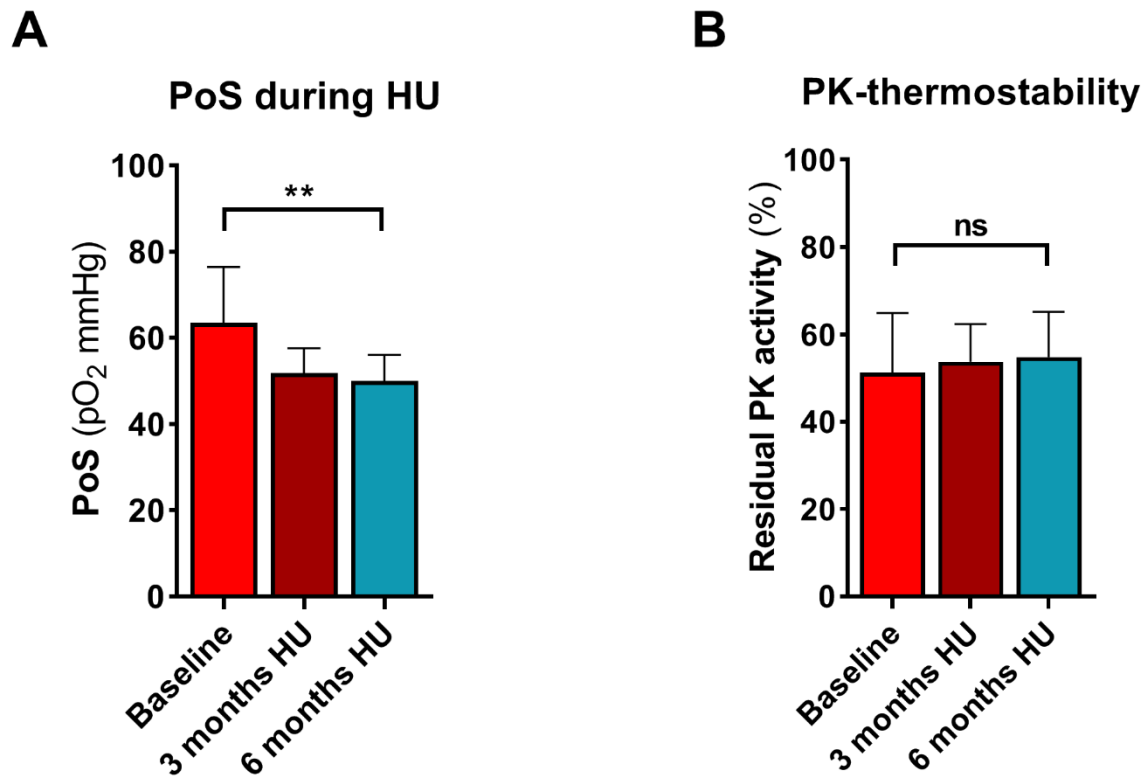


Supplemental Figure 1

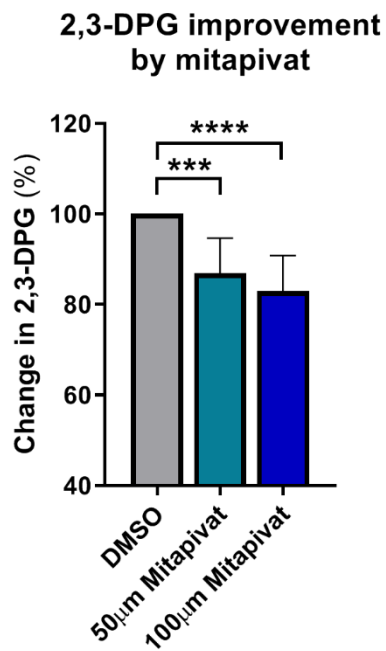


Supplemental Figure 1. Clinical benefit of hydroxyurea (HU) therapy in SCD patients is independent of PK properties

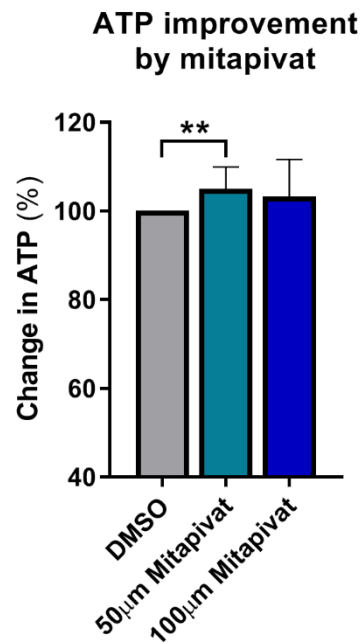
(A) SCD patients (N=5) followed after starting HU therapy show a significant decrease in PoS after 3 (dark red bar) and 6 months (light turquoise), compared to baseline (red). (B) SCD patients (N=5) followed after starting HU therapy show no significant increase in mean PK-thermostability levels. Error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

Supplemental Figure 2

A



B

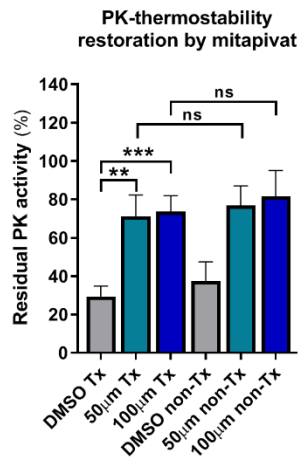


Supplemental Figure 2. *Ex vivo* treatment with mitapivat decreases 2,3-DPG levels

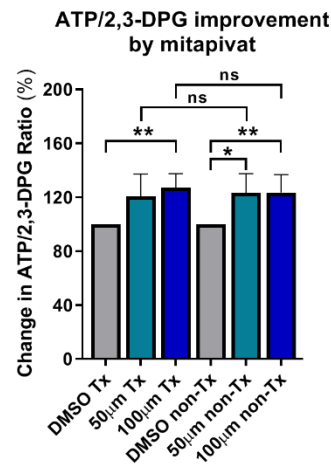
2,3-diphosphoglycerate (2,3-DPG, panel A) and adenosine triphosphate (ATP) levels (panel B) after 22 hours incubation of buffy coat-depleted blood of SCD patients (N=11) in presence of 50 µM (light turquoise) and 100 µM (dark blue) mitapivat shows, respectively, a significant decrease of 17% in 2,3-DPG 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. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$

Supplemental Figure 3

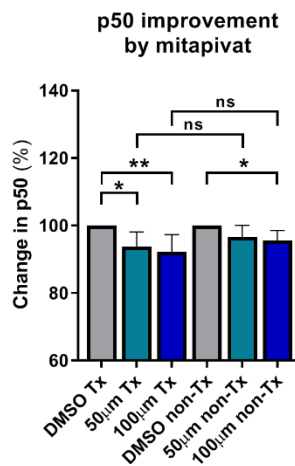
A



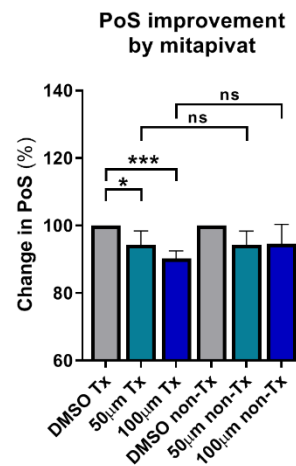
B



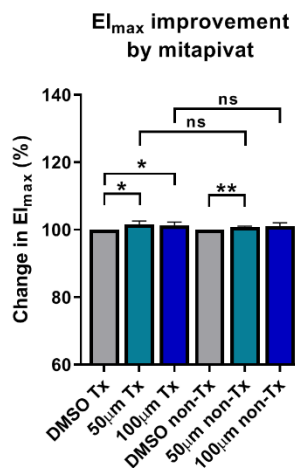
C



D



E



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 µM (light

turquoise) and 100 μ M (dark blue) mitapivat. The increases in PK-thermostability, ATP/2,3-DPG ratio, and $E_{I_{max}}$, 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. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$