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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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 (*n*) 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ 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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ 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 *d*, Pearson's *r*), 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	Transcriptomes were sequenced using the NextSeq 500 and HiSeq 4000 and corresponding Illumina commercial software. Whole-mount embryo images with fluorescence were acquired with LAS v4.6 acquisition software and section images/lightsheet images were acquired with Zeiss Zen imaging software
Data analysis	Cellranger (v2.2.0), R (3.5.1), Seurat (v2.2), Monocle v2, ImageJ v1.51m9, GraphPad Prism 8, Bitplane Imaris software v.9.0.2

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All source data, including sequencing reads and single-cell expression matrices have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO series accession number GSE126128. Data underlying each figure are available in the Source Data Files, Supplementary Information and on the UCSC cell browser at https://mouse-cardiac.cells.ucsc.edu. Users can use the UCSC cell browser to explore the data, view expression of genes of interest in each UMAP plot and download datasets for custom analysis.

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	For single cell RNAseq experiments, the cell capture efficiency of the Chromium technology is ~57%. Thus we loaded all cells dissected from embryos without pre-counting, to minimize cell loss and maximize the number of captured single cells. The sample sizes of embryos used for single-cell transcriptome analysis at each time point was chosen to obtain cell numbers comparable to estimated cell numbers in the cardiogenic region at each embryonic stage. For RNA in situ hybridization experiments, at least 2 embryos per probe, per genotype were tested. For WT vs Hand2-null comparisons, at least 3 independent embryos per genotype were tested.
Data exclusions	All exclusion criteria were pre-established. We captured cells of the endoderm, ectoderm and mesoderm germ layers - but we focused primarily on mesodermal lineages, ectodermal neural crest cells and endoderm cells captured at E7.75 in this study. Of the analyzed cells, those that were of low quality or represented doublets were excluded from our analyses -this was achieved by filtering out cells with greater than 8000 and fewer than 1500 genes in Seurat. After clustering and UMAP projection, one or two cell clusters would emerge that expressed markers representing multiple populations; these were cells that had low transcript (nUMI) and gene (nGene) counts that escaped the first filtering step. These cells were also removed from the analyses.
Replication	Cells from at least 2 somite-matched biological replicate embryos per genotype were captured at E7.75, E8.25 and E9.25. Expression and enrichment of marker genes in clusters derived from the single cell RNA-seq data were validated and reproducible by in situ hybridization experiments (at least n=2). For each in situ experiment comparing gene expression in wild type and Hand2-null embryos, at least 3 embryos per genotype (WT vs Hand2null) were assayed. All attempts at replication were successful.
Randomization	Experimental groups were determined by genotype i.e., Hand2 wild type embryos were compared to Hand2-null embryos. Covariates were not relevant to the analysis of the Hand2-null phenotype as the developmental defect is highly penetrant regardless of embryo sex, and the developmental stages analyzed were prior to the onset of overt heart failure.
Blinding	Investigators were not blinded to allocation of embryos during experiments. Blinding was not possible for the WT and Hand2-null embryo comparisons due to the need to match embryos with the same somite count to control for developmental timing.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Transcriptomes were captured from wildtype (WT) and Hand2-null embryos from intercrossed C57BL/6 mice heterozygous for the Hand2-null allele. The sexes and ages of all embryos used for capture of single-cell transcriptomes are listed in Supplementary Table 1a. Lineage tracing of Cck expressing cells was performed using Cck-ires-Cre mice (JAX stock #012706) and Ai14 mice (JAX stock #007914). Validation of ectopic RA signaling in the Hand2 mutant was done by crossing the mutant line to RARE- hsp68LacZ mice (JAX stock #008477). All animals used for timed matings were aged 6-8 weeks (female) or 8-10 weeks (male) of age.
Wild animals	The study did not involve wild animals

Field-collected samples	The study did not involve samples collected from the field
Ethics oversight	All protocols concerning animal use were approved by the Institutional Animal Care and Use Committee (IACUC) at UCSF and were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

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