**Fig S1** – (A) Cartoon outlining the *in vitro* system used in this study. mRNA coding for the protein of interest is synthesised in a rabbit reticulocyte lysate (RRL) translation system supplemented with [35S] methionine (shown in red). Incubation with ER-derived canine pancreatic rough microsomes (RMs) allows translocated substrates to be identified by N-glycosylation (‘+1g’/+2g’) and/or signal sequence cleavage (‘c’). (B) Cartoon model depicting the role of mycolactone in inhibiting co-translational translocation, but not tail-anchored protein integration into the ER.
Fig S2 - (A) Phosphorimage of SMCC cross-linked PPL86 in the absence of RMs and in the presence or absence of mycolactone. Samples were immunoprecipitated with antisera raised against PPL (‘P’) or SRP54 (‘S’). (B) Phosphorimage of BMH cross-linked PPL75 and PPL86, as well as versions with a C25A point mutation within the signal sequence. (C) Phosphorimages of BMH cross-linked PPL75 that had been in vitro translated in the presence of RMs and in the presence or absence of 500 µM ESI. Samples were immunoprecipitated after carbonate extraction using antisera raised against either PPL (‘P’), Sec61α (‘α’) or Sec61β (‘β’). Phosphorimage is shown at normal exposure (left panel) and high exposure (right panel) to highlight the disappearance of an already faint PPL75- Sec61β cross-link in the presence of ESI. (D) EDTA-treated and salt washed RMs (EKRMs) were incubated with ribosomes in the presence or absence of 1 µg/ml mycolactone, or in the presence of 5 mM EDTA,
and the amount of membrane-bound ribosomes was determined by blotting for ribosomal protein L19 (RPL19). The relative amount of bound ribosomes was then plotted (right panel). Error bars show mean±s.d (n = 3).
Fig S3 – (A) Post-translational translocation of CecZnF and CecZnF N40Q (which lacks the first N-glycosylation site) into RMs in the presence or absence of exogenous Zn\(^{2+}\) (0.5 mM). (B) DSS cross-linking analysis of CecZnF in the presence or absence of exogenous Zn\(^{2+}\) (0.5 mM). Samples were analysed by SDS-PAGE either following isolation of RMs by ultracentrifugation (left panel) or by immunoprecipitation using antisera raised against Sec61\(\alpha\) (‘\(\alpha\)’, middle panel) or calreticulin (‘CRT’, right panel). (C) DSS cross-linking analysis of CecZnF in the presence or absence of exogenous Zn\(^{2+}\) (0.5 mM). Samples were EndoH-treated where indicated to determine whether the CecZnF population that cross-links Sec61\(\alpha\) is N-glycosylated.
**Fig S4** – Cross-linking analysis was performed on RMs in the presence or absence of mycolactone using either BMH (‘B’) or DSS (‘D’), and adducts involving Sec61α, Sec61β, Sec62 and TRAM were detected by Western blotting (A-D). Adducts that appear diminished in the presence of mycolactone are denoted by a asterisk (‘*’) and adducts that appear enhanced in the presence of mycolactone are denoted by a double asterisk (‘**’).