Supporting Information

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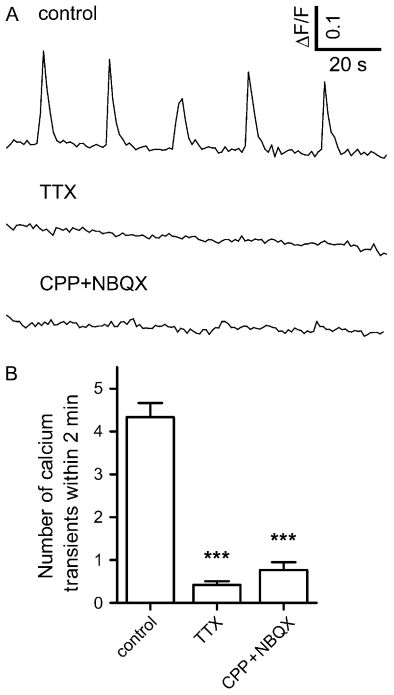

Fig. S1. Excitation of granule cells cultured at the physiological KCl concentration. Granule cells were cultured in medium containing serum for 24 h and then in serum-free medium for 96 h. During the last 30 min of culture, the cells were loaded with Fluo-4 Ca^{2+} indicator. They then were incubated with the fresh medium in a micro CO_2 incubator (Tokai Hit) mounted on a stage of an Olympus BX51WI upright fluorescence microscope equipped with an EM-CCD camera (Andor). Ca^{2+} transients were measured by recording fluorescent changes at 510 nm by excitation at 488 nm and were analyzed by using MetaMorph software (Molecular Devices). Frequencies of Ca^{2+} transients were calculated by counting Ca^{2+} transients that exceeded 3% in changes of fluorescence intensity (ΔF) relative to that of the baseline fluorescence (F). Representative traces (A) and frequencies (B) of Ca^{2+} transients under the different culture condition ($n > 100$ cells) are indicated. *** $P < 0.001$ vs. control. CPP, 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; NBQX, 2, 3-dioxo-6-nitro-1, 2, 3, 4-tetrahydrobenzo [f] quinoxaline-7-sulfonamide; TTX, tetrodotoxin.

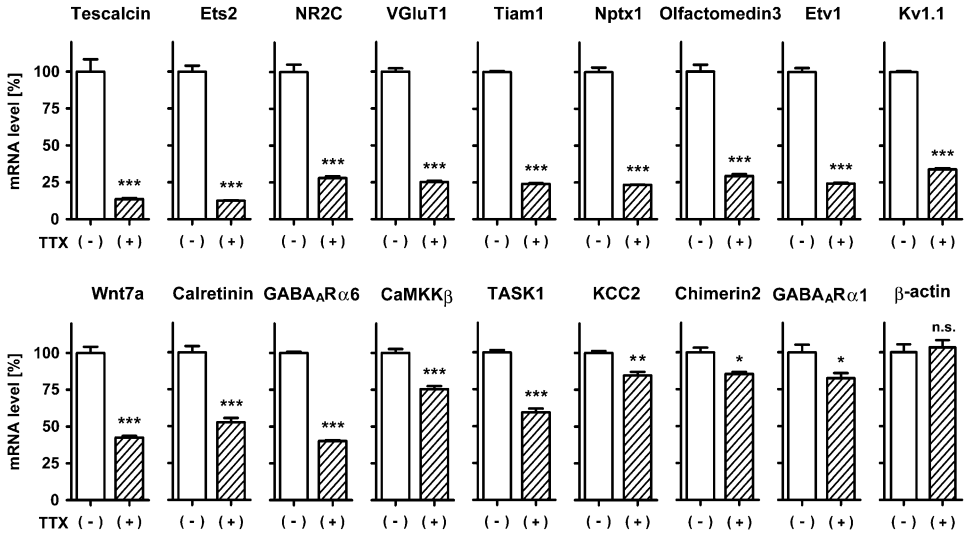

Fig. S2. Inhibition of 17 maturation genes by TTX. Granule cells were cultured in medium containing serum for 24 h and then in serum-free medium in the presence or absence of TTX (5 μM) for 96 h, and mRNA levels were quantified by PCR. Experiments were performed in triplicate. *β-actin* mRNA was quantified as an activity-insensitive control mRNA. Data are expressed as percentages of mRNA levels in TTX-treated cells relative to those in untreated cells (100%) and are shown as mean ± SEM. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ vs. untreated. n.s., not significant. Ets2, E26 avian leukemia oncogene 2; NR2C, NMDA glutamate receptor subunit 2C; VGluT1, vesicular glutamate transporter 1; Tiam1, T-cell lymphoma invasion and metastasis 1; Nptx1, neuronal pentraxin 1; Etv1, Ets variant gene 1; Kv1.1, voltage-gated potassium channel, shaker-related subfamily, member 1; Wnt7a, wingless-related MMTV integration site 7A; $GABA_AR\alpha6$, $GABA_A$ receptor α6; CaMKKβ, calcium/calmodulin-dependent protein kinase kinase β; TASK1, TWIK-related acid-sensitive potassium channel 1; KCC2, potassium-chloride cotransporter 2; $GABA_AR\alpha1$, $GABA_A$ receptor α1.

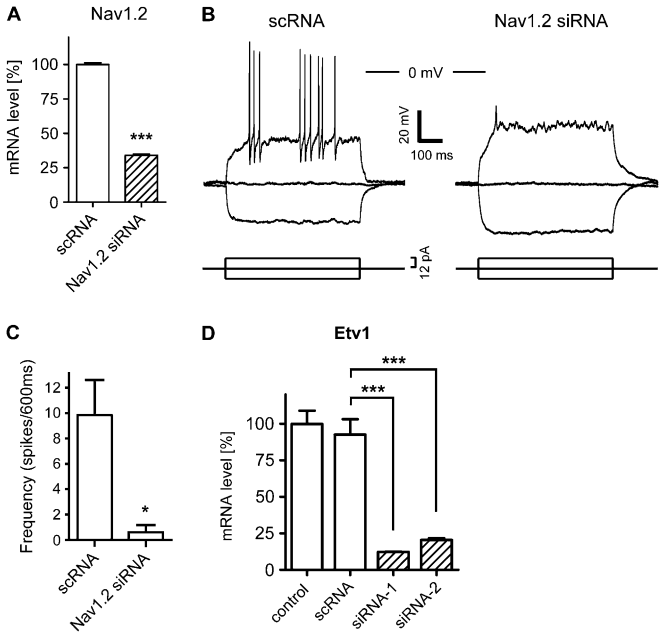

Fig. S3. Knockdown of voltage-gated sodium channel type II (*Nav1.2*) mRNA and Ets variant gene 1 (*Etv1*) mRNA by siRNA treatments and blockade of action potential by Nav1.2 siRNA. (*A*) Nav1.2 siRNA or scrambled siRNA (scRNA) (6 μg each) was electroporated into dissociated granule cells, which then were cultured in medium containing serum for 24 h and in serum-free medium for 96 h. Experiments were performed in triplicate, and mRNA levels were quantified by PCR. Statistical analysis was performed, and data are presented as in Fig. S1. (*B* and *C*) Patch-clamp recordings were performed in granule cells cultured as in *A* for 96–120 h, and action potentials were measured by current injection for 600 ms at the holding potential of −70 mV. Representative voltage traces following current injection of +12 pA, −12 pA, or no injection (*B*) and frequencies of action potentials at +12 pA (*C*) are indicated ($n = 12$ for scRNA; $n = 6$ for Nav1.2 siRNA). (*D*) Etv1 siRNA-1, Etv1 siRNA-2, or scRNA (6 μg each) was electroporated into dissociated granule cells, and culture was conducted as in *A* ($n = 3$). *Etv1* mRNA levels were quantified by PCR. Data are shown as mean ± SEM. $*P < 0.05$; $***P < 0.001$ vs. scRNA.

Table S1. Microarray analysis of TTX-suppressive genes in cultured granule cells

	National Center for Biotechnology Information gene ID#	Gene name	Function	Hybridization signals		Fold reduction
				Control (– TTX)	+ TTX	
●	57816	Tescalcin	Intracellular signaling	984.1	141.4	7.0
●	23872	*Ets2*	Transcription factor	735.4	133.8	5.5
○	14813	*NR2C*	Receptor	76.4	14.3	5.3
●	72961	*VGluT1*	Transporter	1016.1	215.4	4.7
○	21844	*Tiam1*	Intracellular signaling	482.0	102.5	4.7
○	18164	*Nptx1*	Extracellular signaling	1946.7	472.8	4.1
○	229759	Olfactomedin3	Extracellular signaling	1439.8	362.4	4.0
○	14009	*Etv1*	Transcription factor	2886.3	777.6	3.7
	20564	*Slit3*	Extracellular signaling	20.5	6.1	3.3
○	16485	*Kv1.1*	Ion channel	1488.5	529.8	2.8
○	22421	*Wnt7a*	Extracellular signaling	855.4	373.3	2.3
○	12308	Calretinin	Intracellular signaling	1097.8	493.1	2.2
	14403	*$GABA_AR\delta$*	Receptor	732.9	348.6	2.1
	69601	*Dab2ip*	Intracellular signaling	732.3	372.1	2.0
○	14399	*$GABA_AR\alpha6$*	Receptor	5850.2	3006.7	1.9
○	207565	*$CaMKK\beta$*	Kinase	1179.1	720.9	1.6
	64297	*Gprc5b*	Receptor	170.0	108.9	1.6
○	16527	*TASK1*	Ion channel	253.8	172.4	1.5
	105445	*Dock9*	Intracellular signaling	633.3	442.6	1.4
	226251	*Ablim1*	Intracellular signaling	392.3	290.4	1.4
○	57138	*KCC2*	Transporter	2778.3	2129.8	1.3
○	69993	Chimerin2	Intracellular signaling	3483.3	2687.9	1.3
○	14394	*$GABA_AR\alpha1$*	Receptor	3007.1	2607.0	1.2
	20745	Testican	Extracellular signaling	694.8	780.7	–0.9

Microarray analysis was performed twice with RNA samples prepared from different cultures, and the data were averaged. Three genes identified as TTX-suppressive genes in this study are marked by black circles. The other 21 genes were reported previously as developmentally up-regulated genes (1), and all but the *testican* gene were found to be TTX suppressive. The 17 genes indicated by black or white circles were selected for detailed analysis. Slit3, slit homolog 3; $GABA_AR\delta$, $GABA_A$ receptor δ; Dab2ip, Dab2 interacting protein; Gprc5b, G protein-coupled receptor, family C, group 5, member B; Dock9, dedicator of cytokinesis 9; Ablim1, actin-binding LIM protein 1.

1. Sato M, Suzuki K, Yamazaki H, Nakanishi S (2005) A pivotal role of calcineurin signaling in development and maturation of postnatal cerebellar granule cells. *Proc Natl Acad Sci USA* 102:5874–5879.