SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Growth of WT and eIF5Ad strains used for ribosome profiling – Related to Figure 1

(A) Western blot analysis of eIF5A depletion over time. Same number of cells were pelleted, lysed and subjected to western blotting using antibodies against eIF5A (Saini et al., 2009), mAID (MBL International Corporation) and PGK1 (Life Technologies-Novex). (B) Growth of WT and eIF5Ad cells on YPGR and YPDauxin plates. Plates are incubated at 30°C for 2 days. (C) Growth potential of WT and eIF5Ad strains after media change to YPD containing 0.5 mM auxin (0 hr time point) by monitoring growth at OD_{600}. (D) Sucrose gradient sedimentation analysis of cell lysates from WT and eIF5Ad cells. Cells were grown and harvested under the condition in (C), lysed in footprint lysis buffer and subjected to centrifugation through 10-50% sucrose gradients. Polysome profiles were collected by monitoring at 254 nm. P/M denotes polysome/monosome ratio. (E) Scatter plots showing reproducibility of ribosome footprints on coding sequences between biological replicates for WT (left) and eIF5Ad (right). Pearson correlations are shown in each comparison.

Figure S2. Disproportionate distribution of ribosome occupancy in eFI5Ad cells – Related to Figure 1 and 7

(A) Polarity scores of ribosome occupancy on 4,946 genes from Figure 1C are plotted for WT and eIF5Ad cells. Genes in lower left quadrant correspond to those with disproportionate distribution of ribosome occupancy toward their 5'-ends. (B)

In comparison with Figure 1D, distributions of polarity scores for genes containing Pro-Pro dipeptide motifs and genes lacking these motifs from WT cells. (C) Polarity scores of 2,186 genes are plotted for wt and *Δefp E. coli* strains (Woolstenhulme et al., 2015). Genes with strongest Pro-containing stalling motifs before the halfway point are shown in pink.

Figure S3. Metacodon analysis of ribosome stalling in eIF5Ad cells – Related to Figure 2 and 7

(A) Average ribosome occupancy centered at 9,082 pause sites that match the 29 pausing motifs identified in eIF5Ad cells (Figure 2D). Arrow indicates stacked ribosomes. (B) Similar to (A), average plot centered at 1,350 pause sites that match the 4 non-Pro tripeptide motifs (RDK, DVG, DDG, GGT). (C) Analysis of codon optimality effects in eIF5Ad strain. Ribosome occupancy in the E, P and A sites under eIF5A depletion relative to WT. 61 sense codons are colored according to their stAI values (Sabi and Tuller, 2014). (D) Average ribosome density plot centered at the CTCCCG inhibitory codon pair (Gamble et al., 2016).

Figure S4. eIF5A purification and modification, stimulation of Met-Puromycin formation, and analysis of *in vitro* peptidyl-tRNA dropoff – Related to Figures 3 and 4

(A) Coomassie blue stained gel of purified eIF5A proteins. (B) MALDI-TOF analysis of purified eIF5A proteins to confirm hypusine modification. (C) Electrophoretic TLC analysis of *in vitro* Met-Puromycin assay. Time points of Met-Puro formation

were quantified and reaction progression fitted to a single exponential to calculate the observed rate. (D) Electrophoretic TLC analysis of dropoff products in elongation reactions as observed by addition of peptidyl-tRNA hydrolase (PTH).

Figure S5. Effect of C-terminal amino acid and +4 nucleotide on eIF5A-stimulated termination – Related to Figure 5

(A) Violin plot of pause scores at stop codons in WT and eIF5Ad cells sorted by C-terminal amino acid. Fold change indicates the average pause score ratio of eIF5Ad/WT. (B) Similar to (A), but sorted by the nucleotide following the stop codon.

Figure S6. eIF5A is a potent stimulator of peptide release activity, is heat-insensitive, and is a common contaminant of protein purifications – Related to Figure 6

(A) Electrophoretic TLC analysis of eRF1:eRF3 peptide release kinetics in the presence of various amounts of hyspusinated eIF5A, hypusinated eIF5A that was boiled at 95^{o}C for 15 min prior to addition, unmodified eIF5A, and a purified His_6-MBP construct expressed in *S. cerevisiae* containing low-level (though highly active) eIF5A contamination (see protein purification methods for more discussion). (B) Observed rates of peptide hydrolysis calculated for samples presented in (A).

Figure S7. eIF5A stimulates peptide release on pelleted elongation complexes, non-pelleted complexes, and functions through canonical eRF1-GGQ mechanism – Related to Figure 6

(A) Electrophoretic TLC analysis of eRF1:eRF3 peptide release kinetics in the presence of hypusinated eIF5A using pelleted MFK complexes containing UAA stop codon in A site. (B) Same as (A) except using elongation complexes that were not pelleted, containing MFFK peptide and UAA stop codon in A site. TLCs were cropped to include similar time points for comparison. (C) Analysis of eIF5A stimulation of peptide release kinetics in presence of wild-type eRF1 and an eRF1 GGQ→AGQ mutant that is defective for peptidyl hydrolysis.

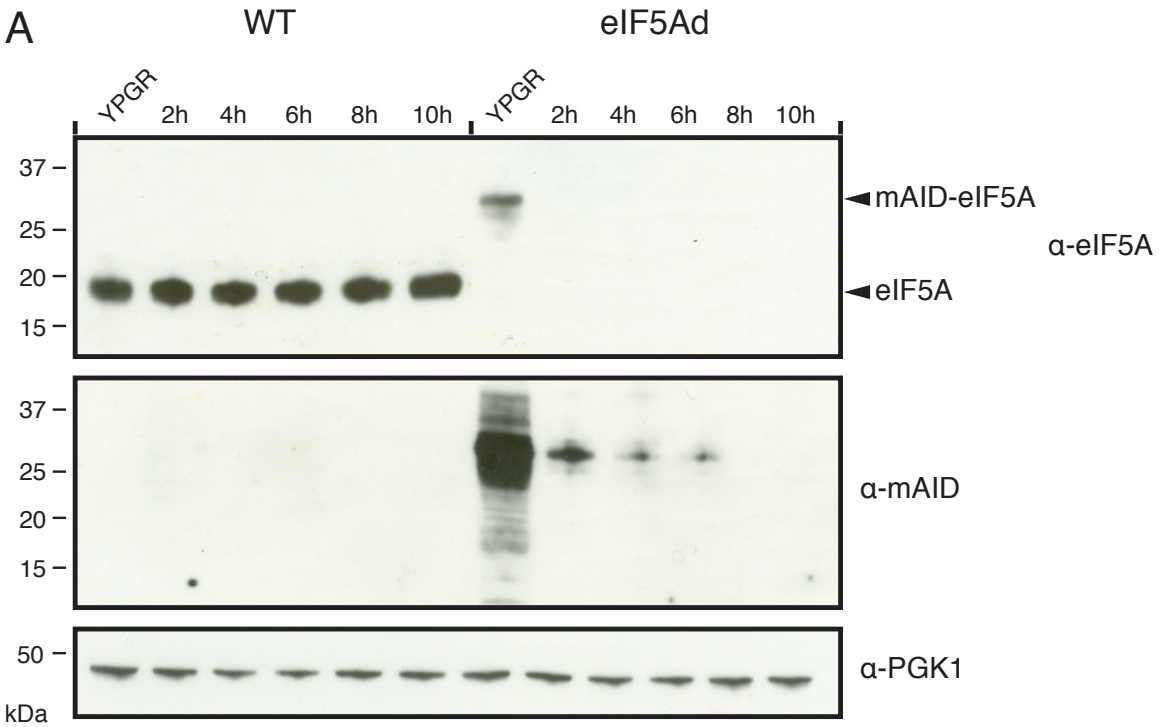
Table S1. Pausing at tripeptide motifs in WT and eIF5Ad cells – Related to Figure 2 and 7

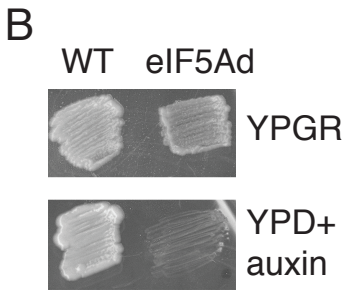
Pause scores of 6,022 tripeptide motifs are computed for WT and eIF5Ad cells, and ranked by the pause scores in eIF5Ad cells. Motifs with less than 100 occurrences in yeast transcriptome were excluded. Ratio denotes pause score ratio of eIF5Ad/WT. Counts are the numbers of motifs included for average.

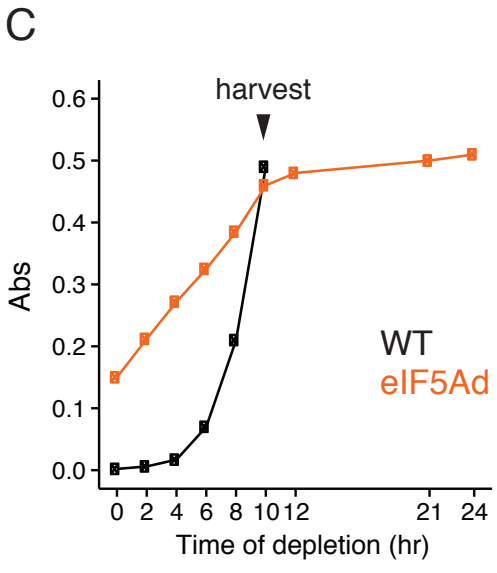
Table S2. Pause scores for dipeptide motifs in WT and eIF5Ad cells – Related to Figure 3

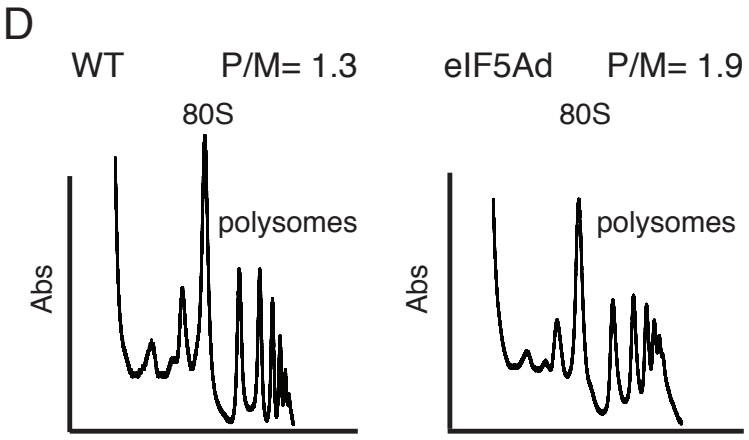
Pause scores of 396 dipeptide motifs, ranked by the pause scores in eIF5Ad cells. Motifs with less than 500 occurrences in yeast transcriptome were excluded.

Table S3. Oligonucleotides used in this study


A
WT
eIF5Ad
YPGR
2h
4h
6h
8h
10h
YPGR
2h
4h
6h
8h
10h
37
25
20
15
mAID-eIF5A
eIF5A
α-eIF5A
37
25
20
15
α-mAID
50
kDa
α-PGK1


B
WT
eIF5Ad
YPGR
YPD+
auxin


C
harvest
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Abs
WT
eIF5Ad
0
2
4
6
8
10
12
21
24
Time of depletion (hr)


D
WT
P/M= 1.3
80S
polysomes
Abs
eIF5Ad
P/M= 1.9
80S
polysomes
Abs

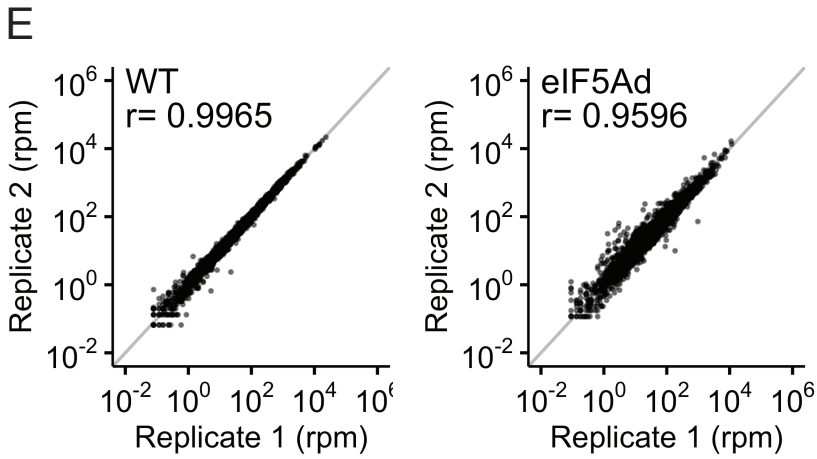
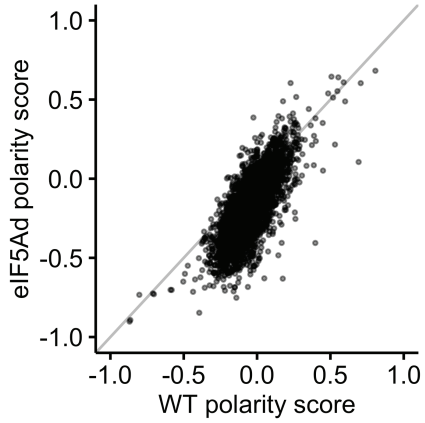
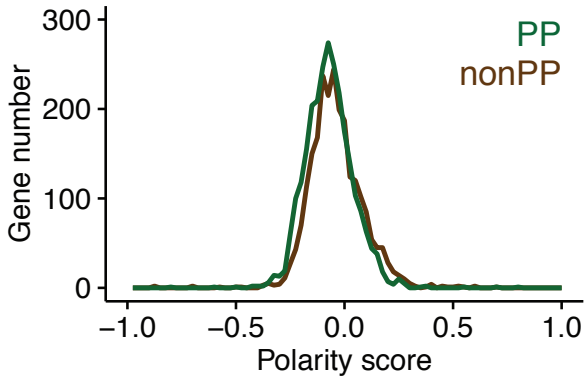

E
WT
r= 0.9965
Replicate 2 (rpm)
Replicate 1 (rpm)
eIF5Ad
r= 0.9596
Replicate 2 (rpm)
Replicate 1 (rpm)

Figure S1

A



B



C

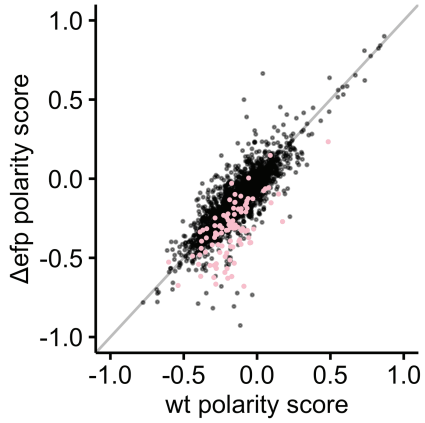


Figure S2

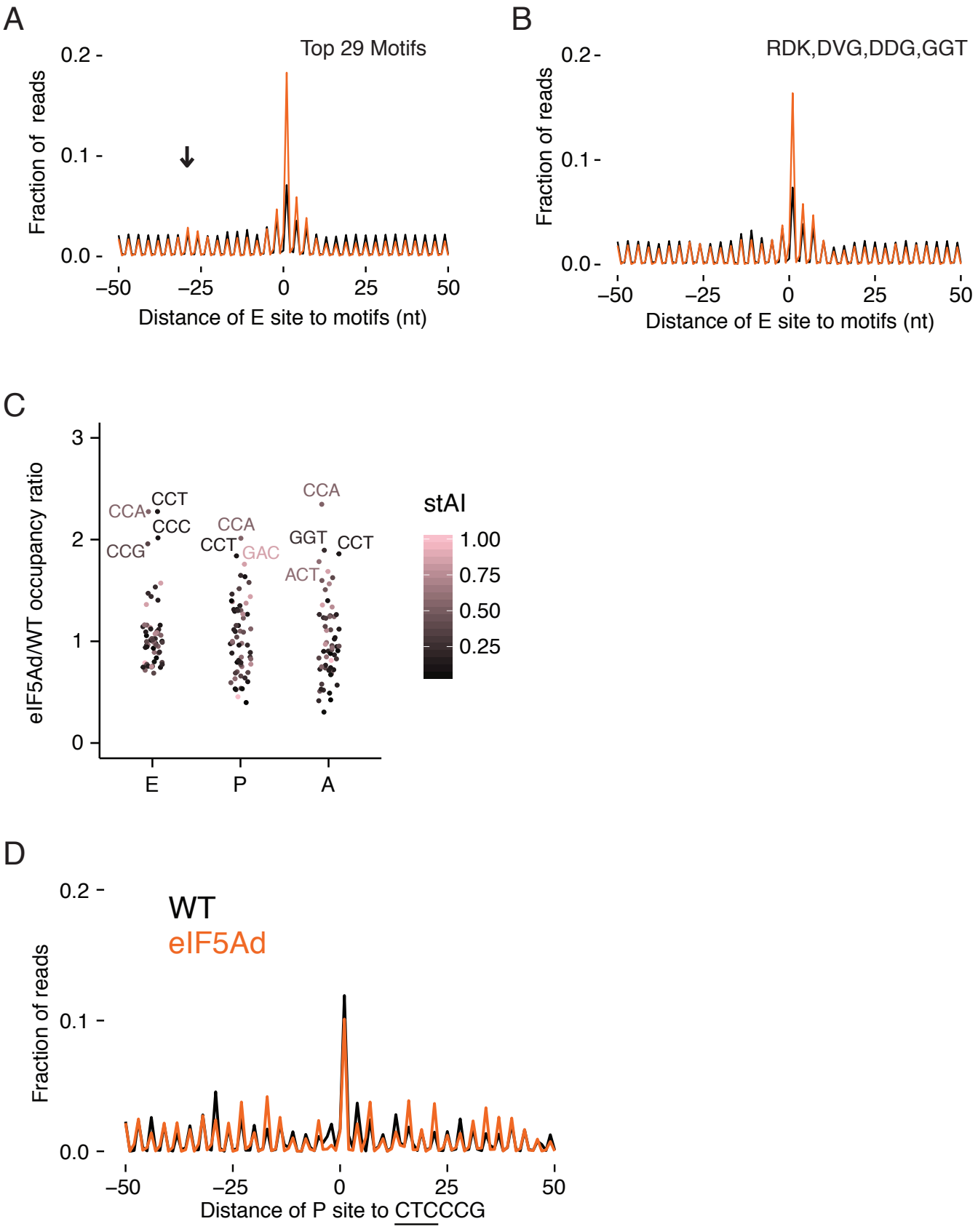

A
Top 29 Motifs
Fraction of reads
0.2
0.1
0.0
−50
−25
0
25
50
Distance of E site to motifs (nt)
B
RDK,DVG,DDG,GGT
Fraction of reads
0.2
0.1
0.0
−50
−25
0
25
50
Distance of E site to motifs (nt)
C
eIF5Ad/WT occupancy ratio
3
2
1
0
CCA
CCT
CCC
CCG
CCA
CCT
GAC
CCA
GGT
CCT
ACT
E
P
A
stAI
1.00
0.75
0.50
0.25
D
WT
eIF5Ad
Fraction of reads
0.2
0.1
0.0
−50
−25
0
25
50
Distance of P site to CTCCCG

Figure S3

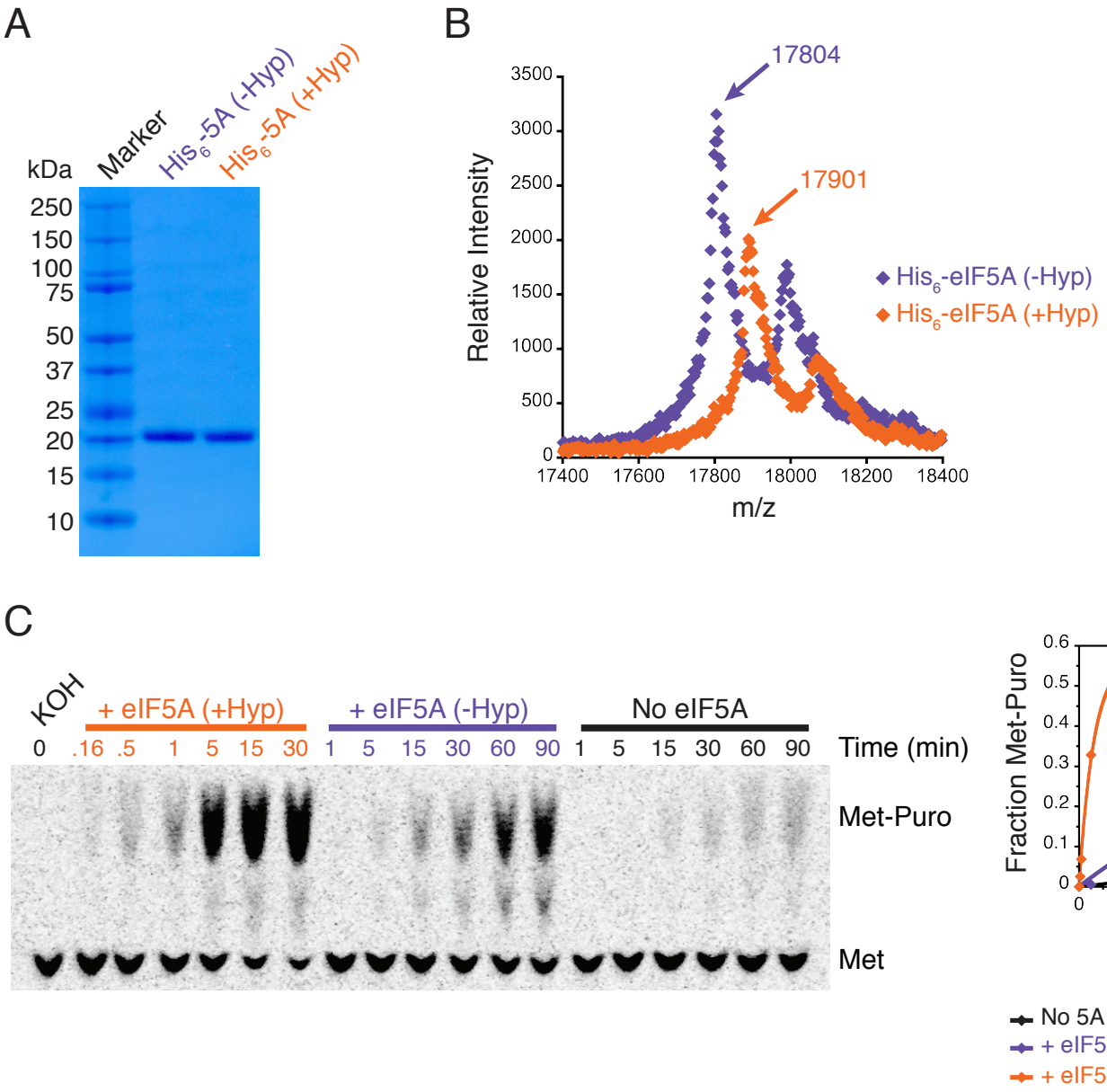
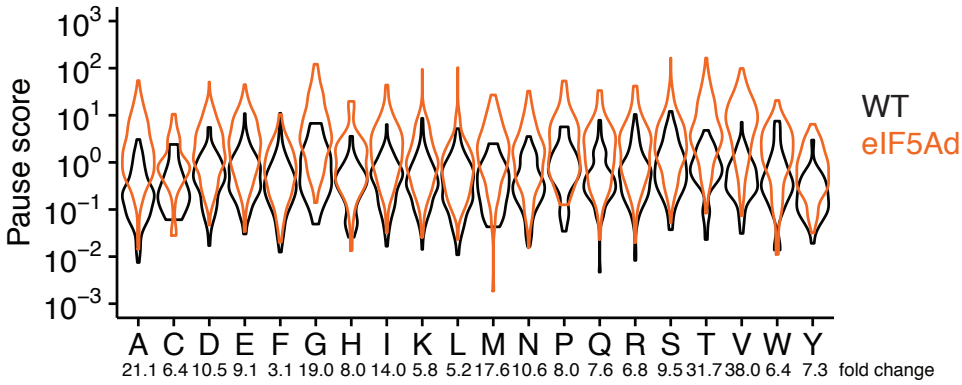

A
kDa
Marker
His6-5A (-Hyp)
His6-5A (+Hyp)
250
150
100
75
50
37
25
20
15
10
B
17804
17901
Relative Intensity
3500
3000
2500
2000
1500
1000
500
0
17400
17600
17800
18000
18200
18400
m/z
His6-eIF5A (-Hyp)
His6-eIF5A (+Hyp)
C
KOH
+ eIF5A (+Hyp)
+ eIF5A (-Hyp)
No eIF5A
0 .16 .5 1 5 15 30 1 5 15 30 60 90 1 5 15 30 60 90
Time (min)
Met-Puro
Met
Fraction Met-Puro
0.6
0.5
0.4
0.3
0.2
0.1
0
0 20 40 60 80 100
Time (min)
kobs
No 5A 0.0015 min-1
+ eIF5A (-Hyp) 0.0121 min-1
+ eIF5A (+Hyp) 0.1613 min-1
D
KOH
Formic
No eIF5A
KOH
+ eIF5A (+Hyp)
KOH
0 0 10 30 60 300 900 900 10 30 60 300 900 900
Time (sec)
Dropoff products
MDDK
MDD
MD
M
Origin

Figure S4

A


Pause score
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
A C D E F G H I K L M N P Q R S T V W Y
21.1 6.4 10.5 9.1 3.1 19.0 8.0 14.0 5.8 5.2 17.6 10.6 8.0 7.6 6.8 9.5 31.7 38.0 6.4 7.3 fold change
WT
eIF5Ad

B

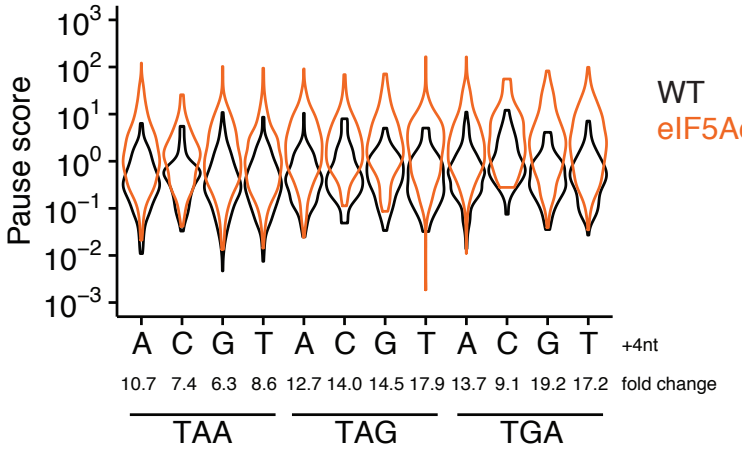
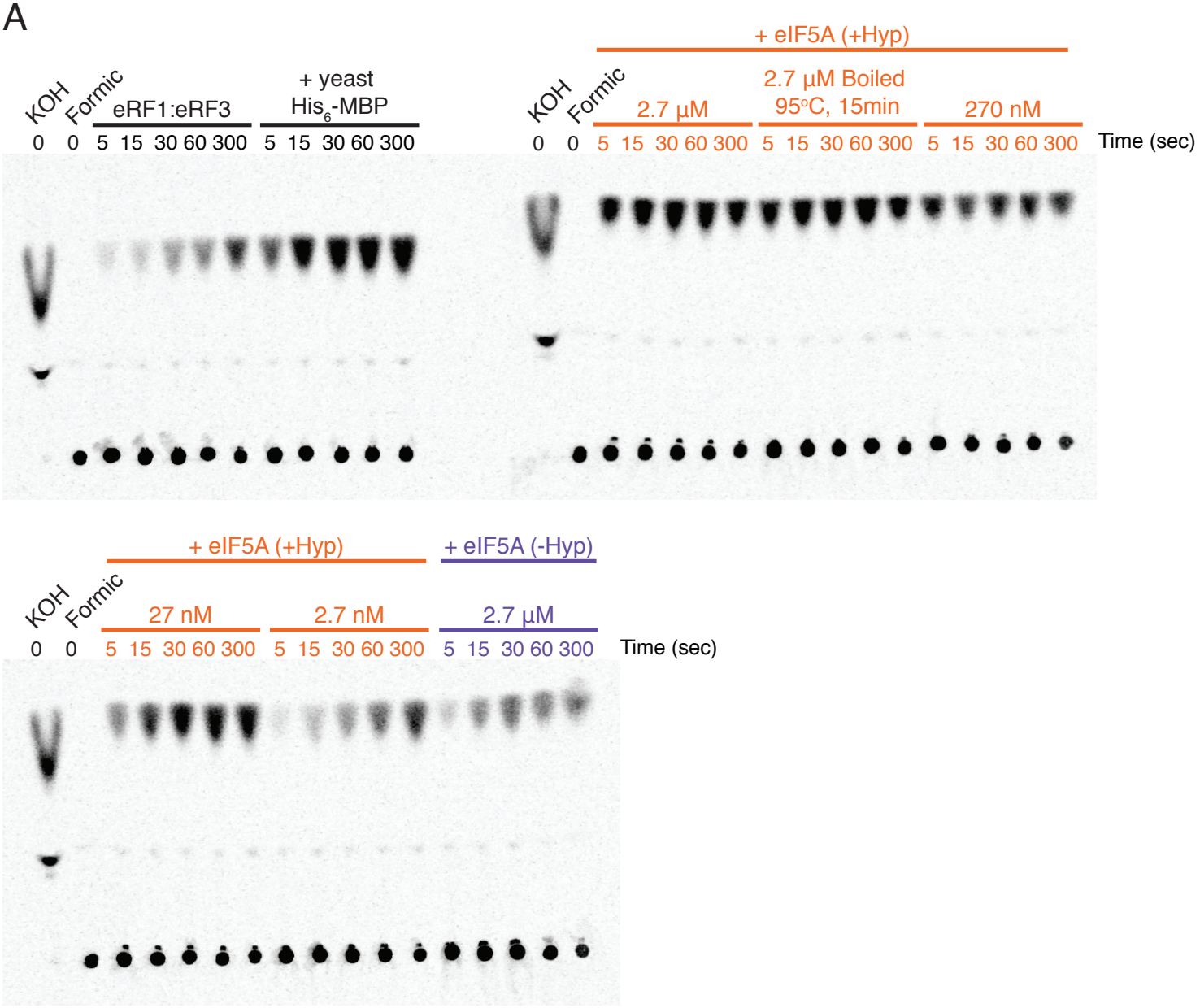

Pause score
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
A C G T A C G T A C G T +4nt
10.7 7.4 6.3 8.6 12.7 14.0 14.5 17.9 13.7 9.1 19.2 17.2 fold change
TAA
TAG
TGA
WT
eIF5Ad

Figure S5

A



B

Sample	k_{obs} release (min^{-1})	
eRF1:eRF3	0.5	
+ yeast His_6-MBP	7.8	
+ 2.7 µM eIF5A (+Hyp)	17	Saturating eIF5A
+ 270 nM eIF5A (+Hyp)	15	
+ 27 nM eIF5A (+Hyp)	3.8	
+ 2.7 nM eIF5A (+Hyp)	0.7	
+ 2.7 µM eIF5A (-Hyp)	1.6	
+ 2.7 µM eIF5A (+Hyp) Boiled	13	

Figure S6

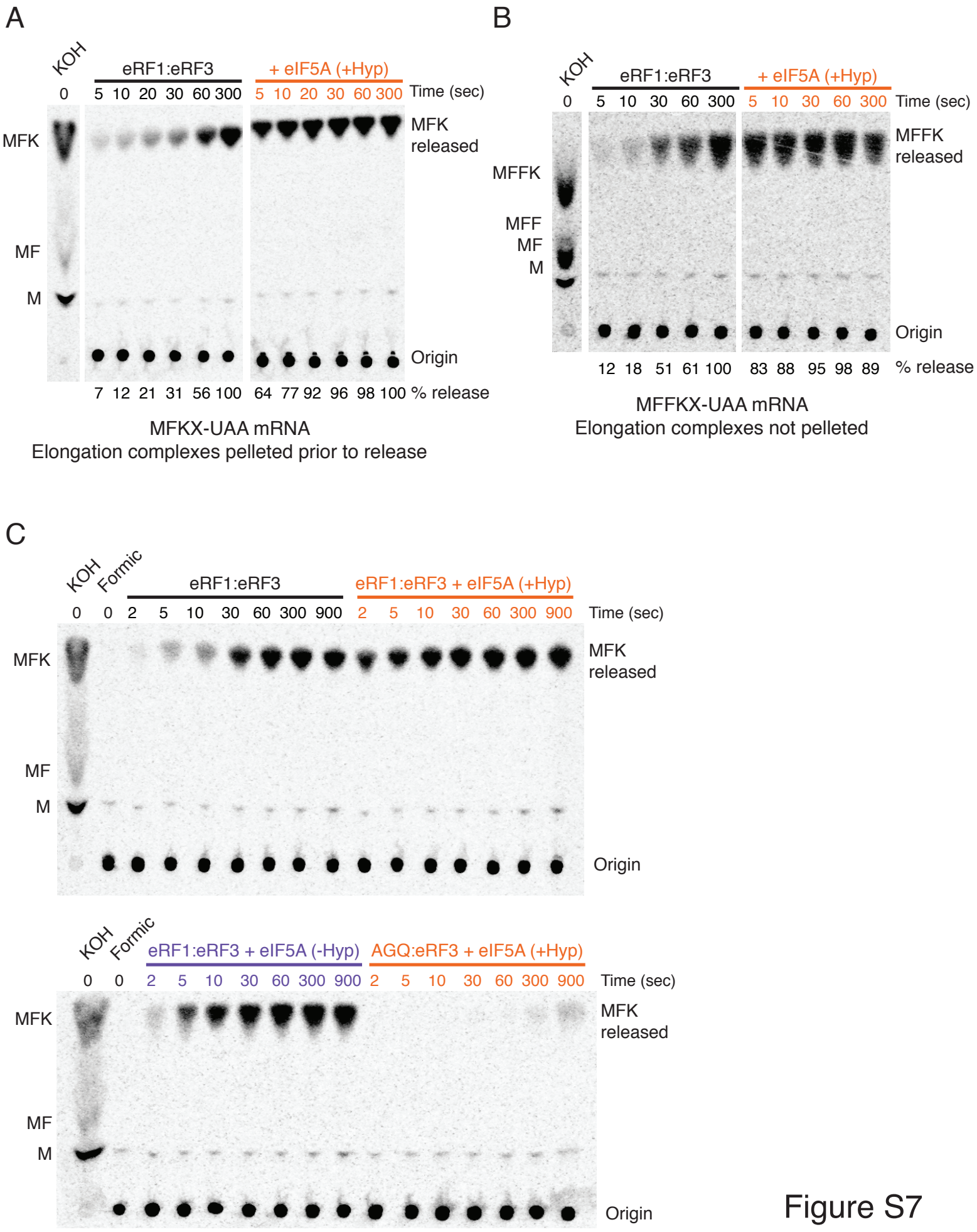

A
KOH
eRF1:eRF3
+ eIF5A (+Hyp)
0
5 10 20 30 60 300
5 10 20 30 60 300
Time (sec)
MFK
MF
M
MFK released
Origin
7 12 21 31 56 100
64 77 92 96 98 100
% release
MFKX-UAA mRNA
Elongation complexes pelleted prior to release
B
KOH
eRF1:eRF3
+ eIF5A (+Hyp)
0
5 10 30 60 300
5 10 30 60 300
Time (sec)
MFFK
MFF
MF
M
MFFK released
Origin
12 18 51 61 100
83 88 95 98 89
% release
MFFKX-UAA mRNA
Elongation complexes not pelleted
C
KOH
Formic
eRF1:eRF3
eRF1:eRF3 + eIF5A (+Hyp)
0 0 2 5 10 30 60 300 900
2 5 10 30 60 300 900
Time (sec)
MFK
MF
M
MFK released
Origin
KOH
Formic
eRF1:eRF3 + eIF5A (-Hyp)
AGQ:eRF3 + eIF5A (+Hyp)
0 0 2 5 10 30 60 300 900
2 5 10 30 60 300 900
Time (sec)
MFK
MF
M
MFK released
Origin

Figure S7