Neuro-2014

Track 7: Novel Research In Neurological Medicine and Trauma

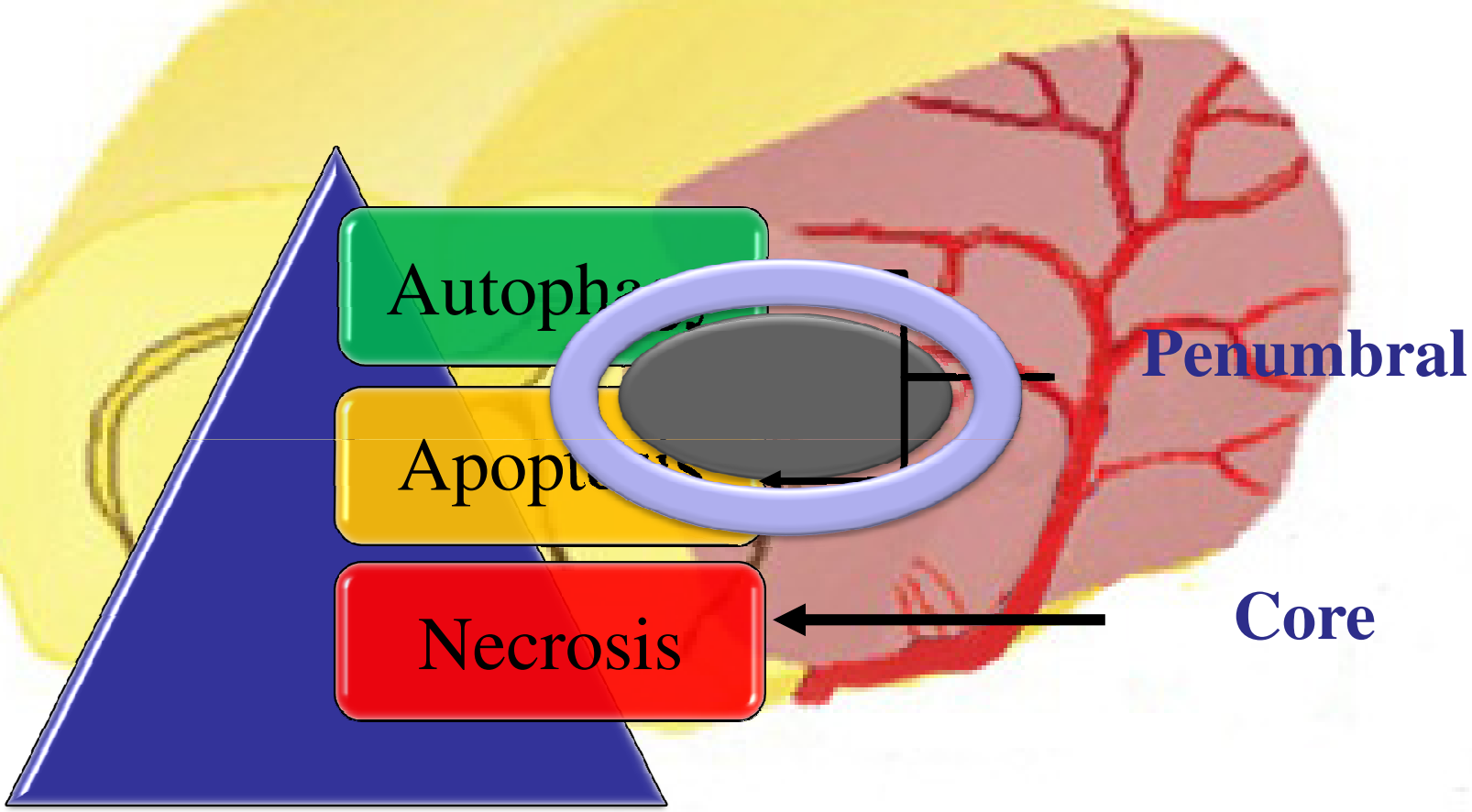
TMEM166 knockdown attenuates ischemic-induced neuronal autophagy via mTOR pathway in mice

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Chinese Society of Anatomical Sciences

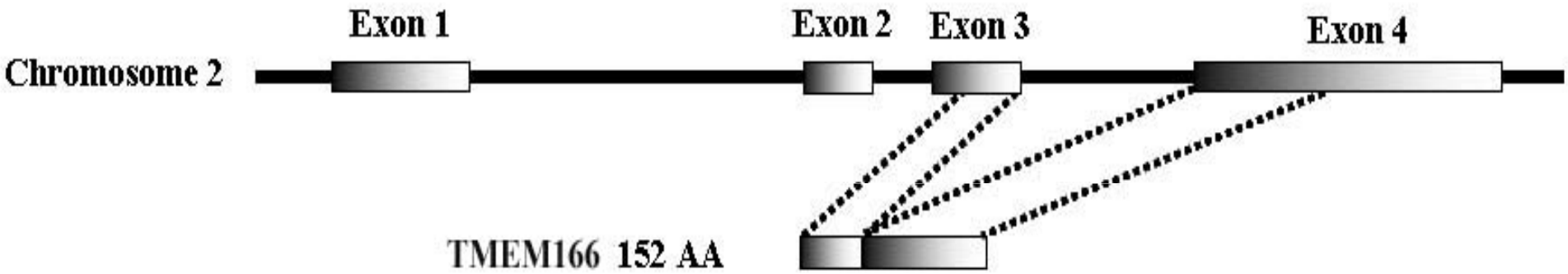
Ischemic lesions



- Three major modes of ischemic cell death.

Backgrounds

Autophagy-related gene TMEM166 :

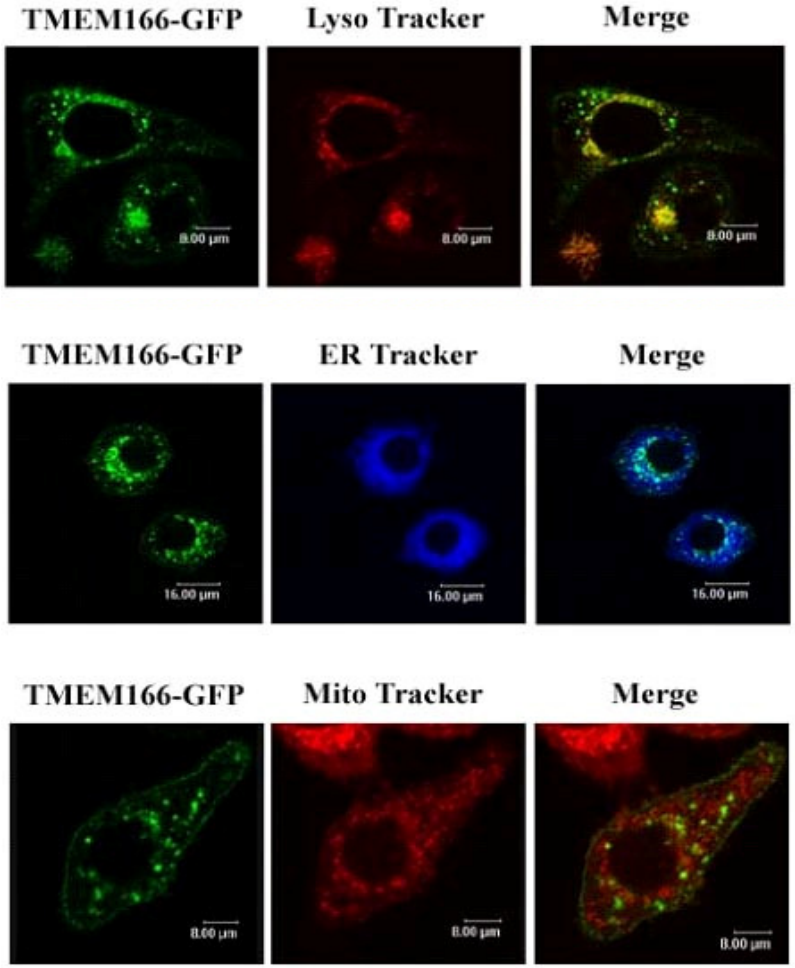


Human *TMEM166* （transmembrane protein 166,molecular weight of 17.5KD,also known as FLJ13391）, which contains 4 exons and 3 introns, is located on chromosome 2p12 and encodes 152 amino acids. Ref Seq：NM_032181； Gene ID：84141； Uni Gene：Hs. 302346。TMEM166 localized to the lysosome and endoplasmic reticulum (ER), which contains a putative TM domain, involving in both autophagy and apoptosis.

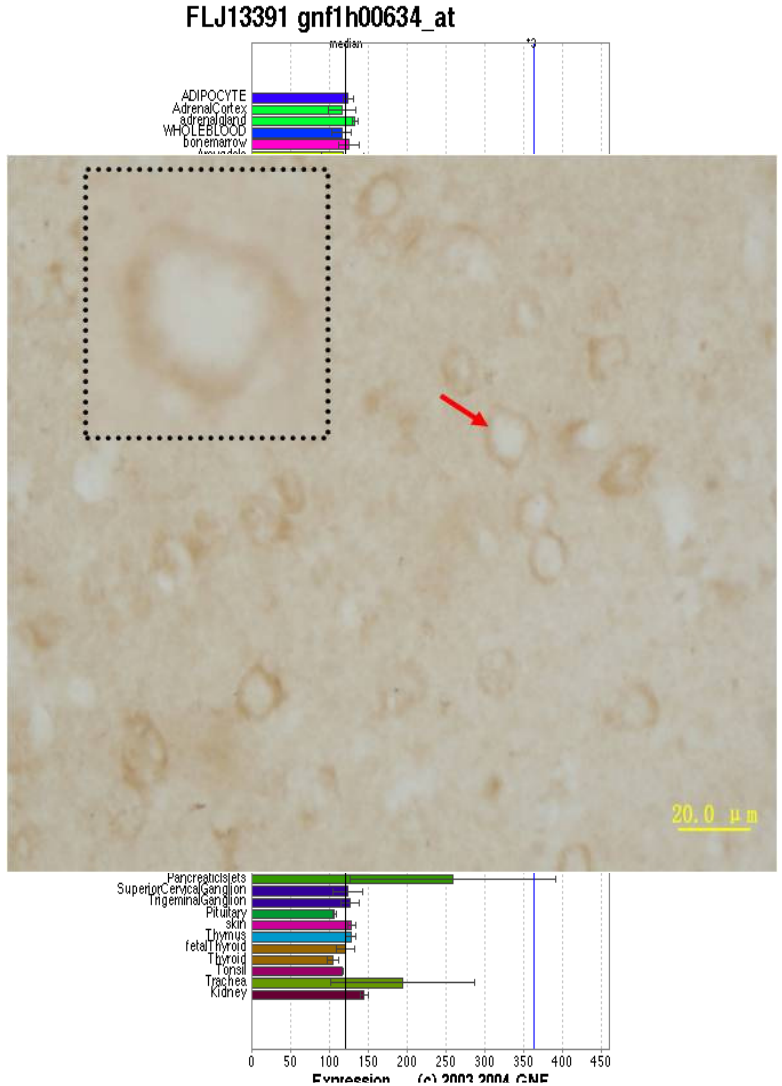
Background

(Section 1)

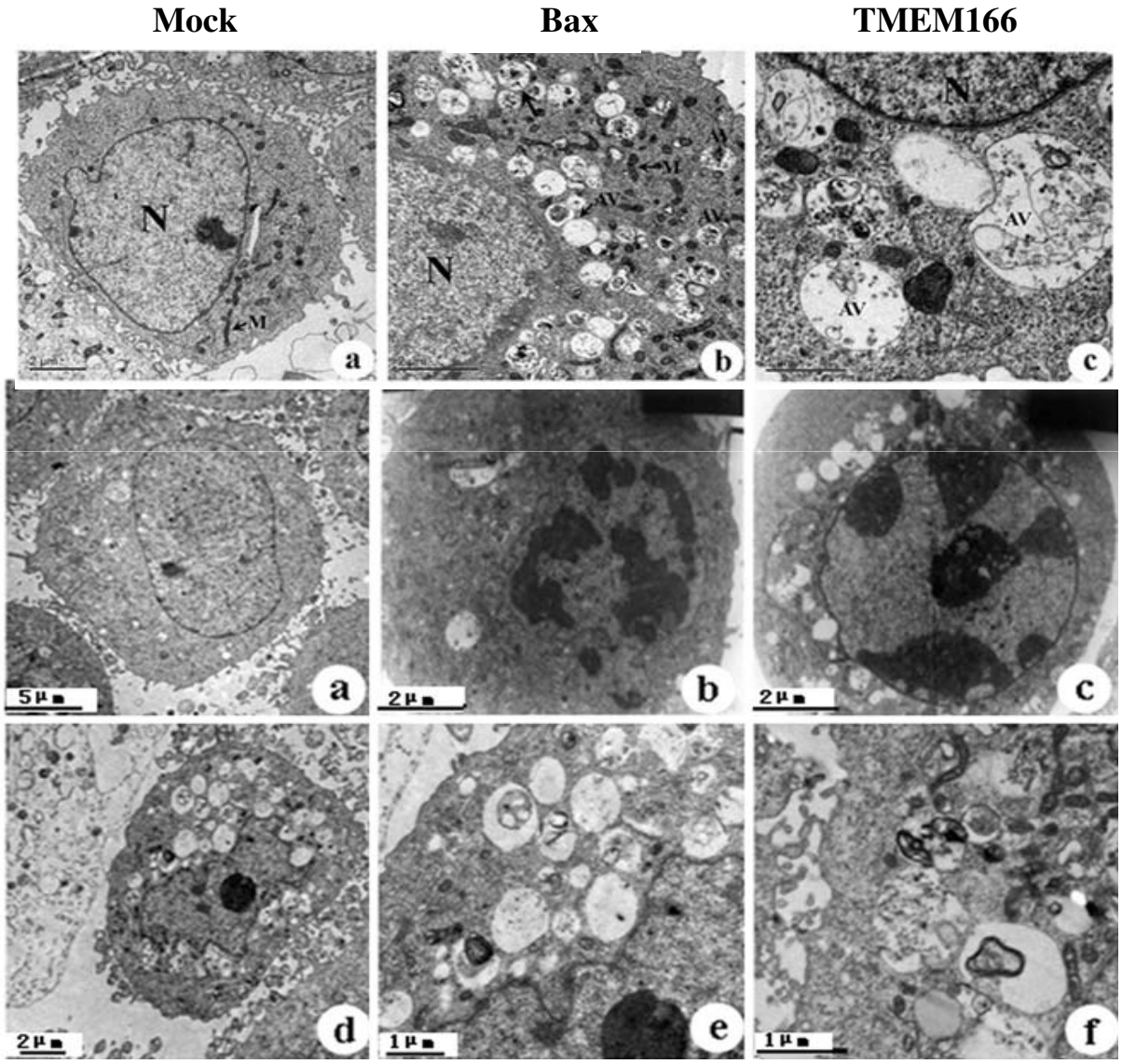
TMEM166 protein localizes to lysosome and endoplasmicreticulum



TMEM166 expression profiles



Overexpression of TMEM166 induced cell death involves autophagy and hallmarks of apoptosis



20h

40h

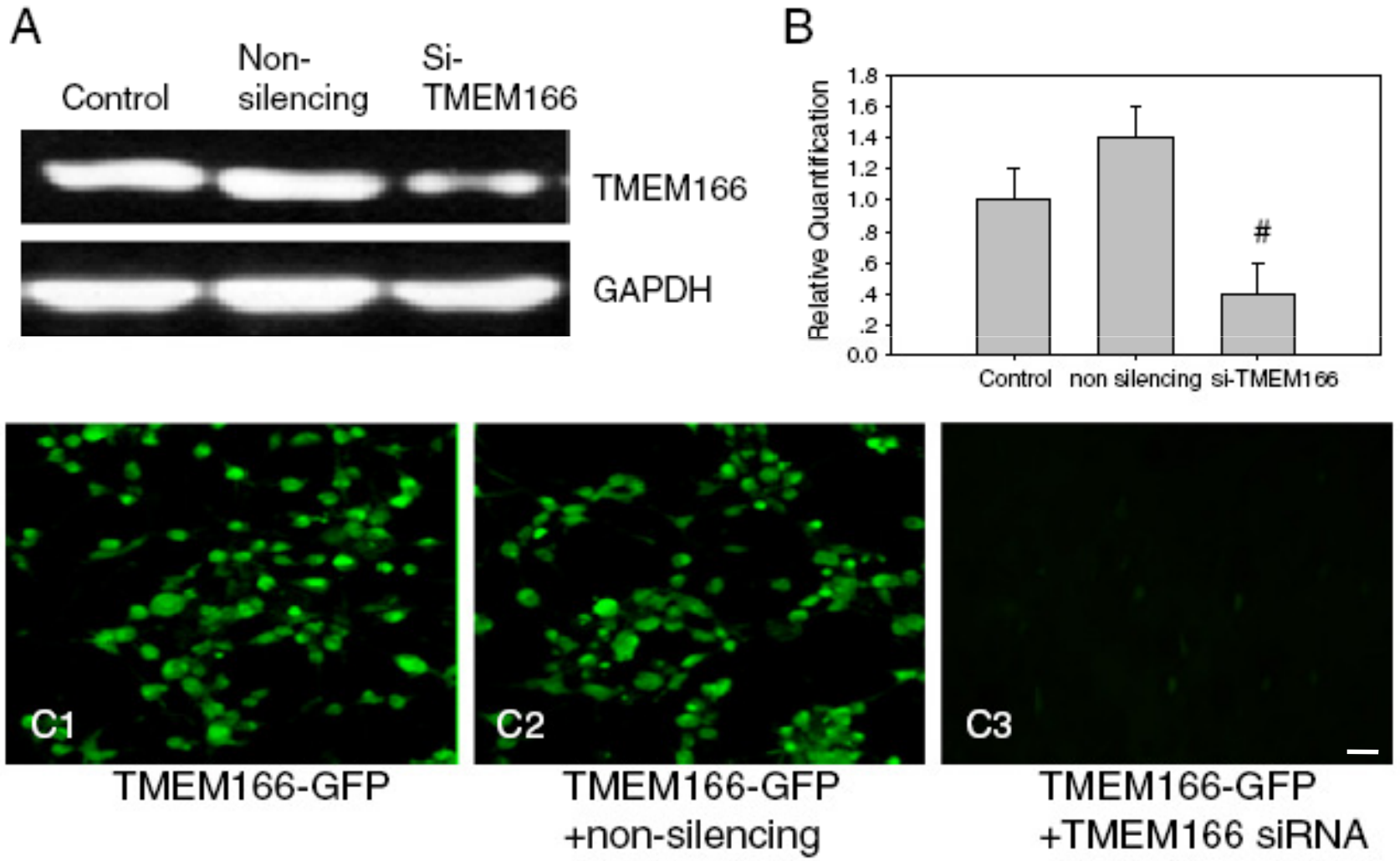
N, nucleus; AV, autophagic vacuole; M, mitochondria. Scale bars, 2 μm (a, b), 1 μm(c).

a, Mock
b, Bax
c, d, e, f TMEM166

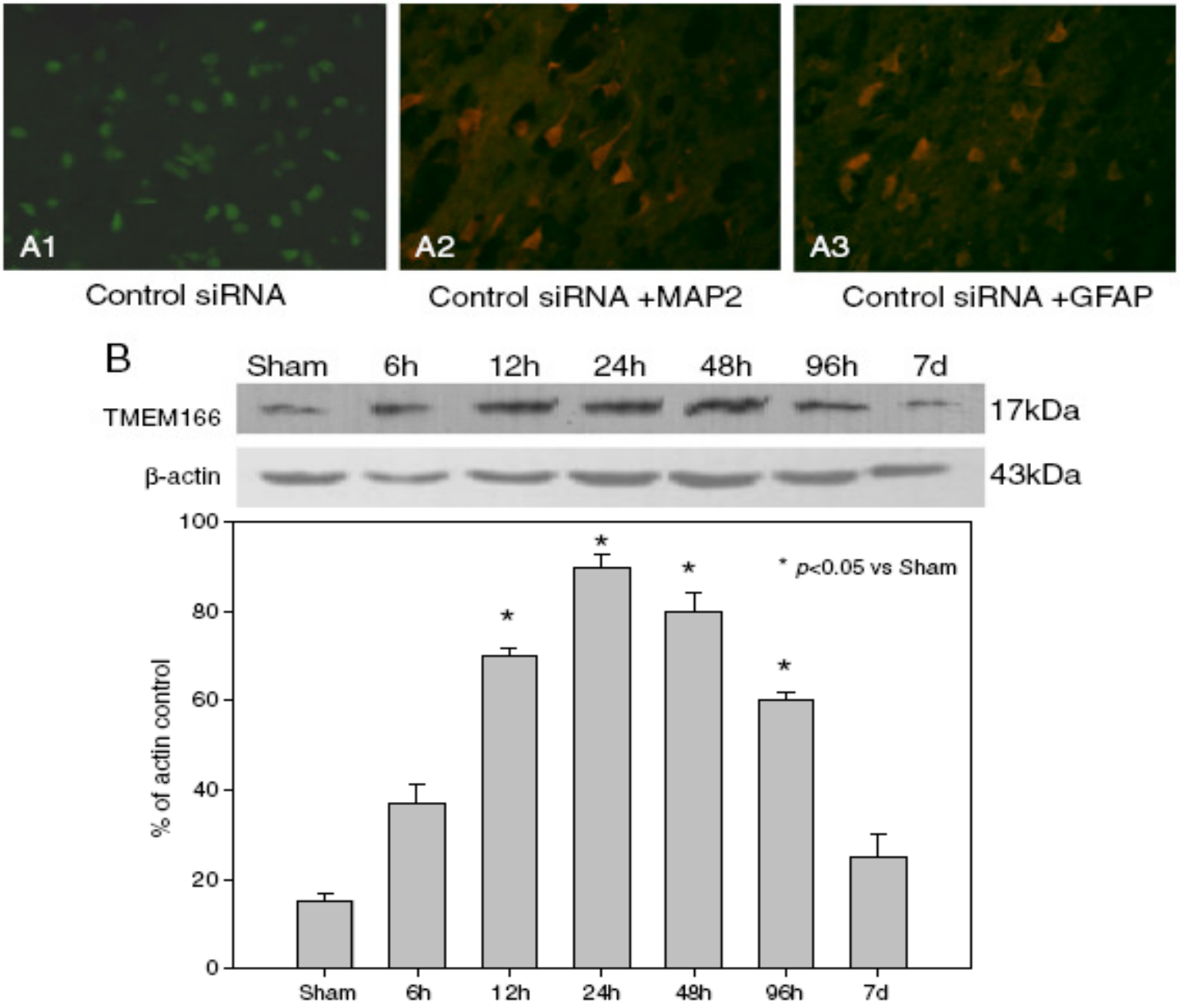
Apoptosis (2007) 12:1489–1502
DOI 10.1007/s10495-007-0073-9

Results

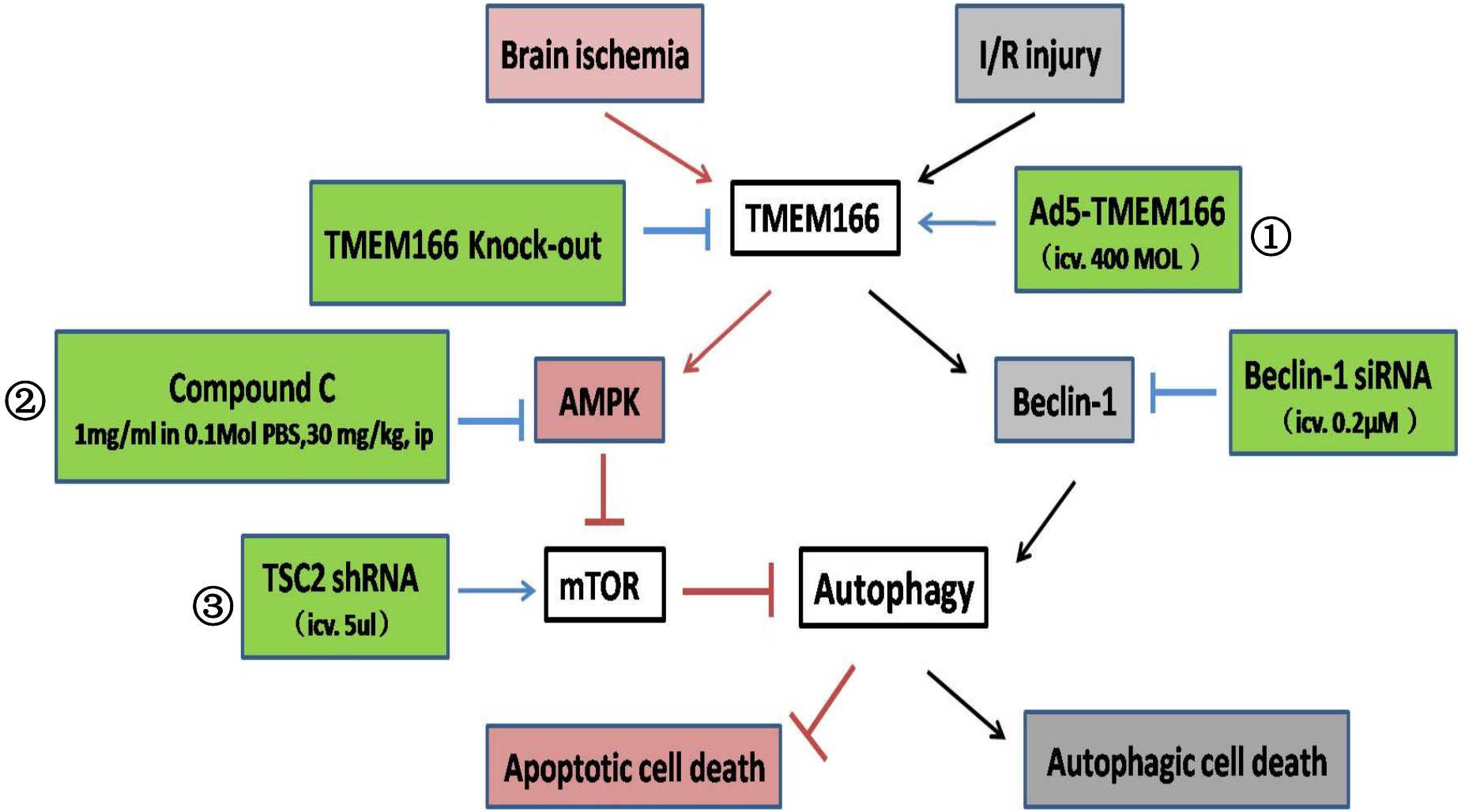
In vitro



Bar=25μm, n=6/3 in each group


A1
A2
A3
Control siRNA
Control siRNA +MAP2
Control siRNA +GFAP
B
Sham
6h
12h
24h
48h
96h
7d
TMEM166
17kDa
β-actin
43kDa
100
80
60
40
20
0
% of actin control
* p<0.05 vs Sham
Sham
6h
12h
24h
48h
96h
7d

Proposed pathway



(①Chang et al. 2013; ②Han et al. 2013; ③Korotchkina et al. 2010)

Experimental Design

Animal:  **C57BL/6 22-28g**

In ischemia phase:

Groups:
- Sham
- Ischemia
- Ischemia+TME166 Knock-out (Ischemia +KO)
- Ischemia+Ad5-TMEM166
- Ischemia+Ad5-TMEM166+Compound C

Measure: (24h after ischemia)

TTC staining; Brain water content; Mortality Neurological scores; Evan`s Blue; electron microscope

Immunoflorences: TMEM166+p-AMPK/p-mTOR; NeuN+TMEM166/p-AMPK/p-mTOR

Western blot: TMEM166, p-mTOR, mTOR, p-AMPK, AMPK, LC3 - Ⅰ, LC3 - Ⅱ, eEF2, p-eEF2,p-p70s6k , p-p70s6k

In I/R phase:

Groups:
- Sham
- I/R(ischemia 1.5h)
- I/R+KO
- I/R + KO + Ad5-TMEM166
- I/R + KO + Ad5-TMEM166+Beclin-1 siRNA

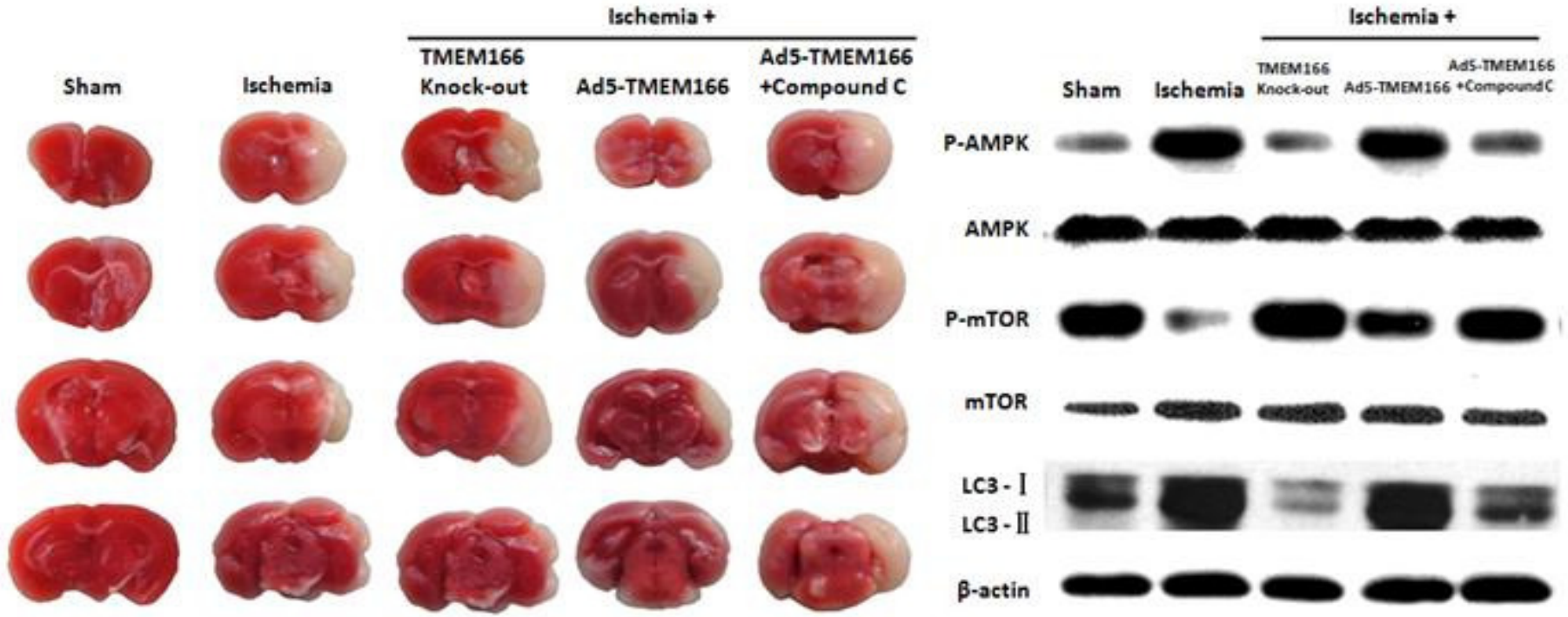
Measure: (24h after I/R)

TTC staining; Brain water content; Mortality Neurological scores; Evan`s Blue; electron microscope

Immunoflorences: TMEM166+Beclin-1; NeuN+TMEM166/Beclin-1

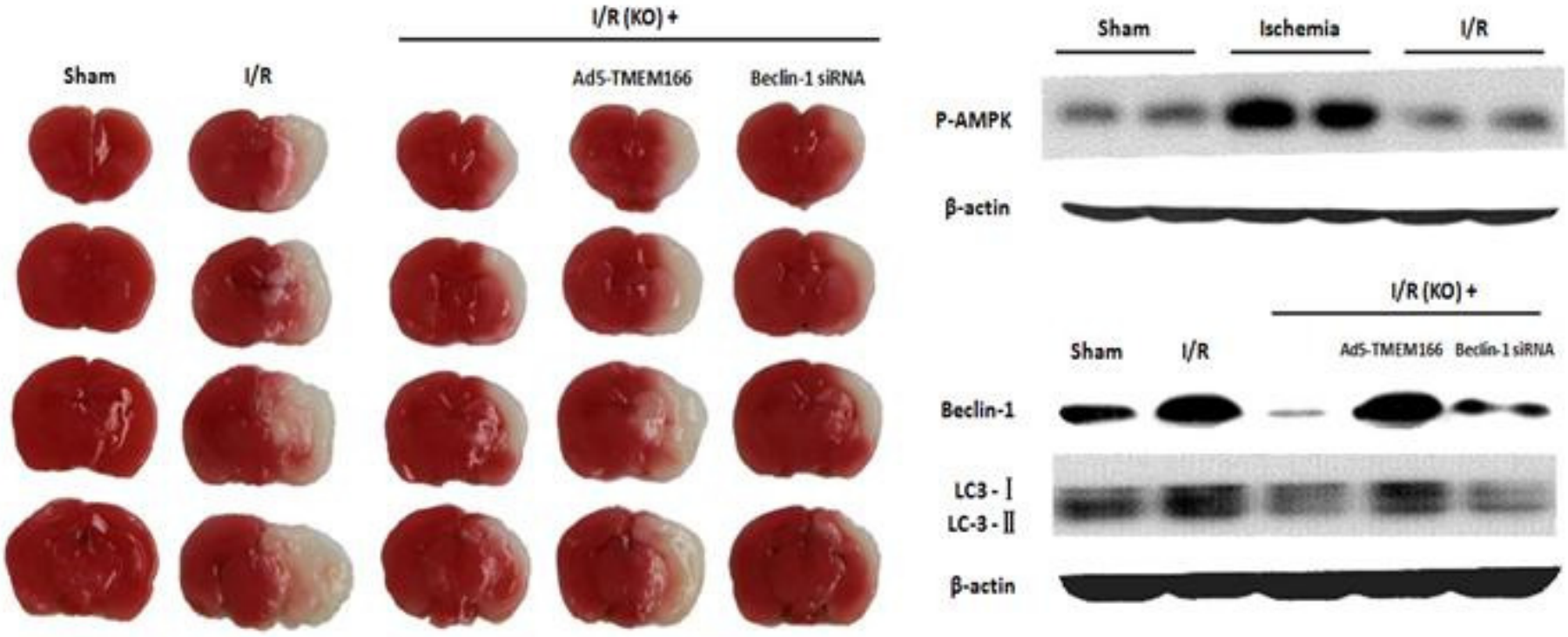
Western blot: TMEM166, LC3 - Ⅰ, LC3 - Ⅱ, eEF2, p-eEF2, Beclin-1

TTC staining and p-AMPK, AMPK, mTOR, LC3 - Ⅰ and LC3 - Ⅱ expression after brain ischemia



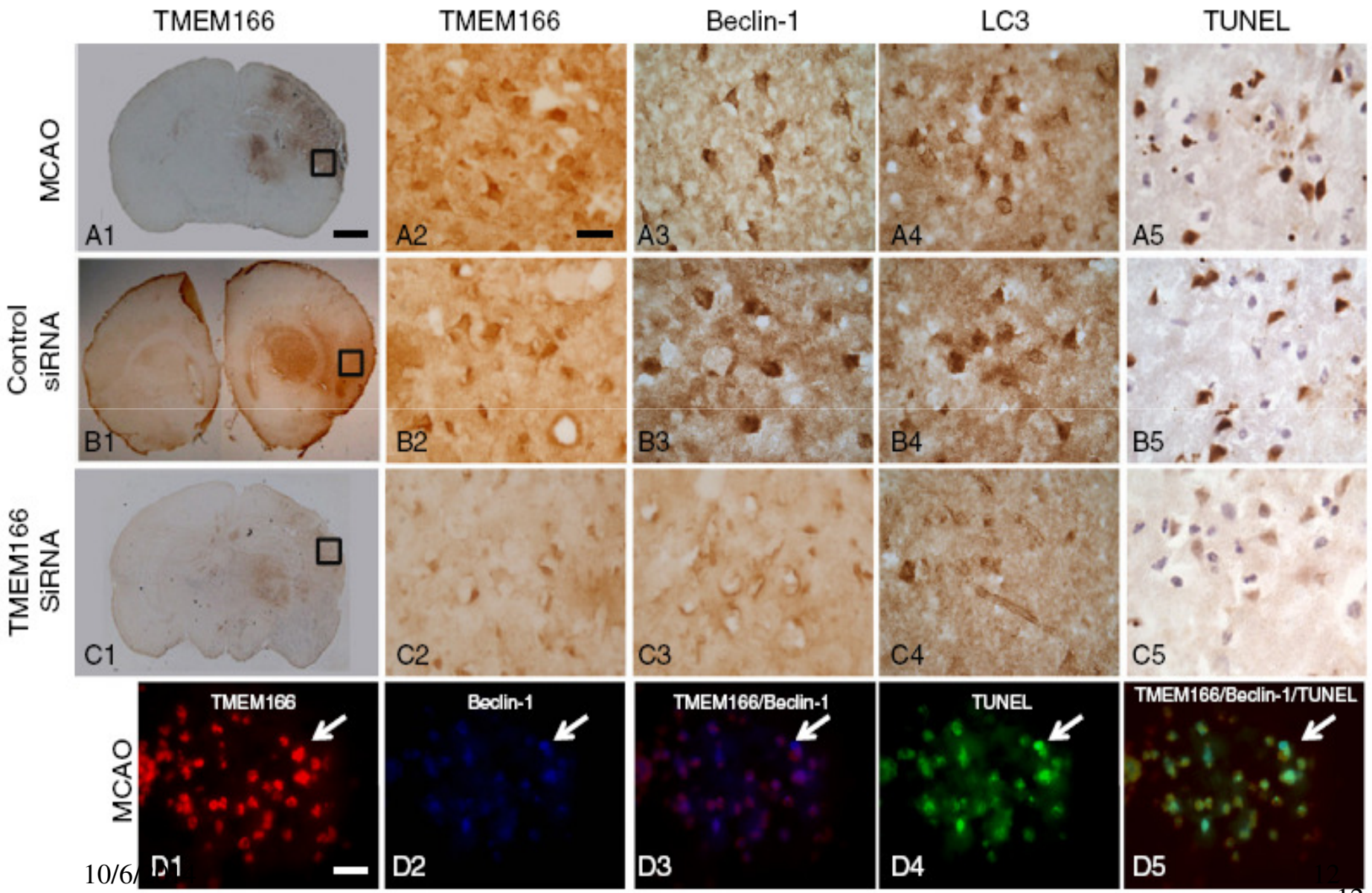
n=6 in each group

TTC staining and p-AMPK, Beclin-1, LC3 - Ⅰ and LC3 - Ⅱ expression after I/R



n=6 in each group

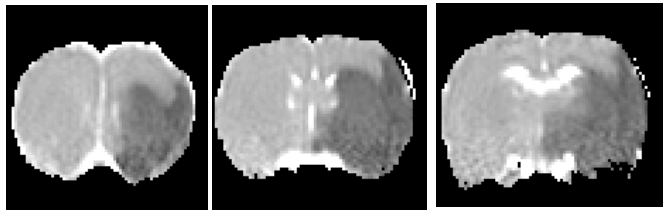
Fig. 5

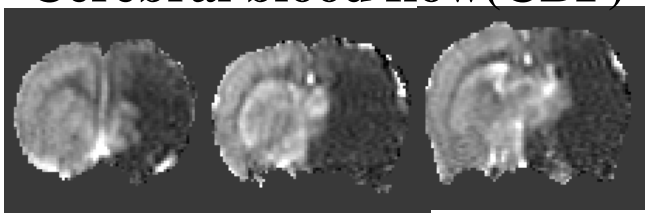

TMEM166
TMEM166
Beclin-1
LC3
TUNEL
MCAO
Control siRNA
TMEM166 SiRNA
A1
A2
A3
A4
A5
B1
B2
B3
B4
B5
C1
C2
C3
C4
C5
MCAO
TMEM166
Beclin-1
TMEM166/Beclin-1
TUNEL
TMEM166/Beclin-1/TUNEL
D1
D2
D3
D4
D5

Apparent diffusion Coefficient (ADC)

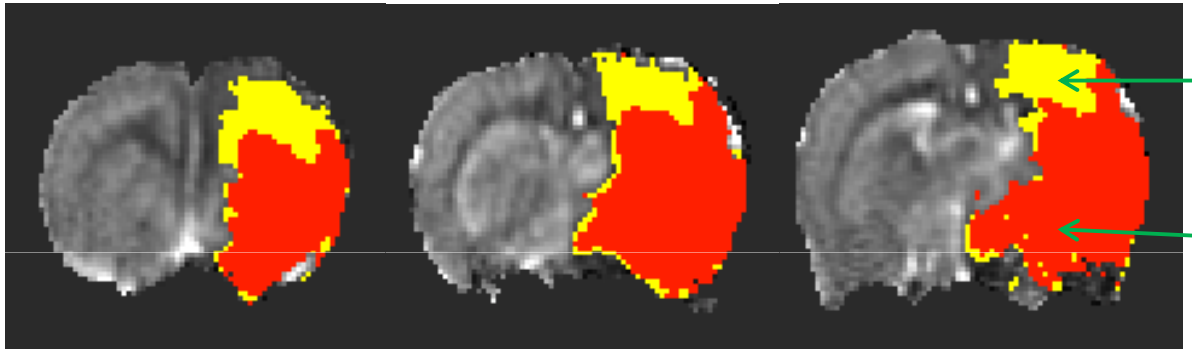
Cerebral blood flow(CBF)

30mins





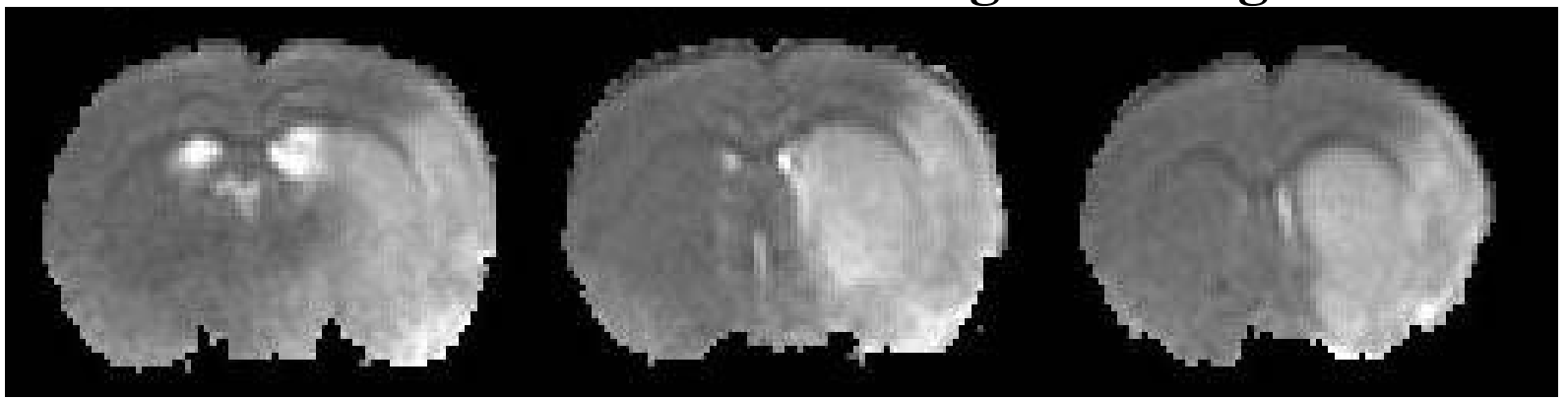
Initial lesion (30mins)



Mismatch

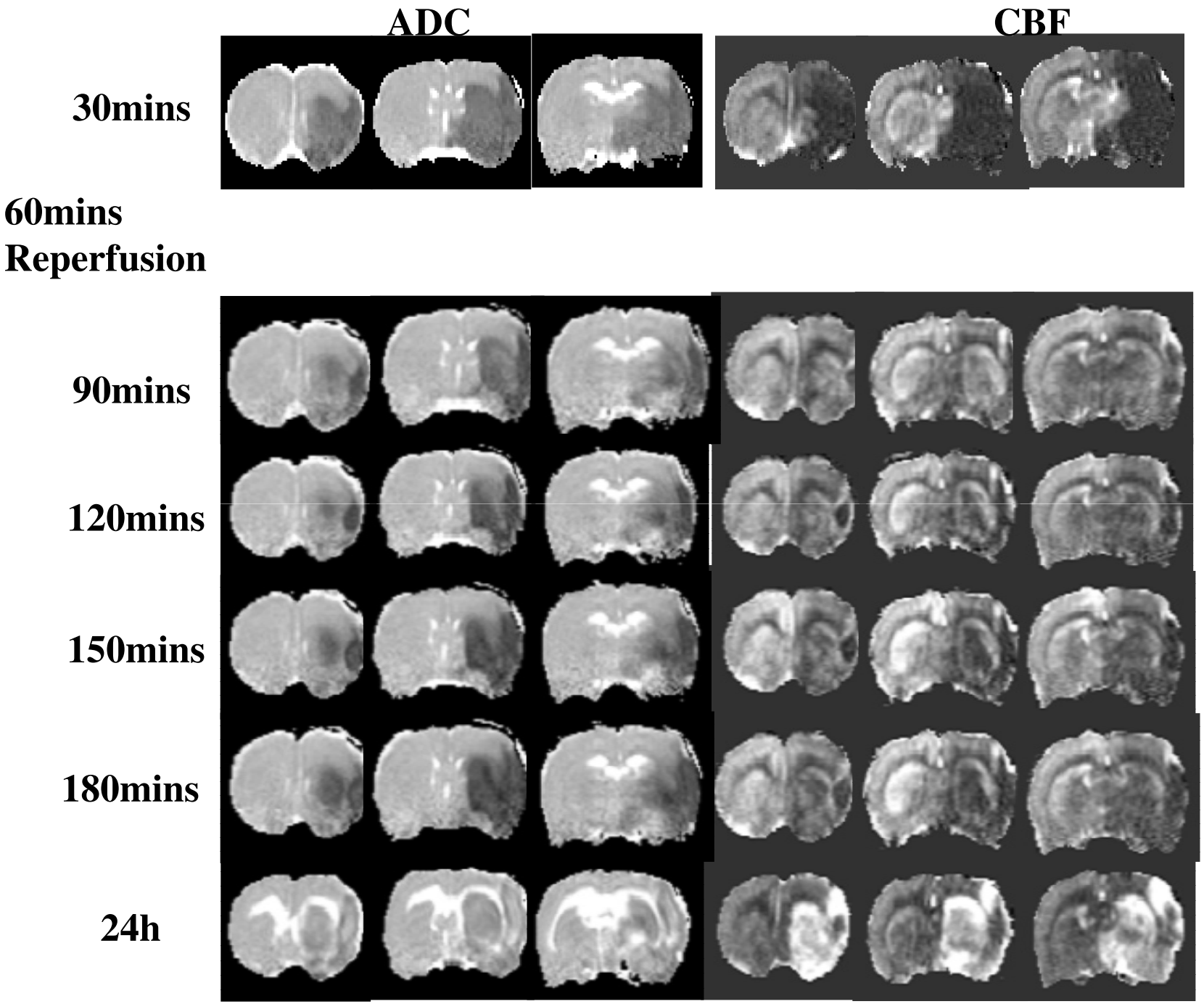
Core

Final infarct 24-hr T2 weighted image



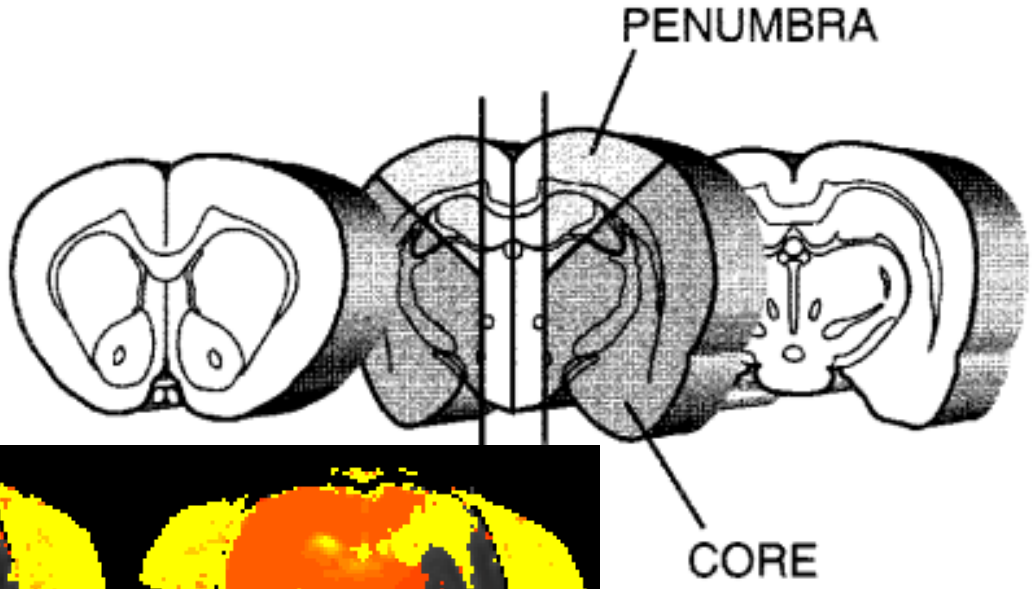
Bruker Biospec 7T/40cm scanner (Billerica, MA)

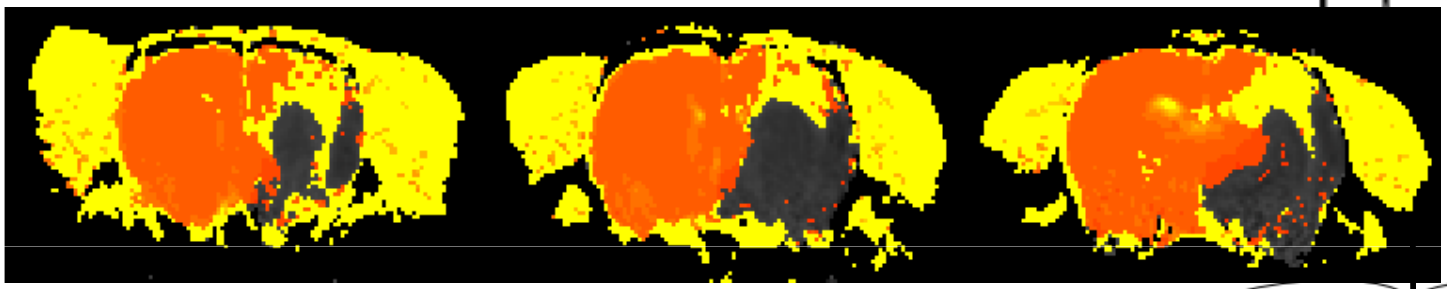
back


ADC
CBF
30mins
60mins
Reperfusion
90mins
120mins
150mins
180mins
24h

back

Core and Penumbra separated samples for Western Blot test.





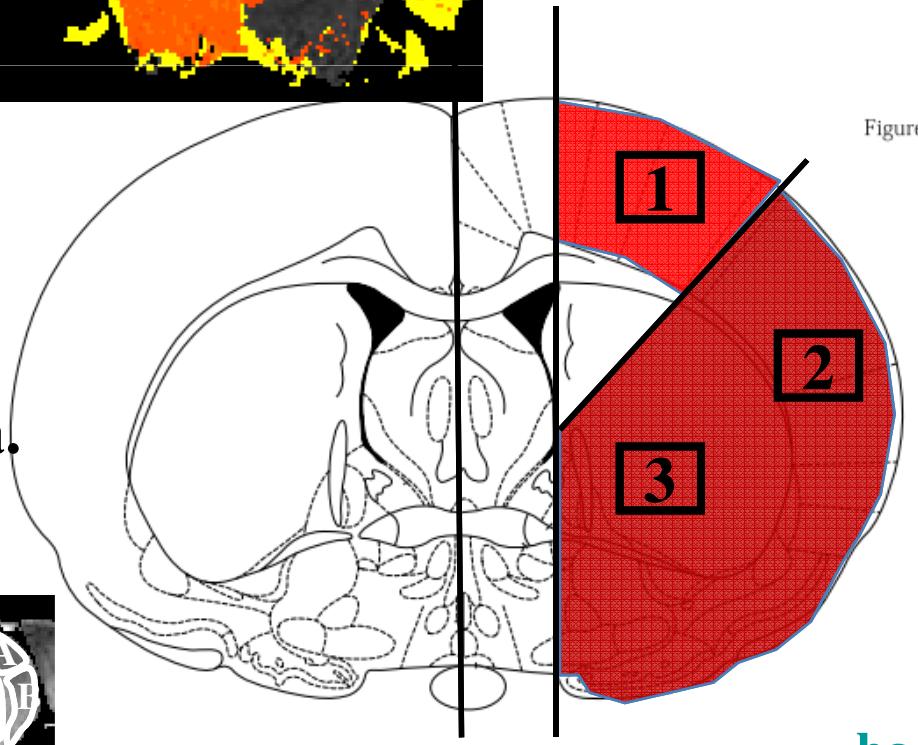
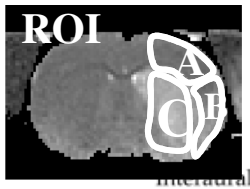


Figure 18

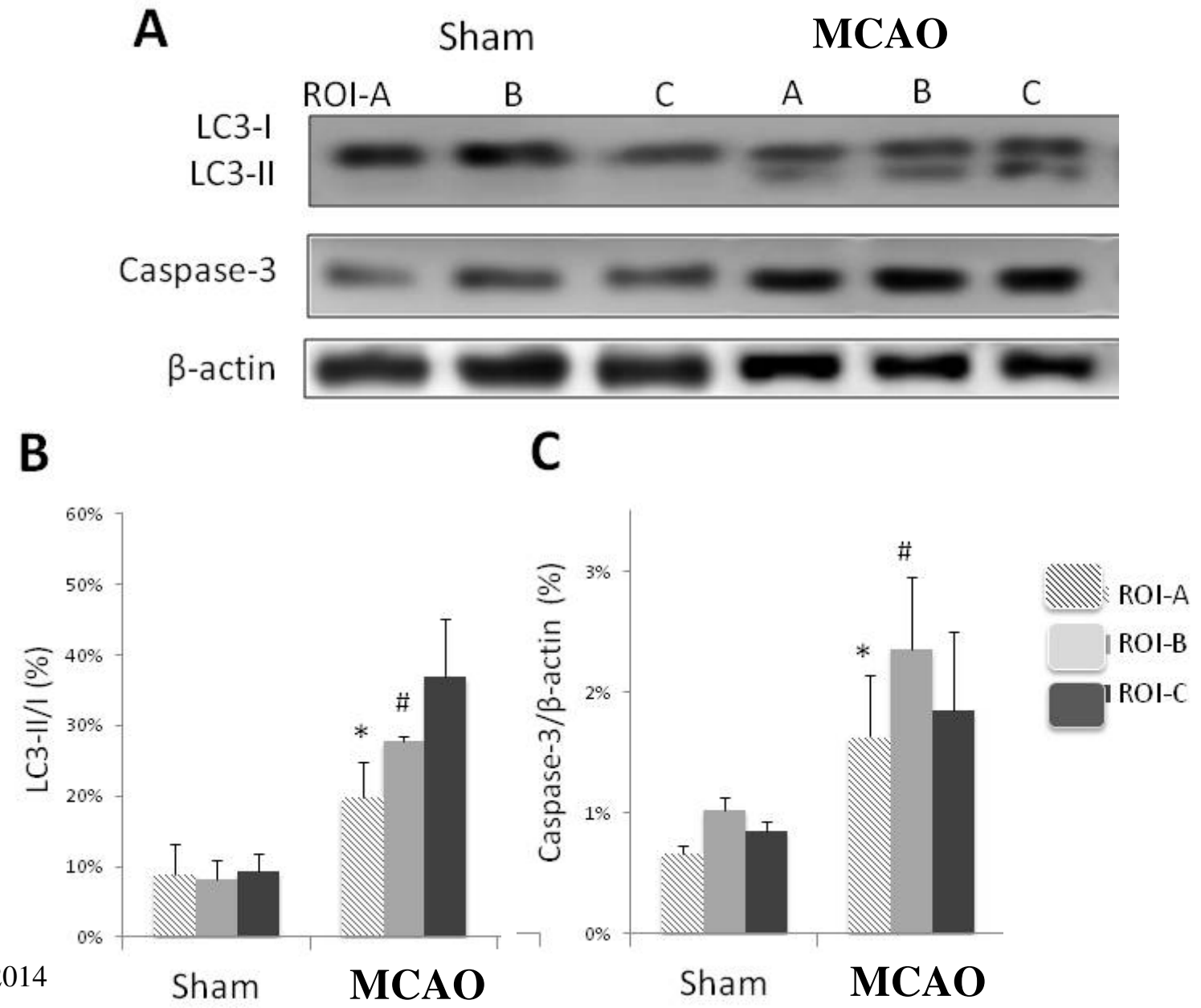
1. Cortex part of the Penumbra.
2. Cortex part of the Core.
3. Central of the Core.

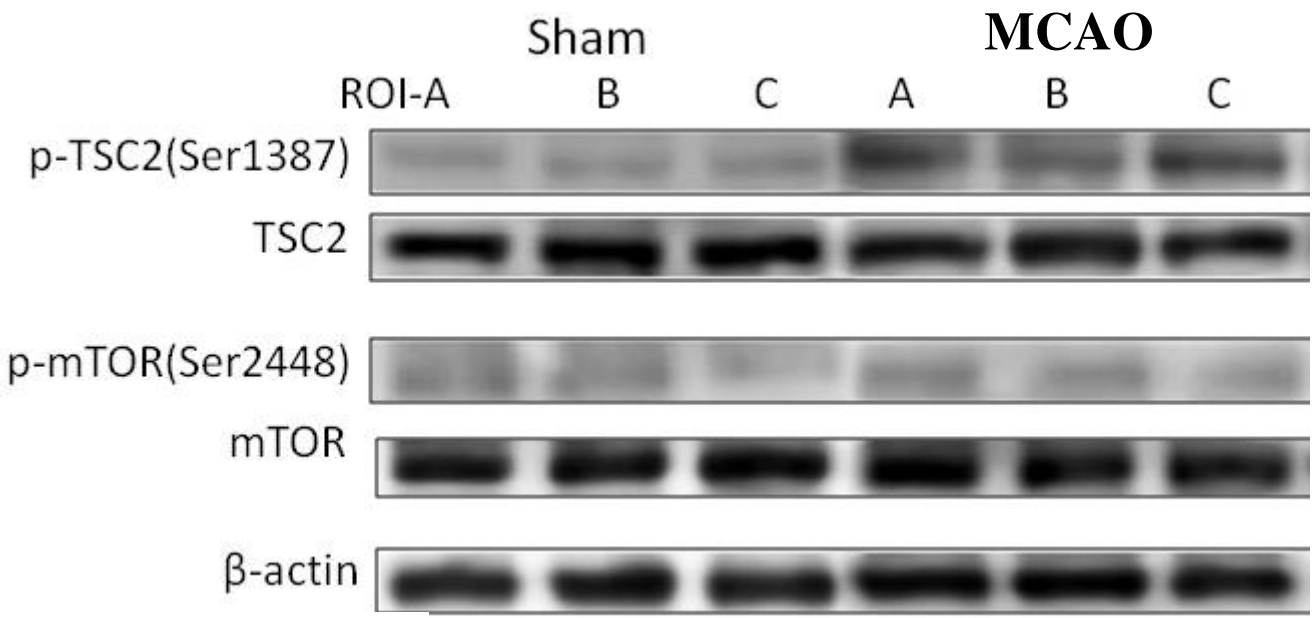


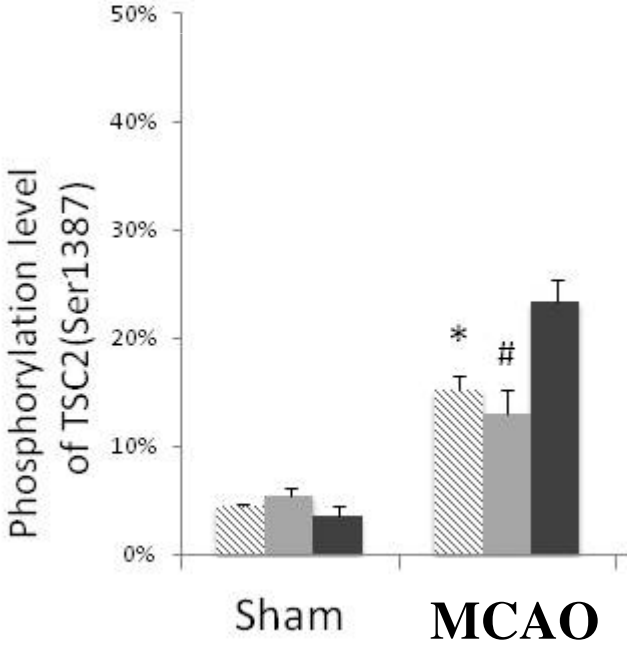
Interaural 8.74 mm

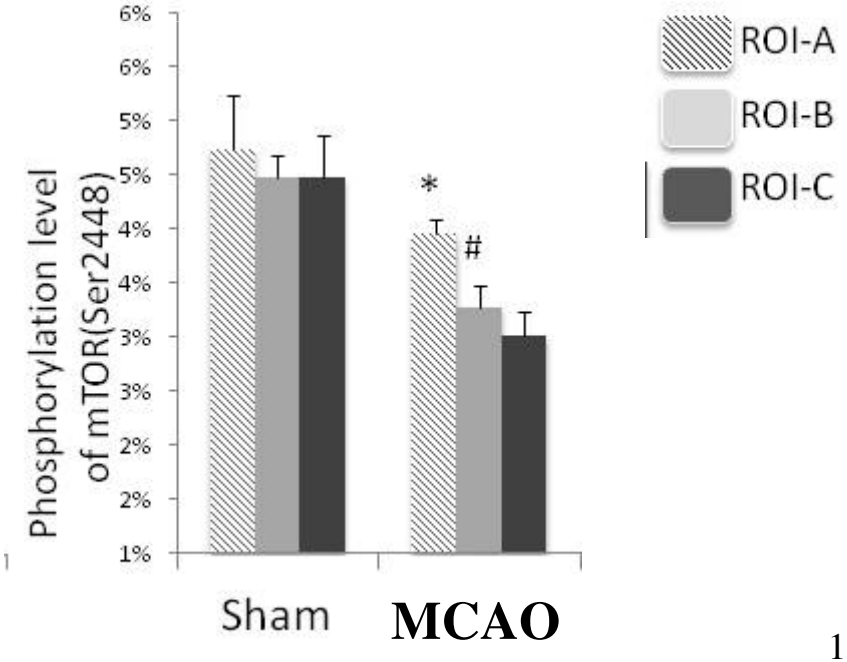
Bregma

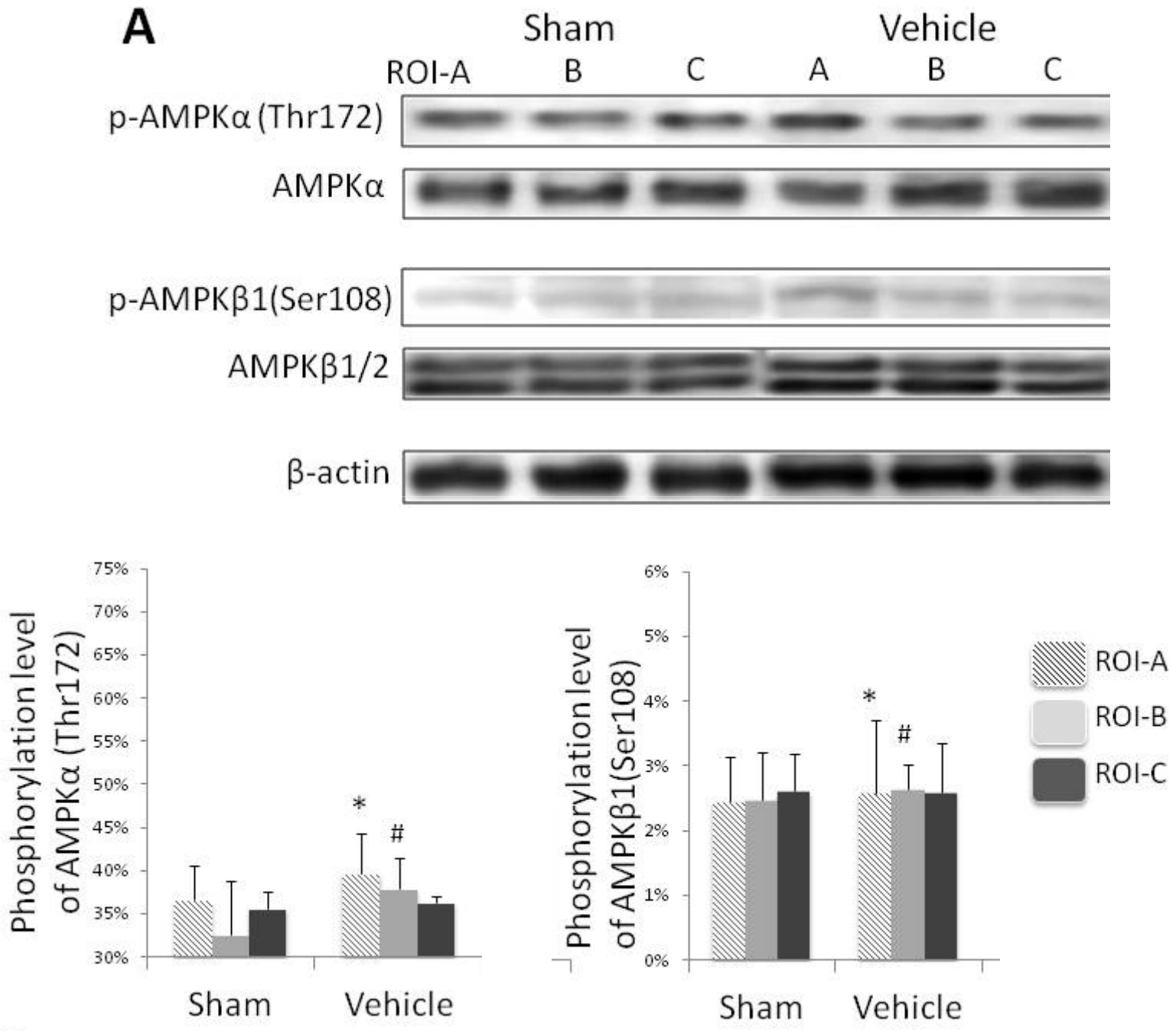
back


A
Sham
MCAO
ROI-A
B
C
A
B
C
LC3-I
LC3-II
Caspase-3
β-actin
B
LC3-II/I (%)
60%
50%
40%
30%
20%
10%
0%
*
#
Sham
MCAO
C
Caspase-3/β-actin (%)
3%
2%
1%
0%
*
#
Sham
MCAO
ROI-A
ROI-B
ROI-C


Sham
MCAO
ROI-A
B
C
A
B
C
p-TSC2(Ser1387)
TSC2
p-mTOR(Ser2448)
mTOR
β-actin


50%
40%
30%
20%
10%
0%
Phosphorylation level of TSC2(Ser1387)
*
#
Sham
MCAO


6%
6%
5%
5%
4%
4%
3%
3%
2%
2%
1%
Phosphorylation level of mTOR(Ser2448)
*
#
Sham
MCAO
ROI-A
ROI-B
ROI-C


A
Sham
Vehicle
ROI-A
B
C
A
B
C
p-AMPKα (Thr172)
AMPKα
p-AMPKβ1(Ser108)
AMPKβ1/2
β-actin
Phosphorylation level of AMPKα (Thr172)
75%
70%
65%
60%
55%
50%
45%
40%
35%
30%
*
#
Sham
Vehicle
Phosphorylation level of AMPKβ1(Ser108)
6%
5%
4%
3%
2%
1%
0%
*
#
Sham
Vehicle
ROI-A
ROI-B
ROI-C

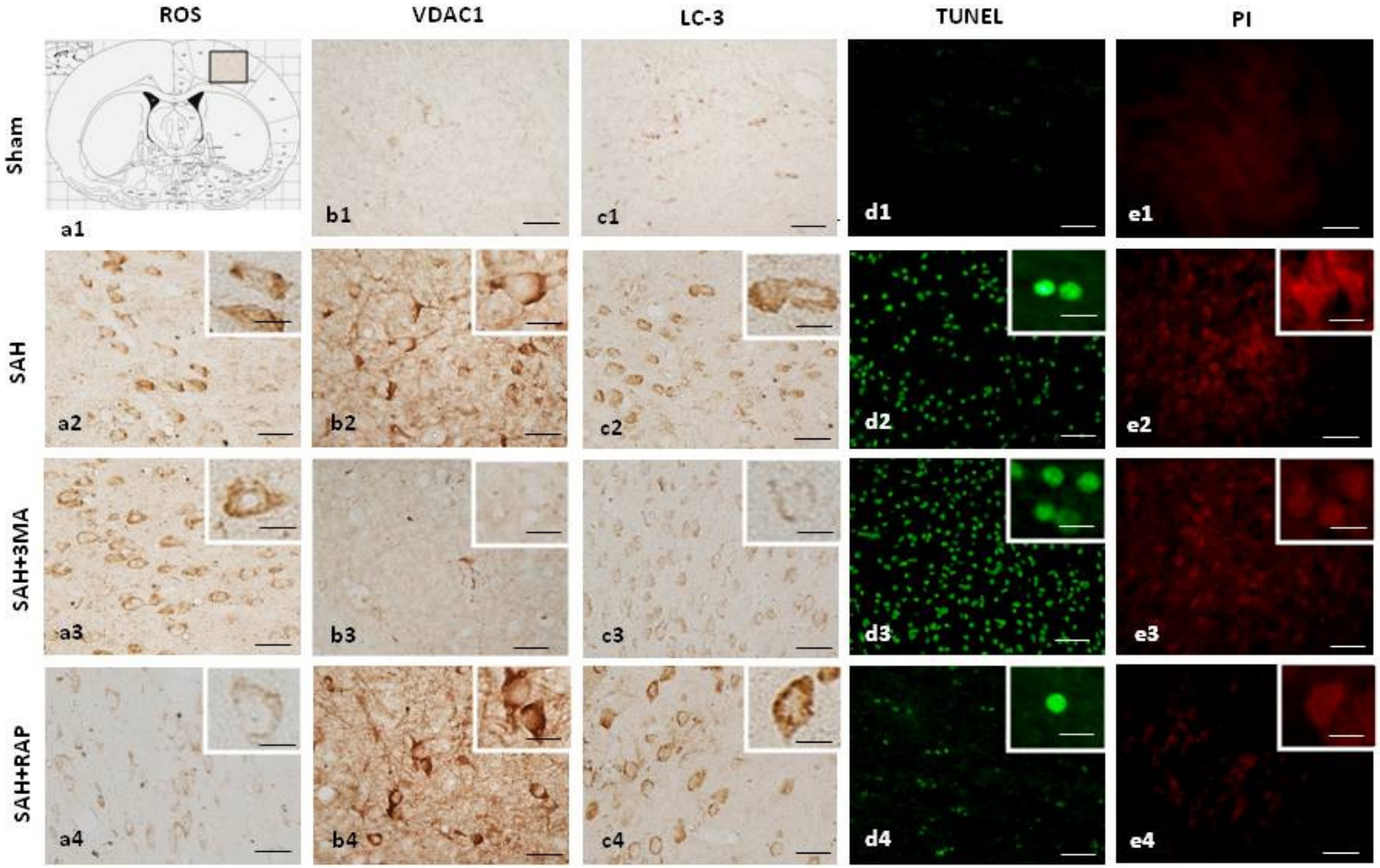
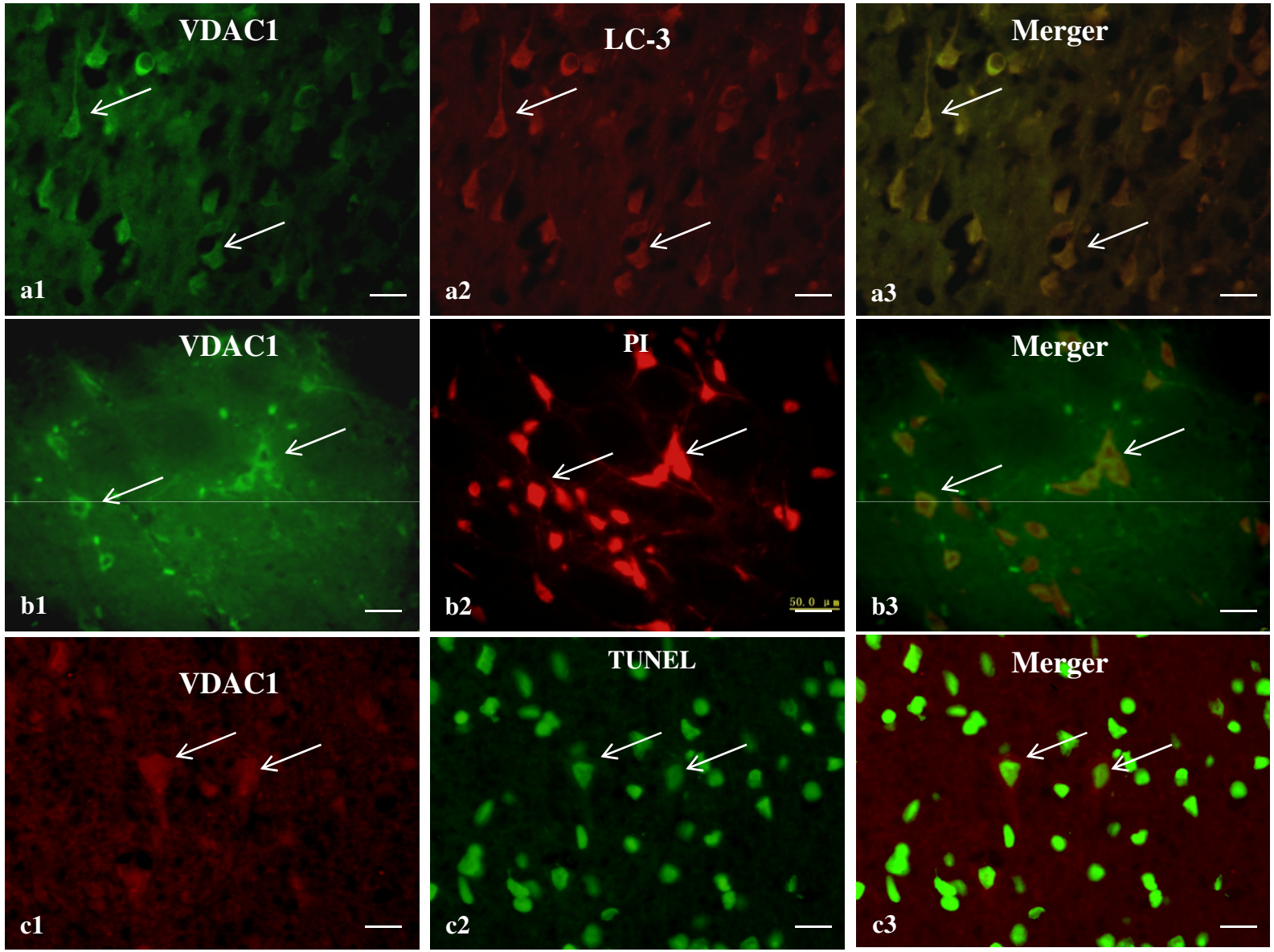
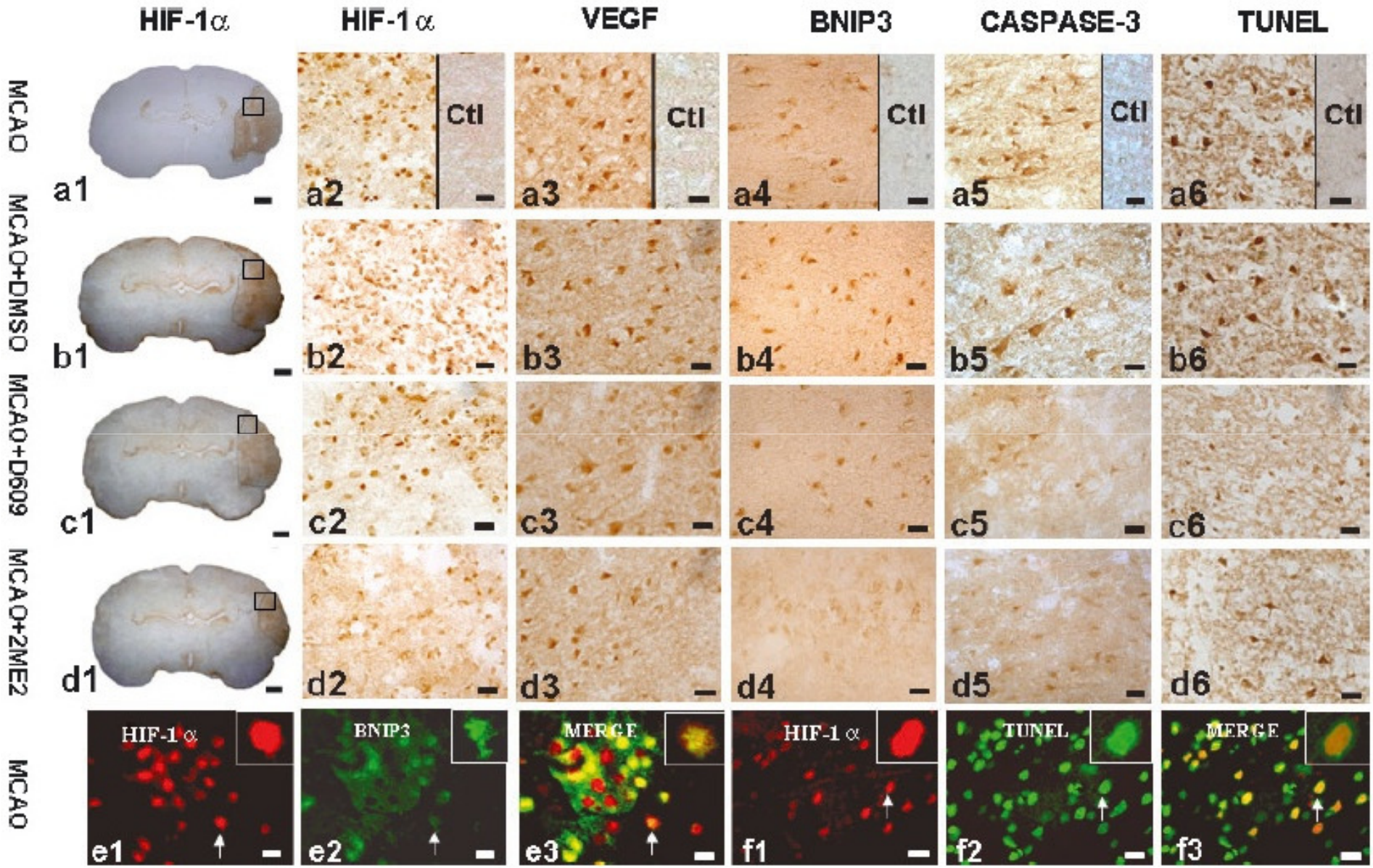


图2：SAH48小时后大脑切片的免疫组织化学染色


VDAC1
LC-3
Merger
a1
a2
a3
VDAC1
PI
Merger
b1
b2
50.0 μm
b3
VDAC1
TUNEL
Merger
c1
c2
c3


HIF-1α
HIF-1 α
VEGF
BNIP3
CASPASE-3
TUNEL
MCAO
MCAO+DMSO
MCAO+D609
MCAO+2ME2
MCAO
Ctl
a1
a2
a3
a4
a5
a6
b1
b2
b3
b4
b5
b6
c1
c2
c3
c4
c5
c6
d1
d2
d3
d4
d5
d6
HIF-1 α
BNIP3
MERGE
HIF-1 α
TUNEL
MERGE
e1
e2
e3
f1
f2
f3

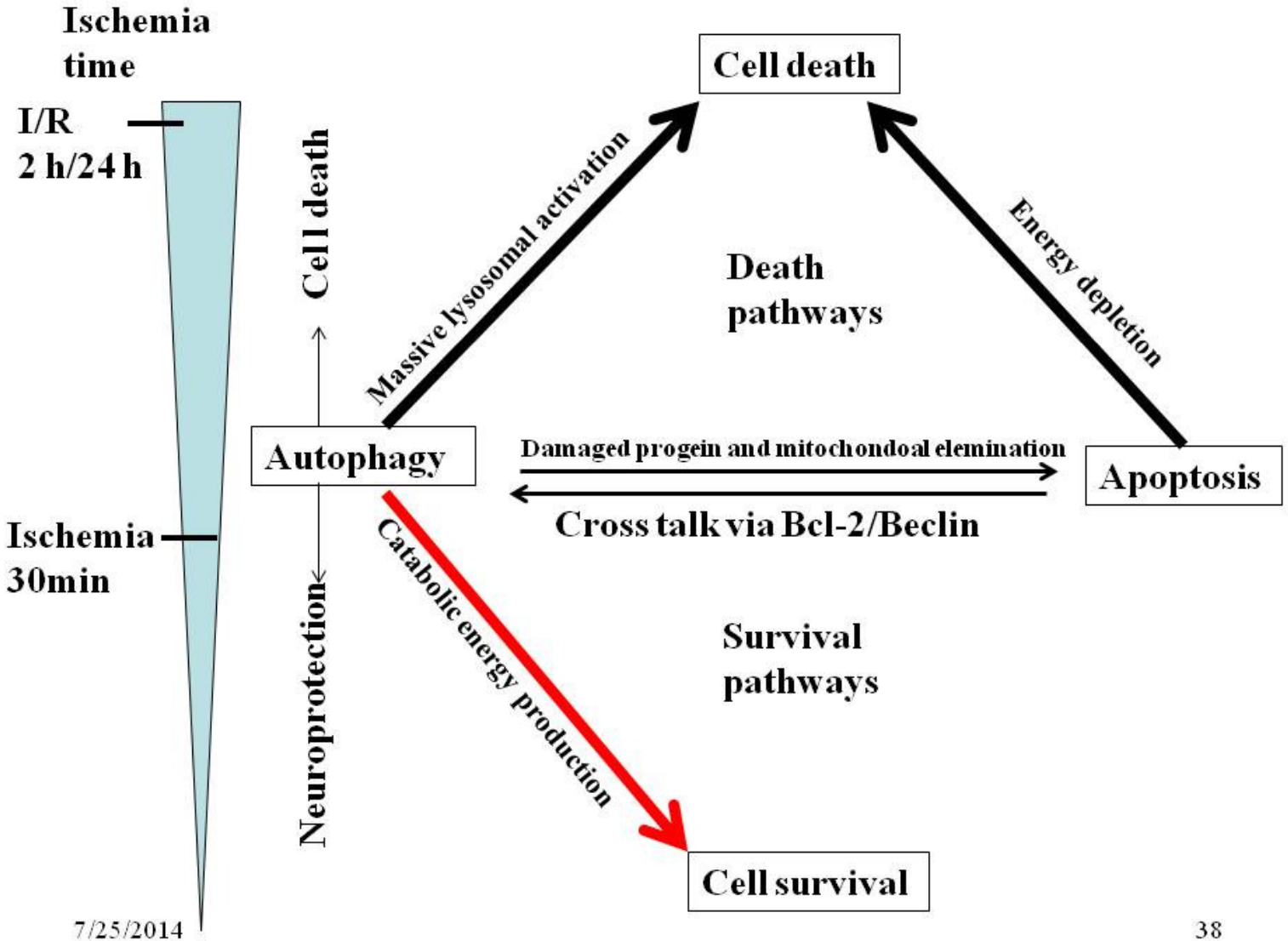
Autophagy

- **What, When and Where?**

- **Autophagy play neuroprotective roly In the early stage ischemic stroke(30 min, hypoxia) in the penumbra and cortex part of the core.**

- **Autophagic cell death in the terminal stage ischemic stroke(24hrs, repurfusion) in the penumbra .**

Conclusion


Ischemia time
I/R 2 h/24 h
Ischemia 30min
Cell death
Neuroprotection
Cell death
Massive lysosomal activation
Energy depletion
Death pathways
Autophagy
Damaged progein and mitochondoal elemination
Cross talk via Bcl-2/Beclin
Apoptosis
Catabolic energy production
Survival pathways
Cell survival