Supporting Information

Shen et al. 10.1073/pnas.1501364112

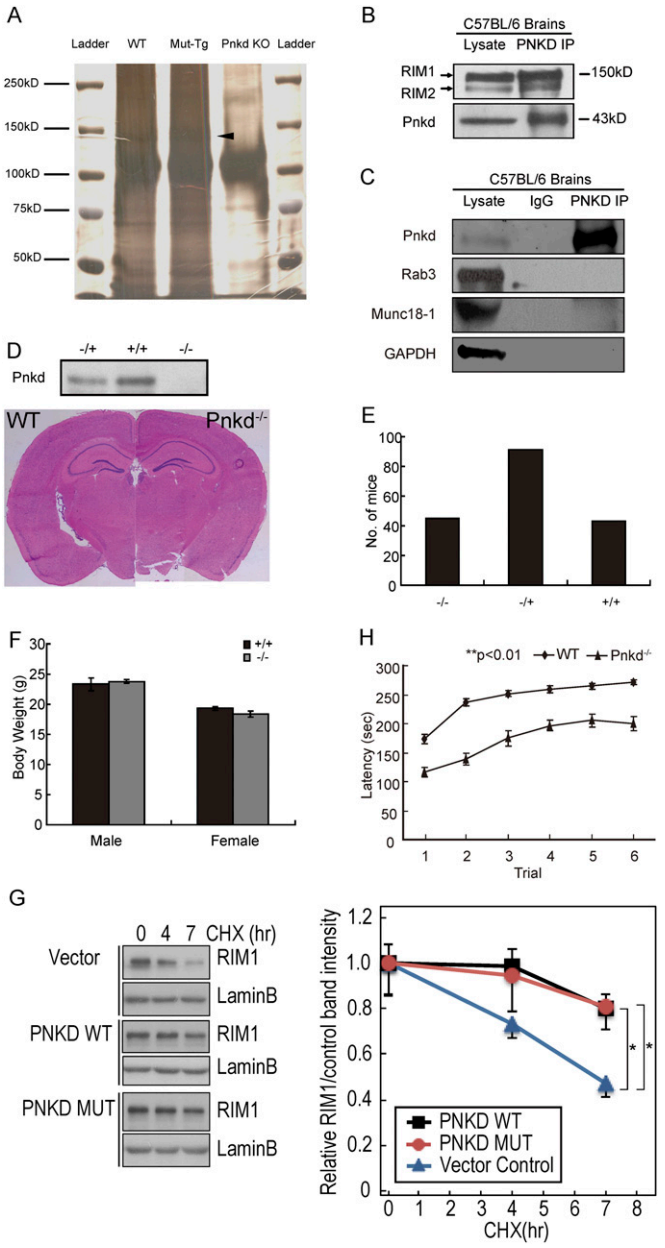

Fig. S1. (*A*) Silverstaining of Pnkd coimmunoprecipitation. Coimmunoprecipitation using a rabbit anti-PNKD antibody in mouse brain lysates from wild-type (*Left*), mut-Tg (*Center*), and Pnkd $^{-/-}$ (*Right*) mice. The arrowhead indicates an ∼150-kD band in wild-type and mut-Tg samples that is absent in the Pnkd$^{-/-}$ sample. (*B*) RIM1 and RIM2 coimmunoprecipitated with PNKD. C57BL/6 mouse frontal cortex lysates were incubated with a rabbit anti-PNKD antibody. Lysate (*Left*) and the immunoprecipitation eluant (*Right*) were blotted with anti-PNKD or a nonspecific anti-RIMS antibodies. (*C*) C57BL/6 mouse frontal cortex homogenates were incubated with rabbit IgG or anti-PNKD antibody. Rab3 and Munc18-1 were not pulled down by Pnkd. (*D*) Immunoblot of Pnkd in adult wild-type, PNKD KO, PNKD heterozygous littermates mice frontal cortex homogenates, and H&E staining of adult wild-type (*Left*) and PNKD KO mouse brain coronal section (*Right*). (*E*) The ratio of genotypes of pups generated by a Pnkd$^{+/-}$ intercross was the expected 1:2:1. Data represent a total of ∼180 newborn pups. (*F*) Weights of 9-wk-old male and female mice were not significantly different between genotypes ($n \geq 10$ for each genotype). (*G*) RIM1 protein stability was increased by PNKD in SH-SY5S cells. Error bars indicate SEs (*$P < 0.05$, Student's *t* test, $n = 3$). LaminB was used as internal control. (*H*) Latency to fall was shorter in Pnkd$^{-/-}$ mice vs. wild-type at all time points (**$P < 0.01$, Student's *t* test). Error bars indicate SEs. (WT, $n = 11$; Pnkd$^{-/-}$ mice, $n = 7$).

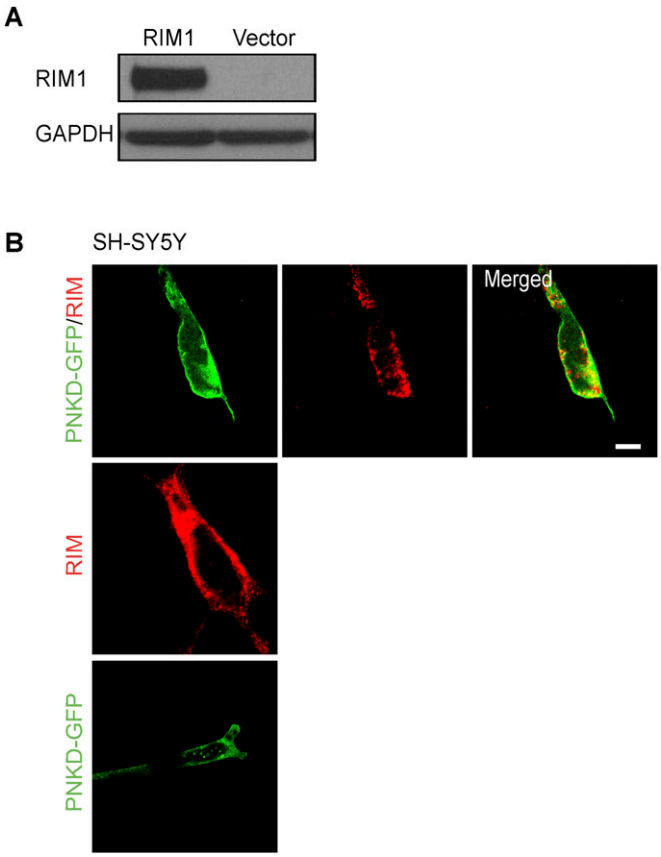

Fig. S2. Expression of PNKD and RIM1 in SH-SY5Y cells. (*A*) Cells were transfected with empty vector or RIM1 expression vector and cell lysates were blotted with anti-RIM and anti-GAPDH antibodies. There was no detectable endogenous RIM protein in SH-SY5Y cells transfected with empty vector. (*B*) Cells were cotransfected with PNKD-GFP and RIM1 expression vectors. RIM1 proteins were immunostained with an anti-RIM antibody. PNKD and RIM1 partially colocalized near the cell membrane. (Scale bar, 10 μm.)

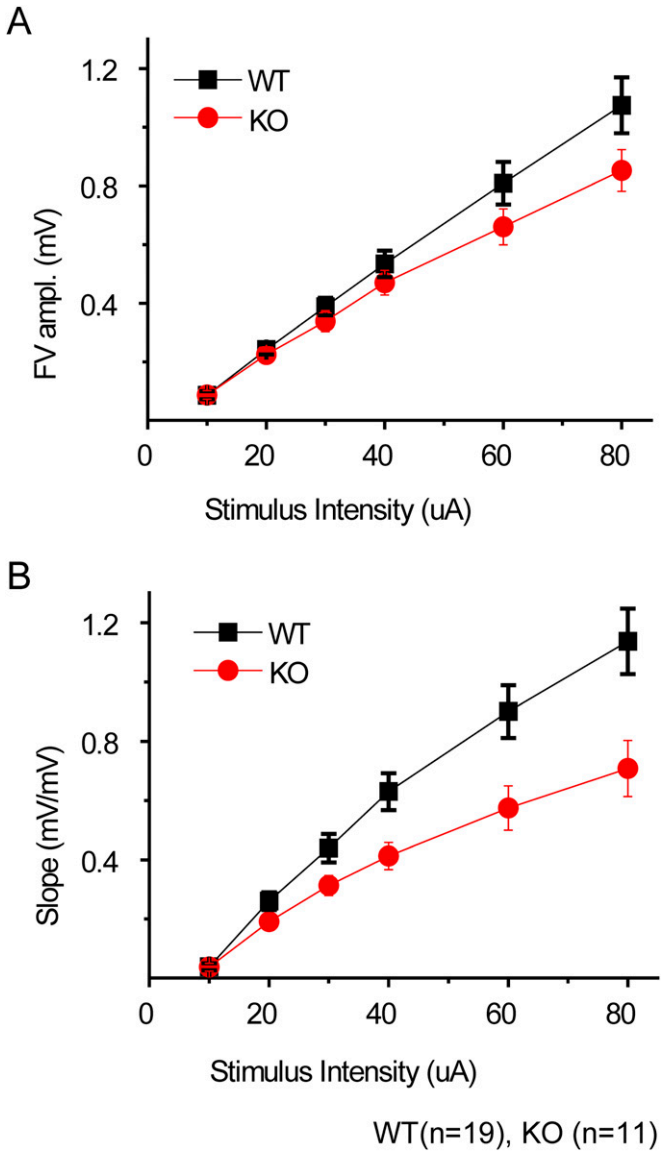

Fig. S3. Synaptic transmission is reduced in Pnkd$^{-/-}$ mice. (*A*) The presynaptic fiber volley (input) is plotted as a function of stimulation current intensity. (*B*) The postsynaptic fEPSP slope (output) is plotted as a function of stimulus intensity. Wild-type (6 mice, 19 slices) and *Pnkd*$^{-/-}$ mice (5 mice, 11 slices).

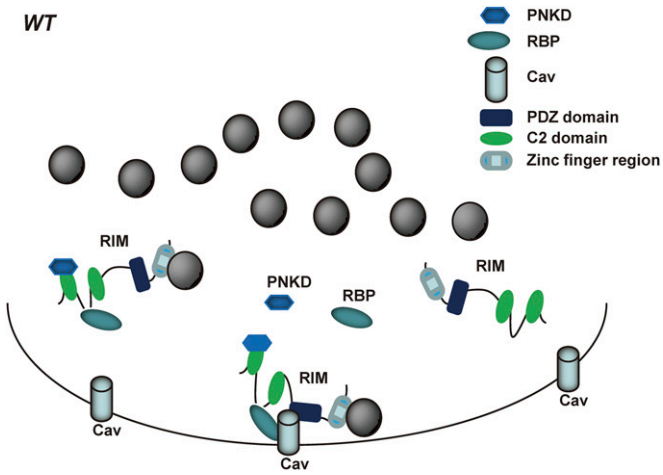

Fig. S4. Model of PNKD function in synapses. In wild-type mice, RIMs interact directly with voltage-gated calcium channels on the presynaptic membrane via RIM binding protein (RBP) and to synaptic vesicles via Rab3. PNKD interacts with RIMs and inhibits neurotransmitter release. When both RIM1 and RIM2 are genetically ablated, calcium triggered spontaneous neurotransmitter release is severely reduced (1). Mutations in PNKD and voltage-gated calcium channel subunits resulted in paroxysmal dyskinesia in humans and mice, respectively.

1. Schoch S, et al. (2006) Redundant functions of RIM1alpha and RIM2alpha in Ca(2+)-triggered neurotransmitter release. *EMBO J* 25(24):5852–5863.

Table S1. Candidate proteins identified by mass spectrometry

Protein name	Molecular weight, kDa	Hits	Rank
Regulating synaptic membrane exocytosis protein 2/Rab3-interacting molecule 2, RIM2	176	14	1
Ankyrin repeat domain-containing protein 27	117	13	2
Actin, cytoplasmic 2	42	7	3
Protein NipSnap3A	28	6	4
TRIO and F-actin-binding protein	223	13	5
Myosin-11	227	14	6
Sentrin-specific protease 6	127	12	7
SH3 and multiple ankyrin repeat domains protein 2	159	11	8
Programmed cell death 1 ligand 2	26	7	9
Orphan nuclear receptor NR1D2	64	9	10
Periplakin	204	13	11
Structural maintenance of chromosomes protein 1A	143	12	12
IFN-activable protein 204	72	8	13
Myosin-9	226	12	14
Structural maintenance of chromosomes protein 1A	143	11	15
Tuberin	201	12	16
Guanine nucleotide-binding protein G(q) subunit alpha	41	6	17
Oxysterol-binding protein-related protein 3	97	9	18
DNA polymerase alpha catalytic subunit	165	11	19
Intraflagellar transport 81	79	9	20

Pnkd was immunoprecipitated from mouse frontal cortex lysates as described in *Experimental Proceedures.* Proteins pulled down by Pnkd were visualized by silverstaining and multiple bands observed in wild-type and mut-Tg samples but not in PNKD KO samples were collected, in-gel digested, and analyzed by MS/MS. Proteins are identified using UniProtKB/Swiss-Prot (www.uniprot.org/uniprot/, Release 53.3, July 2007) and ranked according to their score. The numbers in the Hits columns refer to the number of unique peptides identified in the experiment. Common contaminants (human keratins, porcine trypsin) were removed from the dataset.