Bcl-xL induces Drp1-dependent synapse formation in cultured hippocampal neurons

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Maturation of neuronal synapses is thought to involve mitochondria. Bcl-xL protein inhibits mitochondria-mediated apoptosis but may have other functions in healthy adult neurons in which Bcl-xL is abundant. Here, we report that overexpression of Bcl-xL postsynthetically increases frequency and amplitude of spontaneous miniature synaptic currents in rat hippocampal neurons in culture. Bcl-xL, overexpressed either pre or postsynthetically, increases synaptic vesicle number, the number and size of synaptic vesicle clusters, and mitochondrial localization to vesicle clusters and synapses, likely accounting for the changes in miniature synaptic currents. Conversely, knockdown of Bcl-xL or inhibiting it with ABT-737 decreases these morphological parameters. The mitochondrial fission protein, dynamin-related protein 1 (Drp1), is a GTPase known to localize to synapses and affect synaptic function and structure. The effects of Bcl-xL appear mediated through Drp1 because overexpression of Drp1 increases synaptic markers, and overexpression of the dominant-negative dnDrp1-K38A decreases them. Furthermore, Bcl-xL communoprecipitates with Drp1 in tissue lysates, and in a recombinant system, Bcl-xL protein stimulates GTPase activity of Drp1. These findings suggest that Bcl-xL positively regulates Drp1 to alter mitochondrial function in a manner that stimulates synapse formation.

Bcl-2 | synaptic transmission | mitochondria | cell death | ABT-737

During the initial stages of synaptic development, vesicles containing neurotransmitter aggregate into clusters within the presynaptic axons (1). Although neurotransmitter can be released before contact with the postsynaptic cell (2), enlargement and stabilization of the presynaptic vesicle cluster after postsynaptic contact underlie the development of high-fidelity release of neurotransmitter (3–5). Despite this understanding, many of the molecular players and mechanisms in synapse development are unknown. Here, we detail effects on synapse formation of the Bcl-2 family member, Bcl-xL, and we suggest that dynamin-related protein 1 (Drp1) is a downstream effector of Bcl-xL.

Anti- and proapoptotic Bcl-2 family proteins regulate the permeability of outer mitochondrial membranes during apoptosis, a key control point for amplification of the death pathway (6–8). The antiapoptotic members (such as Bcl-xL, Bcl-2, and Mcl-1) are widely thought to protect against cell death by directly or indirectly counteracting the proapoptotic family members, such as Bax and Bak (9). In addition, a number of studies suggest that Bcl-2 family proteins regulate activities of cells that are not undergoing apoptosis and in particular, may control the strength of synaptic transmission in neurons (10). The amount of Bcl-xL protein in the developing brain increases at approximately the same time that the size of presynaptic vesicle clusters increases (11–13). Synaptic boutons often contain mitochondria to which endogenous Bcl-xL is localized (14). Injection of recombinant Bcl-xL protein into presynaptic terminals of the squid giant synapse potentiates synaptic transmission within minutes and speeds recovery of neurotransmission from tetanus-induced depression (15). Although the mechanisms are unknown, the effects of Bcl-xL on synaptic potentiation at the squid synapse were suggested to result from increased release of ATP from mitochondria, consistent with the observation that injection of ATP into presynaptic terminals mimics the effects of recombinant Bcl-xL protein (15, 16). Increased ATP could facilitate recycling of synaptic vesicles (17) and enhance development of a large “reserve pool” of vesicles to maintain transmission during prolonged or high-frequency firing.

One important aspect of synaptic development is localization of mitochondria near synapses (17, 18). This process occurs in part through activity of the mitochondrial fission protein Drp1, which facilitates mitochondrial division to generate new mitochondria that are inserted into developing synaptic sites (17, 19). In the dendrites of mammalian neurons, inhibition of Drp1 by transfection of a dominant-negative mutant, dnDrp1-K38A, inhibits mitochondrial localization to dendritic spines (19). Drp1 is a large GTPase that is required for normal division of mitochondria in growth and development (20, 21), but it also has a role in promoting programmed cell death in mammals, flies, worms, and yeast (22–25). Drp1 colocalizes with Bax on mitochondria and was reported to interact with Bcl-2 family proteins.

Using electrophysiological measurements and light and electron microscopy, we demonstrate here that Bcl-xL overexpression in cultured hippocampal neurons increases the numbers of mitochondria, synaptic vesicle clusters, and synapses. Furthermore, Bcl-xL increases colocalization of mitochondria and vesicle clusters at synapses. Conversely, depletion/inhibition of endogenous Bcl-xL, using shRNA or treatment with a small molecular inhibitor of Bcl-xL, ABT-737, reduces the number of axonal vesicle clusters and mitochondria. Bcl-xL may act through the mitochondrial fission protein Drp1 to increase synapse formation. Biochemical studies suggest that formation of a Bcl-xL–Drp1 complex increases Drp1 GTPase activity, which is likely to


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play a role in mitochondrial localization to sites of maturing synapses.

**Results**

**Overexpression of Bcl-xL Enhances Synaptic Activity of Rat Hippocampal Neurons in Culture.** To extend our earlier findings in squid, we determined whether Bcl-xL influences synaptic activity in mammalian cells. Low-density cultures of rat hippocampal neurons were transfected at 5 days in vitro (DIV5) with plasmids expressing Bcl-xL tagged at the N terminus with green fluorescent protein (GFP-Bcl-xL) or the Mito-GFP control, in which the mitochondrial targeting sequence of COX4 is fused to GFP. At 12 days posttransfection (DIV17), neurons with green somata were voltage-clamped at their resting potential in the presence of 1 μM tetrodotoxin to block action potentials (26), and miniature postsynaptic currents were recorded (Fig. 1A). Transfection efficacy was low (1–5%), so it is likely that most axons synapsing on the recorded (transfected) cells came from non-transfected cells and that actions on the presynaptic axons were retrograde from the transfected cells. In cells expressing GFP-Bcl-xL, the amplitude of both excitatory and inhibitory spontaneous events was increased compared with Mito-GFP-transfected controls, suggesting that Bcl-xL increases the number of postsynaptic receptors per synapse or possibly the amount of transmitter in presynaptic vesicles (Fig. 1B). Postsynaptic Bcl-xL also decreased the interval between both miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs, respectively; Fig. 1C), indicating that Bcl-xL produces an increase in the frequency of vesicle fusion events, ascribable to an increase in synapse number (see below; Fig. 2H and I).

**Bcl-xL Overexpression Induces Synapse Formation in Cultured Neurons.** To pursue the role of Bcl-xL in regulating synaptic activity, we quantified several synaptic parameters. At 7–8 days posttransfection, the fluorescence patterns of cotransfected GFP-Bcl-xL and Mito-RFP were punctate and nearly completely overlapping in axons (Fig. 2A) and in dendrites (supporting information [SI] Fig. 7), confirming mitochondrial localization of Bcl-xL. During embryonic development and during maturation in culture, presynaptic vesicle clusters can first be mobile precursors or “orphans” that are not associated with postsynaptic densities (PSDs) in an opposing cell before formation of mature excitatory synapses (4, 27). To examine the effects of presynaptic Bcl-xL on formation of either precursor vesicle clusters or synapses, parallel cultures of hippocampal neurons were transfected at DIV5 with GFP-Bcl-xL, or Mito-GFP and immunostained (DIV13) for presynaptic proteins. In three in-
dependent experiments, axons expressing GFP-Bcl-xL had significantly increased immunofluorescence staining for synaptophysin, a component of synaptic vesicle membranes (12) (Fig. 2 B and C) and for the scaffolding protein, bassoon, also found at both precursor vesicle clusters and presynaptic active zones (4) (Fig. 2 D and E). Untransfected axons synapsing on untransfected dendrites did not show the increases in synaptophysin and bassoon seen in nearby axons expressing GFP-Bcl-xL (not shown). Fluorescence was quantified as the number of puncta per unit length of axon and as mean fluorescence intensity (see Materials and Methods and ref. 27). The effects of Bcl-xL on synaptophysin staining were already apparent by 2 days posttransfection (DIV7) but were more obvious at 14 and 21 days in culture after synapses had matured (SI Fig. 8). By this parameter, the controls appeared to reach a lower steady-state level and did not catch up with Bcl-xL-overexpressing cells during the life span of the cultures. These data indicate that presynaptic Bcl-xL overexpression increases number and size of vesicle clusters and of bassoon immunoreactive puncta.

To determine whether presynaptic Bcl-xL overexpression in the axon induces postsynaptic maturation in the dendrites of adjacent untransfected (GFP-negative) cells, cultures were stained for PSD-95, a major protein of the postsynaptic density at excitatory synapses (4). There was a pronounced increase in the number and fluorescence intensity of PSD-95-stained puncta along the axon of GFP-Bcl-xL-transfected cells (Fig. 2 F and G), indicating the presence of mature PSDs in the dendrites of adjacent untransfected cells. In contrast, appositions of untransfected axons to untransfected dendrites showed fewer synaptophysin and PSD-95 puncta than appositions of Bcl-xL-expressing axons to untransfected dendrites (not shown). Expression of GFP-Bcl-xL in dendrites also increased synapse number and size at contacts with untransfected axons (based on synaptophysin staining) (Fig. 2 H and I). This difference could account for the ∼2-fold greater frequency of mPSPs illustrated in Fig. 1. We conclude that overexpression of Bcl-xL in either axon or dendrite significantly increases synapse formation in cultured hippocampal neurons.

Endogenous Bcl-xL Increases the Number and Size of Presynaptic Vesicle Clusters. To verify that endogenous Bcl-xL promotes synapse formation, as does overexpressed GFP-Bcl-xL, hippocampal cultures (DIV5) were treated with 1 μM ABT-737, a small-molecule inhibitor designed to inhibit antiapoptotic activity by occupying the BH3-binding groove of Bcl-xL (28, 29). At DIV14, these cultures showed reduced synaptophysin staining intensity and number of puncta in the axons, indicating that endogenous Bcl-xL is required for normal development of vesicle clusters (Fig. 3 A). Furthermore, ABT-737 did not enhance cell death measured by propidium iodide staining, even at 5 μM, which greatly exceeds the Ki of ≤1 nM (SI Fig. 9) (28).

Although Bcl-xL is reportedly the most abundant Bcl-2 family member expressed in mature neurons and in postnatal brain (11), ABT-737 also binds to the antiapoptotic proteins Bcl-2 and Bcl-w (but not the antiapoptotic protein Mcl-1) (29). To verify the specific role of endogenous Bcl-xL in synapse formation, Bcl-xL protein was depleted by using a short hairpin RNA (shRNA) that effectively depressed endogenous Bcl-xL in mouse IMCD cells (SI Fig. 10) (30). The axons of hippocampal neurons transfected with a bcl-x shRNA plasmid expressing LacZ to mark transfected cells (1 μg), but not the control LacZ plasmid (Fig. 3B), had significantly decreased staining intensity and number of synaptophysin puncta in 14-day cultures (Fig. 3B). Dendrites in which Bcl-xL had been knocked down also showed a marked decrease in synaptophysin puncta, ascribable to a decrease in synapse formation on those dendrites (SI Fig. 11). Using Hoechst stain to mark nuclei and propidium iodide to mark dead cells we confirmed that Bcl-xL knockdown did not compromise cell viability (90.9% for shRNA-Bcl-xL and 92.2% for control, n = 110 cells per group). The ABT-737 and RNAi results strongly suggest that endogenous Bcl-xL participates in synapse formation during maturation of hippocampal neurons in culture.

Bcl-xL Induces Localization of Mitochondria to Presynaptic Sites. Morphological studies have demonstrated that approximately half of all presynaptic terminals contain mitochondria (31, 32). However, mitochondria are mobile and can move along dendrites and into dendritic spines in an activity-dependent manner (19). The mechanisms of mitochondrial recruitment to synapses are currently not understood. Given its mitochondrial localization to synaptic boutons and increases vesicle formation (Fig. 2 F and G), suggest that Bcl-xL induces the targeting of axonal mitochondria to synapses.

To test whether Bcl-xL increases localization of mitochondria to presynaptic sites, lentiviruses encoding GFP-Bcl-xL or GFP alone were used to transduce the neurons (DIV5) with ~100% efficacy, and these cultures fixed at DIV13 were examined by electron microscopy. The percentage of sections through synapses that contained a presynaptic mitochondrion increased from <20% to ~40% with GFP-Bcl-xL (Fig. 4 B and C). In addition, there was a 2-fold increase in the number of vesicles present in the synaptic profiles of GFP-Bcl-xL-containing axons (Fig. 4 D), and both GFP-Bcl-xL and control synapses had a greater number of vesicles if a mitochondrial was present at the synapse (Fig. 4 E). We conclude that Bcl-xL stimulates mitochondrial localization to synaptic boutons and increases vesicle

![Fig. 3.](https://example.com/fig3.png)
Bcl-xL-induced Changes at Synapses Are Facilitated by Drp1. Drp1 is a large GTPase that is required for mitochondrial division (20) and for targeting of mitochondria to newly developing synaptic mitochondrial sites (17, 19, 20). Therefore, we explored the possibility that the effects of Bcl-xL on axonal mitochondria and mitochondrial sites (17, 19, 20). Therefore, we explored the possibility that Bcl-xL could affect the GTPase activity of Drp1, which acts as a positive regulator of Drp1. However, there may be some Drp1 activity in the absence of Bcl-xL, as seen in Fig. 6C. This could be reversed by overexpression of Drp1 (Figs. 4A and 5C). Overexpression of Drp1 did reverse the ABT-737-induced reduction in synaptophysin labeling and increased the number of mitochondria above control, consistent with a downstream mode of action of Drp1 (Fig. 5C).

Bcl-xL Binds Drp1 and Modulates Its GTPase Activity. To determine whether Bcl-xL can be a direct regulator of Drp1, we performed coimmunoprecipitations on lysates of hippocampal neurons transduced with lentiviruses encoding GFP-Bcl-xL or GFP alone. Immunoprecipitation of GFP-Bcl-xL with anti-GFP antibody coprecipitated endogenous Drp1 (Fig. 6A, Top). Drp1 was not coprecipitated from GFP-only samples. In the reciprocal experiment, immunoprecipitation of endogenous Drp1 coprecipitated GFP-Bcl-xL but not GFP alone (Fig. 6A, Middle, and data not shown), despite similar levels of Drp1 in both lysates (Fig. 6A, Bottom). This interaction was verified for endogenous Bcl-xL in adult rat whole brain. Anti-Drp1 antibody coprecipitated endogenous Bcl-xL protein from brain lysate, and conversely, anti-Bcl-xL antibody coprecipitated endogenous brain Drp1 (Fig. 6B).

Drp1 associates with the outer mitochondrial membrane to induce GTP-dependent mitochondrial fission. To investigate the possibility that Bcl-xL could alter the GTPase activity of Drp1, an in vitro GTPase assay (see Materials and Methods) was performed with purified recombinant full-length Bcl-xL and full-length human Drp1 (amino acids 1–699 plus a His tag for number and that the presence of mitochondria increases the size of the vesicle clusters, consistent with the increase in synapse formation and the electrophysiological data (Fig. 1).

Fig. 4. Bcl-xL increases the number of mitochondria per unit length of axon and their colocalization to presynaptic sites. (A) Fluorescence microscopy of transfected axons in hippocampal cultures stained with MitoTracker Red. Neurons were either transfected as indicated or treated with ABT-737 (DIV5 for both). White dots beside the axon images show the axon trajectories. The number of MitoTracker-stained mitochondria per 6 μm of axon (mean ± SEM) was quantified for both control and ABT-737 (n = 6 neurons per group) and for GFP-Bcl-xL (n = 5 in each of three independent experiments for a total of 15 neurons). *, P < 0.05; ***, P < 0.001. (B) Representative electron micrographs of synaptic profiles identified by a synaptic density between neurons (DIV13) expressing GFP only or GFP-Bcl-xL. Data are quantified in C–E. V, vesicles; SD, synaptic density; M, mitochondrion. (Scale bars: 500 nm.) (C) Percentage of synaptic profiles containing a mitochondrion. (D) Number of vesicles per synaptic profile. (E) The number of vesicles per synaptic profile was greater if a mitochondrion was present and if the neuron expressed GFP-Bcl-xL. *, P < 0.05; ***, P < 0.001.

mitochondria were longer in dnDrp1-K38A transfected cells (SI Fig. 12).

One explanation of these data is that Bcl-xL is a positive regulator of Drp1. However, there may be some Drp1 activity in the absence of Bcl-xL (see Fig. 6C). If so, the synaptic effects caused by ABT-737 inhibition of Bcl-xL may be reversed by overexpression of Drp1 (Figs. 4A and 5C). Overexpression of Drp1 did reverse the ABT-737-induced reduction in synaptophysin labeling and increased the number of mitochondria above control, consistent with a downstream mode of action of Drp1 (Fig. 5C).

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coprecipitated with GFP-Bcl-xL, brought down by a GFP antibody. (used for precipitation (IP) and for immunoblots are indicated. Drp1 was transduced with viral vectors (DIV5) as indicated (DIV13) in hippocampal neurons from reciprocal coimmunoprecipitation experiments on hippocampal neurons containing Bcl-xL and Drp1 were identified, and Bcl-xL increases the GTPase activity of Drp1 in vitro. Therefore, Bcl-xL may increase the number of mitochondria in axons and increase their localization to presynaptic domains by activating Drp1. These activities may be rate-limiting in synapse maturation. Bcl-xL overexpression in presynaptic axons also affects the postsynaptic neuron as indicated by PSD-95 induction in adjacent dendrites. Bcl-xL overexpression in dendrites also increases the size and number of syntophysin puncta contacting the dendrite and increases the amplitude of mEPSCs and mIPSCs, consistent with the possibility that Bcl-xL increases responses to transmitter release through effects on postsynaptic receptor insertion. The frequency of mPSCs is also increased consistent with the increase in number of synapses. The augmentation of synaptic transmission and the localization of mitochondria in dendrites suggest that we observe with Bcl-xL overexpression could be caused by enhanced synaptic development or by mechanisms involved in other forms of synaptic plasticity.

Prodeath Factors Have Neuronal Functions Distinct from Cell Death. Despite the contribution of Drp1 to cell death (22), we found that transfection with Drp1 promotes formation of synaptic vesicle clusters and presumably synapses without reducing cell viability. Although a decrease in mitochondrial fission produced by down-regulation of Drp1 can protect cells from death (34), up-regulation of Drp1 can also inhibit apoptosis (33), and in dendrites it induces increased number of dendritic spines (19). Down-regulation of Drp1 also alters mitochondrial targeting to the synapse and compromises synaptic function during high rates of transmission (17). Our data suggest that Drp1 may contribute to formation of vesicle pools in the developing synapse. Several studies have also suggested that both pro- and antideath Bcl-2 family proteins have physiological functions other than regulation of programmed cell death (15, 35–38).

Mitochondrial Targeting May Regulate Synapse Formation and Stabilization. Mitochondria have recently been shown to regulate short-term potentiation of neurotransmitter release after a period of frequent synaptic activity (10) and to influence vesicle replenishment after intense activity (17). Mitochondria arrive at sites distant from the cell body by transport along microtubules (39) and respond to local axonal activation of growth factor receptors that stimulate mitochondrial movement to the site of growth factor stimulation (40, 41). In a growing neurite, mitochondrial localization may occur during the process of synapse formation, where mitochondria would then be in a position to provide energy for future and ongoing synaptic activity. Alterations in mitochondrial biogenesis, fission, and subsequent distribution may play critical roles in metabolic support for synaptic functions (42, 43), and a Bcl-xL–Drp1 complex may distribute new mitochondria to developing synaptic sites.

Bcl-xL May Regulate Availability of ATP for Synaptic Transmission. Synaptic activity increases the rate of both glycolysis and respiration (44), presumably to provide energy for multiple ATP-dependent steps in transmitter synthesis, vesicle preparation, and recycling (45–47). Bcl-xL may increase the availability of ATP by increasing the permeability of the mitochondrial outer membrane or other mechanism (15, 16). We presume that mitochondria preferentially localize to sites of increased activity, in part through action of a Bcl-xL–Drp1 complex, and that ATP production is required for formation of new synapses and maintenance of activity. Furthermore, mitochondrial length and number per unit length along a neurite may be only an approximate measure of mitochondrial function, and long-lasting changes in synaptic transmission and synapse formation depend on changes in mitochondrial bioenergetics and protein interactions mediated by Bcl-xL.

Discussion

We have shown that endogenous and overexpressed Bcl-xL induce synapse formation and increase the number of axonal mitochondria, the size and number of synaptic vesicle clusters, and recruitment of mitochondria to synapses in axons of hippocampal neurons matured in culture. In addition, dnDrp1–K38A, a dominant-negative inhibitor of Drp1, greatly reduces the effects of Bcl-xL on synaptic vesicle clusters and mitochondrial number, suggesting that Bcl-xL-induced synapse formation is mediated at least in part by Drp1. Endogenous complexes containing Bcl-xL and Drp1 were identified, and Bcl-xL increases the GTPase activity of Drp1 in vitro. Therefore, Bcl-xL may introduce the number of mitochondria in axons and increase their localization to presynaptic domains by activating Drp1. These activities may be rate-limiting in synapse maturation. Bcl-xL overexpression in presynaptic axons also affects the postsynaptic neuron as indicated by PSD-95 induction in adjacent dendrites. Bcl-xL overexpression in dendrites also increases the size and number of syntophysin puncta contacting the dendrite.

Fig. 6. Bcl-xL and Drp1 interact. (A) (Top and Middle) Immunoblots of reciprocal communoprecipitation experiments on hippocampal neurons (DIV13) transduced with viral vectors (DIV5) as indicated (n = 2). Antibodies used for precipitation (IP) and for immunoblots are indicated. (Top) Drp1 was coprecipitated with GFP-Bcl-xL, brought down by a GFP antibody. (Bottom) Blot of endogenous Drp1 in the starting lysate for immunoprecipitations above. (B) Immunoblots for endogenous Bcl-xL and endogenous Drp1 from rat adult brain after immunoprecipitation with the antibodies indicated. Each panel shows the starting lysate, control IgG precipitate, and the indicated precipitates probed with Drp1 and Bcl-xL antibodies. (C) Relative amounts of phosphate produced by GTPase activity in 1 h with purified recombinant proteins. Shown are results of three independent experiments (mean ± SEM). BSA had no GTPase activity above background, and this signal was subtracted from the other values. *** P < 0.001. (D) Model of Bcl-xL-induced synaptic function. Black arrows indicate steps addressed in this work.

Materials and Methods

Details beyond the descriptions here and in Results are given in SI Materials and Methods. Low-density cultures of rat hippocampal neurons were prepared as described in refs. 27 and 48 and contained few glial cells. Spontaneous postsynaptic currents at the resting membrane potential (~70 mV) were recorded by whole-cell patch-clamp at 22–25°C. Recording electrodes contained 120 mM potassium gluconate, 8 mM NaCl, 0.5 mM EGTA,
10 mM Hepes, 2 mM MgATP (pH 7.3) with KOH. The external solution [1 μM tetrodotoxin, 150 mM NaCl, 5 mM KCl, 2.5 mM CaCl$_2$, 5.5 mM Hepes-acid, 4.5 mM Hepes-Na, and 10 mM glucose (pH 7.3) with NaOH, 285–300 mosmol$^{-1}$] was perfused continuously over the cells. With these solutions, both mEPSCs and mIPSPs were inward at the resting potential. They were distinguished by the slower decay time of mIPSPs and were sorted on a computer-based template by using pClamp 9.0 (Molecular Devices) according to ref. 26. Axons were identified by using phase contrast (SI Fig. 13). Axons were longer than dendrites and branching occurs in more diameter. Image analysis was performed by using mPlab software (Scanalytics) as described in ref. 27. Background fluorescence of each image was subtracted. Fluorescent puncta with an area up to 0.29 μm$^2$ (10 pixels) were outlined and measured. For every punctum, the software determined a region of interest comprising a maximum of 10 pixels (0.29 μm$^2$) of the highest fluorescence intensity. The summed fluorescence correlates with area (up to 0.29 μm$^2$) and depth of the labeled structure (27). Puncta with <5 pixels were excluded. Mitotracker Red (25 nM; Molecular Probes) or transfection with plasmids encoding a mitochondrionally targeted fluorescent peptide (Mito-RFP) were used to image mitochondria. Analysis of synaptophysin immunofluorescence in axons apposed to transfected dendrites was performed in Adobe Photoshop. For immunofluorescence intensity, in Fig. 2 H and I, a small box of predetermined pixel number was drawn over each punctum, and the average fluorescence within the box was measured.

See SI Materials and Methods for antibodies used in immunolabeling and Western blotting. Western blotting and immunoprecipitation were carried out as described in ref. 49.

For transmission electron microscopy, virally transduced cells in culture were fixed in 2.5% glutaraldehyde, stained with 2% uranyl acetate (SPI Supplies), dehydrated in ethanol, and embedded in Embed 812 epoxy resin (EMS). Ultrathin (60-nm) sections were stained with 2% uranyl acetate and 1% lead citrate and examined in a Tecnai 12 Biotwin electron microscope (FEI Company).

Drp1 GTPase activity was assayed in vitro with recombiant Bcl-xL and Drp1 by Cytosphosphate assay (Cytoskeleton).

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Fig. 7.

Mito-RFP

Bcl-\(x_L\)-GFP

Merge

20 \(\mu\)m
Fig. 10
Fig. 11

- **Actin**
- **BCL-xL**

Bar graph showing relative density with *** indicating statistical significance.
Fig. 12

LacZ vector control

Bcl-x

shRNA

20 μm
Figure 13

Fig. 13
Fig. 8

Axon

Dendrite

20 µm