



Supplemental Figure 1. WT and Nrf2^{-/-} splenic T cells under steady-state were examined from 8-10 week-old female mice. (A) Absolute numbers and (B) flow cytometric analysis of TCR V β repertoire of CD4 and CD8⁺ T cells. Data represent mean + SD (n=8/group combined from 2 experiments). (C) Representative flow cytometric analysis and (D) bar graphs showing the percentage of naïve CD4⁺ T cells polarized towards Treg cells in vitro using α -CD3 (5ug/mL), α -CD28 (2ug/mL), IL-2 (40ng/mL), and hTGF- β (10ng/mL). Data represent mean + SD (n=4/group, 1 of 2 experiments). (E) (Left panel) Absolute number of Treg cells (CD4⁺CD25⁺Foxp3⁺), (Middle panel) percentage of Treg cells within CD4⁺ population, and (Right panel) MFI of intracellular Foxp3 expression within Treg cells. Data represent mean + SD (n> 5/group, 2 experiments). (F) (Left panel) Absolute number of Helios⁺Treg cells, (Middle panel) percentage of Treg cells. Data represent mean + SD (n> 5/group, 2 experiments). (F)

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Supplemental Figure 2



Supplemental Figure 2. Nrf2^{-/-} donor Treg cells display intact apoptosis, survival, and suppressive function. (A-B) Combined flow cytometric histograms showing baseline (A) activated caspase-3 and (B) Annexin-V expression of magnetically purified Treg cells. n>3 animals per group, 2 experiments. (C-E) Lethally irradiated BALB/c recipients received CD45.1⁺ WT B6 TCD-BM (5x10⁶) and CD5⁺ T cells (0.5x10⁶) CD45.2+ Nrf2^{-/-} CD4+CD25+ with WT or Treq cells (0.5x10⁶). Donor Treq cells (CD45.1⁻CD45.2⁺H-Kb⁺CD4⁺CD25⁺) were purified using flow cytometry on day 14 of transplant. Expression of (C) proapoptotic and (D) antiapoptotic mouse gene transcripts were analyzed by quantitative PCR and normalized to Actb expression. (E) Expression of CD39 receptor was analyzed by flow cytometry. Data represent mean + SD of 8-9 animals per group from 2 independent experiments. (F) Thymidine incorporation of T-responder cells co-cultured with Treg cells isolated from WT or Nrf2^{-/-} mice in the presence of anti-CD3/28. Data represent mean + SD of thymidine counts conducted in triplicate from 1 of 2 independent reproducible experiments.