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Supplementary Materials for

Transcription and splicing regulation by NLRC5 shape the interferon response in human pancreatic β cells

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The PDF file includes:

Figs. S1 to S10
Tables S1 to S4
Legends for data S1 to S3

Other Supplementary Material for this manuscript includes the following:

Data S1 to S3

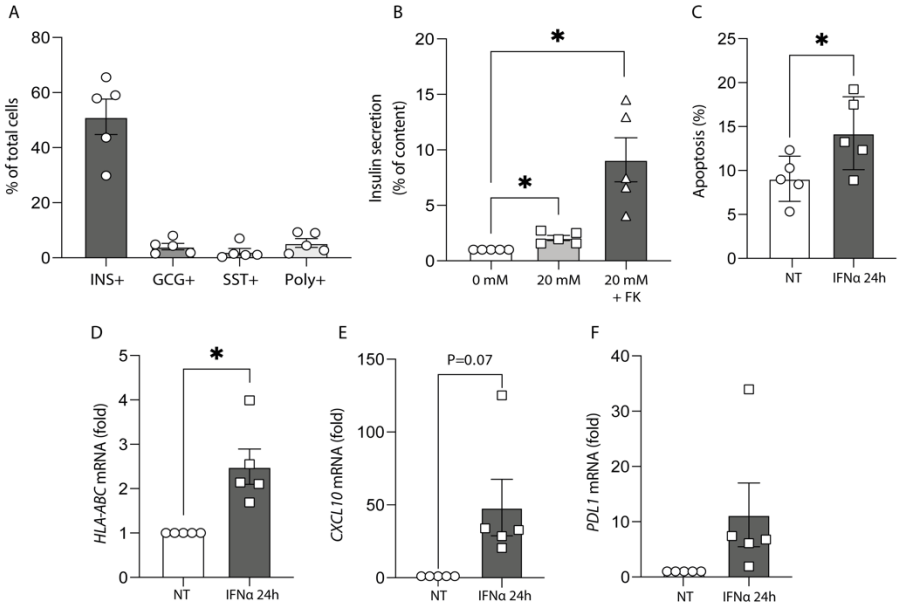


Fig. S1. iPSCs-derived islet-like cells used for the scRNA-sequencing. (**A**) Percentage of insulin (INS), glucagon (GCG), somatostatin (SST) or polyhormonal (Poly) cells per total number of cells. (**B**) Insulin secretion expressed as percentage of total insulin content at the end of the differentiation. (**C-F**) At the end of the differentiation, iPSCs-derived islet-like cells were left untreated (white bars) or treated with IFNα (2,000 U/mL) for 24h (gray bars). (**C**) The percentage of dead cells was counted after Hoechst and propidium iodine staining. Expression of (**D**) *HLA-ABC*, (**E**) *CXCL10* and (**F**) *PDL1* was measured at mRNA level by quantitative RT-PCR and normalized by geometric mean of *GAPDH* and *β-actin* and then by the non-treated (NT) condition considered as 1. Results are means ± SEM of 5 independent experiments. *P < 0.05, as indicated by bars (ANOVA followed by Bonferroni correction for multiple comparisons or paired t-test).

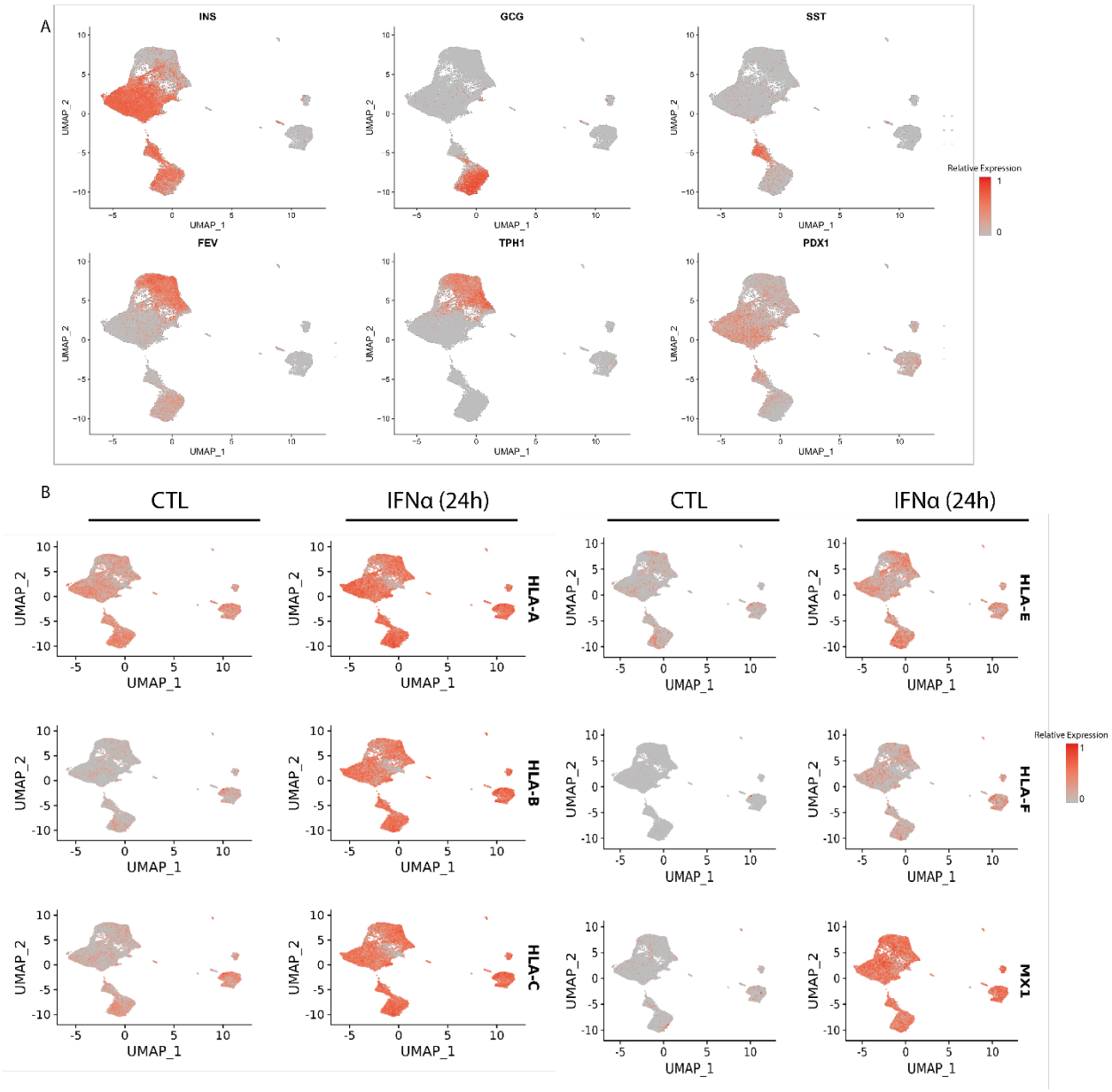


Fig. S2. Composed plots of uniform manifold approximation and projections (UMAP). (**A**) Relative expression of hallmark genes in human iPSC-derived β-like cells as described *(30, 31)*. The expression of those genes is located in specific clusters. (**B**) Induction of HLA Class I-related genes following IFNα exposure is visible in all cell types of iPSC-derived islet-like cells.

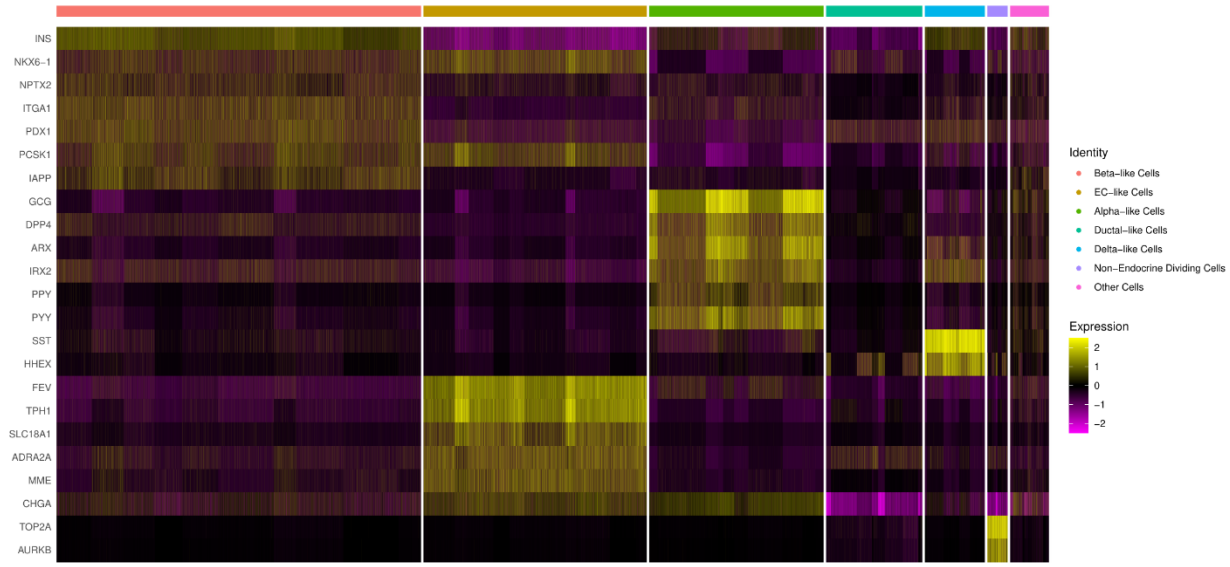
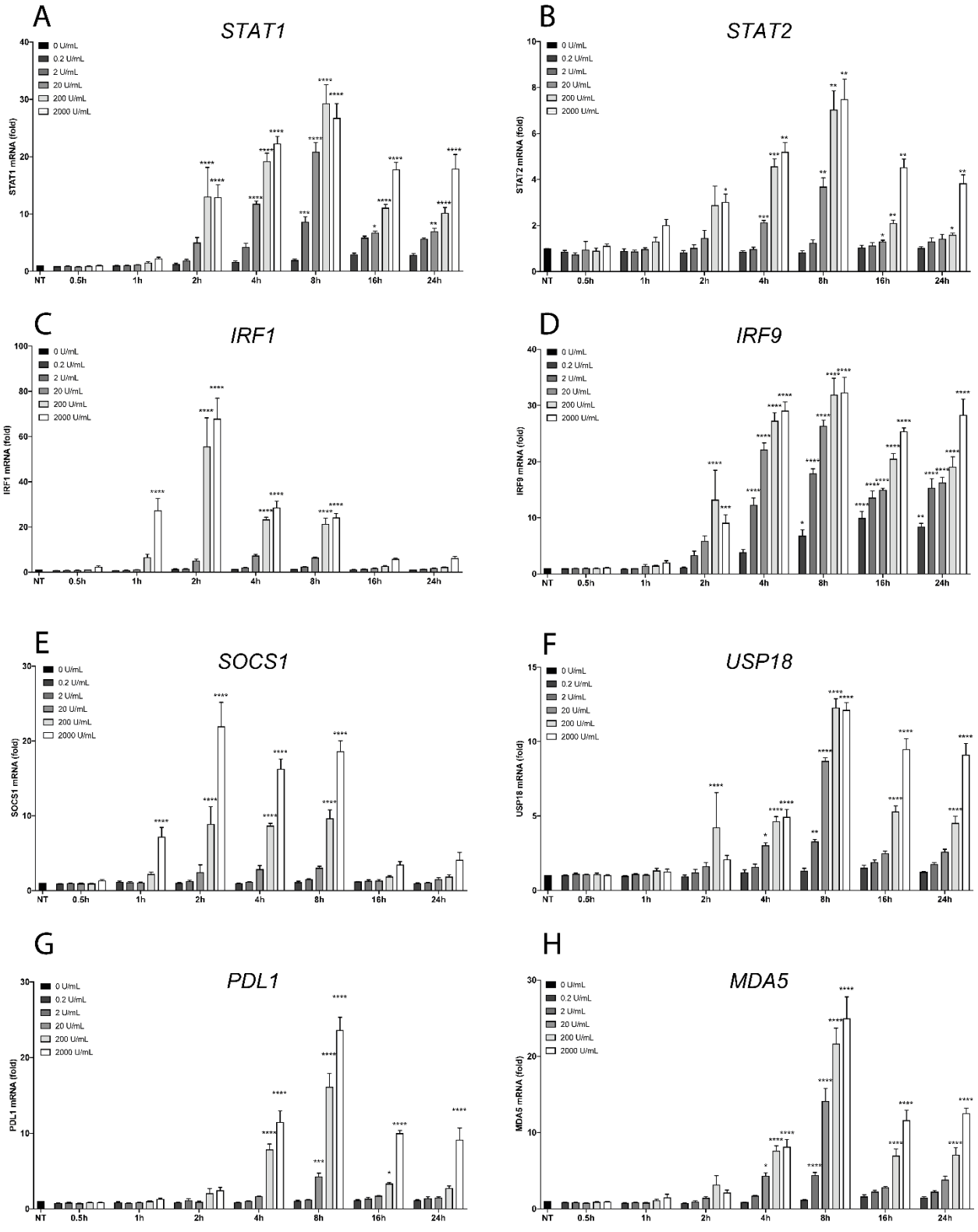


Fig. S3. Heatmap of the hallmark genes of each cell type in the scRNA-sequencing dataset. Gene expression matrix of the scRNA-sequencing data was scaled, and genes described in *(30, 31)* were used to validate the unsupervised clustering. The identified clusters were used to perform differential gene expression analysis between the paired experiments to assess the changes induced by IFNα in each cell type.


A
STAT1
STAT1 mRNA (fold)
B
STAT2
STAT2 mRNA (fold)
C
IRF1
IRF1 mRNA (fold)
D
IRF9
IRF9 mRNA (fold)
E
SOCS1
SOCS1 mRNA (fold)
F
USP18
USP18 mRNA (fold)
G
PDL1
PDL1 mRNA (fold)
H
MDA5
MDA5 mRNA (fold)
0 U/mL
0.2 U/mL
2 U/mL
20 U/mL
200 U/mL
2000 U/mL
NT
0.5h
1h
2h
4h
8h
16h
24h

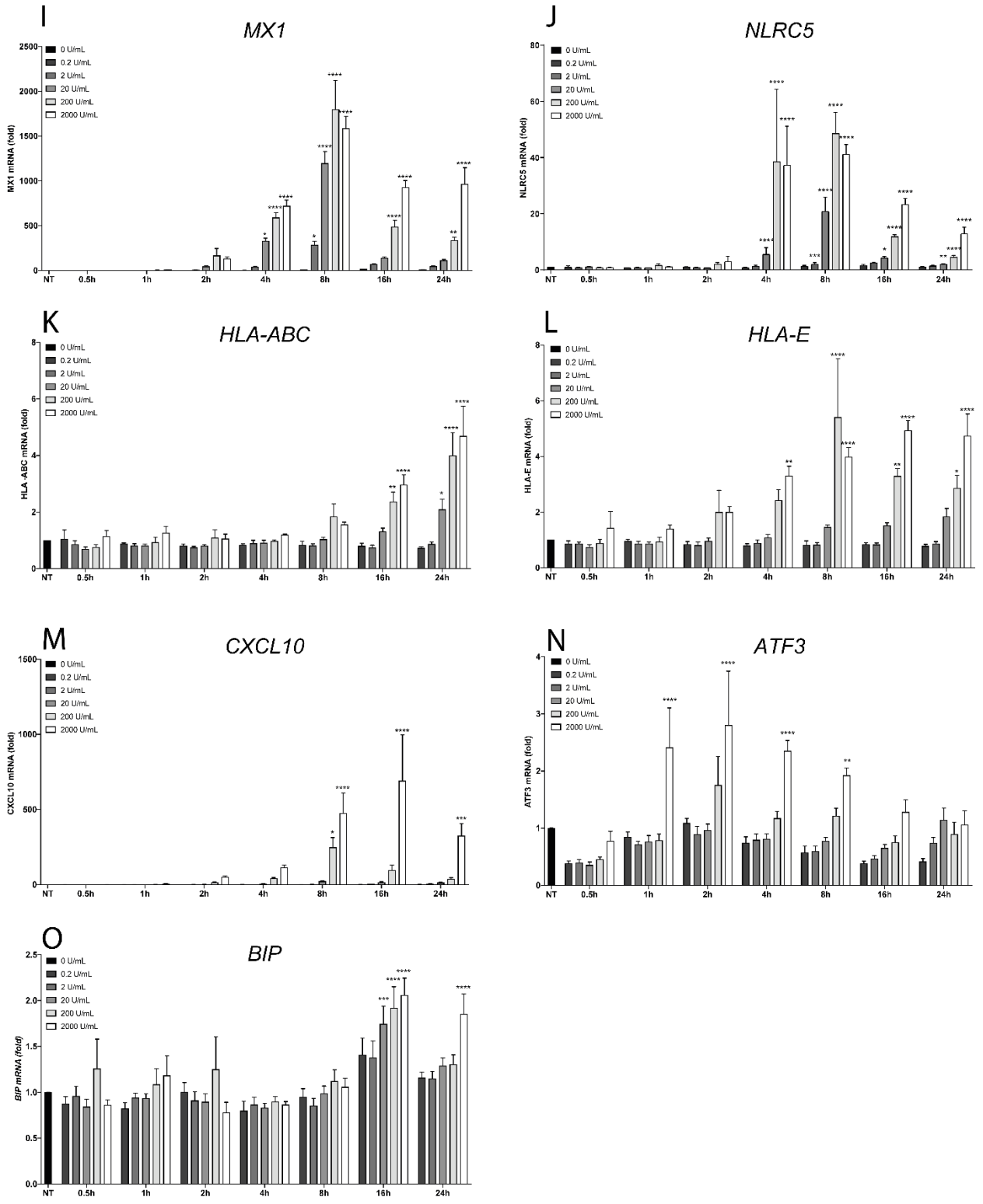


Fig. S4. Time-course and dose-response of IFNα-induced expression of selected mRNAs in EndoC-βH1 cells. EndoC-βH1 cells were exposed to IFNα for the indicated dose (0 to 2000U/mL) and time-points (0.5h to 24h). The mRNA expression of *STAT1* (**A**), *STAT2* (**B**), *IRF1* (**C**), *IFR9*

(**D**), *SOCS1* (**E**), *USP18* (**F**), *PDL1* (**G**), *MDA5* (**H**) *MX1* (**I**), *NLRC5* (**J**), *HLA-ABC* (**K**), *HLA-E* (**L**), *CXCL10* (**M**), *ATF3* (**N**) and *BiP* (**G**) was evaluated by quantitative RT-PCR. The values were normalized by the housekeeping gene *GAPDH* and then by the untreated (NT) conditions of each experiment considered as 1. NT is the mean of NT conditions for all time-points. Data are mean ± SEM of 6 independent experiments, except for NLRC5 experiments where n=3. $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs NT condition (ANOVA followed by Bonferroni correction for multiple comparisons).

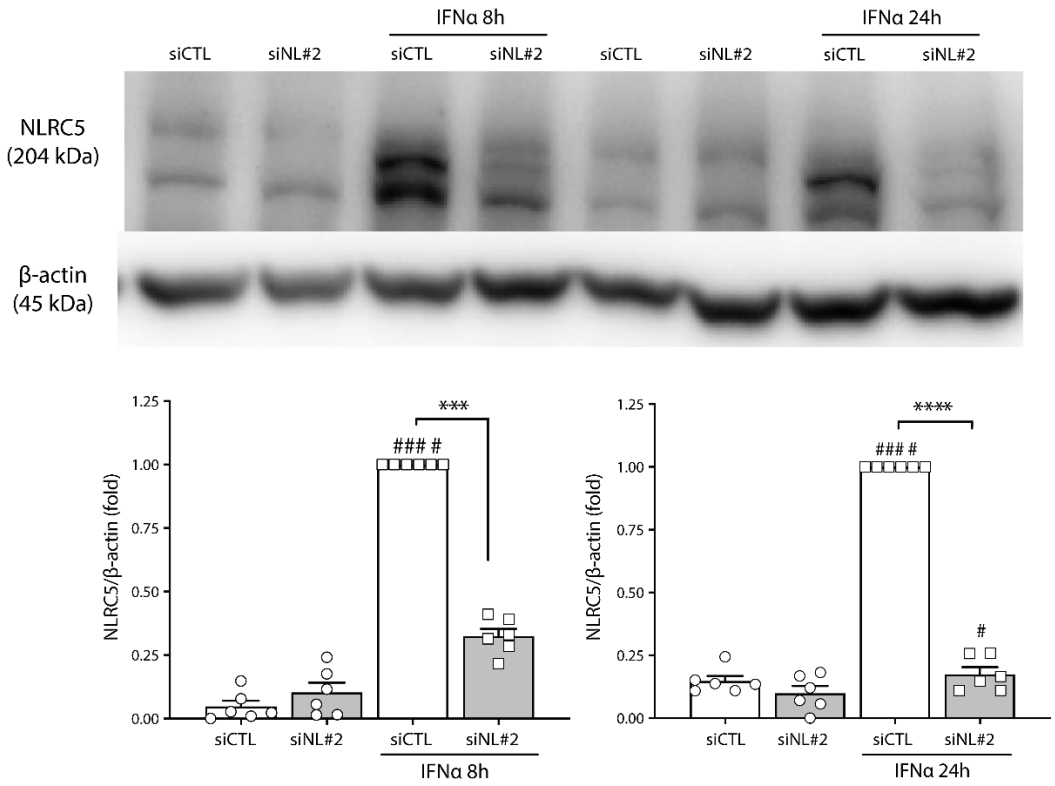


Fig. S5. Validation of the NLRC5 depletion for the RNA-sequencing experiments. EndoC-βH1 were transfected with a siRNA control (siCTL, white bars) or with a siRNAs targeting *NLRC5* (siNL#2, grey bars). The cells were left untreated or treated with IFNα (2,000 U/mL) for 8h and 24h. The efficiency of NLRC5 depletion was confirmed at the protein level by western blotting in all samples ahead of RNA-seq analysis. A representative image of the western blotting is shown together with the respective NLRC5 protein quantification. NLRC5 protein was quantified by densitometry and normalized by the housekeeping protein β-actin and then by the highest value of each experiment considered as 1. Results are means ± SEM of 6 independent experiments. #P < 0.05 and ####P < 0.0001 vs untreated and transfected with the same siRNA; ***P < 0.001 and ****P < 0.0001 as indicated by bars (ANOVA followed by Bonferroni correction for multiple comparisons).

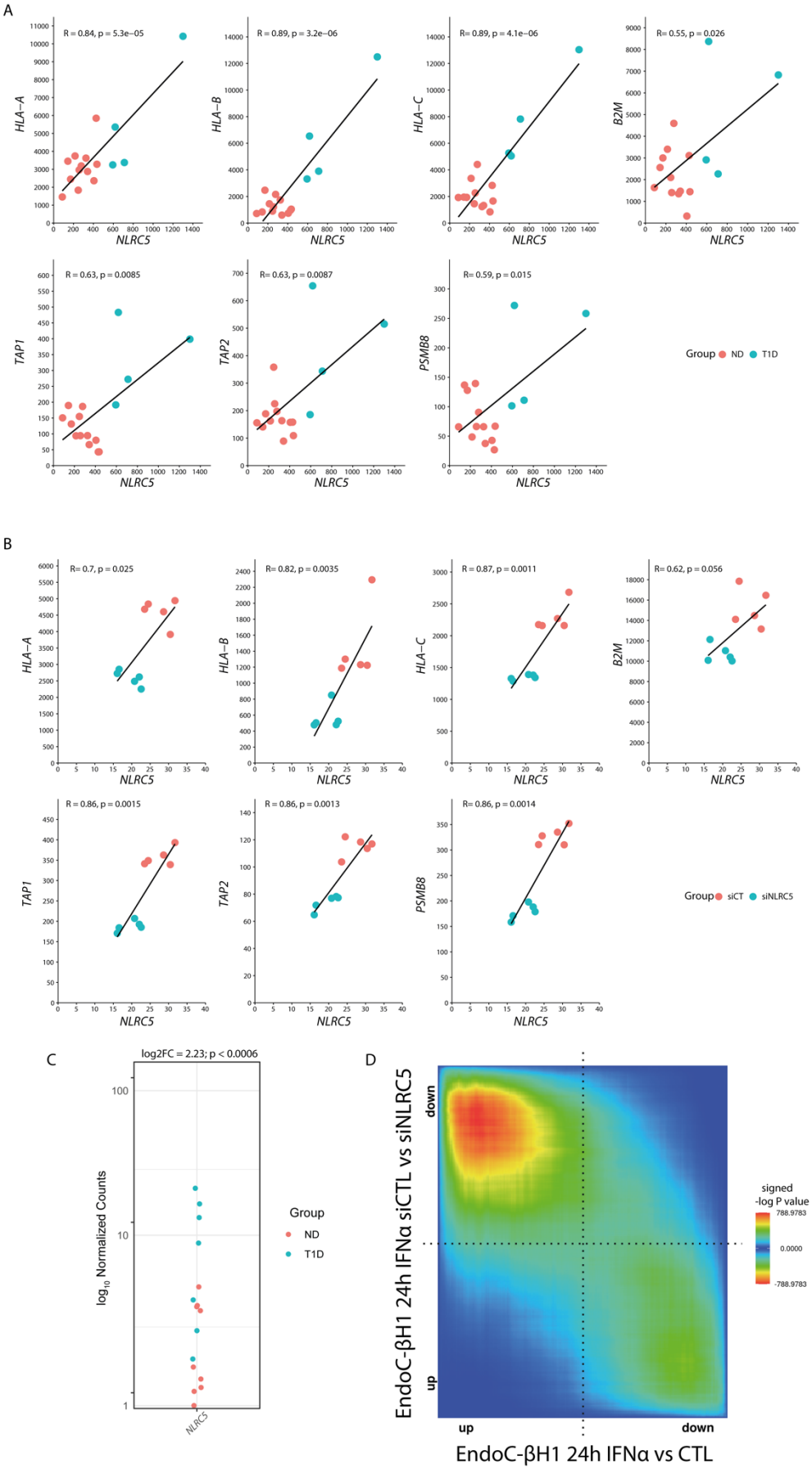

A
HLA−A
R = 0.84, p = 5.3e−05
HLA−B
R = 0.89, p = 3.2e−06
HLA−C
R= 0.89, p = 4.1e−06
B2M
R= 0.55, p = 0.026
TAP1
R = 0.63, p = 0.0085
TAP2
R= 0.63, p = 0.0087
PSMB8
R= 0.59, p = 0.015
NLRC5
Group ND T1D
B
HLA−A
R= 0.7, p = 0.025
HLA−B
R= 0.82, p = 0.0035
HLA−C
R = 0.87, p = 0.0011
B2M
R= 0.62, p = 0.056
TAP1
R = 0.86, p = 0.0015
TAP2
R = 0.86, p = 0.0013
PSMB8
R= 0.86, p = 0.0014
NLRC5
Group siCT siNLRC5
C
log2FC = 2.23; p < 0.0006
log10 Normalized Counts
Group
ND
T1D
NLRC5
D
EndoC-βH1 24h IFNα siCTL vs siNLRC5
down
up
EndoC-βH1 24h IFNα vs CTL
signed
-log P value
788.9783
0.0000
-788.9783

Fig. S6. *NLRC5* expression is positively correlated with HLA Class I-related genes in β-cells from patients affected by T1D and in EndoC-βH1 cells exposed to IFNα. (**A**) Composed scatter plots showing the correlation between the expression of selected HLA class I-related genes and *NLRC5* in FACS-purified β-cells from donors with type 1 diabetes (T1D) or non-diabetic donors (ND). The linear correlation of the normalized counts *(22)* of selected genes and *NLRC5* was generated using Pearson's test. (**B**) Composed scatter plots showing the correlation between the expression of selected HLA class I-related genes and *NLRC5* in EndoC-βH1 cells exposed to IFNα during 24h, with previous exposure to siNL#2 or siCTL. The linear correlation between the expression, measured by transcripts per million (TPM), of selected genes and *NLRC5,* was generated using Pearson's test. **(C)** Scatter plot showing the increase of *NLRC5* expression in β-cells from donors affected by T1D compared to ND donors. scRNA-seq data from T1D and ND donors was obtained via the HPAP (https://hpap.pmacs.upenn.edu/). β-cells were selected, and data was transformed into "pseudo-bulk". Differential gene expression analysis was then carried out as described in the Methods. (**D**) Rank-rank hypergeometric overlap analysis between EndoC-βH1 exposed to IFNα during 24h vs control and EndoC-βH1 exposed to IFNα during 24h following KD of *NLRC5* vs control. The level map colors display the adjusted log P values of the overlap (the P values were adjusted using the Benjamini and Yekutieli method) between genes up-regulated in both datasets (bottom left quadrant), down-regulated in both (top right quadrant), up-regulated in the left-hand dataset and down in the bottom part (top left quadrant), and down in the left-hand dataset and up-regulated in the bottom part dataset (bottom right quadrant).

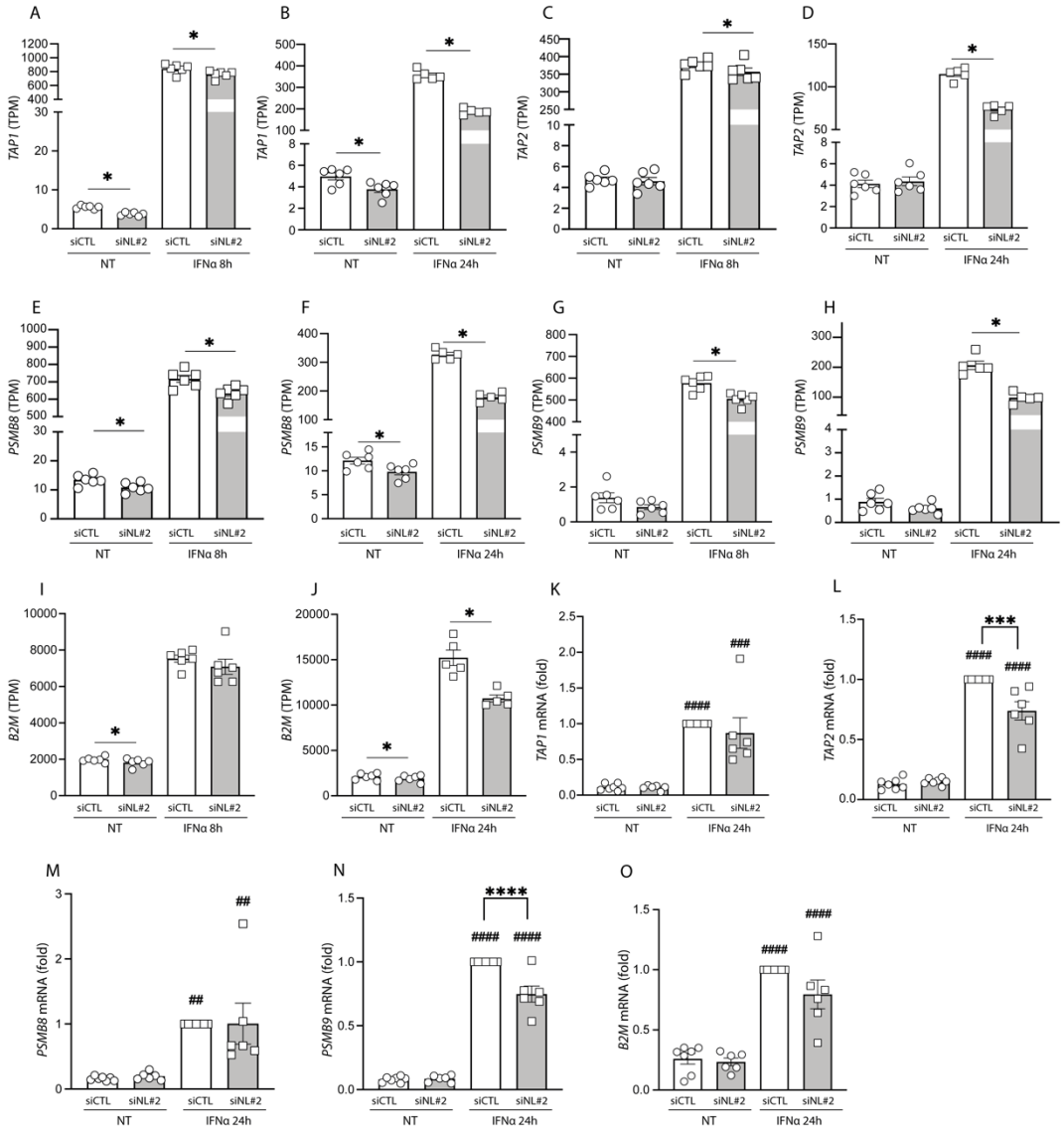


Fig. S7. *NLRC5* depletion prevents the expression of IFNα-induced genes related to the antigen presentation machinery in EndoC-βH1 and dispersed human islets. (**A-J**) RNA-seq analysis evidence that *NLRC5* decreases the mRNA expression levels of *TAP1* (A, B), *TAP2* (**C, D**), *PSMB8* (**E, F**), *PSMB9* (**G, H**), and *B2M* (**I, J**) at 8h and 24h. The mRNA expression levels are expressed as transcripts per million (TPM). (**K-O**) Dispersed human islets were transfected with siRNA control (siCTL, white bars) or with siRNAs targeting *NLRC5* (siNL#2, grey bars). The cells were left untreated or treated with IFNα (2,000 U/mL) for 24h. The mRNA expression level of *TAP1* (**K**), *TAP2* (**L**), *PSMB8* (**M**), *PSMB9* (**N**), and *B2M* (**O**) were evaluated by quantitative RT-PCR and normalized by geometric mean of *GAPDH* and *β-actin* and then by

siCTL treated with IFNα considered as 1. Results are means ± SEM of 6-7 independent experiments. ##P < 0.01, ###P < 0.001 and ####P < 0.0001 vs untreated and transfected with the same siRNA; ***P < 0.001 and ****P < 0.0001 as indicated by bars (ANOVA followed by Bonferroni correction for multiple comparisons).

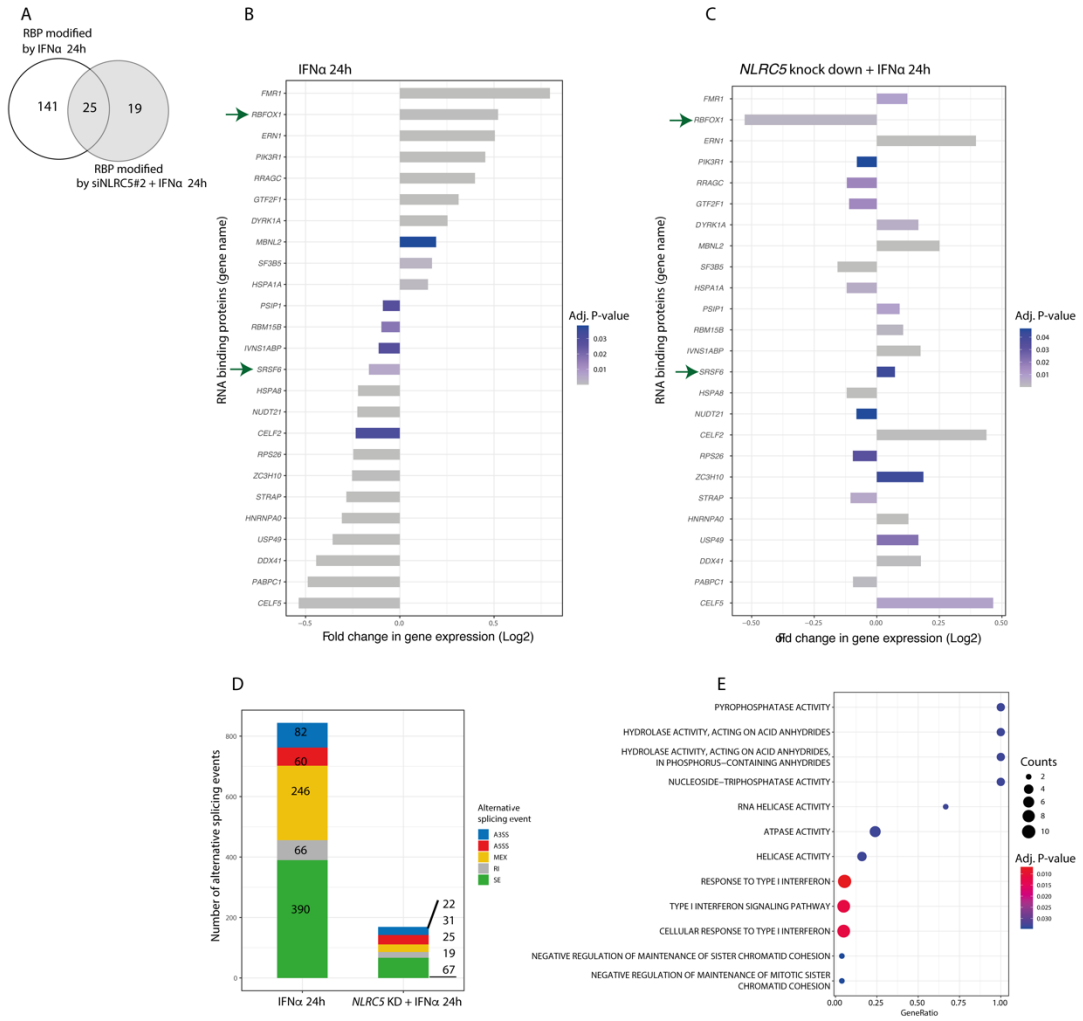


Fig. S8. *NLRC5* depletion attenuates the impact of IFNα-induced alternative splicing modifications in EndoC-βH1 cells at 24h. (**A**) Venn diagram showing the overlap of the RNA binding proteins (RBPs) modified by the exposure to IFNα for 24h with or without previous *NLRC5* depletion. A total of 166 RBPs were modified by IFNα, and 44 RBPs were modified by *NLRC5* depletion followed by IFNα exposure. 25 RBPs were commonly regulated. (**B-C**) Distinct modifications on the mRNA expression level of RBPs proteins in EndoC-βH1 cells exposed to IFNα for 24h (**B**) or EndoC-βH1 cells exposed to IFNα for 24h after *NLRC5* depletion (**C**). The bar charts depict log2-transformed fold-change of the 25 commonly regulated RBPs (Adjusted P value < 0.05). Arrows indicate particularly relevant RBPs that are modified by IFNα (**B**) but restored by *NLRC5* KD (**C**). (**D**) The number of the different alternative splicing events induced by IFNα after 24h exposure was markedly decreased after *NLRC5* depletion. EndoC-βH1 cells

were exposed to IFNα for 24h, and to IFNα for 24h after *NLRC5* depletion (*NLRC5* KD). The number of the alternative splicing events are indicated in the respective dimension of the bar. A3ss, alternative 3' splice site; A5ss, alternative 5' splice site; MEX, mutually exclusive exons; RI, intron retention; SE, skipped exons. (**E**) Gene Ontology (GO) enrichment of the genes with modified alternative splicing events in EndoC-βH1 treated with IFNα for 24h. Gene ratio refers to the percentage of total genes with alternative splicing modifications in the given GO term. Gene count refers to number of genes in the tested set that are associated with a given GO term (Adjusted P value < 0.05).

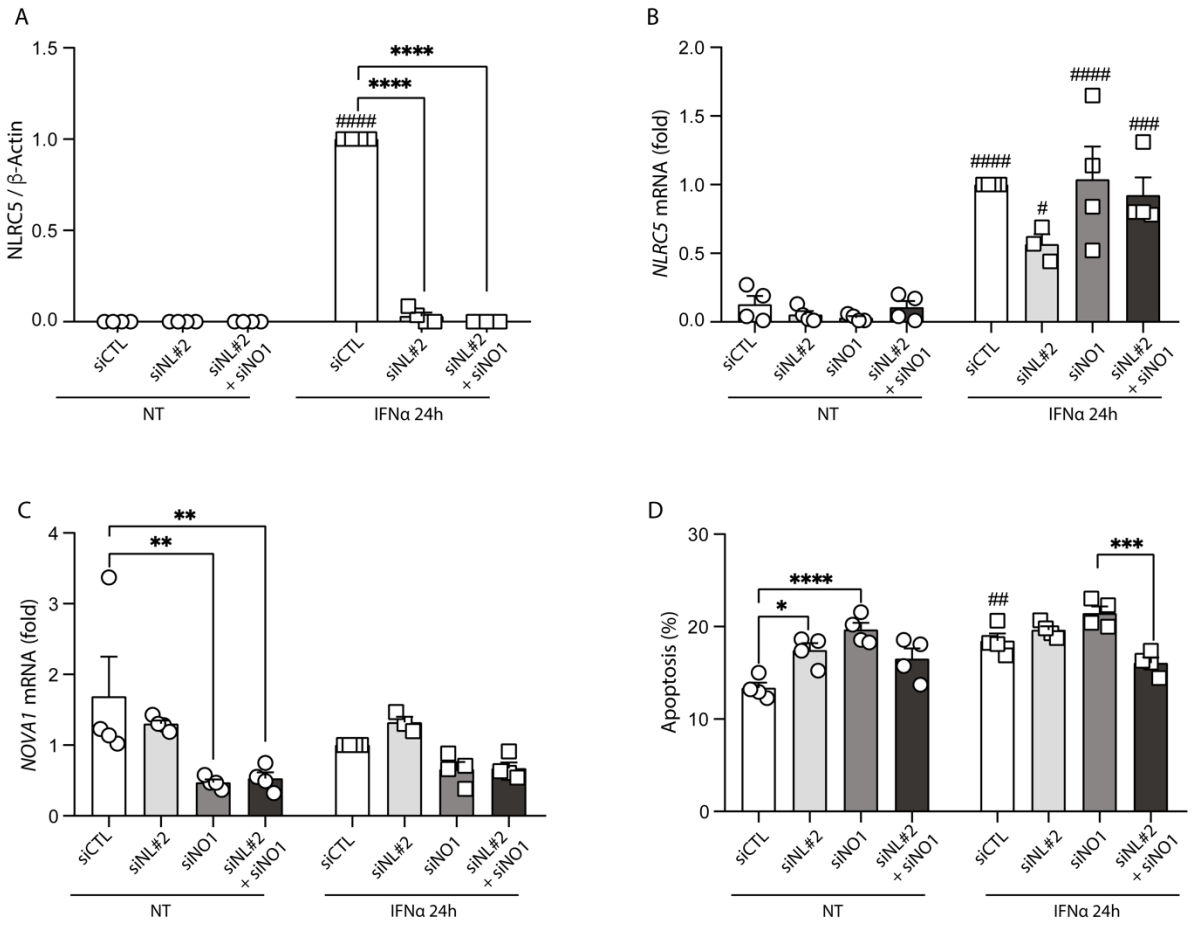


Fig. S9. *NLRC5* depletion decreases cell death induced by siRNA-induced inhibition of *NOVA1*. EndoC-βH1 cells were transfected with: a siRNA control (siCTL, white bars); a siRNA targeting *NLRC5* (siNL#2, light-grey bars); a siRNA targeting *NOVA1* (siNo1, dark-grey bars); or with the combination of both siRNA targeting *NLRC5* and *NOVA*1 (siNL#2+siNo1, black bars). Cells were left untreated or treated with IFNα (2000 U/mL) for 24h. (**A**) NLRC5 protein expression was determined by western blotting and quantified by densitometry and normalized by the housekeeping protein β-actin and then by the highest value of each experiment considered as 1. (**B**) *NLRC5* and (**C**) *NOVA1* mRNA expression was measured by quantitative RT-PCR and normalized by the geometric mean of *GAPDH* and *β-actin* and then by siCTL treated with IFNα considered as 1. (**D**) The percentage of dead cells was counted by Hoechst and propidium iodine staining. Results are means ± SEM of 3-4 independent experiments. $^{\#}P < 0.05$, $^{\#\#}P< 0.01$, $^{\#\#\#}P< 0.001$ and $^{\#\#\#\#}P < 0.0001$ vs untreated cells transfected with the same siRNA; *P< 0.05, **P< 0.01,

***P < 0.001 ****P < 0.0001, as indicated by bars (ANOVA followed by Bonferroni correction for multiple comparisons).

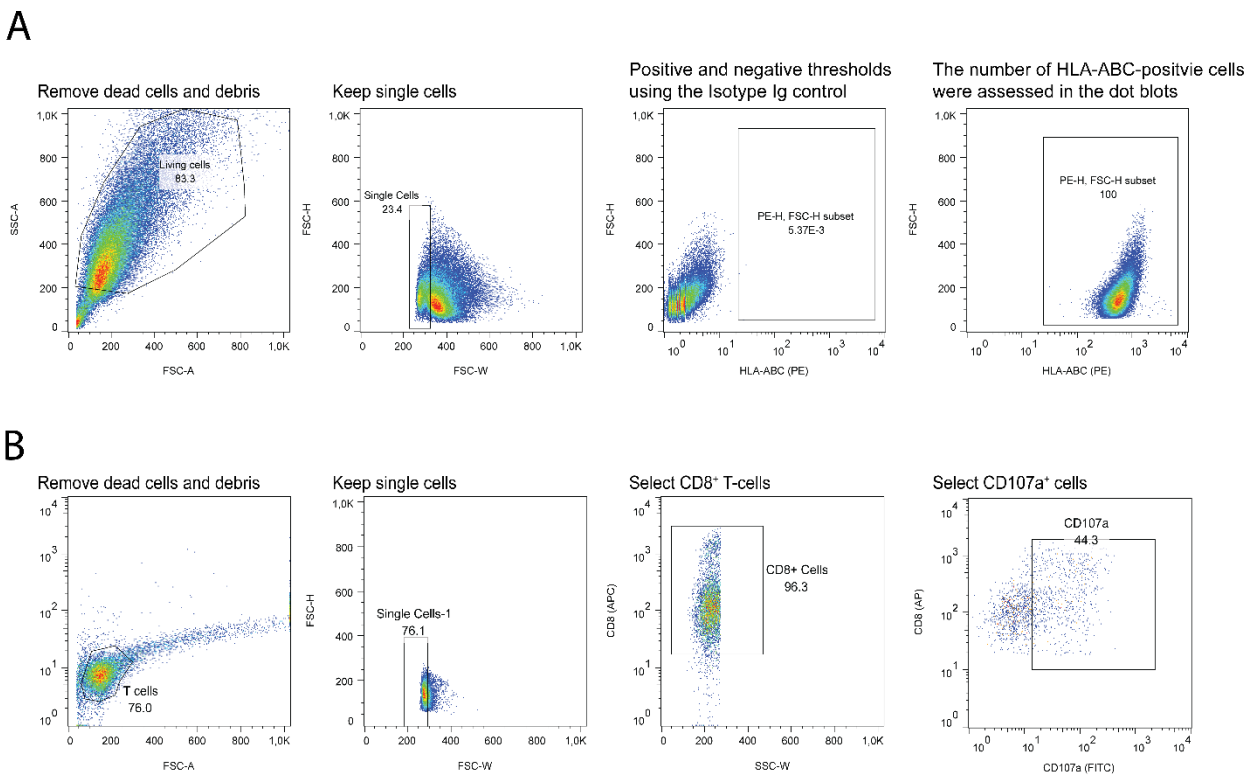


Fig. S10. Gating strategy used to evaluate HLA-ABC expression and CD107a$^+$ T-cells. The cells were first identified based on forward scatter (FSC) and side scatter (SSC) parameters to exclude cell debris and non-viable cells. Next, the relation between forward scatter width (FSC-W) and forward scatter height (FSC-H) was used to eliminate cell doublets. (**A**) The number of HLA-ABC-positive cells were assessed in the dot blots. Positive and negative thresholds were set using an isotype Ig control with the same fluorophore. (**B**) The CD8$^+$ cells were selected first to allow next to calculate the percentage of CD107a$^+$.

Donor ID	Age (years)	Sex	BMI	Clinical Diagnosis	Cause of death
HPAP-020	14	M	13.2	Recent T1DM Unsuspected	Cardiovascular
HPAP-021	13	F	21.4	T1DM	Cardiovascular DKA/IDDM
HPAP-023	17	F	21.35	T1DM	IDDM/DKA
HPAP-024	18	M	24.3	T1D control	Blunt Injury (MVA)
HPAP-026	24	M	20.8	T1D control	Drug Intoxication
HPAP-027	31	F	32.71	T1D control	Cardiovascular
HPAP-028	4	M	17.3	T1DM	Cerebral Edema (DKA)
HPAP-032	10	F	16.3	T1DM	Cardiovascular
HPAP-034	13	M	18.6	T1D control	GSW
HPAP-035	35	M	26.91	T1D control	Seizure
HPAP-036	23	F	16	T1D control	Blunt Injury (Homicide)
HPAP-037	35	F	21.9	T1D control	Intracranial Hemorrhage
HPAP-055	24	M	27.9	T1DM	Cardiovascular DKA
HPAP-056	33	M	32.89	T1D control	Cardiovascular
HPAP-063	45	F	38.41	T2D control	Natural Causes
HPAP-064	24	M	16.98	T1DM	Anoxia
HPAP-071	12	F	15.42	T1DM	Asphyxiation/Suicide
HPAP-072	19	M	23.1	T1D control	Drug Intoxication
HPAP-074	40	F	36.88	T2D control	Drug Intoxication
HPAP-075	35	M	27.52	T2D control	Drug Intoxication
HPAP-077	47	M	32.78	T2D control	Cardiovascular
HPAP-082	25	M	23.96	T1D control	Blunt injury

Table S1: Characteristics of the pancreas donors studied in the HPAP project (https://hpap.pmacs.upenn.edu/) and included in the present study. M, Male; F, Female**.**

	Age (years)	Sex	BMI (kg/m^2)	Cause of death	β-cell purity (%)
Donor 1	74	M	33	Cardiovascular disease	21
Donor 2	76	M	28.4	Cardiovascular disease	43
Donor 3	83	M	25.7	Cardiovascular disease	37
Donor 4	86	F	27.1	Cardiovascular disease	33
Donor 5	62	F	23.5	Cardiovascular disease	56
Donor 6	63	M	29.4	Cardiovascular disease	36
Donor 7	76	F	23.9	Cardiovascular disease	36
Donor 8	60	F	23.4	Postanoxic encephalopathy	24
Donor 9	76	M	26.6	Stroke	63
Donor 10	67	M	25.7	Trauma	48
Donor 11	82	M	22.5	Trauma	70
Donor 12	63	F	27.3	Stroke	58
Donor 13	74	M	26	Cerebral hemorrhage	46
Donor 14	73	M	24.1	Cerebral hemorrhage	11
Donor 15	71	F	31.2	Cerebral hemorrhage	46
Donor 16	72	F	22.9	Cardiovascular disease	45
Donor 17	66	F	26.4	Trauma	45

Table S2. Characteristics of the human islet donors. M, Male; F, Female; β-cell purity was determined by insulin staining, as described in *(62)*.

Target	Catalog number	Company	Application	Dilution	Incubation time (h)	Temperature (°C)
NLRC5	MABF260	Sigma-Aldrich	WB	1:500	overnight	4°C
β-Actin	4967	Cell signaling	WB	1:5000	overnight	4°C
Horse raddish peroxidase-conjugated anti rabbit	711-036-152	Jackson Immuno Research	WB	1:10000	1 h	Room temperature
Horse raddish peroxidase-conjugated anti rat	112-035-003	Jackson Immuno Research	WB	1:10000	1 h	Room temperature
HLA-ABC PE conjugated	560964	BD Biosciences	FC	1:50	30 min	4°C
PE Mouse IgG1 κ Isotype control	555749	BD Biosciences	FC	1:50	30 min	4°C
CD107a (LAMP-1), FITC	11-1079-42	ThermoFisher Scientific	FC	1:50	4h	37°C
CD107a (LAMP-1), FITC	11-1079-42	ThermoFisher Scientific	FC	1:50	4h	37°C
Insulin	IR002	Dako (Agilent)	ICC	Ready to use solution	18 h	4°C
Glucagon	G2654	Sigma-Aldrich	ICC	1:1000	18 h	4°C
Somatostatin	ab108456	Abcam	ICC	1:1000	18 h	4°C
Alexa Fluor 488 AffiniPure Donkey Anti-Guinea Pig IgG (H+L)	706-545-148	Jackson Immuno Research	ICC	1 :500	1 h	Room temperature
Rhodamine Red-X (RRX) AffiniPure Donkey Anti-Mouse IgG (H+L)	715-295-151	Jackson Immuno Research	ICC	1 :500	1 h	Room temperature
Alexa Fluor 647 AffiniPure Donkey Anti-Rabbit IgG (H+L)	711-605-152	Jackson Immuno Research	ICC	1 :500	1 h	Room temperature

Table S3. Antibodies. The table provides information on the antibodies used in this study, including protein target name, company, catalog number, application, dilution, incubation and temperature time. WB Western blotting, FC Flow cytometry, ICC Immunofluorescence.

Gene	Primer sequence (5' -> 3')	Direction	Application	Supplier
ACTB	CTGTACGCCAACACAGTGCT	Forward	qRT-PCR	Eurogentec
	GCTCAGGAGGAGCAATGATC	Reverse	qRT-PCR	Eurogentec
ATF3	GTAGCCCCTGAAGAAGATGAAAG	Forward	qRT-PCR	Eurogentec
	CTTCTCCGACTCTTTCTG	Reverse	qRT-PCR	Eurogentec
BiP			qRT-PCR	Qiagen
	Qiagen QuantiTect primer, cat# QT00096404		qRT-PCR	Qiagen
B2M	TGCTGTCTCCATGTTTGATGTA	Forward	qRT-PCR	Eurogentec
	GACCAAGATGTTGATGTTGGATAAG	Reverse	qRT-PCR	Eurogentec
CXCL10	GTGGCATTCAAGGAGTACCTC	Forward	qRT-PCR	Eurogentec
	GCCTTCGATTCTGGATTCAG	Reverse	qRT-PCR	Eurogentec
GAPDH	CAGCCTCAAGATCATCAGCA	Forward	qRT-PCR	Eurogentec
	TGTGGTCATGAGTCCTTCCA	Reverse	qRT-PCR	Eurogentec
HLA-ABC	GAGAACGGGAAGGAGACGC	Forward	qRT-PCR	Eurogentec
	CATCTCAGGGTGAGGGGCT	Reverse	qRT-PCR	Eurogentec
HLA-E	TGGTTGCTGCTGTGATATGGA	Forward	qRT-PCR	Eurogentec
	GCTCCACTCAGCCTTAGAGT	Reverse	qRT-PCR	Eurogentec
HNRNPLL	GACATGATGGCTATGGATCC	Forward	qRT-PCR	Eurogentec
	CAGAGGGATTTCCTCCATG	Reverse	qRT-PCR	Eurogentec
IRF1	CATTCACACAGGCCGATACA	Forward	qRT-PCR	Eurogentec
	TGGTCTTTCACCTCCTCGATAT	Reverse	qRT-PCR	Eurogentec
IRF9	CTCTTCAGAACCGCCTACTTC	Forward	qRT-PCR	Eurogentec
	GGCTCTCTTCCCAGAAATTCA	Reverse	qRT-PCR	Eurogentec
MBNL2	CTGACAAACTGGAGGTATGC	Forward	qRT-PCR	Eurogentec
	TGTCGATCATGGTGCTGTC	Reverse	qRT-PCR	Eurogentec
MDA5	GAGGAATCAGCACGAGGAATAA	Forward	qRT-PCR	Eurogentec
	TCAGATGGTGGGCTTTGAC	Reverse	qRT-PCR	Eurogentec
MX1	AGACAGGACCATCGGAATCT	Forward	qRT-PCR	Eurogentec
	GTAACCCTTCTTCAGGTGGAAC	Reverse	qRT-PCR	Eurogentec
NLRC5	GTGCTGTGGCAGGTTCACA	Forward	qRT-PCR	Eurogentec
	GCTGAGGTCTTTACACTTGCTCA	Reverse	qRT-PCR	Eurogentec
NOVA1	CCGGTAGCAGGGGCAGGAAC	Forward	qRT-PCR	Eurogentec
	AGCGGCCTTTTCCGCGAGTC	Reverse	qRT-PCR	Eurogentec
NOVA2	CCATCAAGCTCTCCAAGTCC	Forward	qRT-PCR	Eurogentec
	GGGATTTCTCGGACCTTCTC	Reverse	qRT-PCR	Eurogentec
PDL1	CCAGTCACCTCTGAACATGAA	Forward	qRT-PCR	Eurogentec
	ACTTGATGGTCACTGCTTGT	Reverse	qRT-PCR	Eurogentec
PSMB8	CTACTAGATGTATGCGGAGCCC	Forward	qRT-PCR	Eurogentec
	CAATCACTCCATGCTGGAACTTG	Reverse	qRT-PCR	Eurogentec
PSMB9	CATCTACTGTGCACTCTCTGGTT	Forward	qRT-PCR	Eurogentec
	AGGTTCCTCCAGTTCTATCCCAT	Reverse	qRT-PCR	Eurogentec
SOCS1	GACGCCTGCGGATTCTAC	Forward	qRT-PCR	Eurogentec
	GAGGCCATCTTCACGCTAA	Reverse	qRT-PCR	Eurogentec
STAT1	GACCCAATCCAGATGTCTATGA	Forward	qRT-PCR	Eurogentec
	CCCGACTGAGCCTGATTA	Reverse	qRT-PCR	Eurogentec
STAT2	GTTGGCAGTTCTCCTCCTATG	Forward	qRT-PCR	Eurogentec
	GAAGTCAGCCCAGGACAATAA	Reverse	qRT-PCR	Eurogentec
TAP1	TCCAACAGAACCAGACAGGTAAC	Forward	qRT-PCR	Eurogentec
	CACCAGGTACCACAGAAATAAGC	Reverse	qRT-PCR	Eurogentec

TAP2	CTGCACAGCCAGGTGGTTTC	Forward	qRT-PCR	Eurogentec
	CAATGTTGTTCCTCACAGAACCG	Reverse	qRT-PCR	Eurogentec
USP18	CAATCCACCTCATGCGATTCT	Forward	qRT-PCR	Eurogentec
	TTGGAAGGATCTGGCTGAAATC	Reverse	qRT-PCR	Eurogentec
STAT1	GATCCTGCGCGCAGAAAAG	Forward	Splicing	Eurogentec
	AGTTCGTACCACTGAGAC	Reverse	Splicing	Eurogentec
STAT2	GGAAAGGGCAGCAATAAGGGG	Forward	Splicing	Eurogentec
	GAACTGCCAACTGAGAGCAGG	Reverse	Splicing	Eurogentec
HLA-F	CATCCTGAGATGGGAGCAG	Forward	Splicing	Eurogentec
	GACACTGTGACTCCGGAG	Reverse	Splicing	Eurogentec

Table S4. Sequences of the primers used for quantitative RT-PCR and alternative splicing analysis in the present study

Data S1. (See supplementary_table_01.xlsx): Results from the Differential Gene Expression (DGE) analysis performed using MAST *(81)* of each identified cell type in Human iPSC-derived β-like cells exposed or not to IFNα during 24h in the scRNA-sequencing dataset.

Data S2. (See supplementary_table_02.xlsx): Results from the GSEA analysis of each identified cell type in human iPSC-derived β-like cells exposed or not to IFNα during 24h in the scRNA-seq dataset. KEGG *(90)* and REACTOME *(91)* were used as reference databases.

Data S3. (See supplementary_table_03.xlsx): RNA-seq differential expression analysis and GSEA analysis using KEGG *(90)* and REACTOME *(91)* of bulk RNA-seq of EndoC-βH1 exposed to siCTL (control) or siNLRC5 and then treated with IFNα for 8 or 24h with as reference databases (6 sheets). Each experiment has one sheet for differential gene expression analysis and two separate sheets for the results from the fGSEA analysis.