Supplemental Figures

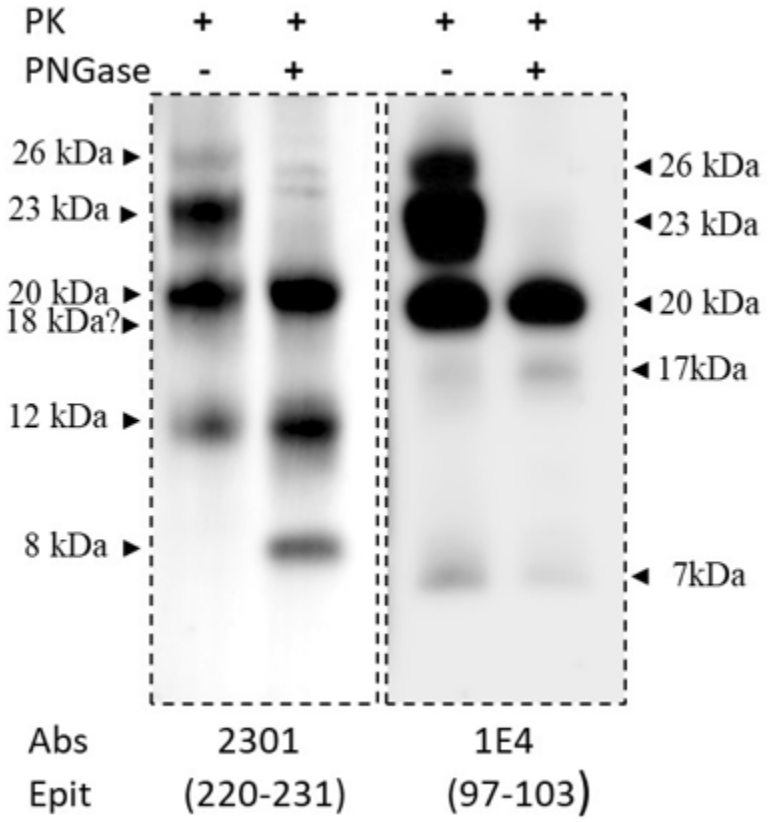

Figure S1. Immunoblots comparing VPSPr fragments belonging to the C- and N-terminus groups. As expected, following deglycosylation with PNGase F, the full-length 20 kDa fragment is detected by both Abs, while the 12 kDa CTF and the 8 kDa fragments are demonstrated only by the antibody to the C-terminus. Note the increase in size of the 12 kDa CTF band following deglycosylation, suggesting that the glycosylated 18 kDa joins the 12 kDa. The apparent increase in the band size may also be consistent with the presence of both the 12 and 13 CTF (see text for details). Purified resPrP^{D} (was used either following PK-treatment alone (PK 8U/ml) (PK+) or also treated with PNGase F (PNGase+).

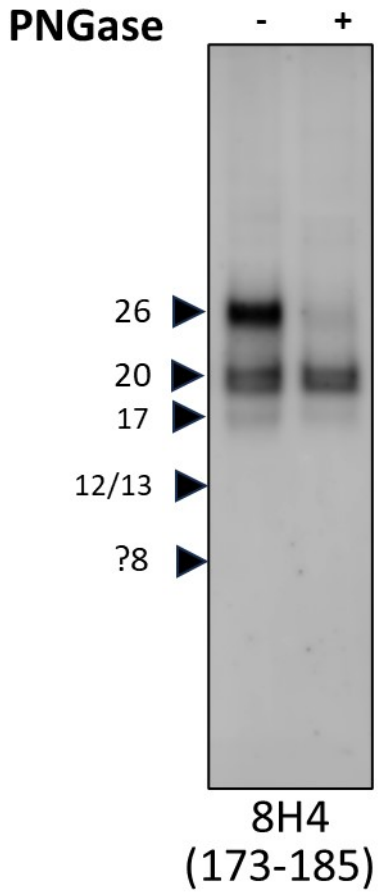

Figure S2. Immunoblotting of VPSPr resPrP^{D} fragments with a monoclonal antibody to the C-preterminal region. Inexplicably, no 12/13 kDa CTF fragments nor 8 kDa and lower molecular weight fragments were detected. See Methods and Materials for details.

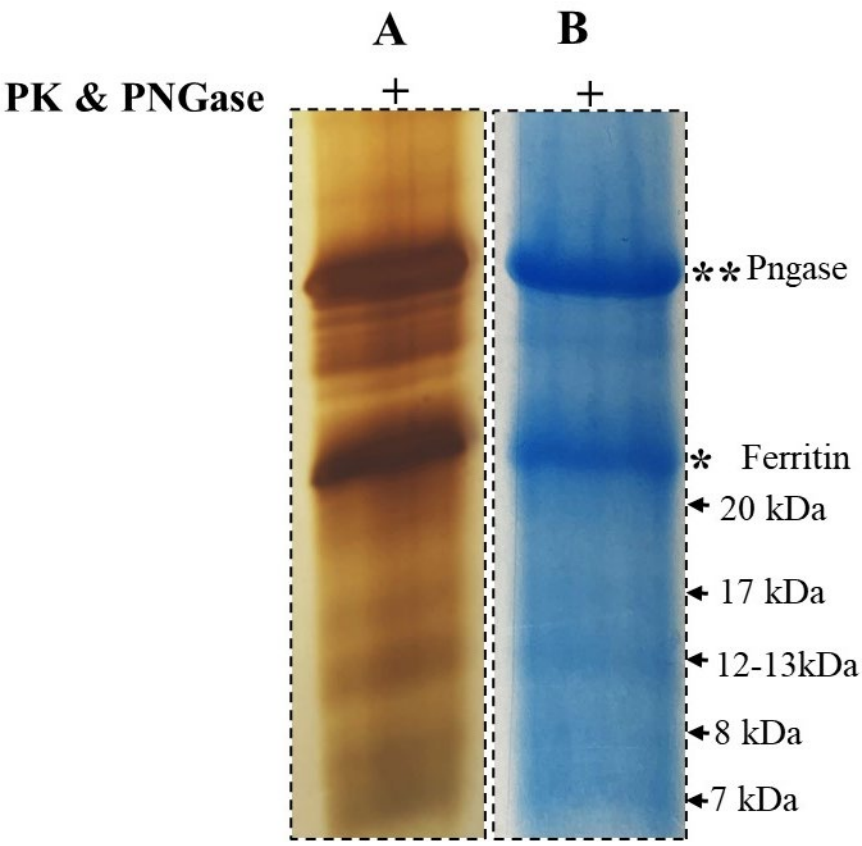

Figure S3: Purified PK and PNGase F-treated PrP was analyzed by SDS PAGE on a 15 cm long gel and stained with (**A)** Silver, and (**B)** colloidal Coomassie as per manufacturer's instructions. For colloidal Coomassie staining, 40 times more purified PrP was loaded compared to Silver staining preparations. After destaining with water, PrP-specific fragments were excised from the Coomassie-stained gel and analyzed by mass spectrometry for N- and C-terminal sequencing. See Methods and Materials for more details.

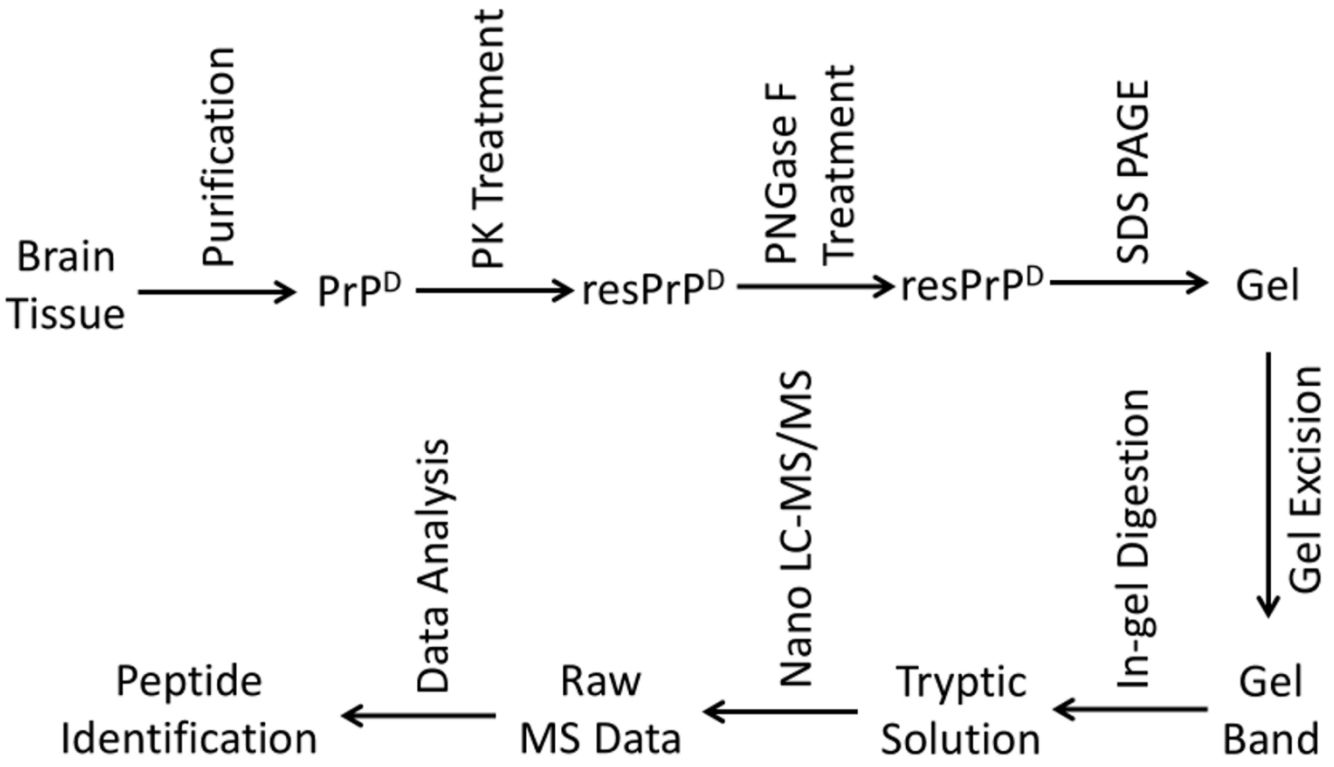

Figure S4. Workflow of MS-based sequencing experiments.

Supplemental Tables

Table S1. Peptides identified by Nano-LC MS/MS for 6 fragments of 7kDa, 8kDa-A, 8kDa-B, 12-13kDa CTF, 17kDa and 20kDa fragments. *(please refer to excel file in Supplementary Material File 2)*

Table S2. Contributions of the post-translational modifications to the molecular weights based on the primary structure of the individual PrP^D fragments as determined by mass-spectrometry.

Post-translational modifications[1]	7 kDa	8 kDa A	8 kDa B	12 kDa	17 kDa	20 kDa
Oxidation	4	2	1	2	5	5
181N/D	0	1	0	1	1	0
197N/D	0	1	1	1	1	1
Sequencing MW + PTM (kDa)	6.9 + 0.064	8.14 + 0.034	4.43 + 0.017	8.37 + 0.034	15.38 + 0.082	15.56 + 0.081

[1] Only modifying post-translational modifications (PTM) are listed. Other PTM examined were ubiquitination, phosphorylation, deamidation and acetylation.

Table S3: Clinical and histopathological data including electrophoretic profiles of the cases used for $resPrP^D$ purification.

Case (No.)	Age at onset (yrs)	Gender	Disease duration (mos)	Pathology and $resPrP^D$ profile
1	78	Male	56	Typical of VPSPr
2	79	Male	24	Typical of VPSPr