Description of Supporting Information

Figure S1: Calculation of Treg function (AUC) for standardized suppression. (A) Raw data showing CFSE-evaluated percent of dividing CD4+ responders in suppression assays from 3 patients, 0110L (very impaired Treg function), 0109L (intermediate Treg function) and 0211L (normal Treg function). (B) To make results of different suppressive assays comparable and to minimize inter-assays variability, the divisions of CD4+ responders pictured at (A) were recalculated as standardized suppressions using formula showed in the figure (B). Below is an example of raw Teffs divisions data and corresponding standardized suppressions for patient 0211L. (C) The same data as (A) showed as standardized suppressions. (D) Area under standardized suppression curve (AUC) for patient 0211L showed as a hatched field. This area, calculated as AUC using statistic software *GraphPad Prism 5.00* (default settings) in the current example is equal 374.8. For comparison, AUC of Tregs from 0110L patient was equal 21.93 and AUC from 0109L patient was equal 170.2. As a result of such calculations, we had results of Treg suppressive functions from all Treg/Teffs ratios from our patients expressed as one single number suitable for further comparisons (more AUC – better suppressive function).

Figure S2: Patterns of FOXP3, CD25 and CD127 expression in healthy donor and patient Tregs. (A) After isolation, aliquots of patient Tregs were stained for CD4, CD25, CD127 and FOXP3, and CD25+ and FOXP3+ expression in those cells (37 samples) were compared with data from 13 healthy donor Tregs isolated using the same kit by the same person. (B) Isolated Tregs were plotted as CD25 vs. CD127 expression and then the FOXP3+ subset (left column) or FOXP3- subset (right column) was back-gated to the current plots to analyze the location of FOXP3+ and FOXP3- in CD25 and CD127 subsets. **Top**: example of healthy donor Tregs, **bottom**: 6 different patient Treg isolates are shown as an example of the altered distributions of cells indicated. CD25+ are gated and percent of CD25+ cells showed.

Figure S3: FOXP3 expression in isolated Tregs has no correlation with suppressive function. No correlations were seen between FOXP3 expression and suppressive function when aliquots of patient Tregs (n=30) were stained for FOXP3 and data plotted versus results of suppressive assays of these cells using CD4 (left) or CD8 (right) responders. This is similar to data we described for healthy donor Tregs (11).

Figure S4: Comparison of Tregs with FOXP3/TSDR ratios > 1 and Tregs with FOXP3/TSDR ratios < 1. Tregs with FOXP3/TSDR ratios >1 were more suppressive (A), and had higher CTLA4+ expression (B), than Tregs with FOXP3/TSDR ratios <1. Ratio between FOXP3+ Tregs to TSDR-demethylated Tregs (FOXP3/TSDR) was calculated in boys whose Tregs contained >40% of FOXP3+ cells after isolation, 9 patients.