Cell Reports, Volume *31*

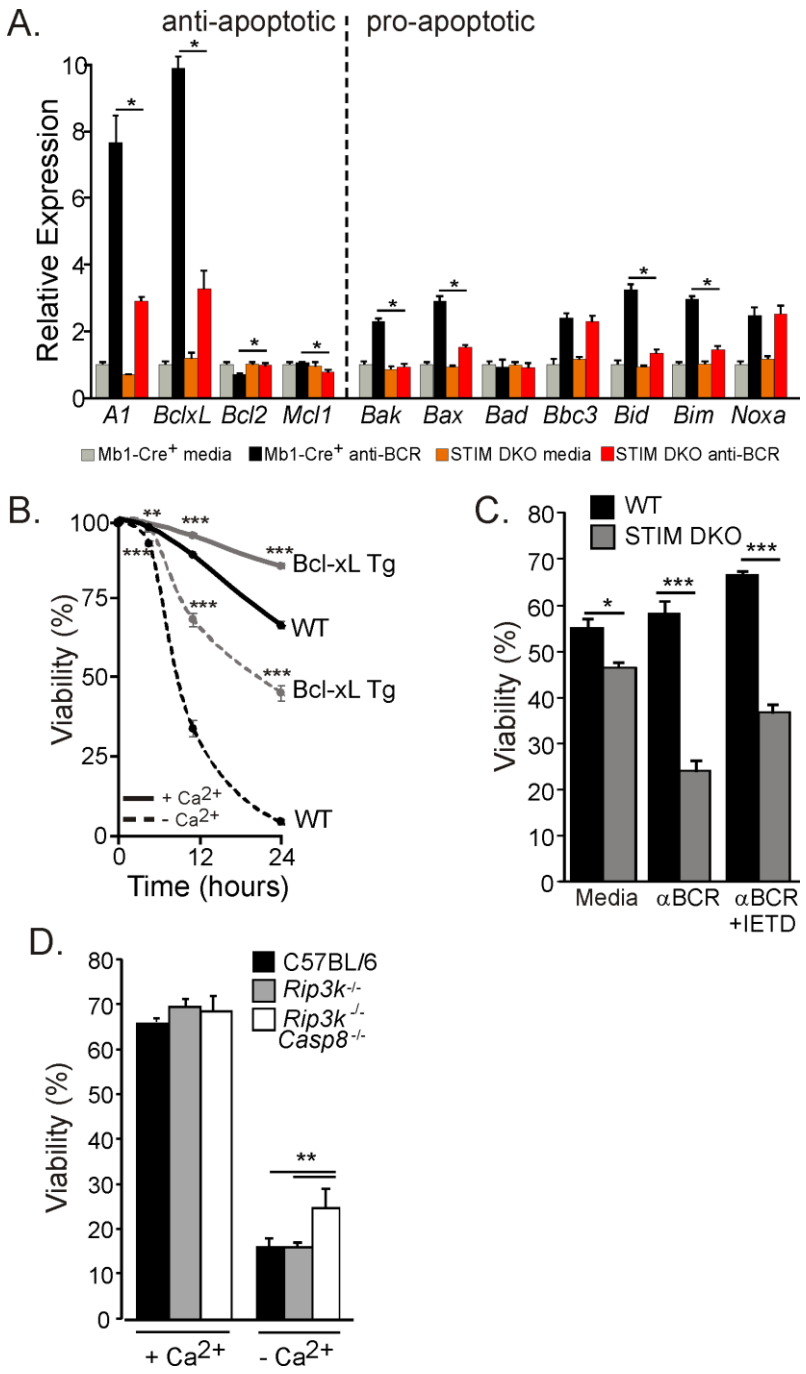
Supplemental Information

BCR-Induced Ca^{2+} Signals Dynamically Tune Survival, Metabolic Reprogramming, and Proliferation of Naive B Cells

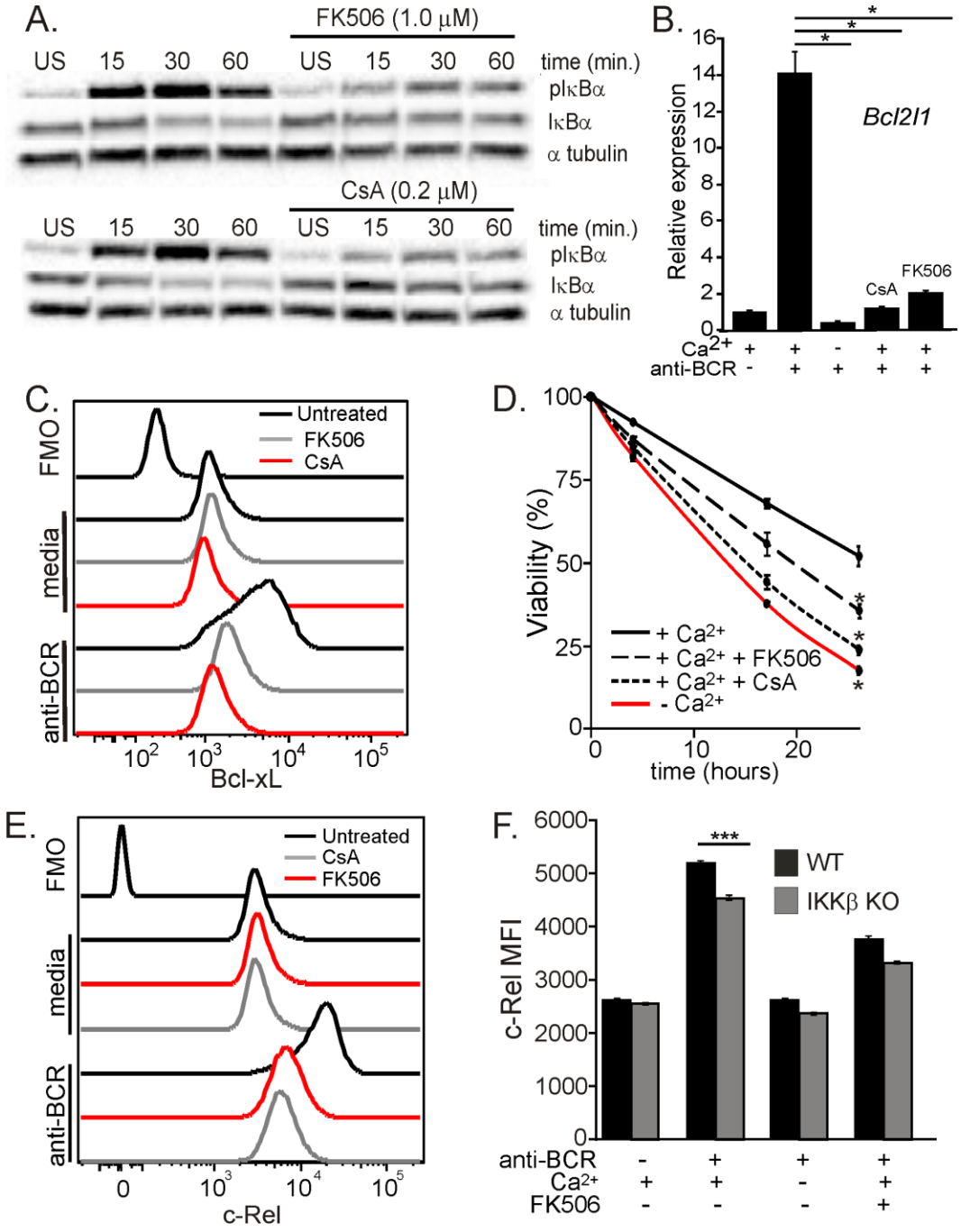
Corbett T. Berry, Xiaohong Liu, Arpita Myles, Satabdi Nandi, Youhai H. Chen, Uri Hershberg, Igor E. Brodsky, Michael P. Cancro, Christopher J. Lengner, Michael J. May, and Bruce D. Freedman

Supplemental Table 1: Oligonucleotides

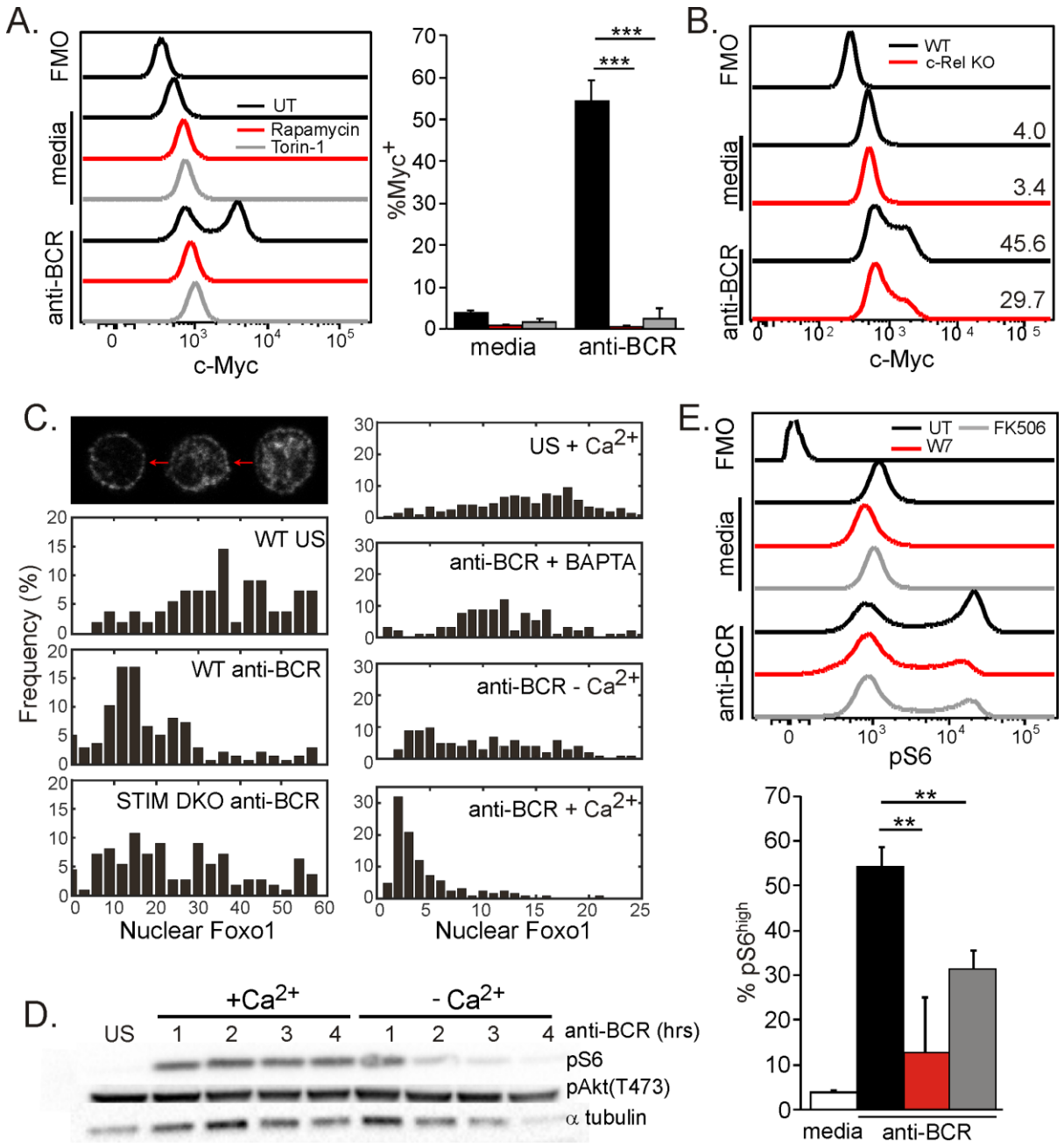
Gene Name	Forward	Reverse
B2m	CGGCCTGTATGCTATCCAGA	GGGTGAATTCAGTGTGAGCC
Bad	GAGGAGGAGCTTAGCCCTTT	AGGAACCCTCAAACTCATCG
Bak1	CTCTCATCGGAGATGATATTAACCG	AGTATGATATCAGCCAAAAAGCAGG
Bax	GGAGATGAACTGGACAGCAATATGG	GTTTGCTAGCAAAGTAGAAGAGGGC
Bbc3	GTGTGGAGGAGGAGGAGTG	TCGATGCTGCTCTTCTTGTC
Bcl2	CAACACAAACCCCAAGTCCT	GTTGAACTCGTCTCCGATCC
Bcl2l1	GGCCTTTTTCTCCTTTGGCG	GATCCACAAAAGTGTCCCAGC
Bcl2l11	TGAGTACCTGAACCGGCATCT	GCATCCCAGCCTCCGTTAT
Bcl2la1	TTGCCTTTGGGGGTGTTCTC	CCAACCTCCATTCTGCCGTA
Bid	AGACGAGCTGCAGACAGATG	GGTCCATCTCATCGCCTATT
Ccne1	GTTCCAAGCCCAAGTCCTGA	TTGCAAAAACACGGCCACAT
E2f3a	CTACGAACCCTTCCACCACG	TTCGCTTTGCCGGTGGG
Irf4	CTTCAAGGCTTGGGCATTGTT	TGGCCATCTGTGTGTCATCC
Mcl1	TGTAAGGACGAAACGGGACT	AAAGCCAGCAGCACATTTCT
Myc	AGCTGTTTGAAGGCTGGATT	AATAGGGCTGTACGGAGTCG
Nfkb2	CAGAAACTTCAGAGGCAGCGTC	GCAAATAAACTTCGTCTCCACCG
Nfkbia	CAGCTGACCCTGGAAAATCT	ATAGGGCAGCTCATCCTCTGT
Noxa	GAGTGCACCGGACATAACTG	CTCGTCCTTCAAGTCTGCTG
Rel	TGACAACCGTGCCCCAAATA	TTGGCGGTGTACATCAGCTT
Ubc	GCCCAGTGTTACCACCAAGA	CCCATCACACCCAAGAACA
CpG (ODN1826)	TCCATGACGTTCCTGACGTT	



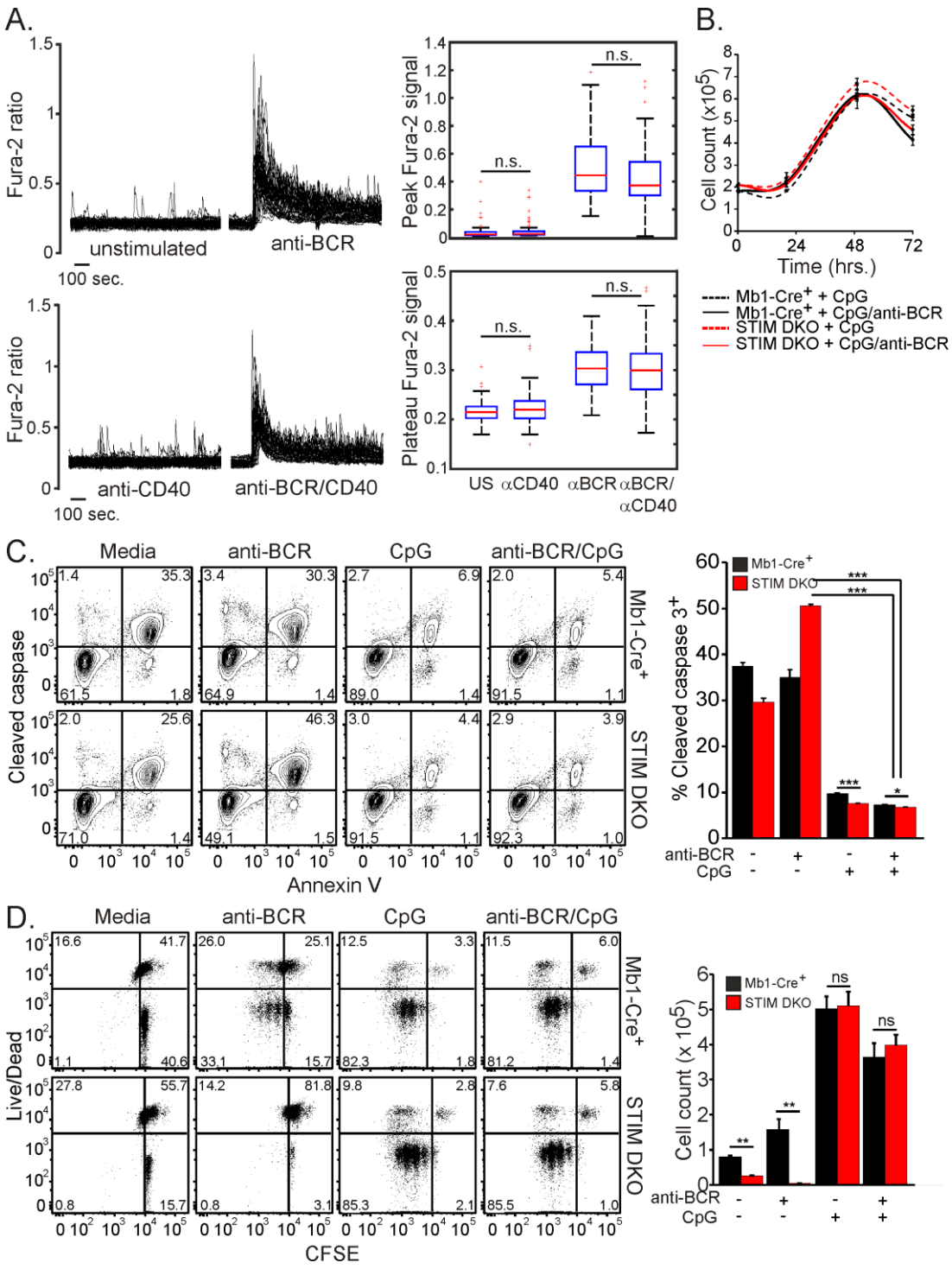
Supplemental Figure 1: BCR-induced Ca^{2+} signals regulate intrinsic but not extrinsic apoptosis. (A) CD23+ B cells from Mb1-cre and STIM DKO mice cultured in complete media in the absence or presence of anti-BCR for 6 hours before RNA isolation. mRNA expression of selected Bcl-2 family genes (mean ± 95% confidence interval based on triplicate measurements, *statistically significant). (B) CD23+ B cells from WT and Bcl-xL Transgenic (Tg) mice were stimulated with anti-BCR in control and Ca^{2+} free (+ 0.5 mM EGTA) media for 24 hours. Percentage of live/dead dye-excluding cells (mean ± SD) from triplicate wells at indicated timepoints after anti-BCR stimulation. (C) Cell viability (mean ± SD of triplicate wells) after 20 hours of stimulation in the absence and presence of caspase 8 selective inhibitor, z-IETD-fmk (100μM). (D) CD23+ B cells from C57Bl/6, *Rip3k*$^{-/-}$, and *Rip3k*$^{-/-}$*Casp8*$^{-/-}$ mice were stimulated for 16 hours in normal or EGTA (0.5 mM) buffered media and viability calculated as the fraction of live/dead dye-excluding cells (mean ± SD from triplicate wells, n = 3-5 mice per group).



Supplemental Figure 2: Calcineurin mediates Ca^{2+} dependent c-Rel activation in mature B cells. (A) Immunoblot of NF-κB activation in splenic CD23+ B cells from anti-BCR stimulated WT mice for indicated times in the absence or presence of Calcineurin (Cn) inhibitors FK506 (1 μM) or Cyclosporine A (CsA, 0.2 μM). (B) *Bcl2l1* mRNA was measured in WT B cells after 3 hours of anti-BCR stimulation in the presence and absence of extracellular Ca^{2+} and Cn inhibitors (mean ± 95% confidence interval, *statistically significant). (C) Bcl-xL expression at 20 hours after anti-BCR stimulation in the presence or absence of Cn inhibitors. (D) B cell viability 24 hours after anti-BCR stimulation in the presence and absence of Cn inhibitors (mean percentage ± SD) (E) c-Rel expression 20 hours after anti-BCR stimulation in the presence or absence of Cn inhibitors. (F) c-Rel expression (mean MFI ± SD from triplicate wells) in CD23+ B cells from *Ikk2*$^{fl/fl}$ x *Mb1cre*- (WT, black line) and *Ikk2*$^{fl/fl}$ x *Mb1cre*+ (IKKβ KO, red line) mice cultured with anti-BCR for 6 hours in the presence and absence of CsA or FK506.



Supplemental Figure 3: Ca^{2+} regulated mechanisms controlling cell cycle entry and proliferation. (A) Intracellular c-Myc levels in anti-BCR stimulated untreated (UT) WT CD23+ B cells and cells treated with rapamycin (25 nM) or Torin-1 (100 nM) for 4 hours (left). Proportion of cells expressing Myc (right, mean +/- S.D.). (B) Intracellular c-Myc in C57Bl/6 (WT) and c-Rel KO CD23+ B cells cultured in the absence (media) or presence of anti-BCR for 6 hours. The proportion of c-Myc+ cells was determined using fluorescence minus one (FMO) staining (representative of 3 independent experiments). (C) Localization of Foxo1 following BCR engagement in WT and STIM DKO B cells treated as indicated. Each histogram represents >50 cells and is representative of 3 independent experiments. (D) Time course of Akt473 and S6 phosphorylation in CD23+ WT and STIM DKO B cells stimulated with anti-BCR in the absence and presence of extracellular Ca^{2+}. Results are representative of at least 3 independent experiments. (E) Phospho-S6 levels 4 hours after anti-BCR stimulation in the presence and absence of the CaM inhibitor W7 (5 μM) or Cn inhibitor FK506 (1 μM) (top). Proportion of pS6 high B cells (bottom, mean +/- S.D. of triplicate measurements).



Supplemental Figure 4: Mechanisms of CD40- and TLR9-dependent rescue of B cell survival and proliferation. (A) Fura-2 ratiometric measurement of Ca^{2+} in B cells stimulated as indicated. Each line depicts the response of a single cell (left). Boxplots of initial peak and steady-state (plateau) fura-2 ratio for samples shown to the left (right, data are representative of 3 independent experiments with >50 cells). (B) Total cell counts (mean +/- S.D. of triplicate wells) following CpG ± anti-BCR stimulation. (C) Intracellular cleaved caspase 3 in live and dead CpG or 24 hours after anti-BCR/CpG stimulation of WT and STIM DKO B cells (left). Percentage (mean +/- S.D., from triplicate wells) of cleaved caspase 3 positive cells (right). (D) CFSE dilution assay for CD23+ B cells from WT and STIM DKO mice stimulated as indicated for 72 hours (left) and total cell counts (mean +/- S.D. from triplicate wells) (right).