From fusion to conquest: how *Plasmodium falciparum* SNARE proteins orchestrate erythrocyte takeover

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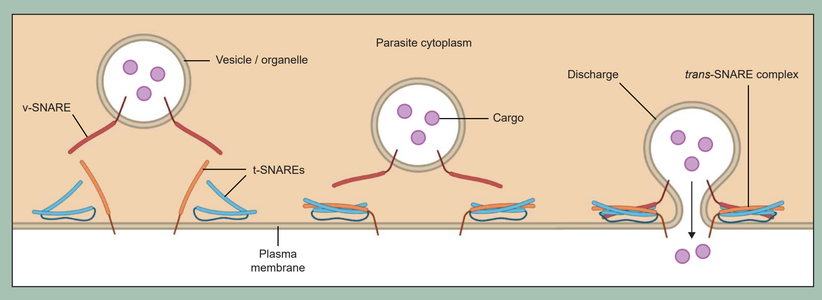
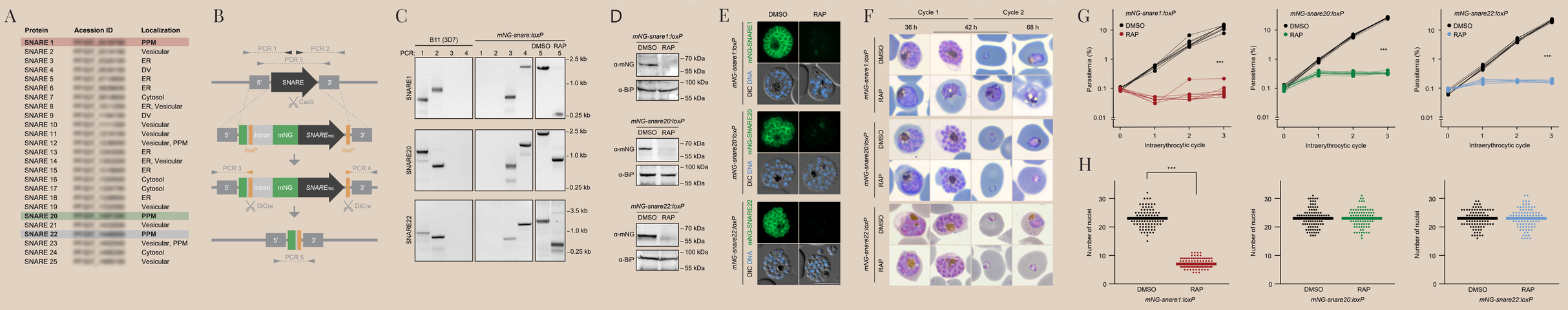
The malaria parasite *Plasmodium falciparum* relies on the coordinated action of specialized secretory systems to invade and replicate within human erythrocytes. During host cell invasion, organelles of the parasite apex – micronemes and rhoptries – fuse with the parasite plasma membrane (PPM) to release proteins that mediate host cell attachment and entry, allowing the parasite to push itself into a parasitophorous vacuole (PV). Maturation of this intracellular niche requires protein secretion from dense granules (DGs), which remodel the newly formed PV and the host environment to support parasite survival and replication. Despite the central role of these secretion events in the establishment of erythrocyte infection, the molecular machinery mediating organelle fusion with the PPM remains poorly understood. Here, we combine conditional reverse genetics with quantitative live-cell imaging and protein interaction studies to investigate the role of a SNARE protein complex that orchestrates the discharge of invasion- and development-associated organelles required for successful red blood cell colonization.



1 | SNARE 1, 20 and 22 localize to the PPM and are essential

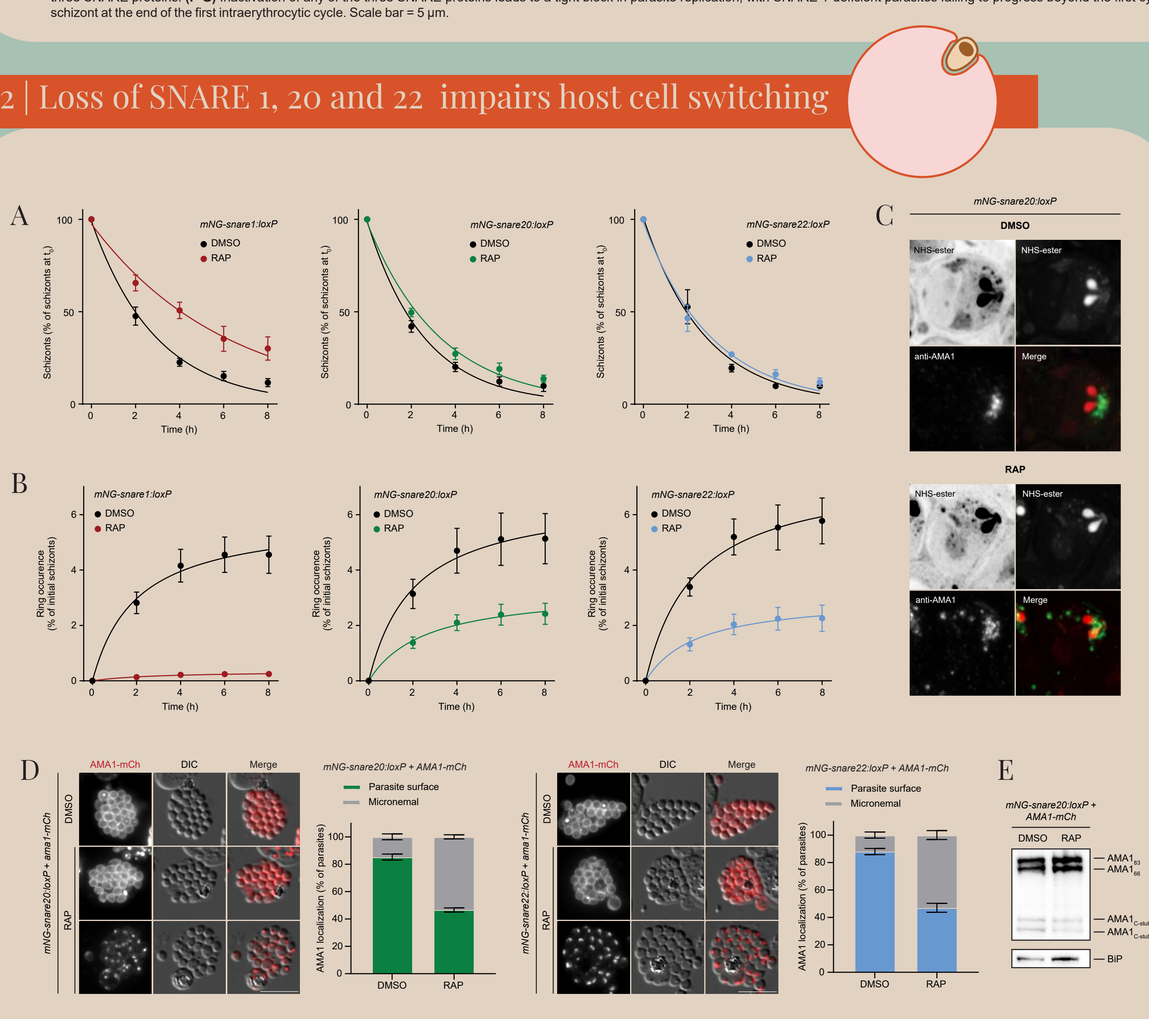
A

Protein	Acession ID	Localization
SNARE 1	[redacted]	**PPM**
SNARE 2	[redacted]	Vesicular
SNARE 3	[redacted]	ER
SNARE 4	[redacted]	DV
SNARE 5	[redacted]	ER
SNARE 6	[redacted]	ER
SNARE 7	[redacted]	Cytosol
SNARE 8	[redacted]	ER, Vesicular
SNARE 9	[redacted]	DV
SNARE 10	[redacted]	Vesicular
SNARE 11	[redacted]	Vesicular
SNARE 12	[redacted]	Vesicular, PPM
SNARE 13	[redacted]	ER
SNARE 14	[redacted]	ER, Vesicular
SNARE 15	[redacted]	ER
SNARE 16	[redacted]	Cytosol
SNARE 17	[redacted]	Cytosol
SNARE 18	[redacted]	ER
SNARE 19	[redacted]	Vesicular
SNARE 20	[redacted]	**PPM**
SNARE 21	[redacted]	Vesicular
SNARE 22	[redacted]	**PPM**
SNARE 23	[redacted]	Vesicular, PPM
SNARE 24	[redacted]	Cytosol
SNARE 25	[redacted]	Vesicular



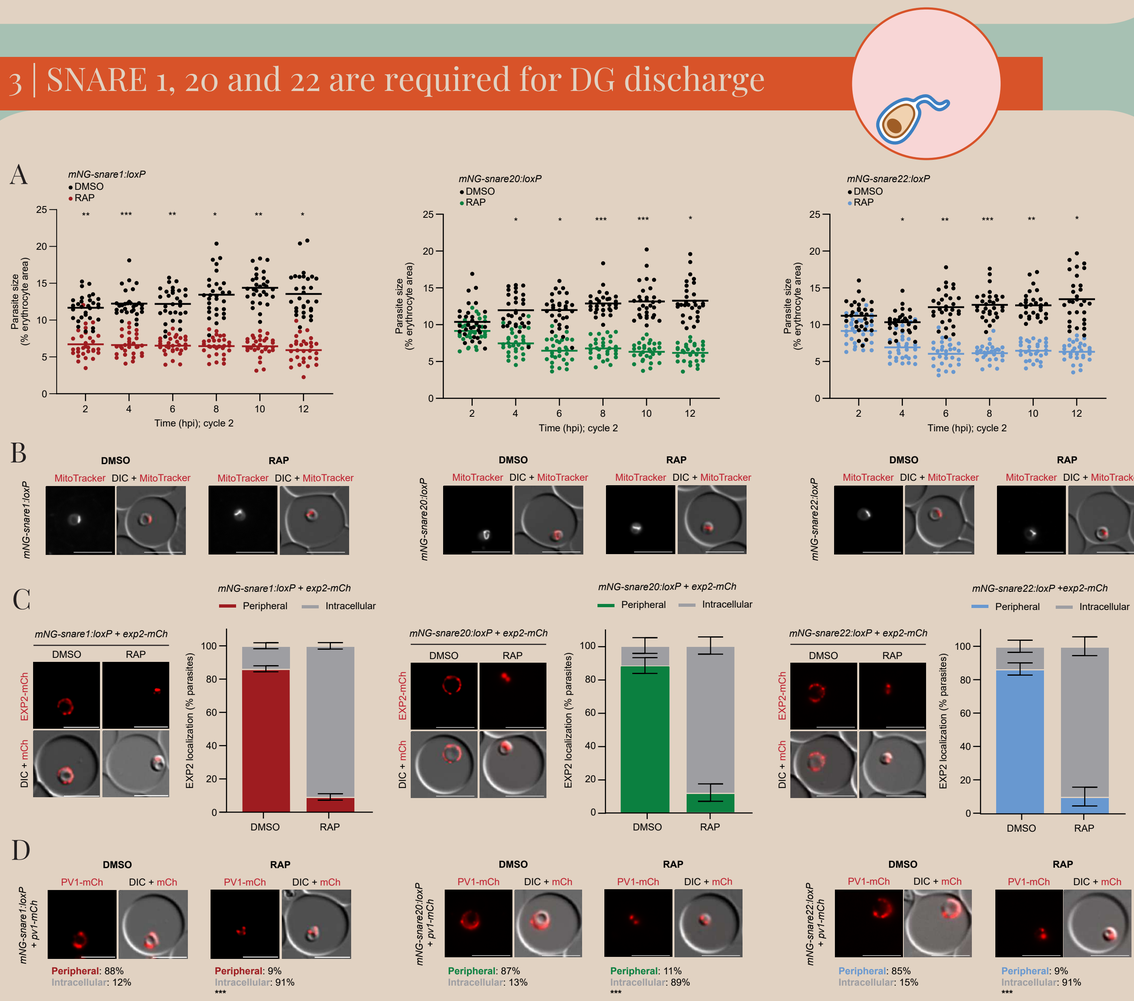
(A) Subcellular localization of the 25 SNARE domain-containing proteins in *P. falciparum* based on episomal expression of mScarlet fusion constructs. **(B)** Genetic strategy for endogenous tagging and conditional deletion of SNARE 1, SNARE 20 and SNARE 22. **(C-E)** Diagnostic PCR (C), Western blotting (D), and live fluorescence microscopy (E) confirm correct integration and RAP-induced loss of the three SNARE proteins. **(F-G)** Inactivation of any of the three SNARE proteins leads to a tight block in parasite replication, with SNARE 1-deficient parasites failing to progress beyond the first cycle, and SNARE 20- or SNARE 22-deficient parasites experiencing a partial defect in cell cycle progression and eventual death in the new host cell. **(H)** Loss of SNARE 1 leads to a reduced number of nuclei per schizont at the end of the first intraerythrocytic cycle. Scale bar = 5 µm.

2 | Loss of SNARE 1, 20 and 22 impairs host cell switching



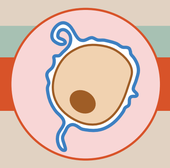
(A-B) Loss of SNARE 1 impairs egress from the host cell (A) resulting in a very small population of parasites that progress successfully to the second cycle (B), whereas loss of SNARE 20 or SNARE 22 leads to a twofold decrease in invasion efficiency (B). **(C)** Ultrastructure expansion microscopy of SNARE 20-deficient parasites shows physiological rhoptry and microneme biogenesis. Inactivation of SNARE 22 reveals a comparable phenotype (data not shown). **(D-E)** SNARE 20- and SNARE 22-deficient parasites experience defective microneme secretion resulting in retention of AMA1 within the micronemes in approximately half of the parasite population (D), and a 45% reduction in the processing of AMA1 (n=1) (E). Scale bar = 5 µm.

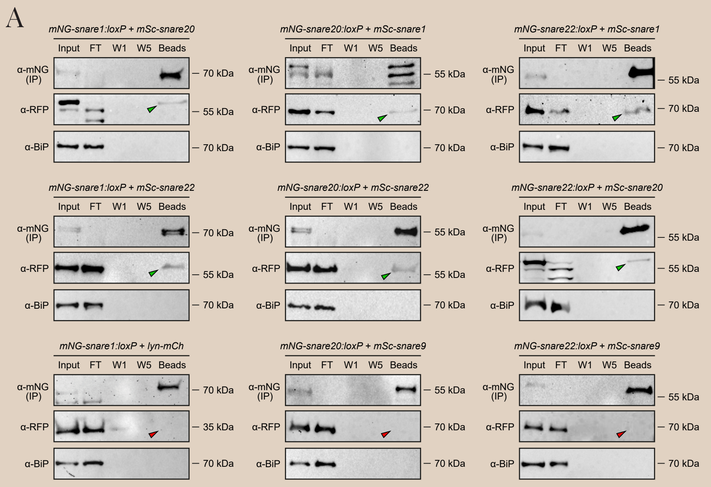
3 | SNARE 1, 20 and 22 are required for DG discharge

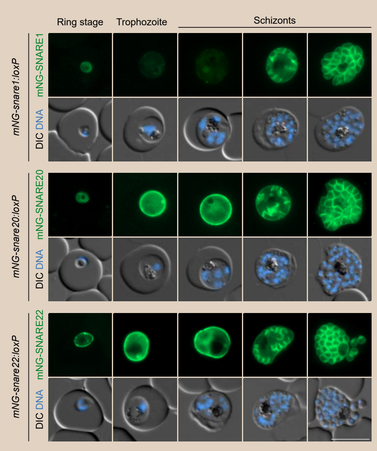


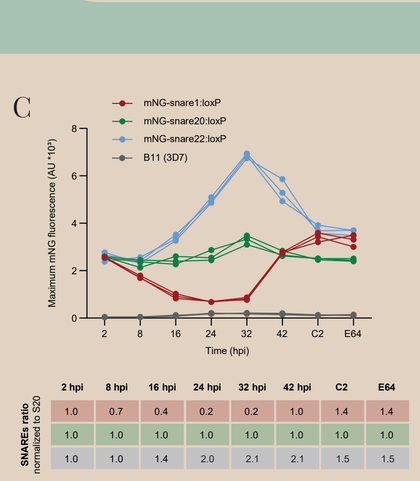
(A-B) SNARE 1-, SNARE 20- and SNARE 22-deficient parasites arrest development shortly after invasion in the second cycle (A) despite metabolically active as shown by MitoTracker staining (B). **(C-D)** DG-resident proteins EXP2 (C) and PV1 (D) remain trapped within the parasite upon depletion of any of the SNARE proteins instead of relocating to the PV or PVM. **(E)** Early exported protein SBP1 fails to relocate to the Maurer's clefts and retains an intracellular localization in SNARE 1-, SNARE 20- or SNARE 22-deficient parasites. Scale bar = 5 µm.

4 | SNARE 1, 20 and 22 interact with one another

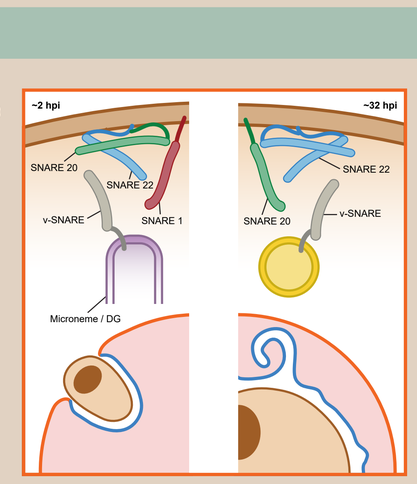








SNAREs ratio normalized to S20	2 hpi	8 hpi	16 hpi	24 hpi	32 hpi	42 hpi	C2	E64
	1.0	0.7	0.4	0.2	0.2	1.0	1.4	1.4
	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	1.0	1.0	1.4	2.0	2.1	2.1	1.5	1.5



(A) Co-immunoprecipitation studies show specific interaction amongst the three PPM-resident SNARE proteins, with no interaction observed for other PPM-localized proteins (Lyn) or SNARE proteins from different compartments (SNARE 9). **(B-C)** Quantification of the maximum fluorescence intensity of SNARE 1, SNARE 20 and SNARE 22 throughout the cycle reveals a balanced stoichiometry during the establishment of erythrocyte infection at 2 hpi, as well as during parasite maturation at 32 hpi. **(D)** Schematic representation of the putative PPM-SNARE fusion complex during early invasion (left) consisting of one SNARE 1, one SNARE 20 and one SNARE 22; and during late-stage development (right) consisting of one SNARE 20 and two SNARE 22. The respective v-SNAREs remain unknown. Scale bar = 5 µm.

Conclusions

- SNARE 1, SNARE 20 and SNARE 22 localize to the PPM and are critical for the survival of *P. falciparum* blood stages.
- SNARE 1, SNARE 20 and SNARE 22 show mutual interactions.
- Inactivation of SNARE 1, SNARE 20 or SNARE 22 impairs microneme and dense granule discharge, as well as early protein export to the host erythrocyte.

Open questions

- Is the invasion deficiency also related to a defect in rhoptry discharge?
- What role do SNARE 1, SNARE 20 and SNARE 22 have during parasite maturation?
- Which are the other components of this SNARE complex?

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