Controlled overexpression of the mitochondria shaping protein Optic Atrophy 1 counteracts cellular damage in vivo

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Supplementary online material

Legends to supplementary figures

Supplementary Figure 1S. Characterization of *Opa1*^{tg} mice

(A) Equal amounts of protein from liver tissue of the indicated genotypes were separated by SDS-PAGE and immunoblotted with the indicated antibodies.

(B) Equal amounts of protein from liver tissue of the indicated genotypes were separated by SDS-PAGE and immunoblotted with the indicated antibodies.

(C) Growth rate of C57/Bl6 Wt and *Opa1*^{tg} males and females during the initial 60 days of life. Data represent average $\pm$ SEM (n=20 per each group).

(D) Body weight is represented as average $\pm$ SEM of 5 months males (n=19 for each group) and females (n= 12 for each group) and 9 months old males (n= 32 for each group) and females (n=18 for each group) SV129 *Opa1*^{tg} and Wt litettermates.

Supplementary Figure 2S. Histological and morphological characterization of heart, liver and kidney

(A) Immunofluorescence analysis on ventricular cryosections from Wt and *Opa1*^{tg} mice stained with an antibody to dystrophin. Images are details from the left ventricle. Scale bars, 25 μm.

(B) Evaluation of cardiomyocyte (CM) area in cryosections from Wt and *Opa1*^{tg} 5 months old hearts. Bars indicate average ± SEM. (n=5 for each group).

(C) Echocardiographic long axis view of hearts from 5 months old Wt and *Opa1*^{tg} littermates. LV: left ventricle; A: aorta.

(D) Haematoxilin-eosin staining in ventricular cryosections from 5 months Wt and *Opa1*^{tg} mice. Scale bars, 100 μm.

(E) Immunofluorescence analysis of heart cryosections from 9 months old Wt and *Opa1*^{tg} mice stained with an antibody to Collagene I (Col.I).

(F) Evaluation of liver weight/body weight ratio in 5 and 9 months old male mice. Wt and *Opa1*^{tg} . Bars indicate average ± SEM (n=10 for each group).

(G) Echographic view of the liver from 9 months old Wt and *Opa1tg* mice.

(H) Echographic short axis view of the left kidney from 9 months old Wt and *Opa1*^{tg} mice.

(I) Morphometric analysis of kidney from 9 months old Wt and *Opa1*^{tg} mice. Error bars indicate SEM (n=6 for each group).

Suplimentary Figure 3S. Autophagic program is not altered in Opa1tg mice

(A) mRNAs expression of atrophy-related and muscle specific genes in *Opa1*^{tg} and Wt littermates, control and denervated muscle (n=5 for each group). Data are average ±SEM. The asterisk denotes $p<0.05$ in an unpaired sample Student's test.

(B) mRNAs expression of autophagic genes in *Opa1*^{tg} and Wt littermates, control and denervated muscle (n=5 for each group). Data are average ±SEM. The asterisk denotes $p<0.05$ in an unpaired sample Student's test.

(C) mRNAs expression of mitochondrial genes in *Opa1*^{tg} and Wt littermates, control and denervated muscle (n=5 for each group). Data are average ±SEM. The asterisk denotes $p<0.05$ in an unpaired sample Student's test.

(D) The time course of LC3 II levels in mouse adult fibroblasts (MAFs) of the indicated genotypes upon autophagy induction by serum deprivation. Actin is shown as a loading control. Densitometric analysis of LC3 II over actin. Data represent average $\pm$ SEM (n=5 independent experiments).

(B) The time course of LC3 II levels when cells were deprived of serum in the presence of 100 μM chloroquine (CQ). Actin is shown as a loading control. Densitometric analysis of LC3 II over actin. Data represent average $\pm$ SEM (n=5 independent experiments).

Suplimentary Figure 4S. *Opa1*^{tg} mitochondria are slightly elongated and contain tighter cristae

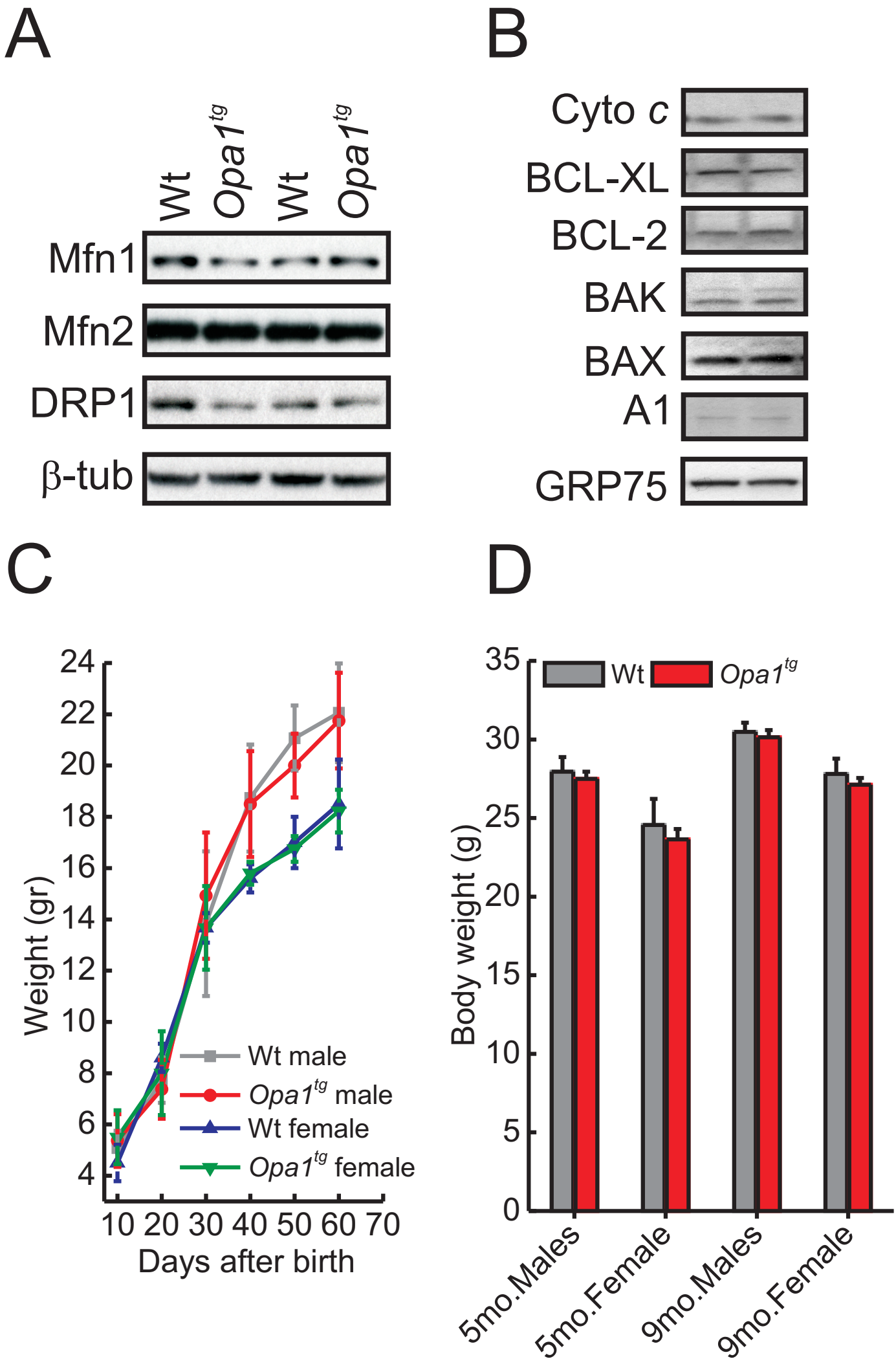
(A) Complex IV dependent respiration of mitochondria isolated from livers of the indicated genotypes. Data represent average ± SEM (n=4 for each group).

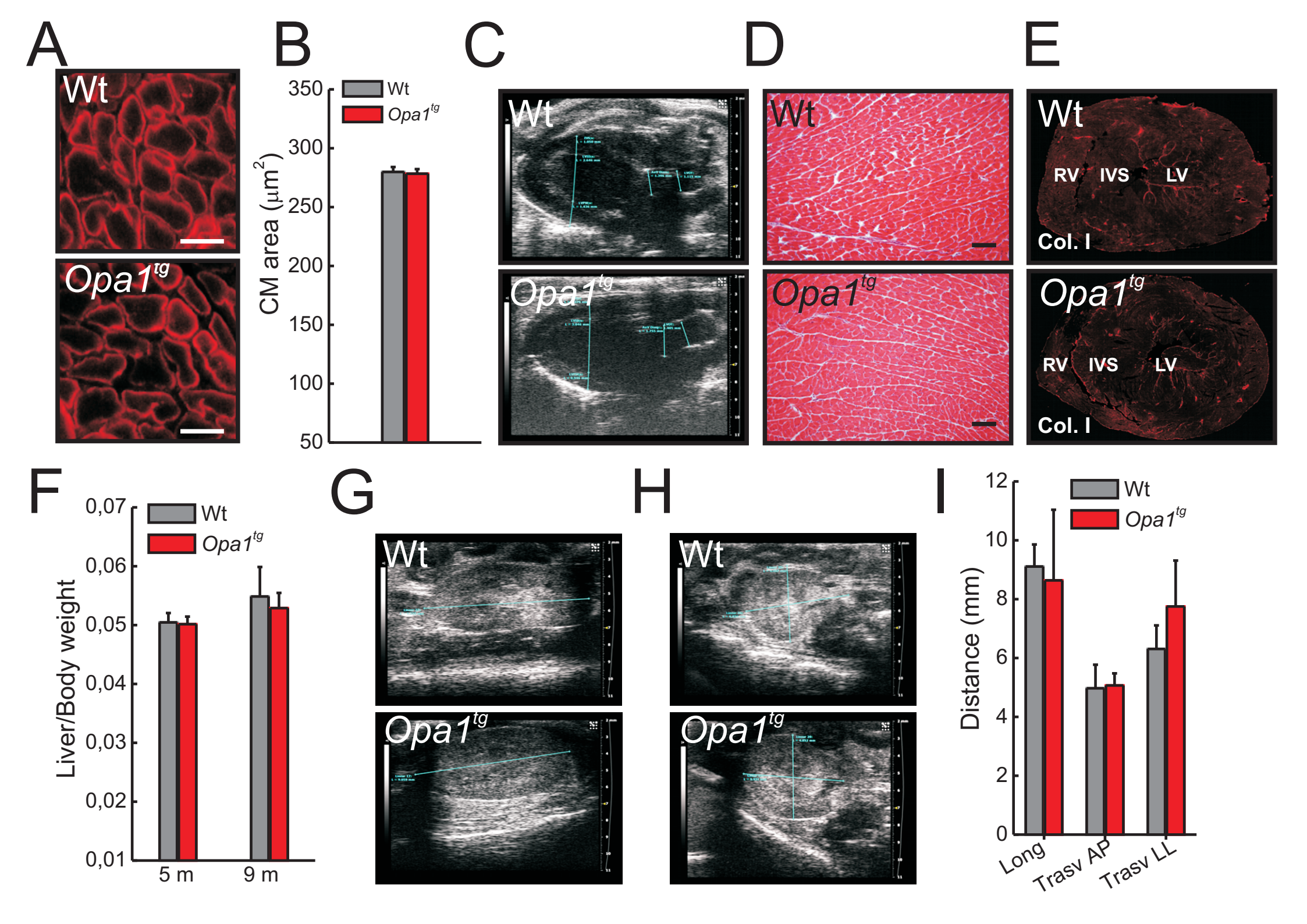
(B) Representative traces of Calcium Retention Capacity (CRC) of liver mitochondria isolated from 5 months old Wt and *Opa1*^{tg} mice. For the assessment of the maximal CRC, 2 μM Cyclosporin A (CsA) was present in the medium.

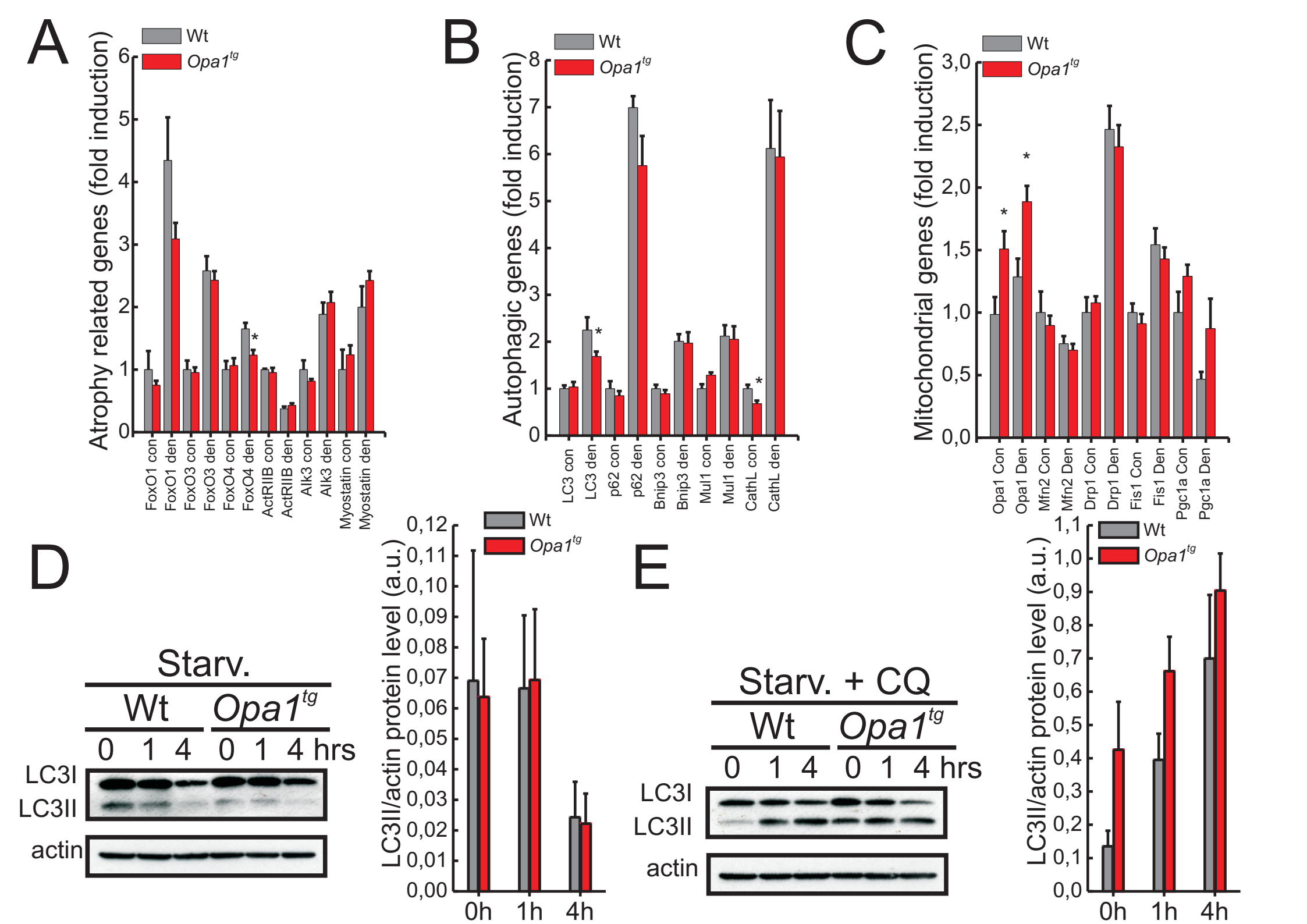
(C) Quantification of CRC in experiments as in (D).

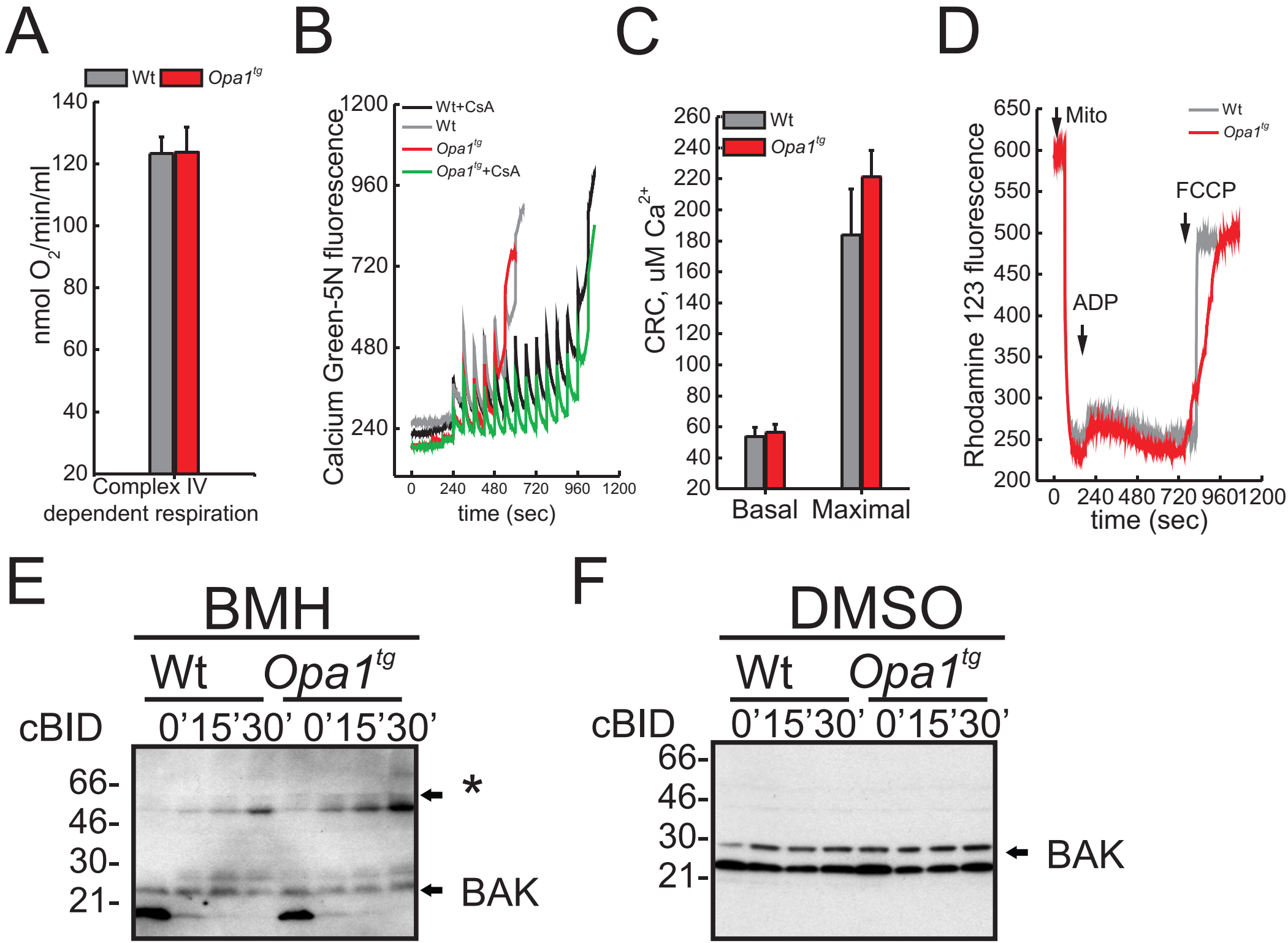
(D) Representative traces of Rhodamine 123 fluorescence. Mitochondria isolated from livers of the indicated genotype (1 mg/mL, MLM) were treated where indicated (arrows) with 300 μM ADP and 200 nM FCCP.

(E) Mitochondria of the indicated genotype were treated with cBID for the indicated times. 10 mM BMH or DMSO was then added and after 30 min the crosslinking reaction was quenched. Equal amounts (40μg) of mitochondrial proteins were analyzed by SDS-PAGE/immunoblotting using anti-BAK antibody. Asterisks indicate BAK multimers.


A
Wt
Opa1tg
Wt
Opa1tg
Mfn1
Mfn2
DRP1
β-tub
B
Cyto c
BCL-XL
BCL-2
BAK
BAX
A1
GRP75
C
Weight (gr)
24
22
20
18
16
14
12
10
8
6
4
10 20 30 40 50 60 70
Days after birth
Wt male
Opa1tg male
Wt female
Opa1tg female
D
Body weight (g)
35
30
25
20
15
10
5
0
Wt
Opa1tg
5mo.Males
5mo.Female
9mo.Males
9mo.Female


A
Wt
Opa1tg
B
Wt
Opa1tg
CM area (µm2)
350
300
250
200
150
100
50
C
Wt
Opa1tg
D
Wt
Opa1tg
E
Wt
RV
IVS
LV
Col. I
Opa1tg
RV
IVS
LV
Col. I
F
Liver/Body weight
0,07
0,06
0,05
0,04
0,03
0,02
0,01
Wt
Opa1tg
5 m
9 m
G
Wt
Opa1tg
H
Wt
Opa1tg
I
Distance (mm)
12
10
8
6
4
2
0
Wt
Opa1tg
Long
Trasv AP
Trasv LL


A
Wt
Opa1tg
Atrophy related genes (fold induction)
FoxO1 con
FoxO1 den
FoxO3 con
FoxO3 den
FoxO4 con
FoxO4 den
ActRIIB con
ActRIIB den
Alk3 con
Alk3 den
Myostatin con
Myostatin den
B
Autophagic genes (fold induction)
LC3 con
LC3 den
p62 con
p62 den
Bnip3 con
Bnip3 den
Mul1 con
Mul1 den
CathL con
CathL den
C
Mitochondrial genes (fold induction)
Opa1 Con
Opa1 Den
Mfn2 Con
Mfn2 Den
Drp1 Con
Drp1 Den
Fis1 Con
Fis1 Den
Pgc1a Con
Pgc1a Den
D
Starv.
Wt
Opa1tg
0 1 4 0 1 4 hrs
LC3I
LC3II
actin
LC3II/actin protein level (a.u.)
0h
1h
4h
E
Starv. + CQ
Wt
Opa1tg
0 1 4 0 1 4 hrs
LC3I
LC3II
actin
LC3II/actin protein level (a.u.)
0h
1h
4h


A
Wt
Opa1tg
nmol O2/min/ml
140
120
100
80
60
40
20
Complex IV
dependent respiration
B
Wt+CsA
Wt
Opa1tg
Opa1tg+CsA
Calcium Green-5N fluorescence
1200
960
720
480
240
0
240
480
720
960
1200
time (sec)
C
Wt
Opa1tg
CRC, uM Ca2+
260
240
220
200
180
160
140
120
100
80
60
40
20
Basal
Maximal
D
Mito
ADP
FCCP
Wt
Opa1tg
Rhodamine 123 fluorescence
650
600
550
500
450
400
350
300
250
200
0
240
480
720
960
1200
time (sec)
E
BMH
Wt
Opa1tg
cBID
0’15’30’ 0’15’30’
66-
46-
30-
21-
*
BAK
F
DMSO
Wt
Opa1tg
cBID
0’15’30’ 0’15’30’
66-
46-
30-
21-
BAK