## SUPPLEMENTAL MATERIAL

**TABLE S1** Primers and probes used for real-time RT-PCR analysis.

| Gene | 5´ primer | Probe | 3´ primer |
|---|---|---|---|
| *Prnp* | 5´-TCTGTGTCCCCCATAGGCTAA-3´ | 5´-CCCCTGGCACTGATGGGCCC-3´ | 5´- AAGCAAAGAGCAACTGGTCTACTGT-3´ |
| *Gapd* | 5´-CTCCACTCACGGCAAATTCA-3´ | 5´-AGGCCGAGAATGGGAAGCTTGTCAT-3´ | 5´-CGCTCCTGGAAGATGGTGAT-3´ |

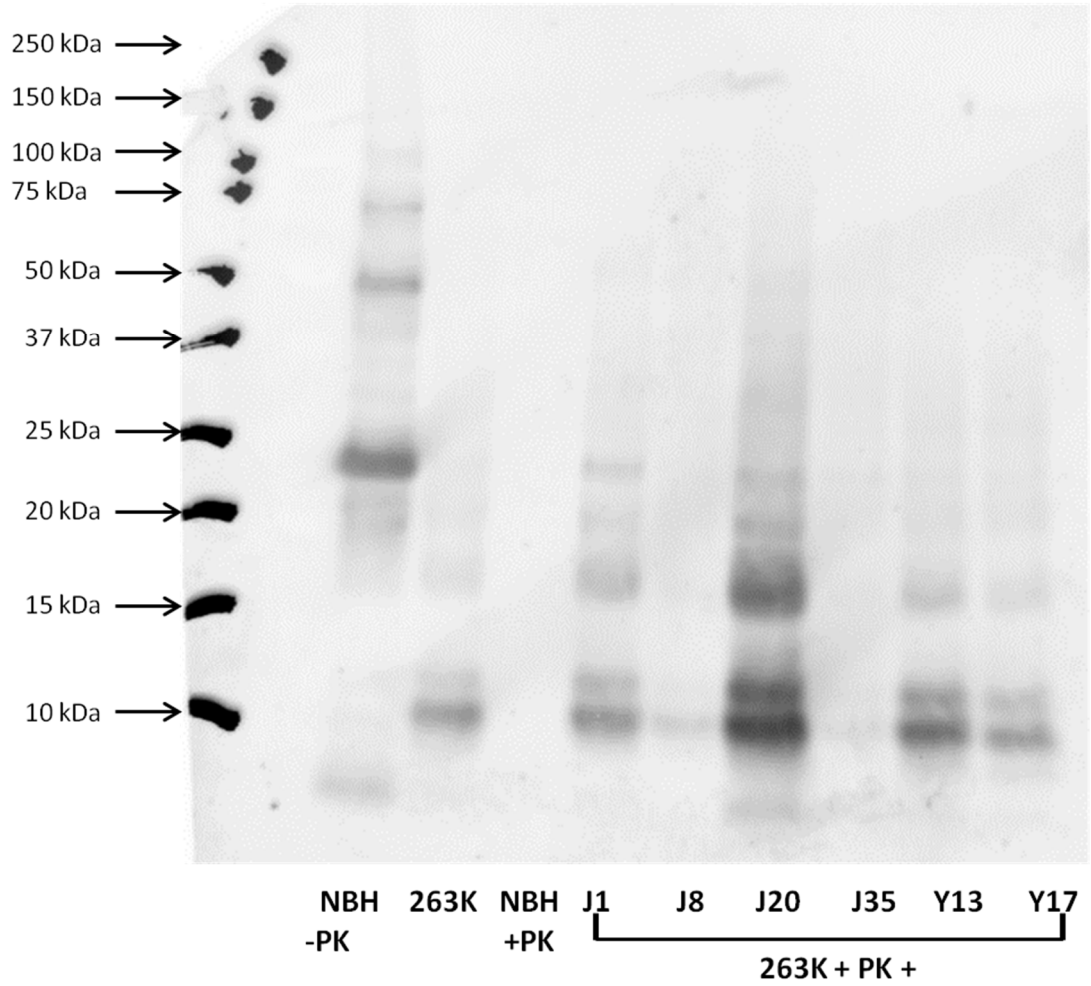


**FIG S1 Whole Western blot image from RT-QuIC with hamster 263K seed.**

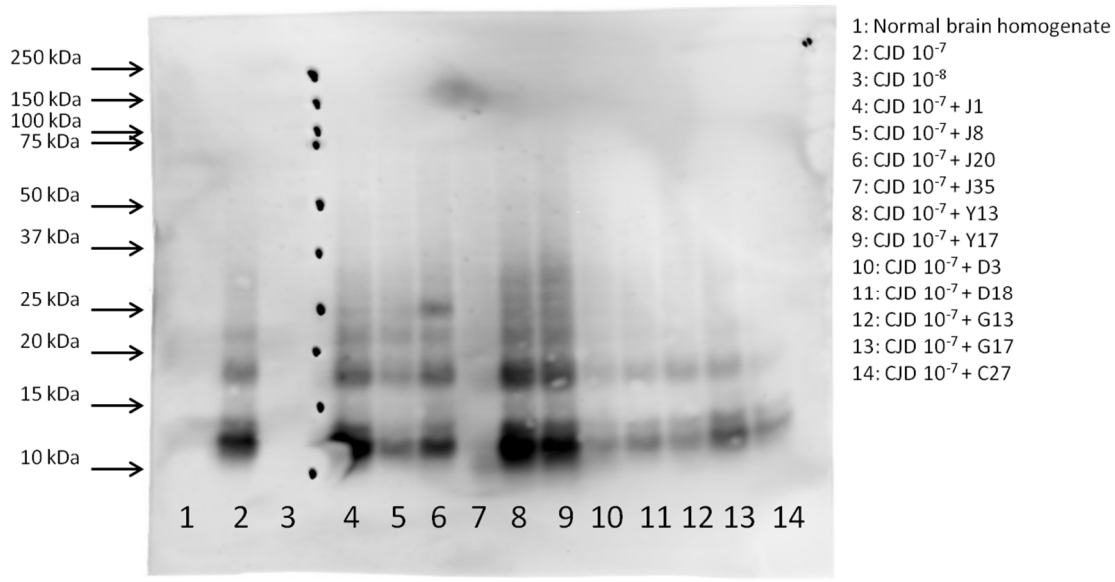


**FIG S2 Whole Western blot image from RT-QuIC with human CJD seed.**

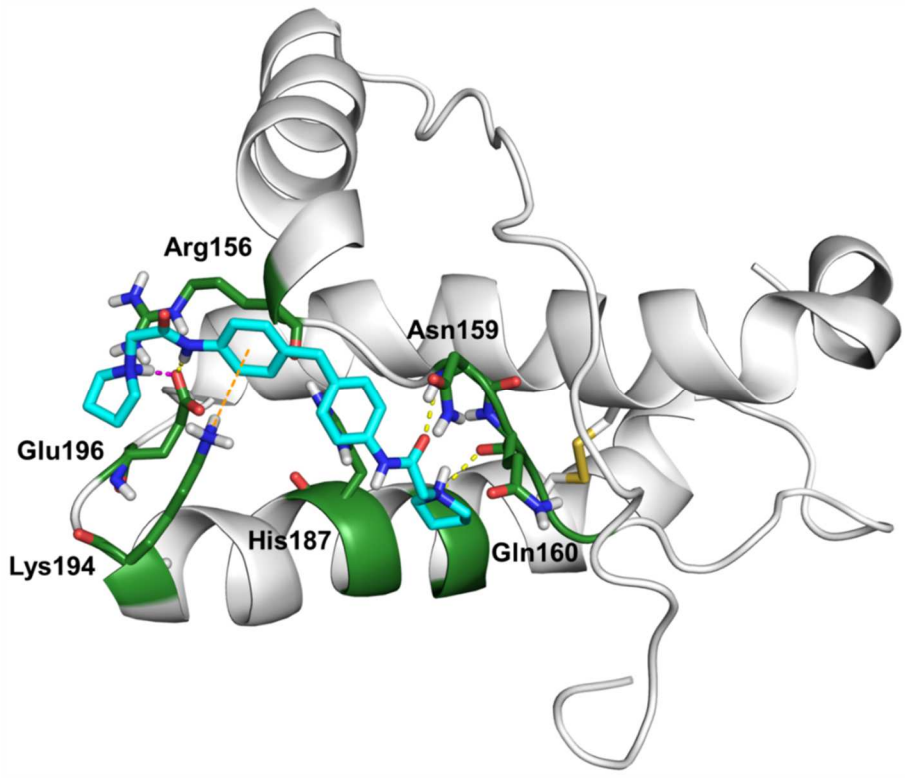


**FIG S3 Molecular docking simulation of GN8 with PrP$^{121–231}$ (PDB: 1AG2).** The carbon atoms in the main residues of PrP$^{C}$ hot spot regions are colored in green, and the carbon atoms in GN8 are colored in cyan. Yellow, magenta and orange dashed lines represent hydrogen bonds, ionic interactions and cation-π interactions, respectively. Electrostatic interactions were formed between the amine portion of a GN8 amide group and Glu196 side chain (2.8 Å); a GN8 charged tertiary amine and Glu196 side chain (2.7 Å); a GN8 phenyl ring and Lys194 side chain (~ 4.0 Å); the carbonyl portion of the other GN8 amide group and Ans159 main chain (2.8 Å); and a GN8 charged tertiary amine and Gln160 main chain (2.8 Å). The binding energy was –8.83 kcal/mol.

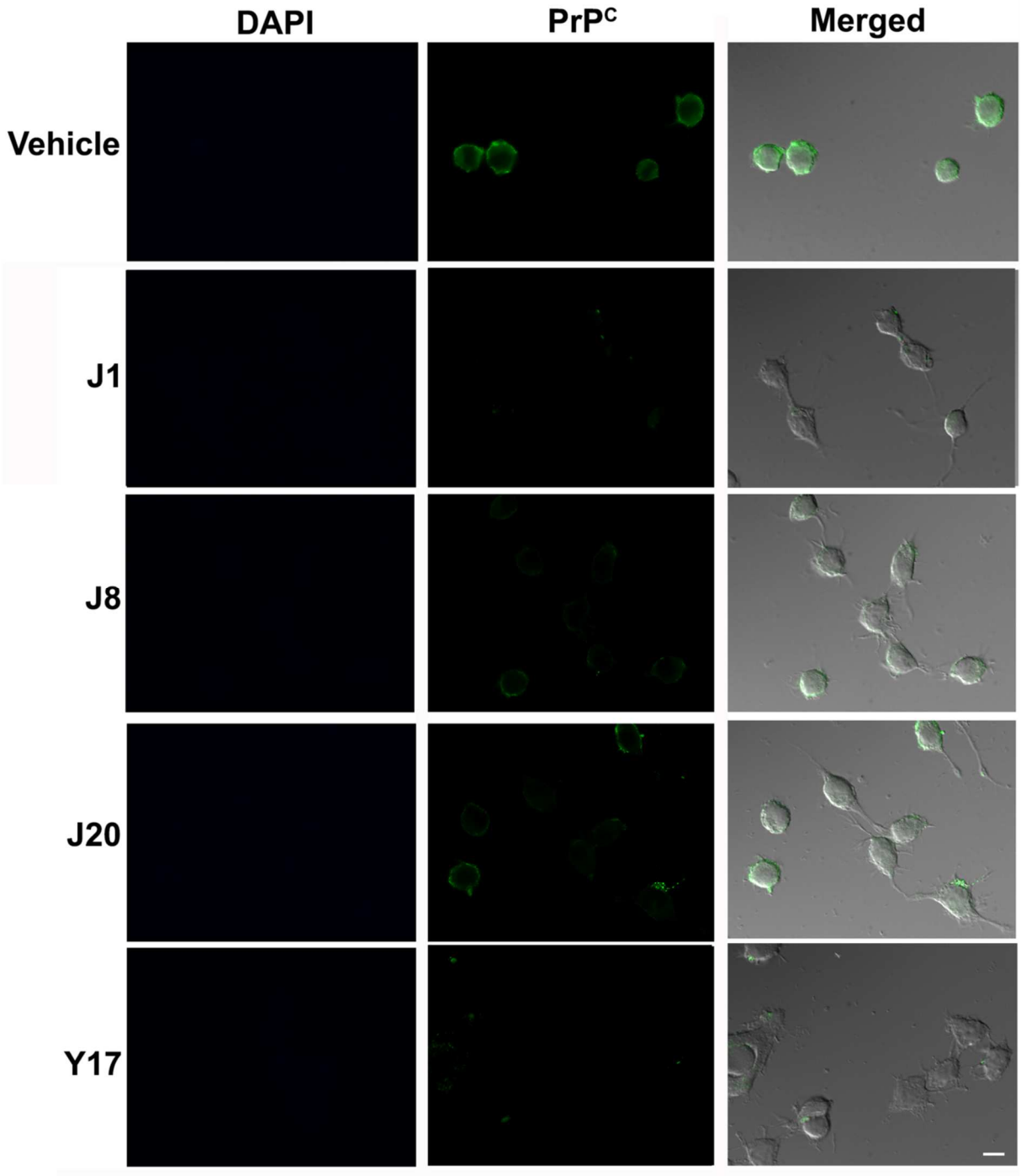


**FIG S4 PrP$^C$ neuronal exposition after test compounds treatment.** Representative fields of immunofluorescence analysis of PrP$^C$ (green), merged with differential interference contrast images. The nuclear staining with DAPI was used as a non-permeability control. Mouse neuroblastoma N2a cells were treated with 10 µM of test compounds (J1, J8, J20, and Y17) or 0.1% DMSO (vehicle). Scale bar corresponds to 10 µm.

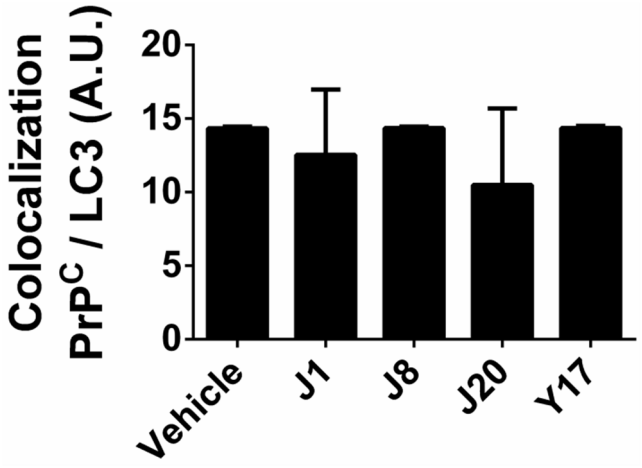


**FIG S5 PrP$^C$/LC3 colocalization after test compounds treatment.** Colocalization analysis was performed with ImageJ software. 20 fields with more than 30 cells each were analyzed per condition. One-way analysis of variance (ANOVA) was performed. Error bars indicate SEM.

**Thermal denaturation followed by circular dichroism spectroscopy**

This assay was executed as previously described (1, 2). Circular dichroism spectra were measured on a J-715 spectropolarimeter (Jasco Corporation, Tokyo, Japan) with 1.0-mm path-length cuvettes. Murine rPrP$^{23–231}$ was diluted to a final concentration of 10 μM in 10 mM sodium phosphate buffer, pH 6.5, in the presence of the compounds at 10 μM and 100 μM. The vehicle for each condition corresponds to 0.1% DMSO and 1% acetonitrile, respectively. The samples were heated from 25 to 80 °C at a rate of 1 °C/min, and ellipticity values were collected at 222 nm with a bandwidth of 2 nm. Data is displayed as fraction denatured (FD), which was calculated by the following equation:

$$FD = \frac{\varepsilon_{measured} - \varepsilon_{initial}}{\varepsilon_{final} - \varepsilon_{initial}}$$

where $\varepsilon_{measured}$ is the ellipticity (222 nm) value measured at any point, $\varepsilon_{inital}$ is the ellipticity value collected at the initial temperature value considered, and $\varepsilon_{final}$ is the ellipticity value recorded at the final temperature value considered. FD plots were then fitted to four-parameter sigmoid regression curves described by the following equation.

$$y = y_0 + \frac{a}{1 + e^{-\left(\frac{x - x_0}{b}\right)}}$$

where $y$ is fraction denatured, $y_0$ is the minimum value of fraction denatured, $x$ is temperature, $x_0$ is the temperature required for 50% of maximum denaturation ($T_{½}$), $a$ is the difference between the maximum and minimum values of fraction denatured, and $b$ is the curve slope.


1. Cordeiro Y, Kraineva J, Gomes MPB, Lopes MH, Martins VR, Lima LMTR, Foguel D, Winter R, Silva JL. 2005. The Amino-Terminal PrP Domain Is Crucial to Modulate Prion Misfolding and Aggregation. Biophys J 89:2667–2676.
2. Vieira TCRG, Cordeiro Y, Caughey B, Silva JL. 2014. Heparin binding confers prion stability and impairs its aggregation. FASEB J 28:2667–2676.

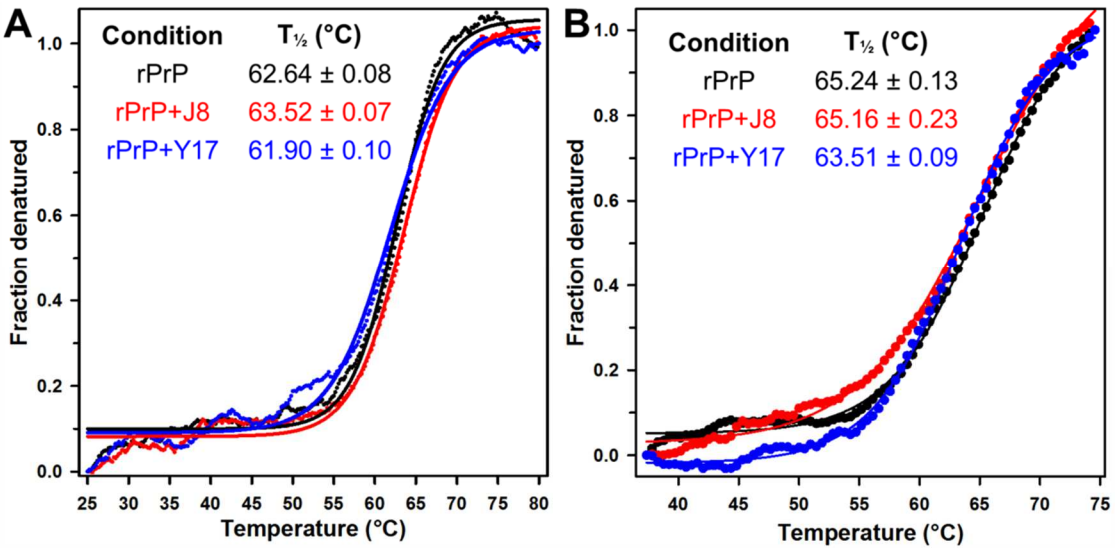


**FIG S6 Temperature-induced denaturation profile of rPrP$^{23–231}$.** The protein was analyzed at 10 μM in the presence of test compounds at (A) 10 μM and (B) 100 μM. The vehicle for each condition corresponds to 0.1% DMSO and 1% acetonitrile, respectively. The results are displayed as fraction denatured (FD). Inset shows $T_{½}$ values calculated for the control (black), J8 treatment (red), and Y17 treament (blue).