



Figure S7 - Comparison of the effect of different proteasome inhibitors on PrP metabolism.

Cells transfected with various PrP constructs (indicated to the left of each blot) were treated with 5 μ M of the indicated proteasome inhibitor for 4 hours prior to analysis of PrP by fractionation and immunoblotting. Non-transfected cells are included as a control ('non-trans.'). The detergent soluble supernatant (S) and insoluble pellet (P) after fractionation (Yedidia et al., 2001; Ma and Lindquist, 2001) of lysates from untreated or proteasome inhibitor-treated cells are shown for each construct. The positions of mature PrP (M) and unglycosylated PrP (U) are indicated. Note that in each case, treatment of wild type PrP results in the accumulation of detergent-insoluble, non-glycosylated PrP aggregates. This is markedly reduced by the use of a more efficient signal sequence (Opn-PrP), but not by mutations that either increase C^{tm} PrP production [e.g., the PrP(AV3) construct] or abolish C^{tm} PrP [e.g., the PrP(G123P) construct]. That C^{tm} PrP was in fact increased or abolished by these mutations was confirmed in these cells by independent experiments (Fig. 7A and Supplementary Fig. S10).