Supporting Information

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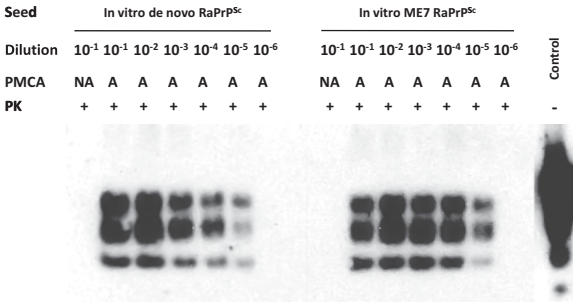

Fig. S1. Replication efficiency studies of in vitro rabbit prion strains. Different dilutions (10^{-1} to 10^{-6}) of in vitro–generated de novo $RaPrP^{Sc}$ and ME7 $RaPrP^{Sc}$ strains were amplified by PMCA using rabbit brain homogenate as substrate. The samples were subjected to 48 cycles (24-h) of standard PMCA. Both in vitro rabbit prion strains showed an excellent level of in vitro amplification of at least 100,000 times in just one round of PMCA. All samples were digested with 100 μg/mL of PK and were analyzed by Western blot using monoclonal antibody D18. A, amplified; NA, nonamplified; Control, normal rabbit brain homogenate.

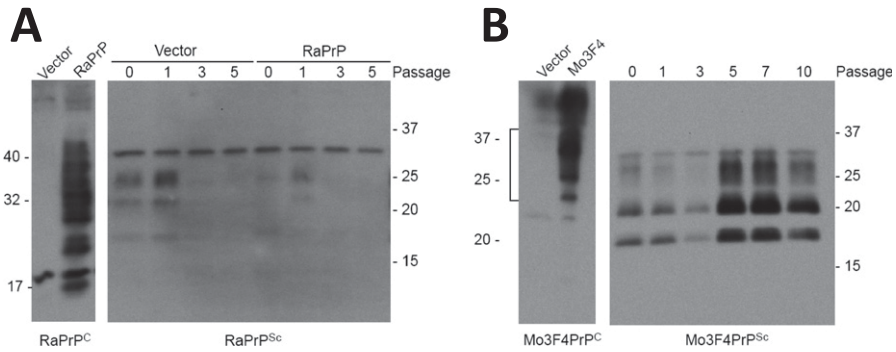

Fig. S2. Rabbit PrP^{Sc} is unable to trigger persistent PrP^{Sc} production in cells susceptible to prion infection. (*A*) (*Left-most panel*) Level of PrP^{C} expression in CF10 cells expressing the pSFF vector alone (Vector) or rabbit PrP^{C} (RaPrP). (*Right-most panel*) Amount of $RaPrP^{Sc}$ detected at different passages following exposure of the cells to $RaPrP^{Sc}$. $RaPrP^{Sc}$ was only detected at passage 0 and 1. Because the L42 antibody cannot distinguish $RaPrP^{Sc}$ in the inoculum from $RaPrP^{Sc}$ newly made in the cell, it is likely that this signal represents residual $RaPrP^{Sc}$ from the inoculum. No $RaPrP^{Sc}$ was detected at passage 3 or 5. Both blots were developed using the L42 antibody. Film exposure times were 15 s (*Left*) and 20 h (*Right*). (*B*) (*Left-most panel*) Level of PrP^{C} expression in CF10 cells expressing the pSFF vector alone (Vector) or Mo3F4 PrP^{C} (Mo3F4, indicated by the bracket). (*Right-most panel*) Amount of Mo3F4 PrP^{Sc} detected at different passages following exposure of the cells to the 22L strain of mouse scrapie. Consistent with previously reported data (1) all passes tested were positive for Mo3F4 PrP^{Sc}, indicating that the cells were persistently infected with mouse scrapie. Blots were developed with the mouse monoclonal antibody 3F4, which detects only PrP^{Sc} made by the cells. Film exposure times were 5 s (*Left*) and 2 min (*Right*). For each panel, molecular mass markers in kilodaltons are shown on the left or right side of the figure.

1. McNally K, Ward AE, Priola SA (2009) Cells expressing anchorless prion protein are resistant to scrapie infection. *J Virol* 83:4469–4475.

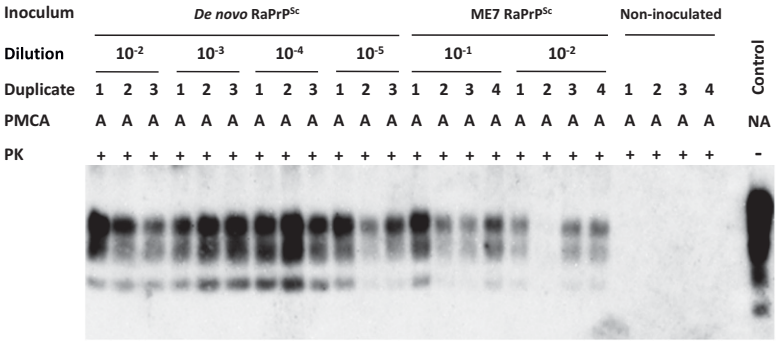

Fig. S3. Prion detection of ME7 RaPrP^{Sc} by serial automated PMCA (saPMCA). The ME7 RaPrP^{Sc} challenged rabbit brain was subjected to saPMCA to determine the presence of PK-resistant PrP^{Sc}. Dilutions 10^{-1} and 10^{-2} in quadruplicate from the ME7 RaPrP^{Sc} brain homogenate were subjected to three rounds of saPMCA using rabbit brain homogenate as substrate. Dilutions from 10^{-1} to 10^{-5} in triplicate were used as a positive control and for semiquantification studies. Noninoculated 10% rabbit brain homogenate was used as negative controls in quadruplicate. The third round of saPMCA showed four of four and three of four PK-resistant ME7 RaPrP^{Sc} positive samples in the dilution 10^{-1} and 10^{-2}, respectively. The number of positive samples versus dilution and rounds of saPMCA obtained using the de novo RaPrP^{Sc} sample as a positive control suggests that the ME7 RaPrP^{Sc} infected brain contains 10^3 to 10^4 times less in vitro replicating PrP^{Sc} than the de novo RaPrP^{Sc}. All samples were digested with 100 μg/mL of PK and were analyzed by Western blot using monoclonal antibody D18. Control, normal rabbit brain homogenate.

de novo RaPrP^{Sc}
challenged NZW rabbit

Movie S1. Clinical signs from de novo RaPrP^{Sc} challenged rabbit at 766 d postinoculation (2 d before death). The rabbit was recorded showing the following clinical signs characteristic of a TSE, respectively: dullness, hyper-response to drinking stimuli, protrusion of the third eyelid, reduced righting reflex, instability of the limbs, intentional tremors, instability resulting in use of the head as a fifth limb, ataxia, and apathy. All of the images were taken the same day. Movie S2 was recorded as healthy control.

Movie S1

Healthy rabbit

Movie S2. Video recording of a 2 y old rabbit used as healthy negative control. The video shows the standard behavior of a healthy rabbit for comparison with the clinically affected rabbit shown in Movie S1. The images show respectively: normal response to stimuli, normal posture, explorative behavior, normal righting reflex, and attempts to escape (normal behavior) and hide. All of the images were taken on the same day.

Movie S2