Supplementary Materials and Methods

Selection of *P. vivax* proteins

The *P. vivax* proteins were selected according to specific sets of criteria, including (1) the presence of IDC transcription evidence, (2) the presence of a signal peptide or transmembrane domains, or (3) both for surface and secreted proteins; as well as (4) a pI < 7.6 for putative cytoplasmic proteins; (5) size, all amplicons were between 300 and 3,000 nt. Multiple exon genes were broken down into single exon fragments and ORFs greater than 3,000 nt were broken down into segments of ≤ 3,000 nt. The multigene variant superfamily termed *vir* (*P. vivax* variant genes) was excluded. A total of 2,233 polypeptides, representing 1,936 *P. vivax* proteins were used.

Protein microarray fabrication

Custom PCR primers comprising 20 base pairs of gene-specific sequence with 20 base pairs of "adapter" sequences were used to amplify target amplicons from *P. vivax* genomic DNA (Sal I strain [MRA-552, MR4]). The adapter sequences, which flank the target amplicons, are homologous to the adapter sequences at the ends of the linearized T7 expression vector pXT7 into which the amplicon will be cloned into. The homology allows the amplified PCR products to be cloned into the expression vector by in vivo homologous recombination in competent DH5α cells. To get an idea of the cloning efficiency a subset of the resulting clone mixtures were then verified by either QCPCR (1/2 of total clones), using sequence specific primers to verify the

correct insert, or sequenced (1/8th of total clones). The target amplicon is flanked by sequences that incorporate a 5' polyhistidine (HIS) epitope and a 3' hemagglutinin (HA) epitope and T7 transcription terminator. Plasmids mixtures, and no DNA controls, are expressed for 5 hours in *E. coli* based cell-free IVTT batch reactions (RTS 100 HY kits from 5 PRIME) according to the manufacturer's instructions. Approximately 1 nl of unpurified IVTT reactions are printed onto nitrocellulose coated glass Oncyte Nova slides (GraceBio Labs) using an OmniGrid Accent microarray printer (DigiLab Global). The *P. vivax* proteome microarrays were prepared by Antigen Discovery Inc. (Irvine, CA).

Once printed and dried, polypeptide expression was verified using monoclonal anti-polyhistidine (clone His-1, Sigma) and anti-hemagglutinin (clone 3F10, Roche). The arrays were rehydrated in 1X Blocking Buffer (Whatman) for 30 minutes and probed with the monoclonals diluted at 1 to 1,000 in blocking buffer overnight at 4°C with constant agitation. The following day, slides were washed 3 times in 1X Tris (hydroxymethyl)-aminomethane (tris) buffer containing 0.05% (v/v) Tween20 (TTBS), and bound antibodies were detected by incubating with Cy5 conjugated goat anti-mouse immunoglobulin (anti-IgG, Jackson Immuno Research), or Cy5 conjugated Goat anti-rat IgG, diluted 1 to 400 in blocking buffer. After washing the slides 3 times in TTBS followed by 3 additional washes in 1X tris (hydroxymethyl)-aminomethane (tris) buffer (TBS) and a final rinse in ultra pure water, the slides were briefly centrifuged and air-dried. The fluorescence was detected and analyzed using Perkin Elmer ScanArray Express HT microarray scanner. All signal intensities were

corrected for spot-specific background, the signal from the substrate surrounding the spots. The expression of the polypeptides is determined based on a cutoff of the mean signal of the “no DNA” control spots plus two standard deviations.