**Supplementary Material**

**Materials and Methods**

**Synthesis of TBL₄K.** (BOC-LL)$_2$ ketone (1) was treated with trifluoroacetic acid following modification of the free amino groups with 1 eq. of 4-(3’-trifluoromethyl-3H-diazirin-3-yl)benzoic acid N-hydroxy-succinimidyl ester (Photoprobes) and 1 eq. of biotinamidocapronic acid N-hydroxysuccinimidyl ester (Molecular Probes).

**Inhibition and photo-labeling of SPP with TBL₄K.** The inhibitory effect of TBL₄K on SPP was tested with in vitro synthesized substrates HLA-A/24 and HLA-A*03/30 as described (1, 2). For analytical labeling, 2 µl of rough ER membrane proteins solubilized with the detergent CHAPS (Anatrace), were diluted with 16 µl of assay buffer (25 mM HEPES-KOH, pH 7.6, 100 mM KOAc, 2 mM Mg(OAc)$_2$, 1 mM DTT) and supplemented with 50 nM TBL₄K (dissolved in DMSO). Samples were incubated at 30 °C for 1 hour and subsequently irradiated with UV light (30 seconds; 350 W high pressure mercury lamp with a Pyrex filter, 10 cm distance to lamp) (3). Proteins were precipitated with 10 % trichloroacetic acid and resolved on Tris-glycine acrylamide gels (10 % acrylamide/bis-acrylamid 37.5:1). Biotinylated proteins were visualized by Western blotting with a polyclonal anti-biotin antibody (Bethyl).

**Preparative labeling and purification.** 15 ml of CHAPS-solubilized ER membrane proteins (prepared from 15-20’000 equivalents rough microsomes (4)) were diluted with 120 ml of assay buffer (see above) and supplemented with 20 µl of 500 µM TBL₄K in DMSO. After incubation at 30 °C for 1 hour, the sample was distributed between four 50
ml polypropylene tubes and irradiated with UV light for 30 seconds. The sample was supplemented with 15 ml of 5 M NaCl and 2.6 ml of 20 % reduced Triton X-100 (Sigma) and applied to a 1 ml Con A-sepharose column (Amersham-Pharmacia), that had been equilibrated with EQ buffer I (50 mM HEPES-KOH, pH 7.6, 500 mM NaCl, 20 mM sucrose, 1 mM DTT, 0.35 % reduced Triton X-100). After washing with EQ buffer I, bound proteins containing TBL₄K-labeled species were eluted with 15 ml EQ buffer I containing 1 M methyl-α-D-glucopyranoside. The eluate was diluted 10 times with 50 mM HEPES-KOH, pH 7.6, 1 mM DTT, 0.35 % reduced Triton X-100 to reduce salt concentration, and applied to a 2.5 ml hydroxyapatite column (BioRad), which had been equilibrated with EQ buffer II (50 mM HEPES-KOH, pH 7.6, 50 mM KOAc, 1 mM DTT, 0.35 % reduced Triton X-100). The column was next washed with 12.5 ml of EQ buffer II, and bound proteins containing TBL₄K-labeled species were eluted with 3 ml elution buffer (50 mM HEPES-KOH, pH 7.6, 500 mM KOAc, 200 mM KP₀, 1 mM DTT, 0.35 % reduced Triton X-100). Proteins were next precipitated by adding 300 µl of 100 % trichloroacetic acid. After centrifugation (Eppendorf centrifuge, 14’000 rpm, 4 °C, 5 min), the protein pellet was washed with acetone and re-suspended in 50 % formic acid. The sample was applied to a RP4 reversed phase HPLC column (CC 125/4 Nucleosil 300-5 C4; Machery Nagel), which had been equilibrated with 50 % formic acid. Proteins were first eluted with a linear gradient of 50 % formic acid in H₂O to 50 % formic acid in acetonitrile. After re-equilibration with 50 % formic acid in H₂O, residual proteins including TBL₄K-labeled species were eluted with a linear gradient of 50 % formic acid in H₂O to 50 % formic acid in propan-2-ol. Fractions containing the TBL₄K-labeled species were pooled and proteins were resolved by SDS-PAGE using a Tris-glycine
acrylamide gel (10 % acrylamide/bis-acrylamid 37.5:1). Proteins were visualized by coomassie blue staining and the TBL_{4K}-labeled protein was identified by Western blotting using anti-biotin antibody. The corresponding coomassie stained band was excised from the gel and subjected to sequencing by mass spectrometry.

**Sequencing by Mass Spectrometry and sequence alignments.** Proteins were reduced, alkylated and in-gel trypsin digested as described (5). Peptides were extracted from the gel and desalted by using ZipTip desalting columns (Millipore). Tandem mass spectrometry (MS/MS) analysis was performed using a nanoelectrospray source (Protana A/S) coupled to the high performance hybrid quadrupole time of flight API QSTAR™ Pulsar mass spectrometer (MDS-Sciex). Doubly or triply charged tryptic parent ion candidates were selected and product ion spectra generated by collision-induced dissociation (CID). The spectra were used to search the NCBInr and dbEST databases (http://www.ncbi.nih.gov/LocusLink/index.html; http://www.ncbi.nih.gov/dbEST/index.html) with the Mascot MS/MS search engine (Matrix Science; http://www.matrixscience.com/cgi/index.pl?page=./home.html). Comparison of the retrieved peptide sequence and masses with the tandem mass spectrum identified the sequences NASDMPETITSR, QYQLLFTQGSGENK, LVFPQDLLEK, and GEVTEMFSYEESNPK in the predicted human protein gi:14772424, FFPANFPNR in the homologous mouse protein gi:14772424 (FFPASFPNR in the human protein), and EEIINYEFATK (EEIINYEFDTK in the human and mouse proteins).
**Plasmid constructs and expression of human SPP in yeast.** Human SPP cDNA is based on human EST fragments from the NCBI database coding for human protein gi:14772424. The cDNA was amplified from a HeLa cell-derived cDNA library with Pfu DNA polymerase (Stratagene) and the PCR primes 5'-'ACGACTAGTTCCACCATGGACTCGGCCCTCAGC-3' and 5'-'TGGAAGCTTCCTGAGAGCTCGGCACCAGC-3'. The resulting 1181bp fragment was cloned into the SpeI/HindIII sites of the yeast expression vector p426gal1 (6) yielding pDAW300. The sequence was confirmed by sequencing and deposited in EMBL databank (AJ420895). SPP mutants D265A and N10Q/N20Q were generated by using the QuickChange Site-Directed Mutagenesis Kit (Stratagene) resulting in pDAW302, and pDAW306. Plasmids were next transformed into *S. cerevisiae* strain BY4742 (MATα; his3Δ; leu2Δ0; lys2Δ0; ura3Δ0) (7) by lithium acetate transformation to give yeast strains DAW300 (MATα; his3Δ; leu2Δ0; lys2Δ0; ura3Δ0; p(spp, gal1,2μ,ura3)), DAW302 and DAW306. Strains were grown at 23 °C in SC-medium with 2 % galactose and appropriate supplements for selective growth until an OD$_{600}$ of ~0.6 was reached. Yeast microsomes (8) were solubilized with CHAPS, tested for activity, and labeled with TBL$_4$K as described above (1).

**In vitro translation and translocation.** *In vitro* translations were performed in reticulocyte lysate (Promega) as described (2). For protein translocation, ER-derived rough microsome were added to translation reactions and incubated with or without the glycosylation inhibitor N-benzoyl-Asn-Leu-Thr-methylamide (2).
References

Supplemental Figures

**Fig. S1.** Multiple sequence alignment (ClustalW 1.4) of predicted amino acid sequences of human SPP (CAD13132.1) and potential orthologues in *Mus musculus* (BAB25172), *Drosophila melanogaster* (AAL48184), *Caenorhabditis elegans* (P49049), and *Arabidopsis thaliana* (AAL38345). Conserved residues are highlighted; the "YD" and "LGLGD" motifs are underlined. Accession numbers refer to the EMBL/GenBank/DDBJ database (http://www.ncbi.nlm.nih.gov/Genbank/index.html).

**Fig. S2.** Glycosylation of asparagines-10 and -20 *in vitro*. The NH$_2$-terminal 150 residues of wt (lanes 1-3) and N10Q/N20Q mutant SPP (lanes 4-6) were translated *in vitro* in the presence of ER-derived rough microsomes isolated from canine pancreas (lanes 2, 3, 5 and 6) and in the presence (lanes 3 and 6) or absence of acceptor tripeptide (N-benzoyl-Asn-Leu-Thr-methylamide) to inhibit glycosylation. Dots indicate glycosylated proteins.
Figure S1
Figure S2

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