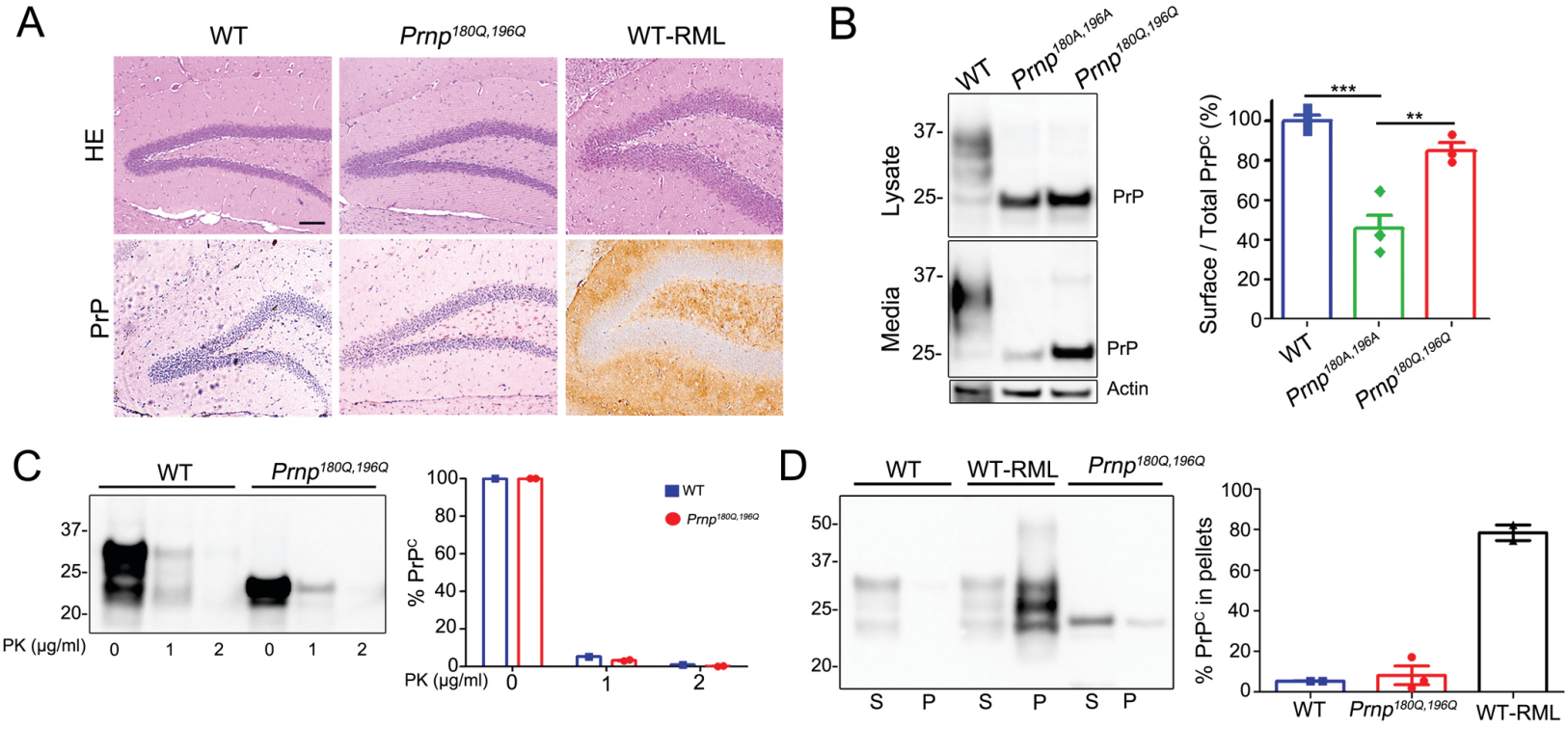
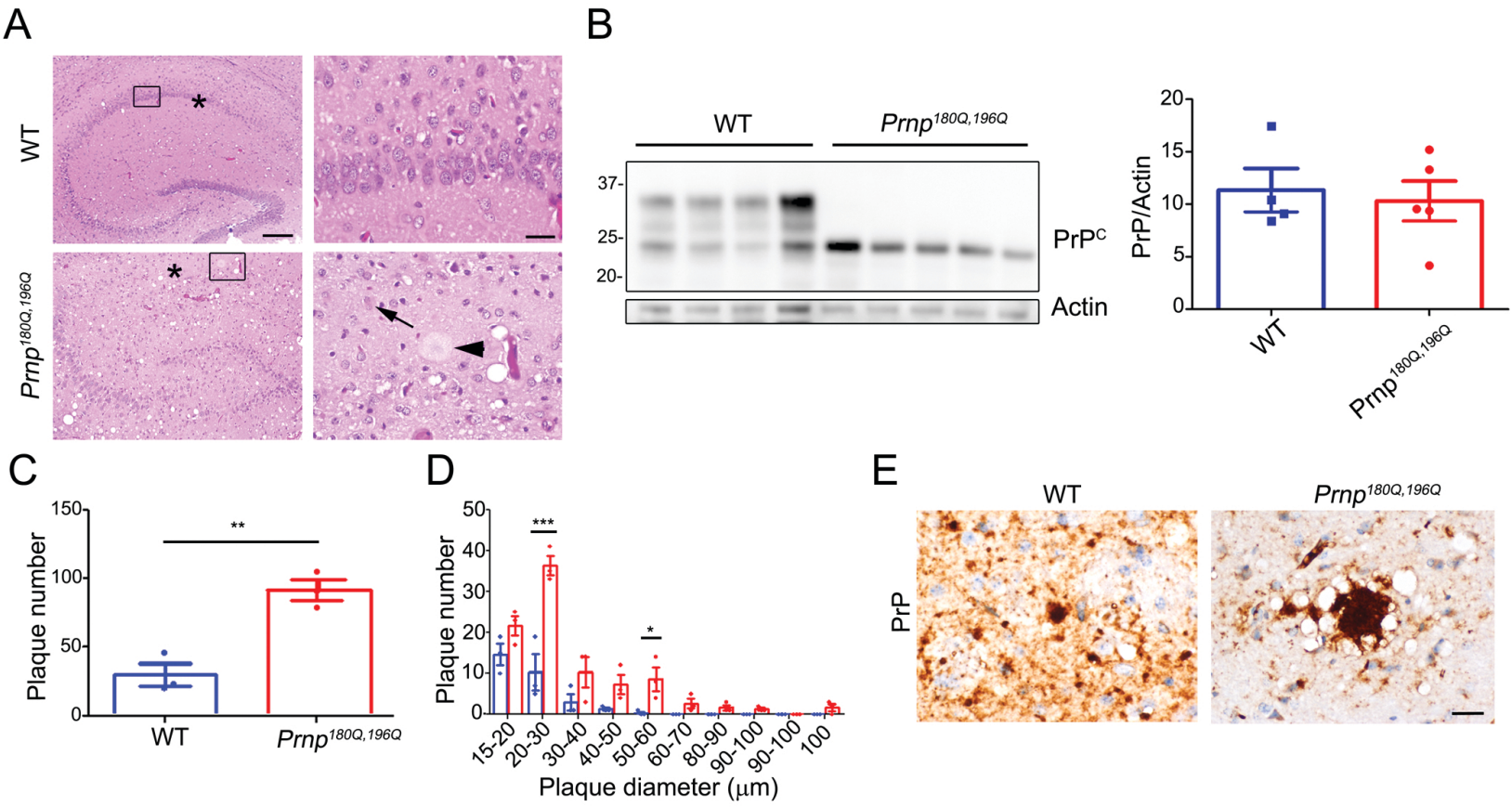
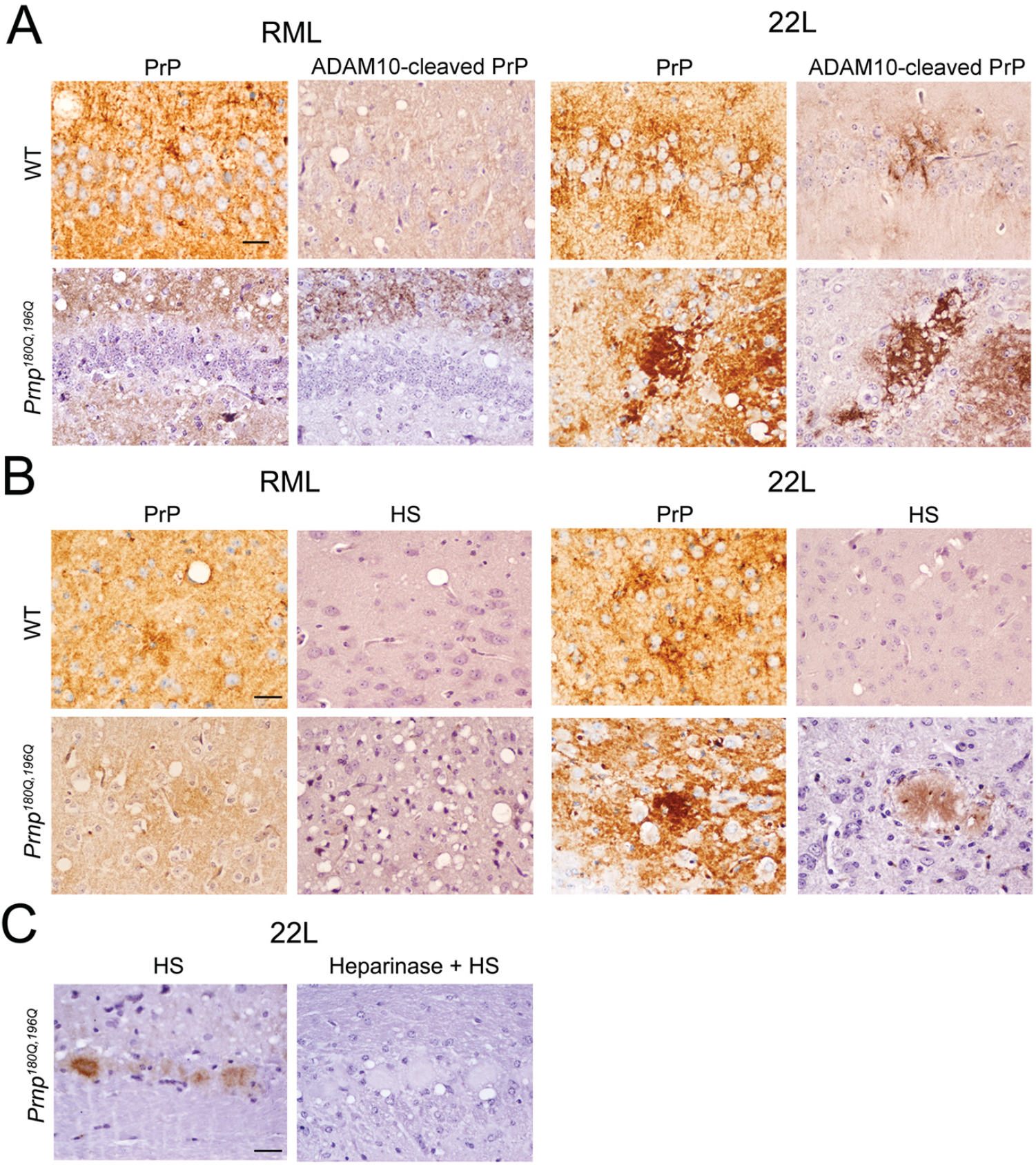


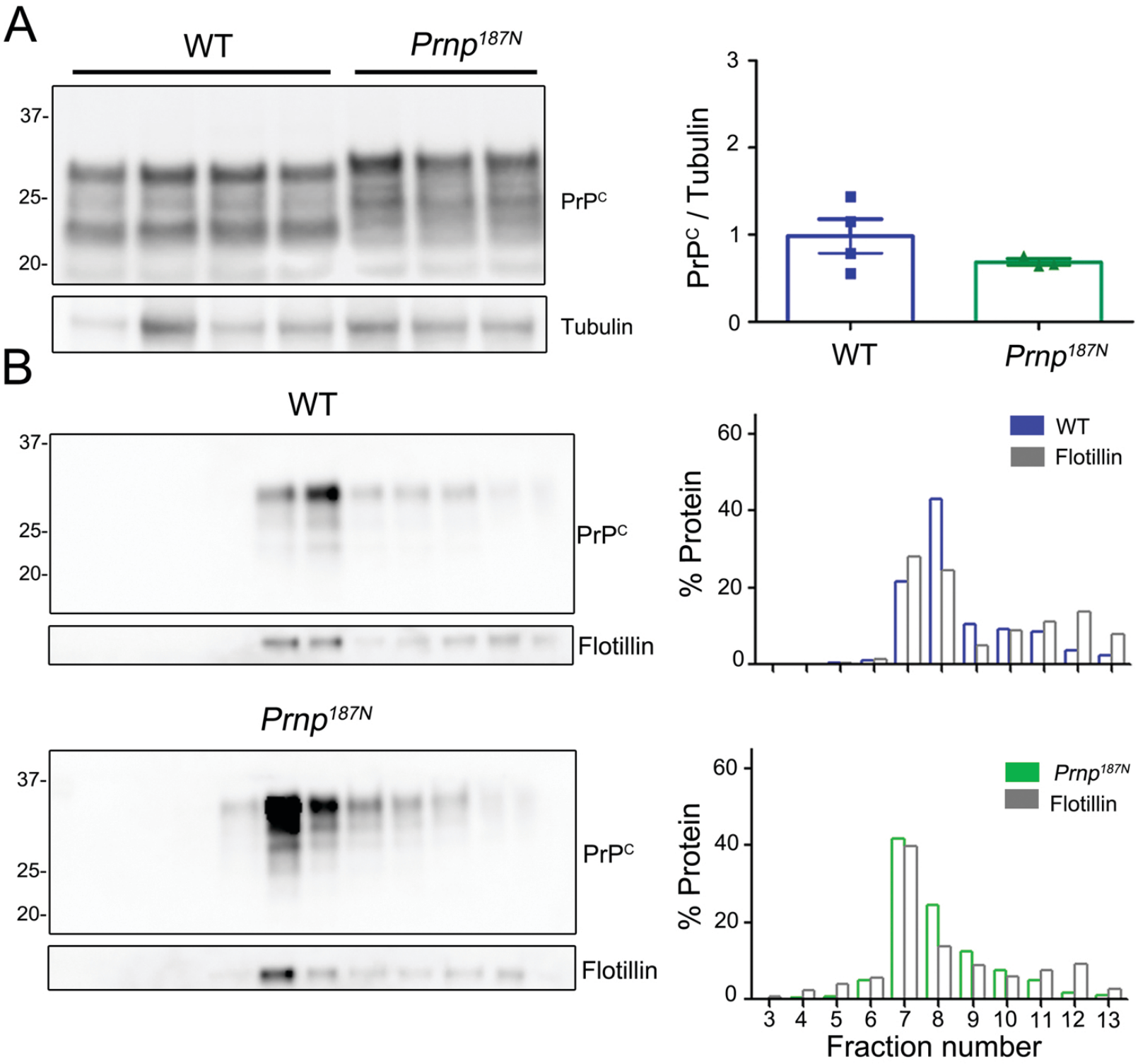
Supplementary figure 1. Characterization of PrP^{180Q,196Q} in cells and in mice. (A) Hippocampal brain sections stained with hematoxylin and eosin (HE) or immunolabelled for PrP revealed no evidence of neuronal loss or PrP accumulation in aged WT and *Prnp*^{180Q,196Q} mice, as compared to prion-infected WT mice (RML strain). **(B)** Representative western blots of cell lysate and media from phospholipase C (PIPLC)-treated WT, PrP$^{180A/196A}$, and PrP$^{180Q/196Q}$ RK13 cells. Quantification relative to WT PrP^{C} shows slightly lower surface PrP$^{180Q/196Q}$ than those from WT PrP^{C}, while PrP$^{180A/196A}$ shows a marked reduction in surface expressed PrP^{C} (n = 3-4 experiments/mutant). **(C)** Western blot of PK-treated uninfected WT and *Prnp*^{180Q,196Q} brain reveal that PrP^{C} is similarly sensitive to proteolytic digestion. **(D)** Western blot of soluble (S) and insoluble (P) PrP after ultracentrifugation shows similarly low levels of insoluble PrP in both uninfected WT and *Prnp*^{180Q,196Q} brain, but high levels in prion-infected (RML) WT brain. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, 1-way ANOVA, Tukey's post-test (panel B). Scale bar = 200 µm.



Supplementary figure 2. WT and *Prnp*^{180Q,196Q} show differences in spongiosis and plaque morphology, but similar cerebellar PrP^{C} levels. (A) Hippocampal brain sections stained with hematoxylin and eosin (HE) from ME7-infected WT and *Prnp*^{180Q,196Q} mice show a mild compared to a severe loss of CA1 pyramidal neurons, respectively (asterisk). Higher magnification of boxed region is shown in right and shows a gemistocytic astrocyte (arrow) and a plaque (arrowhead). **(B)** Western blot of cerebellum shows similar PrP^{C} expression levels in uninfected WT and *Prnp*^{180Q,196Q} mice [n = 4 (WT) or 5 (*Prnp*^{180Q,196Q}) mice]. **(C)** Quantification of the total PrP^{Sc} plaque number (all >15 μm) in the hippocampus of ME7-inoculated WT and *Prnp*^{180Q,196Q} mice. **(D)** Graph showing PrP^{Sc} plaques grouped according to their diameter. **(E)** Brain sections immunolabelled for PrP show parenchymal plaques surrounded by small vacuoles (florid plaques) in ME7-infected *Prnp*^{180Q,196Q} mice only. Scale bar = 50 μm (panel A, left panel, and panel E) and 200 μm (panel A, right panel). $^{**}P \leq 0.01$, unpaired, 2-tailed Student's t test; (panel C) and $^{*}P \leq 0.05$; $^{***}P \leq 0.001$, 2-way ANOVA, Bonferroni post-test (panel D).



Supplementary figure 3. RML- and 22L-infected brain sections immunolabelled for ADAM10-cleaved PrP or HS. (A) PrP- or ADAM10-immunolabelled brain sections show that some ADAM10-cleaved PrP^{Sc} is present in RML- and 22L-infected *Prnp*^{180Q196Q} mice, while in WT mice, less or no ADAM10-cleaved PrP^{Sc} is evident. **(B)** HS-immunolabelled sections show no HS labelling in RML- prion infected WT or *Prnp*^{180Q,196Q} brain, and a rare HS-immunolabelled plaque in 22L-infected *Prnp*^{180Q196Q} brain. **(C)** HS-immunolabelled sections do not show HS labelling when slides were pretreated with a cocktail of heparinases. Scale bar = 50 µm.



Supplementary figure 4. PrP^{C} expression and membrane localization in the *Prnp^{187N}* mouse brain. (A) Western blot shows no difference in PrP^{C} expression in WT and *Prnp^{187N}* brain (*n* = 3-4). **(B)** PrP^{C} co-localizes with flotillin in detergent resistant membranes from WT and *Prnp^{187N}* brain (n=2 mice/group). Graph shows the signals quantified from the western blots.

Additional Methods

PrP^C expression in *Prnp*^{180Q,196Q} and *Prnp*^{187N} brain

To measure total PrP^C levels in WT (C57BL/6) and *Prnp*^{180Q,196Q} brain samples, 30 µg of total protein was deglycosylated using the PNGase F kit (New England Biolabs). In brief, proteins were denatured for 10 minutes at 95 °C, cooled on ice for 10 minutes, and then deglycosylated with PNGase F for 24 hours at 37 °C. Proteins were then briefly centrifuged (30 seconds at 5000 *g*) and pellets were resuspended in NuPage loading dye (Invitrogen) prior to electrophoresis and transfer to nitrocellulose. Non-PNGase treated samples were included as controls. Membranes were incubated in anti-PrP antibody POM1 (epitope in the globular domain, amino acids 121–231 of the mouse PrP)(52), anti-mouse-HRP antibody, and developed using chemiluminescent substrate. The PrP^C signals were captured and quantified using the Fuji LAS 4000 imager and Multigauge V3.0 software.

Quantification of surface PrP^C in primary neurons

Primary cortical neurons from P0 WT or *Prnp*$^{180Q/196Q}$ mouse pups were cultured in neurobasal media (Gibco) containing 2% B27 Supplement (Gibco) and 1X GlutaMAX™ (Gibco) for a minimum of 6 days (500,000 cells in a 6 well plate). In brief, the cerebral cortices were dissected, dissociated with 0.25% trypsin at 37 °C for 20 minutes, treated with DNase, and triturated. Debris was removed by passing the cells through a 70 µm cell strainer. Cells were then centrifuged for 5 minutes and resuspended in neurobasal media with 2% B27 and 1X GlutaMAX™. For the cell surface PrP^C experiment, 7 day old neurons were washed with PBS, and treated with PIPLC (Sigma Aldrich) in Opti-MEM (ThermoFisher Scientific) or PBS in OptiMEM as a control for 20 minutes at 37 °C. Media containing the PIPLC-cleaved PrP was collected, spun down at 2000 *g* for 5 minutes to remove cell debris, and saved for western blotting. The adherent neurons were washed twice with PBS, lysed in 2% N-lauryl sarcosine with benzonase™, collected and maintained on ice for 30 minutes, and centrifuged at 2000 *g* at 4 °C for 5 minutes. The supernatant was collected and the protein concentration measured. The PrP^C levels in the cell lysates and media were measured by western blot and were incubated in anti-PrP antibody POM1, 9, 10, 19 (epitopes in the globular domain, amino acids 121–231 of the mouse PrP)(52). The PrP^C signals were captured and quantified using the Fuji LAS 4000 imager and Multigauge V3.0 software. The PIPLC experiment was independently repeated three times for each genotype on separate days.

PrP^C immunofluorescent staining of primary neurons

Primary cortical neurons from P0 WT or *Prnp*$^{180Q/196Q}$ mouse pups were collected and cultured in neurobasal media containing 2% B27 and 1X GlutaMAX™ for a minimum of 6 days. Cells were plated on glass coverslips treated with poly-L-lysine. Cells were fixed in 4% paraformaldehyde for 30 minutes at 37 °C, washed in PBS, and permeabilized with 0.5% Triton X-100 in PBS for 5 minutes. Cells were then washed, blocked for 1 hour (3% BSA in 0.1% Triton X-100), incubated with primary antibody overnight (POM1) followed by secondary antibody for 1 hour (anti-mouse Alexa488) (Jackson ImmunoResearch). Cells were incubated with DAPI for 5 minutes prior to mounting using ProLong® Gold antifade reagent. Cells were imaged at 63X using a Leica TCS Sp8 confocal microscope. Z-stacks were constructed using the Leica LASX software.

Quantification of PrP^C in detergent resistant membranes

Brain samples from WT and *Prnp*$^{180Q/196}$ mice were homogenized in PBS containing protease inhibitors (Complete TM) (10% w/v) and then centrifuged at 4 °C for 5 minutes at 1000 g. Supernatants (1 mg protein) were lysed in a cold lysis buffer (25 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 2% Triton X-100) (1:1) for 1 hour at 4 °C with rotation. Samples were then mixed with Optiprep™ (40% final), overlayed with 30% optiprep and 5% optiprep in gradient buffer [25 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA], and ultracentrifuged at 163,000 g at 4 °C

for 20 hours (Beckman Coulter SW 50.1, swinging bucket). Fifteen fractions were collected and aliquots analyzed by western blotting, probing membranes with monoclonal anti-PrP POM1 antibody and anti-flotillin antibody (BD Bioscience).

Quantification of surface PrP^C in RK13 cells

PrP-deficient RK13 cells (ATCC) were plated in 10 cm dishes and transfected with plasmid DNA [pcDNA3.1 vector (Invitrogen)] containing mouse WT *Prnp* or the desired glycan mutants (*Prnp*$^{180Q/196Q}$ and *Prnp*$^{180A/196A}$) using Lipofectamine 3000 (Invitrogen). To cleave PrP^C from the plasma membrane through the phosphatidylinositol moiety 24 h post-transfection, cells were washed twice in PBS, and treated with 0.75 milli-units of phospholipase C from *Bacillus cereus* (AG Scientific) for 1 hour at 37 °C. The media was recovered and clarified by centrifugation at 2,000 *g* for 1 minute. Cells were next washed twice in PBS, harvested in 1 ml of PBS, and centrifuged for 1 minute at 1,000 *g*. The pellet was resuspended in PMCA buffer (PBS containing 1% Triton X-100, 150 mM NaCl, and 5 mM EDTA plus CompleteTM protease inhibitors), passed repeatedly through a 27-gauge needle, and clarified by centrifuging at 2,000 *g* for 1 minute. Equal concentrations of protein were analyzed by western blotting using monoclonal anti-PrP antibody POM1 and anti-actin antibody (Genetex).

Proteinase K sensitivity of PrP^C from *Prnp*$^{180Q/196Q}$ brain samples

Equal amounts of protein from 10% (w/v) brain homogenates were solubilized in PBS containing 2% N-lauryl sarcosine for 15 minutes at 37 °C. Samples then were incubated with PBS or proteinase K (Roche) for 30 minutes at 37 °C, and analyzed by western blotting.

Assessment of PrP^C solubility

100 µg of 10% (w/v) brain homogenates from uninfected WT (C57BL/6) and *Prnp*$^{180Q/196Q}$ *and* *Prnp*^{187N} mice were solubilized in an equal volume of 2% N-lauryl sarcosine in PBS and centrifuged at 1,000 *g*. Supernatants were incubated at 37 °C for 30 minutes, transferred to ultracentrifuge tubes, and centrifuged at 100,000 *g* for 60 minutes at 4 °C. Pellets were resuspended in loading dye. Supernatant proteins were precipitated using ice cold acidified acetone/methanol (1:4) and resuspended in loading dye. Supernatant and pellet fractions were immunoblotted using anti-PrP antibody POM19. PrP^C signals were captured and quantified using the Fuji LAS 4000 imager and Multigauge V3.0 software. Brain samples from 2 (WT) or 3 (*Prnp*$^{180Q/196Q}$, *Prnp*^{187N}) mice were measured per mouse line.

Prion transmission experiments in mice

Groups of 4-6 male and female WT (C57BL/6), *Prnp*$^{180Q/196Q}$, and *Prnp*^{187N} mice were anesthetized with ketamine and xylazine and inoculated into the left parietal cortex with 30 µl of 1% prion-infected brain homogenate prepared from terminally ill mice. One group of *Prnp*^{187N} mice was inoculated into the tongue with 10 µl of 1% brain homogenate. Mice were maintained under specific pathogen-free conditions on a 12:12 light/dark cycle, and monitored three times weekly for the development of prion disease, including weight loss, ataxia, kyphosis, stiff tail, hind leg clasp, and hind leg paresis. Mice were euthanized at the onset of terminal disease, and the incubation period was calculated from the day of inoculation to the day of terminal clinical disease. During the necropsy, brain, spinal cord, heart, lungs, spleen, liver, and kidney were collected. One hemi-brain was formalin-fixed, then immersed in 96–98% formic acid for 1 hour, washed in water, and post-fixed in formalin for 2–4 days. Hemi-brains were then cut into 2 mm transverse sections and paraffin-embedded for histological analysis. The remaining hemi-brain was cut and a 2–3 mm transverse section at the level of the hippocampus/thalamus was embedded in optimal cutting temperature (OCT) compound and immediately frozen on dry ice for future h-FTAA staining and fluorescence life time imaging of cryosections. The remaining brain sections were frozen for biochemical analyses. The other organs were also formalin-fixed and immersed in 96–98% formic acid for histological analysis.

The prion strains used were mouse-adapted RML, 22L, ME7, and mCWD. The RML, 22L, and ME7 are cloned prion strains that have been maintained in C57BL/6 mice (1-4), while mCWD inoculum was the fifth passage of a single CWD-infected deer brain (4, 5) propagated in *tg*a20 mice. For the serial passages of RML, 22L, and ME7 prions in *Prnp*$^{180Q/196Q}$ mice, homogenates from individual mouse brains were used. As negative controls, groups of age-matched WT (C57BL/6), *Prnp*$^{180Q/196Q}$, and *Prnp*^{187N} mice (n= 4-6 mice/ group) were inoculated intracerebrally with mock brain homogenate from uninfected WT mice and housed under the same pathogen-free conditions as the prion-infected-mice.

Histopathology and immunohistochemical stains

Four micron sections were cut onto positively charged silanized glass slides and stained with hematoxylin and eosin (HE), or immunostained using antibodies for total PrP (SAF84)(Cayman Chemical), ADAM 10-cleaved PrP [sPrP^{G228} epitope at residue 228G (6)], astrocytes (glial fibrillary acidic protein, GFAP), and heparan sulfate (10E4)(AMS Bioscience). For PrP and sPrP^{G228} staining, sections were deparaffinized and incubated for 5 minutes in 96% formic acid, then washed in water for 5 minutes, treated with 5 µg/ml of proteinase-K (PK) for 7 minutes, and washed in water for 7 minutes. Sections were then placed in citrate buffer (pH 6), heated in a pressure cooker for 20 minutes, cooled for 5 minutes, and washed in distilled water. Sections were blocked and incubated with anti-PrP SAF-84 for 45 minutes followed by anti-mouse biotin (Jackson Immunolabs; 1:250) for 30 minutes and then streptavidin-HRP (Jackson Immunoresearch) for 45 minutes. Slides were then incubated with DAB reagent (ThermoFisher Scientific) for 15 minutes. Sections were counterstained with hematoxylin. GFAP immunolabelling for astrocytes (DAKO; 1:6,000) and additional PrP immunostains were performed on an automated tissue immunostainer (Ventana Discovery Ultra, Ventana Medical Systems, Inc) with antigen retrieval using a protease treatment (P2, Ventana) for 16 minutes, or by heating sections in a Tris-based EDTA buffer at 95 °C for 92 minutes, respectively. For HS immunolabelling, epitope retrieval was performed by heating in a pressure cooker as described for the PrP immunostain. Control slides included substituting isotype control antibodies for the primary, and for the HS stain, control sections were treated with heparin lyases I, II, III at 37 °C for 60 minutes prior to immunolabelling.

For the Congo red staining, slides were deparaffinized, fixed in 70% ethanol for 10 minutes, immersed in an alkaline solution and then stained with Congo red solution overnight.

Lesion profile

Brain lesions from prion-infected WT (C57BL/6) and *Prnp*$^{180Q/196Q}$ mice were scored for the level of spongiosis, gliosis, and PrP immunological reactivity on a scale of 0–3 (0= not detectable, 1= mild, 2= moderate, 3= severe) in 8 regions including grey and white matter: (1) dorsal medulla, (2) cerebellum, (3) hypothalamus, (4) medial thalamus, (5) hippocampus, (6) septum, (7) medial cerebral cortex dorsal to hippocampus, and (8) cerebral peduncle. A sum of the three scores resulted in the value obtained for the lesion profile for the individual animal and was depicted in the 'radar plots'. Two investigators blinded to animal identification performed the histological analyses. Groups of 4-6 mice were analyzed for each strain.

Quantification of ME7 plaques in WT and *Prnp*^{180Q,196Q} mice

Images from ME7-infected brain sections immunolabelled for PrP were collected and the plaques and plaque-like aggregates were counted and measured using the line tool in Image J®. The length of all plaques in a brain section in hippocampus, cortex and the dorsal thalamus were measured. For the plaque counts, only plaques larger than 15 µm diameter were counted.

PK-resistant PrP^{Sc} analyses

80 µg of total protein from prion-infected WT (C57BL/6), *Prnp*$^{180Q/196Q}$, and *Prnp*^{187N} 10% (w/v) brain homogenates was lysed in 2% N-lauryl sarcosine in PBS and digested with 50 µg/ml PK

prior to immunoblotting for PrP. PrP^{Sc} from ME7-infected *Prnp*$^{180Q/196Q}$ mice and mCWD-infected WT mice was concentrated from 10% brain homogenate by performing sodium phosphotungstic acid precipitation prior to western blotting (7). In brief, 100 µl of 10% brain homogenate in an equal volume of 4% sarkosyl in PBS was digested with benzonase™ (Sigma) followed by treatment with 50 µg/ml PK at 37 °C for 30 minutes. After addition of 4% sodium phosphotungstic acid in 170 mM $MgCl_2$ and protease inhibitors (Complete TM, Roche), extracts were incubated at 37 °C for 30 minutes and centrifuged at 18,000 *g* for 30 minutes at 25 °C. Pellets were resuspended in 2% N-lauryl sarcosine prior to electrophoresis and immunoblotting. Membranes were incubated with monoclonal antibody POM19 (8) or polyclonal antibody $sPrP^{G228}$ [(6) followed by incubation with an HRP-conjugated IgG secondary antibody]. The blots were developed using a chemiluminescent substrate (Supersignal West Dura ECL, ThermoFisher Scientific) and visualized on a Fuji LAS 4000 imager. Quantification of PrP^{Sc} glycoforms was performed using Multigauge V3 software (Fujifilm).

PrP^{Sc} solubility assay

10% (w/v) brain homogenates from prion-infected mice were solubilized in 1% N-lauryl sarcosine in PBS (final) and digested with 50 µg/mL of proteinase K (final concentration) at 37 °C for 30 minutes. Protease inhibitors were added (Complete TM™), and samples were layered over 15% Optiprep™ and centrifuged at 18000 *g* for 1 hour at 4 °C. Supernatants were removed and pellets were resuspended in PBS in a volume equivalent to the supernatant. Supernatant and pellet fractions were immunoblotted using anti-PrP antibody POM1. PrP signals were captured and quantified using the Fuji LAS 4000 imager and Multigauge V3.0 software. Brain samples from 4-6 mice were measured per strain.

Conformation stability assay

Prion stability in guanidine hydrochloride (GndHCl) was measured as previously described (9). In brief, 10% brain homogenates were denatured for 1 hour in increasing concentrations of GdnHCl from 0 to 6 M. Samples were then diluted with a tris-based lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 10 mM EDTA, 2% sarkosyl; pH 7.5) to 0.15 M GdnHCl and digested with PK at a ratio of 1:500 (1 µg PK: 500 µg total protein) for 1 hour at 37 °C. The digestion was stopped with 2 mM phenylmethylsulfonyl fluoride (PMSF) followed by centrifugation at 18000 *g* for 1 hour. Pellets were washed in 0.1 M $NaHCO_3$ (pH 9.8) and centrifuged at 18000 *g* for 20 minutes. Pellets were then denatured in 6 M guanidine isothiocyanate (GdnSCN), diluted with 0.1 M $NaHCO_3$, and coated passively onto an ELISA plate. PrP was detected with biotinylated-POM1 antibody (8), a streptavidin HRP-conjugated secondary antibody, and 3,3',5,5'-tetramethylbenzidine (TMB) ELISA substrate (ThermoFisher Scientific). The stability was measured in a minimum of 3 independent experiments for each strain, always comparing WT and *Prnp*$^{180Q/196Q}$ or *Prnp*^{187N}.

h-FTAA staining and fluorescence life time imaging

Sections (10 µm) of OCT-embedded brain samples were cut onto positively charged silanized glass slides, dried for 1 hour, and fixed in 96% then 70% ethanol for 10 minutes each. After washing with deionized water, sections were equilibrated in PBS for 10 minutes. Heptamer-formyl thiophene acetic acid *(*h-FTAA*)* was diluted in PBS to a final concentration of 1.5 µM and added to the sections. The sections were incubated with h-FTAA for 30 minutes at room temperature, washed with PBS, and mounted using Dako fluorescence mounting medium. The detailed procedure of FLIM imaging of h-FTAA stained prion aggregates has been described previously (10). In brief, the fluorescence decay of h-FTAA bound to PrP aggregates was collected using an inverted Zeiss (Axio Observer.Z1) LSM 780 microscope (Carl Zeiss MicroImaging GmbH) equipped with a modular FLIM system from Becker and Hickl. In this setup, the emitted photons were routed through the direct coupling confocal port of the Zeiss LSM 780 scanning unit and detected by a Becker and Hickl HPM-100-40 hybrid detector. Data were recorded by a Becker and Hickl Simple-Tau 152 system (SPC-150 TCSPC FLIM module) with the instrument recording

software SPCM version 9.42 in the FIFO image mode, 256 × 256 pixels, using 256 time channels (Becker and Hickl GmbH). For all acquisitions, a T80R20 main beam splitter was used and the pinhole was set to 20.2 µm. Scanning area was set to 235.7 µm × 235.7 µm, with a scanning resolution of 512 × 512 pixels. A Plan-Apochromat 40 × / 1.3 Oil DIC objective lens was used and a 510 nm longpass filter was positioned in front of the hybrid detector. Excitation utilized the 490 nm laser line from the pulsed tunable In Tune laser (Carl Zeiss MicroImaging GmbH) with a repetition rate of 40 MHz. The acquired decay curves were fitted with a bi-exponential decay function and two components of the fit, and color-coded images, as well as distribution histograms showing the intensity-weighted mean lifetimes were generated with the same software. Decays were collected from 5-10 individual prion deposits from a minimum of three different cases of each prion-infected brain and subsequently analyzed in SPCImage version 3.9.4 (Becker and Hickl GmbH).

Purification of PrP^{Sc} for mass spectrometry studies

PrP^{Sc} was purified from mouse brain following previously described procedures (11), with minor modifications. Briefly, one ml of 10% brain homogenate was mixed with an equal volume of TEN(D) buffer [5% sarkosyl in 50 mM Tris-HCl, 5 mM EDTA, 665 mM NaCl, 0.2 mM dithiothreitol, pH 8.0), containing complete TM protease inhibitors (Roche)]. Samples were incubated on ice for 1 hour followed by centrifugation at 18000 *g* for 30 minutes at 4 °C. All but 100 µl of supernatant was removed, and the pellet was resuspended in 100 µl of residual supernatant and diluted to 1 ml with 10% sarkosyl TEN(D). Each supernatant and pellet was incubated for 30 minutes on ice and then centrifuged at 22000 *g* for 30 minutes at 4 °C. Supernatants were recovered while pellets were held on ice. Supernatants were added to ultracentrifuge tubes with 10% N-lauryl sarcosine TEN(D) buffer containing protease inhibitors and centrifuged at 150000 *g* for 2.5 hours at 4 °C. Supernatants were discarded while pellets were rinsed with 100 µl of 10% NaCl in TEN(D) buffer with 1% sulfobetaine (SB3–14) and protease inhibitors and then combined with pellets and centrifuged at 225000 *g* for 2 hours at 20 °C. The supernatant was discarded and pellet was washed and then resuspended in ice cold TMS buffer containing protease inhibitors (10 mM Tris-HCl, 5 mM $MgCl_2$, 100 mM NaCl, pH 7.0). Samples were incubated on ice overnight at 4 °C. Samples were then incubated with 25 units/ml benzonase™ (Sigma-Aldrich) and 50 mM $MgCl_2$ for 30 minutes at 37 °C followed by a digestion with 10 µg/ml PK for 1 hr at 37 °C. PK digestion was stopped by incubating samples with 2 mM PMSF on ice for 15 minutes. Samples were incubated with 20 mM EDTA for 15 minutes at 37 °C. An equal volume of 20% NaCl was added to all tubes followed by an equal volume of 2% SB3–14 buffer. For the sucrose gradient, a layer of 0.5 M sucrose in buffer [100 mM NaCl, 10 mM Tris, and 0.5% SB3–14 (pH 7.4)] was added to ultracentrifuge tubes. Samples were then carefully loaded on top of the sucrose layer and the tubes topped with TMS buffer. Samples were centrifuged at 200000 *g* for 2 hours at 20 °C. The pellet was rinsed with 0.5% SB3–14 in PBS, resuspended in 50 µl of 0.5% SB3–14 in deionized water, and stored at −80 °C. Gel electrophoresis and silver staining were performed to assess the purity of brain extracts. To quantify PrP levels, samples were compared against a dilution series of recombinant PrP by immunoblotting and probing with the anti-PrP antibody POM19 (8).

Heparan sulfate purification and analysis by mass spectrometry

Heparan sulfate (HS) was extracted from the purified PrP^{Sc} preparation or from whole brain homogenate (10%, w/v) by anion exchange chromatography. First, purified PrP^{Sc} and brain homogenates were denatured in 0.5 M NaOH (final concentration) on ice at 4 °C for 16 hours, neutralized with 0.5 M acetic acid (final concentration), and digested with pronase for 25 hours at 37 °C. Next, the HS was purified by diethyl-aminoethyl (DEAE) sepharose chromatography (Healthcare Life Sciences), and digested with 1 milli-unit each of heparin lyases I, II, and III to depolymerize the HS chains. The disaccharides were then tagged by reductive amination with [$^{12}C_6$]aniline (12) and mixed with [$^{13}C_6$]aniline-tagged disaccharide standards. Samples were

analyzed by liquid chromatography-mass spectrometry (LC-MS) using an LTQ Orbitrap Discovery electrospray ionization mass spectrometer (ThermoFisher Scientific). Internal disaccharides were identified based on their unique mass and quantified relative to the HS weight (12, 13).

Heparin sepharose chromatography

PrP-deficient RK13 cells (ATCC) were plated in 10 cm dishes and transfected with 7.5 µg plasmid pcDNA3.1 containing mouse WT *Prnp* or the glycan mutant (2 plates per WT or mutant: $Prnp^{180Q/196Q}$, $Prnp^{180Q}$, and $Prnp^{187N}$) using Lipofectamine 3000 (Invitrogen). After 24 hours, cells were washed twice in PBS and treated with 0.75 milli-units of phospholipase C from *Bacillus cereus* (Sigma Aldrich) in 1.5 ml of Opti-MEM media in PBS (1:2 dilution) (ThermoFisher Scientific) for one hour at 37 °C. The media was recovered each plate and clarified by centrifugation at 2000 *g* for 1 minute, and the supernatant was transferred into a clean tube. Supernatants from the duplicate plates were pooled into the new tubes and saved for chromatography analysis.

For affinity chromatography, 0.5 ml of Heparin Sepharose 6 Fast Flow beads (Healthcare Life Sciences) were loaded into disposable Bio-Spin® chromatography columns (Bio-Rad) and packed with 2 ml of equilibration buffer (0.15 M NaCl in 25 mM HEPES, pH 7.4). Supernatants (3 ml) containing GPI-cleaved proteins, including unglycosylated, monoglycosylated, diglycosylated, and triglycosylated PrP^{C}, were applied onto the columns. The flow through was recovered, recirculated onto the column two times, and saved in a clean tube marked “flow through”. The column was next washed with 2 ml of 150 mM NaCl in 25 mM HEPES buffer (pH 7.4) and the unbound proteins were recovered in a clean tube. The bound prion protein was step-eluted with 1 ml of elution buffer containing increasing concentrations of NaCl (300 mM - 2M) in 25 mM HEPES. The 150 mM wash and all eluates (45 µl) were analyzed for PrP level by immunoblot using POM19 antibody. At least three experimental replicates were performed for each PrP construct (WT and $Prnp^{187N}$: n= 3; $Prnp^{180Q/196Q}$ and $Prnp^{180Q}$: n= 4).

Brain homogenates from WT (n= 3) and $Prnp^{180Q/196Q}$ (n= 3) mice were also used to study the binding affinity of WT and unglycosylated PrP^{C} to heparin. 100 µl of brain homogenate (10%) were mixed with 150 µl of 2% sarkosyl in PBS, incubated for 30 minutes at 37 °C, and centrifuged at 1000 *g* for one minute. Supernatants were then transferred to ultracentrifuge tubes and centrifuged at 150000 *g* for 1 hour at 4 °C. The supernatant was loaded into disposable Bio-Spin® chromatography columns (Bio-Rad) containing Heparin Sepharose 6 Fast Flow beads (Healthcare Life Sciences), the flow through was collected, the columns were then washed in 150 mM NaCl, and the proteins were eluted with 300 mM – 2.0 M NaCl as described above. Recovered PrP^{C} was immunoblotted using POM19 and $sPrP^{G228}$ antibodies.

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