Supporting Information

Exploiting CD22 to Selectively Tolerize Autoantibody Producing B-cells in Rheumatoid Arthritis

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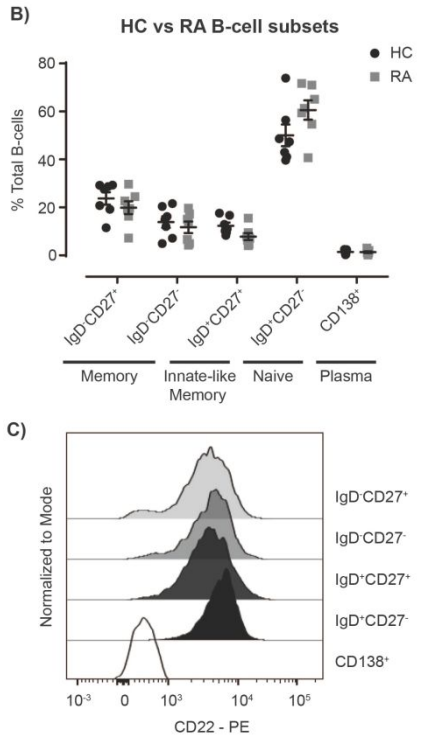
A)

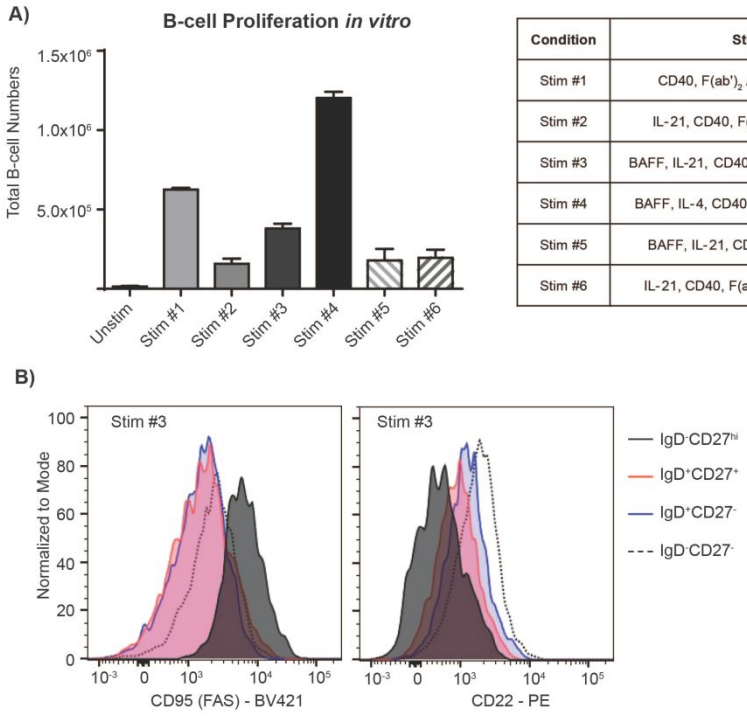
Patient ID	Sex	Age	RF Titer	CCP	HAQ
RA12Jun21D	M	61	16	94	11.1
BHR1243015*	M	57		300	
RA120912D*	F	81	0	36	2.7
RA120912E	M	47	16	100	25.4
RA12Jun12M*	F	82	12	154	5.3
RA12Jun15J*	F	75	0	250	17.5
RA12Jun15H*	F	72	81	172	4

Patients were treated with DMARDs
* Patients were treated with DMARDs and TNF-α inhibitors



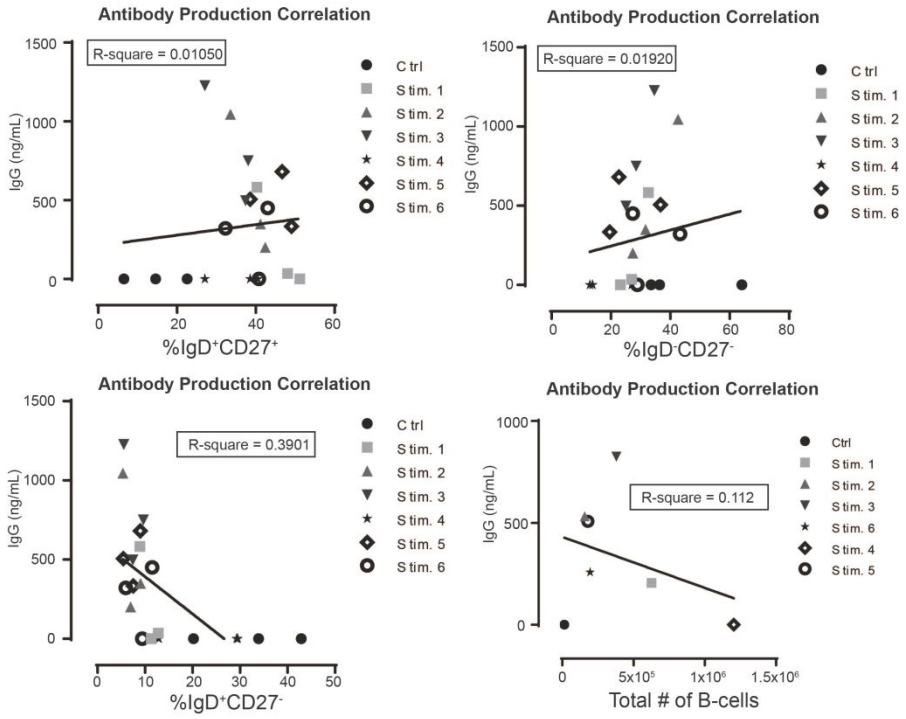
Supplemental Figure 1. RA patient clinical characterization and B-cell subsets. A) Demographics of RA patients and their clinical characteristics, including sex, age, Rheumatoid Factor (RF, IU/mL), anti-citrullinated antibody (ACPA) titer (IU/mL), and health assessment questionnaire disability index (HAQ) (N=7 from 7 individual RA patients). **B)** Healthy control (HC) and Rheumatoid Arthritis (RA) patient B-cells were isolated from total PBMCs and assessed for differences in B-cell subsets by flow cytometry (IgD, CD22, IgG, CD38, CD138, CD20, IgM, CD27) (N=7 individual donors for RA and 7 matched HC donors, 7 independent experiments,

pooled data, unpaired t-test). **C)** A representative histogram for CD22 expression in B-cell subsets from an RA patient.

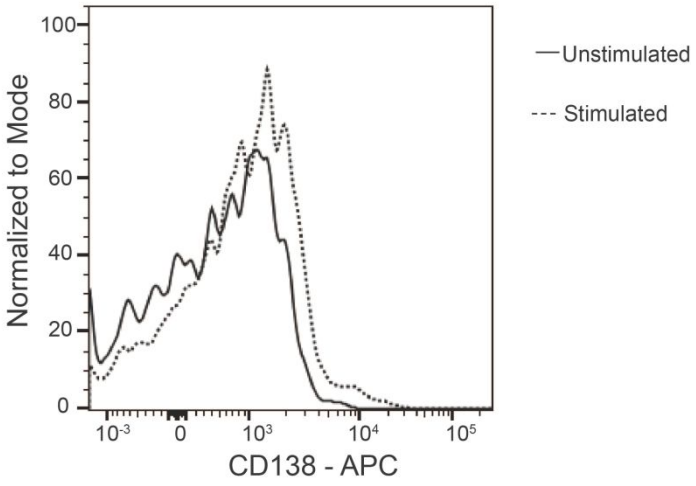


Condition	Stimulation Cocktail
Stim #1	CD40, F(ab')$_2$ Anti-human IgM+IgG, IL-4, LPS
Stim #2	IL-21, CD40, F(ab')$_2$ Anti-human IgM+IgG, LPS
Stim #3	BAFF, IL-21, CD40, F(ab')$_2$ Anti-human IgM+IgG , LPS
Stim #4	BAFF, IL-4, CD40, F(ab')$_2$ Anti-human IgM+IgG, LPS
Stim #5	BAFF, IL-21, CD40, F(ab')$_2$ Anti-human IgM+IgG
Stim #6	IL-21, CD40, F(ab')$_2$ Anti-human IgM+IgG, CD180

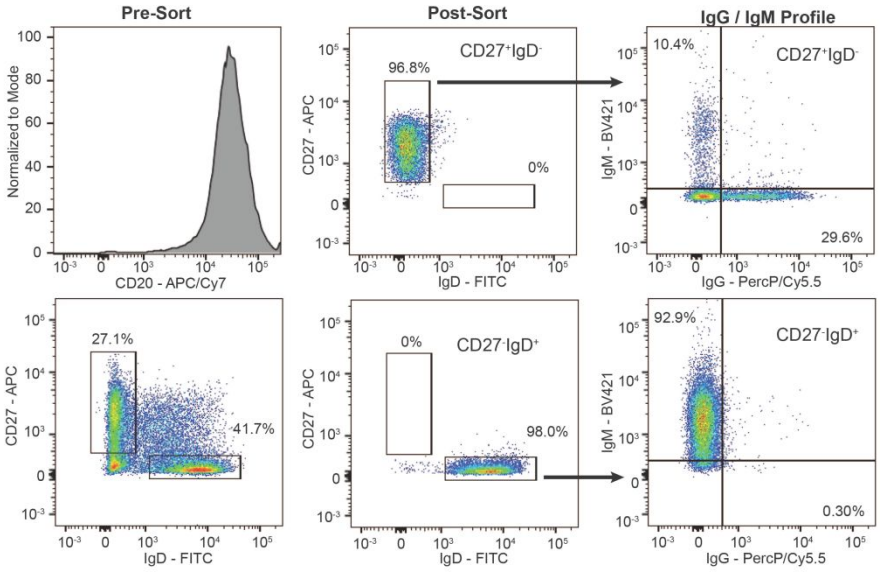
Supplemental Figure 2. B-cell proliferation upon activation and further plasmablast B-cell characterization. A) B-cells isolated from healthy control PBMCs were plated at $2x10^4$ cells/well and stimulated under a variety of stimulating conditions for 7 days using the following factors: F(ab')$_2$ anti-human IgG/IgM, BAFF, anti-human CD40, IL-4, IL-21, LPS, and CD180 as indicated in the table to the right. B-cell proliferation was measured by CellTiter-Glo (N=3 independent experiments from 3 individual healthy control donors, pooled data). **B)** B-cells were treated with Stim-3 and characterized further for a B-cell plasmablast phenotype by flow cytometry, CD95 and CD22 are shown (IgD, CD22, IgG, CD38, CD138, CD20, CD95 (FAS), and CD27) (N=3 independent experiments with 3 individual health donors).



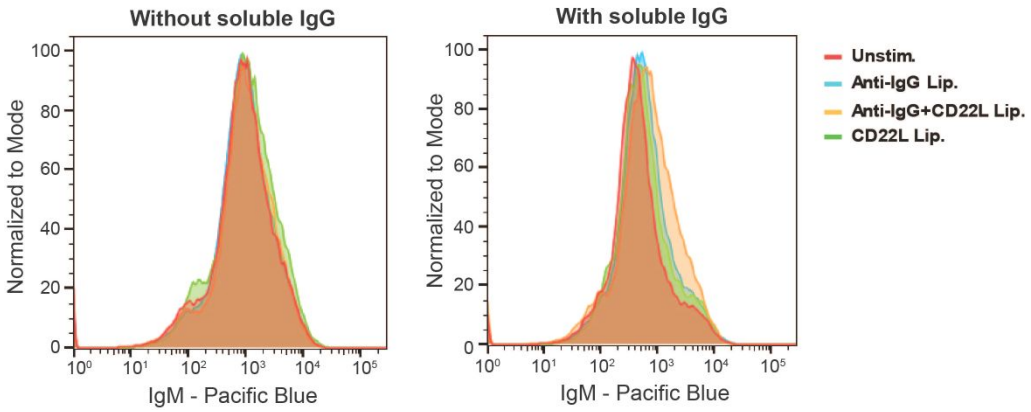
Supplemental Figure 3. No positive linear correlation was found between antibody production and generation of B-cell subsets other than the subset IgD$^-$CD27$^+$ B cells. Total IgG antibody production was determined from culture supernatants of each of the above conditions (**Figure 1B)**, each symbol represents a different healthy control sample (N=3 independent experiments from 3 individual healthy control donors, pooled data, linear regression). B-cells were also stained on day 7 for naïve (CD20$^+$IgD$^+$CD27$^-$) and memory (CD20$^+$IgD$^-$CD27$^+$) B-cell subsets and correlated to IgG production for the subsets shown above (Linear regression analysis) (N=3 independent experiments from 3 individual healthy control donors, pooled data, linear regression) and correlation to total B-cell proliferation.



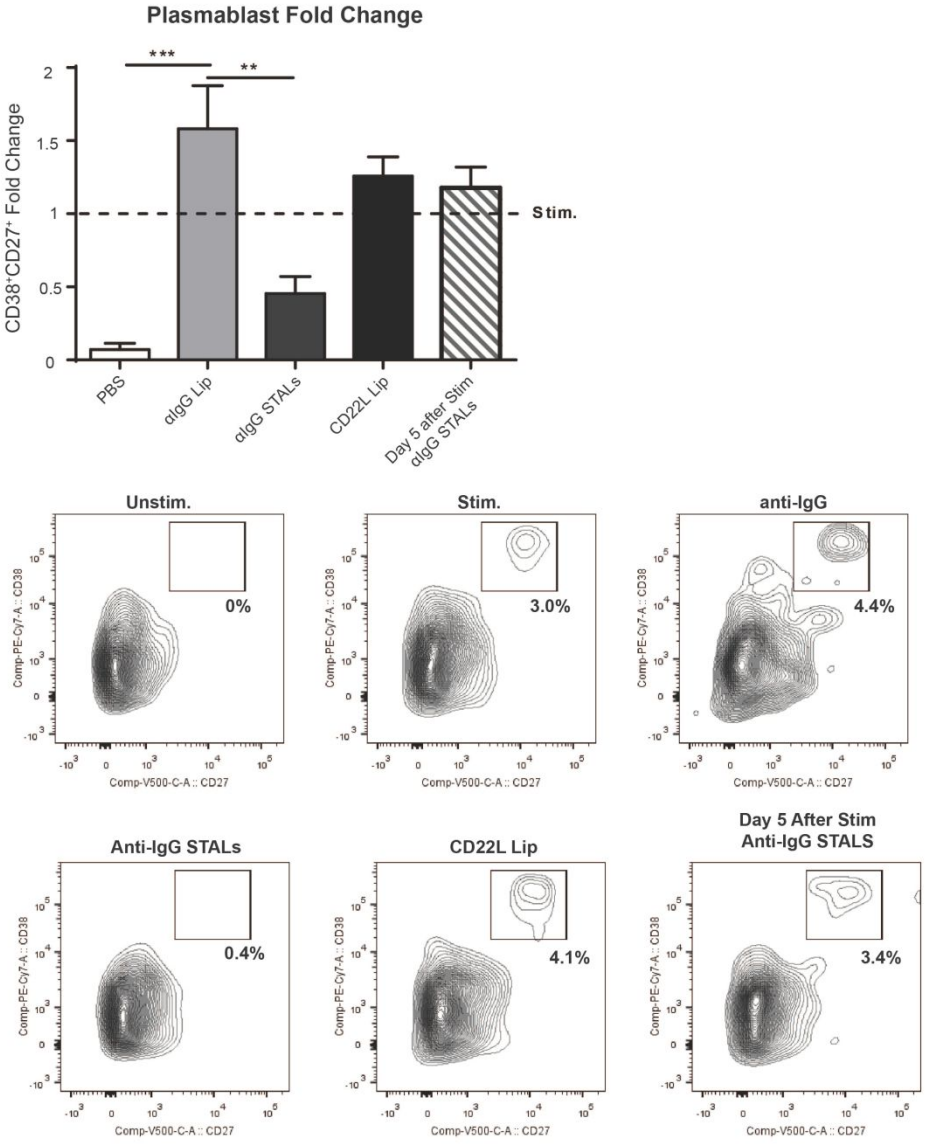
Supplemental Figure 4. Unstimulated and Stimulated Cells do not express the surface marker CD138 after 7 days in culture. B-cells isolated from healthy control PBMCs were plated at $2x10^5$ cells/well and were left unstimulated or stimulated under standard stimulating conditions for 7 days and characterized for analysis of B-cell plasmablast subset markers by flow cytometry (IgD, CD22, IgG, CD38, CD138, CD20, CD95 (FAS), and CD27) (N=3 independent experiments with 3 individual health donors). Plasma B cells were identified by cell surface marker CD138 in FACS analysis.



Supplemental Figure 5. Sorting purity for naïve and memory B-cells. B-cells were isolated from healthy control PBMCs and sorted using BDFacsAria™ for naïve or memory B-cells as described in Methods and Materials. Sorted naïve and memory B-cells were monitored for purity based on cell surface markers CD27, IgD, IgM and IgG. FACS analysis of purify B cells from a representative healthy donor was shown.

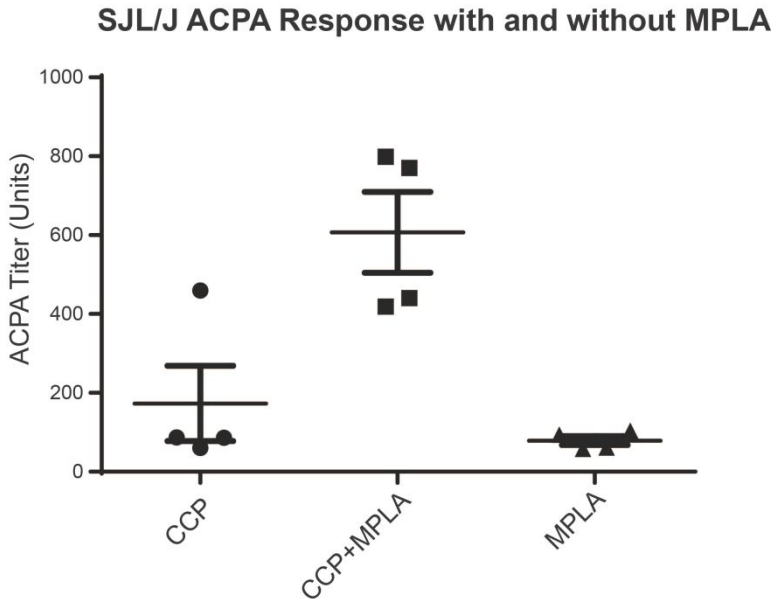


Supplemental Figure 6. IgM expressing B-cells are unaffected by anti-human IgG STALs in the absence and presence of soluble IgG. Human B-cells were isolated from healthy control PBMCs and plated at $1x10^5$/well in 96-well plates under one of the following conditions: unstimulated (PBS), anti-human IgG-liposomes (αIgG Lip), anti-human IgG+hCD22L (αIgG STALs), or CD22L liposomes alone (CD22L Lip) for 24 hrs with or without soluble IgG (5ng/mL) as indicated in the figure. After 24hrs cells were stained for B-cell subsets, including IgM as shown above (Representative figure from N=10 or N=4 independent experiments).



Supplement Figure 7. Plasmablast-like B-cells are not inhibited by anti-IgG STALs after formation on Day 5. Human B-cells were isolated from healthy control PBMCs and plated at $1x10^5$/well in 96-well plate stimulated under standard stimulating conditions for 7 days and under one of the following conditions: unstimulated (PBS), anti-human IgG-liposomes (αIgG Lip), anti-human IgG+hCD22L (αIgG STALs), or CD22L liposomes alone (CD22L Lip) for the entirety of the study or anti-human IgG+hCD22L liposomes were added to the culture on day 5 when

generation of plasmablast-like B-cells in cultures were detectable (N=6 each an independent experiment, pooled data, one-way ANOVA with multiple comparisons). On day 7, each well was harvested for flow cytometry analysis of B-cell subsets as previously described (IgD, CD22, IgG, CD38, CD138, CD20, IgM, and CD27), representative plots are shown (N=6 each an independent experiment, pooled data, one-way ANOVA with multiple comparisons).



Supplemental Figure 8. MPLA is necessary for ACPA titers in SJL/J mice. SJL/J mice were immunized with liposomes decorated with CCP, CCP+MPLA or MPLA alone (N=4 mice from 2 independent experiments). ACPA titers in mouse serum were measured 7 days post-immunization using a CCP ELISA kit as described in Materials and Methods.