Supplementary Figure 1. Immunohistochemial analysis of AATF in islets. Distribution of AATF in rat pancreata analyzed by immunohistochemistry using anti-AATF, anti-insulin, and anti-glucagon antibodies. Merged images show the co-localization of AATF and insulin (upper panel) or AATF and glucagon (lower panel).

Supplementary Figure 2. AATF is regulated by the Perk-eIF2α pathway. (A) Reconstitution of Perk in $Perk^{-/-}$ mouse embryonic fibroblasts recovered AATF gene expression. $Perk^{-/-}$ mouse embryonic fibroblasts were transfected with pcDNA3/Perk and then treated with or without thapsigargin (Tg, 1 μM) for 8 hr. Expression levels of Aatf, Chop, and Perk mRNA were measured by real-time PCR (n = 3; values are mean ± SD). (B) AATF induction by thapsigargin treatment is attenuated by Perk RNAi. Neuro2a cells were transfected with control scrambled siRNA or siRNA against mouse Perk, then treated with or without 0.5 μM of thapsigargin (Tg) for 8 hr. (C) AATF induction by thapsigargin treatment is not attenuated by ATF6 RNAi. Neuro2a cells were transfected with control scrambled siRNA or siRNA against mouse Atf6, then treated with or without 0.5 μM of thapsigargin (Tg) for 8 hr.

Supplementary Figure 3. Glucose deprivation induces ER stress. Glucose deprivation causes ER stress-mediated apoptosis. INS-1 832/13 cells were cultured in glucose-free media for the indicated times. Expression levels of Chop and AATF mRNA were measured by real-time PCR (left panel) (n = 3; values are mean ± SD). Expression levels of caspase-3 (Casp3) and actin were measured by immunoblot. Single and double asterisks indicate uncleaved and cleaved caspase-3, respectively (right panel).

Supplementary Figure 4. AATF is localized to the nucleus and nucleolus.

INS-1 832/13 cells were co-stained with anti-AATF and anti-Nucleolin antibodies as well as DAPI. Merged images show the co-localization of AATF and Nucleolin.

Supplementary Figure 5. Verification of AATF target genes by real-time RT PCR. The expression of 6 AATF targeting candidates in AATF knock-down INS1 832/13 cells with or without thapsigargin (Tg) was examined by real-time PCR (n = 3; values are mean ± SD).

Supplementary Figure 6. Promoter activities of Akt1 intron1 with Stat3 over-expression. Luciferase activity in neuro2a cells transfected with the indicated intron1 region of Akt1 or control mock promoter construct, with or without Stat3 expressing vector (pFlag/STAT3-C). The ratio (Stat3 overexpression/control) of the relative promoter activities was calculated. (n = 3; values are mean ± SD). pGL4.14/Akt1$^{-1323/-32}$ showed the highest induction ratio by Stat3 among the fragments of different length. The numbers on Akt1 intron1 promoter indicates the distance from the start codon.

Supplementary Figure 7. Akt1 is the most protective molecule for ER stress-mediated apoptosis among the AATF targeting gene candidates. (A) We designed siRNAs against each AATF target gene and transfected them in INS1 832/13 cells, then treated them with or without 0.25 μM of thapsigargin (Tg) for 24 hr. The expression of 6 AATF targeting genes was examined by real-time PCR (n = 3; values are mean ± SD). (B) Expression levels of caspase-3 (Casp3) and actin were measured by immunoblot. Single and double asterisks indicate uncleaved and cleaved caspase-3, respectively. The ratio between cleaved caspase-3 and actin was measured using ImageJ software.

Supplementary Figure 8. Mouse primary islets are infected with lentiviruses overexpressing AATF and Akt1. (A) Mouse primary islets were infected with LV-TO/GFP, a

lentivirus overexpressing GFP. Phase constrast, GFP, and merged pictures are shown. All the intact islets showed GFP signals at 48 hr after infection. (B) Mouse primary islets were infected with LV-TO/GFP (GFP), LV-TO/AATF (AATF), or LV-TO/Akt1 (Akt1). An empty lentivirus was used as a negative control (mock). Expression levels of AATF and Akt1 mRNA were measured by real-time PCR at 48 hr after infection (n = 3; values are mean ± SD). (C) Mouse primary islets were infected with LV-TO/AATF (AATF), LV-TO/Akt1 (Akt1), and empty LV-TO (mock) virus. Then, islets were treated with thapsigargin (Tg, 0.5 μM) for 6 hr. After dispersion, cells were fixed and stained with anti-cleaved caspase-3 and anti-insulin antibodies. The ratio of cells with cleaved-caspase-3 signals to those with Insulin signals was calculated as shown in Figure 7F.

Supplementary Figure 9. Both WFS1 and AATF are upstream regulators of Akt1 expression. (A) INS1 832/13 cells were transfected with control scramble siRNA or siRNA against WFS1, then treated with 0.5 μM of thapsigargin (Tg) for 20 hr. Expression levels of WFS1, AATF, and Akt1 mRNA were measured by real-time PCR (n = 3; values are mean ± SD). (B) INS-1 832/13 cells were stably transduced with LV-TO/AATF, an inducible lentivirus expressing mouse AATF. Cells were cultured with or without doxycycline (0.2 μg/ml) to induce AATF for 48 hr, then challenged with thapsigargin (Tg, 0.5 μM) for 20 hr. Cells were also transfected with control scramble siRNA (Cont) or siRNA against WFS1. Expression levels of WFS1, AATF, and Akt1 mRNA were measured by real-time PCR (n = 3; values are mean ± SD). (C) INS1 832/13 cells (left panel) and neuro2a cells (right panel) were transfected with control scramble siRNA or siRNA against AATF, then treated with 0.5 μM of thapsigargin (Tg) for 16 hr. Expression levels of WFS1 mRNA were measured by real-time PCR (n = 3; values are

mean ± SD). Expression levels of AATF, WFS1, and actin proteins were measured by immunoblot (lower panel). (D) Luciferase activity in neuro2a cells transfected with WFS1 promoter (pGL4.13/WFS1$^{-521/+39}$) or control promoter constructs, plus vectors expressing spliced form of XBP1 (n = 3; values are mean ± SD) (right panel).

Supplementary Figure 10. Expression of Puma and Puma is not regulated by the WFS1-AATF pathway. INS1 832/13 cells were transfected with control scramble siRNA or siRNA directed against WFS1 or AATF, then treated with 1 μM of thapsigargin (Tg) for 8 hr. (A) Expression levels of Puma and Bim mRNA were measured by real-time PCR (n = 3; values are mean ± SD). (B) Expression levels of Puma, Bim, WFS1, AATF, and actin proteins were measured by immunoblot.

Supplementary Methods

Plasmid expressing mouse Perk and transfection

A plasmid expressing mouse Perk gene was generated by introducing mouse Perk cDNA in pcDNA3 vector. MEF cells were transfected with pcDNA3/mPerk by using the Amaxa Nucleofector Device.

Small interfering RNA and primers

siRNAs directed against mouse Perk, mouse Atf6 and rat AATF-target genes were designed and synthesized: for mouse Perk, CCATACTACAAGAGAGAAA; for mouse Atf6 AAGAAGTATCCTGCCATTTAA; for rat Noc4l, GCAGCTGGAAGGAGCGAAA; for rat Chn2, GTTTGTTGCACTTCGATAA; for rat Rfc2, AAATAGTGGGAAACGAAGA; for rat Sdc2, CGACCTTGGAGAACGCAAA; for rat Prg1, TGGAAGAGCAAGAACGAGA and for rat Hmga1, GCACAGCCAAGACGCGGAA. siRNA transfections were carried out by using the Amaxa Nucleofector Device.

The following sets of primers and Power SYBR Green PCR Master Mix were used for real-time PCR in supplementary experiments: for mouse Perk, AAGTTTCCGTTGCTGATTGG and ACGCGATGGGAGTACAAAAC; for mouse Atf6a, CCACCCTCACTGTTGGAACT and GAGTATGCTCTGGGCTGAGG; for mouse BiP, TTCAGCCAATTATCAGCAAACTCT and TTTTCTGATGTATCCTCTTCACCAGT; for mouse WFS1, CCATCAACATGCTCCCGTTC and GGGTAGGCCTCGCCATACA; for mouse Chop, CCACCACACCTGAAAGCAGAA and AGGTGAAAGGCAGGGACTCA; for rat Noc4l, CTGTGAACCTACCCCTCCAA and GCTTTCCTCTGCTCCTTCAA; for rat Chn2, TATGAGACCCTGCGCTACCT and CCCAGATTTTCTGCGTTCAT; for rat Rfc2, CCTCAGGAGAACCATGGAAA and

GGACTGGATGGGCTCTATGA; for rat Sdc2, TGGCTTTCTCTTTGCCATTT and CAAGGTCGTAGCTTCCTTCG; for rat Prg1, GGAAAGCTCCGGAGAAGAGT and CTCTTGCGATCTCCAGAACC; for rat Hmga1, CAGGAAAAGGATGGGACTGA and TCACTGGGCTCCTTCTGACT; for rat Puma, GCGGAGACAAGAAGAGCAAC and CTCCAGGATCCCTGGGTAAG; for rat Bim, GCAGTCTCAGGAGGAACCTG and TTGAACTCGTCTCCGATCCT.

Promoter assay

In the experiments of Supplementary Figure 6, the intron1 promoter regions of mouse Akt1 gene were amplified by PCR and cloned it into the KpnI/XhoI site of the pGL4.14 vector (Promega). Fragments of mouse Akt1 intron1 region includes -1629/-32, -1323/-32, -897/-32, -630/-32, -320/-32, and -1629/-1100. Neruo2a cells were transfected with pGL4.14 vectors containing various fragments indicated in Supplementary Figure 6, Stat3 expressing vector (pFlag/STAT3-C) or pFlag empty vector, and pcDNA3/β-galactosidase. In the experiments of Supplementary Figure 9D, Neruo2a cells were transfected with pGL4.13/WFS1$^{-521/+39}$, pFlag/spliced-XBP1 (XBP1), pCS2+/AATF (AATF), and pcDNA3/β-galactosidase. pGL4.13 and pFlag empty vectors were used as negative controls. pGL4.13/WFS1$^{-521/+39}$ was a kind gift from Dr. Kato (30). 24 hrs post-transfection, lysates were prepared using a Luciferase Assay System kit (Promega). The light produced from the samples was read by a standard plate reading luminometer. β-galactosidase activity was measured by β-Gal Reporter Gene Assay, chemiluminescent (Roche). The assay was performed independently three times.

Antibodies

Both anti-Puma and anti-Bim antibodies were purchased from Cell Signaling.

Supplementary Table 1

Genes decreased more than 2-fold in WFS1-deficient β cells (WFS1-RNAi/Control-RNAi).

symbols	ID	Log 2 Ratio (WFS1kd/Cont.)	P.Val
Slc35a1	1374662_at	-1.45	6.47E-05
Spata6	1368643_at	-1.43	0.000107
Aatf	1368330_at	-1.23	0.000125
Pcnp	1389040_at	-1.17	0.000125
Gnb1	1367732_at	-1.16	6.47E-05
Fdx1	1368336_at	-1.13	0.000149
Fmr1	1393459_at	-1.11	0.000396
Cst6	1368162_at	-1.09	0.000431
RGD1565975	1381759_at	-1.07	0.001921
Lrp11	1372443_at	-1.06	0.000107
Gc	1368288_at	-1.04	0.000181
Acbd5	1373507_at	-1.01	0.000273

Supplementary Table 2

Genes decreased more than 2-fold in AATF-deficient β cells (AATF-RNAi/Control-RNAi).

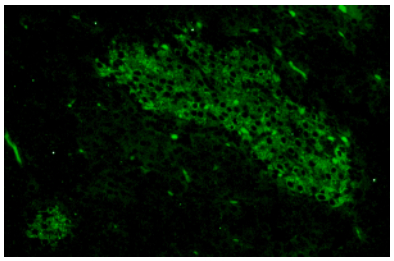
symbols	ID	Log 2 Ratio (AATFkd/Cont.)	P.Val
Aatf	1368330_at	-2.71	0.000706
Rfc2	1370910_at	-1.53	0.000713
Prg-1	1379374_at	-1.40	0.000706
Sdc2	1370166_at	-1.35	0.006119
Hmga1	1388309_at	-1.28	0.001311
Akt1	1383126_at	-1.18	0.006119
Chn2	1376800_at	-1.12	0.001012
Noc4l	1377760_at	-1.03	0.001953

Supplementary Table 3

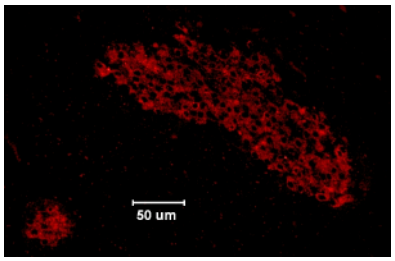
Differentially expressed genes which were commonly seen in the list of two different profiles, WFS1 knock-down and AATF knock-down microarray experiments.

symbols	ID	Log 2 Ratio (WFS1kd/Cont)	P.Val.WFS1	Log 2 Ratio (AATFkd/Cont)	P.Val.AATF
Aatf	1368330_at	-1.23	0.000125	-2.71	0.000706
Wfs1	1368839_at	-0.79	0.000241	-0.69	0.009504
Tmem161b	1374605_at	-0.79	0.001011	0.64	0.008557
Pphln1	1389547_at	-0.77	0.000252	-0.70	0.006119
Cyp4a8	1393894_at	-0.63	0.000987	-0.72	0.006119
Klf2	1376569_at	-0.57	0.002617	0.64	0.007011
unknown locus	1383932_at	-0.48	0.003311	-0.90	0.004514
Rac1	1388332_at	0.43	0.004823	0.77	0.006805
Vav3	1393605_at	0.44	0.003659	1.10	0.001012

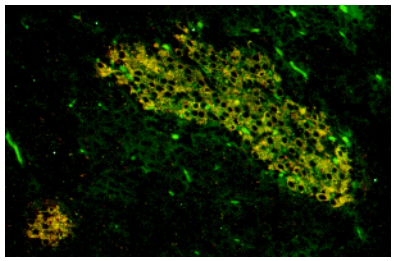
Suppl. Figure 1.



AATF



Insulin



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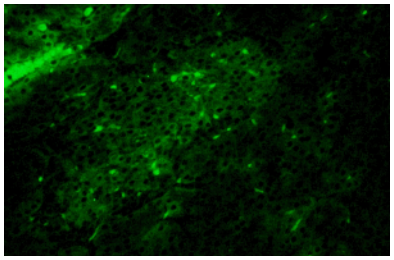
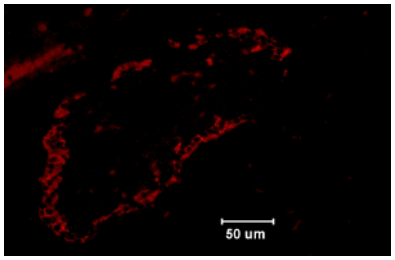


AATF



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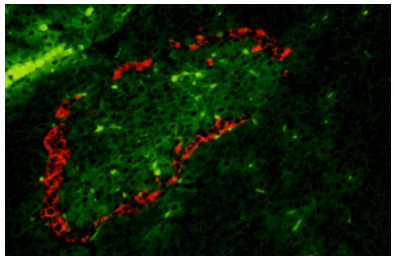
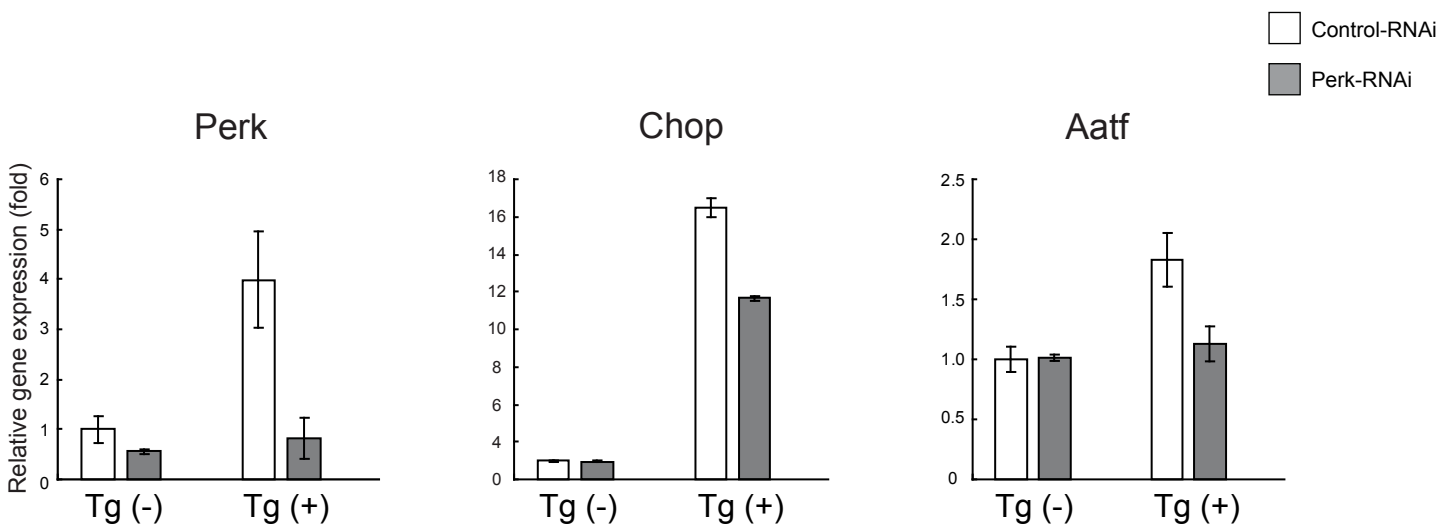
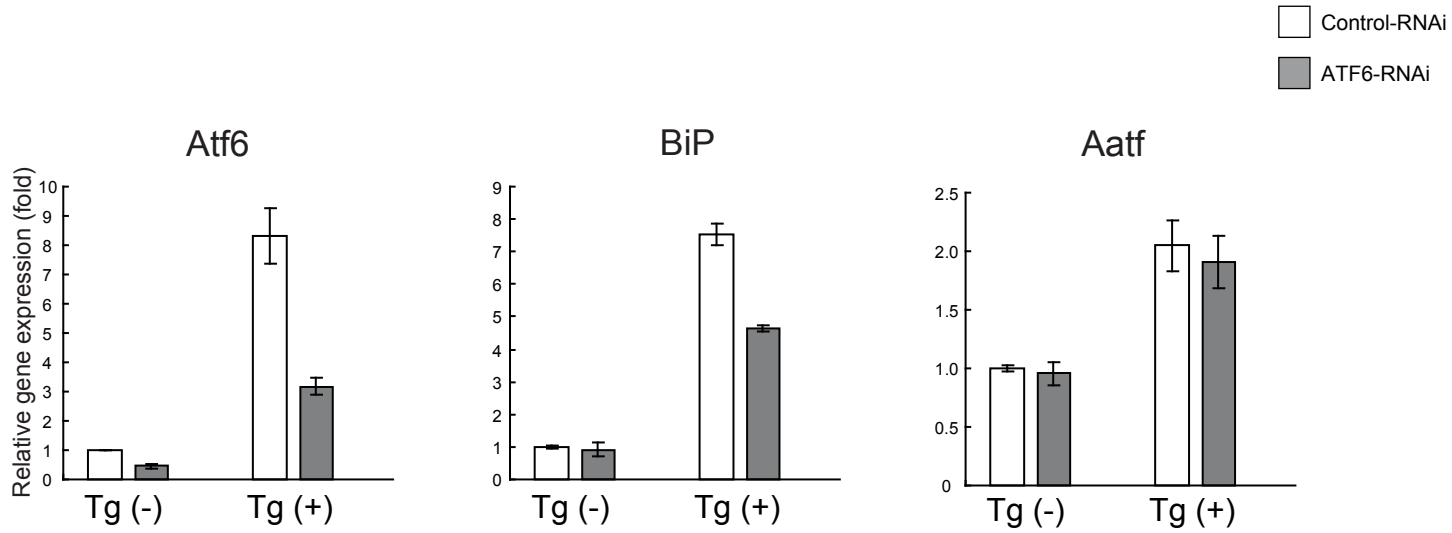


Merge

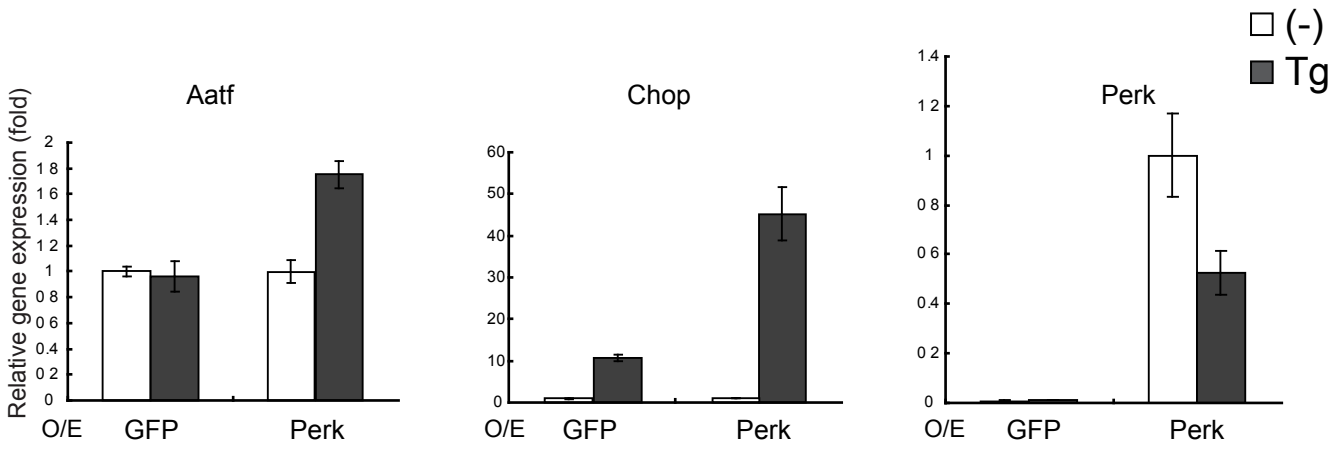
A

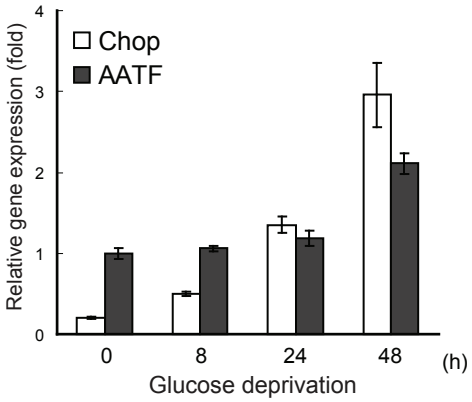

Control-RNAi
Perk-RNAi
Perk
Chop
Aatf
Relative gene expression (fold)
Tg (-)
Tg (+)

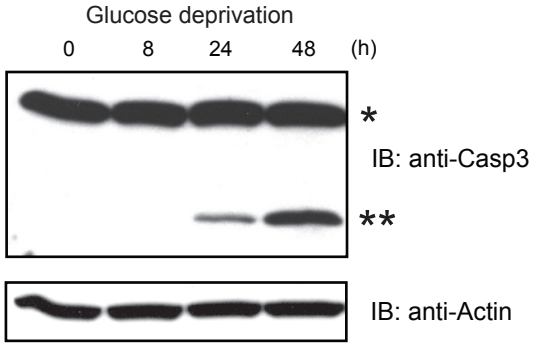
B


Control-RNAi
ATF6-RNAi
Atf6
BiP
Aatf
Relative gene expression (fold)
Tg (-)
Tg (+)

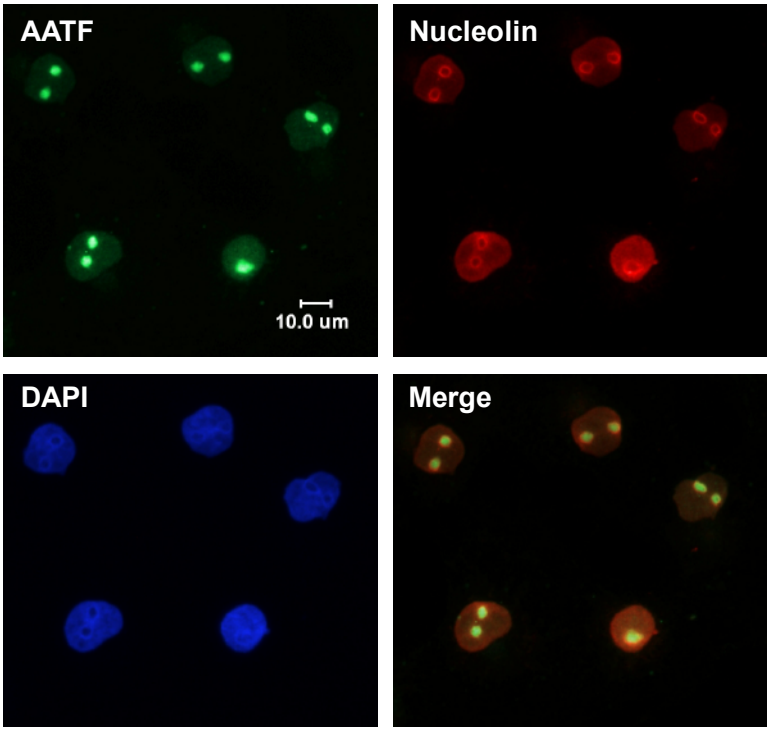
C

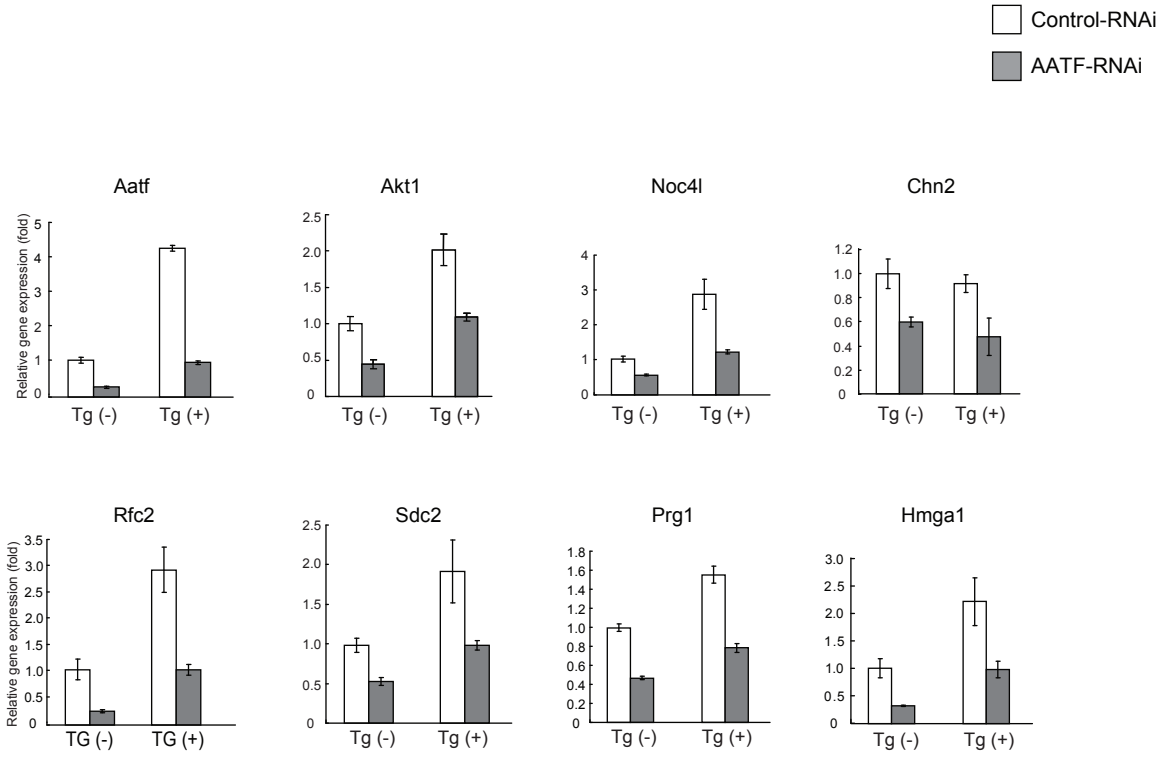

(-)
Tg
Aatf
Chop
Perk
Relative gene expression (fold)
O/E
GFP
Perk

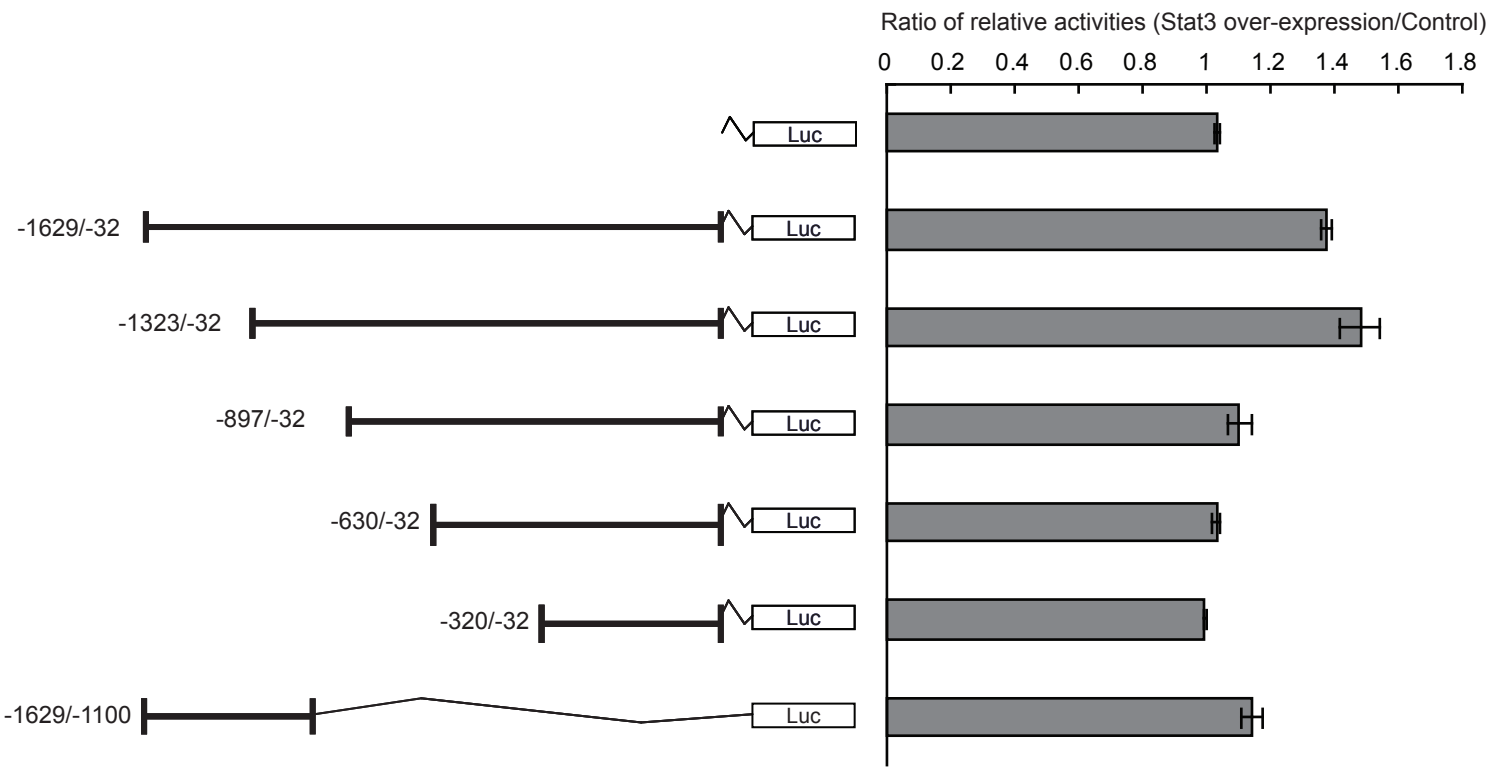

Relative gene expression (fold)
Chop
AATF
0
1
2
3
4
0
8
24
48
(h)
Glucose deprivation


Glucose deprivation
0
8
24
48
(h)
*
**
IB: anti-Casp3
IB: anti-Actin

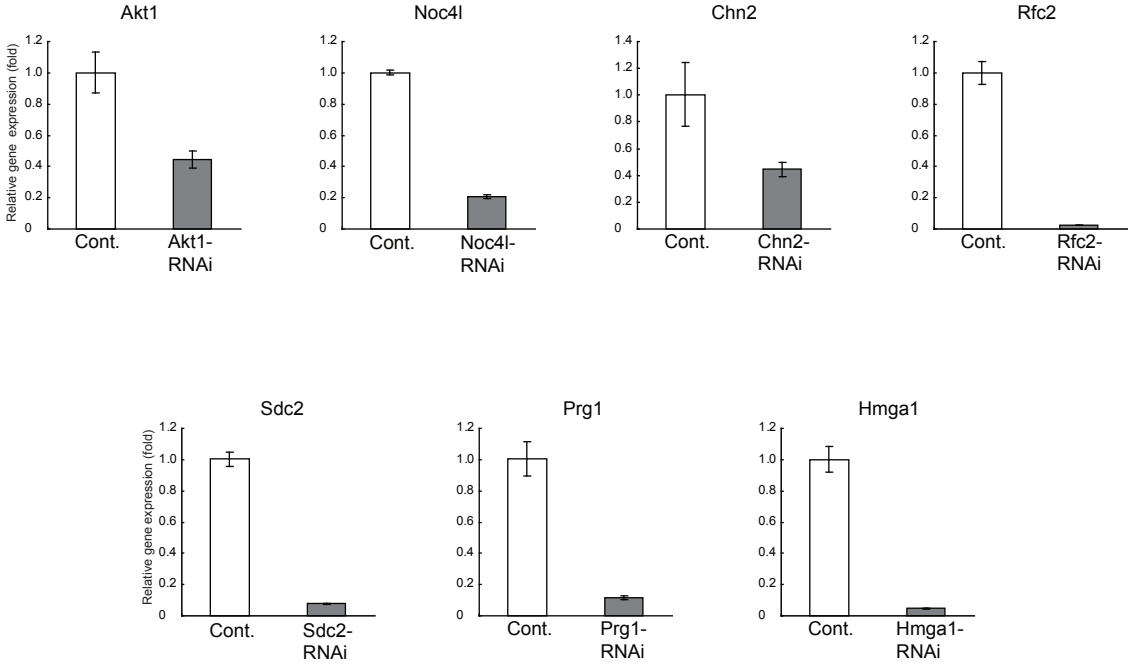
Suppl. Figure 4.



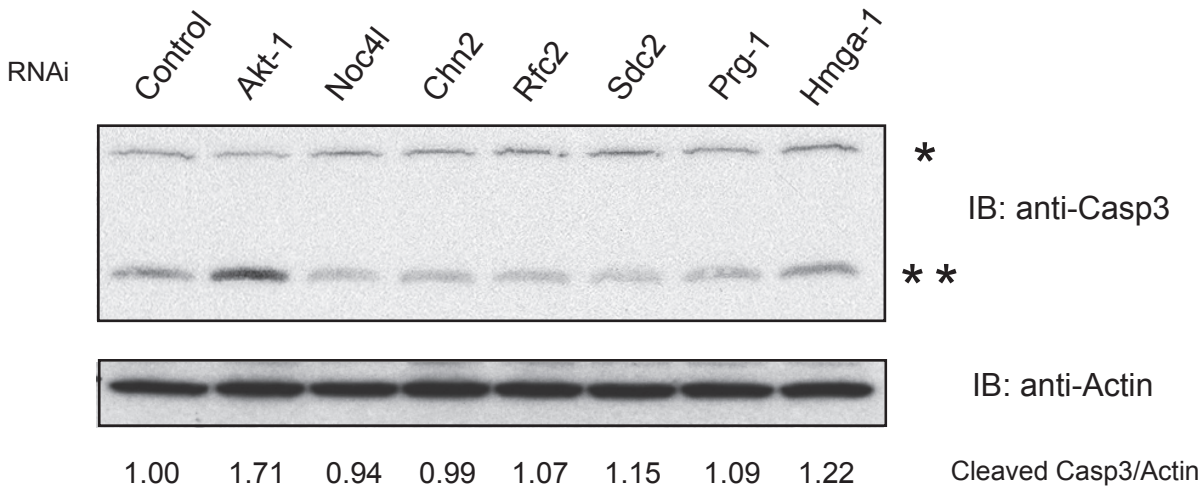

Control-RNAi
AATF-RNAi
Aatf
Akt1
Noc4l
Chn2
Rfc2
Sdc2
Prg1
Hmga1
Relative gene expression (fold)
Tg (-)
Tg (+)
TG (-)
TG (+)


Ratio of relative activities (Stat3 over-expression/Control)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Luc
-1629/-32
-1323/-32
-897/-32
-630/-32
-320/-32
-1629/-1100

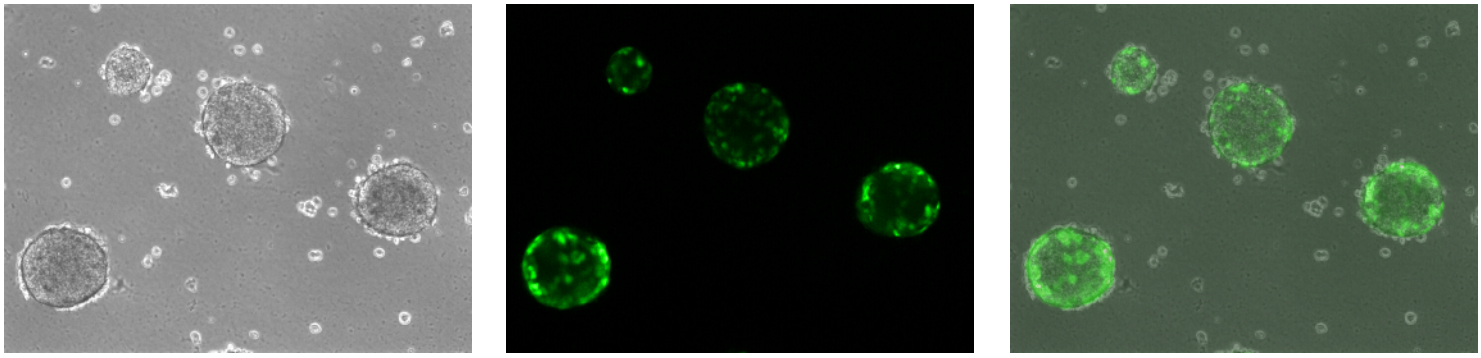
A


Akt1
Noc4l
Chn2
Rfc2
Relative gene expression (fold)
Cont.
Akt1-RNAi
Noc4l-RNAi
Chn2-RNAi
Rfc2-RNAi
Sdc2
Prg1
Hmga1
Sdc2-RNAi
Prg1-RNAi
Hmga1-RNAi

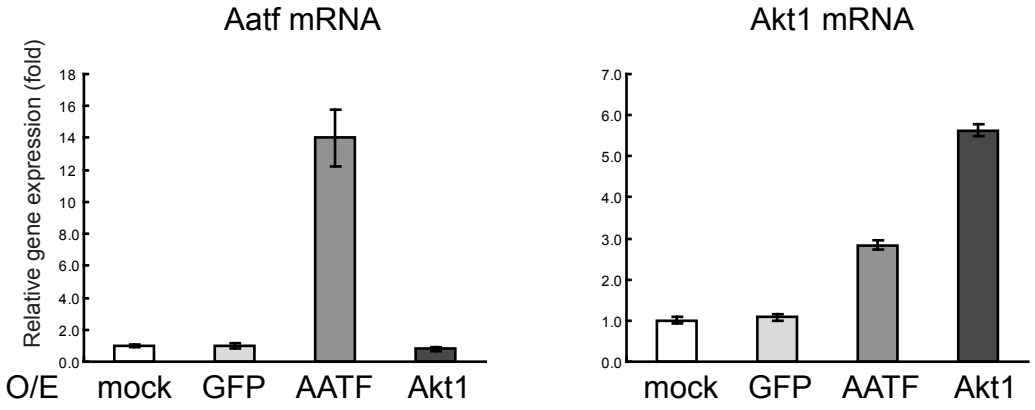
B


RNAi
Control
Akt-1
Noc4l
Chn2
Rfc2
Sdc2
Prg-1
Hmga-1
*
**
IB: anti-Casp3
IB: anti-Actin
1.00 1.71 0.94 0.99 1.07 1.15 1.09 1.22
Cleaved Casp3/Actin

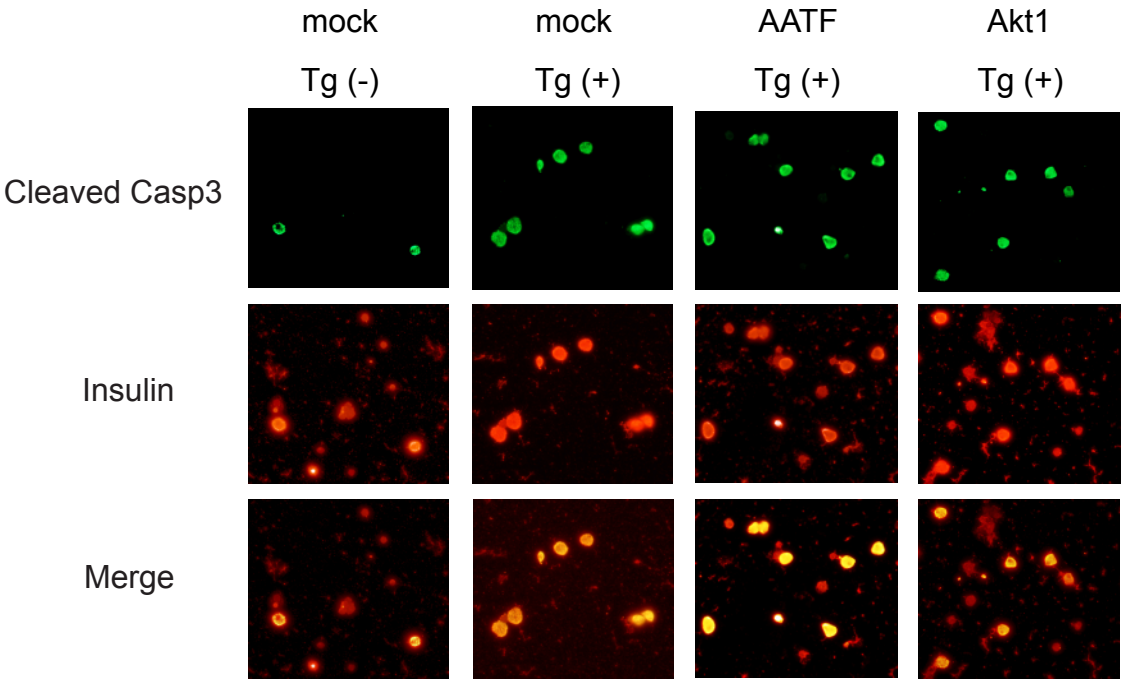
A

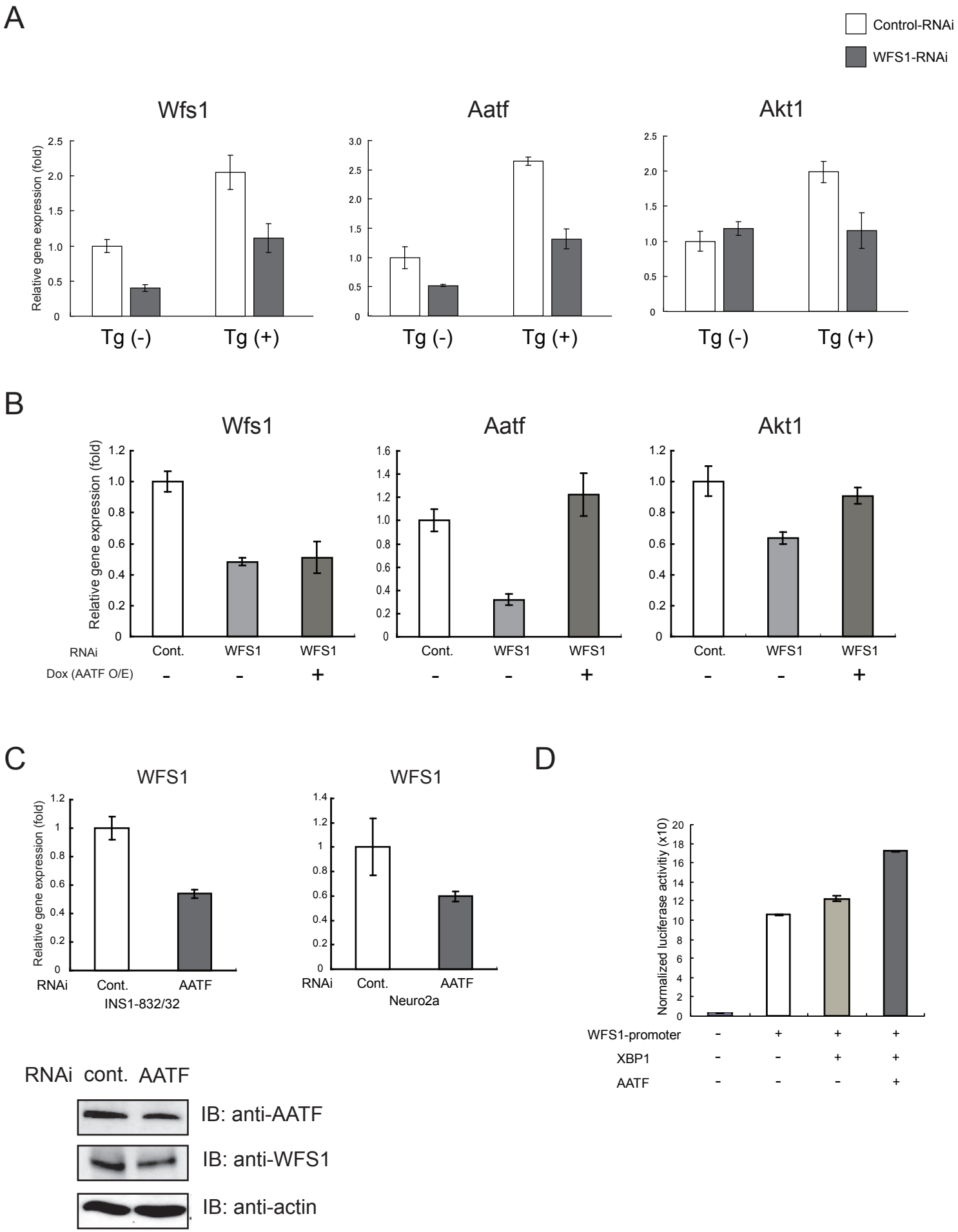

Phase contrast
GFP
Merge

B

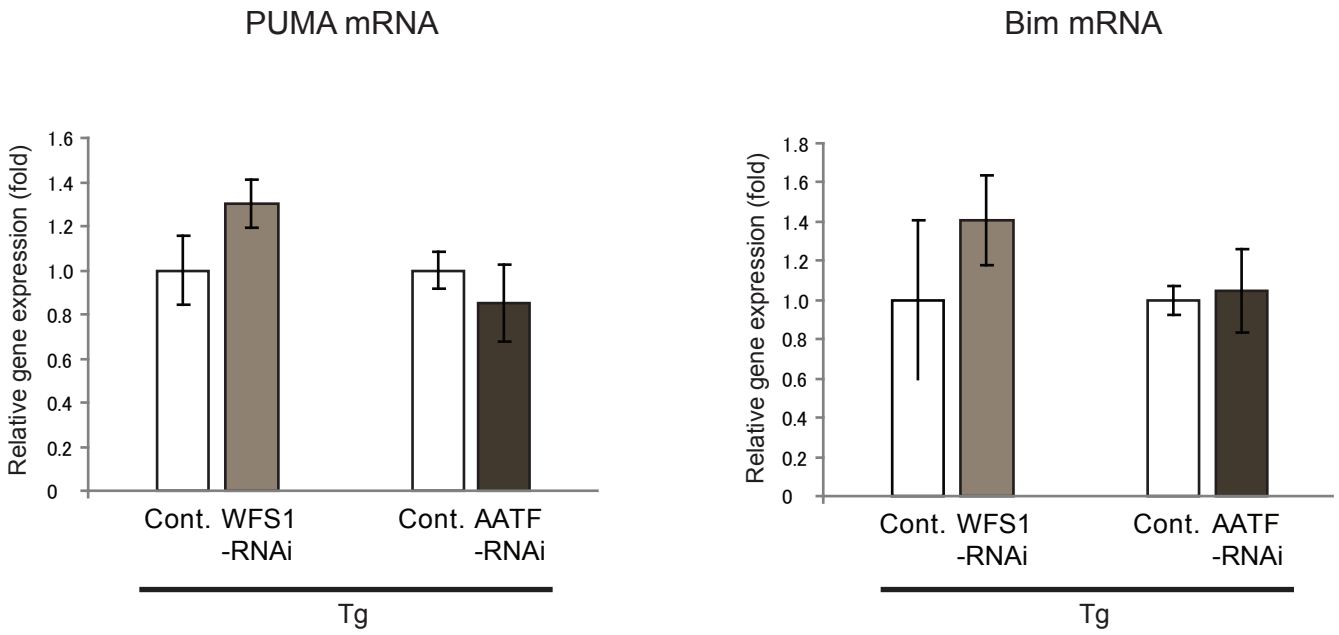

Aatf mRNA
Relative gene expression (fold)
18
16
14
12
10
8.0
6.0
4.0
2.0
0.0
O/E
mock
GFP
AATF
Akt1
Akt1 mRNA
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
mock
GFP
AATF
Akt1

C

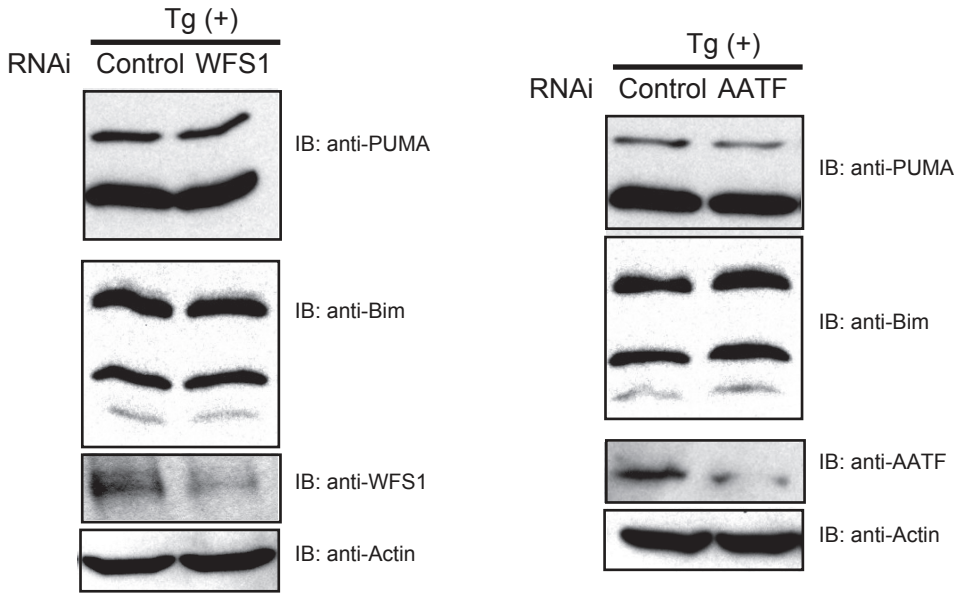

mock
Tg (-)
mock
Tg (+)
AATF
Tg (+)
Akt1
Tg (+)
Cleaved Casp3
Insulin
Merge


A
Control-RNAi
WFS1-RNAi
Wfs1
Aatf
Akt1
Relative gene expression (fold)
Tg (-)
Tg (+)
B
Wfs1
Aatf
Akt1
Relative gene expression (fold)
RNAi
Cont.
WFS1
WFS1
Dox (AATF O/E)
C
WFS1
WFS1
Relative gene expression (fold)
RNAi
Cont.
AATF
INS1-832/32
Neuro2a
D
Normalized luciferase activitiy (x10)
WFS1-promoter
XBP1
AATF
RNAi cont. AATF
IB: anti-AATF
IB: anti-WFS1
IB: anti-actin

A


PUMA mRNA
Relative gene expression (fold)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Cont.
WFS1 -RNAi
Cont.
AATF -RNAi
Tg
Bim mRNA
Relative gene expression (fold)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Cont.
WFS1 -RNAi
Cont.
AATF -RNAi
Tg

B


Tg (+)
RNAi
Control
WFS1
IB: anti-PUMA
IB: anti-Bim
IB: anti-WFS1
IB: anti-Actin
Tg (+)
RNAi
Control
AATF
IB: anti-PUMA
IB: anti-Bim
IB: anti-AATF
IB: anti-Actin