Supplementary Information

Characterization of mitochondrion-targeted GTPases in *Plasmodium falciparum*

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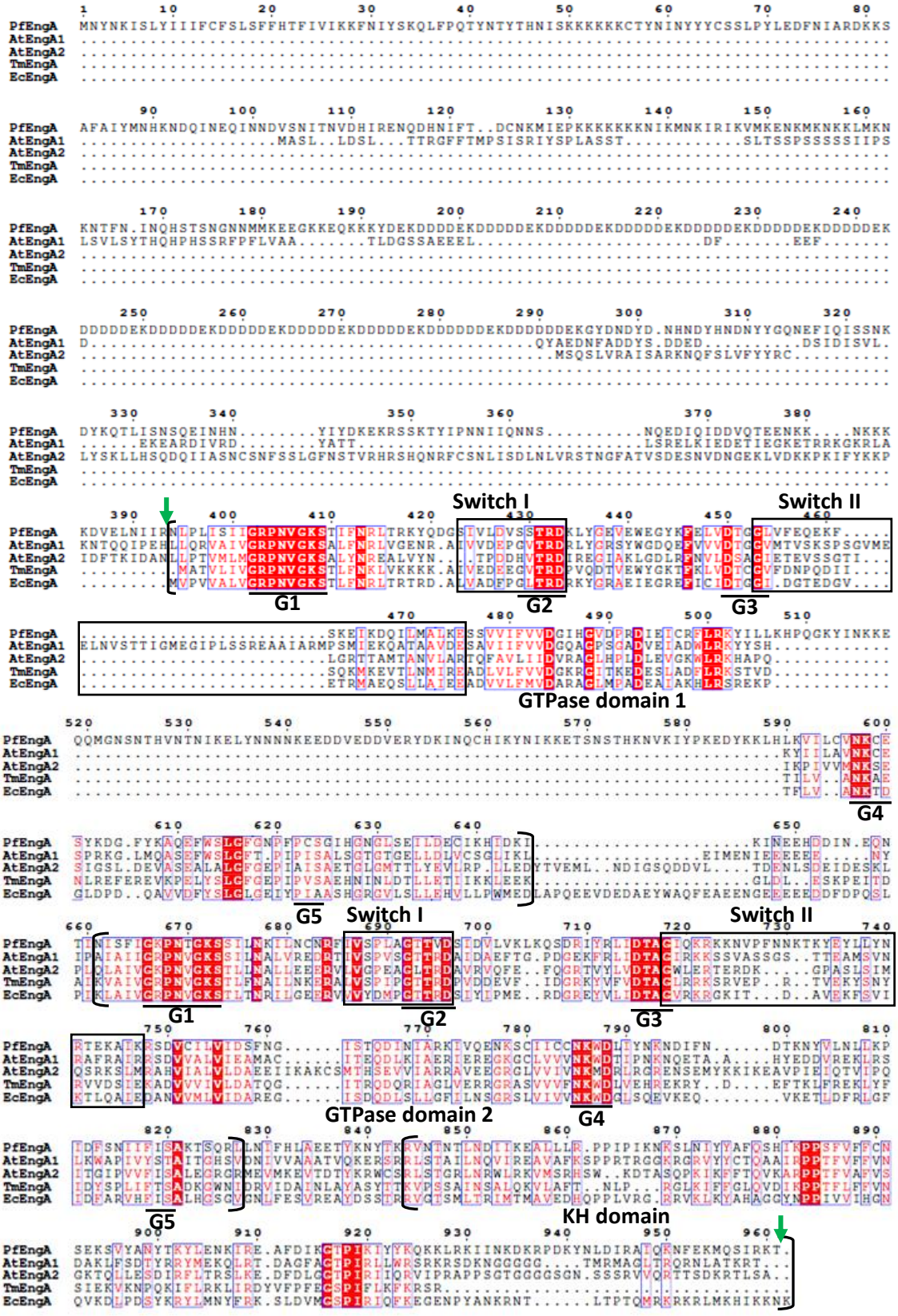
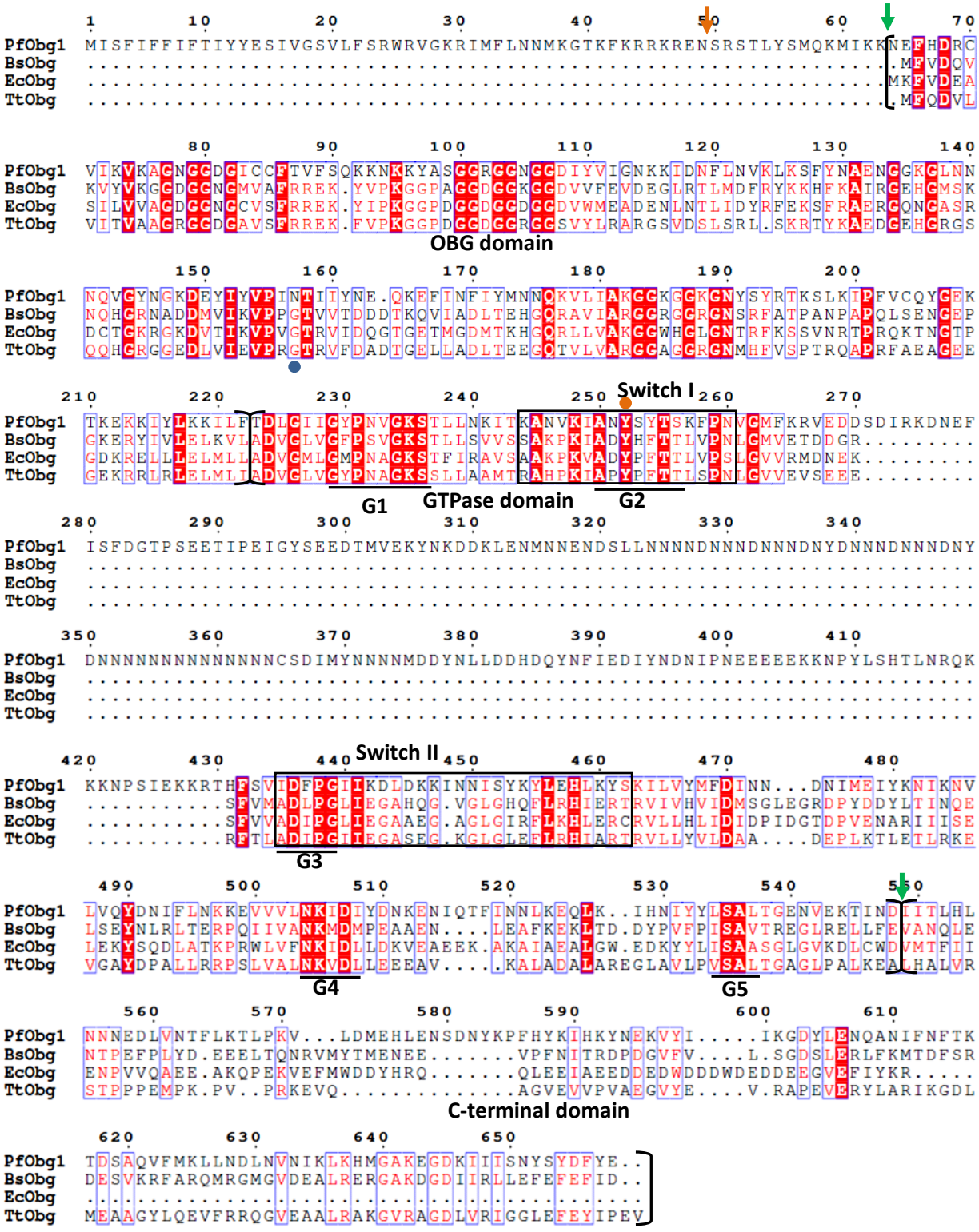
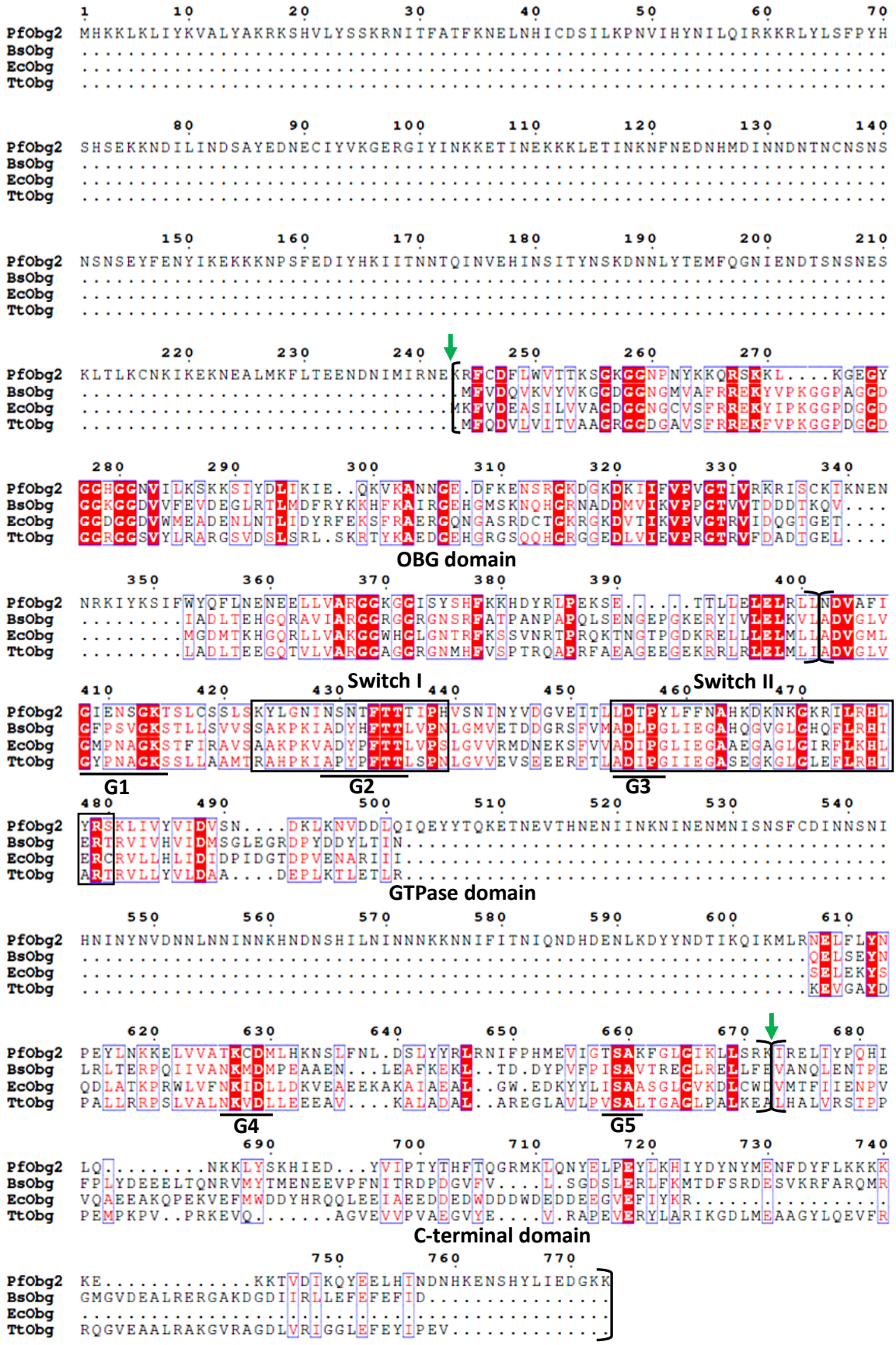


Supplementary Figure S1. ClustalW alignment of EngA homologs from *Escherichia coli* (EcEngA), *Thermotoga maritima* (TmEngA), *Arabidopsis thaliana* (AtEngA1 and AtEngA2) and *Plasmodium falciparum* (PfEngA). The start and end of recombinant *Pf*EngA is marked by green arrows. GTPase domains 1 and 2 and the KH domain are indicated. Black boxes mark switch I and switch II elements. In the ESPript (espript.ibcp.fr/) alignment output format, red filled boxes indicate strict identity, red characters show similarity in a group, blue frame marks similarity across groups. G1-G5 are G motifs of the GTPase domains.



Supplemetary Figure S2. ClustalW alignment of *P. falciparum* Obg1 (PfObg1) with Obg homologs from *Escherichia coli* (EcObg), *Thermus thermophilus* (TtObg), *Bacillus subtilis* (BsObg). The start and end of recombinant *Pf*Obg1 is marked by green arrows. The predicted cleavage site of the mitochondrial targeting sequence is indicated by an orange arrow. The OBG domain, G-domain and C-ter domain are marked. Orange and blue dots mark the conserved tyrosine residue in the G-domain and the glycine residue in the OBG domain.



Supplementary Figure S3. ClustalW alignment of *P. falciparum* Obg2 (PfObg2) with Obg homologs from *Escherichia coli* (EcObg), *Thermus thermophilus* (TtObg), *Bacillus subtilis* (BsObg). The start and end of recombinant *Pf*Obg1 is marked by green arrows.

Supplementary Table S1. Primers used for PCR and double-stranded DNA oligonucleotides for EMSA

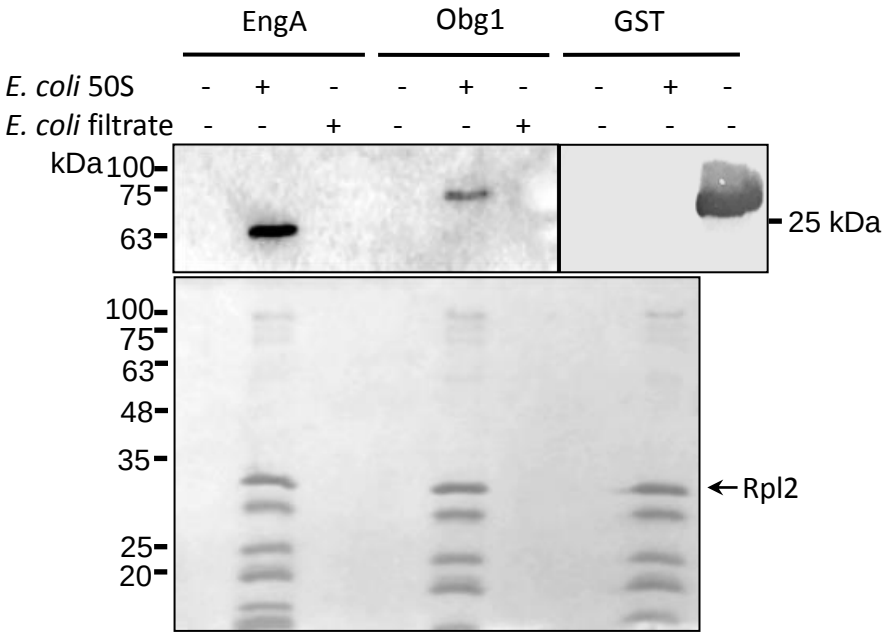
Gene of interest	Primer sequence (5'- 3')		
*Pf*EngA	Forward		CCCGAGCTCAATTTACCATTAATATCAAT
	Reverse		CCCAAGCTTTGTTTTTCTTATAGATTGCATTTTTTC
*Pf*Obg1	Forward	FP-O	AAAGGATCCAATGAATTTCACGATAGGTGTGTAATCAAA
		FP-G	CGCGGATCCACAGATTTAGGTATTATTGGTTATCC
	Reverse	RP-O	CGCGTCGACTCCAACATTAGGAAATTTCGATGTATA
		RP-OI	CGCGTCGACAATTACACTAAAATGAGTTCTCTTCTT
		RP-G	CGCGTCGACATCATTTATGGTTTTTTCAACATTTTC
		RP-C	CGCGTCGACTTCATAAAAATCATAAGAATAATTACTAAT
*Pf*Obg2	Forward		AAAGGATCCAAACGCTTCTGTGATTTTTTATGGGTA
	Reverse		CGCGTCGACTCTACTAAGCAATTTGATACCTAA
Mitochondrial SSUA (for SSU_{mit})	Forward		ATTTGATCCATACAGTCCCAGCG
	Reverse		AGAACGAACGCTTTTAACGCC
Mitochondrial LSUE (for LSU_{mit})	Forward		AACGGTGTAACGACTTCCCC
	Reverse		GTGCTCAGGGTCTTACCGTC
Apicoplast 16S rRNA (for SSU_{api})	Forward		CGTGATAAATATTCCGCCTGAG
	Reverse		AGGTTTATCGTGTTGCATCG
Apicoplast 23S rRNA (for LSU_{api})	Forward		TCCGAGAGTCCATATTGACG
	Reverse		CGAACAGACTTACCCTTAAAAC
Hsp40	Forward		AGAACAACCAGCTGATGCAACAGATTTATTTGAC
	Reverse		TGACCTTCACAATTTGTACATATAATATCTTTACTTATAGC
Putative *Pf*IscA (nuclear, 339 bp)	Forward		CCCGGATCCCCTATCATACAATTAACGAATGATGCT
	Reverse		CGCGTCGACTACAAAGTCAATATCAAAGGAATTTCC
mtDNA Fragment A (535 bp, 5553-5967 bp +1-120 bp)	Forward		CGCGAATTCGTAACCATGCCAACACATAAGAAC
	Reverse		CGCGGATCCAACACAGATAATTCCCAATCA
mtDNA Fragment B (438 bp, 1201-1638 bp)	Forward		CGCGAATTCTACTTAATGGATATGGTGATA
	Reverse		CGCGGATCCAGATATTATTTATTGCAAGGC
Apicoplast Rpl16 (390 bp)	Forward		CGCGGATCCATGACTAATATTATAATAAAAAAA
	Reverse		CGCGTCGACTTATTTTCTAATATATTGTAATTT
EMSA	Probe		5'-FAM-CGGAAATCGGATATTTTTTTT-3' 3'-GCCTTTAGCCTATAAAAAAAA-5'
	Competitor		5'-ÇAGGTGGAAAAGGTGCGCAAAATGTAAATAA-3' 3'-GTCCACCTTTTCCACGCGTTTTACATTTATT-3'

Supplementary Table S2. Peptide mass fingerprint of recombinant *Pf*Obg1.

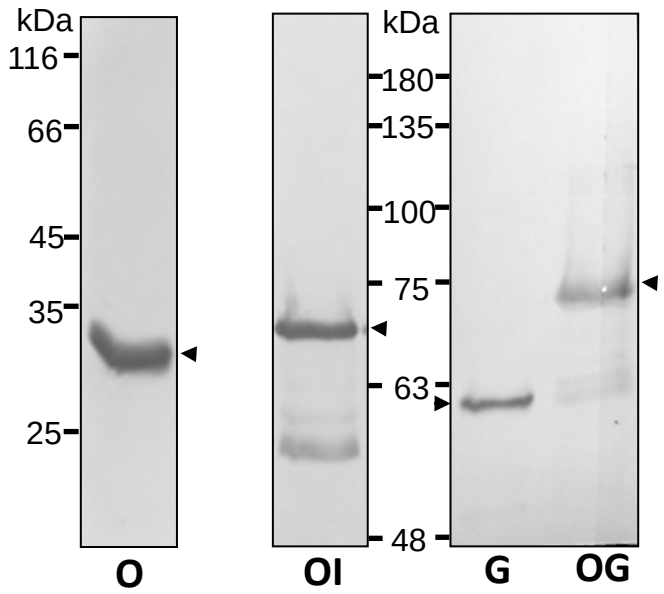
gi|124808333 Mass: 73470 Score: 146 Matches: 9(7) Sequences: 5(4)
GTP binding protein, putative [Plasmodium falciparum 3D7]

Observed	Mr(expt)	Mr(calc)	Start	- End	Peptide
1560.7925	1559.7852	1559.7442	135	- 146	K.EFINFIYMNNQK.V
939.5019	938.4946	938.4684	227	- 234	K.FPNVGMFK.R
1473.8513	1472.8440	1472.8028	399	- 411	R.THFSVIDFPGIIK.D
1579.8850	1578.8777	1578.8406	454	- 466	K.NVLVQYDNIFLNK.K
1964.0552	1963.0479	1963.0051	497	- 513	K.IHNIYYLSALTGENVEK.T

Recombinant *Pf*Obg1 from SDS-PAGE gel pieces was digested with trypsin followed by peptide extraction and MALDI-TOF analysis (AB SCIEX MALDI TOF/TOF 4800 Plus system). The peptide mass fingerprint was searched against the *P. falciparum* peptide database using the Mascot search engine (Perkins *et al.*, 1999). *Pf*Obg1 (gi|124808333; PlasmoDB ID PF3D7_1411600) was the only *P. falciparum* hit in Mascot search result.



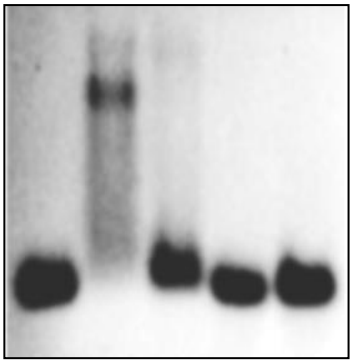
Supplementary Figure S4. Filter-trap assay detects binding of *Pf*EngA and *Pf*Obg1 with *E. coli* 50S ribosome subunit. No ribosome binding was seen with the control protein glutathione-S-transferase (GST). *Pf*EngA and *Pf*Obg1 were not trapped on the filter when incubated with *E. coli* filtrate (bacterial lysate from which large molecular weight complexes were removed by prior filtration through 100 kDa membrane). Upper panel, western blot using anti-His6 Ab to detect *Pf*EngA and *Pf*Obg1 and anti-GST Ab to detect GST; Lower panel, corresponding Coomassie-stained gel. The last lane in the upper panel has GST alone loaded as positive control for western blot.



Supplementary Figure S5. Coomassie-stained SDS-PA gels of purified recombinantly expressed segments of *Pf*Obg1. All migrate to positions higher than predicted from amino acid sequence. O, OBG-domain (expected size, 25kDa); OI, OBG domain with part of the G-domain that includes the insertion (expected size, 45kDa); G, G-domain (expected size, 40kDa); OG, OBG domain and the complete G-domain (recombinant *Pf*Obg1, expected size 59kDa).

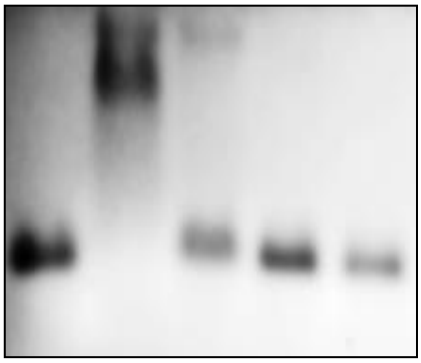
A

Protein	-	O	O	GST	EngA
Probe (21 bp)	+	+	+	+	+
Competitor (20 bp)	-	-	+	-	-



B

Protein	-	O	O	GST	EngA
Probe (36 bp)	+	+	+	+	+
Competitor (40 bp)	-	-	+	-	-



FAM labelled probes:

5’-FAM-CGGAAATCGGATATTTTTTTT-3’ (21 bp)
3‘-GCCTTTAGCCTATAAAAAAAA-5'

5'- GATCTAAAAGACTTGGAAAAATTTTTAAAAAAGATC-FAM-3‘ (36 bp)
3‘- CTAGATTTTCTGAACCTTTTTAAAAATTTTTTCTAG-5'

Competitor – unlabelled *P. falciparum* mitochondrial DNA fragment:

mtDNA1: 5'-ACTGTGAGTGATCCTACAAT-3' (20 bp)
3'-TGACACTCACTAGGATGTTA-5‘

mtDNA2: 5'-GCTATGGGATGTATAGCTGTTTTAGGAAGCTTAGTATGGG-3' (40 bp)
3‘-CGATACCCTACATATCGACAAAATCCTTCGAATCATACCC-5'

Supplementary Figure S6. EMSAs using the recombinant OBG domain (O) using two probe lengths (21 and 36 bp) and *P. falciparum* mitochondrial DNA competitors of similar length confirm that *Pf*Obg1 can bind DNA non-specifically and can interact with parasite mitochondrial DNA. *Pf*EngA does not bind DNA, similar to the control protein GST. Probe and competitor sequences used in the EMSAs are shown.