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SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

FIG. S1. Relative levels of exogenous RBM15-FLAG vs endogenous RBM15 in HEK293 cells. HEK293 cells were transfected with increasing amounts of pRBM15-FLAG plasmid DNA. The expression levels of exogenous (1) vs endogenous RBM15 (2) in the transfected cells were determined by Western blot using anti-RBM15 antibody (A) and each graph (B) indicates changes in protein band intensity as a function of increasing RBM15-FLAG concentrations.

FIG. S2. ORF57, but not RBM15, stabilizes polyadenylated ORF59 RNA. RNA decay analysis of ORF59 mRNA in the presence or absence of ORF57 (A) or RBM15 (B) was determined by qRT-PCR on polyadenylated RNA following actinomycin D (ActD) treatment for the time points shown in (A) and (B). HEK 293 cells were transfected with plasmids expressing ORF59 plus ORF57 (A), RBM15 (B) or an empty FLAG vector control (A and B). Eighteen hours after transfection, 10 ug/ml of ActD was added to stop transcription and polyadenylated RNA samples were prepared at the designated time points post ActD treatment. The amounts of ORF59 and GAPDH RNAs in these samples were quantified by RT-PCR followed by qPCR using Taqman probes. ORF59 was quantified relative to GAPDH and expressed in percentage (%) of remaining mRNA after ActD treatment. Half life of ORF59 RNA was calculated by regression analysis.

FIG. S3. Overexpression of RBM15 promotes the expression of ORF57 mt2+3 and induces nuclear accumulation of the mt ORF57 RNA. (A) RBM15 preferentially increases nuclear accumulation of the ORF57 mt2+3 RNA, but not a wt ORF57 RNA. HEK293 cells were

cotransfected with wild type (wt) or mutated (mt2+3) ORF57 (100 ng) together with with or without 20 ng of an empty FLAG vector (-) or an RBM15 expressing vector. Twenty four hours after transfection, fractionated nuclear and cytoplasmic RNAs were prepared and analyzed by Northern blotting with GAPDH RNA as a loading control. U6 served as an indication of fractionation efficiency. Bar graphs are relative ORF57 RNA levels from each lane after being normalized to GAPGD RNA, with normalized C/N ratios of ORF57 RNA levels over the GAPDH RNA above the bar graphs. (B) Overexpressed RBM15 preferentially promotes the production of ORF57 mt2+3 than wt ORF57 at a higher dose (100 ng) of transfection in HEK293 cells. Protein levels of both wt and mt ORF57-FLAG in cotransfection of HEK293 cells with 20 ng RBM15 were examined by Western blotting.

FIG. S4. RBM15 and OTT3 interact with ORF57, but not with Ref1-II. HEK293 cells were co-transfected with RBM15-or-OTT3-FLAG and either ORF57-HA or Ref1-II-HA. Co-immunoprecipitation was performed under physiological conditions as described in Fig. 8B. Under physiological conditions, RBM15 and OTT3 are shown to co-immunoprecipitate with ORF57, but not with the export cofactor Ref1-II.