## Supplementary Information

## Shear dependent red blood cell adhesion in microscale flow

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Figure S1. Quantification of fluorescently labeled LN and FN immobilized in the shear-gradient microchannels. (A) Shown are the microscope images of non-functionalized, LN-functionalized, and FN-functionalized microchannel surfaces at 10X magnification. The white rectangles indicate the regions along which RBC adhesion analyses were quantified. (B) The calculated fluorescent intensity profiles show that protein coverage along the functionalized surfaces was relatively uniform, while the fluorescent intensity was negligible over the non-functionalized microchannel surface. Scale bars are 4 mm. Error bars represent  $\pm$ SEM (n=3).



Video S1. 3D CFD simulation of the flow of RBC-sized spherical particles through the shear-gradient microchannel.



Figure S2. Results of the 3D CFD simulation of the flow of RBC-sized spherical particles through the shear-gradient microchannel. (A) Shown is the distribution of particles at steady state (40 s) which were introduced to the channel at 2000 particles/second. Shown particles are scaled by 40X. The continuous lines inside the microchannel are the virtual boundaries created to facilitate generation of a structured mesh domain and do not interact with the flow. (B) Mean gray scale intensity analysis of particles in the sub-regions indicates  $\pm$  5.4% variation between the mean and maximum and 7.8% between the mean and minimum, which is consistent with the experimental results presented in Figure S1. CFD simulation of the flow of RBC-sized spherical particles can be seen in Video S1.



Figure S3. Concentration of RBCs and associated mean grayscale intensity profiles during perfusion throughout the shear-gradient microchannel. (A) The microscopic image taken while the blood was flowing at 1.85  $\mu$ l/min shows that RBCs span the entire microchannel surface without any separation that can be seen by the naked eye. The region of interest (ROI) in the area of cell quantification divided into 10 sub-regions (R1-R10) are shown. (B) In order to interrogate the concentration of RBCs throughout the ROI, we perfused blood samples at varying hematocrit (Hct) values and measured the mean grayscale intensity in each sub-region corresponding to that hematocrit. The intensity values were consistently lower when isolated RBCs (100% Hct) were flowed, as highly concentrated RBCs blocked

the light to the microscope objective. Decreased hematocrit levels (50%, 20%, 10%) resulted in less light blockade as well as more light reflection due to flowing RBCs leading to higher mean grayscale intensities. Flowing a whole SCD blood sample and 50% Hct RBC suspension yielded similar intensity values along the sub-regions R1 through R10.



**Figure S4.** Patient specific shear dependent adhesion curves in LN-functionalized microchannels. The high SiGMA, low SiGMA, and control groups were plotted on separate graphs for convenience. Each individual curve represents the change in adherent cell numbers within the shear gradient for a specific SCD subject or control (HbAA) sample. Adherent cell numbers include both deformable and non-deformable RBCs. N=20 for SCD subjects and 2 for the control group.



**Figure S5.** Patient specific normalized shear gradient adhesion curves in LN-functionalized microchannels. The high nSiGMA, low nSiGMA, and control groups were plotted on separate graphs for convenience. The adhesion numbers were normalized using the formula *adherent RBC number in the subregion* 

 $adherent RBC number in the 1st subregion_x 100$ . Each individual curve represents the change in normalized adhesion number within the shear gradient for a specific SCD subject or control (HbAA) sample. The nSiGMA values were calculated based on the normalized adhesion curves. A threshold nSiGMA value of 60 was chosen to differentiate low nSiGMA and high nSiGMA groups.



**Figure S6. Shear dependent RBC adhesion profiles in Laminin, Fibronectin, ICAM-1, and VCAM-1 functionalized channels. (A)** RBCs displayed shear dependent adhesion on all adhesion molecules tested, in all blood samples obtained from subjects with SCD (N=3). **(B)** RBCs from healthy individuals did not exhibit significant adhesion to any of the proteins tested with no shear-dependent profile (n=5). The arrow at the top left corner indicates flow direction through the shear-gradient microchannel.