Supporting Information

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SI Materials and Methods

Gateway-adapted pGBKT7g and pGADT7g vectors were a kind gift of Peter Uetz and Seesandra Venkatappa Rajagopala (J. Craig Venter Institute, Rockville, MD). All yeast two-hybrid media and supplements were acquired from USBiologicals. Mate and Plate normalized universal human library (630481) and the Matchmaker Gold Yeast Two-Hybrid System (630489) were from Clontech. ThermoPol Taq Polymerase (M0267) was purchased from New England Biolabs. All plasticware was purchased from Nalge Nunc International. X-α-gal (5-bromo-4-chloro-3-indoxyl-α-D-galactopyranoside) was from Biosynth International. Max-efficiency DH5α competent cells (12034013) and Zeocin (R250) were from Invitrogen. AH109 and Y187 yeast cells were from Clontech Laboratories. Lyticase (L2524-50KU) and all additional chemicals were obtained from Sigma-Aldrich.

Yeast Two-Hybrid Strains. For all experiments we used Y187 *(MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, met–, gal80Δ, MEL1, URA3::GAL1$_{UAS}$-GAL1$_{TATA}$-lacZ)* and AH109 *(MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3::MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ, MEL1)* strains.

Yeast Two-Hybrid Prey Proteins. In brief, the human ORFeome collection (1) was pooled in 94 pools of 128 ORFs each, using equal amounts of bacterial culture containing entry vector (pDONR221). The pools were grown overnight in terrific broth, cells were harvested by centrifugation, and plasmid was extracted using the QIAGEN R.E.A.L. prep 96 kit. Next, 150 ng of plasmid from each pool was used in a recombination reaction between attL and attR sites (LR reaction) using LR clonase II and pGADT7g destination vector according to the manufacturer's instructions. The LR reaction was then diluted four times with 1× TE buffer and 100 μL of Max-efficiency DH5α-competent cells were transformed with each pool according to manufacturer's instructions and inoculated in 2 mL of terrific broth. After overnight incubation cells were harvested by centrifugation and plasmids extracted using a QIAGEN R.E.A.L. prep 96 kit. Finally, 94 aliquots of AH109 cells were transfected with each of the plasmid pools according to Invitrogen's ProQuest Two-Hybrid System transfection protocol (PQ10001-01).

Yeast Two-Hybrid Bait Proteins. *Plasmodium falciparum* proteins expressed in the late erythrocytic stage or containing PEXEL domain were selected using PlasmoDB query interface. Genomic sequences of the corresponding proteins were downloaded and processed *in silico* to automatically select for nonhydrophobic regions encoded by ~750 bp and completely included within the boundaries of a single exon. Primer pairs were designed to amplify 502 selected regions from 3D7 *P. falciparum* genomic DNA. Primer sequences also included the Gateway compatible recombination sequence. PCR was performed using Pfx Taq Polymerase and amplicons were transferred to pGBKT7g vectors by using BP clonase according to the manufacturer's instructions. The resulting plasmids were used in the transformation of Max-efficiency DH5α-competent cells, propagated, extracted, and sequenced by capillary sequencing. Those clones containing a plasmid insert in the correct frame of translation and less than two nonsynonymous mutations were selected for downstream assays. One hundred forty-five clones passed all quality criteria and were later used in the transfection of Y187 yeast cells according to Invitrogen's ProQuest Two-Hybrid System transfection protocol (PQ10001-01).

Screens. Yeast cells were grown in synthetic complete (SC) media lacking either leucine (–L; Y187 cells with bait protein) or tryptophane (–T; AH109 cells with prey protein) at 30 °C for 48 h and spotted in Omnitray agar plates, using a Biomek FXp liquid handler. Bait plates consisted of 94 spots containing replicas of the same bait. Prey plates consisted 94 spots, each containing a distinct pool of 128 preys. After 2 d, the bait and prey plates were combined in a yeast extract, peptone, dextrose medium supplemented with adenine (YPAD) plate, using metallic pins (V&P Scientific), and mating was allowed to proceed for 12 h, after which the mated cells were transferred to a SC plate lacking leucine, tryptophan, adenine, and histidine (–LTAH combined dropout plate) plus x-α-gal (40 mg/L). After 14 d, we picked the colonies from each of the spots, inoculated SC –LTAH liquid media, expanded the cells for 24–48 h, and performed amplification of the inserts straight from liquid culture by using plasmid-specific primers (pGBKT7g forward, 5′-TAAGTGCGACATCATCATCG-3′; pGBKT7g reverse, 5′-TTTTCGTTTTAAAACCTAAGAGTC-3′; pGADT7g reverse, 5′-AGATGGTGCACGATGCACAG-3′), using ThermoPol Taq Polymerase and zymoliase as described in *Colony PCR*. The amplicons were purified with QIAGEN MinElute 96 HT plates and sequenced by capillary sequencing. To control for proteins that activate the transcription of the reporter genes despite unsuccessful host–parasite complex formation (autoactivators), we spotted each of the positive clones and their respective baits in SC –LTAH solid plates with multiple concentrations of 3-Aminotriazole (3-AT) ranging from 0 to 7.5 mM. By comparing the growth of diploid cells containing only the bait plasmid with the diploid cells containing the interacting pair, we determined whether there was a concentration of 3-AT that was capable of inhibiting growth of the bait alone but not of the cells containing both the bait and the prey. Using a subset of the positive pairs, we then went back to the individual clones and swapped their plasmids (i.e., the bait protein was transferred into a pGADT7g vector and the prey into a pGBKT7g vector). The whole assay was repeated as described above for the swapped bait and prey and the 3-AT titration repeated.

Autoactivatome Screening. To reduce the number of false positives we performed a series of high-throughput screens of human autoactivators. In brief, we mated pools of Y187 cells (1 mL) containing either episomal human ORFs or normalized cDNA library with AH109 cells without plasmids or with plasmids with no insert. Mating was performed in YPAD liquid media (5 mL) with kanamycin, according to the Mate and Plate Normalized human library and Matchmaker Gold manuals. The diploid cells were then plated in either SC –LH + x-α-gal (40 mg/L) (for cells mated with empty AH109 cells) or SC –LTAH + x-α-gal (40 mg/L) (for cells mated with AH109 cells containing empty pGBKT7g plasmids) to select for autoactivators. Cells were incubated at 30 °C for 5 d and positive colonies (i.e., colonies with strong blue halo) were picked from each plate, expanded in liquid media for 4 d, and then, after amplification and purification, sequenced by Sanger capillary sequencing. All proteins identified as autoactivators and paralogs with >60% similarity were filtered out from our positive results dataset.

Colony PCR. Five microliters of Lyticase [dissolved in 50% (vol/vol) glycerol, 10 mM Tris, pH 7.5, at 5 units/μL] diluted in water at

1:10 vol/vol and 5 μL of yeast suspension (with OD between 2 and 7) were mixed. To this mix 20 μL of PCR mastermix was added to obtain a final concentration of 1 mM of forward and reverse primers, 1× ThermoPol buffer, 0.5 mM dNTPs, 0.1 mg/mL BSA, and 100 units/mL of NEB Taq polymerase in a final volume of 25 μL. Cycling reactions were set as follows: denaturing at 94 °C for 2 min; followed by 35 cycles of 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 4 min; and a final extension of 72 °C for 10 min. Five microliters of the PCR product was used to load a 1% (wt/vol) agarose gel to check for PCR amplification.

1. Rual, et al. (2004) Human ORFeome version 1.1: a platform for reverse proteomics. *Genome Res* 14:2128–2135.

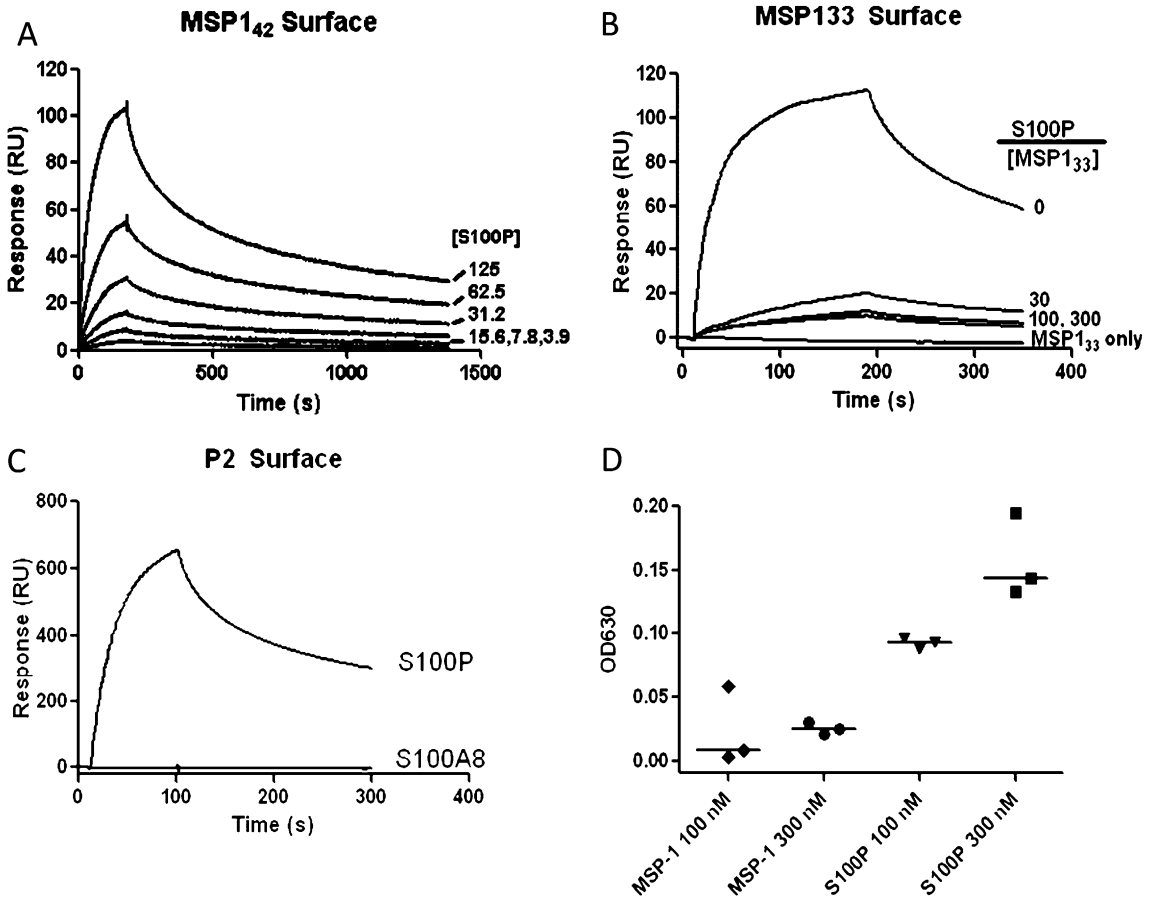

Fig. S1. (*A*) SPR sensogram of the binding of S100P at increasing concentrations (3.9–125 nM) to immobilized 3D7 MSP1$_{42}$. (*B*) SPR sensogram of the binding of S100P to 3D7 MSP1$_{33}$ in the presence of increasing concentrations (0–300 nM) of 3D7 MSP1$_{33}$. (*C*) SPR sensogram of the binding of S100P and S100A8 to immobilized P2. (*D*) MSP1$_{33}$ and S100P were tested for their ability to activate NFκB in the monocyte THP1-XBlue reporter cell line. NFκB activation was determined by SEAP reporter gene assay, using colorimetric detection method. Bars represent the median signal of three replicates.

Other Supporting Information Files

Dataset S1 (XLSX)