Figure S1

A Human islets

Gene	T1D recent-onset	T1D long-lasting	Control-1	Control-2	Control-3	Median (Control)	Mean (Control)	SD (Control)
CD274	15.35	1.88	3.22	2.11	4.39	3.22	3.24	1.14

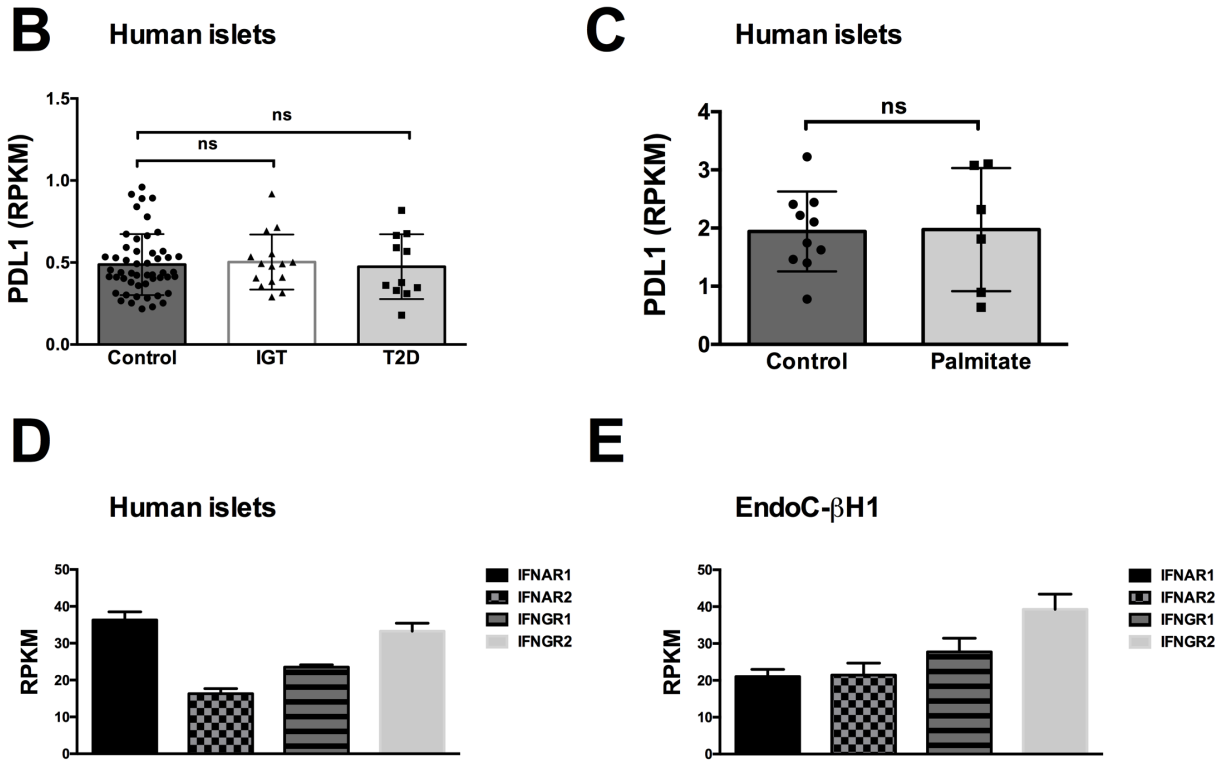

Figure S1. mRNA expression of PDL1 and type I / II interferon receptors in RNAseq of beta cells at different conditions.

RNA sequencing data of human islets **(A to D)** or EndoC-βH1 cells **(E)** were evaluated for the expression of PDL1 (CD274 gene) or type I and II interferon receptor subunits.

(A) RNAseq of pancreatic islets from donors with T1D or healthy controls (1). **(B)** RNAseq of pancreatic islets from donors with different levels of dysglycemia (2). **(C)** RNAseq of pancreatic islets exposed or not to palmitate for 48h (3). **(D and E)** RNAseq of pancreatic islets from healthy donors (4) **(D)** or untreated EndoC-βH1 cells (n=3, unpublished data) **(E)** Values are presented as reads per kilobase million (RPKM; mean ± standard deviation).

Figure S2 - All EndoC-βH1

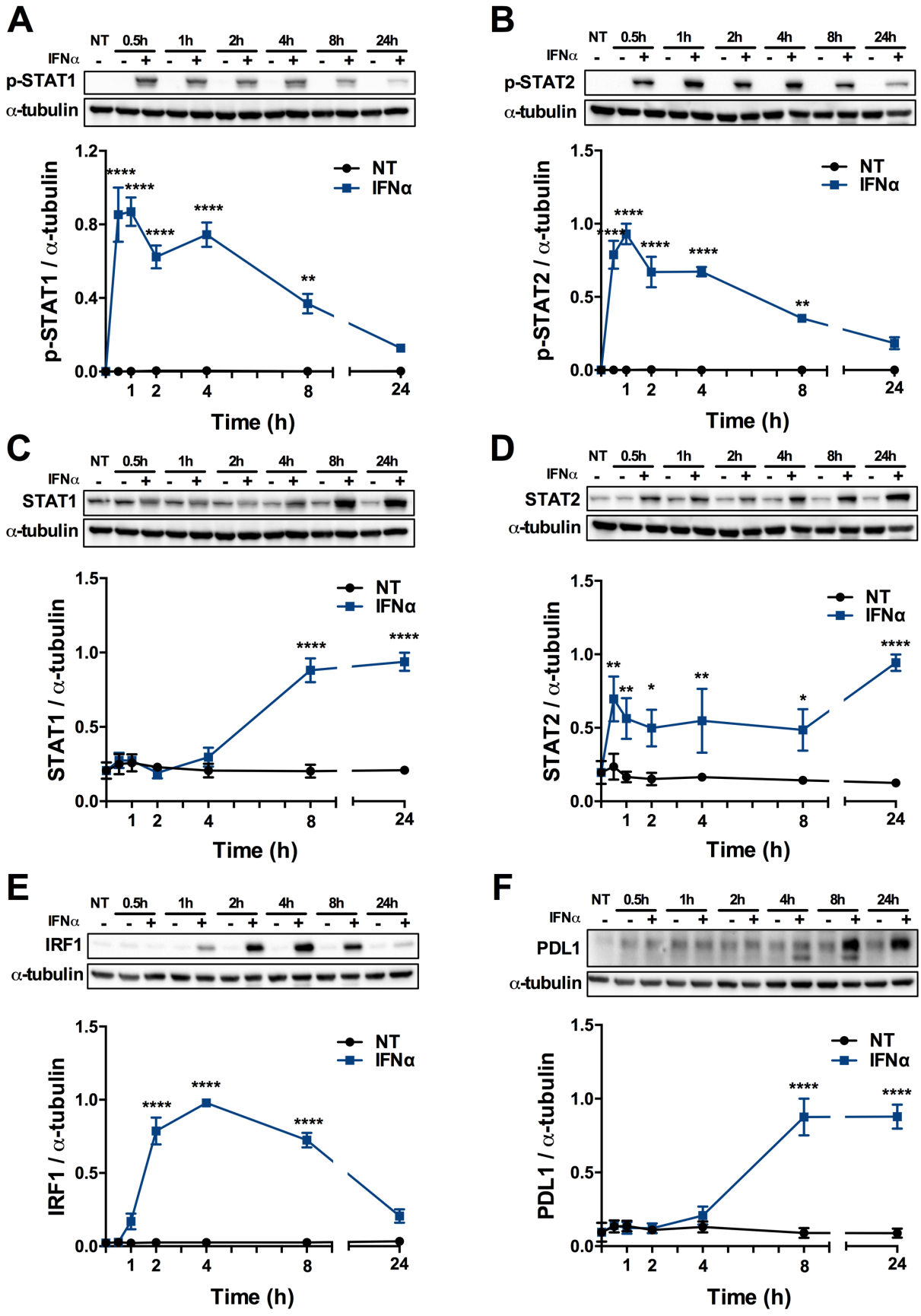

Figure S2. Exposure of beta cells to interferon-α induces an early activation of STAT1/2 and IRF1 followed by up-regulation of PDL1 protein expression.

(A to F) EndoC-βH1 cells were exposed or not to interferon-α (IFNα, 2000U/ml) for the indicated time-points and then collected for protein analysis by Western blot. Phosphorylated STAT1 **(A)**, phosphorylated STAT2 **(B)**, total STAT1 **(C)** total STAT2 **(D)**, IRF1 **(E)** and PDL1 **(F)** were quantified by densitometry and normalized by the housekeeping protein α-tubulin and then by the highest value of each experiment considered as 1 (n=4-5, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, ANOVA with Bonferroni correction). The mean values ± SEM are shown.

Figure S3 - All EndoC-βH1

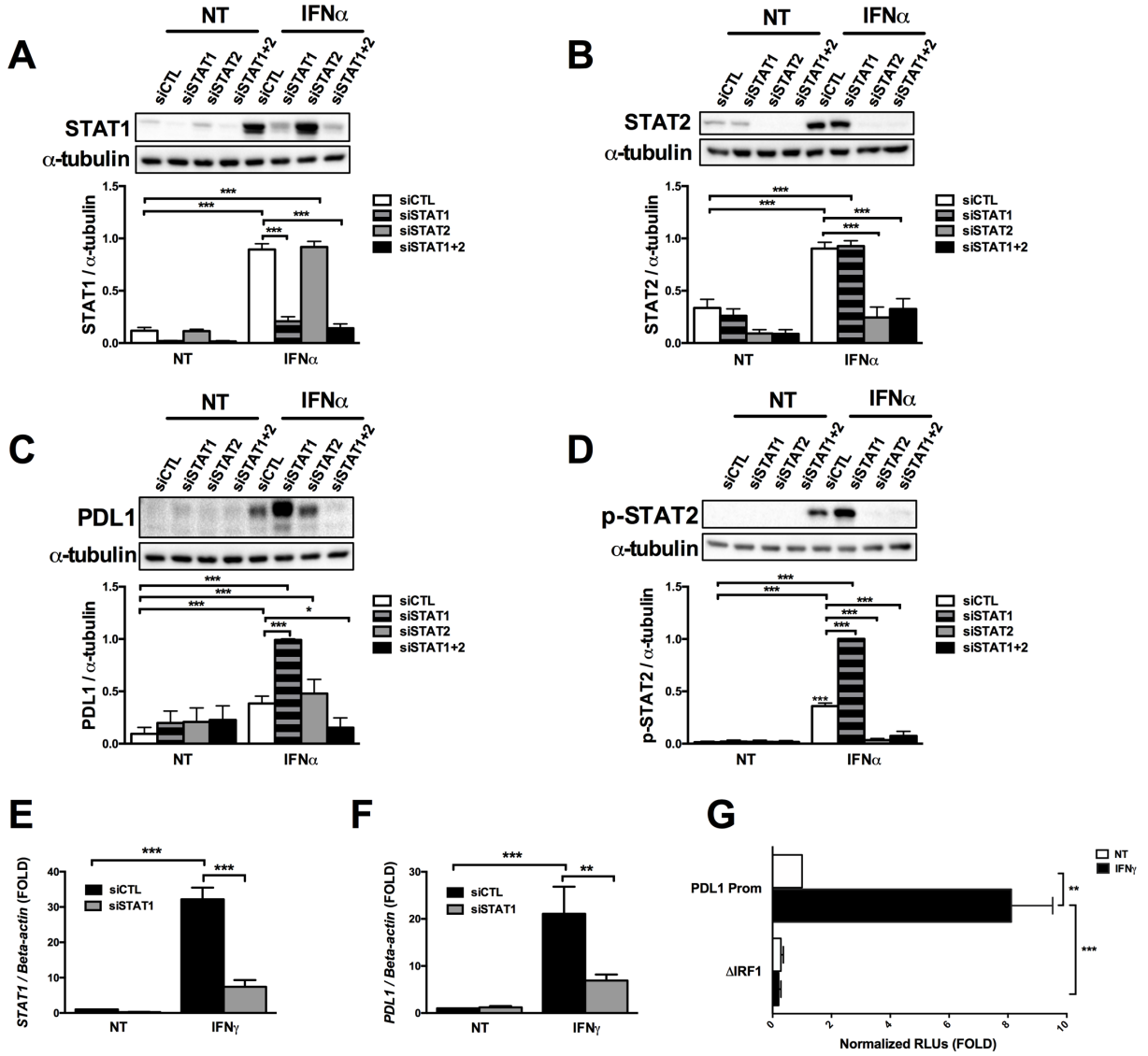

Figure S3. STAT1 silencing alone increases PDL1 expression via compensatory augmentation in phospho-STAT2, while double knockdown STAT1 + STAT2, prevents interferon-α-induced PDL1 up-regulation.

(A to F) EndoC-βH1 cells were transfected with a control siRNA (siCTL) or siRNAs targeting STAT1, STAT2 or STAT1+2. After 48h of recovery, the cells were treated with IFNα **(A to D)** or IFNγ **(E and F)** for 24h. Knockdown efficiency and PDL1 induction were evaluated at protein **(A, B and C)** or mRNA **(E and F)** levels by Western blot and real time RT-PCR, respectively. Protein expression was quantified by densitometry and normalized by the housekeeping protein α-tubulin and then by the highest value of each experiment considered as 1. The mRNA values were normalized by the housekeeping gene *beta-actin* and are represented as fold induction compared to non-treated control cells (NT). (n=6, * $p<0.05$, ** $p < 0.01$, *** $p < 0.001$, ANOVA with Bonferroni correction).

(G) EndoC-βH1 cells were transfected with a luciferase construct reporter containing the wild type human PDL1 promoter or the same construct presenting a deletion in the IRF1 binding site plus a pRL-CMV plasmid (used as internal control); cells were then exposed to IFNγ for 24h and luciferase activity was assayed. The values were corrected for the activity of the internal control (pRL-CMV) and are presented as normalized relative luciferase units (RLUs) in relation to non-treated cells (NT) considered as 1 (n=3, ** $p < 0.01$, *** $p < 0.001$, ANOVA with Bonferroni correction). The mean values ± SEM are shown.

Figure S4 - All Human islets

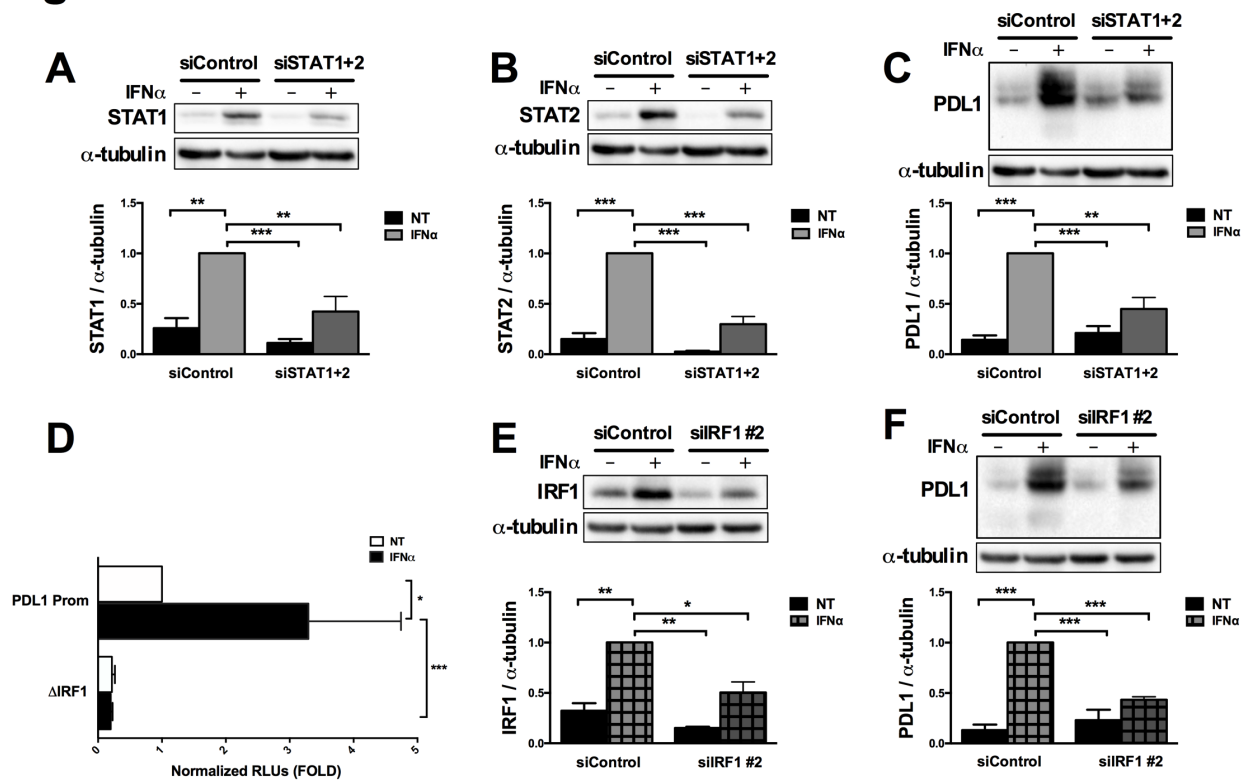

Figure S4. Interferon-α-induced PDL1 expression in primary human islets depends on both STAT1 + 2 and IRF1.

Dispersed human islets were transfected with a control siRNA (siCTL) or siRNAs against STAT1+STAT2 **(A to C)** or IRF1 **(E and F)**. After 48h of recovery, they were exposed to IFNα for 24h. Knockdown efficiency **(A, B and E)** and PDL1 induction **(C and F)** were evaluated at protein level by Western blot. Protein expression was quantified by densitometry and normalized by the housekeeping protein α-tubulin and then by the highest value of each experiment considered as 1. (n=3, * p<0.05, ** p < 0.01, *** p < 0.001, ANOVA with Bonferroni correction).

(D) Dispersed human islets were transfected with a luciferase construct reporter containing the wild type human PDL1 promoter or the same construct presenting a deletion in the IRF1 binding site plus a pRL-CMV plasmid (used as internal control); cells were then exposed to IFNα for 24h and luciferase activity was assayed. The values were corrected for the activity of the internal control (pRL-CMV) and are presented as normalized relative luciferase units (RLUs) in relation to non-treated cells (NT) considered as 1 (n=5, * p < 0.05, *** p < 0.001, ANOVA with Bonferroni correction). The mean values ± SEM are shown.

Table S1: Tissue Samples, Patient Information.

Identifier	Classification	Age (y)	Gender	Duration of Disease
21/89	No diabetes	4	F	
184/90	No diabetes	5	M	
333/66	No diabetes	16	M	
146/66	No diabetes	18	F	
191/67	No diabetes	25	M	
9310/08	No diabetes	58	F	
SC115	Type 1 diabetes	16 months	F	3 days
E308	Type 1 diabetes	3	F	4 weeks
SC41	Type 1 diabetes	4	F	3 weeks
E261	Type 1 diabetes	18	F	3 weeks
E556A	Type 1 diabetes	18	M	4 months
E560	Type 1 diabetes	42	F	18 months
DiViD1*	Type 1 diabetes	25	F	4 weeks
DiViD 2*	Type 1 diabetes	24	M	3 weeks
DiViD 3*	Type 1 diabetes	34	F	9 weeks
DiViD 4*	Type 1 diabetes	31	M	5 weeks
DiViD 5*	Type 1 diabetes	24	F	5 weeks
DiViD 6*	Type 1 diabetes	35	M	5 weeks

* (5)

Table S2: Antibody Details and Immunofluorescence Conditions.

Primary Antibody	Manufacturer and clone	Antigen Retrieval	Antibody Dilution	Incubation time with primary antibody	Secondary Detection System
Step 1: PDL1	Abcam Cat#ab205921 Rabbit monoclonal [28-8]	Abcam Universal buffer (Cat# ab208572)	1:100	Overnight at 4°C	Goat anti-rabbit IgG (H+L) HRP then tyramide Alexa 488 (as per manufacturers instructions; Thermofisher TSA Kit#12; Cat#T20927)
Step 2: Glucagon or IRF1	Abcam Cat#ab82270 Mouse monoclonal	Abcam Universal buffer	1:2000	1h at RT	Goat anti-mouse IgG (H+L) Alexa Fluor™-conjugated secondary antibodies (1/400 for 1hr)
	Cell Signalling Cat#8478 Rabbit monoclonal	Citrate pH6	1:100	Overnight at 4°C then 1h at RT	Goat anti-rabbit IgG (H+L) HRP followed by tyramide amplification (Thermofisher Tyramide SuperBoost Kit (TSA) Cat#B40922) and Alexa Fluor™-conjugated secondary antibodies
Step 3: Insulin	Dako Cat#A0564 Guinea-pig polyclonal	Abcam Universal buffer	1:700	1h at RT	Goat anti-guinea-pig IgG (H+L) Alexa Fluor™ - conjugated secondary antibodies (1/400 for 1hr)

Table S3. Characteristics of the human islets donors.

	Age (y)	Gender	BMI (kg/m^2)	Cause of death	Beta cell purity (%)
Donor 1	63	F	27.3	Stroke	58
Donor 2	64	F	22.2	Cerebral haemorrhage	39.1
Donor 3	71	F	31.2	Cerebral haemorrhage	46
Donor 4	72	F	22.9	Cardiovascular disease	45
Donor 5	73	M	24.1	Cerebral haemorrhage	Not available
Donor 6	82	M	22.5	Trauma	62
Donor 7	87	M	35.1	Trauma	75
Donor 8	60	F	23.4	Postanoxic encephalopathy	23.8
Donor 9	67	M	25.7	Trauma	48
Donor 10	76	M	26.6	Stroke	62
Mean ± SEM	**71.5 ± 2.8**		**26.1 ± 1.4**		**51.0 ± 5.0**

Beta cell purity was determined by immunofluorescence for insulin as described (6).

Table S4. List of siRNAs and primers used in the present study.

	Distributor	Sequence
siRNAs		
siCTL	Qiagen, Venlo, Netherlands	Allstars Negative Control siRNA, sequence not provided
Human si*STAT1*	Invitrogen, Pasley, UK	5'- GGAUUGAAAGCAUCCUAGAACUCAU -3'
Human si*STAT2*	Invitrogen, Pasley, UK	5'- CAGCAGCAUGUCUUCUGCUUCCGAU -3'
Human *siJAK1* #1	Qiagen, Venlo, Netherlands	5'- CCAAAGCAATTGAAACCGATA -3'
Human *siJAK1* #2	Qiagen, Venlo, Netherlands	5'- CACGGATAACATCAGCTTCAT -3'
Human *siIRF1* #1	Qiagen, Venlo, Netherlands	5'- CTGGCTAGAGATGCAGATTAA -3'
Human *siIRF1* #2	Qiagen, Venlo, Netherlands	5'- CAAGCATGGCTGGGACATCAA -3'
Primers		
Human *β-actin – Forward*	ThermoFischer	5'- CTGTACGCCAACACAGTGCT- 3'
Human *β-actin – Reverse*	ThermoFischer	5'- GCTCAGGAGGAGCAATGATC – 3'
Human *STAT1 - Forward*	ThermoFischer	5'- GACCCAATCCAGATGTCTATGA – 3'
Human *STAT1 - Reverse*	ThermoFischer	5'- CCCGACTGAGCCTGATTA – 3'
Human *STAT2 - Forward*	ThermoFischer	5'- GTTGGCAGTTCTCCTCCTATG – 3'
Human *STAT2 - Reverse*	ThermoFischer	5'- GAAGTCAGCCCAGGACAATAA – 3'
Human *JAK1 - Forward*	ThermoFischer	5'- CTCACCAGGATGGGGATAAA – 3'
Human *JAK1 - Reverse*	ThermoFischer	5'- AGTTTCCAAGGTAGCCAAGTAT – 3'
Human *PDL1 - Forward*	ThermoFischer	5'- CCAGTCACCTCTGAACATGAA – 3'
Human *PDL1 - Reverse*	ThermoFischer	5'- ACTTGATGGTCACTGCTTGT – 3'

Table S5. List of antibodies used in the present study.

	SOURCE	IDENTIFIER
Antibodies		
PDL1	Abcam	Cat#ab205921; RRID:AB_2687878
Glucagon	Abcam	Cat#ab82270; RRID: AB_1658481
Insulin	Dako	Cat#A0564; RRID: AB_10013624
α-tubulin	Sigma	Cat#T9026; RRID:AB_477593
IRF1	Cell signaling	Cat#8478; RRID: AB_10949108
CD8	Abcam	Cat#Ab4055 RRID: AB_304247
phospho-STAT1	Cell signaling	Cat#9167 RRID: AB_561284
phospho-STAT2	Cell signaling	Cat#88410 RRID: N/A
total STAT1	Cell signaling	Cat#14495 RRID: AB_2716280
total STAT2	Cell signaling	Cat#72604 RRID: N/A
JAK1	Abcam	Cat#ab133666
Peroxidase- conjugated donkey anti-rabbit IgG	Jackson ImmunoResearch	Cat#715-036-152 RRID:AB_2340590
Peroxidase- conjugated donkey anti-mouse IgG	Jackson ImmunoResearch	Cat#711-036-150 RRID:AB_2340773
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 647	Thermo Fisher Scientific	Cat#A27040; RRID:AB_2536101
Goat anti-Guinea Pig IgG (H+L) Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat#A-11073; RRID:AB_2534117

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