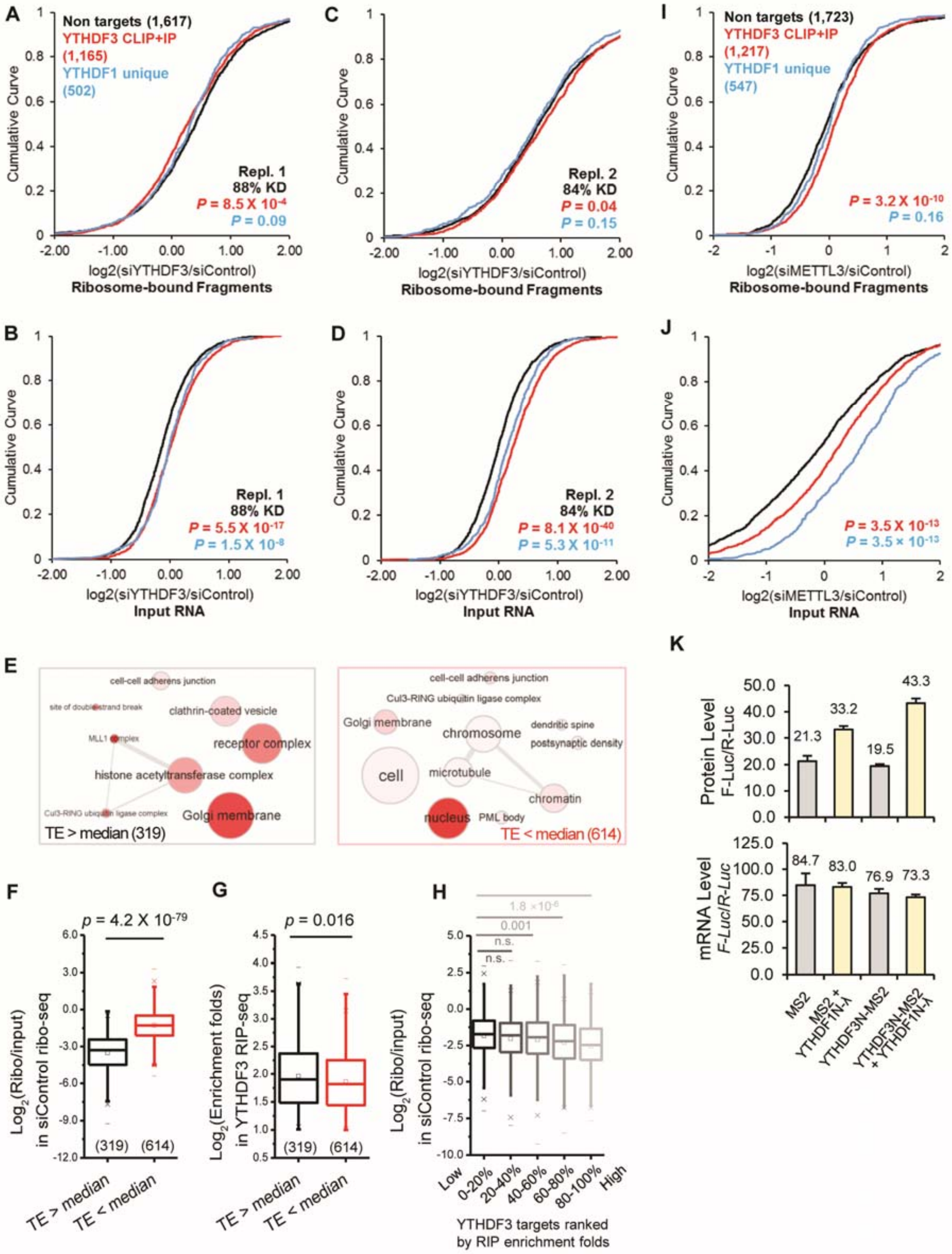A
Non targets (1,617)
YTHDF3 CLIP+IP (1,165)
YTHDF1 unique (502)
Cumulative Curve
Repl. 1
88% KD
P = 8.5 X 10-4
P = 0.09
log2(siYTHDF3/siControl)
Ribosome-bound Fragments
C
Cumulative Curve
Repl. 2
84% KD
P = 0.04
P = 0.15
log2(siYTHDF3/siControl)
Ribosome-bound Fragments
I
Non targets (1,723)
YTHDF3 CLIP+IP (1,217)
YTHDF1 unique (547)
Cumulative Curve
P = 3.2 X 10-10
P = 0.16
log2(siMETTL3/siControl)
Ribosome-bound Fragments
B
Cumulative Curve
Repl. 1
88% KD
P = 5.5 X 10-17
P = 1.5 X 10-8
log2(siYTHDF3/siControl)
Input RNA
D
Cumulative Curve
Repl. 2
84% KD
P = 8.1 X 10-40
P = 5.3 X 10-11
log2(siYTHDF3/siControl)
Input RNA
J
Cumulative Curve
P = 3.5 X 10-13
P = 3.5 × 10-13
log2(siMETTL3/siControl)
Input RNA
E
cell-cell adherens junction
site of double strand break
clathrin-coated vesicle
MLL1 complex
receptor complex
histone acetyltransferase complex
Cul3-RING ubiquitin ligase complex
Golgi membrane
TE > median (319)
cell-cell adherens junction
Cul3-RING ubiquitin ligase complex
Golgi membrane
chromosome
dendritic spine
postsynaptic density
cell
microtubule
chromatin
nucleus
PML body
TE < median (614)
K
Protein Level F-Luc/R-Luc
21.3
33.2
19.5
43.3
mRNA Level F-Luc/R-Luc
84.7
83.0
76.9
73.3
MS2
MS2 + YTHDF1N-λ
YTHDF3N-MS2
YTHDF3N-MS2 + YTHDF1N-λ
F
p = 4.2 X 10-79
Log2(Ribo/input) in siControl ribo-seq
(319)
(614)
TE > median
TE < median
G
p = 0.016
Log2(Enrichment folds) in YTHDF3 RIP-seq
(319)
(614)
TE > median
TE < median
H
1.8 ×10-6
0.001
n.s.
n.s.
Log2(Ribo/input) in siControl ribo-seq
Low
0-20%
20-40%
40-60%
60-80%
80-100%
High
YTHDF3 targets ranked by RIP enrichment folds

Supplementary information, Figure S2 Ribosome profiling and polysome profiling of HeLa cells depleted of YTHDF3 **(A-J)**, and protein and mRNA levels of reporters in the double tethering assay **(K)**. **(A-D, I-J)** Cumulative distribution of log_2-fold changes of ribosome-bound fragments **(A, C, I)** and input RNA **(B, D, J)** between siControl and siYTHDF3, replicate 1 **(A-B)**, siYTHDF3, replicate 2 **(C-D)**, and siMETTL3 **(I-J)**. Non-targets, black; YTHDF3 CLIP+IP targets, red, and YTHDF1 unique targets, blue. *P* values were calculated from a two-sided Mann-Whitney test compared to non-targets. **(E)** Gene Ontology (GO) analysis with DAVID 6.7 [49,50] of the group of genes with increased translation efficiency (TE > median) and that with decreased translation efficiency (TE < median) in absence of YTHDF3. GO terms with $p < 0.05$ were visualized with REVIGO[51]. **(F-G)** Box plot of translation efficiency (Log_2(Ribo/input)) in knockdown control samples **(F)** and YTHDF3 RIP enrichment folds **(G)** of these two groups of genes. Box, 25-75%; "–", max and min; "×", 1% and 99%; "□", median. *P* values were calculated from a two-sided Mann-Whitney test. **(H)** Box plot of the correlation between YTHDF3 RIP enrichment folds and translation efficiency in siControl samples. Box, 25-75%; "–", max and min; "×", 1% and 99%; "□", median. *P* values were calculated from a two-sided Mann-Whitney test compared to the "0-20%" group. **(K)** Normalized protein level and mRNA level of F-Luc 4-hour post F-Luc induction with the expression of effectors indicated in the double tethering assay. Error bars, mean ± s.d., n = 2~4.