

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”

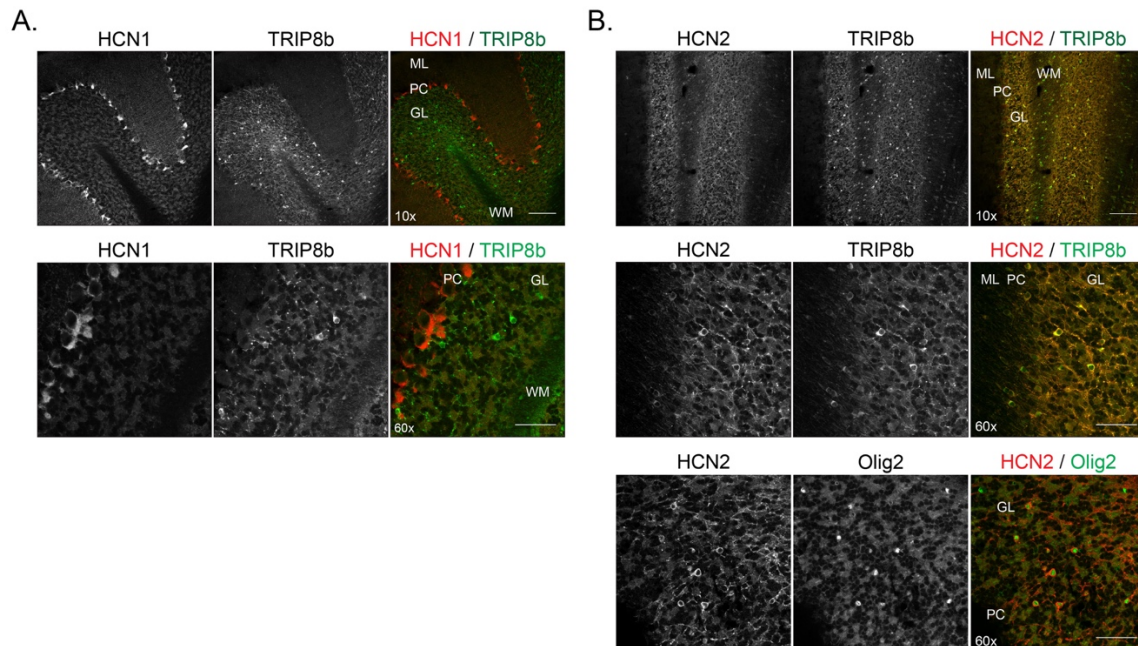


Figure S1. Co-localization of TRIP8b and HCN2 but not HCN1 in the cerebellum.

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= 100 μ m for 10x images and 50 μ m for 60x images.

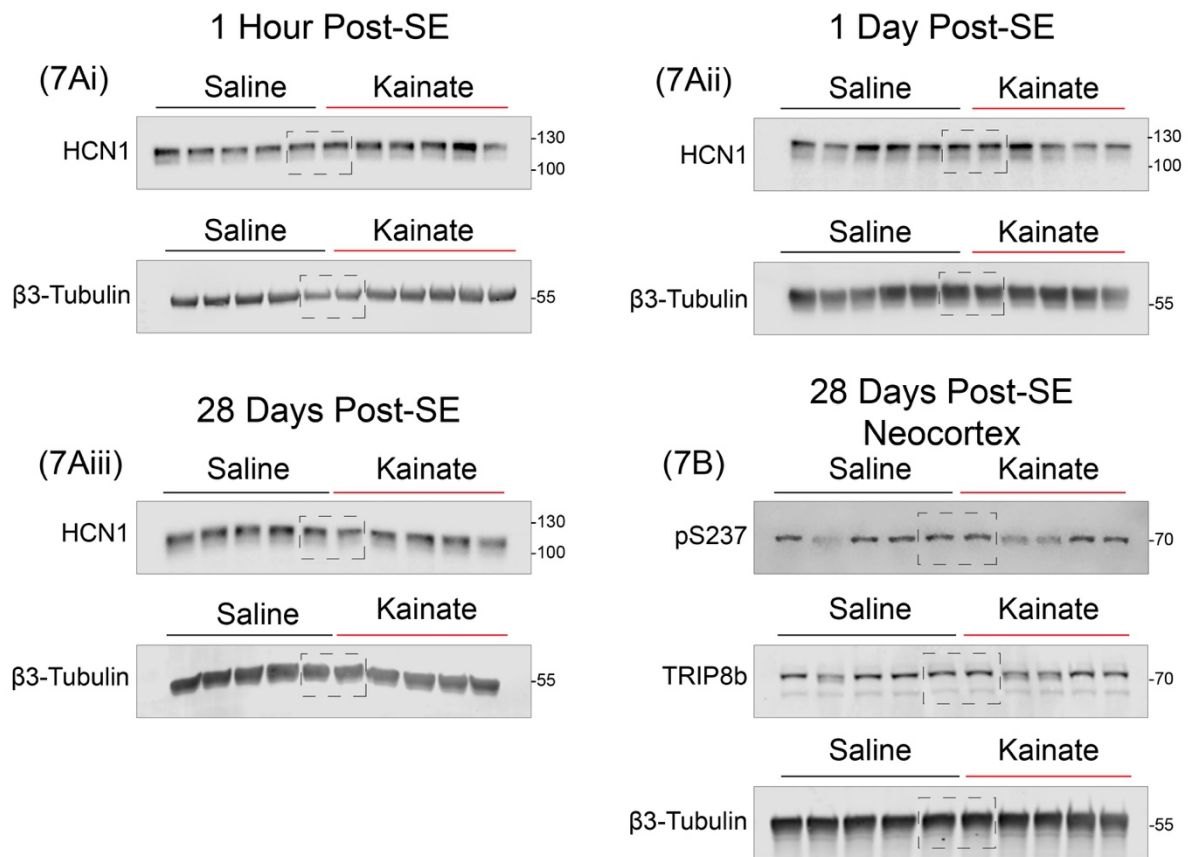


Figure S2. Immunoblots from saline- or KA-treated rats. Immunoblots correspond to Figure 7. A) Hippocampi were harvested from saline- or kainate (KA)-treated rats at (i) 1 hour, (ii) 1 day, or (iii) 28 days post-SE. Immunoblots were probed with antibodies against HCN1 and β 3-Tubulin. B) The neocortex was harvested from saline- or KA-treated rats at 28 days post-SE. Immunoblots were probed with antibodies against pS237, TRIP8b, and β 3-Tubulin.

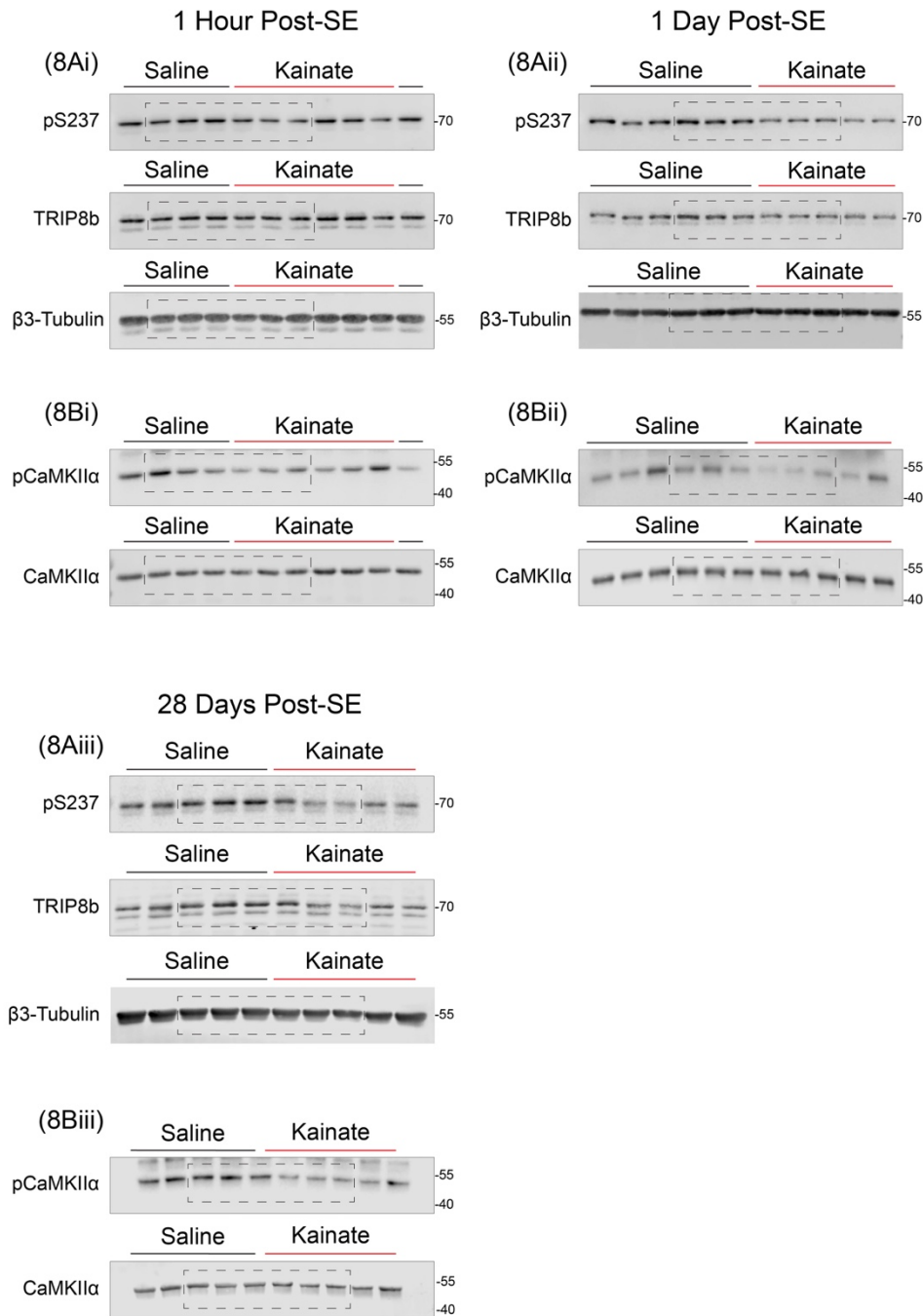


Figure S3. Immunoblots from saline- or KA-treated rats. Immunoblots correspond to Figure 8. Hippocampi were harvested from saline- or kainate (KA)-treated rats at (i) 1 hour, (ii) 1 day, or (iii) 28 days post-SE. Immunoblots were probed with antibodies against pS237, TRIP8b, and β3-Tubulin (A) or pCaMKIIα and CaMKIIα (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/ β 3-Tubulin	1.0 \pm 0.1 N=5	1.1 \pm 0.1 N=6	p=0.7
1 Day	HCN1/ β 3-Tubulin	1.0 \pm 0.1 N=6	0.9 \pm 0.2 N=5	p=0.6
28 Days	HCN1/ β 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 β 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 α / CaMKII α	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 α / CaMKII α	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 α / CaMKII α	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 α 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 α was normalized to total CaMKII α . 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).