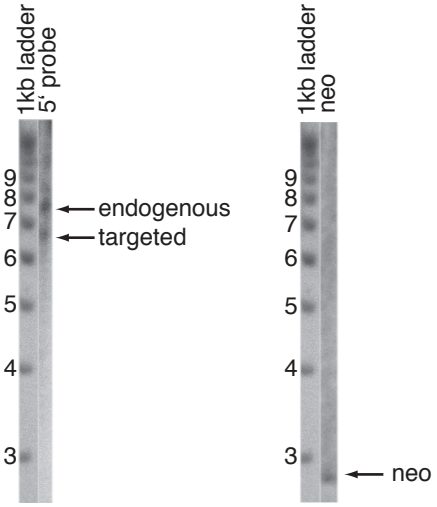
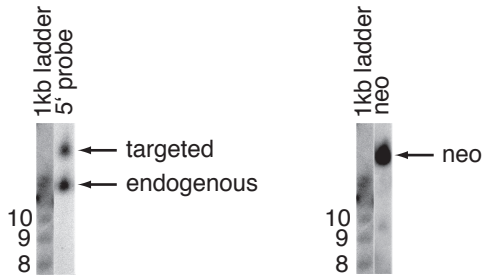
Supplement 1a

Southern blots - *Kcnq2*



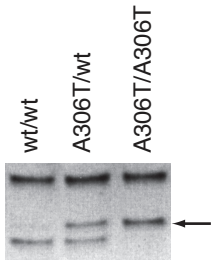
Supplement 1b

Southern blots - *Kcnq3*

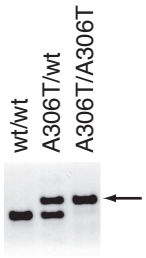


Supplement 1c

SSCP - *Kcnq2*

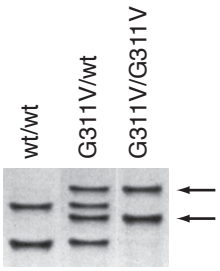


loxP - *Kcnq2*

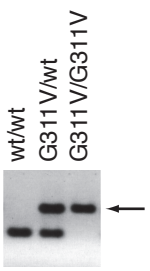


Supplement 1d

SSCP - *Kcnq3*



loxP - *Kcnq3*



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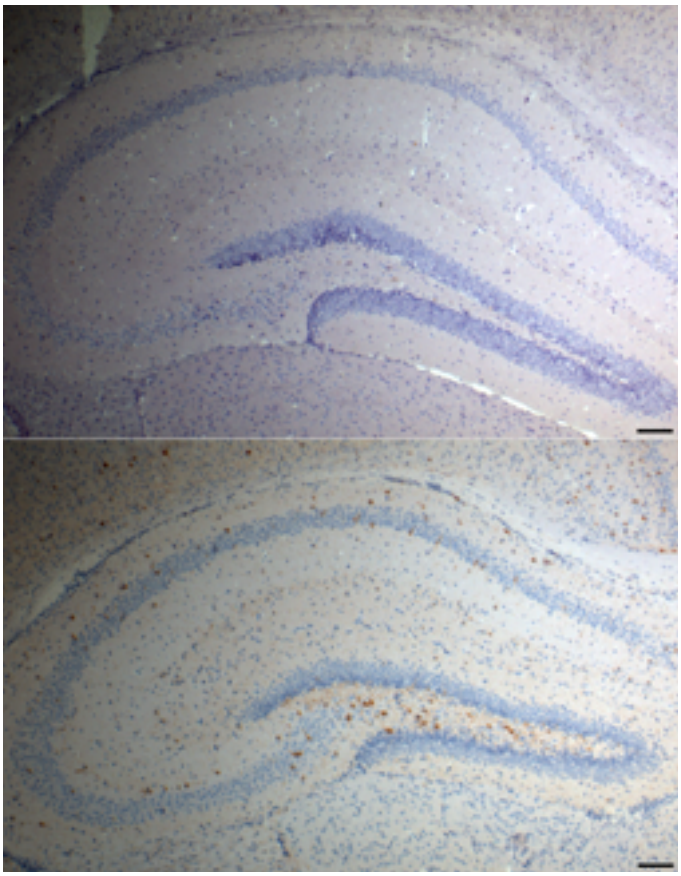
Supplemental Figure 1: Genomic Southern analysis of BamH1 cut embryonic stem cell DNA hybridized with a probe 5' of the targeting vector (left panels) and probed with the neo gene (right panels) confirming homologous recombination in *Kcnq2* (Figure 1A) and *Kcnq3* (Figure 1B) embryonic stem cells. Analysis of PCR-amplified tail DNA on single strand conformational polymorphism (SSCP) gels detected the presence of the A306T mutation in *Kcnq2* exon 6 (Figure 1C left, arrow denotes mutant) and agarose gels detected the corresponding presence of a single loxP site in *Kcnq2* intron 5 (Figure 1C right, arrow denotes mutant). SSCP analysis of PCR-amplified mouse tail DNA demonstrating band shifts (denoted by arrows) caused by the G311V point mutation in *Kcnq3* knockin mice (Figure 1D left, arrows denote mutant). Agarose gel of PCR amplified tail DNA showing the post-excision loxP site in intron 6 of *Kcnq3* (Figure 1D right, arrow denotes mutant).



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Supplemental Figure 2: An absence of NPY upregulation in the stratum lucidum of young homozygous mutant mice. No expression of NPY in the stratum ludicum or hilar mossy fibers of a P30 B6.129 *Kcnq2*$^{A306T/A306T}$ homozygous mutant mouse (top panel). In a P11 FVB.129 *Kcnq3*$^{G311V/G311V}$ homozygous mutant mouse a few hours following a documented seizure, no upregulation of NPY in the stratum lucidum is seen, although a slight increase in expression of NPY is seen in the hilar mossy fibers (bottom panel). In both cases, normal NPY expression is seen in hilar interneurons. Scale bars, 100μm.

Table S1. Number of behavioral seizures over a 12-hour light period as a function of age in FVB.129-Kcnq3$^{G311V/G311V}$ mice

age (days)	P21-25	P26-30	P31-35	P36-40	P41-45	P46-50	P51-55	P56-60	P61-65	P66-70	P71-75	P76-80	P81-85	P86-90	P91-95	P96-100	P100-105	P106-110
1	9*	5*																
2	1*	0	4	7	0*													
3		6*	6*															
4			1*	2	5	6	25	9	21	40*								
5			0*	2	4	21	0	6*										
6			2*	16	19	4	0	15	19	32	67	110	24*					
7				5*	18	18	0	0*										
8					0*	7	19	21	21	23	40	39	25*					
9							0*	18	25	0	0	0	0	0	7	0	5	6*

mice correspond to Figure 4A; *, monitoring was incomplete over the 5-day period

Table S2. Comparison of our grading scale of seizure characteristics to the Racine scale

Grade	**Features**	**approximate Racine Equivalent (see ref 16)**
1	rapid running	stage 8-running and jumping
2	bilateral forelimb clonus, orofacial automatisms, jaw chomping, head clonus, occasional rearing	stage 1-mouth clonus stage 2 head clonus stage 4- bilateral forelimb clonus with rearing
3	Forelimb and hindlimb clonus	stage 5-bilateral forelimb clonus with falling stage 9-running and jumping followed by a tonic clonic seizure
4	Forelimb tonic extension and hindlimb clonus	stage 9-running and jumping followed by a tonic clonic seizure
5	Forelimb and hindlimb tonic extension	stage 9- running and jumping followed by a tonic clonic seizure

Table S3. The *Kcnq2* A306T mutation does not affect the passive membrane properties of CA1 neurons.

Membrane property	Mouse genotype		
	***Kcnq2*$^{+/+}$**	***Kcnq2*$^{A306T/+}$**	***Kcnq2*$^{A306T/A306T}$**
Input Resistance (MΩ)	107 ± 4 (n = 17)	103 ± 5 (n = 9)	103 ± 4 (n = 10)
Series Resistance (MΩ)	14.6 ± 0.3 (n = 15)	13.9 ± 0.3 (n = 9)	14.3 ± 0.3 (n = 8)
Resting Membrane Potential (mV)	-61.1 ± 0.7 (n = 7)	-59.9 ± 0.7 (n = 8)	-59.97 ± 0.8 (n = 10)
Capacitance (pF)	131 ± 5 (n = 11)	134 ± 13 (n = 9)	137 ± 11 (n = 10)

All values expressed as mean ± S.E.M. Input resistance, series resistance, and resting membrane potential were taken from values reported by SealTest and MultiClamp Commander functions.

Table S4. The *Kcnq3* G311V mutation does not affect the passive membrane properties of B6;129 CA1 neurons.

Membrane property	Mouse genotype		
	B6;129-*Kcnq3*$^{+/+}$	B6;129-*Kcnq3*$^{G311V/+}$	B6;129-*Kcnq3*$^{G311V/G311V}$
Input Resistance (MΩ)	107 ± 4 (n = 17)	105 ± 6 (n = 18)	100 ± 4 (n = 16)
Series Resistance (MΩ)	14.6 ± 0.3 (n = 15)	14.0 ± 0.2 (n = 19)	14.3 ± 0.3 (n = 25)
Resting Membrane Potential (mV)	-61.1 ± 0.7 (n = 7)	-59.3 ± 0.5 (n = 15)	-59.3 ± 0.8 (n = 15)
Capacitance (pF)	131 ± 5 (n = 11)	140 ± 4 (n = 21)	132 ± 7 (n = 21)

All values expressed as mean ± S.E.M. Input resistance, series resistance, and resting membrane potential were taken from values reported by SealTest and MultiClamp Commander functions.

Table S5. The *Kcnq3* G311V mutation does not affect the passive membrane properties of FVB;129 CA1 neurons.

Membrane property	Mouse genotype		
	FVB;129-*Kcnq3*$^{+/+}$	FVB;129-*Kcnq3*$^{G311V/+}$	FVB;129-*Kcnq3*$^{G311V/G311V}$
Input Resistance (MΩ)	93 ± 6 (n = 6)	93 ± 5 (n = 7)	95 ± 4 (n = 14)
Series Resistance (MΩ)	13.5 ± 0.5 (n = 7)	13.8 ± 0.3 (n = 7)	13.8 ± 0.3 (n = 16)
Resting Membrane Potential (mV)	-60.1 ± 2.4 (n = 5)	-61.3 ± 1.5 (n = 6)	-58.35 ± 1.58 (n = 10)
Capacitance (pF)	160 ± 15 (n = 6)	162 ± 7 (n = 6)	155 ± 6 (n = 15)

All values expressed as mean ± S.E.M. Input resistance, series resistance, and resting membrane potential were taken from values reported by SealTest and MultiClamp Commander functions.

Supplementary Videos 1 & 2
Spontaneous seizures in N1F2 B6.129-*Kcnq2*$^{A306T/A306T}$ (video 1) and N1F2 B6.129-*Kcnq3*$^{G310V/G310V}$ (video 2) adult mice. Note that in each mutant, behavioral signs of seizure activity precede the onset of abnormal synchronization and cortical epileptic discharges, and may outlast them, consistent with a subcortical origin. Electrode montages are as described in Figure 3.

Supplementary Video 3
Spontaneous generalized tonic clonic seizure in a P24 N5F2 FVB.129-*Kcnq2*$^{A306T/A306T}$ mouse. Note the almost instantaneous onset of a forelimb and hindlimb tonic extension seizure.

Supplementary Videos 4 and 5
Recurrent generalized seizures in a P48 N5F2 FVB.129-*Kcnq3*$^{G311V/G311V}$ mouse. Compared to N5F2 FVB.129-*Kcnq2*$^{A306T/A306T}$, FVB.129-*Kcnq3*$^{G311V/G311V}$ exhibit a longer duration to onset of a forelimb and hindlimb tonic extension seizure. From P33 to P82, over 600 seizures were recorded in this homozygous knockin mouse, including grade 5 (video 4) and grade 3 (video 5) seizures and no mossy fiber sprouting was seen after Timm staining.