Supplemental Material

for

Epilepsy and neurobehavioral abnormalities in mice with
a dominant-negative KCNB1 pathogenic variant

Nicole A. Hawkins1*, Sunita N. Misra2,3*, Manuel Jurado1, Seok Kyu Kang1,
Nicholas C. Vierra4,5, Kimberly Nguyen4, Lisa Wren1,
Alfred L. George Jr.1, James S. Trimmer4,5, Jennifer A. Kearney1

Departments of Pharmacology1 and Pediatrics2,
Northwestern University Feinberg School of Medicine;
3Ann & Robert H. Lurie Children’s Hospital of Chicago
Chicago, IL, USA 60611
Departments of Neurobiology, Physiology and Behavior4, and Physiology and Membrane
Biology5, University of California, Davis, CA 95616

*Denotes equal contribution
**List of Supplemental Materials**

1. **Supplemental Materials and Methods.**

2. **Supplemental Table S1.** Neurological exam scores from wild-type (WT), *Kcnb1*R/+ and *Kcnb1*R/R mice.

3. **Supplemental Table S2.** Echocardiogram data from wild-type (WT), *Kcnb1*R/+ and *Kcnb1*R/R mice.

4. **Supplemental Figure S1.** *Kcnb1*R/R mice have prolonged heart-rate corrected QT interval compared to wild-type (WT) by electrocardiography.

5. **Supplemental Figure S2.** *Kcnb1*R/+ and *Kcnb1*R/R mice do not differ from wild-type (WT) by echocardiography.

6. **Supplemental Figure S3.** Immunolabeling of cultured hippocampal neurons from *Kcnb1*G379R mice.

7. **Supplemental Figure S4.** Immunolabeling in *Kcnb1*G379R brain sections using antibodies to various epitopes.

8. **Supplemental Video S1.** Handling-induced generalized tonic-clonic seizure in a *Kcnb1*R/R mouse at ~3 months of age. <Suppl Video S1 Kcnb1-RR Handling.mp4>

9. **Supplemental Video S2.** Video-EEG of a spontaneous generalized tonic-clonic seizure in a *Kcnb1*R/R mouse at ~2 months of age. <Suppl Video S2 Kcnb1-RR vEEG.mp4>

10. **Supplemental Video S2a.** Enhanced video from Supplemental Video S2 showing behavioral correlates of the generalized tonic-clonic seizure. <Suppl Video S2a Kcnb1-RR vEEG-VIDEO.mp4>

11. **Supplemental Video S3.** Video-EEG of a brief run of rhythmic slow spike and wave complexes (1-2 Hz) during wakefulness in a *Kcnb1*R/R at ~2 months of age. <Suppl Video S3 Kcnb1-RR WakeRun.mp4>

12. **Supplemental Video S4.** Video-EEG of a ~15-minute run of rhythmic slow spike and wave complexes (1-2 Hz) during sleep in a *Kcnb1*R/R at ~2 months of age. <Suppl Video S4 Kcnb1-RR SleepRun.mp4>

13. **Supplemental Video S5.** Video-EEG of a paroxysmal event associated with immobility in a *Kcnb1*R/R mouse at ~2 months of age. <Suppl Video S5 Kcnb1-RR Event.mp4>
Supplemental Materials and Methods

*HEK293T cell culture, immunocytochemistry, and epifluorescence and TIRF imaging.*

HEK293T cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% Fetal Clone III (HyClone Cat# SH30109.03), 1% penicillin/streptomycin, and 1x GlutaMAX (ThermoFisher Cat# 35050061) in a humidified incubator at 37 °C and 5% CO2. Plasmid constructs used include wild-type rat Kv2.1 (rKv2.1) in pRBG4 [1], rat Kv2.1 S586A (rKv2.1 S586A) in pCGN [2], HA-tagged wild-type human Kv2.1 (hKv2.1) (Kang et al, 2019; modified from Addgene #131707), and HA-tagged human Kv2.1 with the G379R mutation (hKv2.1 G379R)(Kang et al, 2019; modified from Addgene #131707). For transfections, cells were split to 15% confluence on number 1.5 glass coverslips coated with poly-L lysine, then transiently transfected using Lipofectamine 2000 following the manufacturer’s protocol within 18 hours of plating. Cells were transiently transfected in DMEM without supplements, then returned to regular growth media 4 hours after transfection. Cells were utilized 40-48 hours post-transfection. For experiments involving immunocytochemistry, fixation was performed as previously described [3, 4]. Briefly, HEK293T cells were fixed in 3.2% formaldehyde (freshly prepared from paraformaldehyde, Sigma Cat# 158127) and 0.1% glutaraldehyde (Ted Pella, Inc., Cat# 18426) for 30 minutes and room temperature, washed 3, 5 minutes in PBS and quenched with 1% sodium borohydride in PBS for 15 minutes at room temperature. Cells were blocked and permeabilized in 4% non-fat milk powder in TBS containing 0.1% Triton-X 100. Primary antibody incubation was performed in blocking solution for 1 hour at room temperature. Primary antibodies used (see Table 2 for details) were mouse monoclonal anti-HA epitope tag antibody 12CA5 (RRID:AB_ 2532070, pure, 5 μg/mL) to detect exogenous hKv2.1, rKv2.1 and hKv2.1 G379R, and mouse monoclonal VAPA antibody N479/24 (RRID:AB_ 2722709, tissue culture supernatant, 1:5) to detect endogenous VAPA [4]. Following primary
antibody incubation and 3, 5 minute washes in blocking solution at room temperature, coverslips were immunolabeled in blocking solution with mouse IgG subclass-specific Alexa Fluor-conjugated goat anti-mouse IgG subclass-specific [5] secondary antibodies (all secondary antibodies from ThermoFisher) at a 1:1500 dilution, and Hoechst 33258 (ThermoFisher Cat# H1399) at 200 ng/mL for one hour, washed 3, 5 min in PBS, and mounted in phosphate buffered saline onto glass depression slides.

Epifluorescence and TIRF imaging of fixed cells and image analysis was performed essentially as described [4, 6]. Images were obtained with an Andor iXon EMCCD camera installed on a TIRF/widefield equipped Nikon Eclipse Ti microscope using a Nikon LUA4 laser launch with 405, 488, 561, and 647 nm lasers and a 100x PlanApo TIRF/1.49 NA objective run with NIS Elements software (Nikon). Images were collected within NIS Elements as ND2 images.

Colocalization analyses were performed within Nikon NIS Elements using ND2 files. An ROI was drawn within a cell and Pearson's correlation coefficient (PCC) was collected, as previously described [4]. Group PCC values were compared using one-way ANOVA with Tukey’s post-hoc comparisons (GraphPad Prism). Measurements of structure sizes were quantified automatically within Fiji previously described [3]. ND2 files collected in TIRF were imported to Fiji, background subtracted, converted into an 8-bit image, and automatically converted into a binary mask using auto local thresholding. An ROI with identical dimensions was drawn within each cell analyzed. The number of individual ER-PM junctions, average ER-PM junction size, and percentage PM occupancy were quantified automatically using the “analyze particles” function in Fiji, as previously described [4]. Signals smaller than 0.04
μm² were excluded from this analysis. Punta size values were compared using one-way ANOVA with Tukey’s post-hoc comparisons (GraphPad Prism).

**Hippocampal Neuron culture, immunocytochemistry, and confocal imaging.** Hippocampal neuronal cultures were derived from postnatal day 0-1 pups that were rapidly genotyped prior to euthanasia. DNA isolated was from tail biopsies by the HotSHOT method [7], PCR amplified, and product was digested with BsiEI resulting in 67 and 137 bp products for WT and a 204 bp product for the G379R mutant allele (New England Biolabs, Ipswich, MA). Cells were plated on PDL-coated coverslips (GG-12-15-PDL; Neuvitro, Vancouver, WA) and grown in Neurobasal medium, supplemented with B-27 and CultureOne (17504044 and A3320201; Gibco, Waltham, MA); half-media changes were performed once a week. Neurons were fixed in Cytofix/Cytoperm (554714; BD biosciences, San Jose CA) containing 4% sucrose for 15 minutes at room temperature, washed 4 times for 5 minutes in dPBS with 0.1% Tween-20 (PBS-T). Cells were blocked using 10% normal goat serum (NGS) in PBS-T. Primary antibody incubation was performed in PBS-T containing 5% NGS overnight at room temperature (mouse-αKv2.1; K89/34 at 1:1000 and rabbit-αMAP2; ab32454 at 1:1000; see Table 2 for additional detail). Coverslips were then incubated in secondary antibodies (A-21121- Goat-αMouse-Alexa 488 at 1:2000; and A-21245- Goat-αRabbit-Alexa 647; at 1:2000; Thermo-Fisher) diluted in PBS-T + 10% NGS, followed by DAPI staining for 5 minutes.

Images were acquired with a Nikon W1 spinning disk confocal and Hamamatsu camera in the Center for Advanced Imaging at Northwestern University. Images collected as ND2 format were processed and analyzed using NIS Elements software (Nikon). Images were identically processed in Photoshop to maintain consistency between samples.
**Immunohistochemistry.** Animals were deeply anesthetized with pentobarbital and transcardially perfused with 4% formaldehyde prepared from paraformaldehyde, in 0.1 M sodium phosphate buffer pH 7.4 (0.1 M PB). Sagittal brain sections (30 µm thick) were prepared and immunolabeled using free-floating methods as previously described [8-10]. Free floating sections were blocked and permeabilized with 10% goat serum, 0.3% Triton X-100 in 0.1 M PB for 1 hour at room temperature (RT) and incubated overnight at 4°C with primary antibodies (Table 2). Following four washes in vehicle for 5 min each at RT, sections were exposed to species- and/or mouse IgG subclass-specific Alexa-conjugated fluorescent secondary antibodies (Invitrogen) and Hoechst 33258 DNA stain (200 ng/mL; ThermoFisher Cat# H21491) diluted in vehicle for 1 hour at RT. After 2, x5 min washes in 0.1M PB and a 5 min wash in 0.05M PB, sections were mounted and air dried onto gelatin-coated microscope slides, treated with 0.05% Sudan Black (EM Sciences) in 70% ethanol for 2 min [11], extensively washed in water, and mounted with Prolong Gold (ThermoFisher Cat # P36930). Images were taken using the same exposure time to compare the signal intensity directly, using an AxioCam HRm high-resolution CCD camera installed on an Axioskop M2 or Axio Observer Z1 microscope with 63x, 1.3 numerical aperture (NA) lens or 20x, 0.8 NA lens, and an ApoTome coupled to Axiovision software, version 4.8.2.0 (Zeiss, Oberkochen, Germany). Images were identically processed in Photoshop to maintain consistency between samples. Labeling intensity within *stratum pyramidale* of hippocampal CA1 was measured using a rectangular region of interest (ROI) of 39 µm x 164 µm. To maintain consistency between samples, ROIs were obtained from a region within CA1 near the border of CA1 and CA2. Data points reflect the mean pixel intensity values of this ~6400 µm² ROI. Values from
multiple immunolabels and of Hoechst dye were simultaneously measured from the same ROI. Background levels for individual labels were measured from no primary controls and mathematically subtracted from ROI values. Data is represented as mean ± SEM. Group values were compared using one-way ANOVA with Tukey’s post-hoc comparisons (GraphPad Prism).

*Electrocardiography (ECG).* At 8-10 weeks of age, WT, *Kcnb1*R/+ and *Kcnb1*R/R mice were anesthetized with isoflurane and surface ECGs were recorded using a digital Dual Bio Amplifier acquisition system and Powerlab software (ADInstruments, Colorado Springs, CO). ECG was recorded using electrodes attached to the two forepaws and the right hindpaw. Baseline ECG was recorded for at least 4 minutes followed by intraperitoneal administration of isoproterenol (1.5 mg/kg) and an additional 10 minutes of recording. Records were analyzed offline using the ECG Module for LabChart software (ADInstruments). Measurement of QT interval was performed on three sequential 10 second intervals at baseline and 6 minutes after the administration of isoproterenol. QT intervals were corrected for heart rate (QTC) using Bazett’s formula. All genotype groups showed ~30% increase in QTC following isoproterenol, presumably due to the elapsed time under isoflurane anesthesia, which has been shown to prolong QT interval [12, 13]. Groups were compared using a two-way repeated measures ANOVA test with Sidak’s multiple comparisons (GraphPad Prism).

*Echocardiography.* Male and female WT, *Kcnb1*R/+ and *Kcnb1*R/R mice were imaged between P74 and P98. Mice were anesthetized with 2-4% isoflurane while on a warming platform with a rectal probe to ensure body temperature was maintained. Imaging duration was 30-40 minutes
per mouse. Echocardiography was performed using Visualsonics Vevo770 (FujiFilm, Toronto, Canada). The following parameters were measured: left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), percent fractional shortening (%FS), calculated as \((\text{LVEDD}-\text{LVESD})/\text{LVEDD} \times 100\), septal thickness (SEPth), posterior wall thickness (PWth), heart rate corrected mean velocity of circumferential shortening (mVcfc), calculated as \(%FS / \text{ejection time} \times \text{the square root of the R-R interval}\). Estimated echocardiographic LV mass (in mg) was calculated as \((\text{LVEDD} + \text{SEPth} + \text{PWth})^3 - \text{LVEDD}^3) \times 1.055\), where 1.055 (mg/mm3) is the density of myocardium. Statistical comparison between groups was made using ANOVA with Tukey’s post-hoc tests.
Supplemental Table S1. Neurological exam scores from wild-type (WT), *Kcnb1*R/+ and *Kcnb1*R/R mice. Individual parameters were compared to WT using multiplicity-adjusted T-tests with a false-discovery rate of 1%. Discoveries are indicated in bold.

| Parameter          | WT (n=32) | Kcnb1*R/+ (n=31) | Kcnb1*R/R (n=23) |
|--------------------|-----------|-----------------|-----------------|
|                    | Mean      | SD              | Mean            | P-value* vs WT | Mean  | SD | P-value* vs WT |
| Transfer Behavior  | 3.8       | 0.5             | 4               | 0.0488        | 4.3    | 0.5 | **0.0002**     |
| Spontaneous Activity| 2.4       | 0.6             | 2.9             | **<0.0001**   | 3.8    | 0.5 | **<0.0001**    |
| Tremor             | 0.1       | 0.4             | 0.4             | 0.0188        | 0.3    | 0.5 | 0.0665         |
| Gait               | 0.0       | 0.0             | 0               | 0.7722        | 0.4    | 0.5 | **0.0004**     |
| Pelvic Elevation   | 2.0       | 0.0             | 2               | 1.0000        | 2      | 0   | 1.0000         |
| Tail Elevation     | 1.0       | 0.0             | 1               | 1.0000        | 1      | 0   | 1.0000         |
| Palpebral Closure  | 0.0       | 0.0             | 0               | 1.0000        | 0      | 0.2 | 0.7200         |
| Piloerection       | 0.0       | 0.0             | 0               | 1.0000        | 0.1    | 0.3 | 0.4735         |
| Escape Behavior    | 0.7       | 0.7             | 1.3             | **<0.0001**   | 1.8    | 0.6 | **<0.0001**    |
| Jumping            | 0.3       | 0.5             | 0.6             | 0.0071        | 0.5    | 0.5 | 0.1720         |
| Air Puff           | 2.4       | 0.9             | 2.7             | 0.0068        | 3.5    | 0.6 | **<0.0001**    |
| Trunk Curl         | 0.8       | 0.4             | 0.4             | **0.0012**    | 0      | 0   | **<0.0001**    |
| Limb Grasping      | 0.3       | 0.5             | 0.2             | 0.2819        | 0.1    | 0.3 | 0.1095         |
| Preyer Reflex      | 0.7       | 0.5             | 0.9             | 0.0981        | 1.3    | 0.8 | **<0.0001**    |
| Salivation         | 0.0       | 0.0             | 0               | 1.0000        | 0      | 0   | 1.0000         |
| Provoked Biting    | 0.6       | 0.5             | 0.8             | 0.0577        | 0.7    | 0.5 | 0.4598         |

*False-discovery rate adjusted p-values (q=0.01).
**Supplemental Table S2.** Echocardiogram data from wild-type (WT), *Kcnb1R/+* and *Kcnb1R/R* mice.

### Ejection Fraction

| Genotype | Sex | HR  | Temp | Diastolic Area mm² | Diastolic Volume ul | Systolic Area mm² | Systolic Volume ul | EF % |
|----------|-----|-----|------|-------------------|-------------------|------------------|-------------------|------|
| WT F     | 521 | 39.1| 21.05| 57.22             |                   | 10.8             | 21.16             | 63.02|
| WT F     | 536 | 37.7| 17.01| 41.05             |                   | 8.83             | 12.55             | 69.43|
| WT F     | 521 | 39.2| 19.51| 50.17             |                   | 10.75            | 17.58             | 64.96|
| WT M     | 523 | 38.3| 21.16| 61.99             |                   | 11.91            | 22.44             | 63.80|
| WT M     | 537 | 36.7| 14.74| 34.49             |                   | 7.65             | 11.45             | 66.80|
| *Kcnb1R/+* F | 483 | 38.3| 18.22| 45.64             |                   | 10.06            | 16.96             | 62.84|
| *Kcnb1R/+* F | 531 | 37.9| 16.09| 40.45             |                   | 8.14             | 12.03             | 70.26|
| *Kcnb1R/+* M | 527 | 38.4| 17.2 | 40.11             |                   | 10.04            | 16.69             | 58.39|
| *Kcnb1R/+* M | 518 | 38  | 15.61| 40.72             |                   | 7.24             | 11.89             | 70.80|
| *Kcnb1R/+* M | 541 | 38.1| 15.36| 31.66             |                   | 8.33             | 11.98             | 62.16|
| *Kcnb1R/+* F | 465 | 37.7| 13.91| 31.62             |                   | 6.2              | 9.76              | 69.13|
| *Kcnb1R/R* M | 512 | 38  | 20.8 | 56                |                   | 9.61             | 17.3              | 69.11|
| *Kcnb1R/R* M | 517 | 37.5| 21.22| 57.91             |                   | 9.74             | 16.43             | 71.63|
| *Kcnb1R/R* M | 553 | 38.5| 20.89| 56.78             |                   | 10.52            | 18.35             | 67.68|
| *Kcnb1R/R* M | 552 | 38.3| 16.02| 38.61             |                   | 9.24             | 16.39             | 57.55|

### Fractional Shortening

| Genotype | Sex | HR  | Temp | Diastolic DD | Systolic DD | SD | FS % |
|----------|-----|-----|------|--------------|-------------|----|------|
| WT F     | 516 | 38.8| 3.79 | 2.35         |             | 37.99|     |
| WT F     | 532 | 37.1| 3.27 | 1.8          |             | 44.95|     |
| WT F     | 521 | 38  | 3.41 | 1.88         |             | 44.87|     |
| WT M     | 523 | 38.3| 4.08 | 2.57         |             | 37.01|     |
| WT M     | 542 | 37.6| 3.56 | 2.01         |             | 43.54|     |
| *Kcnb1R/+* F | n/a | n/a| 3.57 | 2.31         |             | 35.29|     |
| *Kcnb1R/+* F | 521 | 37.3| 3.52 | 1.95         |             | 44.60|     |
| *Kcnb1R/+* M | 532 | 38.1| 3.65 | 2.51         |             | 31.23|     |
| *Kcnb1R/+* M | 521 | 38  | 3.49 | 1.92         |             | 44.99|     |
| *Kcnb1R/+* M | 538 | 38.1| 3.35 | 2.3          |             | 31.34|     |
| *Kcnb1R/+* F | 497 | 37  | 2.92 | 1.82         |             | 37.67|     |
| *Kcnb1R/R* M | 516 | 38.2| 3.85 | 2.61         |             | 32.21|     |
| *Kcnb1R/R* M | 516 | 37.5| 3.87 | 2.48         |             | 35.92|     |
| *Kcnb1R/R* M | 554 | 38.5| 3.64 | 2.42         |             | 33.52|     |
| *Kcnb1R/R* M | 545 | 38.5| 3.56 | 1.89         |             | 46.91|     |
Supplemental Figure S1. Prolonged heart-rate corrected QT (QTC) interval in Kcnb1R/R mice both at baseline and following isoproterenol challenge. We performed an initial screen of cardiac function in Kcnb1G379R mice using electrocardiography (ECG). Surface electrocardiograms (ECG) were recorded in 8-10 week old WT, Kcnb1R/+ and Kcnb1R/R mice under anesthesia. Baseline recording was acquired for at least 4 minutes prior to adrenergic challenge with isoproterenol (1.5 mg/kg, IP) A) Representative recording of a single sinus beat at baseline in WT, Kcnb1R/+ and Kcnb1R/R mice. B) Representative ECG traces from each genotype at baseline (left) and following isoproterenol administration (right). C-D) Both at baseline (C) and 6 minutes following isoproterenol administration (D), QTC interval was affected by genotype (F(2,21)=7.19; two-way repeated measures ANOVA). C) At baseline, Kcnb1R/R mice had a QTC interval of 55.7 ± 2.1 msec, which was prolonged relative to the WT QTC interval of 48.8 ± 1.2 msec (*p=0.0292). D) Following isoproterenol (1.5 mg/kg, IP) administration, Kcnb1R/R mice had a QTC interval of 73.2 ± 2.8 msec, which was prolonged relative to the Kcnb1R/+ QTC interval of 65.1 ± 1.6 msec and the WT QTC interval of 62.0 ± 1.2 msec. (*p=0.0202, ***p=0.0002, Sidak’s). Circles represent individual mice and error bars represent SEM with 4-13 mice per genotype.
Supplemental Figure S2. *Kcnb1*<sup>R/+</sup> and *Kcnb1*<sup>R/R</sup> mice do not differ from wild-type (WT) by echocardiography. There were no genotype-dependent differences in ejection fraction or fractional shortening, indicating the absence of contractile dysfunction at 10-14 weeks of age. A) Representative short-axis M-mode echocardiography images. B) Ejection fraction was not different between groups. C) Fractional shortening was not different between genotypes. D) Average heart rate did not differ between genotypes. Average ± SEM values are shown. Statistical comparison between groups was made using ANOVA with Tukey’s post-hoc comparisons. Detailed underlying data for B-D are shown in Table S2.
Supplemental Figure S3. Immunolabeling of $Kcnb1^{G379R}$ cultured hippocampal neurons. A-C) Representative images of cultured hippocampal neurons from littermate WT (A), $Kcnb1^{R/+}$ (B) and $Kcnb1^{R/R}$ (C) mice at DIV 21. Immunolabeling with Kv2.1 antibody shows Kv2.1 loss in $Kcnb1^{R/+}$ and $Kcnb1^{R/R}$ mice. Cyan-Kv2.1, magenta-Map2 and yellow-DAPI. Scale bar = 10 μm.
Supplemental Figure S4. Immunolabeling in *Kcnb1<sup>G379R</sup>* brain sections. A) Representative images of somatosensory cortex and hippocampus from littermate WT, *Kcnb1<sup>R/+</sup>* and *Kcnb1<sup>R/R</sup>* mice. Immunolabeling with three distinct Kv2.1 antibodies show distinct Kv2.1 loss in *Kcnb1<sup>R/+</sup>* and *Kcnb1<sup>R/R</sup>* mice. Green-D3/71R, red-L105/31 and blue-L80/21. Scale bar = 500 μm. B) Representative images of CA1 from littermate WT, *Kcnb1<sup>R/+</sup>* and *Kcnb1<sup>R/R</sup>* mice. No genotype-specific differences in immunolabeling are apparent. Green- parvalbumin, red-calretinin and blue-Hoechst 33258. Scale bar = 50 μm.
**Supplemental Video S1.** Handling-induced generalized tonic-clonic seizure in a *Kcnb1*<sup>RR</sup> mouse at ~3 months of age.

**Supplemental Video S2.** Video-EEG of a spontaneous generalized tonic-clonic seizure in a *Kcnb1*<sup>RR</sup> mouse at ~2 months of age.

**Supplemental Video S2a.** Enhanced video from Supplemental Video S2 showing behavioral correlates of the generalized tonic-clonic seizure.

**Supplemental Video S3.** Video-EEG of a brief run of rhythmic slow spike and wave complexes (1-2 Hz) during wakefulness in a *Kcnb1*<sup>RR</sup> at ~2 months of age.

**Supplemental Video S4.** Video-EEG of a ~15-minute run of rhythmic slow spike and wave complexes (1-2 Hz) during sleep in a *Kcnb1*<sup>RR</sup> at ~2 months of age.

**Supplemental Video S5.** Video-EEG of a paroxysmal event associated with immobility in a *Kcnb1*<sup>RR</sup> mouse at ~2 months of age.
References

1. Shi, G., et al., *Properties of Kv2.1 K+ channels expressed in transfected mammalian cells*. Journal of Biological Chemistry, 1994. 269(37): p. 23204-23211.

2. Lim, S.T., et al., *A Novel Targeting Signal for Proximal Clustering of the Kv2.1 K+ Channel in Hippocampal Neurons*. Neuron, 2000. 25(2): p. 385-397.

3. Dickson, E.J., et al., *Dynamic formation of ER–PM junctions presents a lipid phosphatase to regulate phosphoinositides*. The Journal of Cell Biology, 2016. 213(1): p. 33-48.

4. Kirmiz, M., et al., *Identification of VAPA and VAPB as Kv2 Channel-Interacting Proteins Defining Endoplasmic Reticulum–Plasma Membrane Junctions in Mammalian Brain Neurons*. The Journal of Neuroscience, 2018. 38(35): p. 7562-7584.

5. Manning, C.F., A.M. Bundros, and J.S. Trimmer, *Benefits and Pitfalls of Secondary Antibodies: Why Choosing the Right Secondary Is of Primary Importance*. PLOS ONE, 2012. 7(6): p. e38313.

6. Bishop, H.I., et al., *Kv2 Ion Channels Determine the Expression and Localization of the Associated AMIGO-1 Cell Adhesion Molecule in Adult Brain Neurons*. Frontiers in Molecular Neuroscience, 2018. 11(1).

7. Truett, G.E., et al., *Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT)*. Biotechniques, 2000. 29(1): p. 52, 54.

8. Palacio, S., et al., *Heterogeneity in Kv2 Channel Expression Shapes Action Potential Characteristics and Firing Patterns in CA1 versus CA2 Hippocampal Pyramidal Neurons*. eneuro, 2017. 4(4): p. ENEURO.0267-17.2017.

9. Rhodes, T.H., et al., *Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy*. Proc Natl Acad Sci U S A, 2004. 101(30): p. 11147-11152.

10. Speca, D.J., et al., *Deletion of the Kv2.1 delayed rectifier potassium channel leads to neuronal and behavioral hyperexcitability*. Genes Brain Behav, 2014. 13(4): p. 394-408.

11. Schnell, S.A., W.A. Staines, and M.W. Wessendorf, *Reduction of Lipofuscin-like Autofluorescence in Fluorescently Labeled Tissue*. Journal of Histochemistry & Cytochemistry, 1999. 47(6): p. 719-730.

12. Nagasawa, Y., et al., *Sensitivity of inhalation anesthetics isoflurane and sevoflurane for the drug-induced QT-interval prolongation in guinea pigs*. J Pharmacol Sci, 2020. 143(1): p. 39-44.

13. Riley, D.C., et al., *Prolongation of the QT interval by volatile anesthetics in chronically instrumented dogs*. Anesth Analg, 1988. 67(8): p. 741-9.