Supplemental Legends and Figures

Heme triggers TLR4 signaling leading to endothelial cell activation and vaso-occlusion in murine sickle cell disease

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Supplemental Figure 1. The structures of heme (ferrous PPIX), hemin (ferric PPIX) and PPIX.
Supplemental Figure 2. Heme induces rapid expression of P-selectin and VWF on the surface of blood vessels in vivo. Normal C57BL/6 and NY1DD sickle mice (n=3/treatment) were infused with saline (12 mL/kg, negative control), histamine (1200 µmols/kg, positive control), or heme (3.2 µmols/kg). Fifteen minutes after infusion mice were sacrificed in CO$_2$ and lungs (A), brain (B), liver (C), and kidneys (D) were removed and frozen in OCT embedding medium for immunofluorescence staining of P-selectin (green) and VWF (red). Representative venules are shown. Overlapping green and red staining (A-D) appears orange. Nuclei were counterstained with DAPI (blue).

Supplemental Figure 2A
Supplemental Figure 2D

<table>
<thead>
<tr>
<th>Saline</th>
<th>Histamine</th>
<th>Heme</th>
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<tbody>
<tr>
<td>C57</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>NY1DD</td>
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50 μm
Supplemental Figure 3. Plasma haptoglobin and hemopexin levels are low in NY1DD and HbSS sickle mice. Equal volumes of plasma (1 µl/lane) from C57BL/6, NY1DD, HbAA-Townes and HbSS-Townes mice (n=4/model) were run on western blots immunostained with anti-haptoglobin (Sigma-Aldrich), anti-hemopexin (Abcam) and anti-IgG (Santa Cruz Biotechnology) antibodies and visualized with the appropriate secondary IgG conjugated to alkaline phosphatase (Santa Cruz Biotechnology). Immunoreactive bands were visualized with ECF™ substrate (GE Healthcare) and a Storm™ Reader (GE Healthcare). Blots were stripped (Restore Stripping Buffer, Thermo Scientific) and re-probed after each visualization.

Supplemental Figure 3