Supplementary information, Data S1 Materials and methods

RNA-seq

Total RNA was extracted from ESCs or wild-type MEF using an RNeasy Plus mini kit (Qiagen). Poly A+ RNA was purified from the total RNA and processed according to manufacturer's recommendations to generate paired-end libraries for an Illumina HiSeq 2000 sequencer.

Generation of Lmna, Lmnb1, and Lmnb2 triple knockout ESCs

DNA fragments for homologous recombination arms of *Lmna* targeting vectors were amplified from mouse BAC clone RP24-265E18.

Primers for the 5' 2.0 kb arm of *Lmna* were

5'-tagggcatcg gcggccgc ATG TTG GCC ACG CAC CAT CCT CTG TG-3' and

5'-gcga aagctt AG TGC ATC TGG GAT GGG ACC TCT CTG-3'

Primers for the 3' 4.0 kb arm of *Lmna* were

5'-gcga ttaattaa GGC AAA GCT GCG TGA CCT GGA GGA CT-3' and

5'-gcca ggcgcgcc GGT AGT GAA TAT ATA GTG AAT GTG TG-3'

The amplified fragments were sequentially cloned into pYZ2150 or pYZ2115, the gene targeting vector containing PGK-$Puro^R$ or PGK-Hyg^R, respectively (full information available upon request). The *Lmna* targeting construct containing PGK-$Puro^R$ was linearized with AsiSI and electroporated into $Lmnb1^{-/-};Lmnb2^{-/-}$ ESCs. Electroporated

ESCs were plated on DR4 MEFs (Applied StemCell), which are resistant to four different antibiotics. After selection with 1 μg/ml puromycin (Life Technologies) and 2 μM ganciclovir (Sigma G2536), ESC clones were screened for homologous recombination by PCR of the flanking regions of the 5' and 3' recombination arms from the genomic DNA with the primers listed below. In the second round of gene targeting, another *Lmna* targeting construct containing PGK-Hyg^R was electroporated into $Lmnb1^{-/-};Lmnb2^{-/-}$; $Lmna^{tm1/+}$ ESCs, selected with 100 μg/ml hygromycin (Life Technologies), 1 μg/ml puromycin, and 2 μM ganciclovir, and screened by PCR as described above. The PCR primers used to screen for the homologously recombined ESC clones are as follows:

5' arm of $Lmna^{tm1}$ or $Lmna^{tm2}$ (a PCR fragment of 2,235 or 2,151 bp indicates homologous recombination for $Lmna^{tm1}$ or $Lmna^{tm2}$, respectively)

pr537: 5'-TGT GAG AAT GTC AGC TTA GAC ACT GC-3' (outside of the 5' recombination arm in the $Lmna^{tm1}$ or $Lmna^{tm2}$ allele)

pr67: 5'-AGC TAG CTT GGC TGG ACG TAA ACT CC-3' ($Puro^R$ specific)

pr538: 5'-GAG CTC CAG CTT TTG TTC CCT TTA GG-3' (Hyg^R specific)

3' arm of $Lmna^{tm1}$ or $Lmna^{tm2}$ (a PCR fragment of 4,249bp or 4,695bp indicates homologous recombination for $Lmna^{tm1}$ or $Lmna^{tm2}$, respectively)

pr142: 5'-GGC TCT ATG GCT TCT GAG GCG GAA AG-3' (inside targeting vector),

pr792: 5'-AGT CTC CCT CAT ACA CAC CTT TAT AG-3' (outside of the 3' recombination arm in the $Lmna^{tm1}$ or $Lmna^{tm2}$ allele)

After PCR screening, *Lmnb1*$^{-/-}$;*Lmnb2*$^{-/-}$; *Lmna*$^{tm1/tm2}$ ESCs were expanded on MEF feeder cells in ESC culture medium [15% FBS, 100 μM β-mercaptoethanol, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 50 μg/ml penicillin/streptomycin in Knockout DMEM (Life Technologies) supplemented with 1,000 units/ml LIF (Millipore)]. The ESCs were cultured for 26 passages on MEF feeder cells and karyotyped using a standard protocol. All procedures involving recombinant DNA have followed the National Institute of Health guidelines.

Feeder-free ESC culture and differentiation

For immunofluorescence staining, proliferation assays, and differentiation into fibroblasts, cardiac and neural lineages, passage 4 ESCs were weaned off the MEF feeder cells for 4 passages in complete GMEM medium [1]. ESCs were differentiated into fibroblast, cardiac, or neural lineages according to published protocols [2-4].

RNA extraction and reverse transcriptase PCR

Total RNA was extracted from ESCs and wild-type MEF, and cDNA were prepared as described previously [1]. To examine the transcription of *Lmna*, the indicated exon regions of *Lmna* were amplified from the cDNA by PCR and the PCR products were analyzed using 1.0% agarose gels. The PCR primers were designed to amplify exon 1 or exonal junctions of *Lmna* as follows:

Lmna exon 1 alone (217 bp PCR fragment)

5'-CTC TGT CCT TCT GTC CAA GTC-3'

5'-GAT CGA TGT ACA CGG CCA G-3'

***Lmna* exons 1-2 (209 bp PCR fragment)**

5'-AAG ACC CTT GAT TCT GTG GC-3'

5'-TCC AAT GTG CGC TTC TCA C-3'

***Lmna* exon2 2-4 (238 bp PCR fragment)**

5'-TTC TCA ACT CCA AGG AAG CTG-3'

5'-TTG GTC TCA CGC AGT TCC-3'

***Lmna* exons 5-6 (205 bp PCR fragment)**

5'-AGT CTC GAA TCC GCA TTG AC-3'

5'-AGC TCC TGG TAC TCG TCC-3'

The PCR primers to amplify *Lmnb1*, *Lmnb2,* and *Gapdh* were as described [1].

Antibodies, Western blotting, and immunofluorescence staining

Western blotting was performed as described [1]. Monoclonal mouse anti-lamin A/C (Active Motif 39287, 1:1,000) raised against 430 - 545th amino acids of human lamin-A was used as the primary antibody and anti-mouse IgG HRP (Pierce) was used as the secondary antibody. Immunofluorescence staining was performed as described [1]. Mouse anti-lamin A/C (Active Motif 39287, 1:1,000), goat anti-lamin B1 (Santa Cruz sc-6216, 1:200), chicken anti-lamin B2 (raised by Aves Labs via a custom order, 1:5,000), mouse

anti-NPC (Abcam ab24609, 1:1,000), and rabbit anti-emerin (Santa Cruz sc-15373, 1:200) were used as primary antibodies. Donkey anti-rabbit IgG Alexa-Fluor 594 (Life Technologies, 1:10,000), and donkey anti-mouse IgG DyLight 488, donkey anti-goat IgG DyLight 594, and donkey anti-chicken IgY DyLight 647 (Jackson ImmunoResearch, 1:10,000) were used as secondary antibodies. DNA was stained with 1 μg/mL Hoechst 33258 dye (Sigma). The images from immunofluorescent cells were obtained using a Leica SP5 confocal microscope using identical exposure times and processed identically using Adobe Photoshop software.

Cell proliferation assay

ESCs were plated at 5.0 x 10^4 cells/cm^2, and subcultured every 48 hours for 8 days. At the end of each 48 hour-period, trypsinized ESCs were stained with trypan blue dye and the number of live cells was determined with a hemocytometer. Fold increase of cell number during a 48 hour-period was determined, and accumulated cell numbers at the indicated time points were calculated by multiplying the accumulated cell number of the previous period by the fold increase. Cell proliferation assays were performed using four replicates at the indicated time points.

Quantitative real-time PCR analysis of pluripotency and neural lineage genes

Total RNA was prepared as described above at the indicated time points. cDNA was reverse transcribed from total RNA using the iScript cDNA synthesis kit (Bio-Rad). Real-time PCR reactions were done using iQ™ SYBR Green Supermix (Bio-Rad) and a CFX96 Real-Time system (Bio-Rad). Primer sequences of Oct4, Nanog, Sox2, Zfp521

and N-Cadherin were as described previously [1, 5]. Primers for Sox1 were 5'-CCA AGG CCA ACC AGG ATC GGG TCA AG-3' and 5'-GTG CAT CTT GGG GTT TTC CTG GGC CA-3'. The amount of GAPDH expression was used to normalize all values.

Teratoma formation assay

Passage 4 ESCs cultured on MEF feeder cells were trypsinized for 5 min at 37°C and neutralized by ESC culture medium. Dissociated cells were then plated on a new culture dish and incubated at 37°C for 10 min to allow MEF feeder cells to attach. ESCs suspended in the medium were collected by centrifugation and resuspended at a density of 1.0 x 10^7 cells/ml in PBS. 2.0 x 10^6 ESCs were subcutaneously injected into immune-deficient mice (Charles River, Strain Code 086). At 5 weeks after injection, teratomas were dissected, and rectangular sizes were measured by a caliper. Dissected teratomas were fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin, and sectioned at 10 μm thickness. Sectioned teratomas were deparaffinized, rehydrated, and stained with haematoxylin and eosin (H&E) as described [1].

References for Supplementary Information

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