Supplemental Information:

Clonal variants of *Plasmodium falciparum* exhibit a hysteresis-like effect and narrow range of rolling velocities to the host receptor CD36 under flow conditions

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Supplemental Methods

Video processing and analysis of particle tracking

All experiments were performed on a Nikon TE2000 microscope (Nikon USA) with a Photometrics CoolSNAP EZ camera. All videos were recorded with a 10x magnification lens with a camera using 2 x 2 binning for a final image of 697 x 520 pixels. Images were recorded using a 30 nm band gap filter with a peak transmission at 420 nm (Chroma) to enhance contrast between rolling pRBCs and the image background. Parasite cultures were loaded into the flow chamber at approximately 20% hematocrit and on average 6.5 ± 2.5% parasitemia.

For flow based studies, microfluidic devices were coated with 50 µg/ml CD36 in phosphate buffered saline overnight at 4°C. Before flow experiments the channels were blocked with 0.5% Albumax/RPMI 1640 media for 2 hours. Loading was accomplished by using a 1mL syringe (BD) to flow culture through one of the two inlets. Once sufficient culture was added, valves to the flow cell outlet were closed and fresh media was pumped through the second inlet hole. This allowed washing the inlets so that no additional pRBCs were added to the flow cell. The parasite culture was then allowed to settle in the microfluidic flow-cell so that all pRBCs in the bottom layer would have a chance to adhere to the substrate. As a control, there was no binding of pRBCs to microfluidic devices coated with 0.5% Albumax alone. For experiments with increasing WSS, the flow rate was started at 0.27 Pa to allow any unbound cells to be removed for about 60 seconds. The flow rate was then increased in stepwise increments of 0.134 Pa from 0.27 Pa to 1 Pa and then in increments of 0.27 from 1 Pa to 4 Pa. The initial small step size gave resolution of where the rolling velocities inflection points occurred. At each flow rate, 10 movies were recorded at specific positions and all of the red blood cells in the field were tracked for 5 second movies at 15 frames per second. Following conclusion of the increasing WSS sequence, the channels were washed at a high rate to remove any bound pRBCs. For experiments with decreasing

WSS, the channels were washed at a high rate to remove any bound pRBCs. The chamber was then loaded again with the remaining malaria culture using an identical loading procedure. After washing the inlets and removing unbound cells using the 60 second 0.27 Pa wash, the shear rate was then increased to 1.88 Pa. The shear stress was then decreased incrementally in 0.134 Pa steps to 0.27 Pa. This allowed the behavior of parasites to be observed under both increasing and decreasing shear stress conditions in the same physiologic range as stresses observed *in vivo*.

For each flow rate, track velocities were calculated for all of the red blood cells in the 10 fields. Fig. S4A shows an example of a single field that was tracked. The rolling velocity for the "raw data ungrouped" was then calculated at each shear stress (Fig. S4B). Next, the pRBC tracks were ordered and grouped together according to their rolling velocity to give "raw data grouped" (Fig. S4C). The number of pRBCs chosen for grouping together was determined so that 50 groups in the initial WSS flow rate would be created. This corresponded to each group representing a 2nd percentile of the pRBC population observed at the lowest WSS. In the example shown (Fig. S4), 55 of the slowest rolling pRBCs were averaged together. Then the next 55 faster pRBCs were averaged together. The process was repeated for all pRBCs observed. The number of red blood cells tracked in each group differed between clonal variants and depended on the initial number of adherent cells. Following grouping of the pRBCs, each group was then related to one another according to their ordered velocities. The slowest rolling group at one shear stress is assumed to be sufficiently identical to the slowest rolling pRBCs at the next WSS. The 2nd slowest group is assumed to be similar to the 2nd slowest group in the next WSS, and so on. In this way the grouped pRBCs could be related between different shear stresses and the lines between groups drawn giving "Data grouped" (Fig. S4D). Color in the plots is assigned as a heat map from blue (lowest velocity) to red (highest velocity).

TABLE S1 Phenotype of parasite clonal parasite variants

Parasite Clone	Var Gene	Number of Domains	Percentage total *var* Expression[a]	Ups	Predicted Extracellular Domain					
P2E11	var33	4	80%	B1	DBLα0.11	CIDRα2.4	DBLδ1	CIDRβ5		
P6G2	var31	4	78%	B1	DBLα0.18	CIDRα2.4	DBLβ3	DBLδ9		
ItG1E7	var31	4	60%	B1	DBLα0.18	CIDRα2.4	DBLβ3	DBLδ9		
P3G5	var10	4	49%	B1	DBLα0.50	CIDRα2.4	DBLβ10	DBLδ1		
	var32	5	40%	unk	DBLα0.23	CIDRα2.4	DBLγ6	DBLδ1	CIDRβ1	
P4H12	var44	5	82%	B1	DBLα0.16	CIDRα3.4	DBLβ13	DBLδ1	CIDRβ6	
P6A1	var11	5	78%	B1	DBLα0.30	CIDRα2.4	DBLβ10	DBLδ1	CIDRβ1	
P6D12	var39	4	66%	B4	DBLα0.50	CIDRα2.5	DBLδ1	CIDRβ6		
	var67	5	28%	B1	DBLα0.10	CIDRα2.2	DBLε2	DBLξ3	DBLε12	
3G8	var1	6	87%	C1	DBLα0.18	CIDRα6	DBLβ5	DBLγ10	DBLδ2	CIDRγ6

[a]Expression is percentage of total *var* genes in panel. All other *var* genes expression level < 2%.

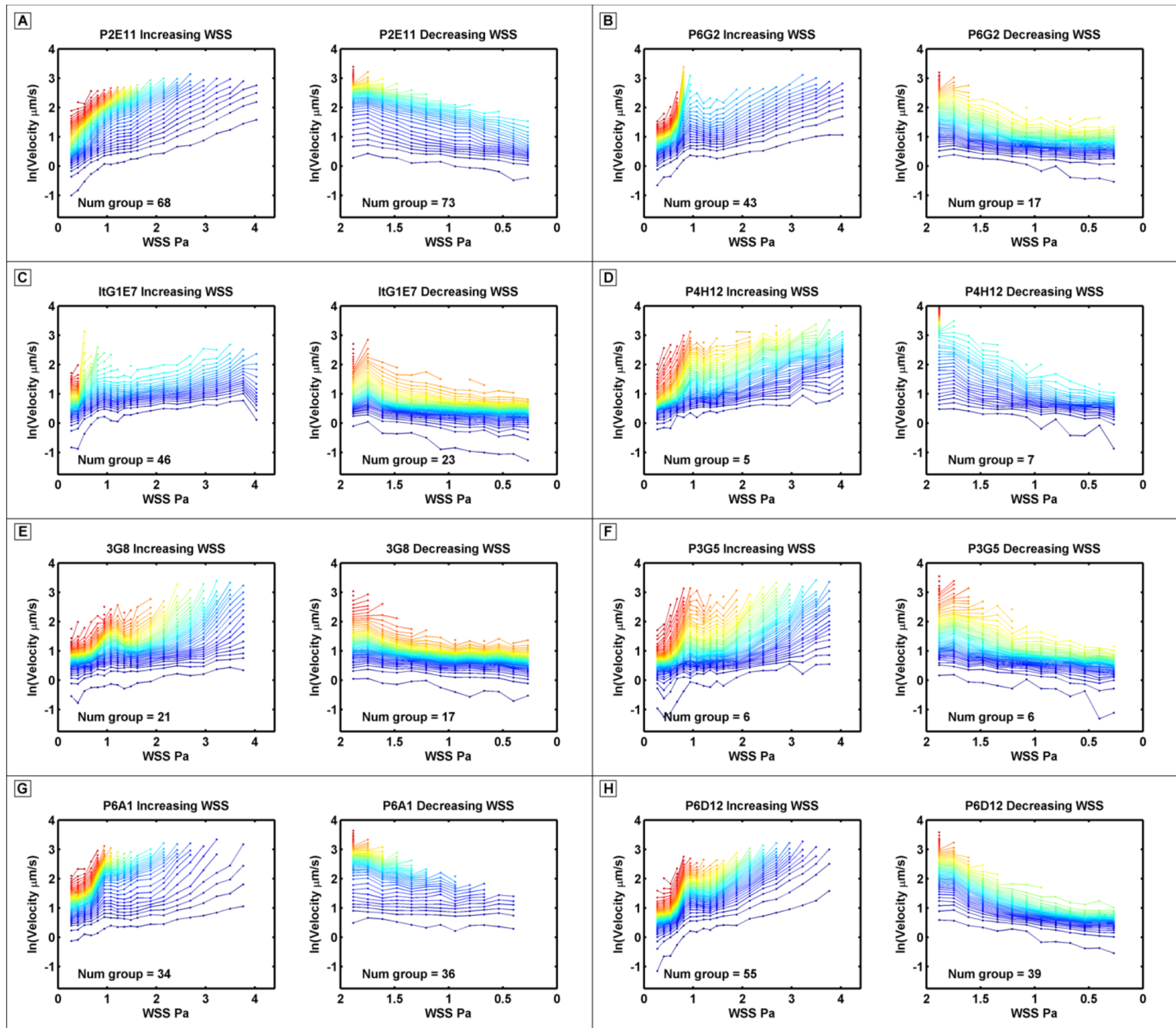


FIG S1 Rolling velocity plots for all clones tested under increasing and decreasing WSS experiments. (A) P2E11. (B) P6G2. (C) ItG1E7. (D) P4H12. (E) 3G8. (F) P3G5. (G) P6A1. (H) P6D12.

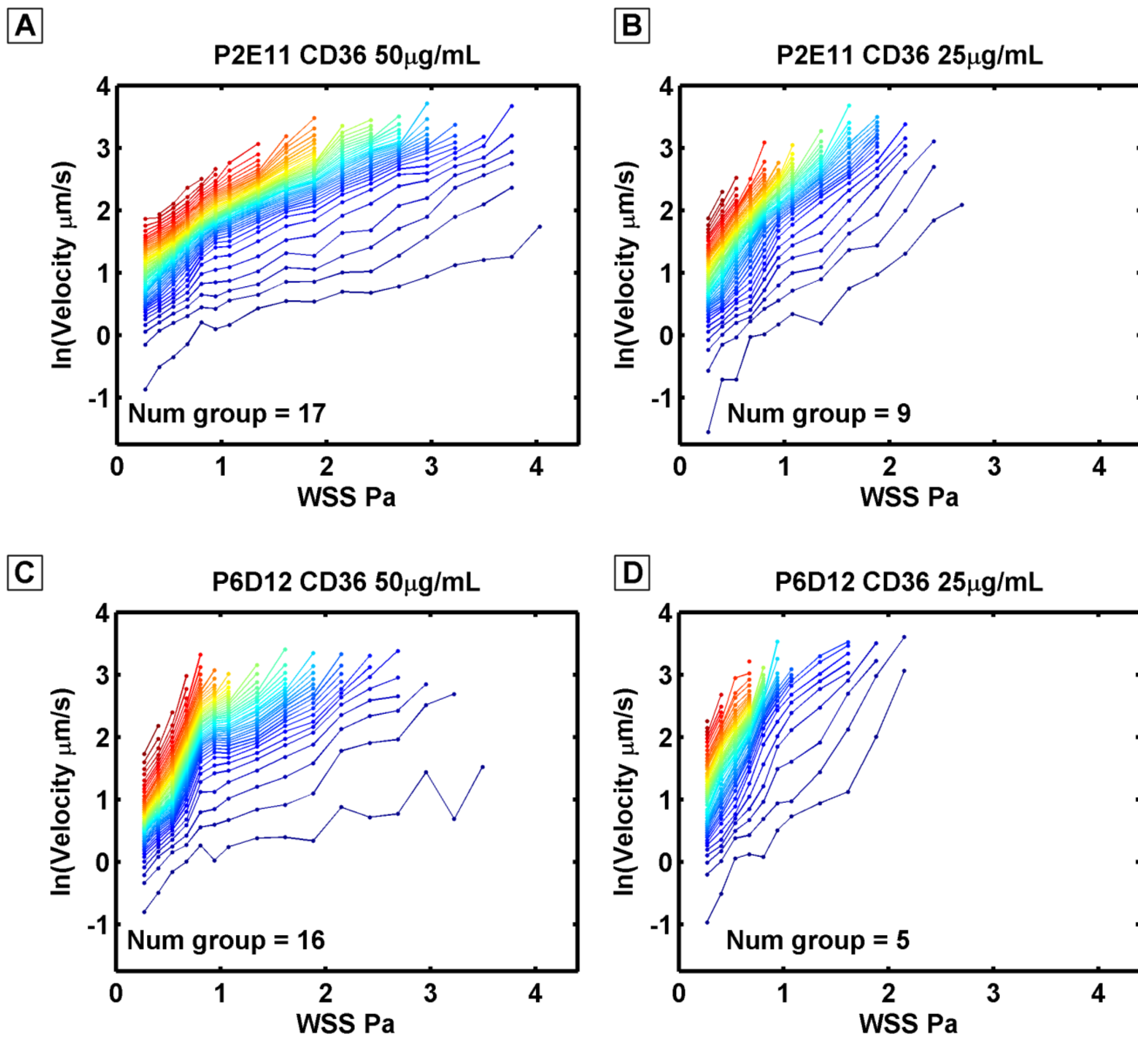


FIG S2 Comparing clones P2E11 and P6D12 rolling velocities on CD36 concentrations of 50μg/mL and 25 μg/mL. While the number of parasites observed rolling on the 25μg/mL CD36 is reduced, the inflection points in the rolling velocity around 0.8-1.3 Pa are still present for both parasite clones. This indicates that the sharp change in rolling velocities in somewhat independent of CD36 concentration.

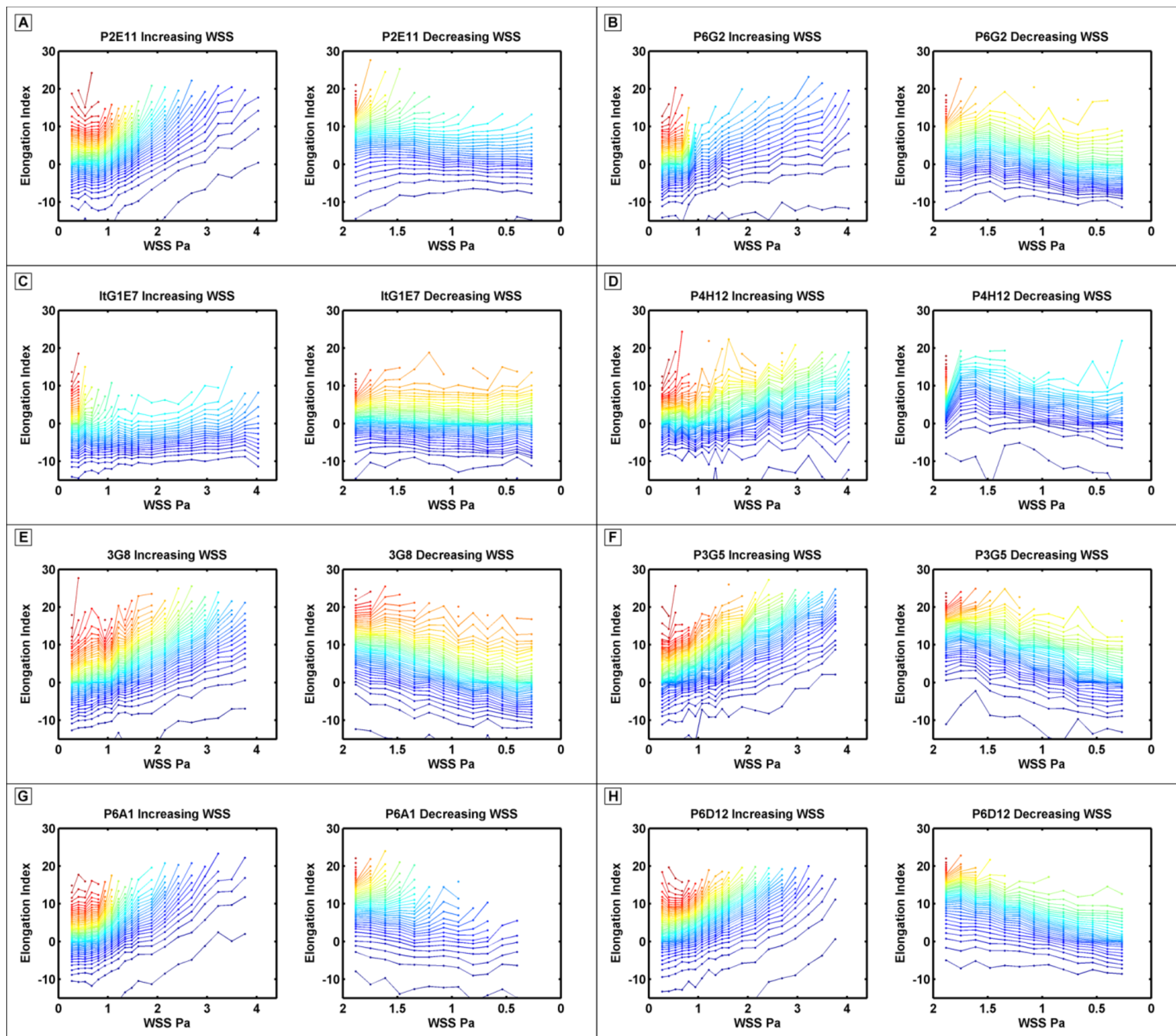
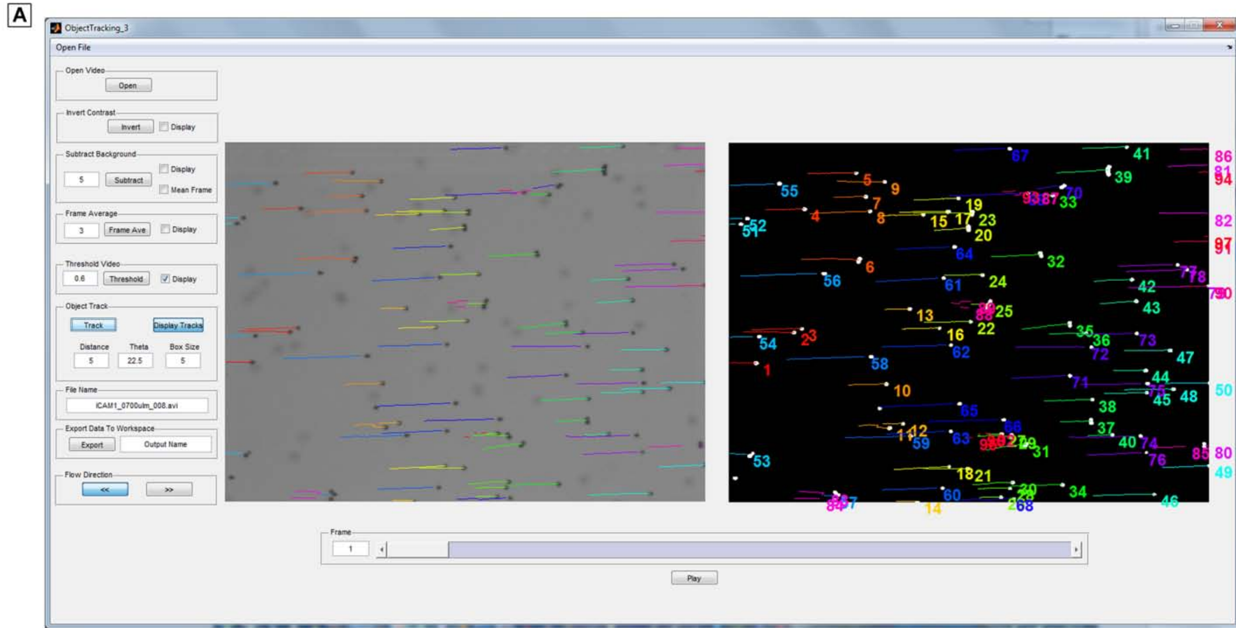


FIG S3 Elongation Index plots for all clones tested under increasing and decreasing WSS experiments.

(A) P2E11. (B) P6G2. (C) ItG1E7. (D) P4H12. (E) 3G8. (F) P3G5. (G) P6A1. (H) P6D12.



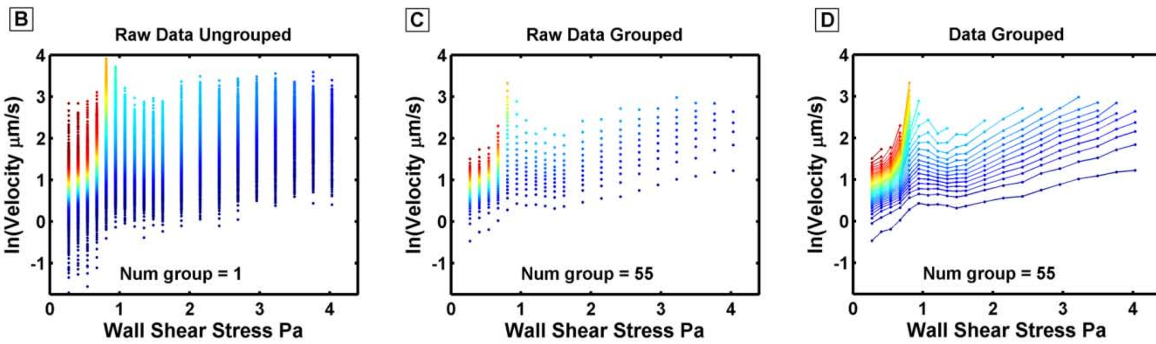


FIG S4 Tracking Objects and Plotting Tracks methodology. (A) Parasitized RBCs (pRBCs) are tracked using MATLAB software developed for the imaging conditions utilized in these experiments. All pRBCs were imaged at 10X magnification using bright field microscopy. All images were taken using a band gap filter which gave the pRBCs a dark appearance on a bright background. The resulting images could easily be threshold into binary images so that cell positions are easily identified using functions in the MATLAB computer vision toolbox. (B) Raw data ungrouped. (C) Raw data grouped. (D) Data grouped and plotted to show group trends with increasing WSS.