Role and mechanism of extracellular heme in organ injury in sickle cell disease

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Sickle Cell Foundation of Ghana

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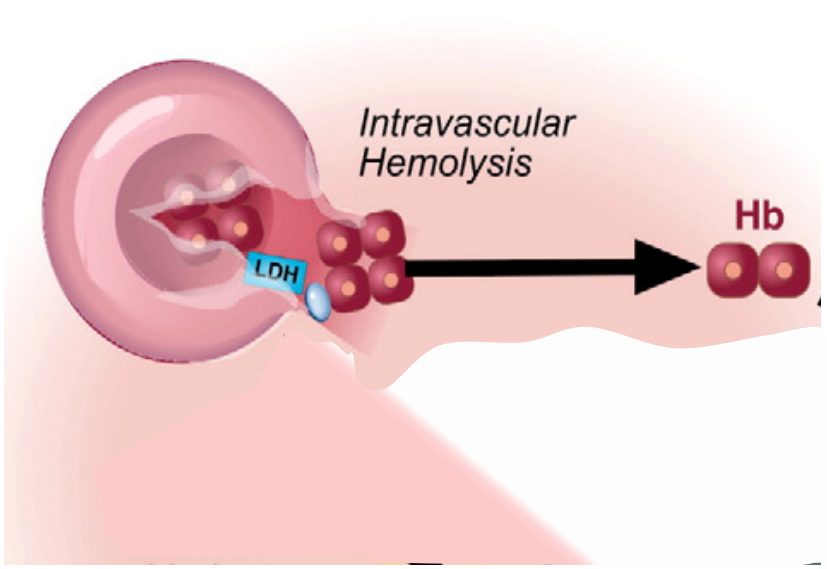
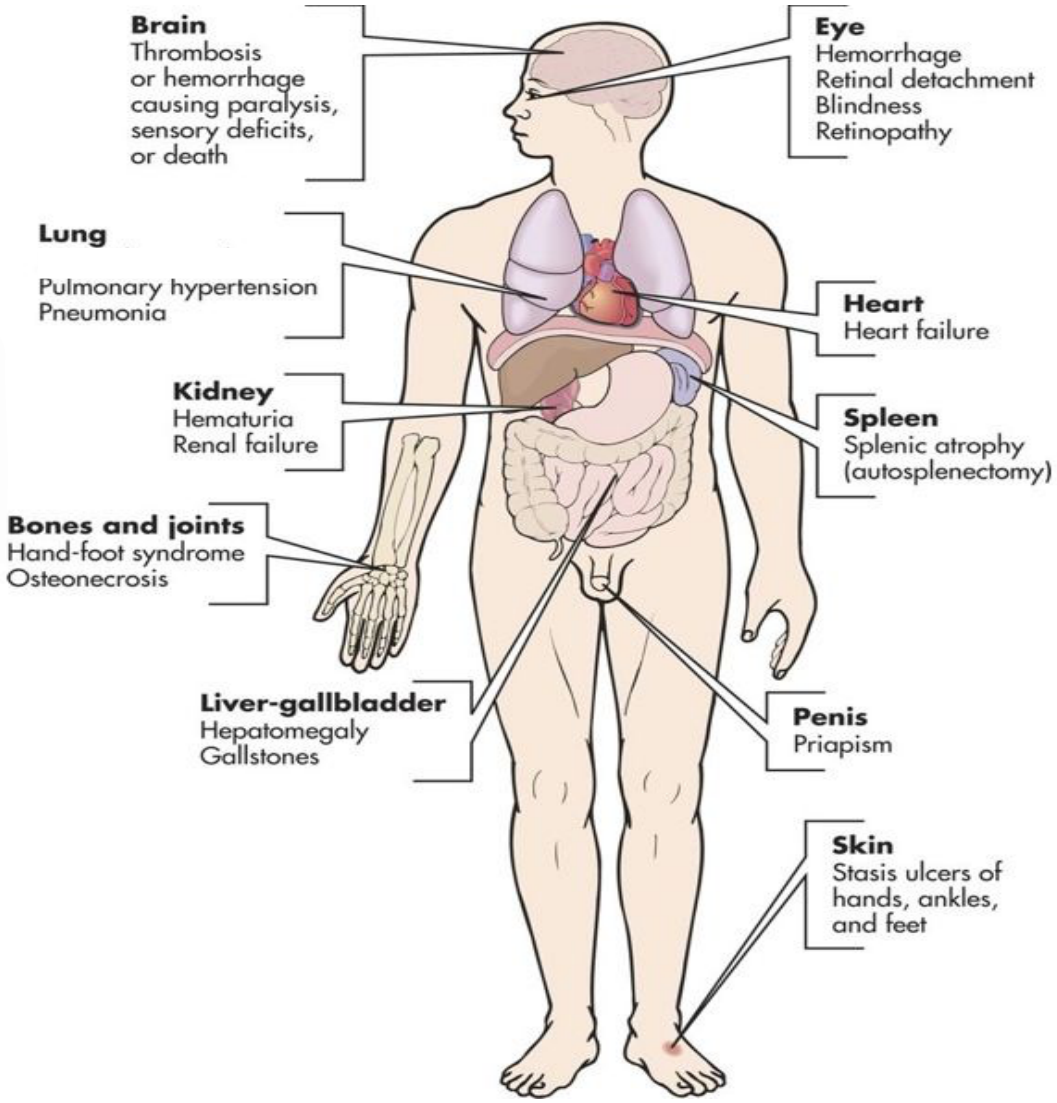
Sickle Cell Disease: The Basics



How does intravascular hemolysis (and vaso-occlusive crises) trigger acute complications of sickle cell disease; what are the mechanisms?



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Acute Lung Injury/Acute Chest Syndrome

- Acute chest syndrome (ACS) is a major concern in sickle cell disease (SCD)
  - ❑ Number one pulmonary complication
  - ❑ Leading cause of intensive care admissions
- Number one cause of death in pregnancy in SCD in low-resource settings
  - ❑ 87% of mortality in a Ghanaian cohort

Asare *et al.,* Am J Hematol 2018

ACS mortality -National Acute Chest Syndrome Study Group

				P value
Age (yrs)	0-9 (n=264)	10-19 (n=145)	≥20 (n=128)	<0.001
Mortality Rate (%)	**<1%**	**2%**	**9%**	

Vichinsky et al., N Engl J Med 342:1855-1865, 2000

- No Targeted therapy
  - ❑ ACS survival is markedly higher in children
    - ➢ potential ACS survival factor
  - ❑ High expression HO-1 promoter variants associate with low risk of ACS

Bean *et al.*, Blood, 2012

The Heme Infusion Story in SCD

Where to inject sickle mice with purified hemin

Intraperitoneal (i.p.)

~2005

Beneficial effect

Induction of Heme Oxygenase-1

The Heme Infusion Story in SCD

HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIA II | NOVEMBER 19, 2010

Acute Chest Syndrome In Transgenic Mouse Models of Sickle Cell Disease Triggered by Free Heme

Samit Ghosh, PhD, Solomon F Ofori-Acquah, PhD

Blood (2010) 116 (21): 944.

https://doi.org/10.1182/blood.V116.21.944.944

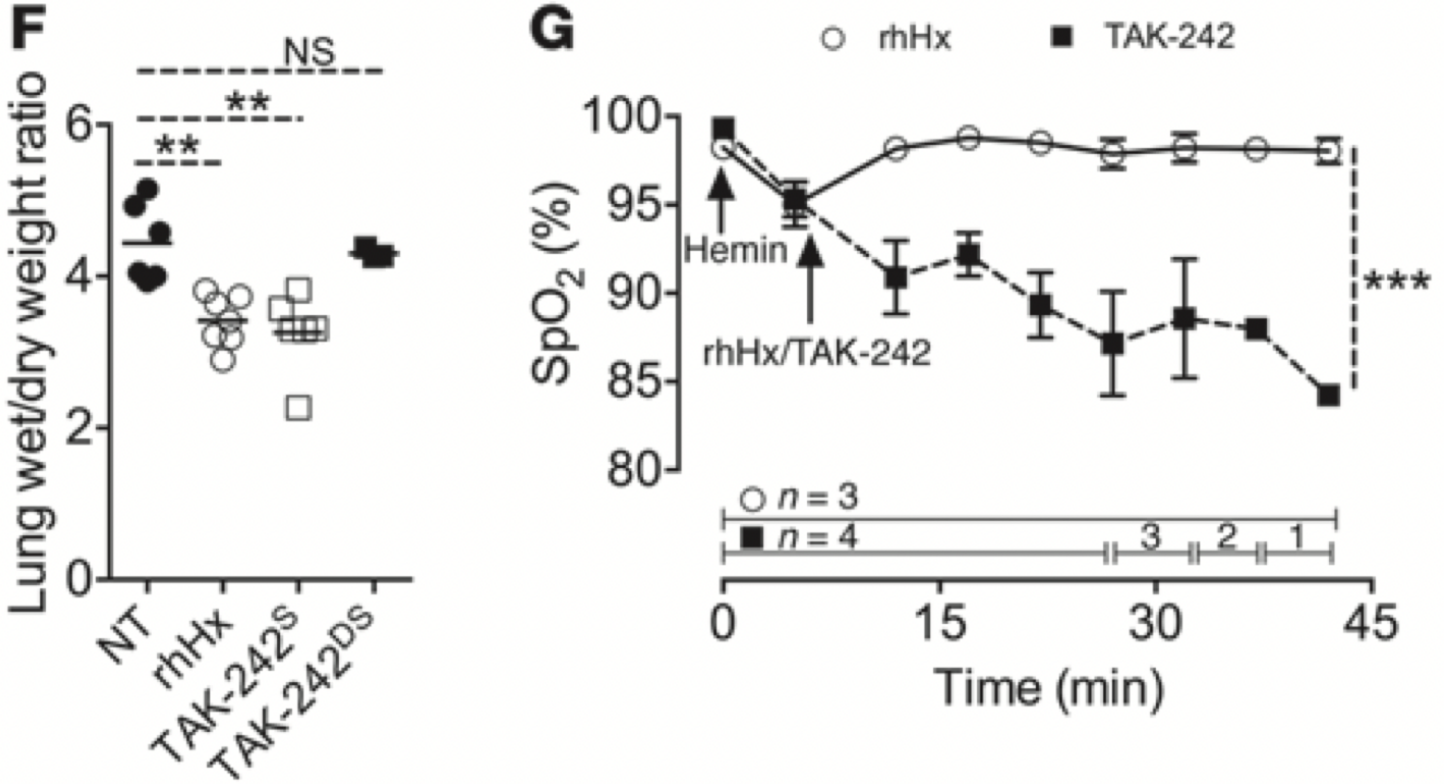
JCI The Journal of Clinical Investigation

Extracellular hemin crisis triggers acute chest syndrome in sickle mice

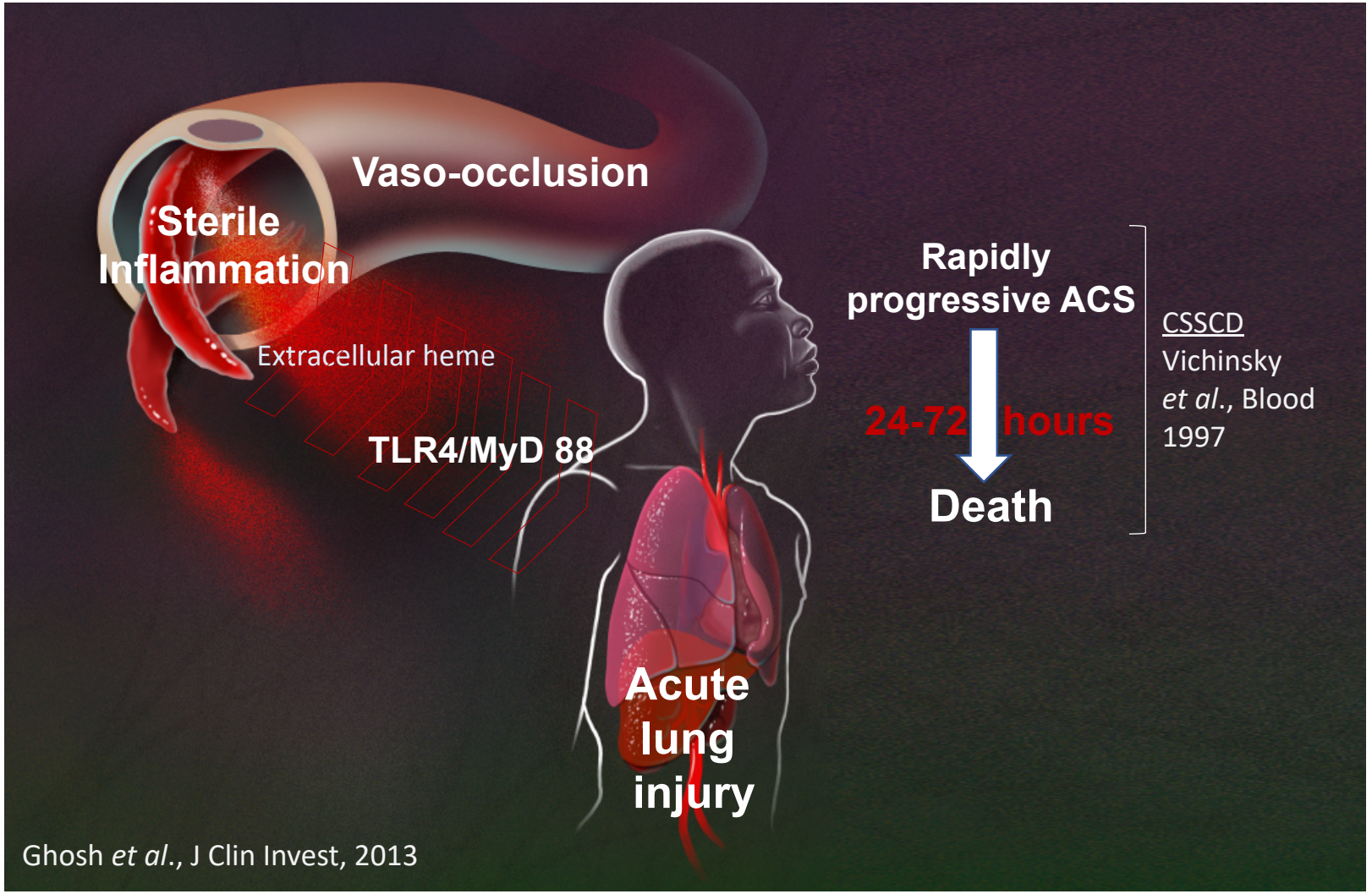
Samit Ghosh, … , David Robert Archer, Solomon Fiifi Ofori-Acquah

J Clin Invest. 2013;123(11):4809-4820. https://doi.org

Research Article | Hematology



Conceptual model of ACS pathogenesis



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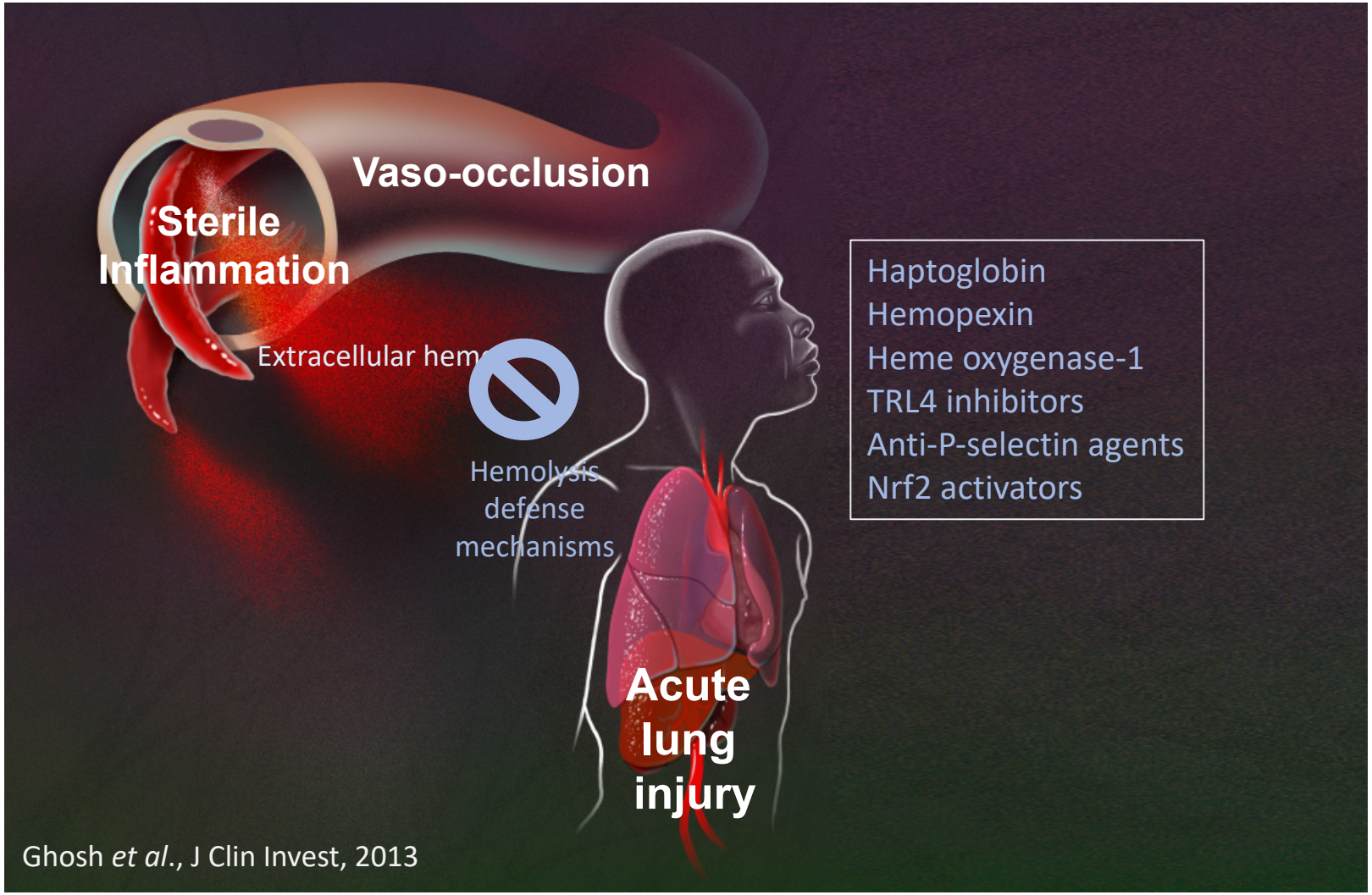


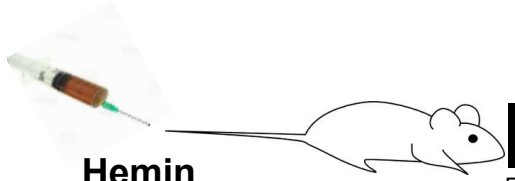
Orphan Drug Designation Granted for CSL Behring's Investigational Plasma-Derived Hemopexin Therapy for Sickle Cell Disease

KING OF PRUSSIA, Pa.

- Both US and European regulators grant special designation
- Phase 1 clinical development underway for CSL889

02 Nov 2020

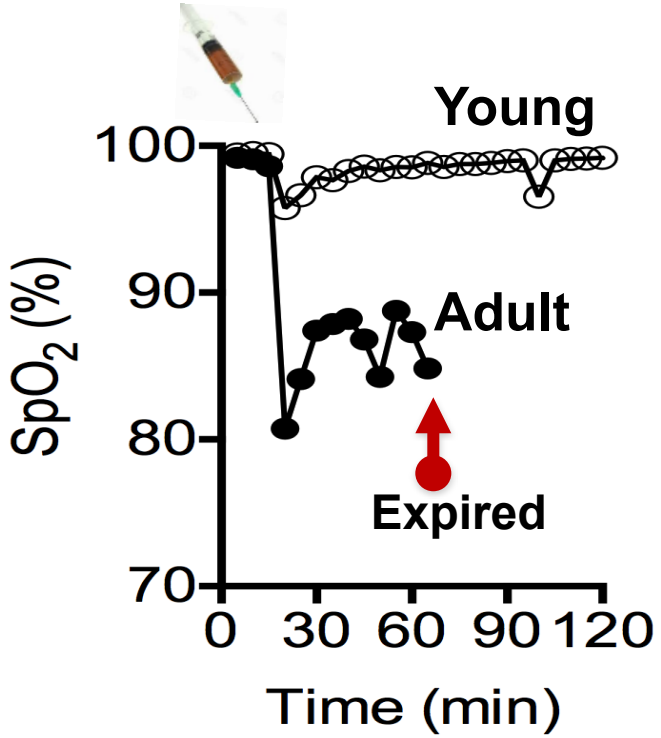
ACS survival rate in transgenic sickle mice phenocopies humans with SCD

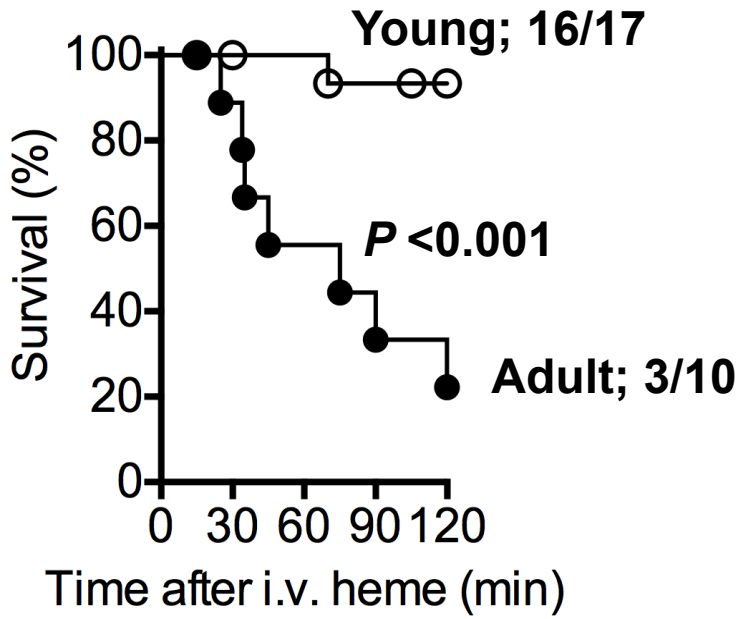


Hemin
(35μmoles/kg)

Continuous monitoring of oxygen saturation and breath rate (2 hours)

Young SS mice: 4-6 weeks
Adult SS mice: 12-14 weeks





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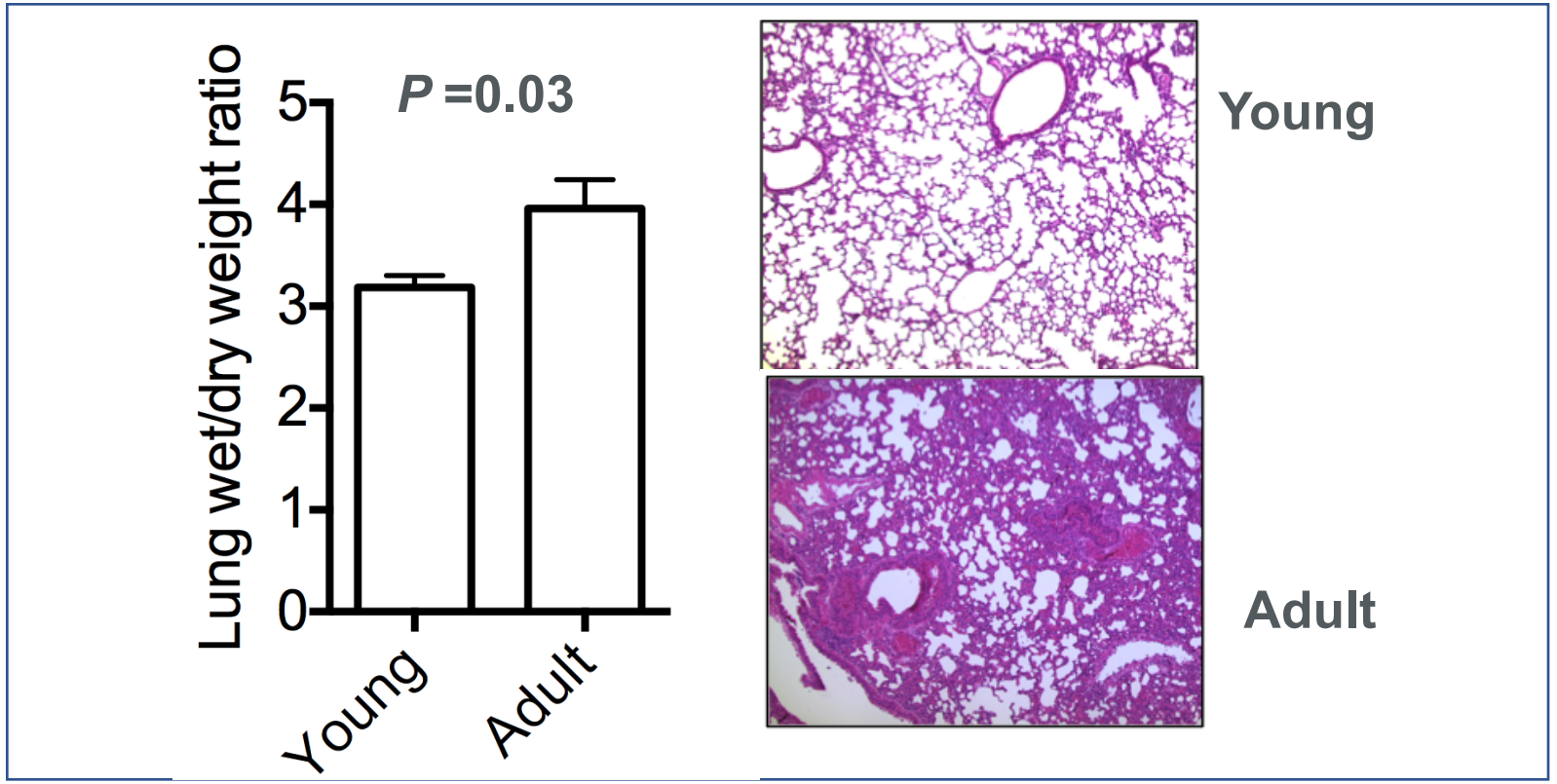
Young sickle mice are protected from heme induced acute lung injury



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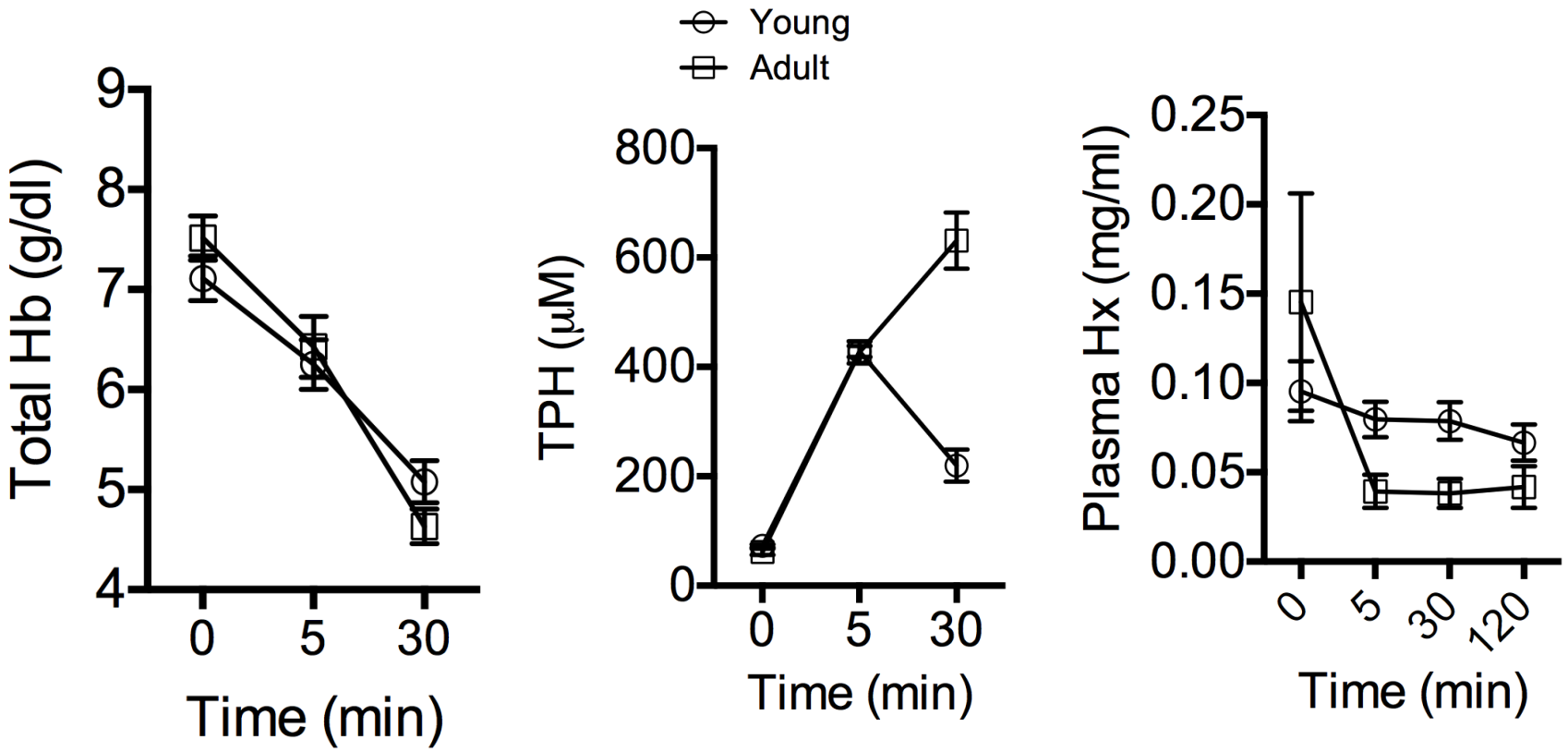
Enhanced heme clearance by young sickle mice



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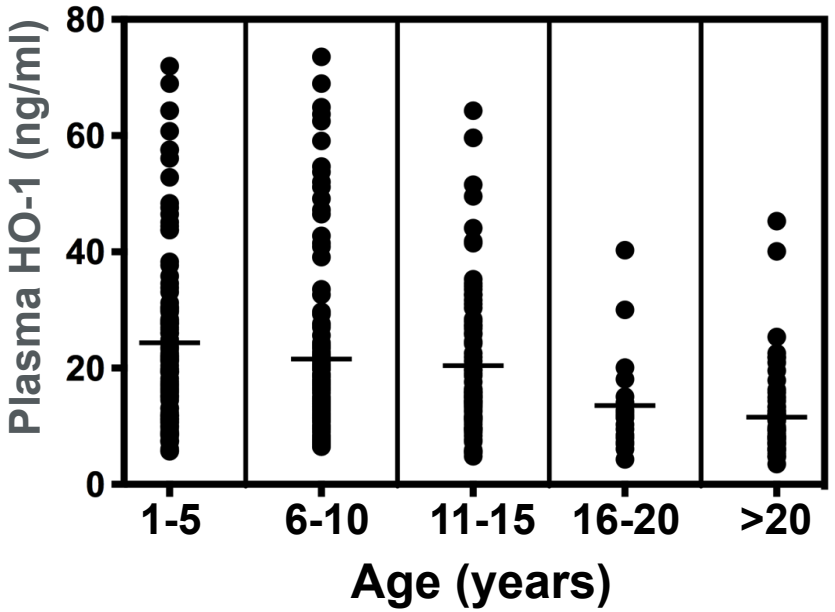
Children and young mice with SCD have raised plasma concentration of HO-1 the rate-limiting heme degradation enzyme

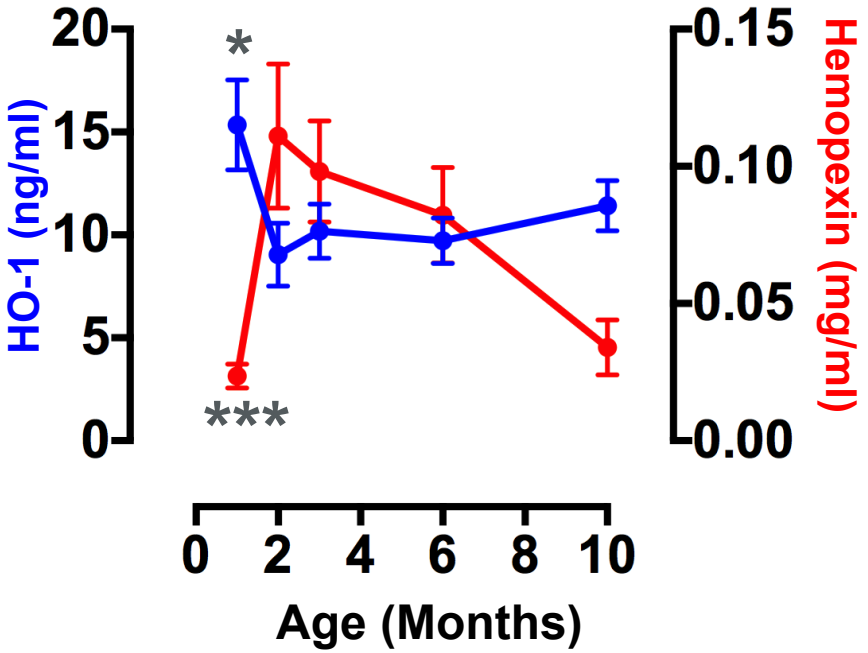
Patients with SCD (n=386)



ANNOVA: All ages, p<0.0001

Control Hb AA adults (17-58 yrs)
HO-1 (ng/ml): 2.3 ±0.1, n=74

Sickle mice (longitudinal)



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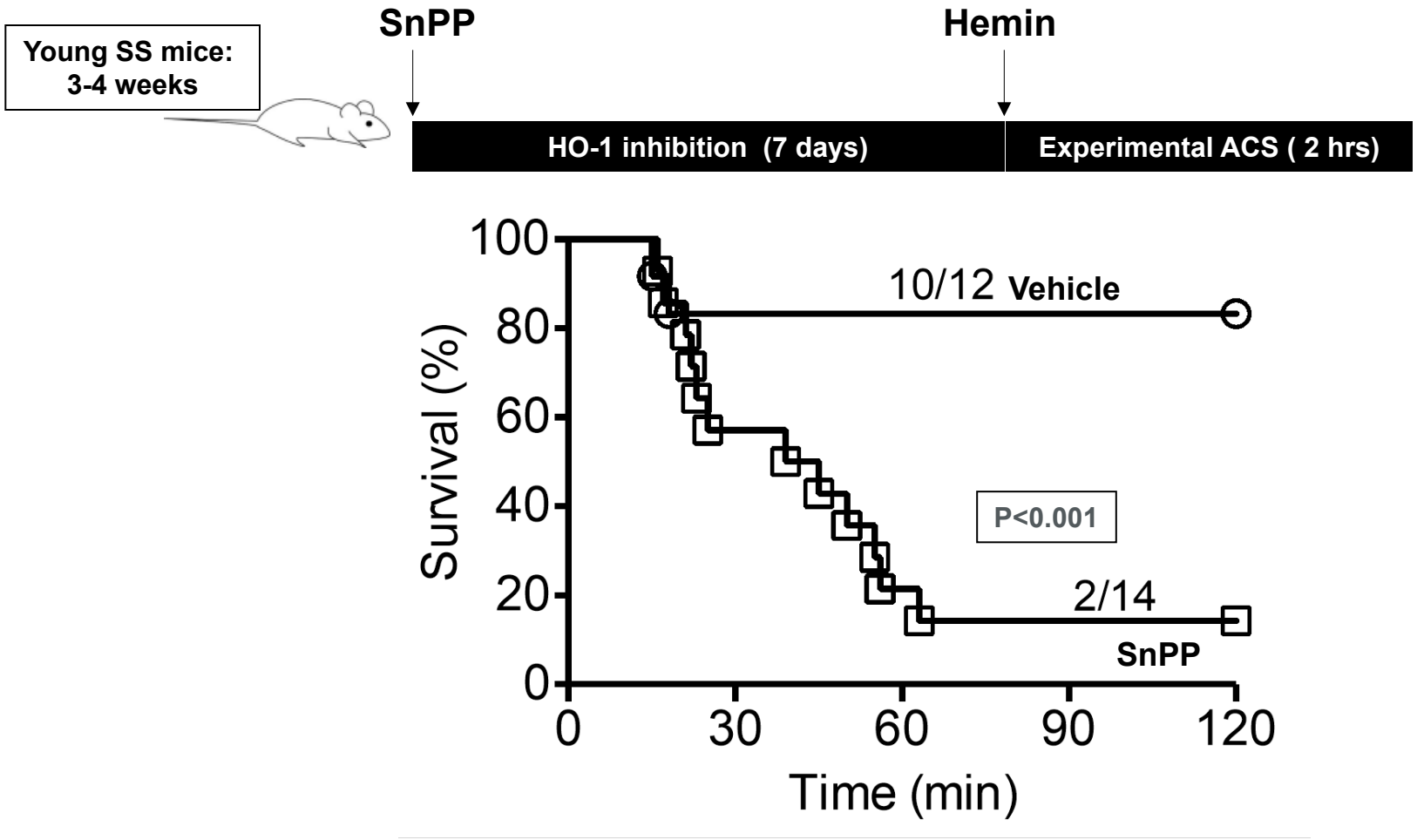
Inhibition of HO-1 activity increases ACS lethality rate in young sickle mice



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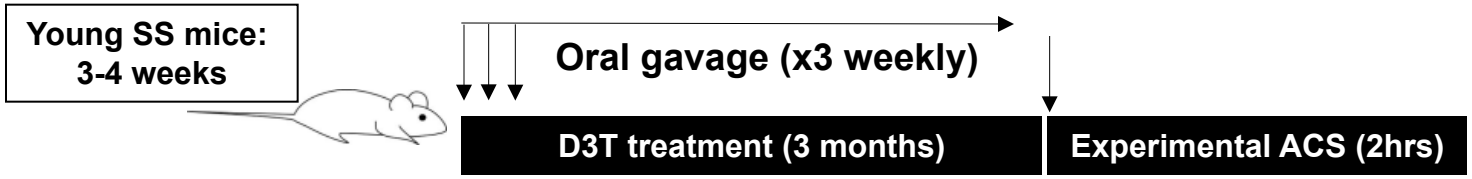
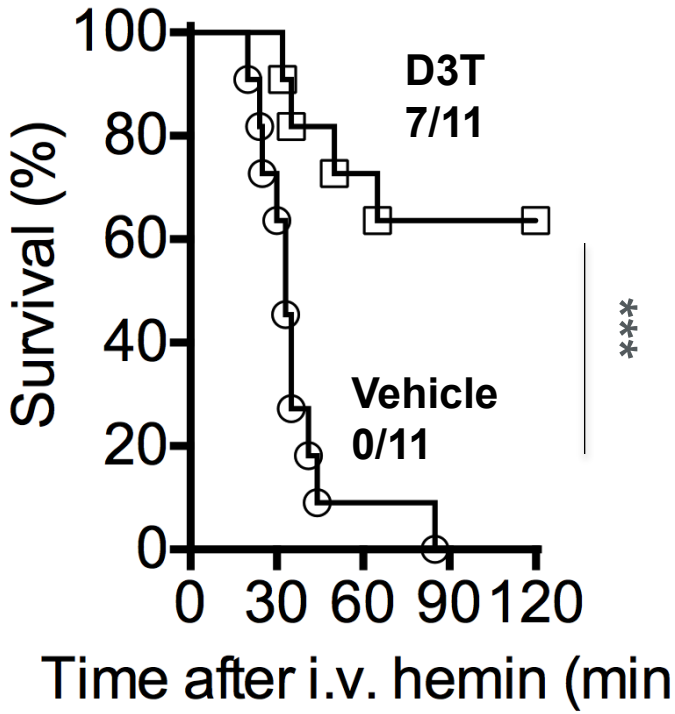
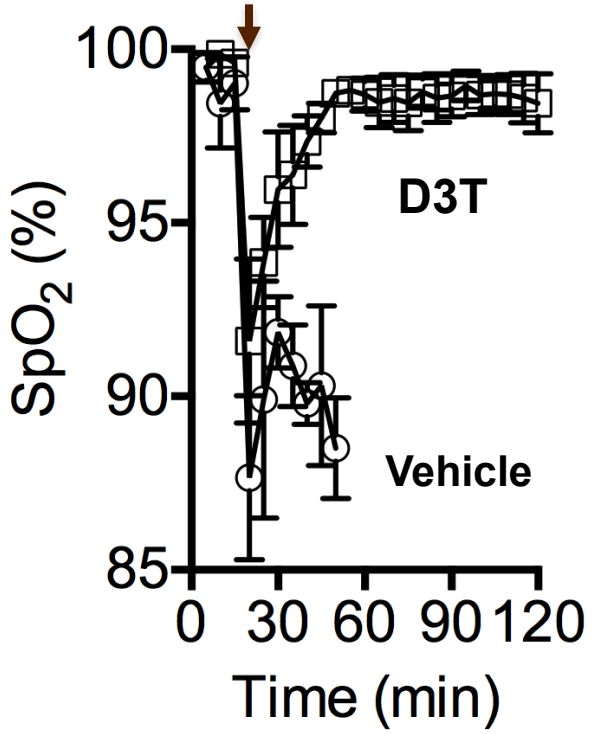
Stabilization of HO-1 decline improves ACS survival in adult sickle mice







Ghosh *et al*., Br J Haematol, 2018

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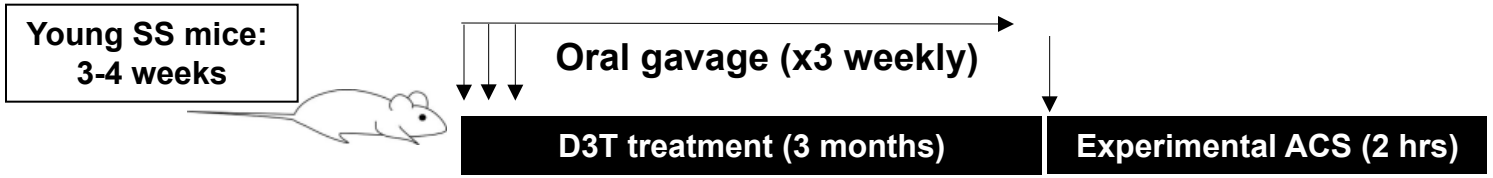
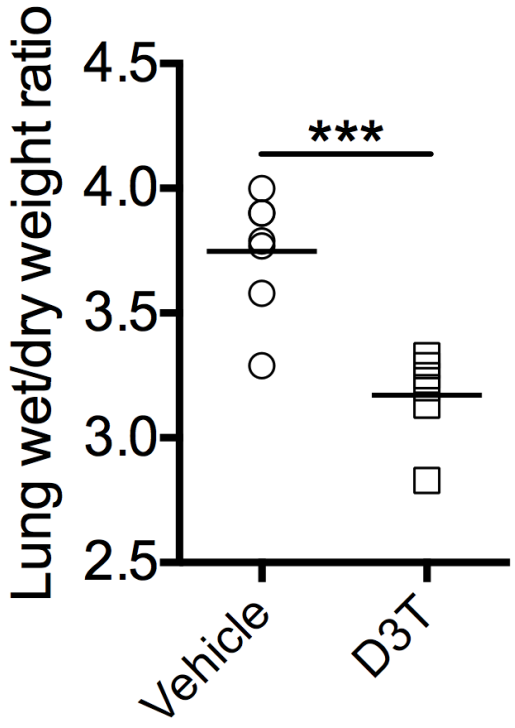
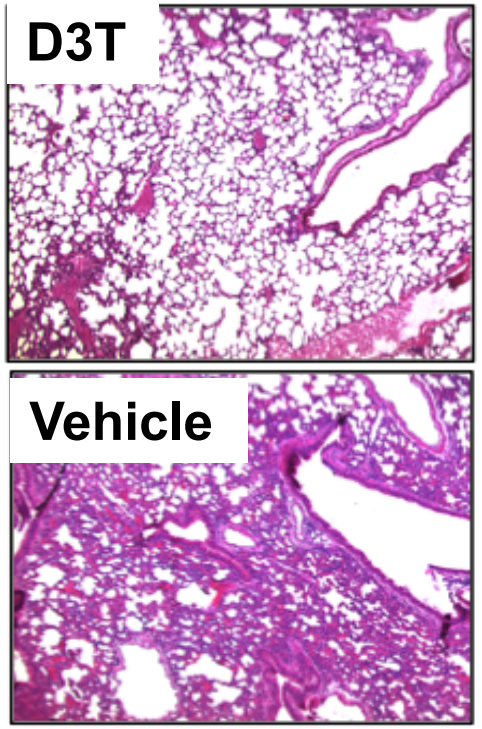
Stabilization of HO-1 decline blocks heme induced acute lung injury in adult sickle mice

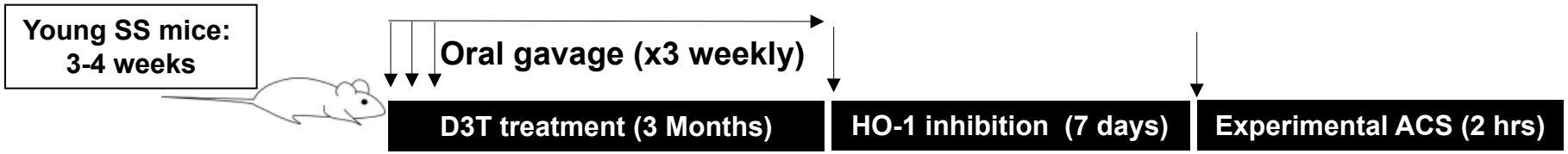


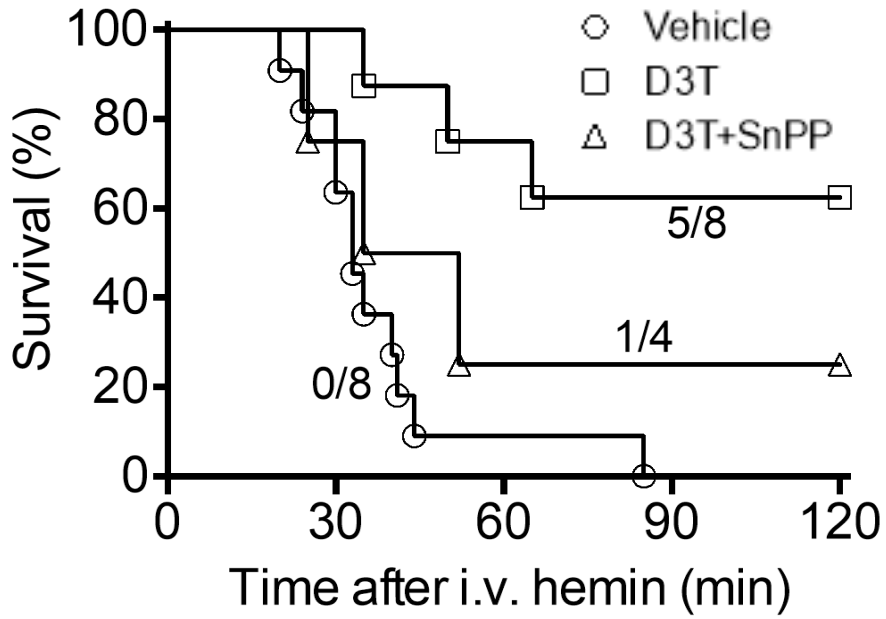


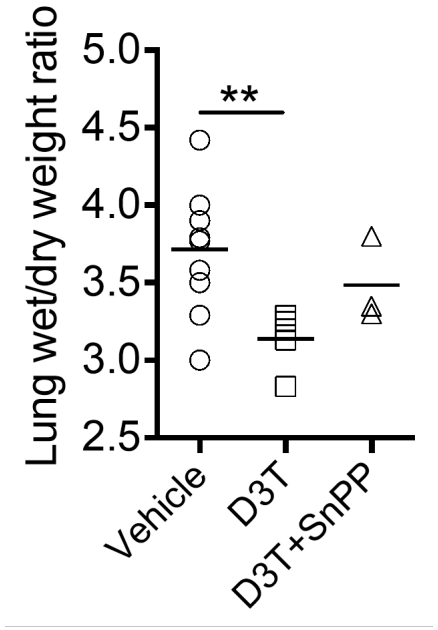


Ghosh *et al*., Br J Haematol, 2018

Inhibition of HO-1 activity abrogates the protective effect of Nrf2 activation in ACS in adult sickle mice







Ghosh *et al*., Br J Haematol, 2018

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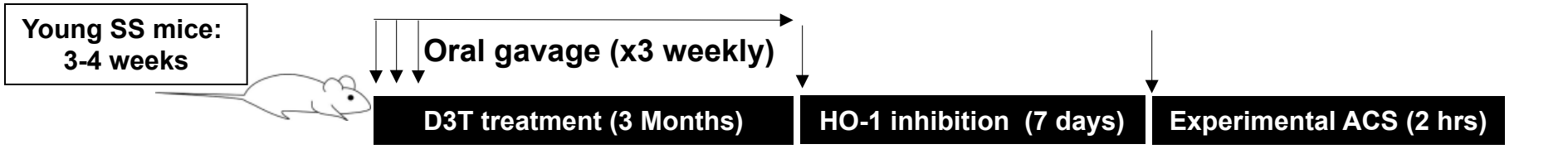

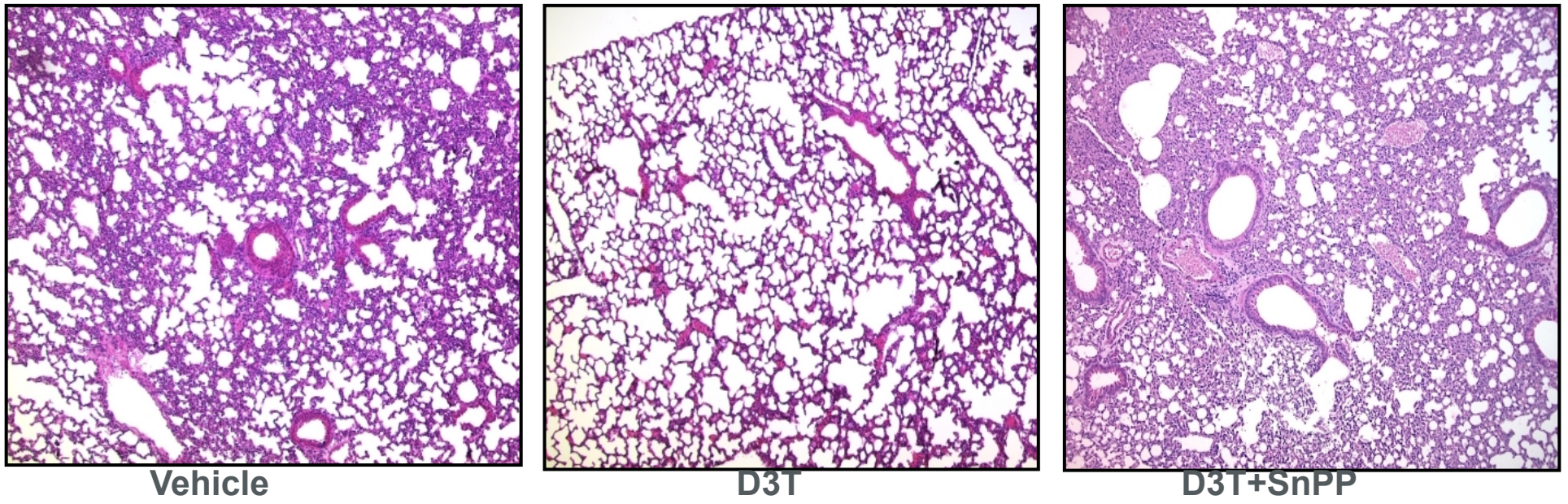

Vehicle D3T D3T+SnPP

Ghosh *et al*., Br J Haematol, 2018

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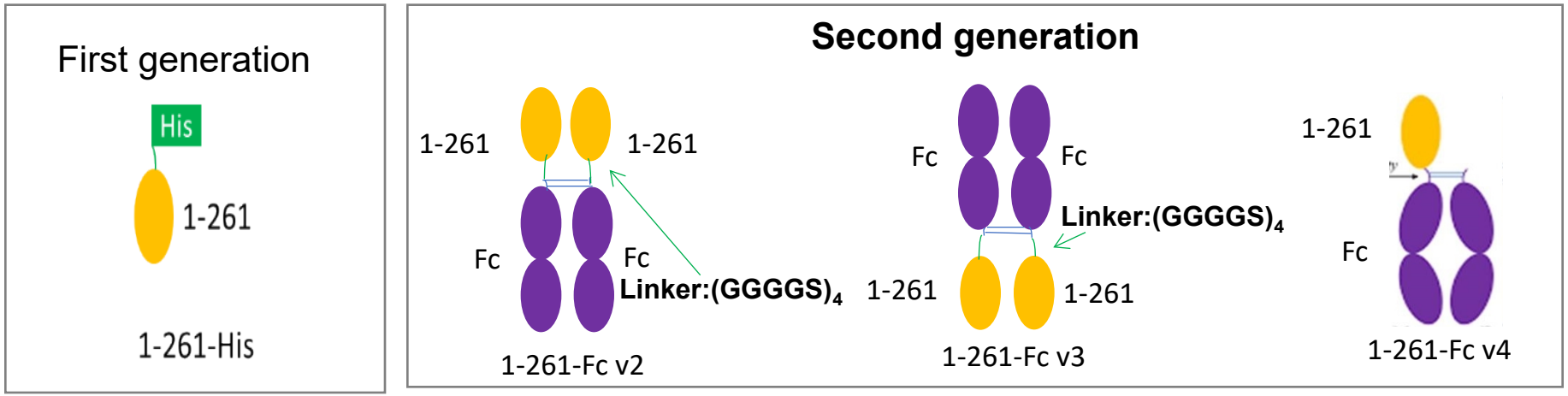
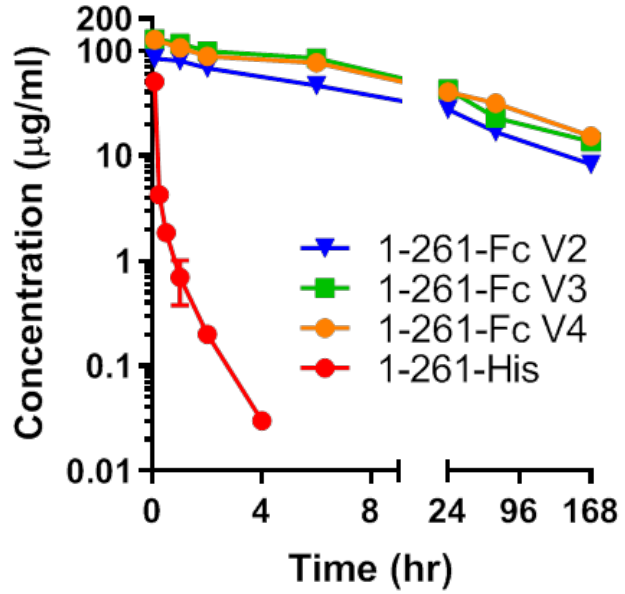
Truncated recombinant human HO-1 molecules

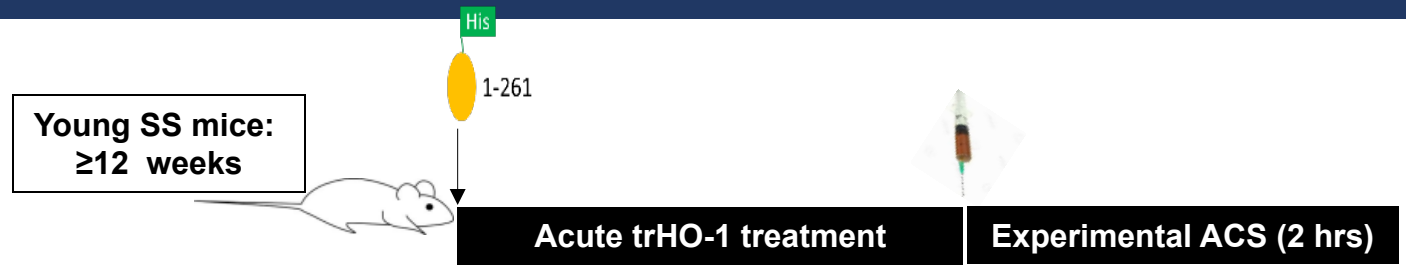


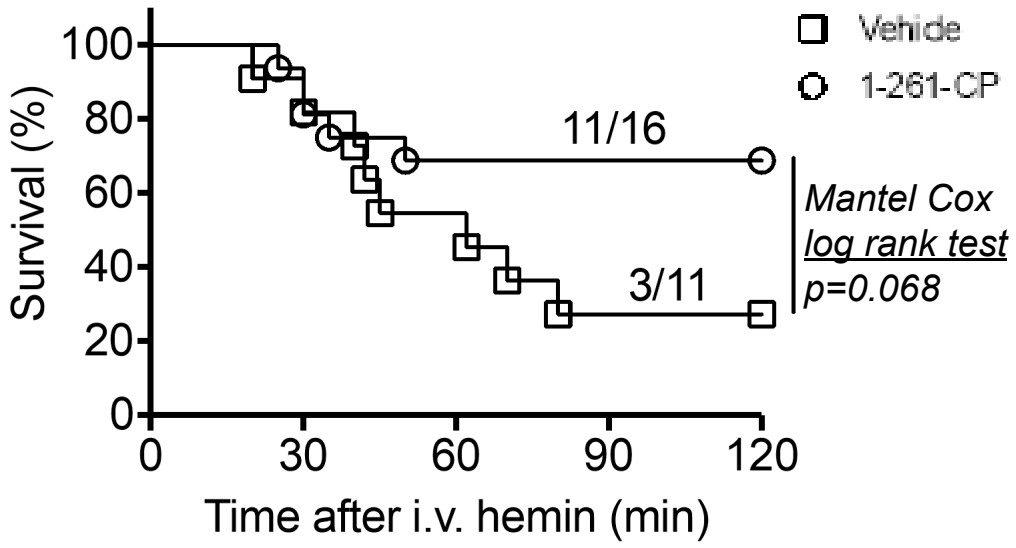


Construct	Half-life (hr)
1-261-Fc V2	85
1-261-Fc V3	92
1-261-Fc V4	101
1-261-His	0.67

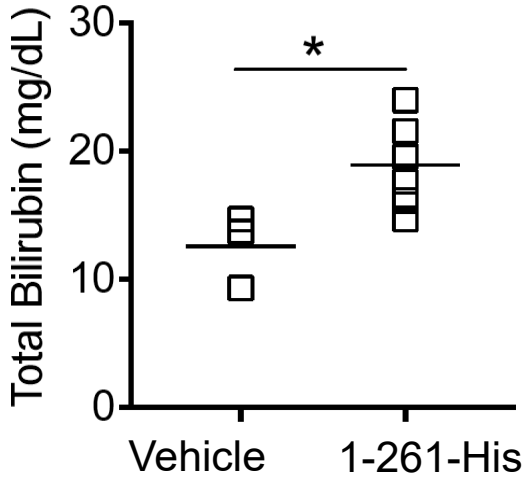
Pharmacokinetic studies: Wild type CD-1 mice

Efficacy of first generation truncated recombinant HO-1 in ACS in adult sickle mice





trHO-1 degrades heme *in situ*



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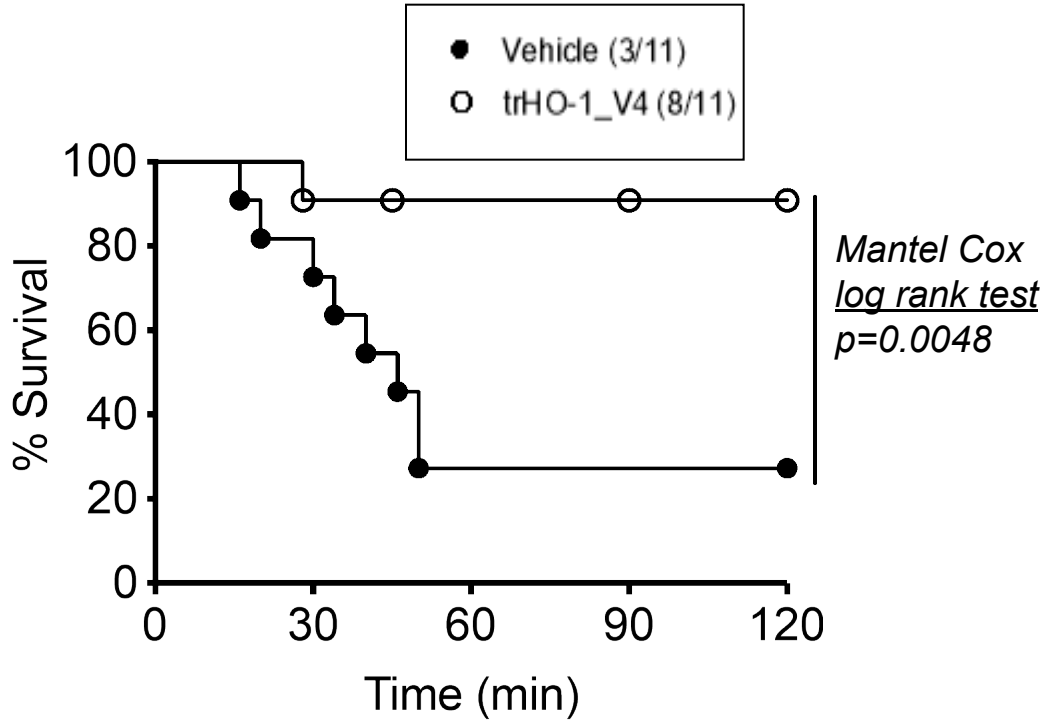
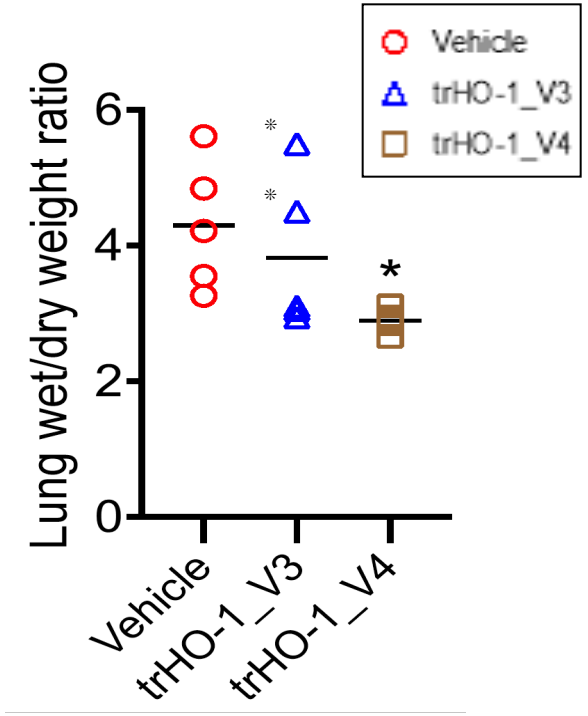
Efficacy of second- generation truncated recombinant HO-1 in ACS in adult sickle mice





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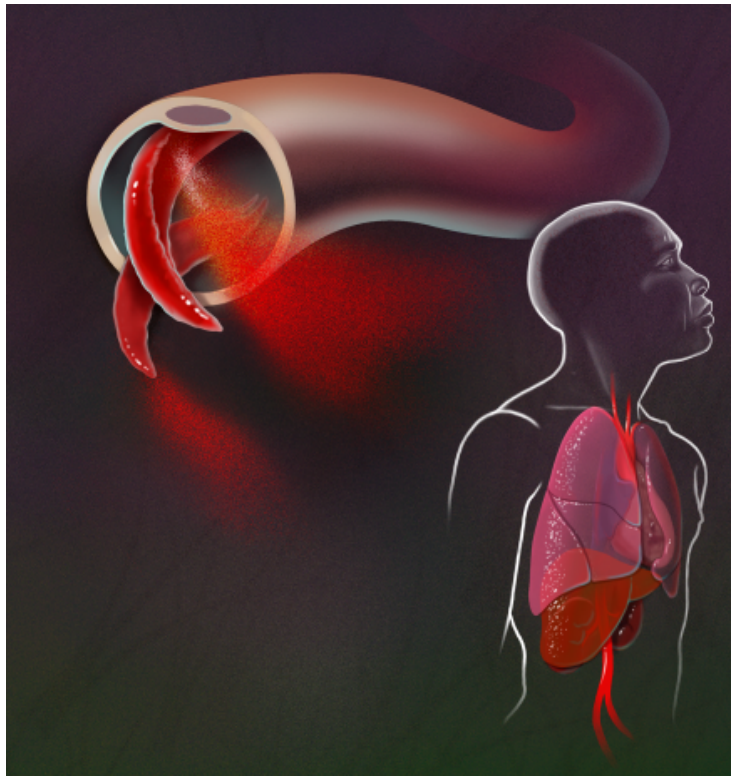
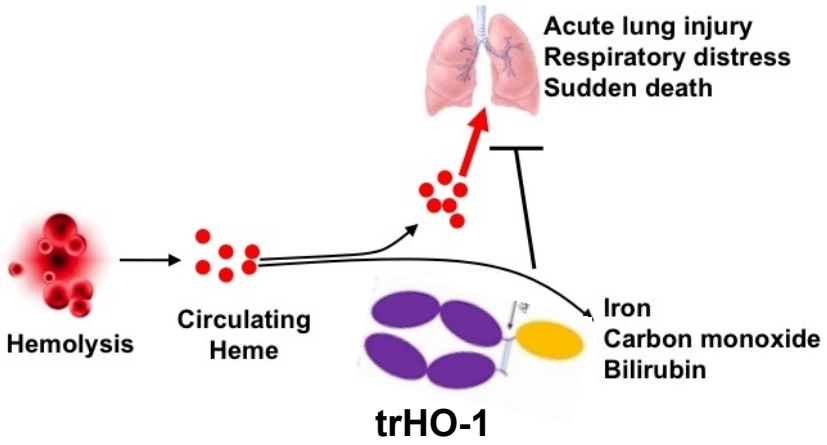
Innovative ACS Therapy: Truncated recombinant human HO-1



Circulating heme oxygenase-1 increases survival in a preclinical acute chest syndrome (ACS)model by degrading extracellular heme, and is thus, a targeted therapeutic option for ACS.



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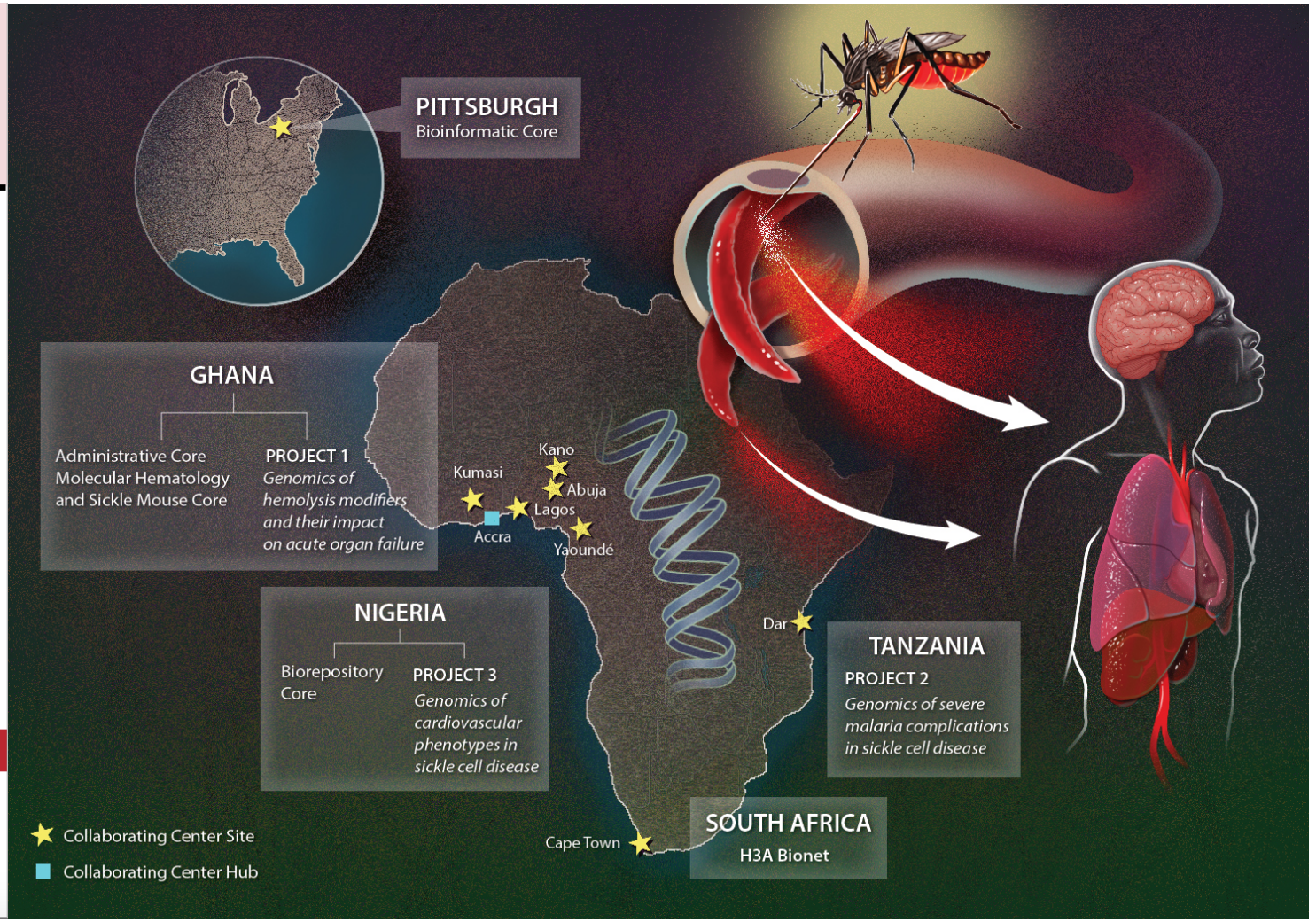
Hemolysis cytoprotective genes: SickleGenAfrica Network



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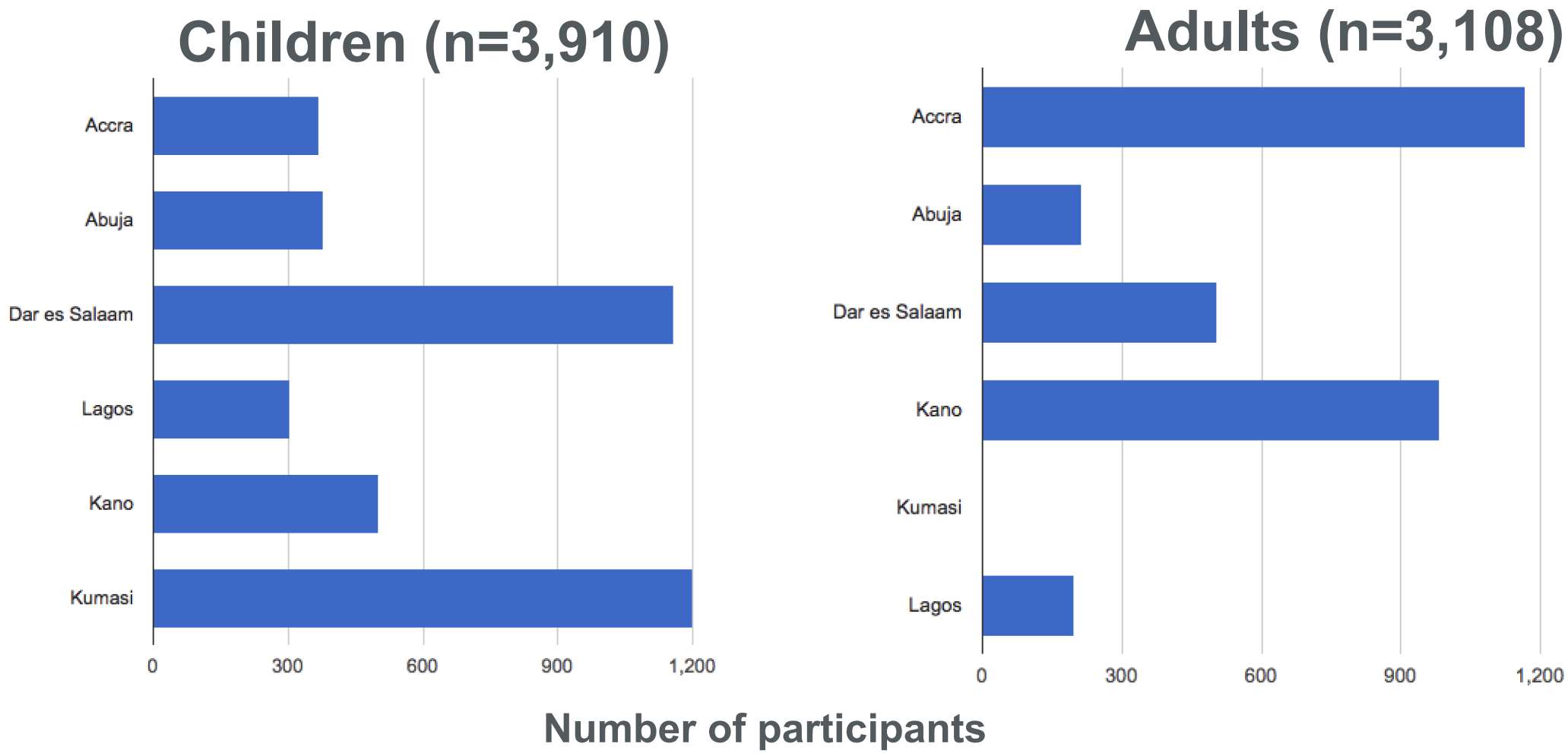
SickleGenAfrica: community engagement workshops

SickleGenAfrica Network: Patient enrolment (November 2021)



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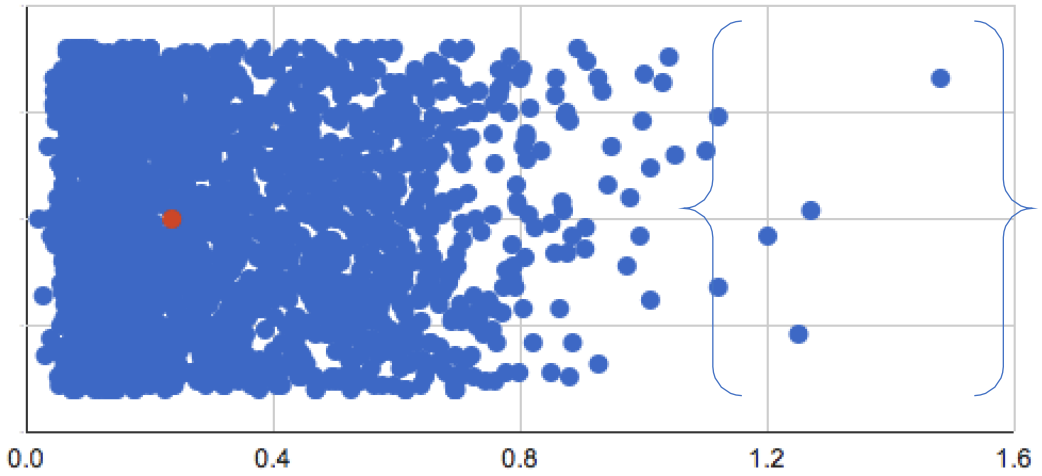
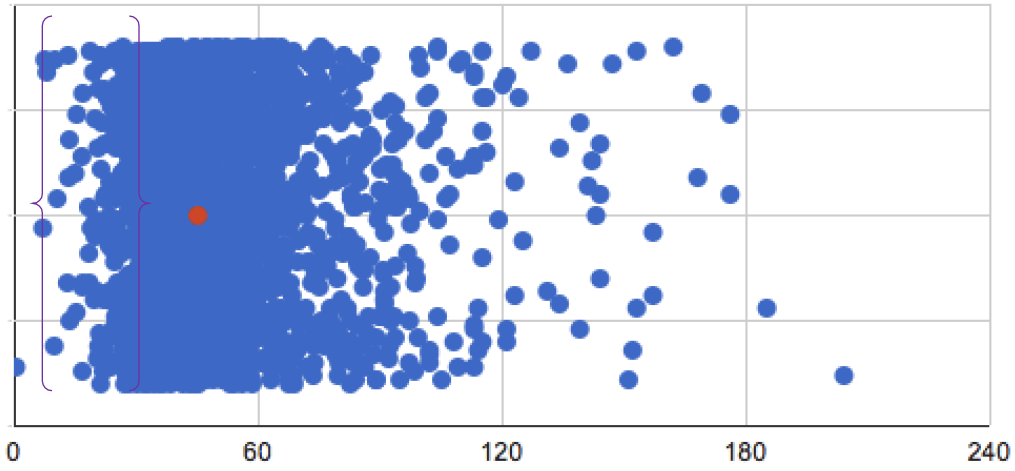
Heme scavenging capacity in the SickleGenAfrica cohort

Ghana cohort (n=2,259)



Hemopexin (g/L)



Alpha-1- macroglobulin (mg/ml)

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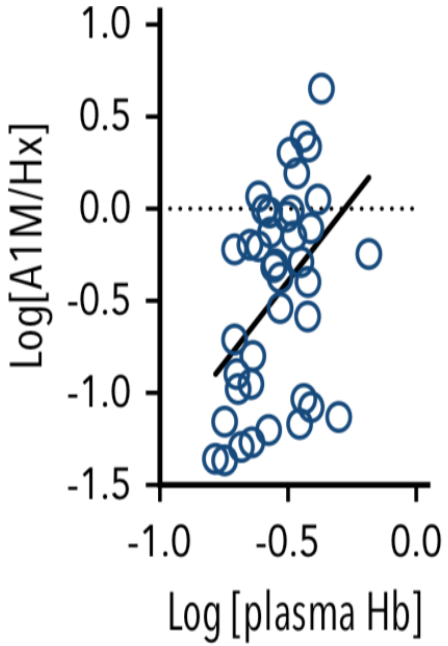
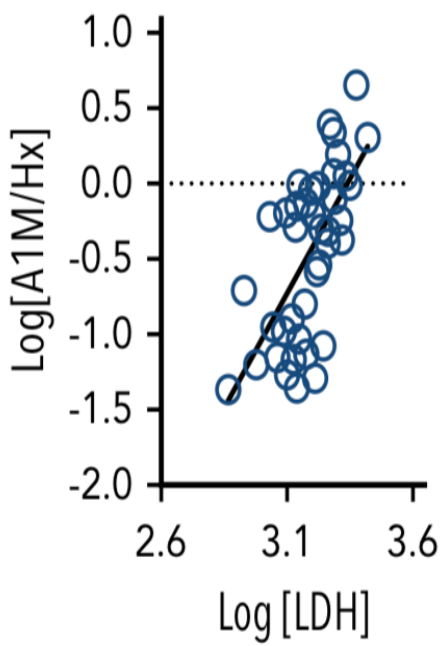
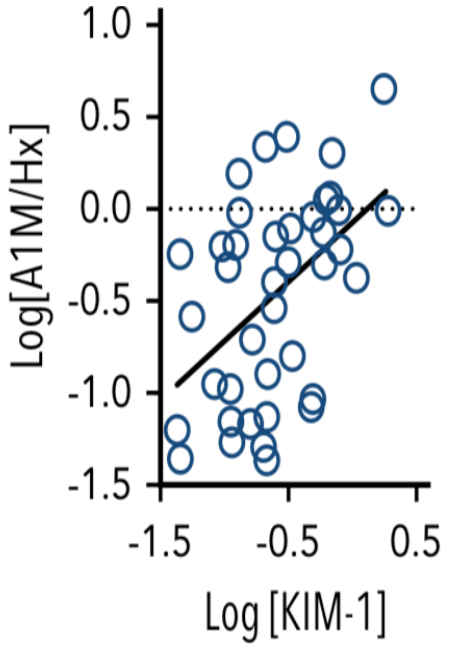
SickleGenAfrica cohort: relationship between hemolysis and kidney injury markers



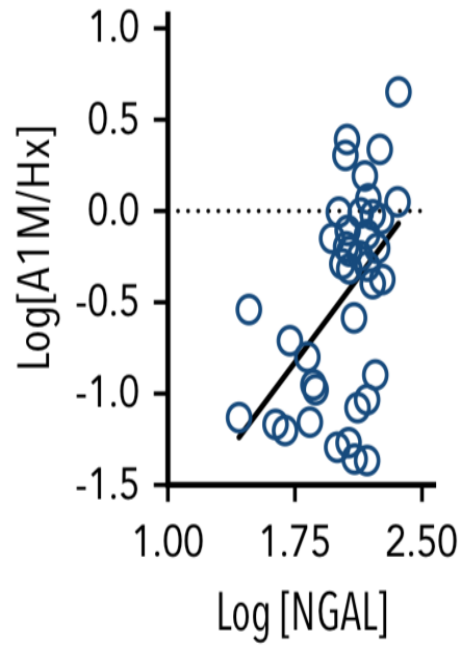
Pearson r= 0.32; p<0.05



Pearson r= 0.62; p<0.001



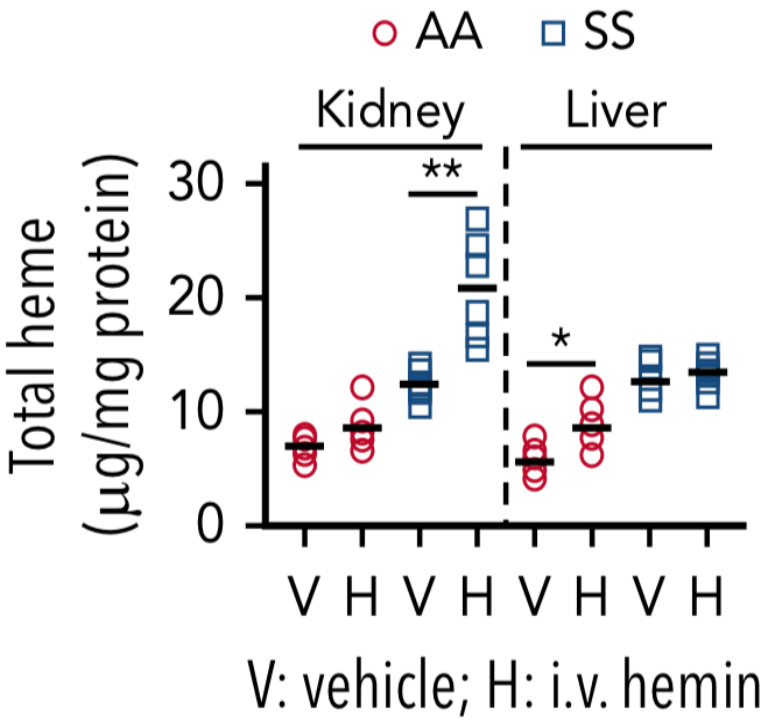
Pearson r=0.54; p<0.001

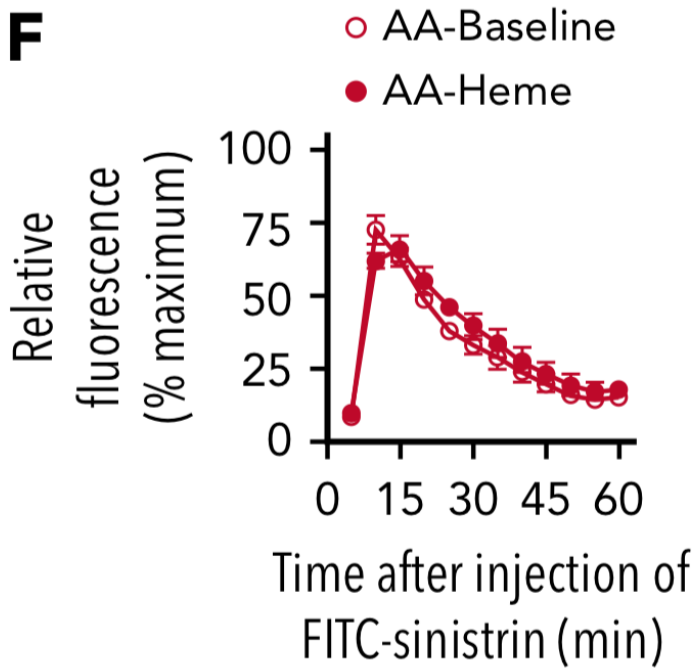


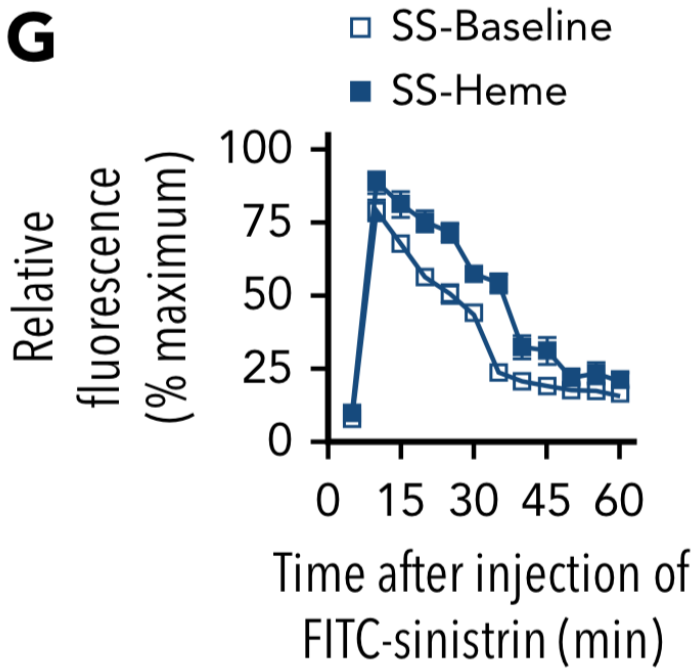
Pearson r=0.50; p<0.001

Ofori-Acquah et al., Blood. 2020; 135:1044-48

Excess Circulating Causes Acute Kidney Injury in Transgenic SCD Mice but in Control Animals







Ofori-Acquah et al., Blood. 2020; 135:1044-48

blood®
Brief Report

RED CELLS, IRON, AND ERYTHROPOIESIS

Hemopexin deficiency promotes acute kidney injury in sickle cell disease

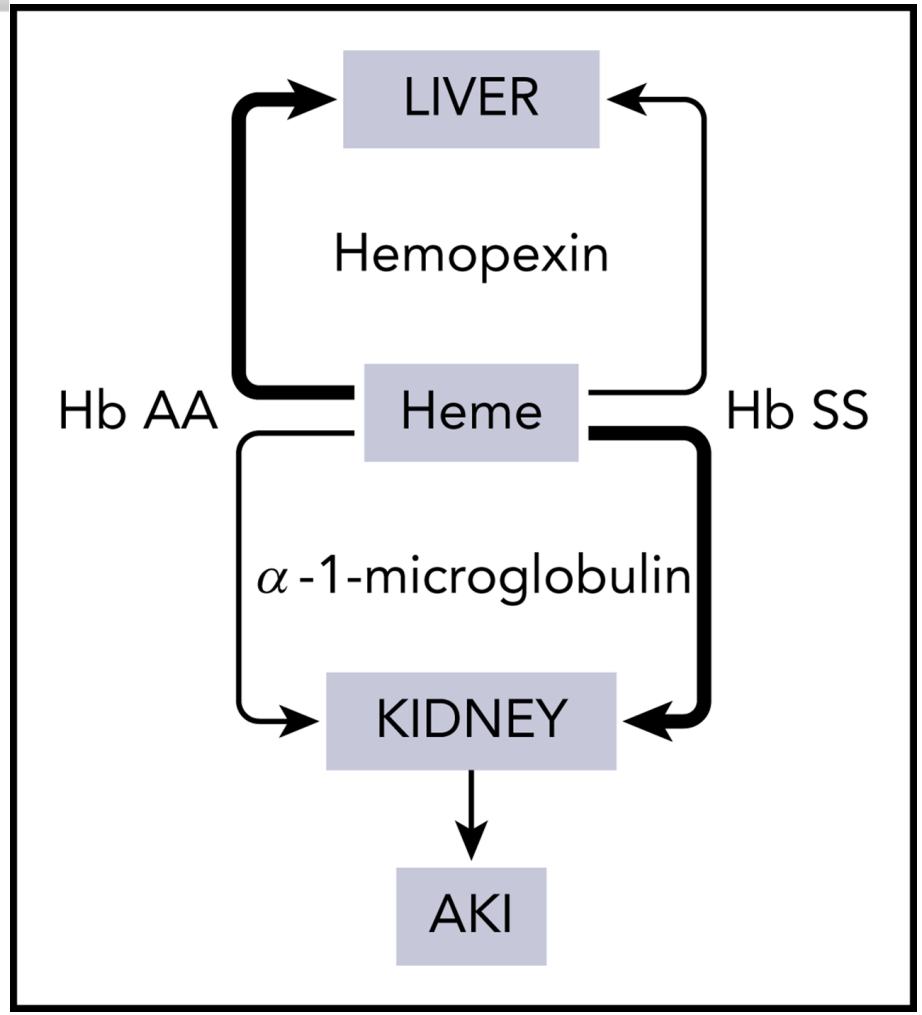
Solomon F. Ofori-Acquah,[1-4] Rimi Hazra,[1-3] Oluwaseun O. Orikogbo,[1] Danielle Crosby,[1-3] Bethany Flage,[1-3] Ezekiel B. Ackah,[5] Diane Lenhart,[1-3] Roderick J. Tan,[6] Dario A. Vitturi,[3,7] Vivian Paintsil,[8] Ellis Owusu-Dabo,[5] and Samit Ghosh,[1-3] in collaboration with the SickleGenAfrica Network

[1]University of Pittsburgh School of Medicine, [2]Center for Translational and International Hematology, and [3]Pittsburgh Heart, Lung, and Blood Vascular Medicine Institute, Department of Medicine, University of Pittsburgh, Pittsburgh, PA; [4]School of Biomedical and Allied Health Sciences, University of Ghana, Accra, Ghana; [5]School of Public Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; [6]Renal-Electrolyte Division, Department of Medicine and [7]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA; and [8]Komfo Anokye Teaching Hospital, Kumasi, Ghana

KEY POINTS

- The ratio of plasma A1M to hemopexin concentration is associated with AKI biomarkers in SCD.
- Enhanced scavenging of circulating heme to kidney triggers AKI in transgenic SCD mice.

Acute kidney injury (AKI) is a major clinical concern in sickle cell disease (SCD). Clinical evidence suggests that red cell alarmins may cause AKI in SCD, however, the sterile inflammatory process involved has hitherto not been defined. We discovered that hemopexin deficiency in SCD is associated with a compensatory increase in α-1-microglobulin (A1M), resulting in an up to 10-fold higher A1M-to-hemopexin ratio in SCD compared with healthy controls. The A1M-to-hemopexin ratio is associated with markers of hemolysis and AKI in both humans and mice with SCD. Studies in mice showed that excess heme is directed to the kidneys in SCD in a process involving A1M causing AKI, whereas excess heme in controls is transported to the liver as expected. Using genetic and bone marrow chimeric tools, we confirmed that hemopexin deficiency promotes AKI in sickle mice under hemolytic stress. However, AKI was blocked when hemopexin deficiency in sickle mice was corrected with infusions of purified hemopexin prior to the induction of hemolytic stress. This study identifies acquired hemopexin deficiency as a risk factor of AKI in SCD and hemopexin replacement as a potential therapy. (*Blood*. 2020;135(13):1044-1048)



Ofori-Acquah et al., Blood. 2020; 135:1044-48

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Conclusions

- Excess circulating heme causes acute injury to multiple organs in sickle cell disease mice

- Acquired deficiency of hemopexin in SCD causes a maladaptive elevation of alpha-1-macroglobulin that promotes delivery of excess circulating heme to the kidneys, instead of the liver to cause acute kidney injury during episodes of hemolytic crisis.

- Over 7,000 patients are enrolled with clinical data and biological samples stored in SickleGenAfrica; a major resource for genomics studies

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