Supplementary Material

Stress-induced premature senescence is associated with a prolonged QT interval and recapitulates features of cardiac aging

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Running title: iPSC-derived cardiomyocytes as a platform for studying myocardial aging

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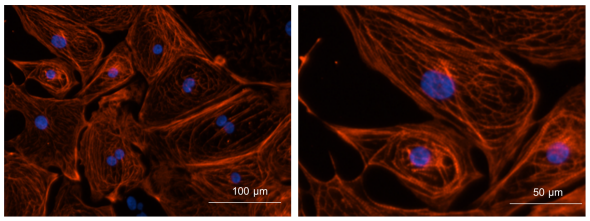
Supplementary Figure S1: (A) Representative images of cardiac specific markers cTnT (red), cTnI (green), a-actinin (red) in iCMs and SenCMs. (B) Quantitative data of eight independent experiments ± SEM. (C) Assessment of chamber-specific differentiation by APD morphology. Representative atrial-like (red) and ventricular-like (blue) APD shapes in iCMs are shown in the left panel. Quantitative data of three independent experiments ± SEM. * $P < 0.05$, *vs* atrial (AT). Right panel shows iCM culture representative of culture heterogeneity myosin light chain (red) and ventricular light chain (green) have been used as specific markers.

Supplementary Figure S2: (A) Apoptosis was assessed by Tunel assay. Nuclei of apoptotic cells were stained in green. Counterstaining of nuclei was performed with Hoechst (blue). Quantitative data (ratio of Tunel positive cells on the total number of nuclei) are shown in the bar graphs representing means of four independent experiments ± SEM. ** $P < 0.01$ *vs* SenCMs (B) Staining assessing cell division in cardiomyocytes. Aurora B Kinases staining (green), EdU incorporation (red), Cardiac Troponin T is stained in gray and nuclei are counterstained with DAPI. bar graphs representing means of four independent experiments ± SEM. ** $P < 0.01$ *vs* SenCMs

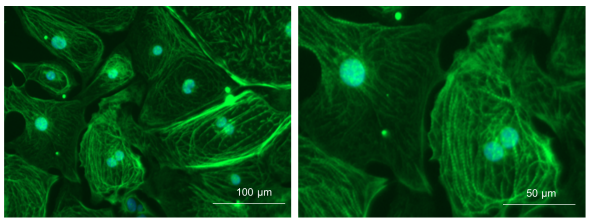
Supplementary Figure S3: (A) Linear regression analysis between KCNH2 expression in atrial tissue and age in thirteen different patients. The linear regression is showing the 95% confidence bands of the best fit trend line.
B data obtained from Human Heart Atlas Database were analysed stratifying patients into three age class (40-50 years old, 50-60 y.o., and 60-70 y.o.). Subset of Ventricular (left) and atrial (right) cardiomyocytes were considered for analysis. For every evaluated gene, percentage of cell expressing such gene is represented by dot size, while colour indicate normalized gene expression in accordance to the reported colour scale. Differential gene expression is statistically evaluated with Weighted Kolmogorov Smirnov (WKS) test followed by FDR correction, **$P < 0.05$.

Supplementary Figure S4: (A) SR Ca^{2+} content estimated through integration of NCX current elicited by caffeine (10 mM) in SenCMs (N=17) *vs* iCMs (N=22). nmol of Ca^{2+} entering the cell through NCX were normalized to cell capacitance. (B) Ca^{2+} sparks characteristics (amplitude, full width at half maximum FWHM, and full duration at half maximum FDHM) in SenCMs (N=410) *vs* iCMs (N=266). ** $P<0.01$ *vs* iCMs.

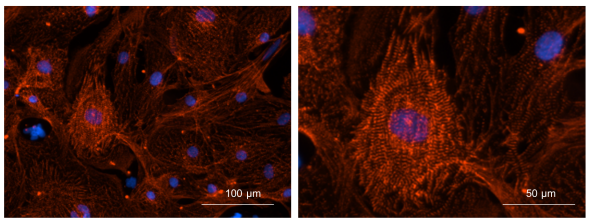
A



Cardiac Troponin T

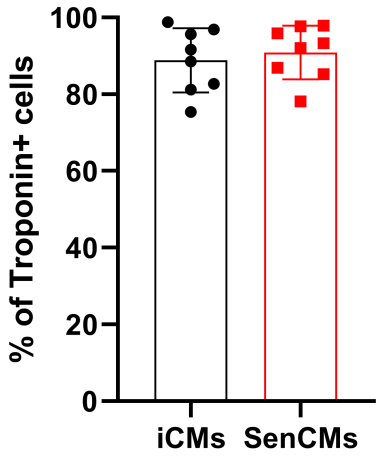


Cardiac Troponin I



α-Sarcomeric Actin

B



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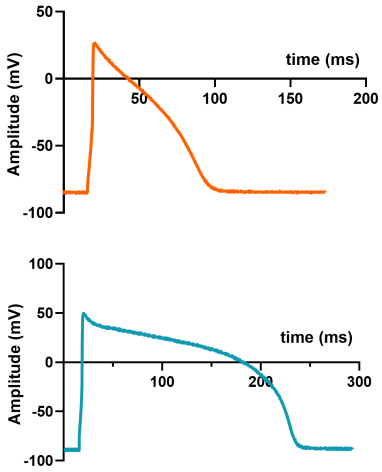
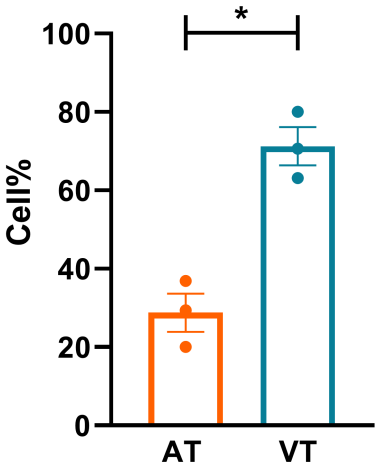
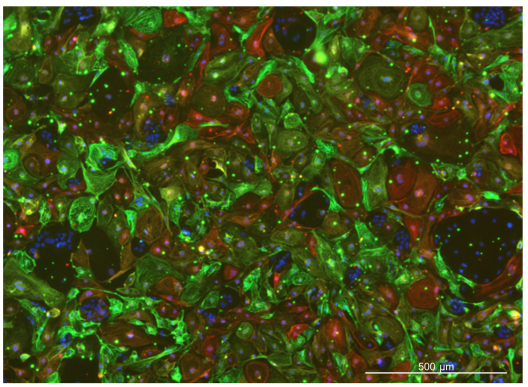
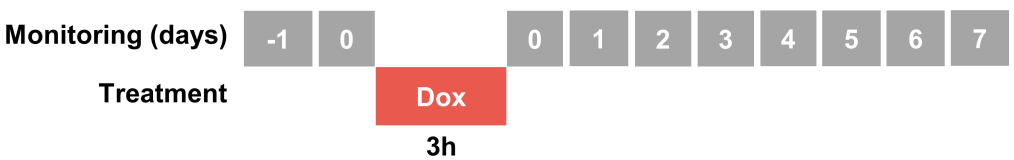






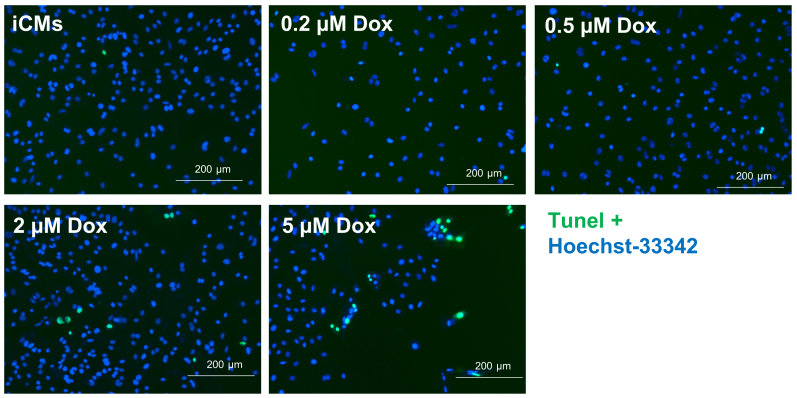
Myosin light chain 2 atrial
Myosin light chain 2 ventricular
Dapi

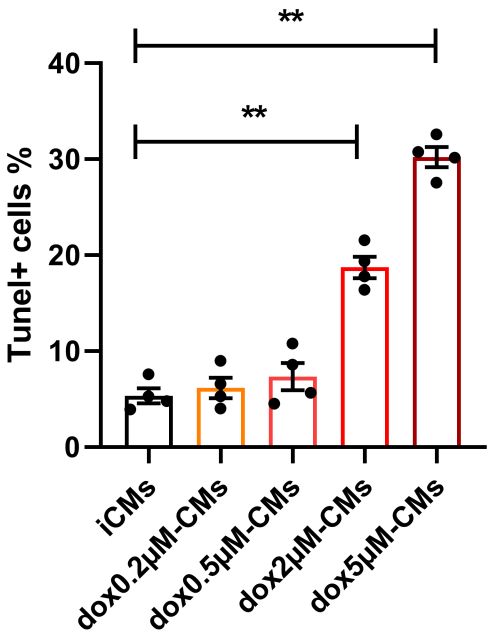
D



Supplementary Figure S1

A





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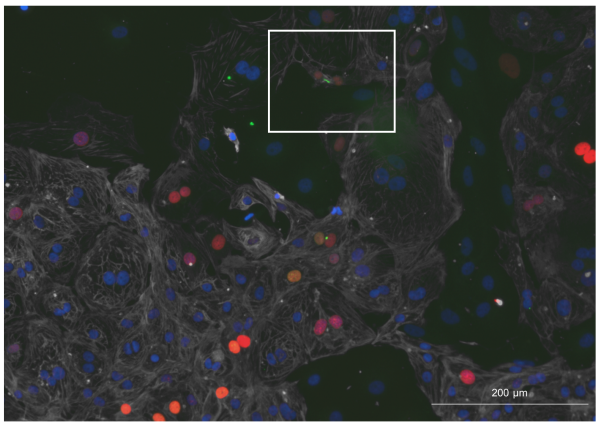
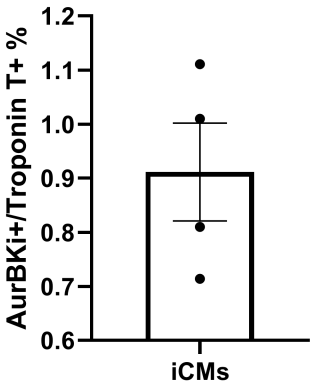
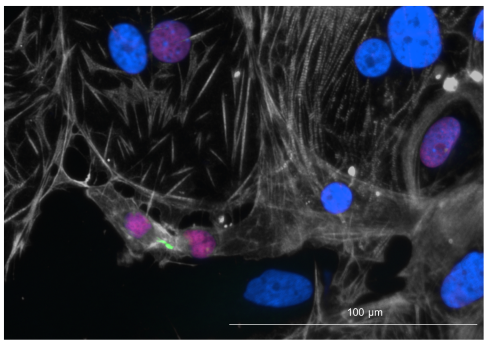


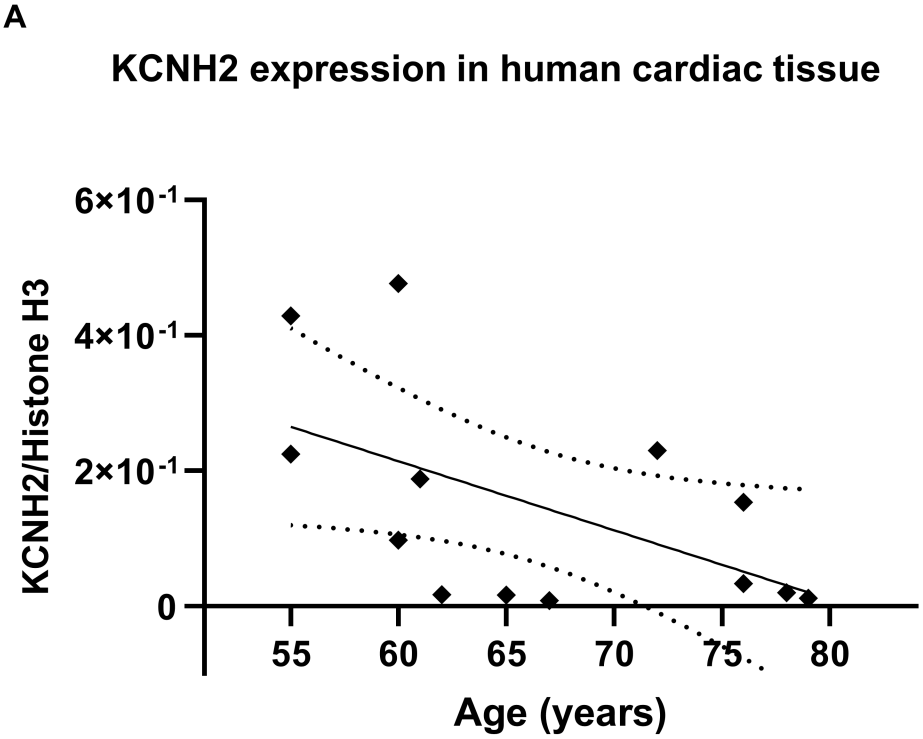
Cardiac Troponin T
Aurora B Kinase
DAPI
EdU





Supplementary Figure S2

A



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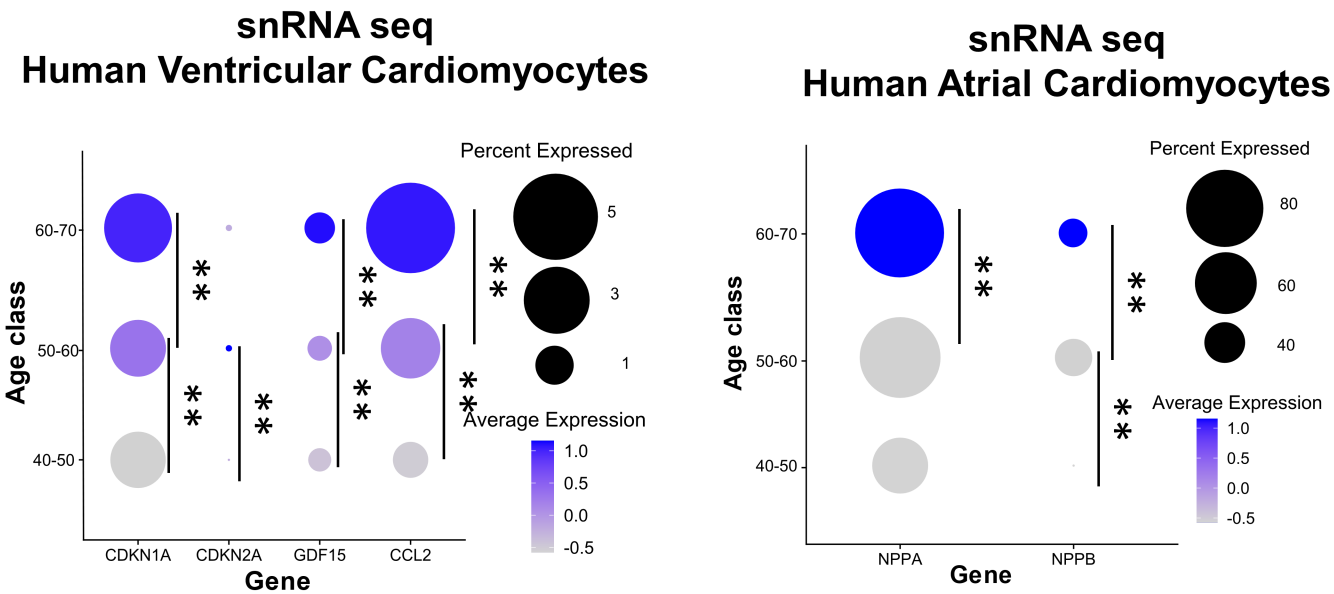
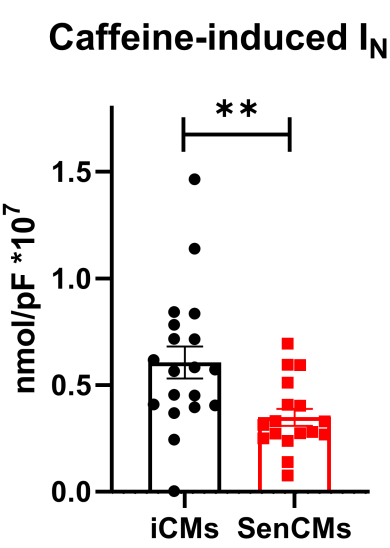


Supplementary Figure S3

A



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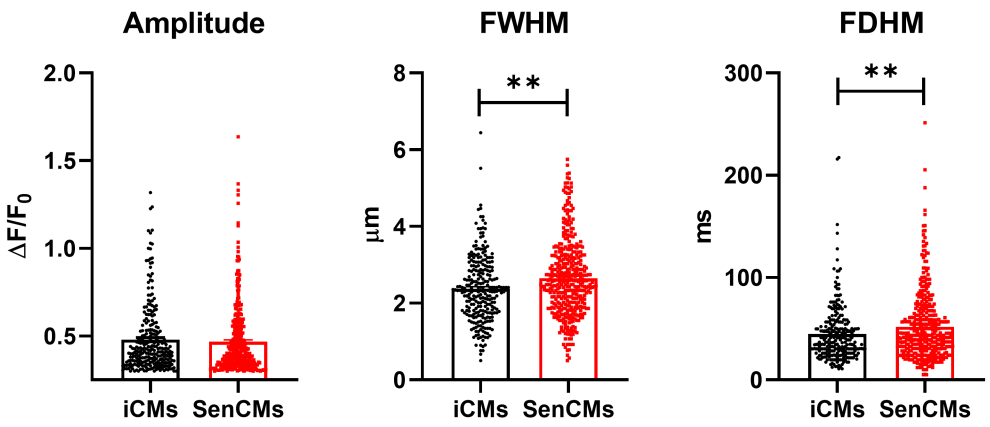

Supplementary Figure S4

Gene	F (5’-3’)	R (5’-3’)
CDKN2A	CTTCGGCTGACTGGCTGG	TCATCATGACCTGGATCGGC
CDKN1A	CACCTCACCTGCTCTGCTGC	GCTGGTCTGCCGCCGTTTT
SERPINE1	TTGCAGGATGGAACTACGGG	GTGGCAGGCAGTACAAGAGT
CXCL8	TCTGGACCCCAAGGAAAACTG	TCACTGGCATCTTCACTGATTCTT
GDF15	AGGTGAGAACCTTCTGGGGTT	CCTGGGAGTCTGTGCTTTTGG
TGFB2	CATCTACAACAGCACCAGGGA	CAACTGGGCAGACAGTTTCGG
CCL2	CCTTCATTCCCCAAGGGCTC	CTTCTTTGGGACACTTGCTGC
MMP3	TGAAATTGGCCACTCCCTGG	GGAACCGAGTCAGGTCTGTG
IL1A	CTTCTGGGAAACTCACGGCA	AGCACACCCAGTAGTCTTGC
IL1B	TTCGAGGCACAAGGCACAA	TTCACTGGCGAGCTCAGGTA
IL6	ATGAACTCCTTCTCCACAAGC	GAATCTTCTCCTGGGGGTACTG
MMP9	GCCACTACTGTGCCTTTGAGTC	CCCTCAGAGAATCGCCAGTACT
NPPA	TGAGCTTCCTCCTTTTACTGG	CCAGCAAATTCTTGAAATCCATC
NPPB	CTCCTGCTCTTCTTGCATCTG	TTGCGCTGCTCCTGTAACC
AMPKA1	ACAGCCGAGAAGCAGAAACA	TTGCCAACCTTCACTTTGCC
AMPKA2	TTGACAGGCCATAAAGTGGCA	TCGAACAATTCACCTCCAGACA