Risks and Tricks of Alloimmunization in Sickle Cell Disease

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Objectives

- Describe the effects of hemolysis on T cells from patients with sickle cell disease on transfusion therapy

- Discuss innate immune response to hemolysis in transfused patients with sickle cell disease

- Describe the effects of transfusions on circulating patrolling monocytes: protection against vaso-occlusion in sickle cell disease

SCD-Transfusion therapy

- An estimated 60-80% will receive at least one transfusion by the age of 20

- Blood transfusion is recommended therapy for SCD complications: stroke, acute chest syndrome, multi-organ failure syndrome and severe anemia and...

Transfusion Complications in SCD

- Alloimmunization
  - Life-threatening transfusion reactions
  - Difficulty obtaining compatible units, resulting in potentially critical delays in blood transfusion
- Higher alloimmunization rates
- Major reason: Differences in red blood cell antigen expression frequencies between the mostly Caucasian donor base and the mostly African-American SCD patients

Antigen-matching for Transfusions in SCD

- Majority of patients do not make antibodies therefore prophylactic matching is costly
- Supply logistics: RBCs of unusual phenotype are a limited resource and should potentially be reserved for patients who require these antigenic specifications
- Identify immune responsiveness of the patient, to predict in advance which patients will make antibodies

Hemolysis in SCD

- Intravascular hemolysis in SCD

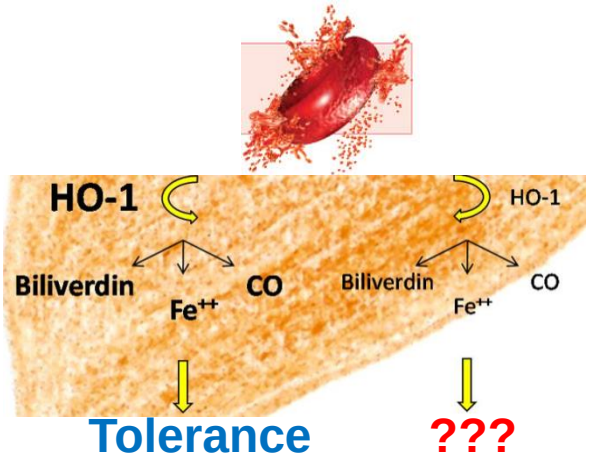
- Heme scavenging/removal system

(hemopexin and haptoglobin) is overwhelmed

- Anti-inflammatory heme oxygenase 1

(HO-1) breaks down heme;

upregulated in SCD



Hypothesis: *Ability of the immune cells to handle ongoing hemolysis is critical in alloimmunization*

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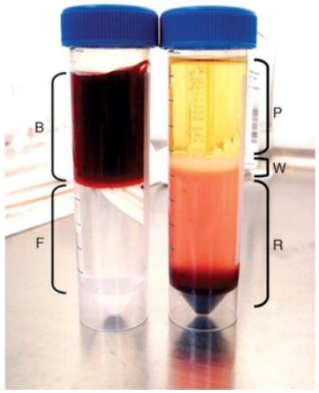
T cells critical for B cell help/Ab production

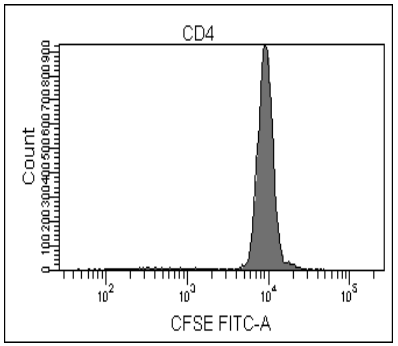
Does heme/hemin alter T cell proliferation/polarization?

Anti-CD3 stimulation assay



PBMC +CFSE

CD4

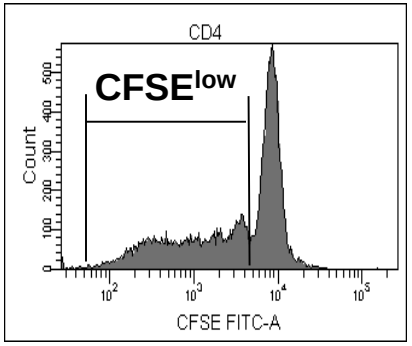


CFSE

Anti-CD3

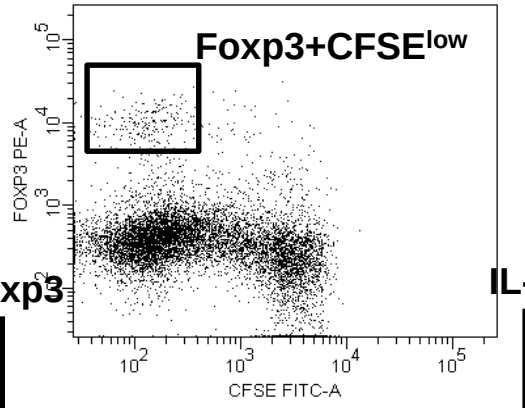
7 days

CD4



CFSE

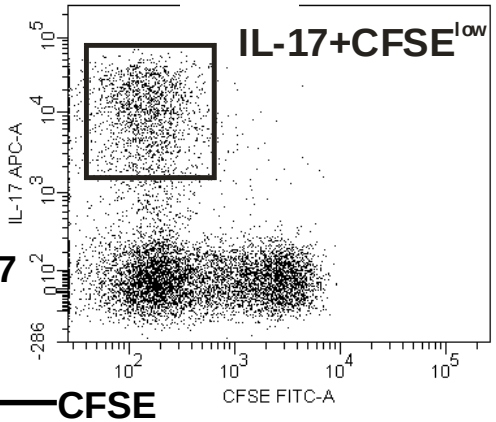
Tregs



Foxp3

CFSE

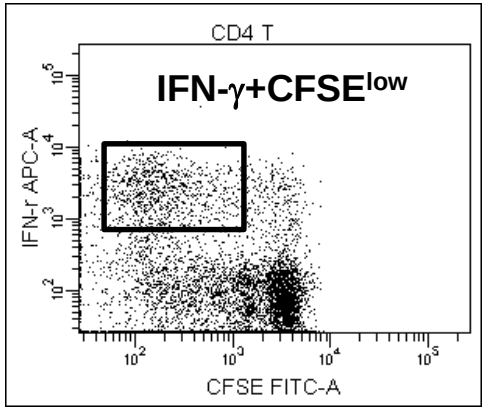
Th17



IL-17

CFSE

Th1



IFN-γ

CFSE

Zhong et al, **Blood. 2012 ;120:3326**
***Zhong and Yazdanbakhsh.* Blood; 2013, 121:2494-502**

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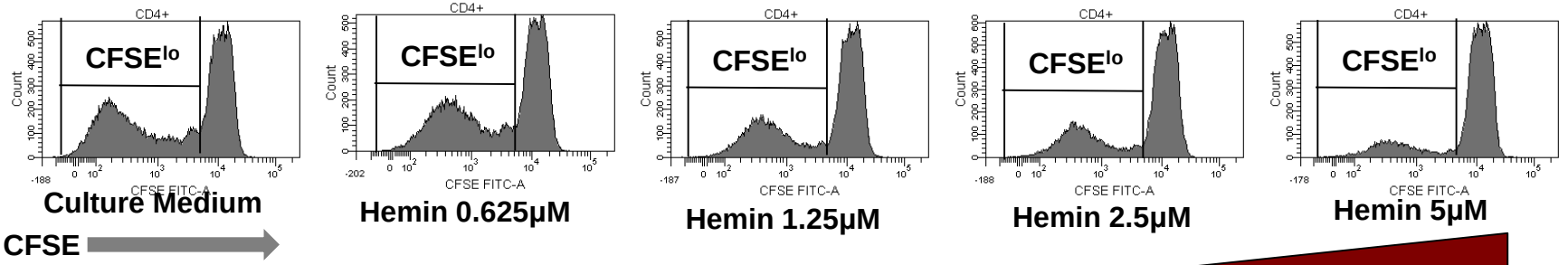
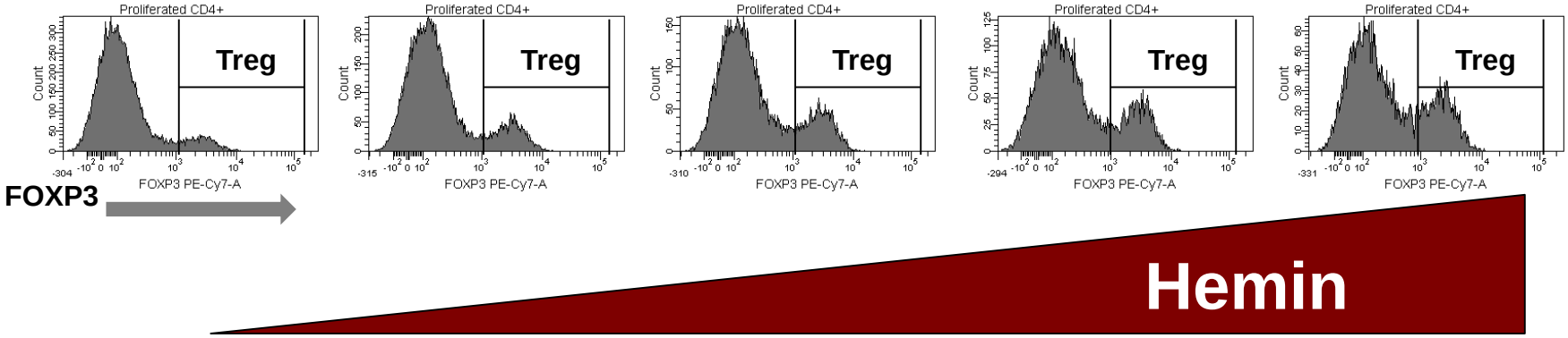
Heme response in Healthy Donors

Proliferation



Treg



Zhong et al, **J Immunol. 2014;193:102.**

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Heme response in Healthy Donors

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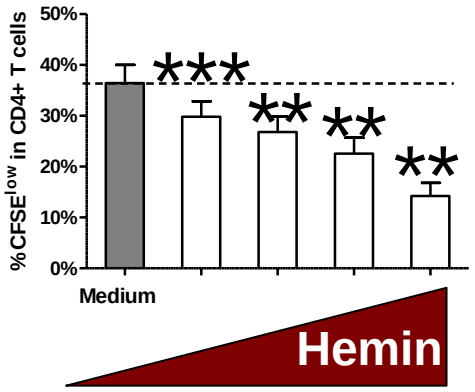
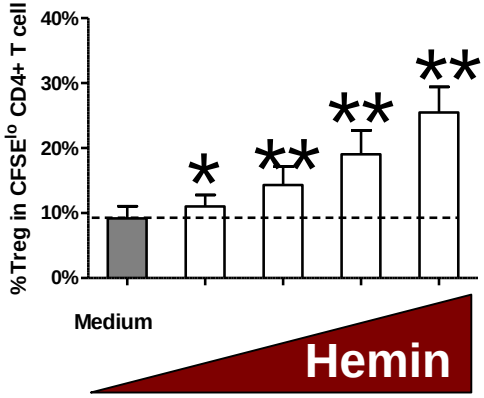
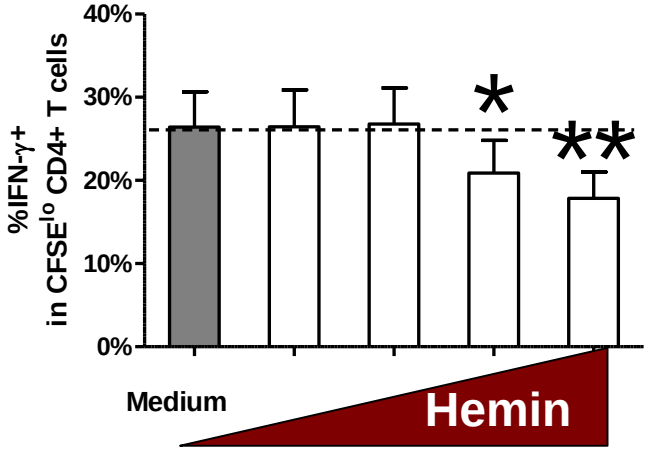


Treg



Th1



Zhong et al, **J Immunol. 2014;193:102.**

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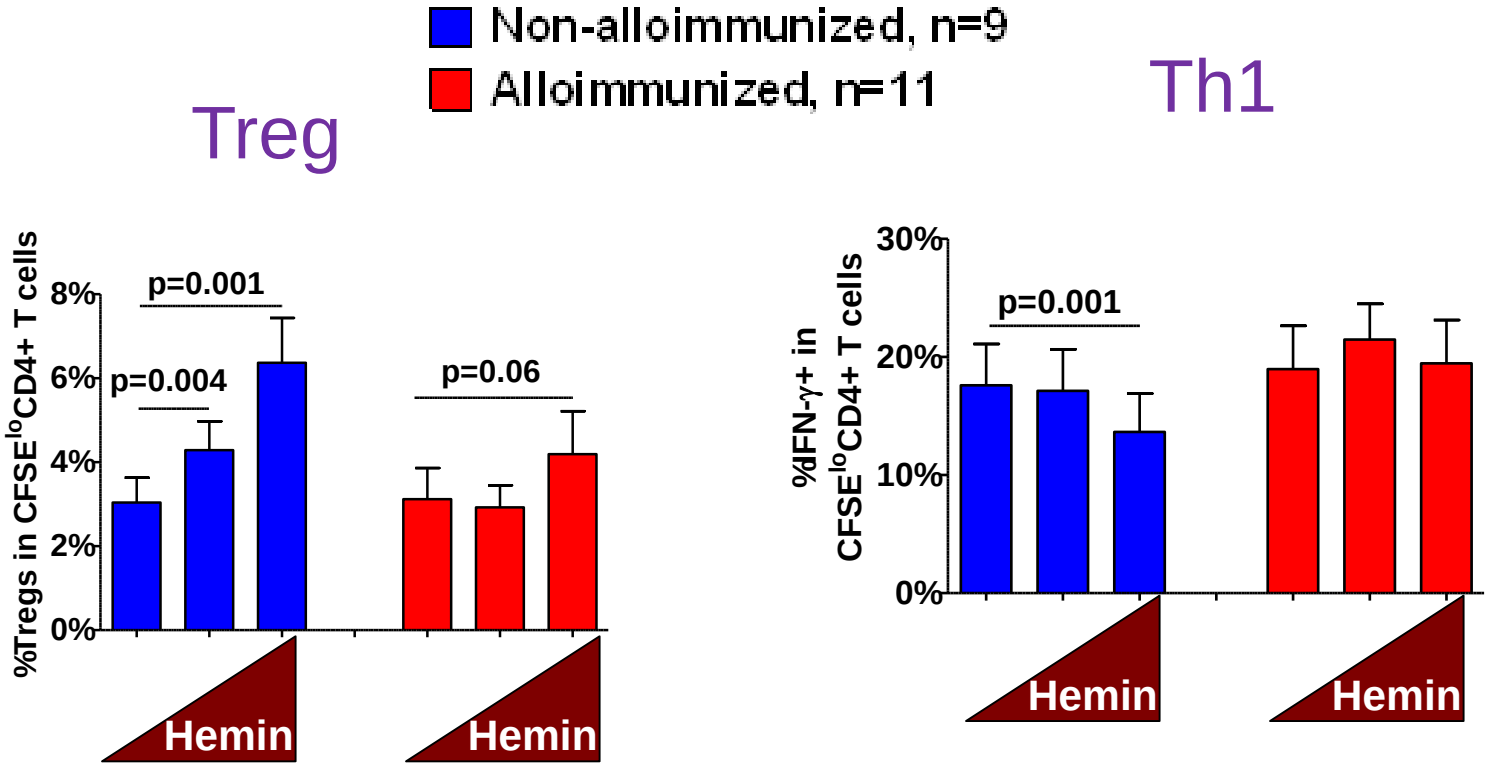
Alloimmunized Patients with SCD have Blunted Treg/Th1 Expansion in Response to Heme



Analysis of T cell Priming by Dendritic Cells (DCs)

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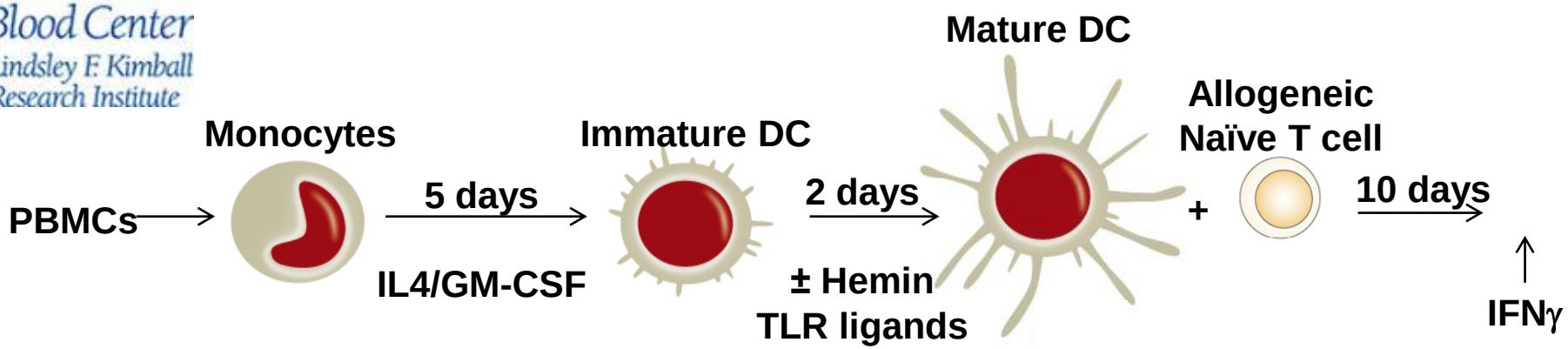
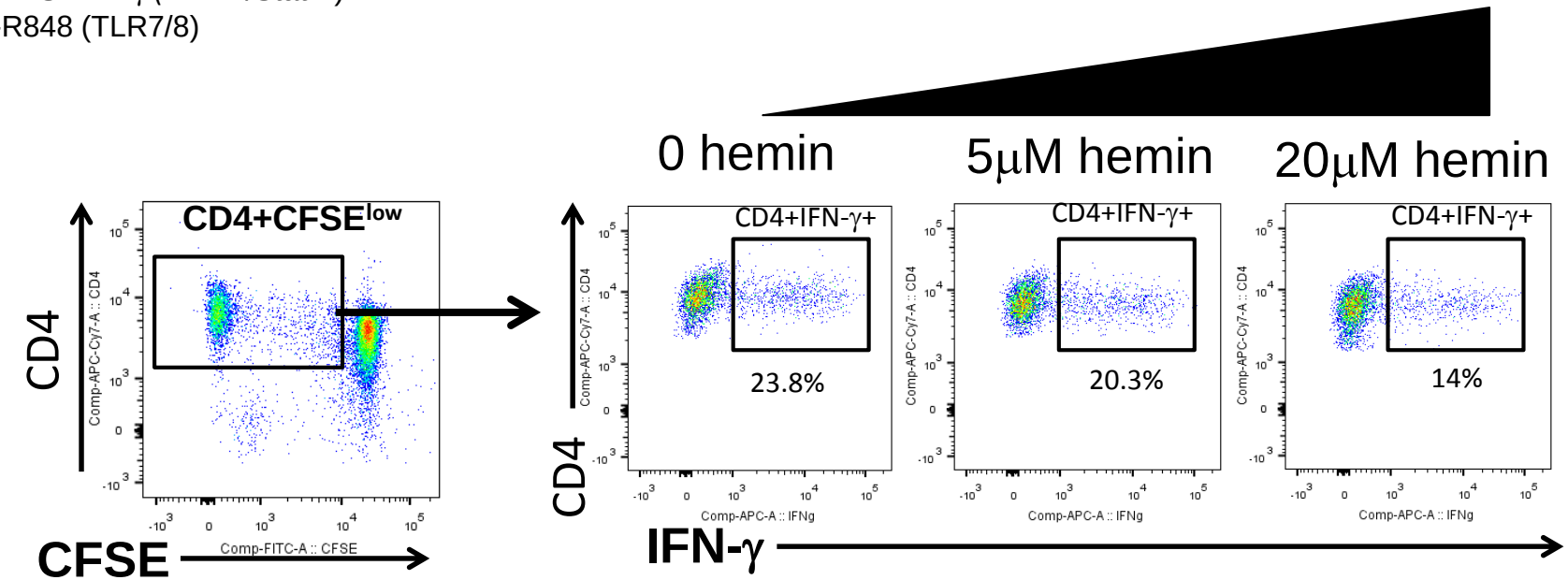
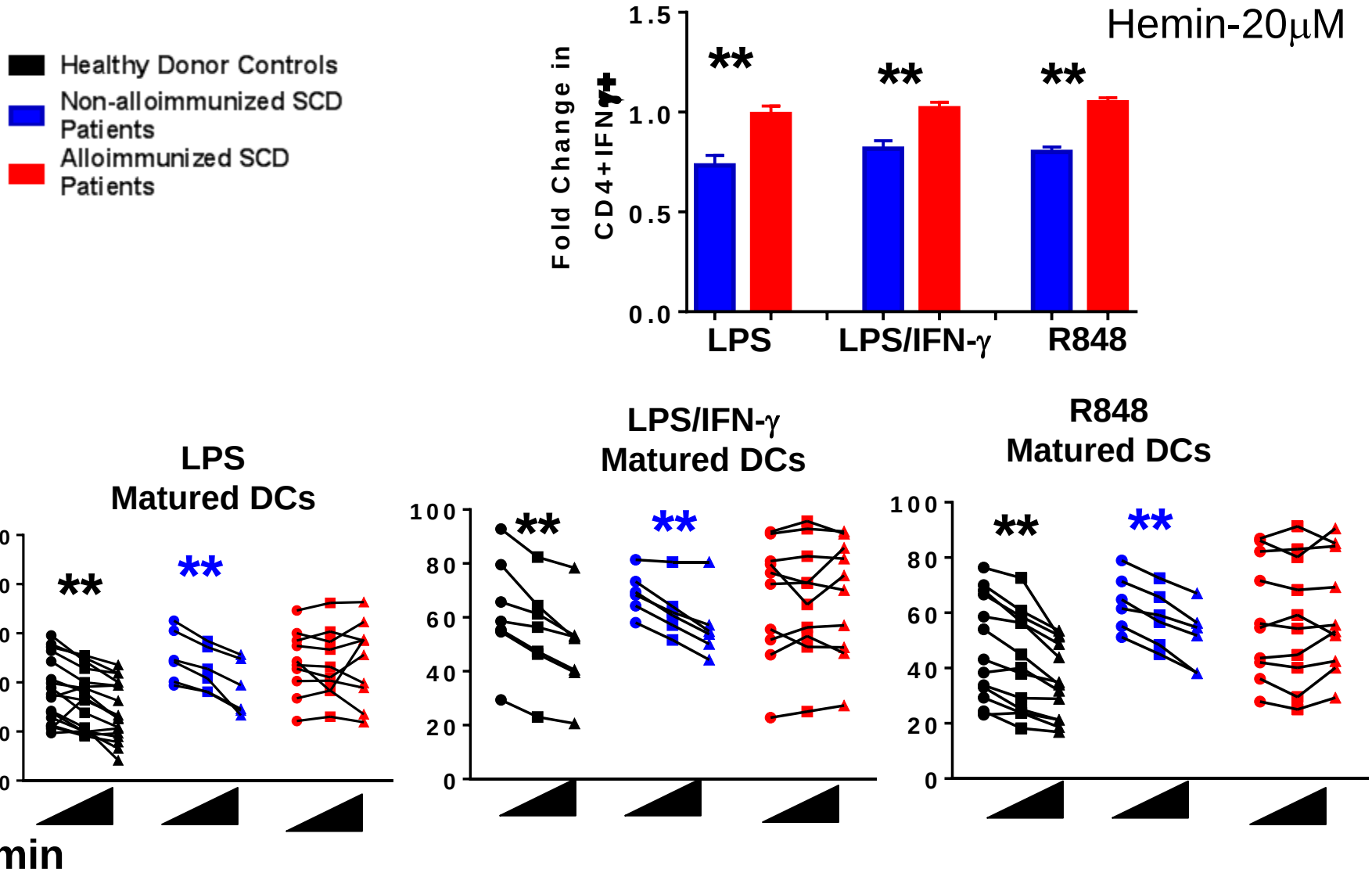


TLR ligands for DC maturation:
-LPS (TLR-4)
-LPS+IFN-γ (TLR-4/Stat-1)
-R848 (TLR7/8)



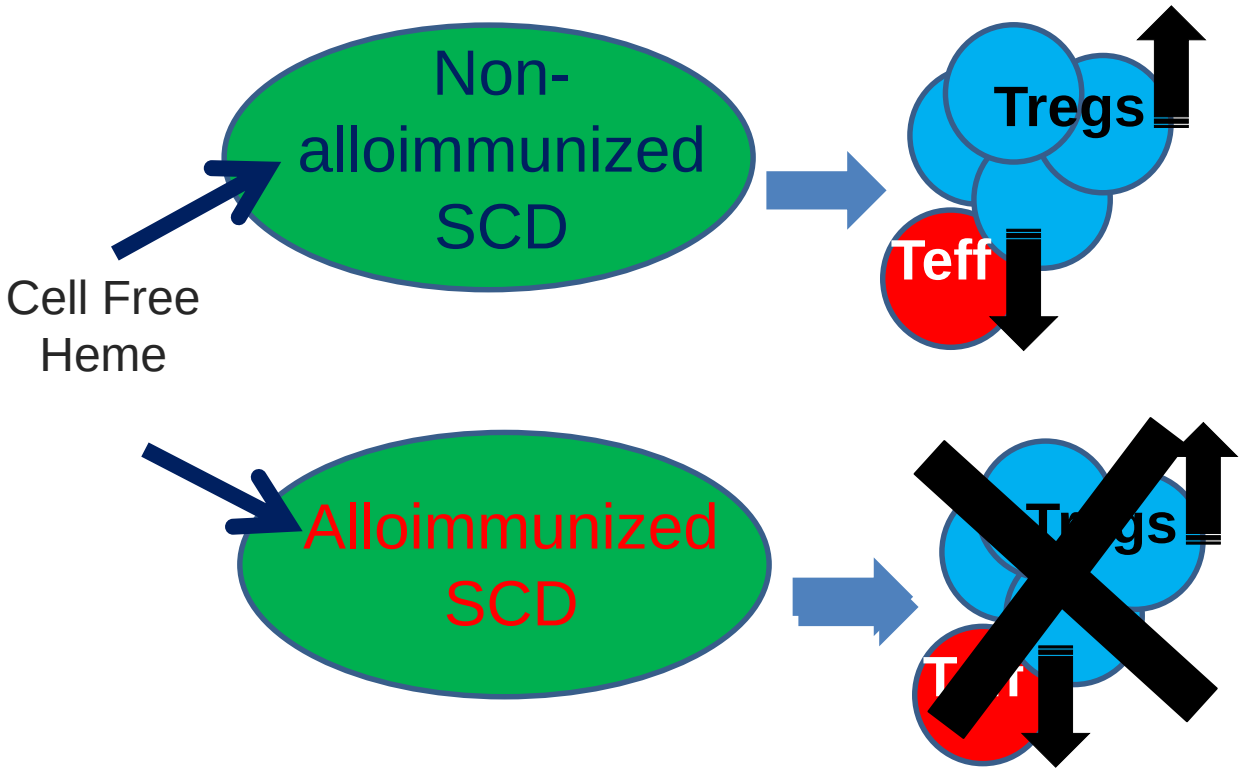
***Godefroy et al*, Hematologica 2016**

Impaired Inhibition of Th1 Priming in Response to Heme in Alloimmunized SCD Patients



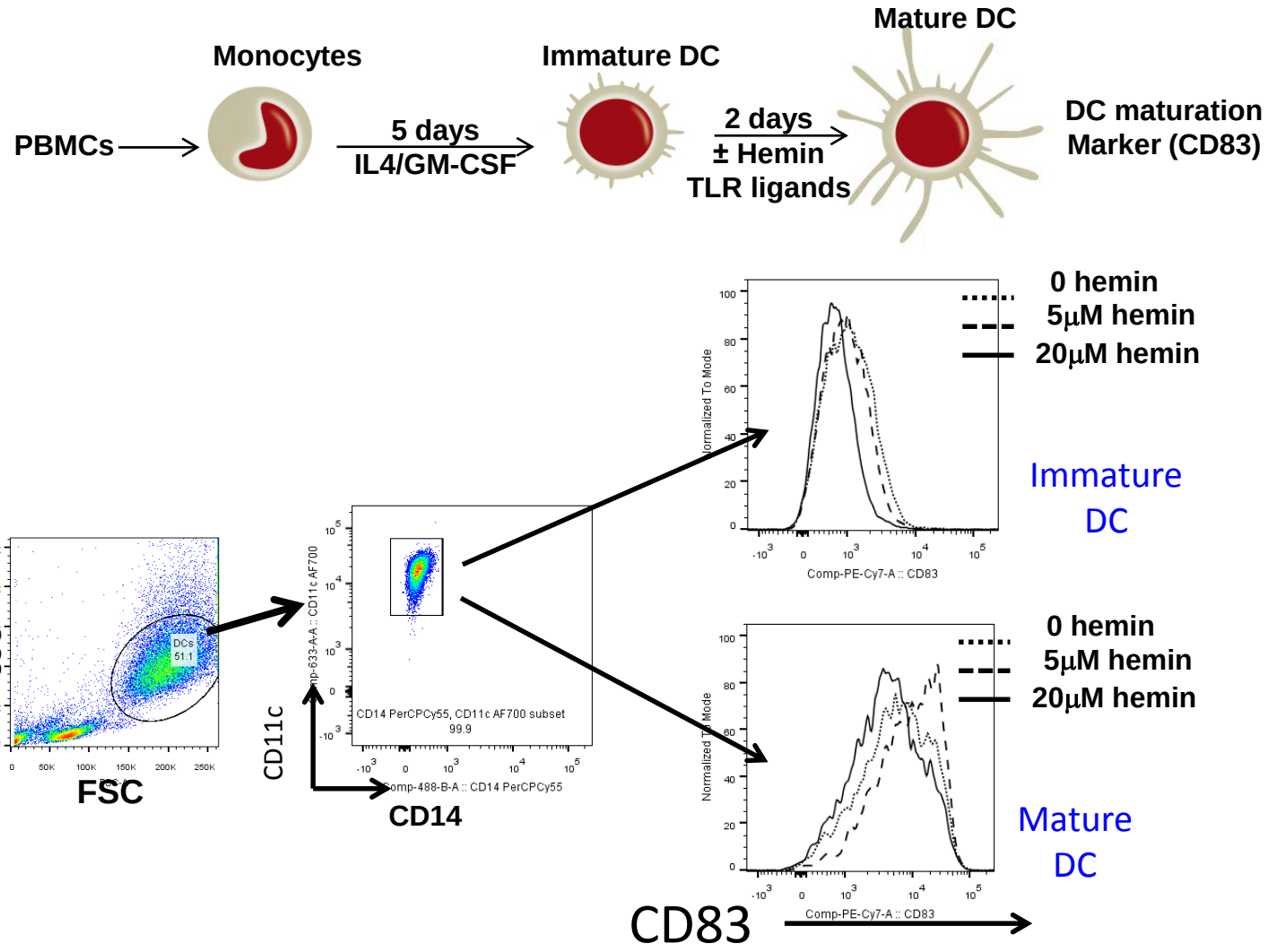
Godefroy et al, **Hematologica 2016**

Hypothetical model



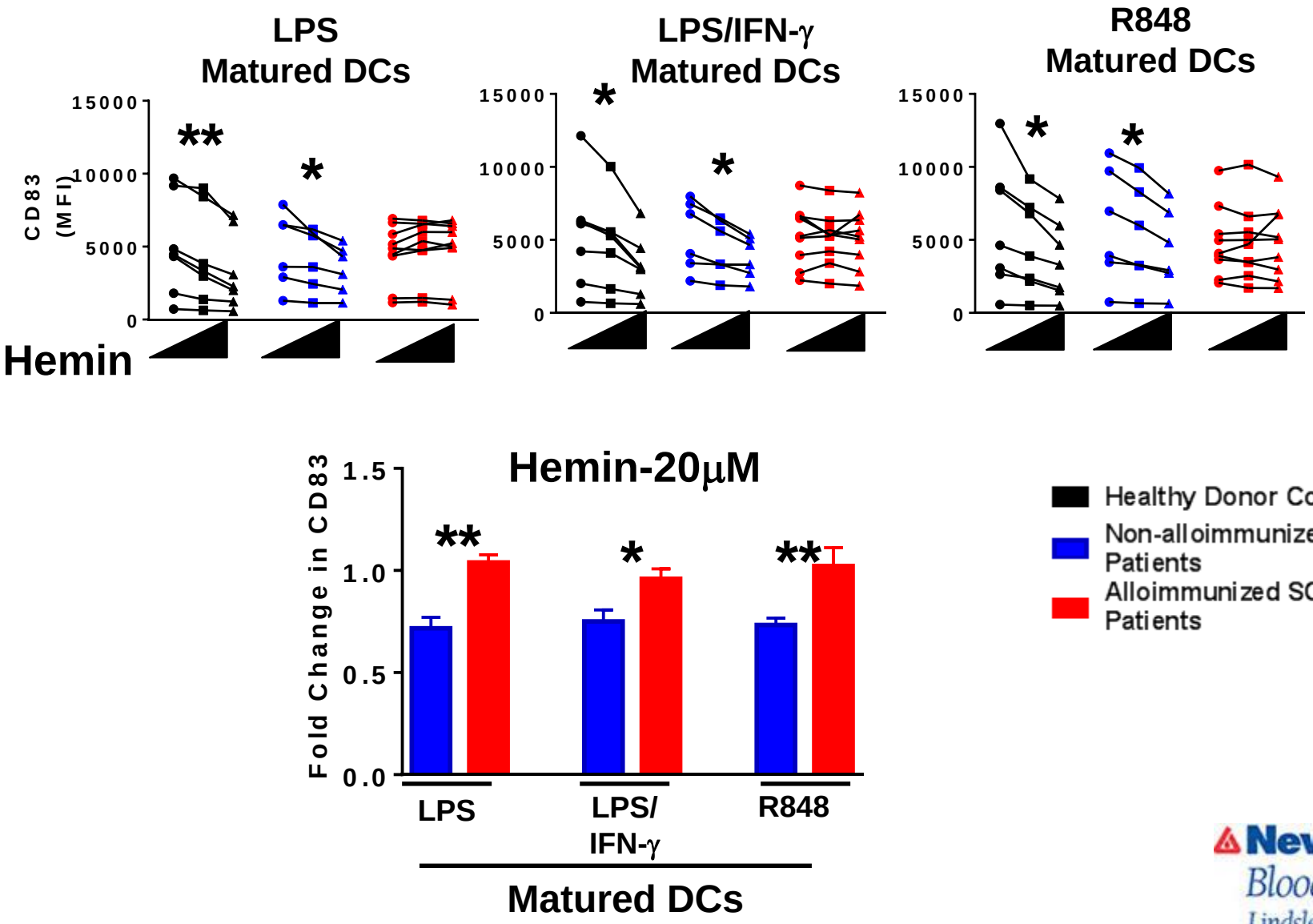
Mechanism of Altered Innate Immune Reactivity by Free Heme

Detection of DC Maturation



***Godefroy et al*, Hematologica 2016**

Impaired Downregulation of CD83 Maturation Marker on DCs in Alloimmunized Patients in Response to Heme



Godefroy et al, **Hematologica 2016**

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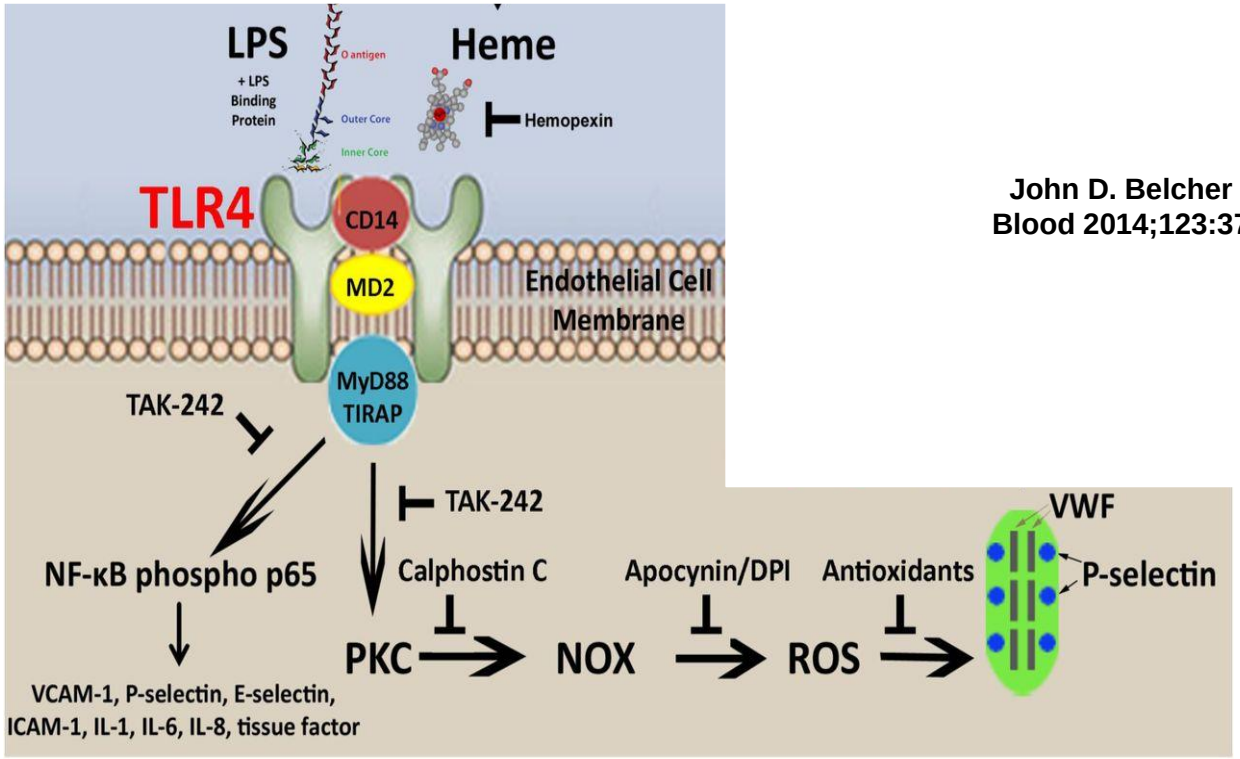
Pro-oxidant and Pro-inflammatory Effects of Cell Free Heme in Endothelial Cells



John D. Belcher et al.
Blood 2014;123:377-390

blood

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Regulation of CD83 Expression by Free Heme is through TLR-4

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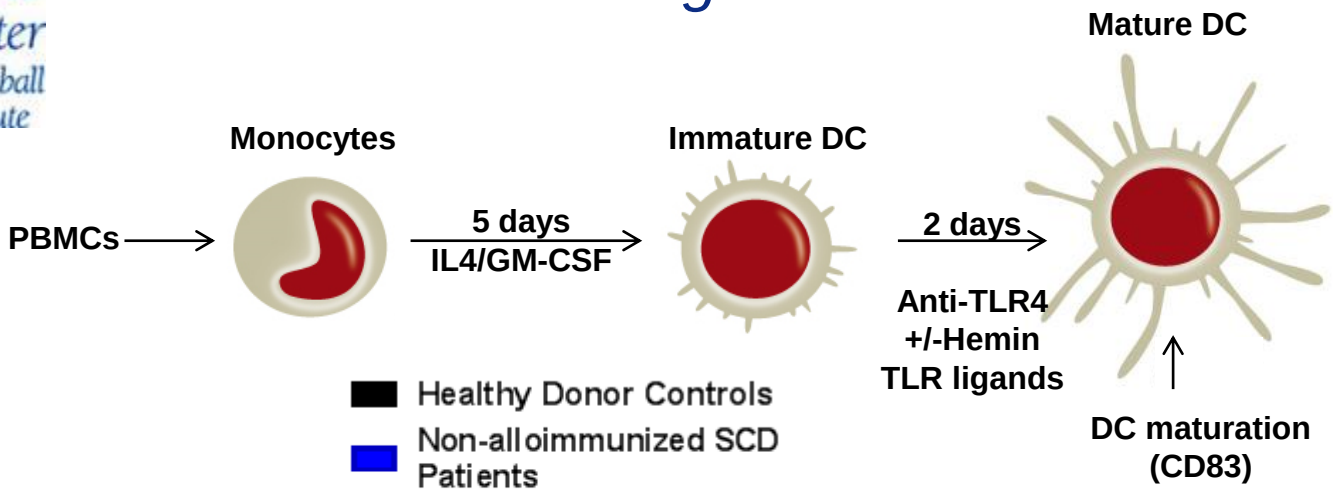
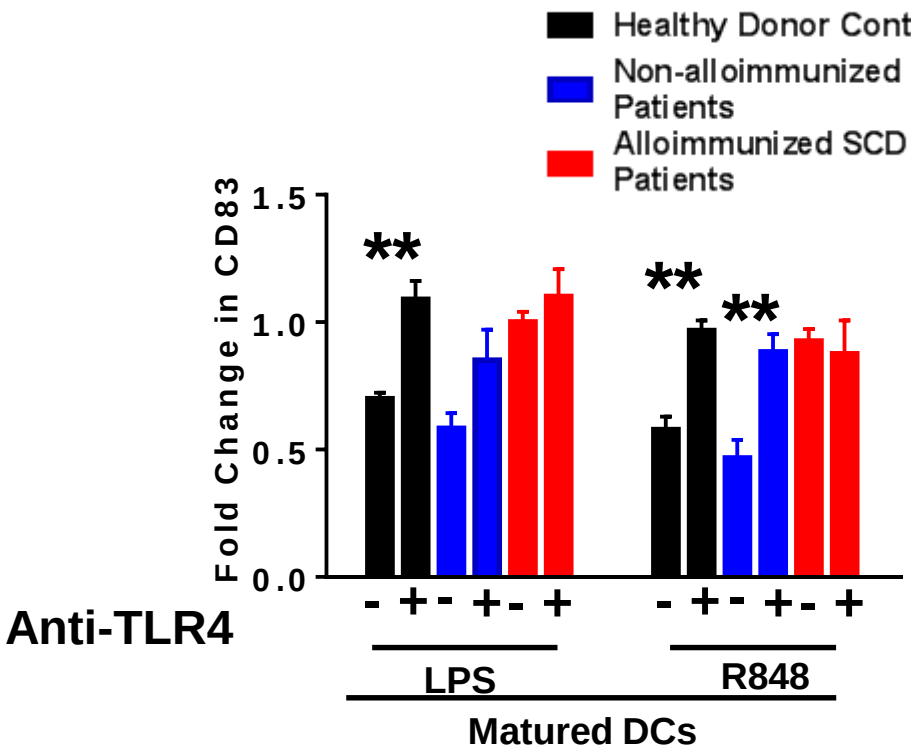
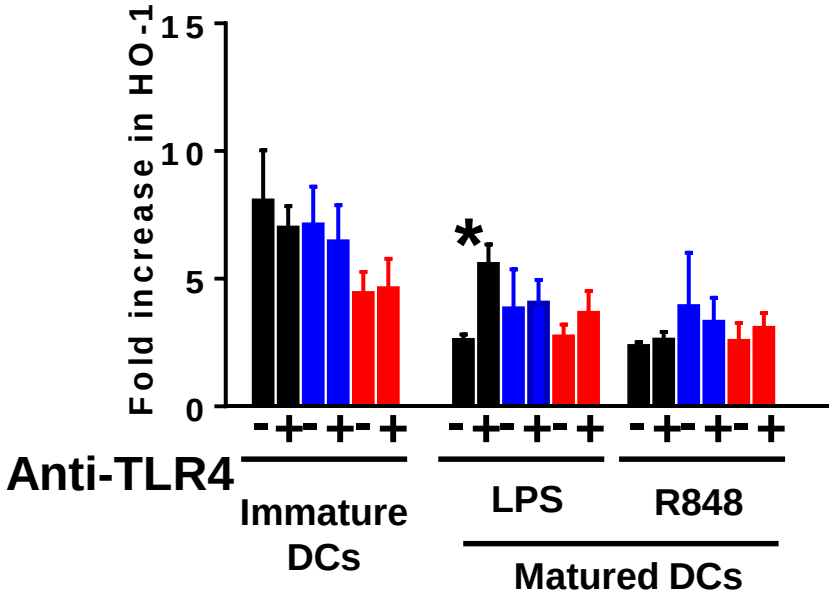
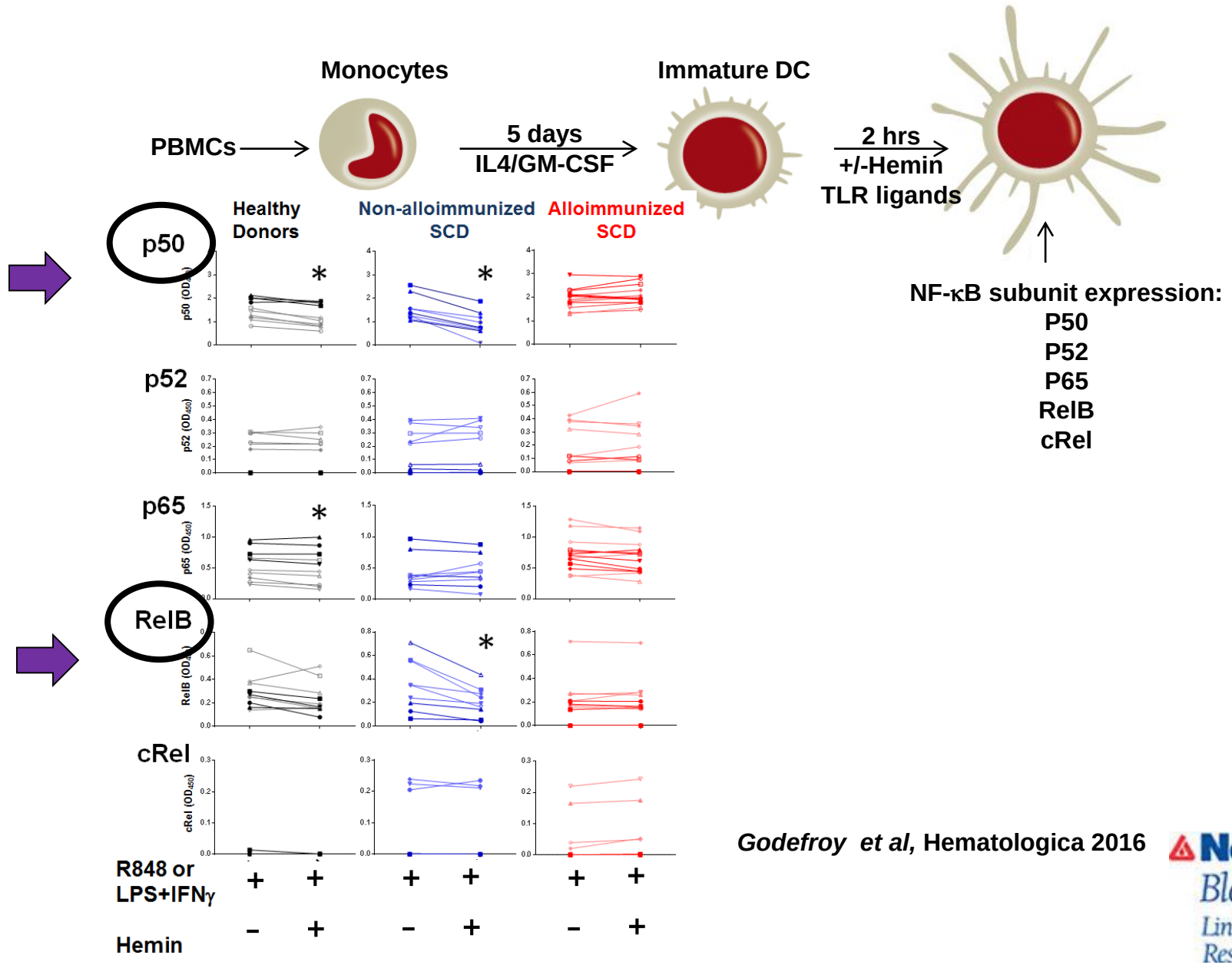






Godefroy et al, Hematologica 2016

Heme-driven NF-κB Expression in DCs



Godefroy et al, Hematologica 2016

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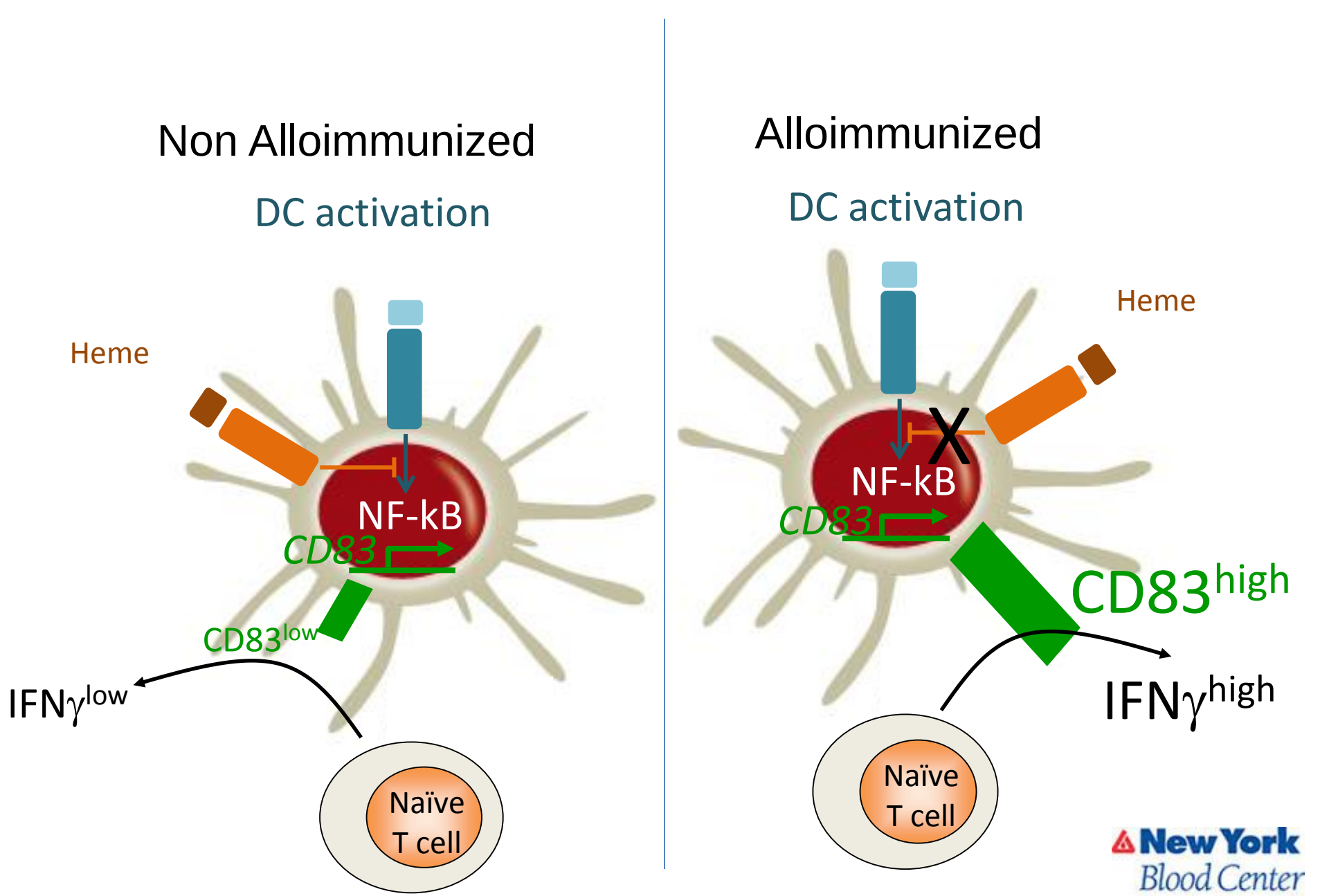

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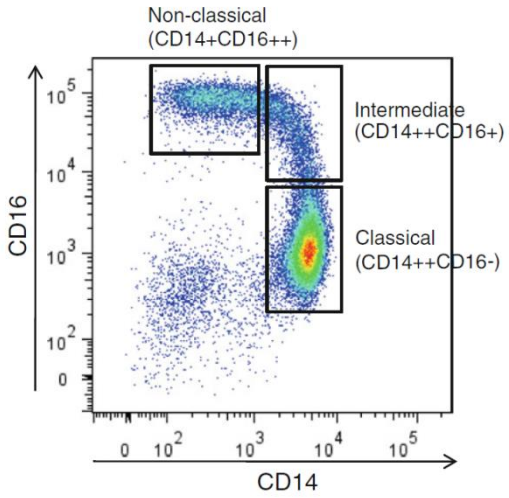
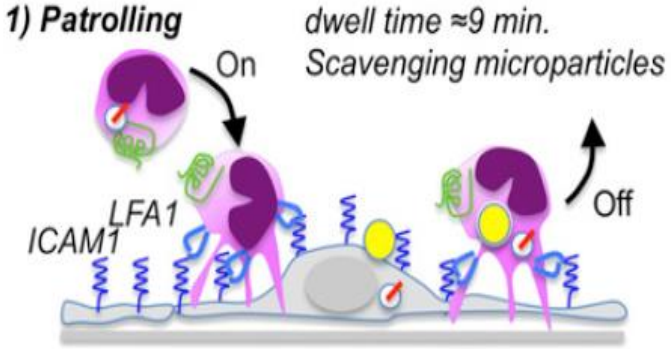
Patrolling Monocytes



- 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.
Michaud et al. (2013) Cell Rep 5(3):646-653.
Zhong …Yazdanbakhsh. (2014). J Immunol 193(1):102-110.

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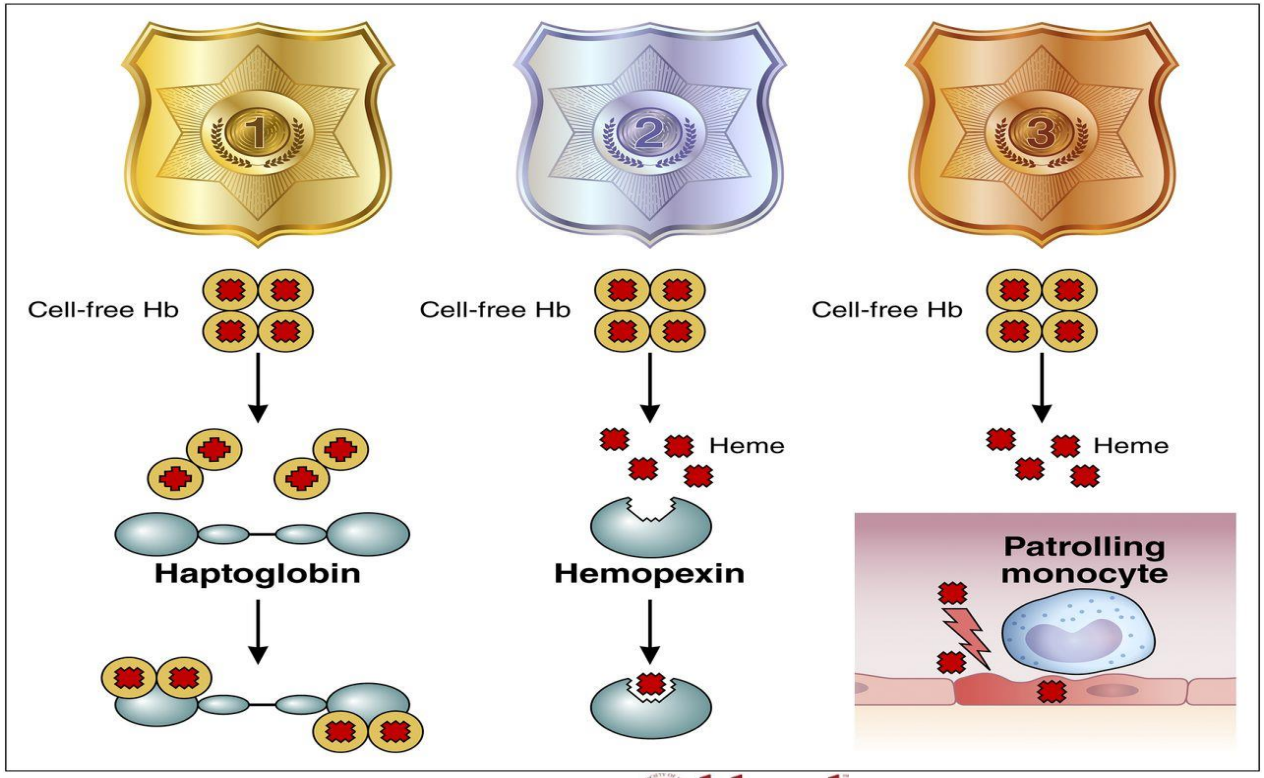
Protection from plasma cell-free hemoglobin and heme in sickle cell disease



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

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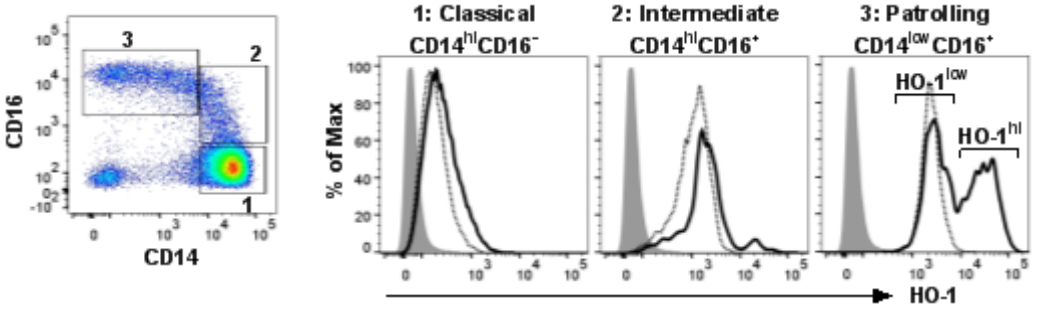
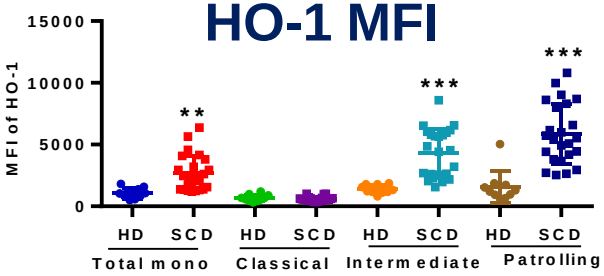
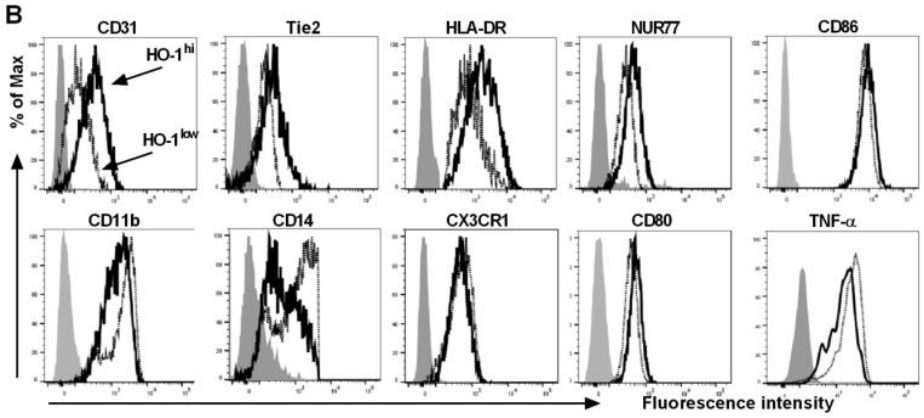
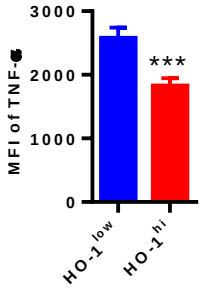
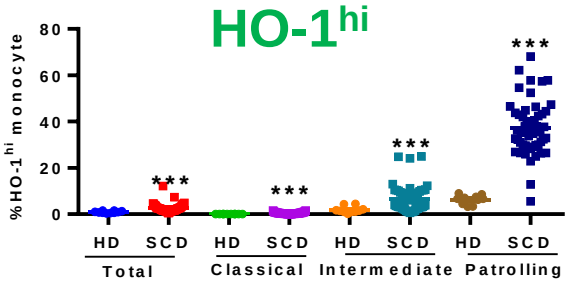
HO-1 expressing Patrolling Monocyte Characterization in SCD







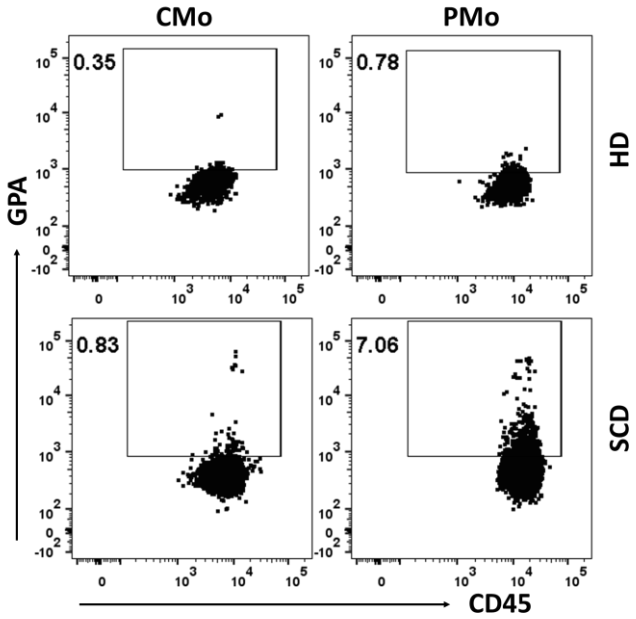


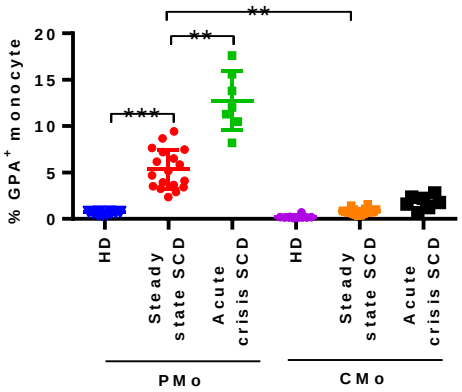


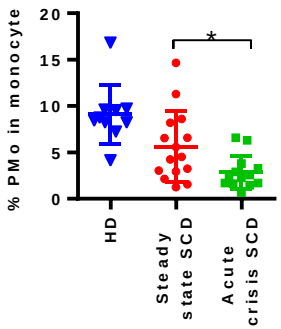
Expanded subpopulation of circulating patrolling monocytes expressing high levels of HO-1 in SCD

Liu …. Yazdanbakhsh. *Blood* 2018, 131(14):1600
Liu et al. *Blood* 2019

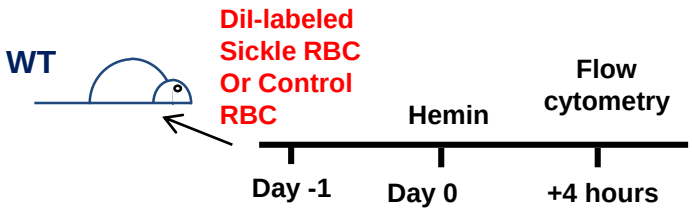
Phagocytosed RBCs in Circulating SCD PMos

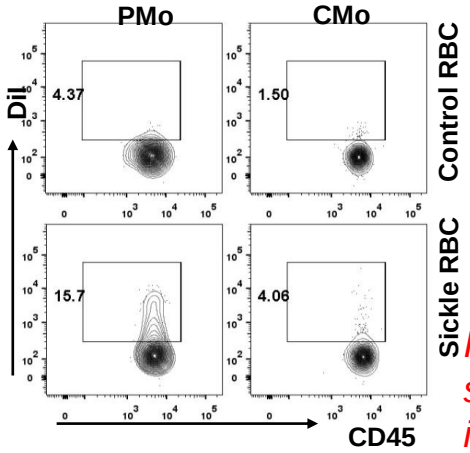






RBC engulfed material is present in the circulating PMos of patients with SCD which is further increased during crisis, and may lead to reduced PMo numbers.

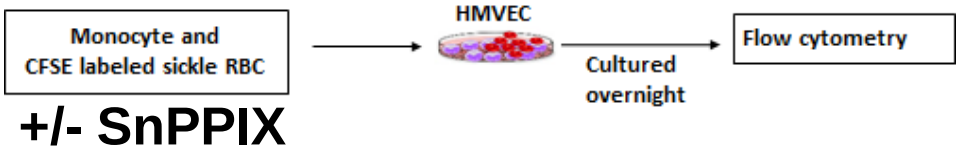


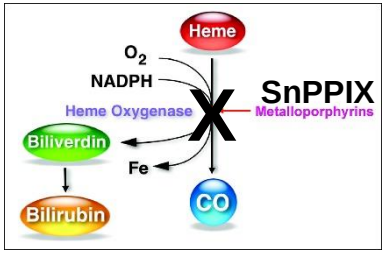


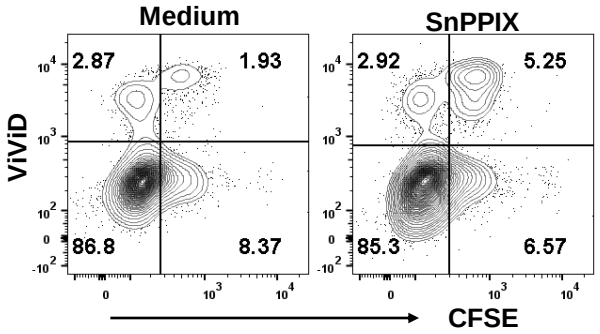
In vivo, PMo uptake sickle RBCs, but not control RBCs in part through monocyte CD11a

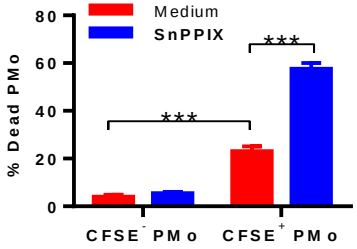
Liu et al. *Blood* 2019

Cryoprotective HO-1 Expression in Sickle RBC Phagocytosed PMo









Sickle RBC engulfed PMo up-regulate HO-1 which in turn is cytoprotective

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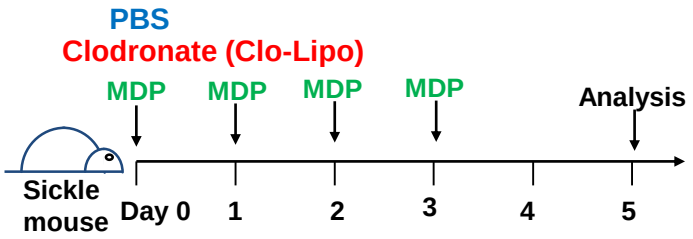
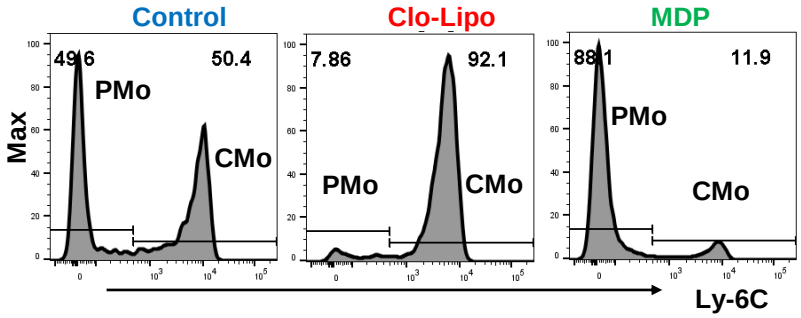
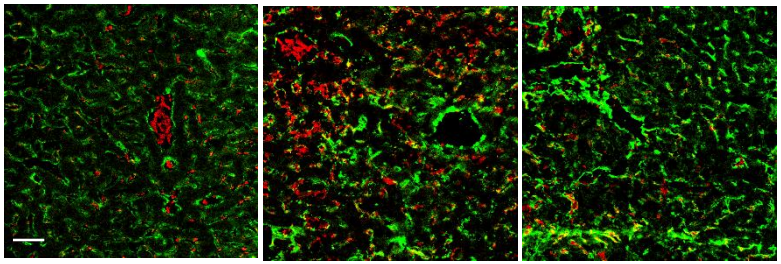
Liu et al. *Blood* 2019

Manipulation of PMo Numbers Affects Sickle RBC Stasis In Vivo

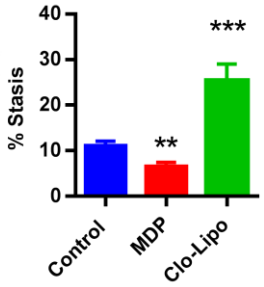


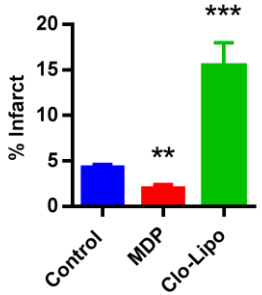


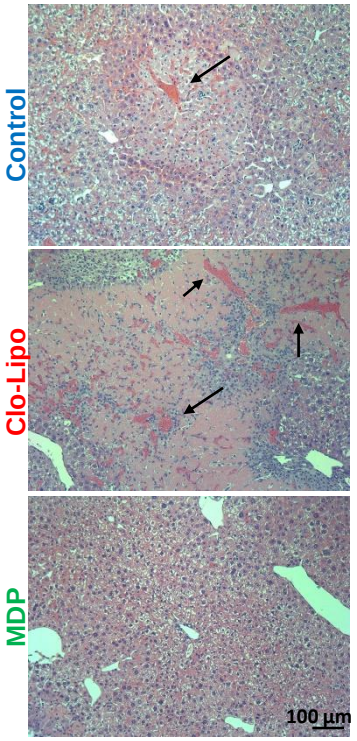


Scale = 50 µm

Red: Ter-119, 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

Liu et al. *Blood* 2019

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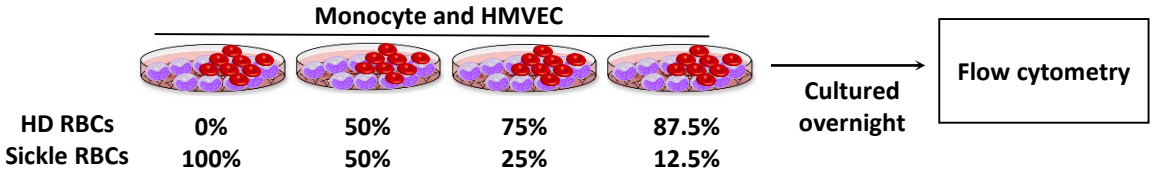
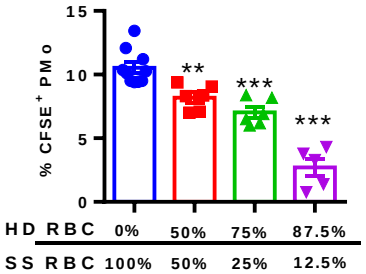
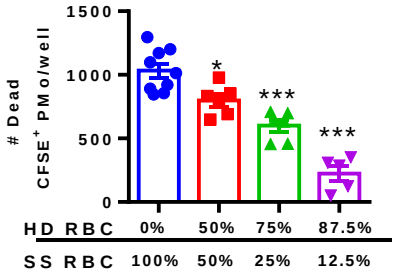
Can transfusions help PMo survival?

Increased Survival of PMos in In Vitro transfusion model







"In vitro transfusions" with healthy donor RBCs reduce PMo uptake of sickle RBCs, leading to improved survival of PMos.

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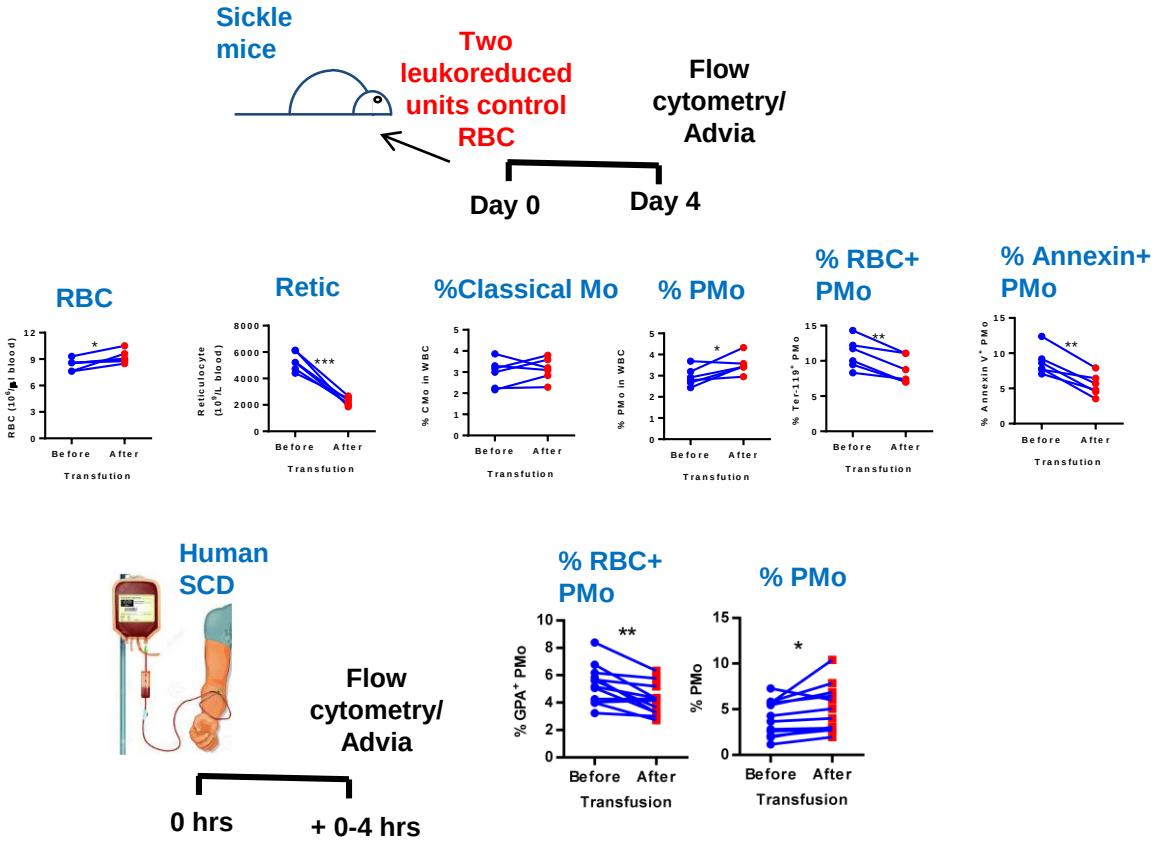
Increased Survival of PMos In Vivo After Transfusion

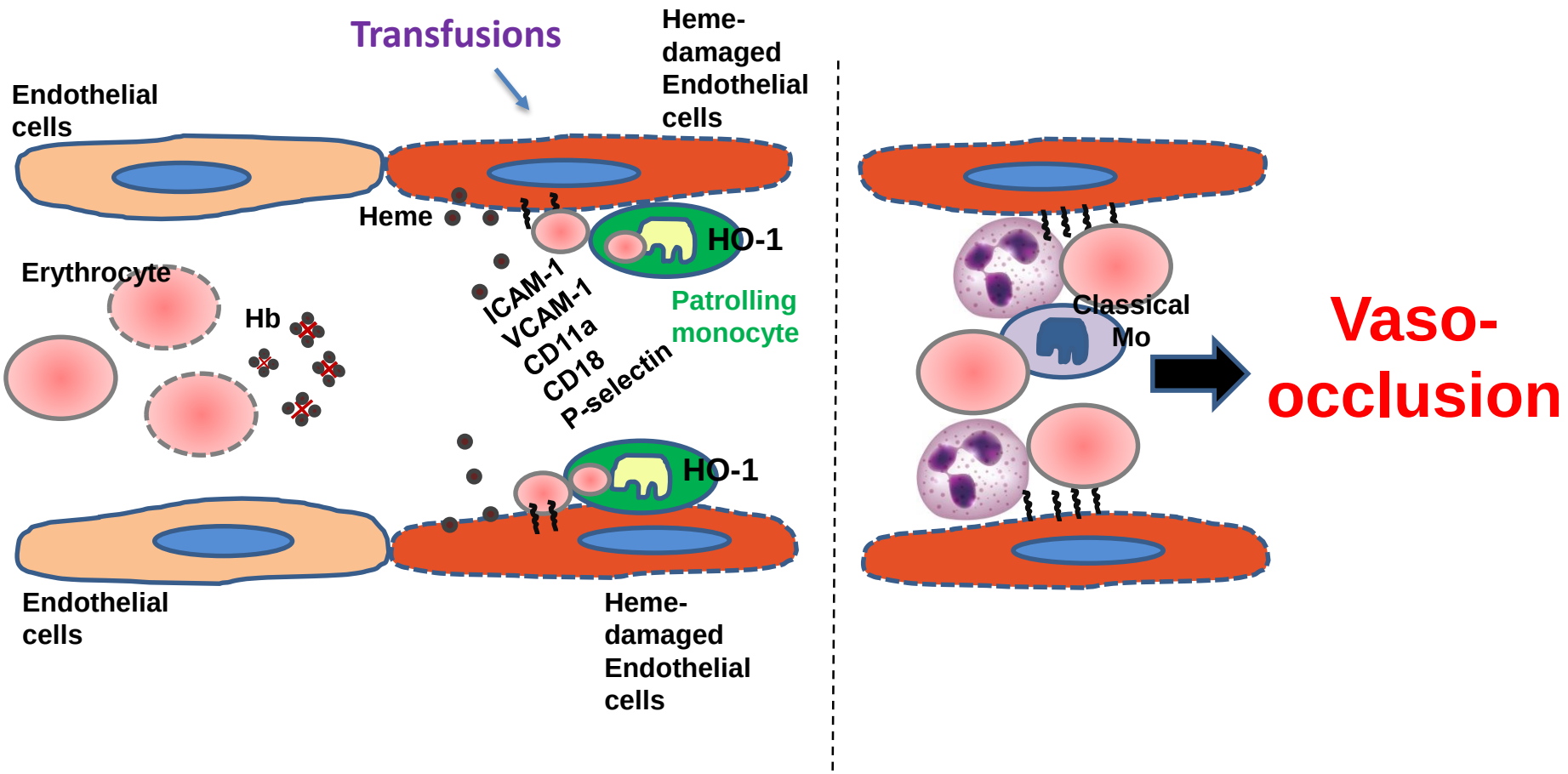


Transfusions with healthy donor RBCs reduce PMo uptake of sickle RBCs, leading to improved survival of PMos.

Liu et al. *Blood* 2019

With Patrolling monocyte

Without Patrolling monocyte



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