

Figure S1. Growth inhibitory activity of chloroquine, azithromycin and azithromycin analogues against drug sensitive and resistant *P. falciparum* parasites.

Ring-stage 3D7 (a) and multi-drug resistant (b) parasites were drug treated for 72 hrs before growth measurement by DNA staining. All values of growth were normalised to that of parasites grown without drug. Dose response curves were generated in GraphPad Prism, with concentrations along the x-axis adjusted to a log₁₀ scale. Error bars represent the SEM for three or more independent experiments each conducted with technical replicates.

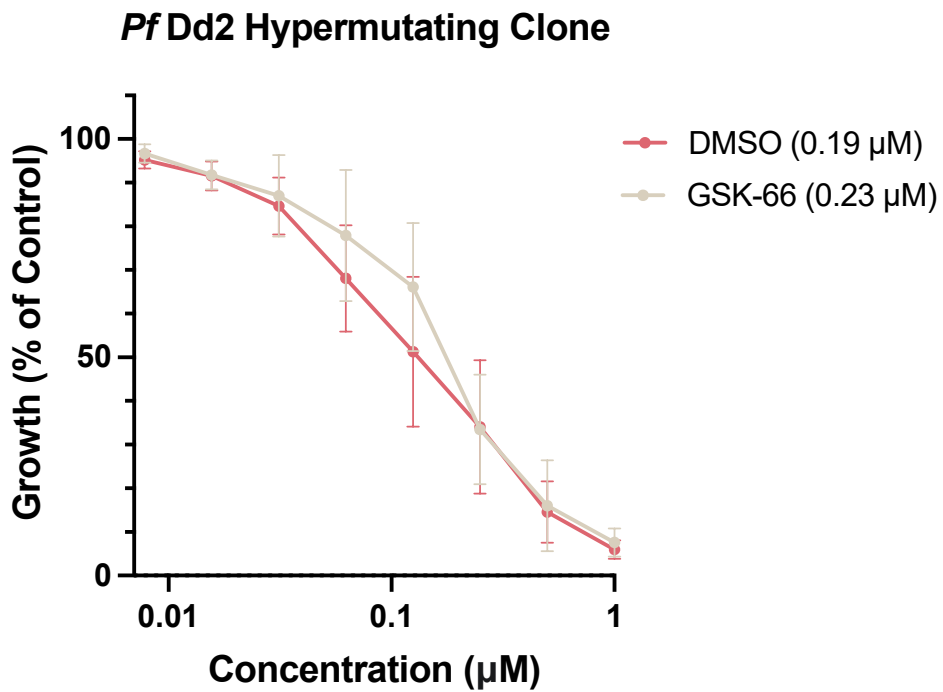
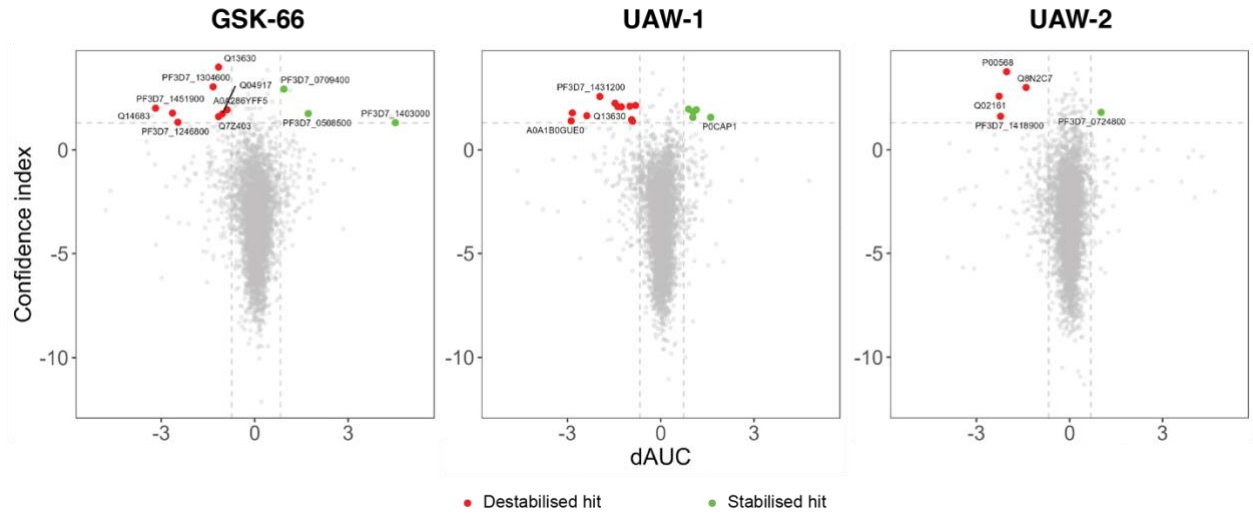


Figure S2. GSK-66 resistance selection in *Pf* Dd2 hypermutating line.

Drug inhibition assays with parasites selected for resistance to increasing drug exposure to GSK-66, or 1:1000 DMSO as a control. IC₅₀ values are indicated in the brackets, showing only a 1.5-fold increase in resistance selected parasites. Error bars represent the SEM for three or more independent experiments each conducted with technical replicates.

a)



b)

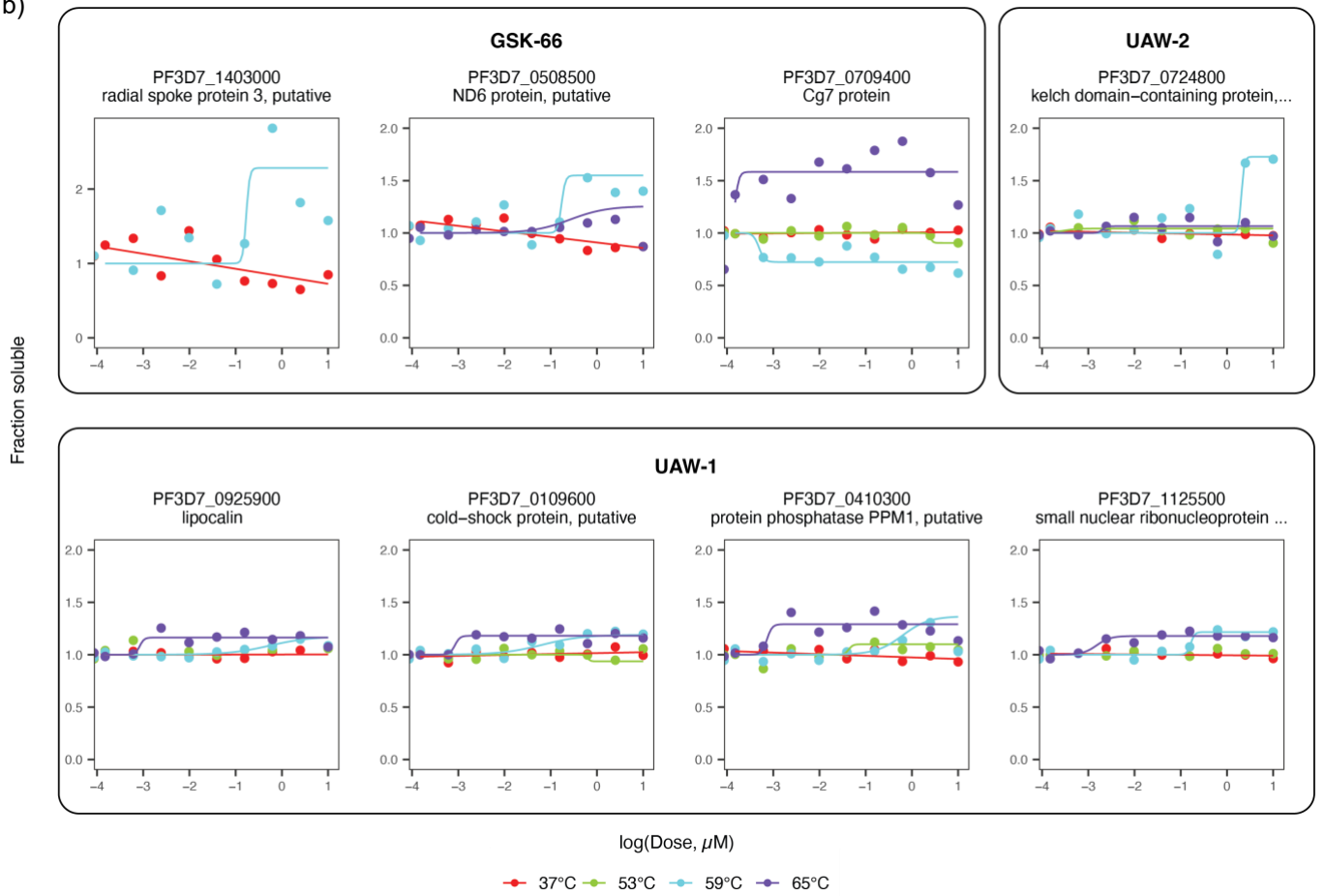


Figure S3. Proteins that exhibited a significant thermal shift with drug treatment in lysate CETSA experiments.

(a) Volcano plots showing significantly stabilised and destabilised human and parasite proteins following CETSA with lysate samples of *Pf* 3D7 trophozoites. Dotted lines represent the cut-off criteria with proteins considered as hits when their confidence index was above $-\log(0.05)$ and the median absolute deviation of ΔAUC was higher than 3. (b) Isothermal dose-response (ITDR) curves for significantly stabilised parasite proteins. While significantly thermal stabilised, these proteins, however, displayed mixed destabilising and stabilising curves at different temperatures, or a low magnitude of response (<1.3). All data representative of one independent experiment.

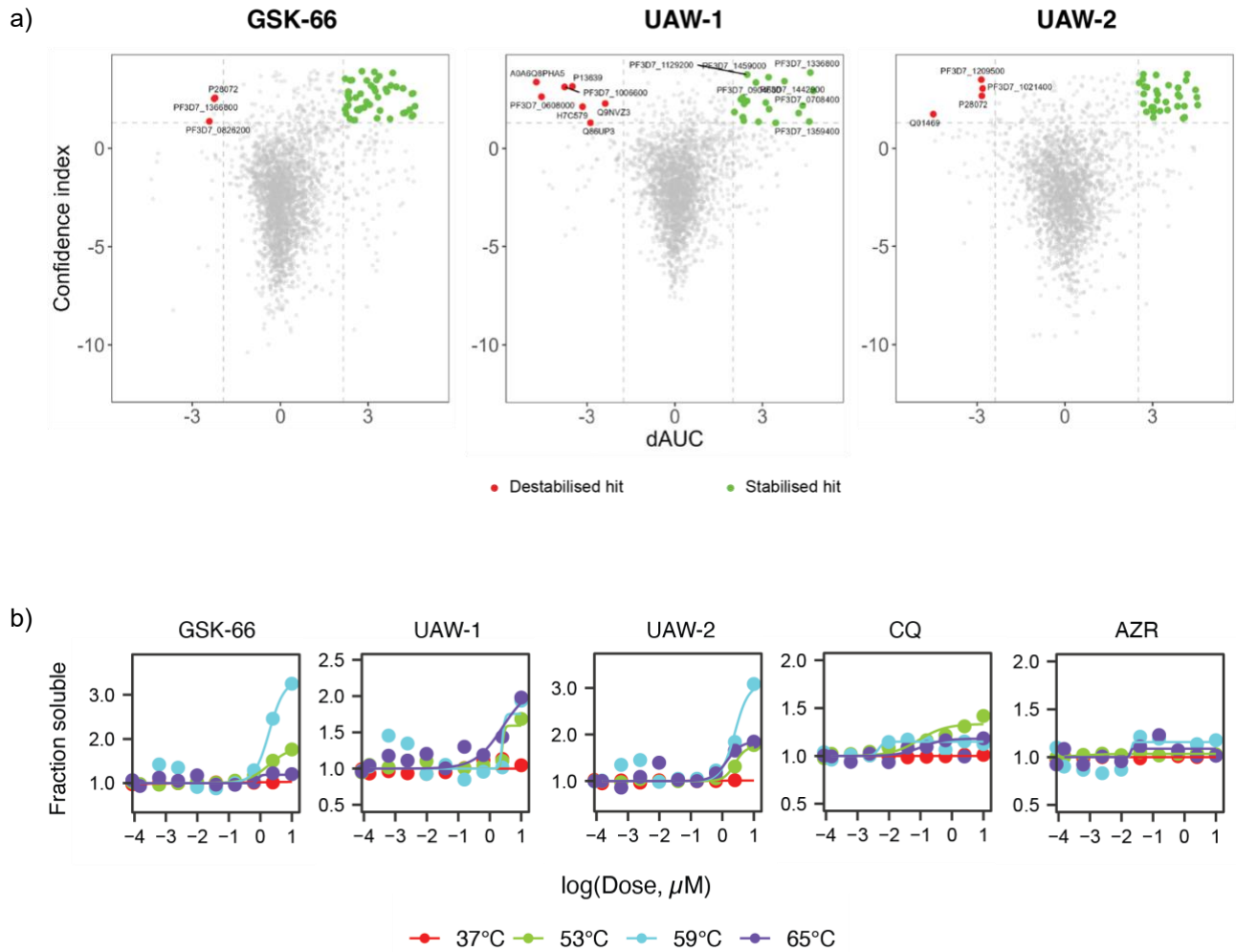


Figure S4. Proteins that exhibited a thermal shift with drug treatment in intact-cell CETSA experiments.

a) Volcano plots showing significantly stabilised and destabilised human and parasite proteins following CETSA of drug-treated *Pf* 3D7 trophozoites. Dotted lines represent the cut-off criteria with proteins considered as hits when their confidence index was above $-\log(0.05)$ and the median absolute deviation of ΔAUC was higher than 3. b) ITDR curves for falcilysin, a previously validated CETSA hit for chloroquine. All but azithromycin demonstrated low levels of thermal

stabilisation relative to the untreated control. Data is representative of at least one independent experiment.

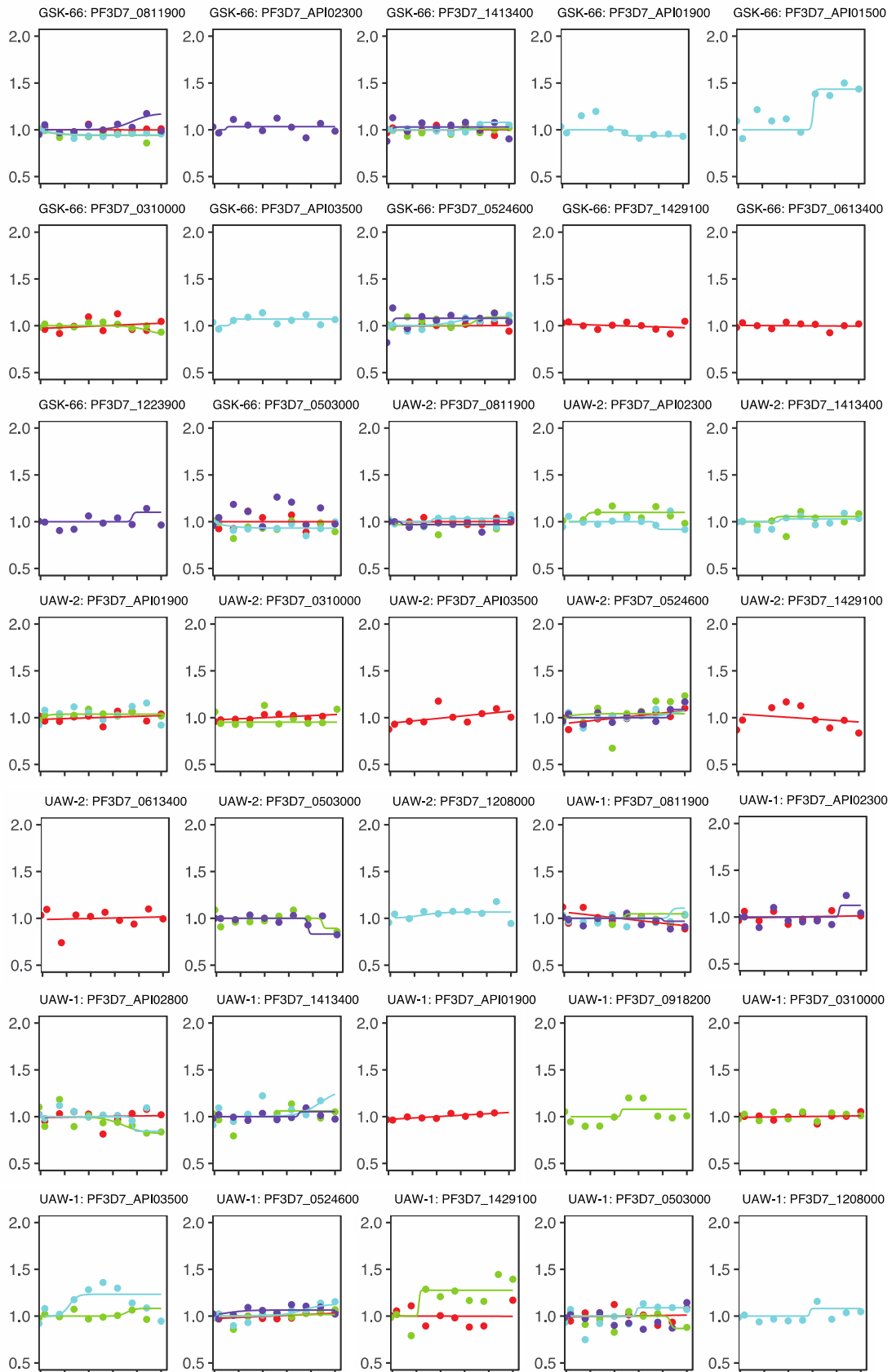


Figure S5. Stabilisation profile of apicoplast proteins detected from lysate CETSA experiments.

Isothermal dose-response (ITDR) curves demonstrate minimal evidence of stabilisation across proteins of the 50s apicoplast ribosome, the known target of azithromycin.

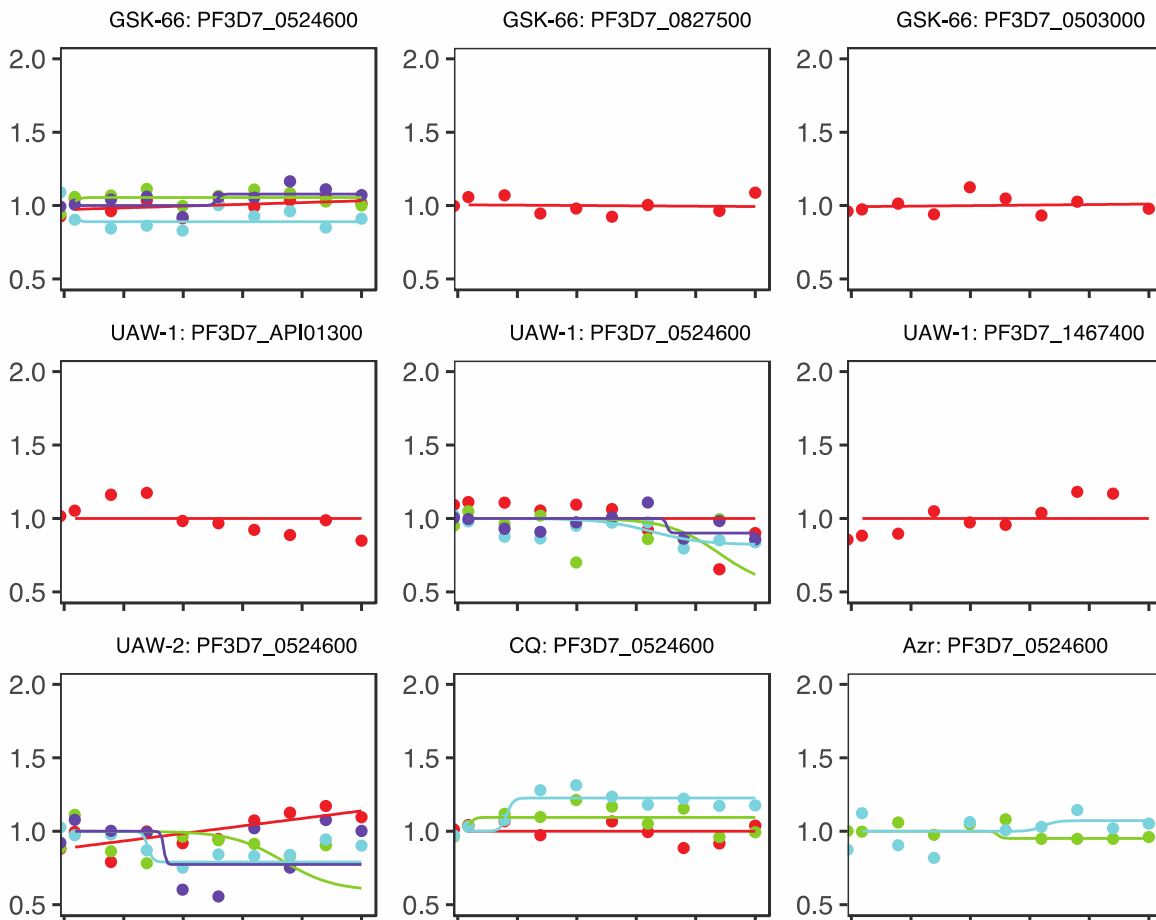


Figure S6. Stabilisation profile of apicoplast proteins detected from intact-cell CETSA experiments.

Isothermal dose-response (ITDR) curves demonstrate minimal evidence of stabilisation across proteins of the 50s apicoplast ribosome, the known target of azithromycin.

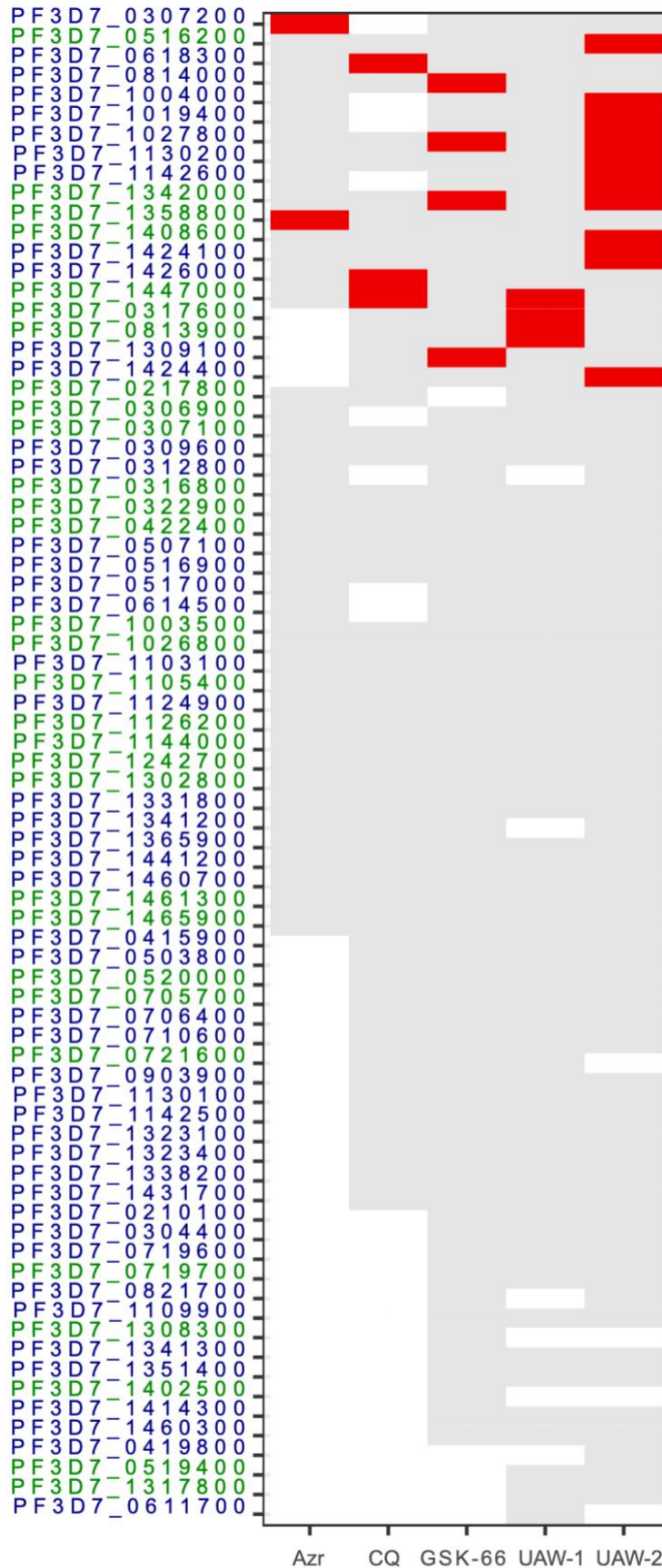
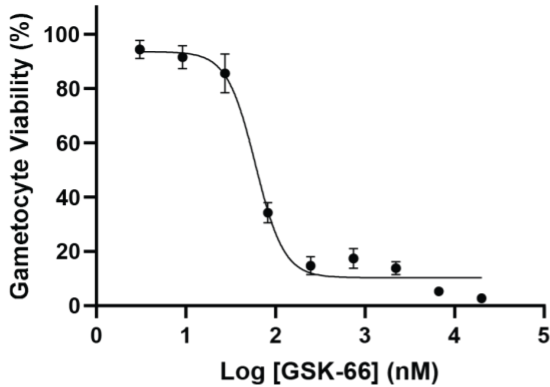


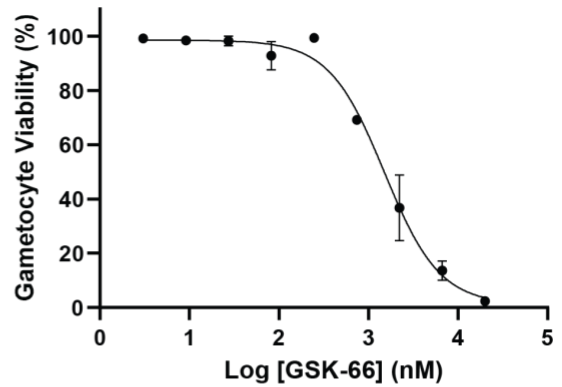
Figure S7. Cytoplasmic ribosome proteins detected for at least one drug in the intact-cell CETSA.

All proteins in blue are components of the 60s ribosomal subunit, whereas those in green constitute the 40s ribosomal subunit. Proteins that were significantly stabilised in the intact-cell CETSA are indicated in red. Proteins in grey exhibited a small thermal shift upon drug treatment but not within the dAUC cut-off of 3 used in this study to be considered a hit.

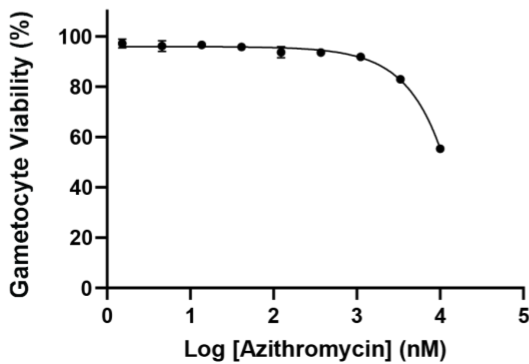
a) Immature gametocytes (>90% stage II/III)



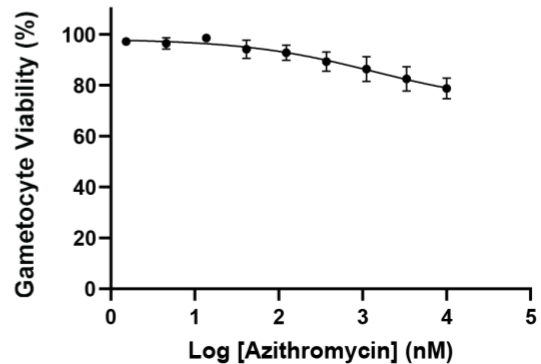
Mature gametocytes (>95% stage V)



b) Immature gametocytes (>90% stage II/III)



Mature gametocytes (>95% stage V)



c)

	GSK-66		Azithromycin	
	Immature	Mature	Immature	Mature
IC ₅₀ (nM ± SEM)	62.29 ± 1.89	1478.7 ± 257.8	ND	ND
Range	3.05 nM – 20 μM	3.05 nM – 20 μM	1.5 nM – 10 μM	1.5 nM – 10 μM
IC ₅₀ n =	3	3	3	3
IC ₅₀ R ²	0.9671	0.9639	0.9736	0.6331
Max inhibition (%)	97.3	97.7	45.2	21.3
Hill slope	-2.727	-1.406	-1.014	-0.5542
IC ₅₀ z-factor	0.90	0.82	0.92	0.84
Methylene blue (% 5 μM)	94.8	90.3	98.6	68.5
MMV048 (% 5 μM)	67.9	65.2	82.9	86.0

Figure S8. Dose response curves of GSK-66 and azithromycin against immature and mature stage gametocytes.

Immature and mature stage gametocytes were treated with GSK-66 (a) or azithromycin (b) for 42 hrs before measurement of luciferase activity. (c) Summary of experimental set-up for IC₅₀ determination and quality control parameters to demonstrate inter-assay reliability across independent experiments for GSK-66 and azithromycin. ND = IC₅₀ not determined.

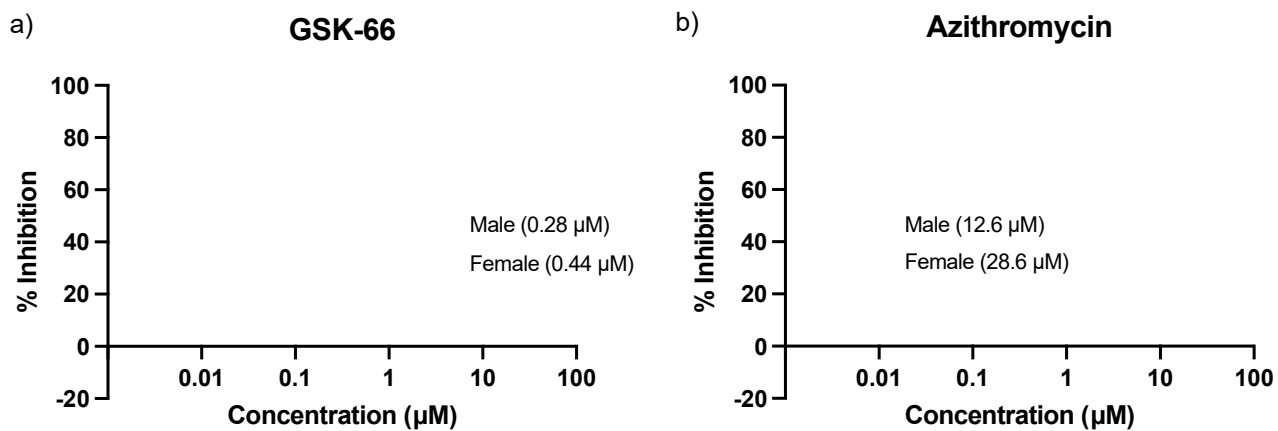


Figure S9. Dose response curves of GSK-66 and azithromycin in Dual Gamete Formation assays (DGFA).

Mature stage V gametocytes were incubated with GSK-66 (a) and azithromycin (b) for 48 hrs before induction of gametogenesis. IC₅₀ values are indicated in the brackets, with error bars representing the SEM for four independent experiments.