

Electronic Supplementary Material

Phenotypic alterations in pancreatic lymph node stromal cells from human donors with type 1 diabetes and NOD mice

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TYPE	SOURCE	REFERENCE	GENDER	ETHNICITY	AGE	AUTOANTIBODIES	DIABETES DURATION
CTRL	NPOD	6413	F	WHITE	10		
CTRL	LIVENY	201	F	WHITE	23		
CTRL	NPOD	6331	F	AFA	27		
CTRL	LIVENY		F	UNK.	32		
CTRL	LIVENY		F	UNK.	39		
CTRL	LIVENY		F	UNK.	45		
CTRL	LIVENY	311	F	WHITE	52		
CTRL	LIVENY	298	F	WHITE	59		
CTRL	LIVENY		F	AFA	70		
CTRL	LIVENY		F	WHITE	73		
CTRL	NPOD	6412	F	WHITE	17		
CTRL	NPOD	6420	M	WHITE	11		
CTRL	NPOD	6431	M	WHITE	13		
CTRL	LIVENY		M	WHITE	18		
CTRL	LIVENY		M	WHITE	21		
CTRL	LIVENY		M	ASIAN	23		
CTRL	LIVENY		M	AFA	24		
CTRL	NPOD	6454	M	WHITE	25		
CTRL	NPOD	6439	M	AFA	26		
CTRL	LIVENY	346	M	WHITE	32		
CTRL	LIVENY	260	M	HISP.	34		
CTRL	LIVENY		M	WHITE	35		
CTRL	LIVENY		M	UNK.	40		
CTRL	LIVENY	259	M	AFA	46		
CTRL	LIVENY		M	UNK.	49		
CTRL	LIVENY	206	M	WHITE	50		
CTRL	LIVENY	480	M	WHITE	55		
CTRL	LIVENY	363	M	WHITE	65		
CTRL	LIVENY	257	M	WHITE	69		
CTRL	NPOD	6428	M	AFA	6		
T1D	NPOD	6457	F	WHITE	9	mIAA+	3 years
T1D	NPOD	6380	F	AFA	11	-	< 1 year
T1D	NPOD	6371	F	WHITE	12	GADA+, IA-2A+, mIAA+, ZnT8A+	2years
T1D	NPOD	6432	F	AFA	16	GADA+ IA-2A+ mIAA+	≥10 years
T1D	NPOD	6456	F	AFA	30	GADA+ ZNT8+	< 1 year
T1D	NPOD	6459	F	WHITE	20	IA2+,IAA+ ZNT+	≥10 years
T1D	NPOD	6458	F	AFA	13	GADA+	6 years
T1D	NPOD	6422	M	WHITE	22	mIAA+	≥10 years
T1D	NPOD	6414	M	AFA	23	GADA+ mIAA+ ZnT8+	< 1year
T1D	NPOD	6449	M	WHITE	24	IA2+,IAA+ ZNT+	2 years
T1D	NPOD	6441	M	WHITE	29	IA2A+ GADA+	5years
T1D	NPOD	6327	M	HISP.	71	mIAA+	≥10 years
AAb	NPOD	6397	F	WHITE	21	GADA+	-
AAb	NPOD	6450	F	WHITE	22	GADA+ ZNT8+	-
AAb	NPOD	6424	M	WHITE	17	GADA+ IAA+	-
AAb	NPOD	6433	M	HISP.	23	GADA+	-

ESM Table 1: List of human donors used for this study. Legend: CTRL (no T1D); AAb (autoantibody positive, not T1D-diagnosed); T1D (type 1 diabetes-diagnosed); LIVENY (LiveOnNY); F (women); M (men); WHITE (European descendant); AFA (African-American); HISP. (Hispanic); UNK. (unknown). Donors from nPOD and LiveOnNY were similarly conditioned, and although LiveOnNY samples were typically fresher than nPOD samples by up to one day, direct comparison of control samples from nPOD vs. LiveOnNY returned no significant differences, suggesting that source and timing were not confounding factors. Furthermore, if only nPOD samples were used, the differences between controls and T1D remained unaffected (with differences in relative FRC frequency or HLA-DR expression, for example, remaining significant).

Reactivity	Protein	Clone	Conjugate	Source	RRID	Dilution
human	CD45	HI30	BV510	Biolegend	AB_2561940	1/400
human	HLA-DR	L243	AF700	Biolegend	AB_493771	1/400
human	PDPN	NC-08	PE	Biolegend	AB_1595457	1/300
human	CD31	WM59	APC/Cy7	Biolegend	AB_10640734	1/300
human	HLA-A,B,C	W6/32	APC	Biolegend	AB_314879	1/400
human	PD-L1	29E.2A3	BV421	Biolegend	AB_2563852	1/300
human	CD3	HIT3a	Biotin	Biolegend	AB_314040	1ul/10 ⁷ cells
human	CD3	HIT3a	FITC	Biolegend	AB_314042	1/200
human	CD19	HIB19	Biotin	Biolegend	AB_314234	1ul/10 ⁷ cells
human	CD20	2H7	FITC	Biolegend	AB_493227	1/200
human	CD235a	HIR2	Biotin	eBioscience	AB_494036	1ul/10 ⁷ cells
human	CD235a	HI264	FITC	Biolegend	AB_10613463	1/200
mouse	CD45	30-F11	APC/Cy7	Biolegend	AB_312981	1/400
mouse	CD45	30-F11	Biotin	Biolegend	AB_312981	1ul/10 ⁷ cells
mouse	I-A ^K (I-A ^{B7})	10-3.6	PE	Biolegend	AB_313457	1/400
mouse	H2-K ^d	SF1-1.1	BV421	Biolegend	AB_2565656	1/400
mouse	PDPN	8.1.1	PE/Cy7	Biolegend	AB_10613648	1/300
mouse	CD31	390	FITC	Biolegend	AB_312901	1/400
mouse	PD-L1	10F.9G2	APC	Biolegend	AB_10612741	1/200
mouse	TER-119	TER-119	Biotin	Biolegend	AB_313705	1ul/10 ⁷ cells

ESM Table 2: List of antibodies used for this study. Note that the anti-I-A^K antibody is used to stain I-A^{B7} (cross-reactive).



JOB ID:	5680_FDGP_15.D1.1	TARGET SPECIES:	Homo sapiens
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Results Explanation:

- I) Assay performance was evaluated via serial dilution of a commercial reference sample.
- II) The ΔCT (or C_q) was calculated for all pairwise combinations of assays in this request and the slope of ΔCT versus \log_2 template dilution calculated and averaged.
- III) The mean slope of ΔCT versus template concentration for cDNA templates indicates relative error in measuring 2-fold changes for the given assay compared to the other assays in this request. If two assays have equal efficiencies and there is no measurement error, the slope will be zero. Typically, 95% of assays fall in the range -0.1 to 0.1.
- IV) NO CALL indicates assays that generated < 3 serial dilution measurements or which typically indicates gene expression too low to be reliably measured in the commercial reference sample.

Reference: Livak KJ, Schmittgen TD. Methods 2001; 25:402-408.

Assay Name (ID)	ΔCT Slope	Assay Name (ID)	ΔCT Slope
ACTB_55733_i2	.034	AIRE_61200_i5	.419
ALDH1A1_3993_i5	.064	AMBP_26385_i0	.029
ARG1_59789_i2	.044	ARG2_24820_i2	.043
CD274_69683_i0	.020	CELA1_24767_i4	-.223
CHGA_24827_i1	-.009	DEAF1_69684_i10	.050
EBI3_20989_i3	.061	ENTPD1_26447_i7	.054
FAS_55622_i0	-.030	FASLG_69305_i2	-.032
FEZF2_56986_i2	-.120	G6PC2_69686_i1	No Call
GAD2_31524_i2	No Call	GAPDH_55153_i3	.026
GUSB_55154_i0	.050	HMOX1_6488_i2	.042
HPRT1_5093_i5	-.029	IAPP_61870_i1	-.048
ICA1_28321_i8	.016	ICOSLG_13662_i3	.015
IDO1_26352_i6	-.049	IL10_12369_i3	-.003
IL12A_13006_i2	.063	IL1RN_6616_i2	.016
IL27_20856_i2	.040	INS_62411_e1	-.026
LGALS1_59844_i1	.042	LGALS9_27386_i1	-.043
LILRB2_61262_i4	.036	LILRB4_62868_i2	.018
MLANA_56562_i2	.011	NOS2_12347_i17	-.081
NT5E_56640_i0	.003	PDCD1LG2_23699_i1	-.020
PTPRN_69685_i20	-.032	SAG_29669_i4	No Call
SEMA4A_29244_i9	-.156	SLC30A8_26364_i3	-.041
TGFB1_7272_i1	.013	TNFAIP3_25385_i1	.036
TNFRSF14_24384_i6	-.066	TSLP_67805_i2	.009
TYR_60959_i0	No Call	VTCN1_60960_i3	-.111

ESM Table 3: Assay performance results of all assays used on Biomark 48x48 IFC. Validation report provided by Fluidigm.

Gene	Protein	Description and rationale for testing
AIRE	AIRE	Autoimmune regulator, controls expression of TSAs in mTECs
DEAF1	DEAF1	Deformed autoregulatory factor 1, controls expression of TSAs in LNs
FEZF2	FEZF2	FEZ family zinc finger 2, controls expression of TSAs in mTECs
INS	Insulin	Insulin, targeted by T cells and antibodies in mouse and human T1D
GAD2	GAD65	Glutamic acid decarboxylase, targeted by T cells and antibodies in mouse and human T1D
PTPRN	IA-2	Insulinoma antigen 2, targeted by T cells in mouse and human T1D
G6PC2	IGRP	Islet-specific glucose-6-phosphatase catalytic subunit-related protein, targeted by T cells in mouse and human T1D
CHGA	CHGA	Chromogranin A, targeted by T cells in mouse and human T1D
SLC30A8	ZNT8	Zinc transporter member 8, targeted by T cells and antibodies in mouse and human T1D
ICA1	ICA1	Islet cell autoantigen 1, targeted by T cells in mouse and human T1D
IAPP	IAPP	Islet amyloid polypeptide, targeted by T cells in mouse and human T1D
CELA1	CELA1	Chymotrypsin-like elastase family member 1, exocrine pancreas TSA
AMBP	AMBP	Alpha-1-microglobulin/bikunin precursor, liver TSA
SAG	Arrestin	S-antigen / arrestin, eye TSA
TYR	Tyrosinase	Tyrosinase, skin TSA, LEC-specific in mice
MLANA	MART1	Melanoma antigen recognized by T-cells 1, melanocyte TSA
TGFB1	TGF-b1	Immunosuppressive cytokine, induces Foxp3 expression in Tregs
IL10	IL-10	Immunosuppressive cytokine, induces IL-10-producing Tregs
IL27	IL-27a	Immune regulatory when combined with EB13 (IL-27), induces IL-10 in T cells and CD39 in DCs
IL12A	IL-12p35/IL-35a	Immune regulatory when combined with EB13 (IL-35), induces IL-35-producing Tregs
EBI3	EBI3	Immune regulatory when combined with IL-27a or IL-12p35
IL1RN	IL-1RA	Anti-inflammatory decoy receptor for IL-1
LGALS1	Galectin-1	Immunosuppressive and anti-proliferative in mice, protective in diabetes (in mice)
LGALS9	Galectin-9	Immunosuppressive for pathogenic Th1 cells, binds Tim3
TSLP	TSLP	Involved with the non-inflammatory maturation of mTECs in the thymus
ENTPD1	CD39	Participates in adenosine production from ATP, promotes Tregs
NT5E	CD73	Participates in adenosine production from ATP, promotes Tregs
CD274	PD-L1/B7-H1	Promotes deletion of activated T cells and supports Treg induction
PDCD1LG2	PD-L2/CD273/B7-DC	Inducible; negatively regulates T cell immune responses.
SMA4A	Semaphorin 4A	Boost Treg function, binds Nrp1
VTCN1	B7-H4	Contributes to tolerance and Treg induction, protective in diabetes (in mice)
ICOSLG	ICOS-L	Amplifies induction of IL-10 in T cells
TNFRSF14	HVEM/CD270	Herpes virus entry mediator, promote Foxp3 expression in T cells.
LILRB4	ILT3	Inhibitory receptor on APCs
LILRB2	ILT4	Inhibitory receptor on APCs
FAS	FAS	Promotes deletion of activated T cells
FASLG	FASL	Promotes deletion of activated T cells
IDO1	IDO1	Indoleamine 2,3-dioxygenase, depletes tryptophan levels, contributes to tolerance and Treg induction
ALDH1A1	ALDH1A1	Aldehyde dehydrogenase 1, rate-limiting enzyme in the production of retinoic acid, which contributes to Treg induction
ARG1	ARG1	Arginase 1, depletes arginine levels, contributes to tolerance
ARG2	ARG2	Arginase 2, depletes arginine levels, contributes to tolerance
NOS2	iNOS	Nitric oxide synthase 2, inducible; immunosuppressive and anti-proliferative
HMOX1	HMOX1	Heme oxygenase 1, inducible. Reduce T cell priming.
TNFAIP3	A20	Tumor necrosis factor, alpha-induced protein 3, inducible. Promotes tolerance.
ACTB	ACTB	Endogenous (housekeeping) gene, normalization control
GAPDH	GAPDH	Endogenous (housekeeping) gene, normalization control
HPRT1	HPRT1	Endogenous (housekeeping) gene, normalization control
GUSB	GUSB	Endogenous (housekeeping) gene, normalization control

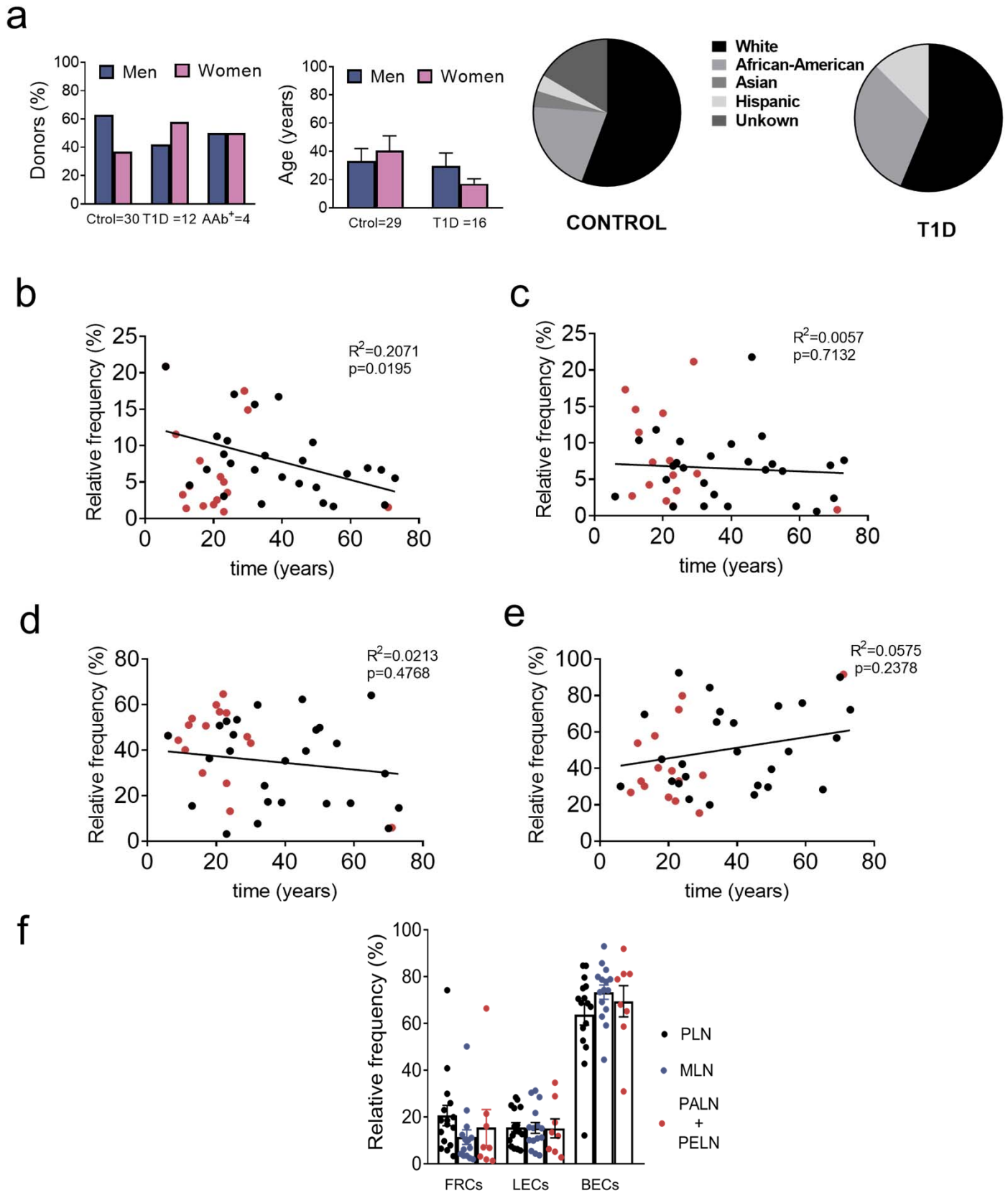
ESM Table 4: List of 48 genes analyzed by RT-qPCR in Fluidigm Biomark chips. Legend: light blue (transcription factors); light orange (beta cell antigens); dark orange (non beta cell antigens); green (secreted inhibitory molecules); red (coinhibitory cell surface ligands or receptors); yellow (tolerance-related enzymes); grey (housekeeping genes).

- Undetected
- Detected in few samples
- Upregulated
- Downregulated
- Not substantially changed

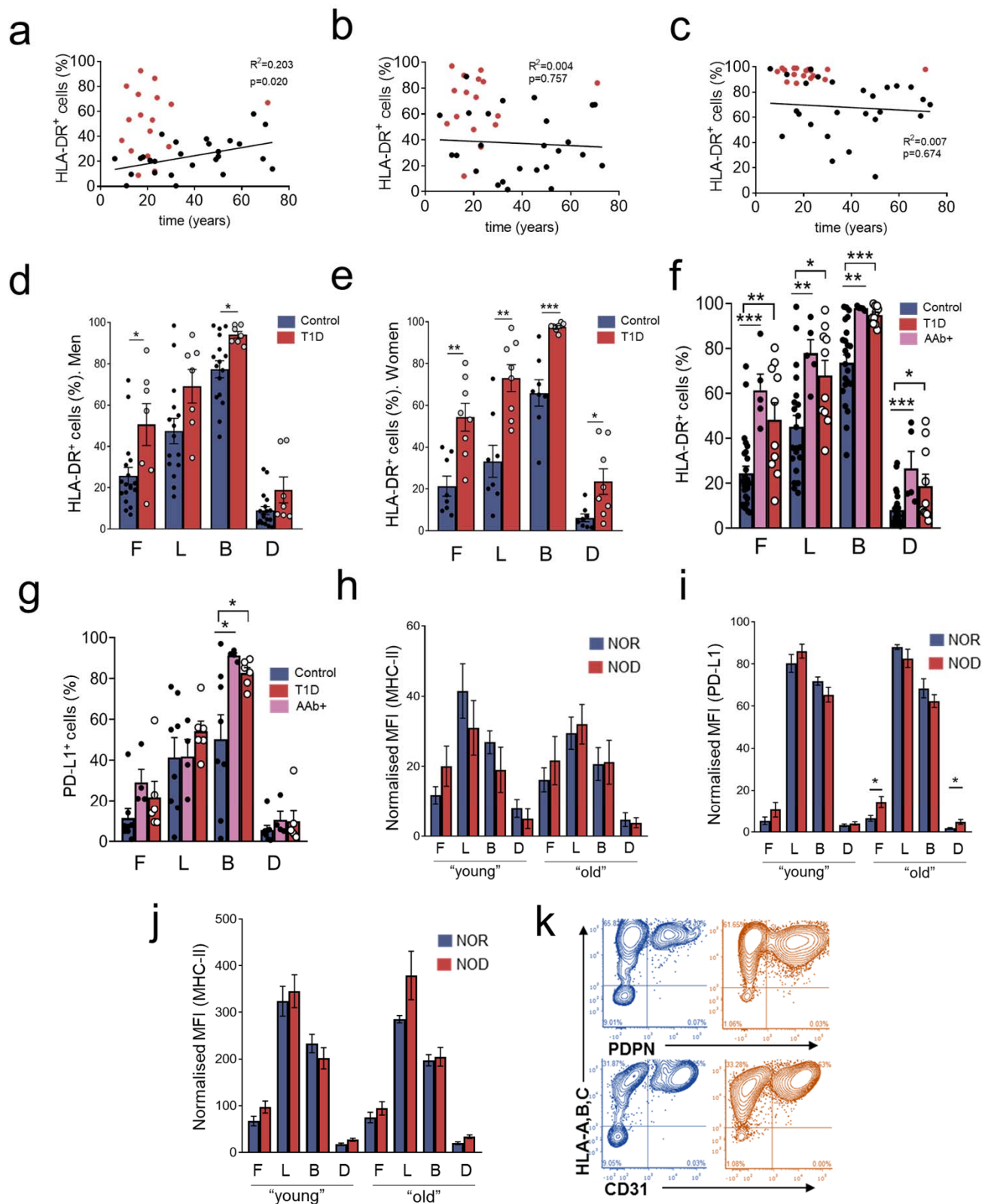
Gene	Protein	FRCs	DNCs	LECs	BECs	HLA-DR ^{hi} CD45 ^{lo}	
AIRE	AIRE	+	+	+	-	↑ 1.15	Transcription regulators
DEAF1	DEAF1	↑ 2.13	↑ 1.5	↑ 1.7	↓ 1.25	↑ 1.13	
FEZF2	FEZF2	-	-	-	-	-	
INS	Insulin	↑ 8.13	↑ 2.13	↓ 1.25	↑ 2.5	↑ 9.0*	
GAD2	GAD65	-	-	+	-	+	Tissue-restricted antigens
PTPRN	IA-2	+	+	+	+	+	
G6PC2	IGRP	-	-	-	-	-	
CHGA	CHGA	-	-	-	-	-	
SLC30A8	ZNT8	-	-	-	-	+	
ICA1	ICA1	↑ 15.23	↓ 1.29	↑ 2.22*	↑ 1.7*	↑ 5.7	
IAPP	IAPP	-	-	+	-	+	
CELA1	CELA1	-	-	-	-	+	
AMBP	AMBP	-	-	-	-	+	
SAG	Arrestin	-	-	-	-	-	
TYR	Tyrosinase	↑ 10.77	+	+	+	+	
MLANA	MART1	↑ 13.27*	↑ 7.37	↑ 2.91	↑ 1.97	↑ 1.94	Secreted molecules
TGFB1	TGF-b1	↑ 1.82	↑ 1.6	↑ 1.58	↑ 1.2	↓ 1.16	
IL10	IL-10	↓ 1.05	↑ 1.5	+	+	↓ 2	
IL27	IL-27a	-	-	-	-	+	
IL12A	IL-12p35	↑ 4.59*	↑ 1.94	↑ 1.67	↑ 2.89	↑ 1.66	
EBI3	EBI3	↑ 3.68	↑ 2.35	↑ 2.91	↑ 1.39	↓ 1.75†	
IL1RN	IL-1RA	↓ 1.06	↑ 1.67	+	+	↓ 2.22	
LGALS1	Galectin-1	↑ 2.7	↑ 1.66	↑ 1.32	↑ 1.25	↓ 1.47	
LGALS9	Galectin-9	↑ 5.05	↑ 3.33	↑ 2.19	↑ 1.99	↓ 1.58	
TSLP	TSLP	↓ 1.45	↑ 1.76	↑ 1.97	↑ 1.38	+	
ENTPD1	CD39	↑ 1.62	↑ 2.2	↑ 2.48*	↑ 1.68	↑ 1.4	
NT5E	CD73	↑ 7.11	↑ 2.7	↑ 1.03	↑ 1.86	↓ 1.35	
CD274	PD-L1	↑ 1.83	↑ 1.27	↑ 2.07	↑ 1.31	↓ 1.05	
PDCD1LG2	PD-L2	↑ 4.22	↑ 2.93	↑ 2.38	↑ 1.44	↑ 1.81	
SMA4A	Sema4A	↑ 1.43	↑ 3.24	↓ 1.12	↑ 9.83	↓ 1.26	
VTCN1	B7-H4	↑ 3.33	↑ 3.35	↓ 2.17	↓ 1.05	↑ 2.91	Surface ligands
ICOSLG	ICOS-L	↑ 8.99	↑ 1.81	↓ 1.03	↓ 1.63	↓ 1.61	
TNFRSF14	HVEM	↑ 2.92*	↑ 1.37	↑ 1.81†	↑ 1.5	↓ 1.16	
LILRB4	ILT3	↑ 2.02	↑ 2.53	↑ 1.73	↑ 3.4	↑ 1.2	
LILRB2	ILT4	↑ 1.4	↑ 2.71	↓ 1.58	↑ 1.65	↓ 1.16	
FAS	FAS	↑ 2.13	↑ 1.88	↑ 1.51	↑ 1.49	↓ 2.3*	
FASLG	FASL	-	+	-	-	+	Enzymes
IDO1	IDO1	↑ 1.8	↓ 1.15	↓ 3.57	↑ 3.84	↓ 3.45*	
ALDH1A1	ALDH1A1	↑ 4.5	↑ 4.89*	↑ 1.78	↑ 1.79	↑ 2.69	
ARG1	ARG1	+	↑ 6.12	+	+	+	
ARG2	ARG2	↑ 2.62	↑ 2.4*	↑ 1.74†	↑ 2.48	↑ 3.56	
NOS2	iNOS	-	-	↑ 3.68	-	+	
HMOX1	HMOX1	↑ 1.19	↑ 1.91	↑ 1.26	↑ 2.34	↓ 2.32	
TNFAIP3	A20	↓ 1.05	↑ 1.68	↑ 1.33	↑ 1.86	↓ 1.44	

Tolerance-related genes

ESM Table 5. Summary of type 1 diabetes-associated changes in gene expression in LNSCs and HLA-DR^{high} CD45^{low} cells. Numbers represent the fold change in gene expression in type 1 diabetes samples relative to control samples (↑ upregulated; ↓ downregulated). Color legend: grey (-), below detection or in less than 20% of the samples; yellow (+), detected only in a fraction of the donors; pink, >2-fold increase OR increase that is significant (*) or would be if it was not for an outlier (†); blue, >2-fold decrease OR decrease that is significant (*) or would be if it was not for an outlier (†); white, no meaningful change in expression.

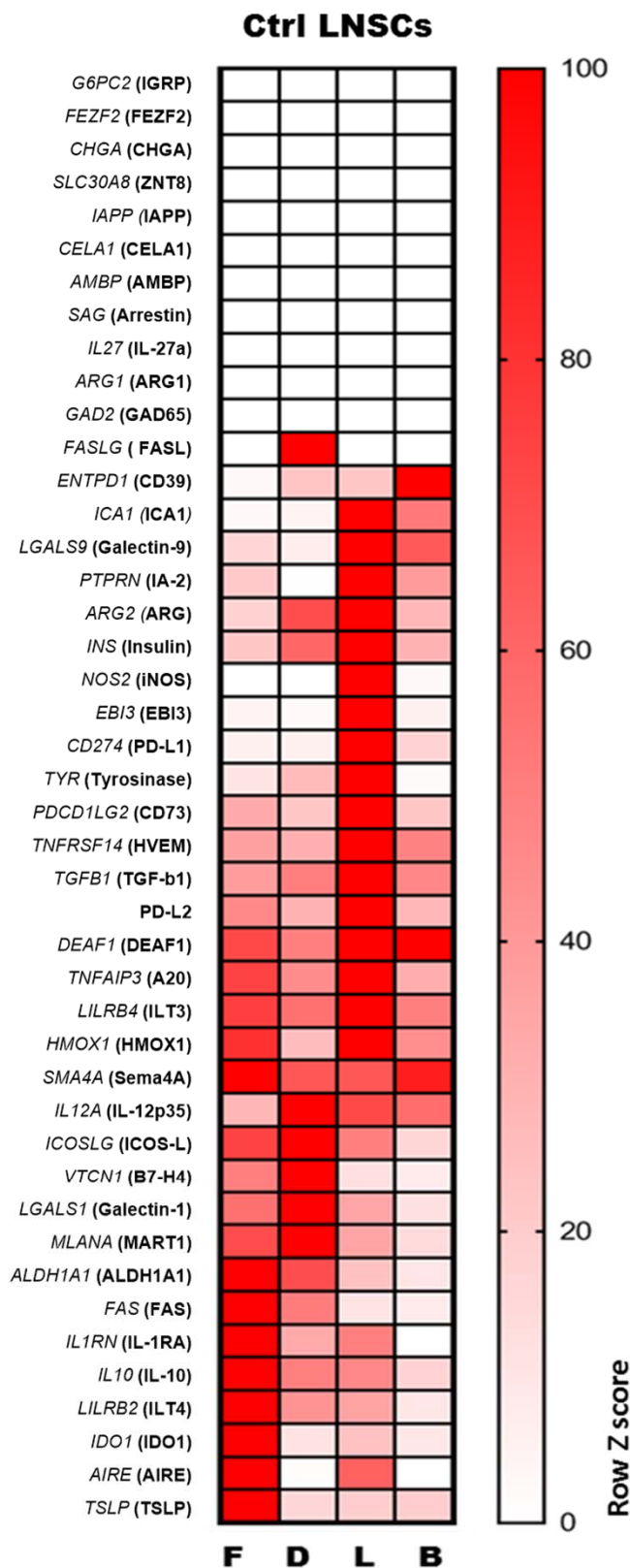


ESM Fig.1. Donor demographics and LNSC subset distribution in human samples. (a) Percentage of men and women, age distribution and ethnicity in control and type 1 diabetes (T1D) donors. (b-e) Distribution of the relative frequencies of LNSCs, FRCs (b), LECs (c), BECs (d) and DNCs (e) in control and T1D human PLNs over the years. (f) Comparison of the relative frequency of FRCs, LECs and BECs between PLNs, mesenteric (MLNs) and para-aortic (PALN) and peri-esophagic (PELNs) LNs from control donors.

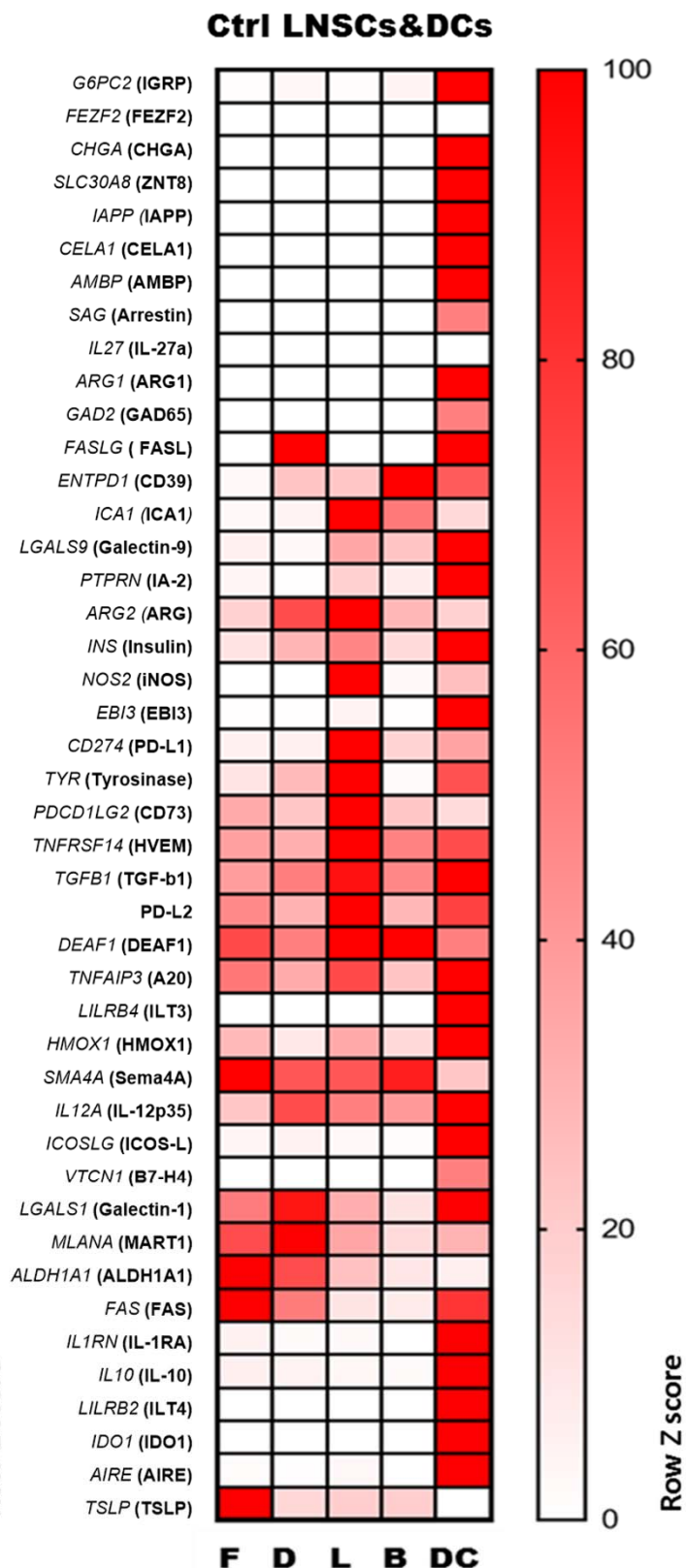


ESM Fig.2. MHC-II, PD-L1 and MHC-I expression in human and mouse LNSCs. (a-c) Distribution of the percentage of HLA-DR⁺ cells among FRCs (a), LECs (b) and BECs (c) in control (black) and type 1 diabetes (T1D) (red) PLNs over the years. (d-e) Percentage of HLA-DR⁺ cells in LNSC subsets in men (d) and women (e) from control and T1D donors. (f,g) Percentage of HLA-DR⁺ (f) and PD-L1⁺ (g) cells in LNSC subsets from the PLNs of control, AAb⁺ and T1D donors. (h-j) Normalized MFI of MHC-II (h), PD-L1 (i) and MHC-I (j) on LNSC subsets in PLNs from NOR and NOD mice from two age groups. (k) Representative dot plots of HLA-A,B,C expression vs PDPN and CD31 in CD45⁻ cells from control (blue) and T1D (red) PLNs. Abbreviations: F, FRCs; L, LECs; B, BECs; D, DNCs.

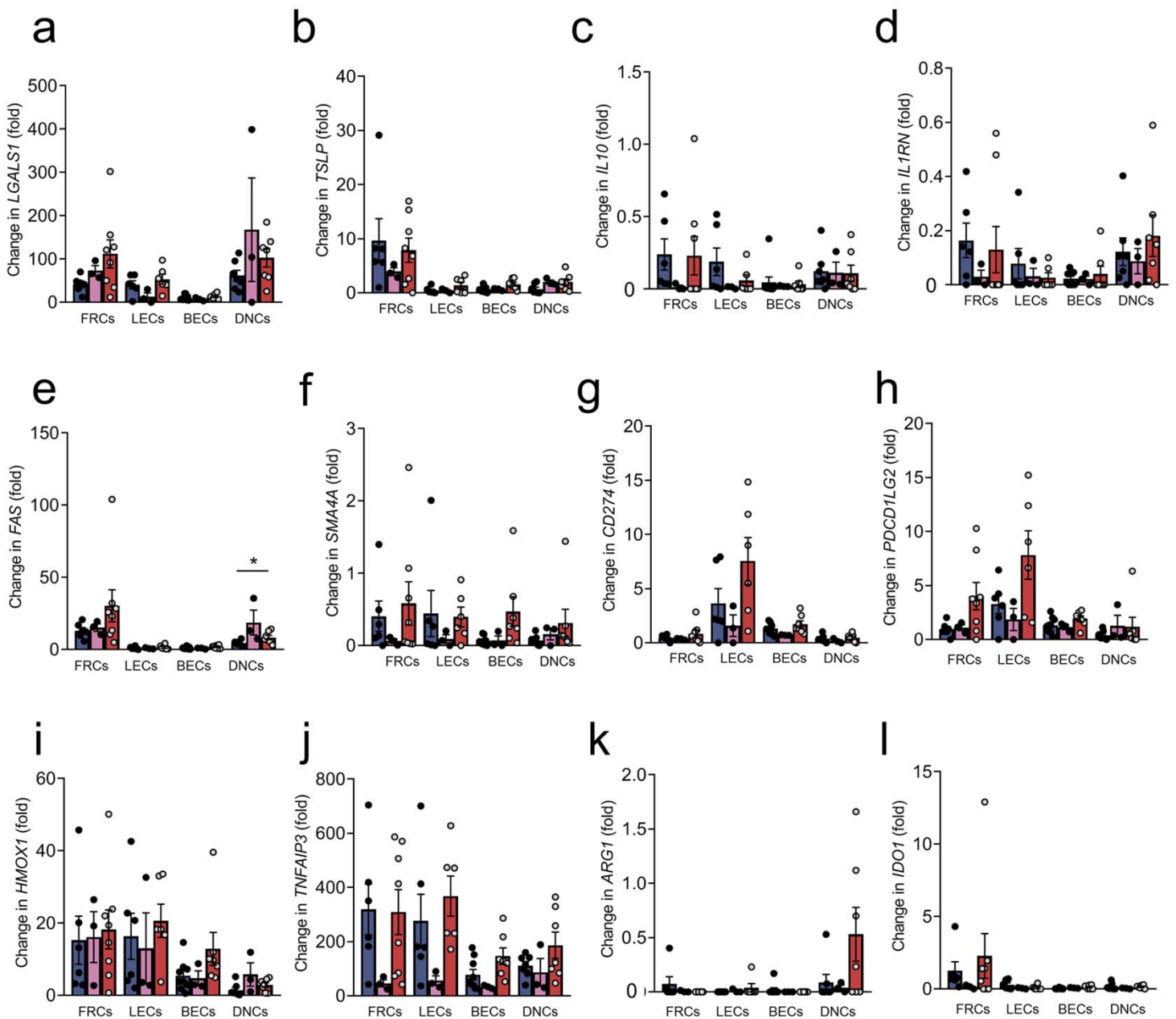
a



b



ESM Fig.3. Gene expression comparison between human LNSCs and AIRE-enriched dendritic cells (DC). (a) Fold change expression of selected genes among the four subsets of LNSCs in PLNs from human control donors, normalized to 100% based on the subset with the maximal expression for each individual gene (row Z score), based on quantitative PCR data. (b) Fold change expression of the same genes (same normalization as in a) with sorted CD45^{low} HLA-DR^{high} cells included in the analysis for comparison. Abbreviations: F, FRCs; D, DNCs; L, LECS; B, BECs; DC, CD45^{low} HLA-DR^{high}.



ESM Fig.4. Type 1 diabetes-associated changes in gene expression in human LNSC subsets. Relative mRNA expression by quantitative PCR in human LNSC subsets between control (black), AAb⁺ (blue) and type 1 diabetes (T1D) (red) donors for: (a,b,c,d) secreted molecules; (e,f,g,h) cell surface ligands and (i,j,k,l) immunosuppressive enzymes. All mRNA levels are normalized against the endogenous control *HPRT1*.