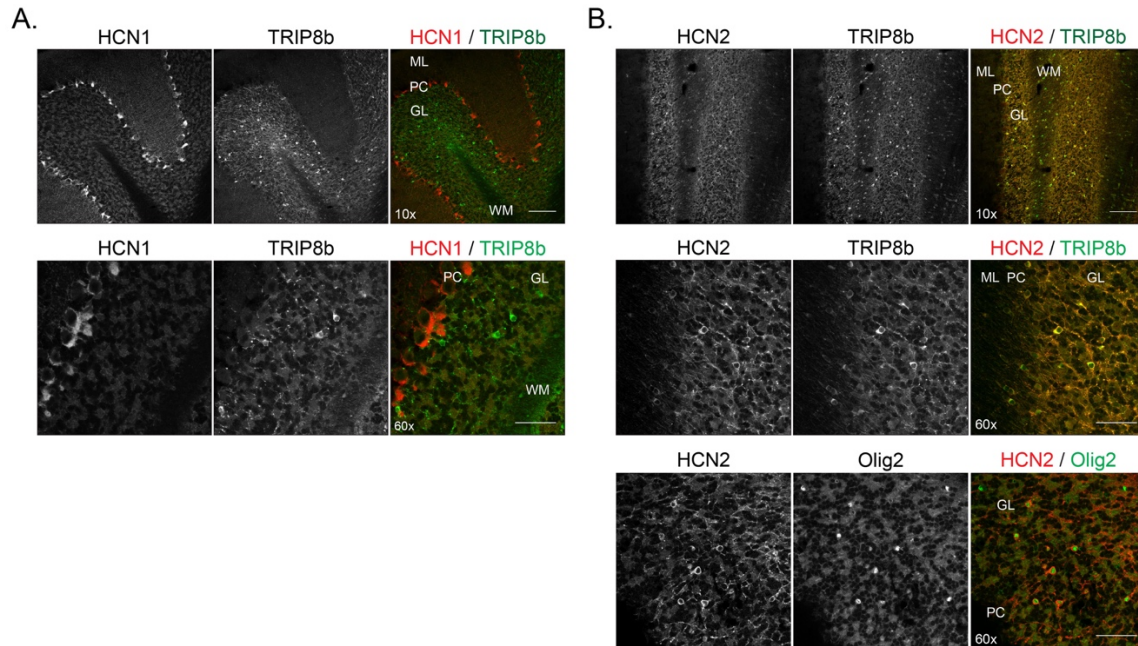


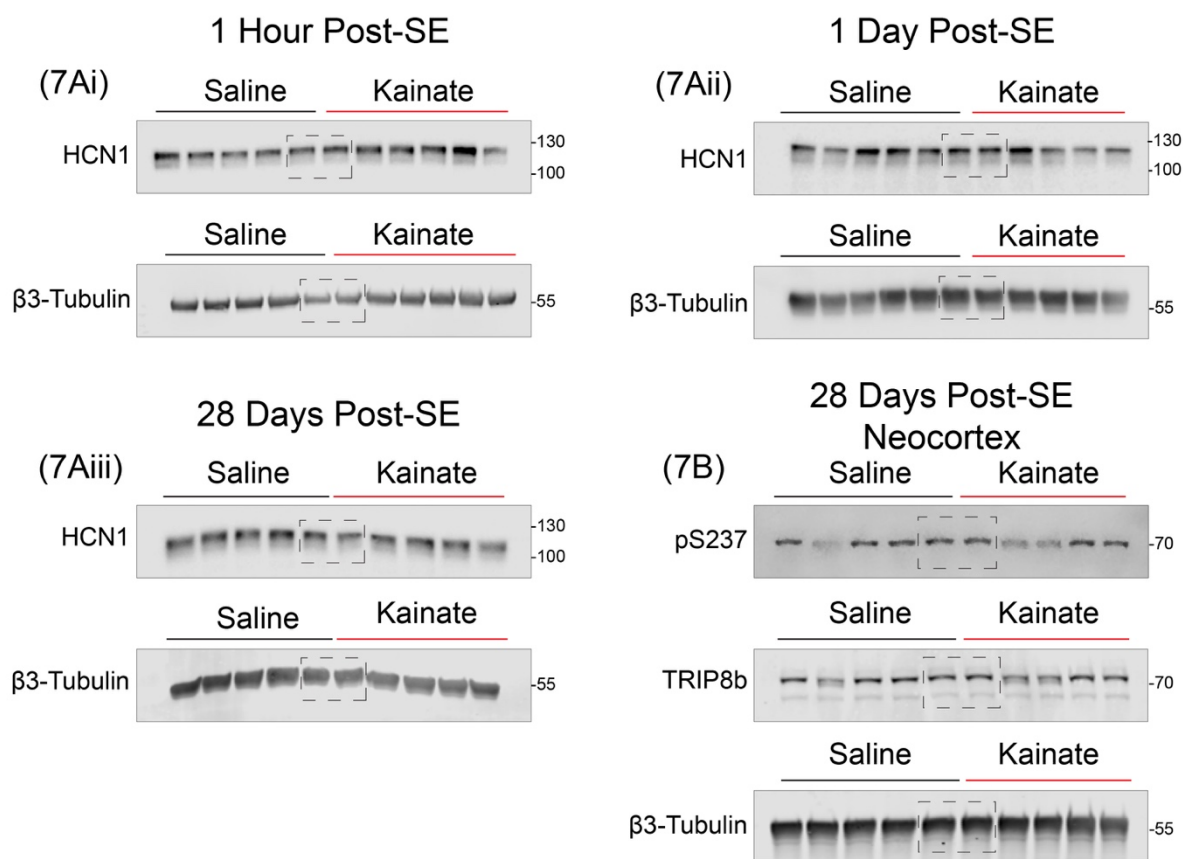
## Supporting Information

For Foote et al., “Phosphorylation of the HCN channel auxiliary subunit TRIP8b is altered in an animal model of temporal lobe epilepsy and modulates channel function”



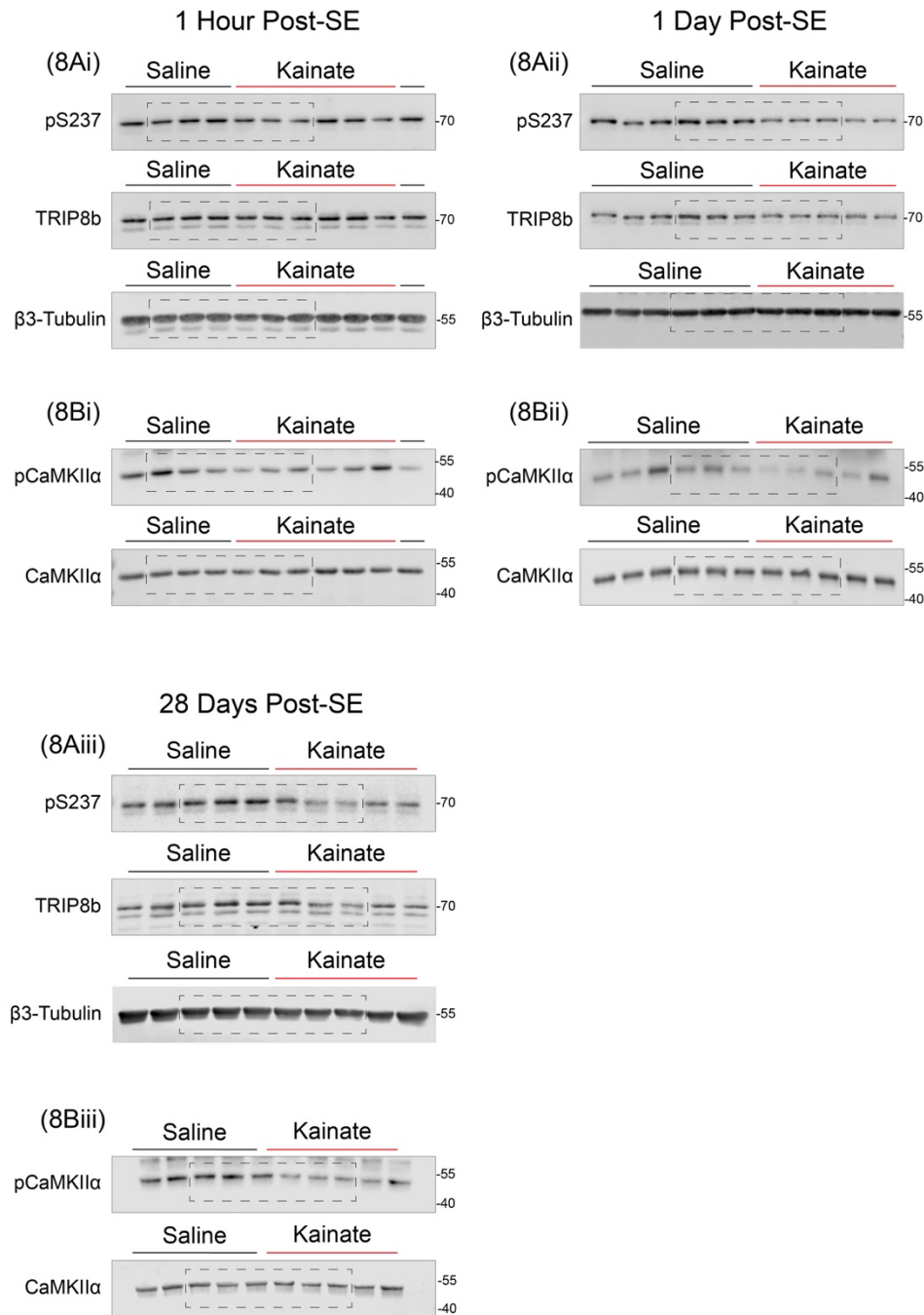
**Figure S1.** Co-localization of TRIP8b and HCN2 but not HCN1 in the cerebellum.

A) Cerebellum stained with antibodies against HCN1 and TRIP8b at 10x or 60x magnification (n=4). B) Cerebellum stained with antibodies against HCN2, TRIP8b, and Olig2 at 10x or 60x magnification (n=4). PC= Purkinje cells, ML= molecular layer, GL= granular layer, WM= white matter. Scale bar= 100µm for 10x images and 50µm for 60x images.



**Figure S2.** Immunoblots from saline- or KA-treated rats.

Immunoblots correspond to Figure 7. A) Hippocampi were harvested from saline- or kainate (KA)-treated rats at (i) 1 hour, (ii) 1 day, or (iii) 28 days post-SE. Immunoblots were probed with antibodies against HCN1 and  $\beta$ 3-Tubulin. B) The neocortex was harvested from saline- or KA-treated rats at 28 days post-SE. Immunoblots were probed with antibodies against pS237, TRIP8b, and  $\beta$ 3-Tubulin.



**Figure S3.** Immunoblots from saline- or KA-treated rats.

Immunoblots correspond to Figure 8. Hippocampi were harvested from saline- or kainate (KA)-treated rats at (i) 1 hour, (ii) 1 day, or (iii) 28 days post-SE. Immunoblots were probed with antibodies against pS237, TRIP8b, and  $\beta$ 3-Tubulin (A) or pCaMKII $\alpha$  and CaMKII $\alpha$  (B).

HCN1 Expression in the Hippocampus				
Time Point (Post-SE)	Normalization Scheme	Normalized Density (Saline) $\pm$ SEM	Normalized Density (KA) $\pm$ SEM	P-Value
1 Hour	HCN1/ $\beta$ 3-Tubulin	1.0 $\pm$ 0.1 N=5	1.1 $\pm$ 0.1 N=6	p=0.7
1 Day	HCN1/ $\beta$ 3-Tubulin	1.0 $\pm$ 0.1 N=6	0.9 $\pm$ 0.2 N=5	p=0.6
28 Days	HCN1/ $\beta$ 3-Tubulin	1.00 $\pm$ 0.04 N=5	1.1 $\pm$ 0.1 N=5	p=0.4

**Table S1.** Total HCN1 protein expression is unchanged in the hippocampus at 1 hour, 1 day, and 28 days post-SE.

Hippocampi from saline- or kainic acid (KA)-treated rats were harvested at 1 hour, 1 day, or 28 days post-status epilepticus (SE). HCN1 was normalized to  $\beta$ 3-Tubulin, and immunoblot band density was quantified using an unpaired Student's t-test,  $p > 0.05$ . Data is presented as mean  $\pm$  standard error of the mean (SEM).

pS237 Expression in the Neocortex				
Time Point (Post-SE)	Normalization Scheme	Normalized Density (Saline) $\pm$ SEM	Normalized Density (KA) $\pm$ SEM	P-Value
28 Days	pS237/ TRIP8b	1.0 $\pm$ 0.1 N=5	0.9 $\pm$ 0.1 N=5	p=0.3

**Table S2.** pS237 is unchanged in the neocortex at 28 days post-SE.

The neocortex from saline- or kainic acid (KA)-treated rats was harvested at 28 days post-status epilepticus (SE). pS237 was normalized to TRIP8b, and immunoblot band density was quantified using an unpaired Student's t-test,  $p > 0.05$ . Data is presented as mean  $\pm$  standard error of the mean (SEM).

A.

Protein Expression at 1 Hour Post-SE			
Normalization Scheme	Normalized Density (Saline) $\pm$ SEM	Normalized Density (KA) $\pm$ SEM	P-Value
pS237/TRIP8b	1.00 $\pm$ 0.04 N=5	0.81 $\pm$ 0.04 N=6	p=0.008
pCaMKII $\alpha$ /CaMKII $\alpha$	1.0 $\pm$ 0.2 N=5	0.9 $\pm$ 0.1 N=6	p=0.8

B.

Protein Expression at 1 Day Post-SE			
Normalization Scheme	Normalized Density (Saline) $\pm$ SEM	Normalized Density (KA) $\pm$ SEM	P-Value
pS237/TRIP8b	1.0 $\pm$ 0.1 N=6	0.7 $\pm$ 0.1 N=5	p=0.0098
pCaMKII $\alpha$ /CaMKII $\alpha$	1.0 $\pm$ 0.1 N=6	0.5 $\pm$ 0.2 N=5	p=0.048

C.

Protein Expression at 28 Days Post-SE			
Normalization Scheme	Normalized Density (Saline) $\pm$ SEM	Normalized Density (KA) $\pm$ SEM	P-Value
pS237/TRIP8b	1.0 $\pm$ 0.1 N=10	0.7 $\pm$ 0.1 N=11	p=0.001
pCaMKII $\alpha$ /CaMKII $\alpha$	1.0 $\pm$ 0.1 N=5	0.63 $\pm$ 0.04 N=5	p=0.005

**Table S3.** Changes in TRIP8b phosphorylation and CaMKII $\alpha$  activity in the KA model of TLE. Hippocampi from saline- or kainic acid (KA)-treated rats were harvested at 1 hour (A), 1 day (B), or 28 days (C) post-status epilepticus (SE). pS237 was normalized to total TRIP8b and pCaMKII $\alpha$  was normalized to total CaMKII $\alpha$ . Immunoblot band density was quantified using an unpaired Student's t-test, p>0.05. Data is presented as mean  $\pm$  standard error of the mean (SEM).