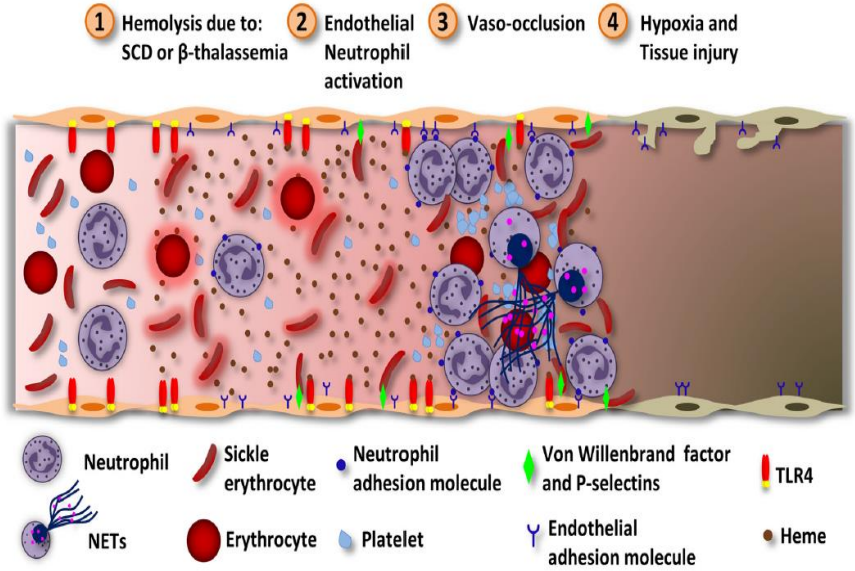
New York Blood Center

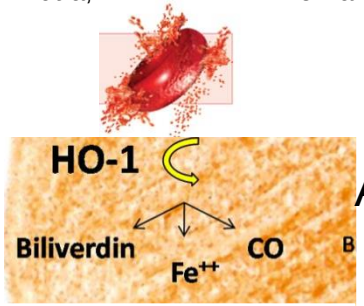
Hemolysis and Immune Activation

Karina Yazdanbakhsh, PhD
Executive Director, Research Institute
New York Blood Center

Hemolysis in Sickle Cell Disease (SCD)



Dutra, F. F. and M. T. Bozza (2014). Front Pharmacol 5: 115.



Anti-inflammatory;
Anti-cytotoxic

- Heme oxygenase 1 (HO-1) breaks down heme upregulated in SCD

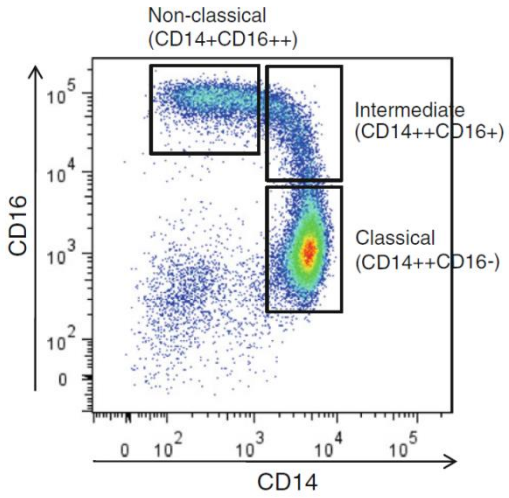
- Hemolysis activates the underlying endothelium: increased expression of endothelial adhesion molecules and apoptotic markers

- Attachment of sickle RBCs and other blood components to the vessel wall; in vivo heme injection induces vascular stasis and acute chest syndrome

Balla et al. 1993 Proc Natl Acad Sci U S A.;90(20):9285-9289.
Belcher et al. 2014 Blood;123(3):377-390.
Camus, et al. 2015 Blood;125(24):3805-3814.
Belcher et al.2006 J Clin Invest; 116(3):808-16
Gosh et al 2013 J Clin Invest: 123(11):4809-20.
Hoover et al. *Blood*. 1979;54(4):872-876
Hebbel et al 1980;302(18):992-995.
Hebbel et al *J Clin Invest*. 1980;65(1):154-160.
Hebbel et al *Blood*. 1981;58(5):947-952.
Mohandas N, Evans E. *Blood*. 1984;64(1):282-287.

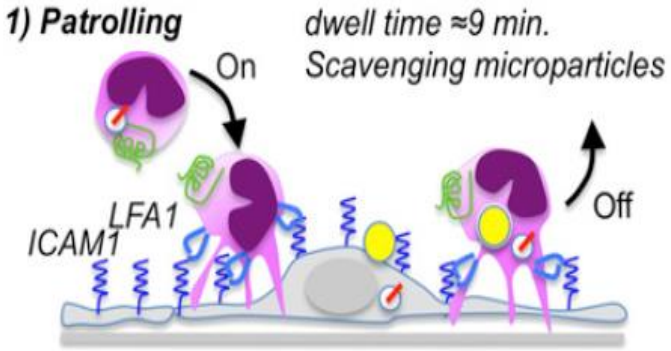
- Heme scavenging/removal system (hemopexin and haptoglobin) is overwhelmed

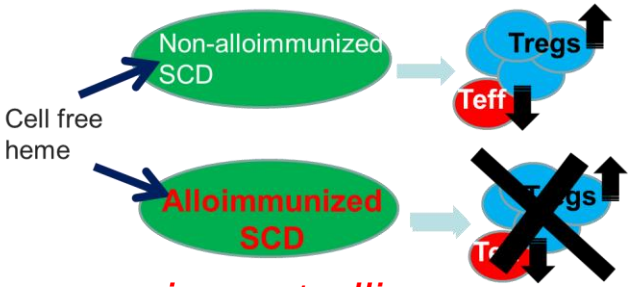
Patrolling Monocytes



- Phagocytose cellular debris derived from damaged endothelial cells
- Control endothelial damage in atherosclerosis models and clear vascular amyloid beta in Alzheimer's disease
- SCD express high levels of HO-1 in patrolling monocytes: control T cell anti-inflammatory profile in SCD under hemolytic conditions

(Zhong… Yazdanbakhsh, (2014) JI 193(1):102-10)

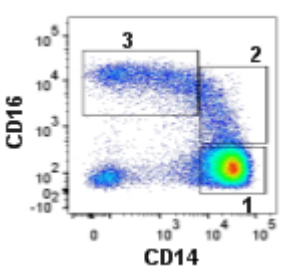


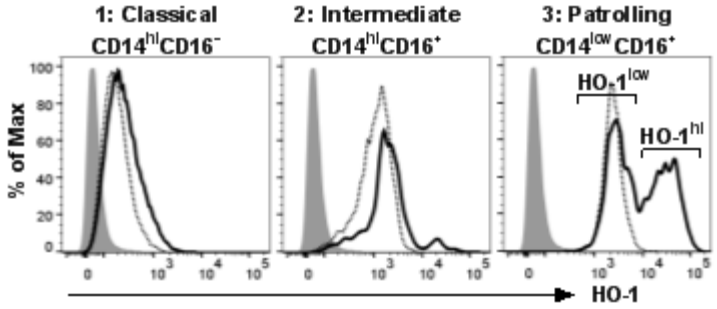


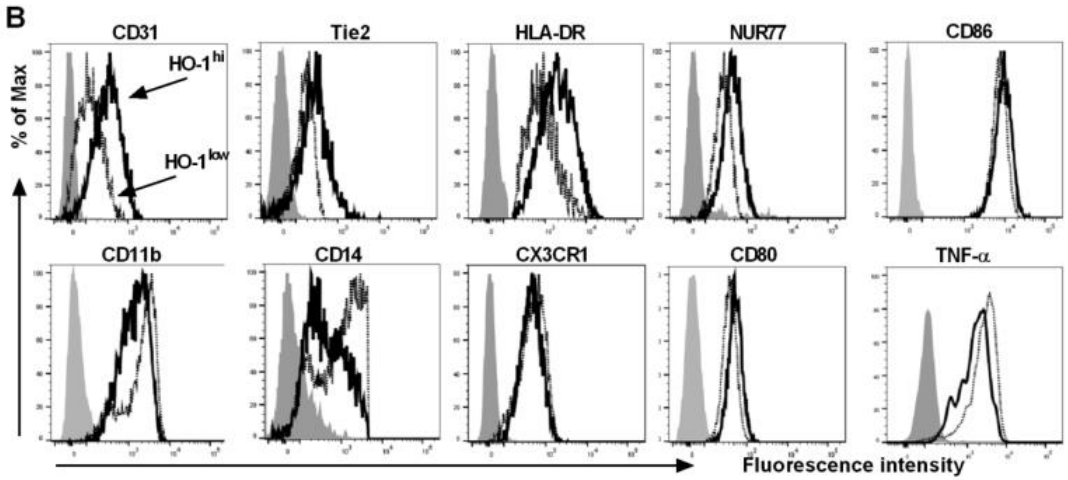
Hypothesis: HO-1 expressing patrolling monocytes clear heme damaged endothelial cells and sickle RBC attached to ECs in SCD, dampening inflammation

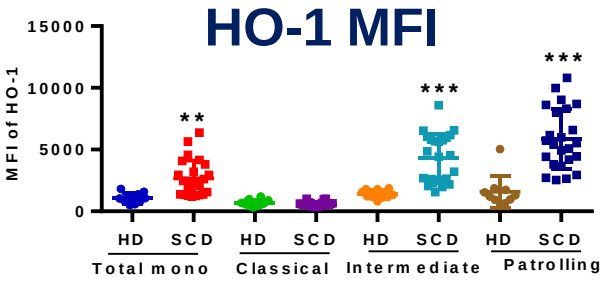
Carlin et al. (2013) Cell 153(2): 362-375.
Quintar et al. (2017) Circ Res 120(11):1789-1799.

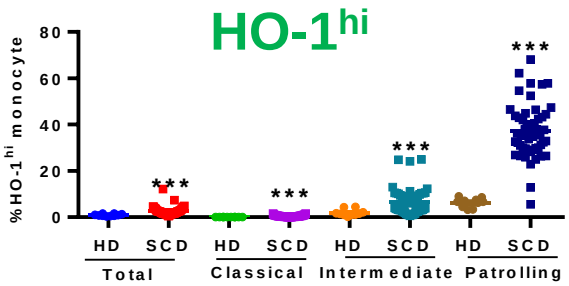
HO-1 expressing Patrolling Monocyte Characterization

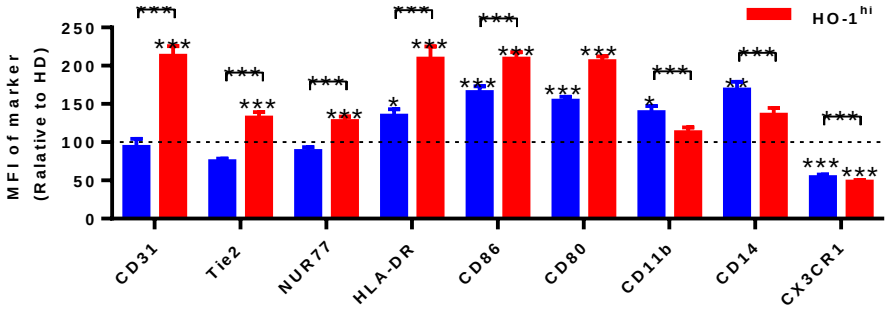


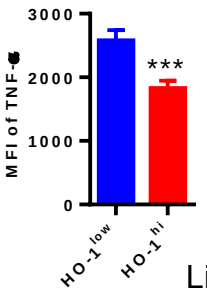










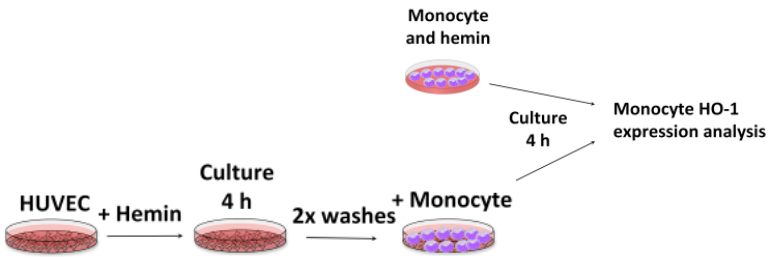


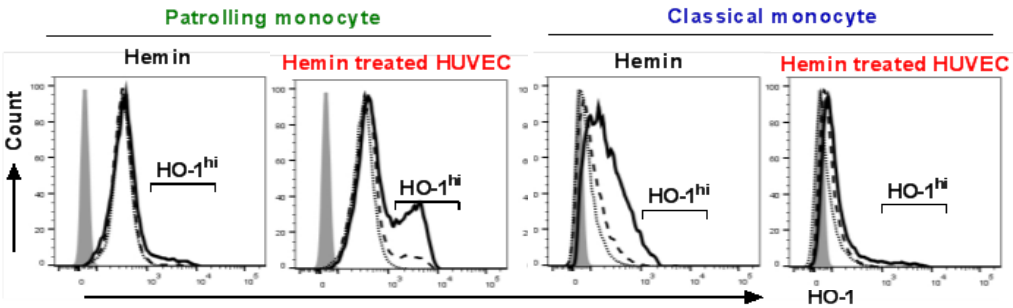
Expanded subpopulation of circulating patrolling monocytes expressing high levels of HO-1 in SCD

Liu …. Yazdanbakhsh. *Blood* 2018, 131(14):1600

Mechanism of HO-1^{hi} Upregulation in Patrolling Monocytes

Liu …. Yazdanbakhsh. *Blood* 2018, 131(14):1600



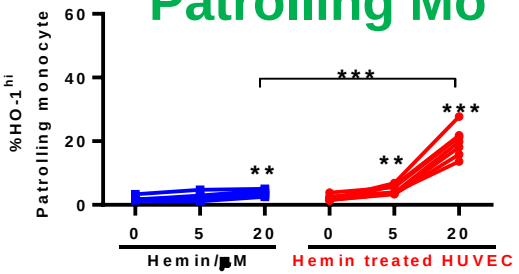


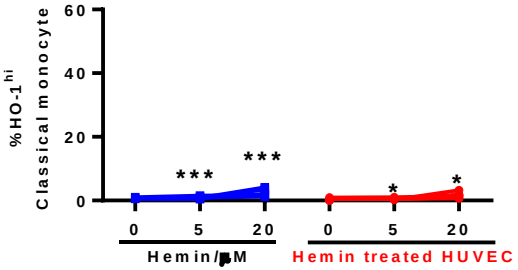
Patrolling Mo

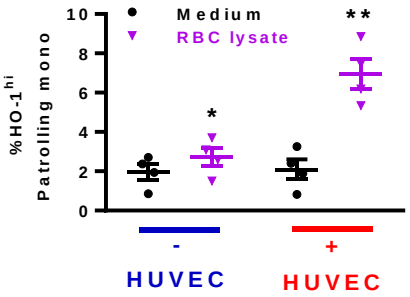
Classical Mo

RBC lysate

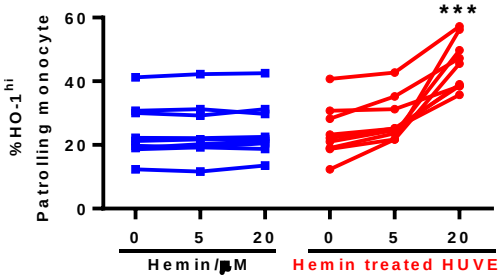
HD

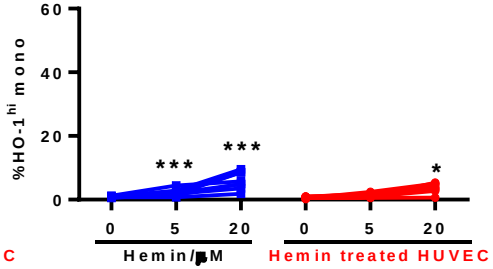




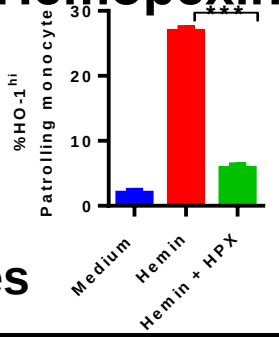


SCD





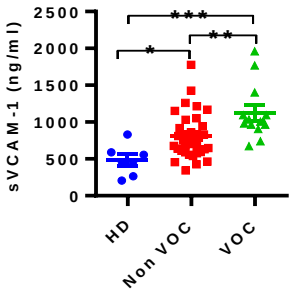
+ Hemopexin



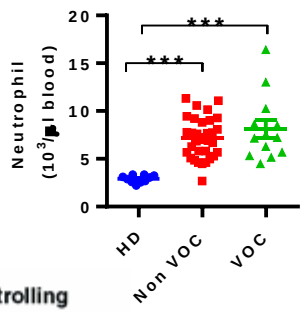
Interaction of cell free heme with endothelial cells is prerequisite for optimal induction of HO-1^{hi} expression in patrolling monocytes

HO-1^{hi} Patrolling Monocytes in SCD patients with Vaso-occlusive Crisis (VOC)

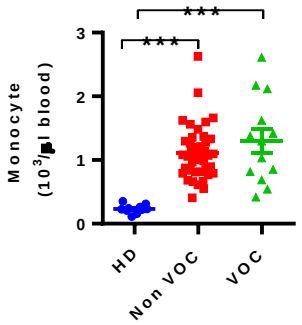
sVCAM-1

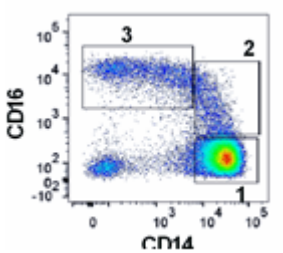


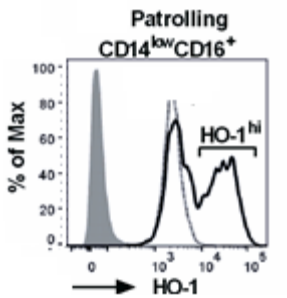
Neutrophils



Total monocytes

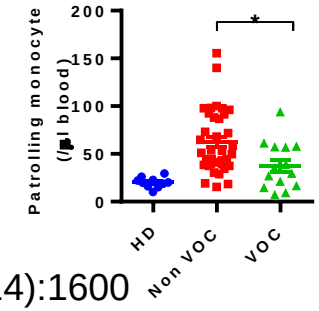




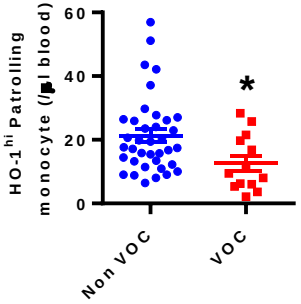


HO-1^{hi} patrolling Mo correlate negatively with VOC in SCD

Patrolling monocyte numbers

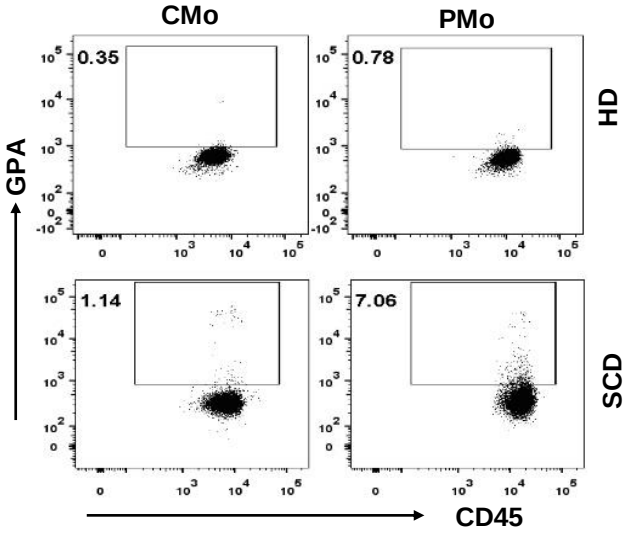


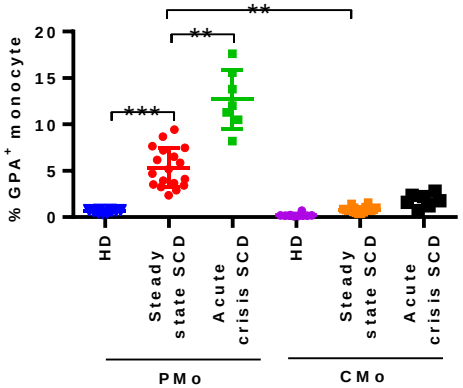
HO-1^{hi} Patrolling monocyte numbers

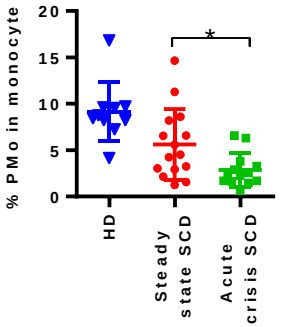


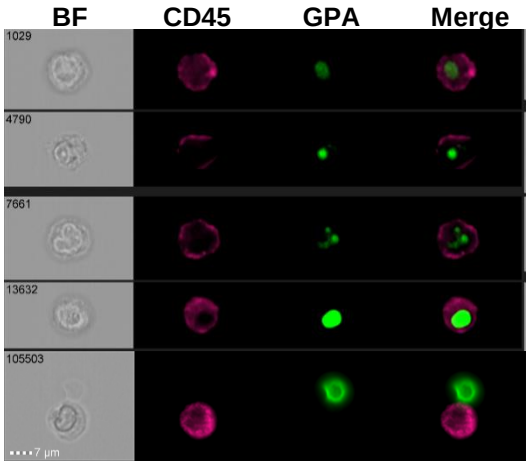
Liu …. Yazdanbakhsh. *Blood* 2018, 131(14):1600

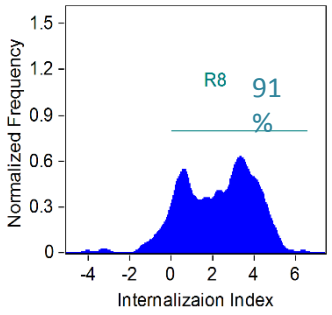
Phagocytosed RBCs in Circulating SCD PMos





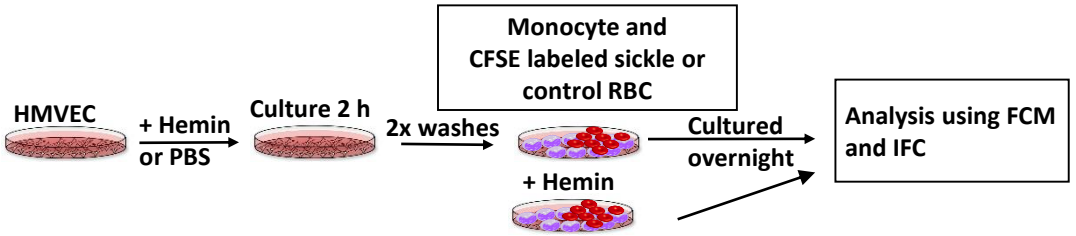




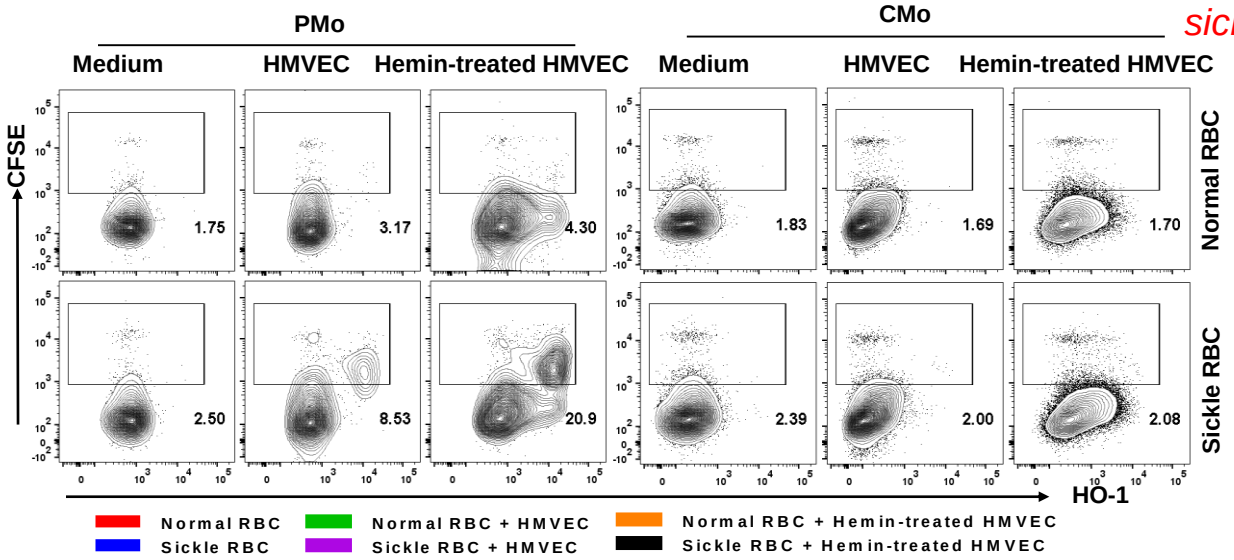


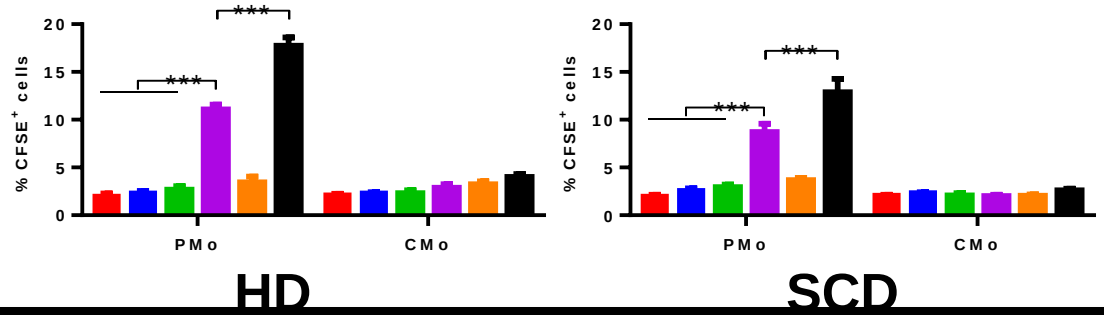
RBC engulfed material is present in the circulating PMos of patients with SCD which is further increased during crisis, and may lead to reduced PMo numbers.

Mechanism of Sickle RBCs Uptake by PMo



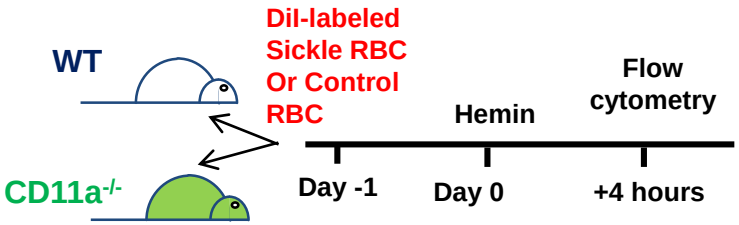
PMo uptake sickle RBC only when attached to ECs mostly through CD11a, CD18 and ICAM1; upregulate HO-1: cryoprotective; heme damaged ECs increase sickle RBC uptake by PMo

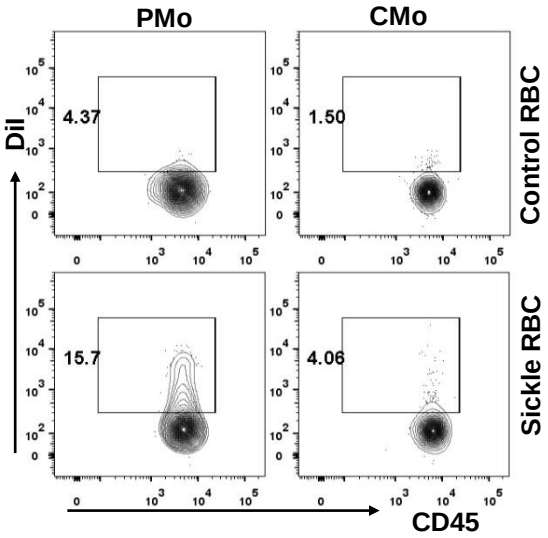


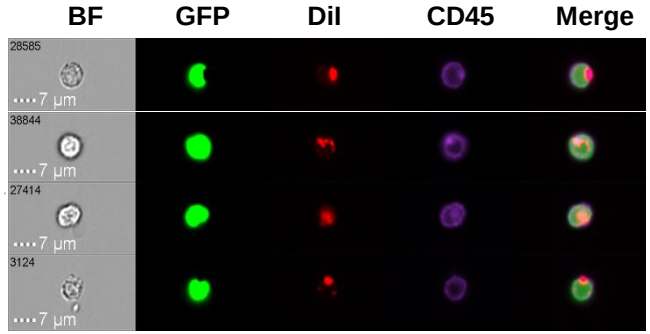


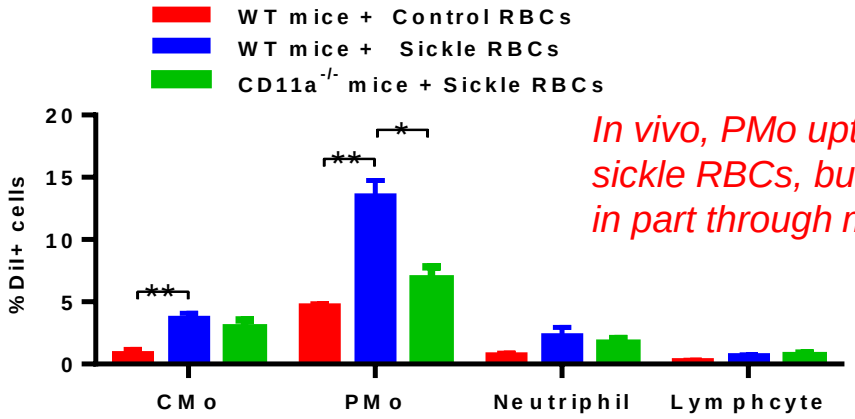
HD SCD

PMo Uptake Sickle RBCs In Vivo



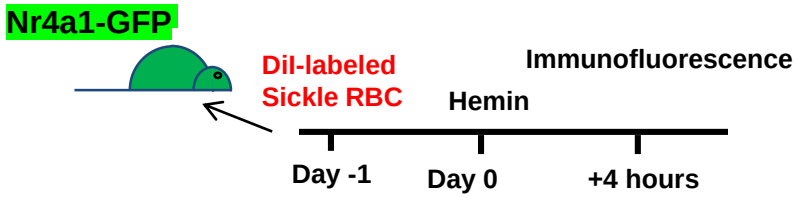


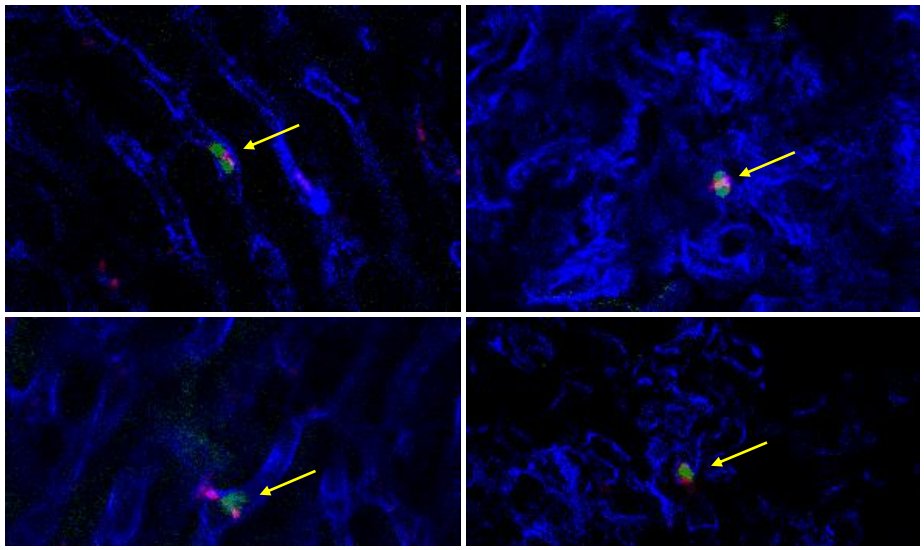




In vivo, PMo uptake sickle RBCs, but not control RBCs in part through monocyte CD11a

PMo Uptake EC-attached Sickle RBCs In Vivo

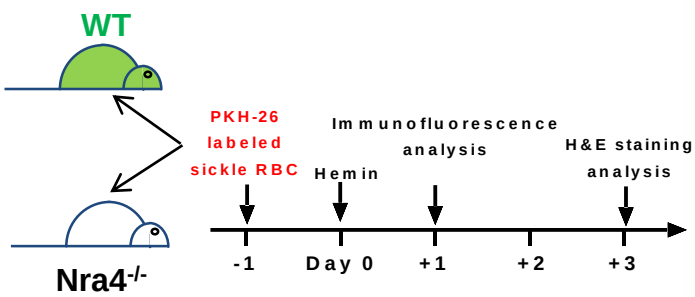


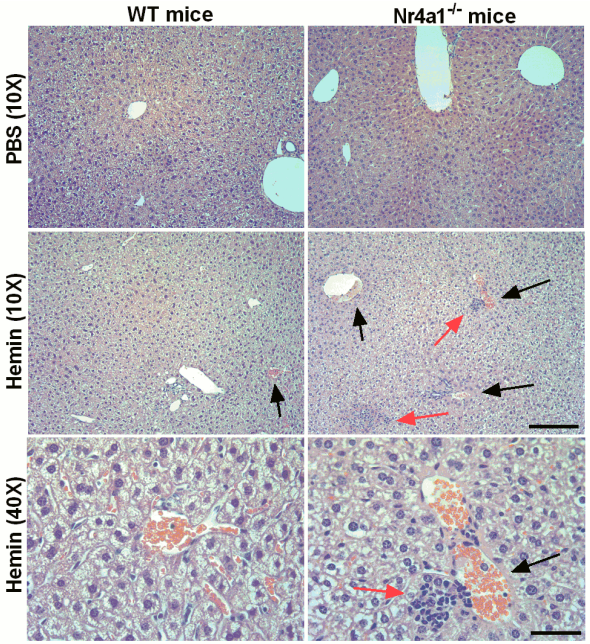


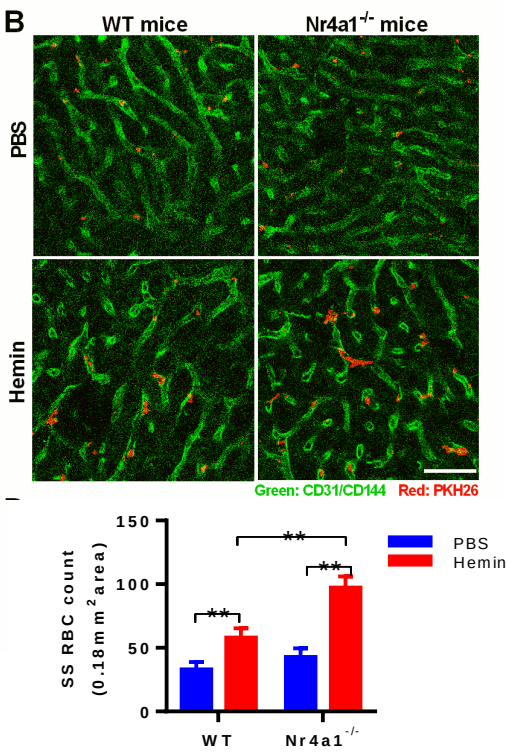
Blue: CD31/CD144, Red: DiI, Green: GFP

Scale = 10 µm

In Vivo Effects of Sickle RBCs and Hemin in Nr4a1-/- mice



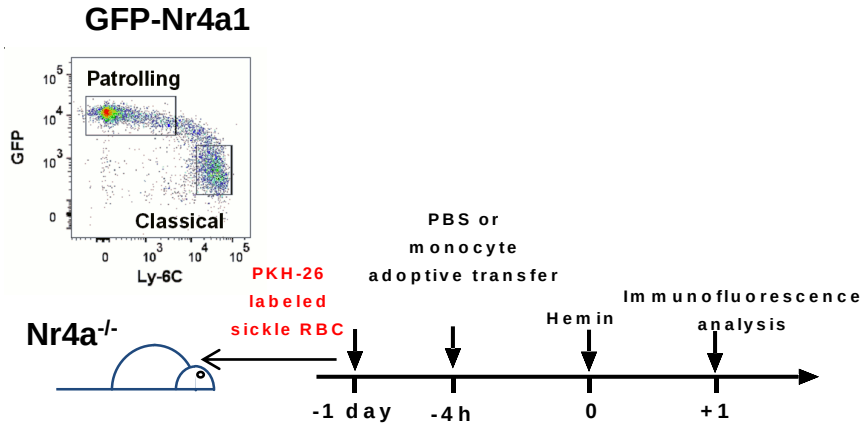


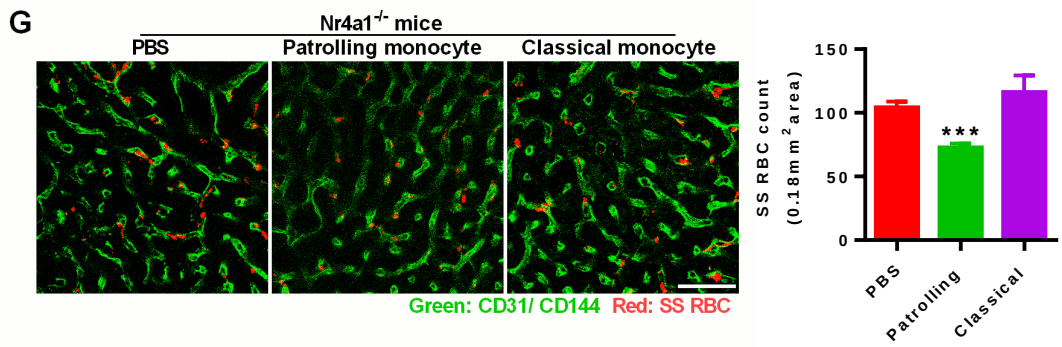


Lack of patrolling monocytes drives heme-mediated endothelial activation and SCD RBC stasis

Liu …. Yazdanbakhsh. *Blood* 2018, 131(14):1600

In Vivo Effects of Sickle RBCs and Hemin in Nr4a1-/- mice



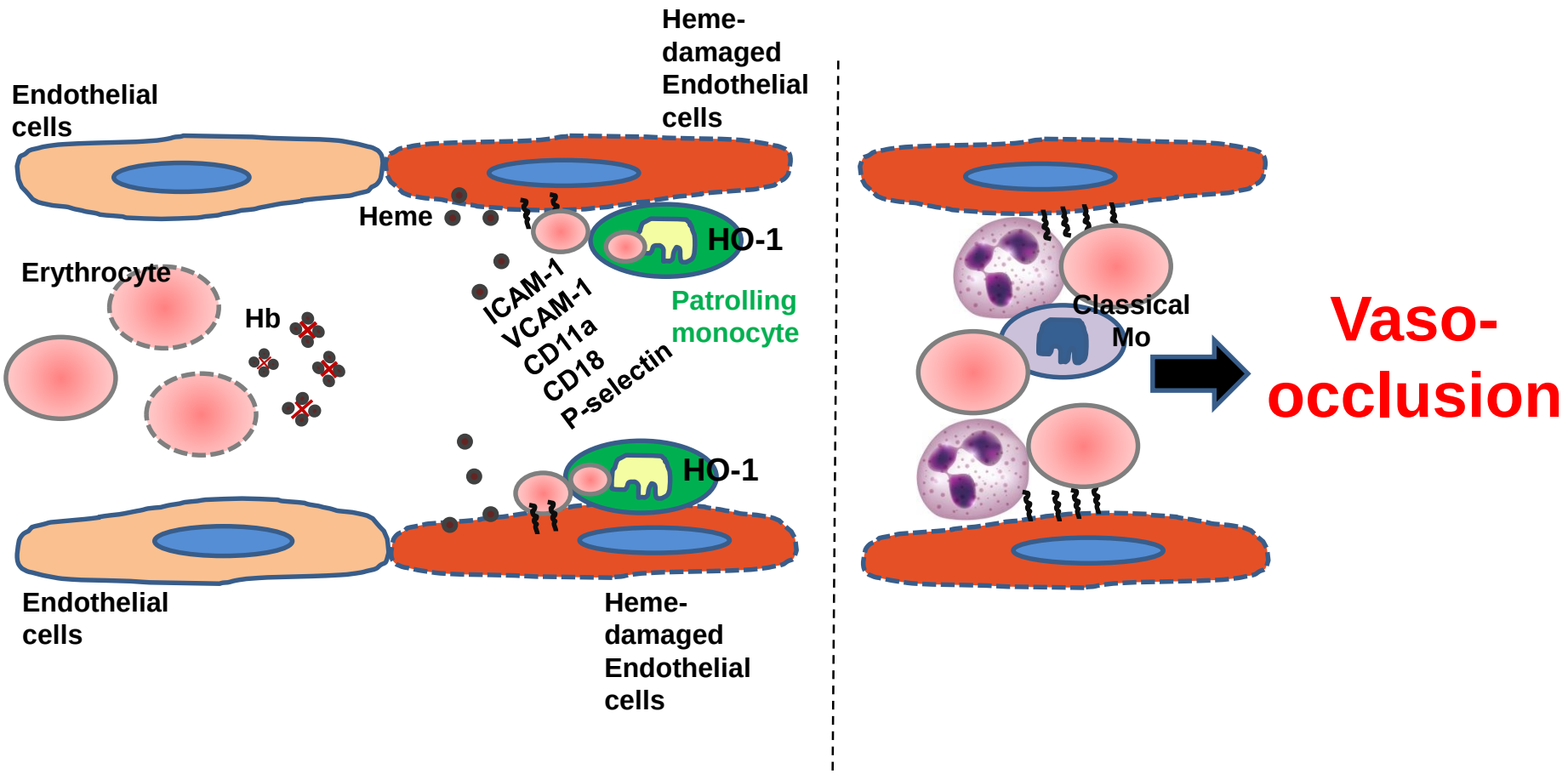


Patrolling monocyte protect against heme-driven endothelial activation and can inhibit hemolysis-driven SCD RBC stasis

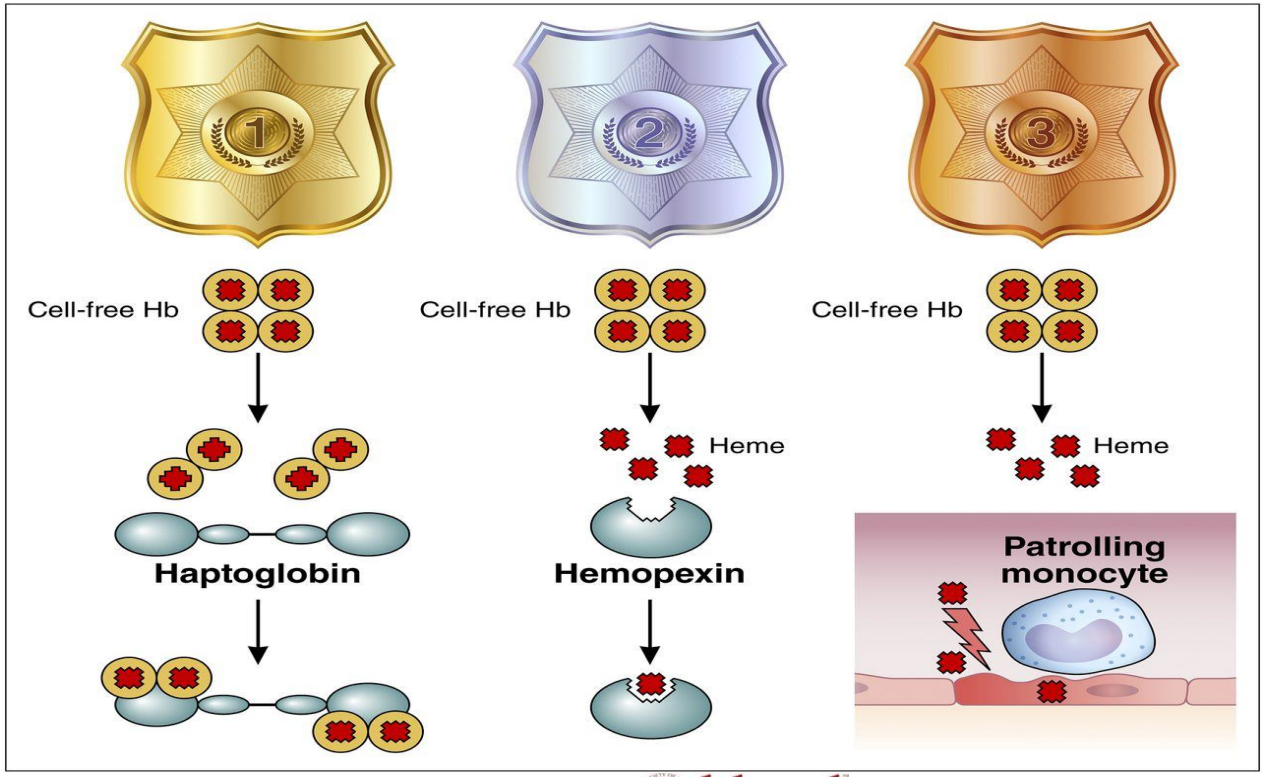
Liu …. Yazdanbakhsh. *Blood* 2018, 131(14):1600

With Patrolling monocyte

Without Patrolling monocyte



Protection from plasma cell-free hemoglobin and heme in sickle cell disease



Victor R. Gordeuk Blood 2018;131:1503-1505 blood

- Yunfeng Liu
- Hui Zhong
- Weili Bao
- Woelsung Yi
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American Heart Association

In Vivo Vascular Effects of Hemin in Nr4a1$^{-/-}$ Mice

PBS
or
Hemin

WT

Nr4a-/-
(no patrolling monocytes)

24hr

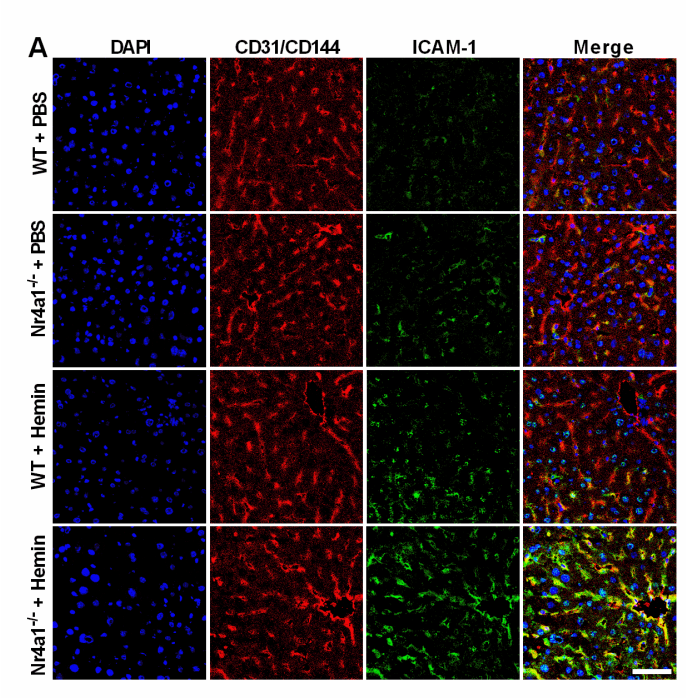
Immunofluorescence
(vascular activation: *ICAM-1 expression on CD31/CD144+ endothelium*)

WT +PBS

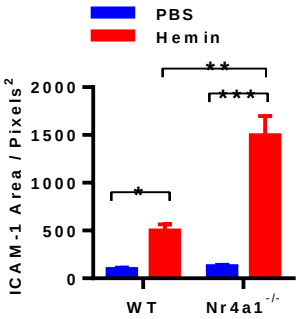
Nra4$^{-/-}$ +PBS

WT +hemin

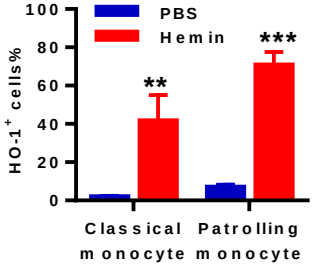
Nra4$^{-/-}$ +hemin



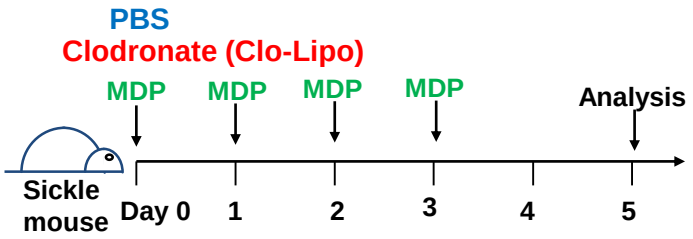
ICAM-1

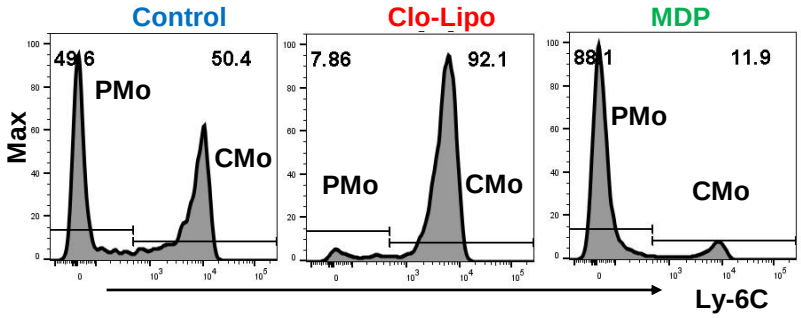


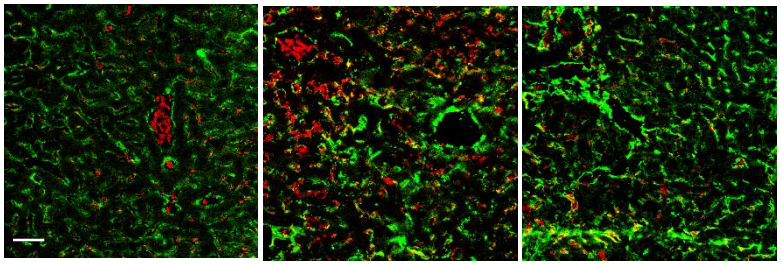
HO-1



Manipulation of PMo Numbers Affects Sickle RBC Stasis In Vivo

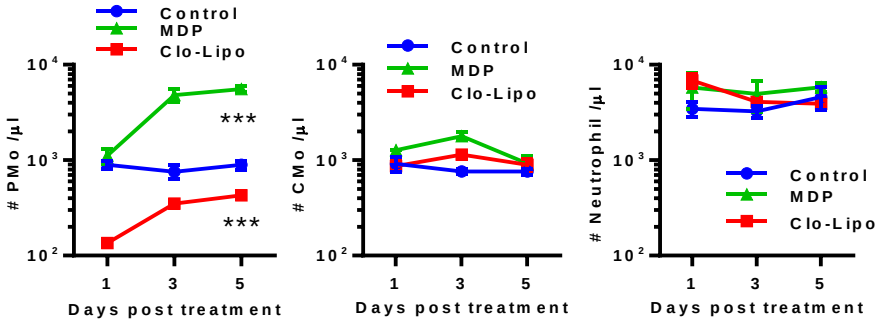


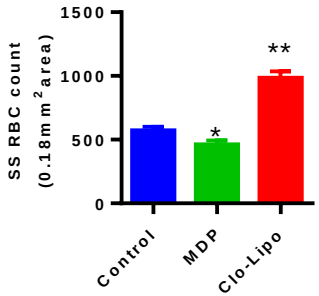


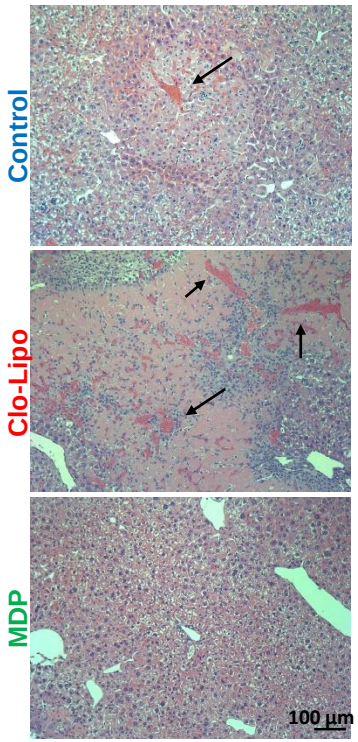


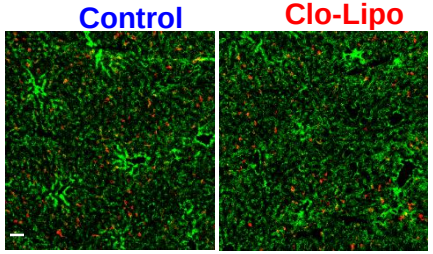
Scale = 50 µm

Red: Ter-119, Green: CD31/CD144









Scale = 100 µm Red: F4/80, Green: CD31/CD144

Depletion of PMo numbers increases sickle RBC attachment to vascular endothelium and RBC stasis in SCD mice, while increasing their nos protects against tissue/organ damage

Lessard, et al. *Cell Rep*. 2017;20:1830.

Biburger et al. *Immunity*. 2011;35:932