SUPPLEMENTAL MATERIALS AND METHODS

Animal preparation. Studies were performed in male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200-250 g. Animal handling and care followed the NIH Guide for Care and Use of Laboratory Animals. The experimental protocol was approved by the UCSD Institutional Animal Care and Use Committee. Briefly, animals were anesthetized using isoflurane (5%/vol for initial induction, 2.5%/vol for maintenance, Drägerwerk AG, Lübeck, Germany) and prepared with: (i) left jugular vein and left femoral artery catheterization, (ii) tracheotomy (polyethylene-190 tube), and (iii) left ventricular (LV) conductance catheter introduced through the right carotid artery. Animals were placed in the supine position on a heating pad to maintain core body temperature at 37 °C. Animals were mechanically ventilated (TOPO ventilator, Kent Scientific, Torrington, CT), with a respiration rate of 70 breaths/min and peak inspiratory pressure of 8-12 cmH_2O. After tracheotomy, isoflurane (2.5%/vol) was administered via the ventilator to preserve the depth of anesthesia during the experimental protocol. Isoflurane was reduced to 1.5%/vol after cessation of surgery. Depth of anesthesia was continually verified via toe pinch and if needed, isoflurane concentration was increased by 0.1%/vol to prevent animal discomfort (**Fig. S4A**)

Inclusion criteria. Animals were suitable for the experiments if: (i) Mean arterial blood pressure (MAP) was above 90 mmHg at baseline, (ii) stroke volume (SV) was above 100 uL at baseline, (iii) systemic hematocrit was above 40% at baseline, and (iv) animals survived the shock period.

Cardiac function. A 2F pressure-volume (PV) conductance catheter (SPR-858, Millar Instruments,

TX) was inserted into the left ventricle (LV) using the closed chested method (48). Briefly, the PV catheter was inserted through the exposed right carotid artery and slowly advanced into the LV. Pressure and volume signals were acquired continuously (MPVS300, Millar Instruments, Houston, TX, and PowerLab 8/30, AD Instruments, Colorado Springs, CO). LV volume was measured in conductance units (relative volume unit, RVU) and converted to absolute blood volume (uL) at the end of the experiment (48). Parallel volume was calibrated via IV injection of 35 uL hypertonic saline (15% NaCl w/v) (48).

Systemic hemodynamics parameters. Mean arterial pressure (MAP) and heart rate (HR) were recorded continuously from the femoral artery (PowerLab 8/30, AD Instruments, CO). Hematocrit was measured from centrifuged arterial blood samples taken in heparinized capillary tubes. Hemoglobin content was determined spectrophotometrically (B-Hemoglobin; Hemocue, Stockholm, Sweden). Arterial and venous blood was collected in heparinized glass capillary tubes (65 uL) and immediately analyzed for oxygen partial pressure (PO_2), carbon dioxide partial pressure (PCO_2), pH, Hb saturation, glucose, and lactate (ABL90; Radiometer America, Brea, CA).

Cardiac pressure-volume indices. Cardiac function was analyzed using PowerLab software (PowerLab 7.8, AD Instruments, Colorado Springs, CO). Cardiac function parameters were calculated from 15 to 20 cardiac cycles at each time point. Stroke volume (SV), stroke work (SW), cardiac output (CO), ejection fraction (EF), cardiac contractility ($dP/dt/V_{ed}$), and arterial elastance (Ea) were directly calculated in the PowerLab software. Systemic vascular resistance (SVR) was calculated as: $\mathrm{SVR} = \frac{\mathrm{MAP}}{\mathrm{CO}}$. The internal energy utilization (IEU) was used as a measure of internal

metabolism of the LV (49) and was calculated as $\mathrm{IEU} = ([\mathrm{V_{es}} - \mathrm{V_0}] * \mathrm{P_{es}})/2$. Where V_{es} is the end systolic volume, V_0 is the ESPV volume axis intercept, and P_{es} is the end systolic pressure. Using the assumption that end-systolic pressure-volume curve intercept is small, V_0 was set to zero for the calculations.

Blood collection and preparation. Briefly, male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 300-450 g were anesthetized with a ketamine/xylazine cocktail (ketamine: 100mg/kg, xylazine: 10 mg/kg) and a femoral artery catheter was implanted. Each donor bled freely into 1.4 mL of CP2D (Taken from an AS-3 blood preparation kit, Haemonetics Corporation, Braintree, MA) until 50% of blood volume was lost. Donor blood was then pooled, and CP2D concentration was adjusted to 14%. Pooled blood was then centrifuged at 1000 g for 7 minutes, and the supernatant was removed. AS-3 (22%/vol) was then added and the blood was mixed gently by inverting the bag for 1 minute. Pooled blood was then passed through a neonatal leukocyte reduction filter (Haemonetics Corporation, Braintree, MA). RBC units intended for conventional storage (**Conventional**) were then stored at 4°C. Units for anaerobic storage (**Anaerobic**) and Anaerobic + CO_2 storage (**AN+CO_2**) were then deoxygenated by filling the storage bag with nitrogen (or 5% CO_2, balance nitrogen), and mixing by rotation at approximately 30 RPM. The gas was replaced every 10 minutes until Hb O_2 saturation measured <8%. Anaerobically stored cells were then placed in a sealed container filled with nitrogen and O_2 sorbent packs and stored at 4°C. For *in vitro* and recovery studies, 2 pools (14 rats each) were split 3 ways and stored for up to 4 weeks. For *in vivo* hemorrhagic shock/resuscitation studies, 2 pools for each storage condition (total n = 6 pools, 10 rats each) were stored for 3 weeks before

infusion.

Histology. Immunohistochemical staining for pimonidazole bound to hypoxic zones in vital tissues during hypoxia was completed via IV injection of the hypoxic marker Hypoxyprobe-1 (40 mg/kg pimonidazole, Hypoxyprobe, Burlington, MA) and 5 mg/kg Hoechst 33342 (Invitrogen, Carlsbad, CA) diluted in PBS (total volume 100 µL) 15 minutes before the animal was sacrificed. Organs were immediately excised after euthanasia and frozen at -80°C. Three parasternal short axis (PSS, cross-sectional "slice") sections of the heart were created for analysis. 6 to 10 random areas per slide were analyzed for positive pimonidazole staining for each organ. Tissues were frozen and processed for cryosectioning followed by immunohistochemical analysis. Sections were fixed with 100% methanol for 20 minutes at -20°C, then blocked and permeabilized with 5% BSA, 5% goat serum, and 0.1% Tween 20 for 1-2 h at room temperature. Sections were then incubated with antipimonidazole (Hypoxyprobe, 1:100) antibodies overnight at 4°C. The sections were mounted in SlowFade DAPI (Invitrogen Corp. Carlsbad, CA) and imaged using a fluorescence digital microscope (VB-6000; Keyence, Osaka, Japan).

Aspartate transaminase (AST), alanine transaminase (ALT), and IL-6 levels were determined in serum samples using ELISA kits (KA1625 and KT-6104 from Abnova Corp, Taiwan, and BMS625 from Thermo Fisher, Waltham, MA). NGAL was determined in the urine using ELISA kits (ERLCN2, Thermo Fisher). Serum Creatinine and Blood Urea Nitrogen (BUN) were measured using colorimetric detection kits (KB02-H2 and K024-H5, Arbor Assays Inc., Ann Arbor, MI). Liver, Lung and spleen CXCL1 are performed on whole tissue homogenates by ELISA assay (ERCXCL1, Thermo

Fisher, Waltham) and corrected for protein concentration using the Pierce™ BCA assay kit (Thermo Fisher).

Positive CD45 neutrophils were quantified in the bronchoalveolar lavage (BAL) fluid collected by instilling sterile PBS into the lung. Percentage fraction of neutrophils in BAL was determined by flow cytometry (FACSCalibur; BD, Franklin Lakes, NJ). Neutrophils are identified by their typical appearance in the forward/side scatter and their expression of CD45 (554875, BD Biosciences).

Volume infused. Blood was infused via a flow-controlled syringe pump at 300 uL/min. Total blood volume infused was calculated as $V = Q * t$, where V is the volume infused, Q is the flow rate of the syringe pump, and t is the time that the syringe pump was active, as measured by a stop-watch. Resuscitation % was calculated as $R = \frac{V}{TBV}$ where R is the resuscitation % and TBV is the total blood volume of the rat, estimated as 7% body weight. Number of units infused was calculated as $N = \frac{R*[Hb]_{donor}}{([Hb]_{human\ donor}*V_{blood\ bag})/Human\ weight}$ where $[Hb]_{donor}$ is the Hb concentration of the rat donor blood, $[Hb]_{human\ donor}$ is the Hb concentration of a typical unit of packed RBCs (assumed 20g/dL), $V_{blood\ bag}$ is the typical volume of a unit of packed RBCs (assumed 500mL) and human weight is the typical weight of a human (assumed 70kg), normalizing the mass of Hb given to a human on a per unit basis to the mass of Hb administered to the animal.

24-hour recovery. Blood was radiolabeled with Technetium-99 (Tc^{99}) as described by Zink et. al. (50). Briefly, RBC samples (1.0 mL) were added to a sterile reaction vial and gently mixed to dissolve the lyophilized UltraTag-RBC (UltraTag-RBC, Mallinckrodt, St. Louis, MO), and allowed to

react for 5 to 7 minutes. Then, UltraTag pH buffers were added to adjust pH, by gently mixing them into the reaction vial by inverting the container. Samples were washed with sterile PBS twice and centrifuged to removed unreacted Tc^{99}, and labeled RBCs were injected. 200 µL of Tc^{99} radiolabeled blood (approximately 2% of blood volume) was delivered I.V. to male Sprague-Dawley rats (n=2 per pool, per storage condition; n=4 per storage condition) and 65 µL samples were drawn at 5 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, and 24 hours post-injection via tail clip. Samples were all run for radioactivity on a Cobra II gamma counter (Packard Instrument Co., Meriden, CT) at the same time so counts reported are independent of sample time and only representative of the still-circulating radio-labeled RBCs.

ATP and 2,3DPG. Aliquots of RBCs were mixed with cold trichloroacetic acid (DiaSys Deutschland, Flacht, Germany) and vortexed for 60 seconds. Aliquots sat on ice for 5 minutes and were then centrifuged at 3600g at 4°C for 10 minutes. The supernatant was removed and frozen at -80°C for later analysis. Supernatants were assayed enzymatically with commercially available kits. DPG was measured with the Roche 2,3-Diphosphoglycerate kit (Roche Diagnostics, Indianapolis IN, USA) according to manufacturer's instruction. ATP was measured by DiaSys ATP Hexokinase FS kit (DiaSys Diagnostic Systems GmbH, Holzheim, Germany) according to manufacturer's instruction.

Statistical analysis. Results are presented as median with 95% confidence interval of the median. The values are presented as absolute values and relative to the baseline. A ratio of 1.0 signifies no change from the baseline, whereas lower or higher ratios are indicative of changes

proportionally lower or higher compared to baseline, respectively. The Grubbs' method was used to assess closeness for all measured parameters at baseline and shock. Sample size was calculated using an α of 0.05 and a 1-β of 0.9, resulting in a minimum acceptable sample size of 5 per group. Additional animals were included due to the complexity of the experimental setup. Statistically significant changes between solutions and time points were analyzed using two-way analysis of variance (ANOVA), followed by *post hoc* analyses using Tukey's multiple comparisons test when appropriate. All statistics were calculated using GraphPad Prism 6 (GraphPad, San Diego, CA). Results were considered statistically significant if $P < .05$.