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Supplemental information

Celsr3 is required for Purkinje cell maturation and regulates cerebellar postsynaptic plasticity

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Supplementary materials

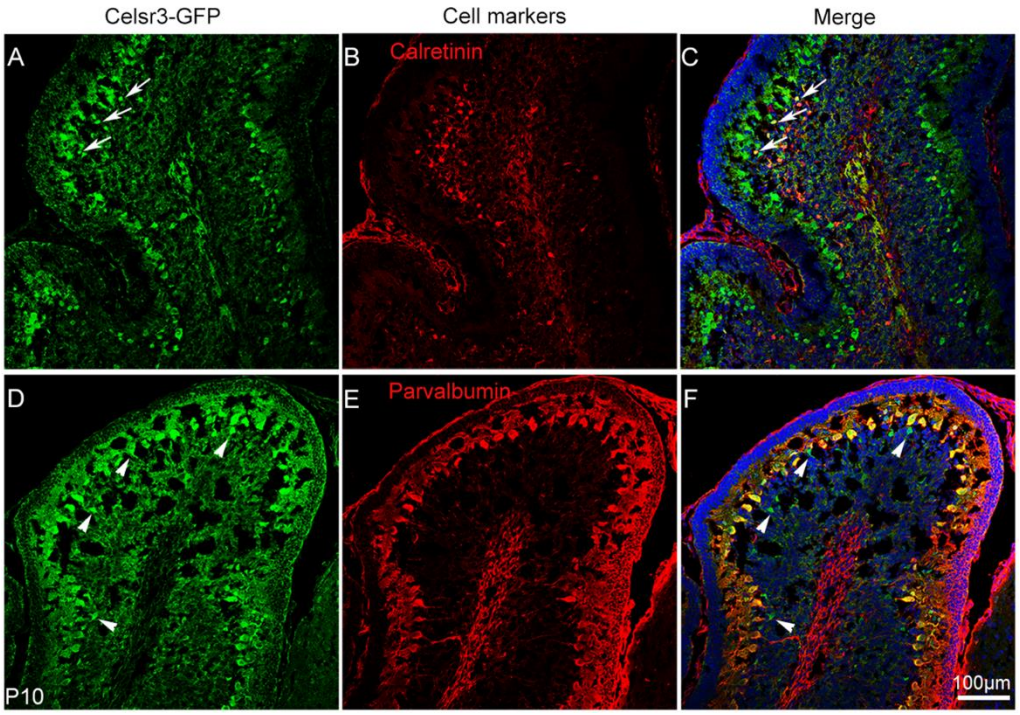

Fig. S1 A few Calretinin-positive cerebellar cells express Celsr3, Related to Figure 1

A-C: P10 cerebellar sections from *Celsr3*-GFP mice immunostained with anti-Calretinin (A, red) and anti-GFP antibodies (B, green). A few GFP-positive cells are positive for Calretinin (C, arrows).

D-F: In P10 sections, all Parvalbumin-positive PCs (red) co-express Ceslr3-GFP (green), but some green cells below the PC layer are negative for Parvalbumin (arrowheads in E and F).

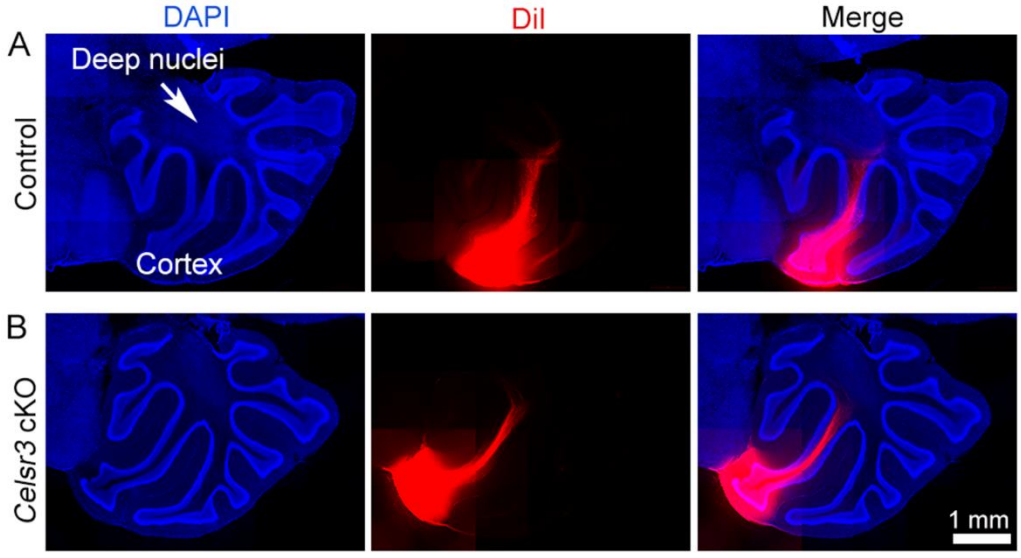

Fig. S2 *Celsr3* cKO PCs have normal axonal projections, Related to Figure 3.

DiI implantation into cerebellar cortex indistinguishably labels PC axons (red) projecting to cerebellar deep nuclei in control (A) and *Celsr3* cKO (B) animals. DAPI (blue) counterstained nuclei.

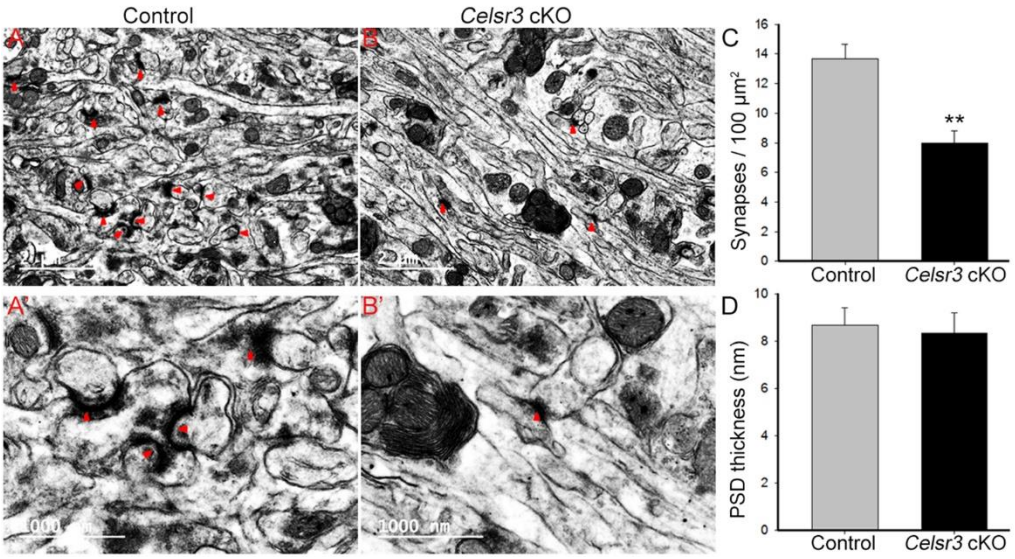

Fig. S3 *Celsr3* cKO in PCs decreases synapse density in cerebellar cortex, Related to Figure 3.

A, B: Ultrastructure of the molecular layer in adult control (A) and *Celsr3* cKO (B) cerebella, 13500x transmission electron microscopy. Red arrowheads indicate synapses.

A', B': Synapses (red arrowheads) at 35000x transmission electron microscopy in control (A') and *Celsr3* cKO (B').

C, D: Significant decrease of synapse density in the mutant compared to the control (7.9 ± 0.85 synapses/100µm^2 *versus* 13.7 ± 0.96 synapses/100µm^2; n = 3 animals in each group, *P*<0.05; Student's *t*-test). The thickness of post synapse density (PSD) is comparable in both genotypes (8.65 ± 0.73nm in the control *versus* 8.32 ± 0.86nm in the mutant, n = 3 animals in each group, *P*>0.05; Student's *t*-test).

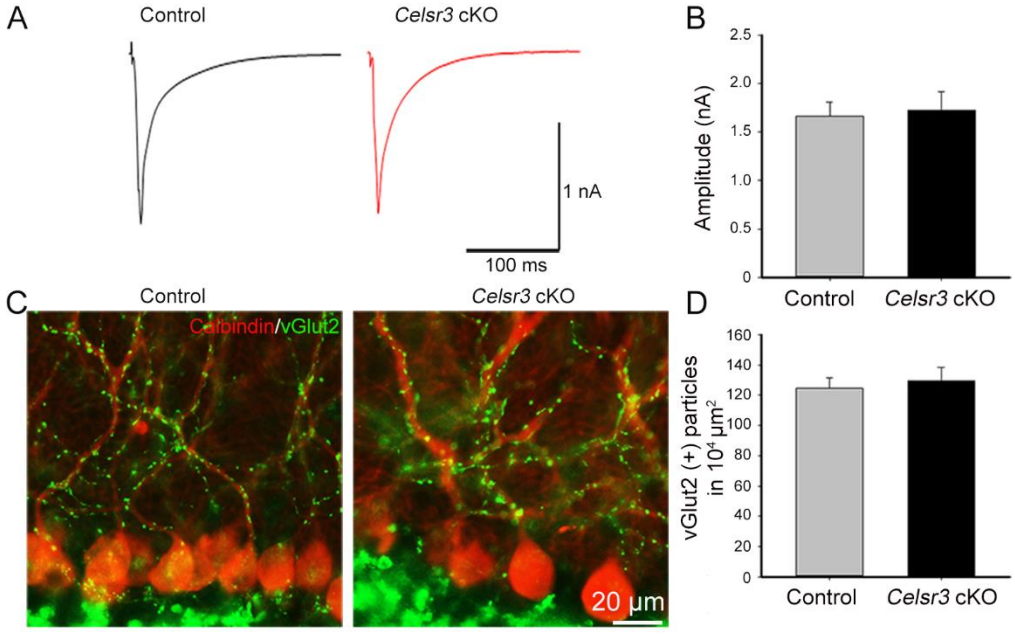

Fig. S4 *Celsr3* cKO PCs have normal CF innervation and CF-PC EPSCs, Related to Figure 5.

A: Representative traces of CF-PC EPSCs in control (black) and *Celsr3* cKO (red) mice.

B: The amplitude of CF-PC EPSC is comparable in two groups (1.66 ± 0.15 nA in the control *versus* 1.72 ± 0.19nA in the mutant; n = 4 animals in each group; *P*>0.05, Student's *t*-test).

C, D: In cerebellar sections, anti-Calbindin and -vGlut2 double fluorescent staining (C) showed that the density of vGlut2-positive particles on Calbindin-labelled dendrites were comparable in two groups (D; 124.5 ± 6.74/10^4μm^2 in the control *versus* 129.7 ± 8.7/10^4 μm^2 in the mutant; n = 3 animals in each group; *P*>0.05, Student's *t*-test).

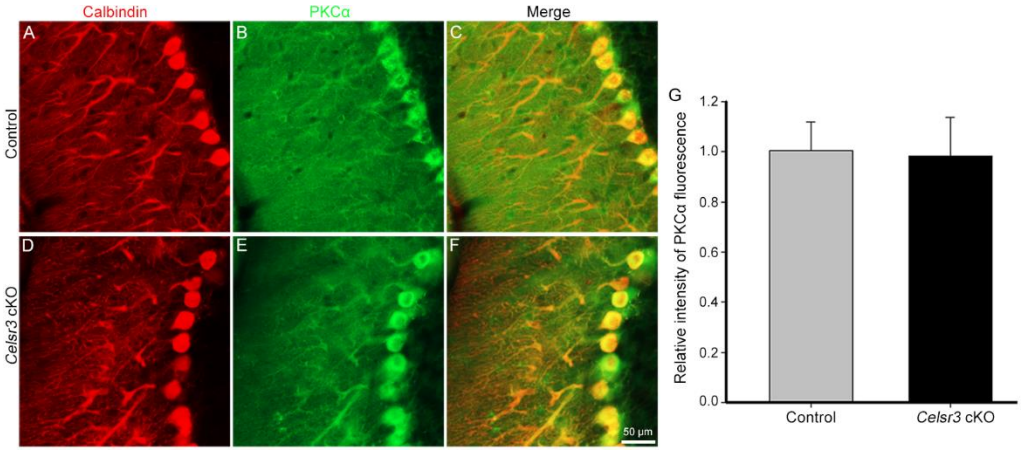

Fig. S5 *Celsr3* cKO in PCs does not affect PKCα basal expression in the cerebella, Related to Figure 7.

A-F: Cerebellar sections were performed for anti-Calbindin (A, D) and - PKCα (B, E) double fluorescent staining in the control (A-C) and the *Celsr3* cKO (D-F).

G: Statistic analysis showed that the relative fluorescent intensity of PKCα was comparable in two groups. *P*>0.05; Student's *t*-test; n = 3 animals in each group.