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A universal erythrocyte receptor for *Plasmodium falciparum* invasion

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Sanger Malaria Programme

Red cells mean different things to different organisms

- Life
- Career
- Food….

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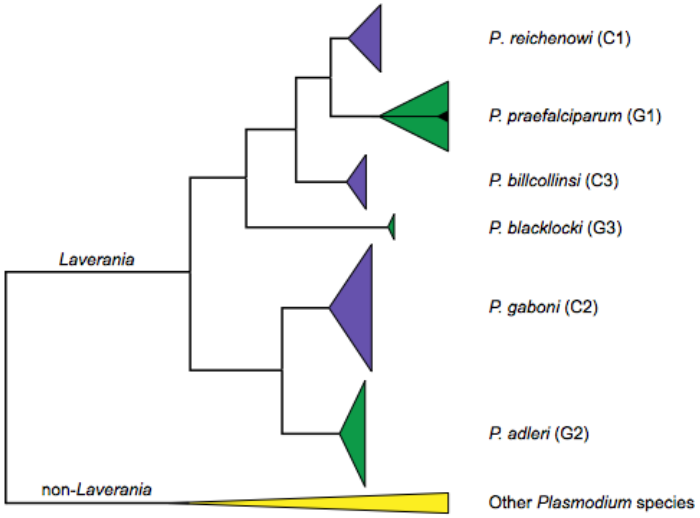
Malaria is caused by multiple *Plasmodium* species

300-500 million clinical cases each year, c. 1 million deaths

Single cell eukaryotic parasite of the *Plasmodium* genus

Four species of *Plasmodium* routinely cause malaria in humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae.* Others can cause zoonotic infections: *P. knowlesi*

Plasmodium species are incredibly successful – there are multiple species adapted to most vertebrate hosts



Rayner *et al.* Trends Parasitol 2011

Two species cause almost all detected human malaria: *P. falciparum* (mortality) and *P. vivax* (morbidity)

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A complex life cycle

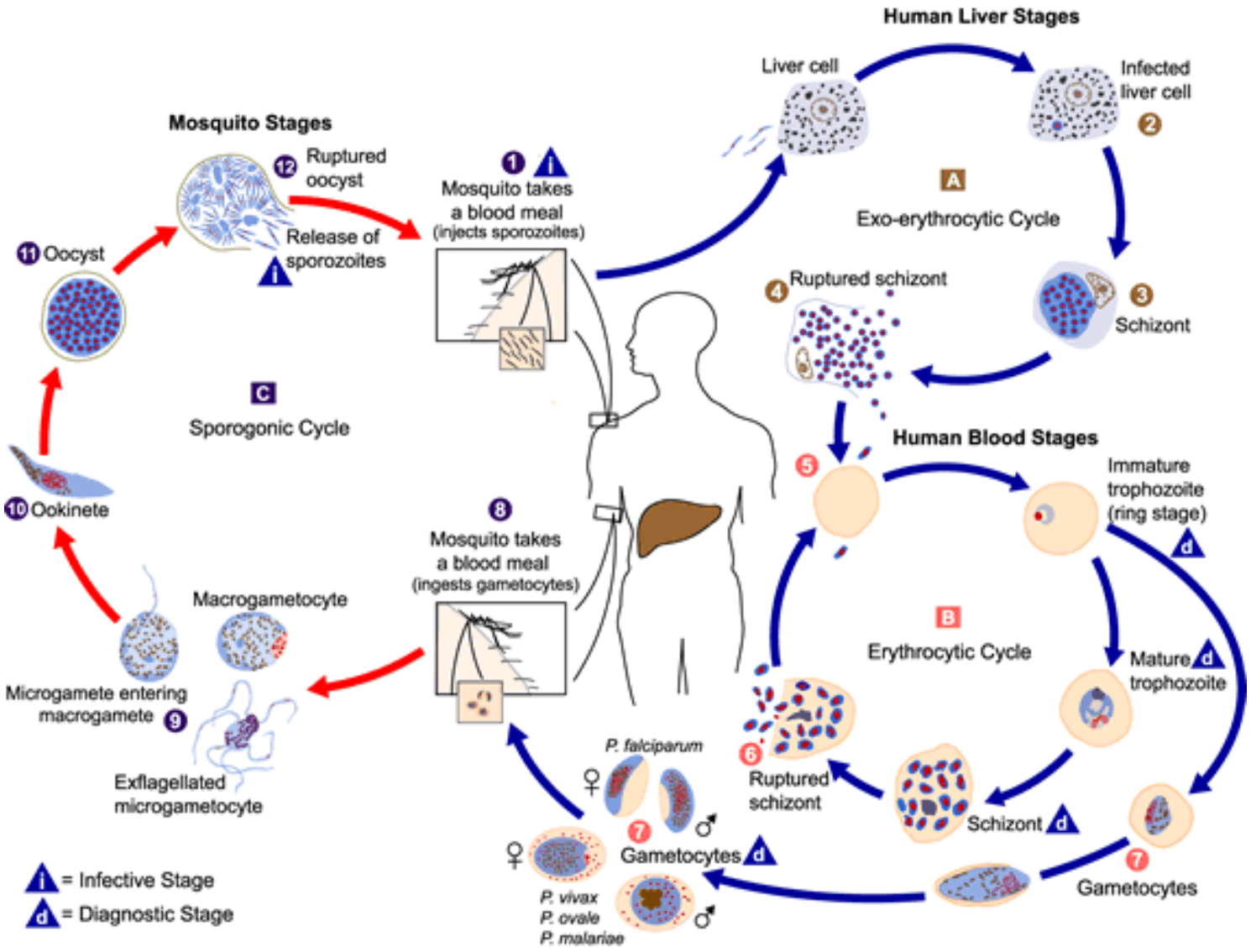

Image source: CDC DPDx

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Erythrocyte Invasion

D. Berry, http://www.wehi.edu.au/wehi-tv/

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Erythrocyte Invasion

Glushakova, S. et al (2005). *Current Biology* 15(18) 1645-50.

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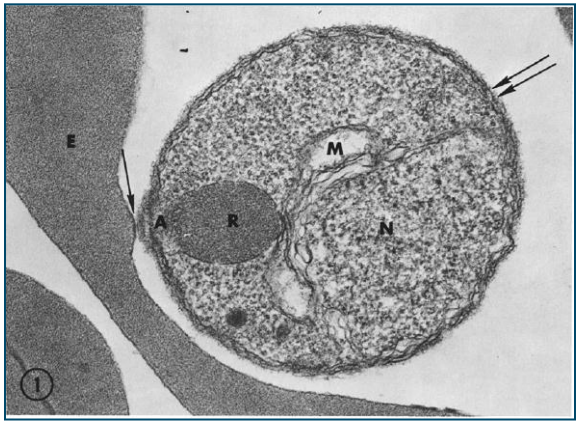
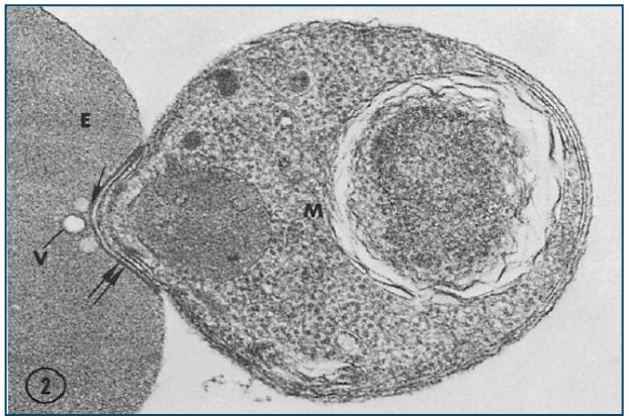
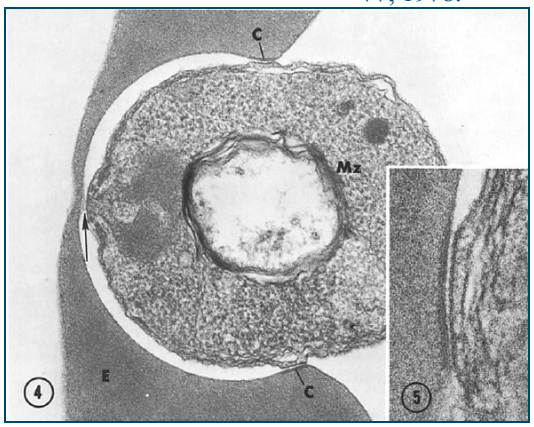
Erythrocyte invasion is a complex, multi-step process

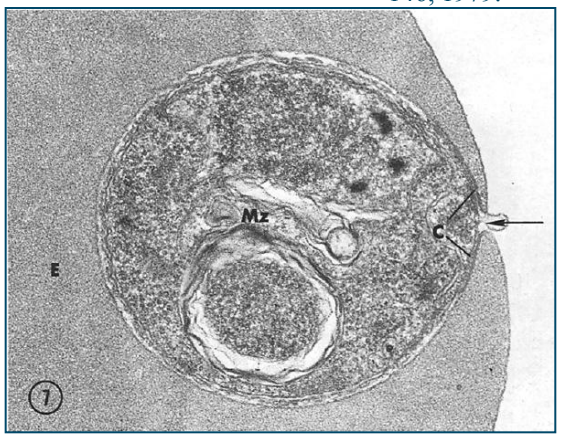


Aikawa *et al.*, J. Exp. Med. 77, 1978.



Miller *et al.*, J. Exp. Med. 146, 1979.





Multiple steps, multiple host-parasite receptor-ligand interactions

What is known about the erythrocyte receptors?

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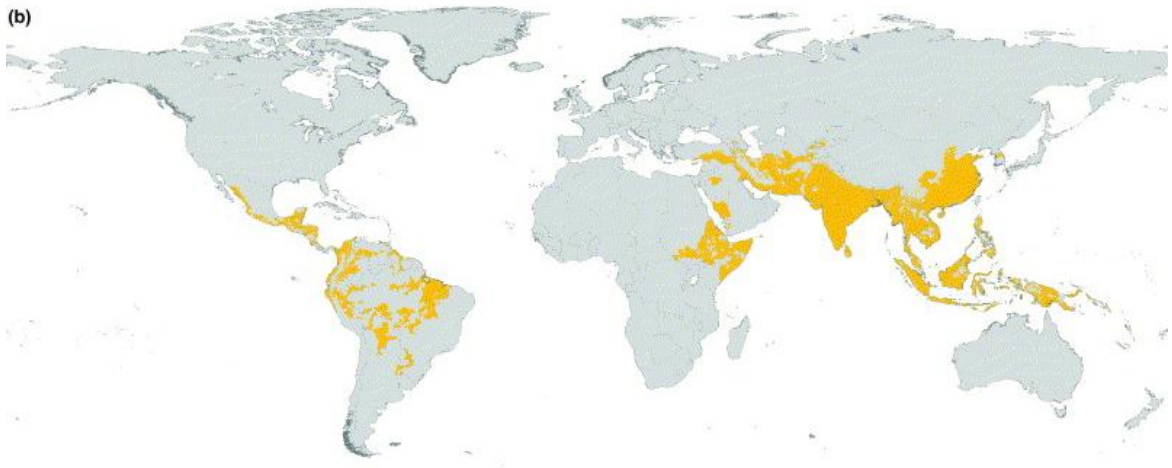
Plasmodium vivax and Duffy antigen

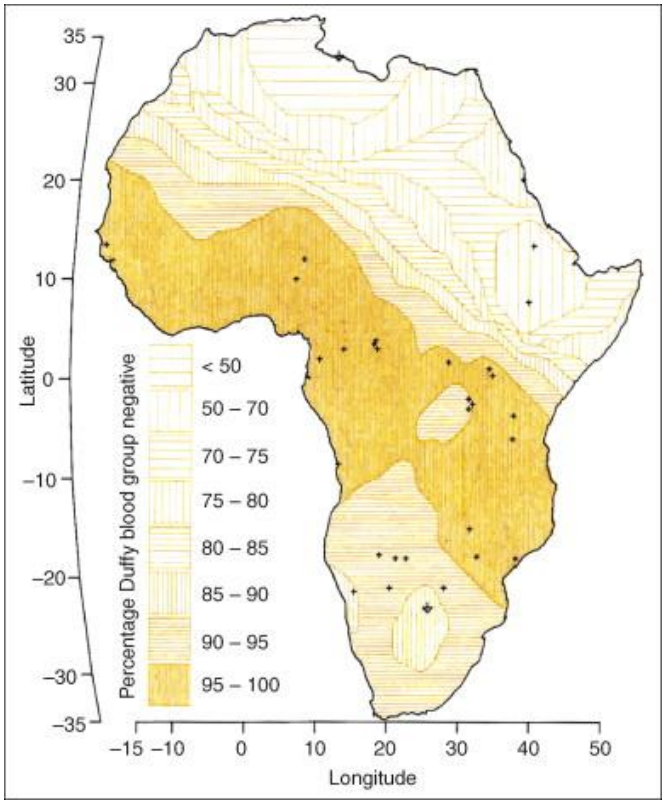
P. vivax merozoites depend on Duffy Antigen Receptor for Chemokines (DARC) to invade

DARC bound by *P. vivax* Duffy Binding Protein

Majority of West/Central Africans are Duffy negative Fy(a-b-). As a result, there is (almost) no *P. vivax* in west and central Africa



P. vivax distribution



Fy(a-b-) distribution

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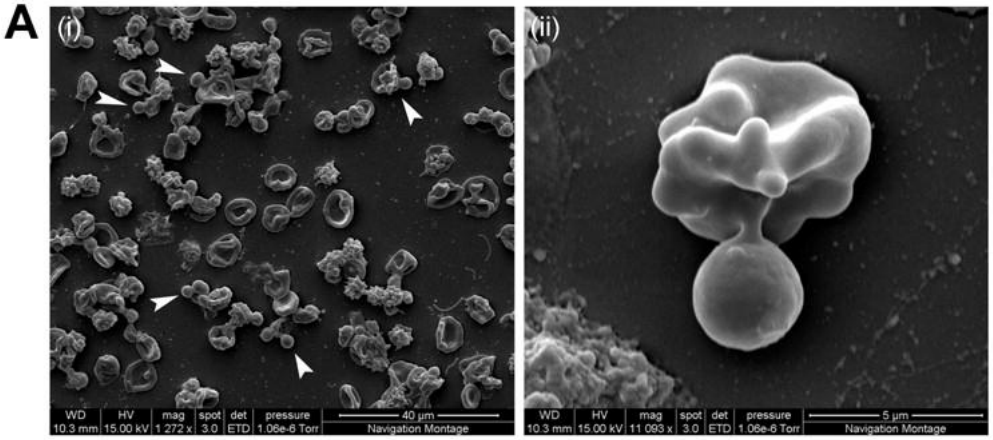
Nothing is quite that simple

P. vivax merozoites won't invade just any Duffy positive erythrocyte

P. vivax merozoites preferentially invade reticulocytes, which are recognised by *P. vivax* Reticulocyte Binding Proteins (PvRBP1 et al)

What are PvRBPs recognising on reticulocytes? Not known



Griffiths *et al.* Blood 2012

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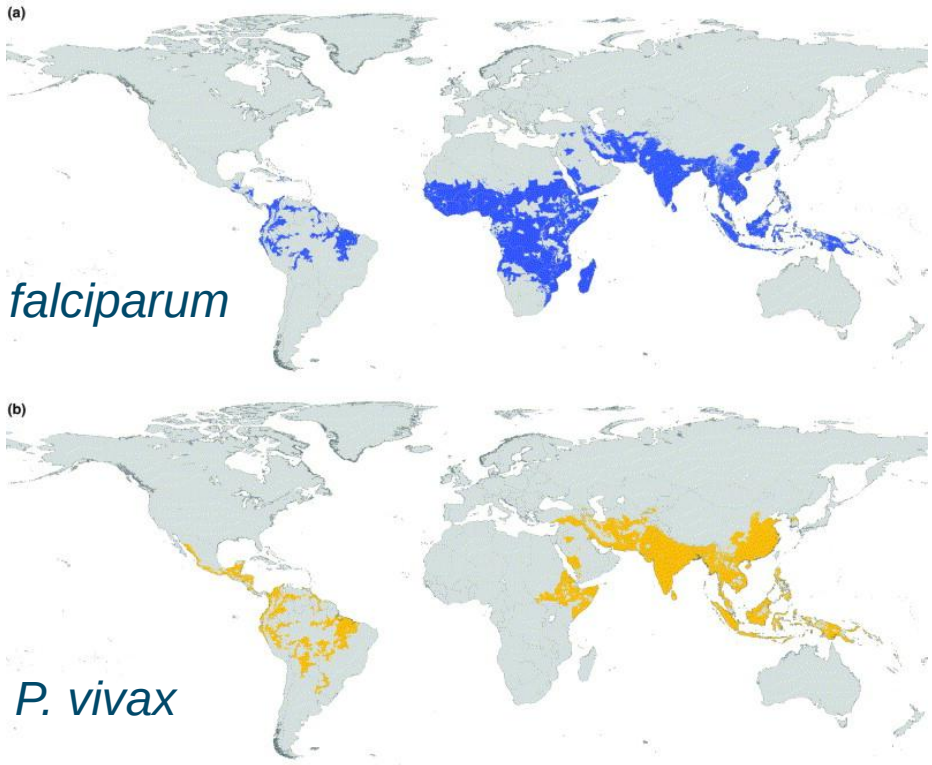
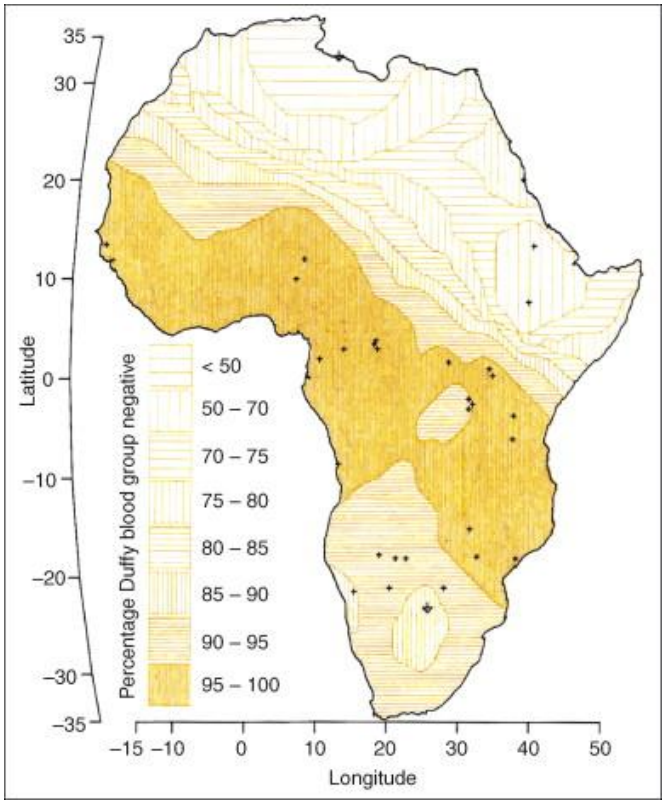
Plasmodium vivax and Duffy antigen



P. falciparum

P. vivax



Fy(a-b-) distribution

What about receptors for *P. falciparum* invasion?

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The *Plasmodium falciparum* receptor?

In 1994, PfEBA175 (the Duffy Binding Protein receptor homologue) was found to bind to Glycophorin A

Glycophorin A is the dominant erythrocyte surface sialoglycoprotein (c. 10^6 copies per cell), MN blood group antigen

Is this it?

Isolate	% Parasitemia ± SD in:		
	WT	FrC	En(a−)
3D7	2.3 ± 0.30[a]	1.5 ± 0.05	0.8 ± 0
ALR	1.4 ± 0.10	1.2 ± 0.08	**0.05 ± 0.01**[b]
JSL	1.8 ± 0	1.6 ± 0.16	0.9 ± 0.06
GVM	1.4 ± 0.04	1.0 ± 0.20	0.9 ± 0.12
FFS	0.8 ± 0.10	0.8 ± 0.10	**0.15 ± 0.04**[b]
04Q	1.3 ± 0.35	1.5 ± 0.23	0.9 ± 0.26
BHZ	1 ± 0.25	0.9 ± 0.06	0.7 ± 0.13
PSS1	1.3 ± 0.16	1.2 ± 0.05	1.2 ± 0.03

Lobo *et al.* Infect Immun 2004

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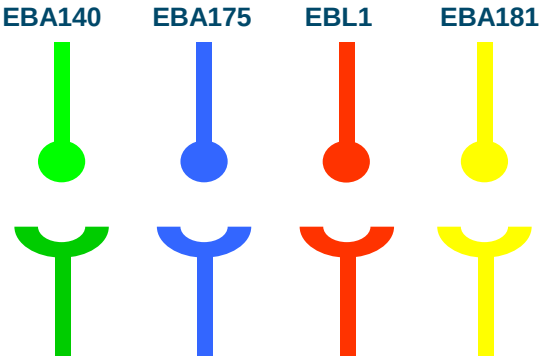
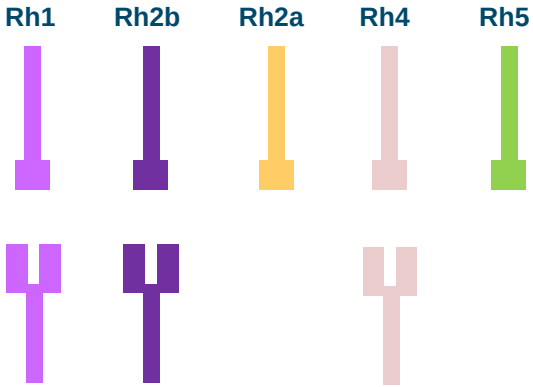
P. falciparum uses many alternative pathways

Erythrocyte-binding like proteins



Reticulocyte-binding like proteins

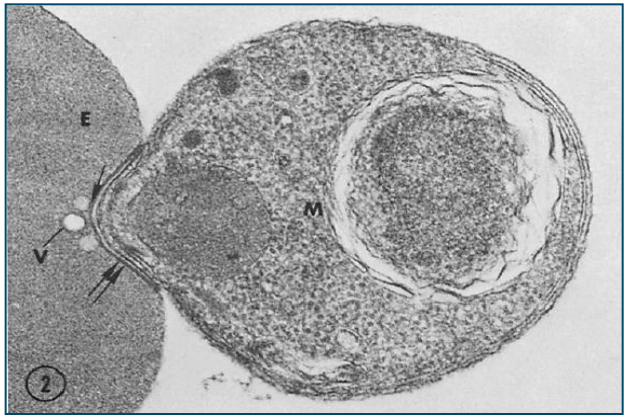


Adapted from Baum *et al*., *PLOS Pathogens* (2005)

P. falciparum has multiple homologues of both PvDBP and PvRBP

Genetic studies suggest that they perform overlapping, redundant functions, and different *P. falciparum* strains rely on different combinations to invade

What do they all recognise?



Miller *et al.*, J. Exp. Med. 146, 1979.

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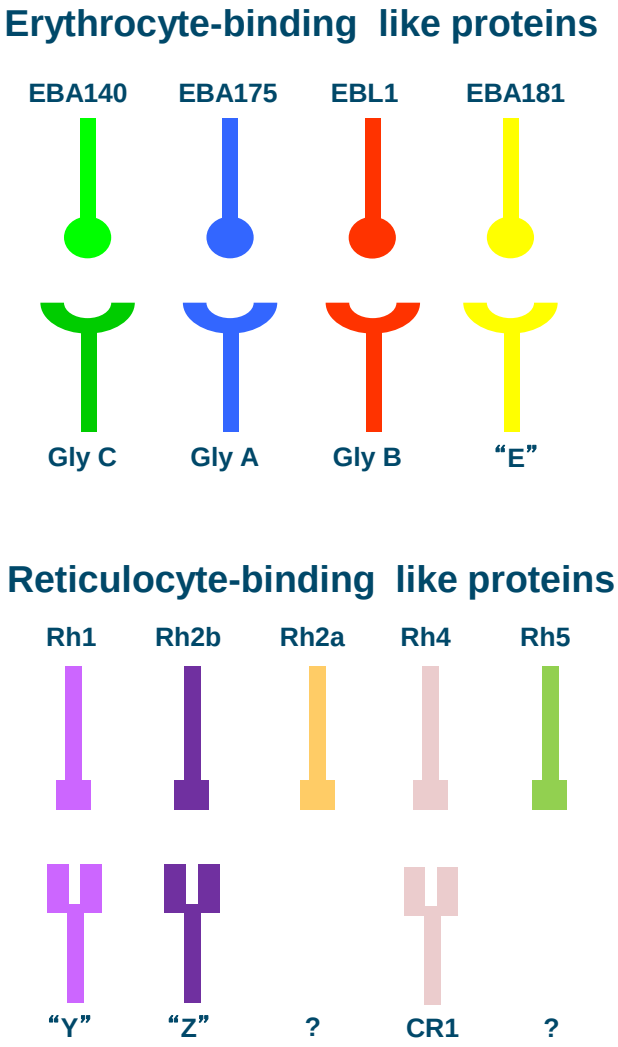
Finding red cell invasion receptors has been slow

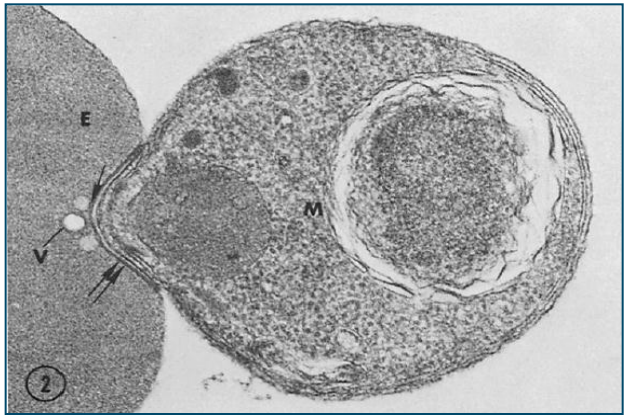


Adapted from Baum *et al*., *PLOS Pathogens* (2005)

Erythrocyte receptors remain somewhat of a black box: Only four identified in past 25 years

All known receptors have been identified on a candidate basis

Roadblock to screening approaches – cell surface interactions are often very low affinity and therefore hard to detect



Miller *et al.*, J. Exp. Med. 146, 1979.

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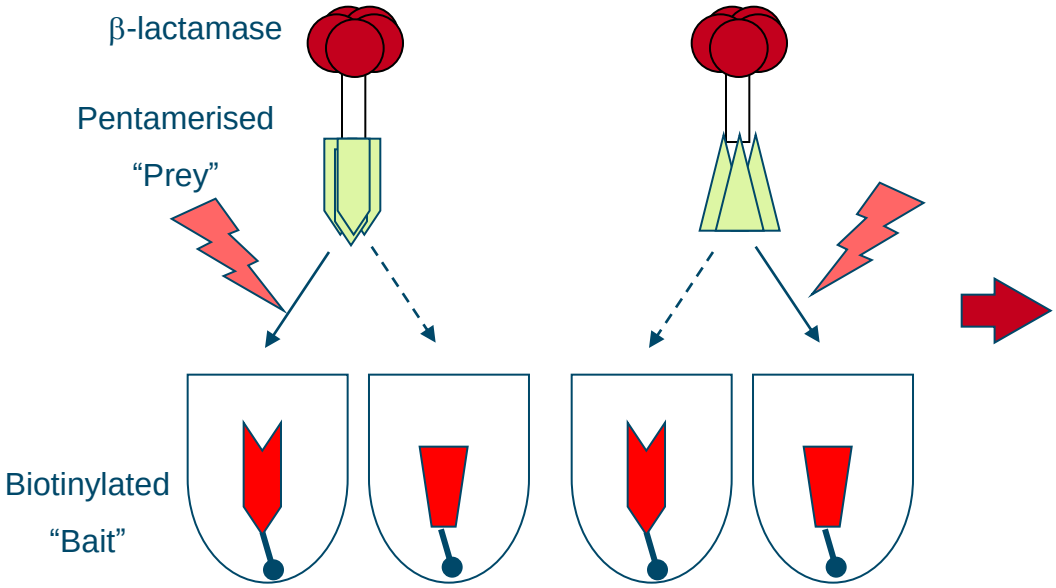
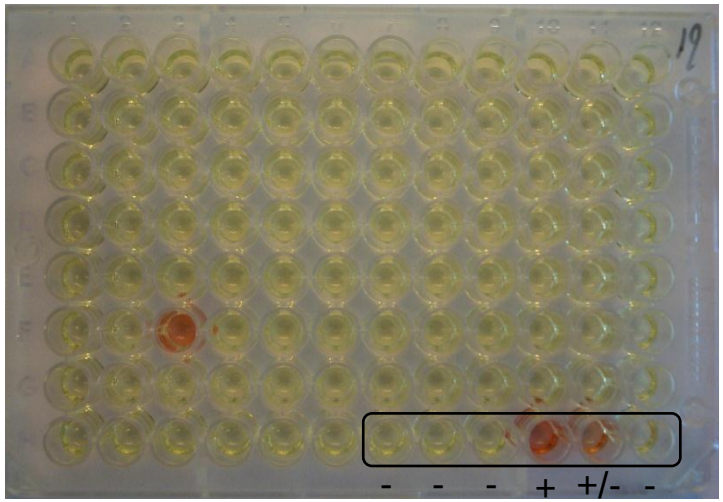
AVEXIS: A scalable system to detect extracellular interactions





Sanger-developed technology: Avidity Based Extracellular Interaction Screen (AVEXIS) – Gavin Wright

Specifically designed to detect low affinity interactions by using multimerised prey to increase avidity

Can we use this approach to identify erythrocyte invasion receptors?

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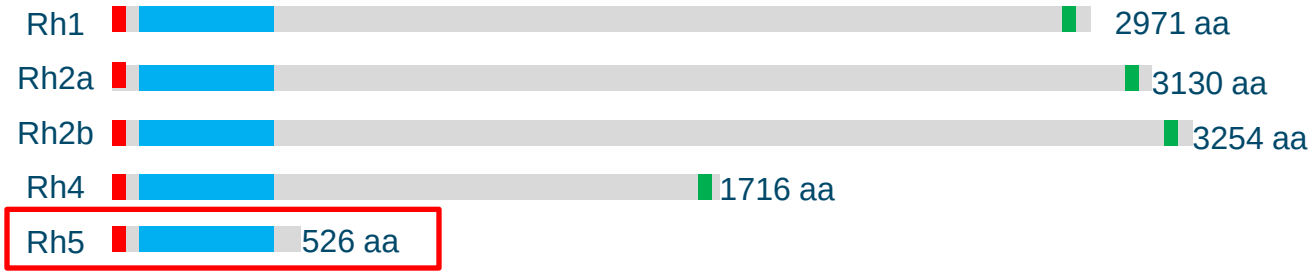
Target: *P. falciparum* Reticulocyte Binding Protein Homologue 5



All EBA proteins and RH1, RH2a, RH2b and RH4 have been successfully disrupted in at least one *P. falciparum* strain, which leads to changes in invasion pathways used

PfRH5 cannot be deleted in any strains tested to date suggesting it is essential

Antibodies against PfRH5 inhibit merozoite invasion *in vitro*.

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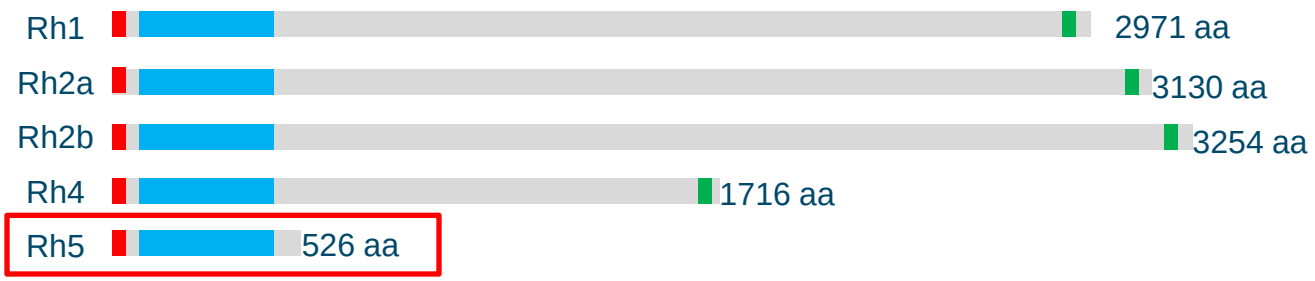
Target: *P. falciparum* Reticulocyte Binding Protein Homologue 5

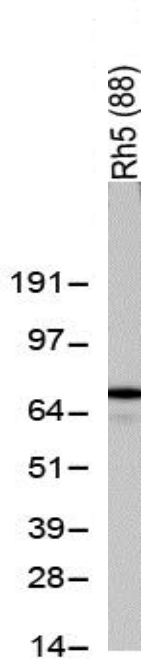


Transient expression of full-length PfRH5 as a secreted protein in HEK293E cells (key – PfRH5 is a eukaryotic secreted protein, expressed as a eukaryotic secreted protein)

Codon optimised, N-linked glycosylation sites removed, heterologous signal sequence

Small amounts needed for AVEXIS, but >1mg can be produced through transient transfections alone

What to screen against?



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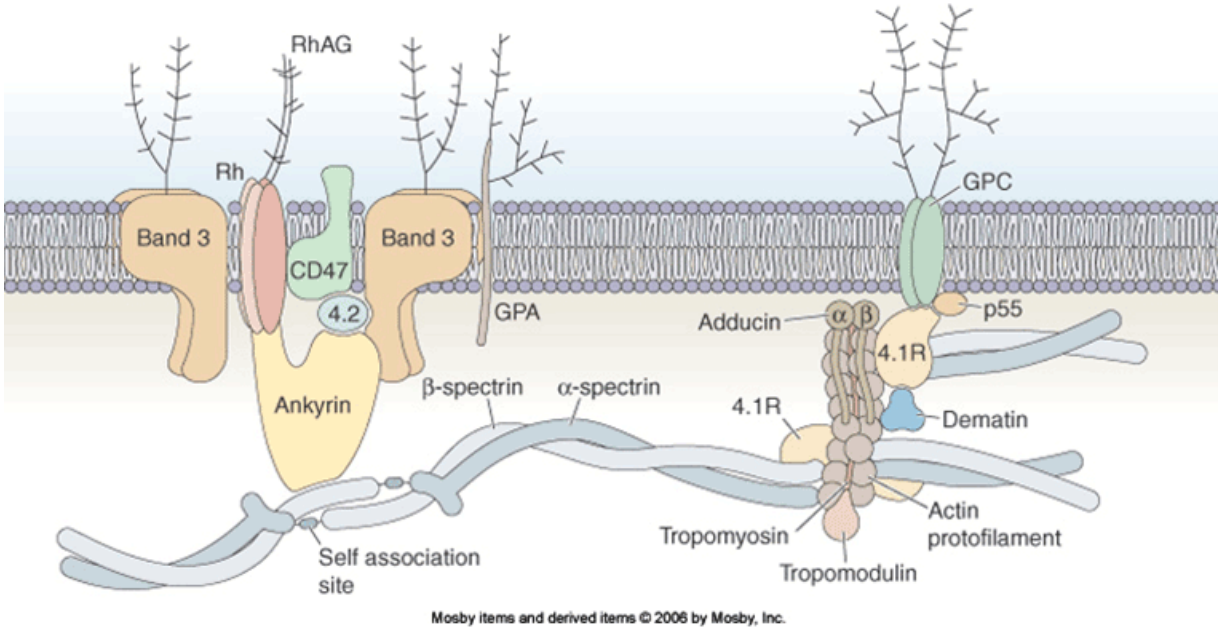
Selecting erythrocyte surface proteins to express

In the HEK293E cell expression system, proteins expressed as soluble ectodomains released into supernatant

We can therefore only express single pass transmembrane, GPI-linked or secreted erythrocyte proteins, not multi-pass proteins like Band 3

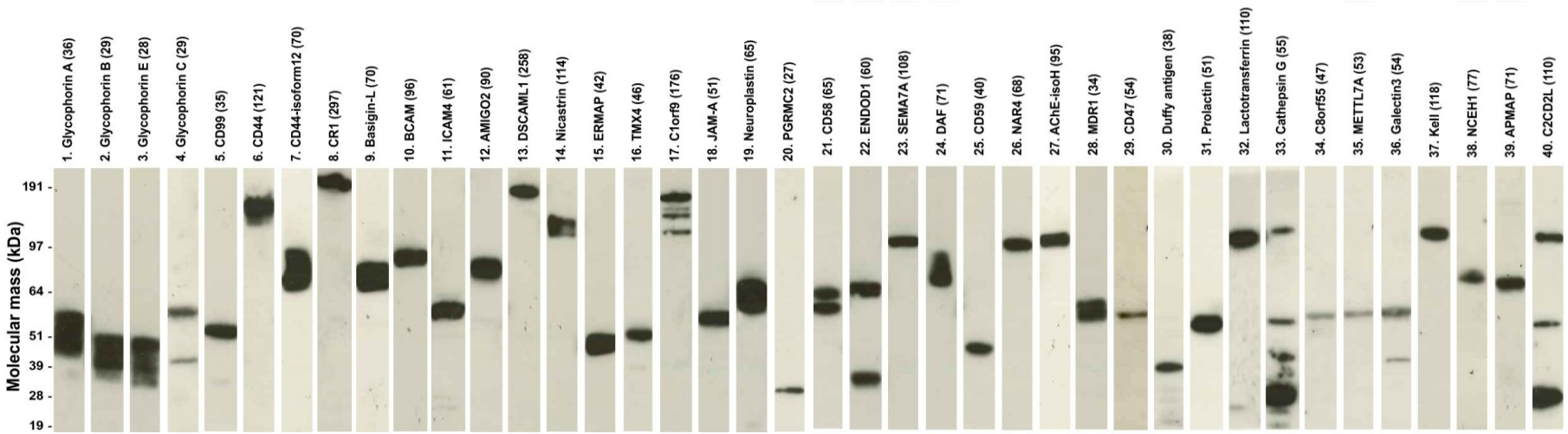
The library is therefore only a subset of possible receptors, other approaches needed for these other classes



Library of erythrocyte receptor ectodomains

40 erythrocyte surface proteins were selected from mass spectrometry data (Pasini et al., *Blood*, 2006)

Protein ectodomains (type-I, type-II, GPI-linked proteins) expressed as baits and preys by transient transfection in the same HEK293E cell expression system.



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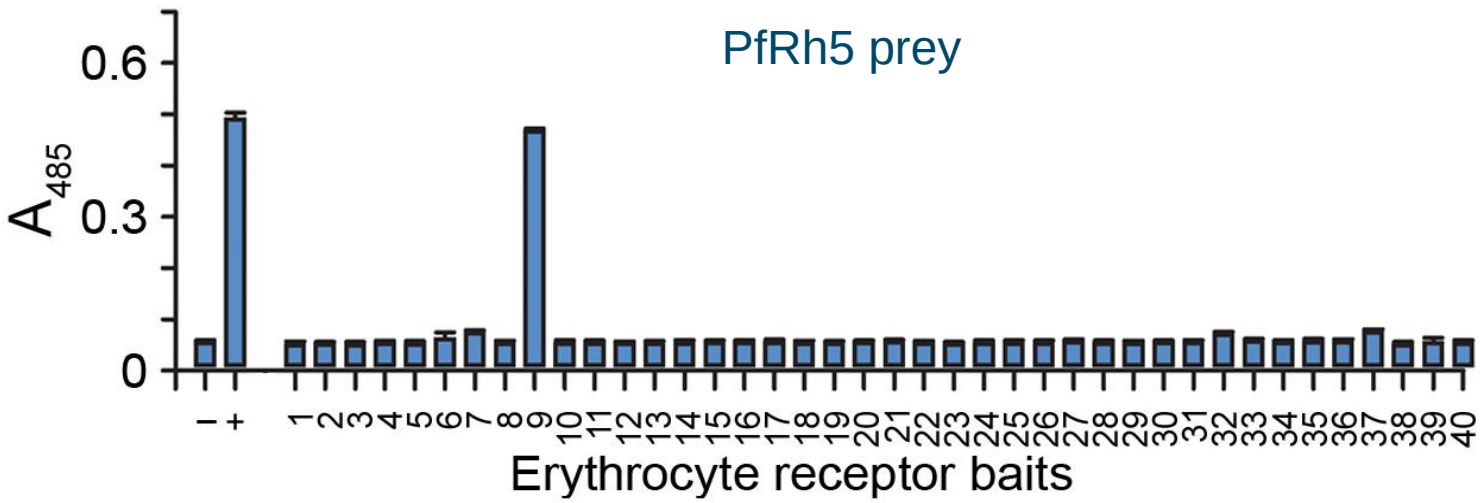
PfRH5 screening by AVEXIS



Single interacting partner, Protein 9 = Basigin

PfRH5-BSG interaction occurs in both orientations

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BSG: Basigin/CD147

Member of the immunoglobulin superfamily (IgSF)

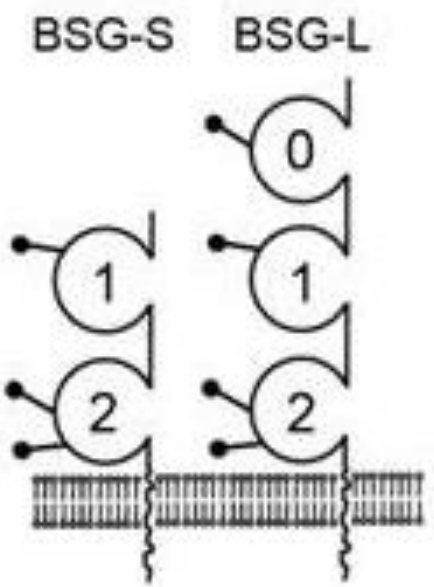
Also known as CD147, EMMPRIN and M6

Ok(a) blood group determinant

Functions include: matrix metalloprotease induction, embryo implantation, spermatogenesis and retinal development

Two splice isoforms with 2 IgSF (BSG-S) or 3 IgSF domains (BSG-L)

Not previously identified as a receptor for *P. falciparum* invasion



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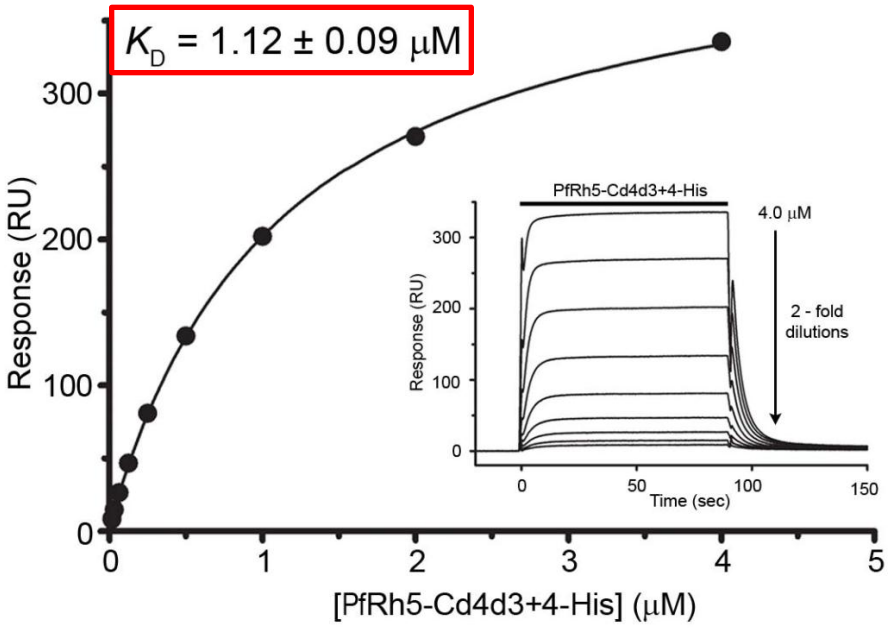
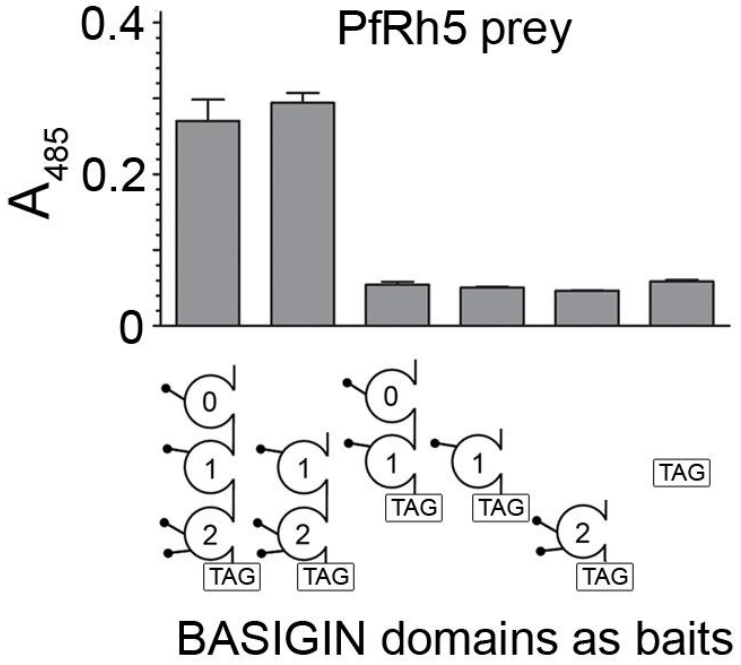
Validation of PfRH5-BSG interaction





Surface Plasmon Resonance – BSG on chip, PfRH5 flowed over

Interaction requires both domains 1 and 2

Does BSG function during invasion?

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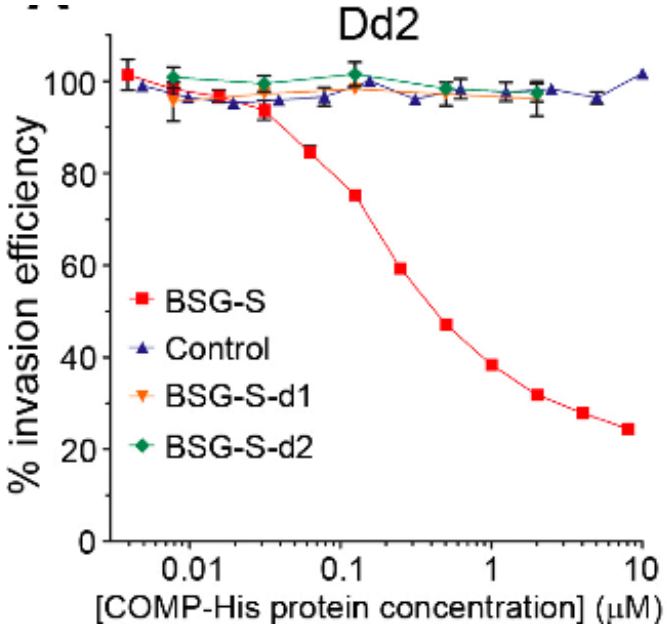
BSG plays a critical role during erythrocyte invasion



Invasion can be inhibited using recombinant BSG-S in a dose-dependent manner

Fragments of BSG that do not bind PfRH5 do not inhibit invasion

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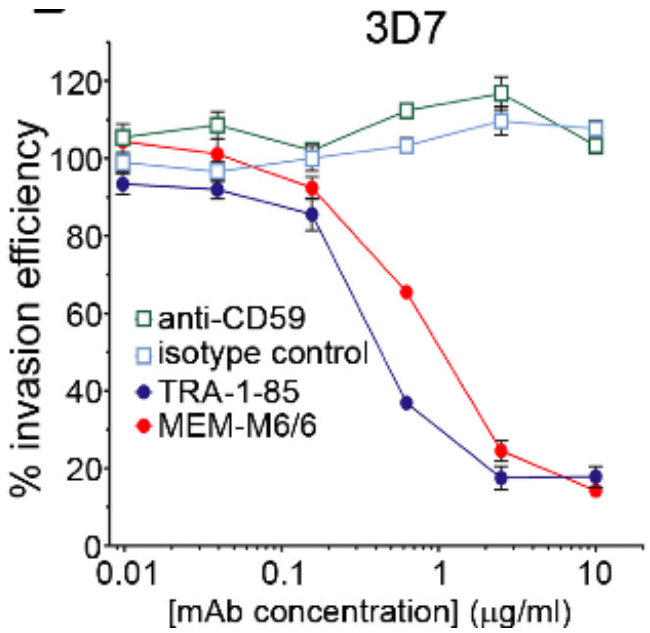
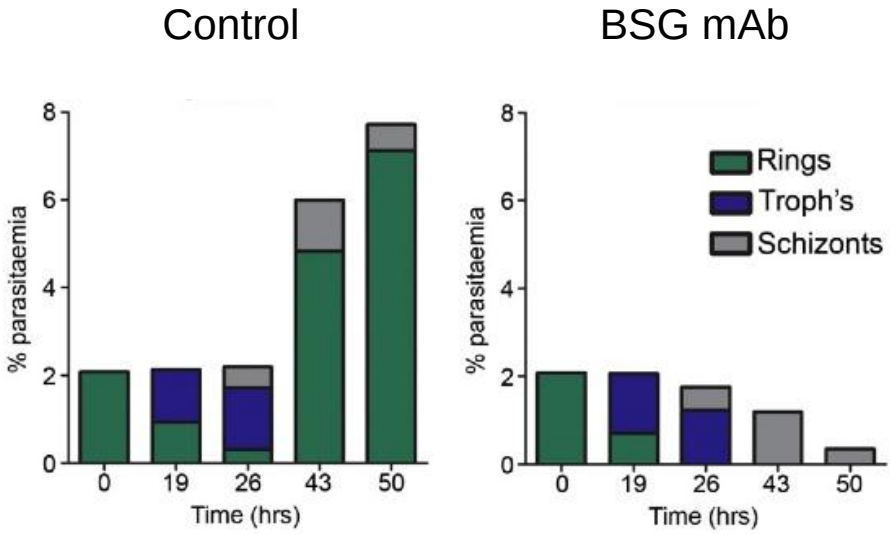
BSG plays a critical role during erythrocyte invasion





Low concentrations of several anti-BSG monoclonals inhibit invasion; mAbs against other erythrocyte surface proteins do not

Anti-BSG monoclonals do not inhibit intra-erythrocytic development, or cause agglutination – inhibition is specific to invasion

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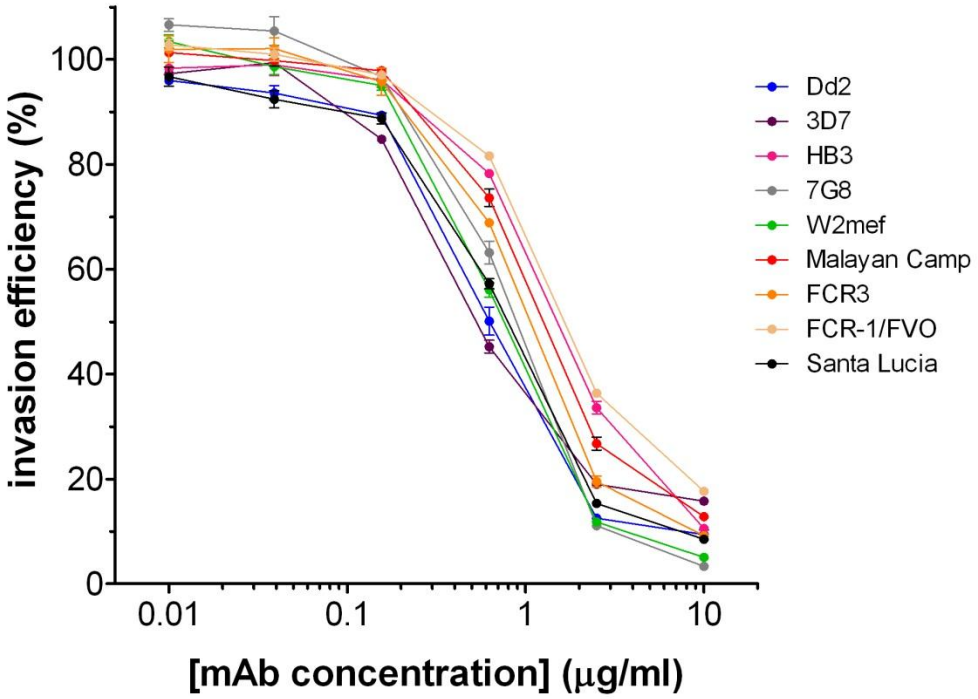
BSG may be a universal receptor



All strains tested to date are inhibited in the same manner – Rh5:BSG interaction may be universal: different to other redundant interactions

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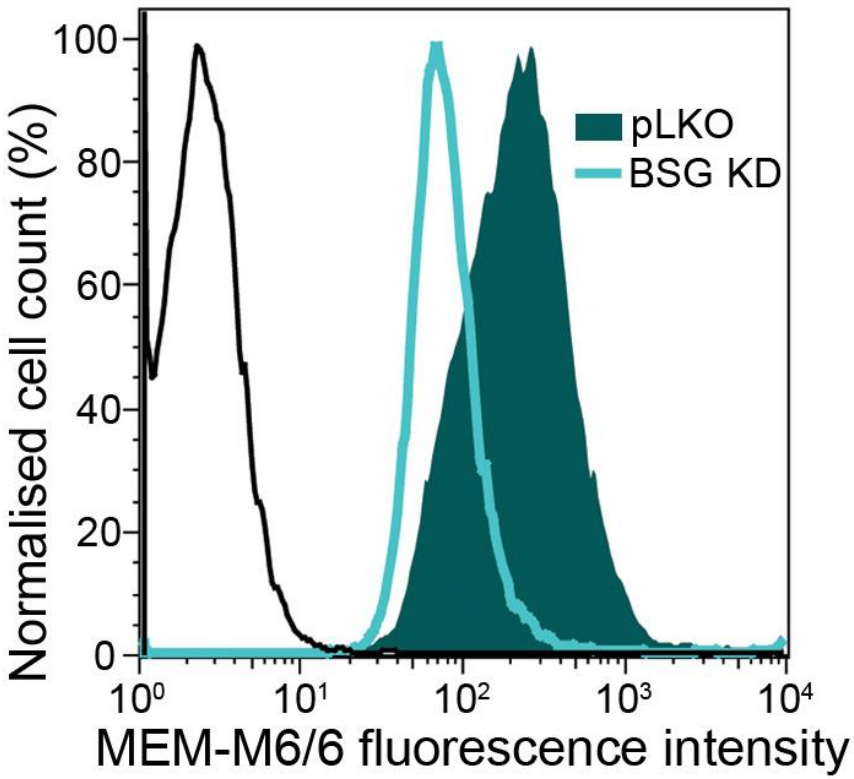
Knockdown of BSG using hSCs

Invasion inhibited using BSG recombinant protein and mAbs. To provide independent validation, BSG expression altered genetically using shRNA in hematopoetic stem cells

Differentiated into mature red cells; BSG levels reduced by 50-60%

Expression of other surface proteins tested (GPA, GPC) unchanged



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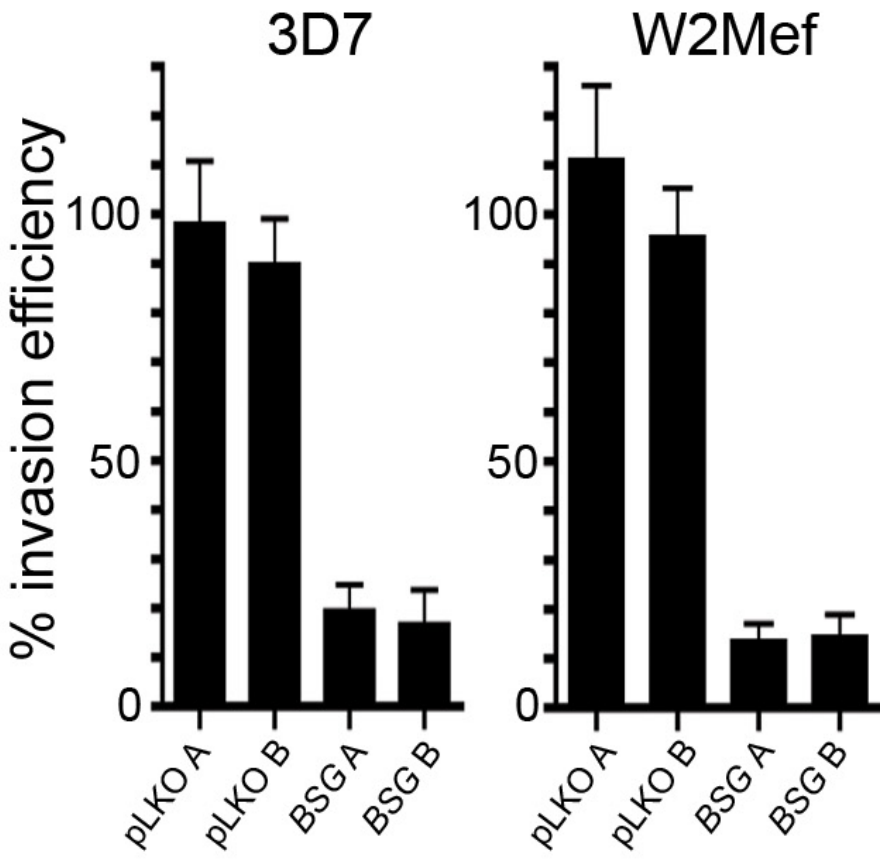
Knockdown of BSG inhibits invasion



Reduction of BSG expression by 50-60% reduces invasion by more than 80% in two *P. falciparum* strains

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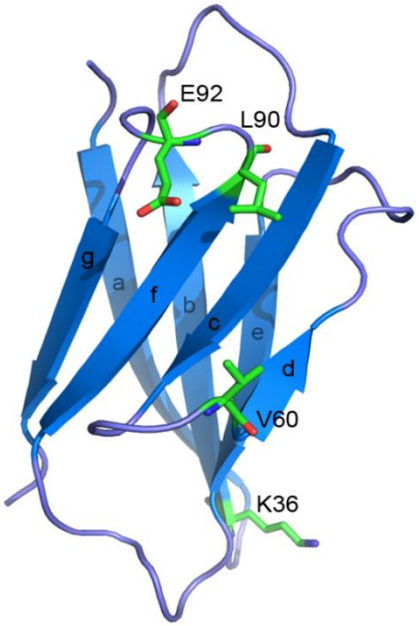
Naturally occurring variants of BSG



SNP number	nucleotide variant	Amino acid polymorphism	Location of polymorphism
rs11551906	A>G	T16A	Signal peptide
rs14704	G>T	K36N	Domain 1
rs2229662	G>T	V60L	Domain 1
rs55911144	T>C	L90P	Domain 1
rs104894669	G>A	E92K	Domain 1
rs1803203	G>T	G153V	Domain 2
rs55805128	C>T	R203C	Membrane proximal

Five nsSNPs in BSG identified in dbSNP,

All on surface of Ig domain; could impact Rh5 binding

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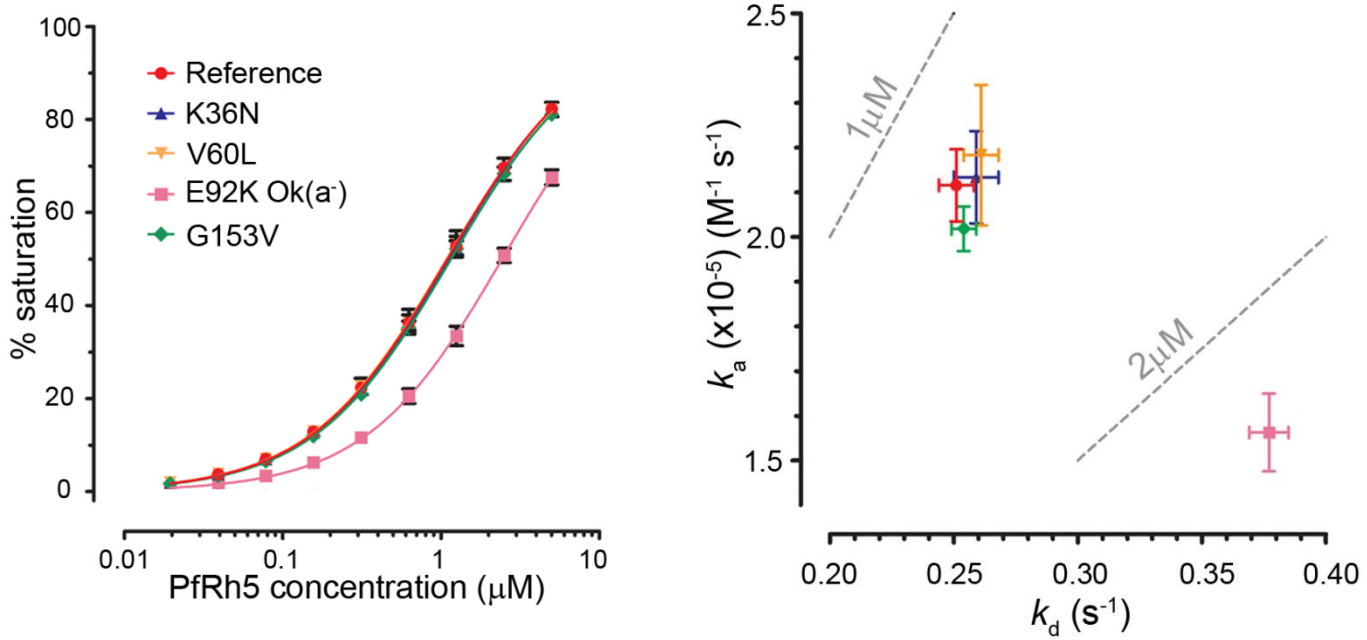
BSG E92K has reduced affinity for PfRH5

Biacore analysis of naturally-occurring polymorphism of BASIGIN



All BSG variants expressed and binding to Rh5 measured

E92K has 2-fold reduced affinity for PfRh5

E92K responsible for Ok(a) blood group

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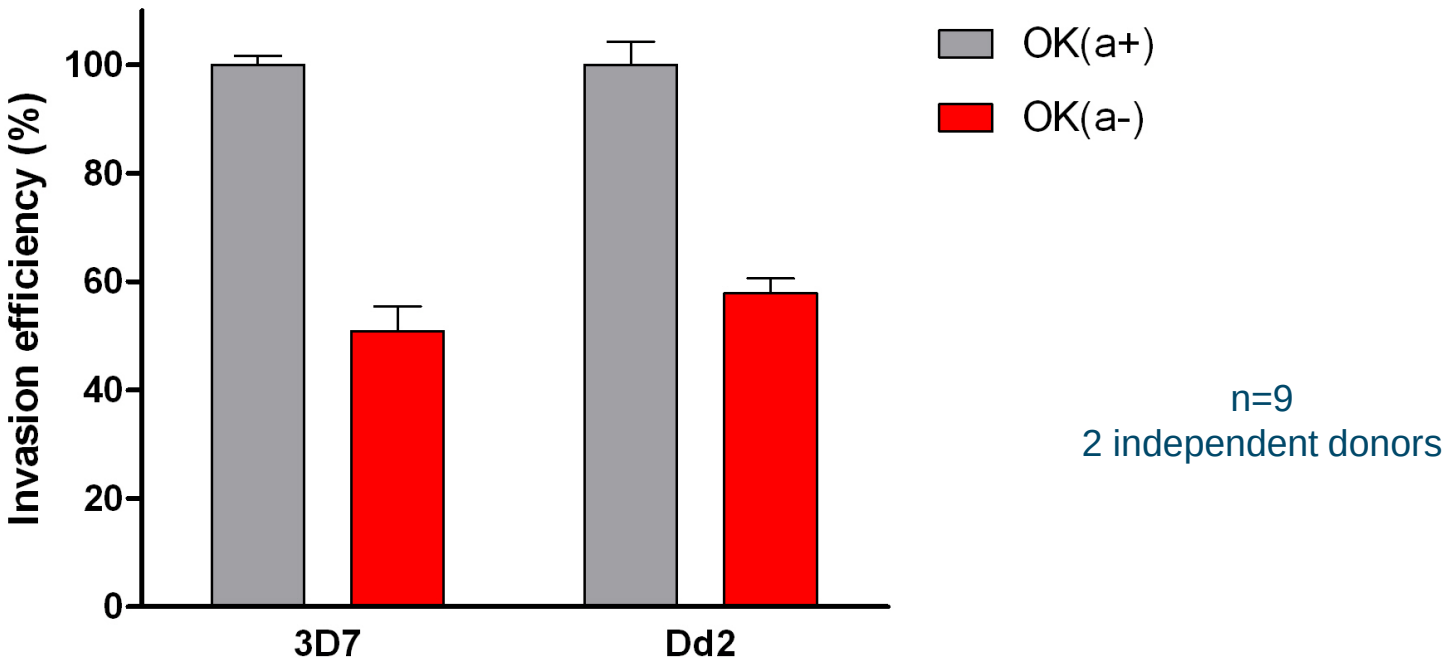
Invasion into Ok(a) variant erythrocytes



n=9
2 independent donors

Invasion into Ok(a) cells reduced approximately 2-fold

Rarity of Ok(a) suggests no major role in resistance to malaria, but search for BSG variants in Africa clearly a priority

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Conclusions

A systematic protein interaction screen (AVEXIS) identified BASIGIN as the receptor for PfRh5.

Anti-BSG antibodies potently block erythrocyte invasion by all *P. falciparum* strains tested and BSG knockdown erythrocytes have reduced invasion efficiency

The BSG-PfRh5 interaction may be essential and universal.

This interaction provides a clearly defined target for the development of an invasion blocking vaccine

However: All the invasion blocking data presented targets the receptor, not the ligand. Can we block invasion with anti-Rh5 antibodies?

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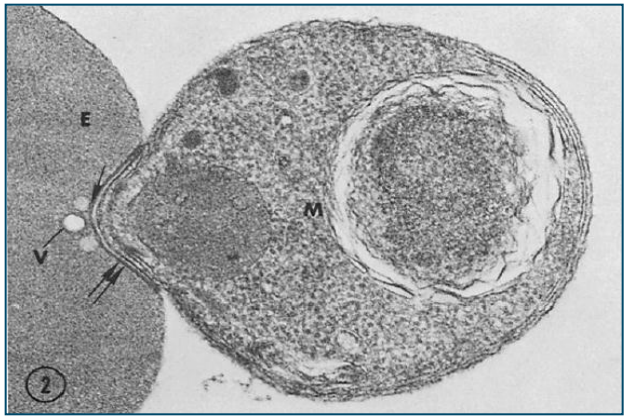
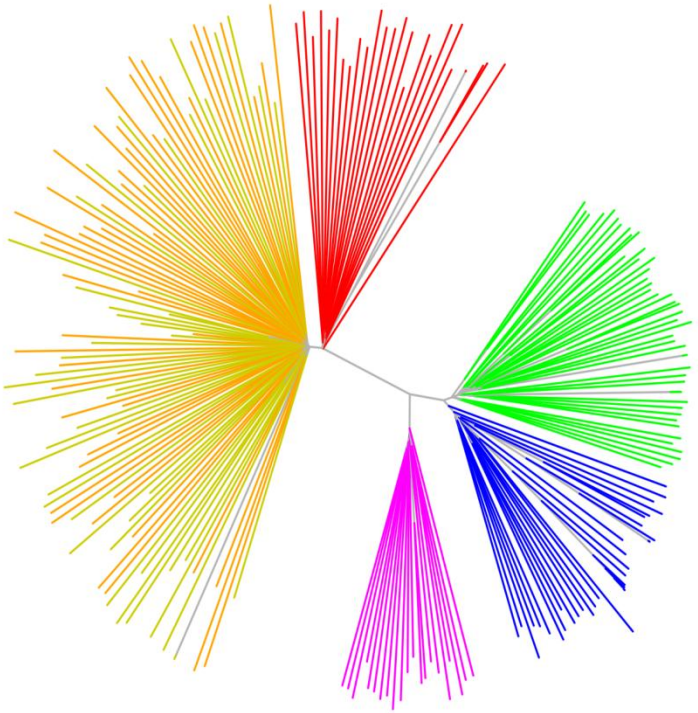
Invasion in the field is more complex



Miller *et al.*, J. Exp. Med. 146, 1979.



Manske *et al.*, Nature, 2012.

In natural populations, receptor-ligand interactions are influenced by genetic variation in both host and parasite

To be effective, any anti-Rh5 vaccine will have to be strain-transcending – able to block all strains. This is where previous blood stage vaccines have failed

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- Eight years for the first *P. falciparum* genome
- More than 1,500 in the last 2 years
- Similar scaling occurring in all areas of genomics

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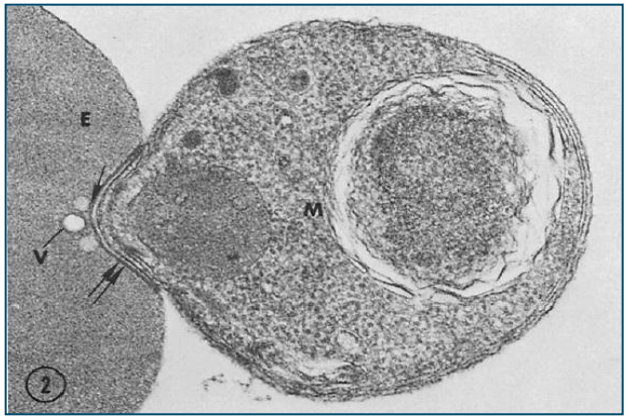
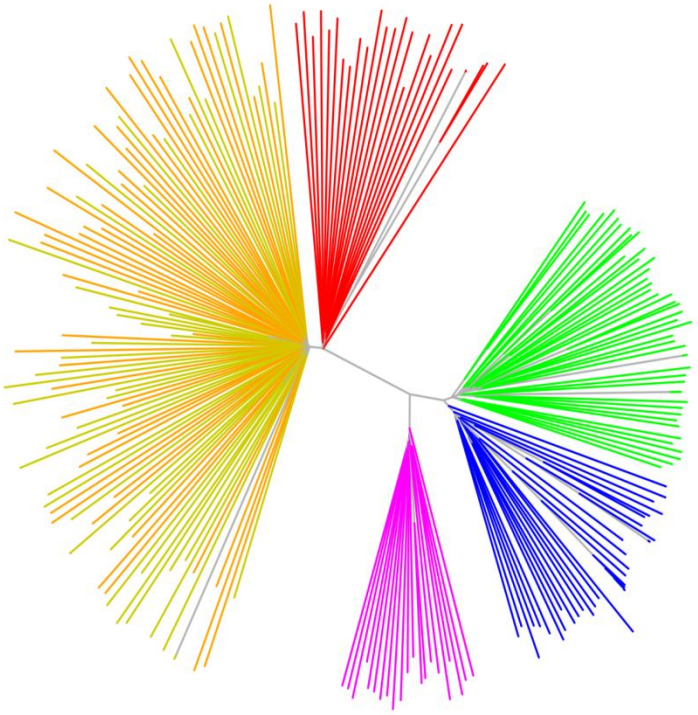
Invasion in the field is more complex



Miller *et al.*, J. Exp. Med. 146, 1979.



Manske *et al.*, Nature, 2012.

In natural populations, receptor-ligand interactions are influenced by genetic variation in both host and parasite

To be effective, any anti-Rh5 vaccine will have to be strain-transcending – able to block all strains. This is where previous blood stage vaccines have failed

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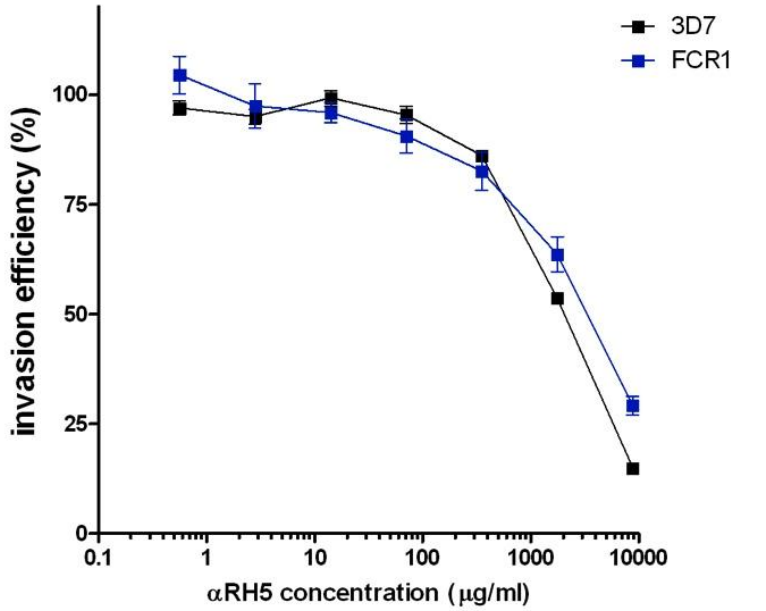
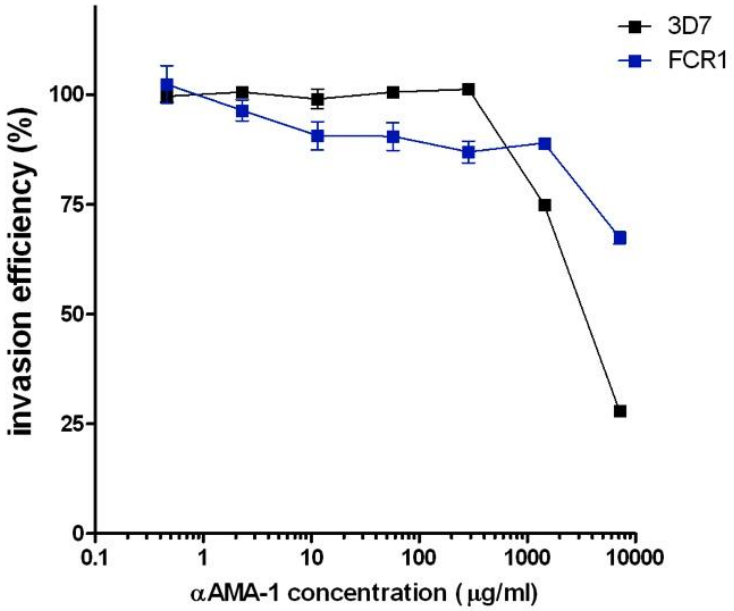
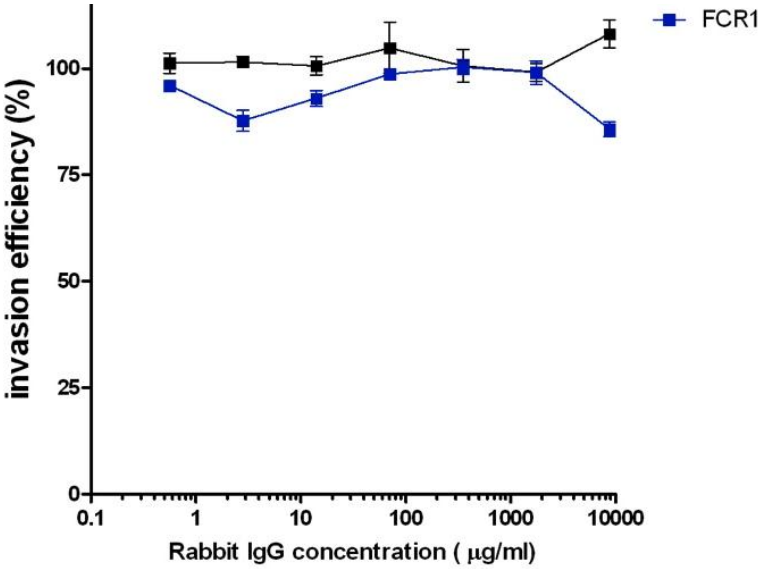
No initial evidence for strain specificity







Data from AMA1 vaccine trials teaches us that we need to do this more comprehensively

Aggregate population genetic data is now available to tackle exactly this kind of problem…

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PfRh5 non-synonymous SNPs

Position	Ref	Non-ref	Freq Africa	Freq Asia	Freq PNG
88	N	D	0.01	0.00	0.00
147*	Y	H	0.09	0.28	0.05
148*	H	D	0.10	0.30	0.05
197*	S	Y	0.00	0.54	0.38
203*	C	Y	0.79	0.62	0.9
233	A	E	0.00	0.00	0.05
365	H	N	0.01	0.00	0.00
371	V	I	0.05	0.00	0.00
407	I	V	0.03	0.00	0.00
410*	I	M	0.00	0.35	0.10
477	Q	H	0.01	0.00	0.00
493	I	V	0.00	0.00	0.00

Data source: MalariaGen whole genome sequencing of 290 samples

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PfRh5 non-synonymous SNPs

Position	Ref	Non-ref	Freq Africa	Freq Asia	Freq PNG	Lab Strain
88	N	D	0.01	0.00	0.00	N/A
147*	Y	H	0.09	0.28	0.05	N/A
148*	H	D	0.10	0.30	0.05	N/A
197*	S	Y	0.00	0.54	0.38	FCR1
203*	C	Y	0.79	0.62	0.9	7G8, GB4
233	A	E	0.00	0.00	0.05	N/A
365	H	N	0.01	0.00	0.00	N/A
371	V	I	0.05	0.00	0.00	N/A
407	I	V	0.03	0.00	0.00	GB4
410*	I	M	0.00	0.35	0.10	Dd2
477	Q	H	0.01	0.00	0.00	N/A
493	I	V	0.00	0.00	0.00	N/A

Data source: MalariaGen whole genome sequencing of 290 samples

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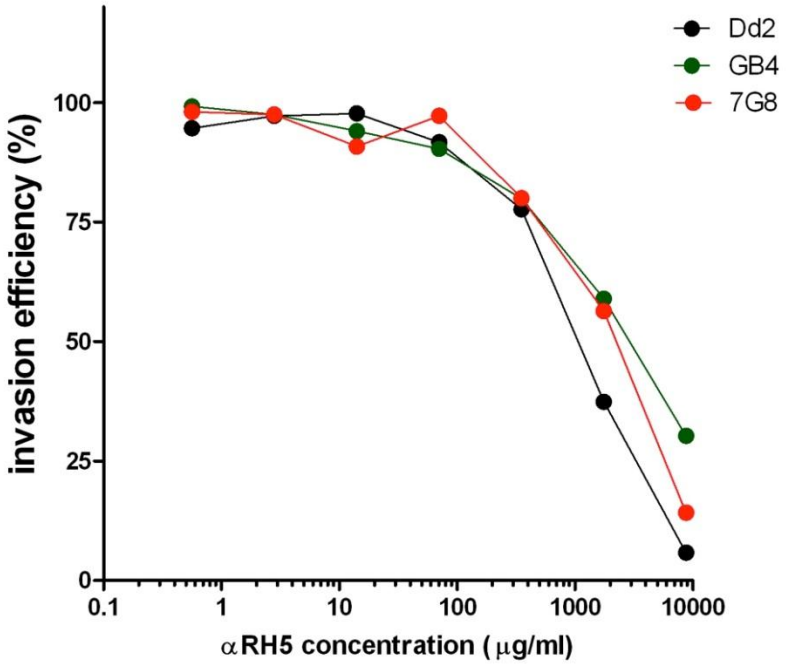
No evidence for strain-specific anti-Rh5 responses



Antibodies raised against 3D7 PfRh5 inhibit invasion of lab strains that contain 3 out of 5 of the common PfRh5 SNPs

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PfRh5 non-synonymous SNPs

Position	Ref	Non-ref	Freq Africa	Freq Asia	Freq PNG	Lab Strain
88	N	D	0.01	0.00	0.00	N/A
147*	Y	H	0.09	0.28	0.05	N/A
148*	H	D	0.10	0.30	0.05	N/A
197*	S	Y	0.00	0.54	0.38	FCR1
203*	C	Y	0.79	0.62	0.9	7G8, GB4
233	A	E	0.00	0.00	0.05	N/A
365	H	N	0.01	0.00	0.00	N/A
371	V	I	0.05	0.00	0.00	N/A
407	I	V	0.03	0.00	0.00	GB4
410*	I	M	0.00	0.35	0.10	Dd2
477	Q	H	0.01	0.00	0.00	N/A
493	I	V	0.00	0.00	0.00	N/A

Data source: MalariaGen whole genome sequencing of 290 samples

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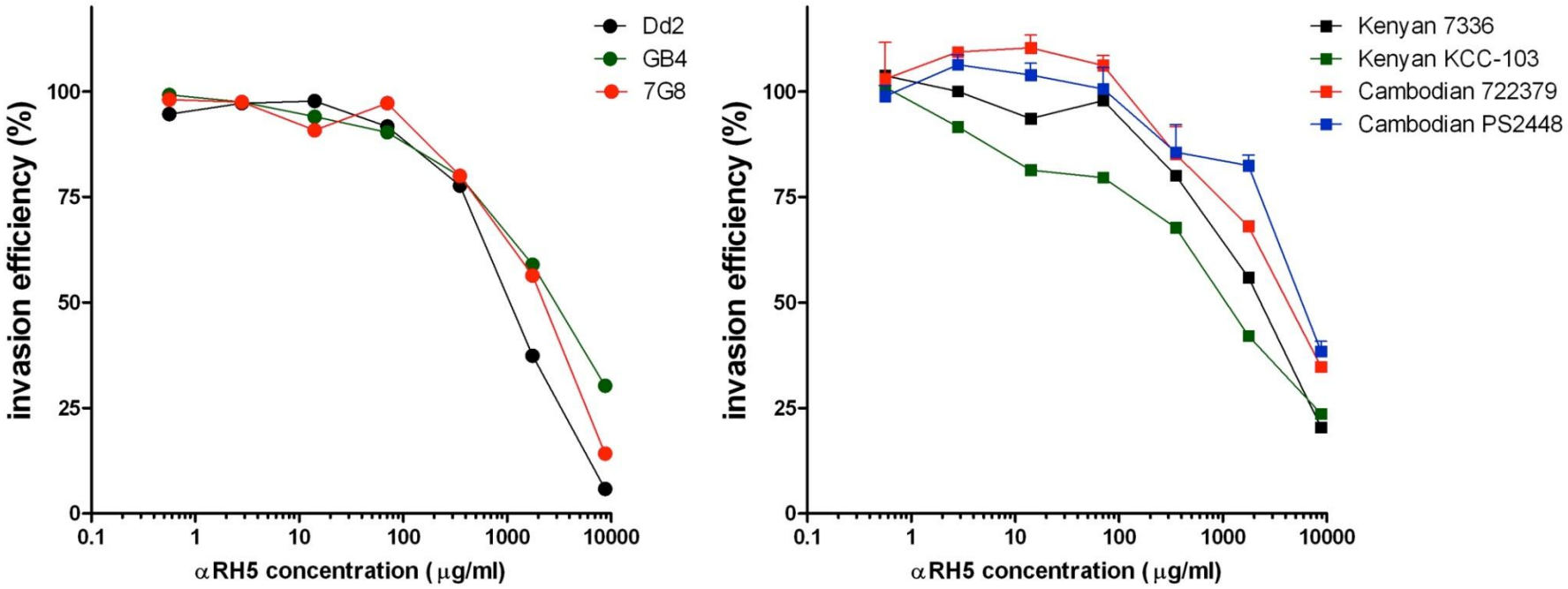
No evidence for strain-specific anti-Rh5 responses



Antibodies raised against 3D7 PfRh5 inhibit invasion of strains that contain all common PfRh5 SNPs (max 1.5-fold shift in IC50)

PfRh5 is a high priority target – currently trying to move to Phase I/IIa trials

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A universal erythrocyte receptor for *Plasmodium falciparum* invasion

Julian Rayner
Sanger Malaria Programme