# Characterising the role of the transcription factor AP2-I in blood stages of the malaria parasite

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Malaria, caused by protozoan parasites of the genus *Plasmodium*, remains a major global health and socioeconomic burden, with over 263 million cases and approximately 597,000 deaths reported annually. Clinical symptoms of malaria arise from repeated cycles of asexual replication of the parasite within red blood cells. Members of the ApiAP2 family of transcription factors (AP2-TFs) represent the most important family of transcription factors in *Plasmodium*, regulating critical biological processes at all developmental stages of the parasite. Among these, AP2-I was initially implicated in regulating erythrocyte invasion based on it binding upstream of invasion-related genes at 40 hours post-invasion (hpi) and its interaction with another bonafide invasion regulator, BDP1. Recent studies however have shown AP2-I to additionally interact with AP2-TFs and multiprotein complexes involved in other processes such as development and sexual commitment. This suggests other potential functions for AP2-I beyond regulating invasion. Multiple attempts to knock out the gene's function has previously failed, preventing further exploration of AP2-I's critical roles during blood stage proliferation.

To remedy this, an inducible knockout line was generated using the DiCre-based SHIFTiKO (frameshift-based trackable inducible knockout) system. Induced knockout of AP2-I expression at different points in their replication cycle surprisingly resulted in a severe developmental defect from as early as 24 hpi, leading to complete ablation of parasite growth. Since AP2-I-null parasites failed to develop into mature schizonts, we were unable to directly assess invasion in these mutant parasites. To address this issue, we then generated a novel inducible "knockout-and-down" line by further tagging *ap2i-shiftiko* gene with aptamer sequences and employing the tetracycline-responsive inducible knockdown system (TetR-DOZI) to enable both complete or tunable depletion of AP2-I. Transcriptional consequences of loss of AP2-I function was analysed by both bulk RNA sequencing at specific timepoints around the onset of developmental arrest and single-cell RNA sequencing across developmental stages following AP2-I depletion. Furthermore, to identify AP2-I's DNA-binding sites and interacting protein partners at trophozoite stages (24 hpi), chromatin immunoprecipitation followed by sequencing (ChIP-seq) and ChIP-coupled mass spectrometry (ChIP-MS) will be performed using a GFP-tagged AP2-I parasite line.

Collectively, this study aims to revise and improve our current understanding of the regulatory function of an important and essential transcription factor AP2-I during asexual blood stages of the malaria parasite.