

Supplemental material

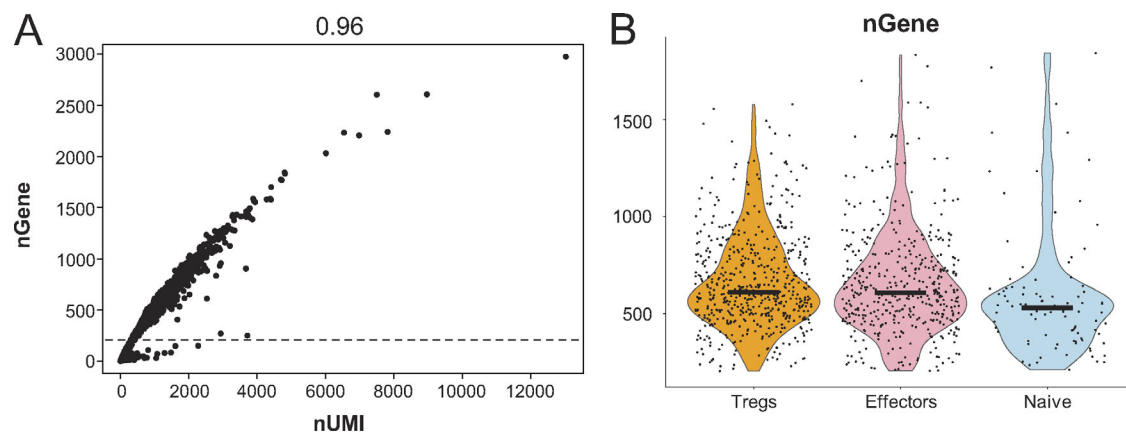
Pratama et al., <https://doi.org/10.1084/jem.20190428>

Figure S1. **scRNA-seq quality control analysis and sequences of repeated TCR clonotypes.** (A) Scatter plot showing the number of unique genes and unique molecular identifiers (UMIs) detected in each cell. Each dot represents a cell. Cells with <200 detected genes (below the dotted line) were excluded from further analysis. The r^2 value of the correlation is shown above. (B) Violin plots showing the number of unique genes detected in cells from different clusters (Treg cells, effectors, and naive). Black bars indicate median values.

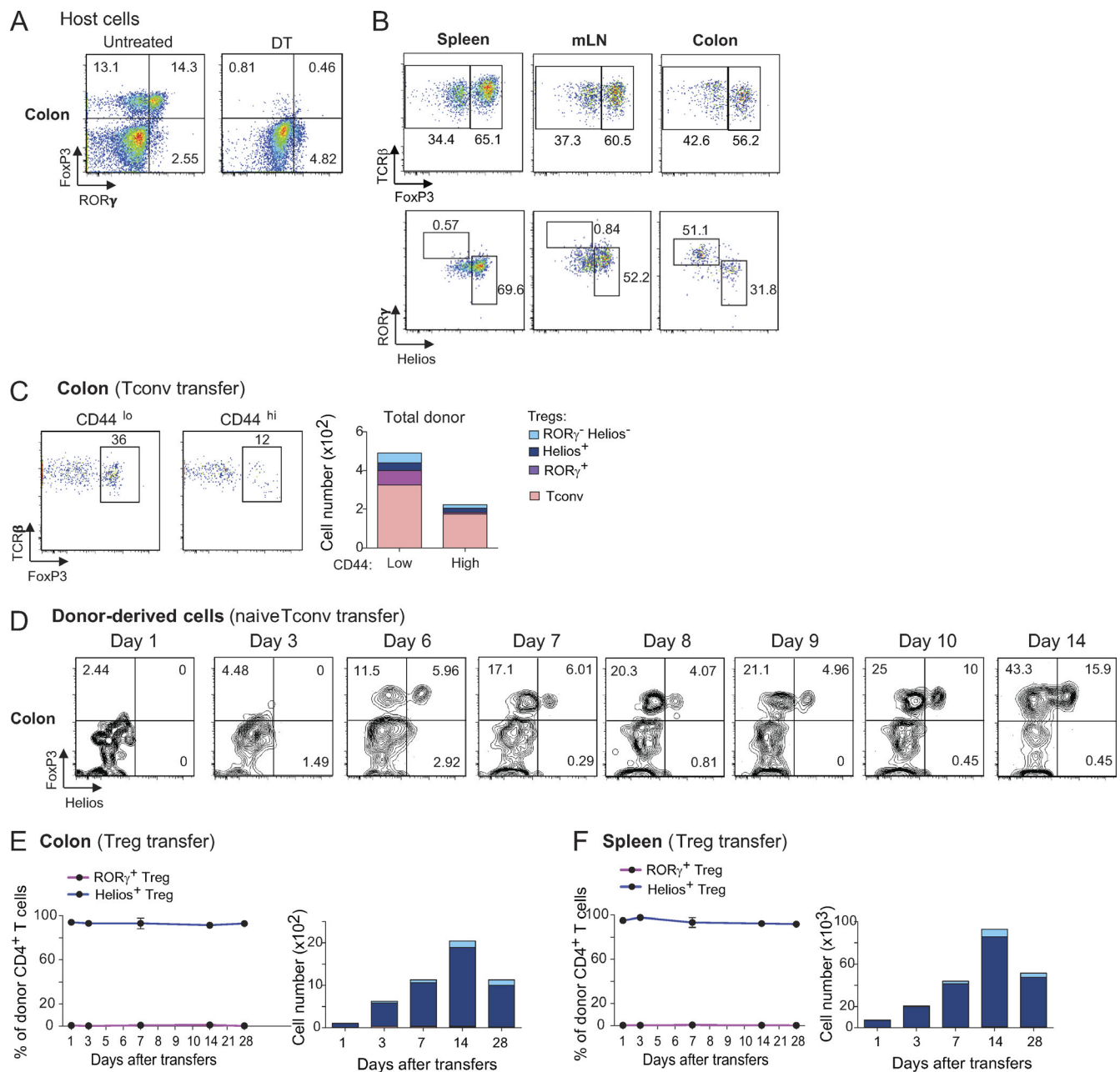


Figure S2. Naive Tconv cells differentiate mostly into RORγ⁺ Treg cells while splenic Treg cells maintain their Helios⁺ phenotypes. (A) Representative dot plots of CD4⁺ T cells in the colon of *Foxp3^{dtr}* mice before (left) and 30 h after (right) DT injection. Frequencies of cells in each quadrant are shown. (B) Representative dot plots of donor CD4⁺ T cells (top) and donor-derived Treg cells (bottom) in the spleen, mesenteric LNs (mLN), and colon of *Foxp3^{dtr}* recipients. Data are representative of two independent experiments. (C) Flow cytometric analysis of a transfer experiment where 0.25×10^6 CD44^{lo} or CD44^{hi} CD4⁺ Tconv cells were transferred into *Foxp3^{dtr}* hosts (left). Average numbers of donor-derived cells are shown (right; $n \geq 8$). (D) Representative dot plots of donor CD4⁺ T cells in the colon of *Foxp3^{dtr}* recipients at different days after transfer. Frequencies of cells in each quadrant are shown. (E and F) LN Treg cells were transferred into *Foxp3^{dtr}* recipients. Frequencies (left) and average numbers (right) of donor-derived cells at different days after transfer in the colon (E) and spleen (F) of recipients ($n \geq 3$ for each time point). Summary plots show data pooled from three independent experiments. Means \pm SD.

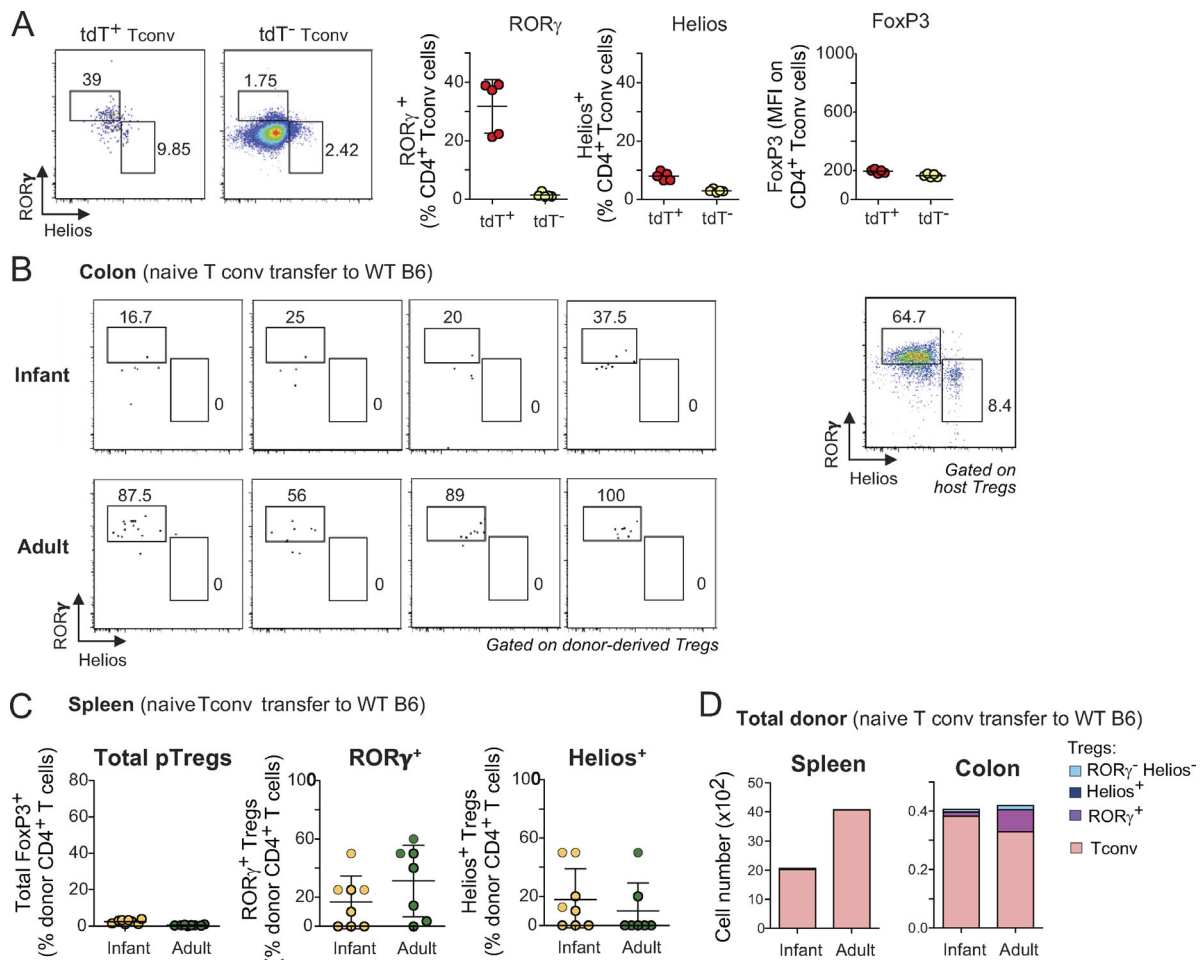


Figure S3. **Pre-existing expression of ROR γ on Tconv cells and availability of a Treg cell niche contribute to the differentiation of ROR γ ⁺ and Helios⁺ pTreg cells.** (A) Representative dot plots (left) and expression of ROR γ , Helios, and FoxP3 (right) on tdT⁺ and tdT⁻ LN Tconv cells ($n = 5$). (B) Flow cytometric analyses of donor-derived Treg cells in the colon of unmanipulated B6 hosts that received naive T cells from infant (top left) or adult (bottom left) donors. Representative dot plot of host colonic Treg cells in the same experiment is shown (right). (C) Frequencies of donor-derived Treg cells in the spleen of unmanipulated B6 hosts that received naive cells from adult or infant donors ($n = 8$). Frequencies of ROR γ ⁺ and Helios⁺ Treg cells are not calculated in mice where no pTreg cells were observed. (D) Average numbers of donor-derived CD4⁺ T cells in the transfers shown in B and C. Data are pooled from two (A) and three (B–D) independent experiments. Means \pm SD.

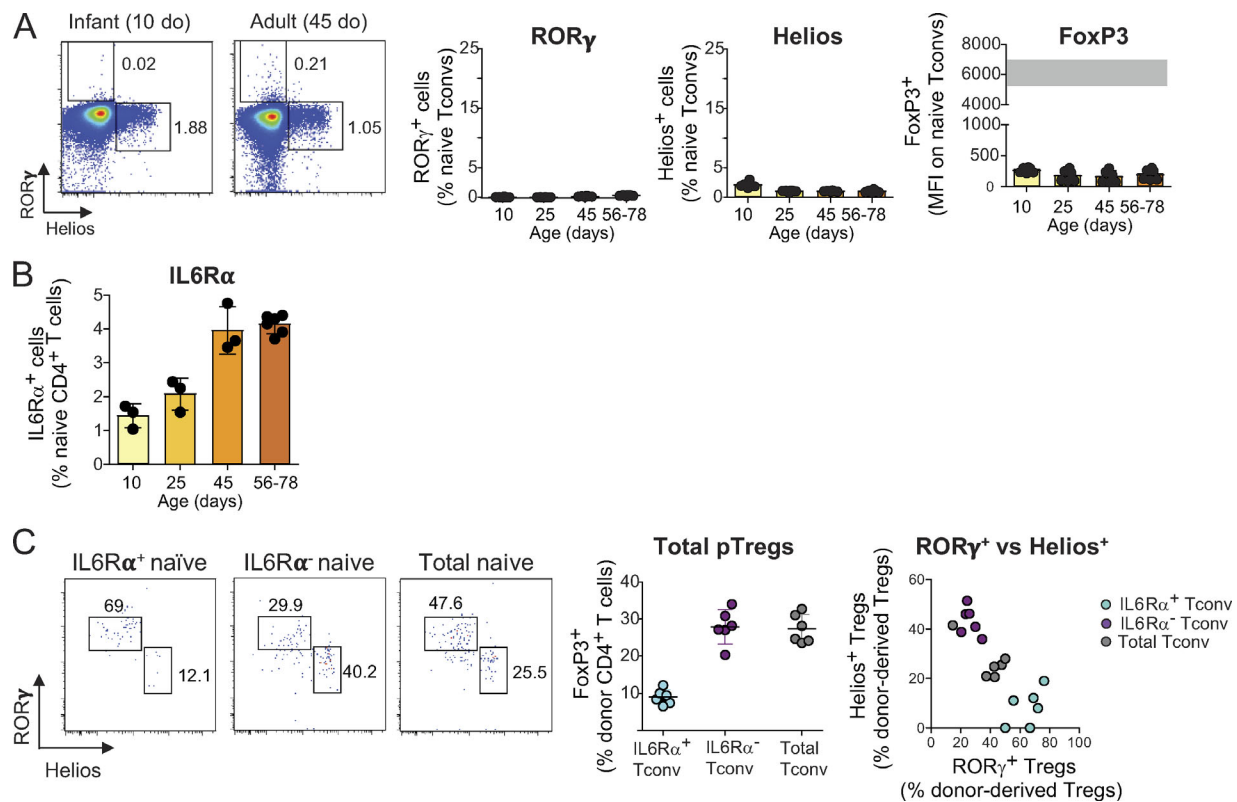


Figure S4. **IL6 signaling contributes to driving the conversion of adult Tconv cells into ROR γ^+ Treg cells.** (A) Representative dot plots (left), frequencies of ROR γ^+ and Helios $^+$ cells, and mean fluorescence intensity (MFI) of FoxP3 on naive CD4 $^+$ T cells from mice of different ages ($n = 6$). The gray bar represents the range of FoxP3 mean fluorescence intensity of Treg cells. (B) Frequencies of IL6R α^+ cells among naive CD4 $^+$ T cells from mice of different ages ($n \geq 3$). (C) Representative dot plots (left) and frequencies and average numbers of donor-derived Treg cells in the colon of Foxp3 dtr hosts that received IL6R α^+ naive T cells, IL6R α^- naive T cells, or unfractionated naive T cells from adult donors ($n = 6$). Summary plots show data pooled from two independent experiments. Means \pm SD. do, days old.

Spleen (naive Tconv transfer)

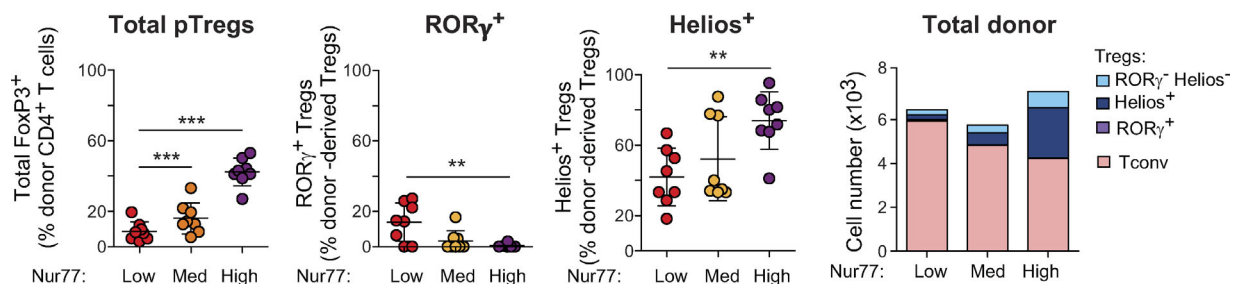


Figure S5. **Tconv cells expressing a high amount of Nur77 preferentially convert to Helios $^+$ pTreg cells in the spleen of recipients.** Frequencies (left) and average numbers (right) of donor-derived Treg cells in the spleen of Foxp3 dtr hosts that received Nur77 low , Nur77 med , or Nur77 high naive Tconv cells from adult mice ($n = 8$). Summary plots show data pooled from three independent experiments. Means \pm SD. **, $P < 0.01$; ***, $P < 0.001$ using Student's t test.

Table S1 is provided online as a separate Excel file and shows lists of genes used to identify the various T cell clusters shown in Fig. 1 A, TCR sequences of the expanded clonotypes shown in Fig. 1 C from monoclonized mice and SPF mice, and lists of primers for TCR sequencing.