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DOI: 10.1038/ncomms8285

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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 2
Figure 3
Figure 4

A. 

B. 

C. 

D. 

MyoA-pS19

Loading Control

MyoA-pS19

Loading Control
Figure 5
Figure 6

A. Western blot analysis showing protein expression levels of CDPK1 and EBA-175 at different time points post-infection (8, 16, 24, 32, 40, 48 hours). The blots are labeled for Total CDPK1, CDPK1-pS64, and EBA-175.

B. Immunofluorescence images comparing control and Compound 2-treated samples. The images show CDPK1-pS64, CDPK1-HA, Merge, and DIC channels.

C. Enlarged view of images from B, focusing on the CDPK1-pS64 and CDPK1-HA channels.

D. Further analysis of CDPK1 and CDPK1-pS64 localization in the treated samples, showing Merge channel images.

E. Immunofluorescence images of Schizont and Free Merozoite samples, showing DAPI, CDPK1, CDPK1-pS64, and Merge channels.

F. Enlarged view of images from E, highlighting the localization of CDPK1 and CDPK1-pS64 in the Schizont and Free Merozoite samples.
Figure 7

A

B
Figure 9

A. Wild type parasites

B. PIPK\textsuperscript{G1618Q} mutant parasites
Figure 10

Key PIPKs
Target Proteins

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

Schizont

Egress

Free Merozoite

Invasion

Second Messenger
Protein Kinase

Ca^{2+}

cGMP

PICDPK1
PISEA-1

PICDPK1
PIPKG

PICDPK1
PIPKG

PICDPK1
PIPKG

Red blood cell
Membrane skeletal protein IMC1-related
Coronin
Coronin-binding protein

PIKAAP40, PIKAAP45, PnMyoA
Dynein-related AAA-type ATPase