Table S1: Antibody list

Target	Clone	Fluorochrome	Vendor
CD1a	HI149	FITC	BD Biosciences
CD1a	HI149	eFluor 450	eBioscience
CD1a	HI149	BV421	BioLegend
CD3	OKT3	eFluor 450	eBioscience
CD3	OKT3	eFluor 650^{NC}	eBioscience
CD3	UCHT1	PE-CF594	BD Biosciences
CD4	SK3	PE-Cy7	BD Biosciences
CD4	OKT4	APC-Cy7	BioLegend
CD7	M-T701	FITC	BD Biosciences
CD8(α)	RPA-T8	APC-eFluor 780	eBioscience
CD8(α)	SK1	FITC	BioLegend
CD8β	2ST8.5H7	PE	Beckman Coulter
CD10	HI10a	PE-Cy7	BD Biosciences
CD25	2A3	FITC	BD Biosciences
CD25	BC96	APC	eBioscience
CD25	BC96	eFluor 450	eBioscience
CD27	L128	PE	BD Biosciences
CD27	L128	APC	BD Biosciences
CD27	eBioRDR5	eFluor 650^{NC}	eBioscience
CD28	CD28.2	APC	BioLegend
CD31	WM59	PE	eBioscience
CD31	WM59	APC	eBioscience
CD31	WM59	eFluor 605^{NC}	eBioscience
CD31	WM59	Alexa Fluor 700	BioLegend
CD34	8G12	FITC	BD Biosciences
CD34	8G12	APC	BD Biosciences
CD34	581	PE-Cy7	BioLegend
CD45RA	HI100	PerCP-Cy5.5	eBioscience
CD62L	SK11	PE	BD Biosciences
CD62L	DREG-56	eFluor 605^{NC}	eBioscience
CD69	L78	FITC	BD Biosciences
CD71	YDJ1.2.2	FITC	BD Biosciences
CD127	R34.34	PE	Beckman Coulter
FoxP3	PCH101	FITC	eBioscience
FoxP3	PCH101	eFluor 450	eBioscience
ICOS (CD278)	ISA-3	FITC	eBioscience
Ki67	20Raj1	FITC	eBioscience
TCRCβ1	Jovi-1	FITC	Ancell
TCRγδ	11F2	FITC	BD Biosciences
TCRγδ	B1	BV421	BioLegend
Fixable Viability Dye		eFluor 455 (UV)	eBioscience

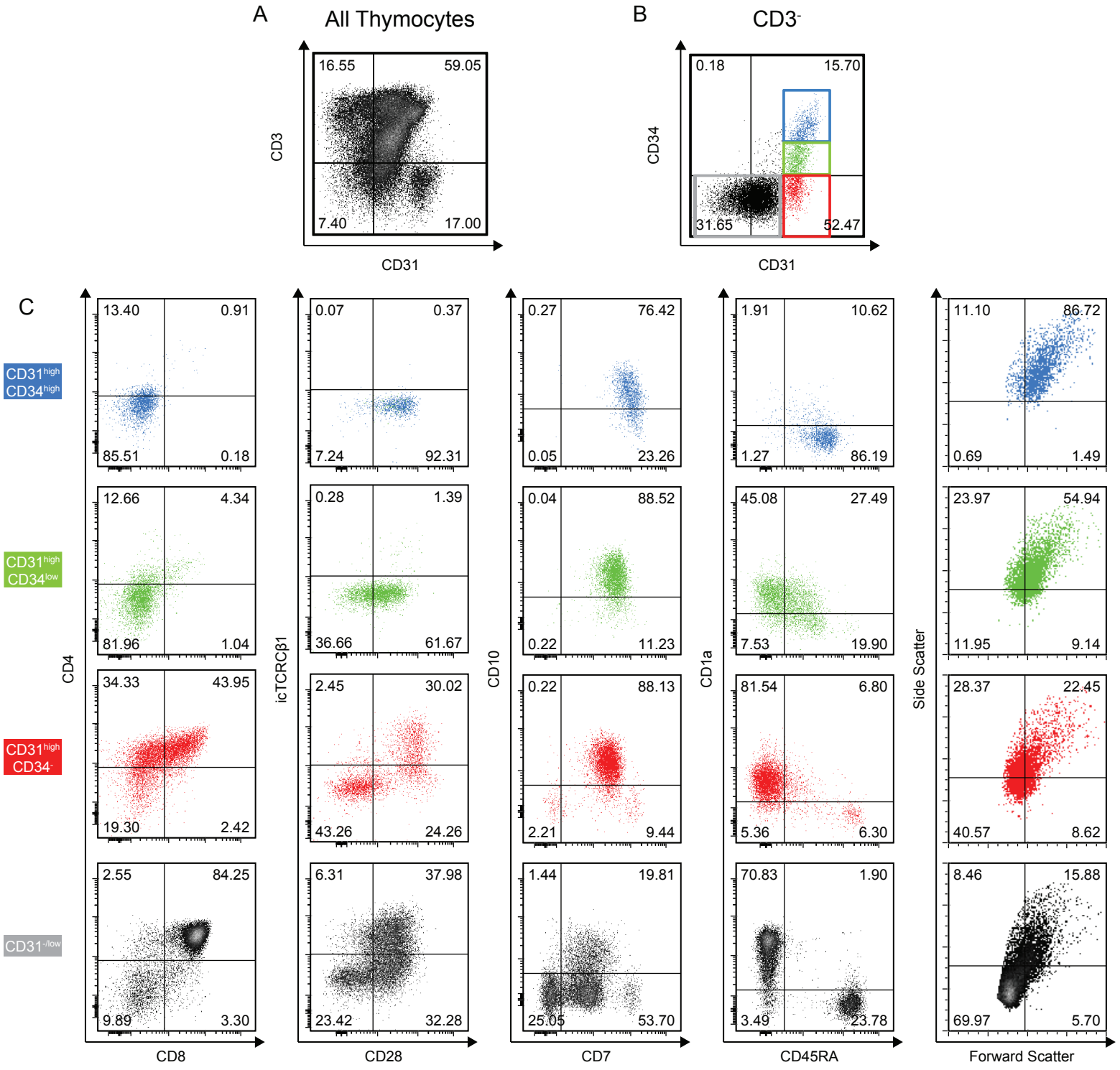

Figure S1: Phenotype of CD3^{-}CD31^{high} thymocytes during down-regulation of CD34 expression. CD3^{-} thymocyte population was gated from all thymocytes (A), then gates were drawn for CD31^{high} CD34^{high}, CD34^{low} and CD34^{-} subsets and the CD31$^{-/low}$ subset. (C) Expression of CD4, CD8, intracellular (ic) TCR Cβ1 chain, CD28, CD10, CD7, CD1a and CD45RA as well as forward and side scatter lights for the subsets described in B.

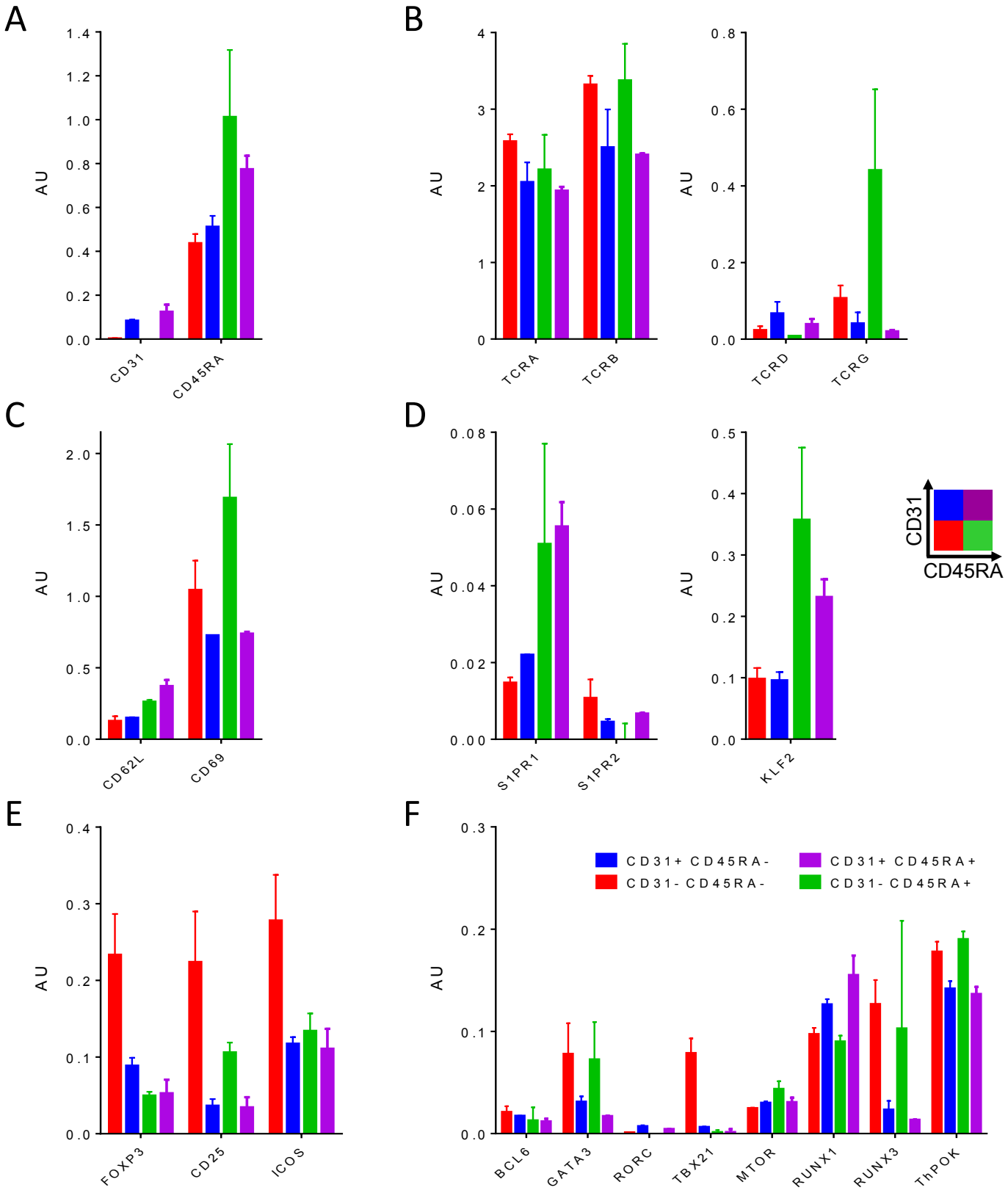

Figure S2: Gene expression quantification was performed on human post-natal thymocytes. Four populations of CD3+ CD27+ CD4+ CD8- cells were sorted based on their expression of CD31 and CD45RA prior to RNA extraction. (A) mRNA levels of CD31 and CD45RA confirm that the cells were properly sorted. (B) Expression of TCRα, TCRβ, TCRγ and TCRδ chains. (C, D) CD62L and CD69 mRNA levels confirm data presented in Figure 5. High expression of S1P1 and KLF2 and reduced expression of S1P2 in CD45RA subsets confirm that these cell populations are ready to egress. (E) Higher expression levels of FoxP3, CD25 and ICOS in the CD31-CD45RA- subset confirms the flow cytometry data presented in Figure 6 and 7. (F) Expression of several transcription factors specific of Th1, Th2, Th17 or Treg lineages or involved in the CD4/CD8 lineage differentiation.

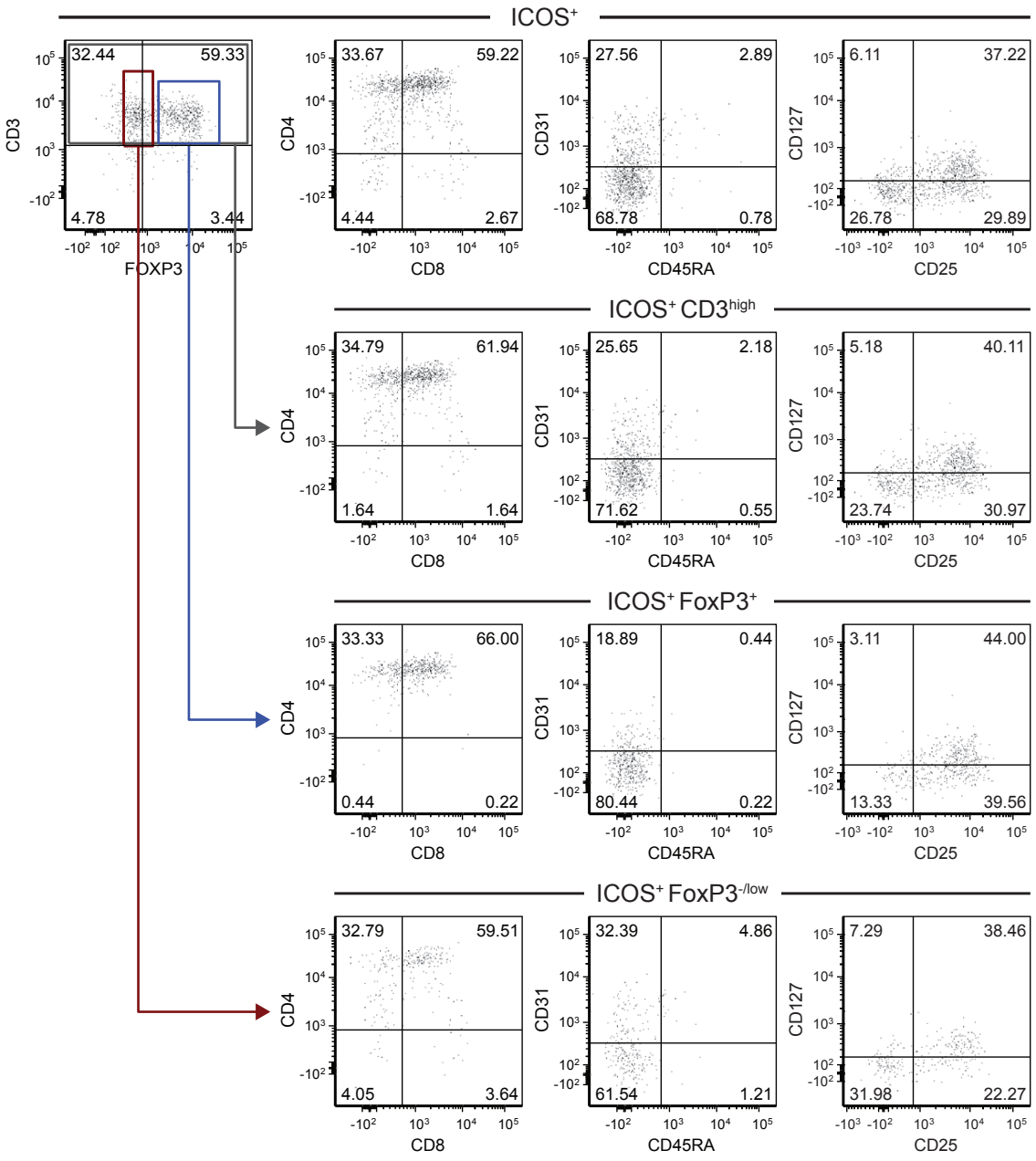

Figure S3: Comparison of the phenotype of FoxP3^{+} and FoxP3$^{-/low}$ subsets of ICOS^{+} thymocytes.
ICOS-expressing thymocytes were gated as in Figure 7 then divided into a FoxP3^{+} subset and a FoxP3$^{-/low}$ subset. Expression of CD4, CD8, CD31, CD45RA ,CD25 and CD127 are displayed for all ICOS cells, ICOS^{+}CD3^{high} cells and FoxP3^{+} and FoxP3$^{-/low}$ subsets of ICOS^{+} thymocytes.