

SUPPLEMENTARY ONLINE DATA

Signal peptide peptidase (SPP) assembles with substrates and misfolded membrane proteins into distinct oligomeric complexes

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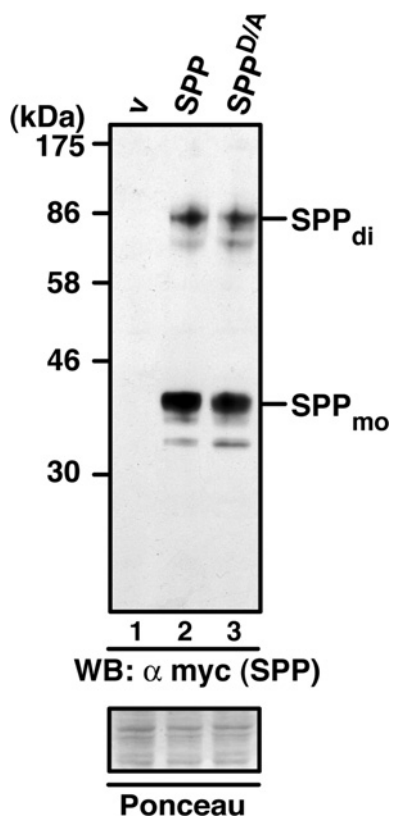


Figure S1 Similar expression levels of SPP and SPP^{D/A}

HEK-293 cells transiently expressing myc-tagged SPP or SPP^{D/A} were lysed in the presence of 1% Triton X-100, and proteins were separated by Tris/glycine SDS/PAGE. SPP monomers (mo) and dimers (di) were identified by Western blotting (WB) using the anti-myc antibody. v, empty vector control. Molecular masses are indicated in kDa.

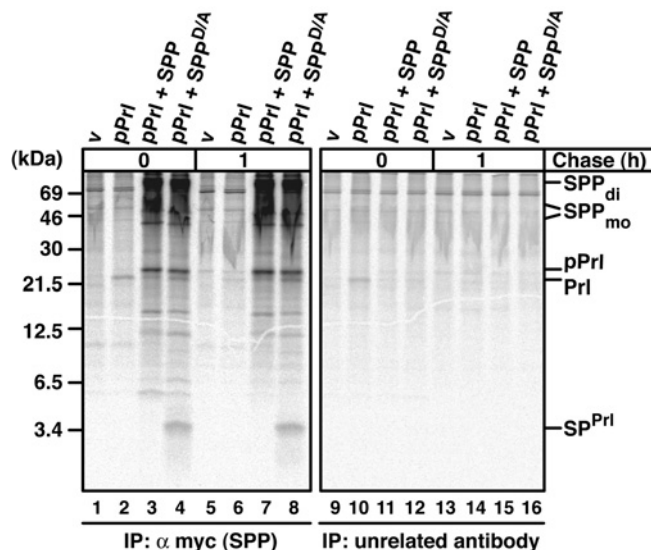


Figure S2 SP^{PrI} and pPrI can be co-immunoprecipitated with an anti-myc antibody, but not with an unrelated antibody

HEK-293 cells transiently expressing pPrI, SPP–myc or SPP^{D/A}–myc were pulse labelled for 30 min and chased for 1 h. Cells were lysed with 1% Triton X-100, and proteins were immunoprecipitated (IP) with a monoclonal anti-myc antibody (left-hand panel) or with an unrelated monoclonal antibody (right-hand panel). Proteins were separated on Tris/Tricine gels and detected by autoradiography. SPP dimer (di) and monomer (mo) are indicated. v, empty vector control. Molecular masses are indicated in kDa.

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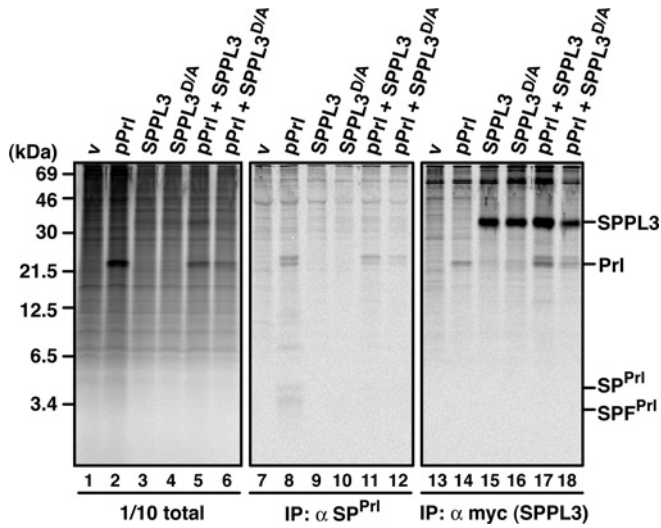


Figure S3 SP^{PrI} does not accumulate or co-immunoprecipitate in the presence of SPPL3^{D/A}

HEK-293 cells transiently expressing pPrI, SPPL3-myc or SPPL3^{D/A}-myc as indicated were pulse labelled for 30 min. Cells were lysed with 1% Triton X-100, and 1/10 of the total lysate was analysed directly by SDS/PAGE, whereas the rest was used for immunoprecipitation (IP) using either anti-SP^{PrI} antibodies or anti-myc antibodies to identify myc-tagged SPPL3/SPPL3^{D/A}. Samples were subjected to Tris/Tricine SDS/PAGE, and labelled proteins were visualized by autoradiography; v, empty vector control; PrI, mature prolactin. Molecular masses are indicated in kDa.

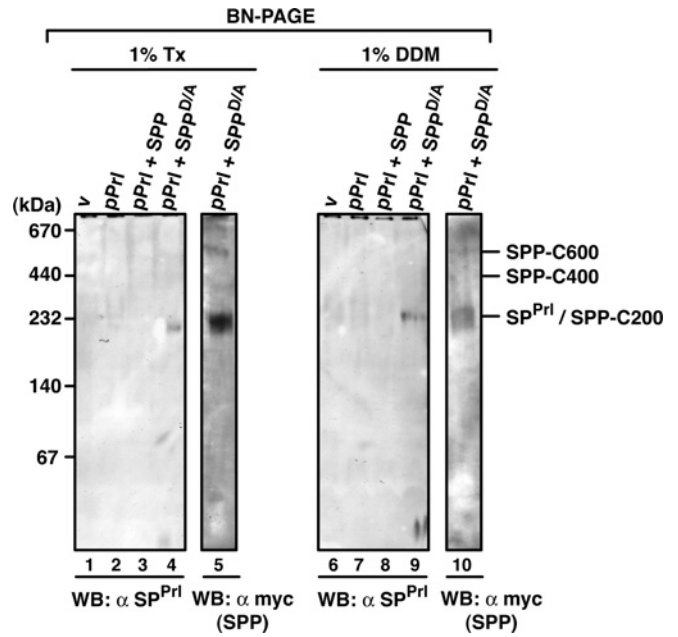


Figure S4 The SP^{PrI}/SPP complex is also stable in 1% Triton X-100 and 1% DDM buffer

HEK-293 cells transiently expressing pPrI, SPP-myc or SPP^{D/A}-myc were lysed with 1% Triton X-100- (Tx) or 1% DDM-containing buffer, and an aliquot of total lysates was applied to BN-PAGE (6–13%). SPP-myc and SP^{PrI} were identified by Western blotting (WB) using anti-myc and anti-SP^{PrI} antibodies respectively. v, empty vector control. SPP complexes of 200 kDa (C200), 400 kDa (C400), 600 kDa (C600) and SP^{PrI} are indicated. Molecular masses are indicated in kDa.

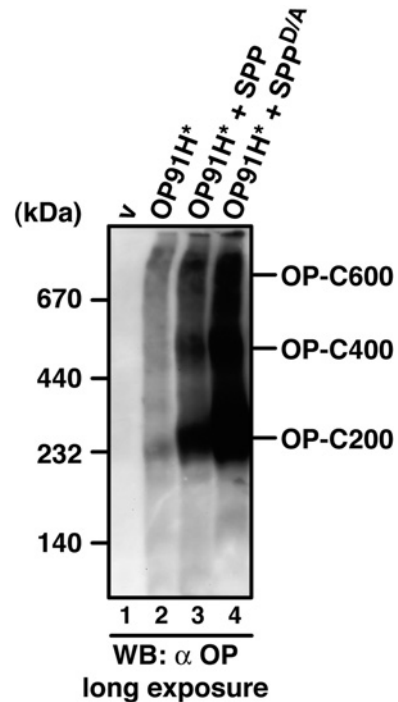


Figure S5 Identification of low amounts of OP91H*/SPP complexes after BN-PAGE and Western blotting using an anti-opsin antibody (long exposures)

A Western blot (WB) as shown in Figure 8(A) of the main text and probed with an anti-opsin antibody was exposed for a prolonged time to visualize faint signals. Opsin complexes of 200 kDa (C200), 400 kDa (C400) and 600 kDa (C600) are indicated. v, empty vector control. Molecular masses are indicated in kDa.

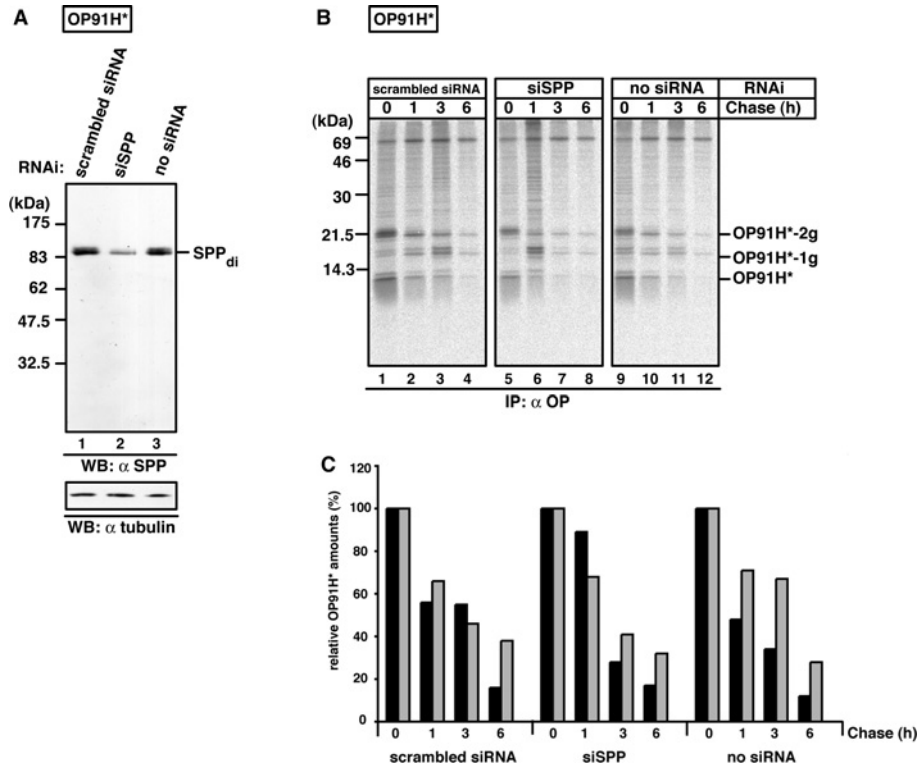


Figure S6 RNA interference-mediated SPP knockdown and the effects on OP91H* accumulation

HEK-293 cells were transiently transfected with 10 nM of either validated SPP-specific siRNA (small interfering RNA) (Silencer Select validated siRNA ID: s37580; Ambion) or a scrambled siRNA (Silencer Select Control siRNA #1; Ambion). After 48 h, the cells were re-transfected with the respective siRNAs as indicated, together with plasmids encoding OP91H*. After an additional 48 h, cells were either lysed in the presence of 1% digitonin and endogenous SPP amounts were identified by Western blotting (WB) using the anti-SPP antibody (**A**) or cells were pulse labelled for 10 min and chased for various times as indicated (**B**). For Western blotting, lysates were mixed with SDS-sample buffer and heated to 37 °C for 10 min. Under these conditions, only the SPP dimer (di) was detected after Western blotting. Upon transfection with SPP-specific siRNAs, endogenous SPP amounts were significantly, but not completely, reduced (**A**, lane 2). After pulse–chase labelling of the cells, proteins were immunoprecipitated (IP) using the anti-opsin antibody, and labelled protein was visualized by autoradiography. Non-, mono- (1g) and bi-glycosylated (2g) forms of OP91H* are indicated (**B**). Molecular masses are indicated in kDa. (**C**) Quantification of OP91H* amounts from two independent pulse–chase experiments. No significant differences in the accumulation of OP91H* upon SPP knockdown (**B** and **C**, middle panel) compared with cells which were transfected with scrambled siRNA (left-hand panel) or without transfection (right-hand panel), could be detected during 6 h of chase.