Chen et al., http://www.jcb.org/cgi/content/full/jcb.201108059/DC1

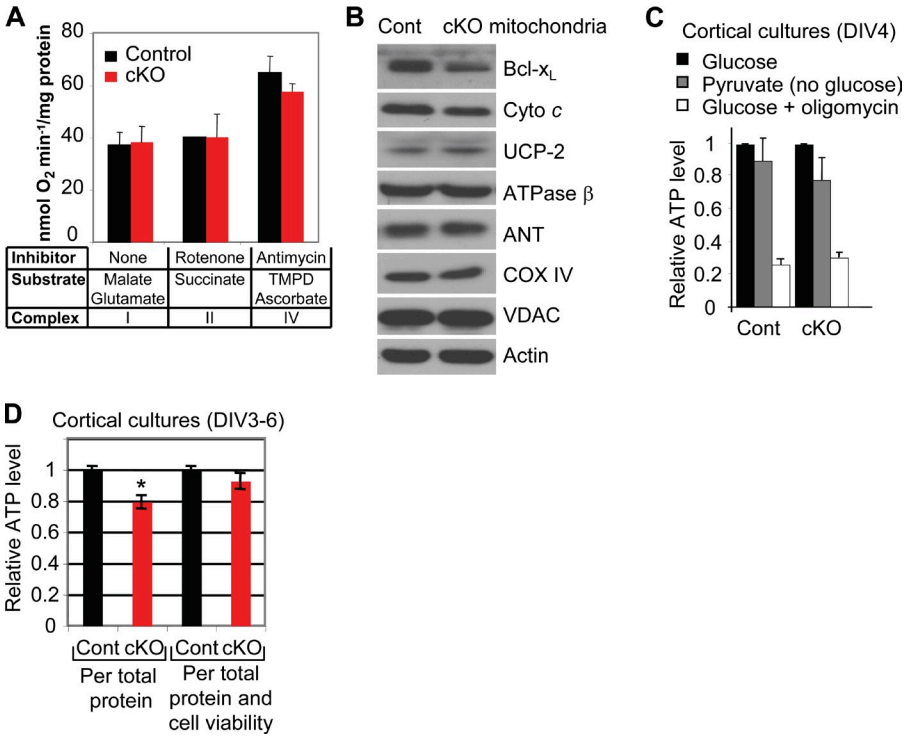

Figure S1. **No defects in *bcl-x*–deficient mitochondria under normal conditions.** (A) 1 mM ADP-stimulated rates of oxygen consumption were determined for mitochondria isolated from the cerebral cortex of P5–7 control and cKO of mouse pups (5 mM malate, 5 mM glutamate, 2.5 mM succinate, 0.5 mM tetramethylphenylenediamine [TMPD], and 2 mM ascorbate) with the indicated inhibitors for the relevant upstream complex (complex I, 2 mM rotenone; complex III, 1 mM antimycin). (B) Immunoblots of mitochondria isolated from dissected P5–7 cerebral cortex (where interneurons and glia still express Bcl-x_L). Cortices from four *bcl-x* cKO and two control (Cont) mice were pooled, and equal protein was loaded based on the BCA assay and analyzed using antibodies to ANT (sc-9299 [1:1,000; Santa Cruz Biotechnology, Inc.]), ATP synthase β subunit (1:1,000; Invitrogen), and other proteins as described in Fig. 2 A. A representative of three independent experiments is shown. VDAC, voltage-dependent anion channel. Molecular mass is indicated in kilodaltons. (C) Relative contributions of mitochondria and glycolysis to whole-cell ATP levels. Cortical neurons cultures (DIV4) were transferred to medium containing 25 mM glucose, glucose-free medium supplemented with 10 mM pyruvate to fuel mitochondria, or medium containing 25 mM glucose and 5 mg/ml of ATP synthase inhibitor oligomycin for 1 h and were then harvested for total ATP determination for each culture dish using a bioluminescence assay (Invitrogen). The absolute value of ATP was calculated from a standard curve generated for each individual experiment using fresh ATP standards (0 nM, 5 nM, 50 nM, 500 nM, 5 µM, and 25 µM) and was normalized by the protein content of each individual sample by BCA assay. Samples were analyzed immediately, or all time points were frozen instantly and analyzed together. Data are presented for three independent experiments, each with two to four different cultures/samples per genotype (control, $n = 9$; cKO, $n = 10$). A *t* test was used; all comparisons were not significantly different ($p > 0.13$). (D) Total ATP levels in cortical neuron cultures (DIV3–6). The mean value for cKO cultures was determined proportional to control and the results from multiple samples (control, $n = 18$; cKO, $n = 19$) in seven independent experiments (left). Relative ATP values for the same cortical cultures were further normalized by the mean difference in cell viability per experiment (mean ratio of cell viability for control/cKO = 1:0.85), determined by nuclear morphology using Hoechst (right). Data are presented as the mean ± SEM. The p value (*, $P < 0.0002$) was determined using a *t* test.

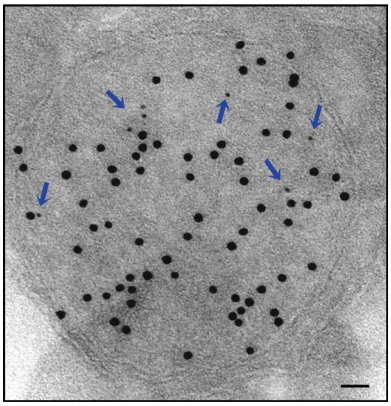

Figure S2. **Cryo-EM of *bcl-x*$^{+/+}$ mouse brain confirms mitochondrial matrix localization of endogenous Bcl-x_L protein.** An example of a mitochondrion co-labeled by immunogold for the β subunit of the ATP synthase (large gold beads) and Bcl-x_L (small gold; arrows) is shown. Note that the size of the antibody–bead complex will not permit colabeling of the same protein complex. Bar, 0.1 mm.

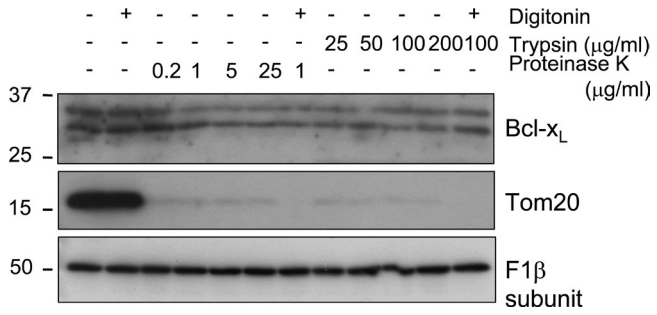

Figure S3. **Protease digestion of mitochondria detected with Bcl-x_L antibody.** Bcl-x_L is protected from protease digestion after permeabilization of the outer mitochondrial membrane of isolated rat brain mitochondria with digitonin. The blotting was performed as described for Fig. 2 E, except with anti–Bcl-x_L antibody. Molecular mass is indicated in kilodaltons.

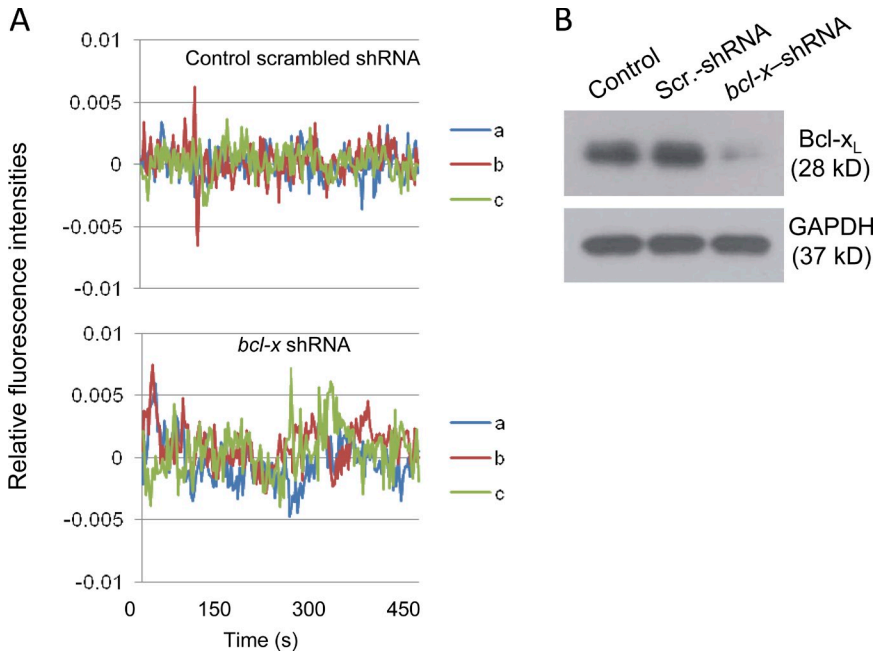

Figure S4. **Example of TMRE traces and Bcl-x_L blots for shRNA knockdowns.** (A) Fluorescence traces for three mitochondria per condition used to calculate SDs shown in Fig. 4 G. (B) Immunoblot analysis verifying shRNA knockdown of Bcl-x_L in hippocampal neurons results in Fig. 4 G. Lentivirus plasmids (pGIPZ vector) express the following shRNAs against rat BCL-x_L: 5′-CGGGCTCACTCTTCAGTCGGAATAGTGAAGCCACAGATGTATTCCGACTGAAGAGT-GAGCCCA-3′ or scrambled (Scr.) control (cat# RHS4346; Thermo Fisher Scientific). GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

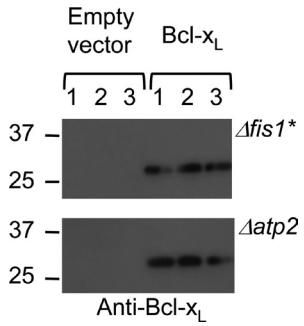

Figure S5. **Immunoblots of human Bcl-x_L in yeast cell lysates.** Three independently transformed strains for each plasmid in each of the indicated yeast knockout strains are shown. Molecular mass is indicated in kilodaltons.