Supplemental Figures and Tables

USP10 Mitigates Ang II-induced Atrial Remodeling and Atrial Fibrillation Susceptibility by De-ubiquitinating NDUFS1

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Supplemental Figure 1

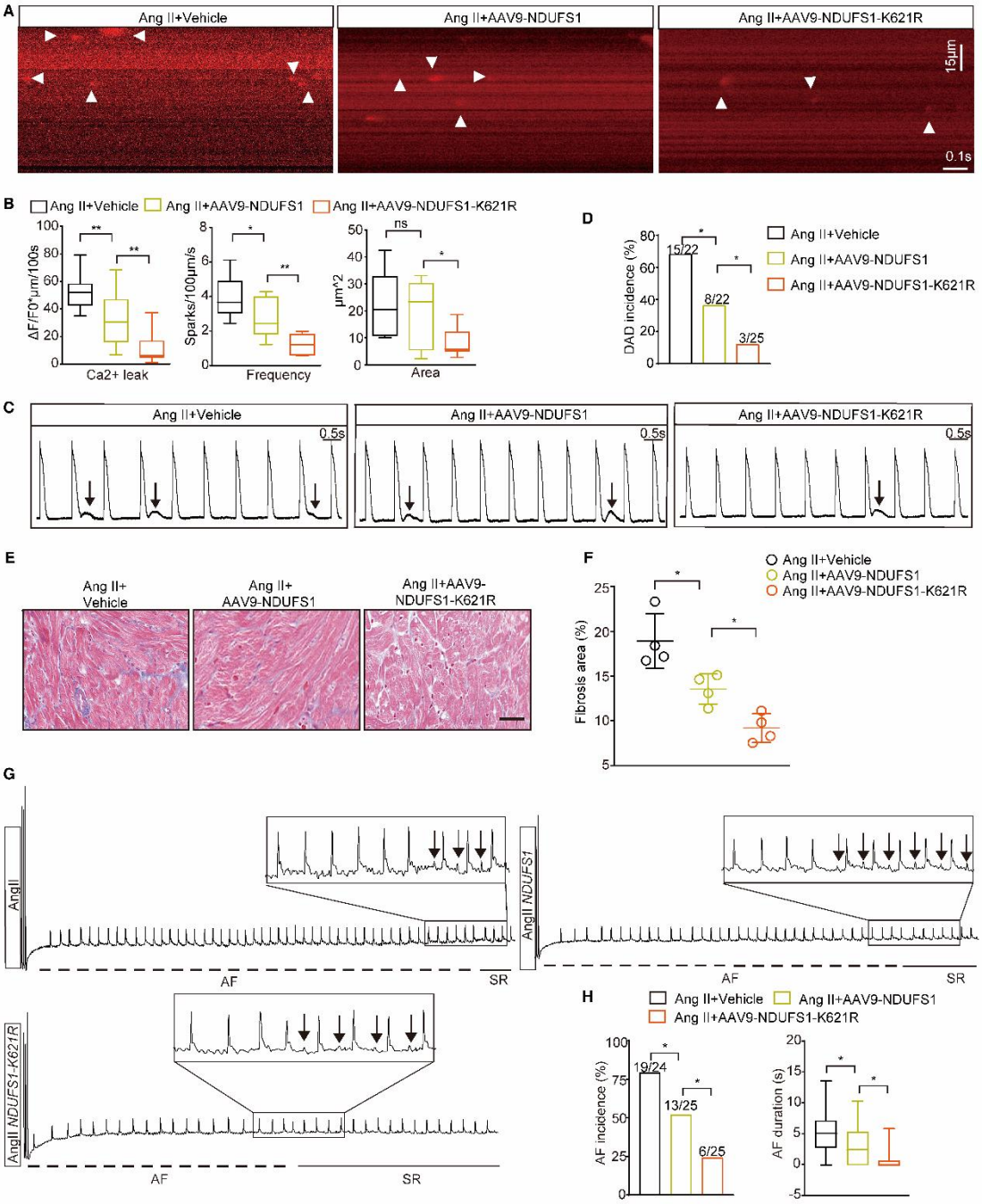

Figure S1. NDUFS1-K621R attenuates abnormal calcium handling, DADs, and AF susceptibility. A, Representative Ca^{2+} spark images obtained from adult mouse atrial cardiomyocytes with AAV-mediated overexpression of NDUFS1 or NDUFS1-K621R in response to Ang II stimulation; **B**, Statistical results of Ca^{2+}

spark-induced Ca^{2+} leak, Ca^{2+} spark frequency, Ca^{2+} spark area in the indicated groups (n=11/group); **C**, Representative DAD recordings in adult mouse atrial cardiomyocytes under Ang II treatment, comparing control and AAV-mediated NDUFS1 or NDUFS1-K621R overexpression groups; **D**, Statistical results of DADs incidence; **E** and **F**, Representative images and quantification of Masson's trichrome staining in left atrial sections from NDUFS1 and NDUFS1-K621R mice treated with Ang II (n=4/group); scale bars, 100 μm; **G**, Representative limb lead ECG traces from NDUFS1, NDUFS1-K621R, and control mice in response to Ang II treatment; **H**, AF incidence (left) and quantification of AF duration (right) in control, NDUFS1, and NDUFS1-K621R mice treated with Ang II (n=24–25/group). Data are presented as mean ± SEM. Data were analyzed using the chi-square test for **D** and **H (left)**, and one-way ANOVA followed by Tukey's multiple comparisons test for **B**, **F**, and **H (right)**. *P<0.05, **P<0.01. USP10, Ubiquitin specific peptidase 10; AF, Atrial Fibrillation; Ang II, Angiotensin II; DADs, delayed afterdepolarization; ECG, Electrocardiogram.

Supplemental Tables：

Table S1: Detailed baseline characteristics of the patient cohort.

Characteristic	SR group(n=5)	AF group (n=5)
Age (years)	58.2 ± 6.5	63.4 ± 7.2
Male (%)	3 (60)	3 (60)
BMI (kg/m^2)	24.3 ± 2.8	25.6 ± 3.4
AF duration (months)	-	18.6 ± 10.2
AF type		
Persistent AF	-	4 (80%)
Paroxysmal AF	-	1 (20%)
Left atrial diameter (mm)	34.8 ± 3.2	44.5 ± 5.6
Left ventricular ejection fraction, %	61.5 ± 4.7	58.4 ± 6.5

Table S2: Details of the antibodies used for this study.

Antibodies	Dilution	Catalog#	Vendor
Anti-Usp10	WB: 1:1000; IHC: 1:250	8501S	CST
Anti-Gapdh	WB: 1:5000	60004-1-Ig	Proteintech
Anti-NDUFS1	WB: 1:4000	12444-1-AP	Proteintech
Anti-K48 Ub	WB: 1:2000	8081S	CST
Anti-Flag	WB: 1:3000	M185-3L	MBL
Anti-Myc	WB: 1:3000	M047-3	MBL
Anti-HA	WB: 1:3000	51064-2-AP	Proteintech
HRP-conjugated anti-rabbit IgG	WB: 1:5000	7074	CST
HRP-conjugated anti-mouse IgG	WB: 1:5000	7076	CST

IHC: immunohistochemistry; WB: western blotting.

Table S3: The sequences of RT-qPCR primers

Species	Primer	Sequence（5’-3’）
Human	*USP10* Forward	ATTGAGTTTGGTGTCGATGAAGT
Human	*USP10* Reverse	GGAGCCATAGCTTGCTTCTTTAG
Human	*GAPDH* Forward	GGAGCGAGATCCCTCCAAAAT
Human	*GAPDH* Reverse	GGCTGTTGTCATACTTCTCATGG
Mouse	*Usp10* Forward	GGACTCCATGAAGAGATGCTGAG
Mouse	*Usp10* Reverse	CTCGTCCTCTATCAAGTCGCTTC
Mouse	*Gapdh* Forward	AGGTCGGTGTGAACGGATTTG
Mouse	*Gapdh* Reverse	TGTAGACCATGTAGTTGAGGTCA