Nyathi and Pool, http://www.jcb.org/cgi/content/full/jcb.201410086/DC1

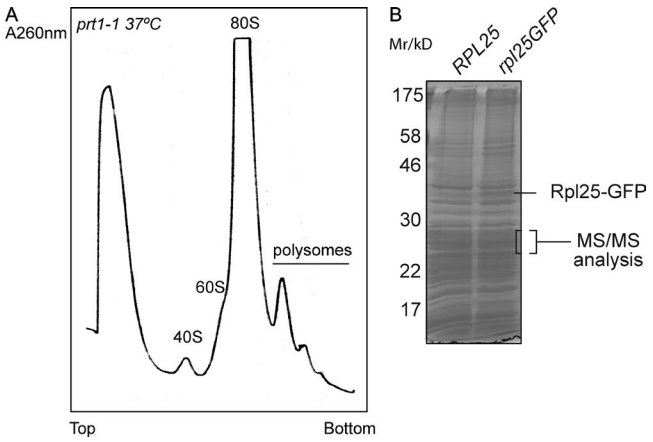

Figure S1. **Analysis of ribosomes from *prt1-1* and *rpl25GFP* strains.** (A) 5 OD_{260nm} units of precleared cellular lysate extracted from cycloheximide-treated *prt1-1* (YMK135) cells shifted to 37°C for 20 min were applied to the top of a 13 ml 10–55% sucrose gradient to separate polysomes. (B) Ribosome-enriched pellets isolated from WT (BY4741) strain (RPL25) and *rpl25GFP* were analyzed by SDS-PAGE and staining with Coomassie Brilliant Blue and bands in 25–27-kD range were excised for mass spectrometry.

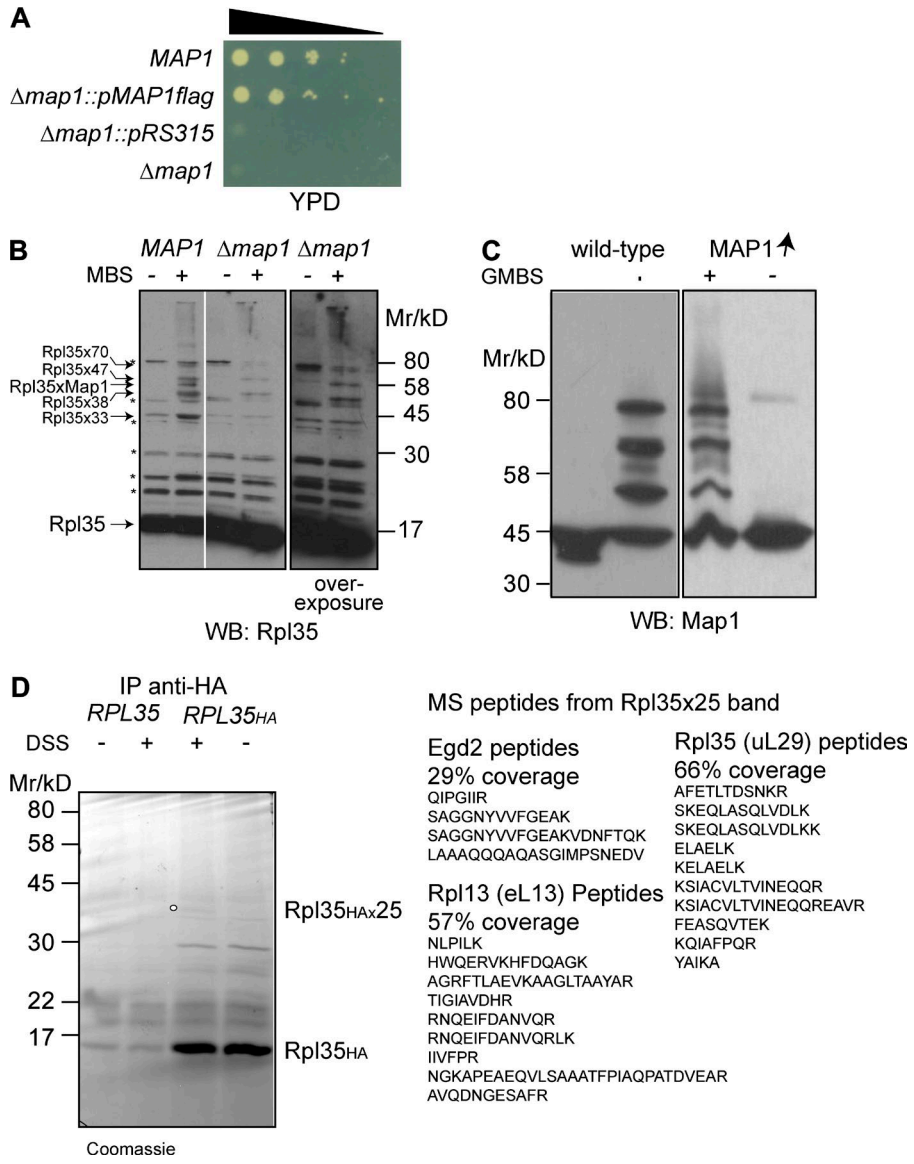

Figure S2. **Map1 and Edg2 are both adjacent to Rpl35 when bound to the ribosome.** (A) WT (BY4741), Δmap1::MAP1FLAG (GFY9::pMP289) strain, Δmap1:: pRS315 (GFY9::pRS315), and Δmap1 (GFY9) strains were grown to an OD of 1. 10-fold serial dilution of each culture were spotted on YPD plates and grown at 30°C for 2 d. (B) A ribosome-enriched pellet from either WT (BY4741) or a *Δmap1* (GFY9) strain was treated where indicated with the cross-linker MBS (250 μM). Samples were analyzed by SDS-PAGE and blotted for Rpl35. Nonspecific cross-reacting bands are indicated by the asterisks. (C) A ribosome-enriched pellet from either WT or strain overexpressing Map1 was treated where indicated with the cross-linker GMBS (250 μM). Samples were analyzed by SDS-PAGE and blotting for Map1. (D) Ribosome-enriched pellets from either WT or a RPL35HA strain were treated with DSS, before denaturing immunoprecipitation with anti-HA resin. The eluted fractions were analyzed by SDS-PAGE and stained with Coomassie brilliant blue. The 38-kD Rpl35HAx25 cross-link adduct was analyzed by tandem mass spectrometry. Identified peptides are shown and indicate a mixture of cross-links between Rpl35HA and two proteins, Rpl13 and Egd2. Rpl13 (eL13) is known to be adjacent to the C terminus of Rpl35 in the 60S ribosome.

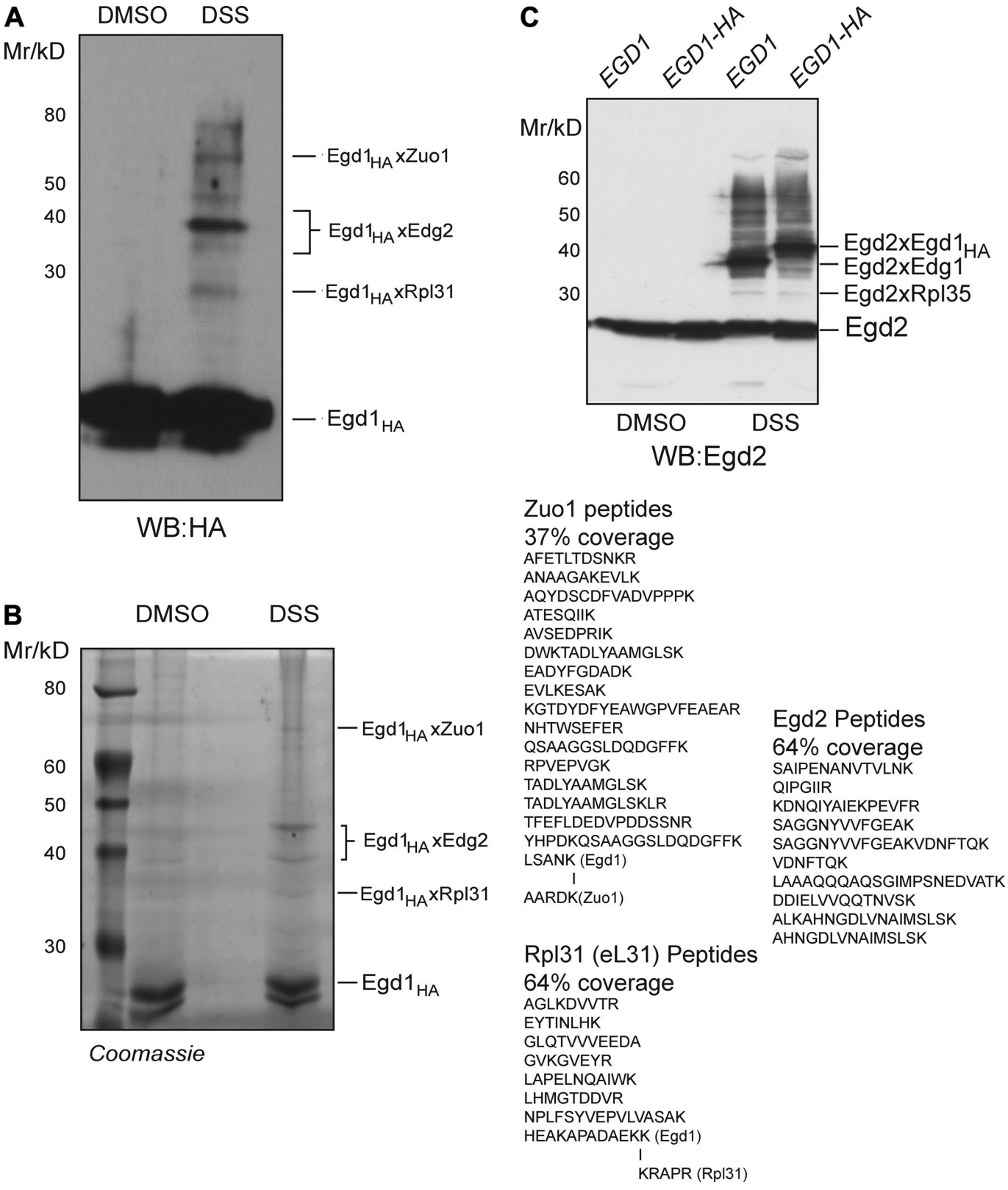

Figure S3. **Cross-link analysis of ribosome-associated Egd1 and Egd2.** A ribosome-enriched pellet from *EGD1-HA* (*MY3612*) strain was treated where indicated with the cross-linker DSS (500 µM). (A) Reactions were analyzed by SDS-PAGE and blotting with anti-HA antibody. (B) After cross-linking samples were denatured and immuno-precipitated with anti-HA resin. Reactions were analyzed by SDS-PAGE and Coomassie staining. The labeled cross-link bands were excised for mass spectrometry analysis. Identified peptides are indicated. (C) Reactions were analyzed by SDS-PAGE and Western blotting with Egd2 antibody.

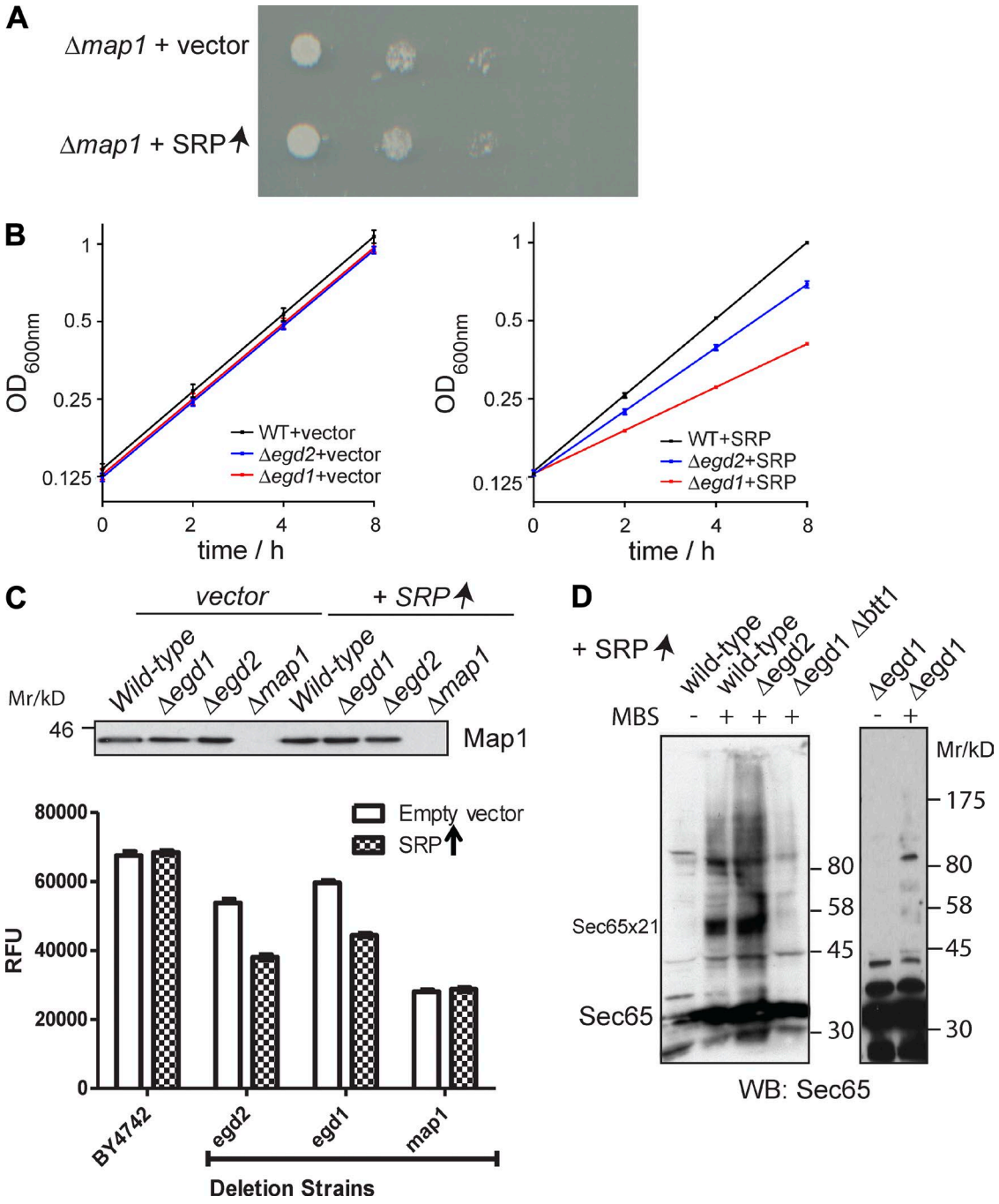

Figure S4. **Overexpression of SRP in *egd1* and *edg2* mutant strains retards growth and reduces MetAP activity.** (A) 10-fold serial dilutions of *Δmap1* (*GFY9*) strain transformed with empty vector or SRP overexpression plasmids (pMW299 and pMW295) were spotted on –URA/LEU plates and grown at 30°C for 5 d. (B) Growth curves of WT (BY4742), *Δegd1*, and *Δegd2* strains transformed with SRP overexpression plasmids (+SRP) or empty vector in liquid minimal media at 30°C. Data are mean with standard error (n = 3). (C) MetAP activity in total lysates from the indicated strains was determined using the fluorogenic substrate Met-AMC as described in Materials and methods. (D) A ribosome-enriched pellet from WT, *Δegd2*, *Δegd1*, or *Δegd1Δbtt1* (YNY2) transformed with SRP-overexpressing plasmids (pMW299 and pMW295) was treated where indicated with the cross-linker MBS (250 μM). Samples were analyzed by SDS-PAGE and blotting with Sec65 antibody.

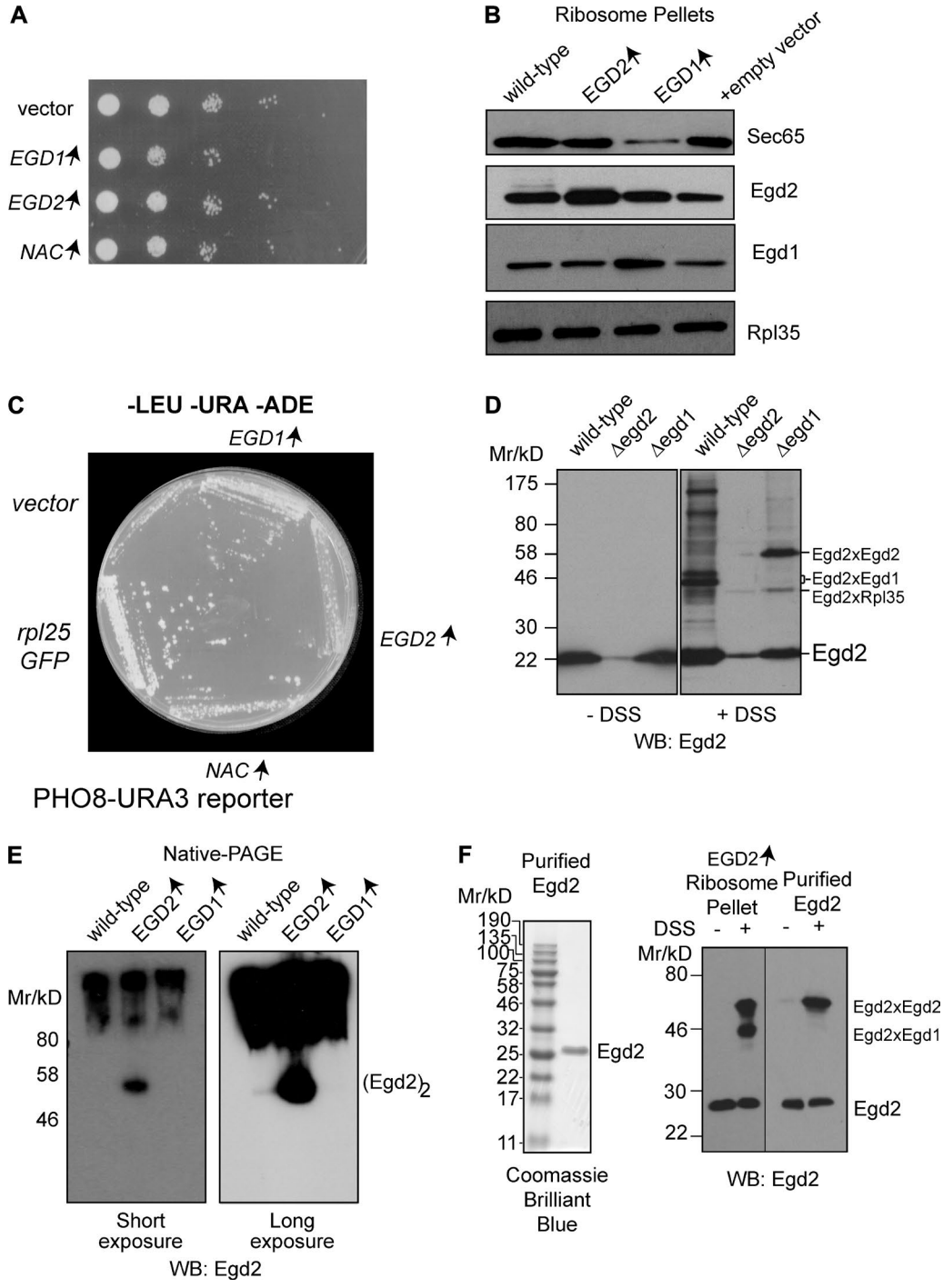

Figure S5. **Overexpression of Egd1 and Egd2 leads to distinct SRP-dependent translocation phenotypes.** (A) 10-fold serial dilutions of WT strain (BY4741) transformed with empty vector (pRS422), *EGD1* (pMP300), *EGD2* (pMP302), or NAC overexpressing plasmids (pMP304) were spotted on –URA/LEU plates. Plates were grown at 30°C for 3 d. (B) Ribosome-enriched pellets from untransformed WT cells or cells transformed with plasmids overexpressing Egd2 (pMP302), Egd1 (pMP300), or empty vector (pRS422) were analyzed by SDS-PAGE and Western blotting with antibodies to Sec65, Egd2, Egd1 and Rpl35. (C) WT cells overexpressing Egd1 (pMP300), Egd2 (pMP302), or NAC (pMP304) were transformed with a *PHO8-URA3* reporter plasmid (*LEU2/CEN*) and then streaked onto SD–leu–ade (selects for plasmid) or SD–leu–ura–ade media (selects for translocation defect) and grown for 5 d at 30°C. WT Strain transformed with empty vector and the Rpl25GFP strains were used as controls. (D) Ribosome-enriched pellets from WT (BY4741), *Δegd1*, and *Δegd2* were treated with DMSO (–DSS) or DSS cross-linking reagent and analyzed by SDS-PAGE and blotting with anti-Egd2 antibody. (E) Cellular extracts from WT cells overexpressing Egd1 (pMP300), Egd2 (pMP302), or empty vector were analyzed on nondenaturing PAGE and blotting with anti-Egd2 antibody. (F) Egd2 was expressed in *E. coli*, purified, and analyzed by SDS-PAGE and staining with Coomassie Brilliant Blue (left). Purified Egd2 and a ribosome pellet from WT yeast cells overexpressing Egd2 were treated with DSS where indicated and analyzed as in D (right).

Table S1. **Yeast strains used in this study**

Strain	Genotype	Reference or source
W303	*MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*	Thomas and Rothstein, 1989
BY4741	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	Euroscarf
Δbtt1	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0* YDR252W*::kanMX4*	Euroscarf
Δegd1	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 YPL037C::kanMX4*	Euroscarf
Δegd2	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 YHR193C::kanMX4*	Euroscarf
Δnac	*MATa; met15 ura3 egd1::HIS3 egd2::LEU2 btt1::ble*	Koplin et al., 2010
ΔssbΔnac	*MATa met15Δ his3Δ ura3Δ leu2Δ ssb1::kanMX4 ssb2::nat1 egd1::HIS3 egd2::LEU2 btt1::ble (BY4741 background)*	Koplin et al., 2010
CSY128	*MATα, leu2-3,-112 ade2 trp1-1 ura3-52 his3-11 sec65-1 (Pro152>Leu)*[a]	Stirling and Hewitt, 1992
CSY221	*MATa leu2 ura3 his4 sec62-1 (D46>G)*[b]	Rothblatt et al., 1989
GFY9	*MATa map1::HIS3MX6 his3Δ1 leu2Δ0 ura3Δ0*	Forte et al., 2011
MPY69	*MATa leu2 his3 trp1 ura3 ade2 rpl25::HIS3 [pYCplac111-RPL25(pMP226)]*	Dalley et al., 2008
MY3612	*MATa ura3-52 trp1 leu2::PET56 gcn4 EGD1-HA3::KanMX4*	Panasenko et al., 2006
RBY175	*MATa his3Δ leu2Δ ura3Δ rpl35A::KanMX4 rpl35B::KanMX4 [pAS24-RPL35A]*	Babiano and de la Cruz, 2010
RPL25GFP	*MATa leu2 his3 trp1 ura3 ade2 rpl25::HIS3 [pYEplac112-rpl25GFP]*	Hurt et al., 1999
RS453	*MATa leu2 his3 trp1 ura3 ade2 his3*	Segref et al., 1997
YMK135	*MATa ade2-1 HIS3 leu2-1, 11 trp1-1 ura3-1 can1-100 prt1-1 (S518>F)*[c]	Hartwell and McLaughlin, 1968; Campbell et al., 2005
YNY1	*MATa his3 leu2 ura3 ade2 sec65-1Δegd2*	This study
YNY2	*MATα his3 leu2Δ0, ura3Δ52 Δegd1Δbtt1*	This study
YNY3	*MATa leu2 his3 trp1 ura3 ade2 rpl25::HIS3 pYEplac112-rpl25GFP: MAP2-HA3::HphNT1*	This study
YNY4	*MATa map1::HIS3MX6 his3Δ1 leu2Δ0 ura3Δ0 MAP2-HA3::HphNT1*	This study
YNY5	*MATa leu2 his3 trp1 ura3 ade2 his3 MAP2-HA3::HphNT1*	This study

[a]Ogg and Walter, 1995.
[b]Wittke et al., 2000.
[c]Evans et al., 1995.

Table S2. **Primers used in the study**

Primer name	Sequence
MAP1F	5′-AATTGGGGGATCCAATTGTATAATGAGCACTGCAACTACAACAGTTAC-3′
MAP1R	5′-CCCAATTGAATTCCTATTTAATTCTCTGTCTTGGGCCACCTG-3′
MAP1 FLAGF	5′-CCCGTCGACGGGAGAACTGCTGCCCATGGC-3′
MAP1 FLAGR	5′-GGGCTGCAGCTACTTGTCGTCGTCGTCCTTGTAGTCTTTAATTCTCTGTCTTGGGCCACC-3′
MAP2 taggingF	5′-CTTGTTGCATGCTCACAAAAAGGAAGTCGTTTCGAAAGGTGATGACTACCGTACGCTGCAGGTCGAC-3′
MAP2 taggingR	5′-GTATTCATATACCTAGTGAGGAGGCCATTTGAAAGCGCATTTTACCATCGATGAATTCGAGCTCG-3′
MAP2 HA_F	5′-AATTGGGACTAGTATGACAGACGCTGAAATAGAAAATTCC-3′
MAP2 HA_R	5′-CCCAATTGAATTCTCAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTAGTAGT CATCACCTTTCGAAACG-3′
EGD2F	5′-AATTGGGGTCGACCCGTCCGAATTTACATATATATGCC-3′
EGD2R	5′-CCCAATTCTGCAGGATTTTGCGTTAGAATAACTACGTACCC-3′
EGD1F	5′-AATTGGGCTGCAGGGCTGCTCTTCCTCTTTTTCGCATATTC-3′
EGD1R	5′-CCCAATTGCGGCCGCGTTTGCCAGGAAGGATGCTCTAAAAAGG-3′
EGD2 pET28F	5′-AAT GGGCATATGATGTCTGCTATCCCAGAAAACG-3′
EGD2 pET28R	5′-CCCAATTGGATCCTTATTTAGACAAGGACATGATAGCG-3′

Table S3. **Plasmids used in the study**

Plasmid	Description	Reference or source
pMP226	*RPL25 cloned into pYCplac111 (CEN, LEU2, RPL25 promoter)*	Dalley et al., 2008
pMP234	*PHO8-URA3* cloned into pRS315 *(CEN, LEU2, PHO5 promoter)*	Dalley et al., 2008
pMP289	$MAP1_{FLAG}$ cloned into pRS415 *(CEN, LEU2, MAP1 promoter)*	This study
pMP299	*MAP1 cloned into* pRS414-*GPD (CEN, TRP1, GPD promoter)*	This study
pMP300	*EGD1 cloned into* pRS422 *(2μ, ADE2, EGD1 promoter)*	This study
pMP301	*EGD2 cloned into* pRS412 *(CEN, ADE2, EGD2 promoter)*	This study
pMP302	*EGD2 cloned into* pRS422 *(2μ, ADE2, EGD2 promoter)*	This study
pMP303	*EGD1 and EGD2* cloned into pRS412 *(CEN, ADE2, EGD1 and EGD2 promoter)*	This study
pMP304	*EGD2 and EGD1 cloned into* pRS422 *(2μ, ADE2, EGD1 and EGD2 promoter)*	This study
pMP305	*MAP2-HA3 cloned into* pRS414-*GPD (CEN, TRP1, GPD promoter)*	This study
pMP306	pET28-*EGD2 (T7 promotor, Kan^r) Bacteria expression vector*	This study
pMR12	YEp351-*CPY-URA3 (2μ, LEU2, PRC1 promoter)*	Dalley et al., 2008
pMW295	*SRP21, SRP72 and SEC65 cloned into* YEp24 *(2μ, LEU2, endogenous promoters)*	Willer et al., 2003
pMW299	*SRP14, SRP54, SRP68, and SCR1 cloned into* YEp13 *(2μ, URA3, endogenous promoters)*	Willer et al., 2003

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