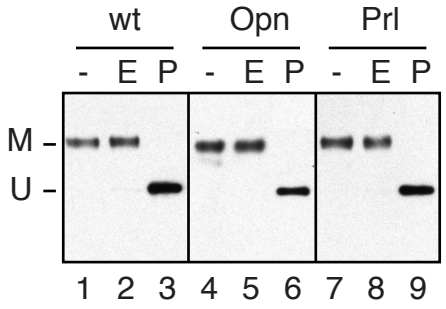

Figure S3 - Effect of the Opn and Prl signal sequences on PrP glycosylation.

Cell lysates from N2a cells transfected with either wild-type PrP, Opn-PrP, or Prl-PrP, were analyzed by immunoblotting with the 3F4 anti-PrP antibody. Prior to analysis, the cell lysates were either left untreated (-), or digested with endoglycosidase H (E) or PNGase F (P). The positions of mature, fully glycosylated PrP (M) and unglycosylated PrP (U) are indicated. Note that for all constructs, the same glycosylation pattern is observed and the majority of PrP is resistant to endoglycosidase digestion, indicative of proper carbohydrate maturation after trafficking to the Golgi. Similar analysis of TRAPα, a resident ER glycoprotein, showed complete sensitivity to digestion with both endoglycosidase H and PNGase F (data not shown).