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_3Rs, 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 IP_3R inhibitors, heparin (4 mg/ml), and IP_3 5-phosphatase (5ppase) had no effects on the CF-evoked response. According to our previous study demonstrating that postsynaptic IP_3 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 antigene 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 μm in C, 1 mm in D, 10 μm 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 τραχεσ οφ ρεπρεσεντατιωε ωολταγε ρεσπονσεσ ιν ριγητ πανελ). Συμμαρψ ηιστογραμ οφ σ ΑΗΠ αμπλιτυδεσ ισ σηοων ιν Φιγυρε 6Β. Σχαλε βαρσ, 50 μm in left images, 10 μm in middle images, 5 μm in right images.