Title: Rhomboids in action: Tracking the roles of rhomboid proteases across the blood stage developmental cycle of the malaria parasite

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Abstract

Plasmodium falciparum is the causative agent of the most virulent form of malaria, resulting in significant global morbidity and mortality. Clinical symptoms arise from blood stage proliferation of these parasites, when they undergo multiple rounds of invasion, replication and egress. These critical steps are governed by tightly regulated proteolytic events which are poorly understood. Amongst these proteases are rhomboid proteases, intramembrane serine proteases that cleave transmembrane substrates and are known to mediate essential processes across evolution. The malaria parasite expresses eight rhomboids, of which at least four are considered essential, but to date their specific functions remain uncharacterised.

To investigate the roles of rhomboid proteases during *P. falciparum* blood-stage development, we employed two conditional gene disruption strategies: the DiCre-loxP system and SHIFTiKO, its scaleable refinement, to inducibly disrupt the function of all eight *Plasmodium* rhomboids. These efforts conclusively identified three rhomboids – ROM4, ROM6, and ROM8 to be essential for blood stage parasite survival. We confirmed ablation of ROM4 caused a complete block in merozoite invasion of RBCs. Disrupting the function of ROM6, a lowly expressed predicted mitochondrial rhomboid, resulted in a developmental arrest at schizont stages, albeit always in the next replication cycle. ROM8 was found localised to the plasma membrane and to be essential for parasite development across the 48-hour cycle. Induced ablation of ROM8 caused limiting defects both during trophozoite and schizont stages resulting in fewer clumped merozoites. This suggests a regulatory role in the parasite's nutrient acquisition. We are currently continuing in-depth characterisation of these mutant lines using proteomics approaches to identify specific substrates of these essential rhomboids and are exploring their potential as drug targets through phenotypic assays using rhomboid-specific inhibitors.