Supplementary Figure Legends

Supplementary Figure 1  Impaired grip force in JP-DKO mice

Body weight (BW) and grip force (GF) were examined in 7-week-old mice. The data indicate mean ± SD (n = 15 for wild-type; n = 7 for JP3-KO; n = 10 for JP4-KO; n = 10 for JP-DHE; n = 9 for JP-DKO). Grip force was examined using a grip strength meter (MK-380S, Muromachi Co., Japan). Significant differences compared with wild-type controls are marked by asterisks (*p < 0.05, **p < 0.01).

Supplementary Figure 2  Normal histology in JP-DKO cerebellum

Cerebellar morphology was examined in JP-DHE (A,C,E,G,I) and JP-DKO (B,D,F,H,J) mice at 6-8-weeks old. (A, B) Hematoxylin staining showing normal cerebellar histology in JP-DKO mice, including cerebellar size, foliation and trilaminar organization of cerebellar cortex. (C-J) Double immunofluorescence for calbindin (marker for PCs) and for VGluT2 (vesicular glutamate transporter 2; marker for CF terminals), VGluT1 (vesicular glutamate transporter 1; marker for PF terminals), or GFAP (glial fibrillary acidic protein; marker for Bergmann glia). JP-DKO mice were normal in monolayer alignment and dendritic branching of PCs, distribution of excitatory and inhibitory terminals, and arrangement of Bergmann fibers. Scale bars, 500 µm in A and B; 20 µm in C-J. For the analysis, mice were pentobarbital-anesthetized and perfused with 4% paraformaldehyde in phosphate-buffered saline. The brains were removed, postfixed for 24 hr in 4% paraformaldehyde, and cryoprotected in 30% sucrose. Cerebellar sections were processed for staining with hematoxylin or double immunohistochemical staining. Images were taken with a confocal laser microscope (FV1000, Olympus).

Supplementary Figure 3  RyRs, but not IP$_3$Rs, contribute to sAHP generation

(A) Representative voltage responses to CF stimuli recorded from JP-DHE PCs in current-clamp
mode. RyR blockers, ruthenium red (RuR, 30 µM) and dantrolene (DTL, 30 µM), abolished sAHP, whereas IP₃R inhibitors, heparin (4 mg/ml), and IP₃ 5-phosphatase (5ppase) had no effects on the CF-evoked response. According to our previous study demonstrating that postsynaptic IP₃ signaling maintains presynaptic functions of PF-PC synapses via BDNF (Furutani et al, 2006), the activity of 5ppase overexpressed in PCs using the viral vector was confirmed by significantly increased paired-pulse ratios of PF-EPSCs (interpulse interval = 100 ms; mean ± SEM: non-infected PCs, 1.688 ± 0.090 (n = 8); infected PCs, 2.090 ± 0.100 (n = 5); p < 0.01, t-test). (B) Average amplitudes of sAHP in JP-DHE PCs treated with the inhibitors or vehicle (control). The data represent mean ± SEM (n = 5 for control, heparin and 5ppase; n = 6 for RuR and DTL). ***p < 0.001, significantly different from control value in t-test.

Supplementary Figure 4  Normal Ca^{2+} transients evoked by CF stimuli in JP-DKO PCs

(Left panel) Typical traces of Ca^{2+} transients recorded from JP-DHE and JP-DKO PCs treated with vehicle (black) or ryanodine (red). No significant differences in amplitude and decay time of the transient profile were detected between the groups (p > 0.05, t-test, lower table). (Center and right panels) Minimal effects of ryanodine on overall Ca^{2+} transient in JP-DHE and JP-DKO PCs. No significant differences (p > 0.05, t-test) in the amplitude (center) and decay time (right) normalized by the value before the application of ryanodine were seen between JP-DHE and JP-DKO PCs. PCs were loaded for at least 20 min with a Ca^{2+} indicator Oregon Green 488 BAPTA-1 (Molecular Probes, 100 µM) through patch pipette filled with the K⁺-based intracellular solution that was composed of (in mM): 130 K D-gluconate, 10 KCl, 10 NaCl, 10 HEPES, 0.5 EGTA, 4 Mg-ATP and 0.4 Na-GTP (pH 7.3, adjusted with KOH). Ryanodine (100 µM), dissolved in high K⁺ (15 mM) solution, was bathly applied. Fluorescence images were acquired by using a high-speed confocal laser-scanning microscope (Oz, NORAN Instruments Inc.) attached to an upright microscope (Olympus BX50WI) as described previously (Hashimoto et al, 2001). The Ca^{2+}-dependent
fluorescence signals from the dendritic regions were background-corrected and expressed as increases in fluorescence divided by the prestimulus fluorescence values ($\Delta F/F_0$) using Igor Pro software (Wavemetrics).

**Supplementary Figure 5  Specificity of RyR1 immunohistochemistry**

(A) Amino acid sequence of RyR1 used for polyclonal antibody production (underlined). This region shows low homology to RyR2 and is lacking RyR3. (B) Immunoblot analysis. Affinity-purified antibody selectively detected of RyR1 in skeletal muscle (SM) and brain (Br) but did not cross-react with RyR2 in cardiac muscle (CM). Pre-absorption with the antigen peptide (abs) abolished immunoreactivity of the antibody. (C) Immunofluorescence labels skeletal muscle (SM), but not cardiac muscle (CM). Immunolabeling was not seen after absorption with the antigen peptide (abs). (D) Overall immunoreactivity of RyR1 in the adult mouse brain. The staining pattern is consistent with that of *in situ* hybridization using a RyR1-specific probe (Mori et al, 2000). (E) Intense immunolabeling for RyR1 in dendrites and perikarya of PCs. Cb, cerebellum; CP, caudate-putamen; Cx, cortex; Hi, hippocampus; Mb, midbrain; MO, medulla oblongate; OB, olfactory bulb; Po, pons; Th, thalamus. Scale bars, 1 $\mu$m in C, 1 mm in D, 10 $\mu$m in E. For antibody production, cDNAs encoding the divergent region 2 of mouse RyR1 (1343-1404, database accession number AY268935) was amplified by polymerase chain reaction and subcloned into BamHI/EcoRI site of the pGEX4T-2 plasmid vector (GE Healthcare Bioscience). Glutathione S-transferase (GST) fusion proteins thus yielded were repeatedly injected subcutaneously into rabbit to prepare antiserum. Specific antibodies were collected by affinity purification using GST-free polypeptides coupled to CNBr-activated Sepharose 4B (GE Healthcare Bioscience).

**Supplementary Figure 6  Specificity of SK2 immunohistochemistry**

Affinity-purified polyclonal antibody to SK2 produced intense signals in the cerebellum of
wild-type mice (A,C,E), but not in that of SK2-knockout mice (B,D,F). DCN, deep cerebellar
nuclei; gc, granular layer; ml, molecular layer; pc, Purkinje cell layer; wm, white matter. Scale bars,
200 µm in A, B; 100 µm in C, D; 50 µm in E, F. For antibody production, cDNAs encoding the
carboxyl-terminus of mouse SK2 (amino acid residues 536-574, database accession number
NM080465) was amplified by polymerase chain reaction and subcloned into BamHI/EcoRI site of
the pGEX4T-2 plasmid vector (GE Healthcare Bioscience). Glutathione S-transferase (GST) fusion
proteins thus yielded were repeatedly injected subcutaneously into guinea pig and rabbit to prepare
antiserum. Specific antibodies were collected by affinity purification using GST-free polypeptides
coupled to CNBr-activated Sepharose 4B (GE Healthcare Bioscience).

Supplementary Figure 7 Expression of JP4-DsRed and DsRed-JP4 in JP-DKO PCs

The sindbis virus vector for expressing JP4-DsRed or DsRed-JP4 was injected into the cerebellar
cortex of JP-DKO mice, and 24-36 hrs later fluorescence signals were detected in infected PCs.
Although both fusion proteins were basically expressed in somatodendritic regions (left image),
JP4-DsRed signals were preferentially detected as puncta along cell periphery in both the soma
(middle image) and dendritic shaft regions (right image), whereas DsRed-JP4 signals were
uniformly distributed to the inside of PCs. The restoration of apamin (Apa, 200 nM)-sensitive
sAHP was observed in JP4-DsRed-expressing PCs, but not in DsRed-JP4-expressing PCs and
nontransfected PCs lacking fluorescence signals (see
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ΑΗΠ αµπλιτυδεσ ισ σηοων ιν Φιγυρε 6Β. Σχαλε βαρσ, 50 µm in left images, 10 µm in middle
images, 5 µm in right images.