

## Supplemental Data

### Disease-Associated Prion Protein

#### Oligomers Inhibit the 26S Proteasome

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#### Supplemental Experimental Procedures

##### Cell culture and prion infection

Murine cerebellar granule neurons (CGN) were prepared from 6 day old FVB mice as described (Schousboe and Pasantes-Morales, 1989).

##### SDS-PAGE and immunoblot analysis

Proteasome subunits were assayed using antibodies against the  $\alpha$ 4,  $\beta$ 2 or  $\beta$ 5 subunits (1:1000, BIOMOL) and goat anti-mouse secondary antibody (1:10000, Biosource) followed by CDP-Star (Tropix) and visualisation on Biomax MR film (Kodak). Equivalent protein loading was confirmed as described (Kristiansen et al., 2005).

##### Enrichment of PrP<sup>Sc</sup> from RML infected mouse brain

One whole RML infected mouse brain was homogenised for 20 strokes in ice-cold PBS, freeze-thawed three times and centrifuged at 500 x g for 10 min at 4°C to remove cellular debris. The supernatant was adjusted with an equal volume of 2 x lysis buffer (100mM Tris, pH7.4, 300mM NaCl, 4mM EDTA, 1% Triton-X-100, 1% deoxycholate) followed by incubation with benzonase (50 U/ml) for 20 min at 4°C. Supernatant was incubated with PK at 5 $\mu$ g/mg protein (37°C, 90 mins), followed by treatment with AEBSF (8mM) for 10 minutes at 37°C and the whole mixture was centrifuged at 100,000 x g for 45 minutes, sonicated in PBS to dissociate the material, and the presence of PrP<sup>Sc</sup> confirmed by immunoblotting.

### **Aggregated $\beta$ -PrP dose response of inhibiting 26S proteasome $\beta$ -subunit proteolysis**

$\beta$ -PrP was aggregated in the presence of 150mM NaCl for 1 hour at 37°C. A range of concentrations of aggregated  $\beta$ -PrP (10, 25, 50, 75, 100, 125, 250ng/ml) were pre-incubated with either 100ng, 300ng, or 900ng pure 26S proteasome (BIOMOL; in 10mM Tris, 25mM KCl, 1.1mM MgCl<sub>2</sub>, 0.1mM EDTA, 1mM DTT, 2mM ATP, pH 7.0 / 35% glycerol) for 1 hour at 37°C before incubation with 1 $\mu$ M dansylAhx3L3VS probe (Berkers et al., 2005) for a further hour at 37°C. Samples were diluted in 2X SDS sample buffer, boiled for 10 minutes at 100°C before running on a 12% gel Tris-Glycine gel (Invitrogen) at 200V for 80 minutes on ice and electrotransferred onto PVDF membranes (35V for 90 minutes). Blocking was performed using 5% BSA in PBS with 0.05% Tween (PBS-T) for 60 minutes. Immunoblotting was performed using a dansyl-sulfonamidohexanoyl polyclonal antibody (1:1000, rabbit, Molecular Probes) which binds to the hapten tag of the peptide vinyl sulfone-based hapten-tagged activity probe, and horse-radish peroxidase-coupled donkey anti-rabbit secondary antibody (1:5000, Southern Biotech) followed by enhanced chemiluminescence (Pierce). Membranes were stripped using Reprobe<sup>TM</sup> western blot recycling kit (Chemicon) and reprobbed with  $\beta$ -actin (1:10000) to check for equal loading.

For cell lysate preparations, a range of concentrations of aggregated  $\beta$ -PrP (10ng/ml, 25ng/ml, 50ng/ml, 75ng/ml, 100ng/ml, 500ng/ml, 1 $\mu$ g/ml, 1mg/ml) were pre-incubated with 100 $\mu$ g of N2aPK-1 cell lysates (1 hour, 37°C) before incubation with 1 $\mu$ M dansylAhx3L3VS probe (1 hour, 37°C) (Berkers et al., 2005). Electrophoresis and immunoblotting were performed as described above.

### **Degradation of the proteasome reporter substrate, Ub<sup>G76V</sup>-GFP in live cells**

Ub<sup>G76V</sup>-GFP constructs were made according to Dantuma *et al* (Dantuma et al., 2000). Transfection of this vector into N2aPK-1 cells was performed using Genejammer (Stratagene). Stably transfected cell lines were generated by selection in 600  $\mu$ g/ml gentamicin (G418) as optimised by kill curves. Ub<sup>G76V</sup>-GFP transfected N2aPK-1 cells (n=6 clones) were treated for 2 hours with 25  $\mu$ M of the reversible proteasome inhibitor

MG132 to accumulate the Ub<sup>G76V</sup>-GFP reporter. Cells were then washed four times in sterile PBS and cultured for 1 hour to allow removal of the MG132 reversible proteasome inhibitor. After one additional PBS wash the fluorescence intensity was measured by FACS (time=0 hours) and was taken to be maximal fluorescence. Measurements were taken on the FL-1 channel of FACSCalibur (BD Biosciences) each hour for 8 hours and analysed using the Cell Quest software. The time required for 50% clearance of accumulated Ub<sup>G76V</sup>-GFP reporter was measured. To determine whether there was a difference in the degradation of the reporter in uninfected versus RML prion infected Ub<sup>G76V</sup>-GFP transfected cells, the protein synthesis inhibitor, cycloheximide was added at a concentration of 20µg/ml for 30 minutes before the addition of MG132.

For visualisation of GFP, pre-infected N2aPK-1 cells, prion infected N2aPK-1 cells or prion infected ScN2aPK-1 cells cured of infection (ScN2aPK-1-18) (all Ub<sup>G76V</sup>-GFP transfected) were grown to ~70% confluence in 22mm glass coverslips, fixed in 4% paraformaldehyde, permeabilised in ice-cold methanol (for 20 minutes at -20°C) and blocked in 10% normal goat serum (30 minutes, room temperature) before mounting in Antifade (DAKO) and visualising under the FL-1 channel of the confocal microscope. Fluorescence images were obtained using a confocal microscope (Zeiss microscope LSM510 META) equipped with “plan-Apochromat” 63 x/1.40 Oil DIC objective at room temperature, controlled by Zeiss LSM software. Fluorescence was recorded at 488 nm using 30 mW Ar-laser for excitation. Zeiss Immersol<sup>TM</sup> 518 F was used as imaging medium.

### **Immunofluorescence and antibodies**

Cells were fixed onto poly-*L*-lysine coated glass coverslips using 4% paraformaldehyde for 20 min at room temperature (RT) and washed three times. To remove PrP<sup>C</sup> and reveal PrP<sup>Sc</sup>, cells were exposed to 98% formic acid for 5 min. Cells were then permeabilised in methanol at -20°C for 15 min before incubating in 10% normal goat serum for 30 min at 37°C. For dual labelling, the primary antibody was applied (1 hour, 37°C) followed by the appropriate fluorochrome-conjugated secondary antibody (45 min, 37°C), and then after washing, the second primary antibody was added (1 hour, 37°C) followed by the appropriate fluorochrome-conjugated secondary (45 min, 37°C). Cells were then washed

several times in PBS and mounted in Antifade (Sigma) containing 1 $\mu$ g/ml 4',6-diamidino-2-phenylindole (DAPI; Sigma). Hsc70 (mouse monoclonal IgG2a, 1:500; Santa Cruz) and LAMP-1 (rat polyclonal, 1:500; Santa Cruz) were both diluted in 1% normal goat serum before application. ICSM18 (mouse monoclonal IgG1, 10 $\mu$ g/ml) was from D-GEN. Isotype specific fluorochromes 488 and 568 were purchased from Invitrogen and were diluted in 1% normal goat serum before use.

### **Image acquisition**

Fluorescence images were obtained using a confocal microscope (Zeiss microscope LSM510 META) equipped with “plan-Apochromat” 63x/1.40 Oil DIC objective at room temperature and is controlled by Zeiss LSM software. Fluorescence was recorded at 488nm using 30mW Ar-laser for excitation or at 543nm using 1mW HeNE-laser for excitation. Zeiss Immersol<sup>TM</sup> 518 F was used as imaging medium.

### **Fluorogenic assays with 26S proteasome**

10ng of pure 26S proteasome (in 10mM Tris, 25mM KCl, 1.1mM MgCl<sub>2</sub>, 0.1mM EDTA, 1mM DTT, 2mM ATP, pH7.0, 35% glycerol) was added to 100 $\mu$ l reaction buffer containing 50mM Tris-HCl, pH7.4, 5mM MgCl<sub>2</sub>, 1mM DTT, 2mM ATP and 100 $\mu$ M Suc-LLVY-AMC (BIOMOL), Boc-LRR-AMC (BIOMOL) or Ac-nLPnLD-AMC (BACHEM). Fluorescence was measured every minute for 30 minutes at 37°C using a TECAN 96-well plate reader ( $\lambda_{ex}/\lambda_{em}$ =360/440nm). All recombinant proteins were incubated for 1 hour at 37°C in the presence of 2mM ATP with 10ng 26S proteasome prior to incubation with fluorogenic substrates in reaction buffer as described above.

### **Fluorogenic assays with 20S proteasome**

Pure 20S proteasome (BIOMOL; in buffer containing 20mM Tris-HCl, pH7.2, 5mM MgCl<sub>2</sub>, 1mM EDTA, 1mM DTE) was activated with 1-4 times molar concentration of PA28 activator (BIOMOL; in buffer comprising 50% (v/v) glycerol, 0.5mM EDTA, 0.5mM DTT, 10mM Tris-HCl, pH7.5). Four times molar concentration of PA28 was used for maximal activation of pure 20S in all experiments. For pure 20S proteasome assays, 10ng (0.014pmol) 20S proteasome was activated with 32ng (0.06pmol) of PA28

activator (BIOMOL) and added to 100µl reaction buffer containing 50mM Tris-HCl, pH7.4, 5mM MgCl<sub>2</sub>, 1mM DTT, 2mM ATP and 100µM Suc-LLVY-AMC (BIOMOL), Boc-LRR-AMC (BIOMOL) or Ac-nLPnLD-AMC (BACHEM). Fluorescence was measured every minute for 30 minutes at 37°C using a TECAN 96-well plate reader ( $\lambda_{ex}/\lambda_{em}=360/440\text{nm}$ ). All recombinant proteins were incubated for 1 hour at 37°C in the presence of 2mM ATP with 10ng PA28 activated 20S proteasome prior to incubation with fluorogenic substrates in reaction buffer as described above.

### **Native gel fluorogenic activity stain**

3µg of pure 26S proteasome (BIOMOL) was incubated with 30µg aggregated  $\beta$ -PrP or with 30µg PrP<sup>Sc</sup> from RML infected mouse brain for 1 hour at 37°C. Samples were run on a 3-12% NativePAGE Bis-Tris gel (Invitrogen) in 1X NativePAGE running buffer (50mM BisTris, 50mM Tricine, pH 6.8; Invitrogen) at 4°C for 1 hour at 150V and then increasing to 200V for a further hour. The gel was then incubated at 4°C for 15 min in developing buffer (50mM Tris HCl, pH 7.4, 5mM MgCl<sub>2</sub>, 2mM ATP). The gel was then overlaid with 100µM Suc-LLVY-AMC (BIOMOL) and visualized on the Biorad Gel Doc 1000 using the Quantity One 4.5.1 (Basic) software. For equal loading of proteins, the native gel activity stain was stained with coomassie. Briefly, the gel was fixed in 40% methanol, 10% acetic acid and microwaved for 45 seconds on high power and then incubated at room temperature for 15 minutes. The gel was then microwaved for 45 seconds in 0.02% R-250 coomassie in 30% methanol, 10% acetic acid and incubated at room temperature for 15 minutes. The gel was destained in 8% acetic acid until the bands were visible.

### **Native gel immunoblotting with pure 26S proteasome and $\beta$ -PrP or PrP<sup>Sc</sup>**

For native gel immunoblotting, 3µg of pure 26S proteasome (BIOMOL) was incubated with 30µg aggregated  $\beta$ -PrP or with 30µg PrP<sup>Sc</sup> from RML infected mouse brain for 1 hour at 37°C. Samples were run on a 3-12% NativePAGE Bis-Tris gel (Invitrogen) in 1X NativePAGE running buffer (50mM BisTris, 50mM Tricine, pH 6.8; Invitrogen) at 4°C for 1 hour at 150V and then increasing to 200V for a further hour. The gel was then immunoblotted onto a PVDF membrane (Millipore) at 25V for 1 hour in 1X

NativePAGE transfer buffer (Invitrogen) in the blotting chamber and double distilled water in the outside chamber. Following transfer, the PVDF membrane was incubated for 20 min with 8% acetic acid to fix the proteins and blocked with 5% milk for 1 hour before overnight incubation with mouse monoclonal  $\alpha 4$  (20S) subunit antibody (1:1000; BIOMOL) and mouse monoclonal Rpt1 (S7) (19S) subunit antibody (1:5000; BIOMOL) in 1X PBST. The blot was incubated with secondary antibody (goat anti mouse 1:10000 in 1X PBST; BIOSOURCE) for 45 min and developed using the CDP Star (Tropix) system.

### **Native gel immunoblotting with cells and brain lysates**

GT-1 and ScGT-1 cells were pelleted and harvested as described above. Protein concentrations were determined using the BCA assay (Pierce). 30 $\mu$ g of GT-1 cells, 30 $\mu$ g of ScGT-1 cells, 70 $\mu$ g of CD-1 brain homogenate and 70 $\mu$ g of RML brain in loading buffer (30mM Tris HCl, pH 7.4, 5mM MgCl<sub>2</sub>, 250mM sucrose, 2mM ATP) were run on a 3-12% NativePAGE Bis-Tris gel (Invitrogen) in 1X NativePAGE running buffer (50mM BisTris, 50mM Tricine, pH 6.8; Invitrogen) for 1 hour at 150V and then 200V for a further hour. The gel was then immunoblotted onto a PVDF membrane (Millipore) at 25V for 1 hour in 1X NativePAGE transfer buffer (Invitrogen) in the blotting chamber and double distilled water in the outside chamber. Following transfer, the PVDF membrane was incubated for 20 min with 8% acetic acid to fix the proteins and blocked with 5% milk for 1 hour before overnight incubation with mouse monoclonal  $\alpha 4$  subunit antibody (1:1000; BIOMOL) and mouse monoclonal Rpt1 (S7) subunit antibody (1:5000; BIOMOL) in 1X PBST. The blot was incubated with secondary antibody (goat anti mouse 1:10000 in 1X PBST; BIOSOURCE) for 45 min and developed using the CDP Star (Tropix) system.

### **Immunohistochemistry antibodies**

The following antibodies were used: rabbit anti-GFP (Molecular Probes), mouse monoclonal anti-GFP (Roche Applied Science), mouse monoclonal anti-ubiquitin (Stressgen), rabbit anti-GFAP (DAKO) and rabbit anti-mouse PrP residues 89-103 (R30) (Raymond et al., 1997).

## Supplemental References

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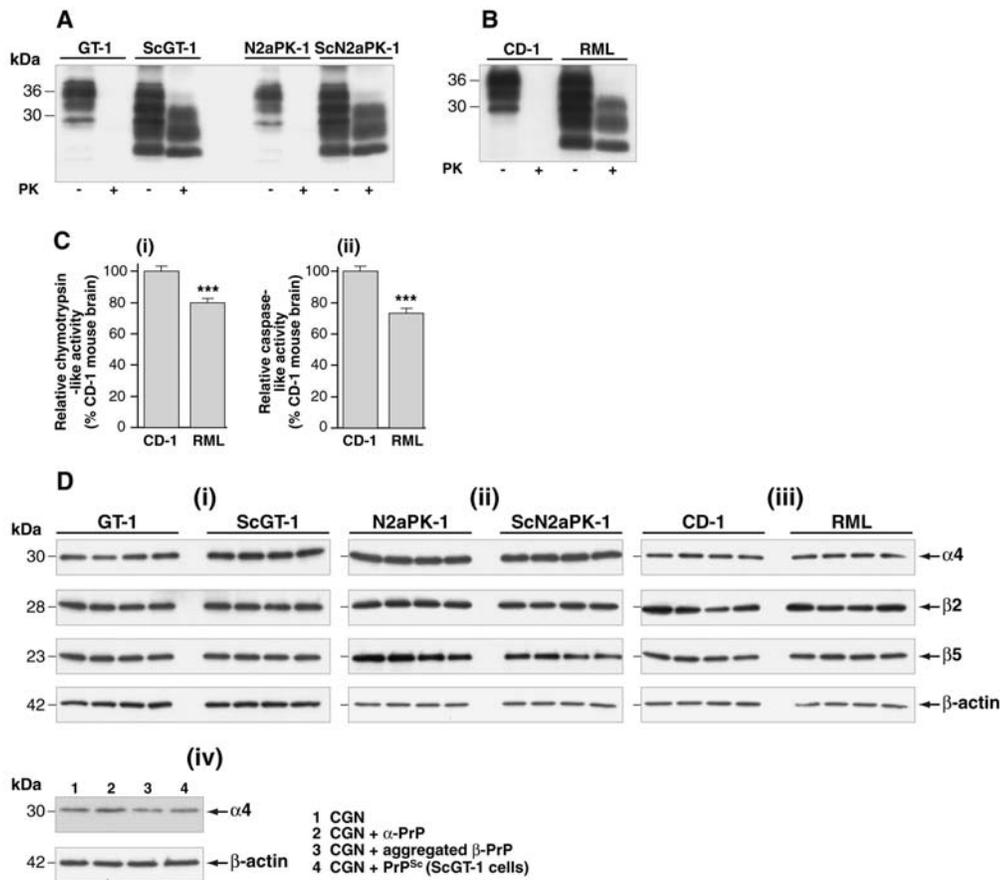
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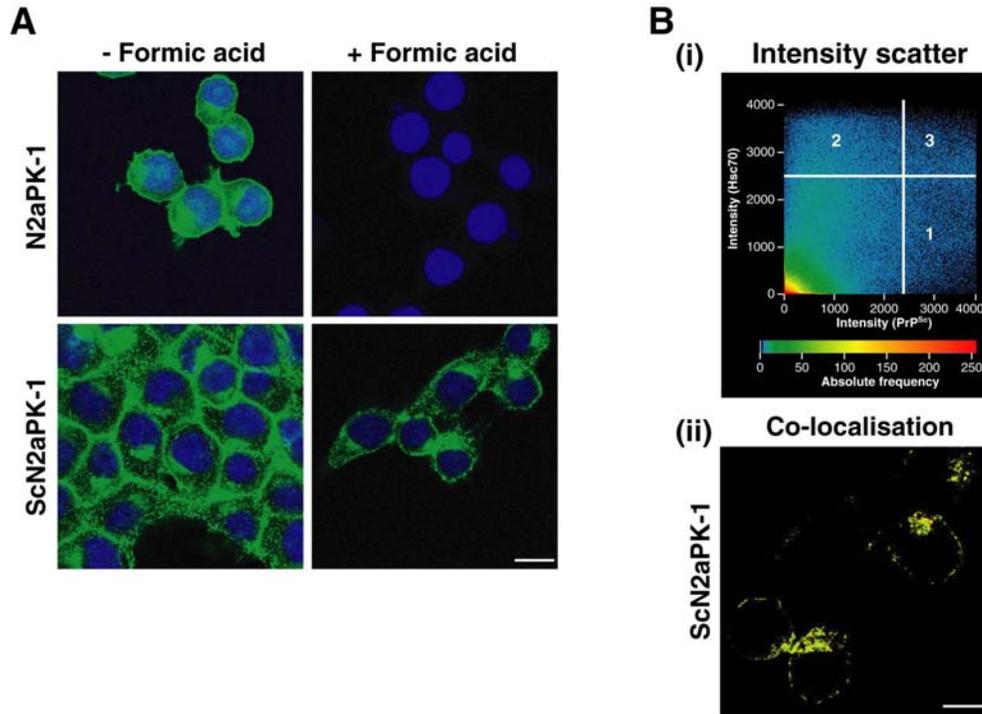
**Figure S1**



**Supplementary figure 1 – Prion infection impairs the proteolytic activity of the 26S proteasome in cells and mouse brain** (A) Western blot showing PK resistant PrP<sup>Sc</sup> in ScGT-1 cell and ScN2aPK-1 cells detected using anti-PrP ICSM18 antibody (B) Western blot showing PK resistant PrP<sup>Sc</sup> in RML prion infected CD-1 mice detected using anti-PrP ICSM18 antibody (C) Scrapie infection significantly reduces chymotrypsin-like and caspase-like proteolytic activities in prion infected CD-1 mouse brain. All data are mean  $\pm$  SEM with n=10. \*\*\* p<0.0001.

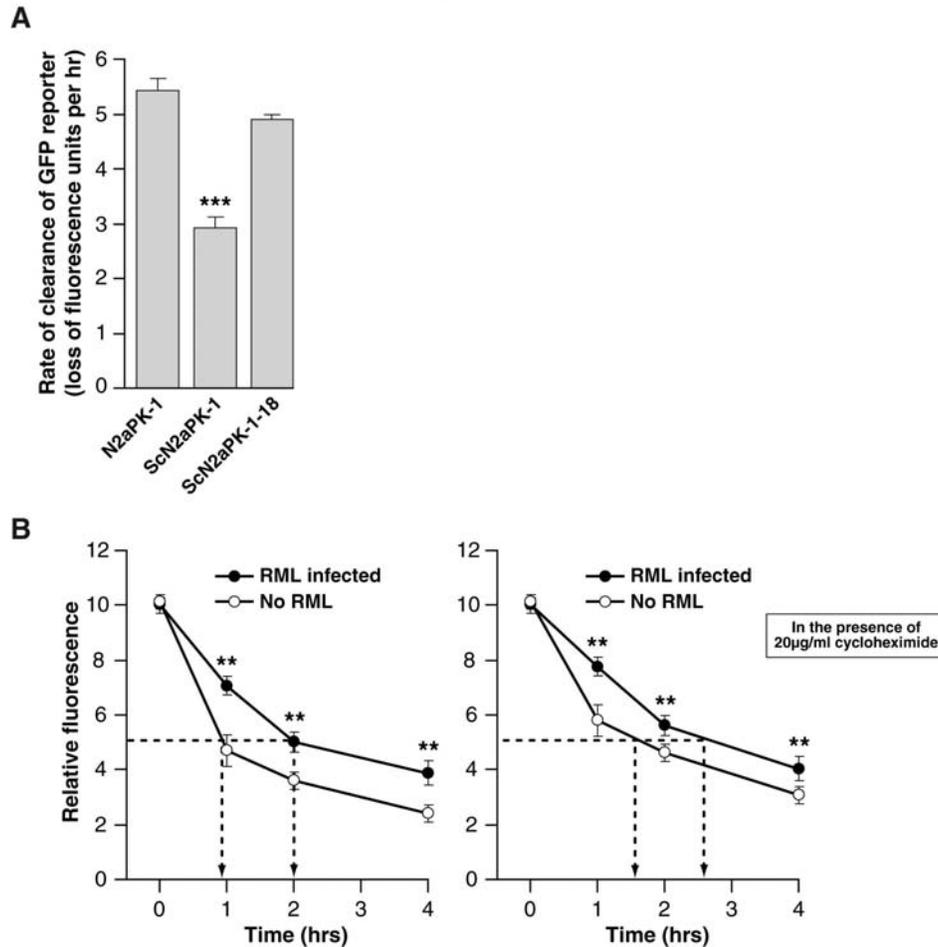
**Decreased  $\beta$ -subunit proteolytic activity is not due to reduced proteasome subunit expression.** Mouse hypothalamic neuronal GT-1 and neuroblastoma N2aPK-1 cells were infected with RML prions and lysed as described (Berkers et al., 2005). (Di) There was no loss of  $\alpha$ 4,  $\beta$ 2 or  $\beta$ 5 subunit levels in ScGT-1 cell lysates compared to uninfected GT-1 cell lysates (n=4). (Dii) There was no loss of  $\alpha$ 4,  $\beta$ 2 or  $\beta$ 5 subunit levels in ScN2aPK-1 cell lysates compared to uninfected N2aPK-1 cell lysates (n=4). (Diii) There was no loss of  $\alpha$ 4,  $\beta$ 2 or  $\beta$ 5 subunit levels in RML infected mouse brain lysates compared to uninfected CD-1 mouse brain lysates (n=4). (Div) Murine cerebellar granule neurons (CGN) were prepared from 6 day old FVB mice (Schousboe and Pasantes-Morales, 1989). There was no difference in expression level of the  $\alpha$ 4 subunit of the 20S proteasome in the CGN lysates incubated with  $\alpha$ -PrP, aggregated  $\beta$ -PrP or PrP<sup>Sc</sup> when compared to untreated CGN lysates. Mouse monoclonal  $\beta$ -actin (1:10,000; Sigma) was used to confirm equal loading in all experiments.

Figure S2



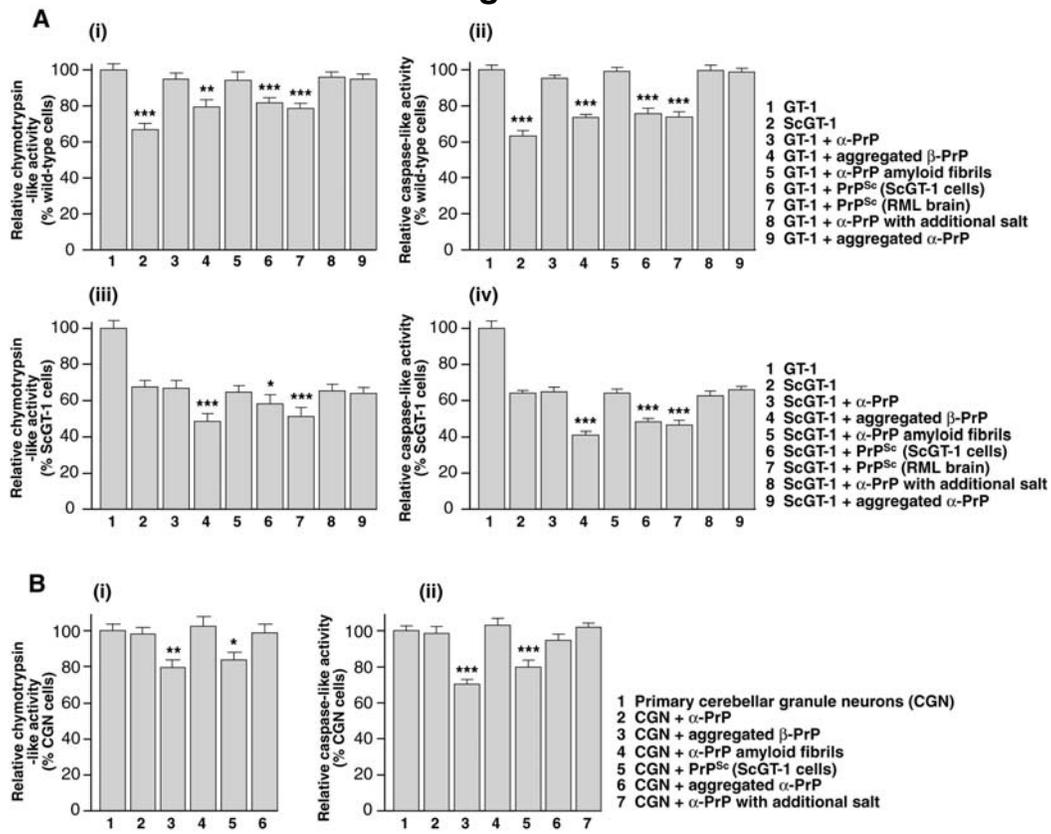
**Supplementary figure 2 - Formic acid treatment removes all detectable PrP<sup>C</sup> and exposes PrP<sup>Sc</sup> in prion-infected neuronal N2aPK-1 cells.** (A) To analyse the subcellular localisation of PrP<sup>Sc</sup>, prion-infected N2aPK-1 cells were treated with 98% formic acid for 5 minutes and then immunostained with anti-PrP antibody (ICSM 18) to reveal PrP<sup>Sc</sup> (as described in (Kristiansen et al., 2005)). In uninfected N2aPK-1 cells, formic acid treatment removed all detectable PrP<sup>C</sup> after 5 minutes (top right panel); in prion-infected ScN2aPK-1 cells, PrP<sup>Sc</sup> is present on the cell surface and intracellularly (bottom right panel). Scale bar = 20 $\mu$ m. **A proportion of formic acid resistant PrP<sup>Sc</sup> colocalises with the cytosolic chaperone Hsc70.** (Bi) The intensity scatter plots the intensity of the pixels stained either for PrP<sup>Sc</sup> (x-axis) or Hsc70 (y-axis). Segment 1 reveals pixels with intense PrP<sup>Sc</sup> staining; segment 2 reveals pixels with intense Hsc70 staining; segment 3 reveals the co-localisation of pixels with both intense PrP<sup>Sc</sup> and intense Hsc70 staining. (Bii) Demonstrates a subtraction image revealing true colocalisation (yellow) between Hsc70 and PrP<sup>Sc</sup>. Scale bar = 20 $\mu$ m.

**Figure S3**



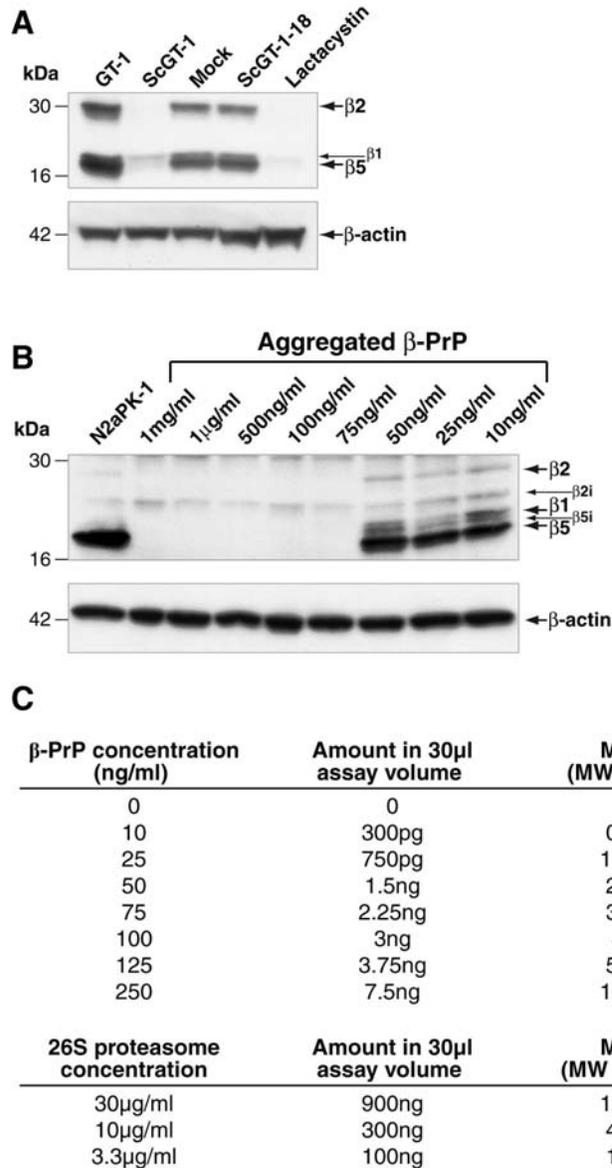
**Supplementary figure 3 – Prion infection results in a delay in degradation of Ub<sup>G76V</sup>-GFP reporter even in the presence of the protein synthesis inhibitor, cycloheximide** (A) ScN2aPK-1 cells had a lower rate of clearance than N2aPK-1 or cells cured of prion infection (ScN2aPK-1-18). Loss of fluorescence units (FU) per hour was 42% less in prion-infected cells. Data are mean ± SEM with n=5. (B) The time required for 50 % clearance of accumulated Ub<sup>G76V</sup>-GFP reporter in prion infected ScN2aPK-1 cells was significantly longer than uninfected N2aPK-1 cells. Cycloheximide was added at a concentration of 20µg/ml for 30 minutes at 37°C prior to fluorescent half life measurement to determine whether the delay in degradation was due to differences in the degradation of the reporter. The presence of cycloheximide confirmed that prion infection results in a significant delay in degradation of the reporter by the UPS (n=6 ± SEM). \*\* p<0.001, \*\*\*p<0.0001

**Figure S4**



**Supplementary figure 4 - PrP<sup>Sc</sup> and aggregated  $\beta$ -PrP inhibit proteolysis by the 26S proteasome.** (A) GT-1 cells, ScGT-1 cells and CGN cultures were lysed in fresh homogenisation buffer and incubated for 1 hour with aggregated  $\beta$ -PrP, PrP<sup>Sc</sup> derived from ScGT-1 cells or RML infected brain,  $\alpha$ -PrP, aggregated  $\alpha$ -PrP,  $\alpha$ -PrP with additional 150mM NaCl, or  $\alpha$ -PrP derived amyloid fibrils. (A*i-ii*) Only aggregated  $\beta$ -PrP and PrP<sup>Sc</sup> caused a significant reduction in chymotrypsin-like and caspase-like activities in GT-1 cells. None of the other prion species ( $\alpha$ -PrP, aggregated  $\alpha$ -PrP,  $\alpha$ -PrP with additional 150mM NaCl, or  $\alpha$ -PrP derived amyloid fibrils) had any inhibitory effect on proteolytic activity. (A*iii-iv*) Concomitant prion infection of GT-1 cells added to the inhibitory effect of aggregated  $\beta$ -PrP and PrP<sup>Sc</sup> as a further marked loss in chymotrypsin and caspase-like activities was observed. A similar pattern of inhibition of predominantly the chymotrypsin and caspase-like proteolytic activities was also seen in N2aPK-1 and ScN2aPK-1 cells (data not shown). (B) Only aggregated  $\beta$ -PrP and PrP<sup>Sc</sup> reduced chymotrypsin-like and caspase-like activities primary CGN lysates whilst none of the other prion species had any inhibitory effect on proteolytic activity. All data are mean  $\pm$  SEM with n=10. \* p<0.01, \*\* p<0.001, \*\*\*p<0.0001.

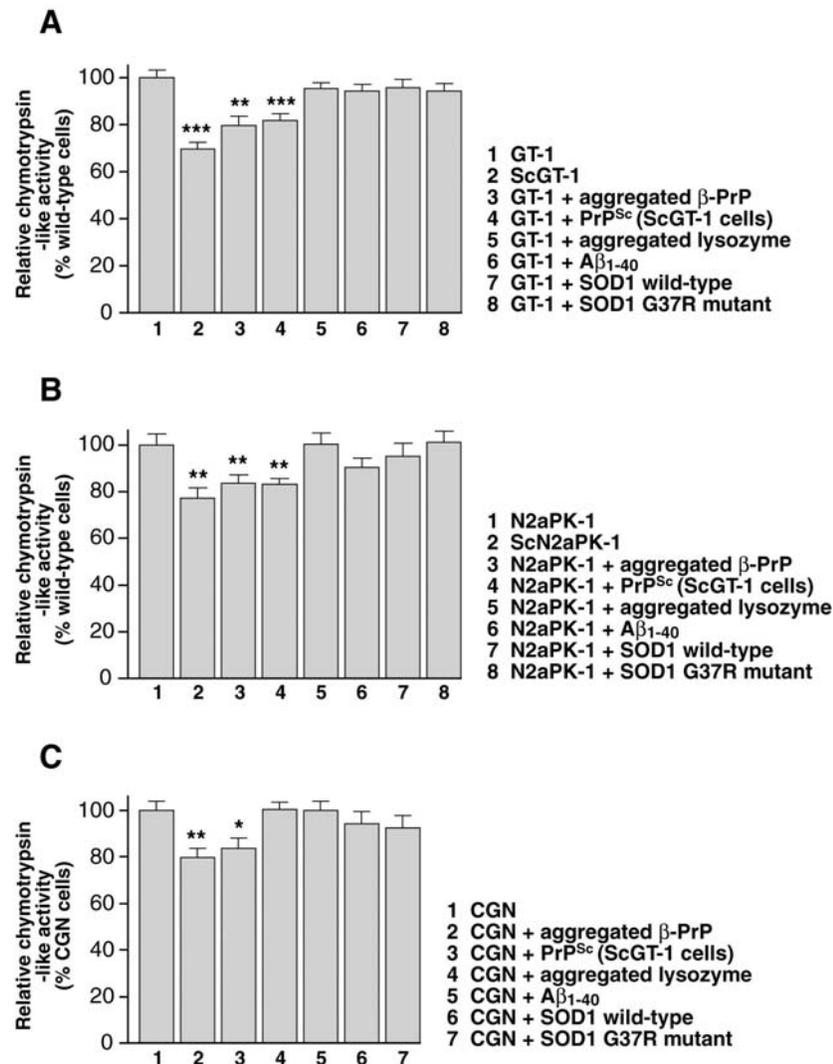
**Figure S5**



**Supplementary figure 5 –  $\beta$ -PrP is a highly potent inhibitor of 26S proteasome  $\beta$ -subunit proteolysis.** (A) Live GT-1 cells were incubated with the cell permeant activity probe in culture for 1 hour, lysed and immunoblotted. Only prion infection of GT-1 cells but not mock infection or ScGT-1 cells cured of infection (ScGT-1-18) resulted in a predominant loss of proteolytic  $\beta$ -subunit activities (B) Serial dilutions of aggregated  $\beta$ -PrP (10ng/ml, 25ng/ml, 50ng/ml, 75ng/ml, 100ng/ml, 500ng/ml, 1 $\mu$ g/ml, or 1mg/ml) were incubated for 1 hour with 100 $\mu$ g cytosolic cell fractions from N2aPK-1 cells before a further 1 hour incubation at 37°C in the presence of 1 $\mu$ M activity probe. Proteolytic  $\beta$ -subunit activity is lost at concentrations up to 75ng/ml but is restored to normal levels at 50ng/ml of aggregated  $\beta$ -PrP (C) Data and calculations of  $\beta$ -PrP and pure 26S proteasome concentrations to determine the

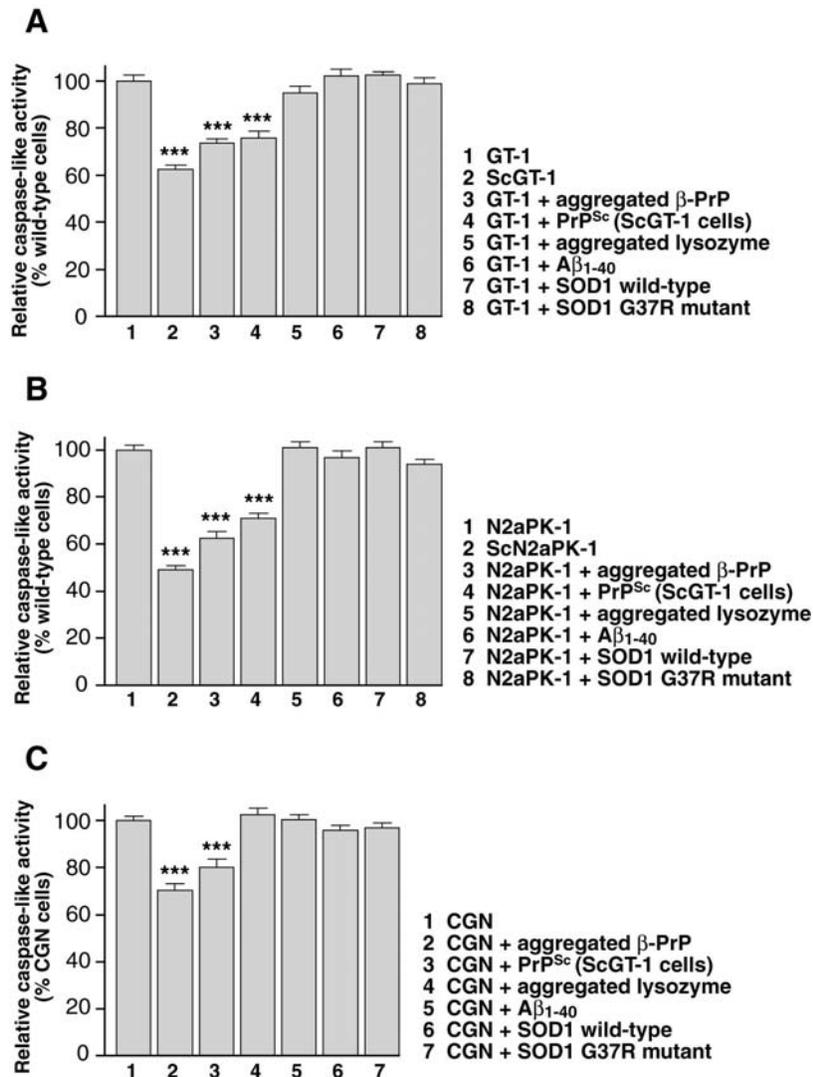
potency of  $\beta$ -PrP in inhibiting the 26S  $\beta$ -subunit activities. These data relate to Fig 4 in the main paper.

**Figure S6**



**Supplementary figure 6 – Non-prion recombinant proteins have no effect on the chymotrypsin-like activity in three different cell lines.** Chymotrypsin-like activities were measured in lysates from GT-1 cells, N2aPK-1 cells, or CGN cultures incubated with aggregated  $\beta$ -PrP, PrP<sup>Sc</sup>, or non-prion recombinant proteins (aggregated lysozyme, A $\beta$ <sub>1-40</sub>, SOD1 wild type or SOD1 G37R mutant). **(A)** GT-1 cell lysates, **(B)** N2aPK-1 cell lysates, or **(C)** CGN lysates incubated with any of the non-prion recombinant proteins had no inhibitory effect on chymotrypsin-like activity. Only prion infection, aggregated  $\beta$ -PrP or PrP<sup>Sc</sup> caused a significant reduction in the chymotrypsin-like activities in these cells, suggesting that inhibition is specific to conformational isoforms of PrP. Data are means of 10 independent experiments  $\pm$  SEM.

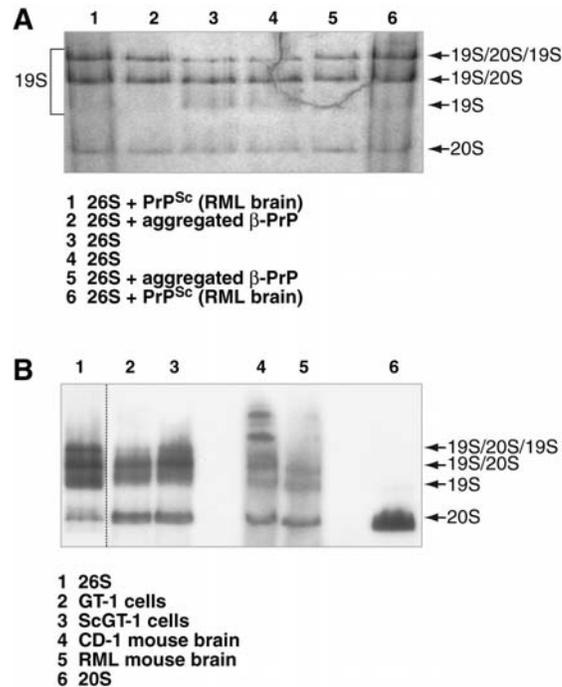
**Figure S7**



**Supplementary figure 7 - Non-prion recombinant proteins have no effect on the caspase-like activity in three different cell lines.** Caspase-like activities were measured in lysates from GT-1 cells, N2aPK-1 cells, or CGN cultures incubated with aggregated  $\beta$ -PrP, PrP<sup>Sc</sup>, or non-prion recombinant proteins (aggregated lysozyme, A $\beta$ <sub>1-40</sub>, SOD1 wild type or SOD1 G37R mutant). **(A)** GT-1 cell lysates, **(B)** N2aPK-1 cell lysates, or **(C)** CGN lysates incubated with any of the non-prion recombinant proteins had no inhibitory effect on caspase-like activity. Only prion infection, aggregated  $\beta$ -PrP or PrP<sup>Sc</sup> caused a significant reduction in the caspase-like activities

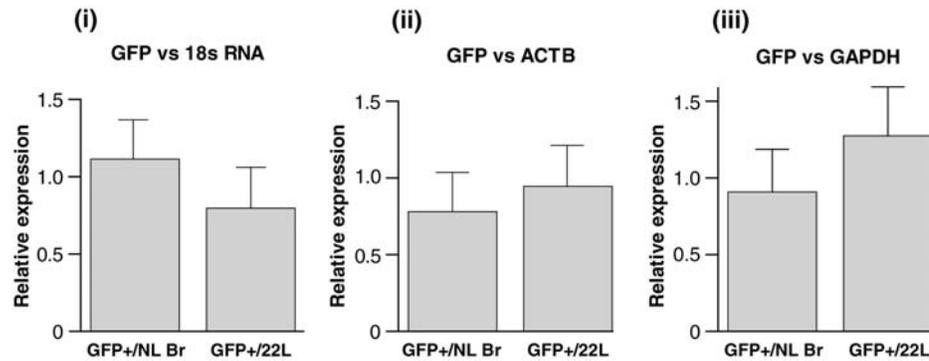
in these cells, suggesting that inhibition is specific to conformational isoforms of PrP. Data are means of 10 independent experiments  $\pm$  SEM.

**Figure S8**



**Supplementary figure 8 – Coomassie stain to confirm equal loading of protein on a native gel activity stain.** (A) Coomassie staining of the native gel activity stain (Fig 6B main paper) was carried out by fixing the native 3-12% gel in 40% methanol, 10% acetic acid and microwaved for 45 seconds on high power and then incubated at room temperature for 15 minutes. The gel was microwaved for a further 45 seconds in 0.02% R-250 coomassie in 30% methanol, 10% acetic acid and incubated at room temperature for 15 minutes. The gel was destained in 8% acetic acid until the bands were present. Equal loading of protein was confirmed in all lanes (B) Native gel immunoblotting of prion infected ScGT-1 cells (lane 3) and prion infected mouse brain (lane 5) showed no dissociation of 26S proteasome into its 19S and 20S components compared with uninfected cell lysates (lane 2) or uninfected mouse brain (lane 4).

**Figure S9**



**Supplementary figure 9** – *Accumulation of the Ub<sup>G76V</sup>-GFP reporter is not due to transcriptional upregulation.* (i-iii) There was no significant difference in expression between prion-infected mice versus mock-infected mice using Real-Time PCR quantified using 3 different house-keeping genes – 18S RNA,  $\beta$ -actin (ACTB) and GAPDH (i-iii). Bars represent mean + SD with n=5.