Supplemental Data

Mutations in Prickle Orthologs

Cause Seizures in Flies, Mice, and Humans

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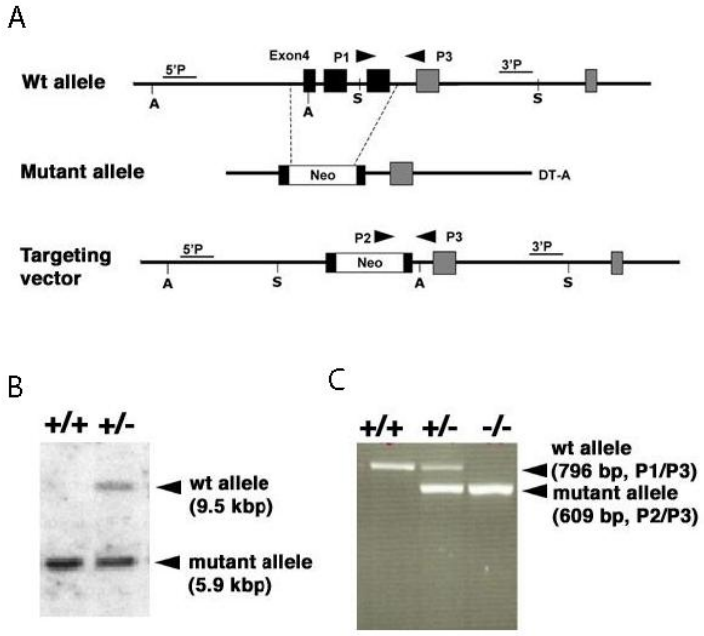


Supplementary Figure 1

Figure S1. Targeted disruption of the mouse *prickle2* (*mpk2*) gene. (PDF, 40 KB) (**A**) Schematic representation of the targeted disruption. Restriction map of the *mpk2* locus (top), the targeting vector (middle), and the mutation-containing locus following homologous recombination (bottom). (**A**), Alw44I; S, SacI. (**B**) Southern blot analysis of targeted ES clones. Genomic DNA from control TT2 ES cells and homologous targeted clones (#134) was digested with SacI and probed with an external probe (5'P). The wild-type (+) and *mpk2* mutant (-) loci generated 9.5 kb and 5.9 kb fragments, respectively. (**C**) PCR analysis. Genotypes of embryos were determined by PCR, using primers P1, P2, and P3 as described in Materials and Methods.

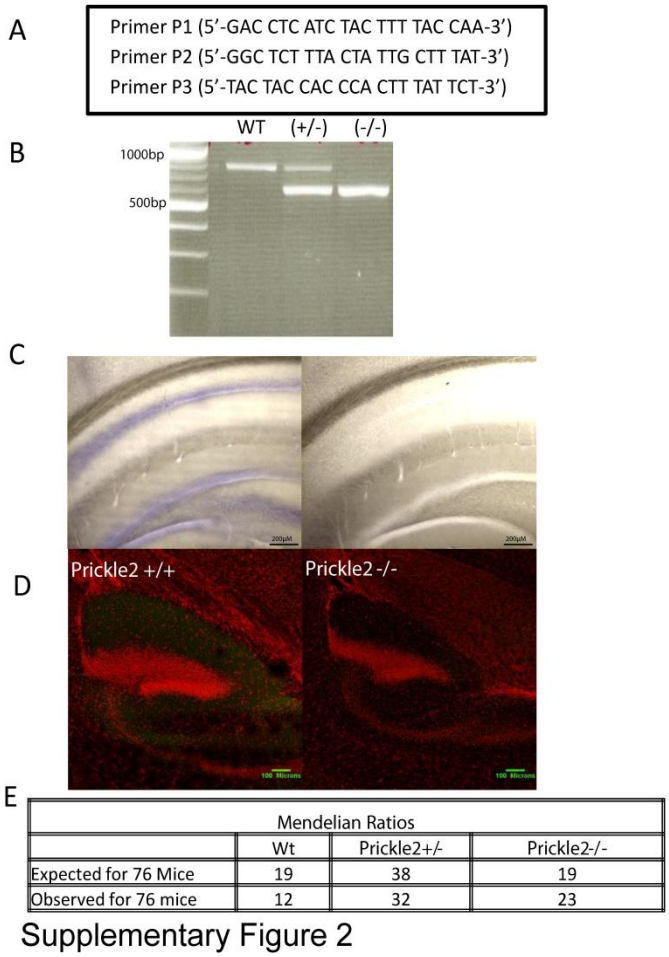
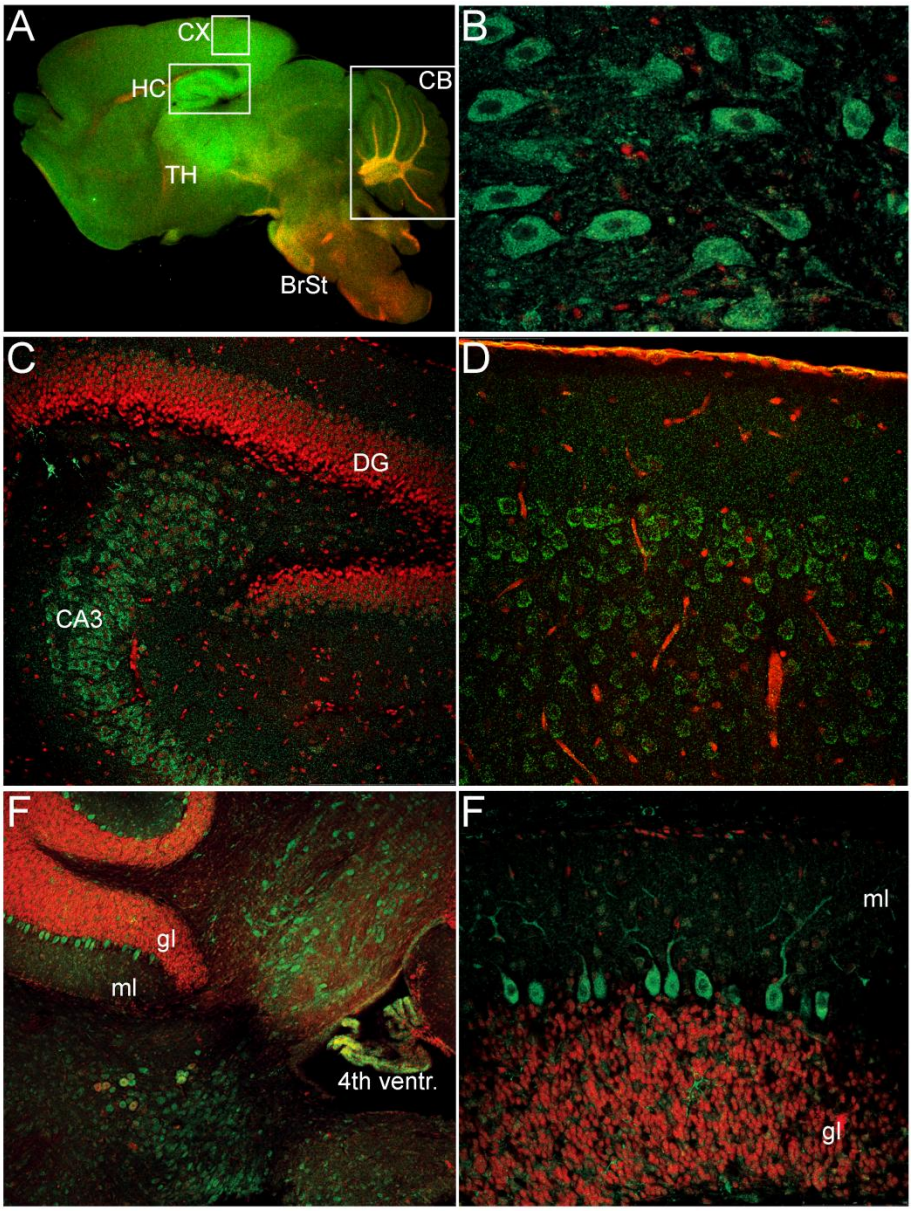


Figure S2. Genotyping of *Prickle2* mice and proof of knockout. (PDF, 304KB). (**A**) Primers used for genotyping of *Prickle2* mice. (**B**) Representative PCR gel of wt, *Prickle2*$^{(+/-)}$, and *Prickle2*$^{(-/-)}$ mice. (**C**) In-situ of a wt cortex and *Prickle2*$^{(-/-)}$ cortex. Prickle2 is widely expressed in the wt cortex. As expected, there is a complete loss of signal in the *Prickle2*$^{(-/-)}$ mouse. (**D**) Green=Prickle2, Red=FarRed DNA stain. (**E**) Expected versus observed genotypes from *Prickle2*$^{(+/-)}$ X *Prickle2*$^{(+/-)}$ matings did not deviate significantly from expected Mendelian ratios.



Supplementary Figure 3

Figure S3. Expression of Prickle2 in P19 mouse brain (JPEG, 3.6MB). Prickle2 (Green), 7AAD (red). (**A**) Prickle2 (green) in P19 mouse brain - image taken with fluorescent dissecting scope. Boxed areas show hippocampus (HC); cortex (CX); and cerebellum (CB). Thalamus (TH) and brain stem (BrSt) are labeled. Boxed areas in A are shown in panels (**B** - **F**) which represent confocal images where Prickle2 is green and 7AAD DNA, staining nuclei of cells, is

red. B. Prickle2 in neurons of the brain stem; (**C**) Hippocampus - dentate gyrus (DG) and CA3 regions are shown; (**D**) Cortex, (**E**) and (**F**). Cerebellum - Prickle2 is expressed in Purkinje neurons.

Table S1. Prickle Resequencing Primers

	Primer Sequence
EXON 1A	F CAG ATG CCG TGA CTG ACT TG
	R GGG AAT TCA CCA AGC AAG AG
EXON 1B	F GGA AGG CGA TCT GAG CAC T
	R TCT GGC TTT CTC ATC CTC CT
EXON 2	F CCA TTG CCA AGG AAT GTG TT
	R CTG GAA TGA GGA AGG ACC AG
EXON 3	F TAC ATC CTG CTG GGG GAA T
	R TTC TTC GGC TAC CTC ATT GG
EXON 4	F TCA GAG TCA TGG GCT TCA GA
	R TGG ATG AAT GAA TGG GTG AA
EXON 5	F CCA GCT AAT GGT GGT TGT CC
	R GTG CAG AAA GCC AGA AGA CC
EXON 6	F GGA CCC CAT AGC TTT CTT CC
	R GGG TTT CTA ACA CTT GCA ATC A
EXON 7 A	F TGG AAT CCT GAG ACA AAC CA
	R CCG TCT TGC CCT TGT TCT T
EXON 7 B	F GAA GAC CCC AAT GGT TCT GA
	R CTT GCC CTC CTG GAC TGT AG
EXON 7 C	F ATG GGA ACA AGA TGG AGC AG
	R TAT TTG GGG ACT TGG ATG CT
EXON 7 D	F ATC AAG GAA TGC CGA GAA GA
	R CCC AGA GAC TAC CCC CTT TC
EXON 8 A	F GAT GAC CAG CAG TGA CAG TGA
	R CCC TCC ATC AAA ATC AAA GC
EXON 8 B	F CCA GCA GTA CCA GGA GAT GG
	R TGG CGC ATA AAT TGG TCA TA
EXON 8 C	F GCC ATC TCC CGG TTA AAA GA
	R GGC CGT AGG AGC TGT ATT TG
EXON 8 D	F TGA CAA CGA GGG CTA TTT CC
	R TTC CCT TTT CTC CCC CAT AA

Table S2.

Subject No.	Sex	Age at study/Onset of symptoms	Family member(s) with homozygous *CSTB* mutation	Myoclonic jerks at onset	Present myoclonic jerks	Clinical examination	EEG	Medication/ day
1	F	32/12	3 brothers	During sleep Frequent feeling of cramp in fingers and toes	None Occasional feeling of cramp in fingers and toes	Normal	Polyspike- and wave discharges	Lamotrigine 500 mg
2	M	18/15	1 brother	During sleep	None	Normal	Normal	None
3	F	36/31	3 siblings	Infrequent focal or generalized jerks	Infrequent focal or generalized jerks	Left eye strabismus	Normal	None
4	M	18/10	1 sister	Frequent nodding of the head	None	Normal	Normal	None
5	M	22/18	1 uncle	Infrequent focal or multifocal jerks in hands and legs	Action-related tremor and jerks in hands	Slight tremor and infrequent jerks in hands	Normal	None
6	M	42/ ?	1 daughter	Infrequent myoclonic jerks in hands and legs and shaking attacks at night	Infrequent myoclonic jerks in hands and shaking attacks at night	Normal	Not done	None

Clinical features in individuals heterozygous for the minisatellite expansion mutation in *CSTB*. Data collected by Drs. Anna-Elina Lehesjoki and Teija Silén, Helsinki, Finland.