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Supplemental Information

Suppression of Ribosomal Pausing by eIF5A Is Necessary to Maintain the Fidelity of Start Codon Selection

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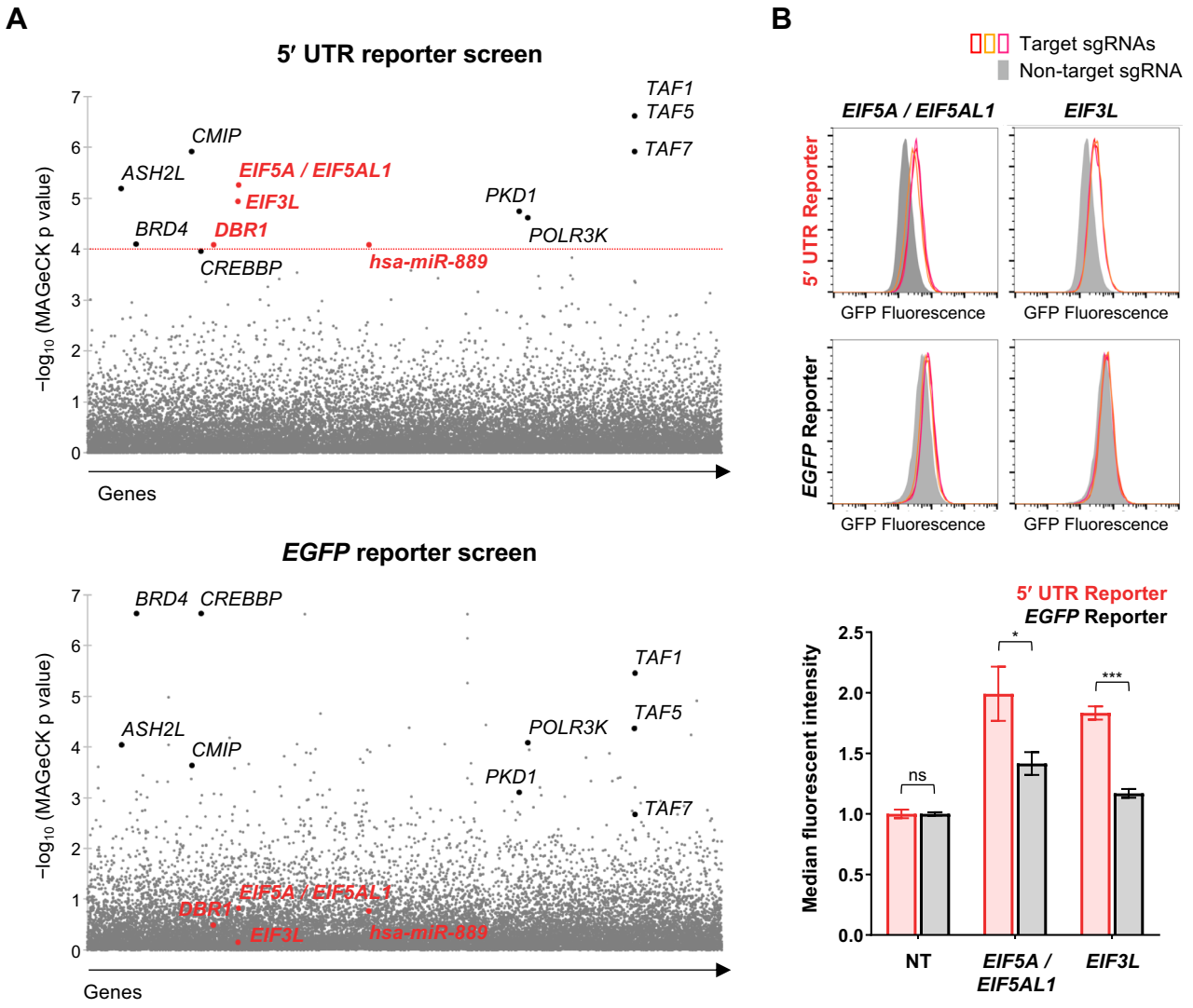
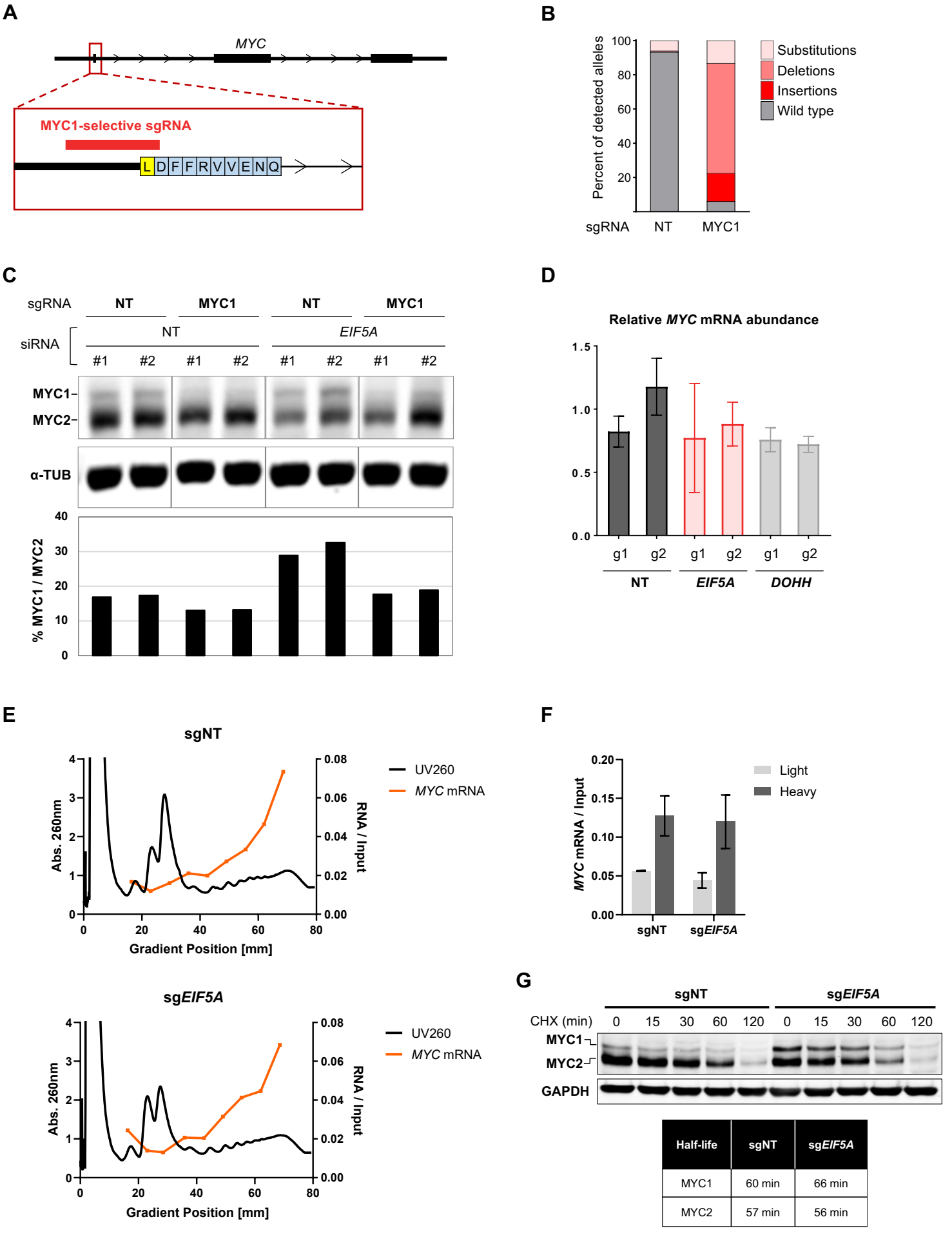

A
5′ UTR reporter screen
−log10 (MAGeCK p value)
Genes
TAF1
TAF5
TAF7
CMIP
ASH2L
EIF5A / EIF5AL1
EIF3L
PKD1
BRD4
DBR1
POLR3K
CREBBP
hsa-miR-889
EGFP reporter screen
BRD4
CREBBP
TAF1
TAF5
ASH2L
CMIP
POLR3K
PKD1
TAF7
EIF5A / EIF5AL1
DBR1
EIF3L
hsa-miR-889
B
Target sgRNAs
Non-target sgRNA
EIF5A / EIF5AL1
EIF3L
5′ UTR Reporter
EGFP Reporter
GFP Fluorescence
5′ UTR Reporter
EGFP Reporter
Median fluorescent intensity
ns
*
***
NT
EIF5A / EIF5AL1
EIF3L
C
sgRNA
NT g2
NT g1
EIF3L g1
MYC
α-TUB
D
Relative expression
EIF5A
EIF5AL1
E
NT
EIF5A
EIF5AL1
EIF5A / EIF5AL1
sgRNA
g1
g2
eIF5A
α-TUB
F
sgRNA
NT
DOHH
EIF5A
MYC1–
MYC2–
α-TUB
DOHH
eIF5A
α-TUB

Figure S1. Analysis and validation of CRISPR/Cas9 screening data, Related to Figure 1

(A) MAGeCK analysis of screening data from *MYC* 5′ UTR reporter cells (top) and *EGFP* control reporter cells (bottom). 5′ UTR-specific hits marked in red, common hits marked in black.
(B) 5′ UTR and *EGFP* control reporter expression assessed by flow cytometry after lentiviral delivery of Cas9 and individual sgRNAs targeting *EIF5A*/*EIF5AL* or *EIF3*.
(C) Western blot analysis of MYC protein levels in HCT116 cells after lentiviral delivery of Cas9 and non-target (NT) or *EIF3L*-targeting sgRNAs.
(D) qRT-PCR analysis of *EIF5A* and *EIF5AL1* expression, normalized to 18s rRNA, in HCT116 cells. n = 3 biological replicates.
(E) Western blot analysis of eIF5A protein levels in HCT116 cells after lentiviral delivery of Cas9 and sgRNAs that target only *EIF5A*, only *EIF5AL1*, or both genes.
(F) Western blot analysis of the indicated proteins in HEK293T cells after lentiviral delivery of Cas9 and sgRNAs.
Error bars represent S.D. *p<0.05, ***p<0.001, calculated by two-tailed Welch's t-test.



Half-life	sgNT	sg*EIF5A*
MYC1	60 min	66 min
MYC2	57 min	56 min

Figure S2. Additional analyses of *MYC* mRNA and protein, Related to Figure 1

(A) Schematic of the MYC1-targeting sgRNA.
(B) HCT116 cells were transduced with lentiviral vectors encoding Cas9 and either the MYC1-selective or non-targeting sgRNAs. The MYC1 sgRNA target site was amplified and the frequency of indels in the population was determined by high throughput sequencing (>100,000 reads obtained for each condition).
(C) Western blot analysis of MYC protein in HCT116 cells following introduction of control or MYC1-targeting sgRNAs followed by transfection with control or *EIF5A*-targeting siRNAs. Quantification of MYC1 as a percentage of MYC2 is shown.
(D) qRT-PCR analysis of *MYC* transcript levels in *EIF5A*$^{-/-}$ and *DOHH*$^{-/-}$ HCT116 cells. Data represented as mean ± S.D. (n = 2 biological replicates per gRNA).
(E) Representative polysome association of *MYC* mRNA in HCT116 cells infected with lentivirus encoding control sgRNA (sgNT) or sgRNA targeting *EIF5A* (sg*EIF5A*). Cell lysates were separated on 5-50% linear sucrose gradients. Ultraviolet (UV) absorbance at 260 nm was measured to assess ribosome distribution along the gradient (black line). *MYC* mRNA levels in nine gradient fractions were measured by qRT-PCR and normalized to the input RNA (orange line).
(F) *MYC* mRNA association with light (sum of fractions 3-5) and heavy (sum of fractions 7-9) polysomes was not significantly altered upon eIF5A loss. Data represented as mean ± S.E.M. (n = 2 biological replicates per sgRNA).
(G) MYC1 and MYC2 protein half-lives were determined in control (sgNT) or *EIF5A* knockout (sg*EIF5A*) HCT116 cells by treating with 50 mg/mL cycloheximide (CHX) and determining protein abundance at the indicated time-points by western blotting.

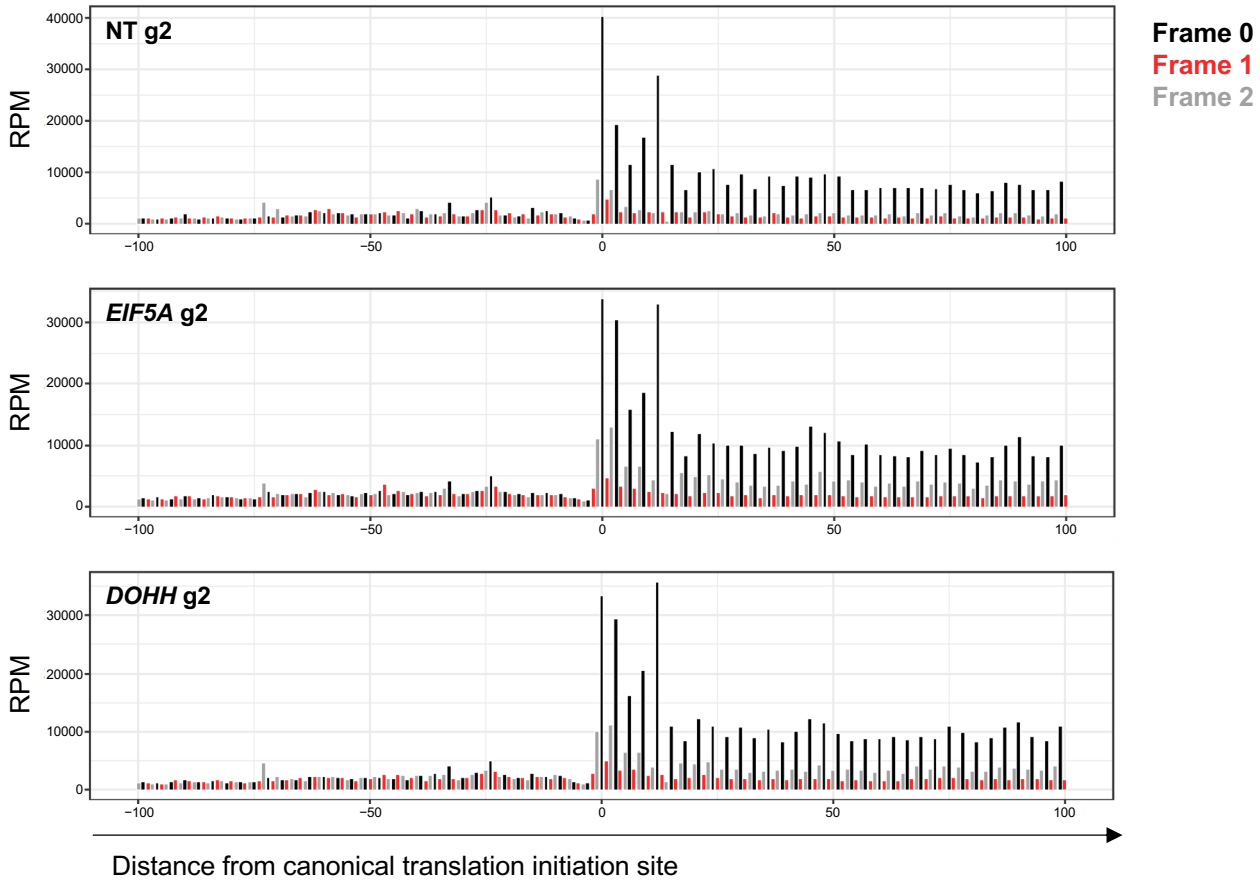
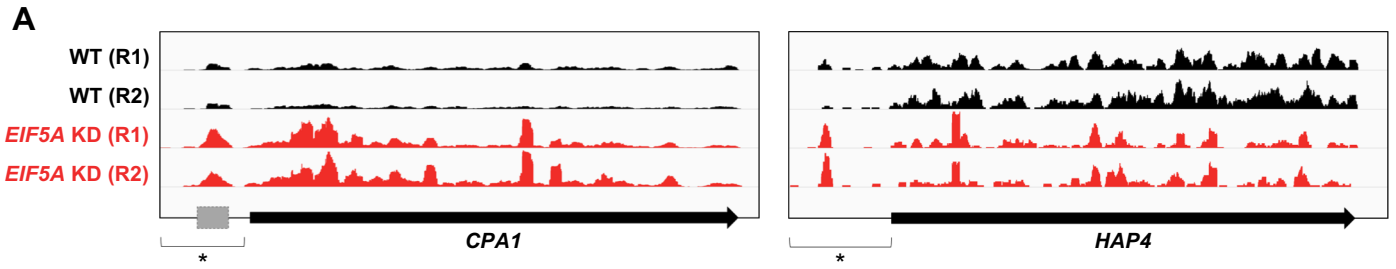
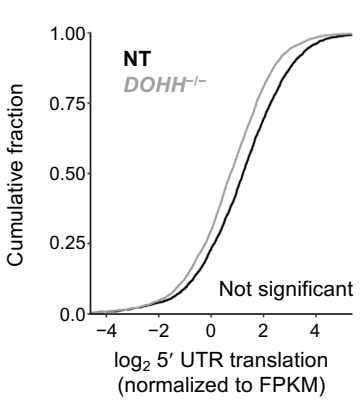
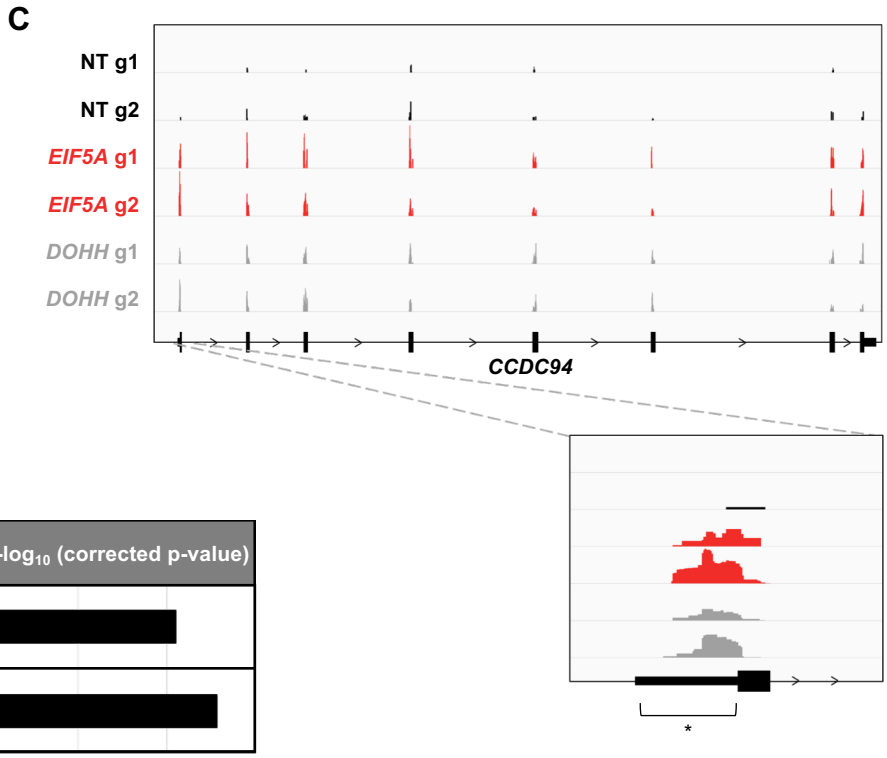


Figure S3. Periodicity of ribosome profiling sequencing reads, Related to Figure 2

Periodicity of ribosome profiling reads visualized by meta-codon plots centered on canonical AUG start codons. Representative samples shown.







D

Organism	GO Ontology term (biological process)	-log10 (corrected p-value)
HUMAN	Cellular response to stress	
YEAST	Response to stress	

2.0 2.2 2.4 2.6

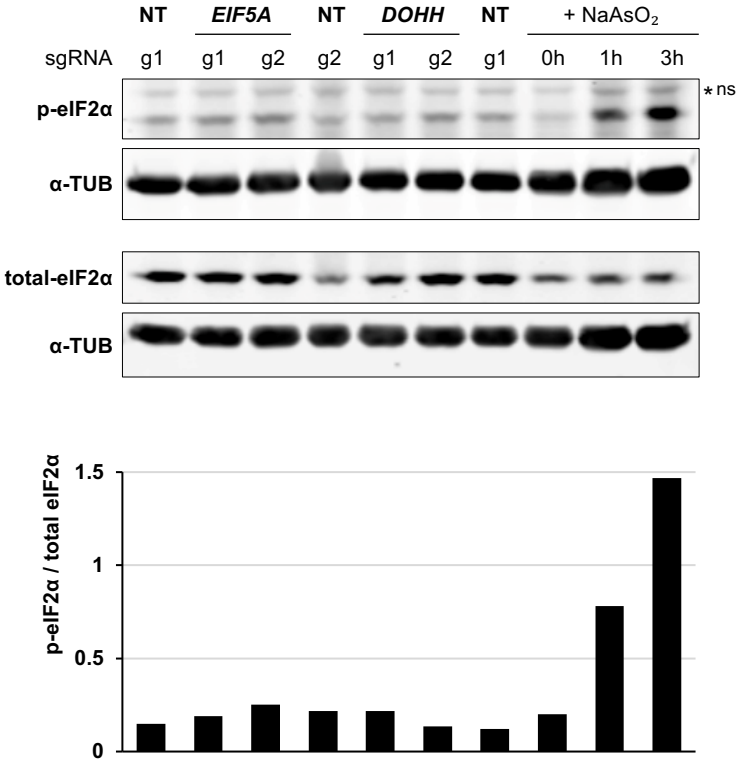


Figure S4. Loss of function of eIF5A increases upstream translation initiation, Related to Figure 3

(A) Ribosome occupancy on the yeast *CPA1* and *HAP4* transcripts in *EIF5A* KD and WT yeast. Region of upstream translation marked with an asterisk. Previously reported uORF in *CPA1* denoted by grey box (Zhang and Dietrich, 2005).
(B) CDF plot of 5′ UTR translation in *DOHH*$^{-/-}$ and control sgNT HCT116 cells. 5′ UTR translation defined as ribosome occupancy (RPKM) in 5′ UTR normalized to transcript expression level derived from RNA-seq (FPKM) (3113 genes included based on minimal coverage criteria).
(C) Ribosome occupancy on the human *CCDC94* transcript in *EIF5A*$^{-/-}$, *DOHH*$^{-/-}$, and control sgNT cells. Region of upstream translation marked with an asterisk.
(D) Enrichment of stress response genes among transcripts that show increased 5' UTR translation in eIF5A-deficient human and yeast cells. Human gene ontology (GO) analysis performed with the DAVID functional annotation tool (Huang et al., 2008; Sherman et al., 2008); yeast analysis performed using the Genecodis webtool (Carmona-Saez et al., 2007; Nogales-Cadenas et al., 2009; Tabas-Madrid et al., 2012).
(E) Serine 51-phosphorylated eIF2α (p-eIF2α) and total eIF2α levels in *EIF5A*$^{-/-}$ and *DOHH*$^{-/-}$ HCT116 cells monitored by Western blot. Sodium arsenite ($NaAsO_2$)-treatment served as a positive control. Asterisk indicates non-specific band recognized by the anti-p-eIF2α antibody.

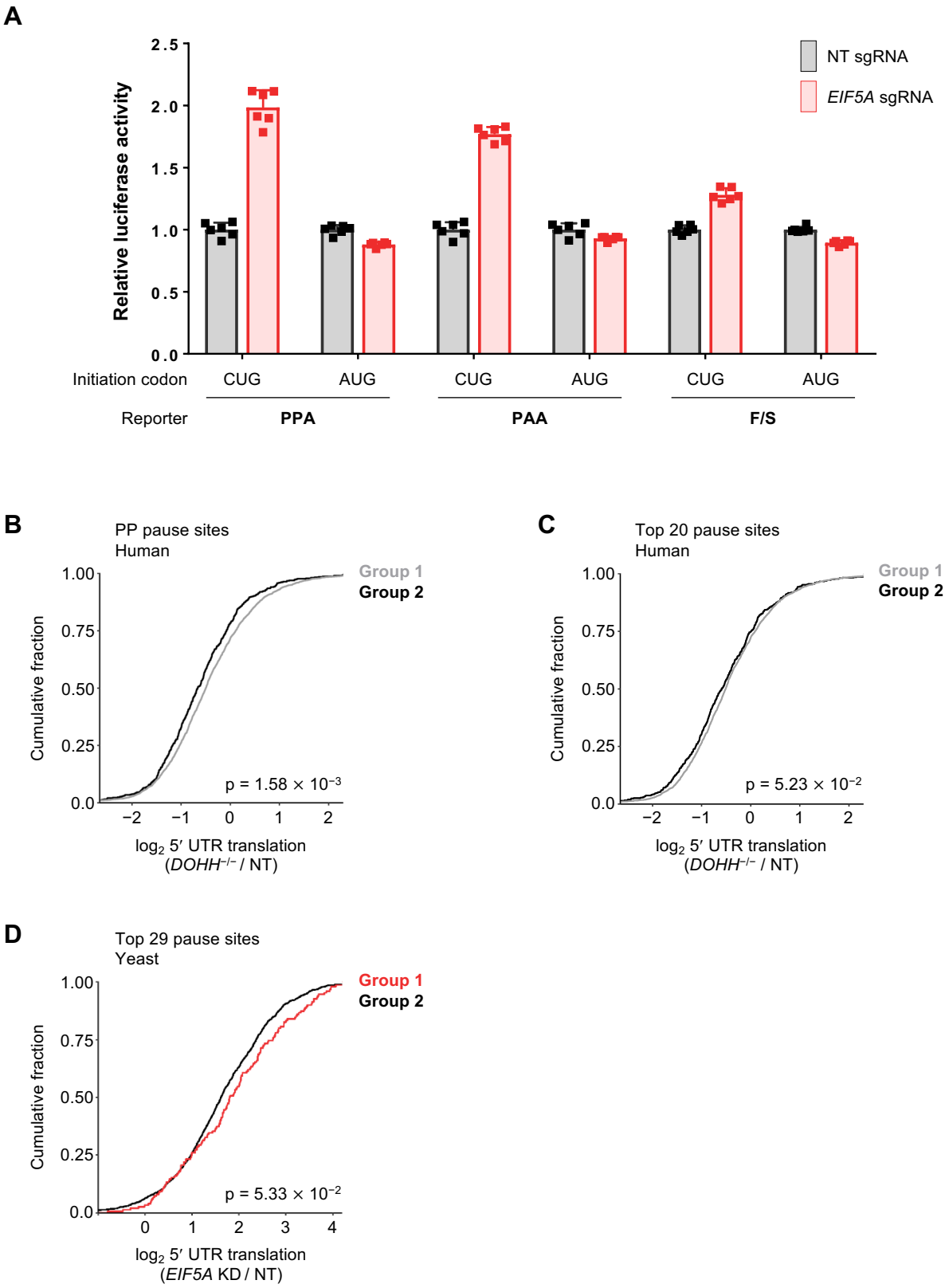

A
Relative luciferase activity
2.5
2.0
1.5
1.0
0.5
0.0
NT sgRNA
EIF5A sgRNA
Initiation codon
CUG
AUG
CUG
AUG
CUG
AUG
Reporter
PPA
PAA
F/S
B
PP pause sites
Human
Group 1
Group 2
Cumulative fraction
1.00
0.75
0.50
0.25
0.0
p = 1.58 × 10−3
−2
−1
0
1
2
log2 5′ UTR translation
(DOHH−/− / NT)
C
Top 20 pause sites
Human
Group 1
Group 2
Cumulative fraction
1.00
0.75
0.50
0.25
0.0
p = 5.23 × 10−2
−2
−1
0
1
2
log2 5′ UTR translation
(DOHH−/− / NT)
D
Top 29 pause sites
Yeast
Group 1
Group 2
Cumulative fraction
1.00
0.75
0.50
0.25
0.0
p = 5.33 × 10−2
0
1
2
3
4
log2 5′ UTR translation
(EIF5A KD / NT)

Figure S5. Proximal ribosome pausing promotes upstream translation on the *MYC* transcript, and broadly throughout the transcriptome, Related to Figures 4 and 5

(A) Activity of the individual CUG-initiated or AUG-initiated *MYC* reporter constructs used in Figure 4B. Following transfection of reporters into *EIF5A*$^{-/-}$ or control sgNT HCT116 cells, firefly luciferase activity was normalized to co-transfected renilla luciferase. Data represented as mean ± S.D. (n = 6 biological replicates).
(B-C) CDF plots of 5' UTR translation in *DOHH*$^{-/-}$ cells compared to sgNT control, with pause sites defined as PP dipeptides (B), or the top 20 tripeptide pause motifs identified in *EIF5A*$^{-/-}$ cells compared to sgNT control (C). 5′ UTR translation defined as ribosome occupancy (RPKM) in 5′ UTR normalized to transcript expression level derived from RNA-seq (FPKM). Genes included based on minimal coverage requirements: 2589 in Group 1 and 524 in Group 2 for (B); 2570 in Group 1 and 543 in Group 2 for (C).
(D) CDF plot of 5′ UTR translation in *EIF5A* KD yeast compared to WT. Pause sites defined as the top 29 tripeptide pause motifs identified in *EIF5A* KD yeast (Schuller et al., 2017). 5′ UTR translation defined as normalized ribosome occupancy (RPKM) in 5′ UTR / CDS. 150 genes included in Group 1 and 1188 genes included in Group 2 based on minimal coverage criteria.