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Supplementary Materials for

A tyrosine sulfation–dependent HLA-I modification identifies memory B cells and plasma cells

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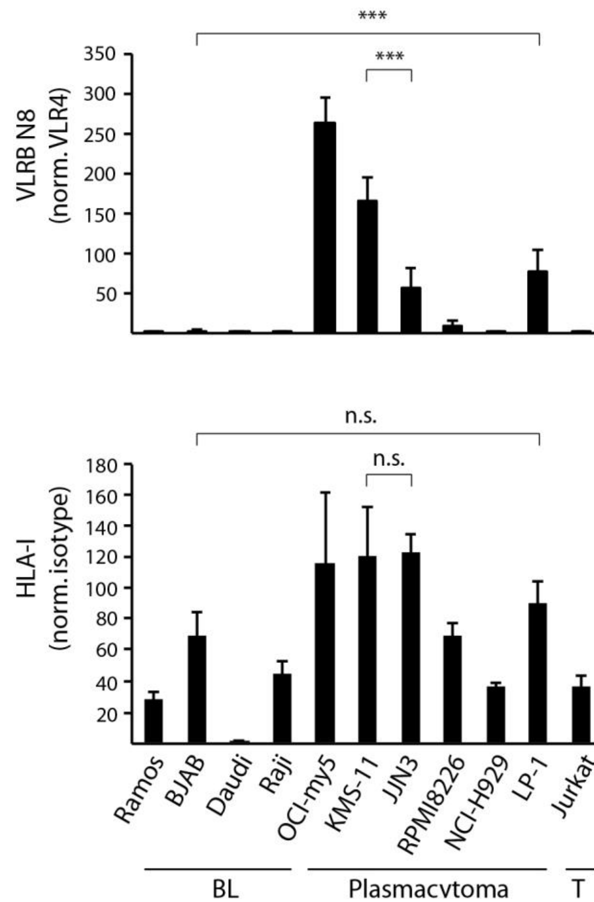


Fig. S1: VLRB N8 binding to cell lines does not correlate with HLA-I cell-surface expression levels. The indicated cell lines were stained with VLRB N8 (top panel) or conventional anti-pan HLA-I antibodies and analyzed by flow cytometry. Shown are mean +/- SD (n=4). Values are normalized to negative control VLR4 or isotype matched control antibodies, respectively. Statistical analysis was performed using a One-way Anova test with Tukey's post-test. Selected pairings (BJAB/LP-1 and KMS-11/JJN3) demonstrating significant differences for VLRB N8 binding but lack of significant differences in HLA-I expression are indicated. (***) indicates $p<0.001$, (n.s.) indicates $p>0.05$.

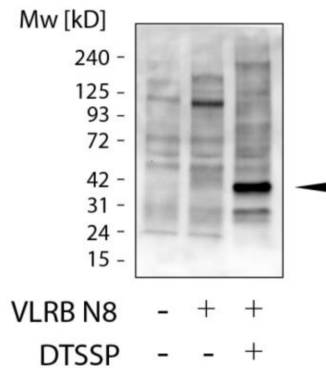


Fig. S2: VLRB N8 immunoprecipitates a prominent 42kD protein antigen. VLRB N8 immunoprecipitates of cell surface-biotinylated KMS-11 cells were resolved on 6-14% SDS-PAGE gradient gels, transferred onto nitrocellulose membranes and dected using HRP-labeled streptavidin. DTSSP indicates the addition of a membrane non-permeable, amine-reactive reducible crosslinker prior to cell lysis and immunoprecipitation with monoclonal anti-VLRB antibody 4C4. Depicted is one of three independently performed experiments.

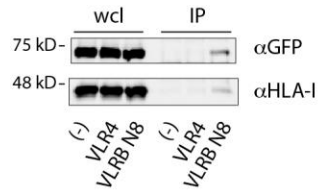


Fig. S3: Immunoprecipitation of HLA-I with VLRB N8. KMS-11 cells expressing exogenous HLA-A*2402-GFP fusion proteins were incubated with VLR4, VLRB N8 or without VLR antibodies, followed by crosslinkage with DTSSP. The cells were lysed in 1% NP40 lysis buffer and the cell lysate subjected to immunoprecipitation using anti-HA epitope tag antibodies. The whole cell lysates (wcl) and immunoprecipitates (IP) were subjected to SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were cut into two sections with the top section incubated with anti-GFP antibodies for the detection of exogenous HLA-A*2402-GFP and the bottom section incubated with anti-HLA-I clone HC-10 for the detection of endogenous HLA-I.

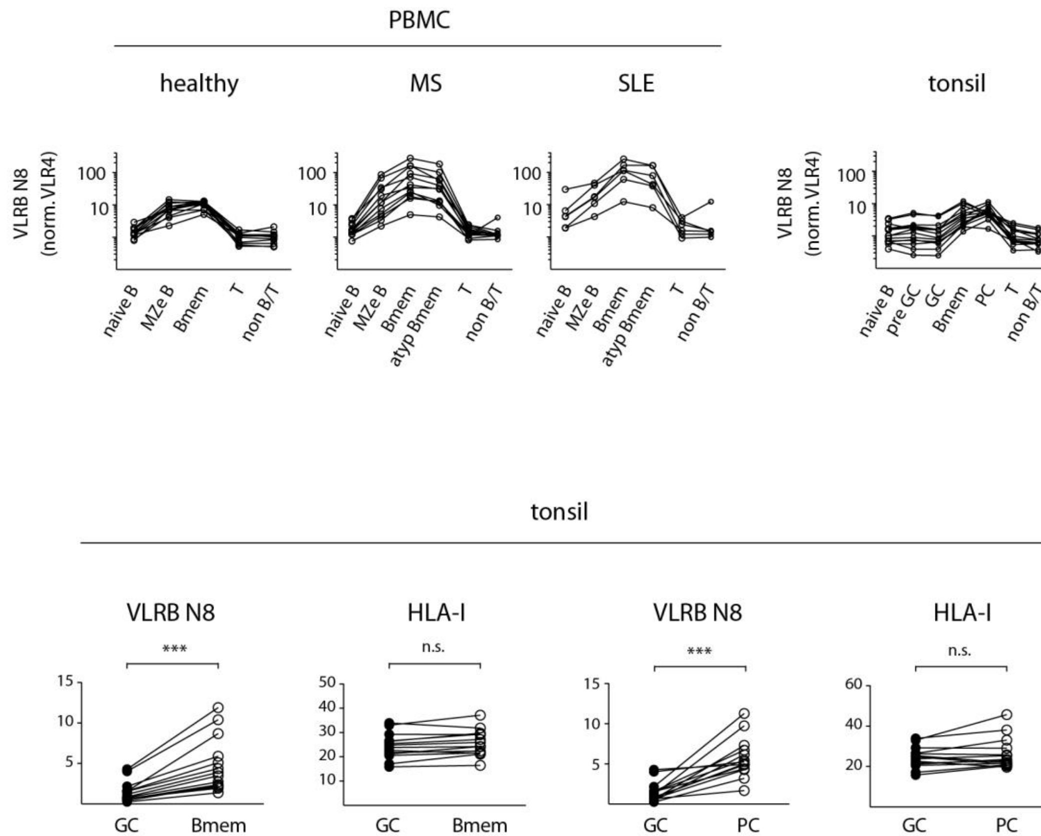
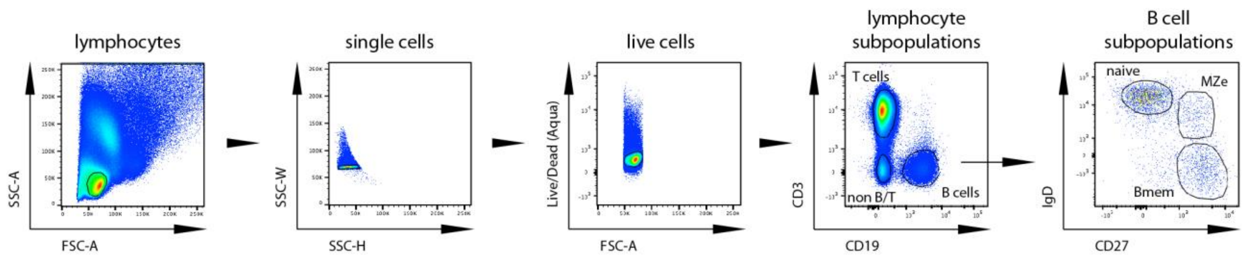
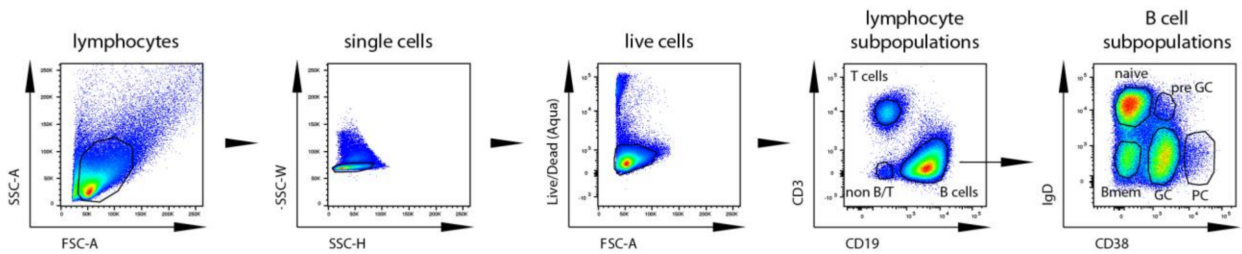


Fig. S4: Recognition of HLA-I by VLRB N8 on memory B cells and plasma cells is independent of HLA-I expression levels. (Top panel) Depicted are MFI values of VLRB N8 normalized to negative control VLR4 for the indicated cell populations from circulating and tissue based lymphocytes. Note that in MS and SLE samples VLRB N8 reactive cells are consistently strongly or moderately reactive within each individual sample. (Bottom panel) Depicted are MFI values of VLRB N8 normalized to negative control VLR4 and HLA-I normalized to isotype-matched control antibodies of tonsilar germinal center B cells (GC) in comparison to memory B cells (Bmem) or plasma cells (PC). Note that VLRB N8 consitently recognized Bmem and PC but not GC while the HLA-I levels of these cell populations remained unchanged. Statistical significance was determined using a Friedman test followed by Dunn's multiple comparison test (n=14) and values of $p<0.001$ are indicated by asteriscs (***); n.s. = not significant.

A Gating strategy lymphocyte subpopulations (PBMC)



B Gating strategy lymphocyte subpopulations (tonsil)



C Gating strategy Bmem subpopulations (tonsil)

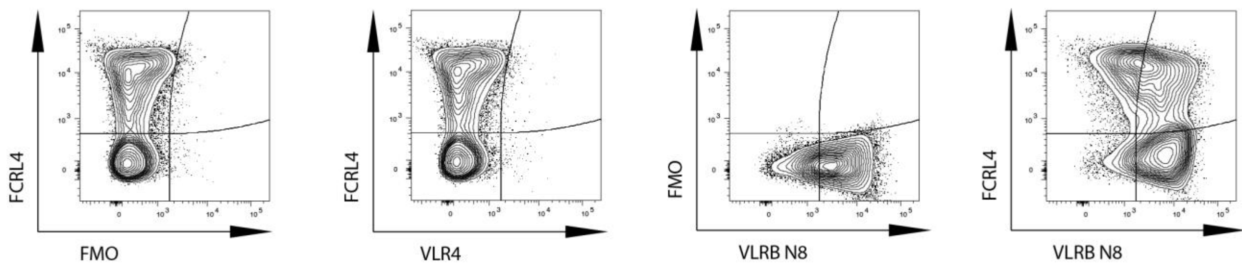


Fig. S5: Gating strategies for evaluation of lymphocyte populations from blood and tonsil. (A) PBMC were purified using ficoll density gradient centrifugation and incubated with anti-CD3, anti-CD19, anti-IgD, anti-CD27 and VLRB antibodies. Shown is the gating strategy for the indicated subpopulations of a representative blood sample. (B) Tonsil were purified using ficoll density gradient centrifugation and incubated with anti-CD3, anti-CD19, anti-IgD, anti-CD38 and VLRB antibodies. Shown is the gating strategy for the FCRL4-positive and FCRL4-negative Bmem. (C) For determination of VLRB N8 reactivity to Bmem subpopulations anti-FCRL4 antibodies were included. Depicted are cells determined as memory B cells using the antibody panel shown in (B).