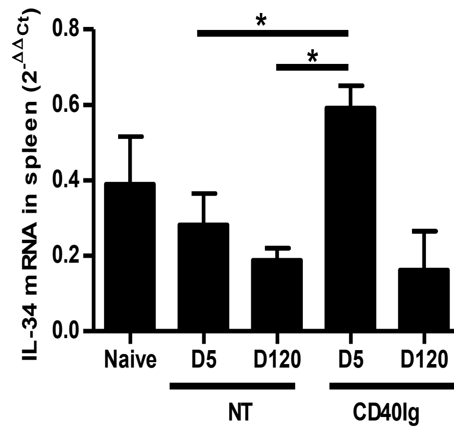
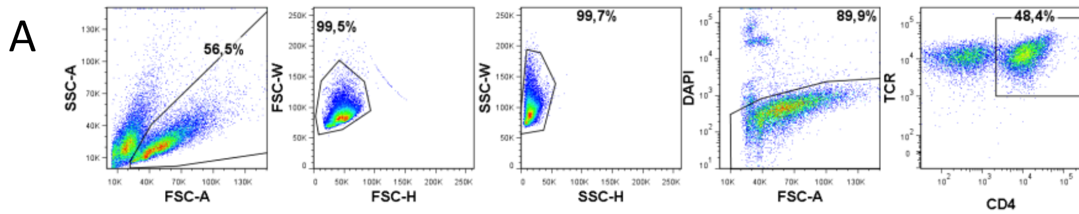
Suppl. Figure 1. Bézie et al.



Suppl. Figure 1. Increased IL34 expression in spleen following CD40Ig-treatment. Spleen from AdCD40Ig-treated recipients at day 5 (n=3) and 120 (n=7) after transplantation were compared with grafts from non-treated (NT)-recipients at day 5 (n=8), day 7 (n=8) and day 120 (n=6) and native hearts from naive animals (n=7) for IL34 mRNA expression. Mann Whitney, *$p < 0.05$.

Suppl. Figure 2. Bézie et al.

A



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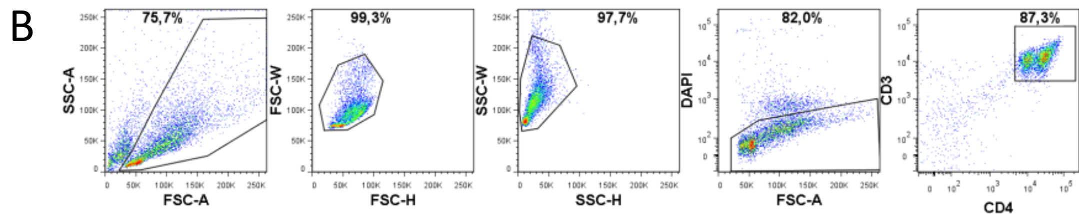
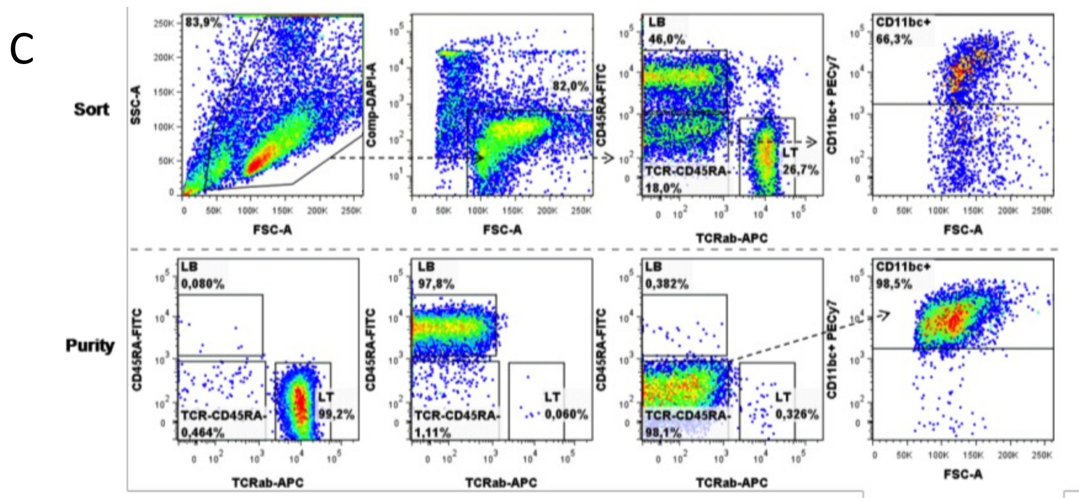
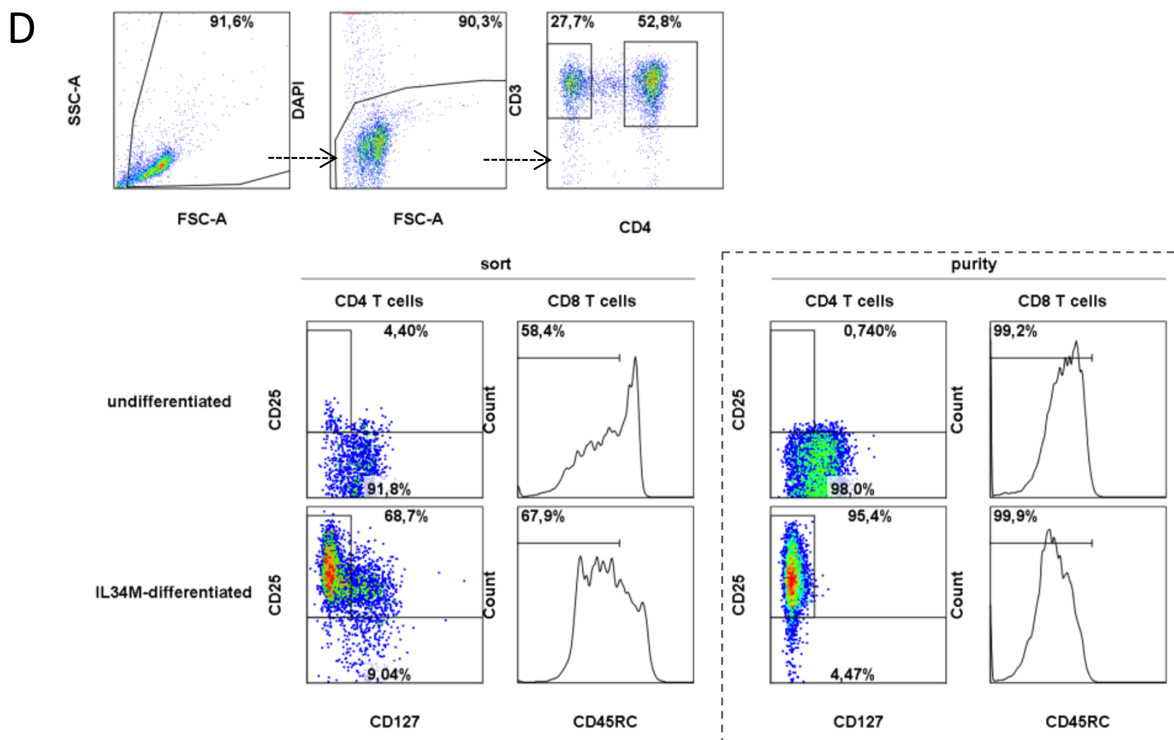


C

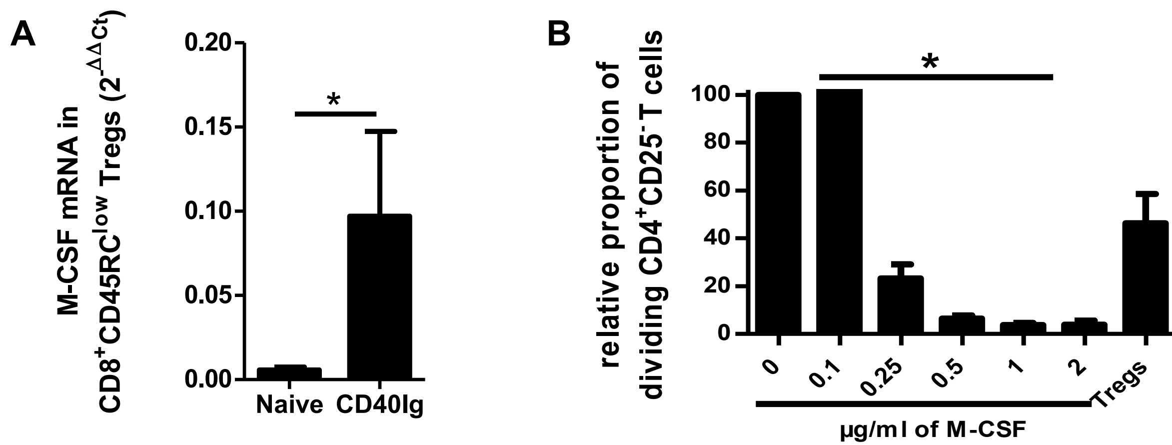


D



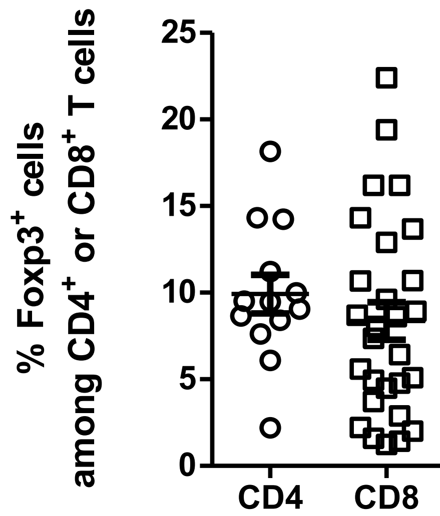
Suppl. Figure 2. Gating strategy of MLR analysis and cell sorting.(A and B) Cells were gated by their morphology, doublets and dead cells were excluded by DAPI expression. CFSE was analyzed on effector $CD4^{+}CD25^{-}$ T cells by gating on CD4 positive cells and $TCRab^{+}$ or $CD3^{+}$ cells for rat and human MLR respectively. **(C)** In the top panel, rat cells were gated by their morphology; dead cells were excluded by DAPI labeling. T cells were sorted by gating on TCRab positive cells, B cells were sorted by gating on $CD45RA^{+}$ cells and macrophages were sorted by gating on $CD45RA^{-}TCRab^{-}CD11b/c^{+}$ cells. Purity after cell sorting (bottom panel) was greater than 97%. **(D)** Cells were gated by their morphology; dead cells were excluded by DAPI expression. $CD3^{+}CD4^{+}CD25^{high}CD127^{low}$ and $CD3^{+}CD8^{+}CD45RC^{low}$ Tregs and $CD3^{+}CD4^{+}CD25^{-}$ effector T cells were sorted from PBMCs from healthy volunteers following culture with IL34-differentiated monocytes and compared to undifferentiated Tregs for suppression assay. Purity after cell sorting was greater than 99% (right bottom panel).

Suppl. Figure 3. Bézie et al.



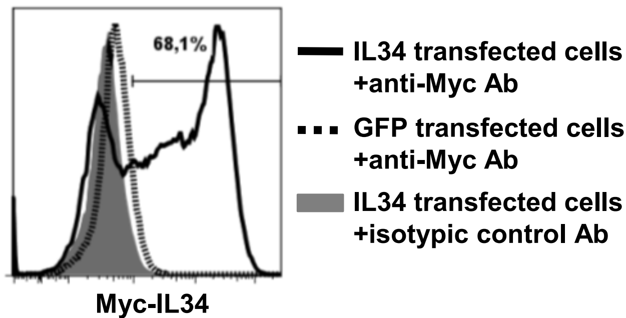
Suppl. Figure 3. M-CSF mediated dose dependent suppression of anti-donor effector CD4$^+$CD25$^-$ T cells responses. (A) FACS Aria-sorted CD8$^+$CD45RC^{low}Tregs from spleen of naive or 120 days old AdCD40Ig-treated recipients (n=6) were analysed for M-CSF mRNA expression by quantitative RT-PCR. Mann Whitney, *; $p < 0.05$.**(B)** Rat M-CSF (0.1 to 2 µg/ml final concentration) was tested for suppressive activity on CFSE-labelled CD4$^+$CD25$^-$ T-cell proliferation after 6 days of culture. CD8$^+$Tregs were used as positive control of suppression. n=3 in triplicates. Results are expressed as mean ± SEM of normalized percentage of proliferation vs. proliferation in the absence of CD8$^+$ Tregs (100 %). Kruskal Wallis and Dunn's post test, *; $p<0.01$.

Suppl. Figure 4. Bézie et al.



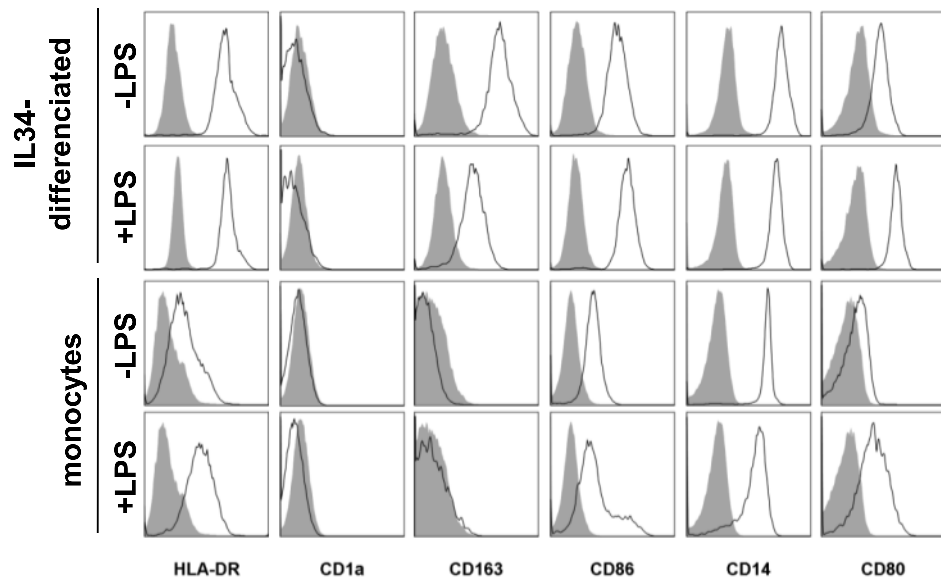
Suppl. Figure 4. Foxp3 is expressed by both $CD4^+$ and $CD8^+$ T cells in healthy individuals. The percentage of Foxp3 positive cells was evaluated in healthy individuals among $CD4^+$ or $CD8^+$ T cells. The mean +/- SEM of >13 healthy individuals was represented.

Suppl. Figure 5. Bézie et al.



Suppl. Figure 5. IL34 protein expression following plasmid transfection. Cells were transfected with the IL34 or GFP-plasmids used to generate the AAV-IL34 and AAV-GFP vectors. IL34 protein was detected by FACS 48h later using anti-myc Ab (solid black line: IL34-transfected cells; dotted black line : GFP-transfected cells; filled grey: IL34 transfected cells labeled with isotypic control Ab; one representative experiment out of 3 performed).

Suppl. Figure 6. Bézie et al.



Suppl.Figure 6. Phenotype of monocytes and IL34-differenciated macrophages. The phenotype of $CD14^{+}$ monocytes from healthy individuals was evaluated 6 days after purification and differentiation or not with IL34. Cells were recovered and stimulated or not 24h with LPS and stained for the expression of several markers. One representative histogram from three independent experiments.