Supplementary information

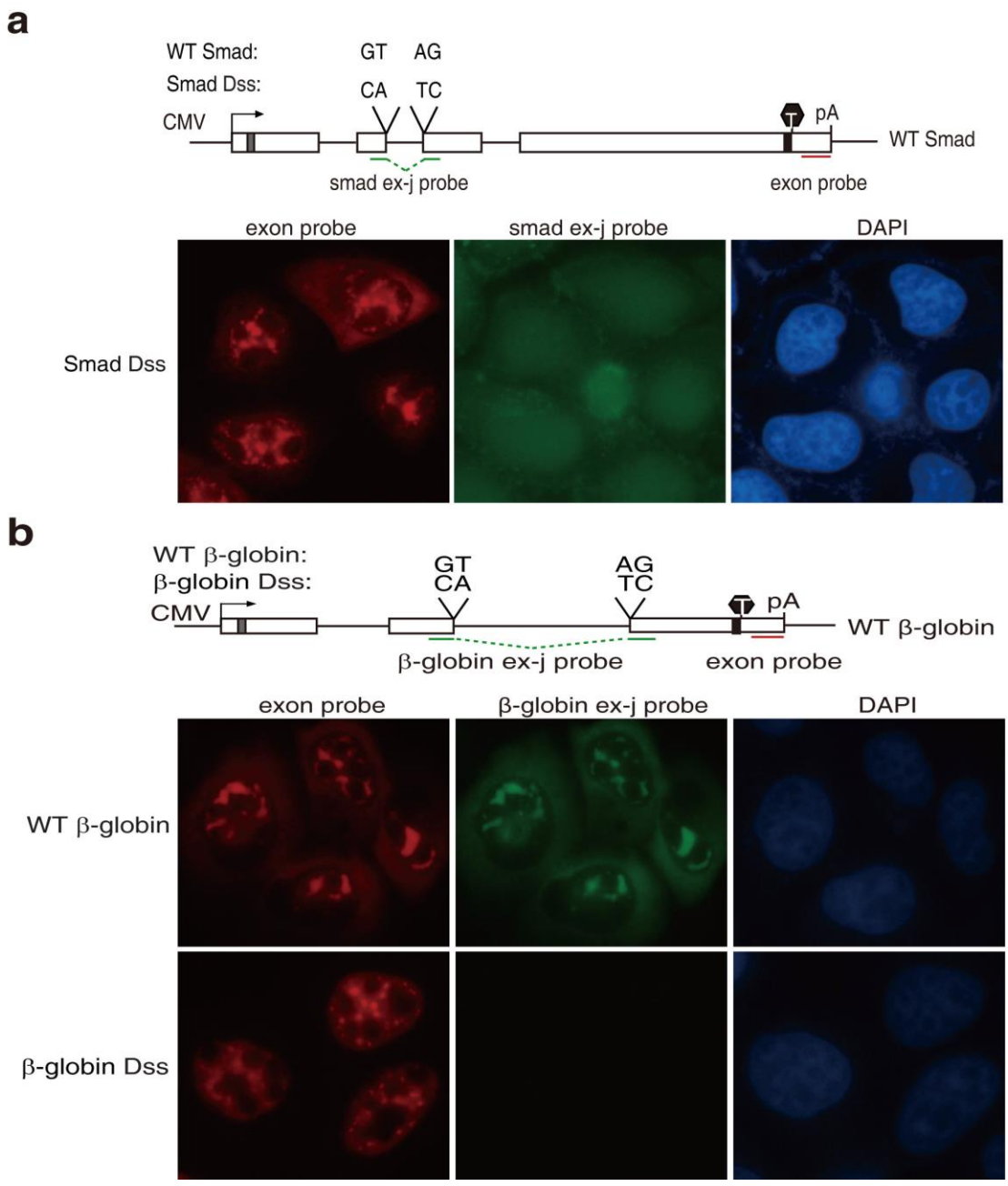

Figure S1. Exon-exon Junction Probes detect spliced mRNA specifically. (a) Schematic of Smad constructs and probe location. The CMV promoter and BGH polyA sites and the location of the FISH probe (indicated by short line) are shown. The grey sand black bars indicate Myc and HA tag respectively. The black triangle indicates stop

codon. The splice sites (GT and AG) are mutated to CA and TC for inhibition of splicing. Smad WT and Dss constructs (50 ng/μl) were microinjected into nuclei of HeLa cell, and α-amanitin was added to block transcription 15 min after microinjection. FISH of was carried out with smad exon-exon junction (ex-j, green) probe and exon probe (red) by 1 hour. (b) Schematic of β-globin constructs and probe location. Same as (a), except that β-globin constructs were injected and FISH was carried out with β-globin ex-j probe and exon probe.

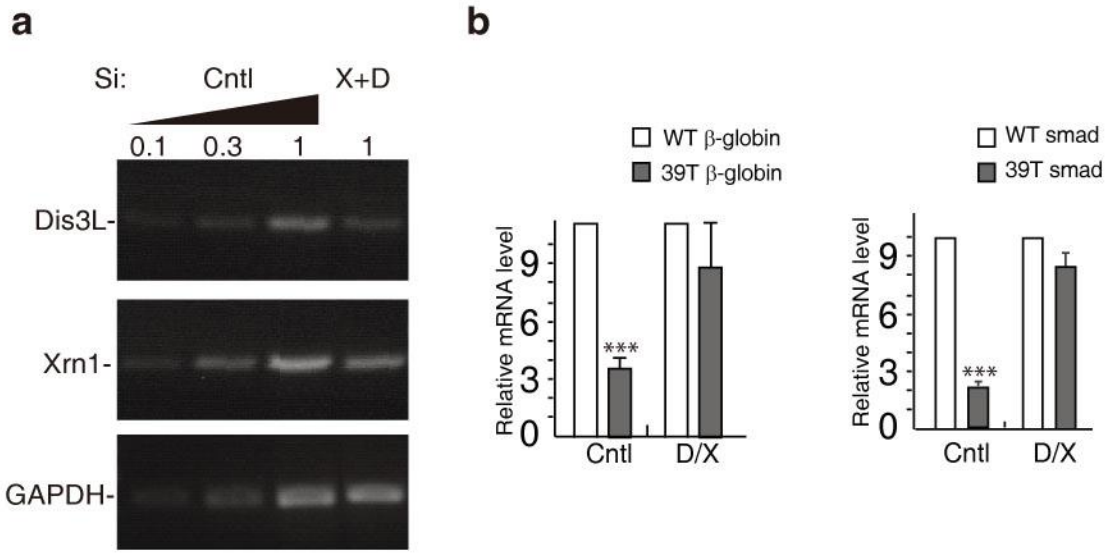

Figure S2. Xrn1 and Dis3L co-knockdown rescued decay of PTC mRNAs. (a) Xrn1 and Dis3L siRNA or non-targeting siRNAs were transiently transfected into HeLa cells, and 48 hrs post-transfection, WT or 39T Smad construct was co-transfected with GFP into these knockdown cells. 24 hrs after transfection, RNAs were extracted followed by RT-PCR using indicated primers. The knockdown efficiencies of Xrn1 and Dis3L were detected by RT- PCR. (b) The ratio of mRNA levels of β-globin and Smad to GFP was quantified and indicated by the bars. Error bars indicate the standard errors from three independent experiments. Statistical analysis was performed using the Student's t test. *P<0.05; **P<0.01; ***P<0.001.

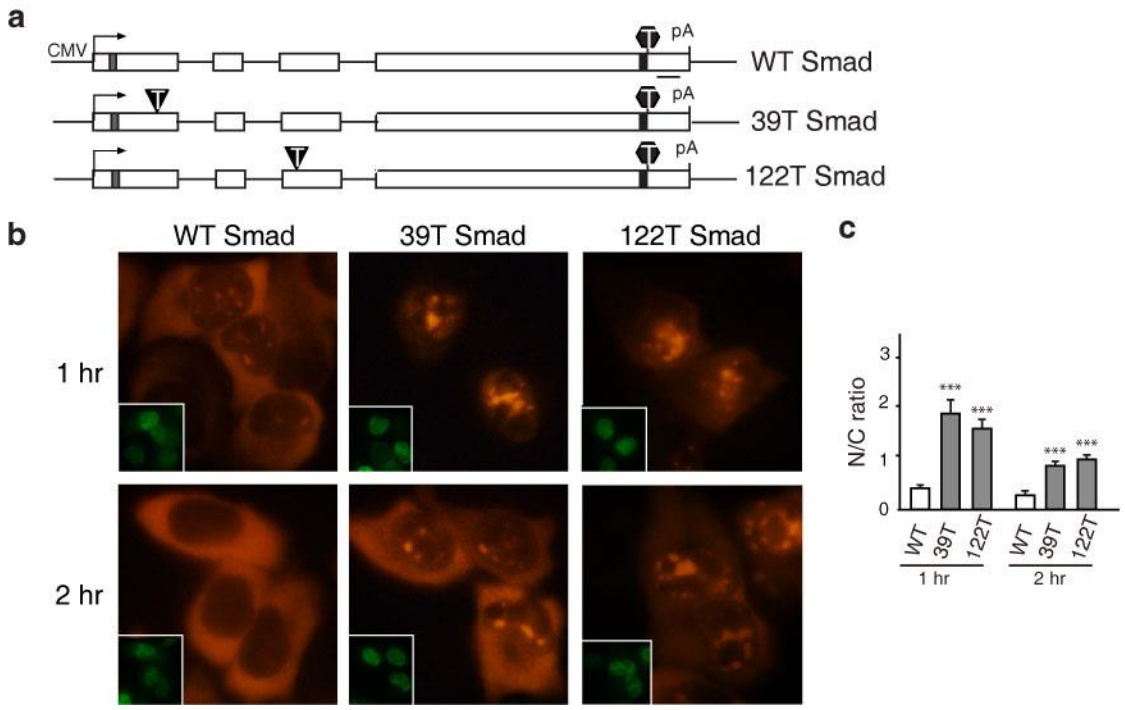

Figure S3. Prolonged Retention of PTC+ Smad mRNAs in the Nucleus, related to Figure 1. (a) Schematic of Smad constructs. The CMV promoter and BGH polyA sites and the location of the FISH probe (indicated by short line) are shown. The grey and black bars indicate Myc and HA tag respectively. The black triangle and hexagon indicate PTC and physiological stop codon respectively. (b) Equal amount of Smad constructs (50 ng/μl) were microinjected into nuclei of HeLa cell, and α-amanitin was added to block transcription 15 min after microinjection. FISH of Smad transcripts was carried out at indicated times after addition of α-amanitin. FITC-conjugated dextran was co-injected as an injection marker. (c) The graph shows the average N/C ratios for Smad mRNAs, and error bars indicate the standard errors among three independent experiments. Statistical analysis was performed using the Student's t test. *P<0.05; **P<0.01; ***P<0.001.

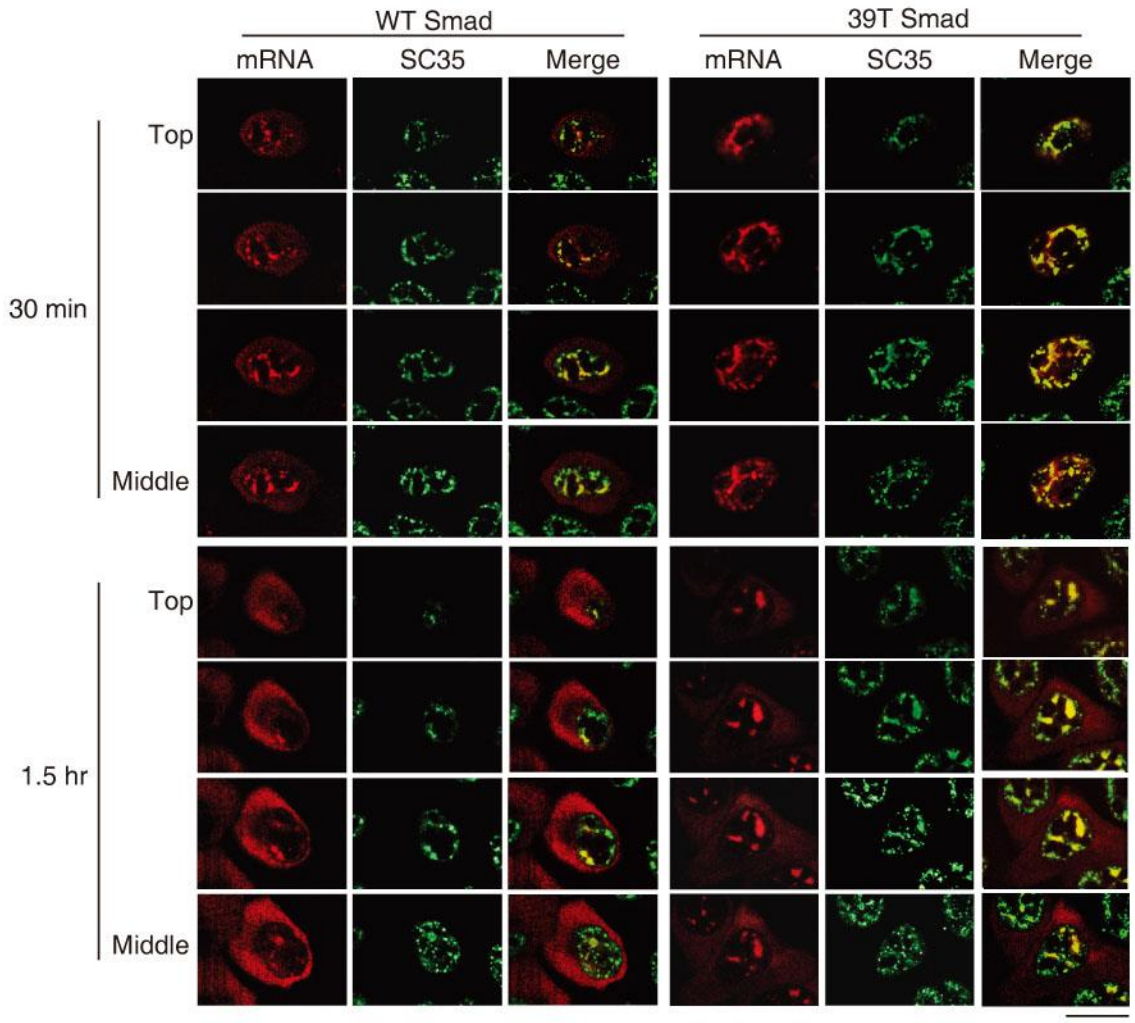

Figure S4. Z-Stack Confocal Analysis of WT and PTC+ mRNAs and SC35, related to Figure 1. Equal amount of Smad constructs (50 ng/μl) were microinjected into nuclei of HeLa cell, and α-amanitin was added 15 mins after microinjection. FISH of Smad transcripts was carried out at indicated time after addition of α-amanitin. Confocal microscopy was used to visualize the cells. Z-stacks were taken in steps of micrometer. The top to middle sections of the Z-stacks are shown. Scale bar, 10 μm.

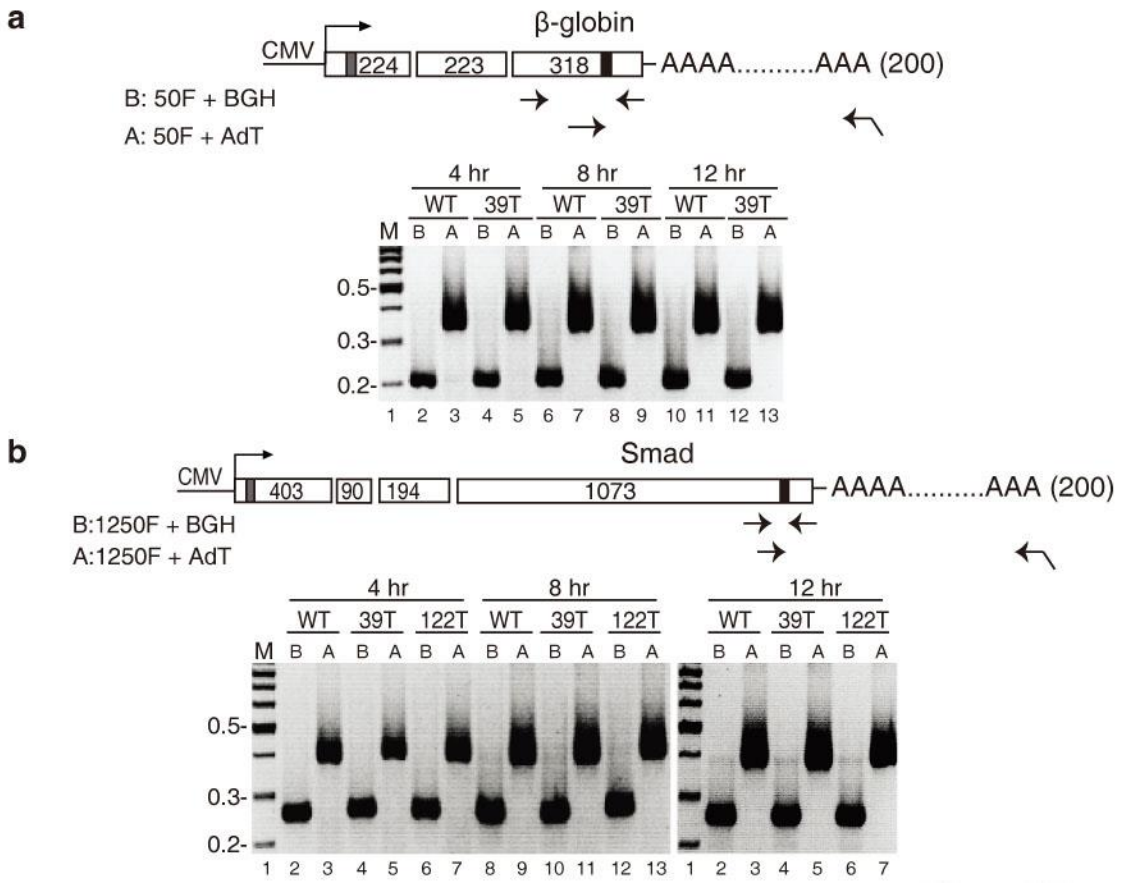

Figure S5. PTC+ β-globin and Smad mRNAs Do Not Have Processing Defects, related to Figure 3. (a) WT and PTC+ β-globin constructs were transfected into HeLa cells. Total RNA was extracted at 4 hrs, 8 hrs and 12 hrs post-transfection and reverse transcription was carried out with an oligo (dT) primer linked to a G/C-rich anchor sequence. PCRs were performed using the indicated primers. (b) Same as (a), except that WT and PTC+ Smad constructs were transfected, and PCRs were carried out using the indicated primers.

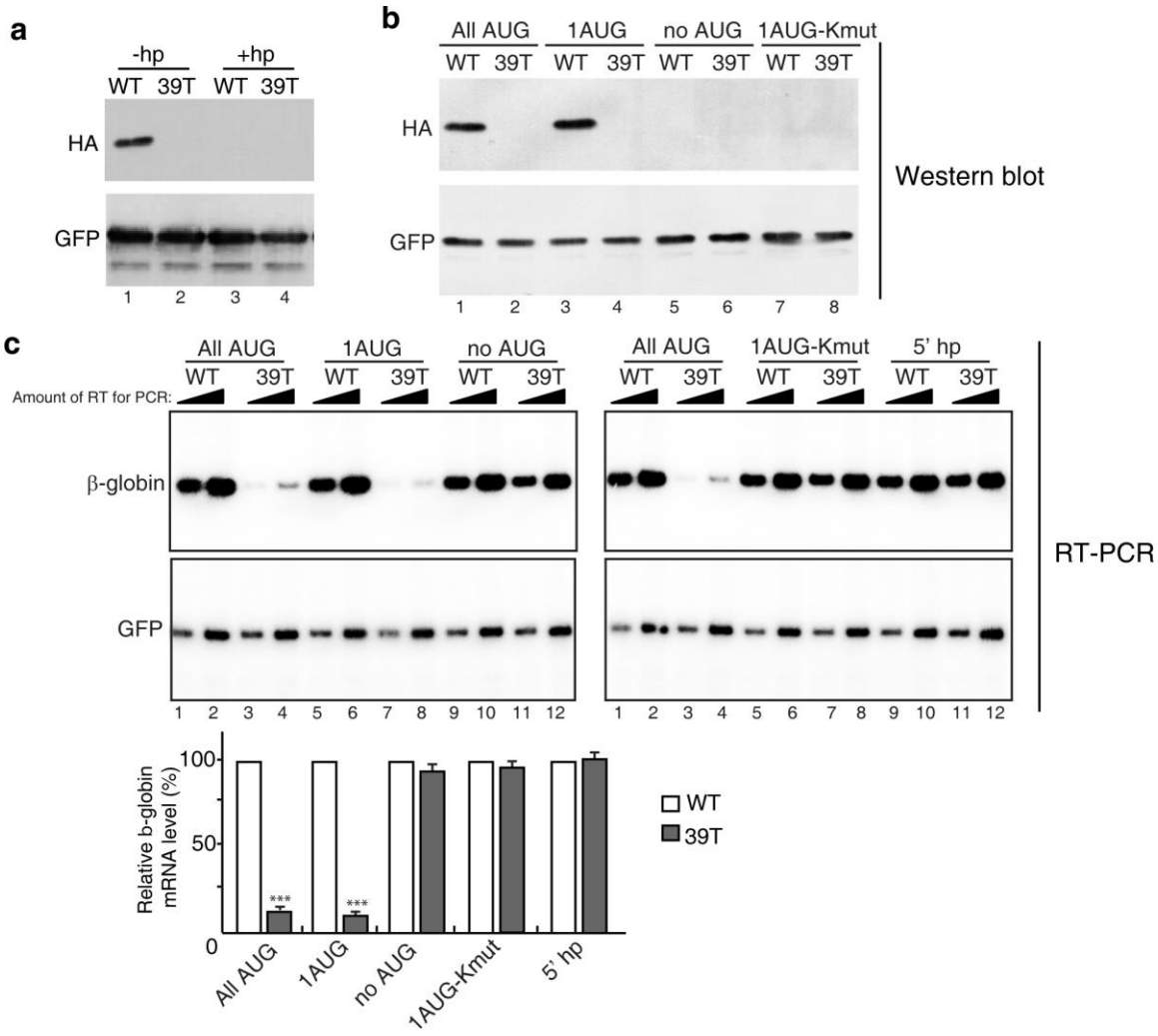

Figure S6. Disruption of the Reading-Frame Efficiently Inhibited Translation and Degradation of PTC+ mRNAs. (a) Equal amount of β-globin constructs that described in Figure 4a were transfected, and 12 hrs after transfection, western blots were carried out using antibodies to HA and GFP. The GFP was shown as transfected control. (b) insertion of a hairpin structure at 5'UTR inhibited translation of β-globin mRNA. Equal amount of the hairpin containing and the hairpin uncontaining β-globin construct was transfected to HeLa cells. After 48h, the western blot was carried with HA antibody to test the expression of β-globin. The GFP was shown as transfected control. (c) Same as

(a), except that the constructs that described in Figure 6a were transfected. (d) the construct that described in (a) and (b) was co-transfected with GFP construct. 24 hrs after transfection, RNAs were extracted followed by RT-PCR using β-globin and GFP primers. The ratio of mRNA level of β-globin to GFP was quantified and indicated by the bars. Error bars indicate the standard errors from three independent experiments. Statistical analysis was performed using the Student's t test. *P<0.05; **P<0.01; ***P<0.001.

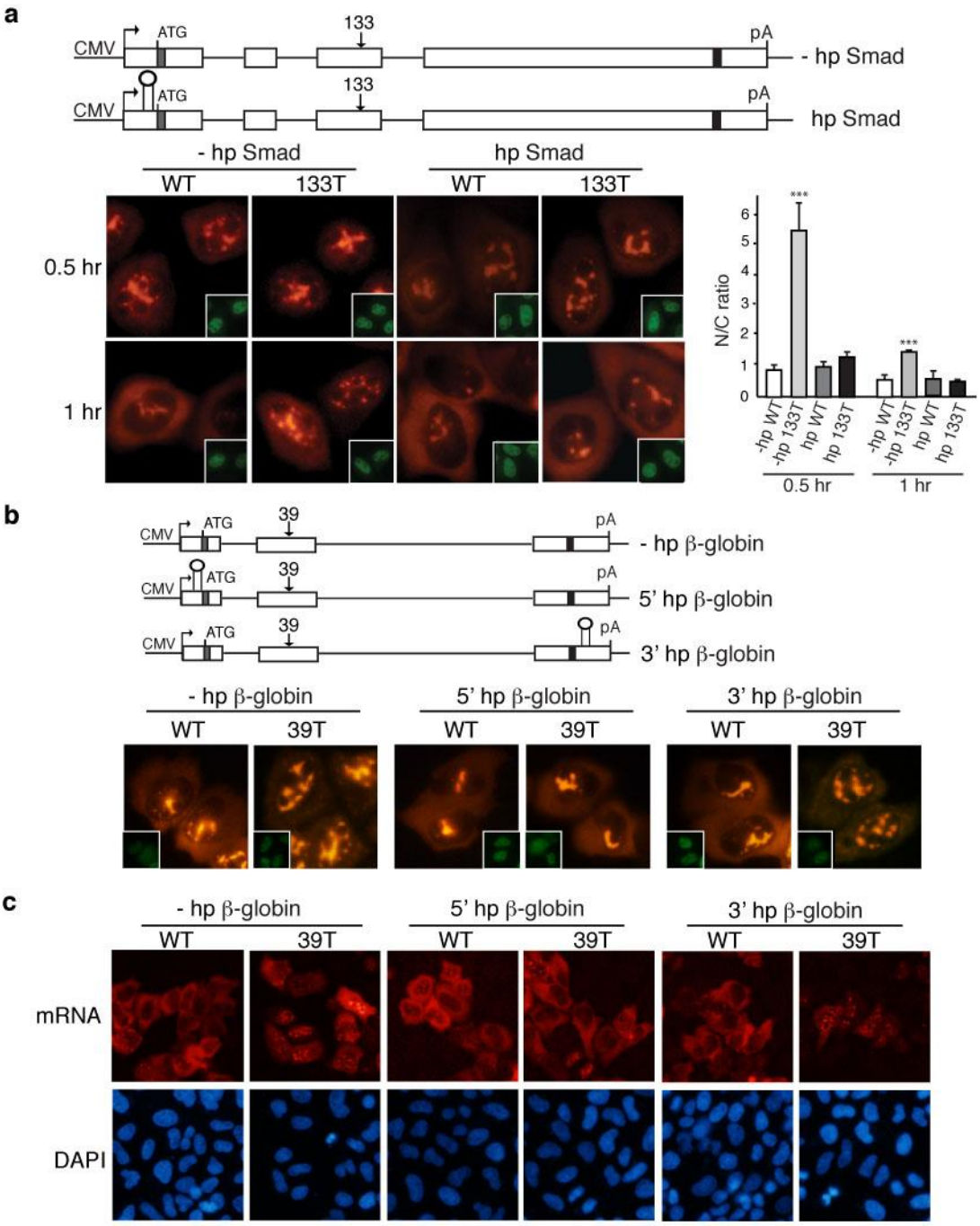

Figure S7. Insertion of A Hairpin Structure at the 5' UTR, but Not at the 3' UTR, Inhibited Nuclear Retention of the PTC+ β-globin mRNA, related to Figure 6. (a) Schematic of Smad constructs is shown. The gray and black boxes indicate the Myc and HA tag respectively. The positions of start codon and the hairpin structure are

marked. Equal amount of Smad constructs were microinjected into the nuclei of HeLa cells, and α-amanitin was added 30 mins after microinjection. 0.5 hr or 1 hr after addition of α-amanitin, FISH was carried out to detect the mRNA. Insets show the injection marker. The graph shows the average N/C ratios for WT and 133T Smad mRNAs at each time point, and error bars indicate the standard errors among three independent experiments. Statistical analysis was performed as in Figure 1C. (b) Schematic of β-globin constructs is shown. The gray and black boxes indicate the Myc and HA tag respectively. The positions of start codon and the hairpin structure are marked. - hp, 5' hp and 3' hp β-globin constructs were microinjected into the nuclei of HeLa cells, and α-amanitin was added 30 mins after microinjection. 1 hr after addition of α-amanitin, FISH was carried out to detect the mRNA. Insets show the injection marker. (c) Equal amounts of β-globin DNA constructs were tranfected into HeLa cells, and 12 hr after transfection, FISH was carried out.

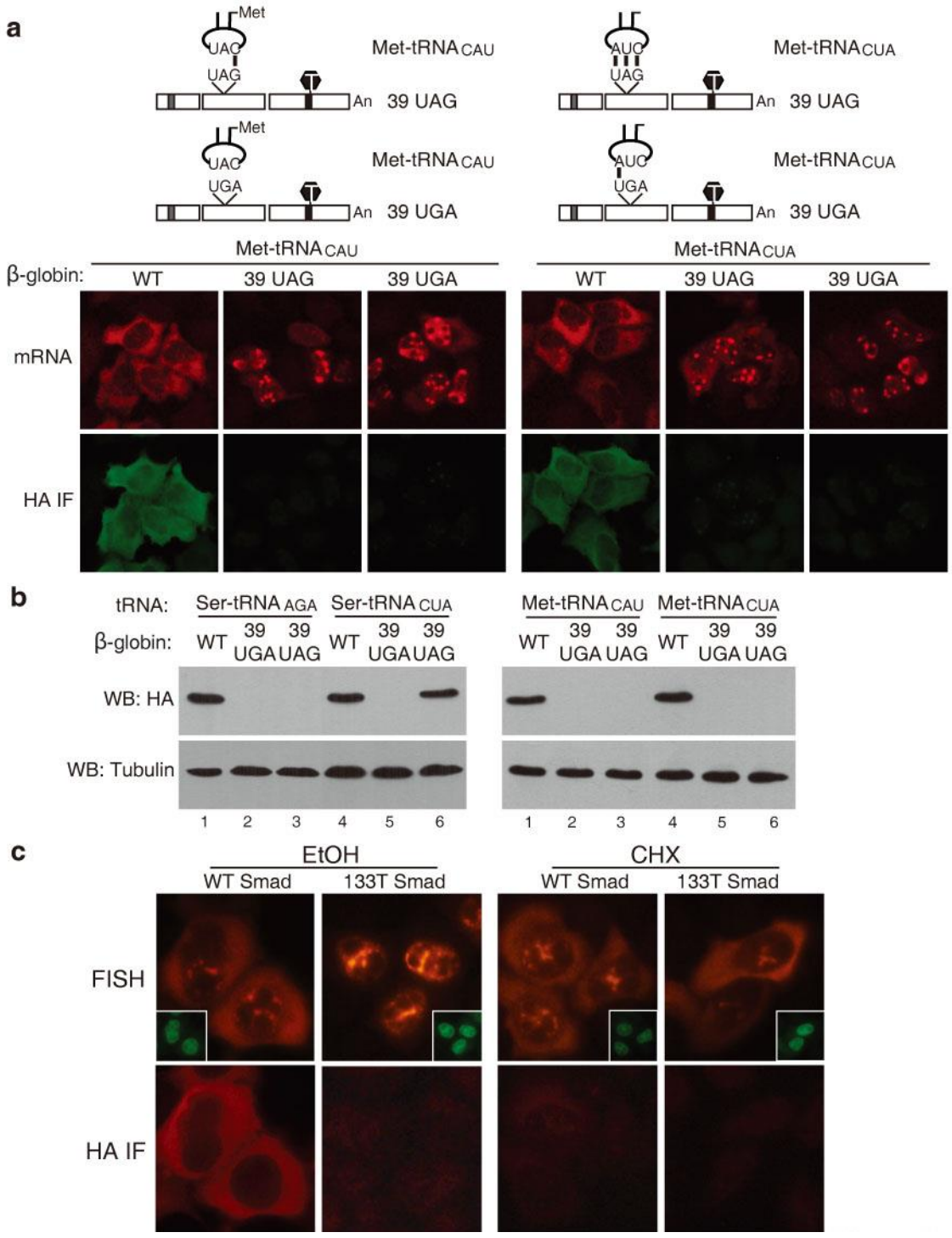

Figure S8. The Ribosome and tRNA Are Involved in Nuclear PTC Recognition, related to Figure 6. (a) Schematic of Met-tRNA constructs whose anticodon either base pair or does not base pair with the PTC. Equal amount of WT and mutant Met-tRNA

constructs were co-transfected with WT or PTC+ β-globin constructs into HeLa cells, 12 hrs later, FISH and immunofluoresence with the HA antibody were carried out. (b) Same as (a), except that 12 hrs post-transfection, cells were lysed and lysates were used for western blots with antibodies to HA and Tubulin. (c) HeLa cells were treated with cycloheximide (CHX; 30 ug/ml) or ethanol, which was used to dissolve CHX, for 2 hrs before microinjection with Smad constructs. α-amanitin was added 15 mins after microinjection and 1 hr later, FISH and immunofluoresence with anti-HA antibody were carried out. Insets show the injection marker.

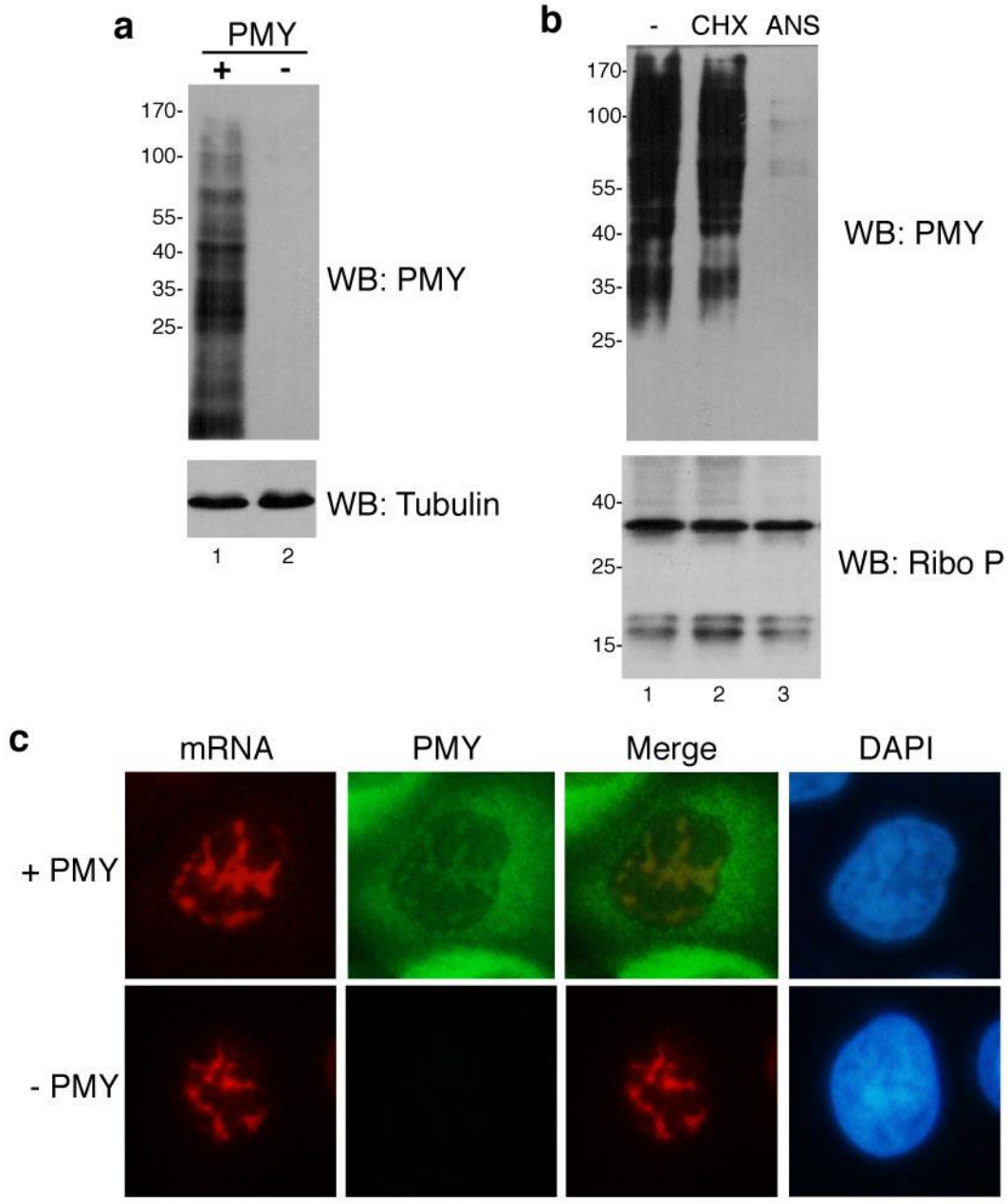

Figure S9. PMY Is Incorporated into the Nascent Peptide Chain. (a) HeLa cells were treated with PMY and emetine (+PMY) or emetine only (-PMY) for 5 mins. Cells were then collected followed by western blot with anti-PMY and anti-tubulin antibodies. (b) HeLa cells were pretreated or not pretreated (-) with cycloheximide (CHX) or anisomycin (ANS) for 15mins followed by PMY and emetine treatment for 5 mins. Cells

were then collected for western blot with anti-PMY and anti-Ribo P antibodies. (c) WT β-globin DNA construct was microinjected into the nuclei of HeLa cells, and 35 mins later, cells were treated with PMY and emetine (+PMY) or emetine only (-PMY) for 5 mins at 37 ℃. Cells were then treated with digitonin for 2 mins followed by FISH and immunofluorescence with the PMY antibody.

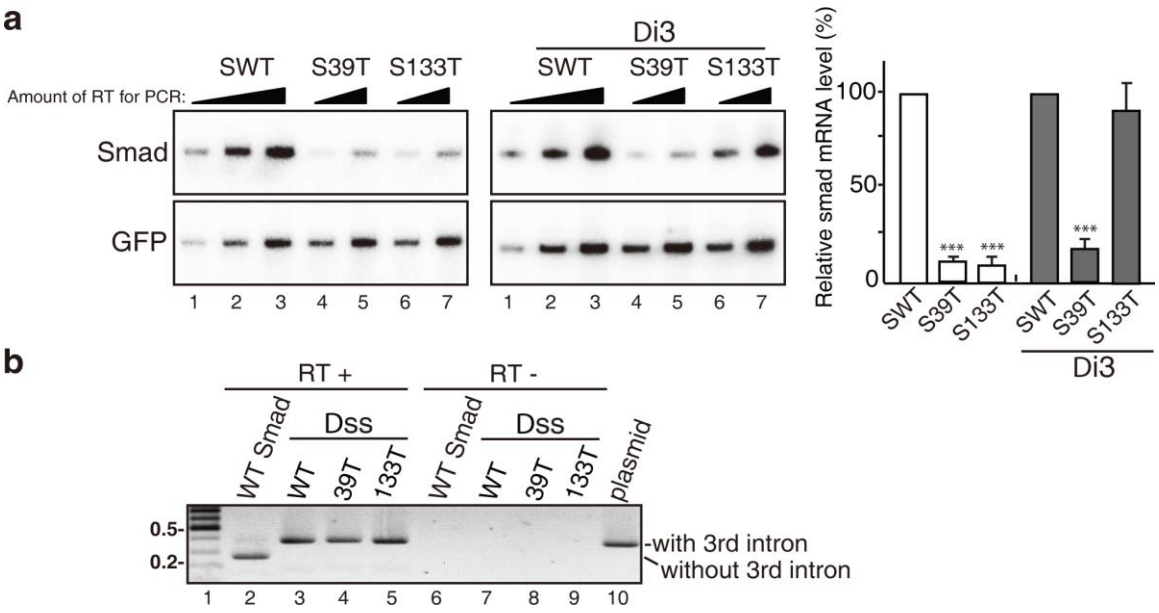

Figure S10. Deletion of the Downstream Intron Inhibited PTC+ mRNA Degradation, related to Figure 7. (a) Smad constructs were co-transfected with GFP, and 24 hrs later, total RNA was extracted followed by RT-PCR using Smad or GFP primers, respectively. The ratio of mRNA level of Smad to GFP was quantified and indicated by the bars. Error bars indicate the standard errors from three independent experiments. Statistical analysis was performed as in Figure 1C. (b) Total RNA was extracted at 12 hr after transfection of the indicated Smad constructs followed by RT-PCR using primers complementary to the third and fourth exons. PCR product from the intron-containing plasmid DNA was used as size a marker for pre-mRNA. The sizes (in kb) of the DNA marker are indicated.

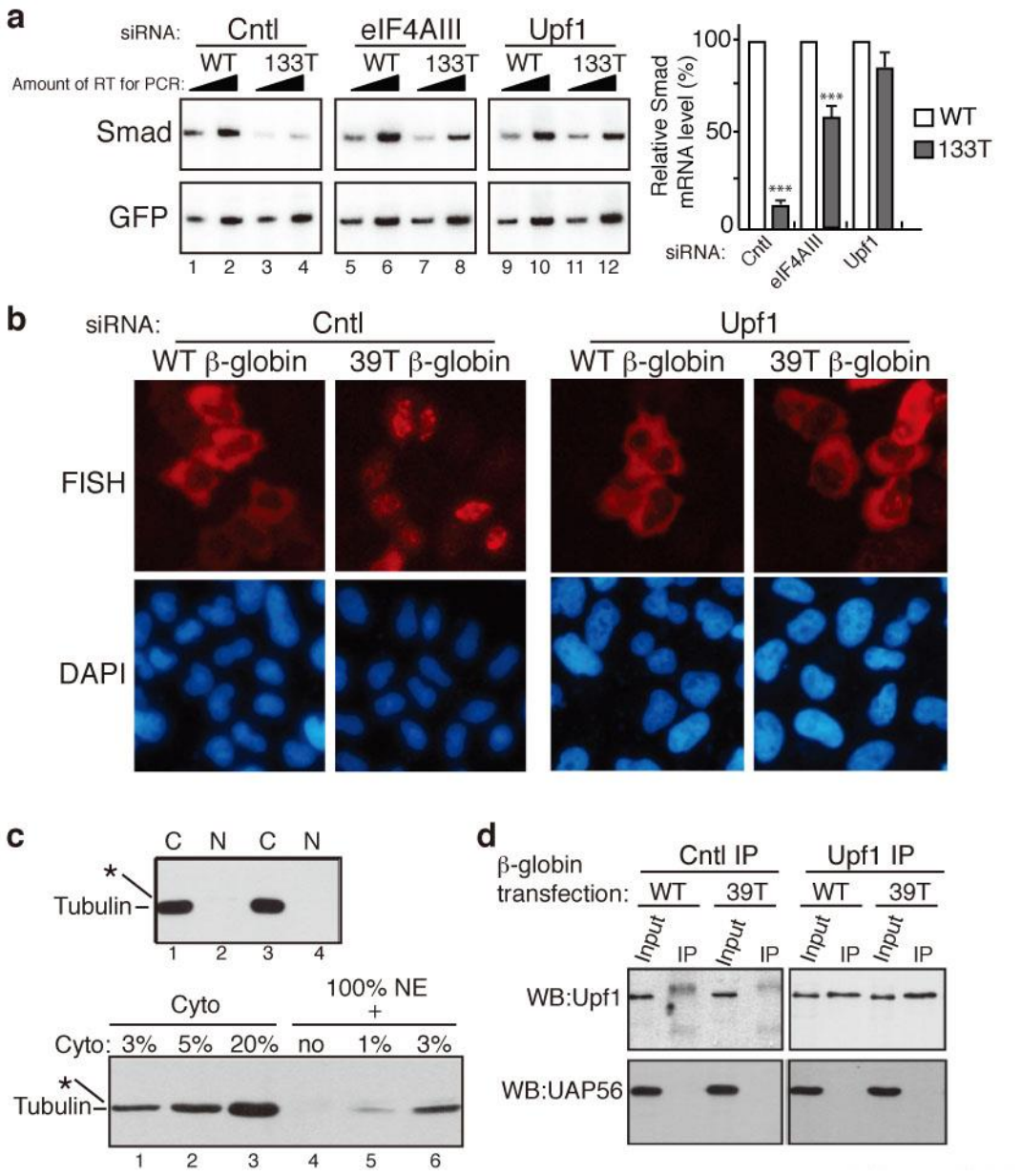

Figure S11. Knockdown of the NMD Factor Upf1 Inhibited the Nuclear Retention of PTC+ mRNAs, related to Figure 7. (a) eIF4AIII, Upf1, or non-targeting siRNAs were transiently transfected into HeLa cells, and 48 hrs post-transfection, WT or 133T Smad construct was co-transfected with GFP into these knockdown cells. 24 hrs after transfection, RNAs were extracted followed by RT-PCR using Smad and GFP primers. The ratio of mRNA level of Smad to GFP was quantified and indicated by the bars.

Error bars indicate the standard errors from three independent experiments. Statistical analysis was performed as in Figure 1C. (b) Upf1 or non-targeting siRNA was transiently transfected into HeLa cells, and 48 hrs post-transfection, WT or 39T β-globin construct was transfected into these knockdown cells. 12 hrs after transfection, FISH was carried out. (c) HeLa cells were transfected with WT or 39T β-globin constructs, and 12 hrs later, nuclear and cytoplasmic extracts were prepared. Upper panel, equal volume of nuclear to cytoplasmic extract was separated by SDS-PAGE followed by western blot using an antibody to Tubulin or UAP56. Lower panel, the purity of nuclear fraction is shown. For comparison with the nuclear fraction, cytoplasmic fractions corresponding to 3, 5 and 20% (lanes 1-3) of the number of cells represented in the nuclear fraction (lane 4) were loaded, and the nuclear fraction was mixed with 1 or 3% cytoplasm (lanes 5 and 6). (d) Using an antibody to Upf1 or an unrelated protein, immunoprecipitations were carried out from nuclear extracts prepared from HeLa cells transfected with WT or 39T β-globin constructs. RNAs were extracted from immunoprecipitates followed by RT-PCR using primers to β-globin or Luciferase. The relative mRNA level of β-globin to Luciferase was quantified and indicated in the graph. Error bars indicate the standard errors from three independent experiments. Statistical analysis was performed as in Figure 1C.

Video 1-4. Upf1 co-localizes with both 39T β-globin and 133T Smad mRNAs deep inside the nucleoplasm. WT β-globin (Video1), 39T β-globin (Video2), WT Smad (Video3) and 133T Smad (Video4) constructs were microinjected into the nuclei of HeLa cells, and α-amanitin was added 15 mins after microinjection. 1 hr after addition of α-amanitin, cells were permeabilized with digitonin (40mg/ml) for 2 mins followed by FISH (Video 1a, 2a, 3a and 4a) and immunofluorescence with the Upf1 antibody (Video 1b, 2b, 3b and 4b). We used confocal microscope to acquire z-stacks through several cells followed by 3D rendering of the images. Merged images were shown in Video 1c, 2c, 3c and 4c.

Supplemental Experiment Procedures

Plasmids and Antibodies

The WT β-globin plasmid was described (Valencia et al., 2008) and 39T β-globin and 39M β-globin plasmids were constructed by mutagenesis from the WT β-globin. The 39T-OutF β-globin plasmid was constructed by mutagenesis twice, first using the primer pair of 39T-OutF F1 and R1, followed by PCR using the primer pair of 39T-OutF F2 and 39T-OutF R2. The 59T-InF β-globin plasmid was also constructed by mutagenesis twice, first using the primer pair of 59T-InF F1 and 59T-InF R1, followed by PCR using the primer pair of 59T-InF F2 and 59T-InF R2. The WT Smad plasmid was described

(Valencia et al., 2008) and PTC-containing Smad plasmids were constructed by mutagenesis from WT Smad. The Smad missense mutants were generated by mutagenesis from the WT Smad plasmid. The SmadΔi3 and Samd Δss plasmids were constructed by mutagenesis from the corresponding Smad constructs. The Smad plasmids containing the hairpin structure were constructed by mutagenesis from the corresponding Smad plasmids without the hairpin. The no hp and hp WT/Mut IRES β-globin plasmids in Figure 6 were kind gifts of Matthias Hentze (Holbrook et al., 2006). The hp β-globin plasmids in Figure 6 were constructed by deletion of the IRES from the WT IRES β-globin plasmid. No AUG, 1AUG and 1AUG-Kmut β-globin plasmids were constructed by mutagenesis from WT and 39T β-globin plasmids. The 5' hp and 3' hp β-globin plasmids in Figure S6 were constructed by mutagenesis from β-globin constructs. The WT Ser-tRNA was amplified from human genomic DNA and cloned into the pSUPER vector. The Ser-tRNA$_{CUA}$ plasmid was constructed by mutagenesis from WT Ser-tRNA. The WT Met-tRNA plasmid was a kind gift of Joseph Sperling (Kamhi et al., 2010). The mutant Met-tRNA plasmid was constructed by mutagenesis from WT Met-tRNA. The sequences of the primers are listed in Table S4.

The UAP56 and GFP antibodies were described previously (Cheng, 2006; Cheng, 2002). For western blot, Upf1 antibody was diluted to 1:500 and antibodies to HA, GFP, CBP80 (ABclonal), UAP56 and Tubulin (Sigma) were diluted to 1:1000.

Table S1 Peptide Sequence of β-globin

Plasmids for transfection	Residue Sequence
WT β-globin	R.LLVVYPWTQR.F
39 UAG β-globin / Ser-tRNA$_{CUA}$	R.LLVVYPWTSR.F

Table S2 siRNA Sequences

CTIF	5’ GCAUCAACCUGAAUGACAU dTdT 3’
eIF3B-1	5’ GGAGAGAAAUUCAAGCAAA dTdT 3’
eIF3B-2	5’ GUGGGAUAUUCCAGAGAAA dTdT 3’
eIF3B-3	5’ GUCCAAAGCCUCAAAGGAA dTdT 3’
eIF3E-1	5’ GAACAUGACUCCAGAAGAA dTdT 3’
eIF3E-2	5’ CCAUGAAUAUUGAGAAGAA dTdT 3’
eIF3E-3	5’ CAACAGGAGUCUUACACAU dTdT 3’
eIF4AIII	5’ GGAAGGGUGUGGCCAUUAA dTdT 3’
Upf1	5’ GAUGCAGUUCCGCUCCAUUdTdT 3’

Table S3 Sequences of FISH Probes

vector	5’AAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGCTGATC AGCGGGT 3’
β-globin	5’CTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTTCTCCTCAGGAGTCAGGTGC ACCAT 3’
Smad	5’CACTTGCAGACTTCTTCCATCCTAGCAGGCGCTTCACTACTGGCGGGGTGAAAGGCAAGATGGAC GACAT 3’
GFP	5’CTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGT GATCG 3’

Table S4 Sequences of Primers

T7	5’ TAATACGACTCACTATAGGG 3’
BGH	5’ TAGAAGGCACAGTCGAGG 3’
CMV-F	5’ TGGAGGTCGCTGAGTAGTGCG 3’
pCDpAR	5’ CCTCAGAAGCCATAGAGC 3’
β-globin exon1F (G1F)	5’ ACGTGGATGAAGTTGGTGGT 3’
β-globin intron2-exon3R (GExExR)	5’ GCCCAGGAGCTGTGGGAG 3’

β-globin exon2-exon3R (GInExR)	5’ GCCCAGGAGCCTGAAGTT 3’
Smad intron3-exon4R (SExExR)	5’ GTGGTAAAACTGTGGAAA 3’
Smad exon3-exon4R (SInExR)	5’ GTGGTAAAACTGGTGTCT 3’
GAPDH F	5’ AGTCAACGGATTTGGTCGTA 3’
GAPDH R	5’ AGGAGGCATTGCTGATGATC 3’
β-globin 1st ATG mu F	5’ CTTGGTACCGCCGCCACCTTTGAACAAAAACTCATCT 3’
β-globin 1st ATG mu R	5’ AGATGAGTTTTTGTTCAAAGGTGGCGGCGGTACCAAG 3’
β-globin 2nd ATG mu F	5’ GGGCAAGGTGAACGTGGACGAAGTTGGTGGTGAGGCC 3’
β-globin 2nd ATG mu R	5’ GGCCTCACCACCAACTTCGTCCACGTTCACCTTGCCC 3’
β-globin 3rd/4th ATG mu F	5’ GGATCTGTCCACTCCTGACGCTGTTCTGGGCAACCCTAAGGTGA 3’
β-globin 3rd/4th ATG mu R	5’ TCACCTTAGGGTTGCCCAGAACAGCGTCAGGAGTGGACAGATCC 3’
β-globin 5th ATG mu F	5’ CCCTAAGGTGAAGGCTCACGGCAAGAAAGTGCTCGGT 3’
β-globin 5th ATG mu R	5’ ACCGAGCACTTTCTTGCCGTGAGCCTTCACCTTAGGG 3’
β-globin 6th ATG mu F	5’ GCTCGGTGCCTTTAGTGACGGCCTGGCTCACCTGGAC 3’
β-globin 6th ATG mu R	5’ GTCCAGGTGAGCCAGGCCGTCACTAAAGGCACCGAGC 3’
β-globin 7th ATG mu F	5’ GGTGGCTGGTGTGGCTAACGCCCTGGCCCACAAGTAT 3’
β-globin 7th ATG mu R	5’ ATACTTGTGGGCCAGGGCGTTAGCCACACCAGCCACC 3’
β-globin kozak mu F	5’ CTTGGTACCGCCTTTTTTATGTAACAAAAACTCATCTCA 3’
β-globin kozak mu R	5’ TGAGATGAGTTTTTGTTACATAAAAAAGGCGGTACCAAG 3’
39M β-globin F	5’ GTGGTCTACCCTTGGACCAAGAGGTTCTTTGAGTCCT 3’
39M β-globin R	5’ AGGACTCAAAGAACCTCTTGGTCCAAGGGTAGACCAC 3’
39T-OutF F1	5’ GTGGTCTACCCTGGACCTAGAGGTTCTTTGAGTCCT 3’
39T-OutF R1	5’ AGGACTCAAAGAACCTCTAGGTCCAGGGTAGACCAC 3’
39T-OutF F2	5’ TCTTTGAGTCCTTTGGGCGATCTGTCCACTCCTGATG 3’
39T-OutF R2	5’ CATCAGGAGTGGACAGATCGCCCAAAGGACTCAAAGA 3’
59T-InF F1	5’ GGATCTGTCCACTCCTGACTGCTGTTATGGGCAACCC 3’
59T-InF R1	5’ GGGTTGCCCATAACAGCAGTCAGGAGTGGACAGATCC 3’
59T-InF F2	5’ CTAAGGTGAAGGCTCATGGCAGAAAGTGCTCGGTGCCTT 3’
59T-InF R2	5’ AAGGCACCGAGCACTTTCTGCCATGAGCCTTCACCTTAG 3’
39M Smad F	5’ AACGGACAGGAAGAGAAGCGGTGCGAAAAAGCGGTAA 3’
39M Smad R	5’ TTACCGCTTTTTCGCACCGCTTCTCTTCCTGTCCGTT 3’
122M Smad F	5’ GTCTCACCGTAAAGGATTACCGCATGTTATCTACTGC 3’
122M Smad R	5’ GCAGTAGATAACATGCGGTAATCCTTTACGGTGAGAC 3’
133M Smad F	5’ TACTGCAGACTGTGGCGCCGGCCAGACCTGCACAGTCAT 3’
133M Smad R	5’ ATGACTGTGCAGGTCTGGCCGGCGCCACAGTCTGCAGTA 3’
Smad Δintron3 F	5’ TCAGAGGGTGGAGACACCAGTTTTACCACCTGTATTAGTT 3’
Smad Δintron3 R	5’ AACTAATACAGGTGGTAAAACTGGTGTCTCCACCCTCTGA 3’
Smad ssm 5’F	5’ AGAGGGTGGAGACACCAGCAGAGTACTCCCTCTCAAAA 3’
Smad ssm 5’R	5’ TTTTGAGAGGGAGTACTCTGCTGGTGTCTCCACCCTCT 3’

Smad ssm 3'F	5' TGTCCCTTTTTTTTCCACTTTTTTACCACCTGTATTAG 3'
Smad ssm 3'R	5' CTAATACAGGTGGTAAAAAAGTGGAAAAAAAAGGGACA 3'
5'hp F	5' GAGAACCCACTGCTTACTGGGGCGCGTGGTGGCGGCTGCAGCCGCCAC CACGCGCCCCGGCTTATCGAAATTAATA 3'
5'hp R	5' AGTAAGCAGTGGGTTCTCTAGTTAGC 3'
β-globin 3'hp F	5' CTACGCTTAAGATATCCAGGGGCGCGTGGTGGCGGCTGCAGCCGCCAC CACGCGCCCCGCACAGTGGCGGCCGCTC 3'
β-globin 3'hp R	5' TCTGGGACGTCGTATGGGTAGTGATA 3'
β-globin RT F	5' TAATACGACTCACTATAGGG 3'
β-globin RT R	5' TGCCCAGGAGCCTGAAGTTC 3'
luciferase RT F	5' GCTGGGCGTTAATCAGAGAG 3'
luciferase RT R	5' AGACTTCAAGCGGCCAACTA 3'
GFP RT F	5' CGAAGCTTGAGCTCGAGATCTG 3'
GFP RT R	5' GCTTGTGCCCCAGGATGTTG 3'
β-globin sense	5' TCTCCTGATGCTGTTATGGGC 3'
Smad sense	5' ACCTTTGCAGTGGTTGGACAAA 3'
Smad RT F	5' TAATACGACTCACTATAGGG 3'
Smad RT R	5' AGCAAGTGCTTGGTA 3'
Actin RT F	5' AGATGACCCAGATCATGTTTG 3'
Actin RT R	5' AAGGAAGGCTGGAAGAGTGC 3'
PTPRF RT F	5' ATGGTGACAGCAAACCTGTC 3'
PTPRF RT R	5' AGCTGTTCCTCTTCGAGCAC 3'
MAP3K RT F	5' AGGCTGAGTGTGAGAATAGCC 3'
MAP3K RT R	5' CTCATCCTCCTGCACTGGGA 3'
ARHGEF18 RT F	5' ACGCAACTCGGACCAATCAC 3'
ARHGEF18 RT R	5' TCTGCATCAGCTCATAAAGG 3'
UPP1 RT F	5' AAGTCACAATGATTGCCCCG 3'
UPP1 RT R	5' CCATACCATGACTGACAGAC 3'
TBL2 RT F	5' AGCTCTGAAGAGCCACAGCG 3'
TBL2 RT R	5' AGGCTACAAATCTGCCACAGG 3'
β-globin 39TGA F	5' GTGGTCTACCCTTGGACCTGAAGGTTCTTTGAGTCCTTT 3'
β-globin 39TGA R	5' AAAGGACTCAAAGAACCTTCAGGTCCAAGGGTAGACCAC 3'
Ser-tRNA$_{CUA}$ F	5' TGGTTAAGGCGATGGACTCTAAATCCATTGGGGTCTCCC 3'
Ser-tRNA$_{CUA}$ R	5' GGGAGACCCCAATGGATTTAGAGTCCATCGCCTTAACCA 3'
Met-tRNA$_{CUA}$ F	5' TAGGTAGCGCGTCAGTCAGTCAGTCTCTAAATCTGAAGGTCGTGAGT 3'
Met-tRNA$_{CUA}$ R	5' ACTCACGACCTTCAGATTTAGAGACTGACGCGCTACCTA 3'
WT Ser-tRNA F	5' CGCCTCGAGGAATTCTCATTTCACCTTAG 3'
WTSer-tRNA R	5' CGCGGTACCGCCAAACAGATGGGTTAAGT 3'

References

Cheng, H., Sugiura, R., Wu, W., Fujita, M., Lu, Y., Sio, S.O., Kawai, R., Takegawa, K., Shuntoh, H., and Kuno, T. (2002). Role of the Rab GTP-binding protein Ypt3 in the fission yeast exocytic pathway and its connection to calcineurin function. Mol Biol Cell *13*, 2963-2976.

Cheng, H., Dufu, K., Lee, C-S, Hsu, J.L, Dias, A, and Reed, R (2006). Human mRNA export machinery recruited to the 5' end of mRNA. Cell *127*, 1389-1400.

Holbrook, J.A., Neu-Yilik, G., Gehring, N.H., Kulozik, A.E., and Hentze, M.W. (2006). Internal ribosome entry sequence-mediated translation initiation triggers nonsense-mediated decay. EMBO Rep *7*, 722-726.

Kamhi, E., Raitskin, O., Sperling, R., and Sperling, J. (2010). A potential role for initiator-tRNA in pre-mRNA splicing regulation. Proc Natl Acad Sci USA *107*, 11319-11324.

Lykke-Andersen, J., Shu, M.D., and Steitz, J.A. (2000). Human Upf proteins target an mRNA for nonsense-mediated decay when bound downstream of a termination codon. Cell *103*, 1121-1131.

Valencia, P., Dias, A.P., and Reed, R. (2008). Splicing promotes rapid and efficient mRNA export in mammalian cells. Proc Natl Acad Sci USA *105*, 3386-3391.