Figures and figure supplements

IgM and IgD B cell receptors differentially respond to endogenous antigens and control B cell fate

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Figure 1. IgD expression enables a dynamic range of IgM responsiveness. (A) GFP expression in mature Fc
splenic B cells (CD19+CD23+CD93-) from Nur77-eGFP BAC Tg reporter mice with either a wild-type BCR
reertoire (left), or harboring IgHEL Tg specific for the cognate antigen HEL (hen egg lysozyme) in the absence
(middle), or presence (right) of endogenous cognate antigen driven by soluble HEL Tg. WT Fc B cells lacking GFP
reporter are included for reference (gray shaded histograms). (B) Surface IgM and IgD expression in splenic Fc B
cells from WT, IgM-/-, and IgD-/- mice expressing the Nur77-eGFP reporter. (C) Splenocytes from WT, IgM-/-,
and IgD-/- mice were loaded with Indo-1 and stimulated with 2.5 μg/mL of F(ab')2 anti-IgM or 1:400 anti-IgD. Fc
B cells with the highest 20% and lowest 20% Nur77-eGFP expression are compared. (D) Representative histograms
and quantification of surface IgK expression in IgM-/- and IgD-/- Fc B cells normalized to WT. (E) Representative
histograms and quantification of Nur77-eGFP expression in IgM-/- and IgD-/- Fc B cells normalized to WT. (F)
Median surface IgK expression of WT, IgM-/-, and IgD-/- Fc B cells was calculated for 200 bins of equal width
across the Nur77-eGFP spectrum. For (A), (B) and (F), data are representative of at least n = 4 independent
experiments. For (C), n = 3 independent experiments for anti-IgM and n = 2 independent experiments for anti-
IgD. For (D) and (E), n = 7 and n = 4, respectively, WT, IgM-/-, and IgD-/- mice. Welch's t test was used to
calculate p values, and mean ±SEM is displayed. ***p<0.001.
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Figure 1—figure supplement 1. Regulation of endogenous Nur77 and Nur77-eGFP reporter expression. (A) Nur77-eGFP expression in mature F0 (B220+CD93-CD23+) B cells from mice with mutations in various signaling pathways (CD40L−/−, Unc93b13d/3d, TLR7−/−). This Unc93b1 mutation abolishes signaling through TLR3, TLR7, and TLR9. (B) Nur77-eGFP and CD69 induction in B220+ splenocytes from Nur77-eGFP reporter mice stimulated with either anti-IgM or IL-4 in vitro for 24 hr. (C) Splenic B cells were stimulated with CXCR4 ligand (SDF-1) in vitro for 18 hr and Nur77-eGFP expression and CXCR4 downregulation were assessed. (D–E) Splenocytes from N = 5 Germ-free and SPF mice were taken directly ex vivo and either permeabilized and stained to detect B220+ cells and endogenous Nur77 by intracellular staining (D), or total Nr4a1 transcript by qPCR (E). (F) PerC (peritoneal cavity) cells from n = 4 MB1-Cre+ MyD88fl/fl and controls were permeabilized and stained immediately ex vivo to detect B1a cells and endogenous Nur77 by intracellular staining. (G) Splenocytes from mice analyzed in (F) were harvested directly ex vivo to detect total Nr4a1 transcript by qPCR. In D–G, Nr4a1−/− splenocytes serve as a control for both Ab and primer specificity. Data in (A) and (C) are representative of n = 2 independent experiments. Data in (B) are representative of n = 3 independent experiments.

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Figure 1—figure supplement 2. Quantification of surface BCR expression on B cell subsets. (A) Surface Igκ expression relative to Nur77-eGFP reporter in Fo B cells from WT, IgM−/−, and IgD−/− mice. (B) Mean surface Igκ expression was calculated for Igκ+ splenic B cell (B220+) subsets in WT, IgM−/−, and IgD−/− mice. T1 (CD93+CD23−); T2/3 (CD93+CD23+); Fo (CD93−CD23+); MZ (CD21hiCD23lo). (C) Surface Igλ MFI of Igλ+ B cell subsets described in (B). (D) Surface Igκ MFI of Igκ+ cells in peritoneal B cell (CD19+) subsets. B1a (CD5+CD23−); B1b (CD5−CD23−); B2 (CD5−CD23+). (E) Surface Igλ MFI of Igλ+ cells in peritoneal B cell subsets described in (D). Data in (A) are representative of n = 3 independent experiments. Values in (B–E) were calculated from n = 3 mice of each genotype. One-way ANOVA with Tukey’s multiple comparisons test (B–E) was used to calculate p values, and mean ± SEM is displayed. *p<0.05, **p<0.01, ***p<0.001.

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Figure 1—figure supplement 3. Induction of Nur77-eGFP and CD69 in TLR-stimulated IgM−/− and IgD−/− B cells. (A) Nur77-eGFP and CD69 upregulation in IgM−/− and IgD−/− splenic B cells stimulated with indicated doses of LPS, CpG, and Pam3CSK4. Histograms compare unstimulated cells with cells incubated with indicated stimuli for 18 hr at 37°C. Histograms are representative of two independent experiments.

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Figure 2. IgD signals strongly in vitro but weakly in vivo. (A) Median intracellular pErk in splenic CD23+ B cells stimulated with anti-Igκ for 15 min. (B) Erk phosphorylation kinetics in splenic CD23+ B cells stimulated with 15 μg/mL anti-Igκ. (C) Splenocytes from IgM−/− and IgD−/− mice were loaded with Indo-1 and stimulated with 2.5 or 5 μg/mL anti-Igκ. B220+CD23+CD93− Fo B cells were compared. (D) CD86 induction in CD23+ IgM−/− and IgD−/− splenocytes stimulated with anti-Igκ for 18 hr. (E) Summary data for CD86 MFI in (D). (F) Nur77-eGFP induction in cells from (D). (G) Nur77-eGFP in cells from (D) incubated with medium alone (0 μg/mL anti-Igκ). (H) Representative histograms and summary data for MHC-II induction in unstimulated cells from (D). (I) Basal calcium in unstimulated IgM−/− and IgD−/− Fo B cells was calculated by normalizing the geometric mean of [Indo-1(violet)/Indo-1(blue)] to WT B cells in the same experiment. For (A), signaling in cells from n = 2 IgM−/− and IgD−/− mice is displayed, and results for 1.5, 3, and 15 μg/mL of anti-Igκ were replicated in n = 3 independent experiments. Data in (B) was compiled from n = 3 independent experiments with n = 3 mice of each genotype in each experiment. Data in (C) are representative of n = 4 independent experiments for 5 μg/mL and n = 2 independent experiments for 2.5 μg/mL. For (D-H), values were calculated for splenocytes from n = 3 mice of each genotype. For (I), basal calcium ratios from n = 6 independent experiments are compiled. Welch’s t test was used to calculate p values, and mean ± sSEM is displayed. *p<0.05, **p<0.01, ***p<0.001.

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Figure 2—figure supplement 1. S6 and calcium signaling in IgM\(^{-/-}\) and IgD\(^{-/-}\) B cells. (A) S6 phosphorylation kinetics in splenic CD23+ B cells following stimulation with 15 \(\mu\)g/mL anti-Ig\(\kappa\). (B) Splenocytes from IgM\(^{-/-}\) and IgD\(^{-/-}\) mice were loaded with Indo-1 and stimulated with 10 or 5 \(\mu\)g/mL anti-Ig\(\kappa\) or anti-Ig\(\kappa\) F(\(\text{ab'}\))\(_2\). Data in (A) and (B) are representative of \(n = 3\) and \(n = 2\) independent experiments, respectively. Welch’s t test was used to calculate p values, and mean ±SEM is displayed. *\(p<0.05\).

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Figure 3. Reduced in vivo antigen sensing by IgD in innate-like B cells. (A) Nur77-eGFP in peritoneal B1a (CD19+CD5+CD23-) and splenic MZ (B220+CD21hiCD23lo) B cells from WT and IgM−/− mice. (B) Nur77-eGFP in PtC-binding peritoneal B1a cells from WT, IgM−/−, and IgD−/− mice. (C) Nur77-eGFP and PtC MFIs were calculated for total B1a and PtC-binding B1a cells. The ratio of the IgM−/− (IgD-only) MFI to the IgD−/− (IgM-only) MFI is displayed with a 95% confidence interval. (D) Representative histograms of Nur77-eGFP in IgM−/− and IgD−/− splenic MZ B cells from mice without (left) and with (right) a BAFF overexpression transgene. (E) Quantification of Nur77-eGFP in MZ B cells from (D). For (A) and (E), n = 3 mice of each genotype were analyzed. For (B), histograms are representative of n = 4 mice of each genotype. For (C), ratios are pooled for n = 4 mice of each genotype from three independent experiments. For (D), histograms are representative of n = 3 mice of each genotype. Welch’s t test (A and E) was used to calculate p values, and mean +SEM is displayed (except in C). *p<0.05.

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Figure 3—figure supplement 1. The effect of BAFF, competition, and allotype on Igκ and Nur77-eGFP expression. (A) Surface Igκ MFI of IgM<sup>−/−</sup> and IgD<sup>−/−</sup> splenic MZ B cells from mice without (left) and with (right) a BAFF overexpression transgene. (B) Nur77-eGFP expression in peritoneal B1a and splenic MZ B cells from IgM-null (IgD<sup>+</sup>) and WT (IgM<sup>+</sup>) loci in IgM<sup>−/−</sup> mice. (C) Nur77-eGFP expression in IgM<sup>+</sup> and IgM<sup>+</sup> splenic MZ B cells in 6-month-old IgH<sup>a/b</sup> mice with a BAFF overexpression transgene. Values in (A) were calculated from n = 3 mice of each genotype. Values in (B) and (C) were calculated in n = 6 and n = 4 mice, respectively. Welch’s t test (A) and a paired t test (B-C) were used to calculate p values, and mean ±SEM is displayed. **p<0.01, ***p<0.001.

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Figure 4. Cell-intrinsic skewing of B cell development by IgM and IgD BCRs. (A) Allelic exclusion leads to a 1:1 mixture of IgM-only and IgD-only B cells in IgM⁻/⁺ IgD⁺/⁻ mice. (B) Proportion of peritoneal B1a (CD19⁺CD5⁺CD23⁻) and splenic MZ (B220⁺CD21hiCD23lo) B cells originating from each Ig locus in IgM⁻/⁺ IgD⁺/⁻ mice. (C) Relative competition between IgM⁺ and IgD⁺ B cells in IgM⁻/⁺ IgD⁺/⁻ mice was calculated for bone marrow, splenic, and peritoneal B cell compartments. Results include data from (B) for reference. Immature (CD23⁻CD93⁺); T2-like (CD23⁺CD93⁺); mature recirculating (CD23⁺CD93⁻); T1 (CD93⁺CD23⁻); T2/3 (CD93⁻CD23⁺); Fo (CD93⁻CD23⁺); MZ (CD21hiCD23lo); B1a (CD5⁺CD23⁻); B1b (CD5⁻CD23⁻); B2 (CD5⁻CD23⁺). (D) Relative competition between WT (IgM⁺b) and IgM-null (IgD⁺a) B cells in IgM⁻/⁺ mice was determined as in (C). (E) Relative competition between WT (IgM⁺b) and IgD-null (IgM⁺a) B cells in IgD⁻/⁺ mice was determined for splenic and peritoneal compartments as described in (C). (F) Competition in peritoneal B1a and splenic MZ compartments in IgM⁻/⁺ mice with or without a BAFF overexpression transgene. Results include data from (D) for reference. For (B) and (C), n = 3–5 mice were analyzed. For (D), n = 3–8 mice were analyzed. For (E), n = 5 mice were analyzed. For (F), n = 4–5 mice of each genotype were analyzed. Welch’s t test was used to calculate p values, and mean ±SEM is displayed. **p<0.01, ***p<0.001.

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Figure 4—figure supplement 1. B cell subset development in IgM<sup>-/-</sup>, IgD<sup>-/-</sup>, WT, and IgH<sup>a/b</sup> mice. (A) Signal strength model of B1a and MZ B cell development. While B1a and MZ cells originate from different precursor populations, their development is thought to be BCR signal strength-dependent. (B) B cell compartments as a percentage of total B220+ splenic B cells. T1 (CD93+CD23-); T2/3 (CD93+CD23+); Fo (CD93-CD23+); MZ (CD21<sup>hi</sup>CD23<sup>lo</sup>). (C) B cell compartments as a percentage of total CD19+ peritoneal B cells. B1a (CD5+CD23-); B1b (CD5-CD23-); B2 (CD5-CD23+). (D) Relative competition between IgM<sup>a</sup>+ and IgM<sup>b</sup>+ B cells in Balb/c-B6 F1 mice was calculated for splenic and peritoneal compartments described in (B–C). For (B), n = 10–12 mice of each genotype were analyzed. For (C), n = 6 mice of each genotype were analyzed. Mice in (D) were 6–14 weeks old. One-way ANOVA with Tukey’s multiple comparisons test (B–C) was used to calculate p values, and mean ±SEM is displayed. *p<0.05, **p<0.01, ***p<0.001. DOI: https://doi.org/10.7554/eLife.35074.020
Figure 5. IgD can drive polyclonal activation and germinal center entry, but not anti-dsDNA IgG2a production, in Lyn−/− mice. (A) Surface CD69 and CD86 expression on CD23+ splenic B cells from each Ig locus in IgM+/− mice on Lyn+/+ and Lyn−/− backgrounds. (B) Percentage of unswitched germinal center (CD19+ Fashi GL-7hi IgM/IgD+) B cells from each Ig locus in IgM+/− and IgD+/− mice on the Lyn−/− background. (C) Anti-dsDNA IgG2a titers from each Ig locus in IgM+/− Lyn−/− mice were calculated by ELISA using pooled IgHα/b autoimmune serum with high-titer autoantibodies from each locus as a reference (titer set at 1000). Each color represents a single mouse tracked over time. (D) Anti-dsDNA IgG2a titers in IgD+/− Lyn−/− mice were calculated as in (C). (E) Paired anti-dsDNA titers from each locus in individual IgM+/− Lyn−/− mice from (C) with additional mice from 24 weeks to 12 months. (F) Paired anti-dsDNA titers from each locus in IgD+/− Lyn−/− mice from (D). (G) Ratio of anti-dsDNA IgG2a[a] to IgG2a[b] from all samples in (E) and (F) with an anti-dsDNA IgG2a titer >250 from either locus, cutoff defined by titers in young WT mice. For (A), n = 4 IgM+/− Lyn−/− mice are compared to a reference IgM+/− Lyn+/+ mouse. Qualitatively similar results were obtained in two independent experiments. For (B) n = 5–6 mice of each genotype were analyzed. For (C) and (D), n = 9 and n = 12 mice were tracked. For (G), n = 36 IgM+/− Lyn−/− and n = 39 IgD+/− Lyn−/− anti-dsDNA IgG2a+ samples were compared. Welch’s t test was used to calculate p values, and mean ± SEM is displayed. **p<0.01.

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Figure 5—figure supplement 1. Splenic B cell subsets in IgH<sup>a/b</sup>, IgM<sup>+/−</sup>, and IgD<sup>+/−</sup> Lyn<sup>−/−</sup> mice. (A) Gating scheme for determining Ig locus of origin for splenic B cell subsets in 6-month-old IgH<sup>a/b</sup> Lyn<sup>−/−</sup> mice. (B) Quantification of compartments in (A). T1 (CD93<sup>+</sup>CD23<sup>−</sup>); T2/3 (CD93<sup>+</sup>CD23<sup>+</sup>); Fo (CD93<sup>−</sup>CD23<sup>−</sup>). (C) Percentage of Fo B cells originating from each Ig locus in IgM<sup>+/−</sup> and IgD<sup>+/−</sup> mice on the Lyn<sup>−/−</sup> background. Percentages in (A-C) were calculated using n = 5–6 mice of each genotype, and mean ± SEM is displayed. DOI: https://doi.org/10.7554/eLife.35074.025
Figure 5—figure supplement 2. Signaling in IgM<sup>−/−</sup> and IgD<sup>−/−</sup> Lyn<sup>−/−</sup> B cells. (A) Splenocytes from IgM<sup>−/−</sup> and IgD<sup>−/−</sup> mice on either Lyn<sup>+/+</sup> or Lyn<sup>−/−</sup> backgrounds were loaded with Indo-1 and stimulated with anti-IgM. WT (IgM<sup>b</sup>+<sup>−/−</sup>) B cells were gated out to isolate IgM-null (IgD-only) and IgD-null (IgM-only) cells. CD19<sup>+</sup>CD23<sup>+</sup> B cells are compared. (B) Intracellular Erk phosphorylation in splenic B220<sup>+</sup>CD23<sup>+</sup> B cells from mice in (A) stimulated with varying doses of anti-IgM for 5 min. Shaded histogram corresponds to IgM<sup>b</sup>+ cells from IgD<sup>+</sup>/Lyn<sup>−/−</sup> mice incubated with medium alone for reference. (A) and (B) are representative of two independent experiments.

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Figure 5—figure supplement 3. Nur77-eGFP in Lyn−/− B cells and the role of BCR allotype in Lyn−/− phenotypes. (A) Nur77-eGFP expression in splenic mature Fo B cells from Lyn+/+ and Lyn−/− reporter mice. (B) Gating scheme for determining the Ig locus of origin for germinal center B cells in 6-month-old IgM−/− Lyn−/− mice. (C) Relative contribution of IgHα+ and IgHβ+ B cells to the splenic unswitched GC (CD19+ Fas+ GL-7+ IgM+) and unswitched PC (CD138+IgM+) compartments of 6-month-old IgHα/β Lyn−/− mice. (D) Anti-dsDNA IgG2a from each Ig locus in 5–6 month old IgM−/−, IgD−/−, and IgHα/β mice on the Lyn−/− background was quantified by ELISA. (E) The OD ratio of knockout (allotype a) to WT (allotype b) anti-dsDNA IgG2a was calculated for all mice in (D) in which either locus produced an OD >0.3. Histograms in (A) are representative of at least n = 3 mice of each genotype. Percentages in (B–C) were calculated using n = 5–6 mice of each genotype, and mean ± SEM is displayed. For (D), n = 10–13 mice of each genotype were analyzed. For (E), n = 5–9 autoantibody-positive mice were analyzed. Welch’s t test (E) was used to calculate p values, and mean ± SEM is displayed. *p<0.05.

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Figure 6. Cell-intrinsic IgM expression is required for unswitched plasma cell expansion in Lyn−/− mice. (A) Representative blot and quantification of Ets1 and GAPDH protein in purified splenic B cells from WT, Lyn−/−, and IgM−/− Lyn−/− mice. (B) Composition of the CD138+ plasma cell compartments in the spleen and bone marrow of WT, Lyn−/−, and IgM−/− Lyn−/− mice was determined by intracellular staining of IgM, IgD, and IgA. (C) Percentages in (B) multiplied by the fraction of live cells positive for CD138 in each tissue. Unswitched cells are positive for either IgM or IgD. Statistics correspond to unswitched plasma cell percentages; differences in IgA+ cells were not significant. (D) Serum IgM in 16-week-old mice was quantified for B6.IgHa (WT) and Lyn−/− mice by ELISA. A sample from an IgM−/− mouse is shown for reference. (E) Serum IgD in 16-week-old mice was quantified for IgM−/− and IgM−/− Lyn−/− mice by ELISA. A sample from a WT mouse is shown for reference. (F) Gating scheme for quantifying the unswitched splenic plasma cell composition of IgM−/− Lyn−/− mice. (G) Percentage of unswitched splenic plasma cells [CD138+B220+ IgM/IgD+] from each locus in IgM−/− and IgD−/− mice on the Lyn−/− background. For (B) and (C), figures are representative of n = 4–5 mice of each genotype. For (D), values from n = 3 WT and n = 4 Lyn−/− mice are averaged. For (E), values from n = 3 IgM−/− and n = 4 IgM−/− Lyn−/− mice are averaged. For (F) and (G), n = 4–5 mice of each genotype were used. Welch’s t test was used to calculate p values, and mean ±SEM is displayed. *p<0.05, **p<0.01, ***p<0.001.

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**Figure 6—figure supplement 1.** BCR signaling in Lyn<sup>−/−</sup> peritoneal B cell subsets. (A) Intracellular Erk phosphorylation in peritoneal B220+ B cells stimulated with anti-Igk for 5 min. B1a (CD5+CD23−); B2 (CD5−CD23 +). Histograms in (A) are representative of cells from n = 2 WT and Lyn<sup>−/−</sup> mice. DOI: https://doi.org/10.7554/eLife.35074.030
Figure 6—figure supplement 2. Lyn restrains unswitched plasma cell differentiation of follicular B cells. (A) Model: Lyn restrains BCR signaling in Fo B cells. Loss of Lyn leads to Btk-dependent downregulation of Ets1 expression and consequent expansion of unswitched (IgM+) plasma cells. DOI: https://doi.org/10.7554/eLife.35074.031
Figure 7. IgD-only cells have intact germinal center responses but impaired IgG1+ SLPC responses. (A) Splenic (CD19+) B cells from WT, IgM−/−, and IgD−/− mice unimmunized or 5 days after i.p. immunization with 200 μL of 10% SRBCs. (B) Quantification of germinal center (Fashi GL-7hi) cells in (A). (C) Splenocytes from mice in (A). (D) Quantification of CD138+ IgG1+ plasma cells in (C). (E) WT (IgM+δ) and IgM-null (IgD+δ) germinal center B cells as a percentage of live splenocytes in unimmunized and IgM+δ/δ mice 5 days after i.p. immunization with 200 μL of 10% SRBCs. (F) WT (IgG1+δ) and IgM-null (IgG1−δ) switched plasma cells in (IgGδδδ) mice unimmunized or 5 days after i.p. immunization with 200 μL of 10% SRBCs. (G) Fraction of unswitched NP-specific germinal center cells (CD19+ Fashi GL-7hi IgM/IgD+) from the IgHδ locus in the spleens of Balb/c-B6 F1 and IgM+δ/δ mice 7–8 days after i.p. immunization with 100 μg NP-RSA. (H) Fraction of IgG1+CD138+ plasma cells from the IgHδ locus in Balb/c-B6 F1 and IgM−δ/δ mice 7–8 days after i.p. immunization with 100 μg NP-RSA. (I) NP-specific IgG1δ and IgG1tδ titers at OD = 0.2 were calculated for the mice in (G–H) by ELISA. The IgG1δ to IgG1tδ titer ratio was calculated for each mouse, and all ratios were normalized such that the average IgG1δ/IgG1tδ ratio in Balb/c-B6 F1 samples = 1.0. For (A–D), statistics from n = 4 unimmunized mice of each genotype and n = 3 WT, n = 6 IgM−/−, and n = 7 IgD−/− immunized mice were pooled. For (E–F), n = 5 unimmunized and n = 5 immunized mice are shown. For (G–I), n = 5 Balb/c-B6 F1 mice and n = 3 IgM−/− mice are shown. One-way ANOVA with Tukey’s multiple comparisons test (B and D), a paired t test (E–F), and Welch’s t test (G–I) were used to calculate p values, and mean + SEM is displayed. *p<0.05, **p<0.01, ***p<0.001.

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Figure 7—figure supplement 1. Role of BCR allotype and generation of unswitched plasma cells in SRBC-immunized mice. (A) Quantification of IgM⁺ and IgM⁺⁺ germinal center B cells (CD19+G6+GL-7⁺) as a percentage of live splenocytes in IgH⁺⁺ mice 5 days after i.p. immunization with 200 μL of 10% SRBCs. (B) Quantification of IgM⁺⁺ and IgM⁺⁺ plasma cells (CD138⁺) as a percentage of live splenocytes in IgH⁺⁺ mice 5 days after i.p. immunization with 200 μL of 10% SRBCs. (C) Quantification of splenic unswitched (IgM⁺ or IgD⁺) CD138⁺ plasma cells from WT, IgM⁻/⁻, and IgD⁻/⁻ mice unimmunized or 5 days after i.p. immunization with 200 μL of 10% SRBCs. (D) WT (IgM⁺⁺) and IgM-null (IgD⁺⁺) unswitched plasma cells (CD138⁺) as a percentage of live splenocytes in IgM⁺⁻ mice unimmunized or 5 days after i.p. immunization with 200 μL of 10% SRBCs. For (A) and (B), n = 3 mice were analyzed. Paired t tests (A-B and D) and one-way ANOVA with Tukey’s multiple comparisons test were used to calculate p values, and mean +SEM is displayed. For (C), cell numbers from n = 4 unimmunized mice of each genotype and n = 3 WT, n = 6 IgM⁻/⁻, and n = 7 IgD⁻/⁻ immunized mice were analyzed. For (D), n = 5 unimmunized and n = 5 immunized were analyzed. **p<0.01, ***p<0.001.

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Figure 7—figure supplement 2. Role of IgM and IgD in regulating rapid antibody responses. (A) Model: Control of peripheral B cell tolerance by IgM and IgD. Selective downregulation of IgM is a well-described feature of autoreactive B cells. This study demonstrates how loss of IgM could desensitize autoreactive B cells towards self-antigens. High and invariant IgD expression provides survival signals to allow these autoreactive cells to be maintained in the repertoire. Upon activation by foreign antigen, the least autoreactive cells receive strong signals through IgM and can quickly differentiate into plasma cells with little danger of autoimmunity. In contrast, the most autoreactive B cells are activated through IgD, which is less efficient at transducing signals in vivo. This weak signaling shunts IgD\(^{hi}\) IgM\(^{lo}\) cells into the germinal center where they can be ‘redeemed’ via somatic hypermutation, as proposed by Goodnow and colleagues. In this way, dual expression of IgM and IgD expression facilitates repertoire diversity and balances immune responsiveness with autoimmune potential. DOI: https://doi.org/10.7554/eLife.35074.037