Supplemental material

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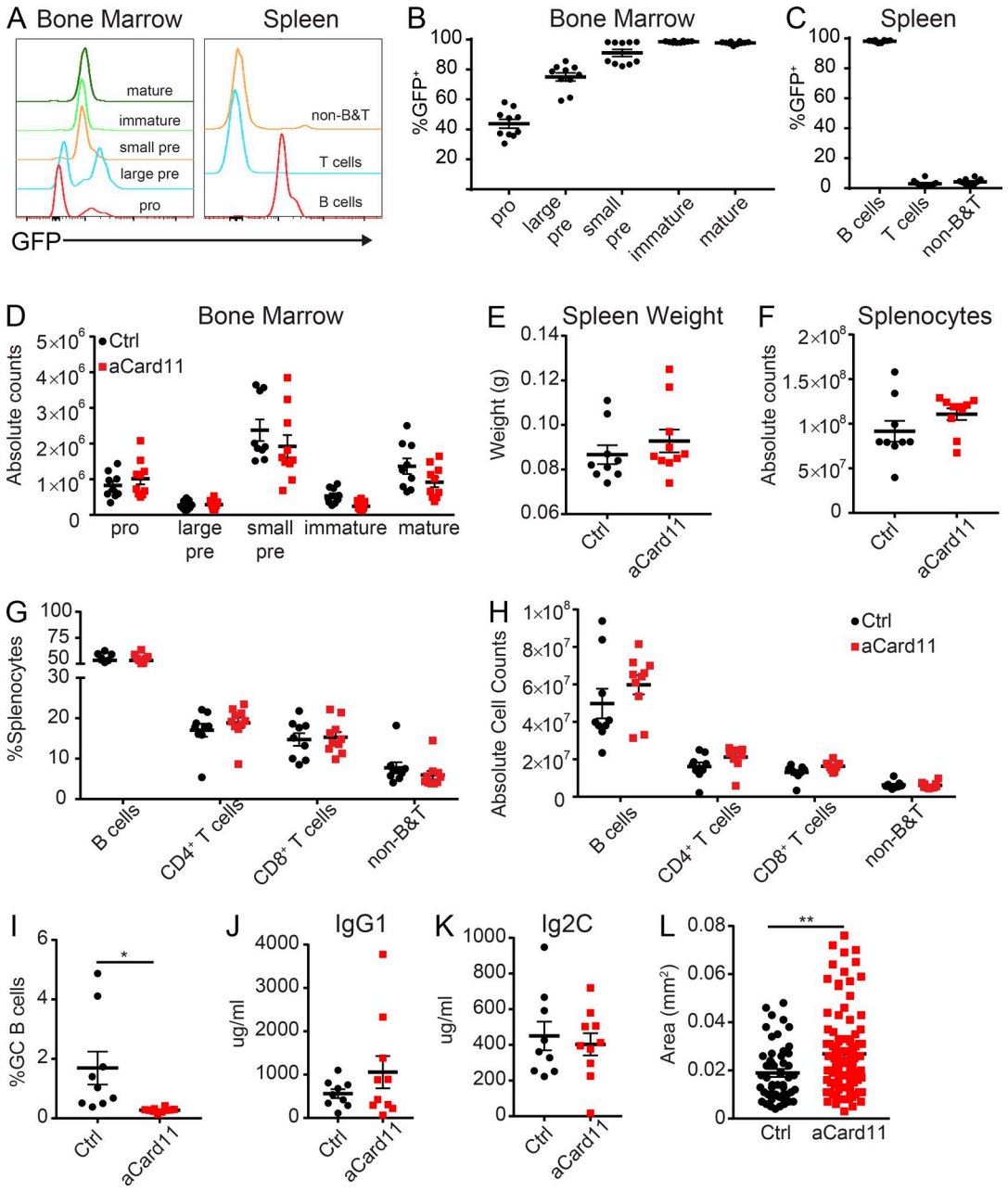

Figure S1. **Mb1-aCard11 mice have mild splenomegaly with unaltered BM B cell development. (A)** Representative histograms of GFP expression in B cell subsets in the BM (left) and spleen (right) of Mb1-Cre-aCard11 mice. **(B)** Frequency of GFP$^+$ cells in BM B cell subsets. **(C)** Frequency of GFP$^+$ cells in splenic B cells (B220$^+$), T cells (CD3$^+$), and non-B&T (B220$^-$CD3$^-$). **(D)** Absolute cell counts of BM B cell subsets from one leg (per animal) in Ctrl and Mb1-aCard11 mice: pro (B220$^+$IgM$^-$CD43$^+$), large pre (B220$^+$IgM$^-$CD43$^-$SSC^{hi}), small pre (B220$^+$IgM$^-$CD43$^-$SSC^{lo}), immature (B220$^+$IgM$^+$IgD$^-$), and mature (B220$^+$IgM$^+$IgD$^+$). **(E)** Spleen weight. **(F)** Total splenocytes. **(G)** Frequency of splenic B cells (B220$^+$), CD4$^+$ T cells (CD3$^+$CD4$^+$), CD8$^+$ T cells (CD3$^+$CD8$^+$), and non-B&T cells (B220$^-$CD3$^-$). **(H)** Absolute cell counts of splenic subsets. **(I)** Frequency of spontaneous GC B cells (B220$^+$CD95^{hi}CD38^{lo}; P = 0.015). **(J)** IgG1 serum titers. **(K)** IgG2c serum titers. **(I–K)** Significance was calculated by Student's unpaired *t* test. **(A–K)** Data are representative of two independent experiments with 9 Ctrl mice and 10 Mb1-aCard11 mice all ∼12 wk of age. **(L)** Area (square millimeters) of individual GCs (PNA$^+$ foci) in splenic tissues sections from Ctrl (littermate controls) and Mb1-aCard11 mice at 5 d after immunization with SRBCs (P = 0.003). Area was measured by ImageJ, with significance calculated by unpaired *t* test. Data are representative of three independent experiments with five Ctrl mice and five Mb1-aCard11 mice. For summary graphs, lines and error bars represent mean ± SEM. *, P < 0.05; **, P < 0.01.

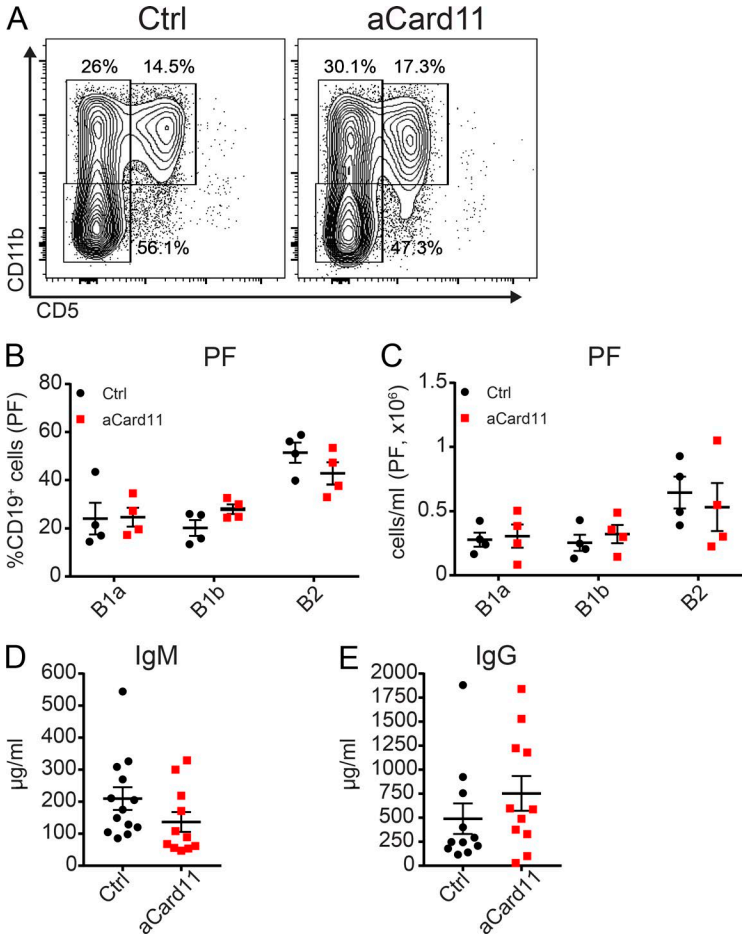

Figure S2. **Cγ1-aCard11 mice have normal peritoneal B cells and serum Ig titers. (A)** Representative flow plots of B cell subsets in PF: B1a ($CD19^+CD11b^+CD5^+$), B1b ($CD19^+CD11b^+CD5^-$), and B2 ($CD19^+CD11b^-CD5^-$). Left: Ctrl (Cre-negative littermate control). Right: aCard11 (Cγ1-aCard11). **(B and C)** Frequency (B) and absolute number (C) of peritoneal B cell subsets (per milliliter of PF). **(D)** IgM serum titers. **(E)** IgG serum titers. **(A–C)** Data are representative of two independent experiments with four Ctrl mice (aCard11) and four aCard11 (Cγ1-aCard11) mice all ∼12–15 wk of age. Significance was calculated by two-way ANOVA. **(D and E)** Data are representative of three independent experiments with eight Ctrl mice and eight Cγ1-aCard11 mice 11–15 wk of age. Significance was calculated by unpaired Student's *t* test. For summary graphs, lines and error bars represent mean ± SEM.

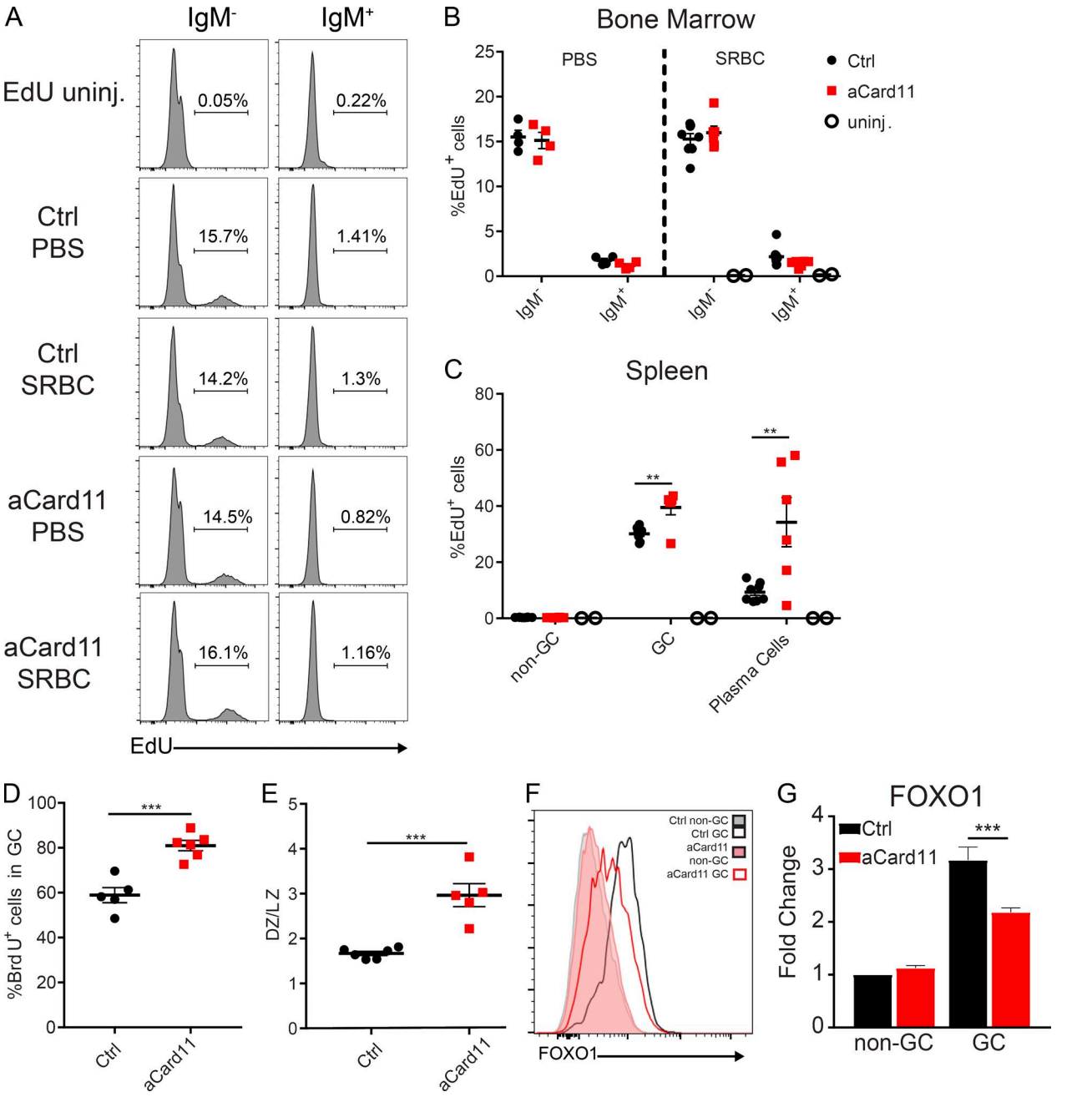

Figure S3. **aCARD11 enhances cycling of GC B cells and PCs, with no impact on cycling in the BM. (A–C)** Ctrl and Mb1-aCard11 mice were immunized with SRBCs and sacrificed on day 5 after immunization for analysis of B cell populations. 1 mg EdU was injected i.p. 1 h before sacrifice. **(A)** Representative histograms showing the relative proportion of EdU$^+$ cells in pro/pre B cells (left column: B220$^+$IgM$^-$) and immature/mature B cells (right column: B220$^+$IgM$^+$) in the BM. **(B)** Percentage of EdU$^+$ cells in pro/pre and immature/mature B cells in the BM from PBS and SRBC-immunized mice at 5 d after immunization. **(C)** Percentage of EdU$^+$ cells in non-GC (B220$^+$CD95^{lo}CD38^{hi}), GC (B220$^+$CD95^{hi}CD38^{lo}; P = 0.002), and PC (B220$^-$CD138$^+$; P = 0.007) compartments. Significance calculated by two-way ANOVA. **(A–C)** Data are representative of two independent experiments using eight Ctrl mice (black circles) and six Mb1-aCard11 (red squares; EdU-injected) and two untreated animals (open circles). **(D and E)** Ctrl and Cγ1-aCard11 mice were immunized with SRBCs and sacrificed on day 5 after immunization for analysis of B cell populations. 1 mg of BrdU was injected i.p. 24 h before sacrifice. **(D)** Percentage of BrdU$^+$ cells in GC (B220$^+$CD95^{hi} CD38^{lo}) at 5 d after immunization with SRBCs (P = 0.0004). **(E)** Ratio of DZ to LZ cells within the GC at 5 d after immunization (as defined in Fig. 4; P = 0.0004). **(D and E)** Data are representative of five Ctrl mice (black) vs. six Cγ1-aCard11 mice (red) from two independent experiments; significance was calculated by Student's unpaired *t* test. **(F)** Representative histogram of total FOXO1 protein in non-GC (B220$^+$CD95$^-$PNA$^-$) and GC (B220$^+$CD95^{hi}PNA^{hi}) B cells. Filled histograms: non-GC B cells (gray, Ctrl; pink, Mb1-aCard11). Open histograms: GC B cells (black, Ctrl; red, Mb1-aCARD11). **(G)** Fold change in FOXO1 median flourescent intensity over Ctrl non-GC B cells (P = 0.0002). Data in F and G are representative of two independent experiments; *n* = 8 Ctrl and *n* = 8 Mb1-aCard11 mice. Significance was calculated by two-way ANOVA. For summary graphs, lines and error bars represent mean ± SEM. For bar graphs, bars represent mean + SEM. **, P < 0.01; ***, P < 0.001.

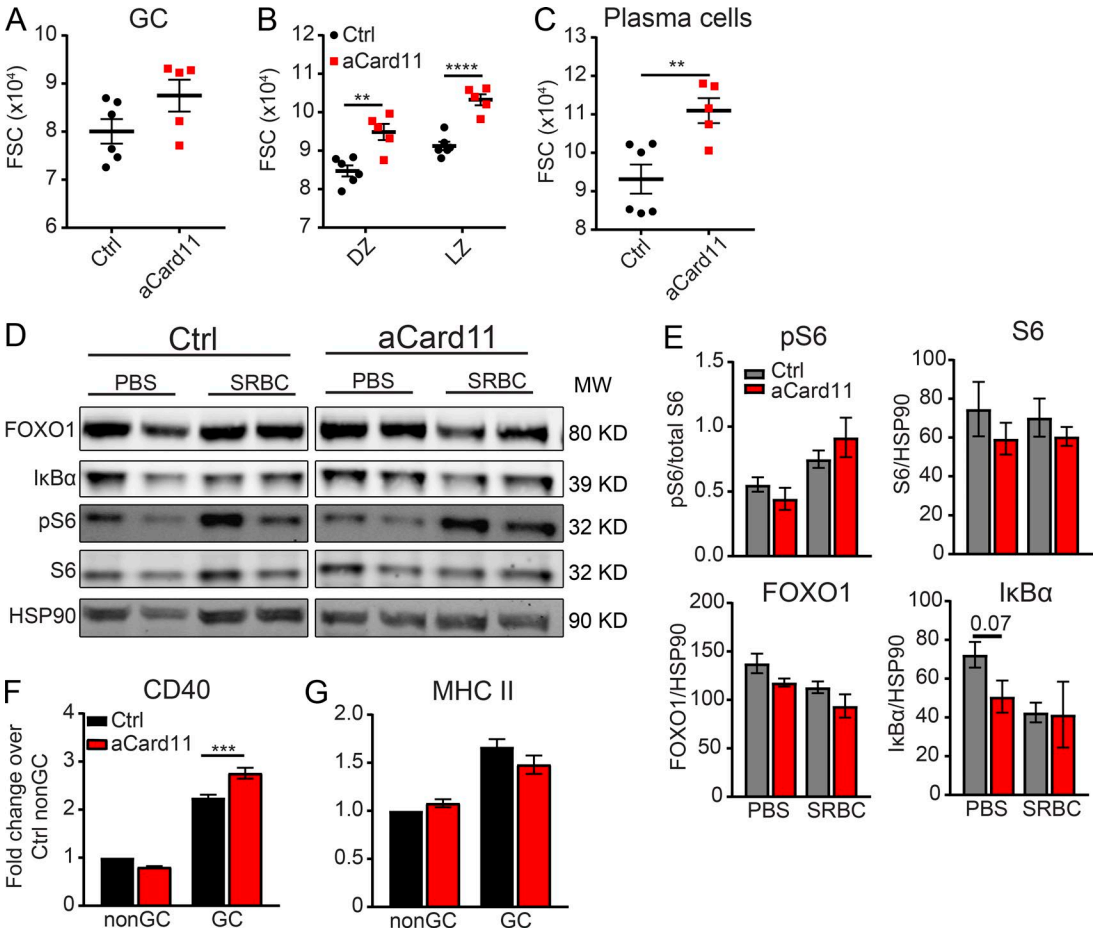

Figure S4. **aCARD11 enhances cell biomass and mTORC1 signaling. (A–C)** Ctrl and Cγ1-aCard11 mice were immunized with SRBCs and sacrificed on day 5 after immunization for analysis of B cell populations. **(A)** Median FSC of GC B cells ($B220^+CD95^{hi}CD38^{lo}$). **(B)** Median FSC of DZ ($CXCR4^+CD86^-$; P = 0.001), and LZ ($CXCR4^-CD86^+$; P = 0.0002) GC B cells (as defined in A). Significance was calculated by two-way ANOVA. **(C)** Median FSC of PCs ($B220^-CD138^+$; P = 0.007). Significance was calculated by Student's unpaired *t* test. **(A–C)** Data are representative of two independent experiments with six Ctrl mice and five Cγ1-aCard11 mice. **(D and E)** Ctrl and Mb1-aCard11 mice were immunized with SRBCs and sacrificed on day 5 for Western blot analysis of splenic B cell populations. For summary graphs, lines represent mean ± SEM. **(D)** Representative immunoblot with purified total splenic B cells and PCs derived from PBS or SRBC immunized mice at 5 d after immunization. Each lane depicts protein data from an individual control or aCard11 animal immunized with PBS or SRBCs as indicated. Graphs to the right indicate quantification of data from two independent experiments, with six Ctrl and five Mb1-aCard11 mice for PBS and five Ctrl and four Mb1-aCard11 mice for SRBCs. **(E)** Quantification of immunoblots by using ImageJ: pS6 normalized to total S6, total S6 normalized to HSP90, FOXO1 normalized to HSP90, and total IκBα normalized to HSP90 (P = 0.07). Significance was calculated by Student's unpaired *t* test. For bar graphs, lines represent mean + SEM. **(F)** Fold change in CD40 median fluorescent intensity over control non-GC B cells. Data are representative of eight littermate controls and eight Mb1-aCard11 mice from two independent experiments (P = 0.0001). **(G)** Fold change in MHC II median fluorescent intensity over control non-GC B cells. Data are representative of 13 littermate controls and 13 Mb1-aCard11 mice from three independent experiments. **(F and G)** Significance was calculated by using two-way ANOVA. For bar graphs, lines represent mean + SEM. **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

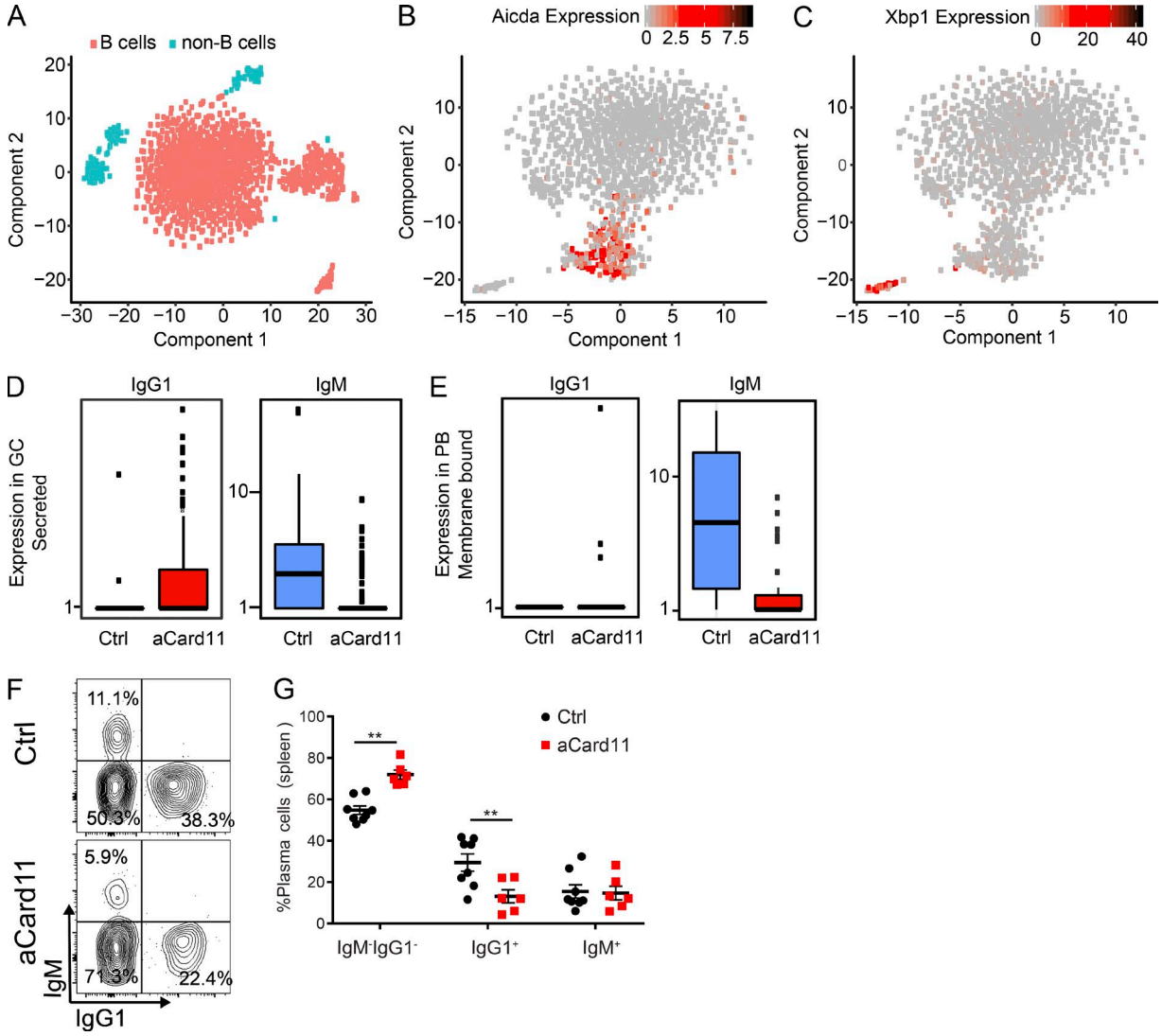

Figure S5. **aCard11 enhances CSR. (A–G)** Ctrl ($Mb1^{Cre/+}$) and Mb1-aCARD11 mice were immunized with SRBCs and sacrificed on day 5 after immunization for scRNA sequence analysis. **(A)** t-SNE plot of 1,954 total cells: blue, non-B cells; pink, B cells. **(B)** t-SNE plot of B cell clusters illustrating expression of AICD primarily in the GC cluster. **(C)** t-SNE plot illustrating that XBP1 expression is primarily found in the PB cluster. **(D)** Expression levels of secreted IgG1 (left; $P = 1.4 \times 10^{-28}$) and IgM (right; $P = 3.8 \times 10^{-36}$) transcripts in GC B cells. **(E)** Expression levels of membrane-bound IgG1 (left) and IgM (right; $P = 2 \times 10^{-24}$) transcripts in PBs. **(D and E)** Data shown are from one of two representative experiments. Blue, Ctrl; red, Mb1-aCard11. P values were calculated by using the likelihood ratio test included within Monocle's differential-GeneTest function. Graphs are box plots showing lower (first) and upper (third) quartiles with the line indicating the median. Lines extending from box plots indicate ±1.5 IQR. Individual data points are outlying points beyond ±1.5 IQR. **(F)** Representative flow plots of cytoplasmic BCR isotype of PCs ($B220^{-}CD138^{+}$). Top: Ctrl; bottom: Mb1-aCard11 from SRBC-immunized animals. **(G)** Percentage of $IgM^{-}IgG1^{-}$ ($P = 0.002$), $IgG1^{+}$ ($P = 0.003$), and IgM^{+} cells within the PC compartment in Ctrl (black) vs. Mb1-aCard11 (red) mice 5 d after immunization. Statistical significance was calculated by two-way ANOVA. **(F and G)** Data are representative of two independent experiments with eight Ctrl and six Mb1-aCard11 mice. For summary graphs, lines represent mean ± SEM. **, $P < 0.01$.