nature portfolio

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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 (*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	No software was used for collection.
Data analysis	TERRA platform: https://app.terra.bio 10x Genomics Cell Ranger (v4.0.0): https://www.10xgenomics.com/support/software/cell-ranger Python (v3.7.9): https://www.python.org/downloads/release/python-379/ CellBender (v0.2): https://github.com/broadinstitute/CellBender scanpy (1.7.0): https://scanpy.readthedocs.io/en/stable/ scVI (1.0.3): https://github.com/scverse/scvi-tools scR-Invex: https://github.com/getzlab/scrinvex Scrublet(v0.2.1): https://github.com/swolock/scrublet Bayesian entropy estimation: https://pypi.org/project/ndd/1.6.3 limma (v3.40.6): https://bioconductor.org/packages/release/bioc/html/limma.html edgeR (v3.26.8): https://bioconductor.org/packages/release/bioc/html/edgeR.html DESeq2 (v1.24.0): https://bioconductor.org/packages/release/bioc/html/DESeq2.html g:Profiler: https://biit.cs.ut.ee/gprofiler/ MiloPy (0.1.1): https://github.com/emdann/milopy MATLAB (R2019b): https://www.mathworks.com/products/matlab.html STAR (2.7.9a): https://github.com/alexdobin/STAR Metascape: https://metascape.org/gp/index.html#/main/step1

ClusterProfiler (version 4.6.2): https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html
ScanMiR (1.5.2) : https://www.bioconductor.org/packages/release/bioc/html/scanMiR.html
MIENTURNet: http://userver.bio.uniroma1.it/apps/mienturnet/
scCODA/PertPy (0.7.0): https://github.com/theislab/pertpy

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

Processed single-nucleus transcriptomic data are available through the Broad Institute's Single Cell Portal (https://singlecell.broadinstitute.org/single_cell) under project ID SCP2489. Raw and processed next-generation sequencing data have been deposited at the NCBI Gene Expression Omnibus with accession number GSE255992.

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	We performed snRNA-seq on a total of 14 males, and 24 females in this study. Age and sex are reported in this study (supplemental Table 1 and Table 2).
Reporting on race, ethnicity, or other socially relevant groupings	The left atrial samples utilized in this study were derived entirely from individuals of European ancestry. No socially relevant groupings were applied in this study.
Population characteristics	18 patients were included that had atrial fibrillation (AF), and 16 were included with no history of atrial fibrillation (Controls). The average age of the patients with AF was 66.3 years old. And the mean age of the control donors was 68.2 years old.
Recruitment	All samples originated from deceased donors with non-failing hearts that were rejected for organ donation. Adult left atrial tissue samples were collected from organ donors by Myocardial Applied Genetics Network (MAG-Net; www.med.upenn.edu/magnet). Control samples were collected from organ donors with no previous history of heart disease or atrial fibrillation AF samples were collected from patients that had a history of atrial fibrillation but no history of other heart disease.
Ethics oversight	Written informed consent for research use of donated tissue was obtained from next of kin in all cases. Research use of tissues were approved by the relevant institutional review boards at the Gift-of-Life Donor Program, the University of Pennsylvania, Massachusetts General Hospital and the Broad Institute.

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 selected the largest sample set available of non-failing (n=16) and patients with atrial fibrillation (AF) (n=18) for single nucleus RNA-seq. No statistical methods were used to predetermine sample size.
Data exclusions	Samples were excluded if single-nuclei RNA-sequencing was low quality based on 1) <50% of reads in cells, 2) < 65% of reads confidently mapping to transcriptome, 3) < 90% valid barcodes, 4) abnormally low Q30, or 5) no ambient plateau in the unique molecular identifier (UMI) decay curve. Nuclei were removed if they 1) were enriched for mitochondrial reads, 2) were enriched for the proportion of reads mapping exclusively to exonic regions, 3) had a high prediction for being a doublet, 4) had extremely high or low UMI, 5) had extremely high or low number of genes detected, or 6) had low entropy
Replication	We selected the largest sample set available of non-failing (n=16) and patients with atrial fibrillation (AF) (n=18) for single nucleus RNA-seq. ATRNL1 immunofluorescence experiments were carried out across 3 different tissue samples from patients with AF and without AF (Figure 3 and Supplemental Figure 2). Bulk RNA-seq experiments were carried out in triplicate for each experimental condition (Figure 4). Archlight

	electrophysiological studies were performed with technical replicates across multiwell plates. To verify the reproducibility of our cell-type specific AF GWAS integration analysis we analyzed data generated by the Human Heart Cell Atlas.
Randomization	No randomization was performed. Age and sex were controlled for in differential expression analysis.
Blinding	Blinding was not relevant for our study and was not performed. Given the nature of the study, we sought to compare transcriptional profiles of AF and non-failing left atrial tissues at single-nucleus resolution.

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

Antibodies

Antibodies used	Antibodies used in this study; ATRNL1, rabbit, Thermo fisher PA5-58234 (1:100), Cx43, mouse, Thermofisher 14-4759-82 (1:100), α-actinin: mouse: abcam ab9465 (1:200). Secondary antibodies: donkey anti-rabbit 488 thermo, A-21206 (1:200); donkey anti-mouse 568 thermo, A10037 (1:200).
Validation	All antibodies are commercially available.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	For lentiviral production HEK293T/17 cells were used. This cell line was acquired from the American Type Culture Collection (ATCC): https://www.atcc.org/products/crl-11268 H9 human embryonic stem cells (hESC) were obtained from WiCell: https://www.wicell.org/home/stem-cells/stem-cells.cmsx
Authentication	differentiated hESC-aCMs are stained for CM markers, and qPCR is conducted to characterize atrial-like status HEK293T cells were obtained directly from ATCC.
Mycoplasma contamination	All cell lines obtained directly from ATCC or WiCell. No Mycoplasma tests were conducted.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.