

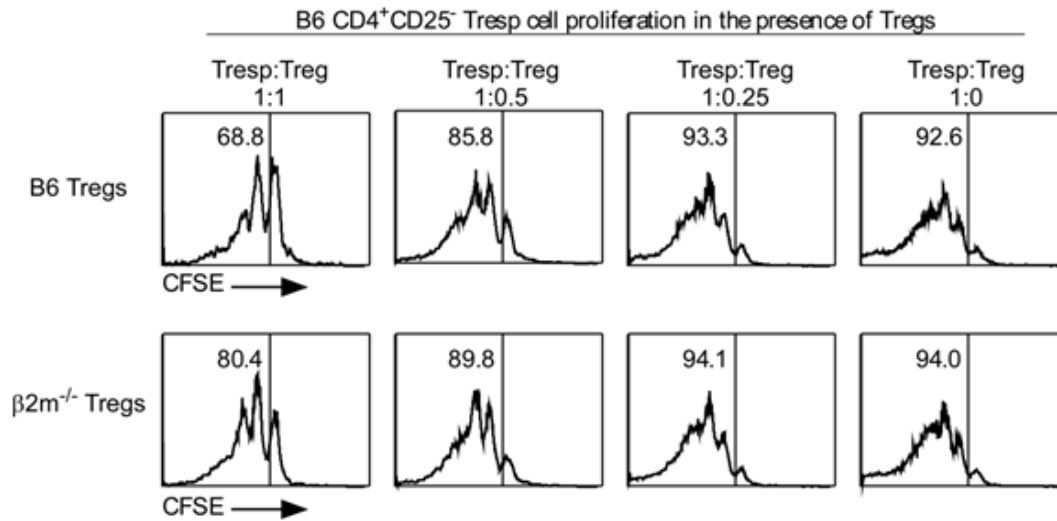
Supplementary Figure 1. MHC class I and beta-2-microglobulin expression in Tregs.

- Beta-2-microglobulin ($\beta 2m$) RNA levels are elevated in $\text{Foxp3}^+ \text{CD4SP}$ thymocytes relative to $\text{Foxp3}^- \text{CD4SP}$ thymocytes. Total RNA was isolated from purified Tregs and Tconv and subjected to RT-PCR.
- Surface expression of MHC class I is equivalent on Tregs derived from WT and $\beta 2m^{+/-}$ mice. Thymocytes were surface stained for H-2K^b, CD4, CD8 and CD25, as described in Materials and Methods. FACS profiles of class I expression on the $\text{CD4}^+ \text{CD25}^+$ (red line) and $\text{CD4}^+ \text{CD25}^-$ (black) populations are shown. Data are representative of two independent experiments.
- Surface expression of MHC class I is equivalent on Tregs derived from WT and $\beta 2m^{+/-}$ mice. Thymocytes were surface stained for H-2K^b, CD4, CD8, fixed and stained for intracellular Foxp3, as described in Materials and Methods. FACS profiles of class I expression on the $\text{CD4}^+ \text{Foxp3}^+$ (red line) and $\text{CD4}^+ \text{Foxp3}^-$ (black) populations are shown. Data are representative of two independent experiments.

Foxp3/IRE

H-2Kb	GGGGTGGGAAGCCCAGGGCTGGGGATTCCCCATCTCCAC <u>AGTTTCACTTCT</u> GCACCT—	482
PD1	TGGGCGGGGAGGCGCGGTGGTGGGGAGTCCCCGTGTCCC <u>AGTTTCACTTCT</u> CCGTCTCG	528
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H-2Kb	—AACCTGGGTCAGGTCCTTCTGTCCGGACACTGTTGACGCGCAGTCAGCTCTTACCCCA	541
PD1	CAACCTGTGTGGGACCGTCCTGCCCGGACACTCGTGACGCGACCCCACTTCTCTCTCCTA	588
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H-2Kb	TTGGGTGGCGGATCACCAAGAA— <u>CCAAT</u> CAGTGTGCGCGGACGCTGGATATAAAGTC	600
PD1	TTGCGTGTCCGTTTCTGGAGAAG <u>CCAAT</u> CGGCGCCACTGCGGTTCCCGTTCTAAACTC	648
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H-2Kb	CACGC—AGCCCGCAGAACT <u>CA</u> GAAGTCGCGAATCG—CCGACAGG—TGCGATGGTACC	654
PD1	TCCACCCACCCGGCTCTGCT <u>CA</u> GCTTCTCCCAGACTCCGAGGCTGAGGATCATGGGGCC	708
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Supplementary Figure 2. The MHC class I promoter elements are highly conserved among species. Alignment of the conserved MHC class I promoter regions in the mouse H-2Kb and swine PD1 genes: red letters, consensus Foxp3 binding site (underlined) and overlapping IRE sequence. (*), conserved nucleotides. The major transcription start sites are boxed.



Supplementary Figure 3. Class I deficiency reduces Treg function *in vitro*.

Treg suppression as assessed by CFSE dye dilution. CFSE-labeled lymph node CD4⁺CD25⁻ Tconv cells from normal B6 mice were either cultured alone or with different numbers of purified CD4⁺CD25⁺ Tregs derived from either B6 or $\beta 2m^{-/-}$ mice and stimulated with anti-CD3 (1 $\mu\text{g}/\text{mL}$) and APCs. The CFSE profiles and percentage of Tconv cells that divide at least once in the cocultures are shown. Data are representative of two independent experiments.