

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

All genotype and cardiac MRI data used as input for the lo-siRF analysis was obtained from the UK Biobank (application number 22282). Data for the gene co-expression networks from 313 explanted human hearts was downloaded from <https://zenodo.org/records/2600420>. Reverse Transcription of RNA was performed using the High-Capacity cDNA Reverse Transcription Kit (Thermofisher) and qPCR of the single stranded cDNA was performed using the Applied Biosystems ViiA 7 Real-Time PCR system with the TaqMan Fast Advanced MM (Thermofisher). Collection of single cell images were performed with a Nikon Ti2-U inverted microscope and a VEO 710S Phantom high-speed camera, controlled by the Phantom PCC v3.4 software.

Data analysis

All code for running the custom lo-siRF analysis and analyzing the experimental results can be found on GitHub (<https://github.com/Yu-Group/epistasis-cardiac-hypertrophy>). This custom lo-siRF analysis was conducted using R v3.6.1, Python v3.6.1, and iRF2.0 (<https://github.com/karlkumbier/iRF2.0>). The LVMi derivation from cardiac MRI images was computed using the ukbb_cardiac package (https://github.com/baiwenjia/ukbb_cardiac). Python v3.9, PLINK (v1.90b5.3) (<https://www.cog-genomics.org/plink/>) and BOLT-LMM (v2.3.4) (https://alkesgroup.broadinstitute.org/BOLT-LMM/BOLT-LMM_manual.html) were used to perform the GWAS dimension reduction. ANNOVAR (downloaded version: June 8, 2020) (<https://annovar.openbioinformatics.org/en/latest/>) was used to map each SNV to a genetic locus within lo-siRF. Comparisons to existing methods were performed using the SKAT R package (v2.2.5), mvMAPIT R package (v2.0.3), GSEA (v4.3.3) (<https://www.gsea-msigdb.org/gsea/index.jsp>), and MAGMA (v1.6). Functional annotation and gene mapping were performed using FUMA v1.5.4 (<https://fuma.ctglab.nl/>), ChromHMM v1.10 Core 15-state (https://egg2.wustl.edu/roadmap/web_portal/chr_state_learning.html), RegulomeDB v2.2 (<https://regulomedb.org/regulome-search/>), CADD v1.4 (<https://cadd.gs.washington.edu/>), and GTEx v8 (<https://www.gtexportal.org/home/>). Circos plot was made using TBtools (v2.210). Gene set enrichment analyses for gene ontology, pathway, and transcription factor were performed using Enrichr (last updated version: June 8, 2023) (<https://maayanlab.cloud/Enrichr/>), ChEA3 (<https://maayanlab.cloud/chea3/>), and MATLAB R2019a. Healthy and heart failure network analyses were performed previously in R v3.5.2. Cell boundary extraction and morphology analysis from single cell images was performed using MATLAB R2019a.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All genotype and cardiac MRI data used as input to the lo-siRF pipeline are available from the UK Biobank (<https://www.ukbiobank.ac.uk/>). This work was conducted under the UK Biobank application 22282. Data for the gene co-expression networks from 313 explanted human hearts is available at <https://doi.org/10.5281/zenodo.2600420>. The RNA-seq data used to establish the healthy and heart failure gene co-expression networks is available in the Gene Expression Omnibus (GEO) under accession number GSE57338. Others databases used in this study include RegulomeDB (v2.2), CADD (v1.4), GTEx (v8), Enrichr (last updated version: June 8, 2023), and ChIP-X Enrichment Analysis Version 3 (ChEA3). All other data have been provided as Supplementary Data files.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Findings are not restricted to a particular sex or gender as these were not used as inclusion/exclusion criteria for defining the cohort under study. In the genome-wide association study, sex was included as a covariate. The sex of the participant was collected by the UK Biobank (field 31) and acquired from the central registry at recruitment, but can be updated by the participant in some cases. Supplementary Table provides the break down of the study cohort by sex with 51% females and 49% males.

Reporting on race, ethnicity, or other socially relevant groupings

The cohort under study includes only White British individuals from the UK Biobank population. This cohort was chosen in order to minimize variability due to population stratification. Specifically, this White British population includes those individuals from the UK Biobank who self-reported as White British and have similar genotypic backgrounds based on principal components analysis as detailed in prior work (Bycroft et al., 2018, PMID: 30305743).

Population characteristics

Covariate-relevant population characteristics of the human research participants are summarized in Supplementary Table 1. This includes a summary of left ventricular mass measurements, age, height, weight, and various cardiac hypertrophy-related diseases and medication.

Recruitment

The UK Biobank recruited over 500,000 UK individuals between ages 40 and 69 years at recruitment from 2006 to 2010. From the UK Biobank participants, we analyzed individuals from the largest ancestry subset (i.e., the White British population described above) who are unrelated and have both genetic and cardiac magnetic resonance imaging data. Related individuals (i.e., third-degree relatives or closer) have been previously described and implemented in Bycroft et al. 2018 (PMID: 30305743) and Morgan et al. 2018 (PMID: 30531825).

Ethics oversight

The UK Biobank received ethical approval from the North West - Haydock Research Ethics Committee (21/NW/0157).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- ☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We report image-based cardiomyocyte morphology measurements for $n > 33000$ single cells per gene-silencing condition per cell line (varying in conditions, exact n numbers provided in Source datasheet). Our sample sizes are much larger than the sample sizes used in other single cell image analysis studies (Wu et al., 2020, PMID: 32010778; Govek et al., 2023, PMID: 37339989; Zhang et al., 2023, PMID: 37095203) to enhance the statistical power for analyzing the non-additive effects of epistatic the proposed gene-gene interactions on single cardiomyocyte morphology.
Data exclusions	Data exclusion of single cell morphologies was performed using three quality control filters to exclude measurements from cell clumps and debris. To remove large cell clumps, the peak-solidity filter removes data outside of the polygonal region defined by $\{(0.9, 0), (0.9, 3.2), (0.934, 8.26), (1, 28), (1, 0)\}$ in the (solidity, peak No.) space. Then, the roundness filter removes cells with weird shapes by excluding data with a roundness error higher than 0.3 or a circularity lower than 0.6. Finally, the small size filter removes cell debris whose major diameter is lower than $15 \mu\text{m}$ ($12 \mu\text{m}$) or minor diameter is lower than $12 \mu\text{m}$ ($10 \mu\text{m}$) for images photographed at the top (bottom) outlet microchannels.
Replication	To verify the reproducibility of our findings, experiments were performed using $n = 3$ independent biological replicates ($n = 4$ for unaffected cells silencing CCDC141). All attempts at replication were successful.
Randomization	Wells of cells were randomly allocated to the six experimental groups (1 scramble control and 5 different gene-silencing conditions) for each biological replicates. Order of single cell imaging experiments for each experimental group were randomized.
Blinding	Investigators were not blinded to allocation during experiments and data analysis. Binding was not possible because these experiments were performed by individual investigators who were aware of the experimental groups and treatment outcomes. However, a different group of investigators who are blinded to allocation re-analyzed the data using the same tool and performed statistical analysis, achieving consistent results.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	This study used cardiomyocytes derived from a patient-specific human induced pluripotent stem cell (hiPSC) line carrying a heterozygous MYH7-R403Q mutation and a healthy control hiPSC cell line, which have been reported by Dainis et al. 2020 (PMID: 32567507).
Authentication	The patient-specific hiPSC cell line was derived from patient fibroblasts collected under Stanford Institutional Review Board approval number 4237 and cultured under Stanford Stem Cell Research Oversight (SCRO) 568. The healthy control cell line was line SCVI273 obtained from the Stanford Cardiovascular Institute Biobank.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable