

1 **Supplementary figure legends**

2 **Figure S1. CDPK7 PH domain binds to monophosphate phosphoinositides.** (A) Purified
3 recombinant GST-PH protein was assessed by SDS-PAGE, Coomassie blue stained gel. The
4 expected mass of GST-PH is 37 kDa. Mass ladder in kilodaltons (kDa). (B) Binding of
5 recombinant GST or GST-PH proteins to varying types of phospho (P)-inositides were
6 assayed. 4 different experiments were performed from 2 different batches of recombinant
7 proteins and one representative experiment is shown.

8

9 **Figure S2. TgCDPK7 knockdown did not affect microneme secretion, egress and gliding**
10 **motility but modestly impacts on parasite host cell invasion.** (A) Invasion assay performed
11 on TATi1-ku80ko or cdpk7i strains \pm ATc. The percentage of host cell invaded parasites is
12 represented on the graph. Values are means \pm SD for three independent experiments.
13 Statistical significance was evaluated using the student's t test. $**P=0.0023$. (B) Western blot
14 analysis of microneme protein MIC2 secretion after induction with ethanol. Dense granule
15 protein-1 (GRA1) shows constitutive secretion of dense granules. MIC2c: cellular and
16 MIC2s: Secreted. (C) A23187 induced egress assay on TATi1-ku80ko or cdpk7i strains \pm
17 ATc. Values are means \pm SD for three independent experiments. (D) Gliding assay performed
18 on TATi1-ku80ko or cdpk7i strains treated with ATc. White arrows show trails from both
19 circular and helical gliding stained with anti-SAG1 antibodies. Scale bars represent 2 μ m.

20

21 **Figure S3. TgCDPK7 knockdown shows intracellular growth defect.** (A, B and C)
22 Intracellular growth of TATi1-ku80ko and cdpk7i cultivated in presence or absence of ATc
23 for 24 hours or 48 hours or 96 hours, respectively. Numbers of parasites per vacuole (X axis)
24 were counted 24 hours after inoculation. The percentages of vacuoles containing varying

25 numbers of parasites are represented on the Y-axis. Values are means \pm SD for three
26 independent experiments.

27

28 **Figure S4. Ultrastructural analysis of TATi1-ku80ko parasites treated with ATc during**
29 **3 days.** (A) Vacuole containing 4 parasites. Rhoptries (R) and micronemes (Mc) are seen at
30 the apical end of the parasites. The residual body (RB) can be seen at the posterior end, and
31 contains no trace of intact apical organelles. (B) and (C) Dividing parasites. Daughter cells
32 (DC) individualizing inside the mother cell in an up orientation.

33

34 **Figure S5. TgCDPK7 knockdown did not disturb the localization of mitochondrion and**
35 **dense granule markers.** *cdpk7i* parasites were incubated with ATc for 3 days. Anti-GRA2
36 and anti-F1-ATPase were used to detect dense granules and mitochondrion respectively. Scale
37 bar = 2 μ m.

38

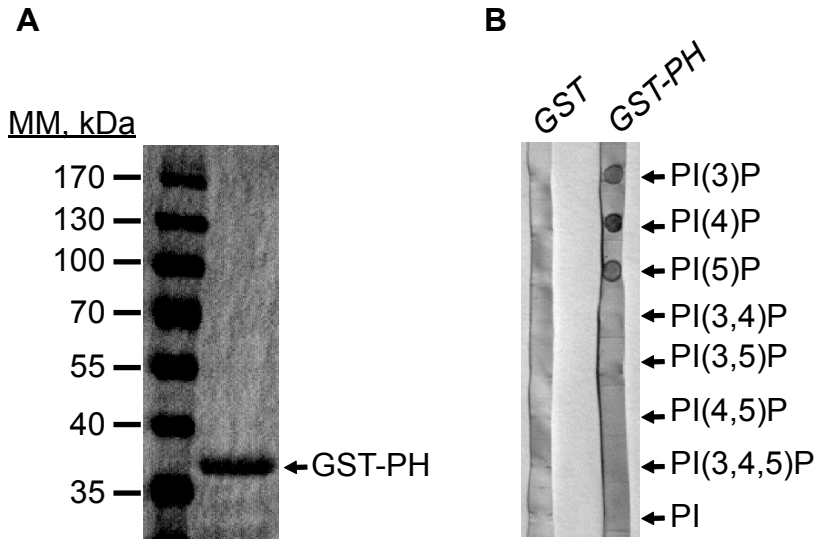
39 **Figure S6. TgCDPK7 knockdown creates unknown parasite bodies and accumulates**
40 **nuclear dense material near or in association with the nuclear envelope.** (A) and (B):
41 IFAs of *cdpk7i* parasites incubated with ATc for 3 days. Anti-ISP1 and anti-IMC1 were used
42 to detect the IMC apical cap and the full IMC respectively. Dashed lines delineate the
43 unknown parasite bodies which are located apically to (in A) or at the opposite end (in B) of
44 developing parasites within a given vacuole. Scale bar = 2 μ m. (C) Thin section electron
45 micrograph was taken from *cdpk7i* that had grown for a total of 3 days in presence of ATc. A
46 vacuole with 1 parasite is presented showing an attached abortive parasite body to the
47 posterior of the parasite. Scale bar, 2 μ m. (D) Scoring of vacuoles showing unknown parasite
48 bodies by IFA using anti-IMC1 antibodies. TATi1-ku80ko or *cdpk7i* parasites were grown in
49 presence or in absence of ATc during 3 days. Data are mean values \pm SD for three

50 independent experiments. Statistical significance was evaluated using the student's t test.
51 ***P=0.0003 (without unknown parasite bodies), ***P=0.0003 (with unknown parasite
52 bodies). (E) Two thin section electron micrographs were taken from *cdpk7i* parasites that had
53 grown for 3 days in presence of ATc. Two nuclei are presented showing the accumulation of
54 nuclear dense materials. Scale bar, 2 μ m and 1 μ m respectively. N: nucleus, N.D.M: nuclear
55 dense material.

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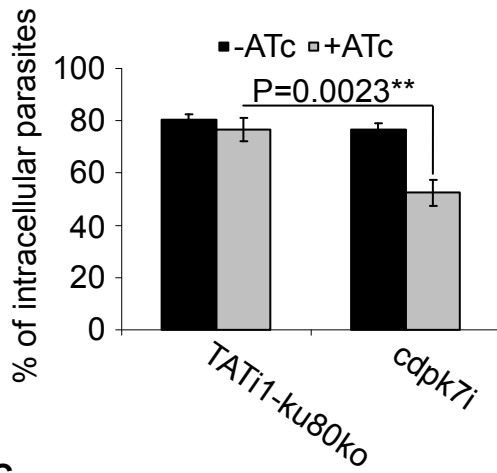
57 **Figure S7. TgCDPK7 knockdown does not impair the DNA replication cycle.** (A) and
58 (B): DNA content analyses of the control parental line TATi1-ku80ko and *cdpk7i* grown for
59 96 hours in the presence and absence of ATc.

Supplementary figure 1

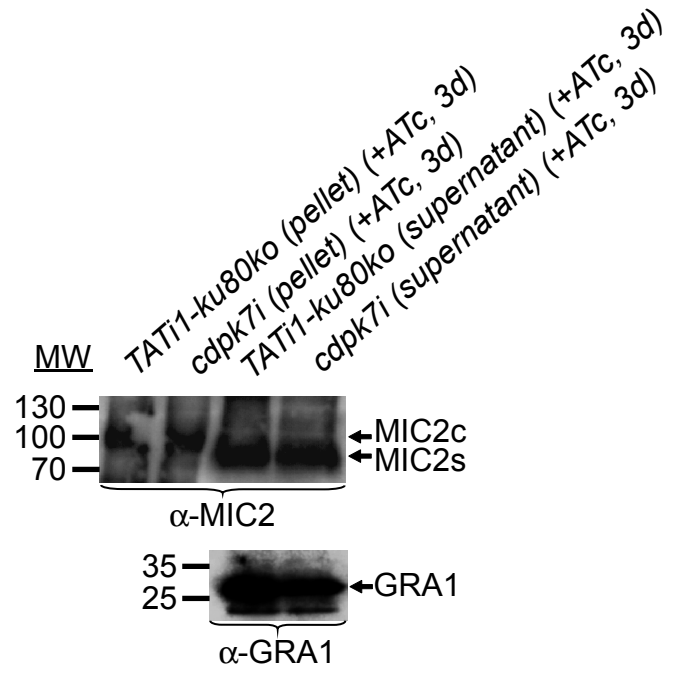


Supplementary figure 2

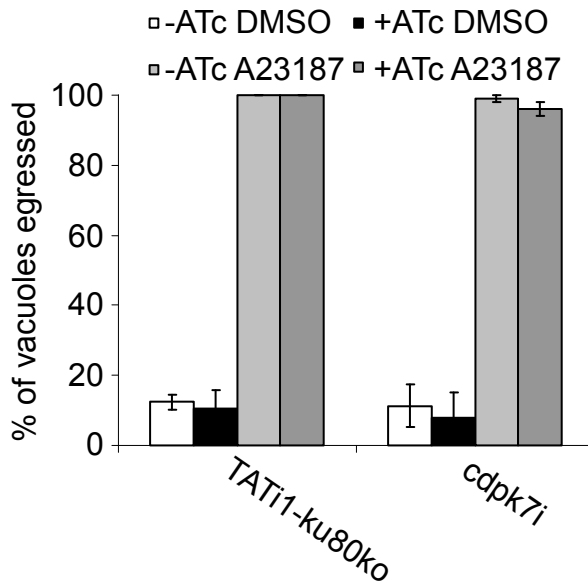
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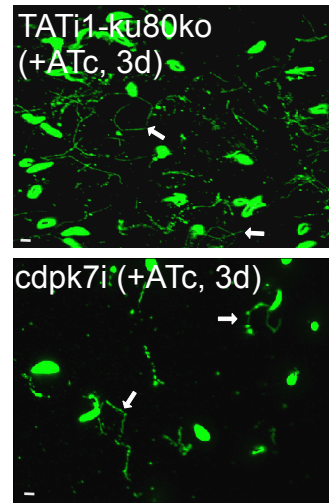
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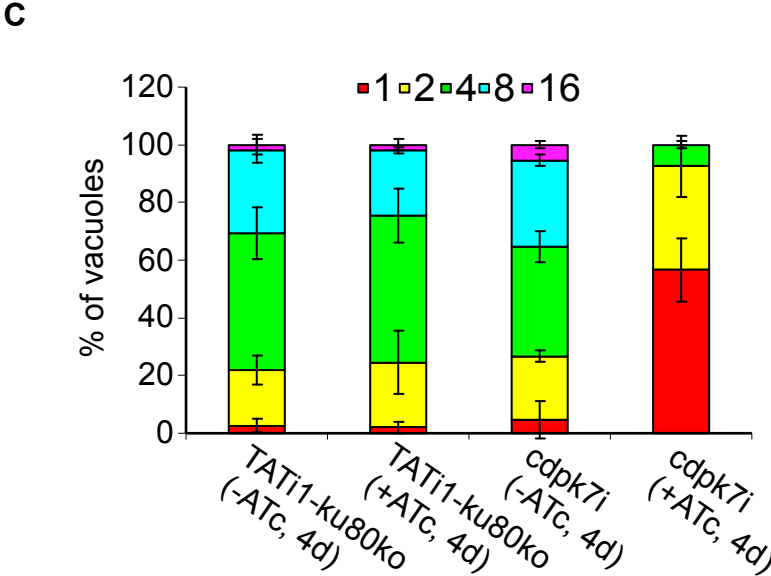
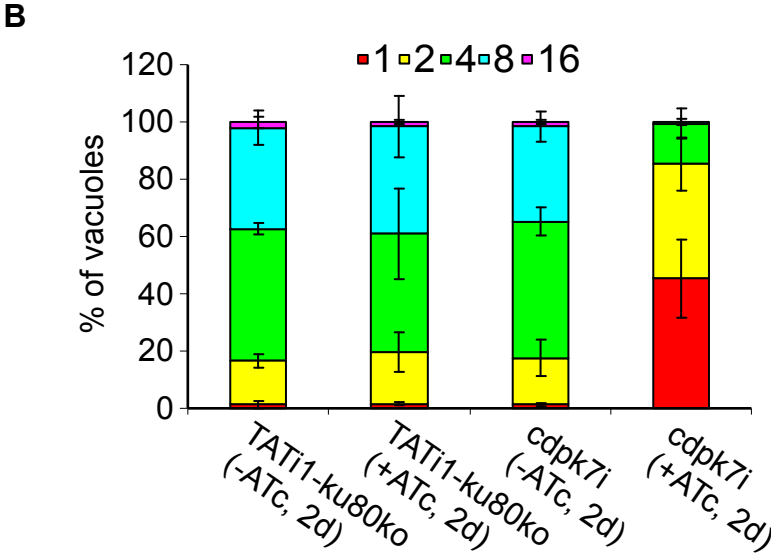
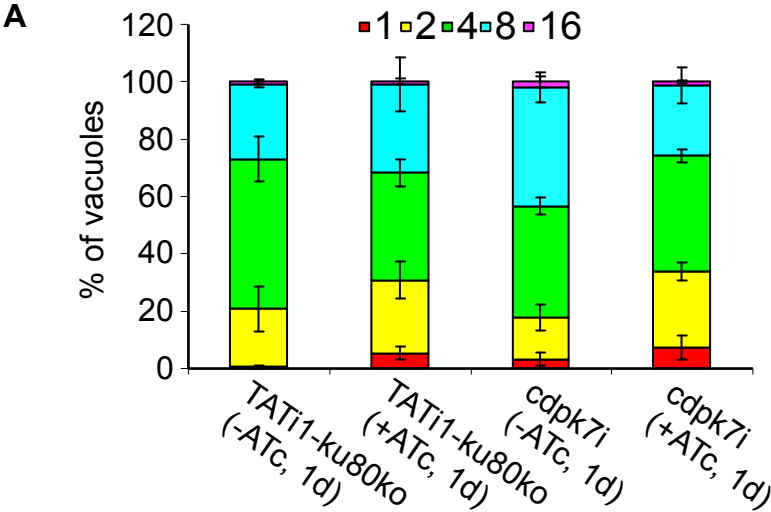
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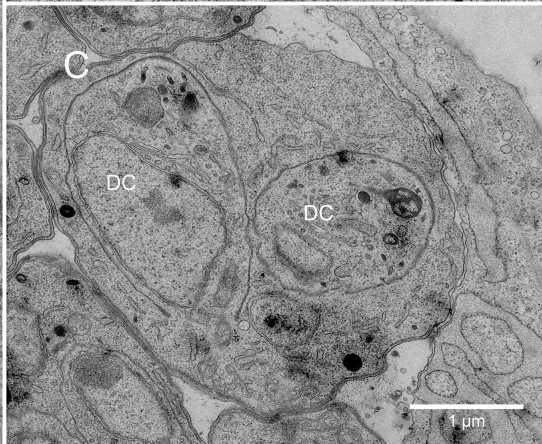
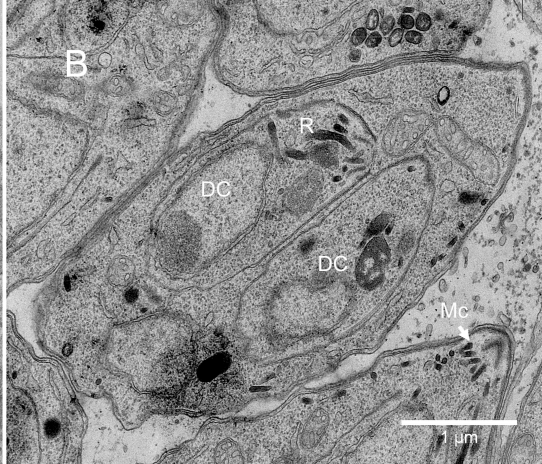
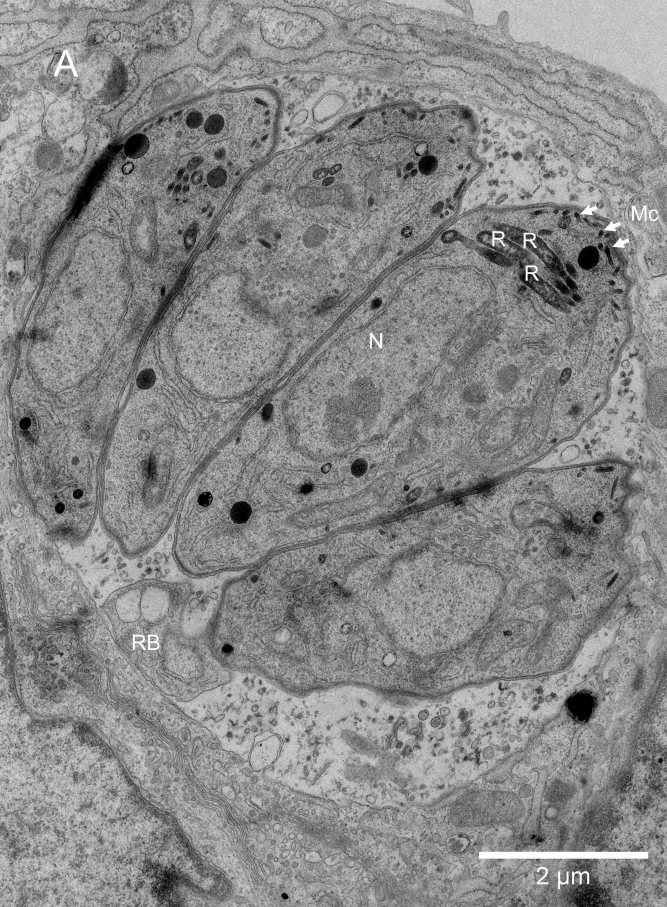


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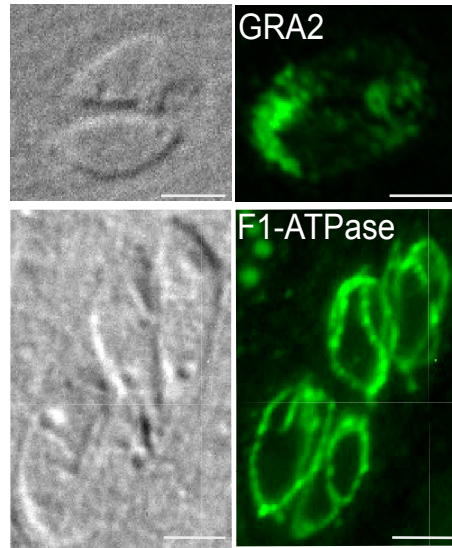


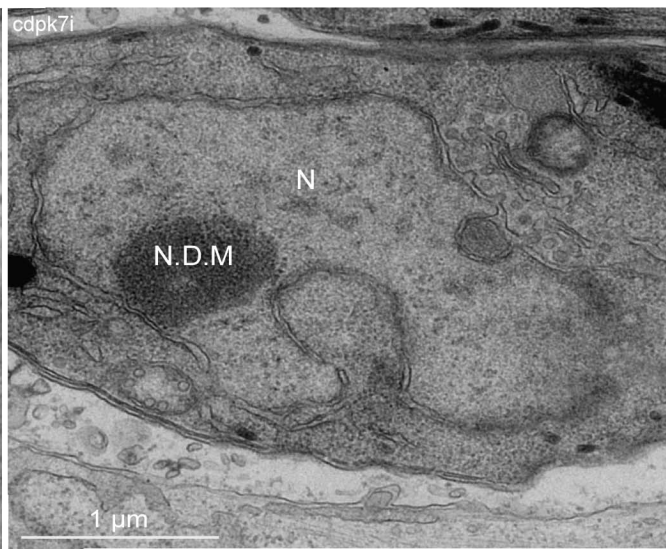
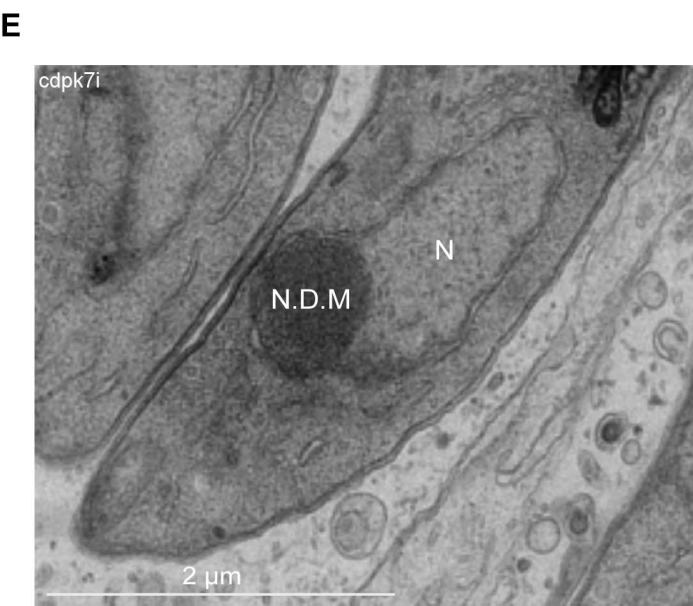
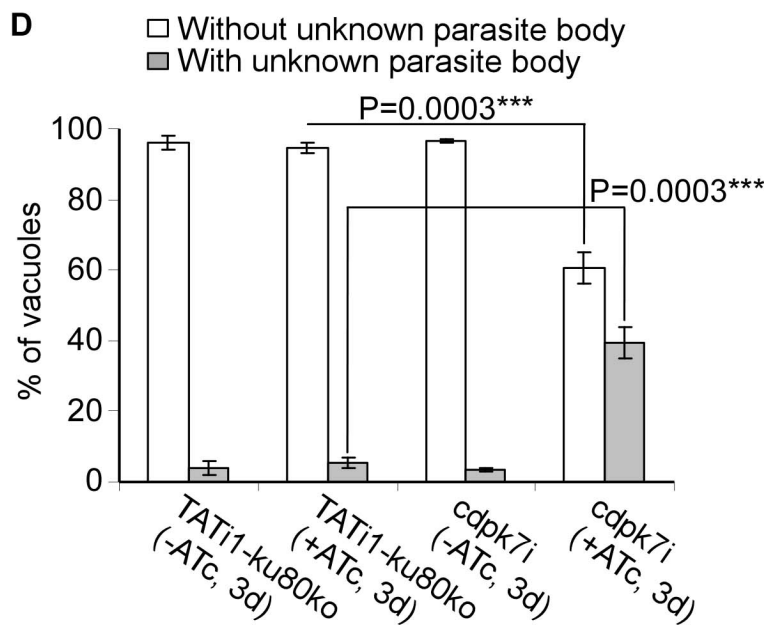
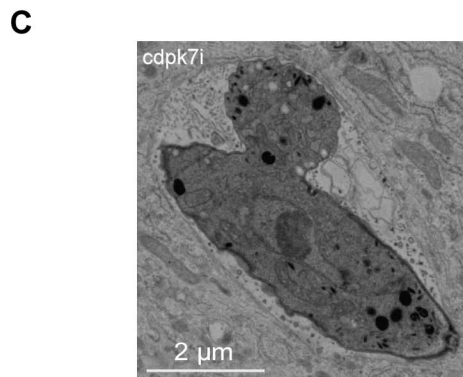
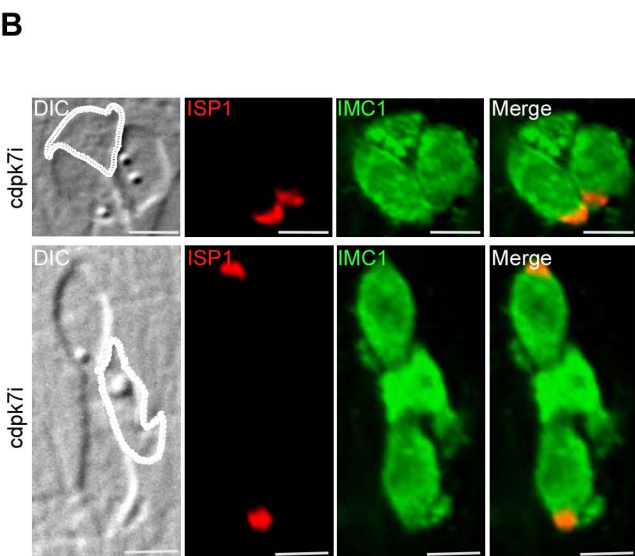
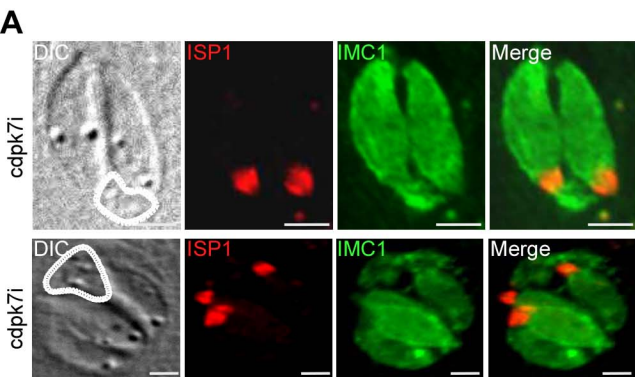
Supplementary figure 3

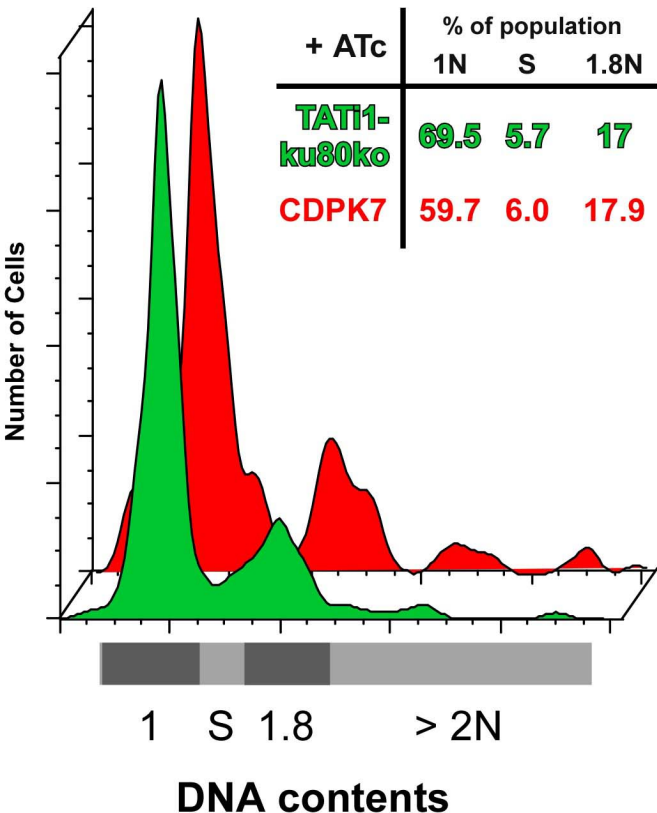




Supplementary figure 5





A**B**