Treatment with bortezomib of human CD4+ T cells preserves natural regulatory T cells and allows the emergence of a distinct suppressor T-cell population

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Online Supplementary Figure S1. Viability of the different types of CD4+ T cells: regulatory (CD25+ PKH+), resting conventional (CD25– PKH–) and activated conventional (CD25– PKH+) T cells, assessed by the percentage of 7AAD- cells of each subpopulation
Online Supplementary Figure S2. Analysis of CD25, FOXP3 and CD127 expression by total CD4+, CD4+CD25– or CD4+CD25+ T cells separated by immunomagnetic selection based on the expression of CD4 and CD25.

Online Supplementary Figure S3. Suppression assays: proliferation and CD25 expression. (A) Schematic representation of 4-day suppression assays analysis by flow cytometry: (a) unstimulated PKH-stained responder T cells; (b) aCD3/aCD28 stimulated PKH-stained responder T cells; (C) aCD3/aCD28 stimulated PKH-stained responder T cells co-incubated with non PKH-stained long-term cultured cells, among which we can distinguish: long-term cultured cells (violet), proliferating CD25+ responder T cells (blue), non-proliferating CD25+ responder T cells (cyan) and non-proliferating CD25– responder T cells (green).
Online Supplementary Figure S4. Suppression assays: IFN-γ and CD40L production after co-culture of responder plus long-term cultured T cells. (A) IFN-γ and CD40L intracytoplasmic expression by both responder (PKH⁺) and long-term cultured (PKH⁻) T cells. One experiment of five is shown.