Supplementary Material

Supplementary Table 1: Antibodies used for IHC and Western Blot analyses.

Immunohistochemistry						
Ab	Clone # or Serum	Species	Source	Antigen Retrieval	Dilution and incubation	Fixation
PNA	#B-1075	peanut	Vector	Citrate steam[1]	5 ug/ml, 1 hr at R.T.	Formalin[3]
Cyclin D3	#sc-182	rabbit	Santa Cruz	1 mM EDTA, pH 7.5, 15 min in steam[2]	1:3000, overnight at R.T.	Formalin or Frozen PFA[4] overnight
CD21/ CD35	#553817	rat	BD Pharmingen	Citrate steam	1:5000, 2 hrs at R.T.	Frozen, 2% PFA 10 min
B220	Clone RA3-6B2	rat	BD Pharmingen	Citrate steam	1:20, 2 hrs at R.T.	Formalin
CD79a	Clone JCB117	mouse	Dako	1 mM EDTA, pH 7.5, 10 min in steam	1:100, 2 hrs at R.T.	Formalin
CD3	Serum	rabbit	Dako	As per insert	Undiluted, 2 hrs at R.T.	Formalin
Bcl6	#sc-858	rabbit	Santa Cruz	1 mM EDTA, pH 7.5, 15 min in steam	0.1 ug/ml, overnight at R.T.	Formalin
Ki67	#NCL-ki67P	rabbit	Novocastra	1 mM EDTA, pH 7.5, 15 min in steam	1 to 500, 2 hrs at R.T.	Formalin
Western Blot						
Ab	Clone/Catalog #		Species	Source	Dilution	
Cyclin D2	sc-593 (M-20)		rabbit	Santa Cruz	1:1000	
Cyclin D3	sc-182 (C16)		rabbit	Santa Cruz	1:1000	
Cyclin E	Sc-481 (M-20)		rabbit	Santa Cruz	1:1000	
Cdk4	Ab-6 (DCS-31 + DCS-35)		mouse	NeoMarker	1:500	
CDK6 (K6.83)	AHZ0232		mouse	Biosource	1:250	
Rb	sc-50 (C-15)		rabbit	Santa Cruz	1:200	
pRb (S807/811)	#9917		Rabbit	Cell Signaling	1:500	
beta-actin	ab8229		goat	abcam	1:500	

[1]Citrate steam indicates Vector Laboratories Antigen Unmasking Solution (cat. #H3300) in a vegetable steamer for 20 minutes, followed by 30-60 minutes of cooling at room temperature.
[2]EDTA indicates 1 mM EDTA pH 7.5 boiled in a microwave for ~ 6 minutes, followed by 15 minutes of simmering on low power.
[3] Formalin indicates 10% Neutral Buffered Formalin overnight.
[4]PFA indicates freshly prepared 2% paraformaldehyde.

Table S2. Sequences of qRT-PCR primers.

Gene		Sequence
HPRT	sense	5′ GCTGGTGAAAAGGACCTCT3′
	antisense	5′ CACAGGACTAGAACACCTGC3′
Bcl6	sense	5′ TATTGTTCTCCACGACCTCACG3
	antisense	5′ ACTGTGAAGCAAGGCACTGG3′
Ccnd2	sense	5′ AGACCTTCATCGCTCTGTGC3′
	antisense	5′ TAGCAGATGACGAACACGCC3′
Trp53	sense	5' GTATCCCGAGCATCTGGAAGA3'
	antisense	5' CCCCCATGCAGGAGCTATT3'
Cdkn1b	sense	5' TCAAACGTGAGAGTGTCTAACG3'
	antisense	5' CCGGGCCGAAGAGATTTCTG3'
Myc	sense	5' GGGCCAGCCCTGAGCCCCTAGTGC3'
	antisense	5' ATGGAGATGAGCCCGACTCCGACC3'

Supplementary Figure Legends

Figure S1. Histological validation of Cyclin D3 antibody and characterization of B cell splenic architecture in KO animals. *A*. Double stain for B220 (brown) and Cyclin D3 (blue) of immunized mouse spleen sections demonstrates that the polyclonal cyclin D3 antibody is specific for this antigen. Intensely staining Cyclin D3$^+$ subcapsular cells are evident in WT spleen (arrowheads) but are absent from the subcapsular and interfollicular regions of *Ccnd3$^{-/-}$* spleen. *B*. Hematoxylin and eosin (H&E) stain of spleens from SRBC-immunized mice indicate that splenic architecture is grossly normal in the absence of cyclin D3, with white and red pulps appropriately organized. *C*. B220 staining (brown) from a separate experiment indicates that the architecture of B cell follicles is preserved in the absence of

cyclin D3. Occasional cyclin $D3^+$ cells outside the follicles (arrowhead) are visible in the WT but not in the KO section. Scale bars are 100 μm in *A* and are 200 μm in *B* and *C*.

Figure S2. Schematic illustration of the immunization and bleeding schedule used to elicit T-cell dependent anti-NP response in Figure 4. Ten-twelve week old littermate were immunized with NP-KLH following a previously described protocol, as were ELISA for resting serum Ab and NP-specific Ab response (1).
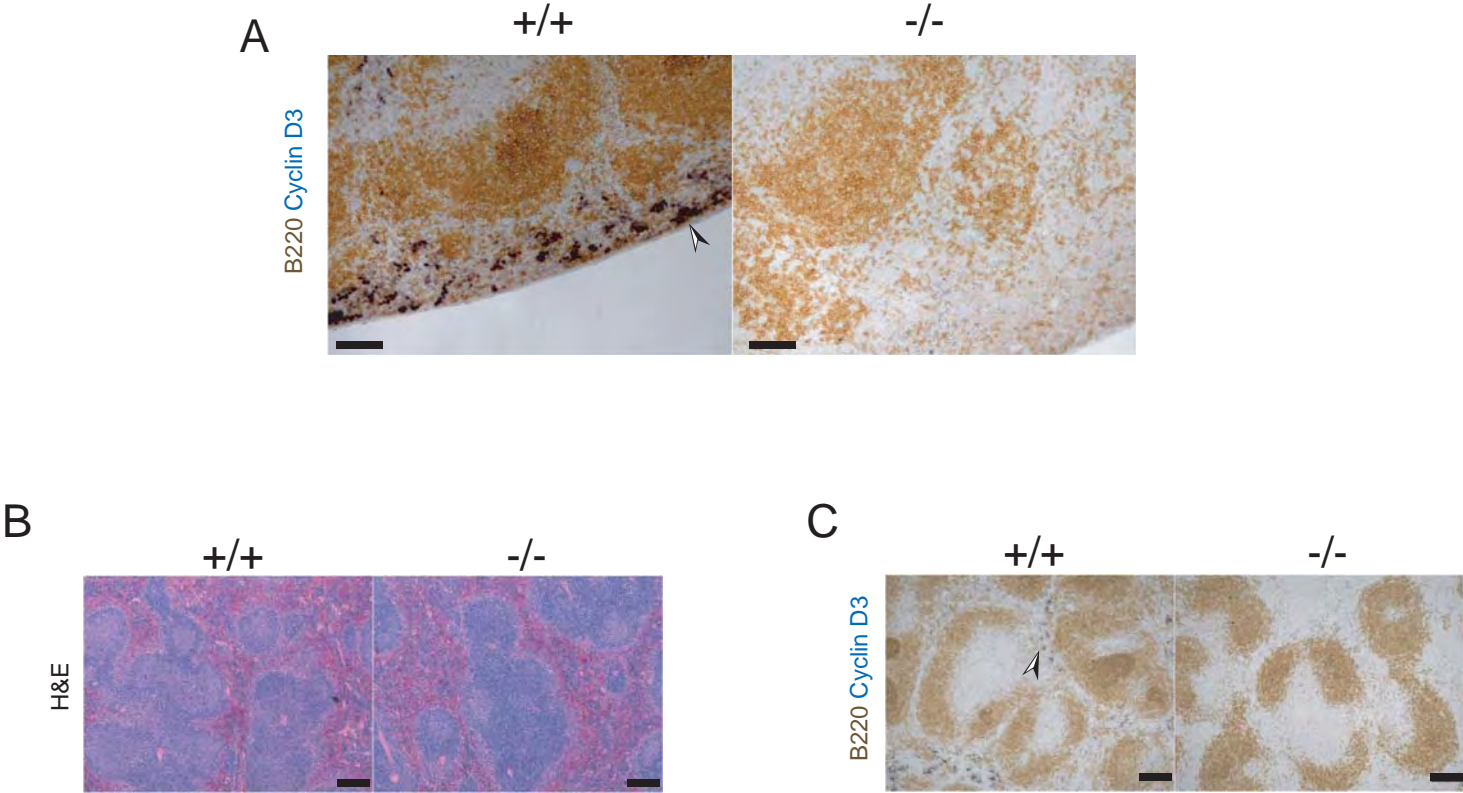
Figure S3. Class Switch Recombination to IgG_1 is moderately impaired in the absence of cyclin D3. Splenic B cells from WT and cyclin D3 KO mice were isolated and stimulated as described (2). After 4 d of culture, CSR was analyzed by surface staining for IgM, IgG_1 and IgG_3, followed by flow cytometry (3). *A*. Representative CSR results showing LPS+IL-4 induced switching from IgM to IgG_1. *B*. Cyclin D3 KO B cells were moderately impaired in their IgG_1 switching efficiency but switched normally to IgG_3 in response to LPS stimulation. Statistical analysis using the linear mixed effects model (described in the MATERIALS AND METHODS of the main text) was performed on the flow cytometry data (the percent of IgG^+ cells in each well). Graph depicts quantification of all CSR experiments (n = 8; IgG_1, $p = 0.02$, IgG_3, $p = 0.46$). Error bars are SEM.
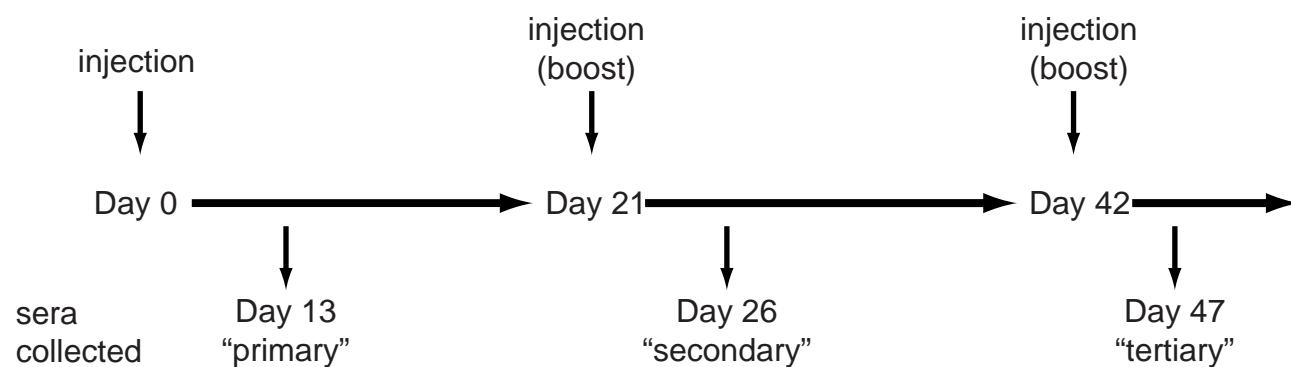
References Cited.

1. Ye, B. H., G. Cattoretti, Q. Shen, J. Zhang, N. Hawe, R. de Waard, C. Leung, M. Nouri-Shirazi, A. Orazi, R. S. Chaganti, P. Rothman, A. M. Stall, P. P. Pandolfi, and R. Dalla-Favera. 1997. The BCL-6 proto-oncogene controls germinal-centre formation and Th2- type inflammation. *Nat Genet* 16:161-170.
2. Bardwell, P. D., C. J. Woo, K. Wei, Z. Li, A. Martin, S. Z. Sack, T. Parris, W. Edelmann, and M. D. Scharff. 2004. Altered somatic hypermutation and reduced class-switch recombination in exonuclease 1-mutant mice. *Nat Immunol* 5:224-229.

3.   Li, Z., C. Zhao, M. D. Iglesias-Ussel, Z. Polonskaya, M. Zhuang, G. Yang, Z. Luo, W. Edelmann, and M. D. Scharff. 2006. The mismatch repair protein Msh6 influences the in vivo AID targeting to the Ig locus. *Immunity* 24:393-403.

Supplementary Figure S1

A

B220 Cyclin D3

+/+   -/-

B

H&E

+/+   -/-

C

B220 Cyclin D3

+/+   -/-

Peled et al.

Supplementary Figure S2

Peled et al.

Supplementary Figure S3

Peled et al.