Title: Resistance to PfGCN5-inhibitor L45 involves a novel mitochondrial carrier protein in *Plasmodium falciparum*

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The increasing emergence of resistance to current antimalarial therapies highlights the urgent need to identify and validate new drug targets. Bromodomain-containing proteins (BDPs) recognise acetylated lysine residues on histones and regulate transcriptional programmes implicated in a wide range of diseases, making them attractive therapeutic targets. In *Plasmodium falciparum*, the bromodomain of General Control Non-repressed 5 (PfGCN5) is essential for parasite invasion and virulence. Using conditional knockout and complementation approaches, we demonstrated that the PfGCN5 bromodomain plays a critical role during the blood stage of parasite development and exhibits rapid parasite-killing activity, identifying it as a potential antimalarial target. The PfGCN5 bromodomain structure has been resolved by Brennan and colleagues in complex with the small-molecule inhibitor L45, which we used as a probe compound to investigate resistance mechanisms targeting this domain. L45 showed potent activity against both blood-stage *P. falciparum* and liver-stage *P. berghei*. Unexpectedly, *in vitro* resistance selection identified point mutations in a mitochondrial carrier protein, PfMCP-R, implicating PfGCN5 in linking transcriptional regulation with mitochondrial function.

Using CRISPR–Cas9 genome editing, we confirmed that mutation of PfMCP-R confers resistance to L45. Notably, L45-resistant parasites exhibited hypersensitivity to other electron transport chain inhibitors, including atovaquone, DSM1, and myxothiazol, indicating altered mitochondrial activity. Metabolomic profiling further revealed accumulation of dUMP following L45 treatment, suggesting broader metabolic perturbations associated with bromodomain inhibition. Collectively, these findings identify a novel escape route used by parasites against PfGCN5 inhibition, offering a roadmap for more effective, rational drug discovery.