Supplementary Information

Infectivity from two sick RML-infected Tg(GSS)12 mice produced disease in inoculated wild type FVB mice with incubation times that were ~40 d longer than the incubation times produced by infection of FVB mice with RML prions derived from wild type mice (Supplementary Table I). The neuroanatomic distribution of PrP^{Sc} assessed by histoblotting coronal brain sections of FVB mice inoculated with RML prions passaged in Tg(GSS)12 mice indicated that the strain properties of RML prions were not altered following passage through Tg(GSS) (Supplementary Fig. 2). While 101LL mice and Tg196 mice have also been shown to be susceptible to mouse adapted strains of scrapie prions (Barron et al., 2003; Manson et al., 1999; Tremblay et al., 2004), the serial transmission experiments reported here demonstrate that the RML-induced disease-associated MoPrP-P101L conformer is capable of causing disease in both Tg(GSS) and wild type mice.

Supplementary References

Barron, R.M., Thomson, V., King, D., Shaw, J., Melton, D.W. and Manson, J.C. (2003) Transmission of murine scrapie to P101L transgenic mice. *J Gen Virol*, **84**, 3165-3172.

Manson, J.C., Jamieson, E., Baybutt, H., Tuzi, N.L., Barron, R., McConnell, I., Somerville, R., Ironside, J., Will, R., Sy, M.S., Melton, D.W., Hope, J. and Bostock, C. (1999) A single amino acid alteration (101L) introduced into murine PrP dramatically alters incubation time of transmissible spongiform encephalopathy. *EMBO*, **18**, 6855-6864.

Taraboulos, A., Jendroska, K., Serban, D., Yang, S.-L., DeArmond, S.J. and Prusiner, S.B. (1992) Regional mapping of prion proteins in brains. *Proc. Natl. Acad. Sci. USA*, **89**, 7620-7624.

Tremblay, P., Ball, H.L., Kaneko, K., Groth, D., Hegde, R.S., Cohen, F.E., DeArmond, S.J., Prusiner, S.B. and Safar, J.G. (2004) Mutant PrPSc conformers induced by a synthetic peptide and several prion strains. *J Virol*, **78**, 2088-2099.

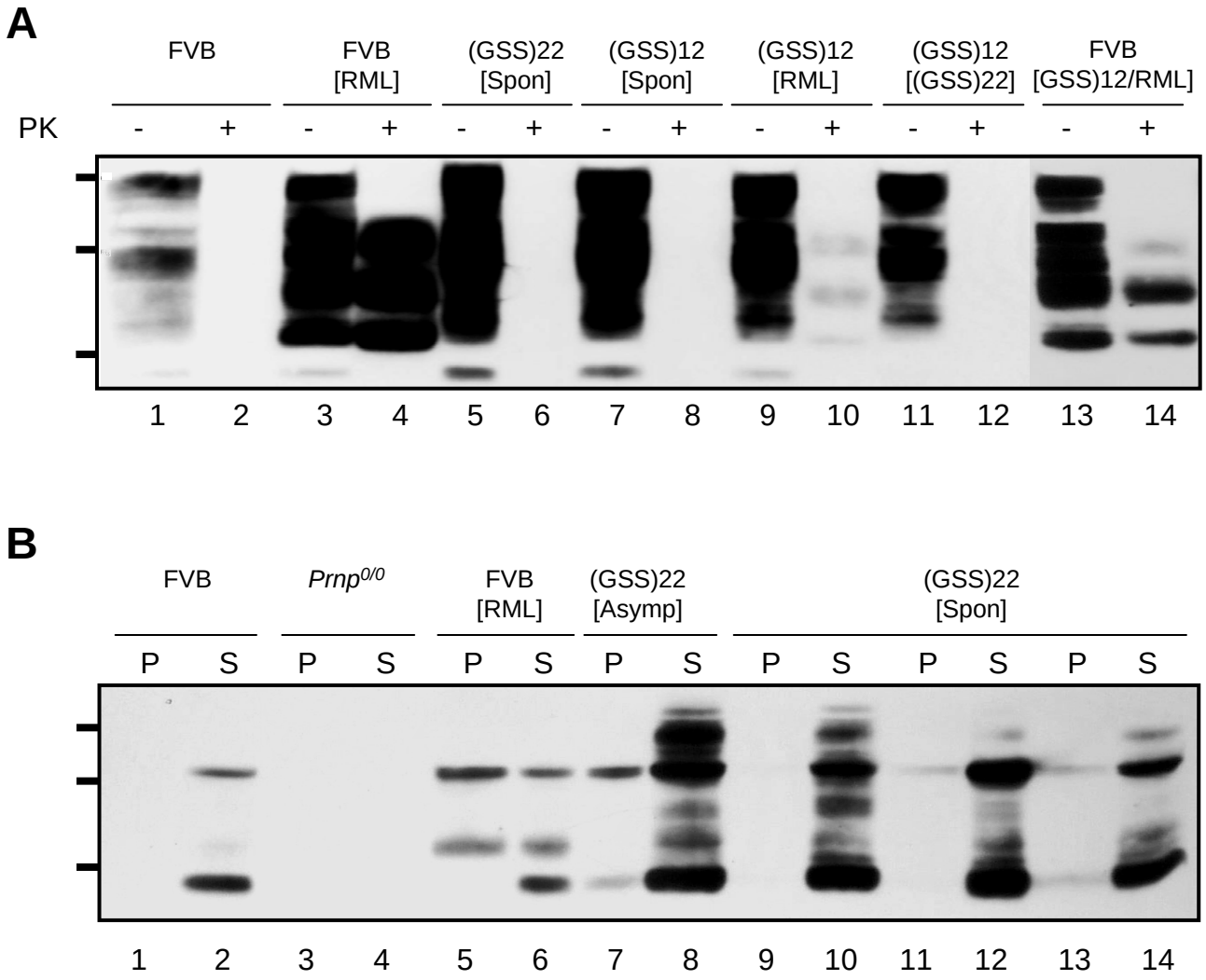
Supplementary Figure Legends

Supplementary Fig. 1*: Attempts to detect disease-associated MoPrP-P101L in the brains of spontaneously sick Tg(GSS) mice on the basis of protease resistance and detergent insolubility (A)* Brain extracts of sick Tg(GSS) mice lack $rPrP^{Sc}$. Brain homogenates were untreated of treated with 20 µg/ml PK at 37ºC as indicated. Lanes 1 and 2, uninoculated FVB mouse; lanes 3 and 4, FVB mouse inoculated with mouse adapted RML scrapie prions; lanes 5 and 6, spontaneously sick Tg(GSS)22 mouse sacrificed at 177 d of age; lanes 7 and 8, spontaneously sick Tg(GSS)12 mouse sacrificed at 488 d of age; lanes 9 and 10, sick Tg(GSS)12 mouse inoculated with mouse adapted RML scrapie prions sacrificed at 195 d post inoculation; lanes 11 and 12, sick Tg(GSS)12 mouse sacrificed at 195 d post inoculation with brain extract from spontaneously sick Tg(GSS)22 mouse; lanes 13 and 14, sick FVB mouse sacrificed at 153 d post inoculation with brain extract from sick Tg(GSS)12 mouse originally inoculated with RML prions. **(B)** Brain extracts of sick Tg(GSS) mice lack detergent-insoluble PrP. For analysis of insoluble and soluble forms of full-length PrP and cleavage products, brain homogenates containing equal amounts of protein were deglycosylated and centrifuged at 100,000-x g for 1 hour Insoluble material in the pellet (P) was suspended in phosphate buffered saline (PBS)/2% sarkosyl while soluble (S) proteins were recovered following methanol precipitation. After immunoblotting the membrane was probed with Mab 8H4, a kind gift from Man-Sun Sy, Case Western Reserve. Lanes 1 and 2, uninoculated FVB mouse; lanes 3 and 4, $Prnp^{0/0}$ mouse; lanes 5 and 6, FVB mouse inoculated with mouse adapted RML scrapie prions; lanes 7 and

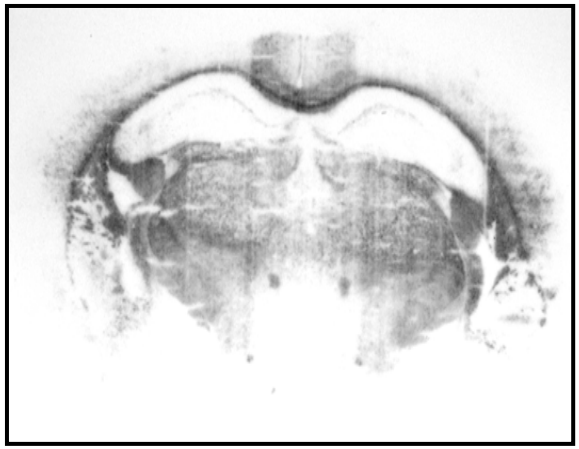
8, asymptomatic Tg(GSS)20 mouse sacrificed at 87 d of age; lanes 9 and 10, spontaneously sick Tg(GSS)22 mouse sacrificed at 152 d of age; lanes 11 and 12, spontaneously sick Tg(GSS)22 mouse sacrificed at 177 d of age; lanes 13 and 14, spontaneously sick Tg(GSS)22 mouse sacrificed at 177 d of age.

Supplementary Fig. 2: ***Regional distribution of PrP^{Sc} in the brains of FVB mice inoculated with RML prions originating from wild type mice or following passage through Tg(GSS)12 mice.*** Histoblots of 10 μm thick cryostat sections through the region of the hippocampus and thalamus were generated as previously described (Taraboulos et al., 1992).To eliminate PrP^{C} from the section, the membranes were air dried, rehydrated for 30 min and exposed for 1 hour at 37^{o}C to 400 mg/ml PK. To enhance immunostaining of PrP^{Sc}, the histoblots were exposed to 3M guanidinium isothiocyanate before immunostaining with Mab 6H4 followed by alkaline phosphatase-conjugated sheep anti-Mo secondary antibody.

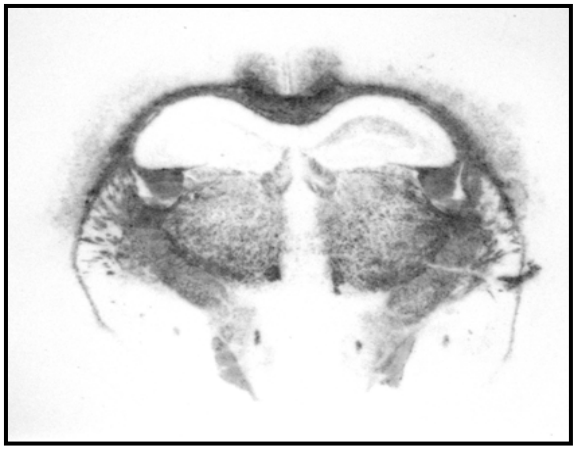
Supplementary Fig. 1



Supplementary Fig. 2



FVB [RML]



FVB [(GSS)12/RML]

Supplementary Table I: Susceptibility of wild type mice to brain materials from spontaneously sick and RML prion infected Tg(GSS) mice

Inoculum	Incubation time mean (d) ± SEM (n/n_0)[a]
(GSS)22-brain 2	>515 d (0/8)
RML	109 ± 2 d (10/10)
(GSS)12 [RML]-brain 1	148 ± 2 d (8/8)
(GSS)12 [RML]-brain 2	142 ± 1 d (7/7)

[a] The number of inoculated mice developing clinical signs of scrapie divided by the original number of mice is shown in parentheses.