Supporting Information

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SI Materials and Methods

Generation of TARP γ-7 KO Mice. The targeting vector was designed as shown in Fig. S1. A 120-bp fragment (from base 16 to base 135 in the protein coding region of *Cacng7*) was replaced by a 6,989-bp cassette, which included an internal ribosome entry site (IRES), β-galactosidase (lacZ) gene, and the neomycin (Neo) resistance gene. The targeting vector was electroporated into murine embryonic stem cells derived from the 129/OlaHsd mouse substrain to generate chimeric mice. Positive ES cell colonies expressing the Neo resistance gene were picked, their DNA was isolated and subsequently screened by Southern blot using an external probe specific for the *Cacng7* gene, selecting for those clones that integrated the *Cacng7*-null allele into the *Cacng7* WT locus by homologous recombination. Electroporated ES cell clones heterozygous for the *Cacng7*-null allele were microinjected into WT 3.5-d-old blastocysts to generate chimeric mice. Genotypes for all subsequent breedings were determined by PCR analysis of digested mice tail samples (Fig. S1*B*). PCR genotyping was performed with the following primers: forward, 5′- GGACTCTGGCCGCTCTCTACTATAG -3′; reverse 1, 5′-AGGATTTTGGGGTGAGTGAGGGCAG-3′; Neo forward, 5′-GGGTGGGATTAGATAAATGCCTGCTCT -3′.

Brain Slice Preparation and Electrophysiology.

Purkinje cell recordings. Parasagittal cerebellar slices (250 μm thick) from postnatal day (P) 17 to 4-mo-old mice were prepared in the cutting solution consisting of (in mM) 110 choline chloride, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 25 glucose, 11.6 sodium ascorbate, 3.1 sodium pyruvate, 7 $MgCl_2$, and 0.5 $CaCl_2$. The slices were cut at 0–4 °C and incubated 30 min at 34 °C in artificial cerebrospinal fluid (ACSF) containing the following (in mM): 125 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 11 glucose, 1 $MgCl_2$, and 2 $CaCl_2$. Whole-cell recordings from visually identified Purkinje cells were obtained at room temperature by using glass electrodes (2–3 MΩ). The pipet solution for synaptic recordings contained (in mM) 115 $CsMeSO_3$, 20 CsCl, 10 Hepes, 2.5 $MgCl_2$, 4 Na_2-ATP, 0.4 Na-GTP, 10 Na-phosphocreatine, 0.6 EGTA, 0.1 spermine, and 5 QX-314, pH 7.2–3, adjusted to 305–315 mOsm. Purkinje cells were voltage clamped at −20 or −70 mV and picrotoxin (100 μM) was always added to block $GABA_A$ receptors. Climbing fiber-EPSCs were evoked by granule cell layer stimulation and were identified by their large-amplitude all-or-none response and paired pulse depression. Kainate receptor EPSCs were isolated in 100 μM 1-(4-aminophenyl)-3-methylcarbamyl-4-methyl-3,4-dihydro-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 53655; Santa Cruz Biotechnology).

Stellate cell recordings. Recording conditions for stellate cells have been described (1). Briefly, transverse cerebellar slices (250 μm thick) were prepared from P17 to 23 mice. Mice were anesthetized with isoflurane, decapitated and their brains rapidly removed and placed in an ice-cold, high-sucrose cutting solution consisting of (in mM): 87 NaCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 25 glucose and 75 sucrose, saturated with 95% O_2/5% CO_2. Slices were cut using a DTK-1000 Microslicer (Ted Pella). Freshly cut slices were then placed in an incubating chamber containing ACSF, containing (in mM): 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 15 glucose, 2 $CaCl_2$, 1 $MgCl_2$, saturated with 95% O_2/5% CO_2, and allowed to recover at 35 °C for ∼30 min. Slices were subsequently maintained for at least 0.5–1 h in ACSF at room temperature before recording. Following recovery, slices were transferred to a submersion chamber on an upright Olympus BX51 microscope, and perfused with ACSF saturated with 95% O_2/5% CO_2, at room temperature (21–22 °C). Cells were recorded with thin-walled borosilicate glass pipettes (WPI), with pipette resistance values of 4–6 MΩ, and filled with an internal solution consisting of (in mM): 140 $CsMeSO_3$, 10 Hepes, 10 EGTA, 2 NaCl, 2 Mg-ATP, 1 QX-314, 5 TEA-Cl, 1 $CaCl_2$, and 0.1 spermine, pH 7.3 with CsOH. Recordings were carried out by using an Axopatch 1D amplifier (Molecular Devices), filtered at 2 kHz, digitized at 20 kHz by using a National Instruments digitizer and were acquired by using custom procedures in Igor Pro (Wavemetrics). Input resistance (R_{in}), series resistance (R_s), and leak current were consistently monitored throughout all experiments and recordings were excluded from the analysis if any of these measures deviated significantly from baseline. All recordings were carried out at room temperature (21–22 °C). mEPSC recording was carried out in the presence of 100 μM picrotoxin (Sigma), 100 μM APV (Ascent), and 500 nM tetrodotoxin (TTX) (Tocris) and at a holding potential of −60 mV. Analysis of mEPSCs was carried out by using custom procedures in Igor Pro (Wavemetrics) (2). Nucleated patches were acquired as described (1). Briefly, nucleated outside-out patches were pulled from the SC somata in transverse cerebellar slices and placed in front of a gravity-fed perfusion device. Control solution consisted of Hepes-buffered ACSF, containing (in mM): 150 NaCl, 2.5 KCl, 10 Hepes, 10 glucose, 2 $CaCl_2$, 1 $MgCl_2$, pH 7.4 with NaOH. Control perfusion solution contained 100 μM picrotoxin, 100 μM APV, 500 nM TTX, and 100 μM cyclothiazide (CTZ), to which 500 μM glutamate or 500 μM kainate was added. KA/Glu ratio experiments were performed by applying kainate and glutamate, in the presence of CTZ, at a V_h of −60 mV. Values for agonist-evoked currents were determined from peak currents. Both mEPSC and nucleated patch recordings from *stg*/γ-7 dKO SCs were interwoven with recordings from WT and *stg* SCs, which were reported (1).

Dissociated cerebellar granule neuron culture and electrophysiology. The dissociation and culture of cerebellar granule neurons were performed following the protocol provided in previous work (2, 3). Whole-cell patch-clamp recordings were made 8–10 DIV as described in previous reports (2–4). To increase the number of mEPSCs recorded from individual neurons, 200 mM sucrose dissolved in recording solution was applied for 2 s every 20 s to each recorded neuron through a local perfusion pencil (Automate Scientific). Only neurons from which more than 20 synaptic events were collected were included in the analyses of mEPSC amplitude and decay to avoid skewing the mean toward data collected from neurons with disproportionately small sample size. mEPSC decay was described by a weighted time constant calculated from the area under the peak-normalized currents, as described in previous work (2). Because all of *stargazer* and *stg*/γ-7 double mutant granule cells showed less than 20 events during the same recording period as WT, these events were not further analyzed. To analyze spontaneous AMPA EPSCs, 100 μM picrotoxin and 100 μM APV were added to the recording solution. To analyze spontaneous NMDA EPSCs, the recording solution contained 100 μM picrotoxin, 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (Tocris Bioscience), 20 μM glycine, and 0 Mg^{2+}.

Virus preparation and infection. To express γ-7–shRNA in cerebellar granule cells, FHUGW (H1-shRNA-pUb-EGFP) lentivirus vector was used as described (4, 5). The targeted sequences against mouse γ-7 (NM_133189.3) were consistent with that of mCherry-γ-7-shRNA construct (MSH037278-mU6, GeneCopoeia);

#1 (5′-CTGCGGCCTGCTCCTTGTG-3′), #2 (5′-GGAGAGTCTGCTTCTTTGC-3′), #3 (5′-CTCAGAGGACCATTCTTGC-3′), and #4 (5′-CTGAGCAGTACTTTCACTA-3′). To transduce dissociated cerebellar neurons, 0.5 μL of concentrated viral supernatant was used for each 24-well dish a day after dissection. Western blot analysis, immunofluorescent staining, and electrophysiological recordings were performed at 8–10 DIV.

Antibodies. In immunofluorescent staining, we used anti-NeuN (clone A60, MAB377; Millipore). In Western blot analysis, we used anti-TARP γ-2, γ-7 (6), anti-GFP (600-101-215S; Rockland), and anti-actin (MAB1501; Millipore).

Immunocytochemistry. Dissociated cerebellar neurons were prepared and grown on coverslips until 8–10 DIV. After wash with PBS, neurons were fixed with 4% (wt/vol) paraformaldehyde in PBS for 20 min at room temperature. Neurons were permeabilized with 0.1% Triton X-100 and 5% (vol/vol) goat serum in PBS for 1 h, then incubated with primary antibodies overnight at 4 °C. The neurons were washed with PBS and incubated for 2 h with Alexa Fluor 594-conjugated secondary antibodies (1: 1,000; Invitrogen). After wash neurons were mounted with SlowFade Gold Antifade Reagent (Invitrogen). Images were taken with a confocal laser scanning microscope (Zeiss LSM 510 Meta; Carl Zeiss Microscopy).

Western blot analysis. After virus infection, dissociated cerebellar neurons were grown for 10 d, then solubilized with lysis buffer (50 mM Tris·Cl, pH 8, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.05% SDS, and protease inhibitor mixture; Roche). Insoluble material was removed by centrifugation at 3,000 × g for 5 min, and supernatant was resolved by using NuPAGE Bis-Tris Gel (Invitrogen) according to manufacturer's instructions.

Statistical analysis of slice electrophysiology data. Data are presented as mean ± SEM. For Fig. 2, statistical significance, defined as $P < 0.05$, was calculated with unpaired nonparametric Wilcoxon rank sum test as appropriate by using KaleidaGraph (Synergy Software). For Figs. 1, 3, and 4, and Fig. S4, statistical analysis was performed by using GraphPad Prism 5 (GraphPad Software, Inc.) by one-way ANOVA, followed by Bonferroni's post hoc tests.

Motor Behavior. ***Behavior scoring.*** All behavioral observations and measurements were performed blind to genotype.

Mice. Both L7Cre$^-$ groups (γ-2 PC WT; γ-7 WT and γ-2 PC WT; γ-7 KO) were made up of either γ-2$^{loxP/loxP}$ or γ-2$^{loxP/+}$ genotypes. L7Cre$^+$ groups (γ-2 PC KO; γ-7 WT and γ-2 PC KO; γ-7 KO) were all γ-2$^{loxP/loxP}$. The only sex differences found within each genotype group were for body weight, but not for any of the motor tests, therefore the results shown in Fig. 6 pool the sexes.

Footprint assay. Footprint test was performed at the age of 3 mo and 5 mo. The hind paws of mice were inked, and the mice were allowed to walk on a sheet of white paper along a narrow runway (30-cm-long, 8-cm-wide, 15-cm-high walls). Each mouse was tested twice per age.

Neurological examination. Brief neurological examinations were conducted to assess mouse body weight, body condition, grooming quality, and whether the mouse had an ataxic gait. Ataxia scoring: A mouse was given a score of 0 if it showed no signs of ataxic gait (i.e., no shaking, normal, and smooth locomotion), or score of 1 if it showed signs of ataxia (i.e., staggering gait, shaking limbs, choppy locomotion). Refer to Movies S1 and S2 for examples of each.

Rotarod. Rotarod assessments were conducted by using the EZrod system (Omnitech Electronics). Mice were first pretrained on the rotarod, a rotating cylinder 30 cm in diameter, raised 13.75 inches above the chamber floor, that can rotate at a fixed or accelerating speed. The mouse is placed on the rod and must continue walking, adjusting its speed if necessary, to avoid falling. Each mouse was subjected to three sessions of four training trials. Each training trial had a maximum duration of 60 s. If a mouse fell off, or rotated clinging to the drum for three rotations or more, the trial ended and that latency was recorded. Training trials occurred at a fixed speed of 20 rpm. Any animal that could not learn to remain on a rod rotating at 20 rpm for 60 s within the 12 trials was excluded from further testing. In the case that a mouse was so impaired that it could not remain on the rod for 5 s or more in any trial of the first session or 10 s or more in the fifth or sixth trials, the mouse was excluded from further testing. Following the training sessions, assessment trials were conducted. Each assessment session consisted of: (*i*) a single fixed speed warm-up trial at 20 rpm with a maximum length of 60 s and (*ii*) three trials of the accelerating rotarod with a maximum length of 360 s. The rod gradually accelerated from 4 to 40 rpm over 300 s. The fixed speed trial and the accelerating trials were separated by at least 30 min. Each mouse had a 1-h rest period in between each accelerating trial. Each trial ended when either the maximum latency was reached, the mouse fell off the rod, or the animal rotated, clinging to the rod, for more than three rotations.

Open field. Spontaneous activity in an open field was used to record general locomotor activity, both in the horizontal and vertical planes (to monitor horizontal motion and rearing, respectively). Mice were placed in a novel open chamber (16 inches × 16 inches) and allowed to explore it freely for 15 min. Horizontal and vertical activity were recorded by infrared beam breaks (PAS-Open Field; San Diego Instruments). This test was repeated for a total of three trials per mouse (one trial per day) to assess the time course of habituation to the environment.

Wire hang. Mice were placed on a wire cage lid and allowed to grasp the wires. The wire lid was then gently inverted and held approximately 20 cm above the home cage floor. The length of time the animal was able to hang on was recorded up to a maximum of 60 s. This procedure was repeated three times and the maximal score was recorded.

Grip strength. Mice were acclimatized to the testing room for at least 30 min before testing. A grip strength meter from Columbus Instruments was used to measure forelimb and hindlimb grip strength. For forelimb testing, mice were held by the base of the tail and lowered toward the horizontal pull bar, allowed to grasp it, and then pulled backward in the horizontal plane. The force applied to the bar at the moment that grasp was released was recorded as the peak tension (*g*). Hindlimb grip strength was measured in a similar manner except that the mice were scruff restrained and allowed to rest their forepaws on a grid held by the experimenter, whereas their hindpaws were allowed to grasp a metal bar. The mice were pulled past the bar and the compression force applied to the bar at the moment the grasp was released was recorded as the peak compression (*g*). Forelimb and hindlimb measurements were alternated so as to provide an intervening rest period for each set of limbs. The test was repeated three times, and the average value recorded as the grip strength for that animal.

Statistical analysis of behavioral data. All analysis was done by using JMP version 11.0.0. For all behavior tests, two-way ANOVA tests were run with Cre status (i.e., γ-2 genotype in Purkinje cells) as one factor and γ-7 genotype as the other, to test which gene was driving effects. The two-way ANOVA also tested whether there was an interaction of these two factors. Individual pairwise comparisons were performed by using the Student's *t* test. For datasets with longitudinal measurements, a two-way repeated measures ANOVA analysis was performed.

1. Jackson AC, Nicoll RA (2011) Stargazin (TARP gamma-2) is required for compartment-specific AMPA receptor trafficking and synaptic plasticity in cerebellar stellate cells. *J Neurosci* 31(11):3939–3952.
2. Milstein AD, Zhou W, Karimzadegan S, Bredt DS, Nicoll RA (2007) TARP subtypes differentially and dose-dependently control synaptic AMPA receptor gating. *Neuron* 55(6): 905–918.
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4. Shi Y, et al. (2010) Functional comparison of the effects of TARPs and cornichons on AMPA receptor trafficking and gating. *Proc Natl Acad Sci USA* 107(37):16315–16319.
5. Suh YH, et al. (2010) A neuronal role for SNAP-23 in postsynaptic glutamate receptor trafficking. *Nat Neurosci* 13(3):338–343.
6. Yamazaki M, et al. (2010) TARPs gamma-2 and gamma-7 are essential for AMPA receptor expression in the cerebellum. *Eur J Neurosci* 31(12):2204–2220.

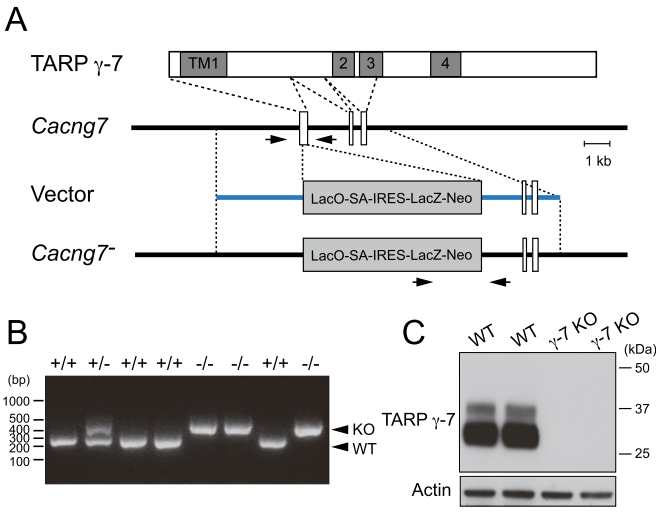


Fig. S1. Generation of TARP γ-7 knock-out mice. (*A*) Schematic representation of TARP γ-7 cDNA, *Cacng7* wild-type allele, targeting vector, and targeted allele. TM1 to 4 represent the transmembrane segments. White boxes represent exons on the *Cacng7* locus. Part of the TARP γ-7 coding region was replaced by LacZ-Neo cassette to eliminate γ-7 protein expression. Arrows indicate the primers used for genotyping. (*B*) Representative example of genotyping PCR for WT ($^{+/+}$), heterozygote ($^{+/-}$), and KO ($^{-/-}$) mouse. (*C*) Western blot analysis for WT and γ-7 KO cerebellum homogenate by using a γ-7–specific antibody. Total protein loaded per lane: 5 μg.

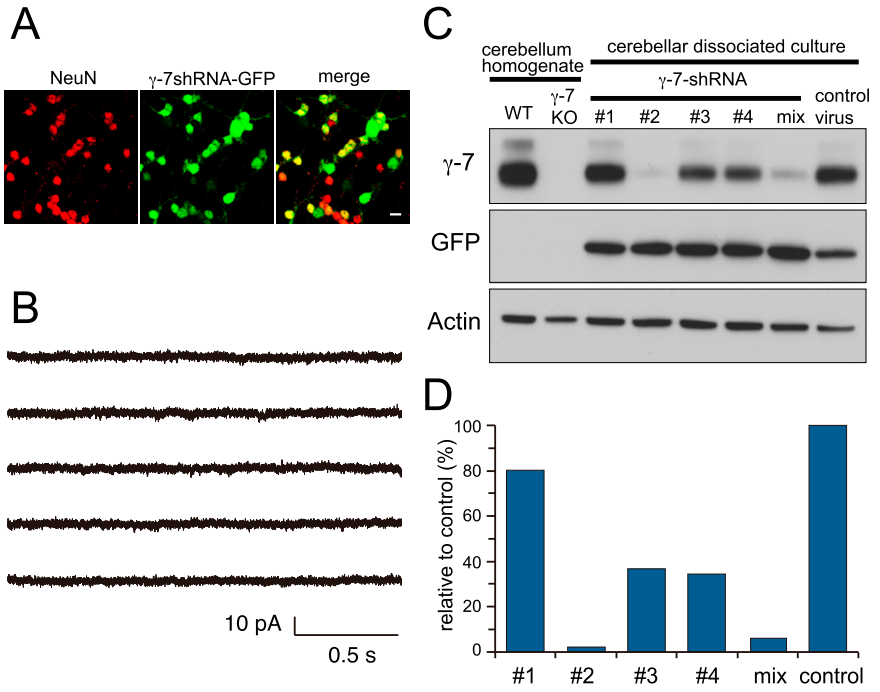


Fig. S2. CGNs from *stg* mice cerebellum were efficiently infected with lentiviruses expressing shRNA against γ-7. (*A*) Immunofluorescent staining after the infection of lentivirus expressing γ-7–shRNA mixture in cultured *stg* cells. Anti-NeuN antibody followed by Alexa 594 anti-mouse secondary antibody (red) were used for detection of CGNs. Lentivirus expressing γ-7–shRNA infected cells were detected by EGFP fluorescence (green). Note that not only CGNs but also non-CGNs have EGFP signal. (Scale bars: 10 μm.) (*B*) Typical current traces recorded from *stg* CGNs infected with Lentivirus expressing γ-7–shRNA–GFP (n = 9). No spontaneous EPSCs were observed in any of the neurons. (*C*) Western blot analysis using γ-7–specific antibody. Dissociated CGNs were infected with lentiviruses expressing shRNA containing four (#1–4) different target sequences against γ-7 or mixture of four viruses the day after dissection and protein samples were prepared at DIV 10. Total protein loaded per lane: 5 μg. (*D*) TARP γ-7 protein level was effectively reduced when either #2 or a mixture of viruses were used.

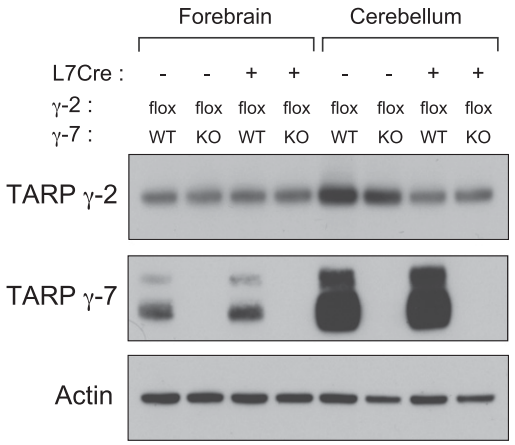


Fig. S3. Assessment of γ-2 gene deletion in PCs. Western blot analysis showing that γ-2 protein was decreased in γ-2 PC KO; γ-7 WT (L7Cre^{+}; γ-2$^{loxP/loxP}$; γ-7$^{+/+}$) and γ-2 PC KO; γ-7 KO (L7Cre^{+}; γ-2$^{loxP/loxP}$; γ-7$^{-/-}$) cerebella on account of Purkinje cell-specific γ-2 gene excision by L7Cre. The homogenate samples were prepared after completion of behavioral tests. Total protein loaded per lane: 10 μg.

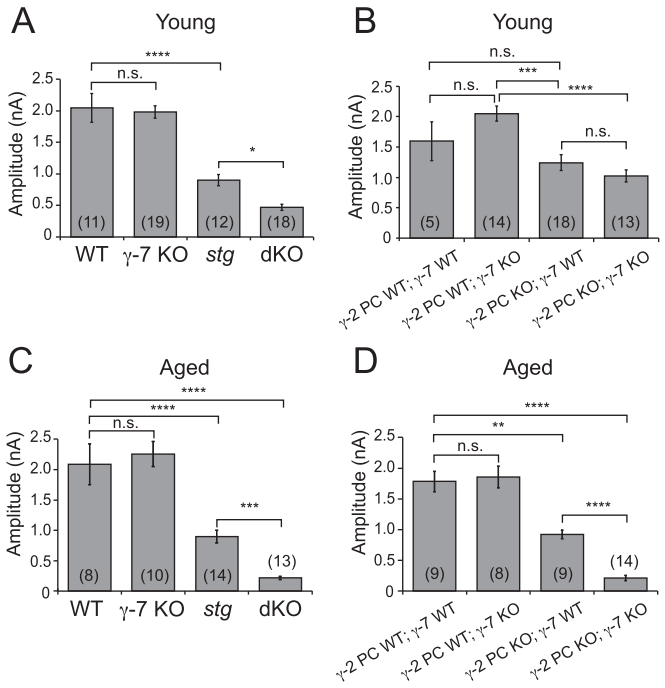


Fig. S4. Climbing fiber responses from young and aged mice. Summary bar graph shows peak amplitudes of cf-EPSCs recorded from young (*A* and *B*, from P17 to P24) and adult (*C*, from P31 to P58; *D*, P63 to 4 mo) mice at the holding potential of −20 mV. Data in *C*, previously shown in Fig. 1, are displayed here for ease of comparison. The numbers of analyzed PCs are indicated in parentheses. The graphs *C* and *D* are the same as Fig. 1*B* and Fig. 4*C* for comparison. Asterisks indicate significance, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $****P \leq 0.0001$. n.s., not significant.

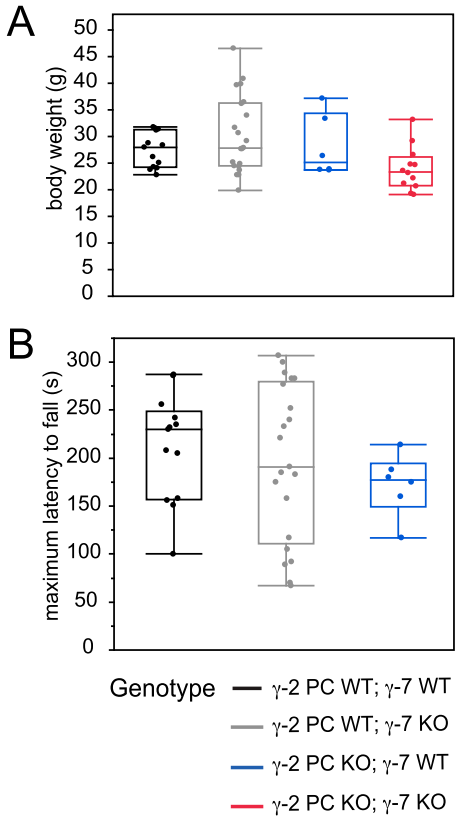


Fig. S5. Body weight measurements. (*A*) Body weight of all mice tested for behavior, at start of battery of tests. *$P = 0.0033$, Student's *t* test between γ-2 PC WT; γ-7 KO (gray) and γ-2 PC KO; γ-7 KO mice (red). (*B*) Final rotarod assessment for all groups that could be trained on the rotarod (except for γ-2 PC KO; γ-7 KO mice for which rotarod testing had been discontinued). There were no significant differences between genotype groups.

Table S1. Behavior cohort composition (by genotype, sex, and age)

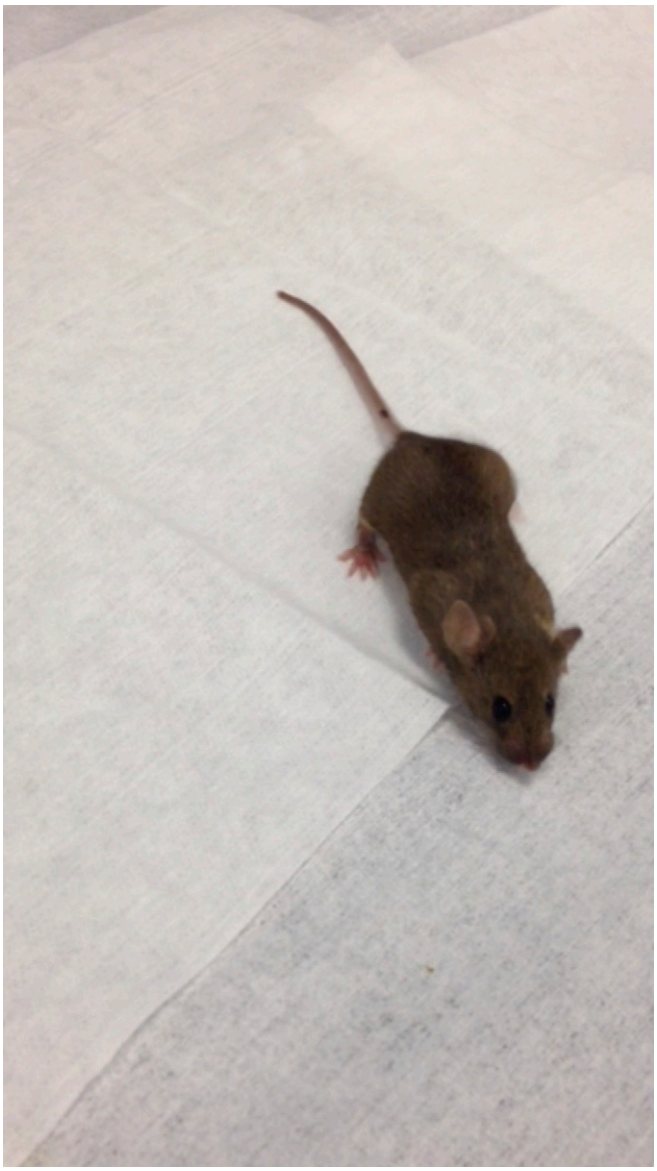
Genotype	Sex	Age at testing, mo	*n*	total *n*	Age range, mo
γ-2 PC WT; γ-7 WT	F	6–7 ($n = 2$); 9–10 ($n = 7$)	9	13	6–10
	M	6 ($n = 3$); 9 ($n = 1$)	4		
γ-2 PC WT; γ-7 KO	F	5–7 ($n = 9$); 11 ($n = 2$)	11	21	5–11
	M	5 ($n = 3$); 8 ($n = 5$); 11 ($n = 2$)	10		
γ-2 PC WT; γ-7 WT	F	8 ($n = 2$); 10 ($n = 2$)	4	6	8–10
	M	9–10 ($n = 2$)	2		
γ-2 PC KO; γ-7 KO	F	5 ($n = 1$); 7 ($n = 2$); 9–10 ($n = 3$)	6	12	5–12
	M	5–7 ($n = 4$); 10 ($n = 1$); 12 ($n = 1$)	6		

For an explanation of genotype nomenclature, refer to Fig. 6*A*.

Table S2. Ataxia scores: *n* per genotype

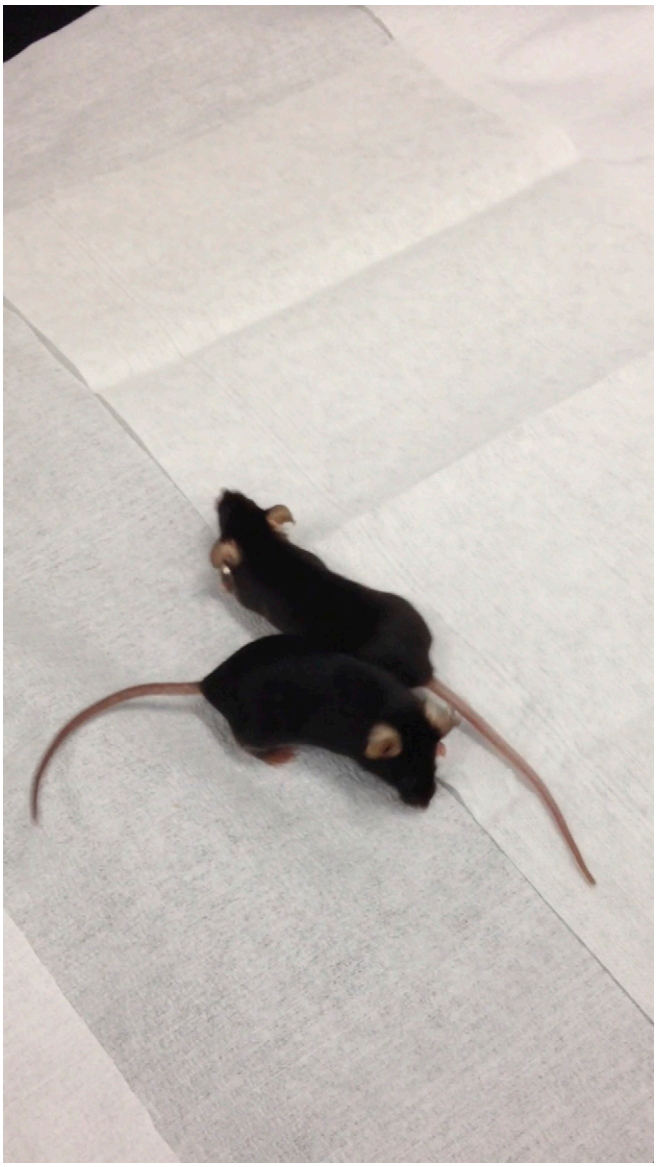
	Ataxia score			
Genotype	0	1	Total ataxic mice	Total % ataxic mice
γ-2 PC WT; γ-7 WT	13	0	0	0
γ-2 PC WT; γ-7 KO	21	0	0	0
γ-2 PC WT; γ-7 WT	6	0	0	0
γ-2 PC KO; γ-7 KO	0	12	12	100

Ataxia score of 0, no ataxia was observed; ataxia score of 1, ataxia was observed. 12/12 mice from the γ-2 PC KO; γ-7 KO group were ataxic, whereas none of the other mice were. For an explanation of genotype nomenclature, refer to Fig. 6*A*.



Movie S1. Example of the ataxia displayed by a γ-2 PC KO; γ-7 KO mouse.

Movie S1



Movie S2. Example of a γ-2 PC KO; γ-7 KO mouse (Cre^{+}) shown alongside a γ-2 PC WT; γ-7 KO littermate (Cre^{-}).

Movie S2