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Supplemental Information

Galectin-8 Favors the Presentation of Surface-Tethered Antigens by Stabilizing the B Cell Immune Synapse

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SUPPLEMENTAL FIGURES

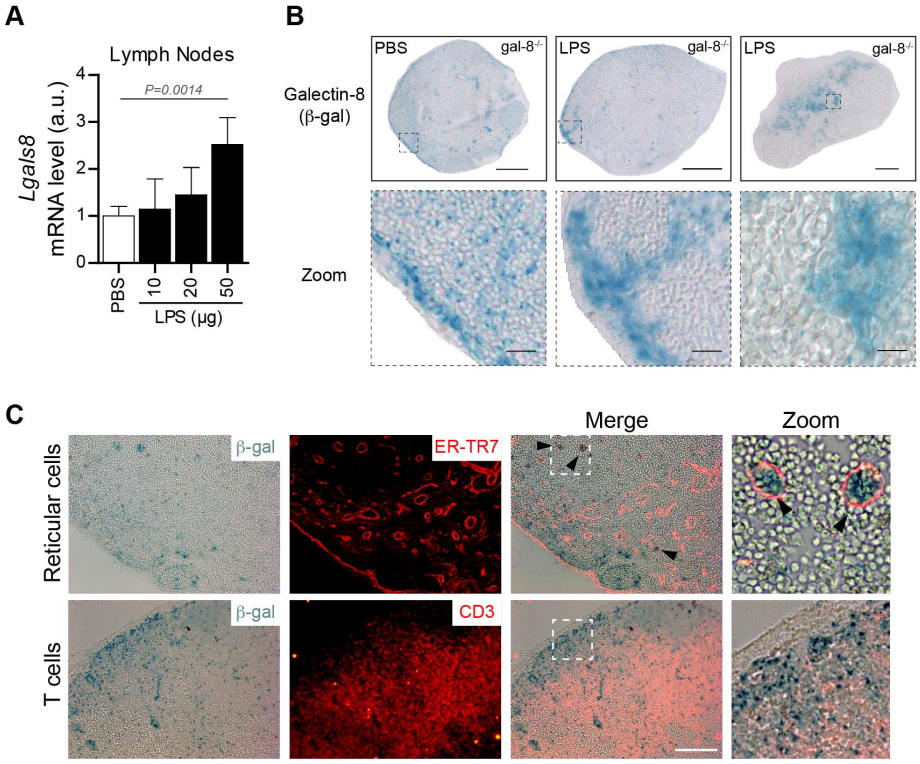

Figure S1. Galectin-8 is expressed in lymphoid tissues. Related to Figure 1. (A) Quantitative RT-PCR analysis of *Lgals8* mRNA levels in peripheral lymph nodes of C57BL/6 mice 6h following retro-orbital injection of PBS (vehicle control) or indicated doses of LPS. Values were normalized with respect to the mean PBS value per replicate. n=3 to 5 mice per condition pooled from N=4 independent experiments. Analysis of variance (ANOVA test) was used to assess statistical significance. **(B)** Representative images of β-galactosidase staining of LN cryosections from mice bearing a LacZ expression cassette at the *Lgals8* locus 6h following injection of PBS (vehicle control) or LPS (50 µg) in the tail vein. Scale bar, 500µm. Zooms highlight the increase in β-galactosidase staining within different areas upon inflammation. Scale bar, 50µm. **(C)** Representative images of popliteal lymph node sequential cryosections from mice bearing a LacZ expression cassette at the *Lgals8* locus stained for β-galactosidase (blue) and either reticular cells (ER-TR7) or T cells (CD3), both shown in red. Scale bar, 200 µm. Arrowheads highlight β-galactosidase staining within the vasculature.

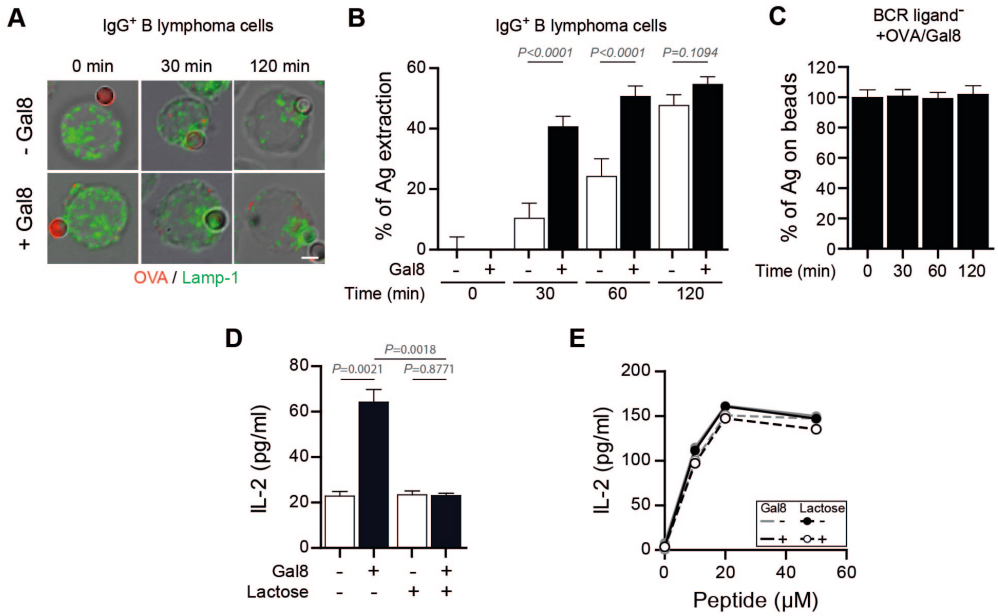

Figure S2. Galectin-8 promotes efficient antigen extraction and presentation by B lymphoma cells *in vitro*. Related to Figure 5. **(A)** Representative images of B lymphoma cells incubated with BCR-ligand^{+} beads plus the model antigen OVA in the presence or not of Galectin-8 for indicated time. Scale bar, 3 μm. **(B)** Quantification of the percentatge of antigen (OVA) extracted from beads following incubation of B lymphoma cells with BCR-ligand^{+} beads plus OVA in the presence or not of Galectin-8 for indicated time. Values were normalized with respect to Ag-coated beads not engaged with B cells. n>40 cells pooled from N=3 independent experiments. **(C)** Quantification of the amounts of antigen (OVA) remaining on beads following incubation of B lymphoma with BCR-ligand^{-} beads plus OVA/Gal8 for indicated time. n>20 cells pooled from 2 independent experiments. **(D)** Antigen presentation assay of B lymphoma cells stimulated with BCR-ligand^{+} beads containing Lack±Gal8 in the presence or not of 100 mM lactose. Data represent the mean±SEM of triplicate and are representative of N=3 independent experiments. Unpaired t-test. **(E)** Peptide control for cells used in the antigen presentation assay shown in (D). Graph shows the mean of duplicates and is representative of 3 independent experiments.

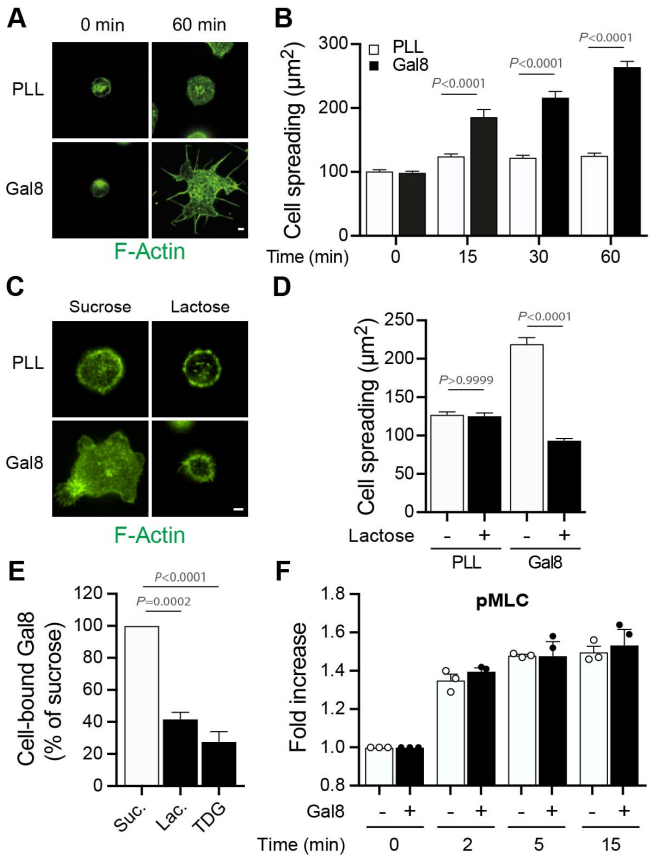

Figure S3. Galectin-8 enhances B cell spreading but not contractility. Related to Figure 5. (A) Representative images of B lymphoma cells seeded on coverslips coated with poly-L-lysine (PLL) or 20 µg Galectin-8 (Gal8) for indicated time and stained for F-Actin (Phalloidin, green) . Scale bar, 3 µm. **(B)** Quantification of the spreading area of cells shown in (A). n>90 cells pooled from N=3 independent experiments. **(C)** Representative images of B lymphoma cells seeded on coverslips coated with poly-L-lysine (PLL) or 20 µg Galectin-8 (Gal8) in presence of 100 mM sucrose (control) or lactose for 60 min and stained for F-Actin (Phalloidin, green) . Scale bar, 3 µm. **(D)** Quantification of the spreading area of cells shown in (C). n>50 cells pooled from N=2 independent experiments. **(E)** AlexaFluor488-conjugated recombinant Galectin-8 was pre-traeted with 20 mM sucrose (Suc.), lactose (Lac.) or thiodigalactoside (TDG) for 30 min prior to be incubated with B lymphoma cells for 60 min, washed and the cell-bound fraction of AlexaFluor488-Gal8 detected by flow cytometry. Values were normalized with respect to the sucrose condition in each experimental replicate. Data are pooled from N=3 independent experiments. **(F)** Flow cytometry analysis of the phosphorylation status of Myosin Regulatory Light Chain (pMLC) upon stimulation of B lymphoma cells with BCR-ligand^{+} beads containing or not Galectin-8 for indicated times. Values were normalized with respect to the time 0 min per condition and experimental replicate. Data are pooled from N=3 independent experiments. An analysis of variance (ANOVA test) followed by a Sidak's multiple comparaison test **(B, D-E)** and a ratio paired t-test **(F)** were used to assess statistical significance.

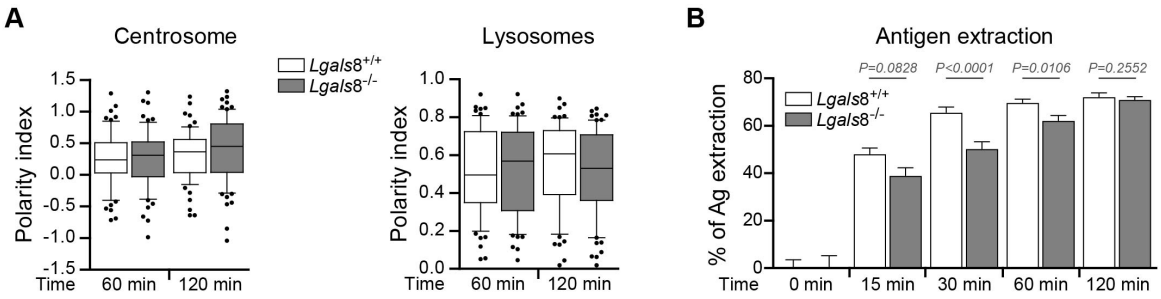

Figure S4. The lack of Galectin-8 does not impair B cell polarization and function. Related to Figure 5. (A) Quantification of centrosome and lysosome polarity indexes of spleen B cells purified from *Lgals8* $^{+/+}$ (white) and *Lgals8* $^{-/-}$ (grey) mice and stimulated for 60 min or 120 min with BCR-ligand^{+} beads. n>60 cells per condition and time point and are pooled from N=2 independent experiments. Unpaired t-test (centrosome) and Mann-Whitney test (lysosomes) were used to assess statistical significance. **(B)** Antigen (OVA) extraction assay with *Lgals8* $^{+/+}$ (white) and *Lgals8* $^{-/-}$ (grey) spleen B cells stimulated with BCR-ligand^{+} beads containing OVA for indicated time. Bars show the mean±SEM with n>55 cells per condition and time point pooled from N=2 independent experiments. Mann-Whitney test was used to assess statistical significance.