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Supplemental Material to:

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Endoplasmic reticulum stress induced by tunicamycin and thapsigargin protects against transient ischemic brain injury: Involvement of PARK2-dependent mitophagy

Autophagy 2014; 10(10)
http://dx.doi.org/10.4161/auto.32136

www.landesbioscience.com/journals/autophagy/article/32136

Figure S1

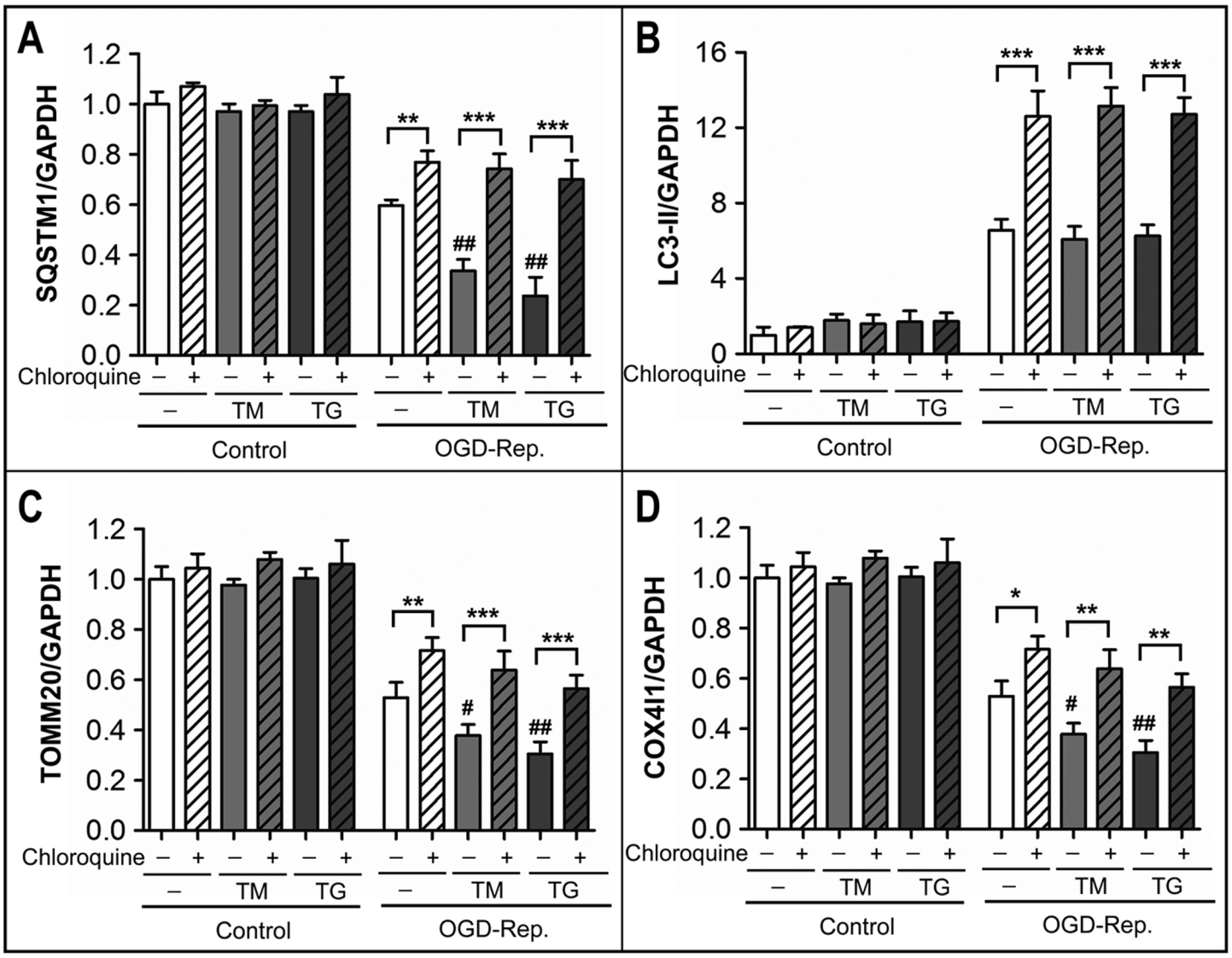
A
SQSTM1/GAPDH
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
***
***
##
##
Chloroquine – + – + – + – + – + – +
– TM TG – TM TG
Control
OGD-Rep.
B
LC3-II/GAPDH
16
12
8
4
0
***
***
***
Chloroquine – + – + – + – + – + – +
– TM TG – TM TG
Control
OGD-Rep.
C
TOMM20/GAPDH
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
***
***
#
##
Chloroquine – + – + – + – + – + – +
– TM TG – TM TG
Control
OGD-Rep.
D
COX4I1/GAPDH
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
**
#
##
Chloroquine – + – + – + – + – + – +
– TM TG – TM TG
Control
OGD-Rep.

Figure S2

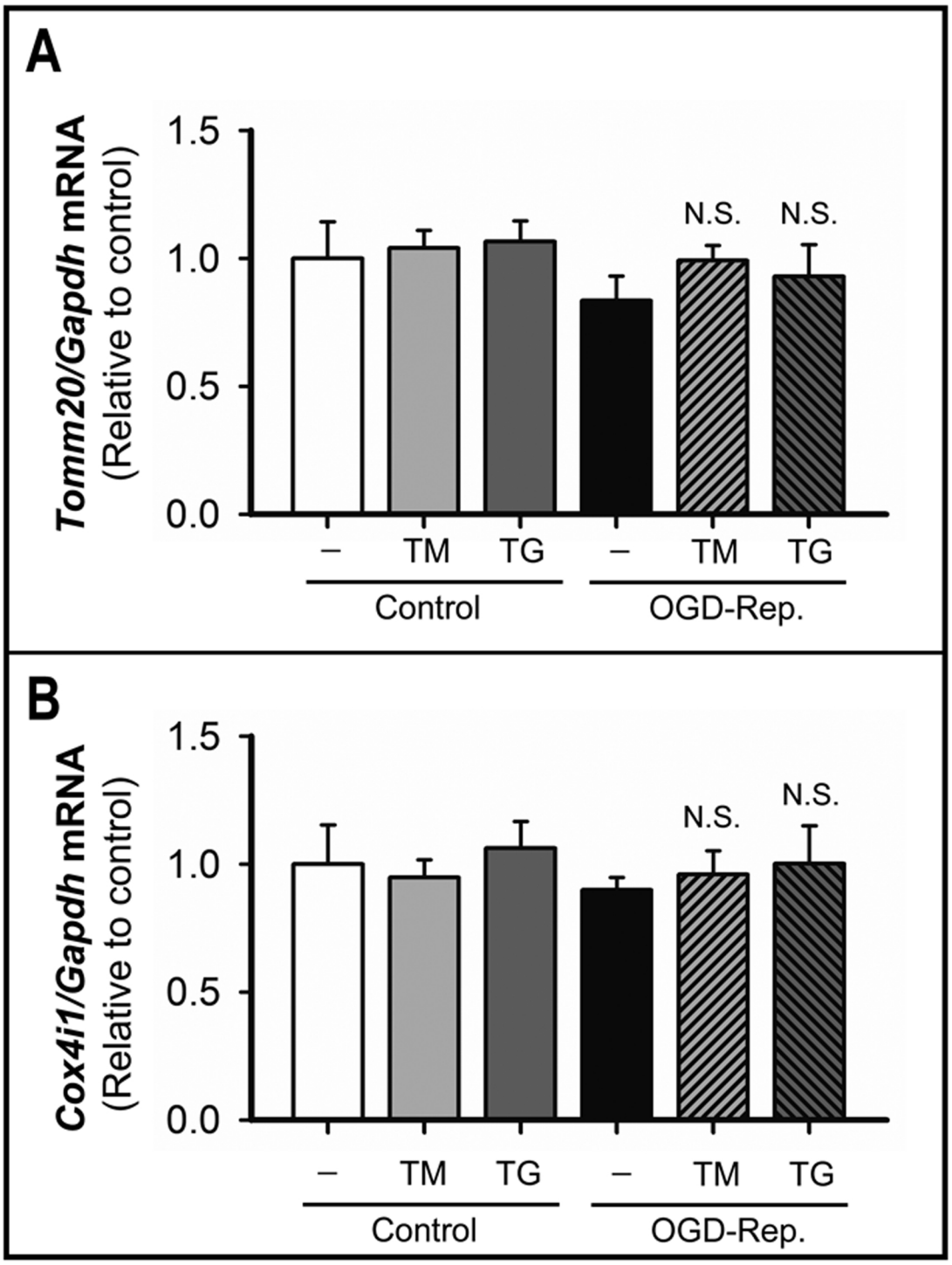
A
Tomm20/Gapdh mRNA
(Relative to control)
1.5
1.0
0.5
0.0
N.S.
N.S.
–
TM
TG
–
TM
TG
Control
OGD-Rep.
B
Cox4i1/Gapdh mRNA
(Relative to control)
1.5
1.0
0.5
0.0
N.S.
N.S.
–
TM
TG
–
TM
TG
Control
OGD-Rep.

Figure S3

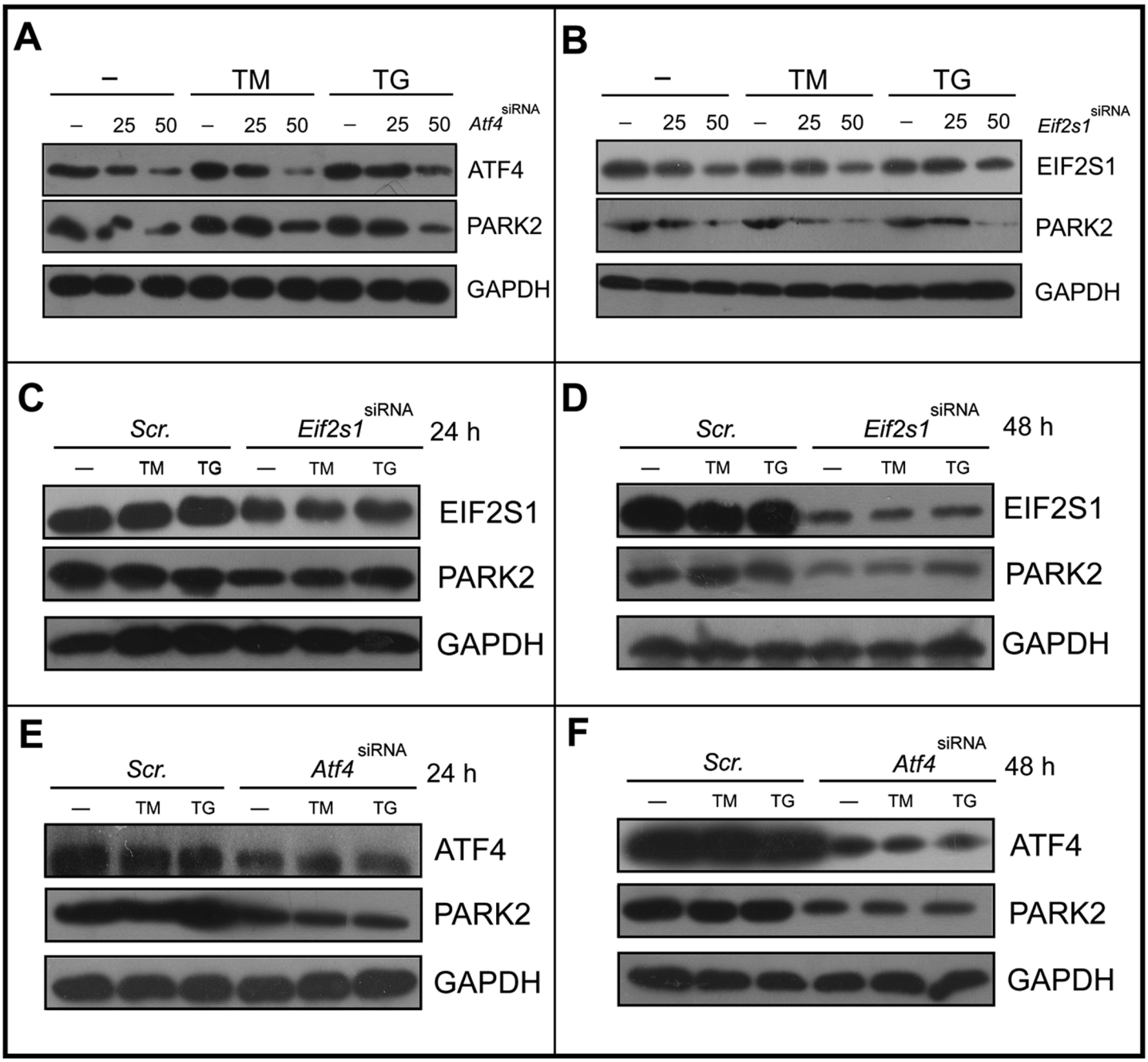
A
–
TM
TG
– 25 50 – 25 50 – 25 50
Atf4 siRNA
ATF4
PARK2
GAPDH
B
–
TM
TG
– 25 50 – 25 50 – 25 50
Eif2s1 siRNA
EIF2S1
PARK2
GAPDH
C
Scr.
Eif2s1 siRNA
24 h
— TM TG — TM TG
EIF2S1
PARK2
GAPDH
D
Scr.
Eif2s1 siRNA
48 h
— TM TG — TM TG
EIF2S1
PARK2
GAPDH
E
Scr.
Atf4 siRNA
24 h
— TM TG — TM TG
ATF4
PARK2
GAPDH
F
Scr.
Atf4 siRNA
48 h
— TM TG — TM TG
ATF4
PARK2
GAPDH

Figure S4

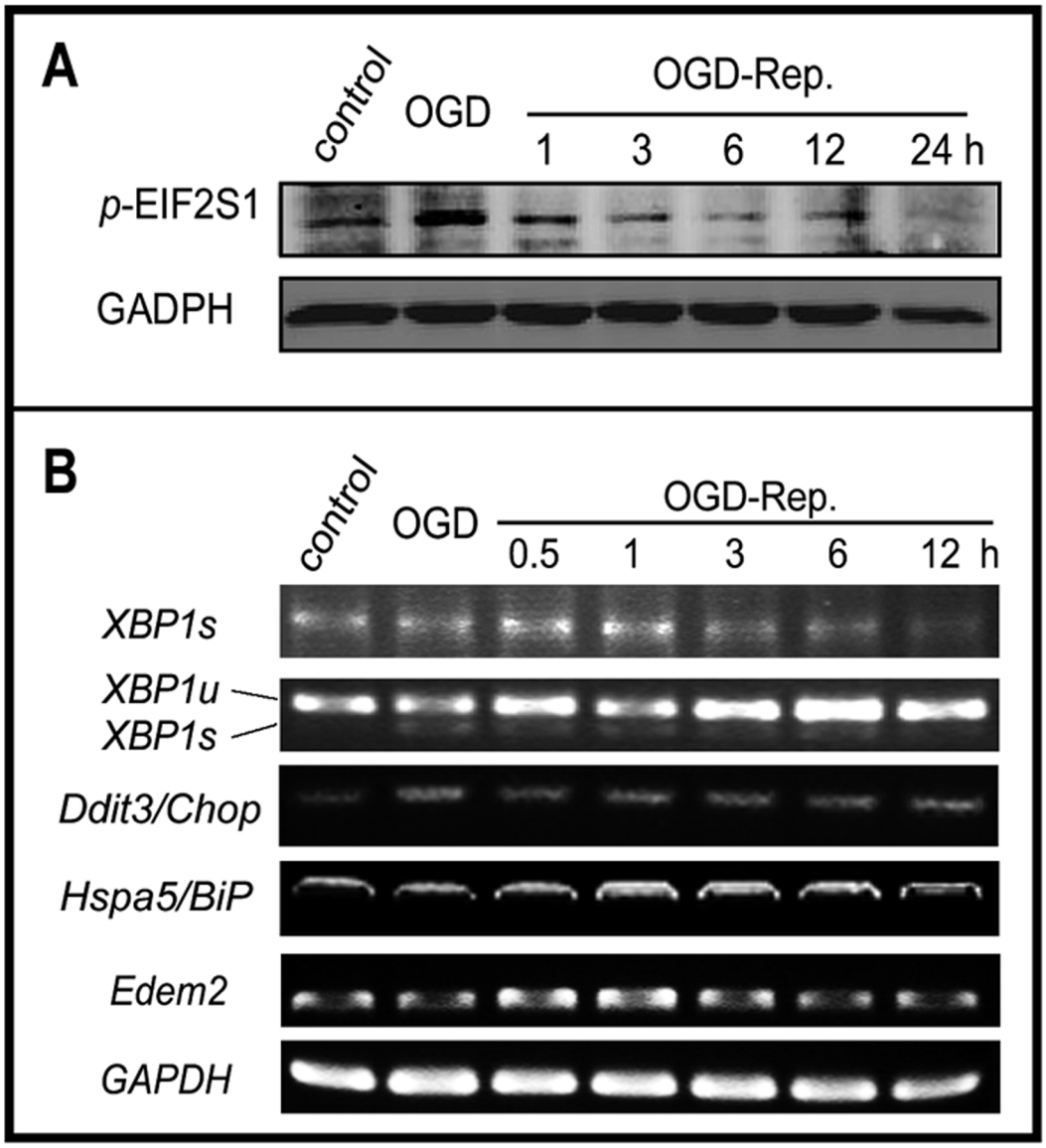
A
control
OGD
OGD-Rep.
1
3
6
12
24 h
p-EIF2S1
GADPH
B
control
OGD
OGD-Rep.
0.5
1
3
6
12 h
XBP1s
XBP1u
XBP1s
Ddit3/Chop
Hspa5/BiP
Edem2
GAPDH

Figure S1. The semiquantitative analysis of western blot bands shown in Figure 3. (**A**) SQSTM1/GAPDH; (**B**)LC3-II/GAPDH; (**C**) TOMM20/GAPDH; (**D**) COX4I1/GAPDH. The data are expressed as mean ± SD. Statistical comparisons were performed with one-way ANOVA followed by the Dunnett *t* test, **P*<0.05, ***P*<0.01, ****P*<0.001 vs. the indicated group. #*P*<0.05, ##*P*<0.01 *vs*. OGD-Rep. alone group.

Figure S2. *Tomm20* and *Cox4i1* mRNA levels were unaffected by tunicamycin (TM) and thapsigargin (TG) treatment in the context of oxygen-glucose deprivation-reperfusion (OGD-Rep.). Primary cultured neurons were subjected to OGD for 2 h, and treated with 0.4 nmol/L TG or 0.2 ng/L TM at the onset of reperfusion. After 6 h of reperfusion, the levels of *Tomm20* and *Cox4i1* mRNA were determined by qRT-PCR analysis. The quantitative levels are shown in the bar chart. The data are expressed as mean ± SD. Statistical comparisons were performed with one-way ANOVA followed by the Dunnett *t* test, relative to vehicle control group. *P* values less than 0.05 were considered statistically significant. The N.S. indicated non-significant vs. indicated group.

Figure S3. Knockdown of *Eif2s1* and *Atf4* reduced PARK2 expression in intact neurons. Primary cultured neuronal cells were previously treated with a lentivirus that delivered shRNA against either *Eif2s1* or *Atf4*. Then the cells were treated with 0.4 nmol/L thapsigargin (TG) or 0.2 ng/L tunicamycin (TM). (**A and B**) *Atf4* and *Eif2s1* were silenced by the indicated MOI of the lentivirus after 1 h of incubation, respectively. 48 h after transfection, the PARK2 levels were detected by western blot. (**C to F**) Cells were subjected to periods of either 24 or 48 h of transfection with *Atf4* or *Eif2s1*-silencing lentiviral vectors, respectively. The PARK2 levels were detected by western blot.

Figure S4. Oxygen-glucose deprivation (OGD)-induced ER stress in neuronal cells was relieved during reperfusion. Primary cultures of mouse brain cortical neurons were subjected to 2 h of OGD followed by the indicated duration of reperfusion. (**A**) The phosphorylated EIF2S1 level was detected by western blot. (**B**) The mRNA levels of spliced (*Xbp1s*), unspliced *Xbp1* (*Xbp1u*), *Ddit3/Chop*, *Hspa5/BiP* and *Edem2* were determined by PCR.