Supplemental Materials

Molecular Biology of the Cell

Giacomini et al.

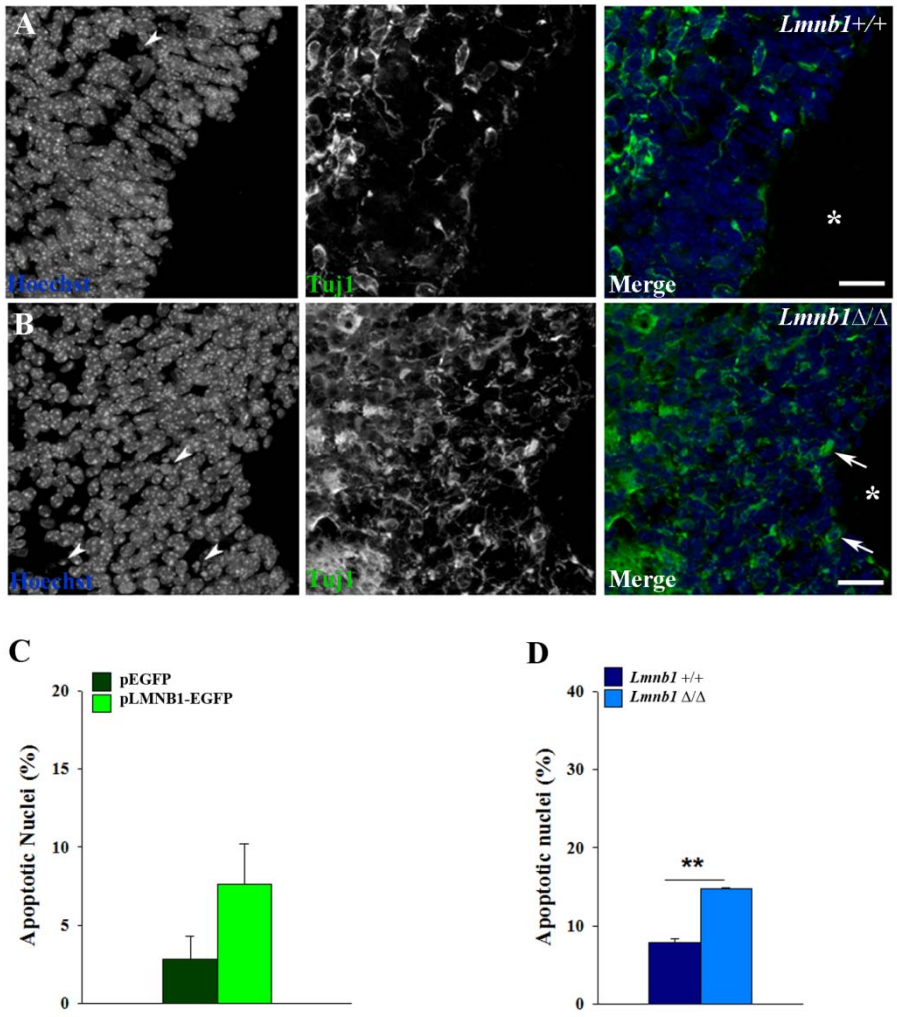
Lamin B1 protein is required for dendrite development in primary mouse cortical neurons

Caterina Giacomini[1], Sameehan Mahajani[1], Roberta Ruffilli[2], Roberto Marotta[2] and Laura Gasparini[1]

[1]Molecular Neurodegeneration Lab, Neuroscience and Brain Technologies Department and [2]Electron Microscopy Lab, Nanochemistry Department, Istituto Italiano di Tecnologia, Via Morego 30, Genova, Italy

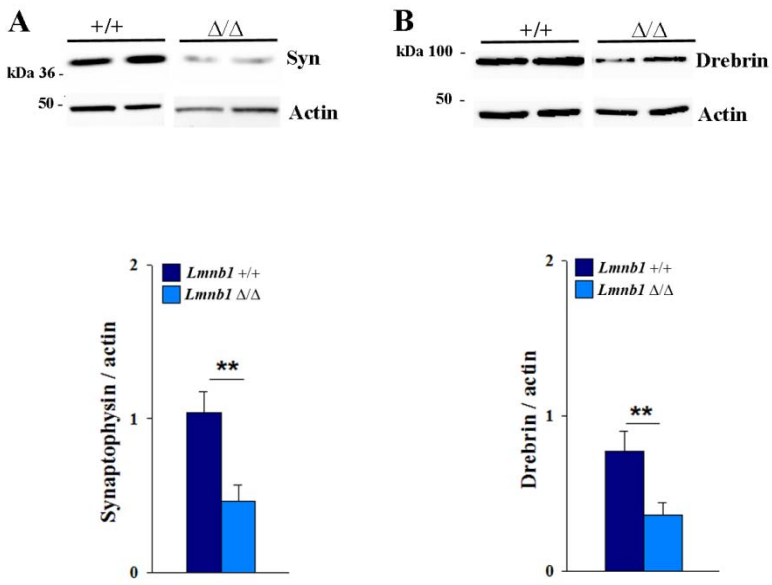
Supplementary Material:

- Supplementary Figures 1- 6
- Supplementary movielegends

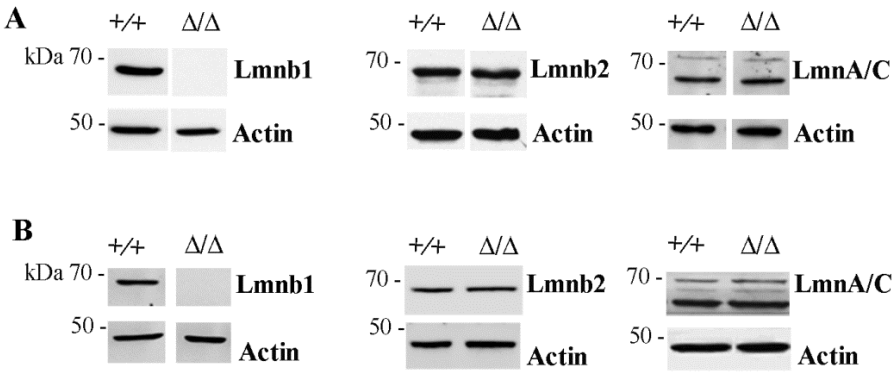


Supplementary Figure 1 - Lmnb1 deficiencyinduces apoptosis in primary cortical neurons and embryonic brain. A-B. Representative maximal projections of confocal z-stack images of βIII-tubulin (green; TuJ1 antibody) immunoreactivity in the SVZ of *Lmnb1*+/+ (A) and *Lmnb1Δ/Δ* (B) E17.5 embryonic brain. Arrowheadsindicate apoptotic nuclei. Arrows indicate βIII-tubulin positive cells in the SVZ. Asterisks indicate ventricles. In all images, nuclei are counterstained with Hoechst 33342 (blue).Scale bars: 20 μm.**C-D.**pLMNB1-EGFP, pEGFP transfected primary cortical neurons and *Lmnb1*+/+, *Lmnb1Δ/Δ*primary cortical neurons were

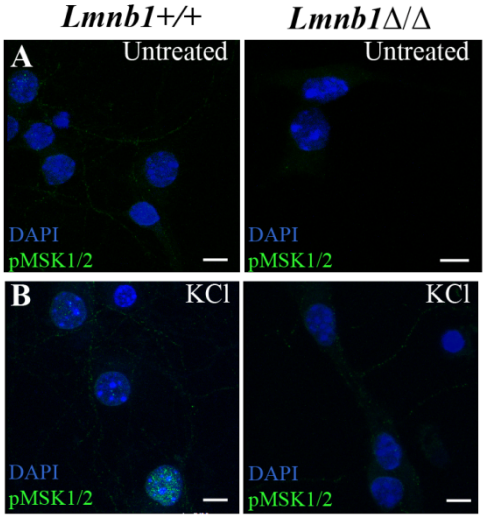
cultured for 7 days and nuclei analyzed as described in the Methods. Quantitative analysis of apoptotic nuclei in pLMNB1-EGFP, pEGFP transfected primary cortical neurons (C) and in *Lmnb1*+/+, *Lmnb1Δ/Δ*primary cortical neurons (D). At least 100 neurons were counted for each experimental condition. Bars represent mean percentage ± SEM of 4 independent experiments. *p< 0.05; Student t-test.



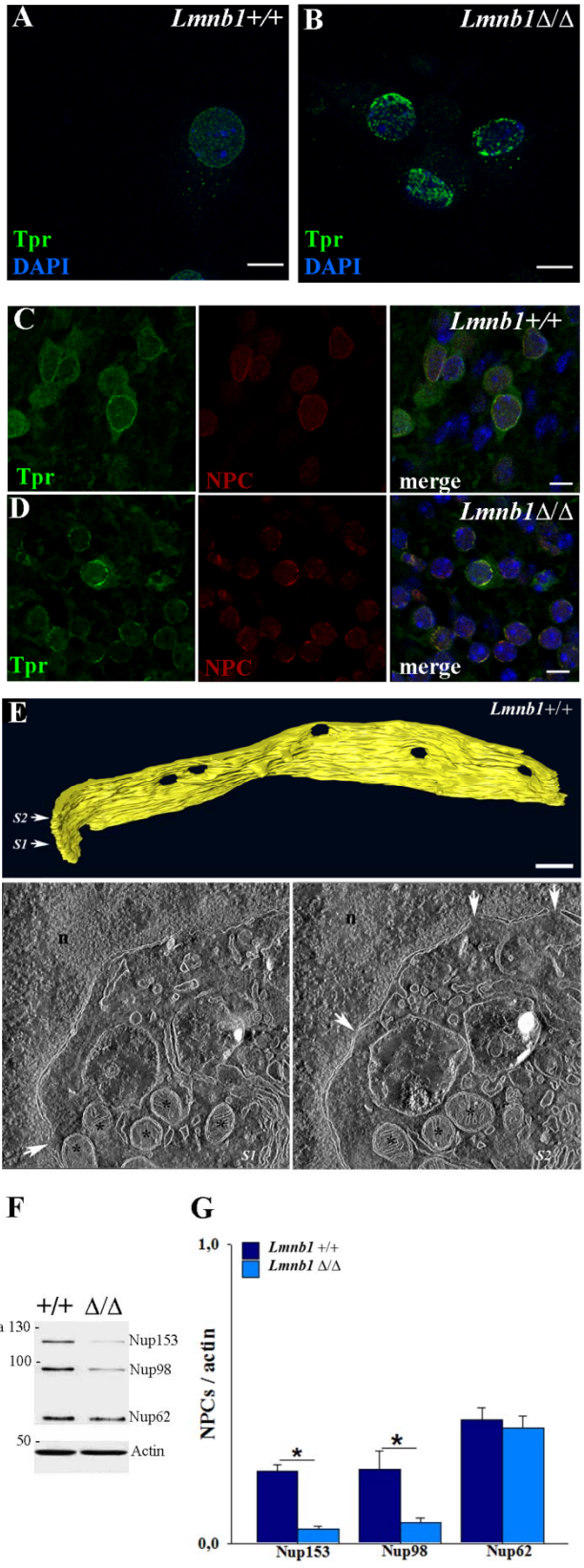
Supplementary Figure 2-Lmnb1-null cortical neurons display reduced expression of synaptophysin and drebrin. A-B. Representative Western blots and quantitative analysis ofsynaptophysin (A), drebrin (B) in lysates of *Lmnb1*+/+ and *Lmnb1Δ/Δ* mature primary cortical neurons (18 DIV). The data are normalized to actin. Bars represent the average ratio ± SEM. *Lmnb1*+/+, n=7;*Lmnb1Δ/Δ,* n=8 embryos. **p< 0.01, Student t-test.



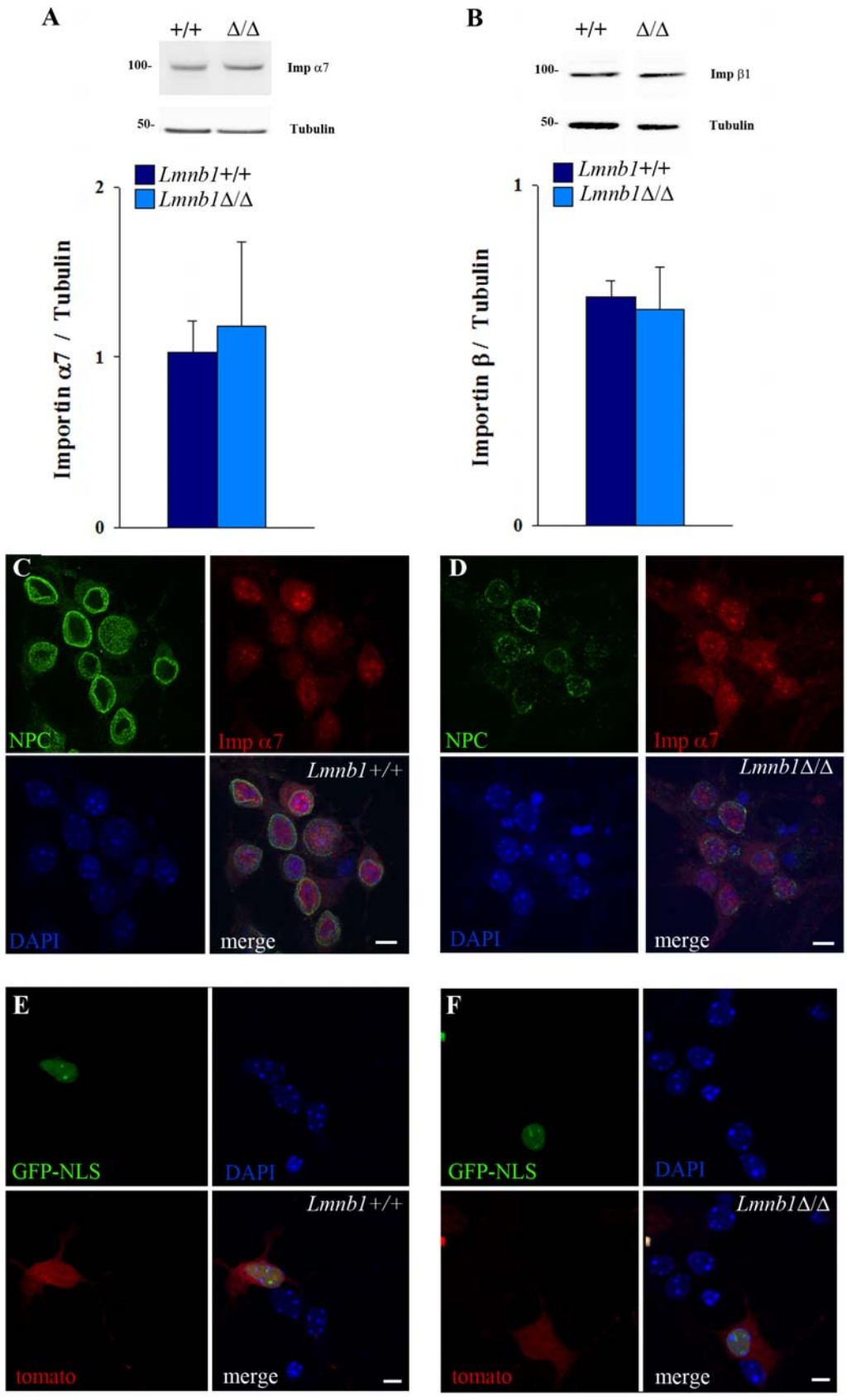
Supplementary Figure 3 -Lmnb1 deficiency does not alter LmnA/C or Lmnb2 expression levels in cultured neurons and embryonic brain.A-B. Representative Western blots of Lmnb1, Lmnb2, LmnA/C and actin in lysates from 7 DIV primary cortical neurons (A) and brain (B) from *Lmnb1*+/+(+/+) and *Lmnb1Δ/Δ*(Δ/Δ) E17.5 embryo.



Suplementary Figure 4- Lmnb1 deficiency impairs pERK nuclear signaling in primary cortical neurons.A-B. Representative maximal projections of confocal z-stack images of pMSK1/2 (green, A, B) immunoreactivity in 7DIV *Lmnb1*+/+ and *Lmnb1Δ/Δ*primary cortical neurons incubated with 50 mMKCl for 1 h (B) or untreated (A). Nuclei are counterstained with DAPI. Scale bars: 5µm.


A
Lmnb1+/+
Tpr
DAPI
B
Lmnb1Δ/Δ
Tpr
DAPI
C
Tpr
NPC
Lmnb1+/+
merge
D
Tpr
NPC
Lmnb1Δ/Δ
merge
E
Lmnb1+/+
S2
S1
n
n
S1
S2
F
+/+ Δ/Δ
kDa 130
100
50
Nup153
Nup98
Nup62
Actin
G
Lmnb1 +/+
Lmnb1 Δ/Δ
NPCs / actin
1,0
0,0
*
*
Nup153
Nup98
Nup62

Supplementary Figure 5 - Lmnb1 deficiency alters distribution and expression of nucleoporins.A-B. Representative maximal projections of confocal z-stack images ofTprimmunoreactivity (green) in *Lmnb1*+/+(A) and *Lmnb1Δ/Δ*(B) primary cortical neurons (7 DIV). **C-D.** Representative maximal projections of confocal z-stack images of *Lmnb1*+/+ and *Lmnb1Δ/Δ* E17.5 embryonic cortex stained against Tpr (green) and NPCs (red). In panels A-D, nuclei are counterstained with DAPI; scale bars5μm.**E.** Top panel: 3D model of a *Lmnb1*+/+ nuclear membrane fragment representing the reconstruction of a 300 nm tomogram acquired in high angular annular dark field (HAADF) scanning TEM (STEM) (see Supplementary Movie 2). Scale bars: 200 nm. Lower panel: singletomogram slices corresponding to sections S1 and S2 in the 3D model. White arrows point to NPCs; asterisks point to mitochondria. Abbreviation: n, nucleus.**F.**Representative Western blot analysis of NPC expression levels in brain lysates from E17.5 Lmnb1+/+ and Lmnb1Δ/Δembryos. **G.**Quantitative analysis of NPC expression levels in E17.5 Lmnb1+/+ and Lmnb1Δ/Δ brains. The data are normalized to actin. Bars represent the average ratio ± SEM. n=3 per genotype. *p< 0.05, Student t-test.


A
+/+
Δ/Δ
100-
Imp α7
50-
Tubulin
Lmnb1+/+
Lmnb1Δ/Δ
Importin α7 / Tubulin
2
1
0
B
+/+
Δ/Δ
100-
Imp β1
50-
Tubulin
Lmnb1+/+
Lmnb1Δ/Δ
Importin β/ Tubulin
1
0
C
NPC
Imp α7
Lmnb1+/+
DAPI
merge
D
NPC
Imp α7
Lmnb1Δ/Δ
DAPI
merge
E
GFP-NLS
DAPI
Lmnb1+/+
tomato
merge
F
GFP-NLS
DAPI
Lmnb1Δ/Δ
tomato
merge

Supplementary Figure 6– Importinα7 and importinβ1 expression and karyopherin-mediated nuclear import of GFP-NLSarenormal in Lmnb1-deficient neurons. A-B. Quantitative analysis of Importinα7 (Imp α7; A) and Importinβ1 (Imp β1; B) expression levels in 7DIV*Lmnb1*+/+ and *Lmnb1Δ/Δ* primary cortical neurons. Protein expression levels were analyzed by sWestern blot and normalized to tubulin. Bars represent the average ratio ± SEM. n = 4 per genotype. **C-D**Representative maximal projections of z-stack confocal images of *Lmnb1*+/+ (C) and *Lmnb1Δ/Δ*(D) primary cortical neurons immunostained against NPC (green) and Importnα7 (red). **E-F.**GFP-NLS nuclear translocation**.**Representative maximal projections of z-stack confocal images of *Lmnb1*+/+ (E) and *Lmnb1Δ/Δ*(F) neurons transfected with pGFP-NLS-IRES-TOMATO. GFP-NLS (green) and Tomato (red) fluorescence are shown. Nuclei are counterstained with DAPI (blue). In C-F, Scale bars: 5 μm.