Supplementary Figure 1. Analysis of T cell apopotosis. (A) OT-II (CD4) or OT-I (CD8) T cells were activated with Ova or SIINFEKL peptide, respecitively and irradiated B7/B7h+/+ (WT) or B7/B7h-/- (TKO) APC for 4 or 7 days. Cells were stained with Vα2-PE antibody and PI, and analyzed by FACS. Histograms represent PI intensity of Vα2+ populations. (B) OT-II or OT-I cells were activated as in (A) for 4 or 7 days. Differentiated cells were restimulated with anti-CD3 for 4 hours and stained with Vα2-PE antibody and AnnexinV/PI for FACS analysis.

Supplementary Figure 2. T cell activation and differentiation in the absence CD28 and ICOS costimulation. (A) OT-II cells were activated with Ova peptide and irradiated B7/B7h+/+ (WT) or B7/B7h-/- (TKO) APC. 4 days later, differentiated T cells were restimulated with anti-CD3 or anti-CD3/anti-CD28 for 24 hours. Proliferation was assayed 24 hours after treatment by adding [^{3}H]-thymidine to the culture for the last 8 hours. Effector cytokine production was measured by ELISA. All cytokines are at ng/ml. (B) OT-I cells were activated with SIINFEKL peptide and irradiated B7/B7h+/+ (WT) or B7/B7h-/- (TKO) APC for 7 days. 7 days later, differentiated T cells were restimulated with anti-CD3 or anti-CD3/anti-CD28 for 24 hours. Proliferation was assayed 24 hours after treatment by adding [^{3}H]-thymidine to the culture for the last 8 hours. Effector cytokine production was measured by ELISA. All cytokines are at ng/ml.

Supplementary Figure 3. TcR signal transduction in effector and tolerant T cells. (A, B) Effector (Eff) and tolerant (Tol) CD4 cells were generated as described in Supplementary Figure 2A and CD8 cells as in Supplementary Figure 2B. These cells were restimulated with anti-CD3 for 30 min and cytoplasmic fractions from nonrestimulated and restimulated cells were probed

with NFATc1, ERK, p65, IkBα antibodies. β-actin was used as loading control. Relative western blotting signals were inducted.

Supplementary Figure 4. Regulation of T-cell transcriptional factor expression by costimulatory molecules. OT-II (A) or OT-I (B) cells were treated with Ova or SIINFEKL peptide, respectively in the presence of irradiated wild-type or B7-/-B7h-/- APC with a control rat IgG or a blocking antibody to B7S1 (S1) or B7H3 (H3) or PD1. Restimulated OT-II T cells were analyzed for GATA-3, T-bet, Grail mRNA expression and OT-I T cells for T-bet and Grail mRNA expression by real-time PCR. The relative expression of each target gene was determined by normalizing it to GAPDH expression.

Supplementary Figure 5. Expression of PD1 and OX-40 on effector and tolerant T cells. OT-II or OT-I cells were activated with Ova or SIINFEKL peptide, respectively, and irradiated B7/B7h+/+ (WT) or B7/B7h-/- (TKO) APC for 4 or 7 days. Differentiated cells were restimulated with anti-CD3 and stained with Vα2-FITC and PD-1-PE or OX-40-PE antibodies for FACS analysis. Histograms represent PD-1 or OX-40 intensity of Vα2+ populations.

Supplementary Figure 6. Regulation of T cell activation by negative costimulatory molecules. OT-II (A) or OT-I (B) cells were treated with Ova or SIINFEKL peptide, respectively, in the presence of irradiated wild-type (WT) or B7-/-B7h-/- (TKO) APC with a control rat IgG or a blocking antibody to B7S1 (S1) or B7H3 (H3) or PD1. IL-2 production was measured by ELISA 24 hrs after activation. Proliferation was assayed 3 days after treatment by adding [^{3}H]-thymidine to the culture for the last 8 hours.

Supplementary Figure 7. IL-2 restores T cell function in the absence of CD28 and ICOS signaling. CD4 OT-II cells (A) or CD8 OT-I cells (B) were treated with Ova peptide or SIINFEKL peptide, respectively, in the presence of irradiated wild-type or B7-/-B7h-/- APC with or without IL-2. After 4 or 7 days days, T cells were restimulated with PMA/Ionomycin for 5 hrs. Intracellular staining of IFNγ was performed, and then analyzed on gated Vα2+ T cells by FACS.

Supplementary Figure 8. Regulation of CD4 T cell tolerance by negative costimulatory molecules. OT-II cells were treated with Ova peptide in the presence of irradiated wild-type (WT) or B7-/-B7h-/- (TKO) APC with a control rat IgG or a blocking antibody to B7S1 (S1) or B7H3 (H3) or PD1 or combination of S1, H3 and PD1 antibodies. 4 days after activation, T cells were restimulated with plate-bound anti-CD3 for 24 hrs. Proliferation was assayed 24 hours after treatment by adding [^{3}H]-thymidine to the culture for the last 8 hours. Effector cytokine production was measured by ELISA with all parameters indicated in Y-axis at ng/ml.