Factor VIII bypasses CD91/LRP for endocytosis by dendritic cells leading to T-cell activation

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Supplementary Figure 1

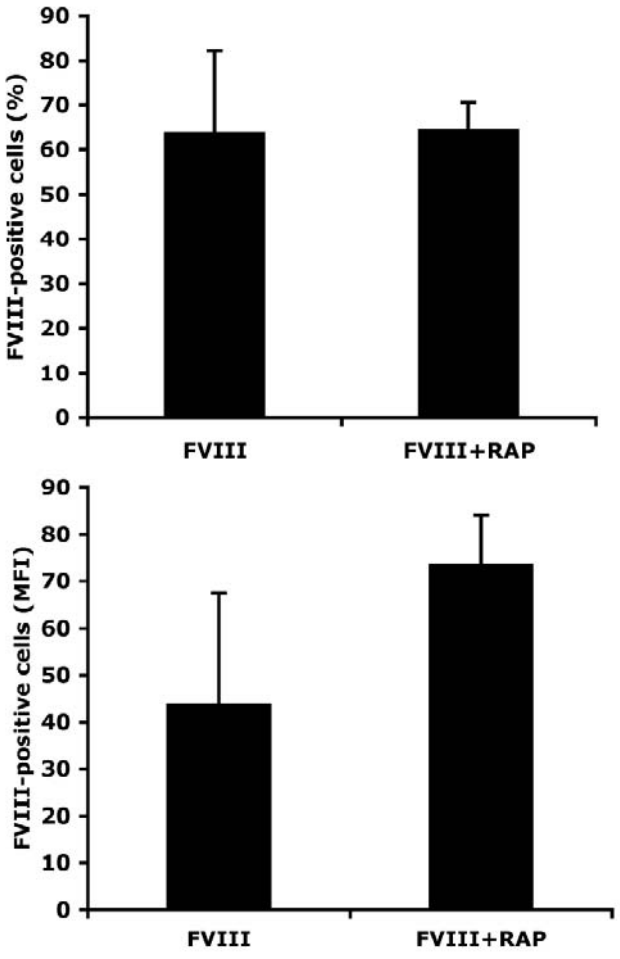

Figure 1. The dendritic cells were prepared in the exactly similar way as done in the case of healthy donors. Briefly, circulating monocytes from two patients (Aged 8 years and 14 years and both were inhibitor positive) were cultured with X-VIVO media supplemented with 1% human AB serum in the presence of human recombinant GM-CSF and IL-4 for 5 days. Immature dendritic cells were collected and incubated with medium alone or 14 μM of Receptor Associated Protein (RAP) at 37°C for 30 min. FVIII-FITC was then added to the wells at 0.14 μM concentration. After 2 hr incubation cells were washed and DC associated fluorescence was analysed by flow cytometry. The results were very similar to the ones we obtained with healthy donor DCs. Hundred molar excess of RAP neither reduced the number of FVIII positive DCs (Figure 1) nor could downmodulate mean fluorescence intensity associated with the DCs for the said incubation period (Figure 1). Results depict average values of the two patient DCs. This indicates that RAP inhibitable CD91/LRP and other members of LDL-receptor family are not implicated in FVIII endocytosis by human DCs derived from circulating monocytes of severe hemophilia A patients.