Supporting Information

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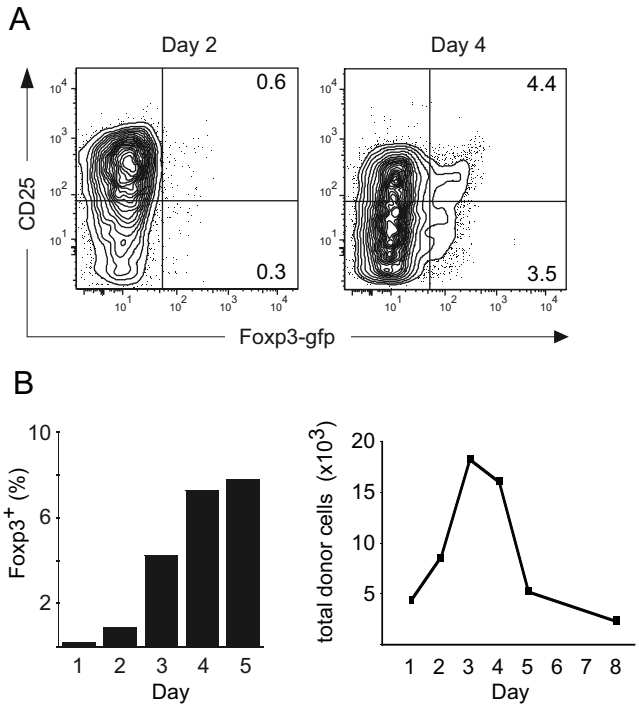

Fig. S1. DO11.10^{+} CD4 SP cells give rise to T_{reg} after intrathymic injection into AIRE-HCO recipient mice. (*A*) CD4 SP thymocytes cells from CD45.1 DO11.10 Rag$^{o/o}$ Foxp3-gfp mice were i.t. transferred into AIRE-HCO mice and analyzed for Foxp3-gfp and CD25 expression at the indicated time points after injection. Numbers indicate the frequency of cells within the respective quadrants. (*B*) Kinetics of intrathymic T_{reg} development. The diagram depicts the percentage of Foxp3^{+} cells (*Left*) or absolute numbers of total donor cells (*Right*) that were recovered at the indicated time points after i.t. injection.

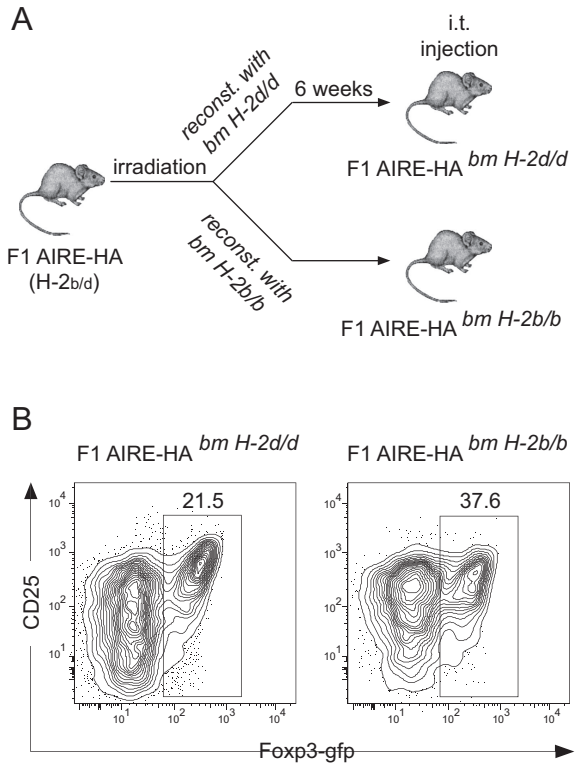

Fig. S2. Antigen presentation by hematopoietic cells is dispensable for T_{reg} induction upon i.t. injection. (*A*) Generation of bone marrow chimeric recipient mice. F1 H-$2^{b/d}$ AIRE-HA mice were lethally irradiated and reconstituted with either H-$2^{d/d}$ or H-$2^{b/b}$ bone marrow to generate F1 AIRE-HA$^{bm\ H\text{-}2d/d}$ or F1 AIRE-HA$^{bm\ H\text{-}2b/b}$ recipients, respectively. Six weeks later, mice were i.t. injected with TCR-HA^{+} CD4 SP thymocytes (CD45.1). (*B*) Injected cells were analyzed for expression of Foxp3-gfp and CD25 5 days after transfer. Numbers indicate the percentage of Foxp3-gfp^{+} cells. Two to three mice per group were injected, and one representative plot per group is shown.

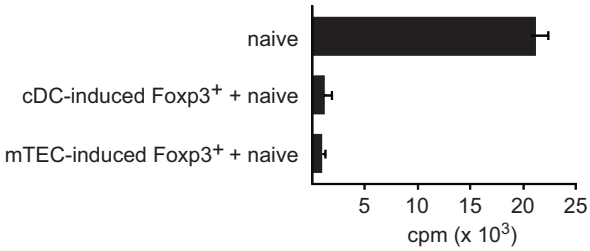

Fig. S3. Foxp3$^+$ cells induced in the presence of mTECs or cDCs are suppressive in vitro. To address the suppressive potential of mTEC- or cDC-induced Foxp3$^+$ cells, 2×10^4 TCR-HA Rag2$^{o/o}$ CD4$^+$ peripheral cells were cultured alone or together with 2×10^4 Foxp3-gfp$^+$ cells sorted from in vitro T_{reg} differentiation assays (as described in Fig. 4) in the presence of syngeneic irradiated splenocytes and HA (107–119) peptide. Proliferation was assessed by scintillation counting after a pulse with [^{3}H]thymidine for the last 24 h of a 96-h incubation period. Data are representative of 2 independent experiments.