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

Running Title: Rosa-Garrido et al.; Remodeling Chromatin Structure in Heart Failure

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Abstract

**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 heart failure patients receiving mechanical unloading via left ventricular assist devices show increased CTCF abundance. Chromatin structural analyses revealed interactions within the cardiac myocyte genome at 5kb resolution, enabling examination of intra- and inter-chromosomal 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.

**Key Words:** epigenetics; genomics; heart failure; gene regulation; chromatin structure, CTCF
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 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.
Heart failure is a devastating condition that affects 6.5 million adults in the USA.\textsuperscript{1} Although it is a multi-organ 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.\textsuperscript{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.\textsuperscript{6} Nucleosomes are differentially positioned along chromosomes, a process controlled by chromatin remodelers and histone modifying enzymes.\textsuperscript{7} Tracks of nucleosomes adopt fiber-like structures that in turn compose the three-dimensional architecture of endogenous chromatin, a process that is necessary for orderly storage and controlled accessibility of genetic information.\textsuperscript{8}

Chromatin modifications are engaged in a developmentally tuned manner to enable both the unidirectional procession of differentiation and transcriptome stability. Cells employ enhancers\textsuperscript{9}—distal regulatory regions that host histone post-translational modifications and have an increasingly appreciated role in cardiovascular disease\textsuperscript{10-12}—and chromatin binding proteins which, together with transcription factors, sculpt the transcriptome. Among chromatin structural proteins, CTCF has been attributed a key role in modulating genome architecture\textsuperscript{13} and maintaining regions of genome accessibility across cell types.\textsuperscript{14} Once cellular lineages have been established, prevailing proteomic programs ensure that upon 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 \textit{in vivo} when cells have exited
the cell cycle. Adult mammalian cardiac myocytes do not readily divide and the heart lacks robust regenerative capacity. While epigenetic transitions in cardiovascular development have been investigated in cell culture, much less is known about the adult heart. Previous studies have demonstrated a role for histone modifying enzymes and the readers of these modifications, but whether three-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 co-regulation of gene expression and are shared across many biological conditions, suggesting they are fundamental features of the genome. 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) utilized 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 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 NIH 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 two 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 endpoints are provided in figure legends). Transcriptome analyses were carried out using RNA-seq to examine the functional readouts of any structural changes in the epigenome. Chromatin structure was then examined at multiple scales to determine structural units, hierarchical organization and three-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 endpoints are provided in figure legends. The statistical tests performed for each endpoint are listed in the individual figure legends, described in the Online Supplemental Methods and are summarized here: Student’s t-test: Figure 1a, 1e, Supplemental 2c, 2e and 4; Tukey test: Figure 1c; Wilcoxon test: Figure 2f; Pearson correlation: Supplemental Figure 3b; RNA-seq data: Cuffdiff was used to determine differential gene expression and assign \( p \)-values and \( q \)-values for each gene; for interaction analyses in Figures 4b-d 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 Supplement.
Results

Chromosome conformation capture
Chromatin was purified from isolated cardiac myocytes and subjected to chromosome conformation capture (Hi-C). Mappability of reads was 63-65% across experimental groups (Supplemental Figure 1A) at a depth of sequencing enabling examination of interactions with 5kb resolution (Supplemental Figure 1B). No significant differences were observed across experimental groups in terms of number of interactions analyzed and run-to-run variability was low (Supplemental Figure 1C). 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 NCBI 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 (Ctcflox/flox mice) were crossed with mice expressing a transgenic tamoxifen-inducible Cre protein under the control of the α-MHC promoter (MerCreMer mice). When administered tamoxifen in the diet for 5 weeks followed by 1 week on regular chow (Supplemental Figure 2A, right panel), Ctcflox/flox-MerCreMer+/− mice display selective excision of the targeted region of the Ctcf gene (Supplemental Figure 2B) and exhibit a gradual loss of the transcript in isolated cardiac myocytes (Supplemental Figure 2C). Further depletion in CTCF levels was associated with poor survival (Supplemental Figure 2D); as a control, treatment of
Ctcf\textsuperscript{wt/wt}-MerCreMer\textsuperscript{+/-} mice with tamoxifen had no sustained effect on cardiac function or morphology (Supplemental Figure 2G-I). The experimental mice—Ctcf\textsuperscript{floxfloxfloxfloxfloxflox/MerCreMer\textsuperscript{+/-}} animals treated with tamoxifen to induce CTCF depletion—had ~80\% reduction in CTCF protein levels (Supplemental Figure 2F) 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, abbreviated TAC) which induces heart failure through clinically-relevant pathophysiological adaptation.

Phenotypic examination of the CTCF-KO mice revealed a striking cardiomyopathy (Figure 1 and Supplemental Figure 2G-I). Loss of CTCF leads to impaired ejection fraction, left ventricular chamber dilation and muscle hypertrophy at the organ and cell level (Figure 1A-C). 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 down-regulation following pathologic stimuli and an inverse correlation with pathologic measurements of heart size (Supplemental Figure 3A and B; interestingly, 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 EF and CTCF depletion in CTCF-KO mice were similar (Supplemental Figure 2C and 2H), and agreed with that for pathologic gene activation (Supplemental Figure 4). CTCF-KO and TAC led to fibrotic deposition (Figure 1B, bottom panel) and activation of known heart failure genes\textsuperscript{2} (Figure 1D; Supplemental Figure 1D shows RNA-seq data quality statistics). Caspase-3 cleavage assays to measure apoptosis showed
no difference between CTCF-KO and control animals (Supplemental Figure 5, 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 devices (LVADs)—which mechanically unload the heart, allowing reverse remodeling of diseased tissue—CTCF levels were increased after device implantation (Figure 1E-G; see Supplemental Figure 6 for patient clinical data; unavailability of healthy human hearts precluded measurement of CTCF expression in these tissues).

**Endogenous chromatin architecture in healthy and diseased myocytes**

We next sought 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 topologically associating domain (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 = 445kb). 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; Supplemental Figure 7A shows all chromosomes in all conditions; Supplemental Figure 8A shows PCA for boundary strength). CTCF-KO or TAC chromatin each hosted new boundaries (Supplemental Figure 8B; “new” include shifted boundaries and de novo formation; note: local rate of change in insulation score determines boundary strength\(^2\)).
Chromatin can be divided into active and inactive regions, 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-E) 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 down-regulated and B to A up-regulated (Figure 2F). Loss of CTCF elicited a transcriptional profile that shared some (41% of genes) features with TAC (Figures 2G-H; Supplemental Figure 8C shows PCA of transcriptome data) as well as known marker gene activation (Figure 1D). Genes determined by ChIP-seq to harbor CTCF in their transcription start sites (TSSs) were enriched in pathways associated with cardiac disease (Supplemental Figure 8D).

**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 were 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 out of 8240; Figure 3E) of loops were flanked by two CTCF peaks (78% ≥1 peak) and loss of one CTCF peak was sufficient to destroy loops in 37% of the cases (326 out of 879; Figure 3F). Within reorganized loops, enrichment for genes in pathways associated with cardiovascular function was observed (Supplemental Figure 8E; 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-H). In agreement with aberrant gene expression in both conditions (Figure 2F-H), lost loops in CTCF-KO and TAC chromatin were enriched in genes associated with cardiac pathology (Supplemental Figure 8F).

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

Notably, 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 one of the two (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 out 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 compared to 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 microenvironment, represented in three example genes (Figure 4D). Expression of
Nppa (Figure 4D, left; top is control interaction matrix, middle has specific interactions colored
by significance and bottom has RNA-seq expression tracks) a known marker for heart failure in
mice and humans,2 was increased in CTCF-KO and TAC. Nearby local interactions are dense but
relatively unchanging, whereas those impinging on the Nppa gene (demarcated by vertical lines)
are decreased. Expression of Kcnd2, a potassium channel implicated in sudden death,22 was
down-regulated and the locus exhibited a notable decrease in long-range interactions in CTCF-
KO and TAC (Figure 4D, center). Lastly, 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 down-stream
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 post-translational modifications. How enhancers are
regulated in the three-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). Remarkably, of
the enhancers that were shared between the two 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 (Supplemental Figure 8G). Rock2, previously implicated in cardiac disease, is an example gene whose interactions with multiple enhancers are depleted in the setting of CTCF-KO or TAC (Figure 5D; Supplemental Tables 1-3). 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 this 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. Interestingly, 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 EF and changes in chamber dimensions), although there were notable differences between these phenotypes (particularly in the extent of LV 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 heart failure patients treated with left ventricular assist devices to unload the heart demonstrated increased CTCF protein and mRNA levels. While 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),\(^2^6\) 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 precise determination of sub-chromosomal genome architecture.\(^8,^{18},^{2^7}\) This information—that is, how the genome is packaged in different cells—is important for basic science reasons (e.g. to answer fundamental questions about the structure-function relationship between an invariant genome and a highly variable transcriptome [amongst different cells in a multi-cellular organism]) as well as for translational implications (e.g. many different 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, i.e. 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 Supplemental Figure 7), 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 non-coding functional units
(i.e. enhancers, which incorporates existing knowledge of post-translational 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-D); 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
post-translational 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.

While CTCF has been associated with TAD boundaries,34–36 its structural role has been
unclear in post-mitotic 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 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 paper, 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 (e.g. cohesins, which behave similarly to CTCF across a population of mice and are modestly down-regulated in CTCF-KO mice [Supplemental Figure 3A], suggesting that cohesins and CTCF are co-regulated in circumstances of global chromatin remodeling) may stabilize TADs, in addition to histone H3 lysine 27 trimethylation (globally unchanged in CTCF-KO, Supplemental Figure 2F) and perhaps DNA methylation. CTCF is essential for development beyond embryo implantation and longer-term depletion in the heart was lethal (Supplemental Figure 2D). 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. Cell death in the heart is an active area of investigation and while 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 one or more forms of cell death to be determined in future studies. Our previous investigations showed that CTCF knockdown was sufficient to induce cell death in HeLa cells but not in neonatal rat ventricular myocytes and other investigations in non-cardiac cells have suggested that aberrant CTCF function leads to abnormal cell death decisions, such as in cancer.

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. In addition, ChIP-seq investigations of histone marks have provided important insights into how these features correlate with gene expression. 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.\textsuperscript{44} The present findings are a resource for examining gene regulation by virtually any known, or to-be-discovered, cardiac enhancer as well as to explore newly identified three-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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Author contributions

Conceptualization, MRG, TMV; Methodology, MRG, DJC, ADS, EB, SR, MP, BR, TMV;
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Disclosures

None

References


Figure Legends

**Figure 1. Loss of CTCF induces cardiac pathology.** (A) Echocardiography measurements of ejection fraction (EF) and LV internal diameter at diastole or systole (LVIDd or LVIDs) demonstrate impairment of EF and chamber dilation in CTCF-KO (purple) and TAC (red) as compared to control (CTRL; green). Heart weight to body weight ratio (HW/BW) indicates cardiac hypertrophy in CTCF-KO and TAC mice (n≥25/group; * p<0.01, Student’s t-test). (B) Picrosirius 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 picrosirius red sections (n=3/group; * p<0.01, Tukey HSD test). (D) Stress response gene expression (log10(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-G indicates separate patients. Lines shifted horizontally in G for ease of viewing.

**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 vs. TAC and control vs. CTCF-KO; Supplemental Figure 7A shows control vs. CTCF-KO for all chromosomes). (C) Quantitation of insulation score differences in control versus KO (left) or TAC (right). Colored dots indicate significant changes (grey dots show range of variation between two 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 panels 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. Log2(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 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 vs. CTCF KO and CTRL vs. TAC. (H) Heatmap depicting log2(KO/CTRL) and log2(TAC/CTRL) FPKM for the differentially expressed genes with q< 0.01 (left). (FPKM) fragments per kb of exon per million mapped reads.

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 two 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 when compared to CTRL (left); overlap of loops that disappear in CTCF-KO and TAC (right). (E) CTRL loops in which zero (green), one (grey), 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, while 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.

Figure 4. Chromatin architecture is remodeled around cardiac genes during disease. (A) As an example cardiac disease gene with changing long range, intra-chromosomal contacts, the interactions emanating from Ppp3ca in control and after CTCF depletion or TAC are shown (q<0.01; 40kb resolution; outer circle, chromosome position; black, mm10 genes). (B) Examining gene expression data for all 5443 genes with shared interaction behavior between CTCF-KO and TAC, 3651 genes were found to be differentially expressed in the same direction in perturbations compared to control. Most (86%) of these gene expression changes were associated with decreased chromatin interactions (green colors), with 1662 up regulated (dark
green) and 1504 down regulated (light green). The remainder of the interaction changes (14%) were distributed between the other possible scenarios: increased interactions and expression (193, dark blue), increased interaction and decreased expression (154, light blue), no change in interactions and either an increase (71, dark orange) or decrease (67, light orange) in expression.

(C) Heatmap showing the number of significant ($q<0.01$) interactions overlapping with differentially expressed genes; top 40 shared differentially expressed genes shown, sorted by number of significant interactions in CTRL (gene name labeling shows direction of expression, where green is up and red is down). (D) Higher resolution mapping of local neighborhood interactions for the example genes $Nppa/Nppb$, $Kcnd2$, and $Mef2c$ gene loci +/- 1Mb ($q<0.01$). Lines revealing precise contact sites are color-coded by $q$-value significance, with red being the most significant. RNA-seq tracks depict gene expression for CTRL (green), CTCF-KO (purple), and TAC (red).

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 (grey) 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.

**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 paper and previous publications²⁻⁵, ¹⁰⁻¹¹, ¹⁵⁻¹⁷, ²⁹⁻³³ as described in text.
A)

B)

C)

D)

E)

F)

G)

H)
High Resolution Mapping of Chromatin Conformation in Cardiac Myocytes Reveals Structural Remodeling of the Epigenome in Heart Failure
Manuel Rosa-Garrido, Douglas J. Chapski, Anthony D. Schmitt, Todd H. Kimball, Elaheh Karbassi, Emma Monte, Enrique Balderas, Matteo Pellegrini, Tsai-Ting Shih, Elizabeth Soehalim, David A. Liem, Peipei Ping, Niels J. Galjart, Shuxun Ren, Yibin Wang, Bing Ren and Thomas M. Vondriska

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Supplemental Material

High resolution mapping of chromatin conformation in cardiac myocytes reveals structural remodeling of the epigenome in heart failure

Rosa-Garrido et al.

This section contains the extended Materials & Methods Section and Supplemental Figures 1-8. Supplemental Tables 1-3, containing enhancer-gene data, are uploaded as separate excel files. Sequencing data used in this paper have been deposited in NCBI with GEO accession number GSE96693.
MATERIALS & METHODS

Hi-C Methodology
For each replicate (n≥3 for all sequencing experiments in this paper), one million isolated adult cardiomyocytes were fixed in 1% formaldehyde and underwent in situ Hi-C as described,¹ using MboI as the restriction enzyme. Hi-C libraries were sequenced on an Illumina HiSeq 4000 instrument. Please see Figure S1 for detailed data on read counts, cis/trans interactions, and mappability. All sequencing data have been uploaded to NCBI with the GEO accession number GSE96693.

Bioinformatics Analyses of Hi-C Data
Raw paired-end 50bp reads were mapped to mm10 using BWA-MEM² as recently described.³ Raw genome-wide contact matrices were generated using a custom Perl script. To normalize genome-wide raw contact matrices, we first removed bin pairs that fell outside the 5th and 99.5th percentiles (that had abnormally low or high coverage, respectively). We then used a custom Perl script to perform iterative correction and eigenvalue decomposition (ICE) on the filtered matrices for 50 iterations.⁴ We then divided all entries in each genome-wide contact matrix by the corresponding matrix sum, and then multiplied them by the arbitrary value of 1 billion for 5kb resolution or 1 million for 40kb and 50kb resolution.⁵ For the main analyses in this study, all biological replicates were combined.

For the individual replicate analyses, we generated replicate-specific contact matrices using the methods described above, and then calculated a Pearson correlation for the ice-corrected and normalized cis bin pairs for which we measured interactions in each replicate pair. We then plotted log₁₀(normalized interactions) for each replicate on a respective axis using the smoothScatter() function in R. Once we determined a high correlation between replicates, we pooled the 3 replicates of each sample type together.
To generate 40kb z-score matrices, we first generated 40kb normalized contact matrices from the combined replicates for each sample type, using the methodologies described above. Z-score cis matrices were then generated using the loess method,\(^5\) using the following function from the HiTC package\(^6\) in Bioconductor: \texttt{normPerExpected(matrix, method="loess", span=0.005, stdev=T)}. Then, \(q\)-values were calculated using the \texttt{zscore2pval.R} script in https://github.com/dekkerlab/cworld-dekker. Significant \(q\)-values \(< 0.01\) were used for downstream analyses and Circos plot generation in R.

Identification of AB compartments at 5kb resolution was performed using the \texttt{prcomp()} function in R and correlating PC1 values to gene density. To generate a heatmap of difference in AB compartmentalization, we first determined the bins on autosomes that underwent compartment change in at least one sample. Then, we performed k-means clustering with \(k = 5\). The AB difference graphs were generated using the following logic: If the PC1 value of a given bin of a diseased sample became more positive or less negative when compared to control, then the difference was deemed “positive” and the magnitude was shown in orange as a positive difference. Contrastingly, if the PC1 value of a given bin of a diseased sample became more negative or less positive, then the difference was deemed “negative” and the magnitude was shown in purple as a negative difference. To compare AB compartment changes with gene expression differences, we determined the AB compartmentalization for the TSS bin for each autosomal gene and then generated boxplots showing compartment change on the x-axis and \(\log_2\) (fold-change) of FPKM on the y-axis.

Insulation scores were calculated using the method,\(^5\) using a 500kb sliding window across each 5kb normalized cis contact matrix. Insulation scores for all samples were normalized together using the \texttt{normalize.quantiles()} function of the \texttt{preprocessCore} package in Bioconductor (originally described\(^7\)). The delta vector for each insulation score profile was generated using a custom R script, using a 500kb sliding window across each 5kb normalized contact matrix, and a 100kb outward distance to the left and right of each bin to calculate the
slope of the insulation score. Insulation boundaries were defined as local minima in the insulation score, and boundary strength was calculated. We discarded boundaries with strength < 0.1. Insulation boundary heatmaps were generated by first calculating the insulation boundaries that overlap between control and CTCF knockout, and between control and TAC. Then, we generated heatmaps of overlapping boundary strength changes per autosome.

To call and visualize chromatin loops, we used Juicer and Juicebox. We first ran juicebox_tools_7.0 pre to generate .hic files for each condition, using reads with mapping quality ≥ 10. We then used juicebox_tools_7.0 hiccups, a functionality of Juicer, to call loops on the .hic files, using default parameters. Juicebox v1.5 was used to visualize the data. We then utilized the InteractionSet library for custom downstream analyses.

**Interaction Calling via Fit-Hi-C**

To call significant interactions in our 5kb cis interaction matrices, we used the Bioconductor version of Fit-Hi-C (https://bioconductor.org/packages/release/bioc/html/FitHiC.html) with the following parameters to analyze mid-range interactions up to 20Mb: distUpThresh=20000000, noOfBins=200. As input, we used concatenated raw autosomal 5kb cis interaction matrices and concatenated autosomal ICE bias vectors generated from our custom ICE implementation. After running Fit-Hi-C, we converted cis interactions with q-value < 0.01 into InteractionSet format for interrogations of significant interactions using R.

**Data Visualization**

All visualizations of Hi-C data were generated either using custom R scripts written in house or using Juicebox. For custom visualizations, we used the Bioconductor package ggbio.
RNA-seq Methodology

Total RNA from isolated adult cardiomyocytes was isolated using the Qiagen RNeasy Mini Kit (Cat# 74104). RNA quality was assessed using the Agilent 2100 Bioanalyzer (Cat# G2940CA). Ribosomal RNA was then removed using an Illumina Ribo-Zero rRNA Removal Kit (Cat# MRZH11124) and the RNA-seq library was prepared at the UCLA Clinical Microarray Core using the KAPA Stranded RNA-Seq Library Preparation Kit (Cat# KK8401). The average insert size of each library was measured using an Agilent 4200 TapeStation (Cat# G2991AA). All libraries ranged from 200 to 400bp in length. RNA-seq libraries were sequenced on two lanes of an Illumina HiSeq 2000 to obtain 2x100bp (paired-end) reads. On average, ~50 million raw pairs were generated per library.

Bioinformatics Analyses of RNA-seq Data

Raw paired-end 100bp reads were sequenced on two lanes of an Illumina HiSeq 2000, and FASTQ files were demultiplexed using a custom Python script. Reads were mapped to the mm10 genome with TopHat v2.1.0,\textsuperscript{13} using mm10 bowtie2 indices and a reference GTF provided by iGenomes (Illumina, downloaded from Ensembl release 81 on July 17, 2015). Transcripts were assembled using Cufflinks\textsuperscript{14} v2.2.1 with the -G parameter (using the same GTF), and merged using the Cuffmerge feature of Cufflinks with the -g and -s parameters (using the same GTF and the reference FASTA provided by iGenomes, respectively).\textsuperscript{14, 15} Cuffquant (from Cufflinks v2.2.1) was performed to determine transcript abundances for all samples, using the -b parameter (with reference FASTA from iGenomes), and the --max-bundle-frags 5000000 and -u parameters. Cuffdiff (also from Cufflinks v2.2.1) was used with identical extra parameters as Cuffquant to determine differential gene expression between conditions and assign $p$-values and $q$-values for each gene. Visualization of RNA-seq data was performed using cummerbund,\textsuperscript{16} ggbiom,\textsuperscript{12} as well as custom R scripts.
**ChIP-seq Methodology**

ChIP for CTCF (Active Motif Cat# 61311) was performed on pooled isolated cardiomyocytes (~10 million total), using the ChIP-IT High Sensitivity Kit as described. Cardiomyocyte pools were obtained from 3 control and 3 CTCF KO hearts. ChIP-seq libraries were prepared using the NuGEN Ovation Ultralow System V2 1–16 kit (Cat# 0344).

**Bioinformatics Analyses of ChIP-seq Data**

ChIP-seq libraries were sequenced on an Illumina HiSeq 2000 instrument. Raw single end 50 bp reads were demultiplexed using a custom Python script and then mapped to the same mm10 Ensembl reference genome as the RNA-seq data, but using Bowtie2\(^\text{18}\) v2.2.6 with default parameters. Samtools\(^\text{19}\) v0.1.19 was used to convert SAM files to BAM format, and peak calling was performed using the macs2.1.1.2016.0309 callpeak function\(^\text{20}\) with default parameters. The CTCF ChIP BAM file was used as the treatment file and input BAM file as the control. A filtering step was added in R to only keep autosomal peaks.

**ChIP-seq Visualizations**

To visualize the distribution of CTCF peak occupancy across features of the mm10 genome, we used the ChIPseeker\(^\text{21}\) package in Bioconductor. We used ngs.plot\(^\text{22}\) to generate coverage heatmaps around TSSs and insulation boundaries.

**KEGG Analysis**

To determine the gene ontology of genes of interest, we used a custom R script that uses KEGG.db in Bioconductor,\(^\text{23}\) with custom visualization.
Cardiomyocyte Isolation

Using an established protocol, adult mice were treated with heparin (100 USP units) for 20 minutes to prevent blood coagulation, and then anesthetized with sodium pentobarbital (100µl of 50mg/ml dilution, intra-peritoneal). Upon loss of rear foot reflex, the heart was removed and instantaneously arrested in ice-cold phosphate buffered saline (PBS) and mounted on a modified Langendorff apparatus. After 5 min of perfusion with Tyrode’s solution (130mM NaCl, 5.4mM KCl, 1mM MgCl2, 0.6mM Na2HPO4, 10mM glucose, and 10mM HEPES, pH 7.37, oxygenated with 95% (v/v) O2-5% (v/v) CO2) at 37°C, the heart was perfused for 15-30 min with 30 ml Tyrode’s containing 20mg collagenase type-II and 3mg protease type-XIV and then washed for an additional 10 min with Krebs buffer (KB) (25mM KCl, 10mM KH2PO4, 2mM MgSO4, 20mM glucose, 20mM taurine, 5mM creatine, 100mM potassium glutamate, 10mM aspartic acid, 0.5mM EGTA, 5mM HEPES, pH 7.18) oxygenated with 95% O2-5% (v/v) CO2. Cardiomyocytes were dissociated in KB solution, filtered (100µm strainer) and centrifuged 2 min at 1000xg for further usage. This method obtained cells that were ≥95% cardiomyocytes by visual inspection of rod-shaped cell morphology.

Post-mortem histology, Cell Size Quantification and Fibrosis Detection

Whole hearts were rapidly excised from animals, perfused with 0.1 M potassium (K+) solution, fixed using 4% paraformaldehyde and then embedded in paraffin. Hearts were sectioned into 4µm thick sections. Cell size was determined using NIS-Elements Advanced Research v4.0, on heart slices labeled with wheat germ agglutinin (Thermo Fisher Scientific Cat# W11262). Fibrotic tissue was visualized using the Abcam Picrosirius Red Stain Kit (Cat# ab150681).

CTCF Knockout Mouse Generation

Ctf-floxed (Ctf\textsuperscript{flox}) mice, in which loxP sites flank exons 3–12, were previously reported. The floxed allele occurs in the C57BL/6 background. To generate cardiac Ctf cKO mice, we
crossed Ctcf<sup>lox/lox</sup> mice with transgenic mice expressing the α-myosin heavy chain promoter that directs expression of a tamoxifen-inducible Cre recombinase (MerCreMer).<sup>26</sup> Adult Ctcf<sup>lox/lox</sup> × α-MHC-MCM<sup>+/−</sup> mice (8 weeks) were administered tamoxifen (Tx) in the chow (0.4mg/g) for 5 weeks plus 1 week on normal chow to deplete CTCF.

**Transverse Aortic Constriction and Echocardiographic Measurements**

All animal studies were approved by the UCLA Animal Research Committee in compliance with the NIH Guide for the Care and Use of Laboratory Animals. Only adult mice were used in this study. Adult C57BL/6 male and female mice were subjected to transverse aortic constriction surgery to induce pressure overload and cardiac function was measured before, directly after and once every 5 days for the duration of the experiment as described.<sup>27</sup> Animals were sacrificed for experiments based on changes in ejection fraction and heart to body weight, indicating the presence of pathology.

**Human Samples**

All human samples used in this study were procured in the Ronald Reagan Medical Center at UCLA following patient consent according to IRB#11-001053-AM-00016. mRNA and protein were isolated from LVAD biopsy cores or explanted hearts using conventional approaches.

**Electrophoresis and Western Blotting**

Cardiomyocytes, neonatal rat ventricular myocytes or human heart tissue were lysed (50mM Tris pH 7.4/10mM ethylenediaminetetraacetic acid [EDTA]/1% Sodium dodecyl sulfate [SDS]/0.1mM phenylmethanesulfonylfouride/protease inhibitor cocktail pellet (Roche)/0.2mM sodium orthovanadate/ 0.1mM sodium fluoride/10mM sodium butyrate), sonicated, and separated via SDS-PAGE using Laemmli buffer. Detection was performed on the LI-COR odyssey. Antibodies were as follows: CTCF mix 1:500 (Abcam, ab70303), (Abcam, ab128909),
(BD, 612148), (Abiocode, R3171-1), (Diagenode, C15410210-50) and (Active Motif, 61311), H3K27me3 1:1000 (Abcam, ab6002), H3K4me3 1:1000 (Abcam, ab8580), Rad21 1:1000 (Abcam, ab992), GAPDH 1:1000 (Santa Cruz Biotechnology, sc20357), Actin 1:1000 (Santa Cruz Biotechnology, sc1616), secondaries 1:10,000 (LI-COR, IRDye conjugated). Relative quantification for CTCF expression normalized with respect to Actin was performed using ImageJ software.

RNA Isolation and qPCR

RNA from left ventricular adult cardiomyocytes were isolated using TRizol (Thermo Fisher Scientific, 15596018) according to the manufacturer's protocol. cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad, 170-8891). qPCR was performed using SsoFast EvaGreen Supermix (Bio-Rad, 172-5201) on a BioRad, C1000 thermocycler.
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Genome-wide chromatin conformation capture datasets and quality control analysis for Hi-C and RNA-seq. (A) Table summarizing read counts and mapping statistics for each sample type. Replicates of the same condition were combined upon verification that interactions and AB compartmentalization correlated with Pearson correlation > 0.90 (at 5kb resolution). (B) Coverage histograms of raw 5kb cis and trans matrices for each sample type (x axis indicates number of raw interactions and y axis indicates the number of bins that had such number of interactions). (C) Normalized interaction frequency per bin is highly conserved between biological replicates, Pearson correlation coefficient of > 0.95 in all comparisons. For this analysis, cis interactions ≤ 2Mb were compared. The normalized cis matrices were generated from ice-normalized cis and trans genome-wide matrices that were divided by matrix sum and multiplied by the arbitrary value of 1 million. (D) Summary statistics of RNA-seq sequencing data, including number of mapped read pairs for each sample.

Supplemental Figure 2. Generation and phenotypic characterization of cardiac-specific CTCF knockout mice. (A) Left, diagram showing the floxed ctf gene from exon 3 to 12. Labeled yellow bars mark exons 1 to 12 and labeled green arrows indicate primers used to verify CTCF depletion via PCR. Primers p8563 and Skas1 should have a PCR product when exons 3 to 12 are excised by the Cre recombinase via the LoxP sites (blue triangles), and Primers p8563 and p8946 should have a PCR product in any scenario in which the Cre recombinase is not expressed. Right, α-MHC-Cre+/− x CTCF^flox/flox mice were fed Tx containing chow (0.4mg Tx/g chow) for 5 weeks and then switched to normal chow for 1 week, resulting in deletion of exons 3-12 of CTCF. α-MHC-Cre+/− x CTCF^flox/flox mice fed normal chow for 6 weeks, and α-MHC-Cre+/− x CTCF^WT/WT mice fed tamoxifen chow for 5 weeks plus 1 week on normal chow, served as controls. (B) PCR genotyping confirms the presence of the floxed CTCF alleles
in our CTCF\textsuperscript{flox/flox} Cre\textsuperscript{+/−} mice (left), as well as presence of the Cre gene in these mice (center). After 5 weeks of tamoxifen treatment followed by 1 week on regular chow, gene excision in the heart was confirmed by PCR using p8563 and Skas1 primers (right). PCR amplified bands at ~500bp in the knockout (red arrows), but did not amplify the ~27kb region of the floxed allele in Cre\textsuperscript{−/−} control mice (white arrows) or normal chow fed mice (black arrows). (Rg) regular chow. 

(C) CTCF KO was confirmed by RT-qPCR (left panel) for α-MHC-MCM\textsuperscript{+/−} x CTCF\textsuperscript{flox/flox} mice that were fed tamoxifen for 1, 2, 3, 4, 5 and 6 weeks. Blue bars show fold enrichment when compared to CTRL, and error bars indicate standard deviation; * indicates p<0.05 Student’s t-test. (D) Kaplan-Meier survival curves of CTCF\textsuperscript{flox/flox} Cre\textsuperscript{+/−} (n=20) (red line), CTCF\textsuperscript{wt/wt} Cre\textsuperscript{+/−} (n=10) (green line), CTCF\textsuperscript{flox/flox} Cre\textsuperscript{−/−} (n=10) (blue line) fed with Tx and CTCF\textsuperscript{flox/flox} Cre\textsuperscript{+/−} (n=10) (orange line) fed with regular chow. Left panel, 5 weeks after Tx treatment, mouse survival decreased 63%, while survival dropped to 8% at 6 weeks. Right panel, Mouse survival stabilizes at 53% after the initial 5 weeks when mice are returned to normal chow. (Rg) regular chow. (E) RT-qPCR revealed mRNA down-regulation of SERCA (Atp2a2) and α-MHC (Mhy6), and upregulation of ANF (Nppa) and β-MHC (Mhy7), in mice undergoing 5 weeks Tx treatment plus 1 week regular chow (Rg). Bars show fold enrichment when compared to CTRL mouse given regular chow, and error bars indicate standard deviation; * indicates p<0.05 Student’s t-test. (F) Western blot confirming CTCF depletion in tamoxifen-treated CTCF\textsuperscript{flox/flox} Cre\textsuperscript{+/−} mice (left), and showing no change in H3K27me3 and H3K4me3 with CTCF depletion. Quantification of average relative CTCF intensity shows a decrease in CTCF protein levels in CTCF\textsuperscript{flox/flox} Cre\textsuperscript{+/−} mice after 5 weeks of tamoxifen treatment. (G) Representative hematoxylin and eosin staining, comparing CTCF\textsuperscript{wt/wt} Cre\textsuperscript{+/−}, CTCF\textsuperscript{flox/flox} Cre\textsuperscript{−/−} and CTCF\textsuperscript{flox/flox} Cre\textsuperscript{+/−} after 5 weeks Tx treatment followed by 1 week on regular chow. CTCF\textsuperscript{flox/flox} Cre\textsuperscript{+/−} treated with regular chow for 6 weeks were used as an extra negative control while TAC samples were used as a positive control for disease. Considerable dilatation of the ventricles is observed in the tamoxifen-treated CTCF\textsuperscript{flox/flox} Cre\textsuperscript{+/−} mice as well as the TAC mice. n = 5 for each condition. (lv) left ventricle, (rv) right ventricle.
right ventricle, (Rg) regular chow. Scale bar: 1mm. (H) Echocardiographic measurements of all the mice used in the study. Notably, CTCF\textsubscript{flox/flox} Cre\textsuperscript{+/-} mice treated with tamoxifen had a pathological phenotype when compared to the CTCF\textsubscript{flox/flox} Cre\textsuperscript{+/-} or CTCF\textsubscript{wt/wt} Cre\textsuperscript{+/-} that were given regular chow as well as the CTCF\textsubscript{wt/wt} Cre\textsuperscript{+/-} or CTCF\textsubscript{flox/flox} Cre\textsuperscript{-/-} mice treated with tamoxifen. \( n = 8-17 \) depending on the survival rate for each condition. Line indicates mean value for the parameter of interest while the error bars indicate standard deviation. (EF) ejection fraction, (LVIDd) LV internal diameter at diastole, (LVIDs) LV internal diameter at systole. (I) Representative B-mode (left) and M-mode (right) echocardiography images at 6 weeks from normal chow CTCF\textsubscript{flox/flox} Cre\textsuperscript{+/-} (upper panel) or tamoxifen fed CTCF\textsubscript{wt/wt} Cre\textsuperscript{+/-}, CTCF\textsubscript{flox/flox} Cre\textsuperscript{-/-} and CTCF\textsubscript{flox/flox} Cre\textsuperscript{+/-} mice. CTCF\textsubscript{flox/flox} Cre\textsuperscript{+/-} mice show pathological phenotype as in (H); a TAC mouse is shown for comparison.

Supplemental Figure 3. CTCF expression is down-regulated by pathologic stress and inversely correlated with heart size. (A) CTCF levels in mice undergoing a model of isoproterenol-induced heart failure across 87 genetically distinct strains. CTCF mRNA levels decrease across 80% of the mouse strains after isoproterenol treatment (top and middle panels; data from \(^{28}\)). Cohesin subunit Smca1 shows coordinate regulation with CTCF in this model. (B) Phenotype data from 87 mouse strains treated with ISO are plotted against CTCF mRNA expression after ISO. Total heart mass, left ventricular mass, and right ventricular mass all show significant negative correlation with CTCF expression, while ejection fraction shows no correlation. p-values were calculated using the Pearson correlation coefficient. (LV) left ventricle, (RV) right ventricle (data from \(^{28}\)). (C) Expression levels of different cohesin subunits decrease after CTCF depletion and TAC (log\(_{10}\)(FPKM+1)).
**Supplemental Figure 4. Cardiac disease associated genes differentially expressed after CTCF depletion.** Real time qPCR measuring expression levels of different cardiac genes after 1, 2, 3, 4 and 5 weeks Tx treatment. Mice fed with regular chow (No Tx) and those fed with Tx were used as controls. Red and blue coloring indicates down- or up-regulation, respectively. (n=4/group; * p<0.05, Student’s t-test).

**Supplemental Figure 5. CTCF depletion does not promote apoptosis.** Cleaved caspase-3 staining of heart sections from CTRL and CTCF KO mice. Human sarcoma tumor cells were used as a positive control (Right panel).

**Supplemental Figure 6. Human subject clinical data.** Available data on patients from whom heart samples were obtained is provided. “Wb quantitation” is the CTCF protein level measured by Western blot expressed as a ratio of after to before LVAD. NICM, non-ischemic cardiomyopathy; ICM, ischemic cardiomyopathy; NYHA, New York Heart Association Classification; EF, ejection fraction at time of LVAD placement.

**Supplemental Figure 7. Differences in boundary strength and A/B compartmentalization per chromosome.** (A) Boundary strength differences between groups (red, higher; blue, lower; Insets: example region on chromosome 16. (B) Percent of genome-wide A/B compartment change with CTCF depletion (left) and TAC (right). Bottom panels highlight only bins that change compartment.

**Supplemental Figure 8. Implications for cardiac phenotype from chromatin structural changes in TAC and CTCF-KO cardiac myocytes.** (A) Principal component analysis of the shared insulation boundary strengths. (B) Number of new boundaries between different experimental groups. Analyses do not distinguish between boundaries that emerge either from
shifting of an existing boundary outside the analysis window (+/-5kb from CTRL boundary center) or *de novo* formation of a completely new boundary. Color codes: Boundaries only found in CTRL (blue), only found in perturbation (beige), and shared between CTRL and perturbation (pink). (C) Principal component analysis of the RNA-seq samples. Control separates from CTCF KO and TAC on PC1. (D) KEGG pathway analysis of up-regulated (top) or down-regulated (bottom) genes bound by CTCF. Terms highlighted in green are cardiac-related. (E) KEGG pathway analysis of genes (2570; Figure 3F) that lie within the loops that lose ≥1 CTCF peak in CTCF-KO. (F) KEGG pathway analysis of genes (Figure 3H, “lost loops”) that lie within the 151 enhancer-promoter loops that are lost in CTCF-KO (left), the 174 lost with TAC (middle), and the 114 loops lost in both diseased conditions (CTCF-KO and TAC; right). (G) KEGG pathway analysis of the differentially expressed genes (Figure 5C, “enhancers with decrease in interactions”) interacting with enhancers that lose interactions after perturbation (left, control vs. CTCF-KO; middle, control vs. TAC; right, shared).
REFERENCES


Supplemental Figure 1.

### A) Supplemental Table 1

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### B) Supplemental Figure 2

Number of reads versus number of interactions for Control, CTCF KO, and TAC samples.

### C) Supplemental Figure 3

Log2 (number of normalized interactions) versus number of interactions for Control, CTCF KO, and TAC samples.

### D) Supplemental Table 2

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<td>0.737</td>
<td>75.3%</td>
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<td>316922</td>
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<td>73.0%</td>
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<td>2709</td>
<td>CTCF KO</td>
<td>461968</td>
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<td>73.0%</td>
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<td>481352</td>
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Supplemental Figure 4.
Supplemental Figure 5.
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<th>Sample</th>
<th>Management</th>
<th>Sex</th>
<th>Age (Year)</th>
<th>Etiology</th>
<th>EF</th>
<th>LVEDD (mm)</th>
<th>NYHA class</th>
<th>BNP (pg/mL)</th>
<th>Comorbidities</th>
<th>Medical History</th>
<th>Medications</th>
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<td>114</td>
<td>Before LVAD</td>
<td>Male</td>
<td>43</td>
<td>NICM</td>
<td>25</td>
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<td>69</td>
<td>4</td>
<td>402</td>
<td>None noted</td>
<td>Pravastatin</td>
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<td>119</td>
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<td>Male</td>
<td>45</td>
<td>NICM</td>
<td>20</td>
<td>2.54</td>
<td>70</td>
<td>4</td>
<td>778</td>
<td>COPD due to 50 years of smoking history</td>
<td>Amiodarone Epinephrine Alteplase</td>
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<tr>
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<td>59</td>
<td>Adenoidectomy Non sustained ventricle tachycardia Heparin induced thrombocytopenia</td>
<td>Amlodipine Atorvastatin Hydralazine Metoprolol Sildenafil Spironolactone Warfarin</td>
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<td>NICM</td>
<td>20</td>
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<td>65</td>
<td>4</td>
<td>548</td>
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<td>68</td>
<td>3B</td>
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Supplemental Figure 6.
Supplemental Figure 7.