Role of Hemolysis

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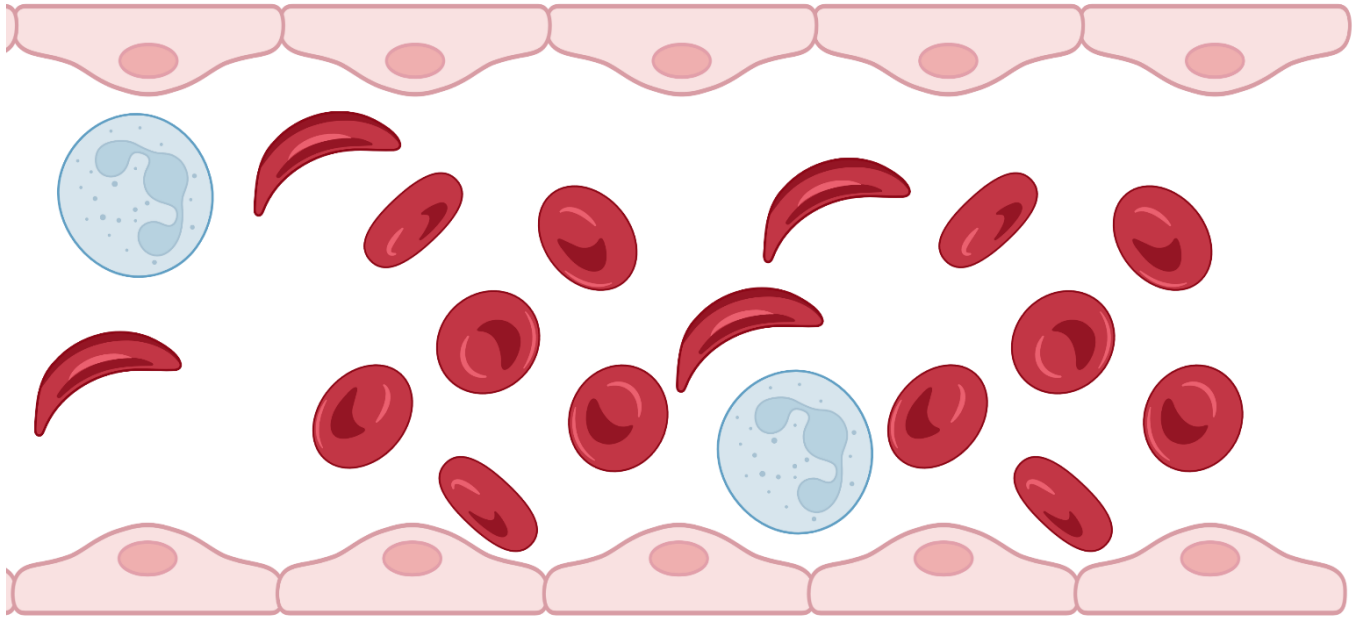
Disclosure

- Advisory Board: Novartis, Vifor
- Research Grant: Agios, Celgene-Bristol

Different Sickle Red Cells Species and Compartments Contribute to SCD Clinical Manifestations

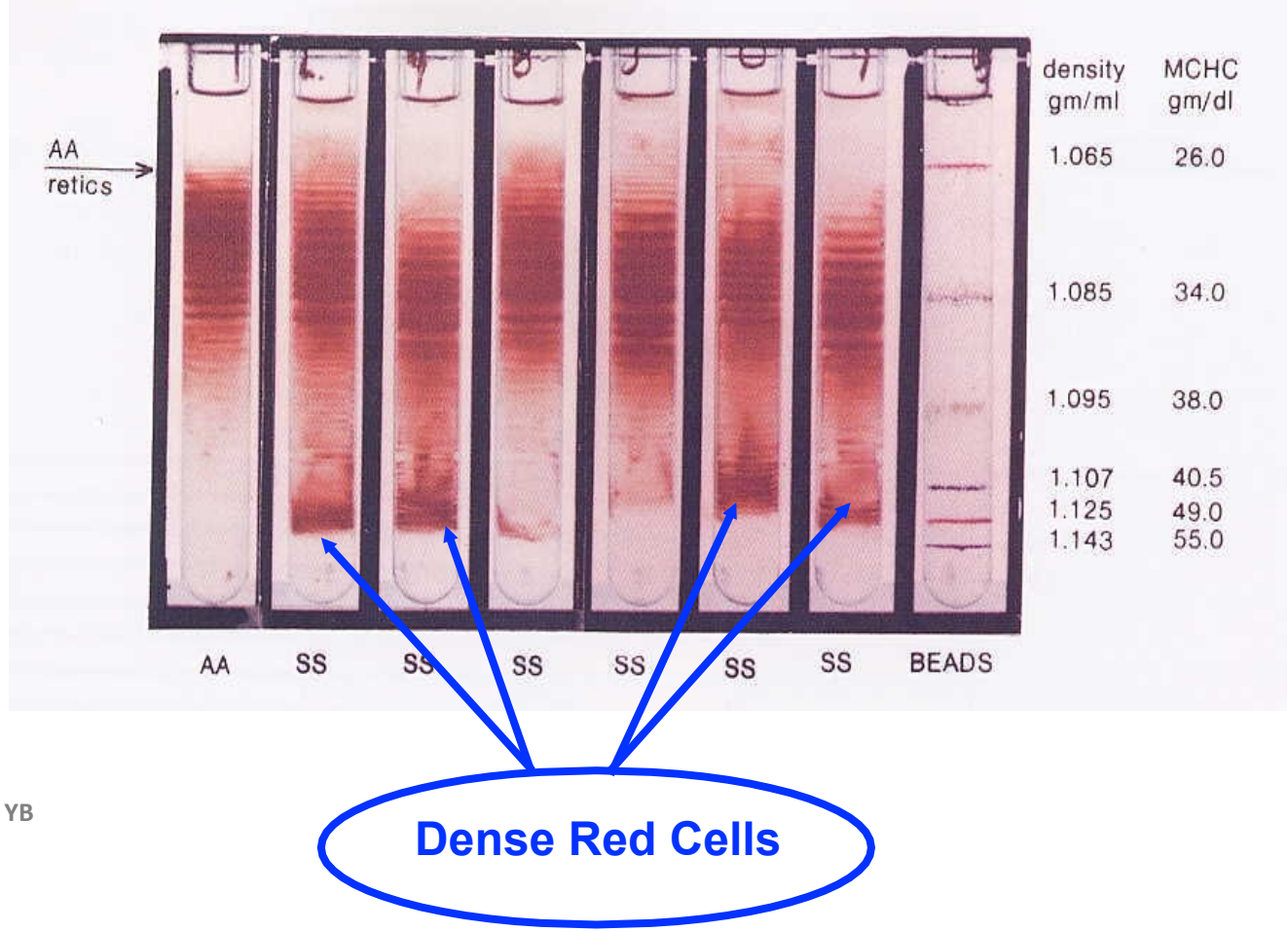


Dense RBCs

Erythroid microparticles

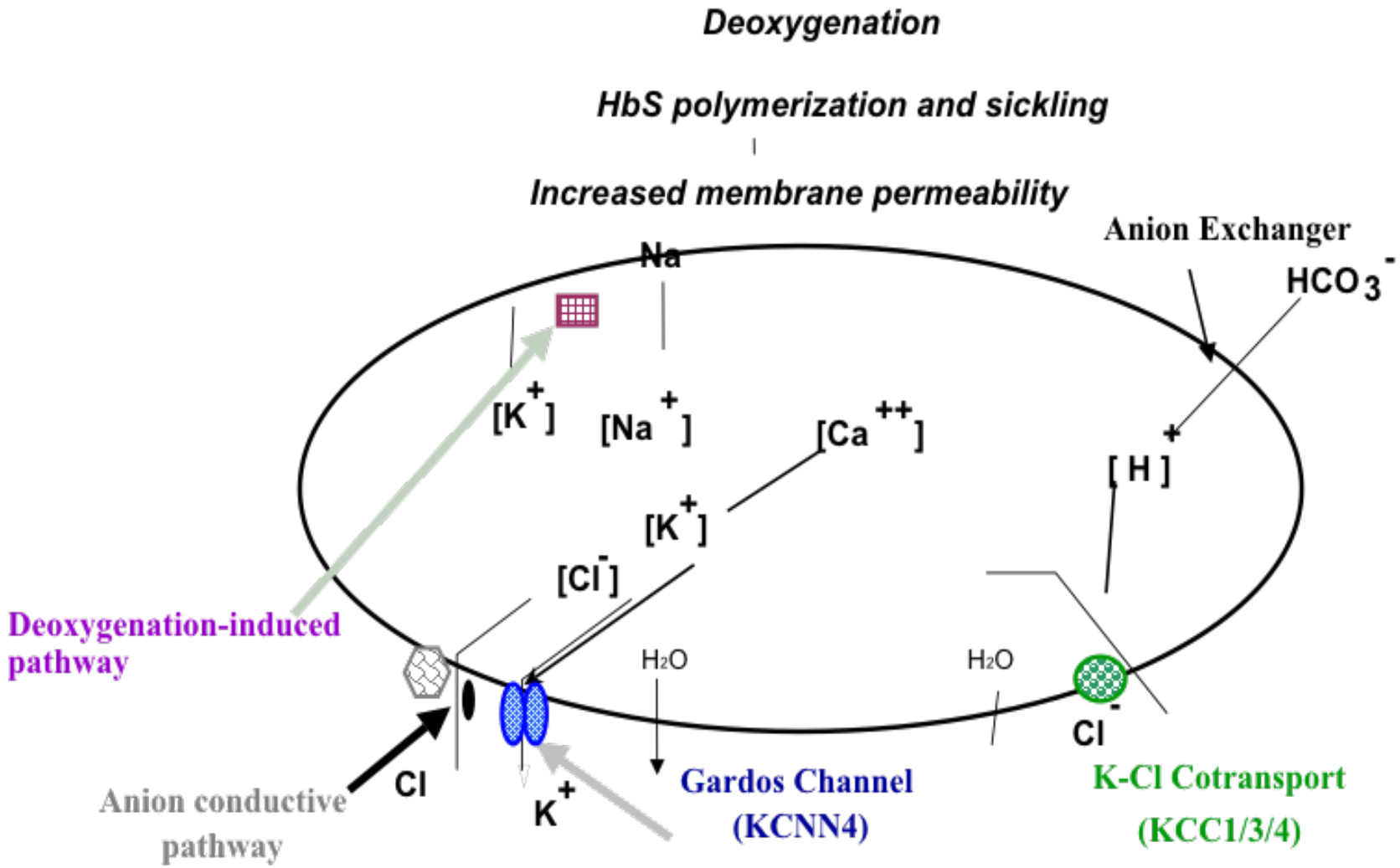
Free heme & free Hb

Heterogeneity of Cell Hb Concentration in Sickle cell Disease plays a central role in both acute and chronic sickle cell related manifestations

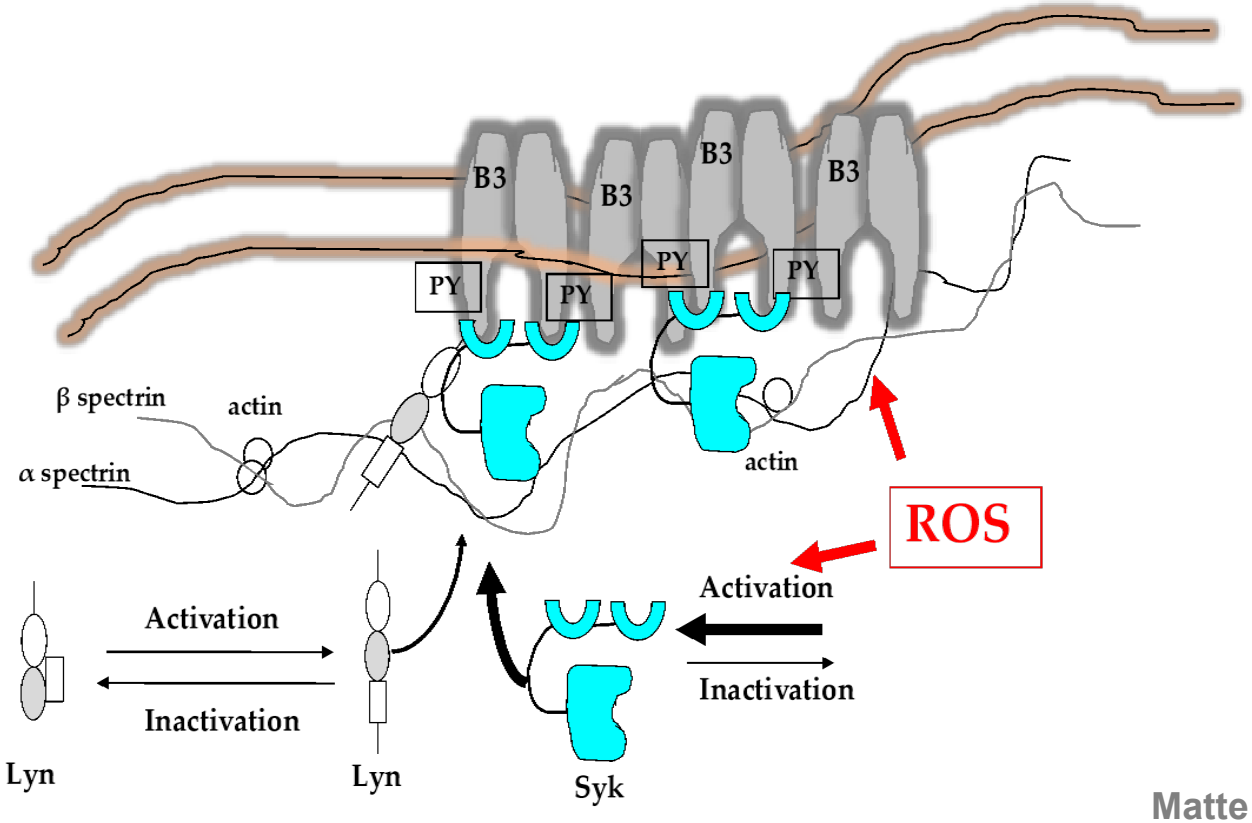


Modified from YB

Abnormal activation of membrane ion systems promotes red cell dehydration



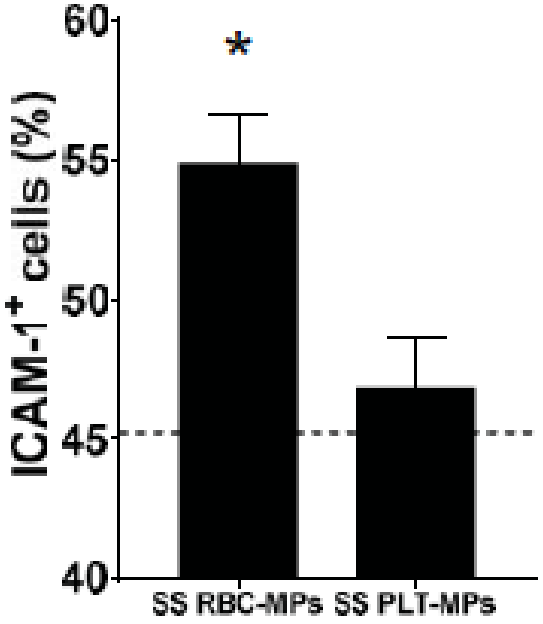
Oxidation Induced Over-activation of Syk Contributes to Sickle Red Cell Membrane Instability -> Erythroid Microparticles



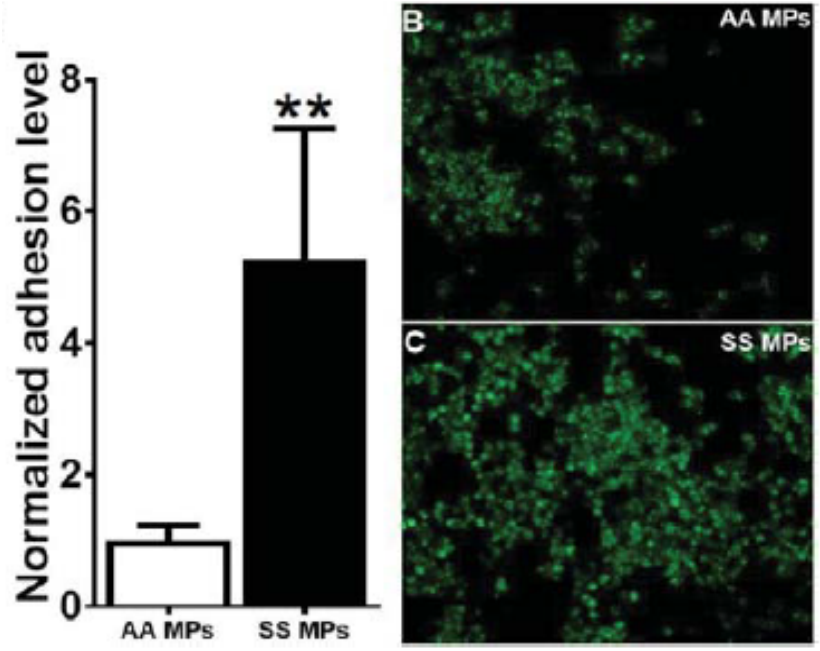
RBCs exposed to oxidation or Sickle RBCs

Matte A et al Antioxidants 2021; Noomuna P et al. Br J Haematol doi: 10.1111/bjh.16671, 2020; Garnier Y et al Blood 10.1182/blood.2020004853, 2020

In SCD, The Release of Erythroid Microparticles Is Increased In SS Patients And Participates To Neutrophil Cell Adhesion



E-MPs increase endothelial ICAM-1 expression

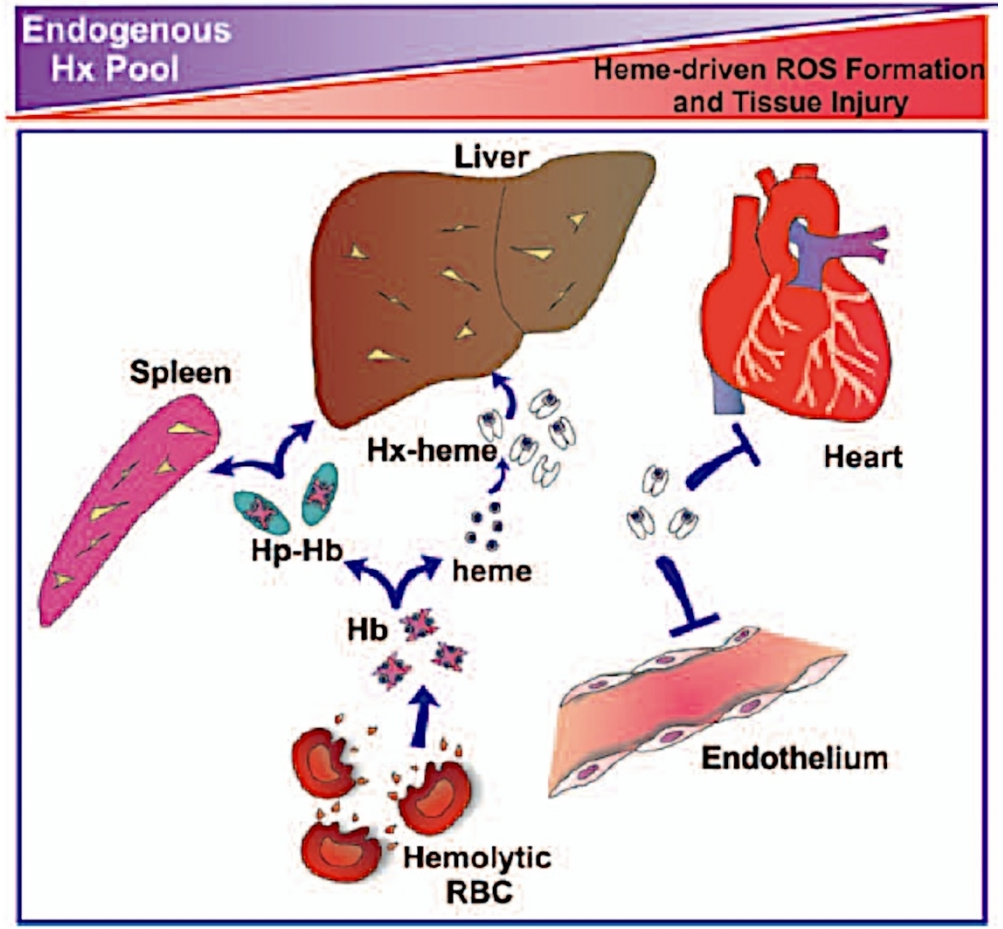


E-MPs favor neutrophil adhesion via PS exposure

Garnier Y et al Blood 10.1182/blood.2020004853, 2020

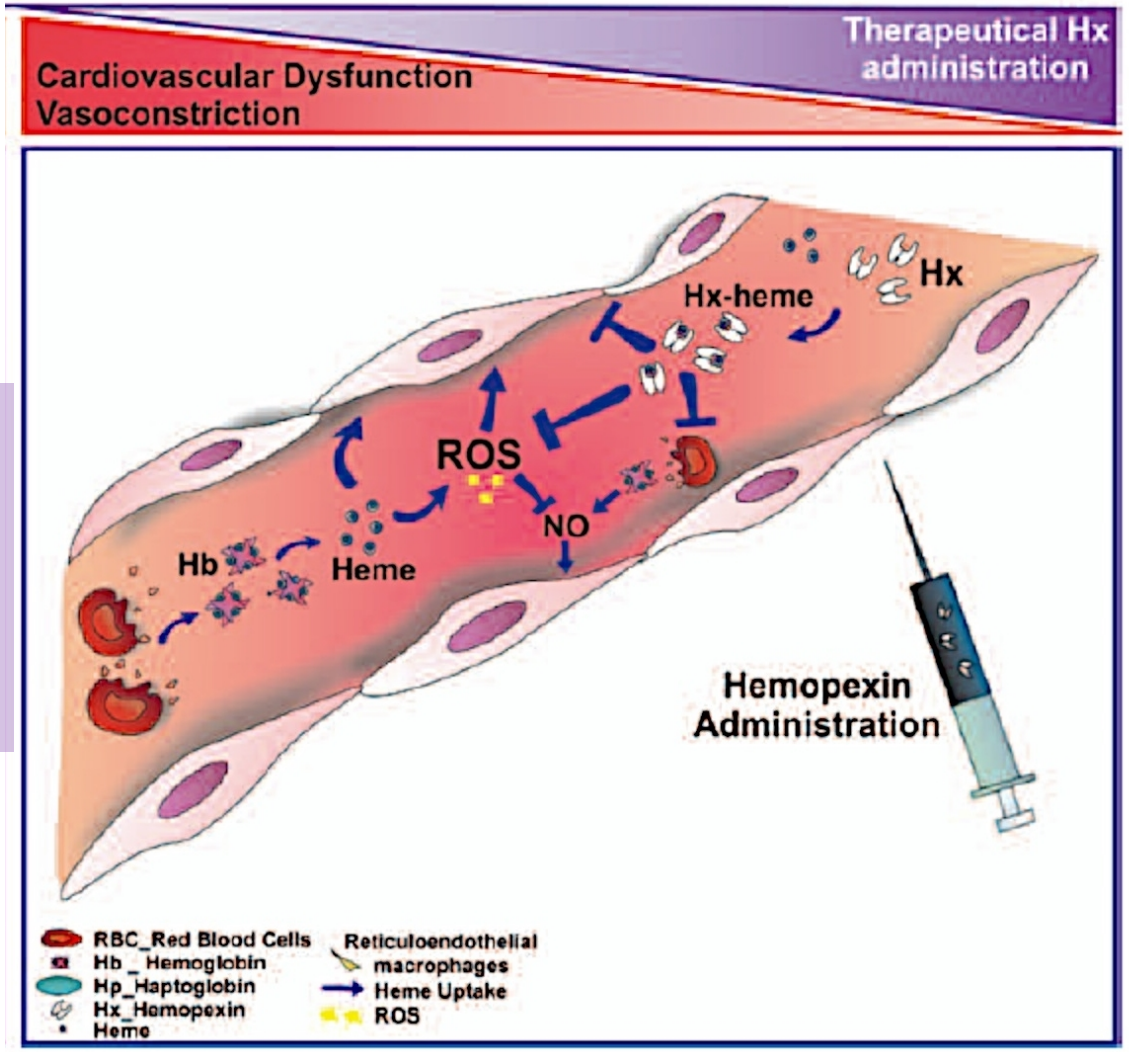
In SCD, Chronic Hemolysis Promotes Vascular Endothelial Activation

- In SCD, chronic hemolytic anemia: **2/3 extravascular hemolysis and 1/3 intravascular hemolysis;**
- ⇧ **plasma free Hb and free heme** is due to the saturation of physiological binding proteins **haptoglobin and hemopexin;**
- Increased **plasmatic pro-oxidant** environment **promotes vascular endothelial activation.**



Vinchi F et al. Circulation 127: 1317, 2013;
Vercellotti GM et al Frontiers in Pharm 5:1; 2014;
Hebbel RP Am J Hematol 86: 123, 2011

NCT04285827. Recombinant Hemopexin-> phase 1 multicenter open label single dose study in pts with SCD (safety and PK) (CLS Behring)



Vinchi F et al. Circulation 127: 1317-29, 2013

SCD, Hemolysis And Nitric Oxide

- In sickle cell disease nitric oxide (NO) metabolites can be decreased during vaso-occlusive episodes associated with severe pain or with acute chest syndrome

- Decrease exhaled NO has been reported in sickle cell patients, suggesting a role for NO in the pathogenesis of sickle pulmonary complications

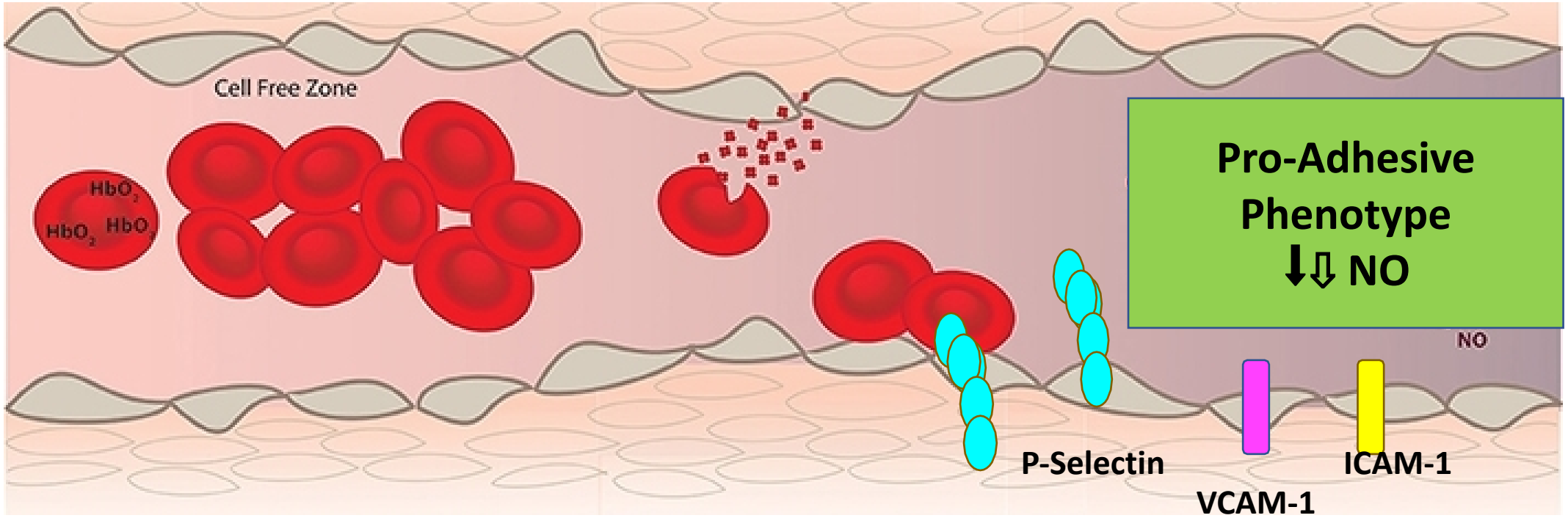
Br. J. Haematol. 91: 834, 1995; Acad. Emerg. Med 3: 1098, 1996; Queshe M et al Blood [abstract] 90: 2571, 2000

- Nitric oxide (NO) is a potent vasodilator and inhibitor of vascular remodeling.

- In SCD, the NO relative deficiency has a detrimental effect on the multistep cascade events that are involved in leukocyte recruitment, including rapid P-selectin dependent leukocyte rolling and ICAM-1, VCAM-1 expression.

Am. J. Physiol. 267: G562, 1994; J. Clin. Invest. 101: 2497, 1998; N.Engl. J. Med. 328:399, 1993;
Helms CC et al Frontiers Physiol 2018

In SCD, vascular endothelial cells reacts to hemolysis as pro-inflammatory stress



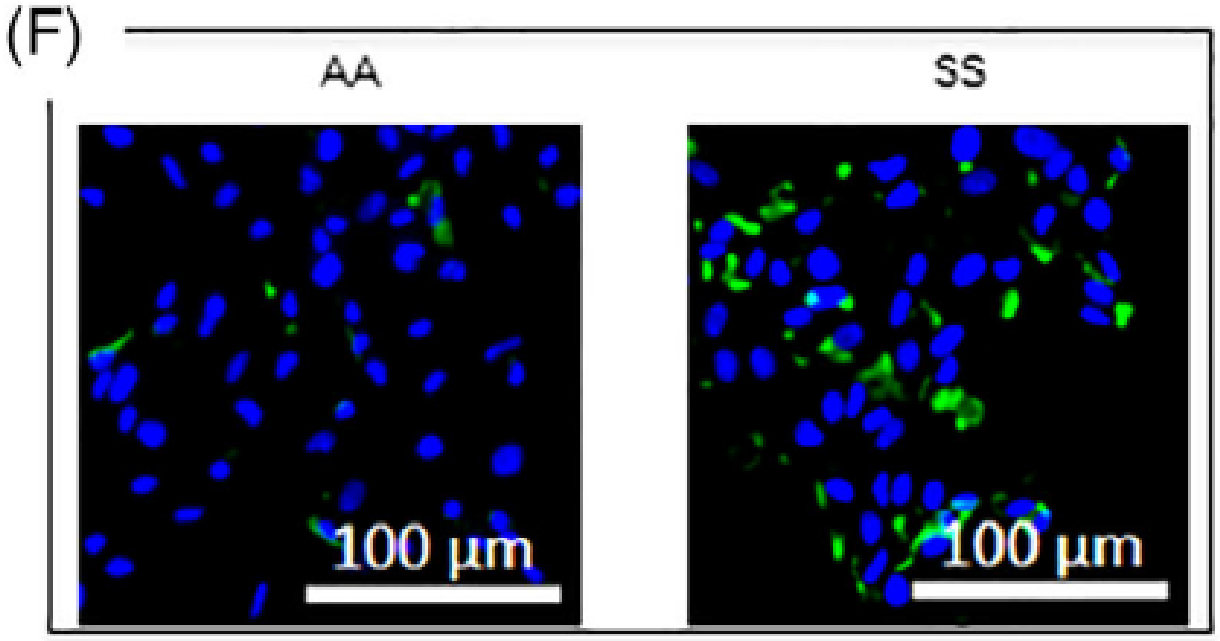
Activation of alternative complement pathway (AP) characterizes SCD

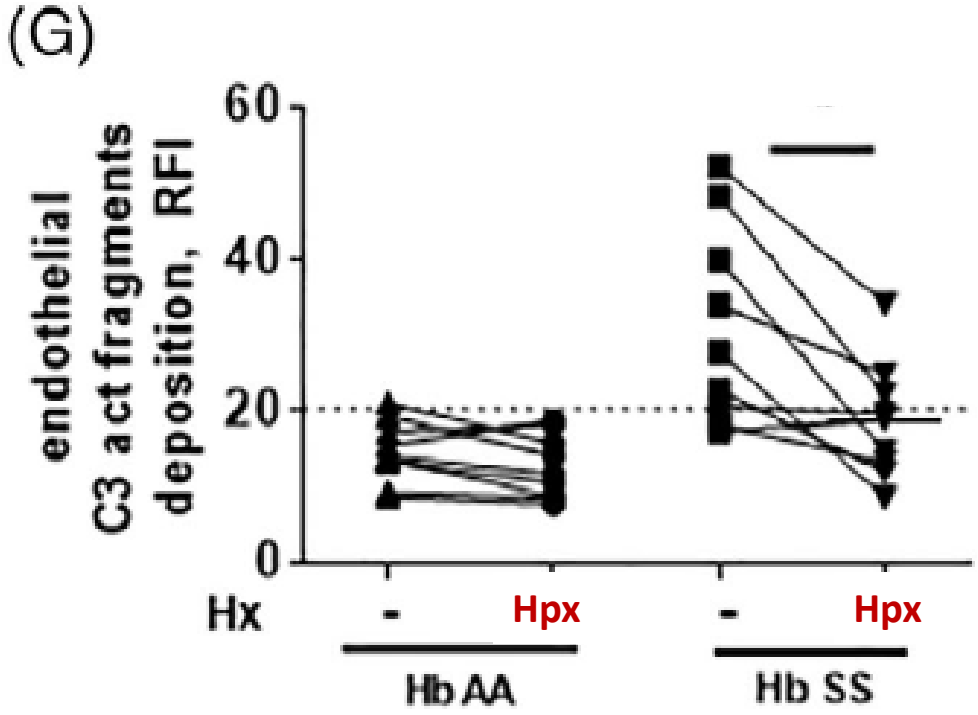
- Free heme and heme loaded microparticles might activate complement

- Chronic inflammation and sickle red cells exposing phosphatidylserine (PS) contribute to AP activation:
  - ⇧ plasma C5a
  - C3 split-fragments bound to the membrane of sickle red cells

Lombardi E et al Haematologica 104: 919, 2019; El Nemer W et al Haematologica 104: 857, 2019; Merle NS et al JCI Insight 3: 2018; Varelas C et al J Blood Med 12: 177, 2021

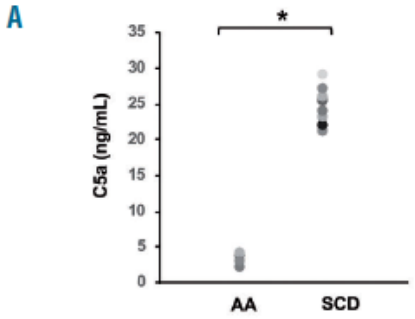
C5b9 complement deposition on ECs is increased in presence of sera from SCD patients, which is prevented by hemopexin

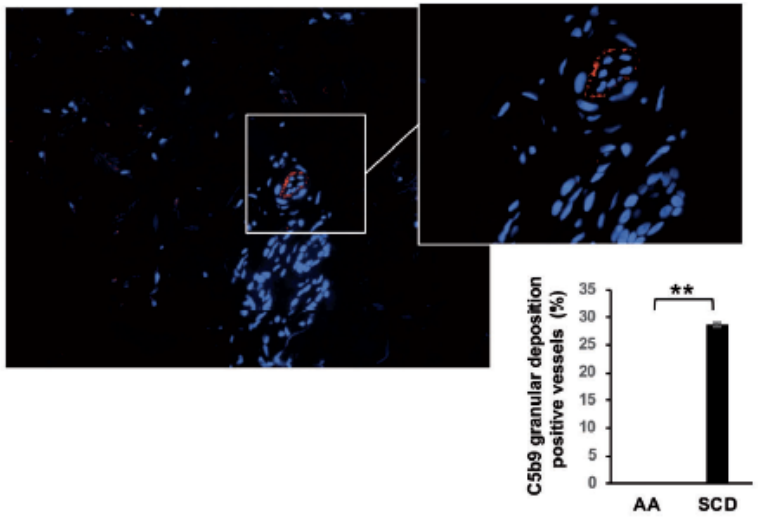


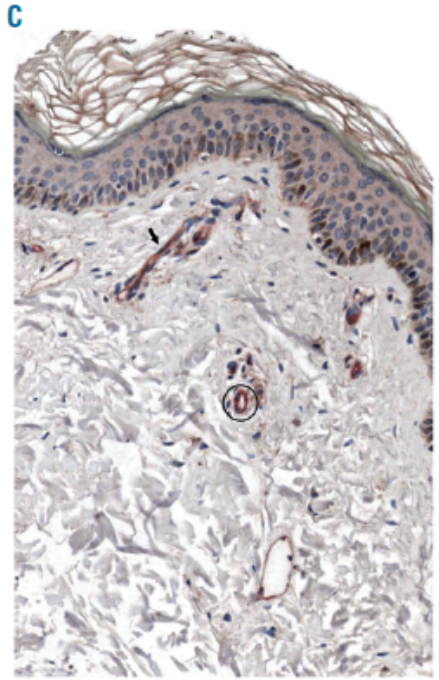


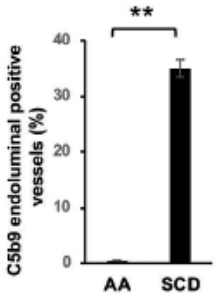
Roumenina LT et al Am J Hematol 95: 456, 2020

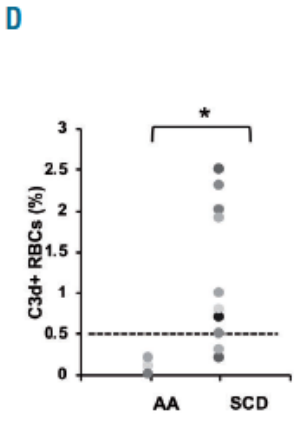
Microvascular focal granular deposition of C5b9 is present in skin of patients with SCD

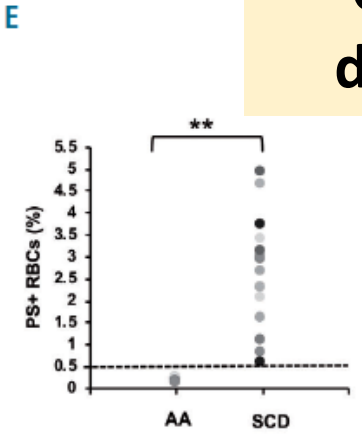








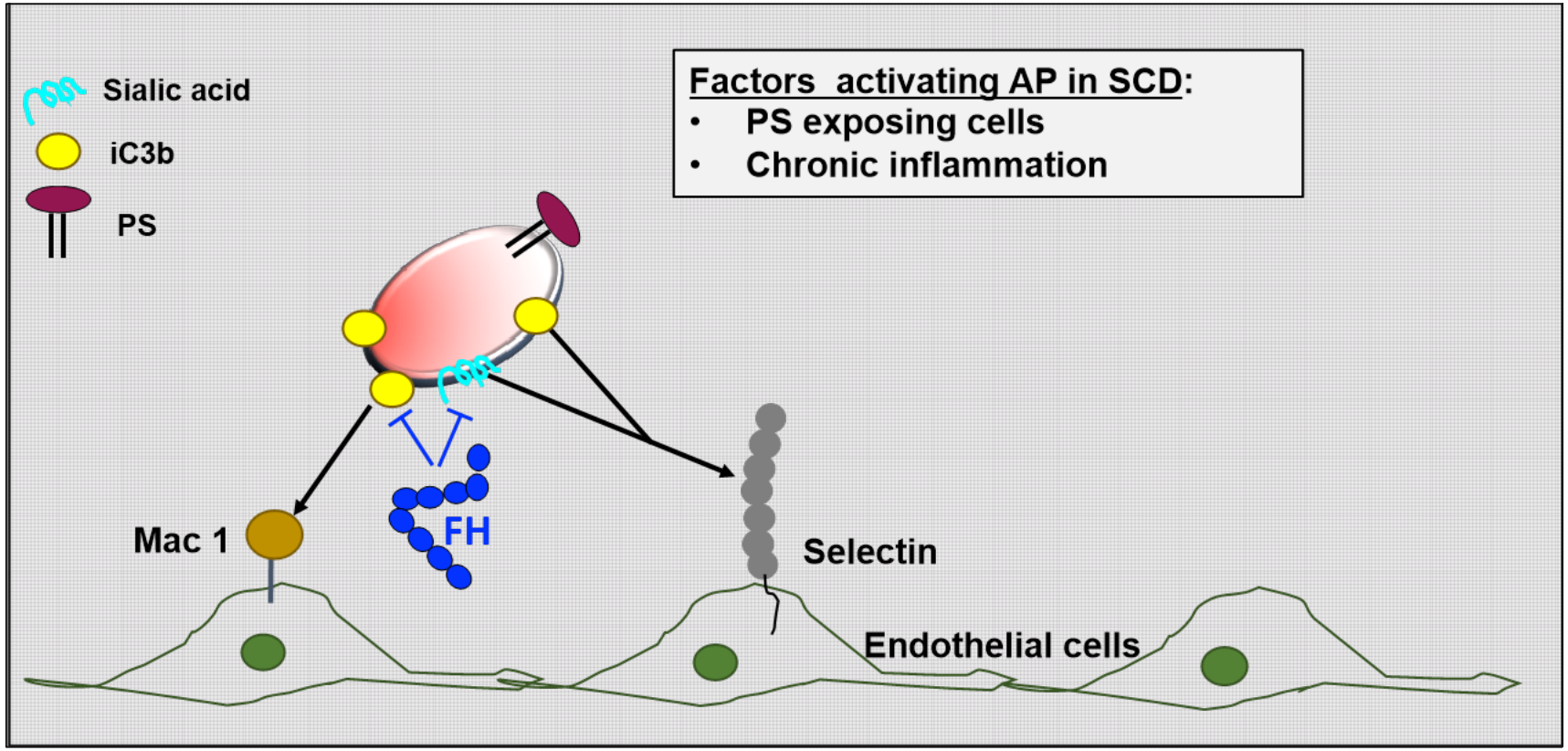




C3 split fragments deposition on RBCs

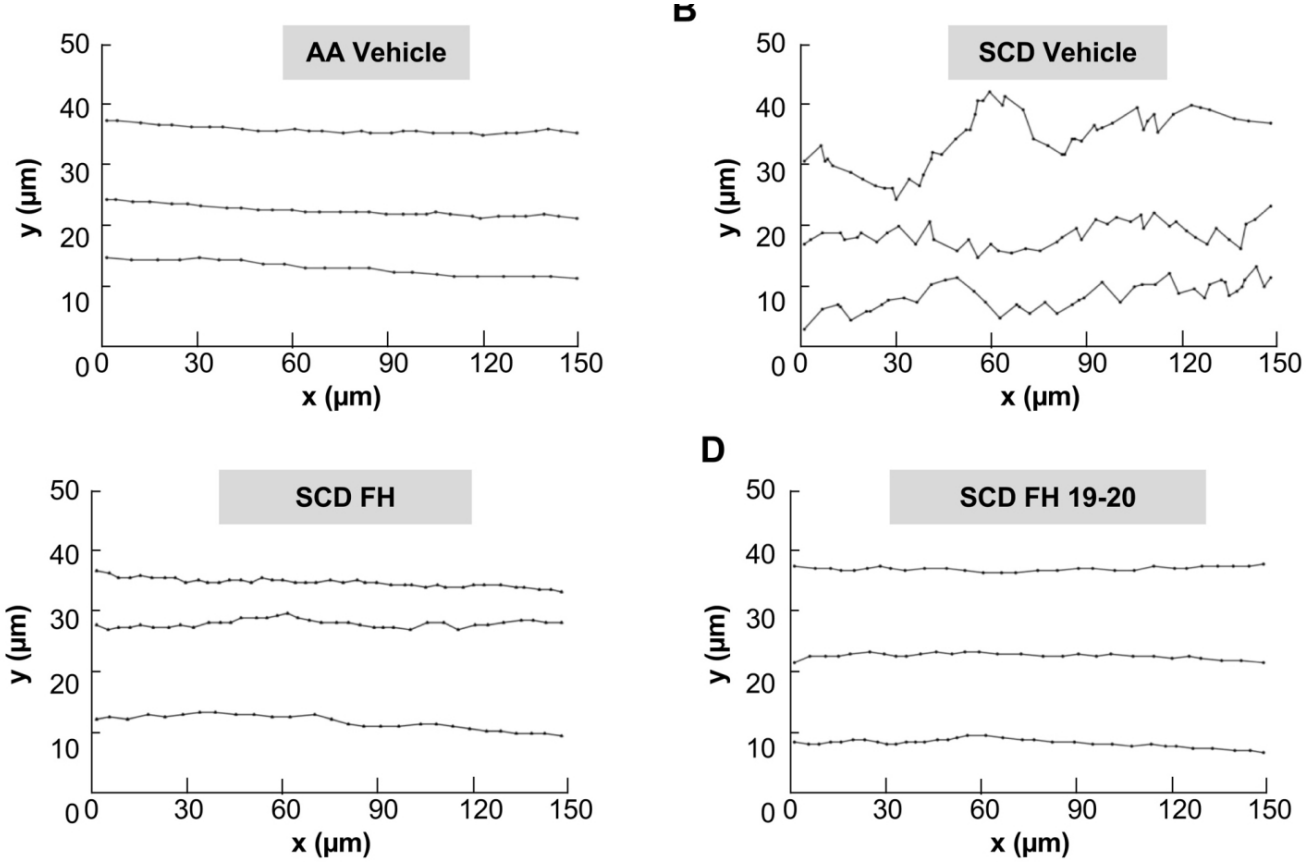
Lombardi E et al
Haematologica 104: 919, 2019

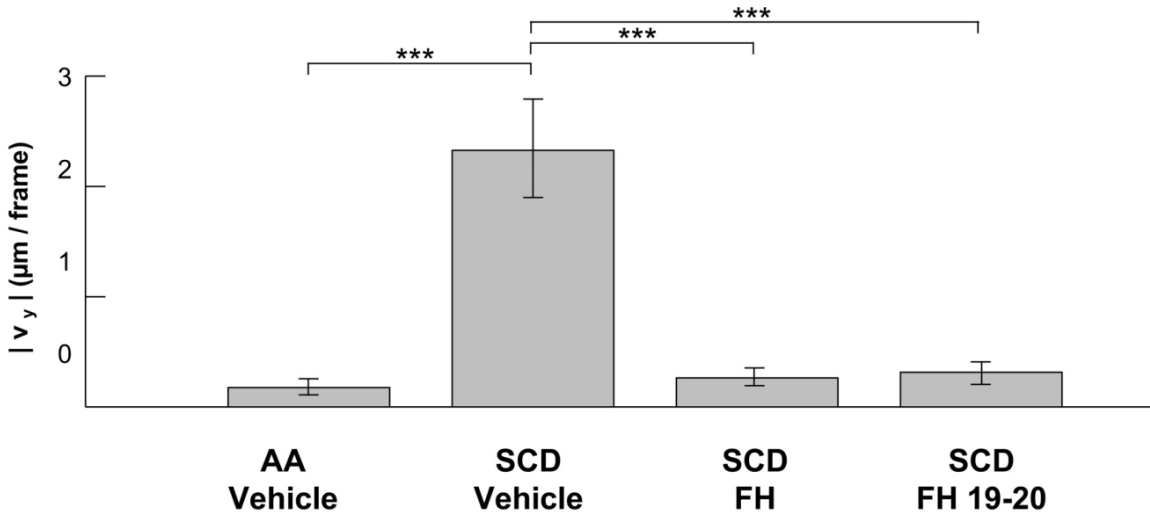
The cross-talk between sickle RBC and endothelium involved C3 split fragments Mac1 and P-selectin interactions



Lombardi E et al Haematologica 104: 919, 2019;

FH, A Regulator of AP, Normalizes the Stop-and-go Motion of Sickle RBCs, Reducing the Transit Time into microcirculation

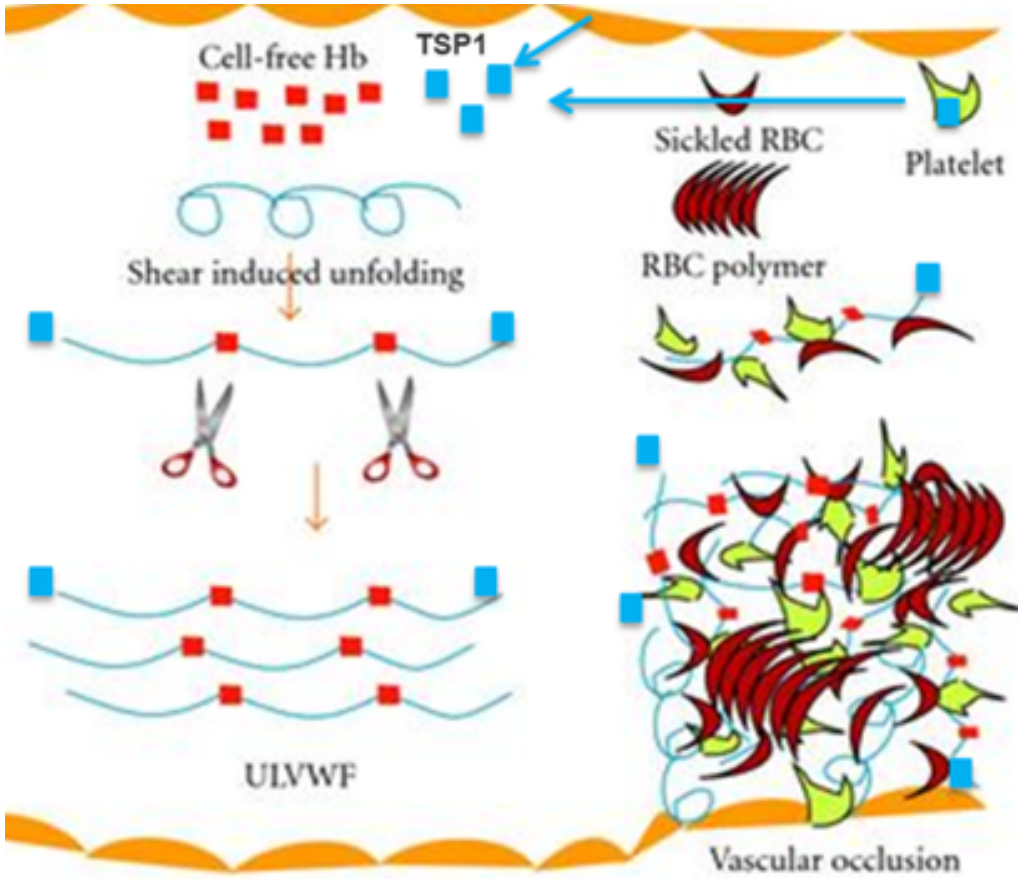




Lombardi E et al Haematologica 104: 919, 2019;

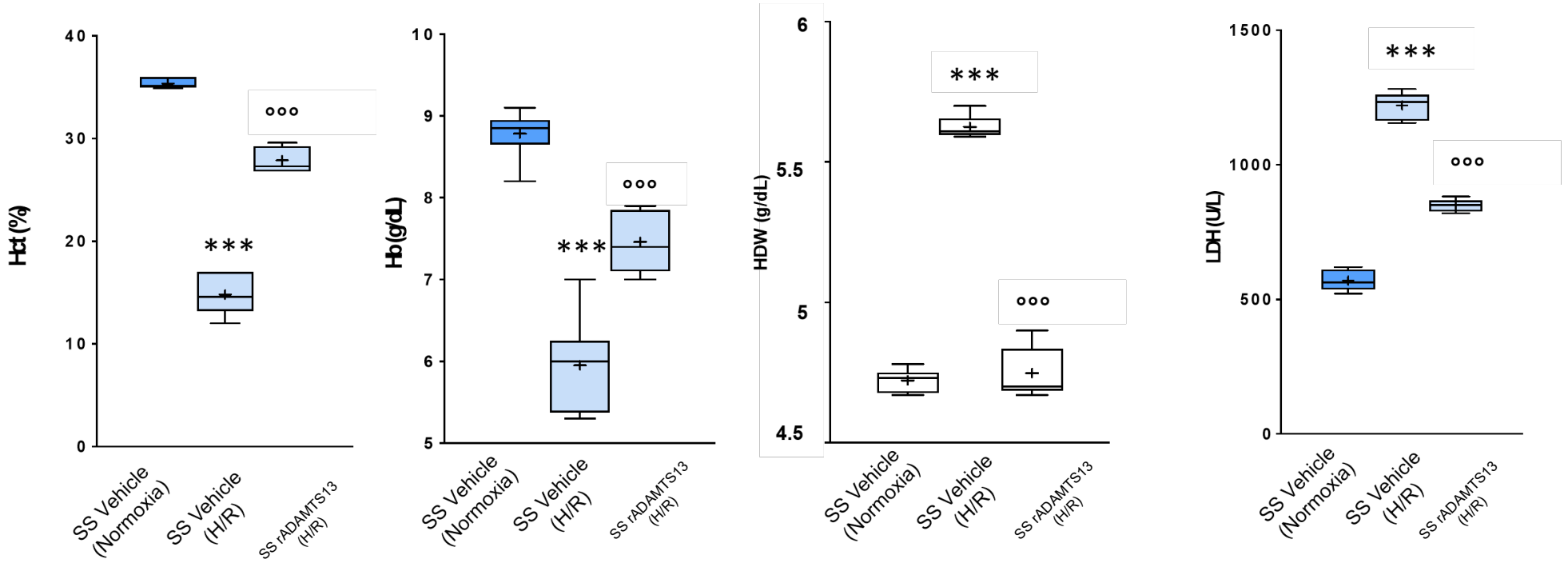
Sickle Cell Disease, Hemolysis and von Willebrand Factor (vWF)

- Increased level of vWF and ultra-large vWF multimers was observed in SCD patients.
- ADAMTS13 activity is inhibited by high plasma concentration of free Hb.
- The reduction of ADAMTS13-to-vWF antigen ratio suggests a possible role of hyper-adhesive ultra-large vWF in SCD vasculopathy.



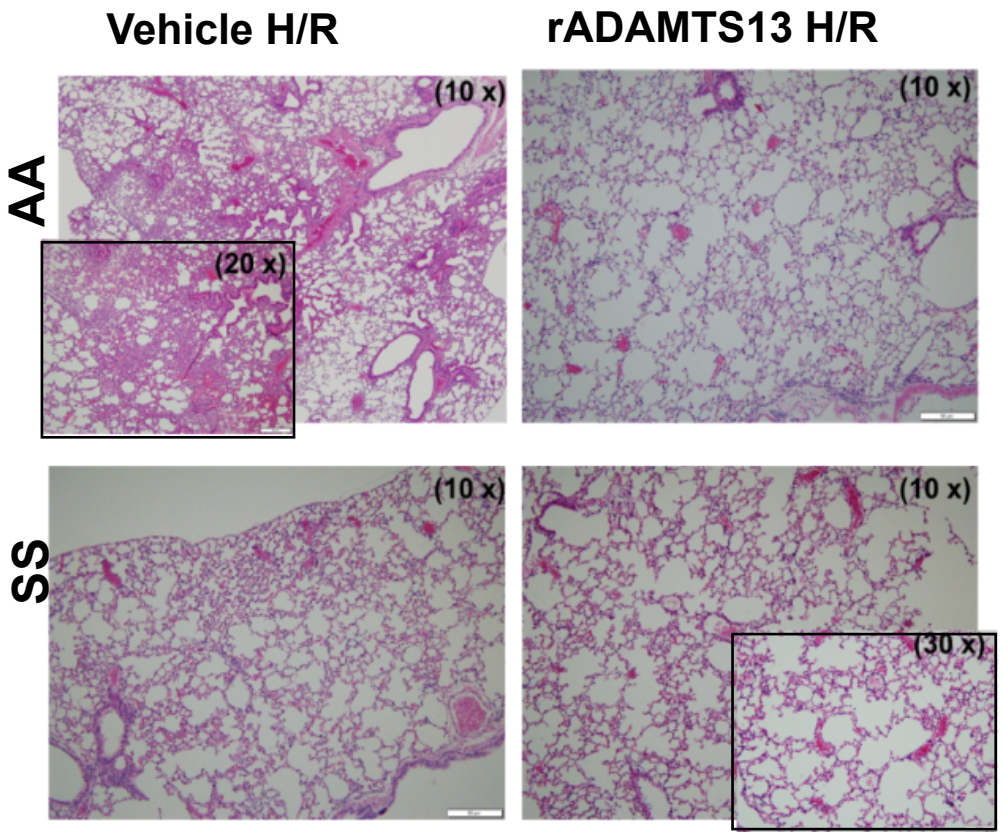
– Schnog JJB et al. Am J Hematol 2006; 81: 492-498; Zhou Z, et al. Anemia 2011; 1-5; Scully M et al. Blood 2017; 130: 2055-2063

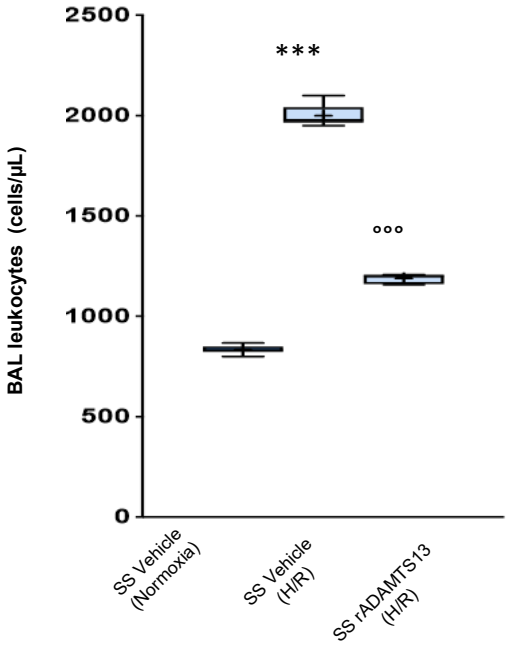
rADAMST13A protects humanized SCD mice against H/R stress Hemolysis

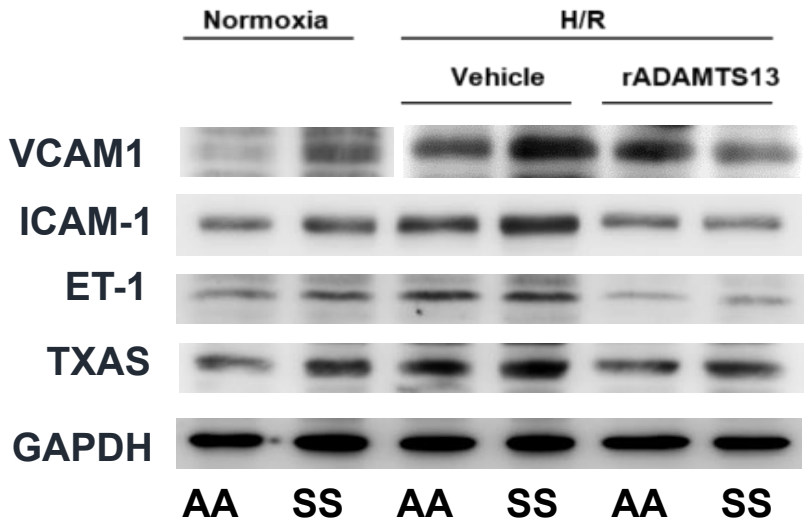


Rossato P et al. Haematologica in press 2022

rADAMST13 Reduces The H/R Induced Acute Lung Damage And Modulates Lung Inflammatory Vasculopathy







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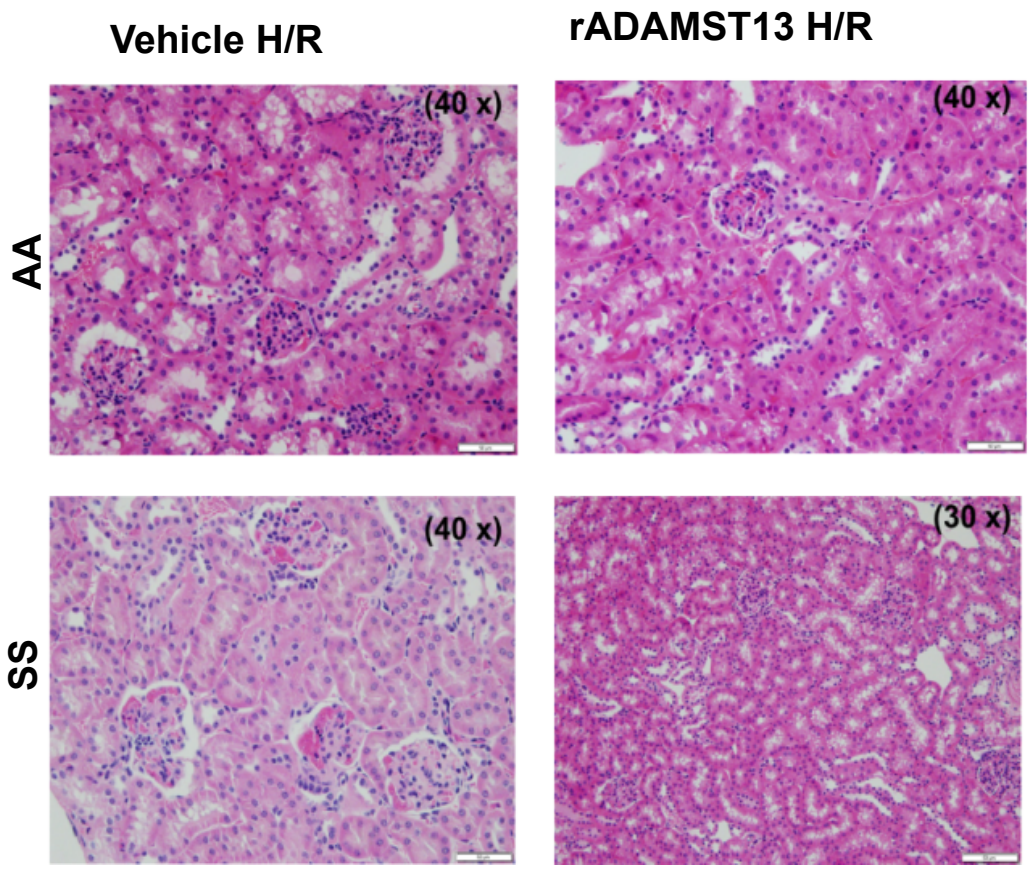
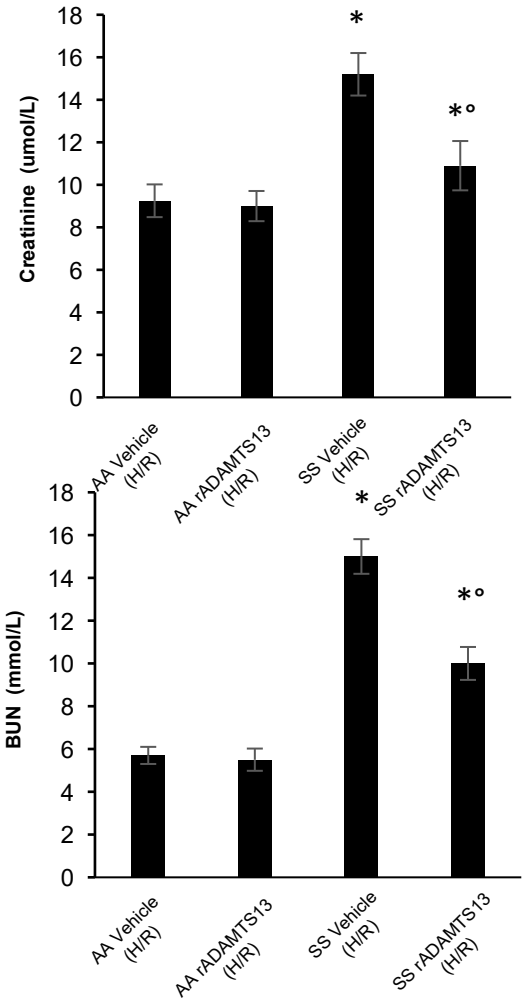
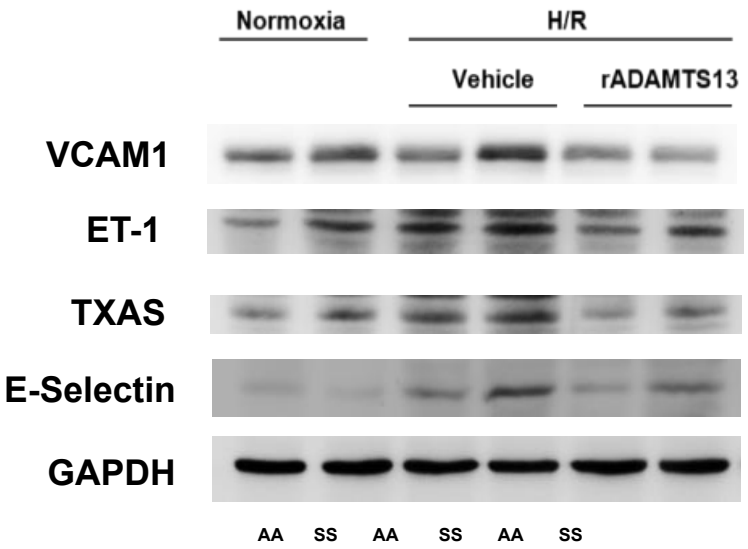
In SCD Mice, rADAMTS13 Prevents H/R Induced Kidney Injury and Modulates Local Inflammatory Response

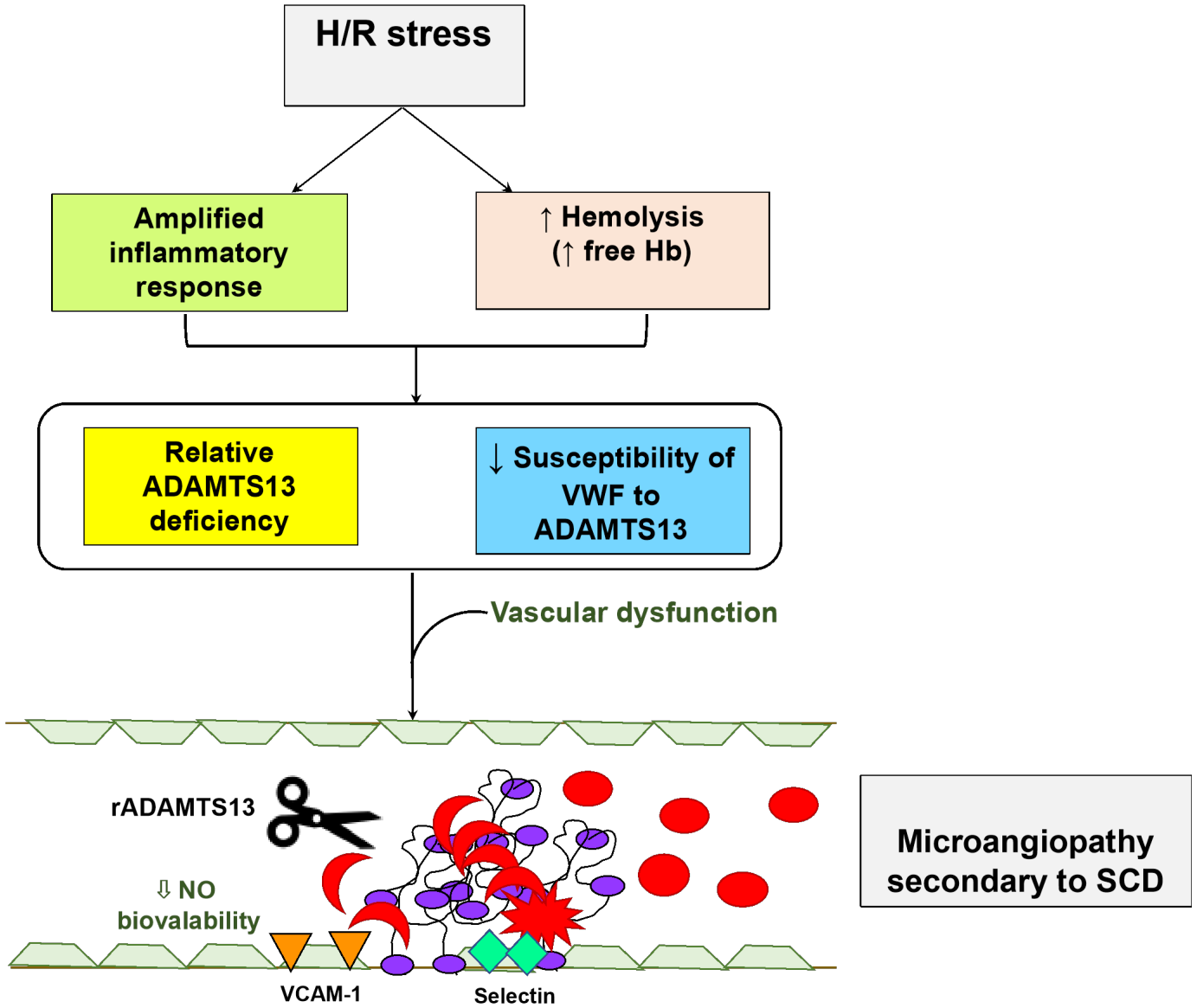






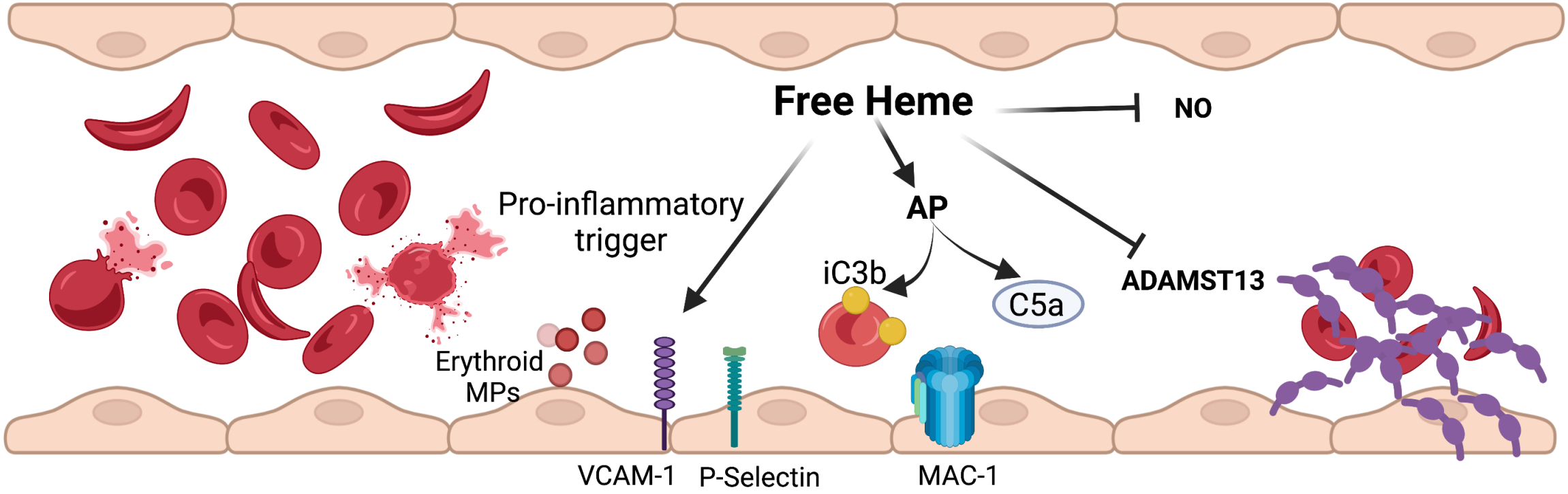
Rossato P et al. Haematologica in press 2022

NCT 03997760. Phase 1 randomized double-blind placebo controlled multicenter ascending single dose study **rADAMST13** in pts with SCD



Rossato P et al. Haematologica in press 2022;
Patwari P et al Blood 3118, 2021

Hemolysis (free heme) is central in the crossroad with inflammatory vasculopathy in SCD



Conclusions

- In SCD, chronic hemolysis and free heme/Hb generate a pro-inflammatory plasma environment
- Endothelium reacts to pro-inflammatory stress, changing into pro-adhesive phenotype
- Free heme contributes to severe endovascular oxidation-> endothelial damage
- Inflammatory vasculopathy plays a crucial role in the pathogenesis of sickle cell related organ damage.

Investigators

- **Matte A, Federti E, Siciliano A, Riccardi V- University of Verona, Verona; Italy**
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- **El Nemer W- INSERM- Marseille, France**
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