Supplementary Material

Glycogen Synthase Kinase 3 Beta Controls Presenilin-1-Mediated Endoplasmic Reticulum Ca^{2+} Leak Directed to Mitochondria in Pancreatic Islets and β-Cells

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SUPPLEMENTARY TABLES:

Supplementary Table 1: List of primer and siRNA sequences for presenilin-1 and GSK3β

Application	Gene	Sequences
Detection PCR	h,r,mPSEN1	5'-GGTCCACTTCGTATGCTGGT-3' 5'- TTGCTGAGGCTTTACCAACC -3'
	hGSK3β	5'- TGTGATTCAGGAGAACTGGTCG-3' 5'-ATCCAACAAGAGGTTCTGCGGT-3'
	rGSK3β	5'-GAGAACTGGTGGCCATCAAGAA-3' 5'-CTGTATCAGGATCCAGCAAGAG-3'
	mGSK3β	5'-GTGATTCTGGAGAACTGGTTGC-3' 5'-ATCCAACAAGAGGTTCTGTGGT-3'
RT-PCR	hPSEN1	QuantiTect Hs_PSEN1 Primer Qiagen
	rPSEN1	QuantiTect Rn_PSEN1 Primer Qiagen
	mPSEN1	QuantiTect Mm_PSEN1 Primer Qiagen
	hGSK3β	5'- ACCTCCTTTGCGGAGAGCTG -3' 5'- TGCCACCACTGTTGTCACCT -3'
	r,mGSK3β	5'- ACCTCCTTTGCGGAGAGCTG -3' 5'- TGCCACCACTGTTGTTACCT-3'
siRNA	hPSEN1 si1	5'-UUG UGU GGU UGG UGA AUA UTT -3'
	hPSEN1 si2	5'- CCA CCU GAG CAA UAC UGU ATT -3'
	m,rPSEN1 si1	5'-UUG UGU GGU UGG UGA AUA UTT -3'
	m,rPSEN1 si2	5'- GGA GAG UAU CCA AAA AUU CTT - 3'

Gene and species specific detection and real time primer and siRNA sequences whereas h = human, m = mouse, r = rat

Supplementary Table 2: List of primer and siRNA sequences

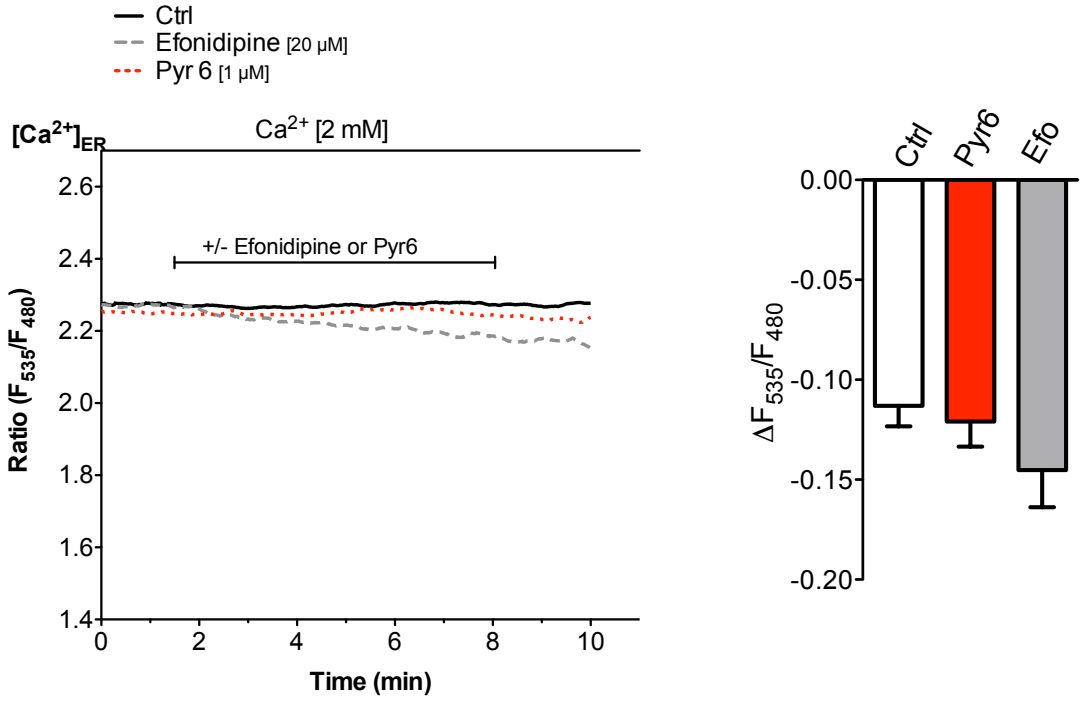
Application	Gene	Sequences
RT-PCR	hIP$_3$R type 1	5'-CACCATCCAGCACTCCTT-3' 5'-GTTCACTGTCACTGTTGC-3'
	hIP$_3$R type 2	5'-AACTACAGCACGCTGCAGAA-3' 5'-GGACACACGCATGGCATTCT-3'
	hIP$_3$R type 3	5'-GCCAAGCAGACTAAGCAGGA-3' 5'-CATGCACCTTCTTGTTCTCC-3'
	rIP$_3$R type 1	5'-CGTTTTGAGTTTGAAGGC-3' 5'-AGCCACACCTCTTCCTCA-3'
	rIP$_3$R type 2	5'-CCTCTTGAGCCCGGTGCA-3' 5'-CGATCCGGTAGTTGTTGC-3'
	rIP$_3$R type 3	5'-CTCTTCCATGCTCAGCCT-3' 5'-CAATCTGCGAGGTGTGGTTC-3'
	rTRPC3	5'-CCAAGCTGGCCAACATAGAG-3' 5'-GGCAAGTTTGACACGACTCA-3'
siRNA	hIP$_3$R type 1	5'-UCA AGC UUU GCU ACA UAA ATT-3'
	hIP$_3$R type 2	5'-CUA UGA GAA UGG AGA AAU ATT-3'
	hIP$_3$R type 3 si1	5'-GCA GAC UAA GCA GGA CAA GTT-3'
	hIP$_3$R type 3 si2	5'-GGA CGU GGA GAA CUA CAA GTT-3'
	rIP$_3$R type 1	5'-GGA AAA ACC UGU CAU GCU GTT-3'
	rIP$_3$R type 2	5'-UGG AAC AAA AAC AGA AUG AGU CA-3'
	rIP$_3$R type 3	5-'GGA UGU GGA GAA CUA CAA ATT-3'
	rTRPC3	5-'CAU UGG CUA UGU CCU UUA U-3'

Gene and species specific real time primer and siRNA sequences

whereas h = human, r = rat

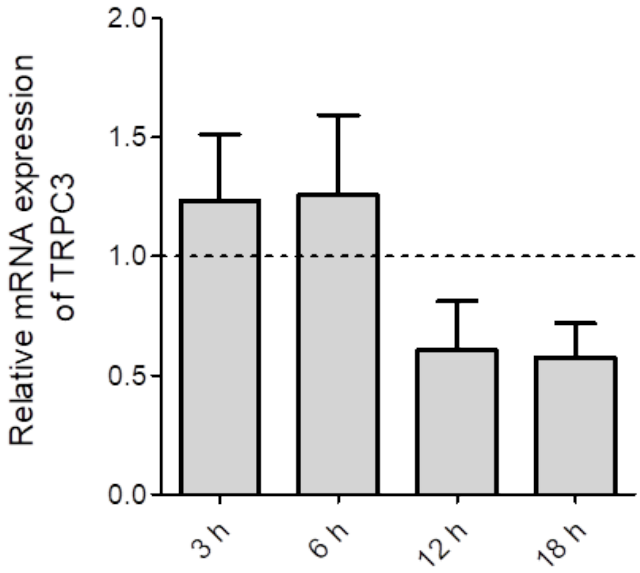
SUPPLEMENTARY FIGURES:

Suppl. Fig. 1



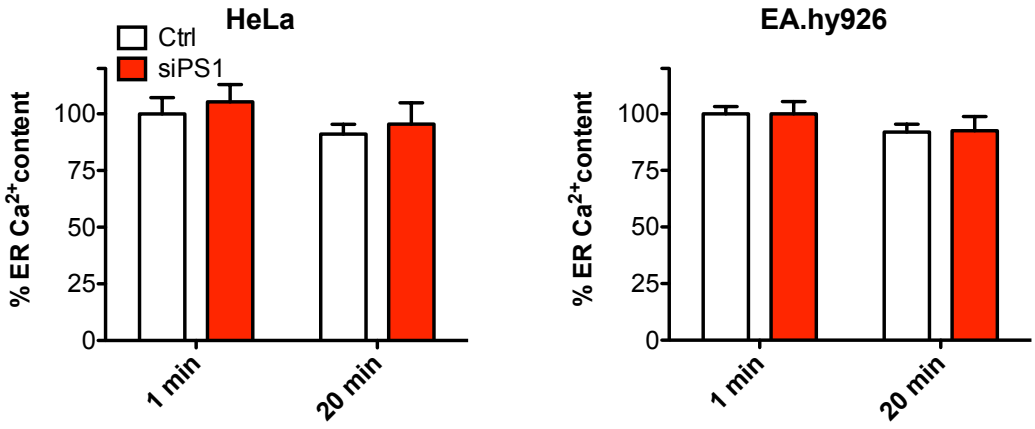
Representative traces for β-cells (depicted INS-1) on the ER Ca^{2+} content measured by the ER Ca^{2+} probe D1ER over time measured in EB containing 2 mM Ca^{2+} under control conditions (black line), with the SOCE inhibitor Pyr6 [1 µM] (red dotted line) or with the L- and T-type Ca^{2+} channel blocker efonidipine [20 µM] (grey dashed line). *Right insert panel:* Bars represent the statistical analysis of the data shown in the graph depicting the absolute change of $[Ca^{2+}]_{ER}$ under control conditions (white bar), after treatment with 1 µM Pyr6 (red bar) or after treatment with [20 µMefonidipine (grey bar). Bars indicate mean ± SEM (n=6). *p<0.05 compared to control using one-way ANOVA.

Suppl. Fig. 2



Quantification of the knock-down efficiency of TRPC3-specific siRNA in INS-1 cells 3, 6, 12 and 18 h after transfection via real-time PCR using GAPDH as a reference gene. Bars represent mean ± SEM (n=3).

Suppl. Fig. 3



Percentage of ER Ca^{2+} content in HeLa cells (upper panel) or EA.hy926 cells (lower panel) after 20 min of incubation under Ca^{2+}-free conditions either under control conditions or after knock-down of presenilin-1. ER stores were depleted after 20 min by applying 100 µM histamine together with the SERCA inhibitor BHQ (15 µM). In each graph the 1 min control value was set to 100% ($n \geq 5$).

Suppl. Fig. 4

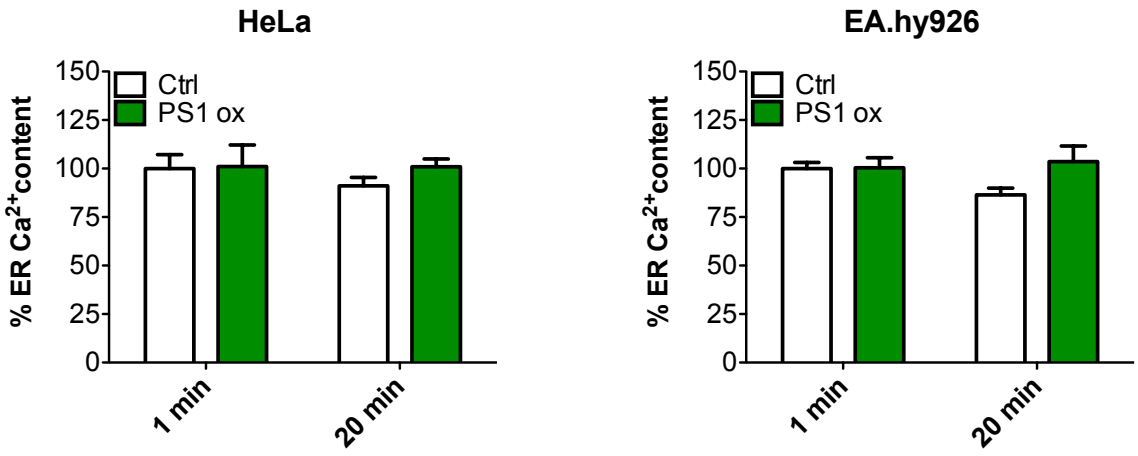
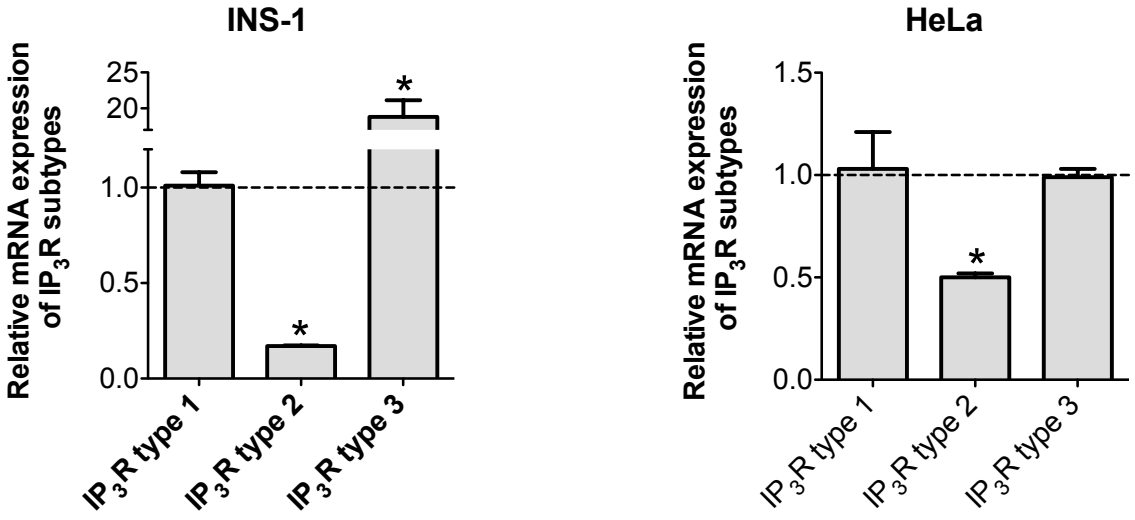


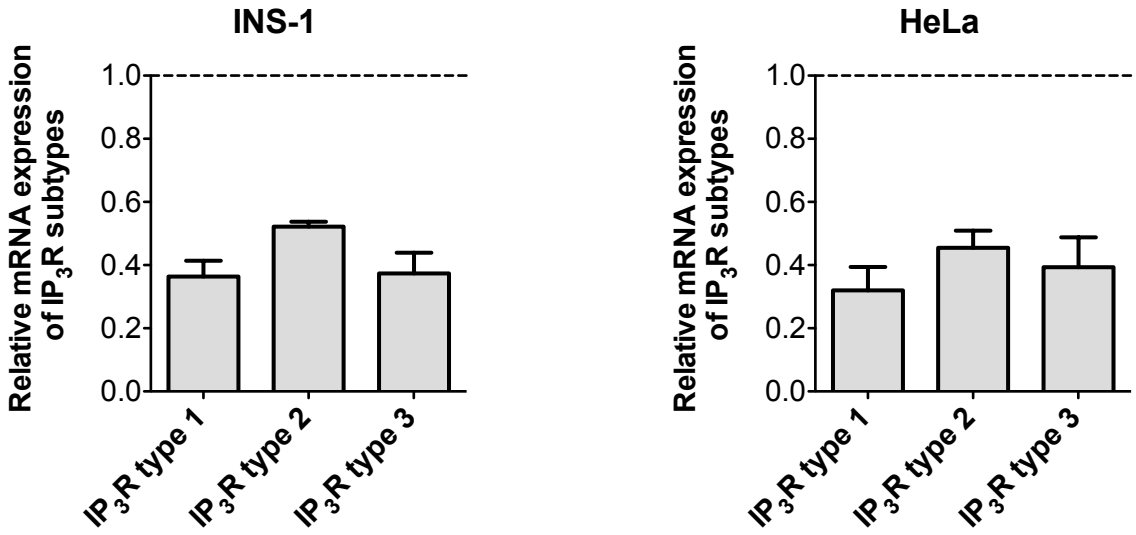
Illustration of the ER Ca^{2+} content of HeLa (l*eft panel*) and EA.hy926 (*right panel*) cells in Ca^{2+}-free EB. At the indicated time points ER Ca^{2+} stores were depleted using the SERCA inhibitor BHQ (15 µM), together with histamine (100 µM). Bars indicate the mean in control cells (Ctrl; white bars) or in cells overexpressing presenilin-1 (PS1 ox; green bars). Control values were set as 100%. Bar charts indicate mean $\pm$ SEM, HeLa (n=7-8), EA.hy926 (n=8-10). *$p<0.05$ vs. control using one-way ANOVA.

Suppl. Fig. 5



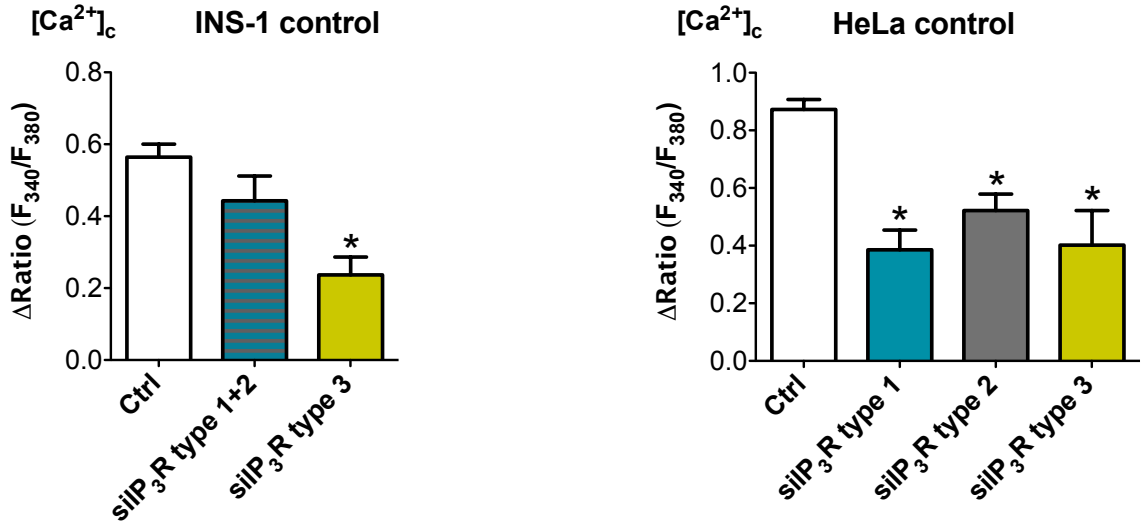
Quantification of mRNA expression levels of the IP_3R subtypes in INS-1 cells (*left panel*) and HeLa cells (*right panel*) compared to mRNA levels of the housekeeping gene GAPDH. For comparison mRNA expression levels were normalized to IP_3R type 1. Bars represent mean ± SEM (n=3). *$p<0.05$ compared to expression level of IP_3R type 1 using one-way ANOVA.

Suppl. Fig. 6



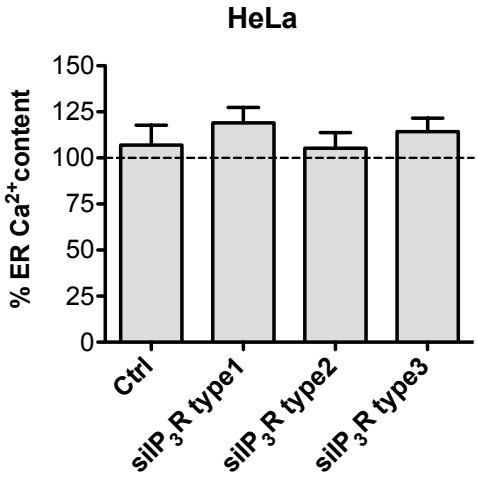
Quantification of knock-down efficiency of the IP_3R subtypes in INS-1 (*left panel*) and HeLa (*right panel*) cells after treatment with specific siRNAs against the indicated IP_3R subtypes via real-time PCR using GAPDH as a reference gene. Bars represent mean ± SEM (n=5).

Suppl. Fig. 7



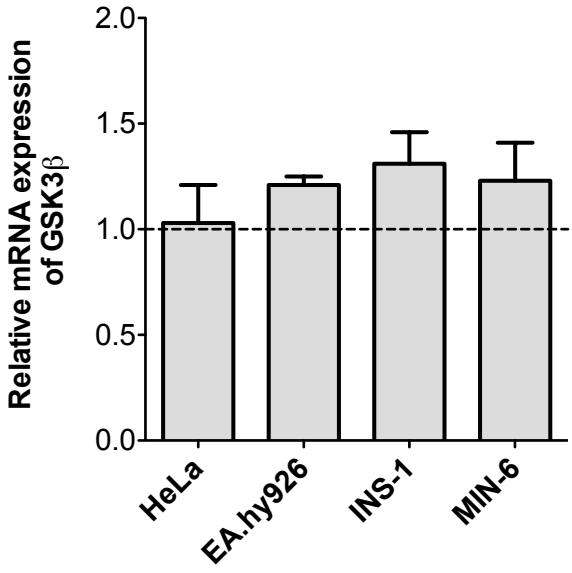
Functional verification of IP_3R subtype knock-down by measuring agonist-induced intracellular Ca^{2+} release INS-1 (*left panel)* and HeLa cells (*right panel*) cells that were sham transfected (Ctrl) or after knock-down of certain IP_3R subtypes with specific siRNAs. Cells were loaded with Fura-2/AM, washed and stimulated with Cch (100 µM; INS-1) or histamine (100 µM; HeLa) together with the SERCA inhibitor BHQ (15 µM) in EB lacking Ca^{2+}. Bars represent mean ± SEM, (n=5-6). *p<0.05 versus control using one-way ANOVA.

Suppl. Fig. 8



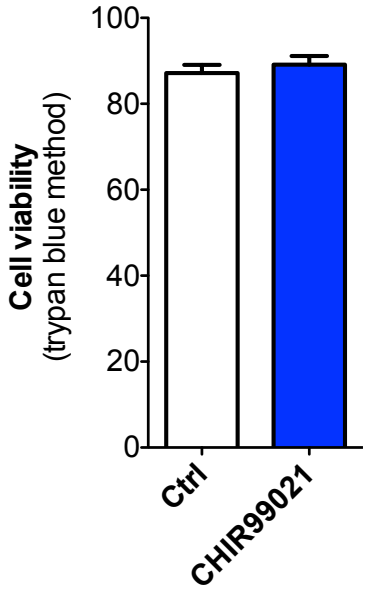
Percentage of ER Ca^{2+} content in HeLa cells after 20 min of incubation under Ca^{2+}-free conditions either under control conditions or after knock-down of the indicated types of IP_3R. ER stores were depleted after 20 min by applying 0.2 µM of ionomycin together with the SERCA inhibitor BHQ (15 µM). In each graph the 1 min control value was set to 100% ($n \geq 5$). *$p<0.05$ versus respective 1 min control or as indicated in the graph using one-way ANOVA.

Suppl. Fig. 9



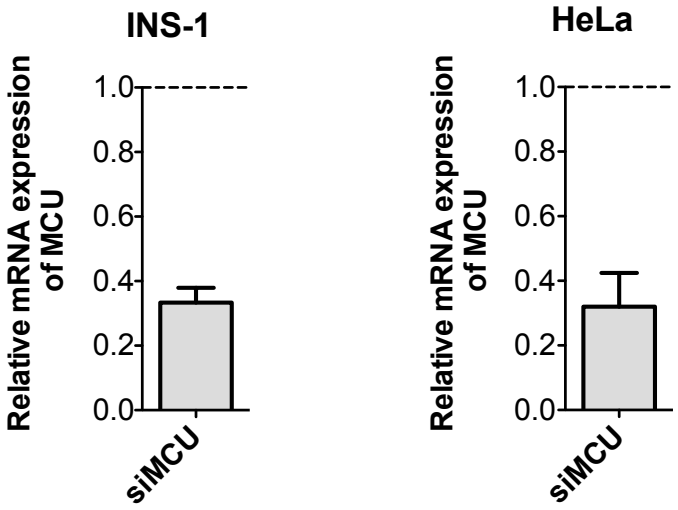
Quantification of mRNA expression levels of GSK3β in HeLa, EA.hy926, INS-1 and MIN-6 cells compared to mRNA levels of the housekeeping gene GAPDH detected with realt-time PCR. mRNA levels were normalized to HeLa expression levels. Bar charts indicate mean ± SEM, (n=3). *$p<0.05$ using one-way ANOVA.

Suppl. Fig. 10



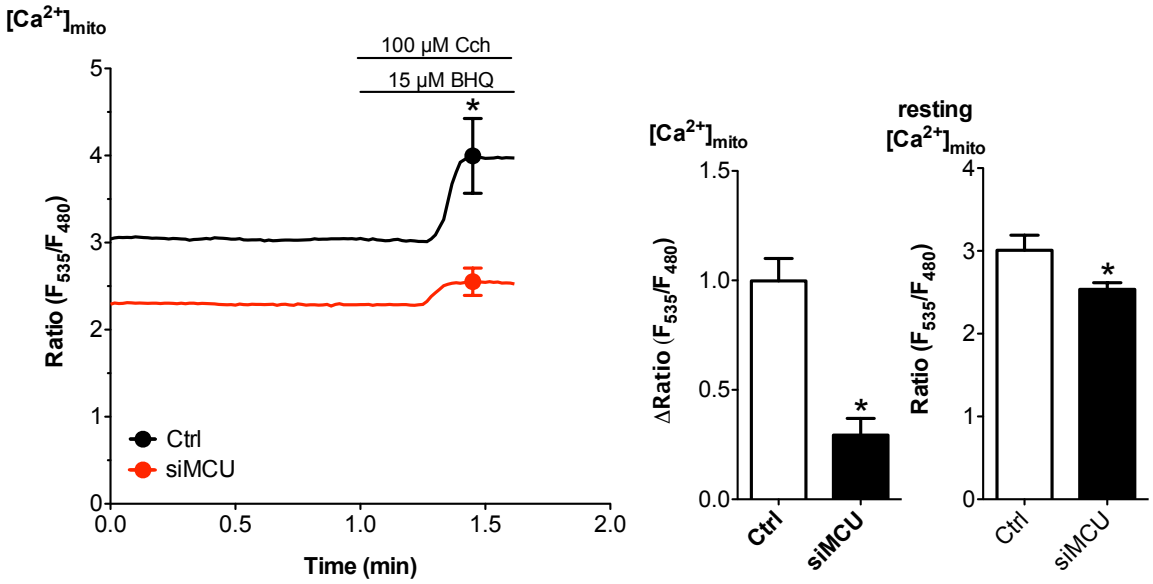
Cell viability of isolated islets was assessed with trypan blue after 24 h incubation with DMSO control (white bar) or 2.5 µM CHIR99021 (blue bar). Bars represent mean ± SEM, (n=3).

Suppl. Fig. 11



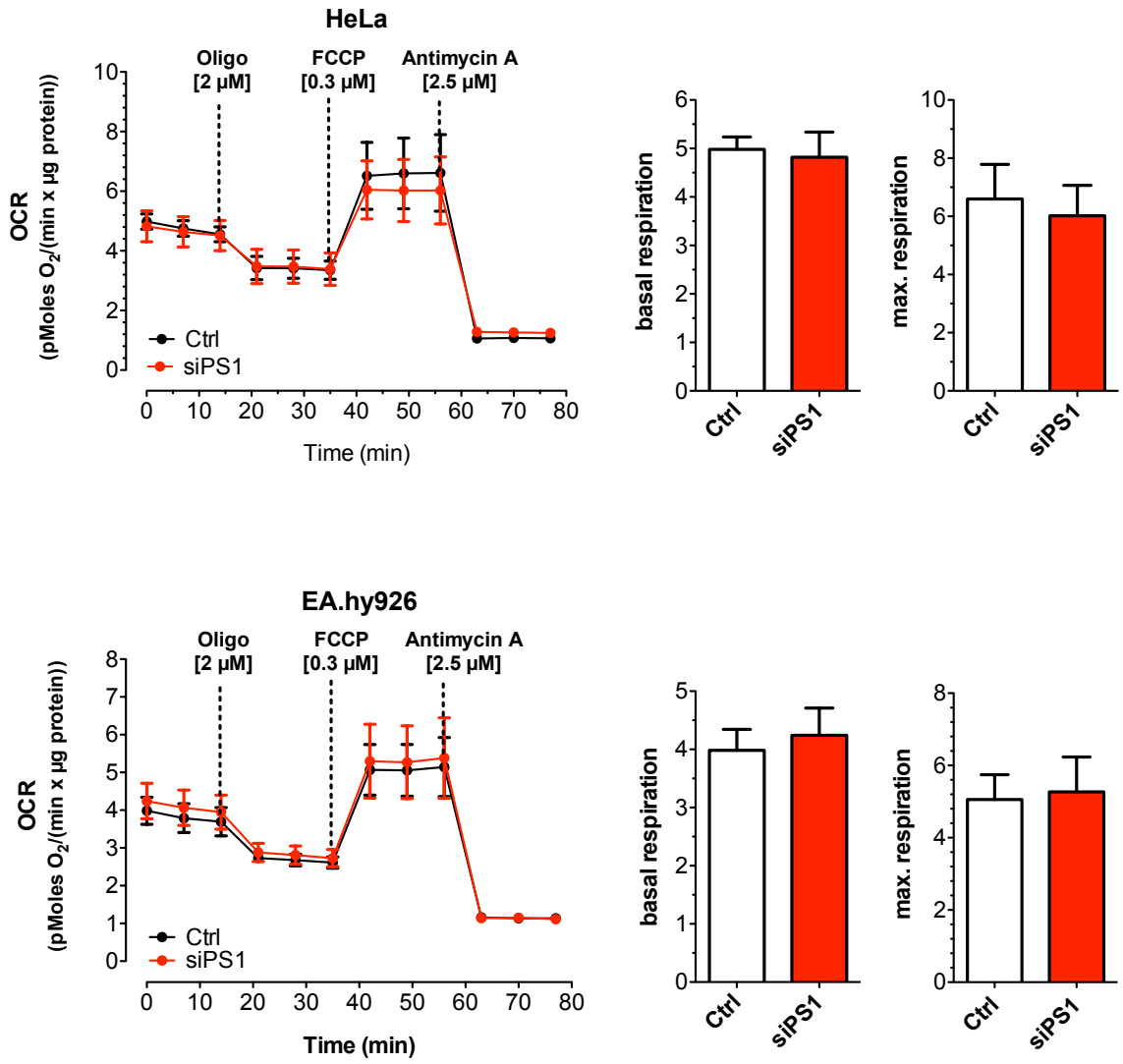
Quantification of knock-down efficiency of MCU in INS-1 (*left panel*) and HeLa (*right panel*) cells after transfection with specific siRNAs via real-time PCR using GAPDH as a reference gene. Bars represent mean ± SEM (n=3).

Suppl. Fig. 12



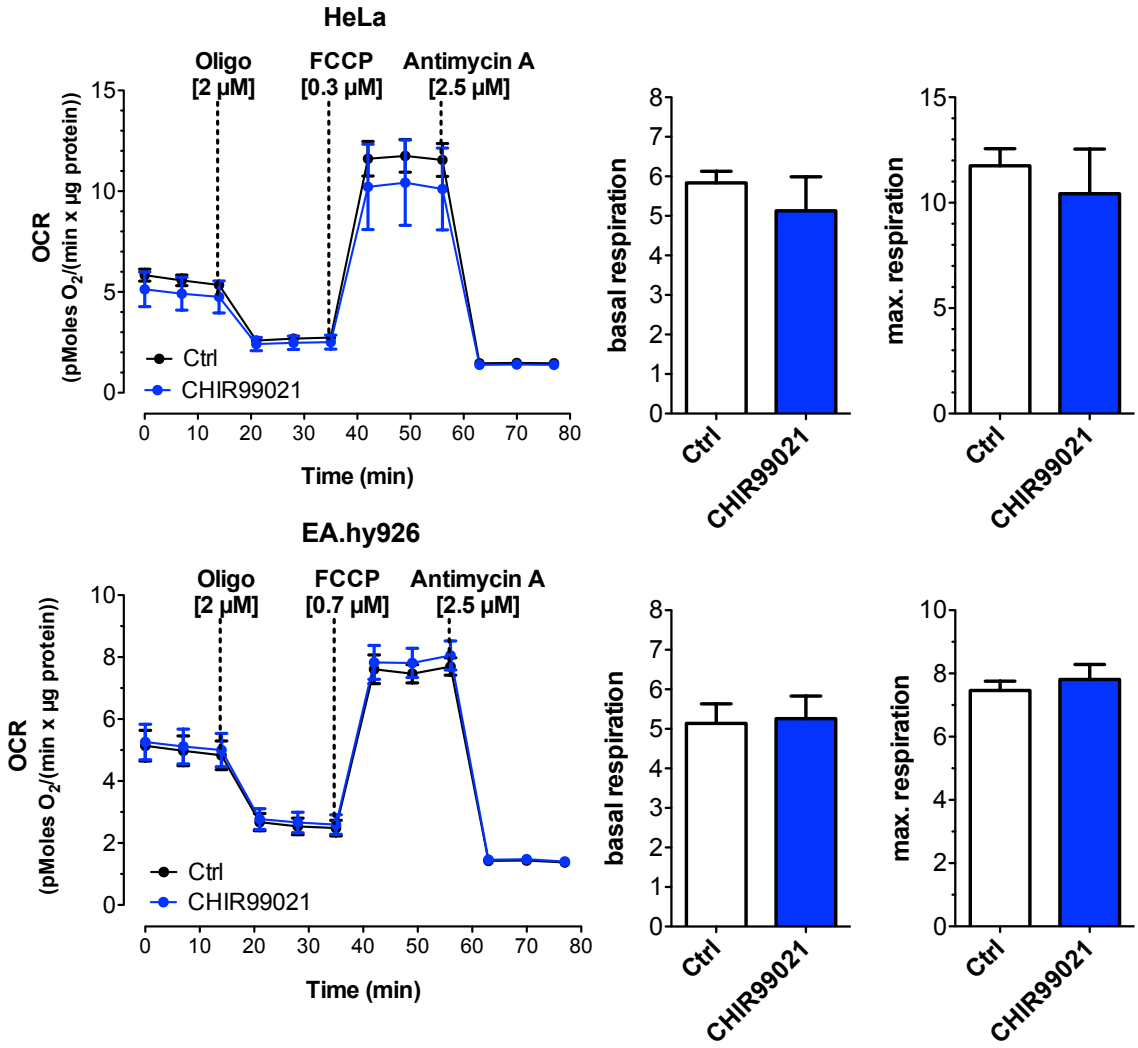
Left panel: Traces represent mitochondrial Ca^{2+} uptake in INS-1 cells measured with the genetically encoded mitochondrial Ca^{2+} indicator 4mtD3cpv under control conditions (Ctrl; black line) or after transfection with MCU-specific siRNA to prevent mitochondrial Ca^{2+} uptake (siMCU; red line). Intracellular Ca^{2+} was mobilized by applying the IP_3-generating agonist Cch (100 µM) and the SERCA inhibitor BHQ (15 µM). *Middle panel:* Corresponding statistical analysis representing maximal mitochondrial Ca^{2+} uptake levels in INS-1 cells under control conditions (white bars) or after knock-down of MCU (black bars). *Right panel:* Basal mitochondrial Ca^{2+} levels in INS-1 cells under control conditions (white bars) or after knock-down of MCU with specific siRNAs measured with 4mtD3cpv. Bars represent mean ± SEM, (n=6). *p<0.05 tested with the unpaired Student's t-test.

Suppl. Fig. 13



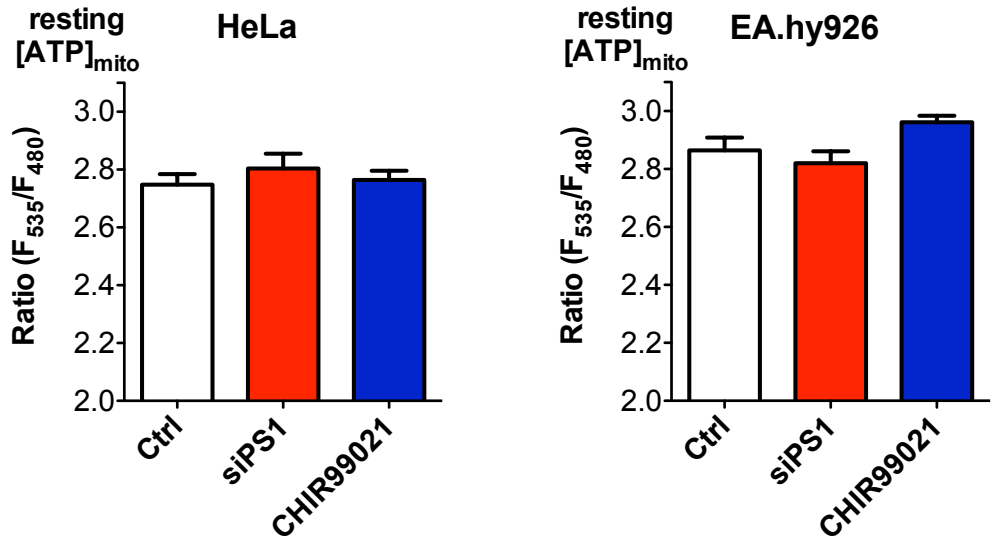
OCR of HeLa (*upper panel*) and EA.hy926 (*lower panel*) cells under control conditions (black lines) or after knock-down of presenilin-1 with specific siRNAs (red lines). Bars on the right represent basal (*middle panels*) and maximal (*right panels*) respiration under control conditions (white bars) or after knock-down of presenilin-1 (red bars). OCR was normalized to protein content. As indicated cells were treated with 2 µM oligomycin, 0.7 µM FCCP for EA.hy926 and 0.3 µM for HeLa, and 2.5 µM antimycin A. *p<0.05 compared to control using the Student's unpaired t-test (n=6).

Suppl. Fig. 14



OCR of HeLa *(upper panel)* and EA.hy926 *(lower panel)* cells under control conditions (black lines) or after pre-treatment with 2.5 µM of the GSK3β inhibitor CHIR99021 (blue lines). Bars represent basal (*middle panels*) and maximal (*right panels*) respiration under control conditions (white bars) or after a 48h pre-treatment with GSK3β inhibitor CHIR99021 (blue bars). OCR was normalized to protein content. As indicated cells were treated with 2 µM oligomycin, 0.7 µM FCCP for EA.hy926 and 0.3 µM for HeLa, and 2.5 µM antimycin A. *p<0.05 compared to control using the Student's unpaired t-test (n=6).

Suppl. Fig. 15



Bars represent resting $[ATP]_{mito}$ levels in HeLa *(left panel)* and EA.hy926 *(right panel)* cells measured with mtAT1.03 under control conditions (white bars), knockdown of presenilin-1 with specific siRNAs (red bars), or after a 48h pre-treatment with 2.5 µM GSK3β inhibitor CHIR99021 (blue bars). Bars represent mean ± SEM (n=6). *$p<0.05$, tested with one-way ANOVA.