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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
Vehicle
Compound 2 (2μM:60mins)

Split into two equal schizont preps
Tryptically digest
Label with isobaric tag
Experiments 1-3 mixed
Phospho-peptide enrichment
LC-MS/MS conducted on an Orbitrap (Velos)

Experiment 1: Isobaric tag 126 = vehicle : 127 = Comp 2 treated
Experiment 2: Isobaric tag 128 = vehicle : 129 = Comp 2 treated
Experiment 3: Isobaric tag 130 = vehicle : 131 = Comp 2 treated

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

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

PIPKG-Dependent Phosphorylation In Schizonts

Key:
- Membrane
- Microtubules
- Rho
- Endomembrane System
- Nuclear
- Mitochondria
- Parasitophorous Vacuolar Membrane
- Red Blood Cell Membrane
Figure 3
Figure 4

A. PMyOA Non-Phosphorylated (I1TQDDNER)

B. PMyOA Phosphorylated (R1VpSNVEAFDK)

C. Comp 2

D. Comp 2
Figure 5
Figure 6

A

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<tr>
<th>Hours post infection</th>
<th>8</th>
<th>16</th>
<th>24</th>
<th>32</th>
<th>40</th>
<th>48</th>
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<td>Total CDPK1</td>
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B

Control

Compound 2 Treated

C

Merge

D

Merge

E

Schizont

F

Free Merozite
Figure 8

A

![High Mwt Complex and Monomer](image)

B

Wild Type

- Percentage of Total
- High Mwt Complex
- Monomer

- Control
- Compound 2

p<0.05

C

PfPKG^{T618Q}

- Percentage of Total
- High Mwt Complex
- Monomer

- Control
- Compound 2
Figure 9

A  Wild type parasites

B  PIPKGT618Q mutant parasites

Percentage Invasion

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<th>Comp 2</th>
<th>Control</th>
<th>Comp 2 Control</th>
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<tr>
<td>B</td>
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Figure 10

Schizont

Key PIPK1
Target Proteins

Second
Mesenger

Protein Kinase

PI3P
PIPK1
PMK1

Ca2+

Egress

Key Merozoite

Apical localisation of CDPK1-pS64

Figure 10