Supplementary Materials

Nrf2 Regulates β-cell Mass by Suppressing Cell Death and Promoting Proliferation

Supplemental Table 1: Primers sequences used in this paper for qPCR

Gene Name	Forward sequence	Reverse Sequence
ACTIN	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA
NRF2	AGGACATGGAGCAAGTTTGG	TTCTTTTTCCAGCGAGGAGA
NQO1	GAAGGAGGCTGCTGTAGAGG	ATCACCAGGTCTGCAGCTTC
KEAP1	GATCGGCTGCACTGAACTG	GGACTCGCAGCGTACGTT
CCNA1	CACAGAGAACCGTGCTAGGG	CACTTTCTTTCCAGCCGCAG
CCNA2	GAGGGCGATCCTTGTGGAT	CACAGCCAAATGCAGGGTCT
CCNB1	TGTGTGTGAACCAGAGGTGGAACT	AGATGTTTCCATCGGGCTTGGAGA
CCNB2	GGCTGGTCCAAGTCCATTCC	GTCCATGATGGCAATGCACA
CCNB3	TTGCTGAACTTCCGATCCCA	TTGCTTGCTCAAAGGAGGGA
CCND1	CAGAAGTGCGAAGAGGAGGTC	TCATCTTAGAGGCCACGAACAT
CCND2	TGTGGATTGTCTCAAAGCCTG	CAACATCCCGCACGTCTGTA
CCNE1	GACACAGCTTCGGGGTGC	AACTCAGACCTGGGAGGACA
CCNE2	TGCTGCCGCCTTATGTCATT	TCGGAGATGTCATCCCATTCC
CDK1	CTGCAGCTCGGAGCACAGTT	CCAGAACACGGAGGCACTTG
CDK2	AGCCAAGTTTCCCCAAGTGG	TTTGGGAAGGGCATCAGAGC
CDK4	AGACCAGGACCTGAGGACAT	TCAGGTCCCGGTGAACAATG
CDK6	GGCTATGGGAAGGTGTTCAA	GGGCTCTGGAACTTTATCCA
CDC25A	CACGGGGAAGATGCTGTTTG	AACAAGACAGGATGCCCAGC
P15	GGGGGCAAGTGGAGACGG	CTTCCCGAGCTGCGTCGT
P16	GTCTTTGTGTACCGCTGGGA	GCCGGATTTAGCTCTGCTCT
P18	ACGTCAACGCTCAAAATGGA	TGGGATTAGCACCTCTGAGGA
P21	GTGGCCTTGTCGCTGTCTT	GCGCTTGGAGTGATAGAAATCTG
P27	GGTGGACCAAATGCCTGACT	TGGCCCTTTTGTTTTGCGAA
P57	GGTGTCCCTCTCCAAACGTG	TGCCCAGCAAGTTCTCTCTG
INS1	TAT AAA GCT GGT GGG CAT CC	GGG ACC ACA AAG ATG CTG T
INS2	TTT GTC AAG CAG CAC CTT T	AGG TTT TCT CGC CCC TTA AC
MAFA	ATCATCACTCTGCCCACCAT	AGTCGGATGACCTCCTCCTT
GLUT2	GGCACAGACACCCCACTTAC	GCCAACATTGCTTTGATCCT
PDX1	CTCCGGACATCTCCCCATAC	ACGGGTCCTCTTGTTTTCCT
NKX6.1	CGCCCGGGCTCTACTTTAG	GTCCAGAGAACGTGGGTCTG

PGD	CTCGGTGCTTGTCCTCTCTG	TTGAGGGTCCAGCCAAACTC
G6PD	CAACAGGATCTTTGGCCCCA	ACGGACATCATCTGAACCTGT
IDH1	AGCTTCATCGCAACCCAGAA	GTAATAAGCCGGCCCCAGTT
HO1	CGCATATACCCGCTACCTGG	TTACCTTGCACCAGGCTAGC
GCLC	GGACAAACCCCAACCATCC	GTTGAACTCAGACATCGTTCCT
GCLM	CTTCGCCTCCGATTGAAGATG	AAAGGCAGTCAAATCTGGTGG
CAT	GGAGGCGGGAACCCAATAG	GTGTGCCATCTCGTCAGTGAA
SOD1	AACCAGTTGTGTTGTCAGGAC	CCACCATGTTTCTTAGAGTGAGG
SOD2	TGGACAAACCTGAGCCCTAAG	CCCAAAGTCACGCTTGATAGC
GPX1	CCACCGTGTATGCCTTCTCC	AGAGAGACGCGACATTCTCAAT
GST-M	CTGAAGGTGGAATACTTGGAGC	GCCCAGGAACTGTGAGAAGA
TRX1	CCCTTCTTCCATTCCCTCT	TCCACATCCACTTCAAGGAAC
TXNRD1	GTGGCGACTTGGCTAATC	ACCAGGAGAGACACTCAC

Supplemental Table 2: Human donor cadaveric islets used in this paper

Donor ID	Age (years)	Sex	Race	%HbA1c (%mmol/mol)	BMI	COVID19	Cause of Death	Used in
SAMN1295912	37	F	C	5.3 (34)	26.3	N/A	S	Fig. 6D-E
SAMN13570019	37	F	C	5.2 (33)	24	N/A	A	Fig. 6D-E
SAMN13881228	34	M	H	5.6 (38)	28.1	N/A	HT	Fig. 6D-E
SMN12713942	41	M	C	5.2 (33)	23.5	N/A	S	Fig. 6F
SAMN12924398	38	M	H	5.4 (36)	28	N/A	HT	Fig. 6F
SAMN1295912	37	F	C	5.3 (34)	26.3	N/A	S	Fig. 6F
SAMN16427178	42	F	C	5.5 (37)	31.2	Negative	S	Fig. 6J-K
SAMN13881228	34	M	H	N/A	28.1	N/A	HT	Fig. 6J-K
HP-20220-01	48	M	AF	5.2 (33)	20.2	N/A	S	Fig. 6J-K
HP-20152-01	21	M	H	5.1 (32)	27.3	Negative	HT	Fig. 6J-K
HP-20199-01	48	F	C	5.8 (40)	30.9	Negative	S	Fig. 6J-K
SAMN21400456	54	M	C	5.3 (34)	29.3	Negative	HT	Fig. 6D-F
SAMN22814513	26	M	C	5.4 (36)	29.2	Negative	HT	Fig. 6D-F

M = males; F = female; H = Hispanic; C = Caucasian; AF = African American; HT = Head trauma; S = Stroke; A = Anoxia

Supplemental Table 3: Antibodies used in this paper

Antibody	Brand and Catalogue number	Used in Figures
Insulin	Dako #A056401 RRID:AB_2617169	1B-D, 1F-H, 4B-D,6A-E
Insulin	Genetex #GTX39371 RRID:AB_11177138	2A-F, 2I-K, 3A-D, 3G-H, 5E-F,5I-J, 5M-R, 6H-I, Supp Fig 3A-B,
Insulin	Developmental Studies Hybridoma Bank, University of Iowa, GN-ID4 RRID:AB_2255626	6J-K, Supp Fig 6E.
Insulin	R&D #MAB1417 RRID:AB_2126533	2N-O, 3E-F
Ki67	Invitrogen #MA5-14520 RRID:AB_10979488	1F-G, 2I-J,4C-D, J,6A-E,-6H-K, Supp Fig 2B
Nrf2 (C-Term)	Santa Cruz sc-722 RRID:AB_2108502	1C, 1H, Supp Fig. 1B
Nrf2 (C-Term)	Cayman Chemicals #10214	2A,2E,5E-F
Nrf2-p	Abcam #ab76026 RRID:AB_1524049	1B,1D, 4B, Supp Fig 1C, Supp Fig 1E, Supp Fig 6B.
Nrf2	Proteintech #16396-1-AP RRID:AB_2782956	2G-H, 5G-H
Nqo1	Abcam #ab2346 RRID:AB_302995	2C
Keap1	Cell Signaling #8047s RRID:AB_10860776	Supp Fig.2A
Keap1	Abcam #ab227828	5C-D
Anti-DNA/RNA Oxidative Damage	Abcam #ab62623 RRID:AB_940049	3G-H
Pdx1	Abcam #AB47308 RRID:AB_777178	3E-F
Cyclin D1	Proteintech #60186-1-Ig RRID:AB_10793718	5K-L
Cre recombinase	Millipore Sigma #MAB3120 RRID:AB_2085748	Supp Fig.1G-H

Supp. Figure 1. Acute exposure of INS1 to high glucose stimulates β-cell proliferation. (A) INS-1 832/13 cells pre-treated with a ROS-sensitive probe, H2DCFDA, were cultured in 20 mM glucose for 5-20 min. 100 μM H_2O_2 was used as a positive control. Generation of intracellular ROS was measured by plate reader (Ex/Em: 495/520 nm) according to the manufacture's instructions. (B) INS-1 832/13 cells were cultured in 20 mM glucose for up to 48 h. The cells were fixed and immunolabeled using an Nrf2 antibody. (C) INS-1 832/13 cells were cultured in 2 mM or 20 mM glucose for 5 min in the presence or absence of the antioxidant agent N-acetylcysteine (NAC) (20 mM). 100 μM H_2O_2 was used as a positive control. The cells were fixed and immunolabeled using an Nrf2-p antibody. (D) INS-1 832/13 cells were incubated in 2 mM or 20 mM glucose for 6 h. RNA was extracted and expression of Nqo1 was measured. (E,F) INS-1 832/13 cells were incubated in 2 mM or 20 mM glucose and with indicated concentrations of brusatol for 72 h. Cells were fixed and immunolabeled using Nrf2 or Ki67 antibodies. (G,H)) Islets from C57Bl/6 mice were isolated, dispersed and transduced with adenovirus expressing either LacZ or Cre, followed by immunostaining with insulin and Cre antibodies. Percentage of insulin and Cre positive cells was calculated. Data shown are the mean +/- SE (*p < 0.05; **p < 0.005; ****p < 0.0001).

Supp. Figure 2. *In vivo* loss of Nrf2 function decreases HFD-stimulated β-cell proliferation. (A) Mice were fed with HFD or RD. After one week, mice were euthanized and their pancreata were stained with insulin and Keap1 antibodies. Mean intensity was then calculated for Keap1. (B) MIP-CreER^{TAM} mice were fed on HFD for one week and their pancreata was immunostained with Ki67 and insulin. β-cell proliferation was then analyzed. Data shown are the mean +/- SE (**p < 0.005).

Supp. Figure 3. Nrf2 deletion decreases insulin content *ex vivo*.
Isolated islets from NRF2$^{lox/lox}$ mice were transduced with LacZ or Cre expressing adenoviruses and incubated in 20 mM glucose. After 72 h islets were (A,B) immunolabeled with insulin and mean intensity was calculated, or (C) measured for insulin content, or (D) RNA was extracted and expression of β-cell identity genes was measured. Data shown are the mean +/- SEM (*p < 0.05; ****p < 0.0001).

Supp. Figure 4. No differences of body weight or fasting blood glucose in βNrf2KO mice after 1 month on a HFD. (A) Body weight, (B) Fasting blood glucose, (C) glucose-stimulated insulin secretion was measured in βNrf2KO mice fed on a HFD for 29 days. (D,E) an insulin tolerance test (ITT) was performed in *ad lib* fed mice and the area under the curve (AUC) was calculated. Data shown are the mean +/- SE (*p < 0.05; **p < 0.005; ***p < 0.0005).

Supp. Figure 5. No differences of plasma insulin, body weight or blood glucose in βKeap1KO mice after 1 month on a HFD. (A) Plasma insulin, (B) Non fasting, (C) fasting blood-glucose, (D) and Body weight were measured. (E,F) Insulin tolerance test (ITT) was performed in *ad lib* fed mice and the area under the curve (AUC) was calculated. Data shown are the mean +/- SE (****p < 0.0001).

Supp. Figure 6. CDDO-Me increases Nrf2 levels and stimulate β-cell proliferation in INS-1 832/13 cells (A) INS1 cells were incubated with the indicated concentrations of CDDO-Me for 72 h, followed by the addition of Trypan blue. Viable cells (unstained cells) were counted under a light microscope. (B) INS1 cells were incubated with 20 nM CDDO-Me for 50 min, followed by immunolabeling using an Nrf2-p antibody. (C) C57BL/6 mouse islets were isolated, dispersed and incubated with 20 nM CDDO-Me. Following 24 h, RNA was isolated and mRNA for known Nrf2 target genes (C) and β-cell identity genes (D) were measured. (E) Immunodeficient mice were transplanted with 500 human islets under the kidney capsule. Following a 17-day recovery, daily IP-injections were made with the indicated doses of CDDO-Me for 1 week and kidney grafts were assayed for TUNEL. The percentage of insulin and TUNEL positive cells was calculated. Data shown are the mean +/- SE (*p < 0.05, **p < 0.005, ****p<0.0001).