SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Effects of different inhibitors of BCR signaling pathways on expression of Pax5, Bcl-6, MITF, Ets-1, Fli-1, Blimp1, Spi-B and IRF-4 mRNA in B lymphocytes from mouse spleen activated by LPS and IL-4. Anti-IgM was added to one half of BAPTA-AM (12.5 μM), TMB-8 (50 μM), Nifedipine (50 μM), TMB-8 plus Nifedipine (Nif.), Bisindolylmaleimide I (Bis.; 0.1 μM), PD98059 (100 μM), KN93 (20 μM) or cyclosporine A (CsA)-treated (0.2 μM) and untreated (-) B lymphocytes for 3 h, followed by quantitative RT-PCR using the mRNA levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for normalization. Values represent the normalized mRNA levels with anti-IgM expressed as a percentage of the levels without anti-IgM. Results are mean ± s.d. (n = 3).

Figure S2. Representative FACS analyses of changes in expression of key proteins in plasma cell differentiation by calmodulin. *A*, effects of calmodulin over-expression on the Pax5 protein level in B lymphocytes from mouse spleen activated with LPS and IL-4 for 60 h followed by BCR stimulation with anti-IgM where indicated for 5 h. Cells were infected with the empty MSCV retrovirus vector or virus encoding calmodulin and green fluorescent protein (GFP). The levels of the proteins were determined by intracellular immunostaining and flow cytometry. Cells with a large increase in GFP fluorescence were considered as infected and used to calculate protein expression levels. *B*, expression level of Bcl-6 in corresponding calmodulin over-expression experiments as in *A* with anti-IgM treatments where indicated for 5 h of B lymphocytes activated with CD40L plus IL-4.

Figure S3. Changes in expression of Id1, Id2, Id3, Id4 and E2A mRNA in B lymphocytes from mouse spleen activated with LPS and IL-4 for 48 h followed by stimulation of the BCR by addition of anti-IgM for the indicated times. The mRNA levels were determined by quantitative RT-PCR and normalized as described in Figure S1. The levels before addition of anti-IgM were set at 100%, and results are mean ± s.d. (n = 3).

Figure S4. Representative FACS analyses of loss of anti-IgM sensitivity of Pax5 and Bcl-6 expression by expression of calmodulin-resistant E12. B lymphocytes from mouse spleen were infected by retrovirus encoding wild-type E12 or calmodulin-resistant m847 or m8N47 mutant. In *A*, the B lymphocytes were activated with LPS plus IL-4 and in *B* with CD40L plus IL-4 as in Fig. S2*A*-*B*. The infected cells were where indicated treated with anti-IgM for 5 h.

SUPPLEMENTAL TABLE

Table S1. Rapid differential gene expression upon BCR activation. A detailed list of the changes in expression of different genes after 3 h of anti-IgM stimulation of the BCR of B lymphocytes from mouse spleen activated with CD40L (200 ng/ml) plus IL-4 (5 ng/ml). Total RNA was isolated from B cells of three independent mice with and without the stimulation and amplified and hybridized on Illumina BeadChips. The fold differential expression of the 4,604 genes with ≥ 1.5 fold change in expression and P-value < 0.05 among the in total 31,492 genes analyzed is listed. The functional classification of genes was done using gene ontology information provided by Illumina.