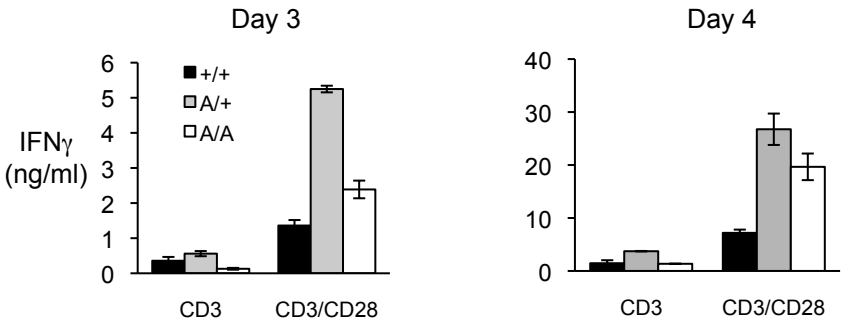
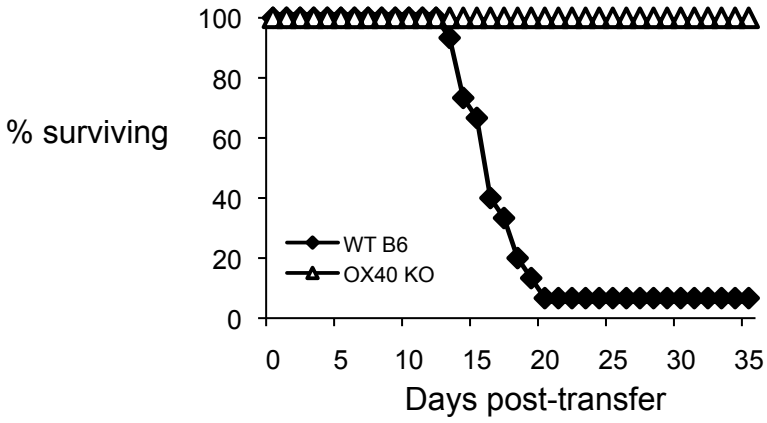
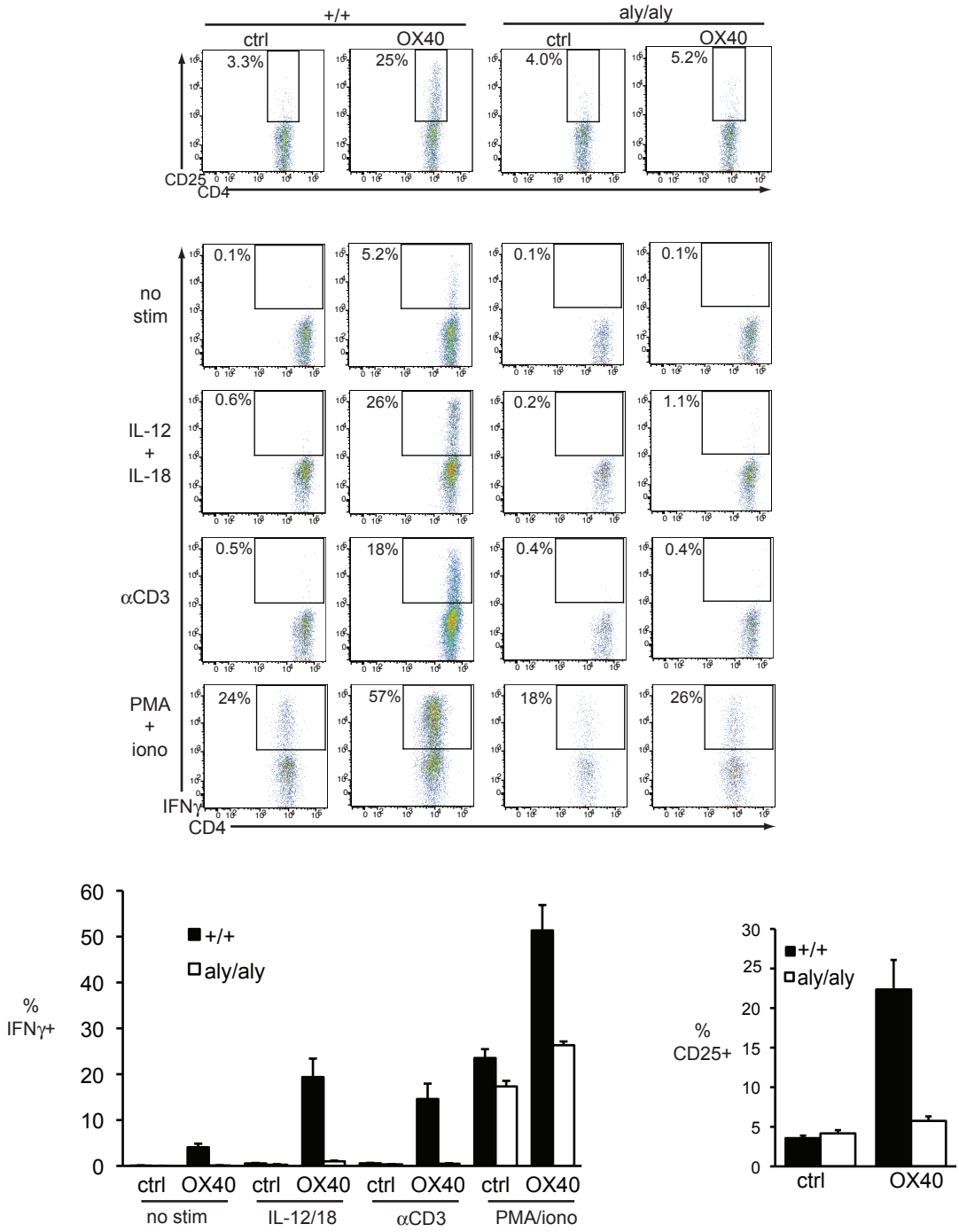


Supplemental Figure 1

Naïve *aly/aly* T cells produce IFNγ upon IL-12 stimulation. Sorted naïve CD4 T cells were stimulated for 3 or 4 days with anti-CD3, anti-CD28, and IL-12. Culture supernatant was assessed for IFNγ by ELISA.

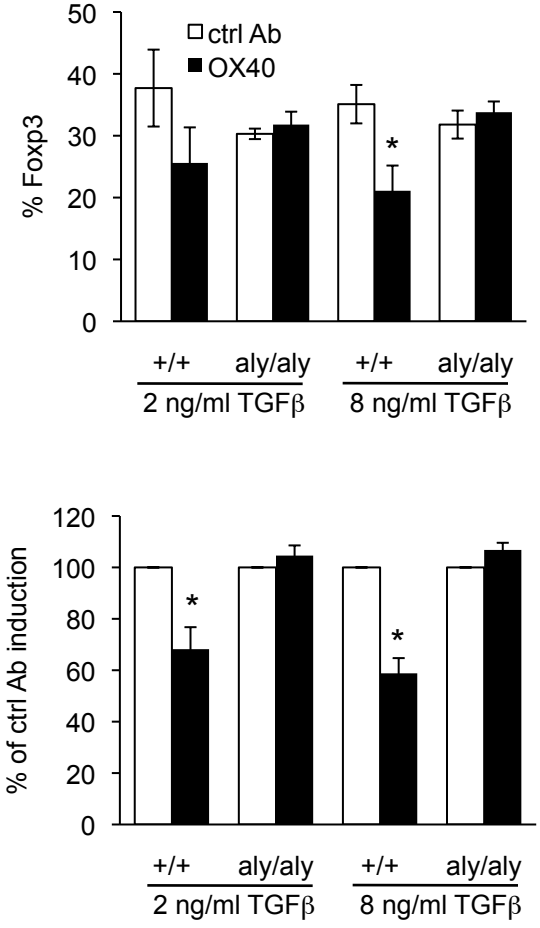


Supplemental Figure 2
OX40-/- CD4 T cells do not cause lethal GVHD. 1 x 10^6 sorted naïve CD4 T cells from OX40-/- or WT mice were injected intravenously into sublethally irradiated (bm12 x CD45.1)F1 recipients.



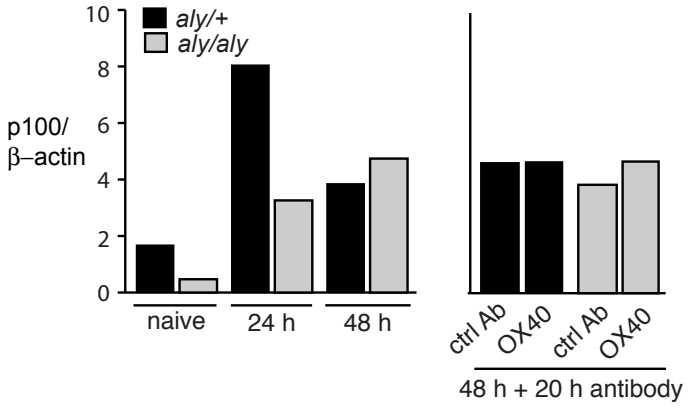
Supplemental Figure 3

NIK-deficient T cells that develop in a NIK-sufficient thymus do not respond to OX40 signaling in a GVHD model. Lethally irradiated B6.CD45.1 mice were reconstituted with *aly/aly* or *+/+* BM. After reconstitution, *aly/aly* or *+/+* naïve CD4 T cells were sorted from these BM chimeras and 3 x 10^6 cells were transferred along with 50 ug control antibody or agonist anti-OX40 into unirradiated (bm12 x CD45.1)F1 recipients. Five days later, recipient spleens were harvested and donor cells were assessed directly ex vivo for CD25 expression and assessed for IFNγ secretion upon 5 hour in vitro stimulation.

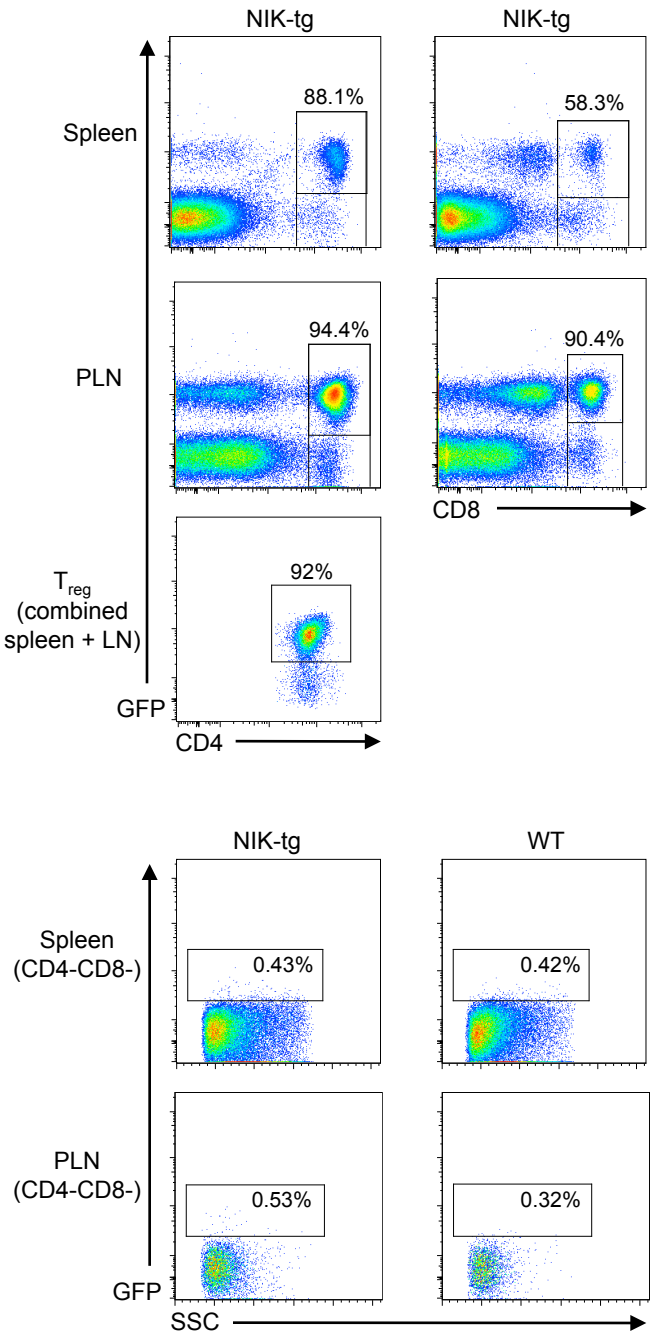


Supplemental Figure 4

OX40 stimulation does not inhibit iT_{reg} induction in NIK-deficient CD4 T cells. Magnetically purified CD4 T cells from *aly/aly* or control +/+ mice (n=3 mice/group) were cultured under iT_{reg} conditions for 3 days with agonist anti-OX40 or control antibody and then assessed by flow cytometry for Foxp3 induction. *p<0.05 versus control antibody.

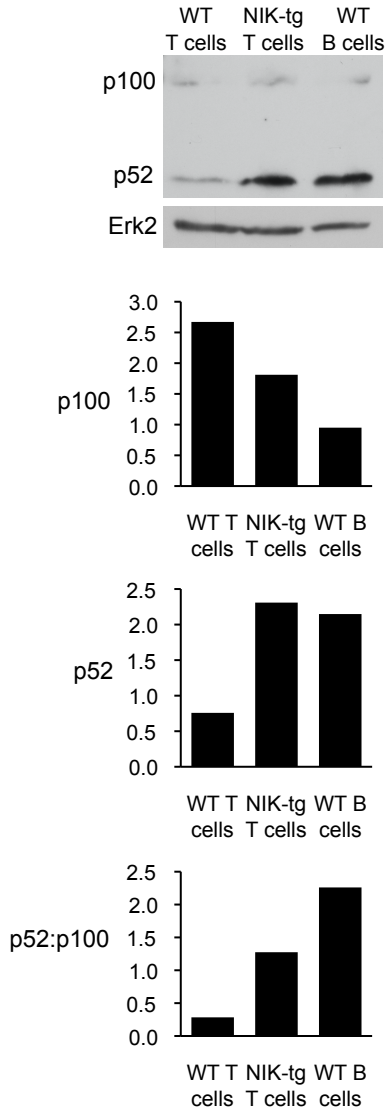


Supplemental Figure 5
p100 quantitation in *aly/aly* and control *aly/+* T cells stimulated *in vitro* with anti-CD3 plus anti-CD28 and anti-OX40 or control antibody. Data are from the experiment depicted in Figure 4.



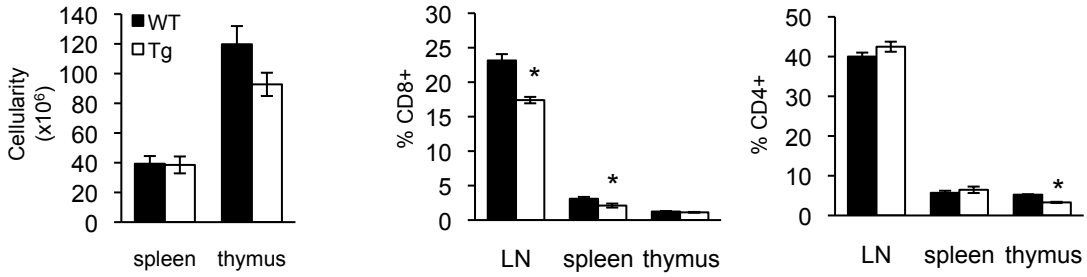
Supplemental Figure 6

GFP expression in T cells and non-T cells from spleen and peripheral lymph node (PLN) of 11 day old CD4-NIKtg and littermate control mice. Numbers refer to the percent of gated T cells that are GFP^+ (upper plots) or the percent of $CD4^-CD8^-$ cells that are GFP^+ (lower plots).

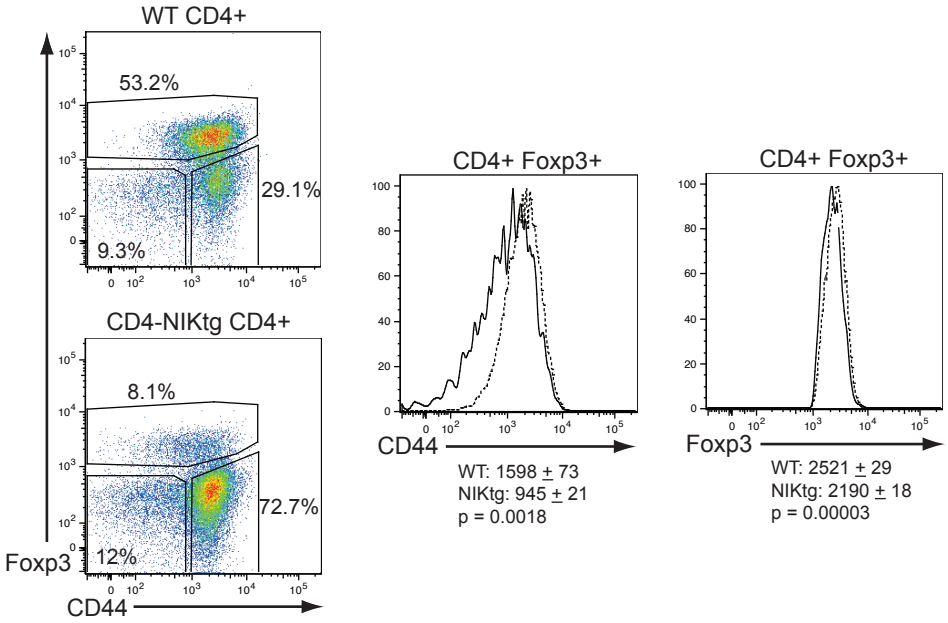


Supplemental Figure 7

NF-κB2 expression and processing in peripheral CD4-NIKtg T cells. Whole cell extracts from spleen and lymph node were assessed for p100 and p52 and normalized to Erk2 loading control. WT T cells and WT B cells were purified by magnetic separation; CD4-NIKtg T cells were obtained from healthy bone marrow chimeras and sorted based on GFP and CD4/CD8 co-receptor expression.

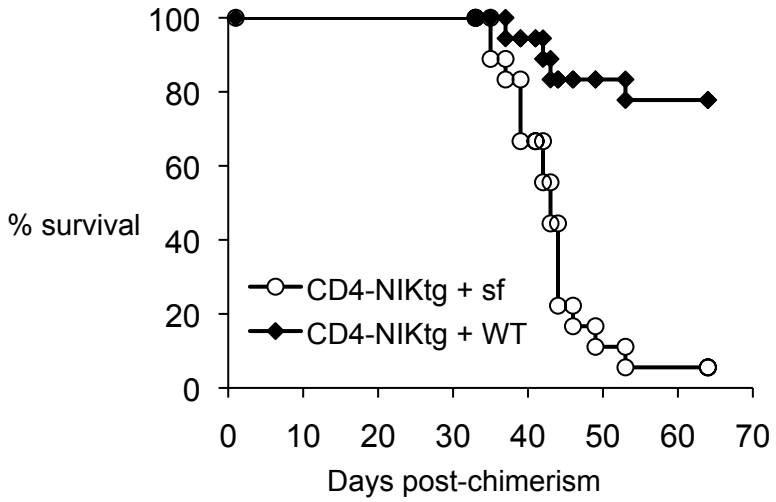


Supplemental Figure 8
Cellularity and T cell proportions in lymphoid organs of 11 day old CD4-NIKtg and littermate control mice. *$P < 0.05$.



Supplemental Figure 9

CD4-NIKtg T_{conv} are activated and WT T_{reg} are expanded in healthy mixed bone marrow chimeras. Lethally irradiated Thy1.1 recipients were reconstituted with a 1:1 mix of CD4-NIKtg and WT B6.CD45.1 bone marrow. Splenocytes from the chimeras were assessed for CD44 and Foxp3 expression. Dot plots are gated on CD4+ cells with the appropriate congenic markers. Histograms are gated on CD4+ Foxp3+ cells; dashed lines are WT and solid lines are NIKtg. Average CD44 and Foxp3 MFIs of the gated populations ± SEM and p values (WT vs. NIKtg) are displayed underneath the histograms.



Supplemental Figure 10
WT, but not NIK-tg, Foxp3^{+} T_{reg} can rescue autoimmunity induced by T cell-specific overexpression of NIK. Rag-/- mice were reconstituted with a 1:1 mixture of CD4-NIKtg BM: WT BM or CD4-NIKtg BM: FoxP3^{sf} BM and survival was monitored for 2 months.