Supplemental Materials

Molecular Biology of the Cell

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Calcium influx assay

Human peripheral blood B cells were analyzed for calcium influx using the Fluo-4 NW Calcium Assay Kit (Invitrogen, Life Technologies). Briefly, the fluo-4 solution was prepared by resuspending the powdered dye in 10 ml of the provided assay buffer and 2.5 mM probenecid. Cells were resuspended in 1 mL of the fluo-4 solution for 30 min at 37°C in 5% CO_2, followed by incubation for 30 min at RT before analysis by flow cytometry. Cells were analyzed on an LSRII for 60 s to establish a baseline, and the cells were then briefly removed from the LSRII and stimulated with Alexa Fluor 647-conjugated F(ab) of antibodies specific for IgM or IgG at 300 nM (Jackson ImmunoResearch), or biotinylated F(ab')2 antibody specific for IgM, IgG, and IgA (10 μg/mL) with streptavidin (Invitrogen). The cells were mixed and immediately placed back on the LSRII and analyzed for additional 600 s.

Immobilization of purified human IgM and IgG

To sparsely immobilize purified human IgM and IgG covalently, clean coverslips were treated with 5% (wt/vol) 3-amino 3-aminopropyl trimethoxysilane (APTMS) in acetone for 15 min at room temperature, washed with acetone and PBS, then incubated with 0.25% (wt/vol) glutaraldehyde in PBS for 30 min. 10 pM of purified IgM and IgG from human serum (Invitrogen) with 5 μM BSA in PBS were added to the functionalized coverslips and incubated for 30 min at room temperature in a humid chamber. After extensive washing in PBS, coverslips were blocked with 10% BSA (wt/vol) in PBS at room temperature for 2 hours, then labeled with Alexa Fluor 647-conjugated F(ab) of antibodies specific for IgM or IgG (Jackson ImmunoReseach) at 300 nM for 10 min on ice and washed extensively with PBS before imaging.

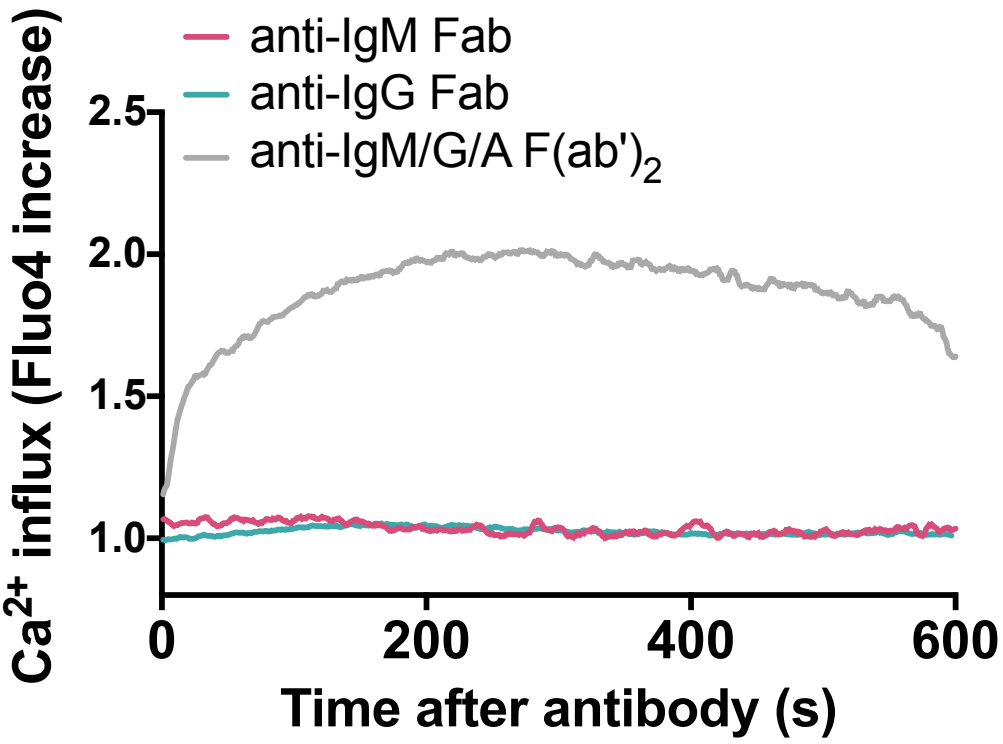

Figure S1. Flow cytometry analysis of Ca^{2+} influx in human peripheral blood B cells. Kinetics of the Ca^{2+} response as Fluo-4 fold increase to baseline in 10 min after the addition of anti-IgM Fab (red line), anti-IgG Fab (blue line), and anti-IgM/G/A F(ab')$_2$ (gray line) are shown.

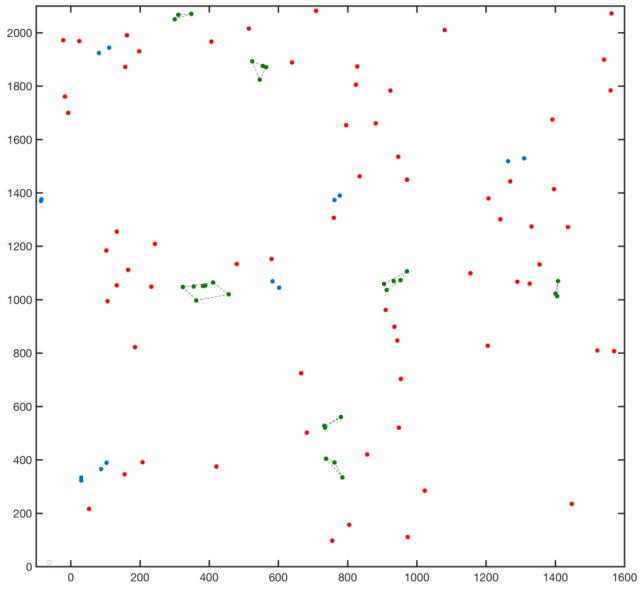

Figure S2. Actual localized peaks of IgG BCRs on a representative contact area of IgG-expressing resting B cells identified as monomers (red), dimers (blue), and protein islands of more than three receptors (green) by the cluster analysis algorithm. Dashed black line shows the protein island perimeter.

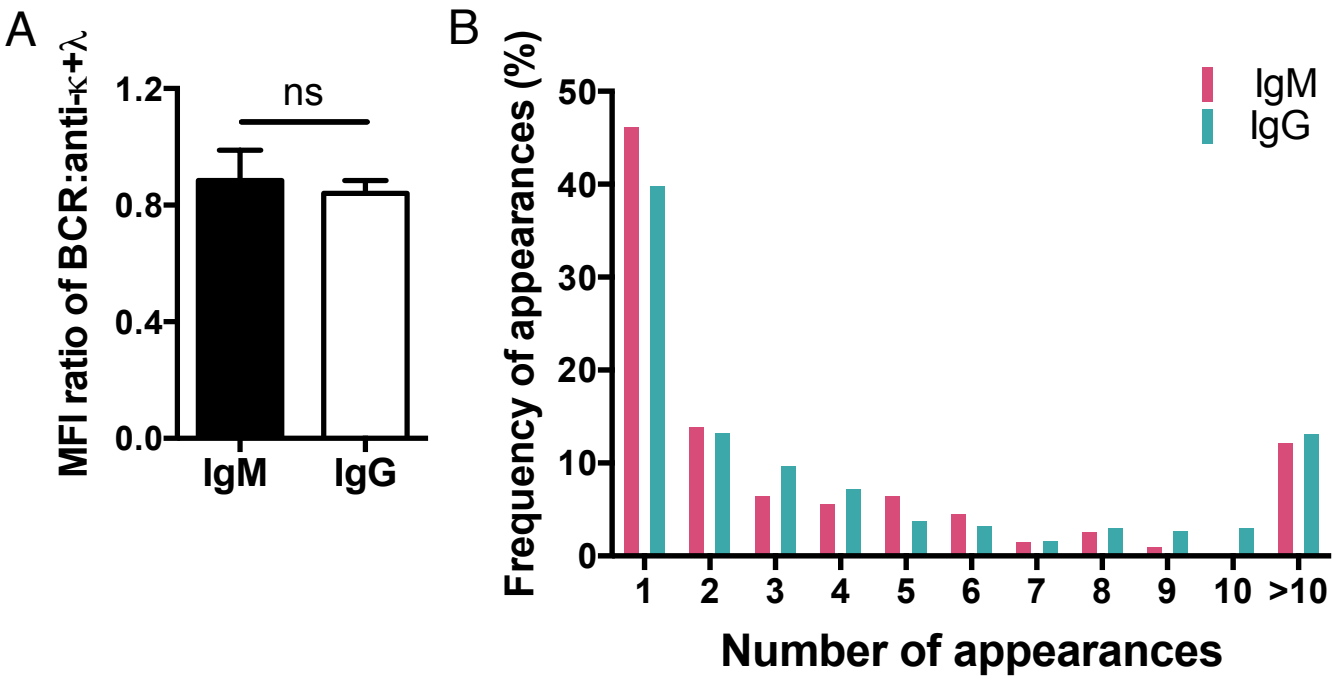

Figure S3. (A) Ratio of mean fluorescence intensity (MFI) of Alexa Fluor 647-Fab anti-IgM:DyLight 488-F(ab')$_2$ anti-κ+λ (IgM) and Alexa Fluor 647-Fab anti-IgG:DyLight 488-F(ab')$_2$ anti-κ+λ (IgG) were quantified and means and SEMs are plotted (*n* = 14 cells for each conditions). Unpaired *t* test were performed comparing the ratio of IgM versus IgG expressing cells. ns, non-significant. (B) Frequency of appearances of Alexa Fluor 647-Fab anti-IgM (red) and -IgG (blue) on surfaces with sparsely immobilized purified human IgM and IgG labeled with saturating conditions of Alexa Fluor 647-Fab anti-IgM and -IgG.