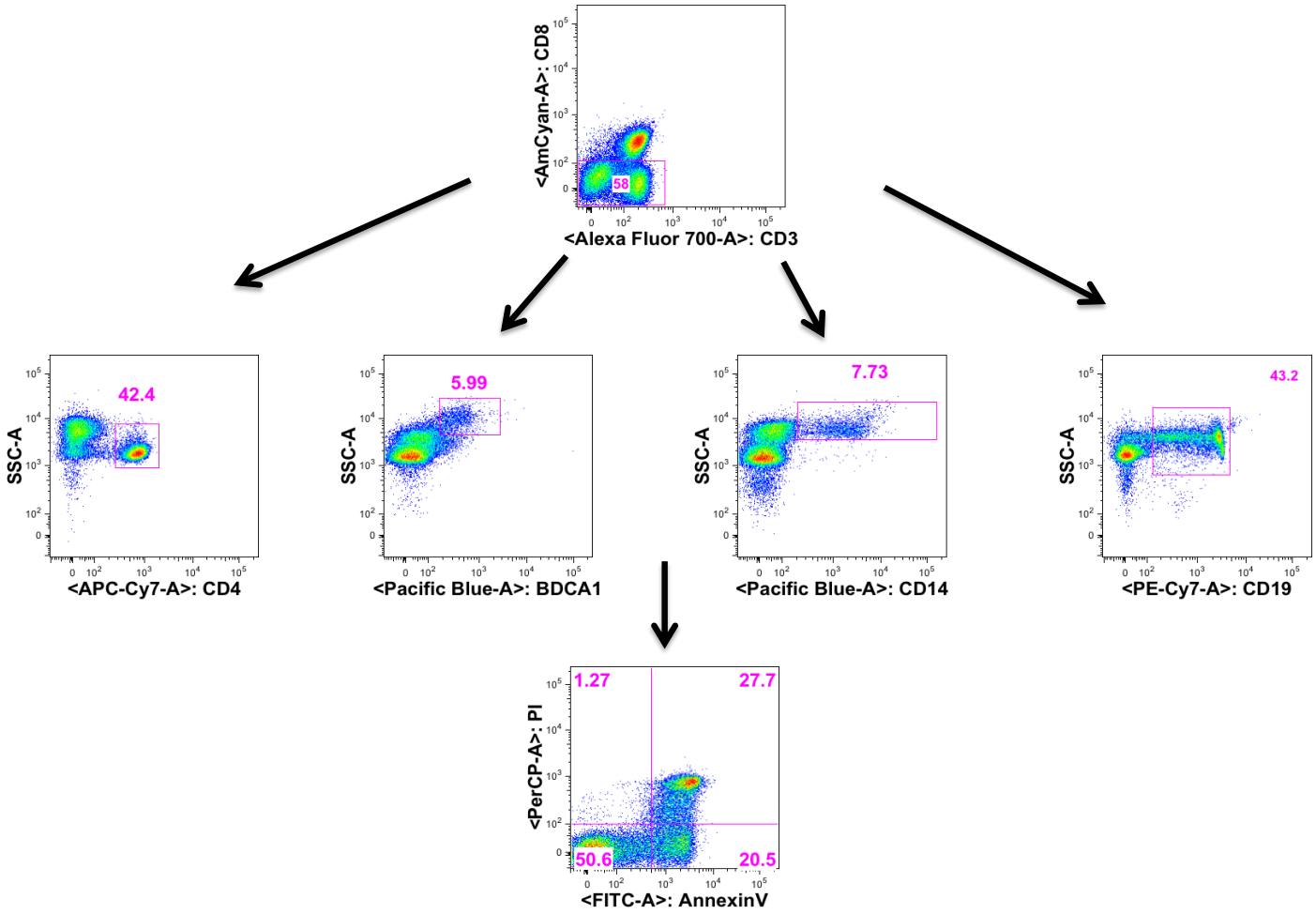
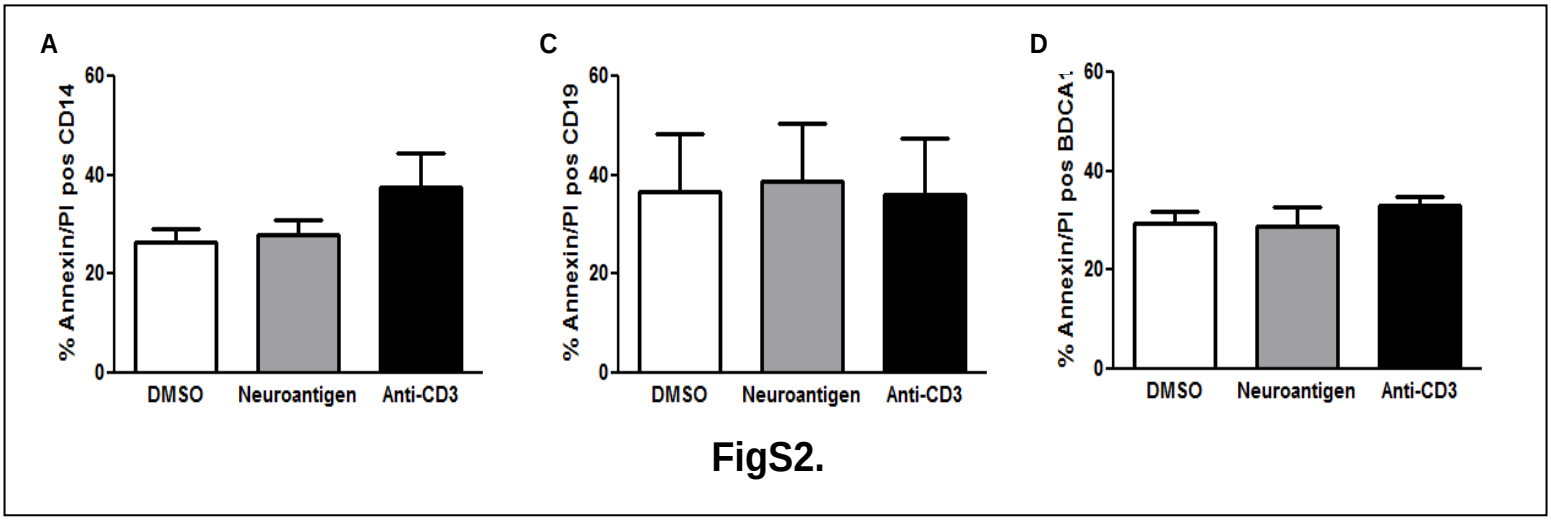


FigS1.

Figure S1: Gating strategy for flow cytometry-based cytotoxic assays. CD8+ T cells CD4+CD25- T cells, monocytes (CD14+), B-cells (CD19+) and myeloid dendritic cells (BDCA1+) were isolated from healthy PBMCs. CD4+CD25- T cells were co-cultured with CD8+ T cells and individual subsets of APCs in the presence or absence of indicated antigenic stimulus. At 72hrs, cultures were collected and stained for CD3, CD8, CD4, CD14, CD19 and BDCA1. Cells were initially gated for CD3+CD8- and CD3-CD8- subsets. CD3-CD8- cells were subsequently gated for either monocytes (CD14+), B cells (CD19+), or myeloid dendritic cells (BDCA1+). CD3+CD8- cells were gated for CD4+ T cells as shown in row2. All selected subsets were finally analyzed for annexin V/PI staining as shown in row 3.



FigS2.

Figure S2: CD8+ Tregs mediated suppression does not involve elimination of APCs. CD4+CD25- T -cells were co-cultured with CD8+ T cells and individual subsets of APCs in the presence and absence of indicated antigenic stimulus. At 72hrs, cultures were collected and frequency of annexing V/PI positive staining was assessed in individual cell subsets. None of the APC subtypes were significantly targeted for apoptosis in the presence of neuroantigen-specific T cells. Five independent experiments were performed with PBMCs from 5 healthy subjects.

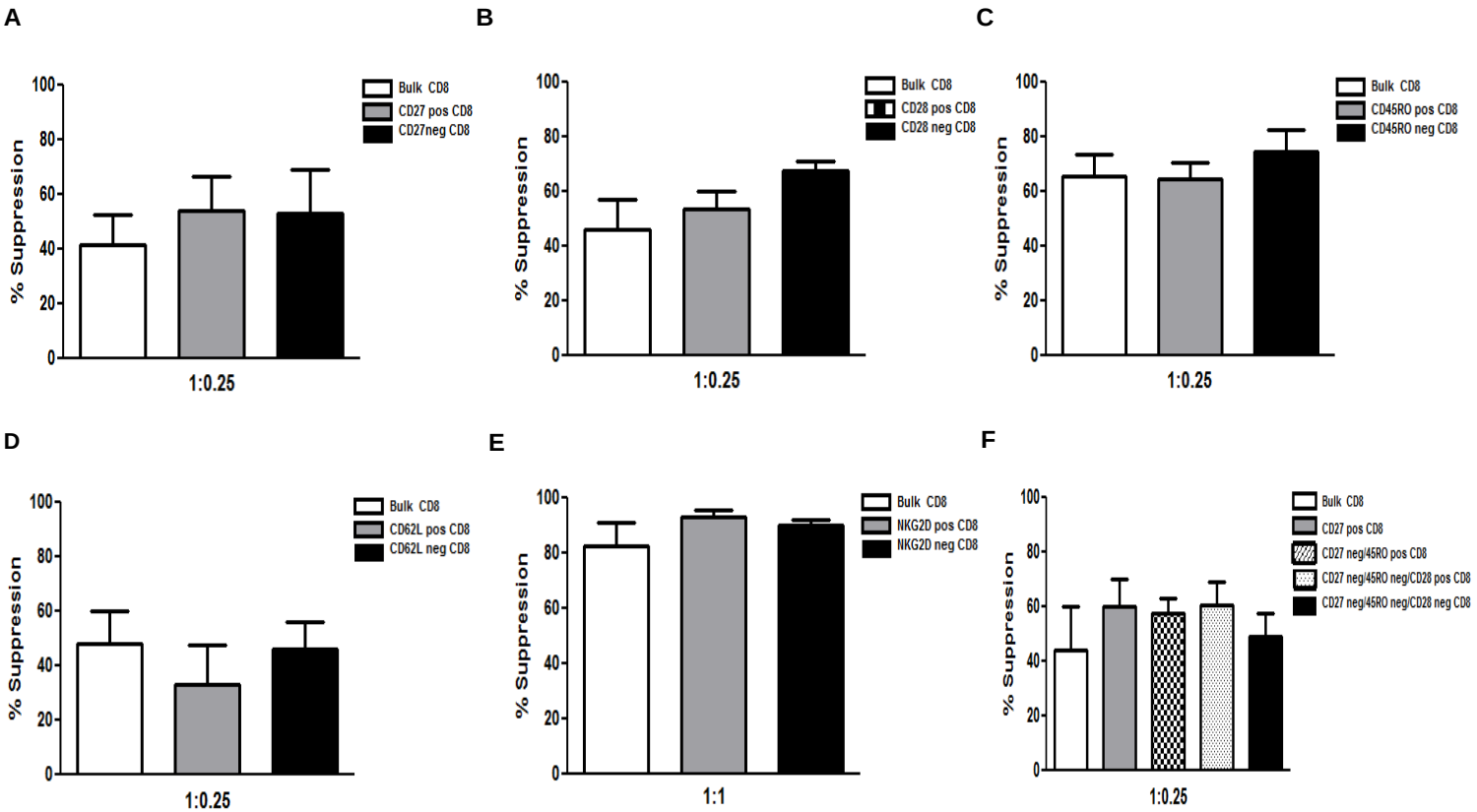
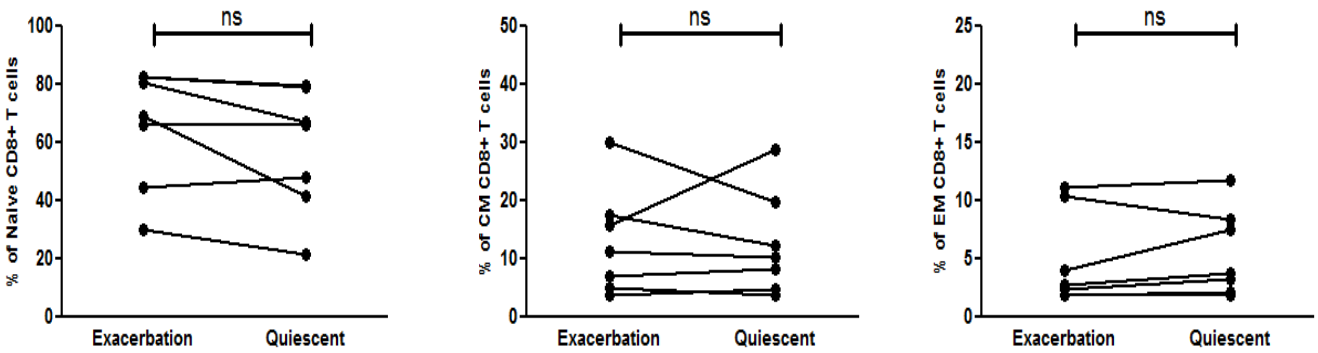


Figure S3: Equivalent suppressive ability of CD8+ T cell subtypes with anti-CD3 activation. Bulk CD8+ T cells were isolated from healthy controls and sorted into (A) CD27+/-, (B) CD28+/-, (C) CD45RO+/-, (D) CD62L+/- and (E) NKG2D+/- CD8+ T cell fractions using magnetic bead-based positive selection kits. Individual CD8+ T cell fractions along with bulk CD8+ T cells were used in suppression assays with autologous APCs and CFSE-stained CD4+CD25- T cells stimulated with anti-CD3. The experiments were performed 4 or more times for each marker selection with PBMCs from 4 independent healthy donors. (F) Positively enriched CD8+ T cells were collected and successively enriched for CD27, CD45RO and CD28 respectively. Individual fractions were used in separate suppression assays activated by anti-CD3 with autologous APCs and responders.*p < 0.05; Four independent experiments were performed with PBMCs from 4 healthy control donors.



FigS4.

Figure S4: Phenotypic differences of neuroantigen-specific CD8+ Tregs during exacerbation vs quiescence. The frequency (A) Naïve, CD27+CD45RO-, (B) Central Memory (CM - CD27+CD45RO-) and (C) Effector Memory (EM - CD27+CD45RO-) CD8+ T cells was assessed in peripheral blood of MS patients during disease exacerbation and quiescence. No significant differences were seen between the two disease states (ns).