Phosphatidic acid homeostasis regulated by a type-2 phosphatidic acid phosphatase represents a novel druggable target in malaria intervention

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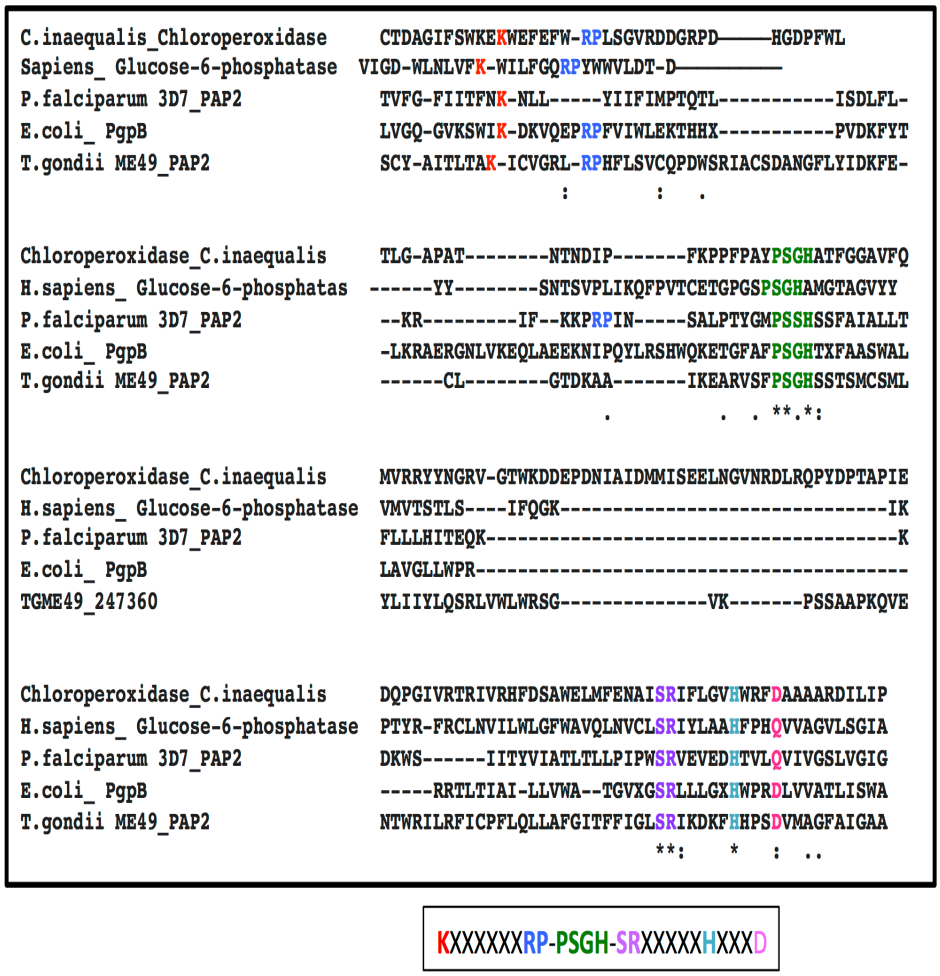
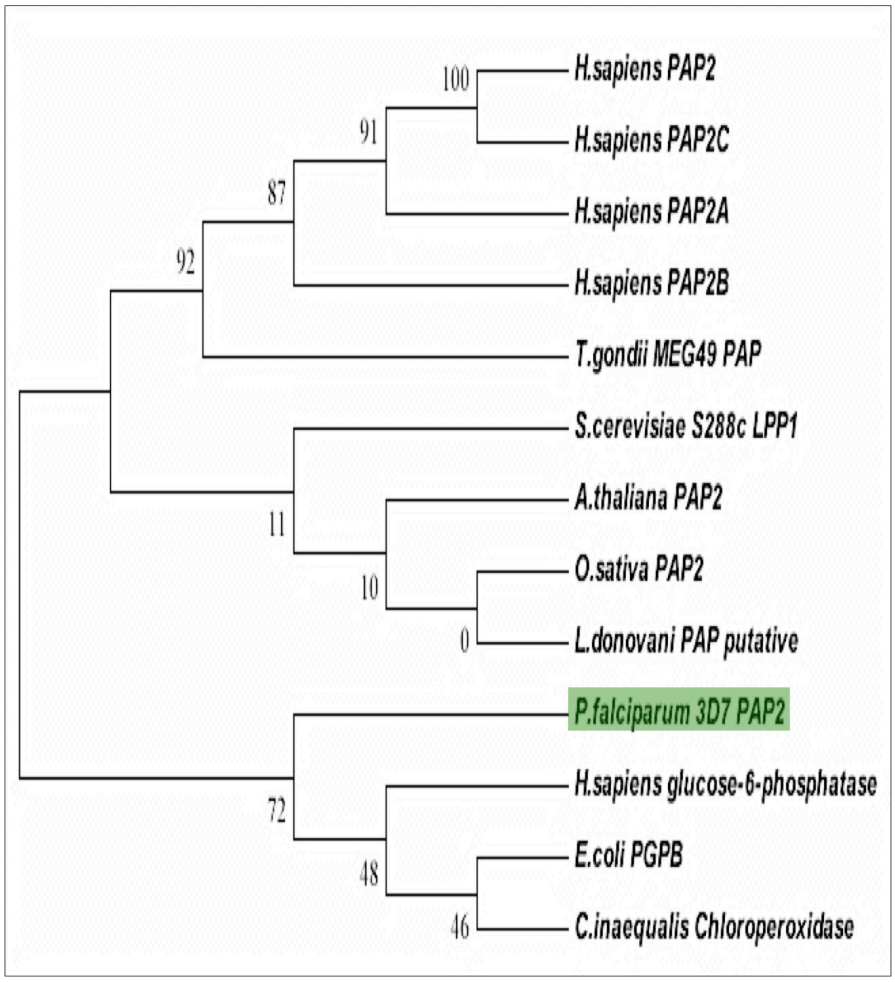
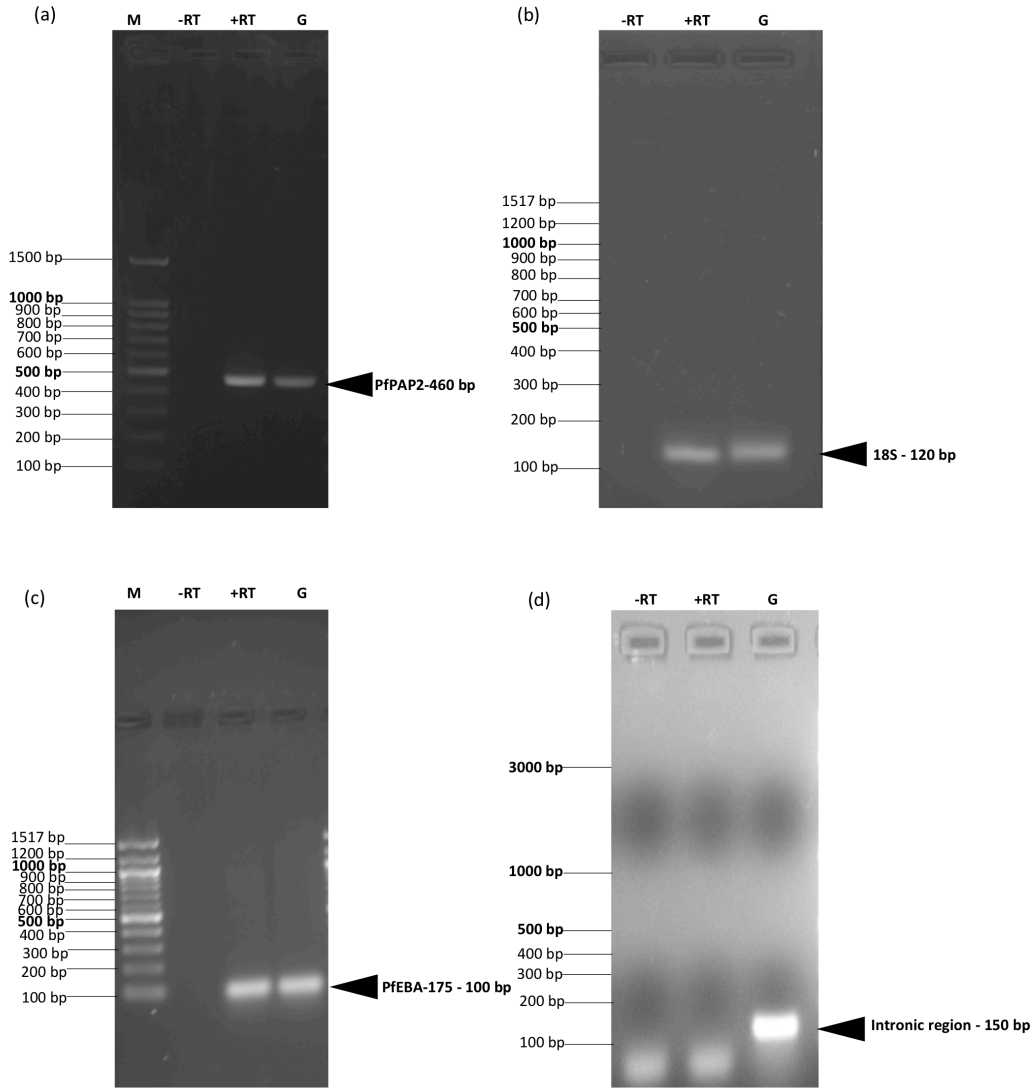
Running title: PfPAP2 is an anti-malarial drug target



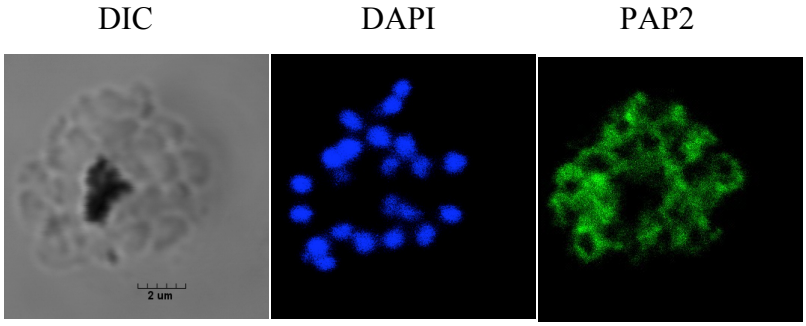
Supplementary figure 1: Multiple sequence alignment of acid phosphatase domain of PfPAP2 with acid phosphatase domain of other known members of PAP2 superfamily. KXXXXXXRP-PSGH-SRXXXXXHXXXD is the characteristic signature motif of acid phosphatase domain, which is conserved across all the members of PAP2 superfamily



Supplementary figure 2: Phylogenetic analysis of PfPAP2. Phylogenetic analysis was performed using protein sequences of known members of PAP2 superfamily using Maximum Likelihood method. Phylogenetic tree was constructed using MEGA 7 software with bootstrap value of 500. Out of all the protein sequences analyzed PfPAP2 is most closely related to the *E.coli* PAP2, ecPgpB.

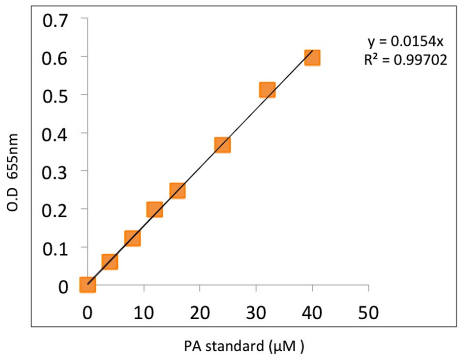


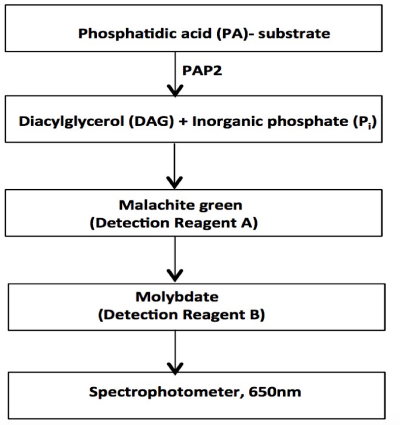
Supplementary figure 3: RT-PCR analysis of PfPAP2 and control genes. (a) A transcript of 460 bp in size confirmed expression of PfPAP2. (b). A 120 bp amplicon of 18S rRNA transcript served as an internal control. (c) PfEBA-175 was used as a late blood stage marker. An amplicon of 100 bp in size confirms EBA-175 expression. (d) Intronic primers were used to amplify a 150 bp region, served as a negative control for genomic DNA contamination.



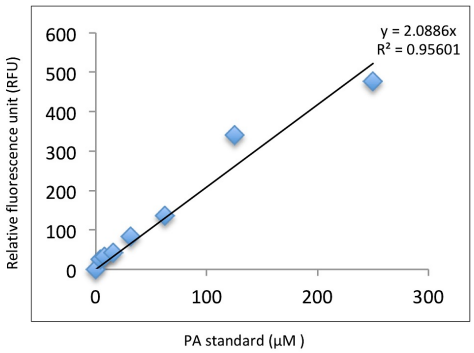
Supplementary figure 4: Immunofluorescence assay of mature schizonts using anti-PfPAP2-peptide antibody. The staining of PfPAP2 is similar to the staining revealed by anti-PfPAP2 antibody.

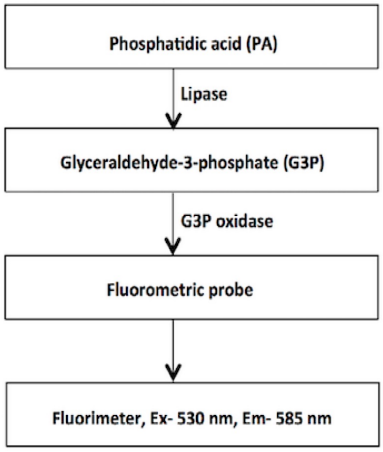
(a)



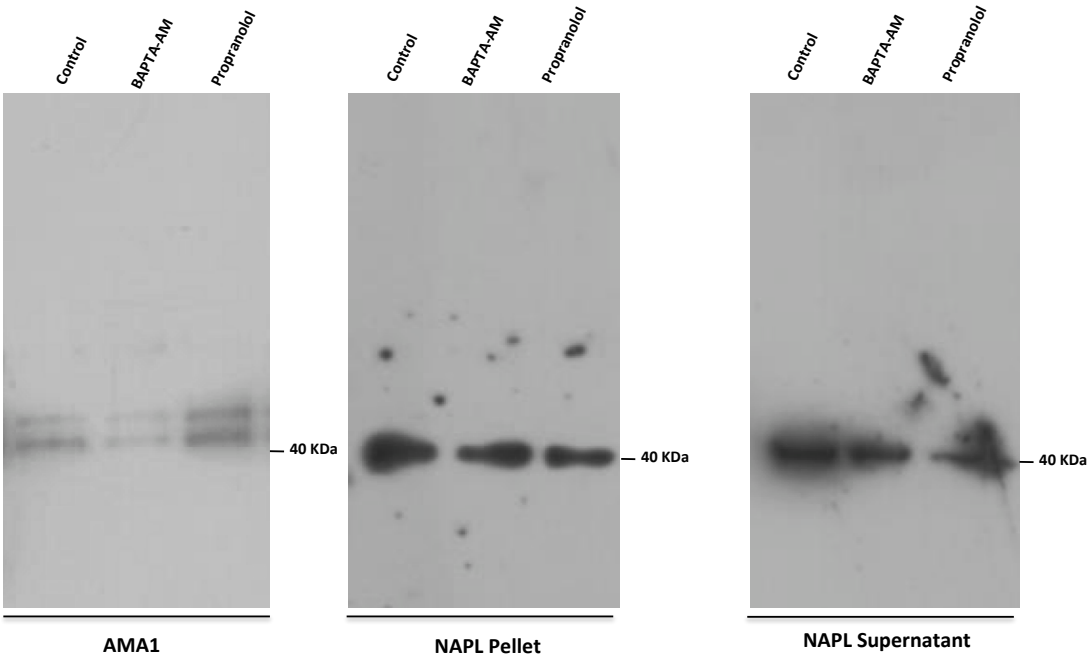


(b)





Supplementary figure 5: Standard curve and schematic representation of the principle for Malachite Green assay (Bioassays systems, USA) (a) and Total PA estimation assay kit (Cell Biolabs, Inc., USA) (b).



Supplementary figure 6: Full blots of merozoite secretion assay for Figure 4 (e).