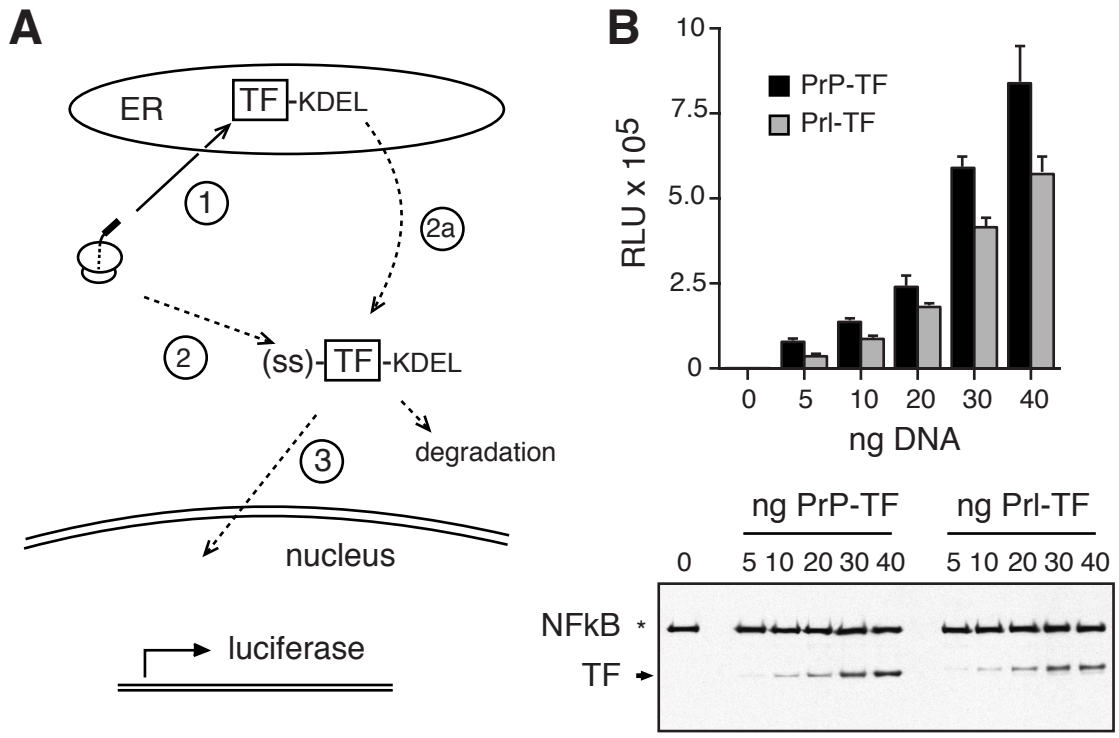

Figure S1 - Transcription factor (TF) sequestration assay for signal sequence function.

(A) Signal sequence-containing nascent polypeptides encoding a TF are co-translationally targeted to and translocated across the ER membrane (step 1), where they are retained by a C-terminal KDEL retention sequence. Inefficient targeting or translocation (step 2) results in cytosolic TF, where it is available to activate transcription of a luciferase reporter (step 3). Cytosolic TF could potentially also be generated if retrotranslocated TF escapes degradation (step 2a). TF is composed of a fusion between the Gal4 DNA binding domain and the transcriptional activation domain from NF-kB (plasmid pBD-NFkB from Stratagene). PrP-TF, Prl-TF, and Opn-TF were made by replacing the initiating methionine of TF with the appropriate signal sequence and appending a sequence encoding KDEL immediately preceeding the stop codon. The luciferase reporter (plasmid pFR-Luc from Stratagene) contains firefly luciferase preceeded by five Gal4 binding sites.

(B) Comparison of luciferase activation by PrP-TF versus Prl-TF at various expression levels. Varying amounts of plasmid encoding PrP-TF and Prl-TF were co-transfected with a constant amount of pFR-Luc and the extent of luciferase reporter activation measured (RLU, relative light units; mean ± SD, n=5). Parallel samples were analyzed by immunoblotting with antibodies against NF-kB (bottom panel). Endogenous NF-kB (asterisk) serves as a loading control. Arrow indicates position of the exogenously transfected TF, which showed equal expression levels for PrP-TF and Prl-TF at each concentration of DNA used. At every level of expression, the Prl-TF consistently showed lower luciferase activity, indicating that it was segregated more efficiently into the ER than PrP-TF. The proportion of cytosolic TF contributed by retrotranslocation (step 2a) is not known, but can at most be the total seen with Prl-TF. Thus, the amount of cytosolic PrP-TF above this level represents the minimum amount that can be attributed to inefficient translocation (step 1). This suggests that for the PrP signal, at least one-third to one-half of all cytosolic TF must result from its inefficient translocation into the ER.