Supplementary Figure Legend:

Figure S1 - Expression pattern of BNIP3 in stroke models.

Primary cortical neurons were treated with OGD for 6 h followed by different time of reperfusion (0-72 h) (A-B). Immunocytochemistry (A) and Western blot (B) were used to demonstrate the time course of BNIP3 expression *in vitro*. Immunofluorescence data verified the 'delayed' expression of BNIP3 in WT neurons, and the complete silencing of BNIP3 in KO tissues after I/H injury. BNIP3 was stained with red, and nuclei was marked with blue. Scale bars = 80 μm. Images were taken at 20· objective in (A). BNIP3 WT mice pups were subjected to the neonatal stroke modeling. Brain tissues were collected and processed after recovery of 1, 3 and 7 days, respectively. Western blot was used to demonstrate the time course of BNIP3 expression *in vivo* (C).

Our data showed both forms of BNIP3 protein, the cytosolic monomer (30 KD) and the mitochondria-localized homodimer (60 KD) expressed in a similar 'delayed' pattern in the brain I/H (B-C). Band densities were measured by Quantity One software. One-way ANOVA analysis and Dunnett's post-tests were used for the statistical analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control group. Controls were sham-operated groups without I/H injuries. N = 3 for each group.

Figure S2 - OGD/RP injury-induced autophagy and autophagic cell death in rat cortical neurons.

Electron micrographs showed morphological changes of neurons undergoing delayed neuronal death after OGD/RP injury (I-IV). Primary rat cortical neurons were subjected to OGD for 6 h then followed by RP for 24 h (II), 48 h (III) and 72 h (IV) and were fixed for EM examination. Normal appearance of cytoplasm, organelles, and nucleus were observed in control neurons (I). Cell shrinkage, nuclear condensation (without fragmentation), loss of cellular organelles, and formation of numerous autophagic vacuoles (black arrows) were shown in the pictures (II-IV), presenting an increased level of autophagy (II) and autophagic cell death (III-IV). Specifically, ultrastructural features of autophagic cell death were evident in neurons treated by OGD 6 h followed by RP 48 h (III) and 72 h (IV). Mitochondria also displayed swelling and dilation as indicated by black arrowheads (II-IV). Mixed morphological features of autophagic cell death and apoptosis (cell membrane blebbing) were found in the same group of neurons treated by OGD 6 h followed by RP 72 h (IV), demonstrating a co-existence of autophagic cell death and apoptosis. An autophagosome was indicated by white box in (IV). N, nucleus; Scale bars = 2 μm in (I-IV).

Figure S3 - BNIP3 regulates the mitophagy pathway in OGD/RP stroke model.

Western blot was used to detect Beclin1, LC3, BNIP3 and Bcl-2 expressions in OGD/RP-challenged neurons with or without presence of 1 mM 3-MA. β-actin (43 KD) was included as internal control. 3-MA (1 mM) effectively inhibited the autophagic activity by significantly decreasing the expression of Beclin1 and LC3-II/I ratio (A), but did not affect the expression

patterns of BNIP3 (A) and Bcl-2 (B). Band densities were measured by Quantity One software. One-way ANOVA analysis and Bonferroni post-tests were used for the statistical analyses. No 3-MA group vs 3-MA-treated group on each time point, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Control groups were without OGD/RP. N = 3 for each group.

This result showed that either with or without the presence of 1 mM 3-MA, BNIP3 was consistently activated and expressed in a 'delayed' manner, and both BNIP3 and Bcl-2 expressed in comparable levels at each time point after OGD/RP. Therefore, BNIP3 was anticipated to be an upstream regulator of the neuronal mitophagy pathway in OGD/RP stroke model.

Figure S4 - BNIP3 regulated excessive mitophagy by interacting with LC3.

Interactions between apoptosis, excessive mitophagy, general non-selective autophagy, cell death, and cell survival when BNIP3 gene is upregulated (left) and downregulated (right) in the stroke models (A). Proposed pathway depicting BNIP3's interaction with LC3 on the isolation membrane and subsequent initiation of excessive mitophagy (B).