Original data

RIPK1 inhibition contributes to lysosomal membrane stabilization in ischemic astrocytes via a lysosomal Hsp70.1B-dependent mechanism

Running head: RIPK1 inhibition stabilizes lysosomal membranes

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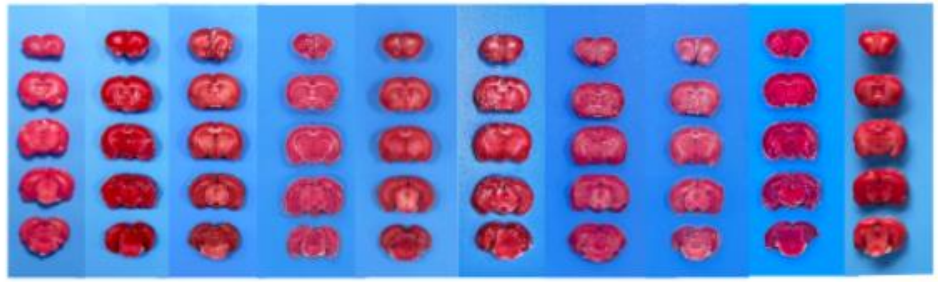
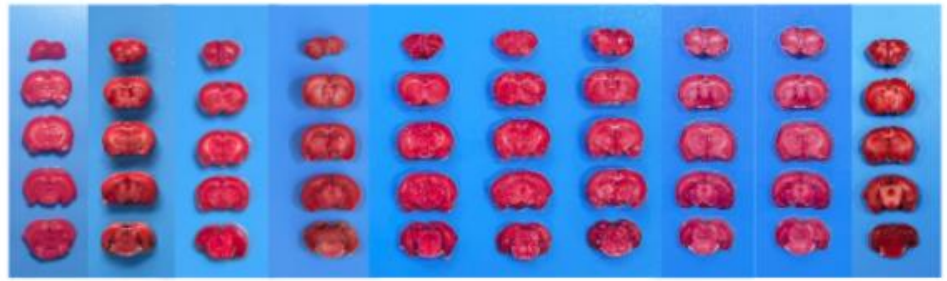
These authors contributed equally: Hua-Ping Du, Yi Guo, Yong-Ming Zhu

All brain slices of TTC scaning in Fig. 5a

sham

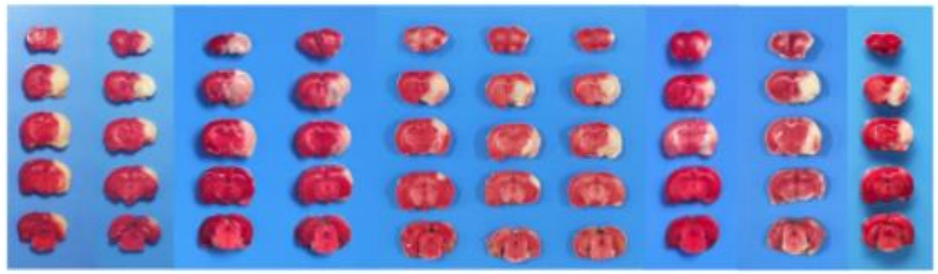


sham+
Hsp70.1B shRNA



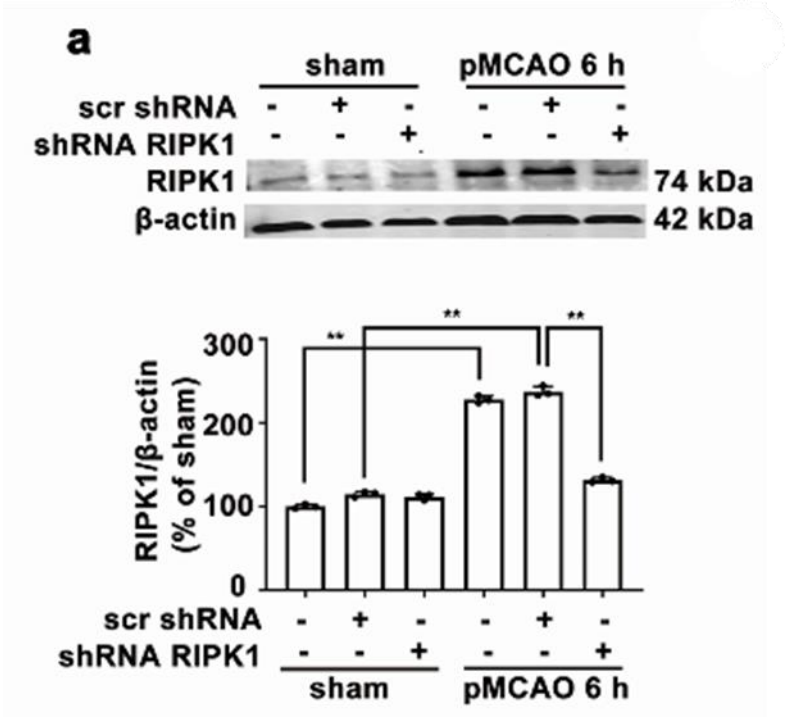
I/R

I/R+
Hsp70.1B shRNA



Original data for all Western blotting bands

Fig. 1a


a
sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
RIPK1
β-actin
74 kDa
42 kDa
RIPK1/β-actin
(% of sham)
300
200
100
0
**
**
**
scr shRNA
shRNA RIPK1
sham
pMCAO 6 h

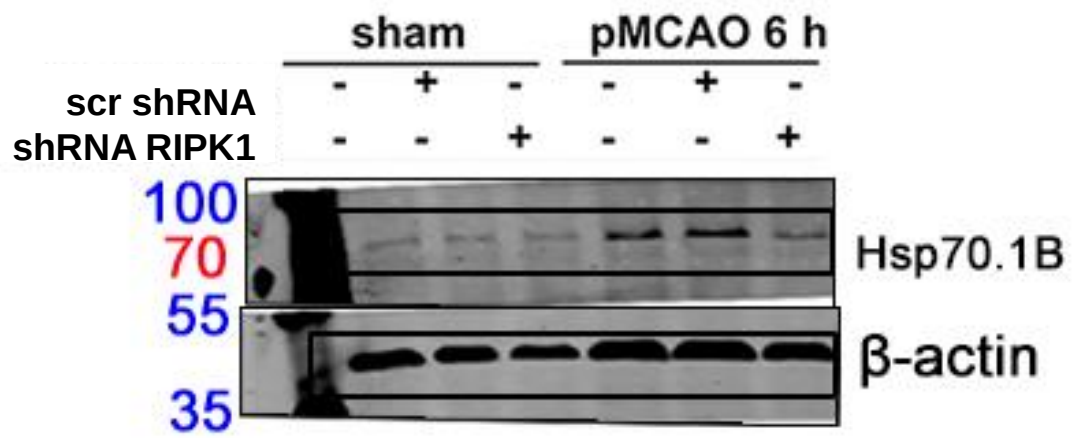
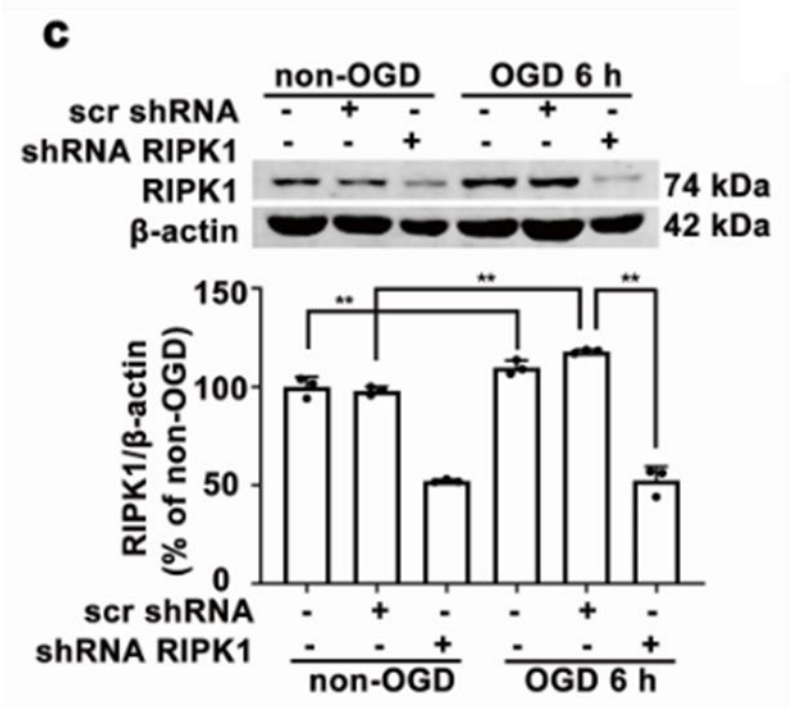

sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
100
70
55
35
Hsp70.1B
β-actin

Fig. 1c


c
non-OGD
OGD 6 h
scr shRNA
shRNA RIPK1
RIPK1
β-actin
74 kDa
42 kDa
RIPK1/β-actin (% of non-OGD)
150
100
50
0
**
**
**
scr shRNA
shRNA RIPK1
non-OGD
OGD 6 h

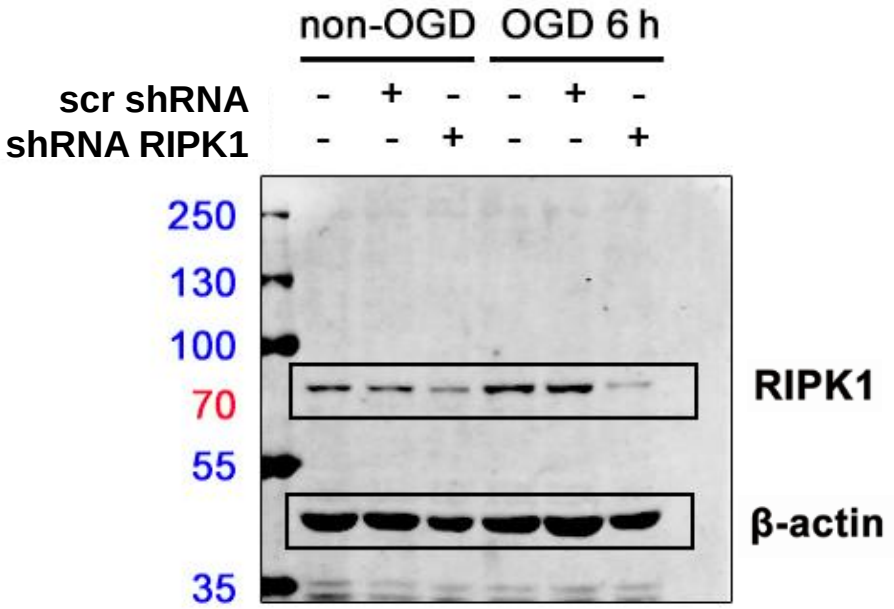
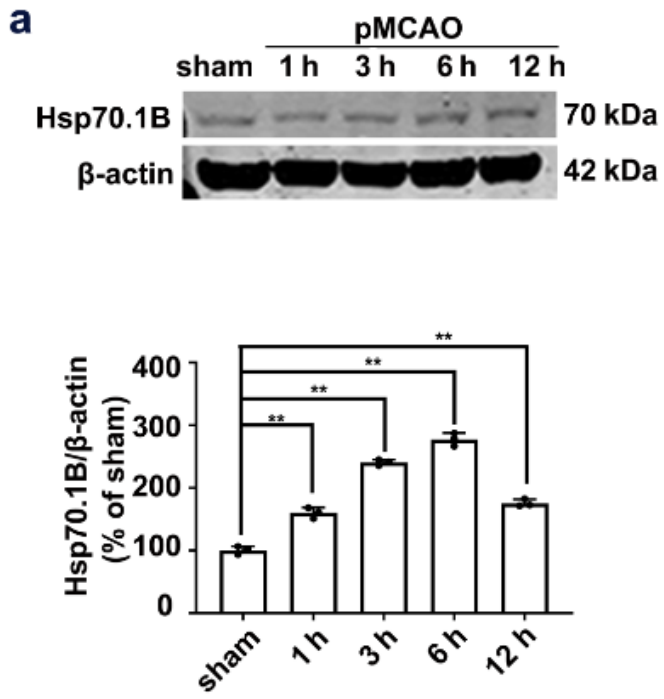

non-OGD
OGD 6 h
scr shRNA
shRNA RIPK1
250
130
100
70
55
35
RIPK1
β-actin

Fig. 2a


a
pMCAO
sham
1 h
3 h
6 h
12 h
Hsp70.1B
70 kDa
β-actin
42 kDa
Hsp70.1B/β-actin
(% of sham)
400
300
200
100
0
**
**
**
**
sham
1 h
3 h
6 h
12 h

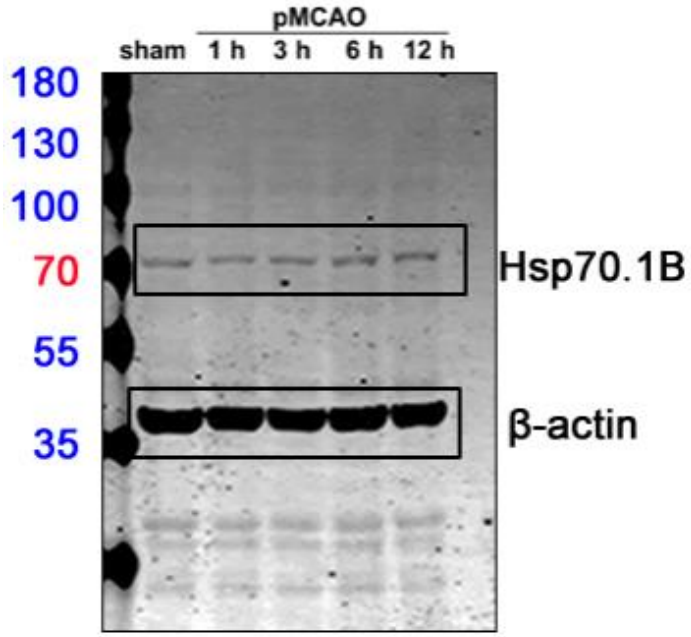
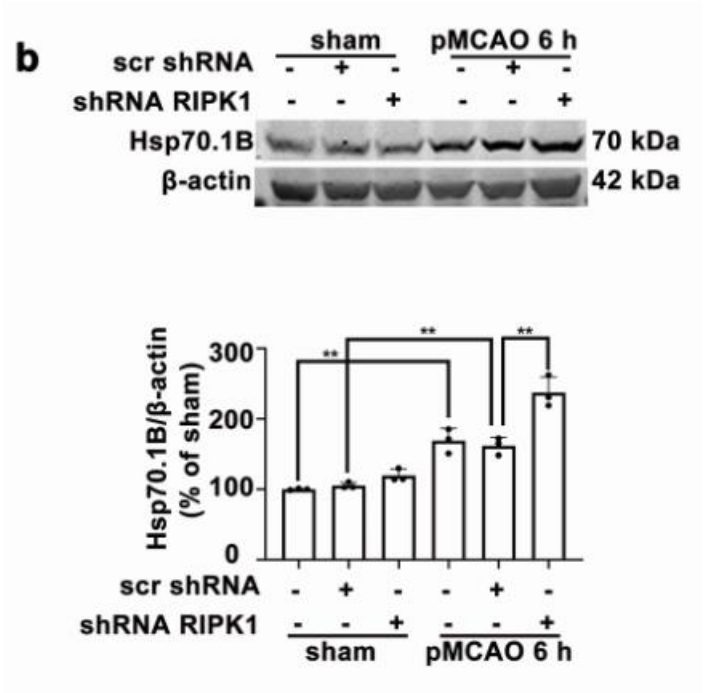

pMCAO
sham
1 h
3 h
6 h
12 h
180
130
100
70
55
35
Hsp70.1B
β-actin

Fig. 2b


b
sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
Hsp70.1B
β-actin
70 kDa
42 kDa
Hsp70.1B/β-actin (% of sham)
300
200
100
0
**
**
**
scr shRNA
shRNA RIPK1
sham
pMCAO 6 h

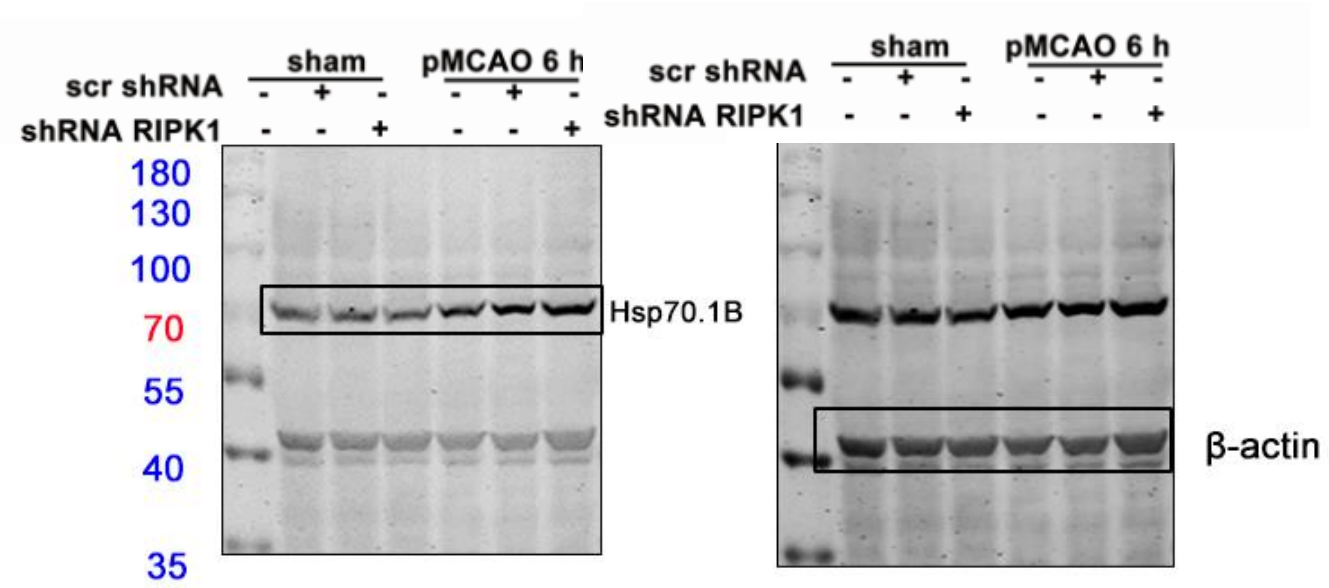

sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
180
130
100
70
55
40
35
Hsp70.1B
β-actin

Fig. 3a

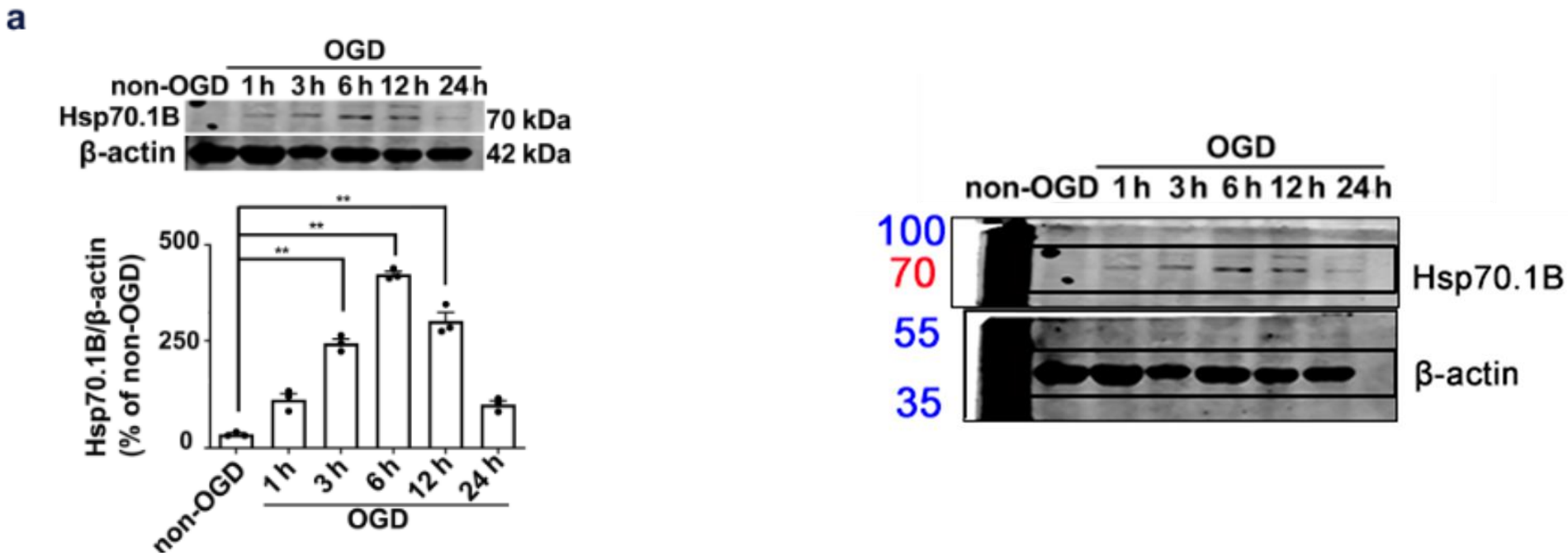
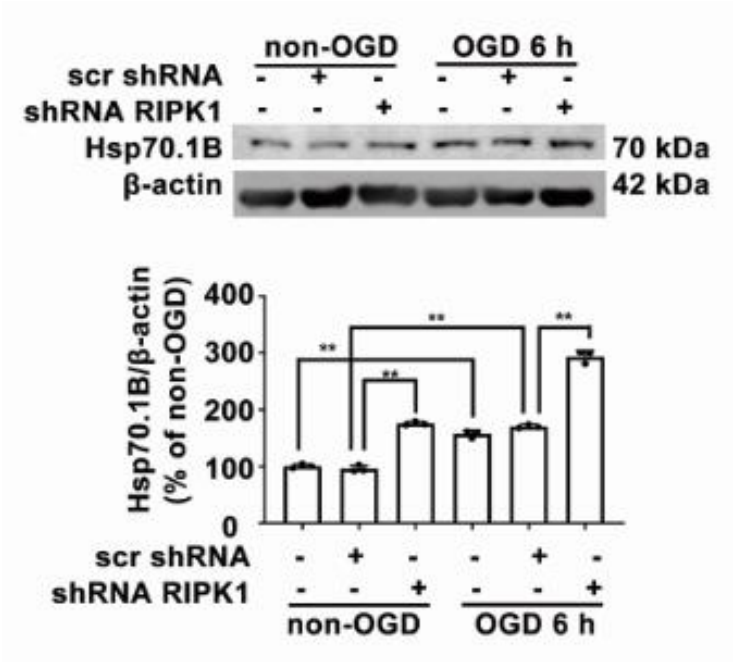

a
OGD
non-OGD 1 h 3 h 6 h 12 h 24 h
Hsp70.1B
β-actin
70 kDa
42 kDa
Hsp70.1B/β-actin
(% of non-OGD)
500
250
0
**
**
**
non-OGD
1 h
3 h
6 h
12 h
24 h
OGD
OGD
non-OGD 1 h 3 h 6 h 12 h 24 h
100
70
55
35
Hsp70.1B
β-actin

Fig. 3b


non-OGD
OGD 6 h
scr shRNA
shRNA RIPK1
Hsp70.1B
β-actin
70 kDa
42 kDa
Hsp70.1B/β-actin
(% of non-OGD)
400
300
200
100
0
**
**
**
**
scr shRNA
shRNA RIPK1
non-OGD
OGD 6 h

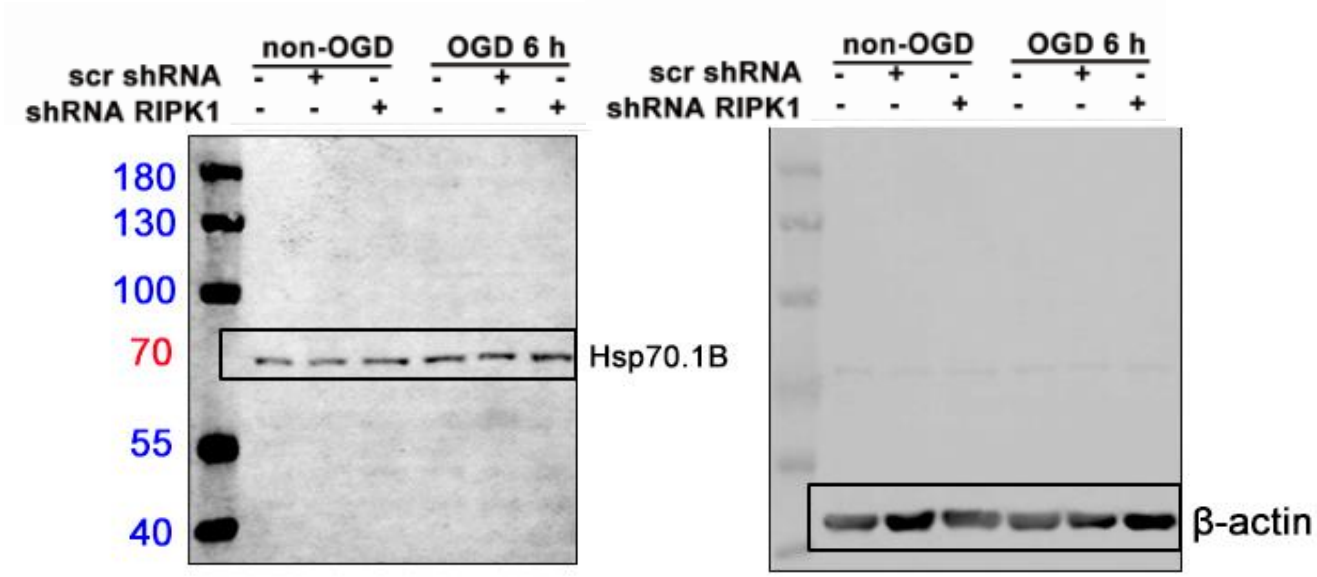

non-OGD
OGD 6 h
scr shRNA
shRNA RIPK1
180
130
100
70
55
40
Hsp70.1B
β-actin

Fig. 4a

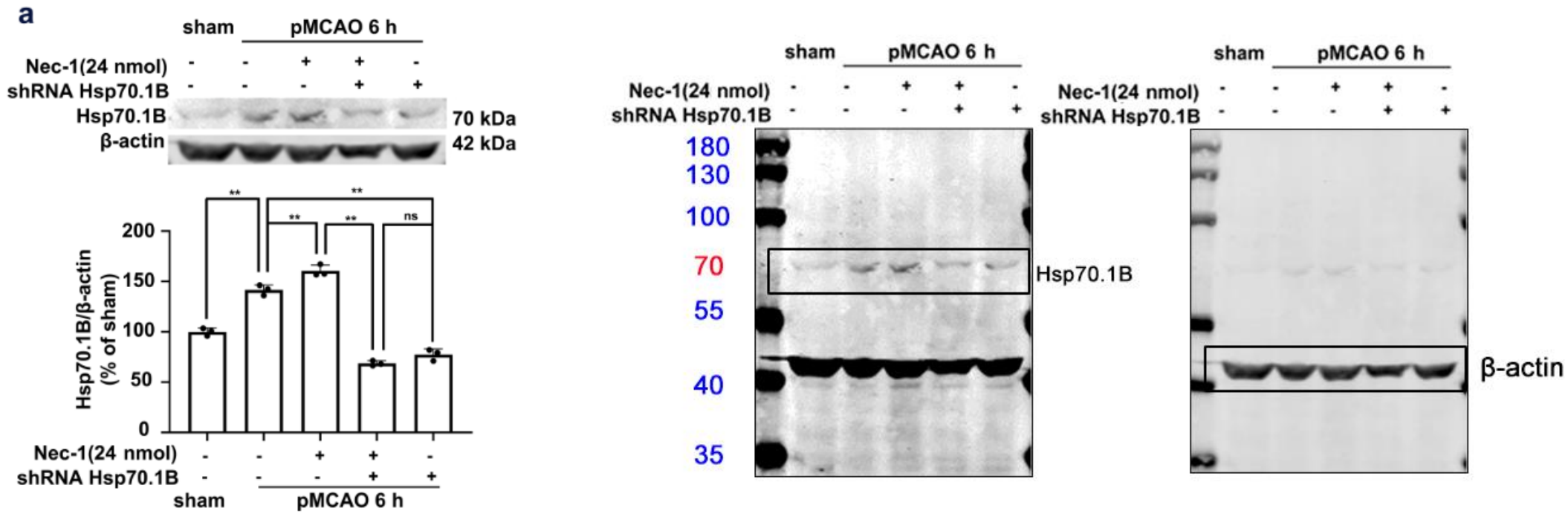

a
sham
pMCAO 6 h
Nec-1(24 nmol)
shRNA Hsp70.1B
Hsp70.1B
β-actin
70 kDa
42 kDa
Hsp70.1B/β-actin (% of sham)
200
150
100
50
0
**
**
**
ns
Nec-1(24 nmol)
shRNA Hsp70.1B
sham
pMCAO 6 h
180
130
100
70
55
40
35
Hsp70.1B
β-actin

Fig. 4b

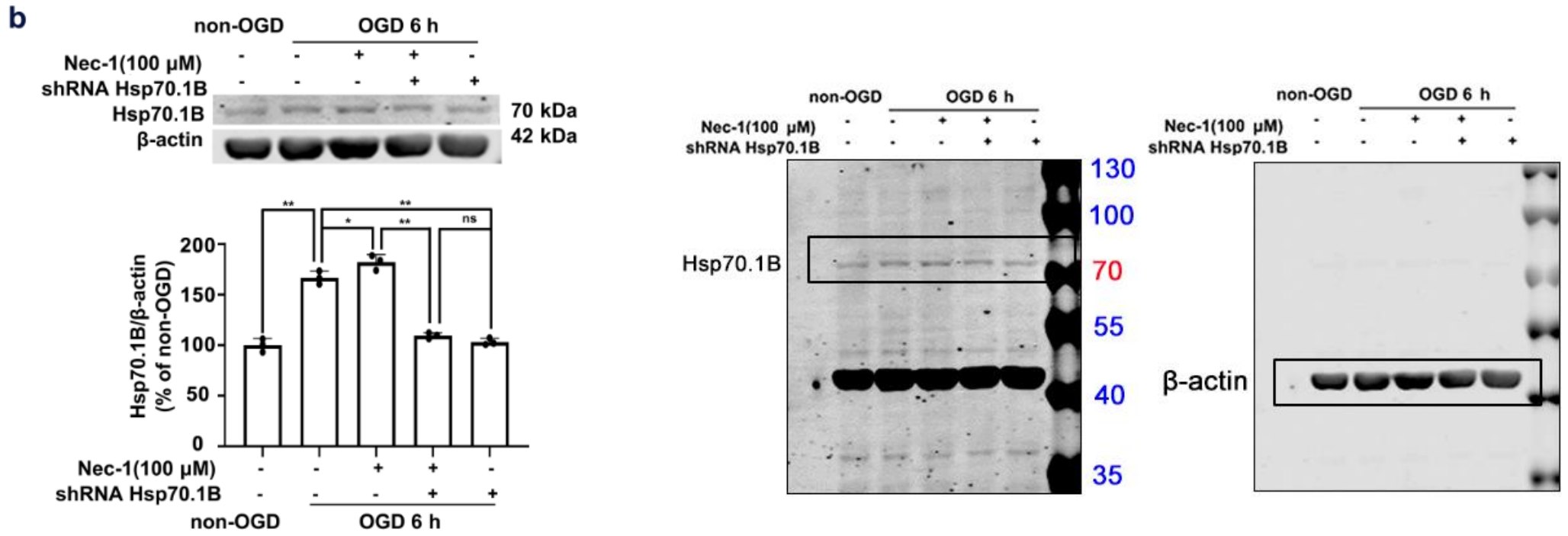

b
non-OGD
OGD 6 h
Nec-1(100 μM)
shRNA Hsp70.1B
Hsp70.1B
β-actin
70 kDa
42 kDa
Hsp70.1B/β-actin (% of non-OGD)
200
150
100
50
0
**
**
*
**
ns
Nec-1(100 μM)
shRNA Hsp70.1B
non-OGD
OGD 6 h
non-OGD
OGD 6 h
Nec-1(100 μM)
shRNA Hsp70.1B
Hsp70.1B
130
100
70
55
40
35
non-OGD
OGD 6 h
Nec-1(100 μM)
shRNA Hsp70.1B
β-actin

Fig. 6a

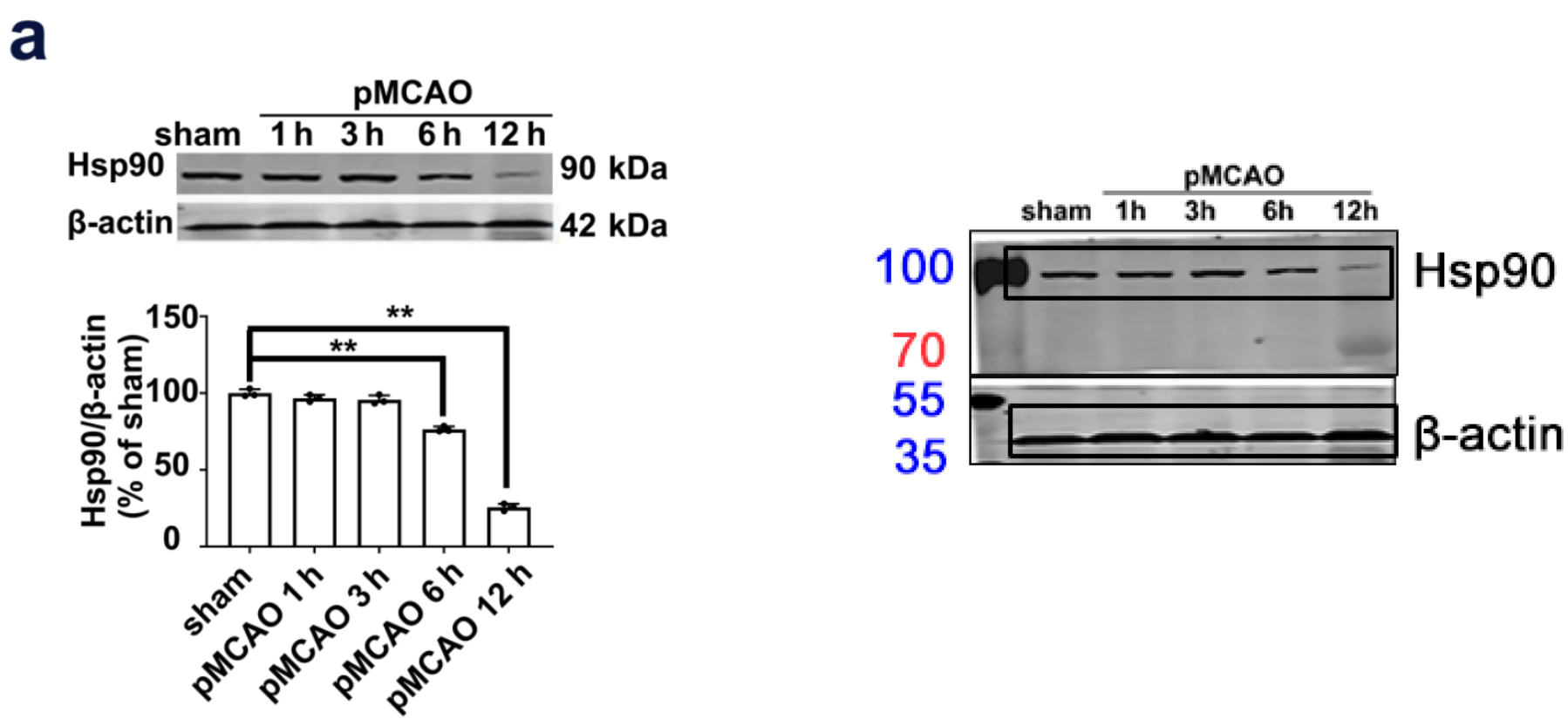

a
pMCAO
sham
1 h
3 h
6 h
12 h
Hsp90
90 kDa
β-actin
42 kDa
Hsp90/β-actin (% of sham)
150
100
50
0
**
**
sham
pMCAO 1 h
pMCAO 3 h
pMCAO 6 h
pMCAO 12 h
pMCAO
sham
1h
3h
6h
12h
100
70
55
35
Hsp90
β-actin

Fig. 6b

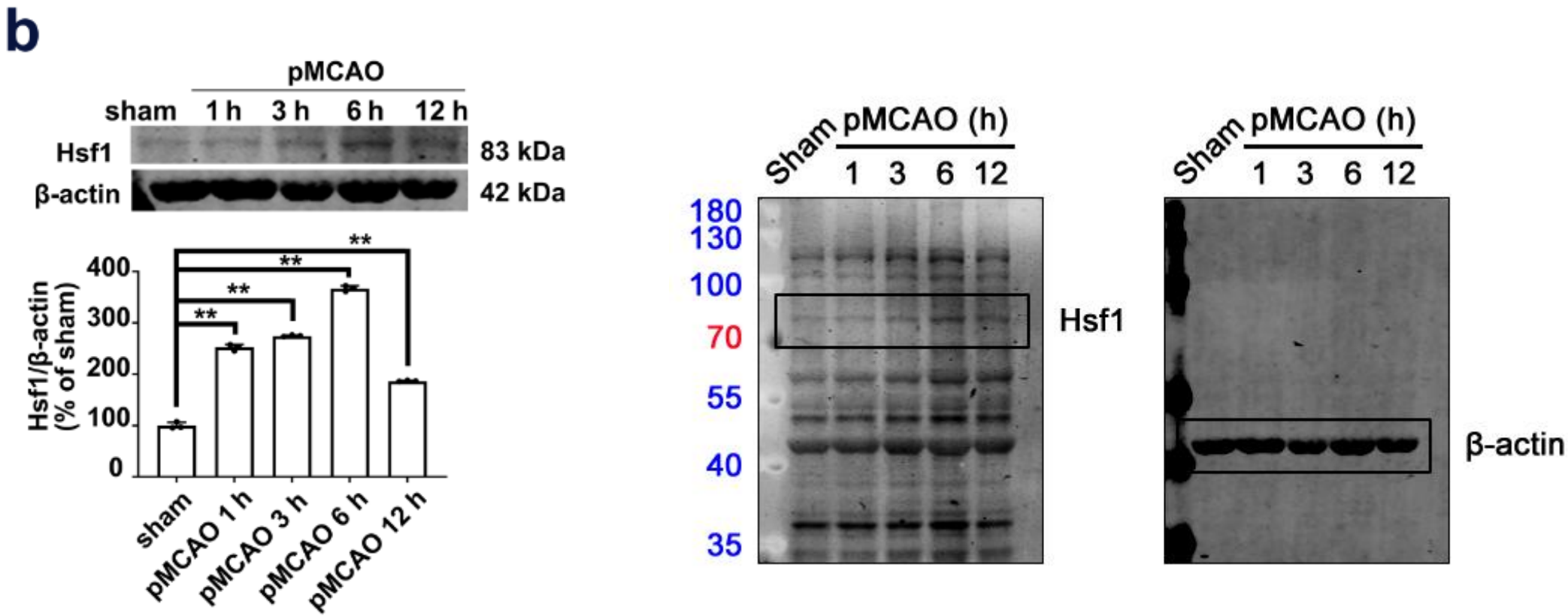

b
pMCAO
sham
1 h
3 h
6 h
12 h
Hsf1
β-actin
83 kDa
42 kDa
Hsf1/β-actin (% of sham)
400
300
200
100
0
**
sham
pMCAO 1 h
pMCAO 3 h
pMCAO 6 h
pMCAO 12 h
Sham
pMCAO (h)
1 3 6 12
180
130
100
70
55
40
35
Hsf1
β-actin

Fig. 6c

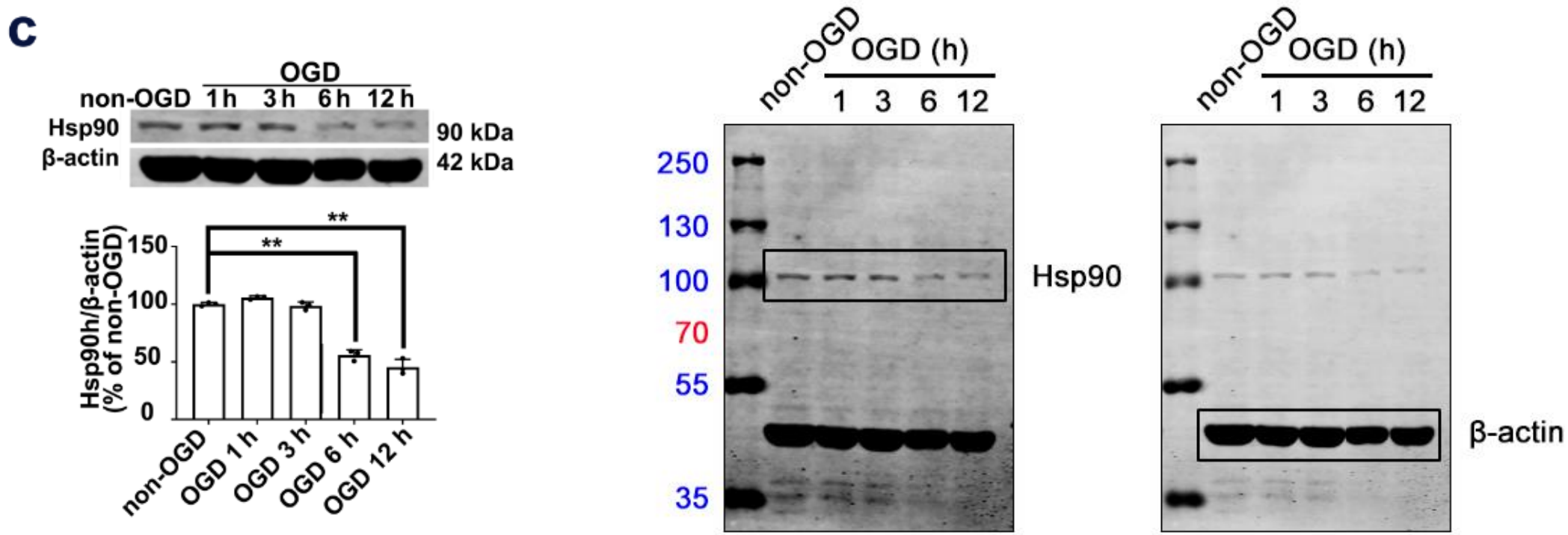

c
OGD
non-OGD 1 h 3 h 6 h 12 h
Hsp90
β-actin
90 kDa
42 kDa
Hsp90h/β-actin (% of non-OGD)
150
100
50
0
**
**
non-OGD
OGD 1 h
OGD 3 h
OGD 6 h
OGD 12 h
non-OGD
OGD (h)
1 3 6 12
250
130
100
70
55
35
Hsp90
non-OGD
OGD (h)
1 3 6 12
β-actin

Fig. 6d

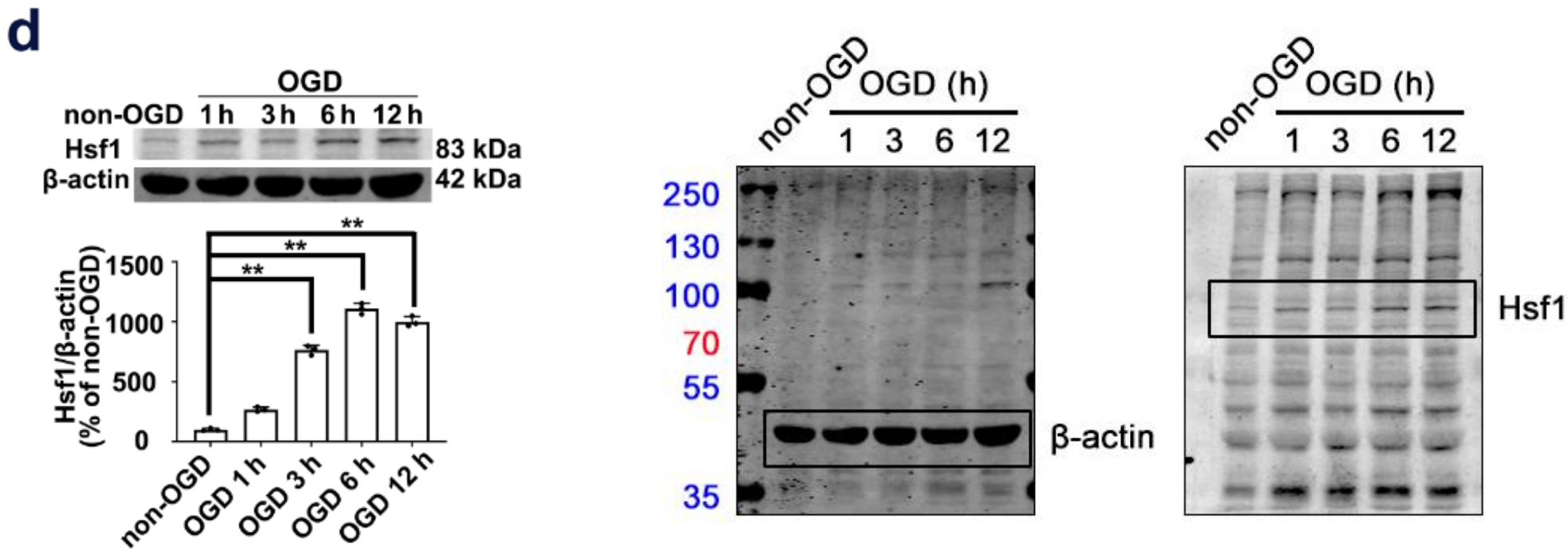
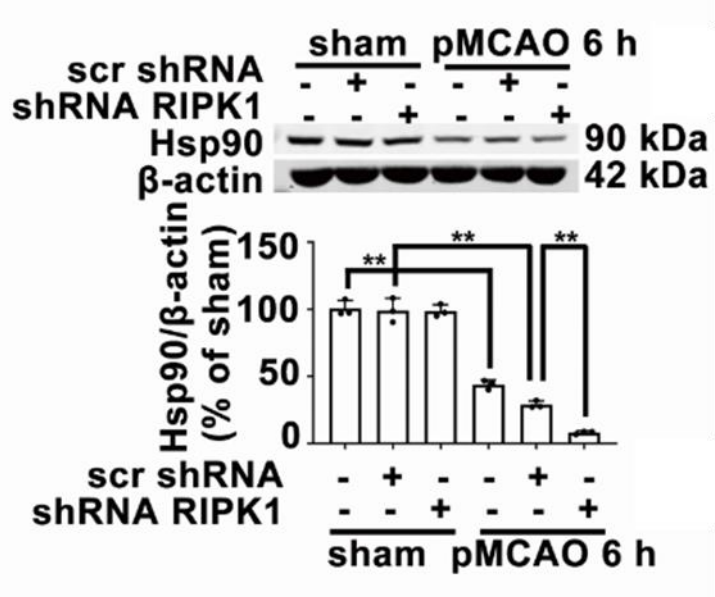
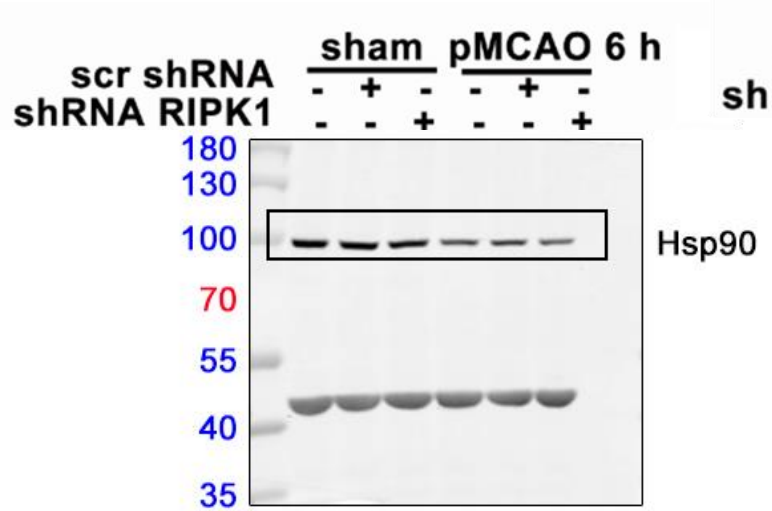

d
OGD
non-OGD 1 h 3 h 6 h 12 h
Hsf1
β-actin
83 kDa
42 kDa
Hsf1/β-actin (% of non-OGD)
1500
1000
500
0
**
**
**
non-OGD
OGD 1 h
OGD 3 h
OGD 6 h
OGD 12 h
non-OGD
OGD (h)
1 3 6 12
250
130
100
70
55
35
β-actin
non-OGD
OGD (h)
1 3 6 12
Hsf1

Fig. 6e


sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
Hsp90
β-actin
90 kDa
42 kDa
Hsp90/β-actin (% of sham)
150
100
50
0
**
**
**
scr shRNA
shRNA RIPK1
sham
pMCAO 6 h


sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
180
130
100
70
55
40
35
Hsp90

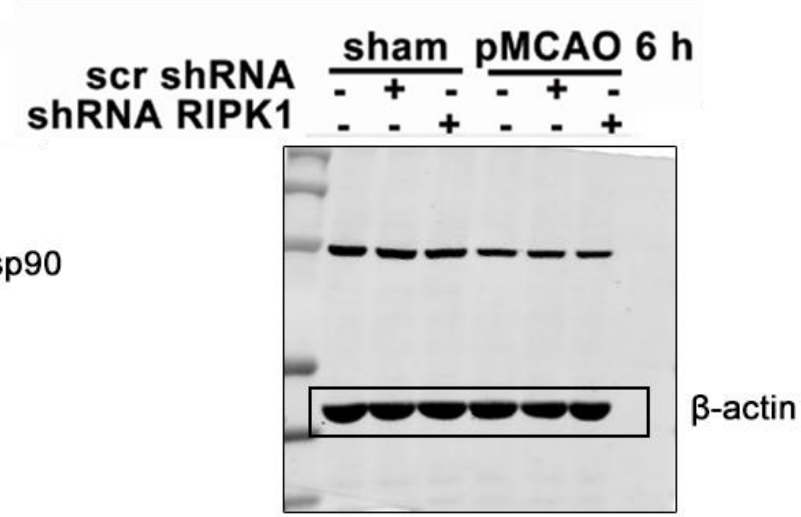
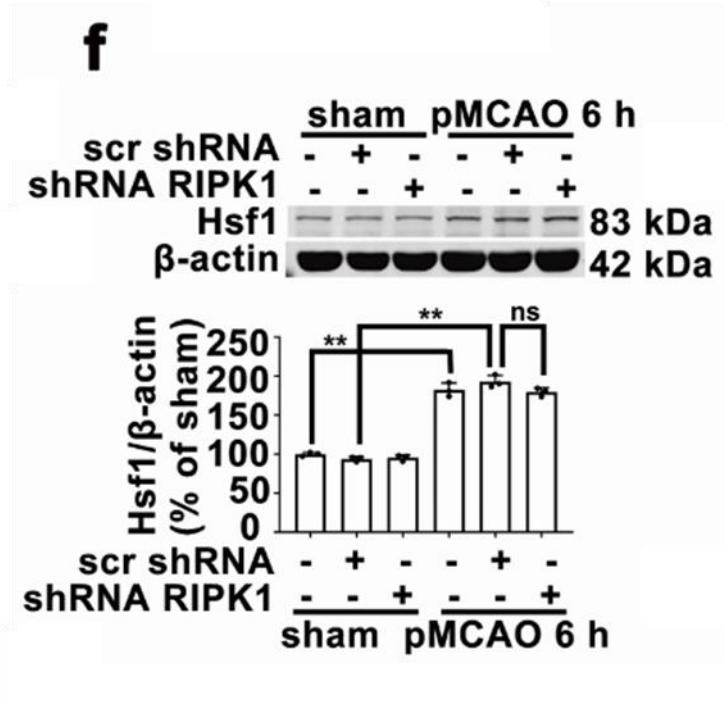
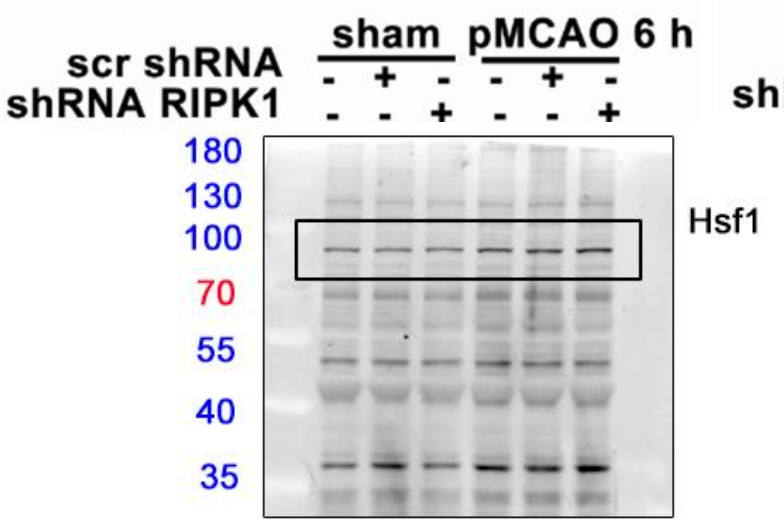

sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
β-actin

Fig. 6f


f
sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
Hsf1
β-actin
83 kDa
42 kDa
Hsf1/β-actin (% of sham)
250
200
150
100
50
0
**
**
ns
scr shRNA
shRNA RIPK1
sham
pMCAO 6 h


sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
180
130
100
70
55
40
35
Hsf1

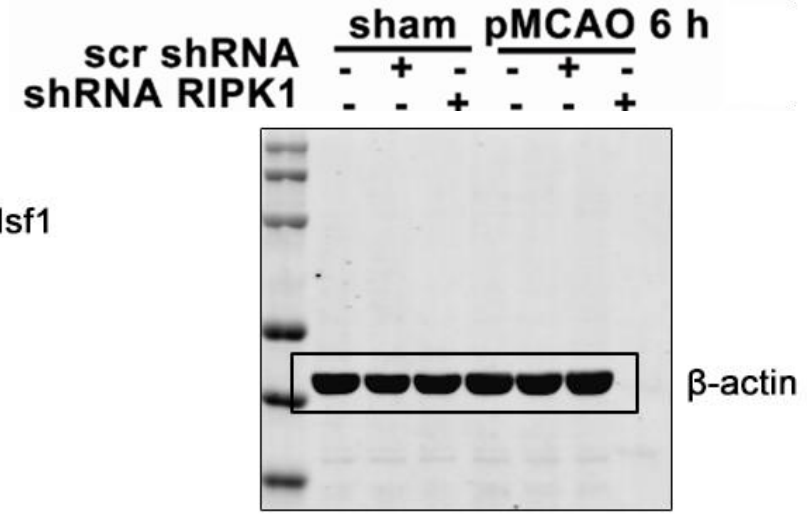
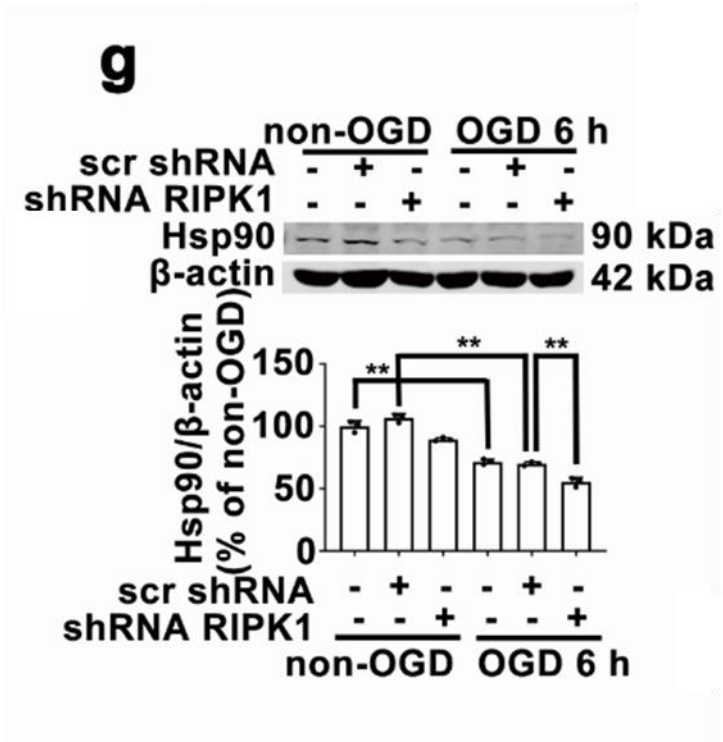
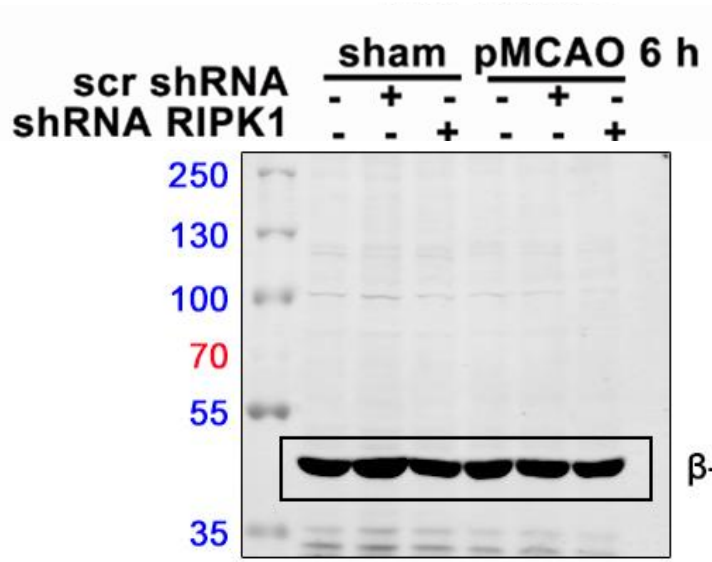

sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
β-actin

Fig. 6g


g
non-OGD
OGD 6 h
scr shRNA
shRNA RIPK1
Hsp90
β-actin
90 kDa
42 kDa
Hsp90/β-actin (% of non-OGD)
150
100
50
0
**
**
**
scr shRNA
shRNA RIPK1
non-OGD
OGD 6 h


sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
250
130
100
70
55
35
β-actin

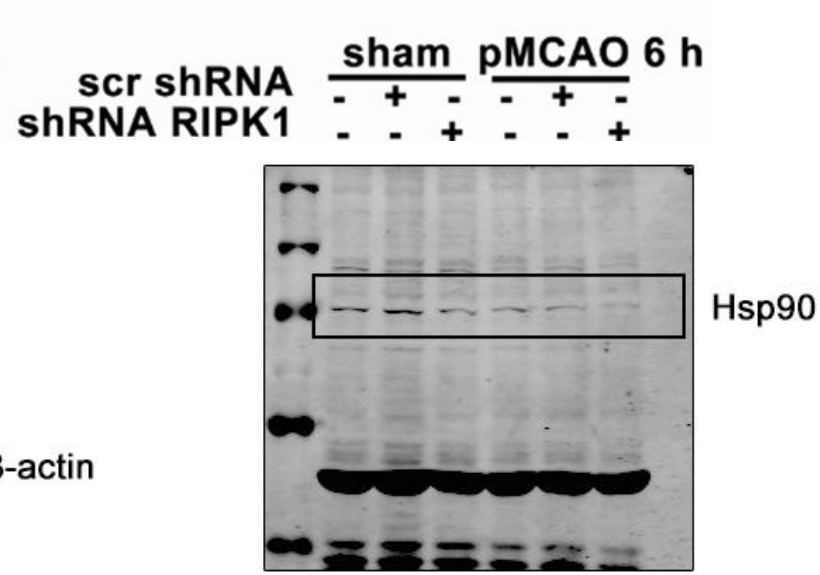
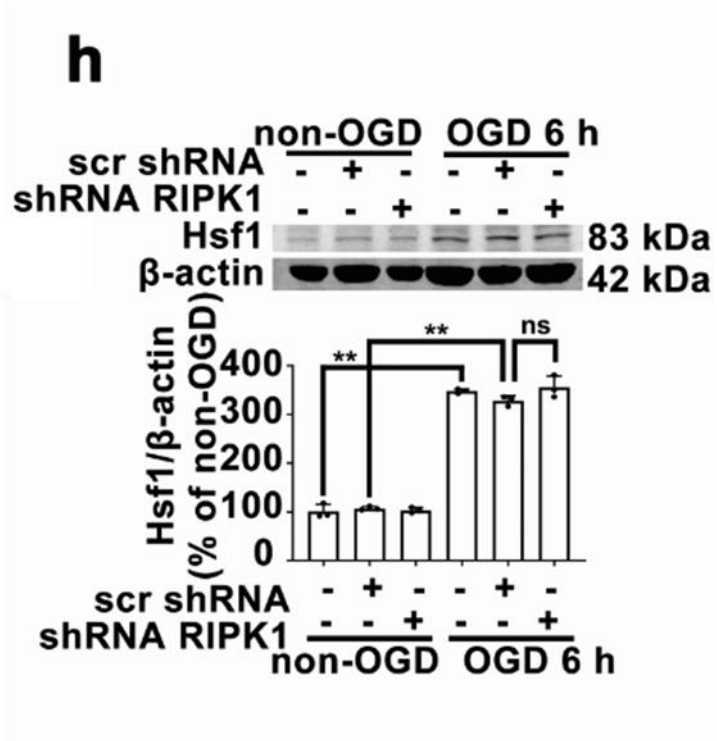
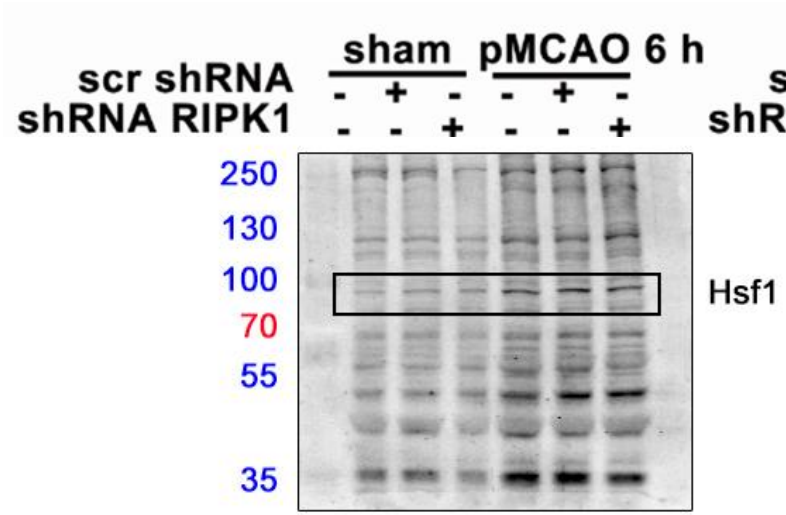

sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
Hsp90

Fig. 6h


h
non-OGD
OGD 6 h
scr shRNA
shRNA RIPK1
Hsf1
β-actin
83 kDa
42 kDa
Hsf1/β-actin (% of non-OGD)
400
300
200
100
0
**
**
ns
scr shRNA
shRNA RIPK1
non-OGD
OGD 6 h


sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
250
130
100
70
55
35
Hsf1

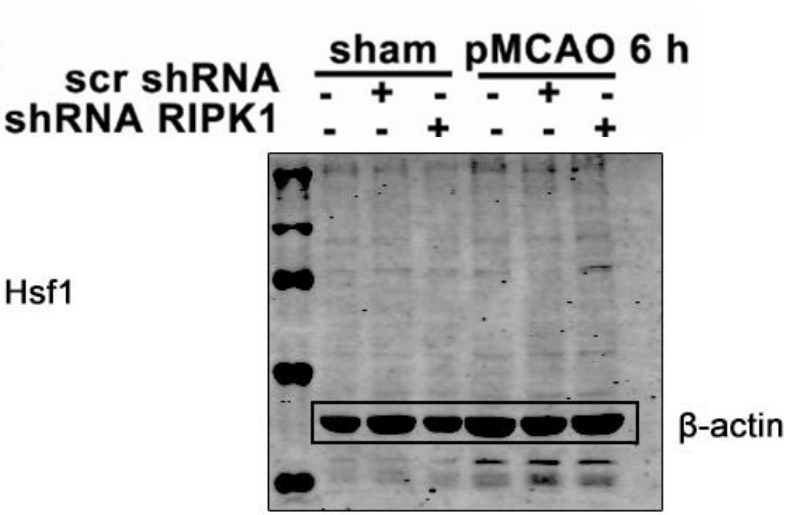
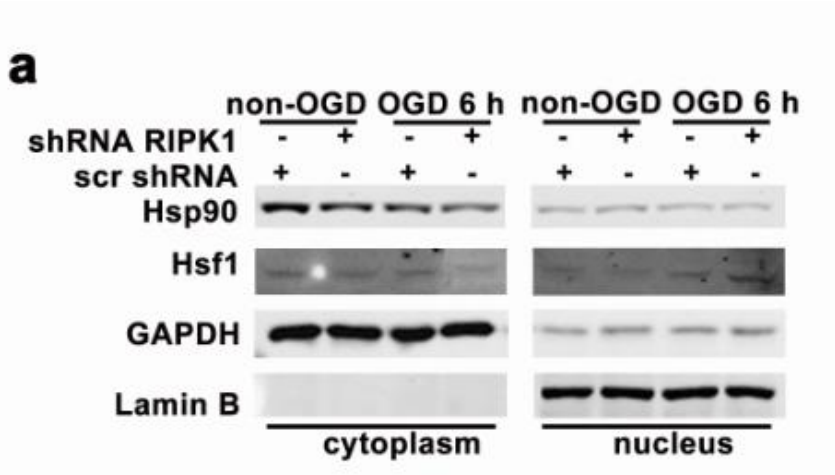

sham
pMCAO 6 h
scr shRNA
shRNA RIPK1
β-actin

Fig.7a


a
non-OGD
OGD 6 h
non-OGD
OGD 6 h
shRNA RIPK1
scr shRNA
Hsp90
Hsf1
GAPDH
Lamin B
cytoplasm
nucleus

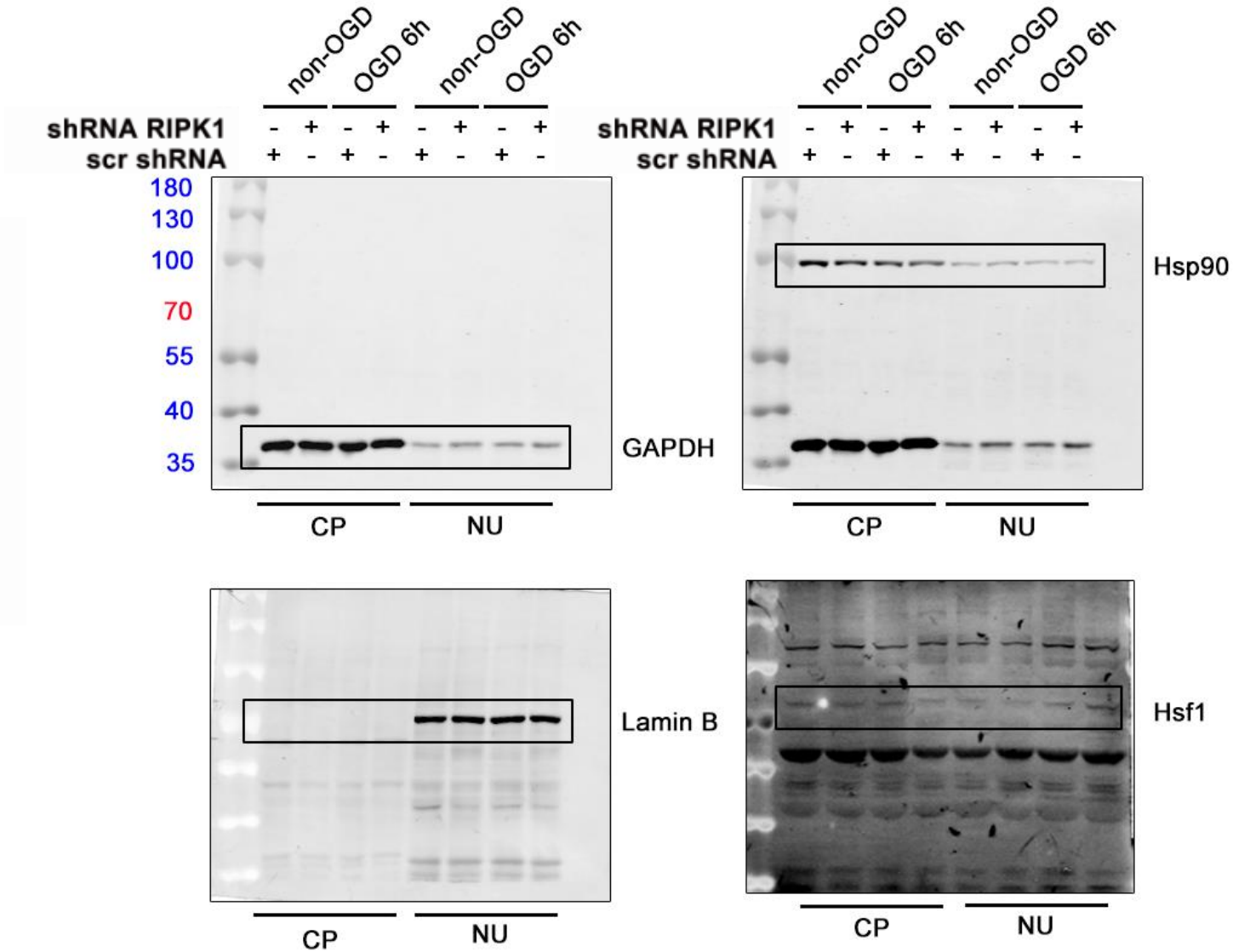
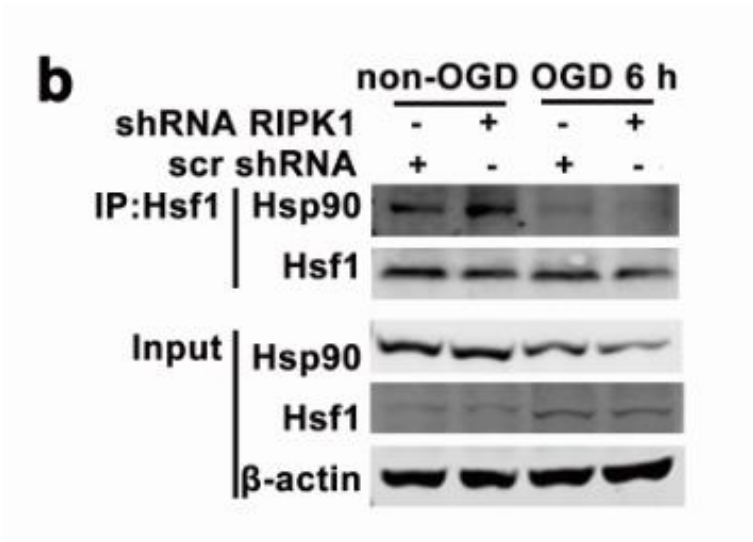
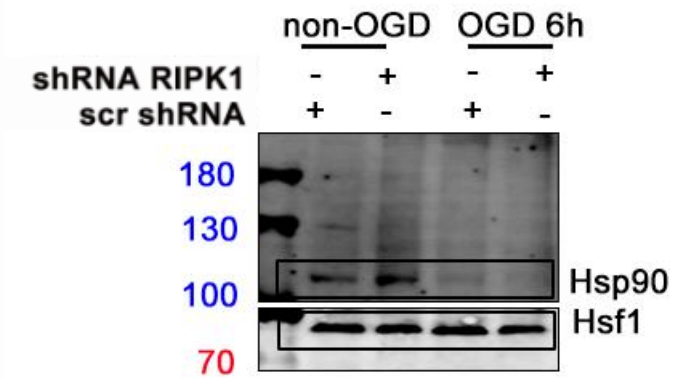
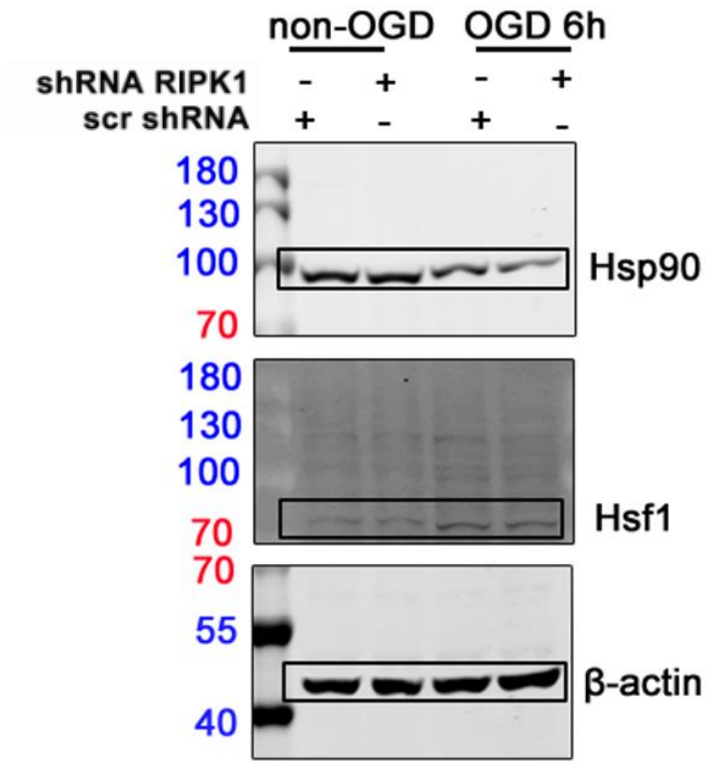

non-OGD
OGD 6h
non-OGD
OGD 6h
shRNA RIPK1
scr shRNA
180
130
100
70
55
40
35
GAPDH
CP
NU
Hsp90
Lamin B
Hsf1

Fig.7b


b
non-OGD
OGD 6 h
shRNA RIPK1
scr shRNA
IP:Hsf1
Hsp90
Hsf1
Input
Hsp90
Hsf1
β-actin


non-OGD
OGD 6h
shRNA RIPK1
scr shRNA
180
130
100
70
Hsp90
Hsf1


non-OGD
OGD 6h
shRNA RIPK1
scr shRNA
180
130
100
70
Hsp90
180
130
100
70
Hsf1
70
55
40
β-actin