

Red blood cell tension controls *Plasmodium falciparum* invasion and protects against severe malaria in the Dantu blood group

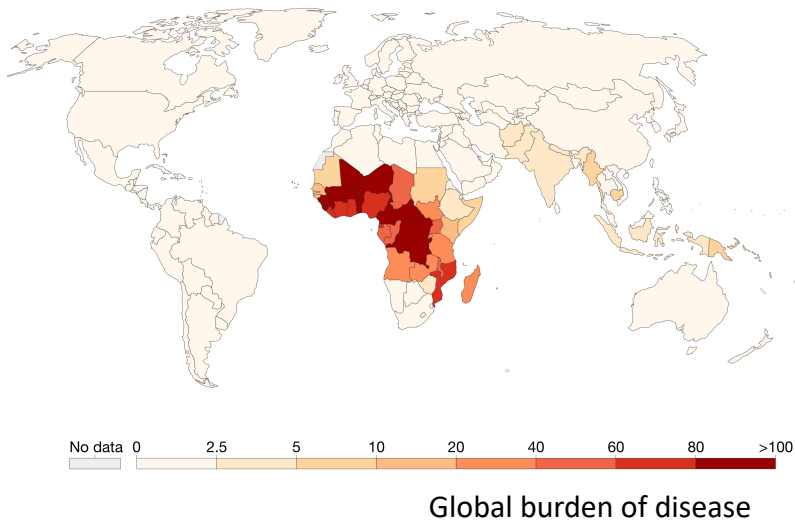
Viola Introini

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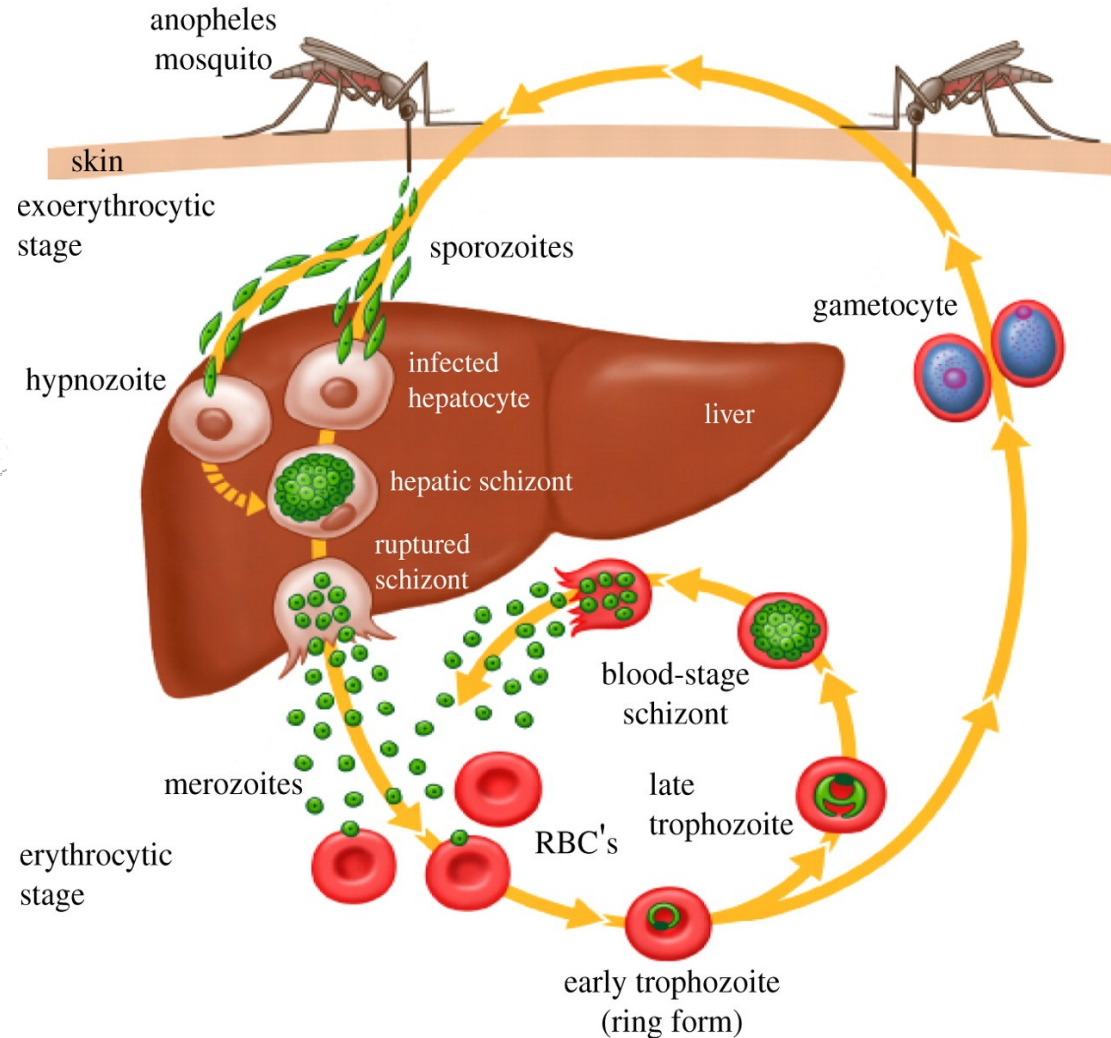
Red Cell SIG - BBTS 2019

Blood stage malaria: target for interventions

Malaria death rate, 2017

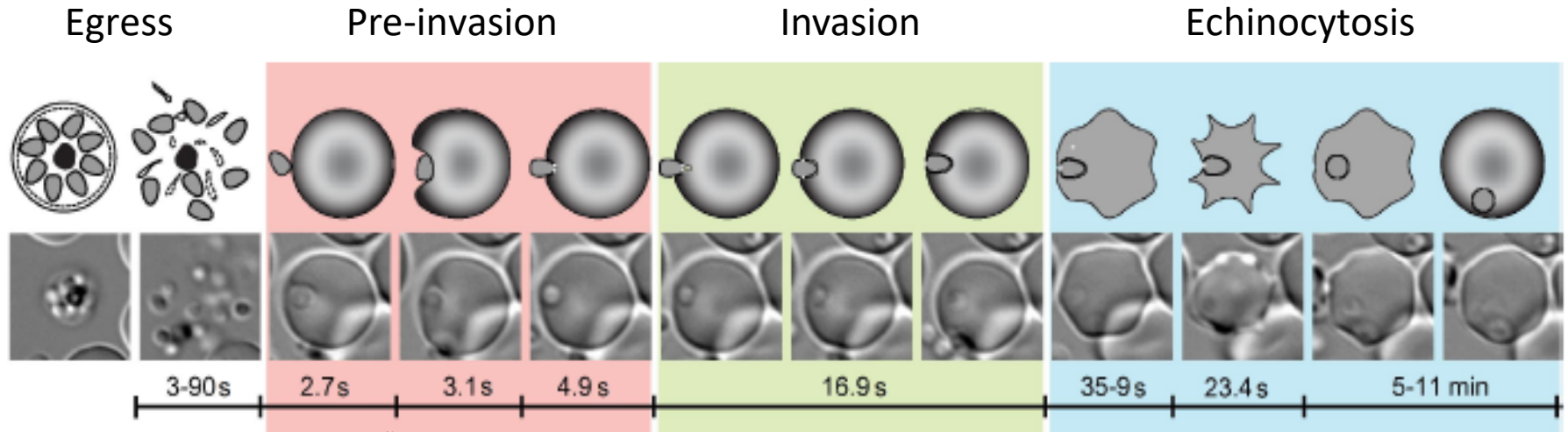


Plasmodium lifecycle

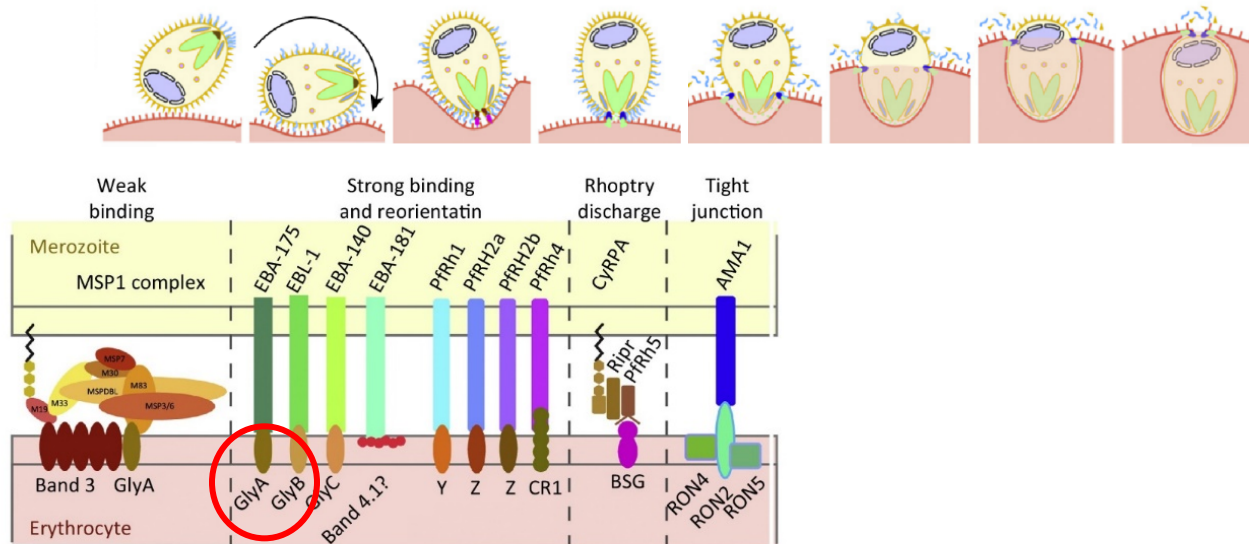


Hill et al., *Philos Trans R Soc Lond B Biol Sci*, 2011

Malaria invasion process

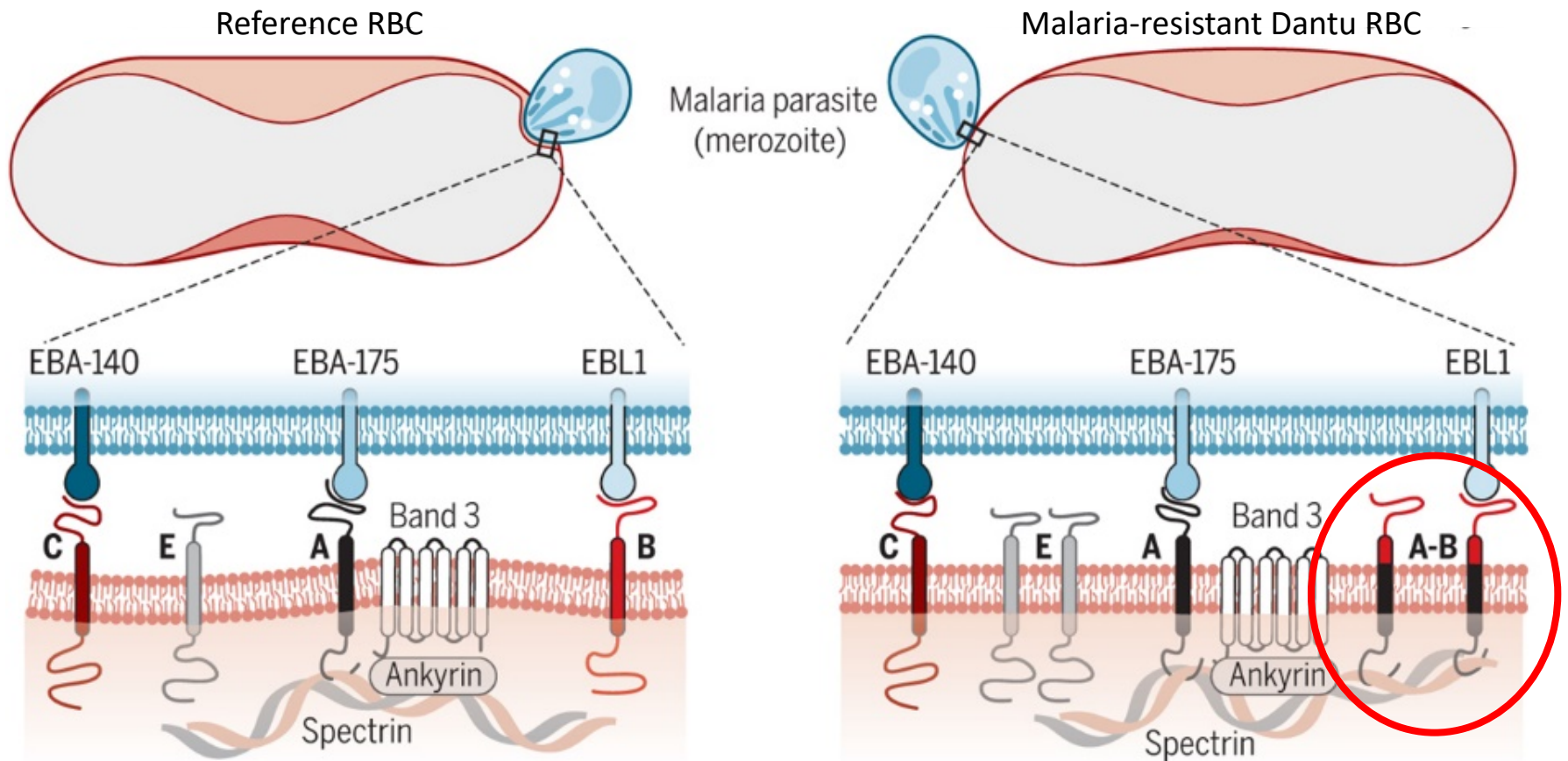


Gilson, Crabb, *Int J Parasitol*, 2009



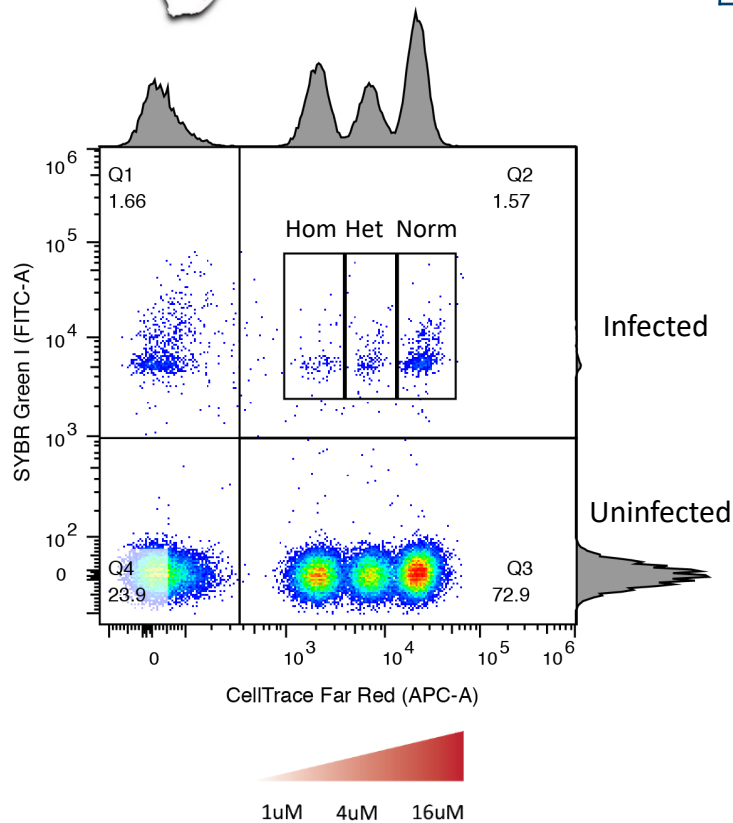
Weiss et al., *Trends Parasitol*, 2016

Structural variation of RBC invasion receptors link to natural malaria resistance

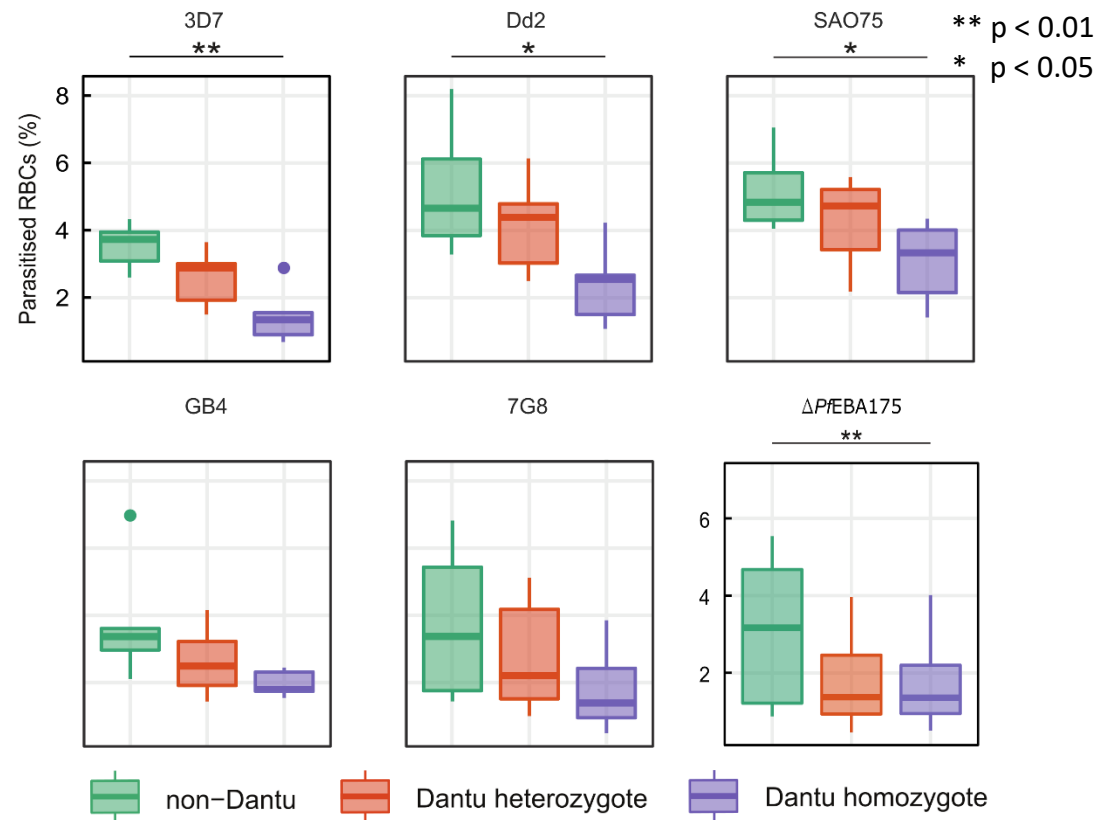


Leffler *et al.*, *Science*, 2017

Invasion efficiency – Flow cytometry



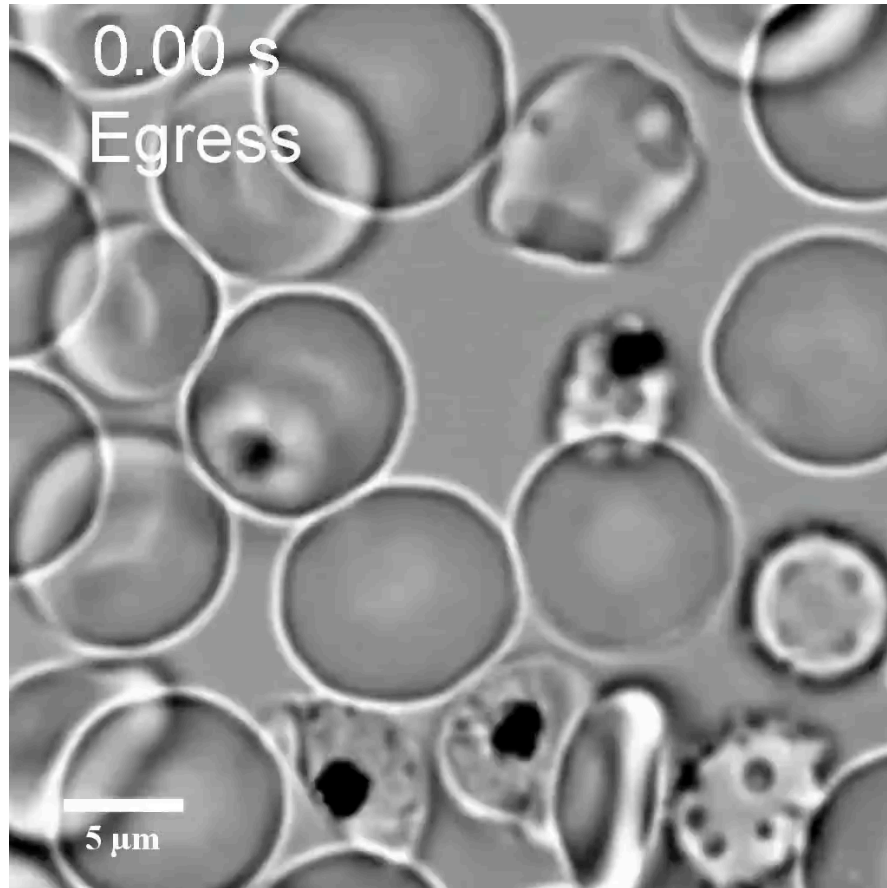
CHARACTERISTICS	NON-DANTU (n=6)	DANTU HETEROZYGOTE (n=6)	DANTU HOMOZYGOTE (n=6)
Median age (range; years)	11 (9-13)	13 (10-14)	7 (1-14)
Gender (% F)	33	67	67
Mean platelets (SD; $\times 10^6/L$)	272.5 (56.0)	275.2 (58.4)	371.3 (130.5)
Mean haemoglobin (SD; g/L)	12.3 (0.5)	12.7 (0.7)	11.5 (1.0)



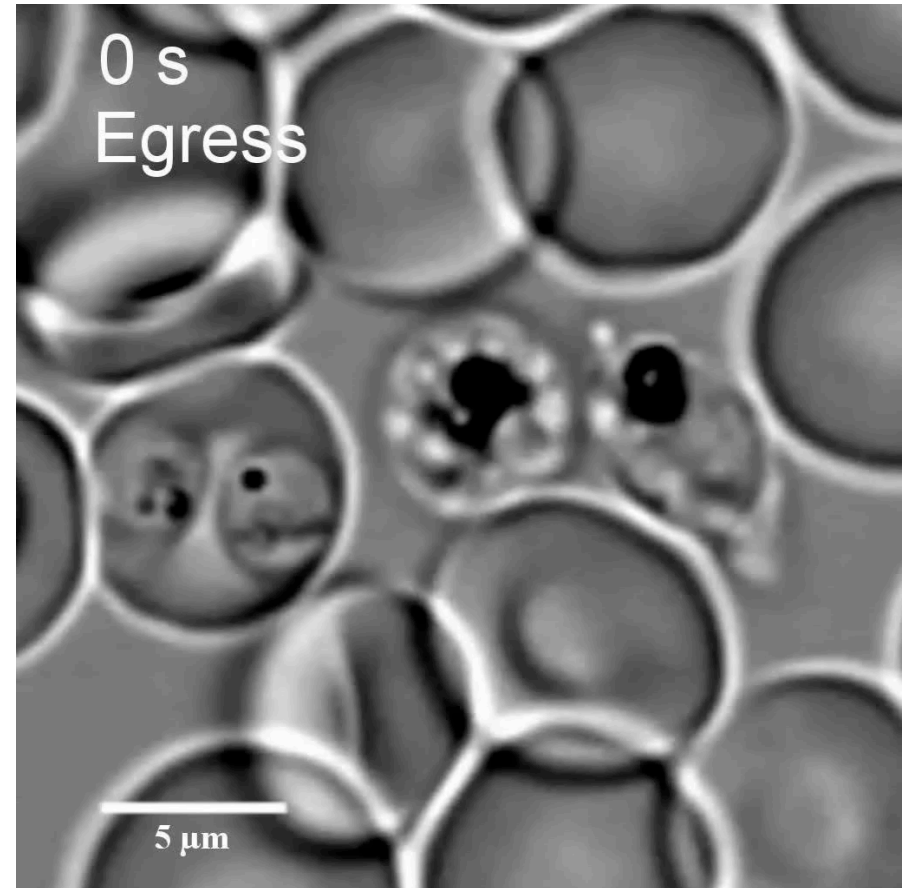
Invasion reduced in Dantu for different parasite strains

Difference in the ability to invade RBCs – Live imaging

Non-Dantu

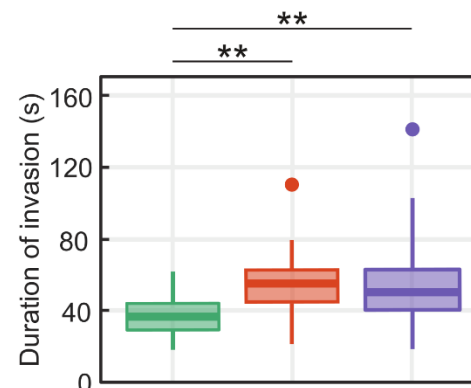
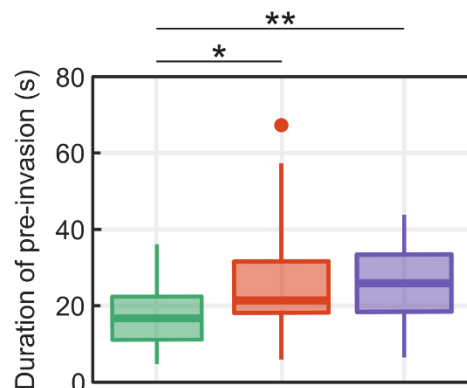
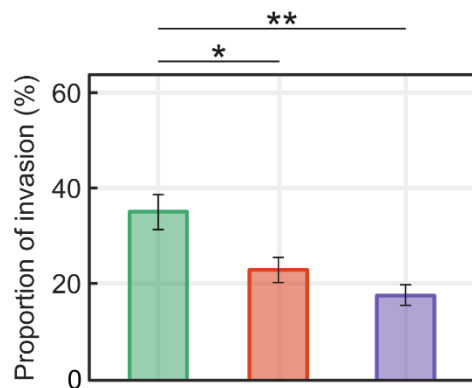
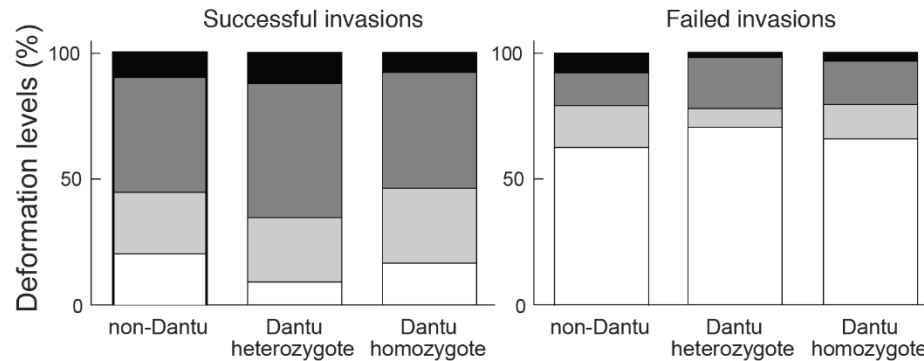
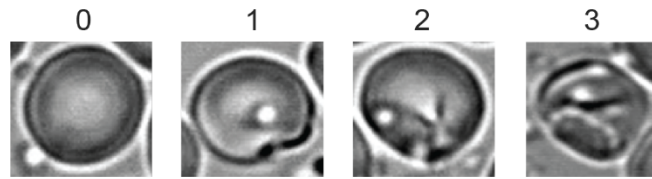


Dantu



Invasion kinetics

RBC membrane deformation scores triggered by parasite contact



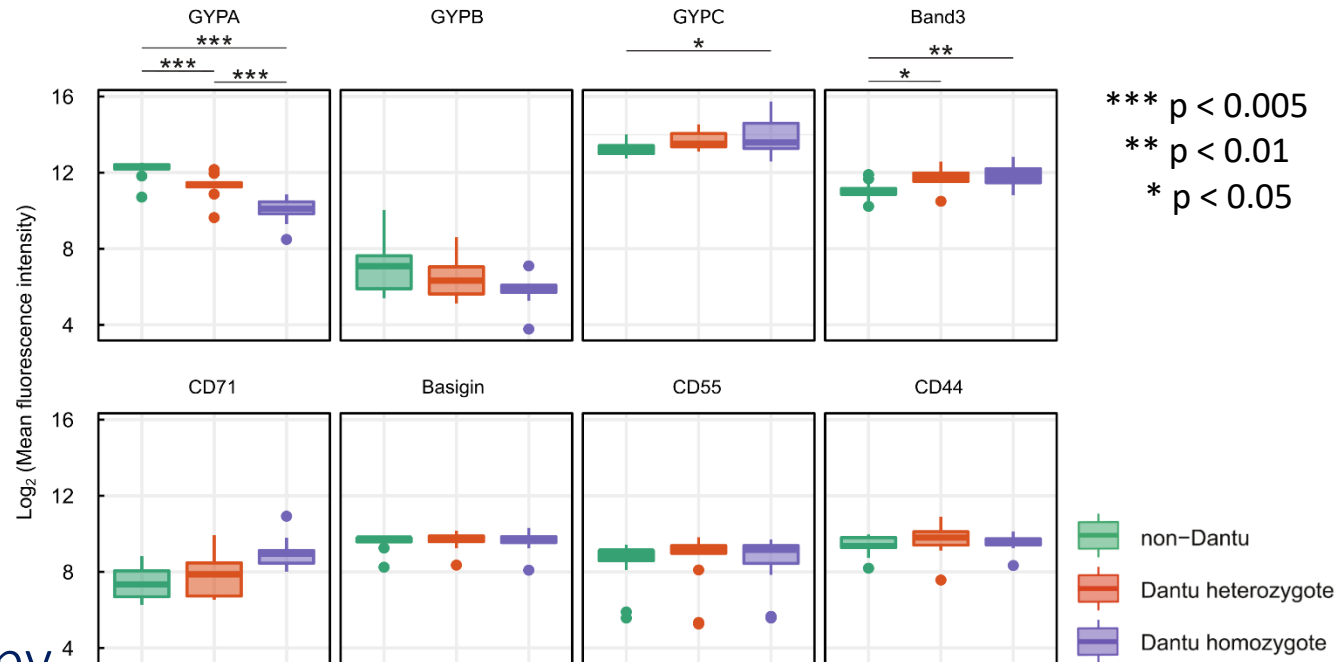
** p < 0.01
* p < 0.05

non-Dantu
Dantu heterozygote
Dantu homozygote

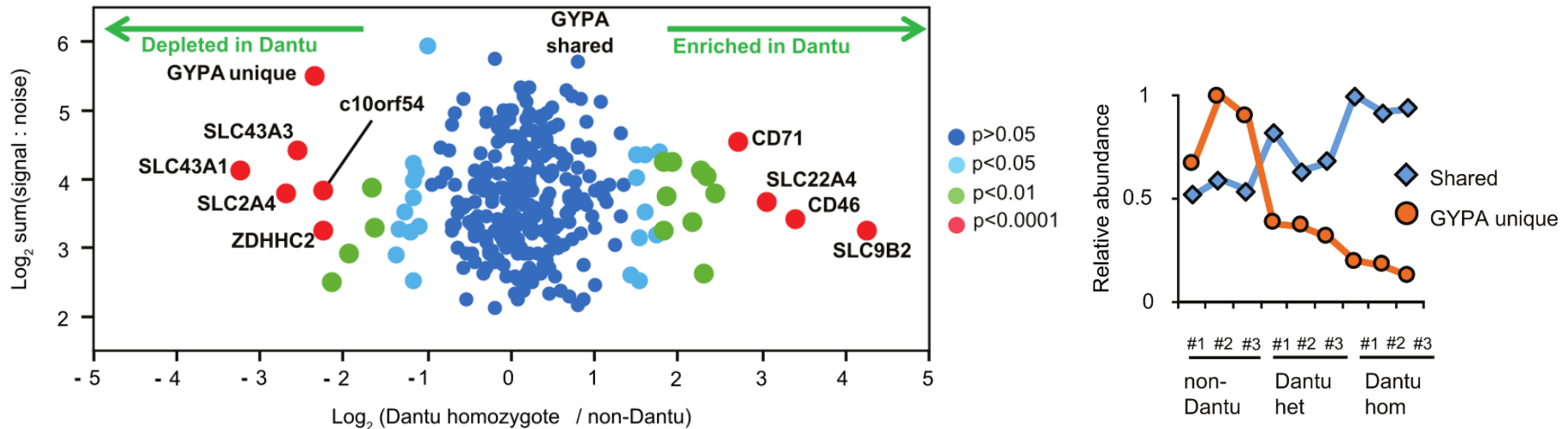
Longer duration of pre-invasion and invasion

RBC membrane protein characterisation

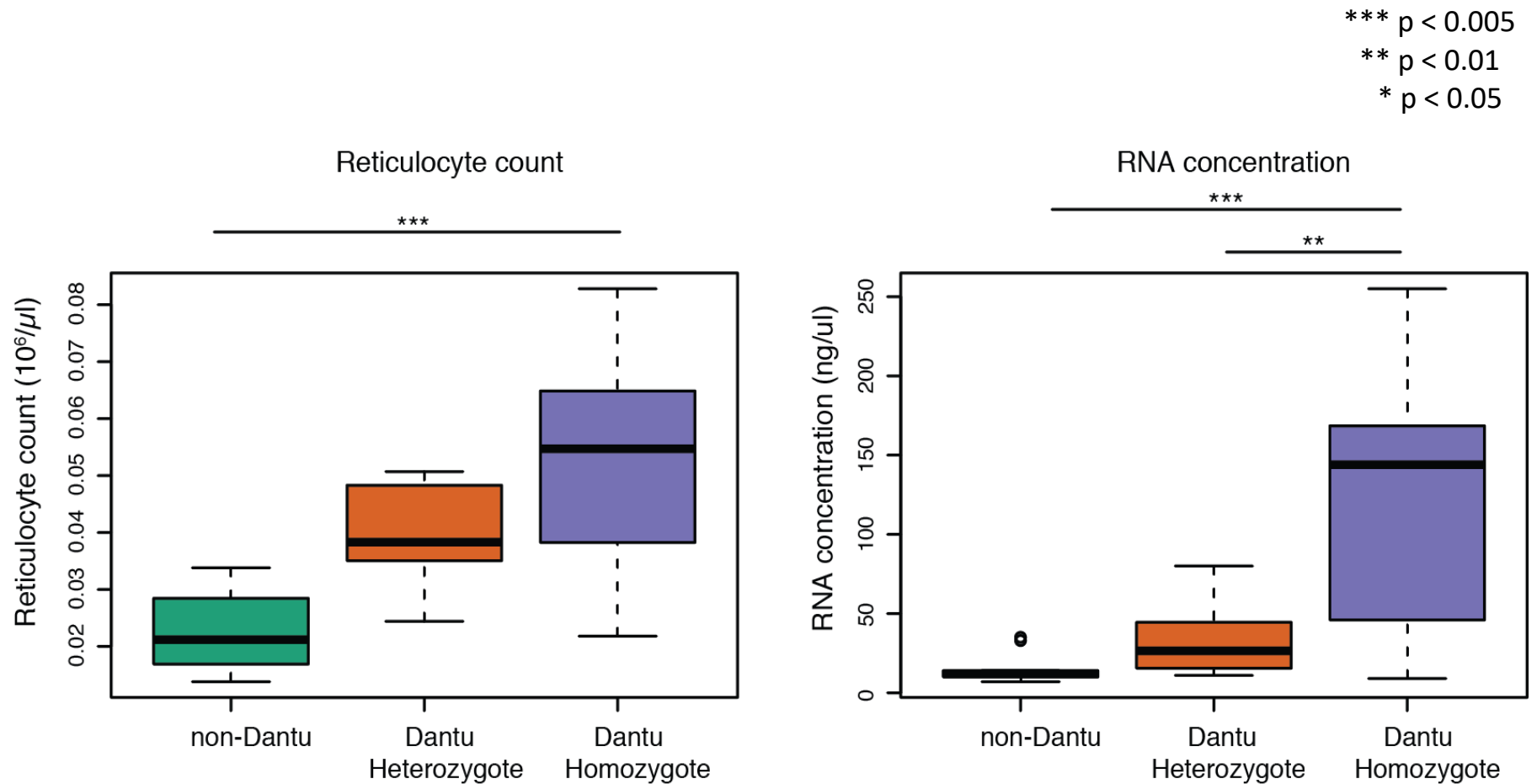
- Antibody Assay



- Mass Spectroscopy

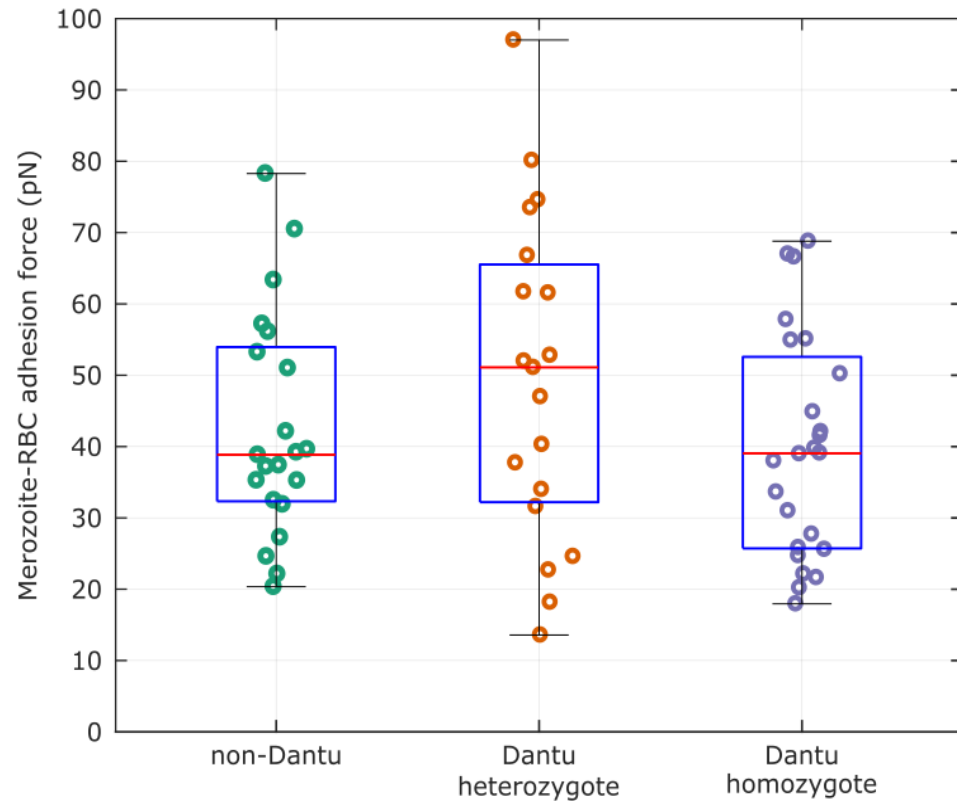
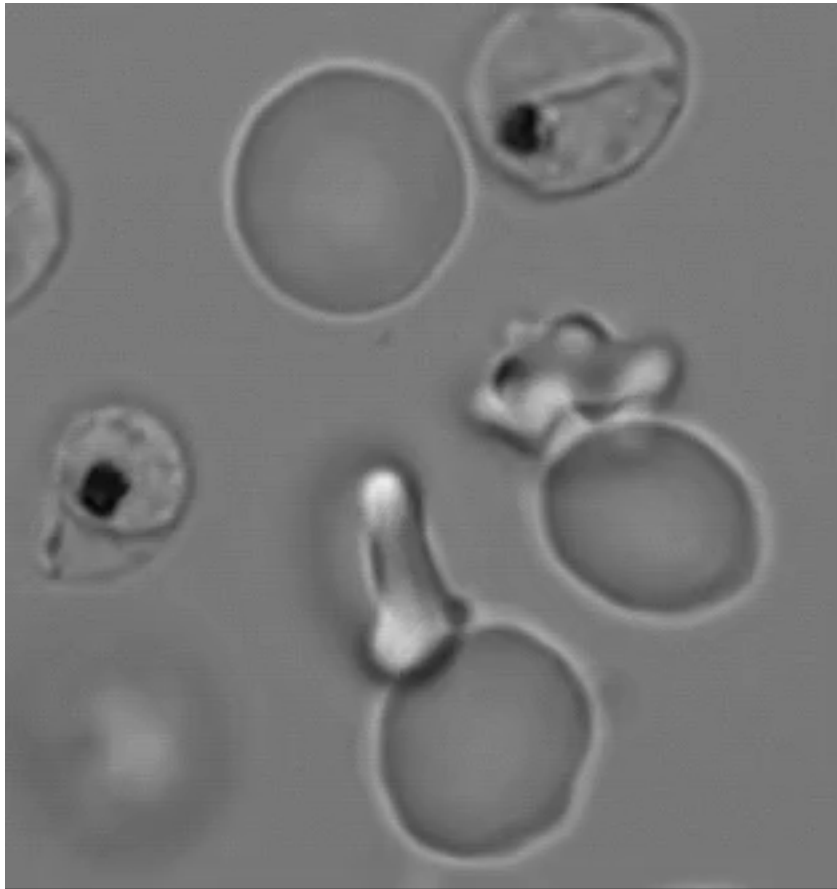


Distribution of reticulocytes and RNA concentration across Dantu genotypes



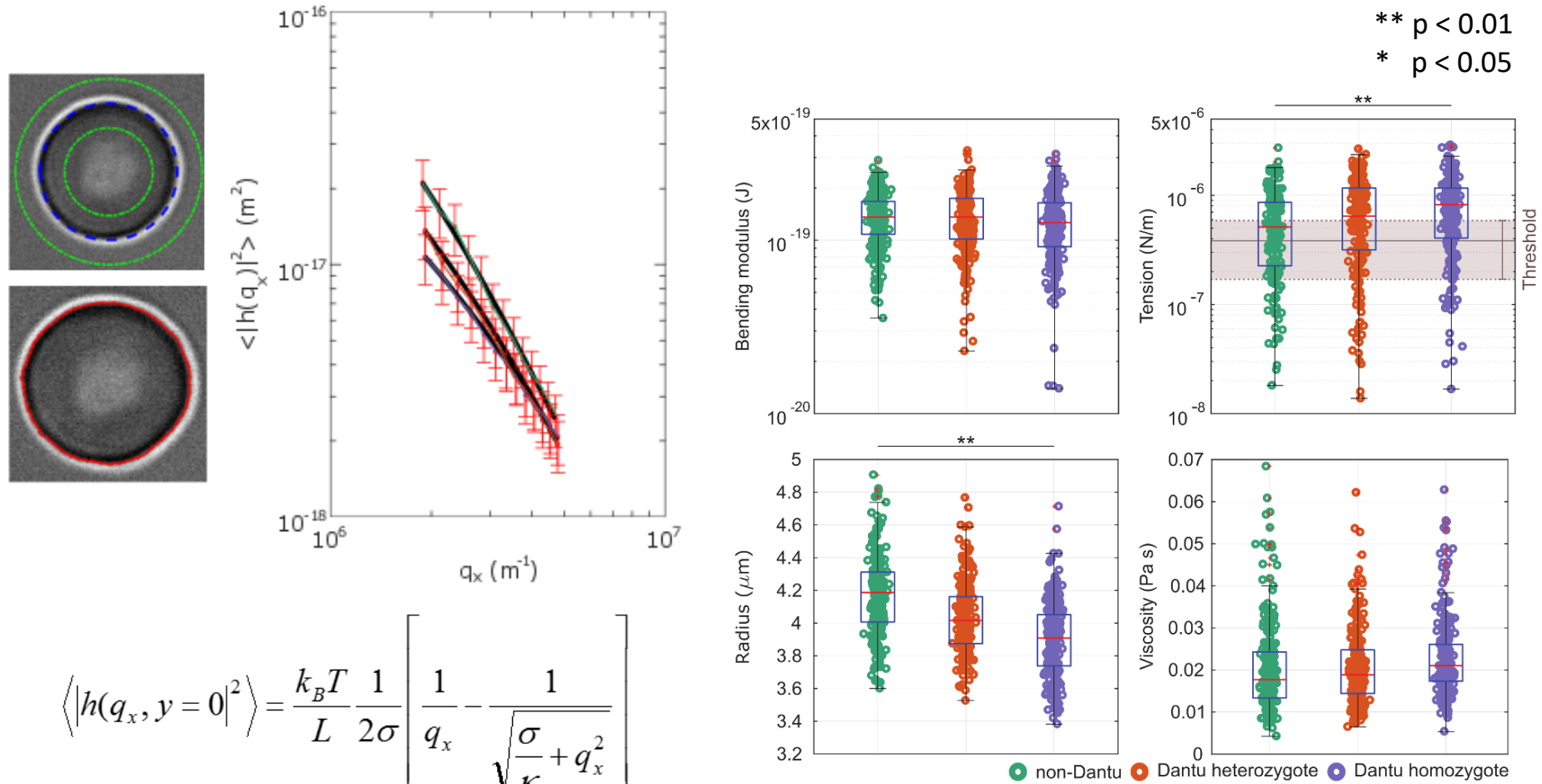
Differences in the biophysical properties – Optical tweezers

Quantification merozoite-RBC adhesion force



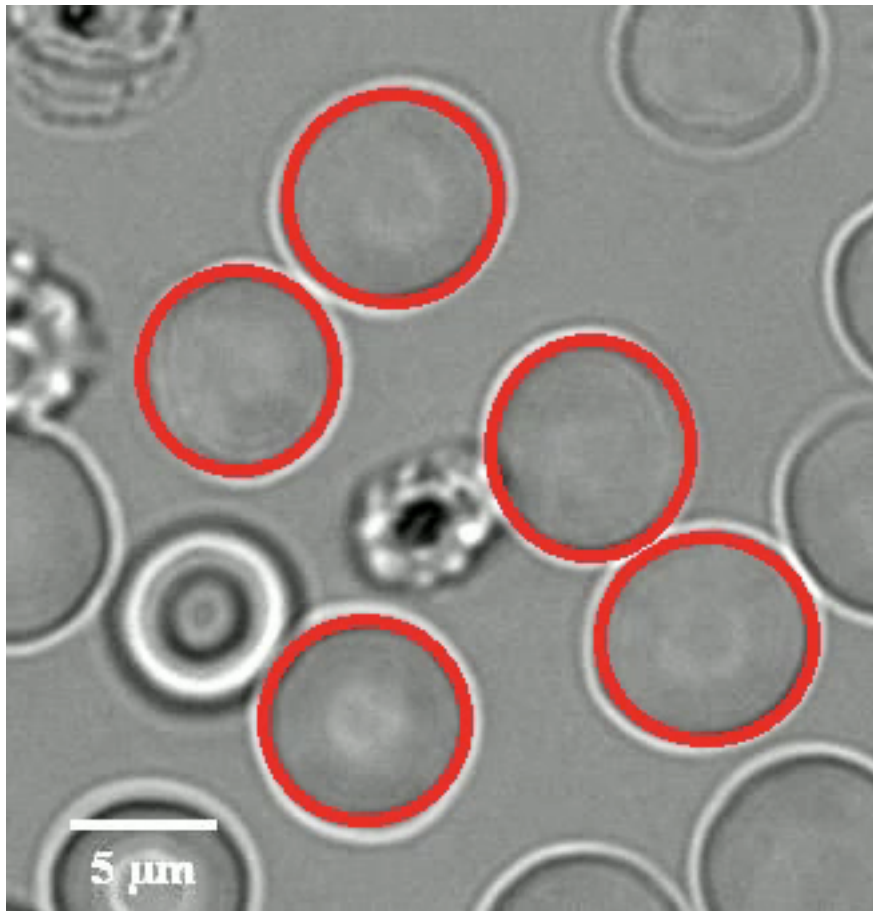
No difference in merozoite-RBC detachment force across genotype groups

Differences in the biophysical properties – Flickering spectroscopy

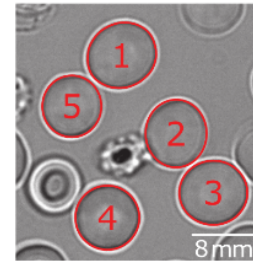


Higher tension and smaller radius in Dantu RBCs

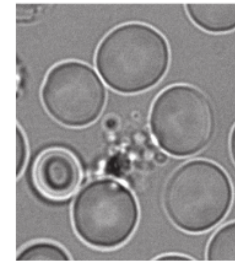
Tension affects invasion success and dynamics



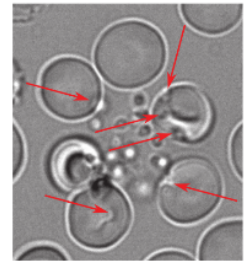
Contour detection



Egress

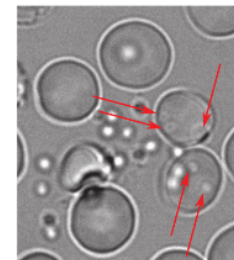


Deformation



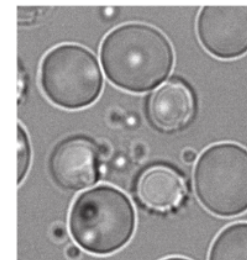
No contact: cell 1

Invasion



No invasions: cells 4,5 Invasions: cells 2,3

Echinocytosis

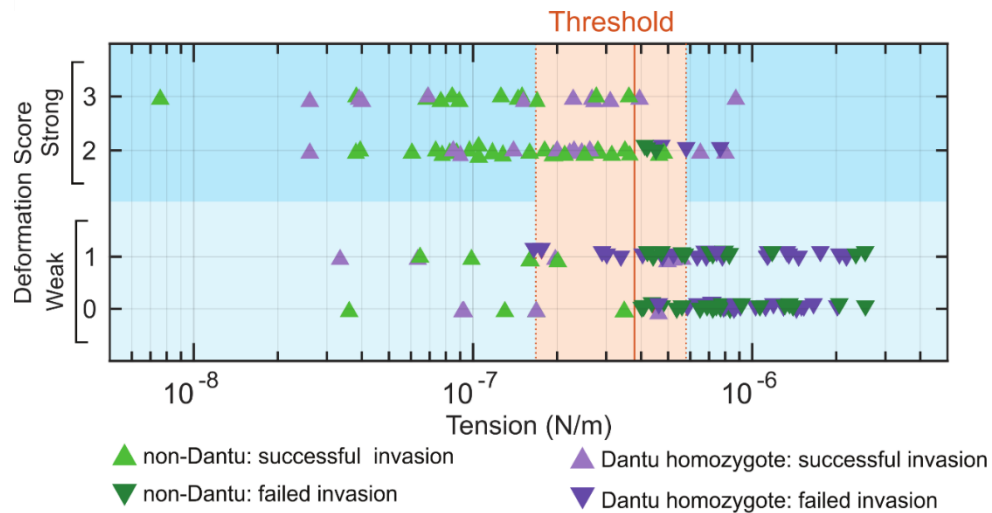
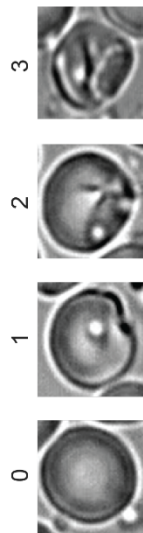
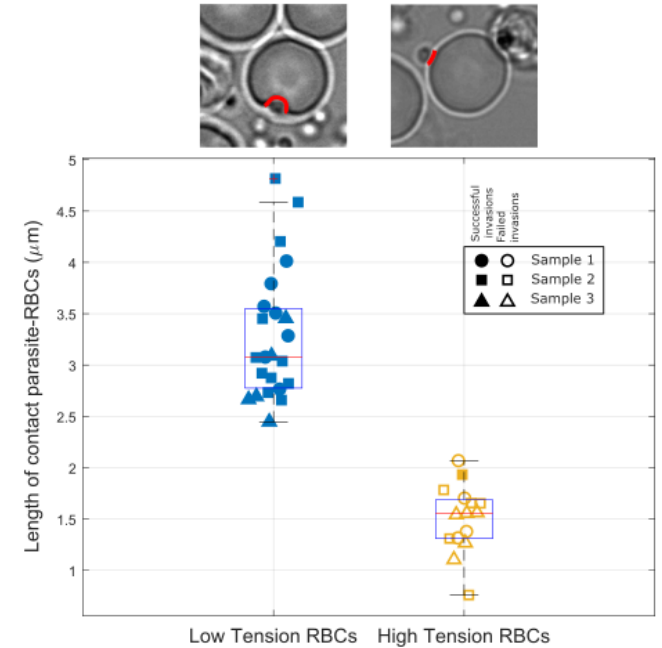


Measure tension of **the same cells** that are then invaded by malaria pathogen
Determine the threshold of protective tension from single cell resolution video analysis

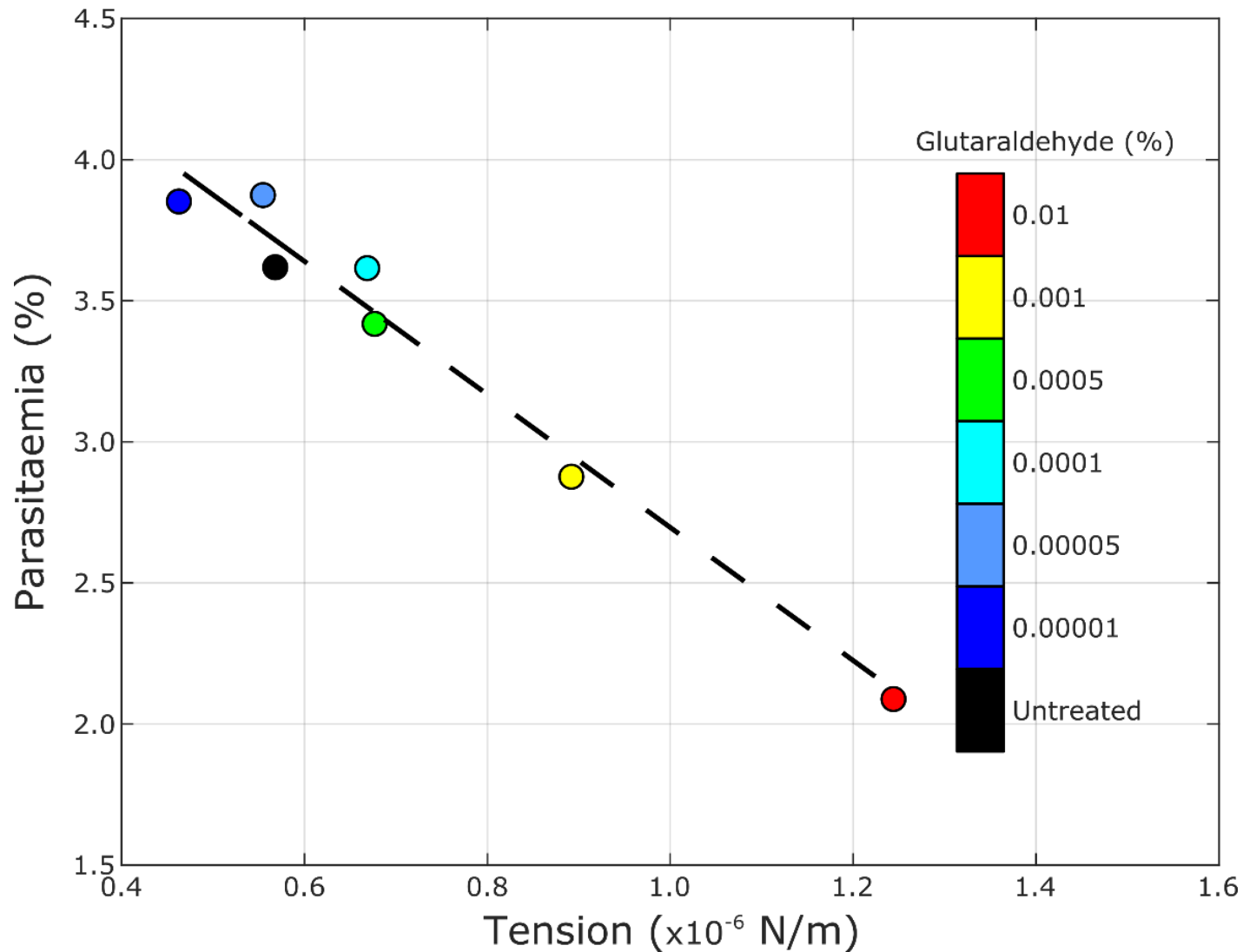
Elevated tension as protective mechanism for Dantu RBCs



Tension threshold: $3.8 (\pm 2.0) \times 10^{-7}$ N/m



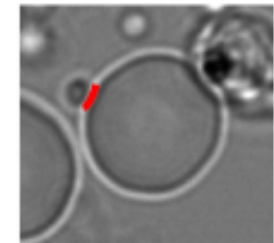
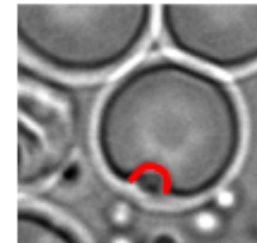
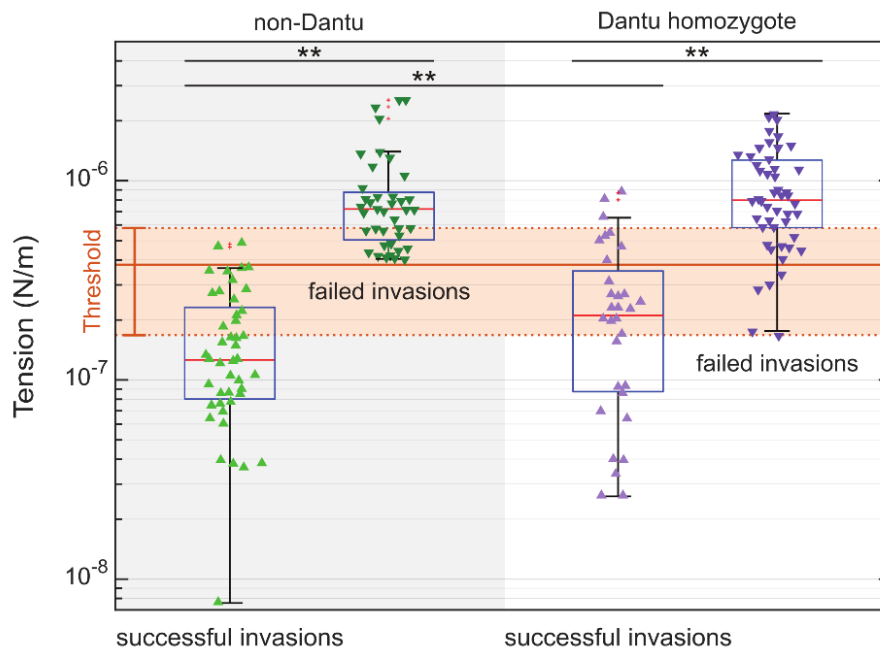
Mimic Dantu: invasion decreases by increasing RBC tension with glutaraldehyde



Conclusions

- Higher tension correlates with lower invasion efficiency in Dantu
- Connection, for the first time, between the RBC tension and protection against malaria observed in a human population
- Tension threshold for successful malaria invasion
- High RBC tension leads to weak RBC deformations and limited parasite wrapping

IN GENERAL



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Cicuta group



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Collaborators



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Thomas Williams
Silvia Kariuki

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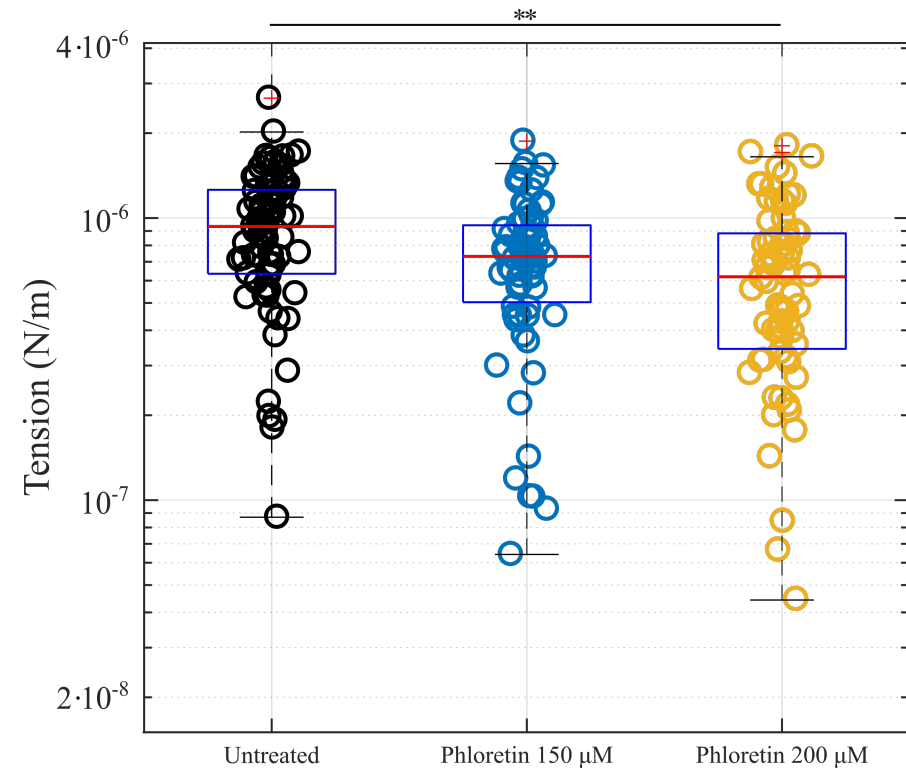
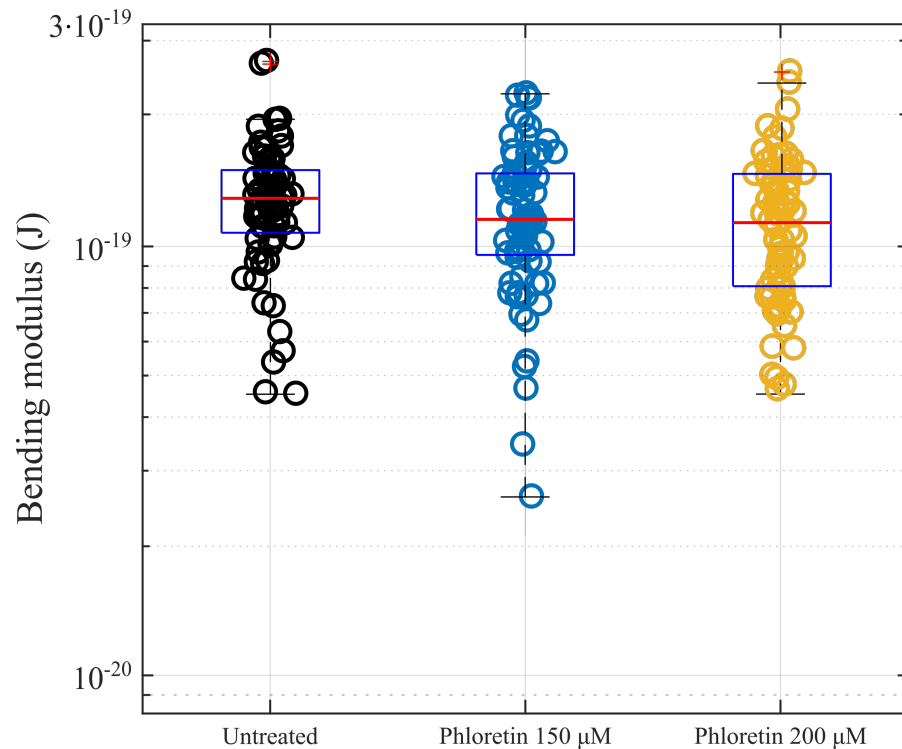
In review: *Kariuki**, *Marin-Menendez**, *Introini** et al. <https://www.biorxiv.org>

Manipulation of Dantu RBC membrane tension with phloretin to imitate non-Dantu cells

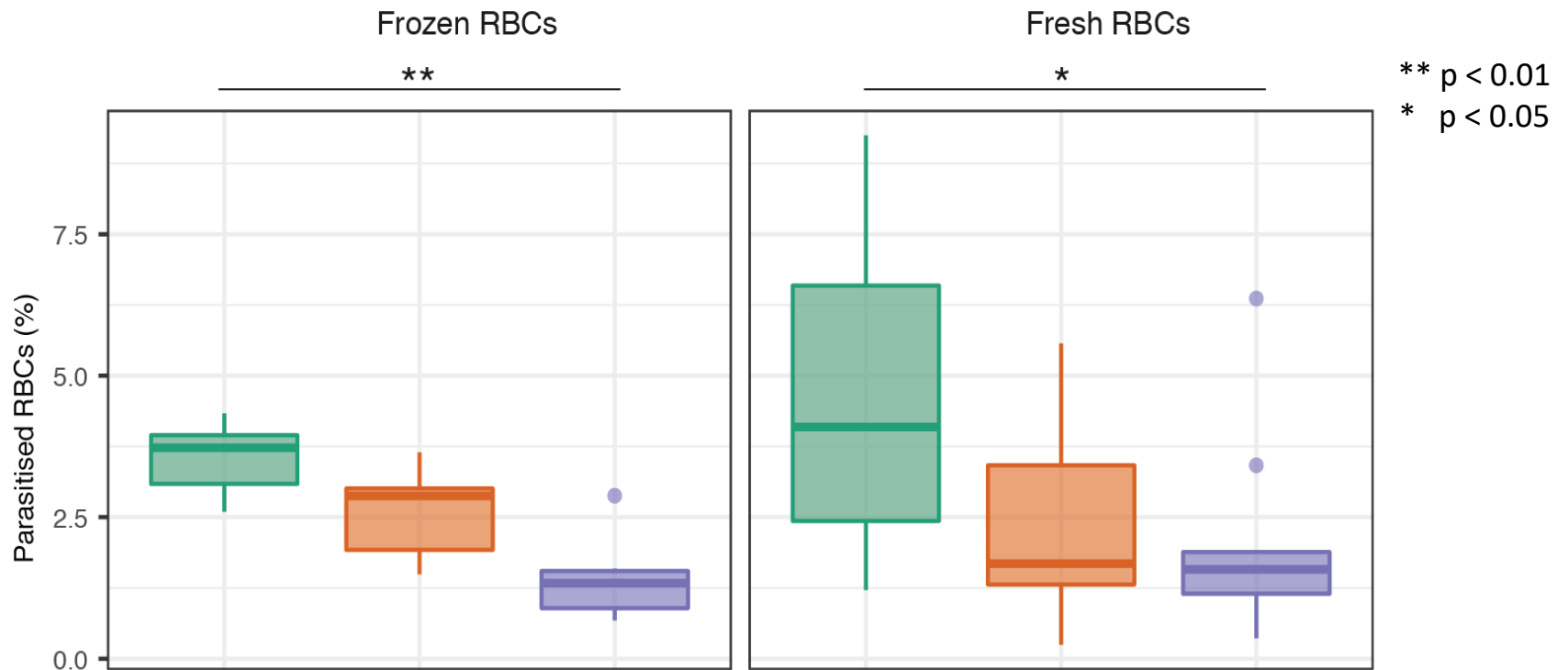
SLC9B2 sodium-hydrogen exchanger upregulated in Dantu homozygotes

** $p < 0.01$

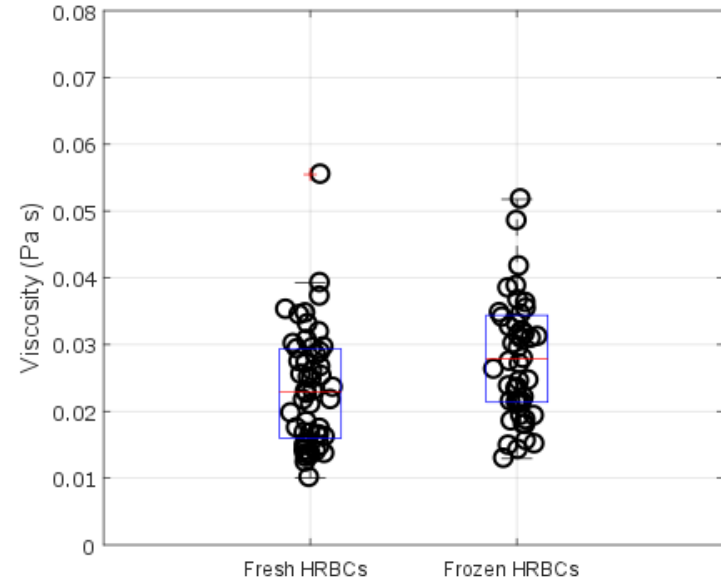
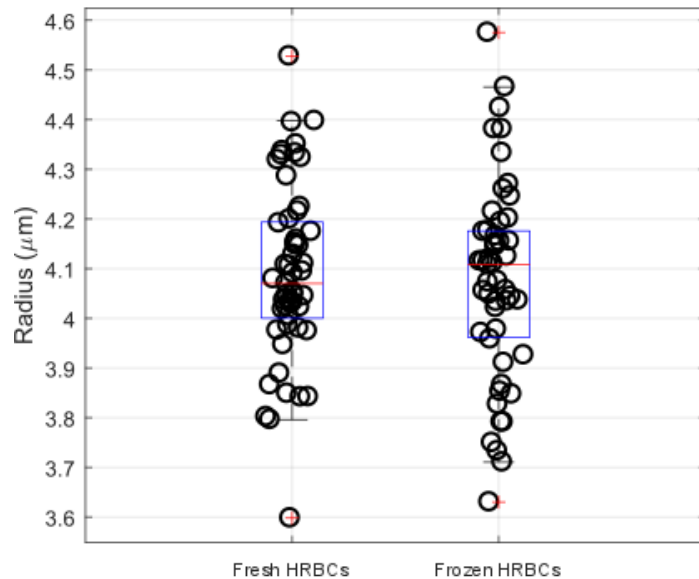
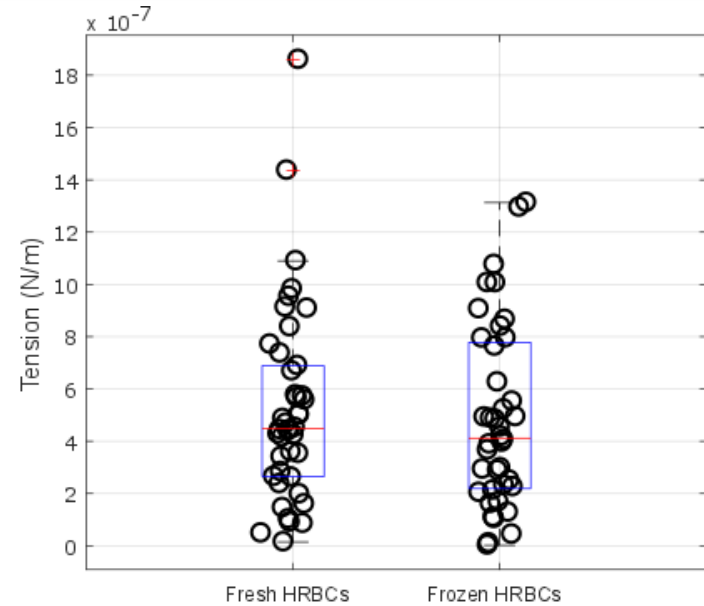
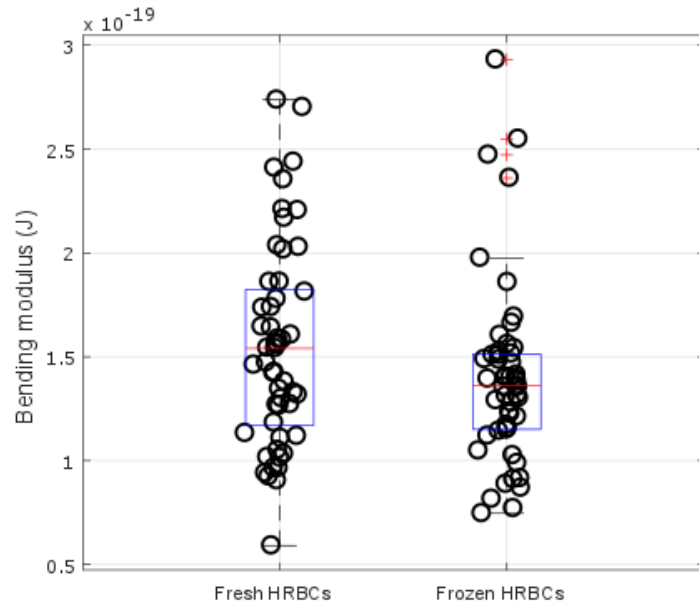
* $p < 0.05$



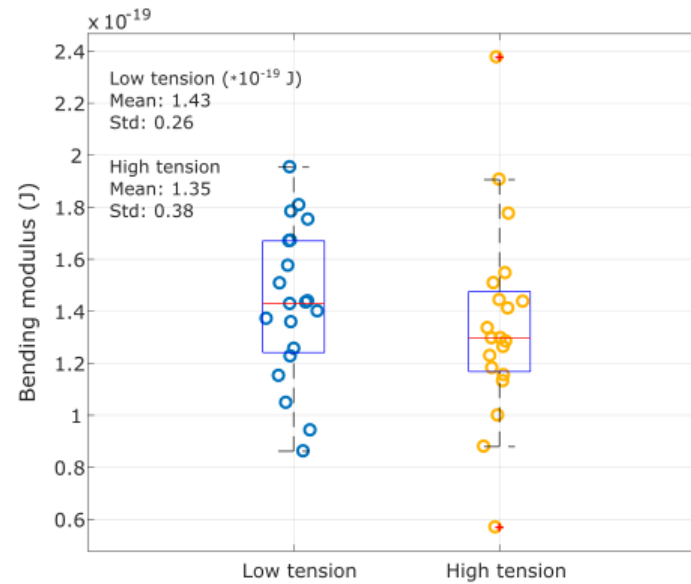
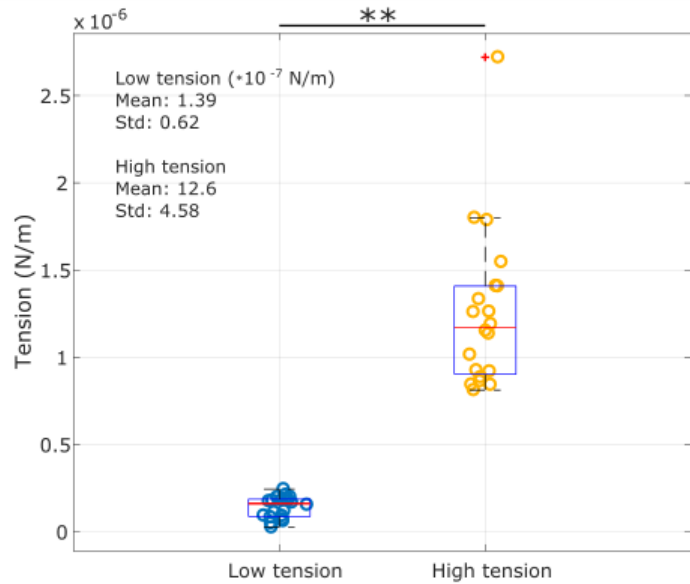
Comparing parasite invasion of frozen and fresh RBCs



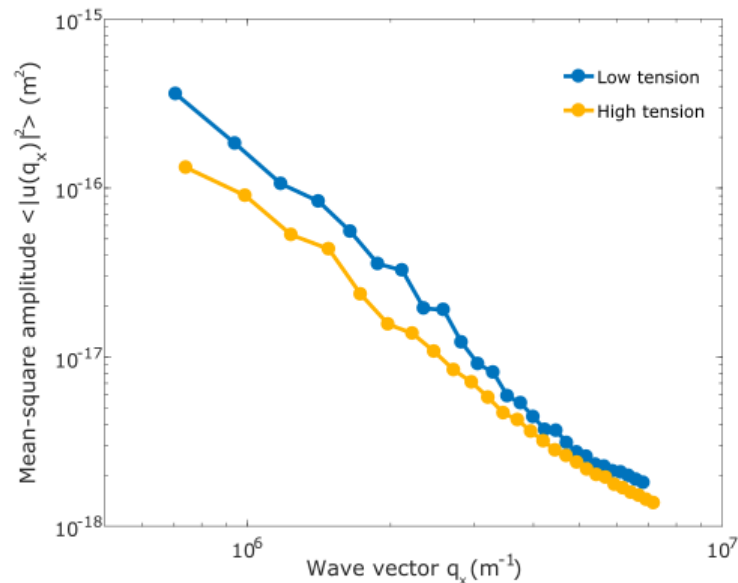
Comparing biomechanical properties of frozen and fresh RBCs



Decoupling tension and bending modulus



** $p < 0.01$
* $p < 0.05$



Correlation RBC radius-tension

