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Supplementary Materials for

Expression of inhibitory receptors by B cells in chronic human infectious diseases restricts responses to membrane-associated antigens

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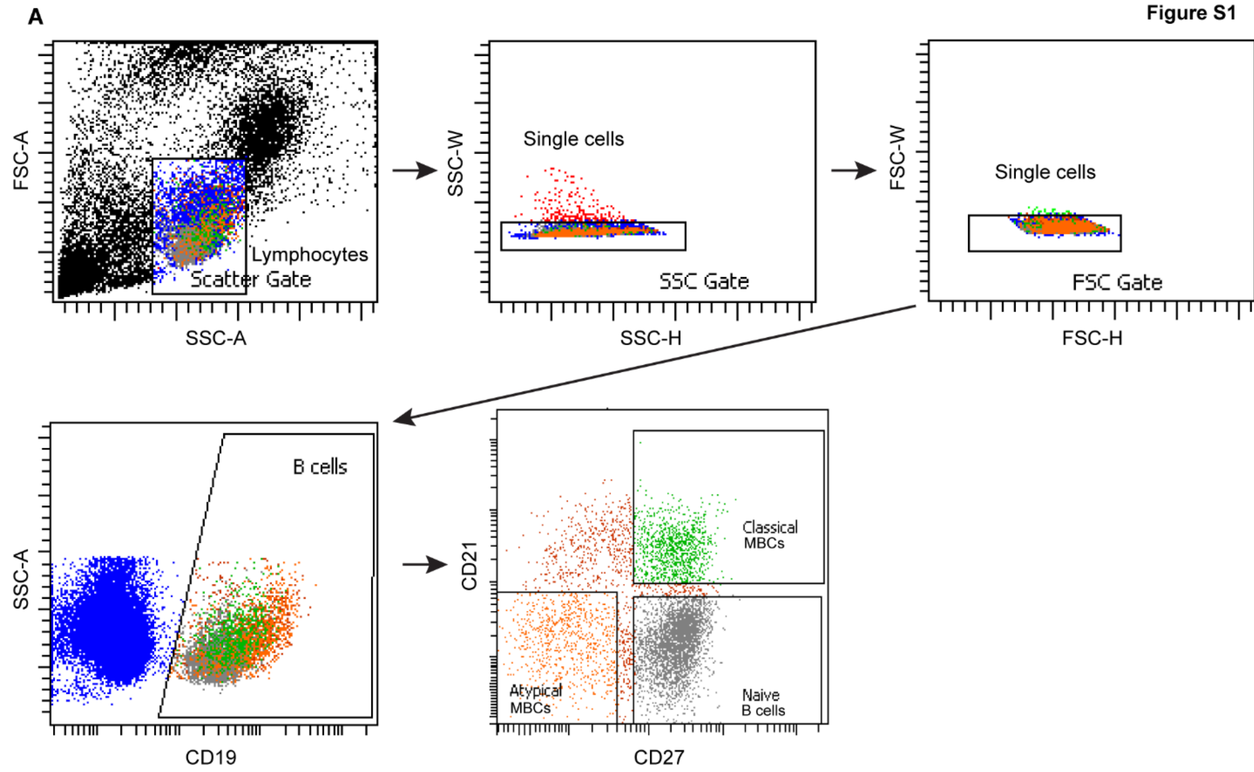
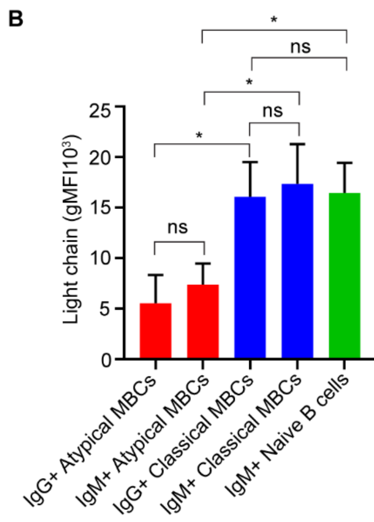
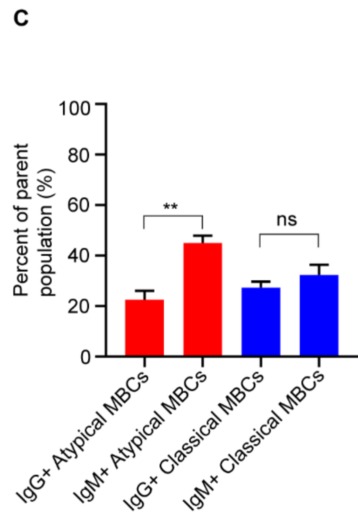
Figs. S1 to S3

SUPPLEMENTARY MATERIALS

Figure S1







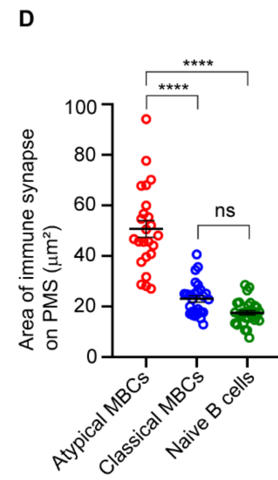


Figure S1. FACS sorting of PBMC into Naïve, Classical MBCs and Atypical MBCs.

(A) Malian PBMC were stained using fluorescently labelled antibodies against CD19, CD21 and CD27 and FACS sorted into atypical MBC (CD19+CD21-CD27-), classical MBCs (CD19+CD21+CD27+) and naïve B cells (CD19+CD21+CD27-).

(B) Comparison of surface BCR (gMFI of light chain) by IgG+ or IgM+ atypical MBCs (red bars), IgG+ or IgM+ classical MBCs (blue bars) and IgM+ Naïve B cells (green bar) ($n = 3$). The error bars indicate S.E.M. Data were analyzed using paired t test. *, $P < 0.05$; ns, not significant.

(C) Comparison of percentage of IgG+ or IgM+ B cells within atypical MBCs (red bars) and IgG+ or IgM+ B cells within classical MBCs (blue bars) ($n = 3$). The error bars indicate S.E.M. Data were analyzed using paired t test. **, $P < 0.005$; ns, not significant.

(D) Quantification of spread area by atypical MBCs (red circles), classical MBCs (blue circles) and naïve B cells (green circles) on PMS containing anti-λ/κ upon incubation for 90 mins at 37 °C. The error bars indicate S.E.M. Data were analyzed using a one-way ANOVA with Tukey's correction. ****, $P < 0.0001$; ns, not significant.

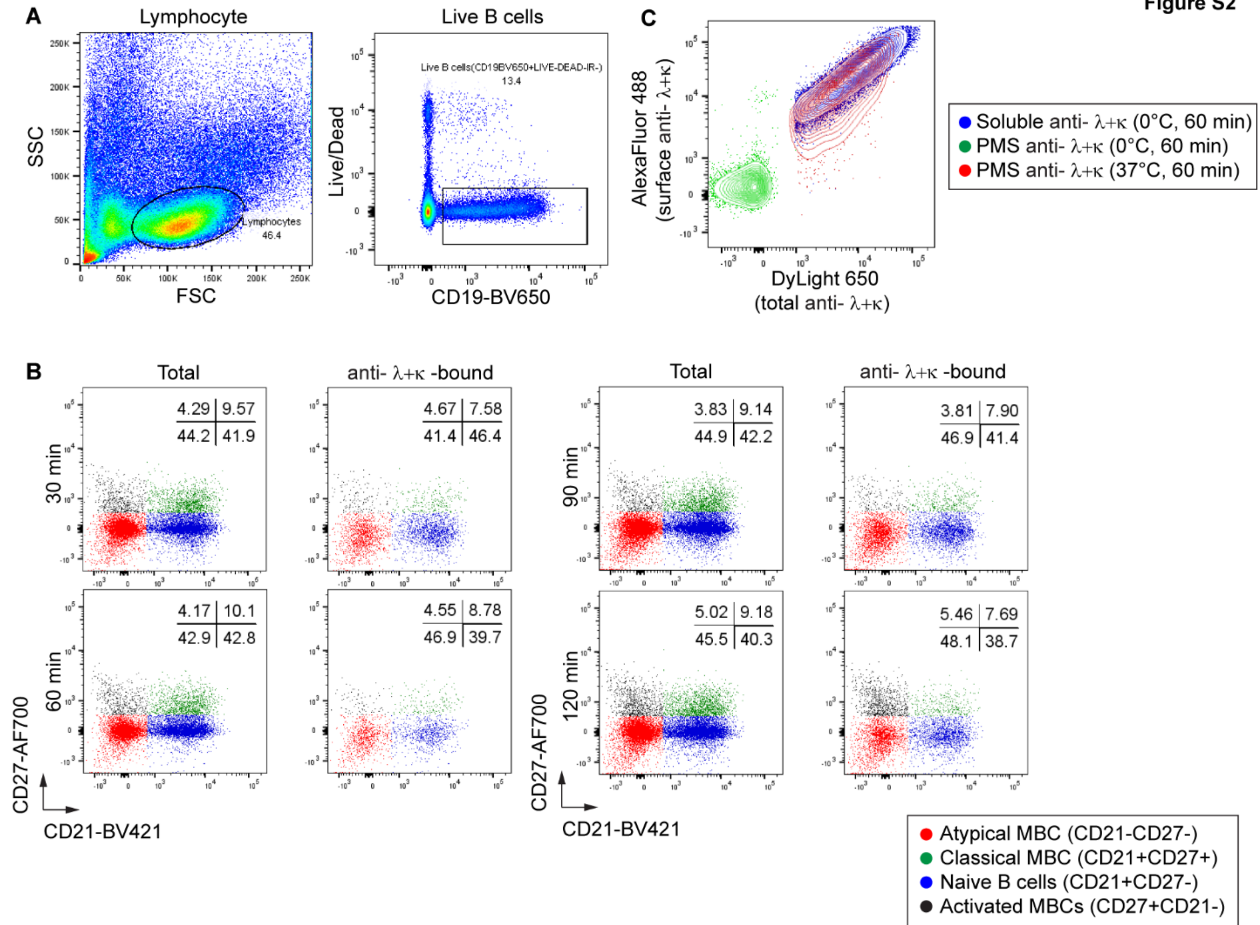


Figure S2
A
Lymphocyte
SSC
FSC
Lymphocytes
46.4
Live B cells
Live B cells(CD19BV650+LIVE-DEAD-IR-)
13.4
Live/Dead
CD19-BV650
C
AlexaFluor 488
(surface anti- λ+κ)
DyLight 650
(total anti- λ+κ)
Soluble anti- λ+κ (0°C, 60 min)
PMS anti- λ+κ (0°C, 60 min)
PMS anti- λ+κ (37°C, 60 min)
B
Total
anti- λ+κ -bound
Total
anti- λ+κ -bound
30 min
4.29 9.57 44.2 41.9
4.67 7.58 41.4 46.4
90 min
3.83 9.14 44.9 42.2
3.81 7.90 46.9 41.4
60 min
4.17 10.1 42.9 42.8
4.55 8.78 46.9 39.7
120 min
5.02 9.18 45.5 40.3
5.46 7.69 48.1 38.7
CD27-AF700
CD21-BV421
CD27-AF700
CD21-BV421
Atypical MBC (CD21-CD27-)
Classical MBC (CD21+CD27+)
Naive B cells (CD21+CD27-)
Activated MBCs (CD27+CD21-)

Figure S2. The proportion of B cell subsets within antigen-bound cells were similar with that of B cell input over the period of incubation on PMS

(A) Malian PBMCs (1×10^6 per well) were added to 8-well lab-tek chamber layered with PMS-anti-λ/κ on the bottom and incubated for given times at 37°C to let the B cells respond to the PMS- anti-λ/κ. At a given time, cells were harvested by brief pipetting and washed once for removal of unbound antigen, stained with CD19-BV650, CD21-BV421 and CD27-Alexa700 antibodies, and analyzed by flow cytometry to identify naïve B cells (CD21+CD27-), classical MBCs (CD21+CD27+), atypical MBCs (CD21-CD27-), and activated MBCs (CD21-CD27+). The flow cytometry plots indicate live B cell gating strategy from the PBMCs after harvesting and staining with B cell subset markers. LIVE/DEAD marker for exclusion of dead cells and CD19-BV650 for B cells were used for live B cell gating.

(B) PBMCs were incubated for 30, 60, 90, and 120 min on PMS- anti-λ/κ at 37°C and harvested, stained and analyzed for the percentage of each B cell subset, naïve B cells, classical MBCs, atypical MBCs, and activated MBCs among B cell population. Shown as numbers in plot is the percentage of each subset, classical MBCs, naïve B cells, atypical MBCs and activated MBCs, in the order of clockwise direction starting from right and top quadrant.

(C) B cells were incubated either with soluble anti-λ/κ on ice or with PMS- anti-λ/κ on ice or 37°C for 60 min. The same anti-λ/κ labeled with biotin and DyLight650 was used for both soluble and membrane-bound. After washing out unbound anti-λ/κ, cells were further stained with streptavidin-Alexa488 to detect anti-λ/κ remained on surface. Contour plots of B cells in which anti-λ/κ bound only on surface (Soluble anti-λ/κ (0°C, 60min)) vs mixed between surface and internal (PMS-anti-λ/κ (37°C, 60min) revealed by Ag-DyLight650 for total bound anti-λ/κ and streptavidin-Alexa488 for anti-λ/κ remained on surface. Membrane-tethered anti-λ/κ binding

on B cells did not occur on ice (PMS anti-λ/κ (0°C, 60min)), but soluble anti-λ/κ did bind (Soluble anti-λ/κ (0°C, 60min)).

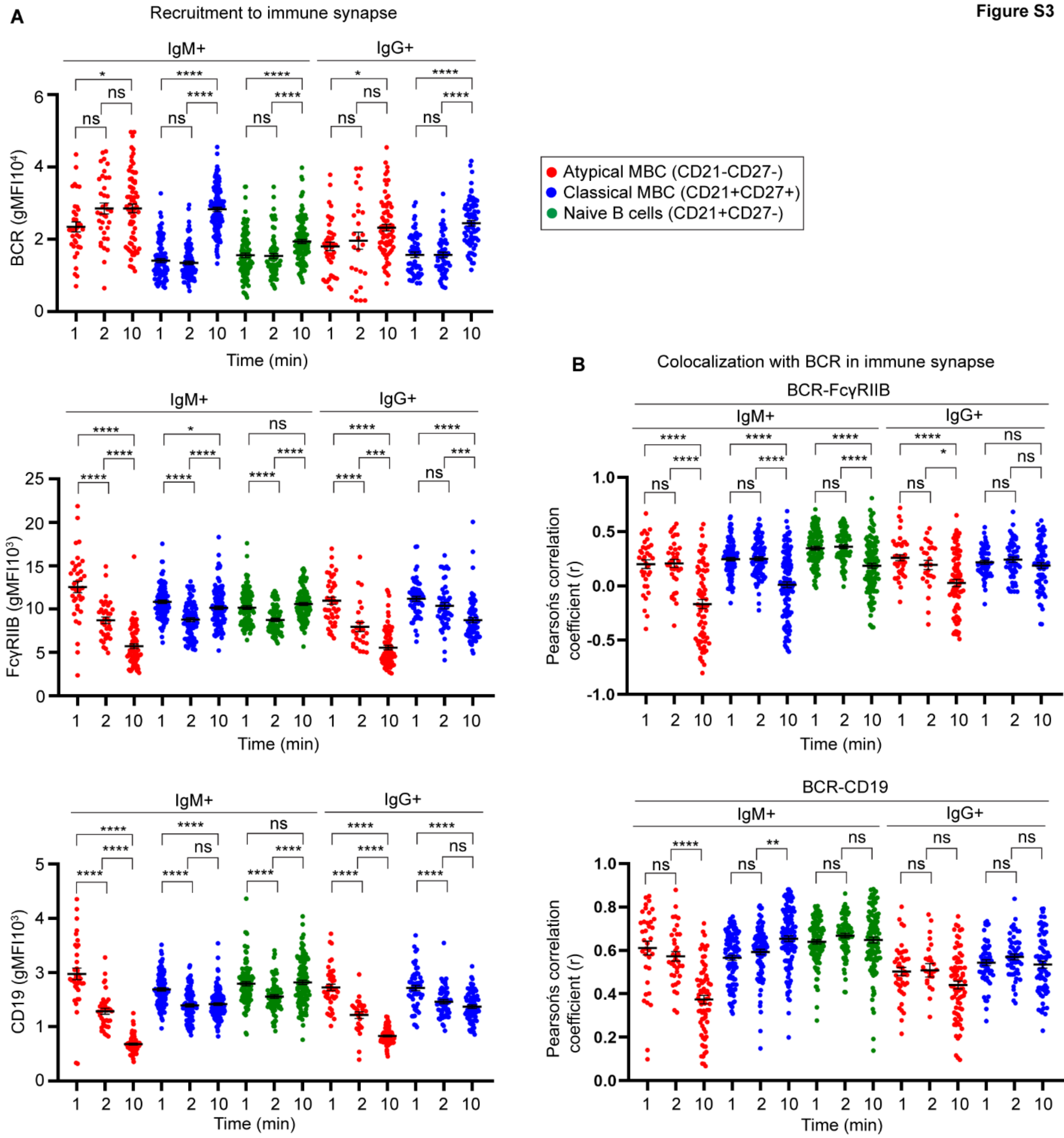

A
Recruitment to immune synapse
IgM+
IgG+
BCR (gMFI10⁴)
FcγRIIB (gMFI10³)
CD19 (gMFI10³)
Time (min)
Atypical MBC (CD21-CD27-)
Classical MBC (CD21+CD27+)
Naive B cells (CD21+CD27-)
B
Colocalization with BCR in immune synapse
BCR-FcγRIIB
BCR-CD19
Pearsońs correlation coefficient (r)

Figure S3. Quantification for recruitment and colocalization of BCR, CD19 and FcγRIIB in the immunological synapse

Atypical MBC, classical MBCs and naïve B cells were FACS sorted from PBMC, stained with DyLight594 conjugated Fab fragments of either anti-IgM or anti-IgG, and AlexaFluor 488 conjugated Fab fragments of anti-CD19 antibodies and activated on PLB containing anti-λ/κ for 1 - 10 min, fixed and stained with antibodies recognizing FcγRIIB and imaged on TIRFM.

(A) Quantification of MFI of BCR, FcγRIIB and CD19 in the immune synapse formed by atypical MBCs (red dots), classical MBCs (blue dots) and naïve B cells (green dots) on PLB containing anti-λ/κ for given time points.

(B) Colocalization of BCR with FcγRIIB or CD19 within the immune synapse formed by atypical MBCs (red dots), classical MBCs (blue dots) and naïve B cells (green dots) upon activation on PLB containing anti-λ/κ.

Data are representative of two experiments. The error bars indicate S.E.M. Data were analyzed using unpaired t test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significant.