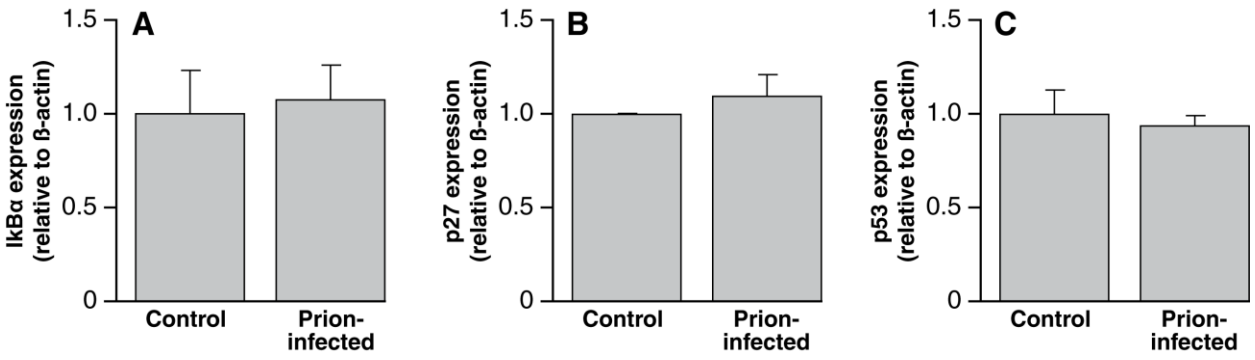
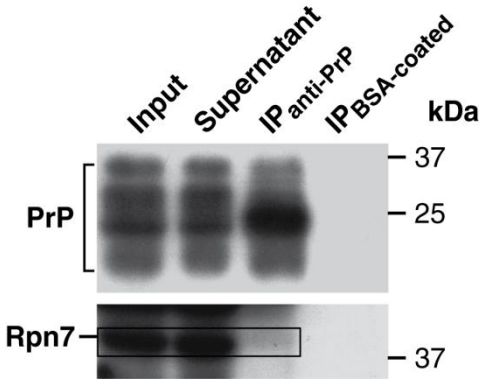
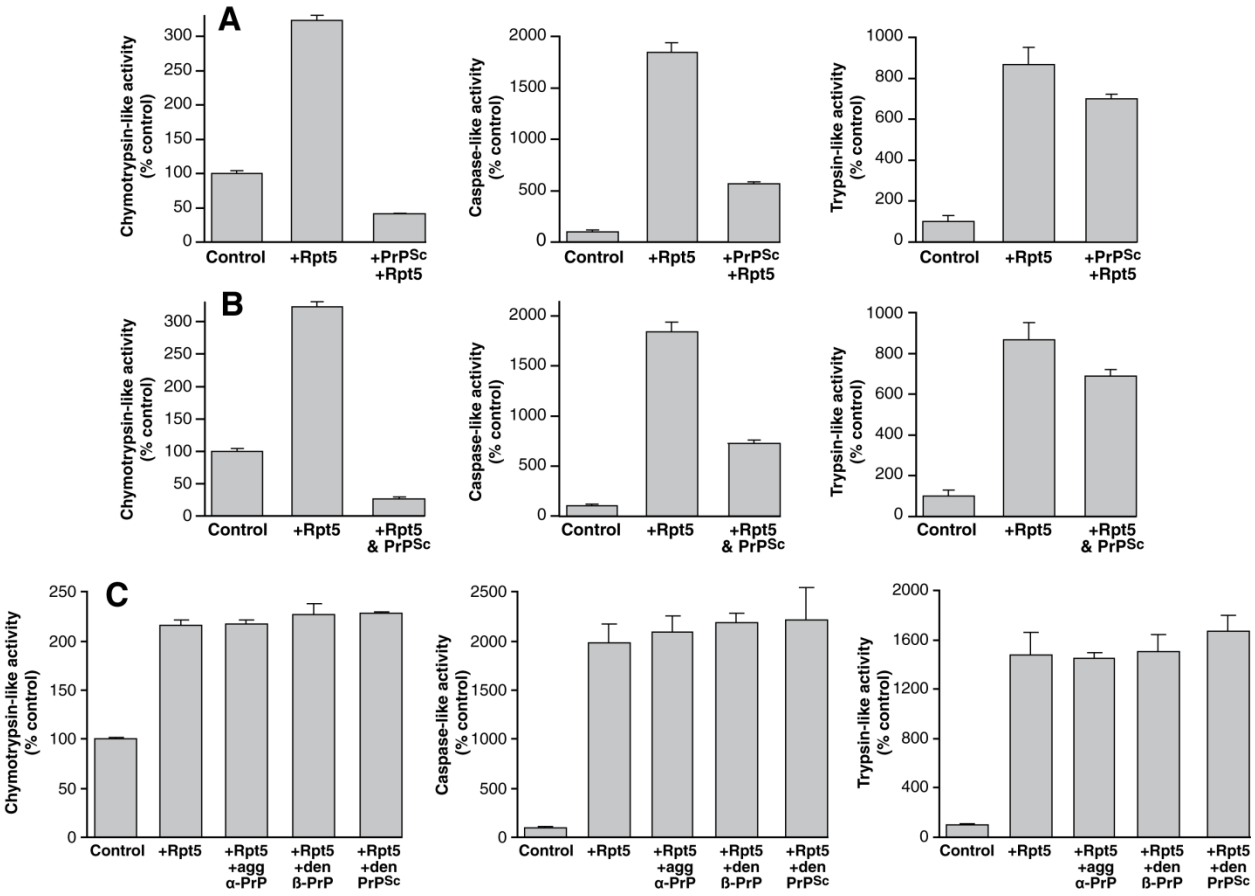


Supplementary figure 1. (A) IκBα, **(B)** p27 and **(C)** p53 mRNA expression levels, assessed by real-time RT-PCR relative to β-actin mRNA expression, in total RNA extracted from homogenates of end-stage prion-infected and uninfected mouse brains (n=2-4±SD).

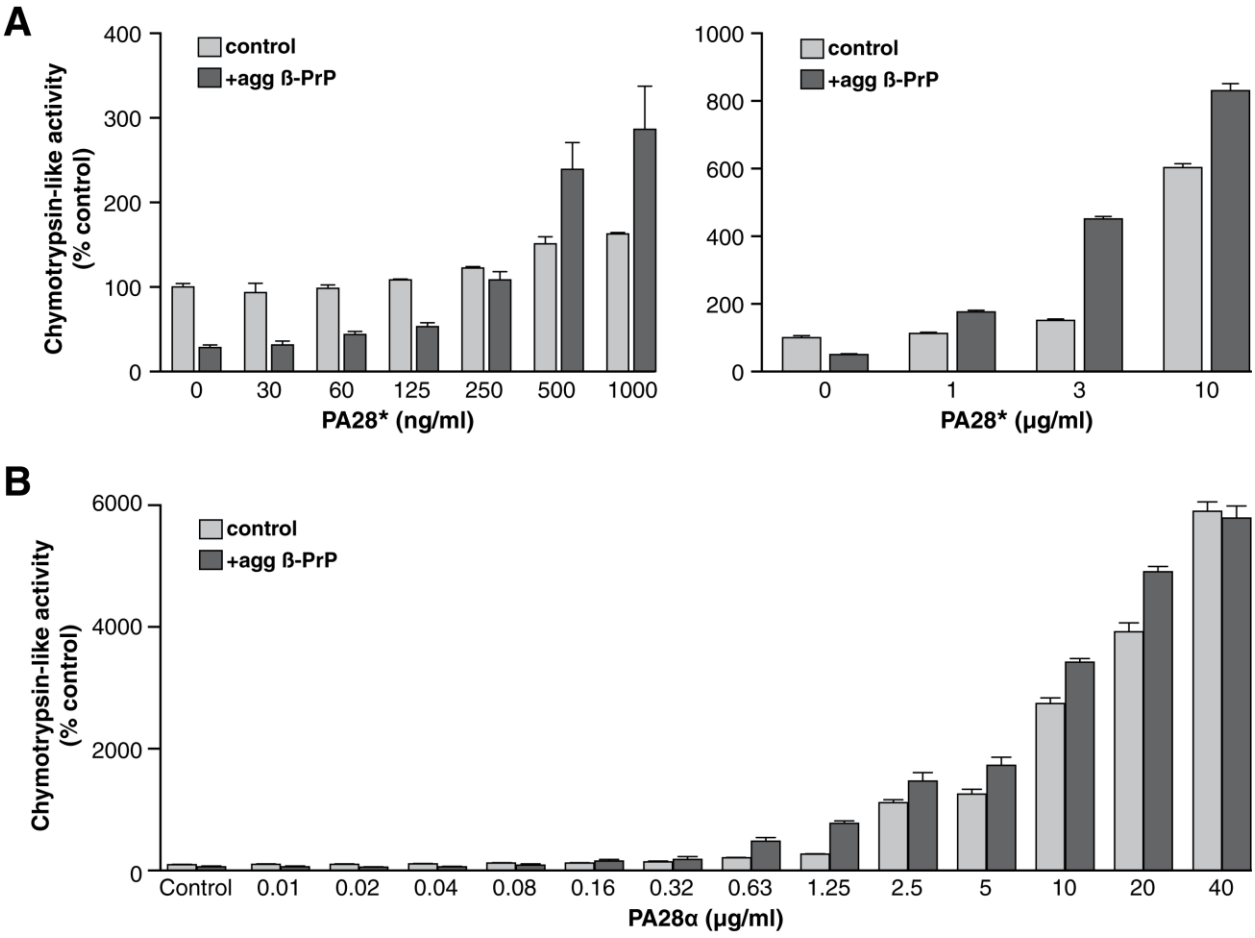


Supplementary figure 2. The 19S component, Rpn 7, co-precipitates with PrP from prion-infected mouse brain using anti-PrP antibody-coated beads. Immunoprecipitated ($IP_{anti\text{-}PrP}$), control (using beads coated with BSA alone; $IP_{BSA\text{-}coated}$) and unbound (supernatant) fractions, together with starting material (input) and antibody-coated beads alone (Ab-coated beads), were immunoblotted with anti-PrP and anti-Rpt1 antibodies. Boxed areas highlight specific bands as distinct from immunoglobulin chains eluted from the antibody-coated beads.



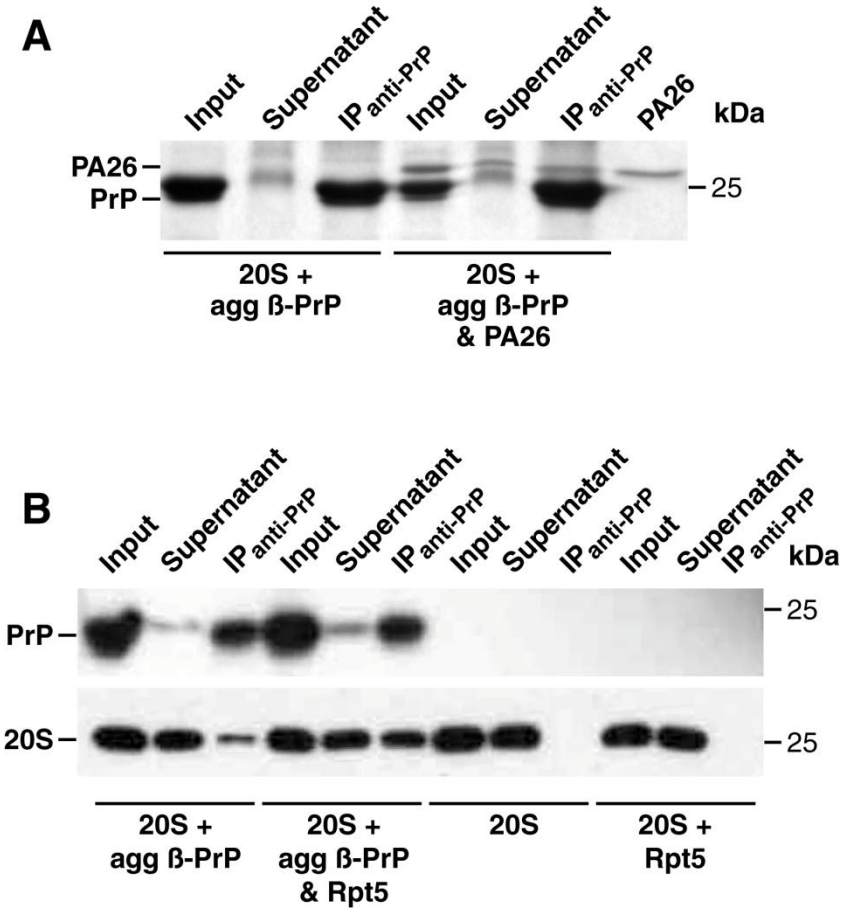
Supplementary figure 3. (A) Pre-incubating human 20S proteasomes with 100 µg/ml semi-purified PrP^{Sc} *before* the addition of Rpt5 prevents Rpt5-mediated gate-opening of the 20S ($p<0.001$, vs. respective Rpt5-activated 20S alone). **(B)** Incubating human 20S proteasomes with Rpt5 and 100 µg/ml semi-purified PrP^{Sc} at the same time results in inhibition of Rpt5-mediated gate-opening in 20S ($p<0.001$, vs. respective Rpt5-activated 20S alone). **(C)** Aggregated β-PrP (50 µg/ml) and PrP^{Sc} (100 µg/ml) do not prevent Rpt5-mediated gate opening in human 20S proteasomes when denatured by ten cycles of freeze-boiling. A recombinant form of PrP^{C} (α-PrP, 100 µg/ml) in an aggregated form (10 min at 70 °C) also does not inhibit Rpt5-mediated

activation of human 20S proteasome.



Supplementary figure 4. **(A)** Incubating human 20S proteasomes with varying concentrations of purified PA28* (0-10 µg/ml) and 50 µg/ml aggregated β-PrP does not reveal any inhibition of PA28-mediated gate opening in the 20S. Inhibition of peptide hydrolysis was observed at concentrations of PA28 that failed to cause activation, but not at concentrations (>250 ng/ml) at which even low levels of activation were seen. (* Denotes PA28 preparation, used previously, that fails even at high concentrations to cause substantial 20S proteasome gate-opening.) **(B)** Incubating human 20S proteasomes with varying concentrations of recombinant PA28α (0-40

μg/ml) and 50 μg/ml aggregated β-PrP also does not reveal any inhibition of PA28-mediated gate-opening in the 20S proteasome.



Supplementary figure 5. **(A)** PA26 was precipitated when added to human 20S proteasome and aggregated β-PrP co-immunoprecipitations. $IP_{anti\text{-}PrP}$, and unbound (supernatant) fractions, together with input starting material, were analysed by Coomassie blue stained SDS-PAGE prior to immunoblotting with anti-PrP and anti-20S antibodies (see Fig 7B). **(B)** Incubating human 20S proteasomes with 250 μM Rpt5 does not displace its binding by co-immunoprecipitation to

aggregated β-PrP (100 μg/ml). $IP_{anti\text{-}PrP}$ and unbound (supernatant) fractions, together with input starting material, were immunoblotted with anti-PrP and anti-20S antibodies.