Supplemental Materials

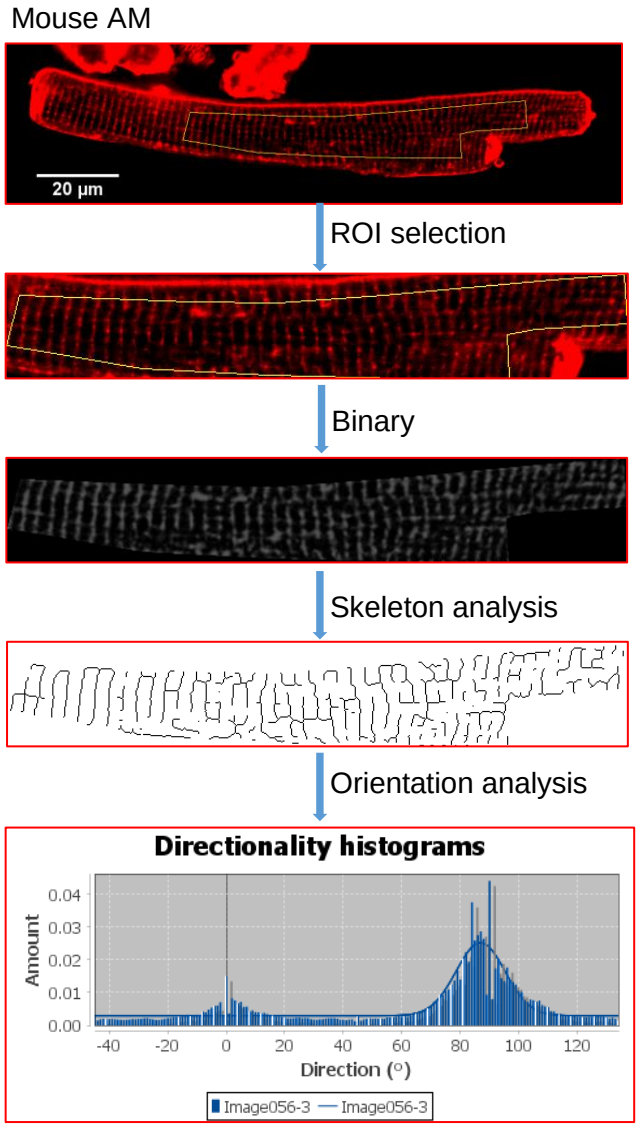
TAT skeleton analysis method

To determine TAT orientation, we used Fiji [1] to analyze the Di-4-ANEPPS confocal images. Briefly, we used the polygon selection tool to manually select the regions of interest (ROIs) while excluding the surface membrane and the nucleus, and measured the area of each ROI. Then we used a 3×3 mean filter in Fiji to reduce noise and binarize the ROIs using the “statistical region merging” function and thresholding [2]. Next we sequentially applied the “Skeletonize” and “Analyze Skeleton” functions to convert the tubular structure into single pixel-wide lines and measured the tubule length to calculate T_{skel} density (i.e. the ratio of total TAT length to ROI area) [2-4]. The angles of the transverse and axial tubules were determined using the “Directionality” plugin in Fiji. Tubule orientations were represented by histograms ranging from -45° to 135° with a bin width of 1°. Tubules oriented at 0° represent those aligned parallel to the long axis (a.k.a. longitudinal axis) of the cell, and tubules oriented at 90° represent those aligned parallel to the short axis (a.k.a. transverse axis), as described previously [3, 5]. The custom-made macro commands are as follows:

```
run("Add to Manager");
run("Measure");
run("Duplicate...", " ");
run("Clear Outside");
run("Subtract Background...", "rolling=5");
run("Enhance Local Contrast (CLAHE)", "blocksize=49 histogram=256 maximum=3 mask=*None*");
run("Smooth");
run("8-bit");
```

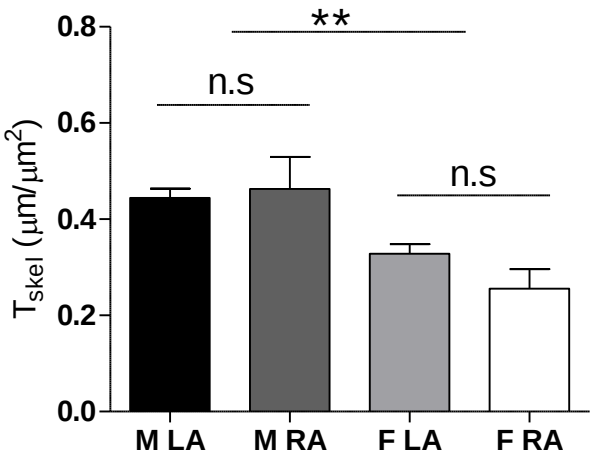
```
run("Statistical Region Merging", "q=100 showaverages");
run("8-bit");
setThreshold(40, 255);
run("Convert to Mask");
run("Skeletonize (2D/3D)");
run("Directionality", "method=[Fourier components] nbins=180 histogram=-45 display_table");
run("Analyze Skeleton (2D/3D)");
selectWindow("Tagged skeleton")
close("Tagged skeleton")
```

Supplemental Fig. 1



Supplementary Fig. 1. Exemplar process of TAT orientation analysis of a mouse atrial myocyte. See text for details.

Supplementary Fig. 2



Supplementary Fig. 2. Summary plot of T_{skel} (TAT density analyzed with skeleton analysis) in left atria (LA) and right atria (RA) from male (M) and female (F). ***P* < 0.001, M *vs.* F, *P* > 0.05, LA *vs.* RA, n = 63 (M LA), 38 (M RA), 62 (F LA) and 30 (F RA) cells from total 8 animals, one-way ANOVA. Note that while there is a reduced T_{skel} density in F compared to M, there is no difference between left and right atria.

References

[1] Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012;9:676-82.
[2] Wagner E, Brandenburg S, Kohl T, Lehnart SE. Analysis of tubular membrane networks in cardiac myocytes from atria and ventricles. J Vis Exp. 2014:e51823.
[3] Brandenburg S, Kohl T, Williams GS, Gusev K, Wagner E, Rog-Zielinska EA, et al. Axial tubule junctions control rapid calcium signaling in atria. J Clin Invest. 2016;126:3999-4015.
[4] Crossman DJ, Young AA, Ruygrok PN, Nason GP, Baddelely D, Soeller C, et al. T-tubule disease: Relationship between t-tubule organization and regional contractile performance in human dilated cardiomyopathy. J Mol Cell Cardiol. 2015;84:170-8.
[5] Wagner E, Lauterbach MA, Kohl T, Westphal V, Williams GS, Steinbrecher JH, et al. Stimulated emission depletion live-cell super-resolution imaging shows proliferative remodeling of T-tubule membrane structures after myocardial infarction. Circ Res. 2012;111:402-14.