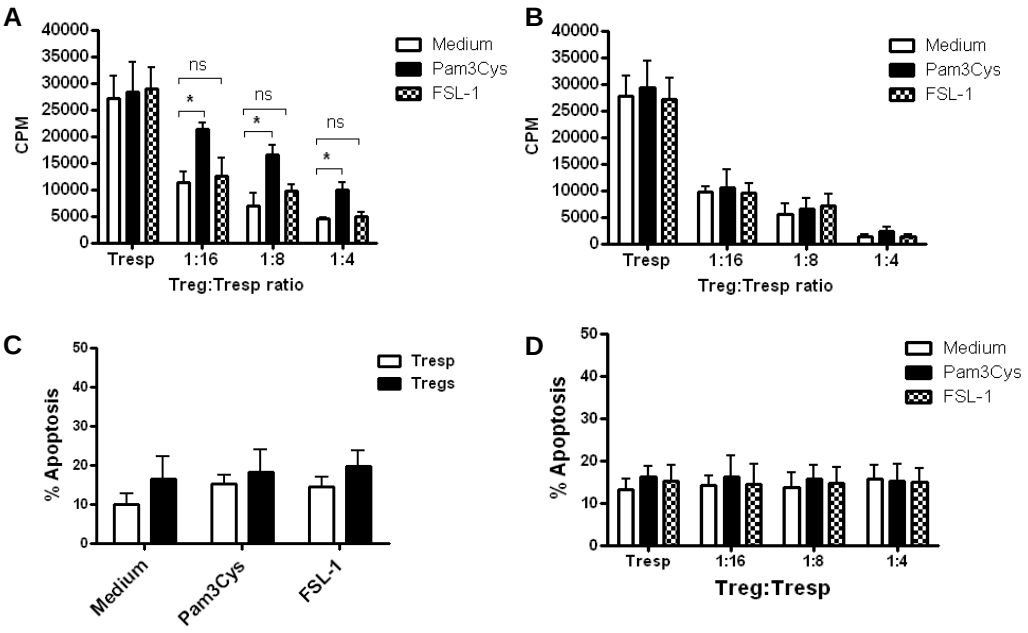
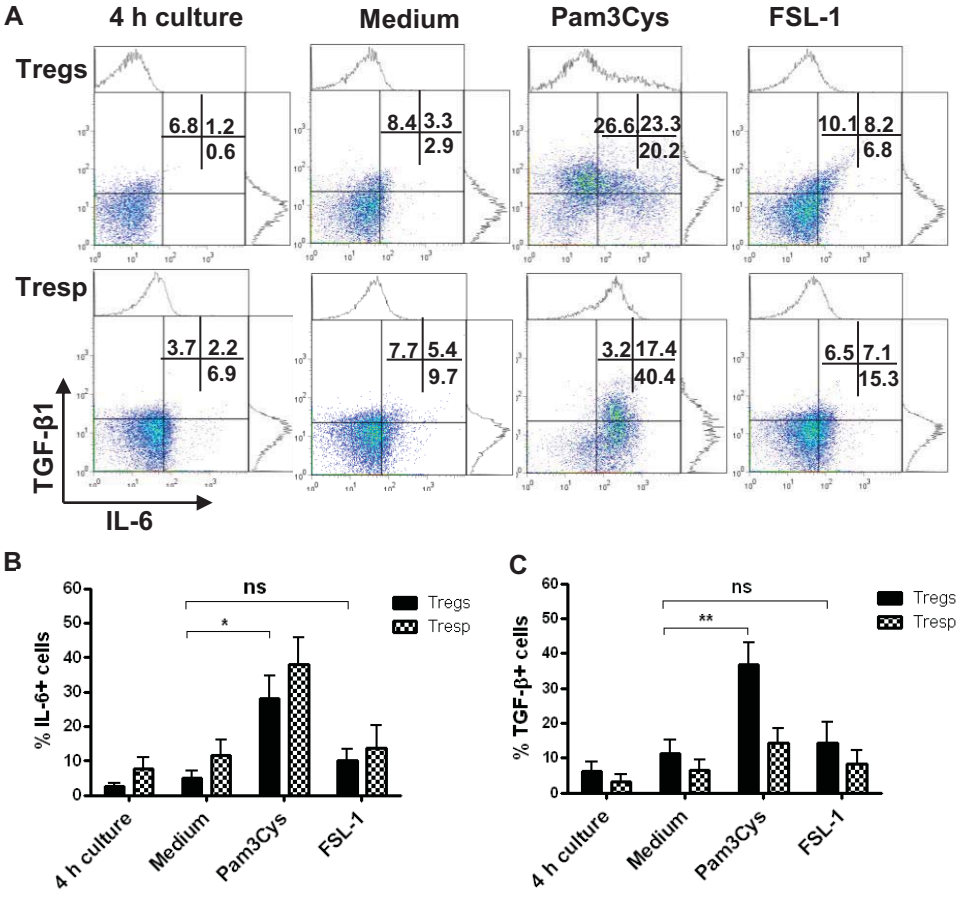
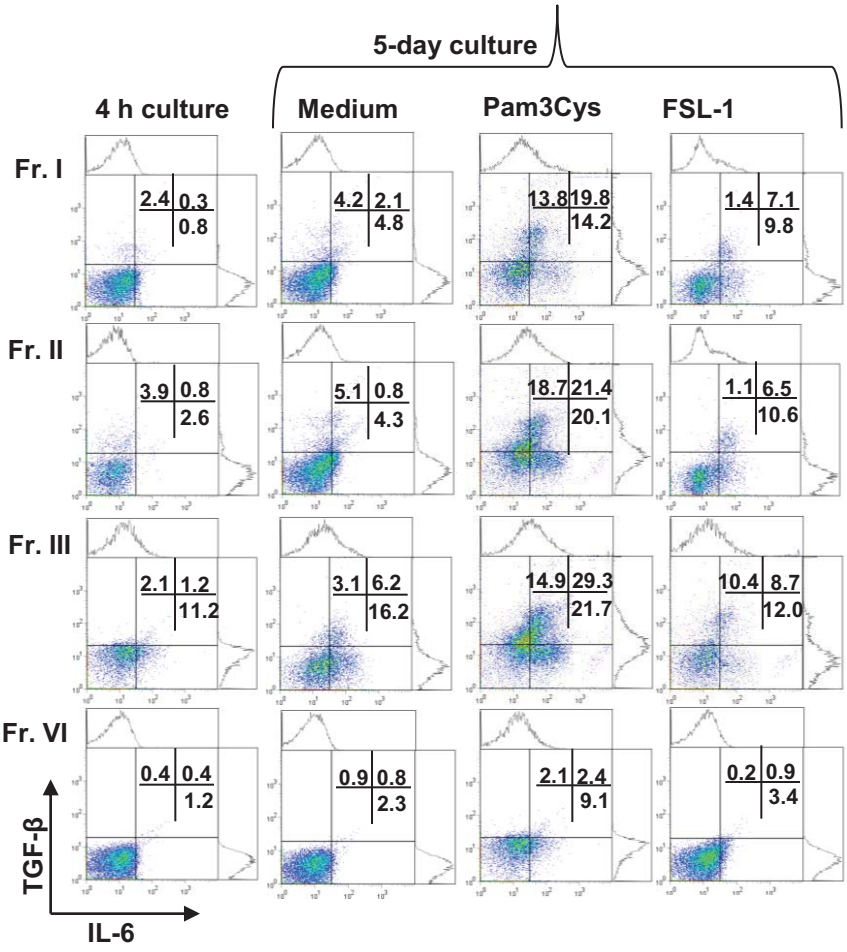


SUPPLEMENTAL FIGURE 1. Preferential activity of Pam3Cys on Tregs. Magnetic bead-purified $CD4^+CD25^+$ Tregs (panel *A*) and $CD4^+CD25^-$ Tresp (panel *B*) (n = 4 each) were pre-incubated with or without Pam3Cys or FSL-1 (5 µg/ml) in separate wells. After 16 h, the cells were washed and then co-cultured with freshly isolated Tresp or Treg cells from the same donor, respectively. Cultures were set in triplicates on plate-bound anti-CD3 and anti-CD28 antibodies (1.0 µg/ml). Cells were incubated for 72 h, then pulsed with [^{3}H]-thymidine and incubated for a further 16 h. Proliferation is expressed as CPM. For cell viability, $CD4^+CD25^+$ Tregs and $CD4^+CD25^-$ Tresp cells were cultured in separate wells (panel *C*) or co-cultured at 1:16, 1:8 and 1:4 Treg/Tresp ratios (panel *D*) on plate-bound anti-CD3 and anti-CD28 antibodies with or without Pam3Cys or FSL-1 (5 µg/ml). After culture for 72 h, cells were harvested and stained with Annexin V and 7-AAD to assess apoptosis by flow cytometry. Numbers represent percentages of Annexin V^{pos}7-AAD^{neg} apoptotic cells. Data represent mean ± SEM of four experiments (*, $P < 0.05$).

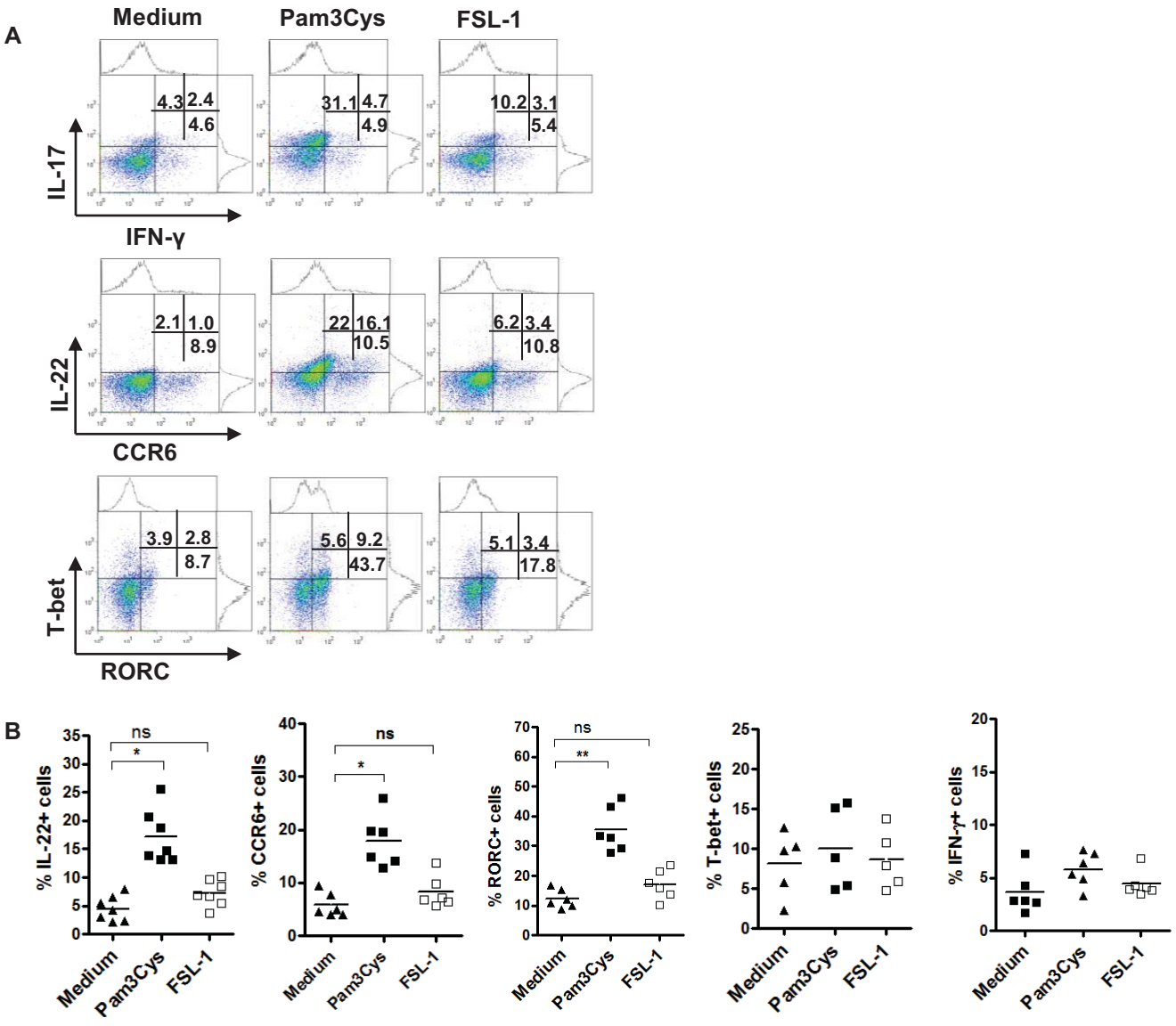


SUPPLEMENTAL FIGURE 2. Expression of IL-6 and TGF-β by Tregs and Tresp.

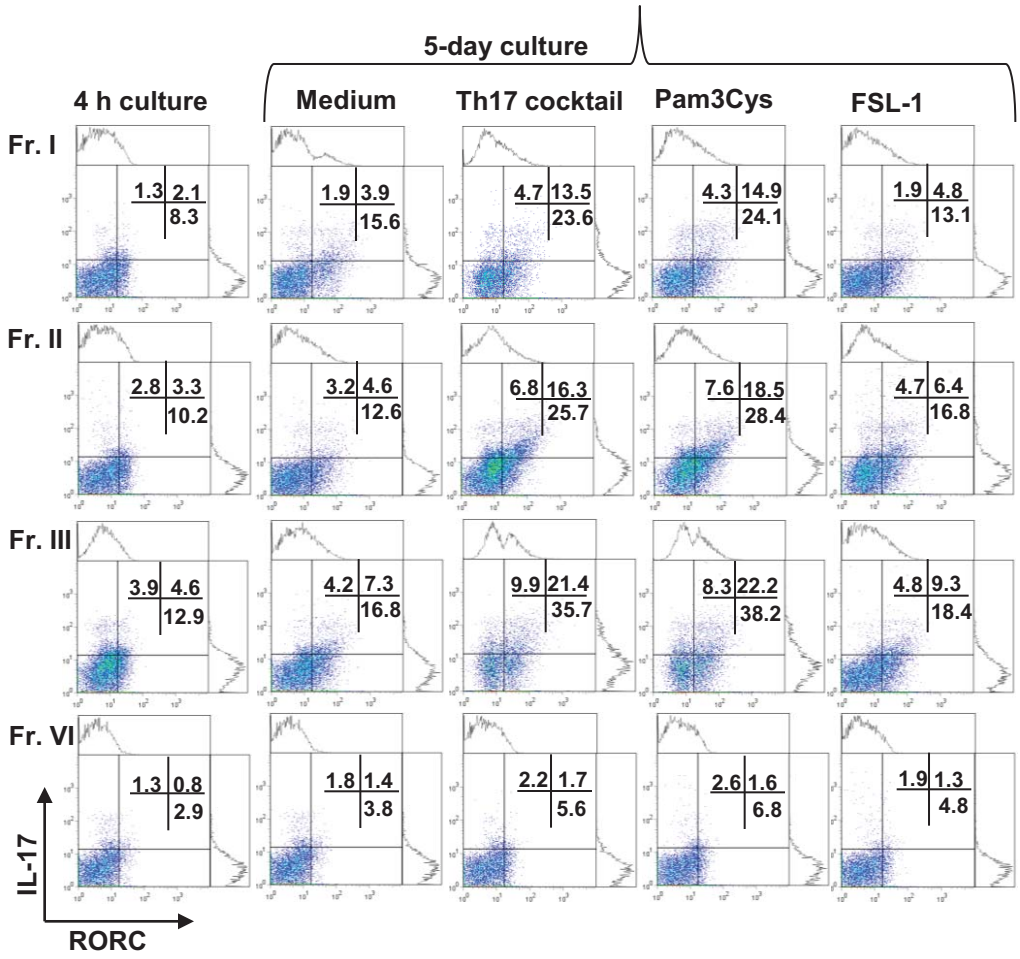
A, FACS-sorted $CD4^+CD25^+CD127^{neg}$ Tregs and $CD4^+CD25^-$ Tresp were cultured for 4 h only (left hand panels, no TLR agonist added) or for 48 h on plate-bound anti-CD3 and CD28 (1µg/ml) in the absence or presence of Pam3Cys or FSL-1 (5µg/ml). Cells were stimulated with PMA/ionomycin in the presence of Brefeldin A during the last 4 h of culture, permeabilized and then intracellularly stained for the expression of IL-6 and TGF-β. One representative experiment out of six is shown. *B-C,* Data represent average percentages of $IL\text{-}6^+$ and $TGF\text{-}\beta^+$ Tregs and Tresp cultured in the absence or presence of Pam3Cys or FSL-1 ($n = 6$; *, $P < 0.05$; ** $P < 0.01$).



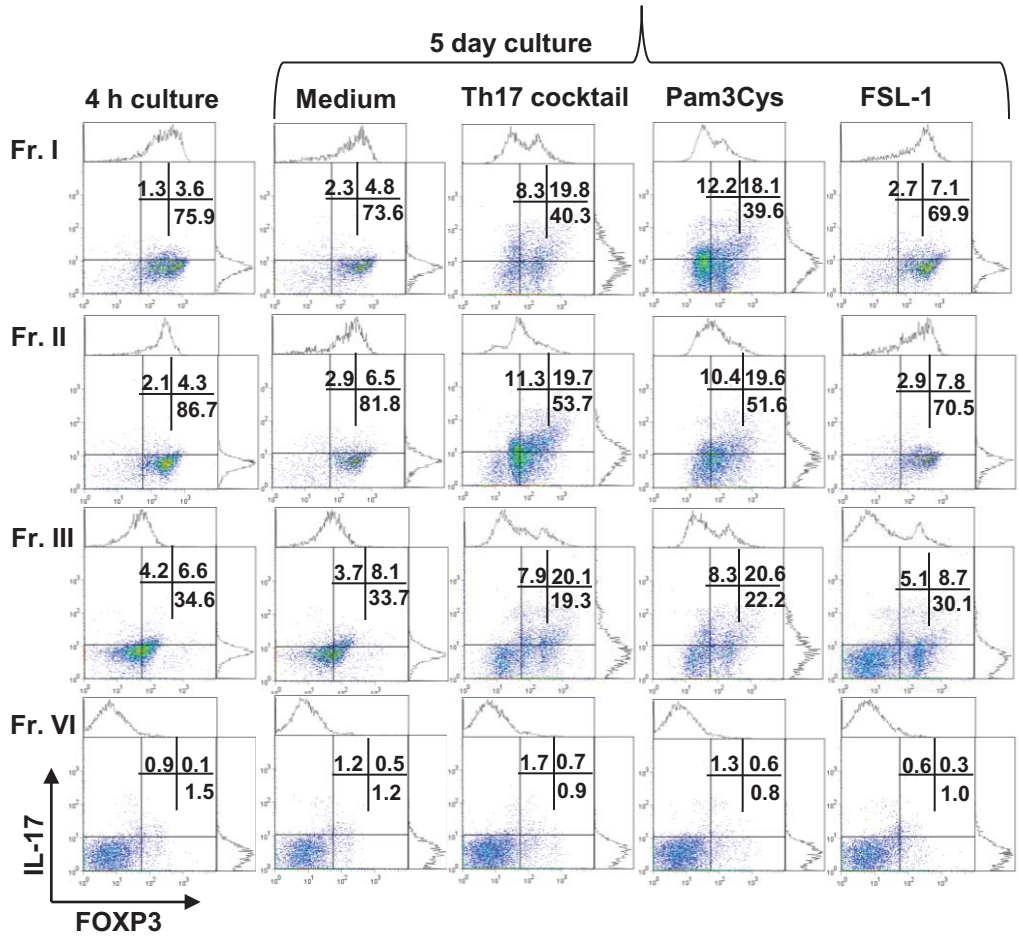
SUPPLEMENTAL FIGURE 3. Expression of IL-6 and TGF-β by subpopulations of $CD4^+$ T cells. FACS sorted $CD4^+CD45RA^+CD25^{++}$ (Fr. I), CD4+$CD45RA^-CD25^{+++}$ (Fr. II), $CD4^+CD45RA^+CD25^{++}$ (Fr. III) and $CD4^+CD45RA^+CD25^-$ (Fr. VI) cells were cultured separately for 4 h or for 72 h on plate-bound anti-CD3 and CD28 (1 µg/ml) in the absence or presence of Pam3Cys or FSL-1 (5 µg/ml). All cells were stimulated with PMA/ionomycin in the presence of brefeldin A during the last 4 h of culture then stained for the intracellular expression of IL-6 and TGF-β. Representative plots of one donor from six subjects (see Fig. 3) are shown.



SUPPLEMENTAL FIGURE 4. TLR2 stimulation induces a Th17-like phenotype in Tregs. *A*, FACS-sorted $CD4^{+}CD25^{hi}CD127^{neg}$ Tregs were cultured for 72 h on plate-bound anti-CD3 and anti-CD28 Abs (1μg/ml) in the absence or presence of 5μg/ml Pam3Cys or FSL-1. PMA / ionomycin and brefeldin A were added during the last 4 h of culture. Cells were then stained for surface expression of CCR6 and intracellular expression of IL-22, IFN-γ, RORC and T-bet. Representative plots of one donor from six subjects is shown. *B*, Data represent percentages of Tregs expressing IL-22, CCR6, IFN-γ, RORγt and T-bet after culture in the absence or presence of Pam3Cys or FSL-1 (n = 6; * $P < 0.05$; ** $P < 0.01$).



SUPPLEMENTAL FIGURE 5. TLR2 stimulation enhances IL-17 production by subpopulations of $CD4^+$ T cells. FACS-sorted $CD4^+CD45RA^+CD25^{++}$ (Fr. I), $CD4^+CD45RA^-CD25^{+++}$ (Fr. II), $CD4^+CD45RA^-CD25^{++}$ (Fr. III) and $CD4^+CD45RA^+CD25^-$ (Fr. VI) cells were cultured separately for 4 h with PMA/ionomycin and brefeldin A (left-hand panels) or for 72 h on plate-bound anti-CD3 and anti-CD28 in the absence or presence of a Th17 cocktail of cytokines and antibodies (10 ng/ml IL-1β, 30 ng/ml IL-6, 10 ng/ml IL-23, 0.5 ng/ml TGF-β and neutralising anti-IL-4 and anti-IFN-γ; 10 μg/ml), Pam3Cys, or FSL-1 (5 μg/ml). Cells were stimulated with PMA/ionomycin in the presence of brefeldin A during the last 4 h of culture. Cells were stained for the intracellular expression of IL-17 and RORC. Representative plots of one donor from six subjects (see Fig. 5) are shown.



SUPPLEMENTAL FIGURE 6. TLR2 stimulation reduces FOXP3 production by subpopulations of $CD4^+$ T cells. FACS-sorted $CD4^+CD45RA^+CD25^{++}$ (Fr. I), $CD4^+CD45RA^-CD25^{+++}$ (Fr. II), $CD4^+CD45RA^-CD25^{++}$ (Fr. III) and $CD4^+CD45RA^+CD25^-$ (Fr. VI) cells were cultured separately for 4 h with PMA/ionomycin and brefeldin A or for 72 h on plate-bound anti-CD3 and anti-CD28 in the absence or presence of a Th17 cocktail of cytokines and antibodies as described in the *Materials and Methods*. Cells were stimulated with PMA/ionomycin in the presence of brefeldin A during the last 4 h of culture then stained for the intracellular expression of IL-17 and FOXP3. Representative plots of one donor from six subjects (see Fig. 5) are shown.