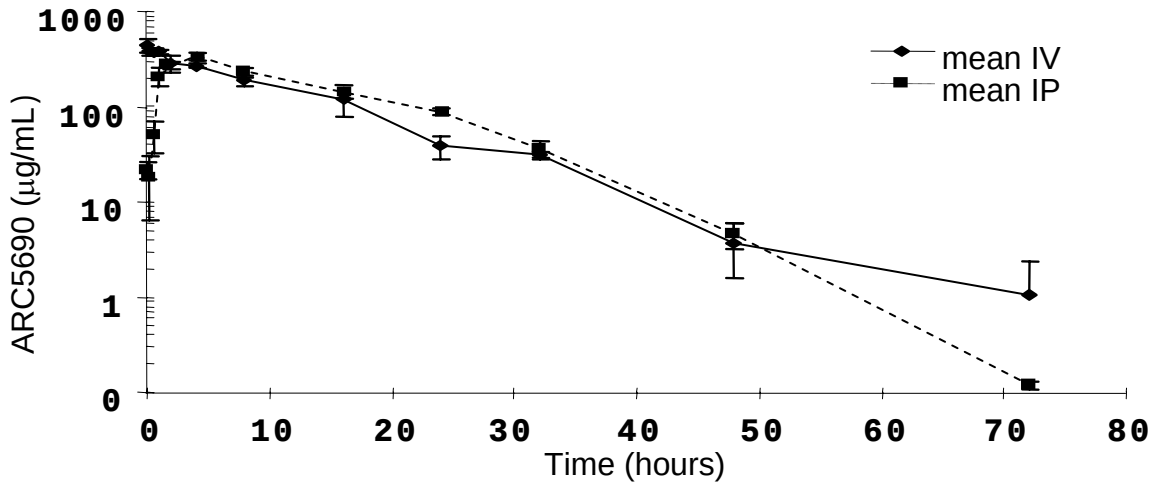
Figure S1. The nucleotide sequence of anti-P-selectin aptamer ARC5690 and scrambled aptamer ARC5694.

ARC5690: 5’-fC-fU-fC-mG-fC-mA-mG-mA-fC-mA-mA-fC-fC-mG-mG-mA-fU-mG-mA-mA-mA-fU-fC-fC-mG-mA-fC-fC-mG-mG-mA-mG-idT-3;

ARC5694: 5’-fC-fU-fC-**fC-mA-mG-mA-fC-mA-fC-**mA-**mG**-fC-mG-mG-mA-fU-mG-mA-mA-mA-fU-fC-fC-mG-**mG**-fC-fC-**mA**-mG-mA-mG-idT-3 ’

fC, 2’-fluoro-cytidine; fU, 2’-fluoro-uridine; mA, 2’-methoxy-adenosine; mG, 2’-methoxy-guanosine, and idT, inverted, 2’-deoxy-thymidine. Bold nucleotides in ARC5694 sequence represent changes relative to the ARC5690 parent sequence.



PK Parameter	Unit	Estimate IV	Estimate IP
Half-life	hr	8.20	5.36
C_{max}	ug/mL	443.14	339.67
T_{max}	hr	0.08	4.00
CL	mL/hr/kg	4.27	3.73

Figure S2. Pharmacokinetic study to determine plasma levels of anti-P-selectin aptamer ARC5690 in mice that were injected either intravenously (IV) or intraperitoneally (IP) at a dose of 20 mg/kg of mouse body weight (bw).
Each point represents the mean ± SD in 3 mice. Cmax, the highest concentration observed; Tmax, time point at which the highest concentration occurred; CL, clearance.

Results: Cmax for IP was achieved 4 hrs after injection and did not differ from Cmax for IV injection of ARC5690. Based on this data, we examined the effect of ARC5690 on cell adhesion by intravital microscopy 4 hours after IP injection of ARC5690 to mice as shown in Figure 2. Four hours after IP injection, no significant differences were noted in the plasma levels of ARC5690 between SCD mice and control mice (data not shown).

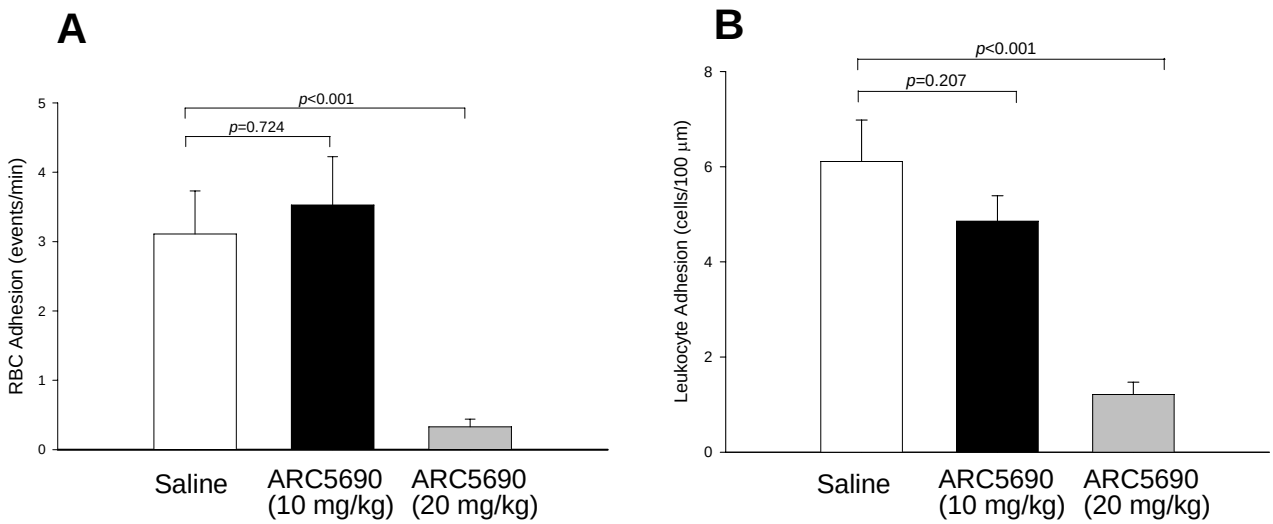


Figure S3. Effects of different doses of ARC5690 on adhesion of sickle RBCs and leukocytes to endothelial cells in skull bone marrow venules in mice subjected to hypoxia/normoxia protocol.

Results: To determine the optimal dose of ARC5690, SCD mice (n= 3 to 5 mice/ group) were injected IP with ARC5690 at doses 10 and 20 mg/kg bw, and subjected to the hypoxia/normoxia protocol as shown in Figure 2. Adhesion of sickle RBCs (A) and leukocytes (B) to the endothelium was examined. Control mice were injected with saline (0.9% NaCl, 10 µl/g bw). Substantial inhibitory effects of the anti-P-selectin aptamer ARC5690 on the adhesion of sickle RBC and leukocytes were observed at a dose of 20 mg/kg bw but not at a dose 10 mg/kg. A dose of 20 mg/kg bw was used for our studies.

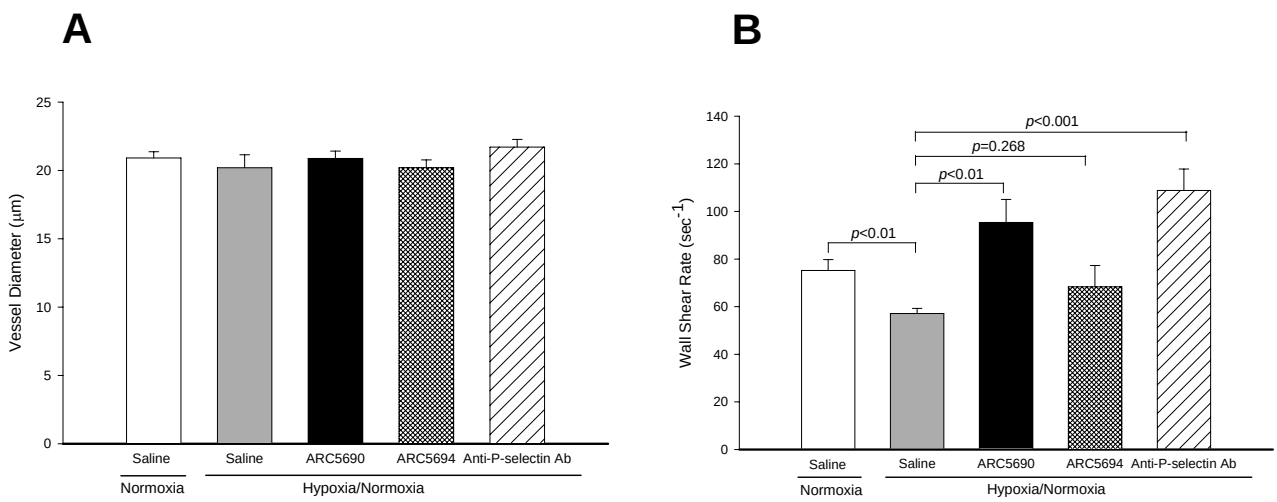


Figure S4. Vessel diameters and wall shear rates in SCD mice pretreated with ARC5690, ARC5694 and anti-P-selectin antibody and subjected to hypoxia/normoxia protocol (RBC adhesion experiments).

Results: Anti-P-selectin aptamer (ARC5690) does not alter vessel diameters (A). Venule diameters were measured using Image Pro-Plus 5.0 software. ARC5690 increases wall shear rates (B). Wall shear rates were calculated using the formula 8Vmean/diameter where Vmean is estimated as center line RBC velocity/1.6 .

Values were mean ± SE obtained from 4-5 mice in each group. The number of venules in experimental groups ranged from 18-21. P-values for statistical analyses are shown on top of the figures.

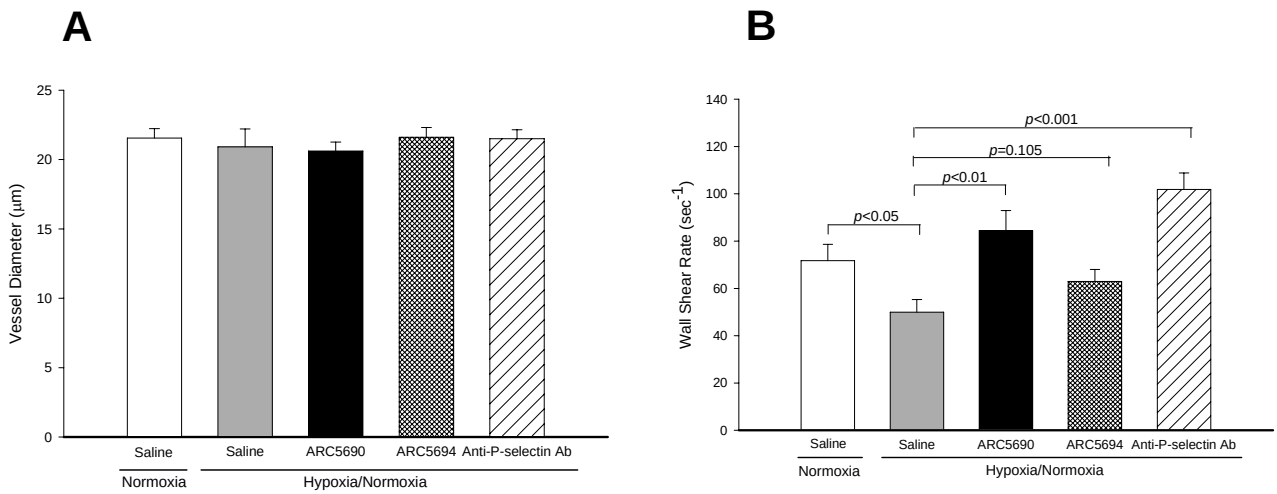


Figure S5. Vessel diameters and wall shear rates in SCD mice pretreated with ARC5690, ARC5694 and anti-P-selectin antibody and subjected to hypoxia/normoxia protocol (leukocyte adhesion experiments).

Results: Similarly to those shown in Supplemental Figure 4, ARC5690 does not change venular diameters (A) and increases wall shear rates (B). Values were mean ± SE obtained from 4 to 5 mice in each group. The number of venules in experimental groups ranged from 18-21. P-values for statistical analyses are shown on top of the figures.