Figure 1

A

Parasite culture
Drug treatment
Protein extraction
Generation of tryptic peptides
Peptide labelling with isobaric tags
Combine experimental replicates
Phospho-peptide enrichment
Mass spectrometry

Synchronised late schizonts
Split into two equal schizont preps
Vehicle
Compound 2 (2µM; 60 mins)
Prepare lysate TCA precipitate
Tryptically digest
Label with isobaric tag

Experiment 1: Isobaric tag 126 = vehicle : 127 = Comp 2 treated
Experiment 2: Isobaric tag 129 = vehicle : 129 = Comp 2 treated
Experiment 3: Isobaric tag 130 = vehicle : 131 = Comp 2 treated
Experiments 1-3 mixed
Resource Q Fractionation
Phospho-peptide enrichment IMAC/TiO2

LC-MS/MS conducted on an Orbitrap (Velos)

B

Example of a phospho-peptide DOWN-REGULATED by Compound 2

C

Example of a phospho-peptide NOT changed by Compound 2
Figure 3
Figure 4

A. PIMyoA Non-Phosphorylated (I1TQDDNER)

B. PIMyoA Phosphorylated (R1VNSVEAFDK)

C. Wild Type + MyoA-pS19

D. TQ-Mutant + MyoA-pS19

Loading Control
Figure 5
Figure 6

A) Image analysis of protein expression over time post infection.

B) Immunostaining images showing CDPK1-pS64 and CDPK1-HA under control and compound 2 treated conditions.

C) Close-up images of CDPK1-pS64 and CDPK1-HA in the merge.

D) Close-up images of CDPK1-pS64 and CDPK1-HA in the merge.

E) Immunostaining images of Schizont and Free Merozite with DAPI, CDPK1, CDPK1-pS64, and Merge.

F) Immunostaining images of Schizont and Free Merozite with DAPI, CDPK1, CDPK1-pS64, and Merge.
Figure 8

A

Fraction

7 8 9 10 11 12 13

High Mwt Complex

Monomer

CDPK1-HA

B

Wild Type

Control

Compound 2

p<0.05

Percentage of Total

High Mwt Complex

Monomer

C

PfPKG_{T618Q}

Control

Compound 2

p<0.05

Percentage of Total

High Mwt Complex

Monomer
Figure 10

Key PPKG
Target Proteins

Key Merozoite
- Red blood cell membrane
- Apical localisation of CDPK1-pS64

Schizont → Egress → Free Merozoite → Invasion

Second Messenger: Ca^2+, cGMP
Protein Kinase

PIODPK1 PSEA-1
PIODPK1 PIPK1
PIODPK1 PIPK1
PIODPK1 PIPK1

Red blood cell

PIODPK1 PIPK1
PIODPK1 PIPK1
PIODPK1 PIPK1
PIODPK1 PIPK1

Dynein-related AAA-type ATPase
Membrane skeletal protein IMC1-related
Coronin-binding protein