

SUPPLEMENTAL DATA

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Immunohistochemistry— One adult male and one 1-day-old (P1) rats were used. The adult male was anesthetized with pentobarbital and perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, and then the brain was removed and post-fixed in 4% paraformaldehyde for 12 hr. The brain of the anesthetized P1 rat was fixed by immersion for 12 hr in 4% paraformaldehyde in PB. After three rinses and overnight incubation in PB containing 20% sucrose, the brains were frozen at -60°C in methyl-butane. Sagittal cryostat sections (14 µm thick) were collected on Superfrost Plus slides (VWR, Strasbourg, France) and stored at -20°C until used. Immunohistochemistry was performed as described (15). Briefly, the tissue sections were incubated overnight at room temperature with either anti-CRMP2 (anti-pep4) (1:75 dilution), or anti-C-ter antibody (1: 300 dilution) followed by Alexa Fluo 488 goat anti-rabbit IgG antibodies. Nuclei were stained using DAPI reagent. Sections were view using the Axioplan II fluorescence microscope.

SUPPLEMENTAL FIGURE LEGEND

FIG S1. Nuclear localization of CRMP2 detected by anti-pep4 antibody in early post-natal rat brain, compared to adult brain Sagittal sections (14 μm thick) of cerebellum and cortex in early post-natal (P1) and adult rat brain were double stained with anti-pep4 antibody and DAPI, and then observed by fluorescence microscopy. **A, Expression of CRMP2 in rat cortex.** a-c, Immunolabeling at stage P1. a- Different layers of the cortex are indicated on the left, CRMP2 (green) is essentially detected in layer 5. b, c- High magnification images from this layer is shown, note that the CRMP2 is highly expressed in the nucleus (arrows). The merge images of anti-pep4 labeling and nuclear staining (blue) with DAPI show that both staining overlapped at P1. d-g, Immunolabeling at adult stage. d- In adult cortex, the CRMP2 expression is low and restricted in the cytosol. e, f, g- High magnification images show that the nucleus is not labeled by anti-pep4 antibody (arrowheads), merge images of CRMP2 labeling and nuclear staining (blue) show, unambiguously, that CRMP2 expression remained cytoplasmic in adult cortex. Scale bars, 200 μm for a and d, 50 μm for b, c, e and f, and 20 μm for g. **B, Expression of CRMP2 in cerebellum.** a-e, Immunolabeling at stage P1. a- CRMP2 is detected in the external part of the EGL (external granular layer), in the Purkinje cells layer, and in the IGL, internal granular layer. b, c, d, e- High magnification images of the Purkinje cells layer indicated by the white rectangle in a, show nuclear expression of CRMP2 (b, c). DAPI staining is shown in d. Merge image indicates, unambiguously, an overlapping labeling of DAPI with anti-pep4 antibody at P1. f-h, Immunolabeling at adult stage. f- In adult cerebellum, the CRMP2 is absent in Purkinje and other layers. g, h- high magnification image showing the absence of labeling by anti-pep4 antibody. Scale bars, 200 μm for a and f, 50 μm for b, g and h, and 20 μm for c-e.

FIG S2. Cytosolic localization of CRMP2 detected by anti-C-ter antibody in early post-natal rat brain. Sagittal sections (14 μm thick) of cerebellum and cortex in early P1 rat brain were double stained with anti-C-ter antibody and DAPI, and then observed by fluorescence microscopy. **A, Expression of CRMP2 in rat cortex at P1.** a- Merge image indicating different layers of the cortex labeled by anti-CRMP2 (anti-C-ter) antibody and DAPI. b- CRMP2 (green) is essentially detected in the cytosol by anti-C-ter antibody. Note the absence of nuclear detection by this antibody at P1. c- Merge image indicated that the anti-C-ter labeling is different from nuclear staining (blue). d,e,f- High magnification images show, unambiguously, that the nucleus is not labeled by anti-C-ter antibody (d), DAPI staining is shown in e, merge images show that at P1 CRMP2 detection by anti-C-ter remained cytosolic (f), while the anti-pep4 antibody detected CRMP2 at the nucleus at identical stage (see Fig. S1A). Scale bars, 100 μm for a, 50 μm for b, c, and, 20 μm for d, e, and f. **B, Expression of CRMP2 in rat cerebellum at P1.** a- CRMP2 is detected by anti-C-ter antibody in the Purkinje cells layer and in the IGL. b,c,d- High magnification images of the Purkinje cells layer indicated by a rectangle in a, show a cytosolic CRMP2 labeling by anti-C-ter antibody (b, d). DAPI staining is shown in c. Merge image clearly indicates that DAPI staining does not overlapped with anti-C-ter labeling at P1. The absence of immunolabeling by anti-C-ter antibody together with the nuclear labeling using anti-pep4 antibody demonstrate that the CRMP2 expressed in the nucleus is the short CRMP2 isoform, devoid of its C-terminal end. Scale bars, 100 μm for a, and, 20 μm for b, c, and d.

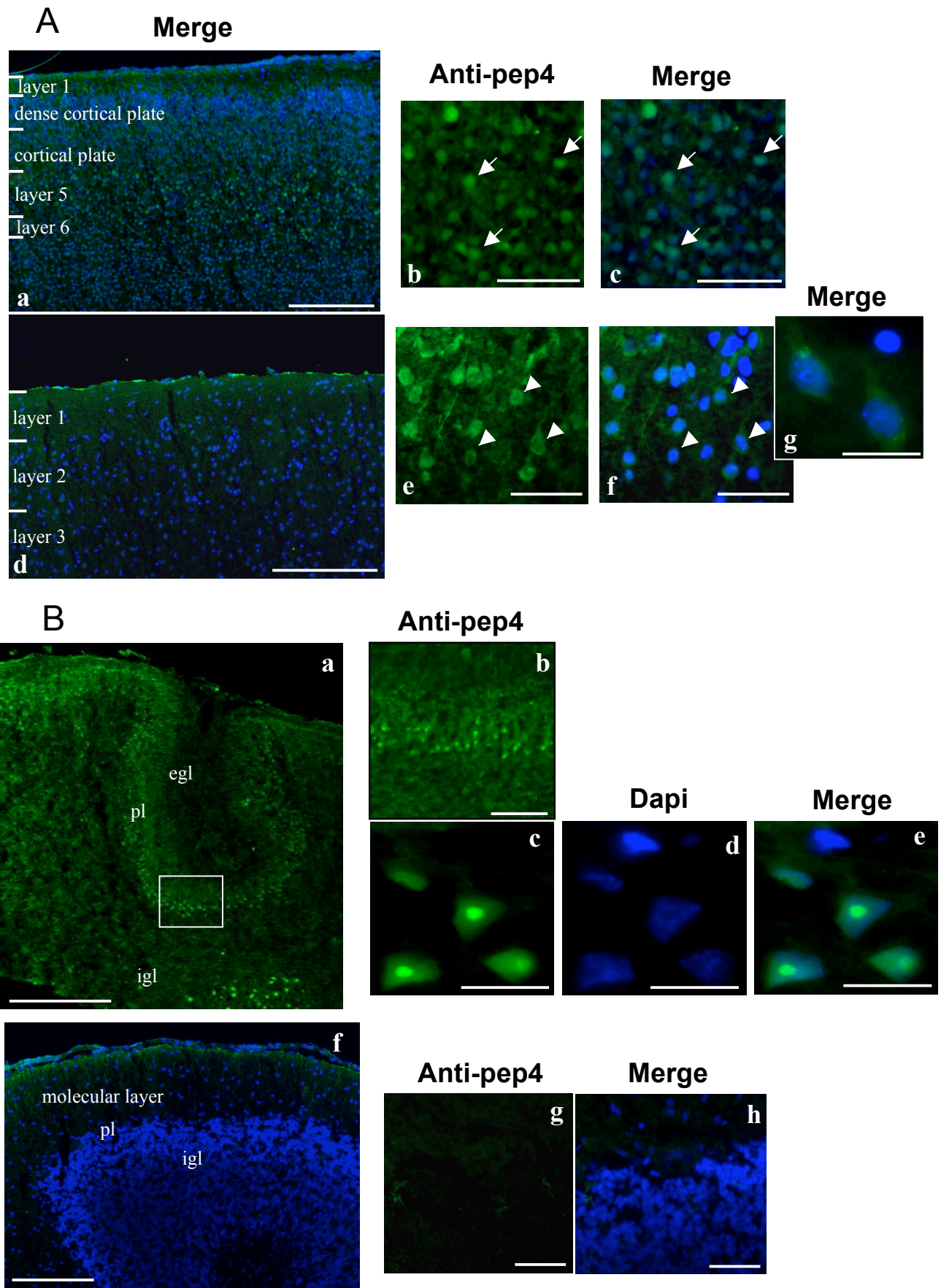


Figure S1

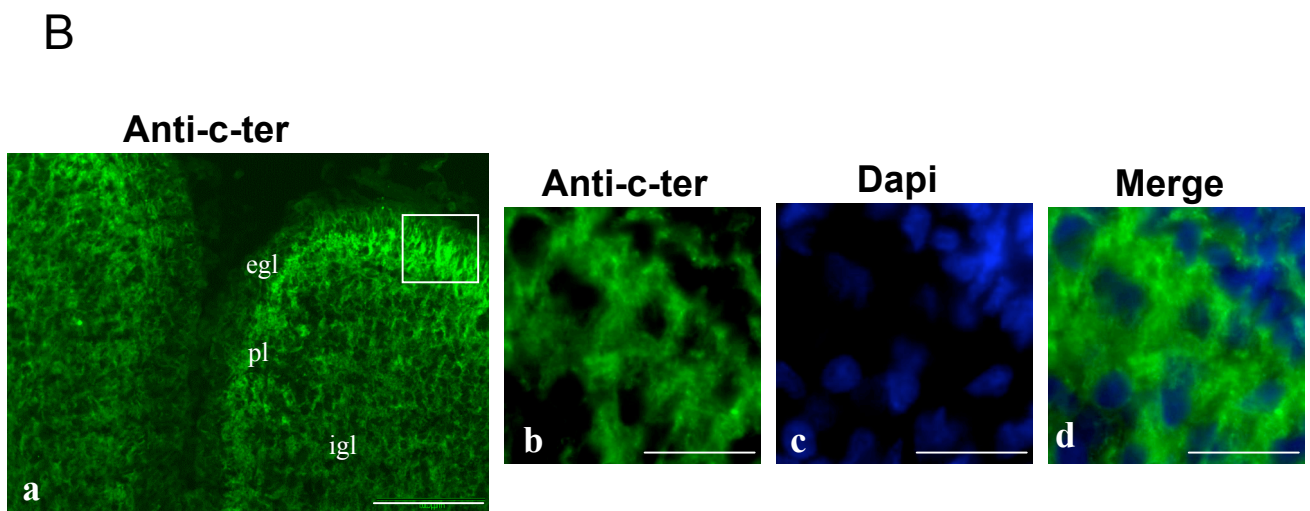
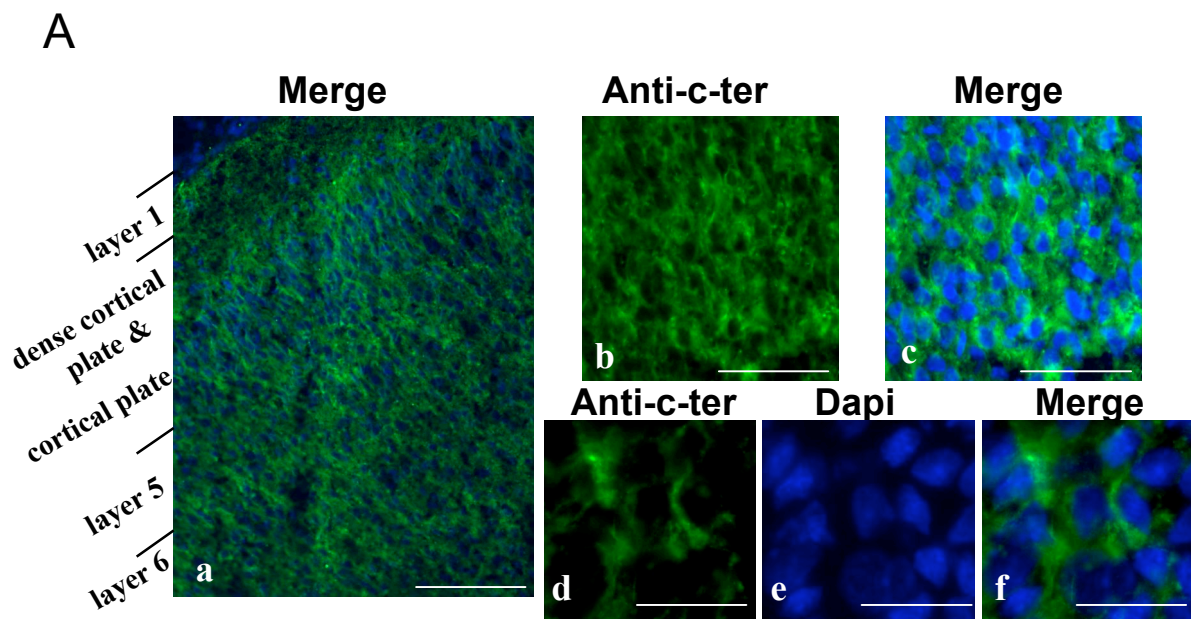


Figure S2