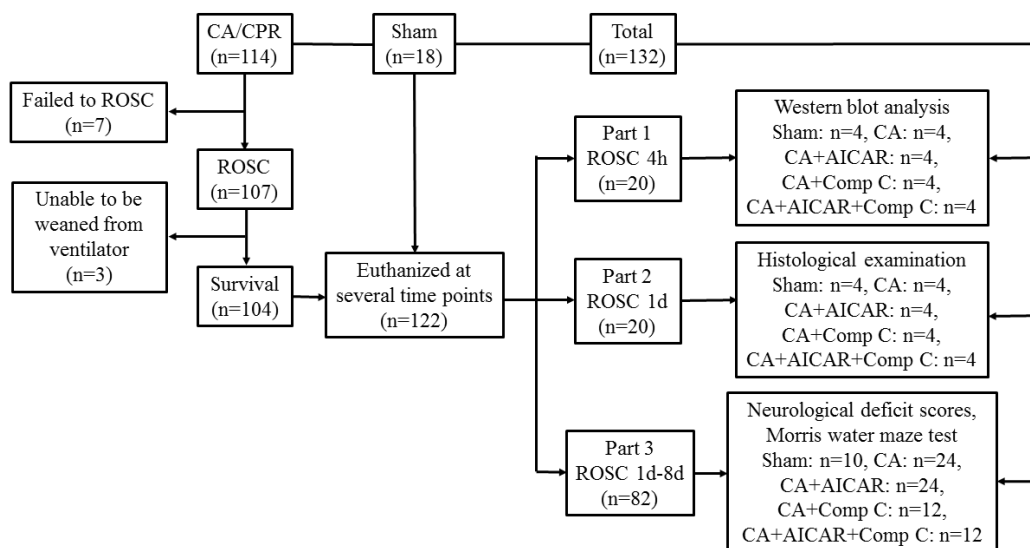


Supplementary Data

Figure S1



Flow chart of the experiment

The whole study consists of 3 parts. In part 1, Western blot was tested at 4 hour after ROSC. In part 2, histological examination (HE, Nissl, TUNEL assay) was analysed at day 1 after ROSC. In part 3, neurological deficit scores and morris water maze test were observed from day 1 to day 8 after ROSC.

Abbreviations: CA: cardiac arrest; CPR: cardiopulmonary resuscitation; ROSC: return of spontaneous circulation; AICAR: 5-aminidazole-4-carboxamide riboside, AMPK activator; Comp C: Compound C, AMPK antagonist; BI 6015: HNF4 α antagonist.

Table S1

Genes		Primers (5'-3')
Actin	F	CACCCGCGAGTACAACCTTC
	R	CCCATACCCACCATCACACC
AMPK	F	CCAAATTATGCAGCACCGGA
	R	CGGTTGAGATACTCCGGGATG
HNF-4 α	F	GGCAATGACTACATCGTCCCTC
	R	GGCTTTGAGGCAGGCGTAT

Table S2. Baseline characteristics among the groups

	Sham (n=5)	CA+Vehicle (n=12)	CA+AICAR (n=12)	CA+Compound C (n=12)
Body weight (g)	393.2±11.26	389.4±30.56	403.7±12.32	405.3±30.02
HR (beats/min)	413.6±26.05	409.3±17.16	420.3±30.53	409.8±25.6
MAP (mmHg)	116.6±7.37	122.1±9.00	114.4±6.50	122.7±6.37
Body temperature (°C)	37.3±0.33	37.5±0.27	37.5±0.44	37.2±0.38
PEA (s)	0	280.3±28.07	276.7±34.40	260.6±36.79
Asystole (s)	0	381.2±35.77	367.9±39.78	354.3±27.7
VF times (%)	0	0	0	0
CPR duration (s)	0	92.0±13.12	91.1±13.26	90.7±15.89
Total epinephrine (μg)	0	11.1±2.50	10.9±2.19	10.8±1.55

Table S3. Baseline characteristics among the groups

	Sham (n=5)	CA+Vehicle (n=12)	CA+AICAR (n=12)	CA+AICAR+BI5015 (n=12)
Body weight (g)	391.6±20.50	385.6±25.26	410.6±12.71	412.9±22.96
HR (beats/min)	408±33.85	407.4±15.26	408.3±25.15	410.8±20.09
MAP (mmHg)	116.4±8.88	116.4±9.90	123.1±10.16	117.1±7.86
Body temperature (°C)	37.5±0.41	37.6±0.30	37.7±0.20	37.4±0.33
PEA (s)	0	267.7±19.07	251.1±12.15	271.9±18.94
Asystole (s)	0	391.1±26.56	379.7±22.33	399.8±12.59
VF times (%)	0	0	0	0
CPR duration (s)	0	99.9±14.85	98.3±12.12	101.7±14.53
Total epinephrine (μg)	0	11.0±2.49	11.0±2.25	11.6±1.88

Note: Values are presented as means ± SDs. There were no statistically significant differences among the experimental groups.

Abbreviations: HR: heart rate; MAP: mean arterial pressure; PEA: Pulseless electrical activity; BP<30 mmHg; Asystole: BP<10 mmHg; VF times: Ventricular Fibrillation times; CPR duration: cardiopulmonary resuscitation duration.

Figure S2. Electron microscope comparison of NGF-induced PC12 cells and neurons

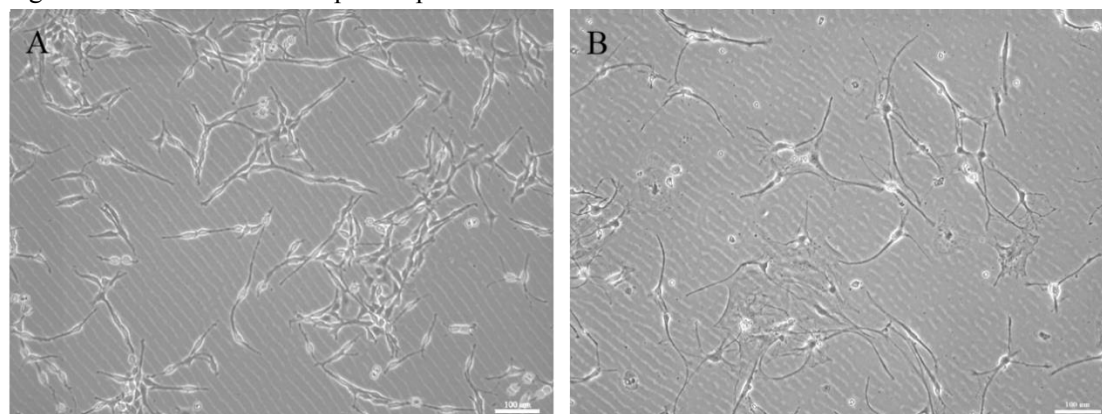


Figure S2. A: Morphology of NGF-induced PC12 cells, B: Morphology of neurons, scale bar: 100μm

Figure S3. Drug concentration and OGD/Reperfusion time exploration

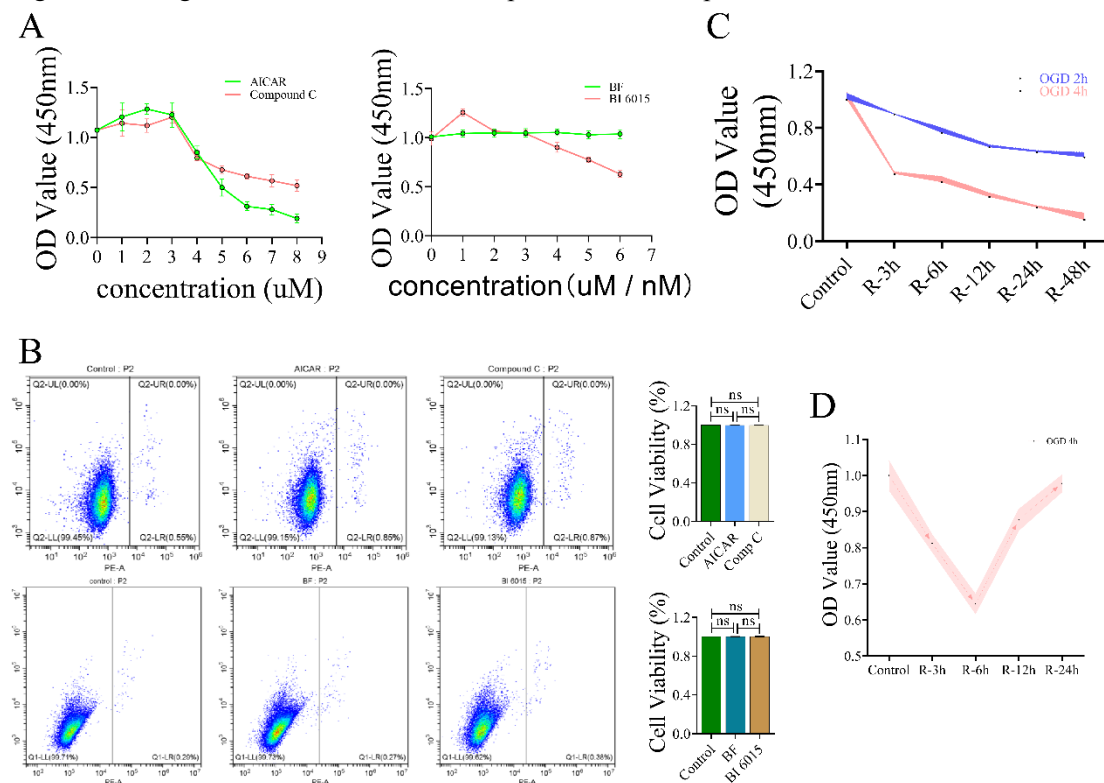
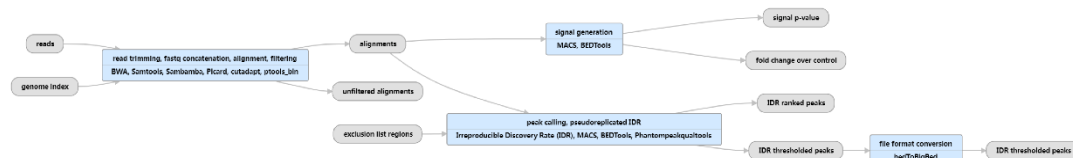


Figure S3. Exploring drug concentration and OGD/R time in cells.

(A) PC12 cells were used to explore the concentrations of AICAR, Compound C, BF and BI 6015 by CCK8 assay. The abscissa numbers 1 to 8 corresponded to the concentrations of AICAR and Compound C, respectively (AICAR: 20umol/L, 50umol/L, 100umol/L, 200umol/L, 400umol/L, 600umol/L, 800umol/L, 1000umol/L. Compound C: 0.2umol/L, 0.5umol/L, 1umol/L, 2umol/L, 4umol/L, 6umol/L, 8umol/L, 10umol/L). The abscissa number 1 to 6 corresponded to the concentration of BF and BI 6015, respectively (BF: 0.01nmol/L, 0.02nmol/L, 0.05nmol/L, 0.1nmol/L, 0.2nmol/L, 0.25nmol/L. BI 6015: 1umol/L, 5umol/L, 10umol/L, 15umol/L, 20umol/L, 25umol/L). (B) flow cytometry was used to detect the viability of primary neuronal cells. (C) OGD/R time exploration of primary neurons by CCK8 assay. (D) OGD/R time exploration of NGF-induced PC12 cells by CCK8 assay. Values are means $\pm$ SD, $n=3$, $ns P > 0.05$.

Figure S4. Computational pipeline schematic



Reference website: <https://www.encodeproject.org/chip-seq/transcription-factor-encode4/>

Figure S5. HNF4 α may combine with Bcl-2 to participate in the regulation of AMPK signaling pathway in brain

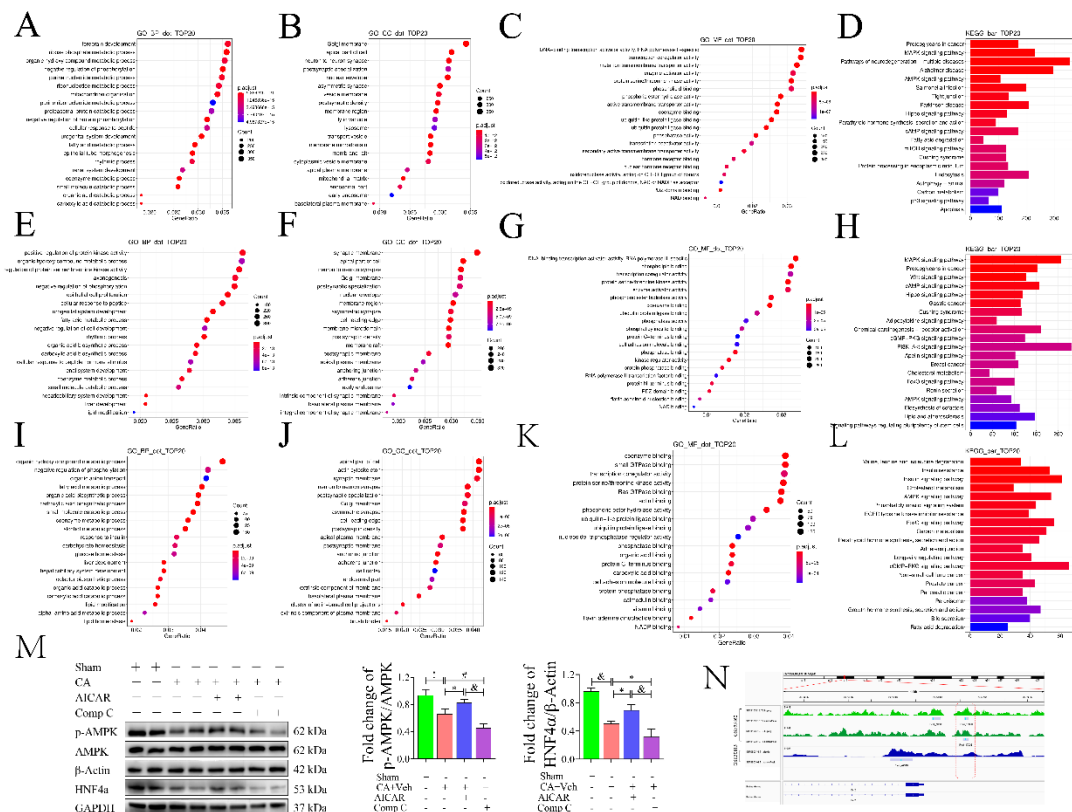


Figure S5. HNF4 α played an important role in vivo and was activated by AMPK

Significantly enriched GO terms and KEGG pathway terms between the chip-seq analysis of kidney tissues and hepatocytes based on their functions. The top 20 GO terms and KEGG pathway terms in the enrichment analysis in three samples SRR980344 (A-D), SRR1103818 (E-H), SRR1103819 (I-L). (A, E, I) The top 20 biological process (BP) terms in the enrichment analysis, (B, F, J) The top 20 cellular component (CC) terms in the enrichment analysis, (C, G, K) The top 20 molecular function (MF) terms in the enrichment analysis. (D, H, L) The top 20 enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway terms of the three samples. (M, N) Representative western blot of P-AMPK and HNF4 α expression in the hippocampus showed a positive correlation between AMPK and HNF4 α , n=4. (N) The genomic region around Bcl-2 promoter was visualized through the Integrative Genome Viewer (IGV). Abbreviations: Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. Values are means $\pm$ SD. & $P < 0.0001$, ! $P < 0.001$, # $P < 0.01$, * $P < 0.05$.

Figure S6. Chip-seq library construction process

1. Quantitative detection of ChIP fragments with qubit
2. Fill in the ends of the fragment and add an A tail to the 3' end
3. Add Adapter
4. 0.8X AMPure beads to remove excess Adapter
5. Library PCR Amplification
6. 1XAMPure beads to remove excess primer
7. qPCR assay library concentration
8. Agilent 2100 detects library fragment size

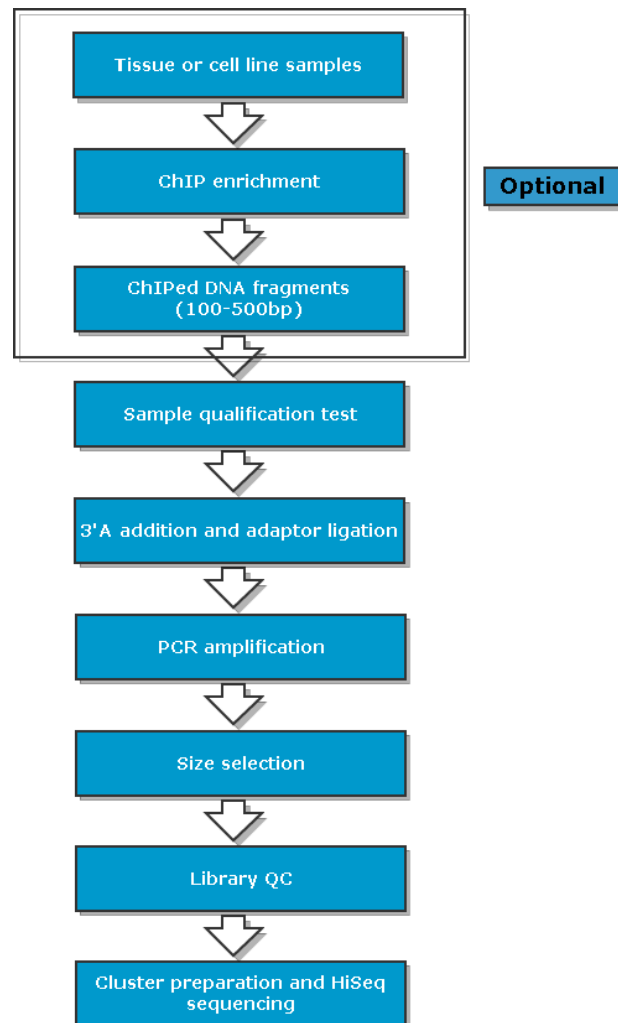


Figure S7. Bioinformatics Analysis Process

The sequencing results were compared with the reference genome, and the sequence at the unique position on the alignment was used for subsequent standard information analysis and personalized analysis.

