Supplemental material to:

P-selectin deficiency promotes liver senescence in sickle cell disease mice

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Supplemental Table

Table 1:

Parameter	**Normal range**	**SS mice**	**SS;Selp-/- mice**
White Blood Cells, $\times 10^9$/L	1.8-10.7	30(±5)	42.5 (20.42-63.7) (p=0.01)
Neutrophils, $\times 10^9$/L	0.1-2.4	8(±3)	26±5.612 (p=0.01)
Lymphocytes, $\times 10^9$/L	0.9-9.3	18(±2)	19.41 (10.36-28.02)
Monocytes, $\times 10^9$/L	0.0-0.4	1.5(±2)	3.46±1.202
Hemoglobin, g/dL	11.0-15.1	6.12±0.399	7.66±2.699 NS
Hematocrit, %	35.1-45.4	26.56±2.057	27.16±4.999 NS
Platelets$\times 10^9$/L	592-2972	311±60	470±90(p=0.01)
Reticulocytes	5.58-9.91	62.43±10	57.35±6 NS

Table 1: *Comparison of hematologic data between SS and SS;SelP$^{-/-}$ mice.* Data shown as mean±SEM for normal distributed data and as median (full range) for skewed distribution. Data are reported for 3 male and 2 female SS mice, 3 male and 3 female of SS;Selp-/- mice. Statistical significance was determined using student T test with unequal variances.

Supplemental Method:

Immunohistochemistry (IHC) and immunofluorescence (IF): Tissue sections (4-6μm) were stained with Prussian blue as described elsewhere[15]. We used primary antibodies against P21 (1:100, Santa Cruz), Ki-67 (1:100, ABCAM) and Ly6G (1:100, ABCAM). Nikon A1 Spectral Confocal microscopes were used to capture images at CBI Pitt.

Western Blot : Immunoblotting was performed as described elsewhere[15]. The following primary antibodies were used: P21 (1:1000, Santa Cruz), P16 (1:1000, Santa Cruz), Ly6G (1:1000, Abcam), Hepcidin ((1:1000, Cell signaling) and pP53 (1:1000, Cell signaling).

mRNA isolation and real time polymerase chain reaction: mRNA was isolated and purified from livers of SS and SS-*Selp*$^{-/-}$ mice (n=4/group). mRNA was isolated using Trizol (Invitrogen). RT-PCR was performed as described elsewhere[15]. 18S and GAPDH were used to normalize the m-RNA expression data. *Sequences of primers used are added below.*

Sequences of primers used in this study

Primer	**Forward**	**Reverse**
18S	CGG CTA CCA CAT CCA AGG AA	GCT GGA ATT ACC GCG GCT
GapDH	AAC TTT GGC ATT GTG GAA GG	ACA CAT TGG GGG TAG GAA CA
p16	GCTCAACTACGGTGCAGATTC	GCACGATGTCTTGATGTCCC
p21	CGAGAACGGTGGAACTTTGAC	CCAGGGCTCAGGTAGACCTT
p53	AGA GTC TAT AGG CCC ACC CC	GCT CGA CGC TAG GAT CTG AC
HO-1	CCTTCCCGAACATCGACAGCC	GCAGCTCCTCAAACAGCTCAA
CD11b	AAACCACAGTCCCGCAGAGA	CGTGTTCACCAGCTGGCTTA
Ki-67	AGAGCCTTAGCAATAGCAACG	GTCTCCCGCGATTCCTCTG
TIMP1	AGGTGGTCTCGTTGATTTCT	GTAAGGCCTGTAGCTGTGCC
ly6g	TGCGTTGCTCTGGAGATAGA	CAGAGTAGTGGGGCAGATGG
CD62L	CTAATTTCCCCTCGCTGATTCAT	GCATTAGCTTCTGTGCTGAATTGA
Col1a1	CATGTTCAGCTTTGTGGACCT	GCAGCTGACTTCAGGGATGT
mCXCR2	GGCGGGGTAAGACAAGAA TC	GGCAAGGTCAGGGCAAAGAA
Hepcidin	TGTCTCCTGCTTCTCCTCCT	CTCTGTAGTCTGTCTCATCTGTTG

Measurement of lactate dehydrogenase: LDH activity in blood serum and liver homogenate of control, SS and SS; *SelP*$^{-/-}$ was determined by a colorimetric kinetic Lactate Dehydrogenase Kit (ABCAM).

Hematological studies: Complete blood counts were obtained with a HemaVet blood analyzer (Drew Scientific, Waterbury, CT). The percentage of red fluorescent reticulocytes (Retic %) was measured by flow cytometry at the Vascular Medicine Institute Flow Cytometry Core Facility that is supported by National Institutes of Health at the University of Pittsburgh school of Medicine.

Supplemental Figure and Legends

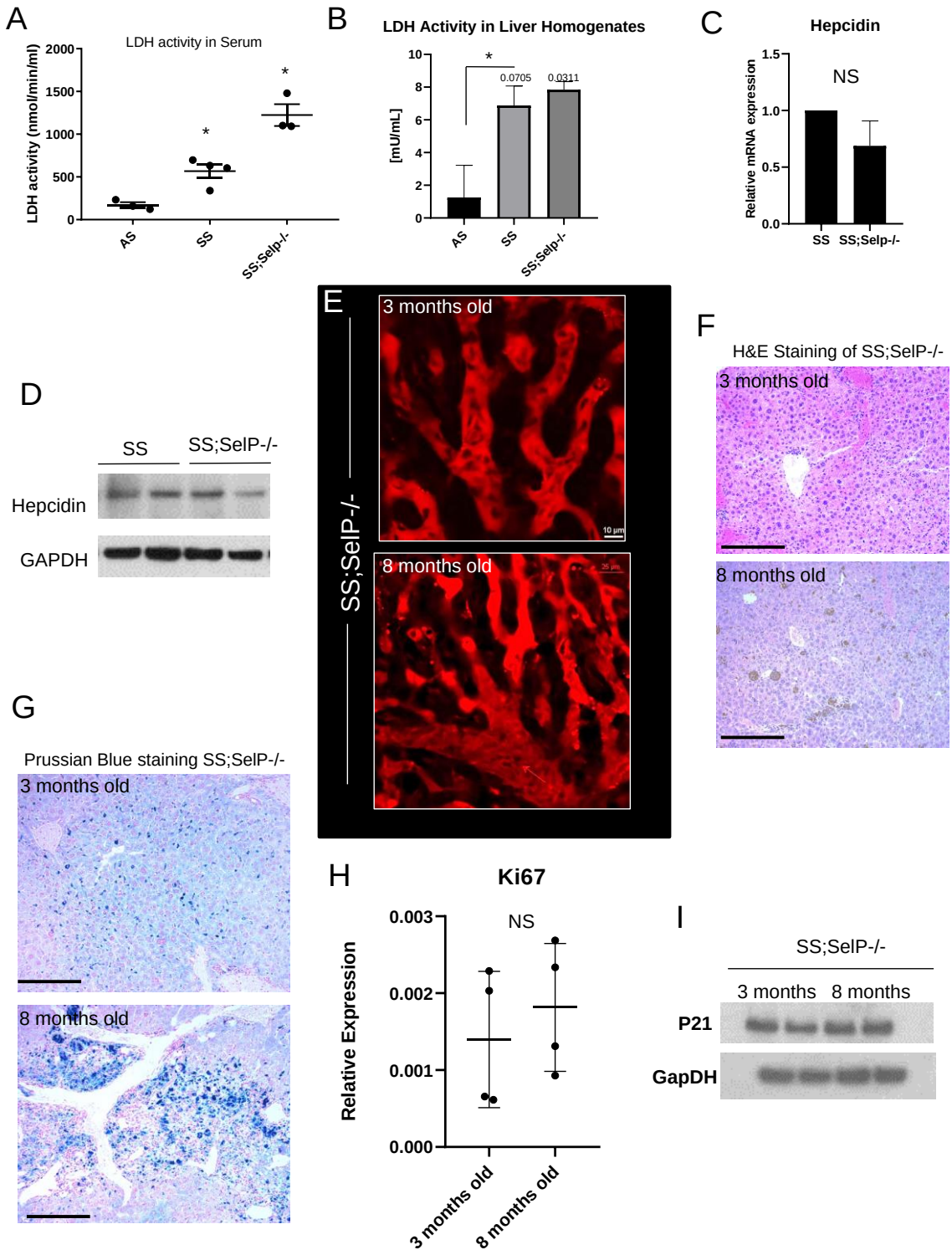

Figure S1: P-selectin deficiency promotes liver injury in sickle cell disease mice. (**A**) Serum lactate dehydrogenase level in AS (control) SS and SS;*Selp -/-* mice. Lactate dehydrogenase is significantly upregulated in SS;*Selp -/-* mice compared to SS. (**B**) Total liver Lactate dehydrogenase level in AS (control) SS and SS;*Selp -/-* mice. Compared to control both SS and SS;SelP-/- mice show significant increase in liver lactate dehydrogenase level. However, no significant change was seen in between SS and SS;SelP-/- mice. (**C**) Analysis of mRNA expression by qRT-PCR showed comparable mRNA expression of Hepcidin in SS and SS-*Selp*$^{-/-}$ liver. (**D**) Western Blot for hepcidin exhibits comparable expression in SS and SS-*Selp*$^{-/-}$ liver. (**E**) qLIM images of 3 and 8 months old SS;*Selp* $^{-/-}$ liver injected with TXR-dextran. Comparable amelioration of vasoocclusion was observed. (**F**) H&E staining of SS-*Selp*$^{-/-}$ liver showed increased liver injury at 8 months timepoint as compared to 3 months timepoint (**G**) Prussian blue staining for iron showed increased iron deposition in SS-*Selp*$^{-/-}$ liver at both 3- and 8-months' time point with significant enrichment of Prussian blue staining at 8 months of age. (**H**) Analysis of mRNA expression by qRT-PCR showed comparable mRNA expression of ki-67 in SS-*Selp*$^{-/-}$ liver at both 3- and 8-months' time point. (**D**) Western Blot for P21 exhibits comparable expression in SS-*Selp*$^{-/-}$ liver at both 3- and 8-months' time point.

Supplemental Movie Legends

Movie S1. Visualization of blood flow in a SCD (SS) mouse after administration of TXR-dextran and CF prior to imaging. The sinusoids in 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 is occluded at a region which appears dark due to lack of flow. Original acquisition rate. Scale bar 20 uM.

Movie S2. Visualization of blood flow in a SCD (SS) mouse after administration of TXR-dextran and CF prior to imaging. The sinusoids in 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 is occluded at various regions which appears dark due to lack of flow. Original acquisition rate. Scale bar 20 uM.

Movie S3. Visualization of blood flow in a SCD (SS) mouse after administration of TXR-dextran and CF prior to imaging. The sinusoids in 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 is occluded at regions which appears dark due to lack of flow. Original acquisition rate. Scale bar 20 uM.

Movie S4. Visualization of blood flow in a* SS-*Selp*$^{-/-}$ *mouse after administration of TXR-dextran and CF prior to imaging. The sinusoids in SS-*Selp*$^{-/-}$ *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 20 uM.

Movie S5. Visualization of blood flow in a* SS-*Selp*$^{-/-}$ *mouse after administration of TXR-dextran and CF prior to imaging. The sinusoids in SS-*Selp*$^{-/-}$ *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 20 uM.

Movie S6. Visualization of blood flow in a* SS-*Selp*$^{-/-}$ *mouse after administration of TXR-dextran and CF prior to imaging. The sinusoids in SS-*Selp*$^{-/-}$ *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. A small region with vasoocclusion is seen (marked by *). Original acquisition rate. Scale bar 20 uM.

Movie S7. Visualization of blood flow in a* SS-*Selp*$^{-/-}$ *mouse at 3 months of age after administration of TXR- dextran prior to imaging. The sinusoids in SS-*Selp*$^{-/-}$ *mouse* liver visualized by carotid artery injection of TXR-dextran (red). Here the blood flow appears continuous. Original acquisition rate. Scale bar 20 uM.

Movie S8. Visualization of blood flow in a* SS-*Selp*$^{-/-}$ *mouse at 8 months of age after administration of TXR- dextran prior to imaging. The sinusoids in SS-*Selp*$^{-/-}$ *mouse* liver visualized by carotid artery injection of TXR-dextran (red). Here the blood flow appears continuous. Original acquisition rate. Scale bar 20 uM.