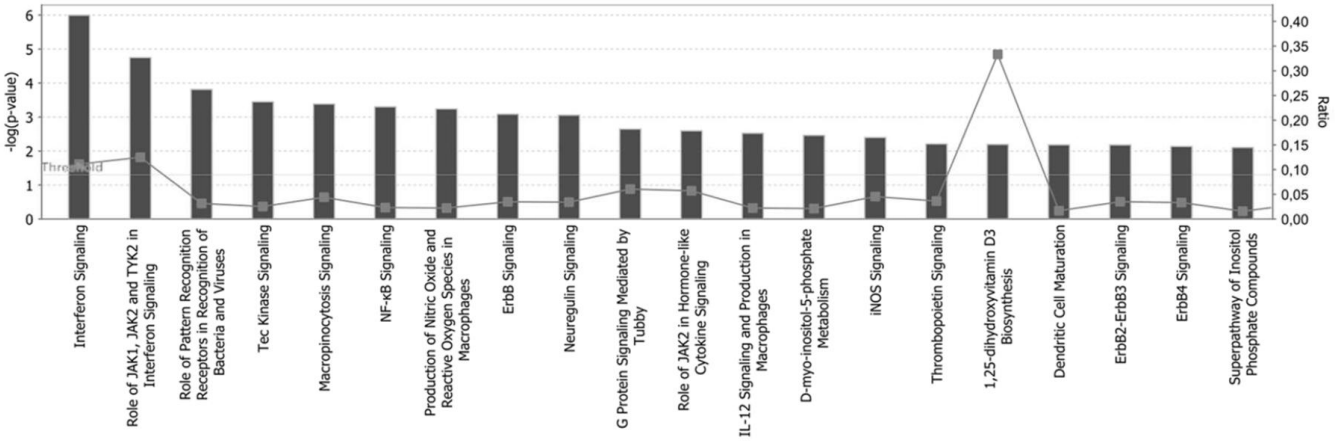
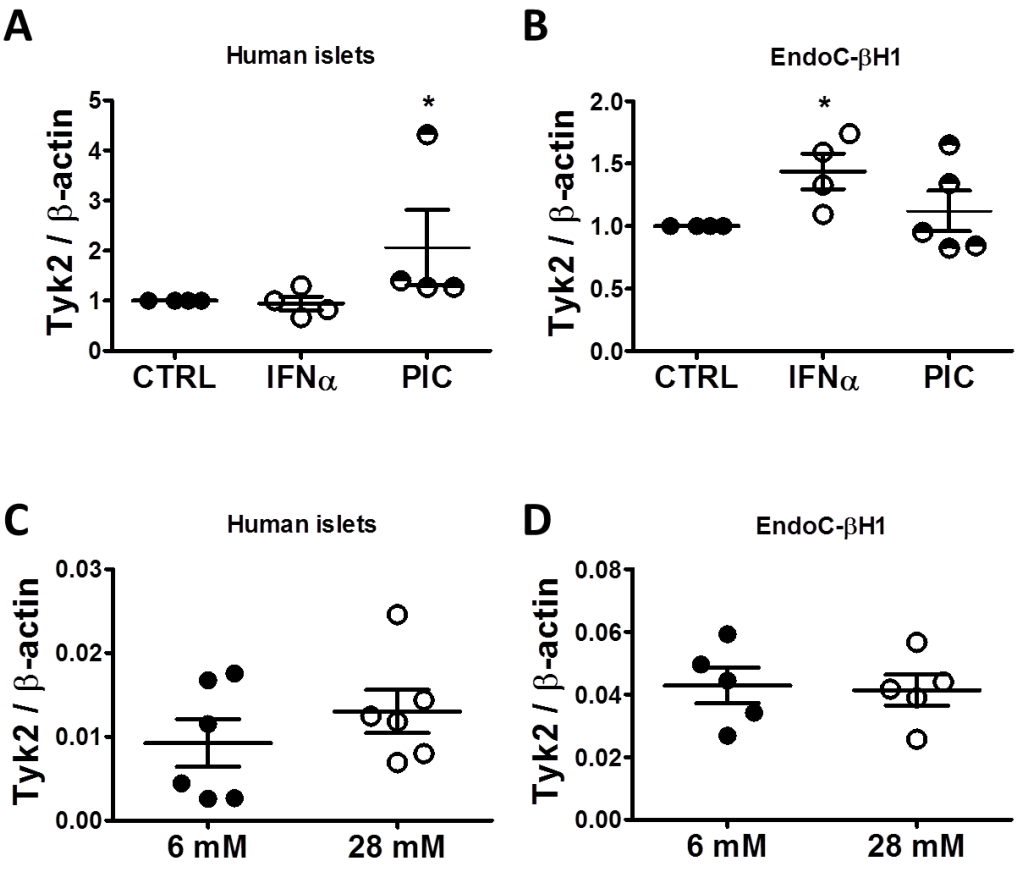
SUPPLEMENTARY DATA

Supplementary Figure 1. Ingenuity Pathway Analysis (IPA) of candidate genes for type 1 diabetes expressed in pancreatic β-cells. The expression of fifty-one candidate genes for type 1 diabetes (see Supplementary Table 4) was compared against our previous RNA-seq data of five human islet preparations exposed to the cytokines IL-1β + IFN-γ (9). Forty-two out of fifty-one genes (82%) were found expressed (i.e. RPKM > 0.5) in dispersed human islets. IPA of the genes expressed in pancreatic β-cells is shown for "Canonical Pathways". The length of the bars indicates the significance of the association between the set of genes and the keyword, and is expressed as minus the logarithm of the probability that a random set of genes from the human genome would be associated with the same keyword. The straight line indicates a threshold of 0.05 (corresponding to a -log(B–H p-value) of 1.3). The line indicates for each pathway the ratio between the number of genes observed in the data set and the total number of genes in the pathway (as annotated in IPA).



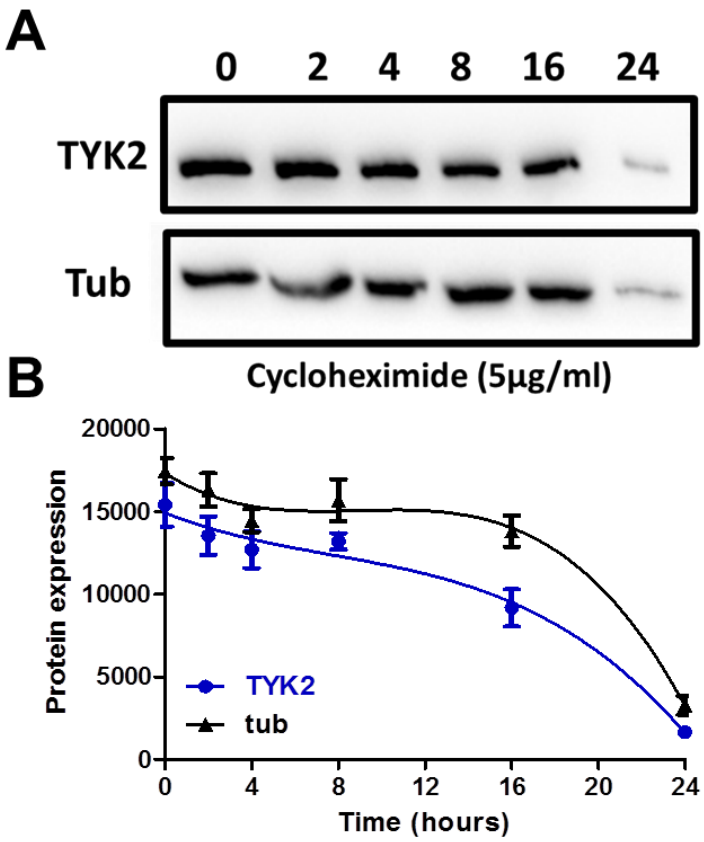
SUPPLEMENTARY DATA

Supplementary Figure 2. TYK2 expression after different treatments. Dispersed human islets (A, C) and EndoC-βH1 cells (B, D) were left untreated or treated with IFNα (2,000 U/ml), intracellular PIC (1 μg/ml) for 24 h or high glucose (28 mM) for 48 h. Results are means ± SEM of 4-6 independent experiments; **P*<0.05 *vs.* untreated. Student's t-test with Bonferroni correction.



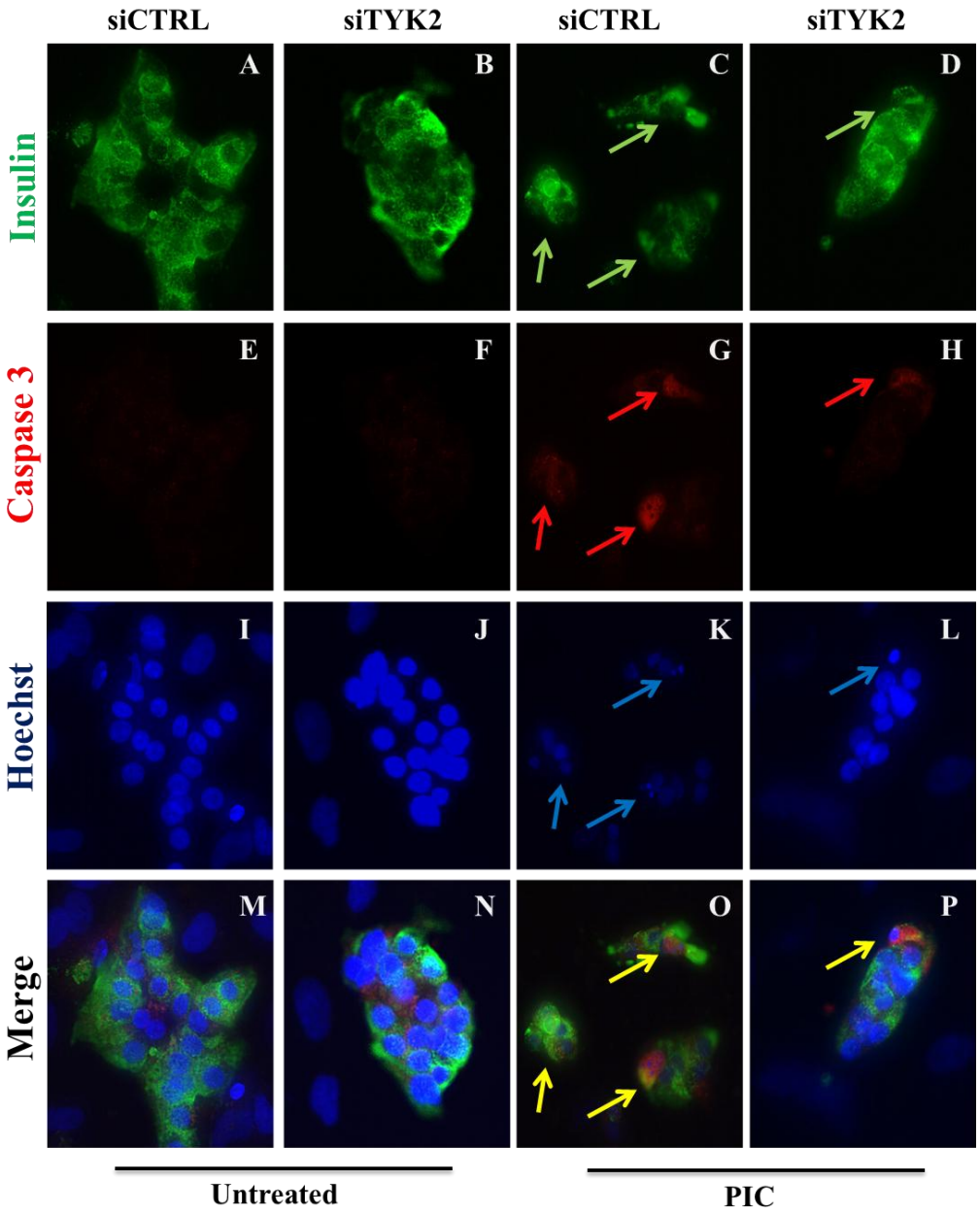
SUPPLEMENTARY DATA

Supplementary Figure 3. TYK2 stability in pancreatic β-cells. INS-1E cells (A and B) were left untreated or treated with cycloheximide (5 µg/ml) and collected at different time points. TYK2 and tubulin expression were assayed by western blot. (A) The figure is representative of 3 independent experiments. (B) Densitometry results of TYK2 and tubulin are means ± SEM of 3 independent experiments.



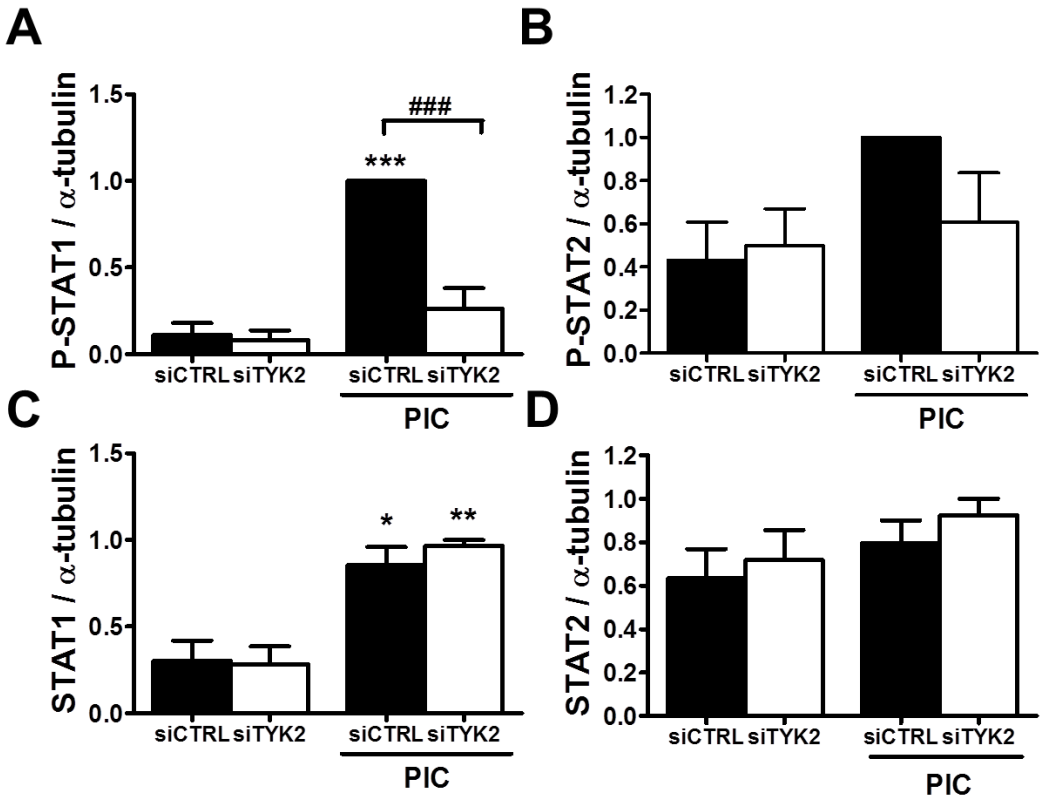
SUPPLEMENTARY DATA

Supplementary Figure 4. TYK2 inhibition prevents PIC-induced apoptosis in human β-cells. Dispersed human islets were transfected with siCTRL or with siRNA targeting TYK2 in a two-round transfection protocol and left to recover during 24 h. After this recovery period, cells were left untreated or treated with intracellular PIC (1 μg/ml) for 24 h. After the treatment, cells were fixed and use for histological studies. Fluorescent microscopy analysis of insulin (A-D, in green) and cleaved caspase 3 (E-H, in red) shows the presence of double-positive cells for insulin and cleaved caspase 3 (O and P merged panels, in yellow) after PIC treatment both in siCRTL and siTYK2 KD cells. There were fewer cleaved caspase 3 positive cells in TYK2 KD (4.3%) insulin positive cells as compared with the siCTRL (7.0%). No double-positive cells (M and N merged panels) were observed in untreated cells. Hoechst staining (I-L in blue) shows the presence of nuclear condensation (K and L) in the apoptotic cleaved caspase 3 positive cells. Double-positive cells for insulin and cleaved caspase 3 are indicated by arrows (C, D, G, H, K, L, O and P).



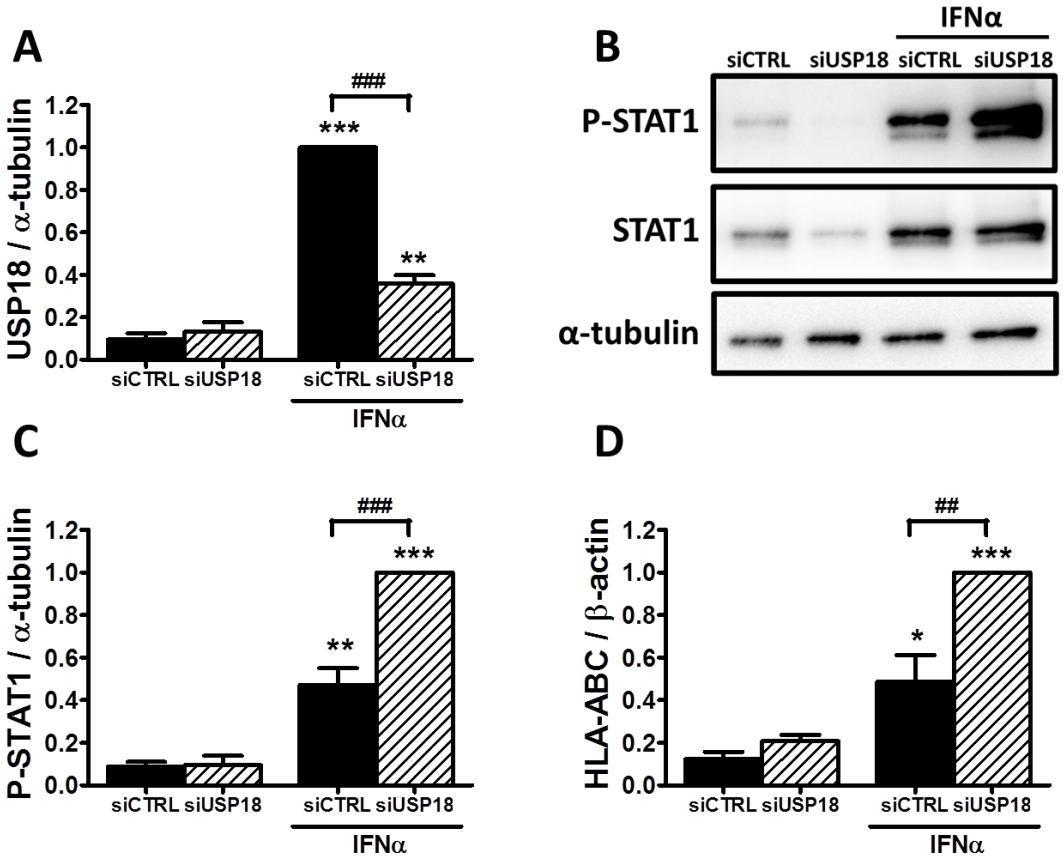
SUPPLEMENTARY DATA

Supplementary Figure 5. Inhibition of TYK2 decreases PIC-induced activation of the type I interferon pathway in EndoC-βH1 cells. EndoC-βH1 cells (A-D) were transfected with siCTRL or with a siRNA targeting human TYK2 as in Fig. 2. Cells were then left untreated or treated with intracellular PIC (1 μg/ml) for 24 h. Expression of phospho-STAT1, total STAT1, phospho-STAT2, total STAT2, TYK2 (for knockdown confirmation) and α-tubulin (used as loading control) were measured by western blot. The densitometry results for P-STAT1 (C), total STAT1 (E), P-STAT2 (D) and total STAT2 (F) in EndoC-βH1 cells are means ± SEM of 3 independent experiments (a representative western blot of 3 experiments appears in Figure 2B); **P<0.*05, ***P<0.*01 and ****P<0.*001 *vs.* untreated (i.e., not treated with PIC) and transfected with the same siRNA; $^{###}P$<0.001, as indicated by bars; ANOVA.



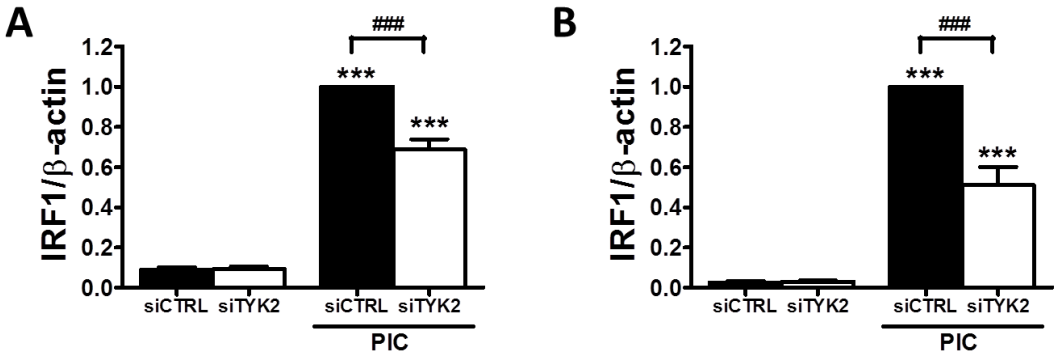
SUPPLEMENTARY DATA

Supplementary Figure 6. Hyperstimulation of the STAT pathway increases IFNα-induced MHC class I expression. Dispersed human islets were transfected with siCTRL or with a siRNA targeting human USP18 as described (22). After this, dispersed human islets were left untreated or treated with IFNα (2,000 U/ml) for 48 h. Expression of USP18 (A), phospho-STAT1, total STAT1 and α-tubulin (B and C) (used as loading control) were measured by western blot. (B) The figure shows a representative blot of 3 experiments in dispersed human islets. (C) Densitometry results of P-STAT1/α-tubulin. (D) mRNA expression of HLA-ABC was analyzed by RT-PCR and normalized by the housekeeping gene β-actin. Results are means ± SEM of 3 independent experiments; ***P<0.05 and *P<0.001 *vs.* untreated (i.e., not treated with IFNα) and transfected with the same siRNA; $^{\#\#}P$<0.01 as indicated by bars; ANOVA.



SUPPLEMENTARY DATA

Supplementary Figure 7. TYK2 knockdown decreases PIC-induced IRF1 mRNA expression. Dispersed human islets (A) or EndoC-βH1 cells (B) were transfected with siCTRL or with a siRNA targeting human TYK2 as in Fig. 2. The cells were then left untreated or treated with intracellular PIC (1 μg/ml) for 24 h. Expression of IRF1 mRNAs was analyzed by RT-PCR and normalized by the housekeeping gene β-actin. Results are means ± SEM of 3-4 independent experiments; ***P<0.001 vs. untreated (i.e., not treated with PIC) and transfected with the same siRNA; ###P<0.001 as indicated by bars; ANOVA.



SUPPLEMENTARY DATA

Supplementary Table 1. Characteristics of the 16 human donors used in the present study.

Date of the islet preparation	Age (years)	Gender	BMI (kg/m2)	Cause of death	Proportion of β-cells in the preparations (%)
18.10.11	72	F	22	Cerebral hemorrhage	62
07.02.12	61	M	32.4	Cerebral hemorrhage	35
27.03.12	50	M	22.4	Cerebral hemorrhage	60
27.07.13	23	F	22.5	Cardiac arrest	46
28.07.13	51	M	NA	Trauma	37
17.09.13	76	M	33.2	Cerebral hemorrhage	68
02.04.14	74	F	27.3	Cerebral hemorrhage	62
02.05.14	81	M	27.8	Cerebral hemorrhage	63
20.05.14	64	F	29.3	Cerebral hemorrhage	67
20.06.14	72	M	23	Cerebral hemorrhage	48
23.07.14	49	F	25.4	Cerebral hemorrhage	72
11.08.14	66	F	19.5	Cerebral hemorrhage	36
01.09.14	47	F	26.3	Cerebral edema	64
02.09.14	69	F	31.2	Cerebral hemorrhage	40
14.04.2015	73	M	22.6	Cerebral hemorrhage	60
22.04.2015	63	M	24.7	Cerebral hemorrhage	49
Mean ± SEM	**62±4**		**25.9±1.0**		**54±3**

M (male); F (female); BMI (body mass index); NA (data not available).

SUPPLEMENTARY DATA

Supplementary Table 2. Primers used in the present study.

	Forward	Reverse
	Sequence (5'-3')	**Sequence (5'-3')**
Human β-actin	CTGTACGCCAACACAGTGCT	GCTCAGGAGGAGCAATGATC
Human TYK2	TGGCTTGGAAGATGGTGGTG	GTTCCGGCCACACACATTACC
Human IFNα	AATTCTGCACCGAACTCTACC	ATGGAGTCCGCATTCATCAG
Human IFNβ	GTTGAGAACCTCCTGGCTAATG	GGTAATGCAGAATCCTCCCATAATA
Human CXCL10	GTGGCATTCAAGGAGTACCTC	GCCTTCGATTCTGGATTCAG
Human HLA-ABC	GAGAACGGGAAGGAGACGC	CATCTCAGGGTGAGGGGCT
Human IRF1	CATTCACACAGGCCGATACA	TGGTCTTTCACCTCCTCGATAT

SUPPLEMENTARY DATA

Supplementary Table 3. Antibodies used in the present study.

Antibody	Company	Reference	Dilution	MW, kDa
TYK2	Abcam, Cambridge, UK	ab52645	1:1000	~140
Cleaved Caspase 3	Cell signalling, Danvers, MA, UK	9661S	1:1000	~17
P-STAT1 (Ser701)	Cell signalling, Danvers, MA, UK	9171	1:1000	~84, 94
STAT1 p84/p91 (E-23)	Santa Cruz, Santa Cruz, CA, USA	Sc-346x	1:1000	~84, 91
P-STAT2 (Tyr690)	Cell signalling, Danvers, MA, UK	4441	1:1000	~113
STAT2 (N-17)	Santa Cruz, Santa Cruz, CA, USA	Sc-839	1:1000	~113
MHC Class I (W6/32)	Enzo Life Sciences, Lausen, Switzerland	ALX-805-711	1:1000	~40-43
Insulin	Sigma, Bornem, Belgium	I2018	1:1000	-
α-Tubulin	Sigma, Bornem, Belgium	T9026	1:5000	~55
Peroxidase-conjugated donkey anti-rabbit IgG	Jackson ImmunoResearch Laboratories, Wes Grove, PA, USA	715-036-150	1:5000	-
Peroxidase-conjugated donkey anti-mouse IgG	Jackson ImmunoResearch Laboratories, Wes Grove, PA, USA	711-036-154	1:5000	-
Alexa Fluor 488 goat anti-mouse IgG	Life technologies, USA	A11029	1:1000	-
Alexa Fluor 568 goat anti-mouse IgG	Life technologies, USA	A11030	1:1000	-
Alexa Fluor 568 goat anti-rabbit IgG	Life technologies, USA	A11036	1:1000	-

SUPPLEMENTARY DATA

Supplementary Table 4. Type 1 diabetes candidate genes used for IPA.

Gene symbol	Gene ID	Reference
INS	ENSG00000254647	Hakonarson et al., Nature, 2007; Todd et al., Nat Genet., 2007; Barrett JC et al., Nat Genet., 2009
PTPN22	ENSG00000134242	Hakonarson et al., Nature, 2007; Todd et al., Nat Genet., 2007; Barrett JC et al., Nat Genet., 2009
ERBB3	ENSG00000065361	Wellcome Trust Case Control Consortium, Nature, 2007; Todd et al., Nat Genet., 2007; Cooper et al., Nat Genet., 2008; Barrett JC et al., Nat Genet., 2009
FUT2	ENSG00000176920	Smyth et al., Diabetes, 2011
SH2B3	ENSG00000111252	Barrett JC et al., Nat Genet., 2009
PTPN2	ENSG00000175354	Wellcome Trust Case Control Consortium, Nature, 2007; Todd et al., Nat Genet., 2007; Hakonarson et al., Diabetes, 2008; Cooper et al., Nat Genet., 2008; Barrett JC et al., Nat Genet., 2009
CTRB1	ENSG00000168925	Barrett JC et al., Nat Genet., 2009; Bradfield et al., PLoS Genet., 2011
CTRB2	ENSG00000168928	Barrett JC et al., Nat Genet., 2009; Bradfield et al., PLoS Genet., 2011
RNLS	ENSG00000184719	Barrett JC et al., Nat Genet., 2009; Bradfield et al., PLoS Genet., 2011
COBL	ENSG00000106078	Barrett JC et al., Nat Genet., 2009
CYP27B1	ENSG00000111012	Bailey et al., Diabetes, 2007
PRKCQ	ENSG00000065675	Cooper et al., Nat Genet., 2008; Barrett JC et al., Nat Genet., 2009
CLEC16A	ENSG00000038532	Hakonarson et al., Nature, 2007; Wellcome Trust Case Control Consortium, Nature, 2007; Todd et al., Nat Genet., 2007; Cooper et al., Nat Genet., 2008; Barrett JC et al., Nat Genet., 2009
CENPW	ENSG00000203760	Barrett JC et al., Nat Genet., 2009; Bradfield et al., PLoS Genet., 2011
GAB3	ENSG00000160219	Barrett JC et al., Nat Genet., 2009
GPR183	ENSG00000169508	Heinig et al., Nature, 2010
IFIH1	ENSG00000115267	Todd et al., Nat Genet., 2007; Barrett JC et al., Nat Genet., 2009
CTSH	ENSG00000103811	Cooper et al., Nat Genet., 2008; Barrett JC et al., Nat Genet., 2009
TYK2	ENSG00000105397	Wallace et al., Nat Genet., 2010
BACH2	ENSG00000112182	Cooper et al., Nat Genet., 2008; Grant et al., Diabetes, 2009; Barrett JC et al., Nat Genet., 2009
ORMDL3	ENSG00000172057	Barrett JC et al., Nat Genet., 2009
SKAP2	ENSG00000005020	Barrett JC et al., Nat Genet., 2009
GLIS3	ENSG00000107249	Grant et al., Diabetes, 2009; Barrett JC et al., Nat Genet., 2009
C1QTNF6	ENSG00000133466	Cooper et al., Nat Genet., 2008; Barrett JC et al., Nat Genet., 2009; Smyth et al., Diabetes, 2011

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RGS1	ENSG00000090104	Smyth et al., N Engl J Med., 2008
STAT4	ENSG00000138378	Fung et al., Genes Immun., 2009
DLK1	ENSG00000185559	Wallace et al., Nat Genet., 2010
TNFAIP3	ENSG00000118503	Fung et al., Genes Immun., 2009
SMARCE1	ENSG00000073584	Barrett JC et al., Nat Genet., 2009
RASGRP1	ENSG00000172575	Qu et al., J Med Genet., 2009; Bradfield et al., PLoS Genet., 2011
LMO7	ENSG00000136153	Bradfield et al., PLoS Genet., 2011
EFR3B	ENSG00000084710	Bradfield et al., PLoS Genet., 2011
CD83	ENSG00000112149	Bergholdt et al., Diabetes, 2012
IFNGR1	ENSG00000027697	Bergholdt et al., Diabetes, 2012
IL17RD	ENSG00000144730	Bergholdt et al., Diabetes, 2012
TRAF3IP2	ENSG00000056972	Bergholdt et al., Diabetes, 2012
IL27RA	ENSG00000104998	Bergholdt et al., Diabetes, 2012
PLCG2	ENSG00000197943	Bergholdt et al., Diabetes, 2012
MYO1B	ENSG00000128641	Bergholdt et al., Diabetes, 2012
CXCR7	ENSG00000144476	Bergholdt et al., Diabetes, 2012
ITGB7	ENSG00000139626	Evangelou et al., Genet Epidemiol., 2014
NRP1	ENSG00000099250	Evangelou et al., Genet Epidemiol., 2014
BAD	ENSG00000002330	Evangelou et al., Genet Epidemiol., 2014
CTSB	ENSG00000164733	Evangelou et al., Genet Epidemiol., 2014
FYN	ENSG00000010810	Evangelou et al., Genet Epidemiol., 2014
UBE2G1	ENSG00000132388	Evangelou et al., Genet Epidemiol., 2014
ITGB1	ENSG00000150093	Evangelou et al., Genet Epidemiol., 2014
IL7R	ENSG00000168685	Evangelou et al., Genet Epidemiol., 2014
UBD	ENSG00000213886	Aly et al., Diabetes, 2008
HTR1A	ENSG00000178394	Asad et al., PLoS One, 2012
OAS1	ENSG00000089127	Field et al., Diabetes, 2005