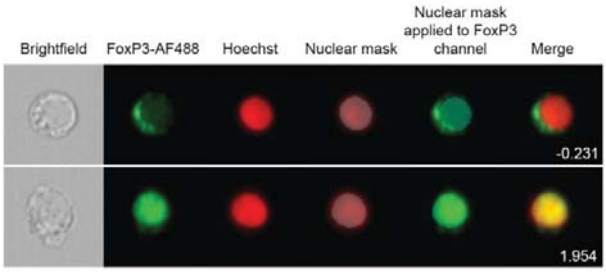
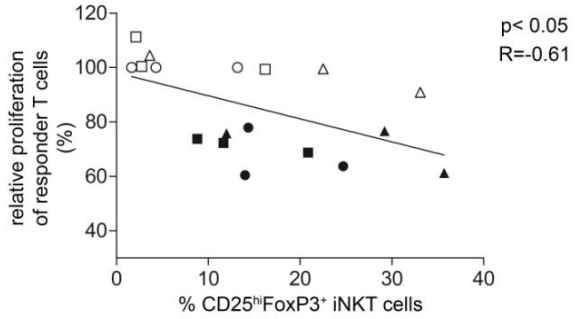
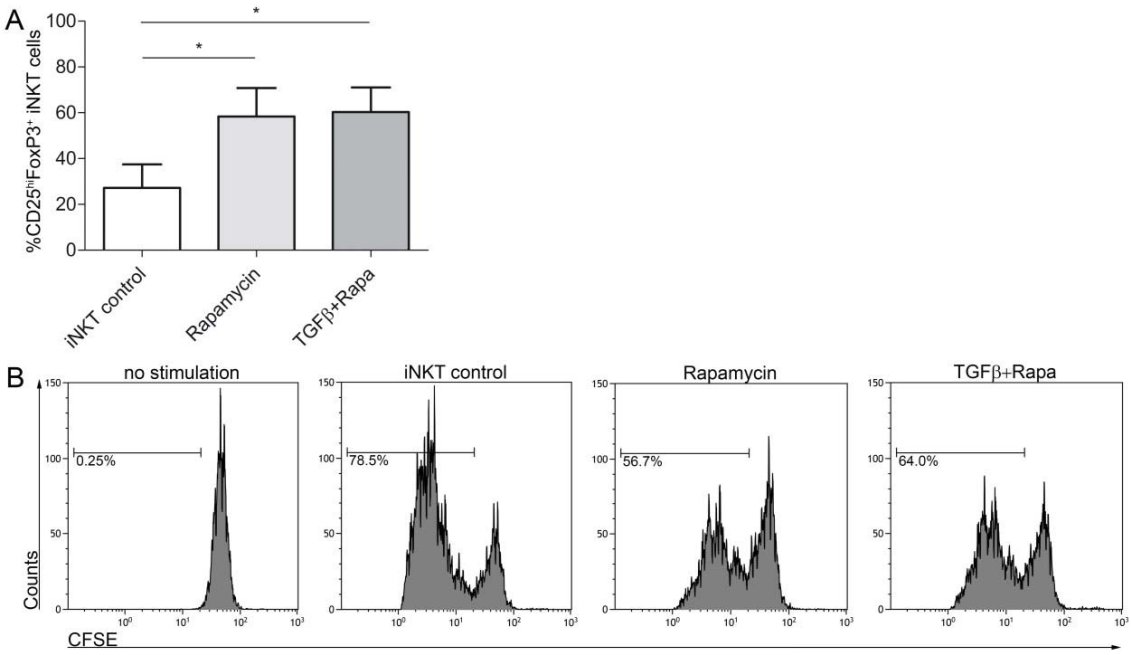


Supplementary figure 1. Determination of FoxP3 nuclear translocation in T cells by imaging flow cytometry. FoxP3 localization was determined by intracellular staining with Hoechst and FoxP3-AF488 using the eBioscience fixation/permeabilization kit. Cells were analyzed using imaging flow cytometry. Based on the Hoechst signal a mask delineating the nuclei was designed. Subsequently the ratio of the amount of FoxP3 in the entire cell *versus* the nuclear mask was calculated. This ratio was log-transformed and termed 'nuclear translocation score'.



Supplementary figure 2. Correlation between the frequency of $CD25^{hi}FoxP3^{+}$ iNKT cells and the relative proliferation of responder T cells. FoxP3 expression was determined after 7 days of co-culture of iNKT cells with α-GalCer loaded immature moDC and IL-2 in the presence of medium alone or supplemented with IL-10, TGFβ and/ or rapamycin. Percentages of $CD25^{hi}FoxP3^{+}$ iNKT cells were assessed by flow cytometry according to the gating strategy shown in Fig. 1A. CFSE dilution of $CD8^{+}$ responder T cells was determined and represented as proliferation of responder T cells relative to the iNKT (medium) control. Each point represents an individual data point, symbols represent the presence of cytokines e.g. no addition of cytokines (iNKT control; ○), addition of TGFβ (□) or IL-10 (Δ). Presence of rapamycin is visualized with filled symbols. p-value <0.05, R=-0.61.



Supplementary figure 3. Induction of FoxP3 and suppressive functionality in *ex vivo* iNKT cells. A, FoxP3 expression was determined after a 7 day co-culture of iNKT cells, purified from peripheral blood, α-GalCer-loaded immature moDC and IL-2 in the presence of medium (iNKT control), rapamycin, or TGFβ and rapamycin. The percentages of $CD25^{hi}FoxP3^{+}$ iNKT cells were assessed by flow cytometry according to the gating strategy shown in Fig. 1A. Means+SEM are shown; p-values are indicated with asterisks; * $p \leq 0.05$, n=4; One way repeated measures ANOVA with Bonferroni post-test. B, Histograms showing CFSE dilution of $CD8^{+}$ responder T cells cultured alone or stimulated using anti-CD3 mAb, anti-CD28 mAb, and IL-2 in the presence of iNKT cells pretreated with medium (iNKT control), rapamycin or TGFβ and rapamycin. Percentages of $CD8^{+}$ responder T cell proliferation are indicated.