

## Supplemental Figure Legends

### Supplemental Figure 1. Flow cytometric analysis of umbilical cord blood CD25<sup>+</sup> naïve T cells

(A) Naïve CD4<sup>+</sup> CD127<sup>+</sup> T cells were gated as CD45RA<sup>+</sup> CD45RO<sup>-</sup> CD62L<sup>+</sup> and analysed for CD25 expression. (B) CD4<sup>+</sup> naïve T cells were analysed by flow cytometry and divided into four subsets on the basis of CD25 and CD31 expression. Two representative examples (UCB-1 and UCB-2) of 9 cord blood samples (UCB: umbilical cord blood) are shown.

### Supplemental Figure 2. Flow cytometric analysis of CD25 expression from the CD4<sup>+</sup> CD45RO<sup>-</sup> CD62L<sup>+</sup> CD122<sup>+</sup> CD95<sup>+</sup> subset of memory cells with stem cell-like properties

Flow cytometry analysis of peripheral blood CD4 memory cells with stem cell-like properties; (A) Gating strategy of naïve and memory CD4 T cells with stem cell-like properties. Combined analysis of CD31 and CD25 or CD31 and CD122 expression among naïve (B) and memory cells with stem cell-like properties (C), (Representative of 3 donors).

### Supplemental Figure 3. Analysis of IL2RA expression differences on naïve CD25<sup>-</sup> vs naïve CD25<sup>+</sup> T cell subsets purified from 7 donors delineated by 96-sample titan Affymetrix Human Gene 1.1 ST array

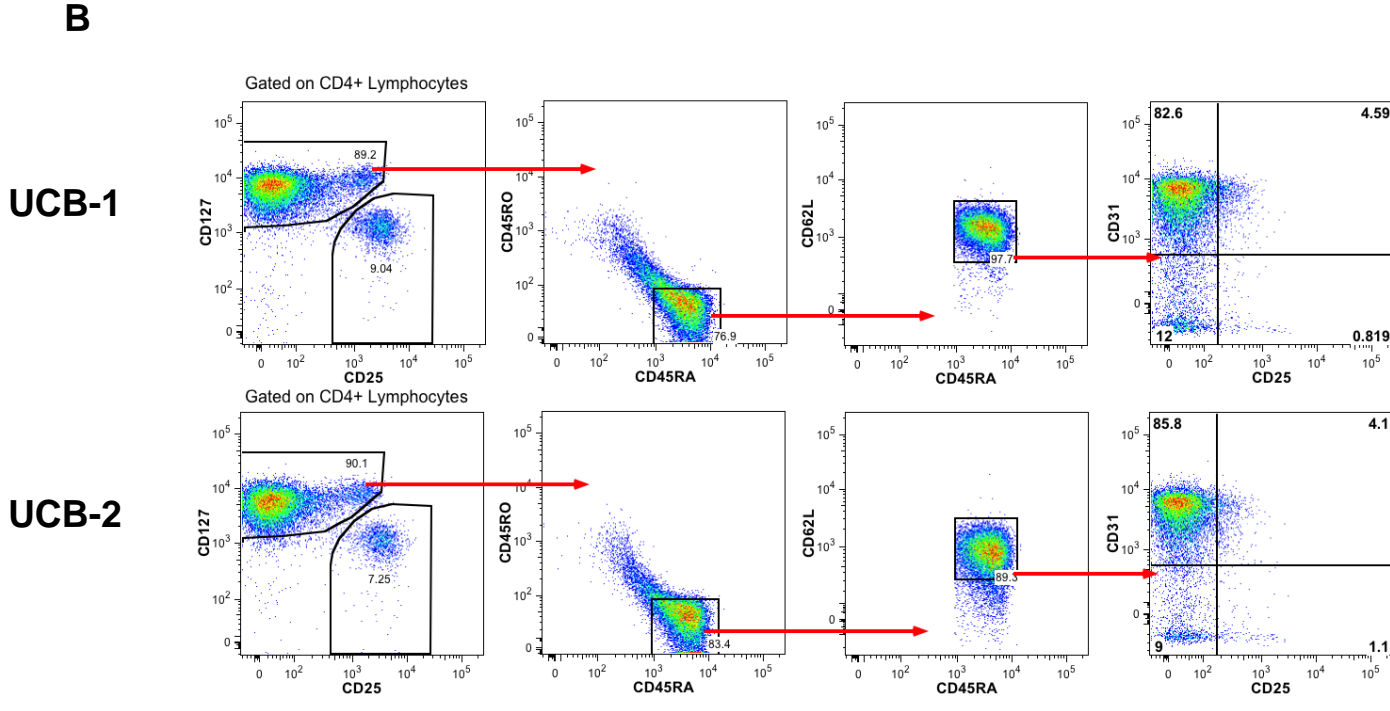
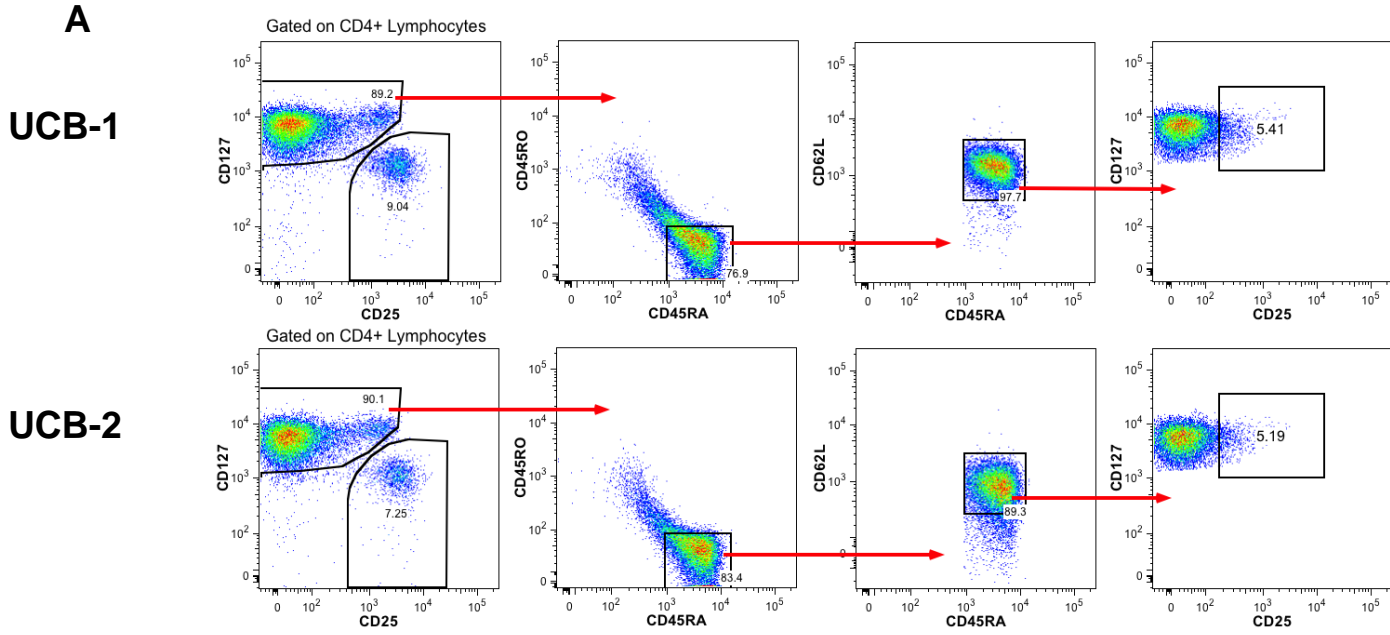
(A) Graphical illustration of Affymetrix GeneST Probesets mapping uniquely to *IL2RA* gene (t1dbase.org). (B) Owing, to the limited sample size tested in the microarray analysis (seven independent donors), modest expression differences observed between the CD25<sup>+</sup> and CD25<sup>-</sup> naïve cells did not reach genome-wide significance. To test the hypothesis that the naïve CD4 T cells sorted on the basis of CD25 protein expression have different *IL2RA* mRNA expression levels accounting for the protein expression difference, results from *IL2RA* probesets were evaluated without a correction for multiple testing and a 5-fold difference in expression was observed using 10 probesets in all seven donors. Analysis of *IL2RA* expression for each *IL2RA* Affymetrix GeneST Probesets demonstrated as fold change in gene expression of naïve CD25<sup>+</sup> vs naïve CD25<sup>-</sup> T cells, n=7. (C) Purity check of sorted TCRαβ<sup>+</sup> CD4<sup>+</sup>, CD127<sup>+</sup>, CD45RA<sup>+</sup>, CD62L<sup>+</sup> CD25<sup>+</sup> and TCRαβ<sup>+</sup> CD4<sup>+</sup>, CD127<sup>+</sup>, CD45RA<sup>+</sup>, CD62L<sup>+</sup> CD25<sup>-</sup> naïve T cells.

**Supplemental Table 1.**

Detailed information about antibodies used for immunophenotyping

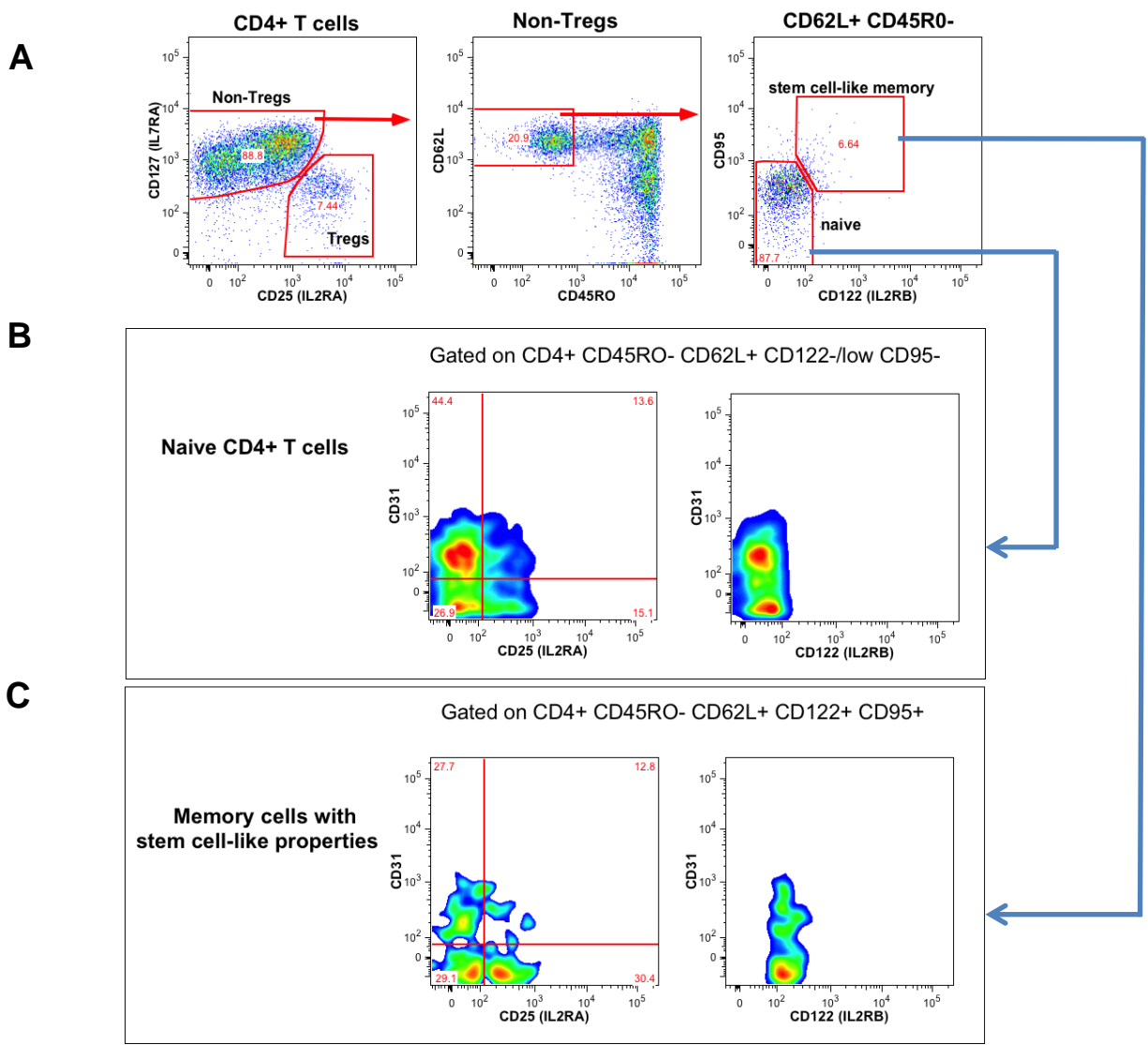
# S-Fig. 1.

## CD25<sup>+</sup> naive T cells are present in cord blood



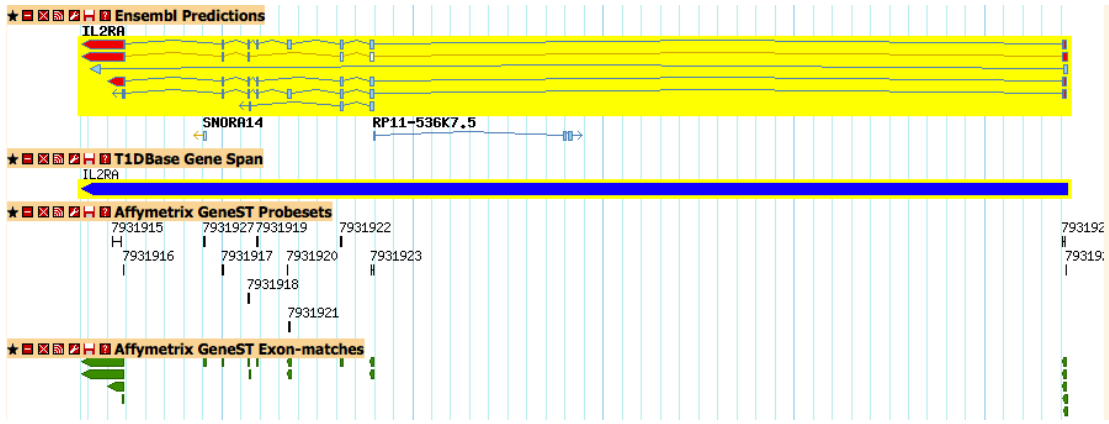
# S-Fig. 2.

## CD4+ stem cell-like memory display heterogeneous CD25 expression



# S-Fig. 3.

A *IL2RA*

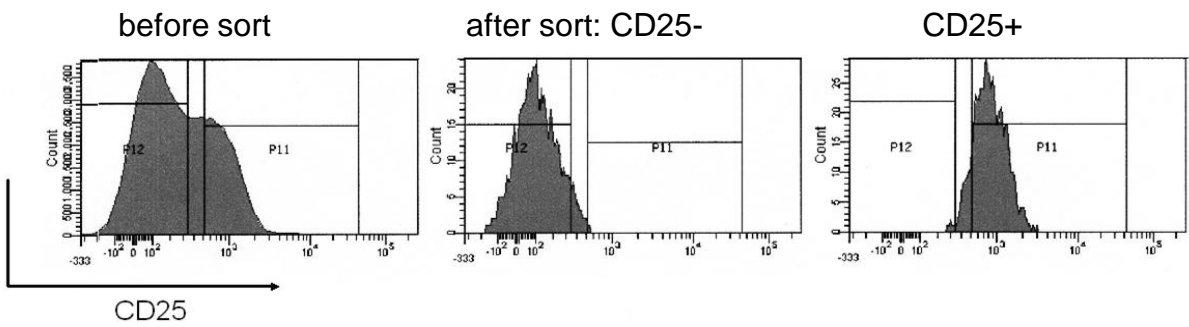


B

probeset.id	P.Value	adj.P.Val	Fold change	CI.025	CI.975
7931915	0.0000032	0.1546776	4.6	1.600	2.823
7931916	0.0000280	0.3790631	5.3	1.533	3.256
7931917	0.0000062	0.1658705	5.6	1.740	3.227
7931918	0.0000059	0.1658705	5.9	1.790	3.307
7931919	0.0000177	0.2854278	3.9	1.295	2.628
7931920	0.0000055	0.1658705	2.4	0.901	1.653
7931921	0.0000162	0.2782334	5.0	1.542	3.104
7931922	0.0000026	0.1546776	4.8	1.645	2.860
7931923	0.0000033	0.1546776	5.7	1.812	3.204
7931924	0.0000005	0.1362445	4.9	1.790	2.822

C

Flow cytometric analysis of sorted CD25 +/- Naïve CD4 T cell populations (CD4+ CD127+ CD45RA+ CD45RO- CD62L+).



# S-Table. 1.

## Antibodies used for immunophenotyping

<u>The anti-human monoclonal abs antibodies used for T-cell surface immunophenotyping</u>		
Fluorochrome	Manufacturer	Clone name
FITC-conjugated anti-TCR $\alpha\beta$	BioLegend	IP26
Alexa Fluor700-conjugated anti-CD4	BioLegend	RPA-T4
APC-conjugated anti-CD25	BD Biosciences	2A3
APC-conjugated anti-CD25	BD Biosciences	M-A251
PE-CY7-conjugated anti-CD127	eBioscience	ebioRDR5
Pacific Blue-conjugated anti-CD45RA	BioLegend	HI100
eFluor605-conjugated anti-CD62L	eBioscience	DREG56
PE-conjugated anti-CD197	BioLegend	3D12
PE-conjugated anti-CD27	BioLegend	323
PE-conjugated anti-CD38	eBioscience	HB7
PE-conjugated anti-CD69	BioLegend	FN50
PE-conjugated anti-CD44	BioLegend	BJ18
PE-conjugated anti-CD137 (41BB)	BioLegend	4B4-1
PE-conjugated anti-CD122	BioLegend	TU27
PE-conjugated anti-CD122	BD Biosciences	Mik- $\beta$ 3
PE-conjugated anti-PD-1	BioLegend	EH12.2H7
PE-conjugated anti-CD95	BioLegend	DX-2
PE-conjugated anti-CD57	BioLegend	HCD57
PE-conjugated anti-CD28	BioLegend	CD28.2
PE-conjugated anti-CD31	eBioscience	WM-59
FITC-conjugated anti-CD31	eBioscience	WM-59
FITC-conjugated anti-CD195	BioLegend	HEK1/85a
FITC-conjugated anti-CD45RO	BioLegend	uch1
FITC-conjugated anti-CD127	BioLegend	A019D5
PerCP-Cy5.5-conjugated anti-CD95	BioLegend	DX-2
PerCP-Cy5.5-conjugated anti-CD45RO	BioLegend	uch1
PerCP-Cy5.5-conjugated anti-CD194	BioLegend	TG6/CCR4
APC-conjugated anti-CD95	BioLegend	DX-2
BrilliantViolet421-conjugated anti-CD25	BioLegend	BC96
eFluor605-conjugated anti-CD45RA	eBioscience	HI100

<u>The anti-human monoclonal abs used for intracellular T-cell immunophenotyping</u>		
Fluorochrome	Manufacturer	Clone name
PerCP-Cy5.5-conjugated anti-KI-67	BD Biosciences	B56
Pacific Blue-conjugated anti-FOXP3	BioLegend	259D

<u>The anti-human monoclonal abs used for intracellular immunophenotyping of pSTAT5 assay</u>		
Fluorochrome	Manufacturer	Clone name
A488-conjugated anti-pSTAT5	BD Biosciences	47/Stat5(pY694)
Pacific Blue-conjugated anti-FOXP3	BioLegend	259D

<u>The anti-human monoclonal abs antibodies used for T-cell sorting</u>		
Fluorochrome	Manufacturer	Clone name
FITC-conjugated anti-TCR $\alpha\beta$	BioLegend	IP26
Alexa Fluor700-conjugated anti-CD4	BioLegend	RPA-T4
APC-conjugated anti-CD25	BD Biosciences	2A3
APC-conjugated anti-CD25	BD Biosciences	M-A251
PE-CY7-conjugated anti-CD127	eBioscience	ebioRDR5
Pacific Blue-conjugated anti-CD45RA	BioLegend	HI100
eFluor605-conjugated anti-CD62L	eBioscience	DREG56
PE-conjugated anti-CD45RO (microarray sorts)	BioLegend	uch1
PE-conjugated anti-CD31 (functional sorts)	eBioscience	WM-59