**Supplemental Methods**

*Hoechst Labeling*

As described in text.

*IL2 mRNA*

As described in text.

*Intracellular Staining*

FOXP3 staining was done as described in text. Cells were fixed in 1.5% PFA and permeabilized with cold 100% methanol prior to staining. Intracellular IL2 cytokine was analyzed using anti-IL2 APC (clone N7.48A, Miltenyi Biotec, Ashland, OR) after incubating cells with Brefeldin A for the last 5 hours of culture. Plots show the comparison between anti-IL2 and respective IgG antibody isotype control using FlowJo 8.8.2 software .

*Protein analysis by Western Blot*

Cell extracts were lysed and fractionated (cytoplasmic and nuclear extracts) using the NE-PER® Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific, Rockford, IL) as per manufacturer's conditions. Quantitation was done using the BCA™ Protein Assay Kit (Thermo Scientific). 10μg of protein was loaded per lane, separated by SDS-PAGE and transferred to an Immobilon™ PVDF Transfer Membrane (Millipore, Bedford, MA), followed by overnight blocking. Primary antibodies were incubated for 2 hours at RT, followed by

several washes and incubation with secondary antibodies for 1 hour at RT. Membranes were developed using the SuperSignal® West Pico Chemiluminescent Substrate as per manufacturer's instructions (Thermo Scientific). p27 Kip1 detection: clone D37H1 rabbit monoclonal antibody (Cell Signaling Technology, Inc., Danvers, MA).

**Supplemental Figure Legends**

**Figure S1. Staining of purified CD4+CD25bright and CD4+CD25- T cells for FOXP3 and CD127.** Freshly isolated CD4+CD25bright and CD4+CD25- T cells were stained for FOXP3 and CD127 expression.

**Figure S2. Time course of nTregs activation and Hoescht labeling.** Freshly isolated nTregs were activated for 5 days and labeled with Hoescht 33342 at 0, 48 and 120 hrs prior to flow cytometry analysis.

**Figure S3. Vitamin D3 does not alter *IL2* mRNA or protein production from CD4+CD25- T cells.** CD4+CD25- T cells were activated under suppression microassay conditions for 6 hrs in the presence or absence of 20 nM vitamin D3 followed by analysis for *IL2* mRNA or IL2 intracellular protein.

**Figure S4. p27 levels in activated nTregs are modulated by vitamin D3.** Purified nTregs were activated in the presence or absence of vitamin D3 for the

indicated times. Cell lysates were quantified for protein content by BCA and equivalent amounts of protein were loaded into each lane prior to western blot analysis for p27 expression