Supplemental Data.

STAT3 regulates mitochondrial gene expression in pancreatic β-cells and its deficiency induces glucose intolerance in obesity

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Patient ID	HP1401	HP1504	HP1602	HP1603	HP1604	HP1606	HP1801	HP1803	HP1805	HP1807	HP1808	HP1901	HP1903	HP1905	HP1906
Age (Years)	83	75	56	62	79	74	62	43	39	78	62	61	78	60	69
Gender	F	M	F	M	F	F	M	M	F	F	F	M	F	M	F
BMI (kg/m²)	31.0	27.0	35.7	33.1	38.8	17.9	29.5	36.8	31.4	30.4	18.9	21.2	37.3	19.1	36.1
Cause of death	trauma	vascular	anoxia	anoxia	vascular	vascular	vascular	anoxia	anoxia	vascular	vascular	trauma	vascular	vascular	trauma
Diabetes (Y/N)	N	Y/ T2D	N	Y / T2D	N	N	Y / T2D	N	N	N	N	N	Y / T2D	N	N
Group	Mod Ob	T2D	Sev Ob	T2D	Sev Ob	Lean	T2D	Sev Ob	Mod Ob	Mod Ob	Lean	Lean	T2D	Lean	Sev Ob

Supplementary Table S1. Pancreas donor characteristics – immunofluorescent staining (Figure 1A, Supplementary Figure S1). Groups were defined using a body mass index (BMI) threshold of the donor as follow: Lean (BMI<30kg/m², n=4), Moderately obese (30 kg/m²<BMI<35 kg/m, n=3), Severely obese (BMI>35 kg/m, n=4) and type 2 diabetic (independently of the BMI, n=4). BMI was significantly different between groups (p<0.05). Donors were not different in age or sex distribution (p>0.05, with a normal frequency distribution). F=female, M=male, N=No, Y=Yes, T2D=Type 2 diabetes, Mod Ob=Moderately obese, Sev Ob=Severely obese. Diabetes treatment, information available for T2D donors: HP1504: Metformin – HP1603: Metformin and Gliclazide – HP1801: Atorvastatin and insulin.

Exp ID	#1	#2	#3	#4	#5	#6
Age (Years)	80	83	75	46	74	76
Gender	M	M	F	F	M	M
BMI (kg/m²)	26.23	31.14	27.3	25.4	33	28.4
Cause of death	vascular	vascular	vascular	anoxia	vascular	vascular
Diabetes (Y/N)	N	N	N	N	N	N
Group	Lean	Mod Ob	Lean	Lean	Mod Ob	Lean

Supplementary Table S2. Pancreas organ donor characteristics for mitochondrial function in isolated human islets (Figure 4F, Supplementary Figure S10). F=female, M=male, N=No, Y=Yes, Mod Ob=Moderately obese.

siRNA name	Company/catalogue number	Sequence
STAT3 #1	(Qiagen, Hilden, Germany, SI02662338)	5'-CAGCCTCTCTGCAGAATTCAA-3'
STAT3 #2	(Qiagen, Hilden, Germany, SI02662898)	5'-CAGGCTGGTAATTTATATAAT-3'
STAT1 #1	(Life Tecnologies-Invitrogen, STAT1HSS110273)	5'GGAUUGAAAGCAUCCUAGAACUCAU-3'
STAT1 #2	(Life Tecnologies-Invitrogen, STAT1HSS110274)	5'-CCUGUCACAGCUGGAUGAUCAAUAU-3'

Supplementary Table S3. List of siRNAs used in the study.

| Stage	Compound	Final concentration	Company
1 (3 days, change medium every day)	MCDB131 no Glutamine		Life Technologies, #10372-019
	GlutaMAX	2 mM	Thermo Fisher, #35050
	NaHCO3	1.5 g/l	Merck Millipore, #1.06329.0500
	BSA fV	0.5%	Sigma, #A7030
	Glucose	10 mM	Sigma, #G8769
	Activin A	100 ng/ml	PeproTech, #120-14E
	CHIR	5 µM (day 1), 0.5 µM (day 2)	Axon Medchem, #1386
2 (3 days, change medium every day)	MCDB131 no Glutamine		Life Technologies, #10372-019
	GlutaMAX	2 mM	Thermo Fisher, #35050
	NaHCO3	1.5 g/l	Merck Millipore, #1.06329.0500
	BSA fV	0.5%	Sigma, #A7030
	Glucose	10 mM	Sigma, #G8769
	L-Ascorbic acid	0.25 mM	Sigma, #A4554
	FGF-7	50 ng/mL	PeproTech, #100-19
3 (2 days, change medium every day)	MCDB131 no Glutamine		Life Technologies, #10372-019
	GlutaMAX	2 mM	Thermo Fisher, #35050
	NaHCO3	2.5 g/l	Merck Millipore, #1.06329.0500
	BSA fV	2%	Sigma, #A7030
	Glucose	10 mM	Sigma, #G8769
	L-Ascorbic acid	0.25 mM	Sigma, #A4554
	FGF-7	50 ng/mL	PeproTech, #100-19
	SANT-1	0.25 µM	Sigma, #S4572
	Retinoic acid (RA)	1 µM	Sigma, #R2625
	LDN-193189	100 nM	Selleckchem, #S2618
	ITS-X	1:200	Thermo Fisher, #51500056
	TPB	200 nM	Santa Cruz, #SC-204424
4 (4 days, change medium every day)	MCDB131 no Glutamine		Life Technologies, #10372-019
	GlutaMAX	2 mM	Thermo Fisher, #35050
	NaHCO3	2.5 g/l	Merck Millipore, #1.06329.0500
	BSA fV	2%	Sigma, #A7030
	Glucose	10 mM	Sigma, #G8769
	L-Ascorbic acid	0.25 mM	Sigma, #A4554
	FGF-7	50 ng/mL	PeproTech, #100-19
	SANT-1	0.25 µM	Sigma, #S4572
	Retinoic acid (RA)	0.1 µM	Sigma, #R2625
	LDN-193189	200 nM	Selleckchem, #S2618
	EGF	100 ng/ml	StemCell Technologies, #78006
	Nicotinamide	10 mM	Sigma, #N3376
	Activin A	10 ng/ml	PeproTech, #120-14E
5 (4 days, change medium every day)	MCDB131 no Glutamine		Life Technologies, #10372-019
	GlutaMAX	2 mM	Thermo Fisher, #35050
	NaHCO3	1.5 g/l	Merck Millipore, #1.06329.0500
	BSA fV	2%	Sigma, #A7030
	Glucose	20 mM	Sigma, #G8769
	ITS-X	1:200	Thermo Fisher, #51500056
	Heparin	10 µg/mL	StemCell Technologies, #07980
	Zinc Sulfate	10 µM	Sigma, #Z0251
	Retinoic acid (RA)	0.05 µM	Sigma, #R2625
	SANT-1	0.25 µM	Sigma, #S4572
	LDN-193189	100 nM	Selleckchem, #S2618
	GC-1	1 µM	Tocris, #4554
	GSiXX	100 nM	Merck Millipore, #565790
	ALK5inhII	10 µM	ENZO, #ALX-270-445
	Betacellulin	20 ng/ml	PeproTech, #100-50
	Penicillin - Streptomycin	100U/ml - 0.1mg/ml	Sigma, #P4333
	ROCK inhibitor Y-27632	10 µM	StemCell Technologies, #72304
	Heparin	10 ng/ml	StemCell Technologies, #07980
6 (7-8 days, change medium every second day)	MCDB131 no Glutamine		Life Technologies, #10372-019
	GlutaMAX	2 mM	Thermo Fisher, #35050
	NaHCO3	1.5 g/l	Merck Millipore, #1.06329.0500
	BSA fV	2%	Sigma, #A7030
	Glucose	20 mM	Sigma, #G8769
	ITS-X	1:200	Thermo Fisher, #51500056
	Heparin	10 µg/mL	StemCell Technologies, #07980
	Zinc Sulfate	10 µM	Sigma, #Z0251
	LDN-193189	100 nM	Selleckchem, #S2618
	ALK5inhII	10 µM	ENZO, #ALX-270-445
	GC-1	1 µM	Tocris, #4554
	GSiXX	100 nM	Merck Millipore, #565790
	Penicillin - Streptomycin	100U/ml - 0.1mg/ml	Sigma, #P4333
7 (8 days, change medium every second day)	MCDB131 no Glutamine		Life Technologies, #10372-019
	GlutaMAX	2 mM	Thermo Fisher, #35050
	NaHCO3	1.5 g/l	Merck Millipore, #1.06329.0500
	BSA fV	2%	Sigma, #A7030
	Glucose	20 mM	Sigma, #G8769
	ITS-X	1:200	Thermo Fisher, #51500056
	Heparin	10 µg/mL	StemCell Technologies, #07980
	Zinc Sulfate	10 µM	Sigma, #Z0251
	GC-1	1 µM	Tocris, #4554
	Trolox	10 µM	Sigma, #238813
	JNKi (SP600125)	20 µM	Selleckchem, #SP600125
	RSV	75 µM	Sigma, #R5010
	R428	2 µM	Selleckchem, #S2841
	N-acetyl-cystein (NAC)	1mM	Sigma, #A9165
	Penicillin - Streptomycin	100U/ml - 0.1mg/ml	Sigma, #P4333

Supplementary Table S4. List of molecules used for iPSC differentiation into β-like cells.

Antibody	Company	Reference	Dilution
Western blot			
STAT3	Cell Signaling	4904	1/1000
STAT3	Cell Signaling	9139	1/1000
p-Ser727-STAT3	Cell Signaling	9134	1/1000
p-Tyr705-STAT3	Cell Signaling	9145	1/1000
TOMM20	Abcam	ab186735	1/1000
VDAC1	Cell Signaling	4661	1/1000
HDAC2	Cell Signaling	57156	1/1000
COX IV	Cell Signaling	4850	1/1000
Cytochrome *c*	BD biosciences	556432	1/500
STAT1	Cell Signaling	9176	1/1000
GAPDH	TACS	2275-PC-100	1/3000
α-tubulin	Sigma	T5168	1/5000
β-actin	Sigma	A1978	1/5000
Immunofluorescence			
STAT3	Cell Signaling	9139	1/250
Insulin	Dako	A0564	1/2000
COX IV	Cell Signaling	4850	1/250
Glucagon	Sigma	G2654	1/1000
OCT4-A	Cell Signaling	2840	1/500
PDX1	R&D system	AF2419	1/400
Anti-Mouse-Alexa 488	Thermo Fisher	A11029	1/1000
Anti-Guinea-Pig-Alexa 568	Thermo Fisher	A11075	1/2000
Anti-Rabbit-Alexa 568	Thermo Fisher	A11036	1/1000

Supplementary Table S5.   List of antibodies used for Western blot and immunofluorescence analysis.

Gene name (specie)	Primer sequences or catalogue number
STAT3 (human)	F: CTTTGAGACCGAGGTGTATCACC R: GGTCAGCATGTTGTACCACAGG
INSULIN (human)	F: CCAGCCGCAGCCTTTGTGA R: CCAGCTCCACCTGCCCCA
MAF-A (human)	F: GCCAGGTGGAGCAGCTGAA R: CTTCTCGTATTTCTCCTTGTAC
NEUROG3 (human)	F: GACGACGCGAAGCTCACCAA R: TACAAGCTGTGGTCCGCTAT
PDX1 (human)	F: AAAGCTCACGCGTGGAAA R: GCCGTGAGATGTACTTGTTGA
mt-ND4 (human)	F: CCTAACAACCCCCCTCCTAAT R: CGTGATAGTGGTTCACTGGATAAG
mt-ND5 (human)	F: GCAGCCTAGCATTAGCAGGAATA R: GCTCAGGCGTTTGTGTATGA
mt-ND6 (human)	F: GATATACTACAGCGATG R: TCATACTCTTTCCTACCCAC
mt-cytB (human)	F: CGTGTTTGTGTGCCTGCTGG R: CGGTCATGTACTTCTCGTCC
mt-TY (human)	F: GTAAAATGGCTGAGTGAAGC R: GCCTAACCCCTGTCTTTAGA
mt-TW (human)	F: ATTTAGGTTAAATACAGACC R: GAAATTAAGTATTGCAACTT
mt-TQ (human)	F: TCTTGTAGTTGAAATACAAC R: TCTCGCACGGACTACAACCA
mt-TL1 (human)	F: ACTTTTAAAGGATAACAGCT R: AATTTTTGGGGCCTAAGA
ATP8 (human)	F: CAACTAAATACTACCGTATG R: GCTTTGGTGAGGGAGGTAGG
ATP6 (human)	F:CATTAACCTTCCCTCTACACT R: GTAGGCTTGGATTAAGGCGA
GAPDH (human)	F: CAGCCTCAAGATCATCAGCA R: TGTGGTCATGAGTCCTTCCA
β-actin (human)	F: CTGTACGCCAACACAGTGCT R: GCTCAGGAGGAGCAATGATC
mt-ND4 (mouse, Taqman)	Mm04225294_s1
mt-ND5 (mouse, Taqman)	Mm04225315_s1
mt-cytB (mouse, Taqman)	Mm04225271_g1
UCP2 (mouse, Taqman)	Mm00627599_m1
β-actin (mouse, Taqman)	Mm00607939_s1

Supplementary Table S6. List of probes used for qPCR. Real-time quantitative PCR was performed using the Biorad CFX96 machine (Biorad, Hercules, CA, USA) and the SYBR green PCR Master Mix (Biorad). F: forward R: reverse.
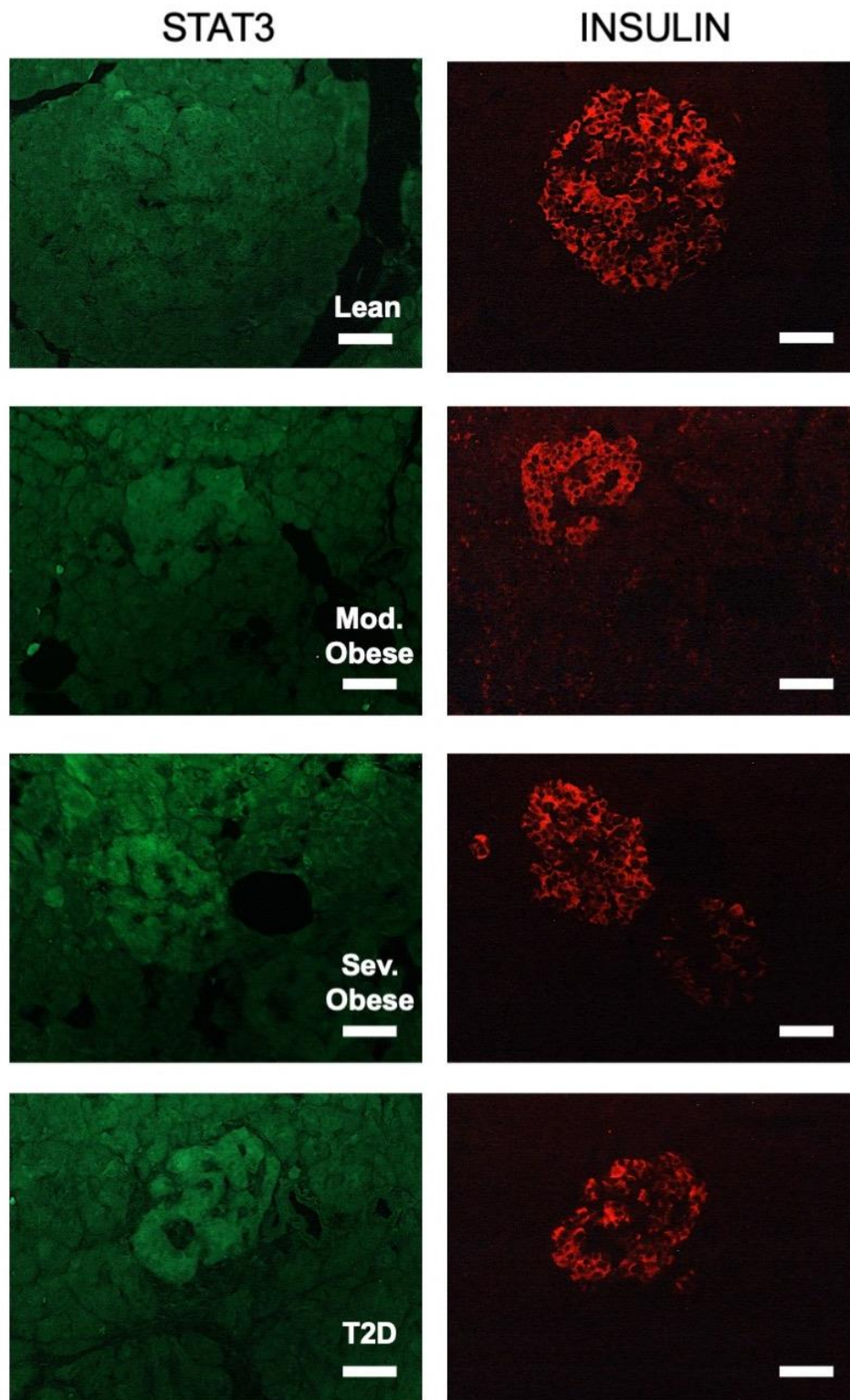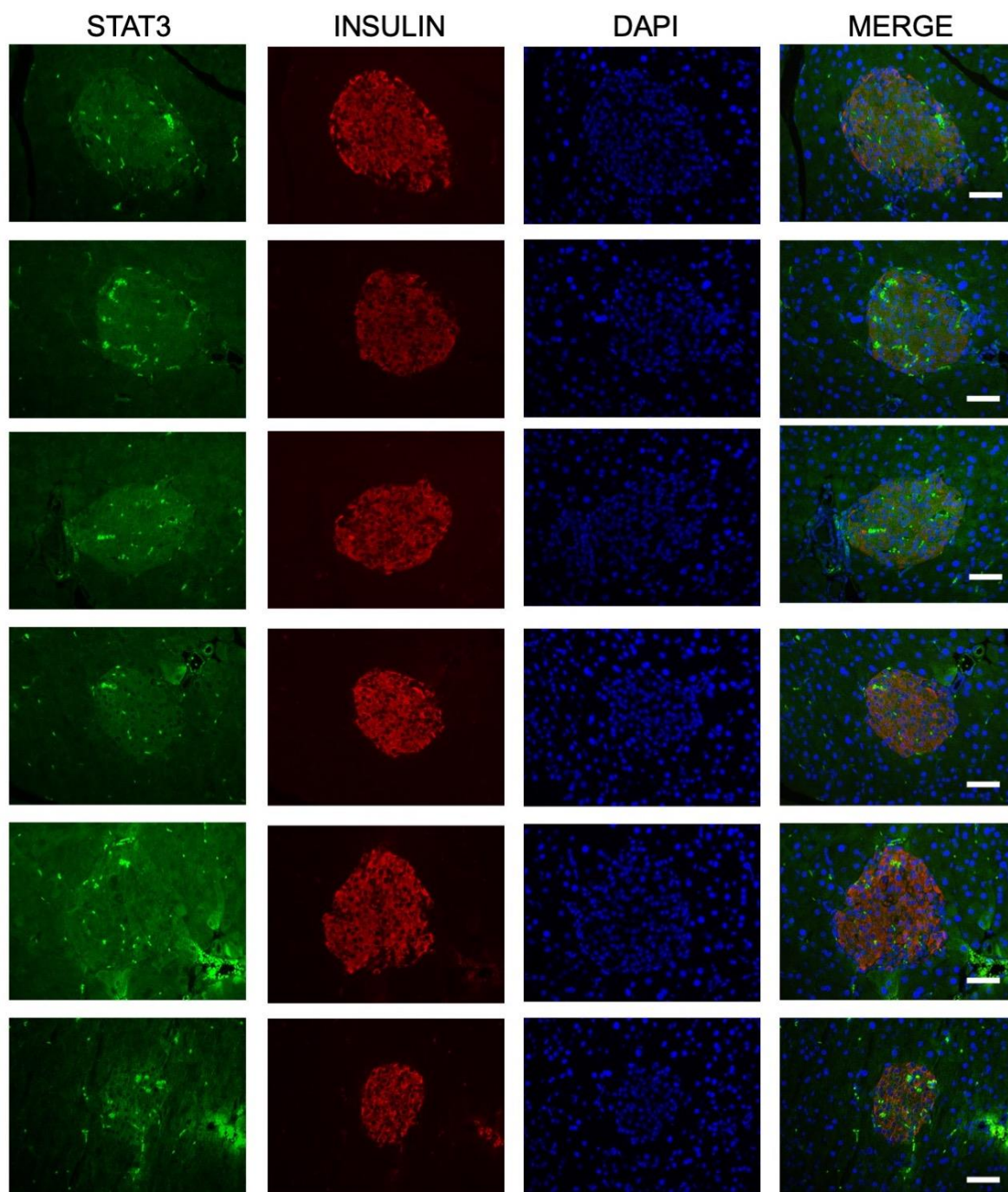
Supplementary Figure S1. STAT3 is expressed in the cytoplasm of islet cells from human subjects. Representative immunofluorescent staining of STAT3 (green) and insulin (red) in sequencing pancreas cuts from Lean (BMI<30kg/m²), Moderately obese (30 kg/m²<BMI<35 kg/m), Severely obese (BMI>35 kg/m) and type 2 diabetes (T2D) organ donors. Scale bar: 50μm.
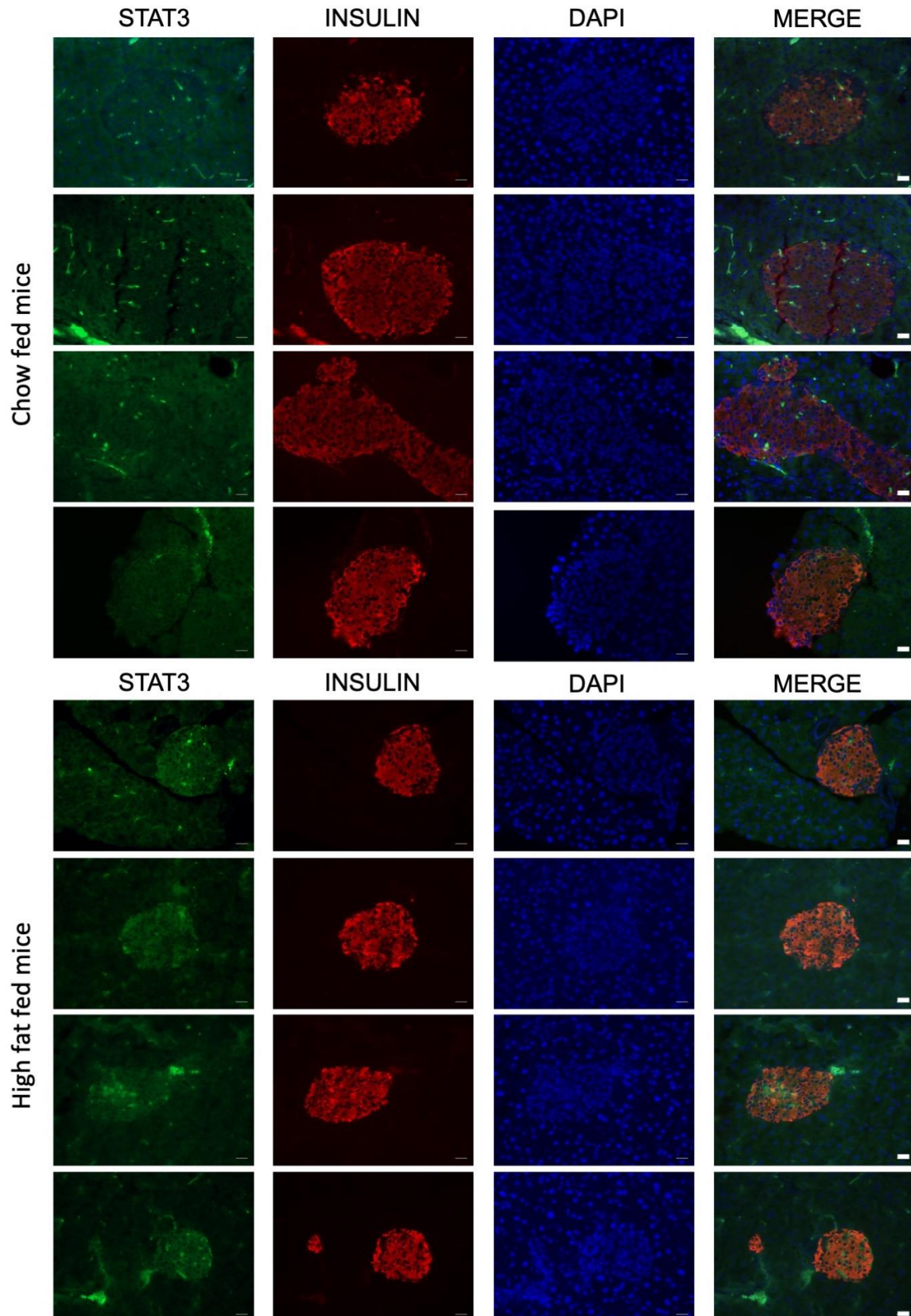
Supplementary Figure S2. STAT3 expression is enhanced in mouse islet cells from obese mice. Representative immunofluorescent staining of STAT3 (green), insulin (red) and DAPI (blue) in pancreas from C57BL/6 mice fed a high-fat diet for 14 weeks or a standard chow diet used as controls (n=3-4). Scale bar: 20µm.

Supplementary Figure S3. STAT3 is expressed in the cytoplasm in mouse islet cells from obese mice. Representative immunofluorescent staining of STAT3 (green), insulin (red) and DAPI (blue) in pancreas from C57BL/6 mice fed a high-fat diet for 14 weeks (n=3). Scale bar: 50μm.
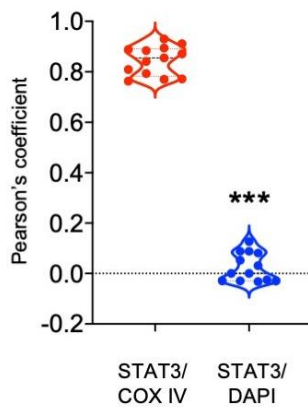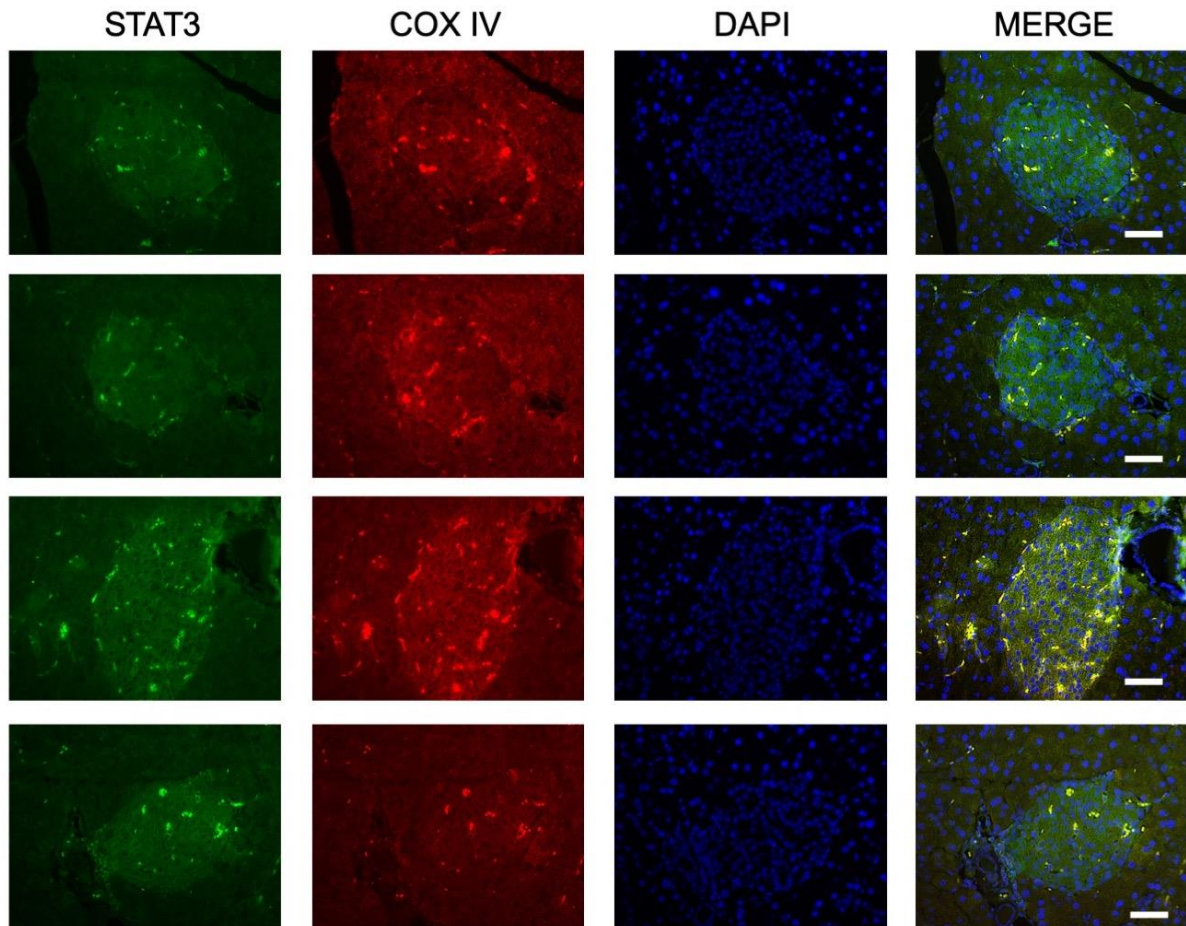
Supplementary Figure S4. STAT3 is localized in the mitochondria in islet cells from obese mice. STAT3 staining colocalized with COX IV, a mitochondrial marker, indicating that STAT3 is present in the mitochondria in the islet cells. Representative immunofluorescent staining of STAT3 (green), COX IV (red) and DAPI (blue) in pancreas from C57BL/6 mice fed a high-fat diet for 14 weeks (n=3). Protein co-localisation was estimated using Pearson's coefficient. Scale bar: 50µm. ***$p < 0.001$.
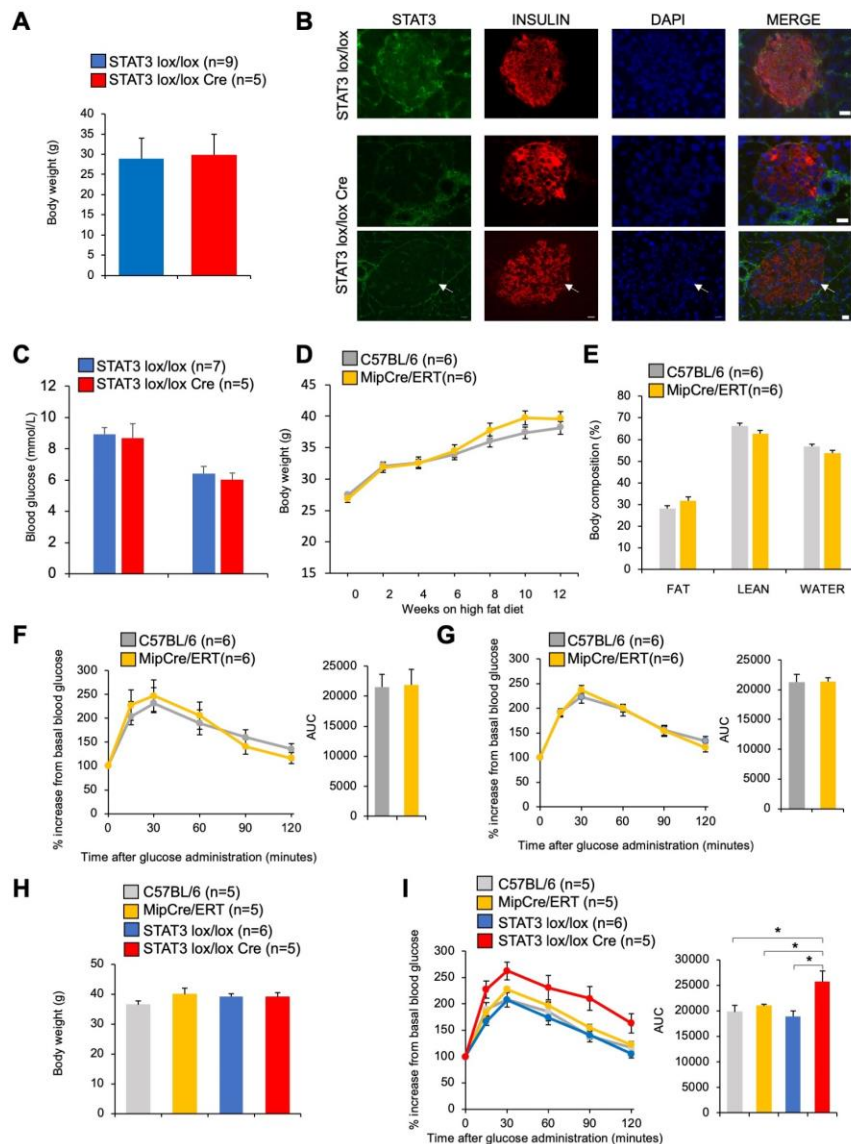
Supplementary Figure S5. The MipCre/ERT promoter insertion did not affect body weights and glucose homeostasis in high fat fed mice. (**A**) Following administration of tamoxifen, 10-week old STAT3$^{lox/lox}$Cre and STAT3$^{lox/lox}$ littermate control male mice were maintained on a chow diet. Body weights were measured at 27 weeks of age (n=6). (**B**) Tamoxifen-treated 10-week old STAT3$^{lox/lox}$ and STAT3$^{lox/lox}$ Cre littermate male mice were maintained on a high-fat diet for 12 weeks. Cryosections of pancreas were stained with antibodies recognizing STAT3 and insulin. STAT3 positive and insulin negative cells is shown (white arrows). Bar, 20 µm. (**C**) At the end of high-fat diet, STAT3$^{lox/lox}$Cre and STAT3$^{lox/lox}$ mice were fasted for 18 hours prior measuring fasted blood glucose. (**D**) Following administration of tamoxifen, 10-week old C57BL/6 and MipCRE/ERT littermate male mice were maintained on a high-fat diet for 12 weeks. Body weights were measured every 2 weeks. (**E**) Body composition (Fat/Lean and water mass) of C57BL/6 and MipCRE/ERT after 12 weeks of high fat feeding. (**F-G**) Intraperitoneal (IP) glucose tolerance test on C57BL/6 and MipCRE/ERT after 4 (F) and 12 (G) weeks of high fat feeding. Glucose at 2g/kg of body weight concentration was IP injected to mice and blood glucose measured for 2h as indicated. Areas under the curve (AUC) were calculated. (**H**) Following administration of tamoxifen, 10-week old STAT3$^{lox/lox}$Cre, STAT3$^{lox/lox}$, MipCRE/ERT and C57BL/6 littermate control male mice were maintained on a high fat diet for 12 weeks and body weights measured. (**I**) Oral glucose tolerance test on 12-week high fat fed STAT3$^{lox/lox}$Cre, STAT3$^{lox/lox}$, MipCRE/ERT and C57BL/6 mice. AUC were calculated. *p< 0.05.
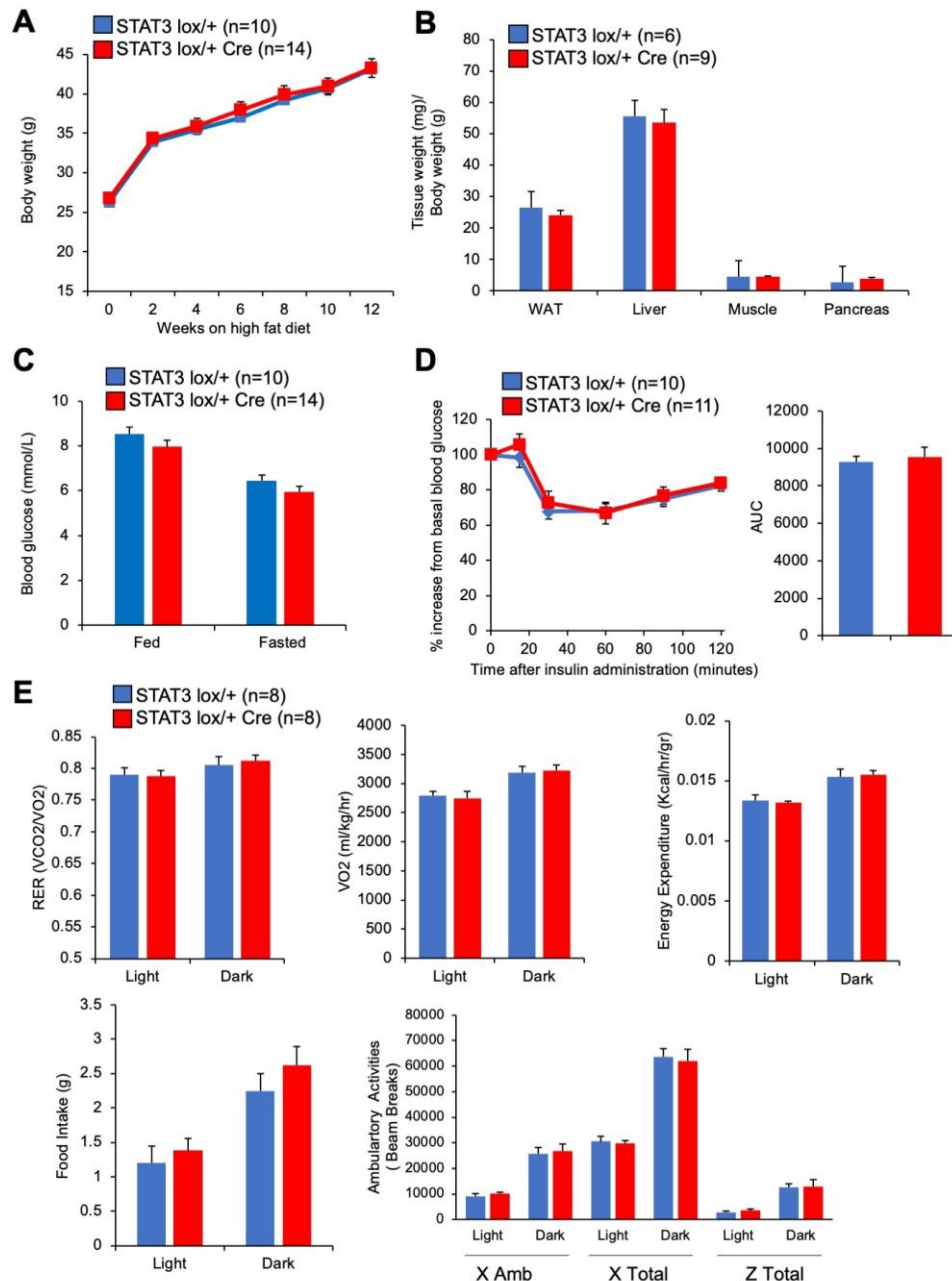
Supplementary Figure S6. Effect of partial STAT3 deletion on mouse energy expenditure.
(**A**) Following administration of tamoxifen, 10-week old STAT3 lox/+ and STAT3 lox/+ Cre littermate male mice were maintained on a high-fat diet for 12 weeks. Body weights were measured every 2 weeks. (**B**) Tissues were harvested from high-fat fed STAT3[lox/+]Cre and STAT3[lox/+] control male mice and the relative weights for white adipose tissue (WAT), liver, gastrocnemius muscle and pancreas were measured. (**C**) At the end of high-fat diet, STAT3[lox/+]Cre and STAT3[lox/+] mice were fasted for 18 hours prior measuring fasted blood glucose. Blood samples were obtained through a tail-nick and glucose concentrations were measured using a glucometer. Fed blood glucose was determined using the same method but without fasting. (**D**) Intraperitoneal insulin tolerance tests were performed on STAT3[lox/+]Cre and STAT3[lox/+] control male mice 12 weeks after high-fat feeding. Mice were fasted for 4 hours and insulin was injected into the intraperitoneal cavity at 0.65mU/g (starting blood glucose: 11.53 ± 1.24mM STAT3[lox/+]Cre and 10 ± 1.49mM STAT3[lox/lox]). AUC were calculated. (**E**) STAT3[lox/+]Cre and STAT3[lox/+] control mice were fed on a high-fat diet for 12 weeks. Oxygen consumption (VO$_2$), respiratory exchange ratios (RER= VO$_2$/VCO$_2$), energy expenditure, daily food intake and ambulatory activity were evaluated in 2 consecutive light and dark cycles using the Comprehensive Laboratory Animal Monitoring System (CLAMS).
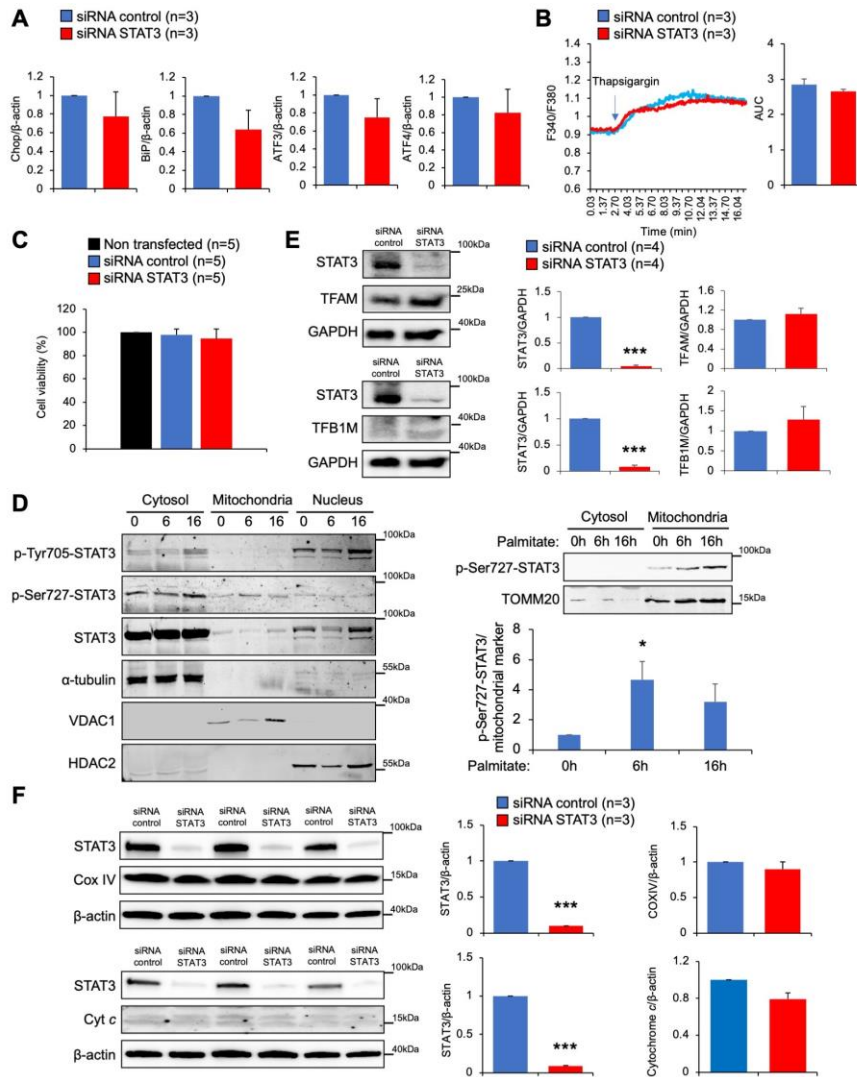
Supplementary Figure S7. Assessment of ER stress, calcium flux, cell viability and nuclear-encoded mitochondrial genes in STAT3 knockdown EndoC-βH1 cells and STAT3 localisation in MIN6 cells. (**A**) qPCR analysis of ER stress markers Chop, Bip, ATF3 or ATF4 on STAT3 knockdown EndoC-βH1. Results were normalized with β-actin and GAPDH as housekeeping genes. (**B**) Assessment of calcium exchange between ER and cytoplasm. Cells were pre-stained with FURA-2M and the baseline was recorded for 4 minutes using a camera-based image analysis system (MetaFluor, Universal Imaging, Ypsilanti, MI). Time-frequency analysis of spectral densities of the Ca^{2+} oscillations was computed using AcqKnowledge Software, (Biopac Systems, Goleta, CA), with a Hamming window. We computed the power spectral density (PSD), the integral below the power spectrum for the frequency band 0–0.17 Hz, and the crest factor for the same frequency band (ratio amplitude of the power spectra/integral 0–0.17 Hz). Thapsigargin, as a SERCA2 calcium pump inhibitor which will deplete calcium for the ER, have been added after the equilibrium period. (**C**) Cell viability analysis after 72h of EndoC-βH1transfected of not with siRNA control or siRNA STAT3. The CellTiter 96® AQueous (Promega) cell viability assay was performed on each sample according to the manufacturer's instructions. (**D**) Cellular fractionation and p-Tyr705, p-Ser727 and total STAT3 localization in palmitate treated MIN6 cells. p-Ser727-STAT3 in the mitochondria fraction was quantified (n=3). (**E**) Western blot analysis of nuclear-encoded mitochondrial transcription factors TFAM and TFB1M in STAT3 knockdown and control EndoC-βH1 cells. GAPDH served as loading control. (**F**) Indirect mitochondrial mass assessment using nuclear-encoded COX IV and cytochrome *c* in STAT3 knockdown and control EndoC-βH1 cells. β-actin served as loading control. *p< 0.05, ***p< 0.001.
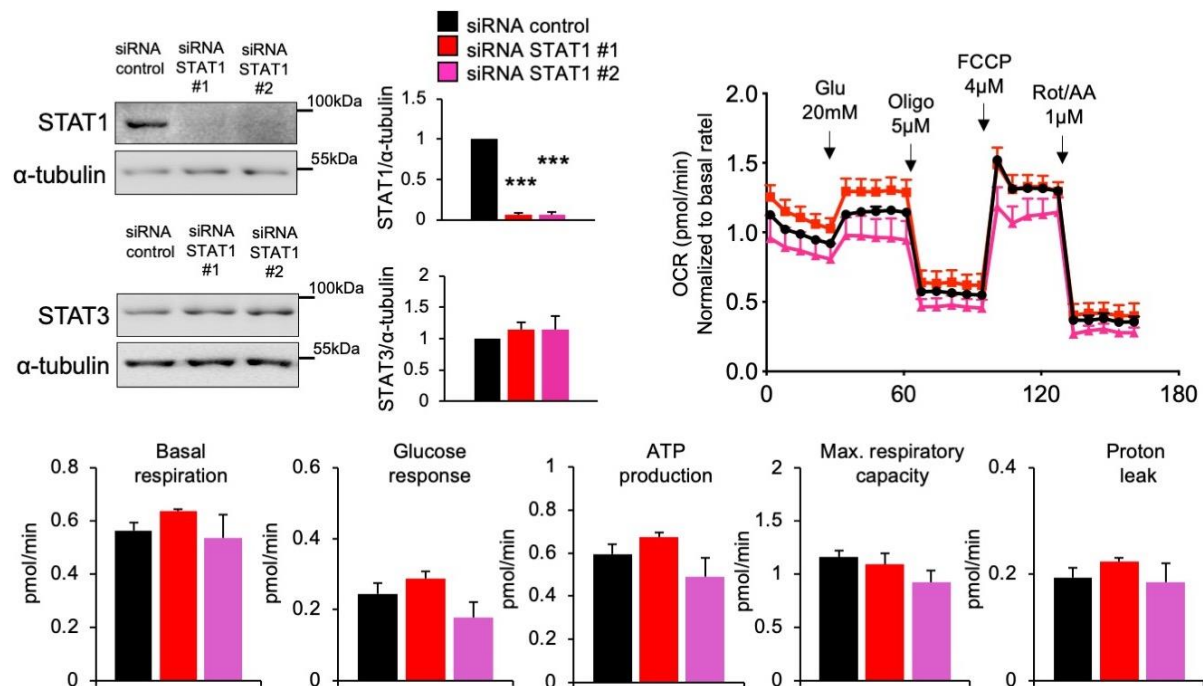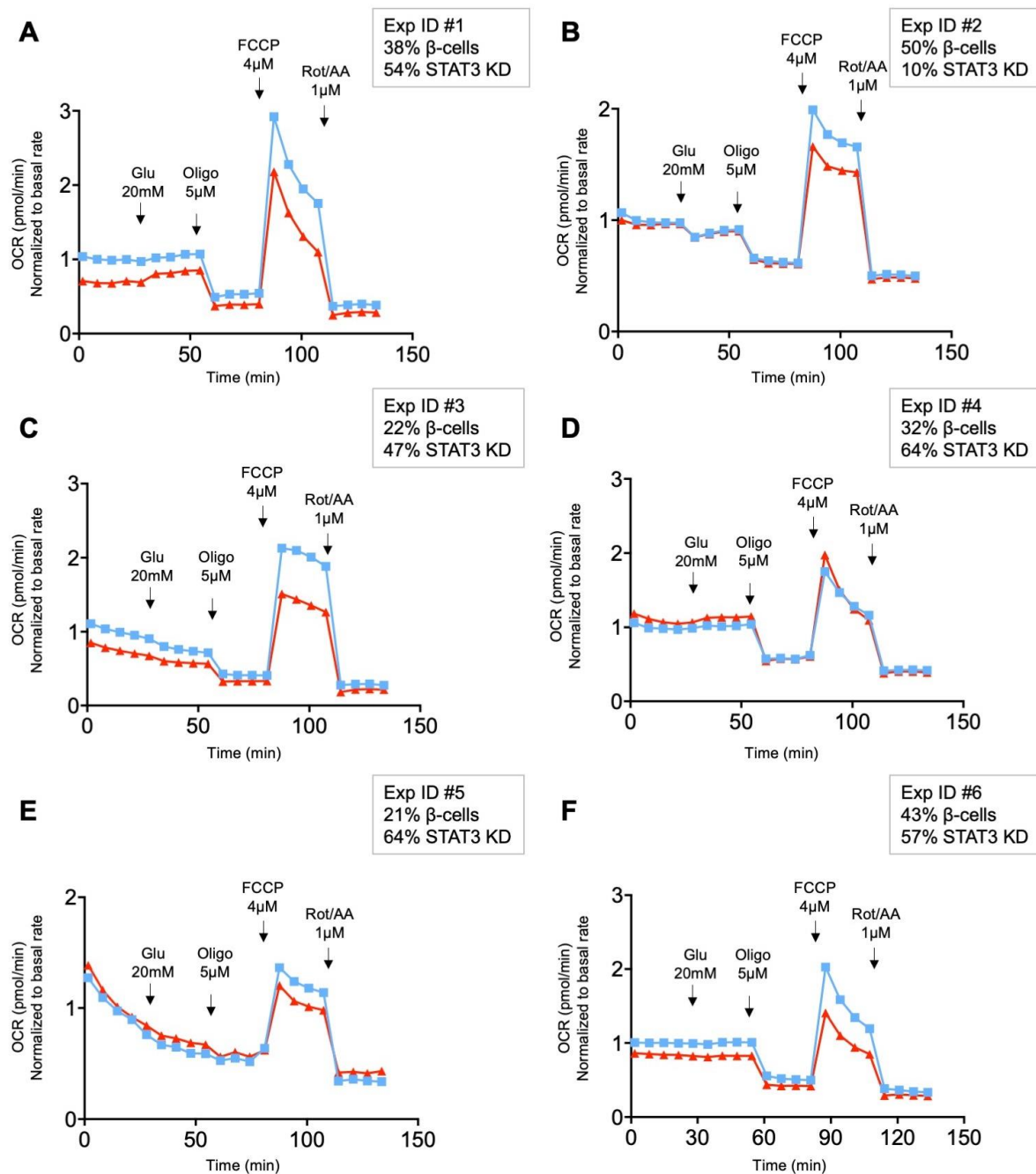
Supplementary Figure S8. STAT1 knockdown does not affect mitochondrial function in EndoC-βH1 cells. Measurement of the oxygen consumption rates (OCR) in STAT1 knockdown EndoC-βH1 cells (n=4). Western blot showing specific knockdown of STAT1 by siRNAs, α-tubulin served as loading control. OCR were measured using a Seahorse analyser and normalized to basal rate of control cells. Glu: Glucose 20mM; Oligo: Oligomycin 5µM; FCCP: Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone 4µM; Rot/AA: Rotenone/Antimycin A 1µM. ***p< 0.001.
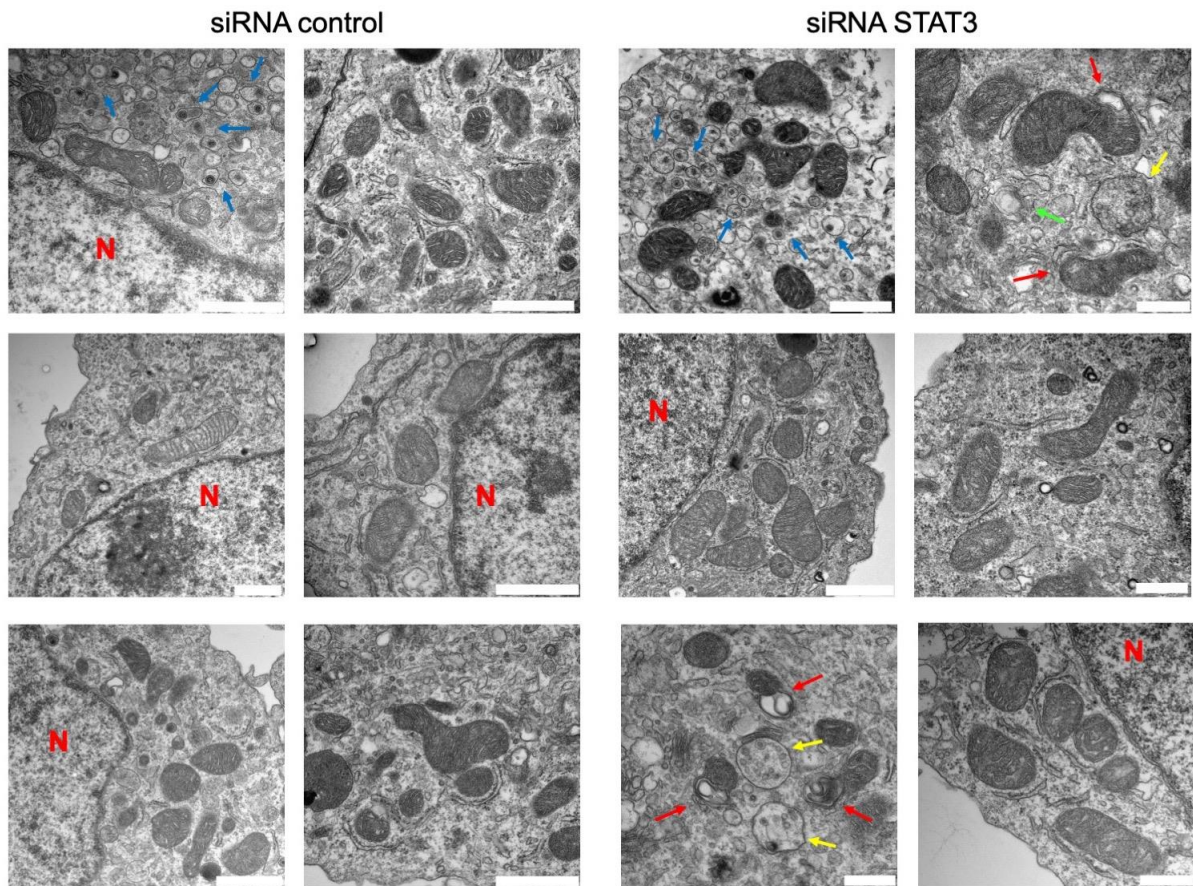
Supplementary Figure S9. TEM of deficient STAT3 EndoC-βH1 cells. Samples transfected for 72h with siRNA control or siRNA for STAT3, were fixed and processed for embedding in epoxy resin, sectioned by ultramicrotomy and analysed by TEM (857-728 mitochondria were quantified). Blue arrows show insulin granules. Red arrows mark the cristae destruction and mitochondria swelling. Yellow arrows show autophagosome structures. Green arrow shows mitophagy. N, nucleus. Scale bar: 500nm.

Supplementary Figure S10. Detailed mitochondrial function following Seahorse analysis of the 6 (A-F) human islet preparations used for STAT3 knockdown (KD). OCR were measured using a Seahorse analyser and normalized to basal rate of control cells. Glu: Glucose 20mM; Oligo: Oligomycin 5µM; FCCP: Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone 4µM; Rot/AA: Rotenone/Antimycin A 1µM.
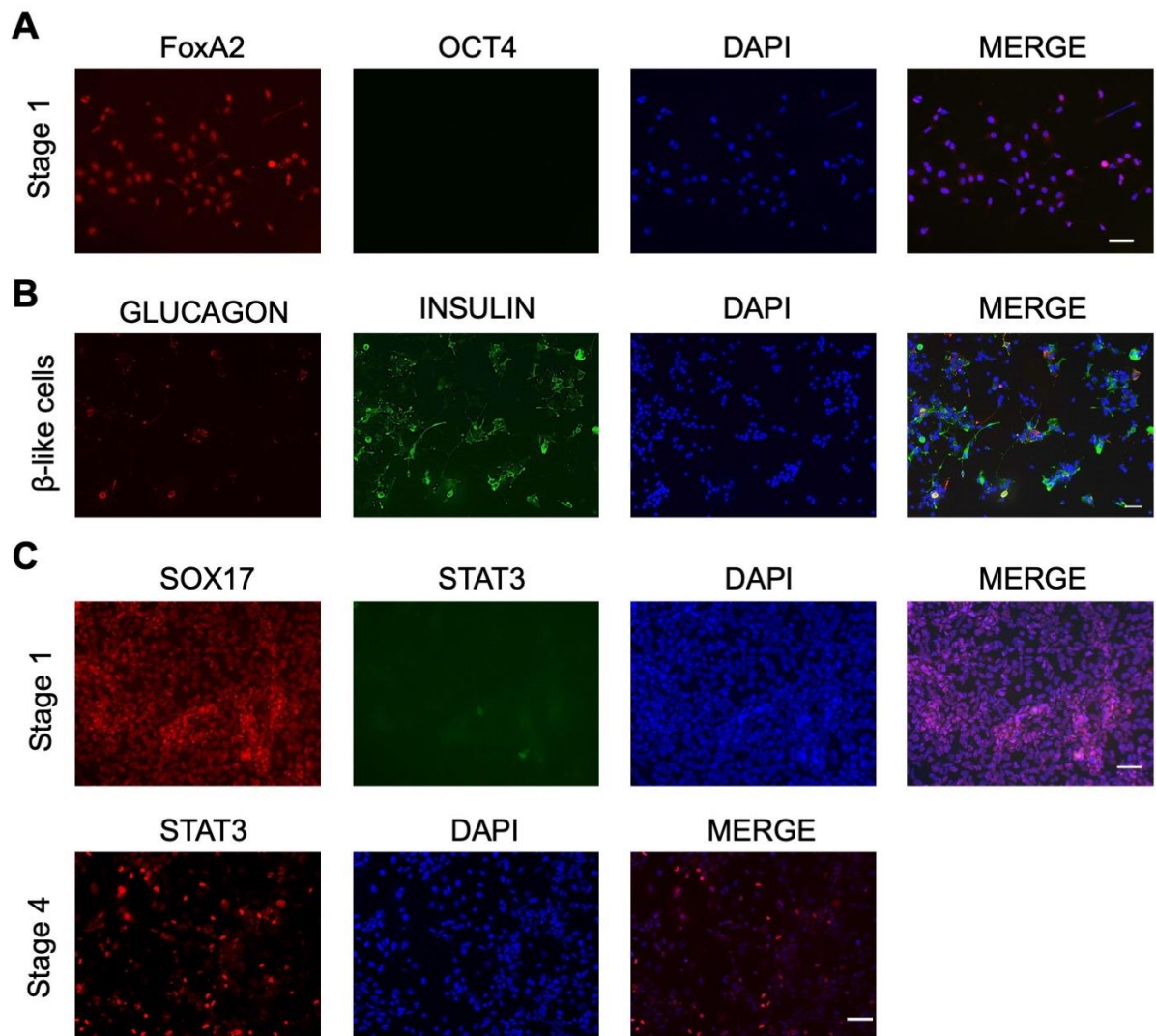
Supplementary Figure S11. Immunofluorescence staining of transition markers in different stages of β-like cell differentiation. (**A-C**). Representative immunofluorescent staining of FoxA2 (red)/OCT4 (green) markers in stage 1 (A), glucagon (red)/insulin (green) in stage 7 (B), SOX17 (red)/STAT3 (green) in stage 1 and STAT3 (red) in stage 4 (C). Scale bar: 50μm