

## **Supplemental information**

### **Targeting *Pf*Prohibitin 2-Hu-Hsp70A1A complex as a unique approach towards malaria vaccine development**

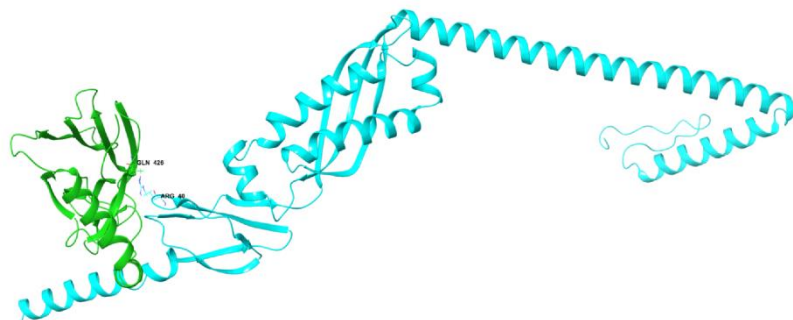
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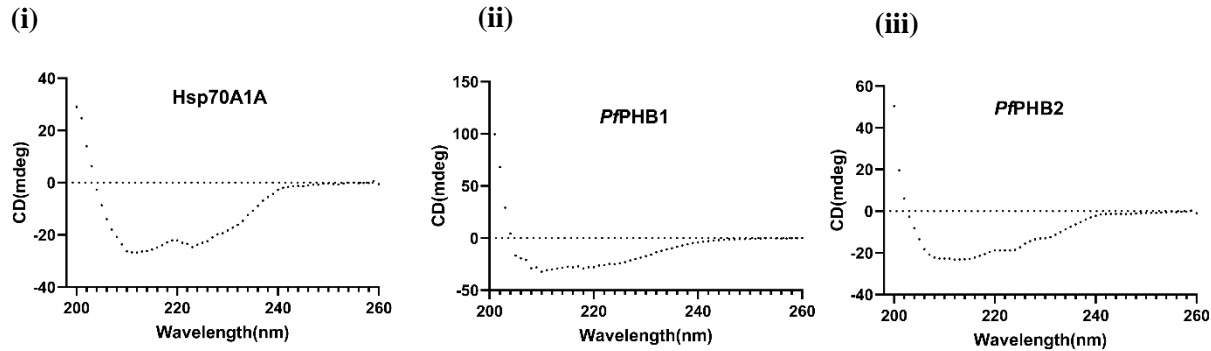


L.major	KFRVEQAEQEKQAAILLAQGEAEAAATLVGNAVKRNPAFLELRGLEAARTIAKTLRDHGNG	268
P.falciparum	KYVVLKAEQEKKSTI IKAQGEAEVAKLIGLAVKDNPAFMELKKIELSREVSNIISKQCN-	289
S.cerevisiae	AFVVDKARQEKQGMVVRAQGEAKSAELIGEAIKKSVDYVELKRLDTARDIAKILASSPN-	286
A.thaliana	KFIVEKAEQDRRSVAVIRAQGEAKSAQLIGQAIANNQAFITLTKIEAAREIAQTIAQSAN-	264
D.melanogaster	VFFVERAKQEKQKIVQAEGEAEAAKMLGLAVKQNPAYLKLRLRAAQSIARTIASSQN-	270
H.sapiens	QFLVEKAKQEQRQKIVQAEGEAEAAKMLGEALSKNPGYIKLRKIRAAQNISKTIATSQN-	269
M.musculus	QFLVEKAKQEQRQKIVQAEGEAEAAKMLGEALSKNPGYIKLRKIRAAQNISKTIATSQN-	269
	: * :*. *::: :: *::*: * ::* * : . : : * : : : : : : * *	
L.major	RYYLSDSDSLYVNVKDLKIDHSGTK-----	292
P.falciparum	KVMLPTDSELLINFTK-----	304
S.cerevisiae	RVILDNEALLLNTVVDARIDGRGK-----	310
A.thaliana	KVYLSNDLLLNLQEMNLEPKK-----	286
D.melanogaster	KVYLSADSLMLNIQDSGFDDMTEKVYKSK-	299
H.sapiens	RIYLTADNLVNLQDESFTTR-----	289
M.musculus	RIYLTADNLVNLQDESFTTRGSDSLIKGKK	299
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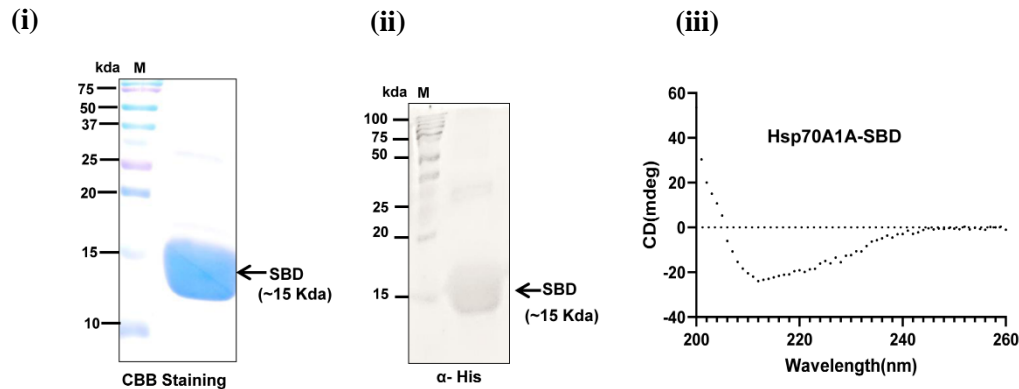
**Figure S2: Multiple sequence alignment of *Plasmodium falciparum* Prohibitin (*Pf*PHB2) with its homologs in other species, related to Figure 1.**



**Figure S3: Cartoon representation of poorly docked complex of *Pf*PHB1 with Hsp70A1A, related to Figure 2.**



**Figure S4: Secondary structure analyses using CD spectroscopy of (i) Hsp70A1A (ii) *Pf*PHB1 and (iii) *Pf*PHB2, related to Figure 2.**

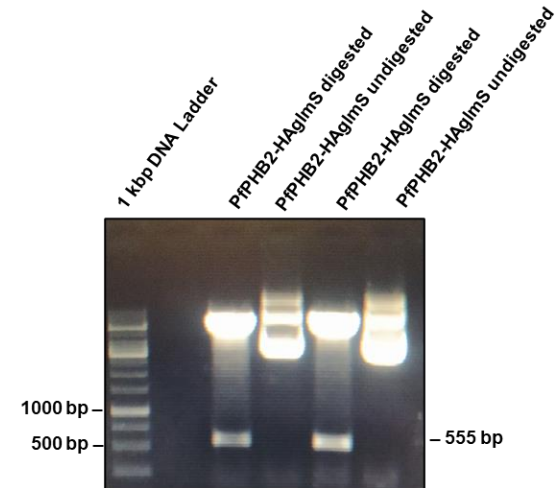


**Fig. S5: Hsp70A1A-SBD protein purification and CD spectroscopy, related to Figure 2.** (i) Coomassie staining (CBB) showing purified recombinant Hsp70A1A-SBD protein construct. (ii) Western blotting to confirm the identity of His tagged Hsp70A1A-SBD protein using mouse anti-His antibody (1:10000) and anti-mice HRP conjugated secondary antibody (1:10000). (iii) The CD spectra of Hsp70A1A-SBD was collected at 260 to 200 nm range and plotted with CD (mdeg) against its wavelength (nm).

(A)

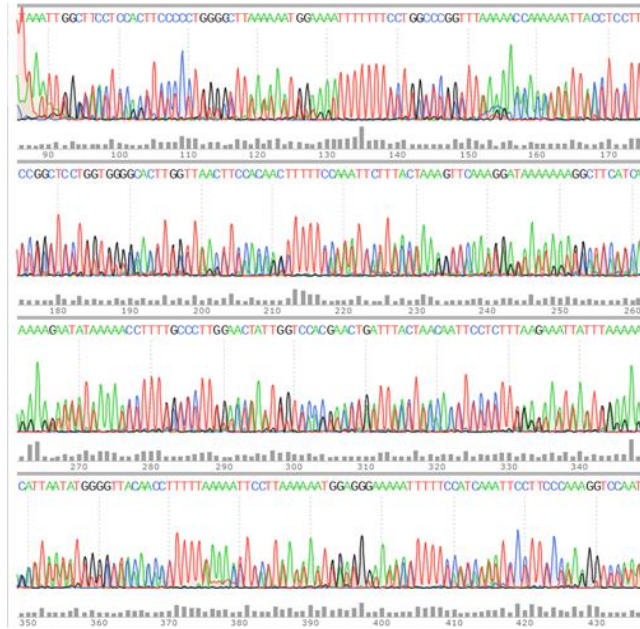
	S. No	Primer Name	Primer sequence (5'-3')
Cloning Primers	1.	PfPHB2_HAglmS_BglII	ACTGAGATCTCAAATGG TAAACATAACATGTCGTG
	2.	PfPHB2_HAglmS_PstI	ACTGCTGCAGTTTTGTAA AATTTATCAACAACG
Episomal and Integration Primers	3.	HAglmS FP	CCGCTAACGTAACAGAC TTAGGAGG
	4.	HAglmS RP	CGGGCGCTATAATTACAG CTGGTCT
	5.	PfPHB2 HAglmS 5' FP	ATGTATAAATTTAAATTT AAATTAAGGAAC

(B)

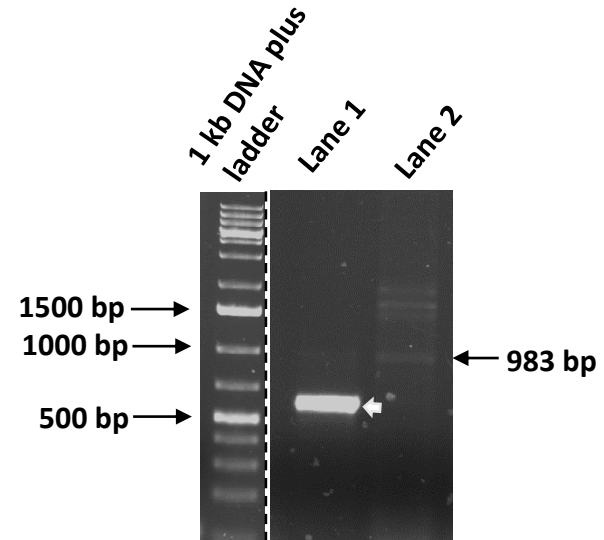


**Figure S6: Cloning of *PfPHB2* in HA-*glmS* vector, related to Figure 5.** (A) Primer details used for cloning of *PfPHB2* in HA-*glmS* vector, and to check for plasmid uptake and its integration in *Pf3D7* genome. (B) Restriction digestion with *Bgl*II and *Pst*I for clone confirmation in HA-*glmS* vector. Desired band size of the fallout (555 bp) was observed.

(i)



(ii)



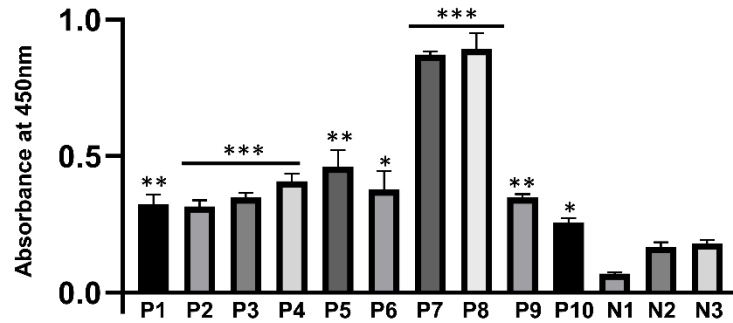
**Figure S7: Validation of *Pf*PHB2 cloning in HA-glmS through sequencing and PCR, related to Figure 5.** (i) Chromatogram plot of the *Pf*PHB2-HAglmS plasmid. (ii) PCR showing confirmation of *Pf*PHB2-HA-glmS plasmid uptake and transfection in *Pf* knock down line. Lane 1 showing the presence of plasmid as episome in transfected *Pf* line. White arrow depict a band of *Pf*PHB2 along with HA-glmS sequence (total band size 657 bp) in *Pf* knock down line using specific primer sets (3 and 4 mentioned in fig. S3 A). Lane 2 showing the integration of *Pf*PHB2 in transfected *Pf* line. Black arrow depicts a band of *Pf*PHB2 (full length; 912 bp) along with HA-glmS sequence (total band size 983 bp) in *Pf* knock down line using primer sets (5 and 4 mentioned in fig. S3 A).

**Table S1: Patient details whose sera were used to detect the presence of anti-*Pf*/PHB2 antibodies, related to figure 7.**

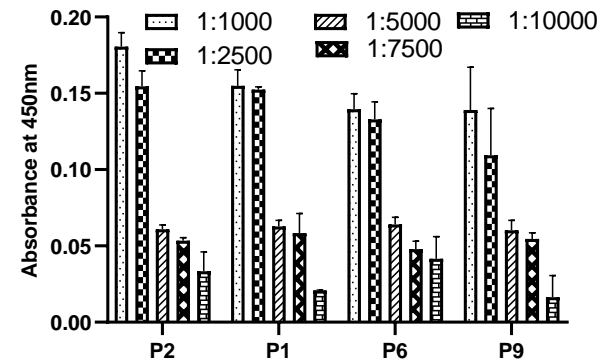
<b>S.NO.</b>	<b>NAME</b>	<b>AGE</b>	<b>PARA (Location)</b>	<b>KIT RESULT (Rapid diagnostic test)</b>	<b>FEBRILE STATUS</b>	<b>MEDICINE STATUS</b>
P1	Dabari Tripura	9	Sumbunath	<i>Pf</i>	3 days fever	Before medicine
P2	Tilyarani Tripura	24	Baharai	<i>Pf + Pv</i>	6 days fever	Before Medicine
P3	Karasa Tripura	35	Khagendra	<i>Pf</i>	1 day fever	Before Medicine
P4	Parenbati Reang	32	Annaram	<i>Pf</i>	2 days fever	After medicine
P5	Kunjari Tripura	26	Dhansingh	<i>Pf</i>	2 days fever	Before medicine
P6	Dhansari Tripura	17	Dhansingh	<i>Pf</i>	1 day fever	Before medicine
P7	Hemali Tripura	8	Acharjee	<i>Pf</i>	3 days fever	Before medicine
P8	Sandali Reang	12	Dongkarai	<i>Pf</i>	2 days fever	Before medicine
P9	Biswajay Tripura	6	Dhansingh	<i>Pf</i>	2 days fever	Before Medicine
P10	Jamirung Reang	32	Dongkarai	<i>Pf</i>	1 day fever	Before medicine



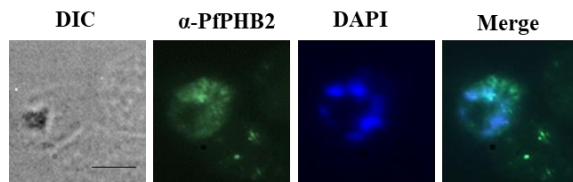
A



B



**Figure S8: Semi-quantitative ELISA showing the presence of anti-MSP1 antibodies and anti-*Pf*PHB2 antibodies in patient sera, related to figure 7.** (A) 200 ng of recombinant MSP1 was coated on ELISA plate followed by its incubation with ten different patient sera (P1- P10) and three naïve sera (N1-N3; 1:1000). Human HRP conjugated secondary antibody (1:10000) was used in the assay. (B) 200 ng of recombinant *Pf*PHB2 was coated on ELISA plates and treated with four different patient sera (P2, P1, P6, P9) at different dilutions (1:1000, 1:2500, 1:5000, 1:7500, 1:10000) followed by secondary human HRP conjugated antibody (1:10000). Error bars represent standard deviation among three replicates.



**Figure S9: Immunofluorescence Assay (IFA) showing the expression of *PfPHB2* in patient derived laboratory adapted parasite line using anti-*PfPHB2* antibodies (1:200), related to figure 7.**