

Single cell transcriptomic meta-analysis uncovers sample-specific molecular heterogeneity in atrial fibrillation

Mohammad-Erfan Farhadieh¹, Olga Bondareva^{2,3,4}, Terrence Tigney², Peter Kohl^{5,6,7}, Ursula Ravens^{5,6,8}, Harald Lahm⁹, Martina Dreßen⁹, Lutz Hein^{2,10}, Achim Lothar^{2,11}, Sebastian Preissl^{1,2}

1 Department of Pharmacology and Toxicology, Institute of Pharmaceutical Sciences, University of Graz, 8010 Graz, Austria
 2 Institute of Experimental and Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Freiburg, Freiburg, Germany
 3 Helmholtz Institute for Metabolic, Obesity and Vascular Research (HI-MAG) of the Helmholtz Center Munich at the University of Leipzig and University Hospital Leipzig, Leipzig, Germany
 4 Division of Angiology, Department of Internal Medicine, Neurology and Dermatology, University Hospital Leipzig, Leipzig, Germany
 5 Institute for Experimental Cardiovascular Medicine, University Heart Center Freiburg Bad Krozingen, University of Freiburg, Freiburg, Germany
 6 Faculty of Medicine, University of Freiburg, Freiburg, Germany
 7 Centre for Integrative Biological Signalling Studies (CIBSS), University of Freiburg, Freiburg, Germany
 8 Institute of Physiology, Medical Faculty TU Dresden, Dresden, Germany
 9 Technical University of Munich, School of Medicine & Health, Department of Cardiovascular Surgery, Institute Insure, German Heart Center Munich, Munich, Germany
 10 BIOSS Centre for Biological Signaling Studies, University of Freiburg, Freiburg, Germany
 11 Interdisciplinary Medical Intensive Care, Medical Center - University of Freiburg, University of Freiburg, Germany

UNIVERSITY OF GRAZ



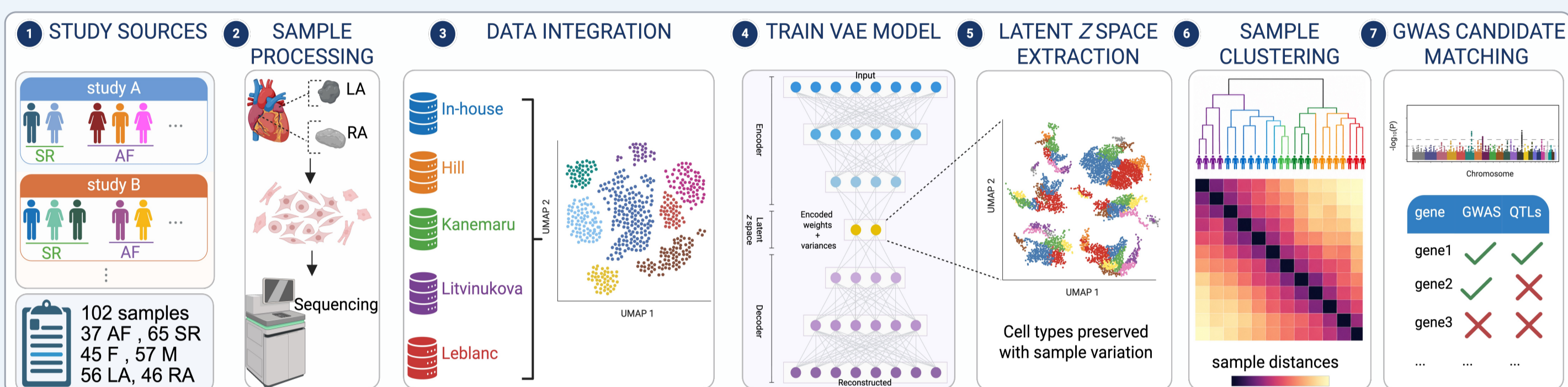
universität freiburg

1. Background and Aim

Atrial fibrillation (AF) is the most common sustained arrhythmia, affecting >59 million people worldwide, and is a major cause of stroke and heart failure. AF is multifactorial, with genetic contributors such as the *PITX2* locus, and progresses from paroxysmal to persistent and permanent disease states. Despite available rate- and rhythm-control therapies, AF remains difficult to reverse or completely cure.

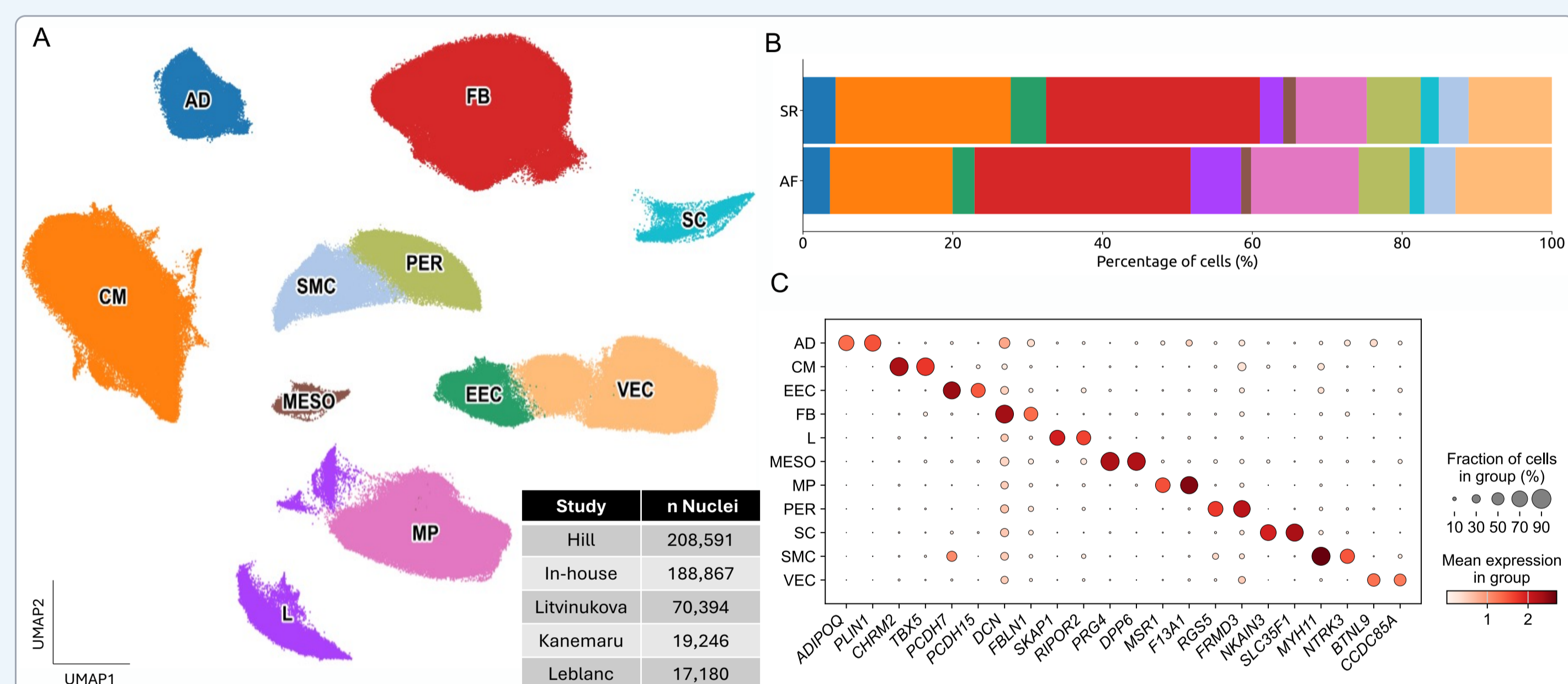
Previous transcriptomic studies reported AF-vs-sinus rhythm (SR) molecular differences, but it remains unclear which signatures are reproducible across cohorts. Here, integrated human atrial snRNA-seq meta-analysis was used to identify shared AF-associated transcriptional programs and characterize cell-type-specific and inter-individual heterogeneity.

2. Study Design



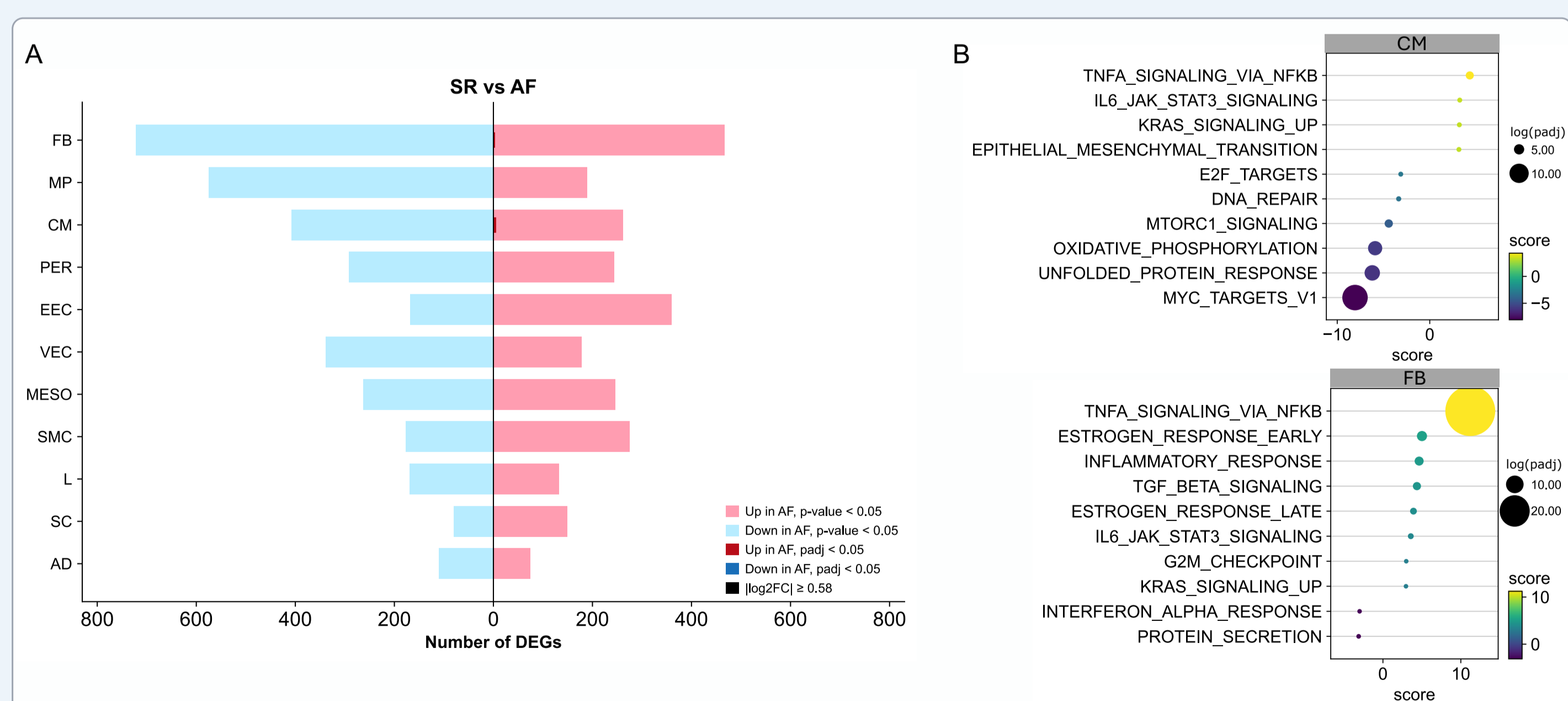
Schematic overview of the study design. Human atrial snRNA-seq datasets from in-house and four public studies (Hill et al. [1], Kanemaru et al. [2], Leblanc et al. [3], and Litvinukova et al. [4]) were integrated across disease status, sex, and atrial region. After sample processing and cell-type annotation, cell-type-specific expression profiles were used to train a variational autoencoder (VAE) model and extract latent representations. Sample-level clustering of the latent space was then used to identify molecularly similar AF/SR samples, define cluster-specific transcriptional programs, and integrate these findings with GWAS and QTL evidence to highlight candidate genes with genetic support.

3. Integrated cell atlas maps major atrial cell populations of heart atria



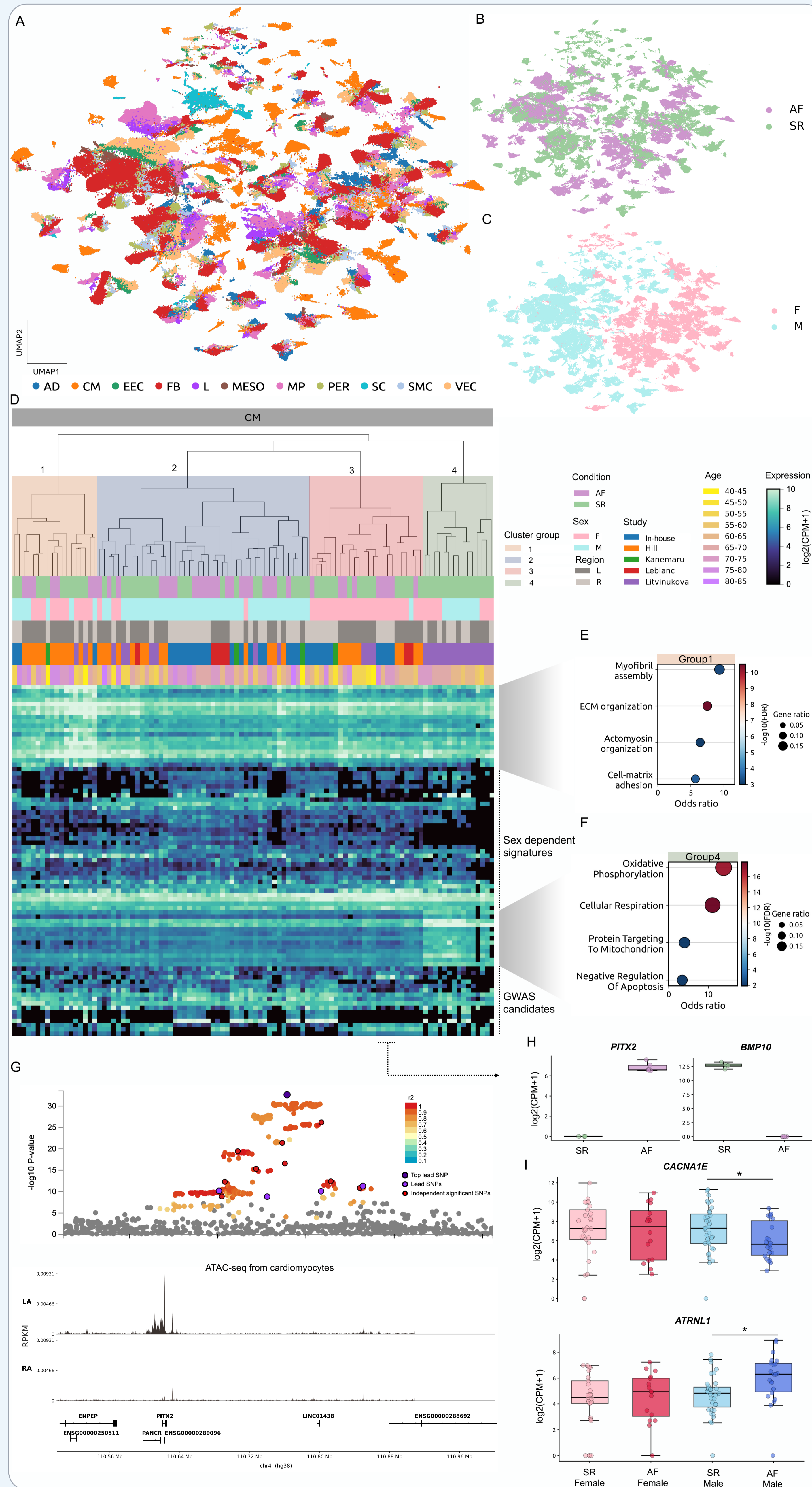
(A) UMAP embedding of integrated human atrial nuclei grouped by major cardiac cell types. (B) Comparison of cell-type proportions between AF and SR samples. (C) Canonical marker gene expression confirming cell-type annotation across atrial cell populations; dot size represents the fraction of expressing cells and color intensity represents mean expression. AD, adipocytes; CM, cardiomyocytes; EEC, endocardial endothelial cells; FB, fibroblasts; L, lymphocytes; MESO, mesothelial cells; MP, myeloid phagocytes; PER, pericytes; SC, Schwann cells; SMC, smooth muscle cells; VEC, vascular endothelial cells.

4. Differential gene expression analysis identifies limited common AF signatures



(A) Cell-type-resolved differential expression showed widespread nominal AF-associated changes, but relatively 10 genes passed FDR correction, indicating that average AF-vs-SR contrasts capture only a modest shared disease signal. (B) Hallmark pathway enrichment in cardiomyocytes highlighted pathway-level trends, including general inflammatory and stress-related programs, and estrogen response in addition to inflammatory program in fibroblasts based on ranked AF-vs-SR transcriptional changes.

5. Latent-space analysis reveals distinct molecular AF subgroups



(A) VAE-derived latent representations reveal sample-specific transcriptional heterogeneity across the dataset while preserving major cardiac cell-type variation. (B, C) AF/SR labels do not form a single global separation, whereas sex shows stronger latent-space organization. (D) Sample-level clustering identifies distinct cardiomyocyte transcriptional programs, and highlights candidate genes with potential links to AF genetic risk loci. (E) Group 1 is enriched for structural remodeling programs (F) Group 4 is enriched for mitochondrial and metabolic programs (G) The AF-associated *PITX2* locus shows stronger chromatin accessibility in LA than RA, indicating an atrial-region-specific regulatory context. (H) Control samples show chamber-restricted expression of *PITX2* in left atrium and *BMP10* in right atrium. However, a subset of right atrial AF samples shows ectopic *PITX2* expression and reduced *BMP10*, suggesting a left atrial-like transcriptional shift. (I) Sex-stratified analysis reveals male-specific differences in *CACNA1E* and *ATRNL1* expression. Asterisk (*) indicates statistical significance ($p < 0.01$)

Summary

Human atrial snRNA-seq meta-analysis enabled cell-type-resolved analysis of AF/SR transcriptional variation across 102 samples.

Average AF-vs-SR analysis showed limited shared transcriptional signal, whereas sample-level latent modeling revealed distinct AF-associated molecular states.

AF samples separated into distinct cardiomyocyte molecular programs associated with sex, structural remodeling, mitochondrial metabolism, and AF-associated loci, including the *PITX2* region.

References

- Hill, Matthew C., et al. "Large-scale single-nuclei profiling identifies role for *ATRNL1* in atrial fibrillation." *Nature communications* 15.1 (2024): 10002.
- Kanemaru, Kazumasa, et al. "Spatially resolved multiomics of human cardiac niches." *Nature* 619.7971 (2023): 801-810.
- Leblanc, Francis JA, et al. "Single-nucleus multi-omics implicates androgen receptor signaling in cardiomyocytes and *NR4A1* regulation in fibroblasts during atrial fibrillation." *Nature Cardiovascular Research* 4.4 (2025): 433-444.
- Litvinukova, Monika, et al. "Cells of the adult human heart." *Nature* 588.7838 (2020): 466-472.

Scan for extended figures

