

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) Pezyzomycotina; (F) Basidiomycota; (G) fungi other than Pezyzomycotina 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

GGGUUUUAUGU	Homo sapiens
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Pan troglodytes verus
UCGUUU <u>AUGU</u>	
UUUUUCAU <u>GU</u>	
GGGUUU <u>AUGU</u>	Macaca fascicularis
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Macaca mulatta
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Pongo abelii
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Canis lupus familiaris
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Ursus americanus
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Equus caballus
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Peromyscus polionotus
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Bos taurus
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Sus scrofa
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Rattus norvegicus
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Mus musculus
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Cavia porcellus
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Ovis aries
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Oryctolagus cuniculus
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Taeniopegia guttata
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Gallus gallus
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Lonchura striata domestica
UUUUUCAU <u>GU</u>	
UCGUUU <u>AUGU</u>	
GGGUUU <u>AUGU</u>	Meleagris gallopavo
UUUUUCAU <u>GU</u>	

UCGUUUUAUGU
GGGUUUUAUGU
UUUUUCAU_G
UCGUUUUAUGU
GGGUUUUAUGU
UUUUUCAU_G
UCGUUUUAUGU
GUGUUUUAUGU
AUUUUCAU_G
UCGUUUUAUGU
GUGUUUUAUGU
AUUUUCAU_G
UUGUUUUAUGU
GUGUUUUAUGU
AUUUUCAU_G
UUGUUUUAUGU
GUGUUUUAUGU
AUUUUCAU_G
UUGUUUUAUGU
GUGUUUUAUGU
AUUUUCAU_G
UCGUUUUAUGU
GUGUUUUAUGU
UUUUUCAU_G
UCGUUUUAUGU
GUGUUUUAUGU
CGACGGGAUGG
UUUUUCAU_G
UUGUUUUAUGU
GUGUUUUAUGU
UUUUUCAU_G
UCGUUUUAUGU
GUGUUUUAUGU
UUUUUCAU_G
CCGUUUUAUGU
GUGUUUUAUGU
UUUUUCAU_G
UUGUUUUAUGU
GUGUUUUAUGU
AUAUUUUAUGG
UUUUUCAU_G
UUGUUUUAUGU
UUGUUUUAUGU

Bungarus multicinctus
Anolis carolinensis
Xenopus tropicalis
Xenopus laevis
Ambystoma mexicanum
Ambystoma tigrinum tigrinum
Cynops pyrrhogaster
Gasterosteus aculeatus
Perca flavescens
Miichthys miuy
Lates calcarifer
Takifugu rubripes
Siniperca chuatsi
Osmerus mordax #1
Osmerus mordax #2
Xiphophorus maculates
Gadus morhua
Oryzias latipes
Lipochromis sp.
Salmo salar #1

UUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
CGACGGGAUGG
UUUUUCUAUGU
UCGUUUUAUGU
UUGUUUAUGU
UUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
CGACGGGAUGG
UUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
UUGUUUAUGU
UUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
CGACGGGAUGG
UUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
UUGUUUAUGU
UUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
UUGUUUAUGU
UUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
CUACGGGAUGG
UUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
CUACGGGAUGG
UUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
CUGCGGAUGG
AUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
UUUUUCUAUGU
UUGUUUAUGU
GUGUUUAUGU
CUGCGGAUGG
AUUUUCUAUGU
UCGUUUUAUGU
GUGUUUAUGU
UUUUUCUAUGU
UUGUUUAUGU
GUGUUUAUGU
CUGCGGAUGG
AUUUUCUAUGU
UCGUUUUAUGU

Salmo salar #2

Oncorhynchus mykiss #1

Oncorhynchus mykiss #2

Oncorhynchus tshawytscha #1

Oncorhynchus tshawytscha #2

Oncorhynchus nerka #2

Thymallus thymallus

Esox lucius

Misgurnus anguillicaudatus

Pimephales promelas #1

Pimephales promelas #2

Danio rerio #2

Ictalurus furcatus #1

Ictalurus furcatus #2

Ictalurus punctatus #1

Ictalurus punctatus #2

GUGUUUAUGU	Cynoglossus semilaevis
UUUUUCAU <u>GU</u>	
UCG <u>UUU</u> AUGU	
GUGUUUAUGU	Squalus acanthias
UUUUUCAU <u>GU</u>	
UUGUUUAUGU	
GUGUUUAUGU	Leucoraja erinacea
UUUUUCAU <u>GU</u>	
UUGUUUAUGU	
GGUUUU <u>AUGU</u>	Petromyzon marinus
AUUUUCAUGU	
GAUUUU <u>AUGU</u>	Eptatretus burgeri
AUUUUCAU <u>GU</u>	
CAGUUUAUGU	

B)

Arthropoda

UUUU <u>AUAUGA</u>	Tribolium castaneum
AGGUG <u>UUAUGA</u>	
UUUU <u>AUAUGA</u>	Dendroctonus ponderosae
AGGUG <u>UUAUGA</u>	
UUUU <u>AUAUGA</u>	Aedes aegypti
UUGUG <u>CAUGA</u>	
UUUU <u>AUAUGA</u>	Anopheles gambiae
ACGUG <u>CAUGA</u>	
UCUU <u>AUAUGA</u>	Drosophila melanogaster
CUGUG <u>CAUGA</u>	
GUACAC <u>CAUGA</u>	
UCUU <u>AUAUGA</u>	Drosophila willistoni
CUGUG <u>CAUGA</u>	
UCUU <u>AUAUGA</u>	Drosophila serrata
CUGUG <u>CAUGA</u>	
GUACAC <u>CAUGA</u>	
UCUU <u>AUAUGA</u>	Drosophila erecta
CUGUG <u>CAUGA</u>	
GUACAC <u>CAUGA</u>	
AAUCUU <u>AUGA</u>	Drosophila ananassae
UGACUU <u>AUGA</u>	
UGGUG <u>CAUGA</u>	
GUACAC <u>CAUGA</u>	
UCUU <u>AUAUGA</u>	Phlebotomus papatasii
UCGUG <u>CAUGA</u>	
UCUU <u>AUAUGA</u>	Phlebotomus sergenti
UCGUG <u>CAUGA</u>	
AUUU <u>AUAUGA</u>	Acyrthosiphon pisum
AGGUG <u>CAUGA</u>	
UUUU <u>AUAUGA</u>	Schistocerca gregaria
ACCU <u>ACAU<u>UGC</u></u>	
AGGUG <u>UUAUGA</u>	
UUUU <u>AUAUGA</u>	Teleopsis dalmanni
UCGUG <u>CAUGA</u>	
UUUU <u>AUAUGA</u>	"Quercus robur"
UCGUG <u>CAUGA</u>	
GCGUG <u>UUAUGA</u>	Bombus terrestris
UUUU <u>AUAUGA</u>	Nilaparvata lugens
AGGUG <u>UUAUGA</u>	
UUUU <u>AUAUGA</u>	Parasteatoda tepidariorum
AGGU <u>UUUAUGC</u>	
UUUU <u>AUAUGA</u>	"Selaginella moellendorffii"
AGGU <u>UUUAUGC</u>	

UUUUAUAUAGA Limulus polyphemus #1
 CCGUUUAUGA
 UUUUAUAUAGA Limulus polyphemus #2
 UUGUUUAUGA Rhipicephalus appendiculatus
 UUUUAUAUAGA
 AGGUUUUAUGC Amblyomma variegatum
 CUUGUAAUGC
 UUUUAUAUAGA
 AGGUUUUAUGC
 UUUGUAAUGC
 UUUUAUAUAGA Loxosceles laeta
 AGGUUUUAUGC
 UUUJUGUAUGC Campodea fragilis
 GAAJUGUAUGC
 AUUUUAUAUGA Aphis gossypii
 AGGUGCAUGA
 UUUUAUAUAGA Locusta migratoria
 GGGUGCAUGA
 CUGUGUAUGU Calanus finmarchicus
 GAUJJUAUGA Polypedilum vanderplanki
 AAGUGCAUGU
 UUUUJUCAUGA Petrolisthes cinctipes
 UCUGUGUAUGU ACUJUCAUGA Penaeus monodon
 UCUUGUUAUGU Callinectes sapidus
 UUUUAUAUAGA Glossina morsitans
 UCGUGCAUGA
 UUUUAUAUAGA Ceratitis capitata
 UCGUGCAUGA
 UCUUUAUAUGA Rhynchosciara americana
 CCUJJUAUGA Chironomus tentans
 CAGUGCAUGA
 UUUUAUAUAGA Spodoptera frugiperda
 GCGUGCAUGA
 UUUUAUAUAGA Spodoptera litura
 GCGUGCAUGA
 UUUUAUAUAGA Gryllus bimaculatus
 ACCUACAUQC
 AGGUGUAUGA
 AACCUUAUGG Pediculus humanus capitis
 ACGUGUAUGA

C)

Nematoda

UUGUGCAUGU Caenorhabditis remanei
 AAUUUUUAUGA
 CGGUGUAUGC
 UUGUGCAUGU Caenorhabditis elegans
 AAUJJUAUGA
 UAGUGUAUGC
 UUGUGCAUGU Caenorhabditis japonica
 AAUUAUAUGA
 CAGUGUAUGC
 UUGUGCAUGU Heterorhabditis bacteriophora
 GAUJJUAUGA
 CAUCGUUAUGC
 AGUUGCAUGU Wuchereria bancrofti
 GAUJJUAUGG

UCACGUUAUGC	
AGUUGCAUGU	Brugia malayi
GAUUUUUAUGG	
UCACGUUAUGC	
AGUUUGUAUGU	Ascaris suum
UAUUUUUAUGG	
CCCGGUUAUGC	
UUGUGCAUGU	Angiostrongylus cantonensis
AAUUUUAUGC	
CAUCGUUAUGC	
UUGUGCAUGU	Ancylostoma ceylanicum
GAUUUUUAUGC	
UAUCGUUAUGC	
AGGUUGUAUGA	Bursaphelenchus mucronatus
CGUUUUUAUGG	
UAACGUUAUGC	

D)

Mollusca

GUUUUUUAUGA	Lottia gigantean
AGGUUUUAUGA	
AUUUUUAUGA	Aplysia californica
GCGUUUUUAUGA	
AUUUUUUAUGA	Aplysia kurodai
GCGUUUUUAUGA	
AUUUUUUAUGA	Lymnaea stagnalis
GCGUUAUGA	
GUUUUUUAUGA	Crassostrea gigas
GGGUUUUAUGA	
GGUUUUAUGA	Crassostrea virginica
GGGUUUUAUGA	
AGUCGUUAUGA	Mytilus californianus
AGUUUUAUGA	

E)

Pezizomycotina

UUGUUUAUGU	Neurospora crassa
GCGUUAUGC	
UACCUUAUGU	
UUGUUUAUGU	Podospora anserine
CUGCCCCAUGU	
CACCACAAUGU	
GAGUUUAUGC	Mycosphaerella graminicola
GAGUUUAUGC	
UGCUGCAUGU	
CAGUUUAUGU	Aureobasidium pullulans
AAUUGCAUGU	
CUGJCUAUGU	Trichophyton rubrum
CUACGCAUGU	
UUGUUUAUGU	Hypocreja jecorina
GAGUUCAUGC	
CGCCACAAUGU	
CUGUUUAUGU	Gibberella moniliformis
UCGJCUAUGC	
UGCCACAAUGU	
CUAJACAUGA	
UUGUUUAUGU	Thielavia terrestris
GCGUUAUGC	
UAAUGCAUGC	
UACCUUAUGU	

CUGJCAUGU Coccidioides posadasii
CUACGCCAUGU Aspergillus niger
UUGUCCUAUGU Trichoderma atroviride
CCACGCCAUGU
CUGUUUAUGU
GAGUJCAUGC
CUCCCUAUGU
CGCJUCAUGC
UUGUUUAUGU
UCGUJCAUGC
CGCCCCUAUGU
CUCUUGAUGC
CGUJAUAUGU Geomyces pannorum
CAACACAUGU
CUCJAUAUGU
GAGUJAUAUGU Cochliobolus heterostrophus
CGCUGAUGC
UUGCGCAUGU
UUGUUUAUGU
CAGCACAUGC
UUGJUCAUG
CAGJUCAUGC
GGGUUUUAUGU
UACCGCAUG
CGUJAUAUGU
CUUCGAUG
UUCUGCAUG
UGUJCCAUG
UUGUUUAUGU
GCGJUAUG
UACCCAUG
CGUCUCAUGA
UUGUUUAUGU
GCGJUAUG
UACCCAUG
UUGUUUAUGU
GCGJUAUG
UACCCAUG
CUGUUUAUGU
UUGJCAUG
UUGUUUAUGU
GCGJUAUG
UAAUGCAUG
UACCCAUG
UUGUUUAUGU
GAGUJCAUG
AACCUCAUG
GUCJUCAUGA
UUGJUCAUG
CGGUJCAUGC
UUGUUUAUGU
GCGJUAUG
UACCCAUG
CUGUUUAUGU
GCGJUCAUG
UCCCCUAUGU
CAGUCCAUG
CUGUUUAUGU
GCGJUCAUG
UCCCCUAUGU
AACJUCAUGU

CUGUUUAUGU Gaeumannomyces graminis
GCGUUCAUGC
UCCCUAUGU
AAGUUCAUGU
UUGUUUAUGU Trichoderma reesei
GAGUUCAUGC
CGCACAUQU
CUCUJGAUGC
CUGUUUAUGU Nectria haematococca
UUGUCUAUGC
UGCACAUUGC
CUCUUCAUGA
CAGUUUAUGU Glomerella graminicola
GAGUUCAUGA
UACCACAUGU
UUCUJUAUGC
CUGUUUAUGU Gibberella zae
CUGUCUAUGC
UGCACAUUGA
CUAUJACAUGA
UUGUCUAUGU Neosartorya fischeri
CUACGCAUGU
CUGUUUAUGU Fusarium oxysporum
UCGUCUAUGC
UGCACAUUGU
CUAUJACAUGA
CUGUUUAUGU Verticillium dahliae
GAGUUCAUGU
UACCCC AUGU
UCUJCCAUGU
UUGUCUAUGU Aspergillus fumigatus
UGCUGUAUGA
CUACGCAUGU
UUGUUUAUGU Grosmannia clavigera
GCCUUCAUGC
UCCCUAUGU
UUCUJUAUGC
UUGUCUAUGU Aspergillus clavatus
CUACGCAUGU
CGUJAUUAUGU Geomyces destructans
CAACACAUGU
CUCUJAUUAUGU
CUGUUUAUGU Metarhizium acridum
GAGUUCAUGC
UGCACAUUGU
ACCUJUAUGC
CUGUUCAUGU Talaromyces stipitatus
UACCGCAUGU
UUGUCUAUGU Aspergillus terreus
UGACCGCAUGU
CGUUJAUUAUGU Botryotinia fuckeliana
AUUCGAAUGU
UUCUGCAUQU
UUGUCUAUGU Aspergillus nidulans
CGGCGUAUGU
CGUJAUUAUGU Blumeria graminis
CGGGCGAAUGU
CAUCAUUAUGU
UUGUCUAUGU Aspergillus flavus
AUACGCAUGU
GAGJAUUAUGU Pyrenophora teres

CGCUGUAUGC
 CUGCGCAUGU
 GAGUUAUAGU Pyrenophora tritici-repentis
 CGCUGUAUGC
 CUGCGCAUGU
 CUGUUCAUGU Penicillium marneffei
 UACCGCAUGU
 CUGUUUAUGU Epichloe festucae
 GAGUUCAUGC
 UGCCCUAUGU
 GUGUUUAUGU
 CGGUUAUAGU Phaeosphaeria nodorum
 UACUGCAUGU
 CGUUUAUAGU
 AUUCGAAUGU Sclerotinia sclerotiorum
 UUCUGCAUGU
 CUGUUUAUGU
 GAGUUCAUGU
 UACCCC AUGU
 UCUCUCC AUGU
 CUGUUUAUGU
 GAGUUCAUGC
 UGCCCAUGU
 UGCUCUCAUGU Metarhizium anisopliae
 AUCUUC AUGC

F)

Basidiomycota

GGAUACAUGC Postia placenta
 GGAAUCAUGC Trametes versicolor
 GGAUUCAUGC Phanerochaete chrysosporium
 GGAUUCAUGC Heterobasidion annosum
 GGAUUCAUGC Pleurotus ostreatus
 GGAUUCAUGC Schizophyllum commune
 GGAUUCAUGC Lentinula edodes
 GAAUUCAUGC Coprinopsis cinerea
 GGAUUCAUGC Gloeophyllum trabeum
 CGGUUCAUGU Leucosporidium scottii
 CAGCUUAUGU Microbotryum violaceum
 UCGUUCAUGU Melampsora larici-populina
 GAGUUCAUGC Tremella mesenterica
 GAGUUCAUGC Cryptococcus neoformans
 UUGUUCAUGC Cryptococcus laurentii
 UCGUUAUAGU Phakopsora pachyrhizi
 GUUUUUUAUGU Ustilago maydis
 AAUUUC AUGC Paxillus involutus

G)

Other fungi

UAGUUCAUGU Cunninghamella elegans
 UUGUACAUGU Mucor circinelloides
 CUGUUCAUGU Phycomyces blakesleeanus
 CGCUUCAUGU Glomus intraradices
 CUGUACAUAGU Piromyces sp.
 CGCUUCAUGA Spizellomyces punctatus

H)

Plants

UCUUUUAUGU Oryza sativa #1
 UGCUGCAUGC

CAAUUUUAUGC
UCUUUUAUGU
UCUUUUAUGU
CACUGCAUGC
UCUUUUUAUGU
CACUGCAUGC
UCUUUUAUGU
GGCUGCAUGC
GCUUUUAUGU
AGCUGUAUGC
UUUUUUUAUGU
ACACAGAUGC
UCUUUUUAUGU
GGCUGUAUGC
UGUUUUUAUGA
GCUUUUAUGU
AGCUGUAUGC
UCUUUUUAUGU
GGCUGCAUGC
UUUUUUUAUGU
AGCUAUUAUGC
UCUUUUUAUGU
GGCUGCAUGC
UUUUUUUAUGU
ACACAGAUGC
AGCUAUUAUGC
GCUUUUAUGU
AGCUGUAUGC
UCUUUUUAUGU
CACUGCAUGC
UCUUUUUAUGU
CACUGCAUGC
UCUUUUUAUGU
UCUJJJUAUGU
CAGUUUAUGC
UCUUUUUAUGU
AGCUGCAUGC
AGGUUUUAUGU
UCUUUUUAUGU
GACUGUAUGC
AGCUGGGAUGC
UCUUUUUAUGU
AGCUGCAUGC
AGGUUUUAUGU
UCUUUUUAUGU
GACUGUAUGC
AGCUGGGAUGC
GCUUUUAUGU
GACUGCAUGA
UCGUJUCAUGC
UCUUUUUAUGU
GACUGUAUGC
UCUUUUUAUGU
GACUGUAUGC
UCUUUUUAUGU
GGCUGCAUGC
UCUUUUUAUGU
GACUUUAUGC
UUUUUUUAUGU
GGCUGCAUGC
UCUUUUUAUGU
ACUCAGAUGC

Oryza sativa #2
Sorghum bicolour #1

Sorghum bicolour #2

Populus trichocarpa #1

Populus trichocarpa #2

Populus trichocarpa #3

Populus tremula #1

Populus tremula #2

Populus tremula #1

Populus deltoides #1

Populus deltoides #3

Populus nigra #1

Populus nigra #3

Populus fremontii #2

Zea mays #1

Zea mays #2

Arabidopsis thaliana

Vitis vinifera #1

Vitis vinifera #2

Vitis shuttleworthii #1

Vitis shuttleworthii #2

Crocus sativus

Nicotiana tabacum #1

Nicotiana tabacum #2

Nicotiana tabacum #3

Nicotiana benthamiana #2

Nicotiana benthamiana #3

Amborella trichopoda

AGAUGCAUGC
ACUUUUAUGC
UCUUUUAUGU
GGCUGCAUGC
UGGUAAAUGA
UCUUUUAUGU
GACUGUAUGC
UCUUUUAUGU
GACUGUAUGC
UCUUUUAUGU
GGCUGUAUGC
AGUUUGAUGG
UCUUUUAUGU
CCCUGCAUGC
UCUUUUAUGU
CCCUGCAUGC
UCUUUUAUGU
CCCUGCAUGC
UCUUUUAUGU
CCCUGUAUGC
UCUUUUAUGU
GGCUGCAUGC
UCUUUUAUGU
AGCUGUAUGC
AGUUUCAUGG
UAUUUUAUGU
AUUUGGAUGC
GGAUGCAUGC
CGGUUUAUGC

Quercus petraea
Petunia axillaris
Petunia x hybrida
Quercus robur
Triticum aestivum #1
Triticum aestivum #2
Triticum aestivum #3
Lolium multiflorum
Lolium perenne
Lolium temulentum
Avena sativa #1
Avena sativa #2
Avena barbata
Festuca pratensis
Festuca arundinacea
Hordeum vulgare #1
Hordeum vulgare #2
Brachypodium distachyon
Lactuca sativa #1
Lactuca sativa #2
Iris brevicaulis
Cynodon dactylon
Zostera marina
Dioscorea alata
Prunus persica #1
Prunus persica #2

UCUUUUAUGU Solanum torvum
GGCUGUAUGC
UCUUUUUAUGU Solanum lycopersicum #1
GACUGUAUGC
UCUUUUUAUGU Solanum lycopersicum #3
UAUUGUAUGU
GGGUJUGUAUGA
GACUGUAUGC
UCUUUUAUGU Solanum tuberosum #1
AACUGUAUGC
UCUUUUUAUGU Solanum tuberosum #2
GACUGUAUGC
UCUUUUUAUGU Solanum tuberosum #3
GACUGUAUGC
UCUUUUAUGU Solanum chacoense
GACUGUAUGC
UCUUUUAUGU Actinidia deliciosa #1
GACUGUAUGC
UCUUUUUAUGU Actinidia arguta #2
GACUGUAUGC
UCUUUUAUGU Actinidia arguta #3
GACUGUAUGC
UCUUUUAUGU Actinidia chinensis #1
GACUGUAUGC
UCUUUUUAUGU Actinidia chinensis #2
GACUGUAUGC
UCUUUUAUGU Liriodendron tulipifera
GGUGUCAU~~G~~
AGUGGC~~A~~
UCUUUUUAUGU Agrostis capillaris
CCCUGUAUGC
UUUUUUUAUGU Jatropha curcas
GGCUGCAUGC
CAGUUUAUGC
UCUUUUUAUGU Ricinus communis
GCCUGCAUGC
CAGUUUAUGC
UUUUCC~~A~~
UCUUUUUAUGU Limnanthes alba
UGCUGUAUGC
AUUUJUAAUGG
UUUUUUUAUGU Carthamus tinctorius
GGCUGUAUGC
UUUUUUUAUGU Euphorbia esula
AUCUGCAUGC
CAGUUUAUGC
UCUUUUUAUGU Coffea arabica
GACUGCAUGC
AGUJUGUAUGA
UCUUUUUAUGU Coffea canephora
GACUGCAUGC
AGUJUGUAUGA
UCUUUUUAUGU Theobroma cacao
AUCUGCAUGC
UUUUUUUAUGU Fagus sylvatica
GGCUGUAUGC
AGUUUGAUGG
UCUUUUUAUGU Triphysaria pusilla
CCGUJAUUAUGU
UCUUUUUAUGU Ficus elastica
AGCUGUAUGC

CUGUUUAUGC
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AGCUGCAUGC
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UAUJUUUAUGU
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AUCUGCAUGC
CAUJUUUAUGC
UCUUUUUAUGU
CAUCUAAUGA
GGCUGCAUGC

Manihot esculenta
Carica papaya
Malus × *domestica*
Citrus clementina #1
Citrus clementina #2
Citrus sinensis
Citrus reticulata
Citrus aurantium
Citrus trifoliata
Diospyros kaki
Beta vulgaris
Euonymus alatus
Rubus ulmifolius
Gossypium raimondii
Gossypium hirsutum #1
Gossypium hirsutum #2
Gossypium hirsutum #3
Gossypium arboreum #1
Gossypium arboreum #2
Gossypium arboreum #3
Gossypium barbadense
Panax ginseng

CAGCUCAUGC
UCUUUUUAUGU
GACUGGUAAUGC
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UAUUUUUAUGU
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UCUUUCAUGU
AGCJJUUAUGC
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AGCUGGUAAUGC
UCUUUCAUGU
AGCUGGUAAUGC
UCUUUCAUGU

Capsicum annuum
Catharanthus roseus
Cannabis sativa
Aquilegia formosa
Ipomoea nil
Striga hermonthica
Cucumis melo
Barnadesia spinosa #1
Barnadesia spinosa #2
Elaeis oleifera
Wrightia tinctoria
Cynara cardunculus
Taraxacum kok-saghyz
Mimulus guttatus
Brassica napus #1
Brassica napus #2
Brassica oleracea #2
Brassica rapa #2
Eucalyptus globulus
Helianthus petiolaris
Helianthus exilis
Glycine max #1
Glycine max #2
Glycine max #3
Glycine max #4
Phaseolus coccineus
Phaseolus vulgaris #1
Phaseolus vulgaris #2
Phaseolus acutifolius #1

AGCUGCAUGC
UCUUUCAUGU Phaseolus acutifolius #2
AGCUGUAUGC
UUUUUUUAUGU Guizotia abyssinica
GGCUGUAUGC
UCUUUCAUGU
AUCUGUAUGC
UCUUUUUAUGU Vigna unguiculata
CCCUGCAUGC
UCUUUUUAUGU Leymus cinereus
GGUUGGAUGC
GGCUGUAUGC
AGUUGGAUGC
UCUUUUUAUGU Pinus taeda
GGUUGGAUGC
GGCUGUAUGC
AGUUGGAUGC
CCUUUUUAUGU Pinus radiate
UUGGUUAUGU
GAAUAAAUGU
UUUJGCAUGU
UCUUUUUAUGU Pinus banksiana
GGUUGGAUGC
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UCUUUUUAUGU
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GCGJAUUAUGA
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GGCUGUAUGC
AGUUGGAUGC
UCUUUUUAUGU Picea engelmannii
GGCUGUAUGC
AGUUGGAUGC
UCUUUUUAUGU Selaginella moellendorffii
UGCUGCAUGC
UCUUUUUAUGU Selaginella lepidophylla
GGCUGCAUGC
GAGUUCUAUGC
UCUUUUUAUGU Physcomitrella patens
GGUUGCAUGU

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

MSLFQENELLRSHWYLYWGNLAKQEEAASSHSGENFMSIEINSCSFLCHPEFSRQLPKNENRSEYPLDQ...AINKPKCISSTAAGTSS|ATRCPV (Vertebrata, 48)

MTEBQREPLWHSEH|EKKFEA,BSSQFSAEMKTYPQKHLSTTSSSRHKFKPssnX|THS|THSTA|RCLGS (Mollusca, 6)

MTHPHNNEH|CRFM|DNPQEGRLSRKKKLKGESTASLSDAEGVCPBPnTnHSP... (Arthropoda, 25)

MYQDQGXXERH|EPPLQAFR|LPKPCSPnHSP... (Pezizomycotina, 52)

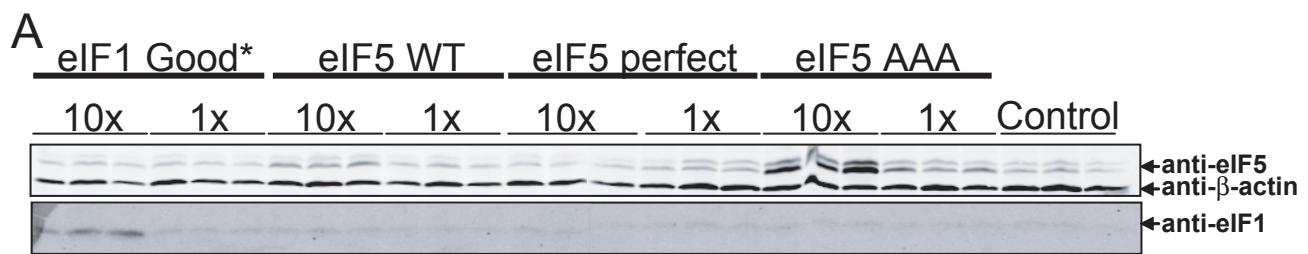
MRRPLAIPRKREFR...RSSTR (Basidiomycota, 10)

MSEKAATYLGSUGLLGEYEPISBKKGEBGSSnCMMHsSTnTnHSP... (Plantae, 135)

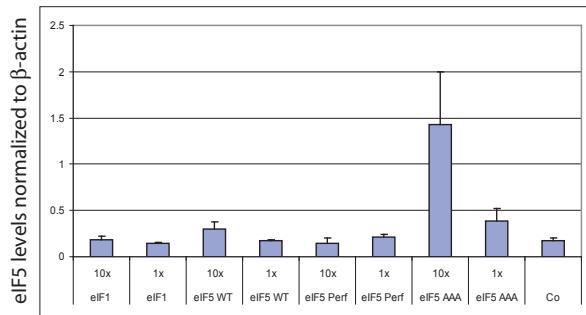
Supplementary Figure S3. Removing eIF5 uORFs results in constitutive eIF5 expression.

(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.

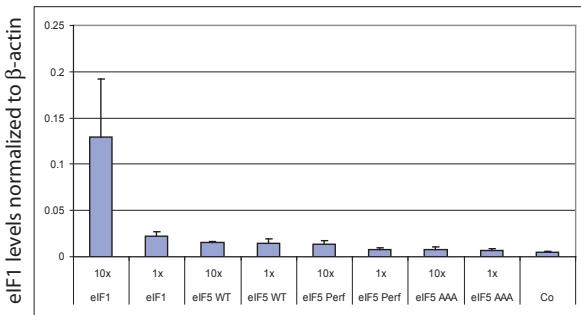
Supplementary Figure S3



B



C



Supplementary Table S1.

Oligonucleotides used in this study.

1	eIF5 wt S PstI	ATAACTGCAGCCAGCCAATGGGCAGTGAGG
2	eIF5 wt AS 5' intron	AACCTTGATACTTACCGTTTCGTCAAATAAGACATAAACCAACGC
3	eIF5 wt S 3' intron	TTTCTCTCCACAGAGCTGTTGCGCAGCCATTGG
4	eIF5 wt AS BamHI	TTATGGATCCAGACATTTGGCTTATTAGTGG
5	eIF5 wt S 5' intron	TTTATTGACGAAAACGGTAAGTATCAAGGTTAC
6	eIF5 wt AS 3' intron	AATGGCTGCGCAACAGCTCTGGAGAGAAAGG
7	eIF5 wt S	AAGCCAAAATGTCTGTCAATGTCAACCGC
8	eIF5 wt stop AS XbaI	TTATTCTAGACCTTAAATGGCATCAATATCG
9	eIF5 wt start S SacI	ATAAGAGCTCGTTAGTGAACGCCAGCCAATGGCAGTGAGG
10	eIF5 wt AS	AGACATTGGCTTATTAGTGG
	eIF5 uORF1 perfect AS	
11	5' intron	AACCTTGATACTTACCGTTTCGTCAAATAAGCCATGGTGGCACCGCTGCTGCCCGGG
	eIF5 uORF1 perfect S	
12	5' intron	CCCGGGCGAGCAGCGTTGCCACCATGGCTTATTGACGAAAACGGTAAGTATCAAGGTT
13	eIF5 uAUG1 S	GTAGGGGTTATGTCCG
14	eIF5 uAUG1 AS	GATCCGGACATAAAACCCCTACTGCA
15	eIF5 uAUG2 S	GTAGTTTCATGTCCG
16	eIF5 uAUG2 AS	GATCCGGACATGAAAAACTACTGCA
17	eIF5 uAUG3 S	GTAGTCGTTATGTCCG
18	eIF5 uAUG3 AS	GATCCGGACATAACGACTACTGCA
19	eIF5 mAUG S	GTAGGCCAAAATGTCCG
20	eIF5 mAUG AS	GATCCGGACATTTGGCCTACTGCA
21	Optimal context S	GTAGGCCACCATGGCCG
22	Optimal context A	GATCCGGCCATGGTGGCCTACTGCA
23	GG/S in worst context	GTAGTTGTTATGGCCG
24	GG/A in worst context	GATCCGGCCATAACAAAACACTGCA
25	CG/S in worst context	GTAGTTCTTATGGCCG
26	CG/A in worst context	GATCCGGCCATAAGAAAACACTGCA
27	TG/S in worst context	GTAGTTTTTATGGCCG
28	TG/A in worst context	GATCCGGCCATAAAAAACACTGCA
29	AA/S in worst context	GTAGTTTATTATGACCG
30	AA/A in worst context	GATCCGGTCATAATAAAACACTGCA
31	AC/S in worst context	GTAGTTTATTATGCCCG
32	AC/A in worst context	GATCCGGGCATAATAAAACACTGCA
33	AT/S in worst context	GTAGTTTATTATGTCCG
34	AT/A in worst context	GATCCGGACATAATAAAACACTGCA
35	GA/S in worst context	GTAGTTGTTATGACCG
36	GA/A in worst context	GATCCGGTCATAACAAAACACTGCA
37	GC/S in worst context	GTAGTTGTTATGCCCG
38	GC/A in worst context	GATCCGGGCATAACAAAACACTGCA
39	GT/S in worst context	GTAGTTGTTATGTCCG
40	GT/A in worst context	GATCCGGACATAACAAAACACTGCA

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 GTAGTTTTTATGACCG
48 TA/A in worst context GATCCGGTCATAAAAAACTACTGCA
49 TC/S in worst context GTAGTTTTTATGCCCG
50 TC/A in worst context GATCCGGGCATAAAAAACTACTGCA
51 TT/S in worst context GTAGTTTTTATGTCCG
52 TT/A in worst context GATCCGGACATAAAAAACTACTGCA
53 AG/S in worst context GTAGTTATTATGGCCG
54 AG/A in worst context GATCCGGCCATAATAACTACTGCA