Activation of PDGF pathway links LMNA mutation to dilated cardiomyopathy

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Lamin A/C (LMNA) is one of the most frequently mutated genes associated with dilated cardiomyopathy (DCM). DCM related to mutations in LMNA is a common inherited cardiomyopathy that is associated with systolic dysfunction and cardiac arrhythmias. Here we modelled the LMNA-related DCM in vitro using patient-specific induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs). Electrophysiological studies showed that the mutant iPSC-CMs displayed aberrant calcium homeostasis that led to arrhythmias at the single-cell level. Mechanistically, we show that the platelet-derived growth factor (PDGF) signalling pathway is activated in mutant iPSC-CMs compared to isogenic control iPSC-CMs. Conversely, pharmacological and molecular inhibition of the PDGF signalling pathway ameliorated the arrhythmic phenotypes of mutant iPSC-CMs in vitro. Taken together, our findings suggest that the activation of the PDGF pathway contributes to the pathogenesis of LMNA–related DCM and point to PDGF receptor–β (PDGFRB) as a potential therapeutic target.

DCM associated with mutations in LMNA (LMNA-related DCM) is an autosomal dominant disorder caused by mutations in the gene that encodes the lamin A/C proteins that constitute the major component of the nuclear envelope1–3. LMNA-related DCM accounts for 5–10% of cases of DCM and has an age-related penetrance with a typical onset4-5 between the ages of 30 and 40. In contrast to most other forms of familial DCM, sudden cardiac death may be the first manifestation of LMNA-related DCM even in the absence of systolic dysfunction, owing to malignant arrhythmias such as ventricular tachycardia and fibrillation6-8. However, the precise mechanisms that link the mutations in LMNA to increased arrhythmogenicity are unknown.

Modelling LMNA-related DCM with iPSC-CMs in vitro

We recruited a large family cohort, members of which carry a frameshift mutation in LMNA that leads to the early termination of translation (348–349insG; K117fs) (Extended Data Fig. 1a–c). Three of the carriers (III-1, III-3 and III-9) presented with atrial fibrillation that progressed to atrioventricular block, ventricular tachycardia (Extended Data Fig. 1d, e) and DCM.

We generated multiple patient-specific iPSC lines using non-integrating reprogramming methods8,12 and derived iPSC-CMs using a chemically defined protocol8-10 to examine the electrophysiological properties at the single-cell level. We found that the LMNA-mutant iPSC-CMs (III-3, III-9, III-15 and III-17) exhibited proarrhythmic activity in both atrial- and ventricular-like iPSC-CMs compared to healthy controls (IV-1 and IV-2) (Fig. 1a and Extended Data Fig. 1f, g). Taken together, these data demonstrate that patient-specific iPSC-CMs recapitulate the disease phenotype associated with LMNA-related DCM in vitro.

Next, we generated a panel of isogenic lines that differed only in this mutation using the iPSC line derived from patient III-3 (who carried one wild-type and one mutant allele (WT/MUT)) through TALEN-mediated genome editing7,11,12. Specifically, we corrected the LMNA mutation to the wild-type allele in the iPSCs (WT/cor-WT), inserted the K117fs mutation in the wild-type allele (ins-MUT/MUT) and generated a knockout iPSC line by targeting the start codon11 (ATG site) of the wild-type allele (del-KO/MUT) (Fig. 1b and Extended Data Fig. 2a–c). We also introduced the K117fs mutation in the healthy control iPSC line (patient IV-1, who carried two wild-type alleles (WT/WT)) to generate a heterozygous mutant iPSC line (WT/ins-MUT). We generated iPSC-CMs from the isogenic lines and observed that the targeted gene correction rescued the electrophysiological abnormalities in WT/cor-WT-derived iPSC-CMs compared to parental WT/MUT, genome-edited ins-MUT/MUT and del-KO/MUT iPSC-CMs (Fig. 1c–g). As expected, the insertion of the K117fs mutation in the line derived from the healthy control individual (WT/ins-MUT) induced arrhythmias (Extended Data Fig. 2d–g). Together, these data suggest that LMNA K117fs is a pathogenic mutation that causes LMNA-related DCM.

As homeostasis of calcium ions (Ca2+) is critical for excitation–contraction coupling in the heart13,14, we analysed the intracellular Ca2+–handling properties of the isogenic iPSC-CMs. Abnormal Ca2+ transients were observed in K117fs iPSC-CMs, whereas the control iPSC-CMs exhibited uniform Ca2+ transients (Fig. 2a). Furthermore, WT/ins-MUT iPSC-CMs displayed abnormal Ca2+ transients when compared to the isogenic WT/WT iPSC-CMs (Fig. 2b). Next, we recorded the calcium transient in the presence of tetrodotoxin, a sodium channel blocker, to inhibit any beating initiated at the plasma membrane15,16. We observed spontaneous Ca2+ cycling at very low extracellular Ca2+ levels in WT/MUT iPSC-CMs in contrast to the
minimal occurrence of Ca$^{2+}$ activity found in the isogenic control line (WT/cor-WT), suggesting that abnormal calcium release from the sarcoplasmic reticulum occurred in the WT/MUT iPS-CMs (Extended Data Fig. 3a–c). Taken together, these findings demonstrated that the dysregulation of Ca$^{2+}$ in the sarcoplasmic reticulum is associated with the electrical abnormalities observed in K117fs iPS-CMs.

Given that hyperphosphorylation of ryanodine receptor 2 (RYR2) by Ca$^{2+}$/calmodulin-dependent kinase II (CAMK2) leads to arrhythmias related to delayed afterdepolarizations\(^3\), as documented in the LMNA-mutant iPS-CMs (Fig. 1d, e), we tested whether the activation of this pathway induces arrhythmias in K117fs iPS-CMs. Notably, phosphorylated RYR2 (pRYR2) and phosphorylated CAMK2D (pCAMK2D) levels were significantly increased in K117fs iPS-CMs (WT/MUT, ins-MUT/MUT and del-KO/MUT) compared to the levels found in the isogenic control iPS-CMs (WT/cor-WT) (Fig. 2c, d). By contrast, expression levels of both CAMK2D and RYR2 mRNA were similar between isogenic control and K117fs iPS-CMs (Extended Data Fig. 3d–g). When the activation of CAMK2D was inhibited in K117fs iPS-CMs using KN93, a specific CAMK2D inhibitor, we observed a significant decrease in the levels of pRYR2 and pCAMK2D as well as a significant decrease in abnormal Ca$^{2+}$ transients (22.2%, \(n = 81\)) compared to K117fs iPS-CMs treated with vehicle (65.38%, \(n = 52\)) or the inactive analogue KN92 (65.30%, \(n = 49\)) (Fig. 2e and Extended Data Fig. 3h–j). Taken together, these data suggest that CAMK2D-mediated RYR2 activation causes abnormal Ca$^{2+}$ handling and arrhythmias in K117fs iPS-CMs.

**Lamin A/C haploinsufficiency in mutant iPS-CMs**

Given that abnormalities in nuclear structures are associated with laminopathies\(^3\), we examined the integrity of the nuclear envelope in K117fs iPS-CMs. Through immunostaining analyses, we demonstrated that K117fs iPS-CMs display abnormal nuclear structures compared to isogenic controls (Fig. 3a and Extended Data Fig. 4a–c). Notably, the expression of lamin A/C proteins was significantly reduced in K117fs compared to isogenic control iPS-CMs. Furthermore, the full-length or truncated lamin A/C were not detected in ins-MUT/MUT and del-KO/MUT iPS-CMs (Fig. 3b, c and Extended Data Fig. 4d–f). These data suggest that the K117fs mutation leads to lamin A/C haploinsufficiency. Furthermore, the total level of LMNA mRNA expression was significantly reduced in K117fs compared to isogenic control iPS-CMs (Fig. 3d and Extended Data Fig. 4g).

Nonsense-mediated mRNA decay (NMD) is a mechanism coupled to translation that selectively degrades mRNAs that contain premature translation-termination codons\(^19,20\). To investigate whether NMD influences the expression levels of LMNA mRNA in K117fs iPS-CMs, we assessed allele-specific expression of LMNA mRNA. We found that 97% and 3% of the total LMNA mRNA was expressed by the wild-type and the K117fs allele, respectively, in the K117fs iPS-CMs (WT/MUT; III-3) (Fig. 3e and Extended Data Fig. 4h). We observed a significant increase in the expression levels of the K117fs allele (18–37%) and the appearance of a 14-kDa band upon inhibition of the NMD pathway in K117fs iPS-CMs (Fig. 3f and Extended Data Fig. 4i, j). In addition, the 14-kDa band was not detected in the isogenic control line (WT/cor-WT) after NMD inhibition (Extended Data Fig. 4k), which suggests that the truncated lamin A/C is translated from the mutant LMNA mRNA. Collectively, these findings indicate that NMD-mediated degradation of mutant LMNA mRNA induces lamin A/C haploinsufficiency in K117fs iPS-CMs.
Increased open chromatin in mutant iPSC-CMs

Lamin A/C interacts with heterochromatin-rich genomic regions at the nuclear envelope called lamin-associated domains (LADs), which have an essential role in the organization of chromatin. We therefore postulate that lamin A/C haploinsufficiency could disturb chromatin distribution, leading to aberrant gene expression in K117fs iPSC-CMs. Using an assay for transposable-accessible chromatin with visualization (ATAC-seq), we observed that the distribution of open chromatin was biased towards the nuclear periphery in K117fs iPSC-CMs, whereas isogenic control iPSC-CMs showed a uniform distribution throughout the nucleus (Extended Data Fig. 5a–g).

To study whether lamin A/C haploinsufficiency results in an abnormal conformation of open chromatin, we investigated the relationship between LADs and gene activation. We compared the LADs in isogenic iPSC-CMs and grouped these LADs into three categories: loss, overlapping and gain (Fig. 4a). The genomic coverage, mean LAD sizes, and the number of LADs in the three categories are shown in Fig. 4b. The genomic regions associated with LADs were more actively expressed in K117fs iPSC-CMs compared to control iPSC-CMs (Fig. 4c). An analysis of chromatin conformation and histone modifications showed that the transcription factor PRRX1 was more actively expressed in K117fs iPSC-CMs compared to control iPSC-CMs (Fig. 4d). We also observed that the distribution of open chromatin was biased towards the nuclear periphery in K117fs iPSC-CMs, whereas isogenic control iPSC-CMs showed a uniform distribution throughout the nucleus (Extended Data Fig. 5a–g).

The PDGF pathway links to arrhythmic phenotype

To identify additional potential target genes that are closely associated with the disease phenotype, we compared the transcriptomes of K117fs mutant and control iPSC-CMs. By comparing the total RNA expression of control iPSC-CMs versus K117fs iPSC-CMs, we found that most of the differentially expressed genes were upregulated in K117fs iPSC-CMs (III-3, 84.87%; IV-1, 70.80%) (Fig. 5a). A cross-analysis of differentially expressed genes based on two different genetic backgrounds (III-3 and IV-1) identified 257 genes for which the expression in K117fs iPSC-CMs significantly differed from that in isogenic control iPSC-CMs (Fig. 5b). As expected, 239 out of 257 genes (93%) were upregulated in K117fs iPSC-CMs compared to isogenic control iPSC-CMs (Fig. 5c). Gene ontology (GO) enrichment analysis revealed that the upregulated genes in K117fs iPSC-CMs were functionally enriched in terms associated with platelet-derived growth factor (PDGF) binding, arylsulfatase activity, protein binding involved in cell–matrix adhesion and PDGF receptor binding (Fig. 5d). The ARCH54 kinase analysis also showed that the upregulated genes in K117fs iPSC-CMs were highly enriched in the PDGF pathway.
The PDGF signalling is highly activated in smooth muscle and endothelial cells, and is initiated through the activation of two major receptors belonging to the PDGF receptor-α (PDGFRA) and PDGF receptor-β (PDGFRB) family.30 During cardiomyocyte differentiation, PDGFRB is highly upregulated in the early stages of differentiation but become downregulated after generating functional cardiomyocytes31 (Extended Data Fig. 9a). In particular, expression of PDGFRB mRNA and protein expression occurred in K117fs iPSC-CMs compared to control iPSC-CMs (Extended Data Fig. 9d–f). In addition, a kinase array showed hyperactivation of PDGFRB in K117fs iPSC-CMs compared to isogenic control iPSC-CMs (Extended Data Fig. 9g). Furthermore, we found that the promoter region of the PDGFRB was more accessible in K117fs iPSC-CMs, as demonstrated by high enrichment of an active histone marker (H3K4me3) and open chromatin in

**Fig. 4 | Lamin A/C haploinsufficiency results in reduced lamin A/C enrichment and increased open chromatin formation of each LAD.**

a. Representative images of chromatin immunoprecipitation followed by sequencing (ChIP-seq). ATAC-seq and RNA-seq of chromosome 20. The sc-376248 anti-LMNA antibody was used for ChIP-seq. b–d, Number (b), genomic coverage (c) and mean of length of LADs (d) in control, mutant, gained, overlapping and lost LADs. e, Location of LADs in the loss or gain category. LADs located within ±100 kb of overlapping LADs are shown as 'partial'. LADs located outside of ±100 kb of overlapping LADs are shown as 'entire'. LADs partially shared with one LAD are shown as 'none'. f, Comparison of normalized ATAC enrichment of each LAD in control and mutant iPSC-CMs. Red, percentage of LADs that showed upregulated normalized ATAC enrichment in mutant iPSC-CMs compared to control iPSC-CMs. Blue, percentage of LADs that showed downregulated normalized ATAC enrichment in mutant iPSC-CMs compared to control iPSC-CMs. g, Normalized ATAC-seq signal intensity around the transcription start site (TSS) of genes located in each LAD category. h, Scatter plot of normalized lamin A/C and ATAC enrichment of each LAD (n = 588). The y axis shows the log2-transformed relative normalized lamin A/C enrichment of each LAD in mutant iPSC-CMs compared to control iPSC-CMs. Note, in the graph, log2(MUT/WT) indicates log2(lamin A/C enrichment of each LAD in MUT/lamin A/C enrichment of each LAD in WT). The x axis shows the log2-transformed relative normalized ATAC enrichment of each LAD in mutant iPSC-CMs compared to control iPSC-CMs (shown as log2(MUT/WT)). j, Percentage of differentially expressed genes located in LADs. False-discovery rate (FDR)-corrected P < 0.01; log2-transformed fold change in expression of >1 or <−1.

k, Representative images of ChIP-seq, ATAC-seq and RNA-seq. Blue boxes, LADs with lower enrichment of lamin A/C and higher expression in mutant iPSC-CMs compared to control iPSC-CMs.
Abnormal activation of PDGFRB is required for the arrhythmic phenotype of mutant iPSC-CMs. a, Number of differentially expressed genes in mutant iPSC-CMs compared to control iPSC-CMs. LMNA WT/MUT and LMNA WT/cor-WT were derived from patient III-3. LMNA WT/WT and WT/ins-MUT were generated form health control IV-1. b, Venn diagram of differentially expressed genes in mutant iPSC-CMs compared to control iPSC-CMs. c, Heat maps of log2-transformed fold change in expression of 257 differentially expressed genes in mutant iPSC-CMs compared to control iPSC-CMs. d, GO and ARCHS4 kinase coexpression analysis of differential expressed genes. Colour codes indicate the combined FDR and Z-score. e, Immunoblot analysis of PDGFRB in control and mutant iPSC-CMs. f, Quantification of signal intensity of LMNA. g, qPCR analysis of PDGFRB expression levels in control and mutant iPSC-CMs. h, Representative Ca2+ transients of mutant iPSC-CMs treated with scramble siRNA or siRNA against PDGFRB. All traces were recorded for 20 s. i, Quantification of cells that exhibit arrhythmic waveforms as shown in h. j, Quantification of cells that exhibit arrhythmic waveforms of Ca2+ transients for mutant iPSC-CMs treated with the PDGFRB inhibitors crenolanib (CB) (100 nM) and sunitinib (SB) (500 nM) for 24 h. k, Immunoblot analysis of pCAMK2D and CAMK2D protein levels after treatment with dimethyl sulfoxide (DMSO), crenolanib or sunitinib. The analyses were independently repeated twice with similar results. l, Hierarchical clustering of amplicon-based sequencing (AmpliSeq) transcriptome data; analysed by one-way ANOVA (P = 0.05). Two different K117fs iPSC-CMs lines treated with crenolanib, sunitinib or DMSO were analysed by RNA-seq. The total number of genes is 915. m, n, GO analysis identified a set of genes that was related with muscle contraction and regulation of cardiac conduction. f, g, Data are mean ± s.e.m.; statistical significance was calculated using one-way ANOVA. The Ca2+ transients shown in h were independently repeated as described in i with similar results.
n = 90) (Extended Data Fig. 10h–j). These data indicate that the abnormal activation of PDGFRB contributes to the arrhythmic phenotype observed in K117fs iPSC-CMs.

To test the effects of the abnormal activation of PDGFRB on the gene-expression profile of K117fs iPSC-CMs, we next evaluated how treatment with crenolanib and sunitinib affected the transcriptome of K117fs iPSC-CMs. We identified a total of 910 genes that were differentially expressed between the treated and the untreated groups (Fig. 5l). GO term analysis of downregulated genes in the treated groups showed a high enrichment of genes related to heart functions, including muscle contraction, the regulation of cardiac conduction and ion transport (Fig. 5m, n and Extended Data Fig. 11a, b). We confirmed significant changes in the expression of genes related to cardiac muscle contraction and actin-mediated cell contraction through the knockdown of PDGFRB in K117fs iPSC-CMs (Extended Data Fig. 11c–e). We found that there were no differences in the lamin A/C level or the nuclear structure after treatment with crenolanib or sunitinib (Extended Data Fig. 11f–h). Taken together, these data confirm that the lamin A/C haploinsufficiency causes the abnormal activation of the PDGFR signalling pathway, leading to the development of arrhythmias in LMNA-related DCM.

Discussion

Lamin A/C proteins are key components of heterochromatin conformation and the gene-silencing machinery, and are expressed in a cell-type-specific manner. Here we elucidate how lamin A/C haploinsufficiency affects chromatin conformation and the gene-expression profile of LMNA-mutant iPSC-CMs. Furthermore, we demonstrate that the inhibition of the PDGFR pathway ameliorates the arrhythmic phenotype of K117fs iPSC-CMs, suggesting a novel therapeutic target for the treatment of LMNA-related DCM (Extended Data Fig. 12). Our study suggests that several FDA-approved PDGFRB inhibitors—such as sunitinib, sorafenib and axitinib—may be repurposed for this condition. However, our previous study using a human iPSC-CM platform also revealed dose-dependent cardiac toxicity that is implicated in most tyrosine kinase inhibitors. Therefore, further studies are warranted to identify the proper dosage or alternatives to these inhibitors that can be safely used in vivo to optimally alter the PDGFR signalling pathway and prevent the fatal arrhythmias that are frequently observed in patients with LMNA-related DCM.

Online content

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METHODS
No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators who performed electrophysiological tests and Ca2+ imaging analysis were blinded to group allocation during experiments and data collection. The studies comply with all ethical regulations.

Patient recruitment. The fibroblasts, PBMCs and heart tissues were obtained from patients using IRB-approved protocols at Stanford University (protocols 17576 and 29904). Informed consent was obtained from all patients who were included in our study. Clinical features of patients are described in the Extended Data Fig. 1d.

Culture and maintenance of iPSCs. iPSC lines were maintained in a chemically defined Essential 8 (E8 medium) medium (Life Technologies) on Matrigel-coated (BD Bioscience) plates at 37 °C with 5% (v/v) CO2.

Pluripotency marker analysis. Human iPSC colonies grown in Matrigel-coated 8-well chamber glasses (Thermo Scientific) were fixed using 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. After blocking samples with 5% goat serum in PBSB (PBS with 0.1% Tween-20), cells were stained with mouse anti-SSEA4 (RD systems) antibodies, rabbit anti-OCT3/4 (Santa Cruz Biotechnology), rabbit anti-NANOG (Santa Cruz Biotechnology) and mouse anti-SOX2 (RD systems) antibodies. Cells were then incubated with Alexa Fluor-conjugated secondary antibodies (Life Technologies) and Hoechst 33342 (Life Technologies) to visualize the specific stains. Image acquisition was performed on an Eclipse 80i fluorescence microscope (Nikon Instruments).

TALEN-mediated homologous recombination. TALEN pair vectors were designed and constructed using the rapid TALEN assembly system as previously described11. In brief, 500 base-pair (bp) fragments of wild-type LMNA exon 1 and adjacent intronic sequences were amplified using PCR in standard 96-well microplates with the cell-permeable calcium-sensitive dye fura-2 AM (2 μM) with the cell-permeable calcium-sensitive dye fura-2 AM (2 μM), and excited at both 340 and 380 nm, and the emission fluorescence signal was measured with an Infinite M1000 PRO microplate reader (Tecan). Gene expression was measured by TaqMan qPCR using mouse-specific primers and probes (Life Technologies). Data were acquired using the CFX96 Real-Time PCR system and analyzed using the CFX Manager software. The expression of the housekeeping gene 18S was normalized to the expression of the target gene, and the fold change was calculated using the 2^(-ΔΔCt) method.

Immunocytochemistry. Cells grown on coverslips were fixed using 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, incubated with primary antibodies and Hoechst 33342, and detected using Alexa Fluor-conjugated secondary antibodies. Primary antibodies include rabbit anti-cardiac troponin T (Abcam), mouse anti-cardiac troponin T (Thermo Scientific), mouse anti-seromucoid α-actinin (Sigma-Aldrich), goat anti-LMNA (Santa Cruz) and rabbit anti-LMNA (Santa Cruz) antibodies. Image acquisition was performed on an Eclipse 80i fluorescence microscope, a confocal microscope (Carl Zeiss, LSM 510 Meta) and ZEN software (Carl Zeiss). Reverse transcription and quantitative PCR. Total mRNA was isolated from iPSC-CMs using the Qiagen miRNeasy Mini kit. Subsequently, 1 μg of RNA was used to synthesize cDNA using the iScript cDNA Synthesis kit (Bio-Rad). Then, 0.25 μl of the reaction was used to quantify gene expression by qPCR using TaqMan Universal PCR Master Mix. Expression values were normalized to the average expression of the housekeeping gene 18S.

Western blotting. Proteins were resolved by SDS–PAGE and were transferred to 0.45-μm nitrocellulose membranes (Bio-Rad) using a mini Bio-Rad Mini PRO Transblot cell (Bio-Rad). Membranes were then blocked in Membrane Blocking Solution (Life Technologies) and incubated with primary antibodies overnight at 4 °C. Blots were incubated with the appropriate secondary antibodies for 1 h at room temperature and visualized using the ECL Western Blotting Analysis System (GE Healthcare). Primary antibodies used were mouse anti-LMNA (Santa Cruz), rabbit anti-LMNA (Santa Cruz), CAMK2D (Abcam), PDGFRB (Cell Signaling), RYR2 (Abcam), pRYR2 (D. M. Bers laboratory) and HRP-conjugated α-tubulin (Cell Signaling).

Patch-clamp recordings. Whole-cell action potentials were recorded using a standard patch-clamp technique. In brief, cultured iPSC-CMs were plated on No. 1 18-mm glass coverslips (Warner Instruments) coated with Matrigel, placed in a RC-260 cell holder (Warner Instruments) and mounted onto the stage of an inverted microscope (Nikon). The chamber was continuously perfused with warm (35–37 °C) extracellular solution (pH 7.4) of the following composition: NaCl (140 mM), KCl (5.4 mM), CaCl2 (1.8 mM), MgCl2 (1 mM), HEPES (10 mM) and glucose (10 mM); pH was adjusted to 7.4 with NaOH. Glass micropipettes were fabricated from standard wall borosilicate glass capillary tubes (Sutter BF 100-50-10, Sutter Instruments) using a programmable puller (P-97; Sutter Instruments) and filled with the following intracellular solution (in mM): 120 KCl, 1.0 MgCl2, 10 HEPES, 10 EGTA and 3 Mg-ATP (pH 7.2). A single-beating cardiomyocyte was selected and action potentials were recorded in whole-cell current-clamp mode using an EPC-10 patch-clamp amplifier (HEKA). Data were acquired using PatchMaster software (HEKA) and digitized at 1 kHz.

Differencing of iPSC-CMs. iPSCs were grown to 90% confluence and subsequently differentiated into beating cardiomyocytes, using a small-molecule-based monolayer method that has previously been described24,25. After 10 days of cardiac differentiation, iPSC-CM monolayers were purified using RPMI-1640 without glucose (Life Technologies) and with B-27 supplement (Life Technologies). The non-glucose culture medium was changed every two days. After five days, iPSC-CMs were cultured in a culture medium containing glucose (Life Technologies). The viable iPSC-CMs were then cycled to end point per the manufacturer's protocol with an annealing temperature of 66 °C over 30 min. After 15 min of washing in 1.8 mmol l−1 NaCl, KCl (5.4 mM), CaCl2 (1.8 mM), MgCl2 (1 mM), HEPES (10 mM) and glucose (10 mM); pH was adjusted to 7.4 with NaOH. Glass micropipettes were fabricated from standard wall borosilicate glass capillary tubes (Sutter BF 100-50-10, Sutter Instruments) using a programmable puller (P-97; Sutter Instruments) and filled with the following intracellular solution (in mM): 120 KCl, 1.0 MgCl2, 10 HEPES, 10 EGTA and 3 Mg-ATP (pH 7.2). A single-beating cardiomyocyte was selected and action potentials were recorded in whole-cell current-clamp mode using an EPC-10 patch-clamp amplifier (HEKA). Data were acquired using PatchMaster software (HEKA) and digitized at 1 kHz.

Droplet digital PCR. Total RNA was extracted from iPSC-CMs at day 30 post-differentiation using the miRNeasy Mini kit (Qiagen) and cDNA preparation was carried out using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories). The concentration of cDNA was reduced to about 0.2 ng μl−1 RNA equivalent, and 1 ng (5 μl of 0.2 ng μl−1) of RNA-equivalent cDNA was mixed with primers, probes and ddPCR Supermix reaction (total volume 20 μl). The final concentrations of the primers and the probe were 900 nM and 500 nM, respectively. The following primers and probes for discriminating allelic expression of LMNA K117 (wild-type allele) from K117fs (mutant allele) were used: forward primer, 5′-GCAAGACCCTTGACTCAGTA-3′; reverse primer, 5′-CTCTTGGAGTGACGACG-3′; wild-type probe: 5′(-FAM)-TGCGCGTTTCAGCTCCTTAA-(Blackhole Quencher)3′; mutant probe, 5′(HEX)-TGCGCGTTTCAGCTCCTTAA-(Blackhole Quencher)3′. Droplet formation was carried out using a QX100 droplet generator. A rubber gasket is placed over the cartridge and loaded into the droplet generator. The emulsion (35 μl in volume) was then slowly transferred using a multichannel pipette to a 96-Well twin-tc PCR Plate (Eppendorf). The plate was heat-sealed with foil and the emulsion was cycled to end point per the manufacturer's protocol with an annealing temperature of 61 °C. Finally, the samples were analysed using a BioRad QX100 reader. Ca2+ imaging. iPSC-CMs seeded on a glass cover slip for 5–7 days were loaded with the cell-permeable calcium-sensitive dye fura-2 AM (2 μmol l−1) for 20 min. After 15 min of washing in 1.8 mmol l−1 Ca2+ Tyrode (135 mmol l−1 NaCl, 4 mmol l−1 KCl, 1 mmol l−1 MgCl2, 5 mmol l−1 glucose and 10 mmol l−1 HEPES, pH 7.4) buffer to allow de-esterification, coverslips were mounted on the stage of an inverted epifluorescence microscope (Nikon Eclipse Ti-S). iPSC-CMs were field-stimulated at 0.5 Hz with a pulse duration of 10 ms. Fura-2-AM-loaded cells were excited at both 340 and 380 nm, and the emission fluorescence signal was collected at 510 nm as previously described24,25. Changes in fluorescence signal were monitored using the ISIS Fluor (D. M. Bers laboratory) and moving the single-cell automatically sampling of multiple cells in one view. Intracellular calcium changes were expressed as changes in the ratio R = F150/F380 and the calcium transient waves were analysed with a previously published method26.
Measuring abnormal calcium release from the sarcoplastic reticulum. Both patient- and healthy individual-derived iPSC-CMs were seeded on coverslips as single cells. After 3–4 days of recovery, the cells were loaded with 5 μM Fluo-4 AM at 37 °C for 10 min and then washed with Tyrode’s solution three times. Ca2+ release events were recorded with a Carl Zeiss confocal (710) in line-scanning mode (512 pixels × 1,920 lines). The extracellular media were prepared with sequential increases of Ca2+ concentration (0, 0.5, 1, 2 and 5 mM), and were used to treat iPSC-CMs during data recording. The Ca2+ imaging data were displayed and analysed using Image J.

Measuring sarcomeric alignments. Immunostaining images of iPSC-CMs were viewed with Image J, and the fluorescent signals along the sarcomere structure were pulled out. A custom-made Interactive Digital Language algorithm was used to analyse the regularity of sarcomere signal distribution with fast Fourier transformation (FFT). The sarcomere length and the regularity of sarcomere distribution were indicated as the position and the height of the first main peak after FFT data processing.

ChIP-seq. LMNA antibodies (Santa Cruz Biotechnology; sc-376248 and Abcam; 8984) were incubated with Dynabeads (Life Technologies; 10003D) for 12 h at 4 °C. A small portion of the crosslinked, sheared chromatin was saved as the input, and the remainder was used for immunoprecipitation using antibody-conjugated Dynabeads. After overnight incubation at 4 °C, the incubated beads were rinsed with sonication buffer (50 mM HEPES pH 7.9, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 0.5 mM PMSF), a high-salt buffer (50 mM HEPES pH 7.9, 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 0.5 mM PMSF) and a LiCl buffer (20 mM Tris, pH 8.0, 1 M LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, 0.5 mM PMSF). The washed beads were incubated with elution buffer (50 mM Tris, pH 8.0, 1 mM EDTA, 1% SDS, 50 mM NaHCO3) for 1 h at 65 °C and then de-crosslinked with 5 M NaCl overnight at 65 °C. The immunoprecipitated DNA was treated with RNase A and proteinase K and purified by ChIP DNA Clean and Concentrator (Zymo Research; D5205). The raw sequencing data were analysed as previously described.

RNA-seq. For each sample in the whole-transcriptome sequencing library, 60–80 million 75-bp paired-end reads were acquired from the sequencer. Base quality of raw reads is high after checking with FastQC 0.11.4. Using STAR 2.5.1b, we aligned the reads to the human reference genome (hg19), with splice junctions defined by the GTF file downloaded from UCSC. On average, 92% of reads were aligned to the reference genome, and 83% of reads were uniquely aligned to the reference genome. Gene expression was determined by calculating the FPKM using Cufflinks 2.2.1. In addition, Cufflinks was used to determine differential expression between each two conditions.

ATAC-seq. The samples were treated and processed as previously described. In brief, 100,000 cells were centrifuged at 500g for 5 min at room temperature. The cell pellet was resuspended in 50 μl lysis buffer (10 mM Tris-C pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.01% Igepal CA-630) and centrifuged immediately at 50g for 10 min at 4 °C. The cell pellet was resuspended in 50 μl transposome mixture (25 μl 2× TD buffer, 22.5 μl DHO2 and 2.5 μl Illumina Tn5 transposase or 100 nM transposase (final concentration) Atto-590-labelled in-house generated Tn5) and incubated at 37 °C for 30 min. After transposition, the mixture was purified with the Qiagen Mini purification kit and eluted in 10 μl Qiangen EB elution buffer. Sequencing libraries were prepared following the original ATAC-seq protocol. The sequencing was performed on Illumina NextSeq at the Stanford Functional Genomics Facility. ATAC-seq reads were trimmed of adapters and then mapped to hg19 genome assembly using Bowtie 2. Following quality control to remove duplicate reads, average read intensities were calculated with the aid of deepTools and R/Bioconductor (v.3.2.1) Promoter regions were defined as ±1 kb around the gene transcription start sites coordinates unless otherwise stated.

ATAC-seeq. The samples were treated and processed as previously described. In brief, iPSC-CMs were fixed with 1% formaldehyde (Sigma) for 10 min and then washed with 0.125 M glycine for 5 min at room temperature. After fixation, the cells (either growing on slides or centrifuged on glass slides with Cytospin) were permeabilized with a lysis buffer (10 mM Tris-C pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.01% Igepal CA-630) for 10 min at room temperature. After permeabilization, the slides were rinsed in PBS twice and placed in a humid chamber box at 37 °C. The transposase mixture solution (25 μl 2× TD buffer, final concentration of 100 nM Tn5-ATTO-590N, adding DHO2 up to 50 μl) was added to the slide and incubated for 30 min at 37 °C. After the transposase reaction, slides were washed with PBS containing 0.01% SDS and 50 mM EDTA for 15 min three times at 55 °C. After washing, slides were mounted using Vectashield with DAPI (H-1200, Vector Laboratories). Fluorescence images were captured on a confocal microscope equipped with an oil-immersion lens. Fluorescent intensity profiles of DAPI and ATAC were exported using ZEN (Zeiss). To find out whether the LMNA mutation led to the specific re-distribution of the epigenetic histone markers in the iPSC-CMs, the correlation between ATAC-seq and DAPI signal of each nucleus was calculated using the Pearson correlation method. Image analysis was conducted using Graphpad Prism v.7.0.

RNA-seq and ChIP-seq analysis. FastQC (v.0.11.5) and MultiQC (v.1.3) were used to assess the quality of the reads. Adaptor and quality trimming of reads were performed with trimmomatic (v.0.36). Reads were mapped to the hg19 reference genome using STAR (v.2.5.3a) with ENCODE long RNA-seq parameters. Uniquely mapped reads were filtered for and bigWig files were generated with samtools (v.1.4). FPKM values were calculated with different expression and compared with cuffdiff (v.2.2.1). ChIP-seq data were processed using the AQUAS pipeline from the Kundaje laboratory at Stanford University (https://github.com/kundajelab/chip-seq-pipeline2), which has an end-to-end implementation of the ENCODE (phase 3) ChIP-seq pipeline. Default parameters were used with the exception of specifying ‘-type histone -species hg19’. Before LAD detection of LMNA data, duplicated reads were removed with ‘mark duplicates’ from Picard tools (v.2.17.3) and ‘DownsampleSam’ was used to downsample the larger of each pair of aligned input and ChIP read files, giving each pair the same read depth and avoiding normalization bias.

LAD detection and analysis. Lamin A/C binding data were analysed using Enriched Domain Detector with an 11-kb bin size, gap penalty of 5, and FDR-adjusted significance threshold of P < 0.05. Gains, losses and intersections in LADs between control and mutant cells were tallied using bedtools (v.2.27.1).

Gene expression changes within each category of LADs (gain, loss or shared in mutant and control) based on RNA-seq data were compared in R v.3.2.1 and Bioconductor using the iRanges and GenomicRanges packages. In deciding whether a gene overlaps with each category, the union of called peaks from the lamin A/C ChIP-seq of two antibodies and the intersection of two cell lines were used. A gene is considered to reside in a particular LAD if any of its hg19-annotated transcription start sites overlaps with the LAD range by genomic coordinates. In cases of ambiguity, intersection (shared between control and mutant cells) regions take precedence over gain and loss regions. ATAC-seq read intensities within each LAD category around genomic features, including transcription start sites and transcription end sites, were visualized using deepTools with feature coordinates from hg19 annotations.

Statistical analyses. Data were expressed as mean ± s.e.m. Immunoblots are representative of at least two independent experiments. All other experiments are the average of at least 2 independent assays, and for cell number calculations in immunostaining assays, at least 100 cells per sample were counted for each independent experiment. Statistical analyses were performed using GraphPad Prism (v.6.0e). An unpaired two-tailed Student’s t-test was used to calculate significant differences between two groups. Multiple comparison correction analysis was performed using one-way ANOVA followed by Tukey’s post hoc HSD test. P < 0.05 was considered statistically significant.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Data are available from the Gene Expression Omnibus (GEO: GSE118885).

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Competing interests H.Y.C. is a advisor to10x Genomics. Stanford University has filed a patent application on ATAC-see technology, in which H.Y.C. is named a co-inventor. J.C.W. is a co-founder of Khloris Biosciences but has no competing interests, as the work presented was performed independently.

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Extended Data Fig. 1 | LMNA-mutant iPSC-CMs can recapitulate arrhythmic phenotype of patients with LMNA-related DCM.

a, Schematic pedigree of the family carrying the mutation in LMNA. Patients (III-1, III-3, III-9, III-15 and III-17) and healthy individuals (IV-1 and IV-2) recruited for this study are numbered. Circles represent female family members and squares represent males. The '+' and '-' signs underneath family members indicate the presence or absence of the mutation in LMNA, respectively.

b, Schematic view of 348-349insG frameshift mutation in LMNA. c, Genotyping of fibroblasts derived from patients and healthy controls. MT, mutant. d, Clinical features of patients and healthy individuals. e, Electrocardiogram of patients III-1 and III-3 and a healthy individual (IV-1). The electrocardiogram data were measured once per individual. f, g, Electrophysiological measurements of spontaneous action potentials in mutant iPSC-CMs (III-15 and III-17) recorded by patch clamp in current-clamp mode. The experiments were repeated three times independently with similar results.
Extended Data Fig. 2 | The mutation in LMNA is a cause of the arrhythmic phenotype in LMNA-mutant iPSC-CMs. a, Gene-editing strategy using the TALEN method. The piggyBac system was used to generate isogenic lines as previously described11,12. b, Genotyping of gene-edited isogenic lines (III-3 corrected, insertion, deletion; IV-1 insertion). For LMNA del-KO/MUT, we used TALEN pairs that target the start codon of LMNA. Genotyping showed the C insertion in the wild-type allele that leads early stop codon. c, Immunostaining of NANOG (red, left), POU5F1 (red, middle) and SOX2 (red, right) in iPSC lines. Blue, DAPI. Scale bars, 10 μm. The experiments were repeated twice independently with similar results. d–f, Electrophysiological recordings of spontaneous action potentials in control (IV-1) and mutant (III-9, isogenic IV-1; WT/ins-MUT) iPSC-CMs measured by patch clamp in current-clamp mode. Red arrows indicate delayed afterdepolarization-like arrhythmias. The experiments were repeated three times independently with similar results. g, Action potential parameters of ventricular-like iPSC-CMs. MDP, maximal diastolic potential; APA, action potential amplitude; APD, action potential duration at 50%, 70%, 90% of repolarization; bpm, beats per min.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3  | Abnormal calcium handling in LMNA-mutant iPSC-CMs. a, Confocal imaging of Fluo-4 AM calcium events in control (III-3; WT/cor-WT) and mutant (III-3; WT/MUT) iPSC-CMs while being treated with increasing extracellular Ca\(^{2+}\) concentrations. All representative traces were recorded from three individual cells (presented as red, blue and black). b, Spontaneous calcium events per 100 s of control and mutant iPSC-CMs for each extracellular Ca\(^{2+}\) concentration. c, Summary of the percentage of cells that have spontaneous Ca\(^{2+}\) release events from the sarcoplasmic reticulum in control and mutant iPSC-CMs. d, qPCR analysis of CAMK2D and RYR2 expression in control and mutant iPSC-CMs. Data are mean ± s.e.m. e, f, Immunoblot analysis of pRYR2, RYR2, pCAMK2D and CAMK2D protein levels in control and mutant iPSC-CMs. Data are mean ± s.e.m.; a two-tailed Student’s t-test was used to calculate P values; n = 3; values above the lines indicate significance. g, qPCR analysis of CAMK2D expression in control and mutant iPSC-CMs. Expression level of GAPDH was used as control. Data are mean ± s.e.m.; n = 8. h, Representative Ca\(^{2+}\) transients of mutant iPSC-CMs (III-3; WT/MUT) treated with 1 μM of KN92 or KN93 for 24 h. i, Quantification of the percentage of cells that exhibit arrhythmic waveforms in mutant iPSC-CMs (III-17 WT/MUT) at baseline, as well as after the treatment with 1 μM of KN92 or KN93 for 24 h. j, Immunoblot analysis of pRYR2, RYR2, pCAMK2D and CAMK2D protein levels after treatment of DMSO, KN92 or KN93 for 24 h. The experiments in a were repeated twice independently with similar results. The Ca\(^{2+}\) transient analyses in h were repeated as described in Fig. 2e independently with similar results. The immunoblot analyses in e and j were repeated twice independently with similar results.
Extended Data Fig. 4 | Downregulation of mutant mRNA through NMD pathway in LMNA-mutant iPSC-CMs. a, Quantification of cells showing abnormal nuclear structures in control and mutant iPSC-CMs. The images were recorded from three differentiation batches. n = 215 (WT/cor-WT), n = 286 (WT/MUT), n = 222 (ins-MUT/MUT) and n = 280 (del-KO/MUT). b, Representative confocal images of control and mutant lines. Micro-patterned iPSC-CMs were stained with specific antibodies against TNNT2 (red), LMNA (white) and LMNB1 (green). Blue, DAPI. Scale bar, 20 μm. The experiments were repeated three times independently with similar results. c, Quantification of cells showing abnormal nuclear structures in control and mutant iPSC-CMs. The images were recorded from three differentiation batches. Data are mean ± s.e.m.; a two-tailed Student’s t-test was used to calculate P values; n = 3 (total number of counted cells, 175 (WT/WT) and 203 (WT/ins-MUT)); the value above the line indicates significance. d, Immunoblot analysis of lamin A/C levels in control and mutant iPSC-CMs. e, Quantification of signal intensity of the lamin A/C band in d. Data are mean ± s.e.m.; statistical significance was obtained using one-way ANOVA; values above the line indicate significance; n = 10 (WT/WT), n = 7 (WT/ins-MUT), n = 5 (WT/MUT). f, Immunoblot analysis of lamin A/C levels in two different clones of control and mutant iPSC-CMs. Two different antibodies that recognize the N terminus of lamin A/C were used. GAPDH was used as loading control. g, Relative mRNA expression of total LMNA in control and mutant iPSC-CMs. Data are mean ± s.e.m.; a two-tailed Student’s t-test was used to calculate P values; the value above the line indicates significance; n = 10 (WT/WT), n = 7 (WT/ins-MUT). h, Confirmation of allele-specific primers using plasmid carrying wild-type LMNA or mutant LMNA. Digital PCR using allele-specific primers detected the ratio of wild-type/mutant LMNA, which was consistent with the ratio of wild-type/mutant plasmids. Data are mean ± s.d.; n = 3. i, Immunoblot analysis of cell lysates from mutant iPSC-CMs treated with emetine and wortmannin. Two different batches of antibodies were used. Red asterisks indicate the truncated lamin A/C with a 14-kDa size. j, Immunoblot analysis of cell lysates from mutant iPSC-CMs treated with wortmannin. k, Immunoblot analysis of cell lysates from control iPSC-CMs treated with emetine and wortmannin. Three different batches of E-1 antibody detect the N terminus of LMNA and the 131C3 antibody detects the C terminus. l, Immunoblot analysis of cell lysates from control iPSC-CMs treated with wortmannin. The experiments in f, i–k were repeated twice independently with similar results.
Extended Data Fig. 5 | Lamin A/C Haploinsufficiency results in an abnormal distribution of open chromatin in LMNA-mutant iPSC-CMs. a–f, Representative images and normalized signal intensity of ATAC-see and DAPI of control and mutant iPSC-CMs. Data were obtained from different patient lines, including the uncorrected and isogenic lines of patient III-3 (a, b); the uncorrected and isogenic line of control IV-1 (c, d); and the line of patient III-15 (e, f) for normalized signal intensity of ATAC-see and DAPI. Data are mean ± s.e.m. g, Correlation of signal distribution between ATAC-see and DAPI. n = 42 (WT/WT), n = 28 (WT/cor-WT), n = 33 (del-KO/MUT), n = 32 (ins-WT/WT), n = 25 (WT/MUT) for normalized signal intensity of ATAC-see and DAPI. Data are mean and minimum to maximum; two-tailed Student’s t-test was used to calculate P values. The experiments in a, c, and e were repeated three times independently with similar results.
Extended Data Fig. 6 | Genomic and chromatin features of LADs in control and mutant iPSC-CMs. a, Normalized enrichment of lamin A/C ChIP–seq signals, histone markers (H3K4me3 and H3K27me3) and ATAC-seq signals within ±0.4 Mb of mapped LAD borders. The genomic locations of LADs were obtained from ChIP–seq on lamin A/C using two different antibodies (Abcam 8984, blue line, sc-376248, green line) in control iPSC-CMs (III-3). b, Representative images of ChIP–seq, ATAC-seq and RNA-seq of chromosome 12 (133 Mb). The red box shows LADs explicitly called in mutant iPSC-CMs (gain); the purple box shows LADs called in both control and mutant iPSC-CMs (overlapping); the blue box shows LADs explicitly called in control iPSC-CMs (loss). c–e, Number (c), genomic coverage (d) and mean of length of LADs (e) in control, mutant, gain, overlapping and loss LADs. ChIP–seq on lamin A/C (Abcam 8984) was used for data analysis. f, g, Average peak intensity of H3K4me3 and H3K27me3 of each LAD. n = 184 (loss), n = 370 (overlapping), n = 184 (gain) for H3K4me3; n = 273 (loss), n = 504 (overlapping), n = 273 (gain) for H3K27me3. Data are mean and minimum to maximum; Wilcoxon matched-pairs signed-rank test was used to calculate P values. h, Scatter plot of normalized lamin A/C, ATAC and histone marker (H3K4me3 and H3K27me3) enrichment of each LAD. The y axis shows the log2-transformed relative normalized lamin A/C enrichment of each LAD in mutant iPSC-CMs compared to control iPSC-CMs. The x axis shows the log2-transformed relative normalized ATAC and histone marks enrichment of each LAD in mutant iPSC-CMs compared with control iPSC-CMs. Each data point represents one LAD. The statistical significance was obtained using one-way ANOVA; n = 587 for sc-376248 and n = 585 for Abcam 8984. i, Percentage of differentially expressed genes in mutant iPSC-CMs compared to control iPSC-CMs. j, Number of differentially expressed genes located in mutant iPSC-CMs compared to control iPSC-CMs. (FDR-adjusted P < 0.01; log2-transformed fold change in expression of >1 or <−1). k, Distribution of log2-transformed fold change in FPKM in control and mutant iPSC-CMs. A non-parametric Kruskal–Wallis (testing for two-sided differences) followed by Dunn’s post hoc test was used to adjust for multiple comparisons; n = 266 (gain), n = 8171 (non-LADs), n = 835 (overlapping), n = 206 (loss).
Extended Data Fig. 7 | Abnormal distribution of H3K9 methylation in mutant iPSC-CMs. a, b, Representative images of immunofluorescence staining of control mutant iPSC-CMs. iPSC-CMs were stained with specific antibodies against H3K9me2 or H3K9me3 (green). Blue, DAPI. Scale bar, 1,000 nm. The experiments were repeated three times independently with similar results. c–e, Representative images of lamin A/C enrichment and LAD distribution of ChIP–seq data. ChIP–qPCR analysis of H3K9me2 and H3K9me3 enrichment on LAD regions. Data are mean ± s.d.; n = 3.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Transcription factors altered by lamin A/C haploinsufficiency contribute to the activation of genes located outside LADs. a, Distribution of absolute distances to the nearest LAD (by nucleotide distance) from the transcription start site of genes that are differentially expressed (top) or that show no significant difference in expression between mutant and control iPSC-CMs (bottom). b, Distribution of median absolute log2-transformed change in expression of genes with relatively long (>7.5 × 10^6 bp) distances to the nearest LAD (top) and genes with relatively short (<2.5 × 10^6 bp) distances to the nearest LAD (bottom). In each category, 500 genes were sampled with replacement over 10,000 times. c, d, Co-occurrence analyses of transcription factors and genes and coexpression analyses of ARCH54 transcription factors of differentially expressed genes located in non-LADs. Genes located in non-LADs are shown in blue; genes with no significant difference in gene expression between control and mutant iPSC-CMs are shown in black; genes located in LADs and highly expressed in mutant iPSC-CMs compared with control iPSC-CMs are shown in red. Top 200 differentially expressed genes located in non-LADs were used for the analysis. e, Representative images of ChIP–seq, ATAC-seq and RNA-seq of the genomic region of PRRX1. f, Relative mRNA expression of PRRX1, PDGFRB, GREM1, LUM and DCN in mutant iPSC-CMs treated with scramble or PRRX1 siRNA. Data are mean ± s.e.m.; a two-tailed Student’s t-test was used to calculate P values; n = 3 (PDGFRB and GREM1), n = 4 (DCN, LUM and PRRX1); values above the lines show significance.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | PDGFRB is upregulated in LMNA-mutant iPSC-CMs. a, Expression levels of PDGFRB and PDGFRB during the human iPSC-CM differentiation process. The data were adapted from previously published data (GSE76523). b, c, Protein and RNA levels of PDGFRB in human tissues. The data were adapted from the Human Protein Atlas Database (data available from http://www.proteinatlas.org/). d, qPCR analysis of PDGFRB expression in LMNA-mutant and control iPSC-CMs. Data are mean ± s.e.m.; a two-tailed Student’s t-test was used to calculate P values; n = 13 (WT/WT), n = 5 (WT/ins-MUT); the value above the line shows significance. e, Immunoblot analysis of PDGFRB protein levels in control versus mutant iPSC-CMs. GAPDH was used as loading control. The experiments were repeated twice independently with similar results. f, Flow cytometry analysis of TNNT2+ PDGFRB+ cells in control and mutant iPSC-CMs. n = 4. g, Kinase array of control and mutant iPSC-CMs. Fifty different protein kinases were presented in each chip. Top, raw images of the blotting membrane. Two dots carried the same antibody in technical duplicates. Bottom, quantification of the signal intensity of each spot. h, Representative images of ChIP–seq, ATAC-seq and RNA-seq on the genomic regions of PDGFRB. The promoter region of PDGFRB is highlighted by a blue box. i, ChIP–qPCR of H3K4me3 and H3K27me3 enrichment at the promoter region of PDGFRB in control and mutant iPSC-CMs. n = 3. j, k, qPCR analysis of LMNA and PDGFRB expression levels in left ventricular heart tissue from health controls (n = 3) and patients with LMNA-related DCM (n = 2). Data are mean ± s.e.m. The kinase data in g were repeated twice independently with similar results. f, i, Data are mean ± s.e.m.; statistical significance was obtained using one-way ANOVA; values above the lines show significance.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10  |  Arrhythmic phenotype in mutant iPSC-CMs is dependent on the activation of the PDGFRB pathway.  
a, qPCR analysis of PDGFRB expression levels in mutant iPSC-CMs (WT/MUT) treated with scramble or PDGFRB siRNAs. The cells were treated with siRNAs for 48 h. Data are mean ± s.e.m.; a two-tailed Student’s t-test was used to calculate P values; n = 3; the value above the line indicates significance.  
b, Representative Ca^{2+} transients of mutant iPSC-CMs (III-17 WT/MUT) treated with scramble siRNA or PDGFRB siRNA.  
c, Quantification of the number of cells that exhibited arrhythmic waveforms in b.  
d, Representative Ca^{2+} transients of mutant iPSC-CMs treated with PDGFRB inhibitors, crenolanib (100 nM) and sunitinib (500 nM), for 24 h. All traces were recorded for 20 s.  
e, Quantification of mutant iPSC-CMs (III-17, III-15 and III-3) that exhibited arrhythmic waveforms with or without the treatment of PDGFRB inhibitors, crenolanib (100 nM) and sunitinib (500 nM), for 24 h.  
f, Representative Ca^{2+} transients of mutant iPSC-CMs (III-17 WT/MUT) treated with PDGFRB inhibitors.  
g, Immunoblot analysis of pRYR2 and RYR2 protein levels with treatment of DMSO, crenolanib or sunitinib. The data were repeated twice independently with similar results.  
h, Immunoblot analysis of PDGFRB, tubulin, pCAMK2D and CAMK2D protein levels in control iPSC-CMs expressing empty and PDGFRB constructs. The signal intensity of the PDGFRB (left) and p-CAMK2D (right) is shown. The experiments were repeated twice independently with similar results.  
i, Representative Ca^{2+} transients of iPSC-CMs expressing empty and PDGFRB constructs.  
j, Quantification of arrhythmic waveforms of iPSC-CMs in i. The Ca^{2+} transients in b, d, f and i were repeated as described in c, e and j independently with similar results.
Extended Data Fig. 11  |  See next page for caption.
Extended Data Fig. 11 | Gene-expression profile of PDGFRB inhibition in LMNA-mutant iPSC-CMs. a, GO analysis of downregulated genes (n = 352) in LMNA-mutant iPSC-CMs treated with PDGFRB inhibitors, crenolanib (100 nM) and sunitinib (500 nM), for 24 h. b, Heat map of the expression profile of the gene set related to the GO function of ion transport. The FDR-adjusted P values were obtained using the GO enrichment analysis tool. c, Hierarchical clustering of AmpliSeq RNA-seq data using one-way ANOVA (P = 0.05; n = 230). Two different siRNAs against PDGFRB and a scramble siRNA were used in LMNA-mutant iPSC-CMs (III-15 WT/MUT). d, e, Heat map of expression profile of gene (n = 25) sets related with the GO function of cardiac muscle contraction (d) and actin-mediated cell contraction (e). The FDR-adjusted P values were obtained using the GO enrichment analysis tool. f, No significant changes in abnormal nuclear structures of mutant iPSC-CMs by inhibition of PDGFRB were found. Representative images of mutant iPSC-CMs treated with PDGFRB inhibitors, crenolanib (100 nM) and sunitinib (500 nM), for 24 h. iPSC-CMs were stained with specific antibodies against LMNB1 (green). Blue, DAPI. Scale bars, 10 μm. The experiments were repeated three times independently with similar results. g, Quantification of cells showing abnormal nuclear structures in mutant iPSC-CMs treated with PDGFRB inhibitors. The images were recorded from three differentiation batches. n = 90 (DMSO), n = 69 (crenolanib), n = 79 (sunitinib). Data are mean ± s.e.m.; statistical significance was analysed using one-way ANOVA; values above the lines indicate significance. h, Immunoblot analysis of lamin A/C and GAPDH protein levels in mutant iPSC-CMs treated with PDGFRB inhibitors. CB, crenolanib; SB, sunitinib. The experiments were repeated twice independently with similar results.
Extended Data Fig. 12 | See next page for caption.
Extended Data Fig. 12 | Proposed disease model of LMNA-related DCM. We recruited a large family cohort with DCM and generated patient-specific iPSCs from several patients (n = 5) and healthy individuals (n = 2). We next used gene-edited isogenic iPSC lines (n = 4) and patient heart tissues to address the question why patients with LMNA-related DCM have increased manifestation of cardiac arrhythmias. The electrophysiological studies of mutant iPSC-CMs demonstrated that a mutation in LMNA was the cause of the increased arrhythmogenicity in LMNA-mutant iPSC-CMs. We also found that the LMNA mutation caused lamin A/C haploinsufficiency, which led to abnormal calcium homeostasis in mutant iPSC-CMs through upregulation of calcium-handling genes. Whole-transcriptome profiling (RNA-seq) further demonstrated an abnormal activation of the PDGF pathway in mutant iPSC-CMs. The inhibition of the PDGF signalling pathway by treatment with siRNA or FDA-approved inhibitors, such as sunitinib and crenolanib, could reverse the arrhythmic phenotype of LMNA-mutant iPSC-CMs. Cross-analysis of ChIP–seq, ATAC-seq and RNA-seq data revealed a possible underlying mechanism that lamin A/C haploinsufficiency could disrupt global chromatin conformation, resulting in abnormal gene expression in mutant iPSC-CMs. These findings were further corroborated by studies in cardiac tissues from healthy individuals and patients with LMNA-related DCM, thus validating a novel mechanism of LMNA-related DCM pathogenesis both in vitro and in vivo.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  
  Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  
  Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- qPCR analysis: CFX Maestro™ Software (Bio-rad)
- Imaging process for ATAC-See: ZEN imaging software (Zeiss)
- Imaging process for kinase array: Image Studio Lite (LI-COR)
- Imaging process for immunoblot: Image Lab (Bio-rad)
- AmpliSeq Transcriptome analysis: Omics Explorer version 3.2 software (Qlucore)
- Ca2+ imaging: NIS Elements AR software

Data analysis

- [RNA-seq] Base quality of raw reads: FastQC 0.11.4
- [RNA-seq] aligned the reads to the human reference genome (hg19): STAR 2.5.1b
- [RNA-seq] calculating fragments per kilobase per million aligned reads (FPKM): Cufflinks 2.2.1
- [ATAC-seq] ATAC-seq reads were mapped to hg19 genome: bowtie 2
- [ATAC-seq] Following QC to remove duplicate reads, average read intensities were calculated: deepTools and R/Bioconductor (v.3.2.1)
- [ChIP-seq] AQUAS pipeline from the Kundaje lab at Stanford University (https://github.com/kundajelab/chip-seq-pipeline2)
- [ChIP-seq] duplicate reads were removed: MarkDuplicates from Picard Tools (v2.17.3)
- [ChIP-seq] LMNA data were analyzed: Enriched Domain Detector (v1.0) with an 11 Kb bin size, gap penalty of 5, and FDR significance threshold of 0.05.
- [ChIP-seq] LAD gain, loss, and intersection were found: bedtools (v2.27.1)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. Github). See the Nature Research guidelines for submitting code & software for further information.
Data
Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

GSE118885

Field-specific reporting
Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

- Sample size: No statistical methods were used to predetermine sample size. We included all patients who provided consent in our study.
- Data exclusions: No data excluded from the analysis.
- Replication: For each experiment, all attempts at replication were successful.
- Randomization: The experiments were not randomized. We allocated our samples into two groups based on genotype of LMNA gene.
- Blinding: The investigators who performed electro-physiological test, Ca2+ imaging analysis and measuring abnormal nuclear structure were blinded to group allocation during experiments and data collection.

Life sciences study design

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Report for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- n/a: Involved in the study
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants
- Clinical data

Methods

- n/a: Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Antibodies

- SSEA4
  - Company (catalog number): R&D systems (MAB1435)
  - Application: Immunostaining
  - Source: Monoclonal Mouse IgG3 Clone # MC-813-70 [Oct-3/4]
- Oct-3/4
  - Company (catalog number): Santa Cruz Biotechnology (sc-5279)
  - Application: Immunostaining
  - Source: mouse monoclonal IgG2b # C-10 [NANOG]
- NANOG
  - Company (catalog number): Santa Cruz Biotechnology (sc-33760)
  - Application: Immunostaining
  - Source: rabbit polyclonal IgG # M-149 [SOX2]
- SOX2
  - Company (catalog number): R&D systems (MAB2018)
-Application: Immunostaining
-Source: Monoclonal Mouse IgG2A Clone # 245610
[Cardiac troponin T]
-Company (catalog number): Abcam (ab45932)
-Application: Immunostaining
-Source: Rabbit polyclonal IgG
[Cardiac troponin T]
-Company (catalog number): Abcam (ab8295)
-Application: Immunostaining
-Source: Mouse monoclonal [IC11]
[α-Actinin (Sarcomeric)]
-Company (catalog number): Sigma-Aldrich (A7811)
-Application: Immunostaining
-Source: Mouse monoclonal EA-53
[LMNA]
-Company (catalog number): abcam (ab8980)
-Application: Immunoblotting, ChIP-seq
-Source: Mouse monoclonal [133A2]
[LMNA]
-Company (catalog number): Santa Cruz Biotechnology (sc-376248)
-Application: Immunoblotting, Immunostaining, ChIP-seq
-Source: mouse monoclonal IgG1 (E-1)
[LMNB1]
-Company (catalog number): abcam (ab16048)
-Application: Immunostaining
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[CaMKII delta]
-Company (catalog number): Abcam (ab181052)
-Application: Immunoblotting
-Source: Mouse monoclonal [EPR13095]
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-Company (catalog number): Abcam (ab32678)
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-Source: Mouse monoclonal [Y92]
[Ryanodine Receptor]
-Company (catalog number): Abcam (ab8984)
-Application: Immunoblotting
-Source: Mouse monoclonal [SSEA4]
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-Ref in manufacturer’s web site: Shevinsky, L.H. et al. (1982) Cell 30:697. Kannagi, R. et al. (1983) EMBO J. 2:2355. [Oct-3/4]
-Ref in manufacturer’s web site: PMID: # 27797132, PMID: # 28159471, PMID: # 27876565
[NANOG]
-Ref in manufacturer’s web site: PMID: # 24550733, PMID: # 24619130, PMID: # 24380431
[SOX2]
-Ref in manufacturer’s web site: Graham, V. et al. (2003) Neuron 39:749., Avilion, A.A. et al. (2003) Genes Dev. 17:126.
[Cardiac troponin T]
-Abcam (ab45932): Ref in manufacturer’s web site: PubMed: 27672365, PubMed: 27226619
-Abcam (ab8295): Ref in manufacturer’s web site: PubMed: 28487635, PubMed: 28490375.
[α-Actinin (Sarcomeric)]
-Ref in manufacturer’s web site: PMID 19668186, PMID 22020047
[LMNA]
-Abcam (ab8980): Ref in manufacturer’s web site: PubMed: 29545600, PubMed: 28737169
-SCBT (sc-376248): Ref in manufacturer’s web site: PMID: # 29684352, PMID: # 29436586
- Abcam (ab8984): Ref in manufacturer’s web site: PubMed: 29580221, PubMed: 29659505
[CaMKII delta]
--Ref in manufacturer’s web site: PubMed: 27084844
[Phospho-CaMKII delta]
--Ref in manufacturer’s web site: PubMed: 29482582, PubMed: 29593308
[PDGF Receptor beta]
-Ref in manufacturer’s web site: PubMed: 28423550, PubMed: 28230073
[Ryanodine Receptor]
-Ref in manufacturer’s web site: PubMed: 26301072, PubMed: 25775120
[LMNB1]
-Ref in manufacturer’s web site: PubMed: 29308302, PubMed: 29333436
[H3K9Me2]
-Company (catalog number): Abcam (ab1220), Active Motif (#39239)
-Application: Immunostaining, ChIP-qPCR

[H3K9me3]
-Application: Immunostaining, ChIP-qPCR
-Ref: PMID: 30143619

[Oct-3/4]
-Company (catalog number): Active Motif (#61013)
-Application: Immunostaining, ChIP-qPCR

[Oct-3/4]
-Ref: PMID: 29033129

[SSEA4]
-Ref in manufacturer’s web site: Shevinsky, L.H. et al. (1982) Cell 30:697. Kannagi, R. et al. (1983) EMBO J. 2:2355.

[Oct-3/4]
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[NANOG]
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[SOX2]
-Ref in manufacturer’s web site: Graham, V. et al. (2003) Neuron 39:749., Avilion, A.A. et al. (2003) Genes Dev. 17:126.
**Eukaryotic cell lines**

Policy information about [cell lines](#)

**Cell line source(s)**
- We obtained dermal fibroblasts or PBMCs from the patients and generated patient specific iPSC lines using Co-MiPs (doi:10.1038/srep08081) or sendai virus method (Thermofisher, CytoTune™-iPS 2.0 Sendai Reprogramming Kit; A16517).
- hESC lines were obtained from WiCell (WAE007-A).
- The iPSC-derived cardiomyocytes were generated by previous protocol (doi:10.3791/52628).

**Authentication**
- Immunofluorescence assay of each iPSC line was performed to check the expression of stem cell markers such as NANOG, POU5F1 and SOX2.
- SNP karyotyping was tested through HuCytoSNP-12 chip (Illumina), and CNV and SNP visualization was performed using KaryoStudio v1.4 (Illumina).

**Mycoplasma contamination**
We confirmed that all cell lines were negative for mycoplasma contamination using MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, LT07-705).

**Commonly misidentified lines**
(See [ICLAC](#) register)
- No commonly misidentified cell lines were used.

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**Human research participants**

Policy information about [studies involving human research participants](#)

**Population characteristics**
The detail information of patients was described in Extended Data Figure 1b.
- Patients III-1, III-3, and III-9 (age 57, 60, and 67, respectively) carried the c.349_350insG frame shift mutation on LMNA gene initially presented with atrial fibrillation that progressed to atrioventricular block and ventricular tachycardia. These patients eventually required implantable cardioverter defibrillators (ICDs) and later developed DCM.
- The other two carriers (III-15 and III-17; ages 38 and 45, respectively) were younger and had exhibited paroxysmal atrial fibrillation prior to the beginning of the study. III-1, III-3, III-9, IV-1, and IV-2 individuals are male. III-15 and III-17 individuals are female.

**Recruitment**
The fibroblasts, PBMCs, and heart tissues were obtained from patients using IRB-approved protocol at Stanford University (Protocol ID 17576 and 29904). Informed consents were obtained from all patients who were included in our study.

**Ethics oversight**
IRB-approved protocols at Stanford University (Protocol ID 17576 and 29904)

Note that full information on the approval of the study protocol must also be provided in the manuscript.
**Data deposition**

- Confirm that both raw and final processed data have been deposited in a public database such as GEO.
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

**Gene Expression Omnibus (GEO): GSE118885**

**Files in database submission**

| Processed Files | Raw Data Files |
|-----------------|----------------|
| RNA_PT3WT_Rep1Aligned.out.sorted.q255.bw | RNA_3_1_1.fq.gz |
| RNA_PT3WT_Rep2Aligned.out.sorted.q255.bw | RNA_3_1_2.fq.gz |
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| RNA_PT5WT_Rep1Aligned.out.sorted.q255.bw | RNA_3W_1_1.fq.gz |
| RNA_PT5WT_Rep2Aligned.out.sorted.q255.bw | RNA_3W_1_2.fq.gz |
| RNA_PT5MT_Rep1Aligned.out.sorted.q255.bw | RNA_3W_2_1.fq.gz |
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| H3K27_18_USPD16084644_HJ3FYBBXX_L1_2.fq.gz | H3K27_18_USPD16084644_HJ3FYBBXX_L1_1.fq.gz |
| H3K27_18_USPD16084644_HJ3FYBBXX_L1_1.fq.gz | H3K27_18_USPD16084644_HJ3FYBBXX_L1_2.fq.gz |
| SC_5_USPD16084631_HJ3FYBBXX_L2_1.fq.gz | SC_5_USPD16084631_HJ3FYBBXX_L2_2.fq.gz |
| SC_5_USPD16084631_HJ3FYBBXX_L2_2.fq.gz | SC_5_USPD16084631_HJ3FYBBXX_L2_1.fq.gz |
### Methodology

**Replicates**

Two parental hiPSCs lines (III-3; WT/MT, IV-1; WT/WT), and two isogeneic lines (III-3; WT/Corr-WT, IV-1; WT/Ins-MT).

Two different antibodies were used for LMNA ChIP-seq. ab8984 and sc-376248.

### Sequencing depth

| Sample     | Raw_Reads     | Clean_Reads    | Raw_Base(G) | Clean_Base(G) | Effective_Rate(%) | Error_Rate(%) | Q20(%)   | Q30(%)   | GC_Content(%) | 
|------------|---------------|----------------|-------------|----------------|-------------------|---------------|----------|----------|----------------|
| H3K4_pt3WT | 54856219      | 42145611       | 16.5        | 76.83         | 0.02             | 95.06         | 88.98    | 56.01    | Yes            |
| H3K4_pt3MT | 59207590      | 39834003       | 17.8        | 67.28         | 0.01             | 95.73         | 90.23    | 57.05    | Yes            |
| H3K27_pt3WT| 41638326      | 31134930       | 12.5        | 74.77         | 0.01             | 95.98         | 90.68    | 46.75    | Yes            |
| H3K27_pt3MT| 53964775      | 40732993       | 16.2        | 75.48         | 0.01             | 96.2          | 91.15    | 47.07    | Yes            |
| ab_pt3WT   | 53766870      | 43207940       | 16.1        | 80.36         | 0.02             | 95.93         | 90.19    | 40.85    | Yes            |
| ab_pt3MT   | 47369372      | 41177310       | 14.2        | 86.93         | 0.02             | 95.56         | 89.41    | 41.44    | Yes            |
| SC_pt3WT   | 57419745      | 51251376       | 17.2        | 89.26         | 0.02             | 95.63         | 89.53    | 40.58    | Yes            |
| SC_pt3MT   | 50700560      | 48187024       | 15.2        | 95.04         | 0.02             | 95.45         | 89.2     | 40.92    | Yes            |

**Antibodies**

- Santa Cruz Biotechnology sc-2025: IgG
- Active Motif 39159: H3K4me3
- Active Motif 39155: H3K27me3
- Abcam ab8984: LMNA
- Santa Cruz Biotechnology sc-376248: LMNA

**Peak calling parameters**

Enriched Domain Detector (v1.0) with an 11 Kb bin size, gap penalty of 5, and FDR significance threshold of 0.05.

**Data quality**

LMNA-sc-376248: EDD peaks (pt3WT; 587, pt3MT; 614)

LMNA-ab8974: EDD peaks (pt3WT; 587, pt3MT; 614)

**Software**

- [ChIP-seq] AQUAS pipeline from the Kundaje lab at Stanford University [https://github.com/kundajelab/chip-seq-pipeline2](https://github.com/kundajelab/chip-seq-pipeline2)
- [ChIP-seq] duplicate reads were removed: MarkDuplicates from Picard Tools (v2.17.3)
- [ChIP-seq] LMNA data were analyzed: Enriched Domain Detector (v1.0) with an 11 Kb bin size, gap penalty of 5, and FDR significance threshold of 0.05.
- [ChIP-seq] LAD gain, loss, and intersection were found: bedtools (v2.27.1)