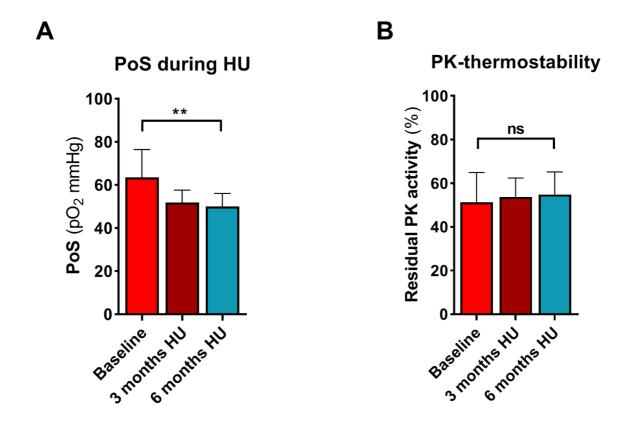
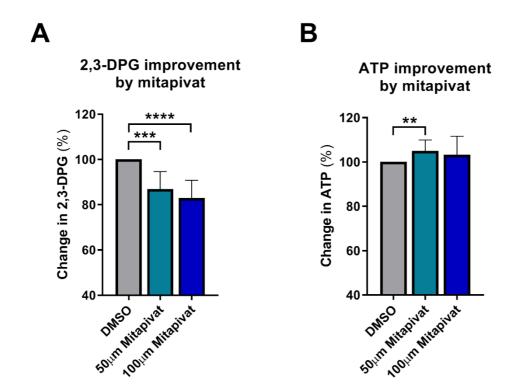
## **Supplemental Figure 1**



# Supplemental Figure 1. Clinical benefit of hydroxyurea (HU) therapy in SCD patients is independent of PK properties

(A) SCD patients (N=5) followed after starting HU therapy show a significant decrease in PoS after 3 (dark red bar) and 6 months (light turquoise), compared to baseline (red). (B) SCD patients (N=5) followed after starting HU therapy show no significant increase in mean PK-thermostability levels. Error bars represent standard deviation. \*\*p<0.01 NS, not significant.

## **Supplemental Figure 2**



### Supplemental Figure 2. Ex vivo treatment with mitapivat decreases 2,3-DPG levels

2,3-diphosphoglycerate (2,3-DPG, panel A) and adenosine triphosphate (ATP) (levels (panel B) after 22 hours incubation of buffy coat-depleted blood of SCD patients (N=11) in presence of 50  $\mu$ M (light turquoise) and 100  $\mu$ M (dark blue) mitapivat shows, respectively, a significant decrease of 17% in 2,3-DPG levels and a non-significant increase of 5% in ATP compared to vehicle controls (DMSO control, gray, N=11). Mean values are reported, error bars represent standard deviation. \*\*\*\*p<0.0001, \*\*\*p<0.001, \*\*\*p<0.001

#### **Supplemental Figure 3** Α В PK-thermostability ATP/2,3-DPG improvement restoration by mitapivat by mitapivat Change in ATP/2,3-DPG Ratio (%) 140 200-Residual PK activity (%) 120 100 80 120 60 80 40 DMSO non.TY South non TY LIN DOUR PORTY Just Populat South Robert Sount TOOM TY July John Pour 14 A MOOIM TA SounT C D p50 improvement PoS improvement by mitapivat by mitapivat 140 140-Change in PoS (%) Change in p50 (%) 120 120 100 100 ADDING TO NOTITE A ROLIN PORT THEO POPLIT 30 Roper Hone TX Philodia Louis Adolul 14 INTROPPET TOP TY ri opin T 60 Sount SOUTH TY DMSOTA Ε El<sub>max</sub> improvement by mitapivat 140 Change in El<sub>max</sub> (%) 120 100 80 THEO POPLIA 3. ROPLIT ROPLIT Andonu It July Bourn non-TX SounT\*

# Supplemental Figure 3. Comparable response to *ex vivo* treatment with mitapivat between non-transfused and transfused patients

PK-thermostability (panel A), ATP/2,3-DPG ratio (panel B), p50 (panel C), PoS (panel D) and  $El_{max}$  (panel E) were measured on RBCs from SCD patients (N=11, except for PoS and  $El_{max}$ : N=9) after 22 hours incubation of buffy coat-depleted blood in presence of 50  $\mu$ M (light

turquoise) and 100  $\mu$ M (dark blue) mitapivat. The increases in PK-thermostability, ATP/2,3-DPG ratio, and El<sub>max</sub>, as well as the decreases in p50 and PoS, compared to vehicle controls (DMSO control, gray, N=11), were comparable and statistically not different between non-transfused and transfused SCD patients. Mean values are reported, error bars represent standard deviation. \*\*\*\*p<0.0001, \*\*\*p<0.001, \*\*p<0.005