Targeting transcription in heart failure via CDK7/12/13 inhibition

Heart failure with reduced ejection fraction (HFrEF) is associated with high mortality, highlighting an urgent need for new therapeutic strategies. As stress-activated cardiac signaling cascades converge on the nucleus to drive maladaptive gene programs, interdicting pathological transcription is a conceptually attractive approach for HFrEF therapy. Here, we demonstrate that CDK7/12/13 are critical regulators of transcription activation in the heart that can be pharmacologically inhibited to improve HFrEF. CDK7/12/13 inhibition using the first-in-class inhibitor THZ1 or RNAi blocks stress-induced transcription and pathologic hypertrophy in cultured rodent cardiomyocytes. THZ1 potently attenuates adverse cardiac remodeling and HFrEF pathogenesis in mice and blocks cardinal features of disease in human iPSC-derived cardiomyocytes. THZ1 suppresses Pol II enrichment at stress-transactivated cardiac genes and inhibits a specific pathologic gene program in the failing mouse heart. These data identify CDK7/12/13 as druggable regulators of cardiac gene transactivation during disease-related stress, suggesting that HFrEF features a critical dependency on transcription that can be therapeutically exploited.
Despite current standard of care, heart failure with reduced ejection fraction (HFrEF) remains associated with excessively high mortality and morbidity, highlighting an urgent need for new therapeutic strategies. Several pharmacotherapies for HFrEF that have been proven to improve outcomes, such as beta-adrenergic receptor antagonists and inhibitors of the renin-angiotensin system, typically interdict signaling at or near the plasma membrane. Ultimately, these stress-activated signaling cascades converge on the nucleus to drive maladaptive changes in gene transcription, which can chronically compromise cardiac function and fuel the vicious cycle of adverse cardiac remodeling and HFrEF pathogenesis. As the gene regulatory machinery functions as a nodal integrator of upstream stress signals, drugging specific components of the transcription apparatus represents a conceptually attractive approach to treat HFrEF.

Previous studies have shown that hyperphosphorylation of RNA polymerase II (Pol II) by multiprotein kinase complexes such as PTEFb (positive transcription elongation factor b) is a hallmark of pathological cardiac stress in rodent models of heart failure and in failing human hearts, prompting us to hypothesize that inhibition of additional upstream Pol II C-terminal domain (CTD) kinase complexes such as TFIIH could attenuate pathologic transcription during HFrEF pathogenesis. THZ1 is a first-in-class small molecule inhibitor of CDK7 (Cyclin dependent kinase 7), a core kinase in the eukaryotic TFIIH complex upstream of Pol II CTD phosphorylation. THZ1 covalently binds Cystein-312 on CDK7 at a site spatially distinct from the catalytic pocket, resulting in potent allosteric inhibition of CDK7 kinase activity. THZ1 also inhibits two related Pol II CTD kinases, CDK12 and CDK13, by covalently binding accessible cysteine residues that are homologous to C312 of CDK7. Preclinical studies across a broad array of tumor types have demonstrated that THZ1 can potently suppress transcription of gene programs that drive cancer progression. These studies prompted us to explore the role of CDK7/12/13-dependent transcription in HFrEF pathogenesis. Here, we demonstrate that CDK7/12/13 are critical regulators of stress-mediated transcription activation in cultured cardiomyocytes and the adult mouse heart that can be pharmacologically inhibited to improve HFrEF.

Results

CDK7/12/13 inhibition in cultured cardiomyocytes inhibits the hypertrophic stress response. We began by studying the effects of inhibiting CDK7/12/13-dependent Pol II CTD phosphorylation via THZ1 (structure shown in Fig. 1A) in cultured neonatal rat ventricular myocytes (NRVM), a well-established in vitro system to probe adverse cardiomyocyte remodeling. THZ1 treatment potently attenuated α1-adrenergic agonist (phenylephrine, PE)-induced cellular hypertrophy (Fig. 1B) and induction of Nppa and Nppb, two hallmark markers of pathological cardiomyocyte stress (Fig. 1C). To determine a specific role for cardiomyocyte CDK7/12/13 activity on phosphorylation of specific serine residues in the C-terminal heptapeptide repeats of Pol II, we performed Western blots using phospho-serine specific Pol II antibodies. We observed increased levels of Ser2P, Ser7P and Ser5P Pol II phosphoforms during PE-mediated hypertrophic stress (Fig. 1D), consistent with increased Pol II CTD phosphorylation in this setting. THZ1 attenuated the PE-dependent increase in Ser2P and Ser7P (Fig. 1D). We did not observe a significant effect on bulk abundance of Ser5P in cardiomyocytes, which may reflect redundant regulation of this phosphoform by other Pol II CTD kinases. The IC50 of THZ1 in these cardiomyocyte assays was 5–10 nM, which reflects a higher sensitivity to THZ1 than what has been observed in studies of growth inhibition of several cancer cell types. THZ1 showed no significant effect on NRVM size, Nppa/Nppb expression, or cell death (Fig. 1B–D and Supplementary Fig. 1B). As a critical negative control, we used THZ1-R, an inactive structural analog of THZ1 with minimal kinase inhibitory activity. THZ1-R did not affect cellular hypertrophy, Nppa/Nppb induction, or Pol II CTD phosphorylation (Supplementary Fig. 1C–E). To validate our findings using THZ1, we also assessed the anti-hypertrophic effect of the small molecule probe YKL-1-116 (Supplementary Fig. 2A), which has substantially lower potency against CDK12/13. YKL-1-116 inhibited cellular hypertrophy and Nppa/Nppb induction but required concentrations 50–100 fold higher than THZ1 (Supplementary Fig. 2B, C). These data suggest that potent and combined inhibition of CDK7/12/13 is required for maximal suppression of the hypertrophic stress response in cardiomyocytes.

To provide orthogonal validation for the on-target molecular pharmacology of THZ1 in our experimental system, we utilized specific siRNA probes to individually knockdown Cdk7, Cdk12, or Cdk13 as well as triple knockdown (TKD) of all three kinases (Supplementary Fig. 3). We found that individual knockdown of Cdk7, 12, or 13 did not consistently attenuate cardiomyocyte hypertrophy. Nppa and Nppb induction, and Pol II CTD hyperphosphorylation with the same potency of THZ1, indicating that depletion of a single kinase did not fully recapitulate the robust effects of THZ1 (Supplementary Fig. 3E, F). In contrast, triple knockdown of Cdk7/12/13 potently attenuated PE-induced hypertrophy, Nppa/Nppb expression, and Pol II CTD hyperphosphorylation in a manner that more closely approximated the effects of THZ1 (Fig. 1E–H). The increased abundance of these specific Pol II phosphoforms during agonist stimulation and its decrease with CDK7/12/13 inhibition using THZ1 or siRNA confirms that the combined activity of these kinases is specifically increased during hypertrophic stress in cardiomyocytes. Together, our complementary data using chemical probes and RNAi establish a role for CDK7/12/13 as druggable transcription co-activators and positive regulators of pathological cardiomyocyte hypertrophy.

To enhance relevance to human HFrEF, we ascertained whether CDK7/12/13 inhibition with THZ1 could also block pathologic remodeling in human cardiomyocytes. We tested the effects of THZ1 in human-induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), a well-validated in vitro experimental platform to study human cardiomyocyte stress signaling, using an iPSC line derived from a healthy human donor [Cell Cardiomyocytes, Cellular Dynamics International Inc. (CDI)]. Previous studies have demonstrated that these cells mount a hypertrophic response to 10 nM endothelin-1 (ET-1) and 3β2,28. We found that THZ1 attenuated ET-1-mediated hypertrophic growth in human iPSC-CMs in a dose-dependent manner (Fig. 1I, J) with an IC50 ~10 nM, a potency that paralleled our rodent cardiomyocyte data. Consistent with the effects on cellular hypertrophy, qRT-PCR demonstrated that THZ1-suppressed transactivation of NPPB/BNP (Fig. 1K) and other typical marker genes induced in human iPSC-CMs during ET-1 stimulation (Supplementary Fig. 4A). Quantitative enzyme-linked immunosorbent assay (ELISA) confirmed that THZ1 attenuated the ET-1-stimulated secretion of N-terminal pro-BNP protein, a widely used clinical biomarker for human HFrEF (Fig. 1L).

THZ1 alters gene expression and transcription in NRVM during hypertrophic stress. We next performed RNA-Seq in NRVM to profile the genome wide transcriptional mechanisms underlying the protective effects of THZ1 (Fig. 2A, Supplementary Data 1). Hierarchical clustering revealed six distinct gene expression patterns, with approximately one-fourth of all expressed genes significantly upregulated or downregulated by THZ1. To validate the on-target gene expression and transcriptional readouts, we performed qRT-PCR for a subset of genes with corresponding RNA-Seq results (Fig. 2B, C). Together, these data strongly support the notion that the anti-hypertrophic effects of THZ1 are mediated through inhibition of CDK7/12/13.
genes falling into cluster 5, representing genes induced by PE stimulation and robustly suppressed with THZ1 co-treatment, such as Nppa, Nppb, and Xirp2/Myomaxin (Fig. 2B). THZ1 also attenuated the PE-mediated downregulation of a subset of genes (cluster 2), further suggesting that primary inhibition of stress-gene transactivation can shift the transcriptome towards the basal state. While THZ1 had no major effect on cardiomyocyte growth or viability at baseline (Fig. 1B–D and Supplementary Fig. 1B), there was a subset of genes for which THZ1 altered baseline expression (clusters 1 and 3). Gene ontology analysis for each cluster is provided in Supplementary Data 2. Given the role of CDK7/12/13 as activators of Pol II and the robust ability of THZ1 to suppress stress-induced cardiomyocyte hypertrophy, we focused our attention on PE-mediated gene transcription by...
performing ChIP-Seq for total Pol II in NRVM after 48 h of PE stimulation. Representative gene tracks for the Nppa/Nppb locus (Fig. 2C) showed that PE stimulation increased gene body enrichment of Pol II, consistent with stress-activated transcription of these genes. THZ1 attenuated enrichment of Pol II at the Nppa and Nppb loci, consistent with inhibition of gene transcription. Genome-scale quantification of Pol II density at this 48-h time point revealed that THZ1 led to a modest increase in Pol II accumulation at transcription start sites (Fig. 2D), a finding that suggests a net effect of impaired transcription elongation at this late time point. We assessed the Pol II trading ratio (defined here as the ratio of Pol II occupancy at the TSS over the gene body; Fig. 2E), a widely used indicator of genome-scale transcription elongation\textsuperscript{30,31}, for genes regulated by THZ1 during PE-mediated stress. THZ1 elicited a rightward shift in the Pol II trading ratio curve, also suggesting a net effect of THZ1 in attenuating Pol II elongation in pathologically stressed cardiomyocytes after 48 h of PE stimulation (Fig. 2E). These ChIP-Seq data are consistent with the biochemical data demonstrating CDK7/12/13 inhibition blocks stress-dependent accumulation of bulk Pol II Ser2P abundance (Fig. 1D, F), a post-translational modification indicative of elongating Pol II. We note that Pol II ChIP-seq in primary cultured newborn cardiomyocytes, which was performed under experimental conditions that are required for robust agonist-induced hypertrophic responses (low plating density and prolonged serum starvation), did not provide signal robustness that would allow us to make reliable correlations between locus-specific Pol II enrichment and differential gene expression by RNA-seq. Furthermore, we cannot exclude a primary and rapid effect of THZ1 on Pol II initiation, which can influence subsequent rates of Pol II elongation and is an event in the transcription cycle that likely occurs at much earlier timepoints after agonist stimulation compared to the 48-h time point used in this experiment. Together, these in vitro data support that CDK7/12/13 are cell-autonomous effectors of transcription activation during cardiomyocyte stress and that CDK7/12/13 inhibition attenuates hallmark features of pathologic cardiomyocyte remodeling.

THZ1 improves cardiac function and inhibits a maladaptive gene expression program in a mouse model of heart failure. Our findings in cultured cardiomyocytes prompted us to test whether interdicting CDK7/12/13-dependent transcription using THZ1 could block HFrEF pathogenesis in vivo. We subjected mice to transverse aortic constriction (TAC), a widely used surgical model of left ventricle (LV) pressure overload and progressive HFrEF\textsuperscript{32}, with administration of THZ1 (20 mg/kg/day, intraperitoneally) or vehicle for 60 days (Fig. 3A). This THZ1 dose has been validated to provide target coverage and efficacy in murine tumor xenograft studies and is generally well tolerated in mice\textsuperscript{4,6,25}, allowing us to test for proof-of-concept in vivo in the context of HFrEF, with the caveat that detailed toxicological analysis has not yet been reported for this chemical probe. We found that THZ1 attenuated several hallmark features of adverse cardiac remodeling and HFrEF pathogenesis including LV systolic dysfunction (Fig. 3B, C and Supplementary Fig. 4B), cardiomyocyte hypertrophy (Fig. 3F), and LV fibrosis (Fig. 3G). THZ1 had no significant effect on cardiac mass, cardiac structure or LV function in the sham group (Fig. 3E, Supplementary Fig. 4 B–D). In addition, THZ1 had no effect on body weight (Supplementary Fig. 4E), consistent with the general tolerability of this compound that has been observed in mouse cancer xenograft studies. THZ1 did not affect systolic or diastolic blood pressure in mice (Supplementary Fig. 4E–G), suggesting that its salutary effect in HFrEF pathogenesis was not simply due to modulation of systemic blood pressure. To confirm on-target bioactivity in vivo, we performed Western blotting for Pol II CTD phosphorylation from LV tissue samples. TAC led to increased abundance of Pol II Ser2P and Ser7P, which were both attenuated by THZ1 (Supplementary Fig. 4H), supporting that cardiac CDK7/12/13 activity is increased during stress and blocked by THZ1 in vivo.

Next, we performed RNA-Seq from LV tissue in each experimental group (Fig. 4A). PCA clustering showed an overlap of Sham-Veh and Sham-THZ1 groups (Fig. 4B), revealing that
intermittent THZ1 exposure at the doses used here had minimal effect on the transcriptome of non-diseased hearts. This finding is consistent with the observation that THZ1 treatment did not alter cardiac mass, cardiac structure, or LV systolic function in the sham group (Fig. 3). TAC-mediated stress shifted the cardiac transcriptome along PC1, and the major effect of THZ1 treatment during TAC was to revert the gene expression profile back towards the sham group along PC1 (Fig. 4B). These shifts along PC1 encompassed a broad program of stress-induced genes whose upregulation was blunted by THZ1 (Fig. 4C). We identified a set of 800 genes that were upregulated during TAC, of which 401 genes (50%) were significantly suppressed by THZ1 (Fig. 4D). We also observed a clear cardiomyocyte signature demarcated by canonical hypertrophic marker genes (Nppb and Nppa) (Fig. 4E; cell compartment-specific heatmaps shown in Supplementary Fig. 5C). We also observed a clear cardiomyocyte signature demarcated by canonical hypertrophic marker genes (Nppb and Nppa) (Fig. 4E; cell compartment-specific heatmaps shown in Supplementary Fig. 5C).

Systemic THZ1 administration affects gene expression in multiple cell compartments in the failing mouse heart. While our in vitro data in primary rodent and human iPSC-derived cardiomyocytes support a cardiomyocyte-intrinsic effect of CDK7/12/13 inhibition, we recognize that administration of THZ1 in vivo may simultaneously act on multiple cell compartments that populate the stressed heart, including cardiomyocytes. Our bulk RNA-seq from adult mouse LV tissue suggests that THZ1 affects genes expressed in both cardiomyocytes and non-cardiomyocyte compartments during TAC. To better understand the relative responsiveness of various myocardial cellular compartments to THZ1 treatment during TAC, we curated two published single-cell transcriptomic datasets from adult mouse hearts to generate a cellular atlas (Fig. 4F, G) that allows for compartment-specific “fingerprinting” of our bulk RNA-seq data from LV tissue. Using the set of 401 genes identified to be upregulated in TAC and suppressed with THZ1 treatment in our bulk LV tissue RNA-seq, we cross-referenced these transcripts against our clustered single cell reference dataset to extrapolate the specific cellular compartments from which these dynamically responsive transcripts originated (Fig. 4G; cell compartment-specific heatmaps shown in Supplementary Fig. 6). Consistent with our cell-culture studies, we observed a clear cardiomyocyte signature demarcated by canonical HF-associated genes (Ankrd1, Xirp2, Nppa, Nppb). We also...
detected a robust signature of genes that were differentially expressed during TAC and sensitive to THZ1 that originated from resident cardiac fibroblasts, particularly the group of Periostin-positive cells that demarcate the myofibroblast sub-population. Other cell compartments in the adult mouse heart that were sensitive to THZ1 included epicardial, endothelial and endocardial cells. Taken together, these results suggest that THZ1 exerts protective effects in the TAC model by inhibiting CDK7/12/13-dependent cell state transitions across cardiomyocyte and non-cardiomyocyte cellular compartments.
Discussion

Collectively, the data presented here demonstrate that CDK7/12/13 activity is a critical effector of stress-dependent cardiac transcription and HFrEF pathogenesis. Using the first-in-class inhibitor THZ1, we provide proof of concept that transcription inhibition can exert effects on the heart during HFrEF pathogenesis in vitro and in vivo. Our in vitro data using chemical inhibitors and siRNA are consistent with a mechanism where CDK7, 12 and 13 have non-overlapping functions in the transcription cycle in cardiomyocytes. In this manner, multi-CDK inhibition of CDK7, 12 and 13 using THZ1 or siRNA is likely exerting additive or synergistic effects on cardiac stress responses by impairing their multiple, non-overlapping functions in transcription control, supporting the concept that inhibition of all
three kinases is required to consistently and potently suppress adverse cardiac remodeling. Future studies using cardiovascular cells engineered to express a CDK7 cysine substitution allele (Cys12Ser) that is resistant to covalent chemical inhibitors like THZ1 (or equivalent cysine substitution alleles for CDK12/13), will also help dissect the non-overlapping functions of these three kinases in cardiac stress responses. While THZ1 can suppress agonist-induced hypertrophy in cultured cardiomyocytes, our transcriptomic profiling of adult mouse hearts demonstrates that the protective effects of THZ1 in vivo are associated with dynamic gene expression changes in both cardiomyocyte and non-cardiomyocyte compartments. Often, dissecting the causality of cell-specific contributions during heart failure pathogenesis can be interrogated using conditional gene-deletion approaches. However, modeling the pharmacology of THZ1 in vivo using conditional gene-deletion approaches will be particularly challenging because it would likely require simultaneous postnatal deletion of multiple loci (Cdk7, Cdk12 and Cdk13). Furthermore, it is expected that gene deletion in any one cellular compartment would only give a partial protection from heart failure pathogenesis and that simultaneous CDK7/12/13 inhibition in multiple cell types, as occurs with THZ1, is required for full therapeutic effects. Finally, in contrast to the intermittent and dose-titratable effects of a chemical antagonist-induced hypertrophy in cultured cardiomyocytes, our finding that THZ1 can ameliorate pressure overload-induced heart failure in adult mice.

Our finding that THZ1 can also suppress pathologic remodeling in human iPSC-CMs at low nanomolar concentration supports the concept that manipulating stress-mediated transcriptional signaling might be an approach to limit adverse cardiac remodeling during human HFpEF pathogenesis. However, we acknowledge that systemic exposure to molecules such as THZ1 may have off-target toxicity in extracardiac organs, including effects on neuronal plasticity36 and possibly in highly proliferative tissue compartments such as the intestinal epithelium or bone marrow. Given the generally high bar for safety required for chronically dosed cardiovascular therapeutics, future studies detailing the on-target liabilities of CDK7/12/13 inhibition and the precise cell compartments mediating therapeutic efficacy will be required to refine such a strategy in the treatment of heart failure. In contrast to several cancer drugs that cause cardiotoxicity37, our data suggest that molecules like THZ1 may be a privileged class of anticancer therapeutics that have cardioprotective properties. More broadly, this work supports the contention that HFpEF pathogenesis, like cancer, features a general dependency on transcription that might be therapeutically exploited.

Methods

All animal studies performed in this study were reviewed and approved by the University of California, San Francisco Institutional Animal Care and Use Committee. All studies were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals38.

Animal models. Studies were conducted with age-matched male C57Bl/6J mice (The Jackson Laboratory, catalog no. 000664). Mice were housed in a temperature- and humidity-controlled pathogen-free facility with 12-h light/dark cycle and ad libitum access to water and standard laboratory rodent chow.

Chemical probes. THZ1, THZ1-R, and YKL-1-116, all of which have been previously described35,37, were generously provided by Nathanael Gray’s lab.

Mouse models of cardiac hypertrophy and heart failure. All mice were male C57Bl/6J mice aged 10 to 11 weeks from The Jackson Laboratory. Mice were placed on a temperature-controlled small-animal surgical table to help maintain body temperature (37 °C) during surgery. Mice were anesthetized with 1–3% isoflurane, mechanically ventilated (Harvard Apparatus), and subjected to thoracotomy. To perform transverse aortic artery constriction, aortic arch was exposed and constricted between the left common carotid and the brachiocephalic arteries using a 7–0 silk suture and a 27-gauge needle as previously described.25 Mice in the sham group were subjected to the same procedures except the placement of constriction. Daily intraperitoneal injections of vehicle solution (0.15 mL of 10% DMSO and 5%
dextrose in water) or 20 mg/kg THZ1 (dissolved in 0.15 mL of the same vehicle solution) were started on postoperative day 1 until postoperative day 60.

Neonatal rat ventricular myocyte isolation and culture. Neonatal rat ventricular myocytes (NRVMs) were isolated from the hearts of 2-3 day old Sprague-Dawley (Strain #001) rat pups from Charles River and maintained under standard conditions as described previously. After overnight digestion with 0.25% trypsin at 4 °C, cells were dissociated from the tissue through a series of digestion with 300 U/mL collagenase II (Worthington Biochemical Corporation, Lakewood, NJ). The dissociated cells were then pre-plated for 1.5 h in cell culture dishes followed by 24 h exposure to BrDU in culture medium to remove contaminating nonmyocytes and inhibit any residual cellular proliferation. Unless otherwise stated, NRVM were plated at a density of 10^5 cells/mL. Cells were initially plated in growth medium (DMEM supplemented with 5% FBS, 100 U/mL penicillin-streptomycin and 2 mM L-glutamine) for 48 h. Prior to stimulation with agonists or chemical probes, NRVM were maintained in serum-free medium supplemented with 0.1% BSA, 1% insulin-transferrin-selenium supplement (Sigma S1316), 100 U/mL penicillin-streptomycin, and 2 mM L-glutamine for additional 48 h.

For individual CDK knockdowns, CDK7, 12, and 13 mRNA were targeted by specific siRNA from Dharmacon (S102088_R01, S102088_R03, S102088_R04, S102088_R07, S102088_R09, S102088_R02, S102088_R05, S102088_R06, and S102088_R10) at a final concentration of 50 nM. For combinatorial triple knockdown of CDK7/12, equal concentrations of each siRNA probe were adjusted to maintain a final siRNA concentration of 50 nM. Transfection was performed with transfection reagent RNAiMAX (Invitrogen) according to the manufacturer’s instructions. Control groups were transfected withMISSION siRNA Universal Negative Control #1 (Sigma) at the same final concentration.

For testing the effects of small-molecule probes in the hypertrophic response, NRVM were incubated with vehicle (DMSO) or inhibitors at indicated concentrations for 48 h followed by stimulation with phenylephrine (100 μM) for various durations. For mRNA level and cell size measurements, NRVMs were stimulated with PE for 48 h. For evaluating the phosphorylation level of RNA polymerase II, NRVMs were incubated with PE for 30 min before harvest.

Human iPSC-CMs culture, qRT-PCR, and BNP enzyme-linked immunosorbent assay (ELISA). Human-induced pluripotent stem cell-derived cardiomyocytes (iCell cardiomyocytes) were purchased from FUJIFILM Cellular Dynamics, Inc. and maintained to the manufacturer’s instructions. LRR review was not required. Cells were seeded on 96-well culture plates coated with 5 μg/mL of fibronectin and maintained in Williams’ E Medium supplemented with 1:25 of Cell Maintenance Cocktail B (Thermo Fisher Scientific, CM4000). For subsequent hypertrophic stimulation, quiescent iPSC-CMs were treated with or without THZ1 at indicated concentrations versus DMSO for 3 h followed by stimulation with either vehicle or 10 nM endothelin-1 (Sigma) for 18 h. The complementary DNA was directly synthesized from RNA in cell lysate using the TaqMan Gene Expression Cells-to-CT kit (TaqMan Gene Expression Cells-to-CT kit (Thermo Fisher Scientific AM1728)) according to the manufacturer’s instructions. qRT-PCR for human NPPB and B2M (normalizer) was performed on the Life Technologies TaqMan assays (NPPB; Hs01073590_m1; B2M, Hs00984230_m1). qRT-PCR for other human genes was performed using TaqMan chemistry including FastStart Universal Probe Master (Roche), and gene-specific oligonucleotide primers (list of qRT-PCR primers and TaqMAN probes are provided in Supplementary Table 3. Relative expression was calculated using the 2^(-ΔΔCt) method with normalization to constitutive genes. Mouse data were mouse heart tissue samples. Mouse hearts were harvested, dried and weighed. Tissue was fixed in 10% neutral-buffered formalin overnight at 4 °C and transferred to 100% ethanol for 24 h. Samples were embedded in paraffin blocks, sectioned, and stained with Picrosirius red (Polysciences) or Wheat Germ Agglutinin 488 (Thermo Fisher Scientific, W11261) according to the manufacturers’ instructions. Images were captured by confocal microscopy, bright field microscopy or high-magnification microscopy on a microscope platform (IN Cell Analyzer, GE Healthcare) as previously described. For cardiac myocyte cross-sectional area from WGA- rhodamine stained LV tissue samples, we quantified area of 50 randomly selected cardiomyocytes per heart from 3 randomly chosen fields at 200X magnification (from tissue sectioned from the mid-papillary region in the LV short-axis), using Image J. This was repeated in 3 different hearts for each experimental group, leading to a total of 150 individual cardiomyocyte cross-sectional area measurements that were pooled for each experimental condition. Quantification of LV fibrosis from picrosirius red-stained heart tissue sections was performed in 5 independent hearts per experimental group as previously described.

Chocardiography and blood pressure measurements. For transthoracic echocardiography, mice were anesthetized with 1-2% isoflurane and imaged using Veo 770 Imaging System (Visual Sonics, Inc.) with RMV-707B probe. Measurements were obtained from M-mode sampling and integrated electrocardiogram-gated kihohertz visualization (EKV) images taken in the LV short axis at the mid-papillary level to generate high spatiotemporal resolution two-dimensional images. LV hypokinetic fraction and ejection fraction from resolved 2D-maps of LV end-diastolic and end-systolic measurements at end-diastole and end-systole.

Non-invasive blood pressure was measured using the BP2000 Blood Pressure Analysis System (Visitech Systems, Inc.) according to the manufacturer’s instructions. Mice received chronic injections of vehicle or THZ1 (20 mg/kg/day) at the same time of day for 1 week. Blood pressure was measured using the 2AΔCt method with normalization to constitutive genes. Mouse data were then pooled and analyzed.

RNA isolation and qPCR. Total RNA was isolated from NRVM using the High Pure RNA isolation kit (Roche 11806650101) with on-column DNAase treatment according to the manufacturer’s instructions. For RNA isolation from mouse tissue, a 10–20 mg piece of mouse heart ventricles was mechanically homogenized in PureZOL RNA isolation reagent (Bio-Rad 7326890) on a Tissue Lyser II (QIAGEN 85300) using stainless steel beads (QIAGEN 69899). Total RNA was purified using the Aurum total RNA purification kit (Bio-Rad 732–8830). The purified total RNA was then reverse transcribed to complementary DNA with iScript RT supermix (Bio-Rad 170–8841) according to the manufacturer’s instructions. A list of qRT-PCR primers and TaqMAN probes are provided in Supplementary Table 3. Relative expression was calculated using the 2AΔCt method with normalization to constitutive genes. Mouse data were normalized to Ppib.

Library preparation and next-generation sequencing for RNA-Seq. RNA isolated from tissue and cells were quantified using Qubit fluorometric quantitation and assessed for quality using Agilent Bioanalyzer Nano RNA Chip. Samples with RNA integrity number larger than 8 were considered high-quality and suitable for RNA-sequencing. Library preparation was performed using the Illumina TruSeq Stranded Total RNA HT Kit (Roche) and the library was sequenced on an Illumina HiSeq 2500 at the Genomics Core Facility of Whitehead Institutes (single-end, 40 base pairs, >50 M reads per sample).
RNA-seq data processing and quantification. Each RNA-seq sample was aligned independently. Specifically, the raw RNA-seq reads from each sample were aligned with TopHat v2.1.1 with options “-p -r 300-library-type fr-firststrand–no-coverage-search”. Each sample was aligned either against the mm9 or mm reference genome as appropriate for the sample.

After alignment, the number of mapped reads per gene per sample using htseq-counts was quantified. Each application of htseq-counts was run with the following options: --stranded --unique --disable-count
diffexpression

A genome as appropriate for the sample.

from the normalized DeSeq2 counts. After row-scaling, individual rows were grouped by their type (e.g., baseline, THZ1-treated) in order to better estimate the sample-level variance for comparison across samples. The standard DeSeq2 workflow followed without modifications, and Benjamin-Hochberg correction was used to control false discovery of differentially expressed genes.

Primary was prepared lists were generated from a matrix of the normalized counts from DeSeq2. Normalized counts were re-centered and scaled per sample prior to the analysis in order to standardize each sample. Heatmaps were generated from the normalized DeSeq2 counts. After row-scaling, individual rows were clustered using hierarchical clustering using a Euclidean distance metric and Ward’s clustering. Bar graphs were generated using calculated fold changes of expression of representative genes from DeSeq2 and the adjusted p-values reported by the software. The numbers of genes in the Venn diagram were generated using adjusted p-value < 0.05 (5% FDR) and Log2 fold change ≥ 1 for comparison between the TAC-Veh group vs Sham-Veh group, and adjusted p-value < 0.05 (5% FDR) and Log2 fold change ≤ −0.89 for comparison between TAC-Veh group vs TAC-THZ1 group. For the TAC samples, the fold change cutoff of ≤ −0.89 was chosen to capture the differential expression of Nppb between TAC-Veh vs. TAC-THZ1 in this n = 4 sample set, so as to maintain a threshold that appropriately captures known functional control gene.

DAVID (v.6.8)41 was used to identify enriched functional annotations in differentially expressed gene IDs relative to the set of “expressed” genes, defined as having sufficient normalized counts for DESeq2 to calculate a differential expression p value, or versus all annotated genes.

For gene set enrichment analysis (GSEA), command line GSEA2 (v.2.2.2, ref.5.) was used with MSigDB v.6.1 gene sets40. Values were pre-ranked by log2 fold change, retaining the absolute maximum change per gene where multiple transcripts were assessed. Significance was expressed as family-wise error rate (FWER). FWER p < 0.250 represents statistically significant enrichment.

Chromatin immunoprecipitation, library preparation and next-generation sequencing. For ChIP experiments, NRVM were plated in 15 cm dishes at 4 ×10^6 cells/dish. Chromatin derived from a total of 15 million cells were used for each immunoprecipitation. After indicated treatment, cells were crosslinked with 1% formaldehyde for 10 min at room temperature followed by quenching with 0.125 M glycine for 5 min. Cells were then lysed and chromatin was extracted. Isolated chromatin was subjected to shearing with BioCutter (Diagenode) for 16 cycles (30 sec on and 30 sec off/cycle) at high intensity. A small volume of sheared chromatin (50 μL) from each was stored at −80 °C as input. The rest of sheared chromatin was then incubated with 5 μg RNAPII antibody (clone 8WG6, Bio- Legend 920102 lot B217159) with 50 μL Dynabeads (Invitrogen) overnight at 4 °C. Chromatin was washed, eluted and reverse crosslinked overnight at 65 °C. Genomic DNA was purified from both immunoprecipitated samples and input samples. The library was prepared with TrueSeq ChIP kit (Illumina), and the library was sequenced on an Illumina HiSeq 2500 at the Genomics Core Facility of Whitehead Institutes (single-end, 40 base pairs, >30 M reads per sample).

ChIP-seq data processing. Each ChIP-seq sample was processed and aligned using bowtie2. Each sample was aligned against the mm9 reference genome using bowtie with options “-k 1 -m 1”. All gene track and ChIP-seq quantifications were normalized by the number of (uniquely) mapped reads to estimate the “reads per million” (rpm) quantity. Gene track displays were made with Integrative Genome Viewer (IGV).

All density plots for Pol II were made using all mm9 gene promoters based on the mm9 transcript reference or the UCSC mm transcript reference, depending on the source of the sample.

The differentially expressed genes were identified by DeSeq2. The quantifications from htseq-counts were imported into DeSeq2, and all samples from the same organism were analyzed together in one analytical run. Unpaired replicate samples were grouped by their type (e.g., baseline, THZ1-treated) in order to better estimate the sample-level variance for comparison across samples. The standard DeSeq2 workflow followed without modifications, and Benjamin-Hochberg correction was used to control false discovery of differentially expressed genes.

From our bulk RNA-seq data from LV tissue of THZ1-treated mice in the TAC Muris cohort35. These two scRNA-seq datasets were filtered, normalized, and integrated by Seurat (v4.0.0) R package62 with canonical correlation analysis. After performing dimensional reduction by Principal Component Analysis (PCA), the top 30 PCAs were used in graph-based clustering based on Louvain with resolution

and cluster-specific gene sets were identified using the FindAllMarkers function by Wilcoxon Rank Sum test in Seurat. Cell type enriched genes were identified using the FindAllMarkers function by Wilcoxon Rank Sum test in Seurat. Cell type enriched genes that were differentially expressed in TAC vs Sham by bulk expression analysis were selected for downstream analysis. TAC altered cell-type enriched signature genes were visualized in a graphic heatmap using the ComplexHeatmap (v2.6.2) R package58.

Statistical analysis. Measurements were taken from distinct samples. Data are reported as mean ± SEM unless otherwise indicated in the figure legend. Statistical analysis of LV fractional shortening as a function of time was performed by ANOVA with Holm-Sidak correction for multiple comparisons using GraphPad Prism. All other statistical analyses were performed using ANOVA with Tukey’s honest significant difference test correction for multiple comparisons using GraphPad Prism. For all analyses, p < 0.05 was considered significant. The statistical methods used in the analyses of RNA-seq and ChIP-seq data are detailed separately above.

Data availability. The data supporting the findings from this study are available within the manuscript and its supplementary information. The RNA-seq datasets generated in this study have been deposited in the Gene Expression Omnibus (GEO) database under accession codes GSE151253 (NRVM RNAseq) and GSE151254 (Mouse heart RNAseq). The ChIP-seq datasets generated in this study have been deposited in the GEO database under accession code GSE1512559. Source data are provided with this paper.

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Author contributions

A.H., Q.D., D.S.D., B.G.B., and S.M.H. conceived the study, interpreted the data, and wrote the manuscript. Y.H. performed heart surgeries and echocardiography. Z.B.F. performed CHIP-seq. A.H. and Q.D. performed primary cell isolations for knockdown and chemical inhibitor experiments and performed molecular and biochemical analyses on in vivo samples. S.M. performed human iPSC-CM studies. T.Z., Y.L., and N.S.G. provided chemical compounds and provided expertise and guidance on studies. D.S.D. performed RNA-seq and CHIP-seq computational analyses. X.L. performed integrated bulk and single-cell RNA-seq analysis. Z.J., M.A., A.P., J.D.R., C.Y.L., and R.A.Y. provided expertise, advisement, and critical revisions to the manuscript.

Competing interests

S.M.H. is an officer, executive and shareholder of Amgen, a scientific founder and shareholder of Tenaya Therapeutics, and serves on the Scientific Advisory Board of the DZHK (German Centre for Cardiovascular Research) of the German Ministry of Health as an uncompensated volunteer. X.L. is an employee and shareholder of Amgen. B.G.B. is a scientific founder and shareholder of Tenaya Therapeutics. R.A.Y. is a founder and shareholder of Syros Pharmaceuticals, Camp4 Therapeutics, Omega Therapeutics, and Dewpoint Therapeutics. N.S.G. is a Scientific Founder, member of the SAB and equity holder in C4 Therapeutics, Syros, Soltego (board member), B2S/Yorozui, Allorion, Lighthorse, Cobroventures, GSK, Larkspur (board member) and Matchpoint. The Gray lab receives research funding from Springerworks and Interline. T.Z. is a scientific founder, equity holder and consultant of Matchpoint. The remaining authors declare no competing interests.

Additional information

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