Electronic Supplemental Tables

ESM Table 1

Genes	**Forward primer (5'→3')**	**Reverse Primer (5→3')**
Mfn1	TGGTAATCTTTAGCGGTGCTC	GGAGGACTTTATCCCACAGC
Mfn2	TTTGGAAGTAGGCAGTCTCCA	CAGGCAGCACTGAAAAGAGA
Pdx1-Cre^{ERT2}	CAGGCGTTTTCTGAGCATACC	CCGGTTATTCAACTTGCACCAT

Sequence of primers used for genotyping *Mfn1* and *Mfn2* flox.

ESM Table 2

Genes	Forward primer (5'→3')	Reverse Primer (5→3')
Mfn1	GCATTTTTTGGCAGGACAAGTAG	GGAGGACTTTATCCCACAGCAT
Mfn2	AGAAGAGTGTCAAGACTGTGAACCA	GCTGCCTGCATGCAACTG
Drp1	TCAGATCGTCGTAGTGGGAA	TCTTCTGGTGAAACGTGGAC
Opa1	ATACTGGGATCTGCTGTTGG	AAGTCAGGCACAATCCACTT
Fis1	AAGTATGTGCGAGGGCTGT	TGCCTACCAGTCCATCTTTC
Ins2	TGGCTTCTTCTACACACCCATGTCCC	ACTGATCTACAATGCCACGCTTCTGCT
Slc2a2	GCAACTGGGTCTGCAATTTTG	CAAGGAAGTCCGCAATGTACTG
Gck	TGGTGGATGAGAGCTCAGTGAA	CATGTACTTTCCGCCAATGATC
Pdx1	CCAAAGCTCACGCGTGGA	TGTTTTCCTCGGGTTCCG
Nkx6.1	GCCTGTACCCCCCATCAAG	GTGGGTCTGGTGTGTTTTCTCTT
Pax6	GCACATGCAAACACACATGAAC	GGTGAAATGAGTCCTGTTGAAGTG
Ucn3	GCTGTGCCCCTCGACCT	TGGGCATCAGCATCGCT
Glp1r	CCCTGGGCCAGTAGTGTG	GCAGGCTGGAGTTGTCCTTA
Nkx2.2	CCTCCCCGAGTGGCAGAT	GAGTTCTATCCTCTCCAAAAGTTCAAA
Gcg	TCACAGGGCACATTCACCAG	CATCATGACGTTTGGCAATGTT
Arx	TCCGGATACCCCACTTAGCTT	GACGCCCCTTTCCTTTAAGTG
Mafa	CTTCAGCAAGGAGGAGGTCATC	CGTAGCCGCGGTTCTTGA
Mafb	TGAATTTGCTGGCACTGCTG	AAGCACCATGCGGTTCATACA
Vdac1	GCTAAGGATGACTCGGCTTTAAGG	AGGTTAAGTGATGGGCTAGGATGG
Vdac2	TCACTGTTGGCTGGTTCCTAGTTG	AAGACCTCGTGGATTATGCTAGGG
Vdac3	CACTTGTCCCTGGAAATGAAGAG	CATGACACTACGTTGTTGCTGAGG
Letm1	TCCTGCGTTTCCAGCTCACCAT	GTCTTCTGTGACACCGAGAGCT
Slc8a1	CCGTGACTGCCGTTGTGTT	GCCTATAGACGCATCTGCATACTG
Trpm5	CCAGCATAAGCGACAACATCT	GAGCATACAGTAGTTGGCCTG
Beclin-1	TGGAAGGGTCTAAGACGT	GGCTGTGGTAAGTAATGGA
Lc3	CACTGCTCTGTCTTGTGTAGGTTG	TCGTTGTGCCTTTATTAGTGCATC
Bnip3	TTCCACTAGCACCTTCTGATGA	GAACACCGCATTTACAGAACAA
p62	CCCAGTGTCTTGGCATTCTT	AGGGAAAGCAGAGGAAGCTC

GabarapL	CATCGTGGAGAAGGCTCCTA	ATACAGCTGGCCCATGGTAG
CathepsinL	GTGGACTGTTCTCACGCTCAAG	TCCGTCCTTCGCTTCATAGG
Pink1	TGAGGAGCAGACTCCCAGTT	AGTCCCACTCCACAAGGATG
Parkin	TGGAAAGCTCCGAGTTCAGT	CCTTGTCTGAGGTTGGGTGT
Atf4	GCAGTGTTGCTGTAACGGACA	CGCTGTTCAGGAAGCTCATCT
Atf6a	GACTCACCCATCCGAGTTGTG	CTCCCAGTCTTCATCTGGTCC
Bip	AGGACAAGAAGGAGGATGTGGG	ACCGAAGGGTCATTCCAAGTG
Chop2	CCACCACACCTGAAAGCAGAA	AGGTGAAAGGCAGGGACTCA
Xbp1	TGGCCGGGTCTGCTGAGTCCG	GTCCATGGGAAGATGTTCTGG
Xbp1s	CTGAGTCCGAA TCAGGTGCAG	GTCCATGGGAAGATGTT CTGG
Cx36	CAGCAGCACTCCACTATGATTG	GTACACCGTCTCCCCTACAA
Ldha	ATGAAGGACTTGGCGGATGA	ATCTCGCCCTTGAGTTTGTCTT
Slc16a1	GCTTGGTGACCATTGTGGAAT	CCCAGTACGTGTATTTGTAGTCTCCAT
Pdgfra	GACCCTGTTCCAGAGGAGGAA	TTCCGAAGTCTGTGAGCTGTGT
Aldh1a3	GGGCCTCAGATCGACCAAAA	CTAGCTTGGCCCCTTCCTTC
Hsd11b	GGAGCCGCACTTATCTGA	TGCCATTTCTCTTCCAATC
Mt9/mt11	GAGCATCTTATCCACGCTTCC	GGTGGTACTCCCGCTGTAAA
Ndufv1	CTTCCCCACTGGCCTCAAG	CCAAAACCCAGTGATCCAGC
Epac1a	GGACAAAGTCCCCTACGACA	CTTGGTCCAGTGGTCCTCAT
Epac2a	TGGAACCAACTGGTATGCTG	CCAATTCCCAGAGTGCAGAT
Epac2b	TCTTTGCTACCTGGGACTGG	AGCAGCCAGCCTTTATCTGA
Adcy3	GTGCTATCATCGTGGGCATC	TCCTTCAGCATCTCGTCAGC
Adcy5	GCCAATGCCATAGACTTCAG	ATCTCCTCCTTCTCTTCTGTG
Adcy6	TAAATGCCAGCACCTATGACC	TGTTCAACCCGA TCTTCA TCTG
Adcy8	TTGGGCTTCCTACACCTTGACT	CGGTAGCTGTATCCTCCATTGAG
Prkar1a	ATGGCGTCTGGCAGTATGG	GCTGCACGATGGAGTCCTTC
Prkar1b	TCTGAAAGGATGCGAGATGTACG	CTGGGAGTTTGACTTCTGCCG
Prkar2a	GAGGAGGATAACGATCCAAGGG	TGCTCGTCAGTTTTGACAATCTT
Prkaca	AGATCGTCCTGACCTTTGAGT	GGCAAAACCGAAGTCTGTCAC
Prkacb	CTCGGGACGGGTTCCTTTG	AGGGACGTATTCCATAACCATGT

β-actin	CGAGTCGCGTCCACCC	CATCCATGGCGAACTGGTG

List of primers used for qRT-PCR.

ESM Table 3

Antibody	Species	Vendor	Catalog number	Dilution/conc	RRID
anti-MFN1	mouse	Abcam	ab126575	1 in 500	RRID:AB_11141234
anti-MFN2	mouse	Abcam	ab56889	1 in 500	RRID:AB_2142629
anti-GAPDH	goat	Cell signalling	#2118s	1 in 10 000	RRID:AB_561053
anti-HRP	goat	Abcam	ab205719	1 in 5 000	RRID:AB_2755049
β*Mfn1/2* dKO IHC					
anti-Insulin	guinea pig	Agilent	#A0564	1 in 500	RRID:AB_10013624
anti-glucagon	mouse	Sigma-Aldrich	G2654	1 in 1 000	RRID:AB_259852
Alexa Fluor 488	goat	Thermo Fisher	#A-11073	1 in 1 000	RRID:AB_2534117
Alexa Fluor 568	goat	Thermo Fisher	#A-11004	1 in 1 000	RRID:AB_2534072
Pdx1CreER IHC					
anti-Insulin	guinea pig	Agilent	#A0564,	1 in 2 000	RRID:AB_10013624
anti-glucagon	rabbit	Abcam	ab92517	1 in 300	RRID:AB_10561971
anti-somatostatin	rabbit	Abcam	ab111912	1 in 1 000	RRID:AB_10903864
Alexa Fluor 488	donkey	Jackson Immuno	#711-545-152	1 in 1 000	RRID:AB_2313584
Alexa Fluor 568	donkey	Jackson Immuno	#706-165-148	1 in 1 000	RRID:AB_2340460

List of antibodies used in immunohistochemistry (IHC) experiments.

ESM Table 4

Condition	Ctrl Average	±SEM	dKO Average	±SEM
3G	225.130794	31.9313528	137.3442788	16.5058023
10G	350.937357	99.2151864	238.6717601	47.844241
17G	1007.68015	144.488618	302.4013885	79.2604199
EX4	1019.23968	204.149417	820.6378323	21.4037722
GLP1	982.176667	3.3427251	833.1233333	55.4545809
GIP	927.603	3.5275689	732.67	20.5050002
FSK	864.981483	8.97886477	783.514548	31.6922934
IBMX	866.672401	23.8144433	711.4497483	15.3320754
KCl	657.561878	107.611853	284.9369204	51.8648557
3G+30mM KCl	238.214722	103.220044	196.2304897	8.12945181
17G+30mM KCl	920.67843	30.819347	907.6114857	5.17694852
10G+H89	513.305815	157.535336	808.3442542	15.0070073
IBMX/FSK	1312.07246	152.327607	1161.305076	13.6682706
IBMX/FSK/H89	1641.45142	83.8140161	1314.467588	173.287437
10G+activ	918.086618	52.9726612	870.8674045	53.8147534
10G+activ+H89	892.469171	48.2800591	1534.612807	90.3019959

Total insulin content measured per GSIS condition (ng/10 islets).

ESM Table 5

Metabolites	Abbreviations	control mean	dKO mean	log_2 (fold change)	Student t-test (p value)
AADA	Aminoadipic acid	1909.1	699.12	-1.4493	0.1347
a(R)-OHB/a(S)-OHB	Alpha-hydroxybutyric acid	2000.2	2105.2	0.0738	0.9064
ADMA/SDMA	(A)symmetric dimethylarginine	151.88	152.32	0.0042	0.9968
Ala	Alanine	50606	35866	-0.4967	0.3161
β-OHB	β-hydroxybutyric acid	3990.9	5578.9	0.4833	0.4692
CA	Cholic acid	1181.3	4177.4	1.8222	0.1482
CDCA	Chenodeoxycholic acid	327.1	348.92	0.0931	0.2608
Cit	Citrulline	6926.6	6273.8	-0.1428	0.5838
DCA	Deoxycholic acid	216.51	252.52	0.222	0.266
GBB	Gamma-butyrobetaine	801.64	760.72	-0.0756	0.6991
GCA	Glycocholic acid	547.92	655.65	0.259	0.0166
Gln	Glutamine	97592	93082	-0.0683	0.7184
Glu	Glutamic acid	21063	17968	-0.2293	0.5822
Gly	Glycine	20756	16462	-0.3344	0.1185
GUDCA	Glycoursodeoxycholic acid	492.84	492.78	-0.0002	0.8557
HCit	Homocitrulline	991.28	979.06	-0.0179	0.3277
Ile	Isoleucine	14195	19491	0.4575	0.0196
IndS	Indoxyl sulfate	8683.7	6507.5	-0.4162	0.119
Kynu	Kynurenine	714.87	729.99	0.0302	0.4022
Leu	Leucine	17020	21708	0.351	0.029
N-MNA	N-methylnicotineamide	547.54	547.58	1E-04	0.3752
Phe	Phenylalanine	11509	11749	0.0298	0.8802
Taurine	Taurine	128076	100512	-0.3496	0.4514
TCA	Taurocholic acid	30302	110668	1.8687	0.0522
TDCA/TCDCA	Tauro(cheno)deoxycholic acid	723.01	1904.5	1.3973	0.0056
Trp	Tryptophan	8152.9	8051.9	-0.018	0.9343
TUDCA	Tauroursodeoxycholic acid	548.74	640.49	0.223	0.3052
Tyr	Tyrosine	12364	12391	0.0031	0.9929
UDCA	Ursodeoxycholic aicd	418.55	437.05	0.0624	0.2671

Metabolite differences found in plasma samples of control vs dKO mice according to metabolic class and both fold-change and t-test criteria.

Supplemental Figure legends

Supplemental Fig.1 Boxplots showing differences between HFHS (yellow) and RC (green) diet in 6 mouse strains over time for *Mfn1* (A) and *Mfn2* (B) genes. The bottom and top of the boxes represent the first and third quartiles, with the horizontal line representing the median. The upper whiskers represent the third quartile plus 1.5x IQR (interquartile range); the lower whiskers represent the first quartile minus 1.5x IQR. Outlier points beyond this range are indicated above or below the whiskers. Statistically significant comparisons following false discovery rate (FDR) correction (FDR <= 0.05) are indicated by a double asterisk. Marginally significant comparisons (raw p value <= 0.05) are indicated by a single asterisk.

Supplemental Fig.2 Pdx1CreER activation has no detectable effect on glycaemia both *in vivo* and *in vitro* and on key beta-cell gene expression and islet morphology. (A) Glucose tolerance measured by IPGTT (2 g/kg body weight) in WT and Pdx1CreER mice (*n*=6 mice per genotype) at 8 weeks of age. (B) Insulin secretion measured during serial incubations in batches in 3 or 20 mmol/l glucose (*n*=6 mice per genotype in three independent experiments) at 8 weeks of age. (C) (a,b) MafA (red) and insulin (green) expression levels in WT and Pdx1CreER islets. (c,d) Typical distribution of beta- (insulin, red), alpha- (glucagon, green), and delta-cells (Somatostatin, SS, green) in 8-week old islet sections (*n*= 50 islets, 3 male mice per genotype). Note that both alpha and delta cells were unidentifiable as these were stained in green. Scale bar: 20 μm. Data are presented as mean±SD. Data assessed by two-way ANOVA test and Sidak's multiple comparisons test.

Supplemental Fig.3 Body weight loss, insulin resistance and increased β-ketone production is observed in β*Mfn1/2* dKO mice. (A) qRT-PCR quantification of *Mfn1, Mfn2* expression in tissues extracted from control and dKO animals relative to *β-actin* (*n*=3-5 mice per genotype in two independent experiments). (B) Measured body weight in control and β*Mfn1/2* dKO mice (*n*=3-6 mice per genotype) at 7-22 weeks of age. (C) Glucose tolerance measured by IPGTT (1 g/kg body weight) in 20-week-old mice in β*Mfn1/2* dKO and control mice (*n*=8 mice per genotype, in 2 independent experiments). (D) Challenging β*Mfn1/2* dKO mice with a 0.75 U/kg body weight insulin injection as compared with control mice at 14 weeks of age (*n*=6 mice per genotype).

Data normalised to baseline (%). (E) Plasma insulin levels during IPGTT of 3g/kg of glucose in dKO and control mice (*n*=5 mice per genotype) (F) Proinsulin to insulin ratio measured in *n*=5 mice per genotype. (G) Glucose and (H) β-ketone bodies measured before or after an overnight (16h) fasting in 14-week control and dKO mice. (I) Plasma insulin levels were quantified under fed and fasted conditions in 14-week dKO and control mice (*n*=6 mice per genotype). Data are presented as mean±SD. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 as indicated, or at the time points indicated analysed by unpaired two-tailed Student's t-test and Mann–Whitney correction or two-way ANOVA test and Sidak's multiple comparisons test. Experiments were performed in 14 or 20-week-old male mice as stated accordingly.

Supplemental Fig.4 β*Mfn1/2* dKO mice show impaired glucose tolerance and insulin secretion at 14 and 20 weeks of age following an IP injection of glucose versus an OG. (A) Glucose tolerance measured by IPGTT (using 1g/kg body weight) in β*Mfn1/2* dKO and control mice at 14 weeks of age. (B) Plasma insulin levels during IPGTT in dKO and control mice. (C) Glucose tolerance measured by IPGTT (using 1g/kg body weight) in β*Mfn1/2* dKO and control mice at 20 weeks of age. (D) Plasma insulin levels during IPGTT in dKO and control mice (*n*=5-6 mice per genotype). (E) Glucose tolerance measured by OGTT (using 1g/kg body weight) in β*Mfn1/2* dKO and control mice at 14 weeks of age. (F) Plasma insulin levels during IPGTT in dKO and control mice. (G) Glucose tolerance measured by OGTT (using 1g/kg body weight) in β*Mfn1/2* dKO and control mice at 20 weeks of age. (H) Plasma insulin levels during IPGTT in dKO and control mice (*n*=5-6 mice per genotype). Data are presented as mean±SEM. *p<0.05 as indicated, analysed by two-way ANOVA test and Sidak's multiple comparisons test.

Supplemental Fig.5 Mitochondrial ultrastructure, glycaemia and beta cell mass are not altered 2 weeks post tamoxifen administration in β*Mfn1/2* dKO mice. (A) Confocal images of the mitochondrial network of dissociated beta cells stained with Mitotracker green; scale bars: 5 μm. (B) Mitochondrial morphology analysis on deconvolved confocal images of dissociated beta cells. A macro was developed to quantify the number of mitochondria per cell and measure the elongation, perimeter, circularity (0: elongated; 1: circular mitochondria), density and surface area of the

organelles in control and dKO animals (*n*=20-50 cells; *n*=3 mice per genotype). (C) Blood glycaemia measured in fed mice before or 2 weeks post-tamoxifen injection in control and dKO mice (*n*=7 mice per genotype). (D) Glucose tolerance measured by IPGTT (3 g/kg body weight) in 10-week-old β*Mfn1/2* dKO and control mice (*n*=7 mice per genotype). (E) Plasma insulin levels were quantified under fasted conditions in 10-week dKO and control mice (*n*=7 mice per genotype). (F) The beta cell and alpha cell surface (G) measured within the whole pancreatic area in control and dKO mice were determined, as well as the beta/alpha cell ratio in (H), (*n*=67-76 islets, 3 mice per genotype; experiment performed in duplicate). (I) The relative mitochondrial DNA copy number was measured by determining the ratio of the mtDNA-encoded gene *mt-Nd1* to the nuclear gene *Ndufv1* (*n*=3 mice per genotype). Data are presented as mean±SD. *p<0.05, **p<0.01 as indicated, analysed by unpaired two-tailed Student's t-test and Mann–Whitney correction or two-way ANOVA test and Sidak's multiple comparisons test. Experiments were performed in 10-week-old male mice.

Suppl. Fig.6 Heatmap of differential gene expression between β*Mfn1/2* dKO and control islet mRNA. Changes in key beta or alpha cell genes, disallowed genes, mitochondrial, ER stress or mito/autophagy genes were assessed by qRT-PCR in control and dKO islets according to the colour coded median values from 0 to 1, white to dark blue respectively (*n*=3-4 mice per genotype; experiment performed in duplicate). Expression values for each gene were normalised to β-*actin*. *p<0.05; **p<0.01, assessed by two-way ANOVA test and Sidak's multiple comparisons test. Experiments were performed in 14-week-old male mice.

Supplemental Fig.7 Impact of *Mfn1/2* deletion on intercellular connectivity. (A) Representative cartesian maps of islets with colour coded lines connecting cells according to the strength of Pearson analysis (colour coded *r* values from 0 to 1, blue to red respectively) under 3mmol/L (3G), 17mmol/L (17G) glucose or 20mmol/L KCl; scale bars: 40 μm.(B) Representative heatmaps depicting connectivity strength (*r*) of all cell pairs according to the colour coded *r* values from 0 to 1, blue to yellow respectively.(C) Percentage of correlated cell pairs at 3G, 17G or KCl (*n*=17-26 islets, 4 mice per genotype).(D) *r* values between beta cells in response to glucose or KCl (*n*=4 mice per genotype).(E) qRT-PCR quantification of *Cx36* expression relative to β-

actin (*n*=3–4 mice per genotype in two independent experiments). Data are presented as mean±SD. *p<0.05, assessed by unpaired two-tailed Student's t-test and Mann–Whitney correction or two-way ANOVA test and Sidak's multiple comparisons test. Analysis and experiments were performed on data collected from 14-week-old male mice.

Supplemental Fig.8 Impaired insulin secretion observed in Clec16a$^{\Delta panc}$ islets can be rescued by GLP-1R agonists *in vitro.* (A) Insulin secretion measured in control (Pdx1-Cre) and Clec16a$^{\Delta panc}$ mice in 3 mmol/l glucose (3G), 17 mmol/l glucose (17G), or 10 nmol/l exendin-4 (ex4) (*n*=4 mice per genotype). (B) Glucose tolerance measured by IPGTT (1.5 g/kg body weight) in 8-week-old male Pdx1-Cre and Clec16a$^{\Delta panc}$ mice or OGTT (1.5 g/kg body weight) in 9–10-week-old animals. (C) The corresponding AUC is shown in (B) (*n*=4-5 mice per genotype). (*p<0.05,**p<0.01, control OGTT vs Clec16a$^{\Delta panc}$; #p<0.05, ##p<0.01, control IPGTT vs Clec16a$^{\Delta panc}$). Data are presented as mean±SD and assessed by two-way ANOVA test and Sidak's multiple comparisons test.

Supplemental Fig.9 Insulin granule density is increased in β*Mfn1/2* dKO beta cells. (A) Confocal images of NPY-Venus fluorescence in dissociated fixed pancreatic beta cells isolated from control and dKO mice. Scale bar: 10 µm.(B) Effect of KCl on exocytosis as reported with NPY-Venus in pancreatic beta cells. Traces represent mean normalised fluorescence intensity over time (F/F_{min}). (C) Confocal images of ZIMIR fluorescence imaging in dissociated pancreatic beta cells isolated from control and dKO mice. Scale bar: 10 µm.(D) Representative time courses of ZIMIR signal fold change above baseline (F/F_{min}) upon KCl-stimulated insulin/Zn^{2+} release and (E) fold change of peaks in dissociated control and dKO cells. (*n*=19 cells from 3 control mice; *n*=12 cells from 3 β*Mfn1/2* dKO mice). Data are presented as mean±SD. *p<0.05,**p<0.01; assessed by unpaired two-tailed Student's t-test and Mann–Whitney correction or two-way ANOVA test and Sidak's multiple comparisons test. Experiments were performed in 14-week-old male mice.

Supplemental Fig.10 Volcano plots showing alterations in metabolites and lipids from plasma samples of control and β*Mfn1/2* dKO mice. (A) Volcano plot summarising both fold-change and t-test criteria for all metabolites. Results are

summarised in a scatter-plot of the negative $\log_{10}$-transformed p values from the t-test plotted against the $\log_2$ fold change. Negative values indicate downregulated metabolites in dKO mice, while positive values reflect upregulated metabolites. Metabolites with statistically significant differential levels according to the t-test lie above a horizontal threshold line (red dots). Metabolites with large fold-change values lie far from the vertical threshold line at $\log_2$ fold change = 0, indicating whether the metabolite is up or downregulated. The list of analysed metabolites with their abbreviations is presented in ESM table 5. (B) Lipids that were found downregulated in dKO mice with statistically significant differential levels according to the t-test are presented above a horizontal threshold line. Plasma samples were isolated from *n*=3 animals per genotype. The most significantly downregulated lipids are annotated. SM, sphingomyelins; CER, ceramide; CE, cholesterol esters; DG, di(acyl/alkyl)glycerols; FA, fatty acids; TG, tri(acyl/alkyl)glycerols; LPC, lysophosphatidylcholines; PC, phosphatidylcholines;LPE,lysophosphatidylethanolamines;PE,phosphatidylethanola mines; PG, phosphatidylglycerols; PI, phosphatidylinositols; PS, phosphatidyserines. Experiments were performed in 14-week-old male mice.

Supplemental Fig.11 Impact of Mfn1/2 deletion on glucose and incretin stimulated-insulin secretion in beta cells. (A) In control animals, glucose is taken up by beta cells through GLUT2 and metabolised by mitochondria (elongated structure) through the citrate (TCA) cycle, leading to an increased mitochondrial proton motive force (hyperpolarised $\Delta\psi_m$), accelerated ATP synthesis and O_2 consumption rate (OCR). Consequently, the cytoplasmic ATP:ADP ratio rises, which causes closure of KATP channels, depolarisation of plasma membrane potential (ψm), opening of VDCCs and influx of cytosolic Ca^{2+}. Elevated $[Ca^{2+}]_{cyt}$ triggers a number of ATP-dependent processes including insulin secretion and improved beta-beta cell communication through connexin 36 (Cx36). (B) Following *Mfn1/2* deletion (β*Mfn1/2* dKO), highly fragmented mitochondria were associated with reduced mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) accumulation, leading to a less polarised $\Delta\psi_m$, weaker OCR, lower mtDNA copy number and decreased ATP synthesis. This is expected to result in weaker ψm depolarisation, cytosolic Ca^{2+} influx and beta-beta cell connectivity due to lower expression of Cx36. Despite observing a higher number of docked insulin granules on the plasma membrane, insulin secretion was highly suppressed in these animals. This was also associated with increased beta cell death and reduced beta

cell mass. (C) In response to incretins, insulin secretion is potentiated through the activation of GLP1-R and cAMP signalling involving PKA- and EPAC-dependent pathways. Elevated $[Ca^{2+}]_{cyt}$ triggers a number of ATP-dependent processes including insulin secretion and Ca^{2+} mobilisation into the endoplasmic reticulum (ER).(D) In β*Mfn1/2* dKO cells, activation of the GLP1-R is linked with a potentiation of the EPAC pathway (inhibited by PKA), an increased ER Ca^{2+} uptake and improved beta-beta cell communication. Red and bold arrows represent enhanced pathways; dashed arrows represent impaired pathways. This figure was produced using illustrations from Servier Medical Art, http://smart.servier.com/

ESM Videos

ESM Video 1

Fluorescence imaging of cytosolic Ca^{2+} oscillations using Cal-520 in control (left) and β*Mfn1/2* dKO (right) whole islets in response to 3G, 3 mmol/l glucose, 17 mmol/l glucose (17G; with or without diazoxide [diaz]) or 20 mmol/l KCl with diaz. Scale bars: 50µm.

ESM Video 2

Fluorescence imaging of mitochondrial Ca^{2+} oscillations using R-GECO in control (left) and β*Mfn1/2* dKO (right) whole islets in response to 3G, 3 mmol/l glucose, 17 mmol/l glucose (17G; with or without diazoxide [diaz]) or 20 mmol/l KCl with diaz. Scale bars: 50µm.

ESM Video 3

Changes in $[Ca^{2+}]_{ER}$ were measured by fluorescence imaging of cytosolic Ca^{2+} oscillations using Cal-520 in control (left) and β*Mfn1/2* dKO (right) whole islets in response to 3G, 3 mmol/l glucose, 17 mmol/l glucose (17G; with or without diazoxide [diaz] or Acethylcholine [Ach]) or 20 mmol/l KCl with diaz. Scale bars: 50µm.

ESM Video 4

Fluorescence imaging of cytosolic Ca^{2+} oscillations using Cal-520 in control (left) and β*Mfn1/2* dKO (right) whole islets in response to 3G, 3 mmol/l glucose, 10 mmol/l glucose (10G; with or without Exendin-4 [ex4]) or 20 mmol/l KCl. Scale bars: 50µm.