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

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C.inaequalis Chloroperoxidase
                             CTDAGIFSWKEKWEFEFW-RPLSGVRDDGRPD-----
                                                              -HGDPFWL
Sapiens_Glucose-6-phosphatase_VIGD-WLNLVFK-WILFGQRPYWWVLDT-D-
P.falciparum 3D7 PAP2
                             TVFG-FIITFNK-NLL-----YIIFIMPTQTL-----ISDLFL-
E.coli_ PgpB
                             LVGQ-GVKSWIK-DKVQEPRPFVIWLEKTHHX-----PVDKFYT
T.gondii ME49 PAP2
                             SCY-AITLTAK-ICVGRL-RPHFLSVCQPDWSRIACSDANGFLYIDKFE-
                                            :
                                                    : .
Chloroperoxidase C.inaequalis
                             TLG-APAT-----NTNDIP-----FKPPFPAYPSGHATFGGAVFO
H.sapiens_ Glucose-6-phosphatas -----YY-----SNTSVPLIKQFPVTCETGPGSPSGHAMGTAGVYY
P.falciparum 3D7 PAP2
                              --KR-----IF--KKPRPIN-----SALPTYGMPSSHSSFAIALLT
                             -LKRAERGNLVKEQLAEEKNIPQYLRSHWQKETGFAFPSGHTXFAASWAL
E.coli PgpB
T.gondii ME49_PAP2
                             -----CL-----GTDKAA-----IKEARVSFPSGHSSTSMCSML
                                                         . . **.*:
                                                .
Chloroperoxidase_C.inaequalis
                             MVRRYYNGRV-GTWKDDEPDNIAIDMMISEELNGVNRDLRQPYDPTAPIE
H.sapiens_ Glucose-6-phosphatase VMVTSTLS----IFQGK------IK
P.falciparum 3D7_PAP2
                             FLLLHITEQK-----K
E.coli PgpB
                             LAVGLLWPR-----
TGME49_247360
                             YLIIYLOSRLVWLWRSG-----VK-----PSSAAPKOVE
Chloroperoxidase C.inaequalis
                             DQPGIVRTRIVRHFDSAWELMFENAISRIFLGVHWRFDAAAARDILIP
H.sapiens Glucose-6-phosphatase PTYR-FRCLNVILWLGFWAVQLNVCLSRIYLAAHFPHQVVAGVLSGIA
P.falciparum 3D7 PAP2
                              DKWS-----IITYVIATLTLLPIPWSRVEVEDHTVLOVIVGSLVGIG
E.coli_ PgpB
                             ----RRTLTIAI-LLVWA--TGVXGSRLLLGXHWPRDLVVATLISWA
T.gondii ME49_PAP2
                             NTWRILRFICPFLQLLAFGITFFIGLSRIKDKFHHPSDVMAGFAIGAA
                                                     **: * : ..
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KXXXXXXRP-PSGH-SRXXXXXHXXXD

Supplementary figure 1: Multiple sequence alignment of acid phosphatase domain of PfPAP2 with acid phosphatase domain of other known members of PAP2 superfamily. KXXXXXRP-PSGH-SRXXXXHXXXD 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.



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).