Supplementary Figures

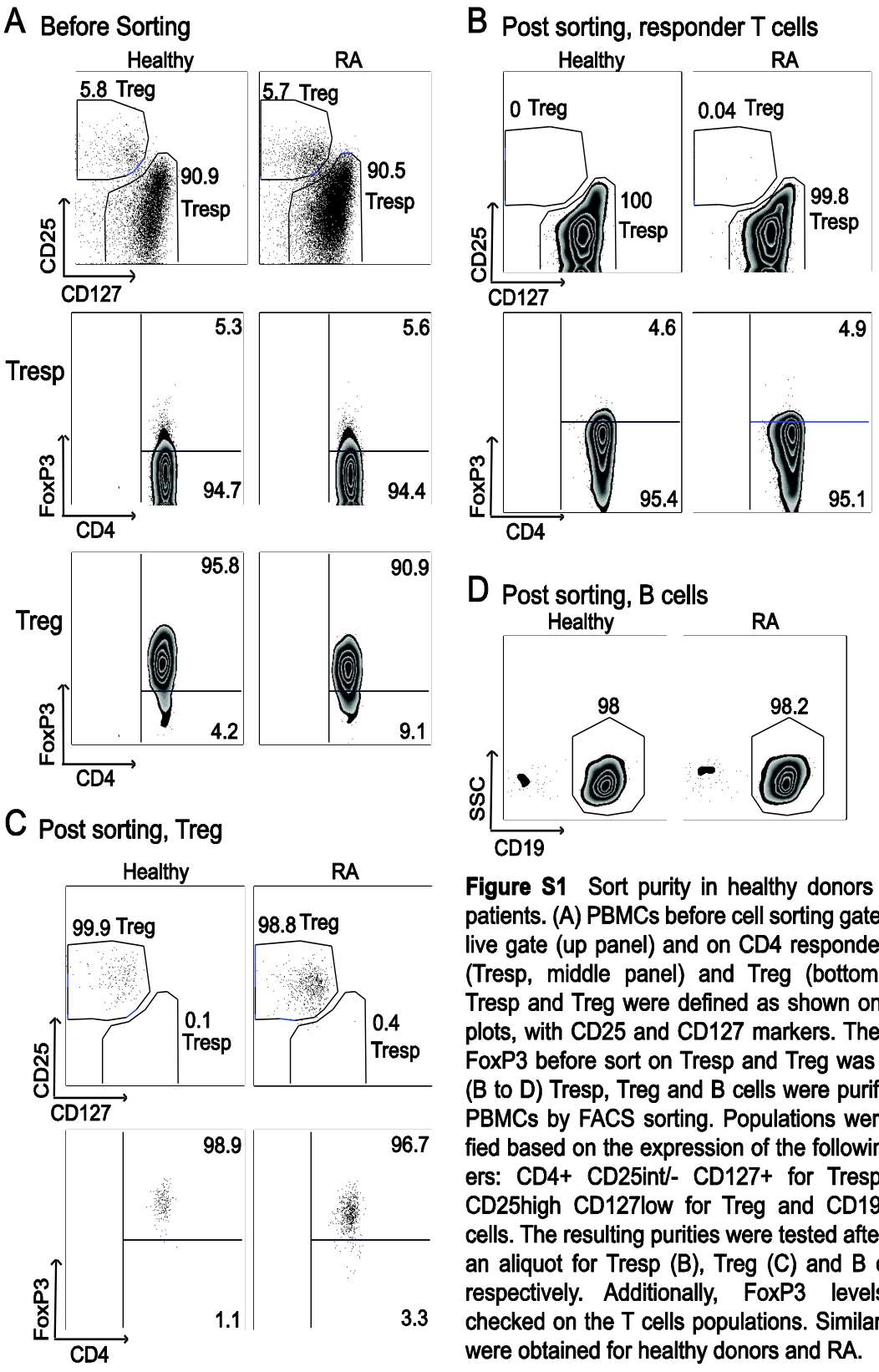

Figure S1 Sort purity in healthy donors and RA patients. (A) PBMCs before cell sorting gated on the live gate (up panel) and on CD4 responder T cells (Tresp, middle panel) and Treg (bottom panel). Tresp and Treg were defined as shown on the dot plots, with CD25 and CD127 markers. The level of FoxP3 before sort on Tresp and Treg was verified. (B to D) Tresp, Treg and B cells were purified from PBMCs by FACS sorting. Populations were identified based on the expression of the following markers: CD4+ CD25int/- CD127+ for Tresp, CD4+ CD25high CD127low for Treg and CD19+ for B cells. The resulting purities were tested after sort on an aliquot for Tresp (B), Treg (C) and B cells (D) respectively. Additionally, FoxP3 levels were checked on the T cells populations. Similar purities were obtained for healthy donors and RA.

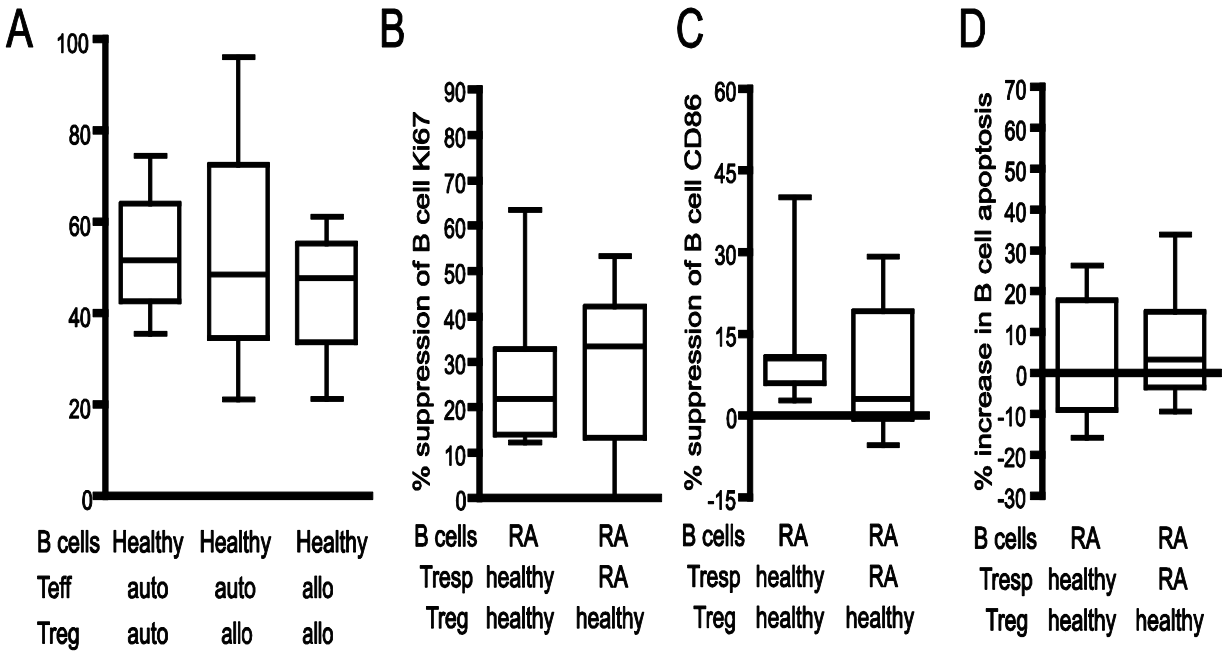

Figure S2 The provenance of responder T cells (RA or healthy) does not influence the suppression of RA B cells by healthy Treg. Suppression assays were performed as described in Figure 2. (A) Comparison of RA B cells suppression by autologous (auto) or allogeneic (allo) healthy Treg in the presence of autologous (auto) or allogeneic (allo) healthy responder T cells. Ki67 suppression (n=4). (B-D) Comparison of RA B cells suppression by healthy Treg in the presence of healthy or RA responder T cells. The same scale as Figure 4 was used to compare them. (B) Ki67 suppression (n=9). (C) CD86 suppression (n=7). (D) Increase in apoptosis (n=9). Cumulative data represent the % suppression of B cell by Treg.The median, the 25th and 75th percentiles, and the minimum and maximum, are represented. The Wilcoxon test was used and no significant differences were observed with respect to suppression of (B) proliferation , (C) activation and (D) increase in apoptosis, using healthy or RA responder T cells.

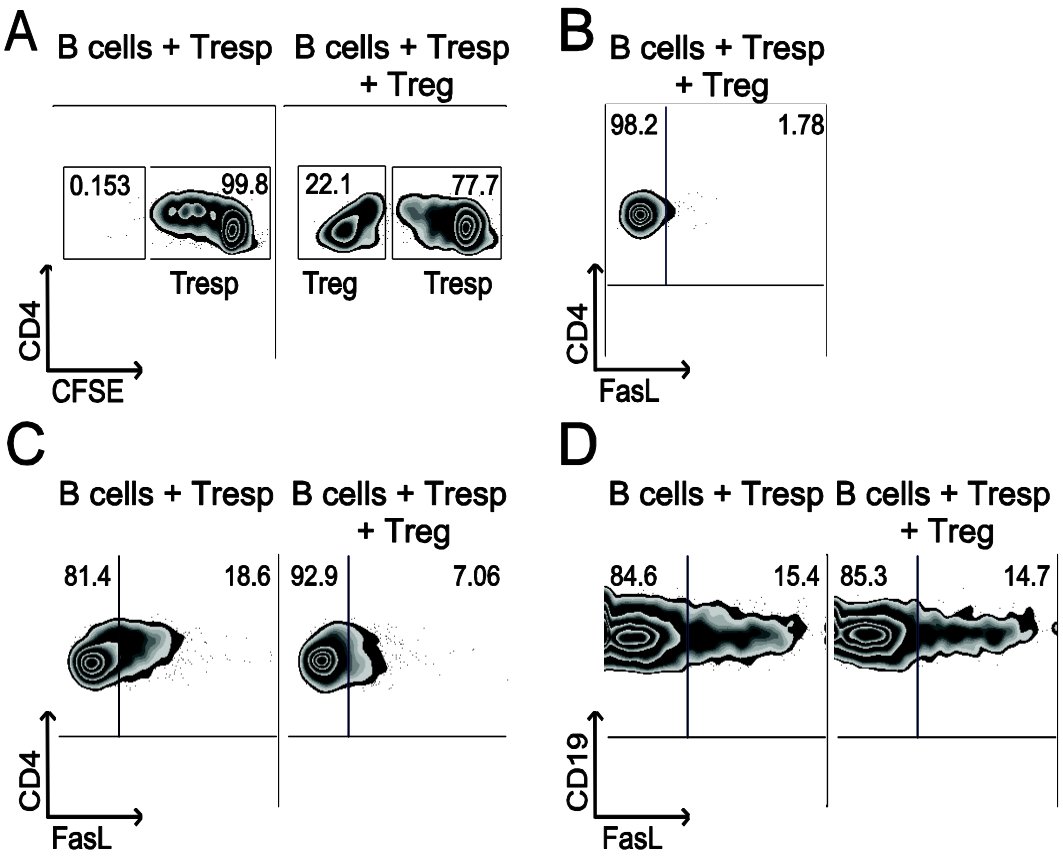

Figure S3 FasL expression on Treg, responder T cells and B cells. To further dissect the mechanism of B cell suppression by Treg in healthy donors, we investigated whether Treg expressed FasL. As recently activated responder T cells express CD25 and down-regulate IL7R, their phenotype is similar to Treg. Therefore to distinguish responder T cells from Treg in the co-cultures, the former were labelled with CFSE after sorting and before their addition to the co-cultures. (A) Representative flow cytometry plots gated on CD4+ T cells, showing CFSE stained Tresp and CFSE unstained Treg. After 4 days of activation, responder T cells were still positive for CFSE and distinct from Treg. Representative flow cytometry plots showing FasL expression on (B) Treg, (C) CFSE stained responder T cells and (D) B cells in the cocultures. Whereas FasL was almost undetectable on Treg, it was expressed on responder T cells and B cells. Addition of Treg modulated surface FasL expression on responder T cells, but not on B cells.