



High-Resolution Mapping of Chromatin Conformation in Cardiac Myocytes Reveals Structural Remodeling of the Epigenome in Heart Failure

Editorial, see p 1626

BACKGROUND: Cardiovascular disease is associated with epigenomic changes in the heart; however, the endogenous structure of cardiac myocyte chromatin has never been determined.

METHODS: To investigate the mechanisms of epigenomic function in the heart, genome-wide chromatin conformation capture (Hi-C) and DNA sequencing were performed in adult cardiac myocytes following development of pressure overload-induced hypertrophy. Mice with cardiac-specific deletion of CTCF (a ubiquitous chromatin structural protein) were generated to explore the role of this protein in chromatin structure and cardiac phenotype. Transcriptome analyses by RNA-seq were conducted as a functional readout of the epigenomic structural changes.

RESULTS: Depletion of CTCF was sufficient to induce heart failure in mice, and human patients with heart failure receiving mechanical unloading via left ventricular assist devices show increased CTCF abundance. Chromatin structural analyses revealed interactions within the cardiac myocyte genome at 5-kb resolution, enabling examination of intra- and interchromosomal events, and providing a resource for future cardiac epigenomic investigations. Pressure overload or CTCF depletion selectively altered boundary strength between topologically associating domains and A/B compartmentalization, measurements of genome accessibility. Heart failure involved decreased stability of chromatin interactions around disease-causing genes. In addition, pressure overload or CTCF depletion remodeled long-range interactions of cardiac enhancers, resulting in a significant decrease in local chromatin interactions around these functional elements.

CONCLUSIONS: These findings provide a high-resolution chromatin architecture resource for cardiac epigenomic investigations and demonstrate that global structural remodeling of chromatin underpins heart failure. The newly identified principles of endogenous chromatin structure have key implications for epigenetic therapy.

Manuel Rosa-Garrido, PhD
Douglas J. Chapski, BS
Anthony D. Schmitt, PhD
Todd H. Kimball, BS
Elaheh Karbassi, PhD
Emma Monte, PhD
Enrique Balderas, PhD
Matteo Pellegrini, PhD
Tsai-Ting Shih, BS
Elizabeth Soehalim, BS
David Liem, MD, PhD
Peipei Ping, PhD
Niels J. Galjart, PhD
Shuxun Ren, MD
Yibin Wang, PhD
Bing Ren, PhD
Thomas M. Vondriska, PhD

Correspondence to: Thomas M. Vondriska, PhD, David Geffen School of Medicine, University of California, Los Angeles, BH 557 CHS, Department of Anesthesiology, 650 Charles Young Drive, Los Angeles, CA 90095. E-mail tvondriska@mednet.ucla.edu

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Clinical Perspective

What Is New?

- Chromatin capture and DNA sequencing were used to determine the endogenous structure of the cardiac myocyte epigenome.
- Physical interactions between regulatory elements and cardiac disease genes have been determined in basal and disease settings.
- The role of the chromatin structural protein CTCF was examined by using an *in vivo* loss-of-function model, revealing its role in chromatin organization and disease.

What Are the Clinical Implications?

- Epigenomic plasticity is identified as a common feature of cardiac pathophysiology induced by distinct stimuli.
- Knowledge of the dynamics of genomic interactions in disease may enable new strategies for therapeutic intervention.

Hear failure is a devastating condition that affects 6.5 million adults in the United States.¹ Although it is a multiorgan disease, heart failure is driven by changes in cardiac myocyte biology, including cell death, calcium handling, myofilament function, metabolism, and other factors. Underlying this complex cellular malfunction are gene expression changes, orchestrated by a network of transcription factors and chromatin-remodeling enzymes.^{2–5} Despite this knowledge, the basic folding principles of the cardiac myocyte epigenome have never been revealed, and the role of chromatin structural changes in cardiovascular disease is unknown.

Correct packaging of the genome within the nucleus determines appropriate gene expression and cellular function.⁶ Nucleosomes are differentially positioned along chromosomes, a process controlled by chromatin remodelers and histone-modifying enzymes.⁷ Tracks of nucleosomes adopt fiberlike structures that in turn compose the 3-dimensional architecture of endogenous chromatin, a process that is necessary for orderly storage and controlled accessibility of genetic information.⁸

Chromatin modifications are engaged in a developmentally tuned manner to enable both the unidirectional procession of differentiation and transcriptome stability. Cells use enhancers,⁹ distal regulatory regions that host histone posttranslational modifications and have an increasingly appreciated role in cardiovascular disease,^{10–12} and chromatin-binding proteins that, together with transcription factors, sculpt the transcriptome. Among chromatin structural proteins, CTCF

has been attributed a key role in modulating genome architecture¹³ and maintaining regions of genome accessibility across cell types.¹⁴ Once cellular lineages have been established, prevailing proteomic programs ensure that, on division, the correct epigenomic landscape is reestablished in daughter cells, maintaining lineage fidelity. An underexplored area of chromatin biology is how epigenomic stability is maintained *in vivo* when cells have exited the cell cycle. Adult mammalian cardiac myocytes do not readily divide, and the heart lacks robust regenerative capacity. Although epigenetic transitions in cardiovascular development have been investigated in cell culture,^{15,16} much less is known about the adult heart. Previous studies have demonstrated a role for histone-modifying enzymes³ and the readers of these modifications,¹⁷ but whether 3-dimensional configuration of the genome contributes to heart disease remains to be determined.

Recent investigations have demonstrated the existence of topologically associating domains (TADs) as regions of chromatin that exhibit a higher level of local interactions. These structural neighborhoods of the genome may facilitate coregulation of gene expression and are shared across many biological conditions, suggesting they are fundamental features of the genome.^{8,18} However, the specific nature of interactions within and between these TADs in cardiac cells, and the additional hierarchical levels of chromatin packaging (including local interactions, long-range interactions, enhancer-gene interactions, and chromatin looping) used in the cardiac myocyte nucleus in health and disease, are unknown.

The findings of the present study establish a high-resolution, genome-wide resource of endogenous chromatin interactions in cardiac myocytes, which can be used by future investigations to examine virtually any genomic locus. In addition, the results demonstrate specific areas of the epigenome that are structurally reorganized in pressure overload-induced heart disease, revealing how enhancers interact with disease-causing genes and the role of chromatin looping in cardiac gene expression. Using a physiologically relevant form of afterload-induced cardiac disease (plus a lineage targeted loss-of-function CTCF knockout [KO] mouse as a unique model for comparison), this investigation examines the role of global epigenome structure in heart failure.

METHODS

All animal studies were approved by the UCLA Animal Research Committee in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Human samples used in this study were procured in the Ronald Reagan Medical Center at UCLA following patient consent according to institutional review board-approved protocol. This study used DNA from adult mouse

cardiac myocytes isolated from control mice or 2 distinct disease models (pressure overload-induced hypertrophy and CTCF-KO) to perform chromosome conformation capture (Hi-C) analysis to determine endogenous chromatin structure (all sequencing-based studies were performed on $n \geq 3$ biological replicates per group; n values for all other end points are provided in figure legends). Transcriptome analyses were performed using RNA-seq to examine the functional read-outs of any structural changes in the epigenome. Chromatin structure was then examined at multiple scales to determine structural units, hierarchical organization, and 3-dimensional regulation of accessibility and transcription.

Statistical Analyses

All sequencing-based studies were performed on $n \geq 3$ biological replicates per group; n values for all other end points are provided in figure legends. The statistical tests performed for each end point are listed in the individual figure legends, described in the [Methods in the online-only Data Supplement](#) and are summarized here: Student's t test (see Figure 1A and 1E, [Figures IIC, IIE, and IV in the online-only Data Supplement](#)); Tukey test (see Figure 1C); Wilcoxon test (see Figure 2F); and Pearson correlation ([Figure IIIB in the online-only Data Supplement](#)). For RNA-seq data, Cuffdiff was used to determine differential gene expression and assign P values and q values for each gene. For interaction analyses, see Figure 4B through 4D and Figure 5, Fit-Hi-C was used to filter for *cis*-interactions with q value < 0.01 , which were then used to map interactions.

Detailed methodology for all experiments and analyses are available in the [online-only Data Supplement](#).

RESULTS

Chromosome Conformation Capture

Chromatin was purified from isolated cardiac myocytes and subjected to chromosome conformation capture (Hi-C). Mappability of reads was 63% to 65% across experimental groups ([Figure IA in the online-only Data Supplement](#)) at a depth of sequencing enabling examination of interactions with 5-kb resolution ([Figure IB in the online-only Data Supplement](#)). No significant differences were observed across experimental groups in terms of number of interactions analyzed, and the run-to-run variability was low ([Figure IC in the online-only Data Supplement](#)). Unless otherwise noted in the figures, all control groups were from myocytes isolated from untreated adult wild-type mice (all mice used in this study are C57BL/6 background). With this high-resolution resource, one can explore the physical environment of virtually any genomic locus in the cardiac myocyte at multiple scales including topologically associating domains, A/B compartmentalization, chromatin looping, enhancer interactions, and gene interactions. Sequencing data have been uploaded to the National Center for Biotechnology Information with the GEO accession number GSE96693 and are publically available.

Loss of CTCF Causes Cardiomyopathy

A mouse line with inducible, cardiac myocyte-specific ablation of CTCF was generated. Mice with a *loxP* flanked *Ctcf* allele (*Ctcf^{flox/flox}* mice) were crossed with mice expressing a transgenic tamoxifen-inducible Cre protein under the control of the α -myosin heavy chain promoter (MerCreMer mice). When administered tamoxifen in the diet for 5 weeks followed by 1 week on regular chow ([Figure IIA, right, in the online-only Data Supplement](#)), *Ctcf^{flox/flox}*-MerCreMer^{+/-} mice display selective excision of the targeted region of the *Ctcf* gene ([Figure IIB in the online-only Data Supplement](#)) and exhibit a gradual loss of the transcript in isolated cardiac myocytes ([Figure IIC in the online-only Data Supplement](#)). Further depletion in CTCF levels was associated with poor survival ([Figure IID in the online-only Data Supplement](#)); as a control, treatment of *Ctcf^{wt/wt}*-MerCreMer^{+/-} mice with tamoxifen had no sustained effect on cardiac function or morphology ([Figure IIG through III in the online-only Data Supplement](#)). The experimental mice, *Ctcf^{flox/flox}*-MerCreMer^{+/-} animals treated with tamoxifen to induce CTCF depletion, had $\approx 80\%$ reduction in CTCF protein levels ([Figure IIF in the online-only Data Supplement](#)) and are henceforth referred to as CTCF-KO mice. A separate cohort of wild-type mice were subjected to pressure overload stress (through surgical application of transverse aortic constriction [TAC]¹⁹) which induces heart failure through clinically relevant pathophysiological adaptation.

Phenotypic examination of the CTCF-KO mice revealed a striking cardiomyopathy ([Figure 1 and Figure IIG through III in the online-only Data Supplement](#)). Loss of CTCF leads to impaired ejection fraction, left ventricular chamber dilation, and muscle hypertrophy at the organ and cell level ([Figure 1A through 1C](#)). The TAC mice, by contrast, also exhibit hypertrophy accompanied by more modest changes in both chamber size and ejection fraction, demonstrating that these animals (CTCF-KO and TAC mice) represent distinct pathophysiological models. Examining CTCF expression in a genetically heterogeneous population of wild-type mice revealed consistent downregulation following pathological stimuli and an inverse correlation with pathological measurements of heart size ([Figure IIIA and IIIB in the online-only Data Supplement](#)); it is interesting to note that CTCF protein and mRNA levels were unchanged after TAC [data not shown], further suggesting that these models of disease, although they share some phenotypic characteristics, have important molecular and pathophysiological distinctions). The time course for chamber dilation, diminished ejection fraction, and CTCF depletion in CTCF-KO mice were similar ([Figure IIC and IIIC in the online-only Data Supplement](#)), and agreed with that for pathological gene activation ([Figure IV in the online-only Data Supplement](#)). CTCF-

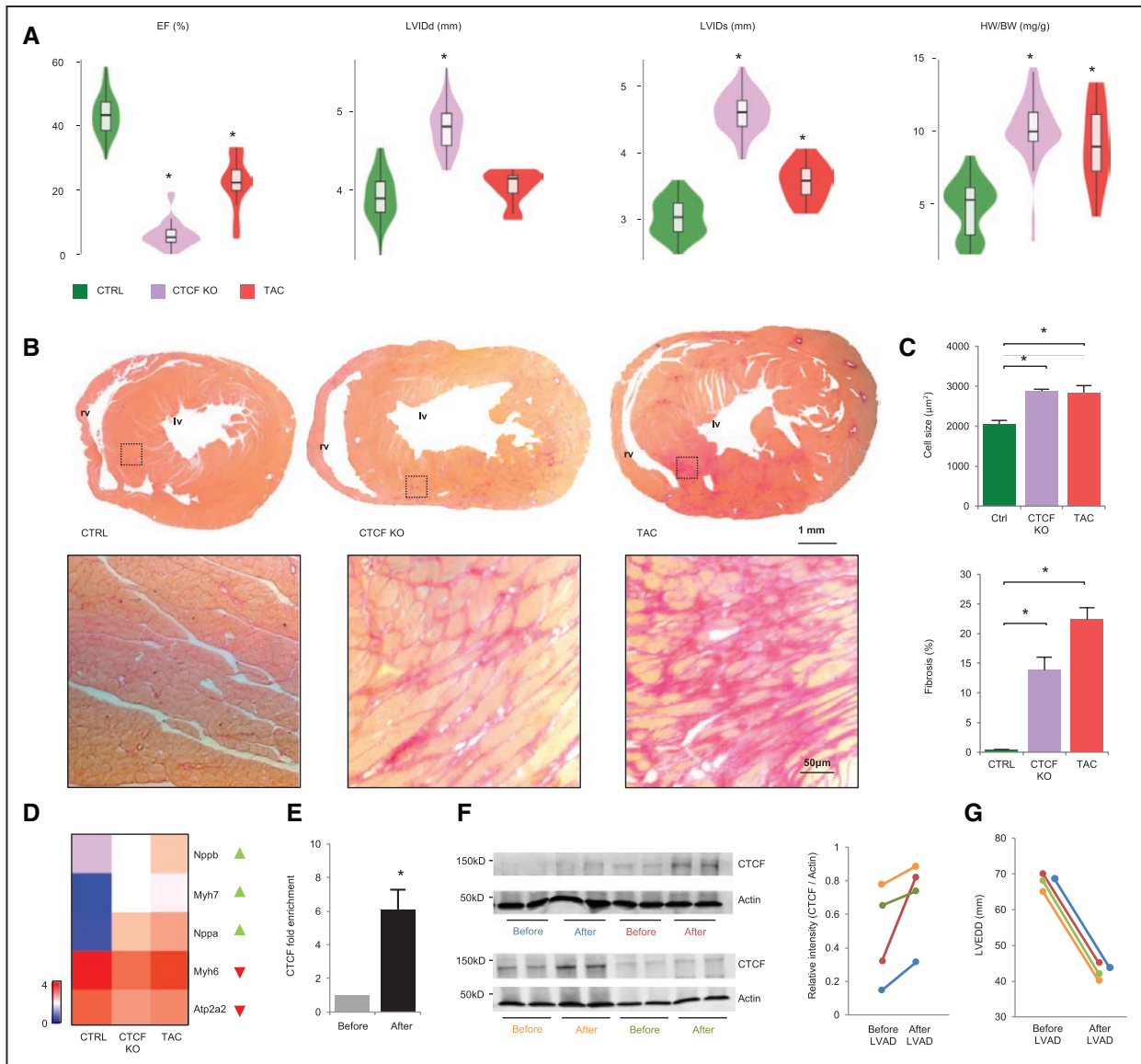


Figure 1. Loss of CTCF induces cardiac pathology.

A, Echocardiography measurements of ejection fraction (EF) and left ventricular internal diameter at diastole or systole (LVIDd or LVIDs) demonstrate impairment of EF and chamber dilation in CTCF-KO (purple) and TAC (red) in comparison with control (CTRL; green). Heart weight to body weight ratio (HW/BW) indicates cardiac hypertrophy in CTCF-KO and TAC mice ($n \geq 25$ /group; $*P < 0.01$, Student's *t* test). **B**, Picosirius red staining shows fibrosis after CTCF depletion or TAC ($n = 3$ /group). **C**, **Top**, mean cardiomyocyte area ($n = 20$ visual fields of wheat germ agglutinin–stained sections across 3 mice per condition); **Bottom**, quantitation of fibrosis from Picosirius red sections ($n = 3$ /group; $*P < 0.01$, Tukey HSD test). **D**, Stress response gene expression ($\log_{10}(\text{FPKM} + 1)$). **E**, Real-time qPCR measurements of CTCF levels in human myocardium before and after LVAD (before values normalized to 1 on a per-patient basis; $n = 4$, $*P < 0.01$ Student's *t* test, bars SD). **F**, Western blots of CTCF levels in individual patients before and after LVAD (**Left**); quantitation of Western blots normalized to actin on a per-patient, per-sample basis (**Right**). **G**, Left ventricular end-diastolic dimension measurements before and after LVAD. Color-coding in **E** through **G** indicates separate patients. Lines shifted horizontally in **G** for ease of viewing. FPKM indicates fragments per kb of exon per million mapped reads; HSD, honest significant difference; KO, knockout; LVAD, left ventricular assist devices; qPCR, quantitative polymerase chain reaction; and TAC, transverse aortic constriction.

KO and TAC led to fibrotic deposition (Figure 1B, bottom) and activation of known heart failure genes² (Figure 1D; Figure ID in the online-only Data Supplement shows RNA-seq data quality statistics). Caspase-3 cleavage assays to measure apoptosis showed no dif-

ference between CTCF-KO and control animals (Figure V in the online-only Data Supplement, suggesting, but not proving, that impairment of cardiac function is secondary to necrosis). In human heart tissues harvested before and after implantation of left ventricular assist

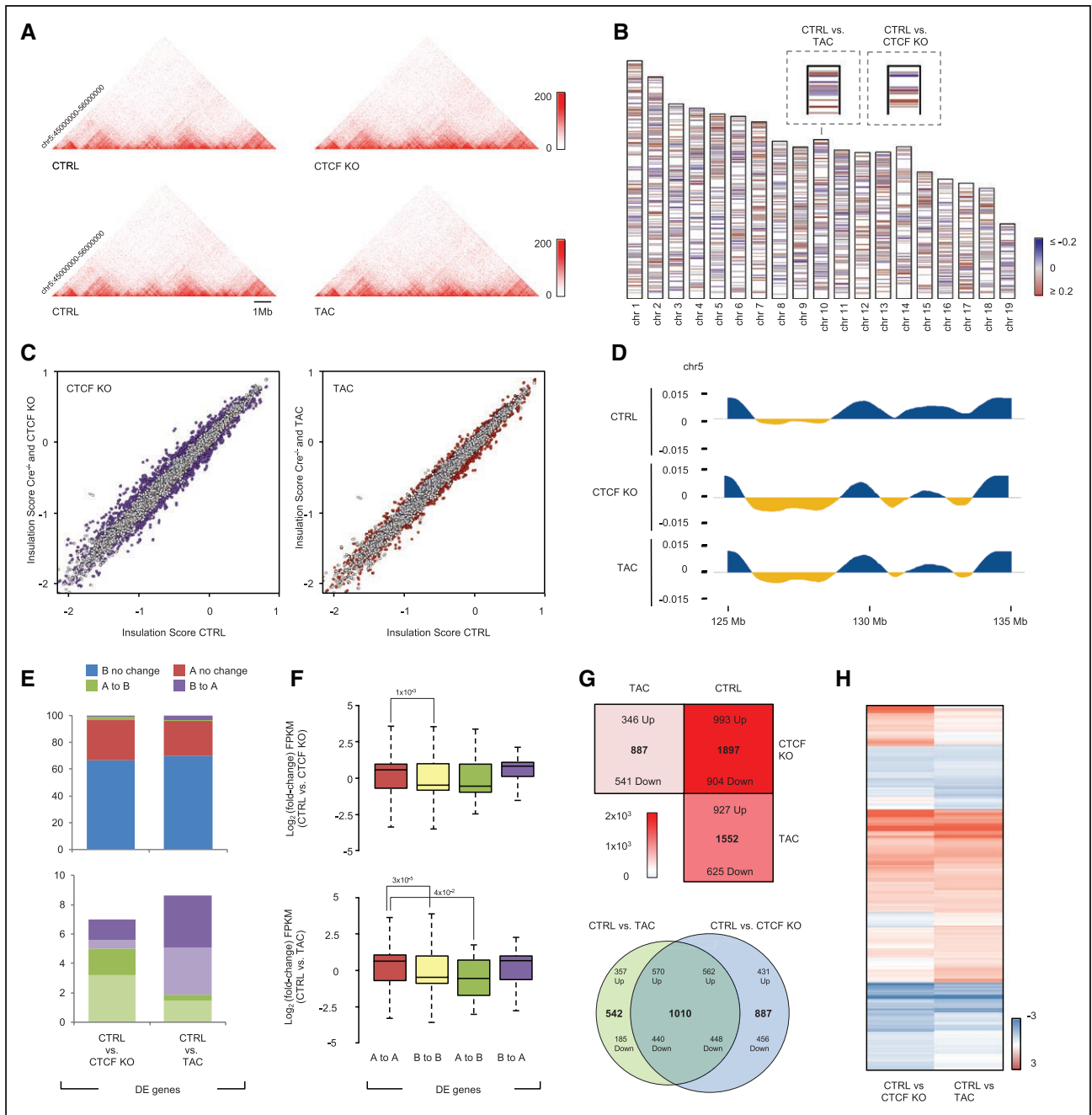


Figure 2. High-resolution cardiac chromatin conformation analyses reveal changes in chromatin compartmentalization and gene expression in pressure overload and CTCF-KO mice.

A, Structure of topological associating domains (TADs) is revealed from contact frequency heatmaps showing *cis*-interaction profile on example chromosome 5 for control, TAC, and CTCF-KO chromatin. **B**, Strength of boundaries between TADs are displayed for all chromosomes comparing control and TAC (red, higher; blue, lower; **Insets**, example region on chromosome 10 in control versus TAC and control versus CTCF-KO; **Figure VIIA in the online-only Data Supplement** shows control versus CTCF-KO for all chromosomes). **C**, Quantitation of insulation score differences in control versus KO (**Left**) or TAC (**Right**). Colored dots indicate significant changes (gray dots show range of variation between 2 control conditions: untreated wild-type mouse and untreated $Cre^{+/+}$ mouse). **D**, A/B compartmentalization, an indicator of genome accessibility at individual loci, for an example region on chromosome 5 is plotted in blue (open, A) and yellow (closed, B): CTRL A/B status on **Top**, CTCF-KO in **Middle**, and TAC on **Bottom**. **E**, Quantification of the genome-wide changes in A/B compartment change with CTCF depletion (**Left**) or TAC (**Right**). **Bottom** highlight only bins that change compartment; dark and light colors represent up- or down-regulated genes, respectively. **F**, Relationship of compartmentalization to gene expression is measured in TAC or CTCF-KO hearts. $\text{Log}_2(\text{fold-change})$ of FPKM for the differentially expressed genes that either remain in the same compartment or that change compartments with CTCF depletion (**Top**) and TAC (**Bottom**). *P* values via Wilcoxon test; whiskers indicate (*Continued*)

devices, which mechanically unload the heart, allowing reverse remodeling of diseased tissue, CTCF levels were increased after device implantation (Figure 1E through 1G; see Figure VI in the online-only Data Supplement for patient clinical data; unavailability of healthy human hearts precluded the measurement of CTCF expression in these tissues).

Endogenous Chromatin Architecture in Healthy and Diseased Myocytes

We next aimed to examine the large-scale alterations in chromatin structure underlying heart disease in the mice subjected to TAC. There are several important differences in the pathophysiology between the CTCF-KO and TAC mice, and thus the former is used herein as a type of alternate disease model to investigate similarities and differences in global chromatin changes in different forms of heart failure. Analyses of TAD architecture in wild-type, CTCF-KO mice, or TAC mice demonstrate TADs to be stable features of chromatin structure (Figure 2A; average TAD size=445 kb). The number of TAD boundaries across the entire genome varied by <2%, with 3686, 3746, and 3705 boundaries called in control, CTCF-KO, and pressure overload, respectively. Boundary strength, however, was differentially altered across the genome (Figure 2B shows the distribution across chromosomes for control versus TAC; Figure 2C shows quantitation; Figure VIIA in the online-only Data Supplement shows all chromosomes in all conditions; Figure VIIIA in the online-only Data Supplement shows principal component analysis for boundary strength). CTCF-KO or TAC chromatin each hosted new boundaries (Figure VIIIB in the online-only Data Supplement; new includes shifted boundaries and de novo formation; note that the local rate of change in insulation score determines boundary strength²⁰).

Chromatin can be divided into active and inactive regions,²¹ called A and B compartments, respectively, which were affected to only a minor degree by loss of CTCF or TAC. Compartmentalization changes were sparse (Figure 2D and 2E) and ranged from <2 to ≈8% of interaction bins genome wide. The scale of the total genome changing compartmentalization was almost identical (≈4%) after CTCF-KO or TAC (Figure 2E). Changes in compartmentalization correlated positively with gene expression; that is, genes moving from A to B were downregulated and moving from B to A were upregulated (Figure 2F). Loss of CTCF elicited a transcriptional profile that shared some (41% of genes)

features with TAC (Figures 2G and 2H; Figure VIIC in the online-only Data Supplement shows principal component analysis of transcriptome data); both also exhibited known marker gene activation (Figure 1D). Genes determined by ChIP-seq to harbor CTCF in their transcription start sites were enriched in pathways associated with cardiac disease (Figure VIID in the online-only Data Supplement).

Chromatin Looping Is Altered With CTCF-KO or TAC

The hierarchical nature of chromatin results in a preponderance of short-range interactions, while simultaneously necessitating long-range looping (Figure 3A). One such long-range interaction affected by CTCF depletion and TAC is shown in Figure 3B. Quantitative analysis shows that loss of CTCF or TAC was associated with a decrease in the total number of long-range loops, without a gross change in loop size (Figure 3C). Of dynamic loops in disease, 51% of those disappearing were shared between CTCF-KO and TAC, whereas only 15% of those appearing were shared; the raw number of loops lost in disease was also greater than the number formed (Figure 3D). In control chromatin, 37% (3056 of 8240; Figure 3E) of loops were flanked by 2 CTCF peaks (78% ≥1 peak) and loss of 1 CTCF peak was sufficient to destroy loops in 37% of the cases (326 of 879; Figure 3F). Within reorganized loops, enrichment for genes in pathways associated with cardiovascular function was observed (Figure VIIIE in the online-only Data Supplement; a caveat here is that analysis of cardiac myocytes may increase the chances that enriched ontology terms are of cardiovascular relevance).

Among total loops, a subset is responsible for bringing together enhancers and promoters (Figure 3G and 3H). In agreement with aberrant gene expression in both conditions (Figure 2F through 2H), lost loops in CTCF-KO and TAC chromatin were enriched in genes associated with cardiac pathology (Figure VIIIF in the online-only Data Supplement).

Heart Failure Involves Altered Enhancer-Gene Interactions

Focusing more deeply on significant interactions that contribute to gene expression can reveal the physiological implications of genomic structure. For example, *Ppp3ca*, a gene implicated in calcium-dependent signaling and cardiac hypertrophy,⁴ exhibited a consistent

Figure 2 Continued. interquartile range. **G**, Heat matrix (**Top**) showing number of differentially expressed genes between CTRL, CTCF KO, and TAC (intensity indicates number of genes). Venn diagram (**Bottom**) showing overlap of differentially expressed genes between CTRL versus CTCF KO and CTRL versus TAC. **H**, Heatmap depicting $\log_2(\text{KO}/\text{CTRL})$ and $\log_2(\text{TAC}/\text{CTRL})$ FPKM for the differentially expressed genes with $q < 0.01$ (**Left**). CTRL indicates control; FPKM, fragments per kb of exon per million mapped reads; KO, knockout; and TAC, transverse aortic constriction.

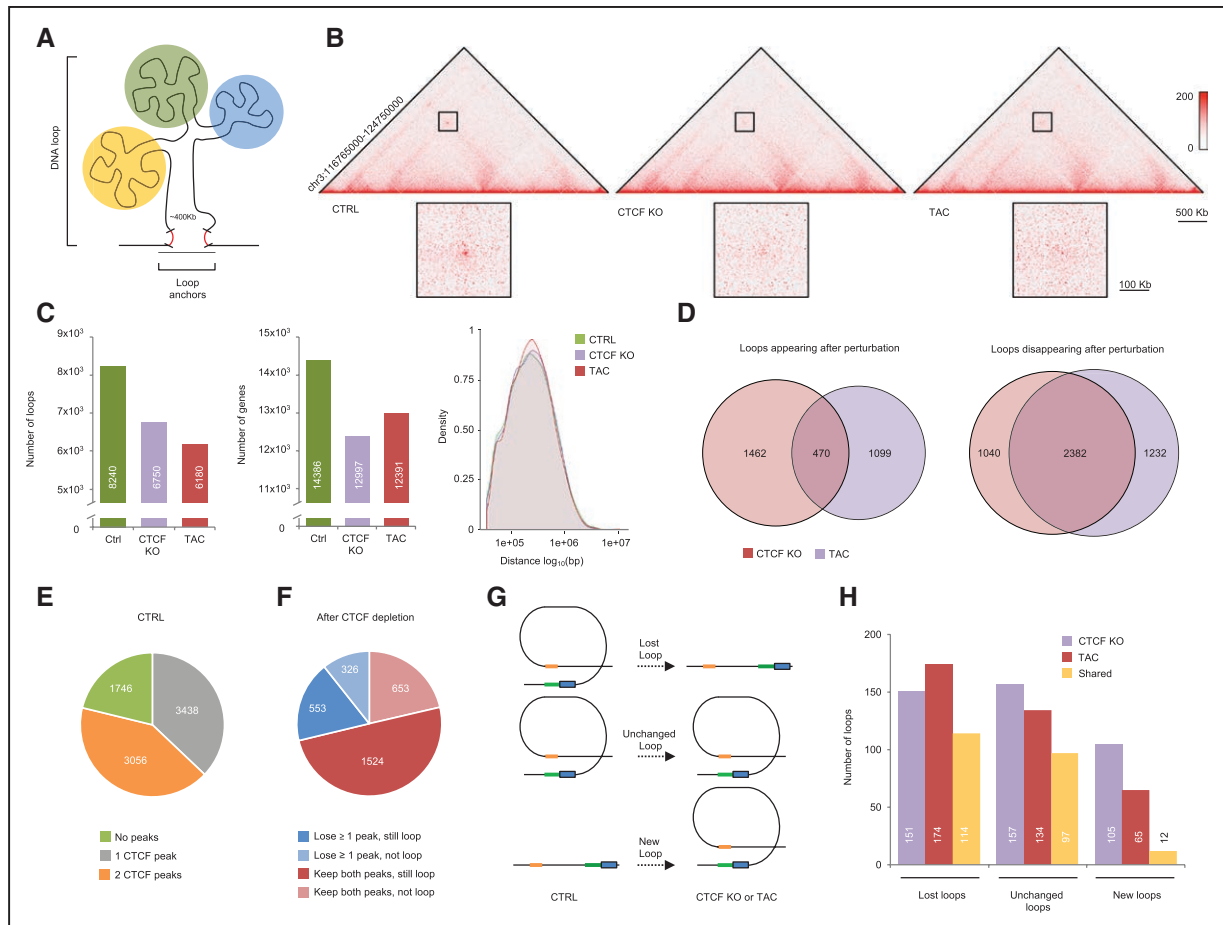


Figure 3. Short- and long-range chromatin interactions, and stable chromatin looping, are altered after pressure overload or CTCF-KO.

A, Schematic displaying that loops are demarcated by 2 anchors and contain regions of high-frequency interactions, indicated by the colored circles. **B**, The bioinformatic tool Juicebox is used to display an example loop that is lost with CTCF depletion (**Middle**) and TAC (**Right**). **C**, Quantitation of the phenomenon in **B** across genome, showing number of chromatin loops (**Left**), number of genes within loops (**Middle**), and loop sizes (**Right**; CTRL, green; CTCF-KO, purple; TAC, red). **D**, Overlap of loops only appearing in CTCF-KO or TAC in comparison with CTRL (**Left**); overlap of loops that disappear in CTCF-KO and TAC (**Right**). **E**, CTRL loops in which zero (green), one (gray), or both (yellow) anchors overlap with a CTCF peak. **F**, CTRL loops that lose ≥ 1 CTCF peak during CTCF depletion (blue) and CTRL loops that keep both CTCF peaks during CTCF depletion (red). Darker shade indicates loops that were preserved with CTCF depletion, whereas lighter color indicates loops that were lost. **G**, Schematic demonstrating types of alterations in looping architecture that can occur, including loss of loops mediating enhancer-promoter interactions (**Top**), no change (**Middle**), or formation (**Bottom**). Enhancers are orange, promoters green, and genes blue. **H**, Quantification of changes in enhancer-promoter loops. CTRL indicates control; KO, knockout; and TAC, transverse aortic constriction.

decrease in total interactions in CTCF-KO or TAC conditions (Figure 4A).

It is noteworthy that, of 9194 expressed genes for which significant ($q < 0.01$) Fit-Hi-C data were available, 60% had the same direction of change of interactions in CTCF-KO and TAC conditions. Of the 40% of genes with unique behavior in the diseased condition, the majority (75%) were different because 1 of the 2 (CTCF-KO or TAC) exhibited no change in interaction; 25% were situations in which the change in interaction was opposite between CTCF-KO and TAC. Of the genes with shared changes in interactions in CTCF-KO and TAC that also underwent differential expression

(3651 of 5443), the vast majority (86%) experienced decreased local interactions (Figure 4B). These findings support a trend in which more fluid chromatin interactions are associated with gene expression and where alterations in expression (when they occur) after perturbation are more likely to be in the same direction in CTCF-KO and TAC in contrast with control. Figure 4C shows the top 40 differentially expressed genes selected for the number of interactions in the control condition, demonstrating a consistent decrease in interactions in CTCF-KO and TAC.

Distinct mechanisms of gene regulation were associated with distinct changes in chromatin microenviron-

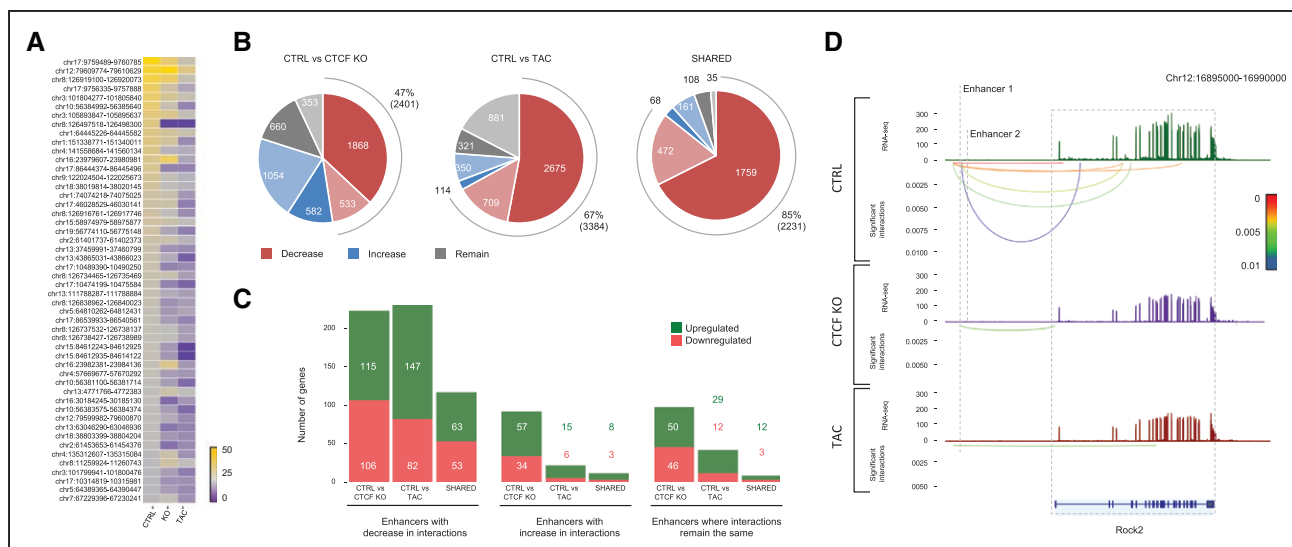


Figure 5. Three-dimensional interactions between enhancers and genes are restructured after pressure overload or CTCF-KO.

A, Number of contact sites per cardiac enhancer are quantified ($q < 0.01$; rows are top 50 enhancers sorted by number of interactions). **B, Top**, enhancers in which the number of significant ($q < 0.01$) interactions (determined by the Fit-Hi-C tool) increases (blue), decreases (red), or remains the same (gray) with CTCF-KO (**Left**), TAC (**Center**), and with consistent changes with CTCF-KO or TAC (**Right**). Darker shading indicates enhancers that interact with genes; lighter colors are regions not annotated as coding. **C**, Number of genes that interact with enhancers from **B**, stratified by whether their expression is upregulated (green) or downregulated (red). Groupings are separated into enhancers whose number of overlapping interactions decreases (**Left**), increases (**Middle**), or remains the same (**Right**) after perturbation. **D**, Contact site mapping of interactions is shown for the example gene *Rock2*, which exhibits decreased interaction with enhancers in CTCF-KO and TAC, concomitant with decreased expression. CTRL indicates control; KO, knockout; and TAC, transverse aortic constriction.

CTCF-KO and TAC (Figure 4D, center). Last, a third class of regulatory scheme is represented by *Mef2c*, whose expression changes with neither CTCF-KO nor TAC, but which undergoes a high degree of splicing regulation²³ and is a central driver of cardiac gene expression²⁴ (Figure 4D, right). This locus underwent a consistent decrease in chromatin interactions up- and downstream of the gene in CTCF-KO and TAC, suggesting a loosening of the local chromatin environment.

Enhancers are regions of the genome that positively influence gene expression and are identified on the basis of diagnostic histone posttranslational modifications. How enhancers are regulated in the 3-dimensional context of the cardiac nucleus is unknown. Of previously identified enhancers from adult cardiac myocytes,¹¹ 5050 contained significant Fit-Hi-C interactions. These enhancers consistently exhibited decreased interactions after perturbation (Figure 5A). Indeed, 47% of enhancers exhibited decreased interactions in CTCF-KO and 67% of enhancers exhibited decreased interactions in TAC (Figure 5B, left and center). It is remarkable that, of the enhancers that were shared between the 2 conditions, 85% showed a decrease of interactions in both CTCF-KO and TAC (Figure 5B, right). Figure 5C further illustrates that CTCF-KO and TAC were both associated with decreased enhancer-gene interactions (the directionality of gene expression change is reported in the

different cohorts of enhancers from Figure 5B), and the affected genes were enriched in cardiac pathology pathways (Figure VIII G in the online-only Data Supplement). *Rock2*, previously implicated in cardiac disease,²⁵ is an example gene whose interactions with multiple enhancers are depleted in the setting of CTCF-KO or TAC (Figure 5D; Tables I through III in the online-only Data Supplement). These findings indicate that a global increase in chromatin fluidity around enhancers is a shared feature of cardiac pathology.

DISCUSSION

This study provides a resource of endogenous chromatin architecture in cardiac myocytes and describes the global changes in chromatin interactions during heart failure. With these data, the basal microenvironment of any genomic locus can be explored in the future, and changes in this microenvironment can be examined in the setting of pressure overload-induced heart failure. As a model of heart disease caused by increased afterload (including hypertension and aortic stenosis), TAC was used in this study to investigate how global changes in the epigenome participate in disease pathogenesis; the CTCF-KO mouse was used as a complementary tool to investigate the role of a critical chromatin structural protein. It is interesting to

note that loss of CTCF or pressure overload–induced heart failure caused greater dynamics of endogenous chromatin structure and specific reorganization of enhancer-gene interactions. Depletion of CTCF resulted in a phenotype that shared some features of that induced by pressure overload (including fibrosis, hypertrophy, changes in ejection fraction, and changes in chamber dimensions), although there were notable differences between these phenotypes (particularly in the extent of left ventricular dilation), meaning that CTCF-KO does not fully recapitulate and is thus not a model of pressure overload hypertrophy, but rather, is a form of dilated cardiomyopathy with hypertrophy and fibrosis. Human patients with heart failure treated with left ventricular assist devices to unload the heart demonstrated increased CTCF protein and mRNA levels. Although this observation is interesting, caution is warranted in the extrapolation of these findings to the clinical arena: future cohort studies in cardiomyopathies of distinct etiology will be required to fully understand the role of CTCF in human heart failure.

Although the pattern of chromosome organization during mitosis is well known, how the genome is structured during interphase or in cells that have exited the cell cycle has remained a mystery until very recently. Interphase nuclei form chromosomal territories (features mainly characterized by microscopy),²⁶ which serve to segregate entire chromosomes apart from each other. The development of genome-wide DNA sequencing–based approaches in the past few years has enabled the precise determination of subchromosomal genome architecture.^{8,18,27} This information, that is, how the genome is packaged in different cells, is important for basic science reasons (eg, to answer fundamental questions about the structure-function relationship between an invariant genome and a highly variable transcriptome [among different cells in a multicellular organism]) and for translational implications, as well (eg, many diseases involve changes in epigenetic machinery, but understanding how these chromatin modifiers exert their control of disease phenotype requires determination of the substrate on which they operate, ie, the structural conformation of the epigenome). With these considerations in mind, the present study investigates each of the various levels of hierarchical organization of cardiac chromatin including the formation of TADs and the compartmentalization of large swaths of the genome (Figure 2 and [Figure VII in the online-only Data Supplement](#)), intermediate structural features, including chromatin loops of various lengths (Figure 3), precise remodeling of interactions around genes involved in disease pathogenesis (Figure 4), and the structural organization of noncoding functional units (ie, enhancers, which incorporates existing knowledge of posttranslational modifications

decorating histones at specific genomic loci) near the genes they modify (Figure 5).

The present study and previous work in the field suggest that heart failure is associated with a plastic epigenome (Figure 6): widespread changes in boundary strength (Figure 2C); decreased formation of chromatin loops (Figure 3C and 3D); decreased interactions in local chromatin environment of differentially expressed genes (Figure 4); and more fluid interactions between enhancers and genes (Figure 5). These findings support previous studies of heart failure showing global DNA demethylation,²⁸ altered histone stoichiometry and a decrease in heterochromatic posttranslational modifications,^{19,29,30} activation of pathogenic noncoding RNAs,^{31,32} and aberrant transcriptional activation.^{3,4,33} Heart disease has been speculated to be the result of a reversion to more primitive gene expression programs²; the present findings extend this hypothesis, identifying shared epigenomic features in response to cellular stress.

Although CTCF has been associated with TAD boundaries,^{34–36} its structural role has been unclear in postmitotic cells in vivo. The present study explores the role of CTCF in cardiac epigenome stability, but cannot unequivocally conclude whether CTCF is dispensable for TAD maintenance, insofar as residual CTCF was present. A recent investigation, using an auxin-inducible degron approach to deplete CTCF protein in mouse embryonic stem cells, demonstrated that near-

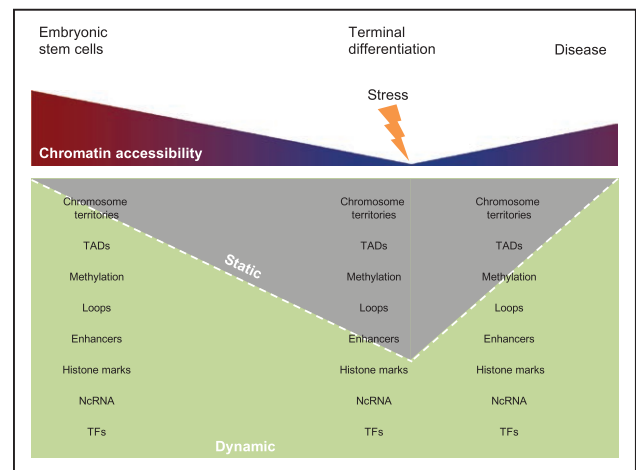


Figure 6. Model for epigenomic changes in development and disease.

Development is accompanied by changes in chromatin to endow terminally differentiated cells with stable transcriptomes. Disease upsets this balance, transitioning select regions of the genome into more dynamic conformations through effects on chromatin structure, enhancer-gene looping, histone modifications, DNA methylation, and other factors. This model is based on findings from this article and previous publications^{2–5,10,11,15–17,29–33} as described in the text. NcRNA indicates noncoding RNA; TAD, topological associating domain; and TF, transcription factor.

complete depletion of CTCF was required to influence gross chromatin organization, with TAD structure still observed with as low as 4% of normal CTCF protein levels.³⁷ In the present article, because many loops did not harbor CTCF, and a large number of loops with unchanged CTCF binding were altered in their conformation, these findings support a model in which other factors stabilize chromatin looping and CTCF participates in gene regulation through mechanisms in addition to anchoring. Other insulator proteins (eg, cohesins, which behave similarly to CTCF across a population of mice and are modestly downregulated in CTCF-KO mice [Figure IIIA in the online-only Data Supplement], suggesting that cohesins and CTCF are coregulated in circumstances of global chromatin remodeling) may stabilize TADs, in addition to histone H3 lysine 27 trimethylation (globally unchanged in CTCF-KO, Figure IIF in the online-only Data Supplement) and perhaps DNA methylation. CTCF is essential for development beyond embryo implantation,³⁸ and longer-term depletion in the heart was lethal (Figure IID in the online-only Data Supplement). This investigation demonstrates that subtle perturbations in the structure of gene expression domains can result in catastrophic malfunction of the myocyte and heart, in agreement with previous findings implicating CTCF in disease-related transcription in other organs.³⁹ Cell death in the heart is an active area of investigation,⁴⁰ and, although our studies found no evidence for apoptotic cell death, it is possible, given the progressive nature of this phenotype, that the CTCF-KO cardiomyopathy is driven by more than one form of cell death to be determined in future studies. Our previous investigations⁴¹ showed that CTCF knock-down was sufficient to induce cell death in HeLa cells but not in neonatal rat ventricular myocytes, and other investigations in noncardiac cells have suggested that aberrant CTCF function leads to abnormal cell death decisions, such as in cancer.⁴²

Recent studies have identified a role for loops in the formation of chromatin neighborhoods to insulate against aberrant, and to coordinate synchronized, expression of genes.⁴³ In addition, ChIP-seq investigations of histone marks have provided important insights into how these features correlate with gene expression.¹¹ With high-resolution chromatin conformation data, the more biologically relevant approach can be taken to examine enhancers in the circumambient context in which they operate.⁴⁴ The present findings are a resource for examining gene regulation by virtually any known, or to-be-discovered, cardiac enhancer, and to explore newly identified 3-dimensional features of the cardiac nucleus.

In summary, these studies point to a common entropic destination for pathologically disturbed chromatin, supporting a model wherein chromatin structural abnormalities underpin the complex cellular networks

that go awry in disease and providing a new conceptual framework to engineer therapies.

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None.

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FOOTNOTES

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