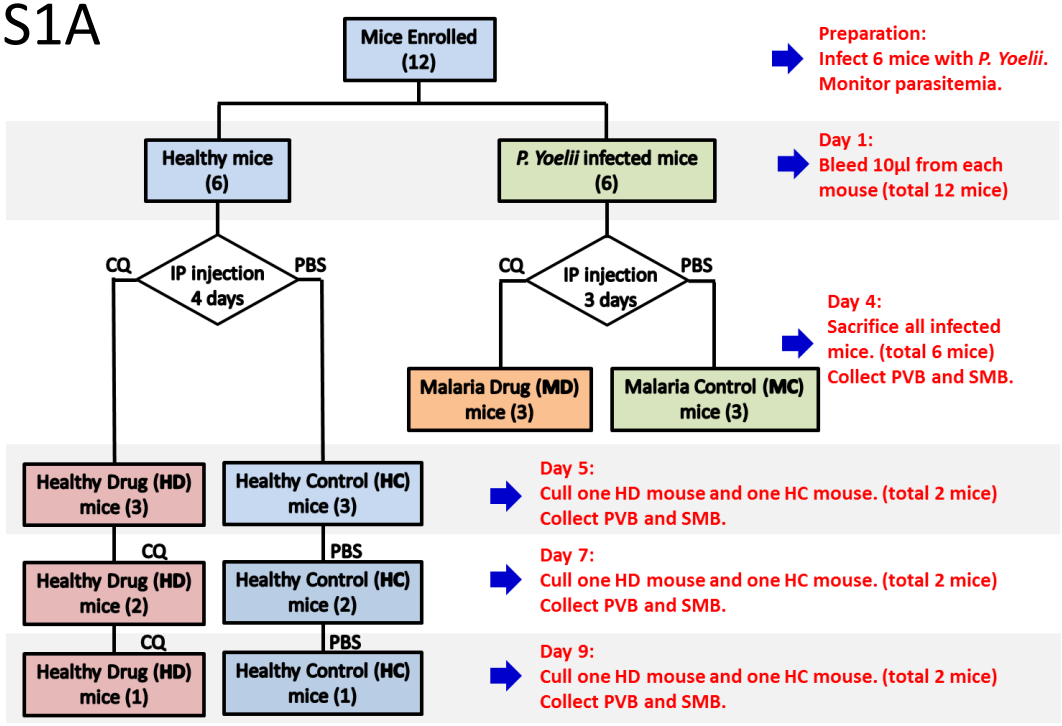
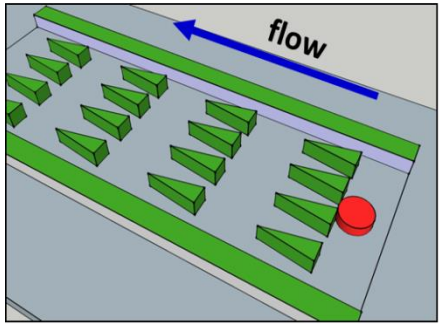
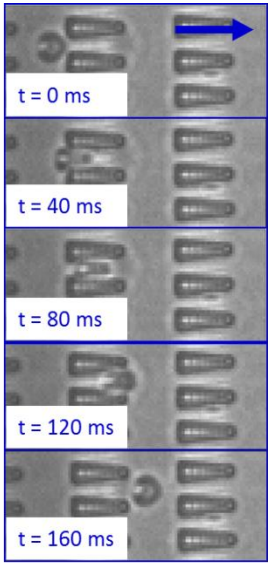
S1A



B



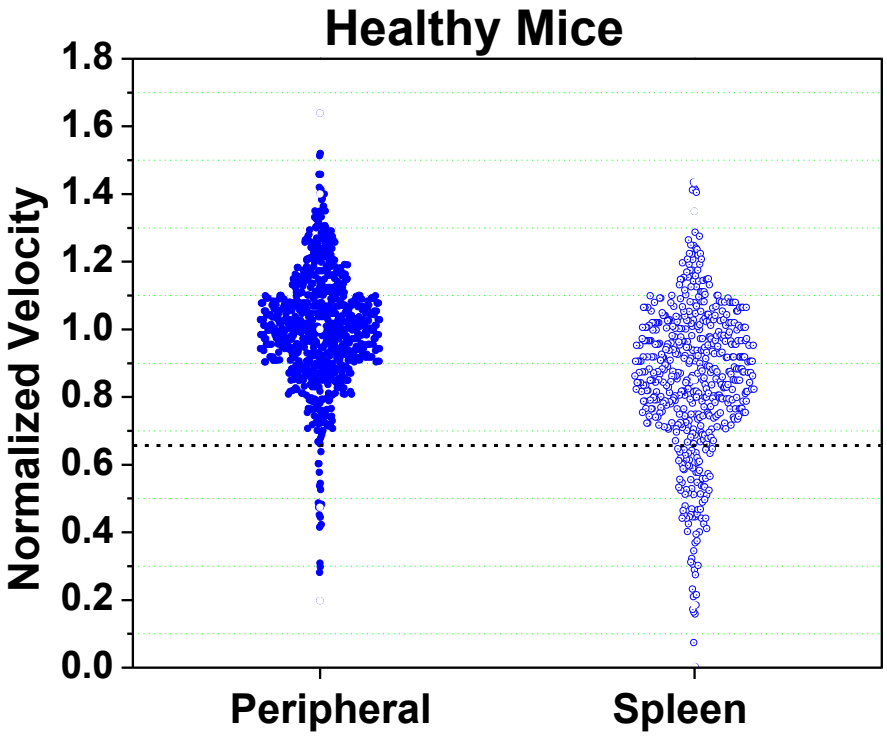


C

	Healthy mice (HC)		Infected mice (MC)		Infected mice + CQ (MD)	
Spleen length (cm)	1.65±0.10		2.62±0.18		3.16± 0.14	
Parasite-free RBCs	PVB	SMB	PVB	SMB	PVB	SMB
Normalized RBC velocity	"1"	0.85	0.91	0.72	0.75	0.54

S1. Flow chart of single experimental round (A), and the microfluidic device layout (B). More than three experimental rounds have been performed separately for healthy and malarial mice during data acquisition. Spleen size and RBC deformability were compared and summarized among healthy mice, infected mice, and infected mice receiving CQ treatment (C).

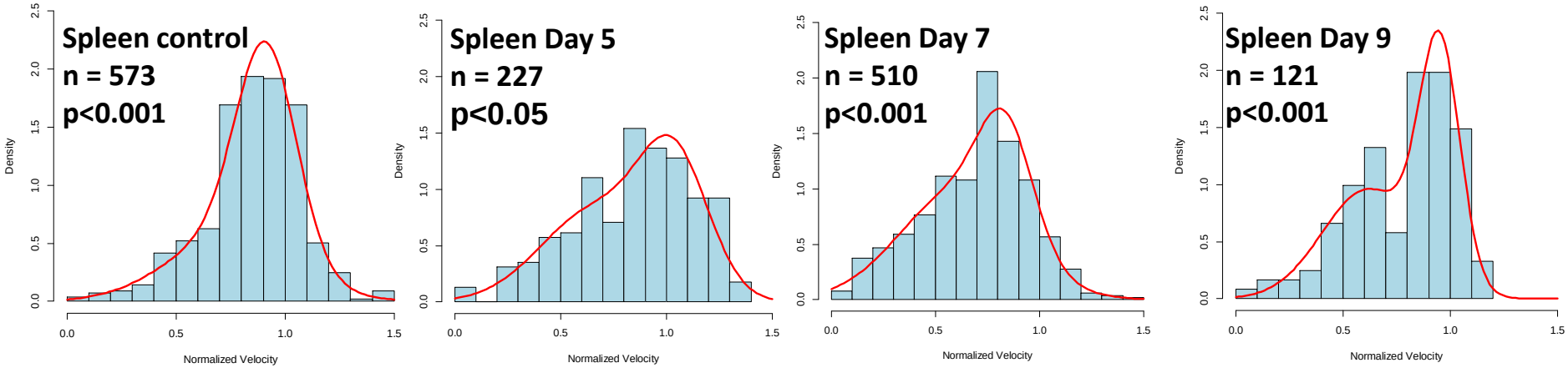
S2



Sample	Control Peripheral	Control Spleen	CQ (days)		
			5	7	9
% retention	2.2	16.1	24.7	39.4	28.1

S2. A hypothetical splenic retention threshold was drawn at 0.65 based on typical RBC lifespan of balb/c mice as well as statistical analysis (details in S3).

S3 A

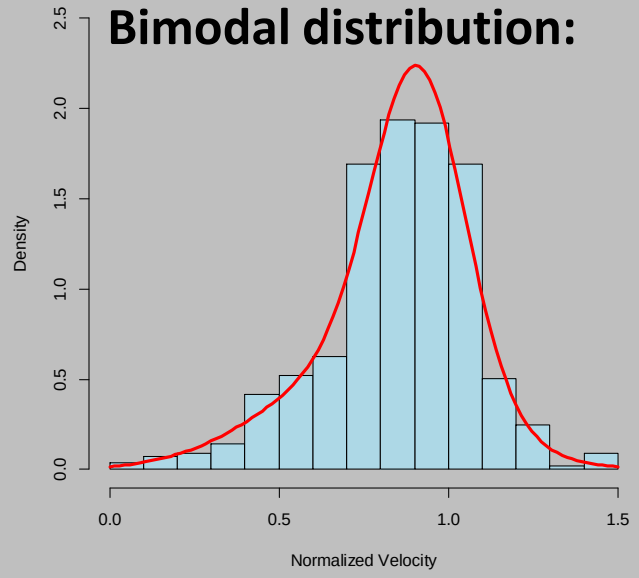


S3. Statistical interpretation on Fig 3C (A). Splenic minced RBC deformability profiles were fitted by using both uni-modal and bimodal distributions (B-E). For all instances, bimodal distribution was determined as a better fitting compared to unimodal normal distribution. ($p<0.05$)

S3 B

Spleen control
Sample size = 573

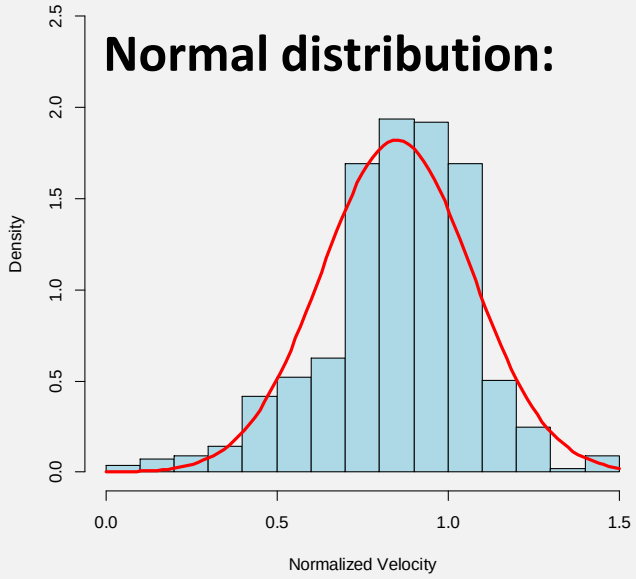
Bimodal distribution:



$$f(x) = \sum_{i=1}^{2} (a_i \frac{1}{\phi(\frac{b_i}{c_i})\sqrt{2\pi}c_i} e^{-\frac{(x-b_i)^2}{2{c_i}^2}})$$

param	estimate	95% CI
a1	0.39	(0.22, 0.56)
b1	**0.75**	**(0.67, 0.82)**
c1	0.28	(0.24, 0.32)
b2	**0.91**	**(0.81, 1.02)**
c2	0.14	(0.11, 0.16)

Normal distribution:



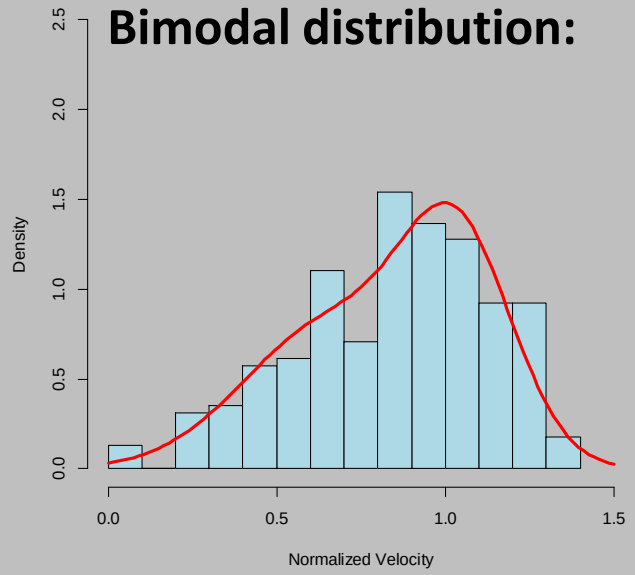
Let a2=0. We reduce the bimodal distribution to one single normal distribution.

param	estimate	95% CI
b1	0.85	(0.83, 0.87)
c1	0.22	(0.21, 0.23)

S3 C

Spleen 5 day
Sample size = 227

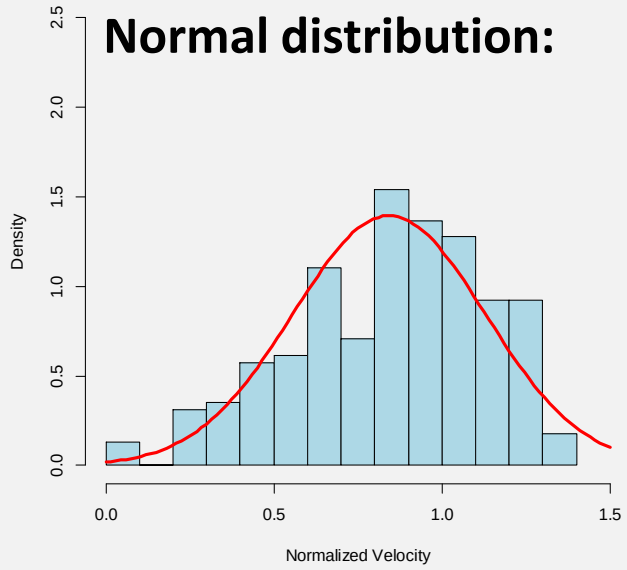
Bimodal distribution:



$$f(x) = \sum_{i=1}^{2} (a_i \frac{1}{\phi(\frac{b_i}{c_i})\sqrt{2\pi}c_i} e^{-\frac{(x-b_i)^2}{2c_i^2}})$$

param	estimate	95% CI
a1	0.53	(0.18, 0.87)
b1	**0.66**	**(0.49, 0.84)**
c1	0.26	(0.19, 0.33)
b2	**1.04**	**(0.82, 1.26)**
c2	0.16	(0.11, 0.21)

Normal distribution:



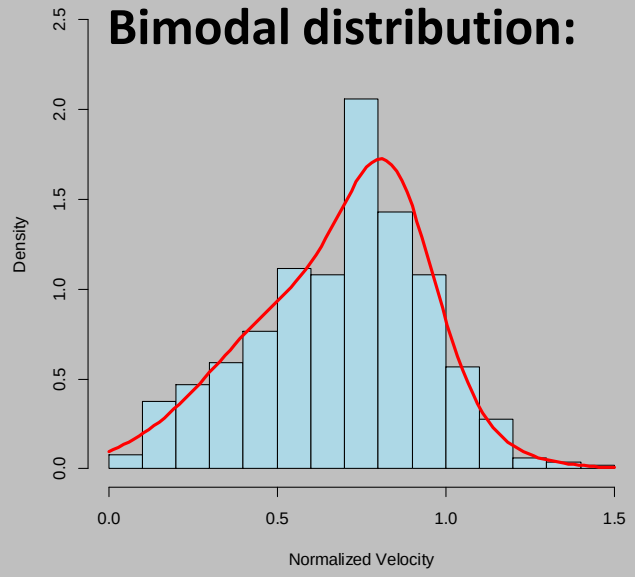
Let a2=0. We reduce the bimodal distribution to one single normal distribution.

param	estimate	95% CI
b1	0.84	(0.80, 0.88)
c1	0.29	(0.26, 0.31)

S3 D

Spleen 7 days
Sample size = 510

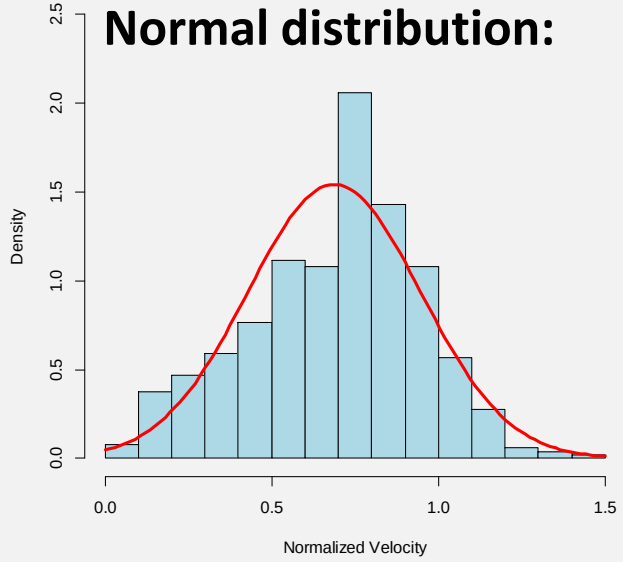
Bimodal distribution:



$$f(x) = \sum_{i=1}^{2} \left(a_i \frac{1}{\phi(\frac{b_i}{c_i})\sqrt{2\pi}c_i} e^{-\frac{(x-b_i)^2}{2{c_i}^2}} \right)$$

param	estimate	95% CI
a1	0.68	(0.42, 0.94)
b1	**0.61**	**(0.51, 0.70)**
c1	0.28	(0.25, 0.31)
b2	**0.84**	**(0.70, 0.97)**
c2	0.13	(0.06, 0.20)

Normal distribution:



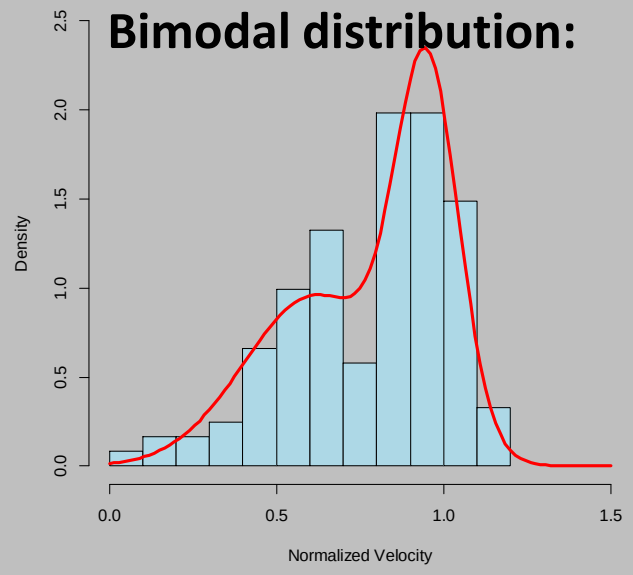
Let a2=0. We reduce the bimodal distribution to one single normal distribution.

param	estimate	95% CI
b1	0.69	(0.66, 0.71)
c1	0.26	(0.24, 0.28)

S3 E

Spleen 9 days
Sample size = 121

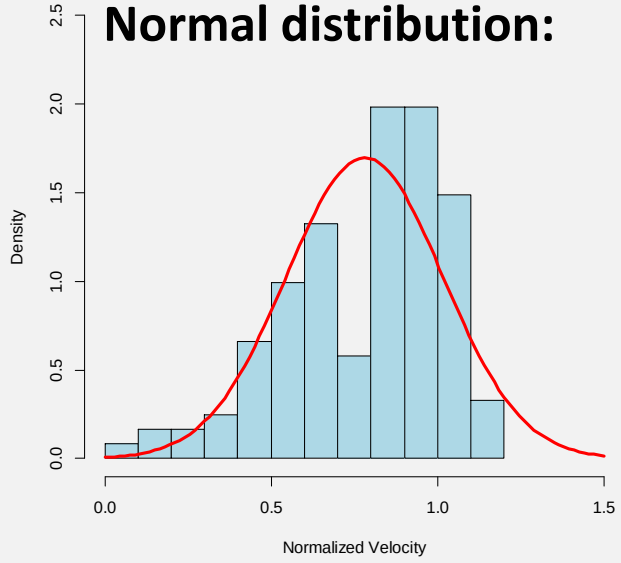
Bimodal distribution:



$$f(x) = \sum_{i=1}^{2} (a_i \frac{1}{\phi(\frac{b_i}{c_i})\sqrt{2\pi}c_i} e^{-\frac{(x-b_i)^2}{2{c_i}^2}})$$

param	estimate	95% CI
a1	0.51	(0.32, 0.70)
b1	**0.62**	**(0.51, 0.72)**
c1	0.21	(0.15, 0.27)
b2	**0.95**	**(0.81, 1.10)**
c2	0.09	(0.07, 0.12)

Normal distribution:



Let a2=0. We reduce the bimodal distribution to one single normal distribution.

param	estimate	95% CI
b1	0.78	(0.74, 0.82)
c1	0.24	(0.20, 0.27)

S4

	Control Spleen	CQ (days)		
		5	7	9
a_1	0.39	0.53	0.68	0.51
b_1	0.75	0.66	0.61	0.62
b_2	0.91	1.04	0.84	0.95
c_1	0.28	0.26	0.28	0.21
c_2	0.14	0.16	0.13	0.09

S4. Parameters were estimated by the maximum likelihood (ML) method, and the fitted results were listed.

S5 A

	Healthy mice + PBS													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	mean	p-value
Before	18.4	19.3	18.9	18.7	17.9	17.1	17.6	21.9	18.3	16.8	18.2	19.8	**18.6**	**P>0.01**
After	18.4	17.5	17.2	17.4	17.6	17.1	18.2	18.6	18.5	17.0	16.3	14.7	**17.4**	

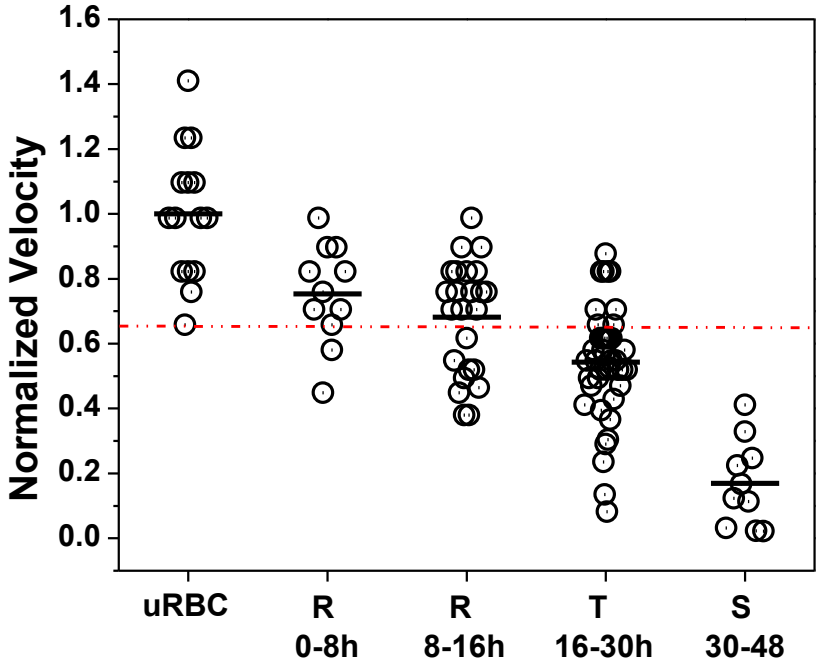
	Healthy mice + CQ													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	mean	p-value
Before	18.8	18.5	17.3	19.0	18.7	18.9	15.3	18.0	19.9	23.6	22.7	20.6	**19.271**	**P<0.01**
After	16.8	18.6	18.3	16.0	18.7	17.2	15.9	16.8	17.5	17.7	15.0	17.0	**17.127**	

B

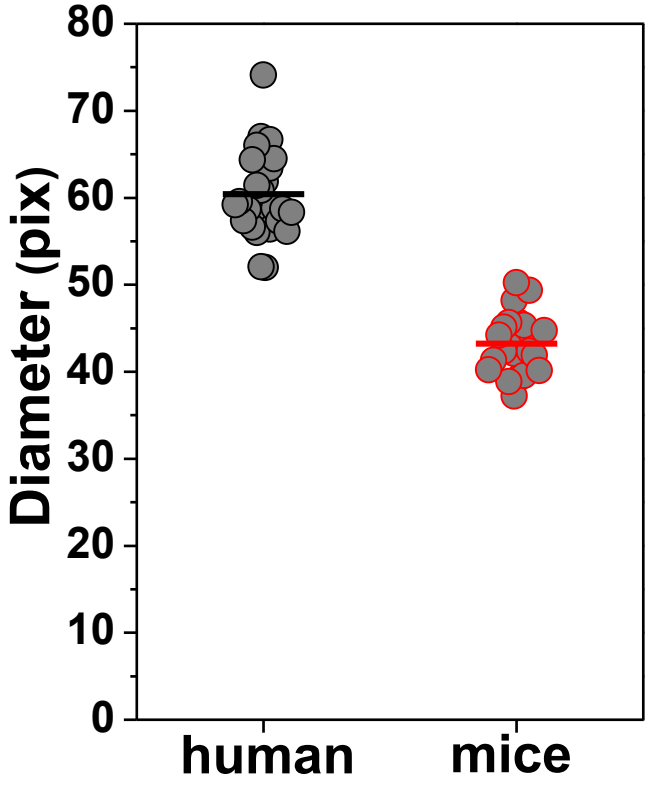
	Healthy mice + PBS							Healthy mice + CQ						
	I	II	III	IV	V	VI	mean	I	II	III	IV	V	VI	mean
Day 1	17.6	21.9	18.3	16.8	17.9	17.1	**18.3**	15.3	18.0	19.9	23.6	18.7	18.9	**19.1**
Day 5	18.2	18.6	18.5	17.0	17.6	17.1	**17.8**	15.9	16.8	17.5	17.7	18.7	17.2	**17.3**
Day 7	15.3	17.7	19.0	18.3	18.4	19.6	**18.1**	17.2	18.47	19.2	17.2	15.9	18.3	**17.7**
Day 9	18.4	15.9	17.6	19.2	19.0	17.5	**17.9**	18.4	16.5	16.3	17.7	18.9	17.0	**17.5**

S5. Hemoglobin concentrations of 12 healthy mice receiving CQ treatment (HD) and 12 healthy mice receiving PBS placebo (HC) were monitored on day 1 and day 5. Significant drop in Hb level was observed only in CQ treatment mice (A). Among these mice, 6 HD and 6 HC mice continued to receive treatment till day 9. No further change in Hb level was observed (B).

S6A



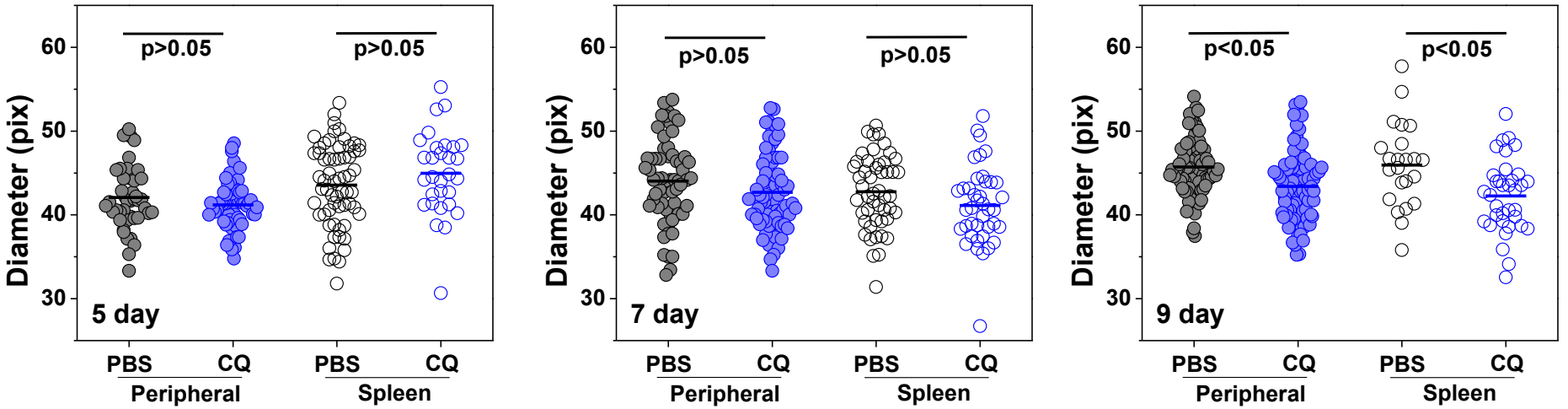
B



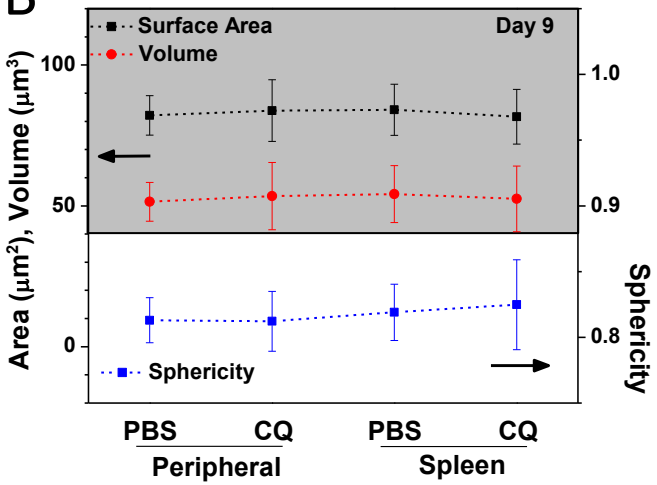
Infection stage	0-8 h	8-16 h	16-30 h	30-48 h
Retention %	30.0	37.5	83.3	100.0

S6. Microfluidic velocity-based deformability measurement is compared with filtration column (reported by Deplaine *et. al*) that mimics iRBC retention in human spleen (A). Synchronized 3D7 culture was used in this study for a matching comparison. Mice RBCs are 25% smaller than human RBCs (B). Therefore, a device with gap size of 4µm was used (instead of 3 µm for mice RBC analysis).

S7 A

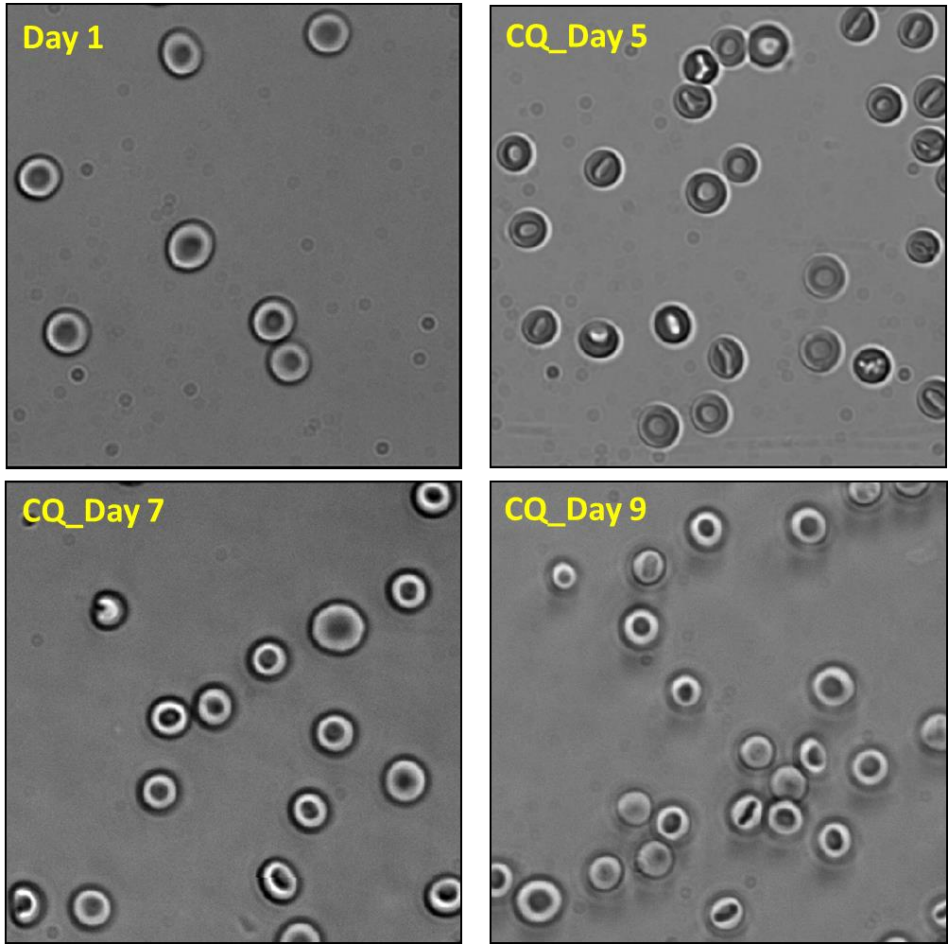


B



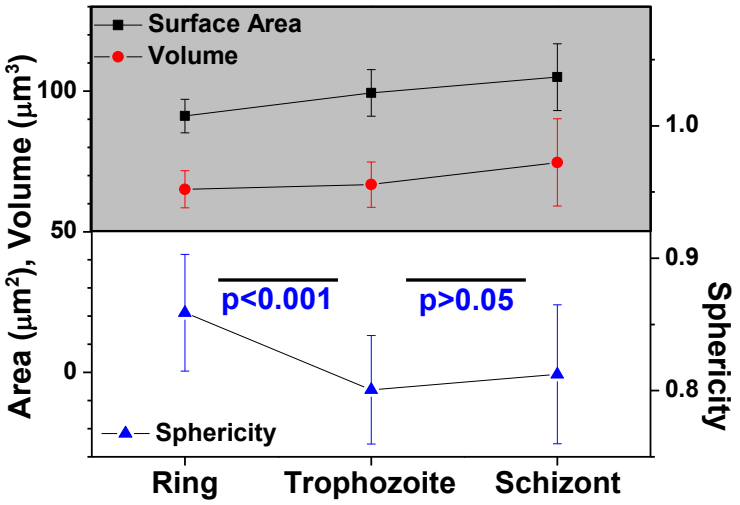
S7. The diameter (A), surface area, volume and sphericity (B) of normal RBCs after CQ treatment *in vivo* are quantified based on microscopic imaging (A) and micropipette aspiration (B).

S8

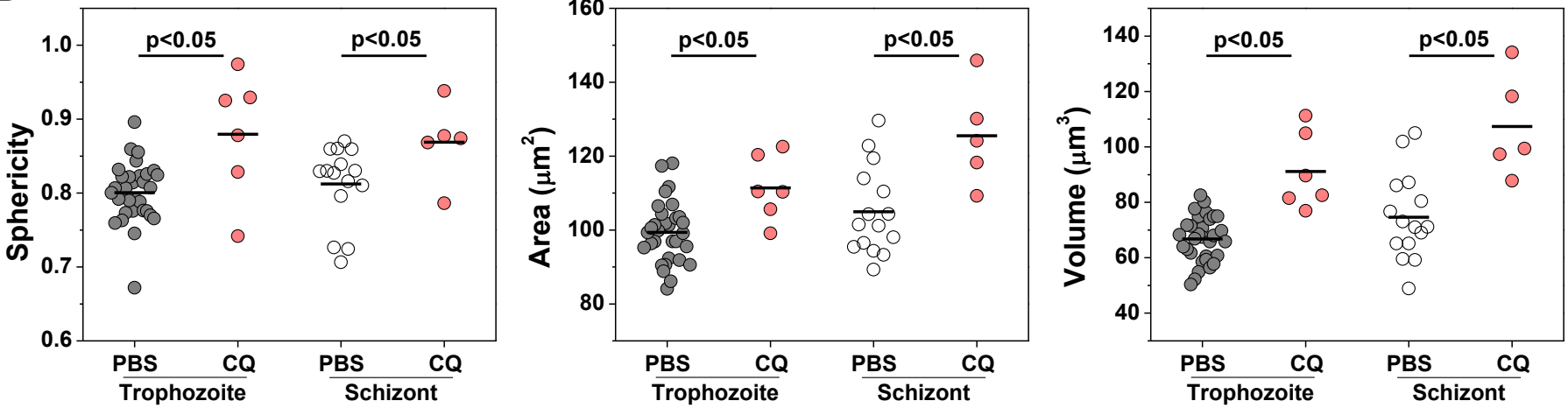


S8. Microscopic images of RBC morphology after *in vivo* CQ treatment.

S9 A



B



S9. Surface area, volume and sphericity measurements on different stages of iRBCs before (A) and after CQ treatment (B).

S10

Shear modulus (pN/µm)	Ring	Trophozoits	Schizonts
Control Peripheral	2.70±1.42	2.18±0.84	1.83±0.67
Chloroquine Peripheral	-	6.24±1.70	16.67±17.0
Control Spleen	2.79±0.96	3.05±0.47	4.65±1.30

S10. Membrane shear modulus of different stages of iRBCs from venous blood, from splenic blood, or from CQ treated venous blood.