# Supplemental Materials

## SUPPLEMENTAL METHODS

### Data and Code Availability

The data has been uploaded to GEO, the accession number for the data is GSE165303. All other relevant data in this study are available from the corresponding authors upon reasonable request.

### Culture of hiPSC-CMs and 293 cells

The human-induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (hiPSC-CMs) obtained from Cellular Dynamics International were plated on fibronectin-coated dishes and maintained with maintenance medium according to the manufacturer's protocol. For adult mouse cardiomyocyte (CM) isolation, the hearts were digested with 15,000 U of type II collagenase (Worthington-Biochem) on a Langendorf apparatus. The 293 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Hyclone) supplemented with 10% FBS.

### 3D-epigenome mapping

### ChIP-seq on heart tissues and hiPSC-CMs

50 mg heart tissues were submersed in PBS with 1% (final concentration) formaldehyde (Thermo Fisher Scientific), cut into small pieces and incubated at room temperature for 15 min. Fixation was stopped by the addition of 0.125 M (final concentration) glycine. The tissue pieces were then treated with a TissueTearer and finally spun down and washed with PBS (twice). The chromatin was isolated by the addition of lysis buffer, followed by disruption with a Dounce homogenizer. For hiPSC-CMs, 10 million cells were fixed by 1% formaldehyde at room temperature for 15 min, followed by cellular lysis and nuclear lysis. The lysates were sonicated and the DNA sheared to

an average length of 300-500 bp. The pellets were resuspended and the resulting DNA was quantified on a Qubit spectrophotometer. An aliquot of chromatin (8 µg) was precleared with protein A/G agarose beads (Thermo Fisher Scientific). Genomic DNA regions of interest were precipitated using 4 µg of antibody against H3K27Ac (Active Motif) or 10 µg of antibody again HAND1 (Abclonal). For HAND1 ChIP-seq in hiPSC-CMs transfected with Ctrl or *HAND1*-overexpressing adenovirus, we employed Active Motif ChIP-seq spike in using Drosophila chromatin (Spike-in Chromatin, Active Motif) and an antibody against Drosophila specific histone variant H2Av (Spike-in Antibody, Active Motif) to normal ChIP-seq signal, as per the manufacturer's instructions. The protein/DNA complexes were washed, eluted from the beads with 1% SDS buffer and subjected to RNase and proteinase K treatment overnight at 65°C. The ChIPed DNA was purified by phenol-chloroform extraction and ethanol precipitation. Genomic DNA (Input) was prepared by treating aliquots of chromatin with RNase, proteinase K in a similar way as ChIPed DNA. Illumina sequencing libraries were prepared from the ChIP products by the standard consecutive enzymatic steps of end-repair, A tailing, and adaptor ligation using the KAPA Hyper Prep Kit (Roche). Following a final PCR amplification step, the PCR products were purified and the size was selected by 0.5X SPRI followed by 0.9X SPRI beads. The resulting DNA libraries were quantified and sequenced on an Illumina NextSeq 500 (75 bp, single end).

**H3K27ac HiChIP (human heart tissues and hiPSC-CMs) and *in-situ* HiC (human heart tissues)**

H3K27ac HiChIP/*in-situ* HiC Library preparation was performed by Arima Genomics (https://arimagenomics.com/). For heart tissues, each HiChIP and *in-situ* HiC library was generated from ~500 mg pulverized and crosslinked (2% formaldehyde, room temperature for 10 min) DCM and NF hearts. For hiPSC-CMs, each HiChIP library was generated from ~10 million crosslinked (2% formaldehyde, room temperature for 10 min) hiPSC-CMs transfected with Ctrl or *HAND1*-overexpressing adenovirus. For mouse-CMs, each HiChIP library was generated from

cardiomyocytes isolated from adult mice transfected with the adenoviruses (Vector Biolabs) carrying cytomegalovirus (CMV) promoter driving *Hand1* (Ad-GFP-*Hand1*, the cells are termed as $mCM^{Hand1}$) or with adenoviruses carrying CMV promoter (Ad-GFP, the cells are termed as $mCM^{Null}$). Initially, biotin-labeled proximally-ligated chromatin was prepared using the Arima-HiC kit (Arima Genomics). Briefly, the crosslinked chromatin was initially digested using a restriction enzyme (RE) cocktail. The 5'-overhangs were subsequently filled in and labeled with a biotinylated nucleotide. Subsequently, the spatially proximal digested ends of DNA were ligated. The resulting proximally-ligated chromatin was fragmented using the Diagenode Bioruptor Pico instrument, bound to an H3K27ac antibody (Abcam) overnight and immunoprecipitated on Protein A magnetic beads (Thermo Fisher Scientific). The immunoprecipitated chromatin was reverse crosslinked and purified using the Arima-HiC kit. Subsequently, the Illumina-compatible sequencing libraries were prepared by initial enrichment for biotin using C1 streptavidin beads (Thermo Fisher Scientific). The samples were tagmented on-bead utilizing Tagment DNA Enzyme and Buffer (Illumina). Subsequently, DNA was PCR amplified utilizing NPM and the indexing primers from the Nextera XT DNA Library Prep kit (Illumina). The PCR products were purified and size selected by 0.5X SPRI beads followed by 0.7X SPRI beads. The purified libraries underwent standard QC (qPCR and Bioanalyzer) and were sequenced on the Illumina HiSeq Xten (150 bp, pair-end) following the manufacturer's protocols. *In situ* HiC was performed in a similar way as HiChIP with the exception of chromatin immunoprecipitation being performed using the H3K27ac antibody and protein A beads.

**RNA-seq on human heart tissues, hiPSC-CMs and adult mouse CMs**

Total RNA was extracted from the heart tissues, hiPSC-CMs, and mouse CMs using Trizol (Thermo Fisher Scientific) according to the manufacturer's instructions. Approximately 20 mg of tissue was converted into powder using liquid nitrogen and the powder samples were transferred into 2 ml Eppendorf tubes containing 1.5 ml Trizol reagent. The mix was centrifuged at 12,000 x

g for 5 min at 4°C. The supernatant was transferred to a new 2.0 ml tube, which contained 0.3 ml Chloroform/isoamyl alcohol (24:1) per 1.5 ml of Trizol reagent. Following centrifugation at 12,000 x g for 10 min at 4°C, the aqueous phase was transferred to a new 1.5 ml tube which contained equal volume of supernatant of isopropyl alcohol. The mix was centrifuged at 12,000 x g for 20 min at 4°C and the supernatant was collected. Following washing with 1 ml 75% ethanol, the RNA pellet was air-dried in the biosafety cabinet and dissolved by addition of 25 µl nuclease-free water. Subsequently, total RNA was qualified and quantified using the Agilent 2100 bioanalyzer. Approximately 1 µg total RNA per sample was depleted from rRNA with the Ribo-Zero™ Magnetic Kit (Epicentre). The retrieved RNA was subjected to fragmentation by adding the First Strand Master Mix (Thermo Fisher Scientific). First-strand cDNA was generated using random primers and reverse transcription, followed by a second-strand cDNA synthesis. The synthesized cDNA was subjected to end-repair and was 3' adenylated. The adapters were ligated to the ends of these 3' adenylated cDNA fragments. A total of 10 rounds of PCR with PCR Primer Cocktail and PCR Master Mix were performed to amplify the cDNA fragments. The PCR products were purified and size selected by 0.5X SPRI beads followed by 0.9X SPRI beads. The identity of the final library was verified by the distribution of the fragments size using the Agilent 2100 bioanalyzer and quantified by the Qubit High sensitivity assay. The Qualified libraries were sequenced pair end on the Illumina HiSeq Xten (150 bp, pair-end).

**ATAC-seq on human heart tissues**

A total of 20-50 mg human heart tissue sample was cut on dry ice for each ATAC-seq assay. Each sample was placed into the corresponding 5 cm petri dish (labeled on bottom of dish) with PBS on ice and minced as finely as possible with a razor (the use of a clean razor was ensured for each sample). The end was cut off with a 1 ml pipette tip at an angle and the samples were transferred to the corresponding conical tubes with PBS (a fresh tip was used for each sample). Subsequently, the tubes were centrifuged at 500 x g for 5 min at 4°C. The PBS was decanted as

thoroughly as possible without losing the pellet and 1 ml ATAC lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% IGEPAL CA-630) was added to each sample. Each sample was added to a 1 ml dounce. The samples were dounced with a pestle 40-50 times slowly in order to avoid foaming. Following douncing, the samples were filtered through a 40 µM mesh strainer into a 1.5 ml tube on ice. Immediately following filtration of each sample, 10 µl aliquots were obtained for counting the nuclei. For each 10 µl of aliquot, 10 µl Trypan Blue was added, mixed, loaded onto the hemocytometer and counted. The tubes were inverted and flicked to mix. Subsequently, 100,000 cells were aliquoted from each sample into a PCR tube and stripped on ice. The ATAC lysis buffer was added to the PCR tube to ensure that the reaction volume was not less than 100 µl. The samples were spun down at 500 x g for 5 min at 4°C. The supernatant was removed very carefully, ensuring not to disturb the cell pellet. The tagmentation reaction was immediately continued. The transposition reaction master mix was prepared and the samples were mixed by pipetting gently, spun quickly and left on ice. The tagmentation master mix included the following reagents: 25 µl 2x TD Buffer (Illumina), 8 µl Tn5 Transposase (Illumina), 17 µl PBS, 5 µl 1% Digitonin (dissolved in 50% DMSO in dH2O) and 5 µl 10% Tween 20. A total of 50 µl tagmentation master mix was added to each sample, which was gently pipetted to resuspend the cells in the transposition reaction mix. The samples were incubated at 37°C for 30 min in a thermomixer at 800 rpm. The purification was conducted using a Qiagen MinElute Kit. The DNA was eluted with a 35 µl EB buffer (10 mM Tris buffer, pH 8.0), allowed to settle at room temperature for 1 min and centrifuged at max speed for 30 sec. Each elution was transferred to a 250 µl PCR tube and the PCR reactions were prepared by adding the components in the order shown. The PCR reaction contained the following components: 5 µl Index mix (Illumina Nextera Kit, 10 µl 5x Phusion HF reaction Buffer, 1.5 µl dNTPs (10 mM), 0.5 µl Phusion HF Polymerase (NEB) and 33 µl tagmented DNA. The reagents were pipetted to mix. The PCR was performed using the following program on a thermal cycler (with heated lid): heating at 72°C for 5 min and at 98°C for 30 sec. A total of 10 cycles were performed at 98°C for 10 sec, at 63°C for 30 sec and

at 72°C for 30 sec. A final extension was performed at 72°C for 1 min. The AMpure double size selection was performed and 80 µl AMpure beads were added to the sample, vortexed briefly to mix and quickly spun. The solution was incubated at room temperature for 5 min. The samples were placed in a magnetic rack and left for 1 min. The liquid was removed with a pipette, taking care not to disrupt the pellet. The samples were washed by addition of 180 µl fresh 70% ethanol to the tube, taking care not to disrupt the pellet. The ethanol was removed with a pipette and the procedure was repeated a second time. The ethanol was removed slowly from the tube. The samples were allowed to dry for 30 sec and the pellet was resuspended with 22 µl Buffer EB (10 mM Tris, pH 8.0) and mixed by pipetting. The sample was allowed to stand for 1 min and placed in a magnetic rack on the magnetic side for 1 min. A total of 20 µl of the eluate were transferred to a new tube, taking care not to collect any magnetic beads. The aliquots of the libraries were run on an Agilent 2100 bioanalyzer and quantified with the Qubit High sensitivity assay. The libraries were sequenced by the Illumina HiSeq Xten (150 bp, pair-end).

## Computational analysis

### Library QC metrics collection

All the QC metrics for ChIP-Seq, HiChIP, in-situ Hi-C, RNA-Seq and ATAC-Seq were collected using MultiQC[60] and were summarized in **Supplementary Table 3**. For HiChIP and Hi-C data, the raw read number, mappability, pairing statistics, filtering and contact statistics were documented. For the ATAC-seq and ChIP-seq data, the raw read number, mappability, duplicated reads and peak number were documented. For the RNA-seq data, the raw read number, average fragment length and mappability were collected.

### ChIP-seq data analysis

Single-end reads were mapped to the hg19 reference genome using the bwa[61] mem. The duplicated reads were removed using the Picard Toolkit (https://broadinstitute.github.io/picard/), whereas the reads with a quality lower than 20 were filtered.

The ChIP-seq peaks were called using the default (narrow) setting in MACS2[62]. The promoter regions were defined as +/- 2.5Kb around the TSSs regions. H3K27ac peaks corresponding to overlapping promoter regions were defined as “proximal promoter peaks” (P), whereas the others were defined as “distal enhancer peaks” (E). The peaks from 10 DCM H3K27ac ChIP-seq and 10 NF H3K27ac ChIP-seq datasets were loaded in R using the DiffBind package (https://bioconductor.org/packages/release/bioc/html/DiffBind.html). The overlapping peaks were merged and a single binding matrix was created. Differential peak analysis was performed using the edgeR[63] with a cutoff FDR < 0.05 and Fold change > 1.

For spike-in HAND1 ChIP-seq from hiPSC-CMs, raw sequencing reads were mapped to human hg19 and drosophila dm6 genome, respectively. Peaks were called using reads mapped to human hg19 genome. Normalization factors for each sample were calculated based on the reads mapped to dm6 genome. Reads mapped to human hg19 genome were then down-sampled based on the normalization factors. For each peak, number of reads were counted using down-sampled bam files and used as intensity for visualization.

**Enhancer breadth analysis**

H3K27ac binding sites (narrowPeak format) in 507 ENCODE cell lines / tissues were downloaded from ENCODE portal (https://www.encodeproject.org)(Data source was provided in **Supplementary Table 2**. H3K27ac enrichment breadth for each DCM-specific enhancer was calculated as the number of cell lines / tissues an enhancer peak overlapped. Common enhancer

peaks were used as a comparison. H3K27ac enrichment breadth were computed for all DCM-specific and common enhancers and visualized using density plot.

**RNA-seq data analysis**

The reads were mapped to hg19 using STAR[64]. Only properly mapped reads pairs were kept. Bam files were indexed and sorted by read names using samtools[65]. The reads mapped to gene exon regions were counted using featureCounts[66]. Differential expressed genes were determined by DESeq2[67] using negative binomial generalized log-linear model followed by Wald Test. Age and gender were added to the model as blocking factors. Normalized read counts were calculated using variance stabilizing transformation (VST) method and then used for visualization in boxplot.

**HiChIP and Hi-C data processing**

HiChIP and Hi-C data were preprocessed using HiC-Pro[68] with default parameters with the exception of the LIGATION_SITE being set to GATCGATC, GANTGATC, GANTANTC, GATCANTC and the GENOME_FRAGMENT that was generated using digest_genome.py in HiC-Pro utilities. The following parameters were used: -r ^GATC G ^ANTC -o hg19_restriction_sites.bed (coordinates of restriction sites) hg19.fasta (reference sequence file). The raw reads were mapped to the hg19 reference genome and were assigned to restriction fragments. The raw reads were filtered for valid pairs (VI). The HiC files were generated using hicprotojuiebox.py utilities in HiC-Pro[68]. All valid pairs served as input files and then imported into Juicebox[69] and Knight–Ruiz (KR) matrix–balanced interaction maps were generated for visualization. For HiChIP data, 1D coverage files for the visualization in bigwig format were converted from bam format using deepTools[70] and RPKM (Reads Per Kilobase per Million reads to normalize the number of reads per bin) normalization was used.

**Identification of chromatin interactions and differential loops**

We first identified a set of significant loops for DCM and NF, respectively. Valid read pairs for each sample served as input to Mango[71] for stage 4 and 5 processing. The "peakinput" parameter was set to the corresponding ChIP-seq peak file. The read pairs were extended 500 bp on each side and subsequently clustered. The clusters in each sample were merged and clustered again to form a union set of clusters for DCM and NF, respectively. Significant loops were identified using the ChIA-PET Tool V3.0[72] with cutoff FDR < 0.00001. The loops with PET counts lower than 10 and spanning less than 5 Kb or larger than 1 Mb were further filtered.

Differential loops were identified following two steps: Initially, loops in DCM and NF were merged and subsequently the PET counts for each loop were counted in all the replicates in DCM and NF. A negative binomial model was fitted using glmFit function in edgeR R package and the loops with p value (<0.1) were determined as differential loops[13]. We acknowledged that while using p value without correction for multiple testing will identify more differential loops, it could potentially generate more false positivity. We further employed 3C-qPCR to validate the fidelity of some differential loops we identified.

**Validation of differential loops by 3C-qPCR**

The 3C-qPCR assay was developed and modified from the previous publications[73, 74]. Briefly, human heart tissues were fixed with 2% formaldehyde for 10 min at room temperature and lysed, and then nuclei were subjected to in-situ digestion (MboI, New England Biolabs) and ligation (T4 ligase, New England Biolabs). The 3C-qPCR primers were designed using unidirectional strategy. Briefly, the primers were designed on the "forward" strand, located near the MboI restriction site (GATC) from 20~100 bp. Loading control (GAPDH) primers were designed on the MboI fragment which does not across MboI restriction site. The primers for 3C-qPCR are as follows: *TBX5* chr12:114819505-114820005: CCAATTCAGGCCAAAGAAGA, *TBX5* chr12:114842979-

114843479: CTCCACCCCCTACTCATCAC; *MEF2D* chr1:156469903-156470403: GCAGGCGCATTATTTTTCTC; *MEF2D* chr1:156478626-156479126; *SMYD2* chr1:214492375-214492875: TGATGGCATCTTCCTGACAA; *SMYD2* chr1:214449791-214450291: CATCTAGGCCCTTTGCTCAC; *MYPN* chr10:69847422-69847922: TAAAGCCATGTGGGAAAAGG; *MYPN* chr10:69869501-69870001: AATTACATATGACGGGACACGA; *GAPDH*-Forward: CCCACACACATGCACTTACC, *GAPDH*-Reverse: CCCACCCCTTCTCTAAGTCC.

**Luciferase reporter gene assays**

To explore the potential regulatory relationship between interacting chromatin loci, the dual luciferase assays for various combinations of promoter and enhancer regions were performed. The fragments of promoter and enhancer regions identified by HiChIP were PCR-amplified and cloned into pGL4.10 vector (Promega). For luciferase assay, cells were co-transfected with the reporter constructs and a constitutive *Renilla* luciferase expressing vector pRL-SV40 (controlling for transfection efficiency) (Promega). Cells were harvested 48 hours after transfection and the luciferase activity was measured using the Dual-Luciferase reporter assay kit (Promega, E1910). For all measurements, firefly luciferase values were normalized to *Renilla* luciferase values.

**Functional enrichment of specific active enhancers or differential loop anchors**

The bed file of specific enhancers or differential loop anchor regions served as input of GREAT (version 4) (http://great.stanford.edu/public/html/). We used whole genome (hg19) as background. The Negative Log10(score) for each GO (gene ontology) terms or Disease Ontology terms was presented as the bar. Functional enrichment of genes network was generated using the ToppFun application of the Toppgene Suite using a corrected (Benjamini and Hochberg) p-value cut-off of 0.05 (https://toppgene.cchmc.org/)[35]. Cytoscape application (https://cytoscape.org/)[75] was used to generate the functional enrichment network.

**Identification of A/B compartments and topologically associated domains (TADs)**

Interaction matrix of .hic format were converted to the cool format using HiCExplorer library hic2cool. Replicates of cool format files with 25 Kb resolution were merged using cooler[76]. A and B compartments were identified using hicPCA command in HiCExplorer (https://hicexplorer.readthedocs.io/en/latest/). The topological associated domains (TADs) were identified using the hicFindTADs command in the HiCExplorer at 25 Kb resolution.

**Differential TAD analysis**

We used two methods to assess the conservation of genome organization at TAD level:1) To define conserved TADs, we require that 70% fraction of reciprocal overlapping TADs in DCM and NF; 2) In addition, we used an alternative metrics, the correlation of insulation score to access the overall TAD boundary conservation between DCM and NF, which has been used as cutoff to define TAD boundaries in multiple studies[77-79].

**ATAC-seq data analysis**

The adapters were removed and the filtered reads were mapped to hg19 using bwa[61] mem. The duplicated reads were removed using Picard (https://broadinstitute.github.io/picard/) and the reads with quality lower than 20 were filtered.

**Transcription factor motif analysis**

The motif analysis was performed using Homer (http://homer.ucsd.edu/homer/, v4.11). We overlapped DCM-specific/NF-specific loop anchor regions with the ATAC-seq peak regions. Subsequently, we extracted the sequences from the overlapped regions using bedtools[80] getfasta and imported them into Homer findMotifs.pl with default setting for motif enrichment analysis.

**Quantitative analysis of ATAC-seq and ChIP-seq**

To calculate the differential chromatin accessibility and HAND1 binding on DCM-specific/NF-specific loop anchors, we generated aggregation plots. The reads were counted in a 6Kb window centered at an anchor with 10bp-bin resolution for the 10 DCM and 10 NF samples. The read counts for all anchors at each bin in each sample were summarized using the median value. Subsequently, the read counts in each bin across the samples were summarized using mean +/- standard error. The Mann–Whitney U test was used to calculate the p-value.

**Sample variation analysis for sequencing data**

For all 1D sequencing data type (RNA-seq, ChIP-seq, ATAC-seq), we performed Principal Component Analysis (PCA) using genes or peaks with the highest variance across samples (top 2000). For 3D sequencing data type (HiChIP, Hi-C), we calculated stratum adjusted correlation coefficient (SCC) score across all chromosomes using HiCRep to quantify the similarity between interaction matrices in 25Kb resolution[81].

**Multiple test correction**

All p-values from 1D high-throughput sequencing data sets were adjusted using Benjamini-Hochberg Procedure. Total number of tests of each dataset are: Human heart tissue RNA-seq (N = 50,762), Human heart tissue H3K27ac ChIP-seq (N = 86,530) and Mouse cardiomyocytes RNA-seq (N = 39,905).

**Visualization**

The chromatin loops were prepared using bedpe format and then uploaded to the IGV[82] for arc-style visualization. Other 1D coverage signals were prepared using bigwig format and uploaded to IGV. The hic files were imported into Juicebox for heatmap visualization.

Statistical analysis

Statistical analysis was performed with R version 3.5.1 or Prism 7.0 (GraphPad Inc). The statistical tests performed for each statistical graph were listed in the individual figure legends. Hypergeometric test implemented in ChIA-PET Tool V3.0 was used to detect significant chromatin interactions in HiChIP data. Negative binomial generalized log-linear model was used to model read counts in sequencing data and quasi-likelihood (QL) F-test or Wald Test was used to identify differentially abundant features (chromatin interactions, expressed genes, transcription factor binding peaks, etc). HiCRep was used to calculate stratum adjusted correlation coefficient (SCC score) of HiChIP and Hi-C data.

## Cardiac function studies

### Plasmid production

Mouse *Hand1* cDNA was cloned into adeno-associated virus serotype 9 (AAV9) expression vector plasmid (Addgene) flanked by inverted terminal repeats (ITRs), which expressed green fluorescent protein (GFP) driven by the cardiomyocyte-specific troponin T (*cTNT*) promoter (AAV9-*cTNT*-GFP). The AAV9 carrying *cTNT* promoter driving GFP was used as control.

### AAV9 production and purification

AAV9 particles were packaged and produced by co-transfecting HEK293 cells (ATCC) with AAV9 expression plasmid, in the presence of other helper-plasmid DNAs. In brief, one day prior to transfection, HEK293 cells (80% confluence) were used. The cells were incubated for 2 days after the transfection and the cell pellets were harvested. The viruses were released by 3x cycles of freeze/thaw (-80°C for 10 min, 37°C for 20 min) in resuspension buffer (50 mM Tris–HCl pH 8.0, 150 mM NaCl, 2 mM $MgCl_2$)[83]. The viruses were purified using CsCl-gradient ultra-centrifugation, followed by desalting. AAV9 was stored at -80°C. For titer quantification, purified virus was

digested with DNAse I (Sigma Aldrich) to remove unencapsidated DNA, followed by proteinase K digestion to release the DNA template from the AAV9 capsid. The viral titers (genome copies/ml) were determined by real-time PCR.

**AAV9 delivery into the heart**

Intraperitoneal delivery of AAV9 was performed accordingly as described[83]. In brief, 8-12 weeks old wild type (WT) C57BL/6 mice (Jackson Laboratory) were anesthetized using light isoflurane and were prepared in a biosafety cabinet. To determine the effects of AAV9 dosage on cardiac phenotype, wild type mice were infected with control AAV9 virus (serial dilution) lacking *Hand1* (Null) and diluted in 50 µl of saline. We defined the mice receiving $5\times10^{11}$ viral genomes (vg), $1\times10^{12}$ vg, and $5\times10^{12}$ vg as *Hand1*_Low, *Hand1*_Mid, and *Hand1*_High, respectively. The control mice received 50 µl of saline with AAV-Ctrl ($1\times10^{12}$ viral genomes). To determine the toxicity of AAV9, another group of WT mice was used that received 50 µl of saline without virus. The animals used after the procedure were kept warm using a heat lamp for recovery.

**Adult mouse CM and fibroblast isolation**

Adult mouse CMs were isolated from adult WT C57BL/6 mice with a modified protocol as described previously[84]. Briefly, the aorta of the excised heart tissue was clamped and mounted on a Langendorf apparatus. The tissue was perfused with calcium-free perfusion buffer (113 mM NaCl, 4.7 mM KCl, 0.6 mM $KH_2PO_4$, 0.6 mM $Na_2HPO_4$, 1.2 mM $MgSO_4$, 10 mM NaOH-HEPES, 12 mM $NaHCO_3$, 10 mM $KHCO_3$, 0.032 mM phenol red, 30 mM taurine, 10 mM 2,3-butanedione monoxime (BDM), and 5.5 mM glucose; pH-7.4.), followed by digestion with 50 ml perfusion buffer containing 15,000 U of type II collagenase (Worthington-Biochem) and 50 µM $CaCl_2$. The digestion was terminated when the heart was slightly pale and flaccid. The heart was gently teased into small pieces with forceps and triturated with a Pasteur pipette to dissociate individual CM in the presence of 5 ml digestion buffer, followed by addition of 5 ml stop buffer (perfusion

buffer, 10% FBS, and 12.5 μM $CaCl_2$) to inhibit the enzyme activity. After passing through a 100-μm filter, the cells were settled by five sequential rounds of gravity in the calcium solution. Then, the cell pellets in the bottom were enriched of highly purified CM fraction, and the supernatant fraction was enriched of non-CMs. To purify the cardiac fibroblasts, magnetic-activated cell sorting approach (MACS) using anti-Thy-1 antibody (Abcam, ab3105, 1: 100) [PMID: 27502479] was performed on the cell pellets collected from non-CM fraction after centrifuge (500 g, 5 mins). The pellets containing the CM fraction and non-CM fraction were subjected to downstream analysis.

**Measurement of contractility and calcium transients in adult mouse CMs**

Mouse CM (mCM) contractility and intracellular calcium transients in response to electrical field stimulation were recorded simultaneously using IonOptix MyoCam System (IonOptix Corp) at room temperature[85]. Freshly isolated mCMs were loaded 1 ml Tyrode's solution (1.8mM $Ca^{2+}$) with 2 μl Fura-2 Am (Stock solution, 1ug/ul in DMSO, Thermo Fisher Scientific), the $Ca^{2+}$ indicator dye in dark at 37˚C with 5% CO2. After 10 mins, the medium was aspirated and the cells were washed twice with Tyrode's solution (10 mins/time). Then, these Fura-2 loaded cells were placed in a perfusion chamber mounted on the stage of an inverted microscope (Nikon eclipse TE2000-U fluorescence microscope) and superfused with Tyrode's solution. Sarcomere shortening was recorded using a video-based sarcomere length detection system. $Ca^{2+}$-bound-Fura-2 fluorescence was collected at an emission wavelength of 515 ± 10 nm that was excited at 340 and 380 nm using a spectrofluorometer (IonOptix). Data were analyzed by IonOptix LLC analyzing software.

**hiPSC-CM culture and transduction**

Functional hiPSC-CMs were obtained from Cellular Dynamics International and cultured according to the manufacturer's protocol. Briefly, hiPSC-CMs were thawed and plated on fibronectin-coated dishes. Following 4 h of incubation, the plating medium was aspirated and replaced with maintenance medium. The CMs were maintained for 7 days until the experiment,

and the maintenance medium was replaced every 48 h. On day 7 post-plating, 4-6 hours prior to the infection, the old medium was aspirated and replaced with fresh maintenance medium. During the adenoviral transduction, the completed medium was removed and replaced with the medium with pre-mixed virus/basic medium (without growth factors and FBS, MOI=100) and incubated for 30 min at standard cell culture conditions. The cell culture was replaced with virus/basic medium and with fresh maintenance medium. HiPSC-CMs were transduced with adenoviruses (Vector Biolabs) carrying cytomegalovirus (CMV) promoter driving *HAND1* (Ad-GFP-*HAND1*, the cells are termed as hiPSC-CM$^{HAND1}$) or with adenoviruses carrying CMV promoter (Ad-GFP, the cells are termed as hiPSC-CM$^{Null}$). The cells were harvested for subsequent experiments 72 h post transduction.

**Measurement of contractility and calcium transients in hiPSC-CMs**

HiPSC-CM contractile function was analyzed after seeding the cells on fibronectin-coated (Sigma Aldrich, 5 µg) cover-slips with a modified protocol using IonOptix MyoCam System (IonOptix Corp)[86,87]. $Ca^{2+}$ transient was measured by Fluo-4-AM (Thermo Fisher Scientific) with a modified protocol as described previously[87, 52]. The dye was dissolved in 20 µl DMSO (Sigma Aldrich) and added to 1 ml of cell culture medium. The myocytes were incubated in 5 µM Fluo-4-AM for 20 min at 37°C and washed three times with Tyrode solution containing 1.8 mM $CaCl_2$ at room temperature prior to $Ca^{2+}$ transient measurements. $Ca^{2+}$ transient recording required myocytes that were paced with field stimulation (Grass S48 stimulator; Grass Instruments) with 2-ms (1.5×) threshold pulses at a rate of 1 Hz at room temperature. Fluorescence signals from Fluo-4–AM-loaded myocytes were imaged for $Ca^{2+}$ transients using an inverted confocal microscope (Zeiss LSM 710). Image processing and data analysis were performed using IDL 6.3 software (Exelis Visual Information Solutions).

**Western blot analysis**

Human or mouse heart samples (50 mg) were lysed with ice-cold cell lysis buffer (Cell Signaling Technology, 9803S) in the presence of protease inhibitor (Sigma Aldrich). Following homogenization with the Bullet Blender Storm homogenizer (MIDSCI), the samples were centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was carefully transferred into a new eppendorf tube and kept on ice. Following determination of the protein concentration by the Bradford assay (Bio-Rad), the protein samples (40 µg) were resolved in 10x NuPAGE reducing agent in the presence of 4x LDS sample buffer and denatured at 70°C for 10 min prior to loading on the NuPAGE™ 4-12% Bis-Tris Protein Gels (Thermo Fisher Scientific). Following electrophoresis, the proteins were transferred from the gel to nitrocellulose membranes using semi-dry transfer (Power Blotter Station, Thermo Fisher Scientific). Equal loading and transfer of proteins was confirmed by the quantitative Ponceau red staining. The membranes were incubated for 60 min with 5% dry milk and Tris-buffered saline was used to block the nonspecific binding sites. The membranes were immunoblotted overnight at 4 °C with antibodies against HAND1 (eHAND) (F-7) (Santa Cruz, SC-390376, 1:500 dilution), GFP (Abcam, ab32146, 1:2000), Histone 3 (H3) (Cell Signaling Technology, 9715S, 1: 2000 dilution) or GAPDH (Sigma Aldrich, G9545, 1: 5000 dilution) on a rocking platform. Following washing for three times for 5 min each with Tris-buffered saline, the membranes were incubated for 60 min with an HRP-conjugated secondary antibody, washed three times with Tris-buffered saline and developed with the SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific).

**Quantitative real-time PCR (qPCR)**

The RNA samples (1.0 µg) were reverse transcribed to cDNA using SuperScript™ the First-Strand Synthesis System for RT-PCR kit (Thermo Fisher Scientific). qPCR was performed on a CFX96 Real-Time PCR system (Bio-Rad) using Power SYBR Green Master Mix (Thermo Fisher Scientific). In each analysis, a 0.5 µl cDNA pool was used for qPCR. The fold changes of each

target gene expression were compared with those of GAPDH under experimental and control conditions and were calculated based on the threshold cycle (CT) as follows: $r = 2^{-\Delta(\Delta CT)}$, where $\Delta CT = CT(target) - CT(GAPDH)$ and $\Delta(\Delta CT) = \Delta CT(experimental) - \Delta CT(control)$. The primers for qPCR are as follows: *Nppa*-Forward: 5' TGCAGCTTCCTGTCAACACT 3' ,*Nppa*-Reverse: 5' GAGGCGAGGAAGTCACCATC 3'; *Nppb*-Forward: 5' TGGAAACGTCCGGGTTACAG 3' *Nppb*-Reverse: 5' CTTCCAGACACCTGTGGGAC 3'; *Myh7*-Forward: 5' AGACACACTTGAGTAGCCCAG 3'; *Myh7*-Reverse: 5' CCACCCTCTCGAGACACGAT 3'; *Gapdh*-Forward: 5' GTCAAGGCTGAGAACGGGAA 3'; *Gapdh*-Reverse:5' AAATGAGCCCCAGCCTTCTC 3'.

**Analysis of cardiac fibrotic area**

Mouse heart tissues were dissected, rinsed with PBS and fixed in 10 % formalin for 48 h. Following dehydration through a series of ethanol solutions, the tissues were embedded in paraffin wax according to standard laboratory procedures. Subsequently, they were sectioned (5 μm thick) using a microtome. Following deparaffinization and rehydration, Masson's trichrome staining (Sigma Aldrich) was performed on the heart sections to quantify the fibrotic area. In brief, the sections were fixed with prewarmed Bouin's solution at 56 to 60 °C for 1 h and stained in Weigert's iron hematoxylin solution for 10 min. Following rinsing in tape water, the sections were stained in Biebrich scarlet-acid fuchsin solution for 10 min and further rinsed in distilled water. The sections were differentiated in phosphomolybdic-phosphotungstic acid solution for 10 to 15 min. Once the red color was removed from the collagen-rich areas, the sections were directly transferred to an aniline blue solution and stained for 5 to 10 min. The sections were rinsed in distilled water and differentiated in 1% acetic acid solution for 2 to 5 min. Following rinsing in distilled water, the sections were dehydrated and cleared with xylene. The images were examined with an Olympus BX41 microscope equipped with a CCD (Magna-Fire TM) camera and the fibrotic area was quantified using Image J software.

**Wheat Germ Agglutinin Staining and Quantify Cell Size**

Following deparaffinization and rehydration, the slides were boiled in citrate buffer (Sigma Aldrich) for 20 min for antigen retrieval. Following cooling down for 30 min in distilled water, the slides were permeabilized with 0.2% Triton, washed with PBS 3 times and incubated with wheat germ agglutinin (WGA) conjugated to Texas Red™-X (Thermo Fisher Scientific) for 1 h at room temperature. Following 3 times washing with PBS, the slides were mounted in antifade mounting medium (Thermo Fisher Scientific). Four fields of each section were examined for quantification using the Image J software. Fluorescent imaging was performed with an Olympus BX41 microscope equipped with an epifluorescence attachment.

**Echocardiography**

Transthoracic echocardiography (Visual Sonics Vevo 2100) was performed with a 40-MHz probe. The hearts were imaged in 2D long-axis view at the level of the greatest LV diameter in animals that were under light general anesthesia. This view was used to position the M-mode cursor perpendicular to the LV anterior and posterior walls. The heart rate and LV dimensions, including diastolic and systolic wall thicknesses and LV end-diastolic and end-systolic chamber dimensions were measured under M-mode tracings at the level of the papillary muscle. LV end-diastolic (LVDd) and end-systolic diameters (LVDs) were measured from M-mode recordings. LV EF was calculated as follows: EF% = [(LVDd)3-(LVDs)3/(LVDd)3 × 100]. LV FS was determined as follows: FS% = [(LVDd–LVDs)/LVDd × 100]. All measurements were performed according to the American Society for Echocardiography leading-edge technique standards and were averaged over three consecutive cardiac cycles.

## SUPPLEMENTAL TABLES

**Supplemental Table 1. Summary of heart sample donors**

| | Diagnosis | |
|---|---|---|
| | Non-failing (NF) | Failing (DCM) |
| No. of donors | 51 | 50 |
| Male | 29 | 38 |
| Female | 22 | 12 |
| Age (yrs) | 43.5 | 57 |
| Ejection fraction (%) | 55 | 15 |

**Supplemental Table 2. Data source of enhancer breadth**

The source of 507 human H3K27ac ChIP-seq data from ENCODE consortium.

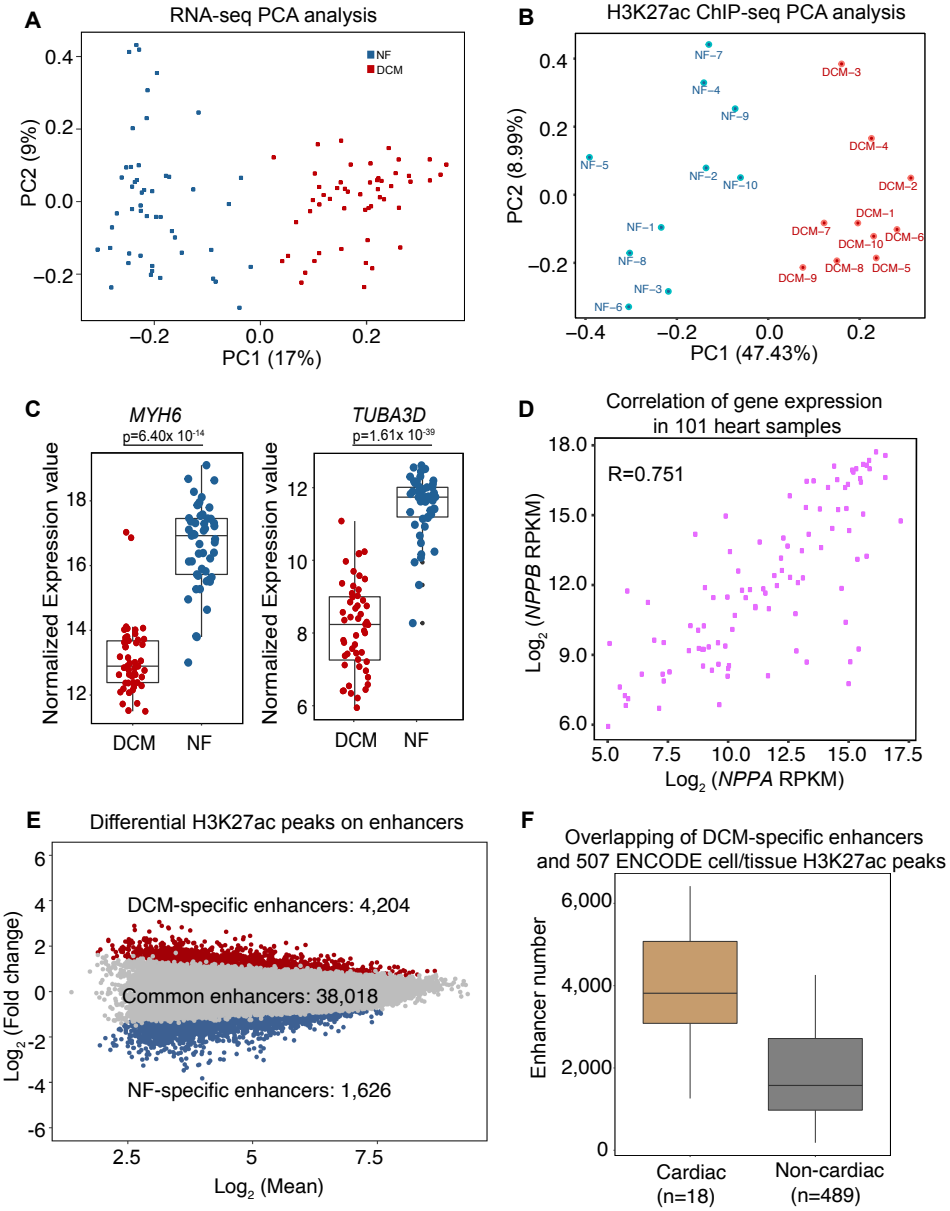
**Supplemental Table 3. Statistics for sequencing data**

Statistics of sequencing data for human heart tissues (H3K27ac ChIP-seq, H3K27ac HiChIP, In-situ Hi-C, HAND1 ChIP-seq, RNA-seq), hiPSC-CMs (H3K27ac ChIP-seq, H3K27ac HiChIP-seq, HAND1 ChIP-seq, RNA-seq) and mouse cardiomyocytes (RNA-seq).

**Supplemental Table 4. Chromatin interactions derived from HiChIP and Hi-C data**

The coordinates of DCM/NF-all H3K27ac HiChIP loop anchors, DCM/NF-enriched H3K27ac HiChIP loop anchors and DCM/NF TADs.

## SUPPLEMENTAL FIGURES



**Supplemental Figure 1. Genome-Wide Identification of Active CREs in DCM and NF Hearts. Related to Figure 1.**

**A**. PCA plot of ensembled 50 DCM and 51 NF RNA-seq RNA-seq profiles using top 2000 variable genes. Red dots represent DCM heart samples. Blue dots represent NF heart samples.

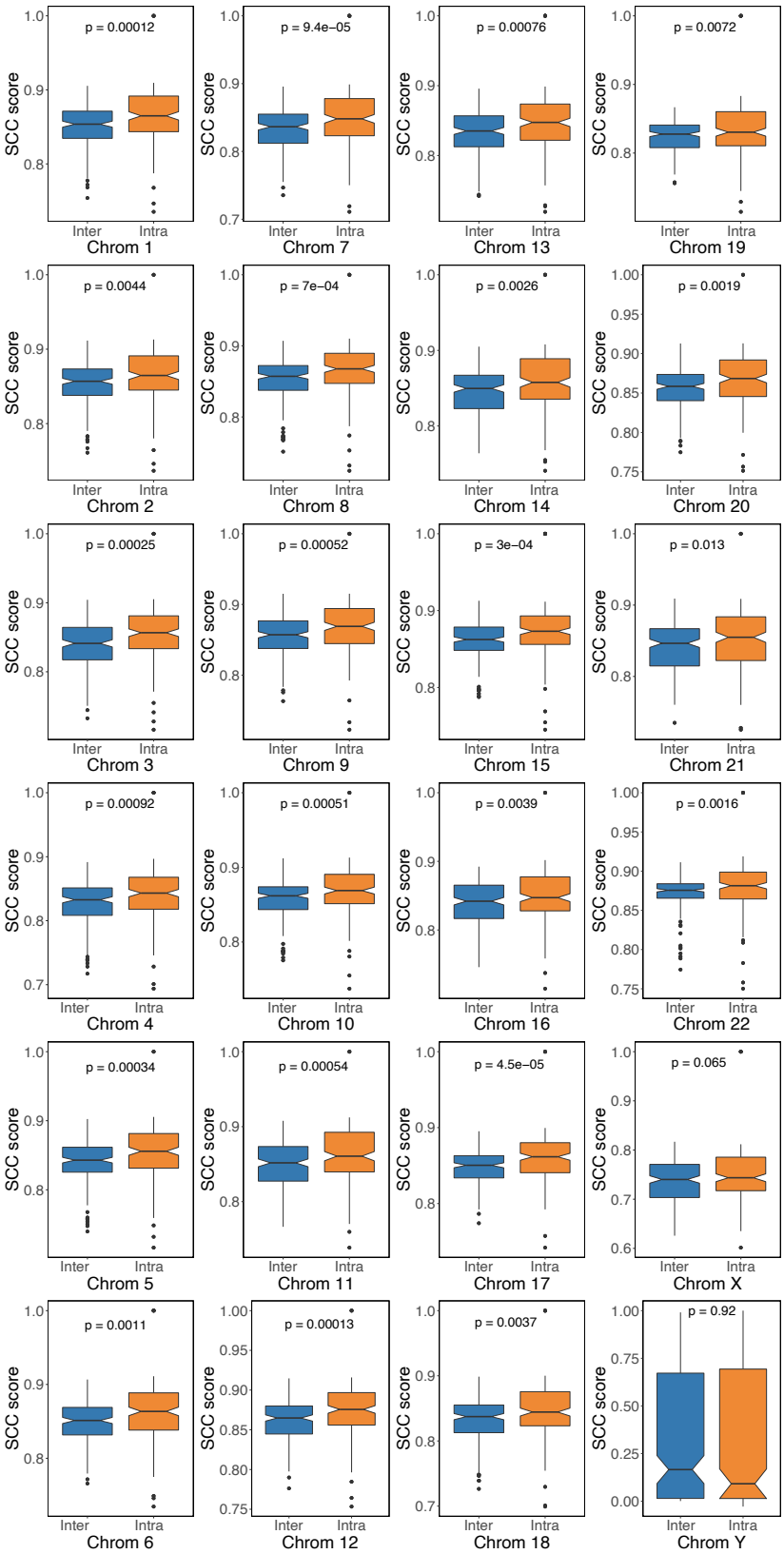
**B**. PCA plot of ensembled 10 DCM and 10 NF H3K27ac ChIP-seq profiles. Red dots represent DCM heart samples. Blue dots represent NF heart samples.

**C**. Expression values ($log_2$(normalized counts)) for *MYH6* and *TUBA3D* in 50 DCM and 51 NF heart RNA-seq datasets. Data were analyzed by Wald test.

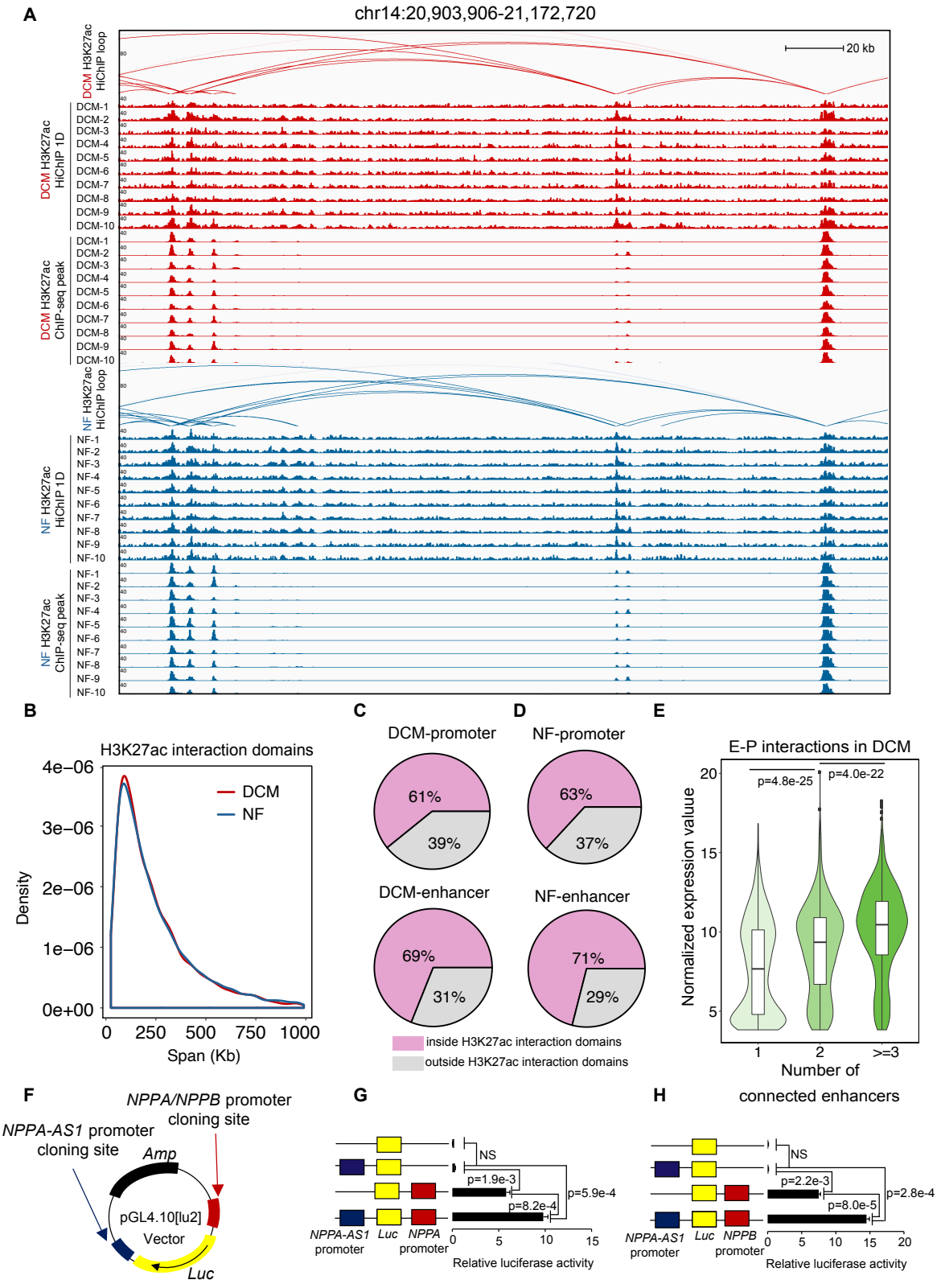
**D**. Correlation between the gene expression of *NPPA* and *NPPB* in 101 DCM and NF heart tissues.

**E.** MA plot showing DCM-enriched, NF-enriched, and common H3K27ac ChIP-seq peak signals in enhancer regions (outside of +/-2.5 kb to known TSSs) of DCM and NF hearts.

**F**. Overlapping between DCM-specific enhancers identified from this study and ENCODE H3K27ac ChIP-seq peaks. Cardiac (left) and non-cardiac (right) cell/tissue-derived ENCODE data were analyzed separately.


p = 0.00012
Chrom 1
p = 9.4e−05
Chrom 7
p = 0.00076
Chrom 13
p = 0.0072
Chrom 19
p = 0.0044
Chrom 2
p = 7e−04
Chrom 8
p = 0.0026
Chrom 14
p = 0.0019
Chrom 20
p = 0.00025
Chrom 3
p = 0.00052
Chrom 9
p = 3e−04
Chrom 15
p = 0.013
Chrom 21
p = 0.00092
Chrom 4
p = 0.00051
Chrom 10
p = 0.0039
Chrom 16
p = 0.0016
Chrom 22
p = 0.00034
Chrom 5
p = 0.00054
Chrom 11
p = 4.5e−05
Chrom 17
p = 0.065
Chrom X
p = 0.0011
Chrom 6
p = 0.00013
Chrom 12
p = 0.0037
Chrom 18
p = 0.92
Chrom Y
SCC score
Inter
Intra

**Supplemental Figure 2. Assessment of the Intra and Inter Group Similarity between Contact Matrices in DCM and NF HiChIP Data. Related to Figure 2.** Stratum adjusted correlation coefficient (SCC) was computed separately for each chromosome.


A
chr14:20,903,906-21,172,720
20 kb
DCM H3K27ac HiChIP loop
DCM H3K27ac HiChIP 1D
DCM H3K27ac ChIP-seq peak
NF H3K27ac HiChIP loop
NF H3K27ac HiChIP 1D
NF H3K27ac ChIP-seq peak
DCM-1
DCM-2
DCM-3
DCM-4
DCM-5
DCM-6
DCM-7
DCM-8
DCM-9
DCM-10
NF-1
NF-2
NF-3
NF-4
NF-5
NF-6
NF-7
NF-8
NF-9
NF-10
B
H3K27ac interaction domains
DCM
NF
Density
4e−06
3e−06
2e−06
1e−06
0e+00
0
250
500
750
1000
Span (Kb)
C
DCM-promoter
61%
39%
DCM-enhancer
69%
31%
D
NF-promoter
63%
37%
NF-enhancer
71%
29%
inside H3K27ac interaction domains
outside H3K27ac interaction domains
E
E-P interactions in DCM
p=4.8e-25
p=4.0e-22
Normalized expression valuue
20
15
10
5
1
2
>=3
Number of
connected enhancers
F
NPPA/NPPB promoter
cloning site
NPPA-AS1 promoter
cloning site
Amp
pGL4.10[lu2]
Vector
Luc
G
NS
p=1.9e-3
p=5.9e-4
p=8.2e-4
NPPA-AS1
promoter
Luc
NPPA
promoter
0
5
10
15
Relative luciferase activity
H
NS
p=2.2e-3
p=2.8e-4
p=8.0e-5
NPPA-AS1
promoter
Luc
NPPB
promoter
0
5
10
15
20
Relative luciferase activity

**Supplemental Figure 3. Characterization of H3K27ac HiChIP Data in DCM and NF Hearts. Related to Figure 2.**

**A**. Genome browser view of comparison of H3K27ac ChIP-seq and H3K27ac HiChIP 1D signals among 10 DCM and 10 NF heart tissue samples (chr14:20,903,906-21,172,720).

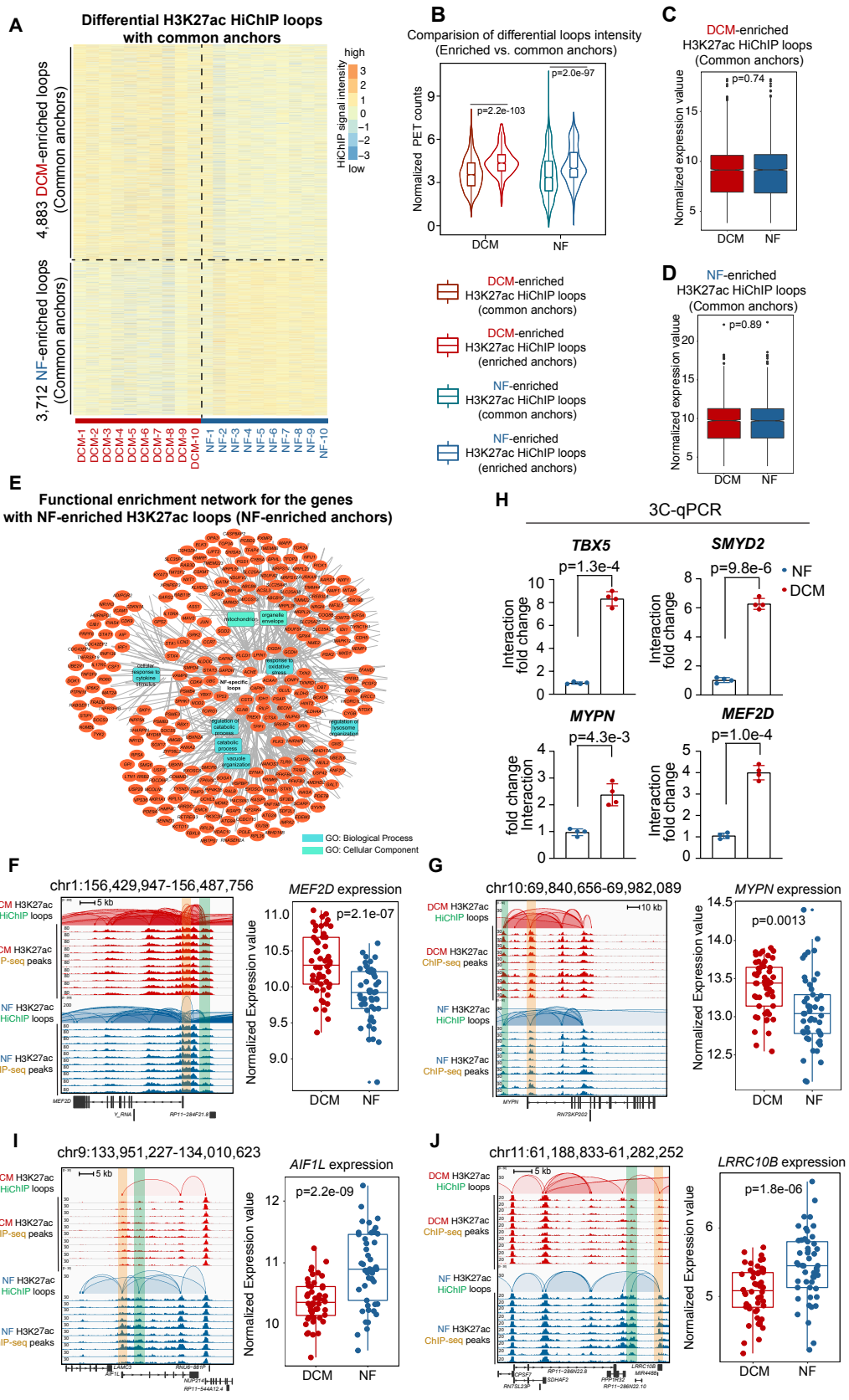
**B**. Genomic span distribution of H3K27ac interaction domains in DCM and NF hearts by density.

**C**. Distribution of all active promoters (upper panel) and active enhancers (lower panel) inside and outside of the H3K27ac interaction domains in DCM hearts.

**D**. Distribution of all active promoters (upper panel) and active enhancers (lower panel) inside and outside of the H3K27ac interaction domains in NF hearts.

**E**. Normalized expression values for genes with different numbers of enhancers connected to promoters in DCM hearts. Data were analyzed by Wilcoxon rank sum test with continuity correction.

**F-H**. Luciferase reporter assays testing the enhancer function of the *NPPA-AS1* promoter. Data were analyzed by One-way ANOVA.


A
Differential H3K27ac HiChIP loops with common anchors
4,883 DCM-enriched loops (Common anchors)
3,712 NF-enriched loops (Common anchors)
HiChIP signal intensity
high
low
DCM-1 DCM-2 DCM-3 DCM-4 DCM-5 DCM-6 DCM-7 DCM-8 DCM-9 DCM-10
NF-1 NF-2 NF-3 NF-4 NF-5 NF-6 NF-7 NF-8 NF-9 NF-10
B
Comparision of differential loops intensity (Enriched vs. common anchors)
Normalized PET counts
p=2.2e-103
p=2.0e-97
DCM
NF
DCM-enriched H3K27ac HiChIP loops (common anchors)
DCM-enriched H3K27ac HiChIP loops (enriched anchors)
NF-enriched H3K27ac HiChIP loops (common anchors)
NF-enriched H3K27ac HiChIP loops (enriched anchors)
C
DCM-enriched H3K27ac HiChIP loops (Common anchors)
Normalized expression value
p=0.74
DCM
NF
D
NF-enriched H3K27ac HiChIP loops (Common anchors)
Normalized expression value
p=0.89
DCM
NF
E
Functional enrichment network for the genes with NF-enriched H3K27ac loops (NF-enriched anchors)
GO: Biological Process
GO: Cellular Component
H
3C-qPCR
TBX5
p=1.3e-4
SMYD2
p=9.8e-6
NF
DCM
MYPN
p=4.3e-3
MEF2D
p=1.0e-4
Interaction fold change
F
chr1:156,429,947-156,487,756
DCM H3K27ac HiChIP loops
DCM H3K27ac ChIP-seq peaks
NF H3K27ac HiChIP loops
NF H3K27ac ChIP-seq peaks
5 kb
MEF2D expression
p=2.1e-07
Normalized Expression value
DCM
NF
G
chr10:69,840,656-69,982,089
10 kb
MYPN expression
p=0.0013
Normalized Expression value
DCM
NF
I
chr9:133,951,227-134,010,623
5 kb
AIF1L expression
p=2.2e-09
Normalized Expression value
DCM
NF
J
chr11:61,188,833-61,282,252
5 kb
LRRC10B expression
p=1.8e-06
Normalized Expression value
DCM
NF

**Supplemental Figure 4. DCM-enriched Enhancer/Promoter Connectome Contributes to DCM-Specific Transcription. Related to Figure 3.**

**A.** Heatmap showing intensity of differential H3K27ac loops with common loop anchors (H3K27ac signal) in 10 DCM and 10 NF heart samples.

**B**. Normalized PET counts for differential loops (enriched anchors vs. common anchors).

**C**. Normalized expression values of the genes with DCM-enriched H3K27ac loops (common anchors). Data were analyzed by Wilcoxon rank sum test with continuity correction.

**D**. Normalized expression values of the genes with NF-enriched H3K27ac loops (common anchors). Data were analyzed by Wilcoxon rank sum test with continuity correction.

**E**. Network representation of functional enrichment of the genes with NF-enriched H3K27ac loops (NF-enriched anchors). Functional enrichment was performed using the ToppFun application. Orange nodes represent NF-enriched genes, while the different colored rectangles represent enriched terms. Only select enriched terms are shown here. Network was generated using the Cytoscape application.

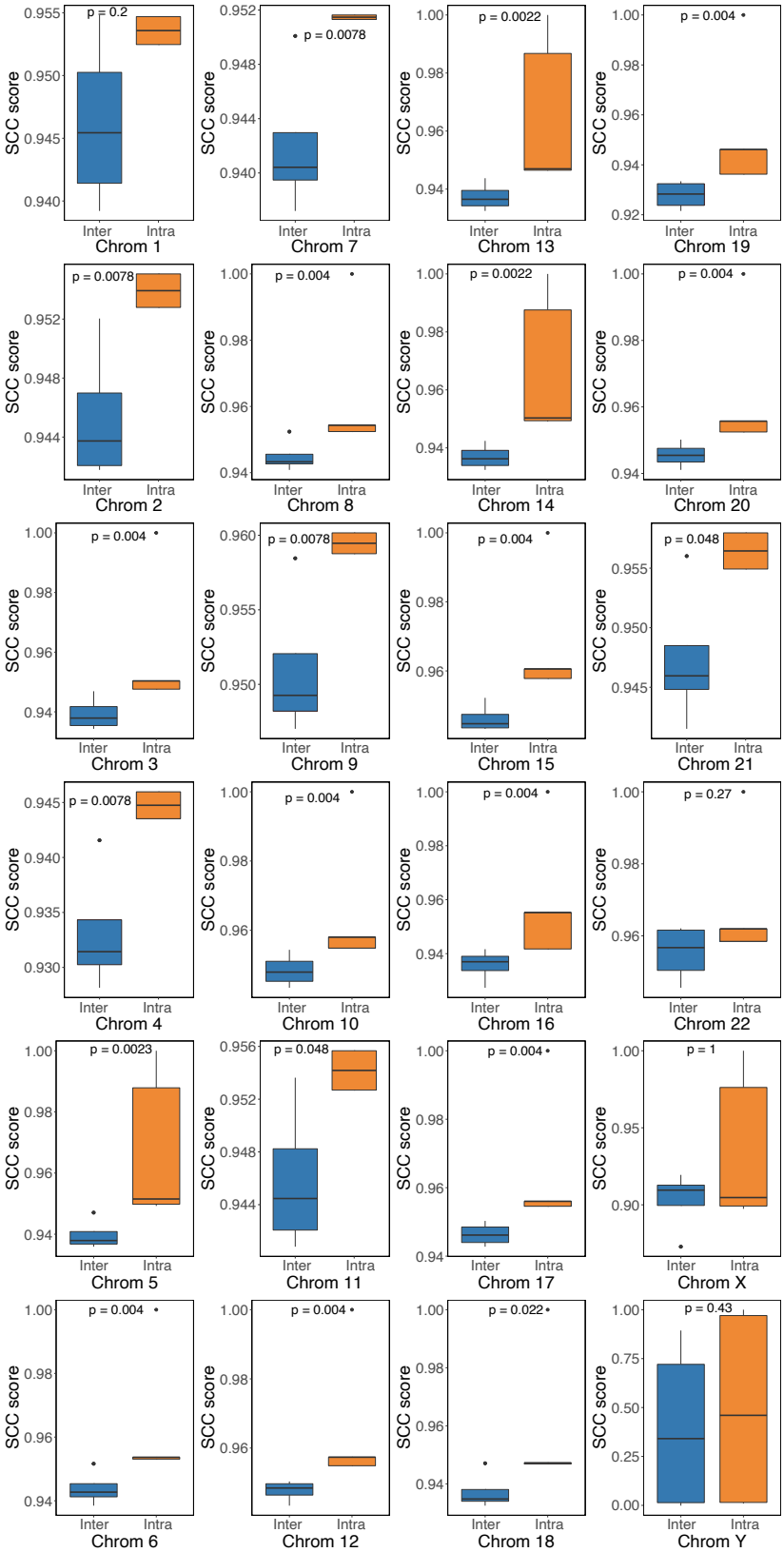
**F**. Left panel: browser screenshot showing *MEF2D* with DCM-enriched E-P interactions (chr1:156,429,947-156,487,756). The top two rows represent the interactions in DCM hearts, and the bottom two rows represent those in NF hearts. The HiChIP loops and ChIP-seq peaks are shown for each. The enhancer is highlighted in the green box, while the promoter is highlighted in the orange box. Right panel: expression values ($log_2$(normalized counts)) for *MEF2D* in 50 DCM and 51 NF heart RNA-seq datasets. Data were analyzed by Wald test.

**G**. Left panel: browser screenshot showing *MYPN* with DCM-enriched E-P interactions (chr10:69,840,656-69,982,089). The top two rows represent the interactions in DCM hearts, and the bottom two rows represent those in NF hearts. The HiChIP loops and ChIP-seq peaks are shown for each. The enhancer is highlighted in the green box, while the promoter is highlighted in the orange box. Right panel: expression values ($log_2$(normalized counts)) for *MYPN* in 50 DCM and 51 NF heart RNA-seq datasets. Data were analyzed by Wald test.

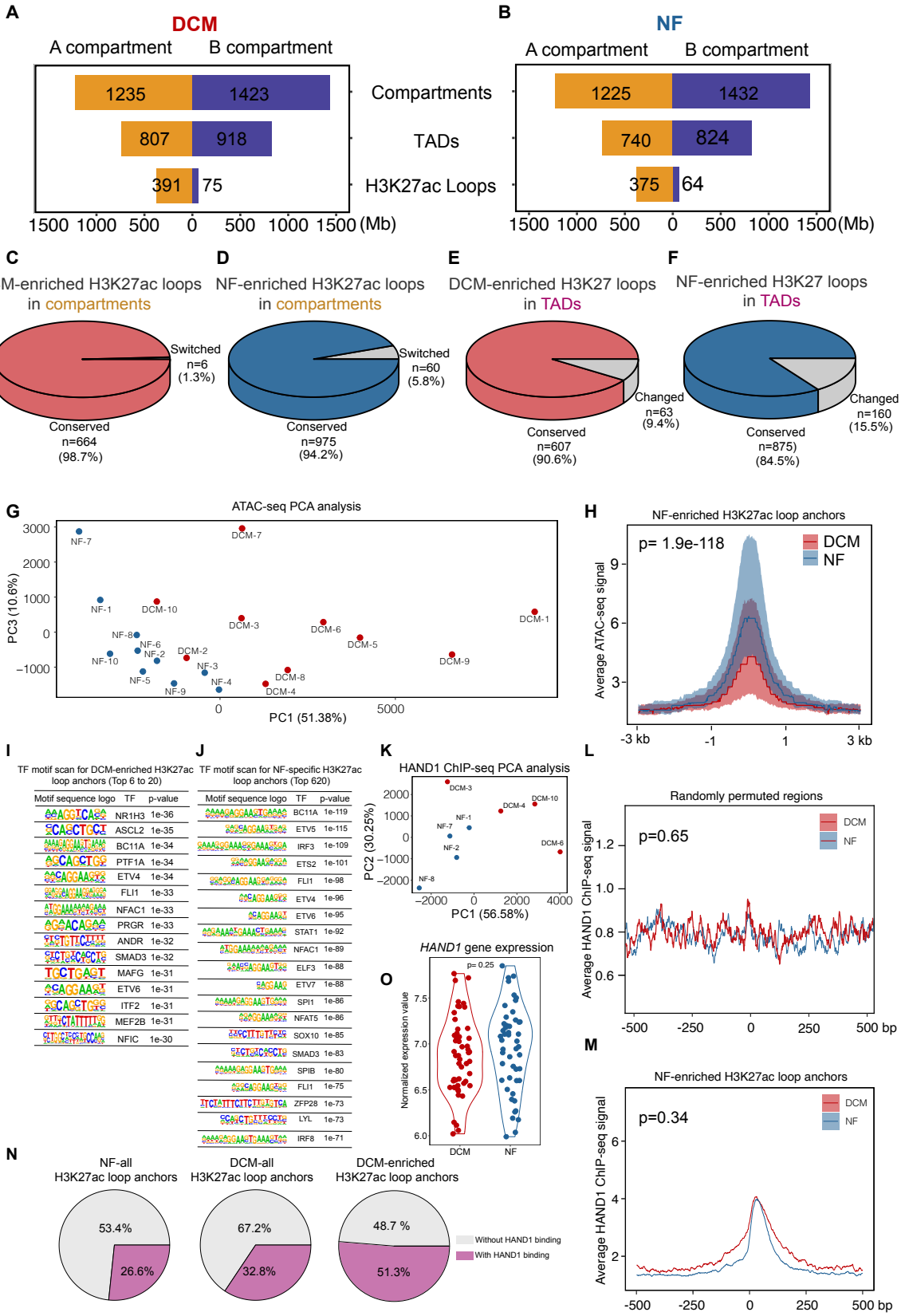
**H**. Validation of E-P interactions with 3C-qPCR analysis in DCM and NF hearts (*TBX5*, *SMYD2*, *MEF2D*, *MYPN*, related to Figure 3E-H). Student *t* test was used for differential analysis.

**I**. Left panel: browser screenshot showing *AIF1L* with NF-enriched E-P interactions (chr9:133,951,227-134,010,623). The top two rows represent the interactions in DCM hearts, and the bottom two rows represent those in NF hearts. The HiChIP loops and ChIP-seq peaks are shown for each. The enhancer is highlighted in the green box, while the promoter is highlighted in the orange box. Right panel: expression values ($log_2$(normalized counts)) for *AIF1L* in 50 DCM and 51 NF heart RNA-seq datasets. Data were analyzed by Wald test.

**J**. Left panel: browser screenshot showing *LRRC10B* with NF-enriched E-P interactions (chr11:61,188,833-61,282,252). The top two rows represent the interactions in DCM hearts, and the bottom two rows represent those in NF hearts. The HiChIP loops and ChIP-seq peaks are shown for each. The enhancer is highlighted in the green box, while the promoter is highlighted in the orange box. Right panel: expression values ($log_2$(normalized counts)) for *LRRC10B* in 50 DCM and 51 NF heart RNA-seq datasets. Data were analyzed by Wald test.


SCC score
Inter
Intra
p = 0.2
Chrom 1
p = 0.0078
Chrom 7
p = 0.0022
Chrom 13
p = 0.004
Chrom 19
p = 0.0078
Chrom 2
p = 0.004
Chrom 8
p = 0.0022
Chrom 14
p = 0.004
Chrom 20
p = 0.004
Chrom 3
p = 0.0078
Chrom 9
p = 0.004
Chrom 15
p = 0.048
Chrom 21
p = 0.0078
Chrom 4
p = 0.004
Chrom 10
p = 0.004
Chrom 16
p = 0.27
Chrom 22
p = 0.0023
Chrom 5
p = 0.048
Chrom 11
p = 0.004
Chrom 17
p = 1
Chrom X
p = 0.004
Chrom 6
p = 0.004
Chrom 12
p = 0.022
Chrom 18
p = 0.43
Chrom Y

**Supplemental Figure 5. Assessment of the Intra and Inter Group Similarity between Contact Matrices in DCM and NF in-situ Hi-C Data. Related to Figure 4.** Stratum adjusted correlation coefficient (SCC) was computed separately for each chromosome.


A
DCM
A compartment
B compartment
1235
1423
807
918
391
75
1500 1000 500 0 500 1000 1500 (Mb)
Compartments
TADs
H3K27ac Loops
B
NF
A compartment
B compartment
1225
1432
740
824
375
64
1500 1000 500 0 500 1000 1500 (Mb)
C
DCM-enriched H3K27ac loops in compartments
Switched n=6 (1.3%)
Conserved n=664 (98.7%)
D
NF-enriched H3K27ac loops in compartments
Switched n=60 (5.8%)
Conserved n=975 (94.2%)
E
DCM-enriched H3K27 loops in TADs
Changed n=63 (9.4%)
Conserved n=607 (90.6%)
F
NF-enriched H3K27 loops in TADs
Changed n=160 (15.5%)
Conserved n=875) (84.5%)
G
ATAC-seq PCA analysis
PC3 (10.6%)
PC1 (51.38%)
NF-7
DCM-7
NF-1
DCM-10
DCM-3
DCM-6
DCM-1
NF-8
NF-6
NF-2
DCM-5
DCM-2
NF-10
NF-3
DCM-9
NF-5
DCM-8
NF-4
NF-9
DCM-4
H
NF-enriched H3K27ac loop anchors
p= 1.9e-118
DCM
NF
Average ATAC-seq signal
-3 kb
-1
1
3 kb
I
TF motif scan for DCM-enriched H3K27ac loop anchors (Top 6 to 20)
Motif sequence logo TF p-value
NR1H3 1e-36
ASCL2 1e-35
BC11A 1e-34
PTF1A 1e-34
ETV4 1e-34
FLI1 1e-33
NFAC1 1e-33
PRGR 1e-33
ANDR 1e-32
SMAD3 1e-32
MAFG 1e-31
ETV6 1e-31
ITF2 1e-31
MEF2B 1e-31
NFIC 1e-30
J
TF motif scan for NF-specific H3K27ac loop anchors (Top 620)
Motif sequence logo TF p-value
BC11A 1e-119
ETV5 1e-115
IRF3 1e-109
ETS2 1e-101
FLI1 1e-98
ETV4 1e-96
ETV6 1e-95
STAT1 1e-92
NFAC1 1e-89
ELF3 1e-88
ETV7 1e-88
SPI1 1e-86
NFAT5 1e-86
SOX10 1e-85
SMAD3 1e-83
SPIB 1e-80
FLI1 1e-75
ZFP28 1e-73
LYL 1e-73
IRF8 1e-71
K
HAND1 ChIP-seq PCA analysis
PC2 (30.25%)
PC1 (56.58%)
DCM-3
DCM-4
DCM-10
NF-1
NF-7
NF-2
DCM-6
NF-8
L
Randomly permuted regions
p=0.65
DCM
NF
Average HAND1 ChIP-seq signal
-500 -250 0 250 500 bp
HAND1 gene expression
p= 0.25
Normalized expression value
DCM
NF
M
NF-enriched H3K27ac loop anchors
p=0.34
DCM
NF
Average HAND1 ChIP-seq signal
-500 -250 0 250 500 bp
N
NF-all H3K27ac loop anchors
53.4%
26.6%
DCM-all H3K27ac loop anchors
67.2%
32.8%
DCM-enriched H3K27ac loop anchors
48.7 %
51.3%
Without HAND1 binding
With HAND1 binding

**Supplemental Figure 6. DCM-enriched H3K27ac HiChIP Loops Largely Reside in Pre-established High-Order Chromatin Architectures and Pre-accessible Chromatin Sites Bound by Reactivated HAND1. Related to Figure 4.**

**A-B**. Bar chart of genomic coverage of A compartment (yellow) and B compartment (blue), TADs, and H3K27ac loops in DCM and NF hearts. Genomic coverage is shown in Mb.

**C**-**D**. Distribution and number of DCM-enriched (C) and NF-enriched (D) H3K27ac loops in switched and conserved compartments.

**E**-**F**. Distribution and number of DCM-enriched (E) and NF-enriched (F) H3K27ac loops in changed and conserved TADs.

**G**. PCA plot of ensembled 10 DCM and 10 NF ATAC-seq profiles. Red dots represent DCM heart samples. Blue dots represent NF heart samples.

**H.** Aggregation plot of NF (blue) and DCM (red) normalized ATAC-seq signals ± 3 kb centered on ATAC-seq peaks overlapped with NF-enriched H3K27ac loop anchors. Data were analyzed by Mann-Whitney U test.

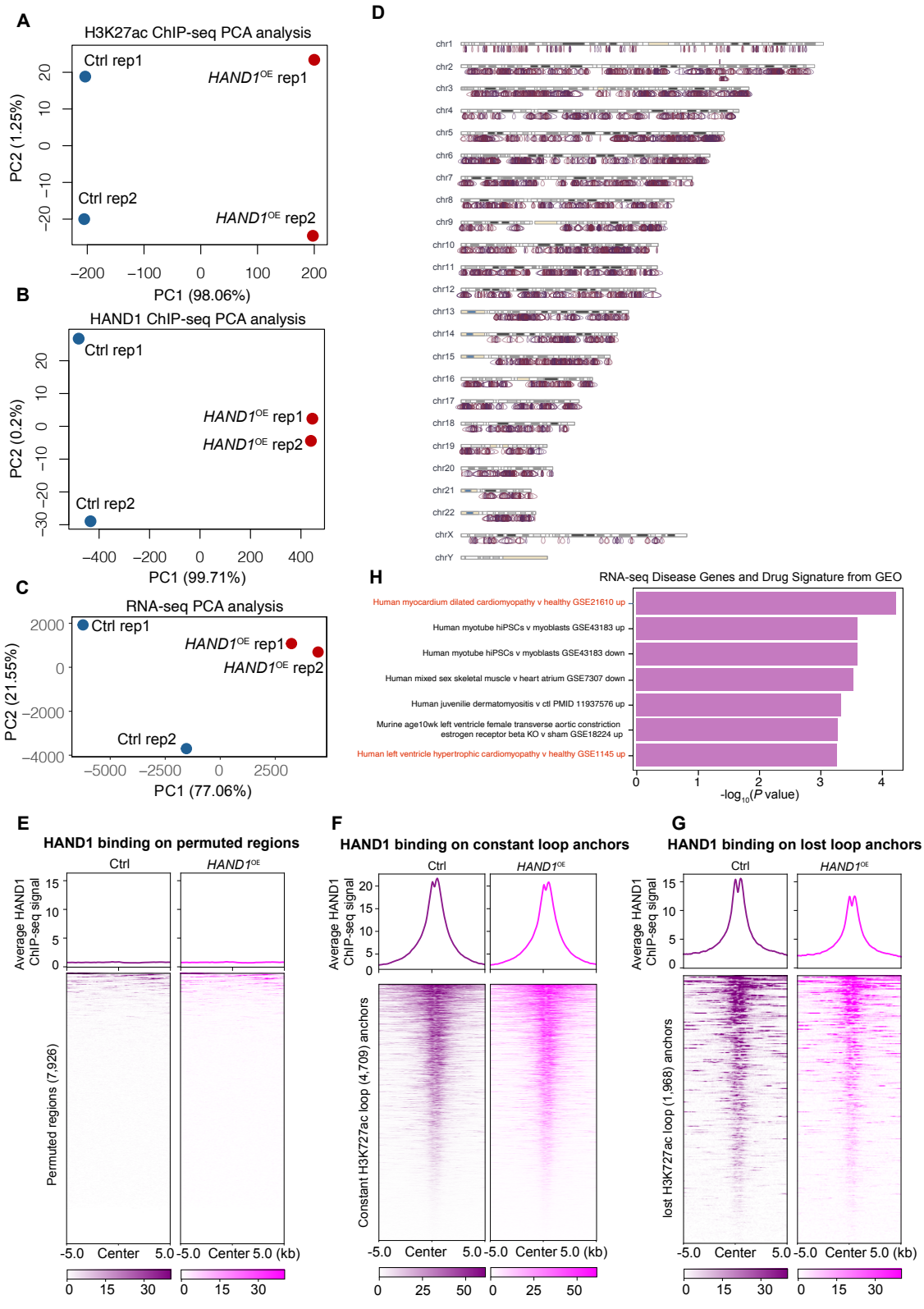
**I**. Transcription factor motif-enriched ATAC-seq peak overlapped with the DCM-enriched H3K27ac loop anchor (top 6 to top 20 transcription factors are shown). The p-values and the motif sequences are also shown.

**J**. Transcription factor motif-enriched ATAC-seq peak overlapped with the NF-enriched H3K27ac loop anchor (Top 20 transcription factors are shown). The p-values and the motif sequences are also shown.

**K**. PCA plot of ensembled 4 DCM and 4 NF HAND1 ChIP-seq profiles. Red dots represent DCM heart samples. Blue dots represent NF heart samples.

**L-M**. Aggregation plot of normalized HAND1 ChIP-seq signals on randomly permuted regions (L) or centered on the ATAC-seq peaks overlapping with the NF-enriched H3K27ac loop anchors (M) in DCM (n=4) and NF (n=4) hearts. Data were analyzed by Mann-Whitney U test.

**N**. Percentage of NF-all H3K27ac HiChIP loops, DCM-all H3K27ac HiChIP loops, DCM-enriched H3K27ac HiChIP loops, the anchors of which do or do not overlap with HAND1 ChIP-seq peaks. **O**. Comparison of *HAND1* gene expression in DCM (n=50) and NF hearts (n=51) from the RNA-seq data in this study.


A
H3K27ac ChIP-seq PCA analysis
Ctrl rep1
HAND1OE rep1
Ctrl rep2
HAND1OE rep2
PC2 (1.25%)
PC1 (98.06%)
B
HAND1 ChIP-seq PCA analysis
Ctrl rep1
HAND1OE rep1
HAND1OE rep2
Ctrl rep2
PC2 (0.2%)
PC1 (99.71%)
C
RNA-seq PCA analysis
Ctrl rep1
HAND1OE rep1
HAND1OE rep2
Ctrl rep2
PC2 (21.55%)
PC1 (77.06%)
D
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22
chrX
chrY
H
RNA-seq Disease Genes and Drug Signature from GEO
Human myocardium dilated cardiomyopathy v healthy GSE21610 up
Human myotube hiPSCs v myoblasts GSE43183 up
Human myotube hiPSCs v myoblasts GSE43183 down
Human mixed sex skeletal muscle v heart atrium GSE7307 down
Human juvenilie dermatomyositis v ctl PMID 11937576 up
Murine age10wk left ventricle female transverse aortic constriction estrogen receptor beta KO v sham GSE18224 up
Human left ventricle hypertrophic cardiomyopathy v healthy GSE1145 up
-log10(P value)
E
HAND1 binding on permuted regions
Ctrl
HAND1OE
Average HAND1 ChIP-seq signal
Permuted regions (7,926)
-5.0 Center 5.0 (kb)
F
HAND1 binding on constant loop anchors
Ctrl
HAND1OE
Average HAND1 ChIP-seq signal
Constant H3K727ac loop (4,709) anchors
-5.0 Center 5.0 (kb)
G
HAND1 binding on lost loop anchors
Ctrl
HAND1OE
Average HAND1 ChIP-seq signal
lost H3K727ac loop (1,968) anchors
-5.0 Center 5.0 (kb)

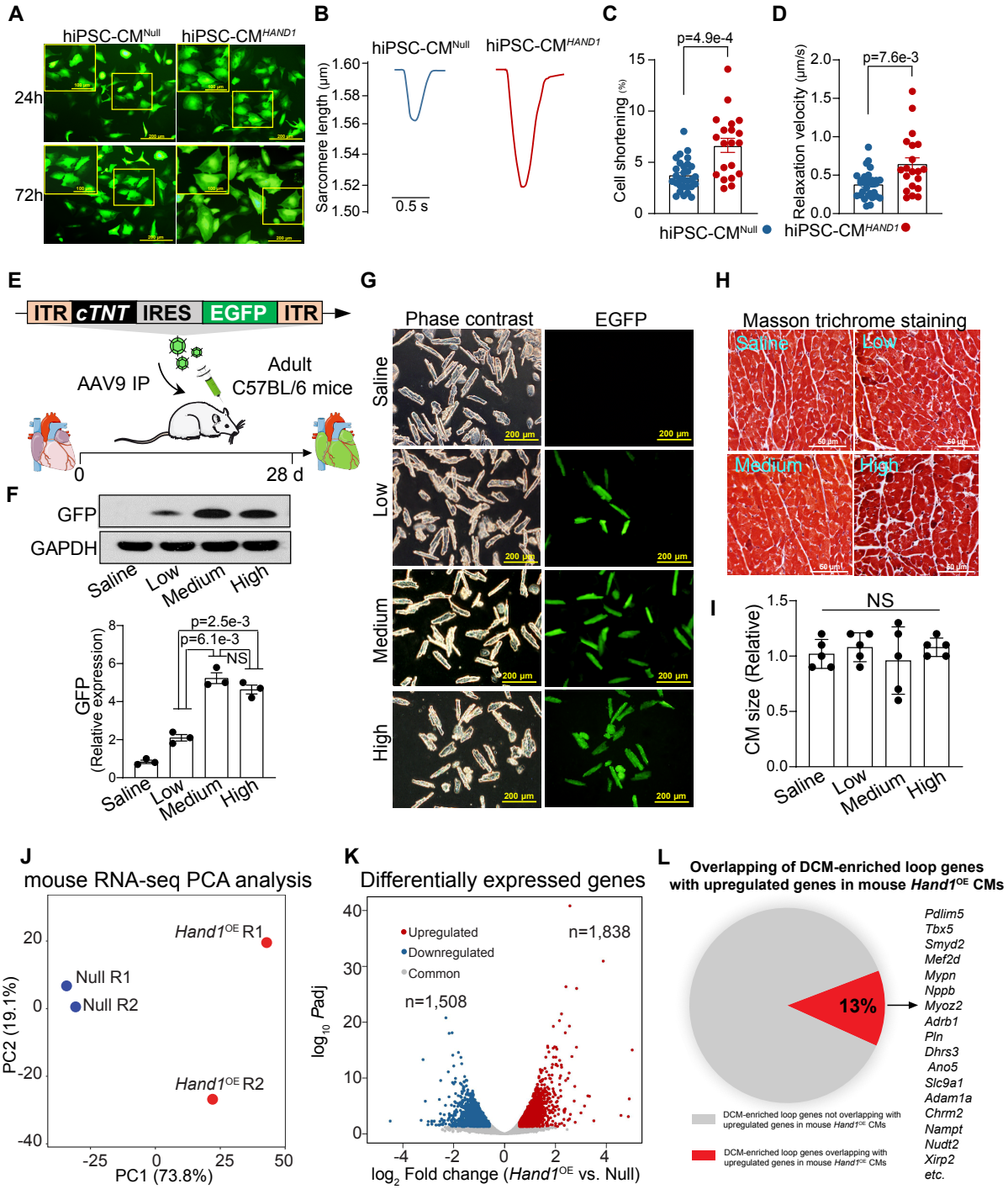
**Supplemental Figure 7. Overexpression of *HAND1* Induces Genome-Wide Gain of Enhancer/Promoter Interactions. Related to Figure 5**.

**A-C.** PCA plot of H3K27ac ChIP-seq (A), HAND1 ChIP-seq (B) and RNA-seq (C) for Ctrl and *HAND1*$^{OE}$ iPSC-CM. Red dots represent Ctrl samples. Blue dots represent *HAND1*$^{OE}$ samples.

**D**. Global view of gained H3K27ac HiChIP loops in hiPSC-CMs induced by HAND1.

**E-G**. Aggregation plot and heatmap showing the intensity of HAND1 ChIP-seq peaks on the permuted regions (E), the constant (F) and lost (G) H3K27ac HiChIP loop anchors (*HAND1*$^{OE}$ vs Ctrl hiPSC-CM)**.**

**H.** Functional annotation (RNA-Seq_Disease_Gene_and_Drug_Signatures_from_GEO) for the overlap between upregulated genes in hiPSC-*HAND1*$^{OE}$ (vs. Ctrl) and upregulated genes in DCM hearts (vs. NF) from this study.


A
hiPSC-CMNull
hiPSC-CMHAND1
24h
72h
B
hiPSC-CMNull
hiPSC-CMHAND1
Sarcomere length (μm)
1.60
1.58
1.56
1.54
1.52
1.50
0.5 s
C
p=4.9e-4
Cell shortening (%)
15
10
5
0
hiPSC-CMNull
hiPSC-CMHAND1
D
p=7.6e-3
Relaxation velocity (μm/s)
2.0
1.5
1.0
0.5
0.0
hiPSC-CMNull
hiPSC-CMHAND1
E
ITR
cTNT
IRES
EGFP
ITR
AAV9 IP
Adult
C57BL/6 mice
0
28 d
F
GFP
GAPDH
Saline
Low
Medium
High
p=2.5e-3
p=6.1e-3
NS
GFP
(Relative expression)
8
6
4
2
0
Saline
Low
Medium
High
G
Phase contrast
EGFP
Saline
Low
Medium
High
200 μm
H
Masson trichrome staining
Saline
Low
Medium
High
50 μm
I
NS
CM size (Relative)
1.5
1.0
0.5
0.0
Saline
Low
Medium
High
J
mouse RNA-seq PCA analysis
Hand1OE R1
Null R1
Null R2
Hand1OE R2
PC2 (19.1%)
20
0
-20
-40
-25
0
25
50
PC1 (73.8%)
K
Differentially expressed genes
Upregulated
Downregulated
Common
n=1,838
n=1,508
log10 Padj
40
30
20
10
0
-4
-2
0
2
4
log2 Fold change (Hand1OE vs. Null)
L
Overlapping of DCM-enriched loop genes
with upregulated genes in mouse Hand1OE CMs
13%
DCM-enriched loop genes not overlapping with upregulated genes in mouse Hand1OE CMs
DCM-enriched loop genes overlapping with upregulated genes in mouse Hand1OE CMs
Pdlim5
Tbx5
Smyd2
Mef2d
Mypn
Nppb
Myoz2
Adrb1
Pln
Dhrs3
Ano5
Slc9a1
Adam1a
Chrm2
Nampt
Nudt2
Xirp2
etc.

**Supplemental Figure 8. HAND1 Induces DCM Phenotypes in Human iPSC-Derived Cardiomyocytes and Mouse. Related to Figure 6.**

**A.** Fluorescence microscopy showing GFP expression of hiPSC-CMs after adenovirus transduction for 24h or 72h. The cells transfected with Null adenovirus (Ad-GFP) were designated as hiPSC-CM$^{Null}$ and the cells transfected with Ad-GFP-*HAND1* were designated as hiPSC-CM$^{HAND1}$.

**B-D**. Contractility mechanic assay of hiPSC-CMs. (B) Representative images of sarcomere shortening tracing. (C) Cell shortening (%) at 0.5 Hz. (D) Relaxation velocity of sarcomere at 0.5 Hz. Data were analyzed by Student's *t* test.

**E**. Schematic representation of the AAV9 transgene (AAV9-*cTNT*-EGFP) used for this study.

**F**. Western blot analysis of EGFP expression in CMs of mice receiving different doses of AAV9-*cTNT*-EGFP or saline (upper panel). The mice receiving the different dose of AAV9 ($5\times10^{11}$, $1\times10^{12}$, and $5\times10^{12}$ viral genomes (vg)) by intraperitoneal injection were defined as Low, Medium, and High group, respectively. The mice receiving saline were defined as the Saline group. The quantification of protein expression in the western blot (lower panel). Data were analyzed by One-way ANOVA.

**G**. Fluorescence microscopy showing EGFP expression in isolated CMs from mice receiving different dosages of AAV9-*cTNT*-EGFP.

**H**. Masson trichrome staining of hearts of mice receiving AAV9-*cTNT*-EGFP or saline.

**I**. Quantification of CM size. Data were analyzed by One-way ANOVA. N.S., no statistical significance.

**J.** PCA plot of RNA-seq for the cardiomyocytes isolated from Null and *Hand1*$^{OE}$ hearts (28 days). Red dots represent Null samples. Blue dots represent *Hand1*$^{OE}$ samples.

**K**. Volcano plot showing the differentially expressed genes in *Hand1*$^{OE}$ cardiomyocytes (vs Null). Red dots represent upregulated genes samples. Blue dots represent downregulated genes.

**L**. Pie chart showing the percentage of DCM-enriched loop genes overlapped with upregulated genes in mouse *Hand1*$^{OE}$ CMs.