

Supplemental Material for

Long-term l-glutamine treatment reduces hemolysis without ameliorating hepatic vasoocclusion and liver fibrosis in a mouse model of sickle cell disease.

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## Supplemental Methods

*Heme assay:* Heme assay was performed as per the manufacturer's instructions (Heme assay kit; ABCAM; ab272534). Briefly, liver tissue was homogenized as directed. The homogenates were centrifuged and heme levels were measured for each sample as per the manufacturer's instructions .

*Immunohistochemistry.* Tissue samples were frozen in OCT compound (Sakura, 4583) on dry ice and stored at - 80°C. Cryopreserved samples were cut into 5 µm sections, washed in PBS, and then fixed in 2% paraformaldehyde for 30 minutes. Following washing, slides were washed with PBS and permeabilized with 0.1% Triton X-100 in PBS for 20 minutes at room temperature. Samples were washed three times with PBS and then blocked with 2% goat serum in 0.1% Tween-20 in PBS (PBST) for 30 minutes at room temperature. Antibodies were diluted in 2% goat serum/PBST and incubated at 4°C overnight. Primary and secondary antibodies used are: Primary antibodies: F4/80 (CST, 70076S, 0.435 µg/ml), CLEF4C (R&D Systems, AF2784, 0.025µg/mL) and (abcam, AB75973, 0.07 µg/ml). Secondary antibodies used are anti-Mouse/Rabbit Cy3/Cy5. Images were taken on a Nikon A1 spectral confocal microscope.

*mRNA isolation and real time polymerase chain reaction:* mRNA was isolated and purified from livers of SCD mice at baseline and post l-glutamine treatment (n=4-5/group). mRNA was isolated using Trizol (Invitrogen). RT-PCR was performed as described elsewhere<sup>27</sup>. 18S and GAPDH were used to normalize the m-RNA expression data. Sequences of primers are as follows:

CD45: F-GAACATGCTGCCAATGGTCT R-TGTCCCACATGACTCCTTTCC;

F4/80: F- GCCCAGGAGTGGAATGTCAA R- CAGACACTCATCAACATCTGCG;

IL1β: F- CCATGGCACATTCTGTTCAAA R- GCCCATCAGAGGCAAGGA;

CLEC4F: F- GGAAAGTCATTCCAGACCCA R- AAGACGCCATTTAACCCACA;

TGFβ: F- GTGTGGAGCAACATGTGGA ACTCTA R- TTGGTTCAGCCACTGCCGTA;

α-SMA: F- GTTCAGTGGTGCCTCTGTCA R- ACTGGGACGACAGGAAAAG;

Col1A1: F- TAAGGGTACCGATGGAGAAC R- CTCCCTGAGCTCCAGCTTCT;

Col3A1: F- TCCCCTGGAATCTGTGAATC R- TGAGTCGAATTGGGGAGAAT;

HBA1: F- ACTCTTCTGGTCCCCACAGACTCAG R- GGGCAGAGCCGTGGCTCAGGTCGAA;

ACS14: F- CGTTTGGCTCATGTGCTGGAAC R- AGTCCAGGGATACG TTCACAC;  
PTGS2: F- GGGAGTCTGGAACATTGTGAA R- GTGCACATTGTAAGTAGGTGGACT;  
GAPDH: F-GACAGTCAGCCGCATCTTCT R- TTAAAAGCAGCCCTGGTGAC;  
18S: FCGGCTACCACATCCAAGGAA R- GCTGGAATTACCGCGGCT.

### Supplemental Movie Supplemental Video Legends

***Video S1. Visualization of blood flow in a SCD (SS) mouse after administration of TXR-dextran.*** The sinusoids in SCD mouse liver visualized by carotid artery injection of TXR-dextran (red). Here the blood flow is occluded at a region which appears dark due to lack of flow. Original acquisition rate. Scale bar 50  $\mu$ M.

***Video S2. Visualization of blood flow in a SCD (SS) mouse after administration of TXR-dextran.*** The sinusoids in SCD mouse liver visualized by carotid artery injection of TXR-dextran (red). Here the blood flow is occluded at various regions which appears dark due to lack of flow. Original acquisition rate. Scale bar 50  $\mu$ M.

***Video S3. Visualization of blood flow in a SCD (SS) mouse after administration of TXR-dextran.*** The sinusoids in SCD mouse liver visualized by carotid artery injection of TXR-dextran (red). Here the blood flow is occluded at regions which appears dark due to lack of flow. Original acquisition rate. Scale bar 50  $\mu$ M.

***Video S4. Visualization of blood flow in l-glutamine treated SCD mouse after administration of TXR-dextran.*** The sinusoids in l-glutamine treated SCD mouse liver visualized by carotid artery injection of TXR-dextran (red) and CF (green; as a marker for liver bile ducts). Here the blood flow appears continuous. Original acquisition rate. Scale bar 50  $\mu$ M.

***Video S5. Visualization of blood flow in l-glutamine treated SCD mouse after administration of TXR-dextran.*** The sinusoids in l-glutamine treated SCD mouse liver visualized by carotid artery

injection of TXR-dextran (red) and CF (green; as a marker for liver bile ducts). Here the blood flow appears continuous. Original acquisition rate. Scale bar 50  $\mu$ M.

***Video S6. Visualization of blood flow in l-glutamine treated SCD mouse after administration of TXR- dextran.*** The sinusoids in l-glutamine treated SCD *mouse* liver visualized by carotid artery injection of TXR-dextran (red) and CF (green; as a marker for liver bile ducts). Here the blood flow appears continuous. Original acquisition rate. Scale bar 50  $\mu$ M.