An absence of lamin B1 in migrating neurons causes nuclear membrane ruptures and cell death

Natalie Y. Chen¹, Ye Yang², Thomas A. Weston³, Jason N. Belling⁴, Patrick Heizer⁵, Yiping Tu⁶, Paul Kim⁷, Lovelyn Edillo⁸, Steven J. Jonas⁹,¹,², Paul S. Weiss⁵,⁶,⁷, Loren G. Fong⁵,¹,², and Stephen G. Young⁴,⁵,¹,²

¹Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90095; ²California NanoSystems Institute, David Geffen School of Medicine, University of California, Los Angeles, CA 90095; ³Department of Chemistry and Biochemistry, David Geffen School of Medicine, University of California, Los Angeles, CA 90095; ⁴Department of Pediatrics, David Geffen School of Medicine, University of California, Los Angeles, CA 90095; ⁵Children’s Discovery and Innovation Institute, University of California, Los Angeles, CA 90095; ⁶El and Eddythe Center Broad of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, CA 90095. ⁷Department of Bioengineering, University of California, Los Angeles, CA 90095; ⁸Department of Materials Science and Engineering, University of California, Los Angeles, CA 90095; ⁹Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA 90095; and ¹ Molecular Biology Institute, David Geffen School of Medicine, University of California, Los Angeles, CA 90095

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Deficiencies in either lamin B1 or lamin B2 cause both defective migration of cortical neurons in the developing brain and reduced neuronal survival. The neuronal migration abnormality is explained by a weakened nuclear lamina that interferes with nucleokinesis, a nuclear translocation process required for neuronal migration. In contrast, the explanation for impaired neuronal survival is poorly understood. We hypothesized that the forces imparted on the nucleus during neuronal migration result in nuclear membrane (NM) ruptures, causing interspersion of nuclear and cytoplasmic contents—and ultimately cell death. To test this hypothesis, we bred Lmnb1-deficient mice that express a nuclear-localized fluorescent Cre reporter. Migrating neurons within the cortical plate of E18.5 Lmnb1-deficient embryos exhibited NM ruptures, evident by the escape of the nuclear-localized reporter into the cytoplasm and NM discontinuities by electron microscopy. The NM ruptures were accompanied by DNA damage and cell death. The NM ruptures were not observed in nonmigrating cells within the ventricular zone. NM ruptures, DNA damage, and cell death were also observed in cultured Lmnb1−/− and Lmnb2−/− neurons as they migrated away from neurospheres. To test whether mechanical forces on the cell nucleus are relevant to NM ruptures in migrating neurons, we examined cultured Lmnb1−/− neurons when exposed to external constrictive forces (migration into a field of tightly spaced silicon pillars). As the cells entered the field of pillars, there were frequent NM ruptures, accompanied by DNA damage and cell death.

Significance

Deficiencies in lamin B1 or lamin B2 in mice result in markedly reduced neuronal density in the cerebral cortex, but the mechanism has been unclear. We found that deficiencies of either lamin B1 or lamin B2 cause nuclear membrane (NM) ruptures in migrating neurons, accompanied by DNA damage and cell death. Our studies were informative because they uncovered large differences in the frequency of NM repair in lamin B1- and lamin B2-deficient neurons, implying unique functions for the 2 nuclear lamins in maintaining NM integrity. Also, our studies uncovered an important role for mechanical stresses in eliciting NM ruptures, helping to explain the increased frequency of NM ruptures in migrating neurons.


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1L.G.F. and S.G.Y. contributed equally to this work.
2To whom correspondence may be addressed. Email: lfong@mednet.ucla.edu or sgyoung@mednet.ucla.edu.

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of the nuclear membranes, resulting in nuclear membrane (NM) ruptures, intermixing of nuclear and cytoplasmic contents, DNA damage, and ultimately cell death. This scenario seemed possible, for 2 reasons. First, reducing nuclear lamin expression in tumor cells or fibroblasts renders the cells more susceptible to NM ruptures in response to external mechanical forces (23–27), and it seemed plausible that the forces imparted on the nucleus during nucleokinesis (or the constrictive forces imparted on cells as they migrate to the cortical plate) could have similar consequences, particularly when the nuclear envelope is weakened by the absence of a B-type lamin. Second, unlike peripheral cell types, migrating neurons in the developing brain do not express lamin A or lamin C (13, 28–30), and the absence of those protein likely renders neurons more susceptible to NM ruptures.

In the current study, we took advantage of both genetically modified mice and cultured cell models to examine the hypothesis that deficiencies in B-type lamin render neurons susceptible to NM ruptures and ultimately to cell death.

Results
NM Ruptures in Neurons of Lmnb1-Deficient Mice. To investigate the reduced density of cortical neurons in Lmnb1-deficient embryos, we stained the cerebral cortex of embryonic day 18.5 (E18.5) Lmnb1-deficient embryos for active caspase 3, a marker of apoptotic cell death. Substantial amounts of caspase 3 staining were observed within the cortical plate of Lmnb1-deficient embryos (Fig. S14). Also, staining with the LIVE/DEAD fluorescent vital dye revealed widespread cell death in the forebrain (but not cerebellum) of forebrain-specific Lmnb1 knockout embryos (Fig. 1B). We suspected that the cell death might be a consequence of NM ruptures and intermixing of nuclear and cytoplasmic contents. To explore that idea, we bred Lmnb1-deficient mice harboring a nuclear-localized fluorescent reporter (SI Appendix, Fig. S1). In neurons of wild-type (WT) mice, the reporter was confined to the cell nucleus (Fig. 1C and D and SI Appendix, Fig. S2, Upper). However, in Lmnb1-deficient embryos, we observed escape of the reporter into the cytoplasm of many neurons (Fig. 1C and E and SI Appendix, Fig. S2, Lower). Interestingly, no NM ruptures were observed in cells of the ventricular zone (Fig. 1C and E and SI Appendix, Fig. S3). By transmission electron microscopy (EM), we had no difficulty identifying discontinuities in the nuclear membranes of cortical plate neurons in Lmnb1-deficient embryos (Fig. 1F and G and SI Appendix, Fig. S1).

NM ruptures in cortical plate neurons of Lmnb1-deficient embryos were accompanied by an inhomogeneous distribution of lamin B2. In cortical plate neurons, lamin B2 was mislocalized to 1 pole of the nuclear rim (SI Appendix, Fig. S4), leaving a large fraction of the nuclear rim devoid of nuclear lamins. In contrast, lamin B2 distribution was uniformly distributed along the nuclear rim in cells of the ventricular zone (where NM ruptures were not observed) (SI Appendix, Fig. S44). The asymmetric distribution of lamin B2 is likely due to the forces of nucleokinesis, given that lamin B2 was largely confined to the leading edge of neurons as they migrated away from cultured neurospheres (SI Appendix, Fig. S4B) (22). In the forebrain of adult, forebrain-specific Lmnb1 knockout mice, where lamin C is expressed and lamin B2 is distributed homogeneously along the nuclear rim, NM ruptures could be detected but were infrequent (SI Appendix, Fig. S5).

NM Ruptures in Cultured Neurons Deficient in B-Type Lamins. To further explore the susceptibility of lamin B1- or lamin B2-deficient cells to NM ruptures, we prepared cultures of Lmnb1-deficient (B1KO) and Lmnb2-deficient (B2KO) neuronal progenitor cells (NPCs). As expected, undifferentiated and differentiated B1KO and B2KO neurons lacked lamin B1 and lamin B2, respectively (Fig. 2A–E). Lamin C expression levels in neurons were very low relative to levels in fibroblasts (Fig. 2A). Prelamin A expression was even lower (Fig. 2A). Prelamin A mRNA expression in neurons is virtually abolished by a neuron-specific microRNA (miR-9) (28, 29). Nuclei in B1KO neurons were slightly smaller and more circular than wild-type or B2KO neurons (Fig. 2F).

To examine the susceptibility of cultured neurons to NM ruptures, we transduced NPCs with a nuclear-localized green fluorescent cell reporter (NLS-GFP). We then quantified NM ruptures (escape of the NLS-GFP into the cytoplasm) in wild-type, B1KO, and B2KO neurons during 50 h of live-cell imaging. NM ruptures were frequent in B1KO neurons (Movie S1), occurring in >60% of neurons examined (Fig. 3B). In many neurons, NM ruptures occurred repetitively, with multiple cycles of NM rupture (escape of NLS-GFP into the cytoplasm) and NM repair (return of NLS-GFP into the nucleus) (Movies S2 and S3). Thus, the total number of NM ruptures was ∼2.7-fold greater than the number of neurons examined (Fig. 3C and SI Appendix, Fig. S6). The average duration of NM ruptures was 2.9 h (Fig. 3D). The increased susceptibility of B1KO neurons to NM ruptures was documented in 5 independent experiments (SI Appendix, Fig. S6). No ruptures were detected in wild-type neurons (Fig. 3B and C). One-third of the B1KO neurons that exhibited a NM rupture (50 of 150 neurons) died during the 50-h period of observation (SI Appendix, Fig. S7). NM ruptures were also observed in B2KO neurons (Fig. 3A), but the pattern was distinct. First, NM ruptures were infrequent in B2KO neurons, occurring in only 7.8% of neurons during 50 h of imaging (25 NM ruptures in 321 neurons examined) (Fig. 3B). Second, NM repair never occurred in B2KO neurons (Fig. 3C and Movies S4 and S5); thus, the mean duration of NM ruptures in B2KO neurons was much longer than in B1KO neurons (38.9 h) (Fig. 3D). Third, NM ruptures in B2KO cells led to cell death (with fragmentation of the nucleus and detachment of the neuron from the plate) (SI Appendix, Fig. S7). The majority of B2KO neurons that exhibited NM ruptures (75 of 91) died during the 50-h period of observation (17 of 25 neurons) (Fig. 3E). The other 8 neurons with a NM rupture remained alive, without evidence of NM repair, when the experiment was terminated after 50 h of imaging.

Cell death in cultured B1KO and B2KO neurons was evident by caspase 3 staining and staining with the LIVE/DEAD vital dye (Fig. 4A and B and SI Appendix, Fig. S8). As expected, the cell death phenotype was more severe in B1KO neurons. We also observed DNA damage in B1KO and B2KO neurons, as judged by staining for γH2AX, a marker for double-stranded DNA breaks (Fig. 4C).

Overexpression of Lamin B2 in B1KO Neurons Does Not Eliminate NM Ruptures. Lee et al. (31) showed previously that overexpression of lamin B2 in Lmnb1-deficient mouse embryos did not prevent neurodevelopmental abnormalities or the perinatal death. However, the overexpression of lamin B2 significantly increased the size of Lmnb1-deficient mouse embryos (31), implying that the surplus lamin B2 improved the viability of Lmnb1-deficient cells. To test the ability of lamin B2 to compensate for the loss of lamin B1 that we used a doxycycline (Dox)-inducible Lmnb1 (DoxOff mice) (32). By transduction of NPCs with a NM rupture reporter, we overexpressed lamin B2 in B1KO and B2KO neurons. In the presence of doxycycline, lamin B2 expression in B1KO neurons was robust (Fig. 5A), such that the lamin B2 covered the entire nuclear rim rather than only 1 pole of the nucleus (Fig. 5B). Overexpression of lamin B2 reduced the frequency of NM ruptures in B1KO cells, but many of the neurons continued to have NM ruptures (Fig. 5C and D). Indeed, some B1KO neurons had repetitive NM ruptures (Fig. 5C and D). In contrast, overexpression of lamin B2 in B2KO cells eliminated NM ruptures (Fig. 5C and D). Lamin B2 overexpression also abolished cell death in B2KO cells (reduced the frequency of cell death to that observed in wild-type neurons) (Fig. 5E and F and SI Appendix, Fig. S9). Overexpression of lamin B2 in B1KO neurons...
reduced but did not eliminate cell death (Fig. 5 E and F and SI Appendix, Fig. S9).

**NM Ruptures and Cell Death in B1KO Cells as They Migrate into and across Tight Constrictions.** We suspected that the NM ruptures and cell death in the cortical neurons of Lmnb1-deficient embryos resulted from mechanical forces on the cell nucleus—both internal forces associated with nucleokinesis and the constrictive forces as cells migrated between other cells within the cortical plate (32). To explore this idea, we plated wild-type and B1KO neurons onto the flat portion of a silicon wafer and observed the cells as they migrated into an adjacent field of tightly spaced pillars (exposing both the cells and cell nuclei to external constrictive forces) (Fig. 6 A and SI Appendix, Fig. S10 A). As B1KO neurons entered the...
field of pillars, we observed NM ruptures (Fig. 6B). No NM ruptures were observed when wild-type neurons entered the field of pillars. Also, we observed widespread caspase 3 staining of B1KO neurons after they had migrated into the field of pillars (Fig. 6C and SI Appendix, Fig. S10B). Cell death was also observed with the LIVE/DEAD vital dye (Fig. 6D).

**Discussion**

In earlier studies, we documented defective migration of cortical neurons in Lmnb1 and Lmnb2 knockout embryos, along with a second abnormality—reduced density of neurons within the cerebral cortex (12). The mechanism for the neuronal migration defect was reasonably well understood, but a mechanism for the reduced cellularity of neurons was not. In the current study, we observed frequent NM ruptures in migrating neurons in the cortical plate of E18.5 Lmnb1-deficient embryos, evident by the escape of a nuclear-localized fluorescent reporter protein into the cytoplasm and by electron micrographs revealing discontinuities in the nuclear membranes, but did not observe NM ruptures in the more stationary cells of the ventricular zone. The regions of the brain with NM ruptures also had many dead and dying neurons, as judged by staining for caspase 3 or by staining with the LIVE/DEAD fluorescent vital dye. Consistent with the findings in Lmnb1-deficient embryos, we observed NM ruptures, accompanied by DNA damage and cell death, in B1KO neurons as they migrated away from cultured neurospheres. Thus, both mouse embryo and cultured neuron observations strongly imply...
that the reduced density of cortical neurons in Lmnb1-deficient mice is due to NM ruptures and the accompanying cell death.

The susceptibility of Lmnb1-deficient neurons to NM ruptures is likely due to several factors. First, neither the cortical neurons in E18.5 embryos nor cultured neurons express Lmna, the gene for lamin A and lamin C. The minimal amounts of lamin A and lamin C expression in cortical neurons probably increase the likelihood of NM ruptures. On the other hand, the high levels of lamin A and C expression in skin and liver likely explains why keratinocyte- or hepatocyte-specific Lmnb1/Lmnb2 knockout mice are free of pathology (8, 9). Second, while lamin B2 is expressed in neurons, it has little ability to compensate for the loss of lamin B1 (30, 31). In migrating neurons of Lmnb1-deficient embryos, lamin B2 is asymmetrically distributed along 1 pole of the nucleus, leaving most of the nuclear rim devoid of any nuclear lamin (SI Appendix, Fig. S4B).

NM ruptures and cell death were never observed in cells of the ventricular zone but were frequent in neurons within the cortical plate. We suspect that the higher susceptibility of cortical plate neurons to NM ruptures relates to deformational forces on the cell nucleus during neuronal migration. Neuronal migration is a saltatory process that depends on nucleokinesis—the motor-driven translocation of the nucleus into the leading edge of the cell. Following translocation of the nucleus, the trailing edge of the neuron is remodeled, resulting in net forward movement of the cell in the direction of the cortical plate. The mechanical forces involved in pulling the cell nucleus forward, together with the virtual absence of nuclear laminas over a large portion of the nucleus, likely triggers NM ruptures. Two observations favor a role for mechanical forces in eliciting NM ruptures. First, NM ruptures were not observed in more stationary cells of the ventricular zone, where the long-distance migration of neurons along glial-directed guides is not in play. Second, NM ruptures, along with cell death, were elicited when cultured B1KO neurons migrated into a field of tightly spaced silicon pillars. The nuclear membrane ruptures in the “silicon pillar experiments” could be a result of nucleokinesis forces and/or a direct consequence of the compressive forces that accompany cellular migration through the field of narrowly spaced pillars.

Lamin B1 and lamin B2 are ~60% identical at the amino acid level (33), and their temporal and spatial patterns of expression in the brain are very similar (12). Those considerations, along with the fact that deficiencies in either protein cause neuronal layering abnormalities in the cerebral cortex, might lead one to suspect that the 2 nuclear lamins have identical functions. Any such view, however, would be inconsistent with other observations. First, the properties of the 2 proteins are different. For example, lamin B1 is essential for a uniform distribution of lamin B2 along the nuclear rim, whereas the distribution of lamin B1 is quite normal in the absence of lamin B2 (12). Second, lamin B1’s farnesyl lipid anchor is required for development of the brain and for survival, whereas lamin B2’s farnesyl lipid anchor appears to be utterly dispensable (22). Lmnb1 knockin embryos that express a nonfarnesylated lamin B1 manifest severe neuronal layering abnormalities in the cerebral cortex as well as perinatal death—like conventional Lmnb1 knockout mice (22). In contrast, Lmnb2 knockin mice that express a nonfarnesylated lamin B2 are entirely healthy, free of both neurodevelopmental abnormalities and perinatal mortality (22). Third, the characterization of “reciprocal Lmnb1/Lmnb2 knockin mice” revealed that lamin B1 and lamin B2, despite high levels of amino acid identity, have minimal capacities to substitute for each other during development. For example, Lmnb2+/−Lmnb1−/+ embryos, where the gene-regulatory elements of Lmnb1 drive the expression of lamin B2 (resulting in substantially increased levels of lamin B2 expression but no lamin B1), manifest severe neuronal migration defects, reduced neuronal density in the cerebral cortex, and perinatal death. However, brain weights in Lmnb2+/−Lmnb1−/+ embryos were ~60% higher than in conventional Lmnb1 knockout embryos (31), implying that greater-than-normal amounts of lamin B2 expression have at least some capacity to improve the survival of Lmnb1-deficient neurons.

Our cultured neuron studies underscored the limited capacity of lamin B2 to substitute for lamin B1. Overexpression of lamin...
B2 in B1KO neurons reduced but did not prevent NM ruptures and cell death, despite the fact that the lamin B2 covered the entire nuclear rim. In contrast, lamin B2 overexpression in B2KO neurons abolished NM ruptures.

Our analysis of NM ruptures in B1KO and B2KO neurons by live-cell microscopy suggested distinct functions for lamin B1 and lamin B2. NM ruptures occurred in most B1KO neurons and repetitively in a subset of neurons, with repeated cycles of NM rupture and repair (escape of the fluorescent reporter into the cytoplasm followed by return of the reporter into the nucleus). The mean duration of NM ruptures in B1KO neurons was 2.9 h. Some B1KO neurons with NM ruptures died, but the majority survived. The behavior of B2KO neurons was different. NM ruptures were much less frequent but when they occurred they were never repaired and resulted in cell death. We do not understand the phenotypic differences, but our studies suggest that lamin B1 is more important for the structural integrity of the nuclear envelope, whereas lamin B2 is crucial for NM repair. Perhaps lamin B2 is the “mortar” that binds together the key building blocks in the nuclear lamina (i.e., lamin B1), and perhaps lamin B2 (or a lamin B2-interacting protein) plays a key role in sealing holes in the nuclear membranes.

The phenotype observed in B2KO neurons—infrequent NM ruptures but an absence of NM repair—may help to explain phenotypes observed in Lmnb2 mouse models. In conventional Lmnb2 knockout embryos, the reduction in the size of the cerebral cortex is modest (only ~5 to 10% smaller than in wild-type embryos) (11). In contrast, the reduction in the size of the forebrain in 4-mo-old forebrain-specific Lmnb2 knockout mice is more substantial (20% smaller than in wild-type mice) (12). The subtle decrease in cortical size in E18.5 Lmnb2 knockout embryos may reflect infrequent NM ruptures (and the resulting cell death), whereas the more substantial decrease in cortical size in the adult forebrain-specific Lmnb2 knockout mice could reflect loss of neurons due to an absence of NM repair. Even if NM ruptures are infrequent, the failure to repair the ruptures would be expected to result in a progressive loss of neurons and brain size.

Finally, we would point out that the 2 principal phenotypes associated with deficiencies of B-type lamins, neuronal layering defects in the cortex and decreased neuronal survival, are mechanistically related. Fundamental to both are a weakened nuclear lamina and mechanical forces on migrating neurons during development. Interestingly, B-type lamin deficiencies have only minor effects on stationary, postmitotic, Lmna-expressing neurons (13, 28). Inactivating Lmnb1 in photoreceptor neurons of the retina early in development leads to neuronal layering abnormalities and a dramatic loss of neurons, whereas inactivating both Lmnb1 and Lmnb2 in postmitotic photoreceptor cells after birth has minimal consequences (minor effects on the positioning of the nucleus within the cell) (34, 35). The B-type lamins are long-lived proteins with a half-life of over 5 mo in postmitotic cells (36), making it challenging to define the functional relevance of the proteins in adult mice (37).
Materials and Methods

Cell Culture Models. NPCs were isolated from E13.5 embryos derived from intercrossing Lmnb1⁺/− mice and used to generate Lmnb1⁺/+Lmnb2⁺/+ (WT) and Lmnb1⁻/−Lmnb2⁺/+ (B1KO) neurospheres. Explants from the cerebral cortex were placed in DMEM/F-12 medium (Corning) and dissociated with TrypLE Select (Gibco); the NPCs were resuspended in DMEM/F-12 (Corning). To generate Lmnb1⁺/+Lmnb2⁻⁻ (B2KO) neurospheres, we intercrossed Emx1-Cre Lmnb2fl/fl mice and isolated forebrain NPCs from Emx1-Cre Lmnb2fl/fl embryos. To be certain that Lmnb2 was completely inactivated, the NPCs were treated twice with Cre adenovirus (26).

Preparing the Heparin–EGF–FGF Supplement. One milligram of FGF was resuspended in 1 mL of FGF buffer (0.1% BSA [Sigma] in PBS) and diluted in 39 mL of DMEM/F-12 (Corning); 1 mg EGF (Thermo Fisher Scientific) was

Fig. 5. Overexpression of lamin B2 reduces cell death and NM ruptures in B1KO and B2KO neurons. (A) Western blots of extracts from WT, B1KO, and B2KO neurons; the neurons had been transduced with a doxycycline-inducible lamin B2 lentiviral vector (pTRIPZ-LMNB2) and then incubated in the presence or absence of Dox. Actin was used as a loading control. The bar graph shows lamin B2 protein levels normalized to actin. (B) Immunofluorescence microscopy of WT, B1KO, and B2KO neurons that had been transduced with pTRIPZ-LMN2B. In the presence of Dox, the cells expressed lamin B2. Immunofluorescence microscopy was performed after staining neurons with antibodies against lamin B2 (red) and LAP2β (green). (Scale bars, 20 μm.) (C) Percentages of B1KO and B2KO neurons exhibiting NM ruptures during 20 h of live-cell imaging. Ratios show numbers of neurons with NM ruptures over the total number of neurons examined. (D) Total numbers of NM rupture events relative to the total number of neurons examined. In these studies, the neurons had been transduced with NLS-GFP and pTRIPZ-LMN2B and incubated in the presence or absence of Dox. NM ruptures (escape of NLS-GFP in the cytoplasm) were observed by fluorescence microscopy. Data show totals from 2 independent experiments. (E) Overexpression of lamin B2 in B1KO and B2KO neurons reduces cell death, as judged by staining with the LIVE/DEAD fluorescent vital dye. WT, B1KO, and B2KO neurons that had been transduced with pTRIPZ-LMN2B were incubated in the presence or absence of Dox for 24 h and then incubated with the LIVE/DEAD dye, which fluoresces green in live cells and red in dead cells. DNA was stained with DAPI (blue). (Scale bars, 50 μm.) (F) Overexpression of lamin B2 in B1KO and B2KO neurons reduces programmed cell death, as judged by staining for caspase 3. WT, B1KO, and B2KO neurons that had been transduced with pTRIPZ-LMN2B were incubated in the presence or absence of Dox for 24 h and then stained with a caspase 3-specific antibody (green). No caspase 3 staining was observed in WT cells. DNA was stained with DAPI (blue). (Scale bars, 50 μm.) The Lower shows caspase 3 staining in black against a white background.
resuspended in EGF buffer (10% BSA in PBS) and diluted in 9 mL of fresh DMEM/F-12; 500 mg of heparin sodium salt (Sigma-Aldrich) was dissolved in 100 mL DMEM/F-12. To prepare 25 mL of the heparin–EGF–FGF supplement, 8 mL of the FGF solution, 5 mL of the EGF solution, and 10 mL of the heparin solution were mixed with 2 mL of DMEM/F-12.

**Neuronal Differentiation.** Cultured neurospheres were carefully removed with polyethylene pipets (Fisher) and pipetted into a single drop of laminin (Sigma-Aldrich) on poly-L-ornithine–coated plates. Neurospheres were allowed to settle for 30 min at 37 °C and then incubated with the DMEM/F-12 containing 2% B-27 supplement, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Neurospheres were differentiated for up to 30 d.

**Quantitative RT-PCR Studies.** RNA was isolated from undifferentiated and differentiated neurospheres, treated with DNase I (Ambion), and reverse transcribed with random primers, oligo(dT), and SuperScript III (Invitrogen). qPCR reactions were performed on a 7900 Fast Real-Time PCR system (Applied Biosystems) with SYBR Green PCR Master Mix (BioLine). Transcript levels were determined by the comparative cycle threshold method and normalized to levels of cyclophilin A. All primers used are listed in the SI Appendix, Table S1.

**Immunocytochemistry.** Neurons that had been differentiated on coverslips were then processed for confocal immunofluorescence microscopy (8) with the antibodies listed in SI Appendix, Table S2. For confocal immunofluorescence microscopy, images were recorded with a Zeiss LSM 700 laser-scanning microscope with a Plan Apochromat 20×/0.80 objective (air) or a Plan Apochromat 100×/1.40 oil-immersion objective. Images along the z axis were processed with Zen 2010 software (Zeiss).

**Western Blots.** Urea-soluble protein extracts from cells were size fractionated on 4 to 12% gradient polyacrylamide Bis-Tris gels (Invitrogen) and then transferred to a nitrocellulose membrane for Western blots. Membranes were blocked with Odyssey Blocking solution (LI-COR Biosciences) for 1 h at room temperature (RT) and then incubated with primary antibodies at 4 °C overnight (antibodies are listed in SI Appendix, Table S2). After washing the membranes with PBS containing 0.1% Tween-20, the membranes were incubated with infrared dye (IR)-labeled secondary antibodies (LI-COR Biosciences) for 1 h at RT. The IR signals were quantified with an Odyssey infrared scanner (LI-COR Biosciences).
Nuclear Shape Analyses. To assess the area and circularity of cell nuclei, WT, B1KO, and B2KO neurosphere cultures were stained with an antibody against the inner nuclear membrane protein LAP2δ, and images of cells were recorded with a Zeiss LSM700 laser-scanning microscope (20× objective), focusing on individual neurons that had migrated away from the main body of the neuron. Nuclear area was assessed using ImageJ software; nuclear circularity (4π × area/ perim²) was assessed by measuring nuclear areas and perimeters for 100 cells/genotype (n = 3 independent experiments). Nuclear circularity reaches a maximum value of 1.0 for a perfect circle (38). Live-Cell Imaging. Neurospheres were plated on a poly-l-ornithine–coated 6-well plate containing 2-mm glass wells (MatTek), and live-cell imaging was performed with a Zeiss LSM 800 confocal microscope equipped with a Plan Achromat 10×/0.45 or a Plan Achromat 20×/0.80 objective at 37 °C with 5% CO₂ (maintained with TempModule S1 CO₂ Module S1 from Zeiss). Z stacks were acquired from both fluorescence and transmission channels. Image sequences were analyzed with ZEN (Zeiss) using linear adjustments applied uniformly to the entire image. For confocal image stacks, images were 3-dimensionally reconstructed and displayed as maximum intensity projections. A nuclear rupture event was defined as NILS-GFP entry into the cytoplasm in interphase cells.

Fluorescent Vital Dye. Neurons were allowed to differentiate on poly-L-ornithine–coated 6-well plates containing 2-mm glass wells (MatTek) for 7 d. After washing cells with PBS (Gibco), cell viability was assessed with the LIVE/DEAD Cell Imaging Kit (Thermo Fisher). Neurons were incubated with the LIVE/DEAD dye for 15 min at 20 to 25 °C and then fixed with 4% paraformaldehyde in PBS and stained with DAPI. Cells were imaged immediately with a Zeiss LSM700 laser-scanning microscope with a Plan Achromat 20×/0.80 objective (air). As a positive control for cell death, cells or tissue sections were treated with 70% ethanol for 15 min before staining.

Plating of Neurospheres on Matrigel. A vial of Matrigel (Corning) was thawed overnight by submerging it in a 4 °C refrigerator. Prechilled pipet tips, Matrigel was pipetted into glass-bottom 6-well culture plates (MatTek) and then incubated at 37 °C for 30 min to allow a gel to form. Neurospheres were resuspended in Matrigel and plated on top of the gel layer, allowing a 30-min incubation at 37 °C for the gel to solidify. Each well was then flooded with DMEM/F-12 medium containing 2% B-27 supplement (Thermo Fisher), 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Neurospheres were allowed to differentiate and migrate into the Matrigel for 7 d. The neurospheres were then prepared for immunocytochemistry as described earlier.

Silicon Pillar Fabrication. Prime quality 4-inch Si (100) wafers (P8, 1 to 10 oz/cm²) were purchased from UniversityWafer (Boston, MA). A quartz photomask (PhotomaskPortal) was designed with LayoutEditor software to fabricate a custom array of circles with 4-μm spacings and used for conventional photolithography. Positive photoresist SPR700-1.2 was spin-coated on the Si surface, followed by a 90-s soft bake at 90 °C on a hotplate. A Karl Suss contact aligner was used to expose the photoresist on the wafer selectively with the photomask via a continuous exposure of 20-min wavelength 365 nm, intensity 12 mW/cm². The exposed wafer was post-exposure baked at 110 °C for 90 s, immersed in MF-26A developer for 1 min (development), rinsed with deionized water, and blown dry with N2. After photolithography, the patterned silicon wafer was reactive-ion etched (Plasma-Thermo FDRIE DSE II) for 6 min and 30 s to remove silicon in the exposed regions, forming 22-μm-deep silicon pillars. The pillar depth, diameter, and pitch were characterized with a Zenica Supra 40VP scanning electron microscope (SEM) and Dektak 6 Surface Profilometer.

Fluorescent Labeling of APTES-Functionalized Pillar Substrates. The silicon micropillar substrates were functionalized with (3-aminopropyl)triethoxysilane (APTES, Sigma-Aldrich) using chemical vapor deposition. Silanol surface silanol functionalities during a 1-h incubation. Substrates were removed from the flask and rinsed with 100% ethanol. The silicon was then labeled with a fluorescein dye (Texas Red). To generate a stock solution, 1 mg of Texas Red-sulfonfyl chloride was mixed with 100 μL of 99% N,N-diethylformamide (Sigma-Aldrich). A total of 50 μL of the stock was added dropwise to 10 mL of a 0.1- to 0.2-M sodium carbonate (Sigma-Aldrich) buffer (pH ~ 9). The APTES-functionalized substrate was then incubated in the buffered dye solution for 2 h at 4 °C on a shaking stage. The silicon substrates were then rinsed with Milli-Q ultrapure H₂O to remove physiosorbed dye and stored at −20 °C until use. To plate substrates for differentiation, laminin (Sigma-Aldrich) was first pipetted onto the silicon wafer substrates; the neurospheres were removed with a polyelectrolyte pipets (Fisher) and pipetted into a drop of laminin. Neurospheres were allowed to settle for 30 min at 37 °C and then incubated with DMEM/F-12 medium containing 2% B-27 supplement, 100 U/mL of penicillin, and 100 μg/mL of streptomycin.

Mouse Studies. Forebrain-specific Lmn1+ and Lmn2 knockout mice (Emx1–Cre Lmn1−/−, Emx1–Cre Lmn2−/−) were generated as described (12) and bred with ROSA26Sor1tm1(CAG-fdTomato–EGFPflh)J from The Jackson Laboratory (Bar Harbor, ME). All mouse studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All mice were fed a chow diet and housed in a virus-free barrier facility with a 12-h light/dark cycle. Animal protocols were reviewed and approved by the Animal Research Committee of UCLA.

Immunohistochemistry. Mouse tissues were prepared for immunohistochemical studies as described (12). Embryonic brains were fixed in 4% paraformaldehyde in PBS for 2 h at room temperature, incubated in 30% sucrose in PBS at 4 °C overnight, then frozen in O.C.T. (Tissue-Tek, Sakura Finetek). Sections (10 μm thick) were fixed for 5 min in 4% paraformaldehyde or ice-cold methanol, followed by 5 dips in acetone and permeabilization with 0.1% Tween-20. Background staining with mouse antibodies was minimized with the Mouse-on-Mouse Kit (Vector Laboratories, Burlingame, CA). Tissue sections were blocked with 2.5% horse serum for 1 h at room temperature and incubated overnight at 4 °C with primary antibodies in the dilutions indicated in SI Appendix, Table S2. Alexa Fluor 488- and Alexa Fluor 568-conjugated secondary antibodies (Molecular Probes, Invitrogen, Carlsbad, CA) were used at a 1:2,000 dilution; Dylight 649-conjugated streptavidin (Vector Laboratories) was used at 5 μg/mL. After counterstaining with DAPI, sections were mounted with Prolong Gold antifade (Invitrogen) and images were obtained with a Zeiss LSM700 laser-scanning microscope with a Plan Achromat 20×/0.80 objective (air) or a Plan Apochromat 100×/1.40 oil-immersion objective. Images along the z axis were processed by Zen 2010 software (Zeiss).

Fluorescent Vital Dye Staining of Embryonic Tissues. Cerebral cortex explants were harvested from E18.5 mouse embryos and placed in PBS containing 1% BSA. Cell viability was assessed by incubating 2-mm-thick pieces of explants with the LIVE/DEAD fluorescent dye (Thermo Fisher) for 15 min at 20 to 25 °C. After fixation with 4% paraformaldehyde in PBS and stained with DAPI, the tissues were immediately imaged with a Zeiss LSM 700 laser-scanning microscope with a Plan Apochromat 20×/0.80 objective (air).

Preparation of Tissues for Electron Microscopy. The cerebral cortex from E18.5 mouse embryos was excised and fixed overnight at 4 °C in a solution containing 4% paraformaldehyde, 2.5% glutaraldehyde, 2.1% sucrose, and 0.1 M sodium cacodylate. After the samples were fixed overnight, they were rinsed 5 times for 3 min each with 0.1 M sodium cacodylate and then incubated in a solution containing 2% osmium tetroxide, 1.5% potassium ferricyanide, and 0.1 M sodium cacodylate for 1 h at 4 °C. Next, the samples were rinsed 5 times for 3 min each with H₂O and then incubated with 1% thiocarbohydrazide for 20 min at room temperature. The samples were then rinsed 5 times for 3 min with H₂O and incubated with 2% uranyl acetate at 4 °C overnight. On the following day, tissue samples were rinsed with H₂O and dehydrated with a series of increasing ethanol concentrations (30, 50, 70, 85, 95, and 100%) for 10 min each, followed by 2-10-min incubations with 100% ethanol. Next, the samples were infiltrated with Embed812 resin (Electron Microscopy Sciences) by incubating samples in 33% resin (diluted in anhydrous acetone) for 2 h, 66% resin overnight, and 100% resin for 4 h. Samples were then embedded in fresh resin using propylene molds (Electron Microscopy Sciences) and polymerized in a vacuum oven at 65 °C for 48 h. After polymerization, samples were removed from the molds; block faces were trimmed, faced, and 65-nm sections were cut using a Leica UC6 ultramicrotome equipped with a Diatome diamond knife. Sections were collected onto glow-discharged copper grids coated with formvar and carbon (Electron Microscopy Sciences) and stained with Reynold’s lead citrate solution for 10 min. Finally, samples were imaged with a FEI Tecnai T12 transmission electron microscope set at 120 kV and equipped with a Gatan CCD camera.
Statistical Analyses. Statistical analyses were performed with GraphPad QuickCalcs (https://www.graphpad.com). Differences in nuclear morphologies (oval versus irregularly shaped nuclei) and the frequency of NM ruptures were analyzed with a $\chi^2$ test. Differences in nuclear circularity and numbers of $\gamma$H2AX foci were assessed with a 2-tailed Student’s t test.

See SI Appendix, SI Materials and Methods for more details on the methods we used.

Data Availability. All data are available in the manuscript and SI Appendix.

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