**SUPPLEMENTARY INFORMATION**

**METHODS**

**Polysome extract preparation and sucrose gradient ultracentrifugation.** Cytoplasmic HeLa cell extracts were prepared as described previously (Hundley, RNA 2008). Briefly, HeLa cells were incubated in the presence of 100 μg/ml cycloheximide for 15 minutes and then scraped from the plate in 1x PBS, pH 7.5. After a brief centrifugation, the cell pellet was resuspended in 1 ml of CBC buffer (20 mM HEPES pH 7.5, 5 mM MgCl2, 10 mM KCl, 1 mM EGTA, 10% glycerol, 0.2 mg/ml heparin, 80 U/ml of RNasin, Roche complete protease inhibitor tablet, 500 μg/ml cycloheximide) + 0.2% NP-40 and vortexed for 30 minutes. Cells treated with puromycin were prepared similarly, except without treatment with cycloheximide. The extracts were cleared of cellular debris by spinning for 5 minutes at 700g in an Eppendorf microfuge at 4°C. The CBC supernatant was immediately loaded onto a 8 ml 10-50% sucrose gradient and centrifuged in an SW41Ti rotor (Beckman) at 41,000 rpm for 3 hours at 4°C. Gradients were scanned at 254 nm and fractionated in 1ml increments with an ISCO gradient collector.

**RNA isolation and qRT-PCR.**

RNA was isolated from cell pellets using Trizol (Invitrogen). RNA was further purified by treating with TURBO DNase (Ambion) followed by RNeasy chromatography (Qiagen). To synthesize cDNA for qRT-PCR, DNase treated total RNA were reverse transcribed with SuperScript II reverse transcriptase (Invitrogen) and gene-specific primers to the coding region of the mRNA (supplementary Table 5). Samples were treated with RNaseH (New England Bioloabs) for 30 minutes. cDNA levels were measured in a Lightcycler 2.0 instrument using the Lightcycler FastStart DNA Master$^{PLUS}$ Syber Green I Kit (Roche) or in an Eppendorf Realplex instrument using KAPA SYBR Fast Universal Master Mix. qRT-PCR primers spanned at least

one exon boundary. Quality of qRT-PCR products was assessed using melting curve analysis and gel electrophoresis.

**SUPPLEMENTARY FIGURE LEGENDS**

**TABLE 1.** Nuclear and cytoplasmic editing levels of endogenous *PSMB2*. HeLa cell genomic DNA and cDNA from total, nuclear and cytoplasmic fractions were amplified and sequenced to detect editing events in the *PSMB2* 3' UTR. Editing levels were determined by estimating peak height of A and G in the sequencing chromatogram.

**TABLE 2.** Nuclear and cytoplasmic editing levels of endogenous *BPNT1*. HeLa cell genomic DNA and cDNA from total, nuclear and cytoplasmic fractions were amplified and sequenced to detect editing events in the *BPNT1* 3' UTR. Editing levels were determined by estimating peak height of A and G in the sequencing chromatogram.

**TABLE 3.** Editing levels of endogenous *PSMB2*. Genomic DNA and cDNA from HeLa, HeLa$^{scr.}$ and ADAR1$^{k.d.}$ cells were amplified and sequenced to detect editing events in the *PSMB2* 3' UTR. Editing levels were determined by estimating peak height of A and G in the sequencing chromatogram.

**TABLE 4.** Editing levels in the firefly-*PSMB2* 3' UTR reporter. Reporter plasmid DNA and cDNA from HeLa, HeLa$^{scr.}$ and ADAR1$^{k.d.}$ cells were amplified and sequenced to detect editing events in the *PSMB2* 3' UTR. Editing levels were determined by estimating peak height of A and G in the sequencing chromatogram.

**TABLE 5.** All primers used in the study are listed.

**FIGURE 1.** Ribosome-association of mRNAs with inverted Alu-containing 3' UTRs. (A and B) Ribosome-association of endogenous HeLa mRNAs. Cytoplasmic extracts from HeLa cells were isolated in the presence of cycloheximide (A) or puromycin (B) and separated by sucrose density sedimentation. The absorbance trace at 254 nm was monitored for each gradient (top panel) and mRNA levels quantified for each fraction (lower graphs). mRNA levels were determined by qRT-PCR for *PSMB2* (purple), *BPNT1* (green), *SNX22* (dark blue), *FCF1* (red), and *GAPDH* (light blue) and plotted as the fraction of mRNA present.

**FIGURE 2.** Sequence of *PSMB2* 3' UTR. The sequence of the endogenous *PSMB2* 3' UTR was determined by RACE. Nucleotides in red correspond to the Alu sequences present in the 3' UTR.

**FIGURE 3.** The *PSMB2* 3' UTR does not affect mRNA stability. Northern blots of total RNA from (A) HEK293 or (B) ADAR1$^{k.d.}$ HeLa cells transfected with the indicated firefly *PSMB2* 3' UTR reporters and a control *Renilla* luciferase reporter, hybridized with indicated probes.

**FIGURE 4.** Sequence of *MPST* 3' UTR. The sequence of the endogenous *MPST* 3' UTR was obtained from the UCSC genome browser. Nucleotides in red correspond to the Alu sequences present in the 3' UTR.

**FIGURE 5.** Sequence of *BPNT1* 3' UTR. The sequence of the endogenous *BPNT1* 3' UTR was obtained from the USCS genome browser. Nucleotides in red correspond to the Alu sequences present in the 3' UTR.

**FIGURE 6.** *BPNT1* 3' UTR does not affect gene expression. (A) For the indicated *BPNT1* reporters, the height of the bar represents the luciferase activity of the firefly inverted Alu (WT) or RC 3' UTR reporters relative to a co-transfected *Renilla* luciferase plasmid, normalized to a wild-type value of 1. Error bars show the SEM for three independent biological replicates. In HEK293 cells, transfection of the *BPNT1* reporters did not reveal any significant differences in luciferase activity between WT and the RC controls. (B) Northern blots of total RNA from the same HEK293 cells analyzed in A, hybridized with indicated probes.