Supplementary Fig. 1 (A) Freshly isolated T cells were stained with anti-CD3 mAb (black line) or an isotype control (grey line). (B) Autologous PKH26-stained T cells were added to pretreated DC (BDD rFVIII) and cocultured for 9 days. Harvested cells were stained with anti-CD3 monoclonal antibody and T cell proliferation was assessed as a decrease in PKH26 fluorescence intensity. Data from 25 different donors and 17 independent experiments are shown (mean). Statistical analysis was performed using Wilcoxon’s matched-pairs signed-rank test (ns, not significant; p = 0.14). (C) Data from Fig. 1C, E were normalized as stimulation index. Stimulation index is the ratio between proliferated T cells in percentage of a given stimulus and proliferated T cells of the cells of the same donor without any stimulus. (D) Autologous T cells were added to untreated DC, cocultured for 9 days, harvested and stained with anti-CD4 or anti-CD8 mAb (black lines) or with the respective isotype controls (grey lines). (E) Autologous T cells were added to pretreated DC and cocultured for 9 days. Cells were harvested and stained with a mAb mix containing anti-CD3 mAb for DC exclusion, as well as anti-CD4 and anti-CD8 mAbs. The percentages of CD3⁺PKH26⁺CD4⁺ and CD3⁺PKH26⁺CD8⁺ T cells were assessed using flow cytometry. Data from different donors and at least five independent experiments are expressed as percentage of proliferated T cells for FVIII⁺ (n = 12), FVIII⁺ LPS (n = 6) and FVIII⁺ LPS (n = 7) (mean ± SEM). (F) Autologous CD4⁺ T cells were added to pretreated DC. After 9 days, proliferation was assessed by flow cytometry. Data from five different donors and five independent experiments are expressed as percentage of proliferated T cells (mean). Statistical analysis was performed using Wilcoxon’s matched-pairs signed-rank test. (G) Autologous PKH26-stained T cells were added to untreated DC, cocultured for 9 days, harvested and stained with a mAb mix containing anti-CD45RA and anti-CD45RO mAbs or the respective isotype controls. Data for the isotype staining of a representative donor are shown. DC, dendritic cell; FVIII, factor VIII; rFVIII, recombinant FVIII; mAb, monoclonal antibody.