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AG-946 Normalizes Glycolysis and Improves Red Cell Indices in a Humanized Sickle Cell Mouse Model

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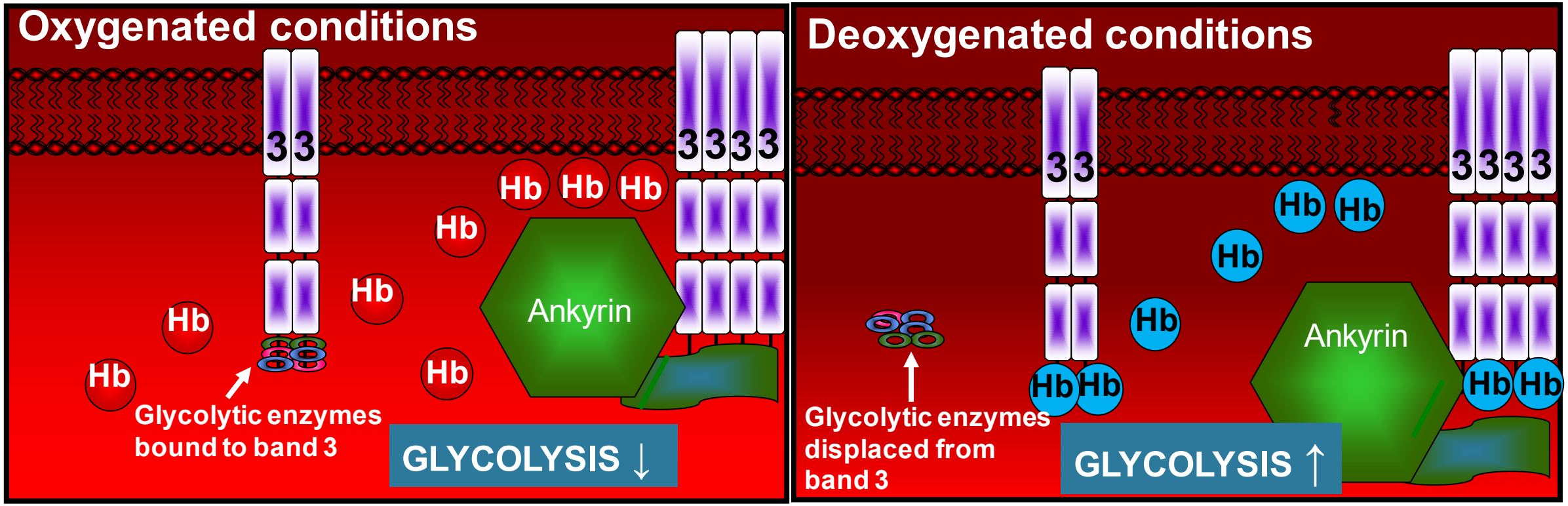
Band 3, a hub for hemoglobin and glycolytic enzyme binding in RBC

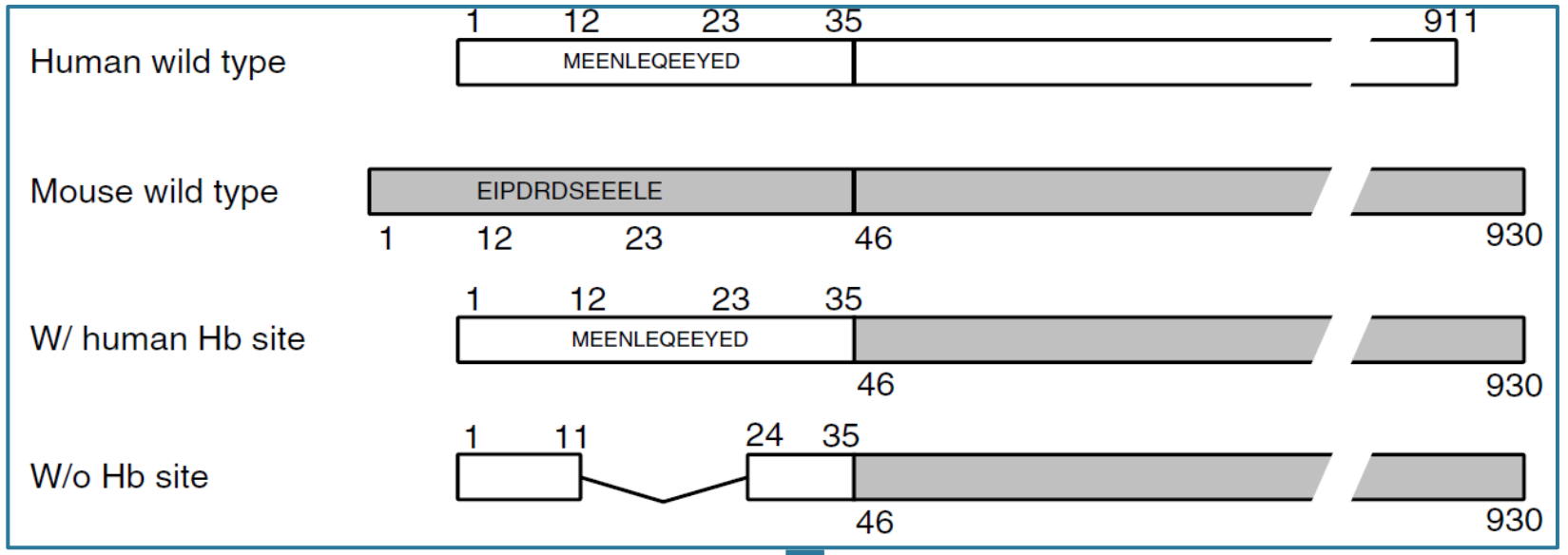


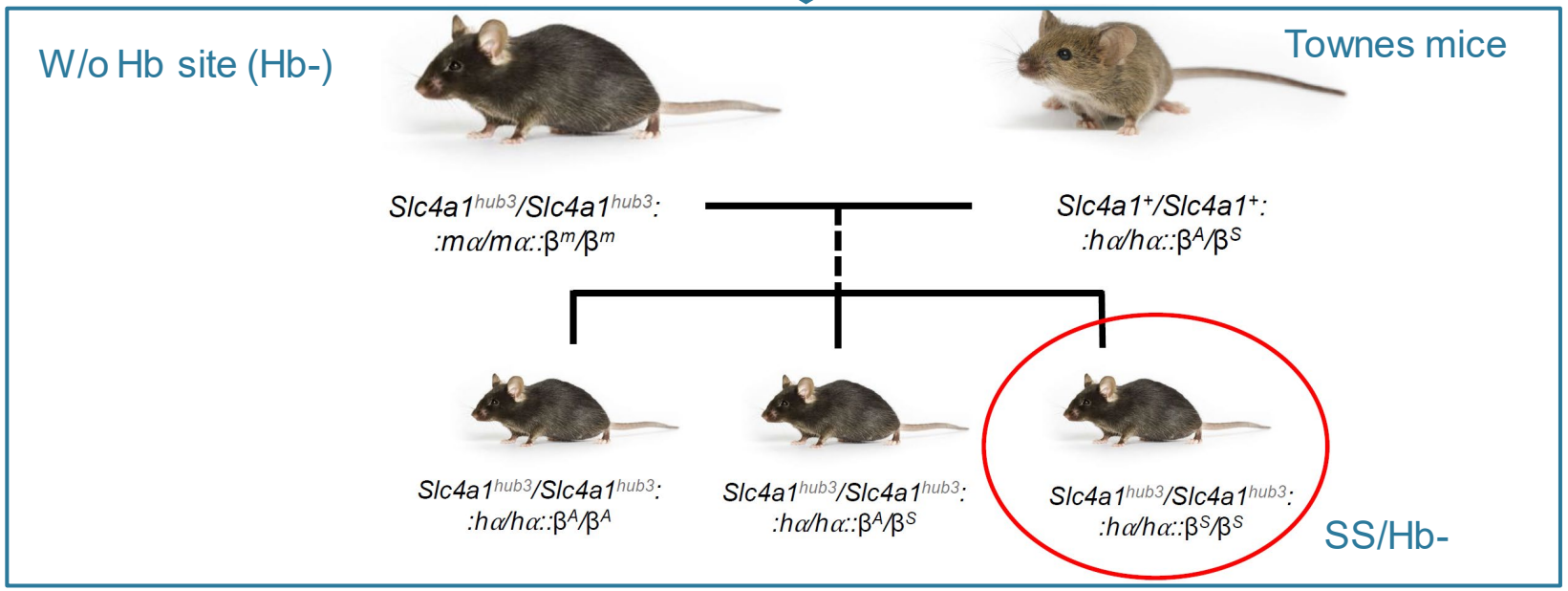
Reversible binding of deoxyhemoglobin to the cytoplasmic domain of Band 3 constitutes a molecular switch regulating assembly of glycolytic enzymes on the erythrocyte membrane based on oxygenation state

Issaian A et al., Hematologica 2021; Chu H et al., Blood 2016

Development of mice with hemoglobin S and mutant human band 3 (SS/Hb-)

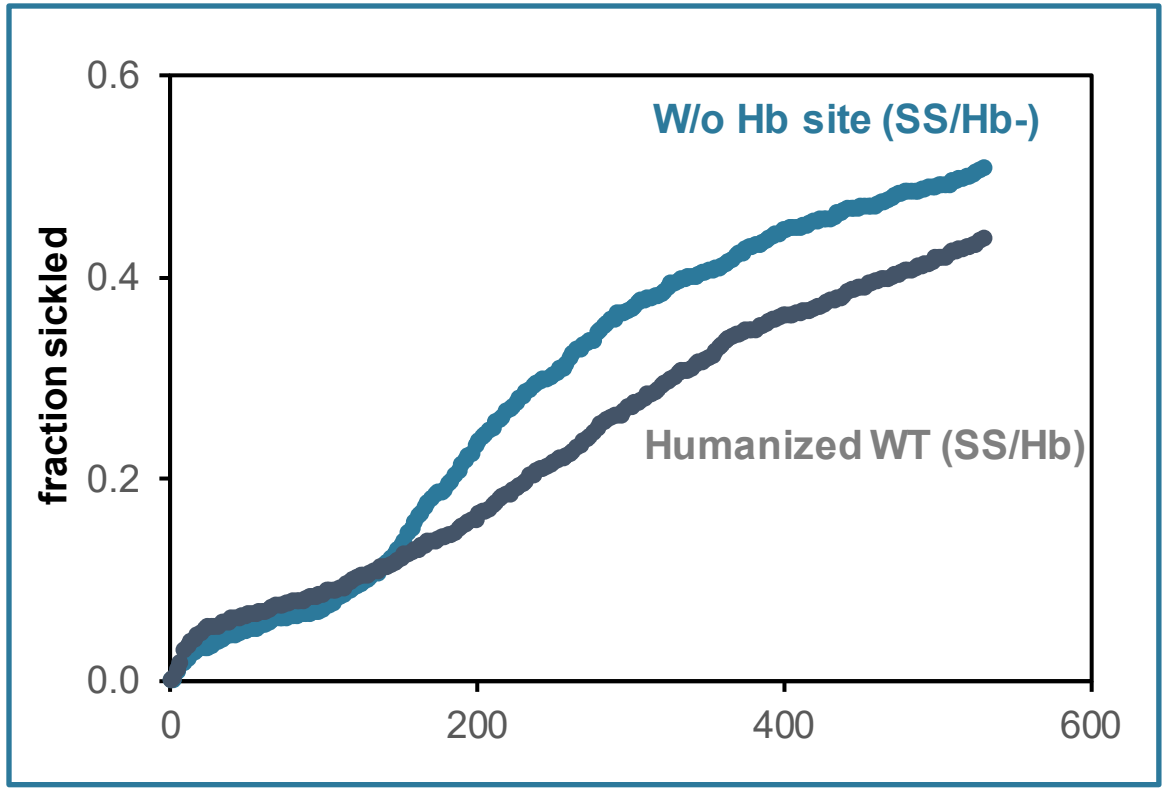
Band 3



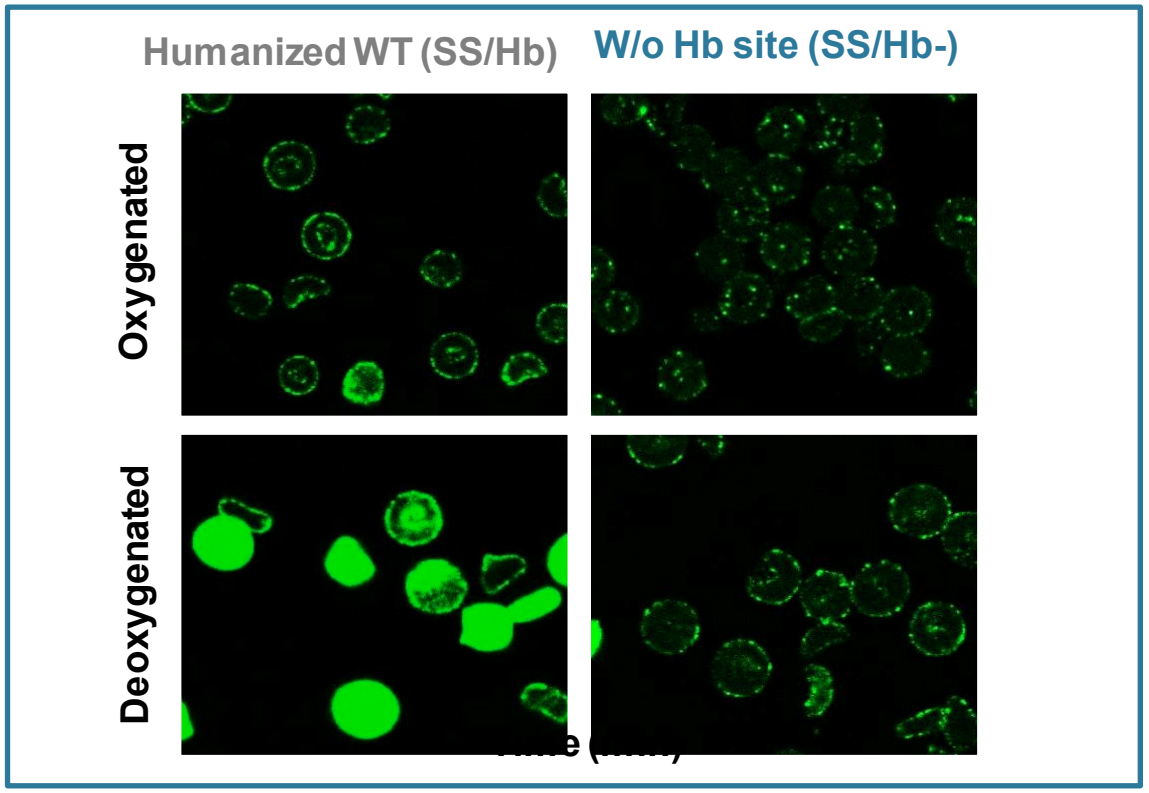


Chu et al., Blood, 2016
Wain et al., Blood, 2021

Townes mice with mutant human band 3 (SS/Hb-) had accelerated sickling



Degree of sickling in murine erythrocytes

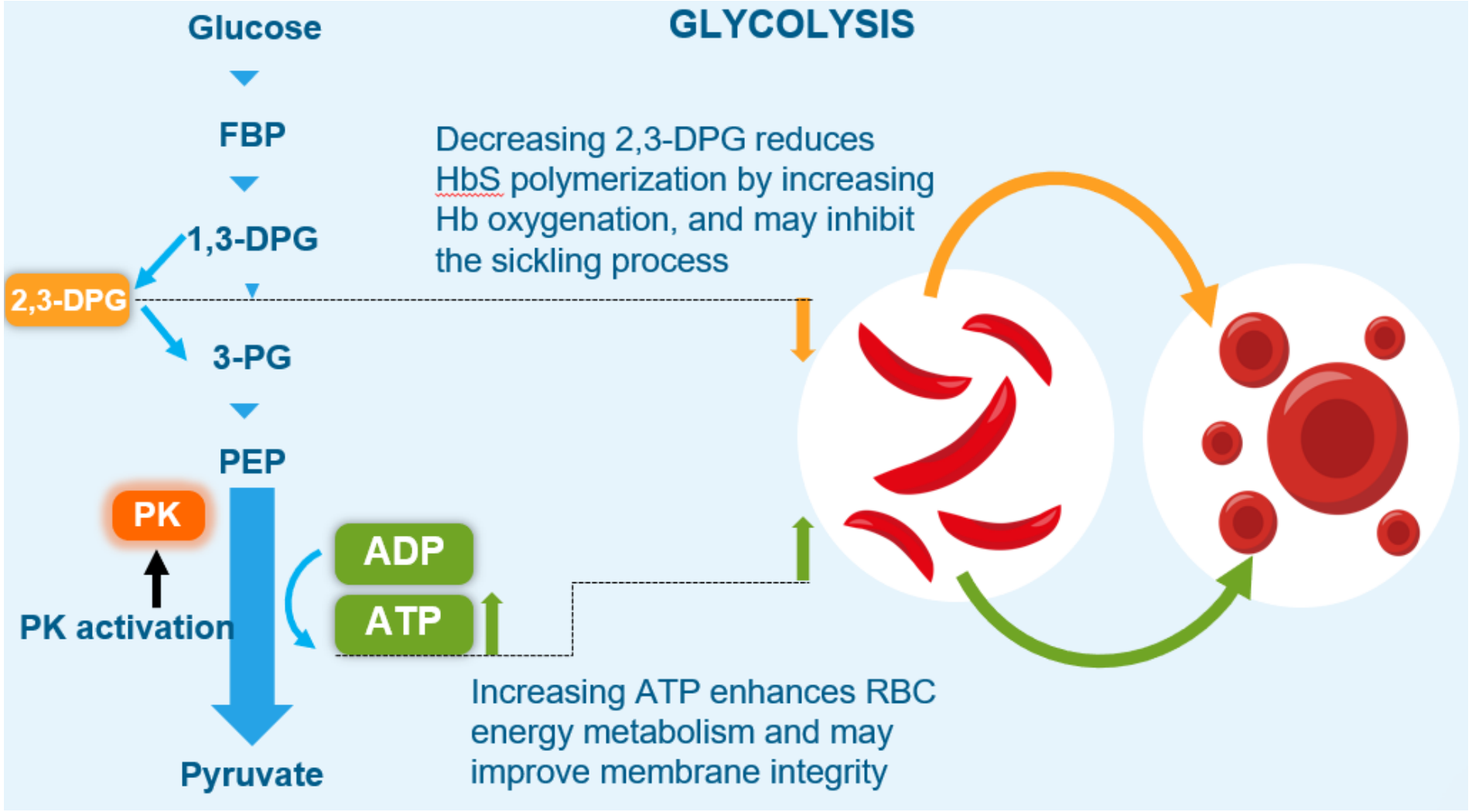


Murine erythrocytes stained for Aldolase

These transgenic mice (SS/Hb-) showed an accelerated rate and extent of sickling along with accumulation of glycolytic intermediates compared to humanized wild-type (SS/Hb)

Chu et al., Blood, 2016; Wain et al., Blood, 2021

PK activation may modulate glycolytic flux in red blood cells (RBCs)



Pilot study design to evaluate the effect of PK activation in SS/Hb- mice

SS/Hb- mice
[human hemoglobin S (Townes) and mutant human band 3]

Control Chow

- 4 week

0 week
Blood collection

AG-946 chow (n=3)
(a potent PK activator)

8 week
Blood collection

0 week
Blood collection

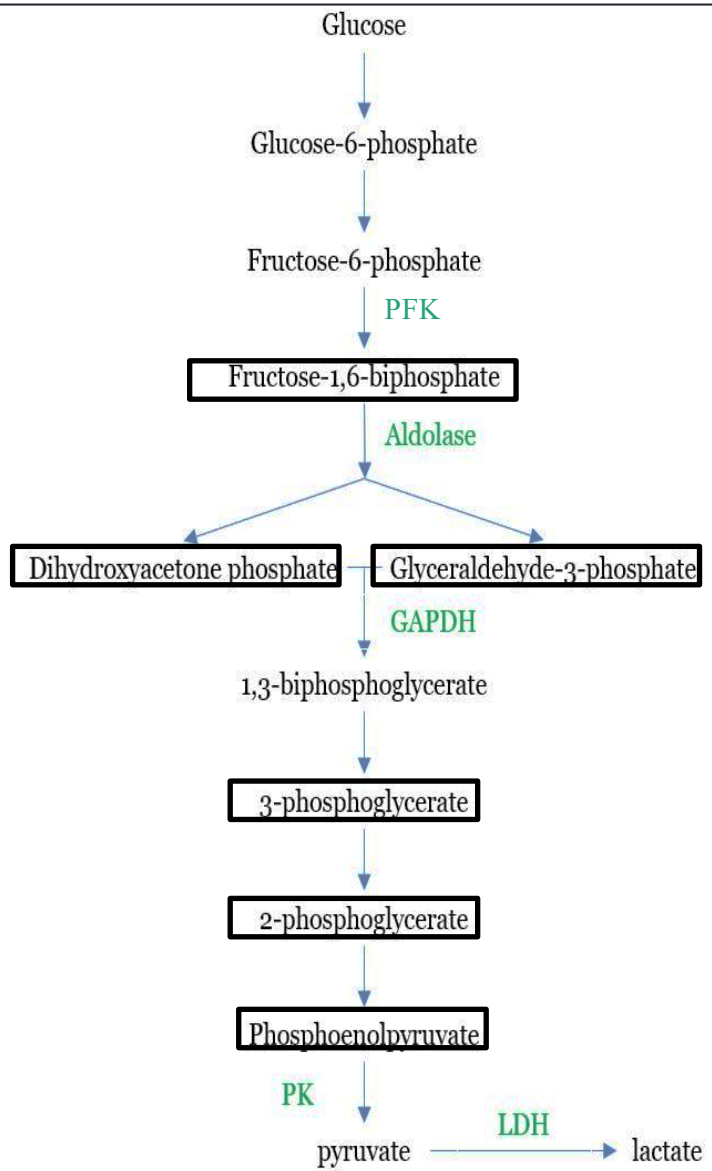
Control chow (n=3)

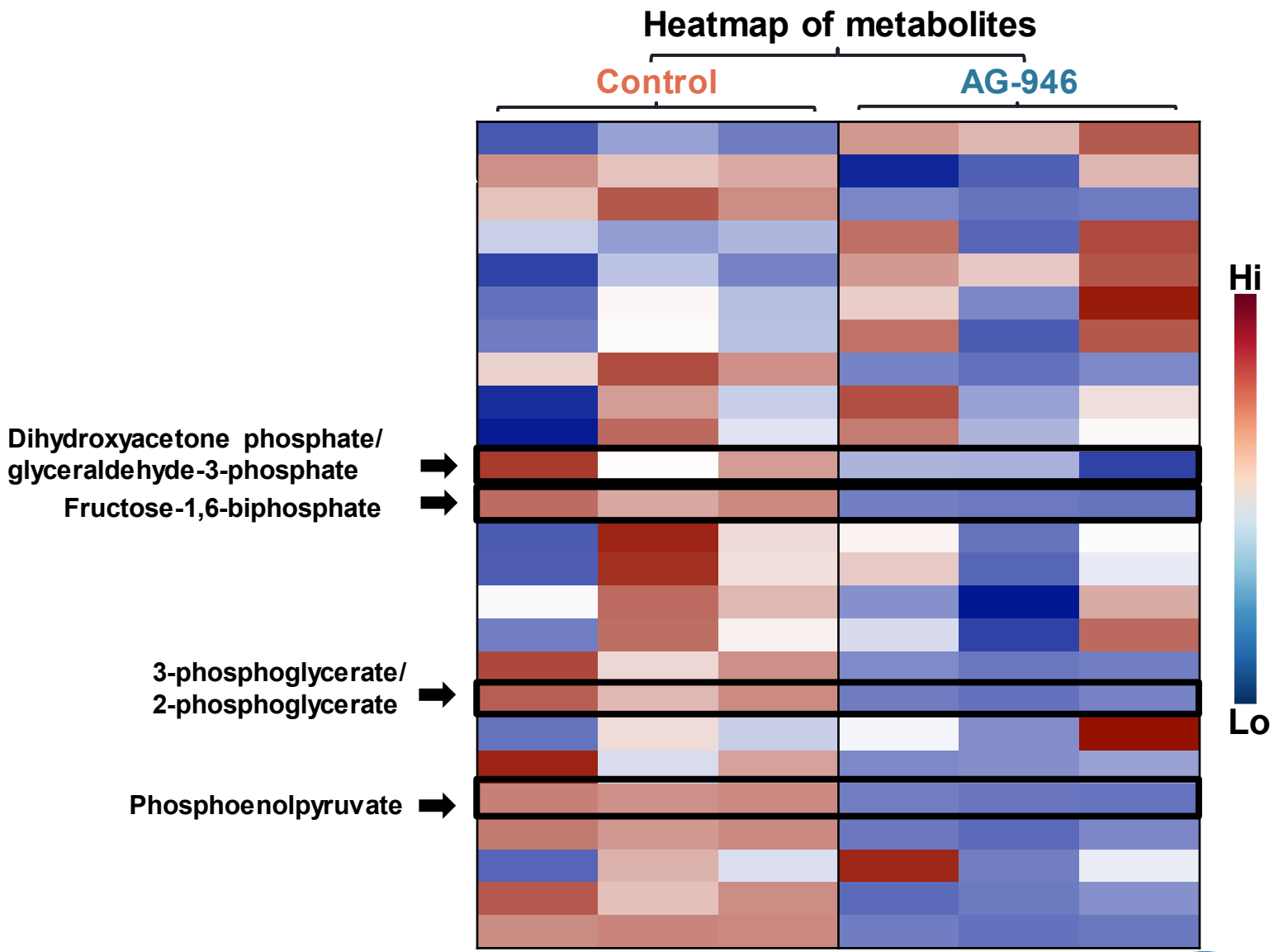
8 week
Blood collection

End points: 28 cellular metabolites; whole blood ATP and 2,3-DPG; CBC (complete blood count); sickling assays

AG-946 chow (~10 mg/kg/day)

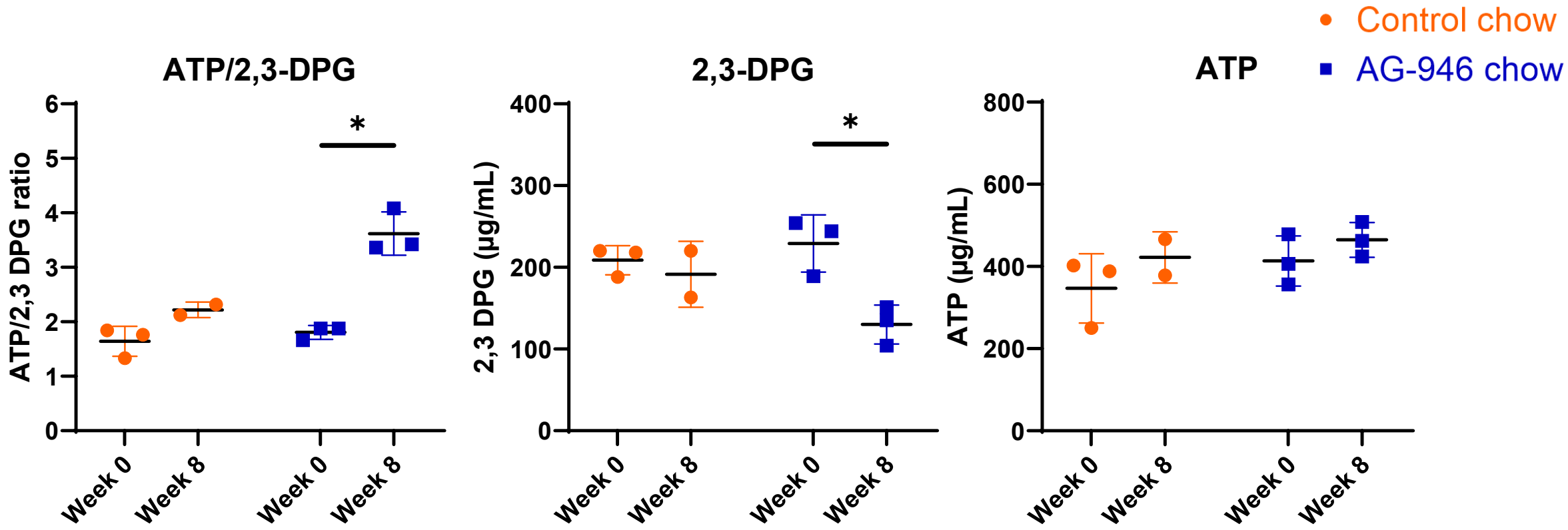
AG-946 normalized the levels of accumulated glycolytic intermediates





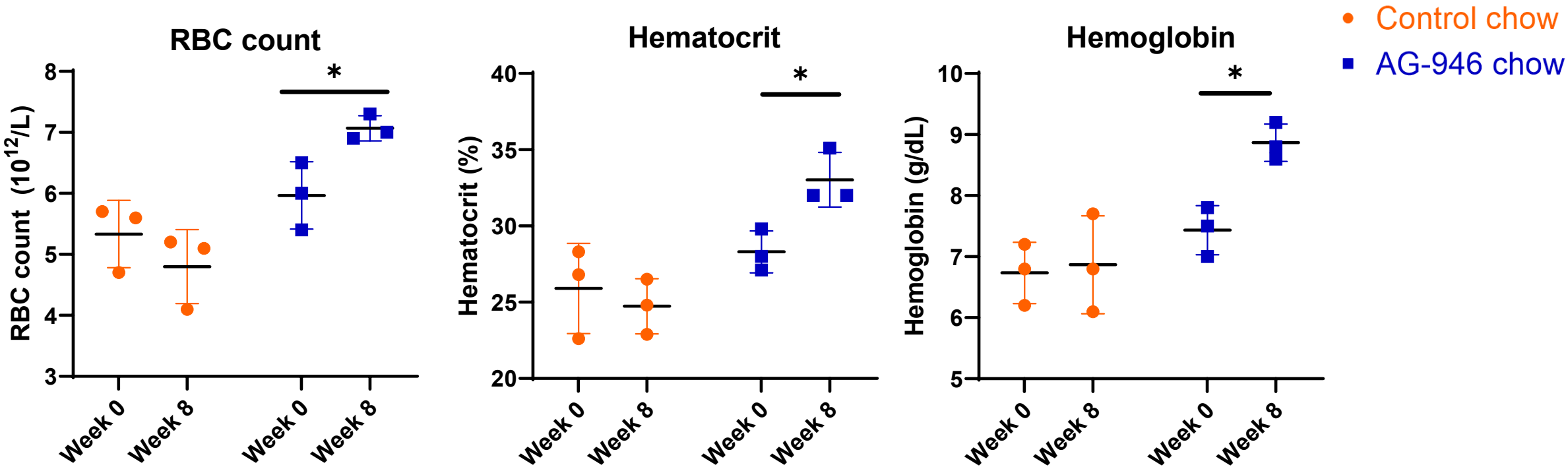
PFK: Phospho-fructokinase; **ALD**: Aldolase; **GAPDH**: Glyceraldehyde 3 phosphate dehydrogenase, **PK:** Pyruvate kinase; **LDH**: Lactate dehydrogenase

AG-946 treatment significantly improved ATP/2,3-DPG ratio in whole blood



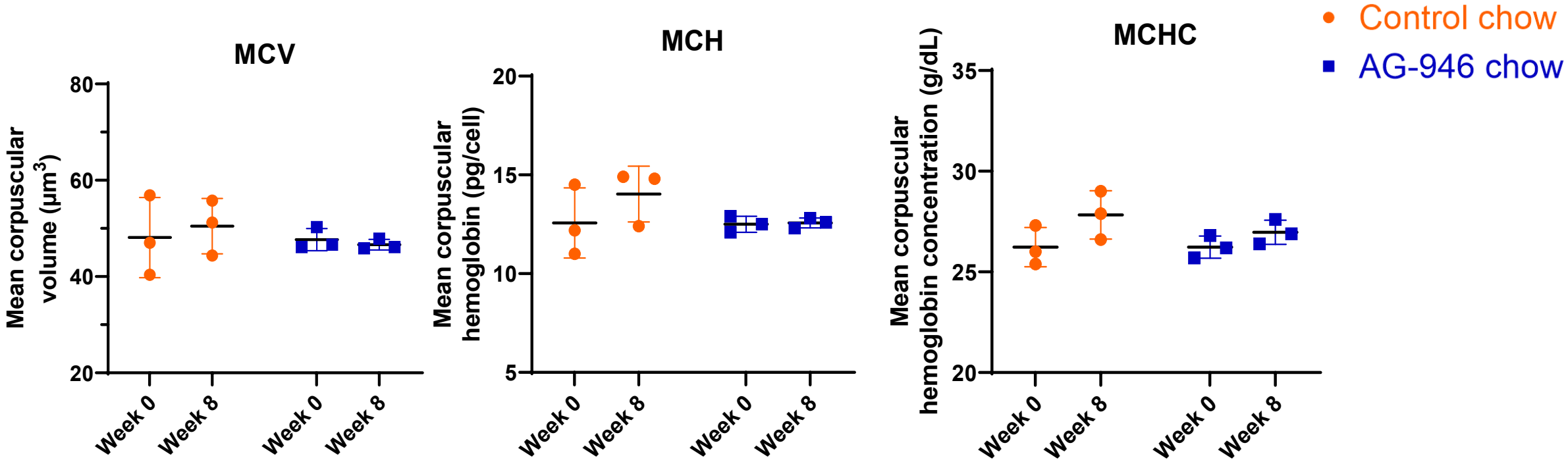
ATP and 2,3-DPG in AG-946 treatment group			
Parameter	**Week 0**	**Week 8**	**p-value (t-test)**
ATP/2,3-DPG ratio	1.81 ± 0.13	3.62 ± 0.40	0.002
2,3-DPG (µg/mL)	229 ± 35	130 ± 24	0.016
ATP (µg/mL)	413 ± 61	465 ± 42	0.298

AG-946 treatment significantly ↑ red cell count, hematocrit and hemoglobin



RBC indices in AG-946 treatment group			
Parameter	**Week 0**	**Week 8**	**p-value (t-test)**
RBC count (10^{12}/L)	5.95 ± 0.55	7.1 ± 0.21	0.030
Hematocrit (%)	25 ± 1.8	33 ± 1.8	0.022
Hemoglobin (g/dL)	7.4 ± 0.40	8.9 ± 0.3	0.008

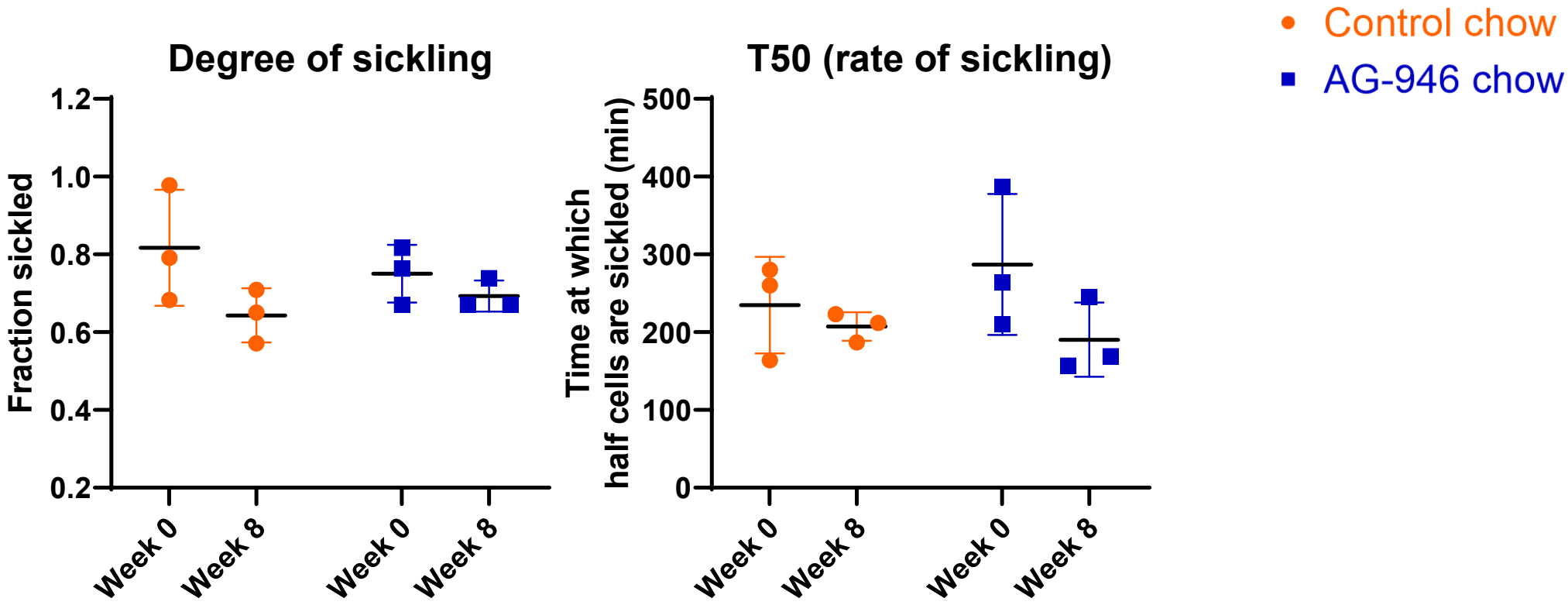
AG-946 treatment had no significant effect on MCV, MCH and MCHC



RBC indices in AG-946 treatment group			
Parameter	**Week 0**	**Week 8**	**p-value (t-test)**
MCV (µm³)	48 ± 2.3	47 ± 1.1	0.521
MCH (pg/cell)	13 ± 0.4	13 ± 0.25	0.819
MCHC (g/dL)	26 ± 0.55	27 ± 0.60	0.195

MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration

AG-946 treatment didn't improve the degree or rate of sickling



Degree and rate of sickling in AG-946 treatment group			
Parameter	**Week 0**	**Week 8**	**p-value (t-test)**
Fraction sickled	0.75 ± 0.07	0.69 ± 0.03	0.304
T50 (min)	25 ± 1.8	33 ± 1.8	0.177

Summary and conclusion

- PK activation effectively enhanced glycolysis in SS/Hb- mice [humanized with hemoglobin S (Townes) and mutant human band 3]
  - AG-946 treatment resulted in the normalization of glycolytic intermediates and was accompanied by significantly reduction in blood 2,3 DPG levels
  - AG-946 treatment led to significant improvement in critical red cell indices (red cell count, HCT, Hb); reduction in the rate or degree of sickling was not significant
- AG-946, a potent PK activator accelerating glycolysis, may offer a treatment for SCD[1,2]

[1]**Publication Number: 2383** Results from the single and multiple ascending dose study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of AG-946 in healthy volunteers
Session Date: **Sunday, December 11, 2022**
Session Time: **6:00 PM - 8:00 PM**

[2]**Publication Number: 2367** Activating Pyruvate Kinase Improves Red Blood Cell Integrity By Reducing Band3 Tyrosine Phosphorylation

Session Date: **Sunday, December 11, 2022**
Session Time: **6:00 PM - 8:00 PM**