Cellular immunological changes in patients with LADA are a mixture of those seen in patients with type 1 and type 2 diabetes

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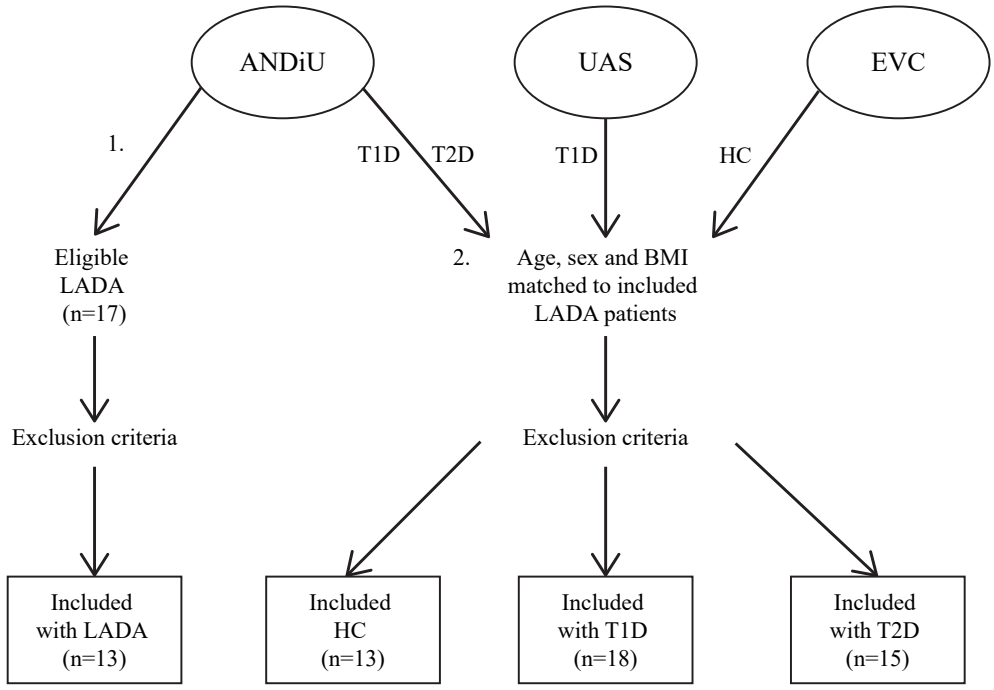
Supplementary Table 1

Antibodies for surface and intracellular antigens used in flow cytometry staining.

Marker	Fluorochrome	Clone	Manufacturer
CD3	APC-H7	SK7	BD
CD4	FITC	RPA-T4	eBioscience
CD8	APC	RPA-T8	BioLegend
CD11b	Brilliant Violet 421	ICRF44	BioLegend
CD11c	Brilliant Violet 421	B-ly6	BD
CD15	PE-Cy7	W6D3	BioLegend
CD16	PE	3G8	BioLegend
CD19	APC-H7	SJ25C1	BD
CD24	FITC	ML5	BioLegend
CD25	APC-H7	M-A251	BD
CD38	Brilliant Violet 421	HIT2	BioLegend
CD40	Brilliant Violet 605	5C3	BioLegend
CD56	APC	HCD56	BioLegend
CD123	PE-Cy7	7G3	BD
CD127	BV605	HIL-7R-M21	BD
Ebi3	APC	607201	R&D
Foxp3	PE-Cy7	PCH101	eBioscience
Helios	Pacific Blue	22F6	BioLegend
HLA-DR	APC-H7	L243	BD
IL-12p35	PE	27537	R&D
Lin 3	FITC		BD

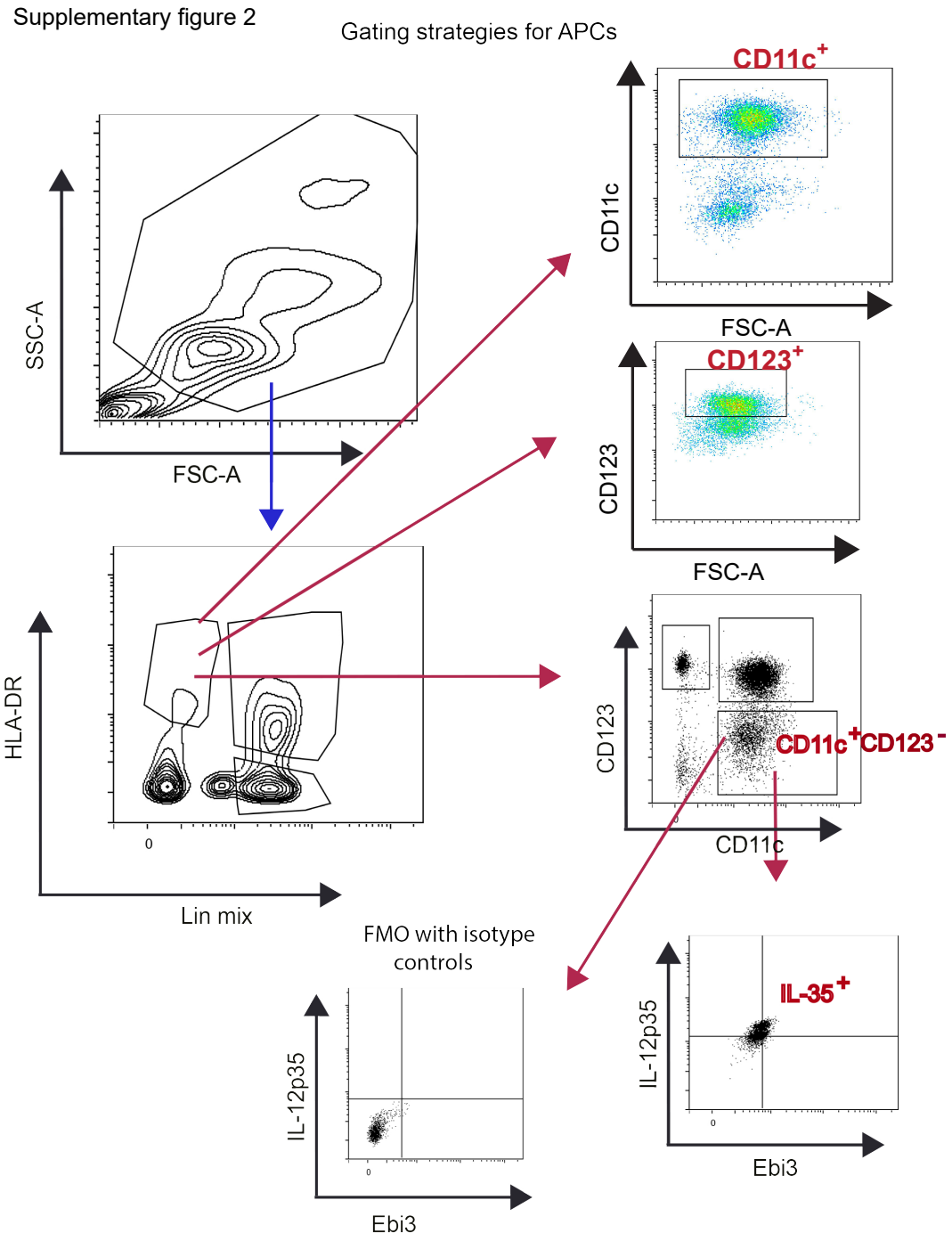
Supplementary figure 1. Flow-chart of the inclusion process of study.
Of the 17 registered LADA patients of the All New Diabetes in Uppsala (ANDiU) study, 13 were included. Additional patients with type 1 diabetes (T1D, n=7), and type 2 (T2D, n=13) diabetes) were recruited from ANDiU. Another 11 participants with T1D were included from the department of Endocrinology, Uppsala University hospital, Uppsala (UAS). Healthy controls (HC, n=13) were recruited upon arrival (with no infection or systemic inflammation) at the Eriksberg Primary Healthcare Centre (EVC). Study subjects were matched for sex, age and body mass index (BMI).

Supplementary figure 1



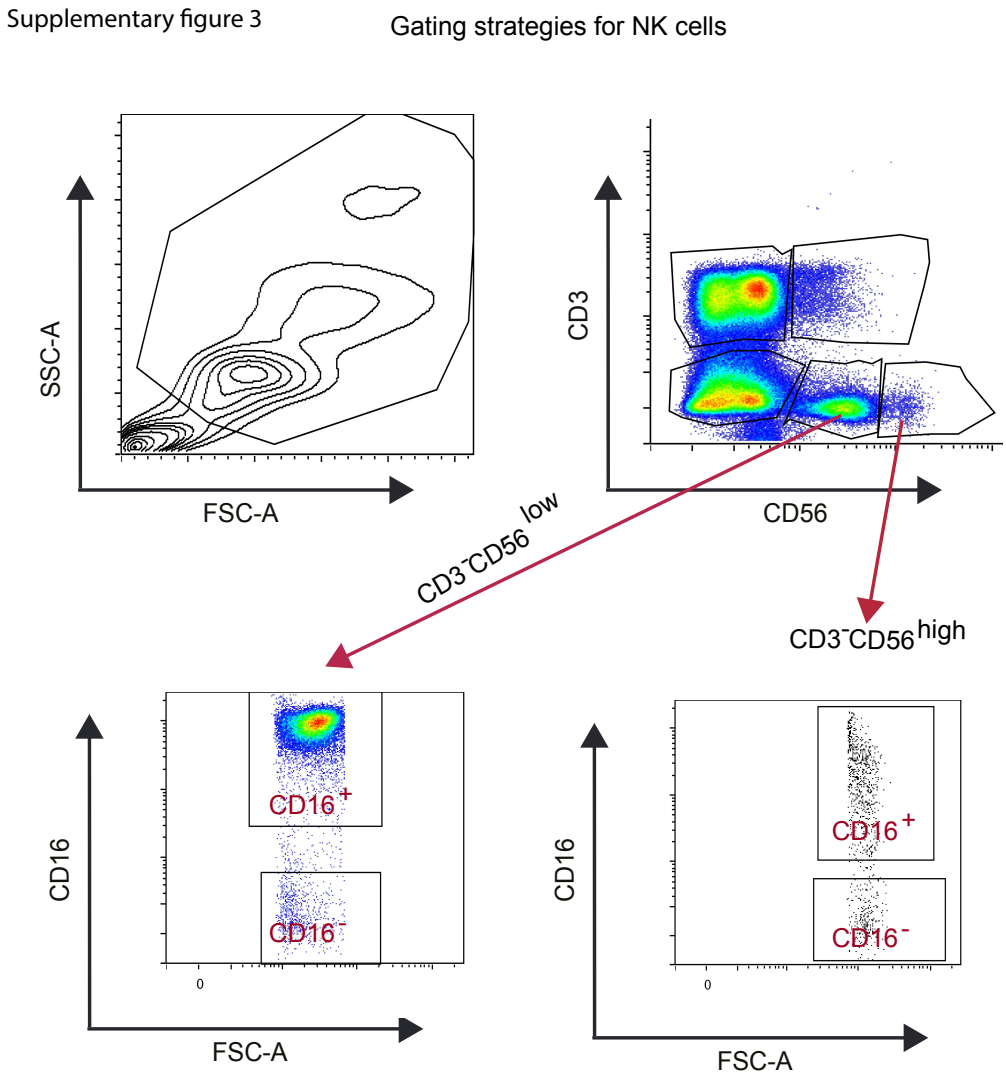
Supplementary figure 2. The representative gating strategies for tolerogenic APCs.

The live cells were gated based on forward scatter (FSC) and side scatter (SSC). Cells were further gated for HLA-DR and Lin mix expression. HLA-DR^{+}Lin^{-} cells were thereafter gated for the expression of CD11c, CD123 or CD11c and CD123. CD11c^{+}CD123^{-}HLA-DR^{+}Lin^{-} cells were gated for IL-12p35 and Ebi3 expression and gate was drawn by using FMO together with isotype controls to analyze IL-35^{+} cells.



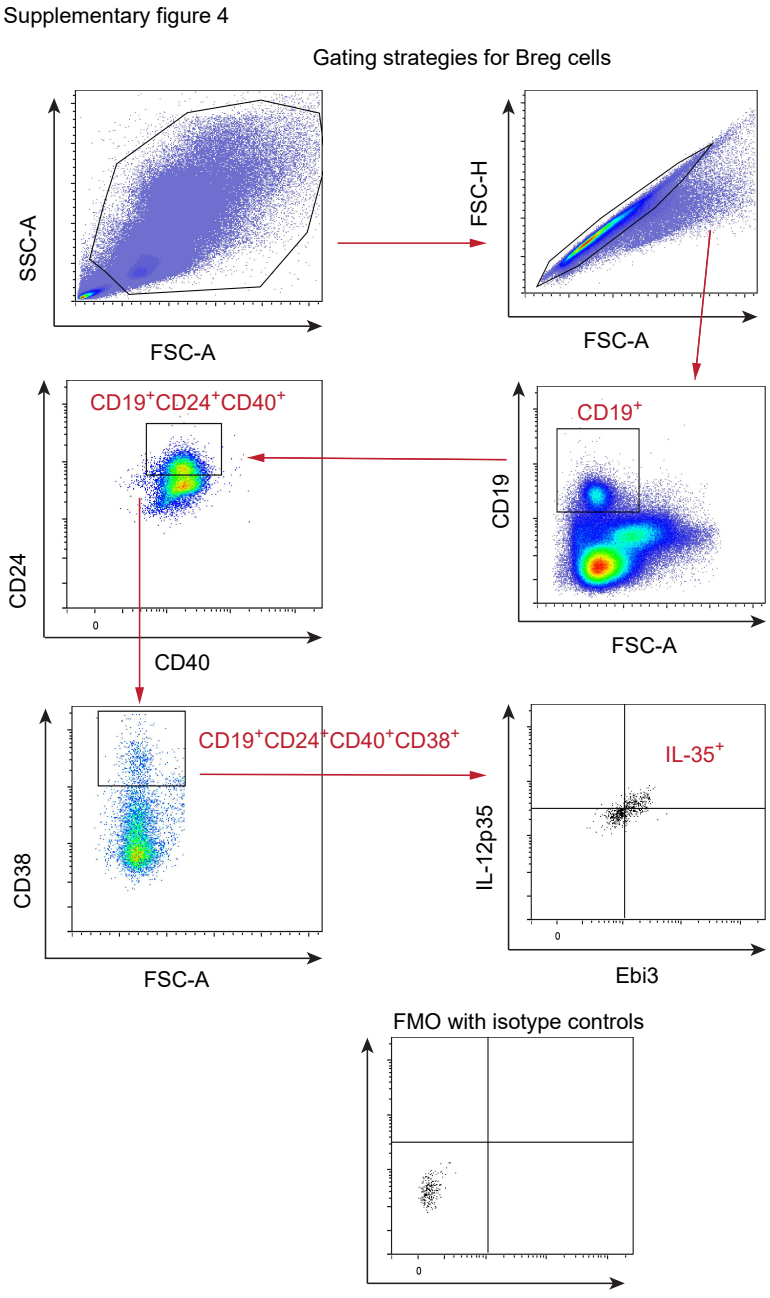
Supplementary figure 3. The representative gating strategies for NK cells.

The live cells were gated based on forward scatter (FSC) and side scatter (SSC). Cells were then gated for CD3 and CD56 expression. $CD56^{high}$ and $CD56^{low}$ cells were thereafter gated for CD16 expression.



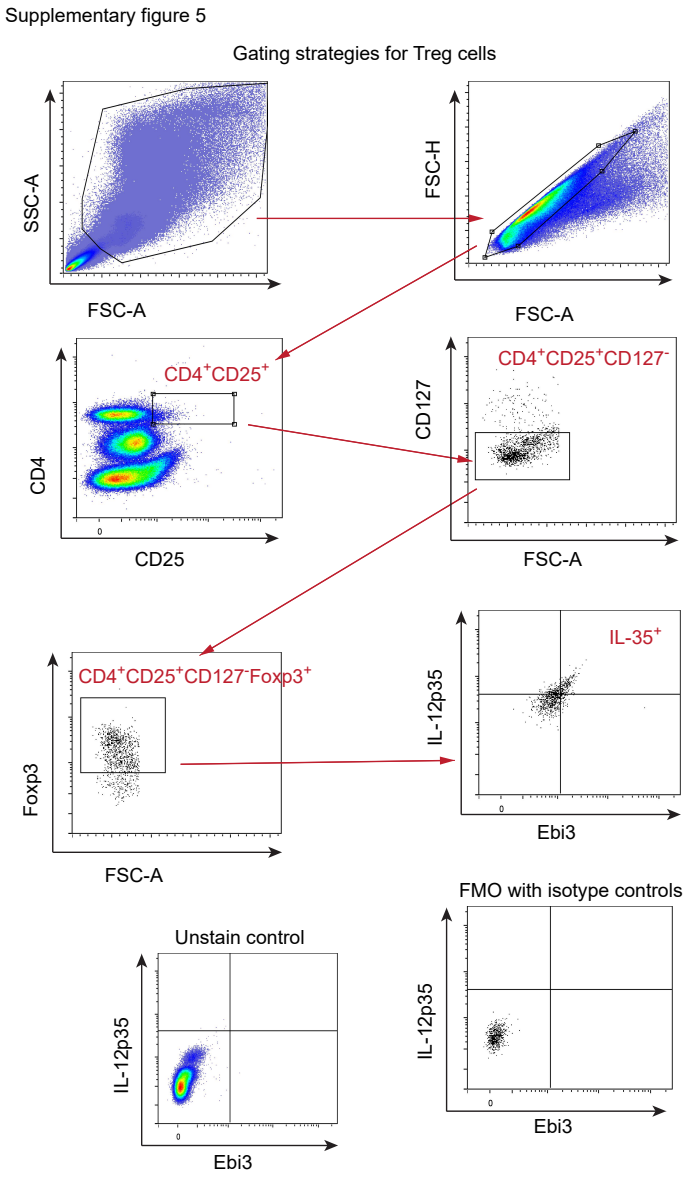
Supplementary figure 4. The representative gating strategies for Breg cells.

The live cells were gated based on forward scatter (FSC) and side scatter (SSC). They were then gated for CD19 expression. $CD19^+$ cells were gated for the expression of CD24 and CD40. $CD19^+CD24^+CD40^+$ cells were gated for CD38 expression, $CD19^+CD24^+CD40^+CD38^+$ cells were thereafter gated for IL-12p35 and Ebi3 expression. FMO together with isotype controls were used for negative control to determine IL- $12p35^+Ebi3^+$ ($IL\text{-}35^+$) cells.

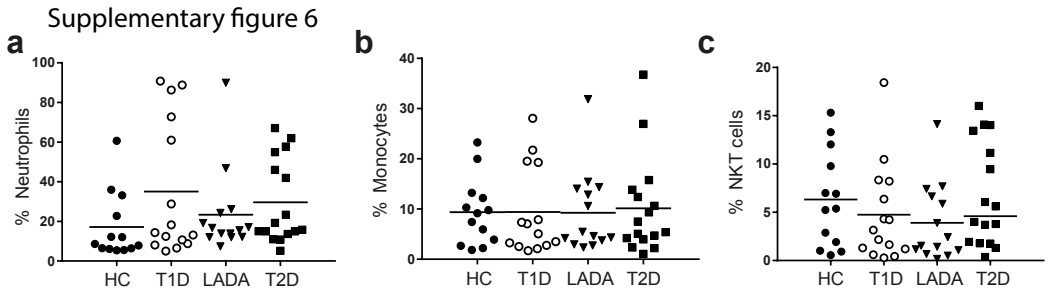


Supplementary figure 5. Representative gating strategies for Treg cells.

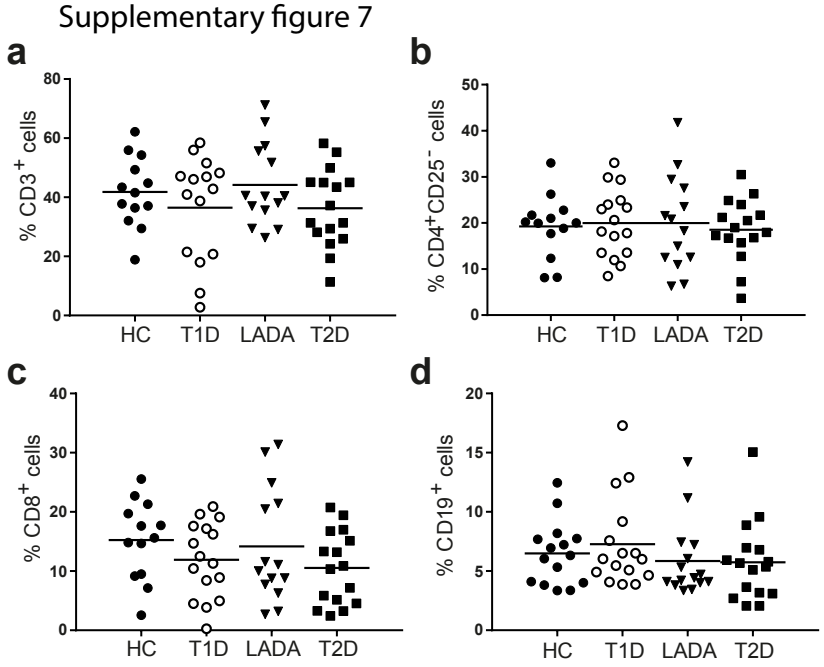
The live cells were gated based on side scatter (SSC) and forward scatter (FSC). The cells were then gated for the expression of CD4 and CD25. $CD4^+CD25^+$ cells were further gated for the expression of CD127. $CD4^+CD25^+CD127^-$ cells were gated for Foxp3 expression. $CD4^+CD25^+CD127^-$ $Foxp3^+$ cells were gated for the expression of IL-12p35 and Ebi3. IL-12p35 and Ebi3 gates were made based on fluorescence minus one control together with isotype controls (this control was used as a negative control).



Supplementary figure 6. The proportions of neutrophils, monocytes and NKT cells. The proportions of (a) $CD15^+$ neutrophils in total live cells, (b) $CD16^+$ monocytes in total live cells and (c) $CD3^+CD56^+$ NKT cells in total live cells, were determined by using a flow cytometer. HC denotes healthy controls. One-way ANOVA with Dunnett's post-hoc test comparison to patients with LADA was used, n = 13-16/group.



Supplemental figure 7. The proportions of T-cells and B-lymphocytes. The proportions of (a) $CD3^{+}CD56^{-}$ T-cells in total live cells, (b) $CD4^{+}CD25^{-}$ T-cells in total live cells, (c) $CD8^{+}$ T-cells in total live cells, (d) $CD19^{+}$ B-lymphocytes in total live cells, were determined by using a flow cytometer. HC denotes healthy controls. One-way ANOVA with Dunnett's post-hoc test comparison to patients with LADA was used, n = 13-16/group.



Supplemental figure 8. The proportions of Treg and tTreg cells among $CD4^+$ T cells. The proportion of **(a)** $CD4^+CD25^+CD127^-Foxp3^+$ Treg cells in total live cells, (b) $CD4^+CD25^+CD127^-Foxp3^+Helios^+$ tTreg cells in total live cells, (c) The proportions of $CD4^+CD25^+CD127^-Foxp3^+$ Treg cells among $CD4^+$ T-cells and (d) $CD4^+CD25^+CD127^-Foxp3^+Helios^+$ tTregs among $CD4^+$ T-cells. The proportions of these cells were determined by using a flow cytometer. HC denotes healthy controls. One-way ANOVA with Dunnett's post-hoc test was used for comparisons, n = 13-16/group.