**N-terminally cleaved Bcl-x<sub>L</sub> mediates ischemia-induced neuronal death**


**Supplemental Figure 1.** Pretreatment with the Bcl-x<sub>L</sub> inhibitor ABT-737 protects against ischemia-induced neuronal death in the CA1. (a) Low (upper) and high (lower) magnification images of Toluidine blue-stained coronal brain sections at the level of the dorsal hippocampus at 6 d after ischemia from rats pretreated with vehicle or ABT-737 (icv, 125 μM) and subjected to sham operation or global ischemia. So: stratum oriens; sp, stratum pyramidale; sr, stratum radiatum. (b) Summary data of neuronal counts showing the number of cells within the region of interest. Number of animals per treatment group is indicated on bars, 4 sections per animal. ttest *, P < 0.05.
Supplemental Figure 2. Pretreatment with the Bcl-x<sub>L</sub> inhibitor ABT-737 blocks ischemia-induced caspase 3 activation. (a) Representative active caspase-staining of the CA1 from coronal brain sections at the level of the dorsal hippocampus at 5 d after ischemia from rats pretreated with vehicle or ABT-737 at 1 hour prior to ischemia (icv, 125 μM) and subjected to sham operation or global ischemia. (b) Summary data of Active caspase-3 intensity in the pyramidal cell layer expressed as a percent of the sham-vehicle treated group, ttest *, P < 0.05.

Ofengeim et al. Suppl. Fig. 2
Supplemental Figure 3. The appearance of ΔN61-Bcl-xL is transient and returns to control levels after 48 h reperfusion. (a) Cleavage of Bcl-xL to generate ΔN-Bcl-xL increased at 24 h post-ischemia and decreased by 48 h post ischemia in rat brain. Similar patterns were not seen with other Bcl-2 family members such as Bcl-2 and Bcl-w. (b) Summary data showing ΔN-Bcl-xL abundance. The abundance of ΔN-Bcl-xL was normalized to that of full-length Bcl-xL by means of ImageJ software. Sham, n = 4; ischemia 24 h, n = 4; ischemia 24 h, n = 4; ttest *, P < 0.05.
Supplemental Figure 4. ABT-737 attenuates ΔN76-Bcl-xL-elicited mitochondrial channel activity. (a) Sample recordings from mitochondria isolated from the hippocampus of control (sham-operated) animals and treated in vitro with ΔN76-Bcl-xL (30 µg/ml) or ΔN76-Bcl-xL+ABT-737 (5 µM) introduced via the patch pipette at V_h = +60 mV. All 10-s recordings (traces) with channel activity were used for analysis. ABT-737 blocks large channel activity and attenuates intermediate channel activity. (b) Histograms showing closed, small, intermediate and large channel activity for recordings like those illustrated in (a). Conductances of channel activity were classified as in Fig. 1 (ΔN76-Bcl-xL, n = 7 mitochondria, 11 traces; ΔN76-Bcl-xL+ABT-737, n = 4 mitochondria, 4 traces). ABT-737 blocks large channel activity.
Supplemental Figure 5. Full-length Bcl-xL does not induce cytochrome c release from isolated brain mitochondria. Mitochondria were treated with recombinant Bcl-xL (1 μM) or ΔN61-Bcl-xL (1 μM) as in Fig. 5 in the presence or absence of ABT-737 (5 μM) for 1 h at 30°C. Protein samples from the mitochondrial pellet and supernatant were subjected to Western blot analysis and probed for cytochrome c. Summary data show abundance of cytochrome c released from mitochondria (n = 3-6 samples per treatment group from 3 independent experiments; blot from same experiment as in figure 5).

Ofengeim et al. Suppl. Fig. 5
Supplemental Figure 6. ∆N61-Bcl-xL induces cell death in single knock out Bax−/− Bak−/− MEFs. (a) ABT-737 does not attenuate ∆N61-Bcl-xL-elicited cell death in single knock out MEFs. Single knockout MEFs were transfected with ∆N61-Bcl-xL or mock-transfected. Summary data show percent cell survival as assayed by MTS solution (see methods) in the absence and presence of 200 nM ABT-737 Bak−/− MEFs: control, n = 9; ABT-737, n = 10; ∆N61-Bcl-xL, n = 7; ∆N61-Bcl-xL + ABT-737, n = 6; Bax−/− MEFs: control, n = 8; ABT-737, n = 8; ∆N61-Bcl-xL, n = 8; ∆N61-Bcl-xL + ABT-737, n = 8). ttest ***, P < 0.001.

Ofengeim et al. Suppl. Fig. 6
Supplemental Figure 7. Scheme for generating caspase-resistant BCL-\(x_L\) (D61/76A) knockin mice.
(a) Diagram of BCL-\(x_L\) protein showing the relative positions of the BH (BCL-2 homology) motifs, unstructured loop, transmembrane anchor (tm), and the caspase cleavage sites (arrow heads).
(b) Diagram and restriction enzyme map of the \(bc\)l-\(x\) gene on mouse chromosome 2. Nucleotide positions for non-coding exons (gray boxes) and coding exons (white boxes) of \(bc\)l-\(x\) are indicated. Exon 2 encodes the first 186 of the 233 amino acid residues of Bcl-x\(L\). The targeting vector with negative (herpesvirus thymidine kinase) and positive (neomycin) selection markers (black boxes), loxP sites, approximate positions of homologous recombination (dashed lines) and two point mutations (D61A* and D76A*) are indicated. (c) Genomic diagram for the completed \(bc\)l-\(x\) knockin mice, which have had the neo cassette deleted by cre recombinase, leaving only a 34 bp loxP site in intron 2 at a disrupted HpaI site.
(d) Southern blot of wild type (+/+), heterozygous (+/ki) and homozygous knockin (ki/ki) \(bc\)l-\(x\) mouse genomic DNA cut with EcoRI and HpaI restriction enzymes and hybridized with the probe indicated in panel C. (e) PCR genotyping results of the indicated mice.

Ofengeim et al. Suppl. Fig. 7
Supplemental Figure 8. Bcl-xL cleavage-resistant mice are protected against ischemia-induced neuronal death. Mice were subjected to sham surgery or 30 min global ischemia. Representative FJ-labeled brain sections at the level of the dorsal hippocampus from wild type and homozygous Bcl-xL cleavage-resistant knockin mice are shown.

Ofengeim et al. Suppl. Fig. 8
Supplemental Figure 9. Bcl-xL cleavage-resistant mice are protected against ischemia-induced neuronal death. (a) Representative images of hippocampal slices from wild-type, heterozygous and homozygous Bcl-xL cleavage-resistant knockin mice treated with propidium iodide and subjected to normoxia or OGD at DIV 8. Cell death was assessed by the fluorescence intensity of propidium iodide (PI) uptake in the CA1. Hippocampal slices from Bcl-xL cleavage-resistant mice exhibit reduced OGD-induced neuronal death. (b) Summary data showing propidium iodide uptake in slices like those illustrated in panel a. n = 6; heterozygotes animals, n = 12; knock-in animals, n = 7; 4-8 slices were used per animal.
Supplemental Figure 10. ABT-737 blocks neuronal death in organotypically-cultured rat hippocampal slice cultures in vitro. (a) Organotypically-cultured rat hippocampal slice were subjected to either control or OGD, homogenized, and protein samples subjected to Western blot analysis to detect the appearance of ΔN-Bcl-xL. Antibodies raised against Bcl-xL detected both full-length and truncated Bcl-xL. (b). Cell death in hippocampal slices by propidium iodide uptake in the CA1. Control, n = 10 slices from 5 animals; OGD, n = 9 slices from 5 animals; control+ABT-737, n = 8 slices from 5 animals; OGD+ABT-737, n = 9 slices from 5 animals. ttest *, P < 0.05.

Ofengeim et al. Suppl. Fig. 10
Suppl. Figure 11. Expanded Views of Western blots. (a) Expanded view of original immunoblots for Fig. 4. Representative lanes selected for presentation are numbered in the order shown in Fig. 4. (b) Expanded view of original immunoblots for Fig. 5 and Supplementary Fig. 5. Lanes 1 and 2 of each set of triplicate samples were arbitrarily selected for presentation in Fig. 5g and h. The adjacent 6 lanes of the Bcl-x\textsubscript{L} samples without and with ABT-737 respectively correspond to the last two lanes for each condition in the blot shown in Suppl. Fig. 5. The last lane is a control lane of whole mitochondria confirming the identity of the indicated endogenous proteins (this lane may have partially contaminated the adjacent Bcl-x\textsubscript{L} sample). Asterisks mark the position of the previous blotting antibody that remains visible. (c) Expanded view of original immunoblots for Fig. 7. The adjacent lanes of wild type and knockin presented in Fig. 7C are indicated. (d) Expanded view of original immunoblots for Supplementary Fig. 3. Representative lanes 2-6, 9 and 10 presented in Supplementary Fig. 3 are indicated, and were rearranged for presentation. Not shown is the accompanying internal control blot of phospho-Akt, which verified ischemic events as expected (available upon request). (e) Expanded versions of the same immunoblots shown in Supplementary Fig. 10.