Protective role of complement C3 against cytokine-mediated β-cell apoptosis

Reinaldo S. Dos Santos[1*], Laura Marroqui[1], Fabio A. Grieco[1], Lorella Marselli[2], Mara Suleiman[2], Stefan R. Henz[3], Piero Marchetti[2], Rasmus Wernersson[3,4], Decio L. Eizirik[1,5*]

Supplemental Data

Supplemental Experimental Procedures

Protein-protein Interaction Analysis

From the total pool of high-confidence interactions we defined the following collections of sub-networks for further investigation:

1) "**1st order networks":** For each protein represented in the filtered interaction dataset a network containing itself and its direct interaction partners were extracted. This yielded 17.000 networks (data not shown).
2) "**C3 network**": A single network containing C3 and its 1st order interaction partners (technically also part of set #1). This network contained 217 proteins in total, including C3.
3) "**C3 neighbor networks**": For each of the 216 proteins interacting with C3 the 1st order network around the protein was extracted (technically also part of set #1).

For the statistical analysis of the network, the previously published RNAseq datasets from Eizirik and collaborators (1) and five new, unpublished datasets from Eizirik's group (similar experimental design as in 1) were prepared as follows:

1) Gene level *p*-value was mapped to UniProtKB AC – in rare cases of one-to-many and many-to-one mappings the lowest *p*-value was kept.
2) The 10 individual pairwise comparisons in the RNAseq data were combined at the *p*-value level by Edington's method. This method was chosen as being the most conservative, and requires genes to be consistently regulated across the majority of the studies in order to receive a strong integrated *p*-value.

For **each** network in a network collection, the following algorithm was applied:

1) The protein-level *p*-values from the RNAseq dataset were mapped onto the nodes of the network. Missing values were handled by reducing the effective size of the network.
2) An original score for the network was calculated using Fisher's methods for combining the individual *p*-values.
3) The significance of the original score was assessed by Monte Carlo simulation: the nodes in the network were resampled from the entire pool of data, and a new integrated score was calculated – the procedure was repeated up to 1,000,000 times. Lastly, the original integrated score was compared to the estimated background from the simulation, and converted to an empirically estimated *p*-value (fraction of simulations getting an equal or better score than the original vs. the total number of permutations).

The network depicted in figure 1 is also available as a Cytoscape (cytoscape.org) session file ("C3_1st_order.cys"; upon request) – the visualization used in Figure 1 is embedded as a style named "C3_network".

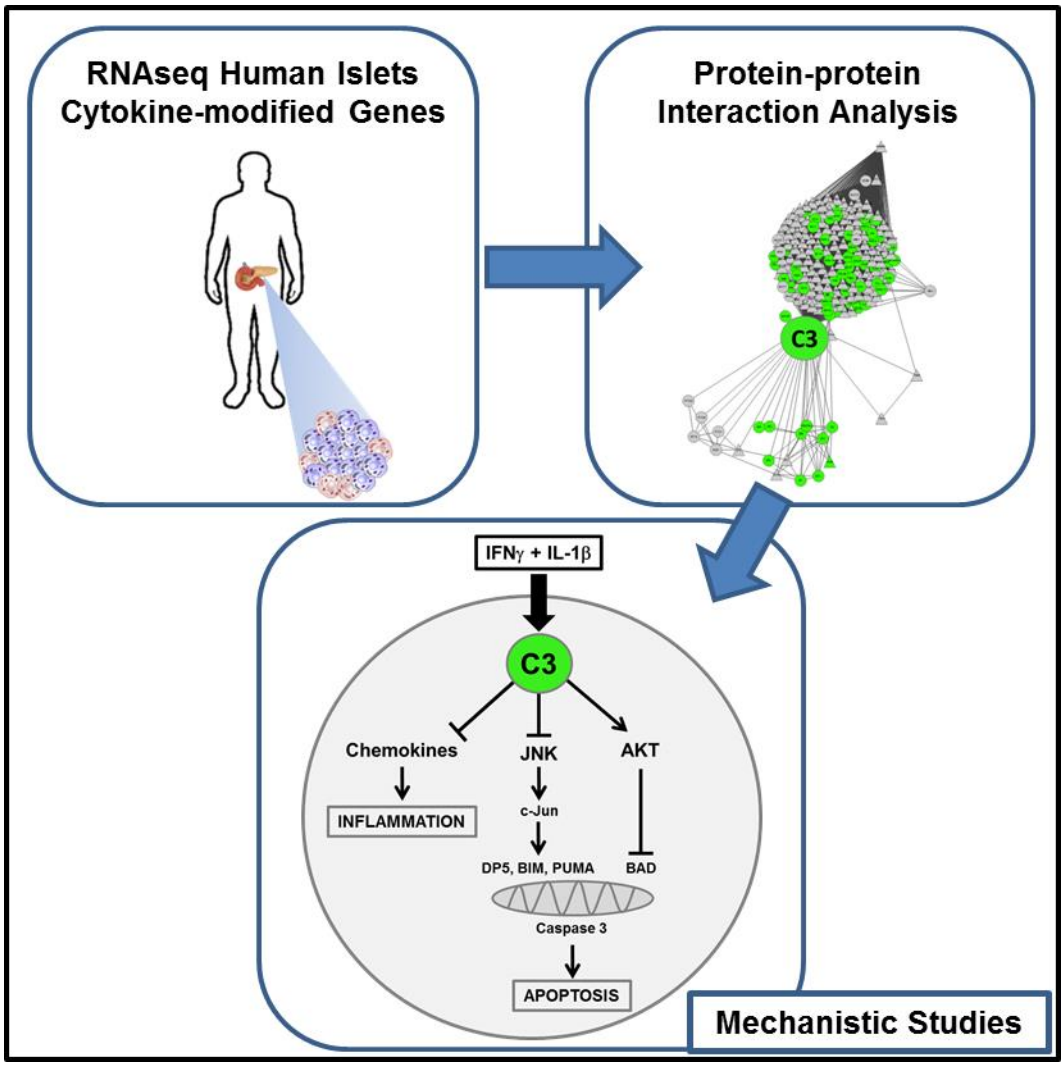
Histology

We performed histology using pancreas sections from two cohorts in two different laboratories, and we thus describe below the methodology used in each laboratory.

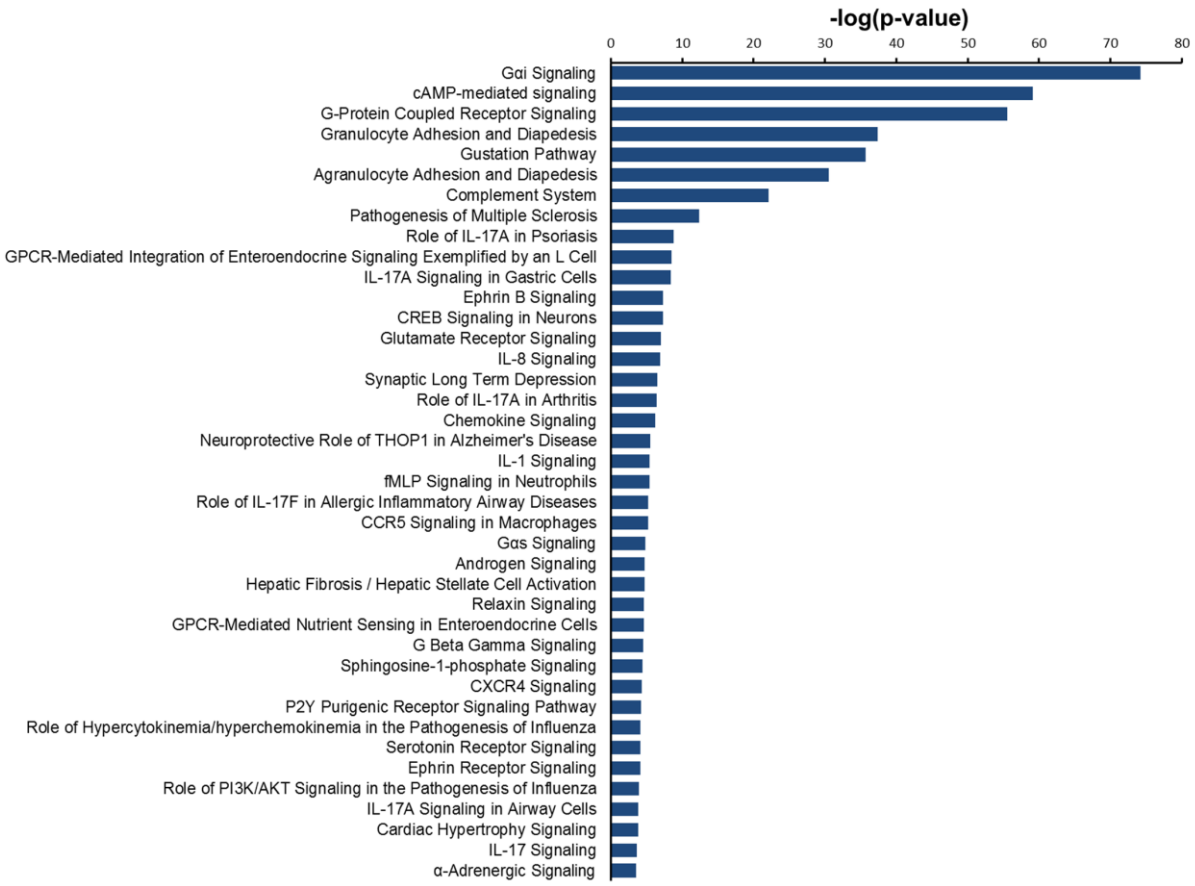
In Brussels, pancreas sections from previously characterized (2) samples from the nPOD collection were subjected to antigen retrieval by boiling the sections in 10 mM citrate buffer (pH 6.0) for 10 min. After overnight incubation at 4°C with primary antibody against C3, slides were double-stained with an anti-insulin antibody (Guinea pig polyclonal antibody DAKO, Glostrup, Denmark; dilution 1:250) for 1 h at room temperature. Alexa Fluor fluorescent secondary antibodies (Molecular Probes-Invitrogen) were applied for 1 h at room temperature (Alexa Fluor 568 Goat anti-rabbit and Alexa Fluor 488 Goat anti-guinea pig; dilution 1:500). After nuclear staining with Hoechst, pancreas sections were mounted with Fluorescence Mounting Medium (DAKO). Immunofluorescence was visualized on a Zeiss microscope (Axio ImagerA1, Zeiss-Vision, Munich, Germany) equipped with a camera (Axio CAM Zeiss), as previously described (2). Images were acquired using Zeiss/EC Plan Neofluar objective lenses at 40x magnification and Axio Vision Ziess software.

In Pisa, pancreas sections obtained at the University of Pisa collection were subjected to antigen retrieval by 3 passages (5 minutes each) in 10 mM citrate buffer (pH 6.0) in microwave at 750 Watt; citrate buffer was replaced at each step. After overnight incubation at 4°C with primary antibody against C3, slides were double-stained with an anti-insulin antibody (Guinea pig polyclonal anti-insulin, Abcam; dilution 1:100) for 1 h at room temperature. Secondary antibodies DyLightTM 594 Donkey anti-guinea pig secondary antibody (DyLightTM 594, Jackson ImmunoResearch; dilution 1:200) and Alexa Fluor 488 Donkey anti-rabbit (Jackson ImmunoResearch Baltimore, PA, USA; dilution 1:200) were applied for 1 h at room temperature. Pancreas sections were mounted with Mounting Medium containing DAPI (VECTASHIELD, Vector Laboratories, Inc. Burlingame, CA, USA). Sections were analyzed using a Leica DM5500 B microscope (Leica, Wetzlar, Germany) equipped with the DFC310 FX camera (Leica). Images were acquired using Leica HCX PL FLUOTAR objective lenses at 10x, 20x, and 40x magnification and Leica MetaMorph® software, v1.8.0.

Supplemental Figures and Tables

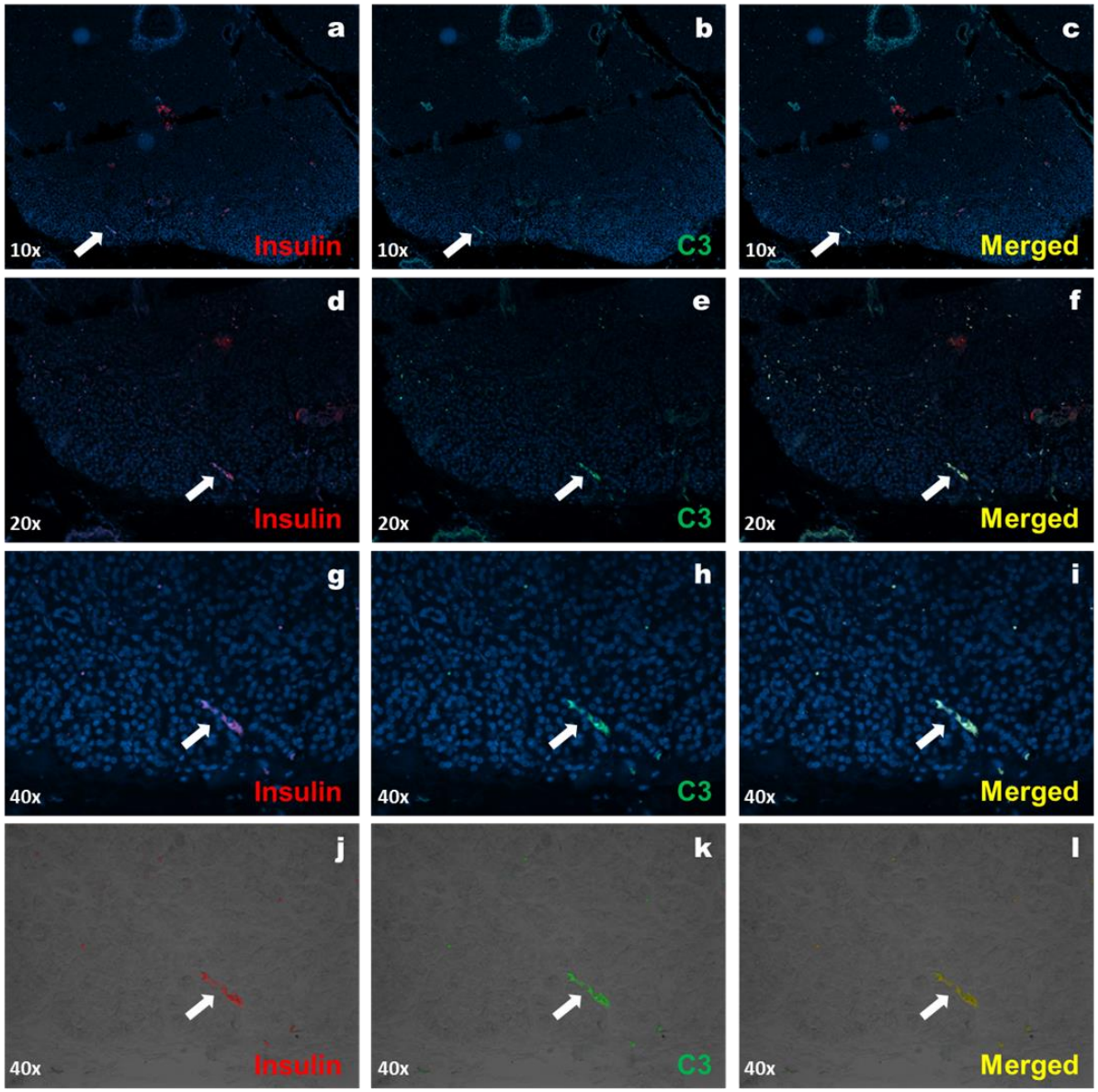


Supplemental Figure 1. Overview of the steps used in the present study. RNA sequencing data of human islets exposed to the proinflammatory cytokines IL-1β + IFNγ were used to perform a protein-protein interaction analysis. Based on the results of the analysis, we performed mechanistic studies to assess C3 role in β-cell survival.



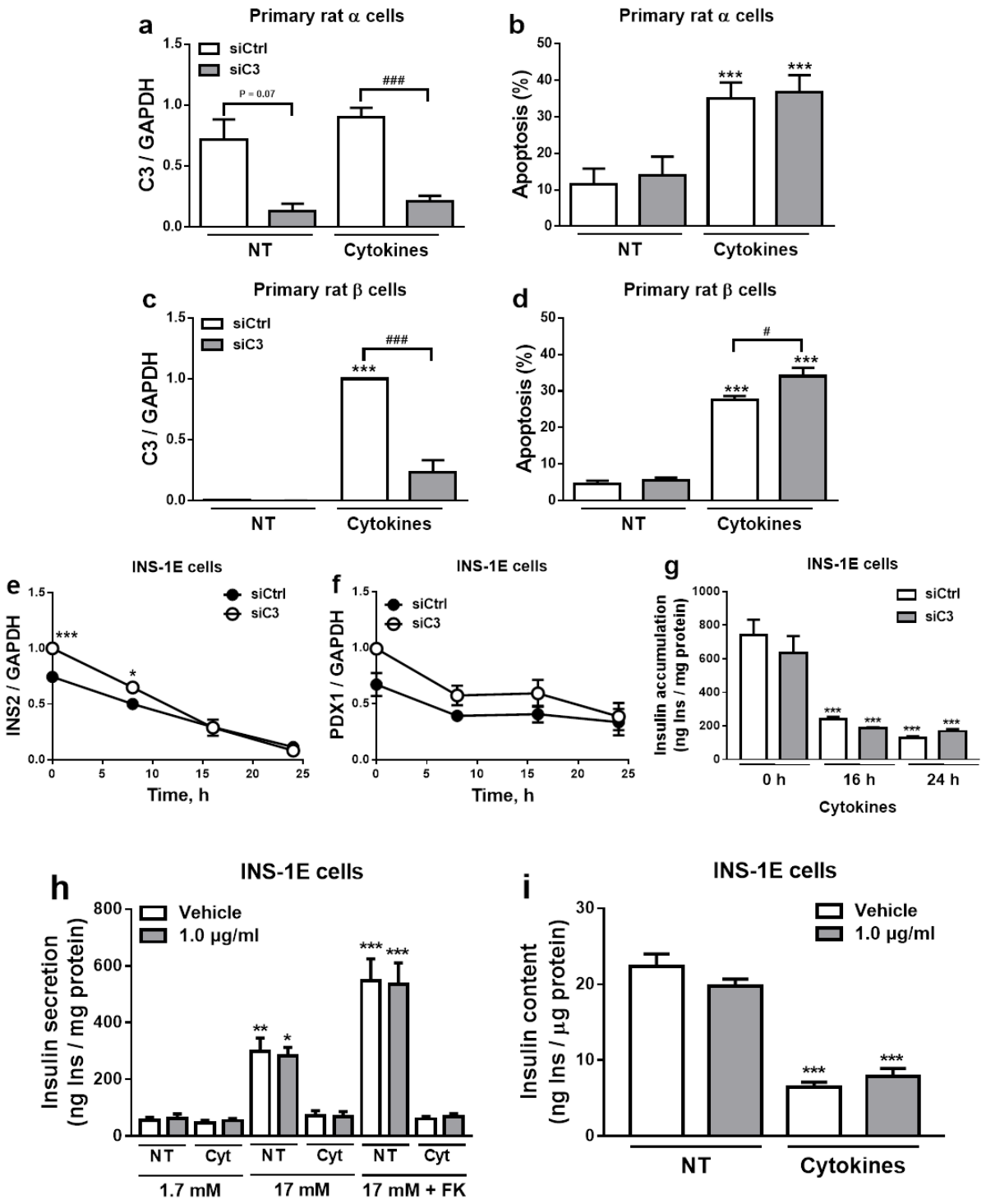
Supplemental Figure 2. Ingenuity Pathway Analysis (IPA) of candidate genes for C3 interacting partners in human islets.

IPA of C3 and its 216 protein partners identified by our protein-protein analysis is shown for the top 40 "Canonical Pathways". The length of the bars designates the significance of the association between the set of proteins 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.



Supplemental Figure 3. C3 expression in pancreas section from a type 1 diabetic donor

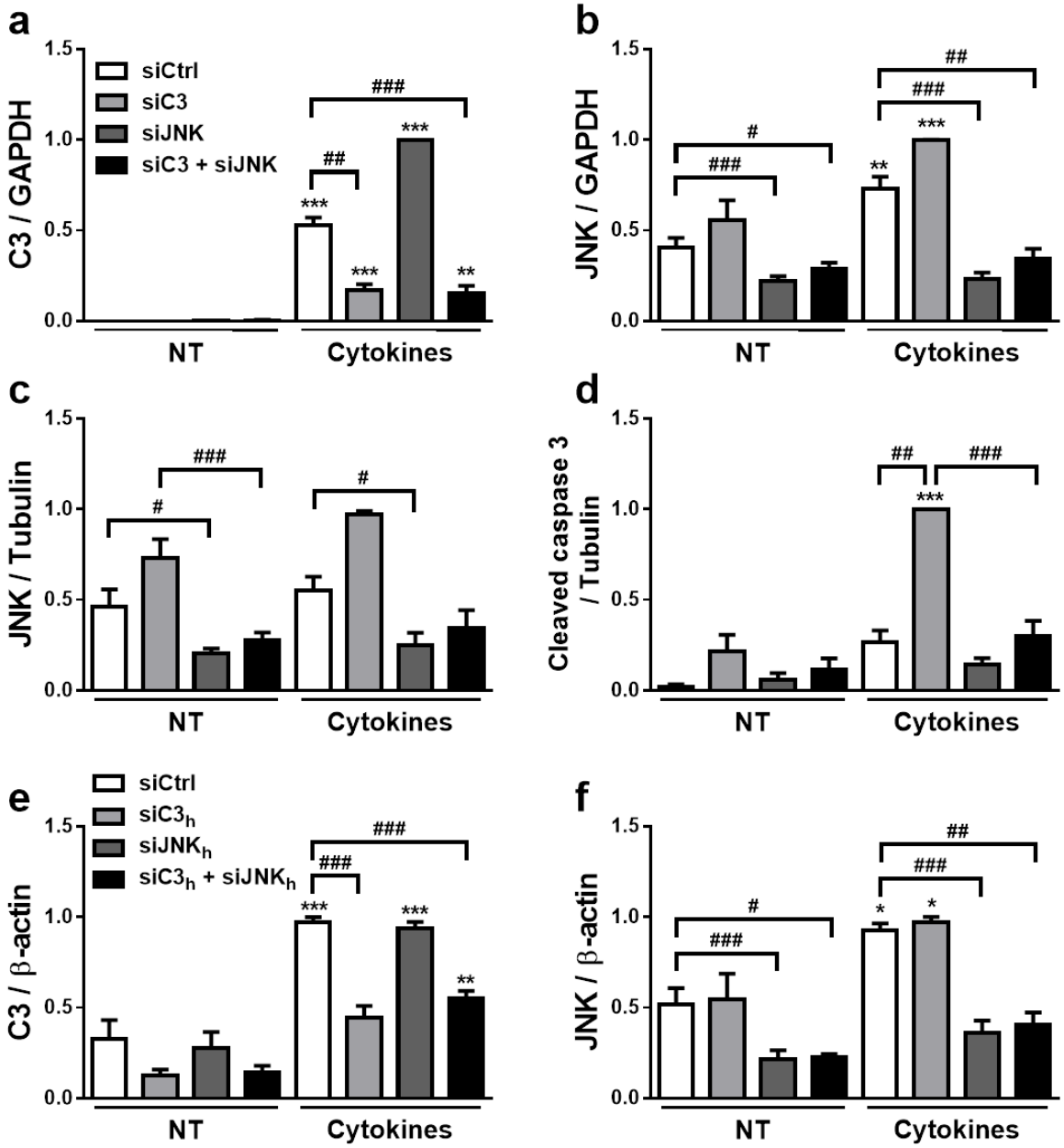
(**a-l**) Pancreas sections from a type 1 diabetic patient from the University of Pisa cohort were stained for insulin (**a,d,g,j**; red), C3 (**b,e,h,k**; green), and merged (**c,f,i,l**; yellow). (**a-i**) DAPI staining is shown in blue. White arrows indicate cluster positive for both insulin and C3. Images are representative of three individuals with type 1 diabetes (same as depicted in the Fig. 3g-i of the main manuscript). Images are shown at 10x (**a-c**), 20x (**d-f**), 40x (**g-i**) magnification, and 40x bright field (**j-l**).



Supplemental Figure 4. C3 inhibition in primary rat α- and β-cells, and β-cell function analysis in C3-silenced cells.

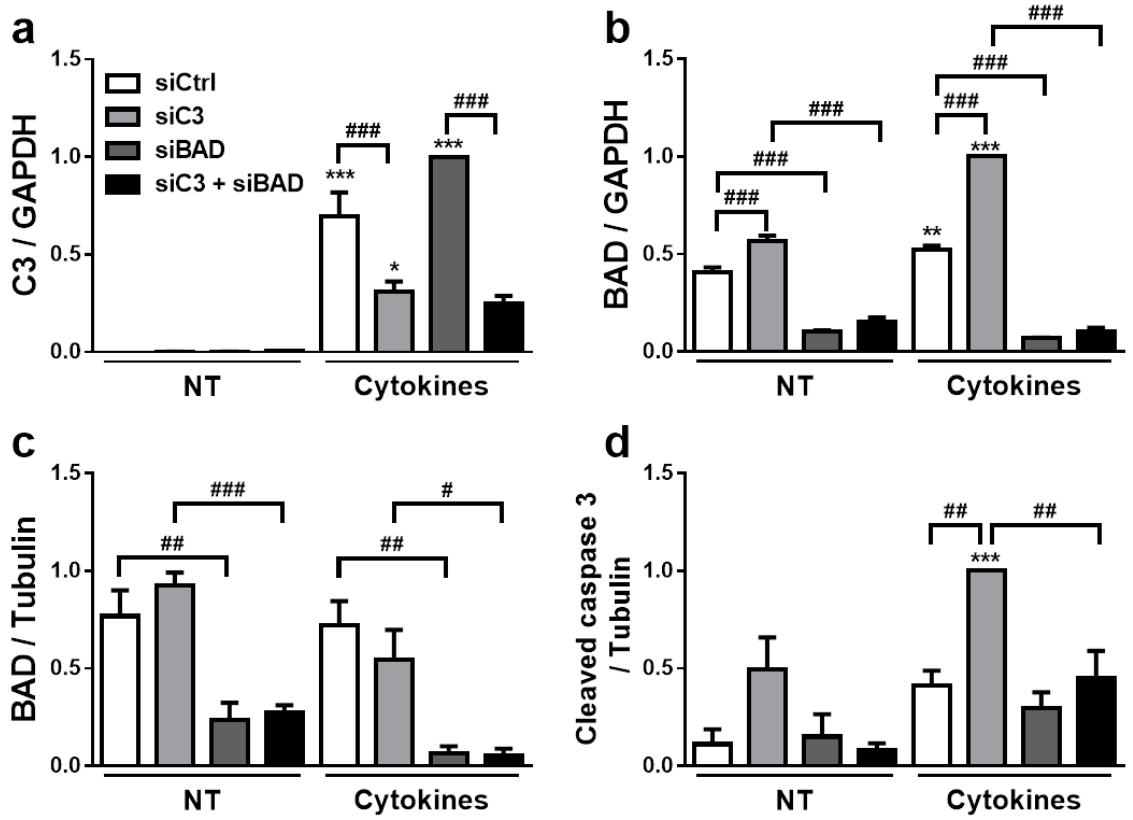
(**a-d**) Primary rat α- and β-cells were transfected with siCtrl or siRNA targeting rat C3 (siC3#1). Cells were then left untreated (NT) or treated with IL-1β + IFNγ (50 and 500 units/ml, respectively) for 48 h. (**a,c**) C3 mRNA expression in primary rat α- (**a**) and β-cells (**c**) was analyzed by RT-PCR and normalized by the housekeeping gene GAPDH. (**b,d**) Apoptosis was evaluated using HO and PI staining in primary rat α- (**b**) and β-cells (**d**). Data represent the means ± SEM of 4 independent experiments; ***P<0.001 vs. non-treated with

cytokines (NT) and transfected with the same siRNA; $^{\#}P \leq 0.05$, and $^{\#\#\#}P < 0.001$ as indicated by bars; ANOVA followed by Student *t* test with Bonferroni correction. (**e-g**) INS-1E cells were transfected with siCtrl (C) or siRNA targeting rat C3 (siC3#1, C3). Cells were then left untreated or treated with IL-1β + IFNγ (10 and 100 units/ml, respectively) as indicated in the figure. (**e,f**) INS2 (**e**) and PDX1 (**f**) mRNA expression was analyzed by RT-PCR and normalized by the housekeeping gene GAPDH during a time-course study. (**g**) Medium insulin accumulation was measured by ELISA. Data represent the means ± SEM of 4-5 independent experiments; $^{*}P < 0.05$ and $^{***}P < 0.001$ vs. transfected with siCtrl (**e,f**) or vs. non-treated with cytokines (0 h) and transfected with the same siRNA (**g**); $^{\#}P \leq 0.05$, and $^{\#\#\#}P < 0.001$ as indicated by bars; ANOVA followed by Student *t* test with Bonferroni correction. (**h,i**) Cells were left untreated (NT) or treated with IL-1β + IFNγ in the absence or presence of exogenously added C3 (1.0 µg/ml) for 24 h. (**h**) Insulin secretion after 30 min of incubation with 1.7 mM glucose, 17 mM glucose, or 17 mM glucose plus forskolin (20 µM). (**i**) Insulin content in cell-free lysates. Insulin secretion and insulin content were measured by ELISA. Data represent the means ± SEM of 3 independent experiments; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. non-treated with cytokines (NT) and incubated at 1.7 mM glucose.



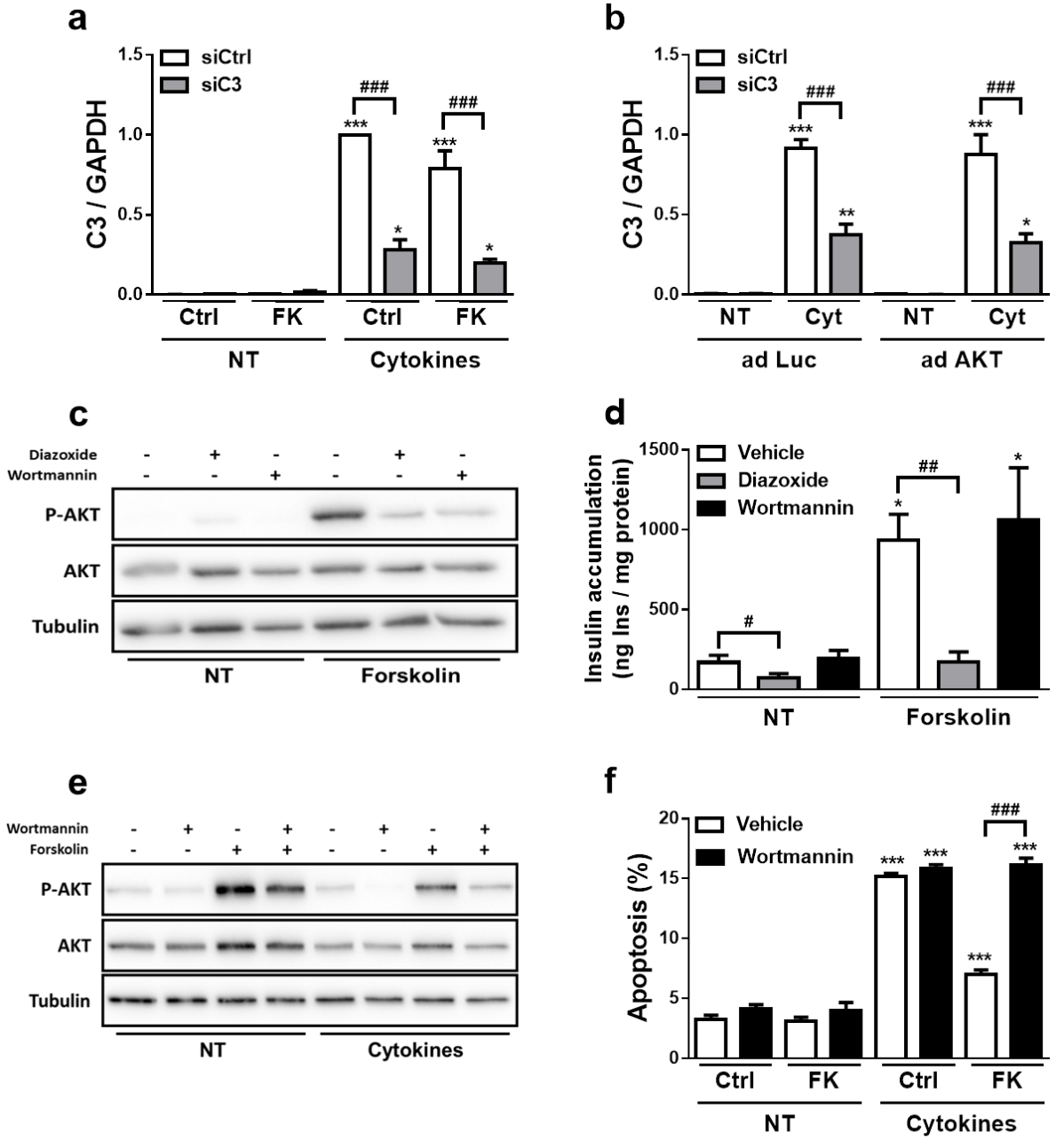
Supplemental Figure 5. Confirmation of C3 and JNK knockdown and cleaved caspase 3 quantification.

(**a-d**) INS-1E cells were transfected with siCtrl, siC3#1, siJNK, or a combination of siC3#1 and siJNK1. Cells were then left untreated or treated with IL-1β + IFNγ (10 and 100 units/ml, respectively) for 16 h. Expression of C3 (**a**) and JNK (**b**) mRNAs was analyzed by RT-PCR and normalized by the housekeeping gene GAPDH. (**c,d**) Quantification of the western blots for JNK (**c**) and cleaved caspase 3 (**d**) shown in Figure 7. (**e,f**) Dispersed human islets were transfected with siCtrl, siC3#2$_h$, siJNK$_h$, or a combination of siC3#2$_h$ and siJNK1$_h$. Cells were then left untreated or treated with IL-1β + IFNγ (50 and 1,000 units/ml, respectively) for 48 h. Expression of C3 (**e**) and JNK (**f**) mRNAs were analyzed by RT-PCR and normalized by the housekeeping gene β-actin. Data represent the means ± SEM of 3-6 independent experiments; **P*≤0.05, ***P*<0.01, and ****P*<0.001 vs. non-treated with cytokines (NT) and transfected with the same siRNA; $^{\#}P\leq0.05$, $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ as indicated by bars; ANOVA followed by Student *t* test with Bonferroni correction.



Supplemental Figure 6. Confirmation of C3 and BAD knockdown and cleaved caspase 3 quantification.

(**a-d**) INS-1E cells were transfected with siCtrl, siC3#1, siBAD, or a combination of siC3#1 and siBAD. Cells were then left untreated or treated with IL-1β + IFNγ (10 and 100 units/ml, respectively) for 16 h. C3 (**a**) and BAD (**b**) mRNA expression were analyzed by RT-PCR and normalized by the housekeeping gene GAPDH. (**c,d**) Quantification of the western blots for BAD (**c**) and cleaved caspase 3 (**d**) shown in Figure 8. (**e**) INS-1E cells exposed to siCtrl or siC3 (siC3#1) were treated with vehicle only (DMSO, Ctrl) or Forskolin (20 µM) in the absence (NT) or presence of IL-1β + IFNγ (10 and 100 units/ml, respectively) for 16 h. (**e**) C3 mRNA expression was analyzed by RT-PCR and normalized by the housekeeping gene GAPDH. (**f**) siCtrl and siC3#1 INS-1E cells were infected with a control adenovirus encoding luciferase (adLuc) or with an adenovirus encoding myr-Akt1 (adAKT). Cells were then left untreated (NT) or treated with IL-1β + IFNγ (10 and 100 units/ml, respectively) for 16 h. (**f**) C3 mRNA expression was analyzed by RT-PCR and normalized by the housekeeping gene GAPDH. Data represent the means ± SEM of 3-4 independent experiments; *$P \leq 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. non-treated with cytokines (NT) and transfected with the same siRNA; $^{\#}P \leq 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ as indicated by bars; ANOVA followed by Student *t* test with Bonferroni correction.



Supplemental Figure 7. Confirmation of C3 knockdown and effects of diazoxide and wortmannin on AKT phosphorylation.

(**a**) INS-1E cells were transfected with siCtrl or siC3 (siC3#1). Cells were then treated with vehicle only (DMSO, Ctrl) or Forskolin (20 µM) in the absence (NT) or presence of IL-1β + IFNγ (10 and 100 units/ml, respectively) for 16 h. (**b**) siCtrl and siC3#1 INS-1E cells were infected with a control adenovirus encoding luciferase (adLuc) or with an adenovirus encoding myr-Akt1 (adAKT). Cells were then left untreated (NT) or treated with IL-1β + IFNγ (10 and 100 units/ml, respectively) for 16 h. C3 mRNA expression was analyzed by RT-PCR and normalized by the housekeeping gene GAPDH. Data represent the means ± SEM of 3-4 independent experiments; **P*≤0.05, ***P*<0.01, and ****P*<0.001 vs. non-treated with cytokines (NT) and transfected with the same siRNA; $^{###}P$<0.001 as indicated by bars; ANOVA followed by Student *t* test with Bonferroni correction. (**c,d**) INS-1E cells were left

untreated or treated with 200 µM diazoxide in the absence (NT) or presence of 20 µM forskolin for 16 h or pretreated or not for 2 h with 100 nM wortmannin and then left untreated (NT) or treated with 20 µM forskolin for 16 h. (**c**) P-AKT, AKT, and α-tubulin were measured by western blot. Images are representative of 4 independent experiments. (**d**) Medium insulin accumulation was measured by ELISA. Results are means ± SEM; **P*<0.05 vs. non-treated with forskolin (NT); $^{\#}P$<0.05 and $^{\#\#}P$<0.01 as indicated by bars; ANOVA followed by Student t test with Bonferroni correction. (**e,f**) INS-1E cells were pretreated or not for 2 h with 100 nM wortmannin and then left untreated (NT) or exposed to IL-1β + IFNγ (10 and 100 U/ml, respectively) and/or 20 µM forskolin for 16 h. (**e**) P-AKT, AKT, and α-tubulin were measured by western blot. Images are representative of 4 independent experiments. (**f**) Apoptosis was evaluated using HO and PI staining in INS-1E cells. Results are means ± SEM; ****P*< 0.001 vs. non-treated with cytokines (NT); $^{\#\#\#}P$< 0.001 as indicated by bars; ANOVA followed by Student t test with Bonferroni correction.

Supplemental Table 1. Characteristics of the human donors used in the present study for islet functional studies.

Date of the islet preparation	Age (years)	Gender	BMI (kg/m^2)	Cause of death	Proportion of β-cells in the preparations (%)
12/12/14	73	F	NA	Cerebral hemorrhage	22
23/01/15	73	M	22.8	Stroke	36
01/04/15	85	F	22.7	Cerebral hemorrhage	51
17/04/15	70	M	22.6	Cerebral hemorrhage	60
09/07/15	49	M	24.7	Cerebral hemorrhage	67
15/07/15	64	F	21.3	CVD	55
24/07/15	51	M	24.7	Trauma	60
19/08/15 (1)	83	F	22.5	Cerebral hemorrhage	48
19/08/15 (2)	54	F	26.3	Cerebral hemorrhage	83
08/04/16	72	M	27.4	Trauma	31
19/05/16	61	M	23.1	CVD	50
13.07.16A	81	F	31.9	Trauma	67
13.07.16B	68	M	21.5	Cerebral hemorrhage	48
29/08/16	79	M	27.7	Cerebral hemorrhage	38
21/09/16	83	M	24.5	Trauma	44
23/09/16	75	M	22.9	CVD	41
Mean ± SEM	**70 ± 3**		**24 ± 1**		**50 ± 4**

M: male; F: female; BMI: body mass index; CVD: cardiovascular disease.

Supplemental Table 2. List of siRNAs used in the present study.

siRNA	Name	Distributor	Sequence
siCTRL	Allstars Negative Control siRNA	Qiagen, Verlo, Netherlands	Sequence not provided
siC3#1 (rat)	Silencer® Selected s235554	Ambion, Life technologies Corporation, CA, USA	5'-GGCUCAACGAGCAAAGAUAtt-3' 5'-UAUCUUUGCUCGUUGAGCCag -3'
siC3#2 (rat)	Silencer® Selected s235555	Ambion, Life technologies Corporation, CA, USA	5'-GGAGUAGACAGAUACAUUUtt -3' 5'-AAAUGUAUCUGUCUACUCCag -3'
siC3$_h$#1 (human)	Silencer® Selected s2161	Ambion, Life technologies Corporation, CA, USA	5'-GGAGUAACCUGGAUGAGGAtt -3' 5'-UCCUCAUCCAGGUUACUCCtg -3'
siC3$_h$#2 (human)	Silencer® Selected s2162	Ambion, Life technologies Corporation, CA, USA	5'-GAUCCGAGCCUACUAUGAAtt -3' 5'-UUCAUAGUAGGCUCGGAUCtt -3'
siJNK1 (rat)	Stealth Selected siRNAi siRNA Duplex Oligonucleotides	Invitrogen, Pasley, UK	5'-AAAGAAUGUCCUACCUUCU-3'
siJNK1$_h$ (human)	Stealth Selected siRNAi siRNA Duplex Oligonucleotides	Invitrogen, Pasley, UK	5'-GGGCCUACAGAGAGCUAGUUCUUAU-3' 5'-AUAAGAACUAGCUCUCUGUAGGCCC-3'
siBAD (rat)	Stealth Selected siRNAi siRNA Duplex Oligonucleotides	Invitrogen, Pasley, UK	5'-GAGUAUGUUCCAGAUCCCAGAGUU-3'

Supplemental Table 3. Primers used in the present study.

Genes	Forward	Reverse
	Sequence (5'-3')	Sequence (5'-3')
Human β-actin	TCTACGCCAACACAGTCGT	GCTCAGGAGGAGCAATGATC
Human C3	CGATAGGAACACCCTCATCATC	CAGGCTGGATAAGCTCTACATT
Human C3aR1	GAAACCAGCCCACTGGATAA	TGGTAGCTCAGACTCGTAGAA
Human JNK1	GGACTGCAGGAACGAGTTTT	CAACTGACCAAATGTCAACG
Rat GAPDH	AGTTCAACGGCACAGTCAAG	TACTCAGCACCAGCATCACC
Rat C3	AGTGCAGAAACGCCCTAAA	CGTGTCCTTCCCAATGATGTA
Rat C3aR1	TTGCCTCCATGGTCATTCTC	TTCACGGTCCTCTTCATCTTTAC
Rat CCL2	TAGCATCCACGTGCTGTCTC	TGCTGCTGGTGATTCTCTTG
Rat CCL5	CCAGAGAAGAAGTGGGTTCA	AGCAAGCAATGACAGGAAAG
Rat CXCL10	GCAAGTCTATCCTGTCCGCAT	GGGTAAAGGGAGGTGGAGAGA
Rat INS2	TGTGGTTCTCACTTGGTGGA	CTCCAGTTGTGCCACTTGTG
Rat c-Jun	CGTTCCTCCAGTCCGAGA	AAGGTCCGAGTTCTTGGCT
Rat JNK	CTAAGCGAGCCTACCGAGAA	GCAAAGATTGCATCCATGA
Rat BAD	CCAATAACAGTCATCATGGAG	GTCCTCGAAAAGGGCTAAG
Rat PDX1	GGTATAGCCAGCGAGATGCT	TCAGTTGGGAGCCTGATTCT

Supplemental Table 4. Antibodies used in the present study.

Peptide/protein target	Antigen sequence (if known)	Name of Antibody	Manufacturer, catalog #, and/or name of individual providing the antibody	Species raised in	Dilution	RRID
C3	NA	Anti-C3 antibody	Abcam, Cambridge, UK, Cat#: ab97462	Rabbit, polyclonal	1:1000 (WB) or 1:100 (ICC)	AB_10679468
Cleaved caspase 3	NA	Cleaved Caspase-3 (Asp175)	Cell signalling, Danvers, MA, UK, Cat#: 9661	Rabbit, polyclonal	1:1000	AB_2341188
Phospho-SAPK/JNK	NA	Phospho-SAPK/JNK (Thr183/Tyr185) Antibody	Cell signalling, Danvers, MA, UK, Cat#: 9251	Rabbit, polyclonal	1:1000	AB_331659
JNK1	NA	JNK1 (2C6) Mouse mAb	Cell signalling, Danvers, MA, UK, Cat#: 3708S	Mouse, monoclonal	1:1000	AB_1904132
Phospho-AKT	NA	Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	Cell signalling, Danvers, MA, UK, Cat#: 4060	Rabbit, monoclonal	1:10000	AB_2315049
AKT	NA	Akt Antibody	Cell signalling, Danvers, MA, UK, Cat#: 9272	Rabbit, polyclonal	1:5000	AB_329827
Phospho-BAD	NA	Phospho-Bad (Ser136) (D25H8) Rabbit mAb	Cell signalling, Danvers, MA, UK, Cat#: 4366	Rabbit, monoclonal	1:500	AB_10547878
BAD	NA	Bad Antibody	Cell signalling, Danvers, MA, UK, Cat#: 9292	Unknown	1:500	AB_331419
Glucagon	NA	Monoclonal Anti-Glucagon antibody produced in mouse	Sigma, Bornem, Belgium, Cat#: G2654	Mouse, monoclonal	1:1000	AB_259852
Insulin	NA	Monoclonal Anti-Insulin antibody produced in mouse	Sigma, Bornem, Belgium, Cat#: I2018	Mouse, monoclonal	1:1000	AB_260137
Insulin (for histology)	NA	Polyclonal Guinea Pig Anti- Insulin antibody	DAKO, Glostrup, Denmark, Cat# A056401-2	Guinea pig, polyclonal	1:250	AB_2617169
Insulin (for histology)	NA	Guinea Pig Anti-Insulin Polyclonal Antibody	Abcam, Cambridge, UK, Cat# ab7842	Guinea pig, polyclonal	1:100	AB_306130
α-Tubulin	NA	Monoclonal Anti-α-Tubulin antibody	Sigma, Bornem, Belgium, Cat#: T9026	Mouse, monoclonal	1:5000	AB_477593

Anti-mouse IgG	NA	Peroxidase AffiniPure F(ab')2 Fragment Donkey Anti-Mouse IgG (H+L)	Jackson ImmunoResearch Laboratories, Wes Grove, PA, USA, Cat#: 715-036-150	Polyclonal	1:5000	AB_2340773
Anti-rabbit IgG	NA	Peroxidase AffiniPure F(ab')2 Fragment Donkey Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch Laboratories, Wes Grove, PA, USA, Cat#: 711-036-152	Polyclonal	1:5000	AB_2340590
Goat anti-mouse IgG	NA	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Life technologies, USA, Cat#: A11029	Goat, polyclonal	1:1000	AB_2534088
Goat anti-guinea pig IgG	NA	Goat anti-Guinea Pig IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Life technologies, USA, Cat#: A11073	Goat, polyclonal	1:1000	AB_2534117
Donkey anti-rabbit IgG	NA	Alexa Fluor 488-AffiniPure Donkey Anti-Rabbit IgG (H+L) Antibody	Jackson ImmunoResearch Laboratories, Wes Grove, PA, USA, Cat#: 711-545-152	Donkey, polyclonal	1:200	AB_2313584
Donkey anti-guinea pig	NA	DyLight 594-conjugated AffiniPure Donkey Anti-Guinea Pig	Jackson ImmunoResearch Laboratories, Wes Grove, PA, USA, Cat#: 706-515-148	Donkey, polyclonal	1:200	The product has been discontinued
Goat anti-rabbit IgG	NA	Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	Life technologies, USA, Cat#: A11036	Goat, polyclonal	1:1000	AB_2534094

NA: Not available

Supplemental Table 5. Characteristics of the non-diabetic and type 1 diabetic donors used for histology.

Patient	Age (years)	Gender	Disease duration (Years)	Cause of death	Source
ND 1	63	F	-	CVD	University of Pisa
ND 2	88	M	-	CVD	University of Pisa
ND 3	32	F	-	Unknown	nPOD, CID: 6034
ND 4	68	F	-	Unknown	nPOD, CID: 6012
T1D 1	73	M	10	CVD	University of Pisa
T1D 2	61	F	Unknown	CVD	University of Pisa
T1D 3	23	M	7	Unknown	nPOD, CID: 6069

ND: non-diabetic donor; T1D: type 1 diabetic donor; M: male; F: female; CVD: cardiovascular disease; CID: case identification number.

Supplemental Table 6. List with the descriptions of the genes/proteins in the C3 1st order network (Network Collection #2).

Gene name	UniProt Acession	UniProt Description
ACKR3	P25106	Atypical chemokine receptor 3
ADORA1	P30542	Adenosine receptor A1
ADORA3	P0DMS8	Adenosine receptor A3
ADRA2A	P08913	Alpha-2A adrenergic receptor
ADRA2B	P18089	Alpha-2B adrenergic receptor
ADRA2C	P18825	Alpha-2C adrenergic receptor
AGT	P01019	Angiotensinogen
AGTR2	P50052	Type-2 angiotensin II receptor
ANXA1	P04083	Annexin A1
APLN	Q9ULZ1	Apelin
APLNR	P35414	Apelin receptor
APP	P05067	Amyloid beta A4 protein
BDKRB1	P46663	B1 bradykinin receptor
BDKRB2	P30411	B2 bradykinin receptor
C2	P06681	Complement C2
C3	P01024	Complement C3
C3AR1	Q16581	C3a anaphylatoxin chemotactic receptor
C4A	P0C0L4	Complement C4-A
C4B	P0C0L5	Complement C4-B
C5	P01031	Complement C5
C5AR1	P21730	C5a anaphylatoxin chemotactic receptor 1
CASR	P41180	Extracellular calcium-sensing receptor
CCL15	Q16663	C-C motif chemokine 15
CCL16	O15467	C-C motif chemokine 16
CCL19	Q99731	C-C motif chemokine 19
CCL20	P78556	C-C motif chemokine 20
CCL21	O00585	C-C motif chemokine 21

CCL23	P55773	C-C motif chemokine 23
CCL25	O15444	C-C motif chemokine 25
CCL27	Q9Y4X3	C-C motif chemokine 27
CCL28	Q9NRJ3	C-C motif chemokine 28
CCL5	P13501	C-C motif chemokine 5
CCR1	P32246	C-C chemokine receptor type 1
CCR10	P46092	C-C chemokine receptor type 10
CCR2	P41597	C-C chemokine receptor type 2
CCR3	P51677	C-C chemokine receptor type 3
CCR4	P51679	C-C chemokine receptor type 4
CCR5	P51681	C-C chemokine receptor type 5
CCR6	P51684	C-C chemokine receptor type 6
CCR7	P32248	C-C chemokine receptor type 7
CCR8	P51685	C-C chemokine receptor type 8
CCR9	P51686	C-C chemokine receptor type 9
CD19	P15391	B-lymphocyte antigen CD19
CD46	P15529	Membrane cofactor protein
CD55	P08174	Complement decay-accelerating factor
CD81	P60033	CD81 antigen
CFB	P00751	Complement factor B
CFD	P00746	Complement factor D
CFH	P08603	Complement factor H
CFHR3	Q02985	Complement factor H-related protein 3
CFHR4	Q92496	Complement factor H-related protein 4
CFHR5	Q9BXR6	Complement factor H-related protein 5
CFI	P05156	Complement factor I
CFP	P27918	Properdin
CHRM2	P08172	Muscarinic acetylcholine receptor M2
CHRM4	P08173	Muscarinic acetylcholine receptor M4

CNR1	P21554	Cannabinoid receptor 1
CNR2	P34972	Cannabinoid receptor 2
CPN1	P15169	Carboxypeptidase N catalytic chain
CR1	P17927	Complement receptor type 1
CR1L	Q2VPA4	Complement component receptor 1-like protein
CR2	P20023	Complement receptor type 2
CXCL1	P09341	Growth-regulated alpha protein
CXCL10	P02778	C-X-C motif chemokine 10
CXCL11	O14625	C-X-C motif chemokine 11
CXCL12	P48061	Stromal cell-derived factor 1
CXCL13	O43927	C-X-C motif chemokine 13
CXCL16	Q9H2A7	C-X-C motif chemokine 16
CXCL2	P19875	C-X-C motif chemokine 2
CXCL3	P19876	C-X-C motif chemokine 3
CXCL5	P42830	C-X-C motif chemokine 5
CXCL6	P80162	C-X-C motif chemokine 6
CXCL8	P10145	Interleukin-8
CXCL9	Q07325	C-X-C motif chemokine 9
CXCR1	P25024	C-X-C chemokine receptor type 1
CXCR2	P25025	C-X-C chemokine receptor type 2
CXCR3	P49682	C-X-C chemokine receptor type 3
CXCR4	P61073	C-X-C chemokine receptor type 4
CXCR5	P32302	C-X-C chemokine receptor type 5
CXCR6	O00574	C-X-C chemokine receptor type 6
DRD2	P14416	D(2) dopamine receptor
DRD3	P35462	D(3) dopamine receptor
DRD4	P21917	D(4) dopamine receptor
FPR1	P21462	fMet-Leu-Phe receptor
FPR2	P25090	N-formyl peptide receptor 2

FPR3	P25089	N-formyl peptide receptor 3
GABBR1	Q9UBS5	Gamma-aminobutyric acid type B receptor subunit 1
GABBR2	O75899	Gamma-aminobutyric acid type B receptor subunit 2
GAL	P22466	Galanin peptides
GALR1	P47211	Galanin receptor type 1
GALR2	O43603	Galanin receptor type 2
GALR3	O60755	Galanin receptor type 3
GNAI1	P63096	Guanine nucleotide-binding protein G(i) subunit alpha-1
GNAI2	P04899	Guanine nucleotide-binding protein G(i) subunit alpha-2
GNAI3	P08754	Guanine nucleotide-binding protein G(k) subunit alpha
GNAT1	P11488	Guanine nucleotide-binding protein G(t) subunit alpha-1
GNAT2	P19087	Guanine nucleotide-binding protein G(t) subunit alpha-2
GNAT3	A8MTJ3	Guanine nucleotide-binding protein G(t) subunit alpha-3
GNB1	P62873	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
GNG2	P59768	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2
GPER1	Q99527	G-protein coupled estrogen receptor 1
GPR17	Q13304	Uracil nucleotide/cysteinyl leukotriene receptor
GPR18	Q14330	N-arachidonyl glycine receptor
GPR55	Q9Y2T6	G-protein coupled receptor 55
GRM2	Q14416	Metabotropic glutamate receptor 2
GRM3	Q14832	Metabotropic glutamate receptor 3
GRM4	Q14833	Metabotropic glutamate receptor 4
GRM6	O15303	Metabotropic glutamate receptor 6
GRM7	Q14831	Metabotropic glutamate receptor 7
GRM8	O00222	Metabotropic glutamate receptor 8
HCAR1	Q9BXC0	Hydroxycarboxylic acid receptor 1
HCAR2	Q8TDS4	Hydroxycarboxylic acid receptor 2
HCAR3	P49019	Hydroxycarboxylic acid receptor 3
HEBP1	Q9NRV9	Heme-binding protein 1

HNF4A	P41235	Hepatocyte nuclear factor 4-alpha
HRH3	Q9Y5N1	Histamine H3 receptor
HRH4	Q9H3N8	Histamine H4 receptor
HTR1A	P08908	5-hydroxytryptamine receptor 1A
HTR1B	P28222	5-hydroxytryptamine receptor 1B
HTR1D	P28221	5-hydroxytryptamine receptor 1D
HTR1E	P28566	5-hydroxytryptamine receptor 1E
HTR1F	P30939	5-hydroxytryptamine receptor 1F
HTR5A	P47898	5-hydroxytryptamine receptor 5A
IFITM1	P13164	Interferon-induced transmembrane protein 1
IFITM2	Q01629	Interferon-induced transmembrane protein 2
IFITM3	Q01628	Interferon-induced transmembrane protein 3
INSL5	Q9Y5Q6	Insulin-like peptide INSL5
ITGAX	P20702	Integrin alpha-X
ITGB2	P05107	Integrin beta-2
KNG1	P01042	Kininogen-1
LPAR1	Q92633	Lysophosphatidic acid receptor 1
LPAR2	Q9HBW0	Lysophosphatidic acid receptor 2
LPAR3	Q9UBY5	Lysophosphatidic acid receptor 3
LPAR5	Q9H1C0	Lysophosphatidic acid receptor 5
MCHR1	Q99705	Melanin-concentrating hormone receptor 1
MCHR2	Q969V1	Melanin-concentrating hormone receptor 2
MT-RNR2	Q8IVG9	Humanin
MTNR1A	P48039	Melatonin receptor type 1A
MTNR1B	P49286	Melatonin receptor type 1B
NMS	Q5H8A3	Neuromedin-S
NMU	P48645	Neuromedin-U
NMUR1	Q9HB89	Neuromedin-U receptor 1
NMUR2	Q9GZQ4	Neuromedin-U receptor 2

NPB	Q8NG41	Neuropeptide B
NPBWR1	P48145	Neuropeptides B/W receptor type 1
NPBWR2	P48146	Neuropeptides B/W receptor type 2
NPW	Q8N729	Neuropeptide W
NPY	P01303	Pro-neuropeptide Y
NPY1R	P25929	Neuropeptide Y receptor type 1
NPY2R	P49146	Neuropeptide Y receptor type 2
NPY4R	P50391	Neuropeptide Y receptor type 4
NPY5R	Q15761	Neuropeptide Y receptor type 5
OPRD1	P41143	Delta-type opioid receptor
OPRK1	P41145	Kappa-type opioid receptor
OPRL1	P41146	Nociceptin receptor
OPRM1	P35372	Mu-type opioid receptor
OXER1	Q8TDS5	Oxoeicosanoid receptor 1
OXGR1	Q96P68	2-oxoglutarate receptor 1
P2RY12	Q9H244	P2Y purinoceptor 12
P2RY13	Q9BPV8	P2Y purinoceptor 13
P2RY14	Q15391	P2Y purinoceptor 14
P2RY4	P51582	P2Y purinoceptor 4
PDYN	P01213	Proenkephalin-B
PENK	P01210	Proenkephalin-A
PF4	P02776	Platelet factor 4
PF4V1	P10720	Platelet factor 4 variant
PMCH	P20382	Pro-MCH
PNOC	Q13519	Prepronociceptin
POMC	P01189	Pro-opiomelanocortin
PPBP	P02775	Platelet basic protein
PPY	P01298	Pancreatic prohormone
PTGDR2	Q9Y5Y4	Prostaglandin D2 receptor 2

PTGER3	P43115	Prostaglandin E2 receptor EP3 subtype
PYY	P10082	Peptide YY
RELA	Q04206	Transcription factor p65
RLN3	Q8WXF3	Relaxin-3
RXFP3	Q9NSD7	Relaxin-3 receptor 1
RXFP4	Q8TDU9	Relaxin-3 receptor 2
S1PR1	P21453	Sphingosine 1-phosphate receptor 1
S1PR2	O95136	Sphingosine 1-phosphate receptor 2
S1PR3	Q99500	Sphingosine 1-phosphate receptor 3
S1PR4	O95977	Sphingosine 1-phosphate receptor 4
S1PR5	Q9H228	Sphingosine 1-phosphate receptor 5
SAA1	P0DJI8	Serum amyloid A-1 protein
SST	P61278	Somatostatin
SSTR1	P30872	Somatostatin receptor type 1
SSTR2	P30874	Somatostatin receptor type 2
SSTR3	P32745	Somatostatin receptor type 3
SSTR4	P31391	Somatostatin receptor type 4
SSTR5	P35346	Somatostatin receptor type 5
SUCNR1	Q9BXA5	Succinate receptor 1
TAS2R1	Q9NYW7	Taste receptor type 2 member 1
TAS2R10	Q9NYW0	Taste receptor type 2 member 10
TAS2R13	Q9NYV9	Taste receptor type 2 member 13
TAS2R14	Q9NYV8	Taste receptor type 2 member 14
TAS2R16	Q9NYV7	Taste receptor type 2 member 16
TAS2R19	P59542	Taste receptor type 2 member 19
TAS2R20	P59543	Taste receptor type 2 member 20
TAS2R3	Q9NYW6	Taste receptor type 2 member 3
TAS2R30	P59541	Taste receptor type 2 member 30
TAS2R31	P59538	Taste receptor type 2 member 31

TAS2R38	P59533	Taste receptor type 2 member 38
TAS2R39	P59534	Taste receptor type 2 member 39
TAS2R4	Q9NYW5	Taste receptor type 2 member 4
TAS2R40	P59535	Taste receptor type 2 member 40
TAS2R41	P59536	Taste receptor type 2 member 41
TAS2R42	Q7RTR8	Taste receptor type 2 member 42
TAS2R43	P59537	Taste receptor type 2 member 43
TAS2R45	P59539	Taste receptor type 2 member 45
TAS2R46	P59540	Taste receptor type 2 member 46
TAS2R5	Q9NYW4	Taste receptor type 2 member 5
TAS2R50	P59544	Taste receptor type 2 member 50
TAS2R60	P59551	Taste receptor type 2 member 60
TAS2R7	Q9NYW3	Taste receptor type 2 member 7
TAS2R8	Q9NYW2	Taste receptor type 2 member 8
TAS2R9	Q9NYW1	Taste receptor type 2 member 9
VSIG4	Q9Y279	V-set and immunoglobulin domain-containing protein 4

Supplemental Table 7. Statistical significance of all 217 1st order networks which contains C3 (Network Collections #2 + #3).

Central protein	Number of edges	Number of proteins	p-value
5HT1A_HUMAN	22683	274	<1e-6
5HT1B_HUMAN	22523	271	<1e-6
5HT1D_HUMAN	22678	271	<1e-6
5HT1E_HUMAN	22678	271	<1e-6
5HT1F_HUMAN	22678	271	<1e-6
5HT5A_HUMAN	18182	197	<1e-6
A4_HUMAN	28148	493	<1e-6
AA1R_HUMAN	22694	273	<1e-6
AA3R_HUMAN	17537	188	<1e-6
ACKR3_HUMAN	18290	205	<1e-6
ACM2_HUMAN	18149	198	<1e-6
ACM4_HUMAN	18161	200	<1e-6
ADA2A_HUMAN	18266	205	<1e-6
ADA2B_HUMAN	17346	197	<1e-6
ADA2C_HUMAN	18191	199	<1e-6
AGTR2_HUMAN	18114	199	<1e-6
ANGT_HUMAN	27009	316	<1e-6
ANXA1_HUMAN	27187	343	<1e-6
APEL_HUMAN	17911	191	<1e-6
APJ_HUMAN	18060	191	<1e-6
BKRB1_HUMAN	26745	299	<1e-6
BKRB2_HUMAN	27009	310	<1e-6
C3AR_HUMAN	18060	191	<1e-6
C5AR1_HUMAN	18078	193	<1e-6
CASR_HUMAN	31463	383	<1e-6
CCL15_HUMAN	25442	290	<1e-6

CCL16_HUMAN	17537	188	<1e-6
CCL19_HUMAN	18073	193	<1e-6
CCL20_HUMAN	18075	196	<1e-6
CCL21_HUMAN	17922	193	<1e-6
CCL23_HUMAN	26982	299	<1e-6
CCL25_HUMAN	18064	192	<1e-6
CCL27_HUMAN	18060	191	<1e-6
CCL28_HUMAN	18060	191	<1e-6
CCL5_HUMAN	17947	199	<1e-6
CCR10_HUMAN	18074	192	<1e-6
CCR1_HUMAN	17962	199	<1e-6
CCR2_HUMAN	18105	198	<1e-6
CCR3_HUMAN	18118	201	<1e-6
CCR4_HUMAN	18118	204	<1e-6
CCR5_HUMAN	18162	213	<1e-6
CCR6_HUMAN	18099	197	<1e-6
CCR7_HUMAN	18095	196	<1e-6
CCR8_HUMAN	18094	195	<1e-6
CCR9_HUMAN	18093	194	<1e-6
CD19_HUMAN	161	32	<1e-6
CD81_HUMAN	129	52	<1e-6
CFAB_HUMAN	37	13	<1e-6
CFAD_HUMAN	4	4	<1e-6
CFAH_HUMAN	13	12	<1e-6
CFAI_HUMAN	30	11	<1e-6
CNR1_HUMAN	22684	276	<1e-6
CNR2_HUMAN	18060	191	<1e-6
CO2_HUMAN	9723	166	<1e-6
CO3_HUMAN	17993	217	<1e-6

CO4A_HUMAN	9666	156	<1e-6
CO4B_HUMAN	9665	155	<1e-6
CO5_HUMAN	18115	205	<1e-6
COLI_HUMAN	22896	290	<1e-6
CR1L_HUMAN	24	8	<1e-6
CR1_HUMAN	26	9	<1e-6
CR2_HUMAN	17	11	<1e-6
CXCL2_HUMAN	17937	195	<1e-6
CXCL3_HUMAN	17927	193	<1e-6
CXCL5_HUMAN	18062	192	<1e-6
CXCL6_HUMAN	18068	195	<1e-6
CXCL7_HUMAN	18062	192	<1e-6
CXCL9_HUMAN	17929	193	<1e-6
CXCR1_HUMAN	17355	187	<1e-6
CXCR2_HUMAN	18081	198	<1e-6
CXCR3_HUMAN	18080	193	<1e-6
CXCR4_HUMAN	18267	224	<1e-6
CXCR5_HUMAN	18211	199	<1e-6
CXCR6_HUMAN	18074	192	<1e-6
CXL10_HUMAN	18096	198	<1e-6
CXL11_HUMAN	17921	193	<1e-6
CXL13_HUMAN	18065	193	<1e-6
CXL16_HUMAN	18060	191	<1e-6
DAF_HUMAN	2378	80	<1e-6
DRD2_HUMAN	18453	228	<1e-6
DRD3_HUMAN	18127	209	<1e-6
DRD4_HUMAN	18232	208	<1e-6
FPR1_HUMAN	17922	193	<1e-6
FPR2_HUMAN	26982	299	<1e-6

FPR3_HUMAN	25833	291	<1e-6
GABR1_HUMAN	23235	315	<1e-6
GABR2_HUMAN	18370	225	<1e-6
GALA_HUMAN	18062	192	<1e-6
GALR1_HUMAN	18060	191	<1e-6
GALR2_HUMAN	18060	191	<1e-6
GALR3_HUMAN	17714	190	<1e-6
GBB1_HUMAN	36904	891	<1e-6
GBG2_HUMAN	32162	433	<1e-6
GNAI1_HUMAN	18437	241	<1e-6
GNAI2_HUMAN	18551	261	<1e-6
GNAI3_HUMAN	18430	239	<1e-6
GNAT1_HUMAN	15680	223	<1e-6
GNAT2_HUMAN	15672	219	<1e-6
GNAT3_HUMAN	18523	242	<1e-6
GPER1_HUMAN	18088	194	<1e-6
GPR17_HUMAN	26982	299	<1e-6
GPR18_HUMAN	18060	191	<1e-6
GPR55_HUMAN	18060	191	<1e-6
GRM2_HUMAN	18148	200	<1e-6
GRM3_HUMAN	18142	198	<1e-6
GRM4_HUMAN	18095	198	<1e-6
GRM6_HUMAN	18080	195	<1e-6
GRM7_HUMAN	18173	210	<1e-6
GRM8_HUMAN	18129	207	<1e-6
GROA_HUMAN	17934	196	<1e-6
HCAR1_HUMAN	18060	191	<1e-6
HCAR2_HUMAN	18110	201	<1e-6
HCAR3_HUMAN	18110	201	<1e-6

HEBP1_HUMAN	18086	194	<1e-6
HRH3_HUMAN	18100	194	<1e-6
HRH4_HUMAN	18060	191	<1e-6
HUNIN_HUMAN	25682	293	<1e-6
IFM1_HUMAN	19	10	<1e-6
IFM2_HUMAN	15	9	<1e-6
IFM3_HUMAN	28	14	<1e-6
IL8_HUMAN	17769	203	<1e-6
INSL5_HUMAN	17909	190	<1e-6
KNG1_HUMAN	27049	321	<1e-6
LPAR1_HUMAN	27015	305	<1e-6
LPAR2_HUMAN	26994	304	<1e-6
LPAR3_HUMAN	26982	299	<1e-6
LPAR5_HUMAN	26982	299	<1e-6
MCHR1_HUMAN	26983	300	<1e-6
MCHR2_HUMAN	26512	296	<1e-6
MCH_HUMAN	26982	299	<1e-6
MCP_HUMAN	55	20	<1e-6
MTR1A_HUMAN	17994	210	<1e-6
MTR1B_HUMAN	22672	285	<1e-6
NMS_HUMAN	26982	299	<1e-6
NMUR1_HUMAN	26336	295	<1e-6
NMUR2_HUMAN	26982	299	<1e-6
NMU_HUMAN	26740	298	<1e-6
NPBW1_HUMAN	18060	191	<1e-6
NPBW2_HUMAN	17355	187	<1e-6
NPB_HUMAN	18060	191	<1e-6
NPW_HUMAN	17909	190	<1e-6
NPY1R_HUMAN	18085	192	<1e-6

NPY2R_HUMAN	18085	192	<1e-6
NPY4R_HUMAN	18085	192	<1e-6
NPY5R_HUMAN	17934	191	<1e-6
NPY_HUMAN	18103	195	<1e-6
OPRD_HUMAN	18280	209	<1e-6
OPRK_HUMAN	22667	275	<1e-6
OPRM_HUMAN	22882	298	<1e-6
OPRX_HUMAN	22628	270	<1e-6
OXER1_HUMAN	17537	188	<1e-6
OXGR1_HUMAN	18060	191	<1e-6
P2RY4_HUMAN	18195	197	<1e-6
P2Y12_HUMAN	18263	202	<1e-6
P2Y13_HUMAN	18263	202	<1e-6
P2Y14_HUMAN	18263	202	<1e-6
PAHO_HUMAN	18060	191	<1e-6
PD2R2_HUMAN	18066	194	<1e-6
PDYN_HUMAN	18093	193	<1e-6
PE2R3_HUMAN	18176	198	<1e-6
PENK_HUMAN	17910	191	<1e-6
PF4V_HUMAN	15926	180	<1e-6
PLF4_HUMAN	17961	203	<1e-6
PNOC_HUMAN	18060	191	<1e-6
PROP_HUMAN	6	6	<1e-6
PYY_HUMAN	17921	193	<1e-6
REL3_HUMAN	22692	281	<1e-6
RL3R1_HUMAN	18060	191	<1e-6
RL3R2_HUMAN	17909	190	<1e-6
S1PR1_HUMAN	22679	278	<1e-6
S1PR2_HUMAN	22676	275	<1e-6

S1PR3_HUMAN	18063	192	<1e-6
S1PR4_HUMAN	18065	193	<1e-6
S1PR5_HUMAN	22624	272	<1e-6
SAA1_HUMAN	26114	300	<1e-6
SDF1_HUMAN	18078	195	<1e-6
SMS_HUMAN	18061	192	<1e-6
SSR1_HUMAN	18074	195	<1e-6
SSR2_HUMAN	18085	199	<1e-6
SSR3_HUMAN	18154	213	<1e-6
SSR4_HUMAN	17922	193	<1e-6
SSR5_HUMAN	22633	273	<1e-6
SUCR1_HUMAN	18192	196	<1e-6
T2R10_HUMAN	18060	191	<1e-6
T2R13_HUMAN	17937	193	<1e-6
T2R14_HUMAN	17909	190	<1e-6
T2R16_HUMAN	17909	190	<1e-6
T2R19_HUMAN	18060	191	<1e-6
T2R20_HUMAN	18060	191	<1e-6
T2R30_HUMAN	18060	191	<1e-6
T2R31_HUMAN	18060	191	<1e-6
T2R38_HUMAN	18060	191	<1e-6
T2R39_HUMAN	18060	191	<1e-6
T2R40_HUMAN	18060	191	<1e-6
T2R41_HUMAN	18060	191	<1e-6
T2R42_HUMAN	18060	191	<1e-6
T2R43_HUMAN	18060	191	<1e-6
T2R45_HUMAN	17205	186	<1e-6
T2R46_HUMAN	18060	191	<1e-6
T2R50_HUMAN	18060	191	<1e-6

T2R60_HUMAN	17909	190	<1e-6
TA2R1_HUMAN	17713	189	<1e-6
TA2R3_HUMAN	18060	191	<1e-6
TA2R4_HUMAN	17909	190	<1e-6
TA2R5_HUMAN	17537	188	<1e-6
TA2R7_HUMAN	18060	191	<1e-6
TA2R8_HUMAN	18060	191	<1e-6
TA2R9_HUMAN	18060	191	<1e-6
TF65_HUMAN	4022	343	<1e-6
VSIG4_HUMAN	1	2	0.00001
ITAX_HUMAN	357	33	0.00046
CBPN_HUMAN	1	2	0.00463
FHR3_HUMAN	1	2	0.00469
FHR5_HUMAN	1	2	0.00478
FHR4_HUMAN	1	2	0.00482
ITB2_HUMAN	1132	106	0.02148
HNF4A_HUMAN	498	82	0.02458

Supplemental Table 8. Ingenuity Pathway Analysis of C3 Interacting Partners. The *Ratio* indicates the number of expressed genes that maps to the pathway divided by the total number of genes that map to the canonical pathway.

Ingenuity Canonical Pathways	-log(p-value)	Ratio	Molecules
Gαi Signaling	74,2	0,442	ADRA2B,GRM3,RXFP4,GNB1,CHRM2,HTR1B,HTR1E,CNR2,GRM6,APLNR,OPRM1,GRM8,CNR1,SSTR3,GNG2,GRM4,DRD2,FPR1,S1PR3,GRM7,ADRA2A,AGTR2,GPR17,GRM2,PTGER3,CHRM4,ADORA3,HTR1D,HRH3,OPRL1,CXCR2,DRD4,ADORA1,DRD3,HCAR2,NPY1R,GNAI1,FPR2,GABBR1,GNAI2,GNAI3,OPRD1,P2RY13,GABBR2,P2RY14,LPAR1,CCR4,OPRK1,S1PR1,P2RY12,ADRA2C,HTR1F,HTR1A
cAMP-mediated signaling	59,1	0,244	ADRA2B,HCAR3,GRM3,RXFP4,CHRM2,HTR1B,HTR1E,CNR2,GRM6,APLNR,OPRM1,GRM8,CNR1,SSTR3,GRM4,DRD2,FPR1,S1PR3,GRM7,ADRA2A,AGTR2,GPR17,GRM2,HTR5A,PTGER3,CHRM4,ADORA3,HTR1D,HRH3,OPRL1,CXCR2,DRD4,DRD3,ADORA1,HCAR2,NPY1R,FPR2,GNAI1,GABBR1,GNAI2,GNAI3,OPRD1,P2RY13,GABBR2,GPER1,P2RY14,LPAR1,CCR4,OPRK1,S1PR1,P2RY12,ADRA2C,HTR1F,HTR1A
G-Protein Coupled Receptor Signaling	55,5	0,204	ADRA2B,HCAR3,GRM3,RXFP4,CHRM2,HTR1B,HTR1E,CNR2,GRM6,APLNR,OPRM1,GRM8,CNR1,SSTR3,GRM4,DRD2,FPR1,S1PR3,GRM7,ADRA2A,AGTR2,RELA,GPR17,GRM2,HTR5A,PTGER3,CHRM4,ADORA3,HTR1D,HRH3,OPRL1,CXCR2,DRD4,DRD3,ADORA1,HCAR2,NPY1R,FPR2,GNAI1,GABBR1,GNAI2,GNAI3,OPRD1,P2RY13,GABBR2,GPER1,P2RY14,CCR4,LPAR1,OPRK1,S1PR1,P2RY12,ADRA2C,HTR1F,HTR1A
Granulocyte Adhesion and Diapedesis	37,4	0,209	FPR3,CXCL12,CCL20,CCL5,CXCL5,CXCL9,HRH3,CXCL10,CXCL3,CXCL13,CXCR2,CCL28,CCL25,CXCL1,CCL16,CCL15,CCL19,CXCL8,CXCL11,C5AR1,CCL23,CXCR4,PF4,PPBP,GNAI1,FPR2,CXCL6,C5,FPR1,GNAI2,ITGB2,GNAI3,CXCL16,CCL27,CCL21,CXCL2,HRH4
Gustation Pathway	35,7	0,262	GNAT3,TAS2R9,TAS2R41,TAS2R31,TAS2R16,TAS2R30,TAS2R10,TAS2R39,TAS2R8,TAS2R3,GNB1,TAS2R38,TAS2R5,TAS2R43,TAS2R1,TAS2R46,P2RY4,TAS2R50,TAS2R19,TAS2R60,GNG2,TAS2R42,TAS2R7,TAS2R4,TAS2R13,P2RY13,P2RY14,TAS2R45,TAS2R40,P2RY12,TAS2R20,TAS2R14
Agranulocyte Adhesion and Diapedesis	30,5	0,175	CXCL12,CCL20,CCL5,CXCL5,CXCR1,CXCL9,CXCL10,CXCL3,CXCL13,CXCR2,CCL28,CCL25,CXCL1,CCL16,CCL15,CCL19,CXCL8,CXCL11,C5AR1,CXCR4,CCL23,PF4,PPBP,GNAI1,CXCL6,C5,GNAI2,ITGB2,GNAI3,CXCL16,CCL27,CCL21,CXCL2

Complement System	22,1	0,432	CFD,C5AR1,C3,CD46,C5,C4A/C4B,ITGB2,CR1,CD55,CFB,CFI,CFH,C3AR1,C2,ITGAX,CR2
Pathogenesis of Multiple Sclerosis	12,4	0,778	CXCL10,CCR1,CXCL11,CCR5,CXCR3,CCL5,CXCL9
Role of IL-17A in Psoriasis	8,77	0,462	CXCL8,CXCL3,CCL20,CXCL1,CXCL5,CXCL6
GPCR-Mediated Integration of Enteroendocrine Signaling Exemplified by an L Cell	8,52	0,139	GNAI2,GNAI3,CHRM2,GAL,SSTR5,GNAI1,NPY2R,SST,GALR1,PYY
IL-17A Signaling in Gastric Cells	8,37	0,280	CXCL10,CXCL8,RELA,CXCL11,CCL20,CXCL1,CCL5
Ephrin B Signaling	7,26	0,123	GNAI2,GNB1,GNAI3,GNAT1,CXCR4,GNG2,CXCL12,GNAI1,GNAT2
CREB Signaling in Neurons	7,26	0,071	GNAI2,GRM7,GNB1,GNAI3,GRM2,GNAT1,GRM8,GRM3,GNG2,GNAI1,GRM6,GNAT2,GRM4
Glutamate Receptor Signaling	6,96	0,140	GRM7,GNB1,GRM2,GRM8,GRM3,GNG2,GRM6,GRM4
IL-8 Signaling	6,91	0,066	RELA,CXCL8,GNG2,GNAI1,CXCR1,GNB1,GNAI2,GNAI3,ITGB2,CXCR2,CXCL1,CR2,ITGAX
Synaptic Long Term Depression	6,51	0,075	GNAI2,GRM7,GNAI3,GRM2,GNAT1,GRM8,GRM3,GNAI1,GRM6,GNAT2,GRM4
Role of IL-17A in Arthritis	6,4	0,119	CXCL8,RELA,CXCL3,CCL20,CXCL1,CXCL5,CCL5,CXCL6
Chemokine Signaling	6,21	0,113	GNAI2,CCR3,GNAI3,CCR5,CXCR4,CXCL12,GNAI1,CCL5
Neuroprotective Role of THOP1 in Alzheimer's Disease	5,52	0,150	KNG1,PDYN,SST,PNOC,AGT,APP
IL-1 Signaling	5,38	0,088	GNAI2,GNB1,GNAI3,RELA,GNAT1,GNG2,GNAI1,GNAT2
fMLP Signaling in Neutrophils	5,38	0,074	GNAI2,GNB1,FPR3,GNAI3,RELA,GNG2,GNAI1,FPR2,FPR1
Role of IL-17F in Allergic Inflammatory Airway Diseases	5,27	0,136	CXCL10,CXCL8,RELA,CXCL1,CXCL5,CXCL6
CCR5 Signaling in Macrophages	5,19	0,101	GNAI2,GNB1,GNAI3,CCR5,GNG2,GNAI1,CCL5

Gαs Signaling	4,8	0,073	GNB1,HCAR3,GPER1,HTR5A,CNR1,OPRK1,GNG2,HCAR2
Androgen Signaling	4,74	0,072	GNAI2,GNB1,GNAI3,RELA,GNAT1,GNG2,GNAI1,GNAT2
Hepatic Fibrosis / Hepatic Stellate Cell Activation	4,72	0,055	CXCL8,RELA,CXCL3,CCR5,CCL21,CXCR3,CCL5,CXCL9,CCR7,AGT
Relaxin Signaling	4,62	0,060	GNAI2,GNB1,GNAI3,RELA,GNAT1,GNG2,GNAI1,GNAT2,RLN3
GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells	4,59	0,082	GNAI2,GNAI3,CASR,GNG2,LPAR5,GNAI1,PYY
G Beta Gamma Signaling	4,49	0,080	GNAI2,GNB1,GNAI3,GNAT1,GNG2,GNAI1,GNAT2
Sphingosine-1-phosphate Signaling	4,44	0,066	S1PR4,GNAI2,S1PR3,GNAI3,S1PR2,S1PR5,GNAI1,S1PR1
CXCR4 Signaling	4,3	0,055	GNAI2,GNB1,GNAI3,GNAT1,CXCR4,GNG2,CXCL12,GNAI1,GNAT2
P2Y Purigenic Receptor Signaling Pathway	4,2	0,061	GNAI2,GNB1,GNAI3,RELA,P2RY4,GNG2,GNAI1,P2RY12
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	4,13	0,116	CXCL10,CCR1,CXCL8,CCR5,CCL5
Serotonin Receptor Signaling	4,13	0,116	HTR1B,HTR5A,HTR1E,HTR1A,HTR1D
Ephrin Receptor Signaling	4,12	0,052	GNAI2,GNB1,GNAI3,GNAT1,CXCR4,GNG2,CXCL12,GNAI1,GNAT2
Role of PI3K/AKT Signaling in the Pathogenesis of Influenza	3,9	0,079	GNAI2,GNAI3,RELA,CCR5,GNAI1,CCL5
IL-17A Signaling in Airway Cells	3,87	0,078	RELA,CXCL3,CCL20,CXCL1,CXCL5,CXCL6
Cardiac Hypertrophy Signaling	3,81	0,043	ADRA2B,GNAI2,GNB1,GNAI3,GNAT1,ADRA2A,GNG2,GNAI1,GNAT2,ADRA2C
IL-17 Signaling	3,63	0,071	CXCL10,CXCL8,RELA,CXCL11,CXCL1,CXCL5
α-Adrenergic Signaling	3,58	0,069	GNAI2,GNB1,GNAI3,ADRA2A,GNG2,GNAI1

Supplemental References

1. Eizirik DL, Sammeth M, Bouckenooghe T, Bottu G, Sisino G, Igoillo-Esteve M, Ortis F, Santin I, Colli ML, Barthson J, Bouwens L, Hughes L, Gregory L, Lunter G, Marselli L, Marchetti P, McCarthy MI, Cnop M. The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. PLoS Genet 2012 8:e1002552

2. Marhfour I, Lopez XM, Lefkaditis D, Salmon I, Allagnat F, Richardson SJ, Morgan NG, Eizirik DL. Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. Diabetologia 2012 55:2417-20