Supplemental Methods

Antibodies and Reagents

Cells were stained with antibodies against Bcl-xL (7B2.5, abcam), Bim (AbD Serotec), and CD122 (TM-β1, Biolegend) where indicated.

IL-2 Treatment

Animals receiving IL-2 support were injected i.p. with 9000 IU (Chiron) every other day for 7 days.

Quantitative RT-PCR

Spleens and lymph nodes were removed and homogenized to produce a single cell suspension. Cells were incubated with Thy1.2 beads (Miltenyi) to distinguish adoptively transferred FH T cells and positively sorted using an AutoMACS pro. Total cellular RNA was isolated using TRIZOL (Invitrogen) per manufacturer's instructions. Contaminating genomic DNA was removed by treatment with RNase-free DNase I, and cDNA was prepared using the SuperScript III First-Strand Synthesis System (Invitrogen). Quantitative real time PCR was performed on cDNA with Titanium *Taq* Polymerase (BD Clonetech) with 1X SYBR Green (Molecular Probes) and 0.4 μM of the primer set of interest in 25-μl reaction mixtures in a MyiQ Single Color Real-Time PCR Detection System (Bio-Rad). Conditions for quantitative RT-PCR were as follows: 95°C for 3 minutes, then 40 cycles of 95°C for 40 seconds, 66°C for 20 seconds, and 72°C for 30 seconds, followed by an extension at 72°C for 1 minute. Melting curve analysis was then performed to ensure equivalent and appropriate melting temperatures. Each sample was normalized relative to the expression of *HPRT* (encoding hypoxanthine guanine phosphoribosyltransferase). Primers used were as follows: *Bid* (forward, 5'-CACAACATCCAGCCCACACT-3'; reverse, 5'-CTCCATGTCTCTGGGGAAGG-3'), *Bad* (forward, 5'-GCGATGAGTTTGAGGGTTCC-3'; reverse, 5'-TCCTTTGCCCAAGTTTCGAT-3'), *Bmf* (forward, 5'-GAGGTGCAGATCGCCAGAAA-3'; reverse, 5'-TGTTCAGGGCGAGGTTTTGA-3'), *Bim(EL)* (forward, 5'-GCCCTGGCCCTTTTGC-3'; reverse,

5’-CCGGGACAGCAGAGAAGATC-3’), *Bim(L)* (forward, 5’-GACAGAACCGCAAGACAGGAG-3’; reverse, 5’-TGGCAAGGAGGACTTGGG-3’) and *HPRT* (forward, 5’-TGCCGAGGATTTGGAAAAAGTG-3’; reverse, 5’-CACAGAGGGCCACAATGTGATG-3’).

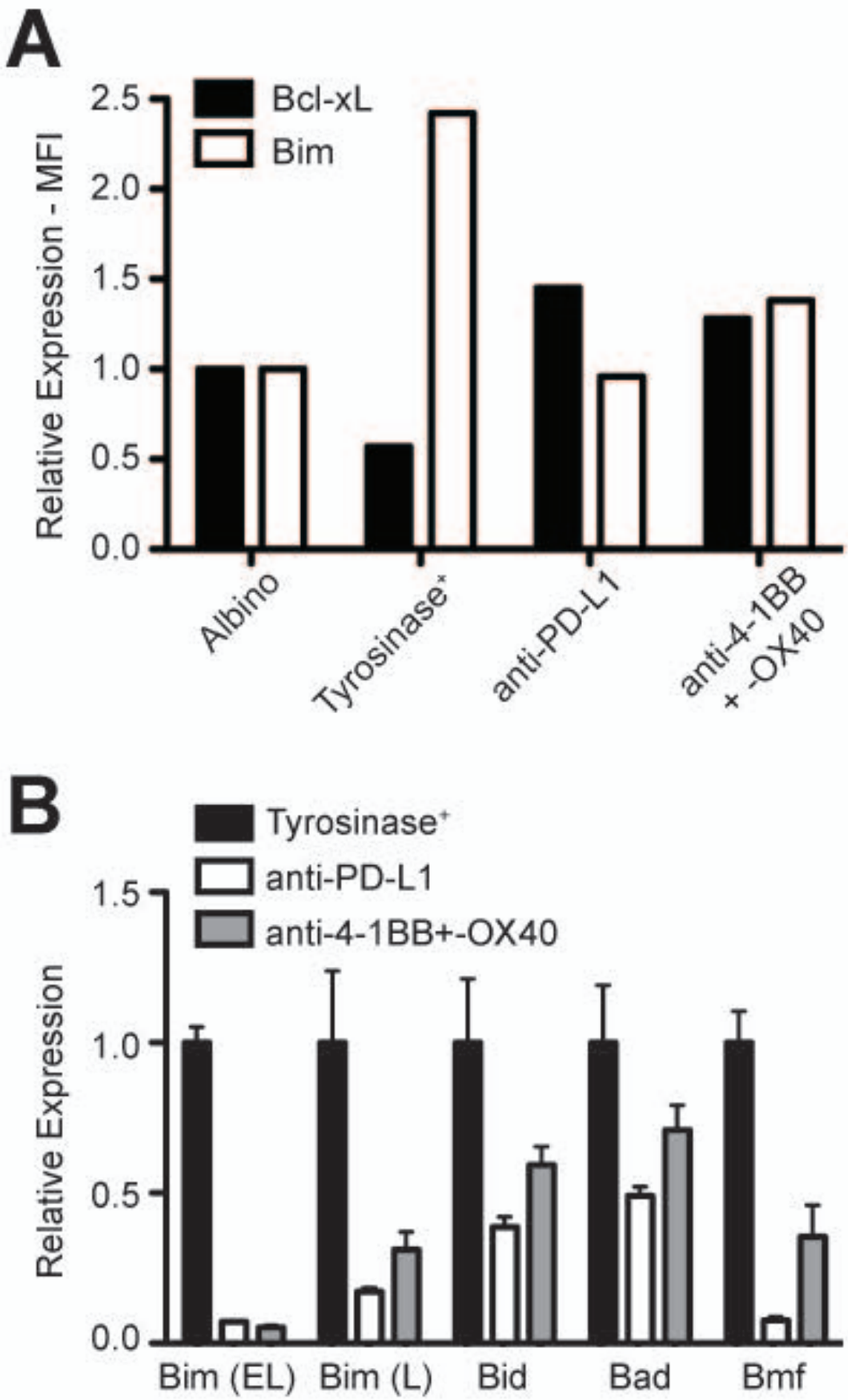
Statistical Analysis

P values on paired samples were calculated by unpaired *t*-tests. Two-way analysis of variance statistical analysis with Bonferonni post-tests was used when multiple groups of samples were compared. Statistics were calculated using GraphPad Prism version 5.0 (GraphPad, La Jolla, CA, USA).

Supplemental Figure 1. PD-L1:PD-1 engagement inhibits the expression of the anti-apoptotic molecule Bcl-xL and increases the expression of pro-apoptotic molecules in deleting FH cells. FH cells were transferred into tyrosinase$^+$ mice left untreated or treated with blocking anti-PD-L1 or anti-4-1BB+-OX40. LN were harvested 3 days post-transfer and FH cells were examined for (**A**) expression of the anti-apoptotic molecule Bcl-xL and pro-apoptotic molecule Bim by MFI or (**B**) expression of mRNA of the pro-apoptotic molecules Bim (EL), Bim (L), Bid, Bad, and Bmf by qRT-PCR. (**A**) Relative expression was calculated by dividing the MFI from each treatment by the MFI of FH cells from control albino mice. Data is representative of 3 mice for each condition from 3 independent experiments for Bcl-xL and 5, 4, and 4 mice left untreated, treated with blocking anti-PD-L1, or with agonist anti-4-1BB+-OX40, respectively, from 4 to 5 independent experiments for Bim. (**B**) Data is representative of 2 mice for each condition from one experiment with each sample run in duplicate.

Supplemental Figure 2. Exogenous IL-2 administration does not rescue FH cells, which express high levels of CD122, from deletion. (**A**) Representative data of FH cells transferred into tyrosinase$^+$ mice, tyrosinase$^+$ mice administered exogenous IL-2, or antigen free albino mice. LN were harvested 7 days post-adoptive transfer. Boxes represent the percent of T_{CD8} that are FH cells in the LN of recipient mice. (**B**) Cumulative data from tyrosinase$^+$ mice, tyrosinase$^+$ mice administered exogenous IL-2, or antigen free albino mice. Data represent 3 mice per condition from 2 independent experiments. **P =.0074 (two-tailed, unpaired *t*-test). (**C**) Data represent the % of FH cells that have undergone the indicated number of divisions that express CD122 3 days post-transfer. Data represents 2 to 3 mice per conditions from 2 to 3 independent experiments utilizing untreated tyrosinase$^+$ mice (●), tyrosinase$^+$ mice treated with blocking anti-PD-L1 (■), or tyrosinase$^+$ mice treated with agonist anti-4-1BB+-OX40 (▲). Differences in CD122 expression were not significant. (Two-way ANOVA, Bonferonni post-test). (error bars (**B, C**), s.e.m.)#

Supplemental Figure 1



Supplemental Figure 2