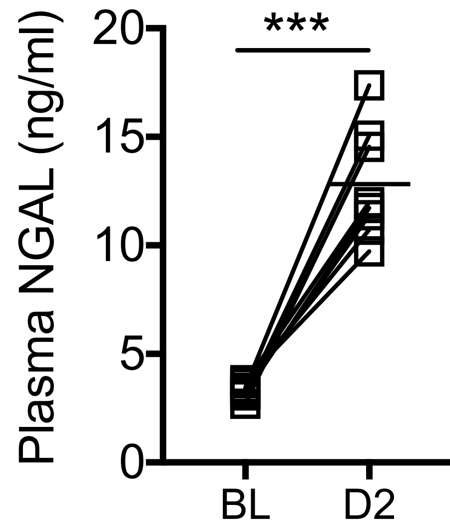
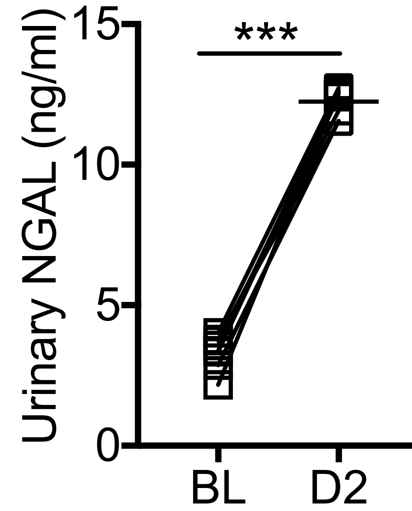
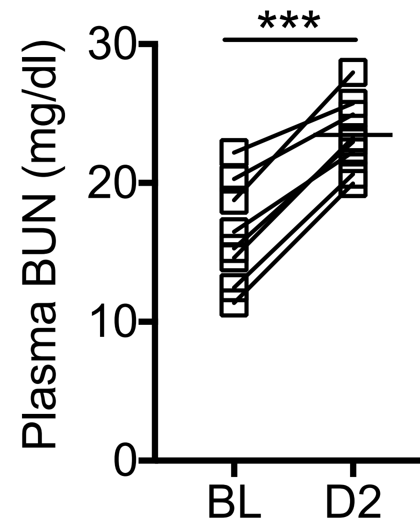
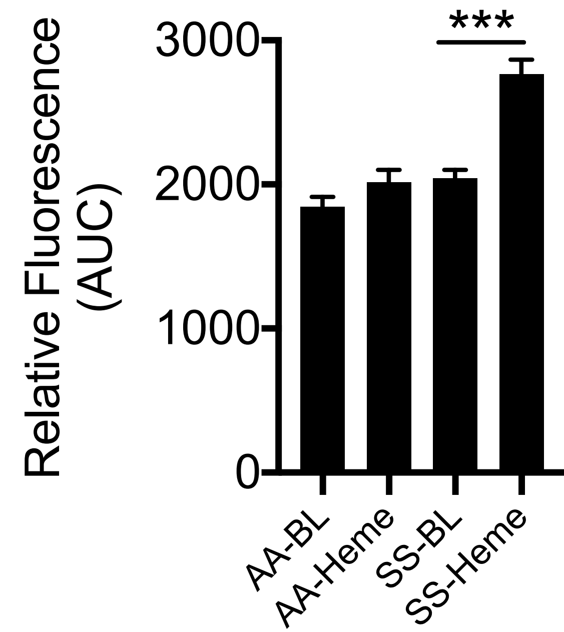
Supplementary Figure 1









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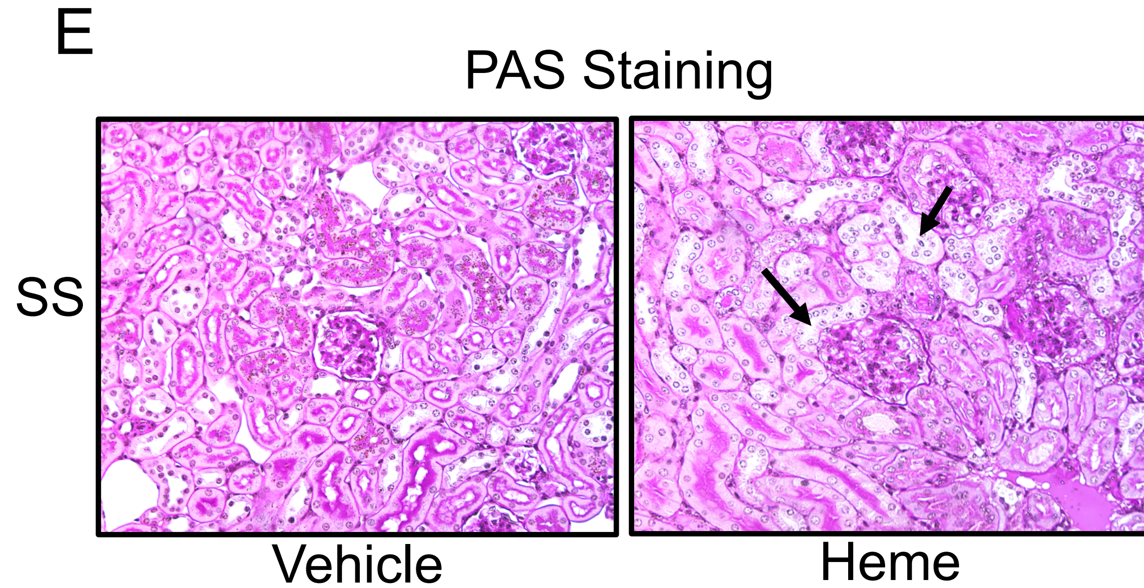
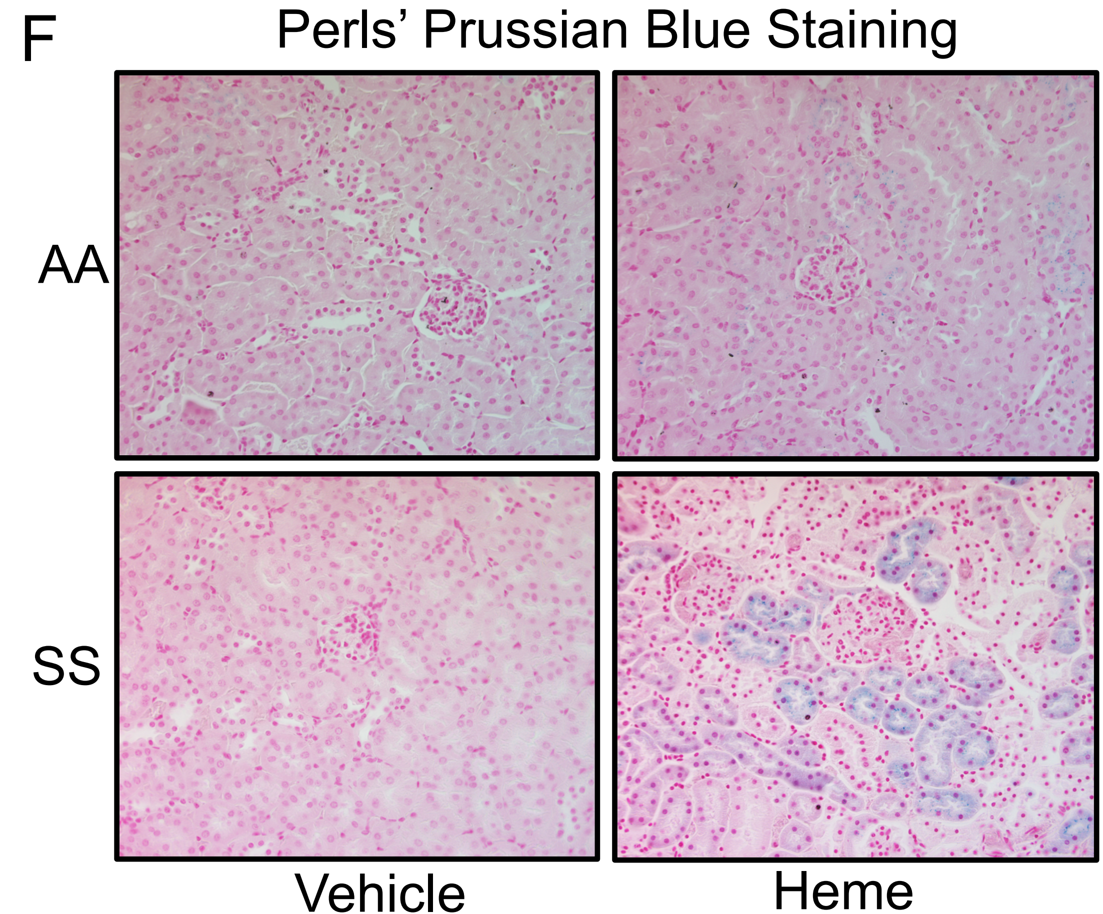
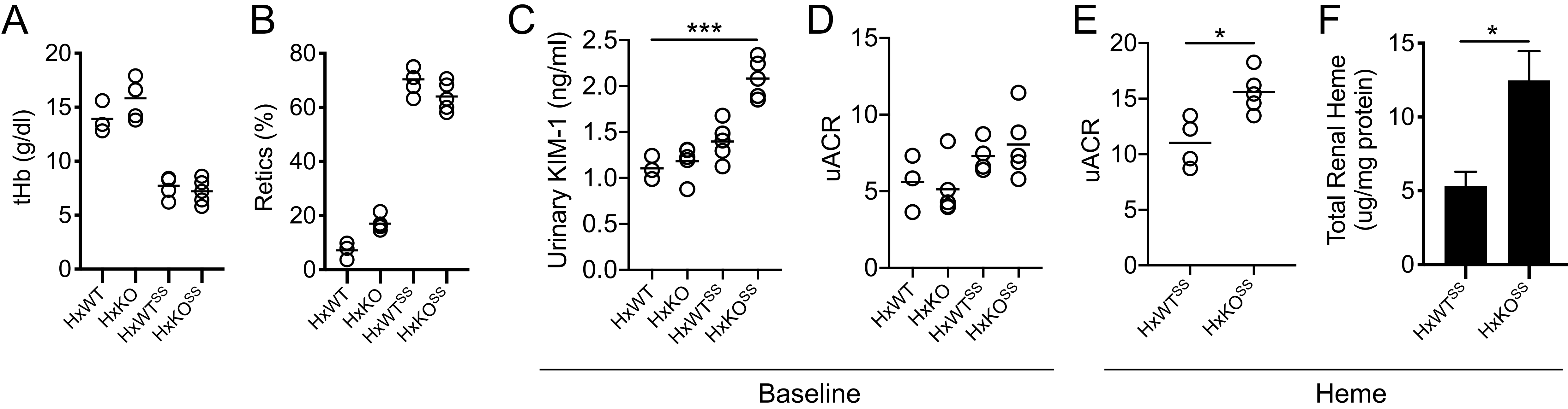




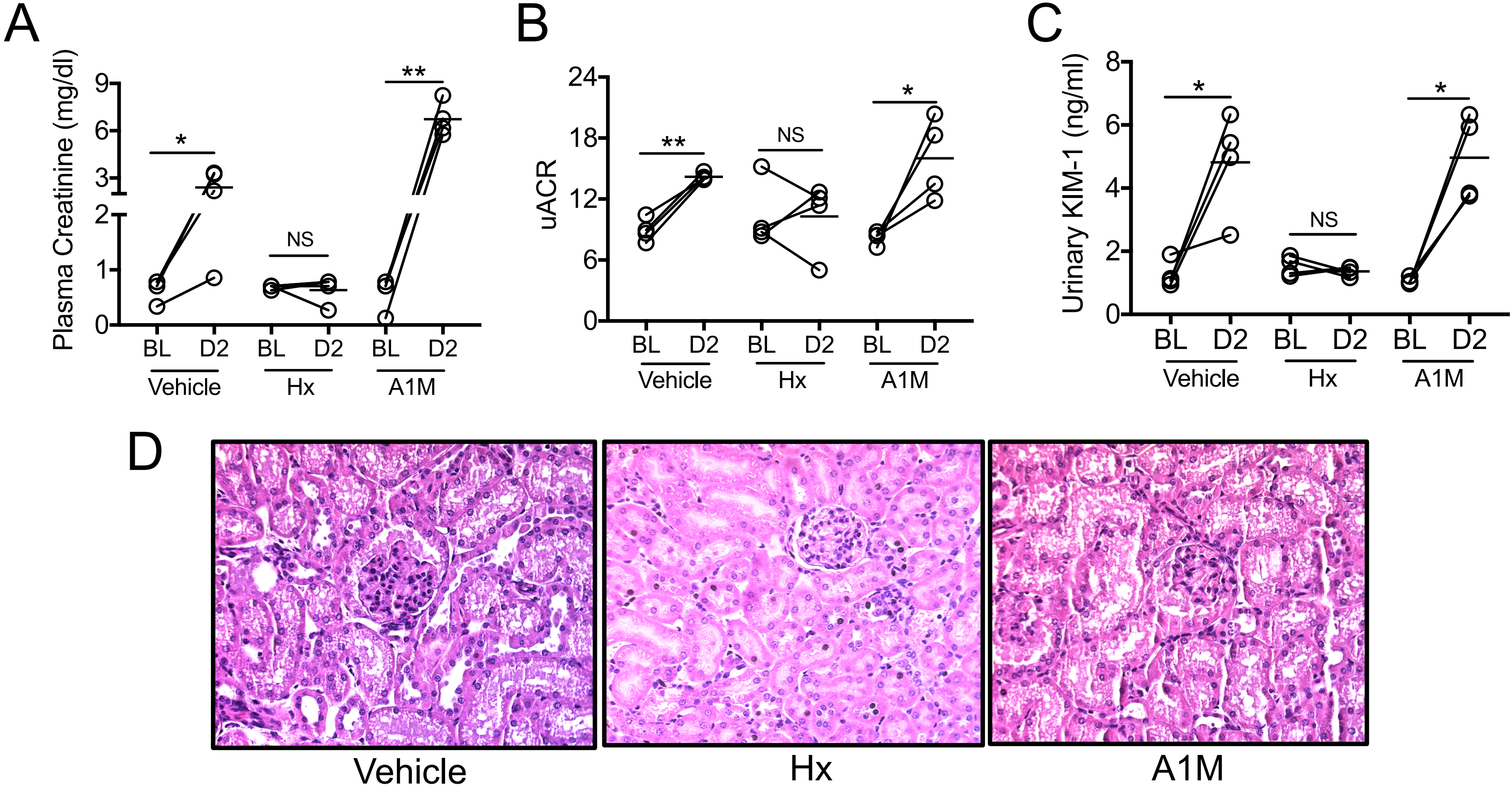
Supplementary Figure 1. Biochemical, functional and histological changes in SS mice kidney following hemin challenge. (A-C) AKI biomarkers- **(A)** plasma NGAL, **(B)** urinary NGAL and **(C)** plasma BUN in SS mice at baseline (BL) and two days (D2) following heme induced AKI. **(D)** Area under curve (AUC) for clearance of infused FITC-sinistrin in the indicated groups showing markedly increased AUC in SS mice following hemin challenge. **(E)** Periodic Acid Schiff (PAS) stained renal sections showing loss (arrow) of tubular epithelial brush borders in hemin infused SS mice kidney. **(F)** Perls Prussian Blue staining showed deposition of iron (indicated by blue stain) in tubular epithelial cells (Original magnification, x400) of SS mice following hemin infusion. ***$p<0.001$.

Supplementary Figure 2



Supplementary Figure 2. Sickle bone marrow chimera lacking non-hematopoietic hemopexin (Hx) had increased albunuria and higher renal heme content. Congenic HxWT and HxKO mice were transplanted with whole bone marrow cells from SS mice to generate $HxWT^{SS}$ and $HxKO^{SS}$ chimera mice. **(A)** Total Hb and **(B)** percent reticulocyte count indicated sickle cell anemia phenotype in bone marrow chimera mice, 2 months following bone marrow transplantation. **(C)** Urinary KIM-1 and **(D)** uACR in chimeric and wild-type mice at baseline. **(E)** Albuminuria represented as uACR and **(F)** Total renal heme in $HxWT^{SS}$ and $HxKO^{SS}$ mice two days following hemin (20 μmoles/kg bw) infusion. *$p<0.05$, ***$p<0.001$.

Supplementary Figure 3



Supplementary Figure 3. Effect of Hx and A1M on AKI biomarkers and kidney tissue after heme induced sickle AKI. Sickle mice were pretreated with either purified hemopexin (Hx), purified alpha-1-microglobulin (A1M) or vehicle prior to hemin (20 μmoles/kg bw) challenge. **(A)** Plasma creatinine, **(B)** Ratio of urinary albumin and creatinine (uACR) and **(C)** Urinary KIM-1 in indicated treatment groups at baseline (BL) and two days following hemin induced sickle AKI (D2). No alteration of these biomarkers in hemopexin treated SS mice showed protection from AKI development following hemin infusion. **(C)** Representative H&E images of renal sections from SS mice showing considerable renal tissue damage in vehicle and A1M treated and fairly normal kidney in Hx treated SS mice following hemin infusion (Original magnification x400). *$p<0.05$, **$p<0.01$, NS, non-significant.

SUPPLEMENTAL METHODS

Methods

Reagents and drugs

Hemin [Fe(III)PPIX; Sigma-Aldrich] was prepared by dissolving in 0.25M NaOH, pH was neutralized to 7.4 with HCl and the solution was adjusted to required concentration with PBS, filter sterilized and used immediately as described previously [1]. Purified human hemopexin (purity >95%) was obtained from Athens Research and Technology, Athens GA. Native human alpha-1-microglobulin was purchased from Bio-Rad.

Human serum, plasma and urine samples

All human samples were obtained from ORDISS bio repository [2]. Whole blood and urine samples were collected when patients attended clinic with no signs of acute illness (steady state). Blood was collected in EDTA and heparin anticoagulant, as well as in serum collection tube, and processed within 4 hours to collect plasma and serum. Urine samples collected during the same day as that of blood were centrifuged immediately at 600 x g for 5 min. Aliquots of each samples were then banked at -80°C locally and shipped to University of Pittsburgh in liquid nitrogen.

Mice and procedures

University of Pittsburgh IACUC approved all animal experiments for this study (#18072763). We have established colonies of both the Townes [3] sickle mice in our laboratory as we have previously reported [4]. Mouse genotypes were confirmed either by PCR or Hb gel electrophoresis. Mice lacking expression of hemopexin gene or HxKO mice (B6.129-*Hpx*^{tm1Altr}/J; stock# 029380) and wild-type C57BL/6 mice, referred to in this study as HxWT (C57BL/6J; stock# 000664) were purchased from The Jackson Laboratory. Mice were injected with **a)** freshly prepared solutions of hemin (20 μmoles/kg bw) or saline as a vehicle (i.v.), **b)** purified human Hx (i.v.; 1mg/mouse). **c)** purified human A1M (i.v.; 10mg/kg bw). Mice were phlebotomized by

retro orbital bleeding using a capillary tube internally coated with heparin/EDTA anticoagulant. Spot urine was collected on a cling wrap placed on a white sheet of paper [5].

Whole blood analysis

Total Hb concentration was measured in venous whole blood using a portable CO-oximeter (AVOXImeter 4000, ITC) as described elsewhere. The device measures the level of different hemoglobin parameters using novel optics and multiple wavelengths [6,7]. Percent reticulocytes were determined by flow cytometric analysis of thiazole orange stained whole blood samples [8] and analyzing data using a FACSDIVA software.

Biochemical analysis (Plasma, urine and tissue)

Freshly collected blood samples in 5% EDTA were centrifuged at 1200 x g for 10 minutes to collect plasma. Freshly collected urine samples were centrifuged at 300 x g, 4°C for 5 minutes. Concentration of plasma hemoglobin and plasma LDH activity was determined by colorimetric method using Quantichrome hemoglobin or LDH assay kit (Bioassay Systems) as described previously [1,9]. Concentration of serum, plasma or urine creatinine, plasma BUN and urine albumin was measured using assay kits purchased from Arbor Assays. Commercial ELISA kits were used to determine plasma concentrations of hemopexin (Kamiya Biomedical Company), A1M (BioLegend), and NGAL (R&D) following instructions provided by vendor. One half of each kidney tissue harvested from mice two days after hemin infusion was snap-frozen in liquid nitrogen. The tissue was homogenized in RIPA buffer (Promega) using protease inhibitor cocktail. Total heme content was measured in tissue homogenate using a colorimetric kit (BioAssay Systems). Total protein was determined using BCA protein assay kit (Pierce) following manufacturer's instructions.

Measurement of glomerular filtration rate (GFR)

GFR was measured in mice prior to and two days following hemin infusion using a non-invasive transcutaneous device (MediBeacon Inc). The procedure has been previously described [10]. Briefly, the animals were placed under isoflurane anesthesia and hairs removed from a portion of dorsal area using a razor and depilatory cream. The fluorescence recording device was then fixed on the depilated region using a double-sided adhesive patch. The device was allowed to measure the background signal (emitted by the skin before the administration of the fluorescent compound) for a few minutes before FITC-sinistrin (7.5 mg/100 g body weight dissolved in NaCl 0.9%) was injected intravenously. Emitted fluorescence was recorded for 60 minutes. The data was then analyzed using a software provided by MediBeacon to obtain half-life ($t_{1/2}$) for clearance of FITC-sinistrin by the individual mouse. GFR was calculated from the half-life and a semi-empirical conversion factor using the formula: GFR [μl/min/100g bw] = 14616.8 [μl/100g bw)] / $t_{1/2}$ (FITC-Sinistrin)[min]. The clearance of FITC-sinistrin was also plotted relative to the maximum fluorescence recorded for each individual mouse.

Bone marrow transplantation

HxWT and HxKO mice were maintained on acidified drinking water for 7 days and subjected to two doses of 600 rads irradiation given 4 hours apart. Irradiated mice were transplanted with 5×10^6 whole bone marrow cells harvested from SS mouse donors. The transplanted mice (designated as $HxWT^{SS}$ and $HxKO^{SS}$) were maintained on medicated (Neomycin: 0.5mg/ml; Polymyxin B: 0.0125 mg/ml) water for one week. Blood samples were collected from transplanted mice 12-weeks post-transplant for complete blood count (CBC) using HemaTrue hematology analyzer (Heska) and to assess reticulocyte count using flow cytometry.

Histopathology

Kidney tissue were harvested two days following low dose hemin infusion and fixed uniformly with 10% buffered formalin. Tissue sections were stained with hematoxylin and eosin (H&E) or periodic acid Schiff (PAS; Sigma-Aldrich) staining. For TUNEL staining, renal tissue sections (5 μm) were processed and labeled with TdT-label followed by counterstaining with nuclear fast-red using a TACS-2 TdT blue label kit (Trevigen Inc). Following staining, tissue sections were examined using Olympus AX70 microscope and images were recorded with an Olympus U-CMAD3 camera at the Center for Biologic Imaging, University of Pittsburgh. Histopathological analysis was performed by a modified extensive scoring system as previously described [11]. Injury was defined by acute tubular necrosis, tubular cell vacuolization and tubular atrophy, loss of proximal tubular brush borders, tubular dilatation and interstitial cast formation. Histopathologic scoring was using the following scale: 0=no damage; 1=1–25%; 2=26–50%; 3=51–75%; and 4=76–100% of field showing injury. TUNEL-positive apoptotic cells and total cells were counted and percentage of TUNEL positive tubules were determined based on ten random fields per slide [12].

Statistical Analysis

Results are reported as both individual data points and mean±SEM. To analyze statistical significance, two-tailed paired or unpaired Student's *t*-test was used as appropriate. Two-way ANOVA analyses were performed while comparing two or more groups. GraphPad Prism 7 software was used for all statistical analyses.

Reference to Supplemental Methods

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SickleGenAfrica Network members

SickleGenAfrica coordinating centre

Korle Bu Teaching Hospital, Accra, Ghana and University of Ghana, Accra, Ghana
Amma Benneh-Akwasi Kuma, Anita Ghansah, Catherine Segbefia, David Nana Adjei, Edeghonghon Olayemi, Gordon Awandare, Solomon F. Ofori-Acquah, William Kudzi

Komfo Anokye Teaching Hospital, Kumasi, Ghana and Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
Ellis Owusu-Dabo, Vivian Painstil

Aminu Kano Teaching Hospital, Kano, Nigeria, Bayero University, Kano, Nigeria and Murtala Mohammad Specialist Hospital, Kano, Nigeria
Aisha Kuliya-Gwarzo, Adullahi Shehu, Baba Musa, Mahmoud Sani, Najibah Aliyu Galandanci

Institute of Human Virology Nigeria, Kano, Nigeria and University of Abuja, Abuja, Nigeria
Alashle Abimiku, Ameh Adeyefa, Obiageli E. Nnodu

Lagos University Teaching Hospital, Lagos, Nigeria and University of Lagos, Nigeria
Michael Akinsete, Olufunto Kalejaiye, Titilope Adeyemo

Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
Floro Ndobho, Furahini Tluway, Julie Makani, Siana Nkya

University of Cape Town
Nicola Mulder

Imperial College London, London, UK
Kofi Anie

Morehouse School of Medicine, Atlanta, USA
Jonathan Stiles

University of Pittsburgh, Pittsburgh, USA
Flordeliza Villanueva, Samit Ghosh, Solomon Ofori-Acquah, Ryan Minster