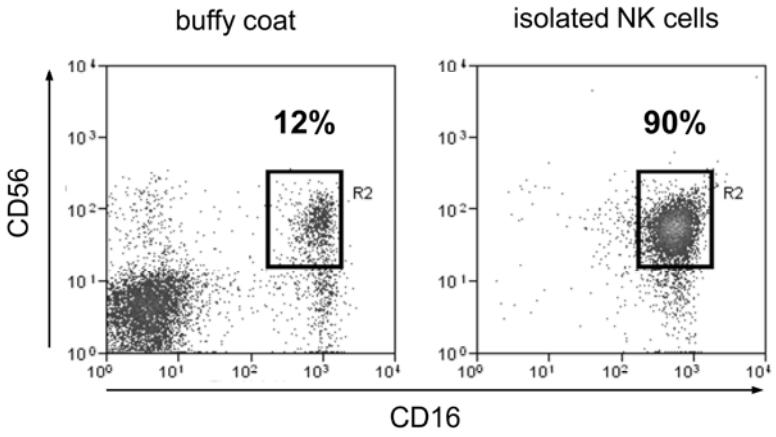
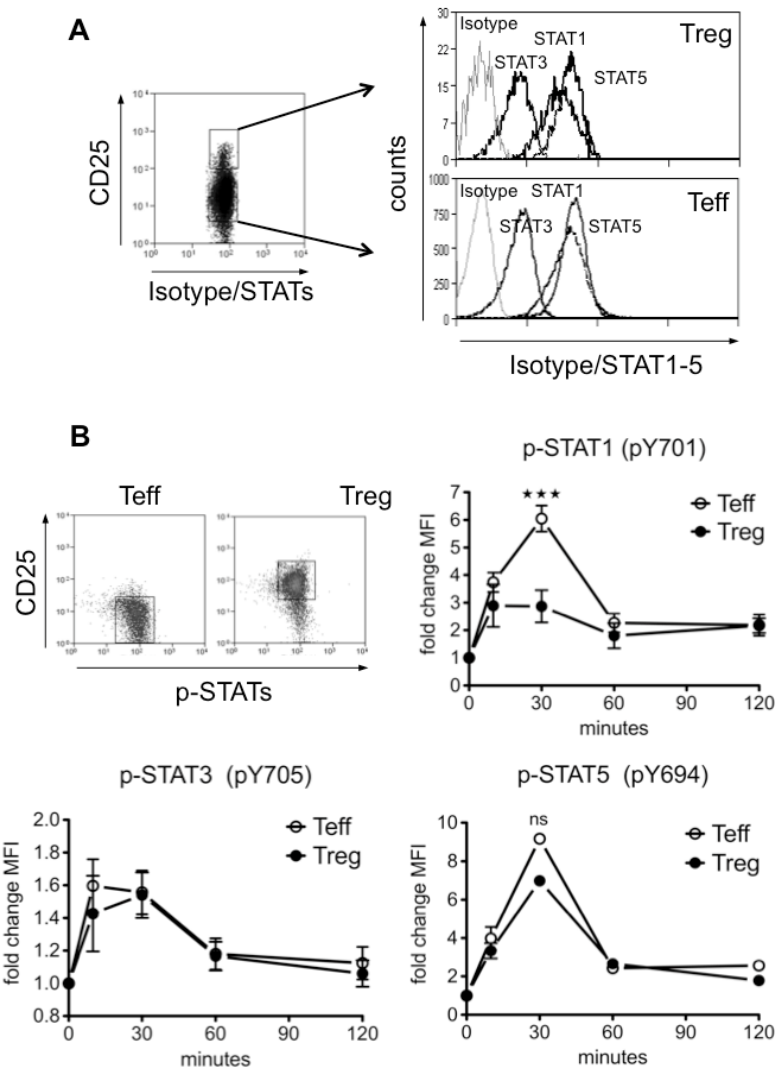
Supplemental Figure Legends

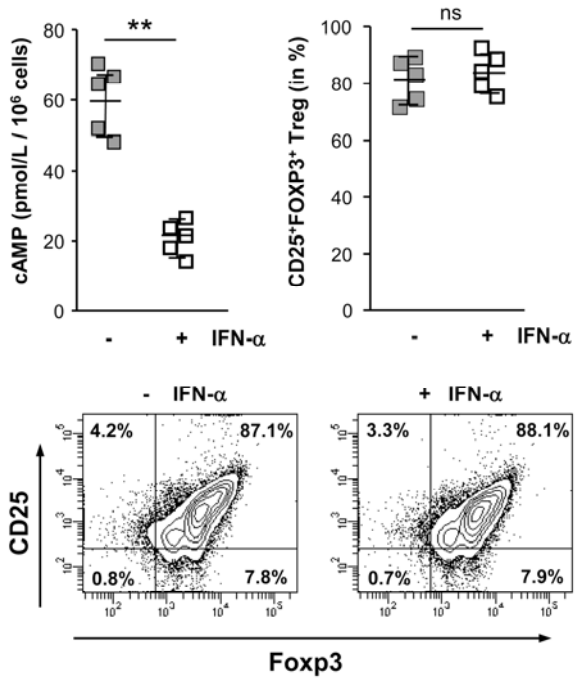


Supplemental Figure 1. $CD56^{+}CD16^{+}$ NK cells isolation from buffy coats. Lymphocytes were gated through SSC and FSC, followed by gating of $CD56^{+}$ and $CD16^{+}$ events. Representative dot plots prior to and after isolation using the $CD56^{+}CD16^{+}$ NK cell isolation kit (Miltenyi Biotec) are shown.



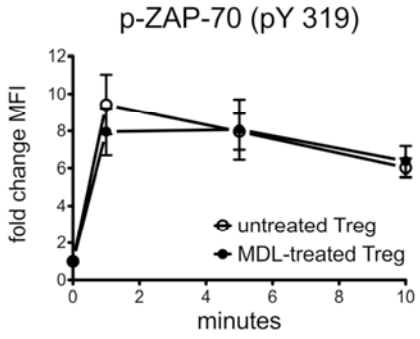
Suppelmental Figure 2. IFN-α treatment impairs STAT1 activation in Treg.

(A) Basal expression of STAT1, STAT3 and STAT5 in Treg ($CD25^{high}$) and Teff ($CD25^{low}$), respectively, in absence of IFN-α. Gates for $CD25^{high}$ and $CD25^{low}$ cells were set as indicated. One representative of three experiments is shown. (B) STAT phosphorylation in response to IFN-α. Teff or Treg were either left unstimulated or stimulated with 10^4 U/ml IFN-α for time intervals indicated, and stained for CD25 and phospho-STAT1 (n=4), phospho-STAT3 (n=3) or phosphor-STAT5 (n=3), respectively. Gates for $CD25^+$ and $CD25^-$ cells were set as indicated. *** $p < 0.001$



Supplemental Figure 3. IFN-α did not affect Foxp3 expression in Treg.

Treg were cocultured with or without of 10^4 U/ml IFN-α overnight and subsequently activated by anti-CD3 and anti-CD28 mAb. After 6 h, T cells were harvested and cAMP levels were analyzed by ELISA (left panel, see also Fig. 5D) and percentages of $CD4^+CD25^+Foxp3^+$ Treg were simultaneously assessed by flow cytometry (right panel). Cumulative results of four experiments and one representative dot blot of $Foxp3^+$ expression are shown. Symbols represent individual cell preparations, and horizontal bars represent mean values ± SD. ** $p < 0.01$, ns: not significant



Supplemental Figure 4. Pharmacological repression of cAMP production in Treg does not affect ZAP-70 phosphorylation.

Treg were either pre-treated with the adenylate cyclase inhibitor MDL-12 (8μM, 30min, 37°C) or left untreated. Upon stimulation with cross-linked anti-CD3 antibodies for times indicated cells were stained for CD25 and phospho-ZAP-70. Gates for $CD25^+$ cells were set based on fluorescence. Fold changes in phospho-ZAP-70 Mean Fluorescence Intensity (MFI) are plotted. Pooled results of four independent experiments shown.