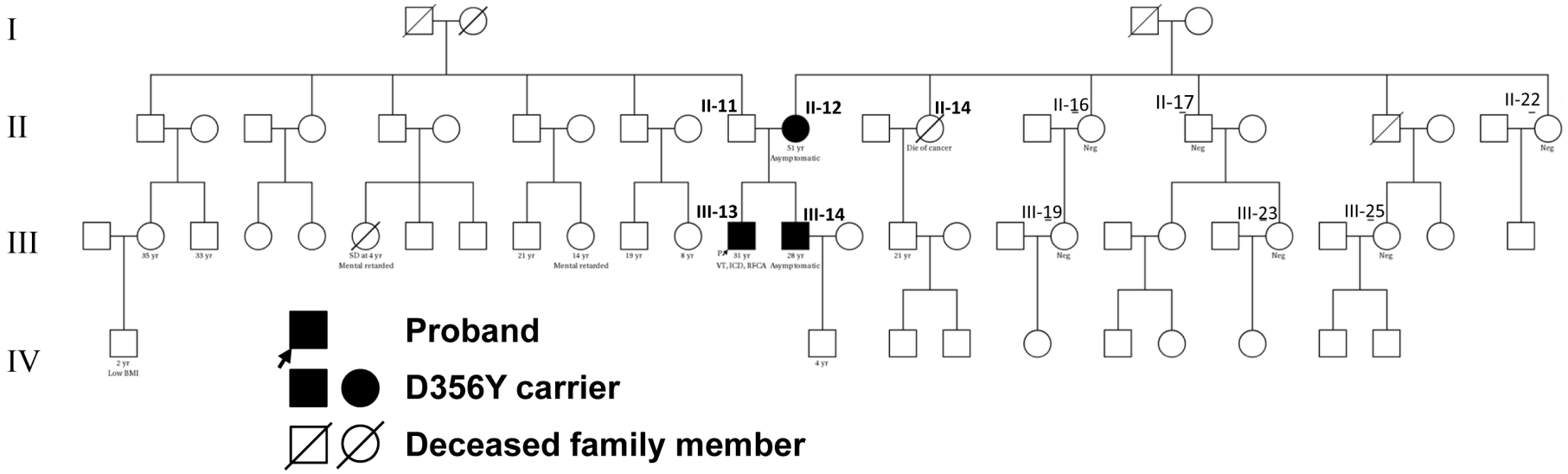
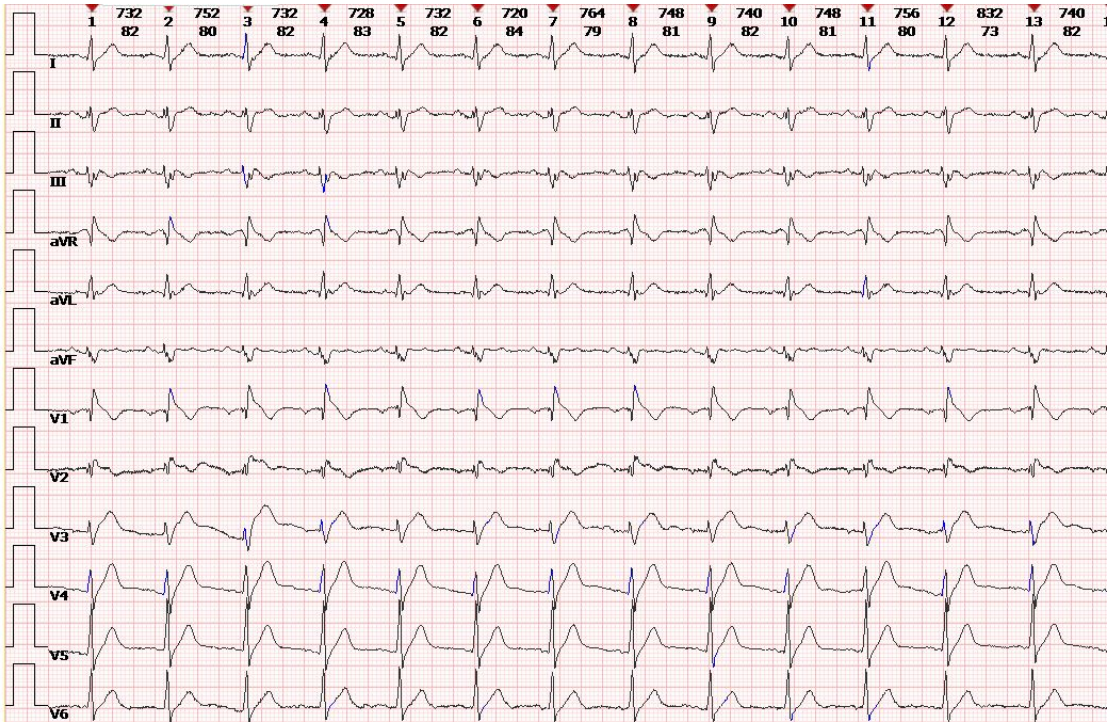
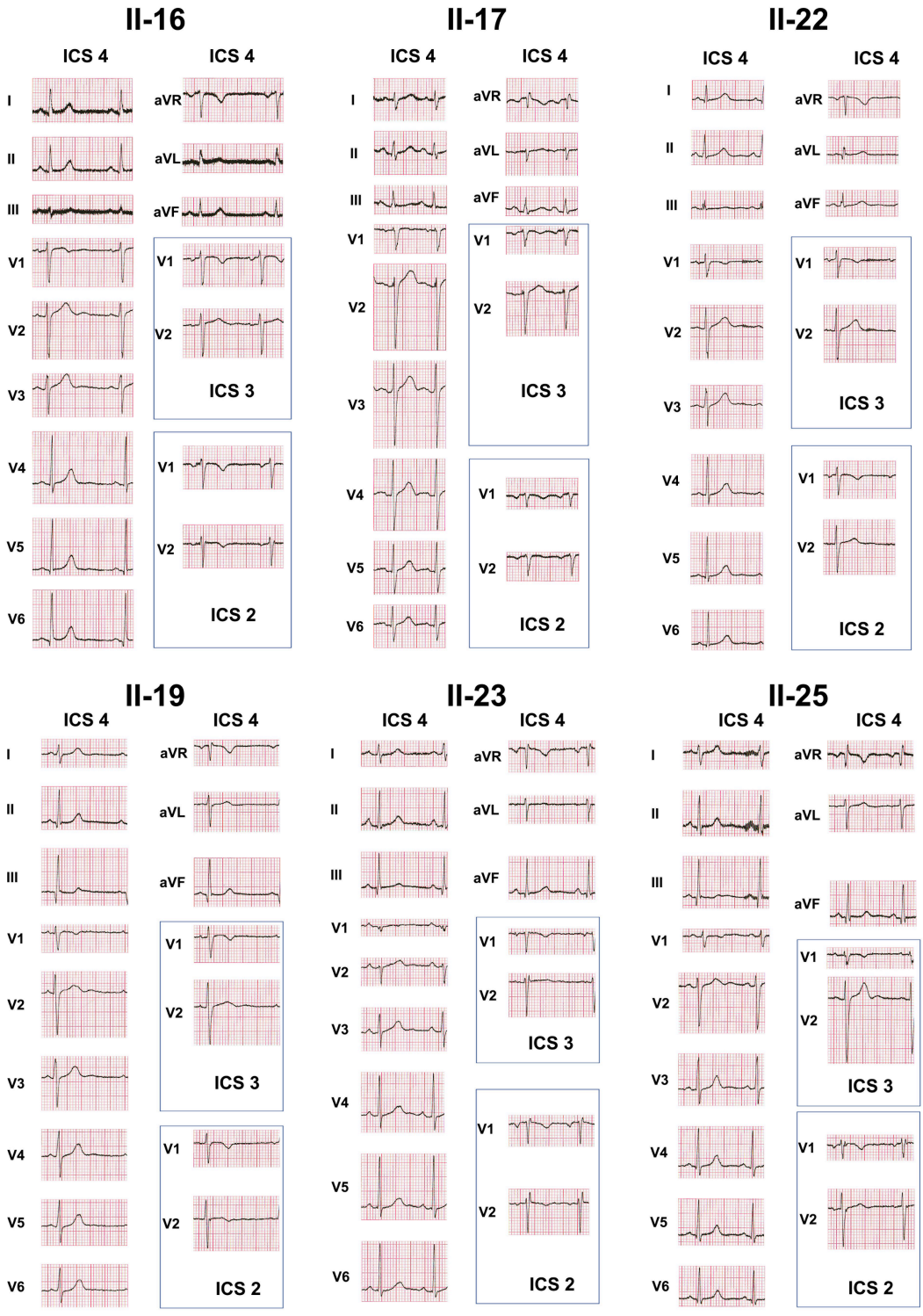


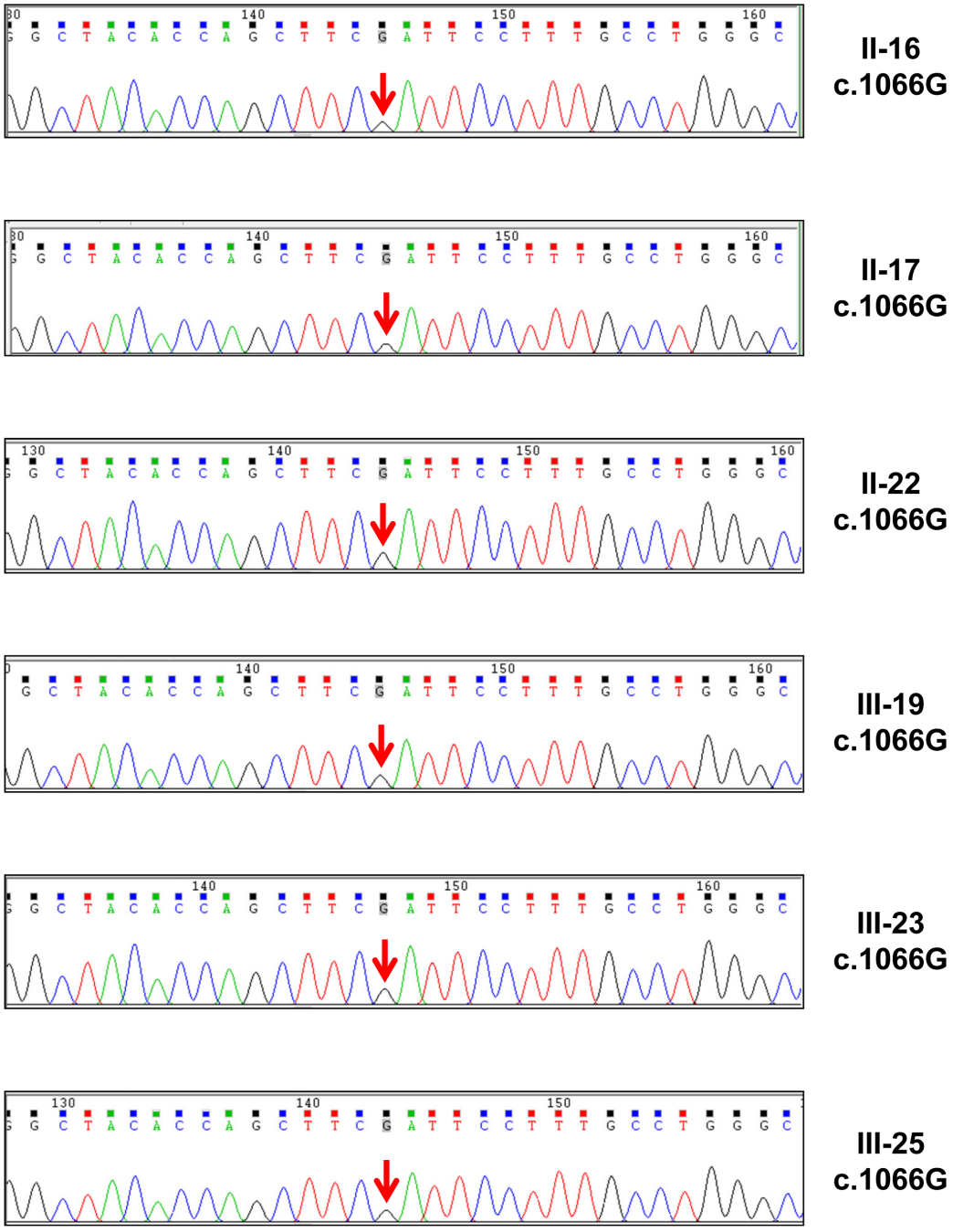
Supplementary Figure 1. The detailed pedigree of the Brugada syndrome family cohort. The mother of the proband (II-12) was a *SCN5A* D356Y mutation carrier. She was asymptomatic. II-14 died of lung cancer. III-6 and III-10 were mental retarded and the former died suddenly at 4 years old. The marker “Neg” means the negative of the *SCN5A* D356Y mutation. BMI: Body mass index. SD: Sudden death. VT: Ventricular tachycardia. ICD: Implantable cardiac defibrillator. RFCA: Radiofrequency catheter ablation.



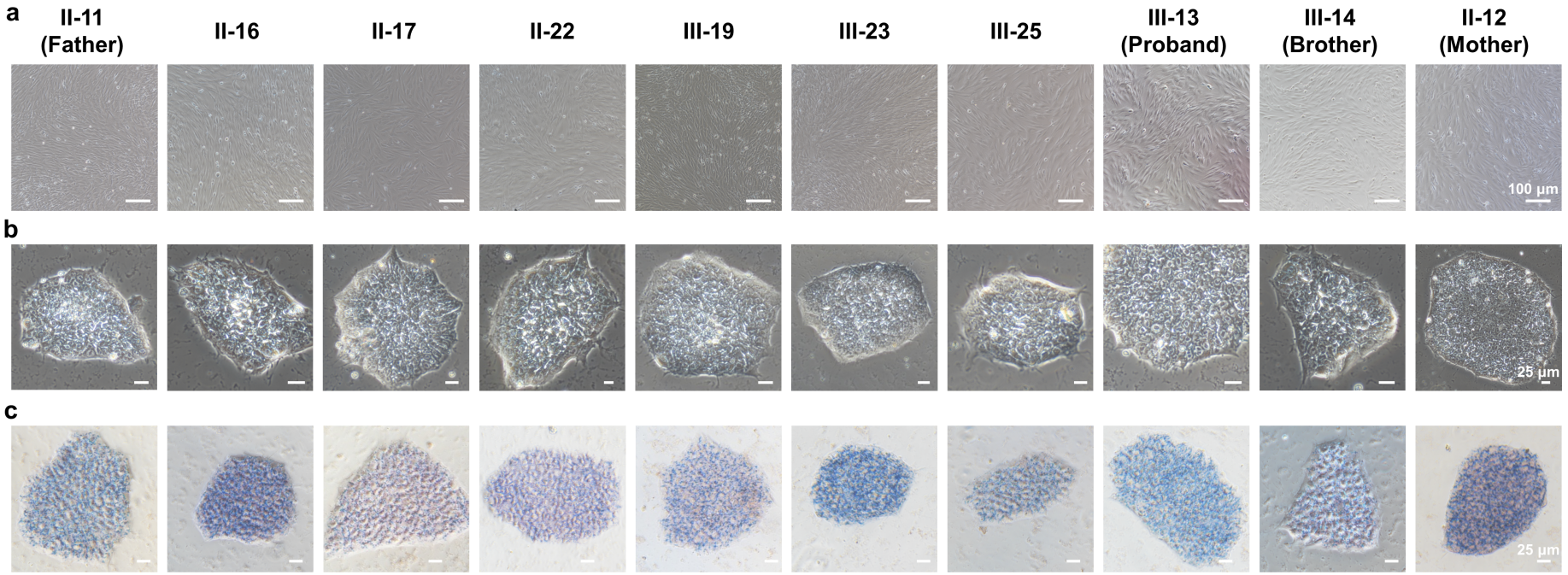
Supplementary Figure 2. The full tracing of the second ICS ECG of the proband.



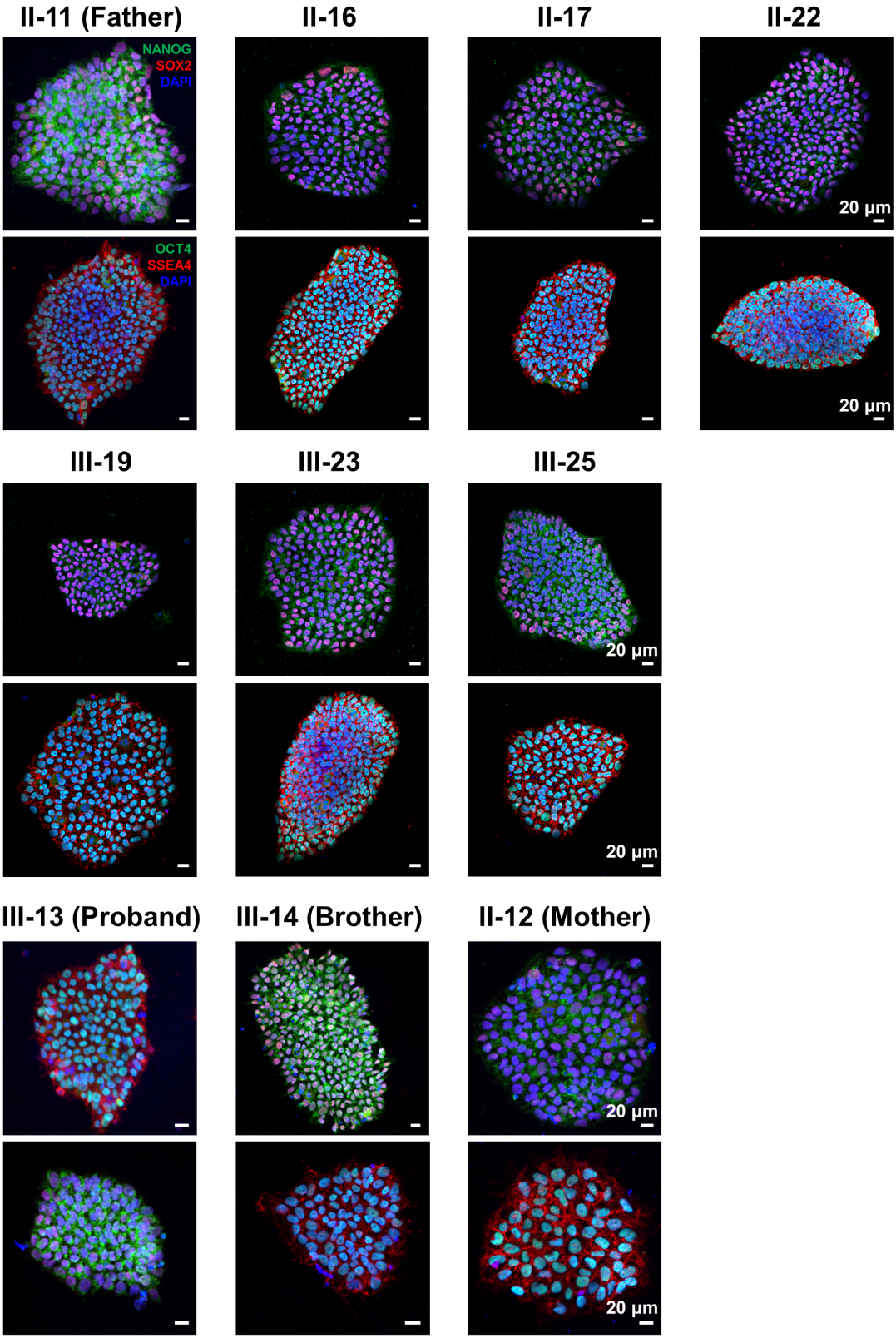
Supplementary Figure 3. The representative ECGs from six healthy control subjects in the BrS family. The representative ECGs from six healthy control subjects, including II-16, II-17, II-22, III-19, III-23 and III-25.



Supplementary Figure 4. Sanger verification of the absence of *SCN5A* D356Y in the six healthy control subjects of the pedigree. Sanger verification of the absence of *SCN5A* D356Y in the six healthy control subjects of the pedigree, including II-16, II-17, II-22, III-19, III-23 and III-25.

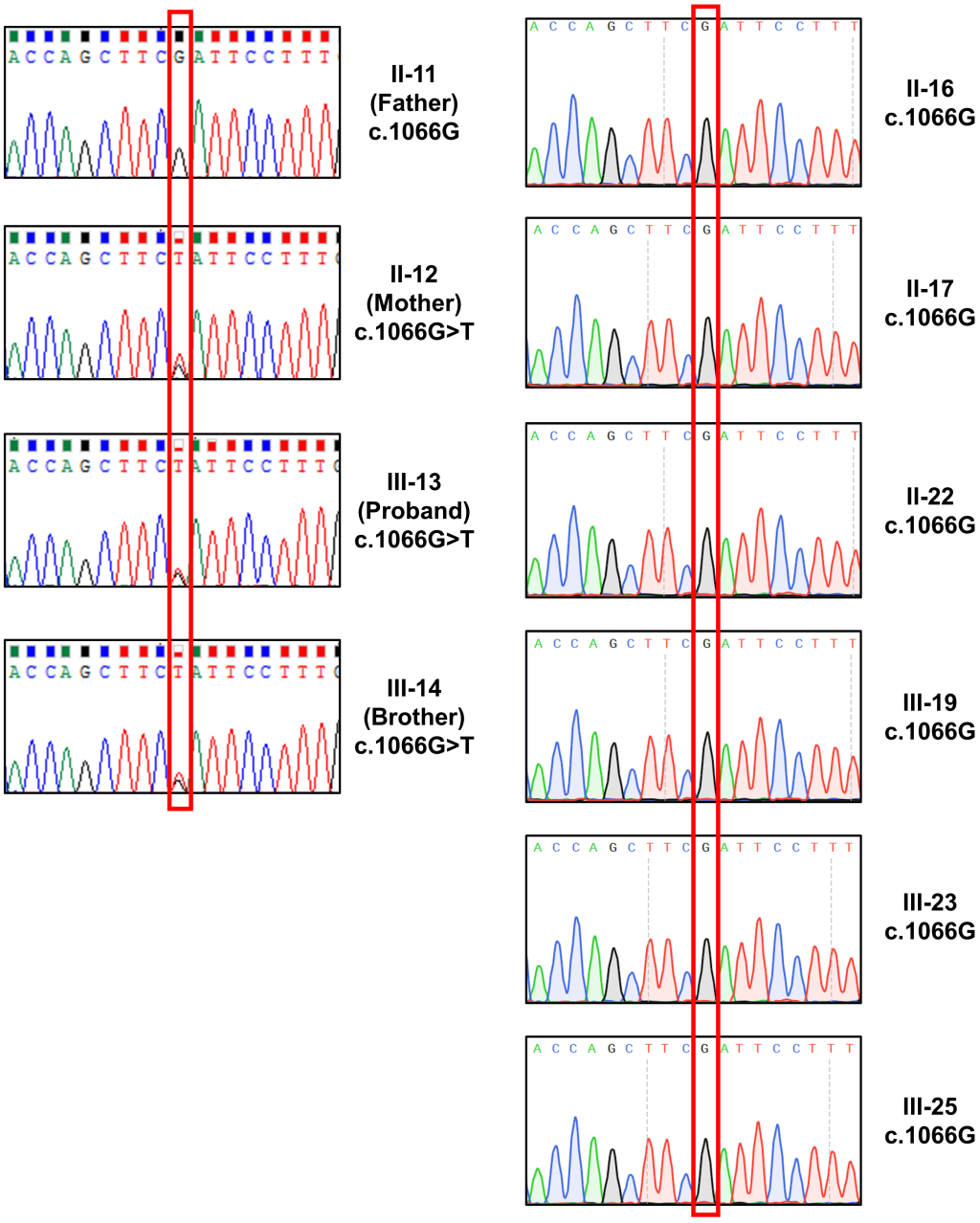


Supplementary Figure 5. Generation and characterization of iPSCs from different individuals in the BrS family cohort. a. Typical morphology of skin fibroblasts derived from healthy control subjects and BrS patients in the same family. Scale bar, 100 μm. **b.** Typical morphology of iPSC colonies derived from healthy control subjects and BrS patients. Scale bar, 25 μm. **c.** Alkaline Phosphatase (ALP) staining of control and BrS iPSCs. Scale bar, 25 μm.

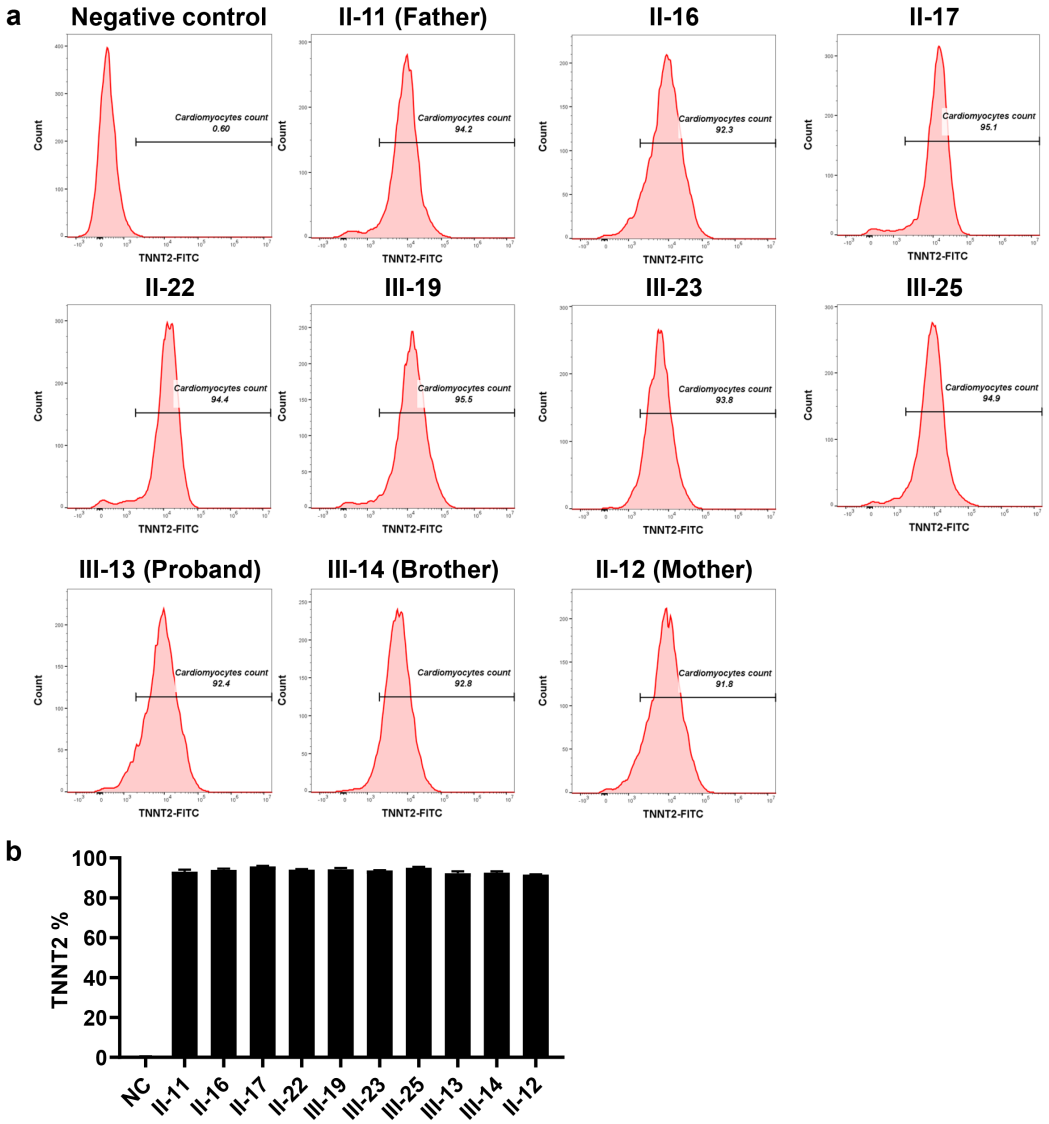


Supplementary Figure 6. Pluripotent staining of generated iPSCs. Pluripotent staining of control and BrS iPSCs using NANOG (green), SOX2 (red), OCT4 (green) and SSEA4 (red). DAPI indicates nuclear staining (blue). Scale bar, 20 µm.

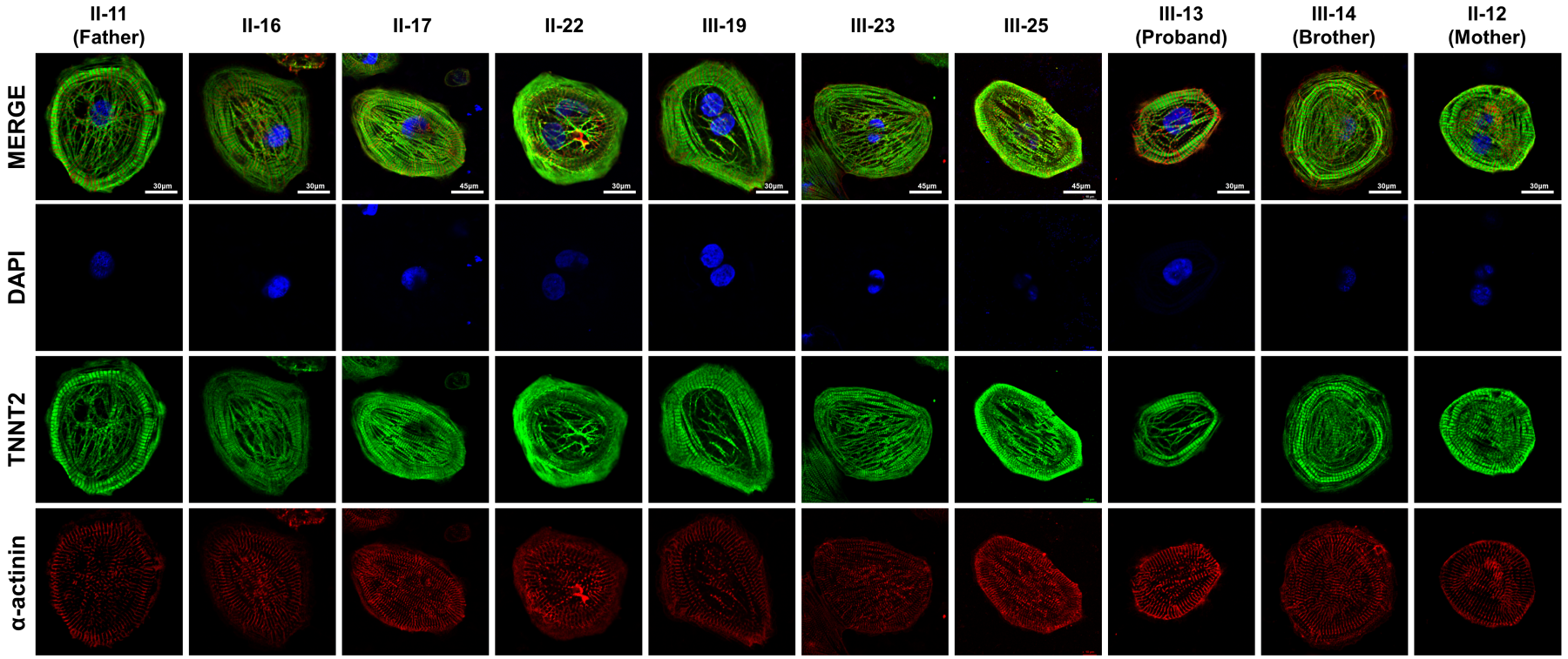
Supplementary Figure 7. Karyotype analysis of generated iPSCs. Representative graphs of karyotype of different generated iPSCs.



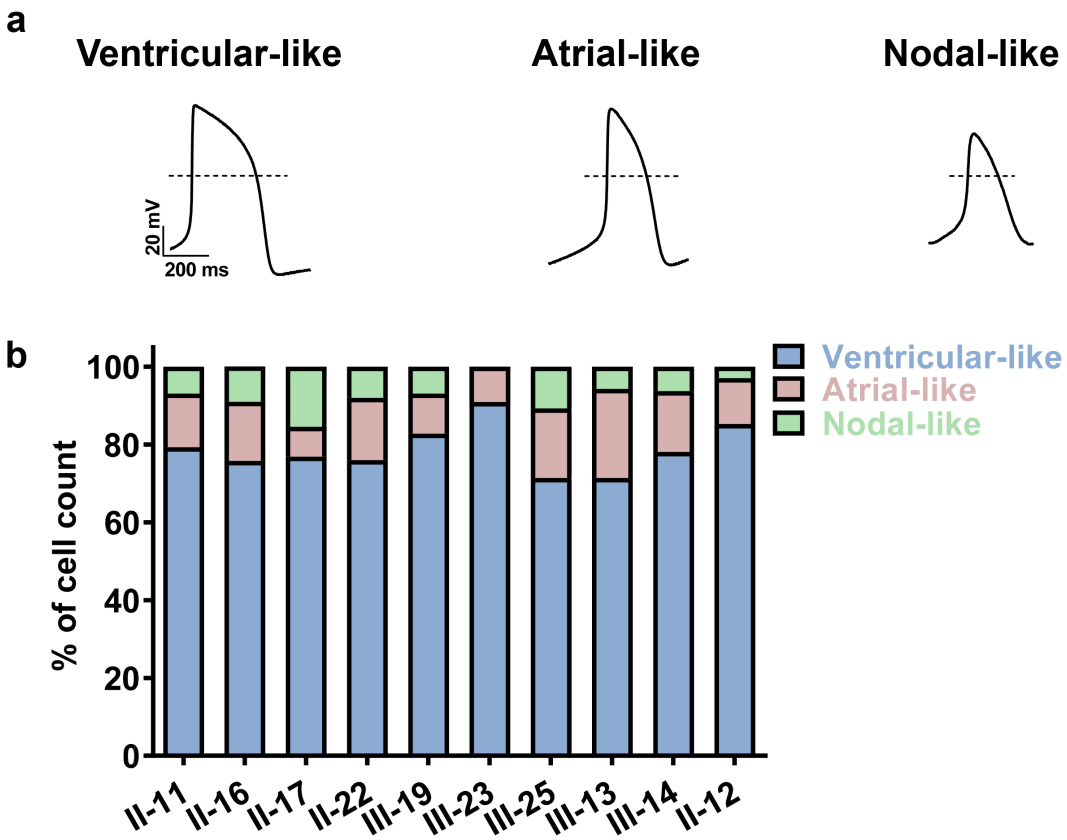
Supplementary Figure 8. Genetic screening of *SCN5A* D356Y in iPSCs from different individuals in the BrS family cohort. Confirmation of the existence of *SCN5A* D356Y variant in proband, proband's brother and mother, but not in proband's father and other healthy control family members at the iPSC level.



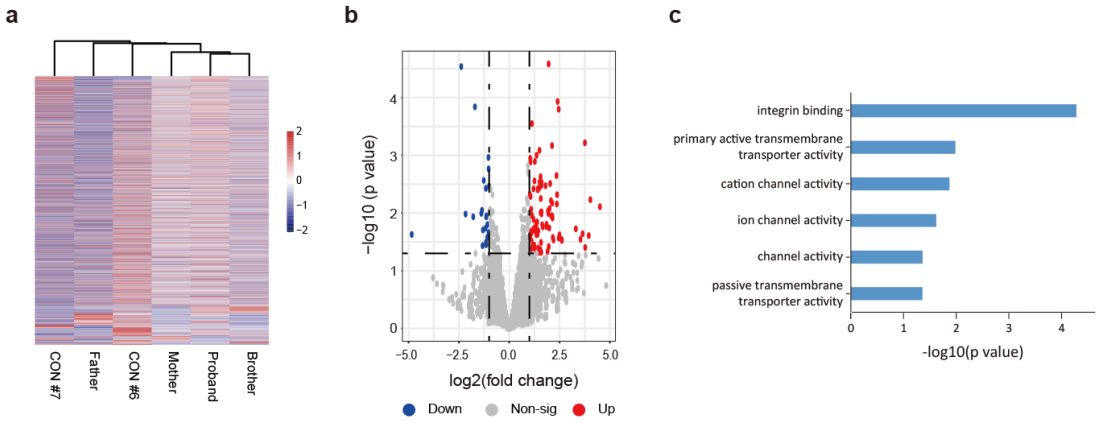
Supplementary Figure 9. Efficient cardiac differentiation of the generated iPSC lines. a. Fluorescence-activated cell sorting (FACS) analysis of TNNT2-positive cells in cardiomyocytes derived from different iPSC lines. **b.** Bar graph to compare the percentage of TNNT2-positive cells between different iPSC-CMs. n= 3.



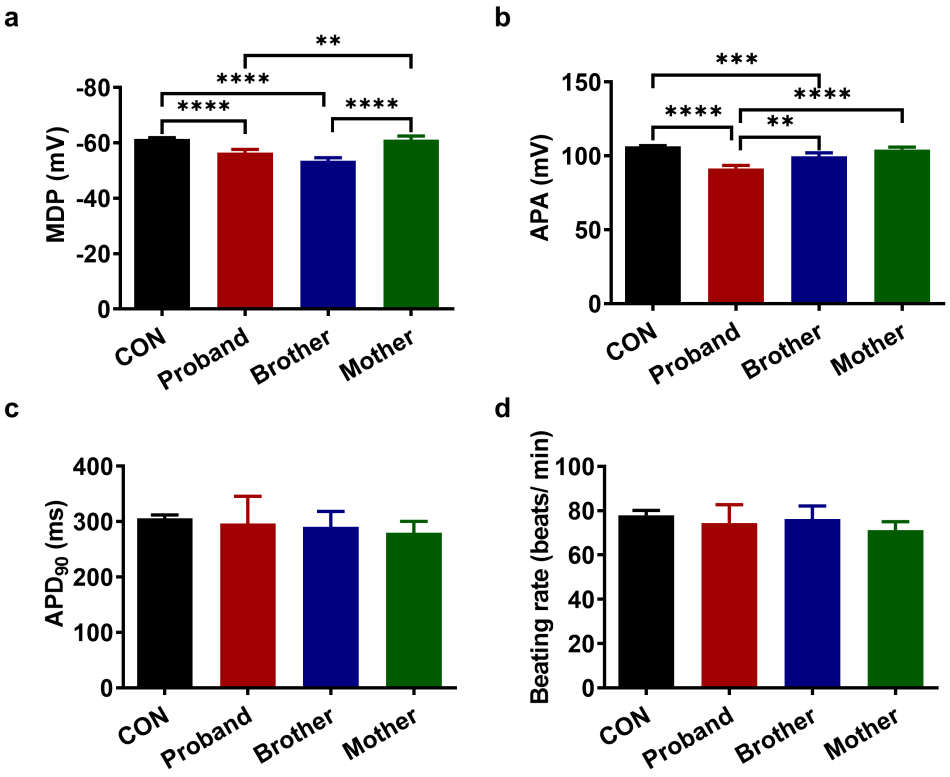
Supplementary Figure 10. Generation and characterization of iPSC-CMs from different individuals in the BrS family cohort. Representative graphs of cardiac-specific staining by TNNT2 (green) and α-actinin (red) in different iPSC-CMs. Scale bar, 5 μm.



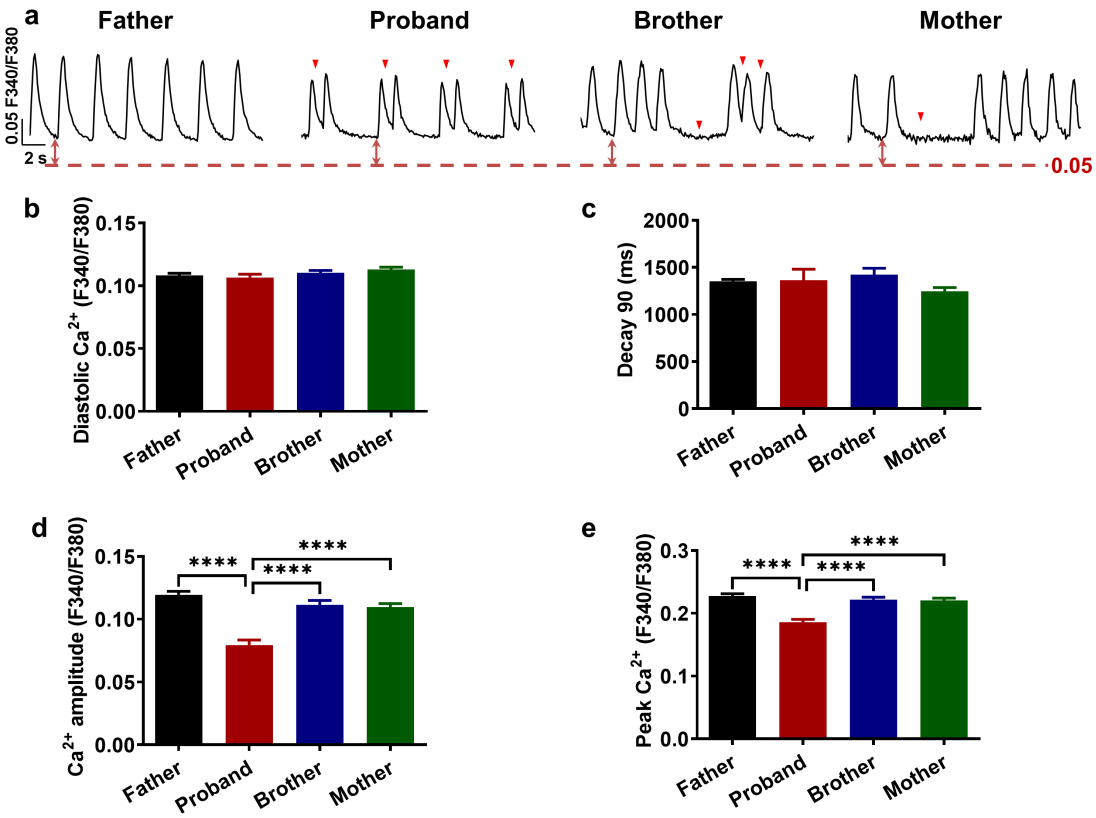
Supplementary Figure 11. Analysis of myocyte subtypes derived from different iPSC lines. a. Representative traces of ventricular-like, atrial-like, and nodal-like action potentials recorded from iPSC-CMs by single-cell patch clamp. **b.** Bar graph to show the percentages (%) of ventricular-like, atrial-like, and nodal-like iPSC-CMs in different groups.



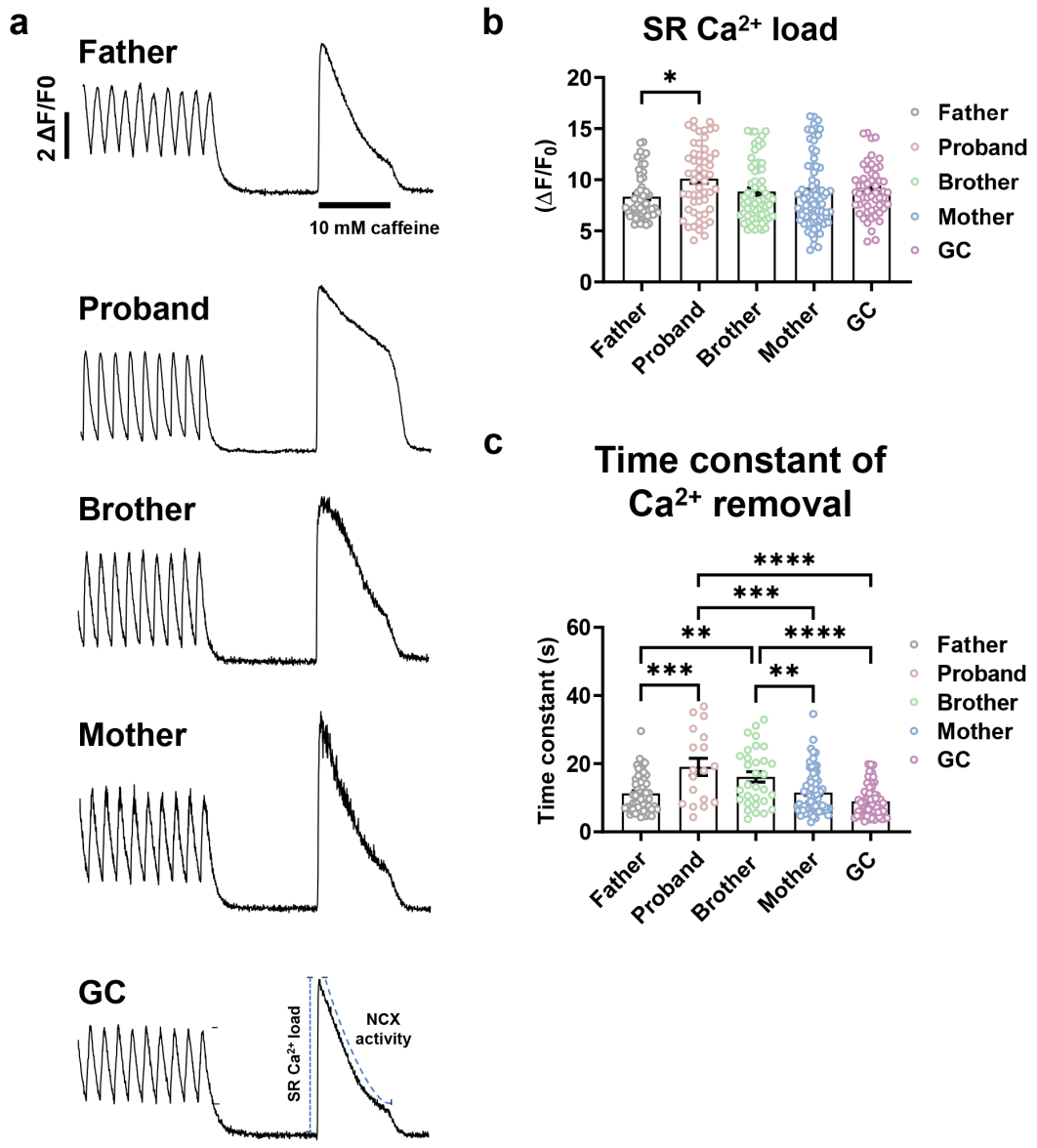
Supplementary Figure 12. Transcriptomic comparison between control and BrS iPSC-CMs. a. Hierarchical clustering based on global expression profile. **b.** Volcano plot showing the DEGs. Control (father, III-23, III-25) and BrS (proband, brother, mother) iPSC-CMs were compared. Up and down indicate genes that are upregulated and downregulated in BrS iPSC-CMs. **c.** GO terms that are enriched in DEGs.



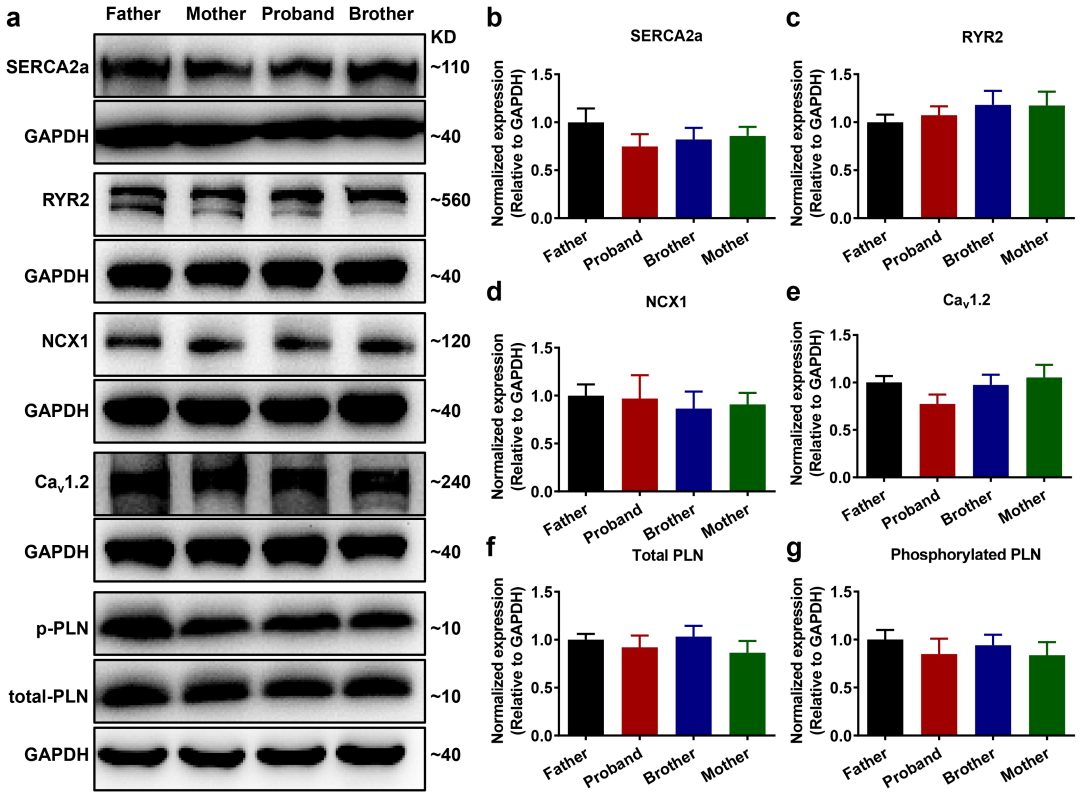
Supplementary Figure 13. Comparison of action potential parameters between control and BrS iPSC-CMs. a-d. Scatter plots to compare MDP, APA, APD_{90} and beating rate between control and BrS (proband, brother and mother) iPSC-CMs by One-way ANOVA (Tukey method). The data collected from seven control iPSC-CMs were averaged and shown as "CON". n= 20-138. $^{**}P< 0.01$, $^{***}P< 0.001$ and $^{****}P< 0.0001$.



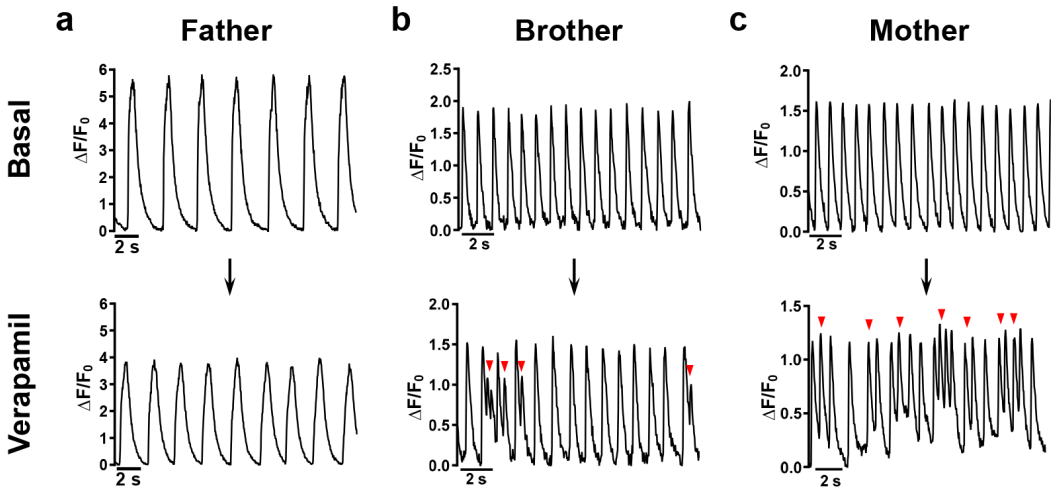
Supplementary Figure 14. Irregular Ca^{2+} signaling. a. Representative Ca^{2+} transient tracings recorded from father, proband, brother and mother iPSC-CMs. Arrhythmia-like abnormal Ca^{2+} transient events are indicated by red arrows. Red dash line indicates 0.05 R_{340}/R_{380}. **b-d.** Bar graphs to compare the diastolic Ca^{2+}, decay 90, Ca^{2+} amplitude and peak Ca^{2+} between father, proband, brother and mother iPSC-CMs by One-way ANOVA (Tukey method). n= 52-84. $^{****}P< 0.0001$.



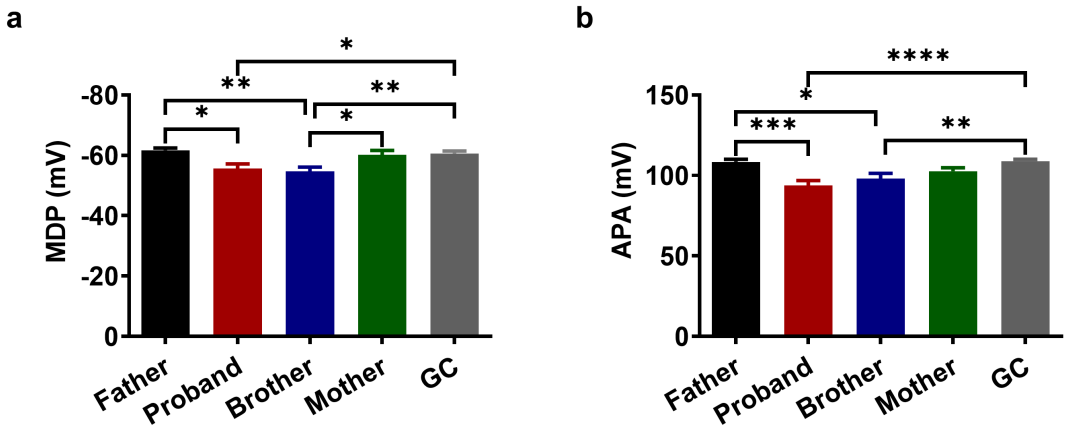
Supplementary Figure 15. Assessment of SR Ca^{2+} load and NCX activity in different iPSC-CMs. a. Representative Ca^{2+} transient tracings following stimulation with 10 mM caffeine in father, proband, brother, mother, and GC iPSC-CMs. **b.** Bar graph to compare the SR Ca^{2+} load between different groups by One-way ANOVA (Tukey method). n= 54-80. $^{*}P< 0.05$. **c.** Bar graph to compare the time constant (τ) of Ca^{2+} removal between different groups by One-way ANOVA (Tukey method). NCX activity (1/τ) was calculated during caffeine application as the inverse of the rate of decrease of cytosolic Ca^{2+} (τ). n= 18-72. $^{**}P< 0.01$, $^{***}P< 0.001$ and $^{****}P< 0.0001$.



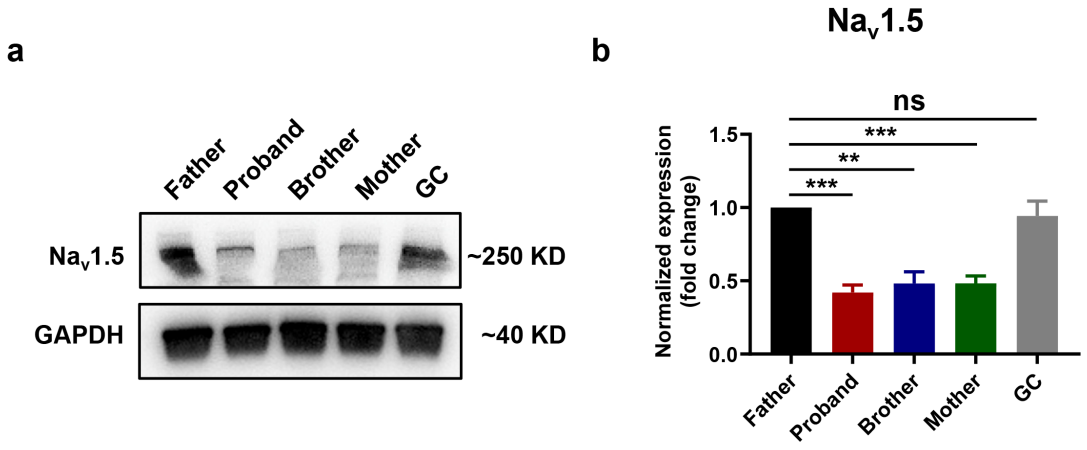
Supplementary Figure 16. Comparable expression of key proteins in Ca^{2+} signaling regulation between father, proband, brother and mother iPSC-CMs. a. Western blot analysis of SERCA2a, RYR2, NCX1, $Ca_v1.2$ and PLN between father, proband, brother and mother iPSC-CMs. GAPDH is used for the loading control. **b-g.** Scatter plots to compare the protein expression of SERCA2a, RYR2, NCX1, $Ca_v1.2$ and PLN between different groups in a by One-way ANOVA (Tukey method). n= 7-19.



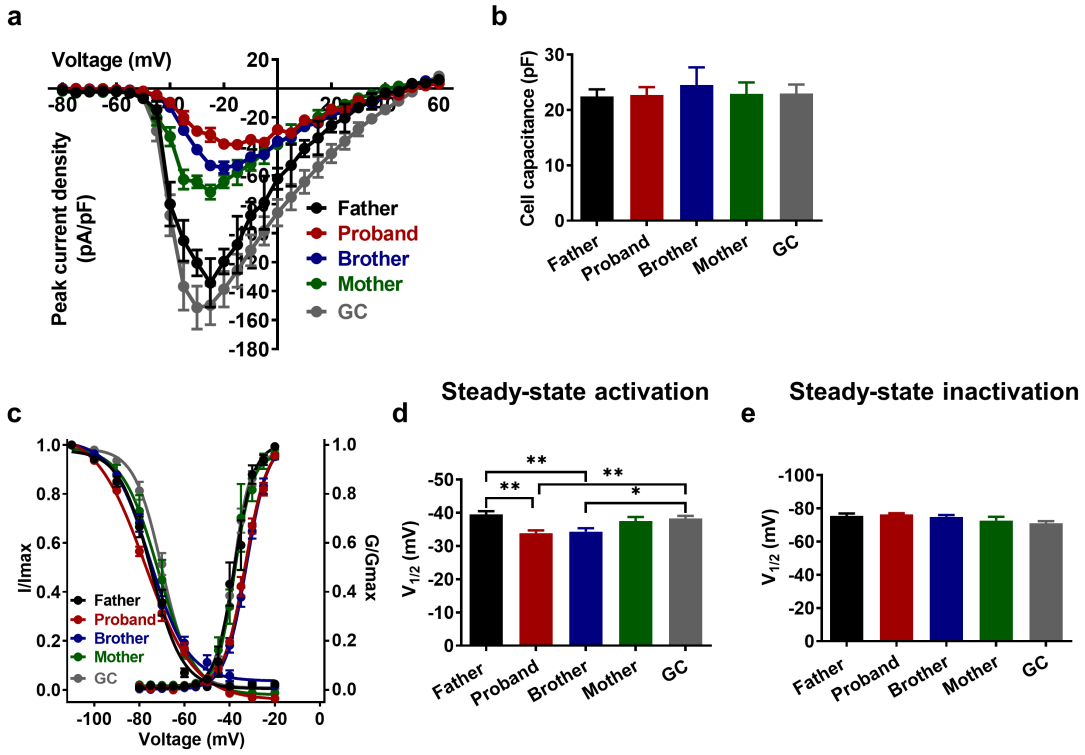
Supplementary Figure 17. Verapamil test unmasks the arrhythmic phenotype in brother and mother iPSC-CMs. a-c. Representative Ca^{2+} transient tracings before and after 100 nM verapamil stress in father, brother or mother iPSC-CMs. Arrhythmia-like abnormal Ca^{2+} transient events are indicated by red arrows.



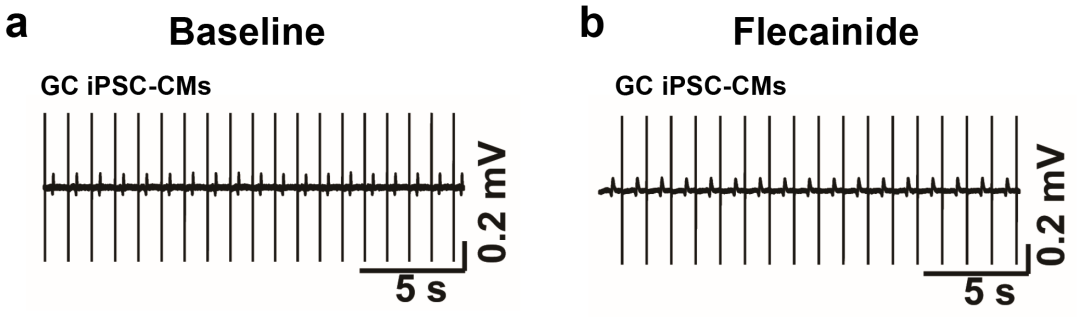
Supplementary Figure 18. GC iPSC-CMs exhibited normal action potential profile. a-b. Scatter plots to compare MDP and APA between father, proband, brother, mother and GC iPSC-CMs by One-way ANOVA (Tukey method). n= 12-37. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ and $^{****}P< 0.0001$.



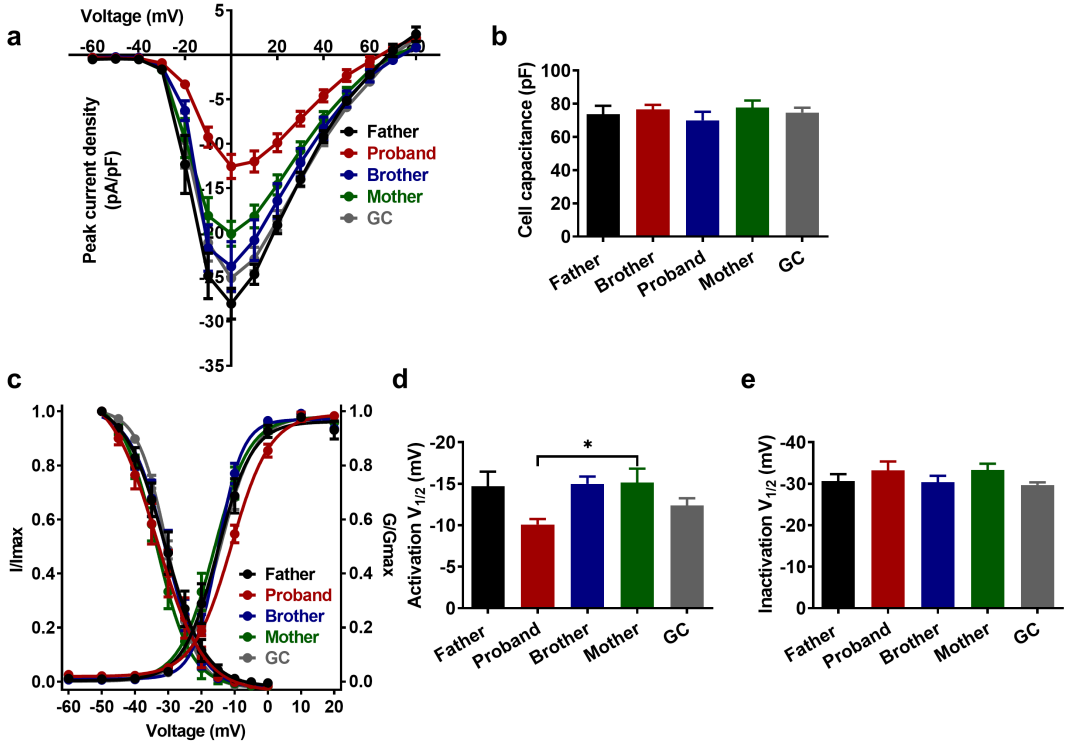
Supplementary Figure 19. Correction of *SCN5A* D356Y in proband iPSC-CMs restores the protein expression of Na$_v$1.5. a. Western blot analysis of Na$_v$1.5 between father, proband, brother, mother and GC iPSC-CMs. GAPDH is used for the loading control. **b.** Bar graph to compare the protein expression of Na$_v$1.5 between different groups by One-way ANOVA (Tukey method). n= 3. $^{**}P< 0.01$ and $^{***}P< 0.001$.



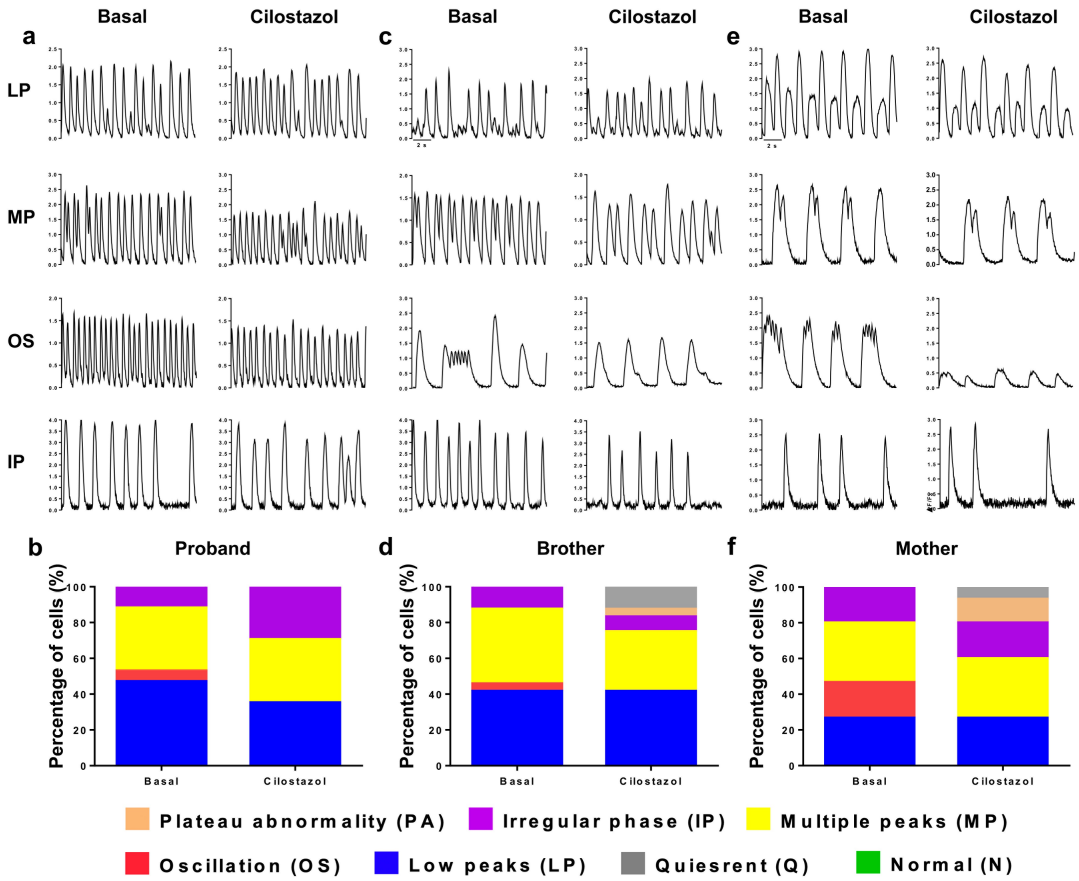
Supplementary Figure 20. Correction of *SCN5A* D356Y in proband iPSC-CMs restored the Na^+ channel function. a. Comparison of Na^+ IV curve between father, proband, brother, mother and GC iPSC-CMs. **b.** Bar graph to compare the cell capacitance between different groups in a by One-way ANOVA (Tukey method). n= 8-12. **c.** Comparison of steady-state activation and inactivation of Na^+ current between father, proband, brother, mother and GC iPSC-CMs. **d-e.** Bar graphs to compare the $V_{1/2}$ of steady-state activation or inactivation between different groups in c by One-way ANOVA (Tukey method). n= 7-18. $^{*}P< 0.05$ and $^{**}P< 0.01$.



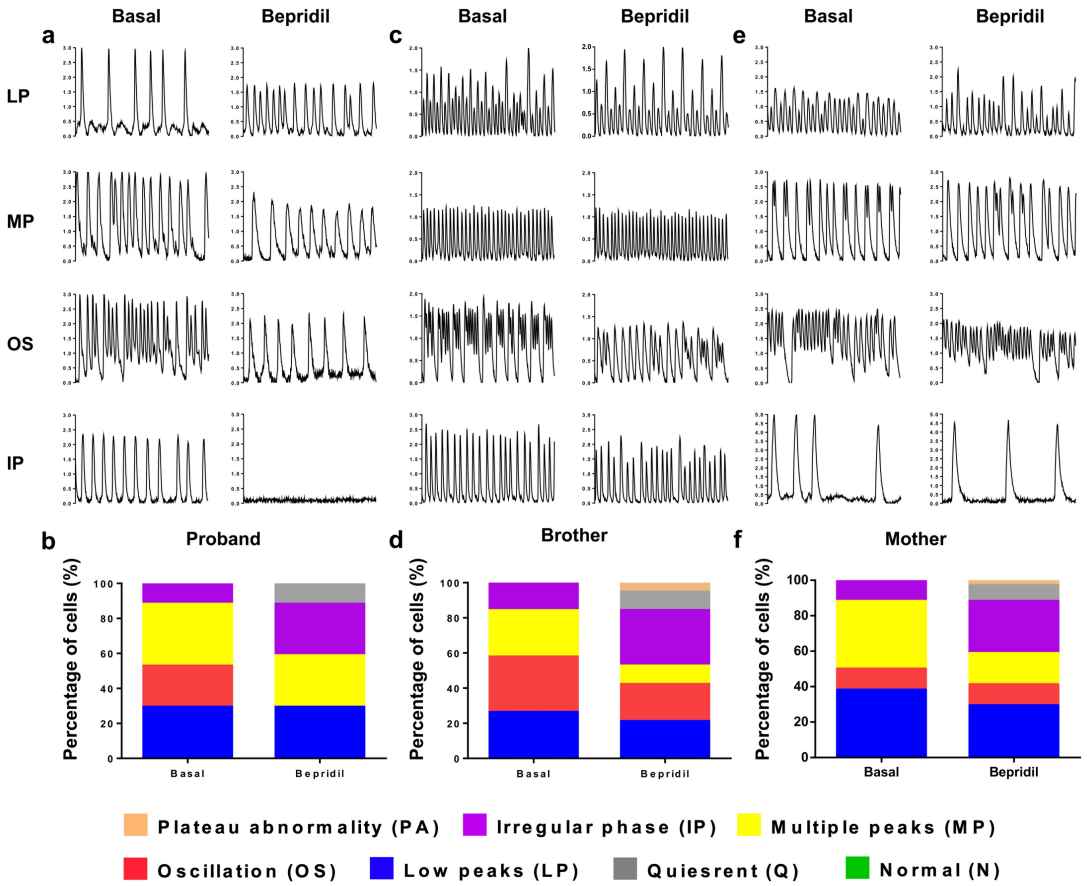
Supplementary Figure 21. Flecainide test on GC iPSC-CMs by MEA. a-b. Representative field potential tracings recorded from GC iPSC-CMs before and after acute treatment of 1 μM flecainide.



Supplementary Figure 22. Correction of *SCN5A* D356Y in proband iPSC-CMs rescued the reduced Ca^{2+} current phenotype. a. Comparison of Ca^{2+} IV curve between father, proband, brother, mother and GC iPSC-CMs. **b.** Bar graph to compare the cell capacitance between different groups in a by One-way ANOVA (Tukey method). n= 8-15. **c.** Comparison of steady-state activation and inactivation of Ca^{2+} current between father, proband, brother, mother and GC iPSC-CMs. **d-e.** Bar graph to compare the $V_{1/2}$ of steady-state activation and inactivation between different groups in c by One-way ANOVA (Tukey method). n= 8-12. $^{*}P< 0.05$.

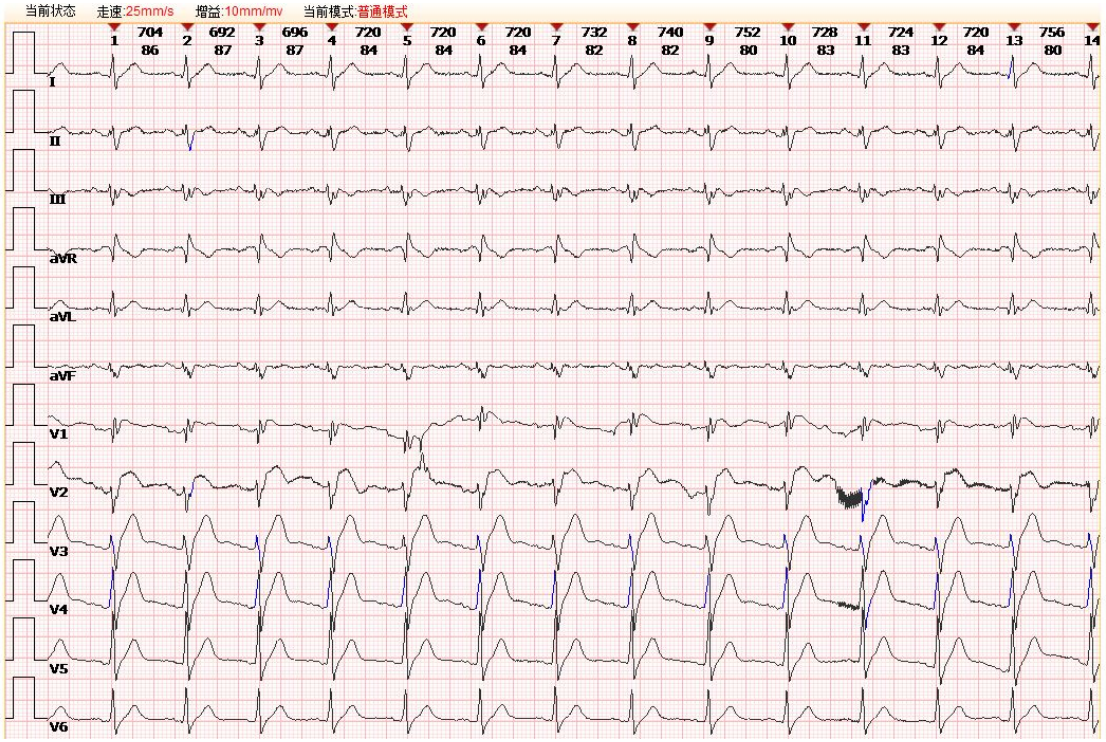


Supplementary Figure 23. Cilostazol test in D356Y iPSC-CMs. a, c and e. Representative Ca^{2+} transient tracings recorded from proband, brother and mother iPSC-CMs by Ca^{2+} imaging using Fluo-4 AM before and after 1 μM cilostazol application. **b, d and f.** Bar graphs to compare percentage of cells exhibiting normal (N) or abnormal Ca^{2+} transient pattern including low peaks (LP), multiple peaks (MP), oscillation (OS) and irregular phase (IP). Plateau abnormality (PA) and quiescent (Q) before and after cilostazol application in proband, brother and mother iPSC-CMs. n= 15-24.

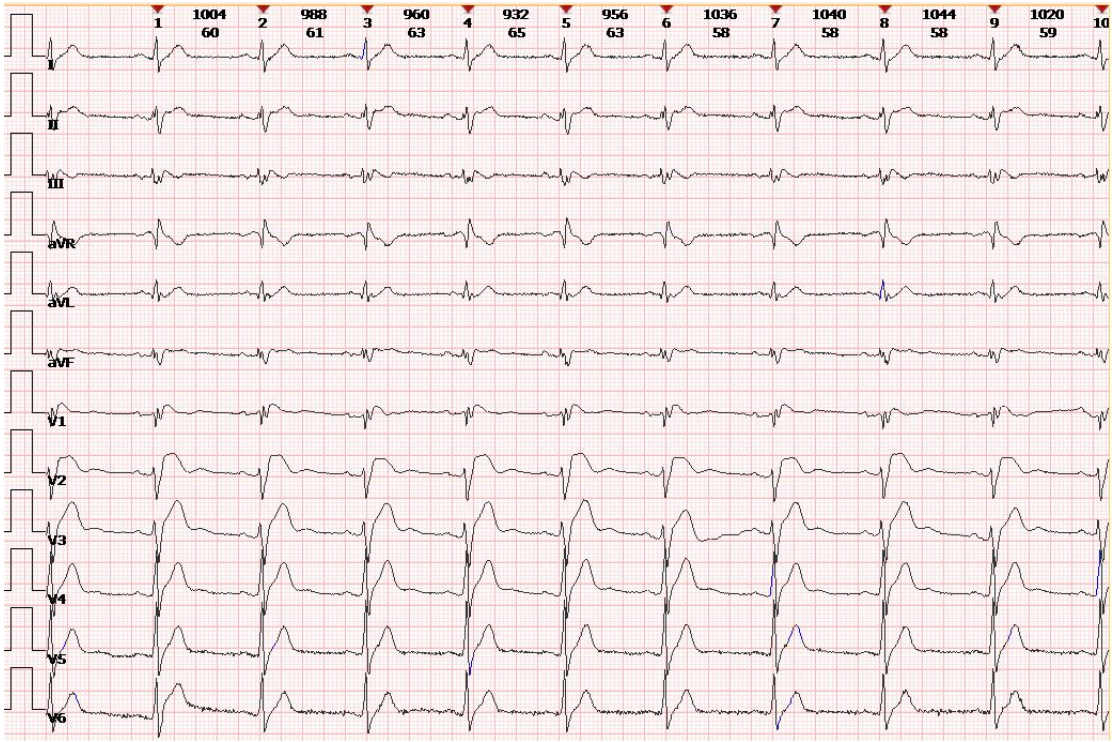


Supplementary Figure 24. Bepridil test in D356Y iPSC-CMs. a, c and e. Representative Ca^{2+} transient tracings recorded from proband, brother and mother iPSC-CMs by Ca^{2+} imaging using Fluo-4 AM before and after 100 nM bepridil application. **b, d and f.** Bar graphs to compare percentage of cells exhibiting normal (N) or abnormal Ca^{2+} transient pattern including low peaks (LP), multiple peaks (MP), oscillation (OS) and irregular phase (IP). Plateau abnormality (PA) and quiescent (Q) before and after bepridil application in proband, brother and mother iPSC-CMs. n= 17-34.

Before Ca^{2+}

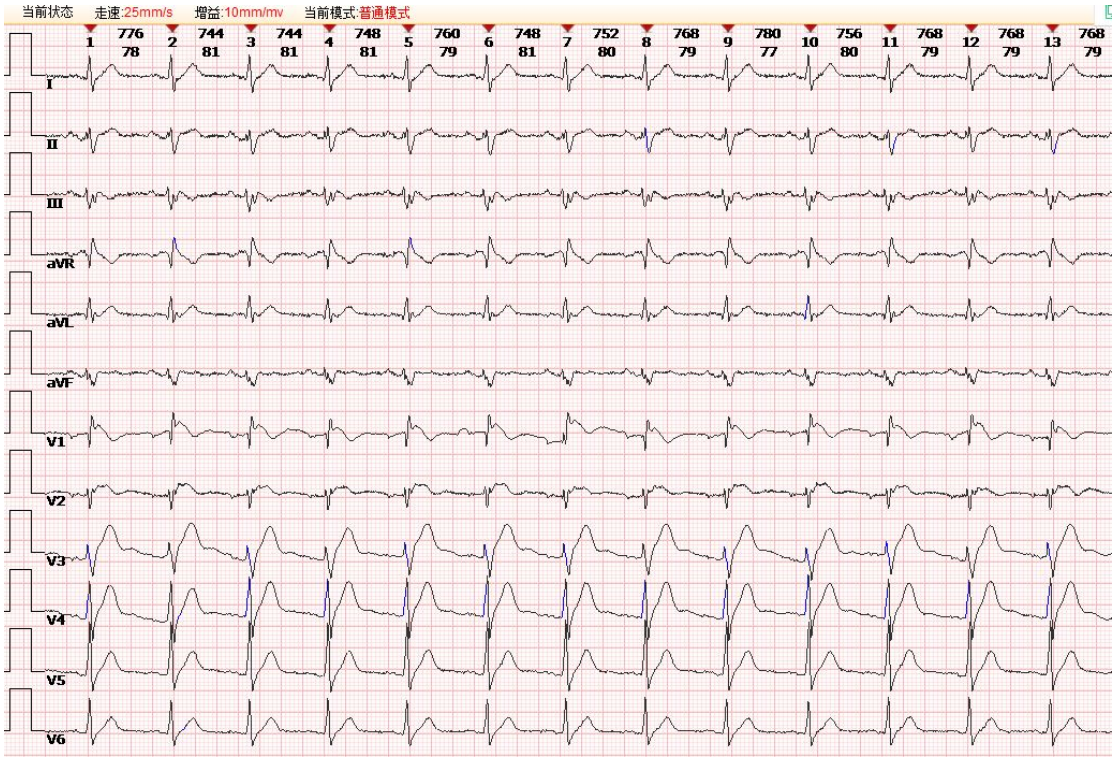


Post Ca^{2+}

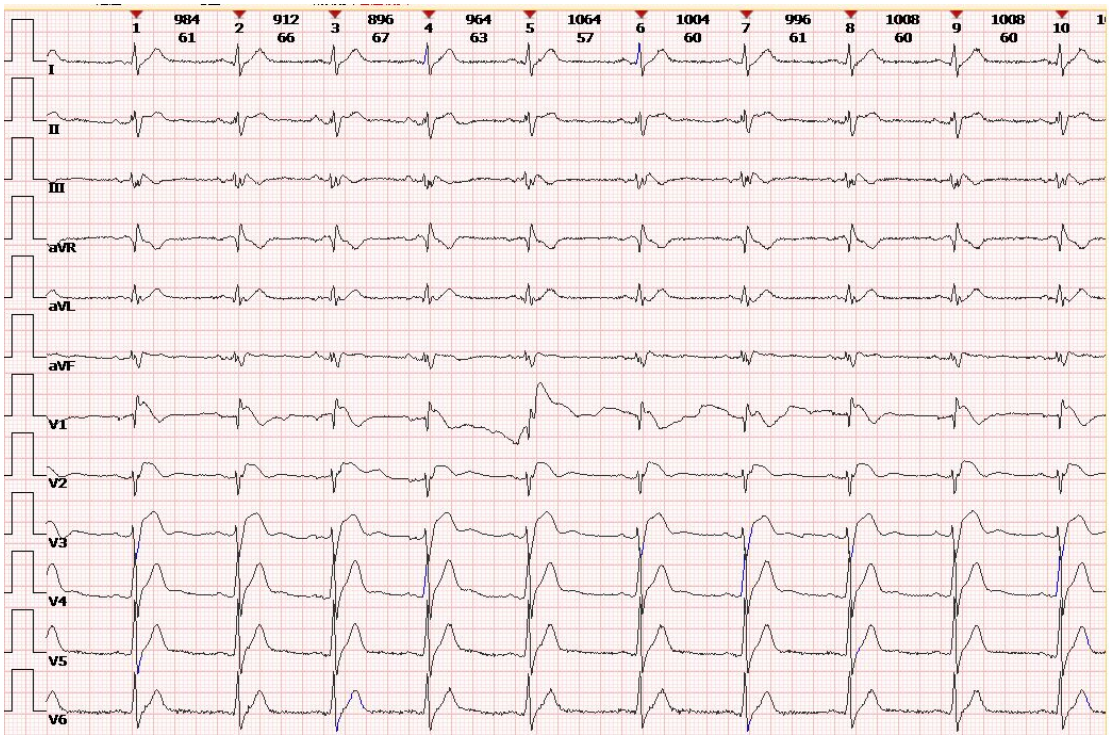


Supplementary Figure 25. The full tracings of regular ECG before and immediately after the application of intravenous calcium.

Before Ca^{2+}



Post Ca^{2+}



Supplementary Figure 26. The full tracings of the third ICS ECG before and immediately after the application of intravenous calcium.

Supplementary Table 1. Clinical information of BrS patients in this study

	Proband	Brother	Mother
Age at presentation	34	31	53
Sex	M	M	F
Age of diagnosis	22	26	49
History of syncope or SCA	Yes	No	No
Age of first syncopal episode or SCA	21	NA	NA
Family history of SD	Yes	Yes	Yes
Deaf-mutism	No	No	No
Spontaneous type 1 Brugada pattern ECG	Yes	Yes	No
QTc of surface ECG (ms)	391	397	432
ICD implantation	Yes	No	No
VT/VF ablation	Yes	No	No
VF/VT inducibility during the EPS	S1S1 270ms RVOT, PMVT	NA	NA
Putative causal genetic site	*SCN5A*	*SCN5A*	*SCN5A*
Mutation type	Inherited	Inherited	NA

SCA: sudden cardiac arrest; SD: sudden death; ECG: electrocardiograph; ICD: implantable cardioverter defibrillator; VT: ventricular tachycardia; VF: ventricular fibrillation; EPS: electrophysiology study; RVOT: right ventricular outflow tract; PMVT: polymorphic ventricular tachycardia

Supplementary Table 2. Summary of control and BrS iPSC lines in this study

	Age	Sex	Ethnicity	Somatic cells	Reprogramming method	iPSC lines established
CON#1 (proband's father, II-11)	52	Male	Chinese Han	Skin fibroblasts	Sendai virus	Clone #7, #12 and #16
CON#2 (II-16)	55	Female	Chinese Han	Skin fibroblasts	Sendai virus	Clone #5, #6 and #9
CON#3 (II-17)	53	Female	Chinese Han	Skin fibroblasts	Sendai virus	Clone #1, #2 and #3
CON#4 (II-22)	47	Male	Chinese Han	Skin fibroblasts	Sendai virus	Clone #2, #3 and #8
CON#5 (III-19)	35	Female	Chinese Han	Skin fibroblasts	Sendai virus	Clone #3, #8 and #10
CON#6 (III-23)	33	Female	Chinese Han	Skin fibroblasts	Sendai virus	Clone #2, #3 and #8
CON#7 (III-25)	21	Female	Chinese Han	Skin fibroblasts	Sendai virus	Clone #2 and #8
BrS#1 (proband, III-13)	29	Male	Chinese Han	Skin fibroblasts	Sendai virus	Clone #3 and #13
BrS#2 (proband's brother, III-14)	26	Male	Chinese Han	Skin fibroblasts	Sendai virus	Clone #4 and #9
BrS#3 (proband's mother, II-12)	48	Female	Chinese Han	Skin fibroblasts	Sendai virus	Clone #8 and #12

Supplementary Table 3. Summary of action potential parameters in control and BrS iPSC-CMs

	MDP	APA	APD_{90}	V_{max}	Beating rate
	(mV)	(mV)	(ms)	(V/s)	(beats/min)
CON#1	-61.66 ± 0.99	106.20 ± 1.30	314.70 ± 17.33	14.81 ± 1.70	80.67 ± 8.36
CON#2	-58.97 ± 1.24	101.70 ± 1.58	297.60 ± 16.12	12.40 ± 0.98	78.67 ± 4.95
CON#3	-63.46 ± 1.02	106.30 ± 1.78	283.20 ± 13.98	12.99 ± 0.95	72.59 ± 6.30
CON#4	-60.59 ± 0.88	104.00 ± 0.77	311.10 ± 19.05	12.06 ± 1.16	75.73 ± 4.84
CON#5	-61.22 ± 1.10	103.70 ± 1.00	275.40 ± 15.07	12.87 ± 1.23	88.38 ± 4.62
CON#6	-63.60 ± 0.7	111.90 ± 0.81	311.90 ± 1.70	12.58 ± 0.81	76.61 ± 3.78
CON#7	-60.88 ± 0.80	108.20 ± 1.35	336.60 ± 15.34	13.00 ± 1.04	74.03 ± 6.01
BrS#1	-56.53 ± 1.13	91.55 ± 2.04	296.50 ± 49.42	8.07 ± 0.77	74.43 ± 8.30
BrS#2	-53.57 ± 1.08	96.67 ± 2.37	290.60 ± 28.09	9.88 ± 0.63	76.23 ± 5.93
BrS#3	-61.19 ± 1.30	103.8 ± 1.78	279.90 ± 20.43	9.63 ± 0.46	71.17 ± 3.90

Supplementary Table 4. Summary of sodium current parameters in control and BrS iPSC-CMs

	Peak current density at -30 mV	**Cell capacitance**	**Steady-state activation**		**Steady-state inactivation**	
	(pA/pF)	(pF)	$V_{1/2}$ (mV)	ƙ (mV/e-fold)	$V_{1/2}$ (mV)	ƙ (mV/e-fold)
CON#1	-141.23 ± 13.63	22.85 ± 0.78	-40.43 ± 0.98	2.88 ± 0.28	-77.64 ± 0.81	6.21 ± 0.096
CON#2	-129.51 ± 10.74	25.40 ± 2.08	-38.17 ± 1.02	2.88 ± 0.16	-80.02 ± 0.64	6.61 ± 0.21
CON#3	-141.57 ± 12.04	26.22 ± 1.89	-42.27 ± 1.36	2.40 ± 0.44	-74.82 ± 1.61	6.42 ± 0.19
CON#4	-117.93 ± 7.97	24.14 ± 1.85	-40.15 ± 1.18	2.47 ± 0.36	-77.78 ± 1.12	6.52 ± 0.21
CON#5	-117.99 ±6.04	20.86 ± 1.74	-41.73 ± 1.10	2.43 ± 0.24	-77.03 ± 0.78	6.34 ± 0.23
CON#6	-125.46 ± 8.86	21.40 ± 1.65	-41.78 ± 1.50	2.33 ± 0.23	-74.39 ± 1.36	7.20 ± 0.25
CON#7	-131.46 ± 11.74	24.76 ± 2.06	-40.98 ± 1.30	2.90 ± 0.26	-79.66 ± 0.86	6.64 ± 0.30
BrS#1	-33.92 ± 3.16	26.38 ± 1.15	-33.79 ± 0.75	4.07 ± 0.16	-74.40 ± 1.65	9.86 ± 0.86
BrS#2	-48.45 ± 4.38	23.87 ± 1.52	-35.24 ± 0.92	3.98 ± 0.16	-76.22 ± 1.41	8.42 ± 0.90
BrS#3	-70.61 ± 3.19	24.47 ± 1.31	-39.05 ± 0.94	3.52 ± 0.23	-76.59 ± 0.89	7.63 ± 0.23

Supplementary Table 5. Summary of Ca^{2+} transient parameters in control and BrS iPSC-CMs

	Diastolic Ca^{2+}	Transient amplitude	Peak Ca^{2+}	Maximal rising rate	Decay 90
	(F340/F380)	(F340/F380)	(F340/F380)	(F340/F380/s)	(ms)
Father	0.108 ± 0.002	0.119 ± 0.003	0.228 ± 0.004	0.424 ± 0.010	1351 ± 21.91
Proband	0.106 ± 0.002	0.079 ± 0.004	0.186 ± 0.004	0.302 ± 0.017	1364 ± 116.8
Brother	0.110 ± 0.000	0.114 ± 0.003	0.222 ± 0.004	0.348 ± 0.011	1422 ± 68.17
Mother	0.113 ± 0.002	0.109 ± 0.003	0.220 ± 0.004	0.381 ± 0.010	1246 ± 40.11

Supplementary Table 6. Summary of calcium current parameters in control and BrS iPSC-CMs

	Peak current density At 0 mV	**Cell capacitance**	**Steady-state activation**		**Steady-state inactivation**	
	(pA/pF)	(pF)	$V_{1/2}$ (mV)	ƙ (mV)	$V_{1/2}$ (mV)	ƙ (mV)
Father	-29.63 ± 1.83	63.30 ± 2.93	-14.26 ± 1.49	4.40 ± 0.32	-29.16 ± 1.50	5.04 ± 0.24
Proband	-14.08 ± 1.41	58.59 ± 4.26	-11.32 ± 1.43	6.67 ± 0.47	-32.96 ± 2.66	7.65 ± 0.77
Brother	-27.81 ± 1.64	56.73 ± 4.19	-14.85 ± 0.96	4.05 ± 0.21	-27.56 ± 1.46	5.87 ± 0.37
Mother	-22.98 ± 1.82	60.68 ± 4.54	-14.79 ± 1.45	4.18 ± 0.37	-30.56 ± 1.46	4.83 ± 0.17