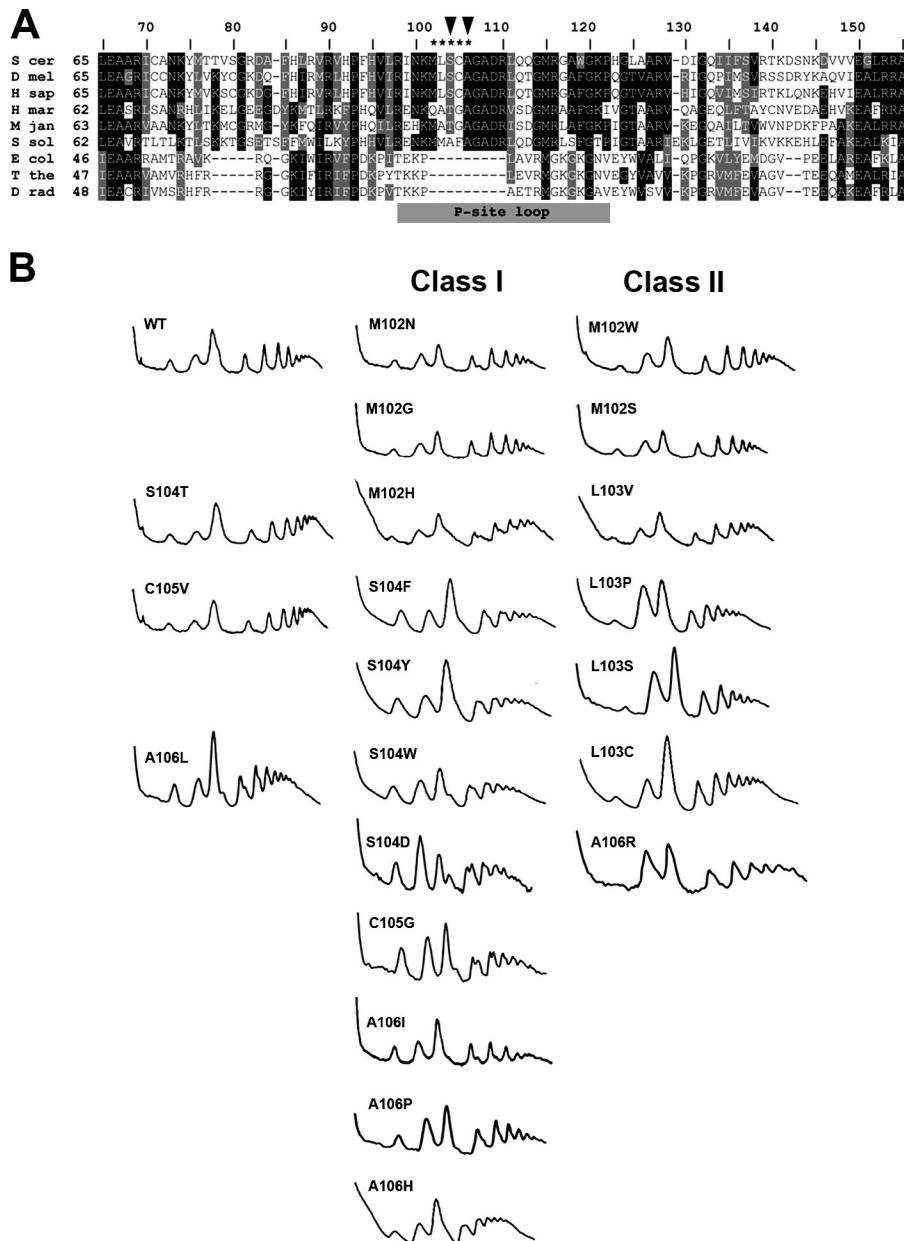
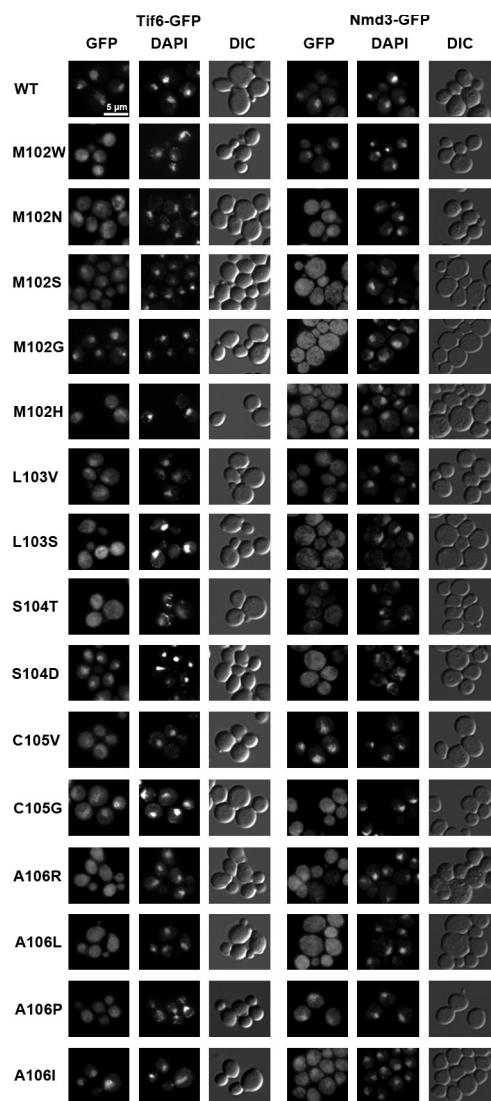
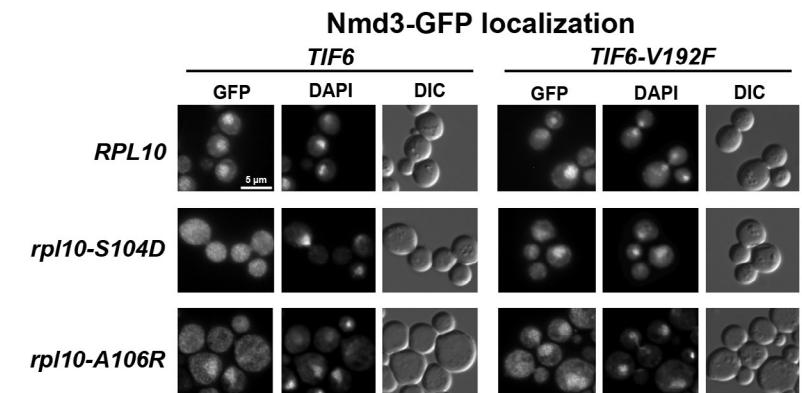
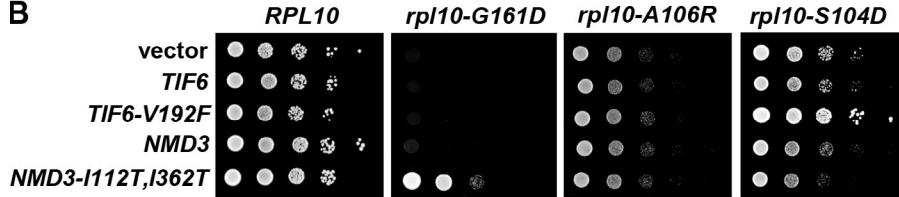
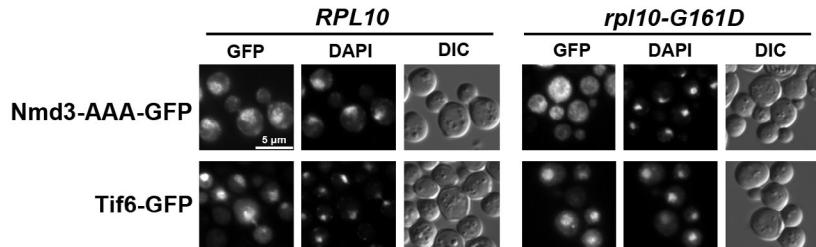


Bussiere et al., <http://www.jcb.org/cgi/content/full/jcb.201112131/DC1>

**Figure S1. Mutation of the P-site loop of Rpl10 yields two classes of mutants.** (A) Multiple sequence alignment encompassing of the P-site loop in Rpl10. Sequences were aligned using T-Coffee, and the image was generated with BoxShade 3.21. aa positions are indicated by numbers. Residues of Rpl10 that were mutated are indicated by asterisks. Arrowheads identify positions S104 and A106 in the yeast protein. Black shading indicates identical residues; gray shading indicates similar residues. S cer, *Saccharomyces cerevisiae*; D mel, *Drosophila melanogaster*; H sap, *Homo sapiens*; H mar, *Halorhacula marismortui*; M jan, *Methanococcus jannaschii*; S sol, *Sulfolobus solfataricus*; E col, *Escherichia coli*; T the, *Thermus thermophilus*; D rad, *Deinococcus radiodurans*. (B) AJY1437 containing WT (pAJ2522) or P-site loop mutant Rpl10 was grown in yeast extract peptone dextrose to mid-log, incubated with cycloheximide, and collected on ice. Crude extracts were made and sedimented through 7–47% sucrose gradients, and  $A_{260}$  was monitored along the gradients.



**Figure S2. The Rpl10 P-site loop mutants trap Tif6 and Nmd3 in the cytoplasm to various degrees.** AJY2765 (*TIF6-GFP rpl10Δ::KanMX*) and AJY1837 (*NMD3-GFP CRM1-T539C rpl10Δ::KanMX*) containing WT (pAJ2522). The indicated *rpl10* P-site loop mutants were grown to mid-log phase, fixed with formaldehyde, DAPI stained, and visualized by microscopy. AJY1837 was incubated with 0.4 pg/ml LMB for 5 min before fixing. DIC, differential interference contrast. Bar, 5  $\mu$ m.

**A****B****C**

**Figure S3. Rpl10 is independently involved in release of Tif6 and Nmd3 from the LSU.** (A) AJY1837 (*NMD3-GFP CRM1-T539C rpl10Δ::KanMX*) containing WT (pAJ2522) or mutant *RPL10* and WT (pAJ2543) or mutant *TIF6* (pAJ2544) was grown in selective media to mid-log phase and treated with LMB before fixation and preparation for microscopy. DIC, differential interference contrast. (B) AJY1657 (*rpl10-G161D*) and AJY1437 with WT (pAJ2522) or mutant Rpl10 were transformed with vector (pRS416), WT (pAJ2665), or mutant (pAJ2240) *TIF6* or WT (pAJ2652) or mutant (pAJ2653) *NMD3*. The strains were grown in selective media, and 10-fold serial dilutions were spotted on plates and incubated for 2 d at 30°C. (C) W303 or AJY1657 (*rpl10-G161D*) was transformed with pAJ758 (*nmd3AAA-GFP*) or pAJ1004 (*Tif6-GFP*). The cells were grown to mid-log phase, fixed with formaldehyde, and stained with DAPI before microscopy. Bars, 5 μm.

*rpl10-S104D*

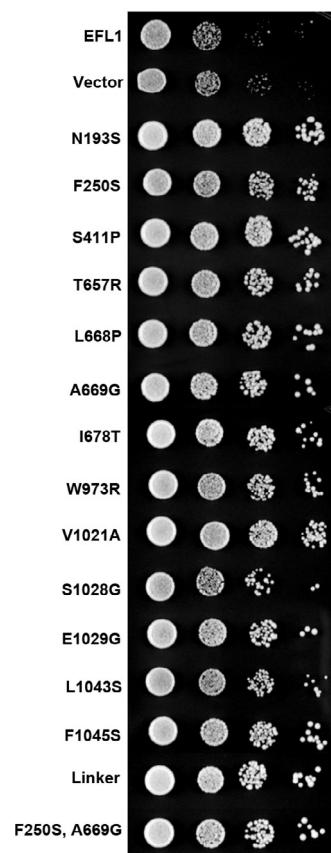
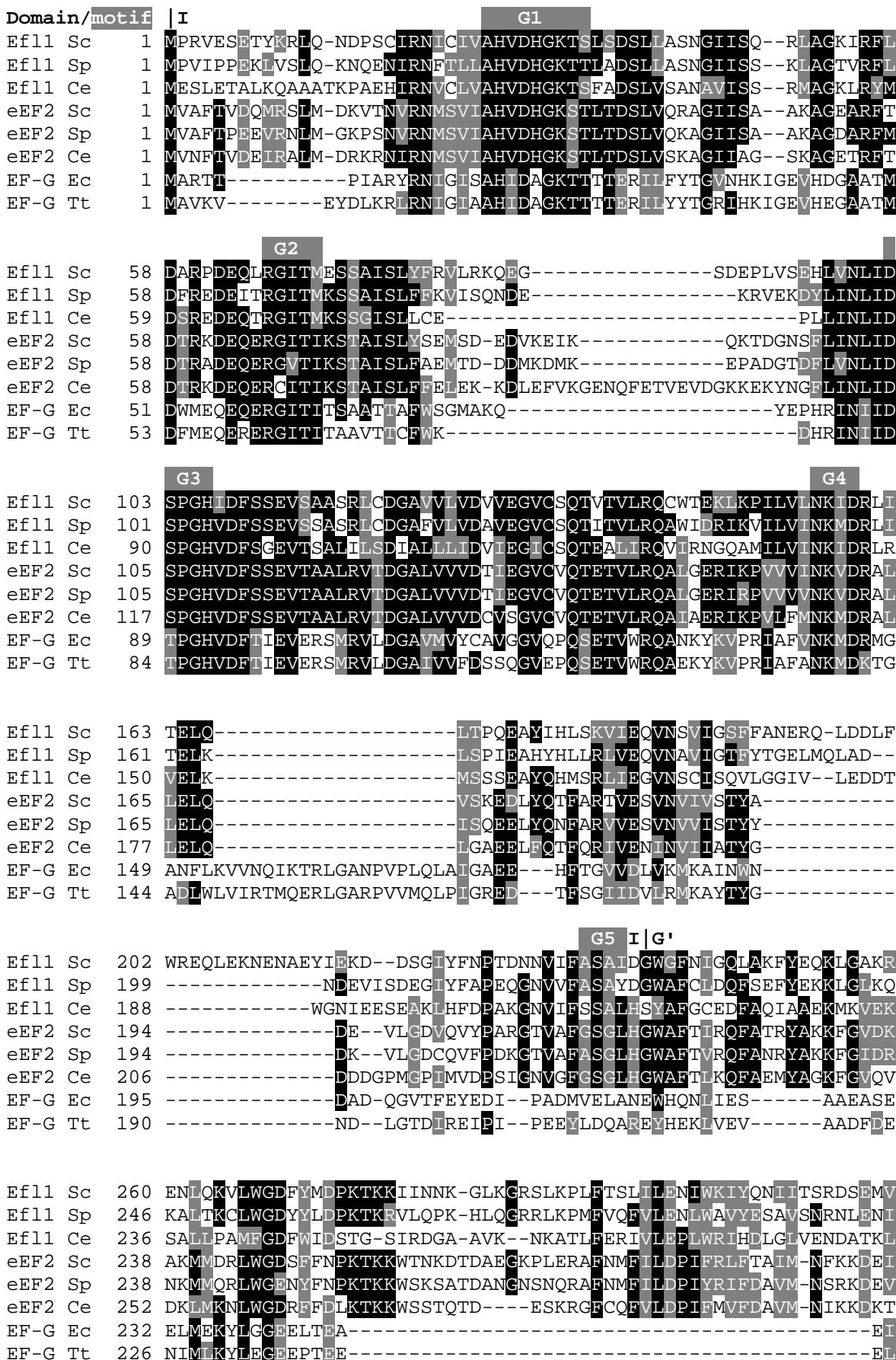


Figure S4. **Mutations in *EFL1* suppress *rpl10-S104D*.** AJY1437 (*rpl10Δ::KanMX*) with *rpl10-S104D* as the sole copy of *RPL10* with WT *EFL1* (pAJ2545) or *EFL1*-suppressing alleles was grown in selective media. 10-fold serial dilutions were spotted on plates and grown for 2 d at 30°C.

## Multiple sequence alignment of Efl1 proteins with eEF2 and EF-G



G' | I                    II

Ef11	Sc	319	EKIAKTLNIKLLARDLRSKDDKQLLRTIMQOWL-PVSTAVLLTIEKLPSPLE--SQTDR		
Ef11	Sp	305	EKLIKALNIKVLPRLDIKSQDPRNLLAIFQQWL-PLSTAAILTAIREIPSPIN--AQANR		
Ef11	Ce	292	AEEAAKKIGINL----KSRRANEAFDELMRWTWLPKASFRAVAR-APNVRSTFDTQHR		
eEF2	Sc	297	PVLLEKLEIVLK-GDEKDLEGKALLKVMVRKFL-PAADALLEMIVLHLPSPVT--AQAYR		
eEF2	Sp	297	FTLLSKLEVTIK-PDEKELEGKALLKVMVRKFL-PAADALMEMIVLHLPSPKT--AQYR		
eEF2	Ce	307	AALVEKIGIKLA-NDEKDLEGKPLMKVFMRKWL-PAGDTMLQMIAFHLPSPVT--AQKYR		
EF-G	Ec	249	KGAL-----RQRVLNNEIILVTCGSAFKNKGVQAMLDAVIDYLPSPVD--VPAIN		
EF-G	Tt	243	VAAI-----RKGTIDLKITPVFTGSALKNKGVQLLLDAVVVDYLPSPLD--IPPIK		
Ef11	Sc	376	LNTILVSESDTAAMDPRLLKAMKTCDK-----EGPVSAVSKMLSIIPREELPVESKRIAS		
Ef11	Sp	362	ARKVLSSTPHYEMIDPDITLAMESCDA---SKEQPVLVYISKMVAEFSERDLPNHRKQLS		
Ef11	Ce	345	LDHLTGHR----IDHPLRKFVLCNP-----EAMTLLVFWVKLLQTEDKNL-----		
eEF2	Sc	353	AEOLYEGP----ADDANCTAIKNCDP-----KADLMLYVSKMVPPTSDK-----		
eEF2	Sp	353	AETLYEGP----MDDECAGVIRNCDA----NAPLMLYVSKMVPPTSDK-----		
eEF2	Ce	363	MEMLYEGP----HDDEAAVAIKTCDP-----NGPLMMYISKMVPPTSDK-----		
EF-G	Ec	297	GIL-----DDGK-DT---PAERHASD-----DEPFPSALAFKIATDPFV-----		
EF-G	Tt	291	GTT-----PEGE-----VVEIHPDP-----NGPLAALAFKIMADPYV-----		
Ef11	Sc	431	SDELMERSRKAREALNAAK-HAGMVENMAMMDLNDNSKNTSDLYKRAKDTVMTPEVGEQ		
Ef11	Sp	419	AEE-MKLIRSKLSESIESGINTISIEENVSST-----		
Ef11	Ce	386	-----		
eEF2	Sc	392	-----		
eEF2	Sp	392	-----		
eEF2	Ce	402	-----		
EF-G	Ec	331	-----		
EF-G	Tt	323	-----		
Ef11	Sc	490	TKPKPSRNNDVFCVVSEPPSSALDLEFYEGEREDDSDSQDNFGLDFVPTIDPNPDLSSMFE		
Ef11	Sp	450	-----		
Ef11	Ce	386	-----		
eEF2	Sc	392	-----		
eEF2	Sp	392	-----		
eEF2	Ce	402	-----		
EF-G	Ec	331	-----		
EF-G	Tt	323	-----		
Ef11	Sc	550	YEEEDPLLESIKQISEDVNDEVDDIFDEKEECLVAFARIYSGTLRVGQEISVLGPKYDPK		
Ef11	Sp	450	--NSDNLEGSTT-----D-MDDDKDILIGFARIYSGTISVGQEVYVYGPKYDPV		
Ef11	Ce	386	-----TSRRAIICRVLSGTLRKGDTLYVLQQQN---		
eEF2	Sc	392	-----GRFYAFGRVFAGTVKSGQKVRIQGPNYVPG		
eEF2	Sp	392	-----GRFYAFGRVFSGTVRSCLKVRIQGPNYVPG		
eEF2	Ce	402	-----GRFYAFGRVFSGKVATGMKARIQGPNYVPG		
EF-G	Ec	331	-----GN-LTFFFRVYSGVNVNSGDTVLN----SVKA		
EF-G	Tt	323	-----GR-LTFIRVYSGTLTSGSYVYN----TTKG		
Ef11	Sc	610	CPEEHIIETAIITHIYLFMGKELVPLDVCPSGNIVGIRGLAGKVLKSGTLIEKGVQGV-NL		
Ef11	Sp	496	NPEKHITKVTVESIYLMMGQELVYLETVPAGNVFAIGGLAGTVLRTATLCCSSPN-GP-NL		
Ef11	Ce	413	NSSE-VTSTKIDRISILRGRDSIPTDTVTSGMICTID-AEILLQNTTCEKPD-FP-CL		
eEF2	Sc	422	KKDDL-FIKAIQRVVLMMGRFVEPIIDCPAGNIIGLVGIDQFLKTGTLTTSET-AH-NM		
eEF2	Sp	422	KKDDL-FIKAIQRVVLMMGSRIEPIEDCPAGNIIGLVGVDQFLVKSGTLLTSEV-AH-NM		
eEF2	Ce	432	KKEDL-YEKTIQRTILMMGRFIEPIEDIPSGNIAGLVGVDQYLVVKGGTITTYKD-AH-NM		
EF-G	Ec	356	AR-----ERFGRIVQMANKREEIKEVRAGDIAAAIGLKDV-TTGDTLCDP-DAPI-IL		
EF-G	Tt	348	RK-----ERVARLLRMHANHREEVEELKAGDLAGVVGKET-ITGDTLVGE-DAPRVL		

**II | III**

Ef11 Sc	669	AGVNFEHTPIVRVAVEPANPVEMSKLVRGLKLLDQADPCVHTYV-ENTGEHILCTAGELH
Ef11 Sp	554	VGVTQQMFPPIVRVALEPVPRFEMNKLVTLGDMLNQADPCVQIAV-EENGHEVIMCAGEIH
Ef11 Ce	468	KIGSQTGEALRVRSISTQCLDDMDDLREKLKLLALLDTSLKVME-LENGEELAMVIAGEVH
eEF2 Sc	479	KVMKFSVSPVVQVAVEVKNANDLPKLVEGLKRLSKSDPCVLTYM-SESGEHIVAGTGEELH
eEF2 Sp	479	KVMKFSVSPVVQVAVEVKNGNNDLPLKLVEGLKRLSKSDPCVLCCTT-SESGEHIVAGAGELH
eEF2 Ce	489	RVMKFSVSPVVVRVAVEAKNPADLPKLVEGLKRLAKSDPMQCIF-EESGEHIIAGAGELH
EF-G Ec	407	ERME-FPEPVISIAVEPKTKADQEKMGLALGRIAKEDPSFRVWTDEESNQTIAGMGEHLH
EF-G Tt	400	ESIE-VPEPVIDVATEPKTKADQEKLSQLARLAEDPTFRVSTHPETGQTIIISGMGEHLH

**III | IV**

Ef11 Sc	728	LERCLKDLTERFAGIEITHSEPAIPYRETFLSASD-MNPPQON-SQL-GRGVHELLLSQLYK
Ef11 Sp	613	LERCLKDLRERFAKIEIQASQPLVPYRETTIATPD-LLAKNK-ELSIGFVTATLPVGGVT
Ef11 Ce	527	LQKCIKDLN-DLGIVDLDVSEPIVPEMETVIEDSV-LSAPQIIEQETECRI---REALY
eEF2 Sc	538	LEICLQDLEHDHAGVPLKISPPVVAYRETVESESS-QTALSKS-P---NKH-----NR
eEF2 Sp	538	LEICLKDLOEDHAGIPLKISPPVVSYRESVSEPPS-MTALSKS-P---NKH-----NR
eEF2 Ce	548	LEICLKDLEEDHACIPLKKSDPVVSYRETVCQSESN-QICLSKS-P---NKH-----NR
EF-G Ec	466	LDIIVDRMKRE-FNVEANVGPQVAYRETIRQKVTDVEGKHA-KQSGGRGQY-----GH
EF-G Tt	459	LEIIVDRMKRE-FKVDANVGPQVAYRETIITKPVD-VEGKFI-RQTGGRGQY-----GH

Ef11 Sc	785	ITFRTFPLSGKVTDLFSQHQ-----NSIKNILKTSTSSMDPVIESTGSSFLDK-K
Ef11 Sp	671	IGITVTPLSGSVVDFLLKHSKTIENVSSNFSKKNRNVVV---SESLTKSMEEVLTPE
Ef11 Ce	581	IKLRVVPGLGDAVVELLDKNSSLISSIRRG-----EAD-----QTEI-D
eEF2 Sc	586	IYLKAEPIDEEV-----SLAIENGIINPRD
eEF2 Sp	586	IFMTAEPMPSEEL-----SVAIETGHVNPRD
eEF2 Ce	596	IHCATAQPMPDGL-----ADDIEGGTVNARD
EF-G Ec	518	VVIDMYPLEPGS-----
EF-G Tt	510	VKIKVEPLPRGS-----

Ef11 Sc	834	SLLVAFEEVINQEE-----KSRELLS--GFKVKLAGFGPSRVCNCNILLSDQNL
Ef11 Sp	725	KFYERLS-KLLEEE-----NSDLGELKNHLDSTIAFGPKRVGPNILFDKTKKM
Ef11 Ce	618	EFQKRFT-SVCLTLPTLKGSWWFRKPKEVIE--SMIDQIWAFGPERARANILFNQVQNY
eEF2 Sc	611	DFKARAR-IMADDY-----GWD-----VTDARKIWCFGPDGNGPNLVIDQTKAV
eEF2 Sp	611	DFKVRAR-IMADEF-----GWD-----VTDARKIWCFGPDGTGANVVVDQTKAV
eEF2 Ce	621	EFKARAK-ILAKEY-----EYD-----VTEARKIWCFGPDGTGPNLMDVTKGV
EF-G Ec	530	-----NPKGY
EF-G Tt	522	-----GF

Ef11 Sc	881	GSLFE----GTPAAFYEYSDSIKNGFQLAVSEGPLANEPVQGMCVLVESVHKMSQEIESI
Ef11 Sp	772	RDFRRQSDETKLIPSDLSEYYVTAFLQLITHQGPLCAEPVQGICVSDQFDI-SDDSEDSK
Ef11 Ce	675	DR-DSVWRKTEFGVRRYDQALVAGFELFCNTGPLCNEIMHGIAVIVEWNV-DEED---
eEF2 Sc	654	QY-----IHEIKDSVVAAFWQATKEGPIFGEEMRSVRVNILDVTL-HADAI---
eEF2 Sp	654	AY-----IYEIKDSVVAAFWASKEGPMFEENLRSCKRFNILDVVL-HADAI---
eEF2 Ce	664	QY-----IYEIKDSVVAGFQWATREGVLSDENMRGVRFNVHDVTL-HADAI---
EF-G Ec	535	EFIGNDI-K-GGVIPGEYIPAVDKGIIQEQQLKAGPLAGYPVVDGMIRLHFGSY-HDVDS---
EF-G Tt	524	EFIGVNAI-V-GGVIPKEYIPAVQKGIEEAMQSGPLIGFPVVDIKVTLYDGSY-HEVDS---

**IV | V**

Ef11 Sc	937	EDPRYQQHIVDLSGRLITSTRDAIHEAFLDWSPRIMWATYSCDIQTSVDVLGKVYAVILQ
Ef11 Sp	831	L---LTINNPQIPGQVISVVKESIRHCFGLGSPRLMLAMYSCDVQATSEVLGRVYGVVSK
Ef11 Ce	729	-----GAIGGQMMTAIKASCASAAKKLALRLVAAMYRCTVTTASQALGKVHAVLSQ
eEF2 Sc	699	-----HRGGGQIIPTRRATYACFLADPKIQEPVFLIVEIQCPEQAVGGIYSVLNK
eEF2 Sp	699	-----HRGGGQIIPTRRPFYASVLTAEPRLLEPVLYIVEIQCPEAAVGGIYGVLNR
eEF2 Ce	709	-----HRGGGQIIPTRRPFYASVLTAEPRLLEPVLYIVEIQCPEAAVGGIYGVLNR
EF-G Ec	589	-----SELAFKL--AASIAFKECFKKAKPVLLEPIMKVEVETPEENTGDVIGDLSR
EF-G Tt	578	-----SEMAFKI--AGSMAIKEAVQKGDPVILEPIMRVEVTTPEYMGDVIGDLNA

Ef11	Sc	997	RHGKI-----I	<b>S E E M K E G T P F F Q I E A H V P V V E A F G L S E D I R K R T S G A A Q P Q L V</b>
Ef11	Sp	888	RRGRV-----I	<b>D E E M K E G T P F F I V K A L I P V V E S F G F A V E I L K R T S G A A Y P Q L I</b>
Ef11	Ce	780	RKSKVGMENCRTFSNIVLSEDINEATNL	<b>F E V V S L M P V V E S F S F C D Q L R K F T S G M A S A Q L Q</b>
eEF2	Sc	750	KRGQV-----V	<b>S E E Q R P G T P I F T V K A Y I L P V N E S F G F T G E L R Q A T G G Q A F P Q M V</b>
eEF2	Sp	750	KRGHV-----F	<b>S E E Q R V G T P L Y N I K A Y I L P V N E S F G F T G E L R Q A T A G Q A F P Q L V</b>
eEF2	Ce	760	RRGHV-----F	<b>E E S Q V T G T P M F V V K A Y I L P V N E S F G F T A D L R S N T G G Q A F P Q C V</b>
EF-G	Ec	638	RRGML-----K	<b>G Q E S E V T -- G V K I H A E V P L S E M F G Y A T Q L R S L T K G R A S Y T M E</b>
EF-G	Tt	627	RRGQI-----L	<b>G M E P R G N -- A Q V I R A F V P L A E M F G Y A T D L R S K T Q G R G S F V M F</b>

V | IV

Ef11	Sc	1045	FSGFEC <del>I</del> DL-DPFWVP	<b>T T E E E L E E L G D T A D R E N I A R K H M N A I R R R K G L F I E E K V V E N A E K</b>
Ef11	Sp	936	FHGFEM <del>I</del> DE-NPFWVP	<b>T T E E E L D L G E L A D R E N I A K R Y M L N V R K R K G L L V E Q K I V E K A E K</b>
Ef11	Ce	840	FSHWQV <del>I</del> IDE-DPYWTP	<b>S T L E E I E F G L K G D S P N H A R G Y M D A V R R R K G L P T E D L I V E S A E K</b>
eEF2	Sc	798	FDHWST <del>I</del> GGS-DPLDPT	<b>S K A -----G E I V L A A R K R H G M K E --E V - P G W --</b>
eEF2	Sp	798	FDHWSPMSG-DPLDPT	<b>S K P -----G Q I V C E A R K R K G L K E --N V - P D Y --</b>
eEF2	Ce	808	FDHWQV <del>I</del> PG-DP <del>L</del> EAGTKP	<b>- - - - - N Q I V L D T R K R K G L K E --G V - P A L --</b>
EF-G	Ec	684	FLKYDEAPS-NV-----	<b>A Q A V I E -----</b>
EF-G	Tt	673	FDHYQE <del>V</del> PK-QV-----	<b>Q E K L -----</b>

IV |

Ef11	Sc	1104	QR-TLKKN
Ef11	Sp	995	QR--TLKH
Ef11	Ce	899	QRNLKKNK
eEF2	Sc	836	Q-EYYDKL
eEF2	Sp	836	T-EYYDRL
eEF2	Ce	846	D-NYLDKM
EF-G	Ec	701	----ARGK
EF-G	Tt	688	----IKGQ

Sequences were aligned using T-Coffee, and the image was generated with BoxShade 3.21. Protein domain boundaries are indicated by roman numerals and GTPase motifs by shaded boxes. Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Ce, *Caenorhabditis elegans*; Ec, *Escherichia coli*; Tt, *Thermus thermophilus*.