

Supplementary Information for

Highly infectious prions are not directly neurotoxic

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Other supplementary materials for this manuscript include the following:

Movies S1 to S6

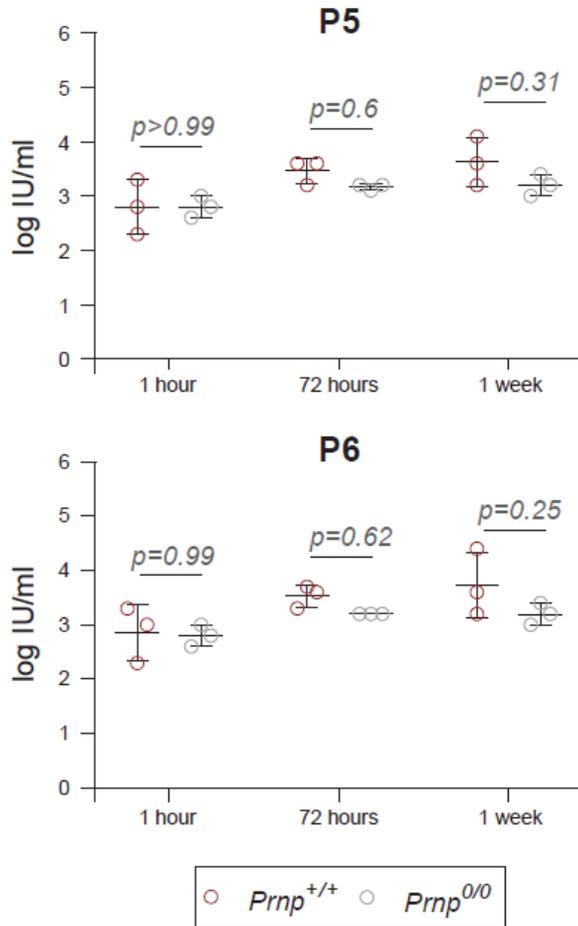


Fig. S1. Primary neuronal cultures do not propagate RML prions after a short treatment with RML prion-infected brain homogenate (RML BH). Three independent cortical cultures from *Prnp*^{+/+} and *Prnp*^{0/0} mice were treated with RML BH (brain concentration 10⁻⁴) for 1 h, 72 h or 1 week. Infectivity titer was determined by Automatic Scrapie Cell Assay in PK1/2 cells after the 5th (P5) and 6th (P6) split, mean ± S.D. Infectivity in *Prnp*^{0/0} cells represents residual infectivity of the inoculum. P values from 2-way ANOVA with Sidak's correction for multiple comparisons reveal no significant difference between genotypes upon any duration of treatment.

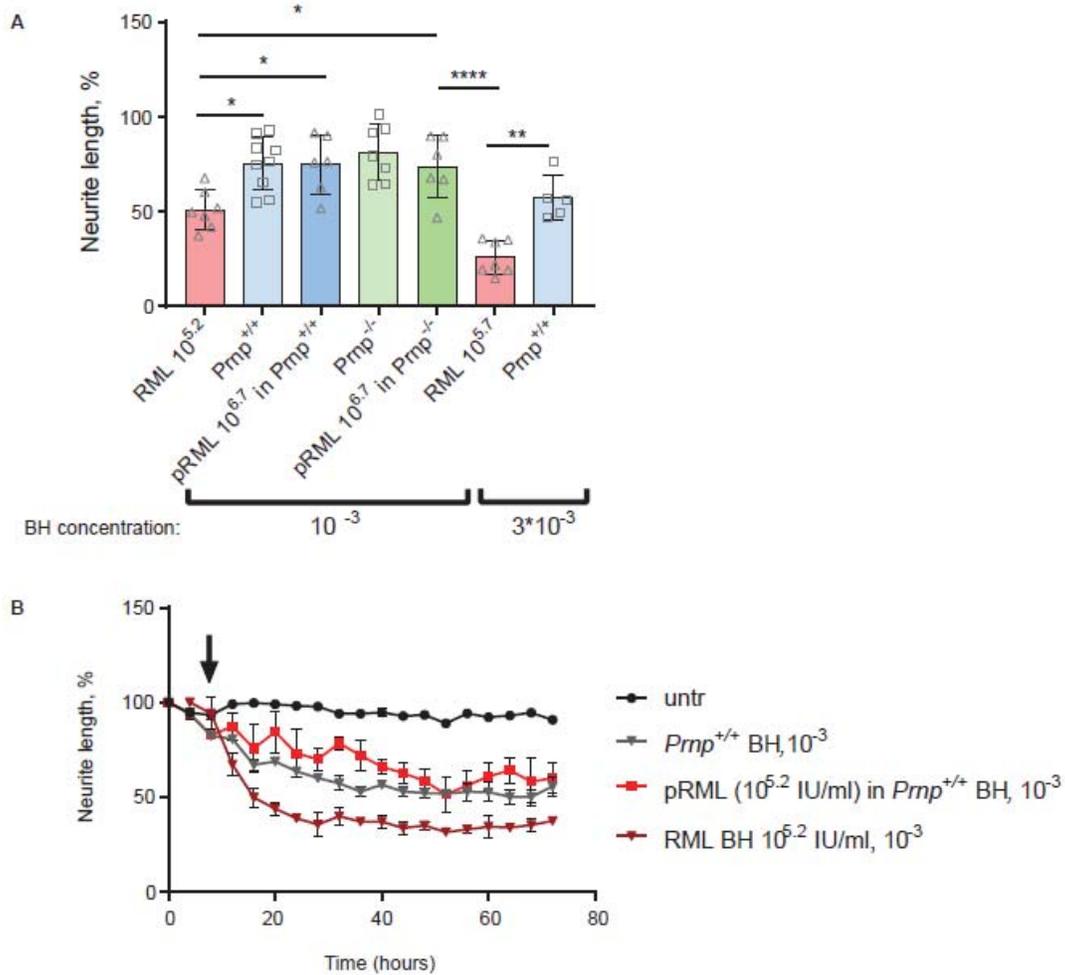


Fig. S2. Purified RML prions at $10^{6.7}$ IU/ml in *Prnp*^{+/+} and *Prnp*^{0/0} mouse brain homogenates at a brain concentration of 10^{-3} are not neurotoxic to primary neurons. **A**, Normalized response at 3 days post-treatment (n=5-9, mean \pm S.D., NeuroTrack analysis parameters: sensitivity 0.4; neurite width: 1 μ m). **B**, Normalized response over 68 h of treatment of primary neurons with pRML at $10^{5.2}$ IU/ml in *Prnp*^{+/+} BH at 10^{-3} (n=3), RML BH at a concentration of 10^{-3} ($10^{5.2}$ IU/ml) (n=3) and uninfected *Prnp*^{+/+} BH at 10^{-3} (n=3), mean \pm S.E.M.

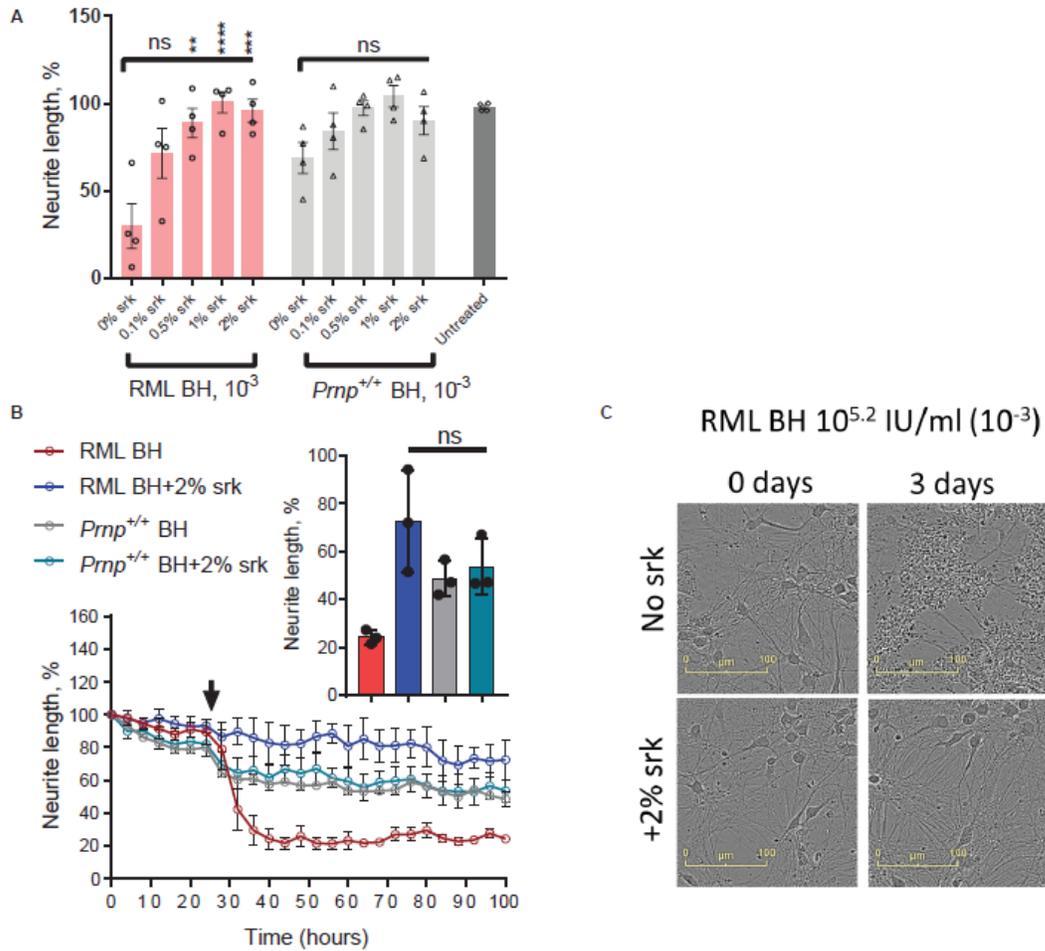


Fig. S3. Sarkosyl does not affect the non-specific toxicity of uninfected *Prnp*^{+/+} brain homogenate. **A**, Normalized response at 12 h post-treatment (n=4, mean \pm S.E.M., **p<0.01, ***p<0.001, ****p<0.0001, “ns”: not significant, 1-way ANOVA with Dunnett’s multiple comparisons test), **B** – Normalized response over 3 days of treatment with 2% (w/v) sarkosyl-pretreated *Prnp*^{+/+} and RML BH at a brain concentration of 10^{-3} (n=3, mean \pm S.E.M.) This dataset is independent of panel **A** and **Fig.5**. **B inset**, normalized response at 3 days post-treatment (n=3, mean \pm S.D., “ns”: not significant in an unpaired two-tailed t-test). **C**, Primary neurons look healthy after 3 days of treatment with 2% (w/v) sarkosyl-pretreated *Prnp*^{+/+} BH at 10^{-3} , scale bar: 100 μ m.

Movie S1 (separate file) Infectious RML brain homogenate (BH) has a pronounced toxic effect on neuronal network. RML BH containing $10^{4.2}$ infectious units (IU)/ml was added to 10 days old cortico-hippocampal mouse neurons after 20 hours of baseline recording. Note acute neurite retraction and loss of neuronal cell bodies.

Movie S2 (separate file) 10 days old neuronal culture treated with a non-infectious *Prnp*^{+/+} brain diluted to a concentration of 10^{-4} (vehicle control for Movie S1). This brain homogenate was added to primary cells after 20 hours of baseline recording.

Movie S3 (separate file) High titre purified RML prions (infectivity $10^{6.7}$ IU/ml) diluted in tissue culture medium are not toxic to primary neurons. Prions were added to 10 days old cortico-hippocampal mouse culture after 20 hours of baseline recording.

Movie S4 (separate file) Non-infectious *Prnp*^{0/0} brain diluted to a concentration of 10^{-4} reconstituted with high titre purified RML prions ($10^{6.7}$ IU/ml) is not toxic to primary neurons. Prions in brain homogenate were added to 10 days old cortico-hippocampal mouse neurons after 20 hours of baseline recording.

Movie S5 (separate file) 10 days old neuronal culture treated with a non-infectious *Prnp*^{0/0} brain at a concentration of 10^{-4} (vehicle control for Movie S4). This brain homogenate was added to primary cells after 20 hours of baseline recording.

Movie S6 (separate file) Non-infectious *Prnp*^{+/+} brain diluted to a concentration of 10^{-4} reconstituted with high titre purified RML prions ($10^{6.7}$ IU/ml) is not toxic to primary neurons. Prions were added to 10 days old cortico-hippocampal mouse neurons after 20 hours of baseline recording.