SUPPLEMENTARY METHODS

1. LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LC/MS) METHODS AND DATABASE SEARCHING

*LCMS/MS Method for Q Exactive*

The samples were run on a Thermo Scientific Q Exactive mass spectrometer connected to a Dionex Ultimate 3000 (RSLCnano) chromatography system. Tryptic peptides were resuspended in 0.1% formic acid. Each sample was loaded onto a fused silica emitter (75 μm ID, pulled using a laser puller (Sutter Instruments P2000)), packed with Reprocil Pur C18 (1.9 μm) reverse phase medium and was separated by an increasing acetonitrile gradient over 27 or 37 minutes at a flow rate of 250 nl/min. The mass spectrometer was operated in positive ion mode with a capillary temperature of 220°C, and with a potential of 2200V applied to the frit. All data were acquired with the mass spectrometer operating in automatic data dependent switching mode. A high-resolution (70,000) MS scan (300-1600 m/z) was performed using the Q Exactive to select the 20 most intense ions prior to MS/MS analysis using HCD.

*LCMS/MS Method for Orbitrap*

The samples were run on a Thermo Scientific LTQ ORBITRAP XL mass spectrometer connected to a Dionex Ultimate 3000 (RSLCnano) chromatography system. Tryptic peptides were resuspended in 0.1% formic acid. Each sample was loaded onto Biobasic Picotip Emitter (120 mm length, 75 μm ID) packed with Reprocil Pur C18 (1.9 μm) reverse phase column and was separated by an increasing acetonitrile gradient. The mass spectrometer was operated in positive ion mode operating in automatic data dependent switching mode. A high resolution MS scan (300-2000 Da) was performed using the Orbitrap to select the 7 most intense ions prior to MS/MS analysis using the Ion trap.

*Database Searching*

The raw data were sequenced de novo and searched against the  the complete database of *P. falciparum*3D7 proteins retrieved from PlasmoDB (EuPathDB Project Team) using the search engine PEAKS Studio 6 (Bioinformatics Solutions Inc., Waterloo, ON, Canada) for peptides cleaved with trypsin. Each peptide used for protein identification met specific Peaks parameters, i.e. only peptide scores that corresponded to a false discovery rate of ≤1% were accepted from the Peaks database search.

2. YEAST-2-HYBRID ANALYSIS WITH PfFKBP35

*cDNA expression library construction*

Library construction was performed commercially by Dualsystems Biotech AG, Zurich, Switzerland from harvested parasites provided as prepared in section 2.2. Briefly, first strand cDNA synthesis started from the 3’-end adapter comprising an oligo (dT) sequence to anneal to the poly(A)+ stretch present at the 3’ end of the mRNA. When the reverse transcriptase (RT) reached the 5’ end of the mRNA, it added several non-template nucleotides, primarily deoxycytidines, to the 3’ end of the newly synthesized first-strand cDNA (Schmidt & Mueller, 1999). This oligo (dC) stretch paired with a complementary oligo (dG) sequence present in the PlugOligo® (5‘-AAGCAGTGGTATCAACGCAGAGTACGGGGG-PHOS-3’). The RT then switched templates and continued first strand cDNA synthesis to the end of the oligonucleotide, thus incorporating the PlugOligo® sequence into the 5’ end of the cDNA. The last 3‘-dG residue of the PlugOligo® is a terminator nucleotide comprising a 3’-phosphate group. This blocking group prevented unwanted annealing and extension of the PlugOligo®. The second adapter pair (3’-end adapter CDS-3M [5'-AAGCAGTGGTATCAACGCAGAGTGGCCGAGGCGGCC(T)20VN -3'] and 5’-end adapter PlugOligo®-3M [5‘-AAGCAGTGGTATCAACGCAGAGTGGCCATTACGGCCGGGGG-PHOS-3‘]) comprised asymmetric sites with *Sfi*I restriction enzyme recognition sites, A & B. The incorporation of SfiIA and SfiIB sites into the 5‘ and 3‘ ends of the cDNA the sites allowed directional cloning of the cDNA library. Following first strand synthesis, the cDNA was amplified by PCR. The use of EasyClone® polymerase and specially designed primers allowed synthesis of full-length enriched cDNA that was flanked by the PlugOligo® and 3’-end adapter sequences. After digestion with *Sfi*I, size selection and purification, the resulting cDNA was ligated into the DUALHYBRID®-library vector using the *Sfi*I sites located in the 5’ and 3’ adapter sequences of the cDNA. *Sfi*I restriction sites are very rare in eukaryotic cDNAs and therefore allowed preferential cloning of full-length cDNAs. Following ligation, the cDNA library was amplified by transformation into *E. coli* for later use.

*Bait functional assay*

This assay determines whether the bait enters the nucleus and binds to the LexA operators situated upstream of the reporter genes (“repression or blocking assay” [Brent:1984]). A bait which enters the nucleus and binds to the LexA operator sites decreases transcription from the downstream *LACZ* gene (repression) and hence decreases β-galactosidase expression on galactose medium. Most LexA fusions that enter the nucleus and bind the operators but do not activate transcription repress β-galactosidase activity from 2 to 20 fold. >2 fold repression indicates >50% operator occupancy by the bait and signifies that the bait can be screened in the DUALhybrid® assay.

*Yeast-2-hybrid screening*

The yeast two-hybrid screen using the PfFKBD-His6 bait was carried out by Dualsystems Biotech. The bait construct for yeast two-hybrid screening was constructed as described in the main text (MATERIALS AND METHODS). The bait construct was transformed into the strain *S. cerevisiae* NMY32 (MATa *his3∆200 trp1-901 leu2-3,112 (lexAop)8-ADE2 LYS2::(lexAop)4-HIS3 URA3::(lexAop)8-lacZ GAL4*) using standard procedures (Gietz & Woods, 2001). Correct expression of the bait was verified by western blotting of cell extracts using a mouse monoclonal antibody directed against the LexA domain (Dualsystems Biotech, Switzerland). The absence of self-activation was verified by co-transformation of the bait together with a control prey and selection on minimal medium lacking the amino acids tryptophan, leucine and histidine (selective medium). For the yeast two-hybrid screen, the bait was co-transformed together with a *P. falciparum* cDNA library (generated by Dualsystems) into *S. cerevisiae* NMY32 (MATERIALS AND METHODS). Transformants were screened, yielding transformants that grew on selective medium. Positive transformants were tested for β-galactosidase activity using a PXG β-galactosidase assay (Dualsystems Biotech). Initial positives which showed β-galactosidase activity were considered to be true positives. Library plasmids were isolated from positive clones. The identity of positive interactors was determined by sequencing.