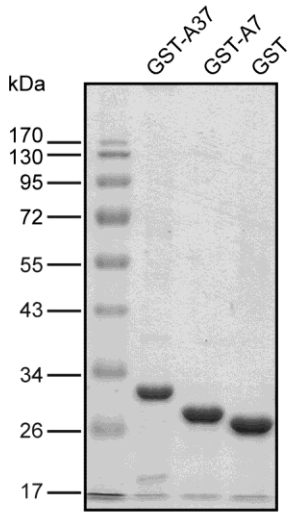
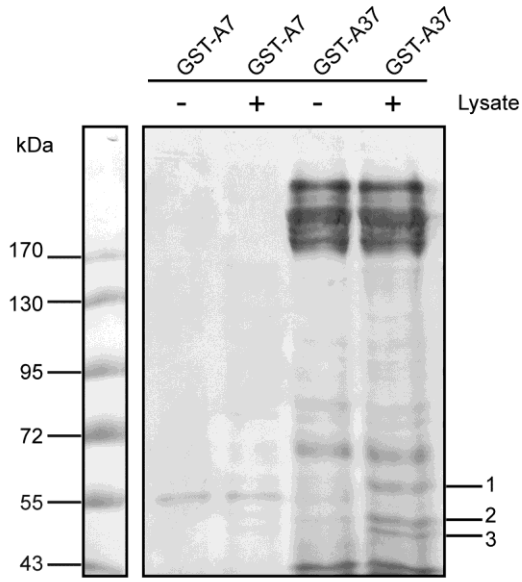
Supplemental Information

A



B



C

Band NO.	Protein Name	GI. NO.	Peptide Count	Protein Score
1	Tubulin, beta	57209813	17	239
2	Eukaryotic translation elongation factor 1 alpha 1	48734966	8	78
3	Actin, beta	14250401	13	149

FIGURE S1. GST pull-down assay and mass spectrometry identified eEF1A1 as an expanded polyA protein interactor.
(*A*) SDS-PAGE analysis of GST-polyA expression. The GST-polyA fusion proteins were expressed in *E. coli* strain *BL21* (*DE3*) and purified by Glutathione Sepharose 4 Fast Flow beads. GST-polyA proteins expression was confirmed by Coomassie blue staining. (*B*) GST pull-down assay identified three protein bands that were exclusively present in the GST-A37 condition. (*C*) Mass spectrometry was performed on band 1, 2 and 3 from (*B*), which were identified to be beta-tubulin, eEF1A1 and beta-actin, respectively. GI no. represents the GenBank molecular biology database identifier. Protein score (a protein with a score >36 is considered as being significant).

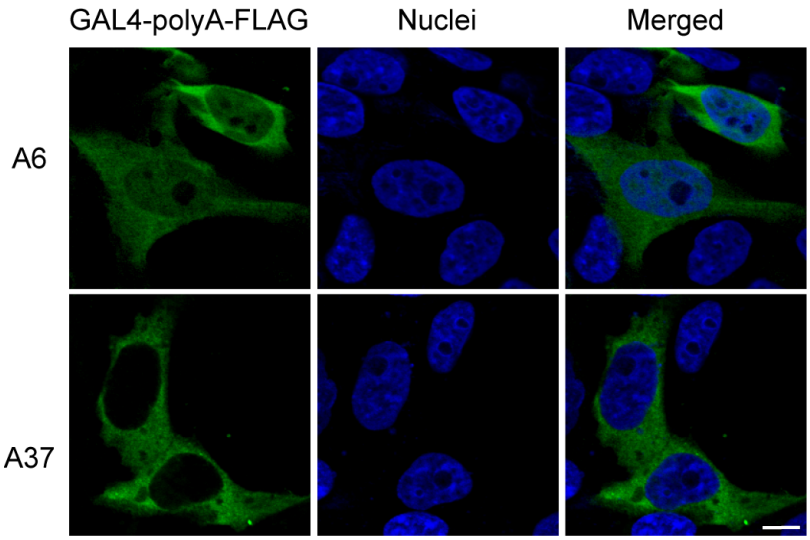


FIGURE S2. GAL4-A6-FLAG showed both nuclear and cytoplasmic localization while GAL4-A37-FLAG localized in cytoplasm.
Subcellular localization of GAL4-polyA-FLAG proteins in HEK293 cells. HEK293 cells were transfected with *pGAL4-polyA-FLAG* constructs and stained nuclei with Hoechst 33342. GAL4-A6-FLAG showed both nuclear and cytoplasmic localization, while GAL4-A37-FLAG was detected only in the cytoplasm. Scale bar represents 10 µm. Three independent experiments were performed.

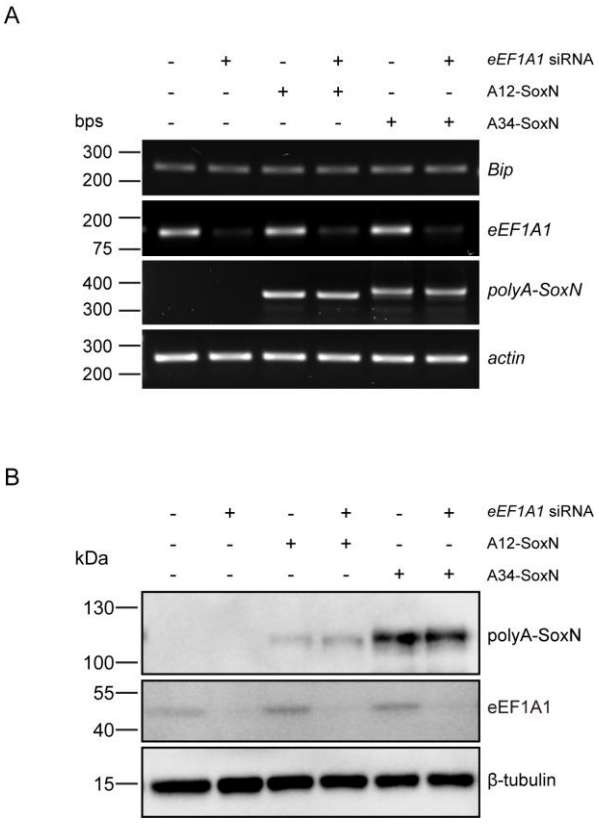


FIGURE S3. Knockdown of *eEF1A1* did not affect folding and degradation of

polyA-containing proteins.
(A) RT-PCR of *BiP* and *polyA-SoxN*. No changes of *BiP* or *polyA-SoxN* transcript levels were detected when *eEF1A1* was knocked down. *actin* severed as the loading control. (B) Detection the expression level of polyA-SoxN using Western blotting. No changes of polyA-SoxN expression levels were detected when *eEF1A1* was knocked down. β-tubulin served as the loading control. Three independent experiments were performed.

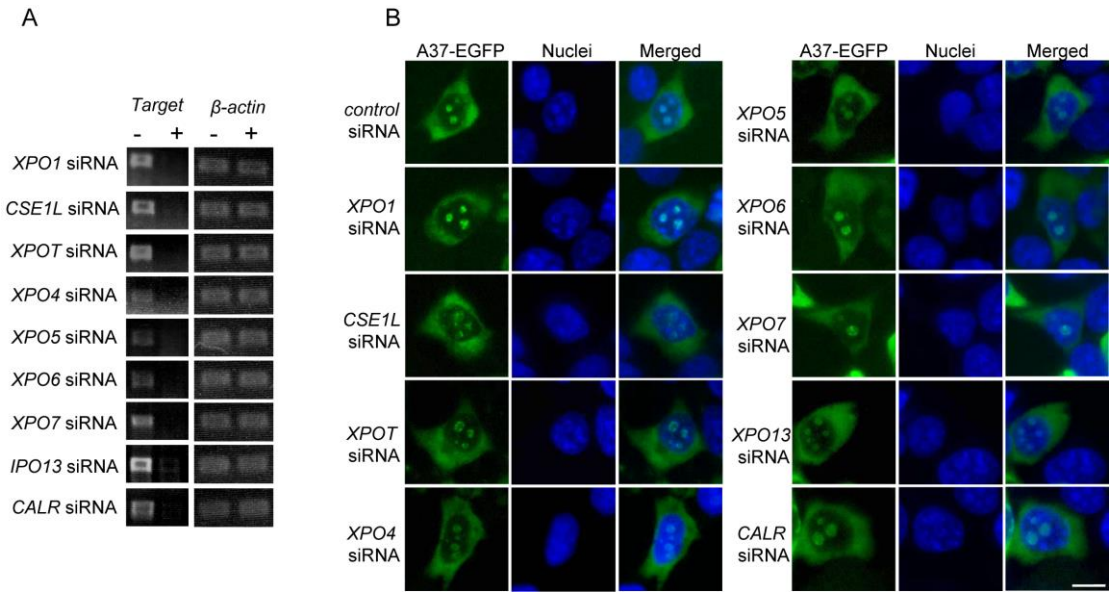


FIGURE S4. Knockdown of candidate genes by siRNAs did not affect nuclear export of expanded polyA tract-containing nuclear export reporter proteins.
(*A*) Knockdown efficiency of siRNAs used in screening of candidate genes in HEK293 cells. Compared with control siRNA (-), treatment of HEK293 cells with specific siRNA (+) caused a reduction in target gene mRNA level. *β-actin* was used as the loading control. (*B*) Subcellular localization of Rev(1.4)-A37-EGFP in HEK293 cells after knockdown candidate genes. The knockdown of candidate genes did not change the subcellular localization of Rev(1.4)-A37-EGFP protein. The cell nuclei were stained with Hoechst 33342. At least 100 transfected cells were analyzed. Scale bar represents 10 μm.

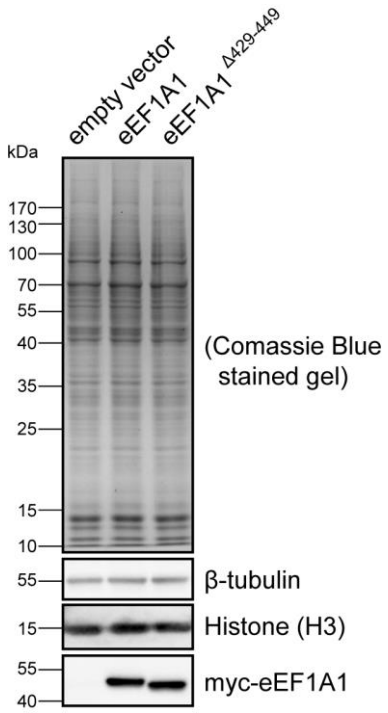


FIGURE S5. Overexpression of eEF1A1 did not affect protein translation.
The cellular protein expression level was examined by SDS-PAGE Coomassie Blue staining. No difference was detected in the cellular protein expression levels among cells overexpressing eEF1A1, eEF1A1$^{\Delta 429\text{-}499}$ and empty vector. β-tubulin and Histone (H3) served as house-keeping protein loading controls. Three independent experiments were performed.

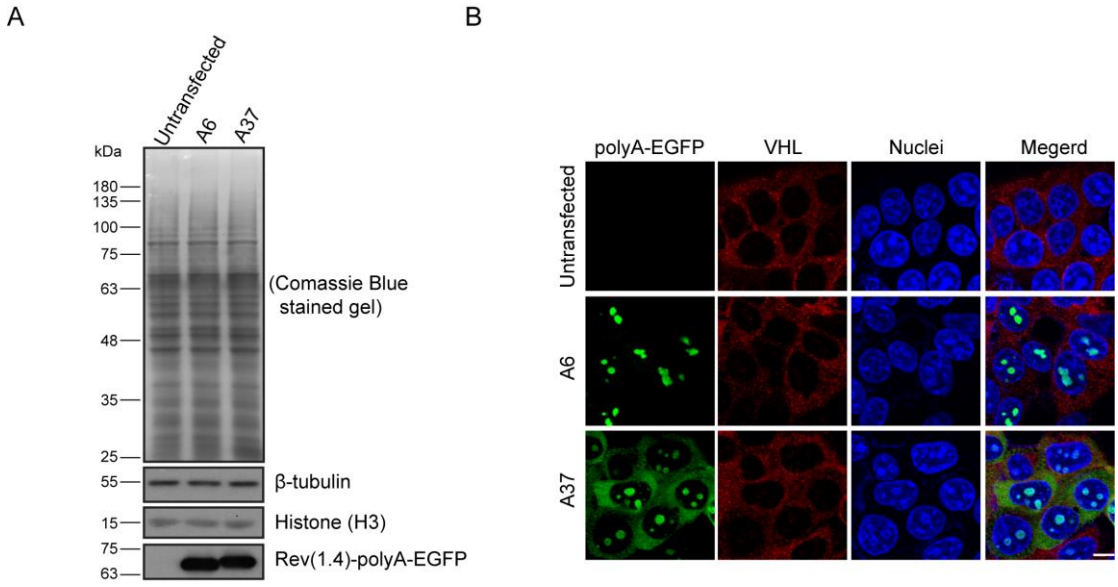


FIGURE S6. Expanded polyA expression did not affect the canonical cellular functions of eEF1A1.
(*A*) The cellular protein expression level was examined by SDS-PAGE Coomassie Blue staining. No difference was detected in the cellular protein expression between cells transfected with the *pRev(1.4)-A37-EGFP* (A37) and *pRev(1.4)-A6-EGFP* (A6) or the

untransfected control. β-tubulin and Histone (H3) served as house-keeping protein loading controls. (*B*) Subcellular localization of VHL, a nuclear export cargo of eEF1A. HEK293 cells were transiently expressing Rev(1.4)-polyA-EGFP. The cell nuclei were stained with Hoechst 33342. VHL protein showed similar cytoplasmic localization in cells expressing Rev(1.4)-A6-EGFP (A6) and Rev(1.4)-A37-EGFP (A37). Scale bar represents 10 μm. Three independent experiments were performed.