



V Simpósio Brasileiro de
Doença Falciforme
e outras
Hemoglobinopatias

Encontro Pan-Americano
para Doença Falciforme
OPAS / OMS

“Role of diminished cAMP-mediated signaling and increased phosphodiesterase 3A activity in augmented platelet adhesive functions in sickle cell disease”

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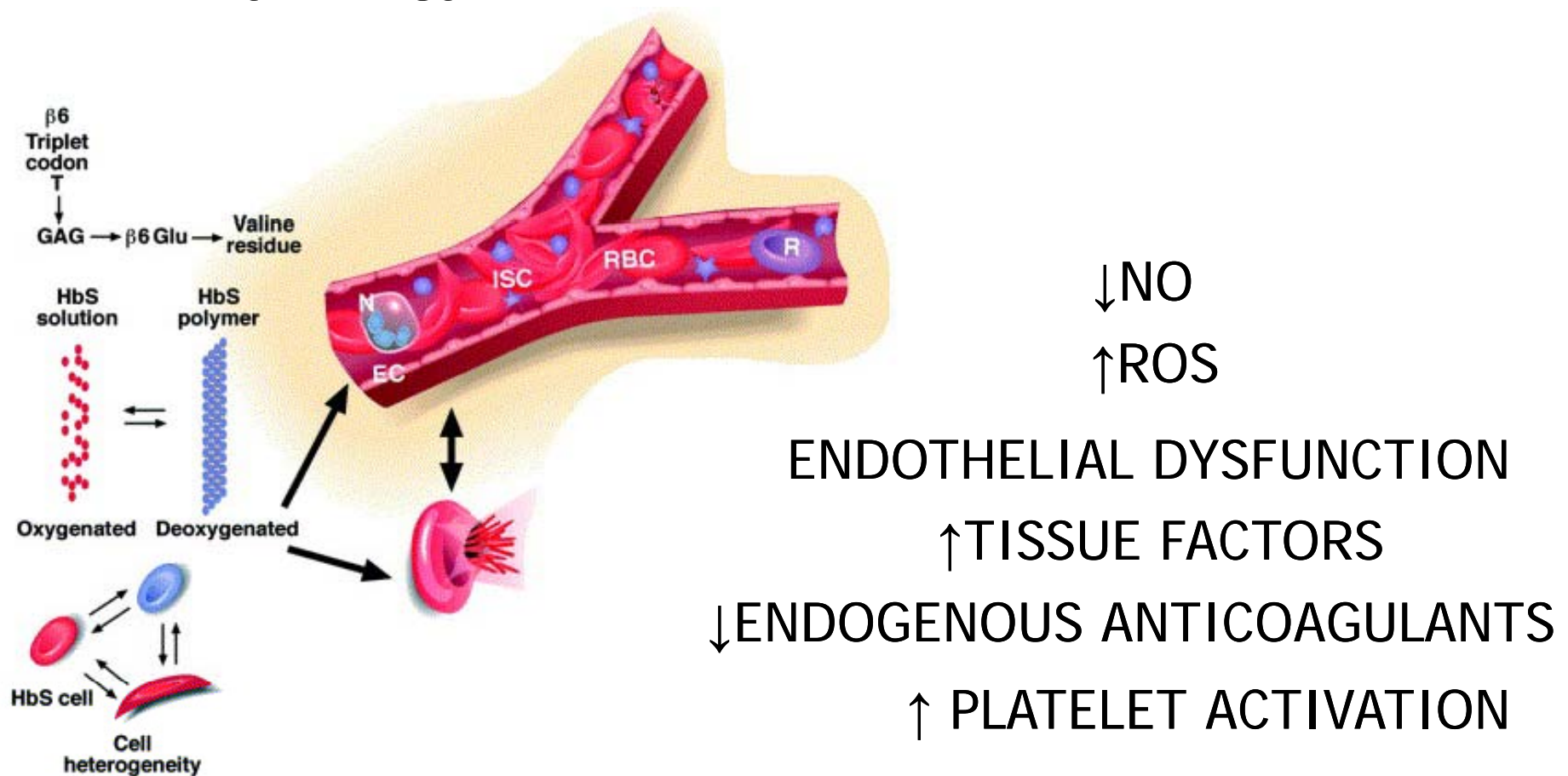
University of Campinas

**Hemocentro / UNICAMP
Belo Horizonte, 6 de outubro de 2009**

FAPESP

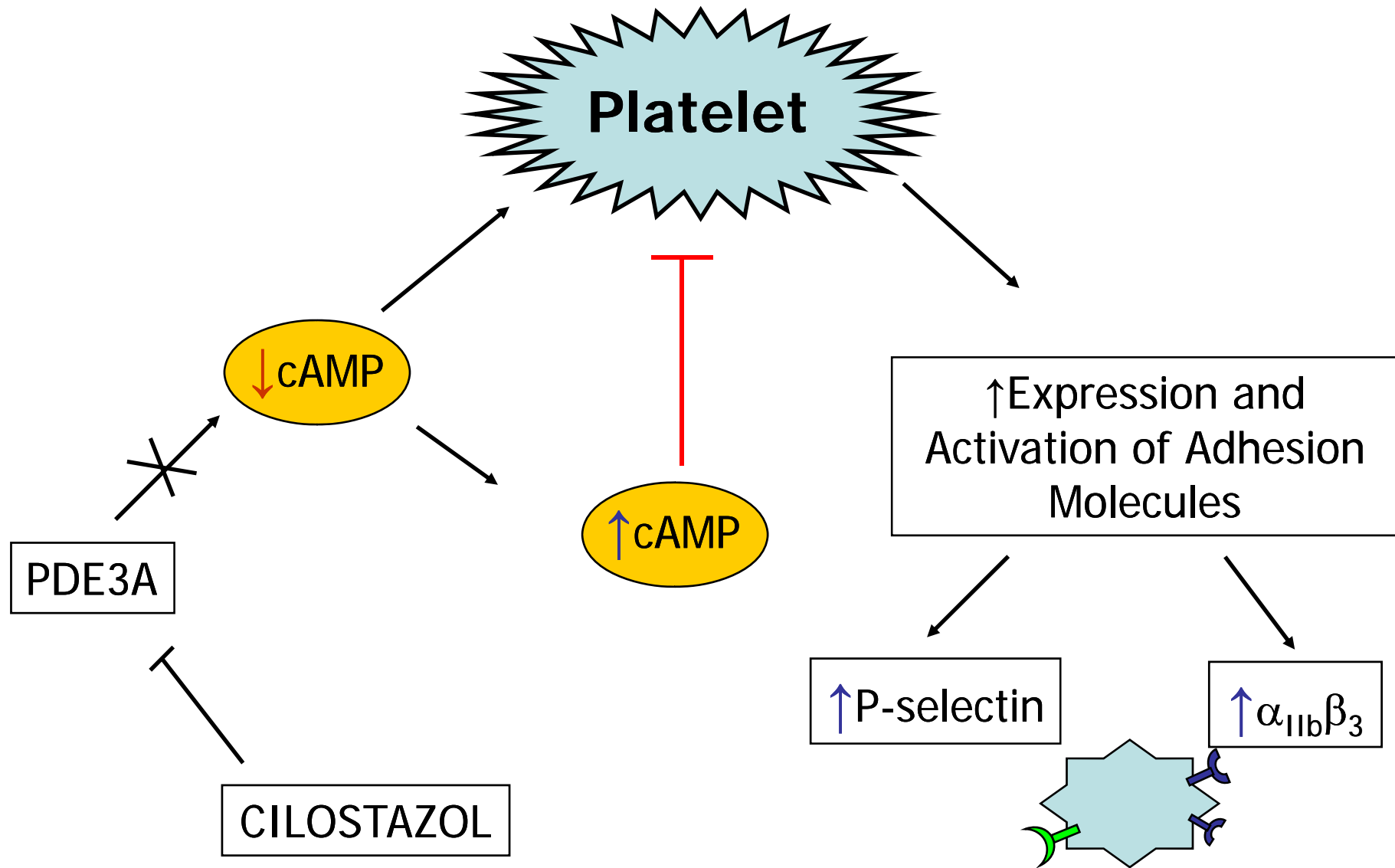
INTRODUCTION

Pathophysiology of Vaso-occlusion



STEINBERG, 2006; SETTY *et al.*, 2001; BROWN *et al.*, 2001; VILLAGRA *et al.*, 2007

PLATELET ACTIVATION



AIM

“Investigation of adhesive properties of platelets of patients with sickle cell anemia, and elucidation of the role of cyclic nucleotides and other signaling pathways as key mediators of the processes of vaso-occlusion and platelet adhesion”

PATIENTS

- Steady-state patients, diagnosed as homozygous for HbS (n=36) → hemoglobin electrophoresis methods and high pressure liquid chromatography
- Patients on hydroxyurea (HU, n=31), 20-30 mg/Kg/day (at least 3 months)

Had not had painful crisis nor received blood transfusion (3 months) and had not taken aspirin during the previous 10 days.

- Healthy volunteers who had not taken any medication within the last 10 days (n=49)

METHODS

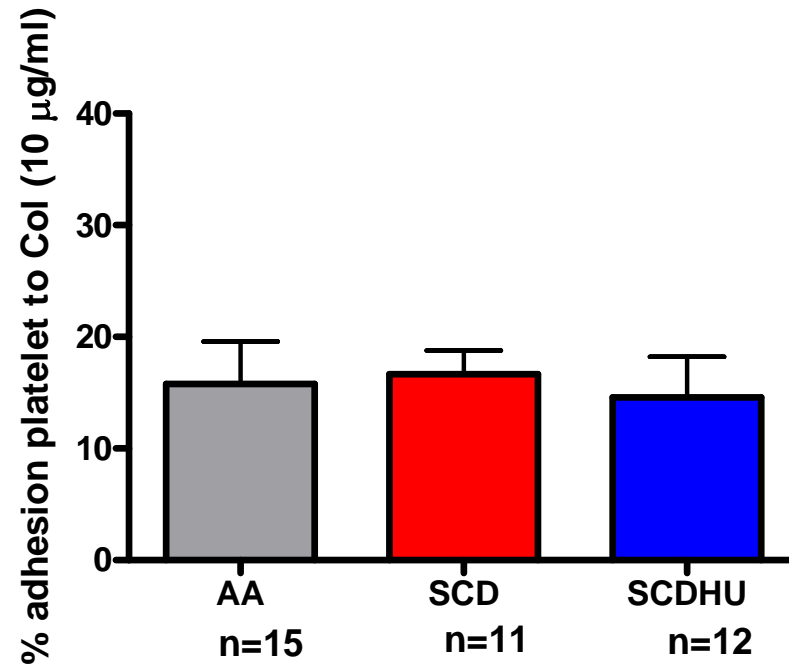
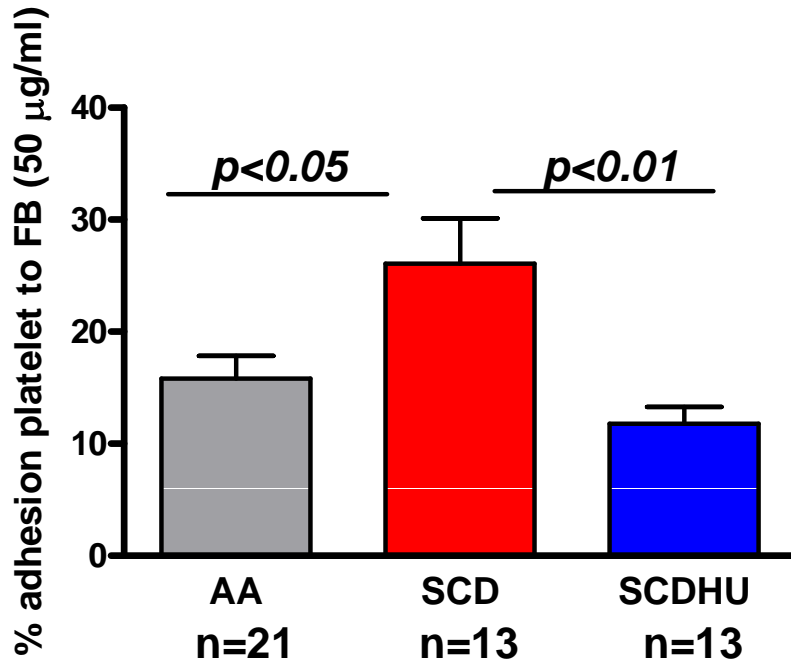
- Separation of platelets (peripheral blood)
 - Platelet rich plasma (PRP)
 - Washed Platelets
- Static Adhesion Assay –96 well plates
- Flow Cytometry
- Extraction and quantification of cyclic nucleotides
 - cAMP (Kits ELISA)

RESULTS

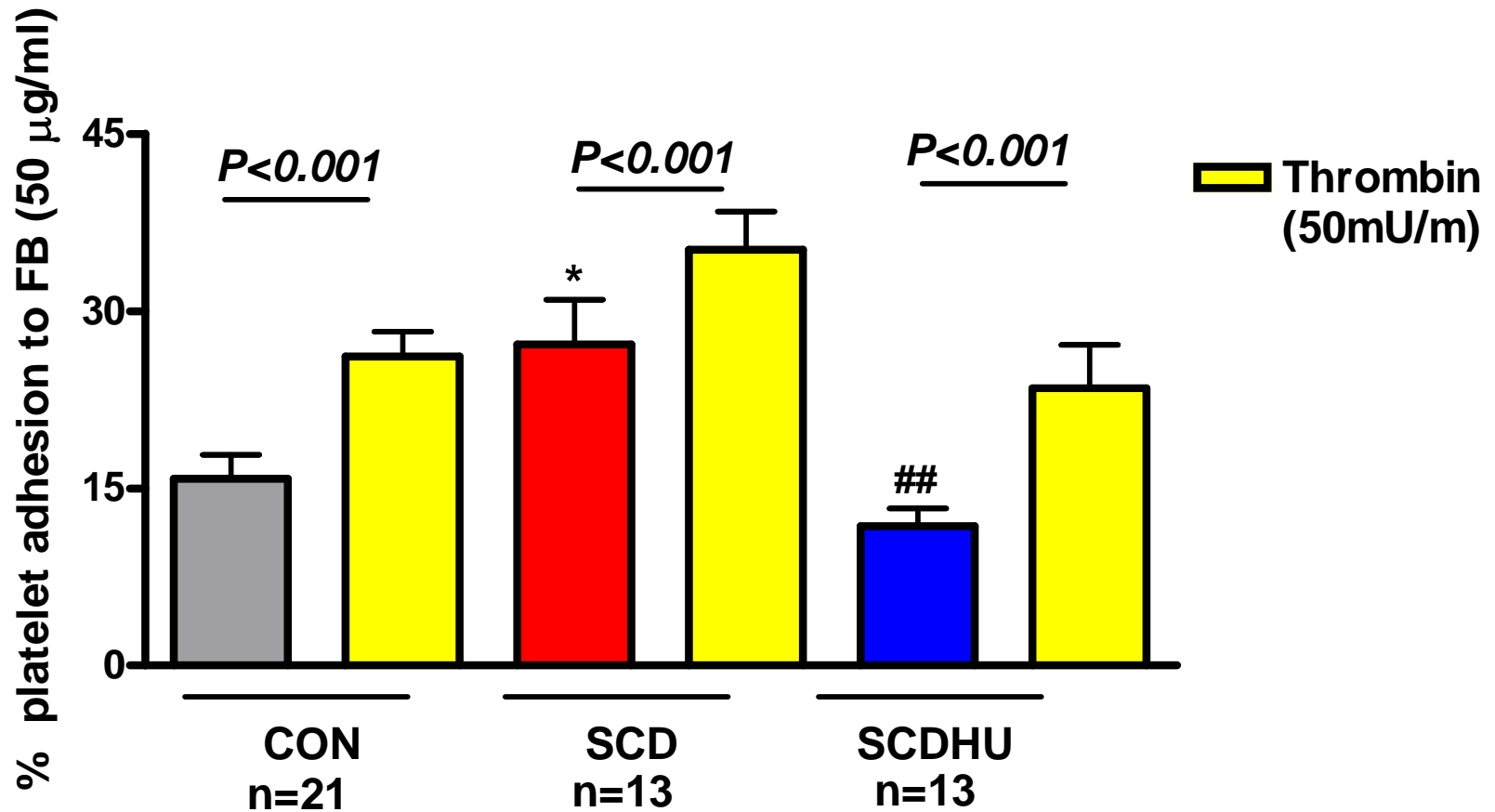
Laboratory details of participating patients and healthy controls

	AA	SCD	SCDHU	<i>P</i> <i>SCD comp.</i> <i>SCDHU</i>
Male/female	20/29	11/25	16/15	
Age (years)	33	39.8	36.4	>0.05
Red blood cells count (10 ⁶ /μl)	N/D	2.7	2.6	>0.05
Hematocrit (%)	44	24.0	27.1	0.005
Hemoglobin (g/L)	N/D	79.5	91.5	0.003
Mean corpuscular volume (fl)	N/D	89.5	102.9	<0.001
Mean corpuscular hemoglobin (pg)	N/D	29.7	34.8	<0.0001
WBC (x10 ⁹ /L)	N/D	9.9	7.9	0.005
Platelets (x10 ⁹ /L)	N/D	470.7	423.1	>0.05
HbF (%)	N/D	8.1	16.3	<0.00001

SCD platelets demonstrate an increased ability to adhere to fibrinogen (FB), but not to Collagen (Col), *in vitro*



A thrombin stimulus significantly increases CON, SCD and SCDHU platelet adhesion to FB

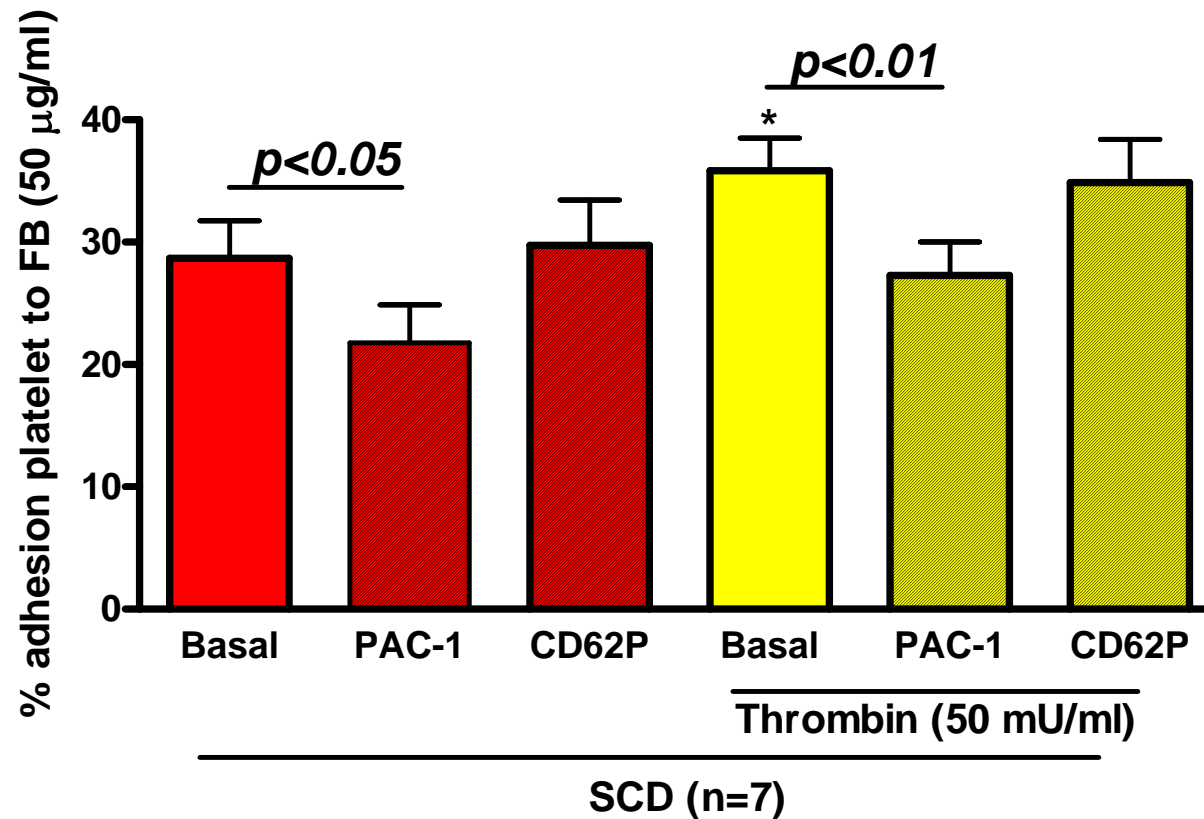


* $p < 0.05$ compared to adhesion basal PLTs CON; ## $p < 0.01$ compared to adhesion basal PLTs SCD

Increased expression of P-selectin molecule and activated $\alpha_{IIb}\beta_3$ on SCD platelets

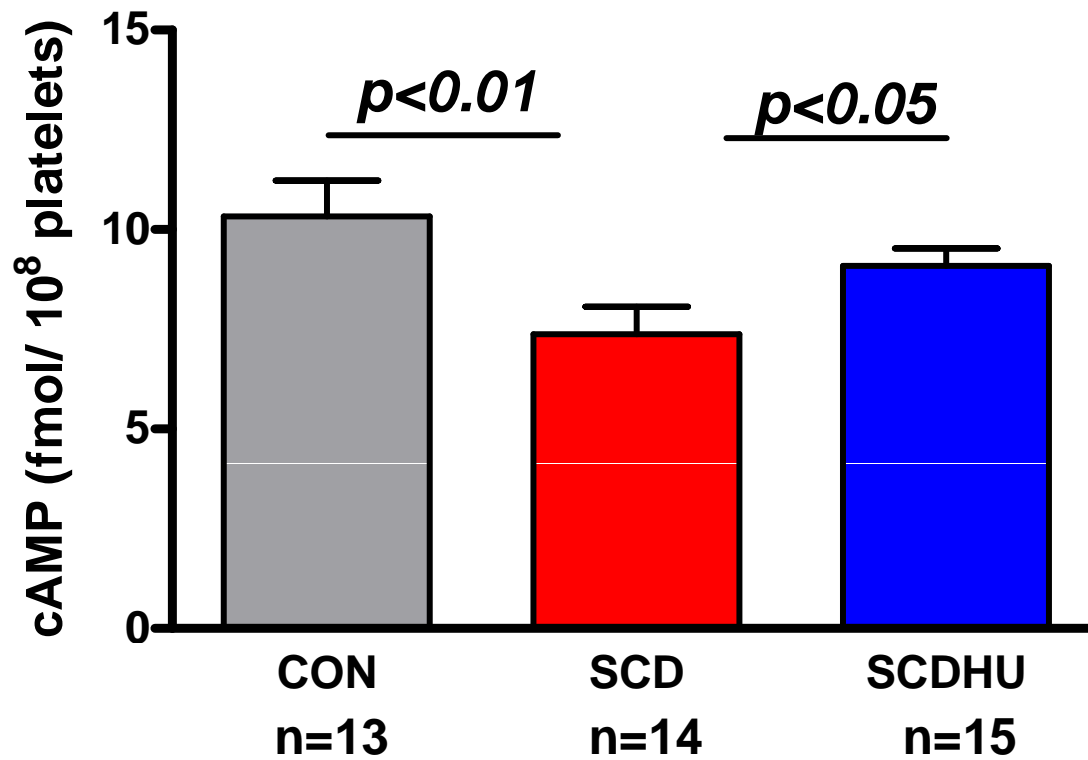
Antibody	CON (n≥18)		SCD (n≥17)		SCDHU (n≥13)		P
	% (+)	MIF	% (+)	MIF	% (+)	MIF	
CD42b-FITC	97.2 ± 0.6	758.5 ± 85.6	98.1 ± 0.4	737.8±47	95.5 ± 1.6	815.6 ± 81.7	
CD62P-FITC	20.2 ± 2.3 **	13.9 ± 1.4 *	29.4 ± 3.6 **	20.7 ± 2.4 *	29.2 ± 4.6	24.2 ± 6.3	0.04** 0.03*
CD49b-FITC	70.5 ± 4.2	27.4 ± 2.0	70.1 ± 4.6	29.4 ± 2.9	68.5 ± 5.2	26.9 ± 2.0	
CD41a-FITC	97.5 ± 0.5	412.9 ± 37.1	98.2 ± 0.4	406.7±37	96.6 ± 1.1	447.0 ± 43.9	
PAC-1-FITC	11.1 ± 3.7 #	8.6 ± 1.5	27.0 ± 6.3 #	14.4 ± 2.6	11.6 ± 2.6	8.6 ± 1.7	0.03#

The anti- $\alpha_{IIb}\beta_3$ antibody (PAC-1), but not anti-CD62P diminishes SCD platelet adhesion to FB

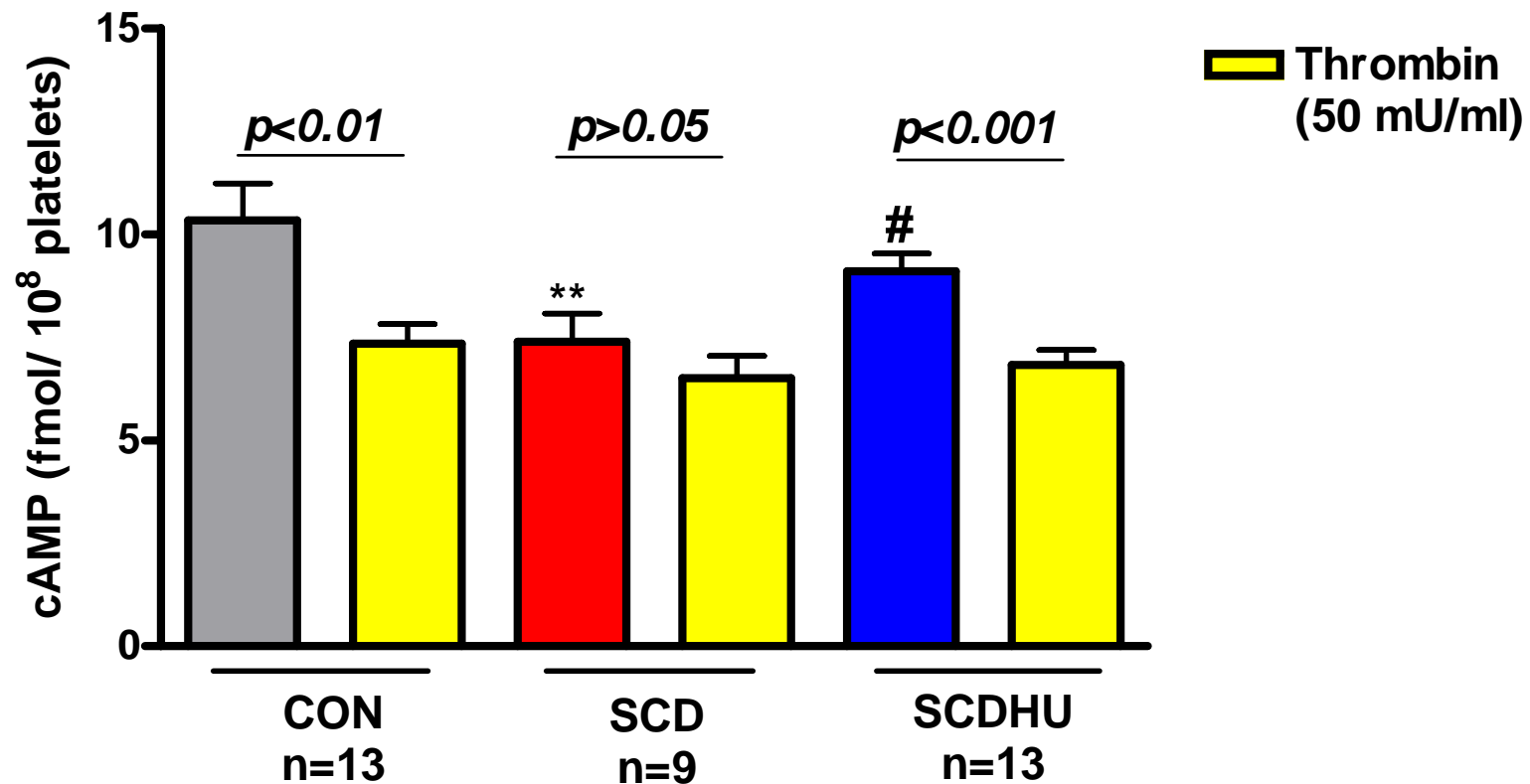


* $p < 0.05$ compared to basal adhesion

Intraplatelet cAMP is decreased in SCD platelets



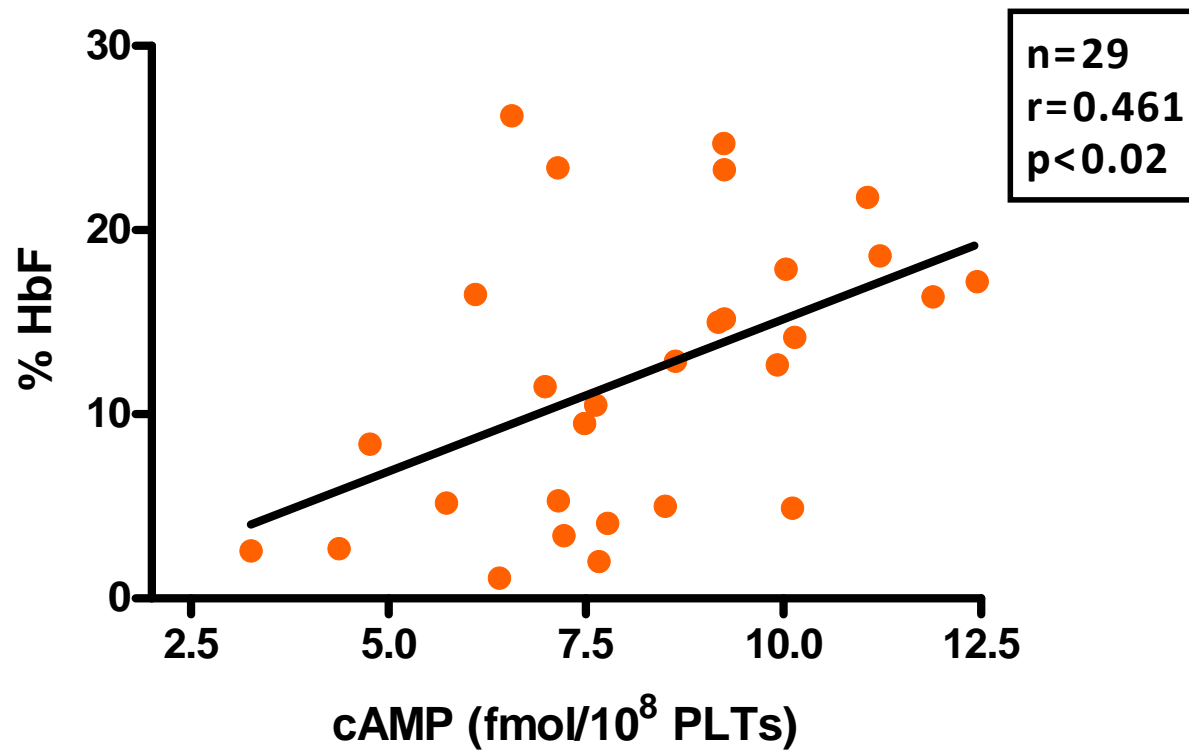
CON and SCDHU Platelet cAMP Levels are Decreased Following Thrombin Stimulation



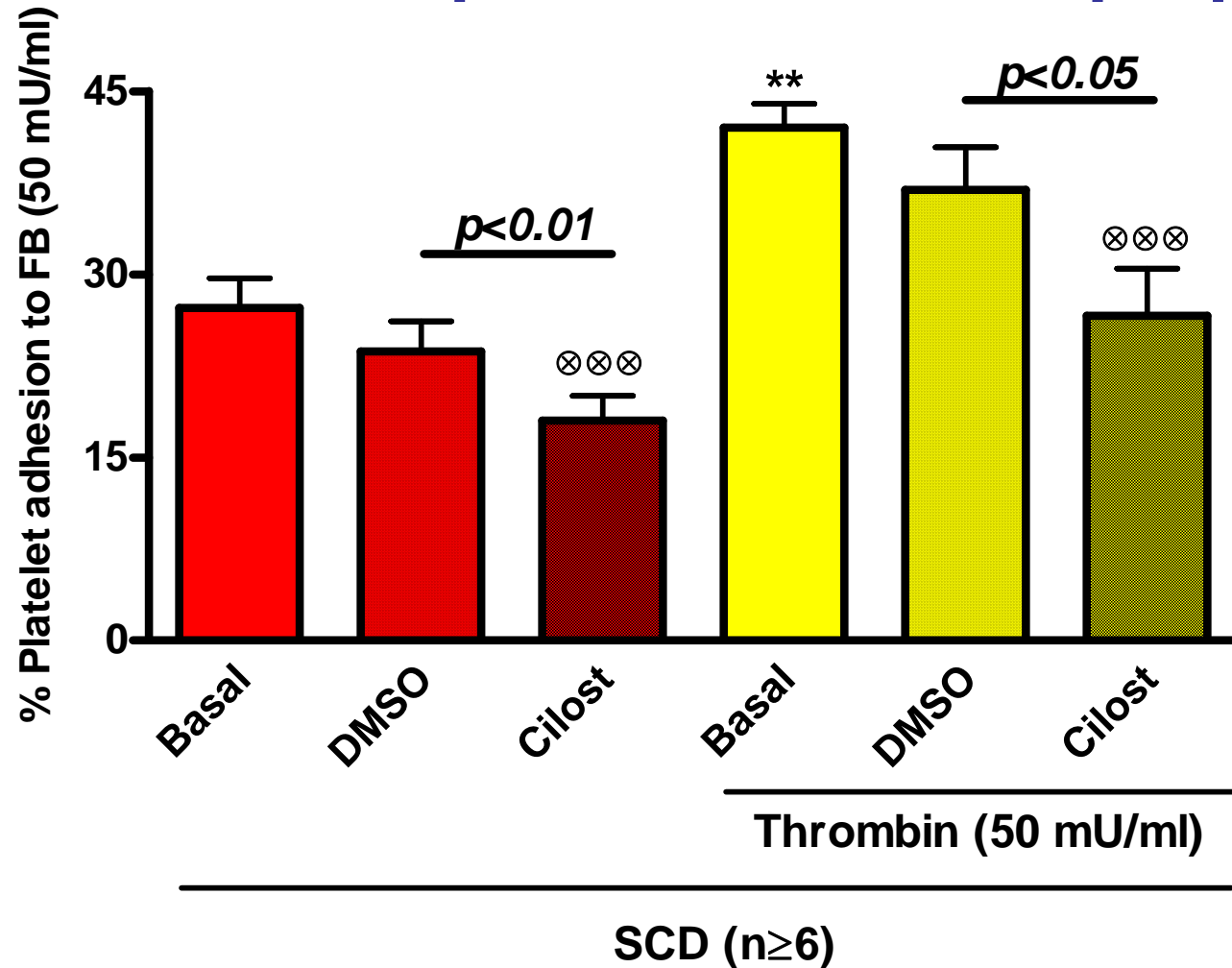
** p < 0.01 compared to CON PLTs

p < 0.05 compared to SCD PLTs

Positive Correlation between Intraplatelet cAMP and levels of HbF in SCD and SCDHU

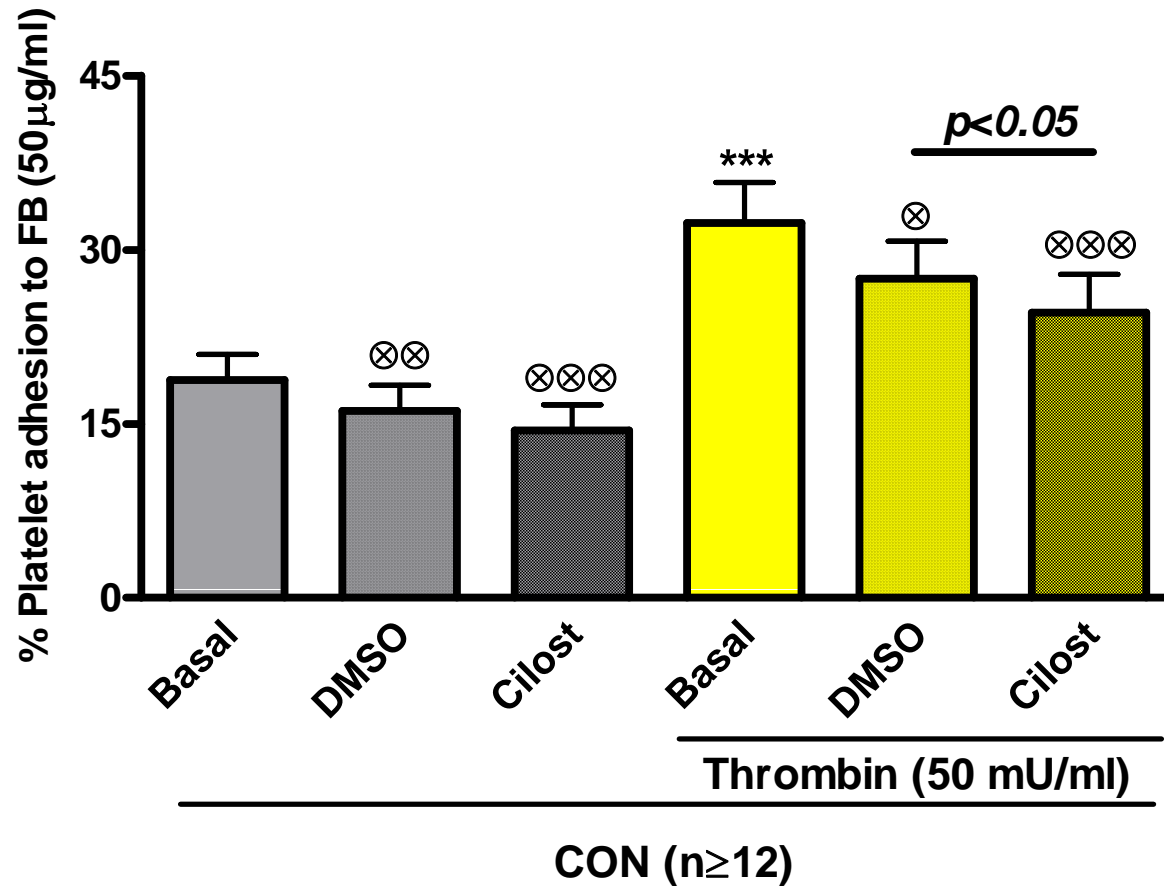


Inhibition of PDE3A (cilostazol) activity diminishes SCD platelet adhesive properties



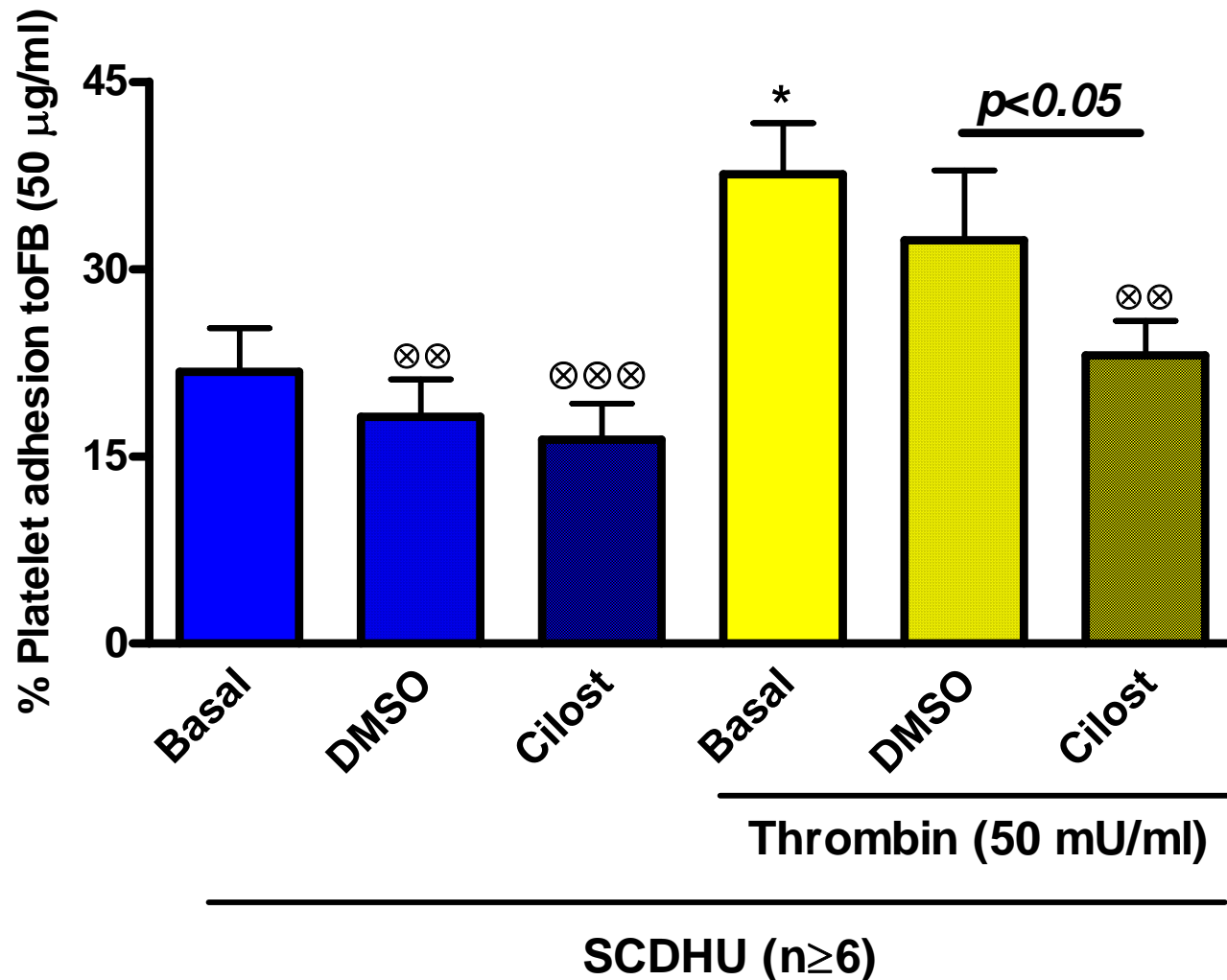
⊗⊗⊗ $p < 0.001$ compared to adhesion basal and/or adhesion TB;
** $p < 0.01$ compared to adhesion basal

Inhibition of PDE3A (cilostazol) activity



⊗⊗ $p < 0.01$ compared to adhesion basal; ⊗⊗⊗ $p < 0.001$ compared to adhesion basal;
*** $p < 0.001$ compared to adhesion basal; ⊗ $p < 0.05$ compared to adhesion TB

Inhibition of PDE3A (cilostazol) activity



⊗⊗ $p < 0.01$ compared to adhesion basal; ⊗⊗⊗ $p < 0.001$ compared to adhesion basal; * $p < 0.05$ compared to adhesion basal

CONCLUSIONS

- SCD Platelets demonstrated a greater capacity to adhere to FB, but not Col, than CON Platelets.
- SCD Platelet adhesion to FB is mediated by activated $\alpha_{IIb}\beta_3$ integrin.
- Reduced cAMP signaling and increased PDE3A activity contribute to increased SCD PLT adhesion.
- HU therapy appears to diminish SCD platelet activation and adhesive properties.
- NO donor or cyclic nucleotide-targeting therapies (cilostazol, sildenafil) may be useful for diminishing PLT activation and adhesion in SCD.

Platelets and Vaso-occlusion in SCD

