B cell receptor repertoire analysis in malaria-naive and malaria-experienced individuals reveals unique characteristics of atypical memory B cells

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Table S1. Donor characteristics.

| Donor | Race | Gender | Age (Years) | Location | Date of Blood Draw |
|-------|------|--------|-------------|----------|-------------------|
| US-B1 | Black | Male   | 24-35       | USA      | February 2018     |
| US-B2 | Black | Male   | 24-35       | USA      | February 2018     |
| US-B3 | Black | Male   | 26          | USA      | November 2018     |
| US-B4 | Black | Female | 45          | USA      | November 2018     |
| US-B5 | Black | Male   | 54          | USA      | November 2018     |
| US-W1 | White | Male   | 44          | USA      | January 2018      |
| US-W2 | White | Male   | 24-35       | USA      | February 2018     |
| US-W3 | White | Male   | 29          | USA      | September 2018    |
| US-W4 | White | Male   | 18          | USA      | October 2018      |
| US-W5 | White | Female | 32          | USA      | October 2018      |
| UG1   | Black | Unknown | Unknown     | Uganda   | June 2017         |
| UG2   | Black | Unknown | Unknown     | Uganda   | June 2017         |
| UG3   | Black | Unknown | Unknown     | Uganda   | June 2017         |
Table S2. Number of sequence reads for naive B cell BCR-seq libraries.

| Donor | Total Reads | Total UMIs | Reads above threshold | UMIs above threshold | Average reads/UMI | Productive rearrangements |
|-------|-------------|------------|-----------------------|----------------------|------------------|---------------------------|
|       |             |            |                       |                      |                  | IgM | IgG |
| US-B1 | 2,379,401   | 119,867    | 890,950               | 53,284               | 16.7             | 31,350 | 117 |
| US-B2 | 2,533,481   | 114,802    | 981,184               | 46,587               | 21.1             | 22,178 | 107 |
| US-B3 | 890,005     | 73,109     | 295,227               | 20,974               | 14.1             | 9,383  | 78  |
| US-B4-1 | 2,137,471 | 126,140    | 809,116               | 46,565               | 17.4             | 22,606 | 54  |
| US-B4-2 | 2,325,328  | 59,517     | 988,485               | 23,691               | 41.7             | 10,942 | 39  |
| US-B5-1 | 1,639,374  | 79,128     | 657,926               | 32,195               | 20.4             | 14,517 | 248 |
| US-B5-2 | 1,885,331  | 52,666     | 788,109               | 19,585               | 40.2             | 6,604  | 152 |
| US-W1  | 2,011,277   | 76,884     | 805,361               | 34,976               | 23.0             | 19,194 | 393 |
| US-W2  | 1,012,129   | 76,590     | 331,288               | 24,582               | 13.5             | 12,069 | 50  |
| US-W3-1 | 838,921    | 81,876     | 242,895               | 20,714               | 11.7             | 11,416 | 200 |
| US-W3-2 | 2,665,490  | 68,597     | 1,120,023             | 26,347               | 42.5             | 12,230 | 176 |
| US-W4  | 2,138,839   | 100,230    | 861,347               | 43,915               | 19.6             | 24,672 | 118 |
| US-W5  | 981,550     | 63,609     | 361,308               | 23,937               | 15.1             | 15,094 | 83  |
| UG1    | 1,120,325   | 117,873    | 299,599               | 25,874               | 11.6             | 6,128  | 297 |
| UG2    | 1,506,121   | 113,838    | 487,709               | 35,328               | 13.8             | 14,241 | 240 |
| UG3    | 887,296     | 62,441     | 301,943               | 18,367               | 16.4             | 5,712  | 281 |

\(^1\)Threshold set at 6 or more reads per UMI.
Table S3. Number of sequence reads for classical memory B cell BCR-seq libraries.

| Donor   | Total Reads | Total UMIs | Reads above threshold<sup>1</sup> | UMIs above threshold | Average Reads/UMI | Productive Rearrangements |
|---------|-------------|------------|-----------------------------------|----------------------|-------------------|----------------------------|
|         |             |            |                                   |                      |                   | IgM | IgG   |
| US-B1   | 645,574     | 29,871     | 259,654                           | 11,839               | 21.9              | 3,851 | 876   |
| US-B2   | 568,075     | 33,284     | 218,259                           | 14,135               | 15.4              | 3,215 | 351   |
| US-B3   | 662,769     | 18,212     | 275,943                           | 6,626                | 41.6              | 1,765 | 559   |
| US-B4   | 1,335,535   | 33,452     | 554,995                           | 9,957                | 55.7              | 1,217 | 765   |
| US-B5-1 | 1,217,532   | 20,229     | 514,364                           | 6,617                | 77.7              | 1,337 | 315   |
| US-B5-2 | 1,387,356   | 25,026     | 599,444                           | 7,620                | 78.7              | 1,047 | 303   |
| US-W1   | 1,622,521   | 68,453     | 665,384                           | 22,020               | 30.2              | 2,096 | 1,957 |
| US-W2   | 1,104,310   | 33,750     | 464,615                           | 12,645               | 36.7              | 4,224 | 678   |
| US-W3   | 1,461,354   | 10,528     | 642,307                           | 2,977                | 215.8             | 637   | 126   |
| US-W4   | 882,366     | 32,231     | 357,739                           | 9,879                | 36.2              | 3,694 | 318   |
| US-W5-1 | 1,461,910   | 46,819     | 587,079                           | 16,005               | 36.7              | 5,425 | 992   |
| US-W5-2 | 1,632,265   | 34,914     | 674,566                           | 12,164               | 55.5              | 4,002 | 802   |
| UG1     | 890,486     | 92,822     | 262,172                           | 16,588               | 15.8              | 465   | 602   |
| UG2     | 1,426,381   | 48,860     | 587,413                           | 16,148               | 36.4              | 2,455 | 2,718 |
| UG3     | 1,124,633   | 59,575     | 435,570                           | 15,714               | 27.7              | 2,275 | 1,164 |

<sup>1</sup>Threshold set at 6 or more reads per UMI.
Table S4. Number of sequence reads for atypical memory B cell BCR-seq libraries.

| Donor | Total Reads | Total UMIs | Reads above threshold\(^1\) | UMIs above threshold | Average Reads/UMI | Productive Rearrangements |
|-------|-------------|------------|------------------------------|---------------------|------------------|--------------------------|
|       |             |            |                              |                     |                  | IgM | IgG   |
| US-B2 | 1,327,581   | 40,378     | 562,190                      | 11,875              | 47.3             | 2,902 | 772   |
| US-B3 | 795,132     | 11,953     | 338,461                      | 3,043               | 111.2            | 139   | 113   |
| US-B4 | 664,545     | 8,386      | 289,330                      | 1,958               | 147.8            | 176   | 41    |
| US-B5 | 972,321     | 10,755     | 422,334                      | 2,586               | 163.3            | 255   | 78    |
| US-W1 | 526,924     | 13,947     | 227,248                      | 3,646               | 62.3             | 757   | 248   |
| US-W2 | 2,267,873   | 19,192     | 1,018,219                    | 3,408               | 298.8            | 459   | 97    |
| UG1   | 2,042,950   | 33,990     | 897,501                      | 8,422               | 106.6            | 631   | 169   |
| UG2   | 1,635,265   | 28,490     | 727,475                      | 6,351               | 114.5            | 1,256 | 399   |
| UG3   | 1,392,734   | 16,074     | 620,859                      | 3,660               | 169.6            | 421   | 306   |

\(^1\)Threshold set at 6 or more reads per UMI.
Table S5. P values for differences in SHM frequencies in B cell subsets.

|        | NBC    | IgM+ cMBC | IgG+ cMBC | IgM+ atMBC |
|--------|--------|-----------|-----------|------------|
| Total R| IgG+ atMBC | 7.4E-04   | 1.5E-03   | 0.03       | 0.02       |
|        | IgM+ atMBC | 1.8E-03   | 1.1E-03   | 1.07E-03   |
|        | IgG+ cMBC  | 1.9E-06   | 1.9E-06   |            |
|        | IgM+ cMBC  | 1.9E-06   |           |            |
| Total S| IgG+ atMBC | 7.4E-04   | 1.5E-03   | 0.17       | 0.02       |
|        | IgM+ atMBC | 3.1E-03   | 1.1E-03   | 1.07E-03   |
|        | IgG+ cMBC  | 1.9E-06   | 1.9E-06   |            |
|        | IgM+ cMBC  | 1.9E-06   |           |            |
| CDR R  | IgG+ atMBC | 7.4E-04   | 5.2E-03   | 0.02       | 0.02       |
|        | IgM+ atMBC | 3.1E-03   | 1.1E-03   | 1.07E-03   |
|        | IgG+ cMBC  | 1.9E-06   | 1.9E-06   |            |
|        | IgM+ cMBC  | 1.9E-06   |           |            |
| CDR S  | IgG+ atMBC | 7.4E-04   | 8.9E-03   | 0.07       | 0.02       |
|        | IgM+ atMBC | 5.1E-03   | 1.1E-03   | 1.07E-03   |
|        | IgG+ cMBC  | 1.9E-06   | 1.9E-06   |            |
|        | IgM+ cMBC  | 1.9E-06   |           |            |
| FWR R  | IgG+ atMBC | 7.4E-04   | 1.5E-03   | 0.17       | 0.02       |
|        | IgM+ atMBC | 1.4E-03   | 1.1E-03   | 1.07E-03   |
|        | IgG+ cMBC  | 1.9E-06   | 1.9E-06   |            |
|        | IgM+ cMBC  | 1.9E-06   |           |            |
| FWR S  | IgG+ atMBC | 7.4E-04   | 1.5E-03   | 0.36       | 0.02       |
|        | IgM+ atMBC | 1.1E-03   | 1.1E-03   | 1.07E-03   |
|        | IgG+ cMBC  | 1.9E-06   | 1.9E-06   |            |
|        | IgM+ cMBC  | 1.9E-06   |           |            |
Table S6. P Values for differences in amino acid properties between B cell subsets.

|                      | NBC     | IgM+ cMBC | IgG+ cMBC | IgM+ atMBC |
|----------------------|---------|-----------|-----------|------------|
| **Basic Amino Acids**|         |           |           |            |
| IgG+ atMBC           | 7.4E-04 | 0.87      | 1.00      | 4.0E-03    |
| IgM+ atMBC           | 1.00    | 4.8E-04   | 4.0E-05   |            |
| IgG+ cMBC            | 1.9E-06 | 1.00      |           |            |
| IgM+ cMBC            | 1.3E-05 |           |           |            |
| **Aromatic Amino Acids**|       |           |           |            |
| IgG+ atMBC           | 1.00    | 0.17      | 2.9E-03   | 1.00       |
| IgM+ atMBC           | 1.00    | 0.01      | 4.0E-05   |            |
| IgG+ cMBC            | 1.3E-05 | 0.44      |           |            |
| IgM+ cMBC            | 2.3E-03 |           |           |            |
| **Acidic Amino Acids**|       |           |           |            |
| IgG+ atMBC           | 1.00    | 1.00      | 1.00      | 1.00       |
| IgM+ atMBC           | 0.60    | 1.00      | 1.00      |            |
| IgG+ cMBC            | 1.00    | 1.00      |           |            |
| IgM+ cMBC            | 1.00    |           |           |            |
| **Hydrophobic Amino Acids**|     |           |           |            |
| IgG+ atMBC           | 2.9E-03 | 1.00      | 0.58      | 0.08       |
| IgM+ atMBC           | 1.00    | 4.0E-05   | 1.00      |            |
| IgG+ cMBC            | 1.00    | 3.7E-05   |           |            |
| IgM+ cMBC            | 1.9E-06 |           |           |            |
| **Aliphatic Amino Acids**|     |           |           |            |
| IgG+ atMBC           | 1.00    | 0.02      | 0.36      | 1.00       |
| IgM+ atMBC           | 1.00    | 0.06      | 1.00      |            |
| IgG+ cMBC            | 0.02    | 1.9E-06   |           |            |
| IgM+ cMBC            | 1.3E-04 |           |           |            |
| **Bulky Amino Acids**|         |           |           |            |
| IgG+ atMBC           | 0.72    | 1.5E-03   | 1.00      | 1.00       |
| IgM+ atMBC           | 1.00    | 1.8E-03   | 1.00      |            |
| IgG+ cMBC            | 1.00    | 3.7E-05   |           |            |
| IgM+ cMBC            | 1.3E-05 |           |           |            |
| **Polarity**         |         |           |           |            |
| IgG+ atMBC           | 0.03    | 0.17      | 1.00      | 0.05       |
| IgM+ atMBC           | 1.00    | 4.0E-05   | 4.0E-05   |            |
| IgG+ cMBC            | 1.9E-06 | 1.00      |           |            |
| IgM+ cMBC            | 1.9E-06 |           |           |            |
| **Charge**           |         |           |           |            |
| IgG+ atMBC           | 0.05    | 1.00      | 1.00      | 1.00       |
| IgM+ atMBC           | 1.00    | 0.96      | 1.00      |            |
| IgG+ cMBC            | 9.7E-04 | 1.00      |           |            |
| IgM+ cMBC            | 7.2E-04 |           |           |            |
Table S7. Primers for BCR-sequencing.

| Name                  | Application                                                                 | Primer | Sequence¹ |
|-----------------------|------------------------------------------------------------------------------|--------|-----------|
| Human IGH             | cDNA synthesis mix                                                           | hIGG_r1| GAAGTAGTCCTTGACCAGGCA |
|                       | Primer for cDNA synthesis, IgG heavy-chain                                  | hIGM_r1| GTGATGGAGTCGGGAAGGAAG |
|                       | Primer for cDNA synthesis, human IgA heavy-chain                             | hIGA_r1| GCGACGACCACGTTCCCATCT |
|                       | Primer for cDNA synthesis, human IgD heavy-chain                             | hIGD_r1| GGACCACAGGGCTGTATTC |
|                       | Primer for cDNA synthesis, human IgE heavy-chain                             | hIGE_r1| AGTCACGGAGGTGCCATTG |
| BCR-seq TSO           | 5' template switch oligo                                                     | TSO    | 5'-Me-isodC/isodG/AAGCAGdUGGTAdUCAACGCAGCGdUNNNNdUNNNNdUNNNNdUCTTrGrG |
| Human IGH             | reverse primer mix                                                          |        |            |
|                       | Nested primer with Z adaptor, IgG/IgE                                       | hIgGE_r2| ATTGGGCAGCCCTGATTARGGGAAGACSGATG |
|                       | Nested primer with Z adaptor, IgA                                            | hIGA_r2| ATTGGGCAGCCCTGATTCAGCGGGAAGACCTTG |
|                       | Nested primer with Z adaptor, IgM                                            | hIGM_r2| ATTGGGCAGCCCTGATTAGGGGAAGACGATG |
|                       | Nested primer with Z adaptor, IgD                                             | hIGD_r2| ATTGGGCAGCCCTGATTATATGGGAAGACGATG |
| PCR 1 primer          | Forward                                                                     | M1SS   | AAGCAGTGGTGATCAACGCA |
| Primer cocktail 1     | Nested primer 1                                                              | M1S1   | NNNNACAATCAGTGATCAACGCAGAG |
|                       | Step-out primer 1                                                            | Z1     | NNNNCGTAAATTTGGGCAGCCCTGATT |
| Primer cocktail 2     | Nested primer 2                                                              | M1S2   | NNNNNGCGAGCAGTGATCAACGCAGAG |
|                       | Step-out primer 2                                                            | Z2     | NNNNTCAATATTGGGCAGCCCTGATT |
| Primer cocktail 3     | Nested primer 3                                                              | M1S3   | NNNNNAGGGACAGTGATCAACGCAGAG |
|                       | Step-out primer 3                                                            | Z3     | NNNNNAGATAATTTGGGCAGCCCTGATT |
| Primer cocktail 4     | Nested primer 4                                                              | M1S4   | NNNNGTTCAGTGATCAACGCAGAG |
|                       | Step-out primer 4                                                            | Z4     | NNNNNNTAGCCATTGGGCAGCCCTGATT |

¹Oligo sequences were obtained from Turchaninova et al. [51] with modifications to the BCR-seq TSO.
Table S8. Antibodies used for B cell sorting.

| Antigen | Antibody           | Clone | Vendor                  | Catalogue Number |
|---------|--------------------|-------|-------------------------|------------------|
| CD19    | CD19-SB645         | HIB19 | Thermo Fisher Scientific | 64-0199-41       |
| CD20    | CD20-BV785         | 2H7   | BioLegend               | 302355           |
| CD21    | CD21-PerCP-eFluor710 | HB5   | Thermo Fisher Scientific | 46-0219-41       |
| CD27    | CD27-PE-Cy7        | O323  | Thermo Fisher Scientific | 25-0279-41       |
S1. Antibody titers against \textit{P. falciparum} antigens in malaria-experienced donors.
S2. Gating strategy for FACS isolation of naive B cells, classical memory B cells, and atypical memory B cells.
S3. Biological replicates show highly similar IGHV gene usage.
S4. Distribution of R mutation frequency in B cell subsets.
S5. Classical and atypical MBCs have different somatic hypermutation frequencies in the CDR and FWR regions.
S6. Differences in HCDR3 physicochemical properties are not influenced by malaria-experience.
S7. IGHV gene usage in B cell subsets.
S8. Difference in IGHV gene usage between malaria-exposed and malaria-naïve donors.