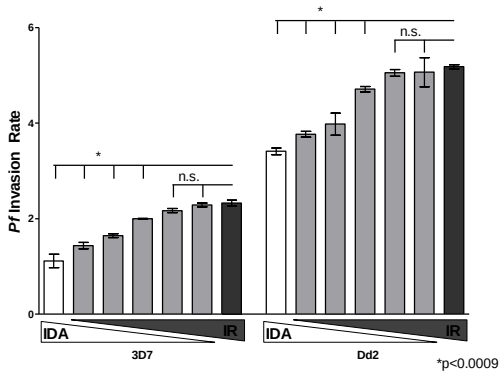
Growth assay	# replications in RBCs of interest	Time (h)												
		0*	***24***	***48****	***72***	***96***	***120***	***144***	***168***	***192***	***216***	***240***	***264***	***288***
1	**1**	Ring	Troph	Ring	Troph	Ring								
2	**3**					Ring	Troph	Ring	Troph	Ring				
3	**5**									Ring	Troph	Ring	Troph	Ring

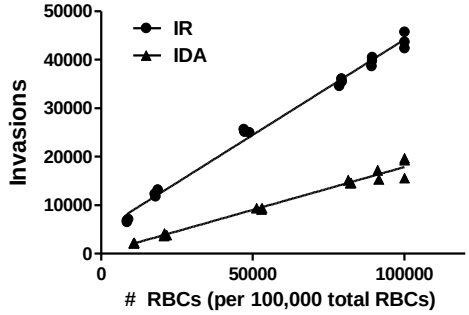
Supplementary Figure 1: Serial *in vitro* growth assay design for *P. falciparum.*

Synchronized *P. falciparum* cultured in RBCs from O+ iron replete donors were seeded as rings at 0.5% initial parasitemia at 1% haematocrit in triplicate into 96 well plates. Parasites were maintained for 96h at 37°C in 5% O_2, and media was changed daily. At 96h, the parasite cultures were split back to 0.5% parasitemia and maintained for an additional 96h in Growth Assay 2. At 192h, the parasite cultures were split back again to 0.5% parasitemia and maintained for an additional 96h in Growth Assay 3. Growth rate was determined for each growth assay independently. *seed experimental cultures with 0.5% ring stage pRBC. **parasites invade experimental cells of interest.

A.



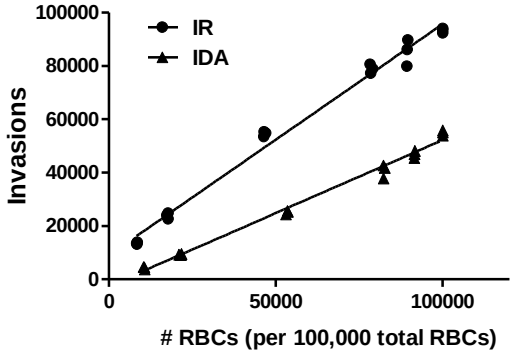
B.



IR R^2=0.9924
IDA R^2=0.9789

p<0.0001

C.

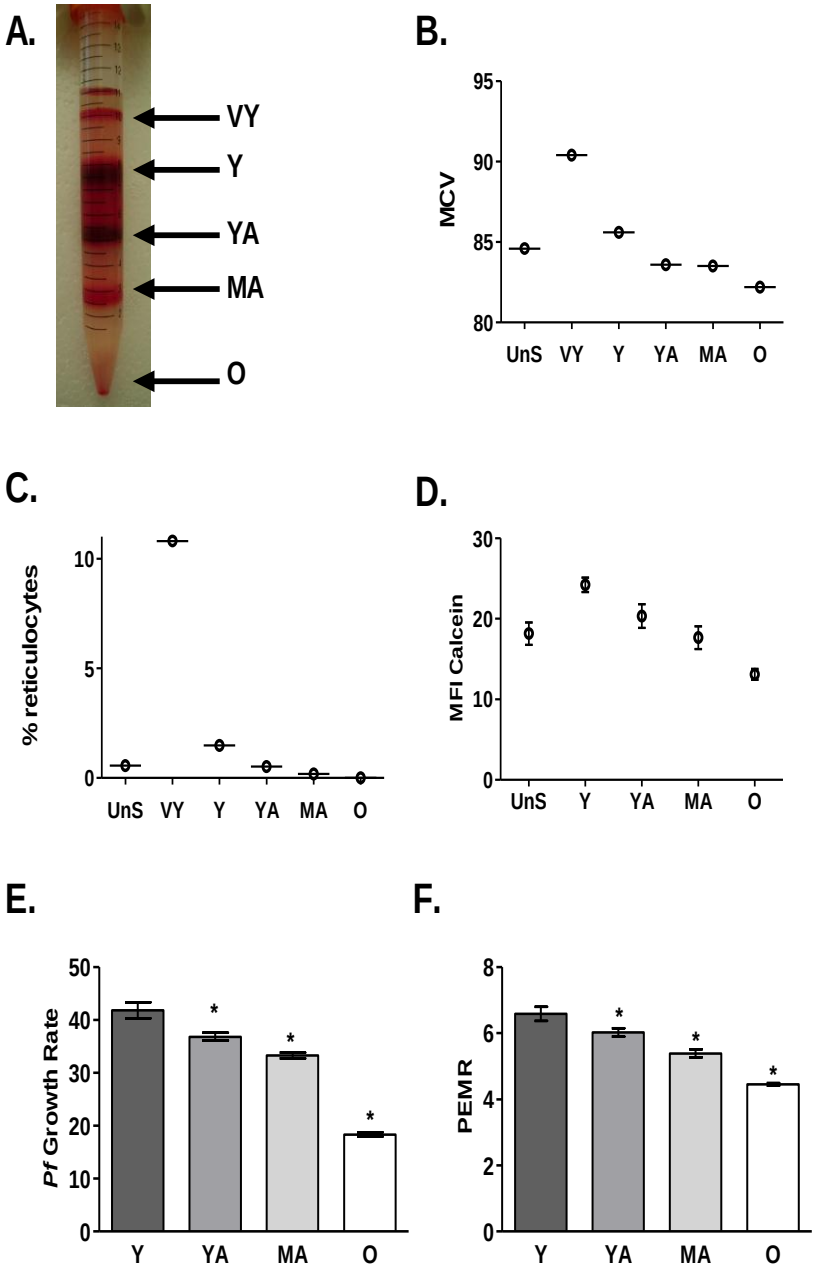


IR R^2=0.9884
IDA R^2=0.9899

p<0.0001

Supplementary Figure 2: Replacement of RBCs from IDA donors with RBCs from IR donors increases the invasion rate of erythrocytic stage *P. falciparum in vitro* (additional strains 3D7 and Dd2).

(A – C) Invasion of *P. falciparum* (strains 3D7 and Dd2) into RBC populations in which DDAO labeled RBCs from an IDA donor were replaced with Violet labeled RBCs from an IR Donor. Differentially labelled RBCs from and IDA and an IR donors were inoculated individually or together in the same wells at different ratios (100% IDA; 90% IDA and 10% IR; 80% IDA and 20% IR; 50% IDA and 50% IR; 20% IDA and 80% IR; 10% IDA and 90% IR; 100% IR). Each invasion condition contained $20x10^6$ total RBCs and was subsequently inoculated with $0.3x10^6$ pRBCs. Data is from a representative experiment of three independent experiments performed in triplicate with RBCs from three different IDA and IR donors. **(A)** Bars represent parasite invasion rates into 100% IDA; 90% IDA and 10% IR; 75% IDA and 25% IR; 50% IDA and 50% IR; 25% IDA and 75% IR; and 100% IR RBCs. Elongated triangles below the X-axis represent the percentage of IDA RBCs (white triangle) and IR RBCs (gray triangle) in the total RBC population. Error bars represent the standard deviation. Significance determined by two-tailed paired Student's *t* test (GraphPad, Prism, v. 5.04, La Jolla, CA). *p<0.0009, compared to *P. falciparum* invasion rate into 100% IR RBCs. **(B and C)** Data shows the number of *P. falciparum* strains 3D7 **(B)** and Dd2 **(C)** invasions events into IDA and IR RBCs as the frequency of each increases in a RBC population containing 100,000 total RBCs. Circles and triangles represent the number of *P. falciparum* invasion events into IR and IDA RBCs respectively as each increases in frequency from 10% to 100% of the total RBC population. Linear regression was used to determine the best fit line for the data, IR R^2=0.9924 and IDA R^2=0.9789 for 3D7 and IR R^2=0.9884 and IDA R^2=0.9899 for Dd2. ANCOVA was performed to compare the slopes of the lines fit to the IDA and IR invasion data. The null hypothesis was no difference between the two RBC types (H_0: $\beta_{Iron\ replete} = \beta_{Iron\ deficient}$, α=0.05). ANCOVA performed with GraphPad, Prism, v. 5.04, La Jolla, CA calculated a p value of <0.0001.

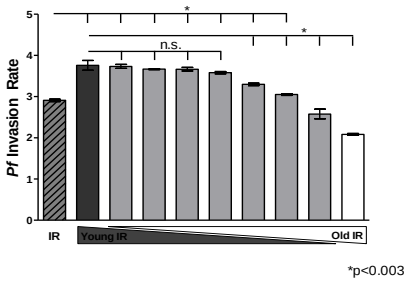


Supplementary Figure 3: Reticulocytes and young RBCs support greater *P. falciparum* growth rates and PEMRs *in vitro* than increasingly older RBCs.

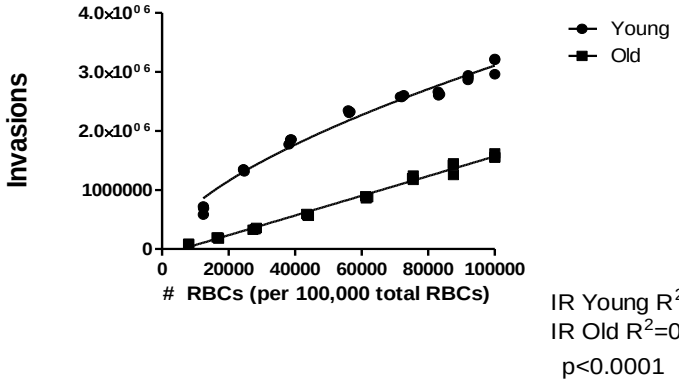
(A) Percoll density separation of RBCs from an IR donor into five fractions of increasing age: very young (VY), young(Y), young adult (YA), mature adult (MA) and old (O). Briefly, 1mL of RBCs at 50% haematocrit in RPMI were layered on top of a discontinuous Percoll gradient and spun at 1075g for 25 min. Layers were removed, washed, and stored at 4°C for up to two days prior to use. Density age separation of RBC was confirmed by: **(B)** MCV, as measured by Beckman Coulter AcT diff2 (Brea, CA), **(C)** percent reticulocytes, as determined by new methylene blue staining and counting, and **(D)** fluorescence of density separated RBCs stained with 5 µM calcein-AM for 30 min and assessed by flow cytometry. For *P. falciparum* experiments the very young RBC population was combined with the young RBC population and subsequently referred to as young.
(E) Growth of *P. falciparum* (strain FCR3-FMG) within age separated RBCs. Data is from a representative experiment of three independent experiments performed in triplicate. Bars represent parasite growth rate after one 96h growth assay in young, young adult, mature adult and old RBCs. Error bars represent the standard deviation. Significance determined by two-tailed paired Student's *t* test (GraphPad, Prism, v. 5.04, La Jolla, CA). *p<0.001, compared to *P. falciparum* growth rate in young RBCs.

(F) Comparison of the Parasite Erythrocyte Multiplication Rate (PEMR) of *P. falciparum* (strain FCR3-FMG) within age separated RBCs. PEMR experiments were performed as described previously for RBCs from IDA and IR donors. Data is from a representative experiment of three independent experiments performed in triplicate. Bars represent PEMR of *P. falciparum* in young, young adult, mature adult and old RBCs. Error bars represent the standard deviation. Significance determined by two-tailed paired Student's *t* test (GraphPad Prism 5, La Jolla, CA). *p<0.001, compared to infectious merozoites produced within young RBCs.

A.



B.



Supplementary Figure 4: *P. falciparum* plateau in invasion rate of RBC populations containing 50% to 100% young RBCs is independent of merozoites inoculum.
(A and B) Invasion of *P. falciparum* (strain FCR3-FMG) into RBC populations in which Violet labeled young IR RBCs were replaced with DDAO labeled old IR RBCs. Differentially labeled young and old RBCs were inoculated individually or together in the same wells at different ratios (100% young; 90% young and 10% old; 80% young and 20% old; 67% young and 33% old; 50% young and 50% old; 33% young and 67% old; 20% young and 80% old; 10% young and 90% old; 100% old). Each invasion condition contained $20x10^6$ total RBCs and was subsequently inoculated with $0.6x10^6$ pRBCs. The invasion rate of *P. falciparum* into total IR RBCs was additionally assessed. Data is from a representative experiment of two independent experiments performed in triplicate with RBCs with *P. falciparum* strains 3D7 and FCR3-FMG. **(B)** Data shows the number of *P. falciparum* invasions events into young and old RBCs as the frequency of each increases in a RBC population containing 100,000 total RBCs. Circles and squares represent the number of *P. falciparum* invasion events into young and old RBCs respectively as each increases in frequency from 10% to 100% of the total RBC population. Linear regression was used to determine the best fit line for young and old RBC data. A linear function best fit old RBC data (R^2=0.9906) and a logarithmic function best fit young RBC data (R^2=0.9764). ANCOVA was performed to compare to determine whether invasion data of old and young RBCs differed significantly. The null hypothesis was no difference between the two RBC types (H_0: $\beta_{Iron\ replete} = \beta_{Iron\ deficient}$, α=0.05). ANCOVA performed with GraphPad, Prism, v. 5.04, La Jolla, CA calculated a p value of <0.0001.