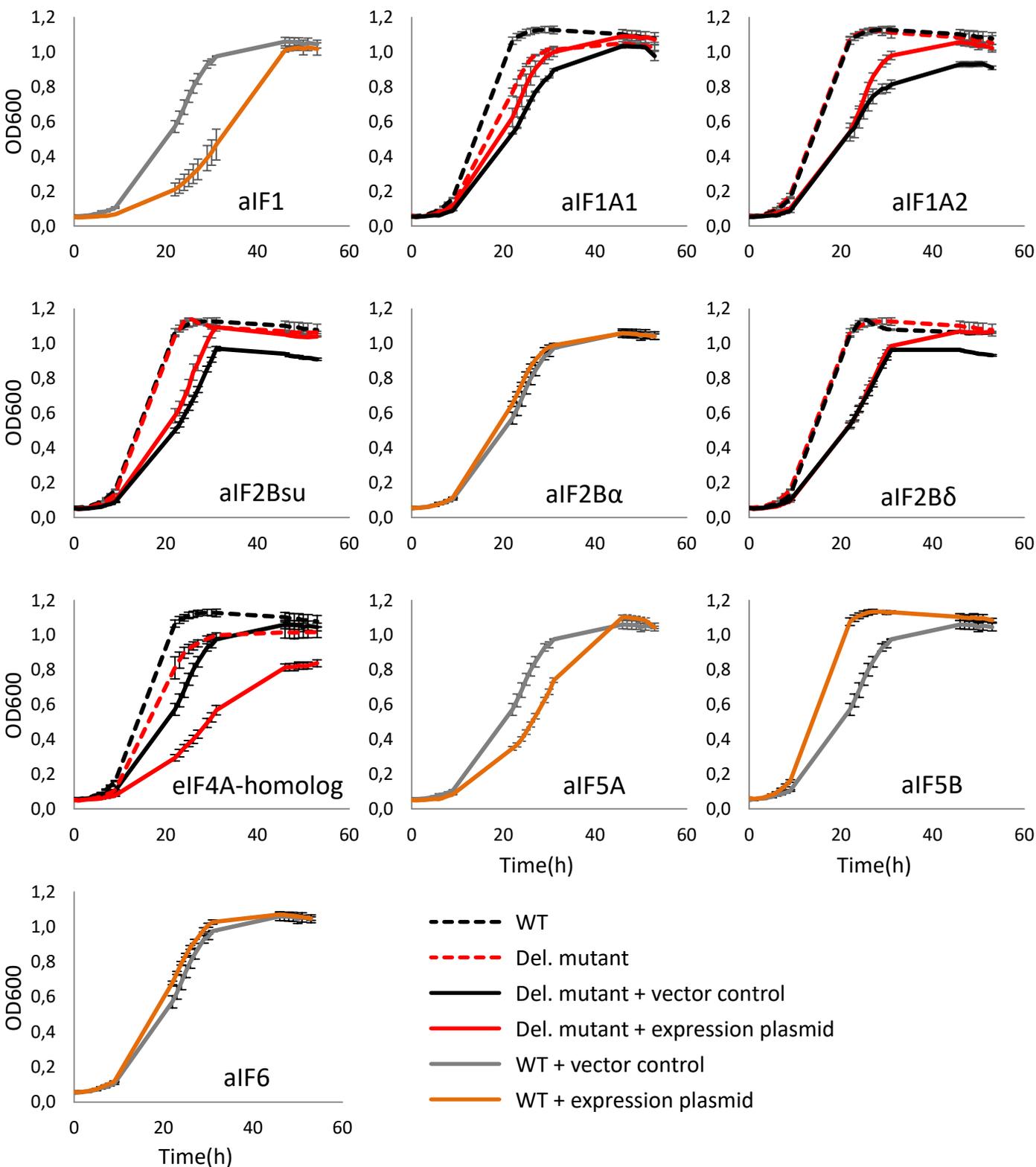


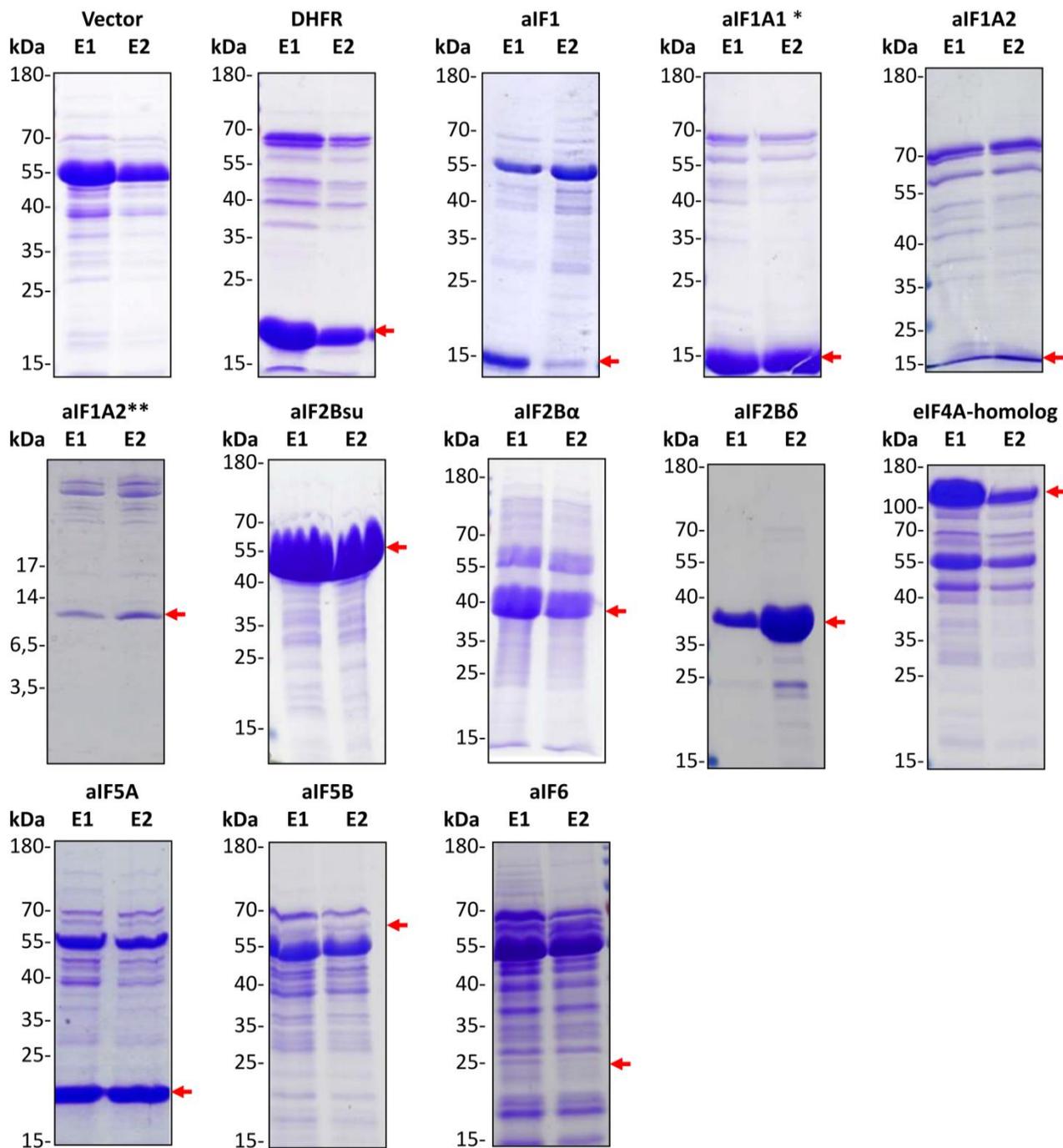
Supplementary Figure 1. Overview of the experimental and bioinformatic workflow.

A) Schematic Overview of the experimental workflow of cell harvest and co-affinity isolation. **B)** Schematic Overview of the bioinformatic workflow used for the identification of co-purified proteins. All steps are shown that have been applied to process the raw data received after LC-MS to identify the co-purified proteins. The Peptide Spectrum Matches (PSMs) served as a semi-quantitative indicator.



Supplementary Figure 2. Growth curves of aIF deletion mutants and overproduction strains.

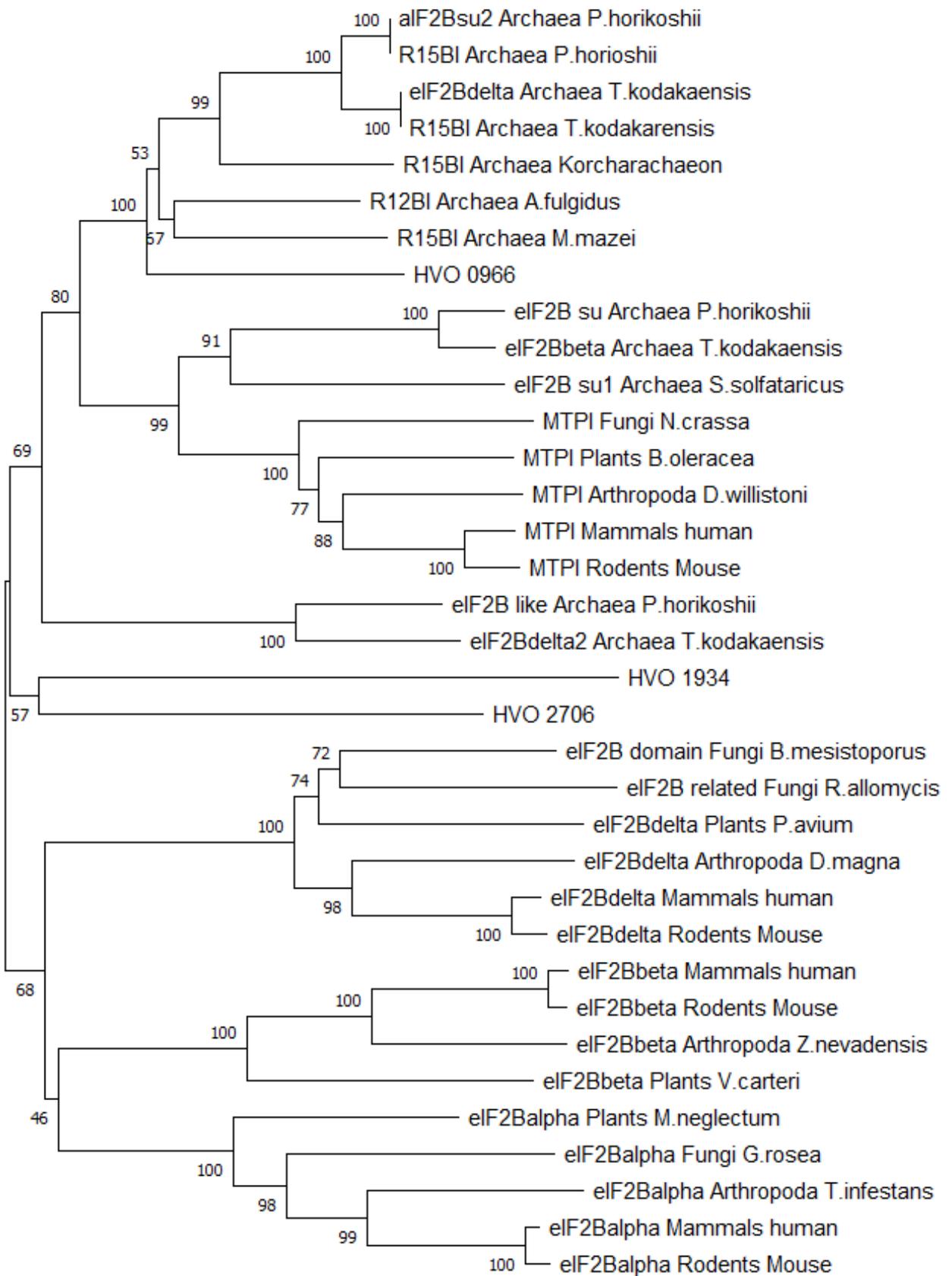
All cells were grown in triplicates under optimal growth conditions in complex media (all vector-containing cultures in the presence of Novobiocin) and in 96-well plates. OD600 was measured frequently, and average values and their standard deviations are shown. Dotted lines – plasmid-free cultures, solid lines – plasmid-containing cultures, red lines – deletion mutants, black lines – wildtype, grey line – wildtype with empty vector, orange line – wildtype with expression plasmid.



Supplementary Figure 3. SDS-Page of the elution fractions 1 and 2 of His₆-tagged aIFs. The red arrow indicates the overexpressed His₆-tagged bait protein. Examples given are representative for three biological replicates.

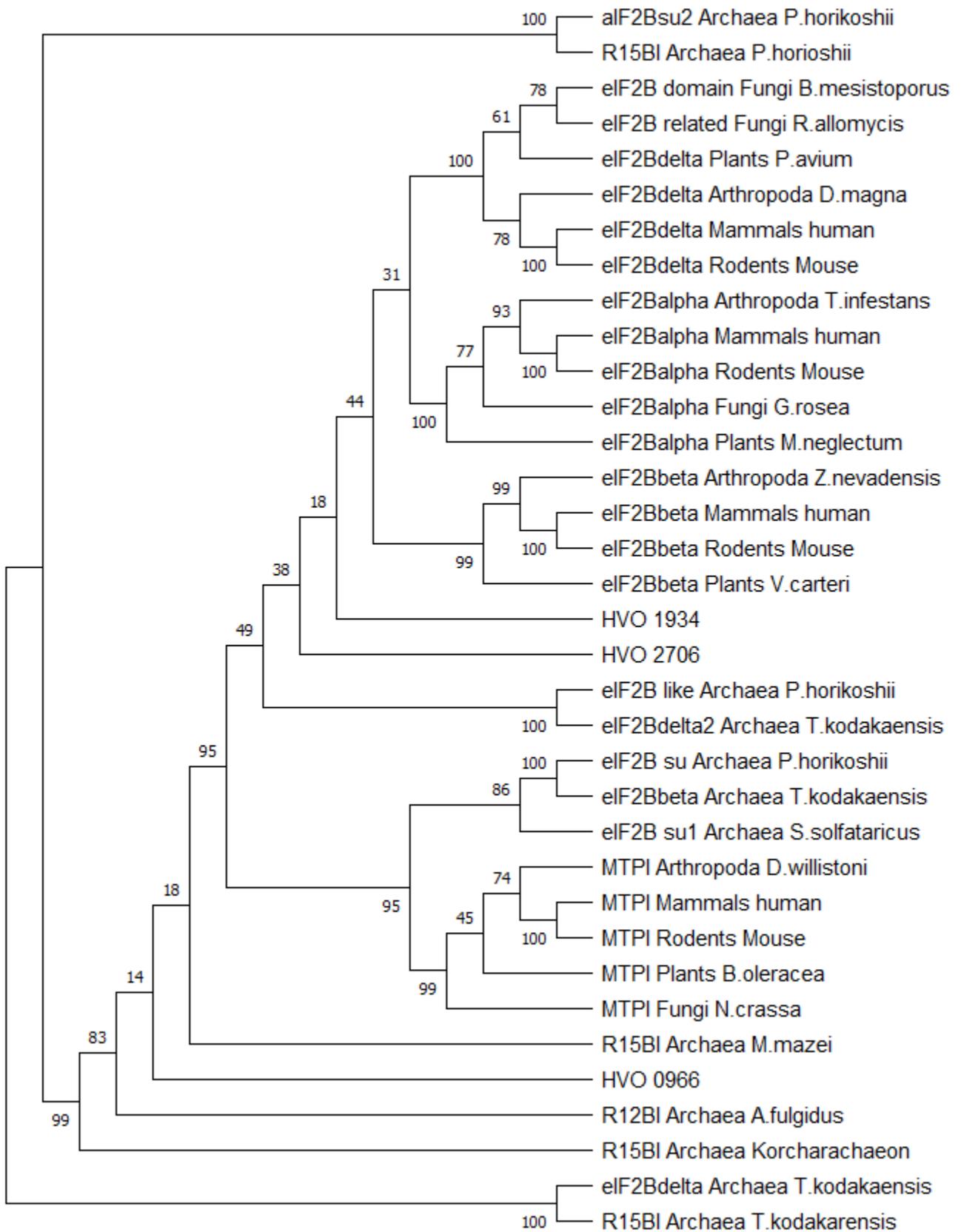
*The aIF1A1 overexpression construct contained the native 83 nucleotides long 3'-UTR after the ORF. Without the UTR, aIF1A1 was not detectable after overexpression.

** Here, a Tricine-based gel was used for better visibility of the aIF1A2 protein.

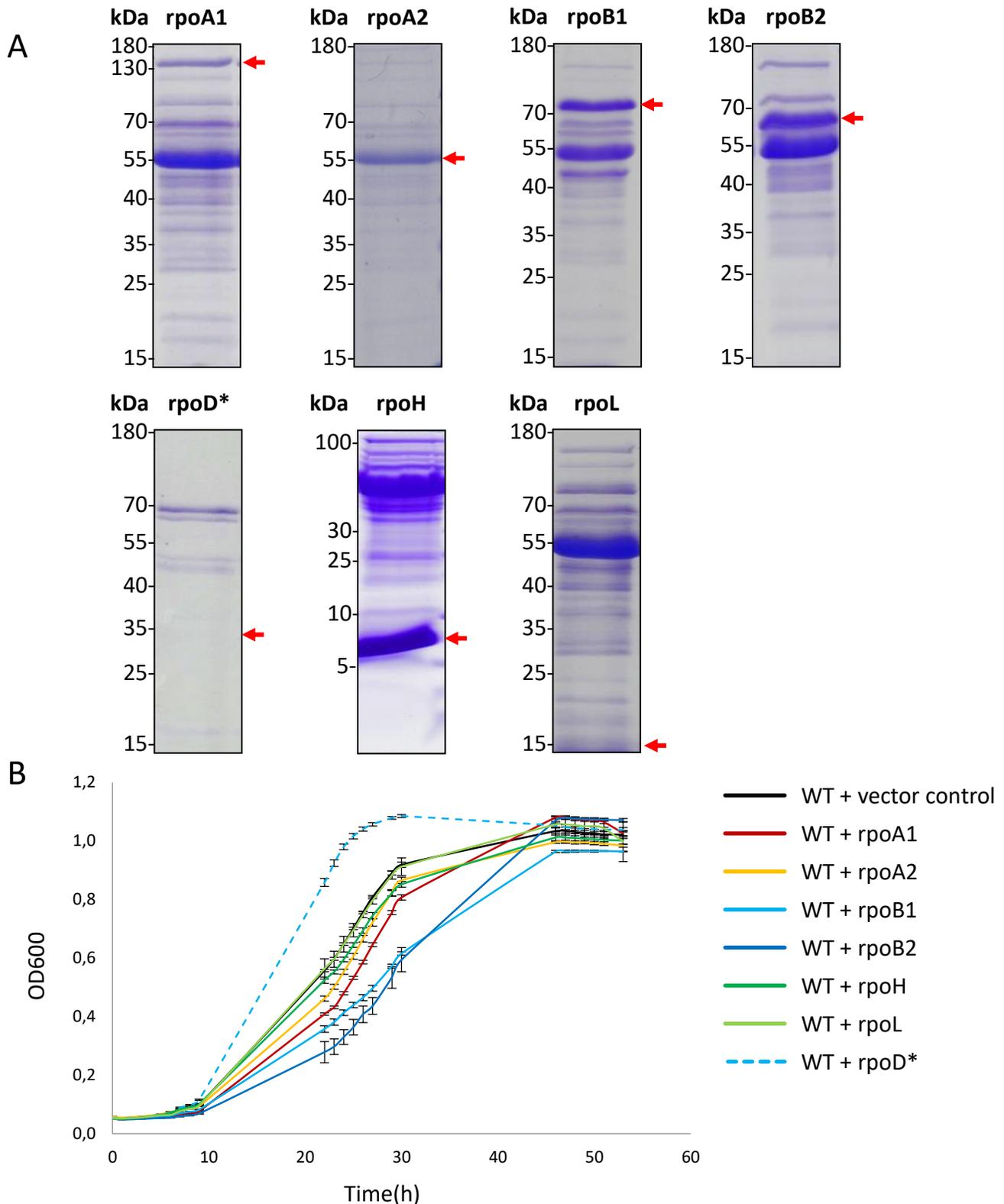


0.20

Supplementary Figure 4. Neighbor Joining (NJ) tree with 1000 bootstrap replications. The bootstrap values (%) are shown at each node.



Supplementary Figure 5. Maximal Parsimony (MP) tree with 1000 bootstrap replications. The bootstrap values (%) are shown at each node.



Supplementary Figure 6. Overproduction of His₆-tagged RNA polymerase subunits.

A) SDS-Page of the elution fraction 2 of indicated Rpo subunits. The red arrow indicates the overexpressed His₆-tagged bait protein. Examples given are representative for three biological replicates.

B) Growth curves of cultures of the wildtype and the strains overproducing the indicated Rpo subunit. All cultures were grown in triplicates under optimal growth conditions in complex media (all vector-containing cultures in the presence of Novobiocin) and 96-well plates. OD600 was measured frequently, and average values and their standard deviations are shown.

* RpoD yield after overproduction was very low.

Sac_cerevisiae	MSSDLAELGFDPAL KKKKKT KKVIPDDFDAAVNGK-----	36
Dro_melanogaster	---MDAEDGFDPTLL KKKKKK TT-FDLDAALGLEDDTK-----KEDP--QDE----	42
Homo_sapiens	---MSGDEMIFDPTMS KKKKKKKK P-FMLDEEGDTQTEETQPSETKEVEPEPTEDKDLEA	56
Rattus_norwegicus	---MSGDEMIFDPTMS KKKKKKKK P-FMLDEEGDAQTEETQPSETKEVEPEPAEEKDVEA	56
Sul_solfataricus	-----	0
Met_jannaschii	-----	0
Mbc_thermoautotrophicum	-----	0
Pco_furiosus	-----	0
Hfx_volcanii_1	-----	0
Hfx_volcanii_2	-----	0
Hqu_walsbii	-----	0
Nal_madadii	-----	0
Hco_morrhuae	-----	0
Hru_sodomense	-----	0
Sac_cerevisiae	-----ENGSGD--DLFAGL KKKKK SKSVSA-----DAAEKEPT	69
Dro_melanogaster	---ASAEGGAAEEDNLDLESFG KKKKKK PFNMDEIEAAIPSGFGDDVAASEPEEEEE	99
Homo_sapiens	DEEDTRKGDASDDLDLNFNFQ KKKKKK TKKIFDIDEAEEGVKDLKIES--DVQEPTEPE	114
Rattus_norwegicus	DEEDSRKGDASDDLDLNFNFQ KKKKKK TKKIFDIDEAEEAIKDVKIES--DAQEPAEPE	114
Sul_solfataricus	-----	0
Met_jannaschii	-----	0
Mbc_thermoautotrophicum	-----	0
Pco_furiosus	-----	0
Hfx_volcanii_1	-----	0
Hfx_volcanii_2	-----	0
Hqu_walsbii	-----	0
Nal_madadii	-----	0
Hco_morrhuae	-----	0
Hru_sodomense	-----	0
Sac_cerevisiae	DDIAEALGELS L KKKK KT KDSSVDAFEKELAKAGLDNVDAESKEGT---PSANSSIIQQ	125
Dro_melanogaster	INLDMDFSM AKKKK SKK-KE-LDELFDQ-----ADDDKSEDKENEDNSSTWFGS	149
Homo_sapiens	DDLDIMLGN KKKK NVKFPD-EDEILEKDEA---LEDEDNKDDGISFSNQTGPWAGS	170
Rattus_norwegicus	DDLDIMLGN KKKK NVKFPD-EDEILEKDEA---LEDEDSKDDGISFSNQTGPWAGS	170
Sul_solfataricus	-----M	1
Met_jannaschii	-----MSDLEN	6
Mbc_thermoautotrophicum	-----	0
Pco_furiosus	-----ME	2
Hfx_volcanii_1	-----	0
Hfx_volcanii_2	-----	0
Hqu_walsbii	-----	0
Nal_madadii	-----	0
Hco_morrhuae	-----	0
Hru_sodomense	-----	0
Sac_cerevisiae	EVGLPYSELLSRFFNILRTNNPELAGDRSGPKFRIPPPVCLR-DGKKTIFSNIQDIAEKL	184
Dro_melanogaster	DRDYTYDELKRVFEIILDKNPDMAGRK-PKFVMRPPQVLRVGTGKTSFANFMDIAKTL	208
Homo_sapiens	ERDYTYEELNRFVFNIMREKNPDMVAGEK-RKFVMKPPQVVRVGTGKTSFVNFTDICKLL	229
Rattus_norwegicus	ERDYTYEELNRFVFNIMREKNPDMVAGEK-RKFVMKPPQVVRVGTGKTSFVNFTDICKLL	229
Sul_solfataricus	SSEKEYVEMLDRLYSKL---PEKGR-KE-GTQSLPNIILN-IGNTTIIRNFAEYCDRI	54
Met_jannaschii	IDYYDYKALKRARSQI---PDYVF-QK-DRFELPEIEILI-EGNRTIIRNFRELAKAV	59
Mbc_thermoautotrophicum	--MDDYEKLLERAIDQL---PPEVF-ET-KRFEVPKAYSVI-QGNRTFIQNFREVADAL	51
Pco_furiosus	IDYYDYEKLLKAYQEL---PENVKHHK-SRFEVPGALVTI-EGNKTIENFKDIADAL	56
Hfx_volcanii_1	---MDYDDQLDRALSAS---PDVAE-GD-DRFSVPEPTVRQ-EGNVTVYENFAATHDRL	50
Hfx_volcanii_2	---MEYQALDRALNVL---PERNV-EQ-ERLTVDPDSGET-DGAFTRLTNLGEIADAL	50
Hqu_walsbii	---MDYTESLNRALDEL---PERRD-EE-SRLSIPDPVGET-DGAFTRLTNLGEIADAL	50
Nal_madadii	---MDYESSLDRAMEDV---PDIGG-DE-ERLQIPDAQTQK-DGAFTRFTNLGEIADVL	50
Hco_morrhuae	---MDYEASLDRAMDSV---PDLDT-GD-SRLDVPDAEAQK-DGAFTRLTNLGNVADAL	50
Hru_sodomense	---MDYESSLDRAMDEV---PDLGG-SD-ERLSVDPPEWQK-DGAFTRLTNLGSAIADAL	50
	* * . : * : * * : . :	

Sac_cerevisiae	HRSPEHLIQYLFAELGTSVSDGQKRLVIKGFQSKQMVNLRRIYILEYVTCKTCKSINT	244
Dro_melanogaster	HRLPKHLLDFFLLAELGTSVSDGQKRLVIKGFQSKQMVNLRRIYILEYVTCKTCKSINT	268
Homo_sapiens	HRQPKHLLAFLLAELGTSVSDGQKRLVIKGFQSKQMVNLRRIYILEYVTCKTCKSINT	289
Rattus_norvegicus	HRQPKHLLAFLLAELGTSVSDGQKRLVIKGFQSKQMVNLRRIYILEYVTCKTCKSINT	289
Sul_solfataricus	RREDKICMKYLLKELAAPGNVDDKLGELVIQKGFSSQVINTLMERFLKAYVECTCKSLDT	114
Met_jannaschii	NRDEEFFAKYLLKETGSAGNLEG-GRLILQRRISPELLKSRINDFLREYVICRECGKPD	118
Mbc_thermoautotrophicum	NRDPQHLLKFLRLRELGTAGNLEG-GRAILQKGFTHFLINERIEDYVNKFCHECNRPDT	110
Pco_furiosus	NRDPQHLLKFLRLREIATAGTLEG-RRVVLQGRFTPYLIANKLKKYIKEYVICPVCGSPDT	115
Hfx_volcanii_1	AREATHVLKFFQTELGTSAQIDDRGRARFTGEFRQRRIADALDEYVESFVLCSECGSPDT	110
Hfx_volcanii_2	SRTPAHLHSAIQRTLTGTSQLEG-DRARYSGSFSINDFEEAIDGYVEEYVICSECGLPDT	109
Hqu_walsbii	GRPAEHVHRNVQELGTNGQLED-ERARYNGSFSVADFEAAIDYVIEYVTECECGLPDT	109
Nal_madadii	SREDEHLHRFVQREMGTSKGFEE-GRGRYNGTFSEQDFNAAVDAYVDEYVLCCECGLPDT	109
Hco_morrhuae	SRDPEHLHRVIQRDLGTNGQFSG-DRARYNGTFSGEDFAAIDSYSQEFVICSECGLPDT	109
Hru_sodomense	SRDPEHVHSKIQQELGTAGQYED-GRARYSGNFRERDFQAAIDSYSIESFVTCSECGLPDT	109
	* : : : : : * * * : *	
Sac_cerevisiae	ELKREQSNRLFVCKSCGSTRSVSSIKTGFQAVTGKRRM-----	285
Dro_melanogaster	ILQK--DTRLFPLQCESCGSRCSVASIKSGFQAVTGKRAAIRAKTT-----	312
Homo_sapiens	ILQK--DTRLFPLQCECHSRCSVASIKTGFQAVTGKRAQLRAKAN-----	333
Rattus_norvegicus	ILQK--DTRLFPLQCECHSRCSVASIKTGFQAVTGKRAQLRAKAN-----	333
Sul_solfataricus	ILKK--EKKSQYIVCLACGAQTPVKPL-----	139
Met_jannaschii	KIIK--EGRVHLLKCMACGAIRPIRMI-----	143
Mbc_thermoautotrophicum	RIIR--EGRISLLKCEACGAKAPLKNV----- -----	135
Pco_furiosus	KIIK--RDRFHFLKCEACGAETPIQHL-----	140
Hfx_volcanii_1	KLTS--QRGADVLRKCDACGALSAPDL-----	135
Hfx_volcanii_2	RLVT--EDGVMLRCCEACGAFRPVQKRSSVSNK-RQEAEEVGGRTYEVEITGTGRKGDGV	166
Hqu_walsbii	RLTT--ENGVDMLRCCEACGAFRPVQKQTTTQQSQSGPAVEEGATYEVEITGTGRKGDGV	167
Nal_madadii	RLVR--EDRTPMLRCCEACGAFRPVTKRSTSAQQQQQDAVEEGKTYTVEITGTGRKGDGV	167
Hco_morrhuae	RLEM--EGRTQMLRCCEACGAFRPVEKNTGSQET-EQRPDVEEGRTYEVEITGTGRKGDGV	166
Hru_sodomense	RLET--ENRTPMLRCCEACGAFRPVAKQNTSNTQ-RQEEAVESGTTYEIVEITGTGRKGDGV	166
	: : * : * : :	
Sac_cerevisiae	-----	285
Dro_melanogaster	-----	312
Homo_sapiens	-----	333
Rattus_norvegicus	-----	333
Sul_solfataricus	-----	139
Met_jannaschii	-----	143
Mbc_thermoautotrophicum	-----	135
Pco_furiosus	-----	140
Hfx_volcanii_1	-----	135
Hfx_volcanii_2	AQRGKYTIFVPGAQEGQTVRIYIKNTSGSLAFARLA-	202
Hqu_walsbii	AEKGKFTIFVSGAEEGQTVRVQIERTSGTLAFAQVI-	203
Nal_madadii	AEKGEYTIFFVPGAQEGDVVDIYIKNISGNLAFARLD-	203
Hco_morrhuae	AEQKTYTIFVPGAQEGVDIYIENISGTLAFARLA-	202
Hru_sodomense	AERGEYTIFFVPGAQEGETVTAYIKNVSGNLAFARRED	203

Supplementary Figure 7. Multiple sequence alignment of a/eIF2 β translation initiation factors from selected archaea and eukaryotes, representing various phylogenetic lineages. The alignment was generated using Clustal Omega at the website of the European Bioinformatics Institute (www.ebi.ac.uk). Positions of increasing degrees of conservation are marked with ., :, and *. The four conserved cysteines in two CXXC motifs, which form a zinc finger, are shown in red. The three polylysine stretches confined to eukaryotic proteins are shown in blue. The C-terminal extensions that are found exclusively in halophilic and methanogenic archaea are shown in purple.

Supplementary Table 1. Primers for generating and/or confirming *H. Volcanii* deletion mutants

Name	Sequence
HVO_0136 P1	CCGACGCGCCGCCGACGCTGT
HVO_0136 P2	GCGGAGCTGGTCGGCAAGGTCTCGGCGGCTCTCGTT
HVO_0136 P3	AGCCGCCGAGACCTTGCCGACCAGCTCCGCCGCGAA
HVO_0136 P4	GACGACCTGTCCCTTGTCTGTG
HVO_0359 P1	GTTTGAGGGAATCCGCAACG
HVO_0359 P2	GCATCGTTATCGCTCGCTCATTGGTGTGGTCACGC
HVO_0359 P3	ACCACACCAATGAGCGAGCGATAACGATGCAACAGGC
HVO_0359 P4	GTTCCGTGCGGCGATTCTG
HVO_0569 P1	CAGCCGCTCAGTGGATGTGC
HVO_0569 P2	GACGGCGAACTCGTCGGCGACGTTCCGTACCATGC
HVO_0569 P3	GTACGGAACGTCGCCGACGAGTTCGCCGTCTGAGC
HVO_0569 P4	CGCTCCCGCGATGTACCAC
HVO_0699 P1	ACACAAAACCCACCACAAGGTTCGATATCTATG
HVO_0699 P2	TTCGCGGTGGTACTCGAGTTCGCCGGGGTCAGGCCA
HVO_0699 P3	GACCCCGCGAACTCGAGTACCACCGGAACGCAGC
HVO_0699 P4	GGGTCAGCTCGGCGACGCC
HVO_1333 P1	ACGCGGGCGAGTCGCTGCC
HVO_1333 P2	GTCAGTCCCAGAAGCCGTCTGGCATCGCCGAGGTTACG
HVO_1333 P3	ATGCCAGACGGCTTCTGGGACTGACGGGGTGGATTCCGGTT
HVO_1333 P4	GTCGCTCGGCTCCGACATCCTCG
HVO_1678 P1	TTCTCGGTTCAGTCGGCTTGCGG
HVO_1678 P2	TCGTTTTTACAGGTAGTCCATGCGCGGCAGTACG
HVO_1678 P3	GCGCATGGACTACCTGTAAAAACGAACGTCCGGCGG
HVO_1678 P4	CGGTCGGCCTCGCCGCCCGCC
HVO_1934 P1	CAGCTGCGAGACAGCGCGACGACG
HVO_1934 P2	GTCGTCCACGCGCGACGTGTGGCATATCGTGACTTACGGC
HVO_1934 P3	GCCACACGTCGCGCGTGGGACGACTGAGTCGGGAGTGC
HVO_1934 P4	CGGTTTCGGGGCTCCGGTTCGAGG
HVO_2242 P1	CGTGATTCGTTCCGGCAGCCCG
HVO_2242 P2	GACCGATTCAGGCCATAGGGGTTTCGAGTGTCTGGC
HVO_2242 P3	CGAACCCTATGGCCTGAATCGGTCGCACGCGTGC
HVO_2242 P4	CGGCTACAAGGCGCTCGACTCC
HVO_2706 P1	CCCATCTGCGAGGCGGGCCAGC
HVO_2706 P2	TCAGAACTCGATGACGGTCTCGTCTATCATACGAGAACGTCGCCGTATCG
HVO_2706 P3	ATGATAGACGAGACC GTCATCGAGTTCGAGCGGGCGGCG
HVO_2706 P4	TCGGTTCCTCGTCAGTGACCGCGCC
HVO_A0637 P1	GTCCGCTGTTCTGGGAGACGACGTAACCG
HVO_A0637 P2	TTACTGAATGTGGCCTTCATTTGACGAGGTCCACGATTCCCCTACAACAC
HVO_A0637 P3	ATGACCTCGTCAAATGAAGGCCACATTCAGTAATCATTTTCTCCGAAAGGAGAG
HVO_A0637 P4	GGGAACCAAGACGGCCGACGACGC
HVO_B0053 P1	GACGCTGGCCGACCGCTATC
HVO_B0053 P2	GCGTTCGTGAAGCGTTCGGAGCGCTTCGAGTCTGC
HVO_B0053 P3	CTCGAAGCGCTCCGAACGCTTCACGACGCCTTCCC
HVO_B0053 P4	ACGACGCTCCGCCAGACTC
Probe 0359 for	TCAACAAGATGGACGTCGTC
Probe 0359 rev	ACTTCTCTGTGGTGCATCTC
Probe 0569 for	CGTTCCGCCAGCGAGACACC
Probe 0569 rev	GCCGCGTAGGCGTCGGCCAT
Probe 0966 for	CACGAACGGGGCGCACCCGGTC
Probe 0966 rev	GCGGTCGTCCATGCGGAACTACGGG
Probe 1333 for	ACCCTTCACCCGCTCTCGAACGGCGTAGC
Probe 1333 rev	GCCGTCTGGCATCGCCCGAGGTTACG
Probe 1934 for	GCGTGGGACGACTGAGTCGGGAGTGC

Probe 1934 rev	CGGTTTCGGGGCTCCGGTCGAGG
Probe 2242 for	GGACGCGCTCTCGCGGACGC
Probe 2242 rev	CTGCGCGCCGGGCACGAAGA
Probe 2706 for	CCCATCTGCGAGGCGGGCCAGC
Probe 2706 rev	ATACGAGAACGTCGCCGTATCG
Probe A0637 for	ACCCAACGACGAACCGGCGA
Probe A0637 rev	CACGTGCGGTACTTCATGCG
Probe B0053 for	TGAACATCACCGAACGACC
Probe B0053 rev	CTCCGAGGTCGTCGAGCAGC

Supplementary Table 2. Primers for generating expression vectors with His₆-tagged gene variants.

Name	Sequence
Oligo-His-Tag_for	CATGGGCCACCACCACCACCACGC
Oligo-His-Tag_rev	CATGGCGTGGTGGTGGTGGTGGCC
NcoI_His_0117_for	TGATGCCCATGGGCCACCACCACCACCACCACGCCACTTCGCGCCTCCTT
KpnI_0117_rev	GAATTGGGTACCTCAGTCGATGTAATCGAG
HVO_0136_His_NheI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGAGCGACGACGAGAACGAG
HVO_0136_KpnI_rev	CGAATTGGGTACCGCTTACTGGATGTGACCTTCG
NcoIH0136mit3UTRKpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCCAGCGACGACGAGAACGAGAG
NcoIH0136mit3UTRKpnI_rev	GAATTGGGTACCGGCGAGATGAAAAGGGCACAGG
HVO_0699_His_NheI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGAAGTACAGCGGATGGCCT
HVO_0699_KpnI_rev	CGAATTGGGTACCGTTACTCTTCGTCGCGCGCTGC
NcoI_His_0966_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGGACGACCGCGTA
NcoI_His_0966_KpnI_rev	CGAATTGGGTACCGCTACGGTTCGGCCCGCGGC
NcoI_1333_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGCCAGACGGCGAGGCCGC
NcoI_1333_KpnI_rev	CGAATTGGGTACCGTCAGTCCCAGAACGACTGG
HVO_1678_His_NheI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGGACTACGACGACCAACTG
HVO_1678_KpnI_rev	CGAATTGGGTACCGTTACAGATCGGGAATCGCTG
NdeI_His_1901_for	ACTAGGACCATATGCACCACCACCACCACCACGTGACGAAAAACCCACAA
1901_KpnI_rev	GGCGAATTGGGTACCTCACTTGAGCGTCCC
NcoI_His_1934_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGCCACACGTGCCACCGT
NcoI_His_1934_KpnI_rev	CGAATTGGGTACCGTCAGTCGTCCCACGCCGC
NcoI_His_1946_KpnI_for	TGATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGTCGGAAGTCTGCTCGA
NcoI_His_1946_KpnI_rev	CGAATTGGGTACCGTCACGCGACGTTGAACCCC
NheI_1963_KpnI_for	ACCACCACGCTAGCGGCATGTCGACACTGATT
NheI_1963_KpnI_rev	CGAATTGGGTACCGCTATTTGCCCGAGAAGGGG
HVO_2242_His_NheI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGAGTATCAGACTGCGCTCGACCGA
HVO_2242_KpnI_rev	CGAATTGGGTACCTCAGGCGAGTCGCGCGAACGC
NcoI_His_2300_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGGCGAAAGAGCAGAAGCA
NcoI_His_2300_KpnI_rev	CGAATTGGGTACCGTTAGACGATCTTTCGCTGG
NcoI_His_2706_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGATAGACGAGACCATCGA
NcoI_His_2706_KpnI_rev	CGAATTGGGTACCGTCAGAACTCGATGACGCC
NcoIHis_A0637_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCCAGTAAAATCAAGGGCGTGC
NcoIHis_A0637_KpnI_rev	CGAATTGGGTACCTTACTGAATGTGGCCTTCGCGCCGAA
NcoI_H0346_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCTAGACGTAAGCCAACACAA
NcoI_H0346_KpnI_rev	CGAATTGGGTACCGTCATTCAATCACCAGTCGGTATACGACT
NcoI_His_0347_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGAACCGACAAGCG
NcoI_His_0347_KpnI_rev	CGAATTGGGTACCGTTAGTCGTCCGCGGGTGCG
NcoI_H0348_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCGCTCAGGCACAGCGAGA
NcoI_H0348_KpnI_rev	CGAATTGGGTACCGTTAGACCGCGTCCTTCAGTTCG
NcoI_His_0349_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGTCAATGCAGACA
NcoI_His_0349_KpnI_rev	CGAATTGGGTACCGTCAGTCATCGGATTTCGACC
NcoI_His_0350_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCATGACTGATACCGAC
NcoI_His_0350_KpnI_rev	CGAATTGGGTACCGTCAGTCGTCCGACGGTTCCG
NcoI_H1042_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCGAACTGCGGGTCATCGA
NcoI_H1042_KpnI_rev	CGAATTGGGTACCGTTACGCGTCGATACCGGCGCGG
NcoI_H2781_KpnI_for	GATGCCCATGGGCCACCACCACCACCACCACGCTAGCGGCGTAAACGACTTCCAGGTCGA
NcoI_H2781_KpnI_rev	CGAATTGGGTACCGTTTAAACTTACAGTGCAGCTTCTCTTC

Supplementary Table 3. Co-isolated proteins with aIF2 subunits.

MW [kDa]	Group	Specification	Genloci	Accession	Description	Bait			
						aIF2 α	aIF2 β -1	aIF2 β -2	aIF2 γ
29.5	Translation	aIFs	HVO_0699	D4GT46	aIF2α	bait	+	+	+
14.9	Translation	aIFs	HVO_1678	D4GZP2	aIF2β-1		bait		
22.2	Translation	aIFs	HVO_2242	L9VAS4	aIF2β-2			bait	+
43.9	Translation	aIFs	HVO_1901	D4GTD4	aIF2γ	+	+	+	bait
34.9	Translation	aIFs	HVO_0966	L9USK7	aIF2Bα	+			+
65.3	Translation	aIFs	HVO_1963	D4GTJ2	aIF5B	+		+	+
45.6	Translation	aEFs	HVO_2413	L9V6J4	aEF1α	+		+	+
57.8	Translation	aEFs	HVO_2575	L9V605	aEF1α-like protein	+			
80.4	Translation	aEFs	HVO_0356	L9UK07	aEF2	+		+	+
24.8	Translation	30S rpS	HVO_1145	D4GWA5	30S rpS3Ae			+	
23.3	Translation	30S rpS	HVO_2544	D4GTX6	30S rpS5	+			
13.4	Translation	30S rpS	HVO_2782	L9V5R1	30S rpS11			+	
23.1	Translation	50S rpL	HVO_2757	P41199	50S rpL1	+			
25.6	Translation	50S rpL	HVO_2561	D4GTZ3	50S rpL2			+	+
37.2	Translation	50S rpL	HVO_2564	L9V5K9	50S rpL3			+	
27	Translation	50S rpL	HVO_2563	D4GTZ5	50S rpL4			+	
36.9	Translation	50S rpL	HVO_2756	P41198	50S rpL10	+			
19.8	Translation	50S rpL	HVO_0484	D4GS24	50S rpL10e	+			
22.5	Translation	50S rpL	HVO_0561	L9UPT6	50S rpL15e	+			
20.2	Translation	50S rpL	HVO_2545	P50563	50S rpL18			+	
12.7	Translation	50S rpL	HVO_2779	D4GWY3	50S rpL18e			+	
16.9	Translation	50S rpL	HVO_2559	L9V5K3	50S rpL22			+	
17.1	Translation	50S rpL	HVO_2543	L9V5X1	50S rpL30	+		+	
81.2	Translation	transferase	HVO_2736	D4GW73	tRNA(Met) cytidine acetyltransferase TmcA	+			
108.8	Transcription	RNAP-subunits	HVO_0349	D4GZX6	rpoA1	+			
46.1	Transcription	RNAP-subunits	HVO_0350	D4GZX7	rpoA2	+			
58.9	Transcription	RNAP-subunits	HVO_0347	L9UK99	rpoB2	+			+
11.7	Transcription	regulator	HVO_1133	D4GW85	HTH domain protein				+
23.8	Transcription	regulator	HVO_A0121	D4GQF3	HTH-10 family transcription regulator	+			
28	Transcription	regulator	HVO_2108	D4GUQ5	IcIR family transcription regulator	+	+	+	
18.1	Transcription	regulator	HVO_1792	D4GSB6	Lrp/AsnC family transcription regulator	+			
8.3	Transcription	regulator	HVO_2723	D4GW47	RNA-binding protein Lsm	+			
19.9	Transcription	regulator	HVO_1727	L9VGC6	TATA-box-binding protein	+			
19.8	Transcription	regulator	HVO_0163	L9UFW5	Transcriptional regulator	+			
28	Transcription	regulator	HVO_0179	L9UGU5	Transcriptional regulator				+
27.5	Transcription	regulator	HVO_2928	L9UK32	Transcriptional regulator	+	+		
30.9	Transcription	regulator	HVO_2636	L9V878	Transcriptional regulator	+			
15.2	Protein folding	chaperone	HVO_0450	D4GRZ1	Hsp20-type molecular chaperone	+			
37.3	Replication and Repair	repair	HVO_0104	Q48328	DNA repair and recombination protein RadA	+			
24.6	Replication and Repair	repair	HVO_2383	L9V6G6	DNA repair and recombination protein RadB	+			
69.6	Replication and Repair	replication	HVO_0415	D4GRV6	Repair helicase UvrD	+			
34.6	Replication and Repair	replication	HVO_0292	L9UJU8	Replication factor A	+			
87.4	Replication and Repair	replication	HVO_1571	L9VEG4	Type 2 DNA topoisomerase 6 subunit B	+			
23.2	RNA turnover	ribonuclease	HVO_2438	L9V6L9	Ribonuclease H I	+			
50.1	RNA turnover	ribonuclease	HVO_2724	L9V5P6	Ribonuclease J			+	
59.3	Protein turnover	thermosome	HVO_0455	O30560	Thermosome subunit 2	+		+	
55.2	Protein turnover	thermosome	HVO_0778	Q9HHA2	Thermosome subunit 3	+		+	

Supplementary Table 7. The RNA polymerase subunits interaction network.
RpoD is shown in brackets as the yield after overproduction was very low.

MW [kDa]	Genloci	Accession	Description	Bait						
				rpoA1	rpoA2	rpoB1	rpoB2	(rpoD)	rpoH	rpoL
108.8	HVO_0349	D4GZX6	rpoA1	bait	+	+	+	+	+	+
46.1	HVO_0350	D4GZX7	rpoA2	+	bait	+	+	+	+	+
67.7	HVO_0348	L9UJM2	rpoB1	+	+	bait	+		+	+
58.9	HVO_0347	L9UK99	rpoB2	+	+	+	bait	+	+	+
28.1	HVO_2781	L9V5W2	rpoD	+	+	+	+	bait	+	+
8.5	HVO_0346	D4GZX3	rpoH	+	+	+	+		bait	+
10.4	HVO_1042	D4GVL8	rpoL	+	+	+	+		+	bait
7.3	HVO_2776	D4GWX5	rpoN	+	+	+	+		+	+
5.1	HVO_0653	L9UMK6	rpoP	+		+	+		+	+
13.6	HVO_2748	D4GWA3	rpoF						+	

Supplementary Table 8. Overview of aIFs co-isolated with RNA polymerase subunits.
RpoD is shown in brackets as the yield after overproduction was very low.

MW [kDa]	Genloci	Accession	Description	Bait						
				rpoA1	rpoA2	rpoB1	rpoB2	(rpoD)	rpoH	rpoL
10.8	HVO_1946	D4GTH5	aIF1							
11.5	HVO_0136	D4GZ79	aIF1A-1							
11.2	HVO_A0637	D4GRU5	aIF1A-2							
29.5	HVO_0699	D4GT46	aIF2 α						+	
14.9	HVO_1678	D4GZP2	aIF2 β -1							
22.2	HVO_2242	L9VAS4	aIF2 β -2							
43.9	HVO_1901	D4GTD4	aIF2 γ							
43.2	HVO_1934	D4GTG3	aIF2B _{su}				+			
34.9	HVO_0966	L9USK7	aIF2B α	+	+	+	+	+	+	+
30.7	HVO_2706	L9V7F9	aIF2B δ							
104.5	HVO_1333	D4GXX1	eIF4A-hom.							
14.2	HVO_2300	L9V7A1	aIF5A							
65.3	HVO_1963	D4GTJ2	aIF5B	+	+	+	+		+	+
22.98	HVO_0117	D4GYW3	aIF6							

Supplementary Table 10. All (non-metabolic) proteins co-purified with RNA polymerase proteins.

Transcription factors that were also co-isolated with aIFs as baits are marked grey-blue.

MW [kDa]	Genloci	Accession	Description	Bait						
				rpoA1	rpoA2	rpoB1	rpoB2	(rpoD)	rpoH	rpoL
108.8	HVO_0349	D4GZX6	DNA-directed RNA polymerase subunit A1	+	+	+	+	+	+	+
46.1	HVO_0350	D4GZX7	DNA-directed RNA polymerase subunit A2	+	+	+	+	+	+	+
67.7	HVO_0348	L9UJM2	DNA-directed RNA polymerase subunit B1	+	+	+	+		+	+
58.9	HVO_0347	L9UK99	DNA-directed RNA polymerase subunit B2	+	+	+	+	+	+	+
28.1	HVO_2781	L9V5W2	DNA-directed RNA polymerase subunit D	+	+	+	+		+	+
8.5	HVO_0346	D4GZX3	DNA-directed RNA polymerase subunit H	+	+	+	+		+	+
10.4	HVO_1042	D4GVL8	DNA-directed RNA polymerase subunit L	+	+	+	+		+	+
7.3	HVO_2776	D4GWX5	DNA-directed RNA polymerase subunit N	+	+	+	+		+	+
5.1	HVO_0653	L9UMK6	DNA-directed RNA polymerase subunit P	+		+	+		+	+
13.6	HVO_2748	D4GWA3	DNA-directed RNA polymerase subunit F						+	
29.5	HVO_0699	D4GT46	Translation initiation factor 2 subunit alpha						+	
43.2	HVO_1934	D4GTG3	Translation initiation factor aIF-2B alpha subunit				+			
34.9	HVO_0966	L9USK7	Translation initiation factor IF-2B subunit delta	+	+	+	+	+	+	+
65.3	HVO_1963	D4GTJ2	Translation initiation factor aIF5B	+	+	+	+		+	+
28.3	HVO_2110	L9V9M0	ArcR family transcription regulator	+						
67.2	HVO_1590	L9VEI0	Chaperone protein DnaK					+		
17.7	HVO_0766	L9UNS7	Hsp20-type molecular chaperone	+						
12.6	HVO_1116	D4GW55	HTH domain protein			+		+		
11.7	HVO_1133	D4GW85	HTH domain protein	+		+	+	+	+	+
13.8	HVO_0962	D4GV69	HTH domain protein						+	
23.4	HVO_1695	D4H040	HTH domain protein	+		+	+		+	+
23.8	HVO_A0121	D4GQF3	HTH-10 family transcription regulator	+						
28	HVO_1501	D4GYE7	HTH-type transcriptional regulator GlpR	+					+	+
15.2	HVO_0450	D4GRZ1	Hsp20-type molecular chaperone						+	
28	HVO_2108	D4GUQ5	IcIR family transcription regulator	+	+	+	+	+	+	+
27.9	HVO_A0583	D4GRP3	IcIR family transcription regulator	+					+	
28.4	HVO_B0114	D4GPB5	IcIR family transcription regulator	+	+	+	+		+	+
28.1	HVO_B0201	D4GPK2	IcIR family transcription regulator	+			+	+		
18.1	HVO_1792	D4GSB6	Lrp/AsnC family transcription regulator	+		+	+	+	+	+
18.5	HVO_B0066	D4GP68	Lrp/AsnC family transcription regulator						+	
11.4	HVO_2869	D4GXH6	PadR family transcription regulator	+			+			+
25.1	HVO_0819	D4GUE2	SirR/DtxR family transcription regulator SirR	+		+	+		+	+
19.8	HVO_0163	L9UFW5	Transcriptional regulator	+		+	+	+	+	+
28	HVO_0179	L9UGU5	Transcriptional regulator	+		+	+		+	+
15.2	HVO_1360	D4GXQ1	TrmB family transcription regulator						+	
39	HVO_0568	L9UN99	Transcriptional regulator	+						
27.5	HVO_2928	L9UK32	Transcriptional regulator	+		+	+	+	+	+
30.9	HVO_2636	L9V878	Transcriptional regulator						+	
48.1	HVO_0853	D4GUK0	DNA double-strand break repair protein Mre11	+			+		+	
100	HVO_0854	D4GUK1	DNA double-strand break repair Rad50 ATPase	+		+	+	+	+	+
94.8	HVO_1573	D4GZ02	DNA gyrase subunit A						+	+
99.2	HVO_0552	D4GSE9	DNA mismatch repair protein MutS	+		+	+		+	+
150.5	HVO_0858	L9US19	DNA polymerase B1	+						
37.3	HVO_0104	Q48328	DNA repair and recombination protein RadA	+	+	+	+	+	+	+
24.6	HVO_2383	L9V6G6	DNA repair and recombination protein RadB	+	+	+	+	+	+	+
53.2	HVO_A0441	L9VPT6	DNA repair helicase Rad25	+						
76.8	HVO_1723	L9VE99	Putative DNA helicase Rad25	+		+	+	+		+

33.4	HVO_2558	D4GTZ0	30S ribosomal protein S3							+	
26.6	HVO_2552	L9V7Y2	30S ribosomal protein S4e	+	+	+	+			+	
23.3	HVO_2544	D4GTX6	30S ribosomal protein S5			+					
13.6	HVO_2373	D4GWM1	30S ribosomal protein S8e							+	
11.5	HVO_0360	L9ULL7	30S ribosomal protein S10	+		+				+	
18.9	HVO_2784	L9V5M6	30S ribosomal protein S13					+			
16	HVO_2560	L9V659	30S ribosomal protein S19P			+	+			+	
11.6	HVO_1896	D4GTC9	30S ribosomal protein S24e							+	
23.1	HVO_2757	P41199	50S ribosomal protein L1	+		+	+	+	+	+	+
25.6	HVO_2561	D4GTZ3	50S ribosomal protein L2	+		+	+	+	+	+	+
37.2	HVO_2564	L9V5K9	50S ribosomal protein L3	+		+	+	+	+	+	+
27	HVO_2563	D4GTZ5	50S ribosomal protein L4			+	+				
36.9	HVO_2756	P41198	50S ribosomal protein L10			+	+			+	
19.8	HVO_0484	D4GS24	50S ribosomal protein L10e			+					
16.8	HVO_2758	P41200	50S ribosomal protein L11	+		+	+	+			+
17.8	HVO_2542	L9V7W8	50S ribosomal protein L15			+					
22.5	HVO_0561	L9UPT6	50S ribosomal protein L15e	+		+	+			+	+
16.9	HVO_2559	L9V5K3	50S ribosomal protein L22	+	+	+	+			+	+
13.4	HVO_2553	L9V5Y5	50S ribosomal protein L24							+	
17.1	HVO_2543	L9V5X1	50S ribosomal protein L30	+		+	+			+	+
25.8	HVO_2547	D4GTX9	50S ribosomal protein L32e			+	+	+			
57.8	HVO_2575	L9V605	Elongation factor aEF-1 subunit alpha-like protein	+		+	+			+	+
45.6	HVO_2413	L9V6J4	Elongation factor 1-alpha	+	+	+	+			+	+
80.4	HVO_0356	L9UK07	Elongation factor 2	+	+	+	+	+	+	+	+
28.9	HVO_1284	D4GXB4	GTP cyclohydrolase III	+		+	+				+
33.5	HVO_2348	D4GWJ7	GTP cyclohydrolase MptA								+
67.4	HVO_0829	D4GUG3	Peptidase S9 family protein	+						+	
48.7		L9UQ37	Proteasome-activating nucleotidase							+	
46.6	HVO_0321	D4GZV0	Peptide chain release factor subunit 1	+		+				+	+
23.6	HVO_1931	D4GTG0	Probable GTP-binding protein EngB	+			+				+
8.3	HVO_2723	D4GW47	RNA-binding protein Lsm	+						+	
59.3	HVO_0455	O30560	Thermosome subunit 2	+	+	+	+	+	+	+	+
55.2	HVO_0778	Q9HHA2	Thermosome subunit 3	+		+	+	+	+	+	
14.3	HVO_3013	D4GYC7	Origin-associated protein							+	
42.2	HVO_0194	L9UGW2	ORC1-type DNA replication protein	+		+	+			+	+
42.4	HVO_1537	D4GYI1	Orc1-type DNA replication protein				+				
86.6	HVO_1703	D4H048	Putative DEAD/DEAH box helicase	+							
23.2	HVO_2438	L9V6L9	Ribonuclease H I	+			+	+	+	+	+
50.1	HVO_2724	L9V5P6	Ribonuclease J	+		+					+
25.6	HVO_1094	D4GW15	Ribonuclease P protein component 3	+			+	+	+	+	+
50.3	HVO_0388	L9UJI9	Ribonuclease R	+		+	+			+	+
34.1	HVO_0144	L9UJH9	Ribonuclease Z	+		+	+			+	+
19.9	HVO_1727	L9VGC6	TATA-box-binding protein			+	+	+	+	+	+