Supplemental Materials

Myocardial Recovery in Recent Onset Dilated Cardiomyopathy: Role of *CDCP1* and Cardiac Fibrosis

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Expanded Materials & Methods

GWAS analysis Patient DNA samples (n=859) were genotyped using Illumina Human 610-Quad BeadChips, as described previously.[13-15] A total of 851 samples passed quality control after removing 8 samples for the following reasons: 4 failed to be genotyped, 2 samples had inconsistent reported gender and genomically determined sex, and 2 samples had low heterozygosity. After excluding patients who had missing clinical information and had not been treated with ACE inhibitors or ARBs and β-blockers (n = 81) or who were not of European ancestry based on genotype analysis (n =84), 686 patients were included in the final GWAS analysis. Genotypes were then used to impute loci not genotyped but found in the Haplotype Reference Consortium Panel[57] using Minimac3 via the University of Michigan Imputation server,[58] dosage R^2 QC metrics were applied to imputed SNPs, and SNPs were discarded with dosage R^2 < 0.5. Imputed estimated allele dosage was used for all analyses. Change in LVEF after drug treatment was used as a phenotype. Approximately 7.87 million observed and imputed SNPs were analyzed using PLINK linear regression, with adjustment of co-variates (baseline LVEF, sex, age, time to follow-up echocardiogram and recruiting site).

Specifically, SNPs with call rates < 98% and samples with call rates < 95% were removed. Reported sex was compared to sex based on X & Y chromosome genotyping. The rate of homozygosity on the X chromosome will be compared to expected based on reported sex, specifically accomplished using the PLINK---sex-check option. As well, call rate of the Y chromosome was incorporated into sex-based QC. Levels of autosomal heterozygosity were compared to what would be expected. The degree of relatedness amongst samples was checked via IBD allele sharing (specifically using---genome option of PLINK). Next a set of SNPs in minimal LD was selected to determine sample race/ethnicity and create covariates to account for population stratification. The STRUCTURE program was used to cluster individuals into 3 clusters using 1000 Genomes Projects[59] samples as anchor samples to help determine race ethnicity. Since the clustering is heavily weighted by Caucasian, African and Asian samples from the 1000 Genomes Projects, study samples, the groups and associated probabilities were assumed to be Caucasian, African and Asian ancestry. Individuals with > 70% estimated probability of Caucasian ancestry were kept for analysis. To further address population stratification, Principal Components Analysis (PCA) was used on the LD thinned SNPs to construct Principal Components (PCs). These PCs are assumed to measure sub-population stratification, and none of these PCs

were found to be associated with LVEF after accounting for co-variates (baseline LVEF, sex, age, time to follow-up echocardiogram and recruiting site), so ultimately these PCs were not included in the GWAS analysis. See **Major Resource Table** for source of code used for data analysis.

Cell culture Cryopreserved adult human cardiac fibroblasts (HCFs) were purchased from two different commercial origins (see **Supplementary Table S1**). Cell culture and subculture were performed following the manufacturer's Instructions. In brief, primary HCFs were cultured in a 37°C, 5% CO_2 humidified incubator. Culture media were changed every 48 hours. The cells were subcultured when the HCF were 80% confluent. HCFs at passages 2-4 were used to perform the *in vitro* experiments. Results were repeated or validated in HCFs of both commercial origins.

Reporter gene assay. The luciferase reporter gene assay has been described in previous study.[56] Specifically, to generate a control plasmid for *CDCP1* transcriptional activity, a 925 base pair (bp) DNA segment that included the *CDCP1* promoter was subcloned to the 5'-end of the *Luc2* gene (encoding firefly luciferase) in the *pGL4.10* vector (cat#: E6651, Promega). To test the effect of the rs6773435 SNP locus on *CDCP1* transcription, an approximately 1.4-kb DNA segment that included the SNP locus was subcloned upstream of the *CDCP1* promoter. Genomic DNA from lymphoblastoid B-cell lines (LCLs) with known genotypes for the SNP of interest was used as template for PCR reactions to amplify DNA fragments containing wild-type (G) or variant (T) SNP alleles. After confirmation of the sequences by DNA sequencing, reporter gene constructs were transfected into cells using Lipofectamine™ 3000 Reagent (ThermoFisher). A *pRL-TK* vector that expresses renilla luciferase (cat#: E2241, Promega) was co-transfected as an internal control. Cells were lysed, and luciferase activity was determined using the Dual-Luciferase® Reporter Assay System kit (cat#: E1910, Promega). Luciferase activity for constructed plasmids were normalized to the *pGL4.10* empty vector.

Transient transfection and RT-qPCR. To knock-down CDCP1 and TMEM158, cells were transfected with specific siGENOME™ SMARTpool siRNAs (Dharmacon) using Lipofectamine™ RNAiMAX Reagent (ThermoFisher). For overexpression of *TMEM158* and *CDCP1*, their open reading frame DNA sequences were cloned into a pCMV-Entry plasmid which encodes a FLAG-tagged fusion protein (OriGene Technologies, Inc). Plasmids were transfected

into cells using Lipofectamine™ 3000 Reagent (ThermoFisher). Cells were harvested for the extraction of total RNA and proteins at different period of time (depend on assays) after transfection. To determine RNA expression levels, quantitative real-time polymerase chain reaction (qRT-PCR) was performed using total RNA extracted by use of the Quick-RNA MicroPrep Kit (Zymo Research, R1051), Power SYBR™ Green RNA-to-CT™ 1-Step Kit (Applied Biosystems™, 50-591-795) on a StepOneTM PCR system (ThermoFisher). Ct values were standardized to the 'housekeeping' gene, *GAPDH*, and relative quantification was calculated by ΔΔCt.

Western blot assay. Total protein lysates from cultured cells were prepared by adding RIPA buffer, followed by centrifugation at 14,000×g for 10 minutes. Protein concentrations were determined by using the Pierce BCA Protein Assay Kit (cat#:23227, ThermoFisher). Equal quantities of denatured protein were loaded onto 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels (Bio-Rad, Hercules, CA) to separate proteins. Proteins were transferred electrophoretically from the gels to PVDF membranes (Bio-Rad, Hercules, CA) which were then blocked with 5% non-fat milk at room temperature for 1 hr. After washing with TBST, membranes were incubated with primary antibodies which were dissolved in 1% BSA prepared in TBST at 4°C overnight with gentle rocking. Following incubation, the membranes were washed vigorously three times in TBST buffer and were then incubated for 1 hr with horseradish peroxidase (HRP)-labelled secondary antibody which was dissolved in 5% non-fat milk at room temperature The SuperSignal West Dura Extended Duration Substrate (cat#:34075, ThermoFisher), a luminol-based enhanced chemiluminescence HRP substrate, was applied to the membranes, and radiographic images were captured by use of the ChemiDoc™ Touch Image System (Bio-Rad, Hercules, CA). GAPDH protein was employed as a loading control. Western blot images were analyzed by using ImageJ2for protein band intensity, which reflects protein level. For comparison of protein levels, triplicate Western blot assays were performed, and protein levels were normalized to GAPDH level of a same sample. One set of Western blot image with best quality (e.g., low background, and no smear bands), and which is most representative to the average value of quantifications from triplicate Western blot assays, was showed in Figures as representative image.

Cell proliferation assay (MTS assay). The HCF cell proliferation assays were performed with the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay Reagent (Promega) as described previously.[15] Cells were seeded in 96 well plates at an initial density of 4,000 cells/mL (100 μL/well). Cells were allowed to attach for 24 hours prior to siRNA transfection (day 0). After the addition of 25 μL of MTS buffer to each well of the 96 well plate and 3-hr incubations at 37°C, plates were read for optical density at 490 nm (OD 490 nm) and 630 nm (reference wavelength) in an Infinite M1000 PRO plate reader (Tecan AG, Switzerland). This measurement was then repeated for subsequent plates until day 5 to derive a cell proliferation curve. Experiments were independently repeated at least twice with triplicate wells at each point.

PDGF-BB and TGF-β1 treatments. Recombinant human PDGF-BB and TGF- β1 proteins were obtained from the R&D System and were reconstituted at 100 μg/mL in sterile reconstitution buffer (BSA/HCL, R&D system, RB04). Before treatment, cells were "starved" in serum-deprived media for 24 hours to remove background growth factors in full-supplemented culture media. Specifically, the HCF growth media was replaced by DMEM supplied with 1% of FBS (serum-deprived media). TGF-β1, PDGF-BB or vehicle (reconstitution buffer) were applied within serum-deprived media. Depending on the experiment, at different timepoints, stimulated HCFs were lysed in a cell lysate buffer for RNA/protein extraction and assay or were fixed using 4% paraformaldehyde for immunofluorescence staining.

Immunofluorescence staining. Cultured in chambered slides, HCFs were washed with PBS and were then fixed for 15 minutes in freshly diluted 4% paraformaldehyde at room temperature. After 30 minutes in a blocking solution (PBS with 10% goat serum, 0.3% Trition X-100), the chambered slides were incubated with specific primary antibody diluted in primary antibody diluting solution (PBS with 3% goat serum, 1% bovine serum albumin, 0.3% Trition X-100) overnight at 4°C at a 1:200 dilution. Same amount of non-specific IgG (host and isotype matched with that of specific primary antibody) was also incubated in a separate chamber, which serve as negative control staining. The next day, the chambered slides were incubated with a 1:1000 dilution of Alexa Fluor 488/594–conjugated goat anti-rabbit/mouse secondary antibody for 2 hours at room temperature. Afterwards, the chambered slides were stained with DAPI at a concentration of 0.1 μg/ml in PBS for 5 minutes at room temperature and were mounted on slides using aqueous mounting medium

(H-1400, Vector Laboratories). Images were acquired with an inverted Nikon A1R confocal microscope using NIS Elements AR 4.13 software. Negative control staining was used to exclude background staining when taking images.

RNA sequencing and pathway enrichment analysis. Total RNA was extracted with TRIzol® Reagent (ThermoFisher) and the miRNAeasy kit (QIAGEN) per the manufacturer's instructions. RNA quality control was performed before RNA-seq, which showed that the RNA integrity number (RIN) was ≥9 for all samples. RNA-seq libraries were generated using the Illumina TruSeq RNA Library Prep Kit v2 (Illumina, San Diego, CA). Paired-end sequencing 2×100bp was performed on an Illumina HiSeq 4000 with approximately 50 million fragment reads per sample. Each sample was sequenced in duplicate. RNA sequencing quality was determined with FastQC (Babraham Institute, Cambridge, UK), and the reads were aligned to hg38 using STAR. Raw counts were generated with featureCounts. Downstream differential expression analysis was conducted with the EdgeR package using R. Significantly differentially expressed genes (DEGs) were defined as having an FDR < 0.05, and fold change of ≥2.0. DEGs were then subjected to pathway enrichment analysis using EnrichR and Gene Ontology. The *p*-value for pathway enrichment was computed from the Fisher exact test which is a proportion test that assumes a binomial distribution and independence for probability of any gene belonging to any set. The adjusted *p*-value was computed using the Benjamini-Hochberg method for correction for multiple hypotheses testing. See **Major Resource Table** for source of code used for data analysis.

Enzyme-linked immunosorbent assay (ELISA) for sST2. HCFs were seeded in a 6-well plate. After reaching 80% confluence, non-targeting control (Ctrl siRNA) and CDCP1 siRNAs (siCDCP1) were transfected using Lipofectamine RNAiMAX. After 24 hours, culture medium with transfection reagent was replaced by fresh serum-free DMEM media (Corning, 10-013-CV). After another 24 hours, 70μl of conditioned medium was collected from each well for ELISA measurement using a Human ST2/IL-33R Quantikine ELISA Kit (R&D Systems, DST200) following the product manual. In brief, 50 μl of Assay Diluent RD1-63 and 50 μl of standard or conditioned medium were mixed and incubated at room temperature for 2 hours. After the washing steps, 100 μl of Human ST2 Conjugate was added to each well and was incubated at room temperature for another 2 hours. After washing, 100 μl Substrate Solution was added to each well

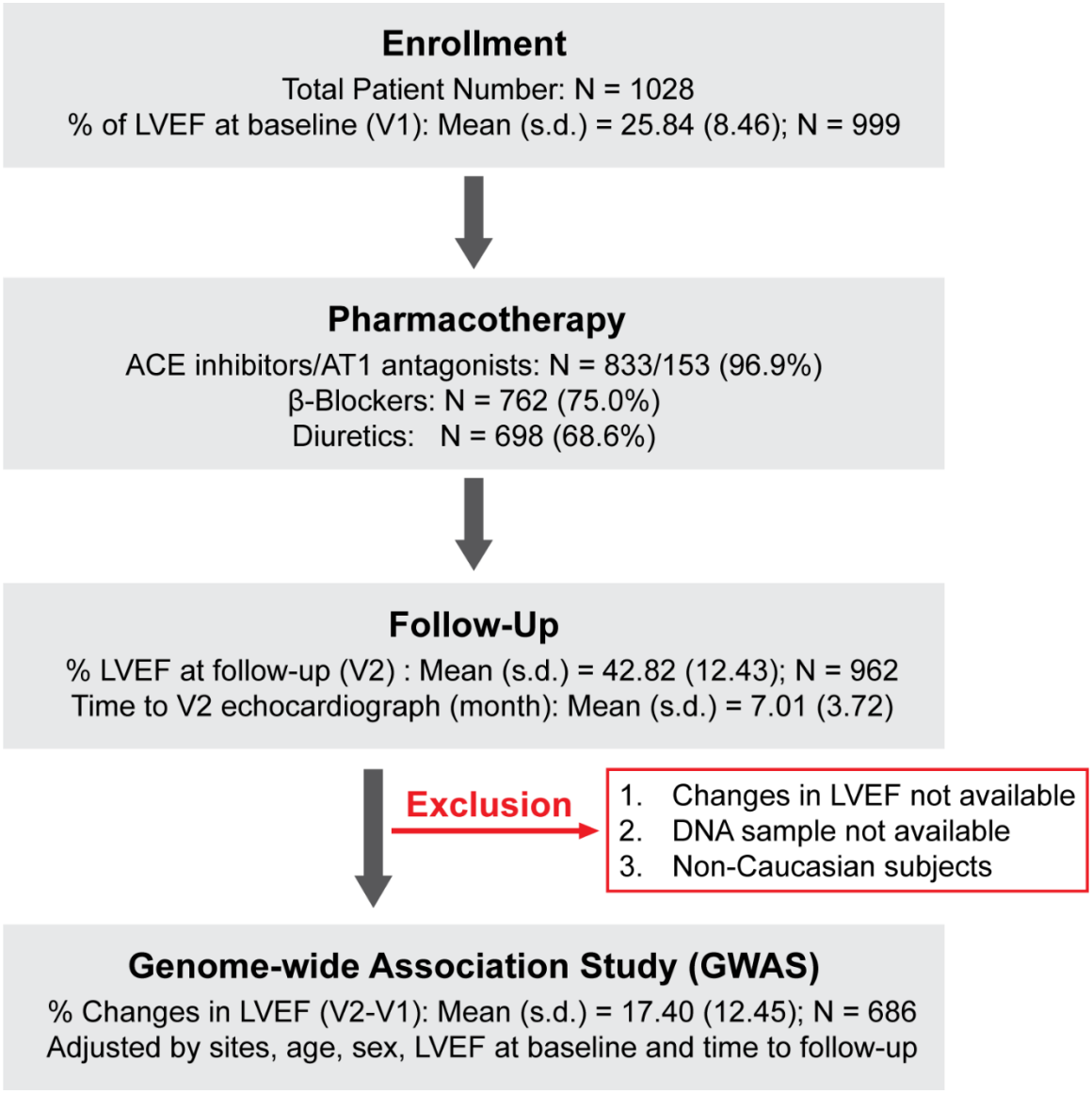
followed by incubation at room temperature for 30 minutes (protected from light). The reaction was stopped by adding 100 μl of Stop Solution and measurement of optical density was performed within 30 minutes by using a microplate reader (infinite M1000 PRO) set to 450 nm.

Drug Treatment. Ramipril, an angiotensin converting enzyme (ACE) inhibitor, and Carvedilol, a β-blocker were reconstituted in DMSO and diluted in growth media at a serial of concentrations that cover the *Ki* values for their targets. HCFs were cultured in 6-well plates with drugs treatment for 24 or 48 hours. Final DMSO concentration in each treatment condition was less than 0.1% (v/v). Total RNA samples were prepared, and RT-qPCR were applied to quantify CDCP1 mRNA level.

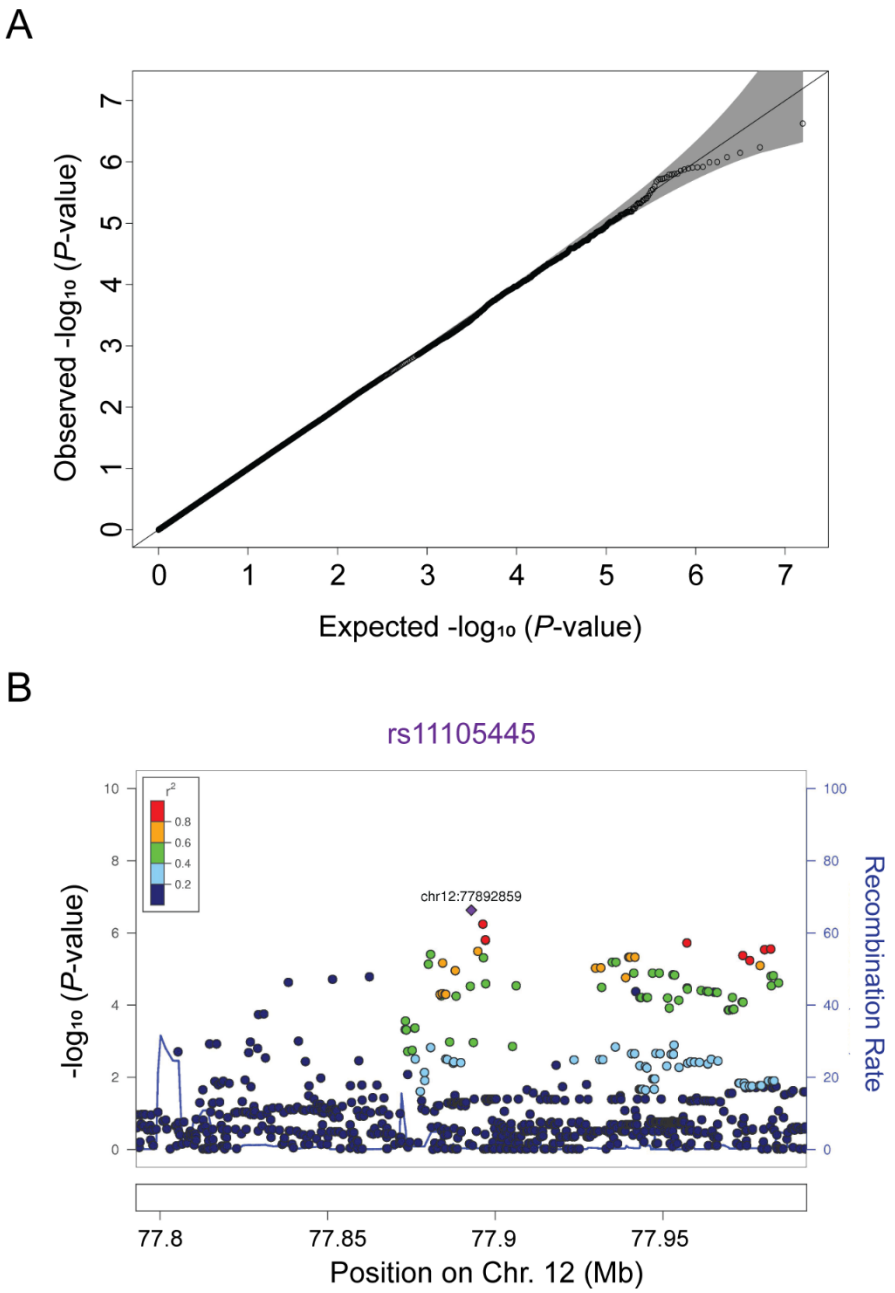
Statistics. Statistical analyses (except for GWAS and RNA-seq data) were performed by using GraphPad Prism 9 (version 9.5.0). Data collected from individual experiments were first tested by Shapiro-Wilk test for normality. When two groups of data were comparing, data passed normality test were compared by using unpaired *t* test (two-tailed), and data that did not pass normality test were compared by Mann-Whitney test. When more than 2 groups of data were comparing, data passed normality test were compared by using ANOVA with *post hoc* multiple comparisons test, and data that did not pass normality test were compared by Kruskal-Wallis test with *post hoc* multiple comparisons test for statistical significance. The specific statistical approaches used for each graph can be found in corresponding figure legends. Statistically analyzed data are presented as mean ± S.D. for all *in vitro* experiments.

Online Supplementary Figures S1-S13

Supplementary Figures S1. DCM patient recruitment and exclusion criteria. Demographic information for the study which lists patient enrollment, pharmacological intervention, patient follow-up and exclusion criteria for the GWAS analysis. N = number of patients; LVEF = left ventricle ejection fraction; V1= the time when patients first visit hospital; ACE = angiotensin-converting enzyme; AT1 = angiotensin II type 1 receptor; V2 = the time when patients were followed-up at their second hospital visiting.



Supplementary Figures S2. (A) Q-Q plot for GWAS analysis. **(B)** Locus zoom plot for the chromosome 12 SNP signal shows no gene is mapping to this SNP locus. Chromosome (Chr.) position is based on the human genome assembly GRCh37/hg19.



Supplementary Figures S3. The rs6773435 SNP is eQTL for both the **(A)** *TMEM158* and **(B)** *CDCP1* genes across human tissues (*p*-value <0.05). Data are generated by the GTEx project. NES = normalized effect size.

A

rs6773435, TMEM158 eQTLs

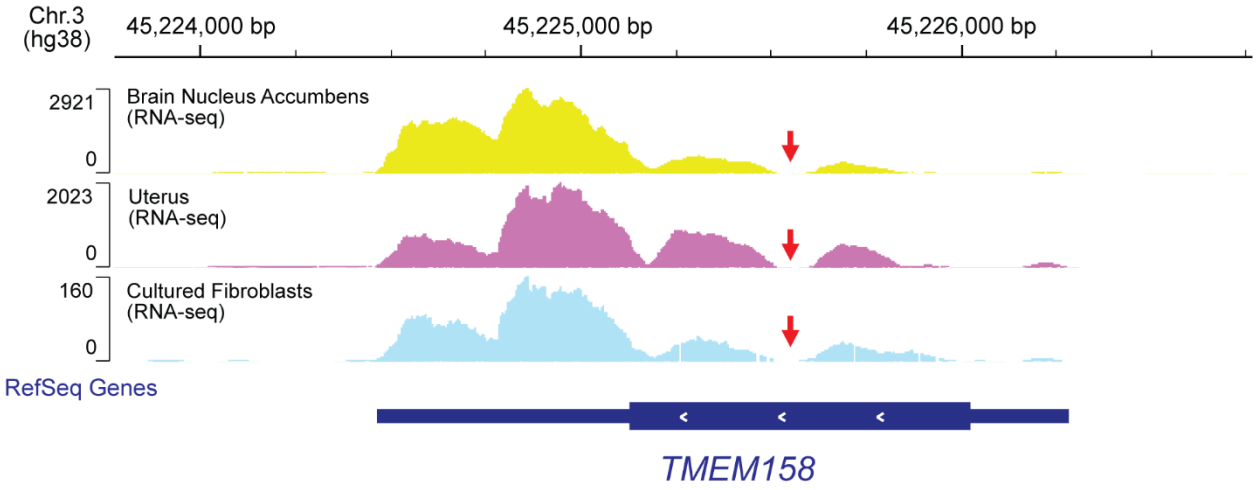
Tissue	Samples	NES	p-value
Whole Blood	670	-0.291	1.2e-8
Brain - Spinal cord (cervical c-1)	126	0.376	2.9e-4
Skin - Not Sun Exposed (Suprapubic)	517	0.179	1.1e-3
Skin - Sun Exposed (Lower leg)	605	0.154	2.2e-3
Brain - Caudate (basal ganglia)	194	0.118	4.7e-3
Kidney - Cortex	73	-0.487	0.01
Artery - Tibial	584	0.127	0.01
Artery - Coronary	213	0.254	0.01
Adipose - Visceral (Omentum)	469	-0.121	0.02
Pancreas	305	-0.177	0.03
Thyroid	574	0.124	0.05

B

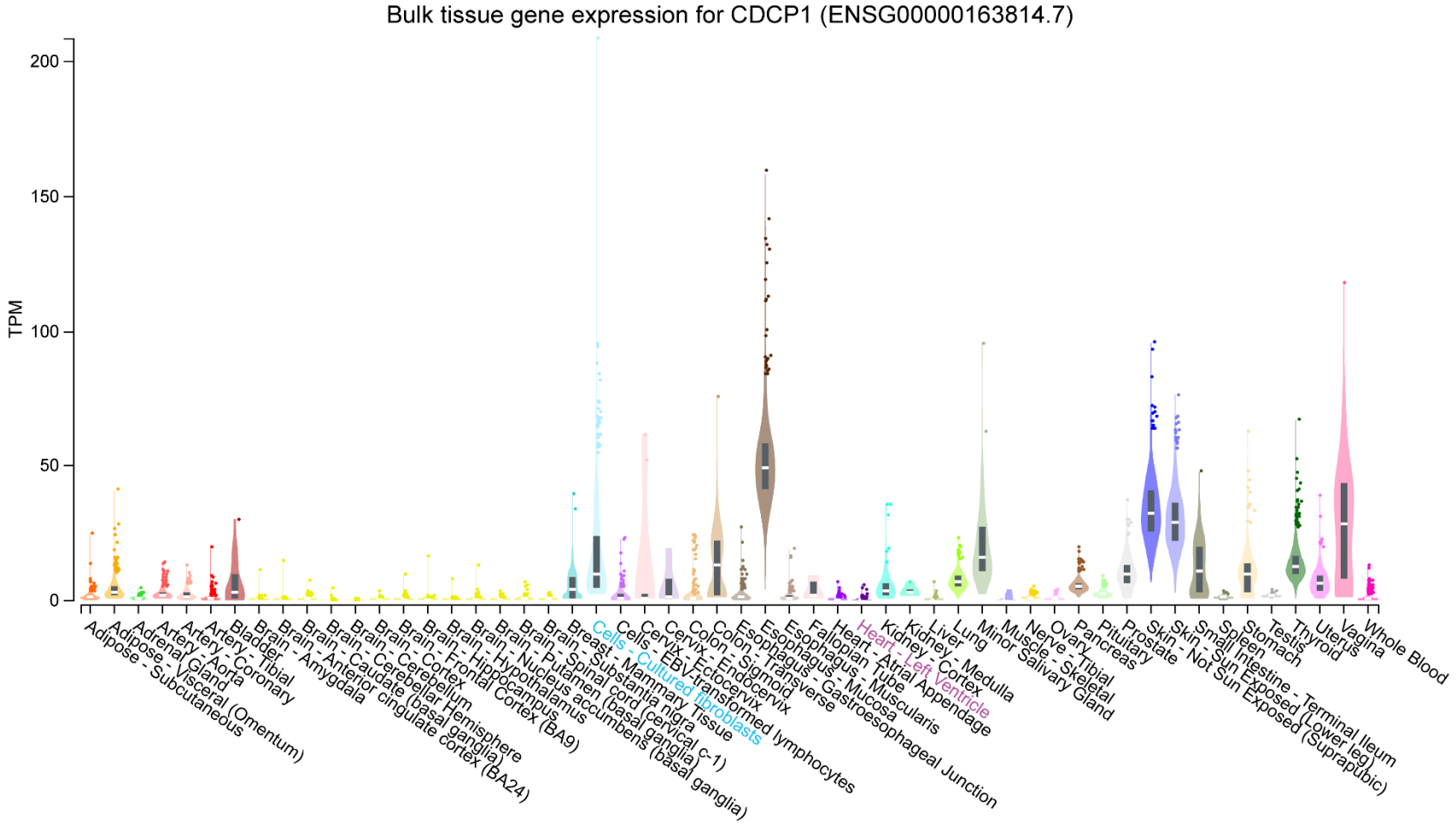
rs6773435, CDCP1 eQTLs

Tissue	Samples	NES	p-value
Pituitary	237	0.413	2.3e-5
Testis	322	0.343	1.8e-4
Nerve - Tibial	532	0.129	0.008
Skin - Sun Exposed (Lower leg)	605	0.0647	0.03
Artery - Tibial	584	0.0918	0.04
Adrenal Gland	233	0.195	0.04
Spleen	227	0.185	0.04
Colon - Sigmoid	318	0.137	0.04

Supplementary Figures S4. Visualization of the GTEx RNA-seq data in the interactive genome viewer (IGV). Panels (from top to bottom) show the physical position of the *TMEM158* gene locus on chromosome 3 (Chr.3) based on human genome assembly hg38; RNA-seq reads generated from human brain nucleus accumbens, uterus tissues, and cultured fibroblasts in which TMEM158 RNA is most highly expressed. RNA-seq reads map to the *TMEM158* gene annotated by RefSeq Genes. The *TMEM158* open reading frame (ORF) and untranslated region (UTR) were depicted by thick and thin lines, respectively. Red arrows indicate a region of the *TMEM158* ORF where no RNA-seq reads were mapped.

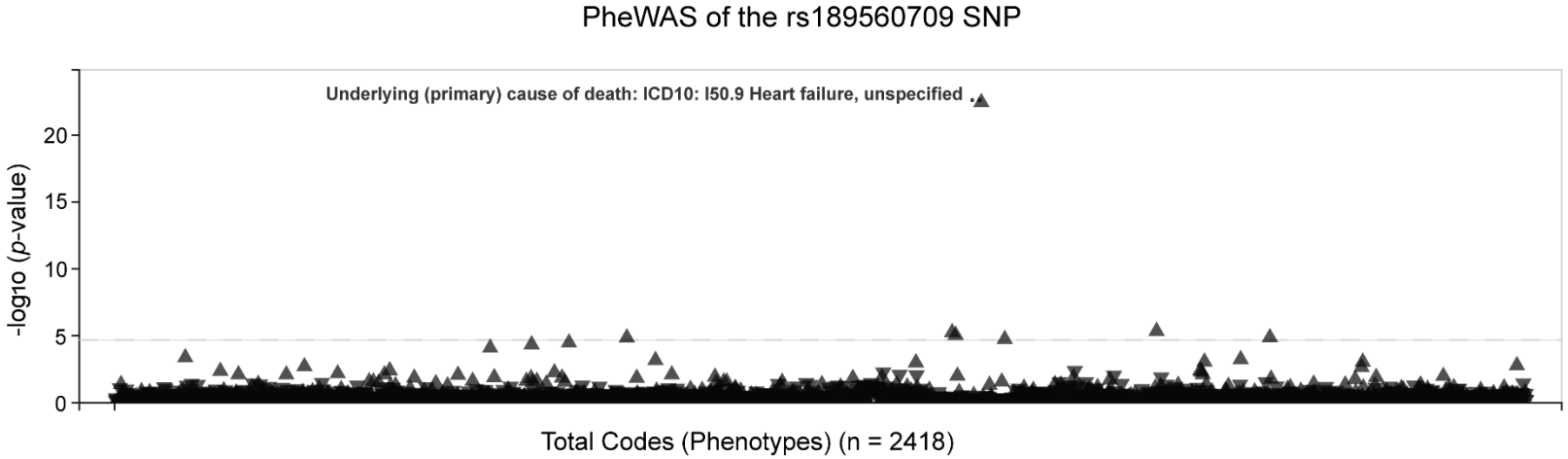


Supplementary Figure S5. CDCP1 expression in human tissues included in the GTEx Project. X-axis lists all human tissue/cell types that were included in the GTEx projects. Y-axis is the CDCP1 expression level quantified by RNA-seq. TPM = transcripts per million. Bars represent median TPM of a certain tissue/cell type from different individuals. Dots represent individual values which are outliers. Data is accessible from the GTEx Portal.



Supplementary Figures S6. SNPs in/near *CDCP1* were associated with HF Mortality. (A) Phenotype-wide association study (PheWAS) of the rs189560709 SNP genotype with 2418 phenotypes in the UK biobank dataset. Each triangle represents a phenotype. (**B**) Locus zoom plot for the *CDCP1* locus shows that the rs189560709 SNP is significantly associated with heart failure mortality in GWAS. Each dot represents a SNP. Purple diamond represents the rs189560709 SNP which is most significantly associated with the phenotype. Data obtained from the UK Biobank PheWeb.

A



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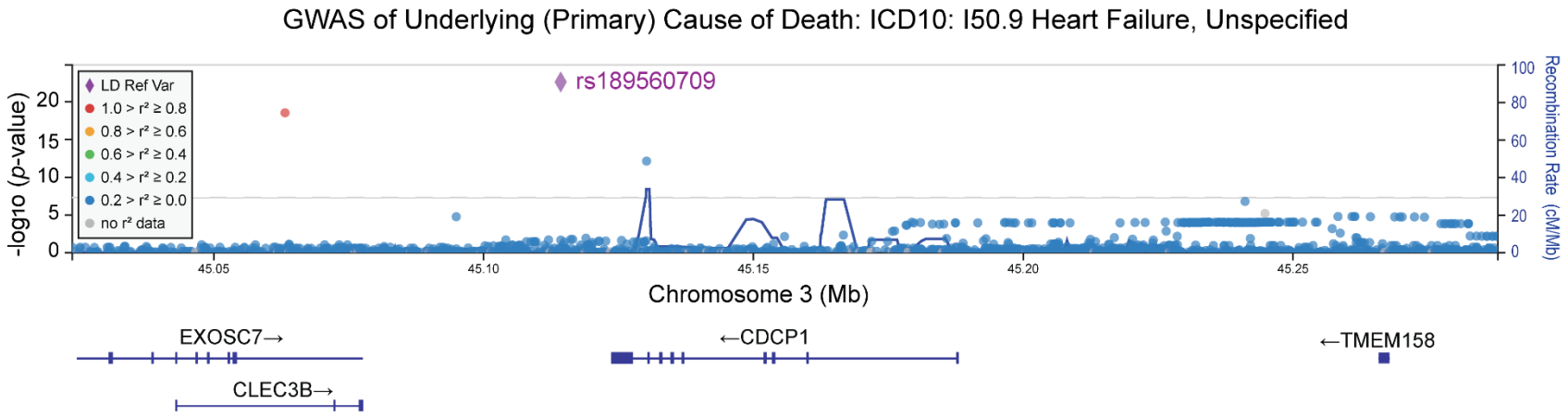
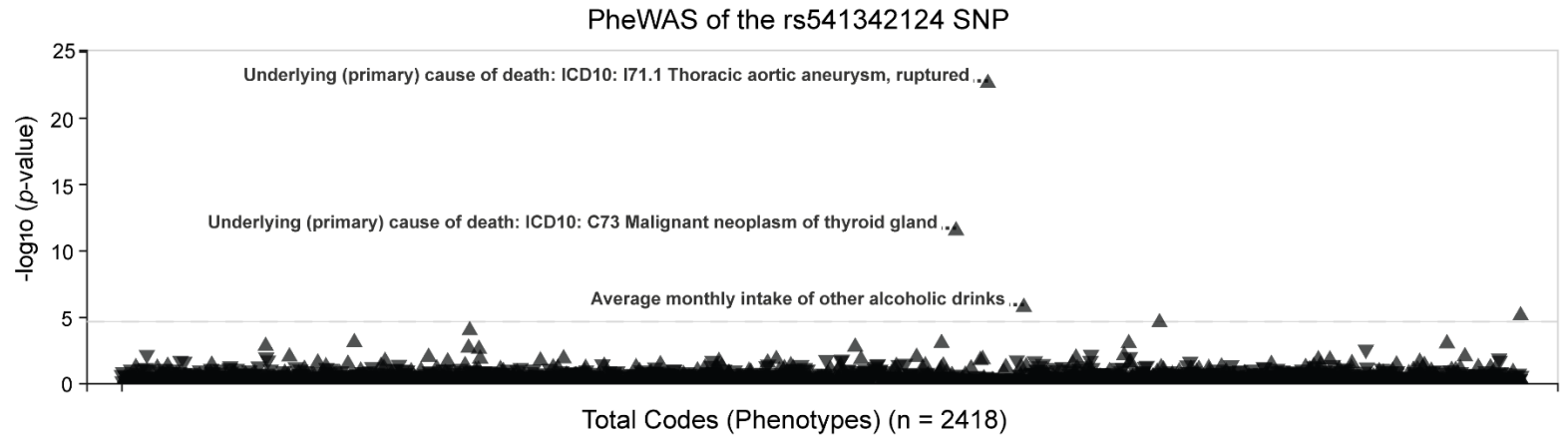


Supplementary Figures S7. SNPs in/near *CDCP1* were associated with death due to Thoracic Aneurysm Rupture. (A) PheWAS of the rs541342124 SNP genotype with 2418 phenotypes in the UK biobank dataset. Each triangle represents a phenotype. (**B**) Locus zoom plot for the *CDCP1* locus shows that the rs541342124 SNP is significantly associated with death due to thoracic aneurysm rupture in GWAS. Each dot represents a SNP. Purple diamond represents the rs541342124 SNP. Data obtained from the UK Biobank PheWeb.

A



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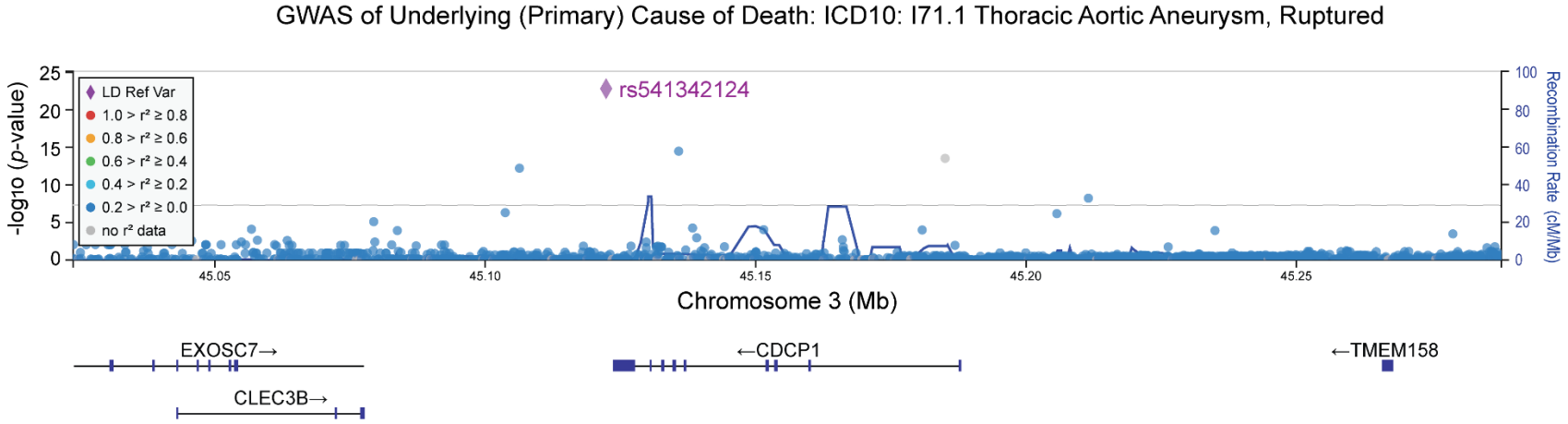
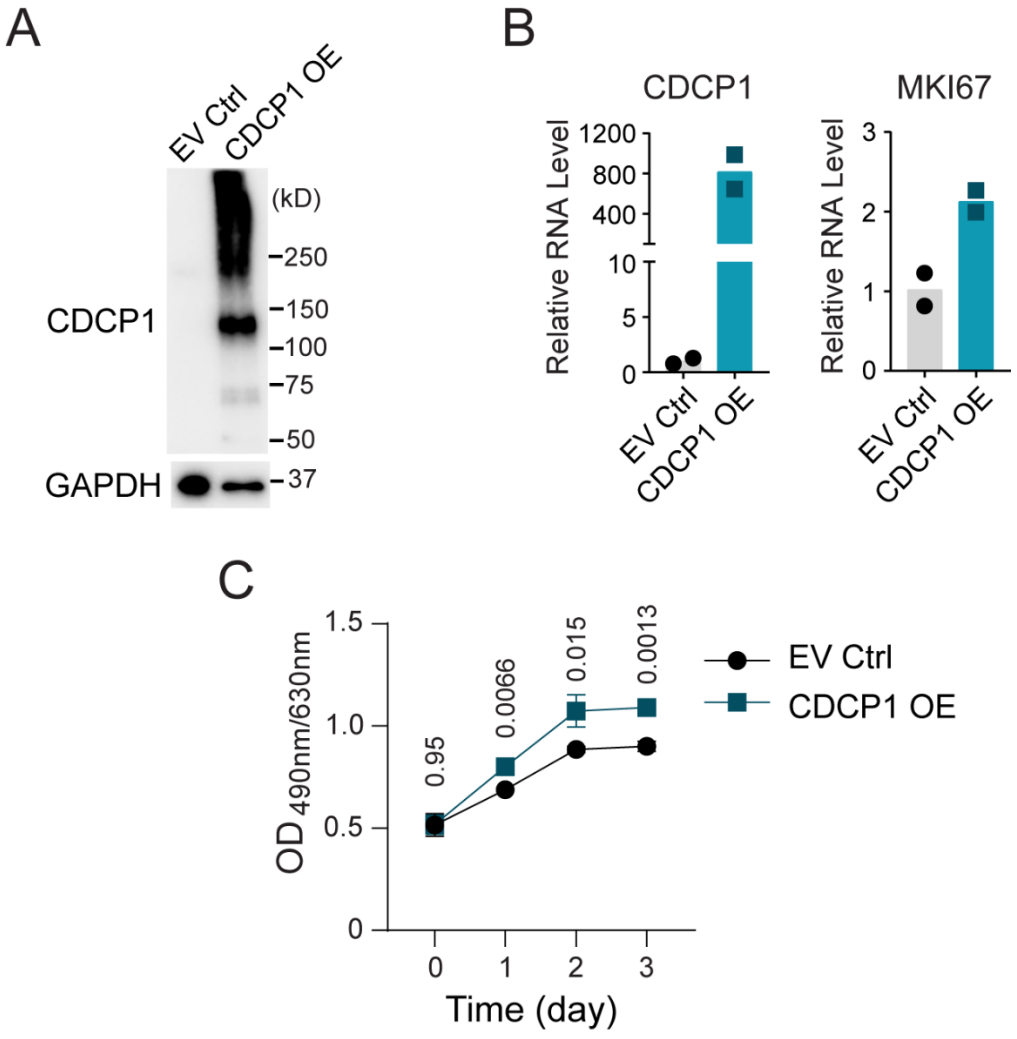
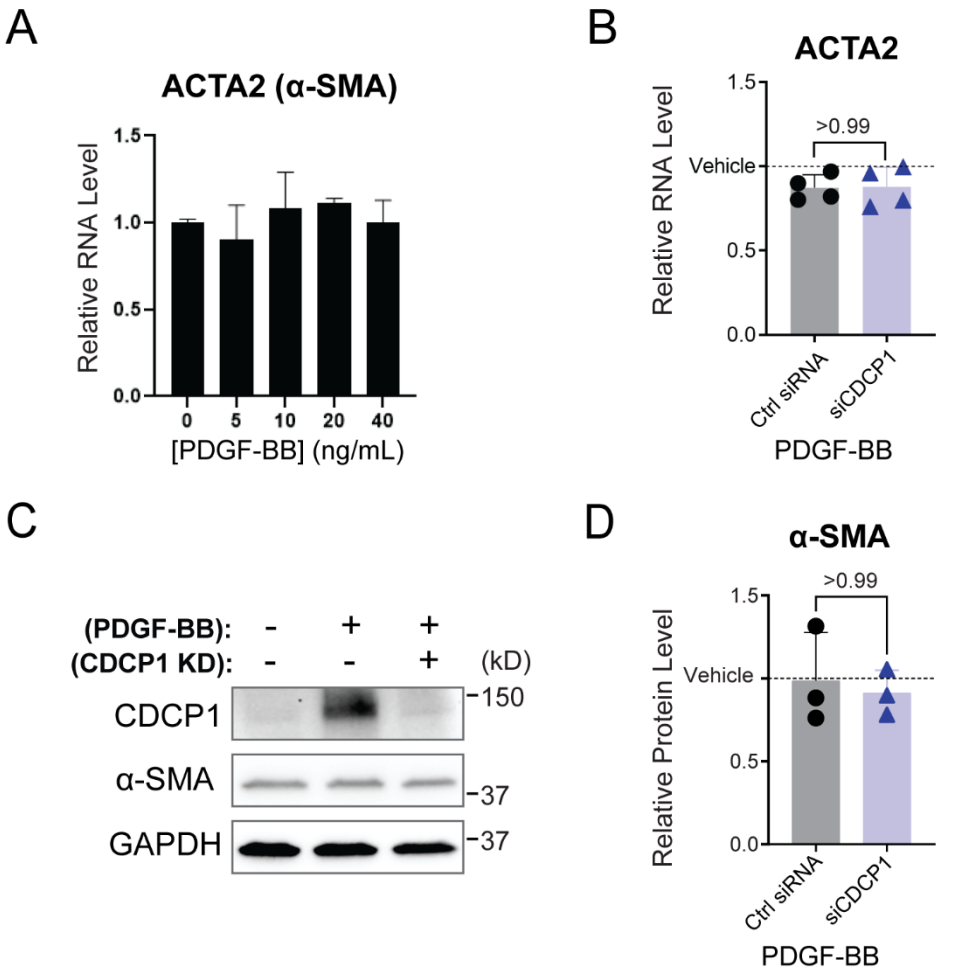


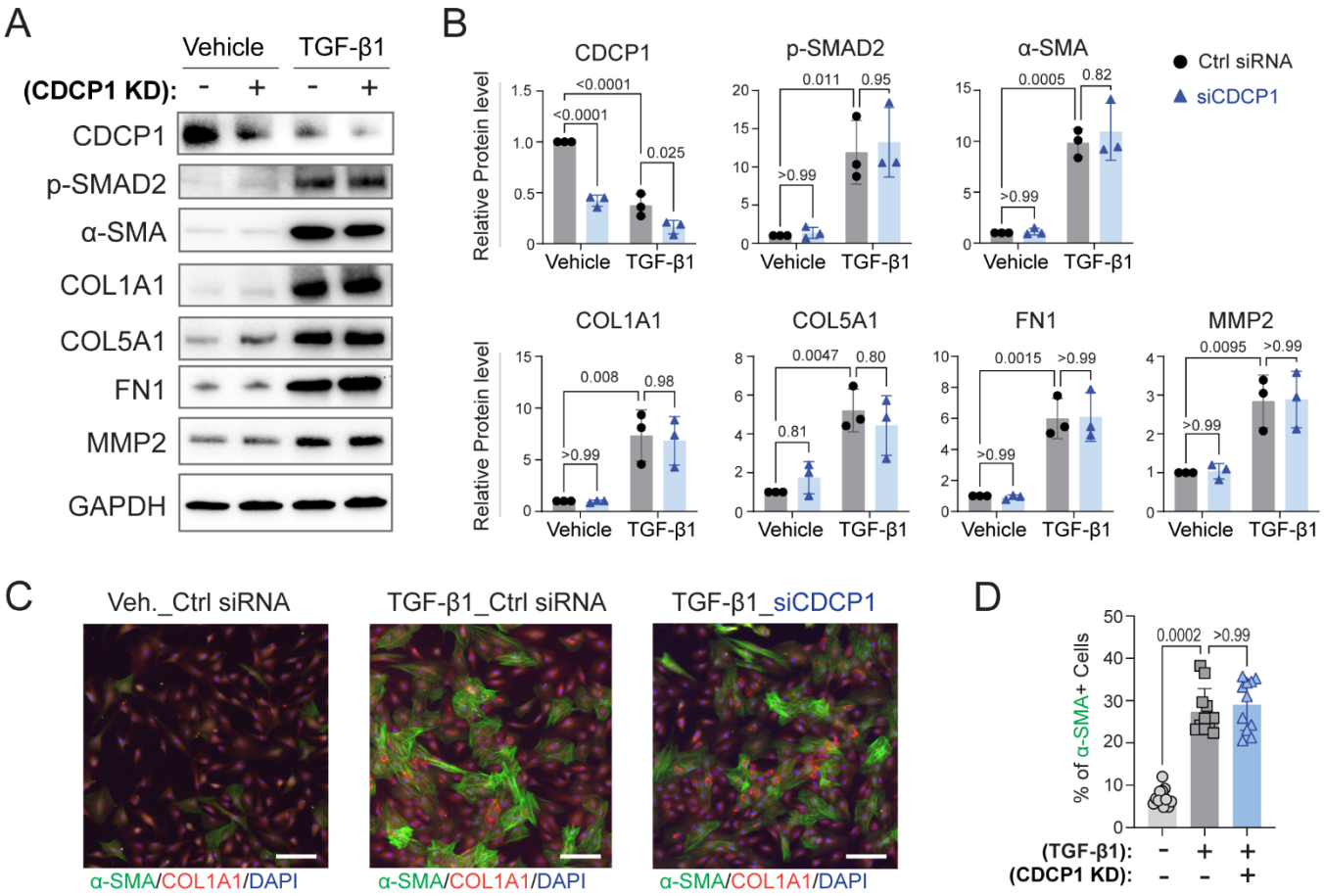
Supplementary Figures S8. CDCP1 overexpression and HCF proliferation. (A) Western blot for CDCP1 in HCFs transfected with empty vector control (EV Ctrl) and CDCP1 cDNA (CDCP1 OE) plasmids. GAPDH was blotted as internal control. CDCP1 has 14 known N-glycosylation sites. Glycosylated CDCP1 will show larger bands in Western blot, which can be easily detected in CDCP1 OE samples. (**B**) RT-qPCR quantified RNA levels for CDCP1 and MKI67 in HCFs with CDCP1 OE. GAPDH mRNA were quantified as internal control. (**C**) HCF cell proliferation measured by MTS assay every 24 hours until day 3. Each dot is a mean value for independent experiments (n=4). Error bars represent standard deviations, *P*-values were calculated by multiple unpaired *t* tests between EV Ctrl and CDCP1 OE at each time point.



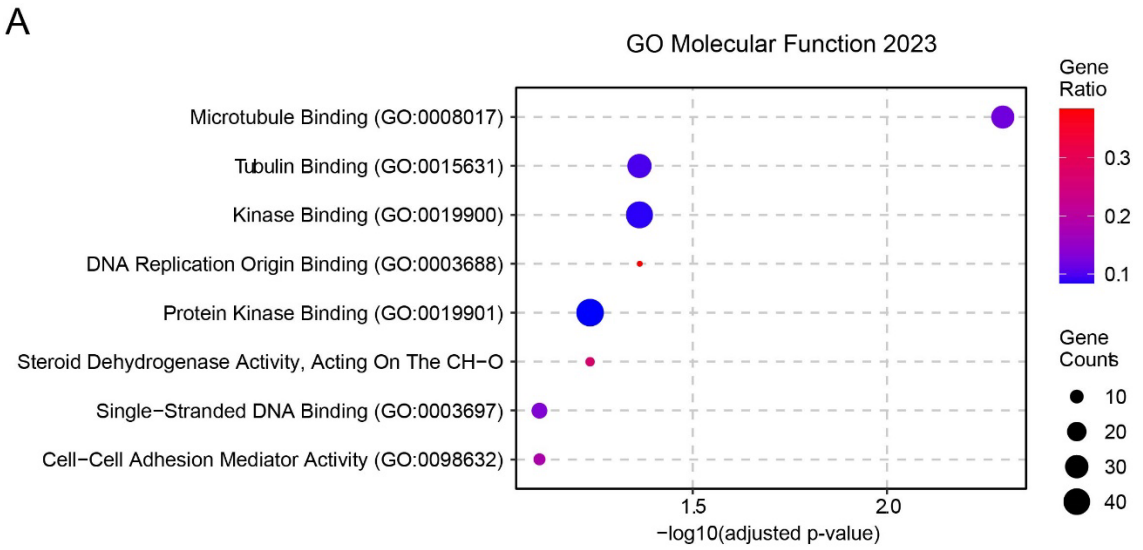
Supplementary Figures S9. Expression of ACTA2 (α-SMA) in HCFs was not affected by PDGF-BB treatment and CDCP1 KD. (A) Relative ACTA2 RNA level in HCFs with treatments of PDGF-BB at different concentrations. RNA level was quantified by RT-qPCR with GAPDH RNA level as internal control. ACTA2 RNA level was normalized to that of vehicle control treatment (0 ng/mL of PDGF-BB). Error bars represent standard deviations of triplicate assays (n=3). **(B)** Relative ACTA2 RNA level in HCFs after transfected with non-targeting control (Ctrl siRNA) and CDCP1 siRNAs (siCDCP1) for knockdown (KD), followed by 24-hour treatment of PDGF-BB at 20 ng/mL. ACTA2 RNA level was normalized to that of vehicle control treatment which showing as dotted line (n=4). (**C**) Western blot shows that CDCP1 protein level was upregulated, but α-SMA level was not changed in HCFs after 48 hours of PDGF-BB treatment. CDCP1 KD did not affect α-SMA level. (**D**). Relative α-SMA protein level quantified from triplicate Western blot assays (n=3) as showing in (C). α-SMA level was normalized to that of vehicle control treatment which showing as dotted line. All data showing were generated from independent experiments. *P*-values were calculated by Mann-Whitney test. Statistical significance was recognized when $p<0.05$.

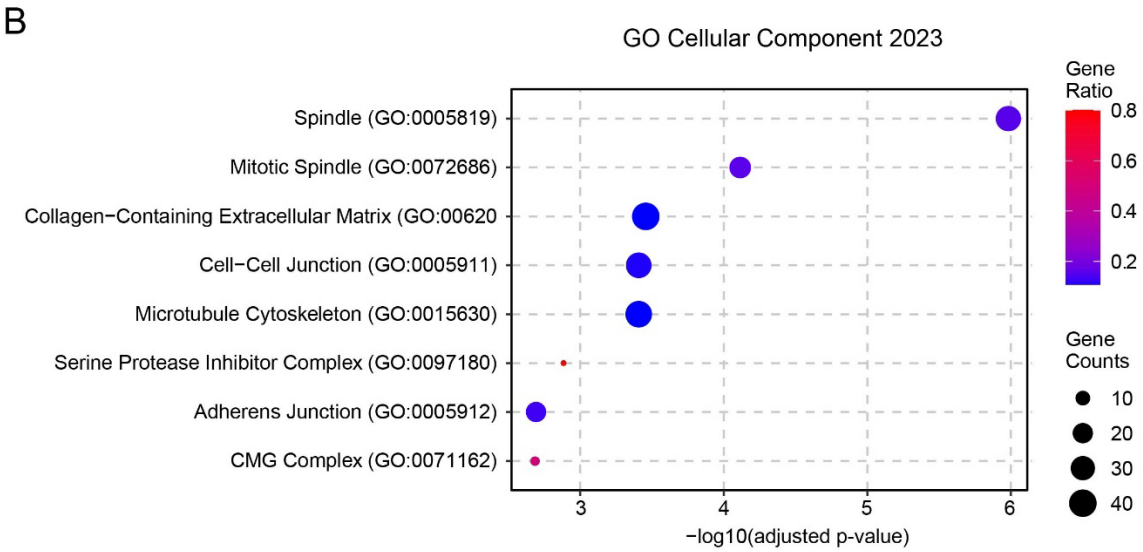


Supplementary Figures S10. CDCP1 Knock-down (KD) did not affect TGF-β1-mediated cardiac fibroblast-to-myofibroblast transdifferentiation. (A) Western blot for CDCP1 and other protein markers in HCFs transfected with non-targeting control (Ctrl; CDCP1 KD -) or CDCP1 siRNA (siCDCP1; CDCP1 KD +), and with 48-hours TGF-β1 treatment (10 ng/mL). Phosphorylated SMAD2 (p-SMAD2) and α-SMA were blotted for control of TGF-β1 treatment and myofibroblast transdifferentiation, respectively. Extracellular matrix (ECM) proteins including collagen type I alpha 1 chain (COL1A1), collagen type V alpha 1 chain (COL5A1), fibronectin 1 (FN1) and matrix metallopeptidase 2 (MMP2) were also blotted. GAPDH were blotted as internal control. (**B**) Relative protein level quantified from triplicate Western blot assays as represented in (A). Protein levels were normalized to GAPDH level and then to vehicle and Ctrl siRNA sample. Error bars represent standard deviations of independent experiments (n=3). *P*-values were calculated by ordinary one-way ANOVA with Tukey's multiple comparisons test. Statistical significance was recognized when $p<0.05$. (**C**) Immunofluorescent (IF) staining of α-SMA and COL1A1 in HCFs after CDCP1 KD and 48-hour treatment with vehicle (Veh.) or TGF-β1. Scale bars represent 200 μm. (**D**) Percentage of α-SMA positive (α-SMA+) cells based on the IF staining as represented in (C). Total cell number was counted by DAPI staining. For each condition, α-SMA+ cells were counted from 12 different fields in triplicate wells. *P*-values were calculated by Kruskal-Wallis test with Dunn's multiple comparisons.

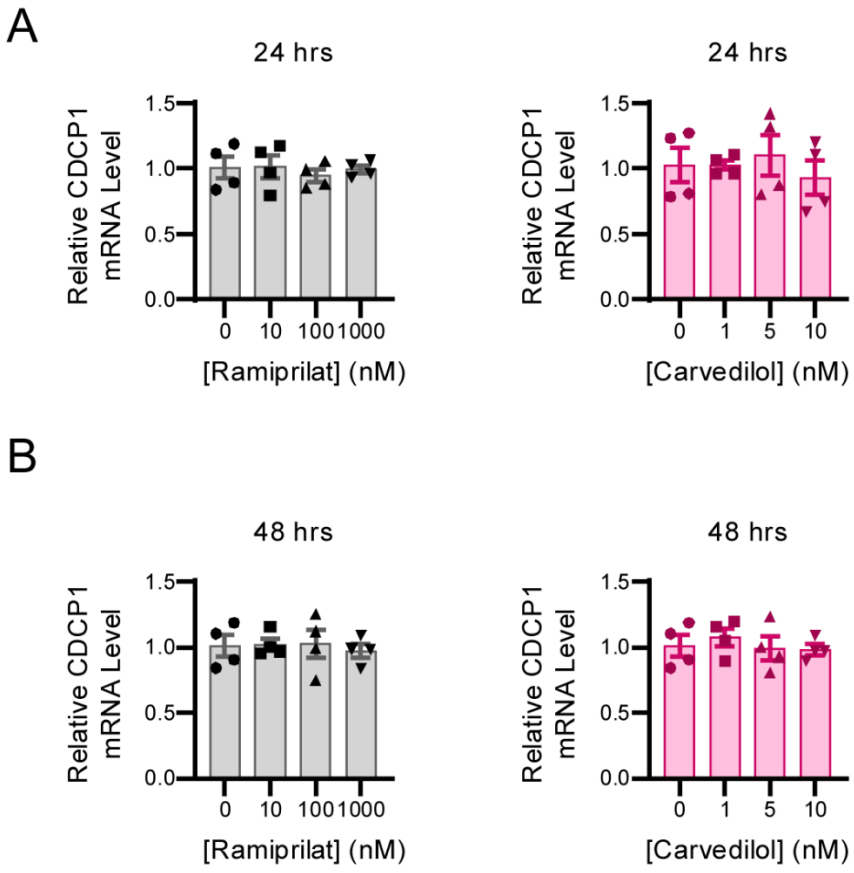


Supplementary Figures S11. Top pathways enriched by DEGs after CDCP1 KD (FC > 2.0; FDR < 0.05) in the Gene Ontology (GO) enrichment analysis of (**A**) Molecular Function and (**B**) Cellular Component. The adjusted *p*-value was computed using the Benjamini-Hochberg method for correction for multiple hypotheses testing.

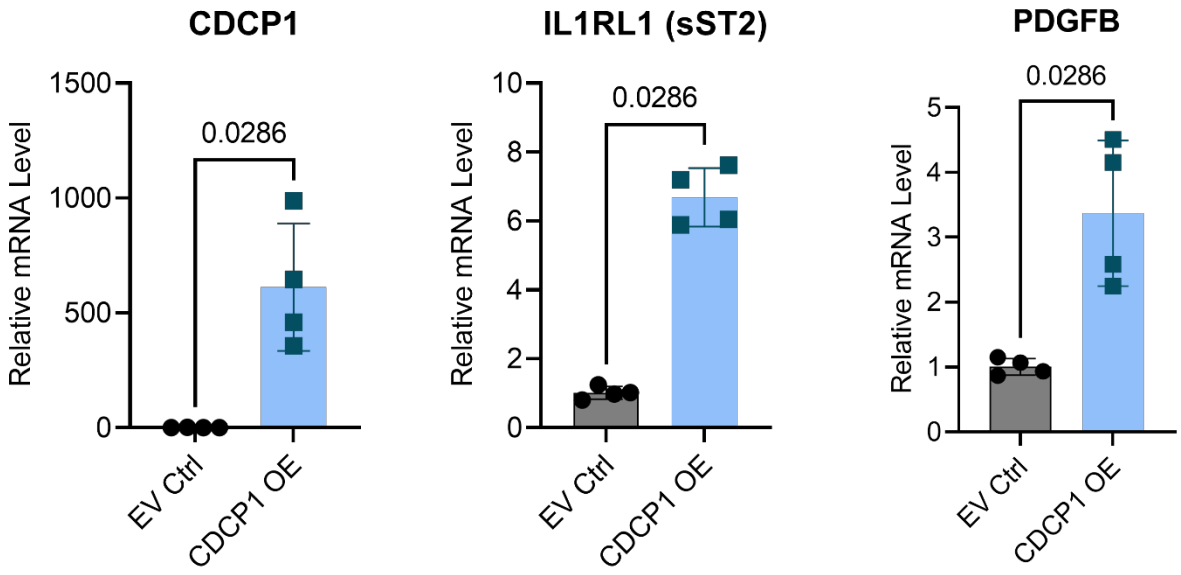




Supplementary Figures S12. CDCP1 mRNA expression in HCFs after exposed to serials doses of Ramiprilat and Carvedilol for (**A**) 24 hour and (**B**) 48 hours. Ramiprilat is an ACE inhibitor and Carvedilol is a beta blocker. RNA level was quantified by RT-qPCR using GAPDH as internal control from independent samples (n=4). *P*-values were calculated by ordinary one-way ANOVA with Dunnett's multiple comparisons test to vehicle treatment (0 nM). No significant *p*-value ($p<0.05$) was obtained.



Supplementary Figure S13. Relative RNA expression in HCFs after CDCP1 overexpression (OE). RNA samples were prepared from HCFs within 24 to 48 hours of cDNA or empty vector (EV) transfection. Each dot represents an independent sample (n=4). RNA level was quantified by RT-qPCR using GAPDH as internal control. Mann-Whitney test was used to calculate the presented *p*-values.



Online Supplementary Tables S1-S3

Table S1. Subject Demographics.

Medical Institute	**Czech**	**Denver**	**Lille**	**Mayo**	**Milan**	**Trieste**	***IMAC-2***	**Total**
Patient Number	(N=66)	(N=25)	(N=286)	(N=34)	(N=50)	(N=56)	(N=169)	(N=686)
Age								
Mean (SD)	49.44 (8.52)	36.52 (12.49)	52.05 (12.71)	65.06 (14.81)	59.76 (13.75)	46.61 (12.49)	46.75 (14.03)	50.69 (13.93)
Range	(30.00-64.00)	(14.00-60.00)	(18.00-82.00)	(40.00-89.00)	(31.00-83.00)	(23.00-71.00)	(18.00-79.00)	(14.00-89.00)
Gender								
F	12 (18.18%)	9 (36.00%)	77 (26.92%)	13 (38.24%)	11 (22.00%)	16 (28.57%)	64 (37.87%)	202 (29.45%)
M	54 (81.82%)	16 (64.00%)	209 (73.08%)	21 (61.76%)	39 (78.00%)	40 (71.43%)	105 (62.13%)	484 (70.55%)
LVEF_baseline								
Mean (SD)	22.73 (6.40)	26.48 (7.41)	27.90 (8.22)	25.56 (10.97)	29.52 (6.18)	26.73 (7.85)	24.08 (8.33)	26.32 (8.29)
Range	(10.00-37.00)	(12.00-40.00)	(10.00-40.00)	(9.00-49.00)	(19.00-40.00)	(8.00-40.00)	(9.00-45.00)	(8.00-49.00)
LVEF_FU								
Mean (SD)	32.30 (10.28)	35.52 (8.83)	52.01 (6.53)	38.97 (14.90)	35.42 (11.19)	36.34 (10.66)	41.21 (11.33)	43.72 (12.11)
Range	(15.00-64.00)	(10.00-53.00)	(40.00-71.00)	(10.57-65.00)	(14.00-63.00)	(14.00-64.00)	(10.00-68.97)	(10.00-71.00)
ChangeLVEF								
Mean (SD)	9.58 (9.18)	9.04 (8.88)	24.11 (9.49)	13.41 (12.62)	5.90 (9.94)	9.61 (10.44)	17.13 (12.88)	17.40 (12.45)
Range	(-12.00-36.00)	(-5.00-31.00)	(6.00-51.00)	(-12.00-39.50)	(-13.00-39.00)	(-9.00-37.00)	(-19.00-45.92)	(-19.00-51.00)
Time Base to FU Echo (months)								
Mean (SD)	6.38 (0.66)	6.06 (3.52)	6.45 (3.51)	7.67 (4.18)	11.48 (6.40)	8.22 (7.08)	6.45 (1.36)	7.00 (3.97)
Range	(3.29-7.85)	(1.97-16.00)	(0.00-16.66)	(2.99-17.74)	(3.06-25.69)	(1.18-49.54)	(3.75-12.12)	(0.00-49.54)
Diuretics Baseline								
No	10 (15.38%)	12 (48.00%)	82 (28.67%)	16 (47.06%)	10 (20.00%)	29 (51.79%)	53 (31.36%)	212 (30.95%)
Yes	55 (84.62%)	13 (52.00%)	204 (71.33%)	18 (52.94%)	40 (80.00%)	27 (48.21%)	116 (68.64%)	473 (69.05%)
Diuretics FU								
No	5 (7.58%)	10 (40.00%)	92 (32.17%)	6 (17.65%)	10 (20.00%)	27 (48.21%)	74 (43.79%)	224 (32.65%)
Yes	61 (92.42%)	15 (60.00%)	194 (67.83%)	28 (82.35%)	40 (80.00%)	29 (51.79%)	95 (56.21%)	462 (67.35%)
ACE Inhibitors Baseline								
No	14 (21.21%)	6 (24.00%)	31 (10.84%)	17 (50.00%)	11 (22.00%)	8 (14.29%)	28 (16.57%)	115 (16.76%)
Yes	52 (78.79%)	19 (76.00%)	255 (89.16%)	17 (50.00%)	39 (78.00%)	48 (85.71%)	141 (83.43%)	571 (83.24%)
ACE Inhibitors FU								
No	5 (7.58%)	7 (28.00%)	21 (7.34%)	8 (23.53%)	17 (34.00%)	10 (17.86%)	33 (19.53%)	101 (14.72%)

Yes	61 (92.42%)	18 (72.00%)	265 (92.66%)	26 (76.47%)	33 (66.00%)	46 (82.14%)	136 (80.47%)	585 (85.28%)
AT1 Antagonist Baseline								
No	60 (93.75%)	23 (92.00%)	272 (95.10%)	30 (88.24%)	40 (80.00%)	51 (91.07%)	124 (73.37%)	600 (87.72%)
Yes	4 (6.25%)	2 (8.00%)	14 (4.90%)	4 (11.76%)	10 (20.00%)	5 (8.93%)	45 (26.63%)	84 (12.28%)
AT1 Antagonist FU								
No	60 (90.91%)	24 (96.00%)	264 (92.31%)	29 (85.29%)	36 (72.00%)	47 (83.93%)	126 (74.56%)	586 (85.42%)
Yes	6 (9.09%)	1 (4.00%)	22 (7.69%)	5 (14.71%)	14 (28.00%)	9 (16.07%)	43 (25.44%)	100 (14.58%)
β-Blockers Baseline								
No	5 (7.58%)	6 (24.00%)	104 (36.36%)	13 (39.39%)	5 (10.00%)	15 (26.79%)	31 (18.34%)	179 (26.13%)
Yes	61 (92.42%)	19 (76.00%)	182 (63.64%)	20 (60.61%)	45 (90.00%)	41 (73.21%)	138 (81.66%)	506 (73.87%)
β-Blockers FU								
No	1 (1.52%)	2 (8.00%)	14 (4.90%)	3 (9.09%)	5 (10.00%)	8 (14.29%)	6 (3.55%)	39 (5.69%)
Yes	65 (98.48%)	23 (92.00%)	272 (95.10%)	30 (90.91%)	45 (90.00%)	48 (85.71%)	163 (96.45%)	646 (94.31%)

LVEF: left ventricular ejection fraction; FU: follow up; ACE: angiotensin-converting enzyme; AT1: angiotensin II type 1 receptor.

Table S2. SNPs Associated with DCM Drug Treatment Response (Changes in LVEF) with *P*-values Less than 1.0E-05.

rsID	Chr.	Position (hg38)	Position (hg19)	CA	MA	MAF	BETA	SE	*P*-value	t	dosR^2	SNP location	Nearest Gene
rs11105445	12	77499079	77892859	G	A	0.36	2.74	0.52	2.37E-07	O	na	5'upstream	*NAV3*
rs10745518	12	77502511	77896291	G	A	0.32	2.68	0.53	5.80E-07	I	1	5'upstream	*NAV3*
rs6773435	3	45227162	45268654	G	T	0.13	3.64	0.73	7.12E-07	O	na	5'upstream	*TMEM158/CDCP1*
rs111882214	3	179241132	178958920	T	C	0.19	3.17	0.64	8.39E-07	I	0.99	intron	*KCNMB3*
rs55748108	3	45221135	45262627	G	A	0.13	3.59	0.73	1.01E-06	I	1	3'downstream/5'upstream	*TMEM158/CDCP1*
rs73829982	3	45220509	45262001	G	A	0.13	3.59	0.73	1.02E-06	I	1	3'downstream/5'upstream	*TMEM158/CDCP1*
rs139134758	2	54847730	55074867	A	G	0.01	12.08	2.47	1.21E-06	I	0.95	intron	*EML6*
rs33752	3	45227641	45269133	T	A	0.13	3.53	0.72	1.23E-06	I	1	5'upstream	*TMEM158/CDCP1*
rs6782888	3	45235258	45276750	G	A	0.13	3.56	0.73	1.24E-06	I	1	5'upstream	*TMEM158/CDCP1*
rs7627300	3	45237466	45278958	T	A	0.13	3.56	0.73	1.28E-06	I	1	5'upstream	*TMEM158/CDCP1*
rs33751	3	45226624	45268116	C	T	0.13	3.52	0.72	1.33E-06	I	1	5'upstream	*TMEM158/CDCP1*
rs1441751	9	116774380	119536659	G	A	0.03	-7.42	1.52	1.39E-06	I	0.82	intron	*ASTN2*
rs28793	3	45230833	45272325	G	A	0.13	3.51	0.72	1.55E-06	I	1	5'upstream	*TMEM158/CDCP1*
rs10777210	12	77503292	77897072	T	C	0.37	2.53	0.52	1.57E-06	I	1	5'upstream	*NAV3*
rs28794	3	45231381	45272873	G	T	0.13	3.51	0.72	1.60E-06	I	1	5'upstream	*TMEM158/CDCP1*
rs7303522	12	77503257	77897037	A	G	0.35	2.52	0.52	1.61E-06	I	1	5'upstream	*NAV3*
rs40417	3	45217775	45259267	A	G	0.13	3.47	0.72	1.85E-06	I	1	3'downstream/5'upstream	*TMEM158/CDCP1*
rs696432	12	77563399	77957179	G	C	0.37	2.51	0.52	1.89E-06	I	0.99	5'upstream	*NAV3*
rs33758	3	45234143	45275635	G	A	0.13	3.48	0.72	1.91E-06	I	1	5'upstream	*TMEM158/CDCP1*
rs33741	3	45216455	45257947	C	T	0.13	3.46	0.72	1.94E-06	I	1	3'downstream/5'upstream	*TMEM158/CDCP1*
rs33735	3	45212204	45253696	C	A	0.13	3.44	0.72	2.09E-06	I	1	3'downstream/5'upstream	*TMEM158/CDCP1*
rs698152	12	77588325	77982105	C	A	0.37	2.47	0.52	2.79E-06	I	1	intron	*NAV3*
rs696451	12	77586565	77980345	G	A	0.37	2.47	0.52	2.92E-06	I	1	intron	*NAV3*
rs10745517	12	77501033	77894813	T	C	0.31	2.49	0.53	3.25E-06	I	1	5'upstream	*NAV3*
rs11922712	3	45238579	45280071	A	G	0.13	3.44	0.74	3.93E-06	I	0.99	5'upstream	*TMEM158/CDCP1*
rs12830941	12	77486970	77880750	C	A	0.23	2.79	0.6	3.94E-06	I	0.98	5'upstream	*NAV3*
rs696440	12	77580037	77973817	T	C	0.37	2.43	0.52	4.24E-06	I	1	intron	*NAV3*
rs57012018	12	77546007	77939787	A	C	0.26	2.57	0.56	4.72E-06	I	1	5'upstream	*NAV3*
rs35564342	12	77546432	77940212	G	A	0.26	2.57	0.56	4.72E-06	I	1	5'upstream	*NAV3*
rs4761385	12	77547903	77941683	C	T	0.26	2.57	0.56	4.72E-06	I	1	5'upstream	*NAV3*
rs61601253	12	77502691	77896471	T	C	0.23	2.7	0.59	4.93E-06	I	1	5'upstream	*NAV3*
rs245206	3	45204986	45246478	T	C	0.13	3.31	0.72	5.77E-06	I	1	3'downstream/5'upstream	*TMEM158/CDCP1*
rs696446	12	77582123	77975903	T	C	0.37	2.4	0.52	5.84E-06	I	1	intron	*NAV3*

rs2195205	3	45200645	45242137	C	T	0.13	3.34	0.73	5.90E-06	I	1	3'downstream/5'upstream	*TMEM158/CDCP1*
rs35693525	12	77541137	77934917	G	A	0.25	2.62	0.58	6.51E-06	I	1	5'upstream	*NAV3*
rs35021134	12	77542208	77935988	A	G	0.25	2.62	0.58	6.51E-06	I	1	5'upstream	*NAV3*
rs77506283	3	45190438	45231930	C	A	0.14	3.27	0.72	6.57E-06	I	0.99	3'downstream/5'upstream	*TMEM158/CDCP1*
rs12422873	12	77541123	77934903	C	G	0.25	2.62	0.58	6.57E-06	I	1	5'upstream	*NAV3*
rs245205	3	45206218	45247710	G	A	0.13	3.27	0.72	6.85E-06	I	1	3'downstream/5'upstream	*TMEM158/CDCP1*
rs4761382	12	77490491	77884271	G	T	0.29	2.53	0.56	6.90E-06	I	0.98	5'upstream	*NAV3*
rs34862346	12	77486323	77880103	A	G	0.24	2.67	0.59	7.36E-06	I	0.97	5'upstream	*NAV3*
rs696448	12	77585213	77978993	T	C	0.31	2.47	0.55	8.04E-06	I	0.99	intron	*NAV3*
rs245207	3	45204252	45245744	A	C	0.13	3.24	0.72	9.01E-06	O	na	3'downstream/5'upstream	*TMEM158/CDCP1*
rs12300650	12	77537682	77931462	T	C	0.32	2.38	0.53	9.33E-06	I	1	5'upstream	*NAV3*
rs61573566	12	77536107	77929887	G	A	0.32	2.38	0.53	9.45E-06	I	1	5'upstream	*NAV3*
rs75803084	3	45185543	45227035	T	C	0.13	3.22	0.72	9.97E-06	I	1	3'downstream/5'upstream	*TMEM158/CDCP1*

rsID: reference SNP cluster identity.
Chr.: chromosome; Position: SNP physical position on chromosome based on human genome assembly versions hg38 or hg19.
CA: common allele; MA: minor allele; MAF: minor allele frequency.
BETA: Beta coefficient, which value is the per unit increase (positive value) or decrease (negative value) in the GWAS phenotype; SE: standard error.
t: type of genotyping, O = observed, I = imputed; dosR^2: R^2 for imputation.

Table S3. Combined Effect of the rs11105445 and rs6773435 SNP Genotypes on Changes in LVEF after Drug Treatment.

		rs6773435 (G>T)		
		GG	**GT**	**TT**
rs11105445 (G>A)	**GG**	Reference [n=206]	2.98 (0.38,5.58) [n=57]	0.1 (-7.09,7.29) [n=6]
	GA	2.39 (0.76,4.01) [n=253]	6.07 (3.81,8.34) [n=82]	15.18 (5.11,25.25) [n=3]
	AA	4.28 (1.74,6.82) [n=61]	10.93 (6.42,15.44) [n=16]	20.95 (8.6,33.31) [n=2]

The change in LVEF was regressed on LVEF at baseline, sex age, time to follow-up echocardiograph, site and all combinations of genotypes for rs6773435 and rs11105445; which allows for non-linear effects of allele dosage for each SNP and interactions of the 2 SNPs. The values in the table for each combination of genotypes is in reference to the GG genotype for both rs6773435 and rs11105445 SNPs. So, the interpretation of the entry "6.07 (3.81, 8.34) [n=82]", for example, would be that individuals with the GA genotype for rs11105445 and GT genotype for rs6773435 (n=82) are estimated to have 6.07 (95 Confidence interval 3.81 to 8.34) units greater of LVEF change from baseline than those with the GG genotype for both rs6773435 and rs11105445 SNPs regardless of their gender, age, time from their follow-up echocardiograph or site they originated from.

Table S4.

Transcriptome-wide Differentially Expressed Genes in HCFs after CDCP1 Knock-down.
(see attached Excel file)

APPENDIX:

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