SUPPLEMENTAL FIGURE 1: Purity of magnetically isolated Treg cells. Treg cells were stained with anti-human FoxP3-PE and appropriate isotype control by intracellular staining and anti-CD25 by surface staining as indicated. Results with 5 donors are shown.

SUPPLEMENTAL FIGURE 2: Treg inhibit proliferation of responder T cells. $1x10^4$ $CD4^+CD25^-$ responder T cells (R) and $2.5x10^3$ Treg (T) or $2.5x10^3$ non-Treg (NT) were co-cultured or cultured alone, and stimulated with T Cell Activation/Expansion Beads. Proliferation was measured by ^{3}H-TdR incorporation after 5 days. One representative experiment out of four is shown.

SUPPLEMENTAL FIGURE 3: TCR stimulation but not TLR pre-treatment increases TLR expression. Untreated or TLR2 ligand pre-treated responder T cells (R, left panel) or Treg (T, right panel) were stained with anti-TLR-1, -2 or -6 mAb or the appropriate isotype controls as indicated before (med) and after TCR stimulation. One representative experiment out of four is shown. The numbers indicate the median fluorescence intensity.

SUPPLEMENTAL FIGURE 4: Treg display different reactivity patterns to TLR2 ligand preincubation. Treg were pre-treated for 24 hours without (black bars) or with 1µg/ml Pam_2CSK4 (light grey bars), FSL-1 (white bars) or Pam_3CSK4 (dark grey bars). For suppression assay, co-culture of $1x10^4$ $CD4^+CD25^-$ responder T cells and $2.5x10^3$ Treg were stimulated with T Cell Activation/Expansion Beads. ^{3}H-TdR-uptake was analyzed after 5 days and suppression was calculated as [(1-cpm of responder with Treg/cpm of responder without Treg) x100]. Ten different experiments with pre-treated Treg are shown ordered by their reaction to Pam_2CSK4 (bars on the left hand side, seven donors), FSL-1 (bars in the middle, four donors) or Pam_3CSK4 (bars on the right hand side, five donors). Significance is indicated as * ($p < 0.05$).

SUPPLEMENTAL FIGURE 5: Responder T cells produce more granzyme B than granzyme A. $1x10^5$ $CD4^+CD25^-$ responder T cells were cultured in medium or stimulated with T Cell Activation/Expansion Beads. Granzyme A and B production were determined by ELISA, and production after stimulation was calculated as fold increase of the medium control. Four different experiments for granzyme A and six different experiments for granzyme B after 72 hours of culture are shown.