SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Norepinephrine does not affect mitochondrial mass content and cell death on cultured rat cardiomyocytes but decreases the respiratory control ratio. (A) Mitochondrial heat shock protein 70 (mtHsp70) levels were determined by Western blot, n=6 and (B) Mitochondrial mass by determined by flow cytometry and Mitotraker Green relative fluorescence in cultured rat cardiomyocytes stimulated with NE for 48 h (n = 6), ns=non-significant. (C) Cell death was measured by flow cytometry using propidium iodide in cardiomyocytes treated with NE for 48 h (n = 3). Hydrogen peroxide (1 mM) was used as positive controls. Representative flow cytometry histograms for cell population distribution on each condition are shown in top region of graphic. (D) Respiratory control ratio (CCCP-uncoupled respiration/ basal respiration) was calculated in cardiomyocytes treated with NE for 0, 24 and 48 h (n= 4). *$p < 0.05$ vs control; ns=non-significant.

Fig. S2. Dominant negative mutant of Drp1 (K38A) promotes mitochondrial fusion in cultured cardiomyocytes. Mitochondrial morphology was assessed by confocal microscopy of Mitotracker green stained cells. (A) Representative confocal images of cardiomyocytes transduced with adenovirus containing LacZ or K38A (n = 4), and (B) mitochondrial volume analysis of these cells. (C-D) Western blot and quantification of Drp1 levels of cells transduced with the above-mentioned adenovirus (n = 4). *$p < 0.05$ vs control; $^{\#}p < 0.05$ vs LacZ.

Fig. S3. Adenovirus containing anti-sense RNA for Mitofusin-2 (AsMfn2) promotes

mitochondrial fission in cultured cardiomyocytes. (A) Representative images of cardiomyocytes transduced with adenovirus containing LacZ or AsMfn2 (n=4) and (B) mitochondrial volume quantification of these cells. Scale bar = 10 μm. (C-D) Western blot and quantification of Mfn2 protein levels of cells transduced with the above mentioned adenovirus. *$p < 0.05$ vs control; $^{\#}p < 0.05$ vs LacZ.

Fig. S4. Expression of inhibitory peptide (CAIN) and a constitutively active form of calcineurin (CN) promotes changes in levels of RCAN 1.4 in cardiomyocytes and AsMfn2 increases parkin mitochondrial localization and does not stimulate synergistic effects with NE or the ATP synthase inhibitor oligomycin. Representative Western blot of RCAN 1.4 protein in cardiomyocytes control or stimulated with NE (10 μM) for 48h and transduced with adenovirus containing LacZ or (A) CAIN and (B) adenovirus containing constitutively active form of CN. (C) Immunofluorescence images of cardiomyocytes treated as indicated, using anti-mtHsp70 (red) or anti-Parkin (green) antibodies. Scale bar=20 μm. (D) Top and bottom graphs show Manders' coefficient and Pearson's coefficients, respectively. Determination of cell area in cardiomyocytes treated with (E) an adenovirus encoding an antisense RNA for Mfn2 (AsMfn2) and then treated the cells with NE or (F) treated with the ATP synthase inhibitor oligomycin (0.2 – 2 μM) for 48 h. n = 3; *$p < 0.05$ vs control.

Figure S1.

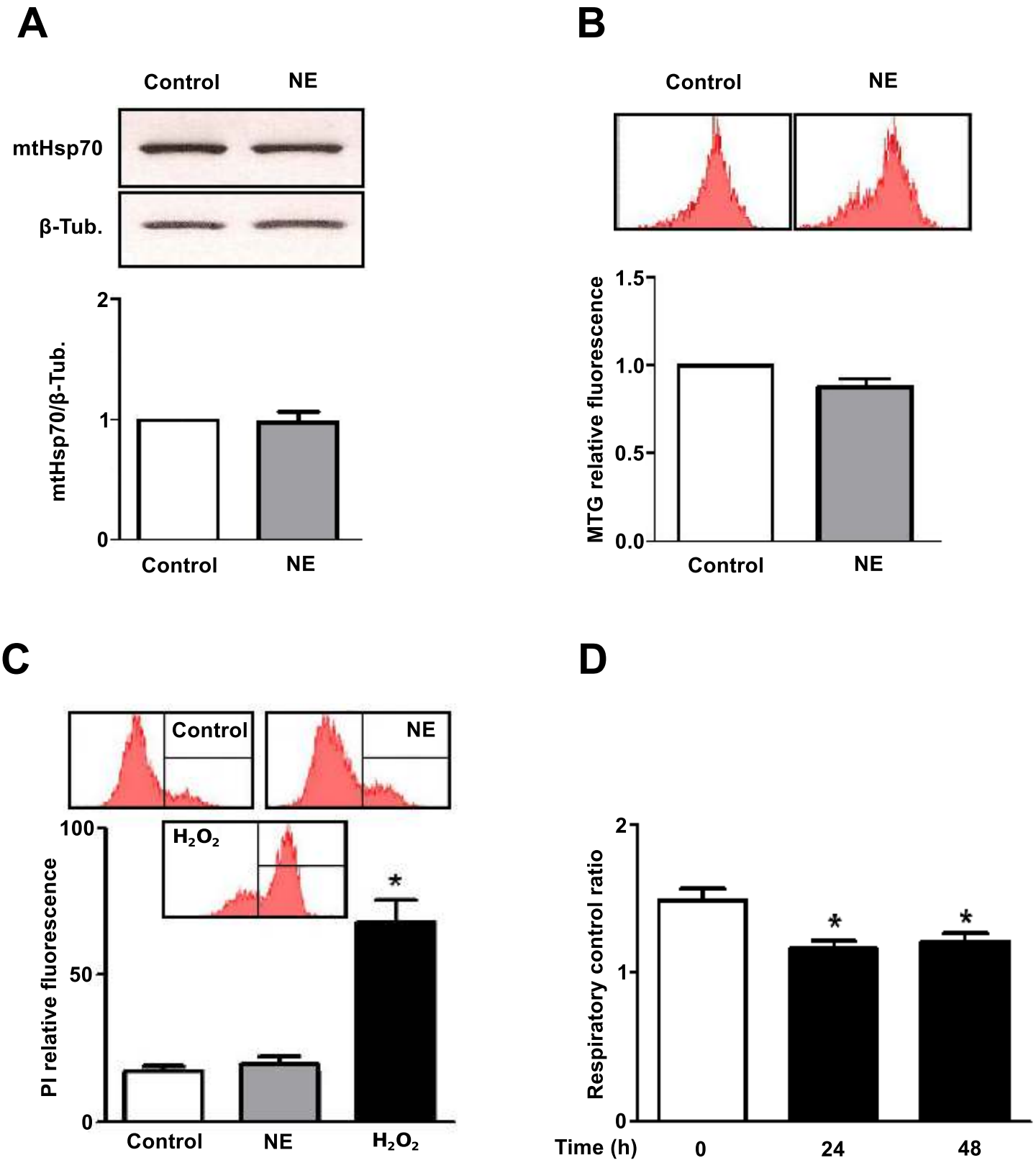

A
Control
NE
mtHsp70
β-Tub.
mtHsp70/β-Tub.
2
1
0
Control
NE
B
Control
NE
MTG relative fluorescence
1.5
1.0
0.5
0.0
Control
NE
C
Control
NE
H2O2
PI relative fluorescence
100
50
0
*
Control
NE
H2O2
D
Respiratory control ratio
2
1
0
*
*
Time (h)
0
24
48

Figure S2.

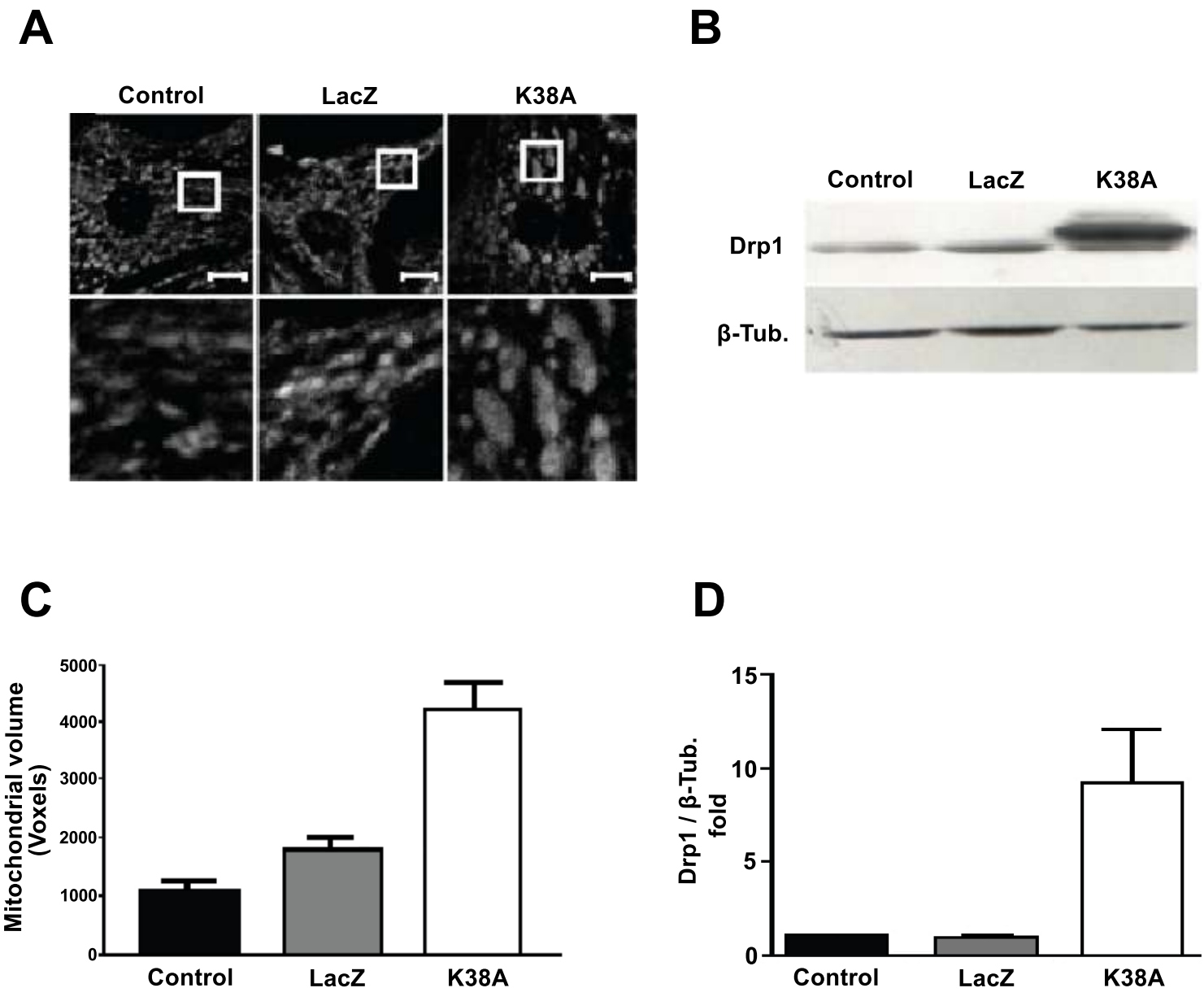

A
Control
LacZ
K38A
B
Control
LacZ
K38A
Drp1
β-Tub.
C
Mitochondrial volume (Voxels)
5000
4000
3000
2000
1000
0
Control
LacZ
K38A
D
Drp1 / β-Tub. fold
15
10
5
0
Control
LacZ
K38A

Figure S3.

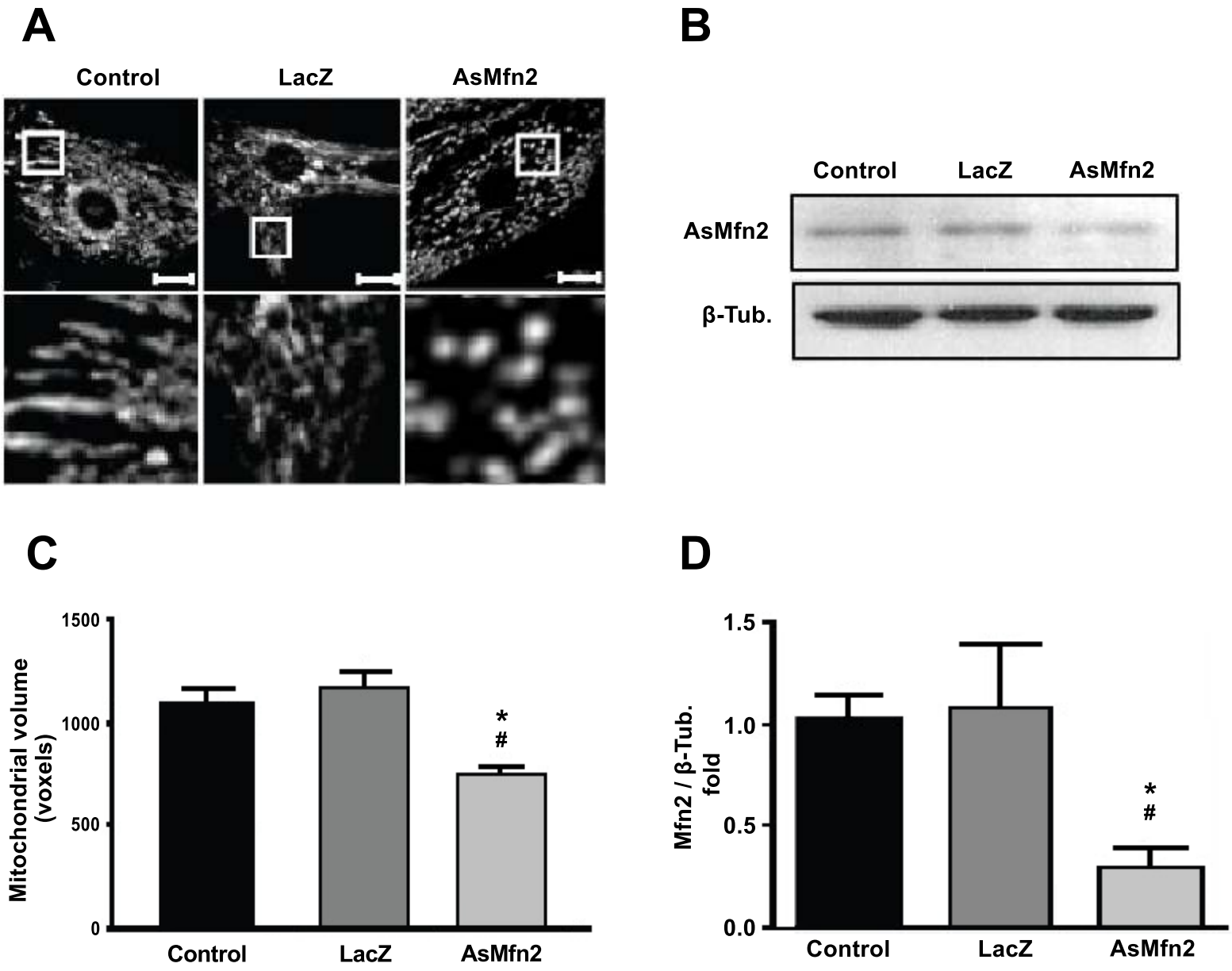
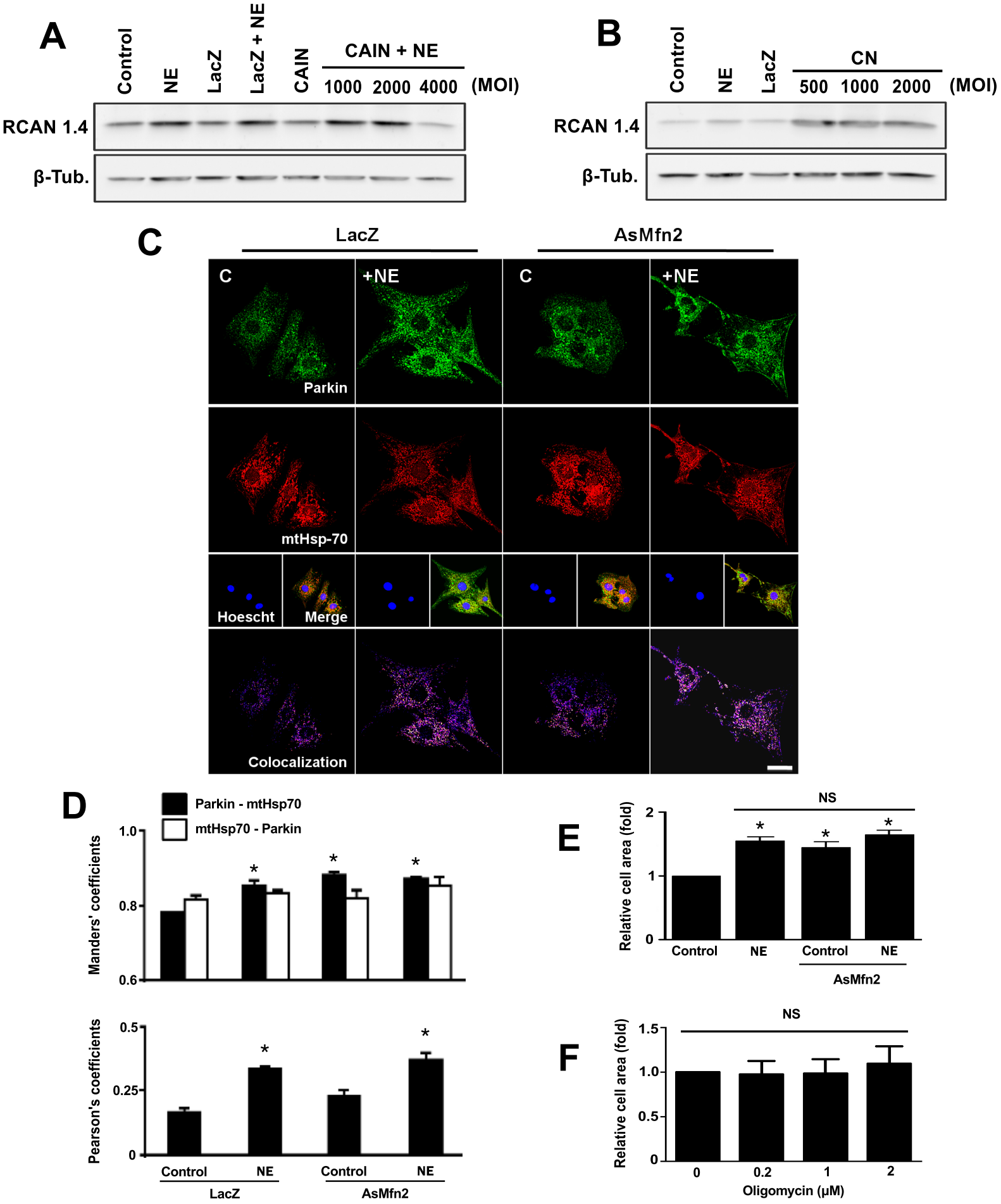

A
Control
LacZ
AsMfn2
B
Control
LacZ
AsMfn2
AsMfn2
β-Tub.
C
Mitochondrial volume (voxels)
1500
1000
500
0
*
#
Control
LacZ
AsMfn2
D
Mfn2 / β-Tub. fold
1.5
1.0
0.5
0.0
*
#
Control
LacZ
AsMfn2

Figure S4.


A
Control
NE
LacZ
LacZ + NE
CAIN
CAIN + NE
1000 2000 4000
(MOI)
RCAN 1.4
β-Tub.
B
Control
NE
LacZ
CN
500 1000 2000
(MOI)
RCAN 1.4
β-Tub.
C
LacZ
AsMfn2
C
+NE
C
+NE
Parkin
mtHsp-70
Hoescht
Merge
Colocalization
D
Parkin - mtHsp70
mtHsp70 - Parkin
Manders' coefficients
1.0
0.8
0.6
Pearson's coefficients
0.5
0.25
0
Control
NE
Control
NE
LacZ
AsMfn2
E
NS
Relative cell area (fold)
2
1
0
Control
NE
Control
NE
AsMfn2
F
NS
Relative cell area (fold)
1.5
1.0
0.5
0
0
0.2
1
2
Oligomycin (μM)