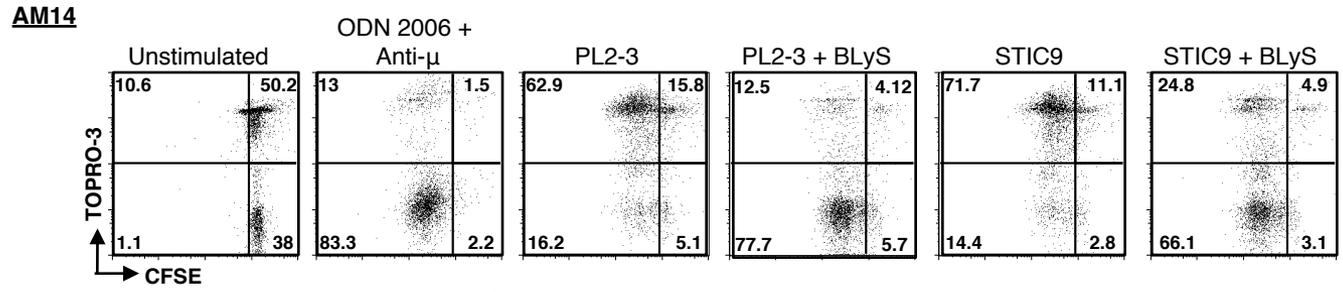
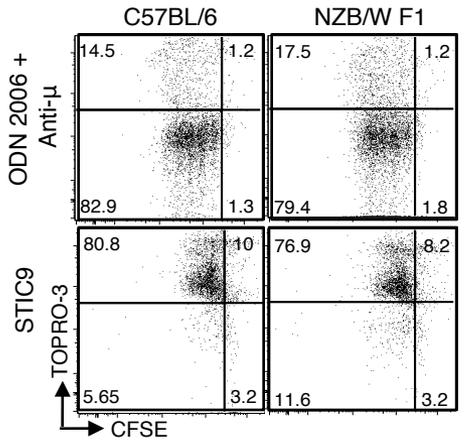


Supplemental Figure 1

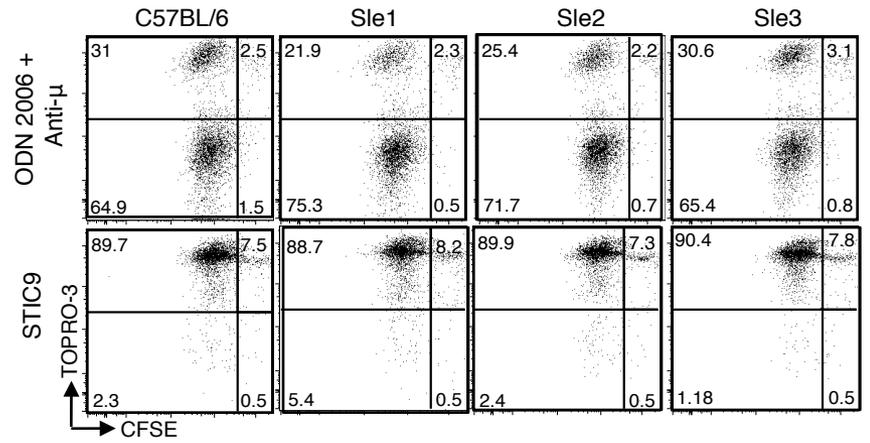
A



B

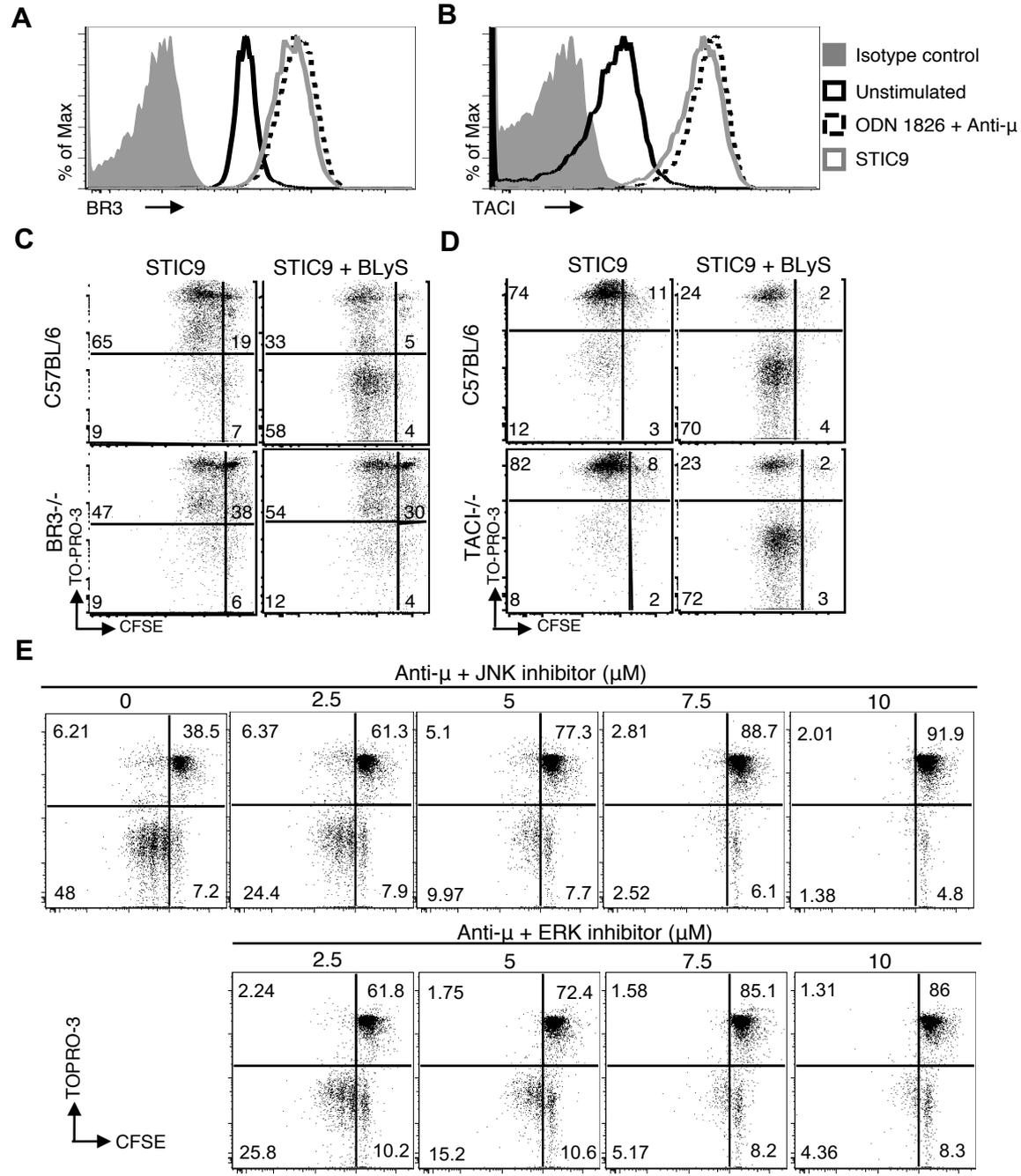


C



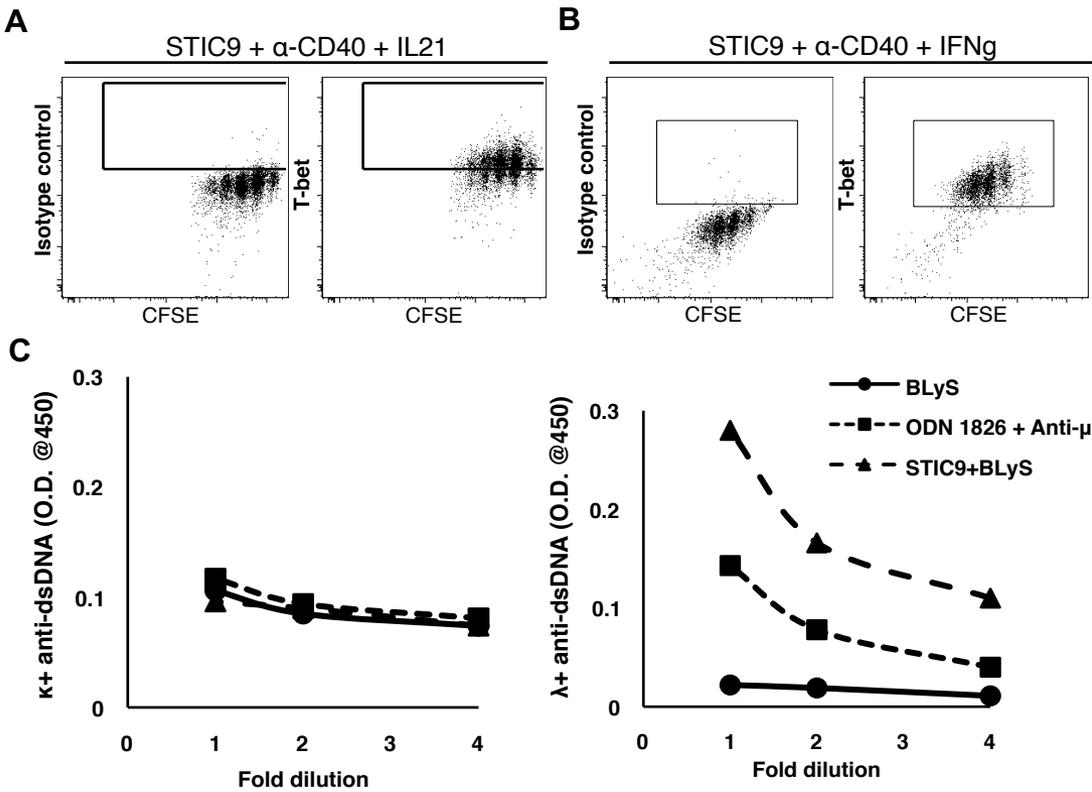
Supplemental Figure 1 (A) CD23⁺ B cells were isolated from the spleens of AM14 mice and stimulated with ODN1826 + F(ab')₂ fragments of anti-IgM, PL2-3, PL2-3 +BLyS, STIC9 or STIC9 +BLyS for 60hrs. Dead cells were stained by TO-PRO-3, while CFSE dilution indicates proliferation. **(B, C)** CD23⁺ B cells were isolated from the spleens of the indicated strains of the mice. FACS analysis of proliferation and survival of CD23⁺ B cells cultured for 60hrs with ODN1826 + F(ab')₂ fragments of anti-IgM or STIC9. Dead cells were stained by TO-PRO-3, while CFSE dilution indicates proliferation. Data are representative of at least three independent experiments.

Supplemental Figure 2



Supplemental Figure 2 (A, B) CD23⁺ B cells were cultured with indicated stimuli for 24hrs. At the end of 24hrs culture cells were probed for either BR3 or TACI with corresponding isotype control antibodies. Plots show **(A)** BR3 and **(B)** TACI expression in live cells. Delta MFIs for BR3 in **(A)** unstimulated, ODN1826 + F(ab')₂ fragments of anti-IgM, or STIC9 are 1262, 6313 and 5114, respectively and for TACI in **(B)** unstimulated, ODN1826 + F(ab')₂ fragments of anti-IgM, or STIC9 are 257, 7437 and 5626, respectively. **(C, D)** Representative FACS plots comparing CFSE dilution and TO-PRO-3 staining after 60hrs of culture with STIC9 or STIC9 + BLYS in B220⁺ **(C)** or CD23⁺ **(D)** cells from C57BL/6 mice and either **(C)** BR3^{-/-} mice or **(D)** TACI^{-/-} mice. **(E)** FACS analysis of proliferation and survival of CD23⁺ B cells from C57BL/6 mice cultured for 60hrs with F(ab')₂ fragments of anti-IgM with or without 2.5 μ M, 5 μ M, 7.5 μ M, or 10 μ M of either the JNK inhibitor SP600125 or the MEK1/2 inhibitor U0126. Dead cells were stained by TO-PRO-3, while CFSE dilution indicates proliferation. Data are representative of at least three independent experiments.

Supplemental Figure 3



Supplemental Figure 3 (A, B) FACS analysis of CFSE dilution and T-bet expression among live cells of CD23⁺ B cells cultured for 60hrs with STIC9 + anti-CD40 in the presence of **(A)** IL21 or **(B)** IFN-gamma. All the data are representative of at least three independent experiments. **(C)** To determine whether STIC9 stimulated autoreactive transitional B cells become ASCs when rescued by BLyS, used 3H9 BCR heavy chain transgenic mice. All lambda-1 bearing B cells in these mice have specificity for self-dsDNA and are therefore eliminated from the repertoire as transitional cells during peripheral selection. Lambda⁺ 3H9 splenocytes were isolated by magnetic cell sorting and cultured for 48hrs with BLyS, ODN 1826 + F(ab')₂ fragments of anti-IgM, or STIC9 + BLyS. Following culture, supernatants were collected and concentrations of either kappa⁺ or lambda⁺ anti-DNA antibodies were detected by ELISA. Each stimulation group (CpG + anti-IgM and STIC9 + BLyS) induced significantly more ($p < 0.05$) lambda⁺ anti-dsDNA antibodies compared to BLyS stimulation alone (right panel). Data are representative of two independent experiments.