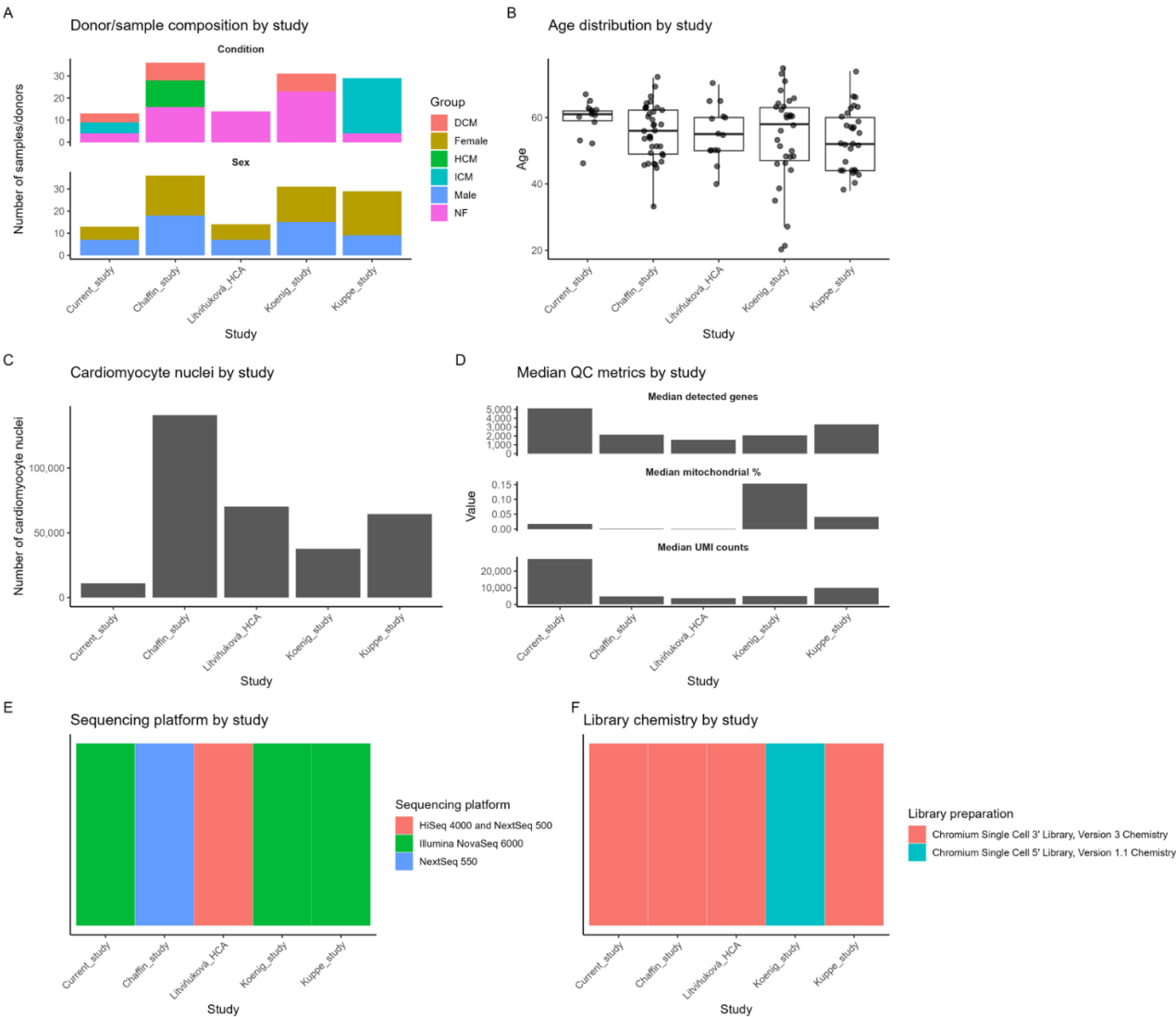


Single-Nucleus RNA-Seq of Human and Rat Cardiomyocytes Identifies Shared and Distinct Regulators and Features of Aging and Disease

Samaneh Ekhteraei-Tousi, Rosa Doñate Puertas, Mohamad Youness, Onne A.H.O. Ronda, Magnus Aronsen, Emil K. S. Espe, Arne Olav Melleby, Ida Marie Hauge-Iversen, Ella Cutsforth, Lirong Li, Emma L. Robinson, Einar Sjaastad Nordén, Mani Sadredini, Filip Rega, Kenneth C. Bedi Jr, Kenneth B. Margulies, Karin R. Sipido, Ivar Sjaastad, H. Llewelyn Roderick

SUPPLEMENTARY DATA

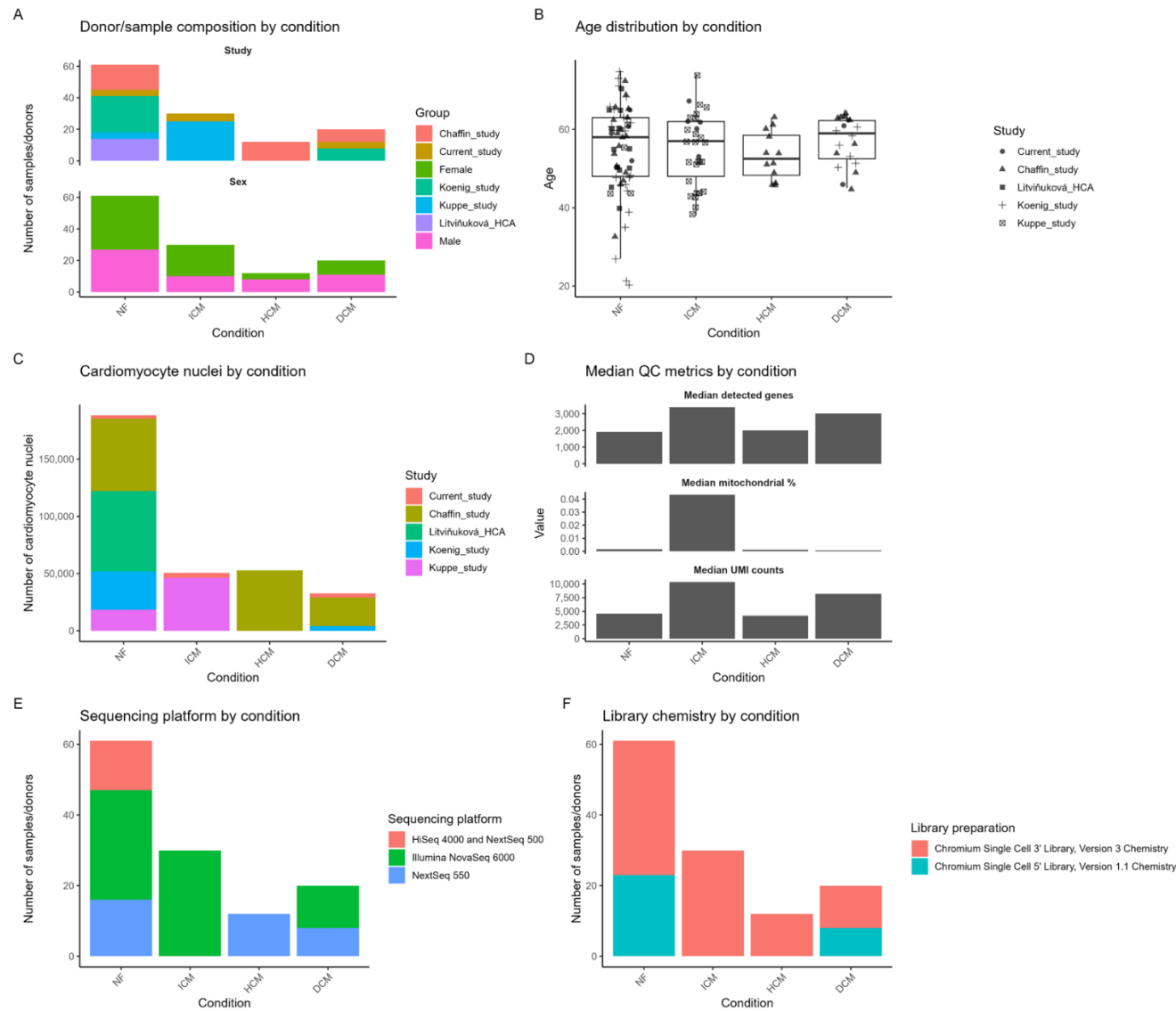
Dataset-level composition and technical heterogeneity of integrated human snRNA-seq datasets



Supplementary Figure 1. Dataset-level composition and technical heterogeneity of integrated human snRNA-seq datasets. A. Donor/sample composition by source study, showing condition and sex distribution. B. Age distribution of donors/samples across studies. C. Number of CM nuclei contributed by each study. D. Median quality-control metrics by study, including detected genes, mitochondrial percentage, and UMI counts. E. Sequencing platform used for each source dataset. F. Library chemistry used for each source dataset.

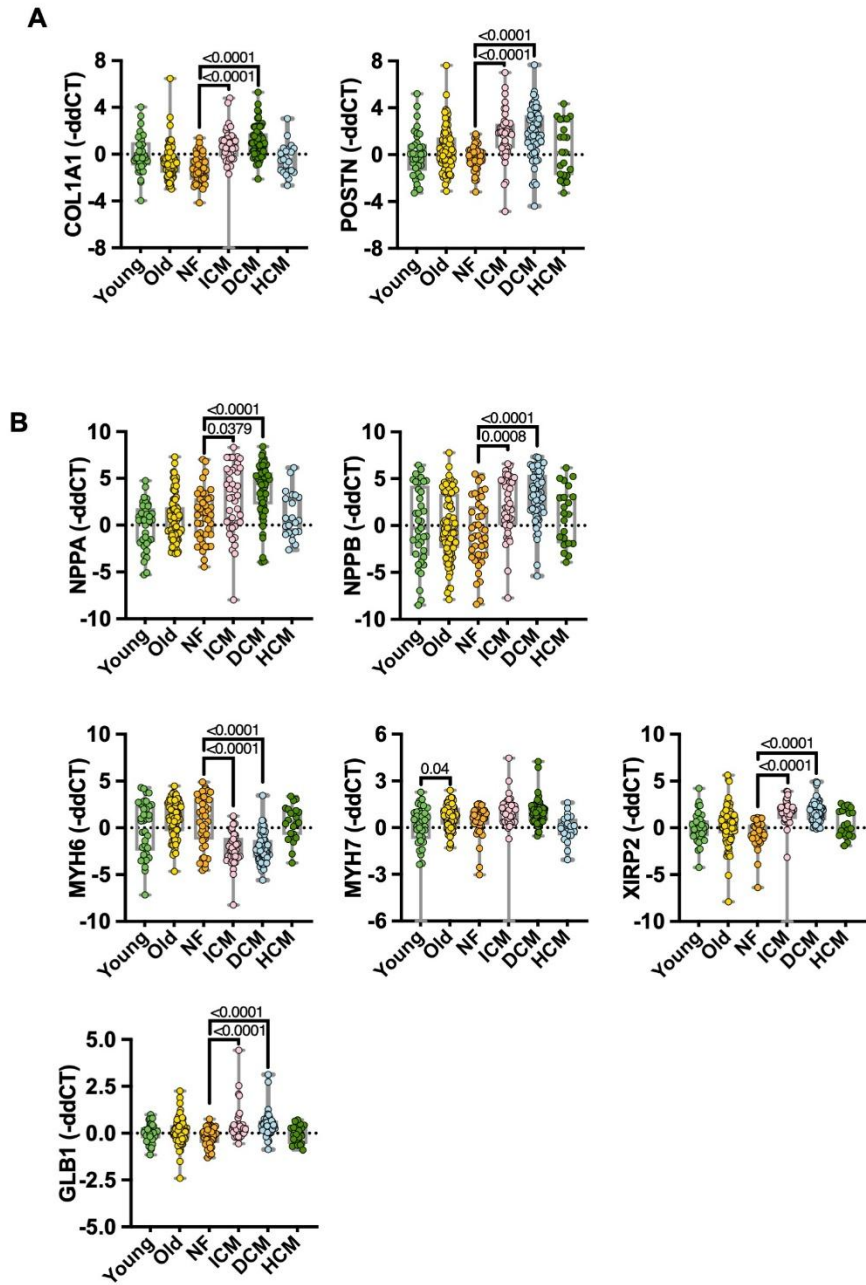
SUPPLEMENTARY DATA

Condition-level composition and technical heterogeneity of integrated human snRNA-seq datasets



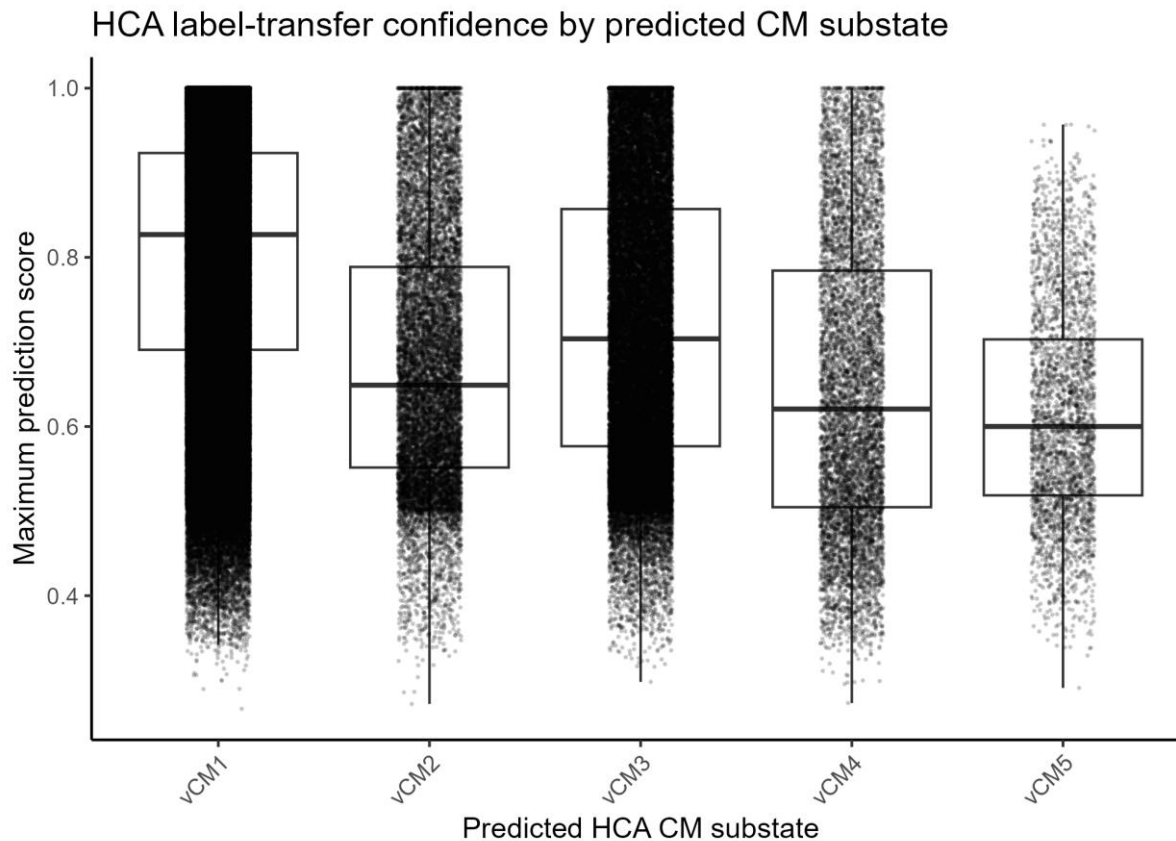
Supplementary Figure 2. Condition-level composition and technical heterogeneity of integrated human snRNA-seq datasets. A. Donor/sample composition by condition, showing source-study contribution and sex distribution for NF, ICM, HCM, and DCM groups. B. Age distribution by condition, with individual donors/samples overlaid and shaped by source study. C. Number of cardiomyocyte nuclei contributed to each condition, stratified by source study. D. Median quality-control metrics by condition, including detected genes, mitochondrial percentage, and UMI counts. E. Sequencing platform represented within each condition. F. Library chemistry represented within each condition.

SUPPLEMENTARY DATA



Supplementary Figure 3. RT-qPCR analysis of fibrotic and hypertrophic marker gene expression in left ventricular tissue from human. A. Expression of fibrotic genes. B. Expression of hypertrophic marker genes. N=37, 88, 41, 41, 62, 22 for Young, Old, NF, ICM, DCM and HCM. Statistical analysis by one way ANOVA.

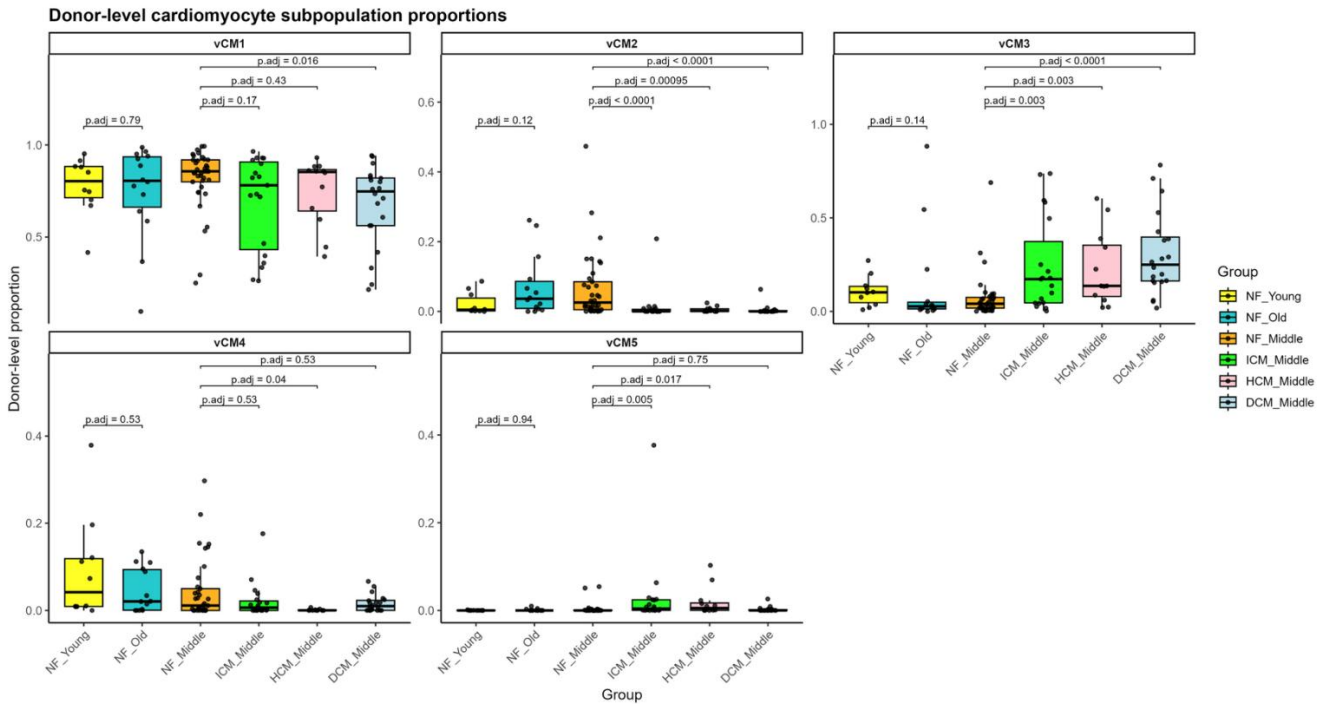
SUPPLEMENTARY DATA



Supplementary Figure 4. Confidence of human cell atlas-based CM substate label transfer.

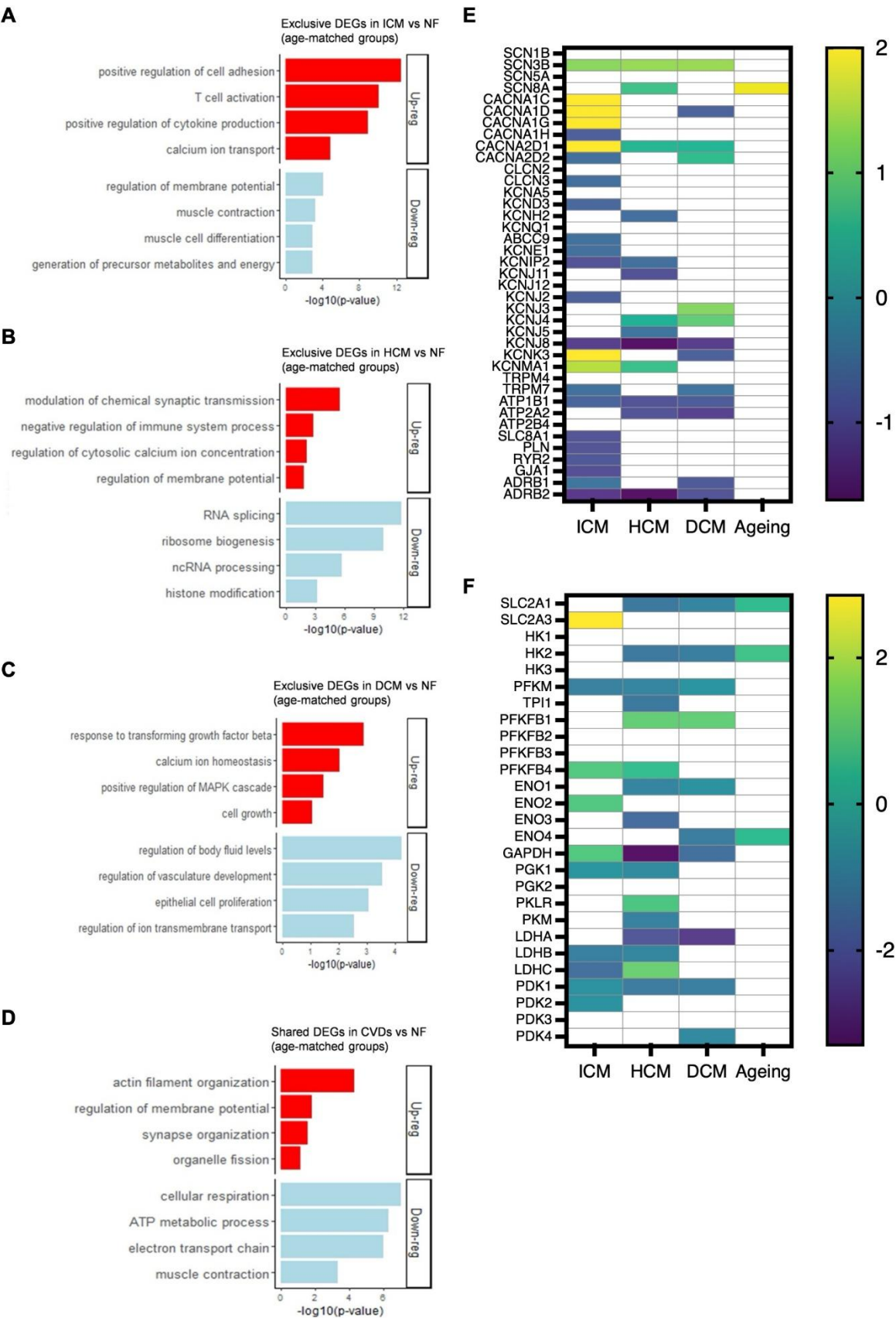
Boxplots show the distribution of Seurat maximum prediction scores for cardiomyocyte nuclei assigned to each Human Cell Atlas-transferred ventricular cardiomyocyte substate. Each point represents one nucleus, and the boxplots summarize the median and interquartile range of prediction.score.max values for each predicted vCM state. Prediction scores were highest for vCM1 and moderate-to-high for vCM2-vCM5, supporting the use of vCM1-vCM5 as HCA-anchored cardiomyocyte transcriptomic state annotations. These labels were used for comparative state-distribution analyses and are interpreted as reference-based transcriptomic states rather than discrete cell types.

SUPPLEMENTARY DATA



Supplementary Figure 5. Donor-level cardiomyocyte subpopulation proportions across aging and cardiomyopathy groups. Boxplots show the donor-level proportion of cardiomyocyte nuclei assigned to each HCA-transferred vCM substate. Each point represents one donor/sample. NF aging was assessed by comparing young and old NF donors, whereas cardiomyopathy-associated changes were assessed by comparing middle-aged ICM, HCM, and DCM donors with middle-aged NF donors at age matched groups. P-values indicate Benjamini-Hochberg-adjusted donor-level Wilcoxon rank-sum tests for the indicated comparisons. This donor-level analysis supports cardiomyopathy-associated shifts in vCM state composition, particularly reduced vCM2 and increased vCM3 in cardiomyopathy groups, while NF aging-associated shifts were more modest.

SUPPLEMENTARY DATA

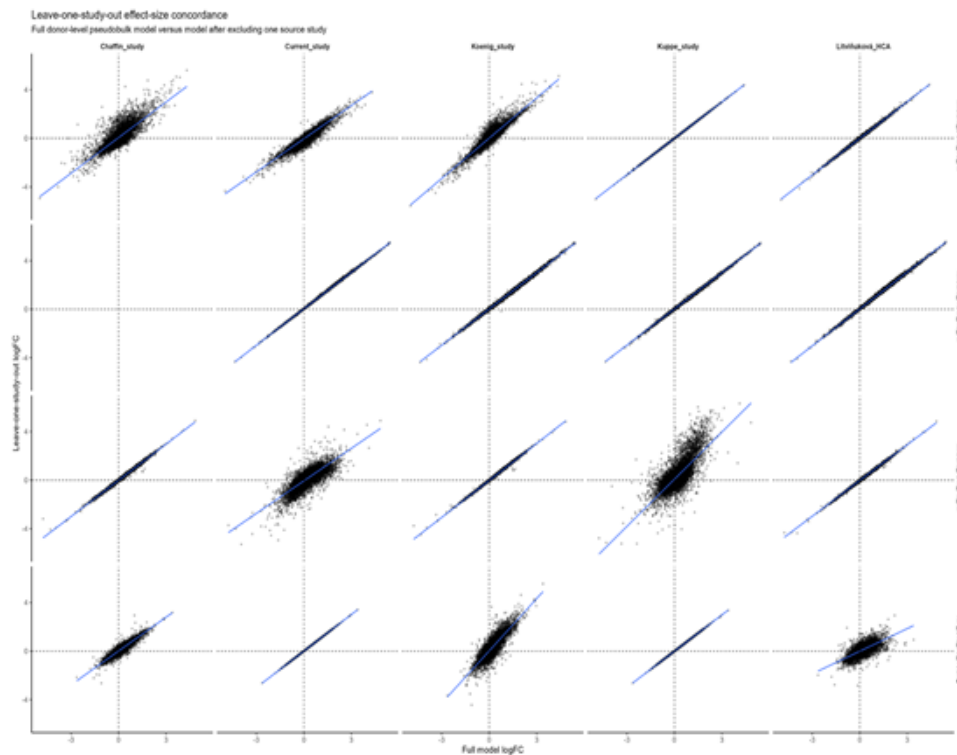


SUPPLEMENTARY DATA

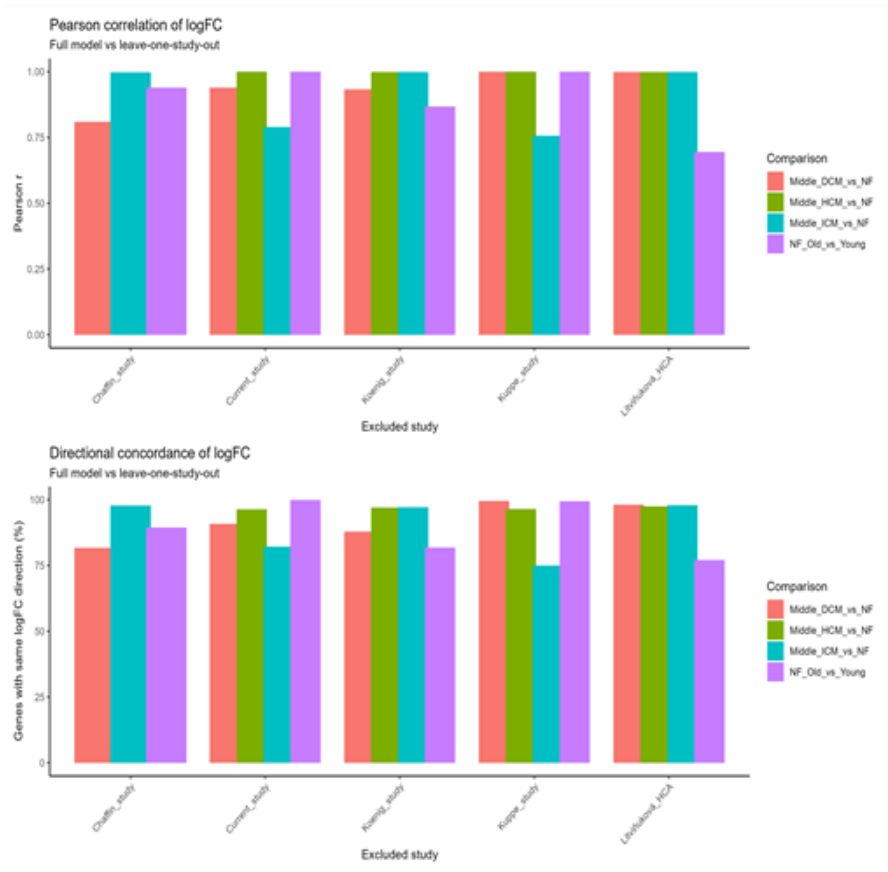
Supplementary Figure 6. Pathways associated with distinct and shared DEGs in each human pathology. A-C. Pathways associated with DEGs unique to each CMP, after subtracting DEGs associated with NF aging. D. Pathways associated with shared altered genes in the pathological conditions. E. Heatmap showing expression of genes (log2 fold change) underlying generation of the action potential and involved in calcium handling. F. Heatmap showing expression of genes (log2 fold change) involved in glycolysis.

SUPPLEMENTARY DATA

A

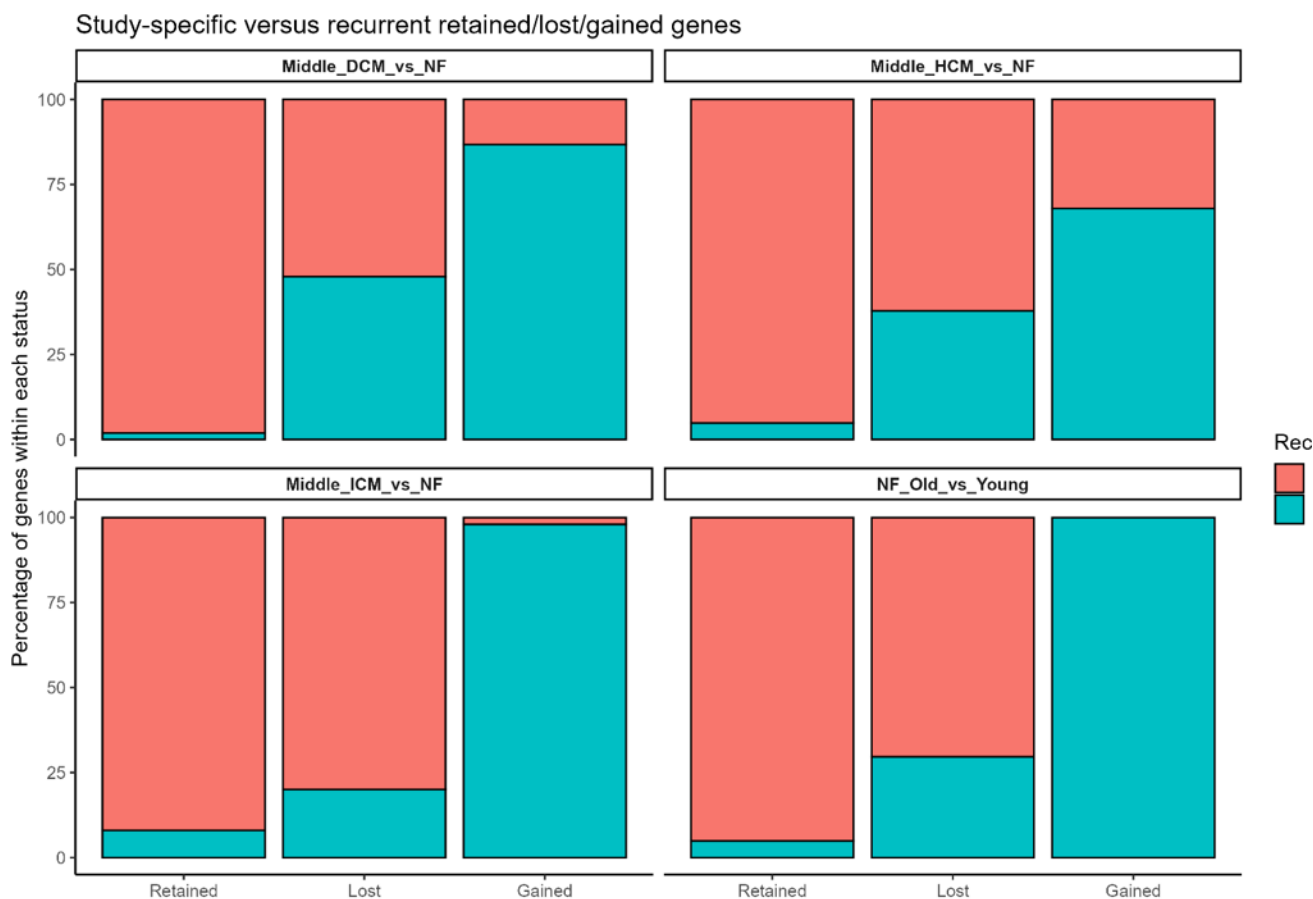


B



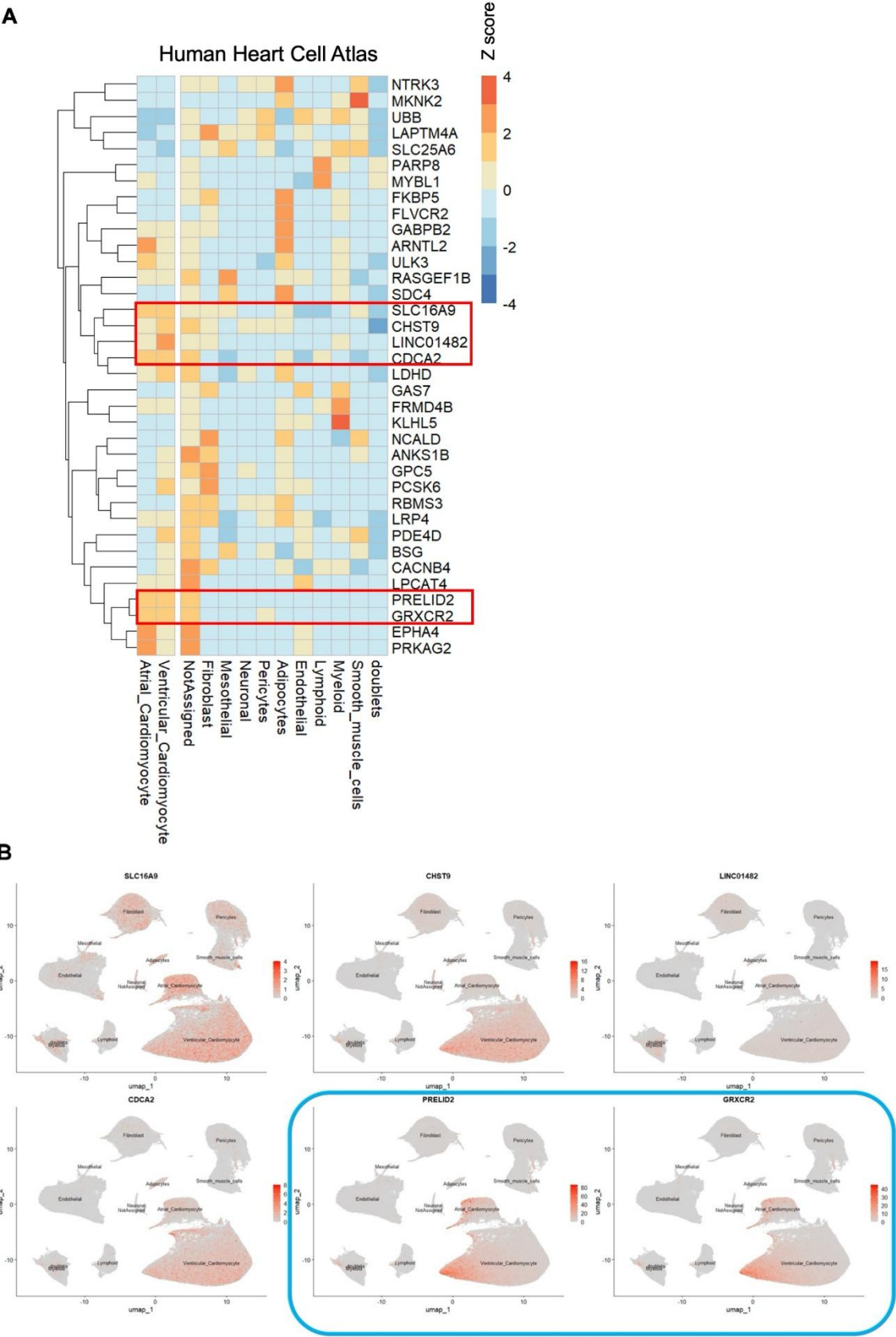
SUPPLEMENTARY DATA

Supplementary Figure 7. Leave-one-study-out sensitivity analysis of donor-level pseudobulk CM differential expression. A. Effect-size concordance between the full donor-level pseudobulk model and leave-one-study-out models. Each panel compares gene-level log2 fold changes from the full model with those obtained after excluding the indicated source study. Columns indicate the excluded study, and rows show the planned comparisons: DCM versus NF, HCM versus NF, ICM versus NF, and NF O vs NF Y. Dashed lines indicate zero log2 fold change, and blue lines show linear fits. B. upper. Pearson correlation of gene-level log2 fold changes between the full model and each leave-one-study-out model for each comparison. B. lower. Directional concordance, shown as the percentage of genes retaining the same direction of log2 fold change in the leave-one-study-out model relative to the full model.



Supplementary Figure 8. Leave-one-study-out sensitivity analysis of donor-level pseudobulk CM differential expression. Differentially expressed genes lost, gained or retained recurrently or in a study specific manner when each of the studies included in the integrated dataset are omitted. Data is shown for DCM vs NF, ICM vs NF, HCM vs NF and O vs Y.

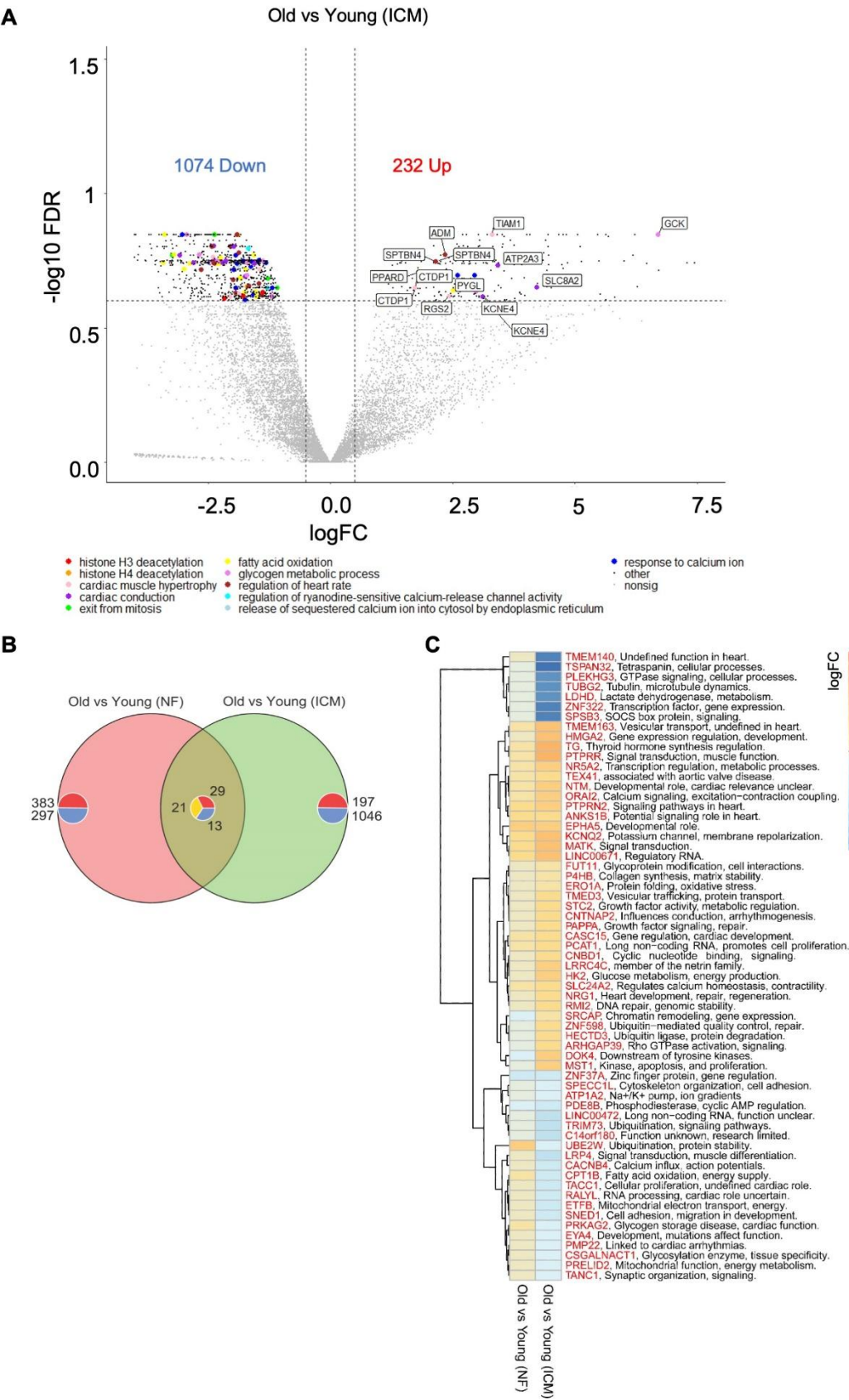
SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 9. Expression profile of the DEGs shared between aging and cardiomyopathies (ICM, HCM, DCM) across various cardiac cell types extracted from human cell atlas. A. The specified genes are predominantly expressed at higher levels in cardiomyocytes. B. Among the specified genes in the heatmap, only those within the blue box exhibit notably higher expression in cardiomyocyte nuclei.

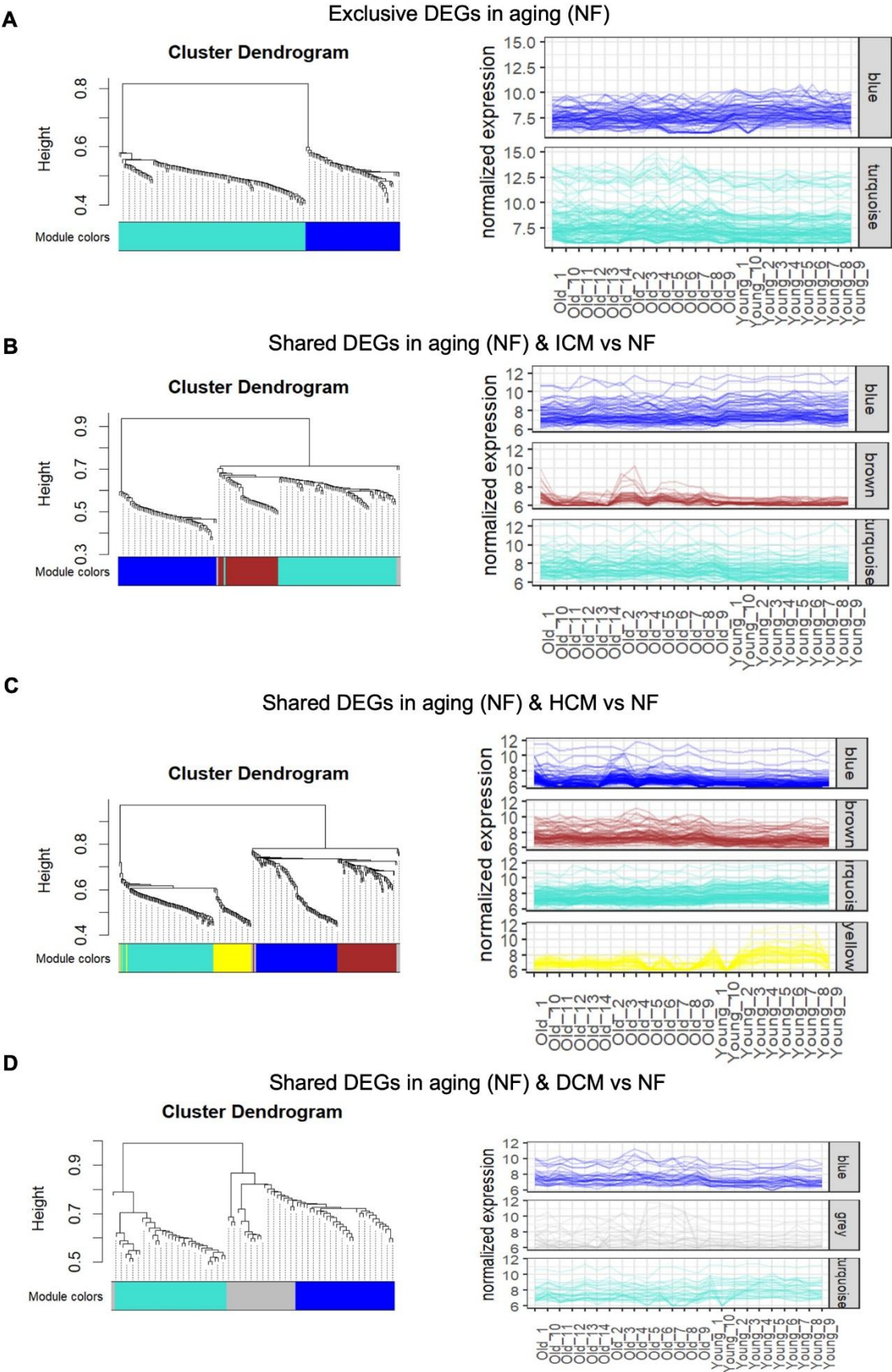
SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 10. Comparison of genes differentially expressed in cardiomyocytes between NF aging and ICM aging. A. DEGs in O versus Y in ICM samples and their associated pathways (pseudobulk-seq data). B. Shared and distinct DEGs between aging in NF and aging in ICM. C. Heatmap of the shared DEGs between the two comparisons in B.

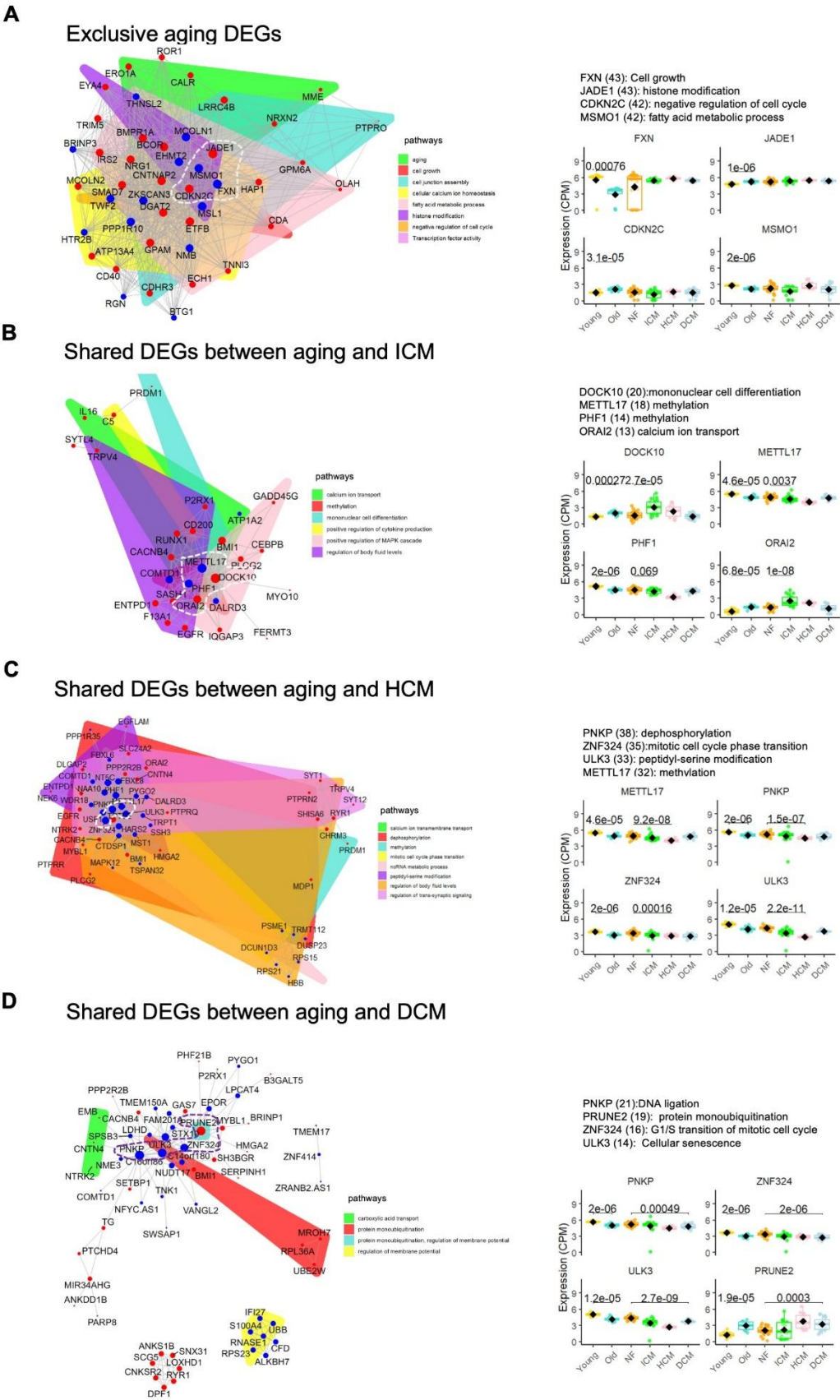
SUPPLEMENTARY DATA



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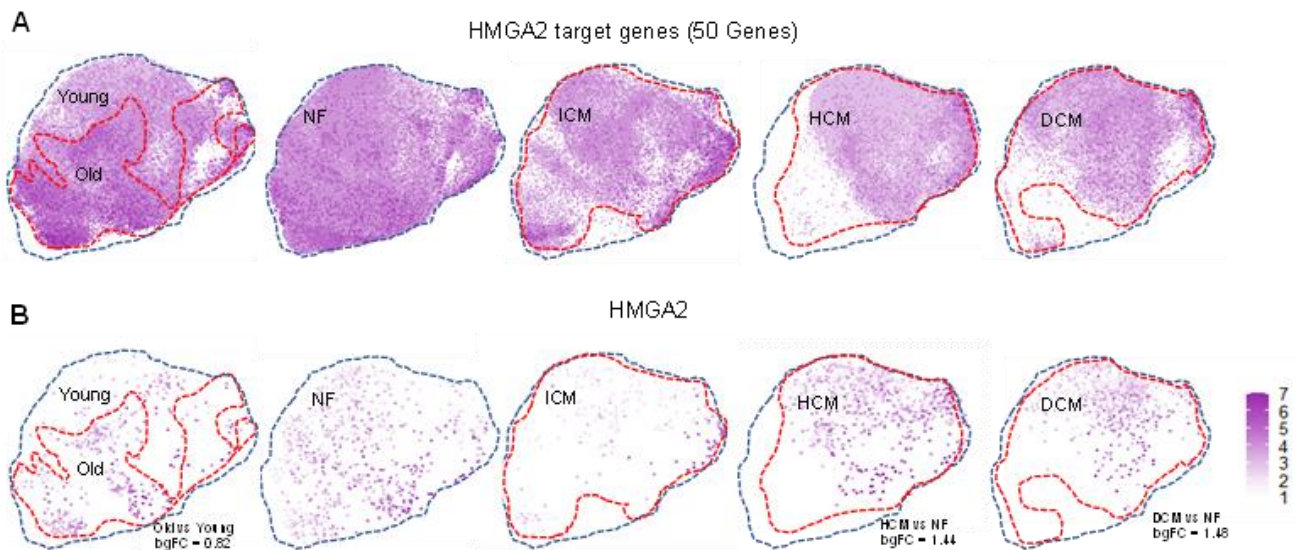
Supplementary Figure 11. WGCNA on the DEGs detected in Y vs O NF CM. A. Co-expressed genes exclusively detected in aging across two modules. B-D. Co-expressed genes shared between aging, ICM, HCM, and DCM, respectively.

SUPPLEMENTARY DATA



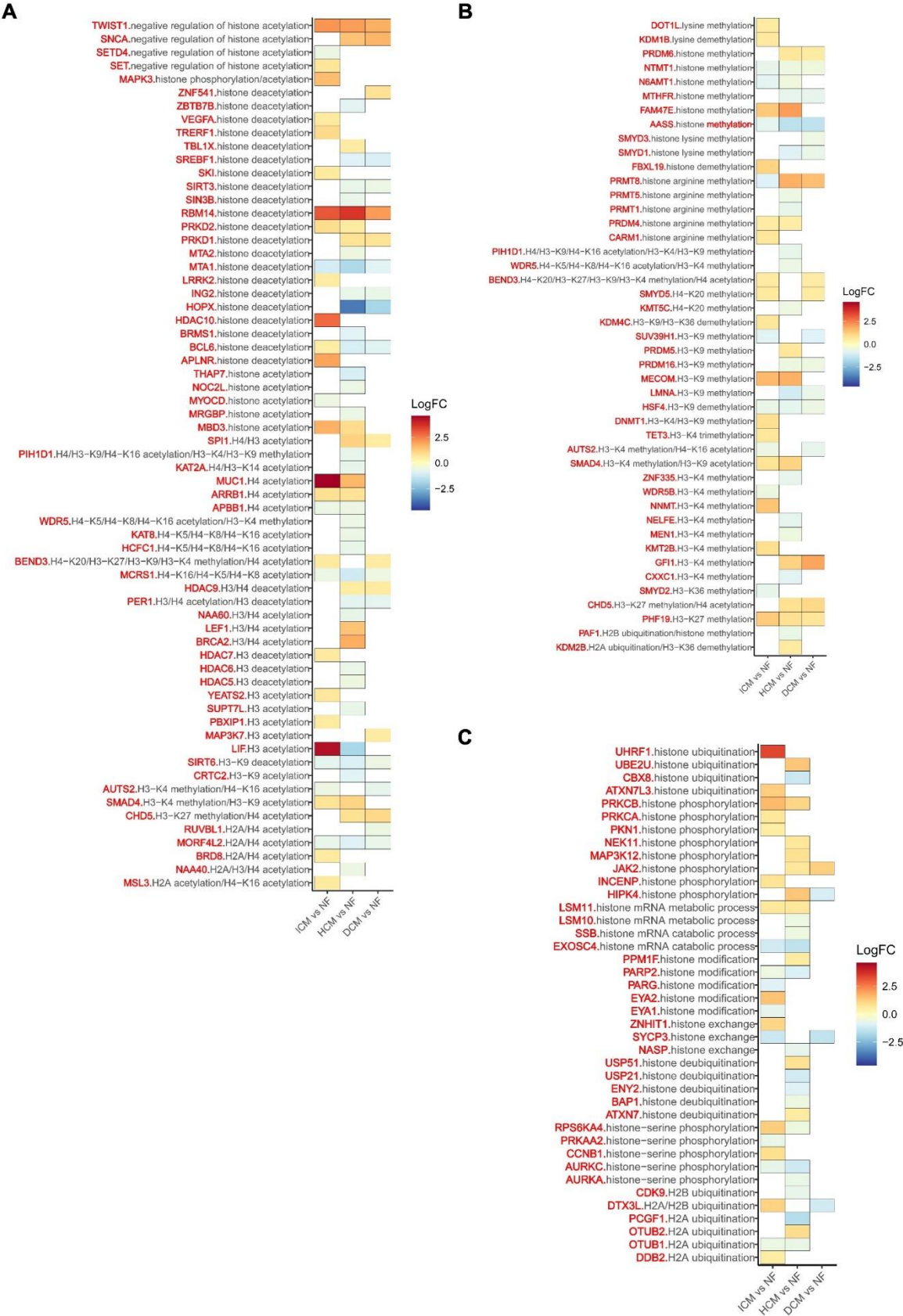
SUPPLEMENTARY DATA

Supplementary Figure 12. Gene-gene expression correlations (calculated by WGCNA) and their interactions in CM nuclei of human samples. The up-regulated and down-regulated differentially expressed genes in O vs Y samples are indicated with red and blue dots close to the gene labels. The size of the dots refers to the number (degree) of gene-gene interactions. The associated pathways of the correlated genes are also indicated by the different colors. The spatial position of the nodes (genes) and edges (interactions) are calculated by igraph and visualized by ggraph. The distinct (A) and common altered genes (B-D). in aging and the CMP, respectively. The top four hub genes per each gene-gene network set are indicated at the top right side, the number of the gene interactions for each hub gene is indicated in the parenthesis. Some hub genes are common between the pathologies (B-D). The normalized expression values (CPM- count per million) of the exclusive and the shared hub genes in aging and cardiomyopathies, respectively are statistically compared between O vs Y in NF and also between the age-matched groups between the NF and the cardiomyopathies.



Supplementary. Figure. 13. Distribution of CM nuclei in the CM nuclei UMAP cluster during aging and pathology in human showing expression of HMGA2 target genes (A) and of (B) expressing HMGA2.

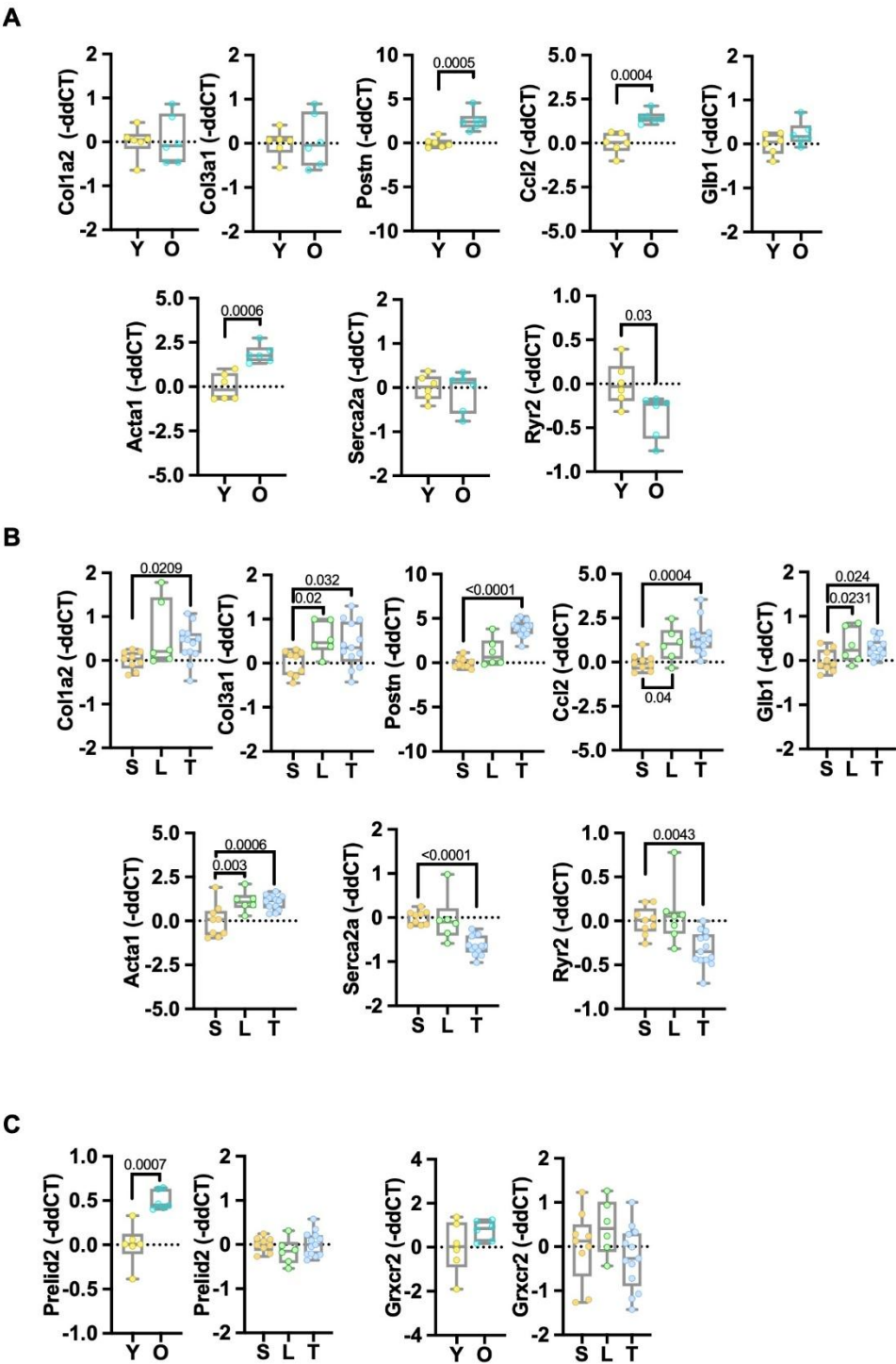
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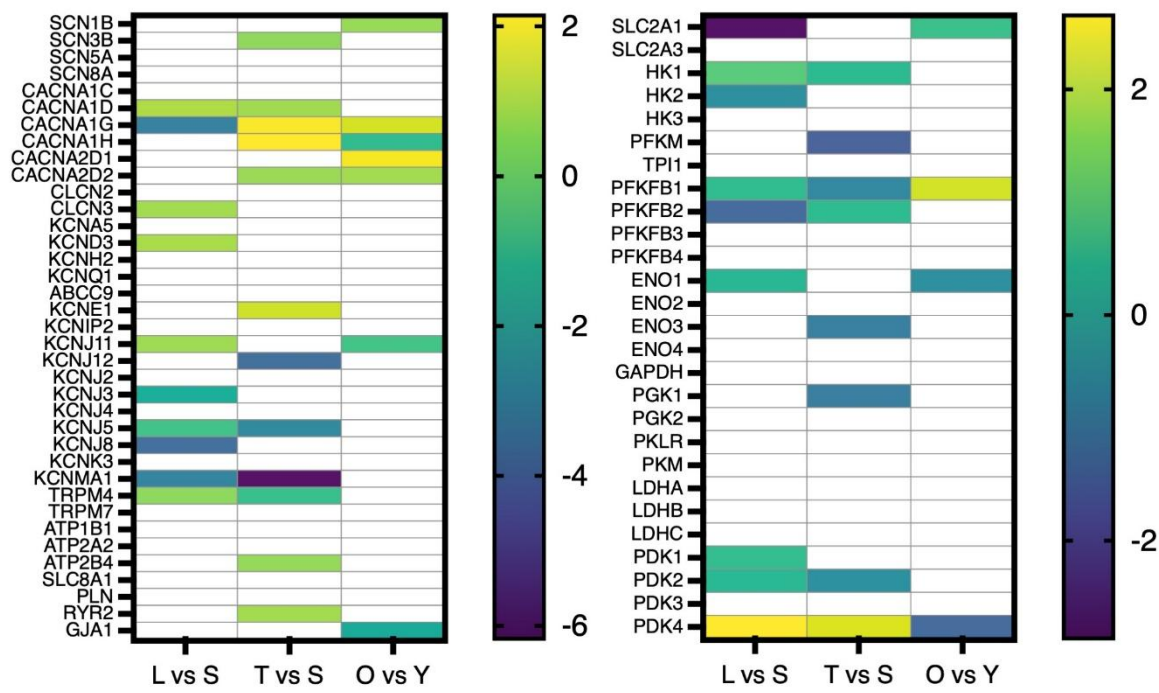
Supplementary Figure 14. Heat maps showing differentially expressed epigenetic regulators in human cardiomyopathies. A. Epigenetic regulators involved in histone (de)acetylation, B. in histone methylation, C. and other histone modifications. The empty tiles indicate genes were not significantly altered in the indicated pathology compared to NF in the age-matched groups.

SUPPLEMENTARY DATA

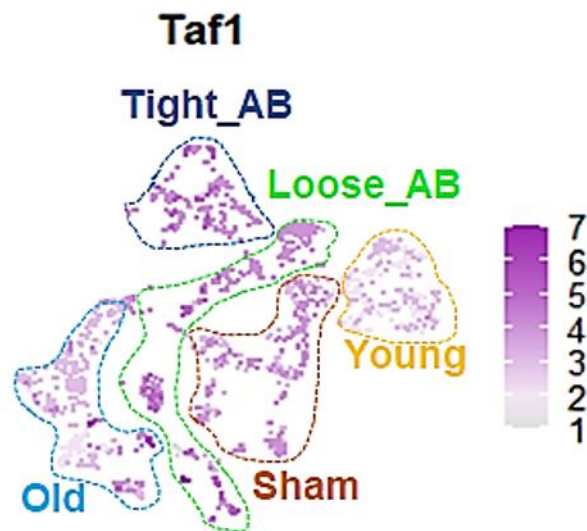


Supplementary Figure 15. RT-qPCR analysis of expression of genes associated with cardiac remodeling in rat LV tissue. A. Comparison between Young (Y) and Old (O). B. Comparisons between Sham (S), Loose_AB (L), and tight_AB (T) groups (qPCR) in B. C. Analysis of *Preli2* and *Grxcr2* in Y vs O and Sham vs Loose AB and Tight AB. For two groups t-test and three groups, ANOVA. P<0.05 considered significant.

SUPPLEMENTARY DATA

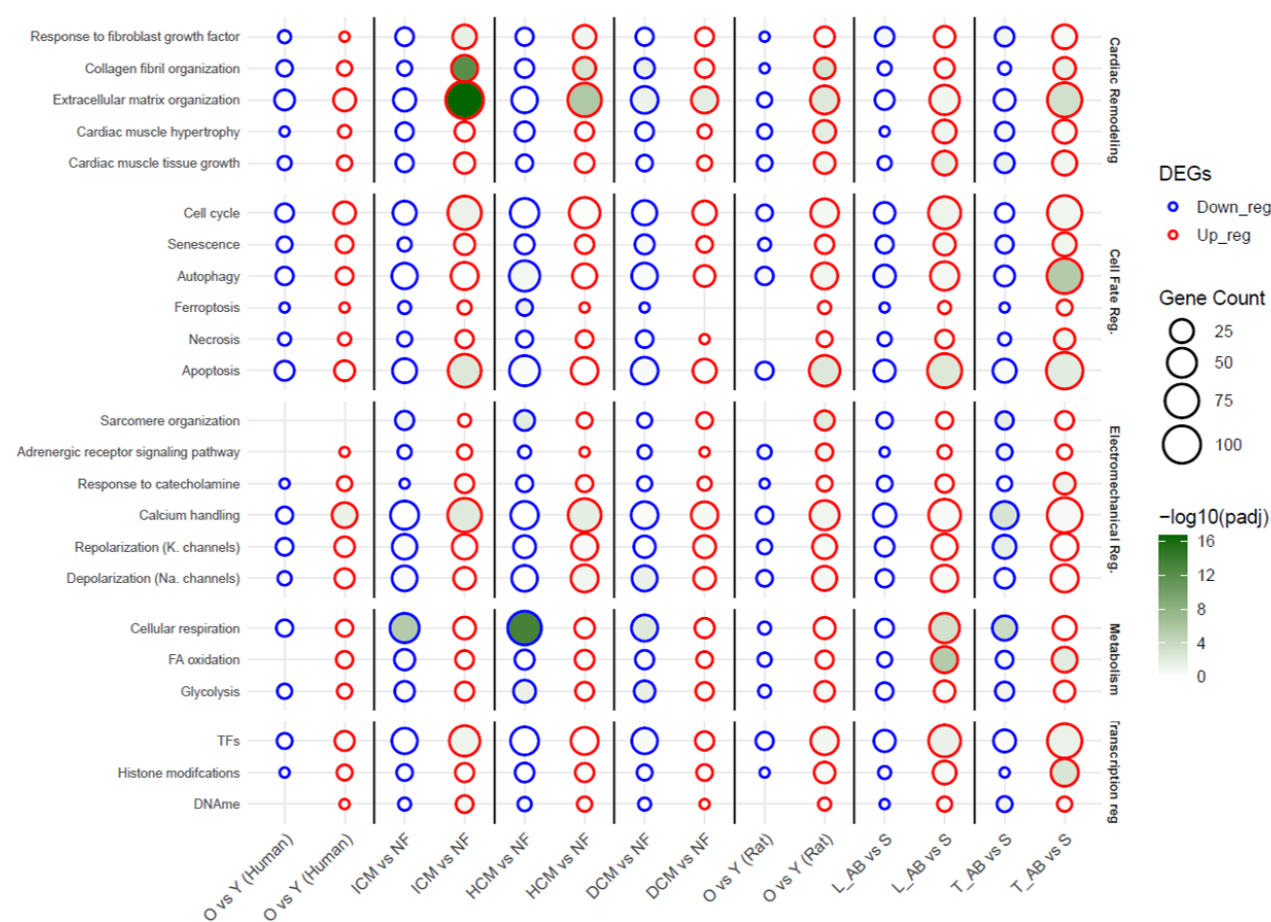


Supplementary Figure 16. Heatmaps showing changes in gene expression between the conditions shown. A. Expression of genes involved in generation of action potential and in calcium handling. **B.** Expression of genes (log2 fold change) involved in glycolysis.



Supplementary Figure 17. Distribution and expression of the Taf1 transcription factor in the UMAP clusters of rat CM nuclei from aging and AB.

SUPPLEMENTARY DATA



Supplementary Figure 18. Shared Gene Ontology (GO) enrichment analysis of DEGs in aging and pathological conditions in human and rat.

SUPPLEMENTARY DATA

Supplementary Tables

Table 1. Characteristics of patient samples used for RT-qPCR analysis.

Group	Young	Old	Age-matched	ICM	HCM	DCM
Total number (female)	37 (19)	88 (43)	41 (19)	41 (5)	22 (9)	62 (21)
Age (years)	34 ± 8	73 ± 6	55 ± 5	58 ± 11	57 ± 11	50 ± 16
Height (cm)	173 ± 10	170 ± 9	171 ± 9	175 ± 11	174 ± 11	172 ± 22
Weight (kg)	74 ± 19	75 ± 15	80 ± 18	84 ± 16	88 ± 16	79 ± 23
BMI (kg/m ²)	25 ± 6	26 ± 4	27 ± 6	27 ± 5	29 ± 5	26 ± 5
EF (%)				19 ± 10	61 ± 10	19 ± 9
Plasma NT-proBNP (ng/L)				2363 ± 1974	2095 ± 1974	4289 ± 8004
LVIDs (mm)				58 ± 8	30 ± 8	57 ± 12
LVIDd (mm)				65 ± 8	42 ± 8	64 ± 11
LVPWd (mm)				9 ± 7	15 ± 7	9 ± 2
LVAD				11 (30)	0 (22)	21 (41)
CRT				32 (9)	5 (17)	49 (13)
ACEi, ARBs				30 (11)	6 (16)	37 (25)
BB				37 (4)	5 (17)	49 (13)
Anti-arrhythmics				19 (22)	3 (19)	29 (33)
Nitrates / hydralazine				10 (31)	1 (21)	8 (54)
Thyroid medication				4 (37)	3 (19)	16 (46)
Anti-coagulants				26 (15)	6 (16)	48 (14)
Anti-aggregant				33 (8)	5 (17)	25 (37)
Lipid lowering				30 (11)	5 (17)	22 (40)
Diuretics				31 (10)	2 (20)	47 (15)

Young (20–44 years), Old (65–99 years), age-matched control group (45–64 years), ICM: ischemic cardiomyopathy, HCM: hypertrophic cardiomyopathy, DCM: dilated cardiomyopathy, BMI: body mass index, EF: ejection fraction, BNP: brain natriuretic peptide, LVIDs: left ventricular internal diameter at end systole, LVIDd: left ventricular internal diameter at end diastole, LVPWd: left ventricular posterior wall diameter at end diastole. Data are shown as mean ± standard deviation (SD). LVAD: left ventricular assist device (Heartmate II, Heartmate III, Heartware, ECMO + Impella), CRT: cardiac resynchronization therapy (CRT-defibrillator, implantable cardioverter defibrillator, dual chamber Implantable Cardioverter Defibrillator), ACEi: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker (perindopril, ramipril, candesartan, LCZ696, telmisartan, captopril, losartan, lisinopril, Zestril), BB: beta blockers (bisoprolol, carvedilol, sotalol, nebivolol, selozok), anti-arrhythmics (amiodarone, diltiazem, digoxin, Procortalan, ivabradine, flecainide, disopyramide), nitrates (isosorbide dinitrate, hydralazine), Thyroid medication (levothyroxine, strumazol), Anticoagulants (warfarin, vitamin K antagonist, apixaban, enoxaparin, dabigatran, rivaroxaban, phenprocoumon), antiaggregants (prasugrel, acetylsalicylic acid, clopidogrel, ticagrelor), lipid lowering drugs (rosuvastatin, simvastatin, pravastatin, atorvastatin, ezetimibe), diuretics (furosemide, bumetanide, acetazolamide, hydrochlorothiazide). Data are shown as number of patients with (number without).

SUPPLEMENTARY DATA

Table 2. Primer sequences used for RT-qPCR in human and rat

Target	Species	Forward	Reverse
36B4	Human	CATATCCGGGGGAATGTGGG	CAGTGACTTCACATGGGGCA
TBP	Human	GGGCACCACTCCACTGTATC	TTTGCAGCTGCGGTACAATC
GAPDH	Human	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
NPPA	Human	TTACTGGCATTCCAGCTCCT	CCAGCAAATTCTTGAAATCCA
NPPB	Human	AACGTCCGGGTACAGGAG	AGGGATGTCTGCTCCACCT
MYH6	Human	CAACAATCCCTACGACTACGC	ACGTCAAAGGCACTATCGGTG
MYH7	Human	TAGACACACTTGAGTAGCCCAG	CTAGCCGCTCCTTCTCTGACT
COL1A1	Human	GTCAGATGGGCCCCCG	GCACCATCATTTCACGAGC
POSTN	Human	GGAGACACACCCGTGAGGAA	CACTGAGAACGACCTTCCCTT
XIRP2	Human	CAGCCAGGAAATGGCAAGAAA	GTTTACTTCCTCGGTGTGCT
SERCA2a	Human	GCTGATGGTGCTGAAATCT	TTAGGAAGCGTTACTCCAG
JADE1	Human	TGAAACGAGGTCGCCTTCC	CGGGAATTCTGGGACCAAGT
GLB1	Human	GGAAGTGTGAGCCCAAAGGA	GTTGGCCCCAGTGATCTAGC
TBP	Rat	GCTGCTAGTCTGGATTGTTCTTCA	CTGCGTTGATCTTCAGTTCTGG
RPL32	Rat	TGTCAAGGAGCTGGAAGTGC	GCTGCTCTTTCTACGATGGC
YWHAZ	Rat	GGGACATCTGCAACGACGTA	CTTGCTTTCTGGCTGCGAA
GAPDH	Rat	AACTCCCTCAAGATTGTCAGCAA	CAGTCTTCTGAGTGGCAGTGATG
18S	Rat	CCAACGTCTGCCCTATCAACTT	CCGGAATCGAATCGAACCTGATT
Nppa	Rat	CGTATACAGTGCAGGTGTCCAAC	CATCTTCTCCTCCAGGTGGTCTAG
Nppb	Rat	AAGTCCTAGCCAGTCTCCAGAACA	TTGAGAGCTGTCTCTGAGCCATT
Myh6	Rat	GGCAAGGTCACTGCCGAA	AGAAGAGGCCTGAGTAGGTATAGA
Myh7	Rat	TGGCACCGTGGACTACAATA	TACAGGTGCATCAGCTCCAG
Acta1	Rat	ACACCATGTGTGACGAGGAC	CCACGATGGATGGGAACACA
Serca2a	Rat	TGGACGAGACGCTCAAGTTT	TGCAATGTTTAGGAAGCGGT
Col1a2	Rat	GCCAAGAATGCATACAGCCG	GACACCCCTTCTGCGTTGTA
Col3a1	Rat	GACCAAAAGGTGATGCTGGC	GACCTCGTGCTCCAGTTAGC
Postn	Rat	TCCGTGTCTTCGTGTATCGG	TGTGAATGGCACCGTTCCTC
Xirp2	Rat	CTCGTAGCAGACCAGCAGAA	TGTCCGTGAAGTGATGCGTA
Grxcr2	Rat	GACAGACCGCACATTTCTCC	TGGCAGTGGAGACAGTTATCC
Preld2	Rat	GTTCCAGTACCCCTTCGAGC	TGACATTTTTATCCATGGGGTTCG
Ccl2	Rat	GCCTGTTGTTACAGTTGCT	CAGCCGACTCATTGGGATCAT
Glb1	Rat	CTTCCGGATACCCCGATTCT	ATATTGTCCTGGCTGGGGTTC
Ryr2	Rat	TCCCGAGAAGCTGGAATACTT	CCATAAATCCATCCATTCGCC

SUPPLEMENTARY DATA

Table 3. Human left ventricular single CM nuclei datasets included.

Study	Sample No.	Single nuclei preparation	Library preparation	Sequencing platform	Read depth	Reference genome	Analysis pipeline
((Youness et al., 2025) , the Current study)	4 NF 5 ICM 4 DCM	DAPI-positive nuclei were separated from cell debris using a BD Influx flow cytometer.	Chromium Single-cell 3' Library Version 3 Chemistry	Illumina Novaseq 6000	80,000 reads per nucleus.	hg38	CellRanger V6.0.2 CellBender V0.2.2 Doublets removed by scDbtFinder
(Chaffin, 2022)	16 NF 8 DCM 12 HCM	Not sorted by FACS, just stained by trypan blue	Chromium Single-cell 3' Library Version 3 Chemistry	Nextseq550	~13,000 reads per nucleus	GRCh38 pre-mRNA human reference (V2020-A)	CellRanger V4.0.0 CellBender V0.2 Doublets removed by Scublet
(Litviňuková, 2020)	14 NF	Hoechst+ nuclei sorted by XDP or FACS Aria (BD Biosciences)	Chromium Single-cell 3' Library Version 3 Chemistry	HiSeq 4000 (Illumina) at Wellcome Sanger Institute, and NextSeq 500 (Illumina) at Harvard Medical School	20,000–30,000 reads per nucleus	GRCh38 V3.0.0	CellRanger V3.0.1 Doublets removed by scrublet
(Koenig, 2022)	23 NF 8 DCM	DRAQ5+ nuclei were sorted into Nuclei Wash Buffer on a BD FACS Melody (BD Biosciences) using a 100-µM nozzle.	Chromium Single-cell 5' Library Version 1.1 Chemistry	Illumina Novaseq 6000	100,000 reads per nucleus	GRCh38 pre-mRNA transcriptome	CellRanger V3 Doublets removed by Scrublet
(Kuppe, 2022)	4 NF 25 ICM	The nuclei were stained with DAPI and FANS sorted using a Sony SH800 to enrich the nuclei.	Chromium Single-cell 3' Library Version 3 Chemistry	Illumina Novaseq 6000	50,000 reads per nucleus	hg38	CellRanger V6.0.2
NF: Non-failing heart, ICM: Ischemic cardiomyopathy, HCM: Hypertrophic cardiomyopathy, DCM: Dilated cardiomyopathy							

SUPPLEMENTARY DATA

- Chaffin, M., Papangelis, I., Simonson, B. et al. (2022). Single-nucleus profiling of human dilated and hypertrophic cardiomyopathy. *Nature*, 608, 174–180.
- Koenig, A. L., Shchukina, I., Amrute, J. et al. (2022). Single-cell transcriptomics reveals cell-type-specific diversification in human heart failure. *Nat Cardiovasc Res*, 1, 263–280.
- Kuppe, C., Ramirez Flores, R.O., Li, Z. et al. (2022). Spatial multi-omic map of human myocardial infarction. *Nature*, 608, 766–777.
- Litviňuková, M., Talavera-López, C., Maatz, H. (2020). Cells of the adult human heart. *Nature*, 588, 466–472
- Youness, M., Ekhteraei-Tousi, S., Nagaraju, C. K., Puertas, R. D., Thienpont, B., Rega, F., Sipido, K. R., & Roderick, H. L. (2025). Location and aetiology are determinants of fibroblast activation and heterogeneity in the failing human heart. *Genome Med*, 17(1), 155. <https://doi.org/10.1186/s13073-025-01580-z>

SUPPLEMENTARY DATA

Table 4. TFs and their targets among the detected DEGs in each pairwise comparison between study groups in humans (10x data).

	DEGs	TFs	NES	#Targets
Old vs Young	Up_reg	HMGA2	2.5	50
	Down_reg	-	-	-
ICM vs NF	Up_reg	TEAD4 BCL6B ETV5 NFATC2 ELF4 ELK3 ERG FLI1 RELA	4.04 3.29 2.92 2.91 2.88 2.88 2.88 2.88 2.73	176 123 116 102 178 178 178 178 339
	Down_reg	-	-	-
HCM vs NF	Up_reg	PRDM6 MECOM BCL6B POU2F2 SPI1 ELK3 ERG ETV4 FLI1	3.05 2.88 2.86 2.73 2.64 2.54 2.54 2.54 2.54	528 76 628 56 189 123 123 123 123
	Down_reg	ETS2 HOXB2 ATF3 ATF4 ELF3 XBP1 JUN	5.90 3.66 3.11 3.11 3.73 2.93 2.87	394 506 528 528 551 405 234
DCM vs NF	Up_reg	-	-	-
	Down_reg	KLF4 CEBPB CEBPD KLF6 FOSL2 JUNB ATF3 KLF5 KLF2	3.94 3.07 3.07 3.03 2.96 2.96 2.86 2.85 2.54	327 43 43 154 63 63 109 365 184

NF: Non-failing heart, ICM: Ischemic cardiomyopathy, HCM: Hypertrophic cardiomyopathy, DCM: Dilated cardiomyopathy, Up_reg: Up-regulated genes, Down_reg: Down-regulated genes, TFs: Transcription factors, NES: Normalized Enrichment Score.

SUPPLEMENTARY DATA

Table 5. Cardiac functional and structural parameters of Y and O rats

	Young (Mean±SEM)	Old (Mean±SEM)	t-test (P-value) Young vs Old
Tissue Parameters			
BW (g)	437.67±8.90 (n=6)	523.67±12.85 (n=6)	0.0003 ***
TL3 (mm3)	145491.73±2962.26 (n=6)	161611.02±1682.76 (n=6)	0.0008 ***
HW (g)	1.89±0.07 (n=6)	2.49±0.09 (n=6)	0.0003 ***
HW/TL (g/mm)	0.04±0.00 (n=6)	0.05±0.00 (n=6)	0.0010 **
HW/TL3 (mg/mm3)	0.01±0.00 (n=6)	0.02±0.00 (n=6)	0.0221 *
LW (g)	1.98±0.04 (n=6)	2.38±0.09 (n=6)	0.0022 **
LW/TL (g/mm)	0.04±0.00 (n=6)	0.04±0.00 (n=6)	0.0078 **
LW/TL3 (mg/mm3)	0.01±0.00 (n=6)	0.01±0.00 (n=6)	
Echocardiography			
IVSd (mm)	1.71±0.07 (n=8)	1.86±0.10 (n=5)	
IVSs (mm)	2.88±0.09 (n=8)	2.75±0.19 (n=5)	
LVIDd (mm)	7.69±0.12 (n=8)	8.18±0.28 (n=5)	
LVIDs (mm)	4.57±0.24 (n=8)	5.84±0.28 (n=5)	0.0062 **
LVPWd (mm)	1.82±0.07 (n=8)	1.90±0.14 (n=5)	
PWs (mm)	3.03±0.15 (n=8)	2.66±0.13 (n=5)	
LAD (mm)	3.41±0.22 (n=8)	4.50±0.37 (n=5)	0.0196 *
FS	0.41±0.03 (n=8)	0.29±0.02 (n=5)	0.0076 **

	Young (Mean±SEM)	Old (Mean±SEM)	t-test (P-value) Young vs Old
Doppler			
PSTV (mm/s)	68.59±2.73 (n=8)	45.15±1.49 (n=5)	<0.0001 ****
e' (mm/s)	72.66±3.25 (n=8)	47.98±2.49 (n=5)	0.0002 ***
E/e'	0.01±0.00 (n=8)	0.02±0.00 (n=5)	0.0108 *
MV e (m/s)	0.98±0.05 (n=8)	0.84±0.04 (n=5)	
MV a (m/s)	0.93±0.06 (n=8)	0.72±0.03 (n=5)	0.0191 *
MitDec (m/s2)	2317.50±99.94 (n=8)	2932.00±153.67 (n=5)	0.0048 **
E/A	1.06±0.03 (n=8)	1.16±0.03 (n=5)	0.0380 *
Pvmax (m/s)	0.85±0.08 (n=8)	0.72±0.03 (n=5)	
PAT (ms)	25.35±1.63 (n=8)	30.90±2.19 (n=5)	
AoVTI (mm)	48.35±2.82 (n=8)	48.55±6.01 (n=5)	
LVOTmax (m/s)	1.07±0.04 (n=8)	0.94±0.16 (n=5)	
LVOTdiam (mm)	3.27±0.16 (n=8)	3.13±0.09 (n=4)	
HR (bpm)	388.50±7.81 (n=8)	335.80±7.49 (n=5)	0.0008 ***
Strain			
GLS (%)	-19.95±1.24 (n=8)	-15.74±1.67 (n=5)	
GCS (%)	-29.73±1.70 (n=8)	-23.72±1.89 (n=5)	0.0429 *
SRs longitudinal (%/s)	828.38±73.40 (n=8)	501.23±34.09 (n=5)	0.0066 **
SRe longitudinal (%/s)	-688.29±36.84 (n=7)	-519.67±54.91 (n=5)	0.0239 *

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SRs circumferential (%/s)	-1411.42±83.71 (n=8)	-661.20±83.92 (n=5)	<0.0001 ****
SRe circumferential (%/s)	1139.54±94.54 (n=8)	654.07±71.45 (n=5)	0.0039 **
Hemodynamics			
SBT (mmHg)	114.00±4.05 (n=8)	181.40±7.03 (n=5)	<0.0001 ****
DBT (mmHg)	83.00±3.83 (n=8)	111.80±6.40 (n=5)	0.0016 **

Table 6. Cardiac functional and structural parameters for rats subjected to AB

				Bonferroni's multiple comparisons test (P-value)	
	<i>Sham (Mean±SEM)</i>	<i>Loose (Mean±SEM)</i>	<i>Tight (Mean±SEM)</i>	Sham vs Loose	Sham vs Tight
Tissue Parameters					
BW (g)	469.67±14.83 (n=9)	453.38±9.67 (n=8)	366.57±8.35 (n=7)		<0.0001 ****
TL3 (mm ³)	58904.47±976.66 (n=12)	59801.32±1281.96 (n=9)	50742.75±543.39 (n=12)		<0.0001 ****
HW (g)	1.23±0.05 (n=12)	1.51±0.04 (n=9)	2.01±0.05 (n=12)	0.0008 ***	<0.0001 ****
HW/TL (g/mm)	0.03±0.00 (n=12)	0.04±0.00 (n=9)	0.05±0.00 (n=12)	0.0009 ***	<0.0001 ****
HW/TL3 (mg/mm ³)	0.02±0.00 (n=12)	0.03±0.00 (n=9)	0.04±0.00 (n=12)	0.0025 **	<0.0001 ****
LVW (g)	0.86±0.03 (n=12)	1.12±0.03 (n=9)	1.20±0.03 (n=12)	<0.0001 ****	<0.0001 ****
LVW/TL (g/mm)	0.02±0.00 (n=12)	0.03±0.00 (n=9)	0.03±0.00 (n=12)	<0.0001 ****	<0.0001 ****
LW (g)	1.71±0.04 (n=12)	1.72±0.05 (n=9)	3.10±0.22 (n=12)		<0.0001 ****
LW/TL (g/mm)	0.04±0.00 (n=12)	0.04±0.00 (n=9)	0.08±0.01 (n=12)		<0.0001 ****
LW/TL3 (mg/mm ³)	0.03±0.00 (n=12)	0.03±0.00 (n=9)	0.06±0.00 (n=12)		<0.0001 ****

Echocardiography

IVSd (mm)	1.54±0.05 (n=12)	1.87±0.05 (n=9)	1.96±0.05 (n=12)	0.0001 ***	<0.0001 ****
IVSs (mm)	2.76±0.11 (n=12)	3.30±0.07 (n=9)	2.92±0.14 (n=12)	0.0089 **	
LVIDd (mm)	8.12±0.12 (n=12)	7.89±0.12 (n=9)	8.81±0.16 (n=12)		0.0020 **
LVIDs (mm)	4.83±0.17 (n=12)	4.25±0.26 (n=9)	6.48±0.24 (n=12)		<0.0001 ****
LVPWd (mm)	1.62±0.03 (n=12)	1.92±0.03 (n=9)	2.07±0.04 (n=12)	<0.0001 ****	<0.0001 ****
PWs (mm)	3.16±0.08 (n=12)	3.58±0.14 (n=9)	3.04±0.11 (n=12)	0.0290 *	
LAD (mm)	3.52±0.06 (n=12)	3.70±0.05 (n=9)	6.17±0.13 (n=12)		<0.0001 ****
FS	0.41±0.02 (n=12)	0.47±0.03 (n=9)	0.27±0.02 (n=12)		<0.0001 ****
SV (μL)	421.45±18.69 (n=12)	410.57±11.36 (n=9)	404.53±16.46 (n=12)		

				Bonferroni's multiple comparisons test (P-value)	
	<i>Sham (Mean±SEM)</i>	<i>Loose (Mean±SEM)</i>	<i>Tight (Mean±SEM)</i>	Sham vs Loose	Sham vs Tight
Echocardiography					
PSTV (mm/s)	50.68±2.06 (n=12)	43.72±1.62 (n=9)	31.66±0.98 (n=12)	0.0133 *	<0.0001 ****
e' (mm/s)	35.82±2.80 (n=12)	35.04±2.27 (n=9)	42.38±3.67 (n=12)		
E/e'	0.03±0.00 (n=12)	0.03±0.00 (n=9)	0.03±0.00 (n=12)		
MV e (m/s)	0.94±0.04 (n=12)	1.01±0.05 (n=9)	1.36±0.05 (n=12)		<0.0001 ****

SUPPLEMENTARY DATA

MV a (m/s)	0.85±0.06 (n=12)	0.95±0.05 (n=9)	1.10±0.07 (n=12)		0.0148 *
MitDec (m/s ²)	2897.00±186.98 (n=12)	2846.67±237.41 (n=9)	6001.33±248.76 (n=12)		<0.0001 ****
E/A	1.14±0.06 (n=12)	1.08±0.05 (n=9)	1.28±0.07 (n=12)		
Pvmax (m/s)	1.00±0.03 (n=12)	1.20±0.07 (n=9)	0.76±0.04 (n=12)	0.0107 *	0.0013 **
PAT (ms)	28.96±1.48 (n=12)	30.29±1.67 (n=9)	18.82±0.62 (n=12)		<0.0001 ****
AoVTI (mm)	59.96±4.18 (n=12)	61.35±2.09 (n=9)	47.20±2.89 (n=12)		0.0174 *
LVOTmax (m/s)	1.30±0.08 (n=12)	1.06±0.05 (n=9)	0.62±0.04 (n=12)	0.0233 *	<0.0001 ****
LVOTdiam (mm)	2.93±0.06 (n=12)	2.79±0.06 (n=9)	2.48±0.06 (n=11)		<0.0001 ****
HR (bpm)	370.25±10.04 (n=12)	351.22±12.36 (n=9)	325.50±6.90 (n=12)		0.0035 **

Strain

GLS (%)	-14.63±0.79 (n=11)	-11.22±1.33 (n=9)	-2.63±0.43 (n=12)	0.0203	<0.0001 ****
GCS (%)	16.86±0.65 (n=11)	15.70±0.99 (n=9)	9.88±0.46 (n=12)		<0.0001 ****
SRs longitudinal (%/s)	317.01±15.15 (n=11)	222.29±20.00 (n=9)	93.46±11.50 (n=12)	0.0004 ***	<0.0001 ****
SRe longitudinal (%/s)	455.33±40.72 (n=11)	363.57±28.25 (n=9)	274.58±22.91 (n=12)		0.0005 ***
SRs circumferential (%/s)	347.51±13.26 (n=11)	281.55±10.60 (n=9)	177.75±4.35 (n=12)	0.0002 ***	<0.0001 ****
SRe circumferential (%/s)	417.15±25.02 (n=11)	375.68±33.10 (n=9)	497.58±24.18 (n=12)		

SUPPLEMENTARY DATA

Table 7. Transcription factors and their targets among the detected DEGs for each comparison of conditions in rats (VASA-Seq data).

	DEGs	TFs	NES	#Targets
Old vs Young	Up_reg	Myef2 Tef Sp3 Gtf2f1 Atf6b Srf Taf1 Sp100 Klf2 Klf12 Trps1	3.05 3.12 3.15 2.87 2.69 2.63 2.60 2.56 2.55 2.52 2.50	147 149 552 100 34 58 35 241 544 103 273
	Down_reg	Pbx1 Rarb Thra	3.39 3.26 3.26	21 13 13
Loose_AB vs Sham	Up_reg	Elk1 Taf1 Gabpb1 Ets1 Elk3 Dnmt3a Epas1 Hcfc1 Atf4 Setdb1 Atf2	3.38 2.86 2.81 2.79 2.78 2.70 2.63 2.56 2.56 2.55 2.5	102 823 587 115 307 365 151 119 129 164 90
	Down_reg	Irf9 Irf1 Stat1 Ezr	4.06 3.98 2.87 2.76	39 55 206 36
Tight_AB vs Sham	Up_reg	Taf1 Kdm5a Sin3a Elk1 Zmiz1 Chd1 Arntl	3.58 3.08 2.82 2.76 2.65 2.58 2.51	969 212 784 213 1061 145 149
	Down_reg	Six4 Zfp3 Egr1 Wt1 Gata5 Myc	2.91 2.84 2.82 2.82 2.72 2.58	76 73 54 54 27 15

NF: Non-failing heart, ICM: Ischemic cardiomyopathy, HCM: Hypertrophic cardiomyopathy, DCM: Dilated cardiomyopathy, Up_reg: Up_regulated genes, Down_reg: Down_regulated genes, TFs: Transcription factors, NES: Normalized Enrichment Score, AB: Aortic banding.

SUPPLEMENTARY DATA

Supplementary Data Files 1-27:

Please find below and for larger files at: <https://doi.org/10.48804/OMC9EK>

Supplementary File 1. Human Samples used for RT-qPCR.

Group	Young	Old	Age-matched	ICM	HCM	DCM
Total number (female)	37 (19)	88 (43)	41 (19)	41 (5)	22 (9)	62 (21)
Age (years)	34 ± 8	73 ± 6	55 ± 5	58 ± 11	57 ± 11	50 ± 16
Height (cm)	173 ± 10	170 ± 9	171 ± 9	175 ± 11	174 ± 11	172 ± 22
Weight (kg)	74 ± 19	75 ± 15	80 ± 18	84 ± 16	88 ± 16	79 ± 23
BMI (kg/m ²)	25 ± 6	26 ± 4	27 ± 6	27 ± 5	29 ± 5	26 ± 5
EF (%)				19 ± 10	61 ± 10	19 ± 9
Plasma NT-proBNP (ng/L)				2363 ± 1974	2095 ± 1974	4289 ± 8004
LVIDs (mm)				58 ± 8	30 ± 8	57 ± 12
LVIDd (mm)				65 ± 8	42 ± 8	64 ± 11
LVPWDd (mm)				9 ± 7	15 ± 7	9 ± 2
LVAD				11 (30)	0 (22)	21 (41)
CRT				32 (9)	5 (17)	49 (13)
ACEi, ARBs				30 (11)	6 (16)	37 (25)
BB				37 (4)	5 (17)	49 (13)
Anti-arrhythmics				19 (22)	3 (19)	29 (33)
Nitrates / hydralazine				10 (31)	1 (21)	8 (54)
Thyroid medication				4 (37)	3 (19)	16 (46)
Anti-coagulants				26 (15)	6 (16)	48 (14)
Anti-aggregant				33 (8)	5 (17)	25 (37)
Lipid lowering				30 (11)	5 (17)	22 (40)
Diuretics				31 (10)	2 (20)	47 (15)

Young (20-44 years), Old (65 -99 years), age -matched control group (45 -64 years), ICM: ischemic cardiomyopathy, HCM: hypertrophic cardiomyopathy, DCM: dilated cardiomyopathy, BMI: body mass index, EF: ejection fraction, BNP: brain natriuretic peptide, LVIDs: left ventricular internal diameter at end systole, LVIDd: left ventricular internal diameter at end diastole, LVPWDd:left ventricular posterior wall diameter at end diastole. Data are shown as mean ± standard deviation (SD).

LVAD: left ventricular assist device (Heartmate II, Heartmate III, Heartware, ECMO + Impella), CRT: cardiac resynchronization therapy (CRT- defibrillator, implantable cardioverter defibrillator , dual chamber Implantable Cardioverter Defibrillator), ACEi: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker (perindopril, ramipril, candesartan, LCZ696, telmisartan, captopril, losartan, lisinopril, Zestril), BB: beta blockers (bisoprolol, carvedilol, sotalol, nebivolol, selozok), anti-arrhythmics (amiodarone, diltiazem, digoxin, Procortalan, ivabradine, flecainide, disopyramide), nitrates (Isosorbide dinitrate , hydralazine), Thyroid medication (levothyroxine, strumazol), Anticoagulants (warfarin, vitamin k antagonist , apixaban, enoxaparin, dabigatran, rivaroxaban, phenprocoumon), antiaggregants (prasugrel, acetylsalicylic acid , clopidogrel, Ticagrelor), lipid lowering drugs (rosuvastatin, simvastatin, pravastatin, atorvastatin, ezetimibe), diruetics (furosemide, bumetanide, acetazolamide, Hydrochlorothiazide). Data are shown as number of patients with (number without).

SUPPLEMENTARY DATA

Supplementary File 2. HCA Label transfer confidence.

Prediction Score Summary.

predicted.id	n_nuclei	median_predi	mean_predict	q25_predictio	q75_predictio	percent_score	percent_score	percent_score_above_0.8
vCM1	255804	0.82694385	0.79816568	0.69068476	0.92334975	97.3084862	73.5856359	55.7094494
vCM2	14229	0.64888905	0.67653565	0.55152677	0.78867378	91.8195235	39.3843559	23.4731886
vCM3	43364	0.70373693	0.71841216	0.5767183	0.85712632	94.0111613	50.6433908	33.8068444
vCM4	7670	0.62068867	0.64688592	0.50463877	0.78435125	76.2451108	36.6101695	23.0638853
vCM5	3072	0.60005878	0.61546885	0.51865014	0.70303841	80.8268229	25.6510417	10.2213542

Label By Study

study	predicted.id	n_nuclei	total_nuclei_study	proportion
Chaffin_study	vCM1	116007	140777	0.82404796
Chaffin_study	vCM2	4061	140777	0.02884704
Chaffin_study	vCM3	18833	140777	0.13377896
Chaffin_study	vCM4	44	140777	0.00031255
Chaffin_study	vCM5	1832	140777	0.01301349
Current_study	vCM1	8185	10954	0.74721563
Current_study	vCM2	170	10954	0.01551944
Current_study	vCM3	2267	10954	0.20695636
Current_study	vCM4	201	10954	0.01834946
Current_study	vCM5	131	10954	0.0119591
Koenig_study	vCM1	26569	37678	0.70515951
Koenig_study	vCM2	1618	37678	0.04294283
Koenig_study	vCM3	5785	37678	0.15353787
Koenig_study	vCM4	3706	37678	0.09835979
Kuppe_study	vCM1	52473	64509	0.81342138
Kuppe_study	vCM2	670	64509	0.01038615
Kuppe_study	vCM3	9113	64509	0.14126711
Kuppe_study	vCM4	1205	64509	0.01867956
Kuppe_study	vCM5	1048	64509	0.0162458
Litviňuková_HCA	vCM1	52570	70221	0.74863645
Litviňuková_HCA	vCM2	7710	70221	0.10979621
Litviňuková_HCA	vCM3	7366	70221	0.1048974
Litviňuková_HCA	vCM4	2514	70221	0.03580126
Litviňuková_HCA	vCM5	61	70221	0.00086869

Label by Condition

condition	predicted.id	n_nuclei	total_nuclei_condition	proportion
DCM	vCM1	21710	32672	0.66448335
DCM	vCM2	68	32672	0.00208129
DCM	vCM3	10618	32672	0.32498776
DCM	vCM4	198	32672	0.00606024
DCM	vCM5	78	32672	0.00238737
HCM	vCM1	41536	52639	0.78907274
HCM	vCM2	396	52639	0.00752294

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HCM	vCM3	9302	52639	0.17671308
HCM	vCM4	25	52639	0.00047493
HCM	vCM5	1380	52639	0.0262163
ICM	vCM1	39347	50495	0.77922567
ICM	vCM2	719	50495	0.01423903
ICM	vCM3	8235	50495	0.16308545
ICM	vCM4	1094	50495	0.02166551
ICM	vCM5	1100	50495	0.02178434
NF	vCM1	153211	188333	0.81351117
NF	vCM2	13046	188333	0.06927092
NF	vCM3	15209	188333	0.0807559
NF	vCM4	6353	188333	0.0337328
NF	vCM5	514	188333	0.00272921

Supplementary File 3. Human vCMClusters. Marker genes.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 4. Donor level CM heterogeneity scores

donor id	study	sample	analysis group	donor median distance	donor mean distance	n_cm nuclei
Chaffin study P1300	Chaffin study	P1300	DCM	7.55806646	8.27618663	2797
Chaffin study P1304	Chaffin study	P1304	DCM	6.60518828	8.1107849	4845
Chaffin study P1371	Chaffin study	P1371	DCM	6.37765308	6.91862639	4782
Chaffin study P1422	Chaffin study	P1422	HCM	7.44988086	8.05439819	7068
Chaffin study P1430	Chaffin study	P1430	DCM	7.55303024	8.4391151	3100
Chaffin study P1437	Chaffin study	P1437	DCM	7.58037306	8.70628797	3268
Chaffin study P1447	Chaffin study	P1447	HCM	11.383011	13.8580584	2171
Chaffin study P1462	Chaffin study	P1462	HCM	8.06028296	9.09882932	4503
Chaffin study P1504	Chaffin study	P1504	DCM	7.79974478	9.00256705	1263
Chaffin study P1508	Chaffin study	P1508	HCM	7.33566779	8.5754955	7946
Chaffin study P1510	Chaffin study	P1510	HCM	8.82160847	11.1876163	3034
Chaffin study P1515	Chaffin study	P1515	NF	6.63944904	7.18648065	3881
Chaffin study P1516	Chaffin study	P1516	Old	7.51544087	9.11159591	2555
Chaffin study P1539	Chaffin study	P1539	NF	6.17661471	6.9025716	3283
Chaffin study P1540	Chaffin study	P1540	Old	6.65492054	7.60462297	5768
Chaffin study P1547	Chaffin study	P1547	NF	6.62448494	7.31406801	3196
Chaffin study P1549	Chaffin study	P1549	NF	7.41709324	8.05179264	3331
Chaffin study P1558	Chaffin study	P1558	NF	6.81726843	7.42438002	4254
Chaffin study P1561	Chaffin study	P1561	Old	6.65115085	7.19574178	2193
Chaffin study P1582	Chaffin study	P1582	NF	6.93821591	7.45182669	5071
Chaffin study P1600	Chaffin study	P1600	NF	7.45243233	8.0197876	6467
Chaffin study P1602	Chaffin study	P1602	HCM	9.28407621	10.5143303	7946
Chaffin study P1603	Chaffin study	P1603	Old	6.84475494	7.46043325	4410
Chaffin study P1606	Chaffin study	P1606	DCM	6.79267181	8.04429595	1090
Chaffin study P1610	Chaffin study	P1610	NF	10.258446	11.0987323	3871

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Chaffin study P1617	Chaffin study P1617	DCM	6.65291501	7.40045246	3807
Chaffin study P1622	Chaffin study P1622	NF	6.70123066	7.30896475	1313
Chaffin study P1630	Chaffin study P1630	HCM	10.9321553	13.010254	4897
Chaffin study P1631	Chaffin study P1631	HCM	9.24471614	11.2771193	2892
Chaffin study P1678	Chaffin study P1678	NF	9.23082403	10.6233866	3019
Chaffin study P1685	Chaffin study P1685	HCM	9.21613766	11.558623	2104
Chaffin study P1702	Chaffin study P1702	NF	5.38296828	5.93350696	8424
Chaffin study P1718	Chaffin study P1718	Young	6.86115469	7.54399862	2150
Chaffin study P1722	Chaffin study P1722	HCM	8.16459909	9.22922574	4382
Chaffin study P1726	Chaffin study P1726	HCM	9.83397405	10.9117558	4952
Chaffin study P1735	Chaffin study P1735	HCM	9.8144398	12.4920386	744
Current study DCM 44	Current study DCM 44	DCM	9.96541139	13.1907873	363
Current study DCM 65	Current study DCM 65	DCM	10.7237708	13.3904045	792
Current study DCM 83	Current study DCM 83	DCM	7.8812343	9.94121026	447
Current study HD 25	Current study HD 25	Old	5.03911239	5.67959777	776
Current study HD 34	Current study HD 34	NF	5.89395724	6.64311694	889
Current study HD 51	Current study HD 51	NF	6.51893447	7.01130084	623
Current study HD 55	Current study HD 55	NF	5.31662292	6.30592724	665
Current study__LAD_IC M 38	Current study LAD ICM 38	ICM	7.05738701	8.21558629	1857
Current study__LAD_IC M 43	Current study LAD ICM 43	ICM	8.72694041	12.4318198	325
Current study__LAD_IC M 45	Current study LAD ICM 45	ICM	7.19285209	8.64522275	426
Current study__LAD_IC M 68	Current study LAD ICM 68	ICM	7.08384836	9.65695072	369
Current study__SCAR_D CM 152	Current study SCAR_DCM_152	DCM	7.5419019	10.428563	2103
Koenig study__H_ZC-11-292	Koenig study H_ZC-11-292	Old	7.54530009	8.14330842	1808
Koenig study__H_ZC-LVAD	Koenig study H_ZC-LVAD	DCM	7.11136678	7.72175427	973
Koenig study__TWCM-10-5	Koenig study TWCM-10-5	DCM	7.1404572	7.72119909	195
Koenig study__TWCM-10-68	Koenig study TWCM-10-68	NF	7.2834311	7.85015974	693
Koenig study__TWCM-11-103	Koenig study TWCM-11-103	NF	7.24261889	7.85902993	2650
Koenig study__TWCM-11-104	Koenig study TWCM-11-104	Old	8.67461655	8.92491281	543
Koenig study__TWCM-11-192	Koenig study TWCM-11-192	NF	6.33563443	6.75904192	1178
Koenig study__TWCM-11-256	Koenig study TWCM-11-256	Young	6.1037704	6.54187382	1732
Koenig study__TWCM-11-264	Koenig study TWCM-11-264	Old	6.32009329	6.80433762	3146
Koenig study__TWCM-11-3	Koenig study TWCM-11-3	DCM	6.85068194	7.60077987	354
Koenig study__TWCM-11-41	Koenig study TWCM-11-41	NF	6.51114733	6.92711959	1834

SUPPLEMENTARY DATA

Koenig_study__TWCM-11-42	Koenig_study	TWCM-11-42	NF	6.46359599	7.15720219	1065
Koenig_study__TWCM-11-74	Koenig_study	TWCM-11-74	Old	8.81499072	9.22128085	439
Koenig_study__TWCM-11-78	Koenig_study	TWCM-11-78	Young	6.60911266	7.30796531	3511
Koenig_study__TWCM-11-82	Koenig_study	TWCM-11-82	Young	5.88995319	6.25520157	855
Koenig_study__TWCM-13-1	Koenig_study	TWCM-13-1	NF	7.96840621	8.82350184	1039
Koenig_study__TWCM-13-101	Koenig_study	TWCM-13-101	NF	5.81073222	6.17933035	2748
Koenig_study__TWCM-13-104	Koenig_study	TWCM-13-104	NF	7.55428257	8.09505817	365
Koenig_study__TWCM-13-132	Koenig_study	TWCM-13-132	NF	6.20738362	7.02065902	427
Koenig_study__TWCM-13-152	Koenig_study	TWCM-13-152	Young	6.35582624	6.88882872	1603
Koenig_study__TWCM-13-168	Koenig_study	TWCM-13-168	Old	7.15120514	7.69685513	529
Koenig_study__TWCM-13-17	Koenig_study	TWCM-13-17	DCM	6.38905399	7.12258699	221
Koenig_study__TWCM-13-192	Koenig_study	TWCM-13-192	Young	7.4723252	7.92715672	769
Koenig_study__TWCM-13-208	Koenig_study	TWCM-13-208	DCM	8.20524599	8.72426152	357
Koenig_study__TWCM-13-36	Koenig_study	TWCM-13-36	Old	5.89151841	6.17960501	2623
Koenig_study__TWCM-13-80	Koenig_study	TWCM-13-80	NF	6.05852651	6.67823813	1071
Koenig_study__TWCM-13-84	Koenig_study	TWCM-13-84	DCM	8.12834798	8.98902013	169
Koenig_study__TWCM-13-96	Koenig_study	TWCM-13-96	Young	6.29274198	6.81245871	2046
Koenig_study__TWCM-14-173	Koenig_study	TWCM-14-173	NF	5.99576018	6.53956278	989
Koenig_study__TWCM-LVAD2	Koenig_study	TWCM-LVAD2	DCM	7.17887416	7.77323161	511
Koenig_study__TWCM-LVAD3	Koenig_study	TWCM-LVAD3	DCM	9.188205	11.1373996	1235
Kuppe_study__CK158	Kuppe_study	CK158	Young	5.84741554	6.30035901	3880
Kuppe_study__CK159	Kuppe_study	CK159	ICM	6.55051421	7.17951418	2653
Kuppe_study__CK160	Kuppe_study	CK160	ICM	8.33736463	8.77331241	3813
Kuppe_study__CK161	Kuppe_study	CK161	ICM	6.6471885	6.91613777	110
Kuppe_study__CK164	Kuppe_study	CK164	ICM	6.63819759	7.19623206	3243
Kuppe_study__CK356	Kuppe_study	CK356	ICM	5.70632477	6.30274333	4346
Kuppe_study__CK357	Kuppe_study	CK357	NF	5.71382285	6.23373679	6478
Kuppe_study__CK358	Kuppe_study	CK358	Young	6.31730609	6.91080361	3956
Kuppe_study__CK360	Kuppe_study	CK360	ICM	6.80486527	7.88939379	8269
Kuppe_study__CK362	Kuppe_study	CK362	ICM	5.35089526	6.22112094	2487
Kuppe_study__CK363	Kuppe_study	CK363	ICM	9.26350338	9.78437296	1123
Kuppe_study__CK365	Kuppe_study	CK365	ICM	9.47661604	9.83557065	41

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Kuppe_study CK366	Kuppe_study	CK366	ICM	6.1651469	6.82885378	3804
Kuppe_study CK367	Kuppe_study	CK367	ICM	9.15181044	10.8517234	314
Kuppe_study CK368	Kuppe_study	CK368	ICM	9.26820808	10.4090704	415
Kuppe_study CK370	Kuppe_study	CK370	ICM	8.33218223	9.10751699	359
Kuppe_study CK372	Kuppe_study	CK372	ICM	6.7444982	7.12983583	578
Kuppe_study CK374	Kuppe_study	CK374	NF	5.9688801	6.39089554	3996
Kuppe_study CK375	Kuppe_study	CK375	ICM	6.36042614	6.67462646	250
Litviňuková HCA D1	Litviňuková_HCA	D1	NF	5.46431782	5.73459418	5000
Litviňuková HCA D11	Litviňuková_HCA	D11	NF	7.14205103	7.81439009	2080
Litviňuková HCA D2	Litviňuková_HCA	D2	NF	6.27951659	6.62349887	6933
Litviňuková HCA D3	Litviňuková_HCA	D3	NF	10.7849413	11.4379545	2216
Litviňuková HCA D4	Litviňuková_HCA	D4	Old	6.04184658	6.45472325	3610
Litviňuková HCA D5	Litviňuková_HCA	D5	Old	5.3590875	5.63797803	3898
Litviňuková HCA D6	Litviňuková_HCA	D6	Old	7.08430286	7.49337135	2806
Litviňuková HCA D7	Litviňuková_HCA	D7	NF	6.54880818	7.0326081	2941
Litviňuková HCA H2	Litviňuková_HCA	H2	NF	6.60581623	7.20021094	3055
Litviňuková HCA H3	Litviňuková_HCA	H3	NF	6.90319702	7.50539266	6965
Litviňuková HCA H4	Litviňuková_HCA	H4	NF	6.30759065	6.66857149	6370
Litviňuková HCA H5	Litviňuková_HCA	H5	NF	6.69517217	7.03274951	9529
Litviňuková HCA H6	Litviňuková_HCA	H6	Young	6.59071108	7.096141	9412
Litviňuková HCA H7	Litviňuková_HCA	H7	NF	6.56502589	6.88992387	5406

Statistical comparison between O vs Y at nucleus level.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif	comparison_t	comparison	p.adj.label
cell_distance	Young	Old	29914	35104	412897090		0	0	****	Nuclei-level Old vs Young	<0.0001

Statistical comparison between NF vs and CMP at donor level.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif	comparison_t	comparison	p.adj.label
donor_media	NF	ICM	37	19	244	0.064	0.064	ns	Donor-level	ICM vs NF	0.064
donor_media	NF	HCM	37	12	33	0.0000117	0.0000351	****	Donor-level	HCM vs NF	<0.0001
donor_media	NF	DCM	37	20	171	0.000903	0.001	**	Donor-level	DCM vs NF	0.001

SUPPLEMENTARY DATA

Supplementary File 5. Variance of human variable genes.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 6. Markers and GO of trajectories in human.

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj	cluster	gene
ANKRD1	4.51E-14	-0.175897249	0.796	0.813	6.46E-10	HCM_Old	ANKRD1
ATRNL1.1	1.25E-11	-0.104442562	0.287	0.32	1.79E-07	HCM_Old	ATRNL1
MYL2	2.24E-08	-0.129220232	0.562	0.585	0.000320822	HCM_Old	MYL2
ACTA1	5.88E-07	-0.153181186	0.295	0.314	0.008428751	HCM_Old	ACTA1
ANKRD1.1	5.51E-41	-0.239902706	0.793	0.816	7.89E-37	HCM	ANKRD1
FGF12	7.91E-38	0.100571628	0.966	0.949	1.13E-33	HCM	FGF12
TMEM178B	6.59E-30	0.105260256	0.835	0.805	9.45E-26	HCM	TMEM178B
ATP2A2	4.23E-29	0.101045885	0.894	0.876	6.06E-25	HCM	ATP2A2
CORIN	6.07E-27	0.110411303	0.597	0.547	8.7E-23	HCM	CORIN
CPNE5	1.04E-20	0.116646399	0.423	0.382	1.49E-16	HCM	CPNE5
PLCE1	4.34E-20	-0.148286874	0.417	0.449	6.22E-16	HCM	PLCE1
CNTNAP3B	6.92E-18	0.111328711	0.327	0.291	9.92E-14	HCM	CNTNAP3B
CREB5	6.08E-16	-0.141880537	0.576	0.598	8.71E-12	HCM	CREB5
ACTA1.1	1.03E-14	-0.210401402	0.29	0.317	1.48E-10	HCM	ACTA1
TNNT1	2.05E-13	-0.109962788	0.345	0.374	2.93E-09	HCM	TNNT1
BDNF	1.07E-12	-0.102383495	0.262	0.288	1.53E-08	HCM	BDNF
ATRNL1.2	1.24E-12	-0.107494938	0.294	0.321	1.78E-08	HCM	ATRNL1
CYP2J2	4.19E-12	-0.102888502	0.568	0.585	6.01E-08	HCM	CYP2J2
MYL2.1	2.65E-11	-0.143007255	0.567	0.585	3.8E-07	HCM	MYL2
RRAS2	1.03E-06	-0.101314227	0.447	0.46	0.014728982	HCM	RRAS2
TNNC1	1.91E-15	-0.150378597	0.177	0.215	2.73E-11	ICM_Old	TNNC1
ADGRL3	1.96E-12	0.12972758	0.443	0.407	2.81E-08	ICM_Old	ADGRL3
ACTA1.2	5.79E-12	-0.163863776	0.28	0.315	8.29E-08	ICM_Old	ACTA1
KCNMB2	7.02E-12	0.12429187	0.661	0.631	1.01E-07	ICM_Old	KCNMB2
TCAP	1.06E-09	-0.110532271	0.385	0.418	1.52E-05	ICM_Old	TCAP
LINC01151	1.03E-08	0.112051976	0.508	0.484	0.000148072	ICM_Old	LINC01151
PDE1A	1.37E-08	-0.127262769	0.391	0.421	0.000196408	ICM_Old	PDE1A
ST7	7.95E-08	-0.139138979	0.482	0.502	0.001139245	ICM_Old	ST7
PLCG2.1	3.69E-33	0.58541926	0.242	0.197	5.28E-29	DCM	PLCG2

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ANKRD1.2	2.51E-20	0.211689744	0.829	0.807	3.6E-16	DCM	ANKRD1
TNNC1.1	4.58E-19	0.124759281	0.242	0.205	6.56E-15	DCM	TNNC1
HSD17B12	1.78E-16	0.107559833	0.231	0.197	2.55E-12	DCM	HSD17B12
MYL2.3	7.27E-16	0.183304202	0.611	0.576	1.04E-11	DCM	MYL2
CNTNAP3B.1	6.46E-15	-0.127458667	0.268	0.305	9.25E-11	DCM	CNTNAP3B
ST7.1	1.96E-14	0.141680281	0.524	0.495	2.81E-10	DCM	ST7
ARL17B	5.66E-14	-0.124928136	0.274	0.305	8.11E-10	DCM	ARL17B
NID1	6.69E-10	-0.106506967	0.405	0.429	9.59E-06	DCM	NID1
ZNF385D	1.13E-09	0.162166221	0.263	0.239	1.62E-05	DCM	ZNF385D
NRXN3	3.79E-08	0.102395579	0.134	0.115	0.000543623	DCM	NRXN3
CHST9	1.17E-07	-0.100423555	0.268	0.291	0.001675232	DCM	CHST9
ACTA1.3	1.36E-07	0.106953819	0.331	0.307	0.001952807	DCM	ACTA1
PKN2.AS1	2.17E-06	0.142182076	0.152	0.136	0.031050267	DCM	PKN2.AS1
PLCE1.1	2.06E-74	0.377359014	0.497	0.43	2.95E-70	Old_CVDs	PLCE1
FGF12.1	1.8E-66	-0.15197593	0.932	0.957	2.58E-62	Old_CVDs	FGF12
CORIN.1	3.33E-65	-0.215802839	0.487	0.573	4.77E-61	Old_CVDs	CORIN
TNNI3K	1.15E-63	-0.162187104	0.796	0.862	1.65E-59	Old_CVDs	TNNI3K
LINC01151.1	3.59E-62	-0.207595817	0.418	0.5	5.14E-58	Old_CVDs	LINC01151
AUTS2	4.14E-59	-0.177056748	0.711	0.77	5.93E-55	Old_CVDs	AUTS2
PDE3A	6.12E-57	-0.113788116	0.966	0.978	8.76E-53	Old_CVDs	PDE3A
ANKRD1.3	2.64E-54	0.277124398	0.83	0.807	3.78E-50	Old_CVDs	ANKRD1
ACTA1.4	6.99E-50	0.376953894	0.358	0.301	1E-45	Old_CVDs	ACTA1
TMEM178B.1	7.12E-49	-0.145501553	0.773	0.82	1.02E-44	Old_CVDs	TMEM178B
IL1RAPL1	3.11E-48	-0.167055267	0.765	0.814	4.45E-44	Old_CVDs	IL1RAPL1
MLIP.AS1	2.44E-44	-0.151215692	0.681	0.742	3.5E-40	Old_CVDs	MLIP.AS1
RBFOX1	1.35E-43	-0.109655154	0.868	0.902	1.93E-39	Old_CVDs	RBFOX1
ADGRL2	1.4E-41	-0.113914438	0.807	0.851	2.01E-37	Old_CVDs	ADGRL2
PSD3	3.05E-39	-0.100368325	0.915	0.944	4.37E-35	Old_CVDs	PSD3
CBLB	1.83E-38	-0.108278845	0.888	0.921	2.62E-34	Old_CVDs	CBLB
FBXO32	4.29E-38	-0.131763907	0.707	0.76	6.14E-34	Old_CVDs	FBXO32
NPPB	7.7E-38	0.222196979	0.131	0.093	1.1E-33	Old_CVDs	NPPB
ATP2A2.1	9.26E-36	-0.115539095	0.853	0.886	1.33E-31	Old_CVDs	ATP2A2
KCNMB2.1	1.15E-35	-0.165397756	0.583	0.645	1.65E-31	Old_CVDs	KCNMB2
PPFIBP1	3.46E-34	-0.114469981	0.834	0.871	4.96E-30	Old_CVDs	PPFIBP1
GRAMD1B	4.08E-34	-0.138361415	0.702	0.747	5.85E-30	Old_CVDs	GRAMD1B

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CHST9.1	1.86E-33	-0.133681747	0.241	0.297	2.66E-29	Old_CVDs	CHST9
TPM1	4.28E-32	0.126005938	0.925	0.926	6.12E-28	Old_CVDs	TPM1
PHACTR1	2.21E-31	-0.112559607	0.835	0.875	3.17E-27	Old_CVDs	PHACTR1
EXT1	6.63E-31	0.214684839	0.722	0.704	9.5E-27	Old_CVDs	EXT1
ATRNL1.3	1.46E-30	0.178078516	0.356	0.306	2.09E-26	Old_CVDs	ATRNL1
CREB5.1	6.85E-30	0.206476204	0.617	0.588	9.81E-26	Old_CVDs	CREB5
SPHKAP	4.08E-29	-0.108228398	0.786	0.826	5.84E-25	Old_CVDs	SPHKAP
NUDT4	8.94E-29	-0.118188275	0.571	0.618	1.28E-24	Old_CVDs	NUDT4
CADPS2	1.57E-28	-0.115242717	0.144	0.187	2.25E-24	Old_CVDs	CADPS2
EDA	5.82E-28	-0.116919665	0.454	0.508	8.34E-24	Old_CVDs	EDA
PDE7B	5.92E-28	-0.118779294	0.402	0.458	8.48E-24	Old_CVDs	PDE7B
C5AR1	3.7E-27	0.111786746	0.17	0.133	5.29E-23	Old_CVDs	C5AR1
CYP2J2.1	6.43E-27	0.161020668	0.605	0.576	9.21E-23	Old_CVDs	CYP2J2
FARP1	8.43E-27	0.134192852	0.208	0.168	1.21E-22	Old_CVDs	FARP1
NALCN	1.22E-26	-0.105795852	0.512	0.566	1.75E-22	Old_CVDs	NALCN
GRK5	2.15E-26	-0.150886854	0.28	0.329	3.08E-22	Old_CVDs	GRK5
ATP13A3	3.34E-26	0.175895545	0.646	0.621	4.79E-22	Old_CVDs	ATP13A3
LINC01473	2.12E-25	-0.103377449	0.537	0.589	3.04E-21	Old_CVDs	LINC01473
DCP1B	7.53E-25	-0.112556037	0.603	0.652	1.08E-20	Old_CVDs	DCP1B
CALD1	9.27E-24	0.13161098	0.913	0.918	1.33E-19	Old_CVDs	CALD1
NEK10	2.66E-23	-0.102765679	0.545	0.588	3.8E-19	Old_CVDs	NEK10
CPNE4	1.41E-22	-0.116245976	0.329	0.374	2.03E-18	Old_CVDs	CPNE4
PRELID2	5.68E-22	-0.104237638	0.492	0.539	8.14E-18	Old_CVDs	PRELID2
SMAD6	6.66E-22	0.143507064	0.247	0.21	9.55E-18	Old_CVDs	SMAD6
SLCO5A1	1.16E-21	-0.103563265	0.528	0.57	1.66E-17	Old_CVDs	SLCO5A1
TNNT1.1	2.26E-21	0.162293581	0.4	0.361	3.23E-17	Old_CVDs	TNNT1
RALYL	2.97E-21	-0.100440689	0.649	0.694	4.25E-17	Old_CVDs	RALYL
PLIN5	1.05E-20	-0.106684784	0.477	0.519	1.51E-16	Old_CVDs	PLIN5
MYL2.4	1.22E-20	0.195089079	0.604	0.577	1.75E-16	Old_CVDs	MYL2
STAT3	1.58E-20	-0.126369427	0.568	0.602	2.27E-16	Old_CVDs	STAT3
LINC00702	2.96E-20	0.140947848	0.318	0.281	4.24E-16	Old_CVDs	LINC00702
EXOC6B	3.81E-20	0.11221998	0.956	0.967	5.45E-16	Old_CVDs	EXOC6B
ACTN2	4.11E-20	0.109968554	0.939	0.948	5.88E-16	Old_CVDs	ACTN2
RRAS2.1	5.74E-20	0.17593952	0.484	0.452	8.22E-16	Old_CVDs	RRAS2
MYO18B	1.19E-19	0.131998918	0.893	0.905	1.71E-15	Old_CVDs	MYO18B

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BDNF.1	5.18E-18	0.132792499	0.312	0.276	7.43E-14	Old_CVDs	BDNF
VCL	6.09E-17	0.169338086	0.781	0.784	8.73E-13	Old_CVDs	VCL
UBE2H	7.21E-17	0.127993677	0.814	0.819	1.03E-12	Old_CVDs	UBE2H
NT5C2	1.94E-16	0.146189812	0.695	0.691	2.78E-12	Old_CVDs	NT5C2
STK38L	2.03E-15	0.168020276	0.61	0.597	2.9E-11	Old_CVDs	STK38L
FLNC	2.84E-15	0.141278473	0.301	0.268	4.06E-11	Old_CVDs	FLNC
NCALD	2.45E-14	-0.105940384	0.244	0.279	3.5E-10	Old_CVDs	NCALD
LTBP1	3.53E-14	0.127536409	0.886	0.898	5.05E-10	Old_CVDs	LTBP1
MYL12A	1.33E-13	0.180516633	0.501	0.478	1.91E-09	Old_CVDs	MYL12A
FHL1	3.02E-13	0.147359079	0.664	0.657	4.32E-09	Old_CVDs	FHL1
ACTN4	1.57E-12	0.124140606	0.657	0.646	2.26E-08	Old_CVDs	ACTN4
MYH9	1.85E-12	0.120655665	0.583	0.566	2.65E-08	Old_CVDs	MYH9
CSNK1E	2.43E-11	0.1075113	0.255	0.228	3.48E-07	Old_CVDs	CSNK1E
ADAM19	2.76E-11	0.108772045	0.371	0.342	3.95E-07	Old_CVDs	ADAM19
SMYD2	6.18E-11	0.108685093	0.701	0.697	8.86E-07	Old_CVDs	SMYD2
MYH7	2.56E-10	0.14643717	0.917	0.922	3.67E-06	Old_CVDs	MYH7
KIF13A	4.11E-10	0.104691249	0.85	0.865	5.89E-06	Old_CVDs	KIF13A
PTPN14	6.17E-10	0.136781579	0.53	0.513	8.83E-06	Old_CVDs	PTPN14
WDPCP	2.51E-09	0.108104204	0.871	0.886	3.59E-05	Old_CVDs	WDPCP
XPR1	4.29E-09	0.103959202	0.885	0.898	6.14E-05	Old_CVDs	XPR1
NUAK1	7.16E-09	0.112513233	0.54	0.525	0.000102608	Old_CVDs	NUAK1
SAMD12	1.48E-08	0.103494932	0.683	0.682	0.000212063	Old_CVDs	SAMD12
CRIM1	1.53E-08	0.126025104	0.592	0.581	0.000218998	Old_CVDs	CRIM1
PRKAR1A	7.66E-08	0.114206487	0.486	0.467	0.001097897	Old_CVDs	PRKAR1A
CTNND2	8.52E-08	0.101592243	0.611	0.598	0.001219828	Old_CVDs	CTNND2
MYPN	8.65E-08	0.10280697	0.819	0.831	0.001239343	Old_CVDs	MYPN
PRICKLE1	9.22E-08	0.121956747	0.527	0.516	0.001320593	Old_CVDs	PRICKLE1
UBAC2	9.84E-08	0.104090102	0.703	0.707	0.001409385	Old_CVDs	UBAC2
ACADVL	5.55E-07	0.128539073	0.399	0.379	0.00794377	Old_CVDs	ACADVL
ST7.2	1.18E-06	0.152960288	0.508	0.498	0.0169688	Old_CVDs	ST7
JAK2	3.17E-06	0.124738267	0.552	0.545	0.04544099	Old_CVDs	JAK2
ANKRD1.4	1.58E-19	0.467134211	0.858	0.81	2.27E-15	Old	ANKRD1
FHL2	8.67E-16	-0.228428563	0.952	0.968	1.24E-11	Old	FHL2
GRAMD1B.1	3.99E-13	-0.251088482	0.632	0.741	5.72E-09	Old	GRAMD1B
NUDT4.1	7.98E-13	-0.246389204	0.483	0.612	1.14E-08	Old	NUDT4

SUPPLEMENTARY DATA

XIST.1	4.3E-11	-0.381083823	0.336	0.436	6.16E-07	Old	XIST
LINC01151.2	3.73E-10	-0.227108681	0.378	0.488	5.34E-06	Old	LINC01151
FSTL1	6.11E-10	-0.237398848	0.504	0.619	8.76E-06	Old	FSTL1
MAP4	7.42E-10	0.183744375	0.956	0.939	1.06E-05	Old	MAP4
RABGAP1L	8.6E-10	0.204241207	0.967	0.954	1.23E-05	Old	RABGAP1L
SLCO5A1.1	6.9E-09	-0.222800015	0.472	0.565	9.89E-05	Old	SLCO5A1
ZNF385D.1	1.15E-08	0.491940126	0.304	0.242	0.000165423	Old	ZNF385D
FKBP5	1.48E-08	-0.239663788	0.484	0.585	0.00021221	Old	FKBP5
ART3	6.43E-08	-0.129167643	0.369	0.462	0.000921142	Old	ART3
TTY10	7.37E-08	0.266431556	0.448	0.382	0.001055356	Old	TTY10
MGAT4C	9.07E-08	-0.198922834	0.431	0.528	0.001298814	Old	MGAT4C
STAT3.1	1.35E-07	-0.178354711	0.499	0.597	0.001930602	Old	STAT3
ACTN2.1	2.17E-07	0.176059926	0.952	0.947	0.003115507	Old	ACTN2
ELL2	3.2E-07	-0.181515609	0.543	0.635	0.00458665	Old	ELL2
TIMP3	3.36E-07	-0.14858281	0.4	0.481	0.00480951	Old	TIMP3
MYH6.1	3.48E-07	-0.258035608	0.431	0.517	0.004991016	Old	MYH6
ARHGAP31	3.59E-07	-0.178804621	0.491	0.581	0.00514041	Old	ARHGAP31
ELF2	3.73E-07	-0.10698858	0.582	0.691	0.005342334	Old	ELF2
UBE2E1	3.91E-07	-0.135463012	0.388	0.477	0.005594562	Old	UBE2E1
FAM227B	3.95E-07	-0.161410154	0.431	0.512	0.005656553	Old	FAM227B
CSGALNACT1	4.28E-07	-0.166865788	0.624	0.724	0.006131523	Old	CSGALNACT1
GRK5.1	4.48E-07	-0.1842035	0.244	0.322	0.006420677	Old	GRK5
GFOD1	4.78E-07	-0.154200253	0.494	0.587	0.006848437	Old	GFOD1
ADGRD1	5.07E-07	-0.135304927	0.413	0.504	0.007266086	Old	ADGRD1
SCPEP1	5.61E-07	-0.111514386	0.261	0.341	0.008029431	Old	SCPEP1
IL1RAPL1.1	6.22E-07	-0.227843905	0.766	0.806	0.008905162	Old	IL1RAPL1
OSBPL8	6.98E-07	-0.174712803	0.542	0.631	0.009996108	Old	OSBPL8
MLF 1.00	8.12E-07	-0.15759238	0.548	0.65	0.011635235	Old	MLF 1.00
CACNB2	8.38E-07	-0.111277349	0.966	0.962	0.011999852	Old	CACNB2
TNNI3	9.42E-07	0.294130017	0.796	0.788	0.013498908	Old	TNNI3
MYL2.5	1.2E-06	0.367536279	0.617	0.581	0.017126086	Old	MYL2
SLC39A14	1.44E-06	-0.145369923	0.394	0.474	0.020589291	Old	SLC39A14
ACTR3	1.55E-06	-0.124271802	0.246	0.317	0.022221457	Old	ACTR3
SLC7A2	1.56E-06	-0.150940831	0.38	0.46	0.022278929	Old	SLC7A2
PLXNA4	1.7E-06	-0.158932969	0.517	0.595	0.024344552	Old	PLXNA4

SUPPLEMENTARY DATA

CYP2J2.2	1.96E-06	0.240015553	0.616	0.581	0.028076309	Old	CYP2J2
ACTA1.5	2.06E-06	0.423781708	0.36	0.31	0.029541998	Old	ACTA1
TMEM178B.2	2.1E-06	-0.156985221	0.739	0.813	0.030078908	Old	TMEM178B
SAMD12.1	2.23E-06	0.217622925	0.717	0.682	0.031969966	Old	SAMD12
INPP4A	2.62E-06	-0.127351526	0.249	0.318	0.037601569	Old	INPP4A
PEBP4	2.67E-06	-0.12199035	0.511	0.601	0.038300406	Old	PEBP4
SLC10A7	2.94E-06	-0.147514824	0.246	0.312	0.042138658	Old	SLC10A7
ITCH	3.05E-06	-0.126484398	0.496	0.583	0.043722792	Old	ITCH
FGD4	3.09E-06	-0.169433171	0.626	0.703	0.044302464	Old	FGD4
ZBTB16	3.19E-06	-0.135155146	0.751	0.822	0.045765617	Old	ZBTB16
PUDP	3.3E-06	-0.163495317	0.239	0.306	0.047283727	Old	PUDP
MAP3K14	3.33E-06	-0.117264815	0.372	0.454	0.047737161	Old	MAP3K14
TMX2	3.35E-06	-0.135501478	0.187	0.252	0.047954752	Old	TMX2
MAX	3.41E-06	-0.138904598	0.36	0.439	0.048803354	Old	MAX
ADGRL3.1	2.64E-13	0.190931321	0.46	0.408	3.79E-09	ICM	ADGRL3
TNNC1.2	4.35E-13	-0.152770948	0.167	0.213	6.22E-09	ICM	TNNC1
PDE1A.2	9.94E-10	-0.196255694	0.375	0.42	1.42E-05	ICM	PDE1A
PIK3R1	8.33E-09	0.107353967	0.827	0.806	0.000119321	ICM	PIK3R1
ITPR1	2.87E-08	-0.118792879	0.258	0.298	0.000411573	ICM	ITPR1
KCNMB2.2	3.49E-08	0.134724774	0.664	0.633	0.000500091	ICM	KCNMB2
MROH7	1.02E-07	0.110347139	0.6	0.569	0.001467051	ICM	MROH7
HSPB7	1.35E-07	-0.107595121	0.526	0.566	0.001933236	ICM	HSPB7
ERBB4.1	3.53E-07	0.101211007	0.922	0.924	0.00506315	ICM	ERBB4
ANKHD1	8.54E-07	0.102857889	0.624	0.593	0.012230803	ICM	ANKHD1
MMP2.1	2.35E-06	-0.10996963	0.162	0.192	0.033619177	ICM	MMP2

SUPPLEMENTARY DATA

Trajectory Markers - GO

ID	Description	setSize	enrichmentScore	NES	pvalue	p.adjust	qvalue	rank	leading_edge	core_enrichment	trajectory
(GO:0048729)	tissue morphogenesis	5	0.8367	3.0146	0.0009	0.0980	0.0848	5	tags=80%, list=9%, signal=80%	ANKRD1/ACTA1/MYL2/TNNI3	Aging
(GO:0006996)	organelle organization	8	0.6124	2.4706	0.0098	0.1785	0.1544	11	tags=62%, list=21%, signal=58%	ANKRD1/ACTA1/MYL2/MAP4/ACTN2	Aging
(GO:0007155)	cell adhesion	22	0.3062	1.6726	0.1039	0.3537	0.3004	54	tags=77%, list=54%, signal=46%	EXT1/MYL12A/VCL/SMAD6/ADGRL3/TPM1/JAK2/ACTN4/PICKLE1/MYH9/PRKAR1A/NLAK1/ACTN2/ADAM19/WDCP/MYPPN/CTNND2	Aging-CVDs-Shared
(GO:0043500)	muscle adaptation	7	0.4130	1.6279	0.1668	0.4346	0.3691	18	tags=33%, list=18%, signal=30%	ACTA1/TNNI1/MYH7	Aging-CVDs-Shared
(GO:0006950)	response to stress	5	0.3333	1.0990	0.3796	0.9476	0.9476	9	tags=100%, list=82%, signal=33%	ANKRD1/HSPB7/MMP2/ITPR1	ICM
(GO:0007154)	cell communication	7	0.3395	1.0935	0.4086	0.9476	0.9476	2	tags=29%, list=18%, signal=64%	ADGRL3/KCNMB2	ICM
(GO:0003015)	heart process	5	0.2891	1.1991	0.3277	1.0000	1.0000	9	tags=80%, list=56%, signal=51%	CORIN/ATP2A2/FGF12/CYP2J2	HCM
(GO:0060047)	heart contraction	5	0.2891	1.1991	0.3277	1.0000	1.0000	9	tags=80%, list=56%, signal=51%	CORIN/ATP2A2/FGF12/CYP2J2	HCM
(GO:0008152)	metabolic process	5	0.7168	1.8154	0.0440	0.1259	0.0899	2	tags=40%, list=14%, signal=63%	PLCG2/ANKRD1	DCM
(GO:0016043)	cellular component organization	8	0.6344	1.5642	0.1069	0.1871	0.1336	3	tags=38%, list=21%, signal=69%	PLCG2/ANKRD1/MYL2	DCM

Supplementary File 7. Dataset sensitivity- Supporting Files.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 8. Human DEG and GO for Y vs O.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 9. Human DEG and GO ICM vs NF.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 10. Human DEG and GO HCM vs NF.

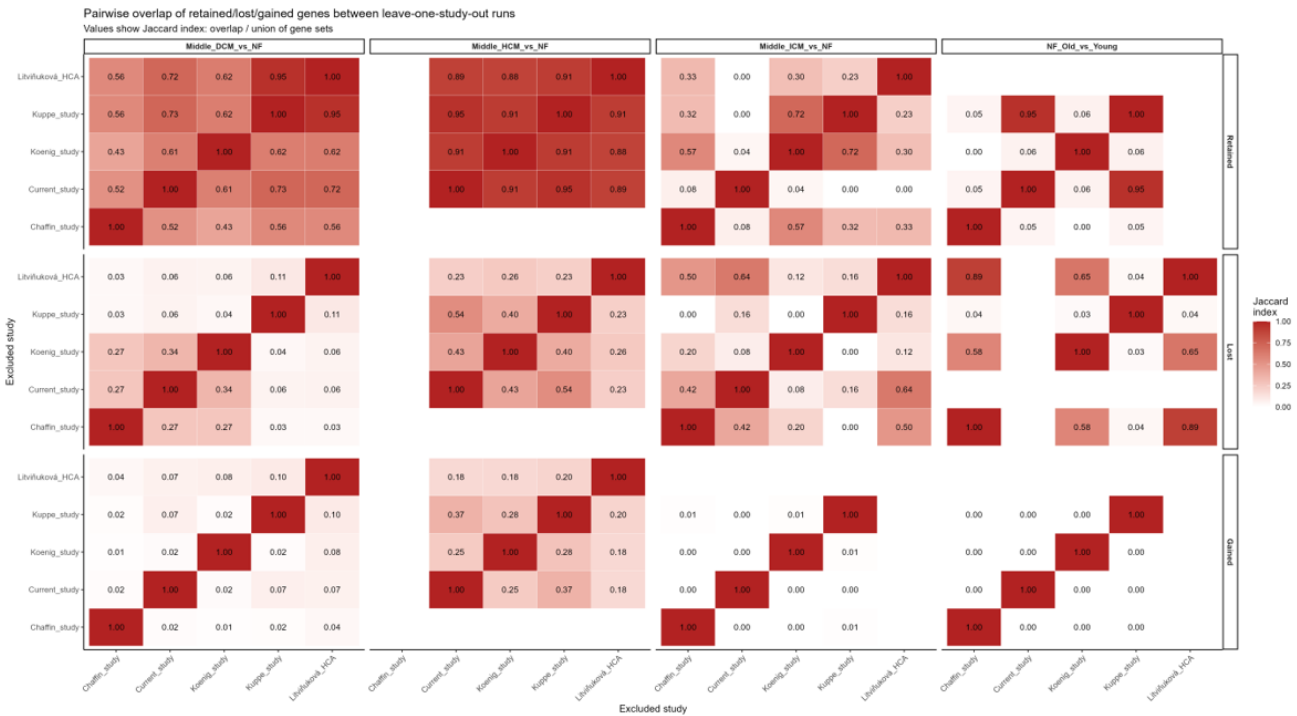
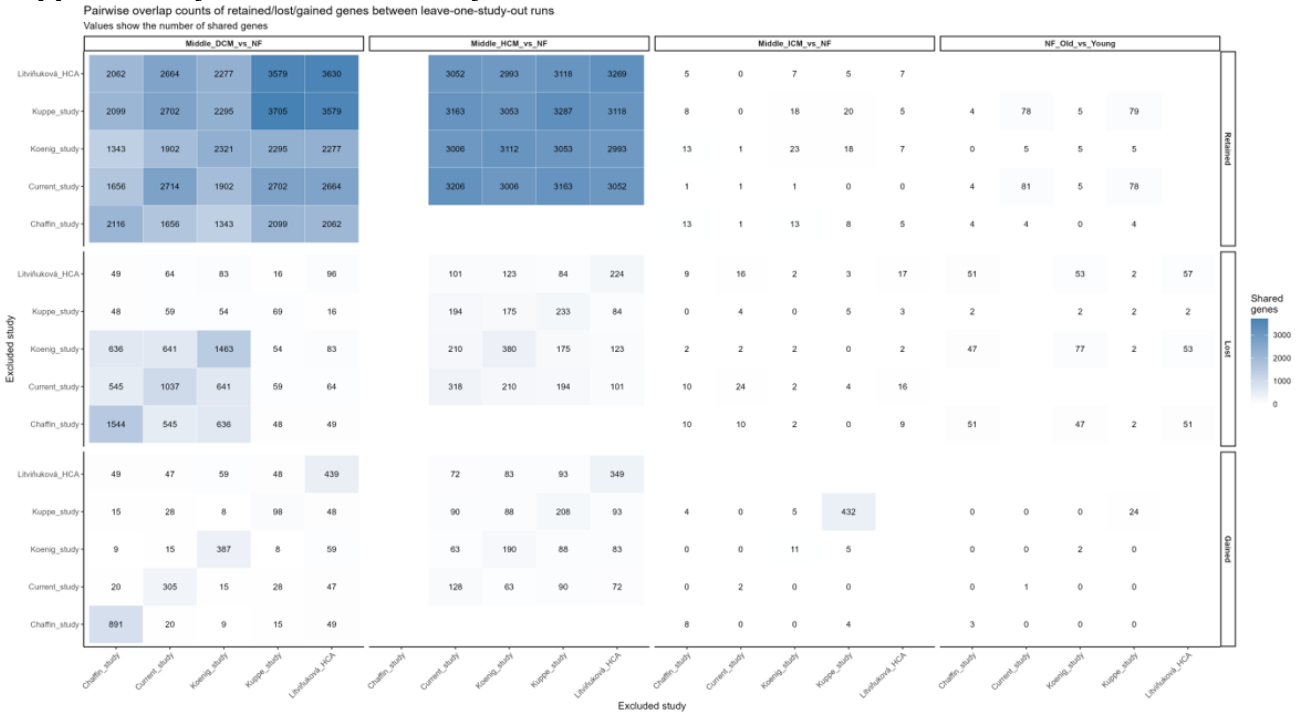
Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 11. Human DEG and GO DCM vs NF.

Link: <https://doi.org/10.48804/OMC9EK>

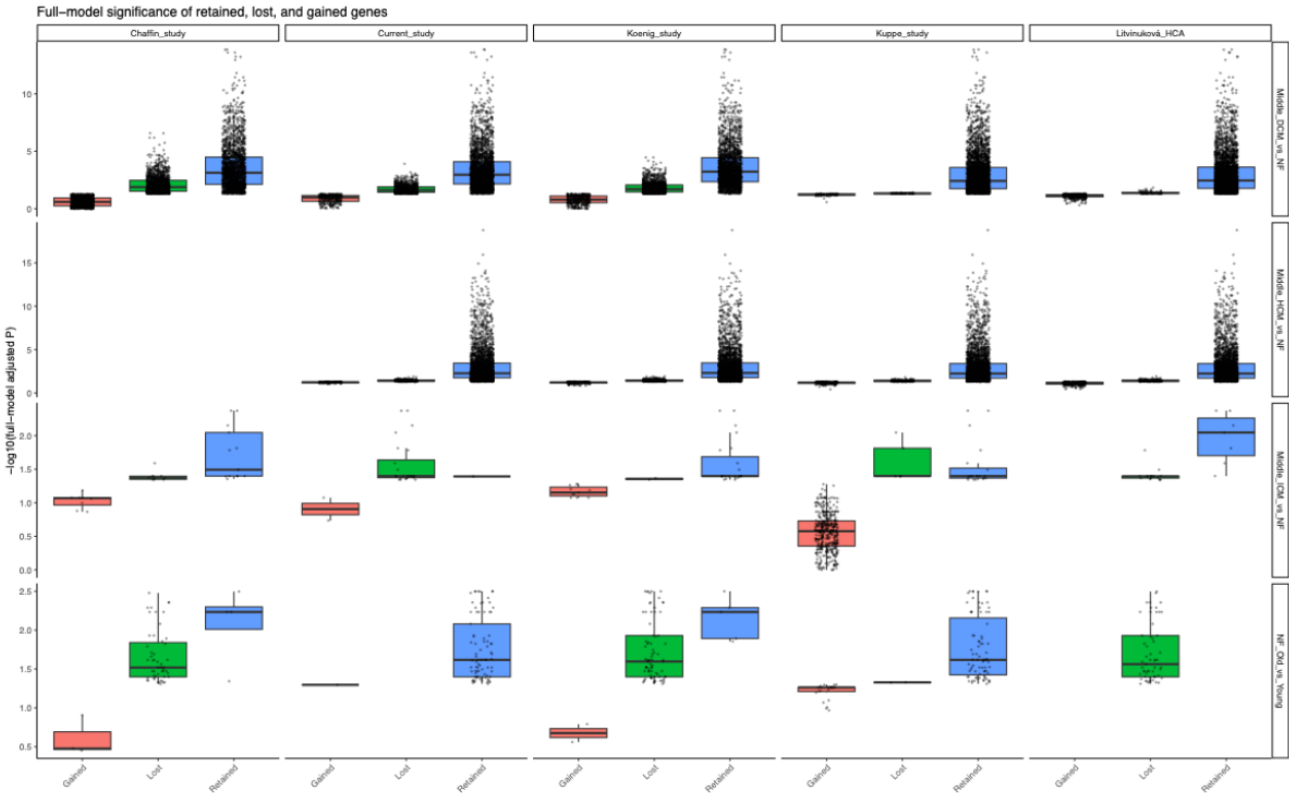
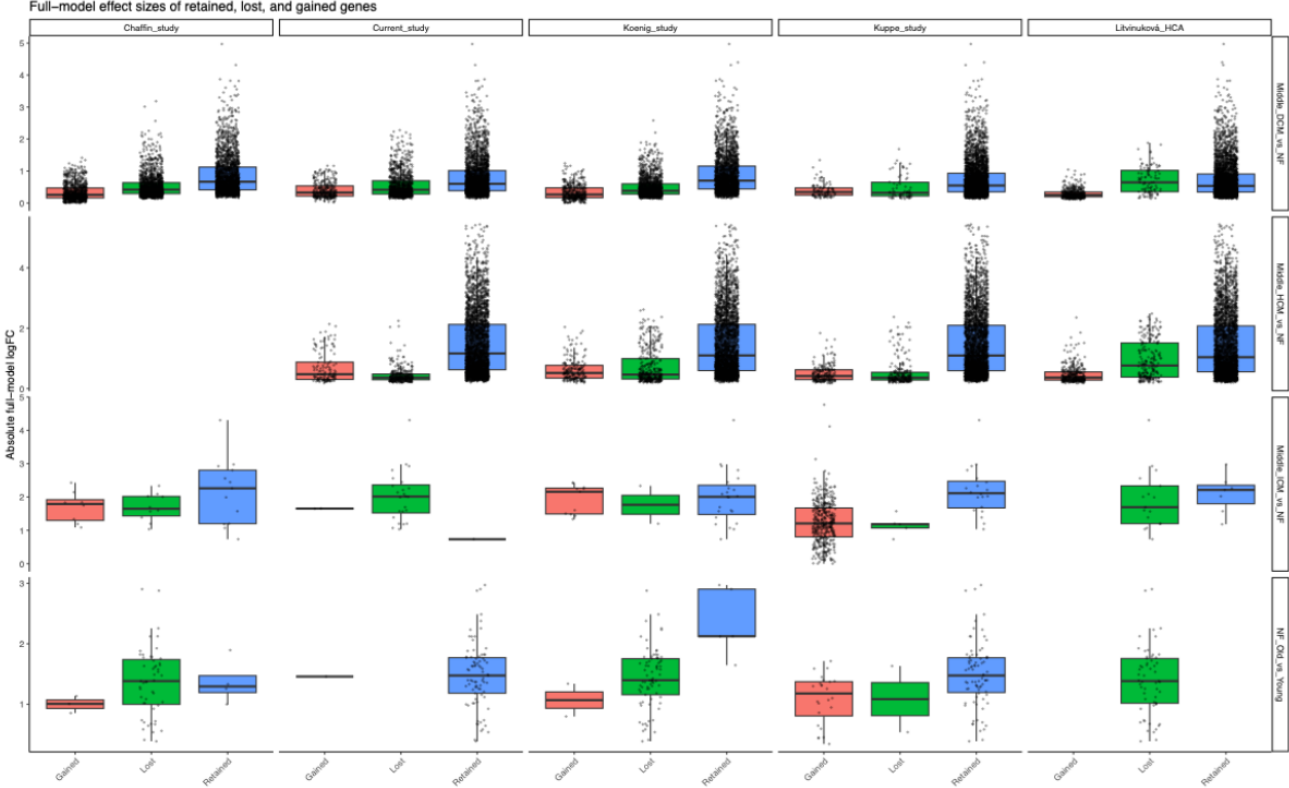
SUPPLEMENTARY DATA

Supplementary File 12. LOO Analysis.



SUPPLEMENTARY DATA

Supplementary File 13. LOO Analysis padj – Fold Change



SUPPLEMENTARY DATA

Supplementary File 14. DEG shared between human pathologies

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 15. DEG in ICM with aging.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 16. snRNAseq_DEGs_GWAS_eQTL_overlaps

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 17. WGCNA_edgelist_pvalue_adj.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 18. Human Enriched TF motifs in DEG.

Link: <https://doi.org/10.48804/OMC9EK>

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Supplementary File 19. HMGA2 O vs Y upregulated targets

X	logFC	logCPM	F	PValue	FDR	gene
ACSL1	0.6209	7.2850	16.9056	0.000368539	0.009794669	ACSL1
AKNAD1	3.7346	1.9752	12.3498	0.001786249	0.025217015	AKNAD1
AQP4	1.0269	2.8518	27.3309	2.02E-05	0.00176073	AQP4
ARHGEF28	1.0880	1.9518	24.1497	4.55E-05	0.002704884	ARHGEF28
C5	1.7998	0.7142	9.2916	0.005344713	0.048953336	C5
CCDC170	2.1829	0.6782	9.6382	0.004675304	0.045029405	CCDC170
CDH22	1.0804	1.6043	16.4892	0.000418117	0.010600942	CDH22
CDKN2C	0.9453	1.4818	28.4542	1.53E-05	0.001512481	CDKN2C
COL25A1	1.4861	3.4584	19.0480	0.000199362	0.006708487	COL25A1
CPT1B	1.0504	3.0011	39.4596	1.37E-06	0.000338211	CPT1B
DGAT2	1.4526	1.1934	19.9644	0.000145329	0.005449833	DGAT2
DPF1	2.1867	-1.5165	11.2985	0.002475661	0.030340587	DPF1
DPP6	1.0122	3.0475	20.8032	0.000114117	0.004779891	DPP6
EGFR	1.5805	1.6265	13.3349	0.001194406	0.01977856	EGFR
ESPN	2.5510	-1.3795	17.7352	0.000283181	0.008442398	ESPN
ESR1	0.6403	2.5857	22.3287	7.44E-05	0.003687981	ESR1
FBLN7	2.3003	-1.1561	26.3291	2.59E-05	0.001994711	FBLN7
FRMD4B	0.9444	6.0078	27.2696	2.1E-05	0.001804031	FRMD4B
GPM6A	3.3191	1.3559	17.7286	0.000293691	0.008603539	GPM6A
HUNK	1.4912	0.6616	9.6455	0.004647064	0.044870399	HUNK
IGF1R	0.7090	8.9109	18.8481	0.000202032	0.006755305	IGF1R
IL6R	0.5390	5.1989	12.1905	0.001793597	0.025263794	IL6R
KAZN	0.9366	2.9675	9.2741	0.005413946	0.049336484	KAZN
LAMA1	3.1901	0.8058	11.9478	0.001979012	0.026821267	LAMA1
LINC00511	1.4237	1.1810	10.6716	0.003130337	0.034988408	LINC00511
LINC01358	0.9011	1.4744	27.7573	1.81E-05	0.001689111	LINC01358
LITAF	0.5118	3.9759	15.1031	0.000655124	0.01382128	LITAF
LOXHD1	1.6579	-0.3041	9.2795	0.005369153	0.049104611	LOXHD1
LRP4	0.7499	5.2800	26.7565	2.33E-05	0.001907851	LRP4
LRRC9	0.6855	2.5856	14.5707	0.000782093	0.01534939	LRRC9
MDP1	2.8879	-0.2331	10.9079	0.002869266	0.03312025	MDP1
MGAT1	0.7312	2.9275	9.7618	0.004442476	0.043808778	MGAT1
MME	2.5335	1.4793	9.3052	0.005428748	0.049407129	MME
NALCN	0.5323	7.3629	32.7271	5.67E-06	0.000780573	NALCN
NCALD	0.7847	6.9972	9.6854	0.004680543	0.045029405	NCALD
NRG1	0.9374	2.5326	21.7035	8.85E-05	0.004116011	NRG1
OLMALINC	0.5980	2.5363	15.4710	0.000580542	0.012953455	OLMALINC
PAPPA	0.7683	3.3084	26.6186	2.41E-05	0.001937704	PAPPA
PDIA6	0.5115	4.6746	13.9302	0.000971342	0.017427838	PDIA6
PHF21B	1.0634	0.7108	15.3170	0.000610583	0.013252417	PHF21B
PRKAG2	1.2193	9.0006	23.0407	6.35E-05	0.003380499	PRKAG2
PRSS23	0.5884	3.7129	14.8585	0.000710431	0.014403183	PRSS23
ROR1	0.7394	9.9304	10.6230	0.003201692	0.035526135	ROR1
RUNX1	1.1111	2.7711	16.1960	0.000462182	0.011278961	RUNX1
SETBP1	1.3105	5.4131	90.3563	8.11E-10	1.32E-06	SETBP1
SMAD7	0.8690	2.8695	14.6363	0.000765087	0.01515888	SMAD7
TACC1	0.9320	5.4695	17.3894	0.000326242	0.009127769	TACC1
TANC1	0.5166	9.3453	9.9230	0.004170357	0.042334113	TANC1

SUPPLEMENTARY DATA

TNFAIP8	0.6266	4.8335	10.7292	0.003085156	0.034754021	TNFAIP8
ZNF385B	1.1999	4.3620	14.1133	0.000953471	0.017223793	ZNF385B

Supplementary File 20. Rat_AB_aging_VASA_cluster_markers_and_GO

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 21. Variance of Rat Variable Genes.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 22. rat_VASA_OldvsYoung_DEGs

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 23. rat_VASA_LooseABvsSham_DEGs.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 24. rat_VASA_TightABvsSham_DEGs.

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 25. Rat - DEG and GO Shared between aging and pathologies

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 26. Rat - TF enriched motifs in DEG

Link: <https://doi.org/10.48804/OMC9EK>

Supplementary File 27. GO shared across rat and human Aging and pathologies

Link: <https://doi.org/10.48804/OMC9EK>

SUPPLEMENTARY DATA

MATERIALS AND METHODS

Human Heart Tissue Collection and Ethics

Human heart samples used for 10x Genomics analysis were from the UZ/KU Leuven Biobank that is generated through a collaboration between the Laboratory of Experimental Cardiology, the UZ transplant team and the Cardiac Surgery Department of UZ Leuven. The study protocol conformed to the Helsinki declaration and was conducted according to national and European Union regulations on the use of human tissues. This study was approved by the ethics committee of UZ Leuven (S58824). The majority of HF hearts used in this study were from patients undergoing cardiac transplantation at UZ Leuven. Non-failing (NF) cardiac tissue samples were from organ donors whose hearts were not allocated for transplant for reasons of age, medical or technical reasons, and/or from hearts from which only valves were harvested. NF hearts were collected as part of a multi-organ retrieval during which cold preservation solution was infused through the aorta for preservation of abdominal and/or thoracic organs. Cardiectomy is performed while systemic cold preservation perfusion is ongoing after which hearts are immediately placed in sterile cardioplegic solution (130 mM NaCl, 6 mM HEPES, 1.2 mM MgSO₄, 27 mM KCl, 10 mM glucose, pH 7.2 in ultrapure water). Tissue samples were taken from the LV anterior and posterior wall, avoiding scar tissue. Mid-myocardium, without epi- and endocardium, was snap-frozen in liquid nitrogen and stored at -80°C for later analysis. Additional transmural LV free wall myocardial samples included in the RT-qPCR analysis were procured from the Human Heart Tissue Biorepository at the University of Pennsylvania. Samples were procured using previously described consent and cardioprotection routines [1, 2]. Briefly, all samples were collected in accordance with protocols and ethical regulations approved by Institutional Review Boards at the University of Pennsylvania and the Gift-of-Life Donor Program (PA, USA). All donors received in situ antegrade cold cardioplegia and were stored on wet ice in cold saline until flash frozen in liquid nitrogen. For the gene expression analysis by RT-qPCR, tissues were from non-failing young (Y; 20-44 years, n=37, 19 female), and non-failing old (O; 65-99 years, n=88, 38 female), ICM (n=41, 5 female), HCM (n=22, 9 female), and DCM (n=62, 21 female). NF middle-aged (45-64 years) served as an age-matched control group for the cardiomyopathies (n=41, 19 female) (Table 1, Supplementary File 1). Samples on which 10x snRNA-Seq was performed are as recently described [GSE298023] [3], and include 4 NF, 5 ICM and 4 DCM samples.

Aging rats

Male Sprague Dawley (SD) rats (Harlan Sprague Dawley Inc. (now Envigo), USA) were housed in a temperature-controlled room with a 12 h/12 h light/dark cycle according to the animal experimental protocol approved by the Norwegian Animal Research Authority (FDU applications 4318 and 20208). At 3 or 26 months, cardiac function and geometry were assessed by echocardiography and magnetic resonance imaging (MRI). Hearts were excised and weighed at time of euthanasia. LVs were immediately snap-frozen in liquid nitrogen before storage at -80 °C. LV samples were also fixed in 10% formaldehyde and then embedded in paraffin for histological studies.

Pressure overload by banding of the ascending aorta in rats

6 week old male SD rats were subjected to constriction (banding) of the ascending aorta (AB) for 6 weeks. The animal experimental protocol used in this study was approved by the Norwegian Animal Research Authority (FDU applications 4318 and 20208) and has been previously described [4]. Rats were anaesthetized and ventilated through endotracheal intubation with a mixture of 69% N₂O, 29% O₂, and 2% isoflurane. Nitrile Rubber O-rings of fixed internal diameter 1.5 mm (mild stenosis; Loose) or 1.07 mm (severe stenosis; Tight) were placed around the ascending aorta proximal to the brachiocephalic trunk. Sham-operated animals (S) served as controls and underwent the same surgical procedure, excluding the placement of the O-ring. Buprenorphine was given as both pre- and post-operative analgesia. Immediately prior to euthanasia, cardiac function and geometry were assessed by echocardiography and MRI. At sacrifice, hearts were excised and weighed. The LV tissue was divided, where one part was snap-frozen in liquid nitrogen and immediately stored at -80 °C, and the other part was fixed in 10% formaldehyde and then embedded in paraffin for histological studies.

SUPPLEMENTARY DATA

Histological assessment of fibrosis in rat cardiac tissue

Paraffin sections (6 µm) were cut using a microtome (Epredia HM 355S, Thermo Fisher Scientific Inc., Waltham, MA, USA). The sections were stained using Masson's trichrome (BenchMark Special Stains, Ventana Medical Systems, Inc., Tucson, AZ, USA). High-resolution images were acquired using an automated slide scanner system. Fibrosis was quantified with QuPath-0.4.3 [5]. Correct masking and fibrosis detection were confirmed by visual inspection. All image analysis was performed by a single operator blinded to animal group.

Isolation of cardiac cell nuclei used in RT-qPCR and VASA-Seq experiments

Debris-free suspensions of intact cardiac cell nuclei were generated by tissue homogenization followed by sucrose gradient centrifugation, and flow sorting with all steps performed at $\leq 4^{\circ}\text{C}$, as we have previously described [6] and with modifications from Preissl et al. [7]. Approximately 50 mg of snap-frozen cardiac tissue was cut on ice with a scalpel into 4-5 smaller pieces after which they were placed into gentleMACS M tubes (Miltenyi Biotec, 130093236) containing 5 mL lysis buffer and thawed on ice (5 mM CaCl_2 , 3 mM MgAc, 2 mM EDTA, 0.5 mM EGTA, 10 mM Tris-HCL pH 8, supplemented prior to use with 1 mM DTT (Sigma-Aldrich, 646563), 1 µg/mL actinomycin D (Sigma-Aldrich, A1410), 0.05% protease inhibitor cocktail (Sigma-Aldrich, P8340), and 0.04 U/µL RNA inhibitors-RNase OUT (ThermoFisher Scientific, 10777019). Tissue was then homogenized using a gentleMACS dissociator (Miltenyi Biotec) on program "protein_01" for 1 min. After homogenization, 5 mL lysis buffer supplemented with 0.1 % NP-40 Surfact-Amps™ (ThermoFisher Scientific, 28324) was added to the homogenate, which was then filtered through a 30 µm filter to remove non-disrupted tissue and cell aggregates (Miltenyi Biotec, 130041407). To recover retained nuclei, the filter was washed with an additional 2 mL lysis buffer containing 0.1 % NP-40 Surfact-Amps™. Nuclei were then harvested from the filtrate by centrifugation (1000 x g, 5 min), after which the pellet was resuspended in and overlaid on sucrose buffer (1 M sucrose, 3 mM MgAc, 10 mM Tris-HCl, pH 8) containing 1 mM DTT, 1 µg/mL actinomycin D, 0.05% protease inhibitor cocktail, 0.04 U/µL RNA inhibitors-RNase OUT, then pelleted by centrifugation (1000 x g, 5 min).

For isolation of CM nuclei, the pellet containing nuclei of all cardiac cell types was resuspended in 370 µL staining buffer (PBS containing 5% BSA (Sigma-Aldrich, A3311), and 0.2 % NP-40 Surfact-Amps™) supplemented with 1 mM DTT, 1 µg/mL actinomycin D, 0.05% protease inhibitor cocktail, 0.2 U/µL RNA inhibitors-RNase OUT and antibodies for 30 min. Samples were stained with 1.5 µL anti-PCM-1 (Sigma-Aldrich, rabbit, HPA 023370 – 100 UL), 1 µL goat anti-rabbit IgG secondary antibody-Alexa Fluor 488 (ThermoFisher Scientific, A-11034), 1 µL PLN (Badrilla, mouse, A010-14), 1 µL goat anti-mouse IgG secondary antibody-Alexa Fluor 568 (ThermoFisher Scientific, A-11031) and 0.02 mg/mL DAPI (Sigma-Aldrich, D9542). After centrifugation (1000 x g, 5 min), the nuclear pellet was resuspended in 300 µL PBS and then passed through a 35 µm strainer.

Nuclei were sorted using a BD Influx flow cytometer either into tubes for qPCR or into 384-well hardshell plates (BioRad), when snRNA-Seq (VASA-seq) was applied. Intact debris-free CM nuclei were harvested by setting gates for DAPI+ve, PCM1+ve, and PLN+ve.

For collection in tubes, CM nuclei were sorted into PBS containing 400 µg/mL UltraPure™ BSA (ThermoFisher Scientific, AM2616). Sorted CM nuclei were flash-frozen with 700 µL Qiazol.

For collection into 384-well plates, before cell sorting, each well was pre-filled with 5 µL of mineral oil (Sigma-Aldrich) and 50 nL of CEL-seq2/VASA-seq [8, 9] primers at a concentration of 0.25 µM. After sorting, plates were sealed and centrifuged at 1,500 x g for 2 min before storage at -80°C .

mRNA isolation and reverse transcription quantitative PCR (RT-qPCR)

Total RNA for RT-qPCR experiments was extracted from the nuclei of CMs and mid-myocardial LV tissue of aging and AB rats using Qiazol/TRIZol, as well as from mid-myocardial LV tissue of humans, but using Qiazol. RNA extracted from the nuclei of aged and AB rat CMs was treated with DNase using the TURBO DNA-free™ kit (Thermo Fisher Scientific) and subsequently reverse-transcribed into cDNA using SuperScript™ IV Reverse

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Transcriptase (Thermo Fisher Scientific). PrimePCR assays were validated by Bio-rad. RT-qPCR was performed using Sso Advanced Universal Sybr Green supermix. For LV tissue samples, total RNA was isolated and 1 µg reverse-transcribed using SuperScript™ III Reverse Transcriptase (Thermo Fisher Scientific). qPCR was performed using GoTaq® qPCR Master Mix (Promega). Primer sequences used in these experiments are detailed in Table 2. Relative quantities were calculated using the $\Delta\Delta CT$ method, normalized to the appropriate reference genes, and expressed as $-\Delta\Delta CT$ values. qPCR reactions were performed using a CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad, Temse, Belgium). For rats, gene expression data were normalized to reference genes 18S, GAPDH, RPL32, SDHA, TBP. The stability of reference genes was established using geNORM [10].

snRNA-Seq - VASA-seq, Vast Transcriptome Analysis of Single Cells by dA-Tailing

High-throughput total RNA sequencing in single nuclei using VASA-seq was performed according to Salmen et al. [11] by Single Cell Discoveries (Utrecht, the Netherlands). The barcoded pooled libraries were sequenced by Illumina Nextseq 500.

snRNA-Seq - 10x Genomics

Human cardiac nuclei were isolated and 10x RNAseq libraries were prepared as outlined by Amoni et al. [3, 12, 13]. Libraries were prepared on ~6,000 nuclei per sample (10x Genomics) and sequenced at a targeted depth of 80,000 reads per nucleus. 10x RNA-Seq data from these samples focusing on FB were recently described [GSE298023] [3].

Bioinformatic analysis

VASaseq: The 60 bp FASTQ read files underwent quality assessment and trimming using fastp [14]. The qualified reads per nucleus, encompassing both intronic and exonic reads, were aligned to the reference genome (GRCh38 for humans and Rnor_6.0 for rats) and enumerated using STARsolo (STAR-2.7.9a) [15]. Reads were deduplicated based on precise reading start/end coordinates. Empty wells and any remaining unqualified nuclei were filtered out using the EmptyDrop algorithm developed by Lun et al [16]. Subsequently, uniquely mapped reads, encompassing both exons and introns per gene, were imported into R (R Core Team, v4.1.2)/ Rstudio (v2021.09.1 Build 372) for downstream analysis. The background reads identified within empty wells were estimated and eliminated from each qualified nucleus using a Bayesian method implemented by DecontX [17]. Nuclei exhibiting less than 2000 detected expressing genes and a mitochondrial/rRNA expression percentage exceeding 5% on each plate were subsequently excluded. The datasets from multiple plates were then integrated using Harmony v 0.1.0 [18], which harmonizes the technical differences to enhance the accuracy of downstream analyses. The data were then analyzed using Seurat v4 [19]. The remaining steps were as for 10x data after harmonization. Following batch harmonization, CM subpopulations were identified by unsupervised graph-based clustering using the Seurat algorithm (FindClusters, algorithm = 3). Cluster identities were subsequently assigned based on established marker gene expression.

10x Genomics: The transcriptome datasets generated by CellRanger v6 [20], including both mapped introns and exons, were subjected to further processing using CellBender to remove ambient RNAs (v 0.2.2) [21] and scDblFinder (v 1.9.4) [22] to identify and remove nuclear doublets, ensuring the inclusion of only singlet nuclei in the subsequent analyses. The singlet nuclei from our dataset described above, along with four other previously published datasets (summarized in Table 3) [3, 23-26], were merged. To minimize data variability within studies, we compiled a gene expression matrix containing all expressed genes across the entire datasets, irrespective of their sparsity or expression level. Nuclei with > 350 detected genes and a mitochondrial/rRNA expression percentage < 5% in each dataset were retained for analysis. Subsequently, the merged datasets were integrated using Harmony [18]. Next, Uniform Manifold Approximation and Projection (UMAP) [27] was applied for dimensionality reduction and visualization of the integrated dataset.

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For broad cell-type composition, nuclei were grouped as CM, fibroblasts, or remaining cell types, and proportions were calculated per donor relative to the total number of nuclei from that donor. The CM subpopulations from the Human Cell Atlas (HCA) [26] were used as a reference to annotate CM nuclei in the harmonized human datasets by identifying transfer anchors between the HCA reference and query datasets. Each CM nucleus was assigned to the reference ventricular CM (vCM) state with the highest prediction score. Since CM substates are transcriptionally continuous, nuclei were not excluded using a hard prediction score threshold. Instead, prediction scores were used as a confidence metric to assess annotation reliability, which validated classification of the CM nuclei to the 5 HCA-anchored transcriptomic state annotations. For calculation of proportions of major cell classes and CM subpopulations in human, data was analysed at the donor level. Group-level stacked bars show mean donor-level proportions, with statistical comparisons performed on donor-level values using two-sided Wilcoxon rank-sum tests followed by Benjamini-Hochberg FDR correction. Owing to the lower number of cells and samples, analysis in rat was at the nucleus level.

Cellular and gene expression heterogeneity analysis

Inter-cellular heterogeneity was assessed at both the cellular (nucleus) and gene expression levels. CM heterogeneity was quantified using Euclidean distances in PCA space. PCA embeddings were extracted from the integrated dataset and the first 20 principal components were used for distance calculations. For the nuclei-level visualization, a group-specific centroid was calculated for each analysis group as the mean coordinate across all cardiomyocyte nuclei in that group, and the Euclidean distance of each nucleus to its corresponding group centroid was computed. These nuclei-level distances were shown as individual points and were used for the young versus old non-failing comparison. For the age-matched disease comparisons, donor-level heterogeneity was also calculated separately by first computing a donor-specific centroid in PCA space, then calculating the Euclidean distance of each CM nucleus to its own donor centroid, and finally summarizing each donor by the median nucleus to donor centroid distance. Donor-level median distances were used for the NF versus ICM, HCM, and DCM boxplots and statistical comparisons. Statistical significance was assessed using two-sided Wilcoxon rank-sum tests, followed by Benjamini-Hochberg FDR correction within the planned comparisons.

Highly variable genes (HVGs) were defined as the 2,000 genes identified in the original merged Seurat object using the variance stabilizing transformation (VST) method implemented in `FindVariableFeatures` Seurat function. These HVGs were retained as a fixed reference set throughout the analysis and were not redefined for individual conditions.

To assess transcriptional variability across aging and disease states, gene-wise variance statistics were recalculated independently for each comparison group. Pre-existing variance metrics inherited from the merged object were discarded prior to recalculation. For each condition or sample, raw UMI counts were log-normalized using `NormalizeData`, and gene specific vst variance standardized values were recomputed using `FindVariableFeatures(selection.method = "vst")`.

For the analysis during aging, non-failing (NF) cardiomyocyte nuclei from Y and O samples were analyzed separately, and distributions of gene-level `vst.variance.standardized` values were compared between age groups. For disease analyses, age-matched middle-aged NF, ICM, HCM, and DCM samples were analyzed. Scatter plots display gene-level standardized variance values calculated from all nuclei within each condition. In contrast, boxplots and statistical comparisons for NF and CMP groups were based on `vst.variance.standardized` values recalculated independently for each biological sample and subsequently pooled within each condition, thereby accounting for inter-sample variability and avoiding pseudo replication due to unequal cell numbers. Boxplots and statistical comparisons for the Y and O groups were based on gene-level standardized variance values calculated from all nuclei within each age group. Genes were classified as HVGs or non-HVGs according to the fixed set of 2,000 HVGs defined in the merged dataset. Statistical significance between groups was assessed using two-sided Wilcoxon rank-sum tests.

Trajectory analysis

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The transcriptome profile shifts across the age groups and pathologies were analysed with the Slingshot (v 2.2.1) package in R [28]. This process involves two main steps: first, identifying the global lineage structure using a cluster-based minimum spanning tree, and second, fitting simultaneous principal curves to describe each lineage.

Pseudo-bulk analysis of RNA-Seq data

To identify DEGs across conditions, gene counts from all nuclei per sample were summed to generate pseudo-bulk RNAseq data. This process mitigates against false positive discoveries, or type 1 errors, arising from the sparsity and heterogeneity inherent in single-cell data [29, 30].

Leave-one-study-out sensitivity analysis.

To evaluate the robustness of the integrated human CM analyses to source-dataset composition, we performed leave-one-study-out sensitivity analyses using donor-level pseudobulk profiles (Supp. File 7, 12, and 13, Supp Fig. 1, 2, 7, and 8). CM nuclei were aggregated by donor/sample within each study, condition, and age group, and raw counts were summed to generate pseudobulk expression matrices. Differential expression was then performed using edgeR with donor/sample-level pseudobulk profiles as the unit of replication.

The sensitivity analysis followed the design of the primary comparisons. For aging-associated changes, old NF samples were compared with young NF samples. For disease-associated changes, middle-aged ICM, HCM, and DCM samples were each compared with middle-aged NF samples. In each leave-one-study-out iteration, one source dataset was excluded and the differential expression model was rerun using the remaining datasets. Robustness was assessed by comparing log-fold changes from the full model with log-fold changes from each leave-one-study-out model. We summarized effect-size stability (using Pearson correlation) and the percentage of genes retaining the same direction of change. Directional concordance was also calculated for genes significant in the full model at $FDR < 0.05$.

Principal Component Analysis (PCA)

Following the preprocessing and batch effect removal, the corrected gene count values were subjected to PCA by 'factoextra' package (v1.0.7) [31] to identify major sources of variation and ensure the absence of any remaining batch effects or outliers.

Batch effect removal & differential gene expression analysis

To minimize the impact of potential technical batch effects in pseudo-bulk data analysis, the "combat" function from "sva" (surrogate variable analysis) package (v 3.42) [32] was employed to harmonize the gene expression profiles between the samples in each experiment set across different batches while preserving the biological differences. The robust DEGs between the specific experimental conditions were identified using edgeR (v 3.36), through glmQLF [33-35]. The age-matched groups included as a covariate in the design matrix of the comparisons of the cardiomyopathies and non-failing heart CMs, allowing us to account for age effects across all comparison groups. We then identified consistently differentially expressed genes (DEGs) by selecting those that were reproducibly significant across the different age groups. DEGs were defined as genes with $FDR < 0.05$ and absolute $\log_2$ fold change > 0.25 . The FDR threshold was used to control for multiple testing, while the modest $\log_2$ cutoff was selected to retain biologically relevant changes in cardiomyocyte single-nucleus pseudobulk data without overemphasizing very small effects. For selected shared-gene heatmaps, genes were further filtered for robust expression, including a threshold of > 100 reads where indicated, to avoid prioritizing low-abundance transcripts whose apparent fold changes may be less stable.

Functional enrichment analyses

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The enriched functional categories ($\text{padj} < 0.05$) associated with the identified DEGs were determined using the "enrichGO()" function from the clusterProfiler v 4.2.0 [36, 37] and enrichR v3.0 [38].

Motif enrichment analyses

RcisTarget (v1.14.0) [39, 40] was used to identify the enriched transcription factor (TFs) binding motifs and their target genes on the list of DEGs per each comparison. The significant motifs for special TFs with a normalized enrichment score (NES) threshold higher than 2.5 were identified for the genes.

Overlap analysis

The shared and exclusive genes between the comparisons were detected and visualized through Venn v1.10 [41] and GOplot v 1.0.2 [42]. UpSetR v1.4.0 package was used to visualise the intersections between more than three categorical vectors [43].

Correlation analysis

The correlation between conditions was assessed using Spearman's Rank-Order correlation of the detected DEGs in each comparison, performed with PerformanceAnalytics v 2.0.4 [44].

Weighted gene correlation network analysis (WGCNA)

To detect potential co-regulated genes that show distinct patterns across the conditions, we applied a weighted correlation analysis using WGCNA v1.70-3 [45], and identified their function by enrichment analysis (enrichR). The spatial positions of the nodes (genes) and edges (interactions) were computed using igraph v1.4.2 [46] and then visualized using ggraph v2.0.5 [47].

GWAS, eQTL, and transcriptome data integration

To identifying DEGs associated with CVD in the aging process, we integrated GWAS, eQTL (expression quantitative trait locus), and our transcriptome data. For this, first GWAS associations were downloaded from the NHGRI-EBI GWAS Catalog for the "cardiovascular disease" trait EFO_0000319 including child traits (https://www.ebi.ac.uk/gwas/efotraits/EFO_0000319). Multi-SNP entries were expanded to one rsID per row and we parsed p-values, effect sizes (OR/BETA), risk-allele frequency, the "strongest SNP–risk allele," and 95% CIs. Cardiac eQTL summary statistics were also obtained from GTEx v8 for Heart-Left Ventricle (https://ftp.ebi.ac.uk/pub/databases/spot/eQTL/imported/GTEx_V8/ge/Heart_Left_Ventricle.tsv.gz). GWAS and eQTL were merged by rsID and harmonized by comparing the GWAS effect/risk allele to the eQTL ALT/REF; eQTL betas were sign-flipped when the GWAS effect matched the REF, and ambiguous A/T or C/G sites were removed only if harmonization was unresolved. Significant genetic instruments (SNPs) were defined as variants meeting genome wide significance in GWAS ($p < 5 \times 10^{-8}$) and nominal significance as eQTL ($p < 1 \times 10^{-5}$), retaining the most significant eQTL per (rsID, gene). Ensembl gene IDs were mapped to HGNC symbols via Ensembl BioMart (version suffixes removed). GWAS traits were heuristically grouped into ICM, HCM, and DCM using keyword matching, excluding congenital terms. Contrasts between snRNA-seq DEG for aging, ICM, HCM, and DCM provided logFC and direction. We defined directional concordance as $\text{risk} \uparrow \text{expr}$ (positive harmonized eQTL beta) with DEG up, or $\text{risk} \downarrow \text{expr}$ with DEG down; true concordance additionally required GWAS trait-class = condition for ICM/HCM/DCM, while aging used the directional rule only. Per gene and condition, we retained the best eQTL p-value. Finally, UpSet plot contrasting disease-class GWAS gene sets with true-concordant snRNA sets was visualized.

Identification of orthologous genes between human and rat

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To integrate the human and rat datasets, orthologous genes between species were identified through biomaRt v2.50.1 [48] and getLDS function.

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