Supplemental Material for

"Oxygen-dependent blood flow of sickle trait blood as an in vitro therapeutic benchmark for sickle cell disease treatments"

X Lu, A Chaudhury DK Wood, JM Higgins

Morphological changes to single RBCs as oxygen tension is decreased is different in sickle cell disease (genotype SS) vs sickle cell trait (genotype AS)

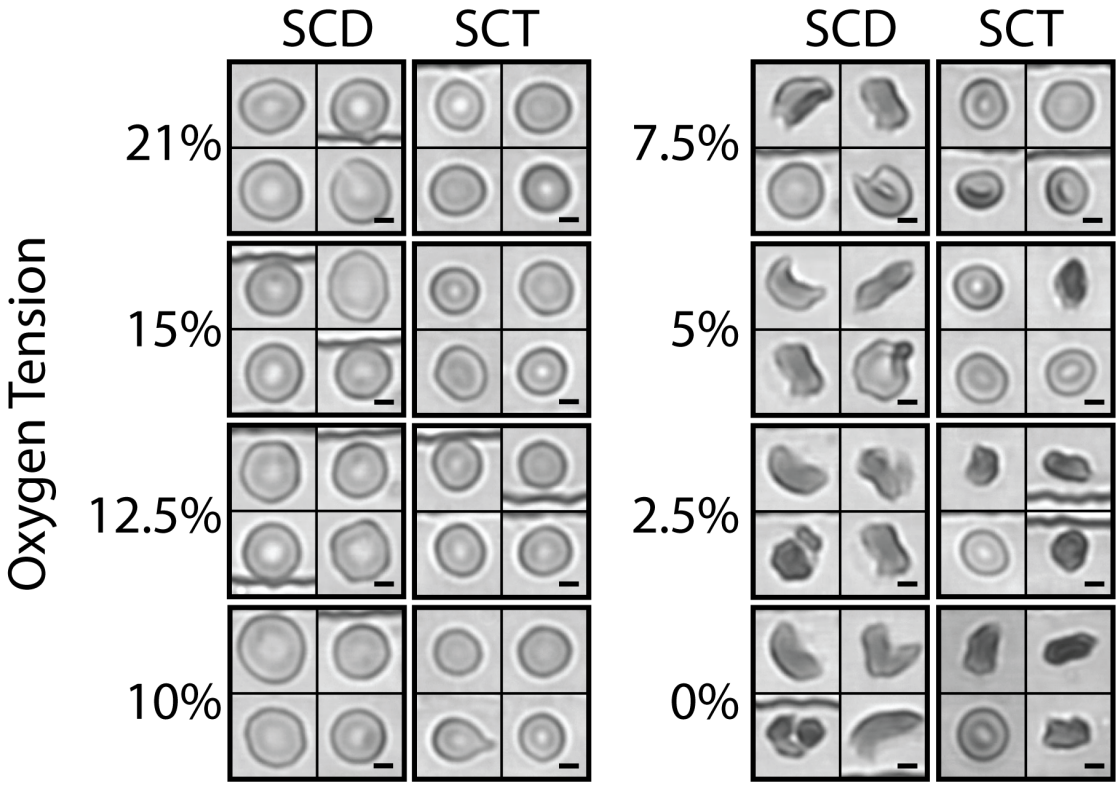

Figure S1. Morphology of individual RBCs from SCD and SCT change in response to oxygen tension. As oxygen tension was varied from an oxygenated environment (ppO_2 = 21%, 160 mmHg) to a severely anoxic environment (ppO_2 = 0%, 0 mmHg) in specific increments, individual RBC morphology was imaged. Here, we used brightfield microscopy to image RBCs resuspsended in PBS at a 1% hematocrit under a 40X magnification. In SCD (genotype SS), individual RBCs established a sickle-like morphology at oxygen tensions as high as 7.5% O_2 (~57 mmHg). In SCT (genotype AS) on the other hand, changes in individual RBC morphology did not occur until a much lower oxygen tension of 2.5% O_2 (~19 mmHg). Scale bars = 3 μm.

Representative blood sample demonstrating flow regimes I, II, and III in sickle cell disease (genotype SS)

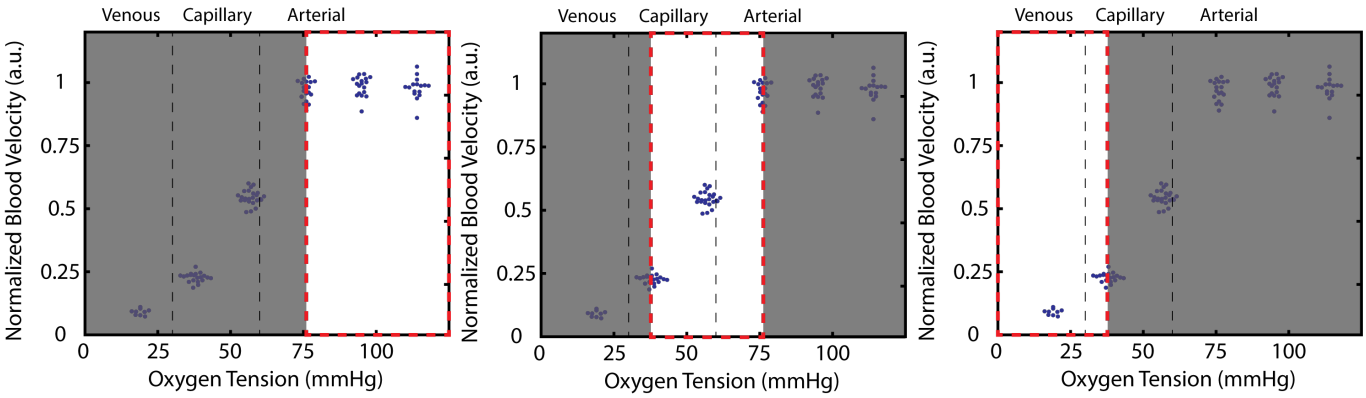

Figure S2. Flow regimes in a representative sample of sickle cell disease. (**Left**) Flow regime I, shown within the red boxed region, demonstrates flow qualitatively similar to non-sickle blood flow (genotype AA). Flow in this regime is oxygen-independent in this regime. (**Middle**) Flow regime II, shown within the red boxed region, demonstrates oxygen-dependent impaired flow velocities. (**Right**) Flow regime III, shown in the red boxed region, demonstrates near-occluded flow behavior at low oxygen tension.

Upon deoxygenation, blood velocity deceleration differs between sickle cell trait (genotype AS) and sickle cell disease (genotype SS) blood samples

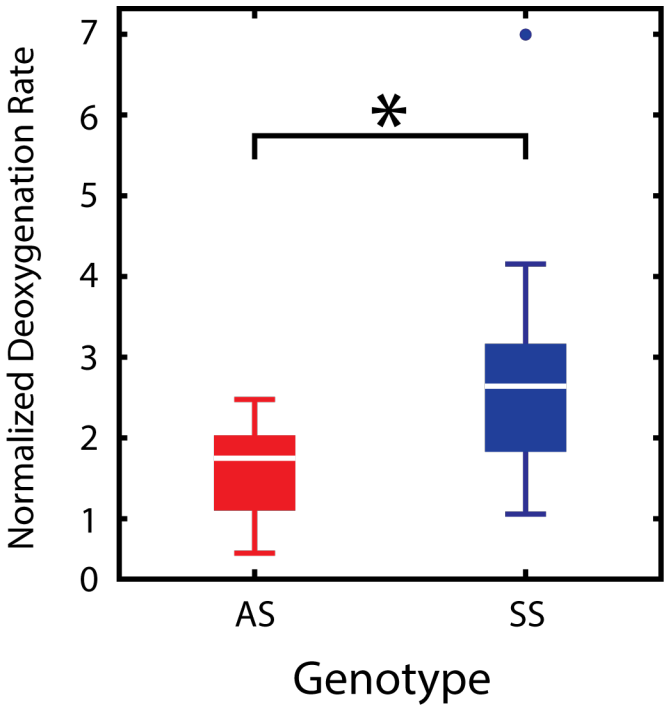

Figure S3. Deceleration rates upon deoxygenation of whole blood from sickle cell trait (genotype AS, n=8) and sickle cell disease (genotype SS, n=11) were compared. As blood was perfused through our microfluidic platform, oxygen tension was changed from oxygenated conditions (160 mmHg) to completely anoxic conditions (0 mmHg), and deceleration rates upon deoxygenation was measured. Deceleration rates from blood samples with sickle cell disease were found to be significantly higher compared to sickle cell trait, with a p-value of P=0.041 as determined through non-parametric Mann Whitney U testing. To determine deceleration rates, deoxygenated blood velocities were fitted to an exponential decay function (shown below), where k_{decel} is the exponential deceleration rate.

$$v = v_o * e^{-k_{decel}*t}$$

Table S1. Blood sample mixing ratios and configurations for simulated transfusion studies

Sample 1						
SS Volume	**AA Volume**	**HbS**	**HbF**	**HbA**	**HbA2**	**HCT**
100	0	73.8	18.2	3.2	4.9	26.9
70	30	47	12.2	37	4	29.6
30	70	18	5.7	73.5	3	33.2
10	90	5.7	3	89	2.5	35
0	100	0	1.7	96.1	2.3	35.9
Sample 2						
SS Volume	**AA Volume**	**HbS**	**HbF**	**HbA**	**HbA2**	**HCT**
100	0	83.1	8.1	4.8	4.1	25.1
70	30	47.1	5.1	44.1	3.7	30.98
30	70	16.1	2.5	78	3.4	38.82
10	90	4.9	1.6	90.3	3.3	42.74
0	100	0	1.2	95.6	3.2	44.7