



Supplemental Figure 1: (A) Effect of B-TRAF2 or TRAF3 KO on marginal zone and follicular B cell development as well as Ab response against TI Ag. (A) Ab response against TI Ag. B-TRAF2 or TRAF3 KO and corresponding LMC mice were immunized with TNP-FICOLL. 14 days after immunization, sera were collected and TNP-specific IgG1, IgG2a and IgE were measured by ELISA as described in Material and Method. Data were representative of 3 independent experiments. P values were calculated by t-test, * $p \leq 0.05$, ** $p \leq 0.01$. LMC: littermate control. KO: knockout. TNP-FICOLL: 2,4,6-trinitrophenol-conjugated FICOLL. **(B) Splenic marginal zone (MZ) and follicular (FO) B cell analysis.** Splenocytes from CD19Cre(+)/TRAF2^{fl/fl} or LMC (TRAF2^{fl/fl}), CD19Cre(+)/TRAF3^{fl/fl} or LMC (TRAF3^{fl/fl}) mice, respectively, were analyzed by flow cytometry. Representative FACS plots of MZ and FO B cells (gated on B220⁺IgM⁺) in B-TRAF2-KO or B-TRAF3-KO mice and corresponding LMC are shown from 3 independent experiments. **(C) The splenic MZ and FO B cell numbers.** Splenic B cells were counted in LMC (n=10) and B-TRAF2-KO (n=10) or LMC (n=16) and B-TRAF3-KO (n=16) mice. Statistical analysis: Student's t-Test. **(D) Evaluation of active NF-κB2 in resting TRAF2 or TRAF3 KO B cells.** Naïve B cells were isolated from spleens of B-TRAF2 or B-TRAF3 KO mice and corresponding LMC mice. Western blot was performed to evaluate NF-κB2 precursor (p100) and active (p52) forms. AKT served as protein loading control. Representative data of three independent experiments are shown.

Supplemental Table I: Abs used in the study

FLOW CYTOMETRY

| Antibody | Clone | Catalogue | Company |
|---|---------|------------|----------------|
| APC-conj. anti-IgG1 | A85-1 | 560089 | BD Biosciences |
| BUV395-conj. anti-IgM | R6-60.2 | 564025 | BD Biosciences |
| BV605-conj. anti-CD19 | 6D5 | 115539 | Biologend |
| BV785-conj. anti-mouse/human B220/CD45R | RA3-6B2 | 103245 | Biologend |
| PE-conj. Anti-mouse IgE | RME-1 | 12-5992-81 | Biologend |
| A647-conj. anti-CD23 | B3B4 | 101612 | Biologend |
| PE-conj. Rat-anti-mouse CD21/CD35 | 7G6 | 552957 | BD Biosciences |

WESTERN / ELISA

| Antibody | Clone | Catalogue | Company |
|----------------------------------|-------------|-------------|------------------------|
| Rabbit anti-panAKT | C67E7 | 4691S | Cell Signaling |
| anti-AID | 30F12 | 4949S | Cell Signaling |
| rabbit-anti-TRAF2 | poly | 4712S | Cell Signaling |
| anti-RelA (p65) | D14E12 | 8242S | Cell Signaling |
| anti-phospho-p65 | S536 (93H1) | 3033S | Cell Signaling |
| anti-I κ B α | L35A5 | 4814P | Cell Signaling |
| anti-NF- κ B2 (p100/p52) | poly | 4882S | Cell Signaling |
| anti- β -actin | C4 | sc-47778 | SantaCruz |
| anti-GAPDH | FL-335 | sc-25778 | SantaCruz |
| anti-TRAF3 | H122 | sc-1828 | SantaCruz |
| anti-I κ B α | C-21 | sc-371 | SantaCruz |
| HRP-conj. Goat-anti-mouse IgG1 | poly | 115-035-205 | Jackson Immunoresearch |
| HRP-conj. Goat-anti-mouse IgG | poly | 115-035-166 | Jackson Immunoresearch |
| HRP-conj. Donkey-anti-rabbit IgG | poly | 711-035-152 | Jackson Immunoresearch |
| HRP-conj. Goat-anti-mouse IgE | poly | 1110-05 | Southern Biotech |
| HRP-conj. Goat-anti-mouse IgG2a | poly | ab97245 | abcam |