SUPPLEMENTAL MATERIALS

Patient-specific and genome-edited induced pluripotent stem cell-derived cardiomyocytes elucidate single cell phenotype of Brugada Syndrome

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SUPPLEMENTAL METHODS

Isolation and maintenance of fibroblast cells. Freshly isolated skin biopsies were rinsed with PBS and transferred into a 1.5 ml tube. Tissue was minced in collagenase 1 (1 mg/ml in Dulbecco's modified Eagle medium (DMEM), Invitrogen, Carlsbad, CA) and allowed to digest for 6 hours at 37°C. Dissociated dermal fibroblasts were plated and maintained with DMEM containing 10% FBS (Invitrogen), Glutamax (Invitrogen), 4.5 g/L glucose (Invitrogen), 110 mg/L sodium pyruvate (Invitrogen), 50 U/mL penicillin (Invitrogen), and 50 g/mL streptomycin (Invitrogen) at 37°C, at 95% air and 5% CO_2 in a humidified incubator. All cells were used for reprogramming within 5 passages.

Culture and maintenance of iPSCs. Control and BrS iPSCs were maintained in chemically defined medium Essential 8 (E8 medium) (Life Technologies) on Matrigel-coated (BD Bioscience, San Jose, CA) plates at 37°C with 5% (vol/vol) CO_2.

Differentiation of iPSC-CMs. Control and BrS iPSCs were differentiated into iPSC-CMs using a 2D monolayer differentiation protocol and were maintained in a 5% CO_2/air environment as previously published (28). Briefly, iPSC colonies were dissociated with 0.5 mM EDTA (Gibco) into single-cell suspension and re-suspended in E8 media containing 10 μM Rho-associated protein kinase inhibitor (Sigma). Approximately 100,000 cells were re-plated into Matrigel-coated 6-well plates. iPSCs were next cultured to 85% cell confluence, and then treated for 2 days with 6 μM CHIR99021 (Selleck Chemicals) in RPMI+B27 supplement without insulin to activate WNT signaling and induce mesodermal differentiation. On day 2, cells were placed on RPMI+B27 without insulin and CHIR99021 (28). On days 3-4, cells were treated with 5 μM IWR-1 (Sigma)

to inhibit WNT pathway signaling and induce cardiogenesis. On days 5-6, cells were removed from IWR-1 treatment and placed on RPMI+B27 without insulin. From day 7 onwards, cells were placed on RPMI+B27 with insulin until beating was observed. At this point, cells were glucose-starved for 3 days with RPMI+B27 with insulin to purify iPSC-CMs. Following purification, cells were cultured in RPMI+B27 with insulin. When re-plating iPSC-CMs for downstream use, cells were dissociated with 0.25% trypsin-EDTA (Life Technologies) into a single-cell suspension and seeded on Matrigel-coated plates.

Genetic screening and analysis. BrS iPSCs were cultured on Matrigel-coated 6-well plates with E8 media and harvested at 80-90% confluence for subsequent analysis. Genomic DNA was extracted using a DNeasy commercial DNA isolation kit (Qiagen, Valencia, CA). Polymerase chain reaction (PCR) was carried out on S1000 Thermal Cycler (Biorad, Hercules, CA). Three single nucleotide polymorphism (SNP) loci within SCN5A gene (2053G>A/R620H, 2626G>A/R811H, 4190ΔA/1397Δ; reference sequence: NM_198056.2) were amplified and analyzed by direct sequencing, and then confirmed by sub-cloning. The primer sequences for SCN5A are listed in **Online Table 6** (reference sequence: NC_000003.12).

Immunofluorescence staining. Immunofluorescence was performed using appropriate primary antibodies and AlexaFluor-conjugated secondary antibodies (Invitrogen, Carlsbad, CA) as described by the manufacturer's protocol. The primary antibodies used in this study were anti-SOX2 (Biolegend, San Diego, CA), anti-NANOG (Santa Cruz, CA), anti-cardiac Troponin T (cTnT) (Abcam, ab10214), anti-sarcomeric α-actinin (Abcam, ab90776), anti-hNav1.5 (Alomone Labs, ASC-005), and anti-SCN7A (Sigma HPA004879).

Karyotype analysis. BrS lines were trypsinized for 5 min and centrifuged at 300 g for 3 min. iPSC pellets were resuspended in 200 μl of PBS and DNA was isolated using the DNeasy blood and tissue kit (Qiagen, Valencia, CA). DNA was submitted to the Stanford Functional Genomics Facility for SNP karyotyping using the HuCytoSNP-12 chip (Illumina). CNV and SNP visualization was performed using KaryoStudio v1.4 (Illumina).

Ca^{2+} imaging. Dissociated control and BrS iPSC-CMs were reseeded in Matrigel-coated 8-well Lab Tek II chambers (Nalge Nuc International). Cells were recovered for 2 days and were loaded with 5 μM Fluo-4 AM with 0.02% Pluronic F-127 (Molecular Probes) in Tyrode's solution for 15 min at 37°C, and were washed with Tyrode's solution afterwards. Ca^{2+} imaging was conducted using a Zeiss LSM 510Meta confocal microscope (Carl Zeiss AG, Göttingen, Germany). Spontaneous Ca^{2+} transients of single beating iPSC-CMs were obtained using a time-lapse line scanning recording mode (512 pixels * 1920 lines) under 40X objective (Plan Apochromat, 0.95 NA) at 37°C, and the raw data was analyzed using customized Interactive Digital Language (IDL) script. Ca2+ signal was normalized to the intracellular basal line (F_0), and transient amplitude was expressed as $\Delta F/F_0$.

RNA Sequencing. Total RNA was extracted from control or BrS iPSC-CMs using the RNeasy Mini kit (Qiagen, Valencia, CA) and DNase treated using a RNase-Free DNase kit (Qiagen). 5 ng of RNA was used as input for cDNA synthesis and amplification using the Ovation RNA-Seq System V2 (NuGEN). Illumina barcodes were ligated to cDNAs using the NEBNext® DNA Library Prep Master Mix Set for Illumina®. The ligated libraries were size selected for an average insert size of 250 bp by agarose gel excision and extraction. The ligated libraries were then

amplified by PCR. Four prepared libraries were pooled and sequenced on one HiSeq2000 (Illumina) lane to obtain an average of 30 million paired-end reads per sample. Reads were mapped to the Ensembl GRCh37 reference genome using Tophat 2.1. Junction BED files generated from Tophat were used to generate an exon coordinate reference file using AltAnalyze 2.0.8 and exon counts were quantified using the reference BED files from Tophat generated BAM files. Finally, AltAnalyze was run using the default settings to calculate RPKM values per Ensembl ID. Differentially expressed genes between control and BrS samples were calculated using an empirical Bayes moderated t-test $p< 0.05$ with a 2-fold expression difference. These genes were imported into Ingenuity pathway analysis (Ingenuity, Redwood City, CA) and the top calls for canonical pathway analysis was reported.

Quantitative real-time PCR (qPCR). Total mRNAs were isolated from control and BrS iPSC-CMs using the Qiagen RNeasy Mini kit. 1 μg of RNA was used to synthesize cDNA using the Superscript II First-Strand cDNA synthesis kit (Invitrogen). 0.25 μl of the reaction was used to quantify gene expression by qPCR using TaqMan Universal PCR Master Mix. Expression values were normalized to the average expression of housekeeping gene 18s (**Online Table 5**).

Patch clamp recordings of iPSC-CMs. Whole-cell patch clamp recordings were conducted using an EPC-10 patch clamp amplifier (HEKA, Germany) from control and BrS iPSC-CMs. Contracting monolayer iPSC-CMs were mechanically isolated, enzymatically dispersed into single cells, and attached to Matrigel-coated glass coverslips (Warner, USA). While recording, the coverslips containing plated iPSC-CMs were transferred to a RC-26C recording chamber (Warner, USA) mounted onto the stage of an inverted microscope (Nikon, Japan). The glass pipettes were

prepared using thin-wall borosilicate glass (A-M System, USA) with a micropipette puller (Sutter Instrument, P-97, USA), polished with a microforge (Narishige, MF830, Japan), and had resistances between 1.5-2 MΩ. Extracellular solution perfusion was continuous using a rapid solution exchanger (Biologic, RC-200, USA) with solution exchange requiring 1 min. Data were acquired using PatchMaster software (HEKA, Germany) and digitized at 1.0 kHz. Data were analyzed using FitMaster (HEKA, Germany), Igor Pro (Wave Metrics), Microcal Origin 6.1 (OriginLab) or Prism (GraphPad). Temperature was kept constant by a TC-324B heating system (Warner, USA) at 36-37°C. To obtain action potentials from iPSC-CMs, current clamp recordings were conducted in normal Tyrode's solution containing 140 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 1.8 mM $CaCl_2$, and 10 mM HEPES (pH 7.4 with NaOH at 25°C). The pipette solution contained 120 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, 3 mM Mg-ATP, and 10 mM EGTA (pH 7.2 with KOH at 25°C). To obtain Na^+ channel currents from control and BrS iPSC-CMs, voltage clamp recordings were conducted at room temperature. External solution contains 50 mM NaCl, 1.8 mM $CaCl_2$, 110 mM CsCl, 1 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES, and 0.001 mM nifedipine (pH 7.4 with CsOH). Pipette solution contains 135 mM CsCl, 10 mM NaCl, 2 mM $CaCl_2$, 5 mM EGTA, 10 mM HEPES, and 5 mM Mg-ATP (pH 7.2 with CsOH). To measure Na^+ channel peak currents, the currents were evoked by depolarizing iPSC-CMs from a holding potential of -80 mV to test potentials ranging from -80 mV to +60 mV with 10-mV increments. All data are expressed as mean ± s.e.m., and t test was used to evaluate statistical significance.

CRISPR/Cas9-mediated genome editing. In order to establish the mutation corrected Brugada patient-specific iPSC lines, guide RNA (gRNA) was designed and constructed in the PX458 plasmid (Addgene, Cambridge, MA) using the protocol as previously described (**Supplemental**

Figure 8A and 9) (1). 500 bp fragments of wild type *SCN5A* exon 23 and adjacent intronic sequences were synthesized as GeneArt® Strings™ DNA fragments (Life Technologies) to make left and right homologous arms and directly cloned into PB-MV1Puro-TK vector (Transposagen, Lexington, KY) to construct a targeting vector carrying a PGK promoter-*puroΔtk* selection cassette flanked by *piggyBac* repeats and the TTAA site as previously described (**Supplemental Figure 8B**). One silence mutation in the homologous arm was inserted in order to create an artificial TTAA site for *piggyBac* transposase (**Supplemental Figure 8B**). Both CRISPR/Cas9 and targeting vectors were delivered into BrS2 iPSCs by Lipofectamine® 3000 (Life Technologies), and subsequently cells with correct targeting vector integration were selected by puromycin (Life Technologies) and genotyped (**Supplemental Figure 10**). To excise the selection cassette, transient expression of *piggyBac* transposase was performed by transfection of excision only *piggyBac*™ transposase mRNA (Transposagen) using Lipofectamine® MessengerMAX™ (Life Technologies). After negative selection using ganciclovir (Sigma Aldrich), the established clones were genotyped by PCR and bidirectional direct sequencing (**Online Table 6 and Supplemental Figures 11 and 12**).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Genetic screening and analysis of BrS iPSCs. A. Genetic screening and analysis of *SCN5A* obtained from iPSCs derived from BrS patient 1 (BrS1) revealed double heterozygous missense mutations in *SCN5A* gene in positions 2053 and 2626 of the coding sequence (2053G>A, 2626G>A), resulting in the substitutions at positions 620 and 811 of the protein (R620H, R811H). **B.** Genetic screening and analysis of *SCN5A* obtained from iPSCs derived from BrS patient 2 (BrS2) revealed a deletion mutation in *SCN5A* gene in position 4190 of the coding sequence (4190Δ), resulting in the deletion at position 1397 of the protein (1397Δ).

Supplemental Figure 2. Karyotype analysis of BrS iPSCs. SNP karyotyping was performed on the BrS lines (BrS1 and BrS2) using the HUCytoSNP-12 chip to demonstrate no large karyotypic abnormalities were present. Green bars represent a gain in copy number, red represents a decrease in copy number and grey indicates a copy-neutral event.

Supplemental Figure 3. Immunofluorescence staining of cardiac markers cTnT (green) and α-actinin (red) in single iPSC-CMs derived from control (CON) subjects or BrS patients. DAPI indicates the nuclear staining (blue). All differentiated cardiomyocytes showed robust expression of cardiac markers with no significant differences between control and BrS iPSC-CMs.

Supplemental Figure 4. Comparison of membrane localization of hNa_v1.5 between control, BrS1, BrS2, and BrS2-GE iPSC-CMs. A. Representative immunofluorescence imaging of the cellular distribution of hNa_v1.5 (green) in single control, BrS1, BrS2, and BrS2-GE iPSC-CMs. Ca_v3 indicates the cell membrane (green), and DAPI indicates the nuclear staining (blue). **B.** Bar

graph comparison of $hNa_v1.5$ membrane localization among control, BrS1, BrS2, and BrS2-GE iPSC-CMs. n=15 cells in 2 lines for each group.

Supplemental Figure 5. Comparison of membrane localization of $hNa_v2.1$ between control, BrS1 and BrS2 iPSC-CMs. A. Representative immunofluorescence imaging of the cellular distribution of $hNa_v2.1$ (green) in single control, BrS1, and BrS2 iPSC-CMs. Ca_v3 indicates the cell membrane (red), and DAPI indicates the nuclear staining (blue). **B.** Bar graph comparison of $hNa_v2.1$ membrane localization among control, BrS1 and BrS2. n=15 cells in 2 lines for each group.

Supplemental Figure 6. Identification of differentially expressed genes between control and BrS iPSC-CMs. A. Principal Components Analysis (PCA) of gene expression between control and BrS iPSC-CMs showing clustering of gene expression profile. Green circles represent 2 samples of control iPSC-CMs, and red square represent 2 samples of BrS iPSC-CMs. **B.** Ingenuity Pathway Analysis (IPA) mapping for significantly altered pathways (log (P value) >3) in BrS iPSC-CMs compared to control cells.

Supplemental Figure 7. BrS iPSC-CMs show differential ion-channel gene expression profile. Bar graph comparison of expression of a panel of 3 ion-channel genes between control and BrS iPSC-CMs detected by RNA sequencing and qPCR, respectively. n=1 repeat for RNA sequencing, and n=6-8 repeats for qPCR. **A.** There is reduced expression of *SCN5A* in BrS lines compared to controls, implying there is a quantitative shift toward reduced expression and not only a qualitative defect in *SCN5A* expressed. **B.** There is markedly reduced gene expression of *KCND3* associated with transient outward potassium current (I_{to}) in BrS iPSC-CMs. **C.** There is markedly reduced

gene expression of *KCNJ2* associated with the inward rectifying potassium current (K_{ir}) in BrS iPSC-CMs.

Supplemental Figure 8. A. CRISPR gRNA recognition sites on *SCN5A* exon 23 of BrS2 iPSC line (yellow box). *SCN5A* c.4190del mutation is shown in red character. **B.** Sequence of left and right arms of the targeting vector. In order to make targeting vector, an artificial TTAA site for *piggyBac* excision was created by introducing a silent mutation near the intended modification site (orange character in left arm). The left and right homologous arms were synthesized by using wild-type *SCN5A* sequence approximately 500 bp upstream and downstream from the TTAA site, respectively. Both arms were cloned into a vector carrying *piggyBac* transposon with PGK promoter-puroΔtk selection cassette to make the targeting vector.

Supplemental Figure 9. Overview of site-specific correction of *SCN5A* c.4190delA mutation using CRISPR/Cas9 and *piggyBac* transposon system. CRISPR/Cas9 and targeting vector mediated homology-directed repair and clones with correct homologous recombination were selected by puromycin. Subsequently, *piggyBac* cassette was excised by transient expression of *piggyBac* transposase. Mutation corrected lines were obtained after negative selection using ganciclovir treatment. Genotyping primers were indicated as WF, WR, 5' Fw, 5' Rv, 3' Fw and 3' Rv.

Supplemental Figure 10. PCR analysis showing *piggyBac* integration after puromycin selection; 5' Fw-Rv, PCR to detect left homology arm using 5' Fw and 5' Rv primers; 3' Fw-Rv, PCR to

detect right homology arm using 3' Fw and 5' Rv primers; WT primers, PCR to amplify *SCN5A* exon 23 using WF and WR primers.

Supplemental Figure 11. PCR analysis showing transposon removal after ganciclovir selection.

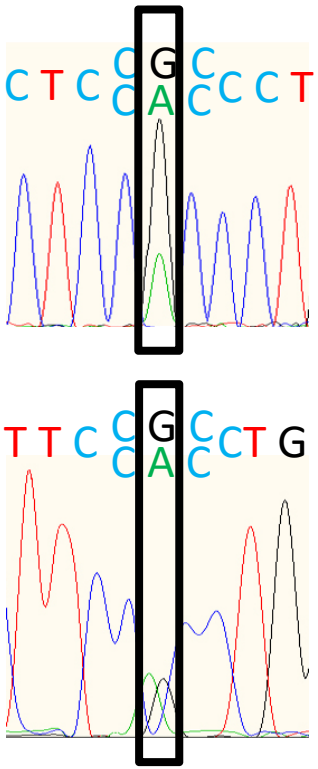
Supplemental Figure 12. A. Precise repair of the deletion mutation site. Mutation corrected iPSC lines showed wild-type sequence (c.4190A, red character) instead of deletion mutation (c.4190delA, red box) and possessed the synonymous mutation acquired from a silent mutation indicated in an orange character. **B.** Comparison of sodium current in BrS2 and BrS2-GE iPSC-CMs. BrS2 iPSC-CMs demonstrate markedly reduced inward sodium current compared to BrS2-GE.

Supplemental References

1. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nature Protocols 2013;8:2281-308.

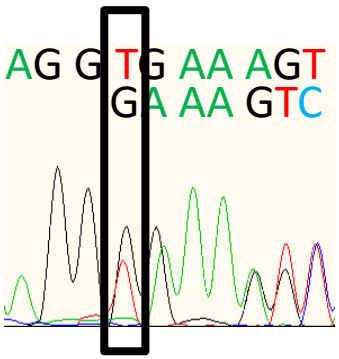
A

BrS1
SCN5A R620H-R811H

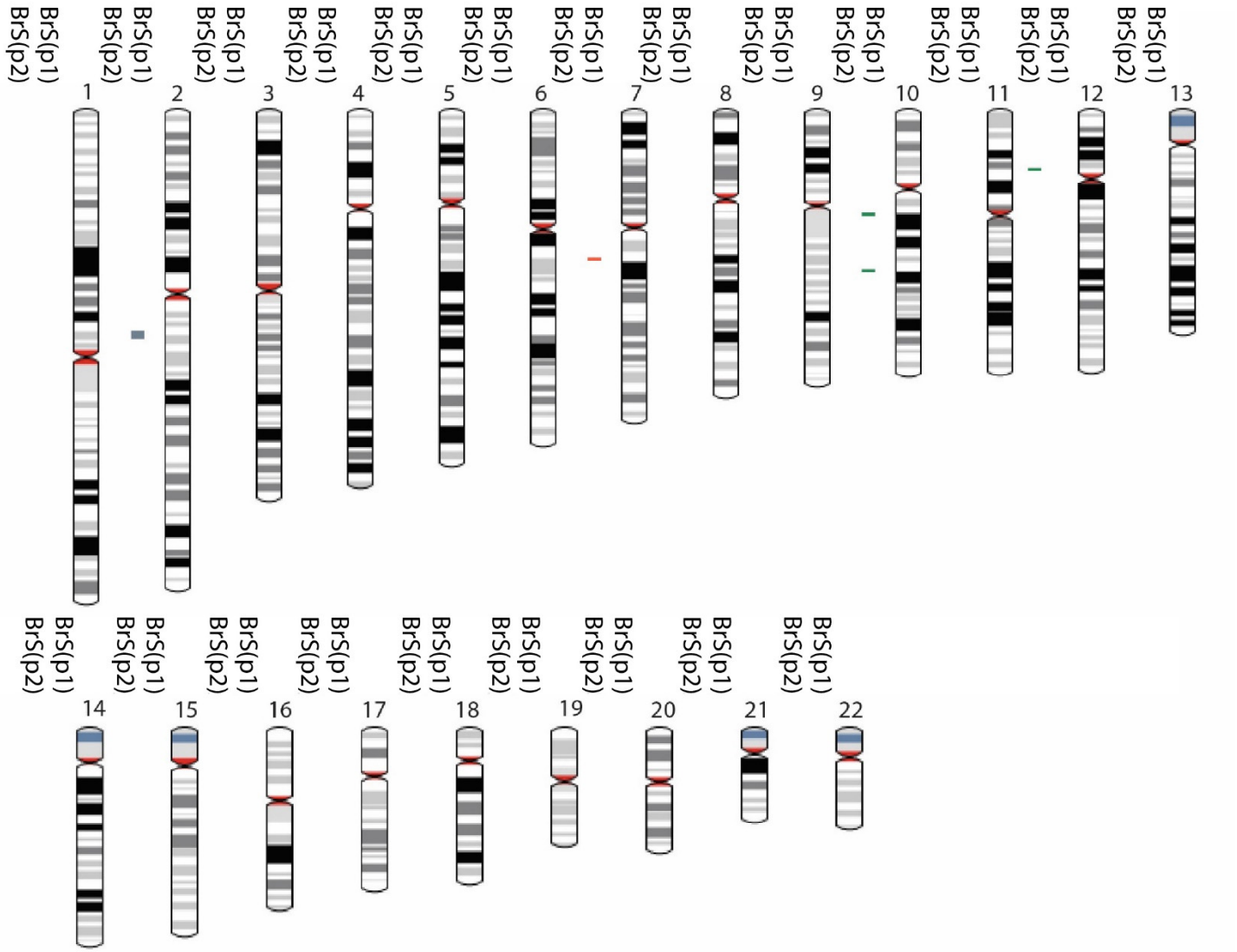


B

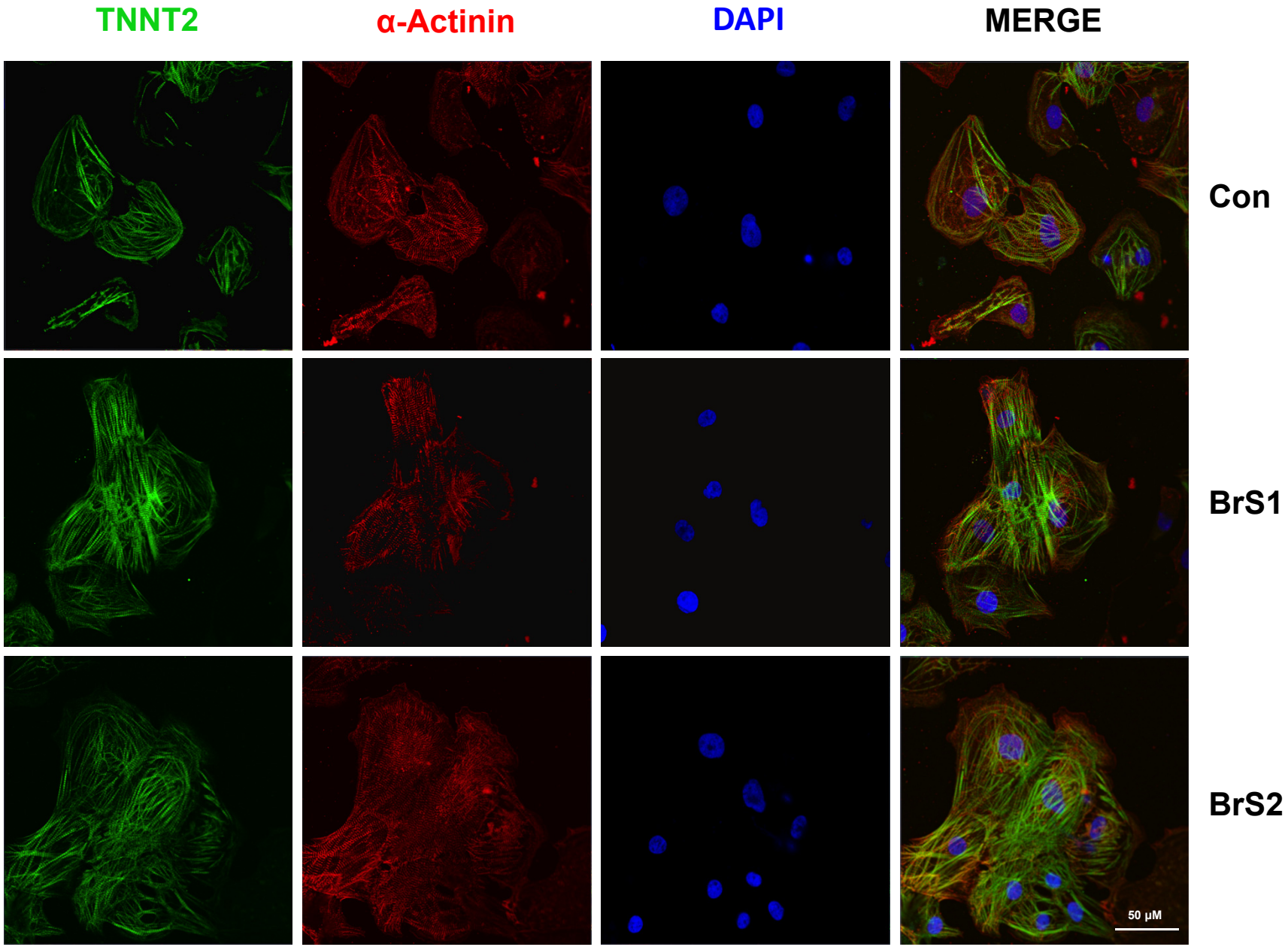
BrS2
SCN5A Δ1397



Supplemental Figure 1



Supplemental Figure 2



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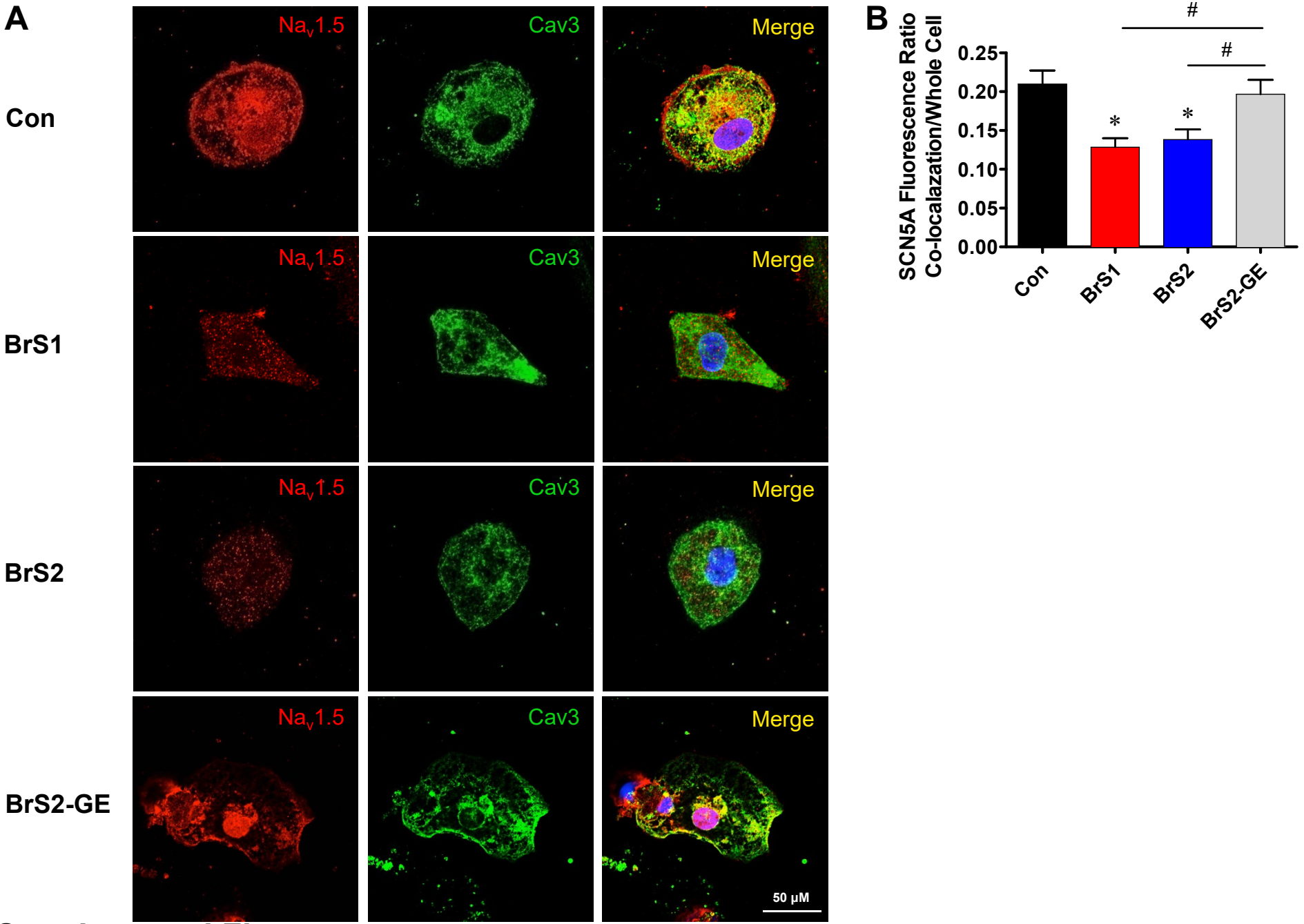

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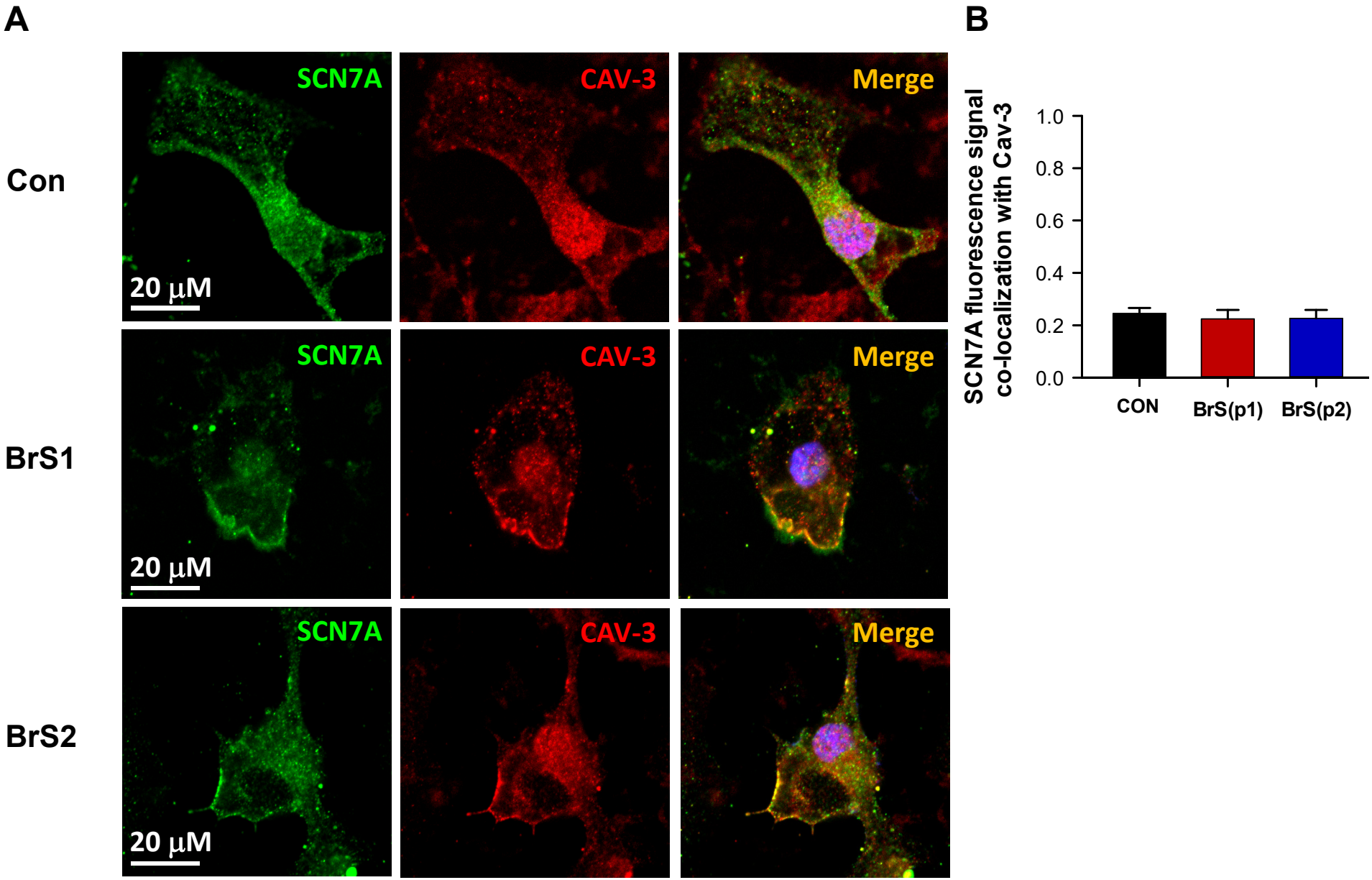
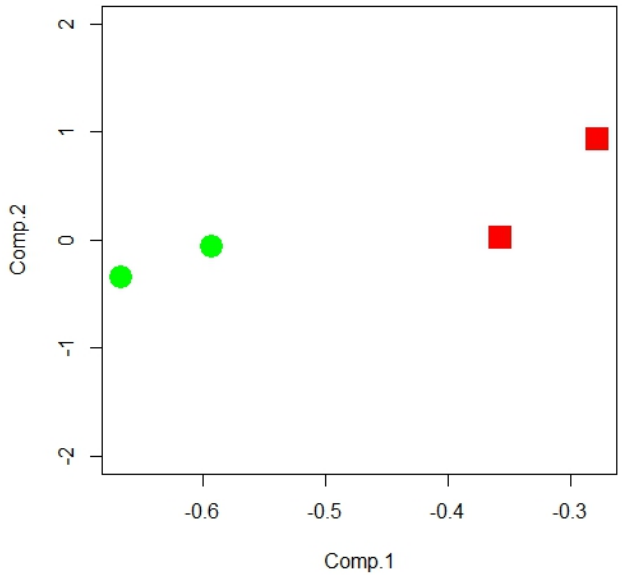
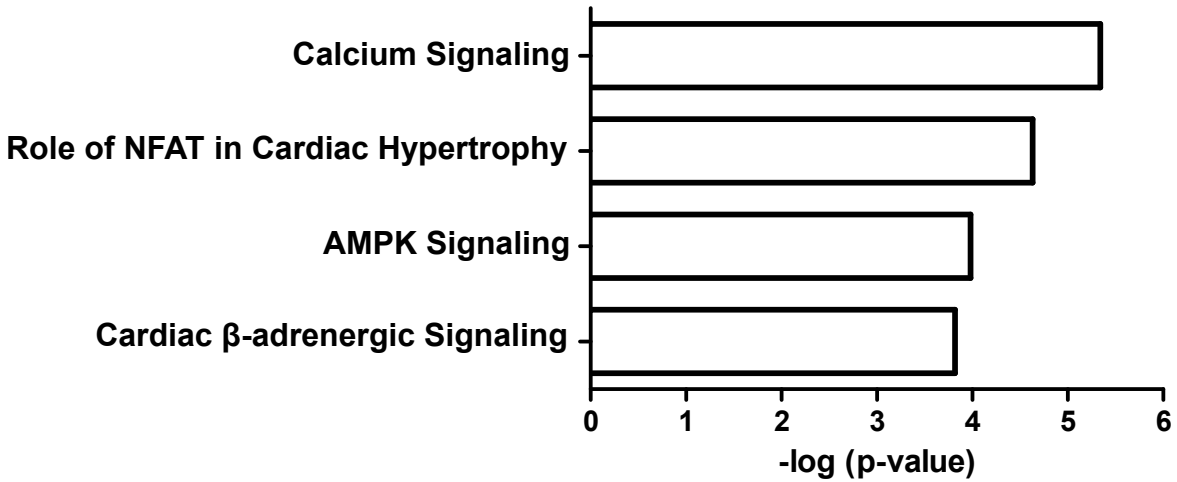


Supplemental Figure 5

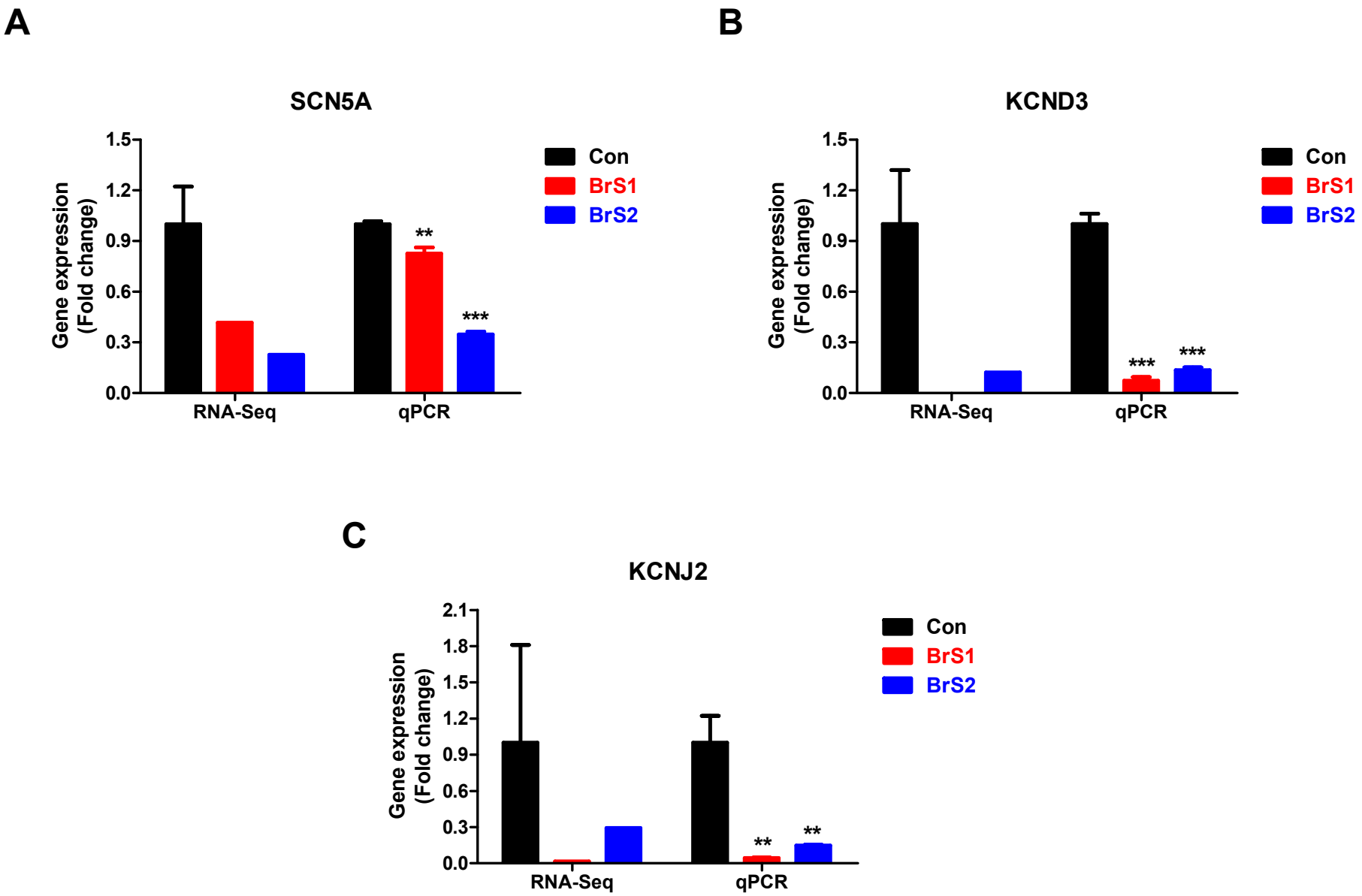
A



B



Supplemental Figure 6



Supplemental Figure 7

A

***SCN5A* exon 23**

MT allele *c.4384del* 5' T G G A C C A _ G G T G A A A G T C A A C T T T G A C A A C G T G G G G G C C G 3'

WT allele 5' T G G A C C A A G G T G A A A G T C A A C T T T G A C A A C G T G G G G G C C G 3'

B

Targeting vector left arm

.....A C T G G A C C A A G G T G A A A G T T A A C C C T A G A A A G A T A A T C A T A T T G T G A

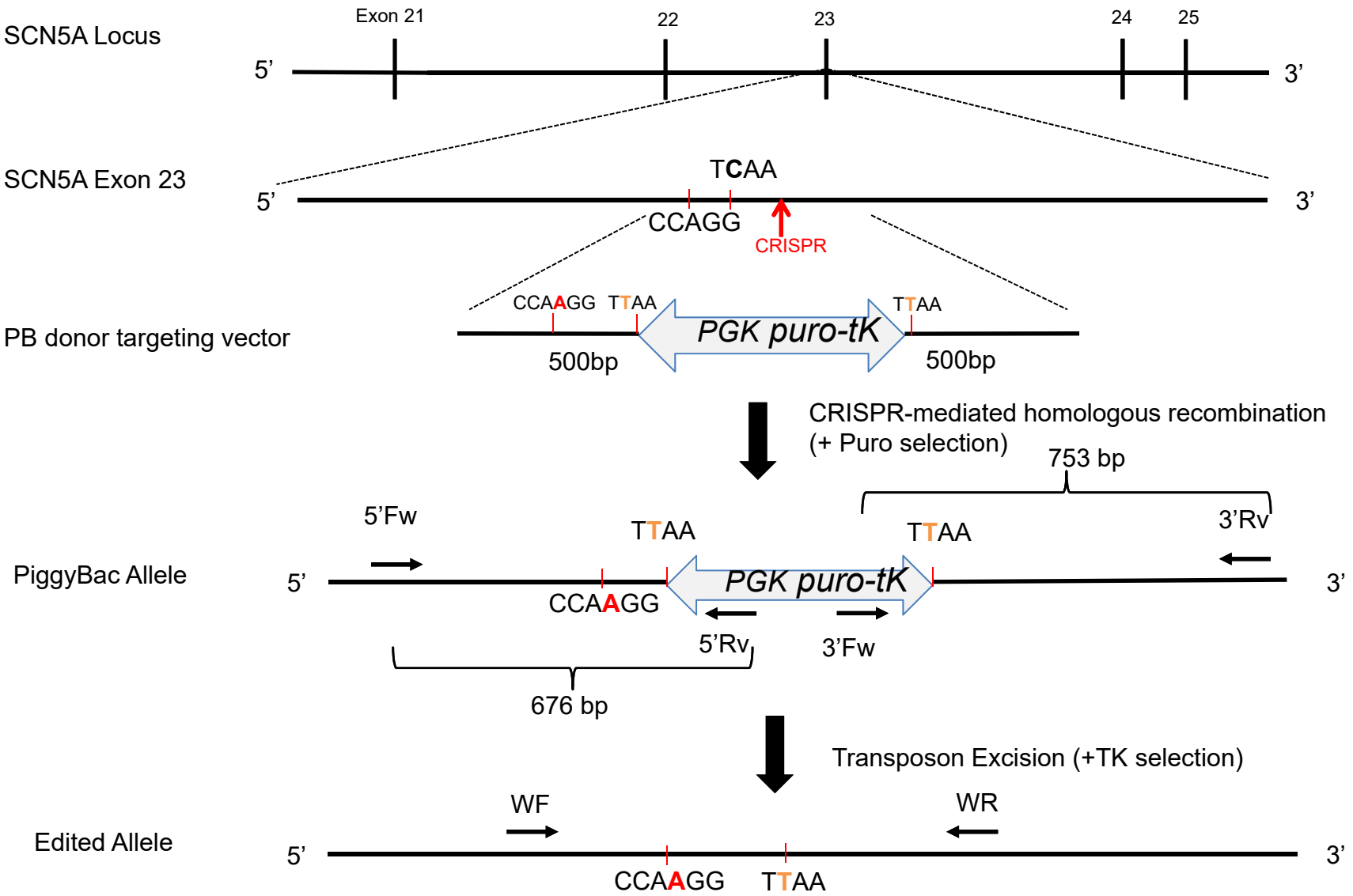
exon 23 | TTAA | *piggyBac-PGK-puroΔtk*

Targeting vector right arm

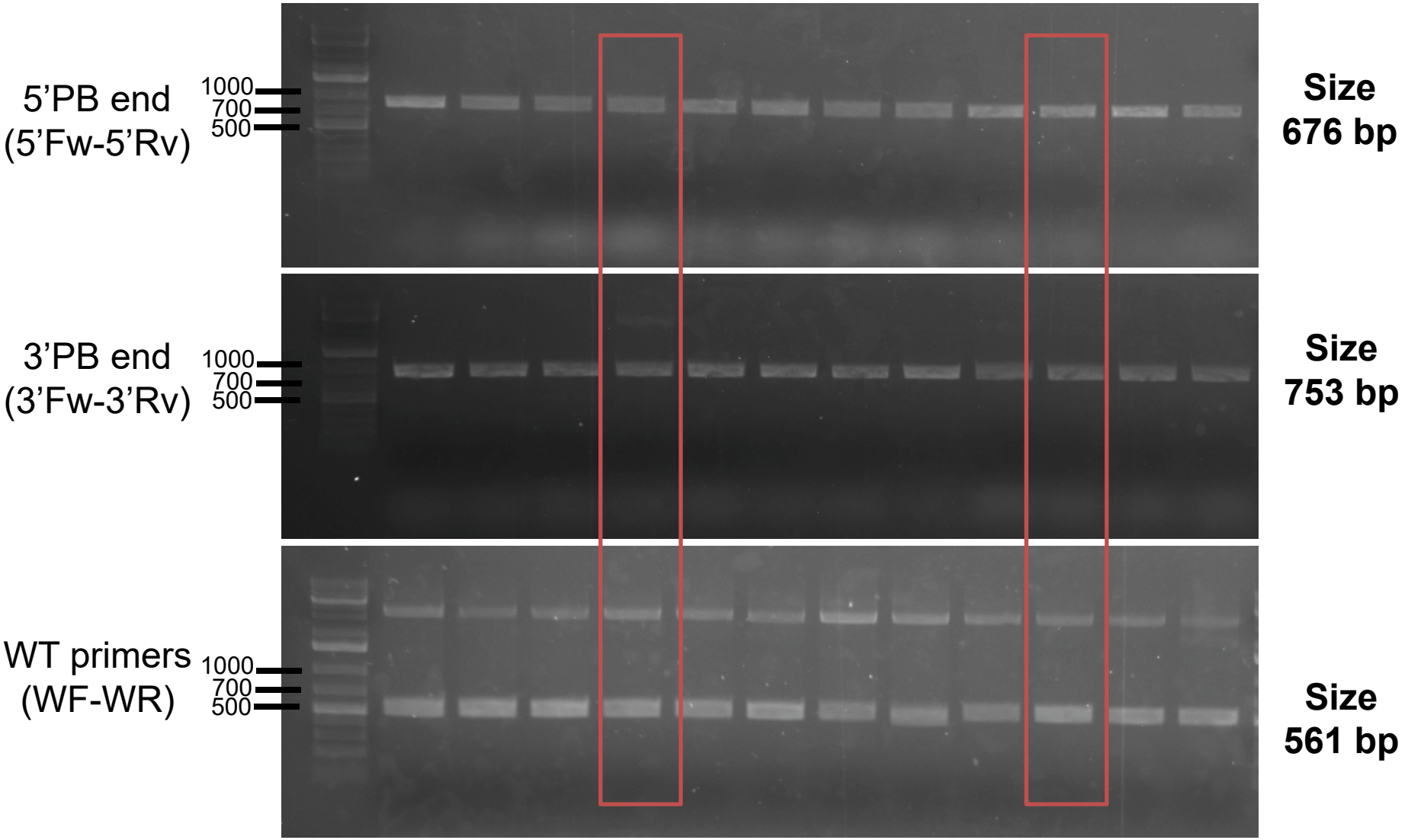
G C G T C A A T T T T A C G C A G A C T A T C T T T C T A G G G T T A A C T T T G A C A A C G.....

piggyBac-PGK-puroΔtk | TTAA | exon 23

Supplemental Figure 8

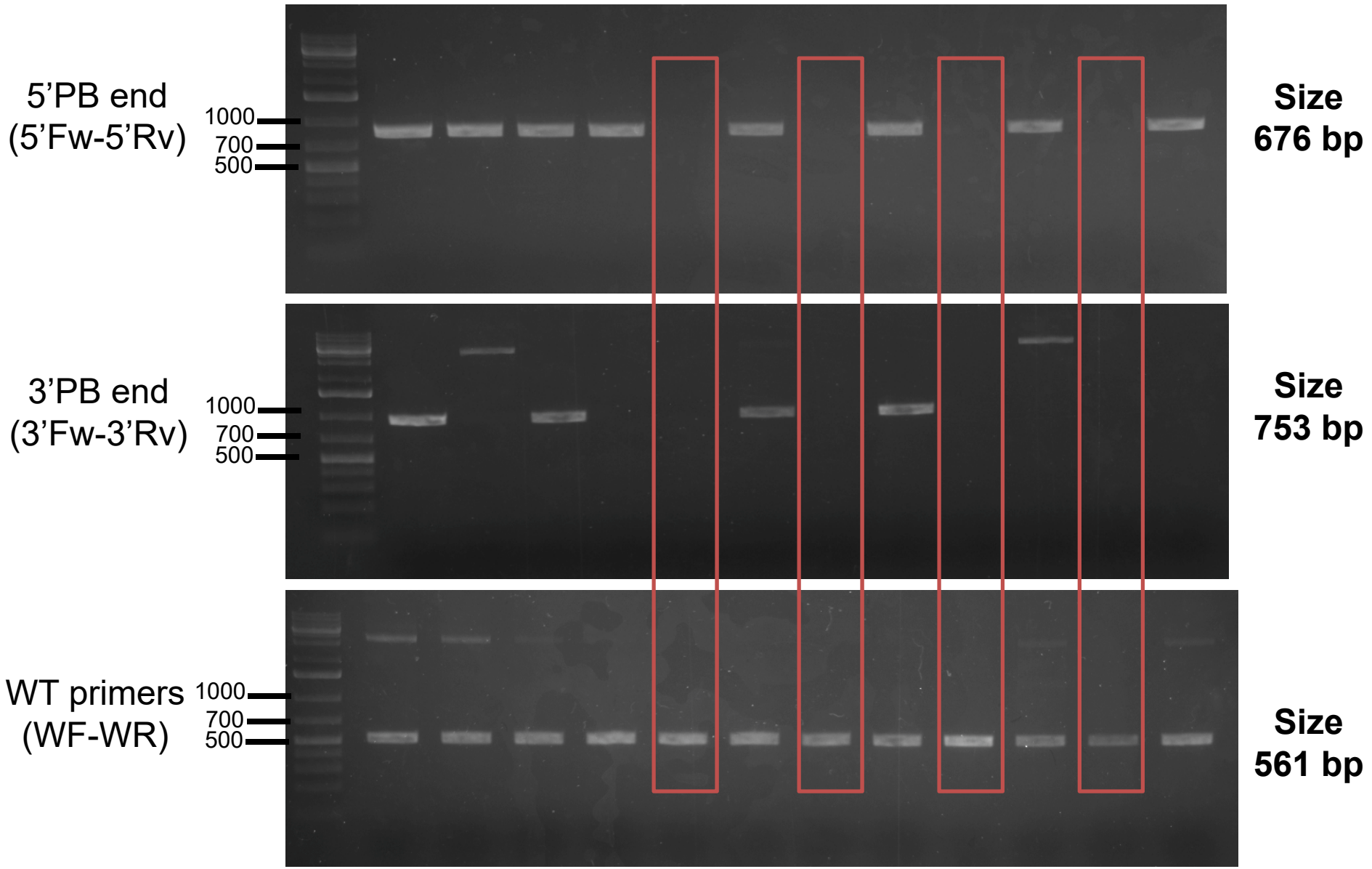


Supplemental Figure 9



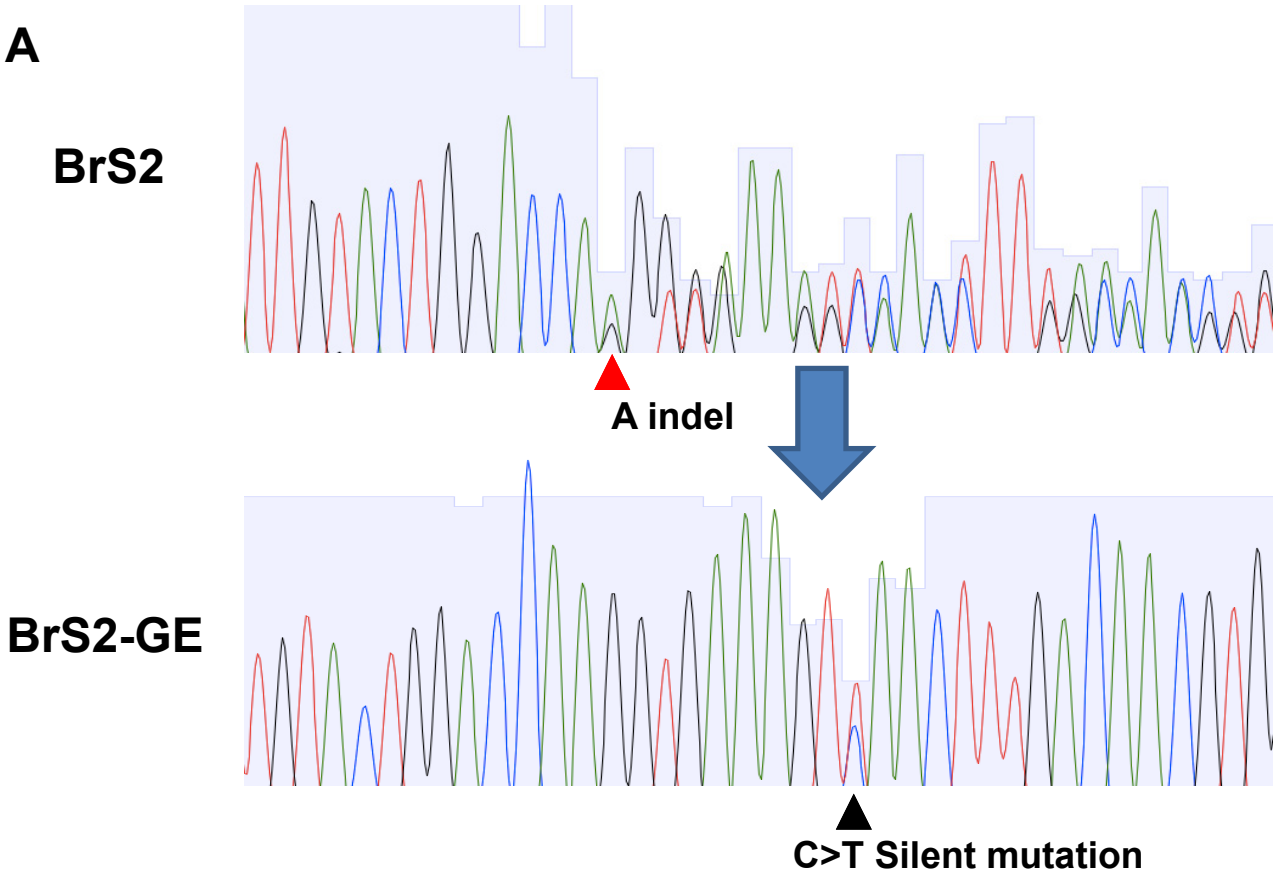
12/12 has piggybac integration, 2 clones are good clones
(unmixed clone and inserted in a correct allele)

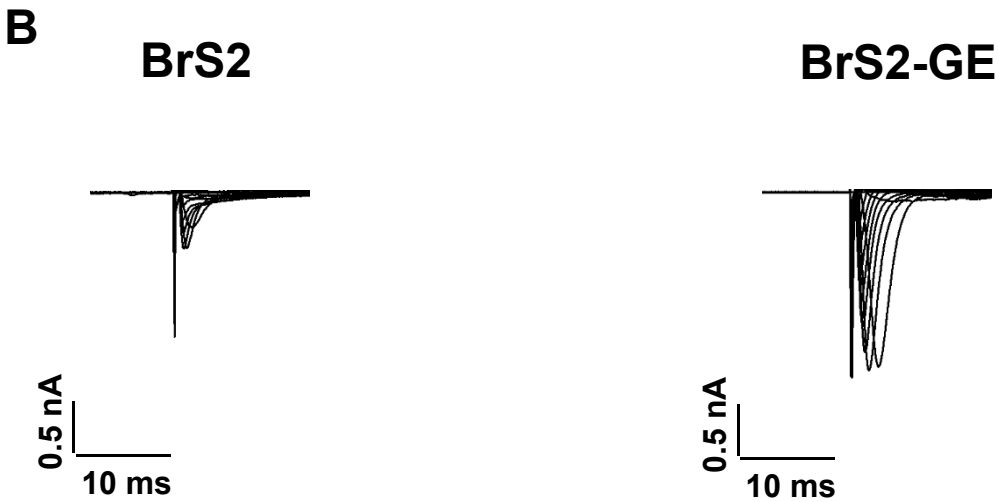
Supplemental Figure 10



4/12 corrected clones (confirmed with sequencing)

Supplemental Figure 11





Supplemental Figure 12

Online Table 1. iPSCs generated from control subjects and BrS patients

	Genetic cause	Number of iPSC lines
Control Subject 1	None	2
Control Subject 2	None	2
BrS Patient 1	*SCN5A* (R620H, R811H)	6
BrS Patient 2	*SCN5A* (Δ1397)	2

Online Table 2. Summary of action potential parameters in control and BrS ventricular-like iPSC-CMs

	Beating rate (bpm)	MDP (mV)	Overshoot (mV)	APA (mV)	APD_{90} (ms)	V_{max} (V / s)	SD of Peak-Peak Intervals (ms)
Control iPSC-CMs	49 ± 4	-58.9 ± 0.8	48.0 ± 0.7	106.4 ± 1.0	443.5 ± 17.3	15.6 ± 0.8	49.4 ± 5.5
BrS1 iPSC-CMs	58 ± 5 (p= 0.101)	-58.1 ± 0.7 (p= 0.4399)	50.1 ± 1.2 (p= 0.110)	107.1 ± 1.6 (p= 0.6756)	459.3 ± 38.2 (p= 0.6743)	11.1 ± 1.0 ($^{**}p$= 0.0015)	380.4 ± 96.2 ($^{***}p$= 0.0007)
BrS2 iPSC-CMs	49 ± 9 (p= 0.974)	-56.5 ± 2.0 (p= 0.3044)	47.8 ± 2.5 (p= 0.5172)	105.0 ± 2.6 (p= 0.6722)	396.2 ± 33.4 (p= 0.4457)	7.2 ± 1.1 ($^{***}p$= 0.0002)	195.5 ± 60.3 ($^{***}p$= 0.0002)
BrS2-GE iPSC-CMs	54 ± 5 (p= 0.412) (p= 0.721)	-64.2 ± 1.0 ($^{***}p$< 0.0001) ($^{\#}p$< 0.0138)	46.1 ± 0.9 (p= 0.0810) (p= 0.0568)	109.3 ± 1.5 (p= 0.0985) (p= 0.3317)	355.8 ± 19.3 ($^{**}p$< 0.0013) (p< 0.4834)	17.4 ± 1.2 (p= 0.245) ($^{\#\#}p$= 0.0013)	48.4 ± 7.4 (p= 0.9161) ($^{\#\#}p$= 0.0037)

Online Table 3. Summary of Na^+ channel currents in control, BrS1, BrS2, and BrS2-GE iPSC-CMs

	Peak Na^+ current density at -20 mV (pA/pF)	Cell capacitance (pF)
Control iPSC-CMs	-122.8 ± 31.3	22.2 ± 4.0
BrS1 iPSC-CMs	-33.7 ± 6.3 ($^{***}p$= 0.0002)	27.0 ± 1.7 (p= 0.2056)
BrS2 iPSC-CMs	-36.8 ± 8.6 ($^{**}p$= 0.0099)	24.9 ± 2.2 (p= 0.5322)
BrS2-GE iPSC-CMs	-63.0 ± 4.3 (p= 0.1295) ($^{\#}p$= 0.0414)	21.9 ± 2.6 (p= 0.9609) (p= 0.3928)

Online Table 4. Summary of Ca^{2+} transient parameters in control and BrS iPSC-CMs

	Transient amplitude (ΔF/F0)	Time to peak (ms)	Transient duration 50 (ms)	Maximal rising rate (ΔF/F0/s)	Decay tau (ms)	Beating rate bpm	SD of beat intervals (ms)
Control iPSC-CMs	6.0 ± 0.3	245.0 ± 9.3	471.3 ± 12.6	92.0 ± 6.6	301.6 ± 15.8	27.1 ± 1.6	108.3 ± 20.4
BrS1 iPSC-CMs	2.6 ± 0.2 ($^{***}p< 0.0001$)	871.1 ± 83.9 ($^{***}p< 0.0001$)	1362.7 ± 124.6 ($^{***}p< 0.0001$)	10.3 ± 0.9 ($^{***}p< 0.0001$)	891.7 ± 104.6 ($^{***}p< 0.0001$)	10.3 ± 0.8 ($^{***}p< 0.0001$)	1894.7 ± 436.5 ($^{***}p< 0.0001$)
BrS2 iPSC-CMs	2.3 ± 0.2 ($^{***}p< 0.0001$)	339.5 ± 31.8 ($^{*}p= 0.037$)	541.5 ± 60.2 ($p= 0.479$)	45.2 ± 4.7 ($^{***}p< 0.0001$)	296.1 ± 31.6 ($p= 0.963$))	38.7 ± 2.6 ($^{***}p= 0.0008$)	313.3 ± 59.1 ($^{***}p= 0.0008$)
BrS2-GE iPSC-CMs	4.7 ± 0.1 ($^{***}p< 0.0001$) ($^{\#\#\#}p< 0.0001$)	396.6 ± 27.6 ($^{***}p< 0.0001$) ($^{\#}p= 0.0446$)	589.3 ± 27.4 ($^{***}p= 0.0007$) ($p= 0.198$)	72.7 ± 5.0 ($^{*}p= 0.0179$) ($^{\#\#\#}p< 0.0001$)	247.2 ± 13.8 ($^{*}p= 0.0112$) ($p= 0.0814$)	28.8 ± 1.1 ($p= 0.353$) ($^{\#\#}p= 0.0012$)	190.7 ± 24.6 ($^{*}p= 0.0145$) ($^{\#\#}p= 0.003$)

Online Table 5. Panel of cardiac ion channel-related transcripts used for quantitative RT- PCR

	Gene	**ASSAY ID**	**Gene description**
1	18s	Hs99999901_s1	18s rRNA
2	*SCN5A*	Hs00165693_m1	Cardiac sodium channel Nav1.5
3	*KCND3*	Hs00542597_m1	Kv4.3 (Cardiac transient outward potassium current, I_{to})
4	*KCNJ2*	Hs00265315_m1	Inward rectifier potassium channel, Kir2.1

Online Table 6. Sequences of primers

Loci	Sense (5’ to 3’)	Antisense (5’ to 3’)	Product Size
2053G>A (WF, WR)	CAGTGGCACAAAAGACAGGC	CAGCACACAGTAGGTGCTCA	561
2626G>A	TCACAGGGATTTTCACAGCA	GGGATGACCAAGTGATGACC	258
4190delA	GCAGCCAGGGAGTTCATTCT	TTTGGGCACTGTGATCCTCC	517
5’Fw, 5’Rv	TAGGAAACCCAGTCTTGGGGAC	GACCGATAAAACACATGCGTCA	676
3’Fw, 3’Rv	GTCCTAAATGCACAGCGACG	CTGGGACTACGGGTGCCC	753