Supplementary Information

Mitochondrial impairment triggers cytosolic oxidative stress and cell death following proteasome inhibition

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Figure S1 | Polyubiquitinated proteins accumulate under proteasome inhibition. Immunoblot of polyubiquitinated proteins using anti-ubiquitin antibody. Samples were 1% NP-40 lysates of CHO cells treated with either DMSO (control) or 1 μM bortezomib (Bort) for 8 h at 37°C.

Figure S2 | Resveratrol reduces cytosolic ROS and improves cell viability under proteasome inhibition. (A) Relative cell viability as determined by the MTT assay. (B) Cytosolic H_2O_2 detection using HyPer under proteasome inhibition. 200 μM H_2O_2 was used as a positive control. Quantitated values are shown as the means ± s.e.m. of three independent measurements. **: $p < 0.01$, *: $p < 0.05$.

Figure S3 | Sesamin functions as a redox modulator in cells under proteasome inhibition. (A) FRET analysis of CHO cells expressing Redoxfluor, (C) cytosolic ROS detection using CM-H2DCFDA, (D) mitochondrial membrane potential detection using Mitotracker® Red CMXRos, and (E) mitochondrial ROS detection using the MitoSOX Red probe, after treatment with DMSO (control), 1 μM bortezomib (Bort), or simultaneous treatment with 1 μM bortezomib and 10 μM sesamin (Bort + Ses). Rotenone was used as a positive control for mitochondrial dysfunction and ROS generation. Fluorescence images (left panel) and quantitated fluorescence intensities (right panel) are shown. (B) Relative cell viability as determined by the MTT assay. All treatments were done for 8 h. Quantitated values are shown as the means ± s.e.m. of three independent measurements. **: $p < 0.01$, *: $p < 0.05$.

Figure S4 | Ascorbic acid did not affect cytosolic redox state in cells under proteasome inhibition. FRET images of CHO cells expressing Redoxfluor (upper panel) and quantitated fluorescence intensities (lower panel) are shown. Treatments were done for 8 h. Quantitated values are shown as the means ± s.e.m. of three

independent measurements. **: $p < 0.01$, NS: not significant.

Figure S5 | SOD expression and cellular localization. (A) Anti-MnSOD immunoblot of 1% NP-40 lysates from CHO cells transfected with either MnSOD construct or empty vector (pcDNA3.1). (B) Mitochondrial localization of overexpressed MnSOD. Immunofluorescence staining of CHO cells transfected with MnSOD. White arrows indicate MnSOD-overexpressing cells. (C) Mitochondrial ROS detection using the MitoSOX Red probe in cells transfected with pcDNA3.1 (mock) after treatment with DMSO (Mock + DMSO), cells transfected with pcDNA3.1 (mock) after treatment with 10 μM MG132 (Mock + MG132), and cells transfected with MnSOD after treatment with 10 μM MG132 (MnSOD + MG132). Under all conditions, cells were transiently transfected with respective plasmids. Fluorescence images (upper panel) and quantitated fluorescence intensities (lower panel) are shown. (D) Anti-HA immunoblot of 1% NP-40 lysates from CHO cells transfected with either HA-SOD1 construct or empty vector (pcDNA3.1).

Figure S6 | Validation of light mitochondrial fraction and ubiquitin localization under proteasome inhibition. (A) Validation of the light mitochondrial fractionation under DMSO (control) and 10 μM MG132 (MG132) treatment along with whole cell extract using a mitochondrial marker (anti-VDAC1), a cytosolic marker (anti-GAPDH), an endoplasmic reticulum marker (Anti-ERp72), and a peroxisome marker (Anti-catalase) (B) Localization of overexpressed HA-Ub. Immunofluorescence staining of CHO cells transfected with HA-Ub after treatment with DMSO (HA-Ub+DMSO), 1 μM Bortezomib (HA-Ub+Bort) and 10 μM MG132 (HA-Ub+MG132) respectively.

Figure S1

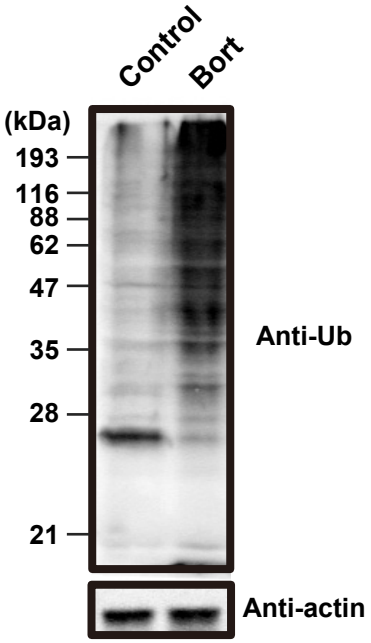


Figure S2

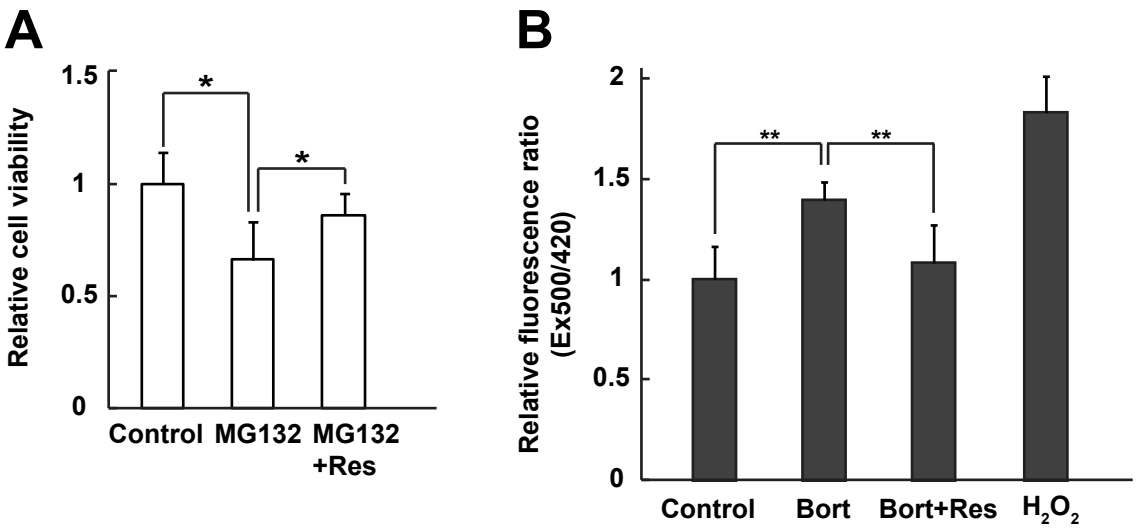

A
Relative cell viability
1.5
1
0.5
0
*
*
Control
MG132
MG132 +Res
B
Relative fluorescence ratio (Ex500/420)
2
1.5
1
0.5
0
**
**
Control
Bort
Bort+Res
H2O2

Figure S3

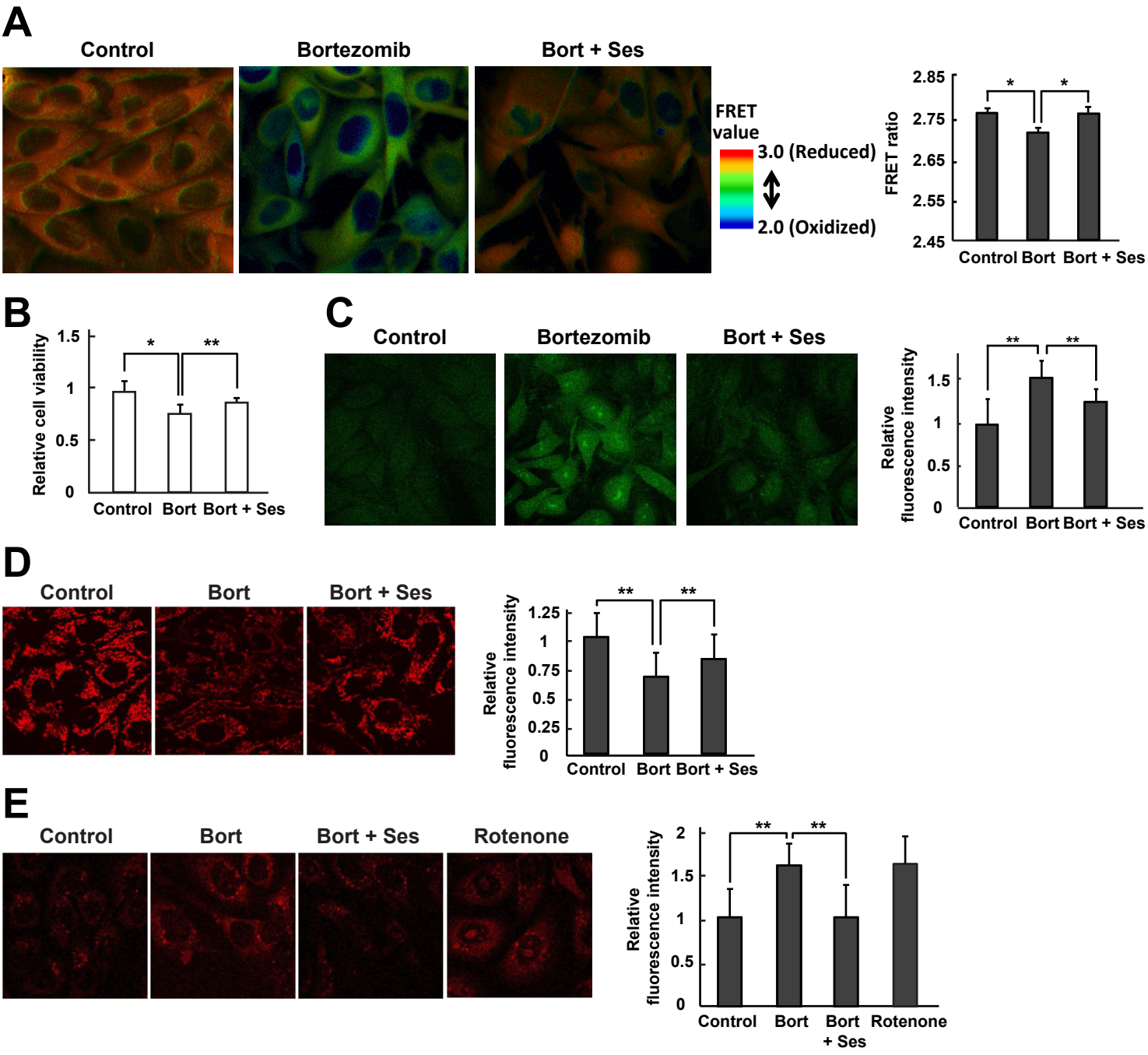
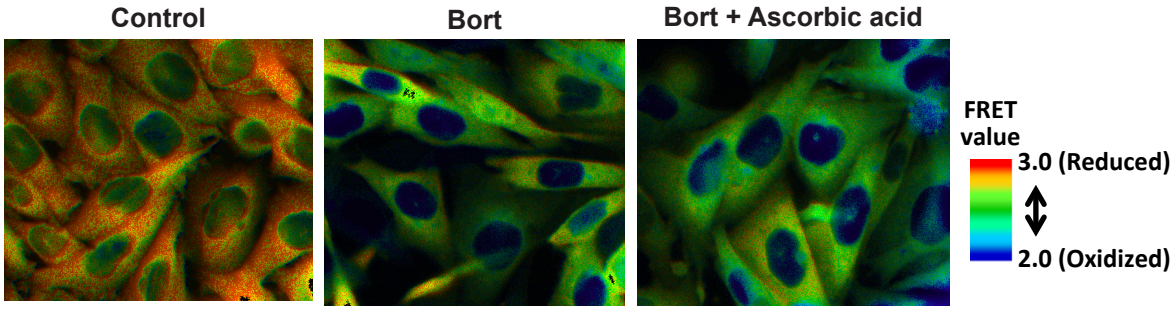

A
Control
Bortezomib
Bort + Ses
FRET value
3.0 (Reduced)
2.0 (Oxidized)
FRET ratio
2.85
2.75
2.65
2.55
2.45
Control Bort Bort + Ses
B
Relative cell viability
1.5
1
0.5
0
Control Bort Bort + Ses
C
Control
Bortezomib
Bort + Ses
Relative fluorescence intensity
1.5
1
0.5
0
Control Bort Bort + Ses
D
Control
Bort
Bort + Ses
Relative fluorescence intensity
1.25
1
0.75
0.5
0.25
0
Control Bort Bort + Ses
E
Control
Bort
Bort + Ses
Rotenone
Relative fluorescence intensity
2
1.5
1
0.5
0
Control Bort Bort + Ses Rotenone

Figure S4


Control
Bort
Bort + Ascorbic acid
FRET value
3.0 (Reduced)
2.0 (Oxidized)

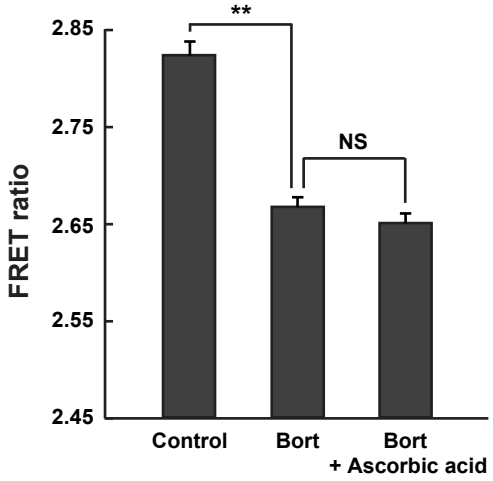

**
NS
2.85
2.75
2.65
2.55
2.45
FRET ratio
Control
Bort
Bort
+ Ascorbic acid

Figure S5

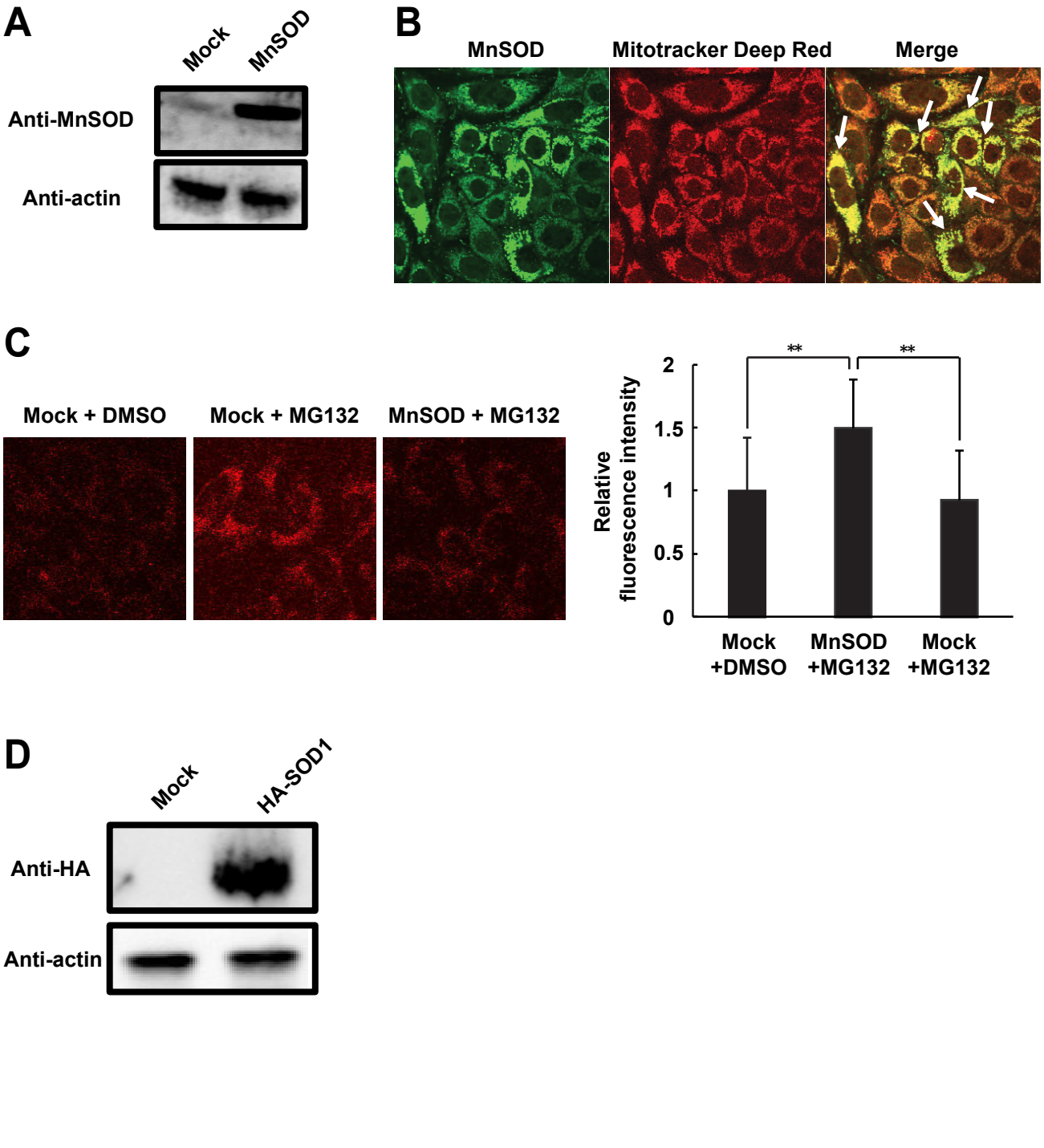
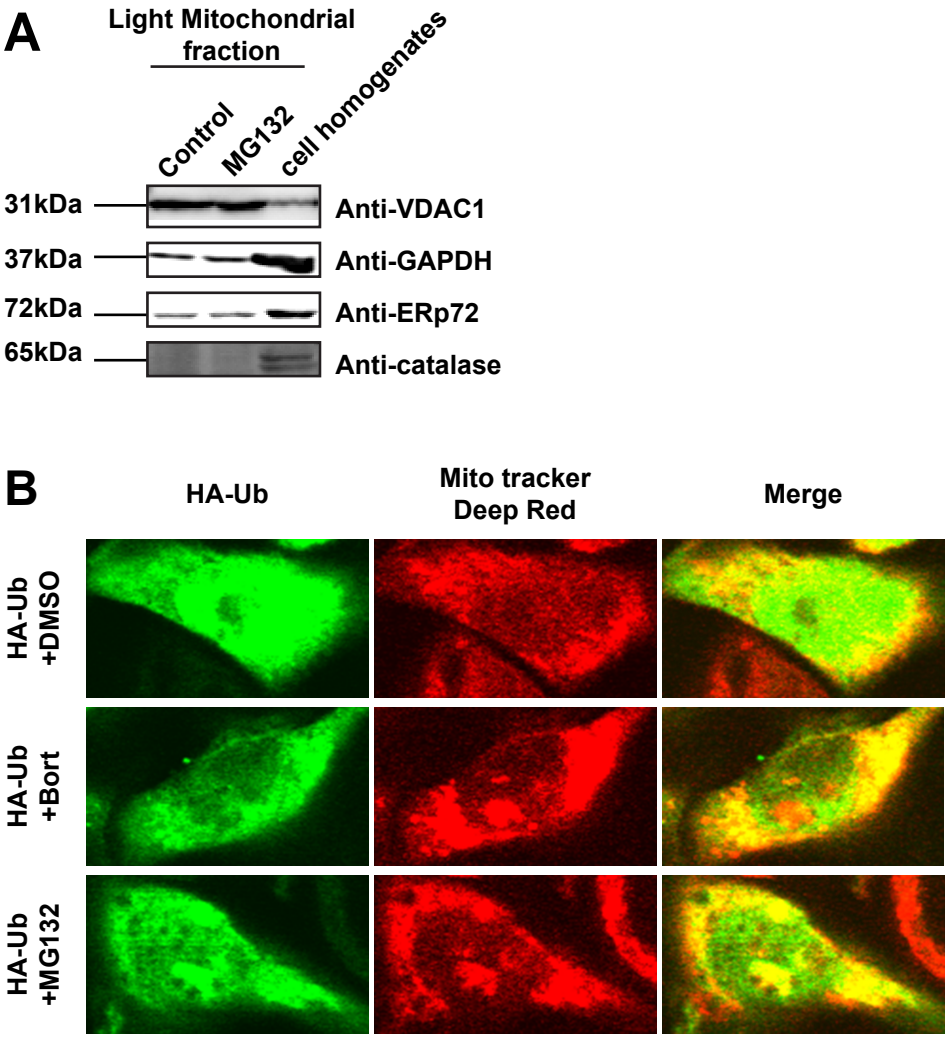

A
Mock
MnSOD
Anti-MnSOD
Anti-actin
B
MnSOD
Mitotracker Deep Red
Merge
C
Mock + DMSO
Mock + MG132
MnSOD + MG132
Relative fluorescence intensity
2
1.5
1
0.5
0
**
**
Mock +DMSO
MnSOD +MG132
Mock +MG132
D
Mock
HA-SOD1
Anti-HA
Anti-actin

Figure S6


A
Light Mitochondrial fraction
Control
MG132
cell homogenates
31kDa
Anti-VDAC1
37kDa
Anti-GAPDH
72kDa
Anti-ERp72
65kDa
Anti-catalase
B
HA-Ub
Mito tracker Deep Red
Merge
HA-Ub +DMSO
HA-Ub +Bort
HA-Ub +MG132