PNAS

Supporting Information for

Heterozygosity for cervid S138N polymorphism results in subclinical CWD in gene-targeted mice and progressive inhibition of prion conversion

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Figures S1 to S5

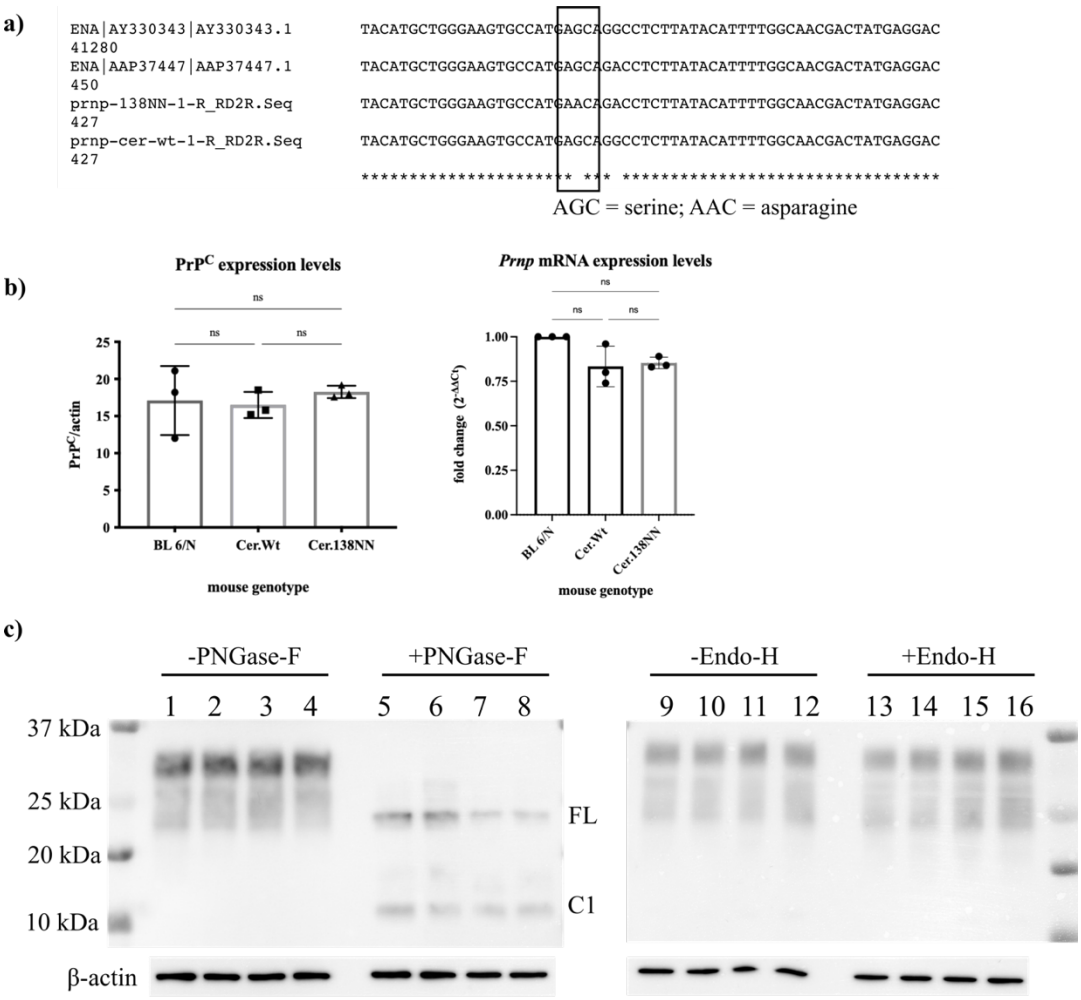

Figure S1. Cervid-*Prnp* knock-in mouse characterization. **(a)** *Prnp*.Cer.Wt and *Prnp*.Cer.138NN gene-targeted mice *Prnp* sequences aligned to mule deer (GenBank: AY330343.1) and white-tailed deer (GenBank: AAP37447.1) *Prnp* sequences using ClustalW. **(b)** *Prnp* mRNA and PrP^C expression levels in the brain presented as fold change and PrP^C/actin levels, respectively, with C57BL/6N as the control. *Prnp* Ct values were analyzed in CFX Maestro (Biorad) and normalized to actin. PrP and actin signals in WB were quantified using Image Lab (Biorad). Statistical analysis was performed using a one-way ANOVA and graphs were generated in Graphpad Prism v9. The x-axis on both graphs

represents the mouse lines. The y-axis represents the fold change and PrP^C levels, respectively. ns: non-significant. **(c)** Representative blot for correct cellular processing of PrP^C as determined by its sensitivity to PNGase-F and resistance to Endo-H digestion. Lanes 1, 5, 9, 13: C57BL/6N BH; lanes 2, 6, 10, 14: *Prnp*.Cer.Wt BH; lanes 3-4, 7-8, 11-12, 15-16: *Prnp*.Cer.138NN BH (2 different mice). Lanes 1-4, 9-12: samples without PNGase-F or Endo-H enzymes; lanes 5-8: samples with PNGase-F enzyme; lanes 13-16: samples with Endo-H enzyme. FL: full-length PrP^C; C1: C1 fragment of PrP^C as a product of endogenous alpha-cleavage.

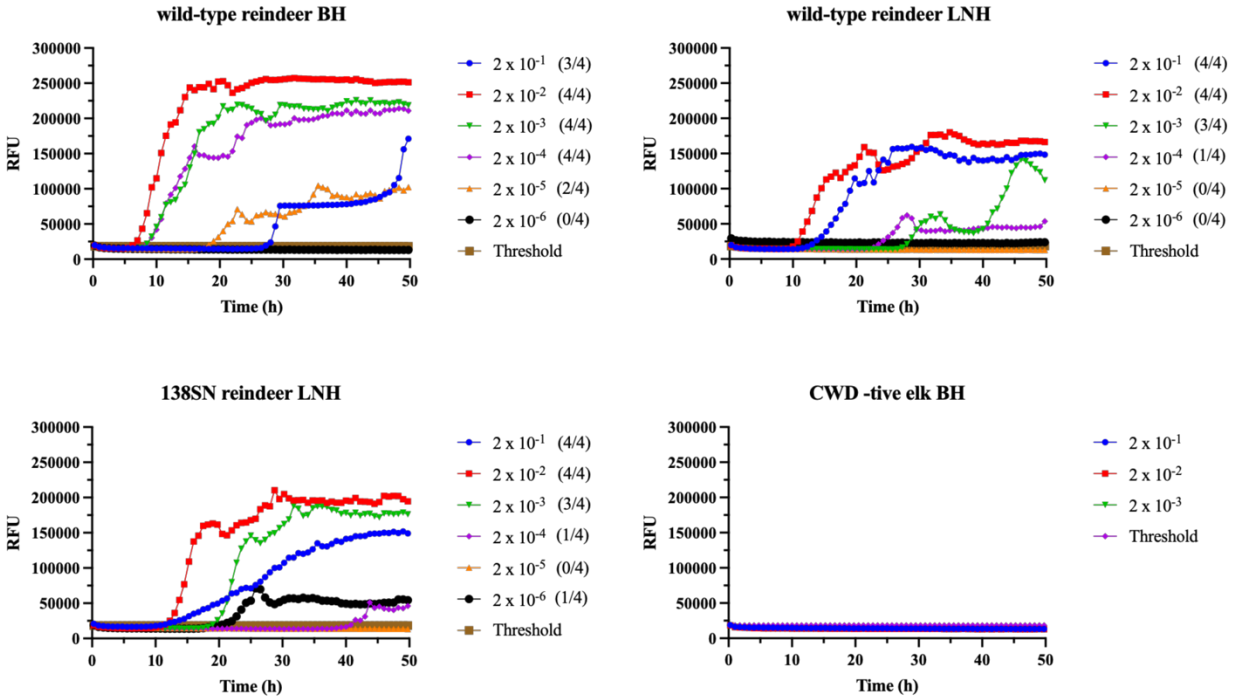

Figure S2. Comparison of reindeer inoculum seeding activity in RT-QuIC using mouse recombinant PrP. Ten percent BH and LNH were subjected to serial dilutions from 2 x 10^{-2} to 2 x 10^{-6} in RT-QuIC seed dilution buffer. Samples were considered positive when a minimum of 2 out of 4 wells crossed the threshold relative fluorescence unit (RFU). Threshold is the average RFU of all negative control wells plus 5 times their standard deviation. Negative control was a CWD-negative elk BH. The wild-type reindeer BH was positive for prion seed activity up to 2 x 10^{-5} dilution, while both the wild-type and 138SN reindeer LNHs were positive for prion seed activity up to 2 x 10^{-3} dilution (100-fold less than the BH). The y-axis represents the RFU, and the x-axis represents time in hours (h). Graphs were generated in Graphpad Prism v9. BH: brain homogenate; LNH: lymph node homogenate; CWD: chronic wasting disease; -tive: negative.

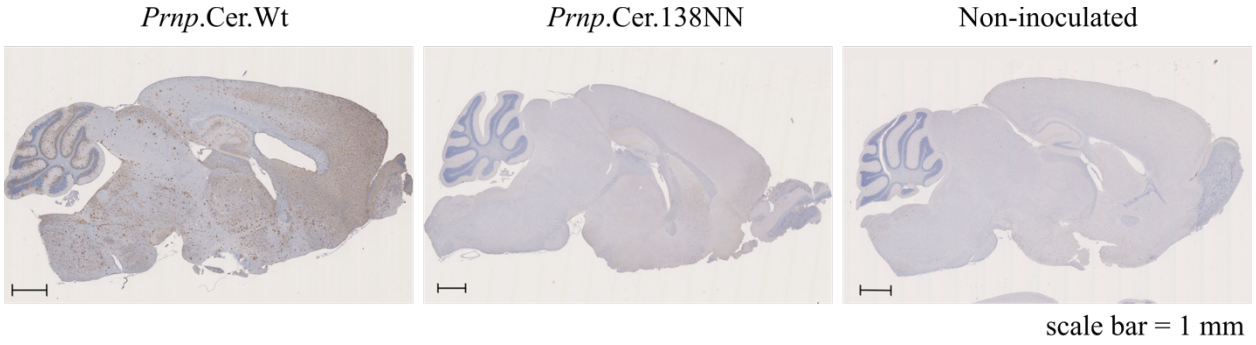

Figure S3. Representative immunohistochemistry of whole brain sections from cervid-*Prnp* gene-targeted mice. Left: CWD-infected *Prnp*.Cer.Wt brain; middle: CWD-infected *Prnp*.Cer.138NN brain; right: non-inoculated *Prnp*.Cer.Wt brain. Abnormal PrP deposits were detected using the BAR224 anti-PrP primary antibody (1:2000; Cayman).

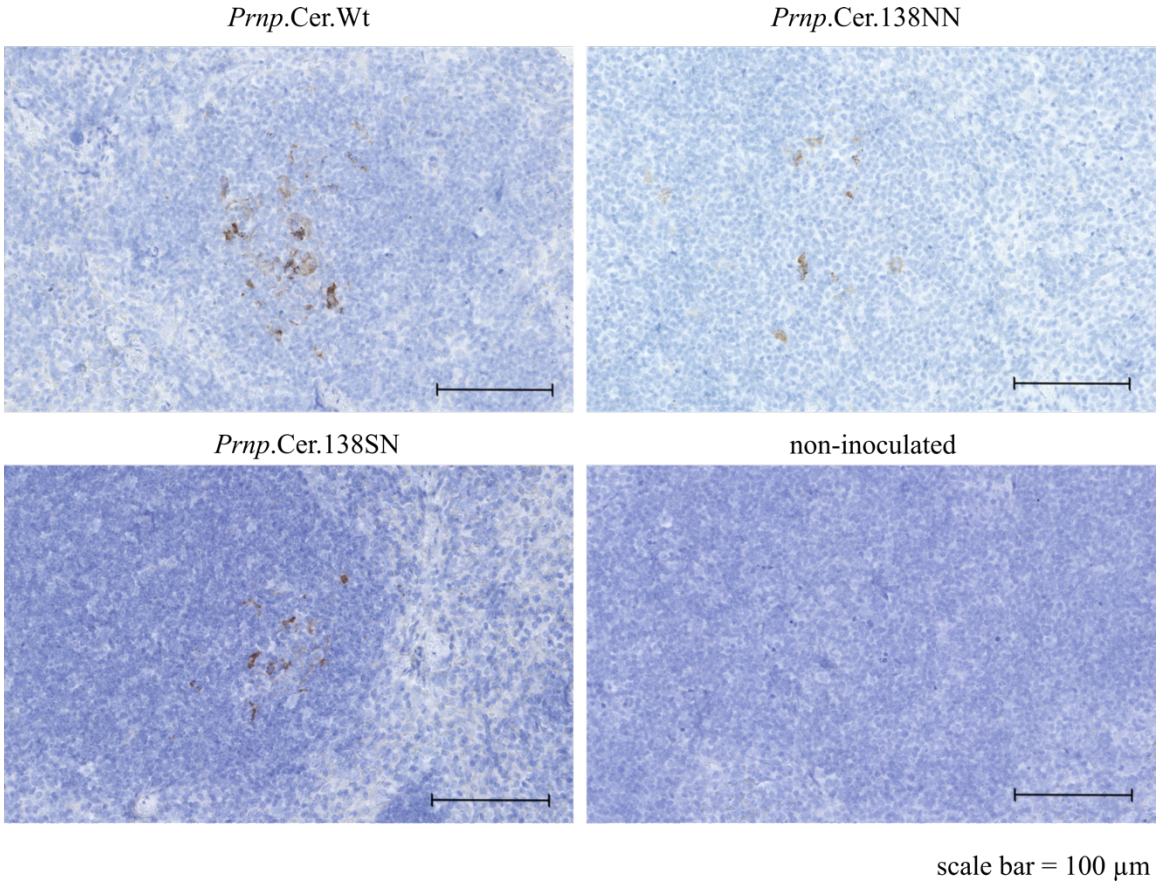

Figure S4. Representative immunohistochemistry of spleen sections from cervid-*Prnp* gene-targeted mice inoculated intracerebrally with the wild-type reindeer brain homogenate isolate. Non-inoculated *Prnp*.Cer.Wt mouse spleen was used as negative control. Abnormal PrP deposits were detected using the BAR224 anti-PrP primary antibody (1:2000; Cayman).

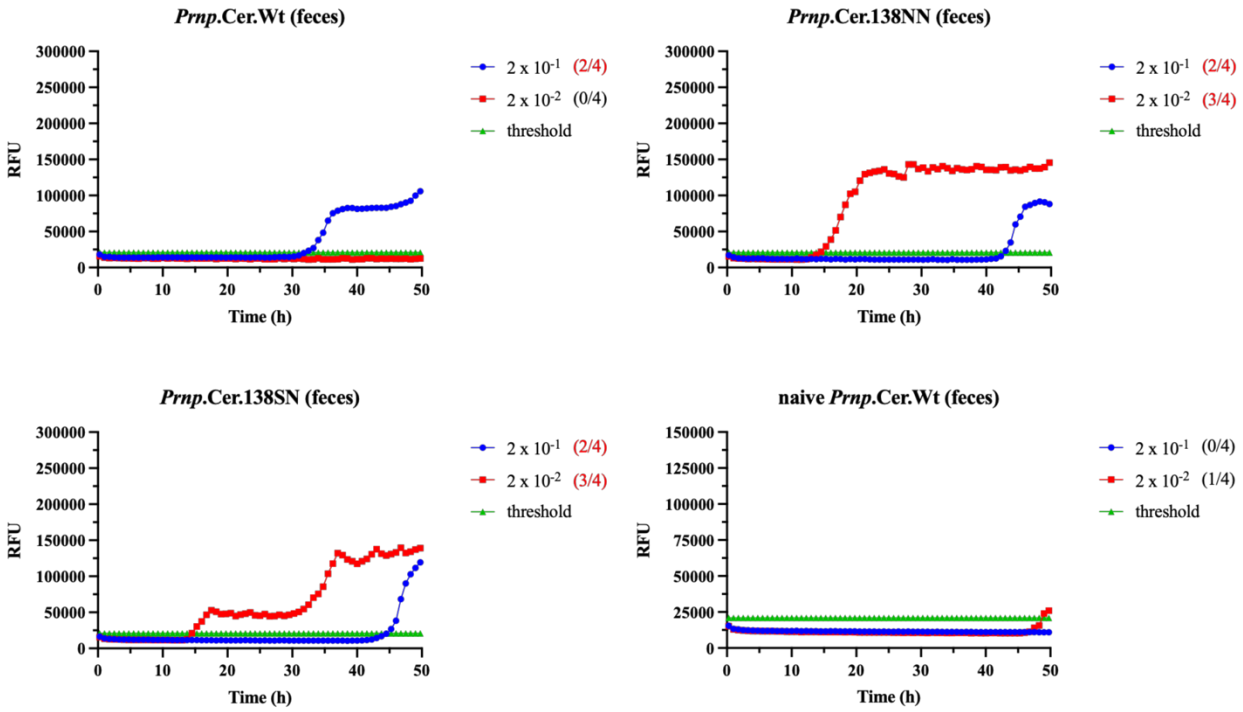

Figure S5. Representative RT-QuIC graphs showing prion seeding activity in fecal samples from *Prnp*.Cer.Wt, *Prnp*.Cer.138SN and *Prnp*.Cer.138NN gene-targeted mouse inoculated intracerebrally with the wild-type reindeer BH. Negative control was pooled feces collected from non-inoculated (naïve) *Prnp*.Cer.Wt mice. Samples were considered positive when a minimum of 2 out of 4 wells crossed the threshold, defined as the average RFU of all negative control wells plus 5 times their standard deviation. Positive sample dilutions with number of positive wells per total number of wells are highlighted in red font.The y-axis represents the RFU, and the x-axis represents time in hours (h). Graphs were generated in Graphpad Prism v9.