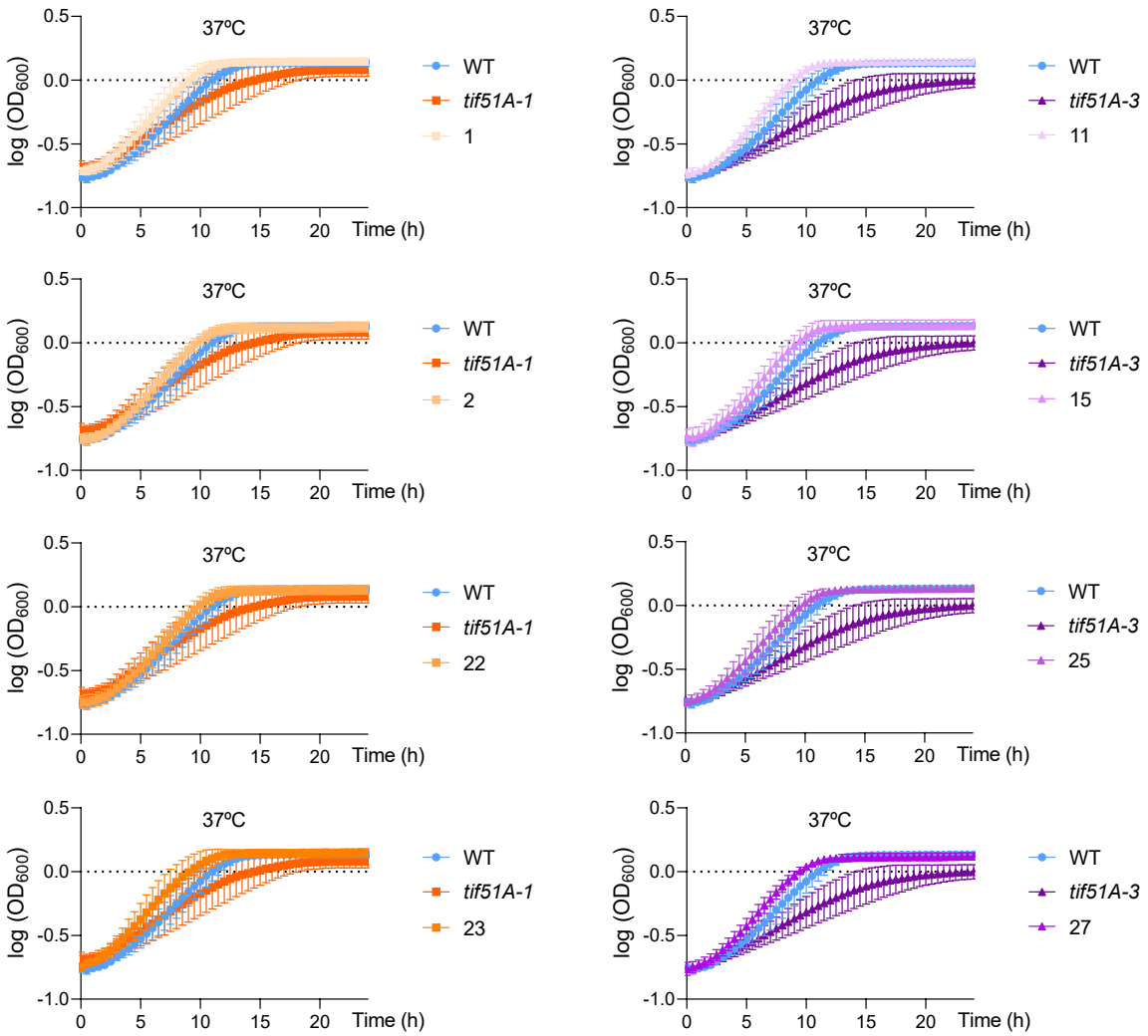
eIF5A is an indispensable protein for eukaryotic cells

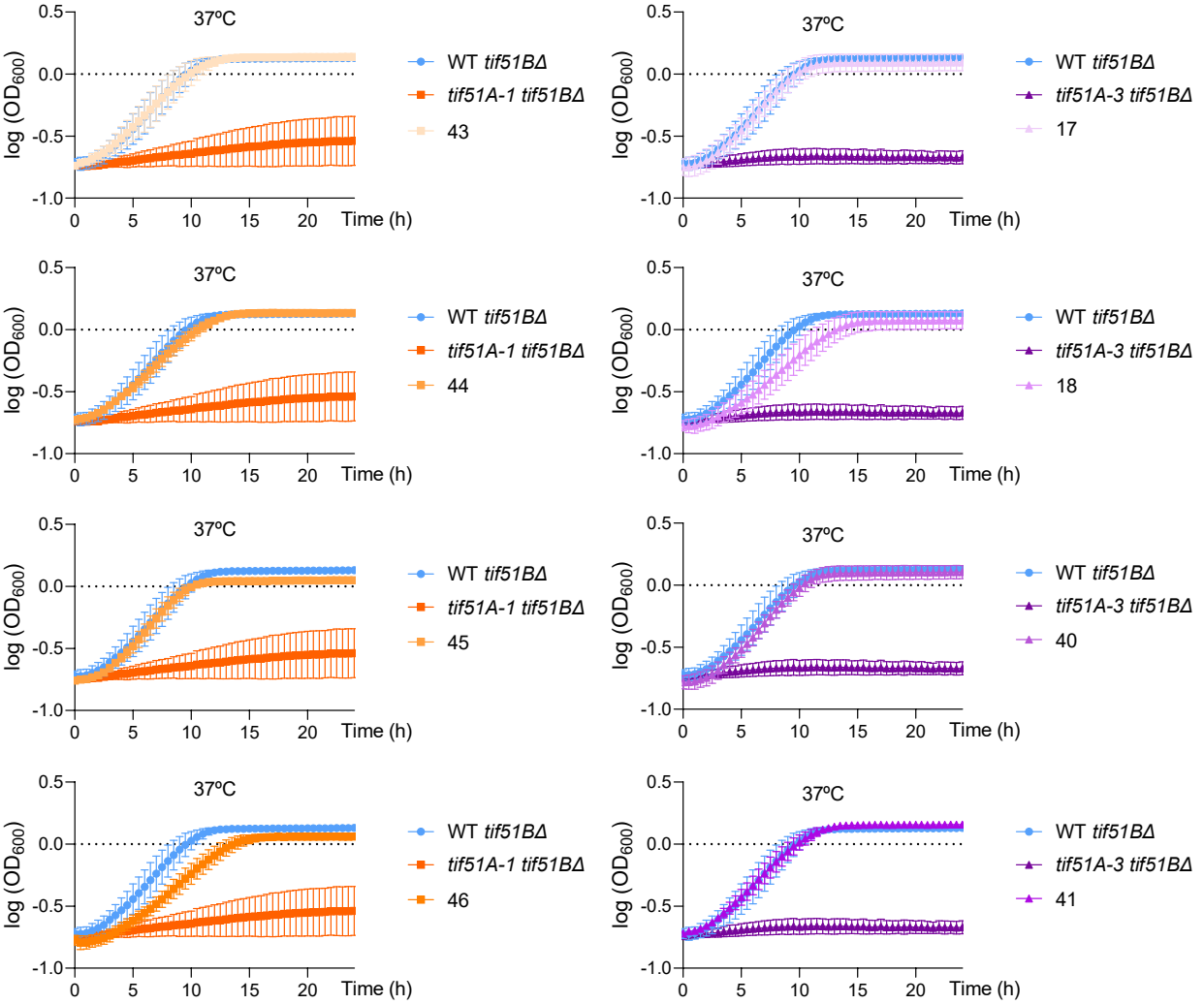
Samoa Prieto-Díez[1,2], Alejandro Aguilar-Díaz[3], Luis Araque[3], David Peris[3,4] and Paula Alepuz[1,2]*

File S3_supplementary Figures S1-S3



Supplementary Figure S1. Growth kinetics of suppressor strains at restrictive temperature. Wild-type (WT), *tif51A-1* and *tif51A-3* strains, along with their respective suppressors, were cultured in YPD at 25ºC. Cultures were then diluted to an OD_{600} of 0.1 and shifted to 37ºC. Growth was monitored by recording OD_{600} every 30 min for 24 h using a SPECTROstar Nano plate reader. Data represent the mean of $\log_{10}$ (OD_{600}) ± standard deviation (SD) of at least three biological replicates.

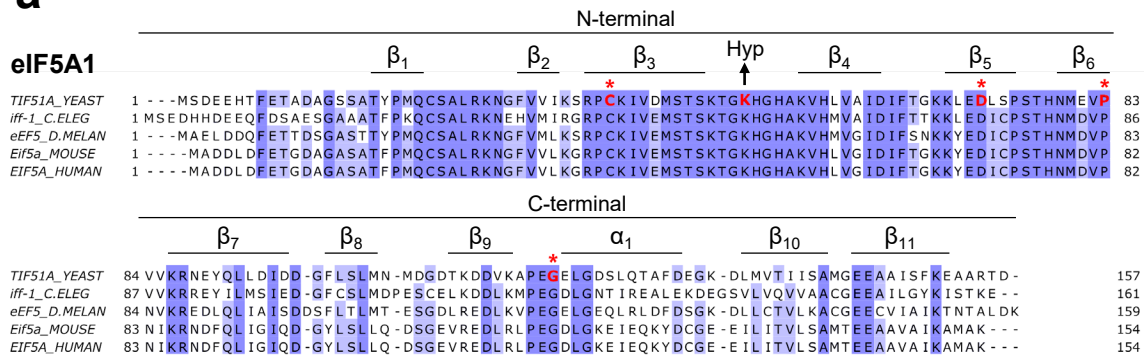
Supplementary Figure S1



Supplementary Figure S2. Growth kinetics of suppressor strains at restrictive temperature. Wild-type (WT), *tif51A-1* and *tif51A-3 tif51BΔ* strains, along with their respective suppressors, were cultured in YPD at 25ºC. Cultures were then diluted to an OD_{600} of 0.1 and shifted to 37ºC. Growth was monitored by recording OD_{600} every 30 min for 24 h using a SPECTROstar Nano plate reader. Data represent the mean of $\log_{10}$ (OD_{600}) ± standard deviation (SD) of at least three biological replicates.

Supplementary Figure S2

a



b

Organism	Protein	Gene	AA	% Identity	% Similarity	Protein	Gene	AA	% Identity	% Similarity
Saccharomyces cerevisiae	**eIF5A1**	*TIF51A*	157			**eIF5A2**	*TIF51B*	157		
Caenorhabditis elegans	**eIF5A1**	iff-1	161	57,7%	78,5%	**eIF5A2**	iff-2	161	58,9%	75,5%
Drosophila melanogaster	**eIF5A1**	eEF5	159	62,3%	77,4%					
Mus musculus	**eIF5A1**	Eif5a1	154	61,8%	82,8%	**eIF5A2**	Eif5a2	153	62,4%	79,6%
Homo sapiens	**eIF5A1**	EIF5A1	154	61,8%	82,8%	**eIF5A2**	EIF5A2	153	62,4%	79,6%

Supplementary Figure S3. Evolutionary conservation of eIF5A across species and orthologous proteins. (a) Multiple sequence alignment of full-length eIF5A1. The alignment includes proteins from *Saccharomyces cerevisiae, Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus* (mouse) and *Homo sapiens* (human). Conserved amino acids in all species are colored in dark blue, and those conserved in most species are colored in light blue. The N- and C-terminal domains, as well as the β-strands (β_1 – β_{11}) and α-helix (α_1), are indicated above the sequences. Amino acid residues mutated in *tif51A-1/-3* suppressors are highlighted in red with an asterisk above, with positions corresponding to the *S. cerevisiae* eIF5A sequence (C39, D72, P83 and G118). The conserved lysine residue targeted for hypusination is highlighted in red and indicated by an arrow (K51 in *S. cerevisiae*). **(b)** Table summarizing the identity and similarity percentages of *Tif51A* (eIF5A1) and *Tif51B* (eIF5A2) across different organisms relative to *S. cerevisiae.*

Supplementary Figure S3