

# Differential endothelial *VCAM1* expression and implications for sickle cell anemia vasculopathy

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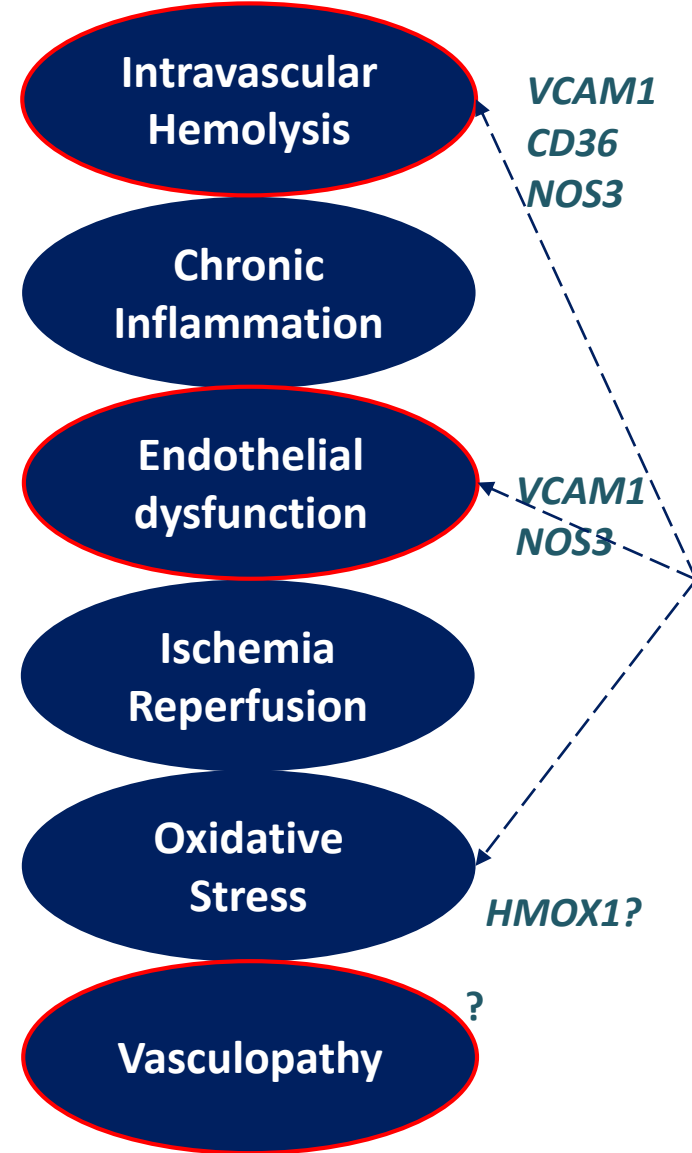
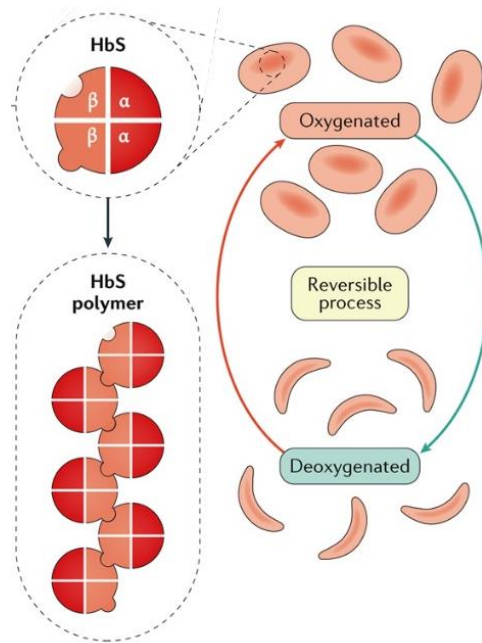
Changing the scene on sickle cell disease

# Sickle cell anemia

Autosomal recessive inheritance → Multifactorial-like presentation

Multi-organ involvement / damage

**HBB:c.20A>T** –  $\beta^S$  mutation



**Genetic modulation**

*BCL11A*  
↓  
**HbF**

# VCAM1 promoter haplotypes previously identified

Gene / Variant	Haplotype						
VCAM1	1	2	3	4	5	6	7
rs1409419 (T>C)	C	C	C	C	C	C	T
rs3917024 (C>T)	C	C	C	C	C	T	C
rs3917025 (CT>delCT)	CT	CT	CT	CT	delCT	delCT	CT
rs3783598 (T>G)	T	T	T	T	T	G	T
rs1041163 (T>C)	T	T	C	C	T	T	T
rs3783599 (C>T)	C	T	C	T	C	C	C

↓  
control

↓  
Severe  
Hemolysis

↓  
Stroke

Coelho A et al, Eur J Haematol 2014:

Silva M et al, Blood Cells Mol Dis 2020:

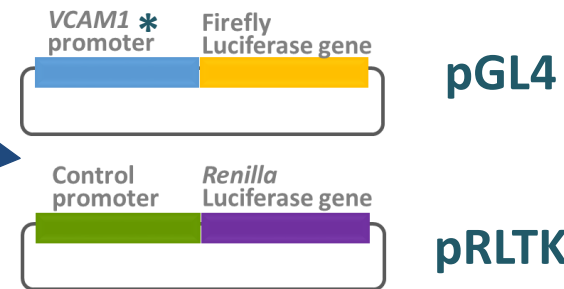
# Dual-reporter luciferase assays – assessment of VCAM1 promoter activity

## Endothelial cell models

EA.hy926 – macrovascular

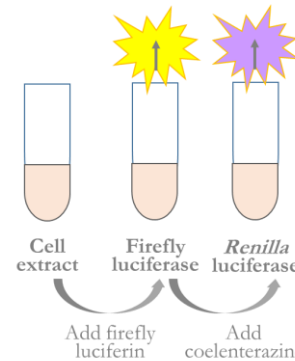
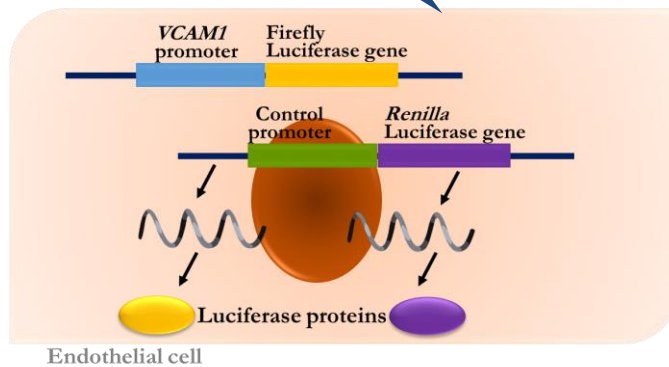
HBEC - cerebral microvascular

## Reporter vectors



\* Hap\_1, Hap\_4, or Hap\_7

TNF- $\alpha$  (20 ng/mL)

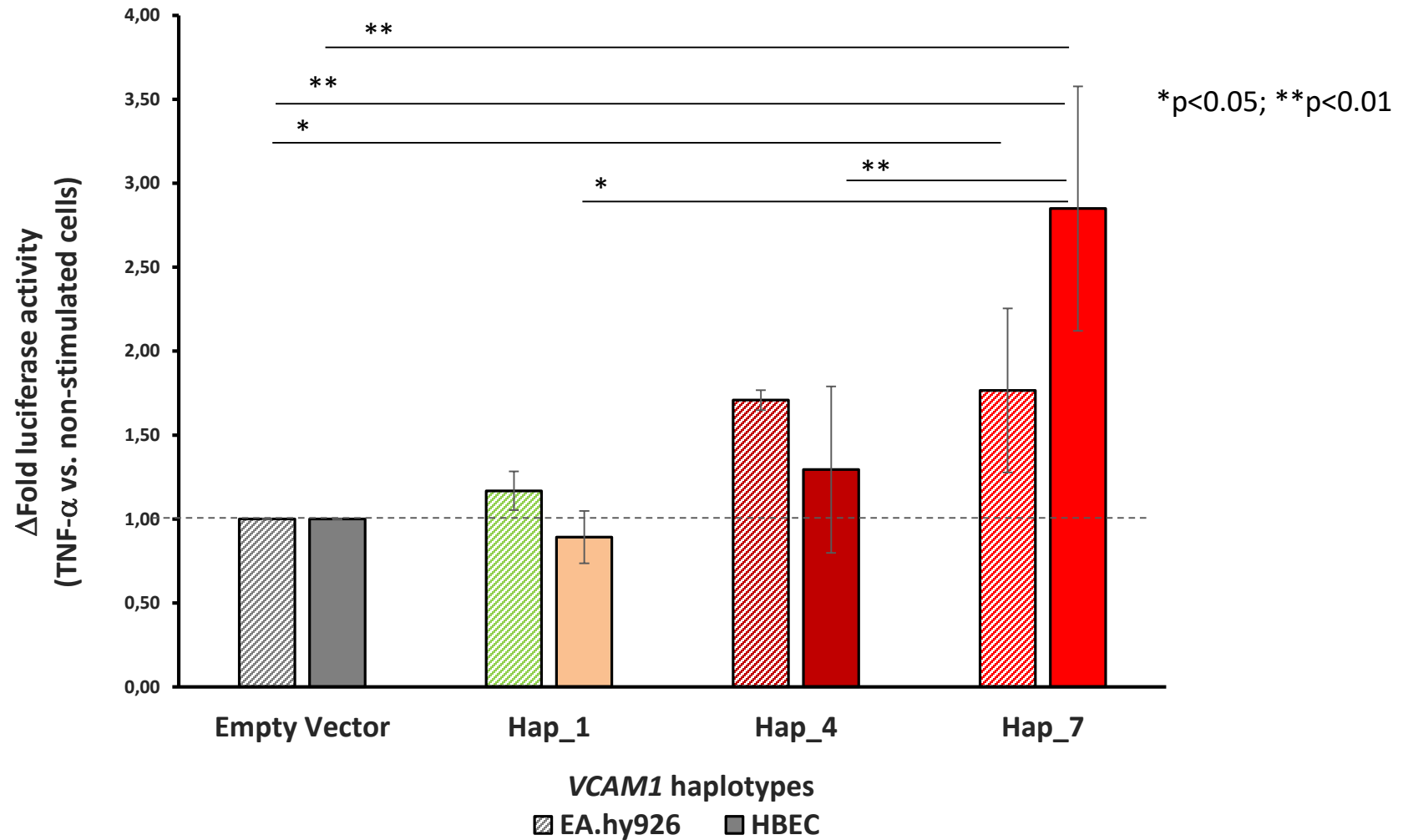


VCAM1 promoter activity

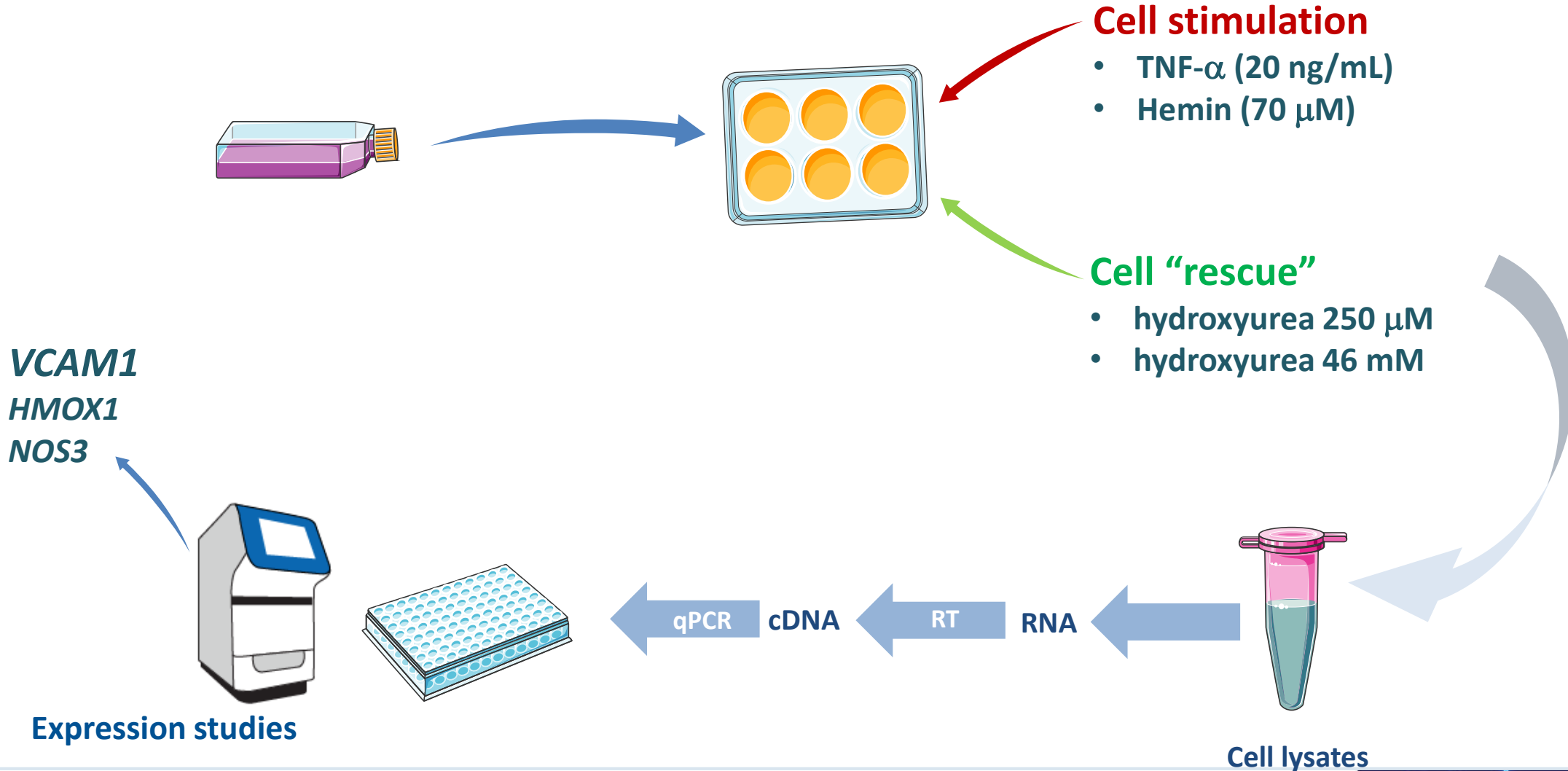
Firefly luciferase activity

Renilla luciferase activity

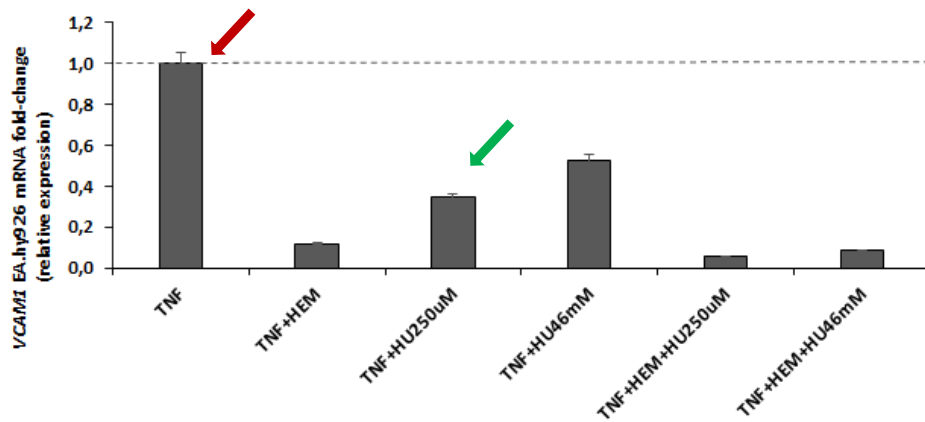
# Haplotypes 4 and 7 increase VCAM1 promoter activity in EA.hy926 and HBEC



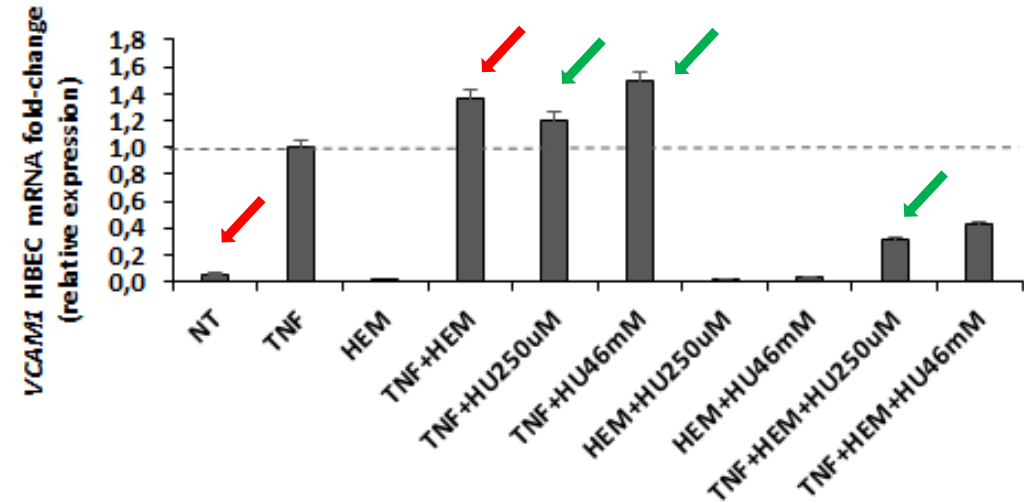
# Assessment of cell response under different stimuli and treatment



**VCAM1 is only expressed in EA.hy926 under TNF- $\alpha$  stimulation**  
**In HBEC expression occurs even without stimulation and increases with combined TNF- $\alpha$  and heme stimulus**



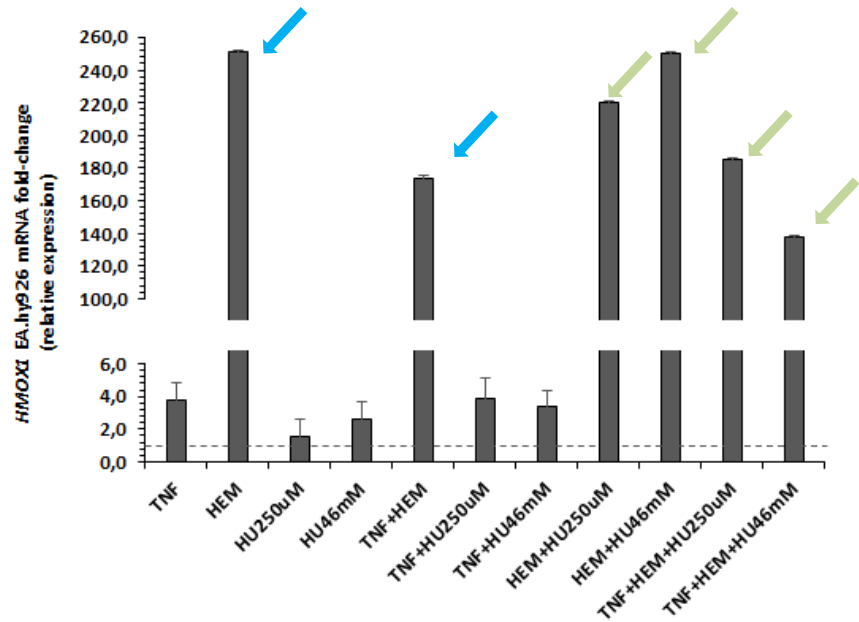
EA.hy926



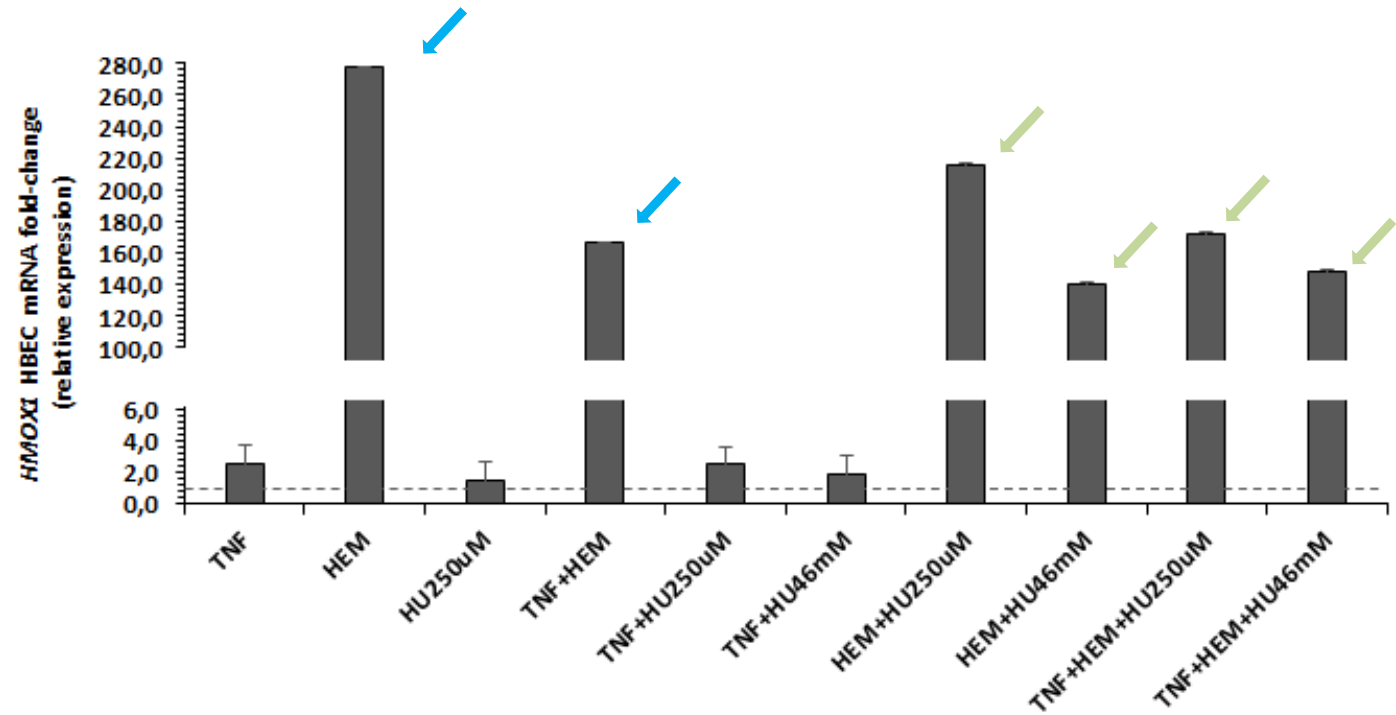
HBEC

**HU reduces VCAM1 expression, however...**

# HMOX1 is highly expressed in ECs only after heme stimulation



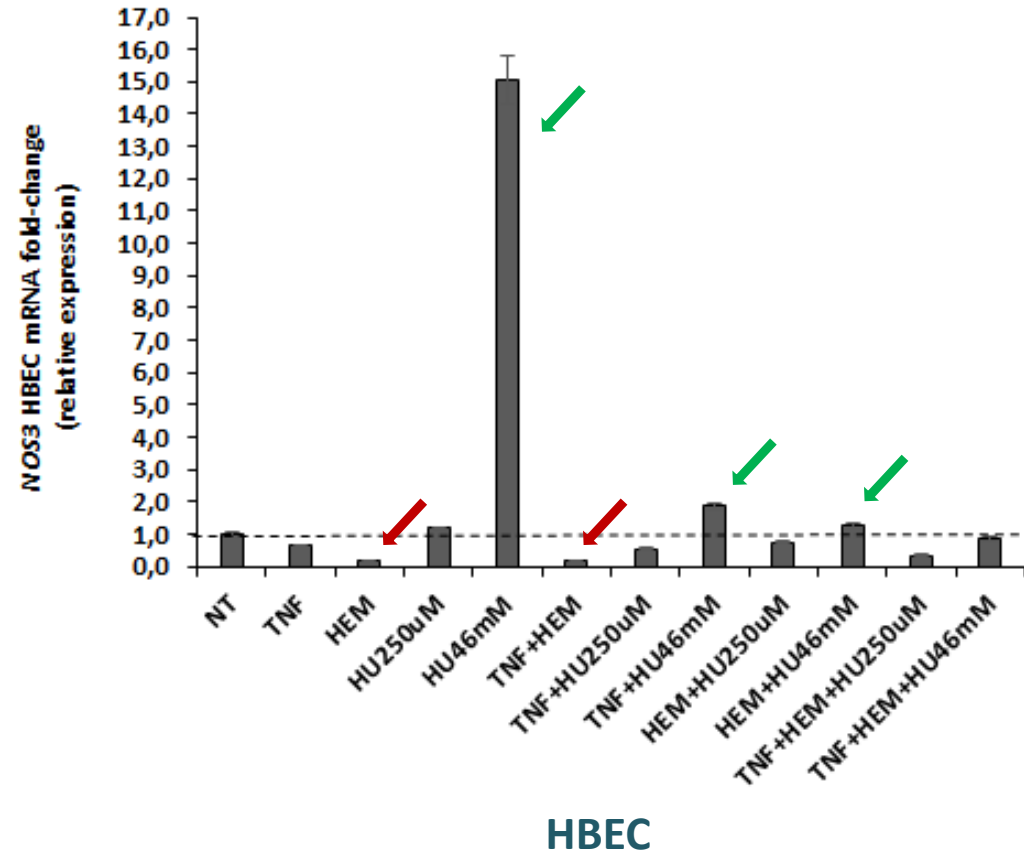
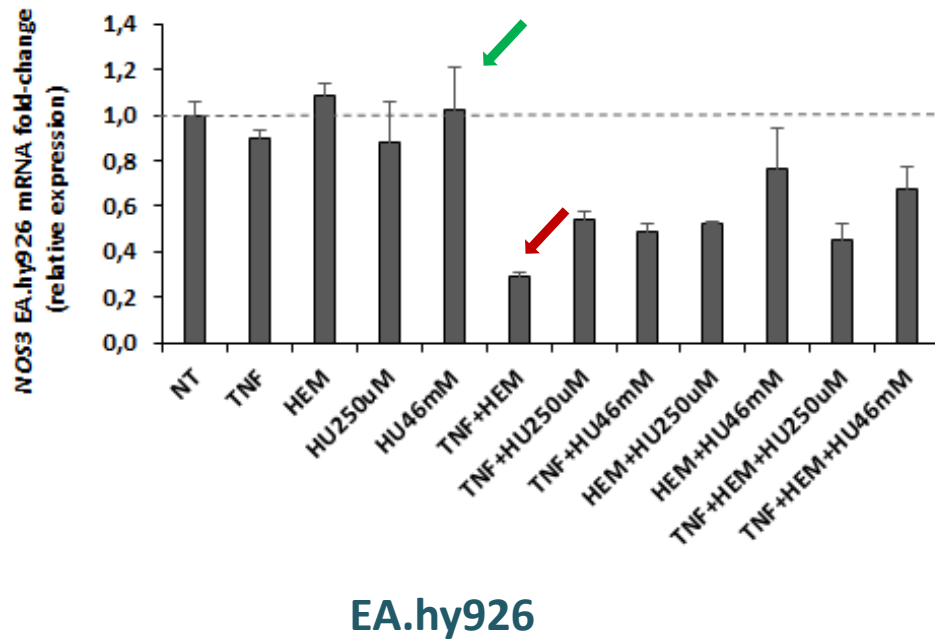
EA.hy926



HBEC

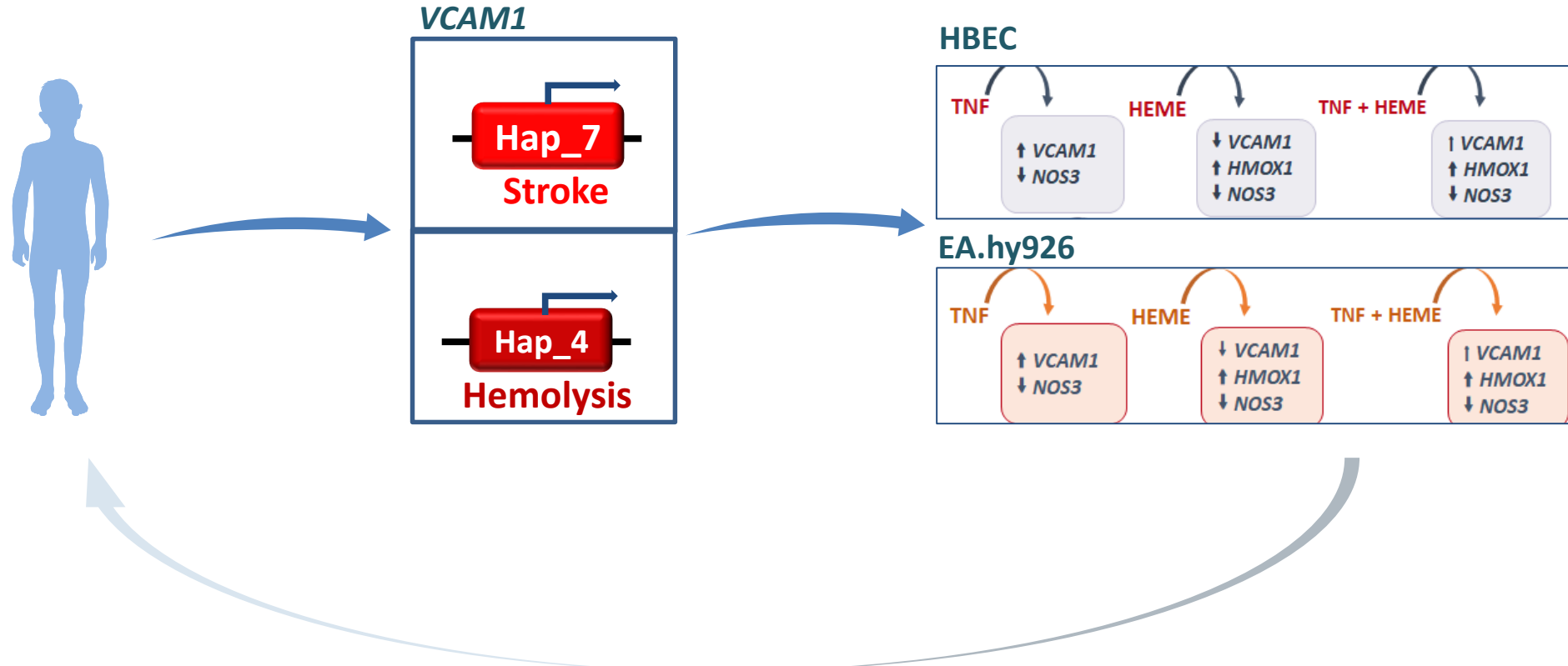
*HU does not affect HMOX1 expression*

# TNF- $\alpha$ and heme significantly reduce *NOS3* expression in both EA.hy926 and HBEC



HU has dose-dependent effect on *NOS3* expression

# VCAM1 promoter haplotypes 7 and 4 potentially increase vasculopathy severity in SCA



VCAM1: therapeutic target?

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**PATIENTS and their FAMILIES**