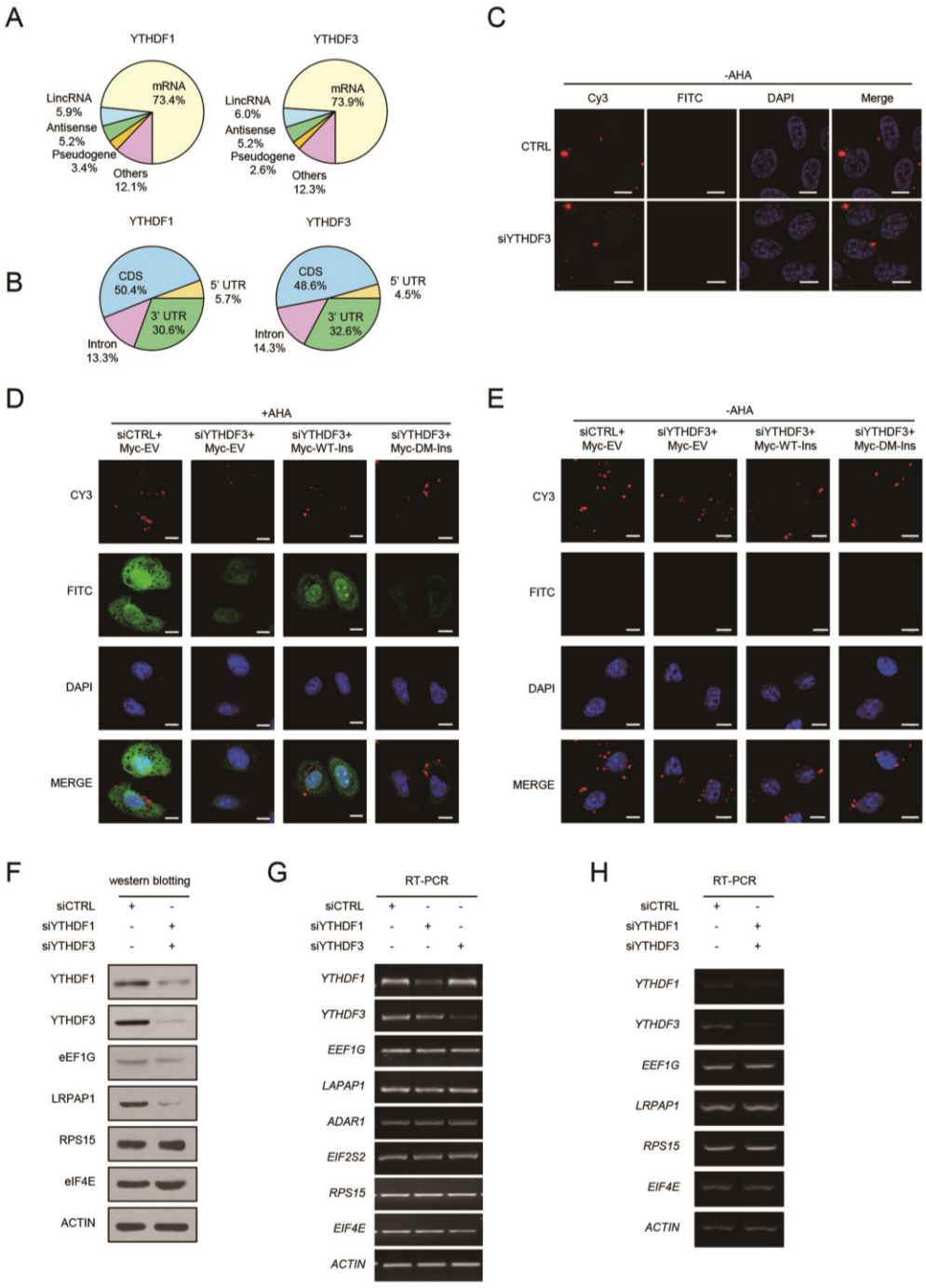

Figure S2. Supporting figure for YTHDF3 in promoting translation.

(A) Percentage of different types of RNAs bound by YTHDF1 or YTHDF3 based on PAR-CLIP-seq analysis. The binding clusters were identified by PARalyzer software with default parameters. Clusters were annotated by Ensembl database (release 72, version hg19) and the majority of binding clusters of both YTHDF1 and YTHDF3 are located on mRNA. (B) Distribution of YTHDF1 and YTHDF3 binding clusters in four mRNA regions: 5 ’UTR, CDS, intron and 3’ UTR. (C) Immunofluorescence analysis

of nascent protein synthesis in control and YTHDF3 deficient HeLa cells (without AHA treatment). FITC (green): nascent synthesized protein staining; CY3 (red): 5'-CY3 labelled siRNAs; DAPI (blue): DNA staining. Scale bar: 10 μm. Representative images from one of the three independent experiments were shown. (D) Immunofluorescence analysis of nascent protein synthesis in YTHDF3 depleted HeLa cells (with AHA treatment) transfected with control Myc-EV, Myc-tagged siYTHDF3-insensitive wild-type YTHDF3 (Myc-WT-Ins) or m^6A binding defective (Myc-DM-Ins) plasmids. FITC (green): nascent synthesized protein staining; CY3 (red): 5'-CY3 labelled siRNAs; DAPI (blue): DNA staining. Scale bar: 10 μm. Representative images from the three independent experiments were shown. (E) Immunofluorescence analysis of nascent protein synthesis in YTHDF3 depleted HeLa cells (without AHA treatment) transfected with control Myc-EV, Myc-tagged siYTHDF3-insensitive wild-type YTHDF3 (Myc-WT-Ins) or m^6A binding defective (Myc-DM-Ins) plasmids. FITC (green): nascent synthesized protein staining; CY3 (red): 5'-CY3 labelled siRNAs; DAPI (blue): DNA staining. Scale bar: 10 μm. Representative images from three independent experiments were shown. (F) Protein levels of YTHDF1 and YTHDF3 common targets (*EEF1G and LRPAP1*), non-targets of both YTHDF1 and YTHDF3 (*RPS15 and EIF4E*) in YTHDF3 and YTHDF1 double knockdown cells. (G) mRNA levels of representative genes (*EEF1G, LRPAP1, RPS15, EIF4E, ADAR1, EIF2S2*) in response to RNAi against *YTHDF1* or *YTHDF3* by semi-quantitative RT-PCR. *ACTIN* was used as loading control. (H) mRNA levels of representative genes (*EEF1G, LRPAP1, RPS15, EIF4E*) in response to RNAi against *YTHDF1* and *YTHDF3* by semi-quantitative RT-PCR. *ACTIN* was used as loading control.