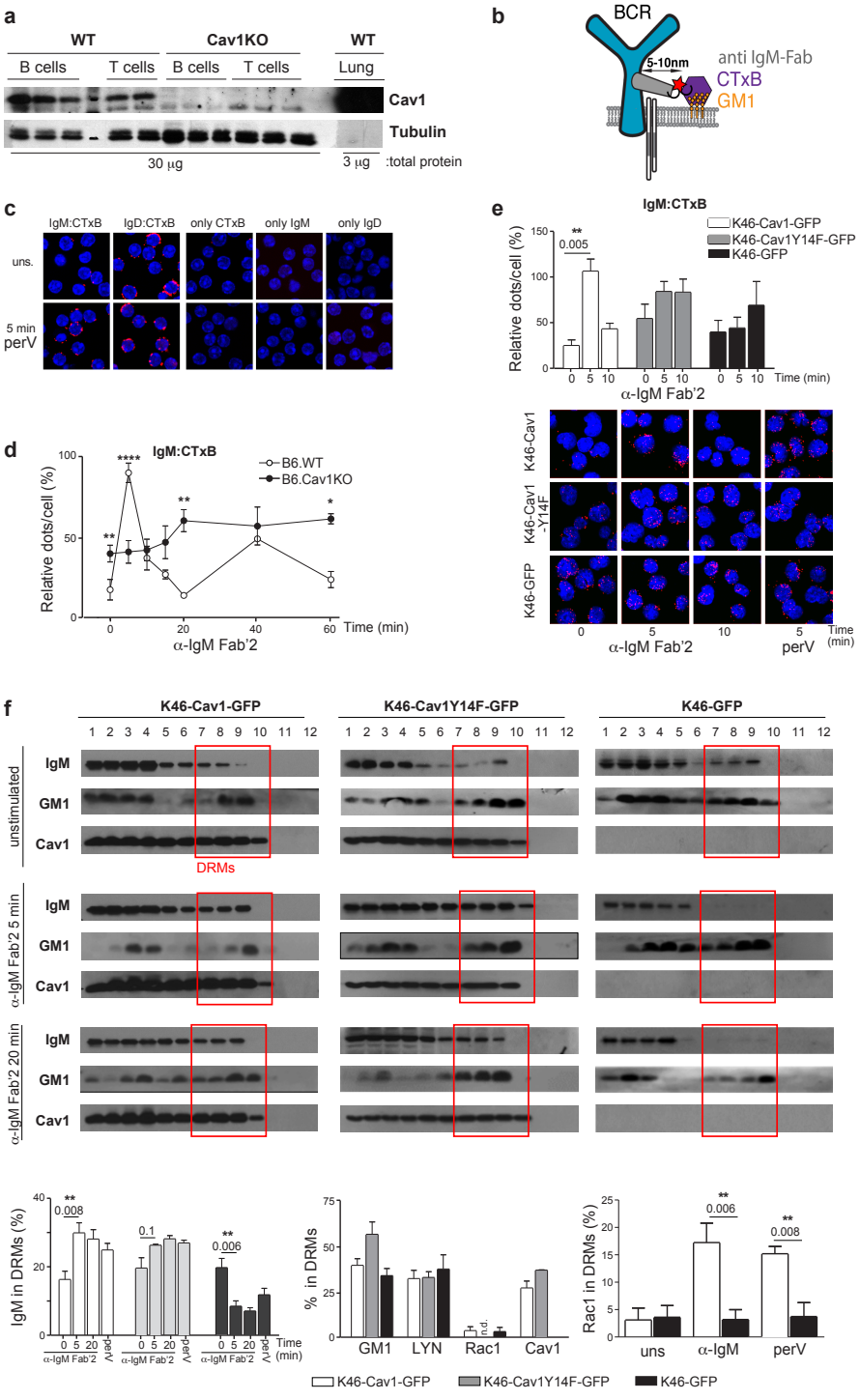
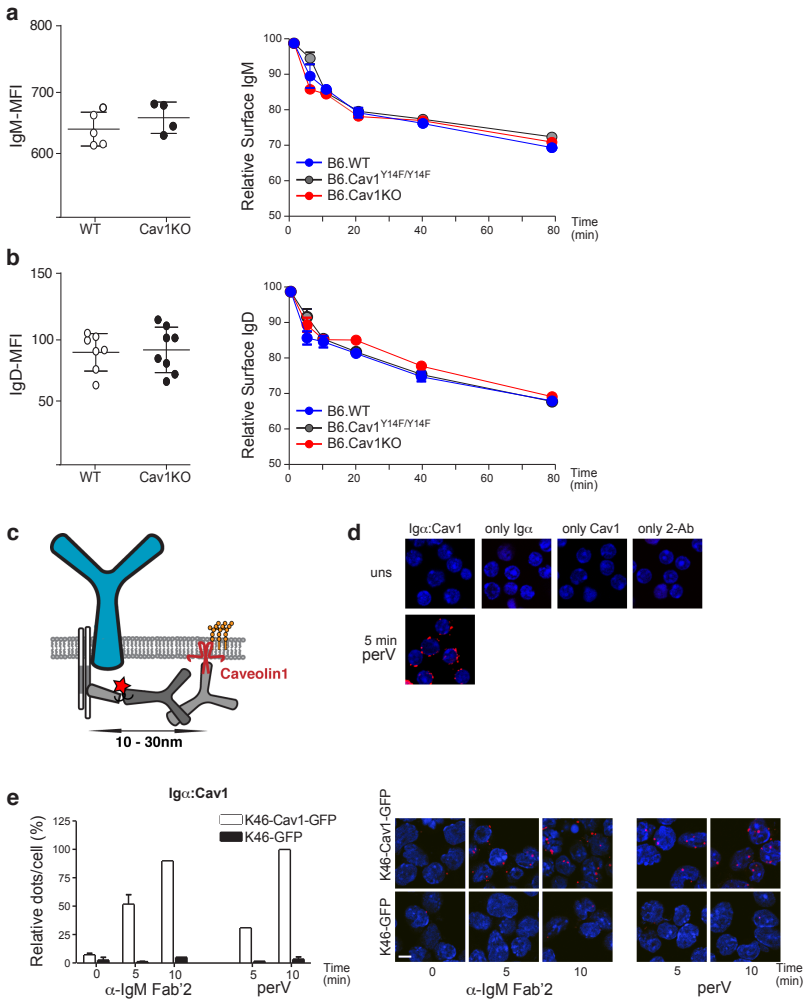


Supplementary Figure 1

Cav1 regulates the proximity of the IgM-BCR to the GM1-ganglioside on the surface of B cells.

(**a**) Purified B and T cells from the spleen of the indicated mice were lysed. As a comparative control, lung tissue was homogenized and lysed. Proteins were then separated by SDS-PAGE, Cav1 and tubulin were detected by immunoblot. The total amount of protein loaded is indicated. A representative experiment out of 2 independently performed experiments is shown. (**b**) Schematics of the IgM:CTxB PLA

experimental setup. Analysis of the proximity of the IgM-BCR to GM1 was performed using an oligo-labeled anti-IgM Fab (grey) and direct oligo-labeled GM1-bound CTxB (purple). PLA signal is indicated with a red star. (**c**) PLA controls show single Fabs or CTxB on unstimulated (top) and 5 min 0.5 mM pervanadate (perV) stimulated (bottom) B6.WT splenic B cells. The PLA was performed as described in (b). Representative microscope images show DAPI stained nuclei (in blue) and PLA signals (in red). A representative experiment out of 7 is shown. (**d**) Purified splenic B cells were stimulated with 10 μg/ml of anti-IgM Fab'2 for the indicated times or left unstimulated. Cells were fixed and *in situ* IgM:CTxB PLA performed. PLA signals were counted and normalized to the PLA signals of WT cells stimulated for 5 min with perV for each individual experiment. (**e**) K46μml cells were lentivirally transfected to express Cav1-IRES-GFP, Cav1Y14F-IRES-GFP or IRES-GFP. GFP^+ cells were sorted. Cells were stimulated with 10 μg/ml of anti-IgM Fab'2 or with 0.5 mM perV for the indicated times or left unstimulated and further treated as in (c). PLA signals were normalized to the PLA signals of K46-Cav1-GFP cells stimulated for 5 min with 0.5 mM perV for each experiment. At least 500 individual cells were quantified per experimental condition and experiment. (**f**) Immunoblot of sucrose density gradient fractions of Cav1-GFP-, Cav1Y14F-GFP- and GFP-transfected K46μml cells. The red boxes denote DRM fractions (7–10). Fractions were analyzed for the distribution of IgM, GM1, LYN, Rac1 and Cav1. The charts show the amount of IgM (left) and Rac1 (right) in the DRMs for each stimulatory condition. The middle chart shows the basal distribution of GM1, LYN, Rac1 and Cav1 in DRMs. Quantified data of (**d**, n=2-9), (**e**, n=2) or (**f**, n=2-3) independent experiments were pooled and statistical analysis performed using unpaired Student's t test. When significant, P values are indicated.

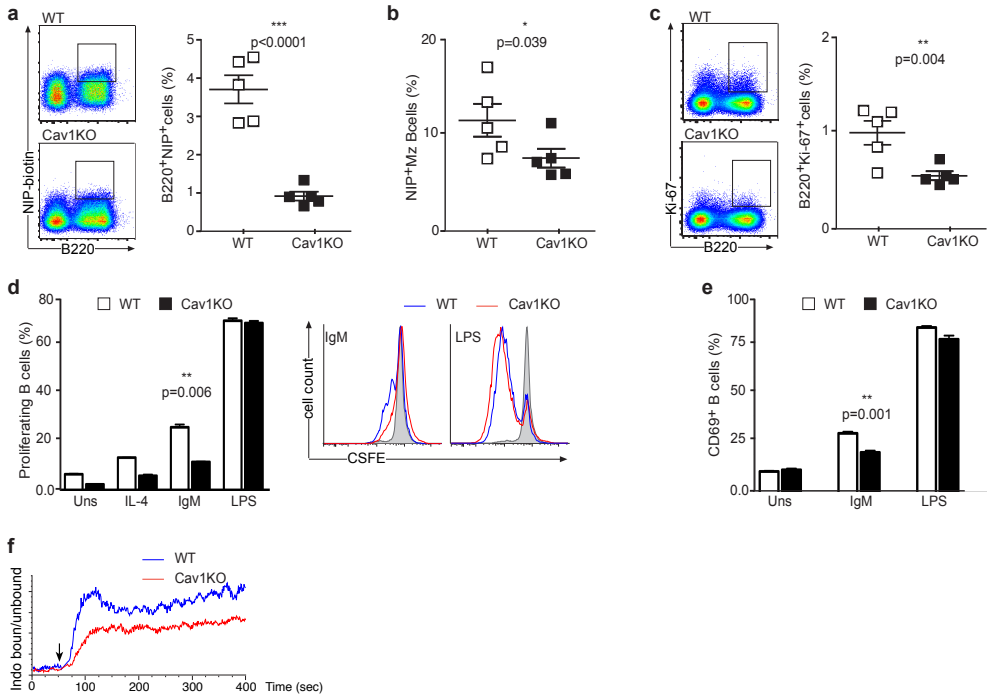


Supplementary Figure 2

Cav1 and the BCR are in close proximity upon BCR stimulation.

(**a**) Basal levels of IgM-BCR expression in splenic $CD19^+$ cells were analyzed by flow cytometry (left panel, n=4-5 animals per genotype). IgM-BCR internalization was assayed by incubating purified B cells from B6.WT or B6.Cav1KO splenocytes with an anti-kappa antibody for 1 hour at 4ºC. Cells were washed once and resuspended in an excess of pre-warmed medium. Samples were taken at the indicated times and mixed with an excess of cold PBS + 0.01% of NaN_3. All samples were simultaneously stained with anti-IgM-APC and analyzed by flow cytometry (n=3-4 animals per genotype). IgM-surface levels were normalized to t=0. (**b**) Basal levels of IgD-BCR expression in splenic $CD19^+$ cells were analyzed by flow cytometry (left panel, n=6-8 animals per genotype). IgD-BCR internalization was assayed as above. Samples were stained with anti-IgD-APC and analyzed by flow cytometry (n=3-4 animals per genotype). (**c**) Schematics of the BCR(Igα):Cav1 PLA experimental setup. Analysis of the proximity of the BCR to Cav1 was done using an anti-Igα-Fab-coupled PLA oligo plus a primary antibody against Cav1 with the corresponding secondary antibody coupled to a PLA oligo. PLA signal is indicated with a red star. (**d**) PLA controls show single antibodies on unstimulated (top) and 5 min perV stimulated (bottom) on B6.WT splenic B cells. Both secondary antibodies were added. Representative microscope images show DAPI stained nuclei (in blue) and PLA signals (in red). An individual experiment out of 7 independently performed experiments is shown. (**e**) Cav1-GFP- and GFP-transfected K46μml cells were stimulated with 10 μg/ml of anti-IgM Fab'2 or with 0.5 mM perV for the indicated times or left unstimulated. PLA signals were normalized to the PLA signals of K46-Cav1-GFP cells stimulated for 5 min perV for each

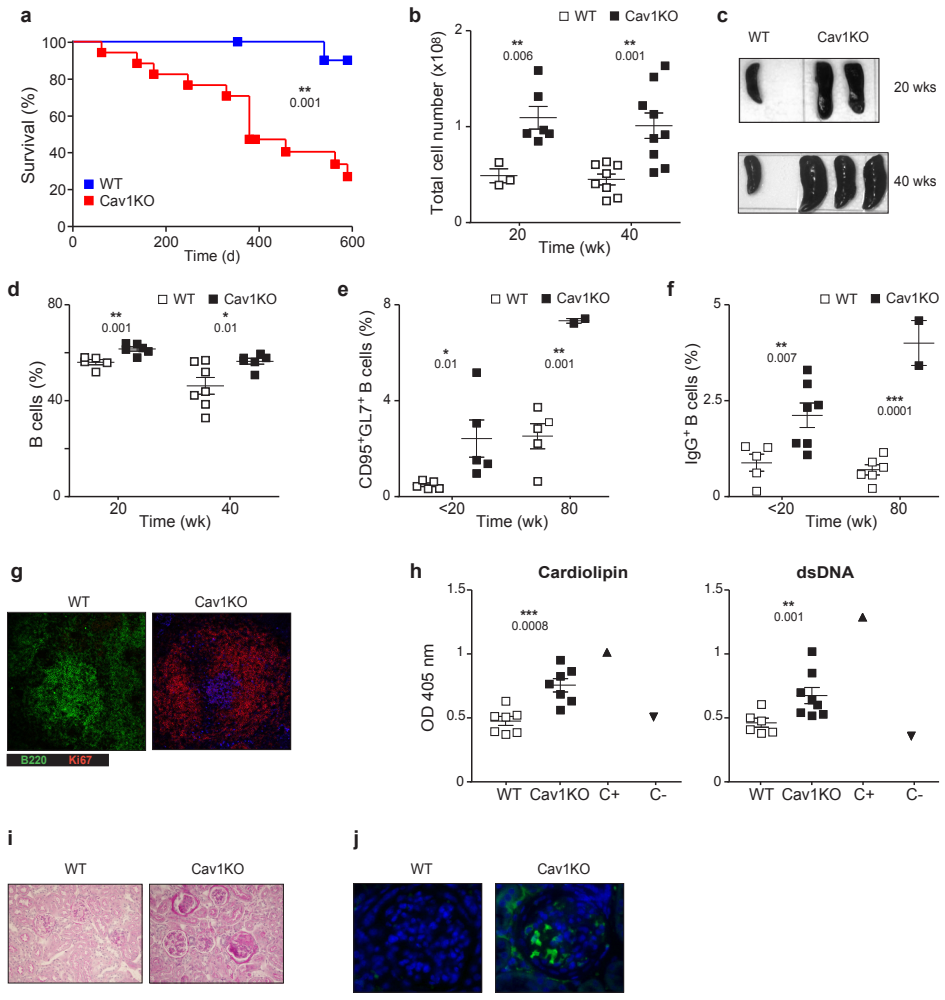
experiment. At least 500 individual cells were quantified per experimental condition and experiment. Quantified data of two independent experiments were pooled. Statistical analysis was performed using unpaired Student’s t test. P values were >0.05.



Supplementary Figure 3

Absence of Cav1 reduces B cell responses of Cav1KO mice.

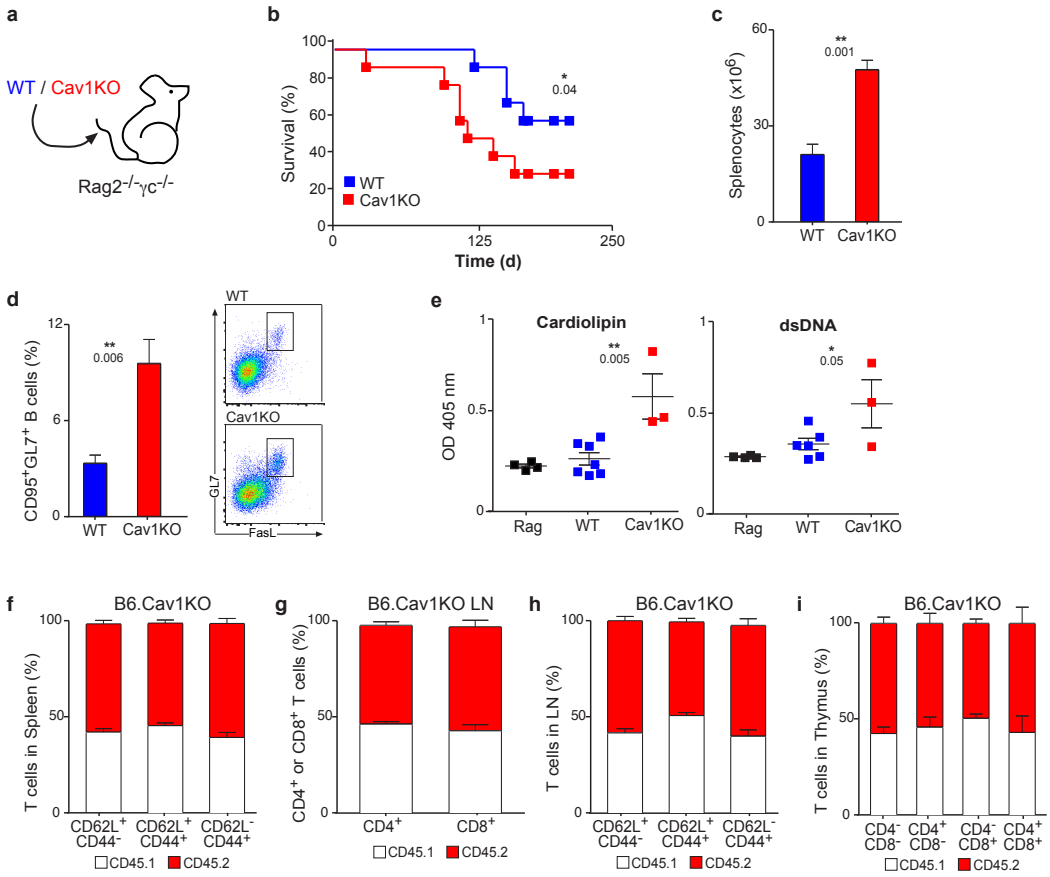
(**a**) WT and Cav1KO littermates were immunized with NP-Ficoll (T-independent immunization) using aluminum hydroxide as an adjuvant. At day 6 after immunization, splenocytes were analyzed for the frequency of NP-reactive B cells. Representative dot plots and the quantification plotted as Mean ± SEM from 5 mice per group are shown. (**b**) The quantification of NP-reactive Marginal zone (Mz) B cells is plotted as in a. (**c**) The frequency of proliferating Ki-67^{+} splenic B cells was analyzed. Representative dot plots and quantification plotted as in (a) are shown. (**d**) Splenic B cells were isolated and labeled with CFSE. Stimulation was done with IL-4 (50 ng/ml) alone or in combination with 3 μg/ml anti-IgM Fab'2 fragments, 1 μg/ml LPS or the cells were left untreated (grey shaded in the representative histogram). After 3 days the proliferation-induced dilution of the CFSE dye was measured by flow cytometry (data are representative of 2 independent experiments). (**e**) Purified splenic B cells were stimulated with the indicated amounts of anti-IgM Fab'2 fragments over night. Cells were then analyzed for CD69 expression by flow cytometry. Quantification shows pooled data from 3 independently performed experiments. (**f**) Splenocytes were stained with the dye Indo-1. The ratio of Ca^{2+}-bound Indo-1 to Ca^{2+}-unbound Indo-1 was measured by flow cytometry. Baseline was acquired 50 sec, and then 8 μg/ml of anti-IgM Fab'2 fragments were added (arrow, n=2). Statistical analysis was performed using unpaired Student's t test. Wen significant, P values are indicated in each panel.



Supplementary Figure 4

Increased B cell numbers, spontaneous GC, autoantibodies and reduced life span in Cav1KO mice of mixed background

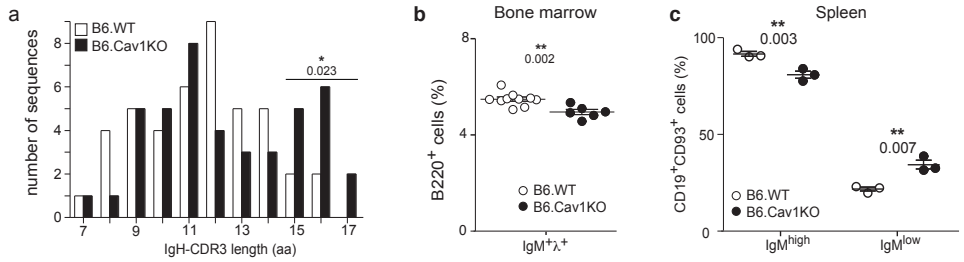
(**a**) The survival curves of WT and Cav1KO littermates are shown. The Log-rank (Mantel-Cox) Test was applied and the P value indicated. (**b**) The total number of splenocytes in WT and Cav1KO littermates was calculated in adult mice (20 weeks) and in older mice (40 weeks). (**c**) A representative picture of the spleens used for the quantification shown in b. (**d**) Relative proportion of B cells in the spleen plotted according to age. (**e**) The proportion of germinal center (GC) B cells in the spleens of untreated adult mice (<20 weeks) and very old mice (80 weeks) is plotted. (**f**) The proportion of splenic class-switched $IgG1^+$ B cells is plotted; mice were grouped according to genotype and age (adult mice, <20 weeks, and very old mice 80 weeks). (**g**) Representative immunofluorescence pictures from the spleen of untreated mice, showing spontaneous proliferation of B cells. (**h**) The presence of anti-cardiolipin or anti-dsDNA IgG-autoantibodies in the serum of old mice (<50 weeks) was assayed by ELISA. The serum from autoimmune MLR/lpr mice was used as positive control and the serum from AIDKO mice was used as negative control. (**i**) Representative photomicrograph of the kidneys of old mice (<50 weeks) positively stained for PAS (n=7). (**j**) Representative immunofluorescence images of the kidneys of old mice (<50 weeks) positively stained for IgG-FITC (n=5). Each square represents an individual animal. Statistical analysis was performed using Student's t test. When significant, P values are indicated in each panel.



Supplementary Figure 5

Increased B cell numbers, spontaneous GC, auto-antibodies and reduced life span in mice reconstituted with Cav1KO BM.

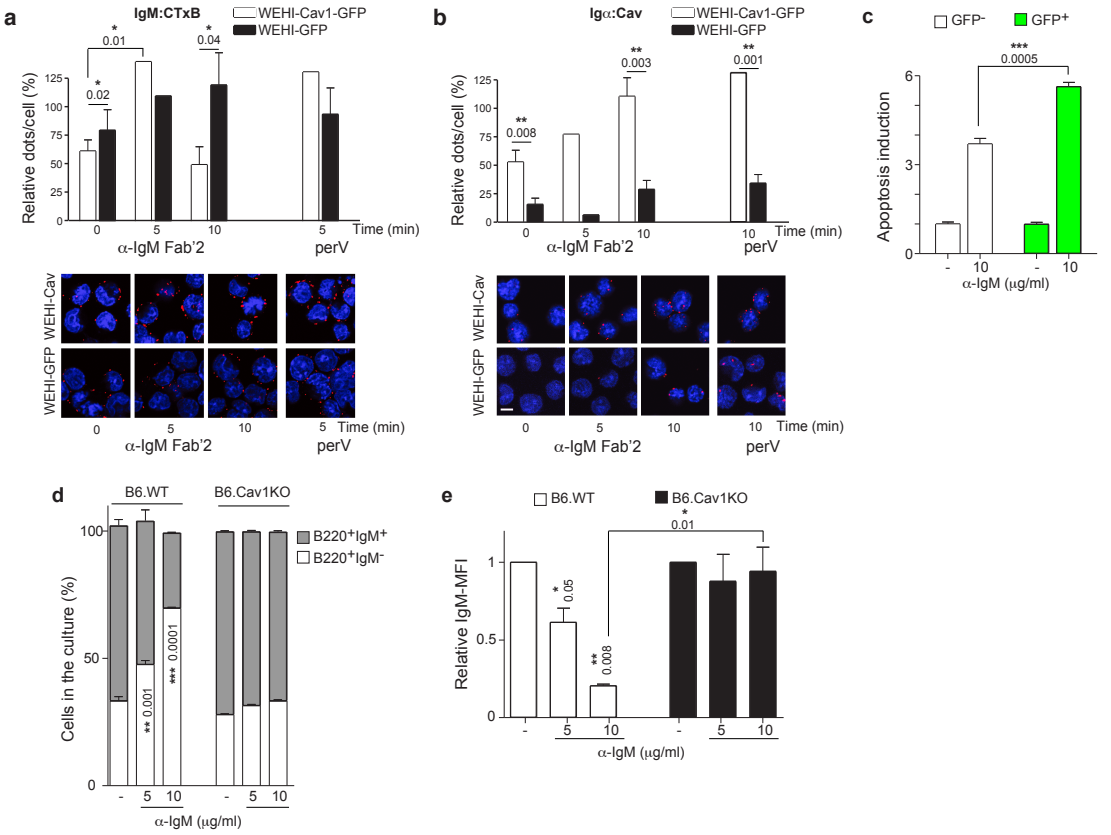
(**a**) Scheme of the repopulation assay. Bone marrow cells obtained from WT or Cav1KO littermates were injected into sub-lethally irradiated recipient $Rag2^{-/-}\gamma c^{-/-}$ mice. Mice were analyzed 25 weeks after injection. (**b**) The survival curves of the reconstituted mice are shown. The Log-rank (Mantel-Cox) Test was applied and the P value are indicated. (**c**) Total number of splenocytes in the reconstituted mice (n=3-7). (**d**) The quantification of the proportion of germinal center (GC) B cells in the spleens of the reconstituted mice and representative dot plots are shown. (**e**) The presence of anti-cardiolipin or anti-dsDNA IgG autoantibodies in the serum of recipient mice 25 weeks after transplantation was assayed by ELISA (n=3-7). As a negative control, serum from uninjected aged matched $Rag2^{-/-}\gamma c^{-/-}$ mice (Rag) was used. (**f**) The percent of $CD45.1^+$ and $CD45.2^+$ cells on gated T cells in the WT(CD45.1):B6.CavKO(CD45.2) chimeras (n=4). Naïve ($CD44^-CD62L^+$), activated ($CD44^+CD62L^+$) and effector memory ($CD44^+CD62L^-$) populations in the spleen are shown. (**g**) Percent of $CD45.1^+$ and $CD45.2^+$ cells on gated $CD4^+$ (left column) or $CD8^+$ (right column) T cells in the lymph nodes (LN) of the WT(CD45.1):B6.CavKO(CD45.2) chimeras (n=4). (**h**) The percent of $CD45.1^+$ and $CD45.2^+$ cells on gated T cells in the LN of the WT(CD45.1):B6.CavKO(CD45.2) chimeras was analyzed as above. (**i**) The percent of $CD45.1^+$ and $CD45.2^+$ thymocytes of the WT(CD45.1):B6.CavKO(CD45.2) chimeras are shown. Each square represents an individual animal. Statistical analysis was performed using Student's t test. When significant, P values are indicated in each panel.



Supplementary Figure 6

Cav1-deficient B cells express a skewed IgH repertoire.

(a) Distribution of the CDR3 length in *Igh* transcripts of B cells from B6.WT and B6.Cav1KO mice. Fischer's exact test for long CDR3s (≥15 aa) P=0.0283 (*). (b) The proportion of IgM^+l^+ cells in the BM of the indicated mice was assayed by flow cytometry, n=6-10 (c) Analysis of $CD93^+$ B cells in the spleen. Transitional B cells are identified as $CD19^+CD93^+IgM^{high}$ and anergic B cells as $CD19^+CD93^+IgM^{low}$, n=3. Each dot represents an individual animal. Statistical analysis was performed using Student's t test. Mean ± SEM are shown.



Supplementary Figure 7

Increased apoptosis and activation-regulated BCR nanoclustering in WEHI cells expressing Cav1.

(**a**) WEHI immature B cells were lentivirally transfected to express Cav1-IRES-GFP or IRES-GFP. GFP^+ cells were sorted and GFP^+ stable lines generated. Cells were stimulated with 10 μg/ml of anti-IgM Fab'2 or with 0.5 mM perV for the indicated times or left unstimulated and the IgM:CTxB PLA performed as previously detailed. The resulting signals were normalized to the PLA signals of WEHI-Cav1-GFP cells stimulated for 5 min with perV for each experiment. Quantified data of three independent experiments were pooled and shown as Mean ± SEM. (b) Cells were stimulated as in (a) and the BCR(Iga):Cav1 PLA was performed. Quantified data of four independent experiments were pooled and shown as Mean ± SEM. At least 500 individual cells were quantified per experimental condition and experiment. (**c**) WEHI immature B cells were lentivirally transfected to express Cav1-IRES-GFP. 24.1% of the cells were GFP^+. Cells were cultured for 48 h in the absence (-) or presence of anti-IgM Fab'2 fragments. Cell viability was measured by 7-ADD staining and analyzed by flow cytometry. Data of triplicates are shown as Mean ± SEM. (**d**) BM cells were grown *ex vivo* for 4 days. IL-7 was initially added to the culture. Every 2 days, half of the medium was replaced with medium without IL-7 to ensure the presence of immature B cells. The relative proportion of pre-B cells ($B220^+IgM^-$) and immature B cells ($B220^+IgM^+$) in the presence of anti-IgM Fab'2 fragments was assayed by flow cytometry. (**e**) The relative MFI of surface IgM from the samples shown in d is plotted as Mean ± SEM. Statistical analysis was performed using Student's t test. When significant, P values are indicated.