SUPPLEMENTAL MATERIAL

Supplementary Figure 1. Generation of iPSCs from three patients with ATS

Immunofluorescence staining for stem cell markers (Tra1-81, NANOG, OCT3/4, and SSEA3) in independent iPSC clones of each ATS case. Nuclei were counterstained with DAPI.

Supplementary Figure 2. Teratoma formation by ATS-iPSCs

Microscopic observation of teratoma sections, showing tissue structures resembling gut (endoderm), cartilage (mesoderm), adipose (mesoderm), and neural tissue (ectoderm).

Supplementary Figure 3. Cardiomyocyte differentiation from iPSCs

Immunofluorescence staining for cardiomyocyte markers (a-actinin, ANP, cardiac troponin T (cTnT), and GATA4) in control- and ATS-iPSC-derived cardiomyocytes. Nuclei were counterstained with DAPI.

Supplementary Figure 4. Isoproterenol responses of ATS-iPSC-derived cardiomyocytes

a. The rates of EBs with arrhythmic events in MEA analyses (control, n = 8; R218W, n = 10; R67W, n=3; R218Q, n=13, Fisher's exact probability test). **b.** Representative MEA recordings after isoproterenol and flecainide administration in ATS-iPSC-derived beating EBs(R67W, R218Q). **c.** The incidences of EBs with arrhythmic events after isoproterenol (1000 nM) and flecainide (5 μM) administration by MEA analysis (control, n = 9; R218W, n = 11; R67W, n=19; R218Q, n=8, Fisher's exact probability test). **d.** Representative MEA recordings after flecainide (5 μM) and isoproterenol (1000 nM) in ATS-iPSC-derived cardiomyocytes.

Supplementary Figure 5. Ca^{2+} transients in ATS-iPSC-derived cardiomyocytes and irregular Ca^{2+} release caused by diastolic Ca^{2+} overload

a. Representative line scan images of spontaneous Ca^{2+} transients in control- and ATS-iPSC-derived single cardiomyocytes (R67W, R218Q). Arrowhead indicates the irregular Ca^{2+} release. **b.** Representative line scan images of Ca^{2+} transients paced at 1 Hz in control- and ATS-iPSC-derived single cardiomyocytes (R67W, R218Q). **c.** The rates of cardiomyocytes with irregular Ca^{2+} release after CPA administration in control- (n = 52) and ATS-iPSC-derived cardiomyocytes (n = 48) (control, 25.0% increase in incidence, $^{**}P < 0.01$ vs. control by Fisher's exact probability test; ATS, 6.3% increase in incidence) **d.** Representative line scan images of spontaneous Ca^{2+} transients in control-iPSC-derived single cardiomyocytes after caffeine administration. Arrow indicates the timing of caffeine administration. **e.** SR Ca^{2+} content after flecainide administration in control- and ATS-iPSC-derived cardiomyocytes, determined by caffeine-induced ΔF/F0 (control; baseline, n = 6, 1.27 ± 0.16, flecainide, n = 7, 1.27 ± 0.28, ATS; baseline, n = 14, 1.31 ± 0.11, flecainide, n = 18, 1.42 ± 0.12, Data are mean ± SEM.). **f, h.** Representative line scan images of spontaneous Ca^{2+} transients at baseline in ATS-iPSC-derived single cardiomyocytes (R67W, R218Q). **g, i.** Representative line scan images of spontaneous Ca^{2+} transients after flecainide (500 nM) administration in

ATS-iPSC-derived single cardiomyocytes (R67W, R218Q).

Supplementary Figure 6. Drug effects on ATS-iPSC-derived cardiomyocytes

a. The incidences of cardiomyocytes with irregular Ca^{2+} release after SEA0400 administration (*P < 0.05 vs. baseline by Fisher's exact probability test. n = 17). **b.** The incidences of cardiomyocytes with irregular Ca^{2+} release after JTV519 administration (vs. baseline by Fisher's exact probability test. n = 13).

Supplementary Table 1. Primer sets for genomic PCR of *KCNJ2*

Forward	Reverse
5’-GAACATTCAAAACTGTTTCTCCAA-3’	5’-AGAGCTATCAACCAAAACACACAG-3’
5’-GTGGATGCTGGTTATCTTCTGC-3’	5’-GCATTGTGACTGAAGACAAGAGTC-3’
5’-CATCATCGATGCTTTCATCATT-3’	5’-ATTTCAAAGTCTGCGTTGTCAAT-3’
5’-CCATGAAATAGATGAAGACAGTCC-3’	5’-CTAGTGCTTTCTGGAACTCCATTT-3’
5’-CTATGAAAATGAAGTTGCCCTCAC-3’	5’-TGGAGACATGGTTAGTGCTTTATG-3’

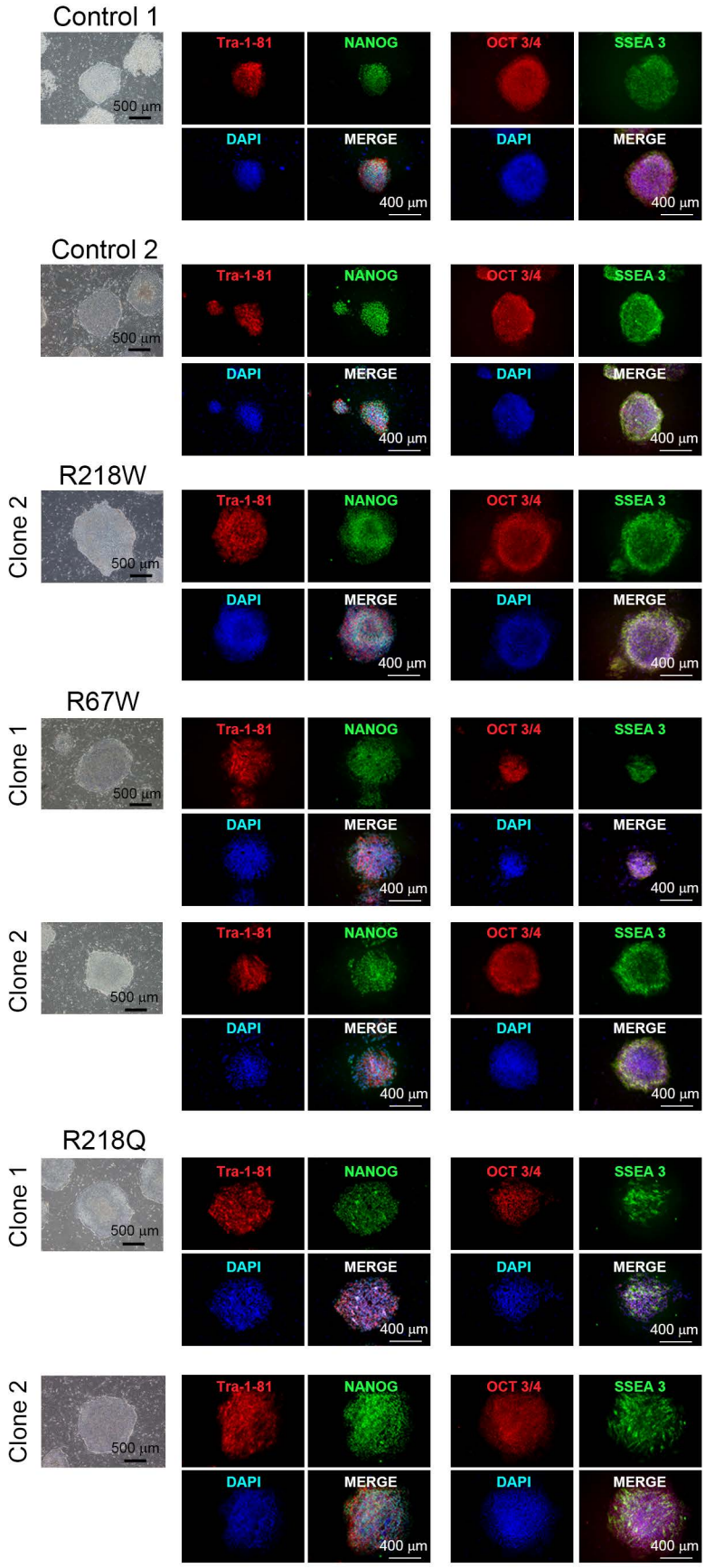
Supplementary Table 2. The clinical characteristics of the patients

	Patient 1	Patient 2	Patient 3
KCNJ2 mutation	R218W	R67W	R218Q
	652C>T	199C>T	653G>A
Frequent PVCs	+	-	-
Periodic paralysis	-	+	+

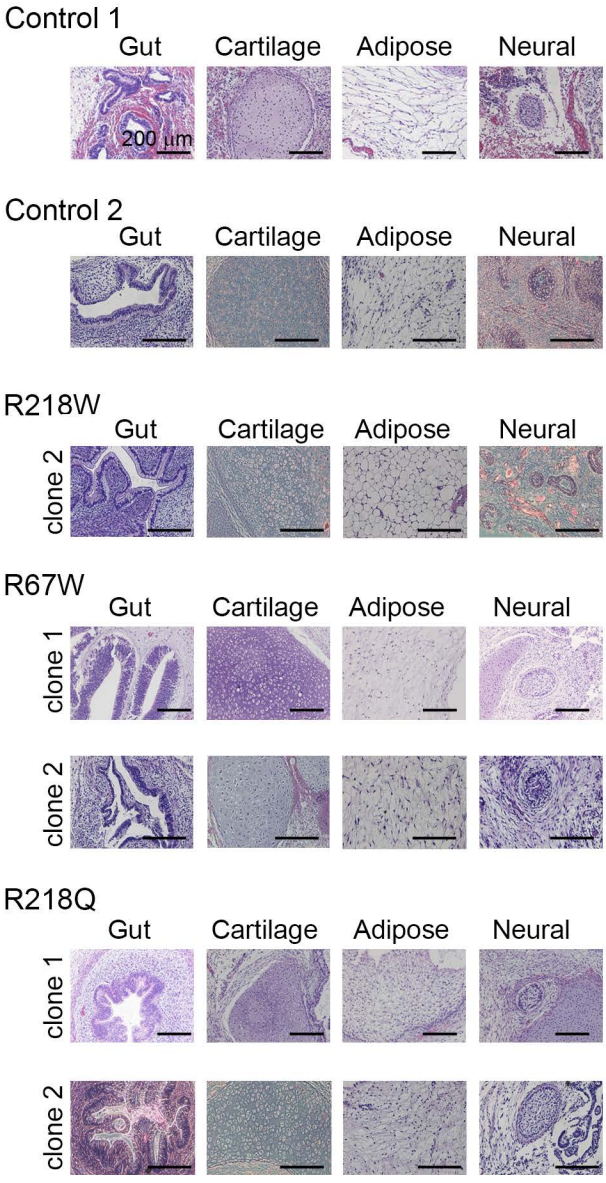
Supplementary Table 3. Drug responses in ATS-iPSC-derived cardiomyocytes

		ATS
Anti-arrhythmic drugs	Flecainide	effective
	Pilsicainide	non-effective
	KB-R7943	effective
	SEA0400	effective
	JVT519	non-effective

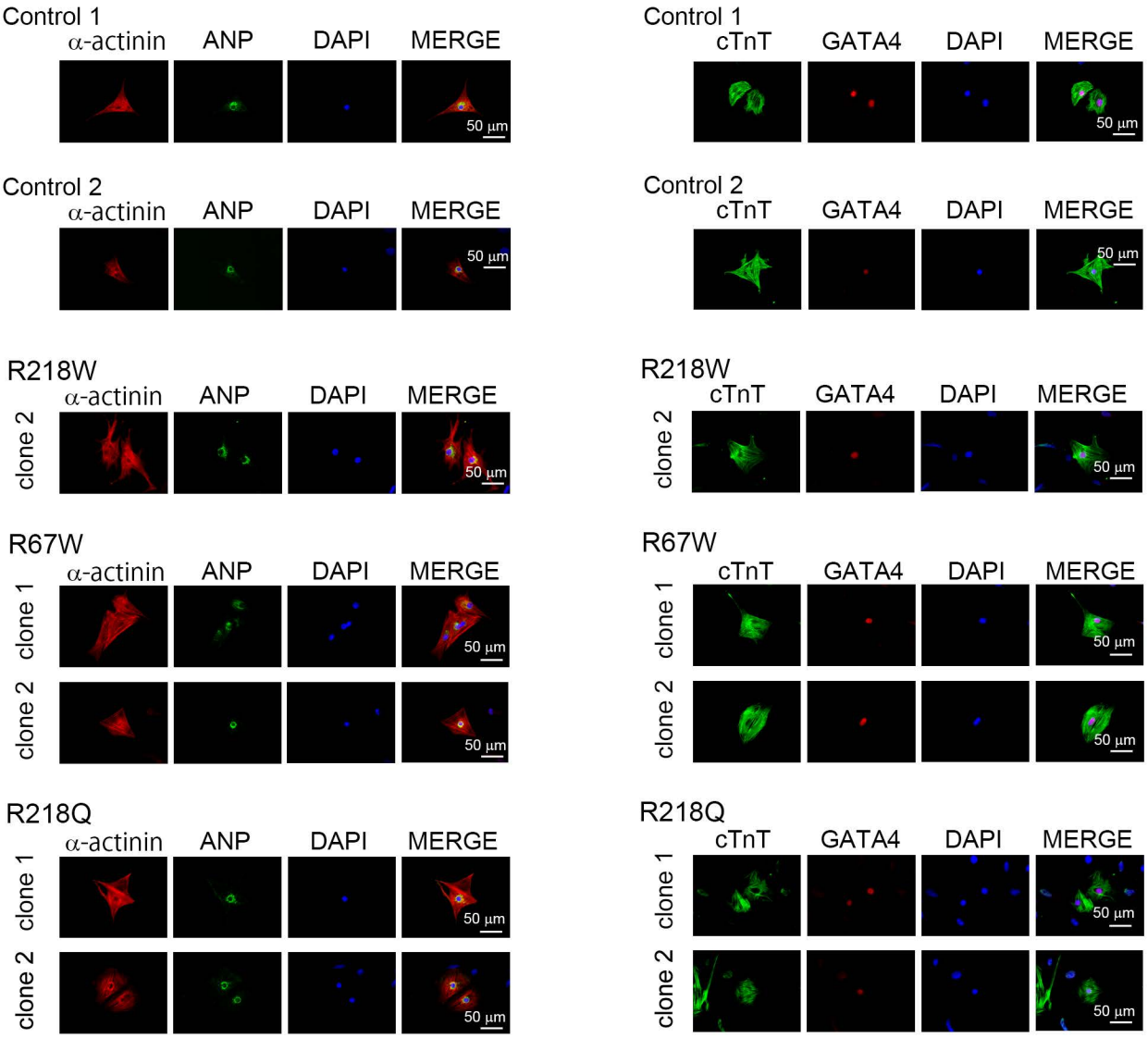
Supplementary Figure 1



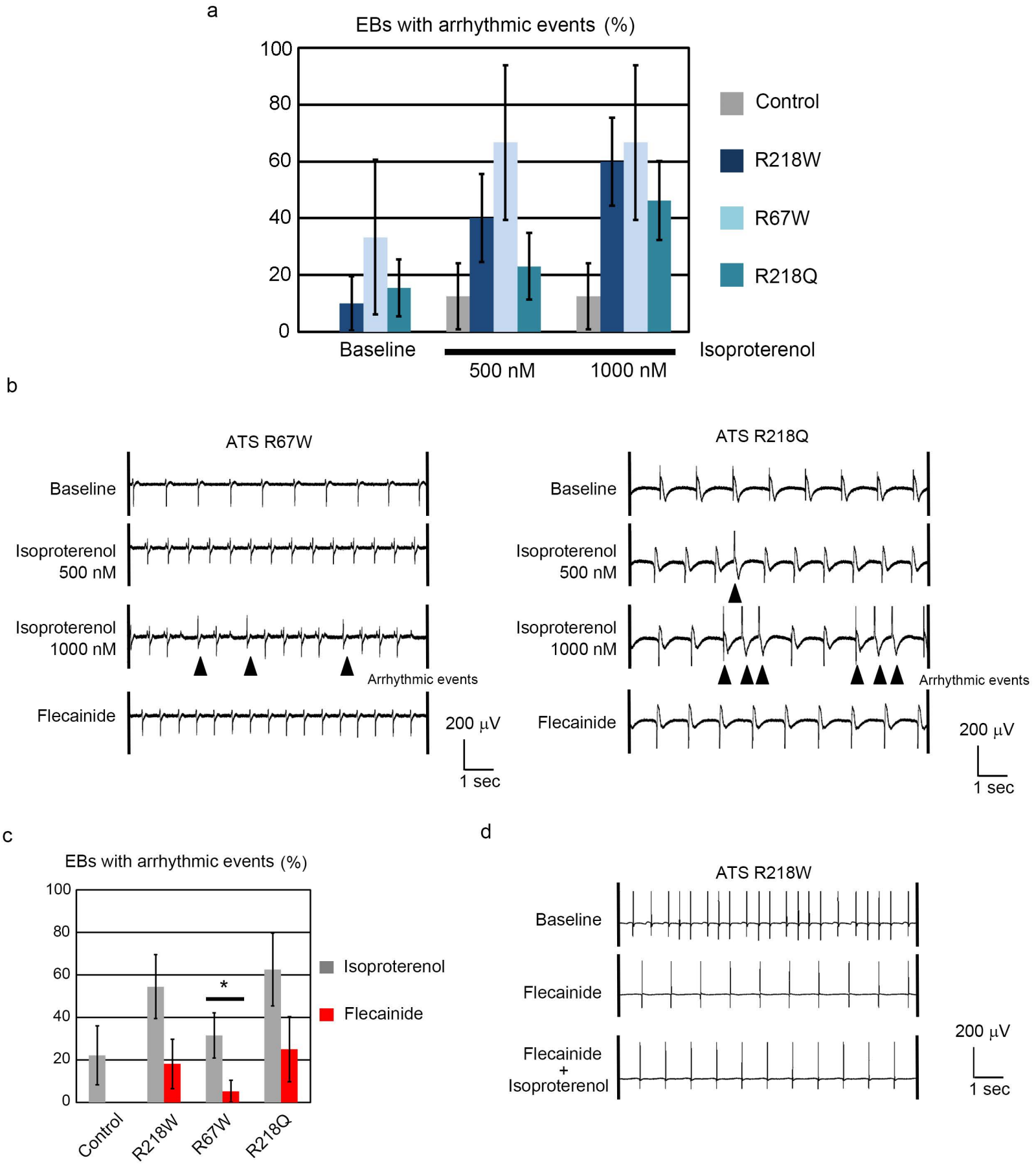
Supplementary Figure 2



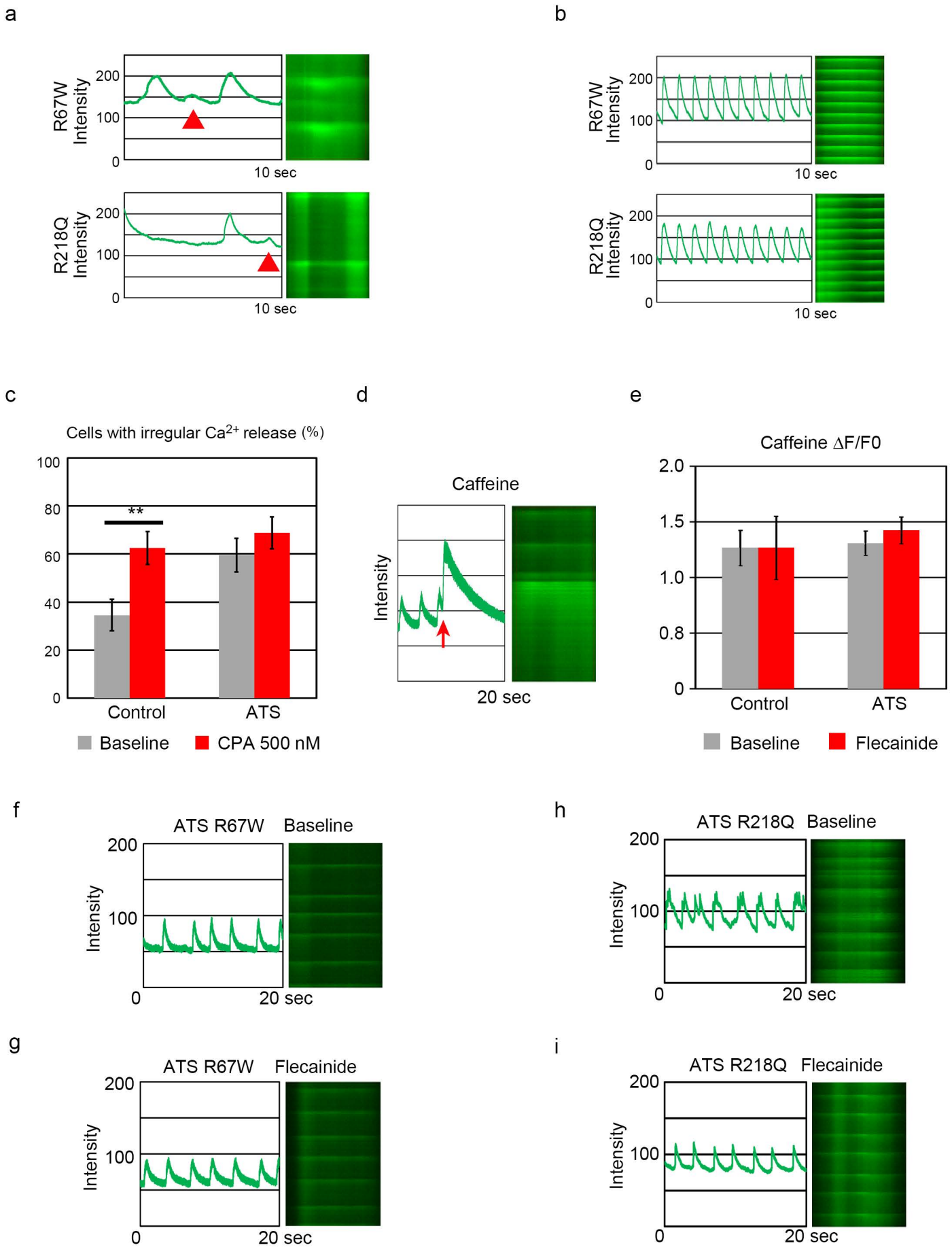
Supplementary Figure 3



Supplementary Figure 4

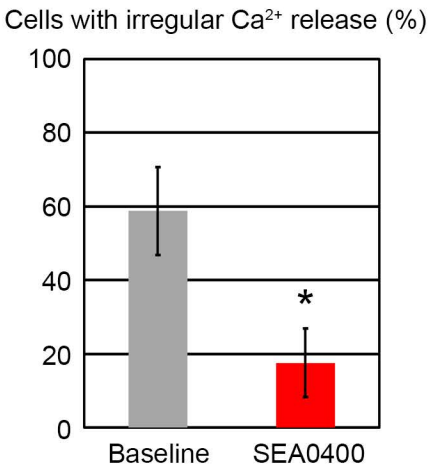


Supplementary Figure 5



Supplementary Figure 6

a



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