Supplemental Data

Sss1p is required to complete protein translocon activation

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Running head: The role of Sss1p in ER protein translocation

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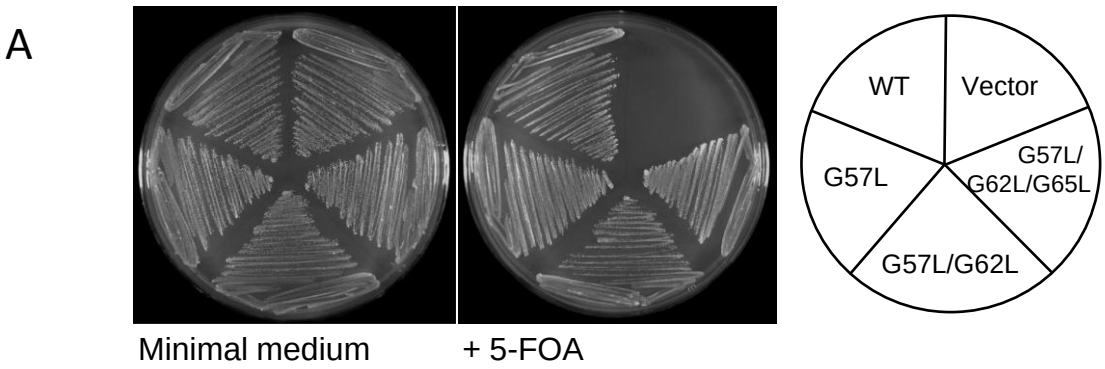

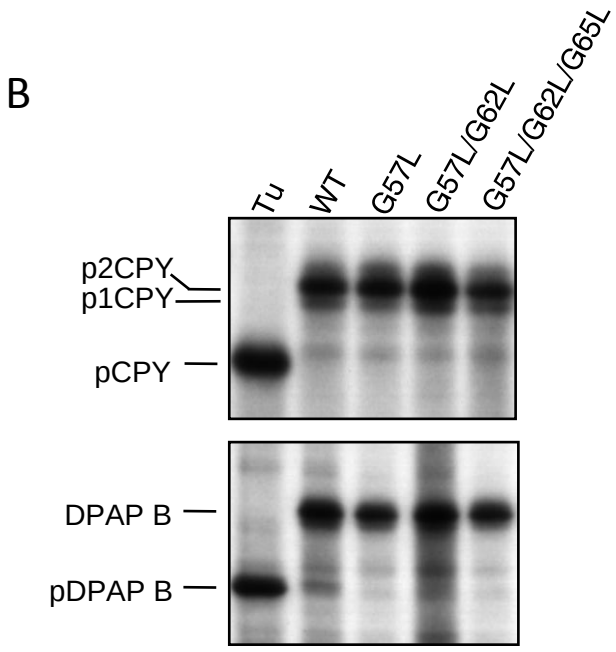

Figure S1. Functional analysis of Sss1p glycine mutants. *A*, Vector (pRS313) and plasmids encoding *SSS1*, the G57L, G57L/G62L and G57L/G62L/G65L *sss1* mutations were transformed into the *SSS1* plasmid shuffle strain BWY530. All tranformants grew normally after 3 days on minimal medium minus uracil and histidine for selection of the *SSS1, URA3* plasmid (FKp53) and the pRS313 (HIS3) transformed plasmids. After 3 days incubation on 5-FOA containing minimal medium, the vector only strain was unable to grow due to the lethality of the *Δsss1* mutation upon FKp53 counter-selection, whilst the transformants containing the wild-type (*SSS1*) and glycine mutation plasmids grew by providing Sss1p function. *B*, After passage on 5-FOA medium, the strains now containing the *SSS1* or glycine mutations on the pRS313 vector as the sole source of Sss1p function were analysed for defects in protein translocation. Whole cell extracts prepared from cells grown at 30^0C and ^{35}S-labelled for 5 min were immunoprecipitated with ppCPY or DPAP B specific antisera and the products were resolved by 10 and 7.5% SDS-PAGE respectively. Upon ER translocation, ppCPY is signal-cleaved and modified by N-linked glycan addition in the ER (p1 CPY) and the Golgi (p2CPY). The type II membrane protein, DPAP B which acquires N-linked glycans upon correct ER membrane integration, and decreases in gel mobility. Tunicamycin (Tu) treatment yields the signal-cleaved, but unglycosylated pro-CPY and the unglycosylated pre form of DPAP B (pDPAP B).

Table S1. Oligonucleotide primers

Name	Sequence (5´-3´)
SSS1-G1	CCAAGATTGTCAAGGCTGTTGGTATTCTATTAATTGCAGTCGGTATCATTGG
SSS1-G1a	CCAATGATACCGACTGCAATTAATAGAATACCAACAGCCTTGACAATCTTGG
SSS1-G2	GGTATTCTATTAATTGCAGTCGGTATCATTCTGTACGCCATCAAGTTGATTC
SSS1-G2a	GAATCAACTTGATGCGTACAGAATGATACCGACTGCAATTAATAGAATACC
SSS1-G3	GGCTGTTGGTATTCTATTAATTGCAGTACTTATCATTCTGTACGCCATCAAG
SSS1-G3a	CTTGATGGCGTACAGAATGATAAGTACTGCAATTAATAGAATACCAACAGCC
SSS1-ΔC	AAGGAATACACCAAGATTGTCAAGTAAGTTGGTATTGGTTTAATTGC
SSS1-ΔCa	GCAATTAAACCAATACCAACTTACTTGACAATCTTGGTGTATTCCTT
UBC6-2	TTGGTTATTTGTGTTCAGAAA
UBC6-3	GGGGGATCCTGAAGAACTATCATTAGGTTC
SSS1-1	TTCAAGGTCGTTGGA GAG
SSS1-4	GGGGGATCCGCTGTTGGTATTGGTTTAAT
USSS1C	GGGATCCATGGTTTATATTGGTATCGCTATTTTTTTGTTTTTGGTTGGCCTTTTTAT GAAGTTGATTCATATTCCAATCAG
USSS1Ca	GCAGTAATATTATATGGGAAG
SSS1-NdeIF	GTATAACATTGAAAAATCATATGGCTAGAGCTAGTG
SSS1-NdeIR	CACTAGCTCTAGCCATATGATTTTTCAATGTTATAC
Δsss1-1	TTTACCAAAGATAAAATCACGTATAACATTGAAAAATAAACGTACGCTGCAG GTCGAC
Δsss1-1a	GTGCCGTTGTTTTTATTTTTTCTCGTCTTTTATCTCTTATCGATGAATTCGAGCTCG
SEC61-121	GCTCAAAAGGTGGCCGCTATTATTCTGATC
SEC61-121a	GATCAGAATAATAGCGGCCACCTTTTGAGC
SEC61-150	CTCGGATTGCCATCGCCTTGTTGTTAATC
SEC61-150a	GATTAACAACAAGGCGATGGGCAATCCGCG
SEC61-373	GTTCTTGGTTCAGCCGCAGTATTTTCC
SEC61-373a	GGAAAATACTGCGGCTGAACCAAGAAC
SEC61tm9C	CATTCCAACTGCTGCATGCTGCTGCGGTGCTACCATC
SEC61tm9Ca	GATGGTAGCACCGCAGCAGCAGCATGCAGCAGTTGGAATG