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Supplemental information

PRRT2 modulates presynaptic Ca^{2+} influx by interacting with P/Q-type channels

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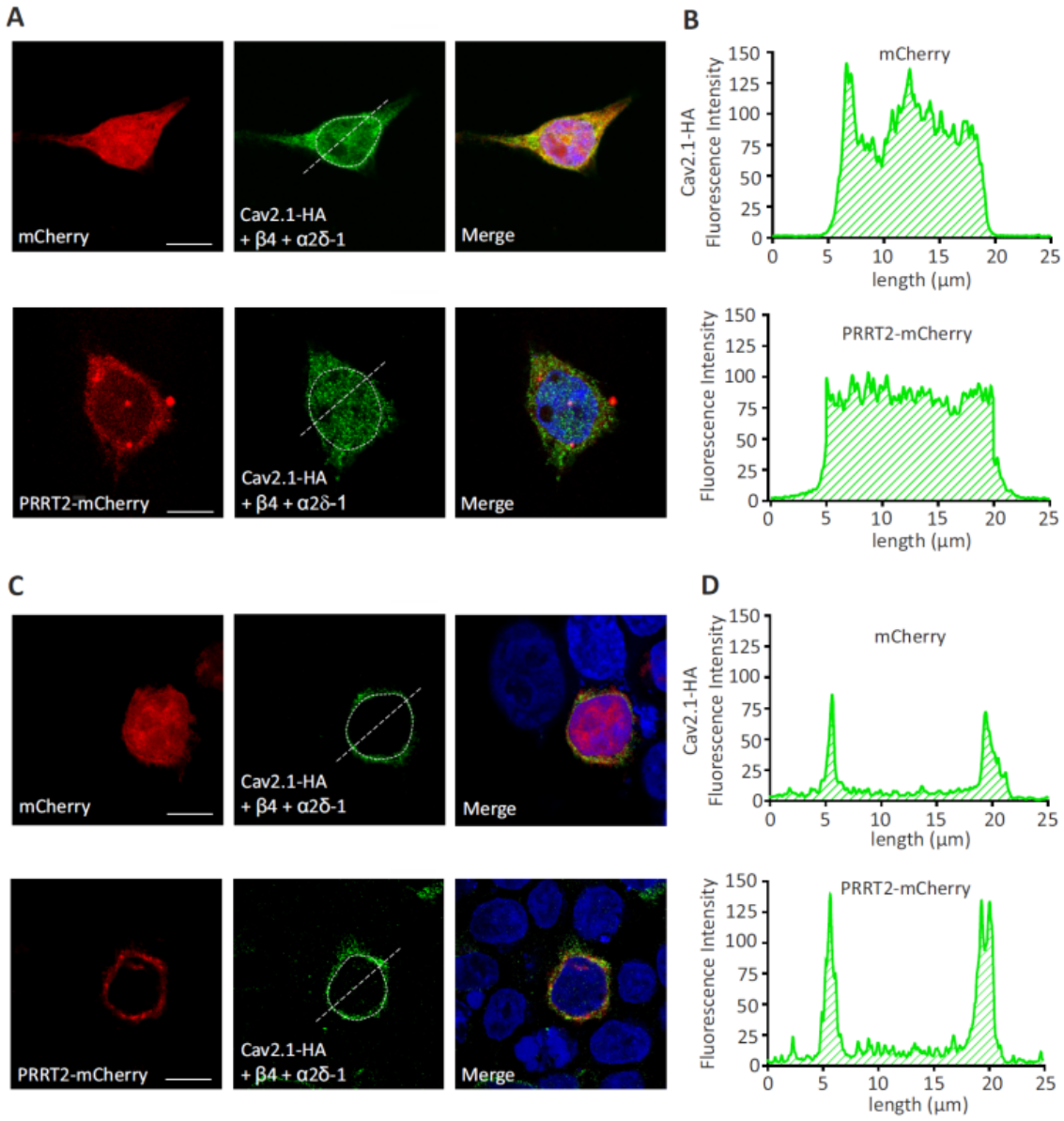

Figure S1. Cav2.1-HA immunostaining in permeabilized and non-permeabilized HEK293 cells. [Related to Figure 4]

A. Representative fluorescence images of permeabilized HEK293 cells transiently co-transfected with Cav2.1-HA/α2δ-1/β4 and either mCherry or PRRT2-mCherry (*red*) and retrospectively stained with anti-HA antibody (*green*). The white line, corresponding to the major axis of the cell, was used to measure the fluorescence intensity of Cav2.1-HA immunostaining. **B.** Intensity profiles of Cav2.1-HA fluorescence in HEK293 cells expressing either mCherry (*upper panel*) or PRRT2-mCherry (*lower panel)*. **C**. Representative fluorescence images of non-permeabilized HEK293 cells treated and analyzed as described in panel as in A. **D.** Intensity profiles of Cav2.1-HA fluorescence in HEK293 cells expressing either mCherry (*upper panel*) or PRRT2-mCherry (*lower panel)*. Scale bar, 10 μm.

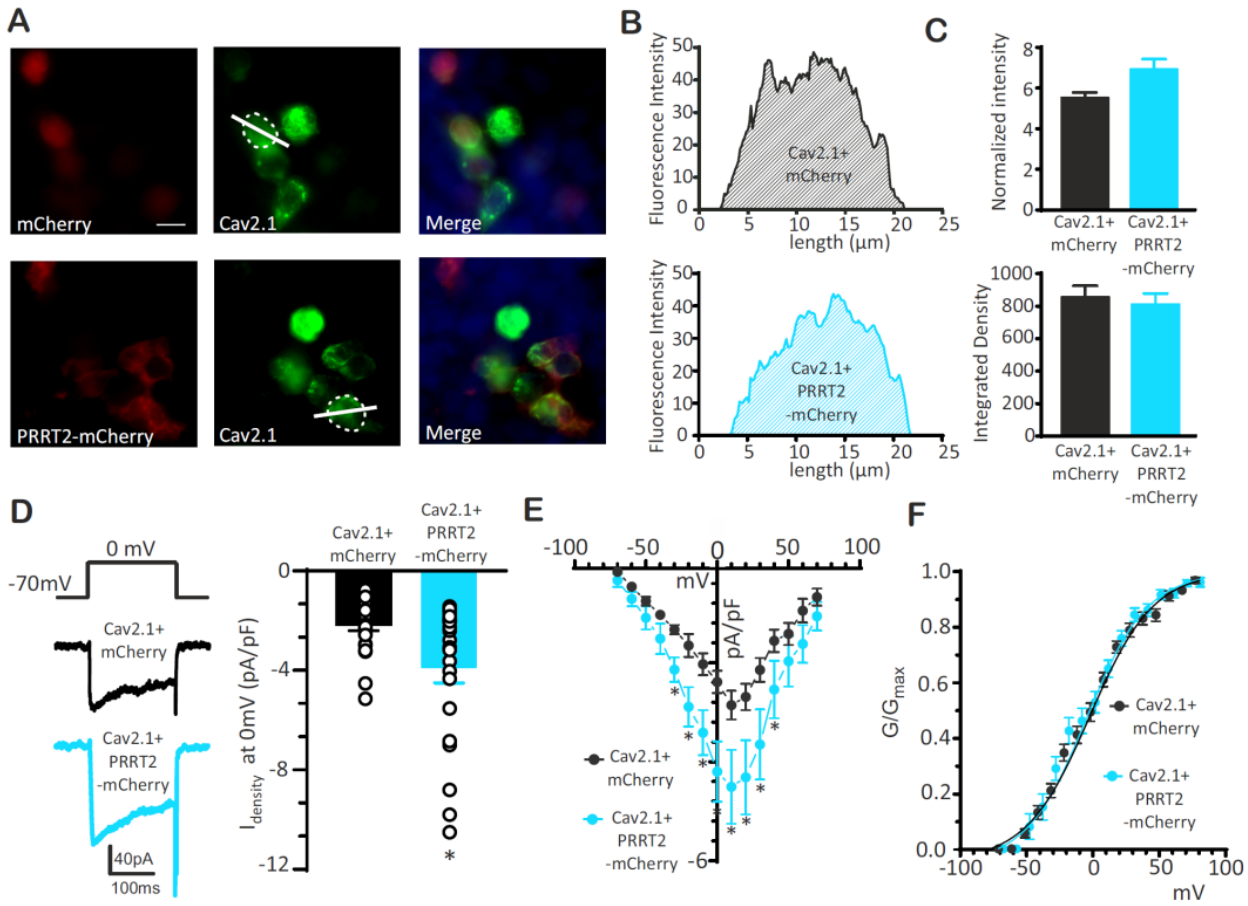

Figure S2. Expression of exogenous PRRT2 increases P/Q-type Ca^{2+} currents in HEK293 cells expressing the Cav2.1 subunit. [Related to Figure 4]

A. Representative fluorescence images of HEK293 cells transiently co-transfected with the Cav2.1subunit and either mCherry alone or PRRT2-mCherry (*red*) and retrospectively stained with Cav2.1-specific antibodies (*green*). The white line, corresponding to the major axis of the cell, was used to measure the fluorescence intensity of Cav2.1 immunostaining. Scale bar, 10 µm. **B.** Intensity profiles of Cav2.1 fluorescence along the major cell axis in HEK293 cells expressing either mCherry (*grey area*) or PRRT2-mCherry (*light blue area*). **C.** Normalized mean fluorescence intensity (*top*; n=48 and 45 cells for mCherry and PRRT2-mCherry, respectively) and integrated fluorescence density (*bottom*; n=52 and 42 cells for mCherry and PRRT2-mCherry, respectively) of Cav2.1 immunoreactivity in HEK293 cells expressing either mCherry (black bars) or PRRT2-mCherry (light blue bars). No PRRT2-induced changes in Cav2.1 expression were observed. Data are means ± sem. **D**. *Left:* Representative traces of voltage-gated Ca^{2+} currents evoked by a 200-ms voltage step at 0 mV (V_h= -70 mV) 2 days after HEK293 cell transfection with Cav2.1 and either mCherry alone (*black*) or PRRT2-mCherry (*light blue*) constructs. *Right:* Individual data and means ± sem of current density ($I_{density}$) values recorded in HEK293 cells expressing Cav2.1 (*black*) or Cav2.1/PRRT2 (*light blue*). **E.** $I_{density}$ *vs* voltage (V) relationships for HEK293 cells expressing Cav2.1 (*black*) or Cav2.1/PRRT2 (*light blue*). **F.** Normalized conductance-voltage curves of HEK293 cells expressing Cav2.1 (*black*) or Cav2.1/PRRT2 (*light blue*). Curves were fitted to the Boltzmann equation. *p<0.05, **p<0.01, unpaired Student's *t*-test/Mann-Whitney's *U*-test (n=19 for both mCherry- and PRRT2-mCherry infected neurons; n=25 and 23 for Cav2.1- or Cav2.1/PRRT2-expressing HEK293 cells).

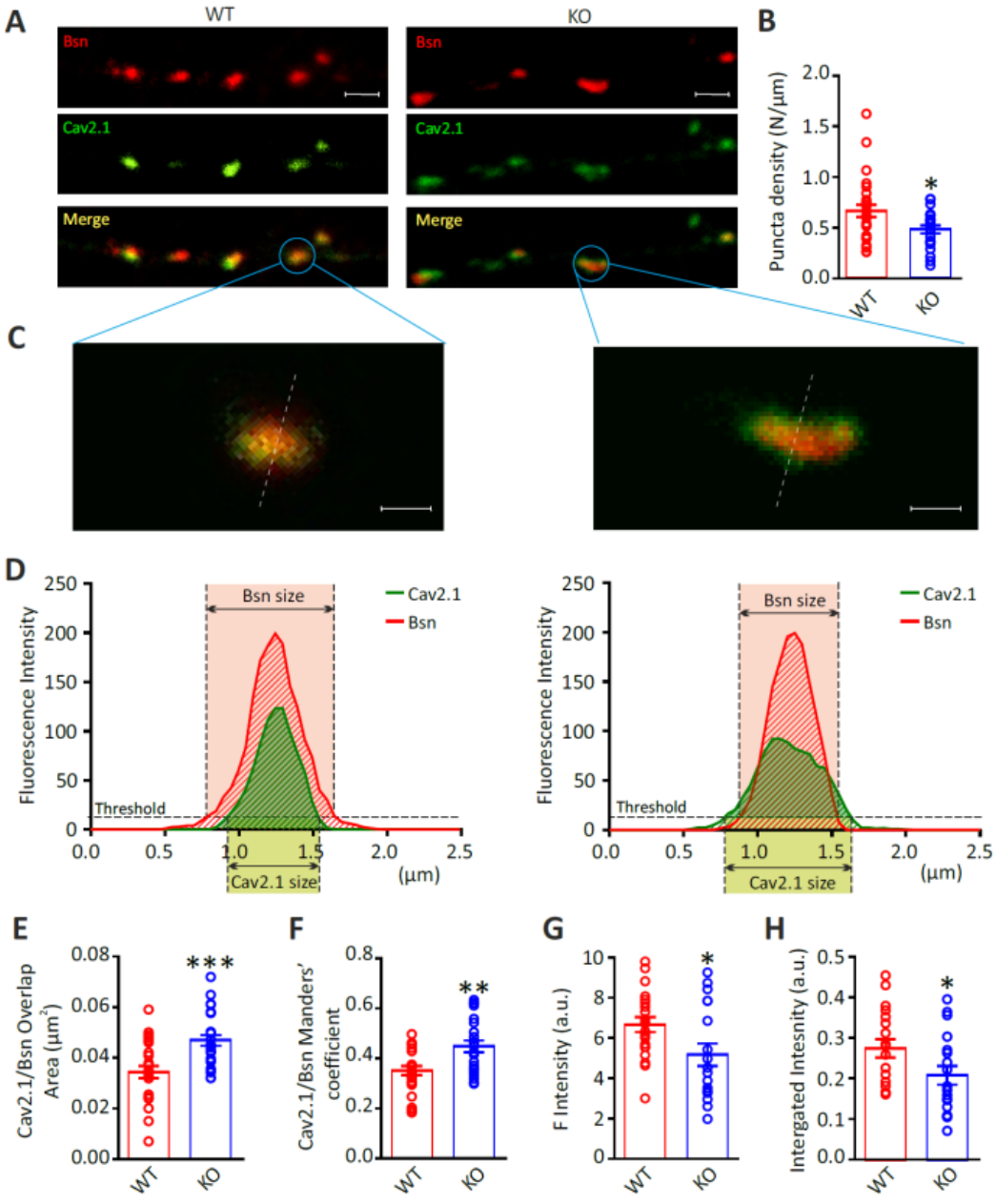

Figure S3. Image scanning microscopy (ISM) confirming the dispersion of P/Q-type Ca^{2+} channels in PRRT2-deficient presynaptic terminals. [Related to Figure 6]

A. Representative ISM images of synaptic contacts from 14 DIV primary hippocampal neurons. Double-immunostaining for Bassoon (Bsn; red) and Cav2.1 (green) was used to analyze the localization of Cav2.1 at Bsn-labeled presynaptic boutons in WT (*left*) and PRRT2 KO (*right*) hippocampal neurons. Merge panels identify synaptic puncta in which Bsn and Cav2.1 colocalize (yellow). Scale bar, 1 µm. **B**. Quantification of the mean (± sem) linear density (puncta/µm) of double-stained synaptic boutons in WT (n=14) and PRRT2 KO (n=15) neurons, each from n=2 independent neuronal preparations. Individual values are superimposed. **C,D**. Representative zoomed detail of single WT (*left*) and PRRT2 KO (*right*) synaptic boutons where Bsn and Cav2.1 stainings colocalize (**C;** scale bar, 0.25 µm) and the corresponding distribution profile (**D**) of the immunofluorescence intensity of Cav2.1 (*green*) and Bsn (*red*). **E-H**. Quantitative analysis of the area of the Cav2.1 immunoreactivity (**E**), Manders' coefficient (**F**), mean (**G**) and integrated (**H**) fluorescence intensities of Cav2.1 measured within Bsn-positive areas. Means (± sem) are shown with superimposed individual values. *p<0.05, **p<0.01, ***p<0.001, Student's *t*-test (n=14 and 15 for WT and PRRT2 KO neurons, respectively, from n=2 independent neuronal preparations).