# Supporting Information

## White *et al.* 10.1073/pnas.0802759105

### SI Text

**siRNA Design.** siRNA sequences corresponding to *Prnp* were designed using the Reynolds' criteria (1) and screened for less than 15/19 nucleotide matches to other mouse genes by Blast searching against murine EST libraries and mRNA sequences (www.ncbi.nlm.nih.gov/BLAST). The best-scoring sequences, 5′-GTACCGCTACCCTAACCAA-3′ (MW1), 5′-GATCAGTACAGCAACCAGA-3′ (MW2) and 5′-TCGTGCACGACTGCGTCAATA-3′ (MW3) of the mouse PrP mRNA (NM_011170), were synthesized as custom RNA duplexes (Invitrogen) with a 3′ dinucleotide thymine overhang and a 6-carboxyfluorescein tag on the 3′ end of the sense strand. A scrambled duplex with matching GC content and no known homology to any vertebrate gene was obtained (Invitrogen).

**Transient Transfection with siRNA Duplexes.** Cells were transfected at ≈30% confluency with 100 nM siRNA duplex using Oligofectamine Transfection Reagent according to manufacturer's instructions (Invitrogen).

**Viral titer Determination.** Viral titer was calculated by serial dilutions in fresh HEK293FT cells. EGFP-positive cells were counted 3 days later by fluorescence microscopy using an Axiovert 200 microscope (Zeiss) with a Nikon Coolpix 995 digital camera.

**RT PCR.** Primers and probes for the *Prnp* transgene in mice were as follows: Forward primer: 5′-GGCCCATGATCCATTTTGG-3′, Reverse primer: 5′-GCGGTACATGTTTTCACGGTAGT-3′, Probe: 5′-FAM-AACGACTGGGAGGACC-3′. Commercially available ABI assays were used to amplify endogenous *Prnp* from cell lines and FVB mice: Mm00448389_ml, FAM-labeled probe, and β-actin: Mouse ACTB Endogenous control, VIC labeled MGB probe. Cycling conditions were: 50°C for 20 min, 95°C for 15 min, then 40 cycles of 94°C for 45 sec followed by 60°C for 45 sec.

**Western Blotting.** Cells were homogenized through a needle in 100–300 μl ice-cold PBS containing 10 units/ml Benzonase (Merck). Protein concentration was determined using a BCA Protein Assay Reagent kit (Pierce). Digestion at 37°C for 45 min with 10 μg/ml Proteinase K (VWR) was performed for detection of $PrP^{Sc}$. 50 μg of total protein from pooled lysates was loaded and immunoblotted as described (2). PrP was detected with primary ICSM18 at 0.5 μg/ml, and binding was detected by incubation with HRP-conjugated secondary antibody (Sigma). The signal was visualized with high-sensitivity ECL (Pierce).

**Mouse Cell Lines.** N2a and GT1 cell lines were maintained in OptiMEM growth medium (Gibco BRL), supplemented with 10% vol/vol FCS (Gibco BRL), and 50 units/ml each of penicillin and streptomycin (Sigma). The HEK293FT cell line (Invitrogen) was propagated in Dulbecco's Modified Eagle Medium containing 4,500 mg/l D-glucose and 4 mM L-glutamine (Gibco BRL), supplemented as above and with 500 μg/ml Geneticin (Gibco BRL) to maintain expression of the large T antigen.

**Flow Cytometry.** Cells were fixed in 1 ml 4% paraformaldehyde in PBS in 5 ml FACS tubes (Becton-Dickinson) on ice for 1 h, diluted in FACS buffer (PBS containing 2% FCS, 0.1% Sodium Azide), then spun at 2,000 rpm for 5 min. PrP was detected with ICSM18 at 12 μg/ml in FACS buffer followed by secondary antibody PE-Alexa Fluor 647 goat anti-mouse IgG at 1:500 dilution (Molecular Probes). Flow cytometry was performed on a FACSCalibur machine (Becton-Dickinson) by using FlowJo software (Tree Star). At least 20,000 cells were counted.

**Novel Object Recognition Task.** This was performed as described (3). Briefly, mice were tested in a dark cylindrical arena (69 cm diameter) mounted with a 96 LED cluster infrared light source and Watec video camera (both Track-sys). Various plastic objects constructed from interlocking plastic building blocks were used in all experiments. On day 1 of each experiment (learning phase), two objects were placed in the center of two 15-cm-diameter circles inside the arena. Each mouse was placed in the arena for two blocks of 10 min for exploration of the objects with an intertrial interval of 5 min. Four hours later, a novel object was randomly exchanged for one of the familiar ones, and retention was tested by placing the mice back in the arena for a 5-min session (test phase). The amount of time spent exploring all objects was measured for each animal with the examiner blind to treatment received and time point for the animals. All objects and arena were cleansed thoroughly between trials to ensure the absence of olfactory cues. Criteria for exploration were based strictly on active exploration, where mice had at least both forelimbs within the circle with their head oriented toward it or touching it with their noses or vibrissae. Mice with overt motor symptoms were not used. New sets of objects were used at each time point.

**Burrowing and Nesting.** Two hours before the start of the dark period, mice (which had not been food-deprived) were placed in individual plastic cages containing a gray plastic tube 20 cm long × 6.8 cm diameter, filled with 200 g of normal food pellets, as described (4). The weight of the pellets remaining in the tube after 24 h was measured, and the percentage displaced (burrowed) was calculated.


1. Reynolds A, *et al*. (2004) Rational siRNA design for RNA interference. *Nat Biotechnol* 22:326–330.
2. Mallucci GR, *et al*. (2002) Post-natal knockout of prion protein alters hippocampal CA1 properties, but does not result in neurodegeneration. *EMBO J* 21:202–210.
3. Mallucci GR, *et al.* (2007) Targeting cellular prion protein reverses early cognitive deficits and neurophysiological dysfunction in prion-infected mice. *Neuron* 53:325–335.
4. Cunningham C, *et al.* (2003) Synaptic changes characterize early behavioural signs in the ME7 model of murine prion disease. *Eur J Neurosci* 17:2147–2155.
5. Ghaemmaghami S, *et al*. (2007) Cell division modulates prion accumulation in cultured cells. *Proc Natl Acad Sci USA* 104:17971–17976.

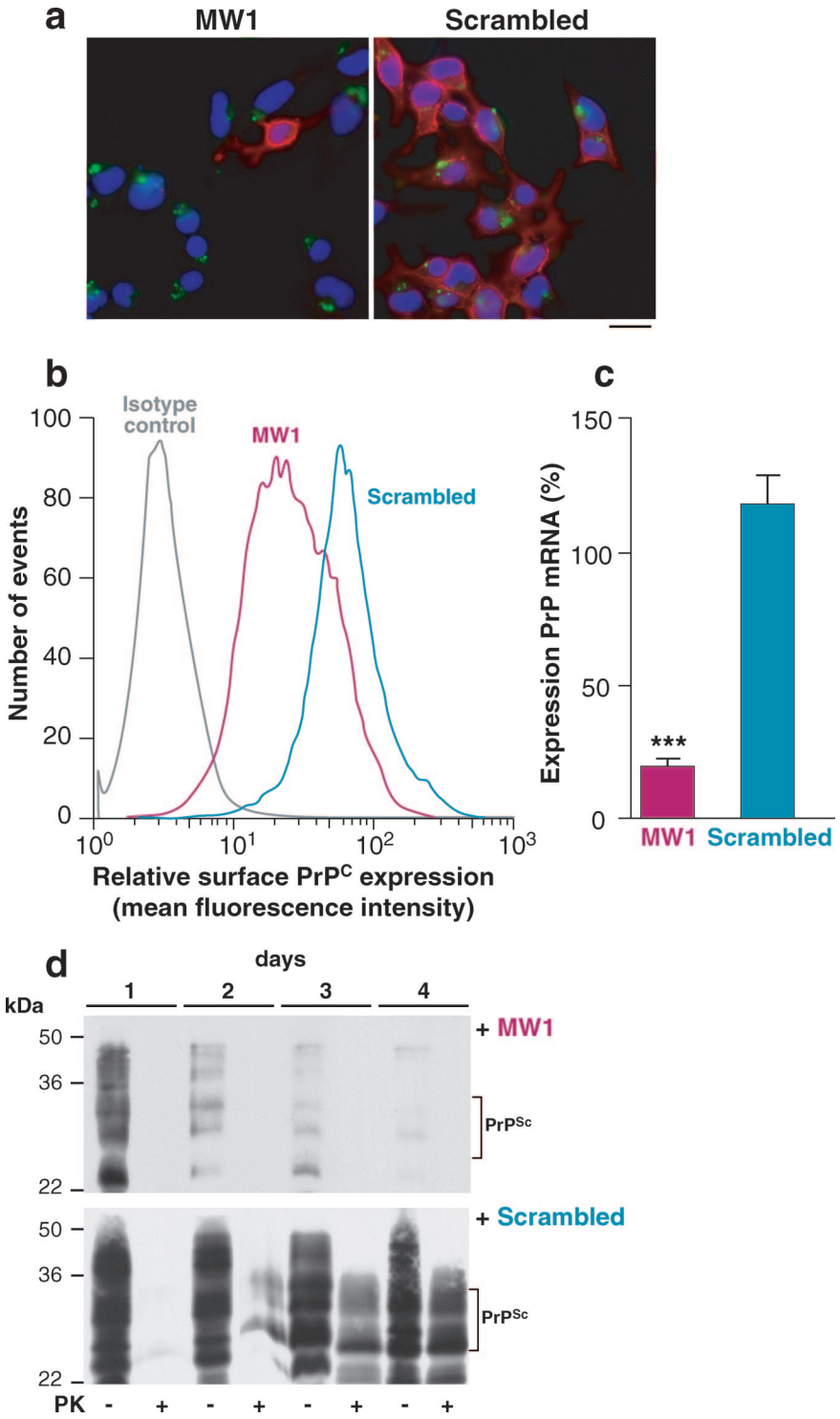


**Fig. S1.** MW1 siRNA duplex reduces PrP protein and mRNA expression in N2a cells and prevent accumulation of PrP$^{Sc}$ in prion-infected cells. (*a*) PrP$^C$ expression detected with anti-PrP antibody ICSM18 (red) is reduced in N2a cells after transient transfection with 100 nM MW1 duplex but not with the Scrambled duplex (shown here 48 posttransfection hours). Fluorescein-tagged siRNA duplexes are shown in green. Cell nuclei were stained with DAPI (blue). (Scale bar, 20 $\mu$m.) (*b*) N2a cells transfected with siRNA duplexes were fixed and labeled with ICSM18 detected with PEA Alexa Fluor 647 goat anti-mouse antibody and analyzed by flow cytometry. Left shift of the curve with MW1 is due to reduction in surface expression of PrP. An isotype control is included (gray curve). (*c*) RT-PCR confirms knockdown of PrP mRNA by duplex MW1, but not the Scrambled duplex, relative to wild-type expression levels. Error bars represent SEM. Three replicates were performed for each sample. (*d*) Time course of Western blots of chronically prion-infected N2a (IPK1) cells transfected with MW1 or Scrambled duplexes show reduction of PrP and prevention of PrP$^{Sc}$ accumulation for 4 days posttransfection for MW1-treated cells, but increasing PrP$^{Sc}$ levels are seen in cells treated with Scrambled duplex, consistent with the accumulation of PrP$^{Sc}$ seen in prion-infected cells as they reach confluence (5). PK, proteinase K.

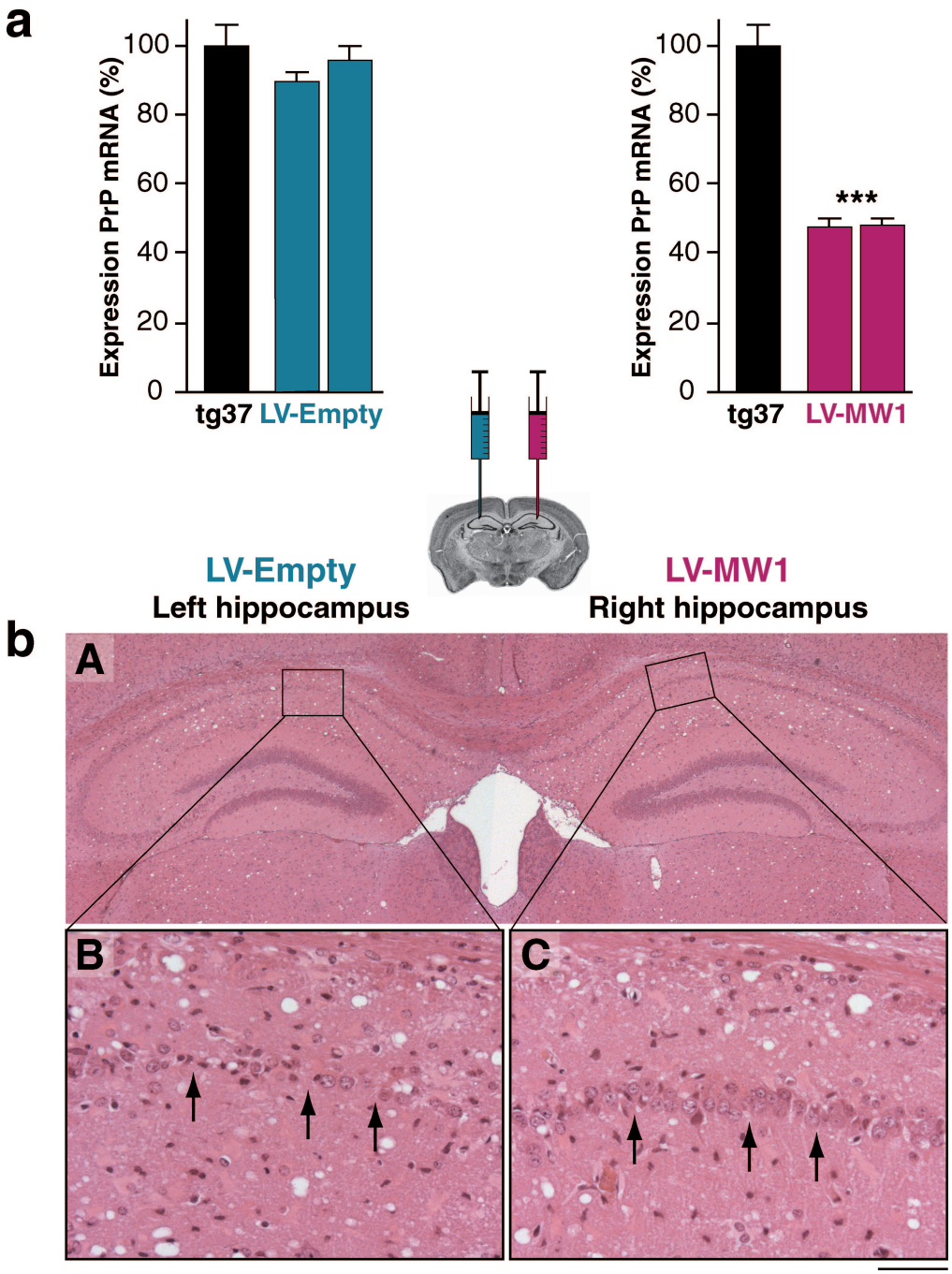


**Fig. S2.** Lentiviral transduction and RNAi of PrP is limited to the side of injection. RML-infected mice were injected with lentiviruses LV-MW1 into the right hippocampus and LV-Empty into the left hippocampus at 8 wpi when early pathology is established. The animals were culled 2 weeks later for RNA quantitation and histopathological examination. (*a*) mRNA was extracted from freshly dissected hippocampi and levels of PrP mRNA were measured by RT-PCR. There was up to 60% knockdown of PrP mRNA levels in LV-MW1 injected right hippocampi but not in LV-Empty injected left hippocampi, where PrP mRNA levels are similar to those seen in uninfected tg37 mice (data from two mice are shown in each case; three replicates per mouse). Error bars represent SEM. ($P = 0.0068$; Student's *t* test; 2 tails). (*b*) Three mice were examined histologically to compare relative effects of each virus on the two sides. Representative sections are shown. The LV-MW1 treated right hippocampus was protected against neuronal loss (*C*), but in the same mouse, there was severe neuronal loss in the LV-Empty-treated left hippocampus, typical of prion pathology at this stage (*B*). Scale bar, 500 $\mu$m.

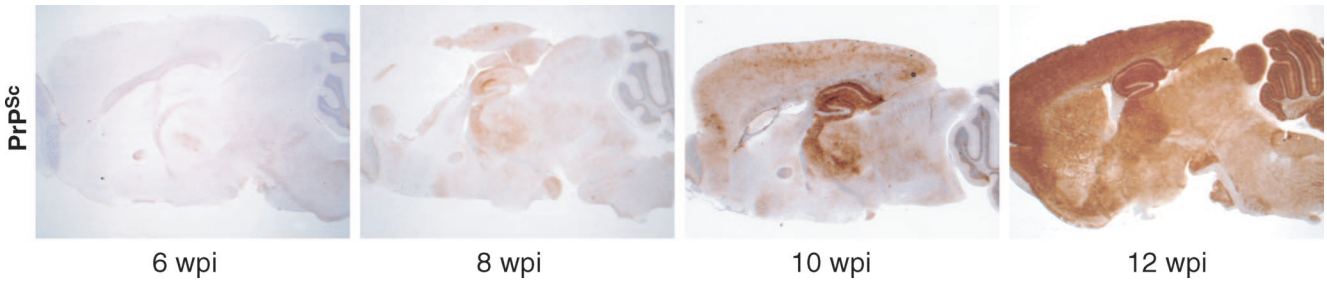


**Fig. S3.** Distribution of $PrP^{Sc}$ over time in RML-infected tg37 mice. $PrP^{Sc}$ staining of RML infected tg37 mice shows hippocampus is the main focus of accumulation of $PrP^{Sc}$ prion replication at early and midstage prion infection. There is widespread deposition of $PrP^{Sc}$ throughout the brain when mice are terminally ill at 12 wpi.

**Table S1. Spongiosis and $PrP^{Sc}$ deposition scores in different brain regions of RML prion infected mice treated with LV-MW1 or LV-Empty**

| | H&E Spongiosis score (0–3), mean ± SD | Hippocampus | Cortex | Thalamus | Cerebellum |
|---|---|---|---|---|---|
| | LV-MW1- treated mice ($n = 9$) | 1.3 ± 0.2 | 1.8 ± 0.6 | 1.6 ± 0.5 | 2.0 ± 0.0 |
| | Student's *t* test, two tails | $P < 0.001$ | $P = 0.10$ | $P = 0.03$ | $P = 0.51$ |
| | LV-Empty- treated mice ($n = 6$) | 2.7 ± 0.2 | 2.4 ± 0.5 | 2.5 ± 0.7 | 1.8 ± 0.6 |
| PrP (ICSM35) Staining intensity (0–3), mean ± SD mean | LV-MW1- treated mice ($n = 9$) | 2.3 ± 0.3 | 2.2 ± 0.5 | 2.3 ± 0.4 | 2.2 ± 0.5 |
| | Student's *t* test, two tails | $P = 0.04$ | $P = 0.40$ | $P = 0.04$ | $P = 0.12$ |
| | LV-Empty treated mice ($n = 6$) | 3.0 ± 0.0 | 2.6 ± 0.4 | 3.0 ± 0.0 | 2.8 ± 0.4 |

Scoring is semiquantitative and performed blind on sections. Range 0–3 where 0 = unaffected, 3 = severely affected. Hippocampus, cortex, and thalamus all appeared less severely affected in LV-MW1-treated mice; significant differences between the two treatment groups were seen in hippocampus and thalamus.