*Supplemental Information*

# Homogeneous Expansion of Human T-Regulatory Cells Via Tumor Necrosis Factor Receptor 2

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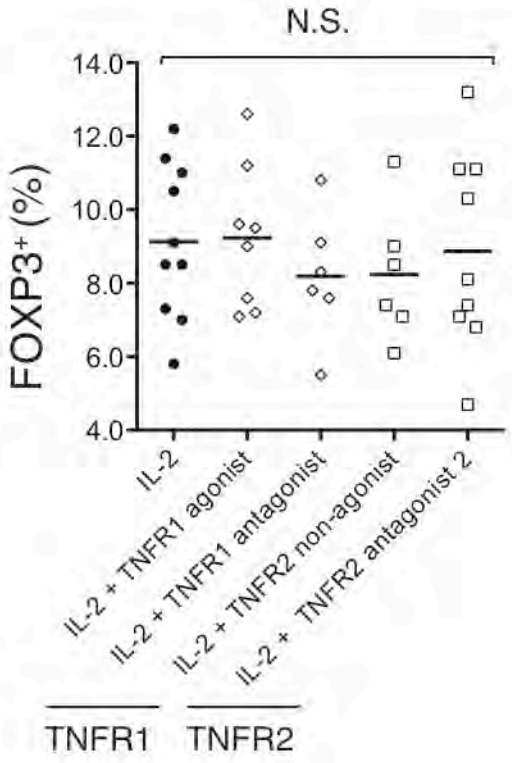



**Figure S1. Induction of FOXP3 expression by different TNFR receptors.** Screening TNFR1 and TNFR2 mAbs reveals that few induce or inhibit FOXP3$^+$ expression.

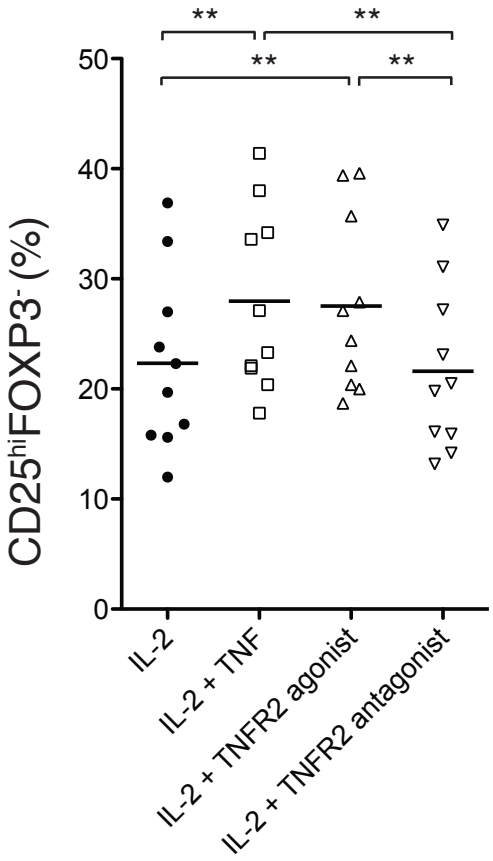



**Figure S2a.** Proportion of $CD25^{+}FOXP3^{-}$ cells after IL-2 overnight incubation with and without the TNFR2 agonist or antagonist. Significant percentage increases were observed if the treatment group was incubated in presence of TNF or TNFR2 agonist. (**; $p < 0.001$). Data are of samples from 10 subjects.

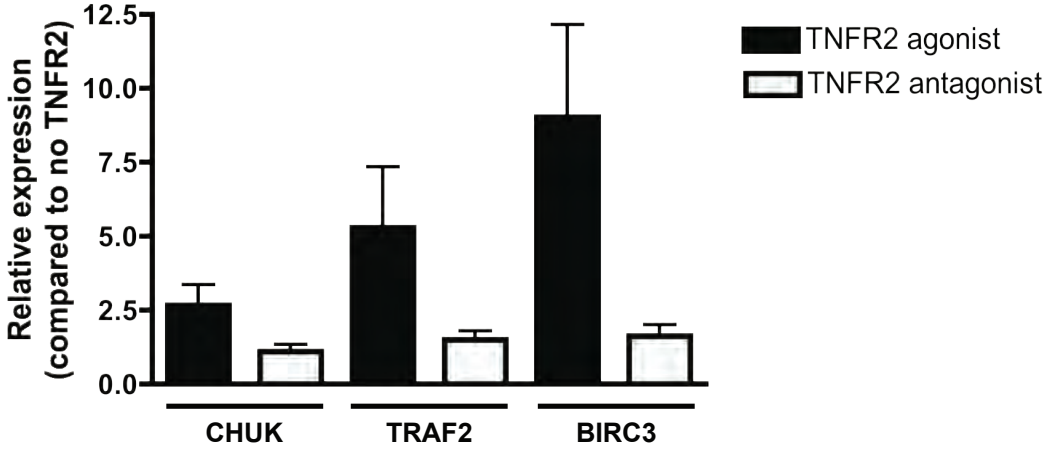



**Figure S2b.** TNFR2 agonist- versus antagonist-treated Tregs show opposing effects in vitro in a signaling pathway assay. Purified CD4+ cells, incubated with IL-2, the TNFR2 agonist and antagonist trigger differences in relative downstream expression of mRNA, especially in signaling proteins CHUK, TRAF2 and BIRC3 (cIAP2) that are preferentially induced by TNFR2 agonism. Data represented are means ± SEM from 32 subjects for the TNFR2 agonist and 8 subjects for the TNFR2 antagonist.

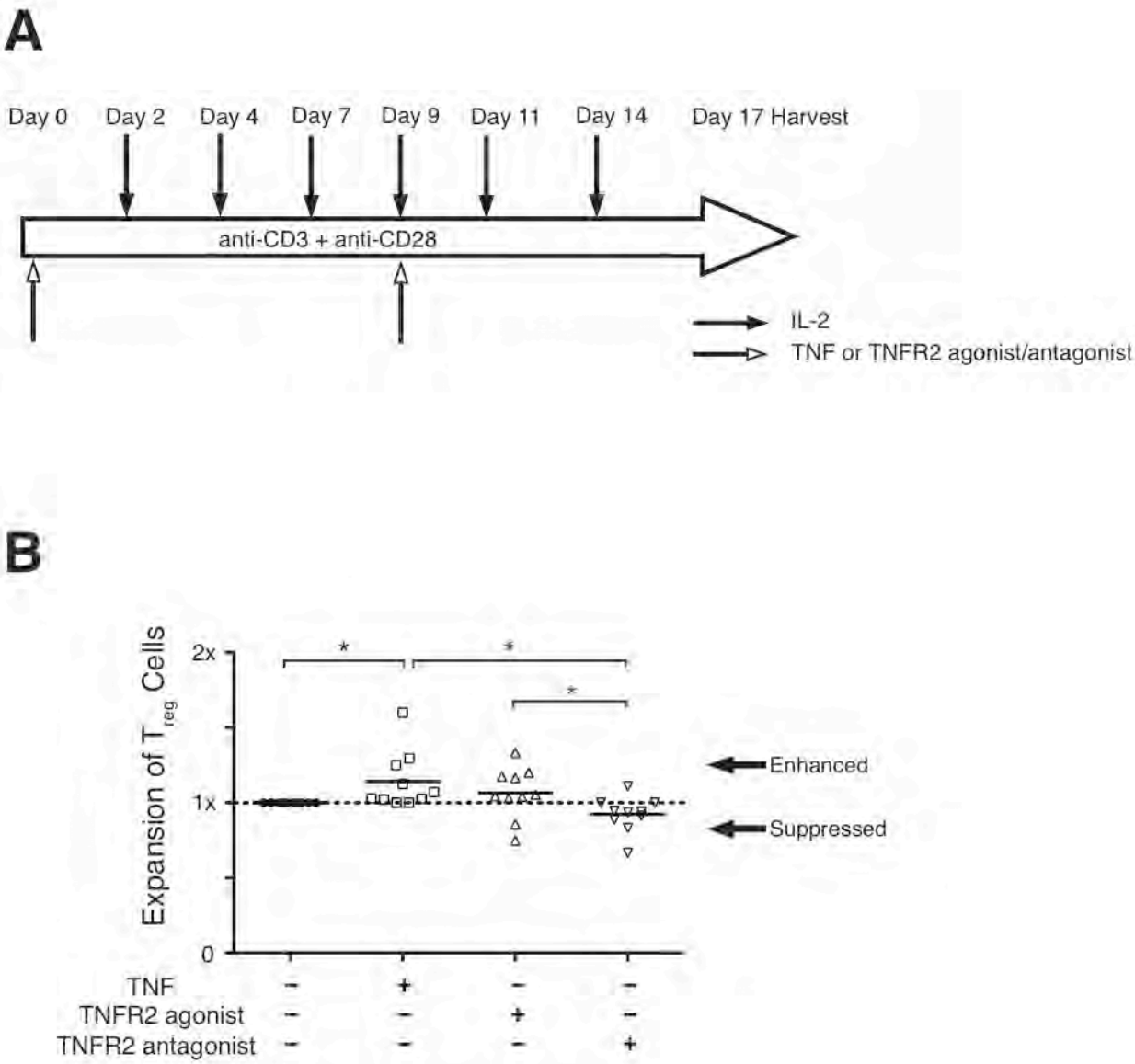



**Figure S3. Expansion of CD4⁺CD25⁺ cells without rapamycin.** Expansion protocol is shown in (A). After 16 days of expansion, Tregs Expander Beads were removed and rested overnight for cell counting. Magnitude of expansion by each treatment group is shown in figure (B). (*; $p < 0.05$, by paired t test) Data in (B) are of samples from10 subjects.

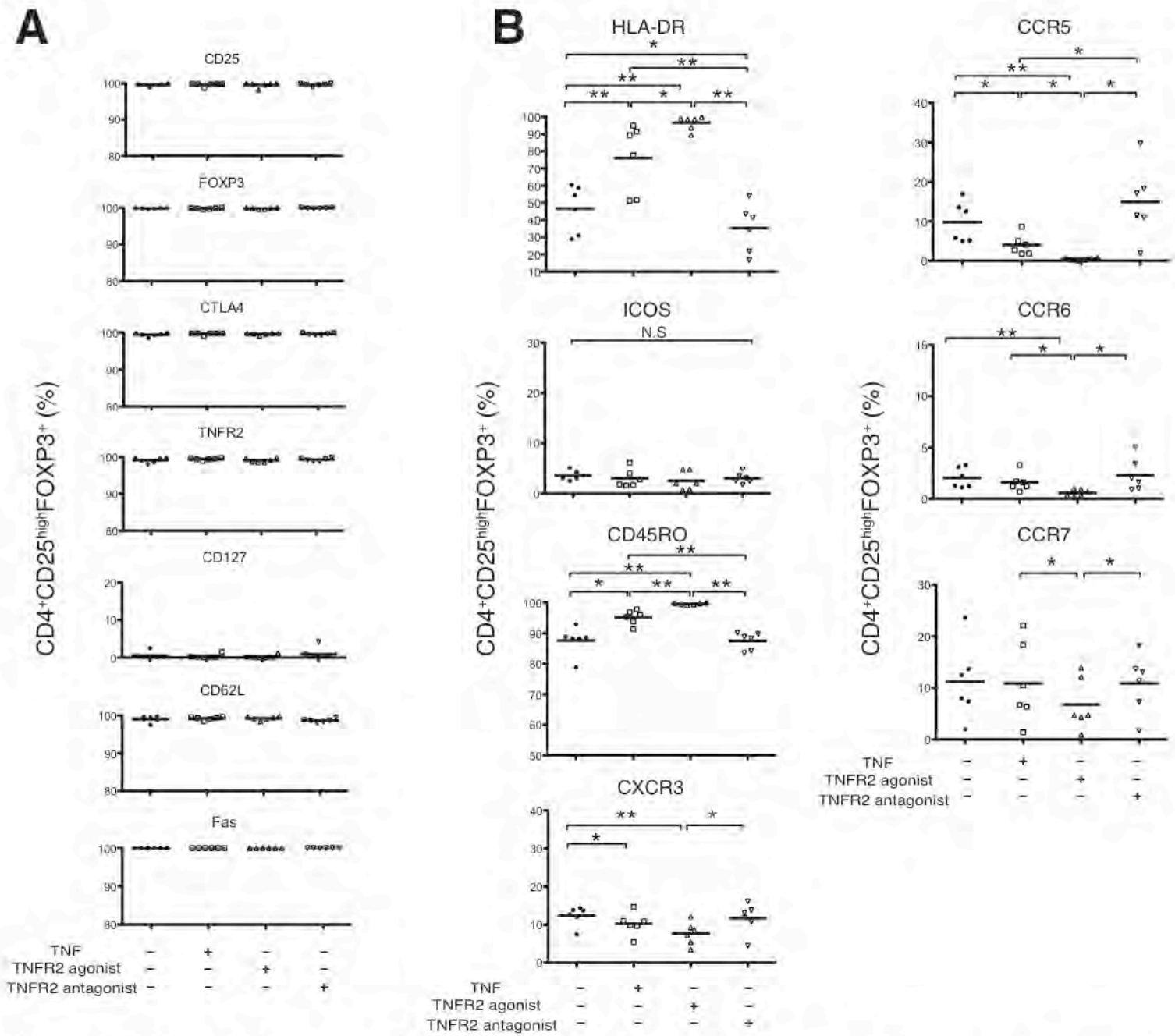
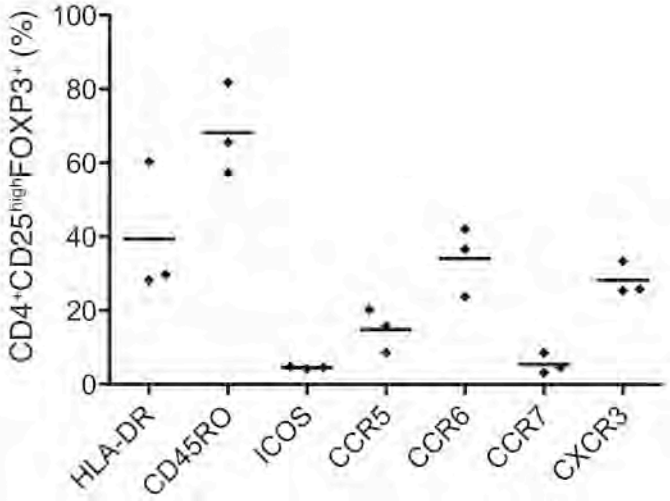



**Figure S4. Phenotypes of expanded Tregs without rapamycin.** All treated groups are positive for Treg markers such as CD25, FOXP3, CTLA4, TNFR2, CD62L and negative for CD127. All cells were positive for Fas (A). Some surface markers showed diverse patterns of expression according to the way the cells were expanded with a similar trend compared to cells expanded using rapamycin (B). (*; $p < 0.05$, **; $p < 0.01$) determined by paired t test). Data are from 6 subjects.

A




B

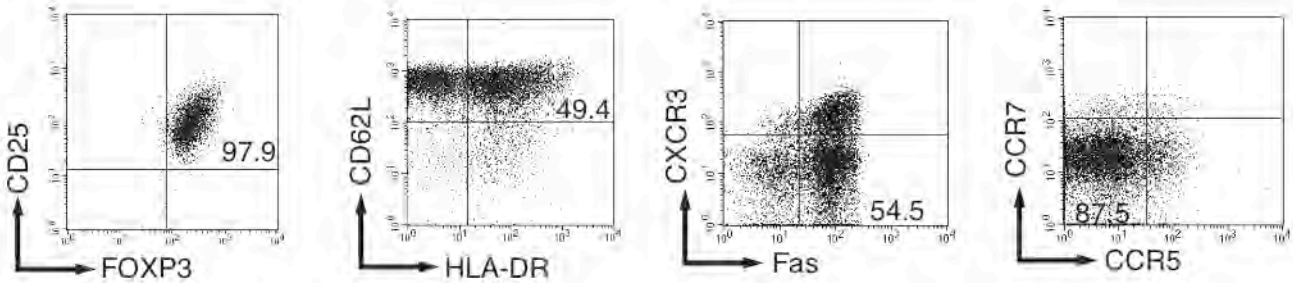



**Figure S5. Representative surface markers on fresh human Tregs.** (A) Phenotypes of freshly separated Tregs before expansion (N=3, samples from 3 subjects). (B) Representative flow diagrams of Tregs markers before expansion.

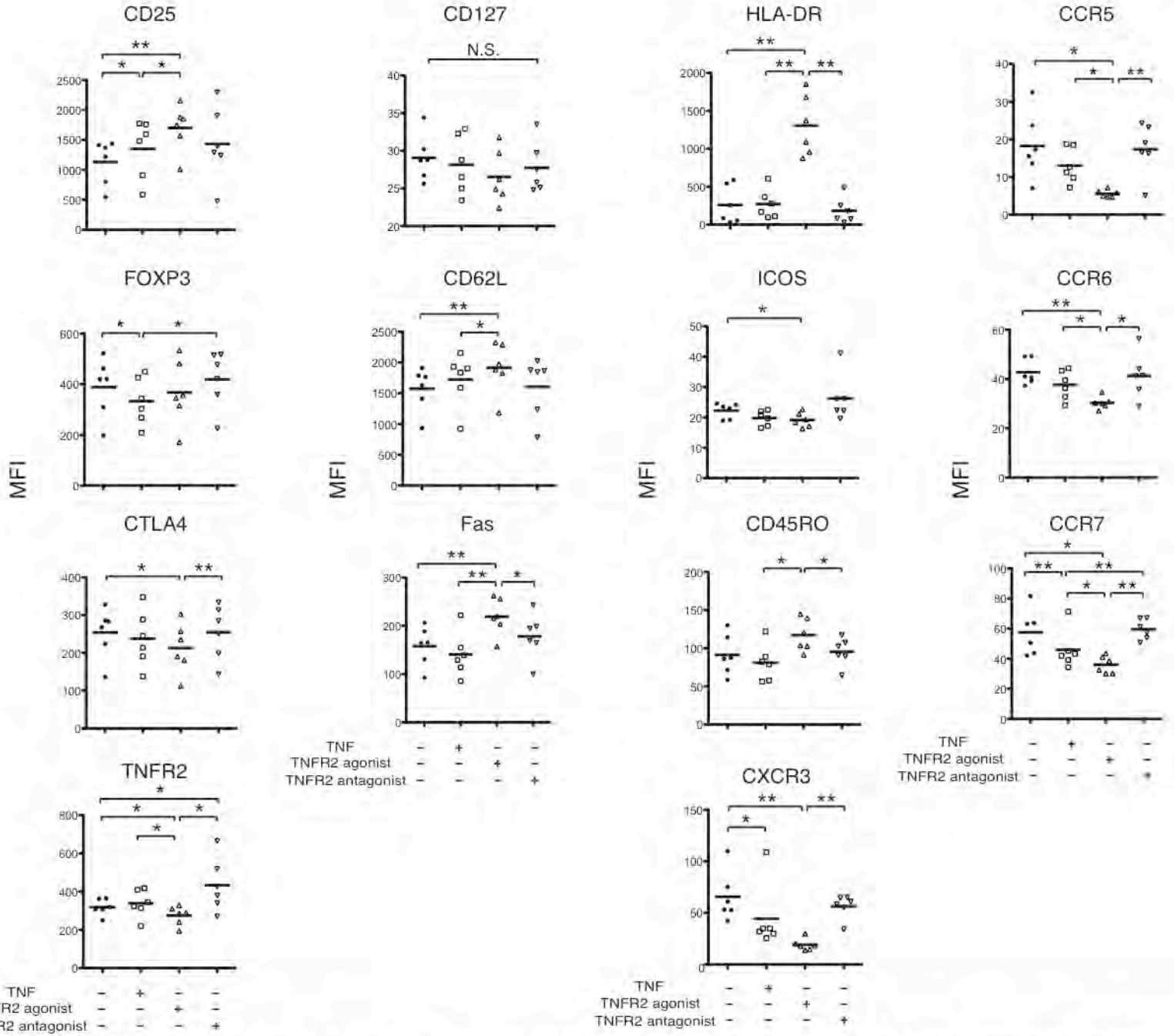



**Figure S6. The density of cell surface markers measured by Mean Fluorescence Intensity (MFI).** MFI of Tregs demonstrates clear differences between TNFR2 agonist expanded cells and TNFR2 antagonist expanded cells. (* $p < 0.05$, ** $p < 0.01$ determined by paired t test)

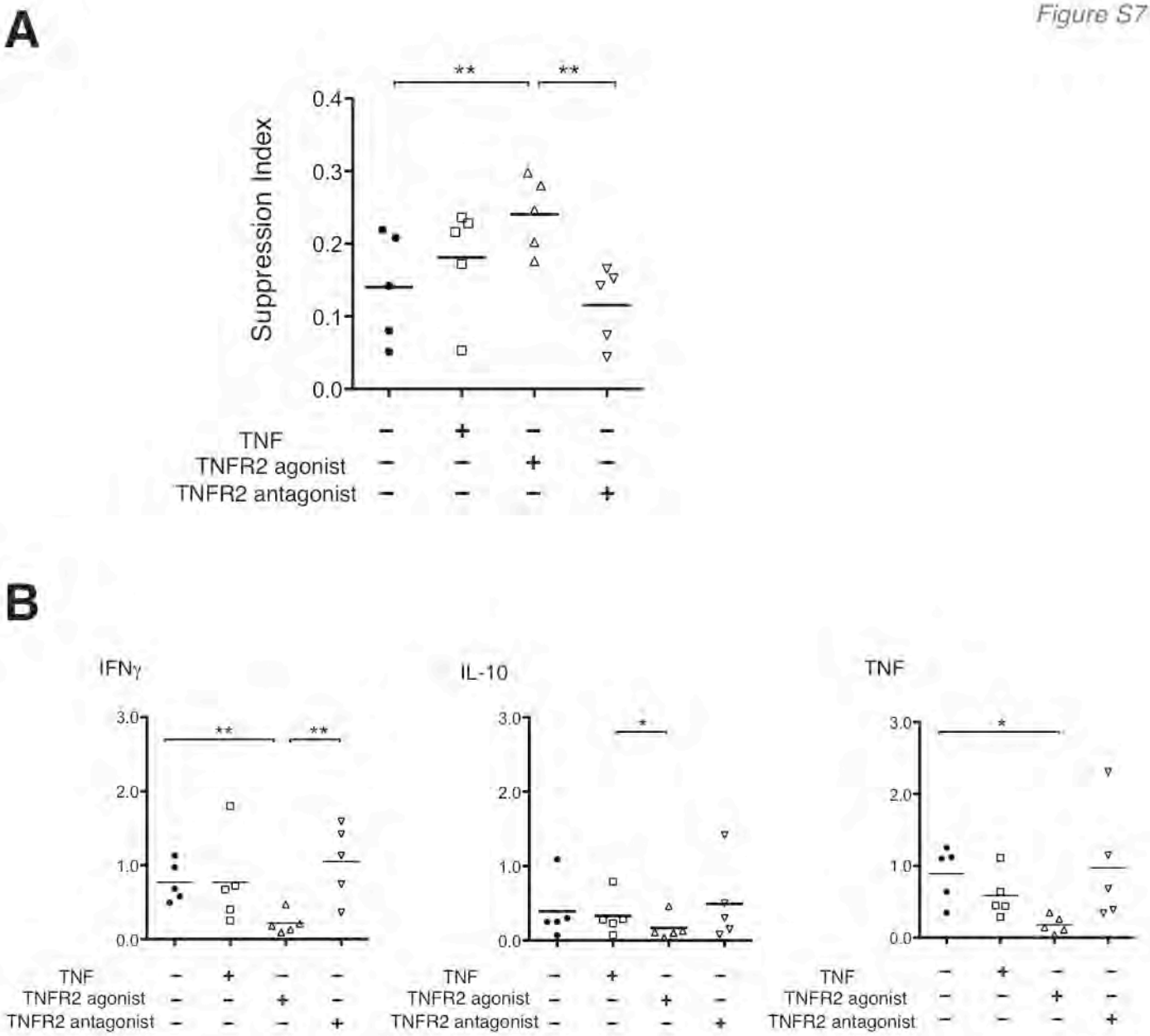



**Figure S7. Functional differences of expanded CD4⁺CD25⁺ cells incubated without rapamycin.** Suppression capacity of expanded CD4⁺CD25⁺ cells was determined by CFSE dilution of CD8⁺ T responder cells. Flow cytometric figures of a typical result and summary of suppression index calculated based upon Responder:Treg of 2 : 1 from four independent experiments is also shown in (A). CD4⁺CD25⁺ cells expanded with TNFR2 agonist exhibited significant enhanced suppression capacity (N=5). Those cells showed lowest cytokine producing capacity. (IFN, IL-10 and TNF after stimulation with PMA and ionomycin for 24 hours (B).(*; $p < 0.05$, **; $p < 0.01$, by paired t test).