Supplementary material online

Supplementary Figure 1 (related to figure 1): Left atrial force transducer experiments. a: Dose-response curves to show change in tension generated on cumulative addition of isoprenaline to stimulated (5Hz) murine left atria preparations under control conditions (circles, n=7) and in the presence of either 100 nM bafilomycin A1 (diamonds, n=9) or 3 µM *trans*-Ned-19 (triangles, n=6). **b**: Comparison of tension force in beating murine left atrial preparations in PSS prior to addition of drug or stimulation with isoprenaline. **c**: Comparison of change in beating force (in %) in beating murine left atrial preparations in PSS on addition of DMSO, 100 nM bafilomycin A1 or 3 µM *trans*-Ned-19, prior to stimulation with isoprenaline. **d**: Dose-response curves to show change in tension generated on cumulative addition of isoprenaline to stimulated (5Hz) murine left atria preparations in WT mice (circles, n=11) and in $TPC2^{-/-}$ mice (squares, n=11). Dose-response curves in panel a and d were fitted using log(agonist) vs. response (three-parameter model) and best-fit values for top (maximum response) and EC_{50} are reported with 95 % confidence intervals (CI, calculated using profile likelihood method). Data in panel b and c are presented as median (interquartile range: 25th–75th percentile). Statistical significance was determined using the Kruskal–Wallis test followed by Dunn's multiple comparisons test, maximal response in panel e was analyzed using unpaired t-test. Asterisks indicate significance level for effect of bafilomycin A1 and *trans*-Ned-19, non-significant (ns)=P>0.05; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

Supplementary Figure 2 (related to figure 4e): 4Pi-SMS super-resolution microscopy analysis. a: 4Pi-SMS super-resolution microscopy analysis; scale bar represents 2 µm. Representative Z-projection of the 4Pi SMS imaged data indicating lysosomes (spheres) which are less than (yellow) or more than (green) 500 nm of their nearest neighbour in cultured and fixed NRAMs. Scale bar 2 µm. **b:** Distribution of nearest neighbor distances between lysosomes in cultured and fixed NRAMs. **c:** Distribution of measured lysosome volumes in cultured and fixed NRAMs.

Supplementary Figure 3 (related to figure 4): characterization of hiPSC-aCMs. a: Representative 3D reconstruction of high-resolution confocal image showing lysosomes (magenta) and nucleus (cyan) in hiPSC-CMs; scale bar represents 4 µm. **b:** Quantification of lysosomal density (N=6 field of views), presented as lysosomes/μm^3. **c:** Summary table of lysosomal measurements in hiPSC-CMs. Values represent the mean distance in µm of the three closest lysosomes to each other per cell, with corresponding minimum (Min), maximum (Max) and standard deviation (Std. Dev., N=6 field of views). Data presented as mean±Std. Dev. Analyses in b and c were performed across N=6 field of views, which corresponds to 2137 lysosomes identified within a cellular volume of 64408 μm^3.

Supplementary Figure 4 (related to figure 4): characterization of hiPSC-aCMs. a: Representative sample traces of potassium currents elicited at +50 mV in control (black) or in the presence of 50 µM

4-AP in hiPSC-aCMs (i) and in hiPSC-CMss (ii). **b**: Current-voltage relationships of 4-AP-sensitive currents in hiPSC-aCMs and in hiPSC-CMss (n= number of cells, N=number of independent differentiations) **c:** Image showing representative staining in hiPSC-aCMs; the plasma membrane localization of $K_v1.5$ (i, green), nuclear Staining DAPI (ii, blue), and merge image (iii, using ImageJ)**. d:** Image showing representative staining in hiPSC-aCMs of pro-ANP localization in perinuclear region e cytosolic granuli (i, red), nuclear Staining DAPI (ii, blue), and merge image (iii, using ImageJ)**.** Scale bar 20μm. Data are represented as mean±SEM and was analyzed using ANOVA followed by Šídák's multiple comparisons test.; not significant (ns); *P< 0.05, **P< 0.01, ***P< 0.001.

Supplementary Figure 5 (related to figure 6): Goat left atrial electron microscopy. Snap shots from goat left atrial sinus rhythm control (column **a**) and atrial fibrillation (column **b**) from 3D tomograms to show proximity of Lysosomes (Ly) to Mitochondria [67] and Sarcoplasmic Reticulum [68]. Scale bars represent 200 nm.

Supplementary Figure 6 (related to figure 2): Rodent isolated cardiomyocyte cytosolic pH measurements. a: Rat ventricular myocytes, loaded with cSNARF1 in control condition and in the presence of 1 μM bafilomycin A1; scale bar represents 100 μm. **b**: Rat ventricular myocytes, loaded with cSNARF1 and measured in HEPES buffer extracellular solution (in mM: NaCl 130, KCl 4.5, HEPES 20, $CaCl_2$ 1, $MgCl_2$ 1, Glucose 11) exposed to 1 μM bafilomycin A1 by dual microperfusion (n=8). Data are represented as mean±SEM.

Supplementary Figure 7: Spatial interaction between lysosome-sarcoplasmic reticulum and lysosome-mitochondria. a-b: Representative 3D electron tomography images of murine SAN including raw images (e and f top panels) and reconstructed organelles in 3D (e and f bottom panels) of lysosomes (red), sarcoplasmic reticulum (blue) and mitochondria (yellow)**.** Model: lysosomes (Ly, red), sarcoplasmic reticulum (SR, blue), mitochondria (Mito, yellow); scale bars represent 200 μm.

Supplementary Figure 8 (related to discussion): IPA analysis of human tissue samples. a: Violin Plot of protein intensity preparations in right atrial appendage human samples from atrial fibrillation patient (N=6) and patient with a normal sinus rhythm (N=7). **B:** Volcano Plot of differential gene expression of human samples from atrial fibrillation patient (N=6) and patient with a normal sinus rhythm (N=7), MaxLFQ is a generic method for label-free quantification, proteins with fold changes greater than 2 are highlighted, Instant Clue v0.12.1. See Supplementary File 1 for protein list. **c:** The highest ranked canonical IPA pathways of the differentially expressed proteins (using a score cut off -log (p values) are greater than 3.8). The horizontal axis shows the pathway names, and the vertical axis is the p-value (-log) of each pathway. Blue bars: negative z-score; orange bars: positive z-score and clear bars are indicative of a z-score of 0, and thus have no difference in activity. Grey shaded bars indicate that there is no activity pattern available identified by IPA, despite highly significant association of the proteins within the pathway. See Supplementary File 2 for list.

Supplementary Figure 9 (related to discussion): Pathway analysis of human tissue samples. a: Pathways analysis in IPA; Bubble plot showing top canonical pathways when comparing AF with sinus rhythm control samples. The intensity of the color depicts the relative increase (red) or decrease [65] in protein counts with the size of the bubble representing the number of proteins found in the pathway. **b:** Integrin Signaling Pathway showing in color all proteins found within the dataset. Red represents upregulation in AF. Whereas Green depicts down regulation **c,** microautophagy top canonical pathway showing in color all proteins found within the dataset. Red represents upregulation in AF. **d:** Plot of differentially expressed proteins (p= 1.98E-05) from the microautophagy regulation pathway showing how each protein changes with AF.

Supplementary Figure 10 (related to discussion): Top 10 networks analyzed in IPA. **a**: Cell-To-Cell Signaling and Interaction, Cellular Assembly and Organization, Cellular Function and Maintenance; **b**: Cell Cycle, Infectious Diseases, Organismal Injury and Abnormalities; **c**: Developmental Disorder, Hematological Disease, Hereditary Disorder; **d**: Cell Morphology, Neurological Disease, Organismal Injury and Abnormalities; **e**: Connective Tissue Disorders, Dermatological Diseases and Conditions, Developmental Disorder; **f**: Energy Production, Nucleic Acid Metabolism, Small Molecule Biochemistry; **g**: Cardiac Arrythmia, Cardiovascular Disease, Hereditary Disorder; **h:** Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance; **i**: Developmental Disorder, Embryonic Development, Organismal Injury and Abnormalities; **j:** Cellular Assembly and Organization, Cellular Function and Maintenance, Infectious Diseases. List of regulated proteins used in IPA can be found in Supplementary File 2 and Supplementary File 4 contains Ingenuity Canonical Pathways Analysis which includes molecules.

Supplementary Figure 11 (related to discussion): The top regulator effects and interaction networks mapped by IPA. a: CALR, DNMT3A, ESRRA (consistency score of 1.152). **b**: ESRRG (consistency score of 0.577). **c**: MMP9 (consistency score of -6.0).

Supplementary Files:

Supplementary File 1 (related to discussion, student T-test): Volcano Plot excel output file. Used to determine significant data points in a data set with a permutation-based FDR, X- axis column displays: log2 fold changes and Y- axis column displays: -log10 p values. Column headers are gene/protein description and columns labelled with the sample IDs are showing the mass spectrometry signal intensities in log2, normalized by MaxLFQ [69].

Supplementary File 2 (related to discussion): List of significant up and down regulated proteins taken from supplementary file 1 for analysis in IPA.

Supplementary File 3 (related to discussion): Canonical Pathways Analysis in IPA.

Supplementary File 4 (related to discussion): List of molecules identified through IPA. The table summarizes molecules with associated scores and top disease and functions linked to each network. Higher scores indicate stronger relevance.

Supplementary Videos:

Supplemental Video 1 (related to Figure 7): Left atrial goat tissue (sham control). 3D animation of tomographic reconstruction of cell in Fig. 7d. Red: Lysosome, Blue: Sarcoplasmic reticulum, Yellow: Mitochondria.

Supplemental Video 2 (related to Figure 7): Left atrial AF goat tissue (atrial fibrillation). 3D animation of tomographic reconstruction of cell in Fig. 7e. Red: Lysosome, Blue: Sarcoplasmic reticulum, Yellow: Mitochondria.

Supplemental Video 3 (related to Figure 6): Mouse Sino-Atrial Node tissue. 3D animation of tomographic reconstruction of cell in Fig. 6e-f. Red: Lysosome, Blue: Sarcoplasmic reticulum, Yellow: Mitochondria.

Supplementary Table:

Supplementary Table 1 (related to Figure 7): Summary table of surgeries underwent by SinusR and ChronicAF patient went biopsies were collected from both University Medical Center Göttingen and Royal Papworth Hospital Research Tissue Bank.