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Supplementary Materials for

TRIBE editing reveals specific mRNA targets of eIF4E-BP in *Drosophila* and in mammals

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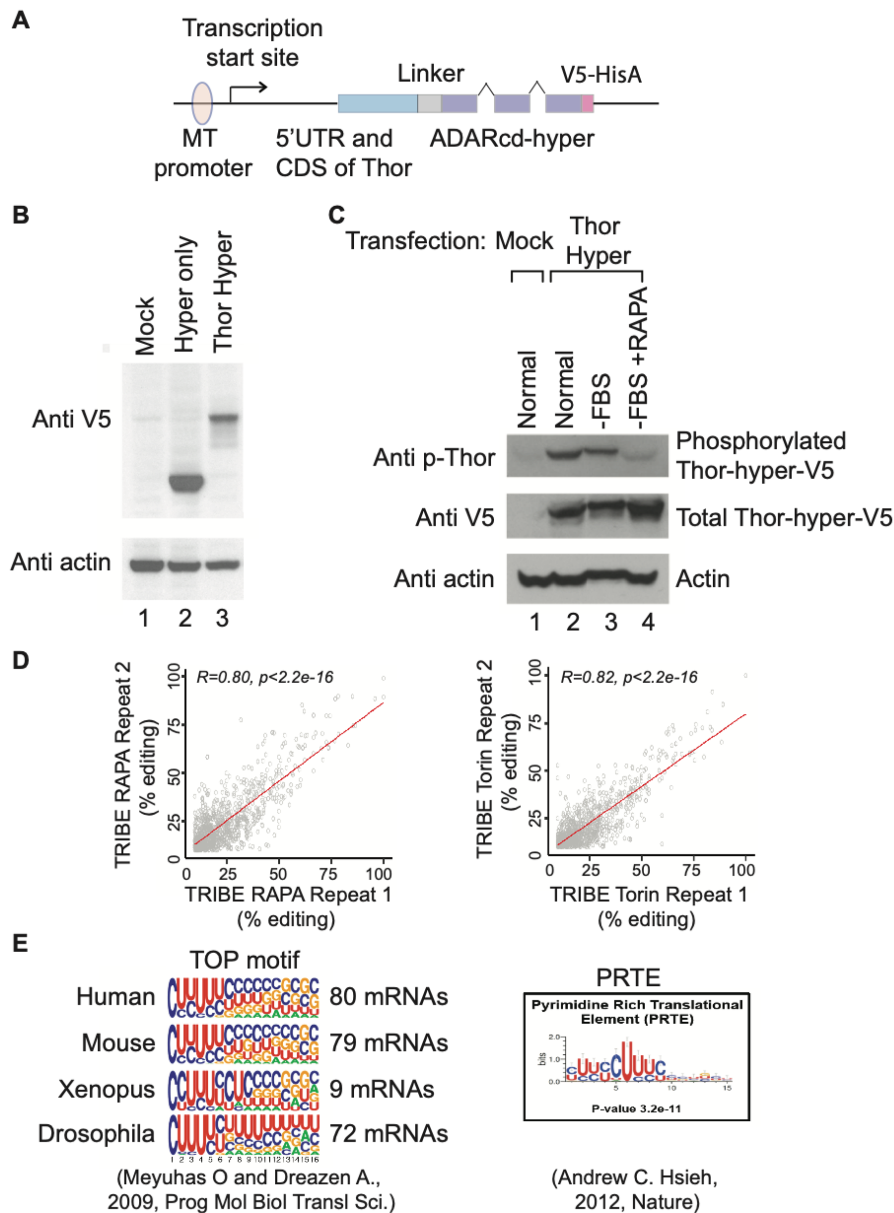
Figs. S1 to S3

Other Supplementary Material for this manuscript includes the following:

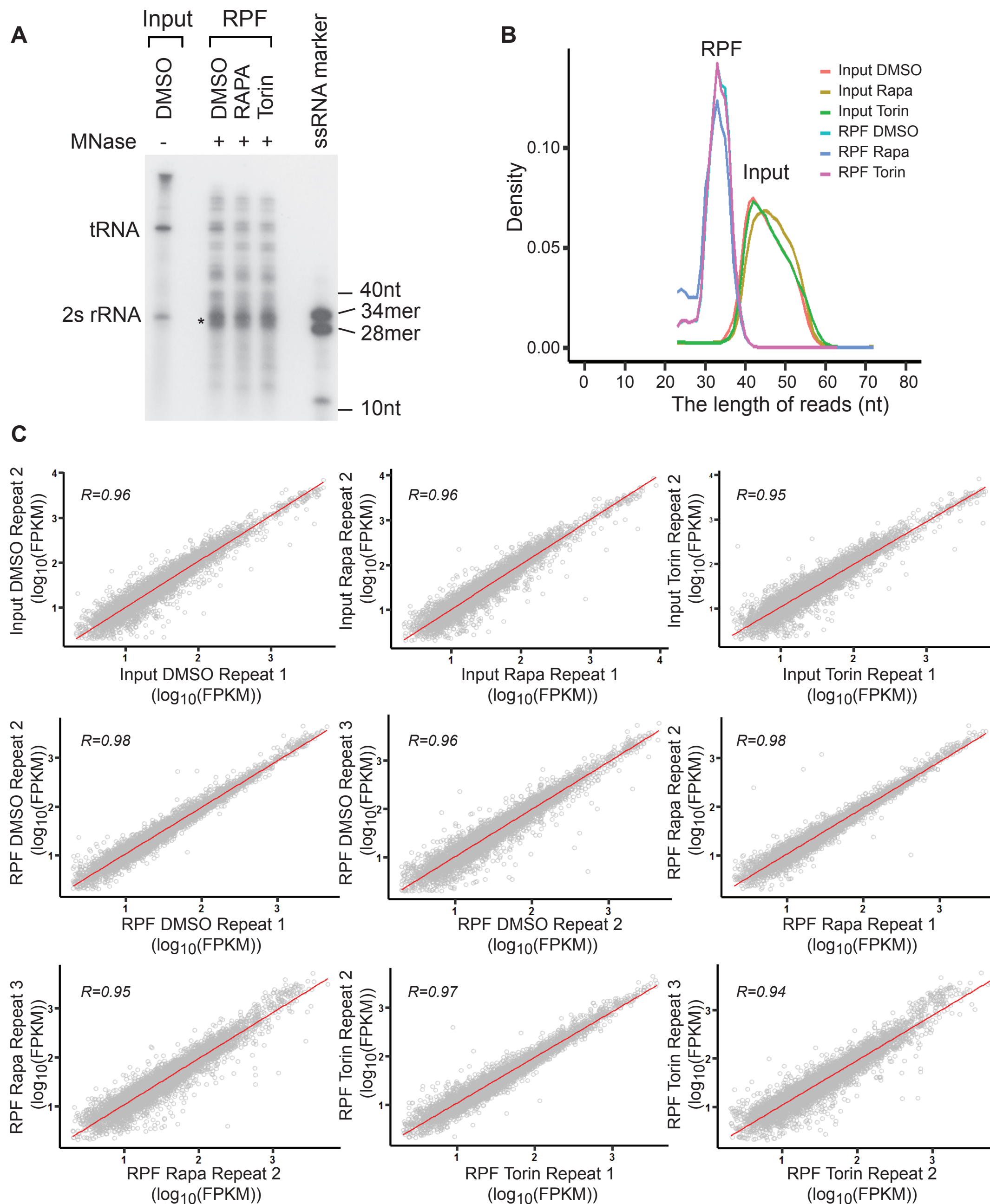
(available at advances.sciencemag.org/cgi/content/full/6/33/eabb8771/DC1)

Tables S1 to S5

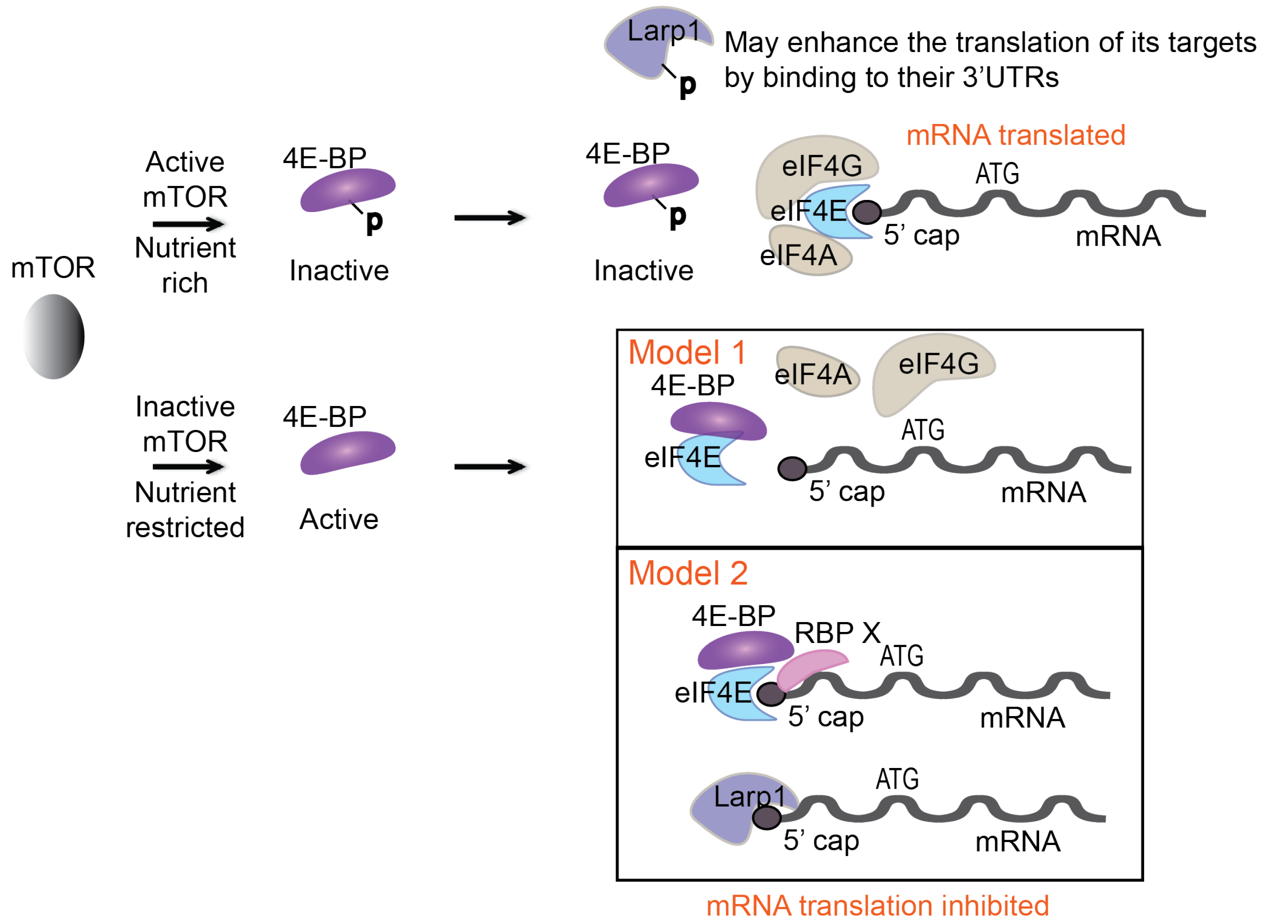


Supplementary Fig. 1 Thor HyperTRIBE construct was successfully expressed in S2 cells and induced consistent editing sites.

(A) Schematic presentation of Thor HyperTRIBE DNA construct. **(B)** The proteins of Hyper (ADARcd-E488Q-V5) and Thor Hyper (Thor-ADARcd-E488Q-V5) were expressed in S2 cells after transfection and induction by copper sulfate. The western blot was carried out using antibody against V5 tag and actin. **(C)** S2 cells were transfect with mock plasmid or the plasmid encoding Thor-ADAR-E488Q-V5 (Thor-Hyper). Cells expressing Thor-ADAR-E488Q-V5 were incubated in media with FBS (Normal), without FBS (-FBS), or with rapamycin in addition to FBS depletion (-FBS +RAPA). Western blot was performed using antibody against phospho-4E-BP1 Thr37/46 (p-Thor), V5 tag, and actin. **(D)** The sites identified in Thor hyperTRIBE in S2 cells are edited to a similar degree between biological repeats. Pearson correlation coefficients (R) are shown. **(E)** Left: The consensus sequences of 5' terminal oligopyrimidine tract (5' TOP) are shown. The TOP motif is conserved in Drosophila rp (ribosomal protein) mRNAs. TOP motif resides from position +1 of the 5'UTR. Right: The consensus sequences of Pyrimidine Rich Translational Element (PRTE) are shown.



Supplementary Fig. 2 Ribosome profiling data are high quality and reproducible.
(A) Ribosome protected fragments (RPFs) were enriched between 28-34nt (marked with an asterisk). S2 cells were treated with 100 nM of rapamycin (RAPA), Torin-1, or DMSO. Cell lysate was treated with MNase to get RPF and passed through Sephacryl S-400 columns to purify monosomes. RPF was purified from the flowthrough of the columns. Input RNA was purified from cell lysate without MNase treatment. RNAs from input and RPF were treated with antarctic phosphatase, then labelled by γ-[32P] ATP at their 5'ends, resolved on a 15% TBE-urea gel and visualized. **(B)** The length distribution of aligned sequencing reads from ribosome profiling is shown. **(C)** Measurements of FPKM are reproducible between replicates in ribosome profiling. Pearson correlation coefficients (R) are shown for FPKM (log_{10} value) of input and RPF between replicates.



Supplementary Fig. 3
The possible acting mechanism of 4E-BP-mediated translational repression.
Under nutrient rich conditions, the mTOR phosphorylates 4E-BP, which prevents it from binding to eIF4E. Under nutrient restricted conditions, inactivation of mTOR results in dephosphorylation and 4E-BP activation. Model 1: eIF4G and hypophosphorylated 4E-BP share the same binding site on eIF4E, active 4E-BP blocks the eIF4G binding site on eIF4E and inhibits eIF4F formation and translation. Model 2: Hypophosphorylated 4E-BP binds to eIF4E and subsequently 4E-BP-eIF4E complex is associated with their target mRNAs. Both hypophosphorylated Larp1 and 4E-BP target PRTE mRNAs to repress their translation.