Lee et al., http://www.jcb.org/cgi/content/full/jcb.201501043/DC1

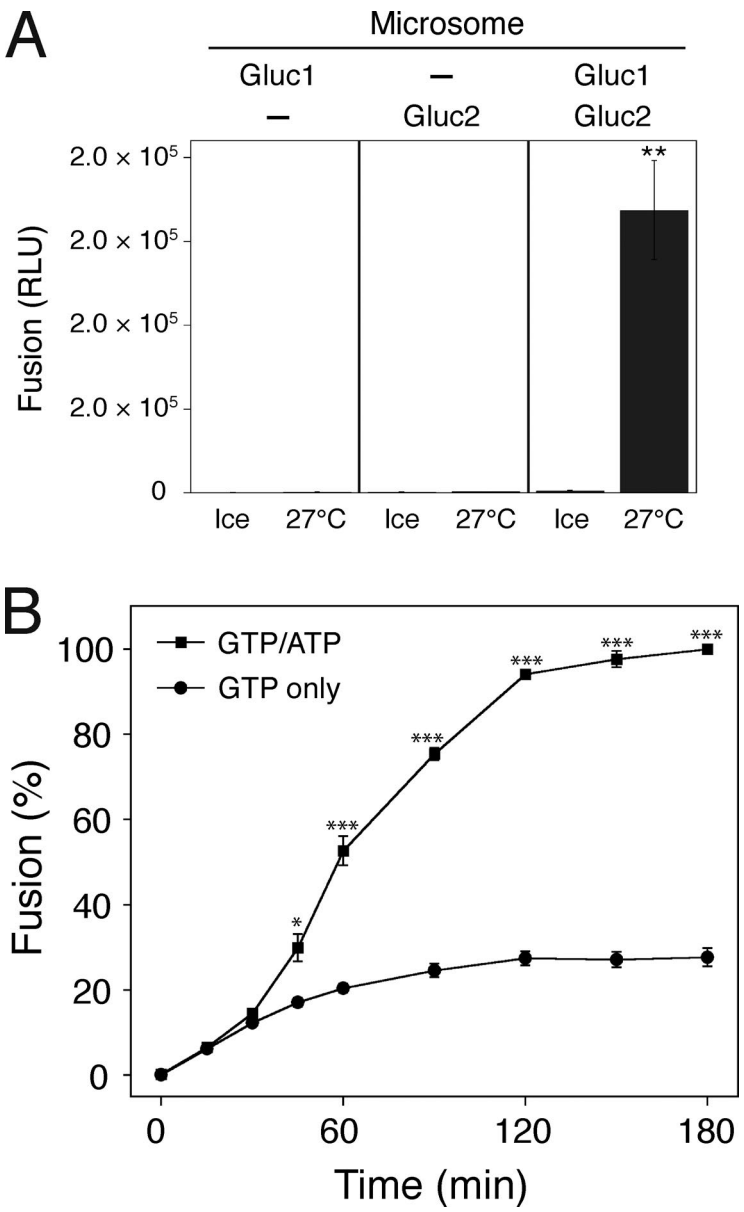

Figure S1. **Characterization of the in vitro ER microsome fusion assay.** (A) Membrane fusion–mediated reconstitution of luciferase activity requires both Gluc PCA fragments. The indicated microsomes were mixed and incubated on ice or at 27°C in the presence of ATP and GTP. After 90 min, luciferase activity was measured using a luminometer and was expressed as relative luminescence units (RLUs). Data represent the means ± SEM (error bars; $n = 3$). **, $P < 0.01$, Tukey's test between samples incubated at 27°C. (B) Comparison of reaction kinetics between ATP/GTP-driven and GTP-only-driven ER fusion reactions. ER fusion reactions were performed in the presence of an energy-regenerating system containing ATP/GTP or GTP only. At the indicated times, a portion of each reaction was placed on ice. Luciferase activity was measured after 180 min. Fusion values were normalized to those obtained using an ATP/GTP-driven reaction placed on ice at 180 min. Data represent the means ± SEM (error bars; $n = 3$). *, $P < 0.05$; ***, $P < 0.001$, Student's *t* test.

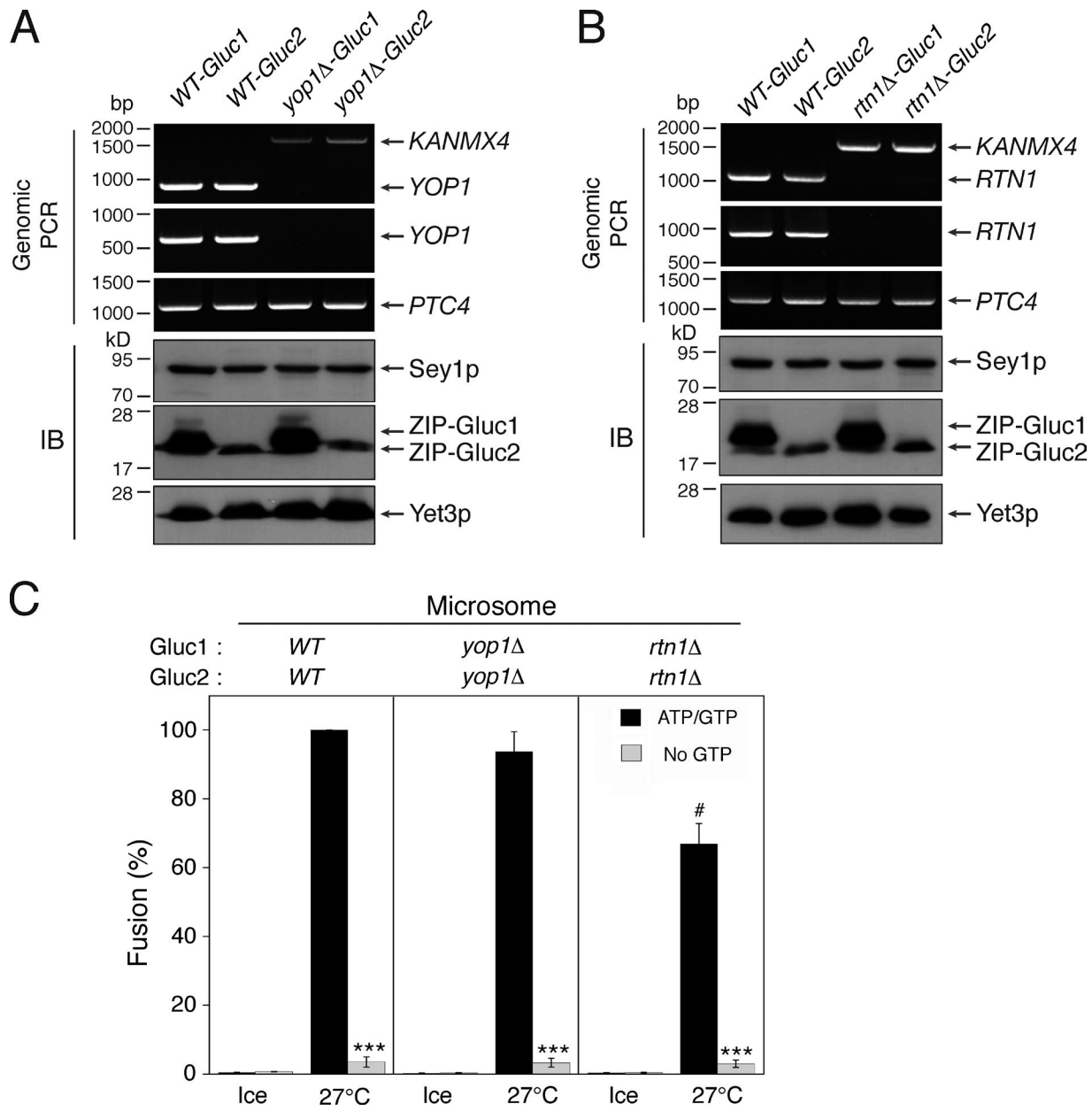

Figure S2. **Reticulons and DP1/YOP may not be directly involved in homotypic ER fusion per se.** (A and B) Deletion of *YOP1* or *RTN1* does not affect the expression level of ZIP-Gluc1, ZIP-Gluc2, or Sey1p in isolated microsomes. Deletion of *YOP1* or *RTN1* was confirmed by genomic PCR. Protein profiles of the indicated microsomes were analyzed by immunoblotting using the indicated antibodies. (C) *yop1*Δ or *rtn1*Δ microsomes support ER fusion. Microsomes isolated from wild-type, *yop1*Δ, or *rtn1*Δ yeast cells were incubated on ice or at 27°C in the presence of ATP and/or GTP. After 90 min, luciferase activity was measured. Fusion values were normalized to those obtained in an ATP/GTP-driven reaction incubated at 27°C. Data represent the means ± SEM (error bars; $n = 3$). ***, $P < 0.001$, Tukey's test between ATP/GTP-driven and GTP-only-driven reactions at 27°C; #, $P < 0.05$, Tukey's test between samples incubated with ATP/GTP at 27°C.

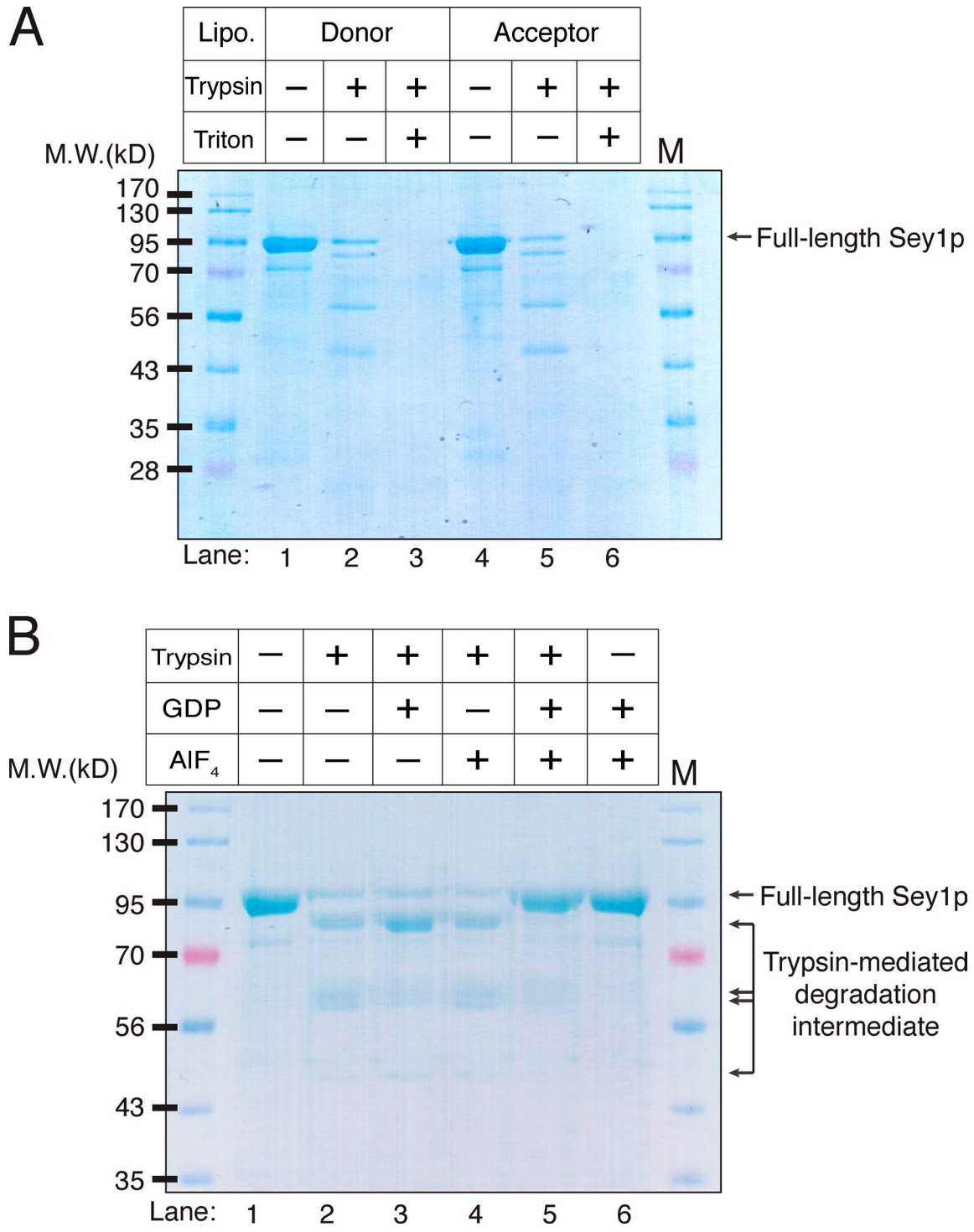

Figure S3. **Characterization of Sey1p reconstituted into preformed liposomes.** (A) A trypsin digestion assay revealed that >95% of Sey1p proteins were incorporated into preformed liposomes in the correct orientation. Sey1p proteoliposomes (donor or acceptor) were treated with trypsin (6 μg/ml) in the absence or presence of Triton X-100 for 30 min at 30°C, and the mixtures were separated by SDS-PAGE. After protein bands were visualized by Coomassie brilliant blue staining, the ratio of the intensities of the full-length Sey1p band before and after trypsin digestion was analyzed by densitometry. (B) The majority of Sey1p proteins incorporated into preformed liposomes became insensitive to trypsin in the presence of GDP and AlF_4. Sey1p proteoliposomes were incubated in the presence of 2 mM GDP and/or AlF_4 (2 mM $AlCl_3$ and 20 mM NaF) for 20 min at 30°C. The mixtures were then further incubated with or without trypsin (2 μg/ml) for 20 min at 30°C, and analyzed by SDS-PAGE and Coomassie brilliant blue staining. All experiments were performed multiple times with similar results, and the data shown are representative of all results.

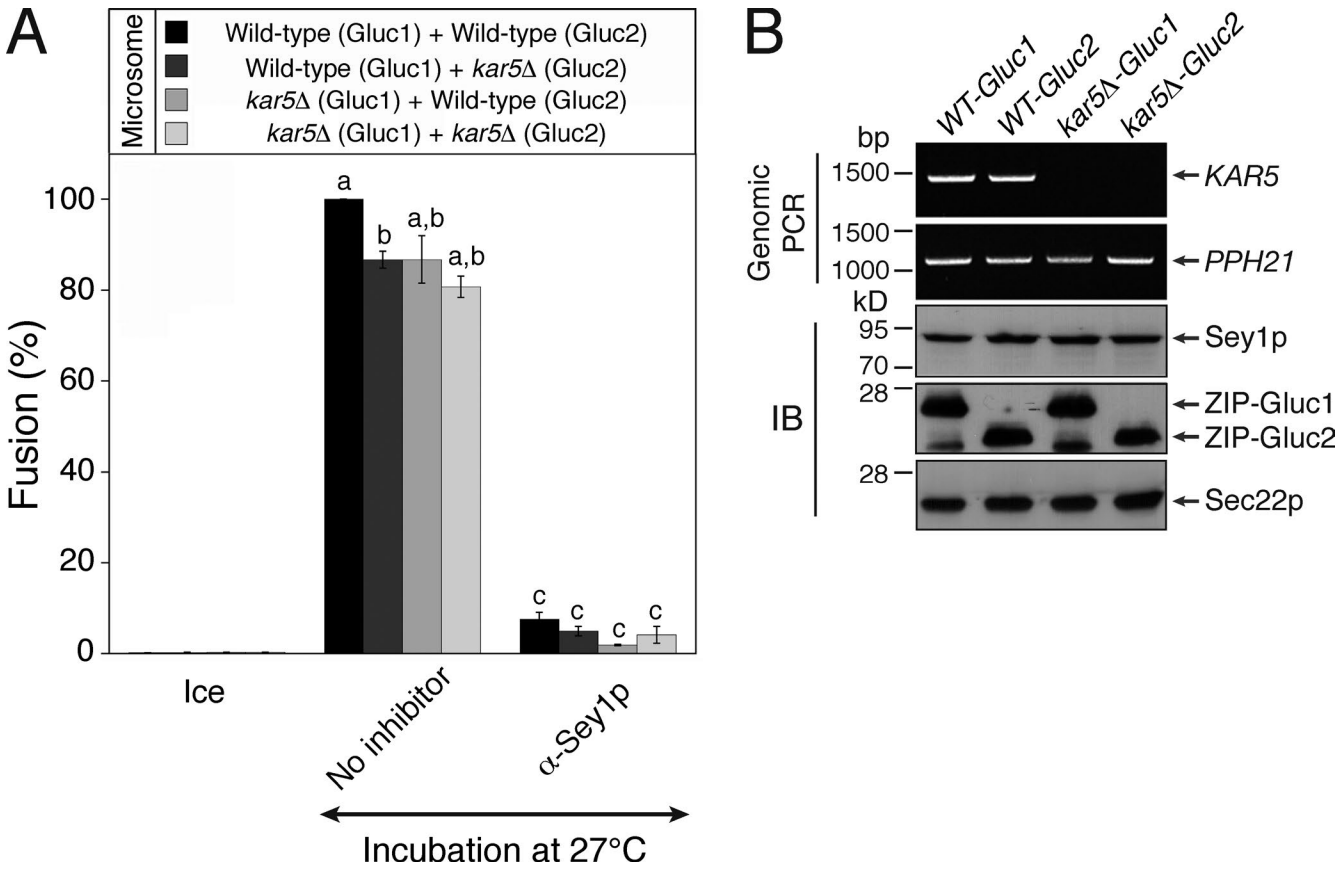

Figure S4. **Nuclear fusion contributes little to the signal of in vitro ER fusion reactions.** (A) Microsomes lacking Kar5p, which is essential for nuclear fusion, support ER fusion. Microsomes isolated from wild-type or *kar5*Δ yeast cells were incubated on ice or at 27°C. After 90 min, luciferase activity was measured. Fusion values were normalized to those obtained in the fusion reaction between wild-type microsomes incubated at 27°C. Data represent the means ± SEM (error bars; $n = 3$). Lowercase letters indicate statistically different groups. Tukey's test, $P < 0.001$. (B) Deletion of *KAR5* does not affect the expression level of ZIP-Gluc1, ZIP-Gluc2, Sec22p, or Sey1p in isolated microsomes. Deletion of KAR5 was confirmed by genomic PCR. Protein profiles of the indicated microsomes were analyzed by immunoblotting using the indicated antibodies.

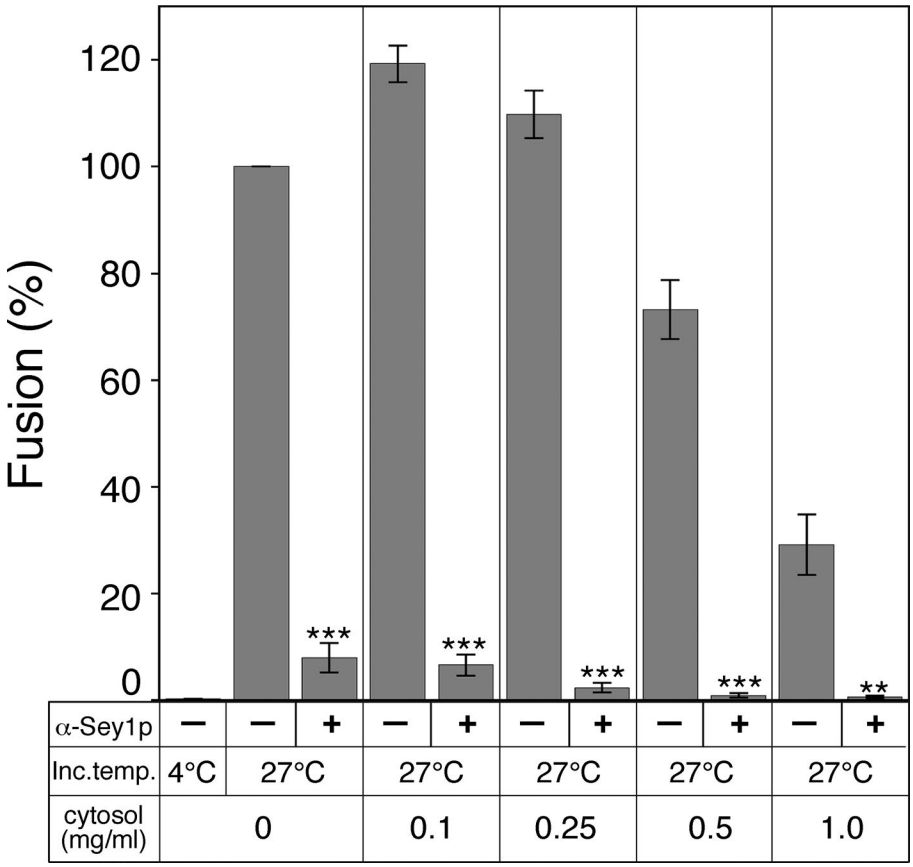

Figure S5. **The Sey1p dependency of ER fusion was unaffected by the addition of varying concentrations of yeast cytosol.** Gluc1 microsomes and Gluc2 microsomes were mixed and incubated on ice or at 27°C in the presence of increasing concentrations of purified yeast cytosol. Some reactions received affinity-purified anti-Sey1p antibodies. Fusion values were normalized to those obtained using an ATP/GTP-driven reaction incubated without anti-Sey1p antibodies at 27°C. Data represent the means ± SEM (error bars; $n = 3$). **, $P < 0.01$; ***, $P < 0.001$, Student's t test between reactions with and without antibodies.

Table S1. **Plasmids used in this study**

Plasmid	Reference
pYJ406-ssZIP-GLuc1-HDEL (*URA3* integrative plasmid, *ADH1* promoter for ssZIP-GLuc1-HDEL expression)	This study
pYJ406-ssZIP-GLuc2-HDEL (*URA3* integrative plasmid, *ADH1* promoter for ssZIP-GLuc2-HDEL expression)	This study
pRS408-pTDH3-SEY1 (*NatMX* integrative plasmid, *TDH3* promoter for Sey1p expression)	This study
pRS408-SEY1-K50A (*NatMX* integrative plasmid, *SEY1* promoter for Sey1p-K50A expression)	This study
pYJ406-ssEGFP-HDEL (*URA3* integrative plasmid, *ADH1* promoter for ssEGFP-HDEL expression)	This study
pGST-SEY1-CD (*AmpR ori* plasmid, tac promoter for GST-Sey1p-CD [1–681 aa] expression in *E. coli*)	This study
pHIS-SEC22-CD (*AmpR ori* plasmid, T7 promoter for His_6-Sec22p-CD [1–188 aa] expression in *E. coli*)	This study
pMBP-UFE1-CD (*AmpR ori* plasmid, tac promoter for MBP-Ufe1p-CD [1–326 aa] expression in *E. coli*)	This study
pHIS-GDI1 (*AmpR ori* plasmid, T7 promoter for His_6-Gdi1p expression in *E. coli*)	This study
pHIS-GYP1-46 (*AmpR ori* plasmid, T7 promoter for His_6-Gyp1-46p [248–647 aa] expression in *E. coli*)	This study
pGST-ZIP (*AmpR ori* plasmid, tac promoter for GST-ZIP expression in *E. coli*)	This study