Supplementary Table and Data Figures:

Table S1: Proteomics discovery data

Table S2: MGH Cohort Discovery- Clinical demographics

Characteristic	Heart Failure (n = 15)	Myocardial Infarction (n = 15)	Control (n = 15)
Male sex	9	10	5
Female sex	6	5	10
Age (years)	76 ± 11	64 ± 12	51 ± 17
SBP (mmHg)	115 ± 14	75 ± 12	-
DBP (mmHg)	70 ± 9	73 ± 12	-
Heart rate (bpm)	73 ± 12	70 ± 10	-
Weight (lbs)	195 ± 50	17[illegible] ± 36	-
Height (in)	67 ± 4	67 ± 4	-
BMI (kg/m²)	31 ± 7	26 ± 4	30 ± 8
LVEF (%)	4[illegible] ± 20	60 ± 9	66 ± 5
Sodium (mmol/L)	140 ± 3	140 ± 3	-
Creatinine (mg/dL)	1.21 ± 0.2	1.0 ± 0.3	-
NT-proBNP (pg/mL)	1685 ± 1900	892 ± 1400	-
Troponin T (ng/mL)		887 ± 1500	-

Table S3: MGH Cohort- validation- Clinical demographics

Characteristic	Heart Failure (n = 15)	Myocardial Infarction (n = 15)	Control (n = 15)
Male sex	7	7	7
Female sex	8	8	8
Age (years)	66 ± 10	64 ± 11	64.5 ± 10.5
SBP (mmHg)	118 ± 14	122 ± 15	
DBP (mmHg)	72 ± 9	74 ± 12	-
Heart rate (bpm)	75 ± 12	72 ± 10	-
Weight (lbs)	195 ± 50	170 ± 36	-
Height (in)	67 ± 4	67 ± 4	-
BMI (kg/m²)	31.0 ± 7	26.5 ± 4	30.4 ± 8
LVEF (%)	55 ± 12	57 ± 10	66 ± 9
Sodium (mmol/L)	140 ± 3	140 ± 3	-
Creatinine (mg/dL)	1.22 ± 0.2	1.13 ± 0.3	-
NT-proBNP (pg/m	1500 ± 600	890 ± 1100	-
Troponin T (ng/mL	6 ± 12	885 ± 1490	-

Table S4: Clinical demographics of donors and individuals with aortic stenosis (single-nuclear RNA-sequencing)

Characteristic	Aortic Stenosis (n=11)	Control (n=7)
Age, years	65.6 ± 19.3	47.6 ± 15.5
Gender, Male, %	55 (6/11)	57 (4/7)
Coronary artery disease	73 (8/11)	-
History of heart failure,	100 (11/11)	-
Hypertension, %	91 (10/11)	-
Hyperlipidemia, %	70 (7/10)	-
Diabetes, %	73 (8/11)	-

Supplementary Figures: 1-6

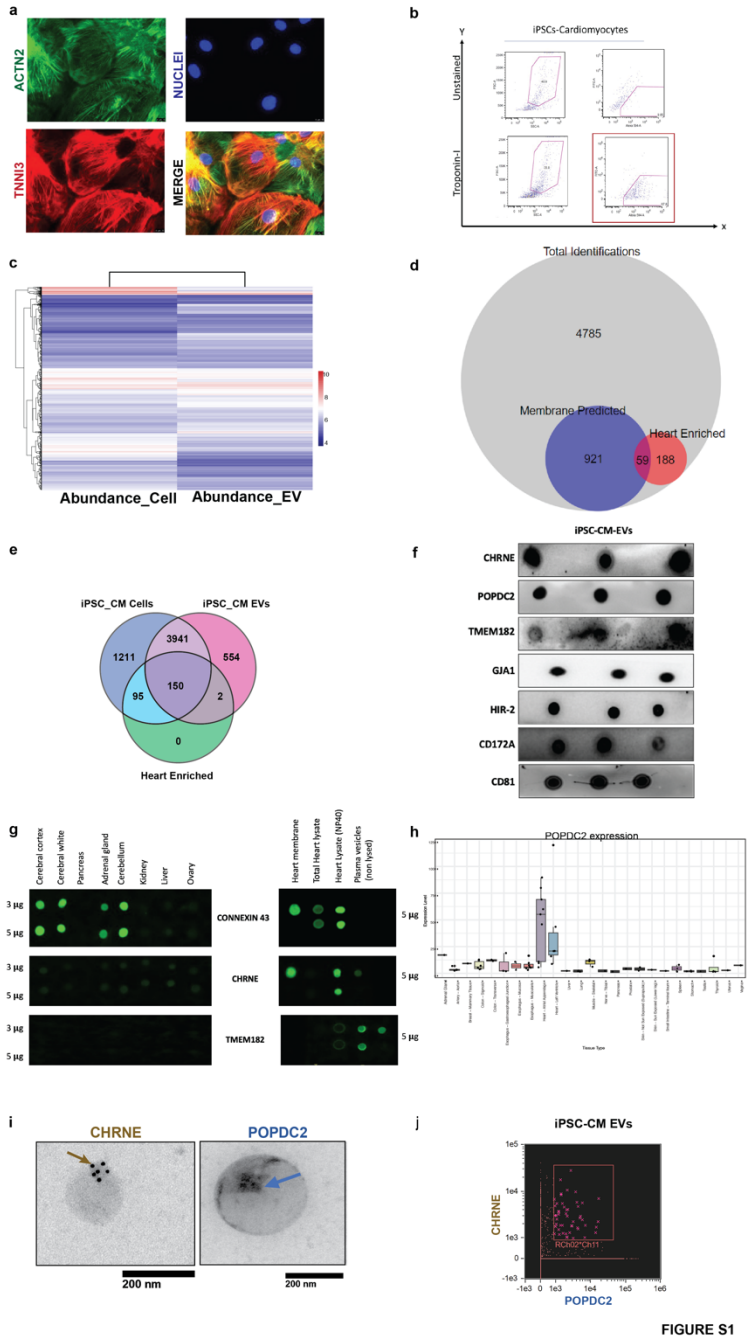

FIGURE S1

Figure S1. Discovery and experimental validation to identify POPDC2 and CHRNE as cardiomyocyte- EV (Extracellular Vesicle) membrane protein candidates. Representative **a.** confocal images (showing ACTN2 and TNNI3 positivity) and **b.** Flow-cytometric data (showing Troponin-1 expression) of iPSC-CM (induced Pluripotent Stem Cell-derived Cardiomyocytes) demonstrating cardiac specificity by their expression of classical cardiac markers such as

ACTN2, TNNI3 and TNNT1. **c.** Heatmap showing the abundance of proteins expressed in iPSC-CMs and iPSC-CM-EVs (iPSC-CM Extracellular vesicles) as identified using LC-MS/MS (Liquid Chromatography with tandem mass spectrometry). **d.** Proportional venn diagram showing the intersection of total proteins identified using LC-MS/MS, membrane -localize and heart enriched. **e.** Venn diagram showing 150 targets that were identified using LC-MS/MS present in iPSC-CM, iPSC-CM-EVs and enriched in the heart (from Human Protein Atlas data). **f.** Representative experimentally validated dot blots in iPSC-CMs for various antibodies against proteins identified using the proteomic approach discovery. **g.** Representative tissue-specific dot blots using brain and heart tissue lysates from human samples tested for CONNEXIN43, TMEM182 and CHRNE derived from Computational approach. **h.** Tissue-wise proteomic expression of POPDC2 using GTEx showing elevated expression in heart tissue. **i.** Representative images of $POPDC2^{+}$ (blue arrows) and $CHRNE^{+}$ (gold arrows) EVs in iPSC-CM-EVs showing single positive ($POPDC2^{+}$ $CHRNE^{-}$; $POPDC2^{-}$ $CHRNE^{+}$) EVs visualized through Transmission Electron Microscopy with gold particle POPDC2 (5 mm) and CHRNE (12 mm). **j.** $POPDC2^{+}$ and $CHRNE^{+}$ EVs in iPSC-CMs demonstrated using ImageStream flow cytometry based-methodology (n=3 replicates).

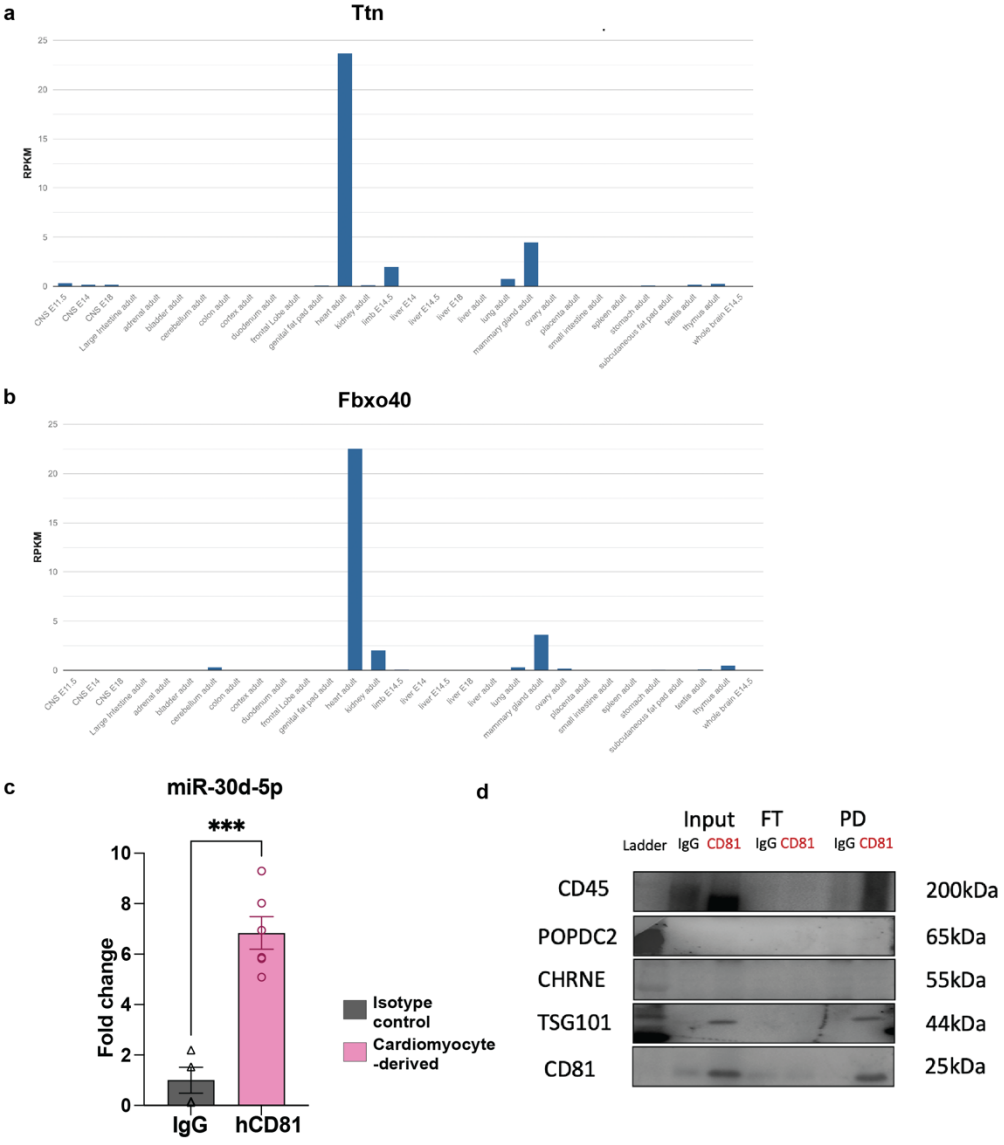

FIGURE S2

Figure S2. Cardiac-specific transcriptome of CD81 EVs in αMHC-Cre driven Exomap mouse model. a,b. Representative tissue-wise expression of heart-enriched transcripts (Ttn, Fbxo40) obtained using Mouse ENCODE transcriptomic data from NCBI. **c.** Enrichment of cardiac-specific small RNA miR-30d -5p in immunocaptured cardiac-enriched humanized CD81 EVs compared to the respective isotype control. **d.** Immune tissue-specific Exomap mouse

model does not express POPDC2 and CHRNE as seen using the western blot image showing isolated hsCD81+ EVs (enrichment for CD81 expression in pulled-down samples as well as other EV markers such as Alix and TSG101) from the plasma of mice derived from crossing vav-cre transgenic mice (cre expression in hematopoietic cells) and ExoMap mice. These EVs were enriched for immune markers (CD45), but not POPDC2 or CHRNE.

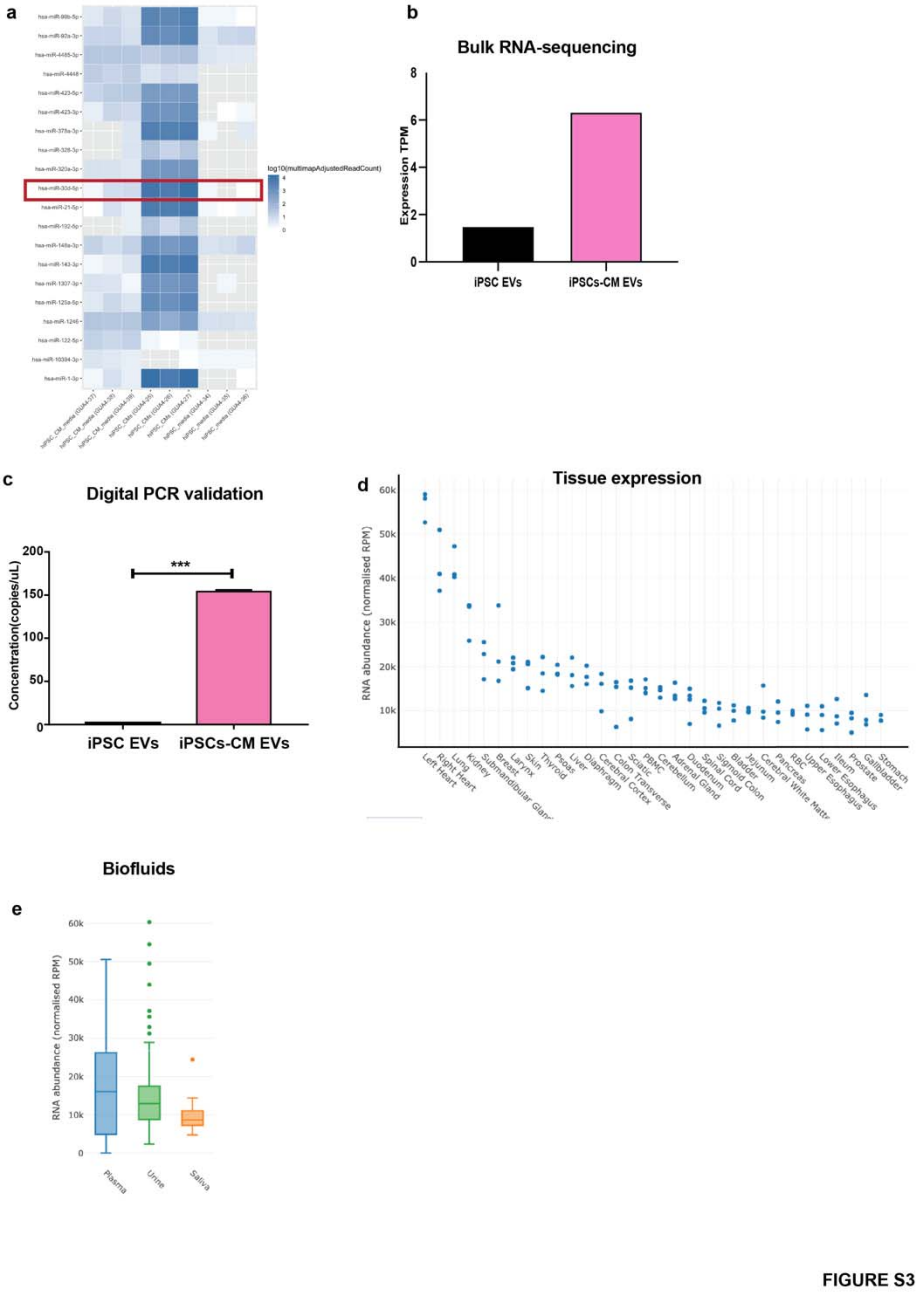

FIGURE S3

Figure S3. Small RNA sequencing of iPSC-CMs (induced Pluripotent Stem Cell-derived cardiomyocytes) and iPSC-CM-EVs (induced Pluripotent Stem Cell-derived cardiomyocyte – Extracellular Vesicles). a. Heatmap of the most significantly different miRNAs between iPSC-CMs and iPSC-CM-EVs with human Pluripotent Stem Cells as controls with miRNA-30d (miR-30d) as one of the most enriched in iPSC-CMs and iPSC-CM-EVs. **b.**

Expression of miR-30d as observed in bulk RNA-sequencing showing enrichment in iPSC-CM-EVs compared to iPSC-CMs. **c.** Validation of miR30d expression using digital PCR showing enrichment in iPSC-CM-EVs compared to iPSC-CMs (p-value calculated using student t-test). **d.** Expression of miR-30d in different human tissues showing cardiac enrichment. **e.** Expression of miR-30d in different human biofluids showing expression in human plasma.

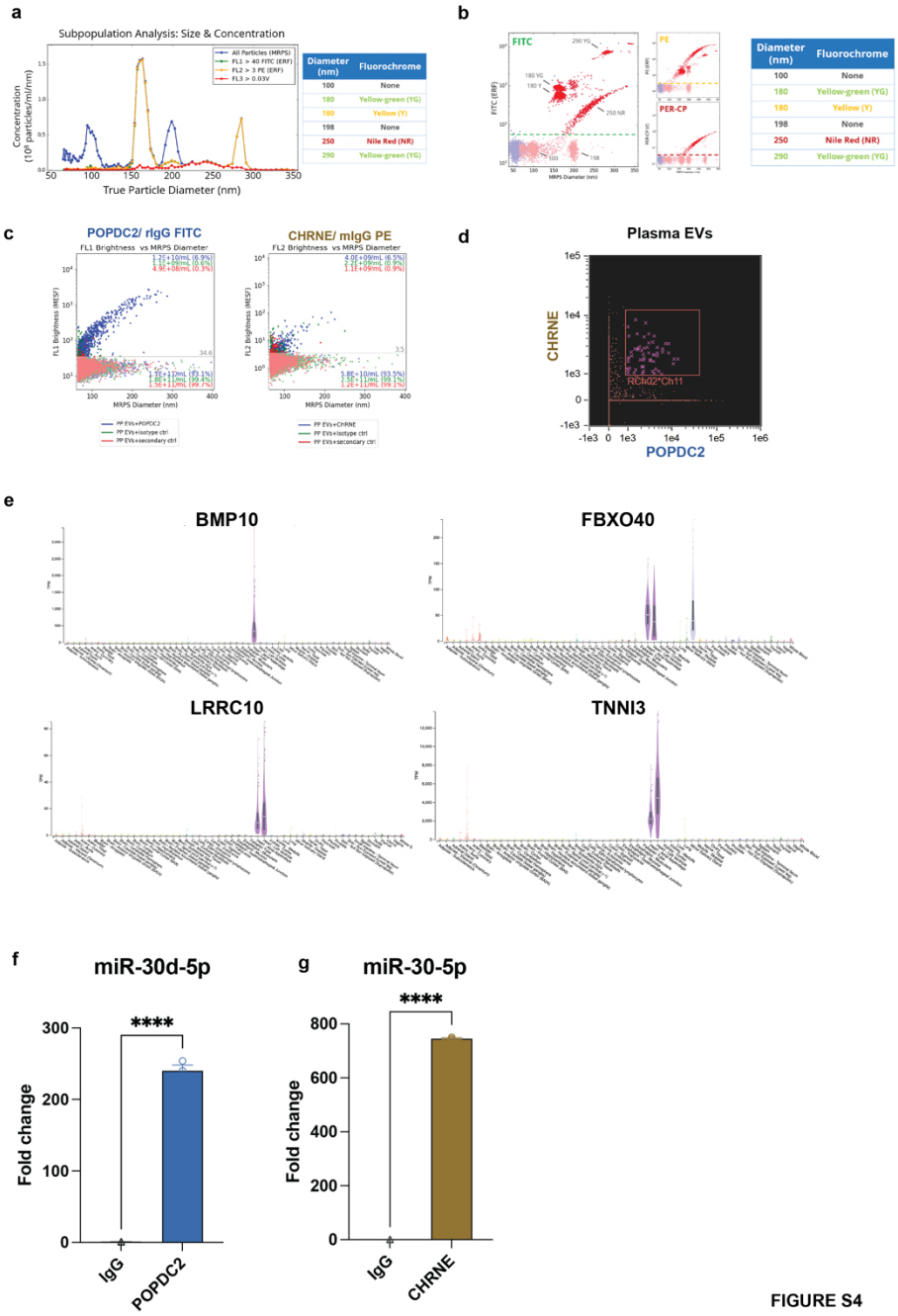

Figure S4. Characterization and profiling POPDC2^{+} and CHRNE^{+} cardiovesicles in healthy human plasma.

Appropriate Controls for Fluorescent- Microfluidic Resistive Pulse Sensing (F-MRPS) **a. b.** demonstrating the size and fluorescence specificity using appropriate bead-controls. **c.** Isotype and secondary antibody only control for POPDC2^{+} and CHRNE^{+} immunolabelling.

d. POPDC2^{+} and CHRNE^{+} EVs in healthy pooled plasma EVs demonstrated using ImageStream flow cytometry based-methodology. **e.** Tissue-wise transcriptomic expression of mRNA transcripts enriched in POPDC2 and CHRNE cardiovesicles (BMP10, FBXO40, LRRC10, TNNI3) using GTEx showed elevated expression in human heart tissue. Enrichment of cardiac-specific small RNA miR-30d -5p in **f.** POPDC2 and **g.** CHRNE immunocapture from healthy human plasma EVs compared to the respective isotype control.

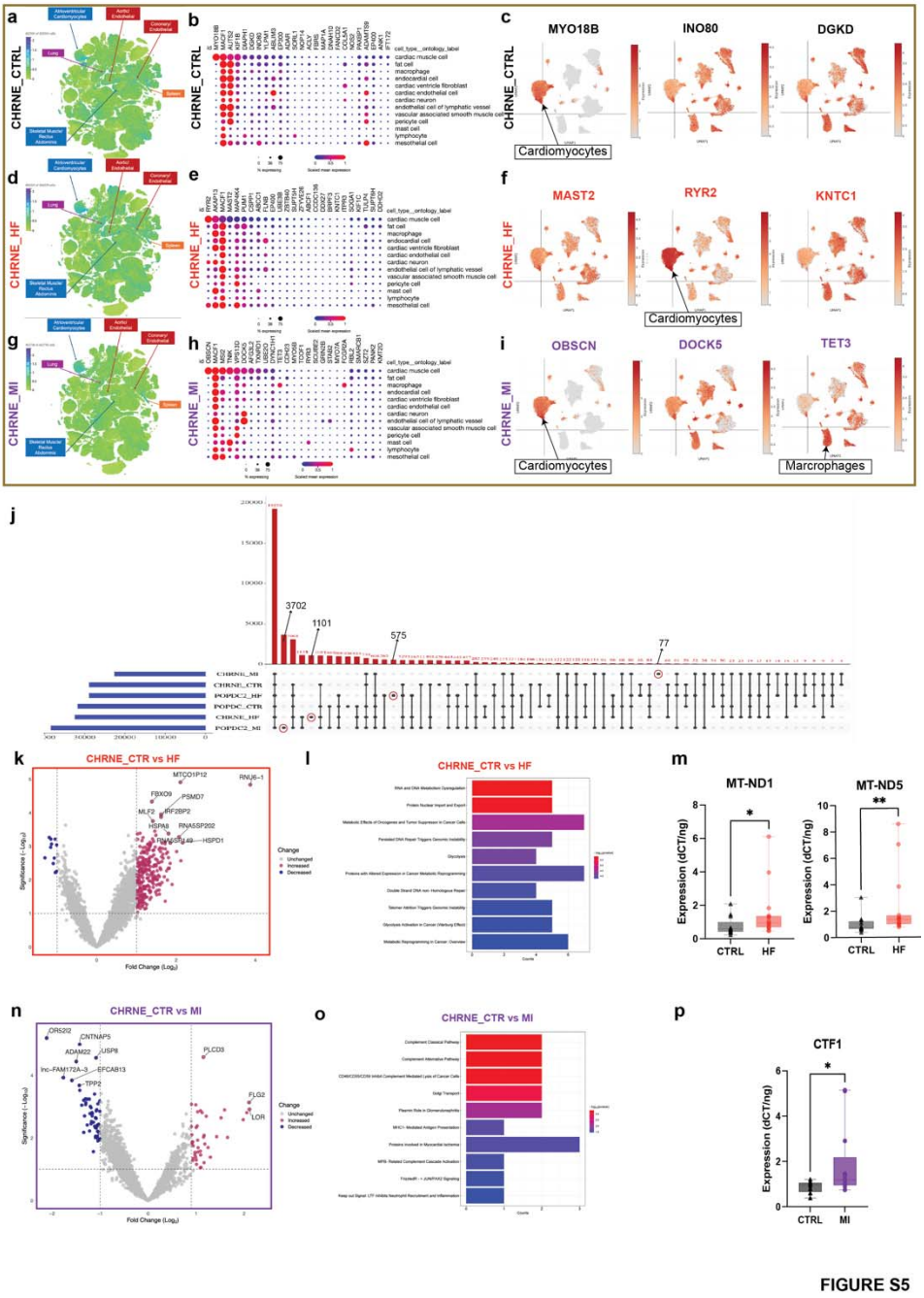

FIGURE S5

Figure S5. Analysis of CHRNE^{+} cardiovesicle transcriptome from HF and MI patients with respect to control patients in the MGH cardiovascular cohort. Top 25 enriched transcripts (with mean expression threefold greater than standard deviation) from CHRNE^{+} Ctrl, HF and MI patients were identified and mapped onto the multiorgan single cell transcriptomic atlas dataset (Tabula Sapiens) (**a, d, g** respectively) and a single-nuclear dataset of human control (no

cardiovascular disease), dilated cardiomyopathy (**b, c, e, f, h, i).** Cardiovesicle transcripts from control patients mapped to the multi-organ atlas is shown as a target UMAP in **a,** with summary dot-plots and individual target UMAPs from the heart single nuclear dataset shown in **b** and **c** respectively. The same representations for the cardiovesicles from HF are shown in **d**, **e**, and **f**, while those from MI are depicted in **g**, **h** and **i**. **j.** Upset plot showing common and unique transcripts in cardiovesicles isolated from Ctrl, HF and MI patients using POPDC2 and CHRNE immunocapture. Genes expressed in 50% of each group of samples are shown. The unique number of genes for each group are indicated using a black arrow with the respective number of genes for that group (3702 genes unique to POPCD2 MI, 1101 unique to CHRNE HF, 575 unique to POPDC2 HF, and 77 genes unique to CHRNE MI).The unique transcripts for each group is indicated using a black arrow with the respective number of transcripts for that group. Transcripts within Cardiovesicles immunocaptured with CHRNE from Ctrls showed high cardiac specificity compared to HF and MI. Volcano plots displaying differentially expressed transcripts (with log FC + 1 and $p < 0.01$) in $CHRNE^{+}$ cardiovesicles from patients with HF compared to control patients (**k**) with pathway enrichment analysis (**l**). **m.** Validation in the MGH validation cohort (n=30) for selected transcripts differentially expressed in HF-$CHRNE^{+}$ EVs (MT-ND1, p=0.0253, MT-ND5, p=0.0061) using qRT-PCR normalized to internal and spike-in controls. Similar analysis including volcano plot for differentially expressed transcripts in $CHRNE^{+}$ EVs in patients with MI compared to controls (**n**), pathway enrichment analysis (**o**), and experimentally validation of select transcripts in the MGH validation cohort (**p,** CTF1, p=0.0398, n= 30. Significance levels are marked as * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$, calculated using the Kruskal-Wallis test.

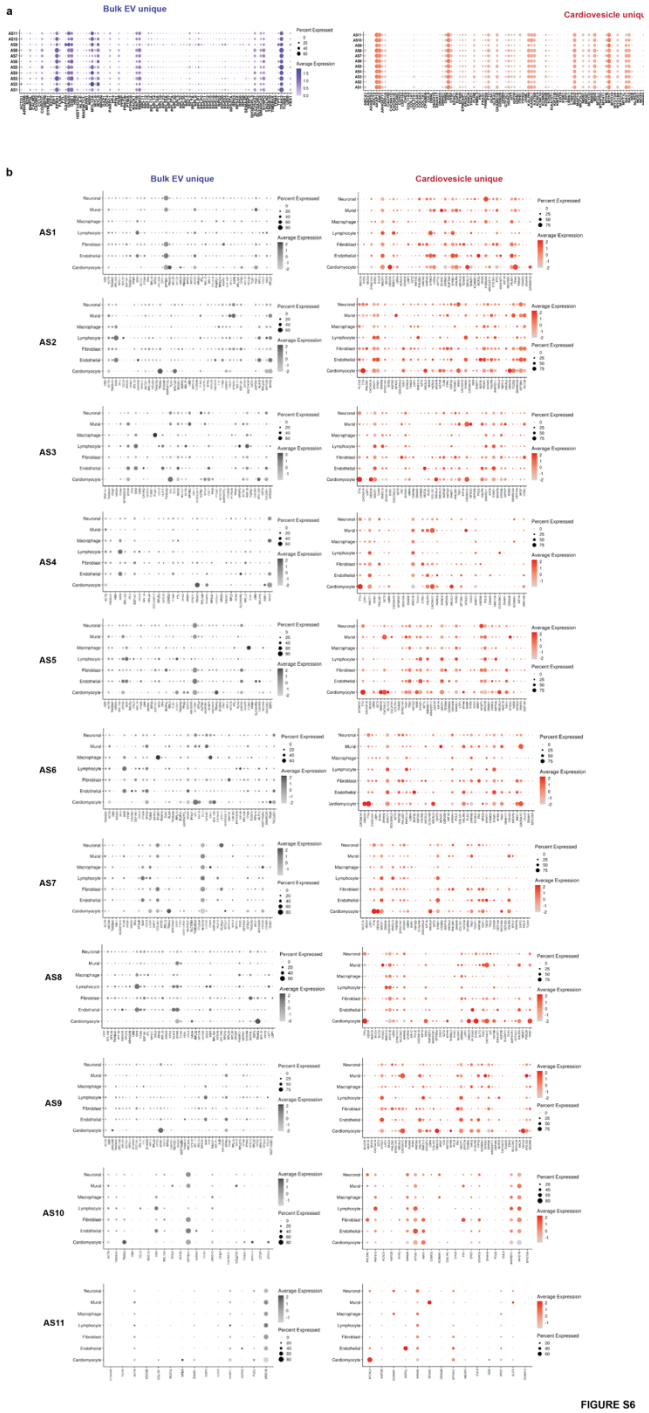

Figure S6. Analysis of cardiovesicle transcriptome from AS patients with respect to control patients in the VUMC cardiovascular cohort. a. Dot plots showing the expression of all “Bulk EV_unique” (EV_AS, 92) and “Cardiovesicle_unique” transcripts (POPDC2_AS and POPDC2_Ctr, 153) in the cardiomyocytes of each AS patient obtained using cardiac tissue

single-nuclear RNA-sequencing demonstrating predominant cardiac specificity of "Cardiovesicle_unique" transcripts compared to "Bulk EV_unique" transcripts. **b.** Dot plots showing the expression of Bulk EV_unique and Cardiovesicle_unique transcripts in different heart cell types of each AS patient.