SUPPLEMENTAL INFORMATION

Supplemental Methods

Cell-surface biotinylation assay

HEK 293 cells transfected with GluRδ2^{Lc}, GluRδ2^{Lc}-V/R, or a *hotfoot-4J* mutant (GluRδ2^{ho}) were treated with a membrane-impermeable biotinylation reagent (EZ-link sulfo-NHS-LC-Biotin; Pierce, Rockford, IL) as described previously (Matsuda & Yuzaki, 2002). Subsequently, cells were solubilized in 500 μl TNE buffer (50 mM NaF, 10 % NP-40, 20 mM EDTA, 1 μM pepstatin A, 2 μg/ml leupeptin, 10 μg/ml aprotinin, 0.1 % SDS, 50 mM Tris-HCl, pH 8.0) and immunoprecipitated using streptavidin-coupled beads (Amersham Pharmacia, Piscataway, NJ) and immunoblotted with anti-GluRδ2 antibody (Chemicon, Temecula, CA).

Electrophysiology

Parasagittal slices (200-μm thick) were prepared from cerebella infected with recombinant Sindbis viruses, and whole-cell patch-clamp recordings were performed using Purkinje cells that emitted nYFP fluorescence. The resistances of the patch pipettes were 3–5 MΩ when filled with an internal solution composed of (in mM): 65 Cs-methanesulfonate, 65 K-gluconate, 20 HEPES, 10 KCl, 1 $MgCl_2$, 4 Na_2ATP, 1 Na_2GTP, 5 sucrose and 0.4 EGTA (pH 7.25, 295 mOsm/kg). The solution used for slice storage and recording consisted of the following (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 D-glucose, bubbled continuously with a mixture of 95% O_2 and 5% CO_2 at room temperature. Picrotoxin (100 μM,

Sigma, St. Louis, MO, USA) was always present in the saline to block inhibitory synaptic transmission. For the recording of PF–evoked excitatory postsynaptic currents (PF–EPSCs), square pulses (50 μs, 20–100 μA) were applied to the PFs through a stimulating glass pipette placed on the molecular layer (~50 μm away from the pial surface).

In the LTD sessions, PF–EPSCs were recorded successively at a frequency of 0.1 Hz from Purkinje cells clamped at –80 mV. After a stable PF–EPSC amplitude had been observed for at least 10 min, a conjunctive stimulation that consisted of 30 single PF stimuli together with a 200-ms depolarizing pulse from a holding potential of –60 to +20 mV was applied for the LTD induction. Access resistances were monitored every 10 s by measuring the peak currents in response to hyperpolarizing steps (50 ms, 2 mV) throughout the experiments; the measurements were discarded if the resistance changed by more than 20% of its original value. Signals were filtered at 1 kHz and digitized at 4 kHz.

Supplemental Figure

Supplemental Figure 1. Cell surface expression of GluRδ2^{Lc} and GluRδ2^{Lc}-V/R. Cells transfected with GluRδ2^{Lc} or GluRδ2^{Lc}-V/R were treated with a membrane-impermeable biotinylation reagent. A *hotfoot-4J* mutant (GluRδ2^{ho}) known to be retained in the endoplasmic reticulum (Matsuda & Yuzaki, 2002) was used as a negative control. Solubilized membranes were precipitated using streptavidin-coupled beads and immunoblotted with anti-GluRδ2 antibody. *A*, Representative results of six independent experiments. Cell lysate fractions (Input), which corresponded to 1% of total lysates, and biotinylated fractions (Biotin), which were precipitated by

streptavidin-coupled beads and corresponded to 18.8% of total lysates, were compared. *B,* A quantitative analysis of the ratio of surface GluRδ2 expression. Band intensities of GluRδ2 in the surface biotinylated fractions were normalized by those in the total lysates. The surface expression ratio of GluRδ2^{Lc} was arbitrarily established as 100%. Error bars indicate the SEM. $^{**}P < 0.01$; $^{*}P < 0.05$; n.s., no significant.

Reference

Matsuda S & Yuzaki M. (2002). Mutation in *hotfoot-4J* mice results in retention of δ2 glutamate receptors in ER. *Eur J Neurosci* **16**, 1507-1516.

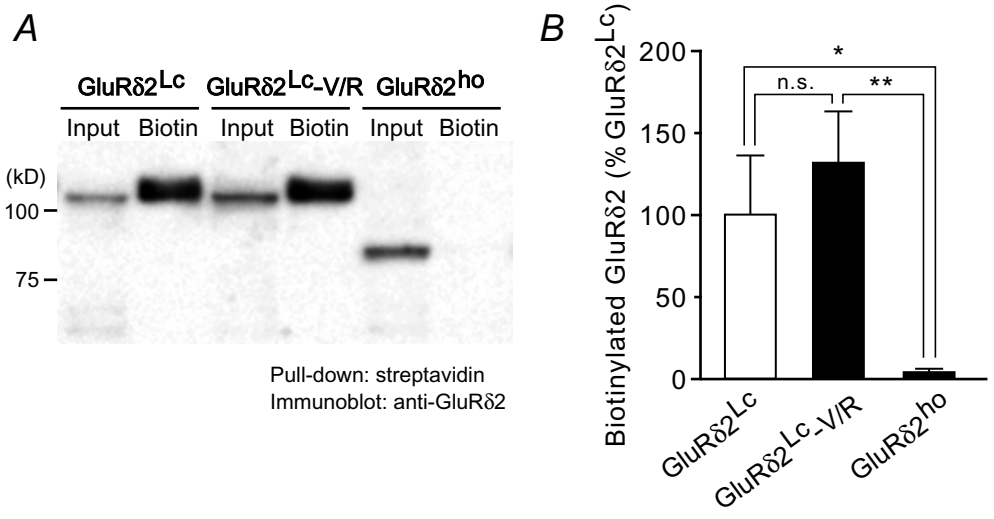

Fig. S1 (Kakegawa et al.)