Supplementary information

The cellular prion protein mediates neurotoxic signaling of β-sheet-rich conformers independent of prion replication

Abbreviated title: Toxic signaling by PrP

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Figure legend

Supplementary Figure 1

(A) Co-cultivation with ScN2a cells increases apoptotic cell death in PrP^C-expressing SH-SY5Y cells. SH-SY5Y cells were co-transfected with EYFP and moPrP. 16 h after co-cultivation, SH-SY5Y cells were fixed on glass cover slips with 3.7% paraformaldehyde for 20 min, washed and permeabilized with 0.2% Triton-X 100 in PBS for 10 min at room temperature. Fixed cells were incubated with an anti-active caspase 3 antibody overnight at 4°C, followed by an incubation with the fluorescently labeled secondary antibody Alexa Fluor® 555 for 1 h at room temperature. Cells were then mounted onto glass slides and examined by fluorescence microscopy using a Zeiss Axiovert 200M microscope. Nuclei were stained with DAPI. **(B) PrP^{Sc} propagation assay.** After co-cultivation, cells were scraped off the plate, the cell pellets were washed twice with cold phosphate-buffered saline (PBS) and lysed in cold detergent buffer (0.5% Triton X-100, 0.5% sodium deoxycholat in PBS). The lysates were centrifuged to generate detergent-soluble and -insoluble fractions. The insoluble fractions were digested with proteinase K (10 µg/ml final concentration) for 0.5 h at 37°C. The reaction was terminated by the addition of PMSF and then both fractions were adjusted to 0.5% sarkosyl. PrP was immunoprecipitated using the monoclonal anti-PrP antibodies 3F4 (specific for the transfected PrP in the SH-SY5Y cells) or 4H11. Immunoprecipitated proteins were boiled with Laemmli sample buffer and analyzed by Western blotting using the monoclonal anti-PrP antibody 4H11. The arrow heads indicate the light chain of the antibody. **(C) The different PrP constructs are expressed at comparable levels**. SH-SY5Y cells expressing the indicated constructs or mock transfected (n.t.) and untransfected N2a cells were lysed in detergent buffer and equal amounts of proteins were analyzed by Western blotting using the anti-PrP antibody 4H11.

Supplementary Figure 2

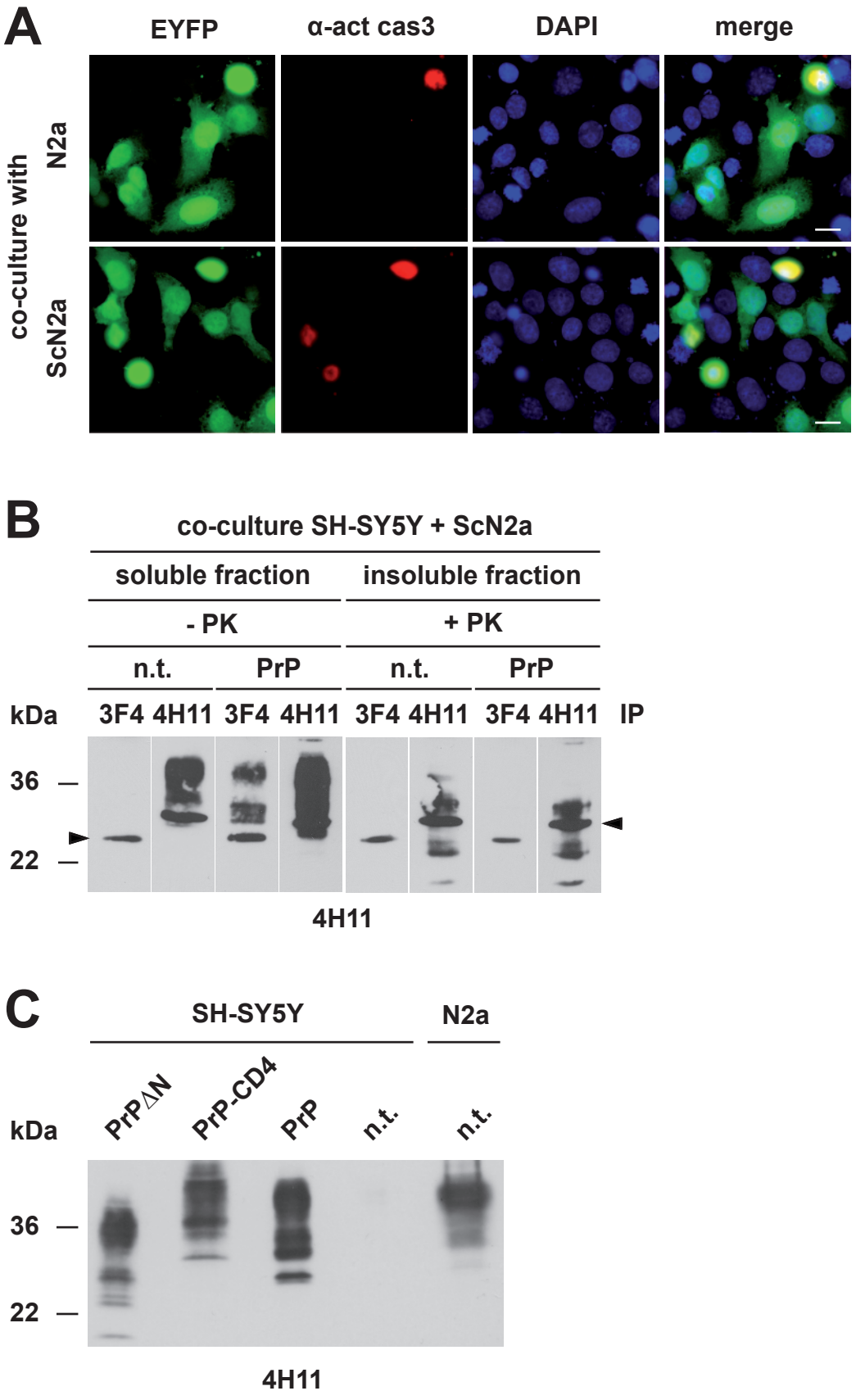
Size separation of Aβ42. Synthetic Aß42 peptides (PSL, Peptide Speciality Laboratories GmbH) were monomerized in formic acid and subsequently re-dissolved in water containing 0.1% ammonia. The aliquots were then loaded onto a Superose 12 (10/300 HR) column (Amersham Bioscience) and 1 ml fractions were collected in PBS as running buffer at a flow rate of 0.5 ml/min. Shown is a chromatogram and a Western blot of the high-molecular-weight- and the oligomer-fraction used for the experiments.

Supplementary Figure 3

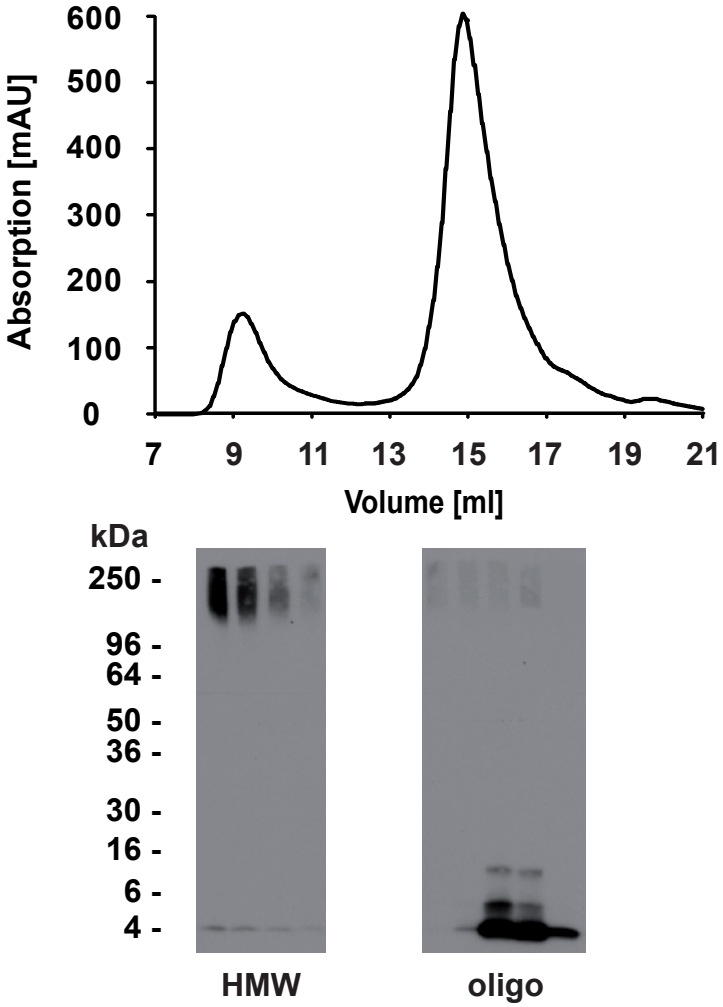
PrP^C expression in primary cortical neurons. (A) Cortical neurons prepared from either $Prnp^{0/0}$ or PrP^C-expressing wild type mice ($PrP^{+/+}$) were cultured for 6 days on poly-L-lysine

coated cover slips. Cells were analyzed by indirect immunoflourescence using the monoclonal anti-PrP antibody 4H11 and a polyclonal anti-β3 tubulin antibody as neuronal marker. The lower panel shows a Western blot analysis of three different preparations harvested after 6 days in culture. **(B)** Equal amounts of protein lysates prepared from transiently transfected SH-SY5Y cells (5Y) or PrP^{C}-expressing wild type primary neurons ($PrP^{+/+}$) cultured for 6 days were analyzed by Western blotting using the anti-PrP antibody 4H11.

Supplementary Figure 1, Resenberger et al., 2011



Supplementary Figure 2, Resenberger et al., 2011



Supplementary Figure 3, Resenberger et al., 2011