Center for Infectious Disease Research

PEOPLE. SCIENCE. HOPE.

JITMM
Bangkok, Thailand
December 2017

"New Tools for the generation of attenuated *Plasmodium falciparum* for vaccine development"

Center for Infectious Disease Research
Seattle WA, USA

Ashley Vaughan

Acknowledgements

Stefan Kappe, Kappe Lab Members Past and Present

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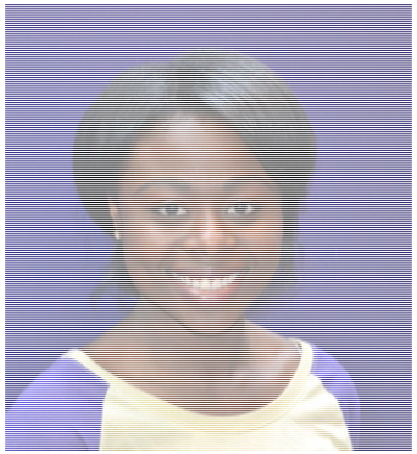
Nelly Camargo

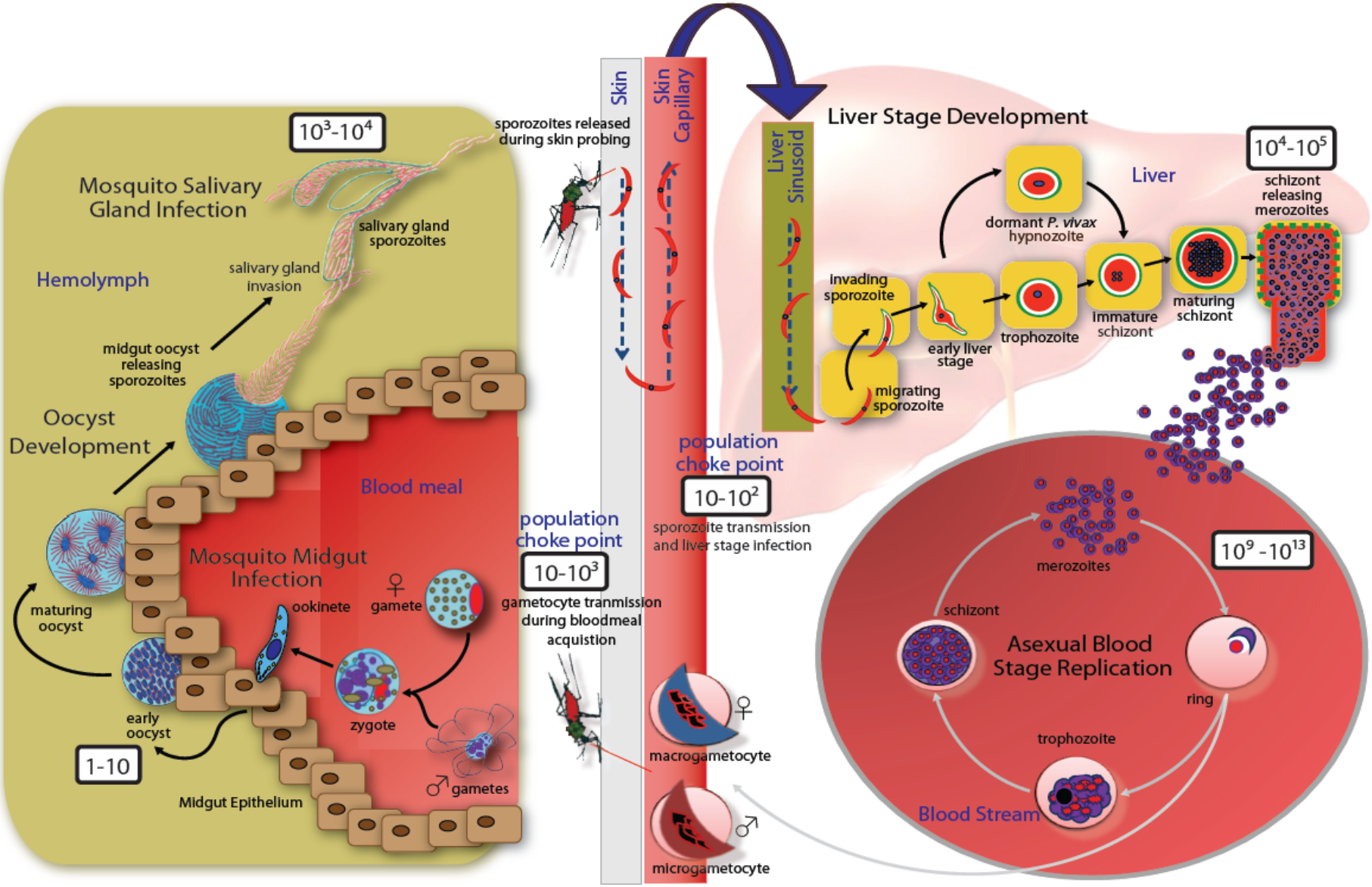


Dorender Dankwa

Deba Goswami

Navin Locham

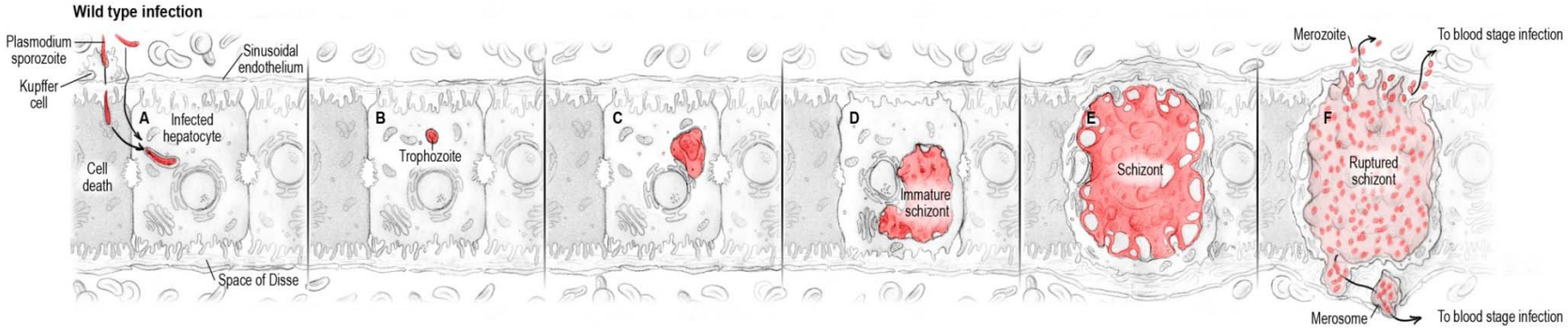
Whole parasite vaccines for malaria



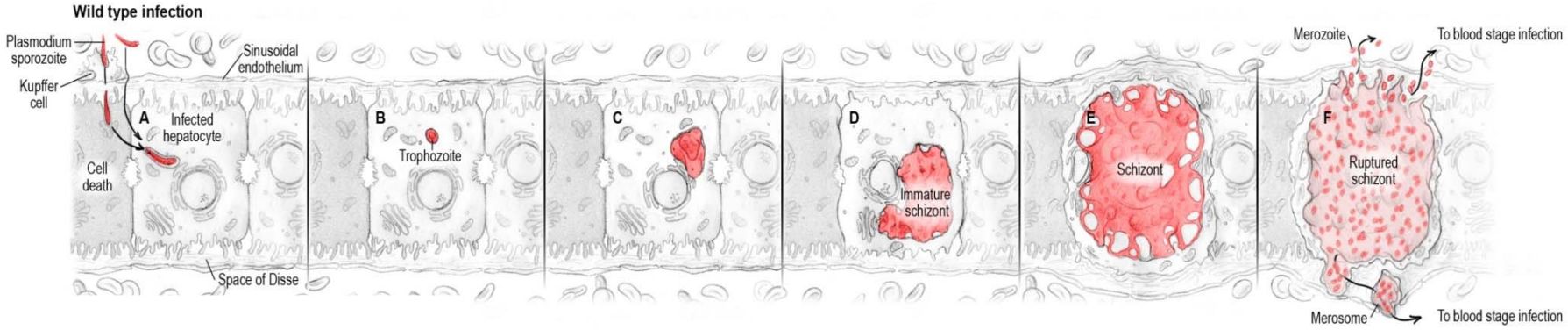
What are genetically attenuated parasite (GAP) vaccines

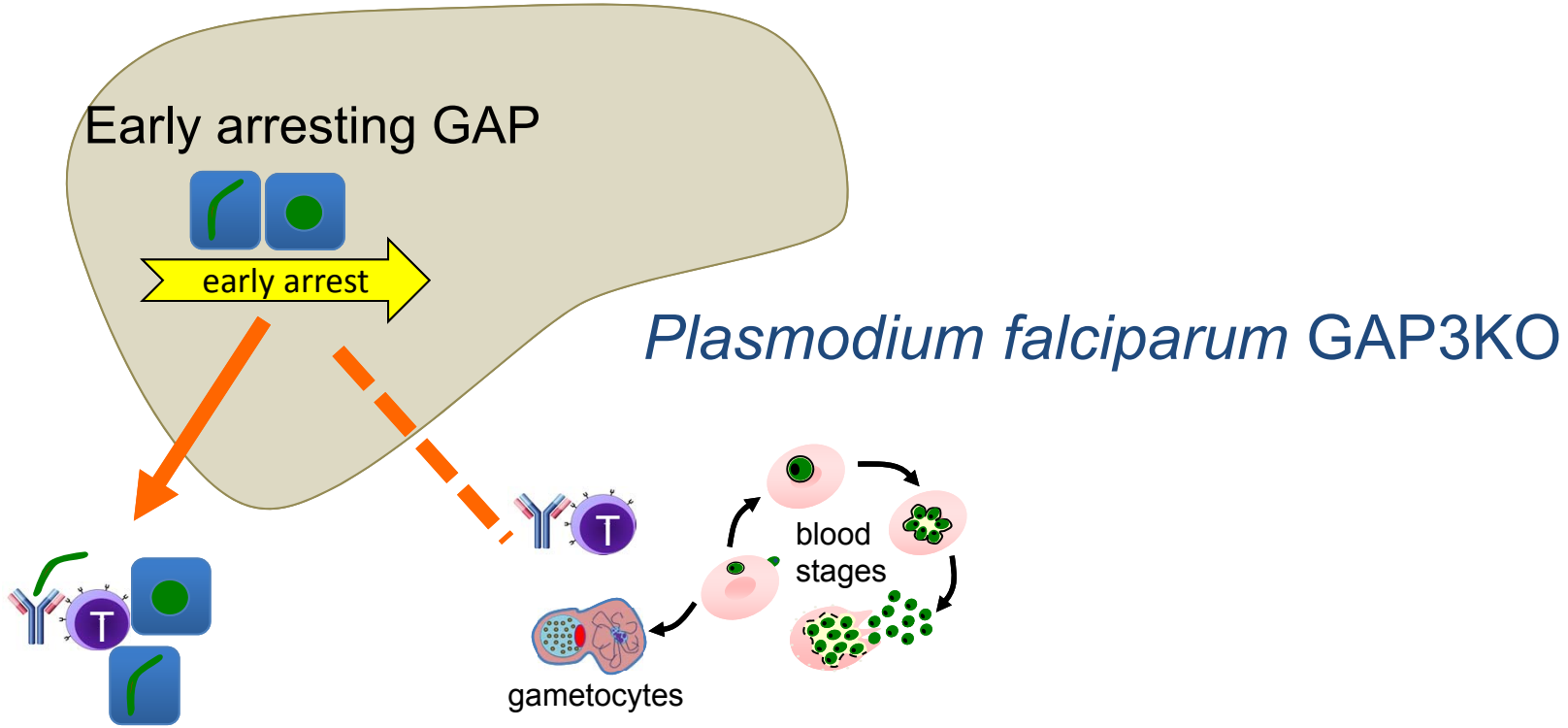
GAP are *Plasmodium* parasites that have been attenuated by targeted deletion of a gene or genes that arrest parasite development at the liver stage of development

Liver stage development and GAP arrest



Liver stage development and GAP arrest

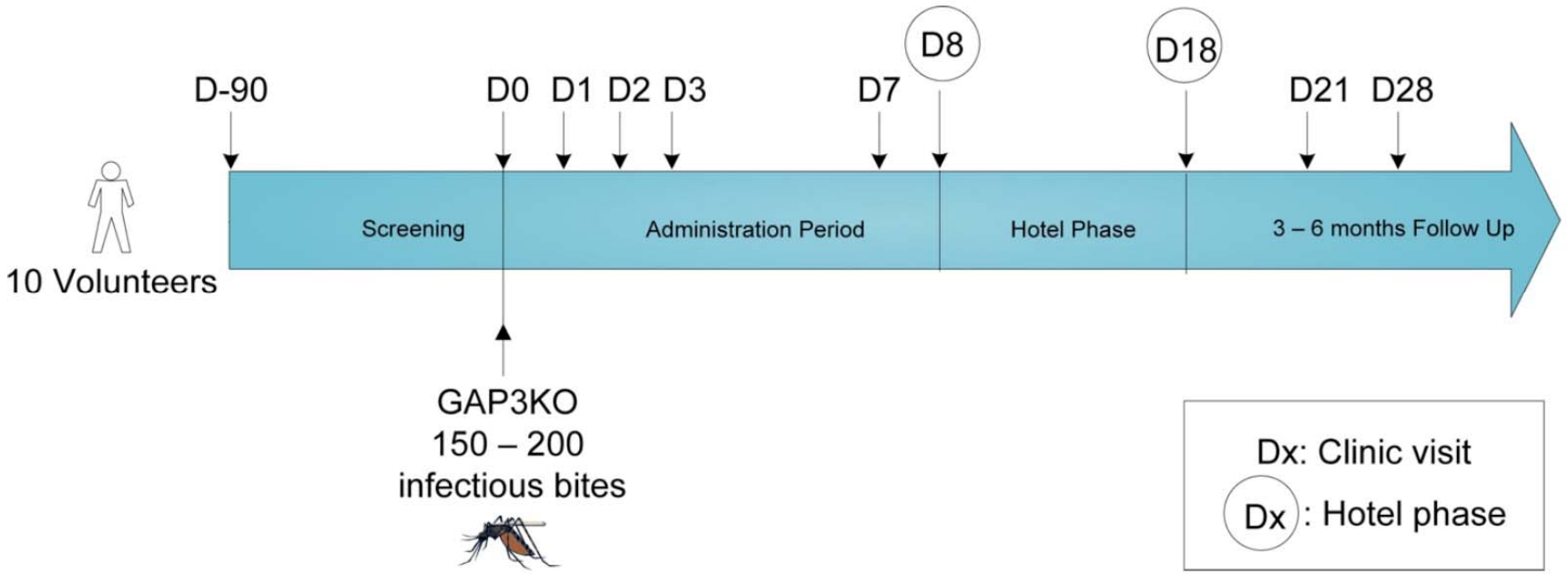




Plasmodium falciparum GAP3KO

Plasmodium falciparum GAP3KO is completely attenuated *in vivo* in humans

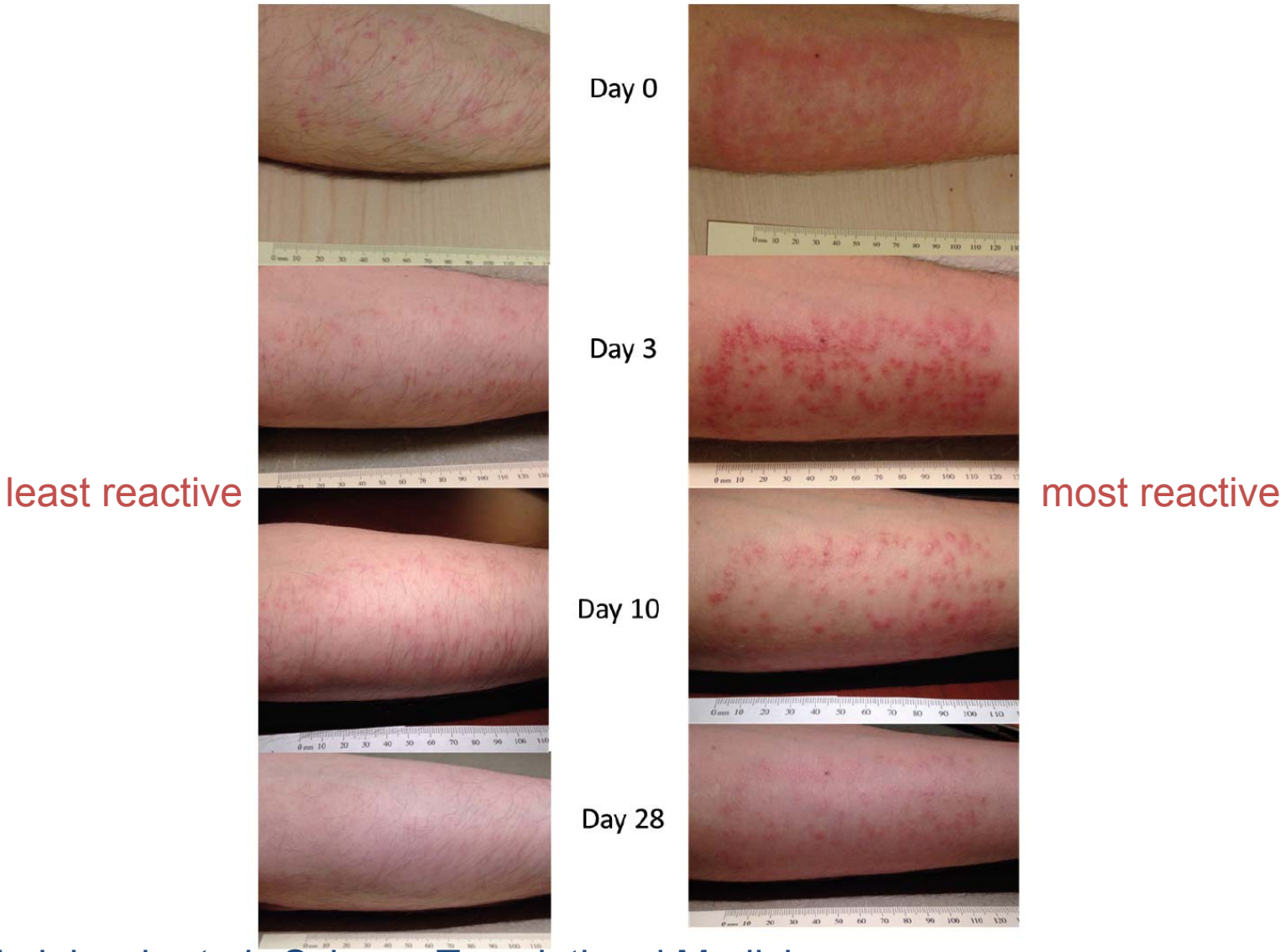
The Phase 1 study of the *Plasmodium falciparum* GAP3KO



No volunteer became patent after infectious mosquito bite – GAP3KO is safe

Kublin, Mikolajczak *et al.*, Science Translational Medicine

Effect on volunteers of GAP3KO infectious mosquito bite administration



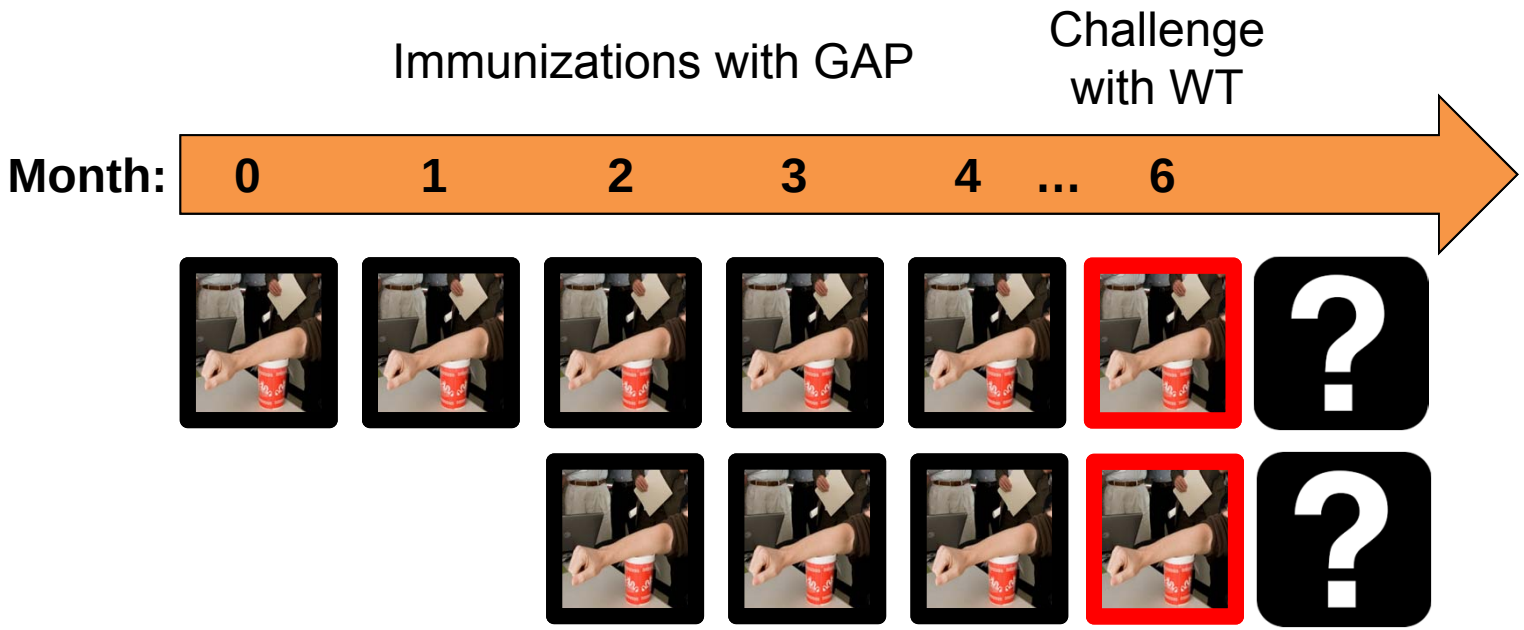
least reactive

most reactive

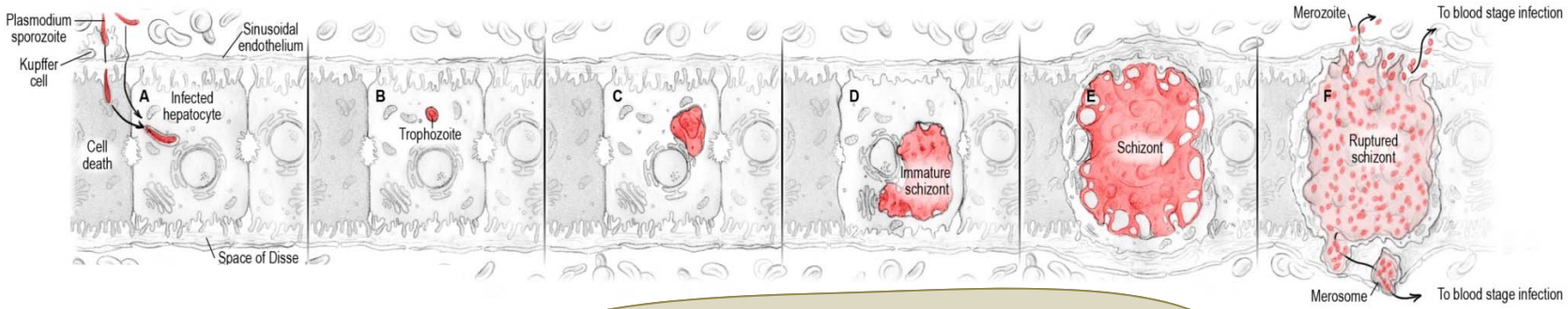
Kublin, Mikolajczak *et al.*, Science Translational Medicine

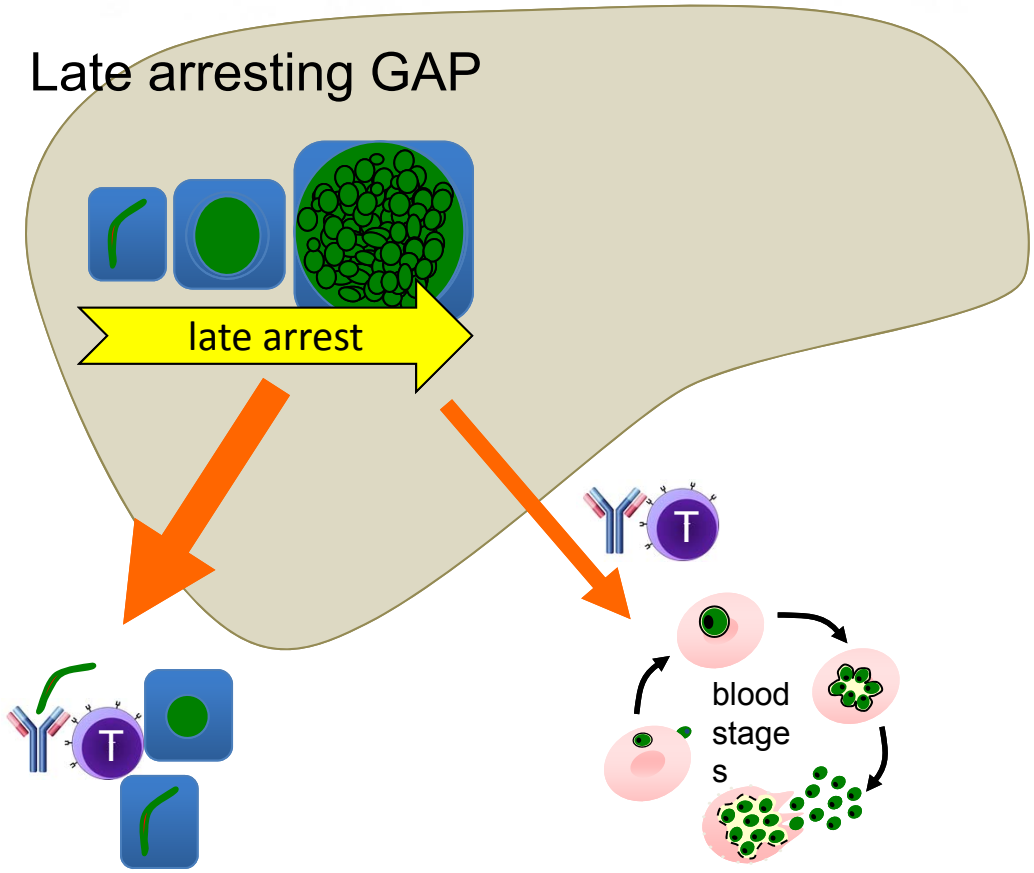
Plasmodium falciparum GAP3KO efficacy trial

- Starting in February 2018

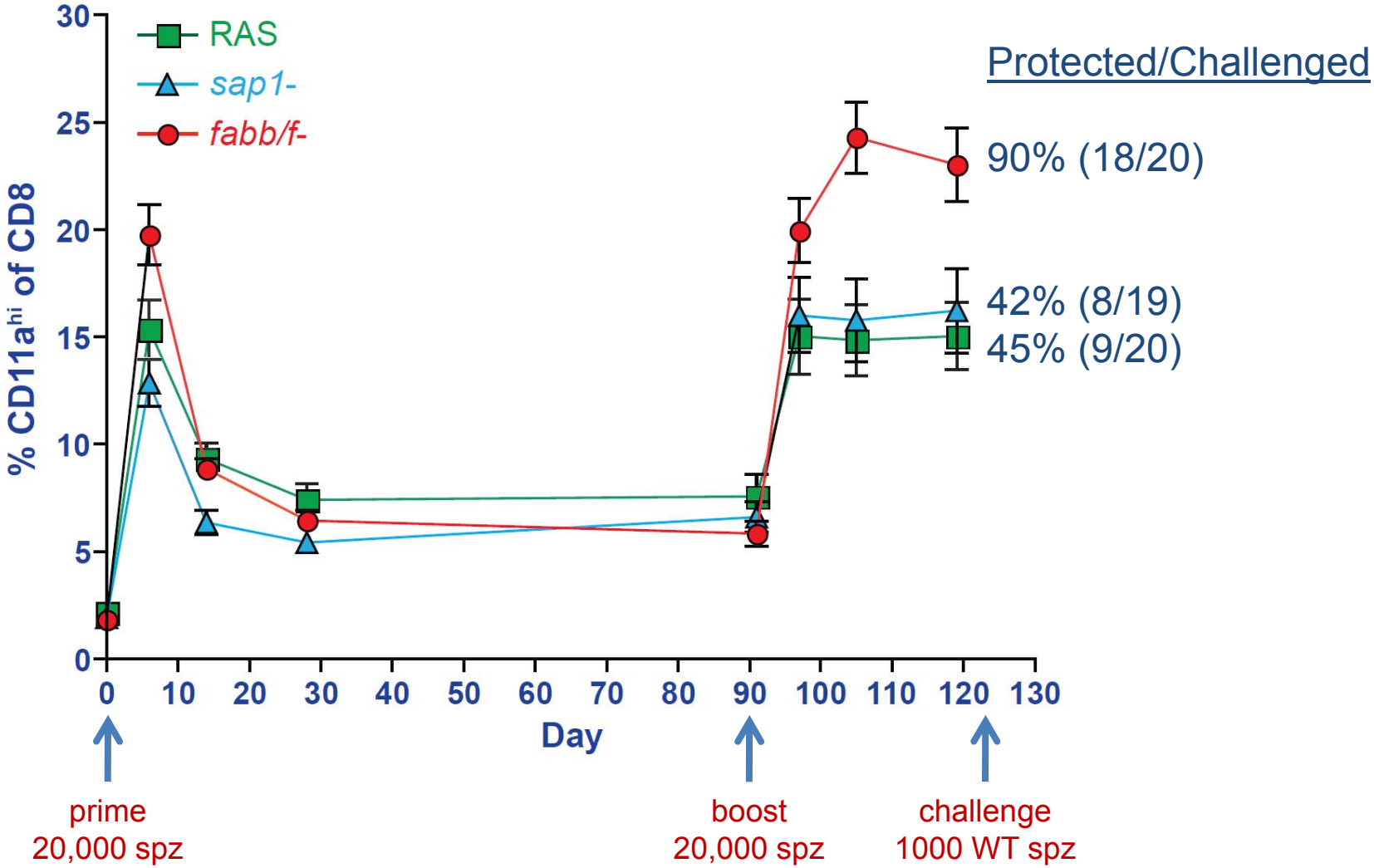


Liver stage development and GAP arrest



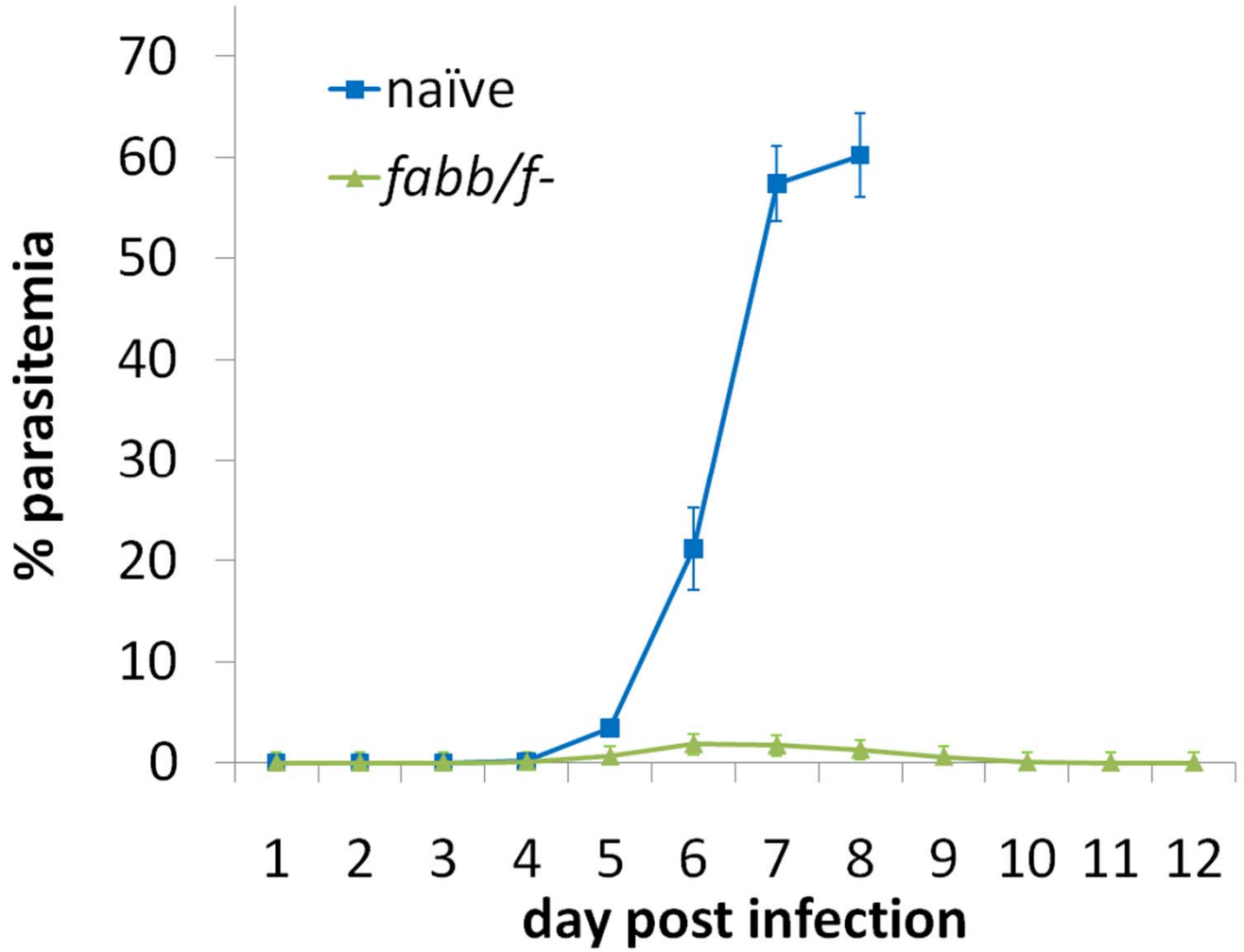


Late arresting GAPs are superior to early arresting GAPs



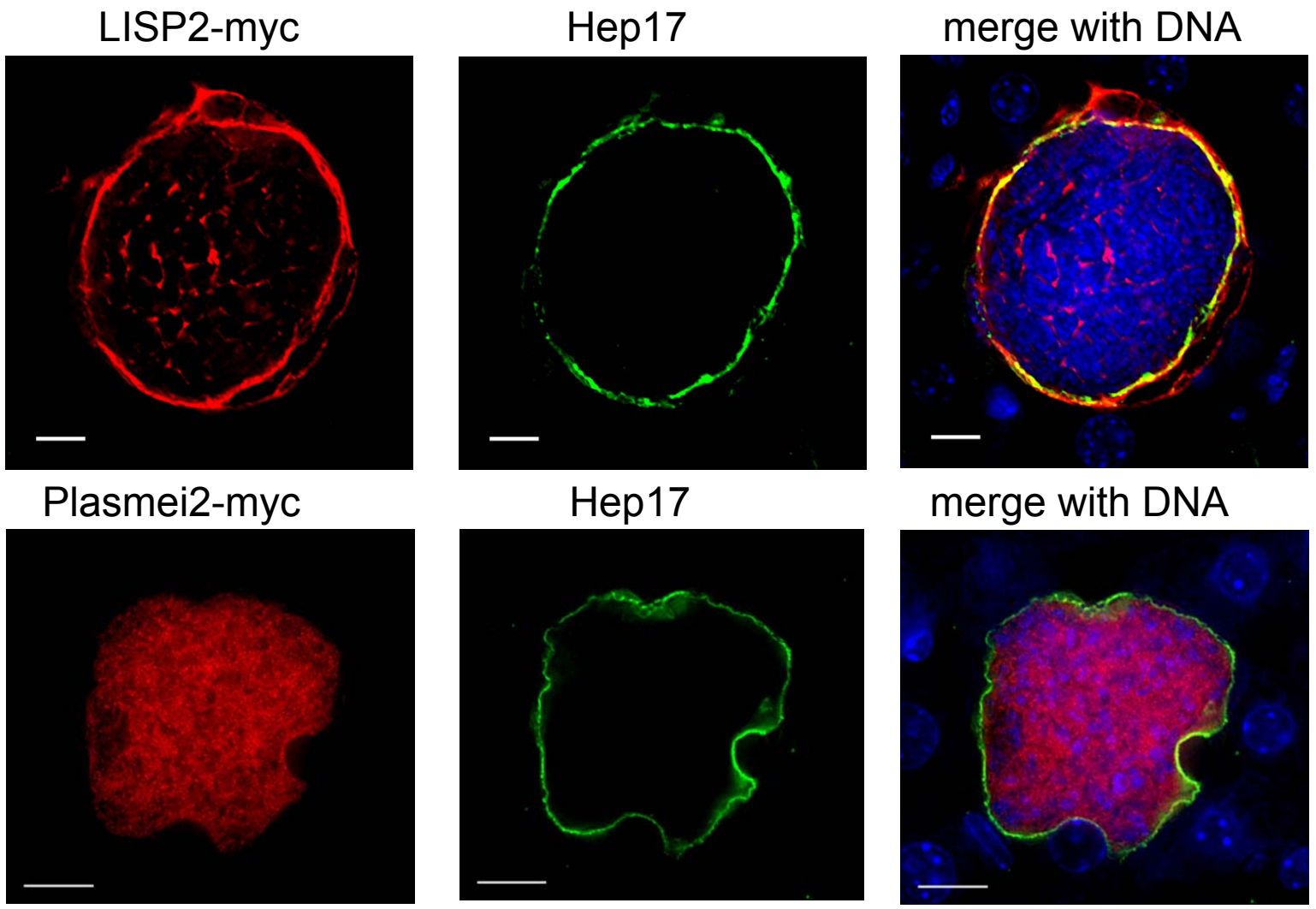
Butler *et al.*, Cell Host and Microbe

Mice immunized with *Plasmodium yoelii fabb/f-* GAPs are protected against a lethal blood stage challenge



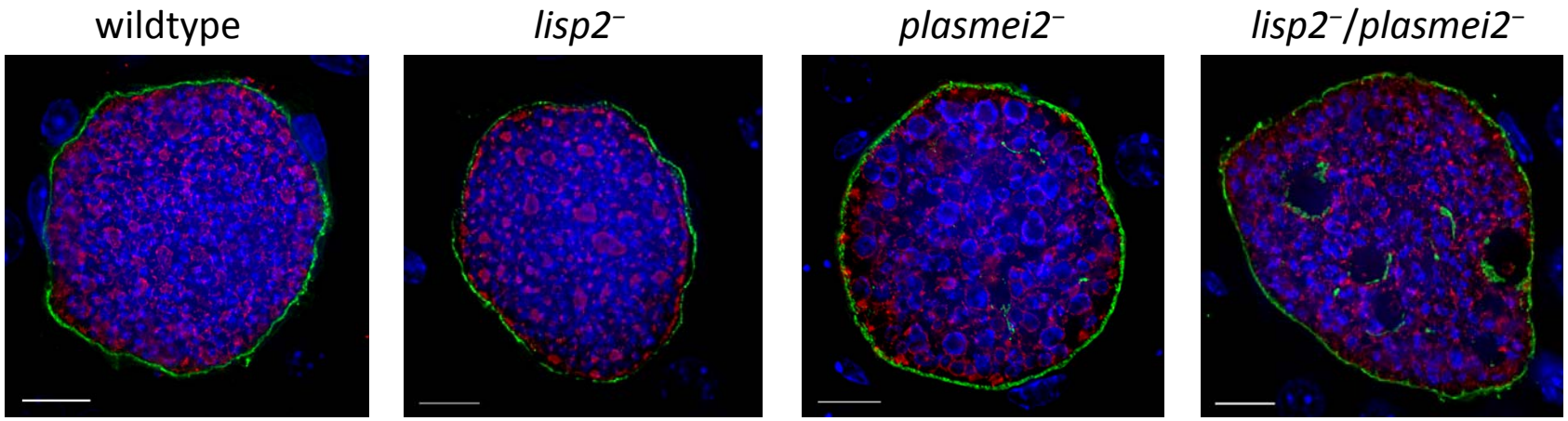
Butler *et al.*, Cell Host and Microbe

Towards a novel late liver stage-arresting GAP



Scale bar: 10 µm

The *Plasmodium yoelii lisp2⁻/plasmei2⁻* GAP persists for at least 44 hours



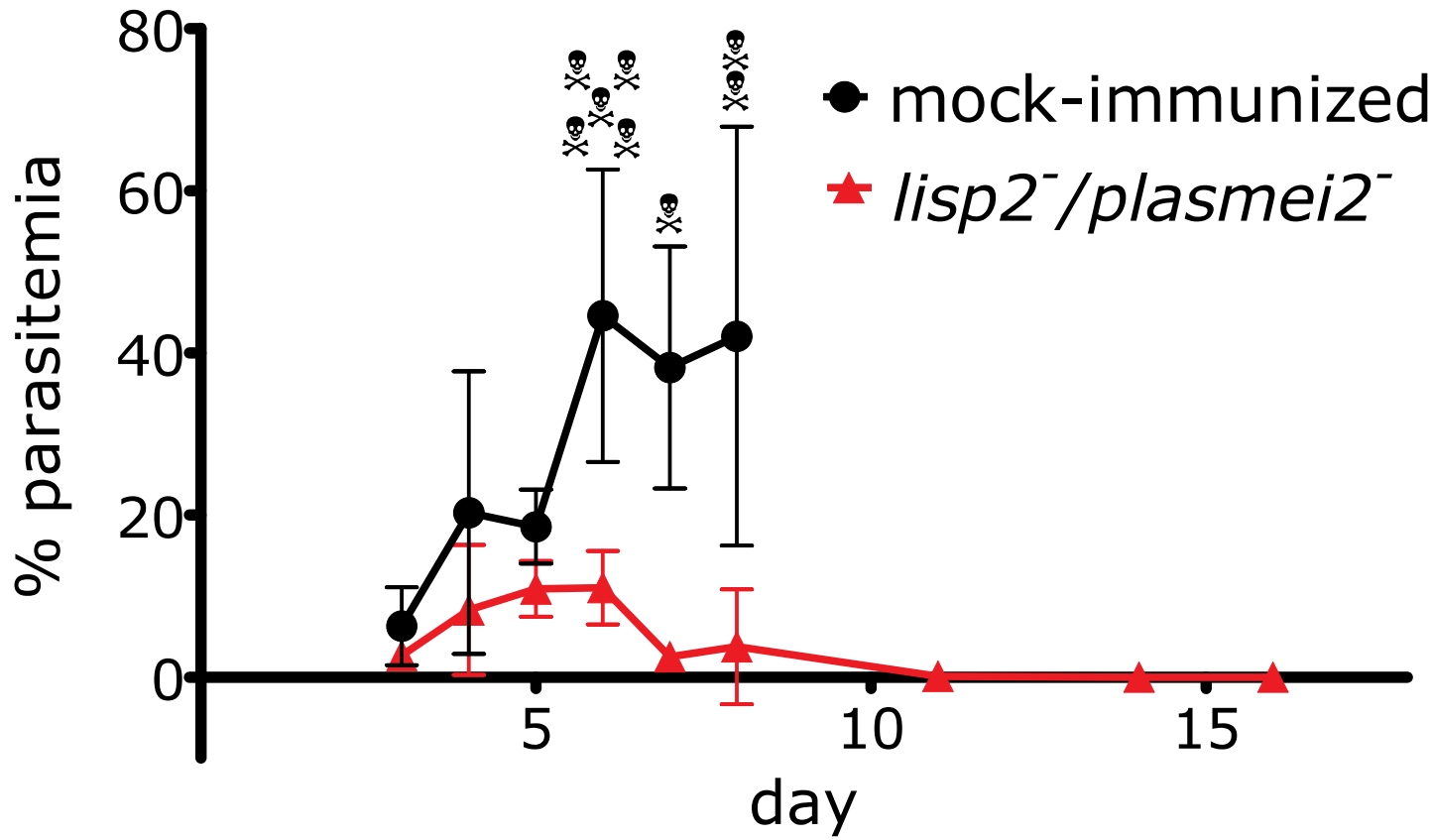
Plasmodium yoelii lisp2⁻/plasmei2⁻ GAP is a synthetic lethal and thus completely attenuated

Mouse	Parasite	Inoculation	Patent
BALB/cJ	*lisp2⁻*	1,000	6/8
BALB/cJ	*lisp2⁻*	10,000	7/7
BALB/cByJ	*plasmei2⁻*	200,000	3/30
BALB/cByJ	*plasmei2⁻*	500,000	4/30
BALB/cByJ	*lisp2⁻/plasmei2⁻*	200,000	0/29
BALB/cByJ	*lisp2⁻/plasmei2⁻*	500,000	0/26

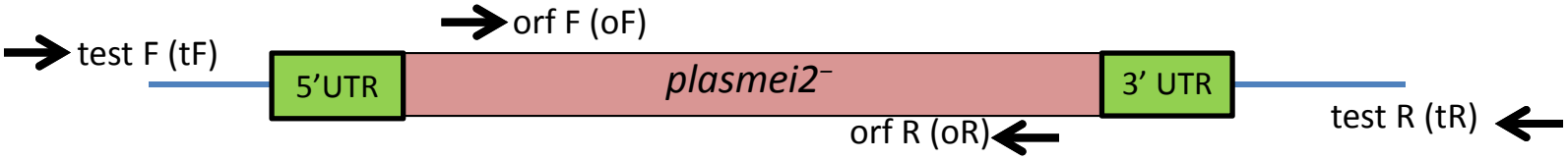
Plasmodium yoelii lisp2⁻/plasmei2⁻ GAP protects against sporozoite challenge in inbred and outbred mice

Mouse	Prime	Boost	Challenge	Patent
BALB/cJ	-	-	10,000	5/5
BALB/cJ	10,000	10,000	10,000 (1 month)	0/19
SW	-	-	15 bites	5/5
SW	50,000	50,000 x 2	15 bites (1 month)	1/10
SW	50,000	50,000 x 2	15 bites (6 months)	1/5

Plasmodium yoelii lisp2⁻/plasmei2⁻ GAP confers stage-transcending protection against blood stage challenge

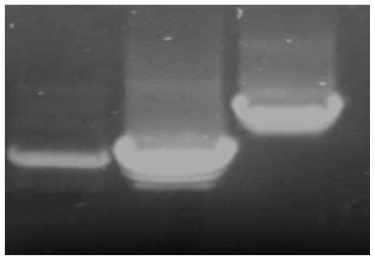


Creation of *Plasmodium falciparum plasmei2⁻* using CRISPR/Cas9 technology



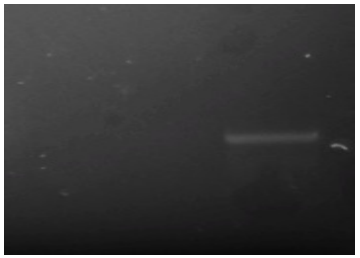
wildtype

tF+oR oF+tR tF+tR

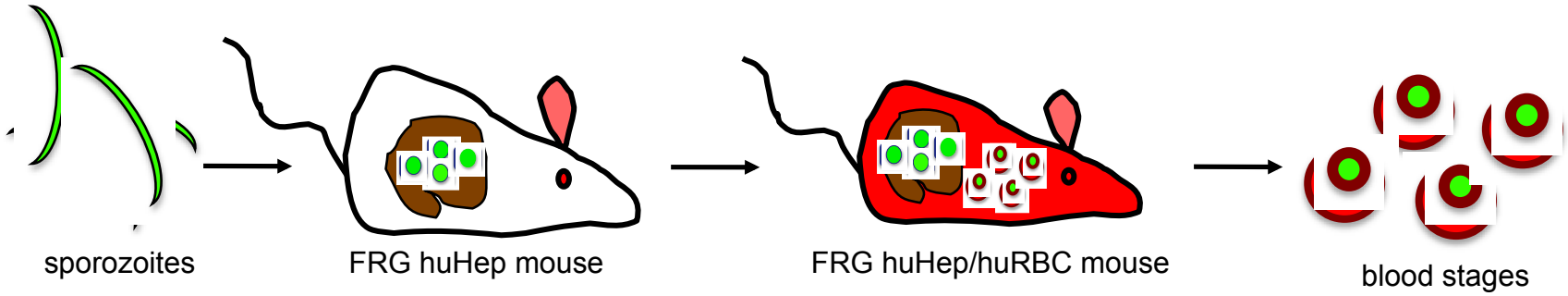


plasmei2⁻

tF+oR oF+tR tF+tR



FRG huHep mice infused with red blood cells (FRG huHep/huRBC) for the *Plasmodium falciparum* liver stage-to-blood stage transition



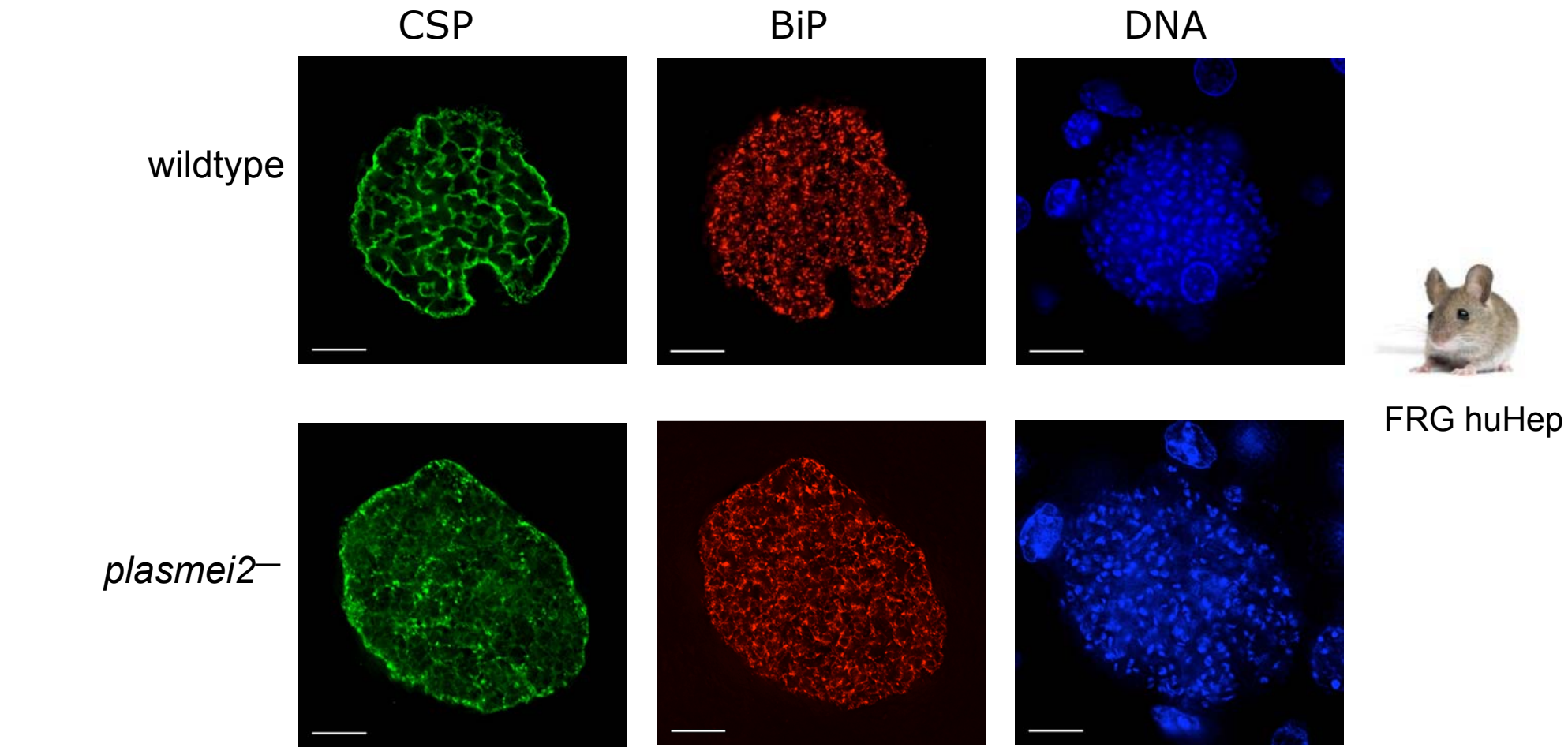
Vaughan, Mikolajczak *et al.*, Journal of Clinical Investigation

Plasmodium falciparum plasmei2⁻ is attenuated at the liver stage

FRG huHep/huRBC mosquito bite inoculation followed by analysis of liver stage-to-blood stage transition both *in vivo* and *in vitro*

Mouse inoculation	qRT PCR Result	*in vitro* culture
wildtype #1	Detected	Detected
wildtype #2	Detected	Detected
wildtype #3	Detected	Detected
plasmei2⁻ #1	Not detected	Not detected
plasmei2⁻ #2	Not detected	Not detected
plasmei2⁻ #3	Not detected	Not detected

Plasmodium falciparum plasmei2^{-} develops to late liver stage schizogony – as late as day 6 of development



Scale bar: 10 µm

Conclusions and continuing studies

- *Plasmodium falciparum* GAP3KO is safe and enters efficacy trials in 2018
- Using the rodent malaria model, we show that late-arresting GAP are more potent than early-arresting GAP and provide stage transcending immunity
- Designing late-arresting *Plasmodium falciparum* GAP has been challenging
- *Plasmodium falciparum plasmei2*$^{-}$ is a late-arresting GAP
- *Plasmodium falciparum lisp2*$^{-}$ has been created and is undergoing phenotypic analysis and evidence for *Plasmodium falciparum lisp2*$^{-}$/*plasmei2*$^{-}$ creation has recently been achieved ad is undergoing cloning