

Supplementary Materials for

Stem cell-associated osteogenic deficiency causes craniofacial deformities with progeroid accumulation of prelamin A

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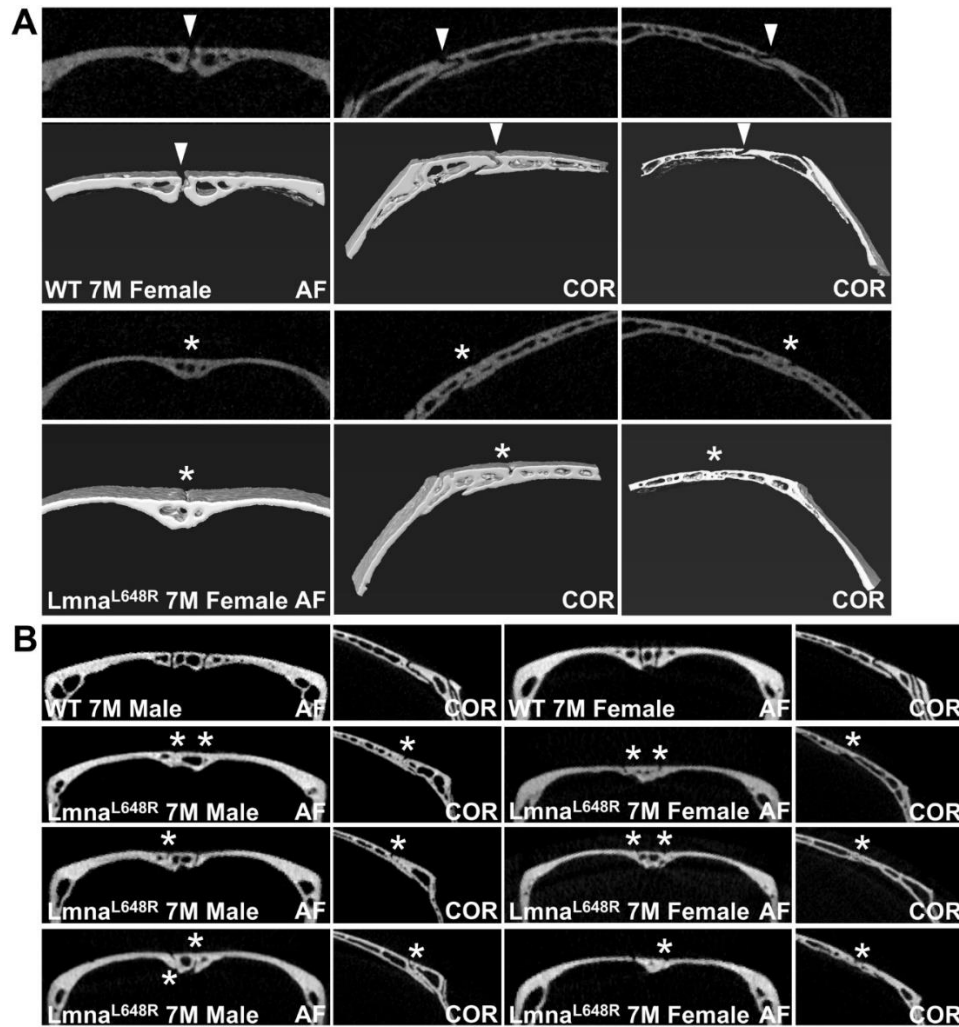
Supplemental Figure 11. Defective assembly of TAN lines in *Lmna*^{L648R} mutant cells.

Supplemental Figure 12. Disruption of the LINC complex in *Lmna*^{L648R} mutant cells.

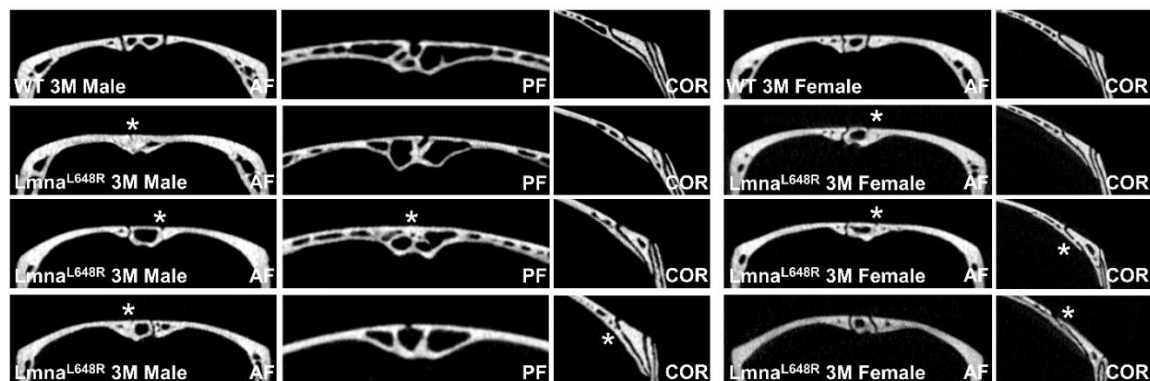
Supplemental Figure 13. *Lmna*^{L648R} mutation causes Golgi dispersal.

Supplemental Figure 14. JAS promotes actin polymerization in *Lmna*^{L648R} osteogenic cells.

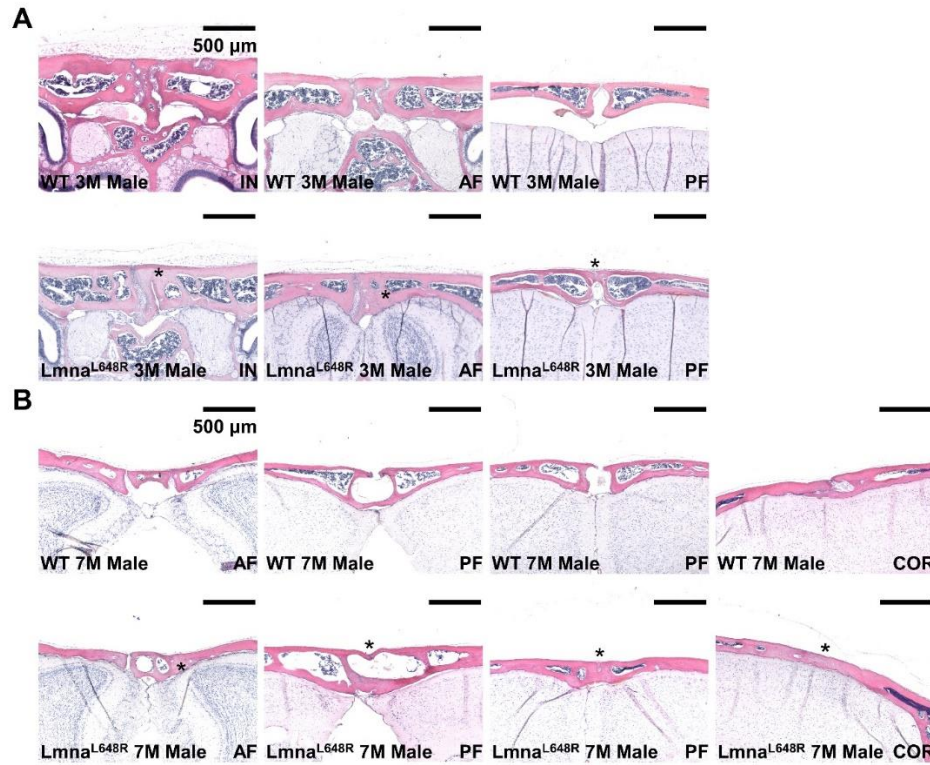
Supplemental Figure 15. The effect of JAS on actin gene expression in *Lmna*^{L648R} osteogenic cells.



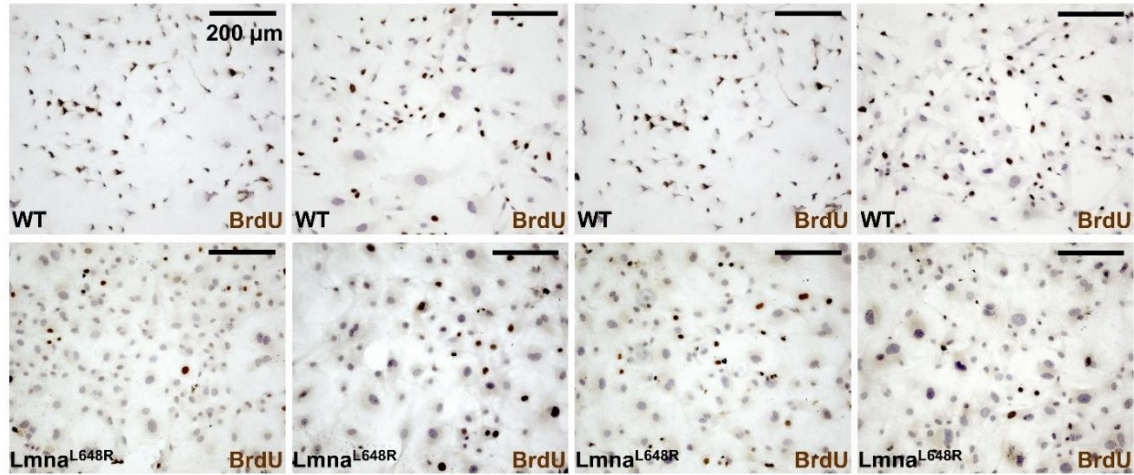
Supplemental Figure 1. Multiple suture synostosis in *Lmna*^{L648R} mice at 7 months of age. (A) Representative μCT-scanned coronal sections and 3D renderings of *Lmna*^{+/+} (WT) and *Lmna*^{L648R/L648R} (*Lmna*^{L648R}) female skulls at 7 months. (B) Images of μCT scanned three different 7-month-old *Lmna*^{L648R} males and females showing synostosed sutures as indicated by asterisks. AF, anterior frontal; COR, coronal.



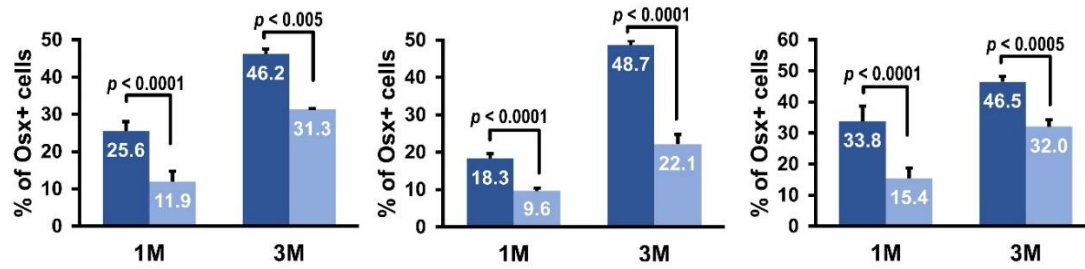
Supplemental Figure 2. Craniosynostosis in the 3-month-old *Lmna*^{L648R} mutant. Images of μ CT-scanned 3-month-old WT and three different *Lmna*^{L648R} males and females, analyzing aberrant suture fusion indicated by the asterisk. AF, anterior frontal; COR, coronal; PF, posterior frontal.



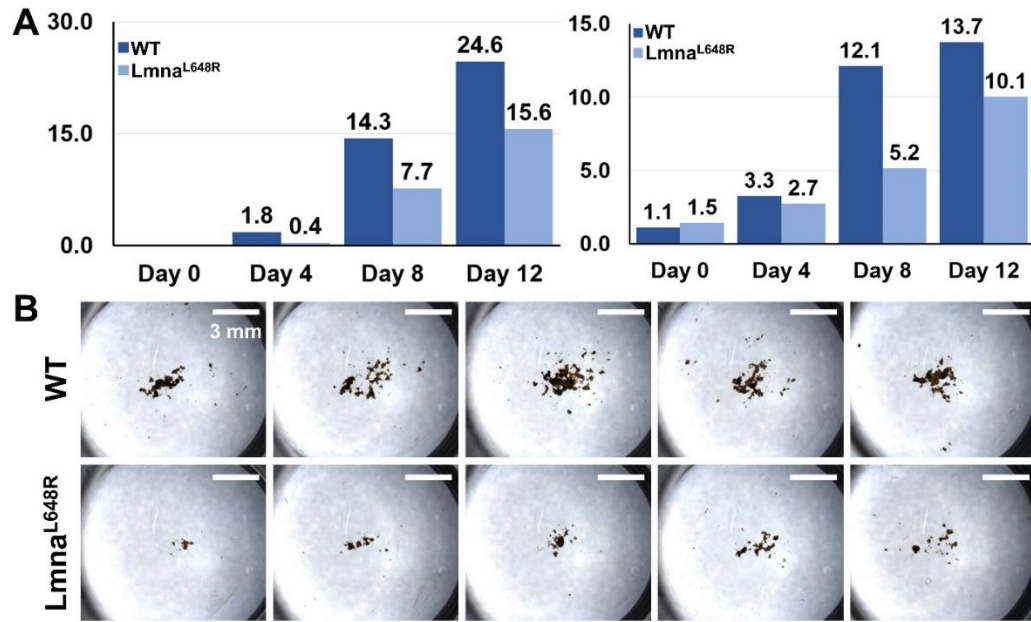
Supplemental Figure 3. Histological evaluation of suture fusion in the *Lmna*^{L648R} mice. Hematoxylin and eosin staining of 3-month-old (**A**) and 7-month-old (**B**) WT and *Lmna*^{L648R} calvarial sections. Asterisks indicate synostosed sutures. AF, anterior frontal; COR, coronal; IN, internasal; PF, posterior frontal. Scale bars, 500 μ m.



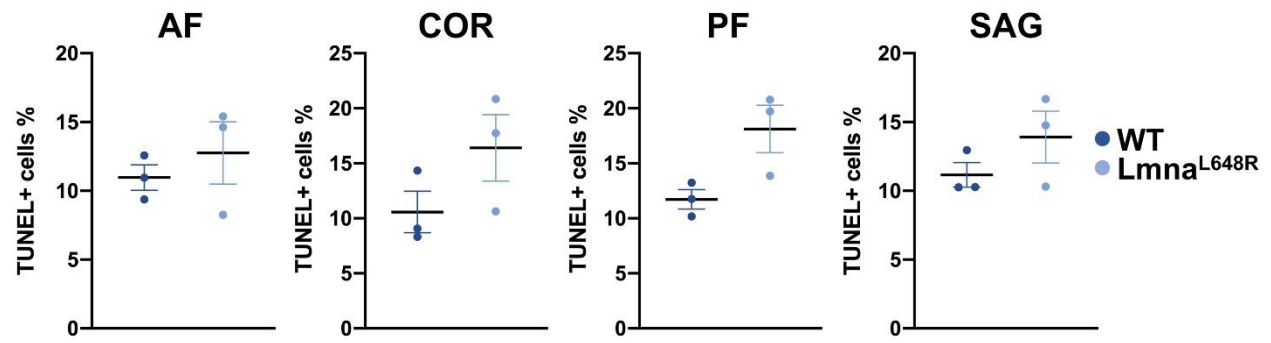
Supplemental Figure 4. Proliferation of calvarial cells decreased by the *Lmna*^{L648R} mutation. Images showing the representative field of views for calvarial cells positive for transient BrdU labeling analysis in the cultured WT and *Lmna*^{L648R} calvarial cells. The average percentage of BrdU-positive cells is then obtained for the quantification study shown in Fig. 2A. Scale bars, 200 μ m.



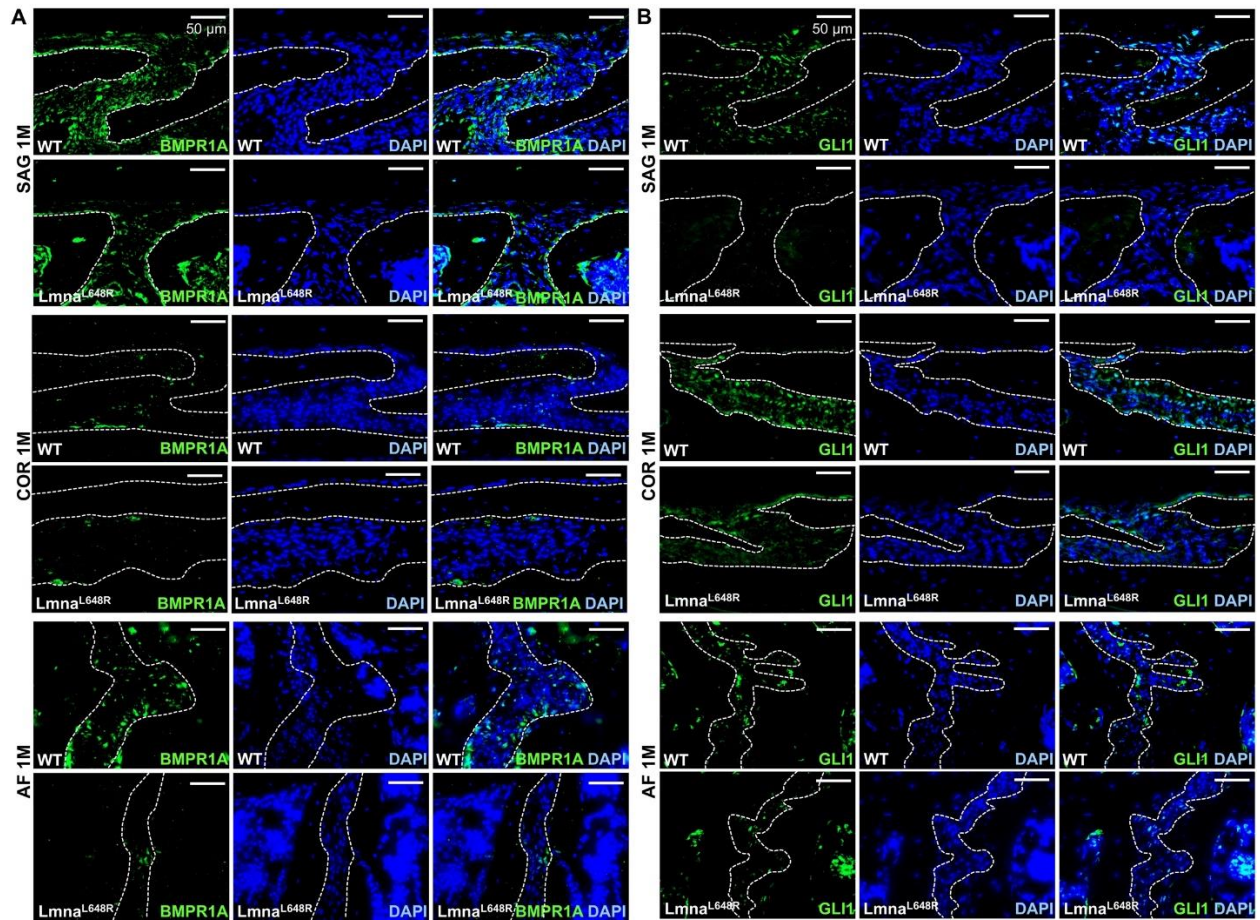
Supplemental Figure 5. Reduction of osteoprogenitor cells in the WT and *Lmna*^{L648R} cranial sutures. Graphs indicate the quantitation of osteoprogenitor cells present in the 1-month and 3-month WT and *Lmna*^{L648R} cranial sutures (1M, 3M). The average percentage of Osx-positive cells was analyzed by the immunostaining of the WT and *Lmna*^{L648R} cranial sutures in three independent experiments (two-sided Student's *t*-test, mean $\pm$ SD).



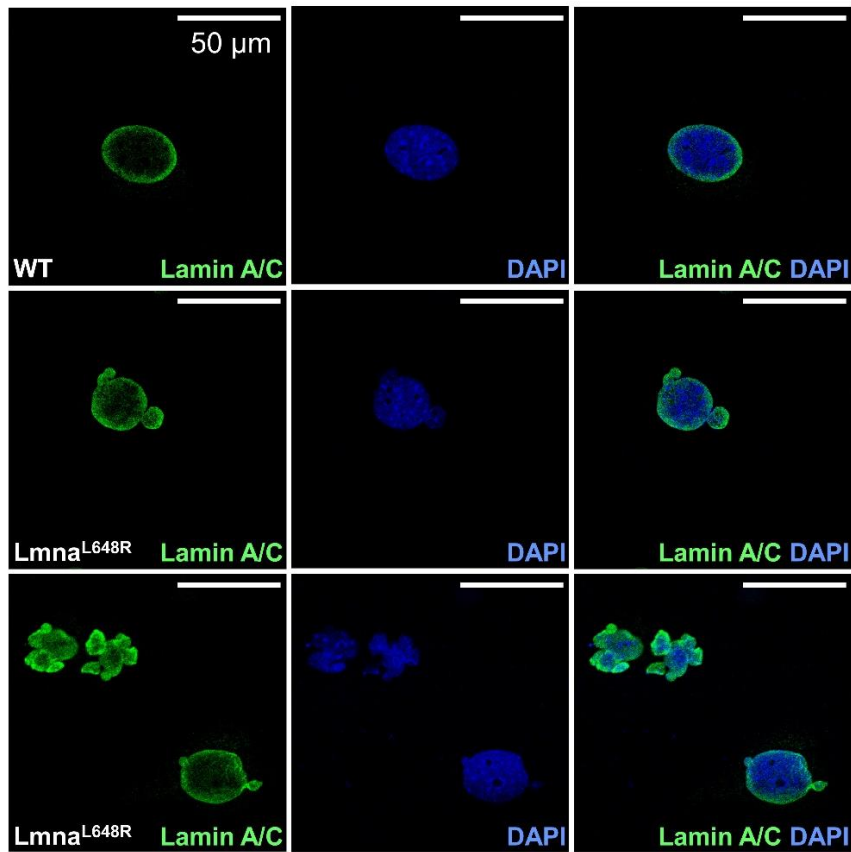
Supplemental Figure 6. Defective osteoblast differentiation and mineralization in premature aging. (A) Graphs show two of three independent experiments analyzing the alkaline phosphatase (ALP) activity of WT and *Lmna*^{L648R} calvarial cells cultured in differentiation media for days as indicated. (B) Images of von Kossa staining show five independent experiments examining mineralized nodule formation in the culture of WT and *Lmna*^{L648R} calvarial cells for 21 days. Scale bars, 3 mm.



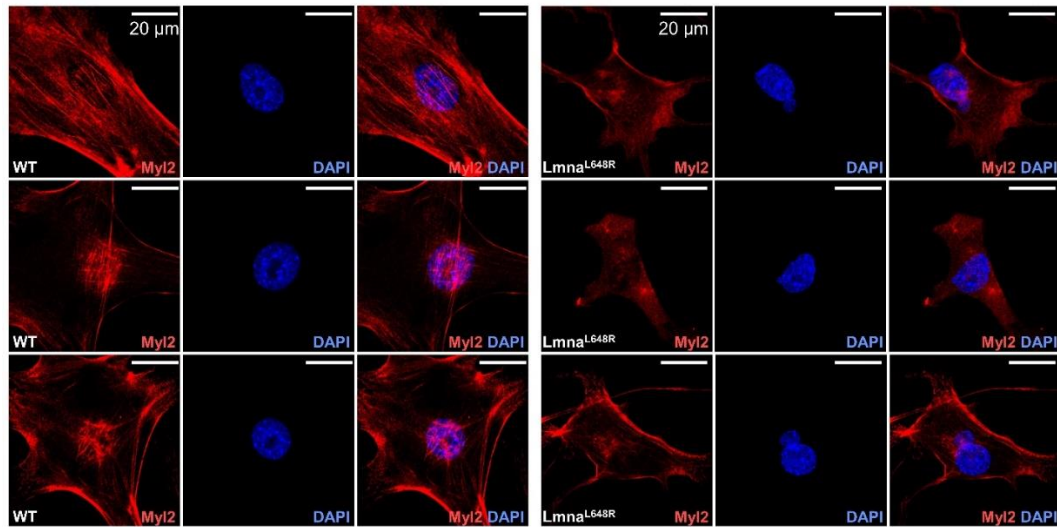
Supplemental Figure 7. No effect of *Lmna*^{L648R} mutation on apoptosis. Graphs indicate the percentage of TUNEL+ cells in the 3-month-old WT and *Lmna*^{L648R} sutures. AF, anterior frontal; COR, coronal; PF, posterior frontal; SAG, sagittal.



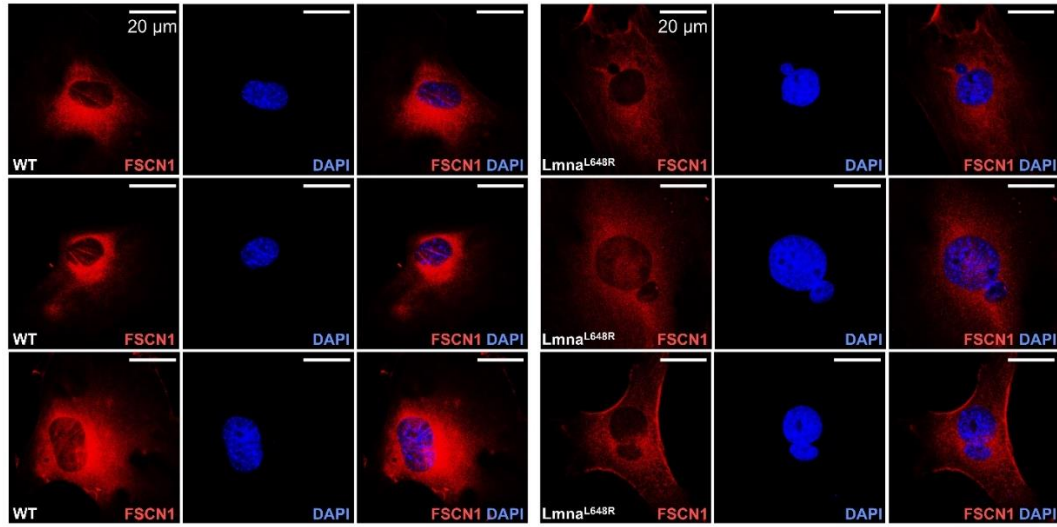
Supplemental Figure 8. Effects of the *Lmna*^{L648R} mutation on stem cells in cranial sutures. Immunostaining images showing cells expressing suture stem cell markers, BMPR1A+ (A) and GLI1+ (B) cells, within the indicated 1-month-old (1M) sutures. AF, anterior frontal; COR, coronal; SAG, sagittal. Scale bars, 50 μ m.



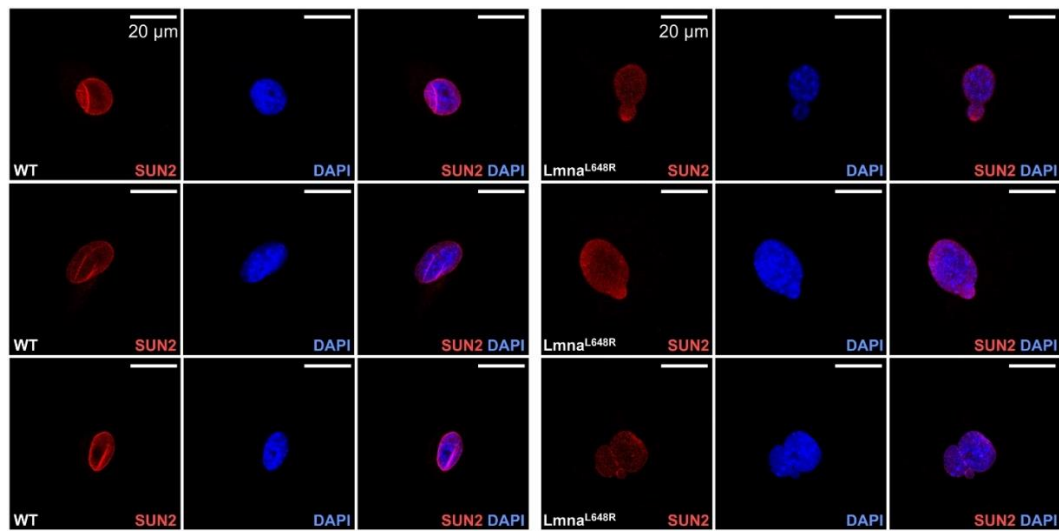
Supplemental Figure 9. Nuclear morphologies of WT and *Lmna*^{L648R} calvarial cells. Images of Lamin A/C staining show representative normal and abnormal nuclear morphologies in cultured calvarial cells. Scale bars, 50 μm.



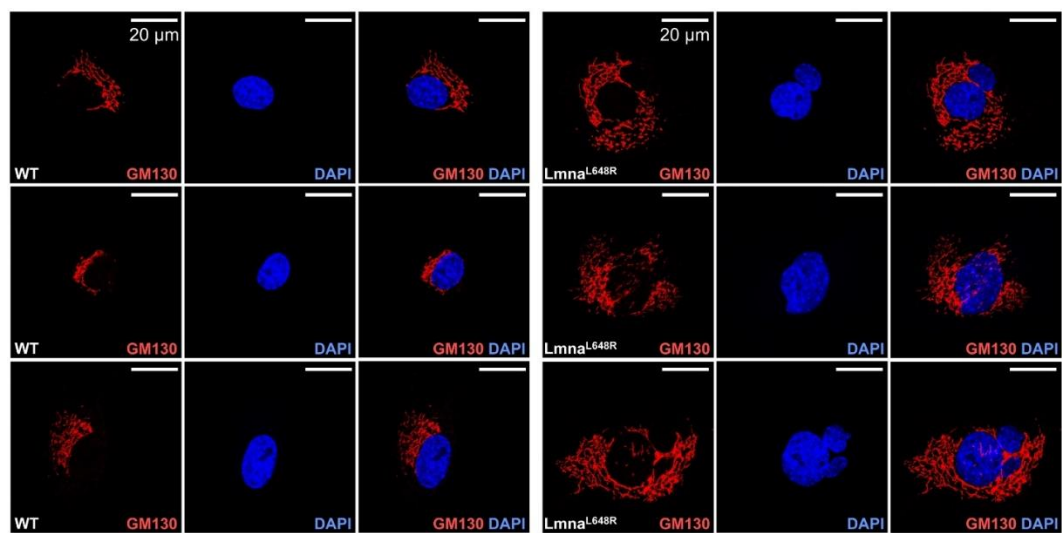
Supplemental Figure 10. Perinuclear actin cap affected by the *Lmna*^{L648R} mutation. Immunostaining of phosphorylated myosin light chain 2 at Ser19 (Myl2), examining perinuclear actin cap fibers in WT and *Lmna*^{L648R} calvarial cells. Scale bars, 20 μ m.



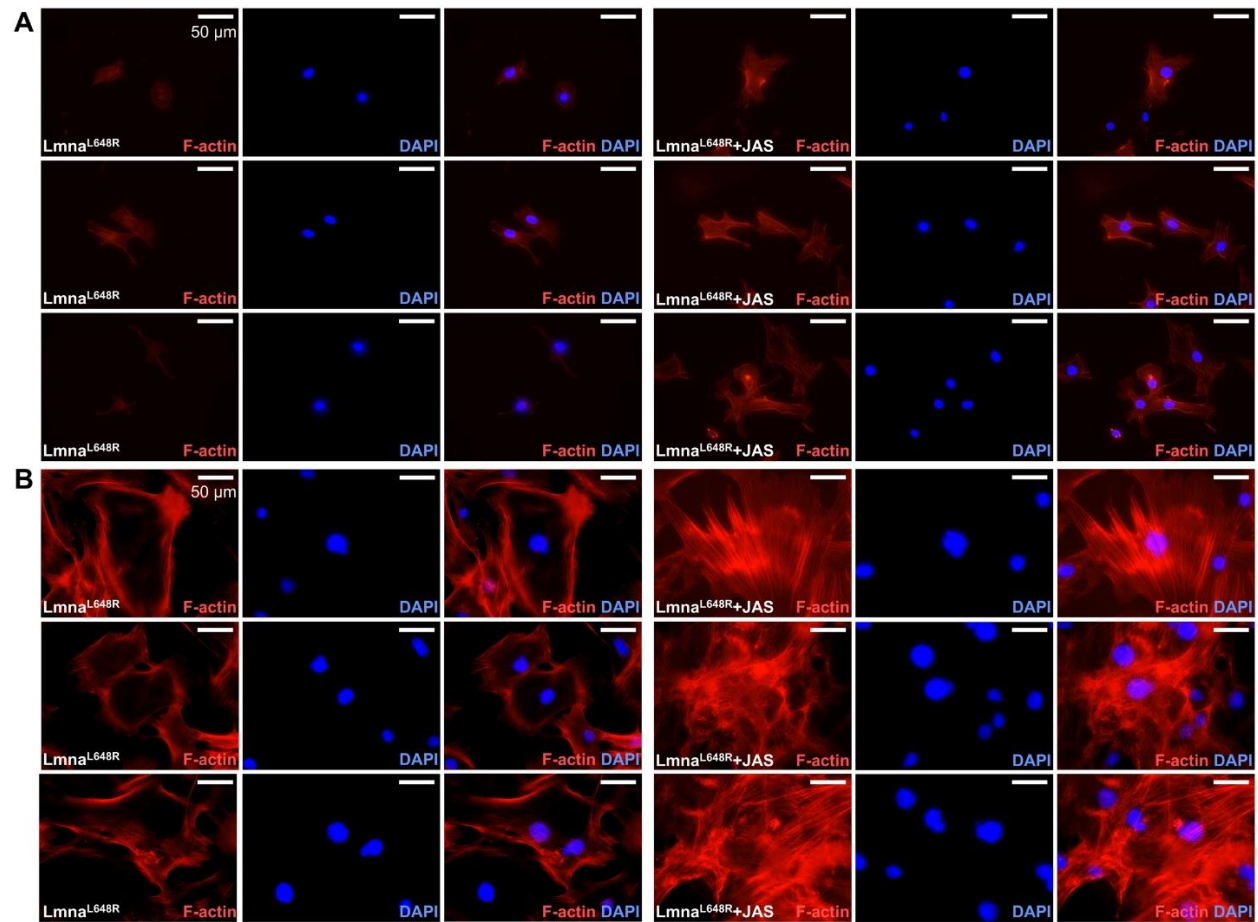
Supplemental Figure 11. Defective assembly of TAN lines in *Lmna*^{L648R} mutant cells.
 Immunostaining of FSCN1 examining Transmembrane Actin-Associated Nuclear (TAN) lines in WT and *Lmna*^{L648R} calvarial cells. Scale bars, 20 μm.



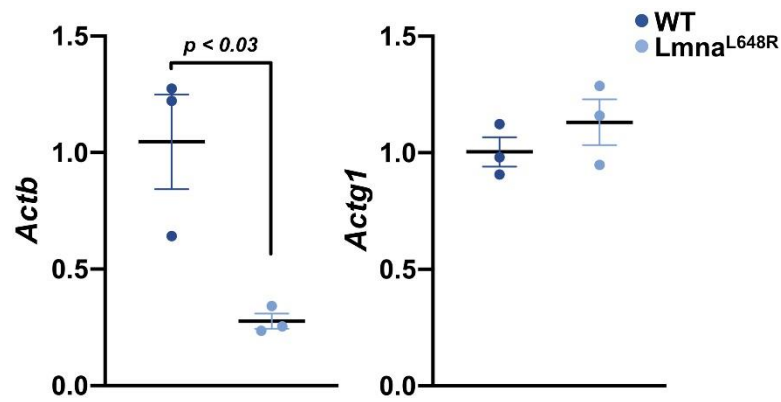
Supplemental Figure 12. Disruption of the LINC complex in *Lmna*^{L648R} mutant cells. Examination of SUN2 residing in the inner nuclear membrane of WT and *Lmna*^{L648R} calvarial cells by immunostaining. Scale bars, 20 μm.



Supplemental Figure 13. *Lmna*^{L648R} mutation causes Golgi dispersal. Immunostaining of GM130, analyzing the Golgi of WT and *Lmna*^{L648R} calvarial cells. Scale bars, 20 μm.



Supplemental Figure 14. JAS promotes actin polymerization in *Lmna*^{L648R} osteogenic cells. Filamentous actin (F-actin) staining analysis of the cytoskeleton in suture (A) and calvarial (B) cells isolated from *Lmna*^{L648R} mice, without or with the addition of JAS. Scale bars, 50 μ m.



Supplemental Figure 15. The effect of JAS on actin gene expression in *Lmna*^{L648R} osteogenic cells. Plots show qRT-PCR results examining the expression of *Actb* and *Actg1* after culture of WT and *Lmna*^{L648R} mutant cells in differentiation media for 12 days (p-value as indicated, n = 3, mean ± SEM, two-sided Student's t-test).