SUPPLEMENTAL DATA for the manuscript:

OVEREXPRESSION OF SPHINGOSINE-1 PHOSPHATE LYASE PROTECTS INSULIN-SECRETING CELLS AGAINST CYTOKINE TOXICITY

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This file contains the following supplementary data:

Table S1, Table S2, Table S3 and Table S4

Table S1. Effects of cytokines on the mRNA level of the most abundant S1P receptor and transporter types in insulin-secreting INS1E cells.

Gene		untreated	IL-1β	Cytokine mixture
S1Pr2	6 h	100 ± 20 (4)	157 ± 33 (4)	268 ± 62 (4)
	24 h	100 ± 22 (3)	463 ± 127 (3)*	375 ± 107 (3)*
S1Pr3	6 h	100 ± 22 (4)	33 ± 12 (4)**	33 ± 15 (4)**
	24 h	100 ± 20 (6)	204 ± 71 (6)*	190 ± 51 (6)**
S1Pr5	6 h	100 ± 10 (4)	95 ± 24 (4)	116 ± 12 (4)
	24 h	99 ± 10 (6)	175 ± 24 (6)**	194 ± 45 (6)*
abca1	6 h	100 ± 7 (4)	71 ± 7 (4)*	53 ± 5 (4)**
	24 h	100 ± 10 (6)	236 ± 28 (6)**	289 ± 27 (6)***

Total RNA was isolated and after reverse transcription real-time RT-PCR was performed. Expression was normalized to the house-keeping gene β-actin. Shown are values as % of untreated. Data are means ± SEM, with the number of experiments indicated in parentheses. *p<0.05, **p<0.01, ***p<0.001 vs. untreated, ANOVA followed by Bonferroni.

Table S2. Concentration-dependent effects of S1P on cell viability in insulin-secreting INS1E cells

S1P [µM]	Cell viability
	24 h
0	100 ± 2 (5)
0.5	94 ± 3 (4)
5	92 ± 3 (4)
10	72 ± 8 (4)
25	47 ± 13 (4)

Cell viability was measured after a 24 h incubation with various concentrations of S1P by means of the MTT assay. Shown are values as % of untreated. Data are means ± SEM, with the number of experiments indicated in parentheses. *$p<0.05$ vs. untreated, ANOVA followed by Bonferroni.

Table S3. Gene expression of enzymes of the sphingolipid pathway in insulin-secreting INS1E cells.

Gene	relative gene expression			
CerS1	1.6E-04	±	4.2E-05	(7)
CerS2	1.9E-02	±	2.6E-03	(8)
CerS3	5.4E-06	±	2.2E-06	(8)
CerS4	9.3E-08	±	4.2E-08	(7)
CerS5	3.4E-02	±	3.8E-03	(8)
spp1	2.0E-04	±	3.6E-05	(8)
spp2	1.2E-03	±	2.6E-04	(8)
sk1	1.0E-04	±	1.5E-05	(11)
sk2	6.3E-03	±	1.5E-03	(8)
CDase acid	1.4E-02	±	2.9E-03	(12)
CDase neutral	5.5E-03	±	1.2E-03	(9)
spl	6.9E-03	±	6.5E-04	(10)
SPT lc1	2.2E-02	±	3.1E-03	(3)
SPT lc2	8.0E-03	±	2.1E-03	(4)
SPT sc	2.8E-05	±	1.0E-05	(4)
SMS acid	1.5E-02	±	2.8E-03	(4)
SMS alkaline	1.7E-05	±	1.3E-06	(3)
SMase	1.2E-03	±	3.0E-04	(7)
Aldh3a2	2.6E-03	±	2.2E-04	(15)

Gene expression was measured in untreated insulin-secreting INS1E cells by means of real-time RT-PCR and normalized to the house-keeping gene β-actin, using the QuantiTect SYBR Green™ technology (QIAGEN, Hilden, Germany) based on a fluorescent dye that binds only double-stranded DNA. The reactions were performed using the DNA Engine Opticon™ Sequence Detection System (Biozym Diagnostik, Hess. Oldendorf, Germany). Data are means ± SEM, with the number of experiments indicated in parentheses.

Table S4 Primers used in real-time RT-PCR analysis (all from Invitrogen)

Gene	FW	REV
S1Pr1	CATCTGCTGCTTGATCATCC	AGCCACTCCTGCTAACAGGT
S1Pr2	CCTTCGTGGCCAACACCTT	ATGGCCAGGAGGCTGAA
S1Pr3	ATCTTGCCCCTCTACTCCAA	GGATCTCTCGGAGTTGTGGT
S1Pr4	TATGGCTGCATCGGTCTGT	AGAGCACATAGCCCTTGGAG
S1Pr5	TGTGCGCTTTCATCGTGC	AGGATGTTGGTGGCGTAGG
Abca1	CCCTCCTGGAGAGTGCTTTG	CTGGTCACAGCGGTATCTCC
Abcc1	GATGGGGCCTTTGCTGAGTT	CCCTAAACCACTGACACCATTC
Abcg2	ATGTTAGGACTGAAGAGGACGG	AGACACTACGCTTTGGCCTG
Aldh3a2	CTCCCAACAGCGAGTCCAAG	ATCACAGCTGATCCTTGACGA
Spns2	CCTCATGCTGTGCCCTTTTG	CTCGTCAGCCTGCTGCTC
Cer Synthase1	TACCCTTTCTTCCATGACCC	CGGTGGCATAGACGGAAT
Cer Synthase2	GCTTGCTTTCTACTGGTCCC	CTGCTCGGACATAATTGGCA
Cer Synthase3	TACGGACTGGCAAAGAAGTG	CAGCCACCGTCATCATGAAA
Cer Synthase4	TGCTGCCTTGCCAATTG	GCACTGGGTTGGGCTTTATC
Cer Synthase5	GCTGGCAGCAGGTCTCTTCT	CCCTTCAGCCTTTTCTCATC
Alkaline CDase	ACAACAACACTTGGGTGGTG	ATCCTGTCAACATGCCCAC
Neutral CDase	ACTCTGGCCCAGCAGGATT	GGACTTTGCCCGGTTTAAGA
Alkaline SMS	TGACTGGGGGTCCTGTCCAA	GCAGGAGTCATGTAGCGAGC
Acid SMS	TCTTTGAGGACGATGTGGTG	GCACTGATGGCAAAGAGATG
sk1	CTTCTGGAGGAGGCTGAGGT	TCAGACCGTCACCGGACAT
sk2	CAAGCCCTACACATACAGCG	GCCACGTGGGTAGGTGTAGA
spp1	CGAGCTGGCCAAAGTGAGCA	AGATGATCACCAGCCTCCGG
spp2	TTCTCCCATTCACCCACTG	TCACAACGGGAGGAGAGGAG
spl	GCCATTCCTGAAGTTGGACA	CCTGAGGCTTTCCCTTCTTG
huSPL	ACGGCCTGGTGGCATTA	CTGACAATTGGGGATTCCC

SpMn	GGCTGCTGGTGCTCCATCTA	TGGATGAACTGGGCCAGTT
SPTlc1	TTGAAGAATGGCAGCCAGAG	CCGAGGAAATTAAAGGAGGC
SPTlc2	GAAAAGTGCCACCATGCAAC	ACAGATGGGTCGATTCCAGT
SPTsc	GAAGGAGTATTTTGCCTGGC	GGGGATGAAGACATAGGCAG
bip	CCACCAGGATGCAGACATTG	AGGGCCTCCACTTCCATAGA
chop	CCAGCAGAGGTCACAAGCAC	CGCACTGACCACTCTGTTTC
β-actin	GAACACGGCATTGTAACCAACTGG	GGCCACACGCAGCTCATTGTA