Expanded View Figures

Figure EV1. $UPF1_{LL}$ has distinct effects on NMD autoregulation and factor requirements.

A Sashimi plot from representative RNA-seq samples of siNT and si$UPF1_{LL}$ knockdown cells. Percent spliced in values and FDR were calculated with rMATS software (Shen *et al*, 2014).

B Density plot of changes in mRNA stability as determined by REMBRANDTS analysis of RNA-seq following isoform-specific $UPF1_{LL}$ depletion (Alkallas *et al*, 2017). mRNAs were binned according to up- or down-regulation in response to si$UPF1_{LL}$. Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons.

C RT–qPCR analysis of indicated transcripts following transfection of HEK-293 cells with siRNAs that target both UPF1 isoform ($UPF1_{total}$) and the $UPF1_{LL}$ isoform. Relative fold changes are in reference to NT siRNA. si$UPF1_{total}$ or si$UPF1_{LL}$ was compared to the NT siRNA control for significance testing. Asterisk (*) indicates $P < 0.05$, as determined by two-way ANOVA. Black dots represent individual data points and error bars indicate mean ± SD ($n = 3$ biological replicates). Dashed lines indicate $\log_2$ (fold change) of ± 0.5. See also Dataset EV3 for *P*-values associated with each statistical comparison.

D Venn diagram (to scale) of overlapping targets identified from RNA-seq following $UPF1_{LL}$ knockdown (this dataset), total UPF1 knockdown, or SMG6/7 double knockdown and rescue (Colombo *et al*, 2017). Depicted are genes that increased in abundance at least 1.4-fold (FDR < 0.05) with $UPF1_{LL}$-specific knockdown and their overlap with genes that increased in abundance (FDR < 0.05) with total UPF1 knockdown or genes that increased in abundance with SMG6/7 double knockdown and were significantly rescued by expression of SMG6 or SMG7 (SMG6/7 targets). *P*-values indicate enrichment of genes that increased in abundance at least 1.4-fold (FDR < 0.05) with $UPF1_{LL}$-specific knockdown among those regulated by total UPF1 and SMG6/7, as determined by Fisher's exact test. Only genes that met read count cutoffs in all conditions were included in the analysis.

E Density plot of changes in relative mRNA abundance as determined by RNA-seq in $SMG7^{ko}/SMG5^{kd}$ cells, relative to a parental cell line treated with control siRNAs (Boehm *et al*, 2021). Genes were categorized as up-regulated by si$UPF1_{total}$ only, si$UPF1_{LL}$ only, or both si$UPF1_{total}$ and si$UPF1_{LL}$. Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons.

F Density plot of changes in relative mRNA abundance as determined by RNA-seq following $UPF1_{LL}$ knockdown in HEK-293 cells. Genes were categorized as up-regulated by $SMG7^{ko}$, $SMG7^{ko}/SMG5^{kd}$, $SMG7^{ko}/SMG6^{kd}$, or $SMG7^{ko}/SMG5^{kd}$ and $SMG7^{ko}/SMG6^{kd}$ (Boehm *et al*, 2021). Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons.

G Box plot of $\log_2$ enrichment for translation at the ER (Jan *et al*, 2014). mRNAs were binned by sensitivity to $UPF1_{LL}$-specific knockdown in HEK-293 cells. Statistical significance was determined by K–S test (****$P = 1 \times 10^{-6}$). Boxes indicate interquartile ranges, horizontal lines represent medians, and bars indicate Tukey whiskers.

Source data are available online for this figure.

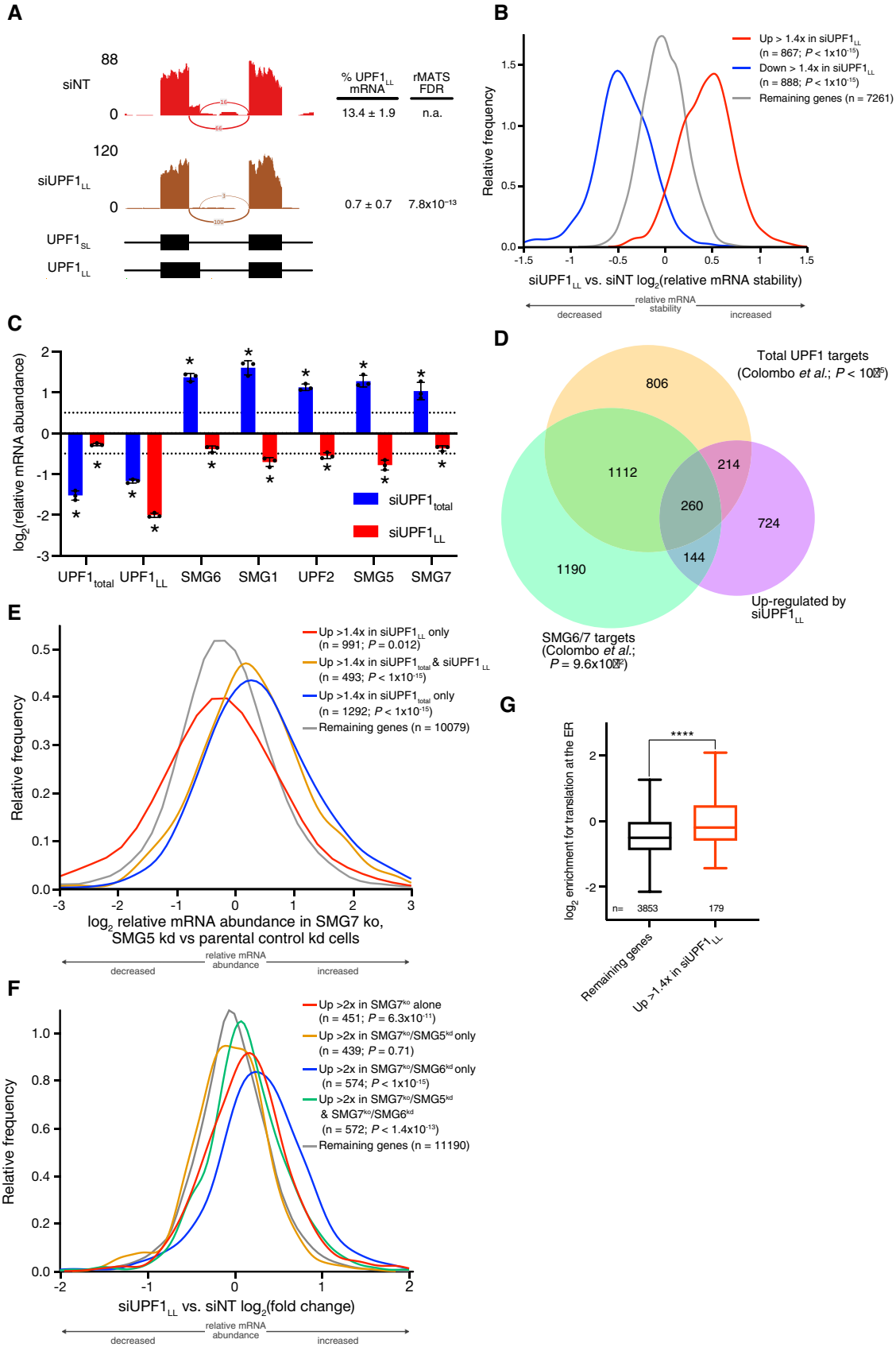


Figure EV1.

Figure EV2. NMD protection can be overcome by $UPF1_{LL}$.

A Western blots of CLIP-$UPF1_{SL}$ and CLIP-$UPF1_{LL}$ overexpression. Membranes were probed with an anti-UPF1 antibody that detects both endogenous and CLIP-tagged UPF1. Wedge indicates serial twofold dilutions of lysate. Mean (± SD) of CLIP-UPF1 overexpression was determined from two replicate membranes.

B RT–qPCR analysis of well-characterized NMD targets following total UPF1 knockdown and rescue with siRNA-resistant CLIP-tagged UPF1. Relative fold changes are in reference to the GFP-expressing control line treated with a NT siRNA. Significance of NMD rescue by CLIP-UPF1 was compared to the GFP-expressing control line treated with total UPF1 siRNA. Asterisk (*) indicates $P < 0.0001$, as determined by two-way ANOVA with multiple comparisons. Black dots represent individual data points and error bars indicate mean ± SD ($n = 3$ biological replicates). PTC+ indicates the use of primers specific to transcript isoforms with validated poison exons (Lareau *et al*, 2007; Ni *et al*, 2007). See also Dataset EV3 for *P*-values associated with each statistical comparison.

C Density plot of recovered mRNAs in CLIP-$UPF1_{LL}$ affinity purifications relative to that of CLIP-$UPF1_{SL}$. Genes were categorized as up-regulated by $siUPF1_{total}$ only, $siUPF1_{LL}$ only, or both $siUPF1_{total}$ and $siUPF1_{LL}$. Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons.

D RT–qPCR analysis of indicated transcripts from UPF1 RIP-seq experiments. Relative fold enrichment was determined by dividing the recovered mRNA by its corresponding input amount. Significance of differential recovery in CLIP-$UPF1_{LL}$ RIP was determined by comparison to that in CLIP-$UPF1_{SL}$. Asterisk (*) indicates $P < 0.05$, as determined by unpaired Student's *t*-test. Black dots represent individual data points, and error bars indicate mean ± SD ($n = 3$ biological replicates). For protected mRNAs, the PTBP1/hnRNP L motif density bin of the 3'UTR is indicated. PTC^+ indicates the use of primers specific to transcript isoforms with validated poison exons (Lareau *et al*, 2007; Ni *et al*, 2007). See also Dataset EV3 for *P*-values associated with each statistical comparison.

E Density plots of changes in relative mRNA abundance as determined by RNA-seq following $UPF1_{LL}$ (top) or $UPF1_{SL}$ (bottom) overexpression. mRNAs were binned according to enrichment in the CLIP-$UPF1_{LL}$ or CLIP-$UPF1_{SL}$ affinity purifications. Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons.

F Density plots of changes in mRNA stability as determined by REMBRANDTS analysis of RNA-seq following $UPF1_{LL}$ (top) or $UPF1_{SL}$ (bottom) overexpression. mRNAs were binned according to enrichment in the CLIP-$UPF1_{LL}$ or CLIP-$UPF1_{SL}$ affinity purifications. Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons.

Source data are available online for this figure.

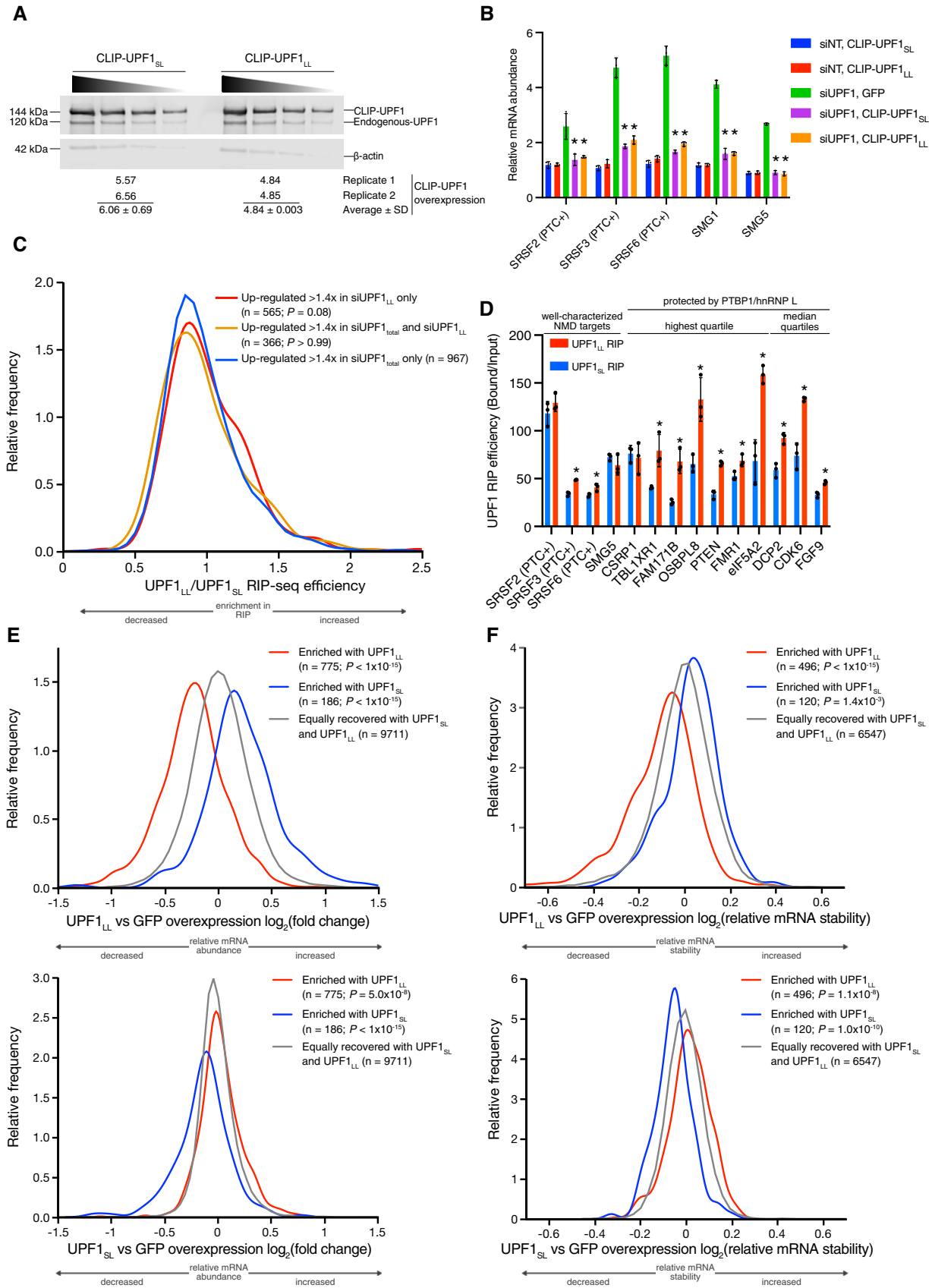


Figure EV2.

Figure EV3. Transcripts targeted by $UPF1_{LL}$ are coordinately down-regulated during ER stress and induction of the ISR.

A Volcano plot of relative mRNA abundance as determined from RNA-seq following treatment of HEK-293 cells with 1 μM tunicamycin for 6 h (Data ref: Park *et al*, 2017). mRNAs were binned by RIP-seq efficiency in CLIP-$UPF1_{LL}$ or CLIP-$UPF1_{SL}$ affinity purifications. Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons. Dashed line indicates the significance threshold $P \leq 0.05$ ($n = 3$ biological replicates).

B Western blot of eIF2α phosphorylation following treatment of HEK-293 cells with 1 μM thapsigargin for 6 h.

C Schematic of the RNA-seq experimental workflow and conditions for $UPF1_{LL}$ knockdown and thapsigargin treatment.

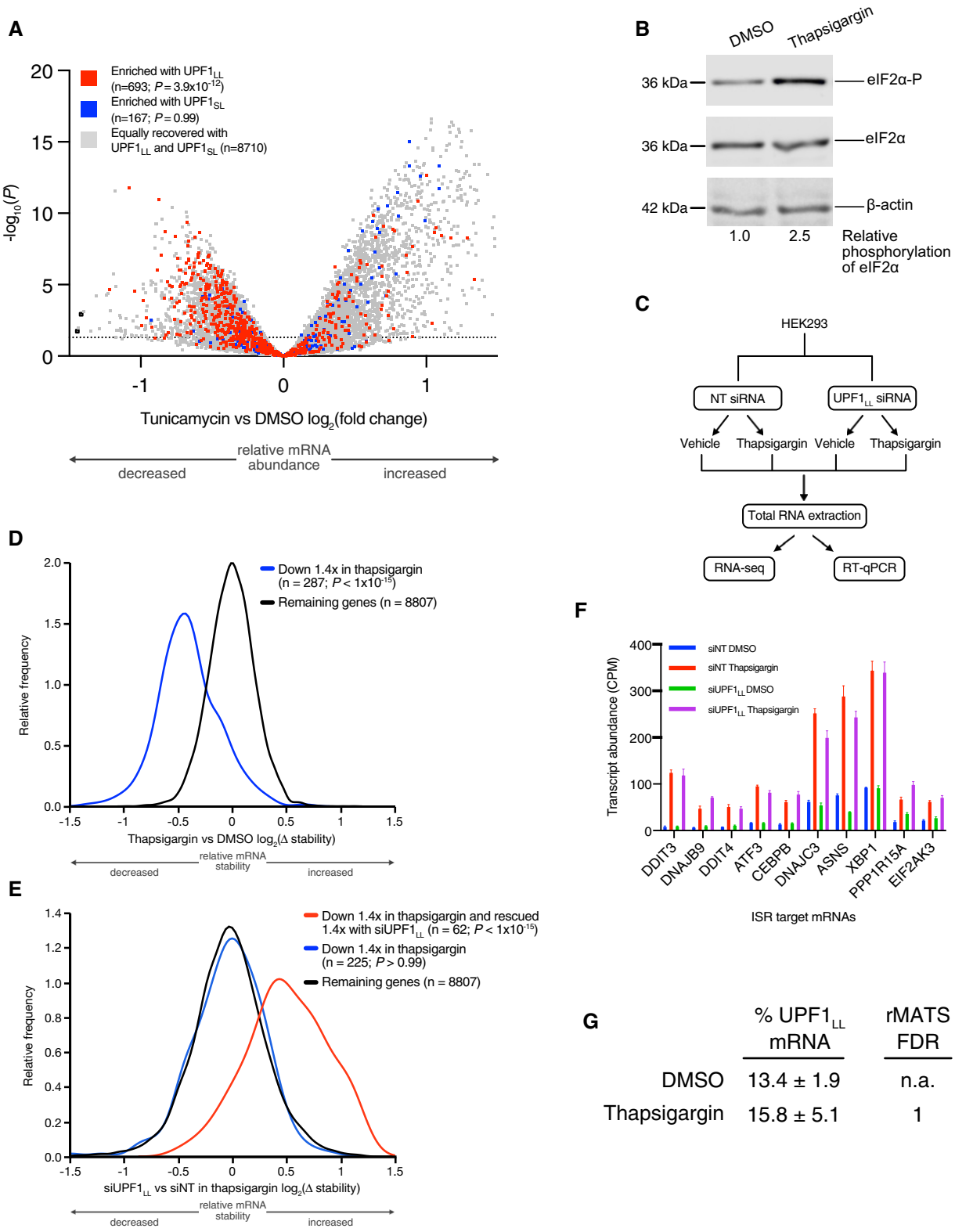
D Density plot of relative mRNA stability as determined by REMBRANDTS analysis of RNA-seq following treatment of HEK-293 cells with 1 μM thapsigargin for 6 h. mRNAs were binned by changes in relative mRNA abundance in thapsigargin. Statistical significance was determined by K–S test.

E Density plot of relative mRNA stability as determined by REMBRANDTS analysis of RNA-seq following $UPF1_{LL}$ knockdown in HEK-293 cells and treatment with 1 μM thapsigargin for 6 h (Alkallas *et al*, 2017). mRNAs were binned by changes in relative mRNA abundance in thapsigargin with $UPF1_{LL}$ knockdown. Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons.

F Quantification of characterized ISR-target transcript abundance in RNA-seq of the indicated conditions. Error bars indicate mean ± SD ($n = 3$ biological replicates).

G Quantification of $UPF1_{LL}$ isoform expression in control and thapsigargin-treated HEK-293 cells from rMATS analyses ($n = 3$ biological replicates).

Source data are available online for this figure.



G

	% UPF1$_{LL}$ mRNA	rMATS FDR
DMSO	13.4 ± 1.9	n.a.
Thapsigargin	15.8 ± 5.1	1

Figure EV3.

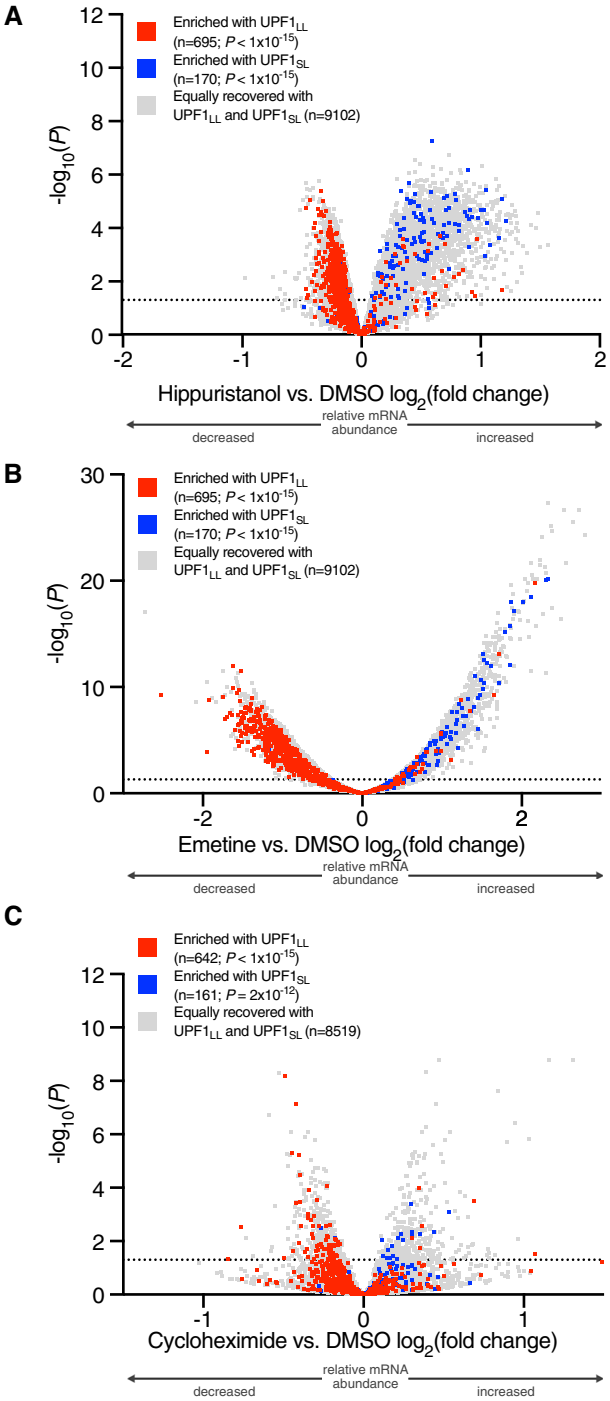


Figure EV4. $UPF1_{LL}$ targets decrease in abundance with translational inhibition.

A Volcano plot of relative mRNA abundance as determined from RNA-seq following treatment of MCF7 cells with 150 nM hippuristanol for 1 h (Data ref: Waldron *et al*, 2019). mRNAs were binned by RIP-seq efficiency in CLIP-$UPF1_{LL}$ or CLIP-$UPF1_{SL}$ affinity purifications. Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons. Dashed line indicates the significance threshold $P \leq 0.05$ ($n = 3$ biological replicates).

B Volcano plot as in (A), following treatment of HEK-293 cells with 50 ng/ml emetine for 4 h (Martinez-Nunez *et al*, 2017). Dashed line indicates the significance threshold $P \leq 0.05$ ($n = 3$ biological replicates).

C Volcano plot as in (A), following treatment of HeLa cells with 100 μg/ml cycloheximide for 15 min (Data ref: Kearse *et al*, 2019). Dashed line indicates the significance threshold $P \leq 0.05$ ($n = 3$ biological replicates).

Figure EV5. Reduced translation efficiency promotes $UPF1_{LL}$ activity.

A Schematic of the RNA-seq experimental workflow and conditions for $UPF1_{LL}$ knockdown and puromycin treatment.

B Density plot of relative mRNA abundance as determined by RNA-seq following treatment of HEK-293 cells with 50 μg/ml puromycin. mRNAs were binned according to destabilization in CLIP-$UPF1_{LL}$ or CLIP-$UPF1_{SL}$ overexpression experiments, as determined by REMBRANDTS analysis (Alkallas *et al*, 2017). Statistical significance was determined by K–S test.

C Quantification of $UPF1_{LL}$ isoform expression in control and puromycin-treated HEK-293 cells from rMATS analyses ($n = 3$ biological replicates) (Shen *et al*, 2014).

D RT–qPCR analysis of indicated transcripts following treatment of HEK-293 cells with indicated concentrations of puromycin for 4 h. Relative fold changes are in reference to vehicle-treated control. Significance of puromycin treatment on relative transcript abundance was compared to the vehicle-treated control. Asterisk (*) indicates $P < 0.05$, as determined by two-way ANOVA. Black dots represent individual data points, and error bars indicate mean ± SD ($n = 3$ biological replicates). Dashed lines indicate $\log_2$ (fold change) of ± 0.5. PTC^+ indicates the use of primers specific to the transcript isoform with a validated poison exon (Lareau *et al*, 2007; Ni *et al*, 2007). See also Dataset EV3 for *P*-values associated with each statistical comparison.

E Density plot of relative mRNA abundance as determined by RNA-seq following treatment of HEK-293 cells with 25 μg/ml or 100 μg/ml puromycin. mRNAs were binned according to sensitivity to 50 μg/ml puromycin and $UPF1_{LL}$ knockdown. Statistical significance was determined by K–S test.

F Density plot of relative mRNA stability as determined by REMBRANDTS analysis of RNA-seq following treatment of HEK-293 cells with 50 μg/ml puromycin for 4 h (Alkallas *et al*, 2017). mRNAs were binned by changes in relative mRNA abundance in puromycin. Statistical significance was determined by K–S test.

G Density plot of relative mRNA stability as determined by REMBRANDTS analysis of RNA-seq following $UPF1_{LL}$ knockdown in HEK-293 cells and treatment with 50 μg/ml puromycin for 4 h (Alkallas *et al*, 2017). mRNAs were binned by changes in relative mRNA abundance in puromycin with $UPF1_{LL}$ knockdown. Statistical significance was determined by K–W test, with Dunn's correction for multiple comparisons.

Source data are available online for this figure.

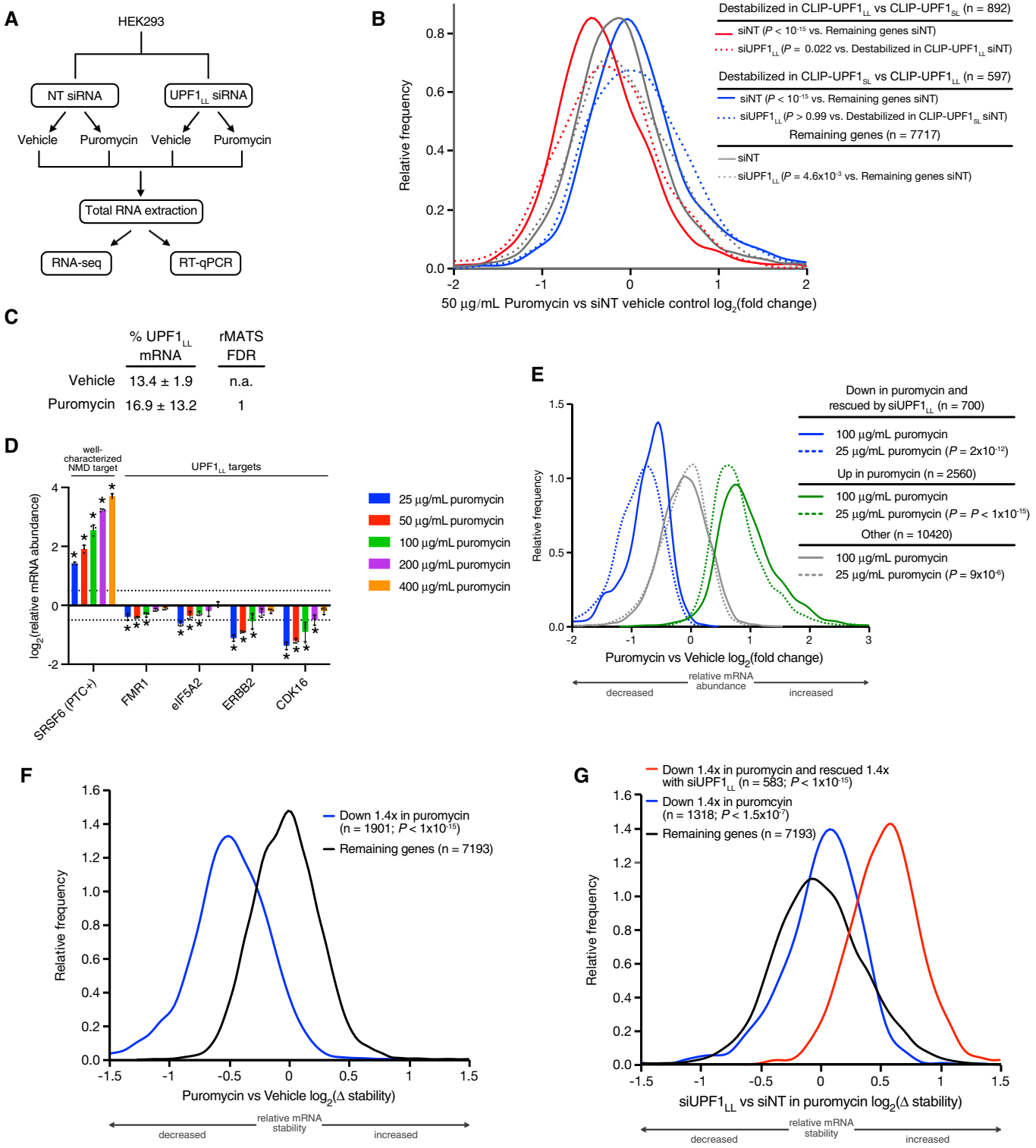

A
HEK293
NT siRNA
UPF1LL siRNA
Vehicle
Puromycin
Vehicle
Puromycin
Total RNA extraction
RNA-seq
RT-qPCR
B
Destabilized in CLIP-UPF1LL vs CLIP-UPF1SL (n = 892)
siNT (P < 10-15 vs. Remaining genes siNT)
siUPF1LL (P = 0.022 vs. Destabilized in CLIP-UPF1LL siNT)
Destabilized in CLIP-UPF1SL vs CLIP-UPF1LL (n = 597)
siNT (P < 10-15 vs. Remaining genes siNT)
siUPF1LL (P > 0.99 vs. Destabilized in CLIP-UPF1SL siNT)
Remaining genes (n = 7717)
siNT
siUPF1LL (P = 4.6x10-3 vs. Remaining genes siNT)
Relative frequency
50 µg/mL Puromycin vs siNT vehicle control log2(fold change)
C
% UPF1LL mRNA
rMATS FDR
Vehicle 13.4 ± 1.9 n.a.
Puromycin 16.9 ± 13.2 1
D
well-characterized NMD target
UPF1LL targets
log2(relative mRNA abundance)
SRSF6 (PTC+)
FMR1
eIF5A2
ERBB2
CDK16
25 µg/mL puromycin
50 µg/mL puromycin
100 µg/mL puromycin
200 µg/mL puromycin
400 µg/mL puromycin
E
Down in puromycin and rescued by siUPF1LL (n = 700)
100 µg/mL puromycin
25 µg/mL puromycin (P = 2x10-12)
Up in puromycin (n = 2560)
100 µg/mL puromycin
25 µg/mL puromycin (P = P < 1x10-15)
Other (n = 10420)
100 µg/mL puromycin
25 µg/mL puromycin (P = 9x10-6)
Relative frequency
Puromycin vs Vehicle log2(fold change)
relative mRNA abundance
decreased
increased
F
Down 1.4x in puromycin (n = 1901; P < 1x10-15)
Remaining genes (n = 7193)
Relative frequency
Puromycin vs Vehicle log2(Δ stability)
relative mRNA stability
decreased
increased
G
Down 1.4x in puromycin and rescued 1.4x with siUPF1LL (n = 583; P < 1x10-15)
Down 1.4x in puromcyin (n = 1318; P < 1.5x10-7)
Remaining genes (n = 7193)
Relative frequency
siUPF1LL vs siNT in puromycin log2(Δ stability)
relative mRNA stability
decreased
increased

Figure EV5.