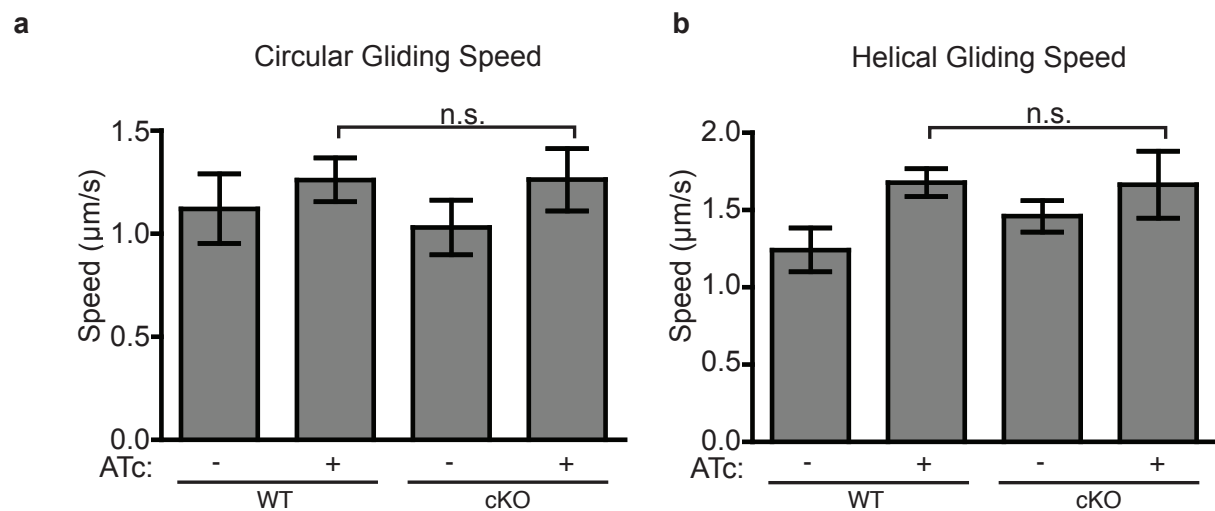
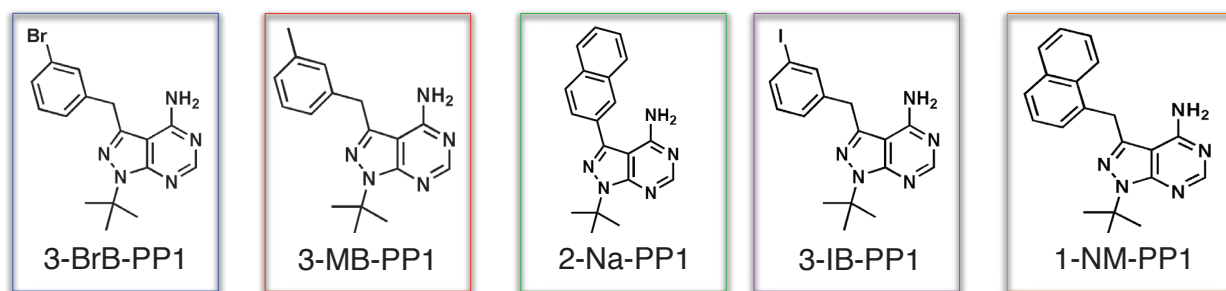
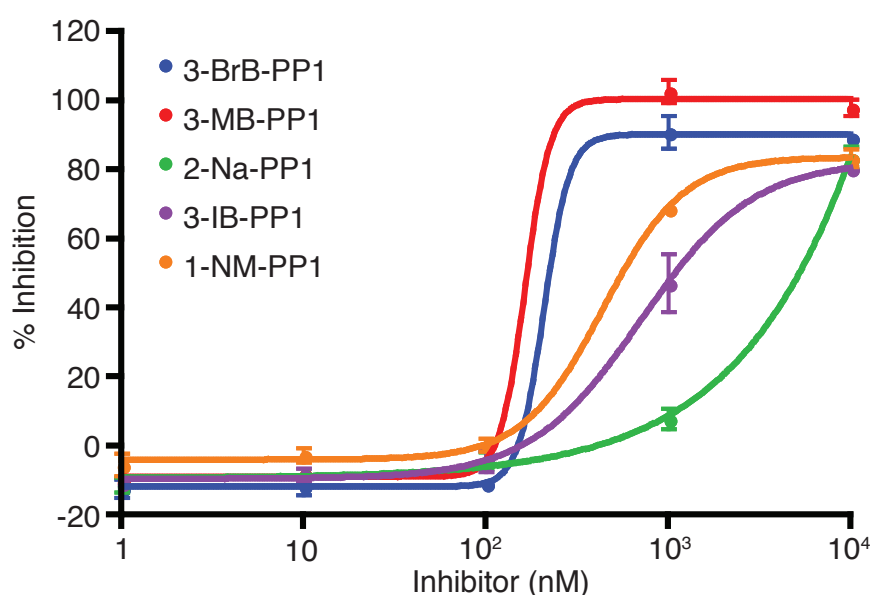


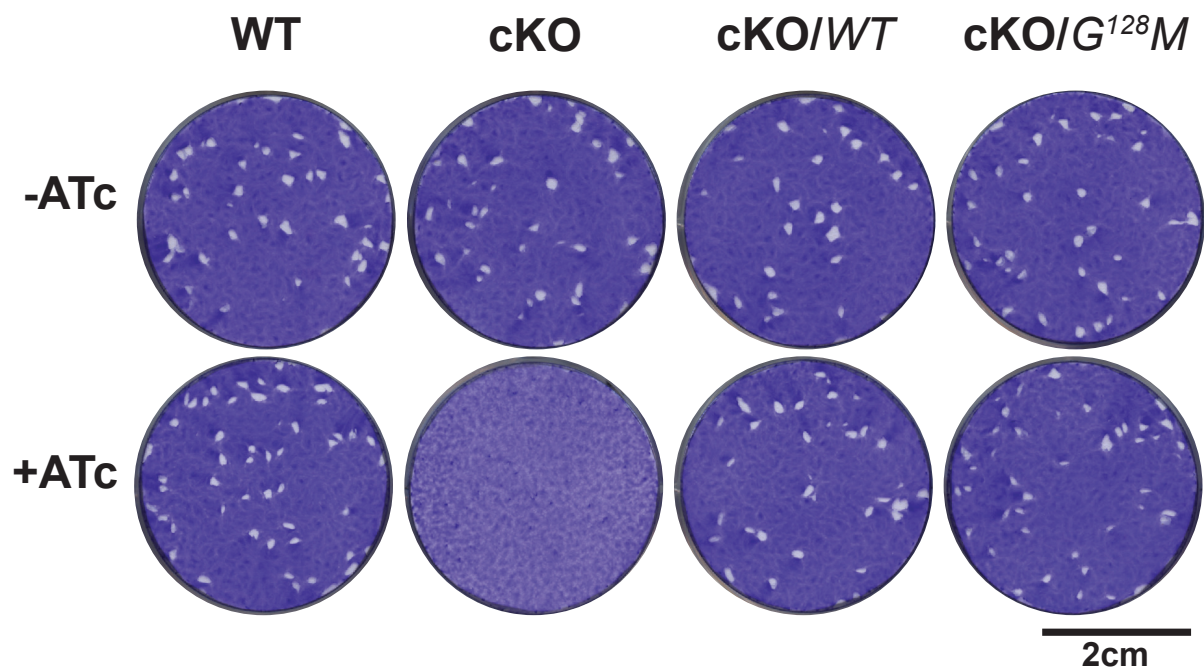
SUPPLEMENTARY INFORMATION



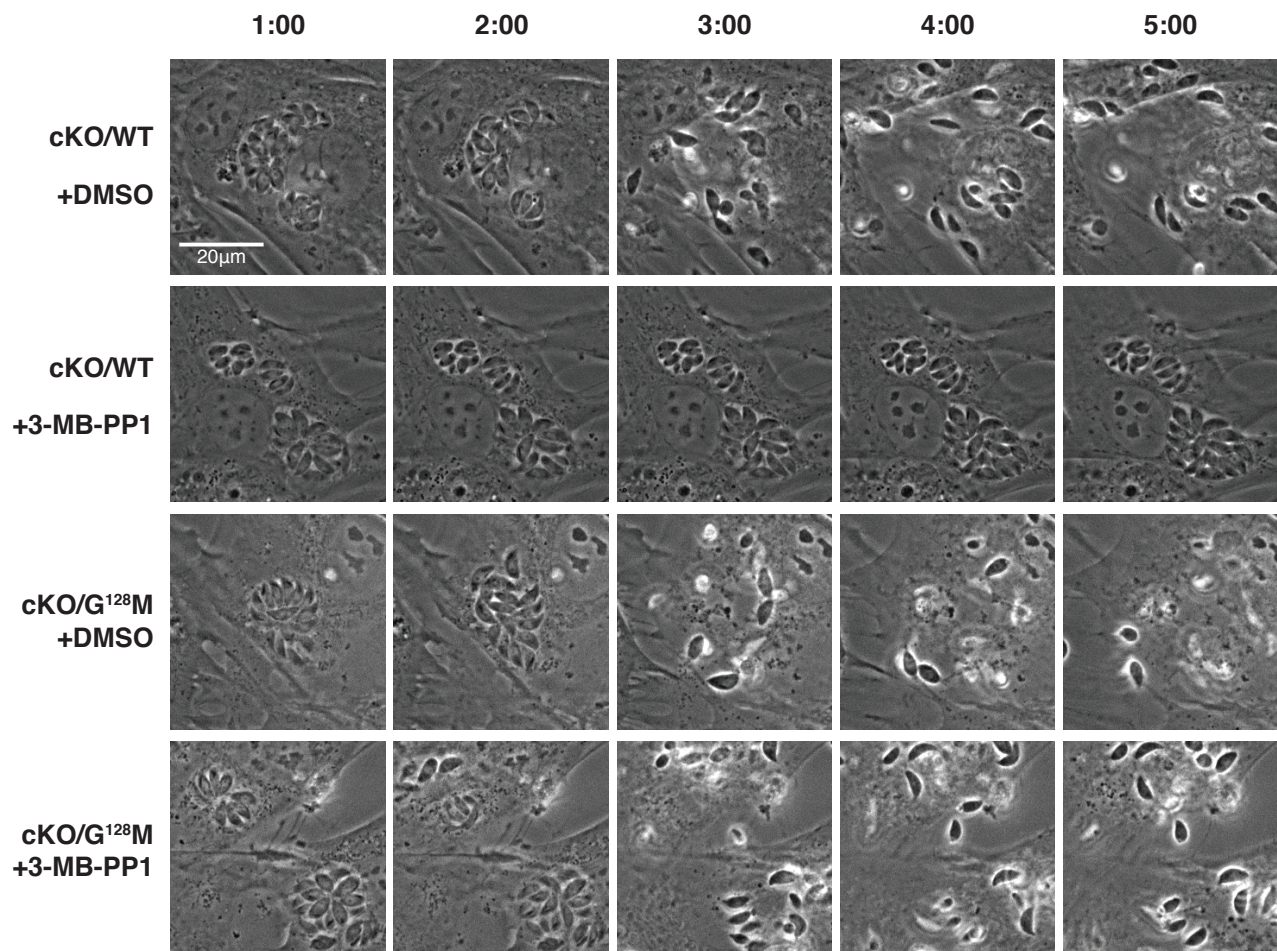
Supplementary Figure 1 | cKO parasites capable of gliding after TgCDPK1 shutdown moved at the same speed as WT parasites under the same conditions. Gliding motility was quantified by video microscopy. Speed was calculated by measuring a parasite's displacement and dividing by the time taken for that displacement during **a**, circular or **b**, helical gliding. Student's *t* test; n.s. > 0.05, mean $\pm$ s.e.m., *N* = at least 5 independent trajectories.

a**b**

Supplementary Figure 2 | PP1-analogues inhibit host-cell lysis by *T. gondii*. **a**, Chemical structure of the PP1 analogues tested. **b**, Lysis of host cells after infection with WT parasites at an MOI of 1 in the presence of different drug concentrations. Values were normalized to uninfected wells (100%) and wells infected in the presence of vehicle (0%); mean $\pm$ s.d., representative experiment. 3-BrB-PP1: 1-tert-butyl-3-(3-bromo-benzyl)-1H-pyrazolo[3,4-d]pyrimidin-4-ylamine; 3-MB-PP1: 1-tert-butyl-3-(3-methyl-benzyl)-1H-pyrazolo[3,4-d]pyrimidin-4-ylamine; 2-Na-PP1: 1-tert-butyl-3-(2-naphthyl)-1H-pyrazolo[3,4-d]pyrimidin-4-ylamine; 3-IB-PP1: 1-tert-butyl-3-(3-iodo-benzyl)-1H-pyrazolo[3,4-d]pyrimidin-4-ylamine; 1-NM-PP1: 1-tert-butyl-3-(naphthalen-1-ylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-ylamine



Supplementary Figure 3 | *WT* and $G^{128}M$ alleles under the regulation of the TgCDPK1 endogenous promoter can complement the TgCDPK1 cKO. Plaque formation in fibroblast monolayers in the presence or absence of ATc for 8 days.



Supplementary Figure 4 | 3-MB-PP1 can block egress in parasites expressing WT but not *G¹²⁸M* alleles of TgCDPK1. Ionophore-induced egress of strains pre-treated with either 3-MB-PP1 or vehicle (DMSO) for 25 min. Time stamp (min:sec) indicates time after calcium ionophore addition.

Supplementary Table 1 | Egress of parasites from host cells following ionophore stimulation

Strain	Experiment	Total Vacuoles	Egressed Vacuoles	Average Time to Egress (min)
cKO - ATc	1	12	12	2.3 ± 0.2
	2	17	17	2.6 ± 0.2
	3	13	13	2.8 ± 0.6
cKO + ATc	1	10	0	> 9.5
	2	17	0	>9.5
	3	18	1	>9.5

Ionophore-induced egress of cKO parasites grown in the presence or absence of ATc for 72 h. Mean average time to egress ± s.d.

Supplementary Table 2 | *In vitro* IC₅₀ of 3-MB-PP1 against recombinant enzymes

Enzyme	Gatekeeper Residue	IC ₅₀ (nM)
TgCDPK1	G ¹²⁸ G	3
	G ¹²⁸ M	>10 ⁴
c-Src	T ³³⁸ T	2000
	T ³³⁸ G	3
	T ³³⁸ A	10

In vitro sensitivity of different kinases to 3-MB-PP1 shows that small gatekeeper residues (glycine or alanine) render kinases significantly more sensitive than those harboring bulkier residues (methionine or threonine). IC₅₀ values were determined for *WT* and mutant recombinant proteins from a dose response curve as described in the materials and methods.