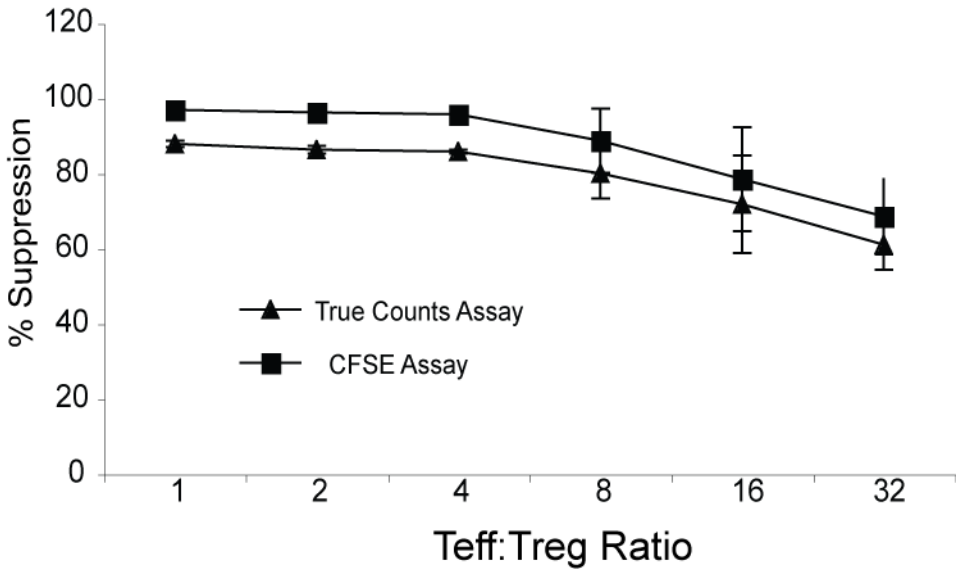

Figure S1: Comparison of bead-based and CFSE-based detection of suppressive activity. Primary human Tregs were isolated from cord blood samples, stimulated with anti-CD3/28 coated beads and transduced with lentiviral vectors encoding the A2-SL9 TCR awt/b6 (43% transduction). Cell were restimulated with K.A2.SL9.41BBL (reaching 78 % tetramer +) and upon resting used in suppression assays. Thawed PBMC were CFSE labeled, mixed with transduced Tregs at various ratios and stimulated with anti-CD3 beads. Five days later, cells were transferred to FACS tubes, anti-CD8 APC labeled mAb, and CountBright absolute counting beads (Invitrogen) were added. The assay was read in two different ways: CFSE dilution of CD8 T cells and the absolute number of CD8 T cells. Percent suppression was calculated using the formula: 1- #daughter cells in suppressed condition/#daughter cells in unsuppressed condition x 100. For CFSE reading, the number of proliferating cells in suppressed and unsuppressed conditions was determined using FloJo software. The error bars indicate standard deviation of 4 replicates. The assays were repeated with 2 donors.