Supporting Information

Kim et al. 10.1073/pnas.0912877107

SI Materials and Methods

Electron Tomography. For electron tomography, sections were cut at a nominal thickness of 0.5 microns and collected on 100:100 clamshell grids. These sections were poststained for 20–30 min in a 2% uranyl acetate solution followed by 15 min in a Sato lead solution. Two sizes of colloidal gold particles, 15 and 20 nm diameter, were deposited on opposite sides of the section to serve as fiducial cues. For stability in the beam, the section was coated with carbon. For each reconstruction, either a single series or double series of images at regular tilt increments was collected with a JEOL 4000EX intermediate high-voltage electron microscope operated at 400 kV. The specimens were irradiated before initiating a tilt series to limit anisotropic specimen thinning during image collection. The illumination was held to near parallel beam conditions and optical density maintained constant by varying the exposure time. Tilt series were recorded using either a slow-scan CCD camera or film at 20,000 magnification. Angular increments of 2° from −60° to +60° about an axis perpendicular to the optical axis of the microscope were achieved using a computer-controlled goniometer to increment accurately the angular steps. The pixel dimensions of the CCD camera were 1,960 × 2,560 and the pixel resolution was 1.1 nm. For film tilt series, negatives were digitized using a Nikon CoolScan with pixel resolution 0.7 nm. For the initial several tilt series, the IMOD software package (1) was used for the complete image processing. After TxBR software became available, IMOD was used for the rough alignment and the fine alignment and reconstruction was performed using the TxBR package (2). Volume segmentation was performed by manual tracing in the planes of highest resolution (X-Y) with the program Xvoxtrace (3). The nuclear reconstructions were visualized using Analyze (Mayo Foundation) and the surface-rendering graphics of Synu (National Center for Microscopy and Imaging Research) as described by Perkins and coworkers (3). These programs allow one to step through slices of the reconstruction in any orientation and to model and display features of interest in three dimensions. Overlays of models with tomographic slices were made with Amira (Visage Imaging).

Cell Culture. MEFs were prepared after removing the head and liver from E13 or E16 embryos. E16 bodies were grossly chopped into smaller pieces and then trypsized by incubating with 0.25% trypsin at 37 °C for 20 min. Tissue was disrupted by 10 passages through a 18-gauge needle, and dissociated cells were plated in DMEM media (Invitrogen) containing 15% FBS (HyClone) and 1% penicillin/streptomycin (Invitrogen). Mouse cortical neurons were prepared from cortex of embryos on E16. Cortex was dissected out and chopped into smaller chunks and incubated in 0.25% trypsin for 25 min at 37 °C. Cells were dissociated by passaging through fire-polished glass pipettes of decreasing diameter, then centrifuged at 200 g for 10 min. Supernatant was removed and cell pellets were resuspended in Neurobasal media (Invitrogen) with B24, glutamine, and penicillin/streptomycin. Cells were plated on PDL/laminin coated coverslips (BD Science) at 100,000 cells/coverslip density. Glial cells were prepared from E18 frontal cortex that was minced, incubated in 0.25% trypsin for 15 min and dissociated by passaging through fire-polished glass pipettes of decreasing diameter. Cells were plated and grown in F12/DMEM media (Invitrogen) with 10% FBS (HyClone) and 1% penicillin/streptomycin (Invitrogen).

Primer Sequences for Real-Time PCR. Primer pair sequences for each gene are: GAPDH 5′-cactgaggaccaggttgtct-3′ (forward), 5′-aattgtgagggagatgctca-3′(reverse); Tor2a 5′-caatgctggtggtgagcagatcaa-3′ (forward), 5′-aactgagccagctcattgagtacg-3′; and Tor3a 5′-aacctcggaggcagtgtcatcaat-3′ (forward), 5′-aatggatttgcagccctgagagga-3′ (reverse).

shRNA Constructs. Mouse *Tor1b* (TRCN0000106487) and *Tor2a* (TRCN0000106460) knockdown shRNA constructs were purchased from Open Biosystems (TRC-6 library). Mouse Tor3a (TRCN0000115441) and mouse *Tor1aip2* (*Lull1*) (TRCN0000196207, TRCN0000179322, TRCN0000180743, and TRCN0000196099) knockdown shRNA and nonsilencing, scrambled control shRNA vectors were purchased from Sigma.

Lentivirus Generation and Transduction. Endotoxin-free shRNA DNA constructs were used to cotransfect 293T cells along with a packaging cassette and an envelope expression cassette by transient transfection using calcium phosphate. Crude viral particles were collected by harvesting the media 60–72 h post-transfection and then centrifuged and filtered through a 0.45-μm filter. Viral particles were titrated using $p24^{gag}$ ELISA. Cultured cortical neurons were transduced at multiplicity of infection (MOI) 5 on DIV 2 and incubated for 4 h. At the end of incubation, the conditioned neurobasal media with B27 and glutamine was added back. Transduction was repeated one more time after 24 h. After a total of 5 days of transduction, cells were collected and analyzed. For mouse embryonic fibroblasts, virus was introduced to trypsinized cells in suspension at MOI 20. After 24 h, transduced cells were selected by puromycin at 5 μg/mL for 4 days. After a total of 5 days of transduction, cells were harvested and analyzed.

1. Kremer JR, Mastronarde DN, McIntosh JR (1996) Computer visualization of three-dimensional image data using IMOD. *J Struct Biol* 116:71–76.
2. Lawrence A, Bouwer JC, Perkins G, Ellisman MH (2006) Transform-based backprojection for volume reconstruction of large format electron microscope tilt series. *J Struct Biol* 154:144–167.
3. Perkins GA, et al. (1997) Electron tomography of large, multicomponent biological structures. *J Struct Biol* 120:219–227.

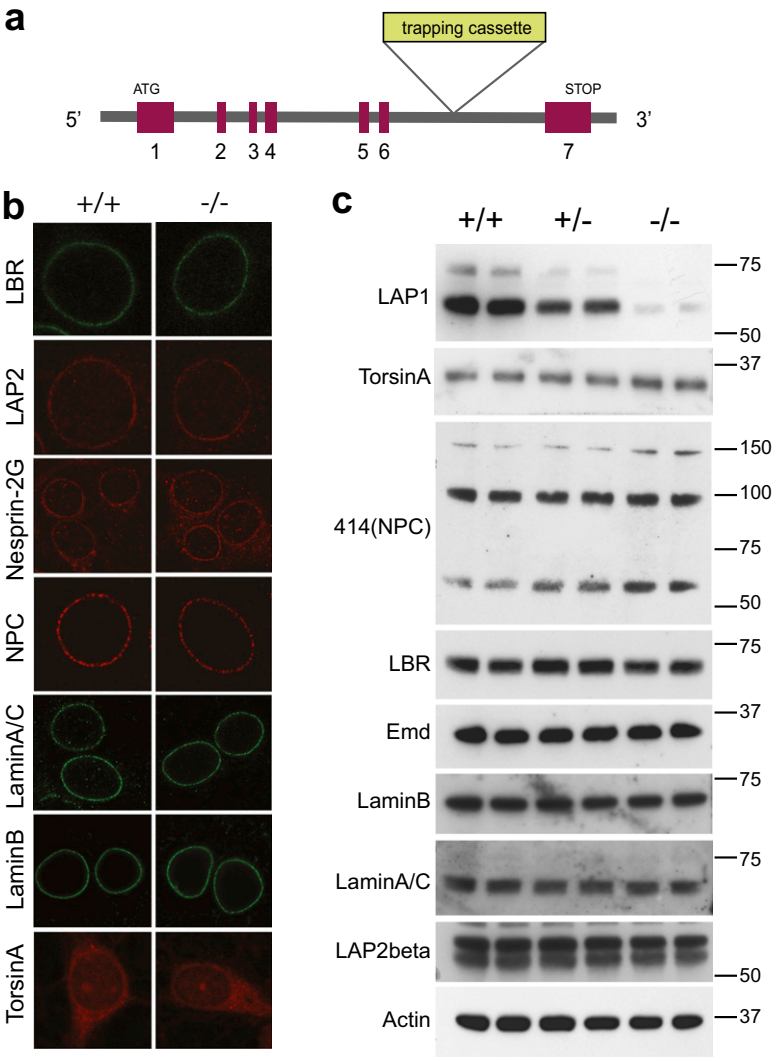

Fig. S1. Nuclear envelope proteome of LAP1 null neurons is grossly normal. (*A*) The gene targeting scheme by gene trap. The trapping cassette is inserted between exon 6 and exon 7 of *Tor1aip1* gene locus. (*B*) The localization of various nuclear envelope proteins and torsinA is unaltered in the absence of LAP1. Cultured cortical neurons from wild-type and *Tor1aip1*$^{-/-}$ embryos were stained with antibodies against several nuclear envelope proteins and torsinA. (*C*) The expression levels of nuclear envelope proteins and torsinA are unaltered in *Tor1aip1*$^{-/-}$ embryonic brain. Protein lysates from embryonic brain probed with various nuclear envelope protein and torsinA antibodies.

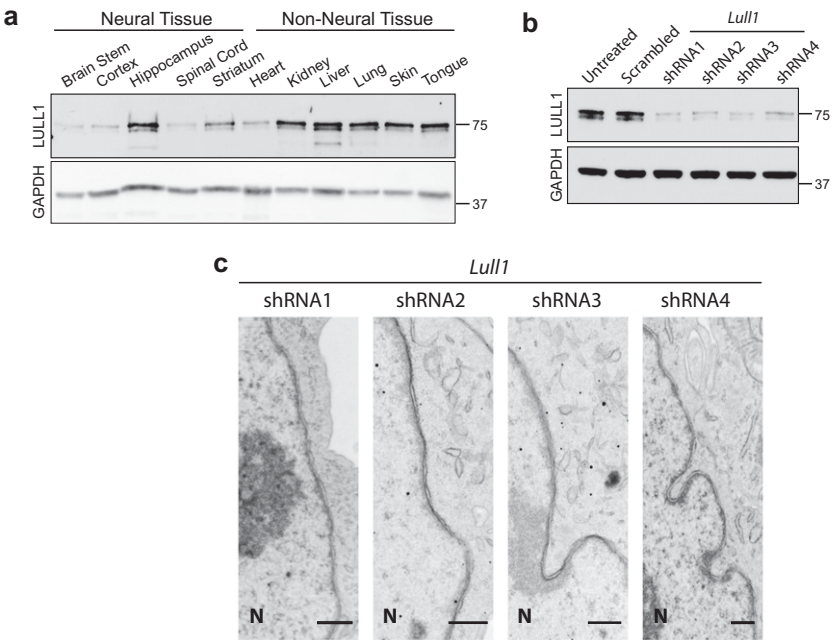

Fig. S2. Down-regulation of LULL1 does not affect the nuclear membrane morphology. (*A*) LULL1 is broadly expressed in neural and nonneural murine embryonic tissue. Protein lysates from E16 wild-type tissues are probed with anti-LULL1 (*Upper*) and anti-GAPDH (*Lower*) antibodies. (*B*) LULL1 is substantially knocked down by four different *Lull1* shRNA constructs in wild-type MEFs. Wild-type MEF cells were electroporated with each construct including the scrambled control shRNA and then selected with puromycin for 4 days. Harvested cell lysates are probed with anti-LULL1 (*Upper*) and anti- GAPDH (*Lower*) antibodies. (*C*) Down-regulation of LULL1 does not induce blebs in MEF cells. The nuclear membrane morphology was assessed by EM. The nucleus (N) is marked. (Scale bar, 0.5 μm.)

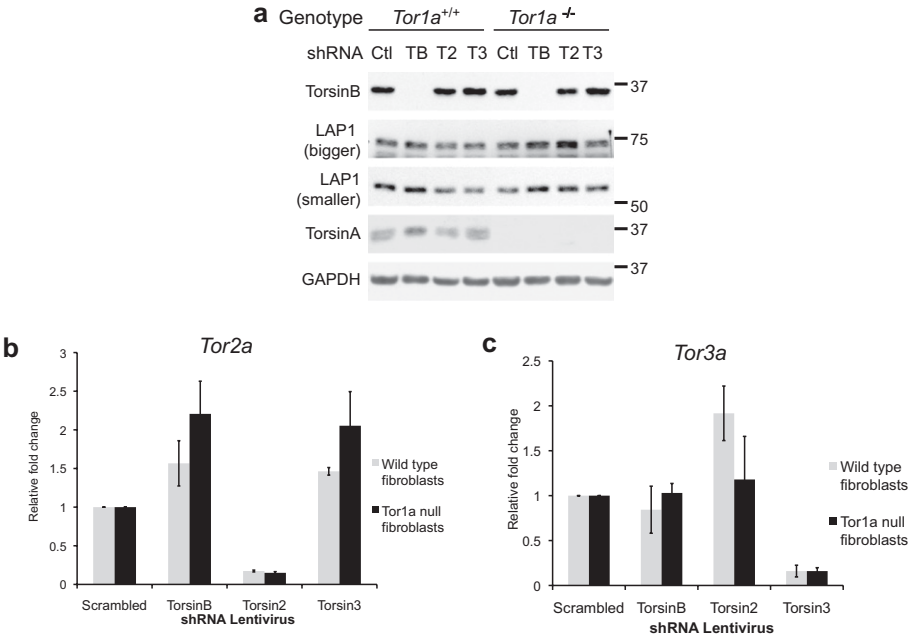

Fig. S3. Lentivirus-mediated shRNA demonstrates substantial and specific down-regulation of each torsin. (*A*) TorsinB is efficiently knocked down by torsinB shRNA. Note that the levels of LAP1 (bigger isoform, second panel; smaller isoform, third panel) and torsinA (fourth panel) are not altered by any of torsin shRNA virus. TorsinB levels are not affected by either torsin2 or torsin3 shRNA. Ctl, scrambled; TB, torsinB; T2, torsin2; T3, torsin3. (*B*) *Tor2a* is selectively and efficiently knocked down by torsin2 shRNA. Quantitative RT-PCR was performed to decide the transcript levels of *Tor2a* using cDNA derived from lentivirus-transduced wild-type and torsinA null MEF cells. Relative fold changes were obtained by comparing the normalized Ct value of each sample to the one transduced with scrambled shRNA virus within the group of same MEF genotype. Note that the mRNA levels of *Tor2a* are not significantly affected by other shRNA. Error bars = SEM of fold change from three independent experiments. (*C*) *Tor3a* is selectively and efficiently knocked down by torsin3 shRNA. Note that the mRNA levels of *Tor3a* are not significantly affected by other shRNA. Error bars = SEM of fold change from three independent experiments. Method was performed as described in *B*.

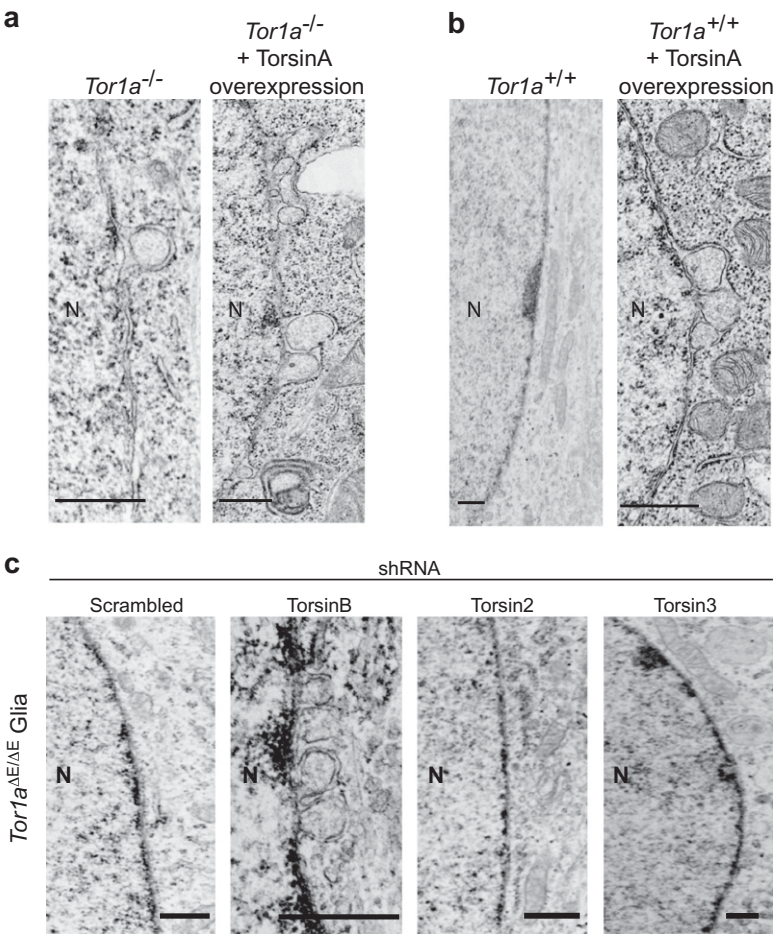

Fig. S4. (*A*) TorsinA overexpression induces blebs in *Tor1a*$^{-/-}$ neurons. The nucleus (N) is marked. (Scale bar, 0.5 μm.) Similar results were found for torsinB overexpression, as described in the text. (*B*) TorsinA overexpression exacerbates blebs in wild-type neurons. The nucleus (N) is marked. (Scale bar, 0.5 μm.) (*C*) TorsinB knockdown in *Tor1a*$^{\Delta E/\Delta E}$ glial cells selectively induces blebs. The nucleus (N) is marked. (Scale bar, 0.5 μm.)