Supplemental material

B cell profiling in malaria reveals expansion and remodelling of CD11c$^+$ B cell subsets

Christopher Sundling[1,2,#,*], Caroline Rönnberg[1,3,4#], Victor Yman[1], Muhammad Asghar[1,2], Peter Jahnmatz[1,5], Tadepally Lakshmikanth[6], Yang Chen[6], Jaromir Mikes[6], Mattias N. Forsell[7], Klara Sondén[1,2], Adnane Achour[1,2,8], Petter Brodin[6,9], Kristina E. M. Persson[3,10], Anna Färnert[1,2]

[1] Division of Infectious Diseases, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden.

[2] Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden.

[3] Department of Microbiology, Tumor, and Cell Biology, Karolinska Institutet, Stockholm, Sweden.

[4] Department of Clinical Microbiology, Karolinska University Hospital, Huddinge, Stockholm, Sweden.

[5] Mabtech AB, Stockholm, Sweden.

[6] Science for Life Laboratory, Department of Women's and Children's Health, Karolinska Institutet, 17121 Solna, Sweden

[7] Division of Infection & Immunology, Department of Clinical Microbiology, Umeå University, Umeå, Sweden.

[8] Science for Life Laboratory, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden.

[9] Department of Newborn Medicine, Karolinska University Hospital, 17176 Solna, Sweden.

[10] Department of Laboratory Medicine, Lund University, Skåne University Hospital, Lund, Sweden.

#Equal contribution

*Corresponding author: christopher.sundling@ki.se

Supplemental methods

Mass cytometry

Cryopreserved PBMCs obtained by ficoll density gradient centrifugation from heparinised blood samples of patients were thawed using RPMI medium (HyClone®) supplemented with fetal bovine serum (FBS), penicillin-streptomycin and benzonase [Sigma-Aldrich, St. Louis, MO, USA] and rested overnight at 37°C in 5% CO_2 for cells to be revitalized. Cells are then counted and checked for their viability. For live-dead discrimination, cells are stained with 2.5μM Cisplatin [Fluidigm, South San Francisco, CA, USA] in RPMI without FBS for 5 min at room temperature, followed by quenching with RPMI containing FBS and washed. Following aspiration of supernatant, cells were washed once again in CyFACS buffer (PBS with 0.1% BSA, 0.05% sodium azide and 2mM EDTA). For surface marker staining, cells were incubated for 30 min at 4°C with a 30ul cocktail of metal conjugated antibodies targeting the surface antigens, washed with CyFACS and fixed with 1% formaldehyde. For intracellular marker (Ki67) staining, cells were permeabilized using an intracellular fixation and permeabilization buffer set [eBiosciences Inc., San Diego, CA, USA] as per the manufacturer's recommendations. To this, 30ul of intracellular antibody cocktail was added and incubated for 45 min at room temperature. Cells were then washed, fixed in 4% formaldehyde overnight, and stained with DNA intercalator (0.125 μM MaxPar® Intercalator-Ir, Fluidigm Inc. South San Francisco) on the following day. Cells were subsequently washed with CyFACS buffer, PBS and milliQ water, filtered through a 35μm nylon mesh, diluted to 500,000 cells/ml and acquired at a rate of 300-500 cells/s using a CyTOF2 [Fluidigm] mass cytometer, CyTOF software version 6.0.626 with noise reduction, a lower convolution threshold of 200, event length limits of 10-150 pushes, a sigma value of 3, and flow rate of 0.045 ml/min.

Mass cytometry antibodies and reagents

Purified antibodies were obtained in carrier/protein-free buffer and then coupled to lanthanide metals using the MaxPar X8 antibody conjugation kit [Fluidigm Inc.] as per the protocol obtained from the manufacturer. Metal conjugated antibodies were also purchased from Fluidigm. The Antibodies used in this study are listed in Supplementary Table 2.

Supplemental Table 1. Descriptive statistics of the study participants.

	Primary infected	**Previously exposed**	***p-value*[3]**
Number of participants	17	34	-
Female sex (%)	4 (23.5)	5 (14.7)	0.46[4]
Age, years, median (range)	33 (20-59)	39 (26-68)	0.31
Severe malaria (%)[1]	1 (5.9)	4 (11.8)	0.65[4]
Cumulative time of residency in endemic area, median years (range)	0 (0-3)	26 (15-39)	<0.0001
Time since residency in endemic area, years, median (range)	-	12 (0-46)	-
Time from symptom onset to diagnosis, days, median (range)	4 (0-13)	3 (0-10)	0.17
Parasitemia, % infected RBCs, median (range)[2]	1.1 (0.01-8.0)	0.6 (0.01-17)	0.07

[1]As determined by WHO 2014 criteria without hyperparasitemia as a single criterion.
[2]Maximum level reached during hospital stay.
[3]Statistical evaluation was done by the Mann-Whitney U-test unless otherwise stated.
[4]Fisher's exact test

Supplemental table 2. Staining panel for mass cytometry

Antibody specificity	Conjugate	Antibody clone	Source	Dilution 1:X[1]
CD45	89Y	HI30	Fluidigm	1:200
CD57	115In	HCD57	BioLegend	1:400
CCR6	141Pr	11A9	BD	1:75
CD19	142Nd	HIB19	Fluidigm	1:200
CD5	143Nd	UCHT2	Fluidigm	1:200
CD16	144Nd	3G8	BioLegend	1:50
CD138	145Nd	DL-101	Fluidigm	1:200
IgM	146Nd	MHM-88	BioLegend	1:75
CD11c	147Nd	Bu15	Fluidigm	1:100
IgA	148Nd	Polyclonal	Fluidigm	1:200
CD123	151Eu	6H6	BioLegend	1:250
CD21	152Sm	BL13	Fluidigm	1:100
CD3e	154Sm	UCHT1	Fluidigm	1:200
CD22	155Gd	HIB22	BioLegend	1:62.5
CXCR3	157Gd	G025H7	BioLegend	1:100
CD14	160Gd	M5E2	BioLegend	1:100
CD24	161Dy	ML5	BioLegend	1:50
HLA-DR	163Dy	L243	BioLegend	1:125
CD44	164Dy	BJ18	BioLegend	1:125
CD127	165Ho	A019D5	Fluidigm	1:200
CD27	167Er	L128	Fluidigm	1:200
CD38	168Er	HIT2	BioLegend	1:200
CD45RA	169Tm	HI100	Fluidigm	1:200
CD20	170Er	2H7	BioLegend	1:125
IgD	172Yb	IA6-2	BioLegend	1:125
CD39	173Yb	A1	BioLegend	1:62.5
CXCR5	174Yb	51505	BioLegend	1:125
CD11b	209Bi	ICRF44	Fluidigm	1:400
Ki-67	162Dy	B56	Fluidigm	1:150

[1]Antibodies diluted as indicated in staining-solution .

Supplemental table 3. Staining panel for flow cytometry

Antibody specificity	Fluorochrome conjugate	Antibody clone	Source	Dilution 1:X[1]
CD38	PerCpCy5.5	HIT2	BD	40
IgD	FITC	IA6-2	BD	20
IgD	BB515	IA6-2	BD	40
CD19	PECy7	HIB19	BD	100
CXCR3	PECy5	1C6/CXCR3	BD	20
FcRL5	PE	509f6	Biolegend	20
IgG	PE	G18-145	BD	40
CD20	APC-H7	2H7	BD	60
CD10	APC-R700	HI10a	BD	60
BAFF-R	AF647	11C1	BD	40
T-bet[3]	AF647	4B10	BD	80
CD11c	BV786	B-ly6	BD	40
CD27	BV650	M-T271	BD	40
Aqua viability dye	V510 channel	N/A	ThermoFisher	300
CD21	BV421	B-ly4	BD	40
CD85j[2]	Biotin	GHI/75	BD	20
Ki67[3]	BUV395	B56	BD	100
Streptavidin	BUV395	N/A	BD	200

[1]Antibodies diluted as indicated in FACS-solution.

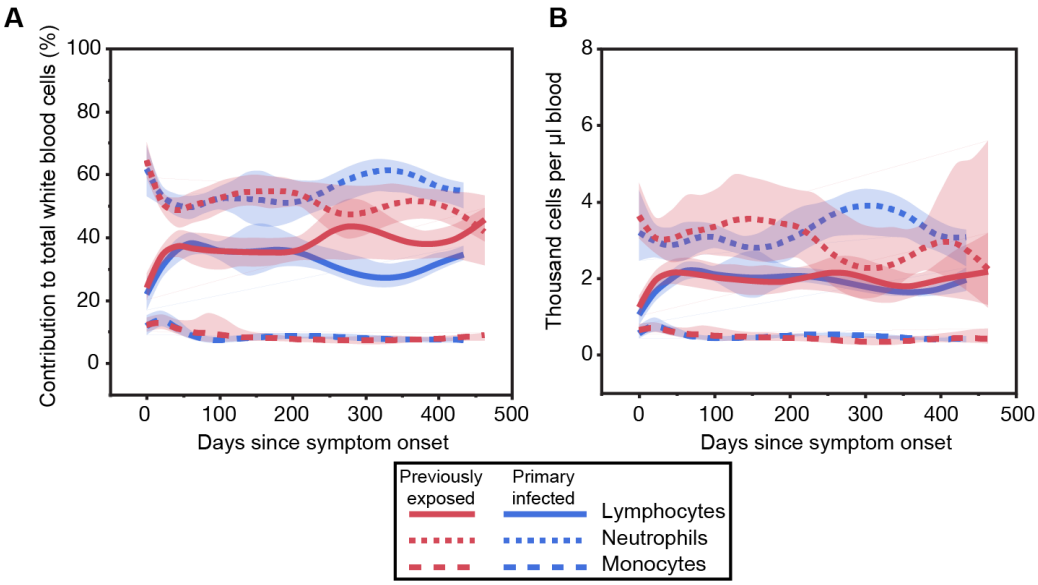
[2]CD85j was biotinylated using the EZ-link Sulfo-NHS-biotin kit.

[3]Used for intracellular staining.

Supplemental table 4. Staining panel for B cell sorting

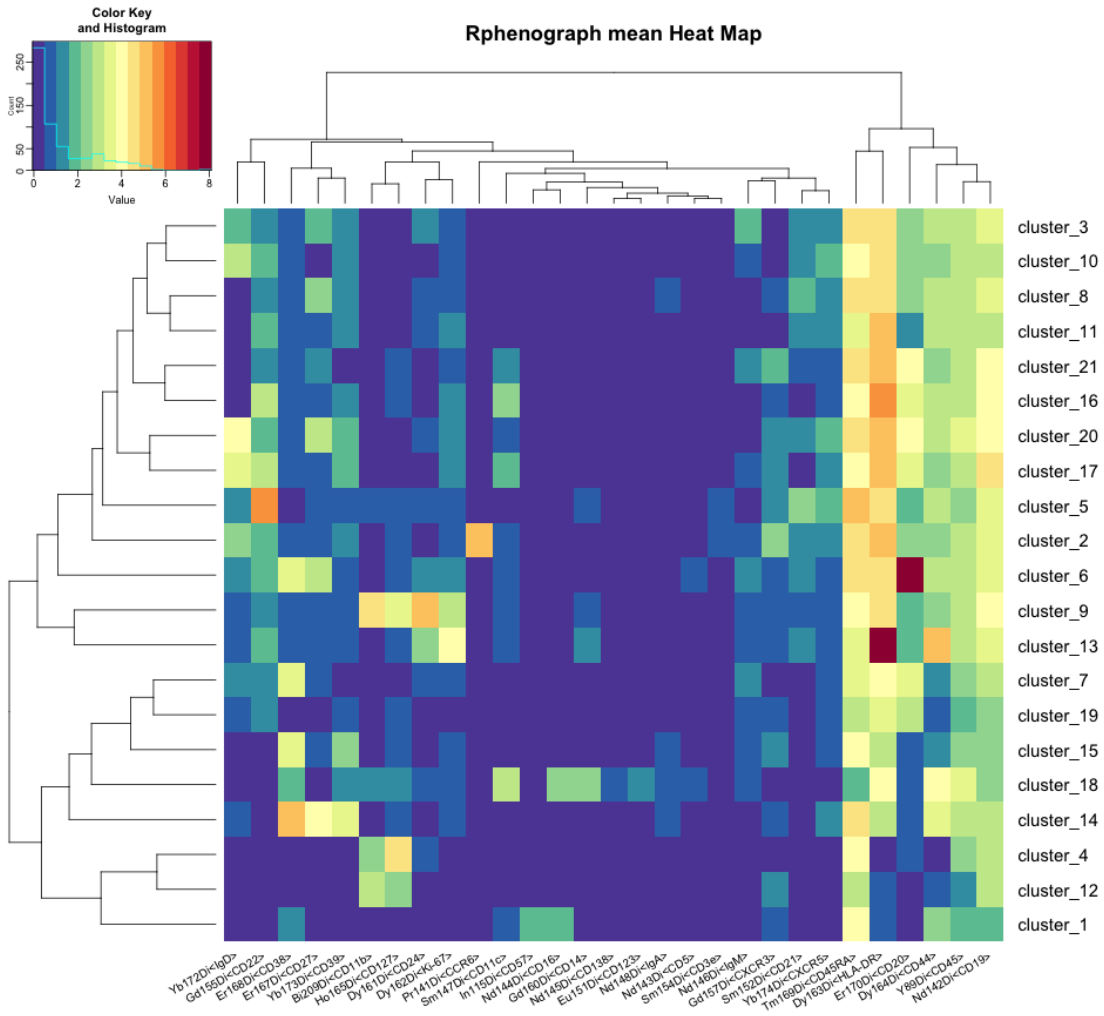
Antibody specificity	Fluorochrome conjugate	Antibody clone	Source	Dilution 1:X[1]
IgA	FITC	Polyclonal	Jackson Immunogresearch	40
IgM	BB515	G20-127	BD	20
IgD	BB515	IA6-2	BD	30
CD19	PECy7	HIB19	BD	100
CD20	APC-H7	2H7	BD	40
CD3	APC	HIT3a	BD	10
CD11c	BV786	B-ly6	BD	30
Aqua viability dye	V510 channel	N/A	ThermoFisher	300

[1]Antibodies diluted as indicated in FACS-solution.



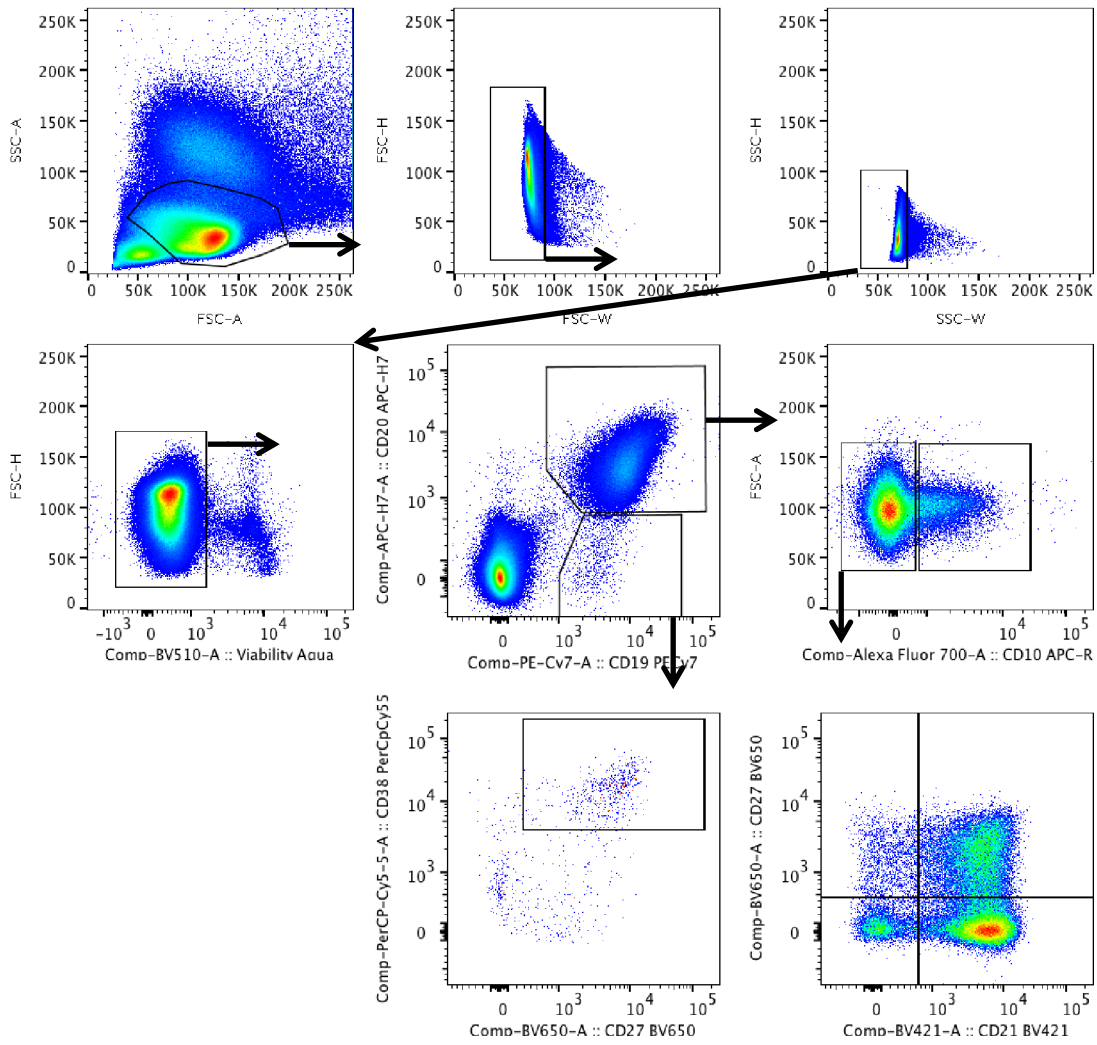
Supplemental figure 1.

White blood cell dynamics following *Plasmodium falciparum* malaria. X-axis corresponds to the number of days since symptoms started. (**A**) Frequency of each cell population to total white blood cells. (**B**) Absolute cell counts per µl blood. Individuals previously unexposed or exposed to malaria are indicated by blue and red color, respectively. The solid line indicate lymphocytes, the dotted line indicate neutrophils and the dashed line indicate monocytes. The shaded area indicates confidence interval with a λ of 0.05.



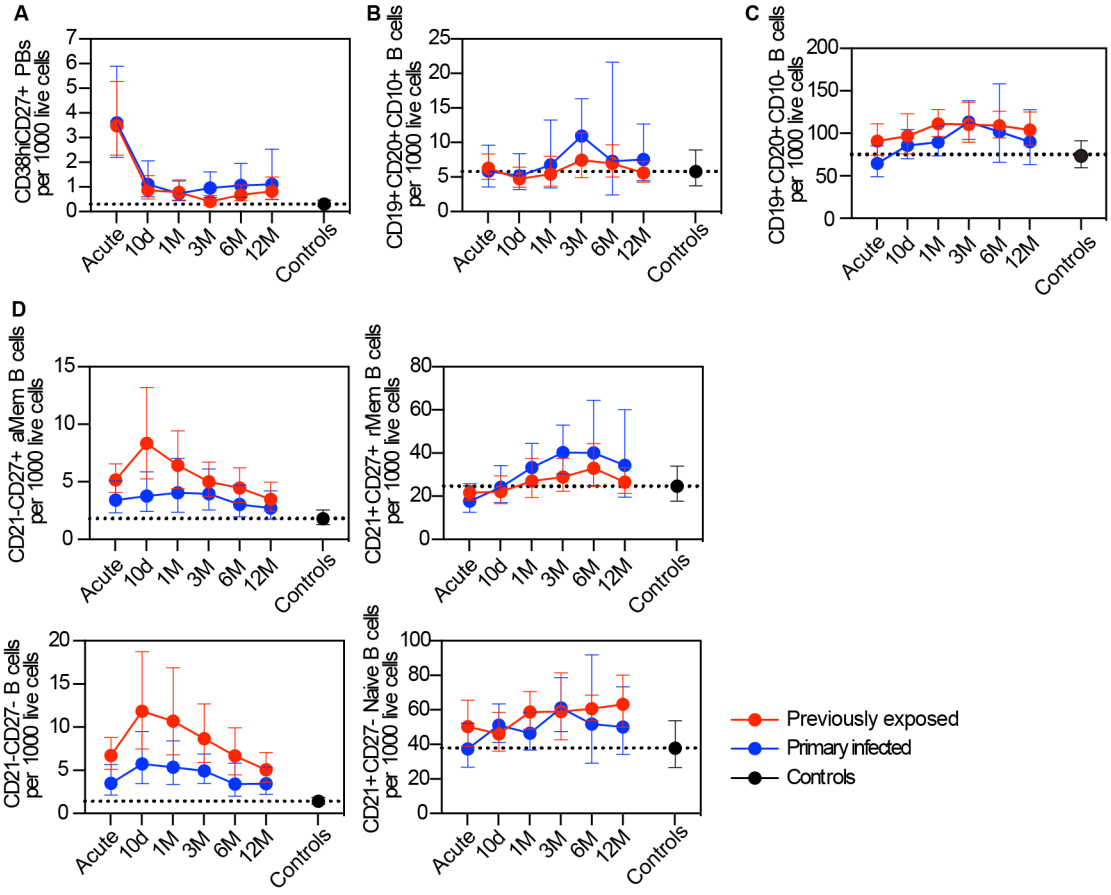
Supplemental figure 2.

Average marker expression in all 21 clusters obtained from mass cytometry data in figure 2.



Supplemental figure 3.

FACS gating for B cell subsets as shown in one representative donor. Arrows indicate gating pathway.



Supplemental figure 4.

Cell counts normalized to total live lymphocytes. Complements main figure 3.

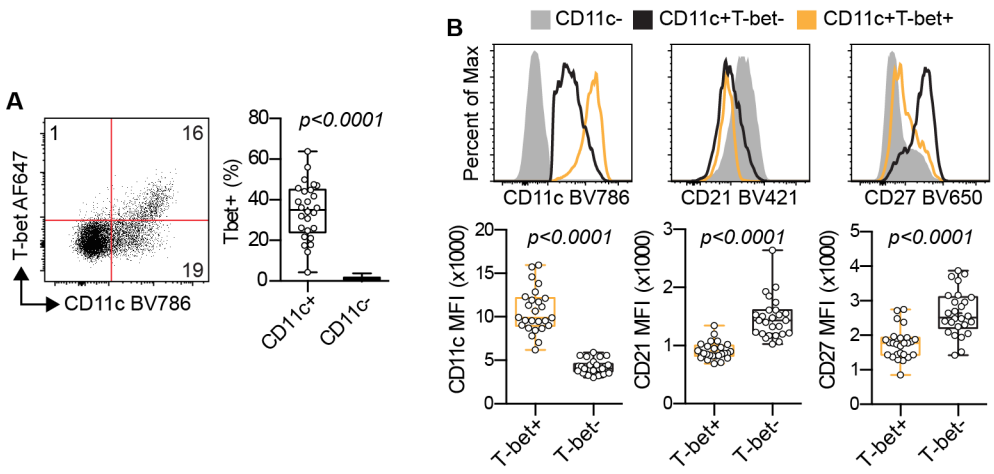
(**A**) Plasmablasts (CD19+CD20loCD38hiCD27+)

(**B**) Immature B cells (CD19+CD20+CD10+)

(**C**) Mature B cells (CD19+CD20+CD10-)

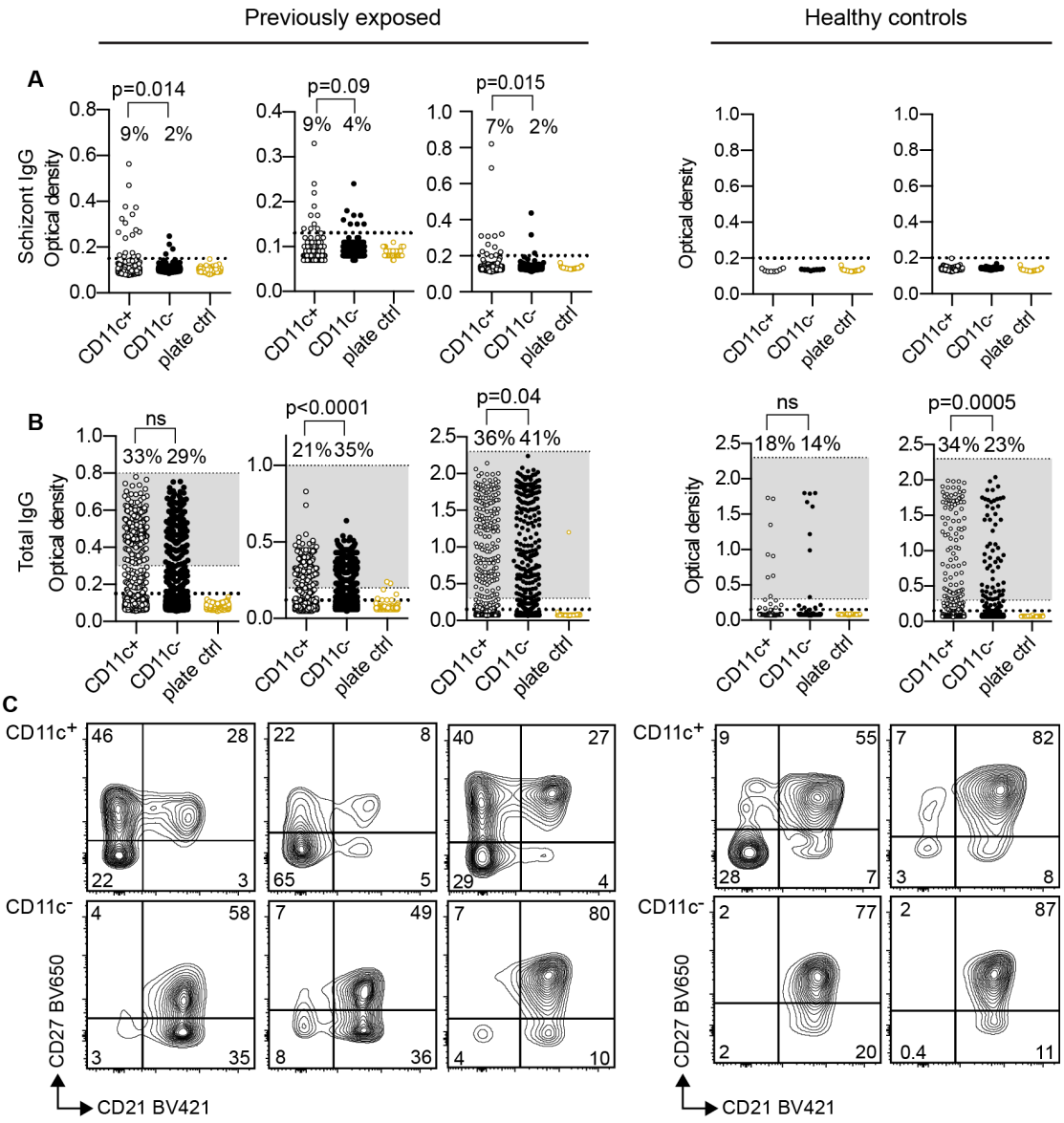
(**D**) Mature B cells (CD19+CD20+CD10-) further divided based on CD21 and CD27.

Red indicates Previously exposed individuals (n=33). Blue indicates Primary infected individuals (n=16). Black indicates healthy controls (n=14). Symbol and error bars indicate geometric mean ± 95% CI. Dotted line indicates geometric mean of controls at one time-point.



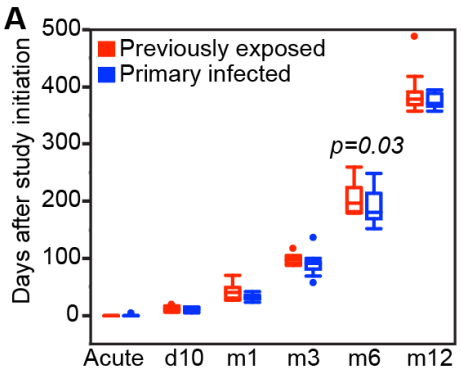
Supplemental figure 5.

(**A**) Representative gating for CD11c and intracellular T-bet in one previously exposed donor at the time of acute infection, followed by comparison of the frequency of T-bet$^+$ cells in CD11c$^+$ or CD11c$^-$ B cells (n=26; Wilcoxon matched pairs test). (**B**) Representative histograms and mean fluorescent intensity of CD11c, CD21, and CD27 expression in CD11c$^+$T-bet$^+$ (orange line), CD11c$^+$T-bet$^-$ (black line), and CD11c$^-$ (grey filled) B cells (n=26; Wilcoxon matched pairs test).



Supplemental figure 6.

CD11c^{+} and CD11c^{-} B cells were sorted from three previously exposed donors at 10 days after acute infection and from two healthy controls. Cells were sorted at 2 cells/well into 384-well plates containing IL2, IL21 and CD40-ligand feeder cells and cultured for ~2 weeks. (**A**) Schizont-specific IgG (clone 3D7) and (**B**) Total IgG was measured in culture wells. The detection limit is indicated by the dotted line. The grey area in total IgG graphs corresponds to wells with an OD > 0.2-0.3 and were further screened for binding to schizont extract. (**C**) Mature B cells were gated as IgD^{-}CD11c$^{+/-}$ and then CD21 vs CD27 to give an indication of which B cell populations that were present in the sorted populations, and therefore could be responsible for antibody production. Statistics was calculated using Fisher's exact test with $p<0.05$ considered significant.



B

Sample summary

	Previous malaria status		
time	**exposed**	**Primary inf.**	**All**
Acute	31	16	47
d10	19	12	31
m1	15	8	23
m3	16	10	26
m6	16	6	22
m12	19	7	26
All	116	59	175

Supplemental figure 7.

(**A**) Time distribution for samples. Individuals were invited for follow-up samples as close to 10 days, 1 month, 3 months, 6 months, and 12 months after the acute sample as possible. (**B**) The number of samples at each time point is indicated in the sample summary. Differences between the groups were evaluated by a linear mixed effects model with restricted maximum likelihood followed by an LSmeans student's t-test for each time-point.