Genetic deletion of C/EBP homologous protein CHOP reduces oxidative stress, improves beta cell function, and prevents diabetes

Supplemental Information

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SUPPLEMENTAL METHODS

All procedures and materials are described in Methods of the main text with the following addition. **Insulin tolerance tests.** Insulin tolerance tests (ITTs) were performed by measurement of blood glucose concentration after I.P. injection of 1 IU/kg insulin into mice fasted for 6 hrs. Glucose was measured using a OneTouch Ultra glucometer (LifeScan Inc., CA) with a sensitivity of 10 mg/dL.

Statistical Analysis. Supplemental data are represented as the mean ± SEM; The statistical significance of differences between groups was evaluated using the Student t-test or the ANOVA one-way test (Tukey) and denoted as: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplemental Figure Legends

Supplemental Figure 1. *Chop*-null mutation prevents glucose intolerance and causes islet hyperplasia in *eIF2α*$^{S/A}$ HF-fed mice. (A) *Chop*-null mutation prevents glucose intolerance. The area under the curve (A.U.C.) for glucose tolerance tests in Figure 1B were quantified; n = 8-10 male mice per condition. The significant differences were determined as described in Methods. (B, C) *Chop*-null mutation causes islet hyperplasia in HF-fed *eIF2α*$^{S/A}$ mice. Mice were fed a HF diet for 9-10 months prior to harvesting of tissue samples. Beta cell mass relative to total pancreas area was quantified from immunofluorescence images as represented in Figure 1C and described in Methods. Total pancreas insulin contents were measured as described in Methods. For statistical analysis, four mice per condition were analyzed.

Supplemental Figure 2. *Chop*-null mutation does not increase insulin sensitivity in (A) HF-fed *eIF2a*$^{S/A}$ or (B) *Lepr*$^{db/db}$ mice. Insulin tolerance tests (ITTs) were performed as described above in Supplemental Methods for mice treated as described in Figure 1 (A) and Figure 3 (B); n = 8-10 mice (A) or 4-6 mice (B) per condition.

Supplemental Figure 3. *Chop*-null mutation reduces apoptosis and preserves insulin content in E18.5 *eIF2a*$^{A/A}$ beta cells. (A) Pancreata islet morphology and insulin content are shown. Pancreas tissue sections were prepared from E18.5 embryos. Total pancreas insulin was measured in acid:ethanol extracts and normalized to glucagon as described in Methods . Data from 6 litters of mice (n = 2-13 per genotype) are expressed versus the mean content of wild-type *eIF2a*$^{S/S}$ and heterozygous *eIF2a*$^{S/A}$ embryos. (B) Co-labeling of pancreas sections for detection of insulin and apoptotic nuclei is shown and quantified. Pancreas tissue sections were prepared from E18.5 embryos and first labeled for immunofluorescence detection of insulin (red) followed by TUNEL assay (green FITC) as described in Methods. TUNEL positive cells are identified in the photographs by white arrows. Quantitation of apoptotic cell number per confocal field from immunofluorescence images is shown, n = 2-5 animals per condition. The white scale bar (A,B) denotes 20 μm.

Supplemental Figure 4. *Chop*-null mutation prevents glucose intolerance and increases beta cell mass in *Lepr*$^{db/db}$ mice. (A) Glucose tolerance was measured in female mice at 6-7 months of age; n = 8-12 mice per condition. Significant differences between *Lepr*$^{db/db}$/*Chop*$^{+/+}$ and *Lepr*$^{db/db}$/*Chop*$^{-/-}$ are indicated. (B) The A.U.C. from the glucose tolerance data in Figure 3B and Supplemental Figure 4A was determined. (C) *Chop*-null mutation causes islet hyperplasia. Morphometric analysis of beta cell mass quantified from digital images like those shown in Figure 3C); n = 4 mice per condition.

Suplemental Figure 5. *Chop* deletion alters gene expression in islets from *Lepr*$^{db/db}$ mice. Relative levels of the indicated mRNAs were measured by real-time RT-PCR as described in Methods. The fold induction of mRNA levels for each gene was then expressed relative to wild-type levels (*Lepr*$^{db/+}$/*Chop*$^{+/+}$); n = 4-6 mice per condition.

Supplemental Results

Chop-null mutation reduces apoptosis and preserves insulin content in E18.5 eIF2a$^{A/A}$ *beta cells.* To study the role of CHOP in the absence of insulin resistance, we analyzed the effect of *Chop*-null mutation in mice with homozygous *Ser51Ala* mutation in *eIF2α.* Embryonic E18.5 *eIF2α*$^{A/A}$ mice display reduced pancreas insulin content and islet mass that is coupled with ER distension,

possibly due to unregulated protein synthesis (1, 2). Although disruption of the *Chop* gene did not rescue the post-natal hypoglycemia-induced lethality of homozygous *eIF2α*$^{A/A}$ mice (data not shown)(1), the beta cells in islets from E18.5 *eIF2α*$^{A/A}$*/Chop*$^{-/-}$ mice were significantly increased in number and insulin content, and displayed reduced apoptosis (Supplemental Figure 3). Heterozygous *Ser51Ala* mutation did not cause a loss of insulin content or increased beta cell apoptosis in these late stage embryos (Supplemental Figure 3). These findings support the hypothesis that a significant portion of the severe beta cell deficiency in *eIF2α*$^{A/A}$ mice is caused by CHOP. It is perhaps surprising that deletion of the *Chop* gene was protective in homozygous *eIF2α*$^{A/A}$ beta cells, as it has been demonstrated that the PERK/eIF2α pathway is a major contributor to regulation of CHOP expression in studies of cultured mouse embryonic fibroblasts. However, recent studies show that both IRE1/XBP1 and ATF6α (3-7) contribute to CHOP induction. Although the beta cell failure in the homozygous *eIF2α*$^{A/A}$ mice might not directly reflect beta cell alterations associated with insulin resistance in adult animals, the ability for *Chop* deletion to rescue beta cell mass and function in embryonic *eIF2α*$^{A/A}$ mice indicates that *Chop* deletion can protect beta cells from ER stress caused by the *Ser51Ala* mutation in *eIF2α* in the absence of insulin resistance.

Supplemental References

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3. Fawcett, T.W., Martindale, J.L., Guyton, K.Z., Hai, T., and Holbrook, N.J. 1999. Complexes containing activating transcription factor (ATF)/cAMP-responsive-element-binding protein (CREB) interact with the CCAAT/enhancer-binding protein (C/EBP)-ATF composite site to regulate Gadd153 expression during the stress response. *Biochem J* 339 (Pt 1):135-141.
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5. Yamamoto, K., Sato, T., Matsui, T., Sato, M., Okada, T., Yoshida, H., Harada, A., and Mori, K. 2007. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. *Dev Cell* 13:365-376.
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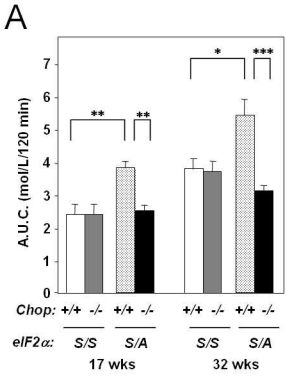
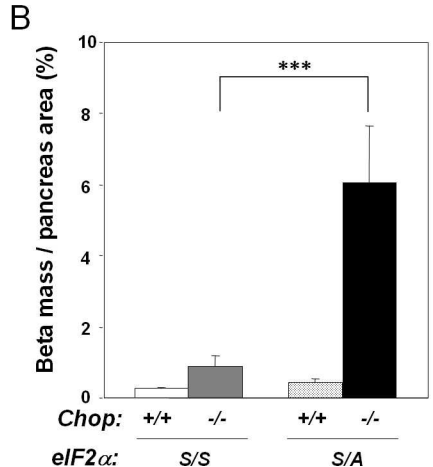
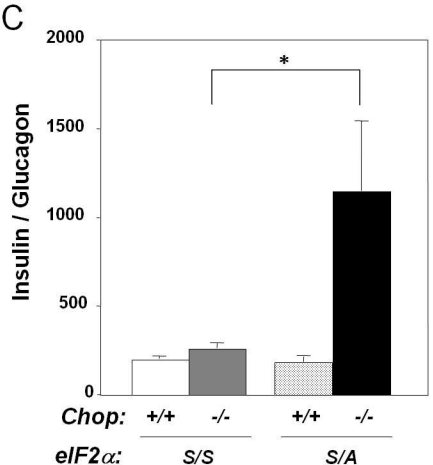
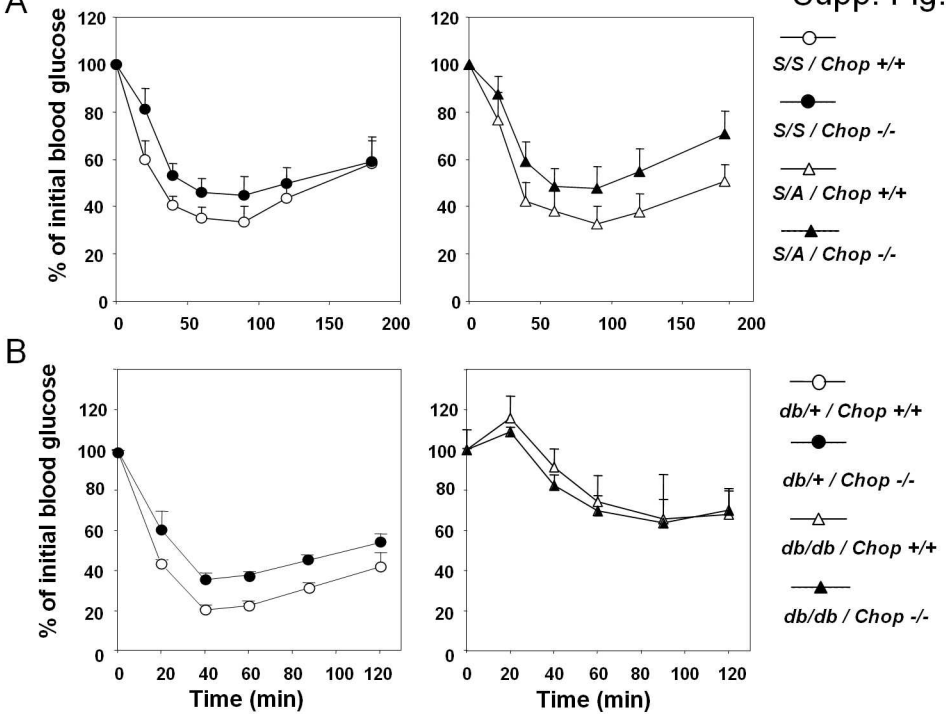
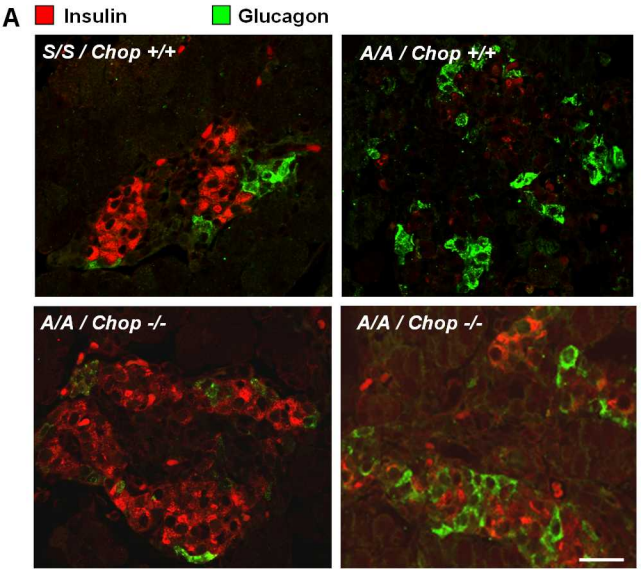

A
A.U.C. (mol/L/120 min)
0
1
2
3
4
5
6
7
**
**
*
***
Chop: +/+ -/- +/+ -/- +/+ -/- +/+ -/-
eIF2α: S/S S/A S/S S/A
17 wks
32 wks

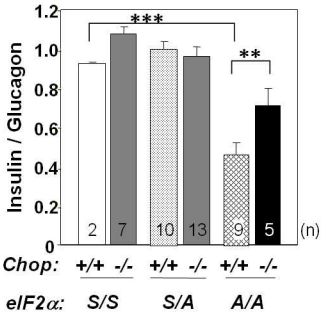

B
Beta mass / pancreas area (%)
10
8
6
4
2
0
***
Chop:
+/+
-/-
+/+
-/-
eIF2α:
S/S
S/A

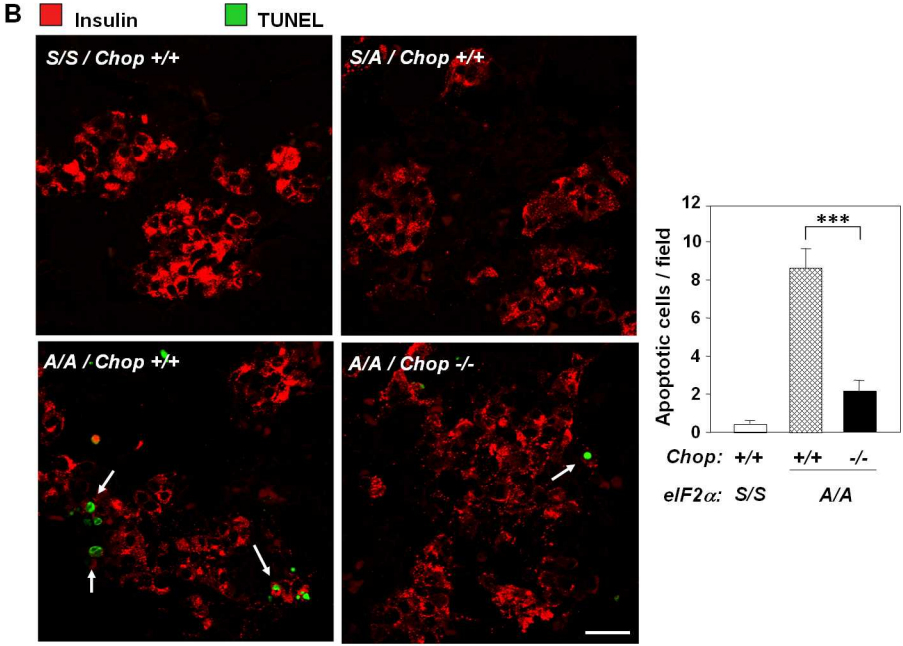

C
Insulin / Glucagon
2000
1500
1000
500
0
*
Chop:
+/+
-/-
+/+
-/-
eIF2α:
S/S
S/A

Supp. Fig. 2

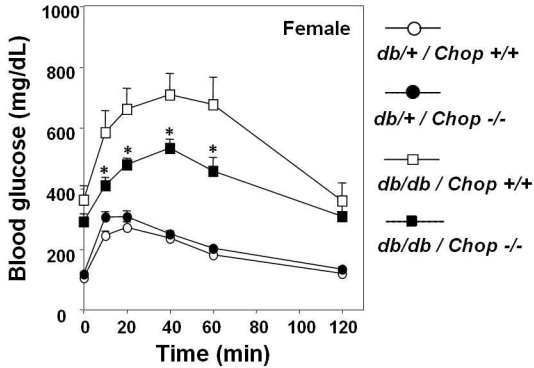

A
% of initial blood glucose
S/S / Chop +/+
S/S / Chop -/-
S/A / Chop +/+
S/A / Chop -/-
B
% of initial blood glucose
db/+ / Chop +/+
db/+ / Chop -/-
db/db / Chop +/+
db/db / Chop -/-
Time (min)
Time (min)


A
Insulin
Glucagon
S/S / Chop +/+
A/A / Chop +/+
A/A / Chop -/-
A/A / Chop -/-

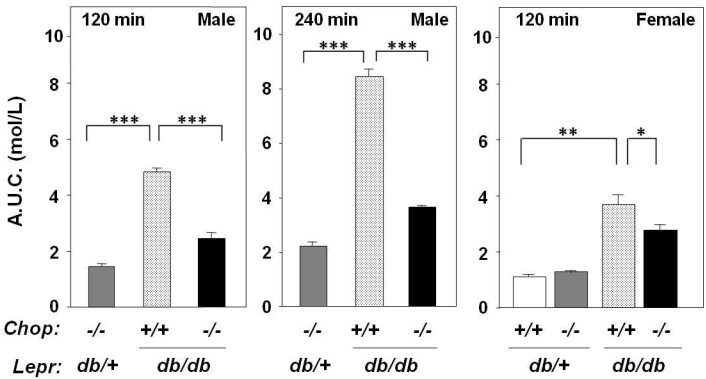

Insulin / Glucagon
1.2
1.0
0.8
0.6
0.4
0.2
0
***
**
2
7
10
13
9
5
(n)
Chop: +/+ -/- +/+ -/- +/+ -/-
eIF2α: S/S S/A A/A

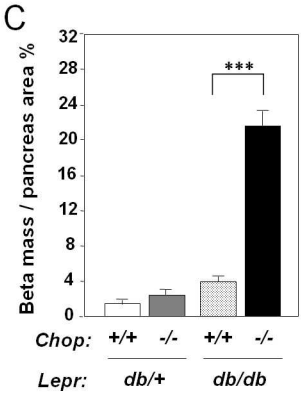

B
Insulin
TUNEL
S/S / Chop +/+
S/A / Chop +/+
A/A / Chop +/+
A/A / Chop -/-
Apoptotic cells / field
12
10
8
6
4
2
0
***
Chop: +/+ +/+ -/-
eIF2α: S/S A/A

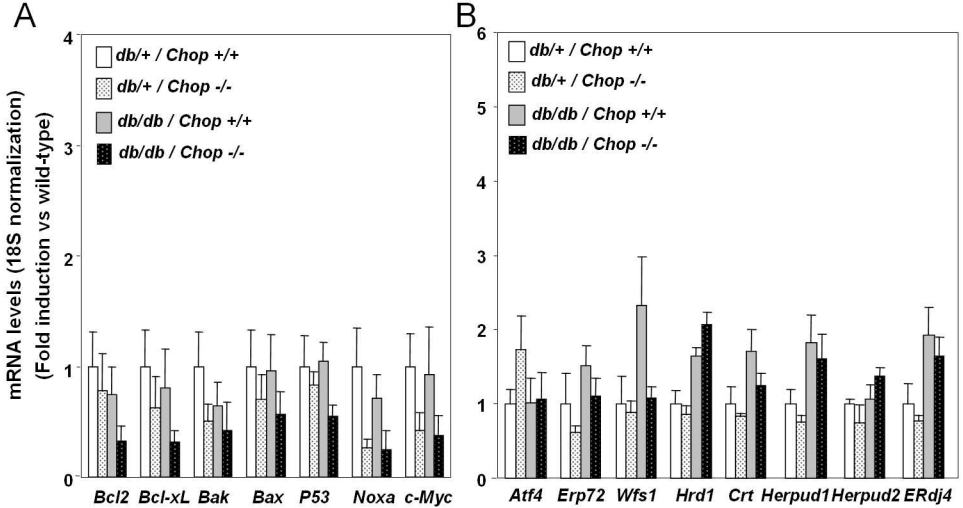
A

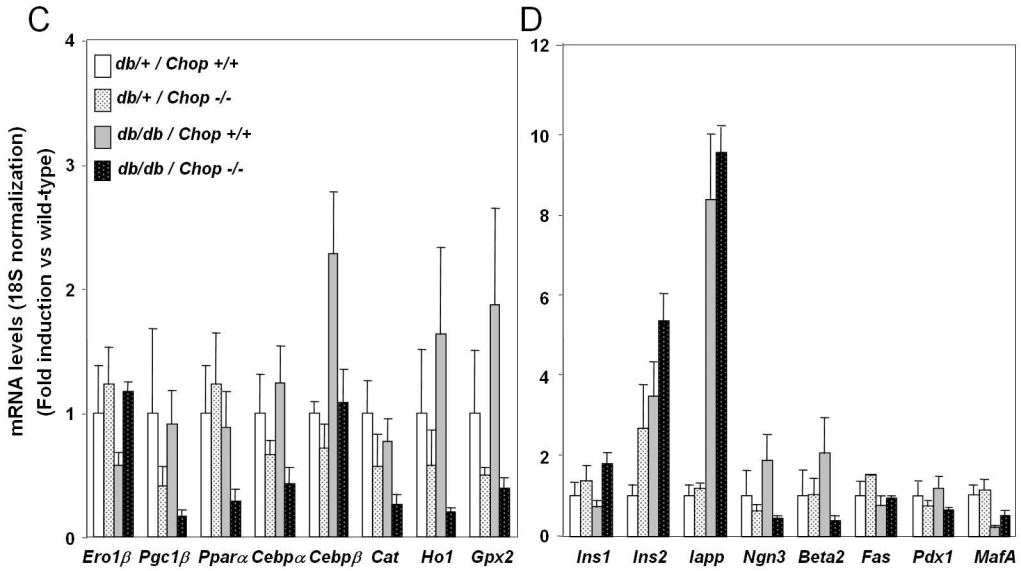

Female
Blood glucose (mg/dL)
1000
800
600
400
200
0
0
20
40
60
80
100
120
Time (min)
*
db/+ / Chop +/+
db/+ / Chop -/-
db/db / Chop +/+
db/db / Chop -/-

B


120 min
Male
240 min
Male
120 min
Female
A.U.C. (mol/L)
0
2
4
6
8
10
***
***
***
***
**
*
Chop: -/- +/+ -/- -/- +/+ -/- +/+ -/- +/+ -/-
Lepr: db/+ db/db db/+ db/db db/+ db/db


C
Beta mass / pancreas area %
32
28
24
20
16
12
8
4
0
***
Chop: +/+ -/- +/+ -/-
Lepr: db/+ db/db


A
B
mRNA levels (18S normalization)
(Fold induction vs wild-type)
db/+ / Chop +/+
db/+ / Chop -/-
db/db / Chop +/+
db/db / Chop -/-
0
1
2
3
4
5
6
Bcl2
Bcl-xL
Bak
Bax
P53
Noxa
c-Myc
Atf4
Erp72
Wfs1
Hrd1
Crt
Herpud1
Herpud2
ERdj4


C
D
db/+ / Chop +/+
db/+ / Chop -/-
db/db / Chop +/+
db/db / Chop -/-
mRNA levels (18S normalization)
(Fold induction vs wild-type)
0
1
2
3
4
Ero1β
Pgc1β
Pparα
Cebpα
Cebpβ
Cat
Ho1
Gpx2
0
2
4
6
8
10
12
Ins1
Ins2
Iapp
Ngn3
Beta2
Fas
Pdx1
MafA

Supplemental Table I.
Real-time RT-PCR primer sequences

Gene name	5' oligonucleotide	3' oligonucleotide
18S rRNA	CGCTTCCTTACCTGGTTGAT	GAGCGACCAAAGGAACCATA
β-actin	GATCTGGCACCACACACCTTCT	GGGGTGTTGAAGGTCTCAAA
Atf3	GCTGCCAAGTGTCGAAACAAG	CAGTTTTCCAATGGCTTCAGG
Atf4	ATGGCCGGCTATGGATGAT	CGAAGTCAAACTCTTTCAGATCCATT
Bak	CCTGAAACCTTGGCCCCT	AGCCGTGCAAAGACGAAGAC
Bax	GGAGCAGCTTGGGAGCG	AAAAGGCCCCTGTCTTCATGA
Bcl2	ACTTCGCAGAGATGTCCAGTCA	TGGCAAAGCGTCCCCTC
Bcl-xl	GTAAACTGGGGTCGCATTGT	TGGATCCAAGGCTCTAGGTG
Beta2	GCAAACTGAAAATCAAAACCAA	GGATTGTTATCAAAAGTTGAAAGATG
Bip/Grp78	GGTGCAGCAGGACATCAAGTT	CCCACCTCCAATATCAACTTGA
Crt	GAGTGGCTTGGACCAGAAGG	GGACCGCAGATGTCCGG
Catalase	ACCCTCTTATACCAGTTGGC	GCATGCACATGGGGCCATCA
CD95/Fas	AACCAGACTTCTACTGCGATTCTCC	CCTTTTCCAGCACTTTCTTTTCCG
Cebpα	TGGACAAGAACAGCAACGAG	TCACTGGTCAACTCCAGCAC
Cebpβ	TCTACTACGAGCCCGACTGC	AGGTAGGGGCTGAAGTCGAT
Chop	CTGCCTTTCACCTTGGAGAC	CGTTTCCTGGGGATGAGATA
c-Myc	TGAGCCCCTAGTGCTGCAT	AGCCCGACTCCGACCTCTT
Cyclophilin	TGTGCCAGGGTGGTGACTTTAC	TGGGAACCGTTTGTGTTTGG
Dr5	ATAAAAAGAGGCTGTGAACGGG	GGTCCAAGAGAGACGA
Edem1	GCAATGAAGGAGAAGGAGACCC	TAGAAGGCGTGTAGGCAGATGG
ERdj4	TCAGAGAGATTGCAGAAGCG	GACTCCCATTGCCTCTTTGT
Ero1α	GCATTGAAGAAGGTGAGCAA	ATCATGCTTGGTCCACTGAA
Ero1β	GGGCCAAGTCATTAAAGGAA	TTTATCGCACCCAACACAGT
Erp72	AGTCAAGGTGGTGGTGGGAAAG	TGGGAGCAAAATAGATGGTAGGG
Fas	GAACCCTGAGGGTCCTACCC	CAAGGAACAGAGGCCGCTC
Fkbp11	ACACGCTCCACATACACTACACGG	ATGACTGCTCTTCGCTTCTCTCCC
Gadd34	CCCGAGATTCCTCTAAAAGC	CCAGACAGCAAGGAAATGG
Glut2	CAATTACCGACAGCCCATCC	TCCTGAGAACTGCTGGGCC
Gpx1	ACAGTCCACCGTGTATGCCTTC	CTCTTCATTCTTGCCATTCTCCTG
Gpx2	GAAAGACAAGCTGCCCTACC	TCCATATGATGAGCTTGGGA
Grp94	AATAGAAAGAATGCTTCGCC	TCTTCAGGCTCTTCTTCTGG
Ho1	CCACACAGCACTATGTAAAGCGTC	GTTCGGGAAGGTAAAAAAAGCC
Herpud1	AGCAGCCGGACAACTCTAAT	CTTGGAAAGTCTGCTGGACA
Herpud2	CCAACAATGTGGACGCTAAC	CTTCACCTGCATCTTCTCCA
Hrd1	TGGCTTTGAGTACGCCATTCT	CCACGGAGTGCAGCACATAC
Iapp	CCTCATCCTCTCTGTGGCAC	CACGTTGGTTGGTGGGAG
Ins1	AGCATCTTTGTGGTCCCCAC	CCCCACACACCAGGTAAAGAG
Ins2	TTTGTCAAGCAGCACCTTTG	GGTCTGAAGGTCACCTGCTC
MafA	GCTGGTATCCATGTCCGTGC	GTCGGATGACCTCCTCCTTG
Ngn3	GTCGTTACCCTTCCCCCAAG	CTAGGGCTTTCCGGTTCACA
Noxa	CGCCAGTGAACCCAACG	TTATGTCCGGTGCACTCCAC
Nrf2	ACATCCTTTGGAGGCAAGAC	GCCTTCTCCTGTTCCTTCTG
p21	CGAGAACGGTGGAACTTTGAC	TCCCAGACGAAGTTGCCCT
P53	AAAACCACTTGATGGAGAGTATTTCA	GCTCCCGGAACATCTCGAA
p58^{IPK}	TCCTGGTGGACCTGCAGTACG	CTGCGAGTAATTTCTTCCCC
Pdx1	GAGCGTTCCAATACGGACCA	TCAGCCGTTCTGTTTCTGGG
Pgc1α	AACCACACCCACAGGATCAGA	TCTTCGCTTTATTGCTCCATGA
Pgc1β	CTTGCTAACATCACAGAGGATATCTTG	GGCAGGTTCAACCCCGA
Pparα	ACGATGCTGTCCTCCTTGATG	GTGTGATAAAGCCATTGCCGT
Pparγ	AGTGGAGACCGCCCAGG	GCAGCAGGTTGTCTTGGATGT
Sod1	GGCCCGGCGGATGA	CGTCCTTTCCAGCAGTCACA
Sod2	GGGTTGGCTTGGTTTCAATAAGGAA	AGGTAGTAAGCGTGCTCCCACACAT
Tnfα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Trb3	TCTCCTCCGCAAGGAACCT	TCTCAACCAGGGATGCAAGAG
Ubc7	TCCTCCAGAAGGAATCGTG	AAGTGGGAAACTCAGGATGG
Ucp2	TACCAGAGCACTGTCGAAGCC	AGTCCCTTTCCAGAGGCCC
Wfs1	GTAGCAAGTGGCCCGTCTTC	TGCAGTTGAGGCAGCTGATG
Xbp1s	GAGTCCGCAGCAGGTG	GTGTCAGAGTCCATGGGA