

Supplemental Fig 3. Viability of WT and KO cells in vitro and in vivo. (A) MACS-purified WT and KO CD4 cells were stimulated with plate-bound anti-CD3 Ab (5 μg/ml) and soluble anti-CD28 Ab (2 μg/ml). After 24 hours, the cells were stained with PI (propidium iodide). The cell death, as indicated by the PI+ staining, was analyzed by FACS. Data shown are summary of two separate experiments (Means±SD, N=6). (2) MACS-purified WT and KO CD4 cells were mixed at a ratio of 1:1, and then were transferred into Rag 1-/- mice (2×10^6 cells). After 4 wks, the CD4 T cells present in the peripheral blood of recipient mice were harvested and analyzed. The cell death, as indicated by positive of LIVE/DEAD® Fixable Near-IR Stain, was analyzed by FACS, gating on CD45+CD4+TCRβ+ cells (KO cells were CD45.2+, and WT cells were CD45.2-). The data shown are representatives of two separate experiments with same results.