SUPPLEMENTARY MATERIALS:

SUPPLEMENTAL VIDEOS:

Supplemental Video 1. Sickle red blood cells under normoxic conditions and exposed to the admixture with a final sodium concentration of 141 mEq/L either transit or occlude a microfluidic device with geometry of the capillary system.

Supplemental Video 2. Sickle red blood cells exposed to the admixture with a final sodium concentration of 141 mEq/L quickly occludes the capillary microfluidic device when going from fully oxygenated to fully deoxygenated conditions.

SUPPLEMENTAL FIGURES AND LEGENDS:

Figure S1.

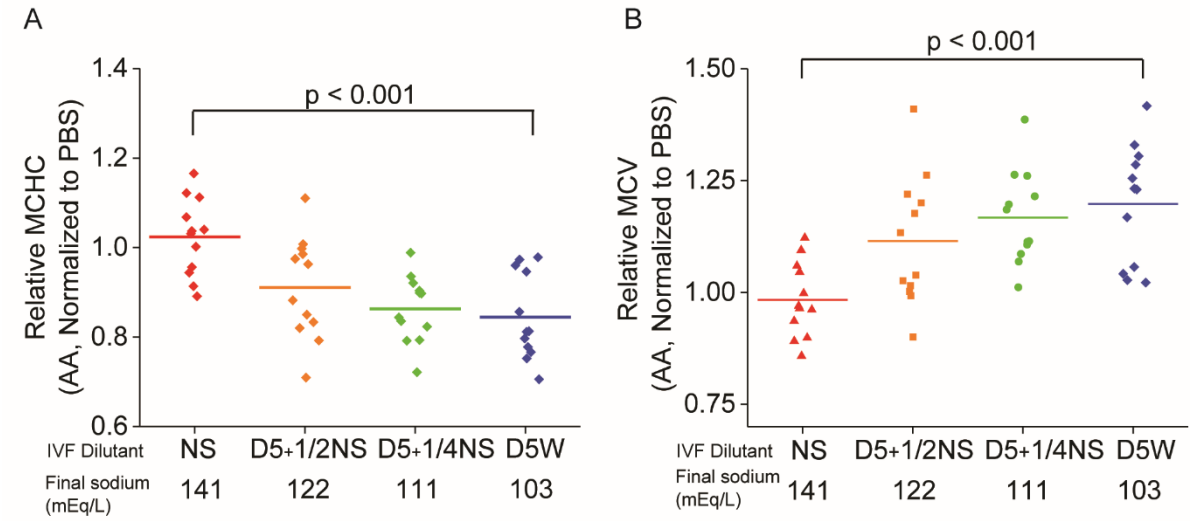

Figure S1. (A) Relative MCHC values of red cells taken from N=3 healthy donors (i.e. AA). Decreased levels were seen in the hypotonic solutions but there was more variability compared to sickle red cell values. **(B)** Relative MCV values of red cells taken from the same donors. Increased levels were seen in the hypotonic solutions but there was more variability compared to sickle red cell values. Lines represent mean values. Measurements were done in quadruplicate for all experiments.

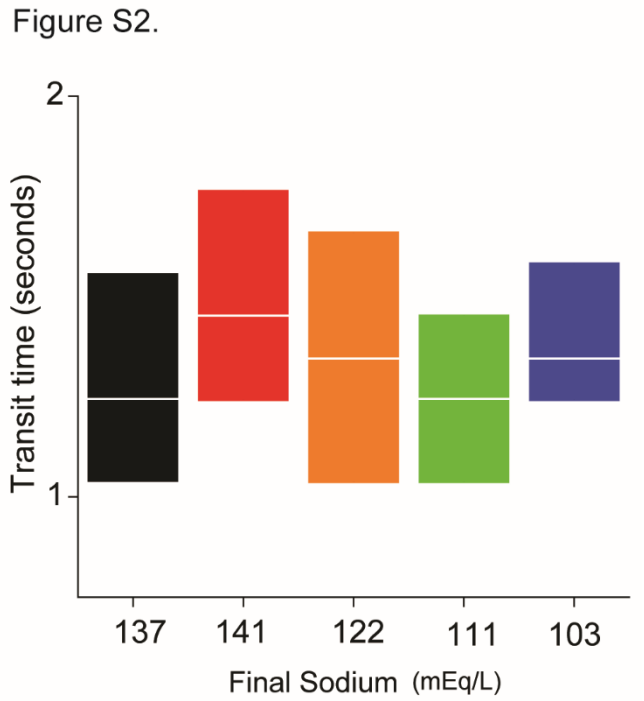

Figure S2. Transit times of sRBCs in the capillary bed model, including those exposed to isotonic PBS buffer only (sodium = 137 mEq/L). Lines and boxes represent medians and 25th and 75th percentiles, respectively.

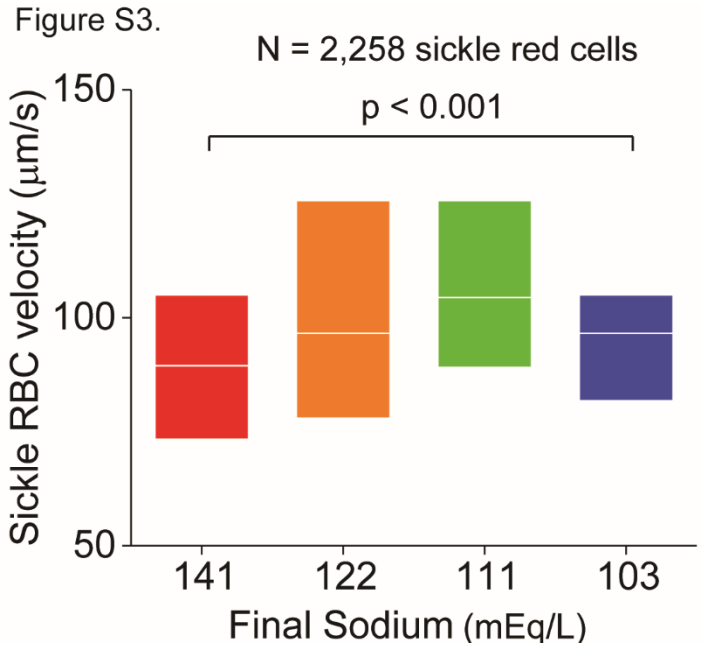

Figure S3. Velocity of sRBCs in the capillary bed model reach physiologic levels. Lines and boxes represent medians and 25th and 75th percentiles, respectively.

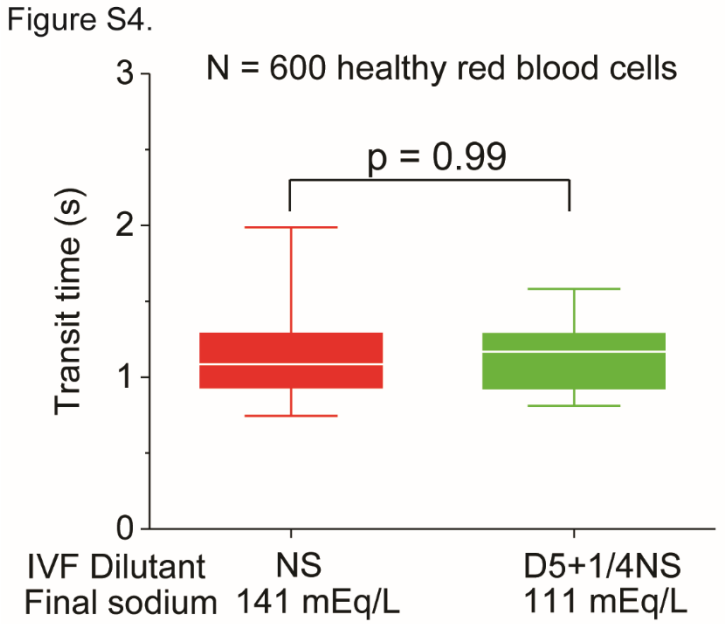

Figure S4. Transit times of red blood cells taken from N=3 healthy donors are decreased compared to sRBCs in the capillary bed model. No difference was noted between the two tonicity conditions, as opposed to sickle red blood cells exposed to similar conditions. Lines, boxes, and whiskers represent medians, 25th and 75th percentiles, and 5th and 95th percentiles, respectively.

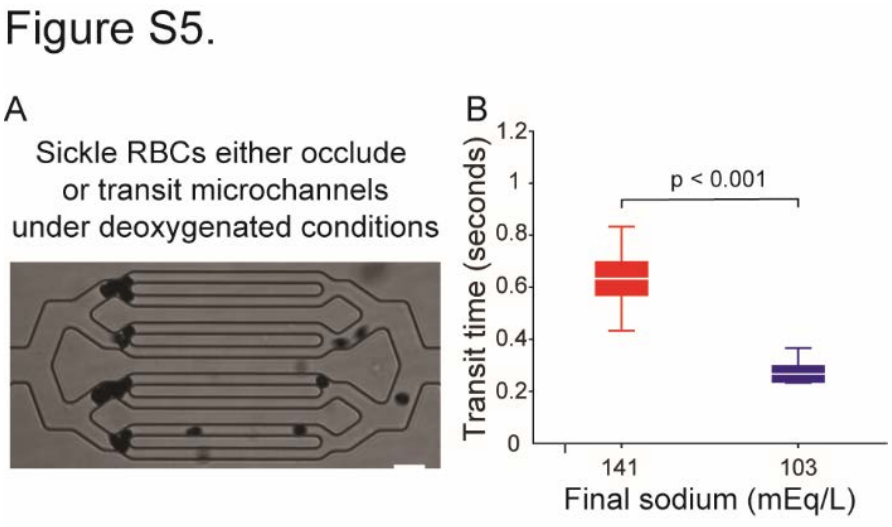

Figure S5. (**A**) Sickle red blood cells (sRBCs) re-suspended in fluid admixtures with a sodium concentration of 141 mEq/L transit or obstruct the capillary device under deoxygenated conditions. Full deoxygenation triggered rapid microchannel occlusion by sRBCs. Scale bar = 20 μm. (**B**) Box plot comparing sickle RBC transit times in deoxygenated conditions and after exposure to admixtures with a sodium concentration of 141 mEq/L (N=72) or 103 mEq/L (N=85). There was an apparent increase in transit times of sickle RBCs that traversed the microchannels after being re-suspended in the higher sodium admixtures and exposed to 0% oxygenation ($p<0.001$).

Figure S6.

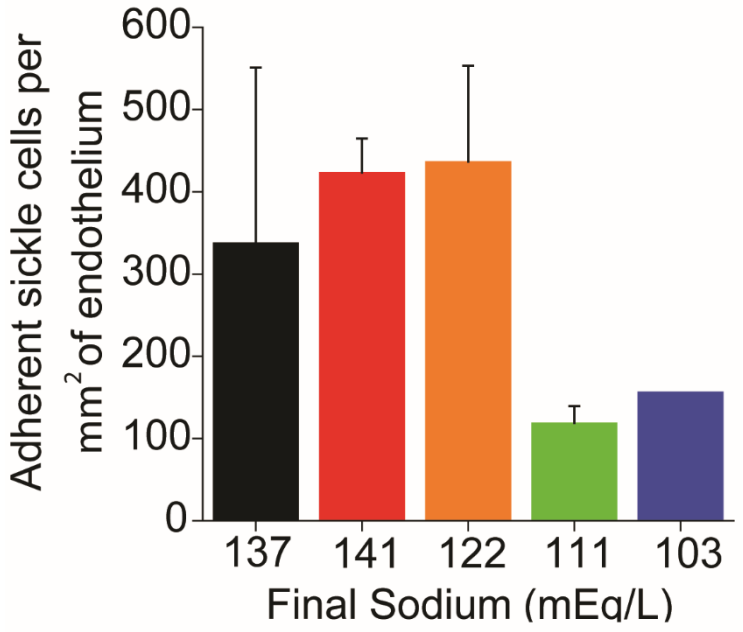

Figure S6. Adhesion of sRBCs to human endothelium under post-capillary venular shear stress, including those exposed to isotonic PBS buffer only. (Bars and lines represent mean ± SD.)

Figure S7.

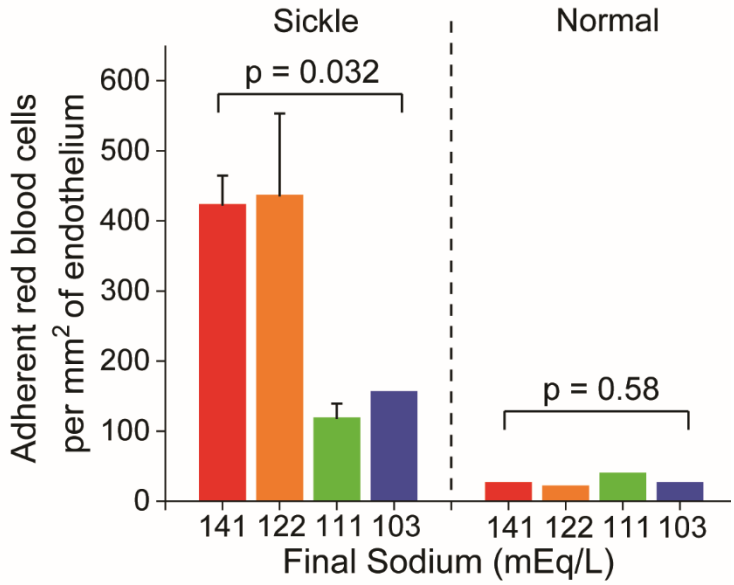

Figure S7. Adhesion of normal red blood cells from N=3 healthy donors to human endothelium (right) under post-capillary venular shear stress in comparison to adhesion of sRBCs under similar conditions (left). Bar heights on the right are representative of composite results averaged from two experiments from two separate donors.

Figure S8.

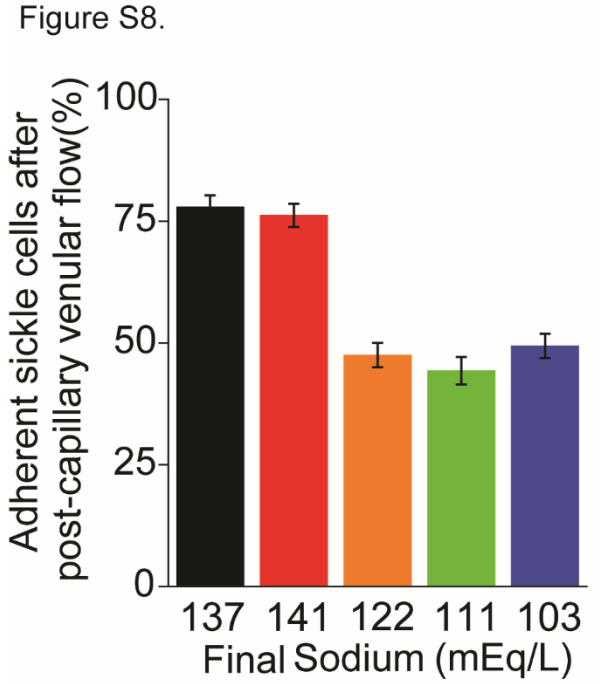

Figure S8. Adhesion of sRBCs to human laminin before and after post-capillary venular flow is affected by fluid admixture tonicity, including those exposed to isotonic PBS buffer. (Bars and lines represent mean ± SD.)

SUPPLEMENTAL TABLE:

Table S1. Effect of commonly used intravenous fluids on the osmolality of plasma from a patient with SCD.

Components of admixture[1]	Average Osmolality (Range)[2] (mmol/kg)
PPP	277 (277-278)
3 parts PPP: 1 part PBS	285 (285-286)
3 parts plasma: 1 part NS	287 (284-291)
3 parts PPP: 1 part D5+1/2NS	317 (313-323)
3 parts PPP: 1 part D5+1/4NS	298 (295-302)
3 parts PPP: 1 part D5W	283 (279-288)

[1]PPP = platelet poor plasma, PBS = phosphate buffered saline, NS = normal saline, D5+1/2NS = 5% dextrose in water and ½ normal saline, D5+1/4NS = 5% dextrose in water and ¼ normal saline, D5W = 5% dextrose in water

[2]Measurements were done in triplicate.

SUPPLEMENTAL METHODS:

MCHC measurements:

Whole blood was drawn into EDTA tubes (BD Biosciences) from N=3 normal donors and from N=3 patients with SCD who had not been transfused in at least 100 days. Baseline hematocrits (HCTs) were determined after spinning in microhematocrit tubes at 15000 rpm for 10 minutes in

a hematocrit centrifuge. For MCHC measurements, 200µL of whole blood was first transferred to 2mL Eppendorf tubes. Each tube was then filled with the various admixtures (i.e. PBS, 3 parts PBS with 1 part NS, 3 parts PBS with 1 part D5+1/2NS, etc.), mixed thoroughly and centrifuged at 5000 rpm for 3 minutes. Cells were washed three times total in this manner. After the third wash, supernatant was removed, leaving a loose red blood cell pellet. HCTs were then determined for each sample as described above in quadruplicate.

Hemoglobin concentrations were also determined in quadruplicate using a spectrophotometer (Beckman Instruments). For each measurement, 10µL of washed, plasma poor packed red cells in the different conditions described above were expelled into 1.50mL of water, mixed and placed in the spectrophotometer, and read at 540nm against a water blank. Paired measurements of hemoglobin and hematocrit were used to calculate MCHC using the formula **MCHC = [OD X 151]/[8.85 X Hematocrit]**, where 151 is the dilution factor, 8.85 the extinction coefficient of hemoglobin, and OD the optical density measured by the spectrophotometer for each sample.

MCV calculations:
To determine relative MCV measurements, we used the following relationship between MCV and MCHC: MCHC = MCH/MCV. Assuming that the average hemoglobin per cell (MCH) was the same regardless of cellular hydration status, we calculated relative changes in MCV from the changes in MCHC produced by suspension in the various fluid admixtures. Comparing measurements of MCHC at baseline ($MCHC_1$) to MCHC at condition 2 ($MCHC_2$), relative MCV at condition 2 can be calculated by the relationship $MCV_2/MCV_1 = MCHC_1/MCHC_2$.

Measuring osmolalities:
Platelet poor plasma was isolated from a patient with sickle cell disease who was seen for a routine clinical visit. We exposed the plasma to the various IVFs in the 3:1 ratio as described in the text (i.e. 3 parts plasma to 1 part IVF), and then used vapor pressure osmometry (Vapro 5500, Wescor Inc., South Logan, UT, USA) to measure changes in osmolality. Measurements were taken in triplicate and averaged.

Deformability and occlusion experiments:
SRBCs were isolated after removing the plasma from spun whole blood, then washed in PBS three times at centrifugation of 150 x *g* for 15minutes. Video recording with phase-contrast microscopy (TE2000-u microscope, Nikon) was used to track TTs and propensity for occlusion of sRBCs under normoxic conditions (Supplemental Video 1).

Adhesion experiments:
HPL: SRBCs were initially allowed to adhere to laminin-coated slides for 15minutes. Syringes filled with 3:1 IVF admixtures were loaded onto a syringe pump and attached via plastic tubing (Cole-Parmer) to the devices. After an additional 15 minutes, adherent sRBCs were counted in each lane along the 4mm straight portion of the microfluidic. SRBCs were then exposed to the different admixtures at post-capillary shear stress for 3 minutes. Post-flow adherent cells were counted in the same microscopic fields, and fractions of cells remaining were calculated.
HUVECs: SRBCs were isolated and washed in PBS, then stained with octadecyl rhodamine b chloride (R18, ThermoFisher Scientific) in PBS and EGM-2 media (Lonza), and then washed once again in PBS before beginning the experiments.

Deoxygenation experiments:
Capillary device:
To determine the effect oxygen gas tension had on sRBC transit times, we modified our existing

microfluidic platform to include an oxygen gas tension reservoir. We diffusively coupled the oxygen gas tension reservoir to the capillary microfluidic device using a multi-layered PDMS approach and controlled the oxygen gas tension in the reservoir off chip through a solenoid valve switch connected to either 159.6mmHg oxygen gas tension (21% O_2, 5% CO_2, balance N_2) or 0 mmHg oxygen gas tension (5% CO_2, balance N_2). SRBCs were then perfused through the microfluidic device at a constant pressure of 300 Pa using a pressure regulator (PCD, Alicat Scientific). A high frame rate camera (XiQ, Ximea; 300 fps) was used to measure sRBC transit times in the device while going from oxygenated to deoxygenated conditions (Supplemental Video 2).

Blood rheology deceleration experiments:

To maintain native hematocrit and to ensure full replacement of blood plasma with the admixtures, we centrifuged 200µL whole sickle blood at 200 x *g* for 10 minutes, carefully replaced approximately 100µL with the 3:1 admixtures, using NS or D5W, gently re-suspended the blood, and repeated the process three times.