Supplemental data

Distinct roles for mTOR in generating versus sustaining humoral immunity

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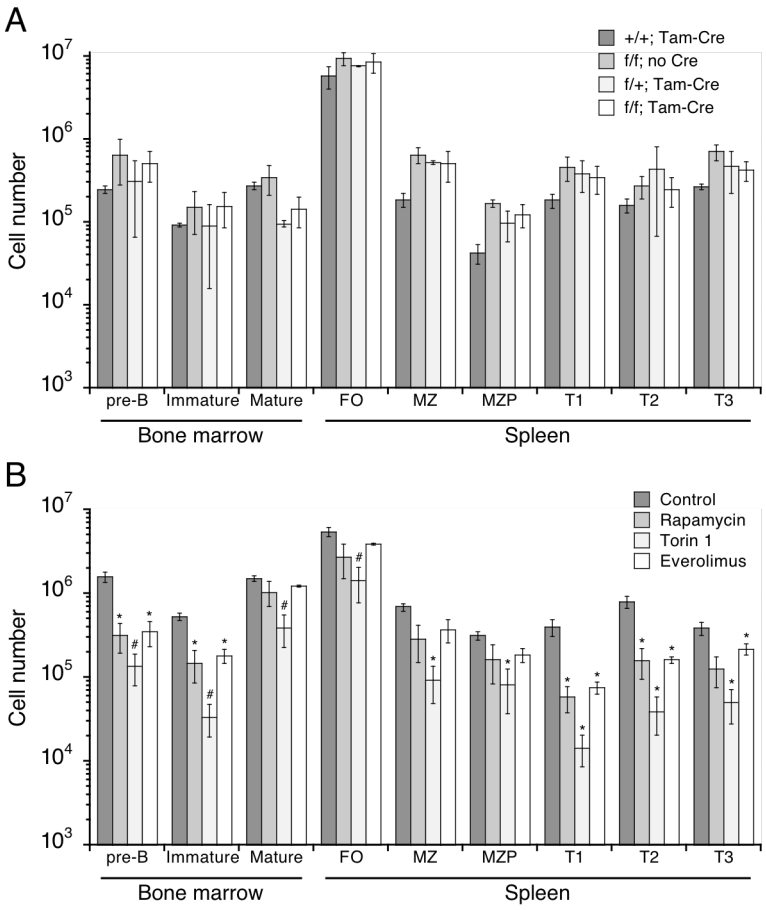
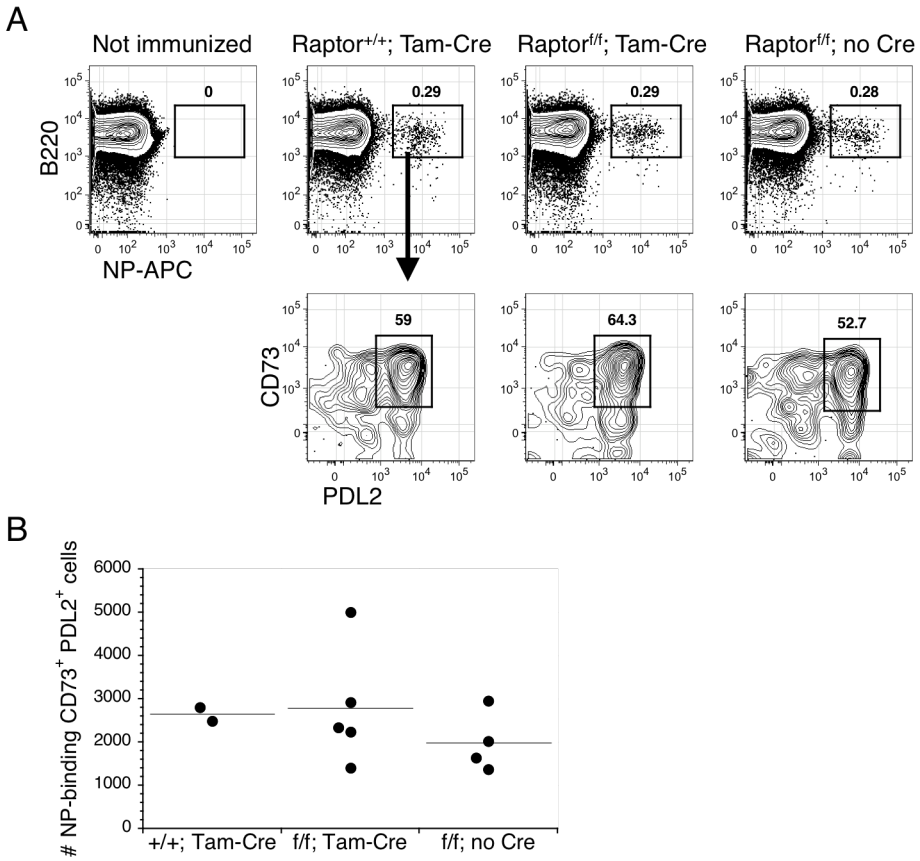
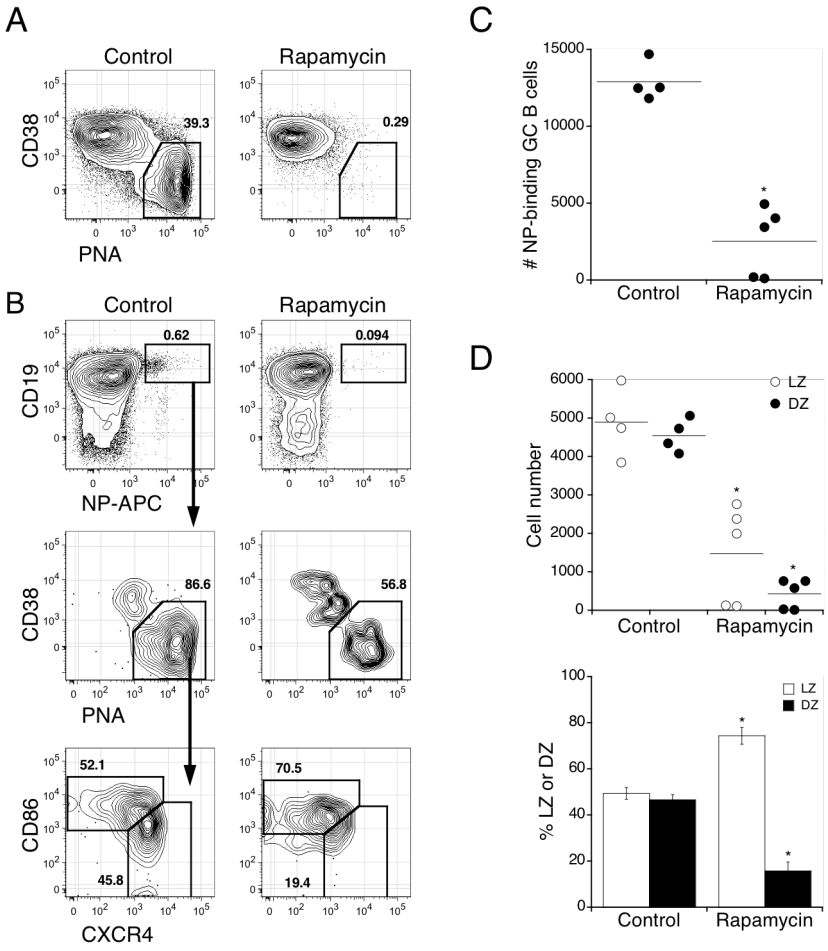


Supplemental Figure 1. Naïve B cell subsets are unaffected by mTOR inhibition. (**A**) Absolute numbers of B cells in the BM or spleen were evaluated for mice of the indicated genotype, following 3 administrations of tamoxifen to induce Raptor deletion. B cell subsets were profiled by analysis of $2.5x10^6$ events by flow cytometry. f/f; Tam-cre (n=4), f/+; Tam-cre (n=3), +/+; Tam-cre (n=3), f/f; no cre (n=4). No significant differences were identified between any genotypes. Pre-B ($B220^+$ $CD43^-$ $AA4^+$ IgM^-); immature ($B220^+$ $CD43^-$ $AA4^+$ IgM^+); mature ($B220^+$ $CD43^-$ $AA4^-$ IgM^+); FO (follicular, $AA4^-$ $CD21^{int}$ $CD23^+$ IgM^{low}); MZ, (marginal zone, $AA4^-$ $CD21^{high}$ $CD23^-$ IgM^{high}); MZP, (marginal zone precursor, $AA4^-$ $CD21^{high}$ $CD23^+$ IgM^{high}); T1, transitional ($AA4^+$ IgM^+ $CD23^-$); T2 ($AA4^+$ IgM^+ $CD23^+$), T3 ($AA4^+$ IgM^{int} $CD23^-$). (**B**) C57BL/6 mice were treated 3 times with rapamycin (20mg/kg), Torin 1 (10mg/kg), or everolimus (10mg/kg), followed by quantitation of BM and spleen B cell subsets as in (A). Four mice were used in each group. * $p<0.05$ as determined using a Kruskal-Wallis test with Dunn's multiple comparison test. Significant differences were only found between control and Torin1-treated mice; no other pairwise comparisons reached significance.



Supplemental Figure 2. TORC1 signaling is dispensable for memory B cell maintenance. (**A**) Mice of the indicated genotype were immunized, and 45 days later splenocytes were stained with the indicated antibodies and NP-allophycocyanin to detect NP-binding cells. Plots were pre-gated for viable IgD^- $Dump^-$ $CD19^+$ $CD38^+$ lymphocytes. (**B**) Absolute numbers per spleen of NP-binding $CD73^+$ $PDL2^+$ cells. Dots represent values for individual mice, and horizontal lines indicate the means. No significant differences were detected between any genotypes. Data are representative of 2 independent experiments.



Supplemental Figure 3. GC centroblasts exhibit enhanced sensitivity to rapamycin. (**A**) Splenocytes from 33-week old NZB/W mice either untreated (control) or treated with twice weekly with rapamycin for 6 weeks were stained with the indicated reagents. Representative plots from 3 independent experiments using 4-6 mice per group. (**B**) Splenocytes from C57BL/6 mice immunized with NP-CγG 17 days previously were stained with the indicated antibodies and NP-allophycocyanin. Rapamycin-treated mice were administered inhibitor on days 12, 14, and 16 post-immunization. The phenotype of NP-binding GC B cells was further evaluated for light zone centrocytes ($CD86^+$ $CXCR4^-$) or dark zone centroblasts ($CD86^-$ $CXCR4^+$). (**C**) Absolute numbers of NP-binding $CD38^+$ PNA^+ B cells were determined for the mice depicted in (B, *middle plots*). Dots represent values for individual mice, and horizontal lines indicate the means. * $p<0.0001$ as determined by Student's t-test comparing control and treated mice. (**D**) *Top*, absolute numbers of light zone (LZ) centrocytes and dark zone (DZ) centroblasts were evaluated in control and rapamycin-treated mice. *Bottom*, average frequency of cells within the LZ or DZ in control (n=4) and rapamycin-treated (n=5) mice; bars indicate the SEM. * $p<0.001$ as determined by Student's t-test comparing control and treated mice. Representative of 2 independent experiments.