**Figure S6** - Time course of PrP accumulation upon proteasome inhibitor treatment.

Cells transfected with wild-type PrP were treated with 5 μM MG132 for between 0 to 8 hours prior to analysis by immunoblotting. Non-transfected cells are included as a control (nt). Lanes 12 and 13 show the detergent soluble supernatant (S) and insoluble pellet (P) after fractionation (Yedidia et al., 2001; Ma and Lindquist, 2001) of lysates from proteasome inhibitor-treated cells (4 hours with 5 μM). The positions of mature PrP (M) and unglycosylated PrP (U) are indicated. Note that in contrast to mature PrP, the unglycosylated PrP that accumulates with proteasome inhibition is largely insoluble, suggestive of its aggregation, as has been observed previously (Yedidia et al., 2001; Ma and Lindquist, 2001).