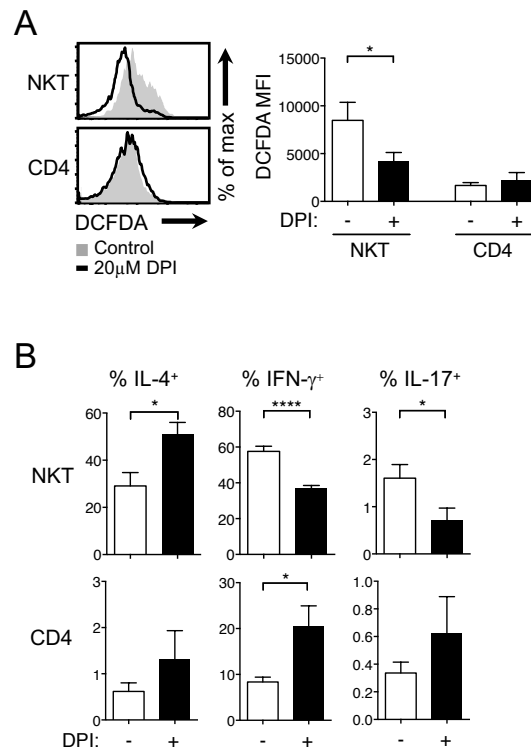
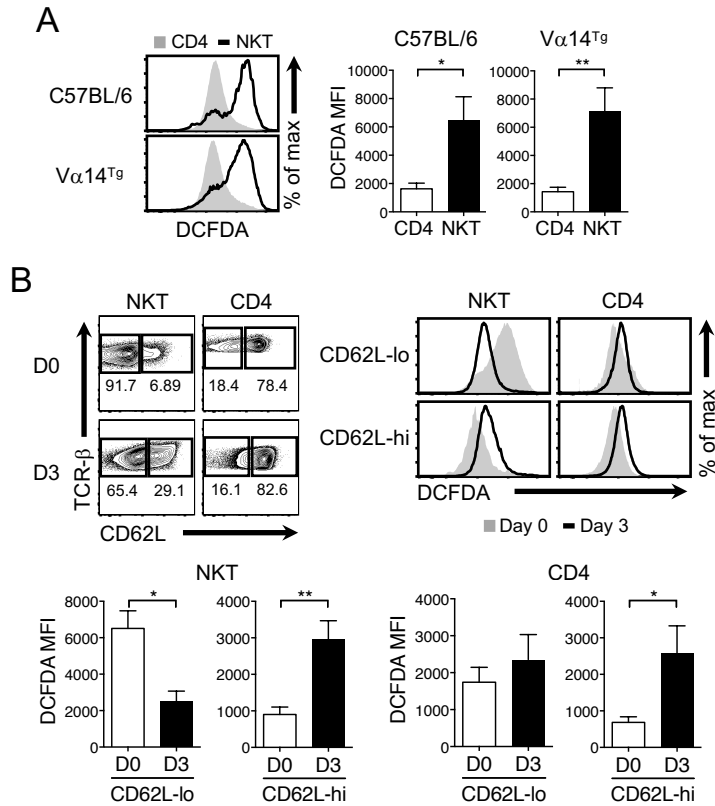


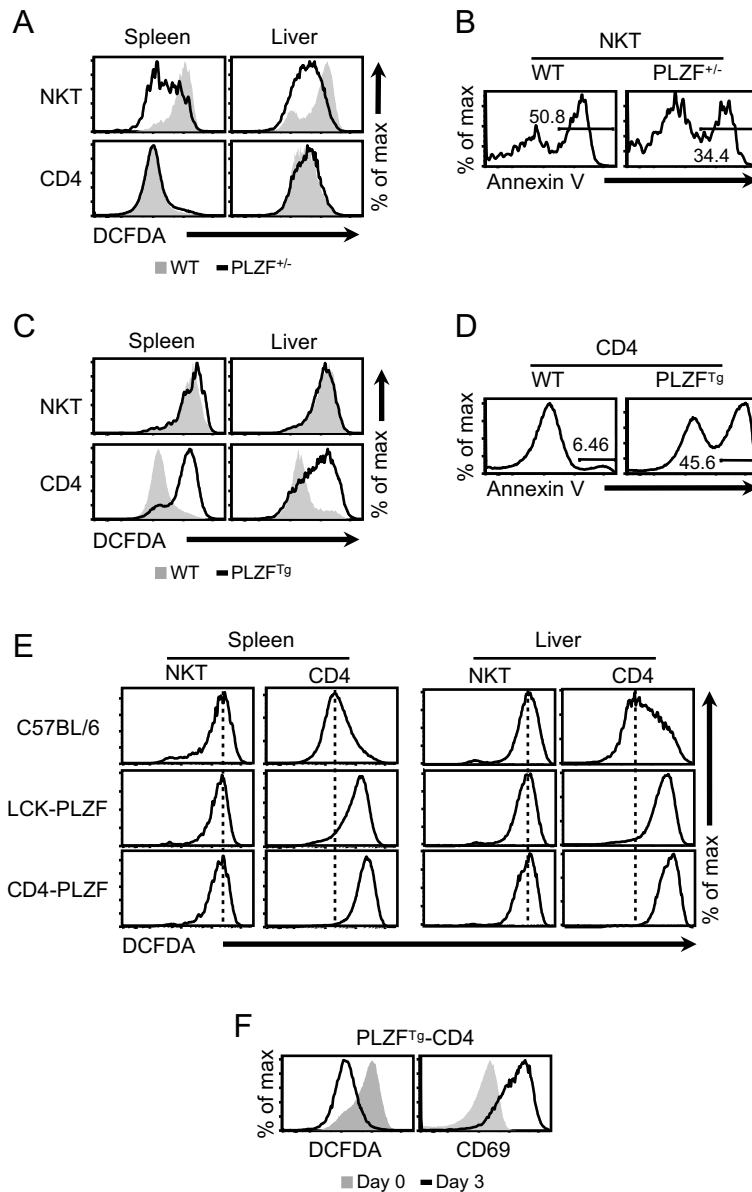
Supporting information



Supplemental Figure 1. ROS levels in hepatic NKT cells affect effector function. **(A)** Total lymphocytes from liver of C57BL/6 mice were treated with 20 μ M DPI for 30 minutes and then measured ROS. N=5 mice per group. **(B)** The cells pretreated with DPI in (A) were washed and then stimulated with PMA (50 ng/ml) and Ionomycin (1.5 μ M) for 5 hours to measure cytokine expression for NKT and CD4 T cells. N=6 mice per group. Each flow cytometry histogram showed one representative experiment. Error bars represent the mean \pm SEM. * p <0.05; **** p <0.0001.



Supplemental Figure 2. Comparison of ROS in V α 14^{Tg} and C57BL/6 NKT, and in CD62L-low and CD62L-high NKT cells. **(A)** NKT and CD4 T cells from spleen of V α 14^{Tg} and C57BL/6 mice were compared for ROS levels. N=5 mice per group. **(B)** CD62L-low and CD62L-high splenic NKT and CD4 T cells were compared for ROS levels before (Day 0) and after stimulation (Day 3). N=5 mice per group. Each flow cytometry histogram showed one representative experiment. Error bars represent the mean \pm SEM. *p<0.05; **p<0.01.



Supplemental Figure 3. PLZF regulates the ROS levels. **(A)** NKT and CD4 T cells from PLZF^{+/-} and WT mice were examined for ROS levels. **(B)** The same cells used in Fig. 4A were treated with 30 μ M of H₂O₂ and compared cell death using Annexin V. **(C and D)** Cells were isolated from WT or PLZF^{Tg} mice and to compare ROS levels (C) and apoptosis (D) as done in Fig. 4 A and B. **(E)** Lymphocytes from spleen and liver of C57BL/6, mice expressing lck promoter driven PLZF (*lck*-PLZF), and cd4 promoter driven PLZF (*cd4*-PLZF) were assessed for ROS level. **(F)** CD4 T cells from PLZF^{Tg} mice were stimulated as in Fig. 3D and compared for the amount of ROS measured by DCFDA and the expression of CD69. Histograms are representative of one experiment and at least three independent experiments were performed.