

Title	Stringency of start codon selection modulates autoregulation of translation initiation factor eIF5
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Supplementary Data

Supplementary Figure S1. Nucleotide sequences used for generating logograms.

Alignments of the 10 nucleotides surrounding the uAUG start codons of eIF5 that define their initiation contexts are shown for: (A) vertebrates; (B) arthropods; (C) nematodes; (D) molluscs; (E) Pezizomycotina; (F) Basidiomycota; (G) fungi other than Pezizomycotina or Basidiomycota; (H) plants. Species names are on the right. Species names in quotation marks specify presumed contaminant sequences that cluster with eIF5 sequences from an unrelated taxonomic group. Some plants have more than one paralog of eIF5. Here they are arbitrarily labelled “#1”, “#2”, “#3” or “#4” and their placement in one of the groups does not imply orthologous clusters. For each species the sequence on top is the context of the first uAUG and subsequent uAUG contexts are shown in descending order. The initiation codon is highlighted in green. Nucleotides in positions -3 and +4 are highlighted in red if they deviate from the Kozak consensus and in gray if they comply with it. Species names in quotation marks represent sequences derived from Expressed Sequence Tags that by similarity analysis of the main open reading frame do not match eIF5 homologs from the phylum to which the species belongs and instead likely belong to contaminating sequences of unknown species. These sequences are grouped with the taxonomic group to which they actually belong determined by reconstructed phylogenetic trees with the entire sequence.

A)

Vertebrata

GGG	UUU	AUGU	Homo sapiens
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Pan troglodytes verus
UCG	UUU	AUGU	
UUU	UUC	AUGU	
GGG	UUU	AUGU	Macaca fascicularis
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Macaca mulatta
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Pongo abelii
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Canis lupus familiaris
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Ursus americanus
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Equus caballus
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Peromyscus polionotus
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Bos taurus
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Sus scrofa
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Rattus norvegicus
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Mus musculus
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Cavia porcellus
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Ovis aries
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Oryctolagus cuniculus
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Taeniopygia guttata
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Gallus gallus
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Lonchura striata domestica
UUU	UUC	AUGU	
UCG	UUU	AUGU	
GGG	UUU	AUGU	Meleagris gallopavo
UUU	UUC	AUGU	

UCG	UUU	AUGU	
GGG	UUU	AUGU	Bungarus multicinctus
UUU	UCAUGU		
UCG	UUU	AUGU	
GGG	UUU	AUGU	Anolis carolinensis
UUU	UCAUGU		
UCG	UUU	AUGU	
GUG	UUU	AUGU	Xenopus tropicalis
AUU	UCAUGU		
UCG	UUU	AUGU	
GUG	UUU	AUGU	Xenopus laevis
AUU	UCAUGU		
UUG	UUU	AUGU	
GUG	UUU	AUGU	Ambystoma mexicanum
AUU	UCAUGU		
UUG	UUU	AUGU	
GUG	UUU	AUGU	Ambystoma tigrinum tigrinum
AUU	UCAUGU		
UUG	UUU	AUGU	
GUG	UUU	AUGU	Cynops pyrrhogaster
AUU	UCAUGU		
UUG	UUU	AUGU	
GUG	UUU	AUGU	Gasterosteus aculeatus
UUU	UCAUGU		
UCG	UUU	AUGU	
GUG	UUU	AUGU	Perca flavescens
UUU	UCAUGU		
UCG	UUU	AUGU	
GUG	UUU	AUGU	Miichthys miiuy
UUU	UCAUGU		
UCG	UUU	AUGU	
GUG	UUU	AUGU	Lates calcarifer
UUU	UCAUGU		
UCG	UUU	AUGU	
GUG	UUU	AUGU	Takifugu rubripes
UUU	UCAUGU		
UCG	UUU	AUGU	
GUG	UUU	AUGU	Siniperca chuatsi
UUU	UCAUGU		
UCG	UUU	AUGU	
UUG	UUU	AUGU	Osmerus mordax #1
UUU	UCAUGU		
UCG	UUU	AUGU	
GUG	UUU	AUGU	Osmerus mordax #2
CGAC	GGAUGG		
UUU	UCAUGU		
UUG	UUU	AUGU	
GUG	UUU	AUGU	Xiphophorus maculatus
UUU	UCAUGU		
UCG	UUU	AUGU	
GUG	UUU	AUGU	Gadus morhua
UUU	UCAUGU		
CCG	UUU	AUGU	
GUG	UUU	AUGU	Oryzias latipes
UUU	UCAUGU		
UUG	UUU	AUGU	
GUG	UUU	AUGU	Lipochromis sp.
AUA	UUU	AUGG	
UUU	UCAUGU		
UUG	UUU	AUGU	
UUG	UUU	AUGU	Salmo salar #1

UUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Salmo salar #2
CGACGGAUGG	
UUUJUCAUGU	
UCGUUU AUGU	
UUGUUU AUGU	Oncorhynchus mykiss #1
UUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Oncorhynchus mykiss #2
CGACGGAUGG	
UUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Oncorhynchus tshawytscha #1
UUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Oncorhynchus tshawytscha #2
CGACGGAUGG	
UUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Oncorhynchus nerka #2
CGACGGAUGG	
UUUJUCAUGU	
UCGUUU AUGU	
UUGUUU AUGU	Thymallus thymallus
UUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Esox lucius
UAACCAAUGU	
UGGCUAAUGU	
AGUUACAUGU	
AUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Misgurnus anguillicaudatus
UUUJUCAUGU	
UUGUUU AUGU	
GUGUUU AUGU	Pimephales promelas #1
UUUJUCAUGU	
UUGUUU AUGU	
GUGUUU AUGU	Pimephales promelas #2
CUACGGAUGG	
CUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Danio rerio #2
CUACGGAUGG	
CUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Ictalurus furcatus #1
UUUJUCAUGU	
UUGUUU AUGU	
GUGUUU AUGU	Ictalurus furcatus #2
CUGCGGAUGG	
AUUJUCAUGU	
UCGUUU AUGU	
GUGUUU AUGU	Ictalurus punctatus #1
UUUJUCAUGU	
UUGUUU AUGU	
GUGUUU AUGU	Ictalurus punctatus #2
CUGCGGAUGG	
AUUJUCAUGU	
UCGUUU AUGU	

GUGUUUAUGU	Cynoglossus semilaevis
UUUUUCAUGU	
UCGUUUUAUGU	
GUGUUUAUGU	Squalus acanthias
UUUUUCAUGU	
UUGUUUAUGU	
GUGUUUAUGU	Leucoraja erinacea
UUUUUCAUGU	
UUGUUUAUGU	
GGUUUUUAUGU	Petromyzon marinus
AUUUUUCAUGU	
GAUUUUUAUGU	Eptatretus burgeri
AUUUUUCAUGU	
CAGUUUAUGU	

B)
Arthropoda

UUUUUAUAUGA	Tribolium castaneum
AGGUGUAUGA	
UUUUUAUAUGA	Dendroctonus ponderosae
AGGUGUAUGA	
UUUUUAUAUGA	Aedes aegypti
UUGUGCAUGA	
UUUUUAUAUGA	Anopheles gambiae
ACGUGCAUGA	
UCUUUAUAUGA	Drosophila melanogaster
CUGUGCAUGA	
GUACACAUGA	
UCUUUAUAUGA	Drosophila willistoni
CUGUGCAUGA	
UCUUUAUAUGA	Drosophila serrata
CUGUGCAUGA	
GUACACAUGA	
UCUUUAUAUGA	Drosophila erecta
CUGUGCAUGA	
GUACACAUGA	
AAUCUUUAUGA	Drosophila ananassae
UGACUUUAUGA	
UGGUGCAUGA	
GUACACAUGA	
UCUUUAUAUGA	Phlebotomus papatasi
UCGUGCAUGA	
UCUUUAUAUGA	Phlebotomus sergenti
UCGUGCAUGA	
AUUUAUAUGA	Acyrtosiphon pisum
AGGUGCAUGA	
UUUUUAUAUGA	Schistocerca gregaria
ACCUACAUGC	
AGGUGUAUGA	
UUUUUAUAUGA	Teleopsis dalmanni
UCGUGCAUGA	
UUUUUAUAUGA	"Quercus robur"
UCGUGCAUGA	
GCGUGUAUGA	Bombus terrestris
UUUUUAUAUGA	Nilaparvata lugens
AGGUGUAUGA	
UUUUUAUAUGA	Parasteatoda tepidariorum
AGGUUUUAUGC	
UUUUUAUAUGA	"Selaginella moellendorffii"
AGGUUUUAUGC	

UUUUAUAUGA	Limulus polyphemus #1
CCGUUUUAUGA	
UUUUAUAUGA	Limulus polyphemus #2
UUGUUUAUGA	
UUUUAUAUGA	Rhipicephalus appendiculatus
AGGUUUUAUGC	
CUUGUAUAUGC	
UUUUAUAUGA	Amblyomma variegatum
AGGUUUUAUGC	
UUUGUAUAUGC	
UUUUAUAUGA	Loxosceles laeta
AGGUUUUAUGC	
UUUUGUAUGC	Campodea fragilis
GAAUUGAUGC	
AUUUAUAUGA	Aphis gossypii
AGGUGCAUGA	
UUUUAUAUGA	Locusta migratoria
GGGUGCAUGA	
CUGUGUAUGU	Calanus finmarchicus
GAUUUUUAUGA	Polypedilum vanderplanki
AAGUGCAUGU	
UUUUUCAUGA	Petrolisthes cinctipes
UCUUGUAUGU	
ACUUUCAUGA	Penaeus monodon
UCUUGUAUGU	
ACUUUCAUGA	Callinectes sapidus
UCUUGUAUGU	
UUUUAUAUGA	Glossina morsitans
UCGUGCAUGA	
UUUUAUAUGA	Ceratitis capitata
UCGUGCAUGA	
UCUUAUAUGA	Rhynchosciara americana
CCUUUUUAUGA	Chironomus tentans
CAGUGCAUGA	
UUUUAUAUGA	Spodoptera frugiperda
GCGUGCAUGA	
UUUUAUAUGA	Spodoptera litura
GCGUGCAUGA	
UUUUAUAUGA	Gryllus bimaculatus
ACCUACAUGC	
AGGUGUAUGA	
AAACUUUAUGG	Pediculus humanus capitis
ACGUGUAUGA	

C)

Nematoda

UUGUGCAUGU	Caenorhabditis remanei
AAUUUUUAUGA	
CGGUGUAUGC	
UUGUGCAUGU	Caenorhabditis elegans
AAUUUUUAUGA	
UAGUGUAUGC	
UUGUGCAUGU	Caenorhabditis japonica
AAUUAUAUGA	
CAGUGUAUGC	
UUGUGCAUGU	Heterorhabditis bacteriophora
GAUUUUUAUGA	
CAUCGUAUGC	
AGUUGCAUGU	Wuchereria bancrofti
GAUUUUUAUGG	

UCACGUAUGC	
AGUUGCAUGU	Brugia malayi
GAUJUUAUGG	
UCACGUAUGC	
AGUUGUAUGU	Ascaris suum
UAUJUUAUGG	
CCCCGUAUGC	
UUGUGCAUGU	Angiostrongylus cantonensis
AAUJUUAUGC	
CAUCGUAUGC	
UUGUGCAUGU	Ancylostoma ceylanicum
GAUJUUAUGC	
UAUCGUAUGC	
AGGUGUAUGA	Bursaphelenchus mucronatus
CGUJUUAUGG	
UAACGUAUGC	

D)

Mollusca

GUUJUUAUGA	Lottia gigantean
AGGUJUUAUGA	
AUUJUUAUGA	Aplysia californica
GCGJUUAUGA	
AUUJUUAUGA	Aplysia kurodai
GCGJUUAUGA	
AUUJUUAUGA	Lymnaea stagnalis
GCGJUAUGA	
GUUJUUAUGA	Crassostrea gigas
GGGUJUUAUGA	
GGUJUUAUGA	Crassostrea virginica
GGGUJUUAUGA	
AGUCGUAUGA	Mytilus californianus
AGUJUUAUGA	

E)

Pezizomycotina

UUGJUUAUGU	Neurospora crassa
GCGJUA AUGC	
UACCCUAUGU	
UUGJUUAUGU	Podospora anserine
CUGCCCAUGU	
CACCACAUGU	
GAGJUUAUGC	Mycosphaerella graminicola
GAGJUUAUGC	
UGCUGCAUGU	
CAGJUUAUGU	Aureobasidium pullulans
AAUUGCAUGU	
CUGUCUAUGU	Trichophyton rubrum
CUACGCAUGU	
UUGJUUAUGU	Hypocrea jecorina
GAGJUUAUGC	
CGCCACAUGU	
CUGJUUAUGU	Gibberella moniliformis
UCGUJUAUGC	
UGCCACAUGU	
CUAJACAUGA	
UUGJUUAUGU	Thielavia terrestris
GCGJUA AUGC	
UAAUGCAUGC	
UACCCUAUGU	

CUGUCUAUGU	Coccidioides posadasii
CUACGCAUGU	
UUGUCUAUGU	Aspergillus niger
CCACGCAUGU	
CUGUUUAUGU	Trichoderma atroviride
GAGUJCAUGC	
CUCCCUAUGU	
CGCUJCAUGC	
UUGUUUAUGU	Trichoderma virens
UCGUJCAUGC	
CGCCCUAUGU	
CUCUJGAUGC	
CGUUAUAUGU	Geomyces pannorum
CAACACAUGU	
CUCUAUAUGU	
GAGUAUAUGU	Cochliobolus heterostrophus
CGCUGUAUGC	
UUGCGCAUGU	
UUGUUUAUGU	Ophiostoma piliferum
CAGCACAUGC	
UUGUJCAUGA	Tuber melanosporum
CAGUJCAUGC	
GGGUUUUAUGU	Cercospora zeae-maydis
UACCGCAUGU	
CGUUAUAUGU	Amorphotheca resinae
CUUCGAAUGU	
UUCUGCAUGU	
UGUJCCAUGU	
UUGUUUAUGU	"Panicum virgatum"
GCGUJAAUGC	
UACCCUAUGU	
CGUCUCAUGA	
UUGUUUAUGU	Myceliophthora thermophila
GCGUJAAUGC	
UACCCUAUGU	
UUGUUUAUGU	Thielavia heterothallica
GCGUJAAUGC	
UACCCUAUGU	
CUGUUUAUGU	Fusarium sporotrichioides
UUGUCUAUGC	
UUGUUUAUGU	"Wrightia tinctoria"
GCGUJAAUGC	
UAAUGCAUGC	
UACCCUAUGU	
UUGUUUAUGU	Cordyceps militaris
GAGUJCAUGC	
AACCUCAUGU	
GUCUJCAUGA	
UUGUJCAUGA	"Picea glauca"
CGGUJCAUGC	
UUGUUUAUGU	Sordaria macrospora
GCGUJAAUGC	
UACCCUAUGU	
CUGUUUAUGU	Magnaporthe oryzae
GCGUJCAUGC	
UCCCUAUGU	
CAGUCUAUGU	
CUGUUUAUGU	Magnaporthe poae
GCGUJCAUGC	
UCCCUAUGU	
AAGUJCAUGU	

CUGUUUAUGU	Gaeumannomyces graminis
GCGUUCAUGC	
UCCCUUAUGU	
AAGUUCAUGU	
UUGUUUAUGU	Trichoderma reesei
GAGUUCAUGC	
CGCCACAUGC	
CUCUUGAUGC	
CUGUUUAUGU	Nectria haematococca
UUGUCUAUGC	
UGCCACAUGC	
CUCUUCAUGA	
CAGUUUAUGU	Glomerella graminicola
GAGUUCAUGA	
UACCACAUGC	
UUCUUAUGC	
CUGUUUAUGU	Gibberella zeae
CUGUCUAUGC	
UGCCACAUGA	
CUAUACAUGA	
UUGUCUAUGU	Neosartorya fischeri
CUACGCAUGU	
CUGUUUAUGU	Fusarium oxysporum
UCGUUCUAUGC	
UGCCACAUGC	
CUAUACAUGA	
CUGUUUAUGU	Verticillium dahliae
GAGUUCAUGU	
UACCCCAUGU	
UCUUCCAUGU	
UUGUCUAUGU	Aspergillus fumigatus
UGCUGUAUGA	
CUACGCAUGU	
UUGUUUAUGU	Grosmannia clavigera
GCGUUCAUGC	
UCCCUUAUGU	
UUCUUCAUGC	
UUGUCUAUGU	Aspergillus clavatus
CUACGCAUGU	
CGUUUAUGU	Geomyces destructans
CAACACAUGC	
CUCUAUAUGU	
CUGUUUAUGU	Metarhizium acridum
GAGUUCAUGC	
UGCCCAUGU	
ACCUUCAUGC	
CUGUUCAUGU	Talaromyces stipitatus
UACCGCAUGU	
UUGUCUAUGU	Aspergillus terreus
UGACGCAUGU	
CGUUUAUGU	Botryotinia fuckeliana
AUUCGAAUGU	
UUCUGCAUGU	
UUGUCUAUGU	Aspergillus nidulans
CGGCGUAUGU	
CGUUUAUGU	Blumeria graminis
CGGCGAAUGU	
CAUCAUAUGU	
UUGUCUAUGU	Aspergillus flavus
AUACGCAUGU	
GAGUAUAUGU	Pyrenophora teres

CGCUUAUGC	
CUGCGCAUGU	
GAGUAUAUGU	Pyrenophora tritici-repentis
CGCUUAUGC	
CUGCGCAUGU	
CUGUJCAUGU	Penicillium marneffeii
UACCGCAUGU	
CUGUUUAUGU	Epichloe festucae
GAGUJCAUGC	
UGCCCUAUGU	
GUGUUUAUGU	
CGGUUAUAUGU	Phaeosphaeria nodorum
UACUGCAUGU	
CGUUAUAUGU	Sclerotinia sclerotiorum
AUUCGAAUGU	
UUCUGCAUGU	
CUGUUUAUGU	Verticillium albo-atrum
GAGUJCAUGU	
UACCCAUGU	
UCUJCCAUGU	
CUGUUUAUGU	Metarhizium anisopliae
GAGUJCAUGC	
UGCCCAUGU	
AUCUJCAUGC	

F)
Basidiomycota

GGAUACAUGC	Postia placenta
GGAUJCAUGC	Trametes versicolor
GGAUJCAUGC	Phanerochaete chrysosporium
GGAUJCAUGC	Heterobasidion annosum
GGAUJCAUGC	Pleurotus ostreatus
GGAUJCAUGC	Schizophyllum commune
GGAUJCAUGC	Lentinula edodes
GAAUJCAUGC	Coprinopsis cinerea
GGAUJCAUGC	Gloeophyllum trabeum
CGGUJCAUGC	Leucosporidium scottii
CAGCUUAUGU	Microbotryum violaceum
UCGUJCAUGC	Melampsora larici-populina
GAGUJCAUGC	Tremella mesenterica
GAGUJCAUGC	Cryptococcus neoformans
UUGUJCAUGC	Cryptococcus laurentii
UCGUUAUAUGU	Phakopsora pachyrhizi
GUUUUAUGU	Ustilago maydis
AAUJCAUGC	Paxillus involutus

G)
Other fungi

UAGUJCAUGC	Cunninghamella elegans
UUGUACAUGU	Mucor circinelloides
CUGUJCAUGC	Phycomyces blakesleeanus
CGCUJCAUGC	Glomus intraradices
CUGUACAUGU	Piromyces sp.
CGCUJCAUGA	Spizellomyces punctatus

H)
Plants

UCUUUAUGU	Oryza sativa #1
UGCUJCAUGC	

CAAUUUAUGC	
UCUUUUUAUGU	Oryza sativa #2
UCUUUUUAUGU	Sorghum bicolour #1
CACUGCAUGC	
UCUUUUUAUGU	Sorghum bicolour #2
CACUGCAUGC	
UCUUUUUAUGU	Populus trichocarpa #1
GGCUGCAUGC	
GCUUUUAUGU	Populus trichocarpa #2
AGCUGUAUGC	
UUUUUUUAUGU	Populus trichocarpa #3
ACACAGAUGC	
UCUUUUUAUGU	Populus tremula #1
GGCUGUAUGC	
UGUUUUUAUGA	
GCUUUUAUGU	Populus tremula #2
AGCUGUAUGC	
UCUUUUUAUGU	Populus deltoids #1
GGCUGCAUGC	
UUUUUUUAUGU	Populus deltoides #3
AGCUAUUAUGC	
UCUUUUUAUGU	Populus nigra #1
GGCUGCAUGC	
UUUUUUUAUGU	Populus nigra #3
ACACAGAUGC	
AGCUAUUAUGC	
GCUUUUAUGU	Populus fremontii #2
AGCUGUAUGC	
UCUUUUUAUGU	Zea mays #1
CACUGCAUGC	
UCUUUUUAUGU	Zea mays #2
CACUGCAUGC	
UCUUUUUAUGU	Arabidopsis thaliana
CAGUUUAUGC	
UCUUUUUAUGU	Vitis vinifera #1
AGCUGCAUGC	
AGGUUUUAUGU	
UCUUUUUAUGU	Vitis vinifera #2
GACUGUAUGC	
AGCUGGAUGC	
UCUUUUUAUGU	Vitis shuttleworthii #1
AGCUGCAUGC	
AGGUUUUAUGU	
UCUUUUUAUGU	Vitis shuttleworthii #2
GACUGUAUGC	
AGCUGGAUGC	
GCUUUUAUGU	Crocus sativus
GACUGCAUGA	
UCGUUCAUGC	
UCUUUUUAUGU	Nicotiana tabacum #1
GACUGUAUGC	
UCUUUUUAUGU	Nicotiana tabacum #2
GACUGUAUGC	
UCUUUUUAUGU	Nicotiana tabacum #3
GGCUGCAUGC	
UCUUUUUAUGU	Nicotiana benthamiana #2
GACUUUAUGC	
UUUUUUUAUGU	Nicotiana benthamiana #3
GGCUGCAUGC	
UCUUUUUAUGU	Amborella trichopoda
ACUCAGAUGC	

AGAUGCAUGC	
ACUJUAAUGC	
UCUJUUAUGU	Quercus petraea
GGCUGCAUGC	
UGGJAAAUGA	
UCUJUUAUGU	Petunia axillaris
GACUGUAUGC	
UCUJUUAUGU	Petunia x hybrida
GACUGUAUGC	
UCUJUUAUGU	Quercus robur
GGCUGUAUGC	
AGUJUGAUGG	
UCUJUUAUGU	Triticum aestivum #1
CCCUGCAUGC	
UCUJUUAUGU	Triticum aestivum #2
CCCUGCAUGC	
UCUJUUAUGU	Triticum aestivum #3
CCCUGCAUGC	
UCUJUUAUGU	Lolium multiflorum
CCCUGUAUGC	
UCUJUUAUGU	Lolium perenne
CCCUGUAUGC	
UCUJUUAUGU	Lolium temulentum
CCCUGUAUGC	
UCUJUUAUGU	Avena sativa #1
CCCUGUAUGC	
UCUJUUAUGU	Avena sativa #2
CCCUGUAUGC	
UCUJUUAUGU	Avena barbata
CCCUGUAUGC	
UCUJUUAUGU	Festuca pratensis
CCCUGUAUGC	
UCUJUUAUGU	Festuca arundinacea
CCCUGUAUGC	
UCUJUUAUGU	Hordeum vulgare #1
CCCUGCAUGC	
UCUJUUAUGU	Hordeum vulgare #2
CCCUGCAUGC	
UCUJUUAUGU	Brachypodium distachyon
CCCUGCAUGC	
UUUJUUAUGU	Lactuca sativa #1
CUUJAAUUGC	
AUUJUUAUGU	Lactuca sativa #2
ACCUJUAUGC	
GCUJUUAUGU	Iris brevicaulis
CGCUGCAUGC	
UCGUJUUAUGC	
UCUJUUAUGU	Cynodon dactylon
CACUGCAUGC	
UCUJUUAUGU	Zostera marina
UGUUGCAUGA	
AGUUGGAUGC	
UCUJUUAUGU	Dioscorea alata
GGCUGCAUGC	
UCUJUUAUGU	Prunus persica #1
AGCUGUAUGC	
AGUJUCAUGG	
UAUJUUAUGU	Prunus persica #2
AUUUGGAUGC	
GGAUGCAUGC	
CGGUJUUAUGC	

UCUJUUAUGU	Solanum torvum
GGCUGUAUGC	
UCUJUUAUGU	Solanum lycopersicum #1
GACUGUAUGC	
UCUJUUAUGU	Solanum lycopersicum #3
UAUUGUAUGU	
GGGUGAUGA	
GACUGUAUGC	
UCUJUUAUGU	Solanum tuberosum #1
AACUGUAUGC	
UCUJUUAUGU	Solanum tuberosum #2
GACUGUAUGC	
UCUJUUAUGU	Solanum tuberosum #3
GACUGUAUGC	
UCUJUUAUGU	Solanum chacoense
GACUGUAUGC	
UCUJUUAUGU	Actinidia deliciosa #1
GACUGUAUGC	
UCUJUUAUGU	Actinidia arguta #2
GACUGUAUGC	
UCUJUUAUGU	Actinidia arguta #3
GACUGUAUGC	
UCUJUUAUGU	Actinidia chinensis #1
GACUGUAUGC	
UCUJUUAUGU	Actinidia chinensis #2
GACUGUAUGC	
UCUJUUAUGU	Liriodendron tulipifera
GGUGUCAUGC	
AGUUGCAUGC	
UCUJUUAUGU	Agrostis capillaris
CCCUGUAUGC	
UUUJUUAUGU	Jatropha curcas
GGCUGCAUGC	
CAGJUUAUGC	
UCUJUUAUGU	Ricinus communis
GCCUGCAUGC	
CAGJUUAUGC	
UUUCCAUGC	Limnanthes alba
UCUJUUAUGU	
UGCUGUAUGC	
AUUJUAUGG	
UUUJUUAUGU	Carthamus tinctorius
GGCUGUAUGC	
UUUJUUAUGU	Euphorbia esula
AUCUGCAUGC	
CAGJUUAUGC	
UCUJUUAUGU	Coffea arabica
GACUGCAUGC	
AGUUGUAUGA	
UCUJUUAUGU	Coffea canephora
GACUGCAUGC	
AGUUGUAUGA	
UCUJUUAUGU	Theobroma cacao
AUCUGCAUGC	
UUUJUUAUGU	Fagus sylvatica
GGCUGUAUGC	
AGUUGAUGG	
UCUJUUAUGU	Triphysaria pusilla
CCGUAUUGU	
UCUJUUAUGU	Ficus elastica
AGCUGUAUGC	

CUGUUUAUGC	
UCUUUUUAUGU	Manihot esculenta
GGCUGCAUGC	
CAGUUUAUGU	
UCUUUUUAUGU	Carica papaya
AGCUGCAUGC	
CAGUUUAUGC	
UAUUUUUAUGU	Malus x domestica
UACUUGAUGA	
AGUUGAAUGC	
CAAUUUUAUGC	
UCUUUUUAUGU	Citrus clementina #1
GGCUGUAUGC	
UUUUUUUAUGU	Citrus clementina #2
GGCUGCAUGC	
CUGUUUAUGC	
UCUUUUUAUGU	Citrus sinensis
GGCUGUAUGC	
UCUUUUUAUGU	Citrus reticulata
GGCUGUAUGC	
UCUUUUUAUGU	Citrus aurantium
GGCUGUAUGC	
UCUUUUUAUGU	Citrus trifoliata
GGCUGUAUGC	
UCUUUUUAUGU	Diospyros kaki
GGCUGCAUGC	
UCGUUAUUGC	
UUUUUUUAUGU	Beta vulgaris
AGUUGUAUGC	
AAUUUUUAUGC	
UCUUUUUAUGU	Euonymus alatus
GCCUGCAUGA	
CGUUUUUAUGC	
UAUUUUUAUGU	Rubus ulmifolius
AGUUGAAUGC	
CAAUUUUAUGC	
UCUUUUUAUGU	Gossypium raimondii
AUCUGCAUGC	
CAUUUUUAUGC	
UCUUUUUAUGU	Gossypium hirsutum #1
AUCUGCAUGC	
CAUUUUUAUGC	
UCUUUUUAUGU	Gossypium hirsutum #2
AGCUGCAUGC	
CGUUUUUAUGC	
UCUUUUUAUGU	Gossypium hirsutum #3
AUCUGCAUGC	
UCUUUUUAUGU	Gossypium arboreum #1
AUCUGCAUGC	
CAUUUUUAUGC	
UCUUUUUAUGU	Gossypium arboreum #2
AGCUGCAUGC	
CGUUUUUAUGC	
UCUUUUUAUGU	Gossypium arboreum #3
AUCUGCAUGC	
UCUUUUUAUGU	Gossypium barbadense
AUCUGCAUGC	
CAUUUUUAUGC	
UCUUUUUAUGU	Panax ginseng
CAUCUAAUGA	
GGCUGCAUGC	

CAGCUC	AUGC	
UCU	UUUAUGU	Capsicum annuum
GAC	UGUAUGC	
UCU	UUUAUGU	Catharanthus roseus
GAC	UGUAUGC	
UAU	UUUAUGU	Cannabis sativa
GGC	UGUAUGC	
AAG	UGUAUGC	
UCU	UUUAUGU	Aquilegia formosa
GAC	UGCAUGC	
UAU	UUUAUGU	
CCU	UUUAUGU	Ipomoea nil
GGC	UGCAUGC	
UCU	UUUAUGU	Striga hermonthica
GGC	UGCAUGC	
CAU	UUUAUGU	
CCU	UUUAUGU	Cucumis melo
GGC	UGCAUGC	
CAU	UUUAUGU	
UUU	UUUAUGU	Barnadesia spinosa #1
GGC	UGUAUGC	
UCU	UUUAUGU	Barnadesia spinosa #2
GGC	UGUAUGC	
UCG	UUUAUGU	Elaeis oleifera
GGC	UGCAUGC	
UAU	UGUAUGC	
UUU	UUUAUGU	Wrightia tinctoria
GAC	UGUAUGC	
AGU	UCAUGG	
UUU	UUUAUGU	Cynara cardunculus
GGC	UGUAUGC	
UUU	UUUAUGU	Taraxacum kok-saghyz
ACC	UGCAUGC	
UCU	UUUAUGU	Mimulus guttatus
AGC	UGCAUGA	
UCU	UUUAUGU	Brassica napus #1
UCU	UUUAUGU	Brassica napus #2
UCU	UUUAUGU	Brassica oleracea #2
UCU	UUUAUGU	Brassica rapa #2
UCU	UUUAUGU	Eucalyptus globulus
UGU	UGGAUGC	
GGG	UUUAUGC	
AUU	UUUAUGU	Helianthus petiolaris
GGC	UGUAUGC	
AUU	UUUAUGU	Helianthus exilis
GGC	UGUAUGC	
UCU	UCAUGU	Glycine max #1
AGC	UGUAUGC	
UCU	UCAUGU	Glycine max #2
AGU	UGUAUGA	
UCU	UCAUGU	Glycine max #3
AGU	UGUAUGA	
UCU	UCAUGU	Glycine max #4
ACC	UGUAUGC	
UCU	UCAUGU	Phaseolus coccineus
AGC	UUUAUGC	
UCU	UCAUGU	Phaseolus vulgaris #1
AGC	UGUAUGC	
UCU	UCAUGU	Phaseolus vulgaris #2
AGC	UGUAUGC	
UCU	UCAUGU	Phaseolus acutifolius #1

AGCU	GCAUGC	
UCUU	JUCAUGU	Phaseolus acutifolius #2
AGCU	JGUAUGC	
UUUU	JUUUAUGU	Guizotia abyssinica
GCCU	JGUAUGC	
UCUU	JUCAUGU	Vigna unguiculata
AUCU	JGUAUGC	
UCUU	JUUUAUGU	Leymus cinereus
CCCU	JGCAUGC	
UCUU	JUUUAUGU	Pinus taeda
GGUU	JGGAUGC	
GCCU	JGUAUGC	
AGUU	JGGAUGC	
UCUU	JUUUAUGU	Pinus radiata
GGUU	JGGAUGC	
GCCU	JGUAUGC	
AGUU	JGGAUGC	
CCUU	JUUUAUGU	Pinus banksiana
UUGU	JUUUAUGU	
GAAU	JAAAUGU	
UUUU	JGCAUGC	
UCUU	JUUUAUGU	Pinus contorta
GGUU	JGGAUGC	
AGUU	JGGAUGC	
UCUU	JUUUAUGU	Picea sitchensis
GCCU	JGUAUGC	
GCCU	JAUUAUGA	
AGUU	JGGAUGC	
UCUU	JUUUAUGU	Picea glauca
GCCU	JGUAUGC	
AGUU	JGGAUGC	
UCUU	JUUUAUGU	Picea engelmannii
GCCU	JGUAUGC	
AGUU	JGGAUGC	
UCUU	JUUUAUGU	Selaginella moellendorffii
UGCU	JGCAUGC	
UCUU	JUUUAUGU	Selaginella lepidophylla
GCCU	JGCAUGC	
GAGU	JUCAUGC	
UCUU	JUUUAUGU	Physcomitrella patens
GGUU	JGCAUGC	

Supplementary Figure S2. Weblogo representation of amino acid conservation in eIF5 uORF1 encoded peptides.

Letter heights are proportional to the frequency of conservation of each amino acid at each position. Each line represents a different eukaryotic branch which is indicated in parentheses on the right followed by the number of sequences used in each alignment. The sequences used to generate the alignment presented in this figure are available upon request.

Supplementary Figure S2

MSLFPENELLRSHHWXLYWGNIAYKQDAEAFSSSGEFVFFMSIEIEHNSCSRLCHPEFSRQKIPKSNRSEFLADKATNKKKCLSTSTAAQTSSVATRCPV (Vertebrata, 48)

MTLRLRPRPLVWHSYIIEAKLEARRSLRSLAFMNFVQYDQLKLSKIQSSLRHKRKF...LXINERSSWLSQVNFATsVTHSTθ | RCLGS (Mollusca, 6)

MTLESLRLDGLDQNSLQVLP...RPFEPQ...V...LRHRKLRPPFET... (Arthropoda, 25)

MFLEP...CRFILVDINPQEGRLSRKRKLLK...EST...L...DA...R...YCYLP...R...L...V...R...R...L...D...L...N...R...G...S...Q...R...S...L...R...L...Q...N...S...R...L...N...Q...S... (Nematoda, 9)

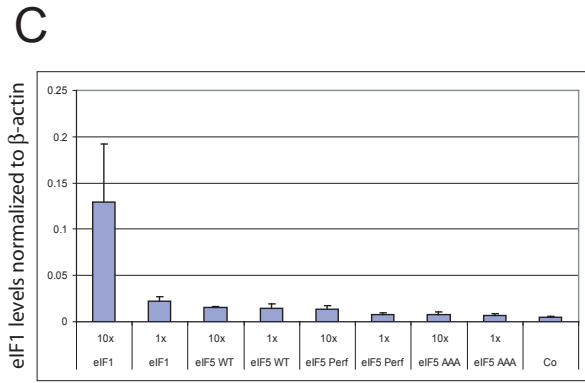
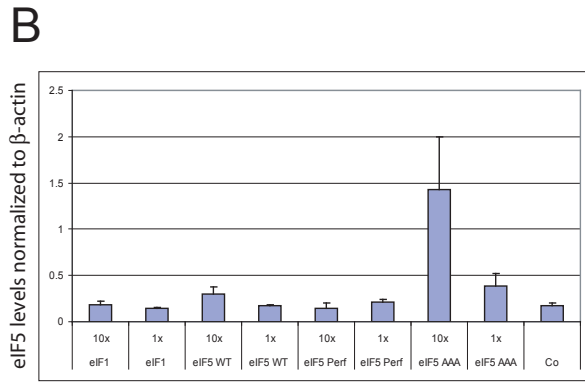
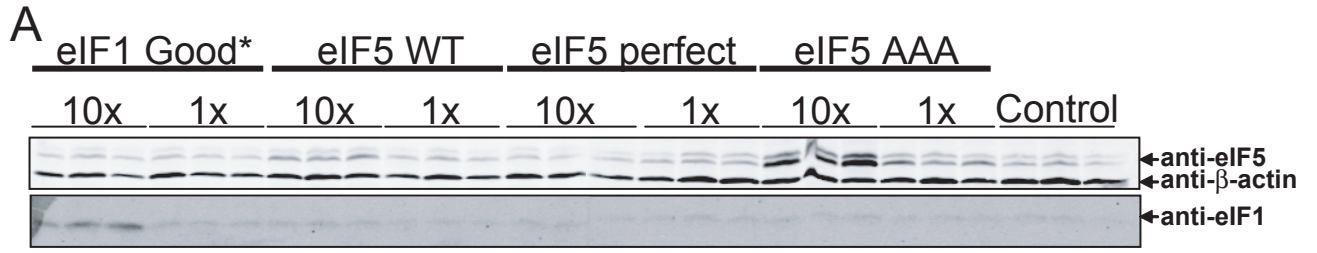
MV...R...R...V...R...R...L...R...K...P...S...R...G... (Pezizomycotina, 52)

MLRPP...LR...R...R...E...R...R...S...H...R... (Basidiomycota, 10)

MSE...R...L...Y...L...G...S...J...G...L...L...G...E...Y...C...P...R...S...B...K...K...V...R...R...C...M...H...L...R...S...R...R...L...S...R...R...S...S...R...S... (Plantae, 135)

Supplementary Figure S3. Removing eIF5 uORFs results in constitutive eIF5 expression. (A) Western blots of protein lysates from cells transfected in triplicate with the eIF5 or eIF1 overexpression constructs indicated in Figure 2B of the main text. The eIF1 overexpression construct is the “eIF1 good*” described previously (2). In lanes marked “10×”, 10-fold more vector with insert was transfected compared with lanes marked “1×,” where the difference in the amount of transfecting DNA is made up with the inert vector pcDNA3 (2). The control cells are transfected with “10×” amount of pcDNA3. The blot shown was probed with anti-eIF5 and anti-β-actin followed by anti-eIF1. The corresponding detected proteins are indicated by arrows. (B & C) Densitometry analysis of the western blot shown in A. Relative eIF5 levels (B) and eIF1 levels (C) were determined by normalizing to β-actin levels.

Supplementary Figure S3



Supplementary Table S1.

Oligonucleotides used in this study.

1	eIF5 wt S PstI	ATAACTGCAGCCAGCCAATGGGCAGTGAGG
2	eIF5 wt AS 5' intron	AACCTTGATACTTACCGTTTTTCGTCAAATAAAGACATAAACCCAACGC
3	eIF5 wt S 3' intron	TTTCTCTCCACAGAGCTGTTGCGCAGCCATTGG
4	eIF5 wt AS BamHI	TTATGGATCCAGACATTTTGGCTTATTAGTGG
5	eIF5 wt S 5' intron	TTTATTTGACGAAAACGGTAAGTATCAAGGTTAC
6	eIF5 wt AS 3' intron	AATGGCTGCGCAACAGCTCTGTGGAGAGAAAGG
7	eIF5 wt S	AAGCCAAAATGTCTGTCAATGTCAACCGC
8	eIF5 wt stop AS XbaI	TTATCTAGACCTTTAAATGGCATCAATATCG
9	eIF5 wt start S SacI	ATAAGAGCTCGTTTAGTGAACCGCCAGCCAATGGGCAGTGAGG
10	eIF5 wt AS	AGACATTTTGGCTTATTAGTGG
11	eIF5 uORF1 perfect AS 5' intron	AACCTTGATACTTACCGTTTTTCGTCAAATAAAGCCATGGTGGCAACGCTGCTCGCCCCGGG
12	eIF5 uORF1 perfect S 5' intron	CCCCGGCGAGCAGCGTTGCCACCATGGCTTTATTTGACGAAAACGGTAAGTATCAAGGTT
13	eIF5 uAUG1 S	GTAGGGGTTTATGTCCG
14	eIF5 uAUG1 AS	GATCCGGACATAAACCCCTACTGCA
15	eIF5 uAUG2 S	GTAGTTTTTTCATGTCCG
16	eIF5 uAUG2 AS	GATCCGGACATGAAAACTACTGCA
17	eIF5 uAUG3 S	GTAGTCGTTTATGTCCG
18	eIF5 uAUG3 AS	GATCCGGACATAAACGACTACTGCA
19	eIF5 mAUG S	GTAGGCCAAAATGTCCG
20	eIF5 mAUG AS	GATCCGGACATTTTGGCCTACTGCA
21	Optimal context S	GTAGGCCACCATGGCCG
22	Optimal context A	GATCCGGCCATGGTGGCCTACTGCA
23	GG/S in worst context	GTAGTTTGTTATGGCCG
24	GG/A in worst context	GATCCGGCCATAACAACTACTGCA
25	CG/S in worst context	GTAGTTTCTTATGGCCG
26	CG/A in worst context	GATCCGGCCATAAGAACTACTGCA
27	TG/S in worst context	GTAGTTTTTTTATGGCCG
28	TG/A in worst context	GATCCGGCCATAAAAACTACTGCA
29	AA/S in worst context	GTAGTTTATTATGACCG
30	AA/A in worst context	GATCCGGTCATAATAAACTACTGCA
31	AC/S in worst context	GTAGTTTATTATGCCCCG
32	AC/A in worst context	GATCCGGGCATAATAAACTACTGCA
33	AT/S in worst context	GTAGTTTATTATGTCCG
34	AT/A in worst context	GATCCGGACATAATAAACTACTGCA
35	GA/S in worst context	GTAGTTTGTTATGACCG
36	GA/A in worst context	GATCCGGTCATAACAACTACTGCA
37	GC/S in worst context	GTAGTTTGTTATGCCCCG
38	GC/A in worst context	GATCCGGGCATAACAACTACTGCA
39	GT/S in worst context	GTAGTTTGTTATGTCCG
40	GT/A in worst context	GATCCGGACATAACAACTACTGCA

41 CA/S in worst context GTAGTTTCTTATGACCG
42 CA/A in worst context GATCCGGTCATAAGAAACTACTGCA
43 CC/S in worst context GTAGTTTCTTATGCCCG
44 CC/A in worst context GATCCGGGCATAAGAAACTACTGCA
45 CT/S in worst context GTAGTTTCTTATGTCCG
46 CT/A in worst context GATCCGGACATAAGAAACTACTGCA
47 TA/S in worst context GTAGTTTTTTATGACCG
48 TA/A in worst context GATCCGGTCATAAAAAACTACTGCA
49 TC/S in worst context GTAGTTTTTTATGCCCG
50 TC/A in worst context GATCCGGGCATAAAAAACTACTGCA
51 TT/S in worst context GTAGTTTTTTATGTCCG
52 TT/A in worst context GATCCGGACATAAAAAACTACTGCA
53 AG/S in worst context GTAGTTTATTATGGCCG
54 AG/A in worst context GATCCGGCCATAATAAACTACTGCA