Supplementary Information

Large-scale Single-nuclei Profiling Identifies Role for *ATRNL1* in Atrial Fibrillation

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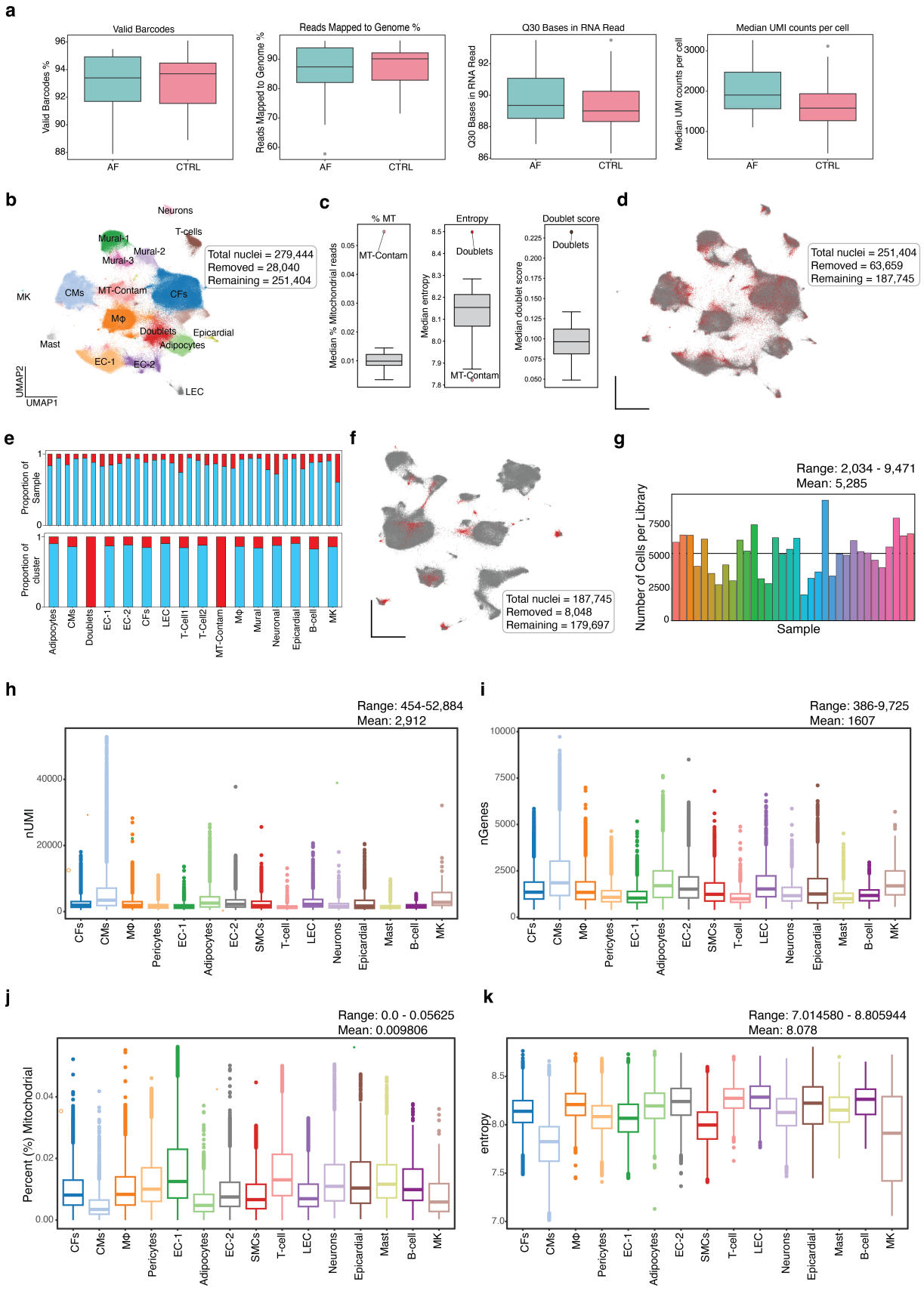
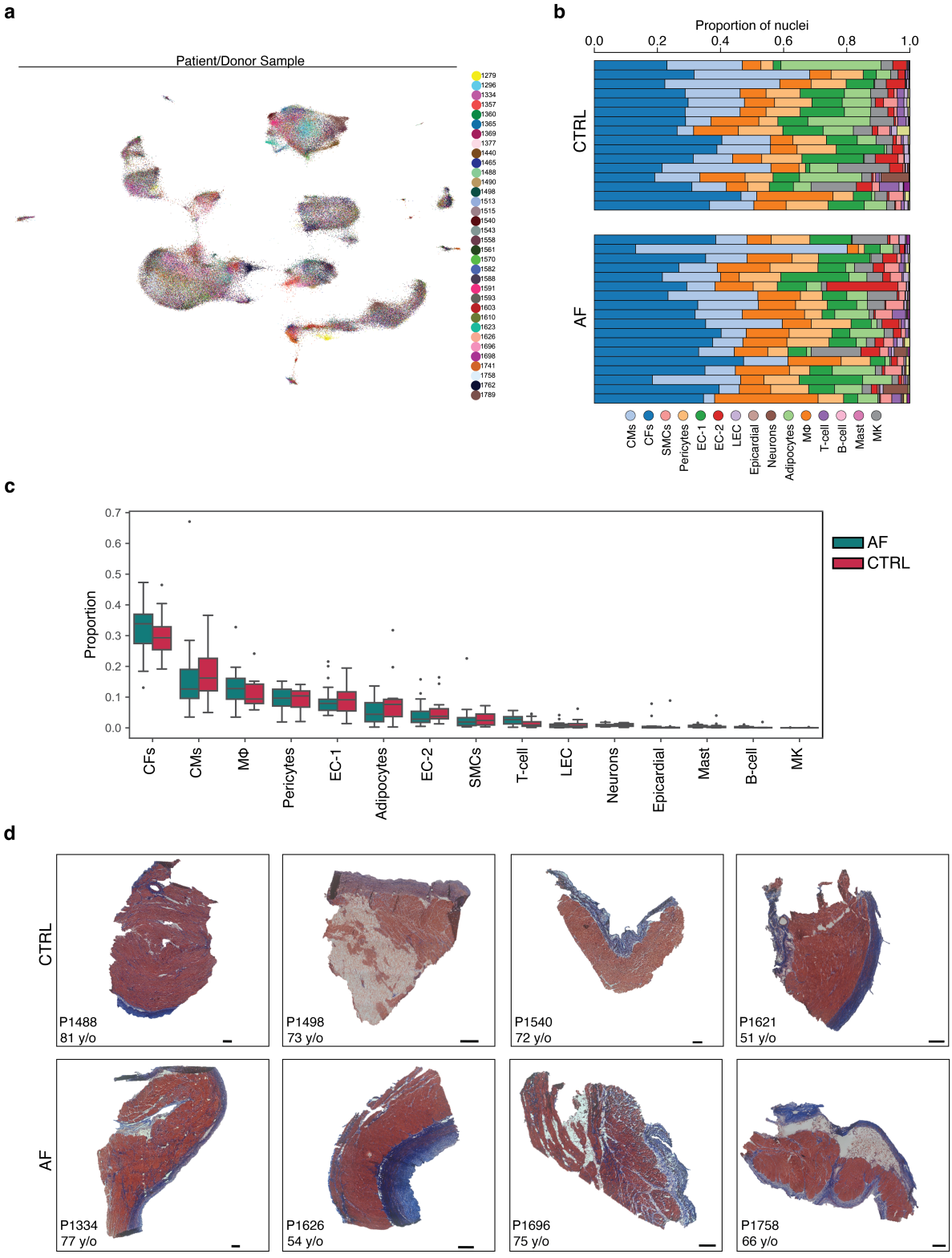
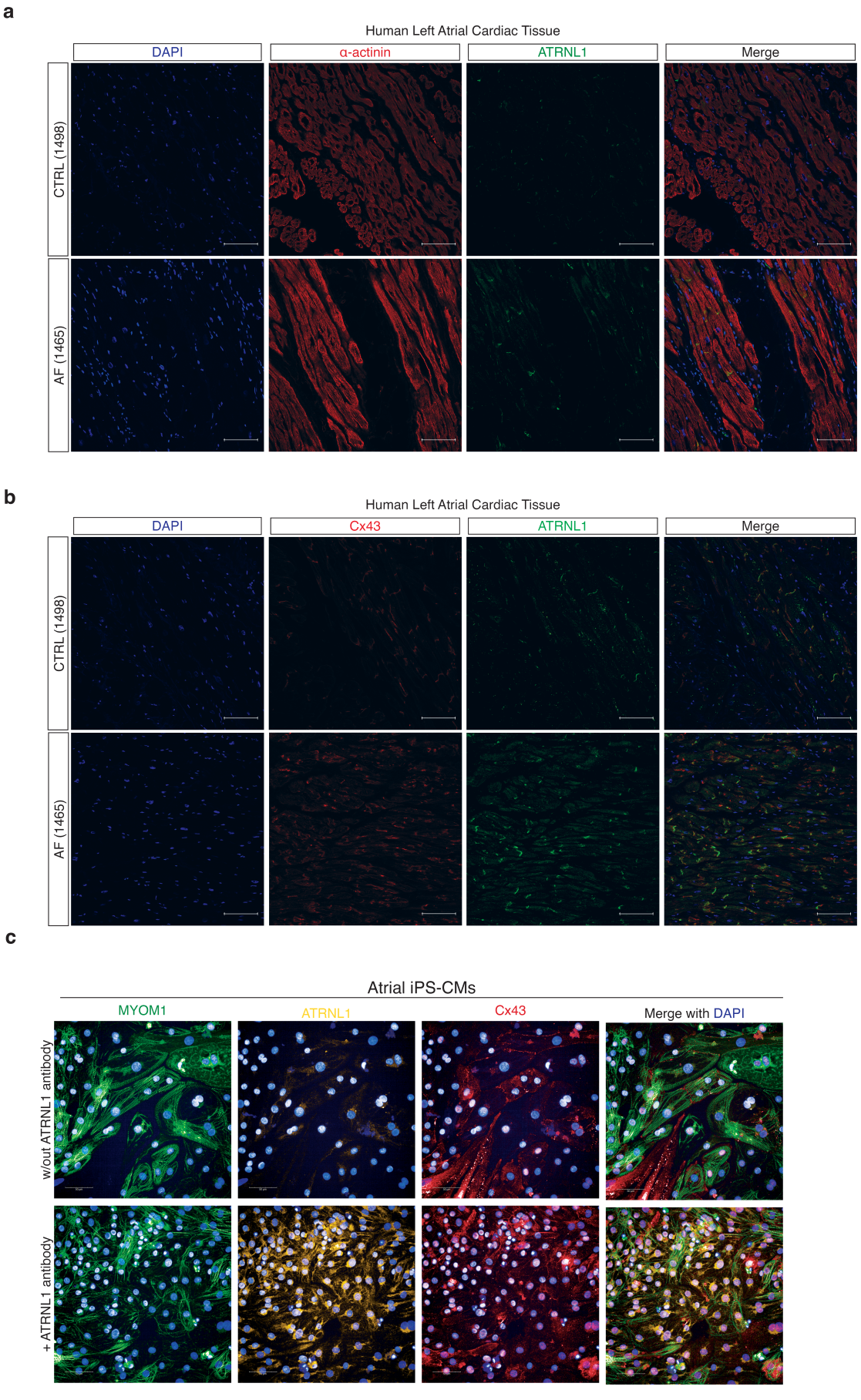

a
Valid Barcodes
Valid Barcodes %
AF
CTRL
Reads Mapped to Genome %
Q30 Bases in RNA Read
Median UMI counts per cell
b
Neurons
T-cells
Mural-1
Mural-2
Mural-3
MK
CMs
MT-Contam
CFs
MΦ
Doublets
Epicardial
Adipocytes
Mast
EC-1
EC-2
LEC
UMAP2
UMAP1
Total nuclei = 279,444
Removed = 28,040
Remaining = 251,404
c
% MT
Median % Mitochondrial reads
MT-Contam
Entropy
Median entropy
Doublets
Doublet score
Median doublet score
d
Total nuclei = 251,404
Removed = 63,659
Remaining = 187,745
e
Proportion of Sample
Proportion of cluster
Adipocytes
CMs
Doublets
EC-1
EC-2
CFs
LEC
T-Cell1
T-Cell2
MT-Contam
MΦ
Mural
Neuronal
Epicardial
B-cell
MK
f
Total nuclei = 187,745
Removed = 8,048
Remaining = 179,697
g
Range: 2,034 - 9,471
Mean: 5,285
Number of Cells per Library
Sample
h
Range: 454-52,884
Mean: 2,912
nUMI
CFs
CMs
MΦ
Pericytes
EC-1
Adipocytes
EC-2
SMCs
T-cell
LEC
Neurons
Epicardial
Mast
B-cell
MK
i
Range: 386-9,725
Mean: 1607
nGenes
j
Range: 0.0 - 0.05625
Mean: 0.009806
Percent (%) Mitochodrial
k
Range: 7.014580 - 8.805944
Mean: 8.078
entropy

Supplemental Figure 1. snRNA-seq sample quality control metrics. a Distribution of snRNA-seq quality control metrics for each library derived from CellRanger count as box boxplots grouped by patient category. **b** UMAP embedding of all nuclei post-sample QC (n = 279,444) colored by Leiden clustering and labelled according to predicted cell type. **c** Distribution of the median of three quality control metrics for each cluster shown as boxplots, including percent of unique molecular identifiers (UMIs) mapping to mitochondrial genes (% MT),entropy, and the Scrublet estimated doublet score. Clusters containing cells to be removed are highlighted in red (n= 28,040 nuclei). **d** UMAP embedding of all nuclei after removal of low-quality clusters identified in **b** and **c** (n = 251,404). Additional low-quality nuclei as detected per cluster and per-sample are colored in red. **e** Proportion of each sample and cluster removed during the quality control procedure shown as stacked bar graph. **f** UMAP representation of remaining nuclei after removal of low-quality clusters and per-cluster quality control. Red nuclei were deemed as misclassified or low-quality following sub-cluster analysis. **g** Number of nuclei per library included in final snRNA-seq embedding. **h** Distribution of number of unique molecular identifiers (nUMI). **i** Distribution of number of unique genes (nGene). **j** Distribution of percentage of mitochondrial transcripts per cell. **k** Distribution of entropy across nuclei of each unique cell type. Center line, median; box limits, upper and lower quartiles; whiskers,1.5x interquartile range.

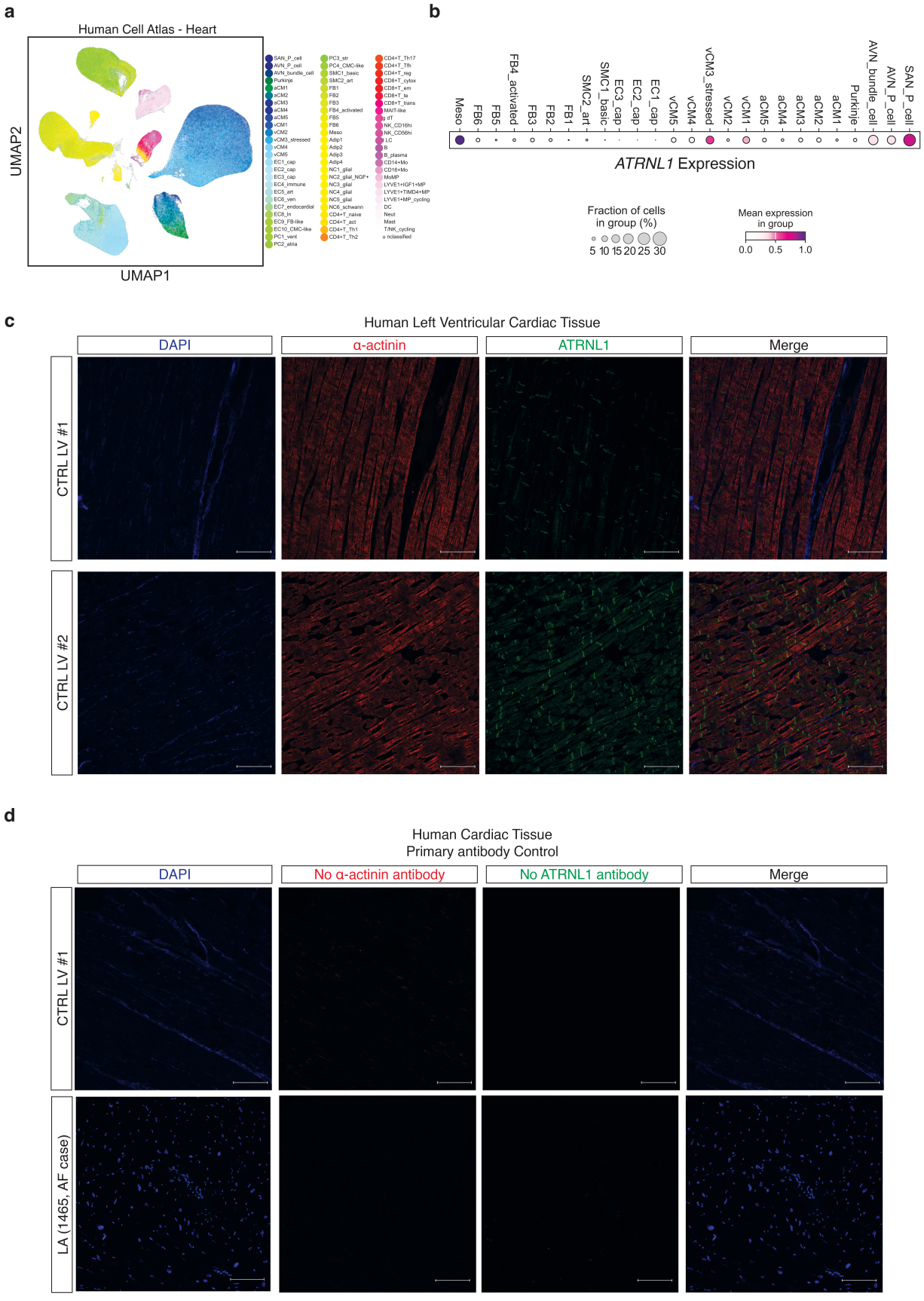


Supplemental Figure 2. Cellular composition of non-AF and AF patients LA tissue. a UMAP embedding of 179,697 cardiac nuclei colored by patient sample ID. **b** Cell-type composition of each individual human sample with cell-type colored according to our cluster annotations. **C** Box plot displaying the differences in cell composition

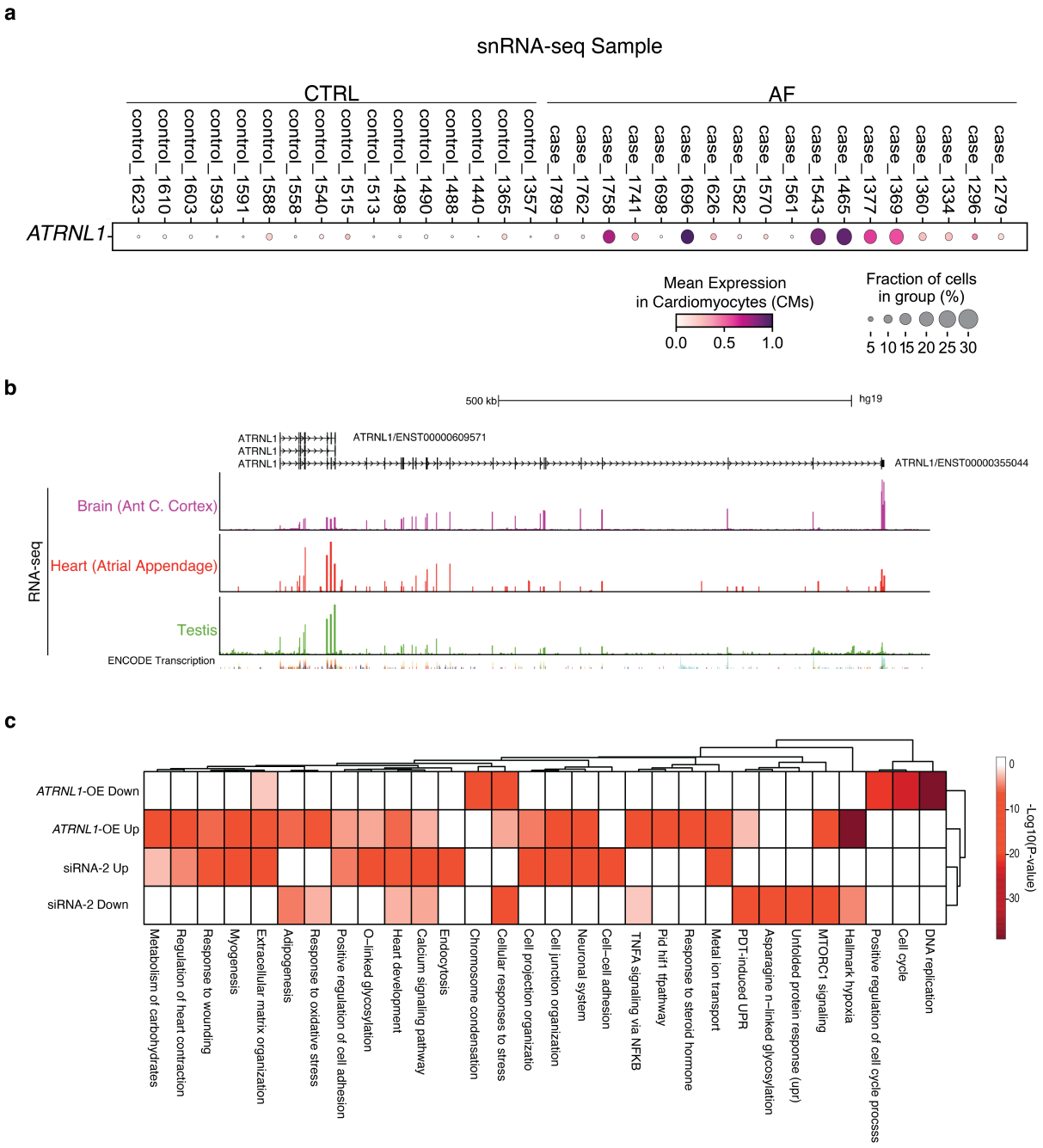
calculated with ScCODA for each cardiac cell type in controls (red) and patients with atrial fibrillation (AF) (blue). Boxplot represented as: center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers. **d** Mason's trichrome staining of LA samples from patients included in snRNA-seq analysis.


a
Human Left Atrial Cardiac Tissue
DAPI
α-actinin
ATRNL1
Merge
CTRL (1498)
AF (1465)
b
Human Left Atrial Cardiac Tissue
DAPI
Cx43
ATRNL1
Merge
CTRL (1498)
AF (1465)
c
Atrial iPS-CMs
MYOM1
ATRNL1
Cx43
Merge with DAPI
w/out ATRNL1 antibody
+ ATRNL1 antibody

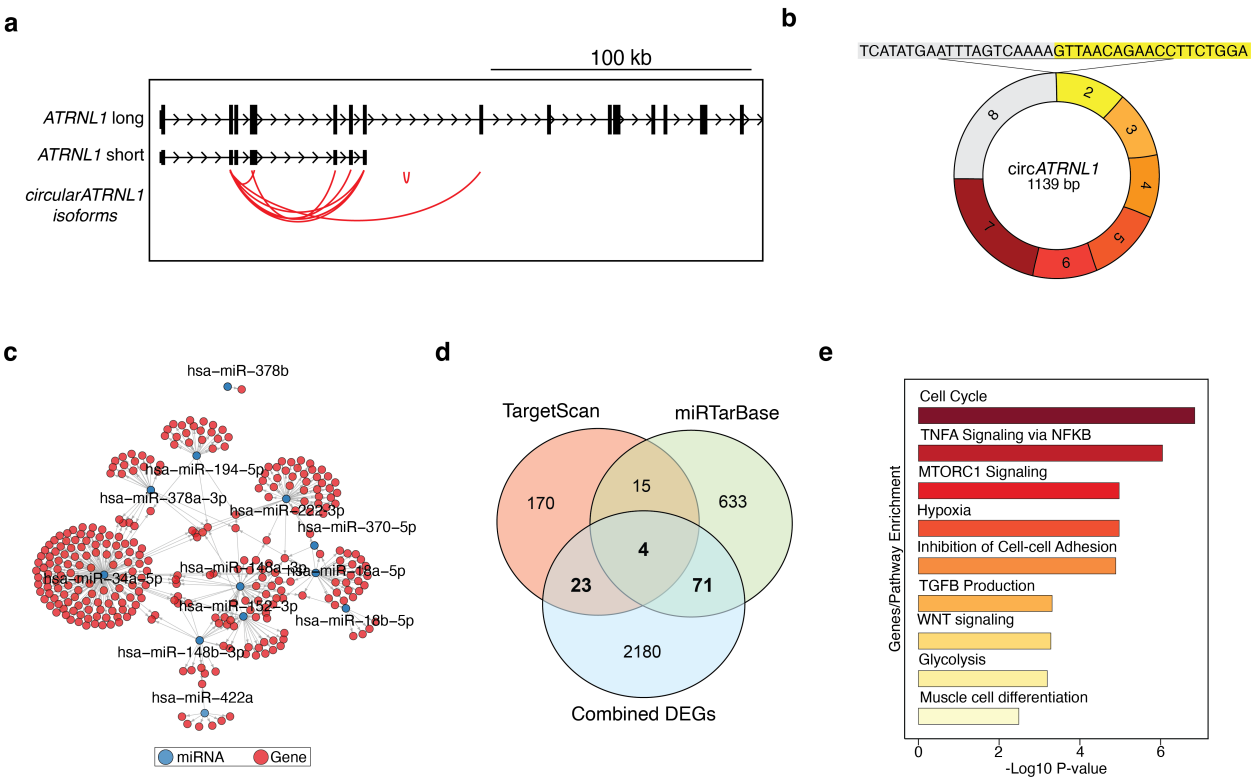
Supplemental Figure 3. Subcellular localization of ATRNL1 in human LA tissue. a Tissue immunohistochemistry for nuclei/DAPI (blue), α-actinin (red), and ATRNL1 (green) in control and AF LA tissue. **b** Tissue immunohistochemistry for nuclei/DAPI (blue), Cx43 (red), and ATRNL1 (green) in control and AF LA tissue. Scale bars = 100 μm. **c** Immunohistochemistry for nuclei/DAPI (blue), MYOM1 (green), Cx43 (red) ,and ATRNL1 (gold) in hESC-aCMs. Scale bars = 50 μm.


a
Human Cell Atlas - Heart
UMAP2
UMAP1
SAN_P_cell
AVN_P_cell
AVN_bundle_cell
Purkinje
aCM1
aCM2
aCM3
aCM4
aCM5
vCM1
vCM2
vCM3_stressed
vCM4
vCM5
EC1_cap
EC2_cap
EC3_cap
EC4_immune
EC5_art
EC6_ven
EC7_endocardial
EC8_ln
EC9_FB-like
EC10_CMC-like
PC1_vent
PC2_atria
PC3_str
PC4_CMC-like
SMC1_basic
SMC2_art
FB1
FB2
FB3
FB4_activated
FB5
FB6
Meso
Adip1
Adip2
Adip3
Adip4
NC1_glial
NC2_glial_NGF+
NC3_glial
NC4_glial
NC5_glial
NC6_schwann
CD4+T_naive
CD4+T_act
CD4+T_Th1
CD4+T_Th2
CD4+T_Th17
CD4+T_Tfh
CD4+T_reg
CD8+T_cytox
CD8+T_em
CD8+T_te
CD8+T_trans
MAIT-like
g dT
NK_CD16hi
NK_CD56hi
LC
B
B_plasma
CD14+Mo
CD16+Mo
MoMP
LYVE1+IGF1+MP
LYVE1+TIMD4+MP
LYVE1+MP_cycling
DC
Neut
Mast
TiNK_cycling
u nclassified
b
Meso
FB6
FB5
FB4_activated
FB3
FB2
FB1
SMC2_art
SMC1_basic
EC3_cap
EC2_cap
EC1_cap
vCM5
vCM4
vCM3_stressed
vCM2
vCM1
aCM5
aCM4
aCM3
aCM2
aCM1
Purkinje
AVN_bundle_cell
AVN_P_cell
SAN_P_cell
ATRNL1 Expression
Fraction of cells in group (%)
5 10 15 20 25 30
Mean expression in group
0.0 0.5 1.0
c
Human Left Ventricular Cardiac Tissue
DAPI
α-actinin
ATRNL1
Merge
CTRL LV #1
CTRL LV #2
d
Human Cardiac Tissue
Primary antibody Control
DAPI
No α-actinin antibody
No ATRNL1 antibody
Merge
CTRL LV #1
LA (1465, AF case)

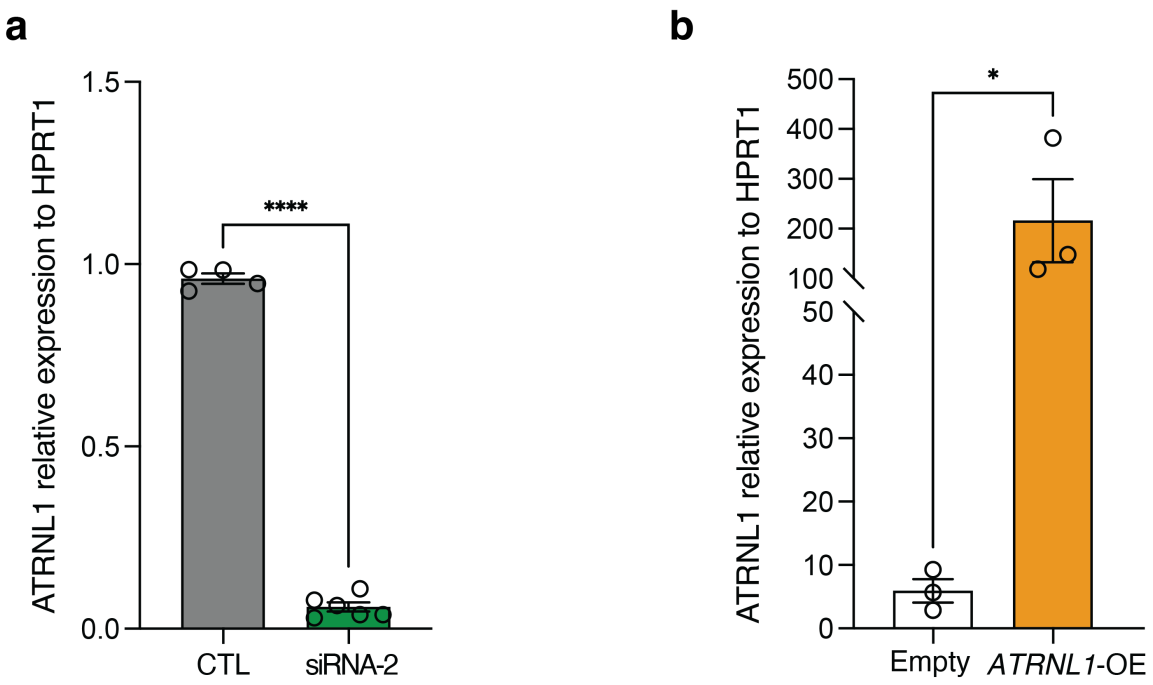
Supplemental Figure 4. Tissue expression of ATRNL1. a UMAP embedding of Human Heart Cell Atlas colored by cell state. **b** Dot plot showing expression of *ATRNL1* across all common cardiac cell states (non-immune cells). **c** Immunostaining of human left ventricular (LV) tissue. **d** Immunostaining of human left ventricular (LV) tissue and left atrial (LA) tissue controlling for primary antibody. Samples treated stained with DAPI and secondary antibodies only.



Supplemental Figure 5. Transcriptional profiling of hESC-aCMs. a Dot plot displaying the expression of *ATRNL1* in cardiomyocytes across all samples. **b** Genome browser tracks displaying GTEx RNA-seq reads mapped to the human genome (hg38). **c** Heatmap displaying pathway analysis colored by significance of enrichment (-Log10 P-value). Differentially expressed genes from the indicated conditions were used for pathway enrichment analysis.



Supplemental Figure 6. Circular *ATRNL1* isoforms in hESC-aCMs . a Genome browser tracks displaying detectable *circATRNL1* isoforms uncovered from our total RNA-seq analysis in human hESC-aCMs (hg38). **b** Diagram of the most common *circATRNL1* isoform detected in hESC-aCMs. **c** Target gene prediction network of miRNAs with significant binding scores to *circATRNL1*. **d** Venn diagram displaying the intersection of TargetScan, miRTarBase with the combined differentially expressed genes identified in **Fig. 5h**. **e** Pathway enrichment analysis for all 98 common genes identified in **Supplemental Fig. 6d**.



Supplemental Figure 7. Manipulation of *ATRNL1* levels in hESC-aCMs. **a** qPCR results of *ATRNL1* knockdown in hESC-aCMs with siRNA-2. **b** qPCR results of *ATRNL1* overexpression in hESC-aCMs transduced with a lentiviral *ATRNL1*-OE vector.

Supplemental Figure 8. Expression of AF GWAS loci from Human Heart Cell Atlas. Dot plot displaying the mean expression of AF GWAS genes across all cell states identified by the Human Heart Cell Atlas. Top, bars are colored according to the clusters presented in our study (**Figure 6c**). SAN, sinoatrial node; AVN, atrioventricular node; P cell, pacemaker cell; aCM, atrial cardiomyocyte; vCM, ventricular cardiomyocyte; EC, endothelial cell; PC, pericyte; SMC, smooth muscle cell; FB, cardiac fibroblast; meso,

mesothelial cell (epicardial cell); Adip, adipocyte; NC, neural cell; NK, natural killer cell; Mo, monocyte; MP, macrophage; DC, dendritic cell; Neut, neutrophil.