Supporting Information

Deleault et al. 10.1073/pnas.1206999109

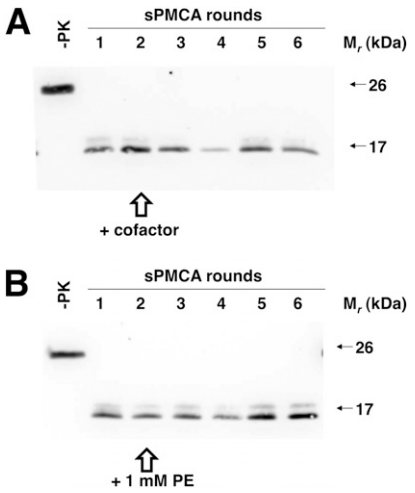

Fig. S1. Effect of cofactor molecules on the propagation of protein-only PrP^{Sc} molecules. Western blots of reconstituted serial protein misfolding cyclic amplification (sPMCA) reactions. –PK, samples not subjected to proteinase K digestion; all other samples were proteolyzed. All reactions initially were seeded with OSU (recombinant prion strain originally generated de novo as previously described in ref. 1) protein-only PrP^{Sc} (misfolded prion protein) molecules and subsequently were propagated in substrate mixtures without added cofactor in the first round and subsequently with added purified cofactor preparation (*A*) or 1 mM plasmalogen phosphatidylethanolamine (PE) (*B*) in rounds 2–6.

1. Wang F, Wang X, Yuan CG, Ma J (2010) Generating a prion with bacterially expressed recombinant prion protein. *Science* 327:1132–1135.

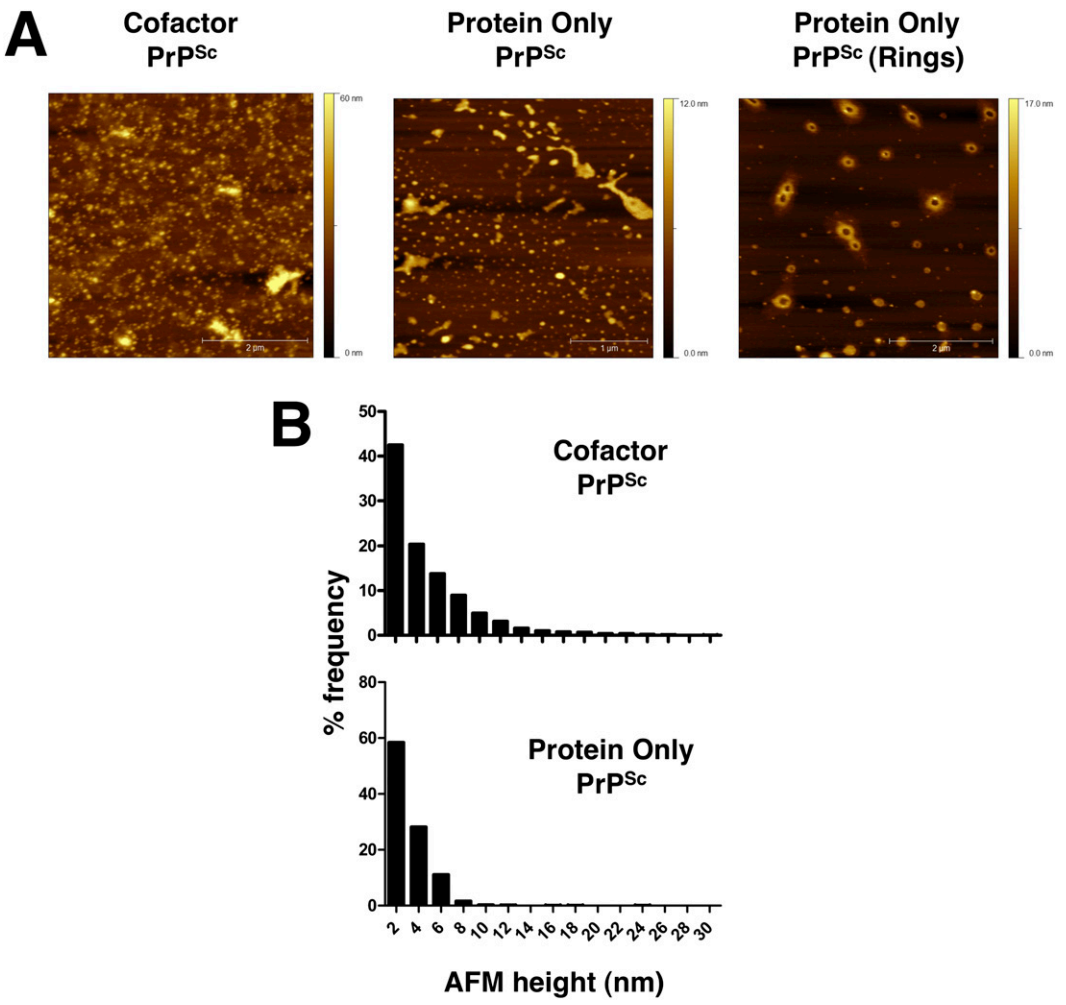

Fig. S2. Atomic force microscopy. (*A*) Representative 2D atomic force microscopy amplitude images of OSU cofactor PrP^{Sc} (*Left*) and OSU protein-only PrP^{Sc} (*Center* and *Right*) samples. The right panel contains ring forms occasionally observed in the protein-only PrP^{Sc} sample. (*B*) Graphs showing the frequency distribution of heights from cofactor PrP^{Sc} (*Upper*) or protein-only PrP^{Sc} (*Lower*) samples. The data were collected by analyzing atomic force microscopy images collected in tapping mode. More than 1,000 individual particles from each group were analyzed.

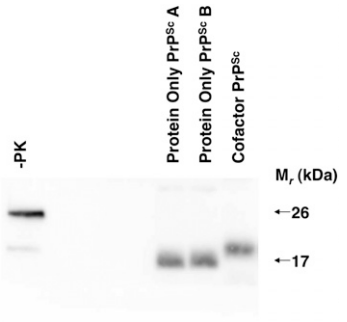

Fig. S3. Comparison of inocula. Western blots showing the final product of 18-round sPMCA reactions containing either OSU cofactor PrP^{Sc} or OSU protein-only PrP^{Sc} molecules. −PK, samples not subjected to proteinase K digestion; all other samples were proteolyzed.

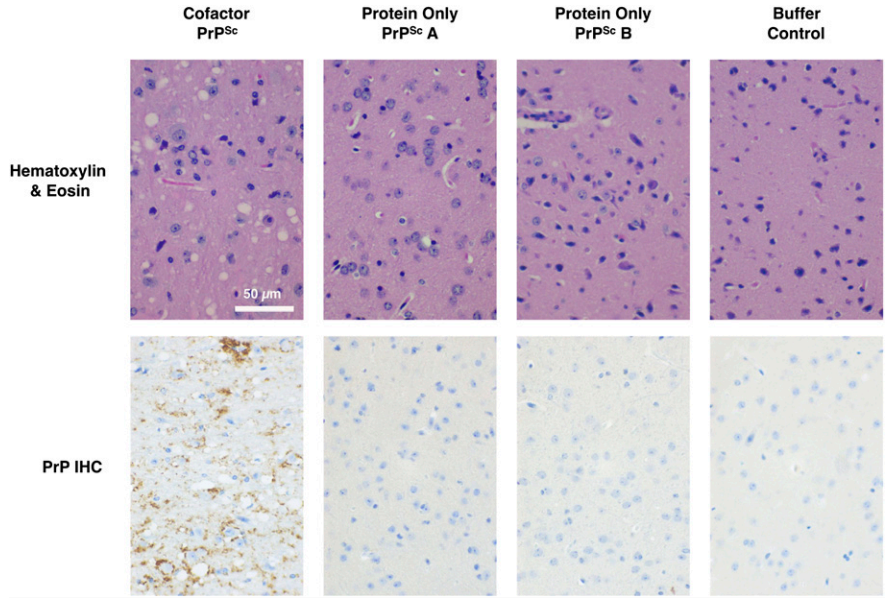

Fig. S4. Neuropathology in inoculated mice. H&E and anti-PrP immunohistochemical (IHC) stains of brain sections taken from mice inoculated with OSU cofactor PrP^{Sc} molecules, OSU protein-only PrP^{Sc} molecules, or control buffer.

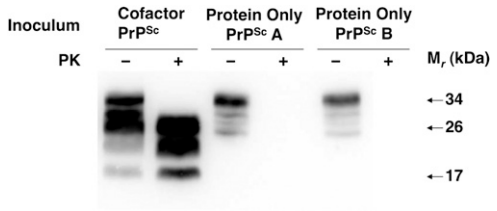

Fig. S5. PrP^{Sc} detection in inoculated mice. Western blot to detect proteinase K-resistant PrP^{Sc} in brain homogenates of mice inoculated with OSU cofactor PrP^{Sc} or OSU protein-only PrP^{Sc} molecules, as indicated. –PK, samples not subjected to proteinase K digestion; all other samples were proteolyzed.

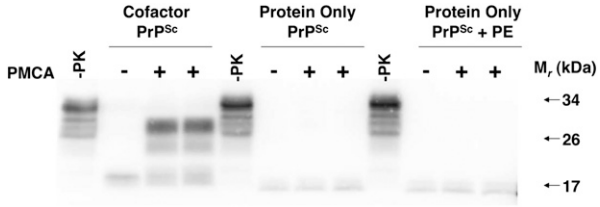

Fig. S6. Seeding of brain homogenate sPMCA reactions. Western blot of one-round sPMCA reactions using normal mouse brain homogenate substrate, seeded with various samples, as indicated. –PK, samples not subjected to proteinase K digestion; all other samples were proteolyzed. Duplicate samples were subjected to one-round (24 h) PMCA as indicated (+). Protein-only PrP^{Sc} + PE indicates a sample produced by using OSU protein-only PrP^{Sc} to seed four rounds of sPMCA in a substrate mixture containing recPrP plus 1 mM plasmalogen PE.

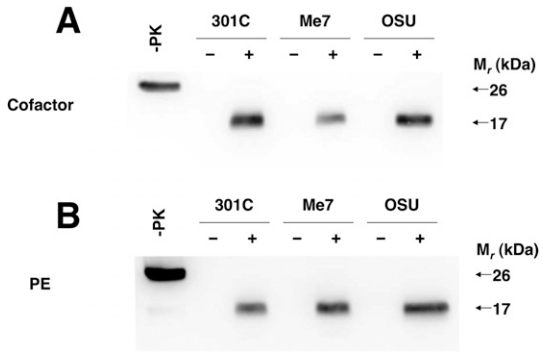

Fig. S7. Comparison sPMCA-generated PrP^{Sc} inocula. Western blots showing the final product of 18-round sPMCA reactions containing either (*A*) cofactor PrP^{Sc} or (*B*) PE PrP^{Sc} molecules that originally were seeded with prion strains 301C, Me7, or OSU as indicated. –PK, samples not subjected to proteinase K digestion; all other samples were proteolyzed.

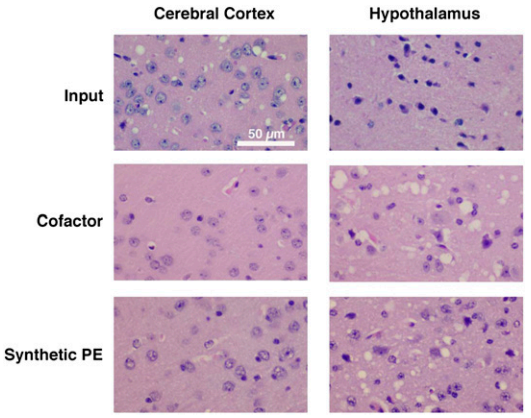

Fig. S8. Selective patterns of vacuolation in infected mice. H&E-stained brain sections taken from mice inoculated with OSU input, OSU cofactor PrP^{Sc}, and OSU PE PrP^{Sc} molecules, as indicated.

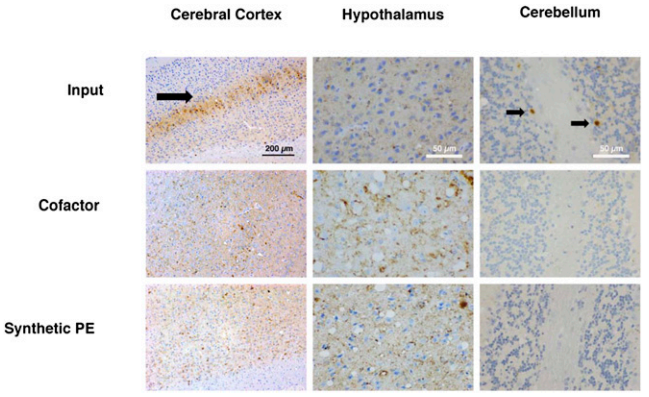

Fig. S9. Selective PrP deposition in infected mice. Anti-PrP–immunostained brain sections taken from mice inoculated with 301C input, 301C cofactor PrP^{Sc}, or 301C PE PrP^{Sc} molecules, as indicated. Arrowheads indicate cortical layers III–IV within the cerebral cortex and discrete PrP deposits within the cerebellum.

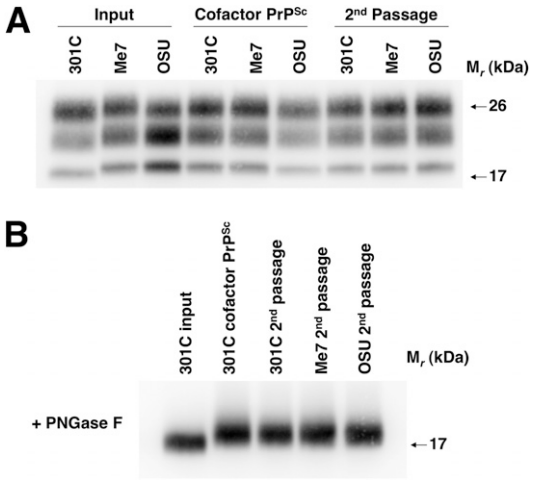

Fig. S10. Glycoform distribution and electrophoretic mobility of PrP^{Sc} molecules in the brains of infected mice. (*A*) Western blots of brain homogenate samples prepared from animals inoculated with samples containing input prions, cofactor PrP^{Sc} molecules, and serial (second)-passage (of cofactor PrP^{Sc} molecules) prions derived from different prion strains, as indicated. All samples were subjected to limited proteolysis. (*B*) Samples also were deglycosylated by treatment with peptide:N-glycosidase F (PNGase F), as indicated (+), before SDS/PAGE.