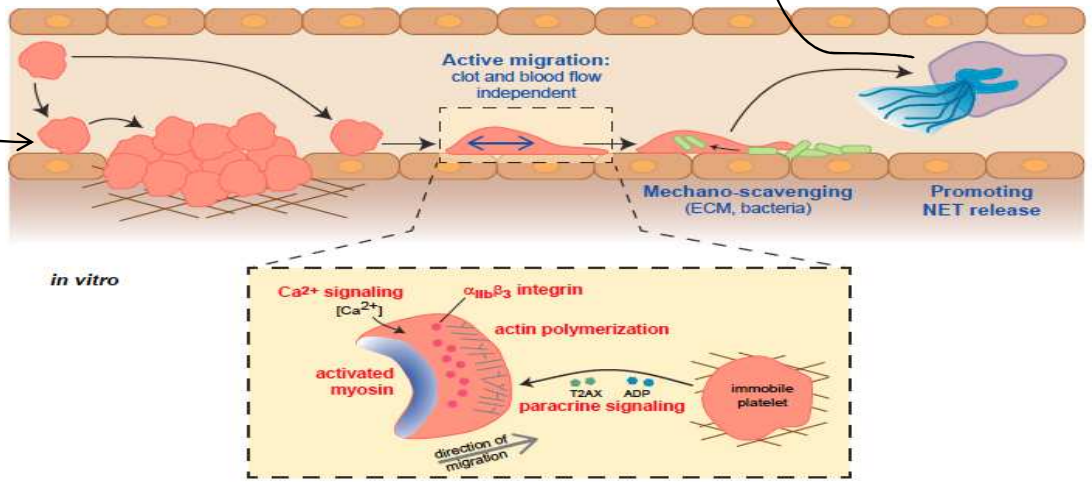






Promotion of atherothrombosis

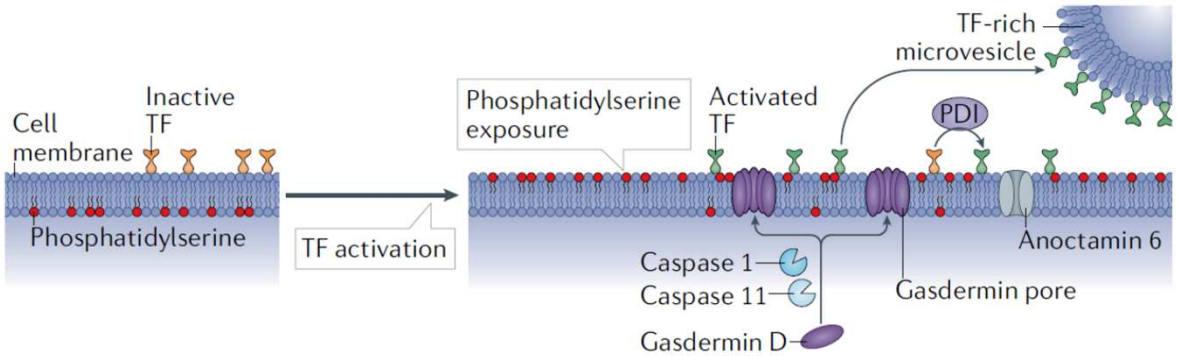
…and boost neutrophil activation



Gärtner et al., Cell 2017
Rosowski & Huttenlocher, Immunity 2018

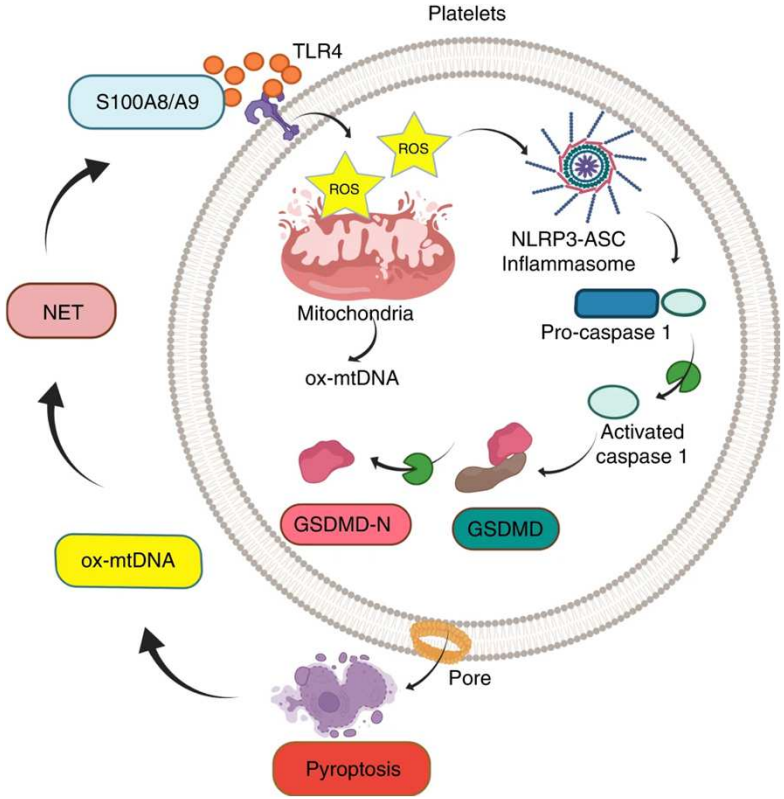
Pyroptosis as regulator of coagulation and platelet activation

Pyroptosis is invovled in tissue factor activation on the cell membrane



Stark et al., *Nature Reviews Cardiology* 2021

Platelet pyroptosis exacerbates NET formation and inflammation in severe sepsis



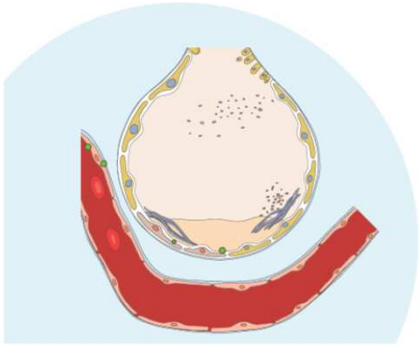
Meiling Su, et al., *Nature Cardiovascular Research* 2022

Dysregulated immunothrombosis as driver of disease severity in COVID-19

Clinical and pathophysiological staging of COVID-19

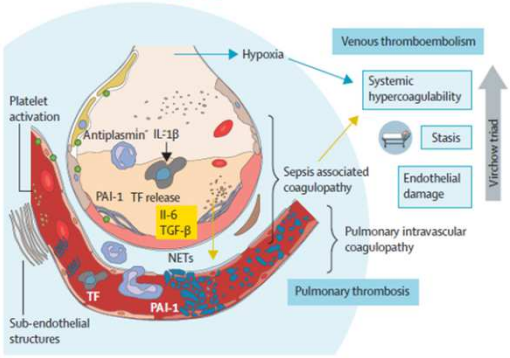
Stage 1

- Ambulatory patients
- Mild Symptoms
- **No inflammatory thrombi**



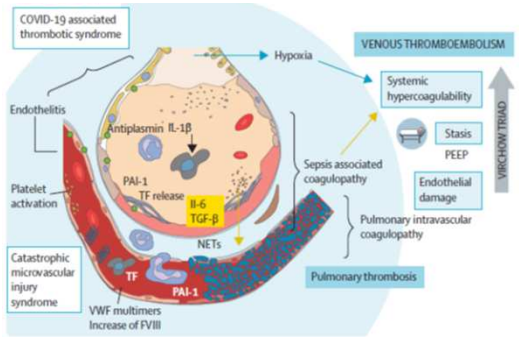
Stage 2

- Hospitalized patients
- Need oxygen supply
- **Evolving inflammatory microthrombi and macrothrombi**



Stage 3

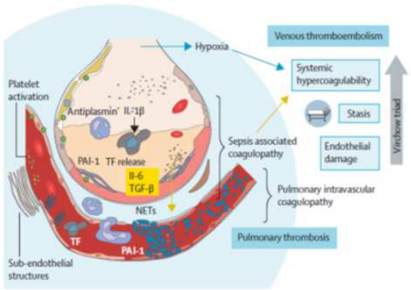
- Critically ill patients
- Mechanical ventilation
- **High incidence of inflammatory microthrombi and thrombotic events**



Leentjens et al.; *Lancet Haematol* 2021

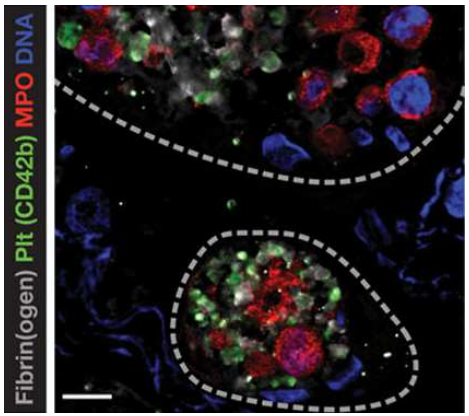
The link between inflammation and Thrombosis in disease progression of COVID-19

Stage 2

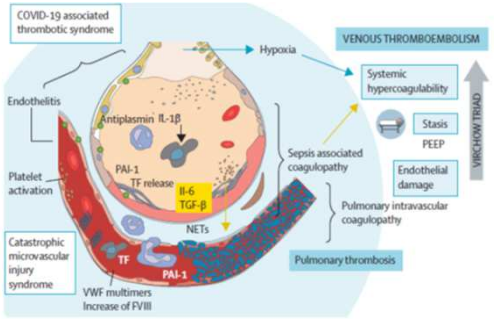


- Evolving inflammatory microthrombi and macrothrombi

Immunothrombotic dysregulation links Coagulopathy to respiratory failure



Stage 3

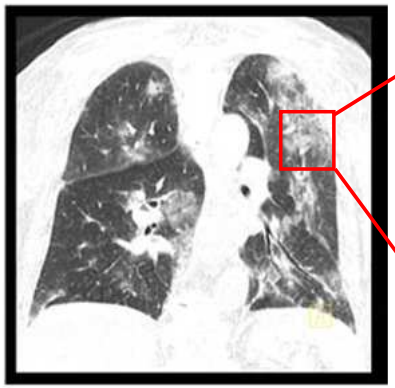


- COVID-19 associated thrombotic syndrome
- Vessel occlusion by pulmonary microthrombi
- Systemic coagulopathy and endotheliopathy
- Increased incidence of venous thromboembolism, myocardial infarction, ischaemic stroke

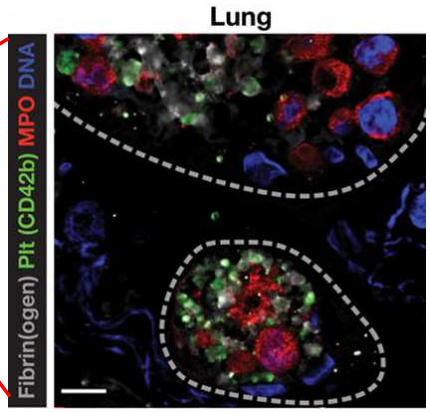
Nicolai,…., Stark, *Circulation* 2020

Dysregulated immunothrombosis in COVID-19

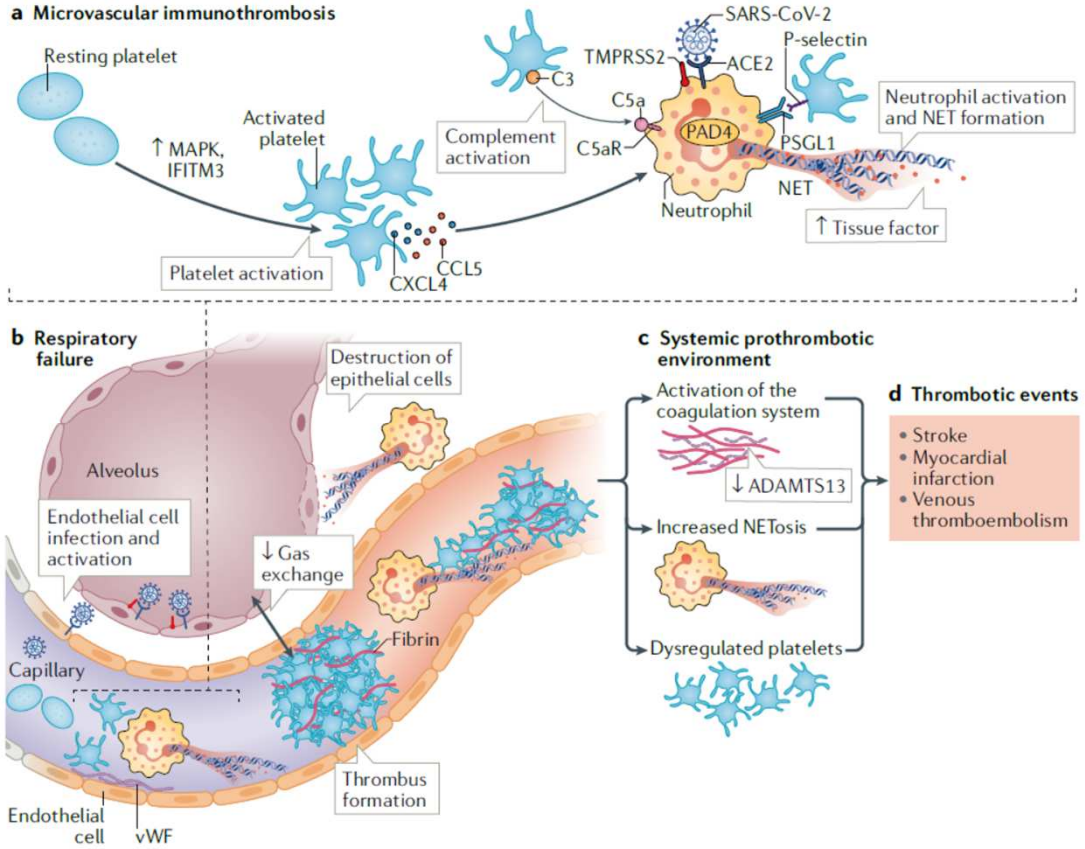
Linking respiratory failure to systemic coagulopathy



CT scan of COVID-19 pneumonia



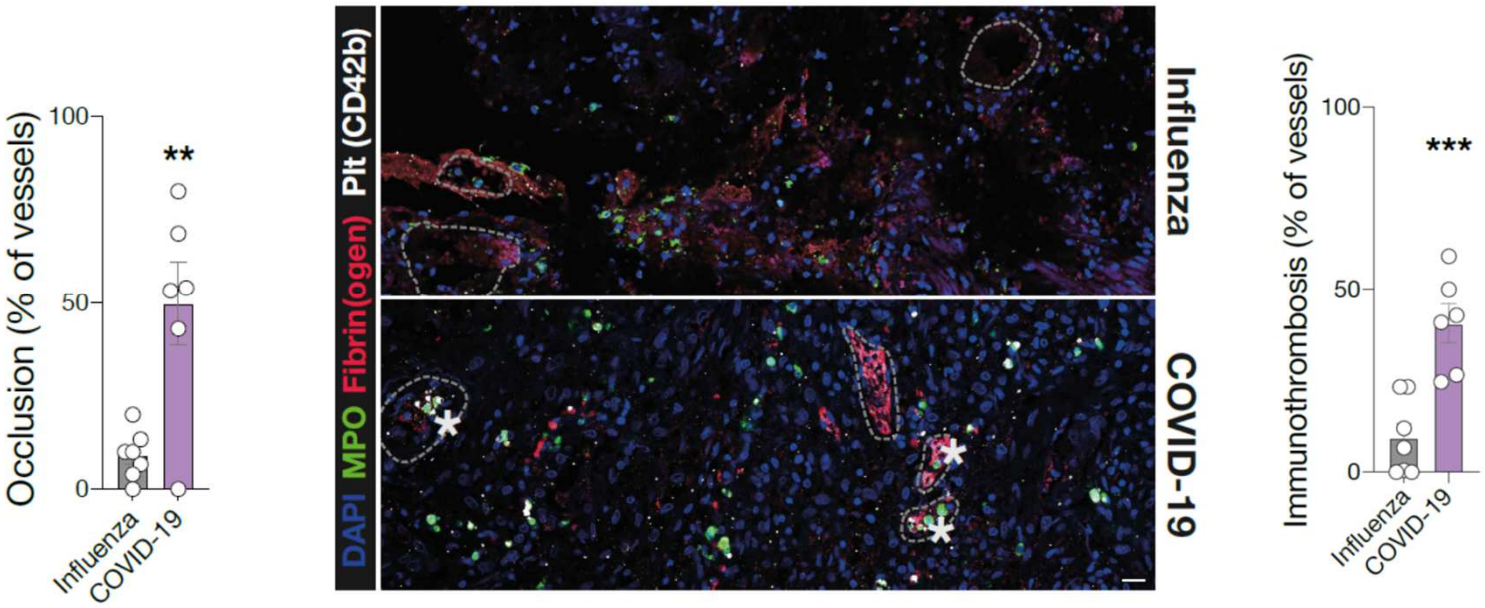
Autopsy specimens of COVID-19 lungs



Nicolai, Leunig,…Pekayvaz, Stark, *Circulation* 2020

Stark & Massberg, *Nature Reviews Cardiology* 2021

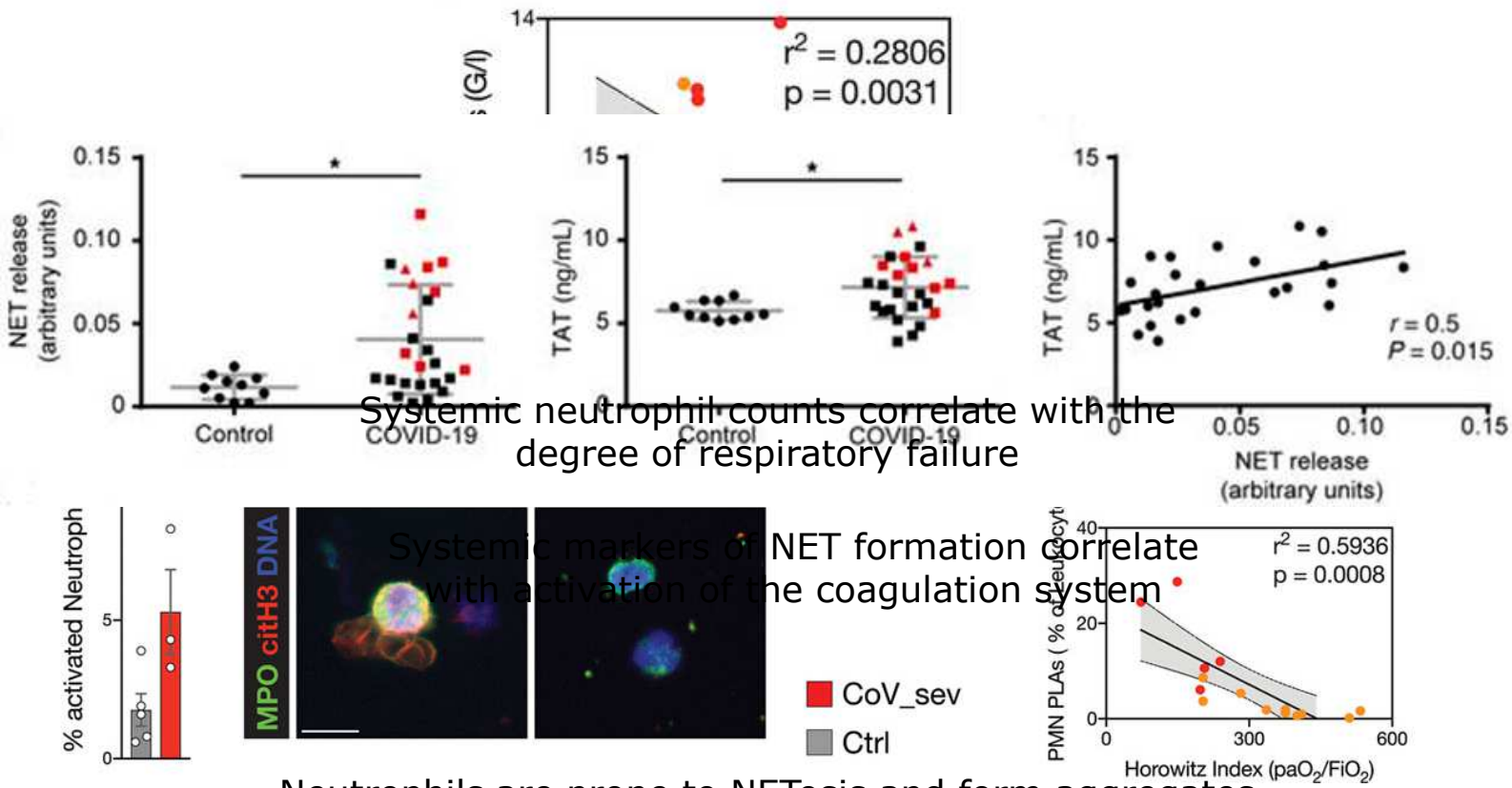
Immothrombotic vessel occlusions are more frequent in COVID-19 than in H1N1 influenza



Autopsy specimens of lungs from COVID-19/influenza patients

Nicolai; Leunig,…Pekayvaz; Stark, J Thromb Haemost 2020

Neutrophils are activated in COVID-19 and correlate with respiratory failure



Systemic neutrophil counts correlate with the degree of respiratory failure

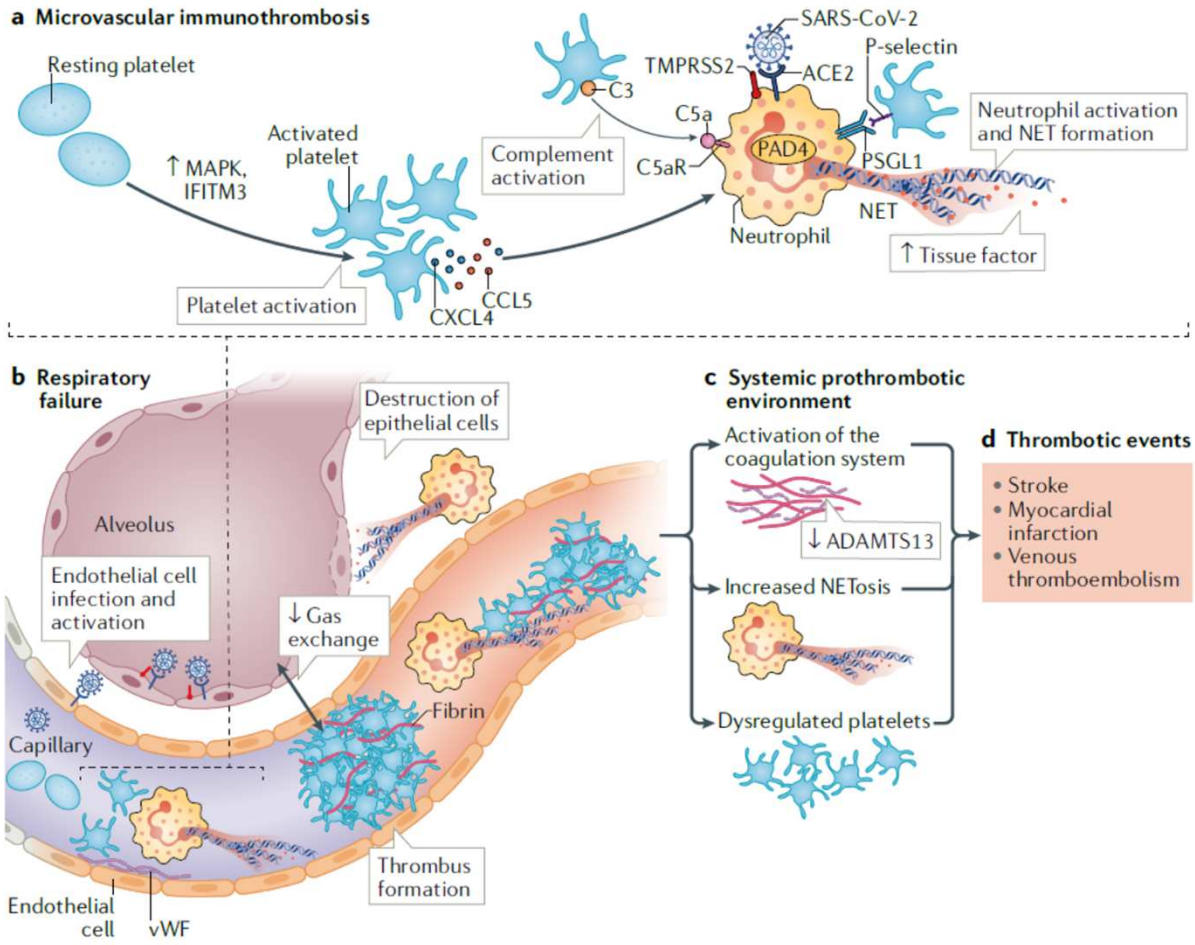
Systemic markers of NET formation correlate with activation of the coagulation system

Neutrophils are prone to NETosis and form aggregates with platelets

Nicolai; Leunig,…Pekayvaz, Stark, Circ, 2020

Skendros, Stark, Circ, 2020

Immunothrombosis links respiratory failure and systemic coagulopathy in COVID-19



Stark & Massberg, *Nature Reviews Cardiology* 2021

Anticoagulation in COVID-19
Selecting the right time, Drug, and Dose

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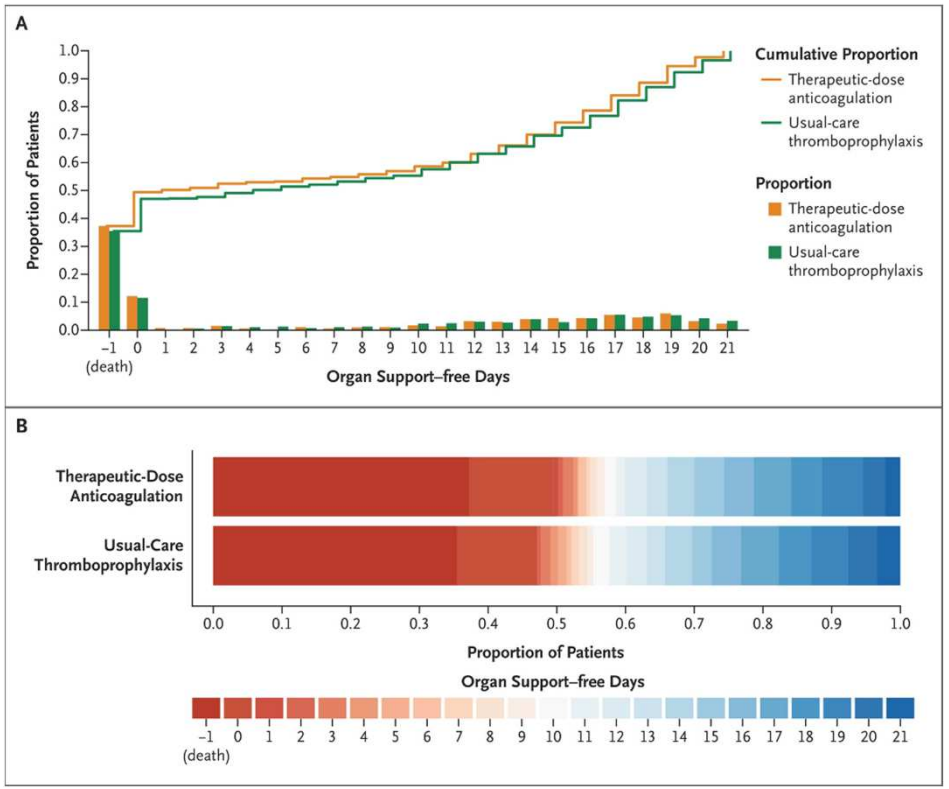
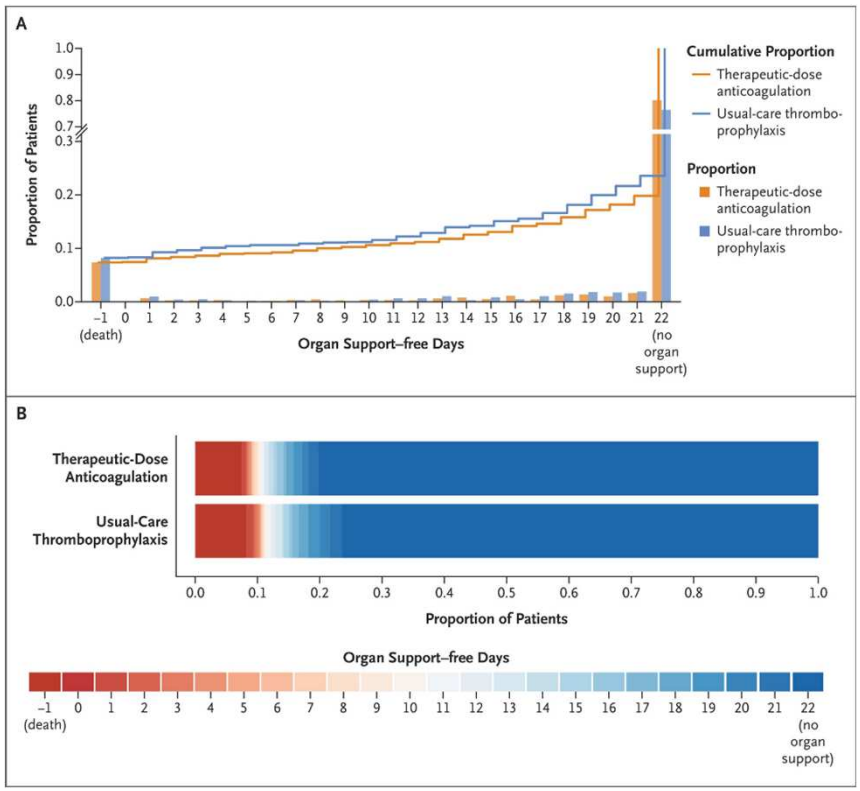
Critically ill patients (stage 3)



Noncritically ill patients (stage 2)



Therapeutic-dose anticoagulation in noncritically ill patients:
Increased rate of survival to hospital discharge and reduced use of of organ support

ATTACC, ACTIV-4a, REMAP-CAP investigator, *NEJM* 2021

Inflammation translates reduced blood flow velocity into venous thrombosis

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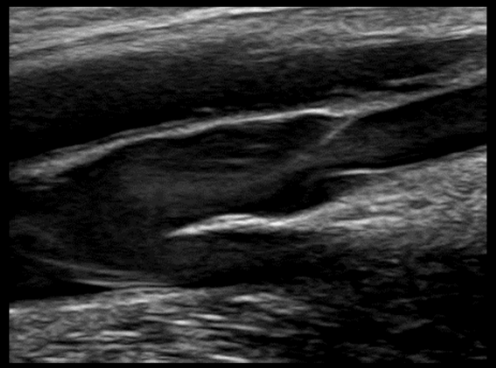
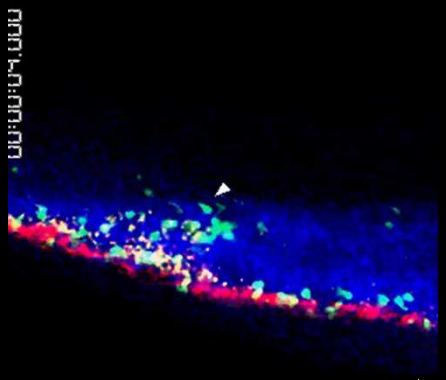
Stasis **Inflammation** **Thrombosis**

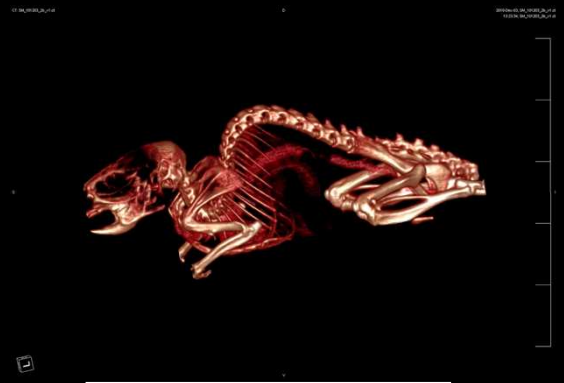


Stasis

Ultrasound of human vein



- Neutrophils
- Vessel wall
- Platelets
- Plasma

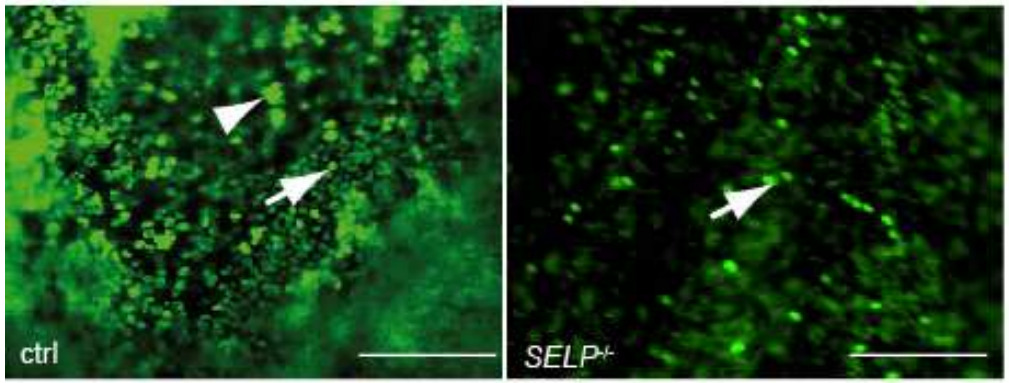


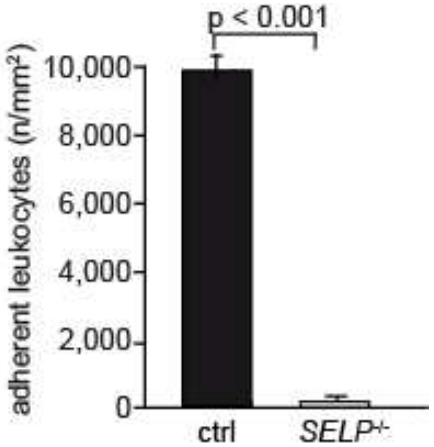
Thrombosis

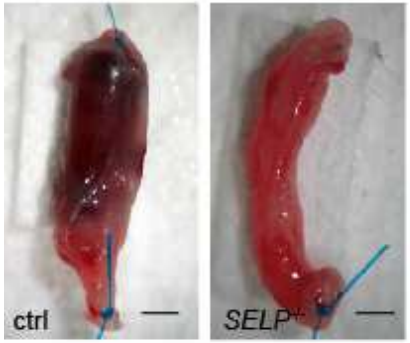
Mouse model of thrombosis in the inferior vena cava

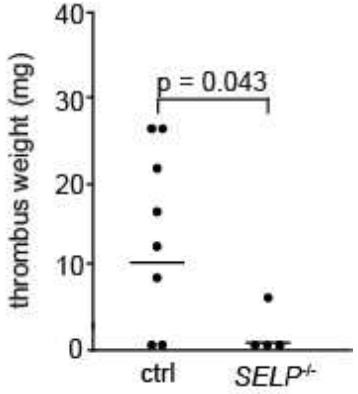
Von Brühl & Stark et al. JEM 2012

DVT formation depends on leukocyte recruitment



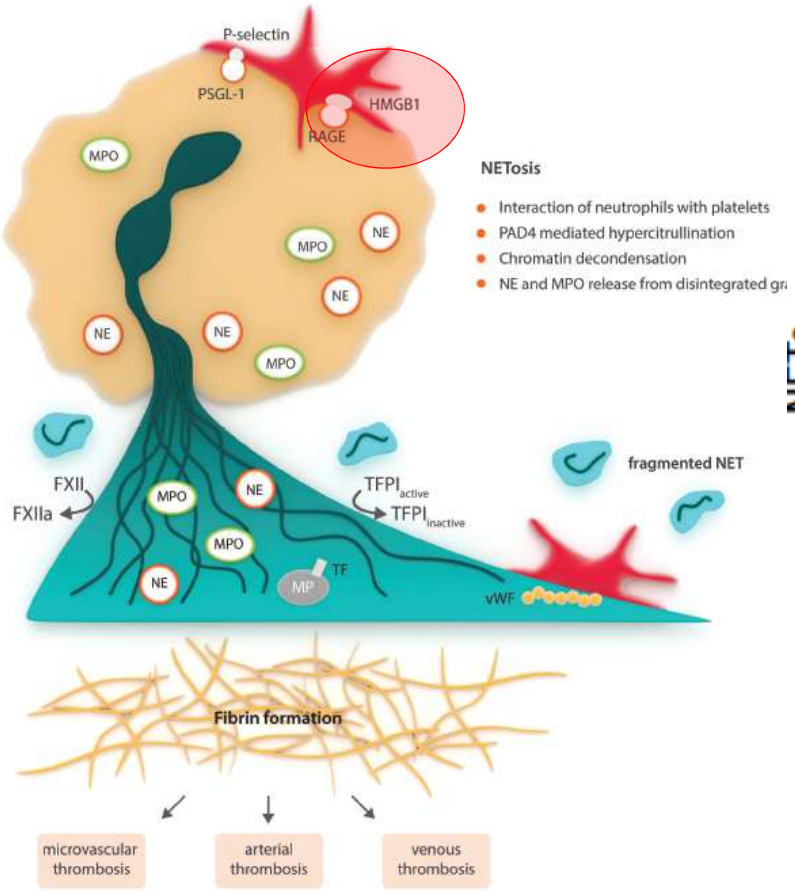


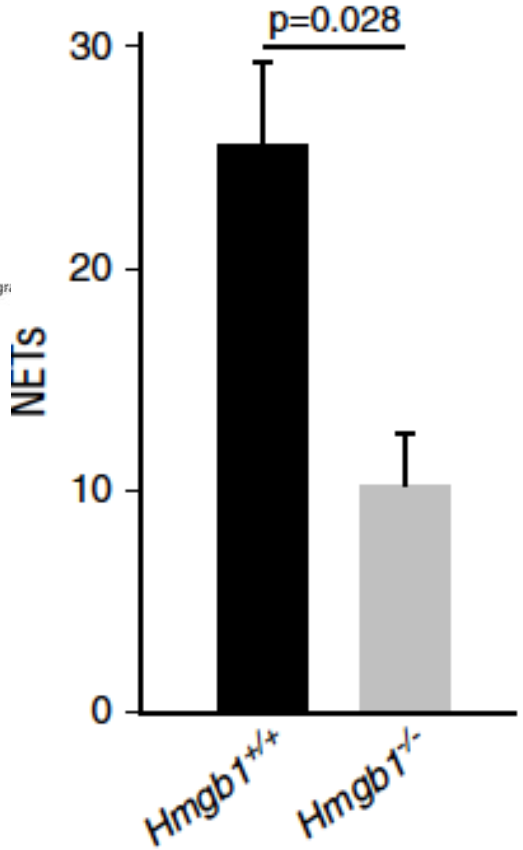


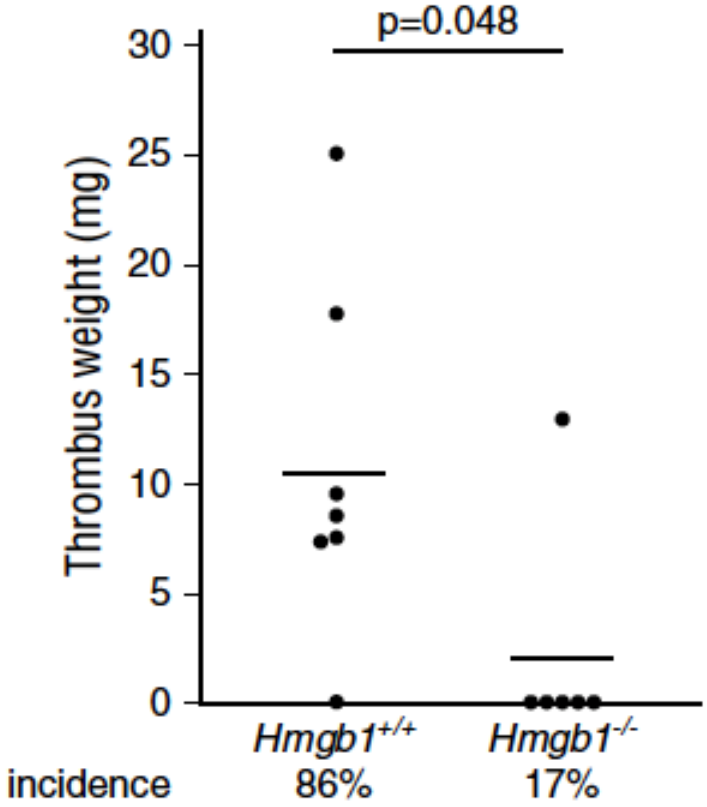


Von Brühl & Stark et al. JEM 2012

Death signals released by platelets support NETosis



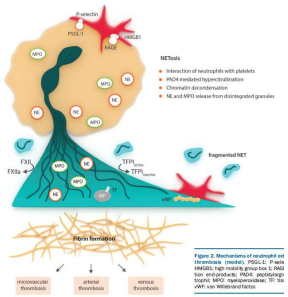


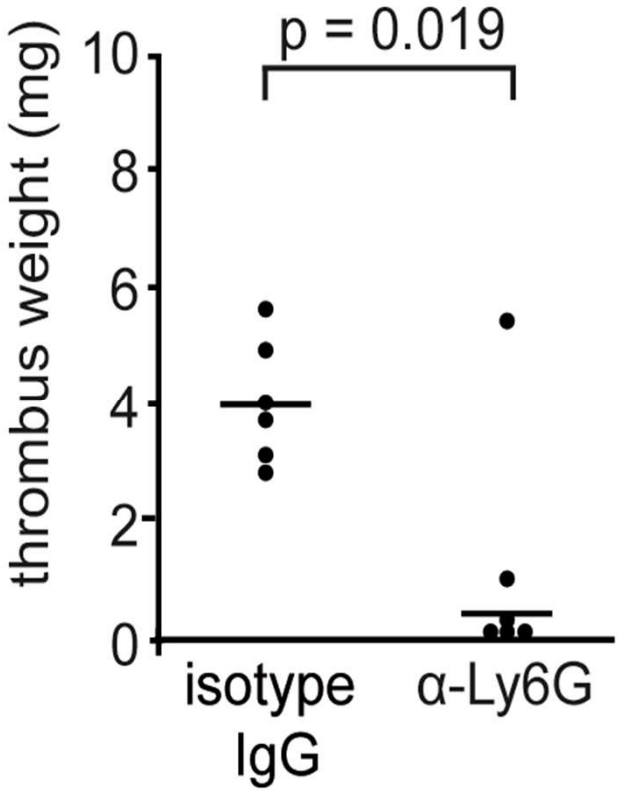


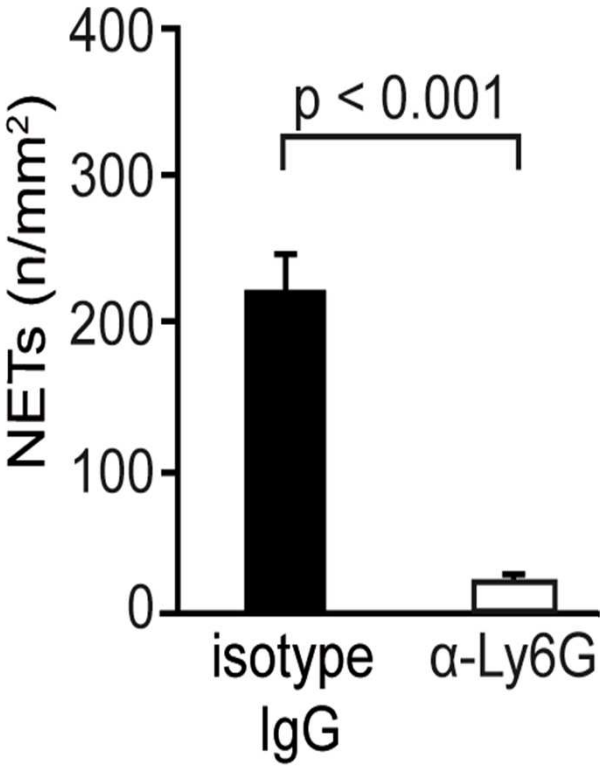
Pfeiler&Stark et al., Haematologica 2017

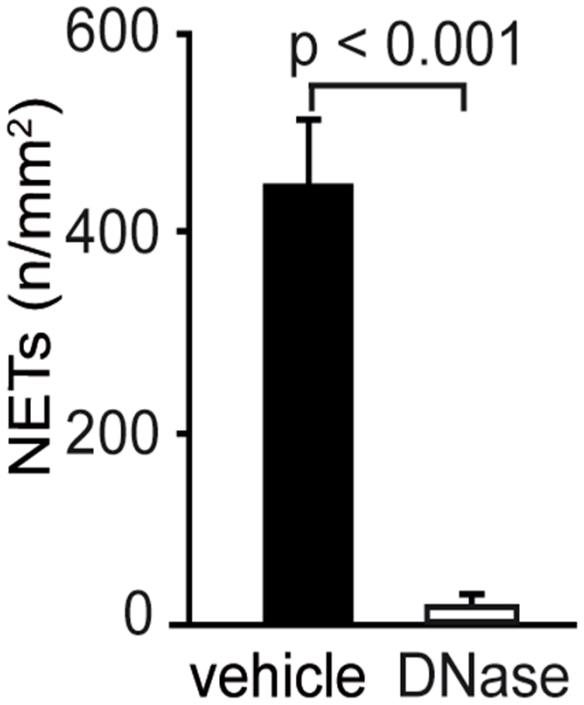
Stark et al., Blood 2016

NETTING NEUTROPHILS ARE ESSENTIAL FOR PROPAGATION OF DVT



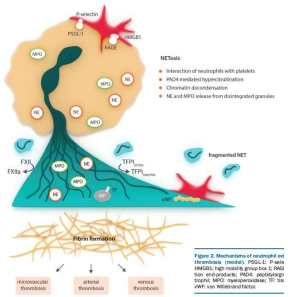


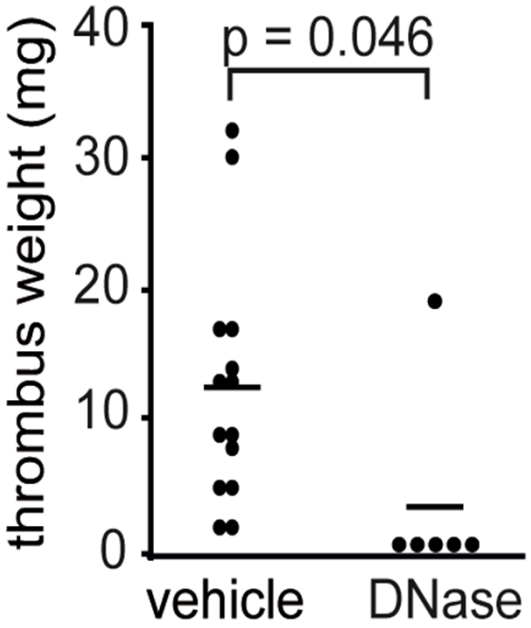


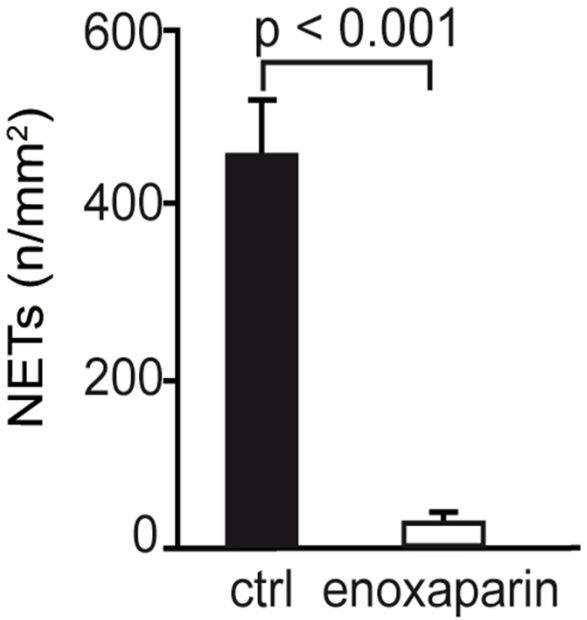


Von Brühl & Stark et al. JEM 2012

NETTING NEUTROPHILS ARE ESSENTIAL FOR PROPAGATION OF DVT

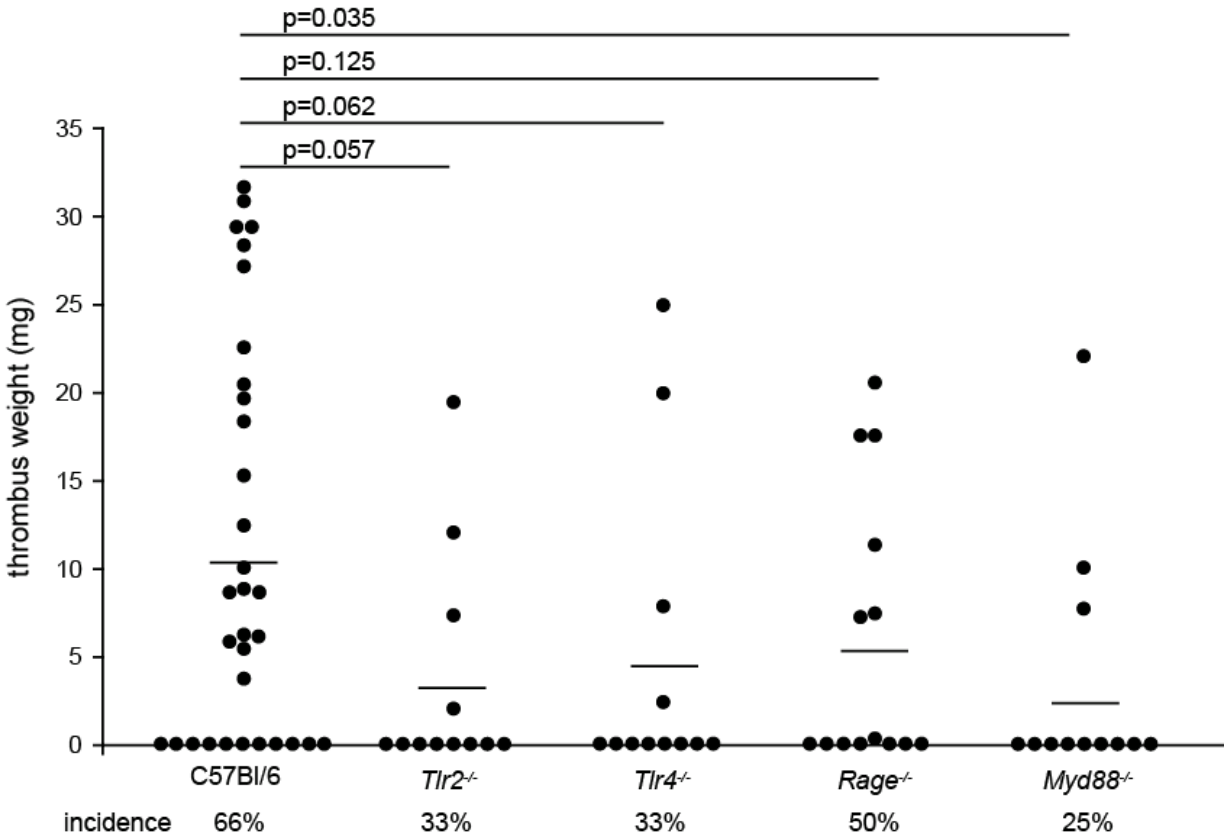






Von Brühl & Stark et al. JEM 2012

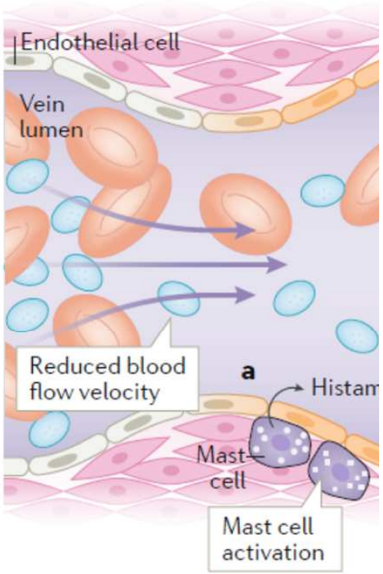
Redundant pattern recognition receptors mediate the effect of HMGB1 in venous thrombosis



Stark et al., Blood 2016

Inflammatory mechanisms of thrombosis

Aberrant activation of immunothrombosis in venous thrombosis

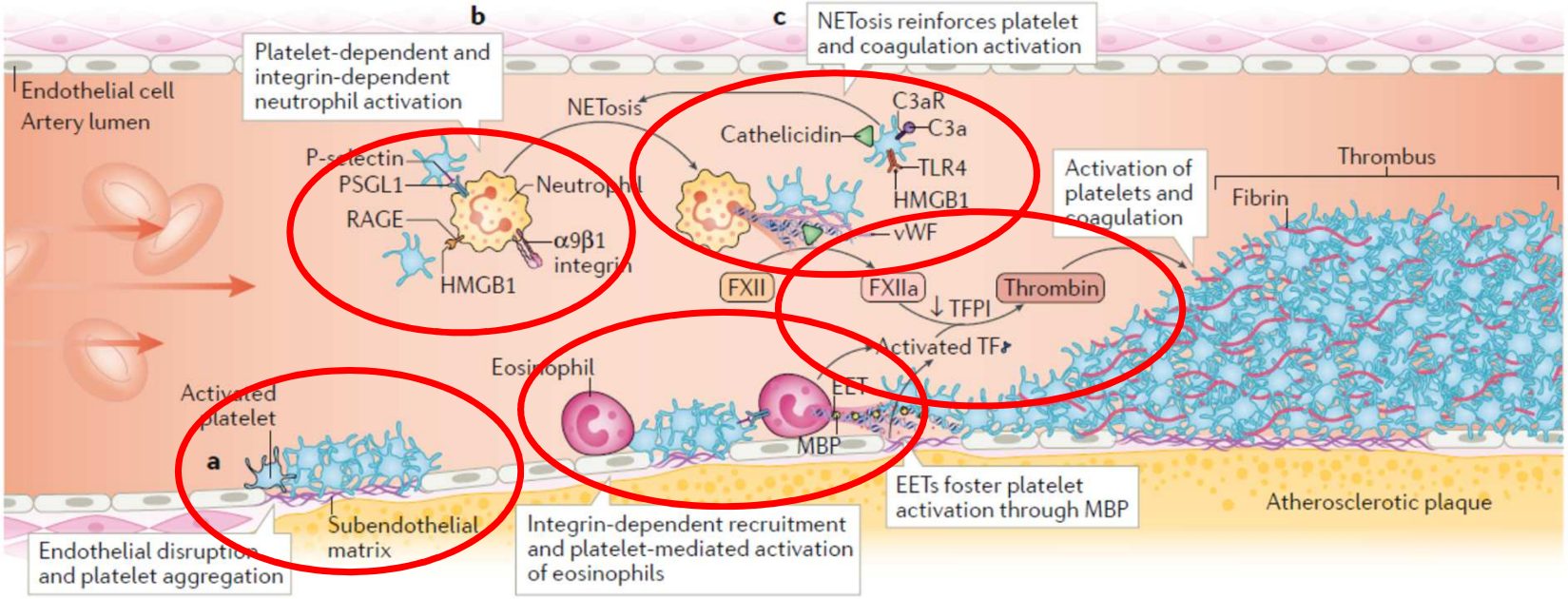


Blood flow reduction **Inflammation** **Thrombosis**

- **Venous blood flow reduction triggers a local immune response**
- **The interplay between innate immune cells and platelets triggers the activation of the coagulation cascade**

Stark & Massberg, *Nature Reviews Cardiology*, 2021

Inflammatory mechanisms of thrombosis
Innate immune cells stabilize arterial thrombi



Myocardial infarction

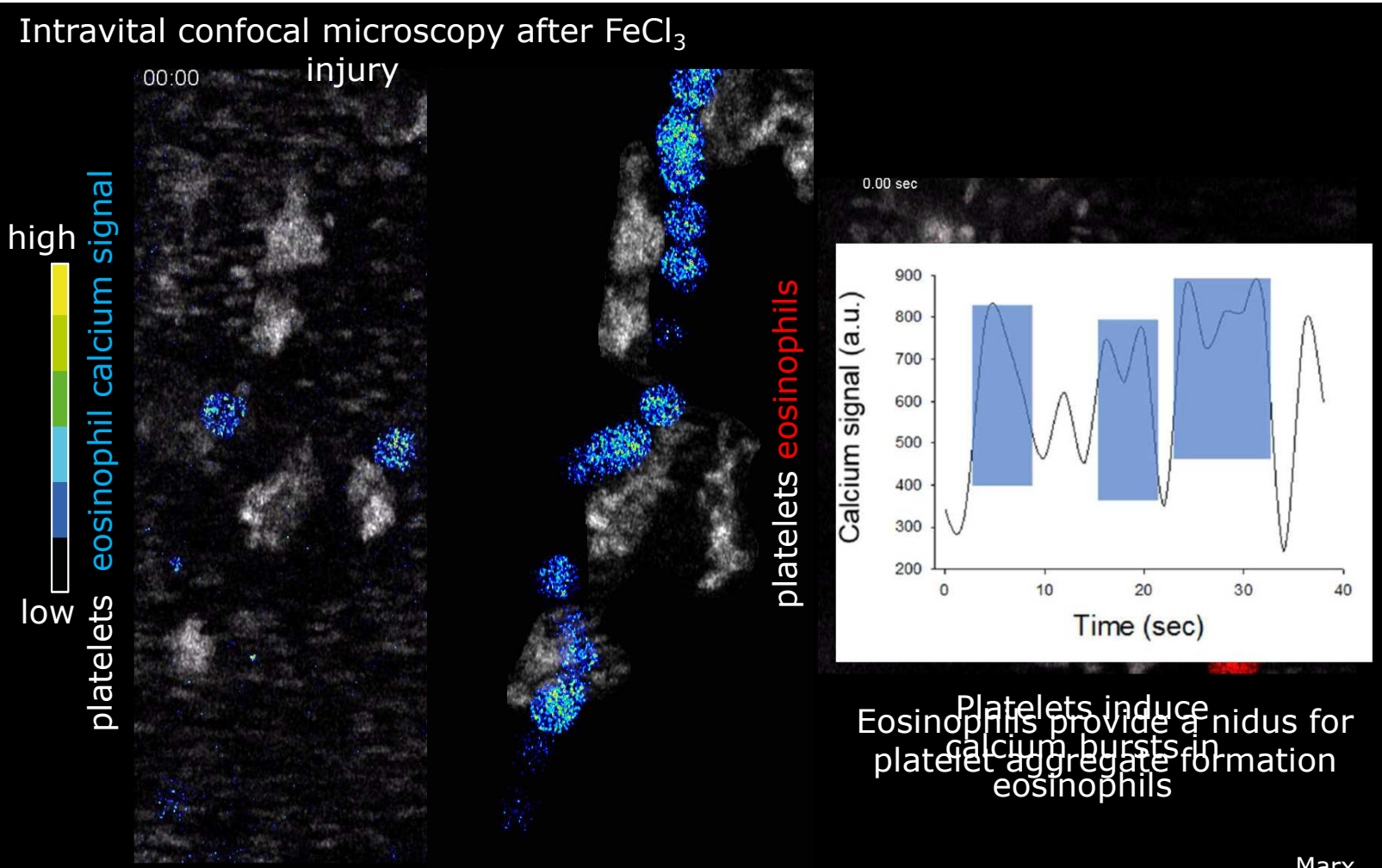
Stroke

Plaque rupture **Platelet activation** **Inflammation** **Thrombosis**

- **Plaque rupture triggers platelet adhesion and aggregation**
- **Thrombus stabilization by myeloid leukocytes**

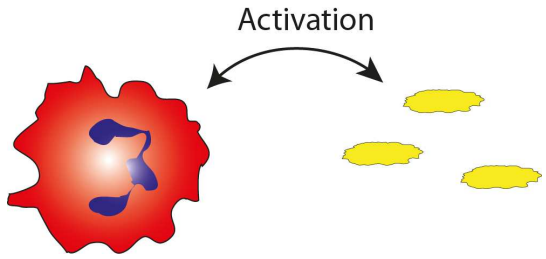
Stark & Massberg, *Nature Reviews Cardiology*, 2021

Eosinophils interact with platelets resulting in mutual activation

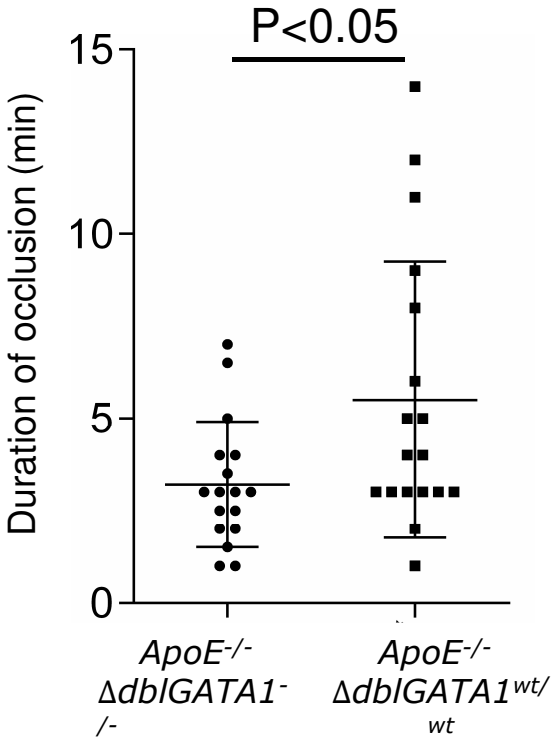


Marx, …..,Stark, Blood 2019

Eosinophils promote platelet accumulation in thrombosis

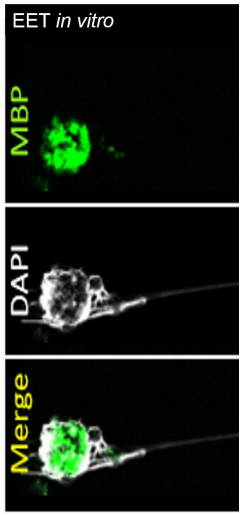


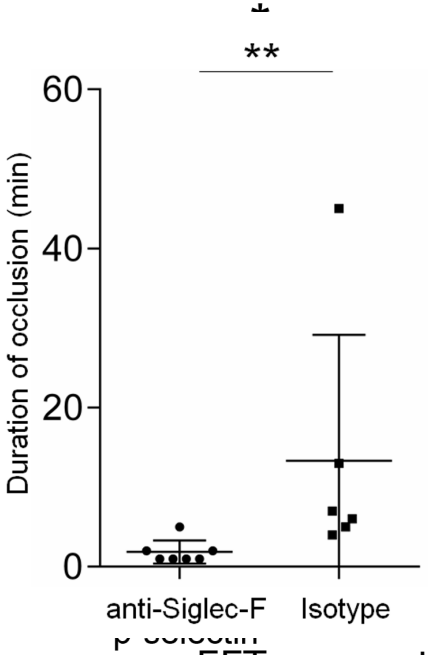
Intravital microscopy of platelet accumulation in the carotid artery after $FeCl_3$ injury

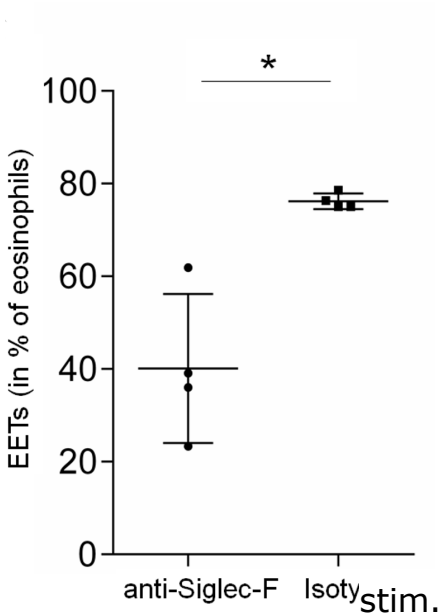


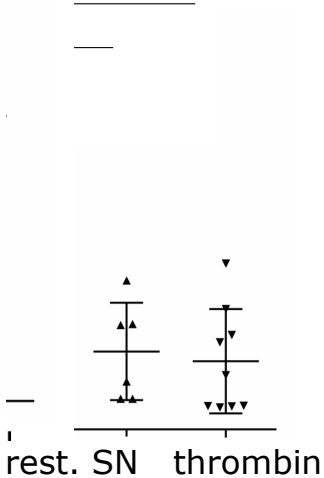
Marx, …..,Stark, Blood 2019

Platelets induce Eosinophils extracellular trap (EET) formation









EETs are present in human arterial thrombi

EETs promote arterial thrombosis *in vivo*

Platelets induce EETs through P-selectin and Siglec-8

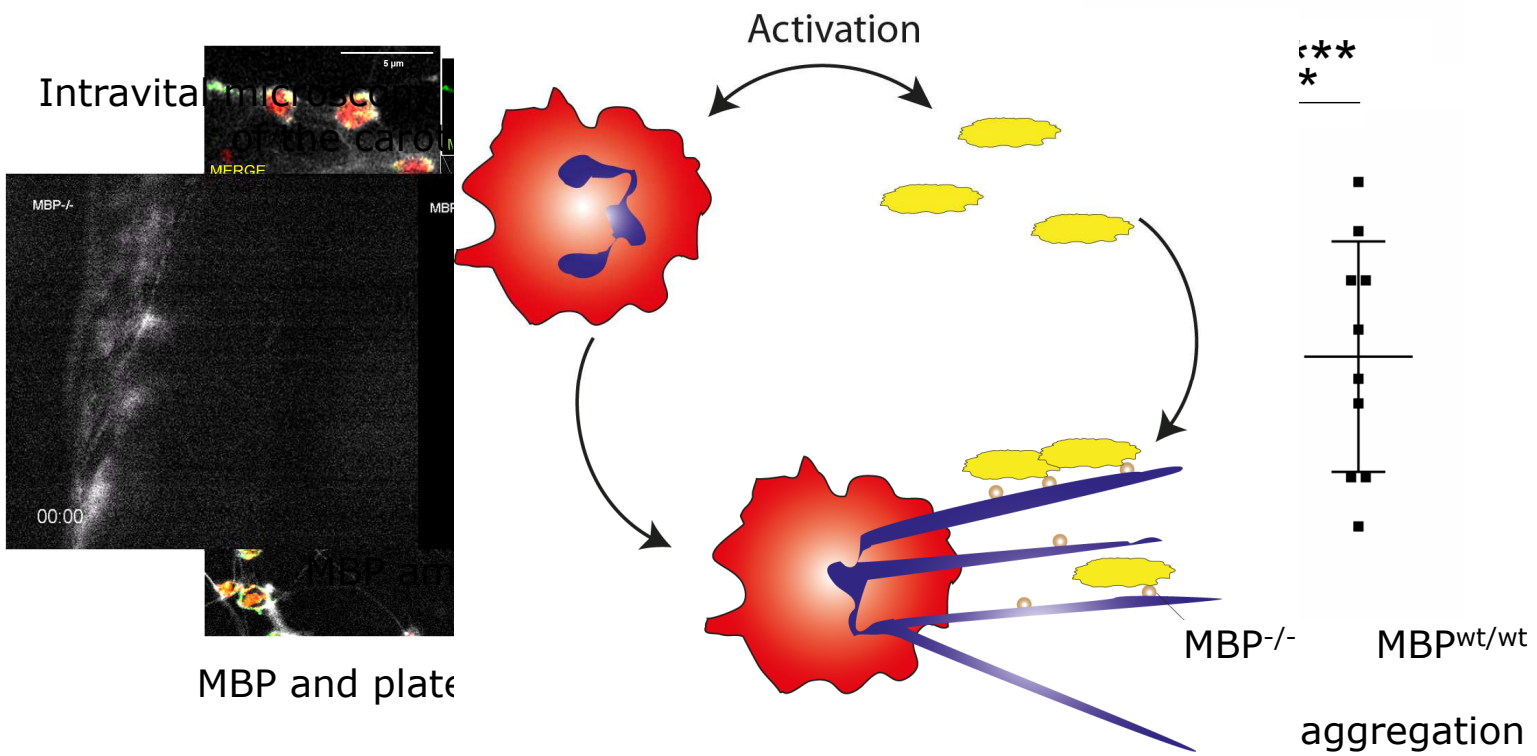
Direct contacts between activated platelets and eosinophils induce EETs

Marx, …..,Stark, Blood 2019

Diapositive 29

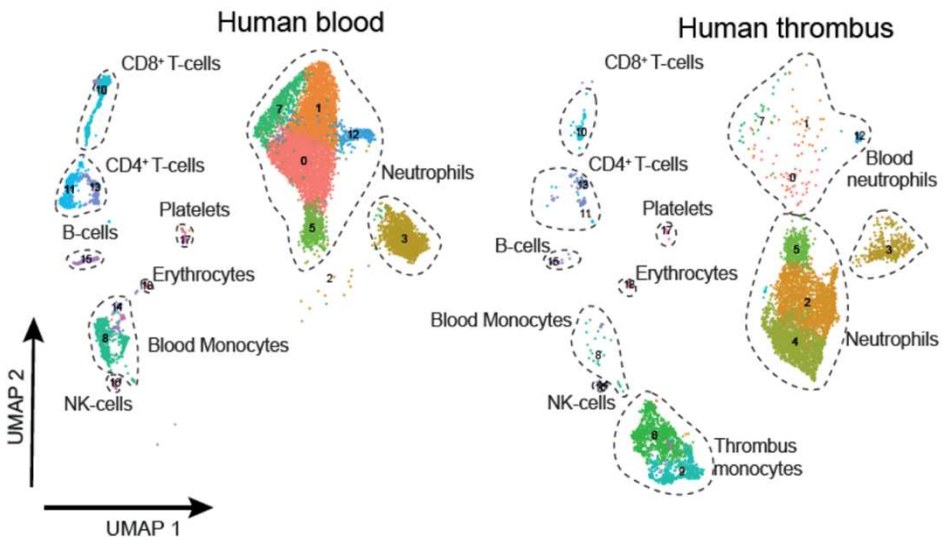
KS1 Konstantin Stark; 15/02/2019

Platelets induce Eosinophils extracellular trap (EET) formation

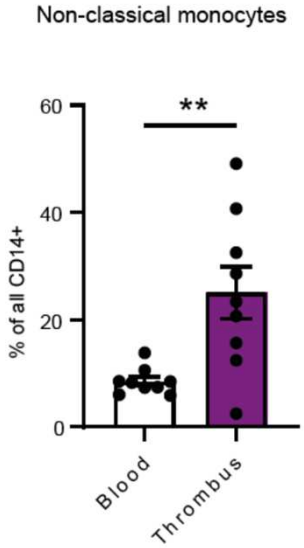


Marx, …..,Stark, Blood 2019

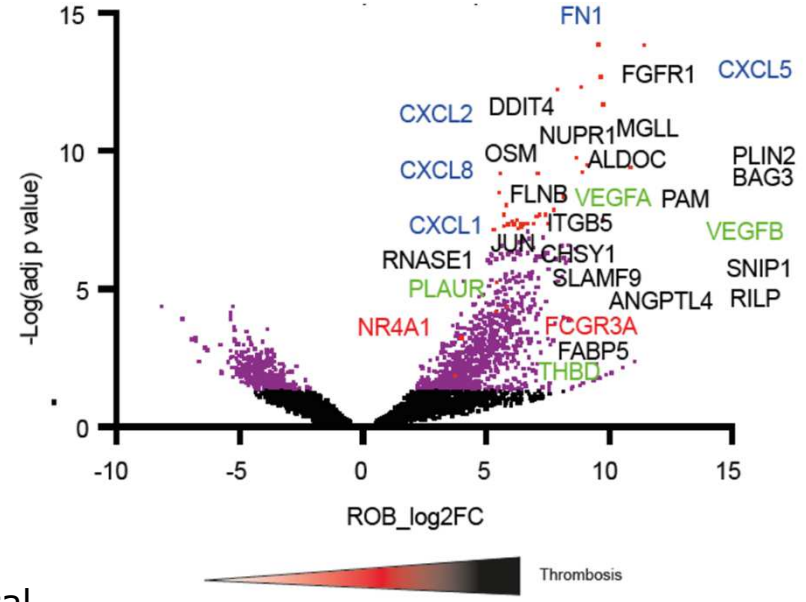
Functional insights into immunothrombosis by scRNAseq of human stroke thrombi



scRNAseq of human stroke thrombi and peripheral blood



Enrichment of non-classical monocytes in thrombi



Upregulated genes in thrombus monocytes compared to blood monocytes

Pekayvaz, …..,Stark, unpublished

Thank you!

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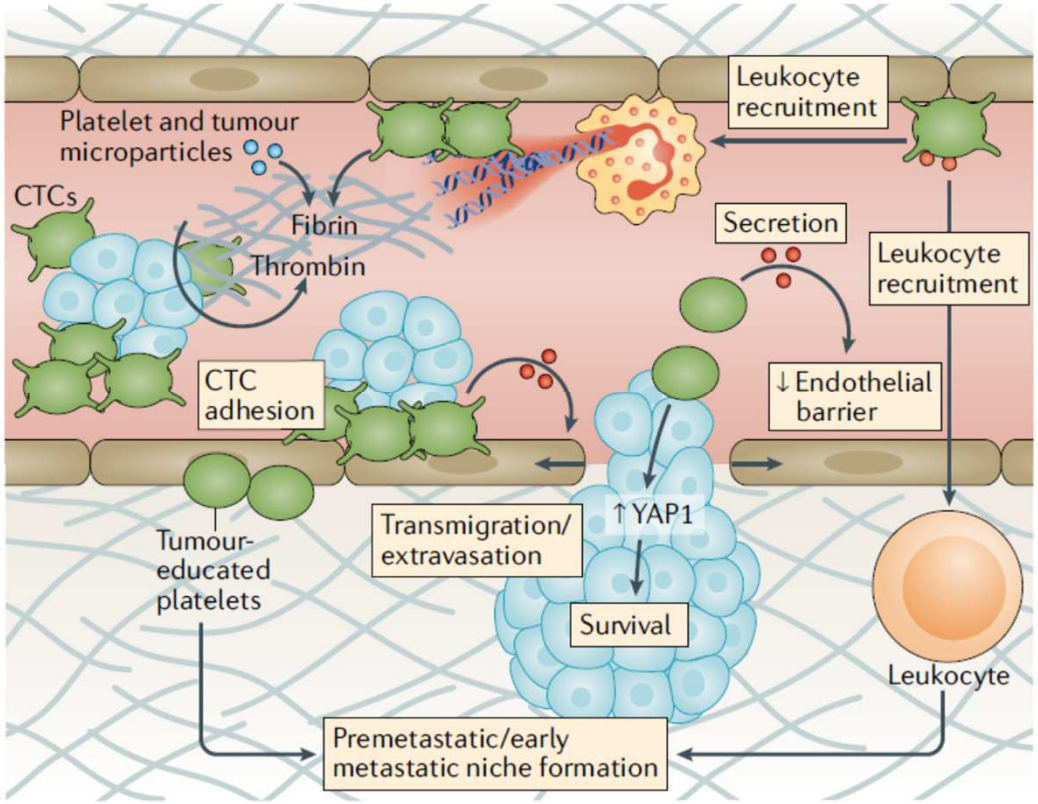
Cancer affects the interplay between thrombosis and inflammation

Differential effects on tumor growth and metastasis formation



- The interplay of platelets, myeloid leukocytes and coagultion is invovled in metastasis formation
- Cancer associated coagulopathy causes thrombotic complications
- Aberrant activation of immunothrombosis affects cancer blood supply

Gärtner & Massberg, *Nature Reviews Immunology*, 2019