SUPPLEMENTAL METHODS

Subject recruitment

Subjects were recruited for participation in our cardiomyopathy biobank. Patients undergoing cardiac procedures, as well as non-cardiac patients with known genetic mutations (as identified by their health care provider) were targeted. In the latter case, we had 4 DCM subjects (two of which exhibit reduced ejection fraction <45%), 2 HCM subjects (with IVSd >=1.8 cm but wall thickness 0.9 and 1.2 cm), and 4 LVNC subjects who lacked a clinical diagnosis. These samples were used for WGS but not the cardiomyocyte differentiation and subsequent RNA-seq with the exception of subject 969 (DCM, reduced LVEF of 23.8%), subject 544 (HCM, IVSd 1.8cm), subject 603 (HCM, IVSd 2.1cm). Their data is included in the WGS figures except where indicated.

Healthy subjects without known genetic mutations and lacking a progressive condition were recruited from our cardiovascular prevention clinic. An additional category of control patients (referred to as “other” in Table S1) represent patients with non-cardiac conditions who were recruited at the clinic and over the phone, with permission of their providers. Two patients with known cardiac conditions other than cardiomyopathy (long QT syndrome and Fabry disease) were also recruited. Echocardiogram assessment of left ventricular ejection fraction (LVEF) and interventricular septum thickness, end diastole (IVSd) from the most recent measurement in the electronic medical record were queried and populated in RedCap when available.

iPSC reprogramming

Induced pluripotent stem cells were reprogrammed from PBMCs using Sendai virus (CytoTune iPS 2.0 Sendai Reprogramming Kit) as previously described.[39] Three clones were generated per subject, karyotyped (KaryoStat, ThermoFisher Scientific), determined to be mycoplasma-free, and evaluated by immunohistochemistry for expression of pluripotency markers TRA-1-60 (LifeTech MA1023) and SSEA4 (LifeTech MA1021). Cells were maintained under feed-free conditions in mTeSR (STEMCELL Technologies, 5850) or Essential 8 media (Fisher, A1517001) and stored in liquid nitrogen.

To assess pluripotency of our cohort, we compared our RNA-seq data from 102 iPSC lines to 196 iPSC lines from the HipSci project (human induced pluripotent stem cell initiative) of the Wellcome Sanger Institute and EMBL (Expression Atlas ID for dataset: E-MTAB-4748)[40]. The HipSci dataset also contained 5 fibroblast samples and 4 PBMC samples for control. Expression from the HipSci project was publicly available as an expression matrix with expression tabulated as transcripts per million (TPM). To enable equal comparison, we used our raw RNA-seq data to tabulate TPM for our cohort (tabulated using DESeq2). (Note that we used salmon-aligned

[ensemble90] RNA-seq data versus STAR, as this initial quality control assessment of the biobank was done prior to designing our subsequent RNA analysis workflow.) The joint HipSci-Stanford TPM dataset was log2 transformed. Stanford iPSC lines were all derived from blood while HipSci lines were derived from either blood or skin tissue, both of which are from the mesoderm lineage. We selected both pluripotency and mesoderm genes for examination based on the iPSCORE resource (genes taken from Figure 2A of the iPSCORE paper).[41] The pheatmap package in R was used to generate a heatmap (samples and genes clustered using Euclidean distance). We confirmed our iPSC cohort exhibited similar expression profiles as the HipSci iPSCs and did not cluster with PBMC samples (Figure S1).

Cardiomyocyte differentiation and drug treatment

As previously described,[42] iPSCs were plated on Matrigel and cultured in StemMACS iPS-Brew XF (MACS Miltenyi Biotec, 130-104-368) until the final passage in Essential 8 media (Fisher, A1517001). Cardiomyocyte differentiation was induced at 60-80% confluency, with culture in RPMI media (Gibco/LifeTech 11875-119) plus B27 supplement lacking insulin (Gibco/LifeTech A1895601). 6μM of CHIR-99021 (Fisher, NC0976209) was added on day 0 and 6 μM IWR1 (Fisher, NC1319406) was added on day 3. Beginning on day 7, media was changed every other day using RPMI media supplemented with B27 containing insulin (Gibco/LifeTech 17504-044). Upon commencement of beating (around day 15), cells underwent purification via a three-day glucose starvation (RPMI media without glucose [Gibco/LifeTech 11879-020] supplemented with insulin-containing B27), a one-day recovery in glucose-containing media, and subsequent replating (dissociated in TrypLE, Fisher, 50-591-353). Cells were then maintained in RPMI media supplemented with insulin-containing B27 until approximately day 30. After differentiation, drug treatment occurred at 0 hours and 24 hours and samples assayed at 48 hours. Cells were treated with 250nM MYK-461 (Cayman Chemical, 19216-5mg), 400nM or 1uM omecamtiv mecarbil (Selleckchem via Fisher, NC1069600), or DMSO.

Additionally, at approximately day 24, one to three wells of the ongoing differentiation were replated (dissociated with TrypLE) into 96-well plates for immunohistochemistry (two wells, ~40,000 cells/well) or 384-well plates (Thermo, 142761, ~20,000 cells / well) for contractility assays and maintained in parallel until the end of differentiation. Cardiomyocytes were analyzed by immunohistochemistry to assess purity as previously described,[42] via staining for cardiac troponin T (Rabbit cTnT, Abcam, ab45932, 1:100). Cells were imaged on the Cytation5 Image Reader (BioTek) running the accompanying software (Gen5 Image+ version 3.03) to screen differentiations for a minimum of 90% cTnT positive cells.

Whole genome sequencing

Library preparation and sequencing was performed by Macrogene (first 10 samples) and Novogene on genomic DNA we extracted from iPSC cells (Qiagen DNeasy kit). Paired-end 150bp reads were acquired on the Illumina HiSeq X Ten for a minimum of 90 gigabases of data. Reads were processed using Sentieon's FASTQ to VCF pipeline (Sentieon version 201808.07).[43] This pipeline is a drop-in replacement for a BWA[44] plus GATK best-practices[45] pipeline for germline SNVs and indels, but has been highly tuned for optimal computational efficiency. BWA alignment to hg38 was followed by deduplication, realignment, base quality score recalibration,

and variant calling to generate g.vcf files for each sample. Coverage was assessed (GATK version 3.7) (Tables S2 and S3). Individual sample g.vcf files were joined and variant quality score recalibration performed.

Curation of candidate pathogenic mutations

To manually curate pathogenic and likely pathogenic variants we first created an overly-broad list of potential cardiomyopathy genes (referred to as our "panel genes" in the figures) (Table S4). The rationale was to include genes posited to play a role in cardiomyopathy, even where the data supporting a causal role was sparse to create a more comprehensive list of candidate mutations that we then filtered further. This included genes from six clinical genetic testing panels for HCM and DCM, the American College of Medical Genetics (ACMG) recommended list of genes to test for in HCM or DCM,[46] any gene annotated for HCM, DCM, or LVNC in the Human Genome Mutation Database, and genes evaluated for HCM or DCM pathogenicity in two systematic studies from the literature.[5, 6] We used ANNOVAR[47] to apply various filters, generating different pools of mutations (Figure S2) for manual interpretation.

Others have suggested a maximum minor allele frequency of 1×10^{-4} for cardiomyopathy.[5] For pool 1, we set a more inclusive filter for a minor allele frequency less than 0.01, which is the threshold for a rare variant, (frequency in ExAc, version November 2015), and required the variant be an exonic (excluding synonymous SNVs) or splicing mutation or have a CADD phred score greater than or equal to 20. Thus, pool 1 represents rare variants with the potential to alter protein sequence in our “panel genes”. For the sake of thoroughness, we also sought to capture mutations regardless of their likeliness to alter protein sequence if they were rare enough. These were curated separately in pool 0. For pool 0, we filtered for variants with a minor allele frequency less than or equal to 0.001 in ExAC or 1000 Genomes (version August 2015). Pool 0 (15.9 million mutations) and pool 1 (6082 mutations) were too large to examine manually. We thus further filtered for a ClinVar designation of pathogenic or likely pathogenic (for any disease) as curated by ANNOVAR (and thus a reflection of the latest ClinVar information in the ANNOVAR database). We found a large number of rare GATA4 variants in introns (933 mutations) or untranslated region (270 mutations) that had been flagged in ClinVar for congenital heart disease (and not cardiomyopathy). After removing these for lack of relevance to HCM and DCM, we had 159 mutations in pool 5. We call pool 5 “WGS_P” for pathogenic, to demarcate this filtering strategy was dependent on a pathogenic or likely pathogenic ClinVar designation. These represent our first strategy for filtering for candidate variants. We evaluated each of these manually and with CardioClassifer, an online research tool for annotating pathogenicity of cardiomyopathy mutations.[48] However, we then went back and applied additional filtering strategies to overcome some of the technical limitations of this strategy. Below is a brief description. See Figure S2 for the full filtering workflow.

The first complication we addressed was that our variant calling workflow had the potential for a larger indel to be miscategorized as two neighboring smaller indels or SNVs. We thus created pool 10 to merge nearby mutations and evaluate the resulting larger mutation for pathogenicity. This step was performed only for the diseased samples and not the control subjects. We started by flagging any mutation within 40 bp of another mutation in the same subject (365 mutations). We

removed individual indels greater than 50 bp since this could have represented a sequencing error. (This was applied before merging neighboring mutations). For SNVs, we merged SNVs if they occurred within 2 bp of each other (ie could be on the same codon, and thus their expected effect on protein sequence would only be properly determined when analyzed together). We also merged SNVs within 5 bp of an indel to expand the indel. We then confirmed that the neighboring mutations had the same zygosity and were on the same allele, thus justifying our analysis of them in tandem. We call pool 10 "WGS_merge' to indicate it represents manually merging of nearby mutations.

The second complication we addressed is that our first filtering strategy was dependent on ClinVar flags. This could lead to many false negatives due to many variants not being listed in ClinVar. We thus took any of the pool 1 variants (rare variants with the potential to alter protein sequence of "panel genes") that hadn't had a ClinVar flag and kept them in the analysis if they met the more stringent allele frequency of less than 0.001 (pool 13). Note that for Pool 13, unlike the previous frequency filters, here we used the maximum frequency in 1000 Genomes and any individual ethnic group in ExAc (to screen out mutations that while rare in genomic datasets as a whole, are more abundant in specific ancestral backgrounds). We needed to further curate pool 13 to a list that was feasible for manual evaluation. We applied two separate additional filters. First, we kept any mutation in pool 13 that was in a gene for which the CardioClassifer tool could be applied, given that this overcame the technical limitation of manual curation and would allow us to first screen mutations via the tool. This created pool 14. (CardioClassifier is an expert-developed tool incorporating cardiomyopathy specific knowledge to apply ACMG guidelines.) The CardioClassifier genes for HCM are MYH7, TNNT2, TPM1, MYBPC3, PRKAG2, TNNI3, MYL3, MYL2, ACTC1, CSRP3, PLN, TNNC1, GLA, FHL1, LAMP2, and GAA. The CardioClassifier genes for DCM are LMNA, TNNT2, SCN5A, TTN, TCAP, MYH7, VCL, TPM1, TNNC1, RBM20, DSP, and BAG3. For LVNC we used the 12 DCM genes. The CardioClassifer genes for long QT syndrome were KCNQ1, KCNH2, SCN5A, and KCNE1. For pool 14, we required that the mutation fall in a CardioClassifer gene associated with the disease of the subject. We call pool 14 "WGS_freq" to indicate these are mutations that lacked a ClinVar flag but were kept in the analysis due to their low frequency.

Given that truncating variants can have an especially dramatic effect on protein sequence, we separately evaluated pool 13 for mutations that may change the length of the protein sequence to create pool 15. For pool 15, we included stop-gain, stop-loss, frameshift insertion, or frameshift deletion mutations. (Note that for stop-loss and frameshift insertions, they could act to increase protein sequence rather than truncate.) We removed indels greater than 50 bp due to the possibility they represent sequencing artifact. There were 95 mutations, but removing those already identified in pool 14 left 46. We call pool 15 "wgs_trunc" for truncation, to indicate they may alter protein length. For variants most likely to be pathogenic truncating variants (heterozygous, stop-gain mutations) we performed additional characterization, using the RNA-seq data from the iPSC-derived cardiomyocytes where available. First we used our combat-corrected processed data (see Supplemental Methods section for RNA-seq) to compare gene expression in the mutation-carrying line to the other cardiomyopathy (HCM or DCM depending on the disease of the mutation-carrying line) or control lines. Second, we re-processed the RNA-

seq fastq files to get allelic expression via STAR, setting the waspOutputMode as SAMtag and inputting a vcf file for the line containing the mutation of interest.

Pools 14 and 15 generated candidates with less definitive annotation data. Thus as a control to provide confidence on the likeliness for false positives, we applied the same filters to the control subjects to evaluate the rate of detecting mutations with these filters in a cohort that should have few true pathogenic or likely pathogenic mutations (pool 16). We filtered for CardioClassifier's "cardiomyopathy" gene list: ACTC1, BAG3, CSRP3, DSP, FHL1, GAA, GLA, KCNE1, KCNH2, KCNQ1, LAMP2, LMNA, MYBPC3, MYH7, MYL2, MYL3, PLN, PRKAG2, RBM20, SCN5A, TCAP, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL. We call pool 16 "WGS_healthyfreq" and pool 17 "WGS_healthyTrunc" to indicate it is the same filters from WGS_freq and WGS_trunc applied to the control subjects.

We also pulled any variant listed in the electronic medical record (EMR). For many of these we had already assessed pathogenicity as part of our WGS workflow. However, some variants in the EMR had not passed our WGS filters and had not been annotated yet. We collected these into pool 8 for evaluation. Often, pathogenicity classification for the variant was provided in the EMR, however we always classified them ourselves as well in case the original annotation pre-dated new information in the literature. We call pool 8 "Clin_research" to indicate they are variants that came from the clinical genetic testing for which we needed to research their potential pathogenicity.

Our "final pool" represents all the mutations from all of these filtering strategies. For any variant in our final pool that was only found in WGS data and not listed in the EMR (not clinically validated to be present in the subject's genome), we further examined the mutation in the vcf file for quality metrics to confirm confidence that the mutation was present. The final pool became Table S5. Column K indicates which filtering strategy resulted in identification of the mutation. Note that if a mutation was identified from our first filtering strategy "WGS_P" it will be listed as such in Column K. Even if the variant is truncating or rare, it won't be listed as "WGS_trunc" or "WGS_freq" because these additional filtering approaches were not necessary to identify the mutation. Thus column K represents the minimal filtering we needed to identify the variant.

Comparison of mutation burden in cardiomyopathy genes with echocardiogram measurements
We first analyzed the distribution of pool 1 variants (Figure S2) between HCM, DCM, and control lines. We found six samples (control lines 820, 822; HCM lines 543, 598; DCM line 596, 969) were outliers (z score > 3) for having a large number of pool 1 variants. Thus the subsequent analysis of mutation types in the control, HCM, and DCM cohort were done on the full cohorts and after removing these six samples to ensure there were no differences in the results. Starting with the pool 1 variants, we removed mutations with frequency > 0.001 in 1000 genomes or any ExAc ethnicity. (Previous ANNOVAR filter used to generate pool 1 used the mutation frequency in ExAc as a whole, while here we used the maximum frequency in any ethnicity.) We removed indels > 50bp as these could be due to a sequencing error. We removed mutations shared by more than 10 patients. (Only 3 mutations fit this description. They were shared by 171, 46, 31 patients). The next most common mutations were shared by 7 patients. This is also the max frequency for a

mutation we annotated as pathogenic or likely pathogenic. Finally, we grouped the mutations into two categories. The first category was mutations that could change protein length (frameshift insertion, frameshift deletion, stop-gain, stop-loss). The second category was all other exonic or splicing mutations. For calculating mean and standard deviation values, the two "other" samples with known cardiac conditions (long QT syndrome and Fabry's disease) were excluded from the control cohort. P-values for figure 2A are calculated using t-test.

Pucklewartz et al.[16] defined a set of 102 cardiomyopathy genes whose cumulative burden of nonsynonymous SNVs correlates with LVEF in DCM. We replicated this analysis by summing the instances of a nonsynonmous SNV in the 102 genes. This was done by going back to the original Annovar output files for SNVs (we did not include indels) and using R to identify all nonsynonymous SNVs regardless of allele frequency (as opposed to starting from our pooled of filtered rare variants). We set a cutoff of DP (depth of coverage) >=8 and GQ (genotype quality) >=20. Zygosity was not incorporated. The total burden was plotted against LVEF and linear regression computed. We did this for both the HCM and DCM lines. We the repeated the analysis for DCM after setting an additional threshold of maximum allele frequency of 0.5 (using the maximum frequency in 1000 Genomes and any individual ethnic group in ExAc). This was done separately on DCM samples with a known pathogenic or likely pathogenic variant (P/LP) and those without (nopatho). Finally, we applied a further filter for the variants, restricting variants to 20 core DCM genes with greater evidence for pathogenicity (as defined by appearing in at least one of the following: [4] or DCM genes only[5, 15]). The core genes are: ACTC1, ACTN2, BAG3, DES, DSP, FLNC, JPH2, LMNA, MYH7, NEXN, PLN, RBM20, SCN5A, TCAP, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL.

To assess mutational burden in HCM samples within the promoter regions of the 102 Puckelwartz et al genes, we defined a promoter as 2000 bp upstream and 500 bp downstream of the transcription start site and collected all SNV variants (not indels) regardless of frequency and regardless of mutation type. DP (depth of coverage) >=8 and GQ (genotype quality) >=20 filters were applied. Unlike the analysis of LVEF versus coding variants in DCM, for promoter analysis we did not restrict the variants to nonsynonymous SNVs. For each gene we computed the mean number of variants in the control and HCM cohorts separately. 54 genes had higher mean in control than promoter. These were: A2ML1, ALPK3, BAG3, CACNA1C, CALR3, CASQ2, CAV3, CHRM2, CSRP3, CTNNA3, DES, DOLK, EMD, EYA4, FHL1, FKTN, GATA6, GATAD1, JUP, KRAS, LAMP2, LDB3, LMNA, LRRC10, MAP2K1, MYL2, MYOM1, MYOZ2, MYPN, NEBL, NEXN, NKX2.5, NRAS, PDLIM3, PRDM16, PRKAG2, PTPN11, RAF1, RASA1, RBM20, RRAS, SCN5A, SHOC2, SLC22A5, TAFAZZIN, TCAP, TGFB3, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TRDN, TXNRD2. We performed regression on the total mutation count in these promoters compared to IVSd for HCM samples with and without known pathogenic mutations and accounting for RNA subgroup (steep or moderate). Finally, we applied the published polygenic risk score[29] to the HCM samples. The dbSNP IDs were used to convert from hg37 to hg38 coordinates and search the Annovar output files for overlapping variants. In instances where a variant was not returned for the loci, we assumed the sample had the reference allele. For each variant we determined presence or absence of the risk allele (ignoring zygosity) and multiplied by the published beta values, summing across all variants to get the final risk score. The score is composed of 36 SNVs.

RNA-seq library preparation, sequencing, quality control, and expression matrix generation

RNA was extracted from iPSCs or cardiomyocytes (RNeasy, Qiagen). Illumina RNA-seq libraries (TruSeq Stranded Total RNA LP Gold) were prepared on the Bravo (Agilent; 3 samples prepared manually as indicated in Table S6), pooled (Table S6), and sequenced (NovaSeq-6000, paired-end, 100bp). Where possible drug treatment conditions for the same differentiation were kept together in batches, while replicate differentiations for the same iPSC lines were split apart, and HCM, DCM, and control samples were distributed across batches. Reads were aligned to hg38 (STAR). Principal component analysis on cardiomyocyte and iPS samples separately returned no outlier samples (as defined as Zscore of principal component 1 > 3). Library quality control was assessed via fastp, fastQC, STAR, and Picard metrics. Samples were flagged for poor QC by the following metrics: GC content after filtering outside of 20-80% (fastp), duplication rate greater than 40% (fastp), uniquely mapped read pairs (fragments) < 20 million (STAR), mean reads (average of forward and reverse) <20 million (fastQC), ribosomal RNA bases > 20% (Picard), coding plus UTR (untranslated region) < 50% (Picard), uniquely mapping fragments <60% (STAR). Samples with more than one flag were removed. Cardiomyocyte and iPSC samples were subsequently processed separately. Reads were computed as CPM (edgeR) and corrected for library preparation batch (combat-seq) and TMM normalized (edgeR) to generate the final expression matrix. For samples with biological replicates, TMM counts were averaged. Principal component analysis was performed and principal component 1 assessed for spearman correlation with the following metadata: percent GC content (fastp), mean reads (average of forward and reverse) in millions (fastQC), percent ribosomal RNA bases (Picard), uniquely mapped fragments in millions (STAR), duplication rate (fastp), percent coding or UTR (picard), library preparation batch, and sequencing pool. The maximum absolute value for spearman correlation between PC1 and the library metadata was 0.08 for cardiomyocyte samples, indicating good quality control with technical artifacts having minimal influence on the dataset. iPSC samples had higher correlation for three metrics (0.26 with GC content, 0.22 with duplication rate, and 0.11 with percent coding or UTR), with the remaining less than an absolute value of 0.04.

DESeq2 analysis of differential expression

Raw data was input into DESeq2 (as required for DESeq2) with library preparation batch included in the design (in line with the combat-seq correction strategy we used for generating our final expression matrix). We assessed baseline (without drug) control vs HCM cardiomyocytes and control vs DCM cardiomyocytes separately and determined significance (Benjamin-Hochberg corrected p-value < 0.05). Drug treatment was compared to DMSO using all samples regardless of disease. Geno ontology analysis for differentially expressed genes (or for *ADCY5* connected genes in the network, see below) was performed using DAVID bioinformatics,[49] with enriched ontologies defined as Benjamin-Hochberg corrected p-value < 0.05.

Personalized co-expression network construction using lionessR

Linear interpolation to obtain network estimates for single samples was performed using lioness[18, 19] implemented in R (lionessR package). This was done separately on the HCM versus control cohort and the DCM versus control cohort. First, a cohort-level network was built using the control and diseased samples. The finalized cardiomyocte expression matrix (TMM

normalized, batch-corrected) was input. The dataset was refined to the top 2000 most variable genes (greatest standard deviation between all samples, diseased and control samples combined). For the control and diseased samples separately a co-expression matrix was computed using Pearson correlation for each gene-by-gene comparison. The control matrix was subtracted from the diseased matrix to assess differential co-expression between the control and diseased cohorts, and the network was trimmed to the 200 most differential edges (LIMMA) between control and disease. Doing this for both the HCM and DCM data, we thus built two networks: an HCM network (reflecting differential co-expression between HCM and control samples) and a DCM network (reflecting differential co-expression between DCM and control samples). Personalized co-expression networks were inferred for each sample individually through an iterative process where lionessR removed one sample from the cohort, recalculated the cohort edge strengths, and determined the difference in cohort edge strength with and without the sample, and then applyied a linear model to estimate the edge weights of the sample.

Node strength calculation

Node strength represented the total weight of all edges surrounding a gene. We calculated this in two ways. For Figure 3B, this was calculated by summing the weights of all edges surrounding a gene. This was displayed by plotting the summed weight on the x-axis for different genes along the y-axis. Samples were colored from light shades (small ADCY5 node strength) to dark shades (large ADCY5 node strength) and maintained the same color when displaying nodes strengths of other genes. This was useful for visualizing the variability across our diseased cohorts. For subsequent analyses of node strength in Figures 4 and 5, we modified the calculation such that greater node strength would indicate greater difference from non-diseased samples. Each edge surrounding a gene were previously determined by lionessR to be red (stonger in disease) or green (stronger in control). This was colored based on behavior of the full cohort. To calculate node strength in each sample, we subtracted the sum of the green edges from the sum of the red edges.

Assessment of co-modulation of edges around a common hubnode

We defined hubnodes as genes that were connected to at least three other genes. We tested which hubnodes represented units of network activation, in that higher co-expression of one of the edges co-occurred with higher co-expression of the other edges. For each disease network, we analyzed the disease (HCM or DCM) and control cohorts separately. We first calculated the Pearson correlation coefficient for each edge-by-edge comparison. Second, we subset all edge-edge pairs surrounding a shared hubnode, called the “All” dataset. We also created a “background” dataset with all edge-edge pairs expect those for which the same gene was shared in both edges. We randomly sampled the All and Background datasets and calculated the difference in Pearson correlation coefficient (All – Background). We did this 10,000 times to obtain the mean and 95% confidence intervals. Nodes whose 95% confidence interval bars do not cross zero are concluded to exhibit co-modulation of the edge strengths for their surrounding edges.

Principal component analysis

The edge weights for the HCM cohort, control cohort, and individual HCM samples were analyzed by principal component analysis (prcomp, scale=TRUE, in R). Separately, the same was done for

the DCM cohort, control cohort, and individual DCM samples. Linear regression compared principal component 1 (PC1) to echocardiogram measurements (LVEF for DCM, IVSd for HCM) (Figures 4B and 4D). The contribution of *ADCY5* edges to PC1 for the HCM network (Figure 5A) was computed. For each sample, the scaled edge weights were multiplied by the loadings for PC1 and all *ADCY5* edges summed.

The relative contribution of each edge to the principal component was calculated as: (loadings^2) / sum(loadings^2). This was done for the full network (not on individual samples). Edges were ordered from highest to lowest relative contribution. Visual inspection of the list revealed enrichment of *ADCY5* edges at the top of the PC1 list for highest contribution and *ANLN* edges at the top of the PC3 list. This was confirmed by plotting the cumulative contribution (sum of relative contributions) with each successive edge added (Figure 5B) alongside the cumulative contribution specifically from *ADCY5* or *ANLN* edges only. (Note that for clarity, in Figure 5B, the cumulative contribution for either ADCY5 or ANLN was not plotted as a smooth curve, but only displayed at the *ADCY5* or *ANLN* edges.)

Pearson correlation was used to obtain R^2 values for correlation of hubnode strength to PC1 and to echocardiogram measurements and as well as to compare *ADCY5* node strength to *ADCY5* expression and *MEF2A* expression in HCM samples.

Kinetic imaging cytometry to measure contractility

Contractility was assessed as previously described.[50, 51] On approximately day 24, cells were dissociated (TrypLE, Fisher, 50-591-353) and replated in Matrigel-coated 384 well plates (20,000 cells/well, 8 wells per drug condition), and maintained in parallel for the remainder of the differentiation. The four perimeter rows and columns of wells were not used, and filled with PBS to minimize the effect of temperature fluctuation on the assay. At the time of assay, 400nM tetramethylrhodamine, methyl ester (TMRM, Marker Gene Techonologies), a live cell stain of mitochondria, was added to the cardiomyocyte cell culture, and the cells were returned to the 37°C incubator for approximately 15 min to restabilize their temperature. Contractility was analyzed on the IC200 Kinetic Imaging Cytometer (Vala Sciences) running CyteSeer software (Vala Sciences), using a 0.75 NA 20x Nikon Apo VC objective. Cells were maintained at 37C°, 5% CO_2 throughout the assay. Time series images were acquired over 10 second at 33 Hz frequency. A custom MatLab script was used to further process the outputs of CyteSeer and extract key metrics of contractility, including averaging multiple contractions per well into a representative peak and extracting the area under the curve (AUC) as well as average time between peaks (T.peak) .[52] AUC divided by T.peak represented the total amount of deformation normalized to time. Each differentiation was assayed in 8 wells per condition (DMSO and drugs).

***ADCY5* hub node correction with drug treatment**

For each edge surrounding *ADCY5* in the HCM and DCM networks respectively, we calculated the mean edge weight in disease at baseline and after drug treatment as well as in control samples at baseline. We computed the difference as such: HCM_{DMSO} – $Control_{DMSO}$; HCM_{MYK} – $Control_{DMSO}$; DCM_{DMSO} – $Control_{DMSO}$; DCM_{OMEC} – $Control_{DMSO}$. We then converted the differences to absolute value. Box and whisker plots displayed these values for all edges in each comparison.