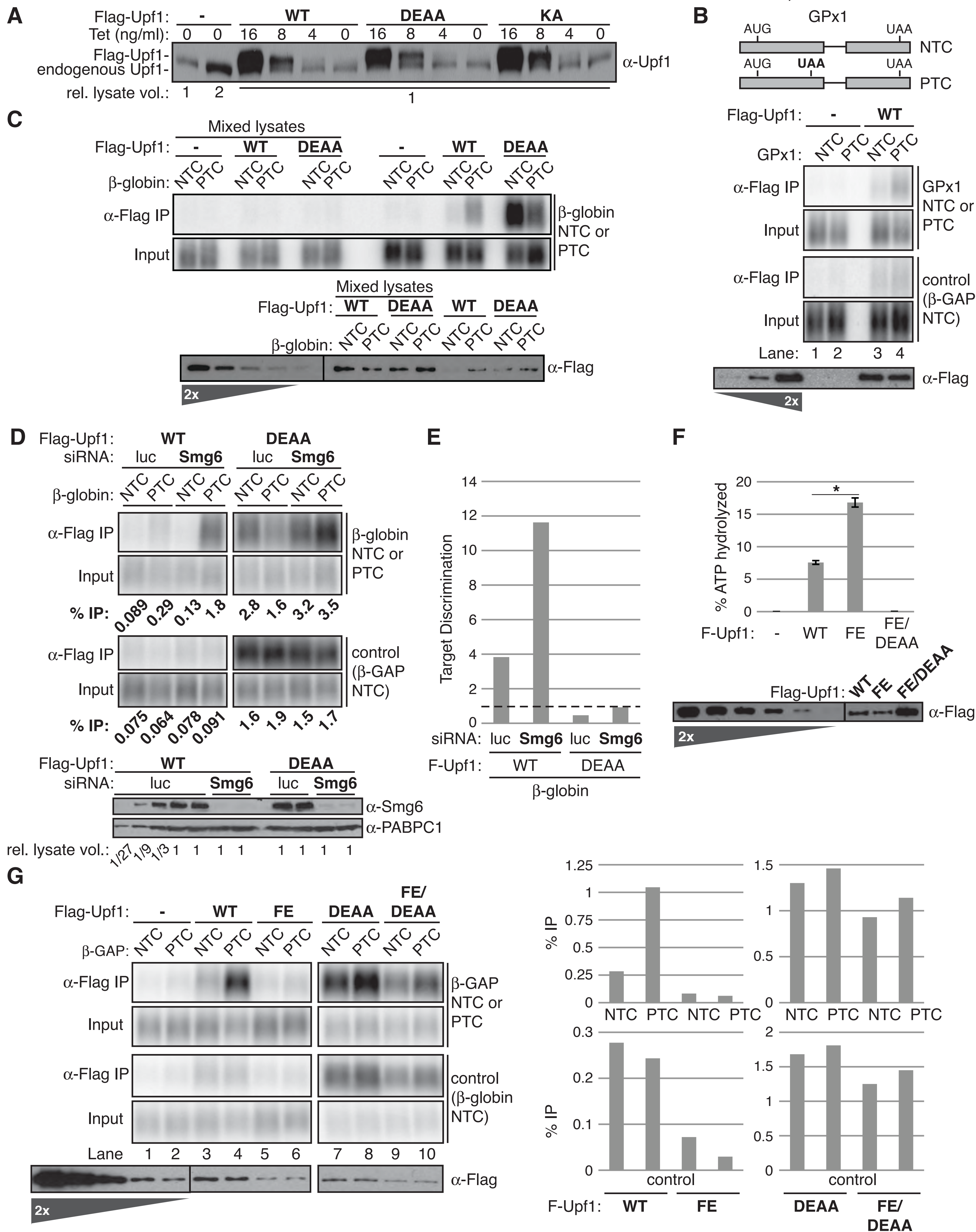
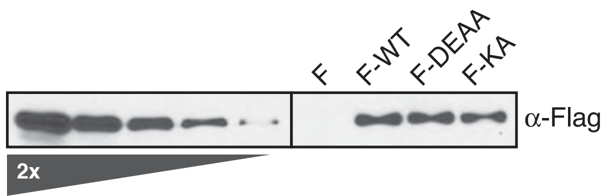
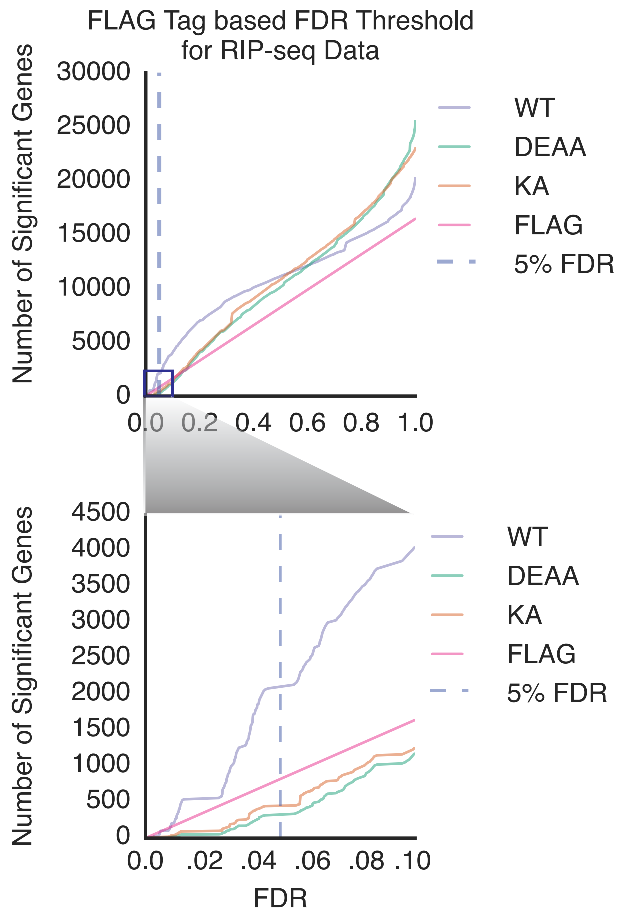
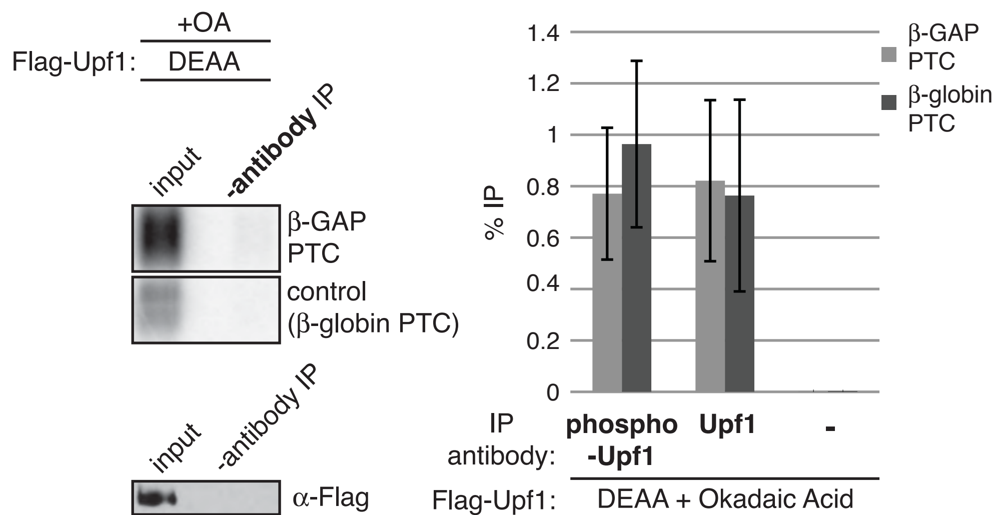
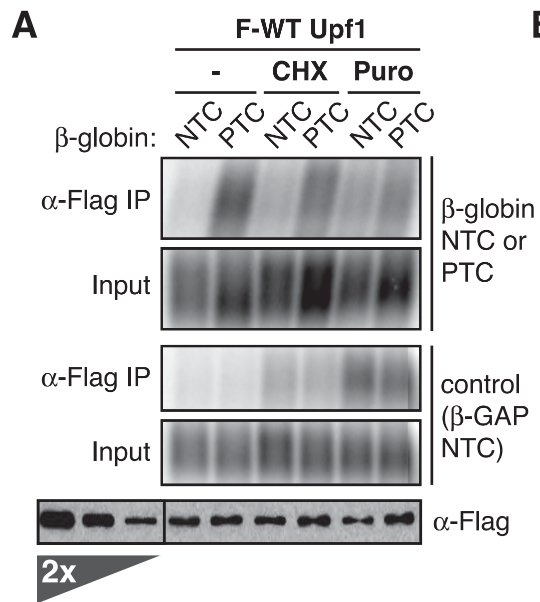
Lee at al FIGURE S1, RELATED TO FIGURE 1

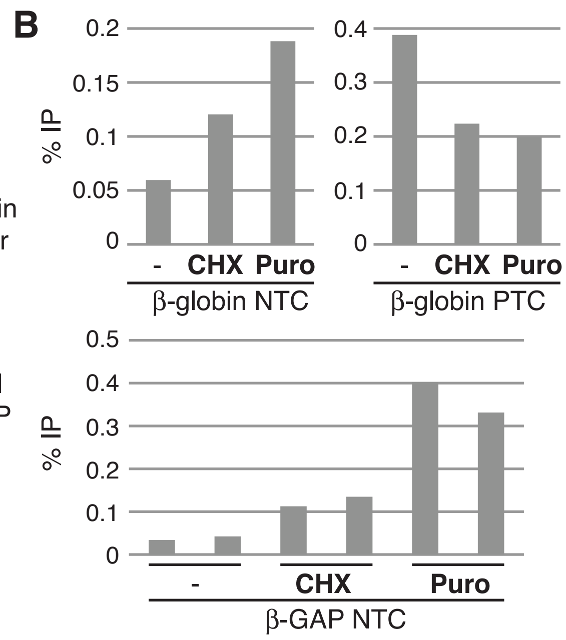



A
F
F-WT
F-DEAA
F-KA
α-Flag
2x

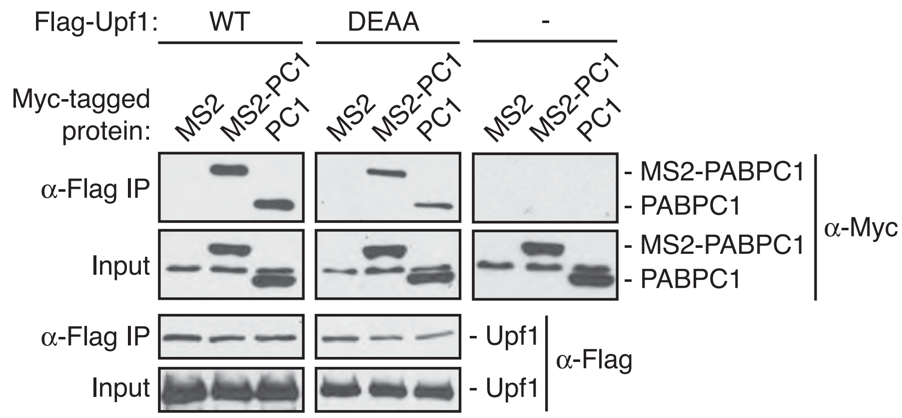

B
FLAG Tag based FDR Threshold
for RIP-seq Data
Number of Significant Genes
30000
25000
20000
15000
10000
5000
0
0.0 0.2 0.4 0.6 0.8 1.0
WT
DEAA
KA
FLAG
5% FDR
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0.0 .02 .04 .06 .08 .10
FDR


+OA
Flag-Upf1:
DEAA
input
-antibody IP
β-GAP
PTC
control
(β-globin PTC)
input
-antibody IP
α-Flag
1.4
1.2
1
0.8
0.6
0.4
0.2
0
% IP
β-GAP
PTC
β-globin
PTC
IP
antibody:
phospho
-Upf1
Upf1
-
Flag-Upf1:
DEAA + Okadaic Acid

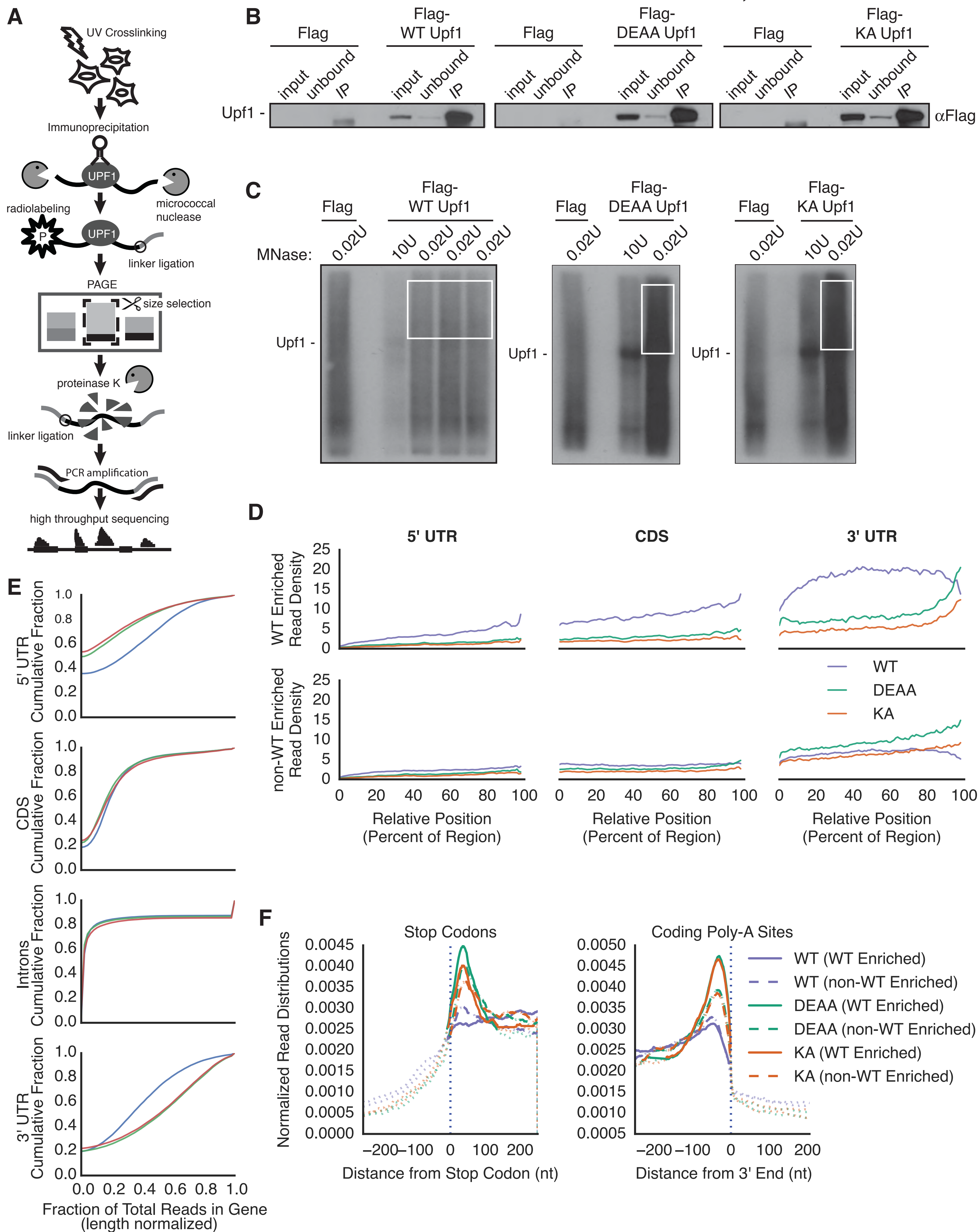

A
F-WT Upf1
-
CHX
Puro
β-globin:
NTC
PTC
NTC
PTC
NTC
PTC
α-Flag IP
Input
β-globin NTC or PTC
α-Flag IP
Input
control (β-GAP NTC)
α-Flag
2x

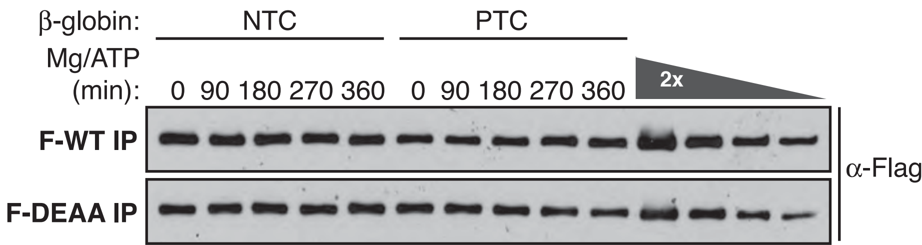

B
% IP
0.2
0.15
0.1
0.05
0
-
CHX
Puro
β-globin NTC
0.4
0.3
0.2
0.1
0
-
CHX
Puro
β-globin PTC
0.5
0.4
0.3
0.2
0.1
0
-
CHX
Puro
β-GAP NTC

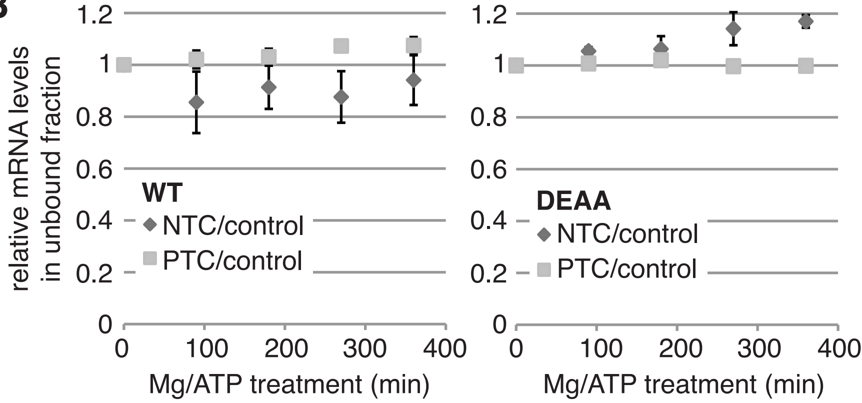
Lee at al FIGURE S5, RELATED TO FIGURE 5

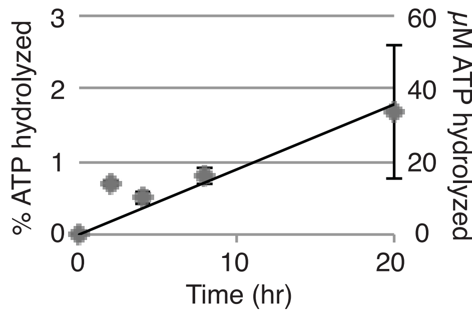


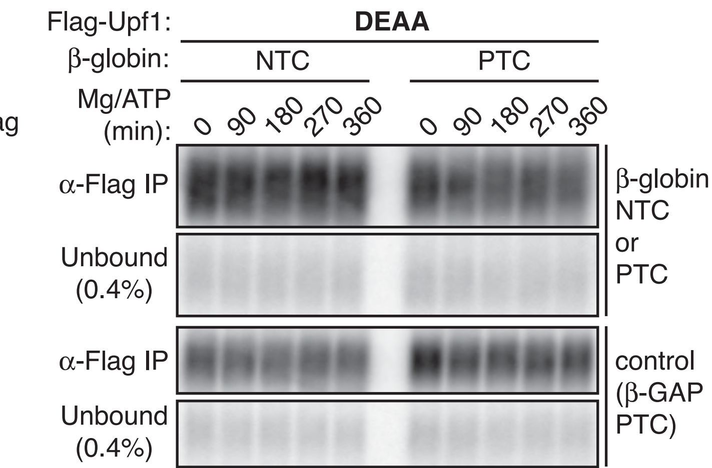
Lee et al FIGURE S6, RELATED TO FIGURE 6

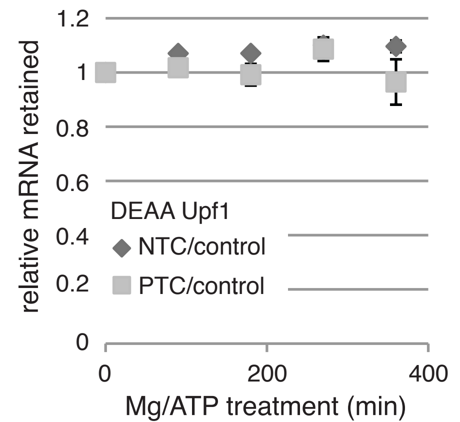


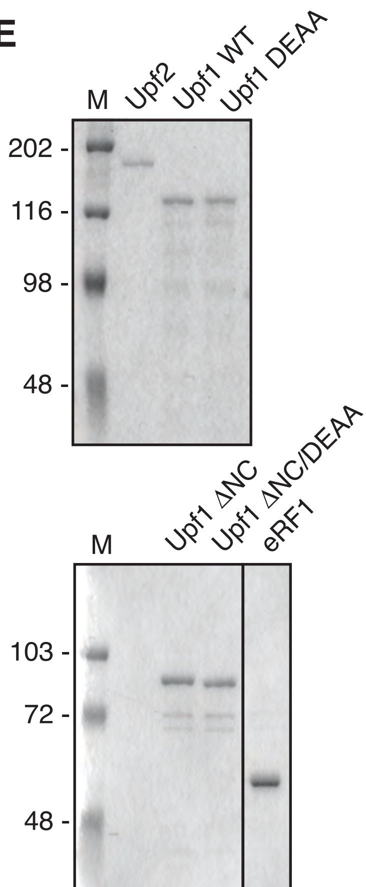

A
β-globin:
NTC
PTC
Mg/ATP (min):
0 90 180 270 360
0 90 180 270 360
2x
F-WT IP
F-DEAA IP
α-Flag

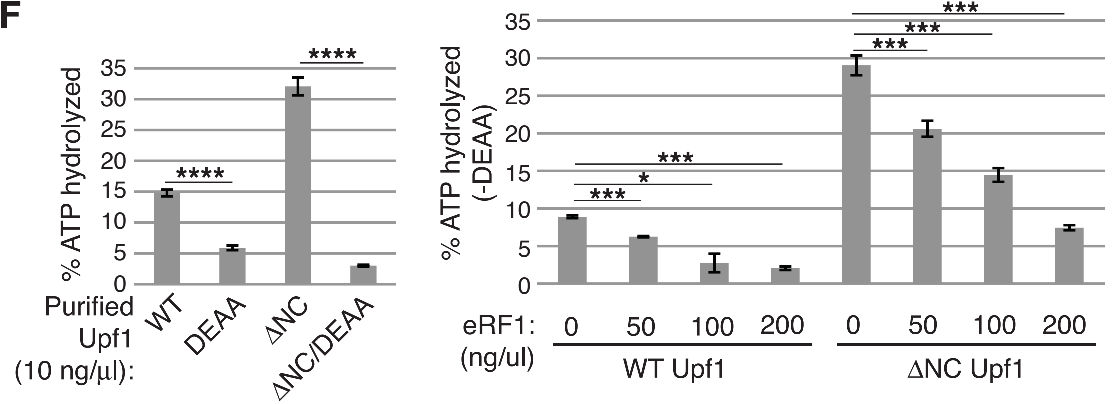

B
relative mRNA levels in unbound fraction
WT
NTC/control
PTC/control
DEAA
NTC/control
PTC/control
Mg/ATP treatment (min)

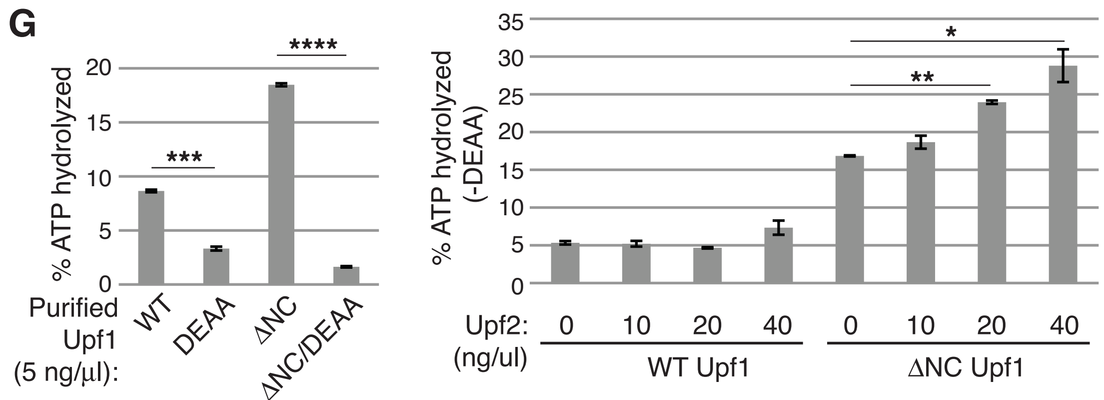

C
% ATP hydrolyzed
μM ATP hydrolyzed
Time (hr)


D
Flag-Upf1:
DEAA
β-globin:
NTC
PTC
Mg/ATP (min):
0 90 180 270 360
0 90 180 270 360
α-Flag IP
Unbound (0.4%)
β-globin NTC or PTC
α-Flag IP
Unbound (0.4%)
control (β-GAP PTC)


relative mRNA retained
DEAA Upf1
NTC/control
PTC/control
Mg/ATP treatment (min)


E
M
Upf2
Upf1 WT
Upf1 DEAA
202
116
98
48
M
Upf1 ΔNC
Upf1 ΔNC/DEAA
eRF1
103
72
48


F
% ATP hydrolyzed
Purified Upf1 (10 ng/μl):
WT
DEAA
ΔNC
ΔNC/DEAA
% ATP hydrolyzed (-DEAA)
eRF1: (ng/ul)
0 50 100 200
0 50 100 200
WT Upf1
ΔNC Upf1


G
% ATP hydrolyzed
Purified Upf1 (5 ng/μl):
WT
DEAA
ΔNC
ΔNC/DEAA
% ATP hydrolyzed (-DEAA)
Upf2: (ng/ul)
0 10 20 40
0 10 20 40
WT Upf1
ΔNC Upf1

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Upf1 RIP supplemental assays, Related to Figure 1

(A) Western blot for Upf1 in whole cell lysates from cell lines induced with varying concentrations of tetracycline (Tet) to express no Flag-tagged Upf1 (-), Flag-Upf1 WT, Flag-Upf1 D637A/E638A (DEAA) and Flag-Upf1 K498A (KA). In Flag-Upf1 lanes, slower migrating band represents Flag-tagged Upf1 and faster migrating band represents endogenous Upf1.

(B) Northern blots of GPx1 and control mRNAs in inputs (0.4%) or coprecipitated with Flag-Upf1 using anti-Flag antibody (α-Flag IP). Flag-Upf1 recovered in IPs is shown alongside a two-fold titration of input lysate on an anti-Flag Western blot under Northerns.

(C) Northern blots of β-globin mRNAs in inputs (0.9%) or α-Flag IPs from reporter transfected, Flag-Upf1-expressing cells (lanes on the right) or from a mix of untransfected, Flag-Upf1 expressing cells and reporter-transfected parental cells (lanes on the left; mixed lysates). Western blots of Flag-Upf1 recovered in IPs are shown alongside a two-fold titration of input under Northerns.

(D) Northern blots of β-globin mRNAs in inputs (0.5%) or α-Flag IPs from Flag-Upf1 WT or DEAA expressing cells treated with control luciferase siRNA or Smg6 siRNA, with Western blots for Smg6 and PABPC1 in inputs under Northerns. Relative volume of lysate in Western blots is indicated for lane comparison (rel. lysate vol.).

(E) Target discrimination for RNA-IP shown in panel E, calculated as described in Figure 1E.

(F) Graph representing mean percent ATP hydrolyzed in *in vitro* ATPase assays of Upf1 WT, FE, and FE/DEAA purified from FITR-based stable cell lines +/- SEM from three repeats using the same protein preparation. Asterisk denotes P-value: *≤0.03 (paired student t-test, two-tailed). Western blot under graph shows levels of Flag-Upf1 used in the ATPase assays, alongside two-fold titration of purified recombinant GST-Flag for quantification.

(G) Northern and Western blots and quantification of a biological replicate of the RNA-IP assays shown in Figure 1F.

Figure S2. Supplemental data related to RIP-RNAseq, Related to Figure 2

(A) Western blot of Flag-Upf1 recovered in RNA-IPs used for the RNA seq in Figure 2 alongside a two-fold titration of Flag-Upf1 WT input lysate.

(B) Plot showing derivation of empirical false discovery rate (FDR) based on IP/input ratios for FLAG only, with X-axis as FDR and Y-axis as the number of genes remaining at that FDR threshold for all experiments. Inset depicts region around 5% FDR cut-off (0.05) used for designating genes identified in WT Upf1 RNA-IP as Upf1-enriched.

Figure S3. Background association of mRNA and protein to IP resin without antibody is minimal, Related to Figure 3

Representative Northern blots of β-GAP and control reporter mRNAs in inputs (0.3%) or coprecipitated with protein A sepharose resin lacking antibody from okadaic acid-treated cells expressing Flag-Upf1 DEAA. Western blot of Flag-Upf1 in inputs (0.2%) and IPs is shown below Northerns. Graph shown on right represents mean percent IP recovery +/- SEM of duplicate biological repeats for β-GAP PTC and control mRNAs in RNA-IPs with resin lacking antibody (-) compared to resin with α-phospho-Upf1 and α-Upf1 antibodies.

Figure S4. Translation elongation inhibitors increase Upf1 association with non-targets and reduce association with target mRNAs, Related to Figure 4

(A) Northern blots of β-globin and control reporter mRNAs in inputs (0.5%) and α-FLAG IPs from cells stably expressing Flag-Upf1 and treated with no translation inhibitor (-), or with 100 μg/ml cycloheximide (CHX), or 500 μg/ml puromycin (Puro) for two hours or 30 minutes, respectively. Western blots for Flag-Upf1 recovered in IPs alongside a two-fold titration of input lysate are shown under Northerns.

(B) Percent recovery of β-globin and control reporters in RNA-IPs shown in panel A.

Figure S5. Recovery of PABPC1 with and without MS2 fusion in Upf1 RIPs, Related to Figure 5

Western blots of Myc-tagged proteins and Flag-Upf1 in inputs (4%) and IPs from RNA-IP assays shown in Figure 5A. Asterisks denote P-values: *≤0.1, ** ≤0.01, *** ≤0.001 (Paired student t-test, two-tailed).

Figure S6. Supplemental data related to CLIP-seq, Related to Figure 6

(A) Overview of workflow for CLIP-seq performed with Flag-Upf1 WT, DEAA and KA using α-Flag antibodies for immunoprecipitation.

(B) Anti-Flag Western blots of Flag-protein purification for CLIP-seq.

(C) Film exposure of polyacrylamide resolved, ^{32}P-end-labeled RNA that remained associated with Flag-Upf1 WT, DEAA and KA compared to Flag epitope-only, after 0.02U or 10U MNase digestion. The prominent band made apparent with 10U MNase treatment only in Flag-Upf samples migrates at a size consistent with Flag-Upf1. White box delineates region excised for CLIP-seq library construction.

(D) Mean read density across the metagene, shown as a percentage of total reads in CLIPs for WT enriched and non-WT enriched genes, as defined in Figure 2, not normalized to the total number of reads per gene.

(E) Cumulative fraction of genes with regional read abundance represented as a fraction of total reads in the gene, normalized to nucleotide length. UTR, untranslated region; CDS, coding sequence

(F) Read density around stop codons and mRNA 3' ends for WT-enriched and non-WT enriched genes. Solid and long-dash lines represent regions where differences were found to be significant with a P-value <0.05 (Bonferroni corrected).

Figure S7. Upf1 release and ATPase assays, Related to Figure 7.

(A) Western blots of Flag-tagged Upf1 recovered in IPs in release assay shown in Figures 7A and S7D alongside two-fold titrations of input lysate.

(B) Graphs showing mean ratios in unbound fractions of β-globin mRNA levels to internal control β-GAP PTC mRNA levels after indicated time of $MgCl_2$-ATP-treatment for the experiments shown in Figures 7A and S7D.

(C) Graph showing percent (left) and μM (right) hydrolyzed ATP over time of incubation with Upf1 at 4˚C in release assay buffer using 200 ng/μl recombinant His_6-tagged Upf1 ΔNC (aa 115-914) and 2 mM ATP. Values for each data point were calculated by subtraction of background activity observed with a Upf1 DEAA counterpart. The estimated rate of hydrolysis based on the shown trend line is ≈one molecule of ATP hydrolyzed per hour per molecule of Upf1.

(D) Release assays for Upf1 DEAA similar to those for Upf1 WT in Figure 7A.

(E) Coomassie-stained, SDS-PAGE-resolved recombinant full-length Upf2 and full-length Upf1 WT and DEAA (left panel), and recombinant Upf1 ΔNC WT and DEAA, and eRF1 (right panel) used in Figures S7D, E.

(F) Graphs showing percent of ATP hydrolyzed in *in vitro* ATPase assays by 10 ng/μl purified recombinant Upf1 variants (left) and Upf1 WT or Upf1 ΔNC in the presence of increasing amounts of eRF1 (right). Values in graph shown on the right were calculated by subtraction of background activities observed with Upf1 DEAA counterparts. Data is represented as mean +/- SEM for triplicate repeats from single protein preparations.

(G) Same as panel F, but using 5 ng/μl Upf1 variants in the absence or presence of increasing amounts of Upf2 as indicated.

Asterisks denote P-values: *≤0.1, **≤0.05, *** ≤0.01, ****≤0.001 (paired student t-test, two-tailed).

SUPPLEMENTAL TABLES AND TABLE LEGENDS

Table S1. Gene types represented by transcripts in Upf1 RIP-seq compared to inputs, Related to Figure 2

Percent of RNA-IP-seq reads mapping to different gene types in FLAG only and FLAG-Upf1 WT, DEAA and KA IPs and input samples, along with percent representation in the human genome.

Type	Wild Type Input	Wild Type IP	DEAA Input	DEAA IP	KA Input	KA IP	FLAG Input	FLAG IP	Genome
antisense	4.24%	0.41%	5.47%	0.25%	4.94%	0.24%	3.82%	2.77%	4.17%
lincRNA	1.46%	0.61%	1.52%	0.98%	1.55%	1.03%	0.97%	3.90%	5.62%
misc_RNA	14.85%	0.29%	17.65%	0.60%	16.04%	0.79%	13.82 %	10.40%	0.35%
Processed transcript	0.92%	1.11%	0.89%	1.16%	0.91%	1.11%	0.95%	0.87%	3.02%
Protein coding	67.86%	92.73%	63.60%	80.76%	65.05%	79.77%	69.86 %	69.15%	75.57%
pseudogene	9.01%	4.42%	8.72%	15.62%	9.75%	16.26%	9.68%	8.93%	9.71%
snoRNA	0.80%	0.19%	0.85%	0.26%	0.90%	0.33%	0.43%	3.12%	0.14%

Table S2. Gene types represented by transcripts in Upf1 CLIP-seq, Related to Figure 4

Percent of CLIP-seq reads mapping to different gene types in FLAG-Upf1 WT, DEAA and KA CLIPs.

Type	WT CLIP	DEAA CLIP	KA CLIP
antisense	0.52%	0.42%	0.42%
lincRNA	1.15%	1.34%	1.39%
misc_RNA	0.27%	0.50%	0.56%
processed_transcript	1.04%	0.92%	0.92%
protein_coding	94.67%	94.12%	93.57%
pseudogene	1.43%	1.80%	2.12%
snoRNA	0.28%	0.21%	0.27%

EXTENDED EXPERIMENTAL PROCEDURES

RNA-immunoprecipitation (RIP) and release assays

Stable cell lines or HEK293T cells were transfected with CMV promoter-driven reporter mRNA constructs, as well as protein expression constructs if used, 48-72 hrs before cell harvest (see below for details on stable cell lines and constructs). Protein depletions, if used, were achieved through transfection with siRNAs (Dharmacon, see below for sequences). Flag-Upf1 in stable cell lines was induced with tetracycline (Sigma) and okadaic acid-treated cells were treated with 200 nM (Calbiochem) at 20-24 and 3-3.5 hrs, respectively before harvest. Cells were harvested by scraping into phosphate-buffered saline (PBS) and centrifugation at 1,500 rpm in a clinical centrifuge at room temperature, followed by 1-2 rinses with PBS.

Except for RIPs involving pre-treatment with λ protein phosphatase, cell lysates were prepared from cell pellets with 1 ml per 10 cm plate of cells of ice-cold isotonic lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, 0.1% Triton-X100) containing 0.1 μg/ml total yeast RNA, 2 μg/ml aprotinin and leupeptin, 1 mM phenylmethanesulfonyl fluoride and 2 μl/ml RNaseOUT (Invitrogen) and clarified by microcentrifugation at 15,000 rpm at 4°C. Phosphatase Arrest I (G Biosciences) was included in lysis buffer at 1x final concentration for all lysates used in Figure 3 except if lysate was treated with λ protein phosphatase. λ protein phosphatase and mock treatments involved preparation of clarified cell lysates with 150 μl isotonic lysis buffer containing yeast RNA and inhibitors as described above, except with 12 μl/ml

RNaseOUT. These lysates were then incubated for 2 hours at 30°C in 1x PMP buffer (NEB), 1 mM $MnCl_2$ (NEB), and 2000 units of λ protein phosphatase (NEB) or nuclease-free water (Ambion), before dilution to 1 ml total volume with additional lysis buffer.

Immunoprecipitations (IPs) were performed at 4°C from one 10-cm plate equivalent of lysates with 25-50 μl anti-Flag M2 agarose (Sigma), in the presence of 1 μg/ml FLAG peptide to reduce non-specific background, or protein A Sepharose CL-4B (GE Healthcare) reconstituted in wash buffer (5 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Triton-X100) and preconjugated with 10 μl anti-Myc 9B11 (mouse monoclonal, Cell Signaling Technology), 5 μg anti-phospho-Upf1 (Ser 1127, rabbit polyclonal, EMD Millipore), or 10 μl anti-Upf1 (rabbit polyclonal, (Lykke-Andersen et al., 2000)) antibody at 4°C overnight in the presence of protease inhibitors. For RIP assays, IPs were carried out for 1-3 hours, while for release assays, IPs were carried out for 6-7 hours, during which 4 mM $MgCl_2$ and 2 mM ATP or AMP-PNP were added at regular intervals. Resin was recovered by microcentrifugation at 2,000 rpm followed by eight rounds of washes in ice-cold wash buffer and subjected to analysis by Western, Northern or RNA-seq (see below).

Endogenous Upf1 depletion was initially used for all RNA-IP experiments, but later omitted from replicates shown in Figures 2 and 4 through 7 after we found that endogenous Upf1 depletion had no significant impact on reporter mRNA recovery (data not shown). Flag-Upf1 expression was induced in FITR-based stable cell lines to levels close to endogenous Upf1 with media containing 6-8 ng/ml tetracycline (Sigma) for all experiments except those shown in Figures 1F and 7, where 1 μg/ml tetracycline was

used; while the higher induction conditions improved the consistency of results between biological replicates, no difference between near endogenous and higher expression levels was observed in the average recovery of mRNA reporters (data not shown).

Stable cell line establishment

Gene cassettes encoding full-length Upf1 (amino acids 1-1118) were subcloned into the BamHI and NotI sites of pcDNA5 modified with an N-terminal FLAG epitope tag inserted between the AflII and HindIII sites for integration into Flp-In T-Tex (FITR) 293 cell lines as described by the manufacturer (Life Technologies). Upf1 DEAA, KA, and FE mutations were as described previously and introduced by site-directed mutagenesis (Chakrabarti et al., 2011; Franks et al., 2010).

SiRNA sequences

SiRNA target sequences used were (5' to 3'): GCUGCAGGUUACUUACAAG (Smg6, Eberle et al., 2009) and CCAAGAUGCAGUUCCGCUCCA (Upf1, Mendell et al., 2002).

Construction of mammalian mRNA and epitope-tagged protein expression plasmids

All plasmids sequences created in this work are available upon request. In brief, a DNA cassette encompassing the CMV promoter was used to replace the existing promoter in previously described NMD target and non-target mRNA expression constructs (Singh et al., 2008) to enable constitutive expression. For 5' hairpin bearing mRNA expression

constructs, the hairpin derived from previously described constructs (Franks and Lykke-Andersen, 2007) was introduced upstream of the β-globin coding region. PABPC1 constructs were generated by subcloning the PABPC1 cassette derived from a previously described construct (Singh et al., 2008) downstream of Myc or Myc-MS2 tags in CMV-driven plasmids.

RIP sample preparation for Western, Northern or RNA-seq analysis

To monitor protein depletions, if used, and recovery of Flag- or Myc-epitope tagged proteins and/or coprecipitating proteins, a fraction of the input lysate and IP was set aside for Western blotting in 1x SDS loading buffer (60 mM Tris-HCl, pH 6.8, 2% SDS, 4% β-mercaptoethanol, 10% glycerol, 0.1% bromophenol blue and xylene cyanol). To monitor RNA, Trizol (Ambion) was added to input, unbound, and IP samples. RNA was isolated according to the manufacturer, with 25 μg linear polyacrylamide used as carrier for either library preparation for RNA-seq analysis (see below) or Northern blotting.

Western blotting

Western blots were performed using 8% or 10% SDS-PAGE gels for protein resolution before transfer to nitrocellulose. Primary antibodies used were: anti-Myc (rabbit polyclonal, Sigma-Aldrich, C3956; 1:1000 or 9E11 mouse monoclonal, Cell Signaling Technology 1:1000), anti-FLAG (rabbit polyclonal, Sigma, F7425; 1:1000), anti-Smg6 (rabbit polyclonal, Abcam, ab87539; 1:1000), anti-Upf1 (rabbit polyclonal, 1:1000; (Lykke-Andersen et al., 2000), and PABPC1 (mouse monoclonal, Abcam, ab6125;

1:1000). Secondary antibodies were anti-rabbit or anti-mouse horseradish peroxidase-coupled antibodies (Thermo Scientific). Blots were developed using SprayGlo ECL (Denville Scientific) or SuperSignal West Femto (Thermo Scientific) and exposed to film.

Northern blot preparation and imaging

Northern blotting for purified RNA has been described previously (Lykke-Andersen et al., 2000). Blots were visualized and quantified using a PhosphoImager (Typhoon Trio; Amersham Biosciences) and ImageQuant TL software. Two-tailed student's *t* test was used to calculate statistics in Excel (Microsoft).

Preparation of recombinant proteins from E. coli

Bacterial expression plasmid for C-terminally His_6-tagged Upf1 ΔNC was a generous gift from Dr. Le Hir (Chamieh et al., 2008); the plasmid for Upf1 ΔNC DEAA expression was generated by site-directed mutagenesis. Expression plasmid for N-terminally His_6-tagged eRF1 (pET-His-eRF1) was generated by inserting PCR-amplified eRF1 coding region between *Bam*HI and *Not*I sites of a pET-His-PL vector (Wagner et al., 2007). Expression plasmid for full-length Upf1 (pGEX-TEV-Upf1-Flag) and Upf2 (pGEX-TEV-Upf2-Flag), each containing an N-terminal GST-tag followed by a TEV protease cleavage site and a C-terminal Flag-tag, were generated by inserting PCR-amplified coding regions of human Upf1 (between *Bam*HI and *Not*I sites) and Upf2 (between *Xho*I and *Not*I sites) of a pGEX-4T1 vector (GE Health Sciences) modified to encode a TEV cleavage site (ENLYFQG) in frame with GST, upstream of the *Bam*HI site, and a Flag

epitope (DYKDDDK) downstream of the *Not*I site. Expression plasmid for Upf1 DEAA (pGEX-TEV-Upf1 DEAA-Flag) was generated by site-directed mutagenesis of pGEX-TEV-Upf1-Flag.

BL21/DE3 cells containing a pRI952 plasmid expressing rare arginine and isoleucine tRNAs (Tito and Ward, 1995) were transformed with expression constructs and plated on LB plates containing ampicillin (for Upf1, Upf1 DEAA, Upf2 and eRF1) or kanamycin (for Upf1 ΔNC and Upf1 ΔNC DEAA). Colonies were picked and grown up at 37˚C in 200 ml LB containing chloramphenicol (to maintain the pRI952 plasmid) and ampicillin or kanamycin. Protein expression was induced with 0.1 mM IPTG during log-growth (A_{600} = 0.3-0.4) overnight at 15°C.

For His-tagged protein purification, cell pellets were harvested for resuspension in 6 ml ice-cold TKET buffer (10 mM Tris-HCl, pH 7.5, 100 mM KCl, 0.1 mM EDTA, 0.05% Triton X-100) containing 20 mM imidazole, pH 7.0 and 1 mM PMSF. The same conditions were used for GST/Flag-tagged proteins, but in the absence of imidazole. Resuspended cells were sonicated six to eight times in 30 second bursts at 30 second intervals at 6.5 amplitude on ice. Triton X-100 was added to 0.5% and sonicates were nutated at 4°C for 15 minutes before centrifugation at 1,500 rpm for 5 minutes at room temperature, collection of lysate, and centrifugation at 15,000 rpm for 15 minutes at 4°C. Proteins were then purified from supernatants over columns containing 250 *μ*l Ni-agarose resin (Qiagen; for His-tagged proteins) or 250 *μ*l glutathione-sepharose (GE Health Science; for GST/Flag-tagged proteins) pre-washed two times with 10 ml TKET. Resin was washed four times with 10 ml TKET (containing 20 mM imidazole, pH 7.0 for

Ni-agarose) and proteins eluted three times with 250 μl ml TKET containing 200 mM imidazole, pH 7.0 (for Ni-agarose) or 20 mM glutathione (for glutathione-sepharose). For GST-TEV/Flag tagged proteins, pooled elutates were incubated with 100 μg His_6-tagged TEV protease at 4˚C for 2 hours, followed by addition of 50 μl anti-FLAG M2 agarose, nutation at 4˚C for 1 hour, six washes with 1 ml TKET, followed by elution three times with 50 μl ml TKET containing 0.2 mg/ml Flag peptide. Eluted proteins were aliquoted, flash-frozen in liquid nitrogen and stored at -80˚C.

ATPase assays

Proteins were purified from either *E.coli* (for experiments shown in Figure 6 and S7) or stably expressing FITR cell lines (Figure S1), and quantified by Bradford (BioRad) against a bovine serum albumin standard (Fraction V, Sigma-Aldrich) or by Western blot against a recombinant GST-FLAG standard purified from transformed *E. coli*, respectively. *E. coli* expressed proteins were checked for purity by Coomassie staining after resolution by SDS-PAGE. ATPase assays were performed as previously described (Fiorini et al., 2012). ATPase assays in Figure S7C were performed with 200 ng/μl Upf1 ΔNC and 2 mM ATP at 4˚C in the same buffer used for release assays (see above) with the addition of 0.2 mg/ml BSA and 0.2 mg/ml total yeast RNA.

Library construction for RIP-seq and CLIP-seq

For RIP-seq libraries, approximately 1000 ng of input RNA and 1000ng of RNA isolated from RIPs performed as above, were obtained from four 10 cm plates of cells. Libraries

were prepared using an Illumina TruSeq Stranded Total RNA HT Sample Prep Kit with Ribo-Zero Gold for ribosomal RNA depletion. Sequencing was performed on an Illumina HiSeq 2500 instrument. Single end 100 base pair reads were obtained.

For CLIP-seq libraries, CLIP was performed as described previously (Yeo et al., 2009), with slight modifications (see also Figure S6A). Briefly, two 10 cm plates expressing Flag only or Flag-Upf1 variants were irradiated twice with 100 mJ/cm^2 of 254 nm UV light in PBS. Cell pellets were recovered by scraping and centrifugation in a clinical centrifuge at 4°C, flash frozen in liquid nitrogen, and stored at -80°C until library preparation. Each library was prepared from one of the cell pellets, except in the case of wild-type Upf1, where three pellets were used to compensate for the low amount of RNA bound (Figure S6). Each pellet was treated as a separate sample until the membrane cutting step (see below) at which point all wild-type lanes were cut and pooled together. Cell pellets were lysed and immunoprecipitated in CLIP wash buffer (1x PBS, 0.1%SDS, 0.5% deoxycholate, 0.5% NP-40) using anti-FLAG M2 magnetic beads (Sigma). Immunoprecipitates were further washed with high salt wash buffer (5x PBS, 0.1%SDS, 0.5% deoxycholate, 0.5% NP-40) and again with wash buffer. Samples were digested with either 0.02U or 10U per sample of micrococcal nuclease (New England Biolabs) and the 3' RNA linker was ligated. Complexes were labeled with γ-^{32}P-ATP, size fractionated by SDS-PAGE, transferred to nitrocellulose membranes, and visualized by autoradiography. Slices of membrane were cut extending from the 150 kDa marker to about 250 kDa. The cut membranes were digested with proteinase-K and the RNA was recovered by further treatments with urea and phenol-chloroform. The 5'

RNA linker was ligated and library preparation was completed by reverse transcription and 24 cycles of PCR amplification. Fifty base-pair single end reads were obtained using an Illumina HiSeq 2500 instrument.

RIP-seq and CLIP-seq analysis

Sequence alignment of CLIP-seq and RIP-seq data to the human genome.

Sequencing reads from CLIP-seq and RIP-seq libraries were first trimmed of polyA tails, adapters, and low quality ends using cutadapt with parameters --match-read-wildcards --times 2 -e 0 -O 5 --quality-cutoff' 6 -m 18 -b TCGTATGCCGTCTTCTGCTTG -b ATCTCGTATGCCGTCTTCTGCTTG -b CGACAGGTTCAGAGTTCTACAGTCCGACGATC -b TGGAATTCTCGGGTGCCAAGG -b AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA -b TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT. Reads were then mapped against a database of repetitive elements derived from RepBase18.05. Bowtie version 1.0.0 with parameters -S -q -p 16 -e 100 -l 20 was used to align reads against an index generated from Repbase sequences (Langmead et al., 2009). Reads not mapped to Repbase sequences were aligned to the hg19 human genome (UCSC assembly) using STAR (Dobin et al., 2013) version 2.3.0e with parameters --outSAMunmapped Within –outFilterMultimapNmax 1 –outFilterMultimapScoreRange 1.

CLIP-seq Cluster Identification and analyses.

Reads that were PCR replicates were removed from each CLIP-seq library using a custom script. Briefly one read was kept at each nucleotide position when more than one read's 5' end was mapped. Clusters were then assigned using the CLIPper software with parameters --bonferroni --superlocal --threshold- software (Lovci et al., 2013). Clusters that overlap by at least one base pair are considered overlapping clusters, as determined using bedtools (Quinlan and Hall, 2010) and pybedtools (Dale et al., 2011).

Read Distribution Region Counting and comparisons.

Genic features were defined using gencode v17 annotations (Harrow et al., 2006). For each gene, all annotated transcripts for that gene were combined and gene level features were generated. 5' UTR, CDS, and 3' UTR regions from gencode genes were identified and merged at the gene level. The number of reads mapping to each annotated meta-region for each gene was counted. Each gene region (3' and 5' UTRs and CDS) was binned into 100 bins, and the mean of reads in each bin was calculated. Then for all genes, the mean of each bin was calculated and plotted. Finally, for each experiment the distribution was normalized to compute a probability density across each bin. To compare different regions, the total number of reads within each region was totaled. For each region the RPK (reads per 1,000 bases) was calculated. To find the percent of reads falling into each region, the percent of RPK normalized reads that were contained within a given region per gene was calculated.

Read Distribution Feature Counting.

For read distributions around specific genic features (termination codons and 3' transcript ends), features from gencode v17 were selected, the number of reads around each feature was counted, and the mean for each base was calculated across all features. Finally, for each experiment the distribution was normalized to show a probability density across each bin. A background model was generated assuming a uniform distribution of reads distributed to each base, and reads were normally distributed. Each feature at each base is then an independent observation and a z-test was applied to each base (Bonferroni corrected) to see if the reads at that base differed significantly from the background distribution.

RIP-seq Analysis.

RPKMs for each gene annotated in gencode v17 were calculated from RIP-seq data using custom scripts. We determined the fold-change (log2) threshold by which at most 5% of genes in the FLAG RIP-seq sample were "enriched". This threshold reflected a false discovery rate (FDR) of 5%, and was applied to both WT Upf1 and mutant Upf1 RIP-seq samples to identify Upf1 target genes. Non-targets were defined as having a log2 fold change in WT Upf1 RIPs of between -0.05 and 0.05 RPKM.

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