Supplementary Material

1 Supplementary Data

Supplementary Data Files are attached separately.

Supplementary Data 1: All RAU data from the PNG child cohort and the negative control panels. “Type_antibody” specifies the biomarker (i.e. total IgG, IgG1 etc).

Supplementary Data 2: All RAU data from the Peruvian cohort (log10 transformed). Sheet 1 contains IgG data and sheet 2 contains IgG3 data. Sheet 3 includes the data dictionary.

2 Supplementary Figures and Tables

2.1 Supplementary Figures
Supplementary Figure S1. The kinetics of total IgG against 30 P. vivax antigens following asymptomatic P. vivax infections in PNG children.

Total IgG antibody responses against 30 P. vivax antigens following asymptomatic P. vivax infections in PNG children (n=31) over 36 weeks were measured using a multiplex assay. Data is presented in RAU (in log 10). Each dot point shows the individual antibody response to each P. vivax antigen measured at each timepoint. Locally estimated scatterplot smoothing (LOESS) lines and 95% confidence intervals were plotted over time. Negative control (grey line) was the average antibody response of naïve individuals (n=274) and the grey ribbon was set as 2 SD from the average negative control. Anti-antigen antibody with LOESS-smoothed line that was seropositive at week 0 and became seronegative within 9 months are indicated by an asterix (*). Anti-antigen antibody with LOESS-smoothed line that was seropositive for at least 36 weeks is indicated by an up arrow (^).
Supplementary Figure S2. The kinetics of IgM against 32 *P. vivax* antigens following asymptomatic *P. vivax* infections in PNG children.

IgM antibody responses against 32 *P. vivax* antigens following asymptomatic *P. vivax* infections in PNG children (*n*=31) over 36 weeks, measured using a multiplex assay. Data are presented in RAU (in log 10). Each dot point shows the individual IgM antibody response to *P. vivax* antigens measured at each timepoint. LOESS-smoothed line and 95% confidence intervals were plotted over time. Negative control (grey line) was the average antibody response of naïve individuals (*n*=260) and the grey ribbon shows 2 SD from average negative control. No anti-antigen antibody with LOESS-smoothed line that was seropositive at week 0 and became seronegative within 9 months.
Supplementary Figure S3. The kinetics of IgG1 against 29 *P. vivax* antigens following asymptomatic *P. vivax* infections in PNG.

IgG1 antibody response against 29 *P. vivax* antigens following asymptomatic *P. vivax* infections in PNG children (n=31) over 36 weeks, measured using a multiplex assay. Data are presented in RAU (in log 10). Each dot point shows the individual antibody response to *P. vivax* antigens measured at each timepoint. LOESS-smoothed lines and 95% confidence intervals were plotted over time. Negative control (grey line) was the average antibody response of naïve individuals (n=248) and the grey ribbon shows 2 SD from average negative control. Anti-antigen antibody with LOESS-smoothed line that was seropositive at week 0 and became seronegative within 9 months are indicated by an asterix (*). Anti-antigen antibody with LOESS-smoothed line that was seropositive for at least 36 weeks is indicated by an up arrow (^).
**Supplementary Figure S4.** The kinetics of IgG3 against 29 *P. vivax* antigens following asymptomatic *P. vivax* infections in PNG.

IgG3 antibody response against 29 *P. vivax* antigens following asymptomatic *P. vivax* infections in PNG children (n=31) over 36 weeks, measured using a multiplex assay. Data are presented in RAU (in log 10). Each dot point shows the individual antibody response to *P. vivax* antigens measured at each timepoint. LOESS-smoothed lines and 95% confidence intervals were plotted over time. Negative control (grey line) was the average antibody response of naïve individuals (n=256) and the grey ribbon shows 2 SD from average negative control. Anti-antigen antibody with LOESS-smoothed line that was seropositive at week 0 and became seronegative within 9 months are indicated by an asterix (*).
Supplementary Figure S5. Total IgG responses to 28 P. vivax antigens in Peruvian cohort based on time since last infected with P. vivax.

Total IgG responses to 28 P. vivax antigens were measured at the end of study of a Peruvian cohort (n=590). Antibody responses were presented in relative antibody unit (log 10) in median with interquartile range. Individuals were grouped based on the last P. vivax infection status as measured by qPCR. Antigen-specific IgG were ordered based on decreasing seropositivity to IgG3 to reflect Fig 3. Anti-antigen antibody that was significantly higher in individuals with infections in the prior 9 months compared to individuals with no infections or infections >9 months ago are indicated by an
asterix (*) (student’s t-test, p<0.05). Anti-antigen antibody with consistent IgG levels above background, regardless of recency of infection are indicated by ^.
Supplementary Figure S6. Correlation of total IgG antibodies to 20 *P. vivax* antigens measured in Peruvian cohort using magnetic and non-magnetic bead in multiplex assay.

Total IgG antibodies to 20 *P. vivax* antigens were measured in Peruvian cohort using magnetic and non-magnetic beads in the Peruvian cohort (n=590). Antibodies level is represented in relative antibody unit. Correlation is analysed using a Spearman’s rank correlation.
Supplementary Figure S7. Correlation of total IgG antibodies to 19 *P. vivax* antigens measured in negative controls using magnetic and non-magnetic bead in multiplex assay

Total IgG antibodies to 19 *P. vivax* antigens were measured in negative controls using magnetic and non-magnetic beads in the Peruvian cohort (n=590). Antibodies level is represented in relative antibody units. Correlations were analysed using a Spearman’s rank correlation.
**Supplementary Figure S8.** Comparison of RBP2b\textsubscript{1986-2653} classification performance for identifying individuals with *P. vivax* infections in the last 9 months generated using non-magnetic beads and magnetic beads in Peruvian individuals.

(a) ROC curves for RBP2b\textsubscript{1986-2653} generated using non-magnetic beads and magnetic beads; (b) Bioplex RBP2b\textsubscript{1986-2653} classification performance; (c) Magpix RBP2b\textsubscript{1986-2653} classification performance. The size of the bar corresponds to the number of samples in each category. The darker colour represents the proportion of correctly classified individuals, and the lighter colour represents the proportion of mis-classified individuals.
### Supplementary Tables

**Supplementary Table S1.** List of *P. vivax* antigens used for detecting antibodies in multiplex assay. Protein amount signifies the amount of protein added to a bulk coupling of magnetic beads as described in the methods. In the current study double the optimised amount of protein was added compared to usual (see Mazhari et al PLOS One 2020 in main reference list), as a modification required to measure the presence of functional antibody responses. Log-linear standard curves were still achieved for all proteins. Abbreviations: aa=amino acid; WGCF= wheat germ cell-free system. Annotations: a=PlasmoDB.

| Protein                                           | PlasmoDB IDa | Construct aa (size) | Protein amount (ug) | Expression System   |
|---------------------------------------------------|--------------|---------------------|---------------------|---------------------|
| sexual stage antigen s16                          | PVX_000930   | 31-end (110)        | 4                   | WGCF expression    |
| exported protein                                  | PVX_003555   | 434-1075 (642)      | 2                   | WGCF expression    |
| merozoite surface protein (MSP) 5                 | PVX_003770   | 23-365 (343)        | 0.08                | WGCF expression    |
| MSP7                                              | PVX_082645   | 23-end (355)        | 32                  | WGCF expression    |
| MSP7B                                             | PVX_082650   | 24-end (429)        | 16                  | WGCF expression    |
| MSP7F                                             | PVX_082670   | 24-end (388)        | 2                   | WGCF expression    |
| MSP7L                                             | PVX_082700   | 23-end (397)        | 3                   | WGCF expression    |
| thrombospondin-related anonymous protein (TRAP/SSP2)| PVX_082735   | 26-493 (468)        | 4                   | WGCF expression    |
| PTEX150                                           | PVX_084720   | 24-908 (885)        | 4                   | WGCF expression    |
| rhoptry-associated membrane antigen (RAMA)        | PVX_087885   | 462-730 (269)       | 2.4                 | WGCF expression    |
| Pv-fam-a associated protein 2 (SIAP2)             | PVX_088860   | 33-end (380)        | 16                  | WGCF expression    |
| cysteine-rich protective antigen (CyRPA)          | PVX_090240   | 27-366 (340)        | 4                   | Baculovirus        |
| Pv-fam-a                                          | PVX_090265   | 1-326 (326)         | 32                  | WGCF expression    |
| hypothetical protein                              | PVX_090970   | 20-254 (235)        | 24                  | WGCF expression    |
| hypothetical protein                              | PVX_091710   | 26-884 (859)        | 4                   | WGCF expression    |
| apical membrane antigen 1 (AMA1)                 | PVX_092275   | 1-463 (464)         | 4                   | Expi293            |
| Pv-fam-a reticulocyte binding protein 2b (RBP2b) | PVX_092990   | 1126-1414 (289)     | 32                  | WGCF expression    |
| (n-terminal fragment)                             | PVX_094255   | 161-1009 (849)      | 4                   | WGCF expression    |
| RBP2b (c-terminal fragment)                       | PVX_094255   | 1986-2653 (667)     | 16                  | WGCF expression    |
| Pv-fam-a                                          | PVX_096995   | 61-end (420)        | 6                   | WGCF expression    |
| MSP8                                              | PVX_097625   | 24-463 (440)        | 2.8                 | WGCF expression    |
| Protein Name                             | Accession   | Start-End       | Score | Method         |
|-----------------------------------------|-------------|-----------------|-------|----------------|
| MSP3b                                   | PVX_097680  | 21-end (996)    | 1.6   | WGCF expression|
| hypothetical protein                    | PVX_097715  | 20-end (431)    | 6     | WGCF expression|
| MSP3a                                   | PVX_097720  | 25-end (828)    | 4     | WGCF expression|
| MSP1-19                                 | PVX_099980  | 1622-1729 (108) | 8     | WGCF expression|
| exported protein                        | PVX_101530  | 38-end (330)    | 2     | WGCF expression|
| Pv-fam-a                                | PVX_112670  | 34-end (302)    | 4.52  | WGCF expression|
| rhoptry neck protein 2 (RON2)           | PVX_117880  | 21-198 (178)    | 32    | WGCF expression|
| circumsporozoite protein 210 (CSP 210)  | PVX_119355  | 1-348 (349)     | 4     | Expi293        |
| CSP 247                                 | PVX_119355  | 1-330 (331)     | 4     | Expi293        |
| Pv-fam-a                                | PVX_125728  | 30-end (250)    | 36    | WGCF expression|
Supplementary Table S2. Antibody seropositivity in the PNG child cohort at week 0. Seropositivity cut-off was defined as the average of negative controls plus two-times the standard deviation. NA= The antibody responses in the children and/or the negative controls were not measured due to antigen unavailability, so seropositivity cannot be determined.

| Antigen                                      | IgG  | IgG1 | IgG3 | IgM  |
|----------------------------------------------|------|------|------|------|
| AMA 1                                        | 36.40% | 63.60% | 15.20% | 0.00% |
| CSP210                                       | 9.10% | 69.70% | 57.60% | 0.00% |
| CSP247                                       | 3.00% | NA    | NA    | 3.00% |
| CyRPA exported                                | 9.10% | 45.50% | 9.10% | 33.30% |
| hypothetical protein                         | 9.10% | 84.90% | 39.40% | 6.10% |
| MSP5                                         | 18.20% | 33.30% | 51.50% | 18.20% |
| MSP7                                         | 30.30% | 24.20% | 36.40% | 9.10% |
| MSP1-19                                      | 48.50% | 33.30% | 36.40% | 9.10% |
| MSP3a                                        | 48.50% | 63.60% | 60.60% | 24.20% |
| MSP3b                                        | 42.40% | 66.70% | 54.60% | 15.20% |
| MSP7B                                        | 45.50% | 57.60% | 42.40% | 12.10% |
| MSP7F                                        | 33.30% | 42.40% | 21.20% | 72.70% |
| MSP7L                                        | 51.50% | 51.50% | 39.40% | 48.50% |
| MSP8                                         | 21.20% | 72.70% | 36.40% | 18.20% |
| PTEX150                                      | 39.40% | 57.60% | 33.30% | 12.10% |
| Pv-fam-a (PVX_088820)                        | 36.40% | 51.50% | 33.30% | 9.10% |
| Pv-fam-a (PVX_090265)                        | 24.20% | 51.50% | 51.50% | 0.00% |
| Pv-fam-a (PVX_092990)                        | 6.10% | 27.30% | 21.20% | 12.10% |
| Pv-fam-a (PVX_096995)                        | 30.30% | 75.80% | 51.50% | 3.00% |
| Pv-fam-a (PVX_112670)                        | 21.20% | 39.40% | 42.40% | 30.30% |
| Pv-fam-a (PVX_125728)                        | 21.20% | 45.50% | 69.70% | 6.10% |
| PVX_090970                                   | 15.20% | 15.20% | 36.40% | 0.00% |
| PVX_091710                                   | 24.20% | 21.20% | 36.40% | 12.10% |
| PVX_101530                                   | 54.60% | 69.70% | 36.40% | 75.80% |
| RAMA                                         | 54.60% | 60.60% | 48.50% | 15.20% |
| RBP2b161-1009                                 | NA    | 97.00% | 33.30% | 9.10% |
| RBP2b1986-2653                                | 84.90% | 93.90% | 6.10%  | 3.00% |
| RON2                                         | 12.10% | 30.30% | 39.40% | 9.10% |
|       | 15.20% | 54.60% | 30.30% | 9.10% |
|-------|--------|--------|--------|-------|
| s16   |        |        |        |       |
| SIAP2 | 45.50% | 57.60% | 39.40% | 30.30%|
| TRAP/SSP2 | 21.20% | NA     | NA     | 33.30%|
Supplementary Table S3. Summary table of epidemiologic data of the Peruvian cohort. The Peruvian cohort study was a longitudinal study performed from December 2012 to December 2015 in Cahuide and San José de Lupuna, Loreto, Peru. Serum samples analyzed in this project were collected at the end of the study period (n=590).

Epidemiologic characteristics of Peruvian cohort (n=590)

| Months of follow-up, n (%) | 13 months | 240 (40.68%) |
|----------------------------|-----------|--------------|
|                            | 37 months | 350 (59.32%) |
| Age, year (range, mean ± SD) | 3.8-85.6 years, 30.1±20.8 |
| <15 y, n (%)                | 212 (35.93%) |
| 15-39 y, n (%)              | 192 (32.54%) |
| ≥40 y, n (%)                | 186 (31.53%) |
| Sex, n (%)                  | Male      | 246 (41.69%) |
|                            | Female    | 344 (58.31%) |
| Community, n (%)            |           |              |
|                            | Cahuide   | 289 (48.98%) |
|                            | Lupuna    | 301 (51.02%) |
| Total P. vivax infections in 13 months by qPCR, n (%) |           |              |
|                            | 0         | 146 (24.75)  |
|                            | 1-2       | 287 (48.64)  |
|                            | >=3       | 157 (26.61)  |
| Time since last P. vivax infection, n (%) |           |              |
| Currently infected (<1 month) | 160 (27.12) |
| 1-9 months                  | 228 (38.64) |
| 9-13 months                 | 56 (9.49)  |
| None 13 months              | 75 (12.71)  |
| 13-37 months                | 59 (10.00)  |
| None 37 months              | 12 (2.03)   |
**Supplementary Table S4.** Seropositivity of IgG3 and total IgG in all the Peruvian cohort (n=590) and in those infected within the last 9 months (n=388). Negative control of PVX_094255(RBP2b<sub>161-1009</sub>) was not measured, so the seropositivity cannot be determined. Abbreviation: NA=not available.

| Antigens                  | IgG3 seropositivity in cohort, n (%) | IgG3 seropositivity in infected within 9 mo, n (%) | IgG seropositivity in cohort, n (%) | IgG seropositivity in infected within 9 mo, n (%) |
|---------------------------|--------------------------------------|-----------------------------------------------------|-------------------------------------|--------------------------------------------------|
| AMA 1                     | 206 (34.92%)                         | 159 (40.98%)                                        | 463 (78.47%)                        | 313 (80.67%)                                     |
| CSP210                    | 76 (12.88%)                          | 56 (14.43%)                                        | 70 (11.86%)                         | 52 (13.40%)                                      |
| exported                  | 18 (3.05%)                           | 13 (3.35%)                                         | 168 (28.47%)                        | 132 (34.02%)                                     |
| hypothetical protein      | 81 (13.73%)                          | 63 (16.24%)                                        | 119 (20.17%)                        | 90 (23.20%)                                      |
| MSP5                      | 243 (41.19%)                         | 189 (48.71%)                                        | 241 (40.85%)                        | 190 (48.97%)                                     |
| MSP7                      | 73 (12.37%)                          | 53 (13.66%)                                        | 308 (52.20%)                        | 217 (55.93%)                                     |
| MSP1-19                   | 149 (25.25%)                         | 108 (27.84%)                                        | 481 (81.53%)                        | 315 (81.19%)                                     |
| MSP3a                     | 188 (31.86%)                         | 138 (35.57%)                                        | 285 (48.31%)                        | 219 (56.44%)                                     |
| MSP3b                     | 82 (13.90%)                          | 65 (16.75%)                                        | 478 (81.02%)                        | 321 (82.73%)                                     |
| MSP7B                     | 200 (33.90%)                         | 148 (38.14%)                                        | 542 (91.86%)                        | 358 (92.27%)                                     |
| MSP7F                     | 48 (8.14%)                           | 39 (10.05%)                                        | 65 (11.02%)                         | 54 (13.92%)                                      |
| MSP7L                     | 106 (17.97%)                         | 77 (19.85%)                                        | 252 (42.71%)                        | 177 (45.62%)                                     |
| MSP8                      | 67 (11.36%)                          | 52 (13.40%)                                        | 499 (84.58%)                        | 340 (87.63%)                                     |
| PTEX150                   | 85 (14.41%)                          | 64 (16.49%)                                        | 254 (43.05%)                        | 190 (48.97%)                                     |
| Pv-fam-a (PVX_088820)     | 72 (12.20%)                          | 52 (13.40%)                                        | 205 (34.75%)                        | 147 (37.89%)                                     |
| Pv-fam-a (PVX_090265)     | 35 (5.93%)                           | 30 (7.73%)                                         | 436 (73.90%)                        | 301 (77.58%)                                     |
| Pv-fam-a (PVX_092990)     | 13 (2.20%)                           | 8 (2.06%)                                          | 47 (7.97%)                          | 32 (8.25%)                                       |
| Pv-fam-a (PVX_096995)     | 89 (15.08%)                          | 63 (16.24%)                                        | 259 (43.90%)                        | 204 (52.58%)                                     |
| Pv-fam-a (PVX_112670)     | 49 (8.31%)                           | 35 (9.02%)                                         | 254 (43.05%)                        | 192 (49.48%)                                     |
| Pv-fam-a (PVX_125728)     | 366 (62.03%)                         | 261 (67.27%)                                        | 566 (95.93%)                        | 375 (96.65%)                                     |
| PVX_090970                | 46 (7.80%)                           | 30 (7.73%)                                         | 466(78.98%)                         | 309 (79.64%)                                     |
| PVX_091710                | 23 (3.90%)                           | 16 (4.12%)                                         | 476 (80.68%)                        | 317 (81.70%)                                     |
| PVX_101530                | 17 (2.88%)                           | 12 (3.09%)                                         | 530 (89.83%)                        | 354 (91.24%)                                     |
| RAMA                      | 26 (4.41%)                           | 18 (4.64%)                                         | 187 (31.69%)                        | 140 (36.08%)                                     |
| RBP2b161-1009             | 42 (7.12%)                           | 29 (7.47%)                                         | NA                                  | NA                                               |
| RBP2b1986-2653            | 12 (2.03%)                           | 7 (1.80%)                                          | 387 (65.59%)                        | 288 (74.23%)                                     |
| RON2                      | 45 (7.63%)                           | 29 (7.47%)                                         | 151 (25.59%)                        | 110 (28.35%)                                     |
| s16                       | 113 (19.15%)                         | 84 (21.65%)                                        | 141 (23.90%)                        | 110 (28.35%)                                     |
| SIAP2                     | 14 (2.37%)                           | 10 (2.58%)                                         | 123 (20.85%)                        | 98 (25.26%)                                      |
**Supplementary Table S5.** Comparison of AUC value of each antigen-specific IgG3 and total IgG in all individuals in Peruvian cohort (n=590) vs in Lupuna only (n=301) in classifying *P. vivax* infections within the last 9 months.

| Antigens                        | IgG3        | Total IgG     |
|---------------------------------|-------------|---------------|
|                                 | 9 mo (All)  | 9 mo (Lupuna) | 9 mo (All)  | 9 mo (Lupuna) |
| Pv-fam-a (PVX_125728)           | 0.764       | 0.864         | 0.800       | 0.886         |
| Pv-fam-a (PVX_096995)           | 0.740       | 0.779         | 0.828       | 0.904         |
| MSP5                            | 0.724       | 0.813         | 0.718       | 0.895         |
| RBP2b_1986-2653                  | 0.722       | 0.787         | 0.838       | 0.878         |
| MSP3b                           | 0.714       | 0.777         | 0.819       | 0.893         |
| MSP3a                           | 0.703       | 0.771         | 0.835       | 0.912         |
| PVX_101530                      | 0.686       | 0.728         | 0.825       | 0.895         |
| Pv-fam-a                        | 0.685       | 0.725         | 0.824       | 0.890         |
| MSP1-19                         | 0.676       | 0.734         | 0.805       | 0.873         |
| RBP2b_161-1009                   | 0.675       | 0.738         | 0.734       | 0.898         |
| MSP7L                           | 0.673       | 0.704         | 0.811       | 0.892         |
| MSP7B                           | 0.672       | 0.741         | 0.835       | 0.910         |
| CSP210                          | 0.663       | 0.716         | 0.584       | 0.843         |
| AMA1                            | 0.655       | 0.700         | 0.805       | 0.878         |
| hypothetical protein             | 0.655       | 0.708         | 0.651       | 0.871         |
| PVX_090970                      | 0.654       | 0.704         | 0.795       | 0.882         |
| RAMA                            | 0.652       | 0.712         | 0.761       | 0.873         |
| Pv-fam-a (PVX_092990)           | 0.648       | 0.692         | 0.700       | 0.830         |
| MSP 8                           | 0.647       | 0.710         | 0.820       | 0.893         |
| PTEX150                         | 0.646       | 0.691         | 0.762       | 0.868         |
| s16                             | 0.639       | 0.692         | 0.744       | 0.898         |
| RON2                            | 0.637       | 0.694         | 0.751       | 0.875         |
| exported                        | 0.634       | 0.663         | 0.729       | 0.854         |
| Pv-fam-a (PVX_088820)           | 0.625       | 0.652         | 0.763       | 0.886         |
| Pv-fam-a (PVX_112670)           | 0.623       | 0.642         | 0.808       | 0.896         |
| MSP 7                           | 0.621       | 0.680         | 0.777       | 0.879         |
| PVX_091710                      | 0.617       | 0.652         | 0.800       | 0.879         |
| SIAP2                           | 0.602       | 0.631         | 0.757       | 0.850         |
| MSP7F                           | 0.571       | 0.589         | 0.720       | 0.870         |