SUPPLEMENTARY FIGURE LEGEND

Supplementary Figure 1: Effect of MG132 on sporozoite viability. Viability of sporozoites after treatment with different concentrations of MG132 was determined, in two independent experiments. Approximately 50-100 sporozoites were microscopically examined for each condition. Percentage of viable sporozoites was determined as the fraction of total that excluded propidium iodide. Heat-killed sporozoites were used as positive control (data not shown).

Supplementary Figure 2: Inhibition of PfPKG does not have a global effect on protein degradation. Western Blot analyses for total levels of ubiquitinated proteins in tightly synchronized late stage *P. falciparum* 3D7 schizonts treated with vehicle (untreated), MG132 (100 nM) or TSP (2 μM) for 8h under standard culture conditions. *P. falciparum* EF1α was probed as loading control.

Supplementary Figure 3: Consensus phosphorylation site of PfPKG. A) A positional scanning peptide library approach was used to determine amino acid residues preferred by PfPKG at each of 9 positions surrounding the phosphorylation site in peptide substrates. The library consists of 182 peptide mixtures, each of which contains the indicated amino acid fixed at the indicated position, while the remaining 8 positions are degenerate mixtures of amino acids. Results shown are from two independent screens of the peptide libraries. B) Reaction rates of PfPKG, using the BAD WT peptide and its derivatives (left), demonstrate a strong preference for Arg at the -5 position and a weaker preference for basic amino acids at the -2 position Reaction rates of PfPKG, using Aktide peptides containing either Ser or Thr as the phosphorylated residue (right), demonstrate a preference for Thr as the phosphoacceptor residue. Phosphorylation by PfPKG is cGMP-dependent and inhibited by TSP (50 nM). Results shown are from a representative experiment. C) Reaction rates of peptides from RPT1 (left) and PB_090940 (right) that contain phosphorylation consensus sites for PfPKG. In the RPT1 peptide, substitution of Arg at the -2 position or a hydrophobic residue at the +1 position significantly decreases phosphorylation by PfPKG. In

contrast, substitution of a Thr at the +2 position does not have a significant effect. Phosphorylation of the PB_090940 peptide is dependent on the Ser_{450}, as predicted by the PfPKG consensus. Results shown are from a representative experiment.

SUPPLEMENTARY TABLE LEGEND

Potential direct substrates identified in the PfPKG-dependent phosphoproteome of schizonts (12) and PbPKG-dependent phosphoproteome of ookinetes (10), PfRPT1 and PfHSP90. Proteins that undergo phosphorylation in a PfPKG or PbPKG dependent manner were identified from data reported in PfPKG-dependent phosphoproteome of schizonts (12) and PbPKG-dependent phosphoproteome of ookinetes (10). Sites within the putative *P. falciparum* substrate proteins that match the minimal consensus sites for PfPKG are listed. GeneIDs of *P. berghei* proteins that undergo PKG-dependent phosphorylation and carry a potential phosphosite that matches the *Plasmodium* PKG consensus site are listed, followed by the position in the amino acid sequence of the predicted phosphoacceptor Ser or Thr residue.