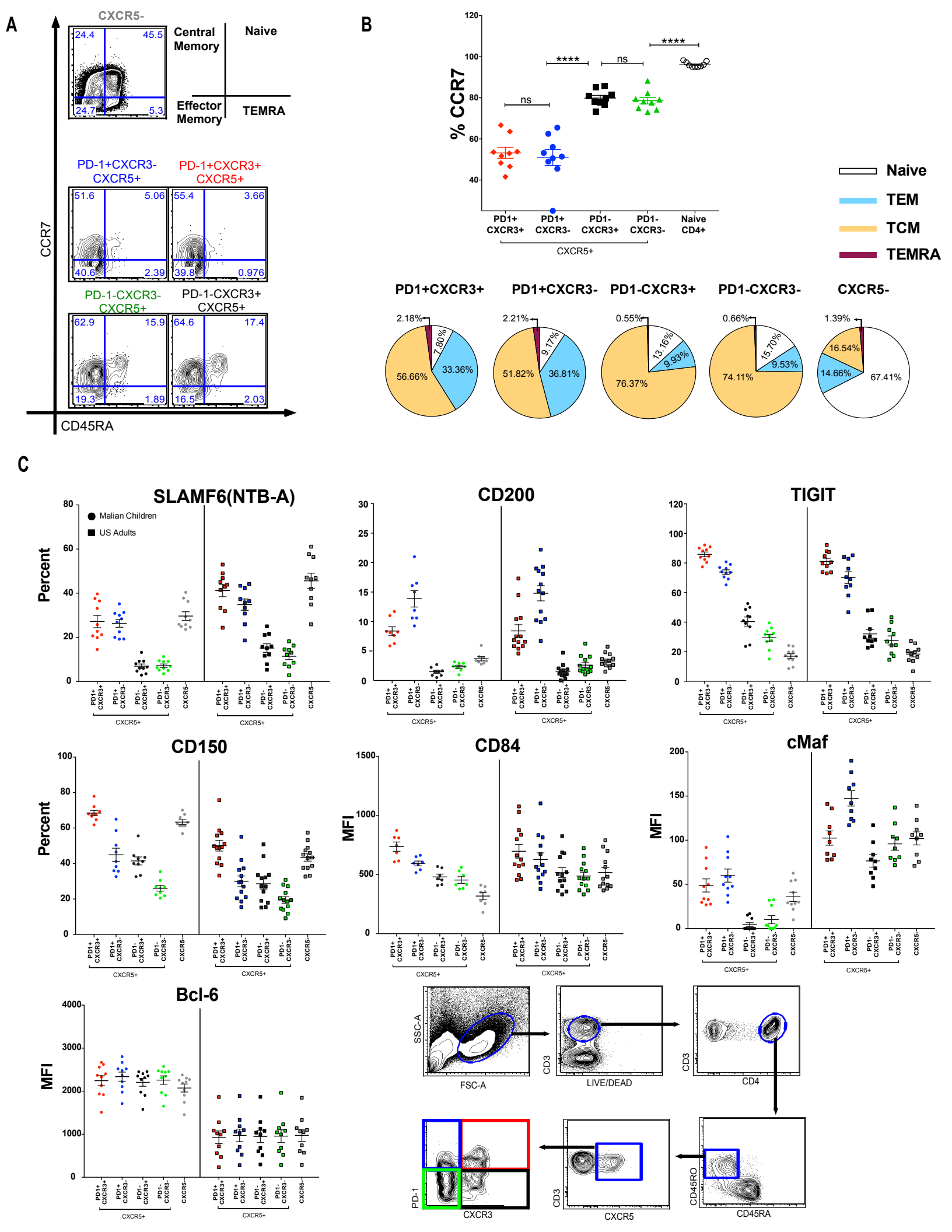
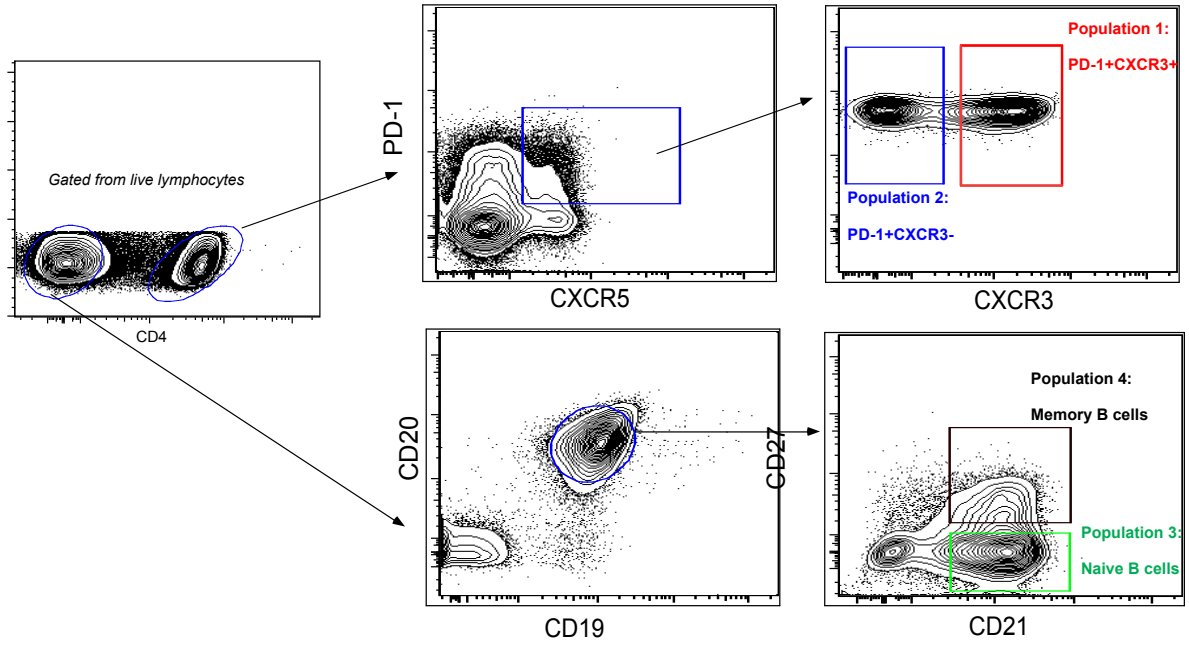
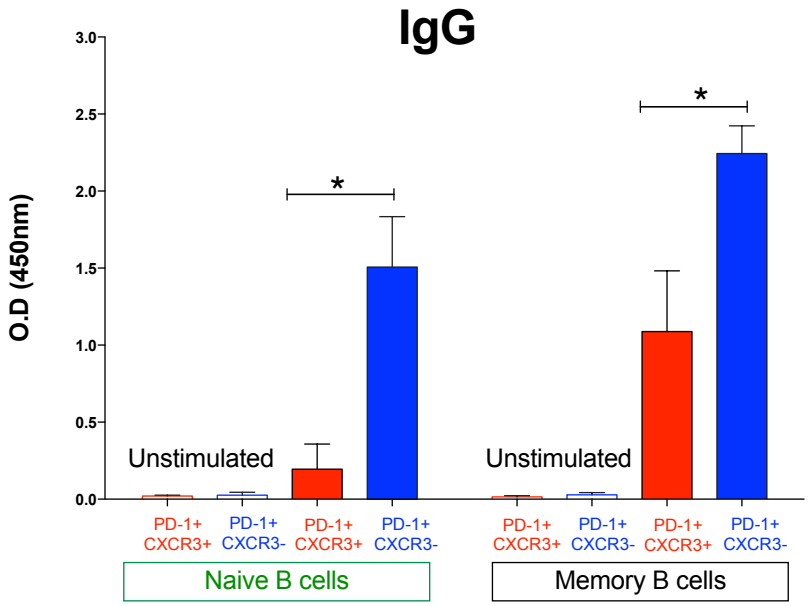

A
CXCR5-
24.4
45.5
24.7
5.3
Central Memory
Naive
Effector Memory
TEMRA
PD-1+CXCR3- CXCR5+
PD-1+CXCR3+ CXCR5+
51.6
5.06
40.6
2.39
55.4
3.66
39.8
0.976
PD-1-CXCR3- CXCR5+
PD-1-CXCR3+ CXCR5+
62.9
15.9
19.3
1.89
64.6
17.4
16.5
2.03
CCR7
CD45RA
B
% CCR7
ns
****
****
ns
PD1+ CXCR3+
PD1+ CXCR3-
PD1- CXCR3+
PD1- CXCR3-
Naive CD4+
CXCR5+
Naive
TEM
TCM
TEMRA
PD1+CXCR3+
PD1+CXCR3-
PD1-CXCR3+
PD1-CXCR3-
CXCR5-
2.18%
7.80%
33.36%
56.66%
2.21%
9.17%
36.81%
51.82%
0.55%
13.16%
9.93%
76.37%
0.66%
15.70%
9.53%
74.11%
1.39%
16.54%
14.66%
67.41%
C
SLAMF6(NTB-A)
Malian Children
US Adults
Percent
CD200
TIGIT
CD150
CD84
MFI
cMaf
Bcl-6
SSC-A
FSC-A
CD3
LIVE/DEAD
CD4
PD-1
CXCR3
CXCR5
CD45RO
CD45RA

Supplemental Figure 1, Related to Figure 1. Characteristics of blood CXCR5$^+$CD4$^+$ T cells from Malian children. (A) Gating strategy to identify memory and naïve cells within the CXCR5$^+$CD4$^+$ subsets. (B) Percent expression of CCR7 and the distribution of naïve, terminal differentiated effector memory (TEMRA), effector memory (TEM) and central memory (TCM) T cells across CXCR5$^+$ subsets in Malian children. (C) Expression of GC Tfh cell-related molecules by CXCR5$^+$ subsets in Malian children and U.S. adults.

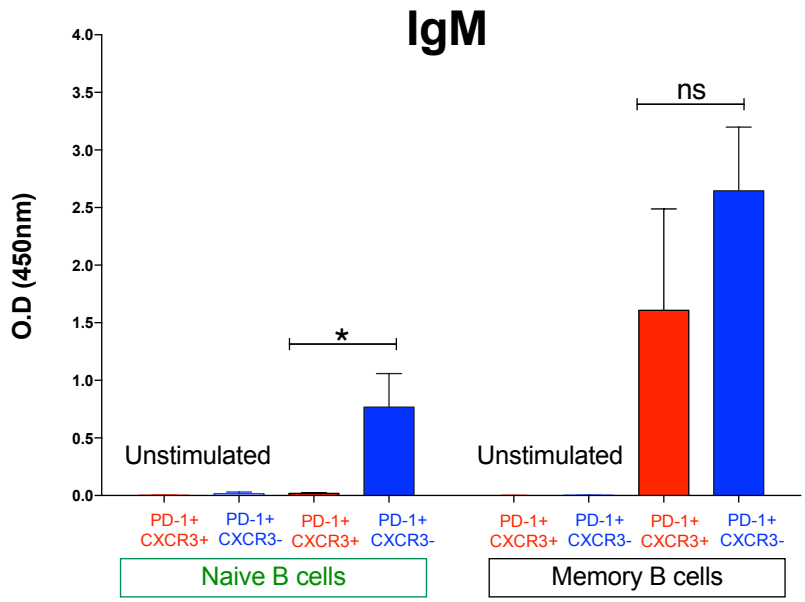
A


Gated from live lymphocytes
CD4
PD-1
CXCR5
Population 1:
PD-1+CXCR3+
Population 2:
PD-1+CXCR3-
CXCR3
CD20
CD19
CD27
Population 4:
Memory B cells
Population 3:
Naive B cells
CD21

B

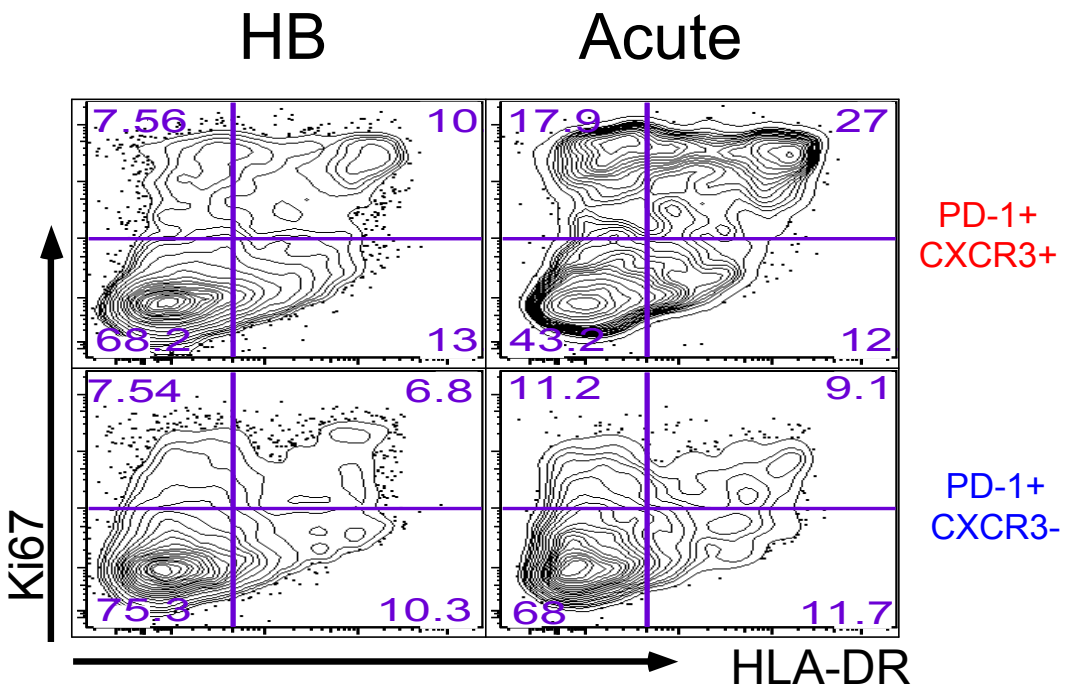

IgG
O.D (450nm)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
Unstimulated
Unstimulated
PD-1+ CXCR3+
PD-1+ CXCR3-
PD-1+ CXCR3+
PD-1+ CXCR3-
PD-1+ CXCR3+
PD-1+ CXCR3-
PD-1+ CXCR3+
PD-1+ CXCR3-
Naive B cells
Memory B cells

C

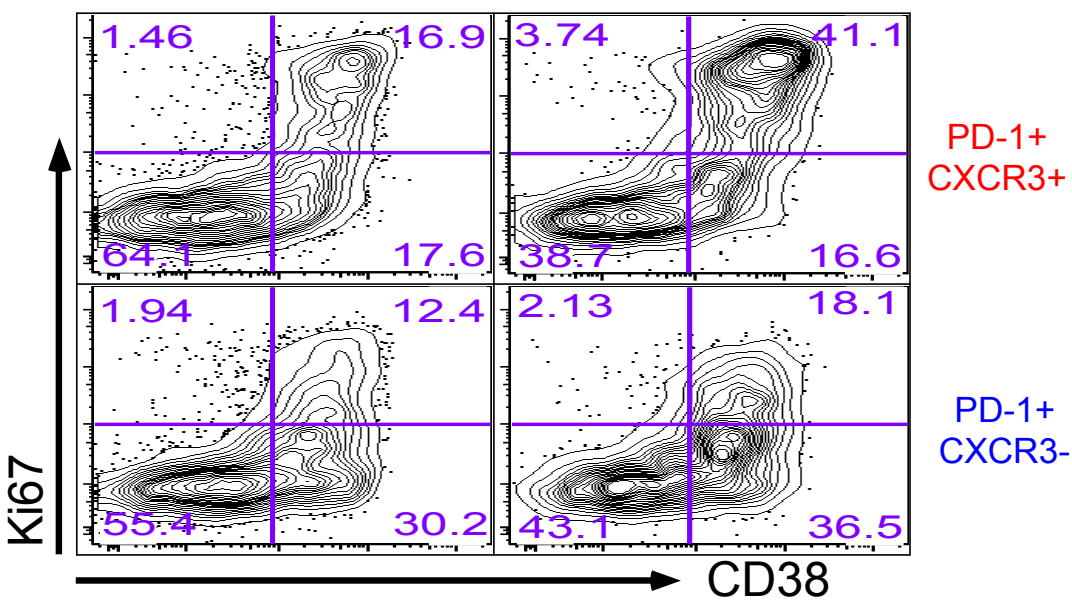

IgM
O.D (450nm)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
ns
Unstimulated
Unstimulated
PD-1+ CXCR3+
PD-1+ CXCR3-
PD-1+ CXCR3+
PD-1+ CXCR3-
PD-1+ CXCR3+
PD-1+ CXCR3-
PD-1+ CXCR3+
PD-1+ CXCR3-
Naive B cells
Memory B cells

Supplemental Figure 2, Related to Figure 2. PD-1^{+}CXCR3^{-}CXCR5^{+}CD4^{+} Tfh cells from healthy U.S. adults provide superior B cell help. (A) Gating strategy for FACS sorting of circulating Tfh and B cell populations. (B and C) Sorted Tfh subsets from healthy U.S. adults were cultured with autologous naïve or MBCs in the presence or absence (unstimulated) of SEB, and secreted total IgG (B) and IgM (C) antibodies were measured at day 6 and 12 for MBCs and naïve B cells, respectively. P values determined by paired *t* test. **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns = not significant

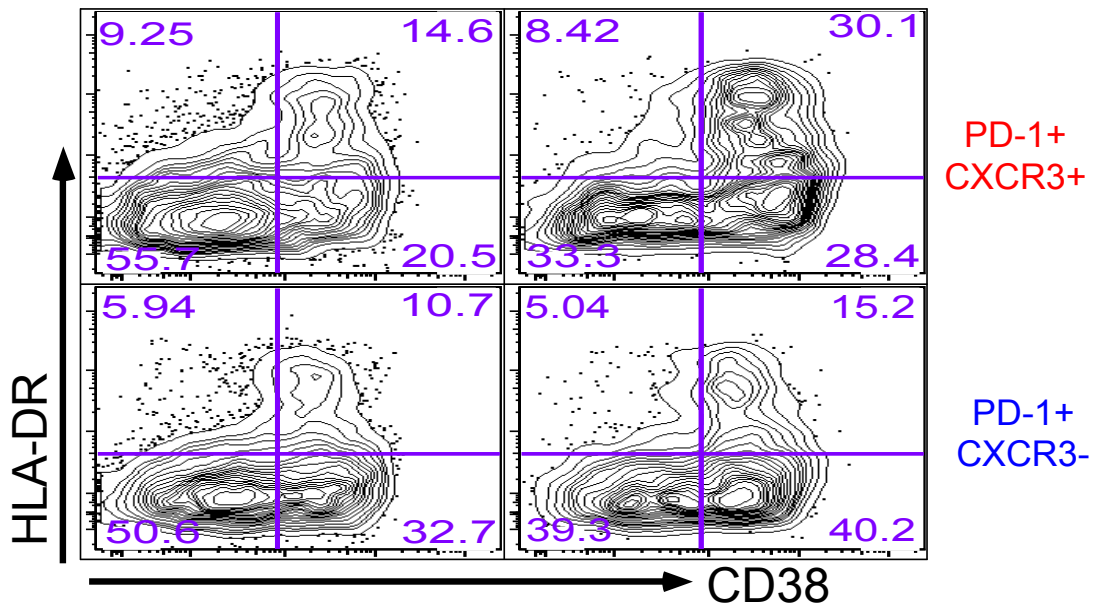
A


HB
Acute
7.56
10
68.2
13
17.9
27
43.2
12
7.54
6.8
75.3
10.3
11.2
9.1
68
11.7
PD-1+ CXCR3+
PD-1+ CXCR3-
Ki67
HLA-DR

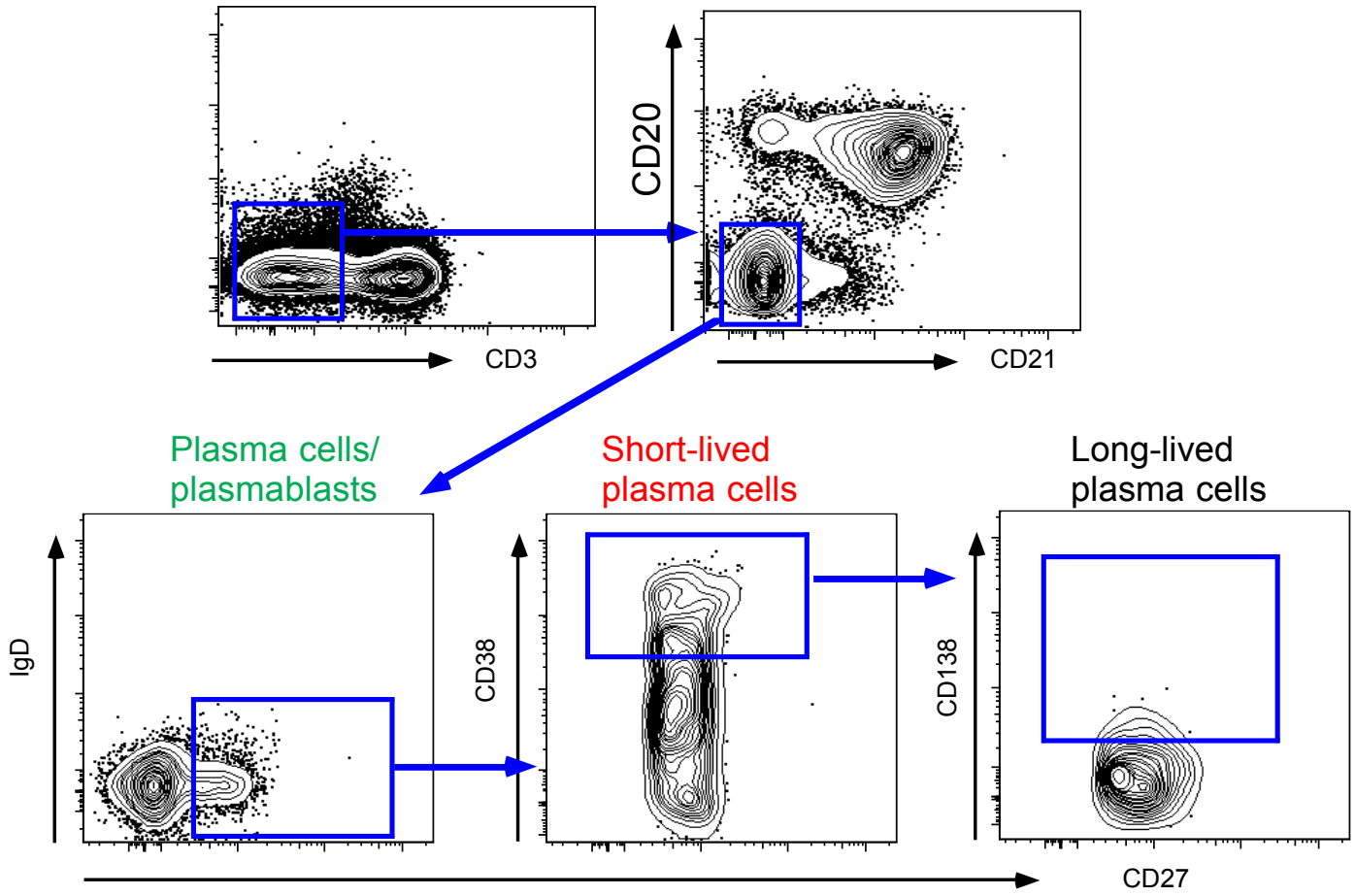
B


1.46
16.9
64.1
17.6
3.74
41.1
38.7
16.6
1.94
12.4
55.4
30.2
2.13
18.1
43.1
36.5
PD-1+ CXCR3+
PD-1+ CXCR3-
Ki67
CD38

C


9.25
14.6
55.7
20.5
8.42
30.1
33.3
28.4
5.94
10.7
50.6
32.7
5.04
15.2
39.3
40.2
PD-1+ CXCR3+
PD-1+ CXCR3-
HLA-DR
CD38

Supplemental Figure 3, Related to Figure 4. Acute malaria preferentially activates PD- 1^{+}CXCR3^{+}CXCR5^{+}CD4^{+} Tfh cells. Representative flow cytometry plots showing staining of (A) Ki67 and HLA-DR, (B) Ki67 and CD38, and (C) HLA-DR and CD38 at the healthy pre-infection baseline and during acute malaria for PD-1^{+}CXCR3^{-}CXCR5 $^{+}$CD4^{+} and PD-1^{+}CXCR3^{+}CXCR5^{+}CD4^{+} Tfh subsets.


CD3
CD20
CD21
Plasma cells/
plasmablasts
Short-lived
plasma cells
Long-lived
plasma cells
IgD
CD38
CD138
CD27

Supplemental Figure 4, Related to Figure 7. Gating strategy for plasma cells. Representative flow cytometry plot of plasma cell gating strategy in a Malian child.

Table S1, Related to Experimental Procedure. **Demographic and clinical data of study subjects and assays in which PBMC and sera samples were used.**

Table S2, Related to Experimental Procedure. Antibodies used for T and B cell characterization.

Table S3, Related to Experimental Procedure Antibodies used for intracellular staining and sorting.

Supplemental Experimental Procedures, Related to Experimental Procedure.

Human samples and Mali study site

The field study was conducted in the rural village of Kalifabougou, Mali where intense *P. falciparum* transmission occurs from June through December each year. The cohort study has been described in detail elsewhere (Tran et al., 2013). Briefly, healthy children and adults aged 3 months to 25 years were enrolled in an ongoing cohort study in May 2011. Exclusion criteria at enrollment included a hemoglobin level <7 g/dL, axillary temperature ≥37.5°C, acute systemic illness, underlying chronic disease, or use of antimalarial or immunosuppressive medications in the past 30 days. For this study we randomly selected 56 malaria-susceptible children aged 6 – 12 years who were asymptomatic and not infected with *P. falciparum* (by PCR) in May 2013, and who also had plasma and PBMC samples at the following three time points over an 8-month period: at the end of the 6-month dry season (May 2013), during the first febrile malaria episode of the ensuing 6-month malaria season and 7 days after treatment of the same malaria episode. Clinical malaria was defined as ≥ 2,500 asexual parasites/μL, an axillary temperature of ≥37.5°C or self-reported fever within 24 hours, and no other cause of fever discernible by physical exam. All individuals with signs and symptoms of malaria and any level of parasitemia detected by microscopy were treated according to the Malian National Malaria Control Program guidelines.

Detection of P. falciparum infection

Thick blood smears were stained with Giemsa and counted against 300 leukocytes. Parasite densities were recorded as the number of asexual parasites per microliter of blood based on a mean leukocyte count of 7500 cells/μL. Detailed methods

for the detection and quantification of *P. falciparum* by PCR have been described (Tran et al., 2013).

PBMC and plasma processing

At each time-point blood samples were drawn by venipuncture into sodium citrate-containing cell preparation tubes (BD, Vacutainer CPT Tubes) and transported 45 km to the laboratory where PBMCs and plasma were isolated and frozen within three hours according to the manufacturer's instructions. Plasma was frozen at −80°C. PBMCs were frozen in fetal bovine serum (FBS) (Gibco, Grand Island, NY) containing 7.5% dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO), kept at −80°C for 24 hours, and then stored at −196°C in liquid nitrogen. For each individual, PBMCs from all time points were thawed and assayed at the same time. The trypan blue dye exclusion assay consistently demonstrated >80% viability of PBMCs after thawing.

Antibodies, flow cytometry and intracellular cytokine staining

For cell surface staining and sorting, PBMCs were washed in PBS with 4% heat-inactivated FCS and incubated with Live/dead fixable Aqua (Invitrogen) and fluorescently-labeled antibodies as indicated. Briefly, for intracellular cytokine staining, lymphocytes were stimulated with Cell Stimulation Cocktail (ebioscience) in the presence of protein transport inhibitors (BD) and were treated with Transcriptional Factor Fixation/Permeabilization kit (ebioscience). The antibodies used are listed in Table S2 and S3. FACS analyses were performed on a BD LSR II flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star, Inc).

Isolation and in vitro co-culture of B and T cell subsets

B and T cell subsets were isolated from PBMCs of Malian children by a FACS sorter. Live cells were sorted into the following four populations: $CD4^+CXCR5^+PD\text{-}1^+CXCR3^+$, $CD4^+CXCR5^+PD\text{-}1^+CXCR3^-$, $CD4^-CD19^+CD21^+CD27^-$ (naïve B cells) and $CD4^-CD19^+CD21^+CD27^+$ (MBCs). Each sorted Tfh cell population (2.5×10^4) was co-cultured with naïve B cells (2.5×10^4) for 12 days and MBCs (2.5×10^4) for 6 days in complete medium with or without staphylococcal enterotoxin B (SEB) (1.5μg/ml; Sigma-Aldrich) in 96 round U-bottomed plates. At the end of each co-culture, cells were stained with monoclonal antibodies and Ig levels in supernatants were measured with ProcartaPlex Human Antibody 7plex Isotyping Panel (ebioscience).

Preparing *P. falciparum-infected red blood cell lysate for in vitro stimulation of PBMCs*

As previously described (Portugal et al., 2014), 3D7 *P. falciparum* parasites were maintained in fresh human O^{Rh+}erythrocytes at 3% hematocrit in RPMI 1640 medium (KD Medical) supplemented with 10% heat-inactivated O^{Rh+}human serum (Interstate Blood Bank, Memphis, Tennessee), 7.4% Sodium Bicarbonate (GIBCO, Invitrogen) and 25 μg/ml of gentamycin (GIBCO, invitrogen), at 37°C in the presence of a gas mixture containing 5% O_2, 5% CO_2 and 90% N_2. Parasite cultures were shown to be free of mycoplasma and acholeplasma using an ELISA-based Mycoplasma Detection Kit (Roche) which contains polyclonal antibodies specific for *M. arginini*, *M. hyorhinis*, *A. laidlawii*and *M. orale*. *P. falciparum* schizont iRBCs were isolated in RPMI 1640 medium supplemented with 0.25% Albumax (GIBCO, Invitrogen) and 7.4% Sodium Bicarbonate (GIBCO, Invitrogen) using magnetic columns (LD MACS Separation

Columns, Miltenyi Biotec). Control preparations of naïve red blood cells (nRBC) from the same blood donor were obtained and tested in all experiments. Lysates of *P. falciparum*-infected and naïve RBCs were obtained by three freeze-thaw cycles in liquid nitrogen and 37°C water bath.

In vitro stimulation of PBMCs with P. falciparum-infected red blood cell lysate

PBMCs were cultured in complete RPMI in round-bottom 96 well plates at 37°C in 5% CO_2 atmosphere. 500,000 PBMCs were stimulated with *P. falciparum*-infected RBC lysate or naive RBC lysate at a ratio of 3 RBCs per PBMC for 5 days. The 3:1 ratio of RBC to PBMC was used on the basis of titration experiments (from 5:1 to 1:1) as described previously (Portugal et al., 2014). Following stimulation, cells were centrifuged and supernatants were recovered and used immediately or frozen at −80°C for cytokine analysis. The stimulated cells were centrifuged, washed and recovered for surface and intracellular flow staining and analysis.

Measuring cytokines in plasma and supernatants of stimulated PBMCs

Plasma or supernatants from cultured cells were thawed and immediately analyzed with 17 Bio-plex human cytokine assays (Bio-Rad Laboratories, Inc.) as recommended by the manufacturer. Briefly, 100 µL of plasma at 1:2 dilution or 100 µL of supernatant were incubated with anti-cytokine antibody-coupled magnetic beads at room temperature shaking at 300 RPM in the dark. After several washes, the beads were then incubated with a biotinylated detector antibody at room temperature before incubation with streptavidin-phycoerythrin. Finally, the complexes were resuspended in 125 µL of detection buffer and 100 beads were counted with a Luminex 200 device (Bio-Rad Laboratories, Inc.). Final concentrations were calculated from the mean fluorescence

intensity and expressed in pg/mL using standard curves with known concentrations of each cytokine.

Antibody profiling by protein microarray

We used a protein microarray (Antigen Discovery Inc., Irvine, CA) containing 1087 sequence-verified *P. falciparum* polypeptides that were generated in an in vitro transcription translation (IVTT) reaction (RTS 100 *Escherichia coli* HY kits; Roche) as described previously (Davies et al., 2005). Due to gene length, some proteins were printed on the microarray in multiple spots of overlapping polypeptides representing 871 unique full-length *P. falciparum* proteins. The protein expression efficiency of the in vitro reactions was 98.7%. The proteins included on this array were down-selected from larger microarray studies in which these proteins were consistently immunoreactive in adults living in malaria endemic areas (Crompton et al., 2010; Tan et al., 2011; Trieu et al., 2011). For probing, plasma samples were diluted 1:100 in Protein Array Blocking buffer (Whatman Inc, Sanford, ME) supplemented with DH5α *E. coli* lysate (MCLAB, San Francisco, CA) at 20% (vol/vol) for IgM probing and 10% for IgG, and incubated on arrays overnight at 4°C. Pre-absorption with *E. coli* lysate is necessary to block anti-*E. coli* antibodies as described previously (Davies et al., 2005). Microarray slides were then incubated in biotin SP-conjugated affinity-purified goat anti-human IgG (Fcγ-fragment specific) secondary antibodies (Jackson ImmunoResearch, West Grove, PA; cat #s 109-065-043 and 109-065-098, respectively), and detected by incubation with streptavidin-conjugated SureLight P-3 (Columbia Biosciences, Columbia, MD) both diluted 1/400 in blocking buffer without lysate. The slides were washed and air-dried by brief centrifugation. Probed array slides were scanned in a GenePix 4200 confocal laser

scanner at a wavelength of 670 nm, at 30% laser power and 330 PMT. The output grey scale TIFF files generated by the scanner were quantitated using ProScanArray Express software (Perkin Elmer, Waltham, MA) with spot-specific background correction.

Statistical analysis

Protein microarray data were analyzed using the R Project for Statistical Computing (R-Core-Team, 2013). Median foreground intensity 635nm and mean background intensity 635nm were imported separately from 9 raw data files generated by the ProScanArray Express software. Mean slide background intensity and the average NoDNA negative control intensity from each slide were background subtracted from the median foreground intensity of each antigen spot on the array prior to transformation and normalization with variance stabilizing normalization (VSN) algorithm from the R package library vsn (Huber et al., 2002). NoDNA spots are negative controls on the array containing the products from an empty *E. coli* vector used to estimate background noise and cross-reactions of antibodies to *E. coli* antigens. VSN was fit to the NoDNA negative control spots as well as the human-IgG and antihuman-IgG positive control spots, but the resulting normalization was applied to all spots, including both controls and antigen spots. Boxplots, histograms, density plots and PCA plots of the data were made for quality control assessments immediately after import and after VSN to assess the impact on any batch effects or specific groups of spots. We separated all target spots from control spots on the protein array and used the mean and standard deviation of the NoDNA control spots to determine which target spots were reactive. Any target spot with a VSN-intensity value greater than two standard deviations around the mean of the similarly transformed NoDNA spots was considered reactive. The number of reactive

antigens per subject represents each subject's antibody "breadth". We used a Negative Binomial-family generalized linear model to compare the profile breadths at each time point. Differences in the level or "magnitude" of antibody responses between time points were tested using linear model comparisons of average of the positive VSN-intensities from all antigens per sample. Differences in antibody breadth and magnitude were visualized using bee swarm scatter plots and heat maps.

References

Crompton, P.D., Kayala, M.A., Traore, B., Kayentao, K., Ongoiba, A., Weiss, G.E., Molina, D.M., Burk, C.R., Waisberg, M., Jasinskas, A.*, et al.* (2010). A prospective analysis of the Ab response to Plasmodium falciparum before and after a malaria season by protein microarray. Proc Natl Acad Sci U S A *107*, 6958-6963.

Davies, D.H., Liang, X., Hernandez, J.E., Randall, A., Hirst, S., Mu, Y., Romero, K.M., Nguyen, T.T., Kalantari-Dehaghi, M., Crotty, S.*, et al.* (2005). Profiling the humoral immune response to infection by using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery. Proc Natl Acad Sci U S A *102*, 547-552.

Huber, W., von Heydebreck, A., Sultmann, H., Poustka, A., and Vingron, M. (2002). Variance stabilization applied to microarray data calibration and to the quantification of differential expression. Bioinformatics *18 Suppl 1*, S96-104.

Portugal, S., Moebius, J., Skinner, J., Doumbo, S., Doumtabe, D., Kone, Y., Dia, S., Kanakabandi, K., Sturdevant, D.E., Virtaneva, K.*, et al.* (2014). Exposure-dependent control of malaria-induced inflammation in children. PLoS Pathog *10*, e1004079.

R-Core-Team (2013). R: A Language and Environment for Statistical Computing.

Tan, X., Traore, B., Kayentao, K., Ongoiba, A., Doumbo, S., Waisberg, M., Doumbo, O.K., Felgner, P.L., Fairhurst, R.M., and Crompton, P.D. (2011). Hemoglobin S and C heterozygosity enhances neither the magnitude nor breadth of antibody responses to a diverse array of Plasmodium falciparum antigens. J Infect Dis *204*, 1750-1761.

Tran, T.M., Li, S., Doumbo, S., Doumtabe, D., Huang, C.Y., Dia, S., Bathily, A., Sangala, J., Kone, Y., Traore, A.*, et al.* (2013). An intensive longitudinal cohort study of Malian children and adults reveals no evidence of acquired immunity to Plasmodium falciparum infection. Clin Infect Dis *57*, 40-47.

Trieu, A., Kayala, M.A., Burk, C., Molina, D.M., Freilich, D.A., Richie, T.L., Baldi, P., Felgner, P.L., and Doolan, D.L. (2011). Sterile protective immunity to malaria is associated with a panel of novel P. falciparum antigens. Mol Cell Proteomics *10*, M111 007948.