Bax Inhibitor-1-Mediated Inhibition of Mitochondrial Ca^{2+} Intake Regulates Mitochondrial Permeability Transition Pore Opening and Cell Death

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Supplementary Figure 1

Ca^{2+} induces cytochrome c release from mitochondria more potently in BI-$1^{-/-}$ liver tissues than in $^{+/+}$ tissues. Mitochondria from BI-$1^{-/-}$ liver and BI-$1^{+/+}$ liver were incubated with 100 μM $CaCl_2$ for various times (0, 10, 30, and 60 min). Absorbance at 540 nm was measured after incubation at 30°C. The data shown represent the mean ± S.E. (n = 4). $^{*}p < 0.05$ versus Ca^{2+}-exposed mitochondria from BI-$1^{+/+}$ tissue (**A**). The released cytochrome c in supernatants was detected by western blotting with anti-cytochrome c antibody (**B**). Sup, supernatants.

Supplementary Figure 2

A549/Neo and A549/BI-1 cells were treated with 5 μM thapsigargin (arrow) and analyzed for Rhod II AM fluorescence (upper) and erAEQ luminescence (lower), respectively (A). A549/Neo and A549/BI-1 were loaded with Fura2-AM and treated with the mitochondrial inhibitor CCCP (1 μM). Representative Ca^{2+} traces are shown. Individual cells were imaged (n = 16) and their fluorescence intensities (Fura2) recorded; the peaks of Ca^{2+} release were also shown (B). Mitochondria from A549/Neo and A549/BI-1 were loaded with 1 μM Rhod II AM and treated with 2 mM $CaCl_2$. Rhod II fluorescence was monitored (C). A549/Neo and A549/BI-1 cells were treated with 5 μM thapsigargin in the presence or absence of 5 μM CsA or 1 μM Ru360 for 48 hrs. Cell viability was measured by trypan blue exclusion (D) A549/Neo and A549/BI-1 cells were treated with 5 μM thapsigargin in the presence or absence of 100 μM diazoxide, 500 μM 5-HD, 100 μM NS1619, or 2 μM paxilline for 48 hrs. Cell viability was measured by trypan blue exclusion (E). *, $p < 0.05$ versus thapsigargin-treated A549/Neo; $^{\#}$, $p < 0.05$ versus thapsigargin-treated A549/BI-1. $[Ca^{2+}]_i$, intracellular Ca^{2+}; Tg, thapsigargin; CCCP, carbonylcyanide m-chlorophenylhydrazone; A549/Neo, neomycin-resistant pcDNA3-transfected A549 cells; A549/BI-1, HA-BI-1-pcDNA3-transfected A549 cells.

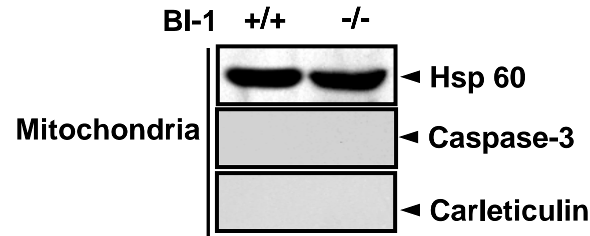
Supplementary Figure 3

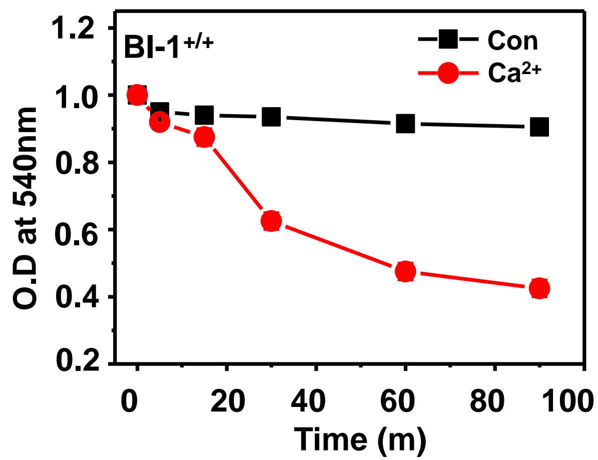
These experiments used HeLa cells in which BI-1 expression was conditionally driven using a doxycycline-inducible system. Cells were treated with 5 μM thapsigargin (arrow) and loaded with Rhod II AM or transfected with erAEQ and the fluorescence (upper) and erAEQ luminescence (lower) was monitored (A). Doxycylcine-treated or non-treated Hela-Tet-on BI-1 cells were loaded with Fura2-AM and exposed to the mitochondrial inhibitor CCCP (1 μM). Representative Ca^{2+} traces are shown with or without doxycycline. Individual cells were imaged (n = 15) and their fluorescence intensities (Fura2) were recorded; the peaks of Ca^{2+} release are also shown (B). Mitochondria from doxycylcine-treated or non-treated Hela-Tet-on BI-1 cells were loaded with 1 μM Rhod II AM and treated with 2 mM $CaCl_2$. Rhod II fluorescence was monitored (C). Cells were treated with 5 μM thapsigargin in the presence or absence of 5 μM CsA or 1 μM Ru360 for 48 hrs. Cell viability was measured by trypan blue exclusion (D). Cells were treated with 5 μM thapsigargin in the presence or absence of 100 μM diazoxide, 500 μM 5-HD, 100 μM NS1619, or 2 μM paxilline for 48 hrs. Cell viability was measured by trypan blue exclusion (E). *, $p < 0.05$ versus thapsigargin-treated without doxycycline; $^{\#}$, $p < 0.05$ versus with doxycycline. $[Ca^{2+}]_i$, intracellular Ca^{2+}; Tg, thapsigargin; CCCP, carbonylcyanide m-chlorophenylhydrazon; Con, Doxycycline-non-treated; BI-1, Doxycycline-treated.

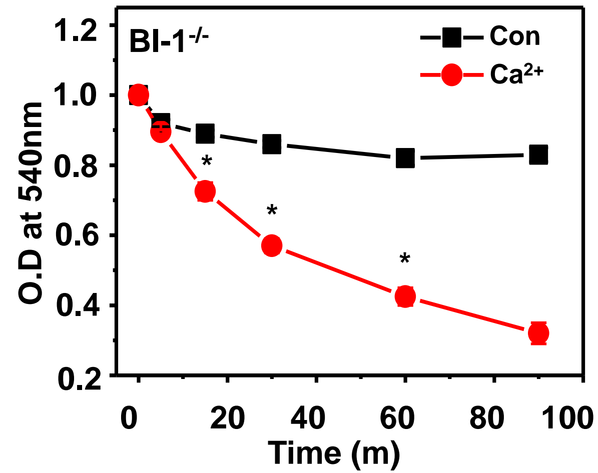
Supplementary Figure 4

Mitochondrial membrane potential in HT1080/Neo and HT1080/BI-1 cells was measured by JC-1 (5, 5´, 6, 6´-tetrachloro-1, 1´, 3, 3´-tetraethylbenzimidazol-carbocyanine iodide) staining and analyzed by PTI. The mitochondrial uncoupler, CCCP (1 μM) was added to collapse the mitochondria membrane potential.

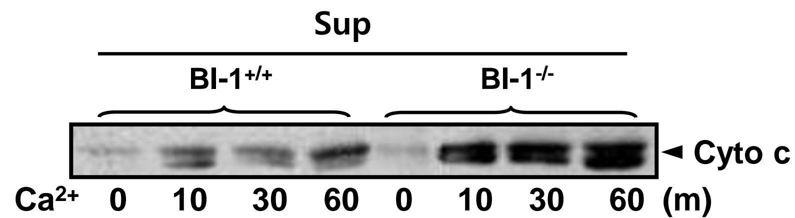
A

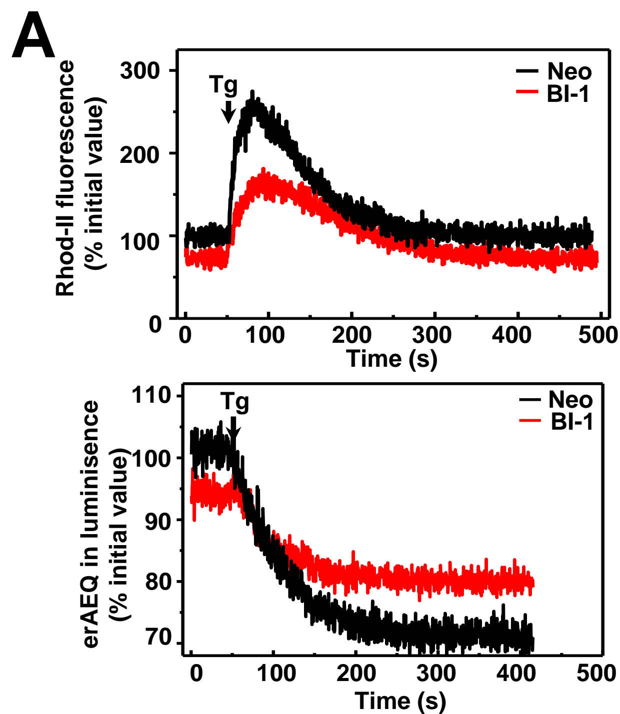


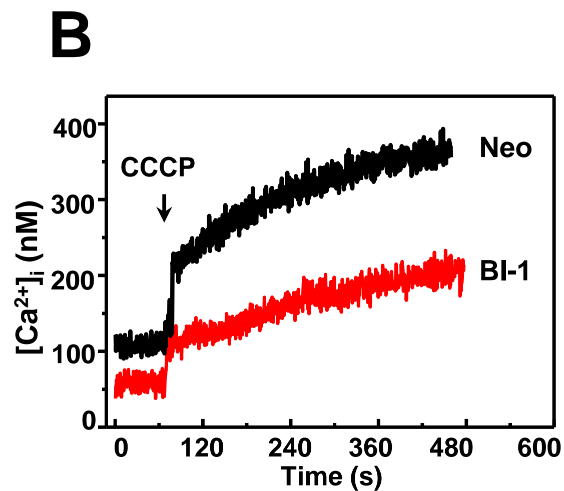


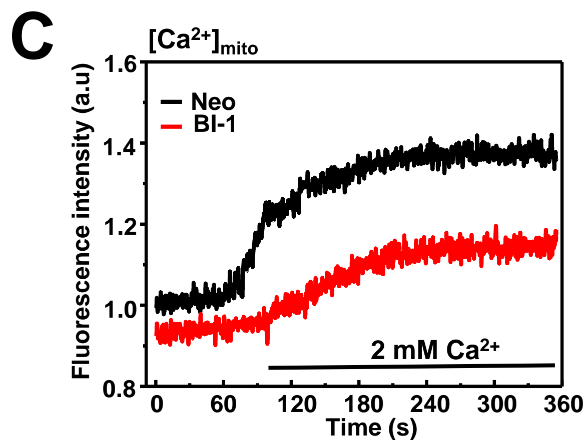


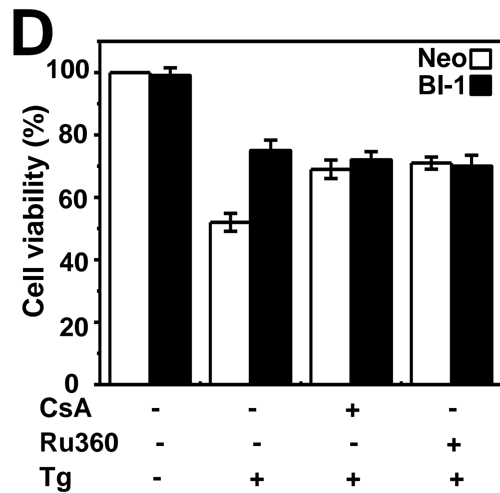
B

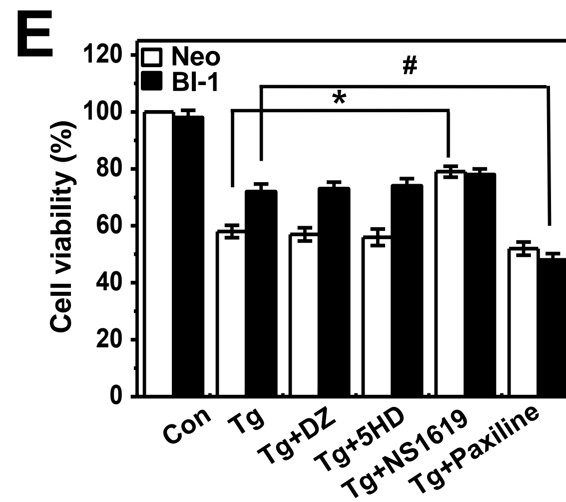


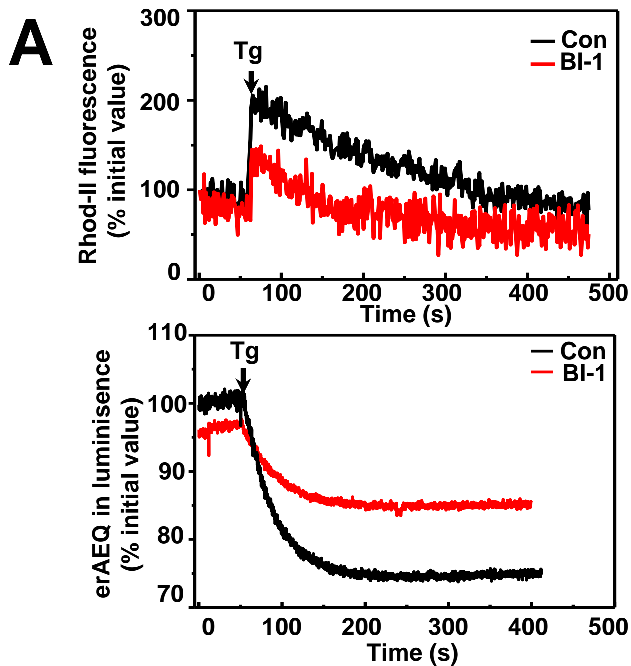

A
Rhod-II fluorescence (% initial value)
Tg
Neo
BI-1
Time (s)
erAEQ in luminisence (% initial value)
Tg
Neo
BI-1
Time (s)

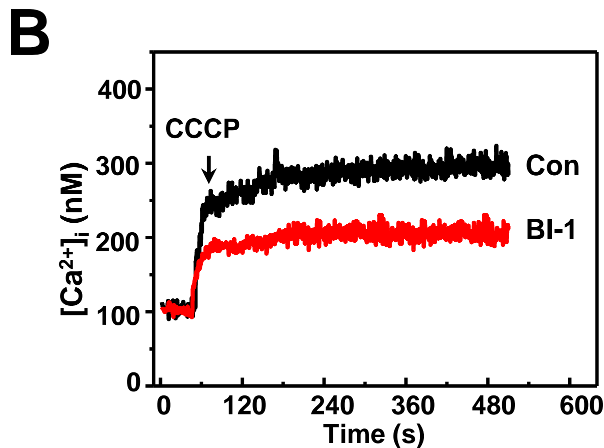

B
$[Ca^{2+}]_i$ (nM)
CCCP
Neo
BI-1
Time (s)

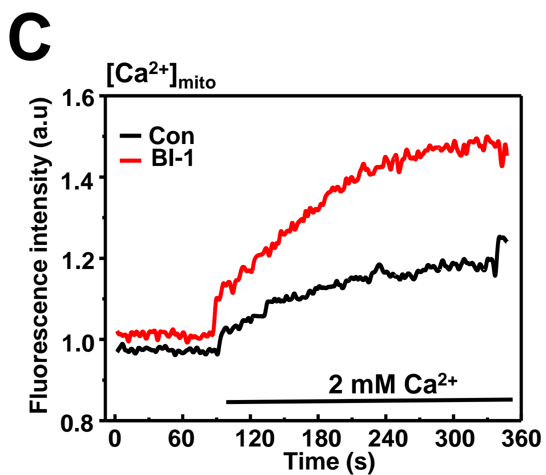

C
$[Ca^{2+}]_{mito}$
Fluorescence intensity (a.u)
Neo
BI-1
2 mM Ca^{2+}
Time (s)

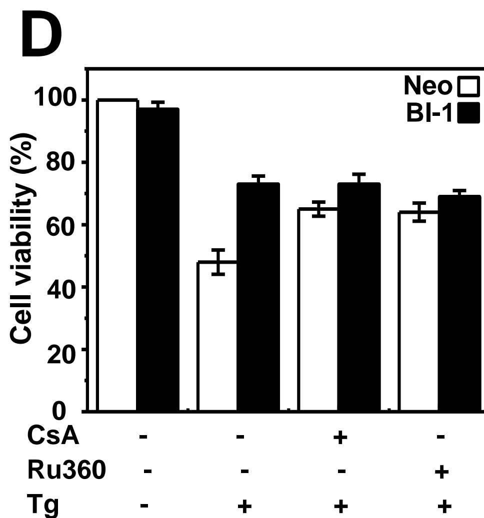

D
Cell viability (%)
Neo
BI-1
CsA
Ru360
Tg

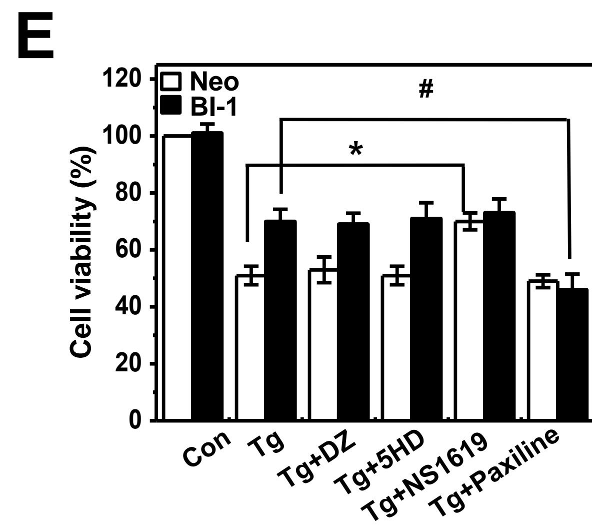

E
Cell viability (%)
Neo
BI-1
#
*
Con
Tg
Tg+DZ
Tg+5HD
Tg+NS1619
Tg+Paxiline

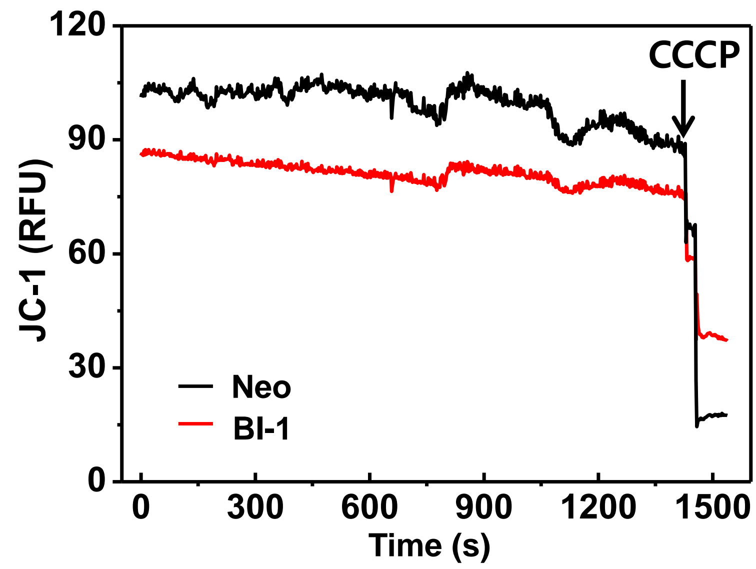

A
Rhod-II fluorescence (% initial value)
Tg
Con
BI-1
Time (s)
erAEQ in luminisence (% initial value)


B
CCCP
Con
BI-1
$[Ca^{2+}]_i$ (nM)
Time (s)


C
$[Ca^{2+}]_{mito}$
Con
BI-1
Fluorescence intensity (a.u)
2 mM Ca^{2+}
Time (s)


D
Neo
BI-1
Cell viability (%)
CsA
Ru360
Tg


E
Neo
BI-1
#
*
Cell viability (%)
Con
Tg
Tg+DZ
Tg+5HD
Tg+NS1619
Tg+Paxiline



CCCP
120
90
60
30
0
JC-1 (RFU)
Neo
BI-1
0
300
600
900
1200
1500
Time (s)