

Supplementary Figures

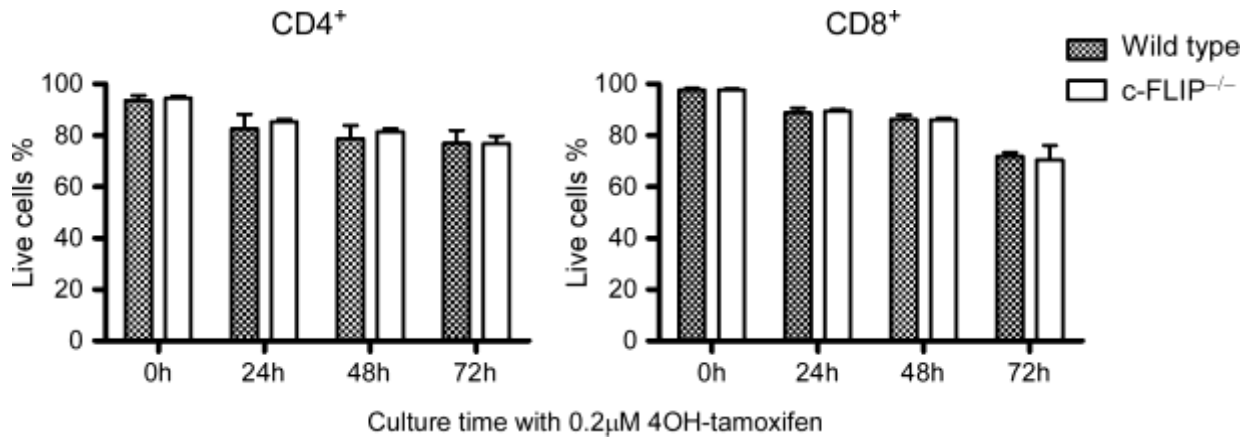


Figure S1: Comparable survival rates of c-FLIP^{fl/fl} and c-FLIP^{fl/fl} ER-Cre⁺ T lymphocytes during *in vitro* deletion. Shown are percent of live cells in T cells during *in vitro* deletion. Lymphocytes pooled from spleen and lymph nodes from *c-Flip^{fl/fl}* and *c-Flip^{fl/fl} ER-Cre⁺* mice were cultured for 3 days with 200nM 4-hydroxy-tamoxifen. Cell death was measured by Annexin V and 7-AAD staining at the beginning of the culture (0 hour), and after 24, 48 and 72 hr culture. Live cells were analyzed by gating on the Annexin V⁻ 7-AAD⁻ population within the total CD4⁺ or CD8⁺ populations (n=3). All error bars represent the standard error of the mean (s.e.m.).

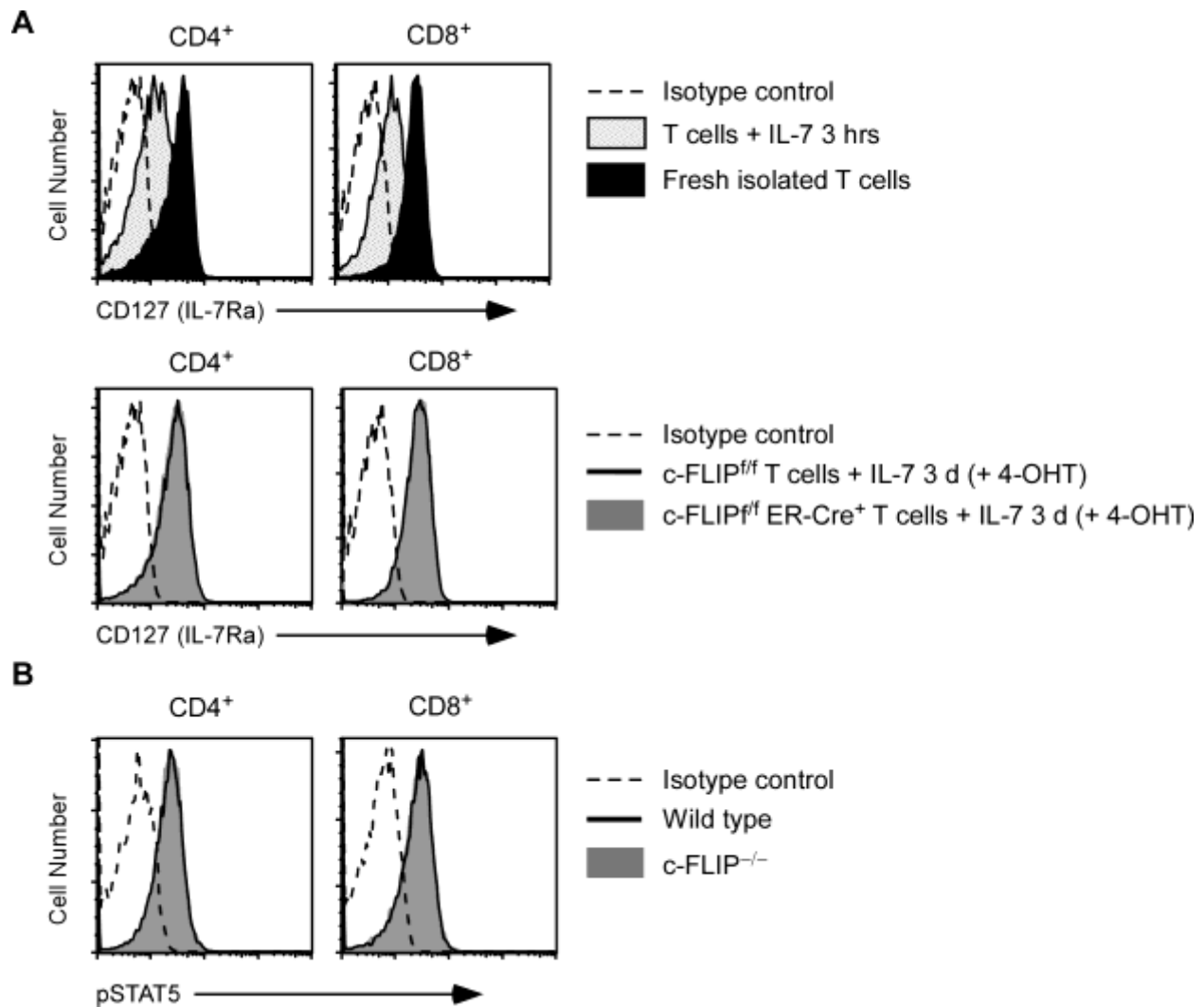


Figure S2: Intact IL-7 signaling in purified c-FLIP^{fl/fl} and c-FLIP^{fl/fl} ER-Cre⁺ T lymphocytes after *in vitro* deletion. (A) The impact on surface IL-7 receptor level of short time (3 hours) and long term (3 days) culture with IL-7. T cells from *c-Flip^{fl/fl}* and *c-Flip^{fl/fl} ER-Cre⁺* mice were cultured with 1 ng/ml IL-7 from 3 days. Fresh isolated T cells from spleen were treated with 1 ng/ml IL-7 for 3 hours or immediately stained for surface CD127 (IL-7R α). Surface staining of CD127 on CD4⁺ and CD8⁺ T cells were analyzed by flow cytometry (n \geq 3). (B) Comparable IL-7 signaling in wild type and c-Flip^{-/-} T cells. Live T cells were enriched after *in vitro* induced deletion and treated with 1 ng/ml IL-7 for 30 min. T cells were fixed by methanol and stained for phosphorylated Y694 of STAT5a (47, BD Bioscience).

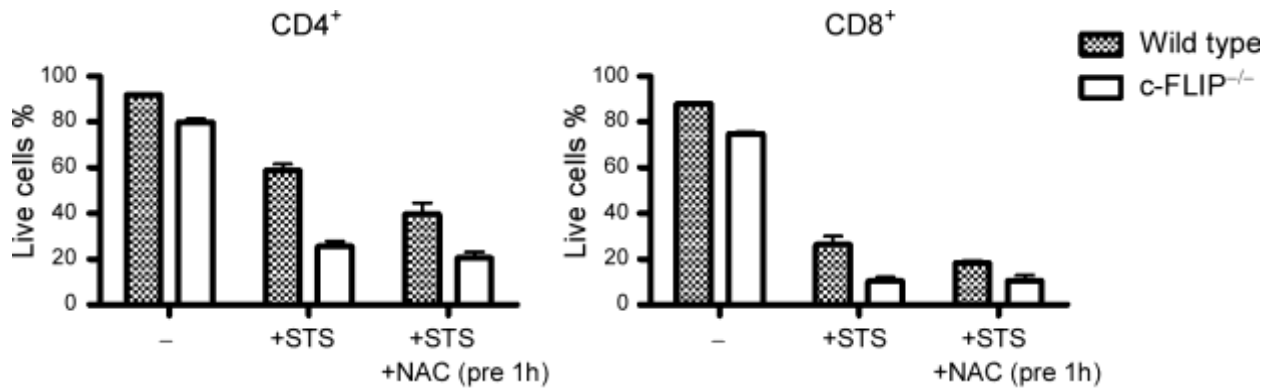


Figure S3: ROS-independent apoptosis in c-FLIP-deficient T cells upon staurosporine treatment. For acetylcysteine (NAC) group, live resting T lymphocytes were pretreated with NAC for one hour to build up glutathione levels. Untreated and treated T cells are cultured in the absence or presence of staurosporine for 16 hours. Cell death rates of T cells were analyzed by flow cytometry. Live cells were analyzed by gating on the 7-AAD⁻ population within the CD4⁺ or CD8⁺ populations. All error bars represent the standard error of the mean (s.e.m.) (n>=3).