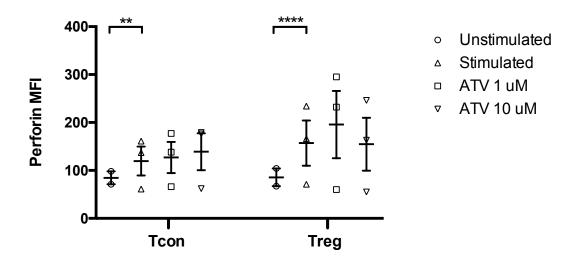


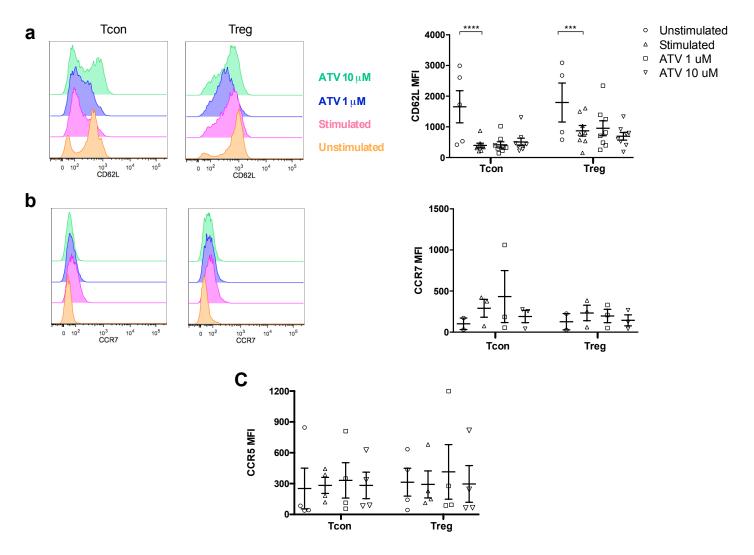
Tcon

Treg

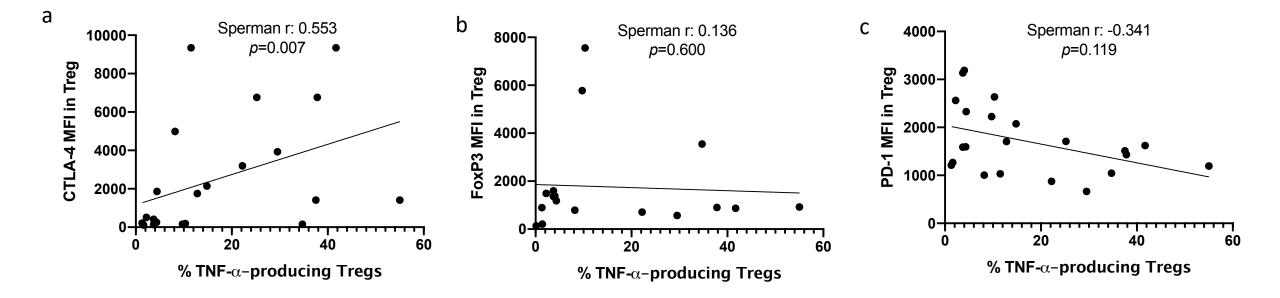
through magnetic separation from PBMCs of healthy donors and cultured during 48 h under TCR activation as it was mentioned on material and methods. Viable cells were determined as 7-AAD and DIOC-6+ cells (n=3) donors) (a). Representative flow cytometry plot showing live and dead isolated T cells (Tcon and Treg) by using Live/dead fixable cell stain (b). Percentage analysis of live cells in subsets of T con and Treg (c). No statistical significant differences were found (n=4).



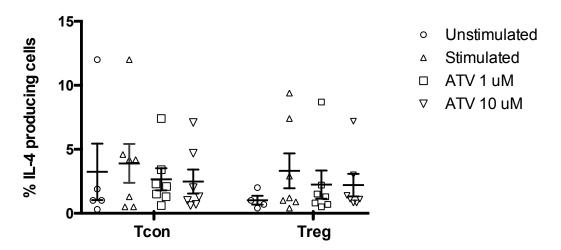
Supplementary Figure 2. Mean fluorescence intensity (MFI) analysis of perforin (n=3 donors) on Tcons and Tregs in basal conditions and after activation with anti-CD3, anti-CD28 and IL-2 in the absence or presence of the indicated concentrations of ATV for 48 h. No significant differences were found between groups.



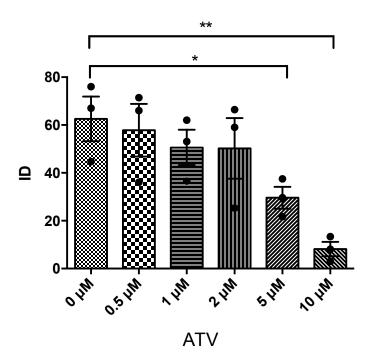
Supplementary Figure 3. Representative flow cytometry histogram and MFI analysis of CD62L (n=8 donors) (a), CCR7 molecules (n=3 donors) (b), and CCR5 (n=4 donors) in Tcons and Tregs in basal conditions and after activation with anti-CD3, anti-CD28 and IL-2 in the absence or presence of the indicated concentrations of ATV for 48 h. Statistical analyses were performed using the GLM ANOVA, Dunnett's post-hoc tests. Mean and SEM. ****p<0.0001, ***p<0.0002. Each dot represents one individual in the graph.



Supplementary Figure 4. Correlation of TNF-a-producing Tregs percentage with CTLA-4 MFI (a), FoxP3 MFI (b), and PD1 MFI (c) in Treg cells. Spearman's rank correlation coefficients (r) and p values (P) are indicated.



Supplementary Figure 5. Toons and Tregs were isolated by cell sorting and stimulated with anti-CD3, anti-CD28 and IL-2 in the absence or presence of the indicated concentrations of ATV for 48 h. Cells were re-stimulated with PMA/ionomycin in the presence of brefeldin A for 5 h. Then, they were stained with monoclonal antibodies to detect the percentage of cells expressing IL-4 (n=7 donors). No significant differences were found between groups.



Supplementary Figure 6. Untouched total CD4⁺ T cells were isolated through magnetic separation from PBMCs of healthy donors and they were labeled with CFSE at 1.25 μ M . Cells were washed twice with PBS and put in co-cultured in 96-well round-bottom plates at a ratio 1:1 in the presence of CD3/CD28/IL-2 for 72 hours and different atorvastatin (ATV) concentrations. Proliferation of CD4⁺ T cells was detected as the dilution of CFSE on flow cytometry and Index division (ID) was calculated using FlowJo software. (n=3 donors). Statistical analyses were performed using One-Way ANOVA, Dunnett's post hoc test. Mean and SEM. *p=0.011, **p=0.001.