Supplementary

RNAe (RNA enhancement) enhances translation by recruiting ILF3 and eIF4A1 to the target mammalian mRNA

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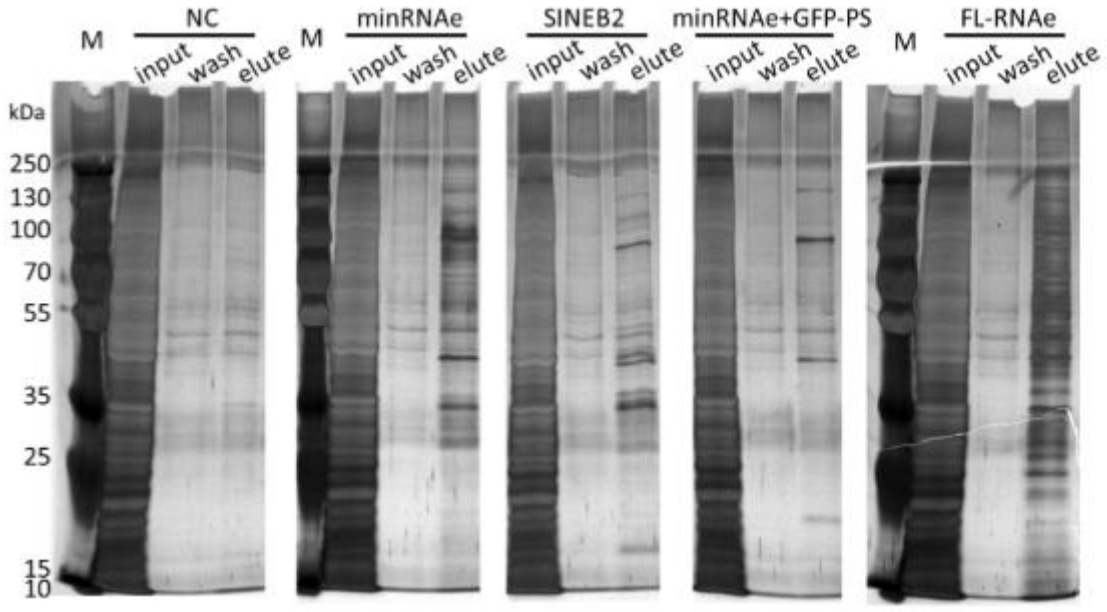
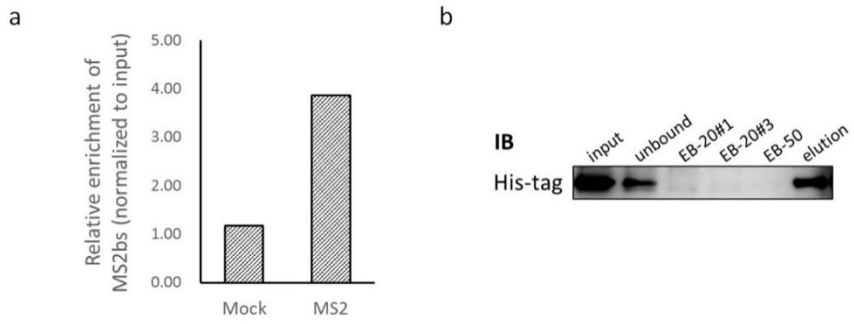
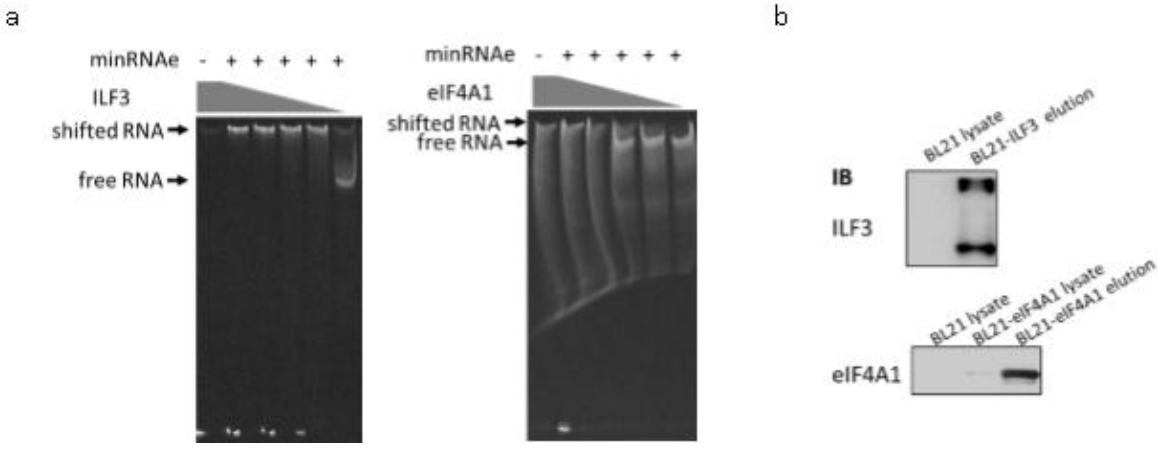
Figures



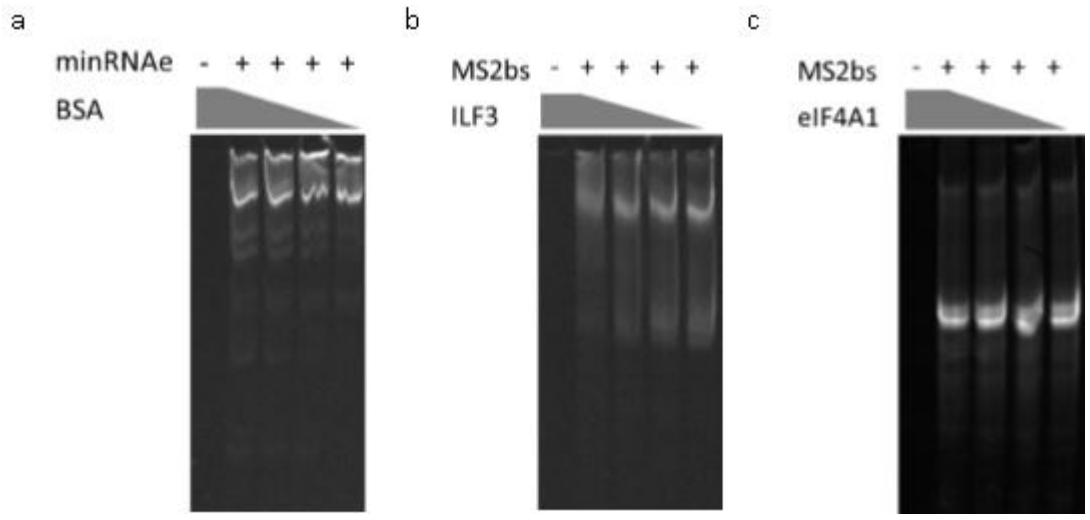
Supplementary Figure 1 SDS-PAGE and silver staining of fractions from the RNA pull-down protein experiments.



Supplementary Figure 2 the positive control of the RNA-binding protein affinity system. a, RT-PCR results showing the relative enhancement of MS2bs by the MS2 protein. 18S rRNA was used as the internal reference, and each group was normalized to the individual input RNA. b, WB analysis of the purification process of the target proteins in a.



Supplementary Figure 3 EMSA used to detect the interactions of RNAe lncRNA and ILF3 and eIF4A1 (expressed in *E. coli*) *in vitro*. a, SYBR Green staining of RNA after incubating with ILF3 or eIF4A1 and separation by 6% native PAGE. There was no minRNAe lncRNA in the "-" lanes and 0.5 μg in the "+" lanes. The amounts of ILF3 and eIF4A1 proteins were 6.0 6.0, 3.6, 1.8, 0.6, and 0.0 μg, respectively. b, WB analysis of the purification process of the target proteins in a.



Supplementary Figure 4 EMSA used to detect the unspecific combination of minRNAe lncRNA with ILF3 and eIF4A1. SYBR Green staining of RNA after incubating RNA (minRNAe lncRNA in a, MS2bs in b and c) and protein (BSA in a, ILF3 in b and eIF4A1 in c), and separating by 6% native PAGE. There was no RNA in the "-" lanes and 0.5 μg in the "+" lanes. The protein amounts were 6.0 6.0, 3.0, 1.0, and 0.0 μg, respectively.

Supplementary Table

Supplementary table 1 plasmids used in the article.

Name	Source	Description	Cloning strategy
pRNAe-mock	pRNAe-mock in (Yao *et al.*, 2015)	Vector for negative control of RNAe lncRNA	——
pFL-RNAe	pRNAe-egfpc1 in (Yao *et al.*, 2015)	Vector expressing RNAe lncRNA targeting pEGFP-C1	——
pminRNAe	pminRNAe-egfpc1 in (Yao *et al.*, 2015)	Vector expressing minRNAe lncRNA targeting pEGFP-C1	——
pminRNAe-HindIII	This paper	Vector expressing minRNAe lncRNA targeting pEGFP-C1 with one HindIII enzyme site behind SINEB2	Relative sequence was amplified from pFL-RNAe by primers (ATTATCTCGAGCCGGTGAACAGCT & ATTATaagcttACTGGAGCTAAAGAGATGGCTCA) and cloned into pFL-RNAe lncRNA with XhoI/HindIII.
pSINEB2	This paper	Vector expressing SINEB2 RNA	Relative sequence was amplified from pFL-RNAe by primers (ATTATCTCGAGCAGTGCTAGAGGAGGTCAGAAGAG & ATTATaagcttGAGCTAAAGAGATGGCTCAGCAC) and cloned into pFL-RNAe with XhoI/HindIII.
pGFP-PS	This paper	Vector expressing 72-nt segment of egfpc1 mRNA pairing with RNAe-egfpc1	Relative sequence was amplified from pFL-RNAe by primers (ATTATCTCGAGTAGTGAACCGTCAGATCCGCTAG & ATTATaagcttCCGGTGAACAGCTCCTCGC) and cloned into pFL-RNAe with XhoI/HindIII.
p8×MS2bs	This paper	Vector expressing 8×MS2-binding sites	Relative sequence was amplified from template plasmid (gifted from Jianzhong Xi lab Peking university, China) by primers (ATTATctcgagACACGACGCTCTTCCGATCT & attatAAGCTTCACCATGGAAACAGACT) and cloned into pFL-RNAe with XhoI/HindIII.
pEGFP-C1	Clonetech, USA	Vector expressing EGFP in HEK 293T cells	——
p2×MS2-dTomato	This paper	Vector expressing 2×MS2-dTomato in HEK 293T cells	Relative sequences were amplified from template plasmid (gifted from Jianzhong Xi lab Peking university, China) by primers (attatACCGGTgccaccatggtaCATCATCACCACCATCATGCT TCTAACTTTACTCAGTTCGTTCTCG & attatagatctGCTAACCACGACTACGGAGTTTG, and attatagatctGCTTCTAACTTTACTCAGTTCGTTCTC & ATTATctcgagCTTGTACAGCTCGTCCATGCC), and digested by BshT1/BglII and BglII/XhoI, separately, and then ligated into BshT1/XhoI-digested pEGFP-C1.
p6×His-ILF3	This paper	Vector expressing 6×His-ILF3 in HEK 293T cells	Relative sequence was amplified from pILF3-ORF (ViewSolid Biotech, China) by primers (attatACCGGTgccaccatggtaCATCATCACCACCATCATCGT CCAATGCGAATTTTTGTG & ATTATagatctCTAGGAAGACCCAAAATCATGATAGC) and cloned into pEGFP-C1 with BshT1/BglII.
p6×His-NCL	This paper	Vector expressing 6×His-NCL in HEK 293T cells	Relative sequence was amplified from pILF3-ORF (ViewSolid Biotech, China) by primers (attatACCGGTgccaccatggtaCATCATCACCACCATCATGT GAAG CTCGCGAAGGCA & ATTATagatctCTATTCAAACTTCGTCTTCTTTCCTT) and cloned into pEGFP-C1 with BshT1/BglII.
p6×His-eEF1A1	This paper	Vector expressing 6×His-eEF1A1 in HEK 293T cells	Relative sequence was amplified from pILF3-ORF (ViewSolid Biotech, China) by primers (attatACCGGTgccaccatggtaCATCATCACCACCATCATGG AAAGG AAAAGACTCATATCAACAT& ATTATagatctTCATTTAGCCTTCTGAGCTTTCTG) and

			cloned into pEGFP-C1 with BshT1/BglII.
p6×His-eIF4A1	This paper	Vector expressing 6×His-eIF4A1 in HEK 293T cells	Relative sequence was amplified from pILF3-ORF (ViewSolid Biotech, China) by primers (attatACCGGTgccaccatggtaCATCATCACCACCATCATTC TGCG AGCCAGGATTCC & ATTATaagcttTCAGATGAGGTCAGCAACATTGA) and cloned into pEGFP-C1 with BshT1/HindIII.
p6×His-ILF3-BL21	This paper	Vector expressing 6×His-ILF3 in BL21 (DE3)	Relative sequence was amplified from p6×His-ILF3 by primers (TTAAGAAGGAGATATACatATGGTACATCATCACCAC CATCAT & ATTATagatctCTATTCAAACTTCGTCTTCTTTCCTT) and cloned into pEGFP-C1 with BshT1/BglII.
p6×His-eIF4A1-BL21	This paper	Vector expressing 6×His-eIF4A1 in BL21 (DE3)	Relative sequence was amplified from p6×His-eIF4A1 by primers (TTAAGAAGGAGATATACatATGGTACATCATCACCAC CATCAT & ATTATagatctCTATTCAAACTTCGTCTTCTTTCCTT) and cloned into pEGFP-C1 with BshT1/HindIII.

Reference

Yao, Y., Jin, S., Long, H., Yu, Y., *et al.* (2015). RNAe: an effective method for targeted protein translation enhancement by artificial non-coding RNA with SINEB2 repeat. Nucleic Acids Res *43*, e58.