Figure S1. RiboToolkit analyses showed significant Ribosome pausing at histidine codons in yeast treated with 3-amino-1,2,4-triazole (3-AT). (A) Codon occupancy changes. **(B)** Cumulative occupancy distribution of histidine codons. **(C)** Cumulative occupancy distribution of individual histidine codons and AAC (Asparagine) is used as negative control. **(D)** Peaks of occupancy change in codon metagene plot.
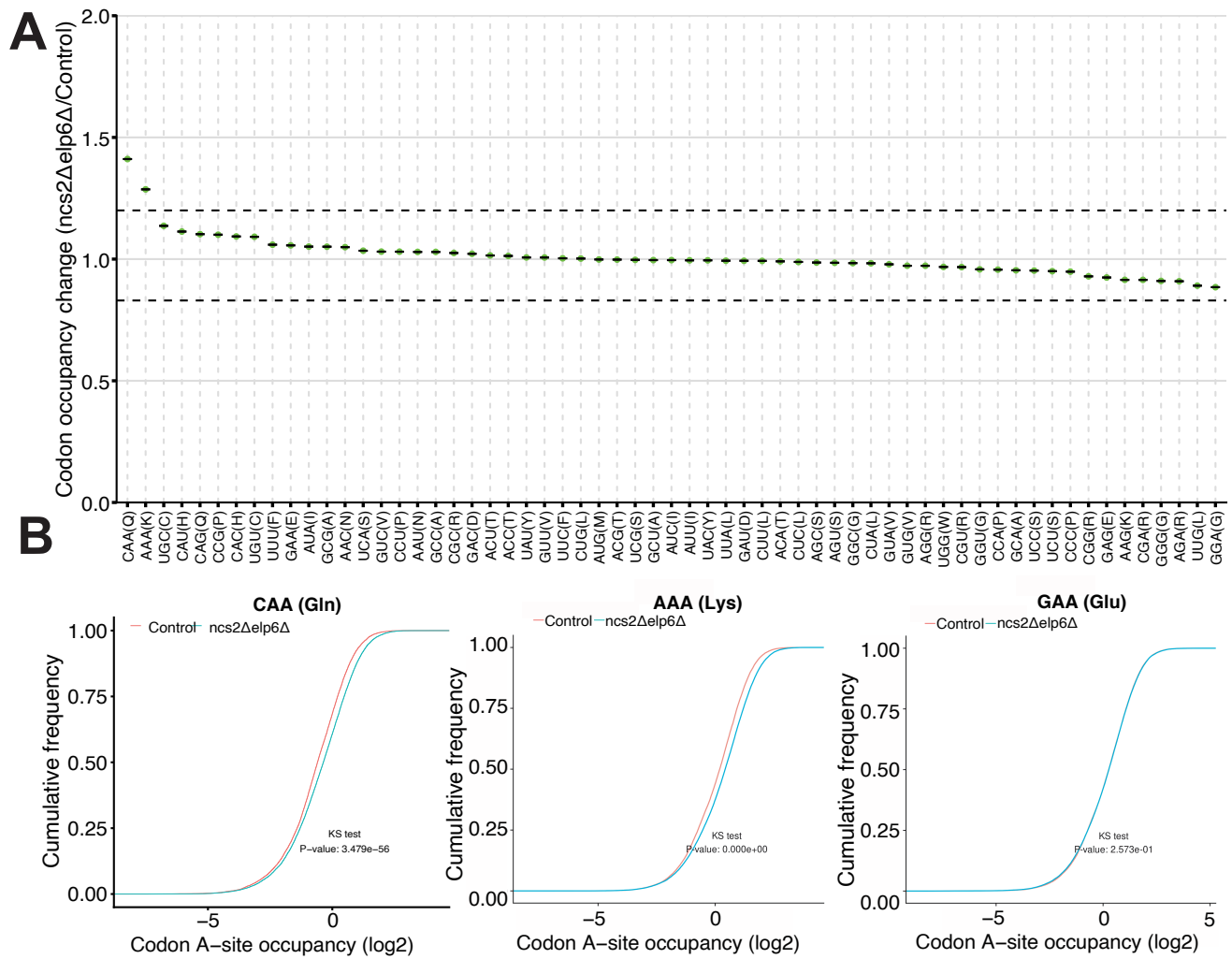
Figure S2. Codon analyses by RiboToolkit of yeast ncs2Δelp6Δ mutant (lacking all U34 modifications) showed strikingly distinct effects of U34 modification loss on codon occupancy.

(A) Codon occupancy changes between yeast ncs2Δelp6Δ mutant and wide type; **(B)** Cumulative occupancy distribution of individual codons decoded by U34 modification tRNAs.

Table S1. Source of dataset used for the 16 supported model species

	Homo sapiens (hg19, hg38)	Mus musculus (mm10)	Rattus norvegicus (rn6)	Danio rerio (GRCz11)	Drosophila melanogaster (Dm6)	Caenorhabditis elegans (WBcel235)	Saccharomyces cerevisiae (R64)	Arabidopsis thaliana (TAIR10)	Oryza sativa (MSU7.0)	Zea mays (B73_RefGen_v4)	Glycine max (Wm82.a2)	Solanum lycopersicum (SL3.0)	Escherichia coli (k-12)	Bacillus subtilis (strain 168)	Pyrococcus furiosus (DSM 3638)	Halobacterium salinarum (NRC1)
Reference genomes & mRNAs																
GENCODE	√	√														
Ensembl Genome			√	√	√	√	√	√	√	√	√	√	√	√	√	√
tRNAs																
GtRNAdb	√	√	√	√	√	√	√	√	√	√	√		√	√	√	
Ensembl ncRNAs												√				√
rRNAs																
Ensembl ncRNAs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
snRNAs																
Ensembl ncRNAs	√	√	√	√	√	√	√	√	√	√	√	√			√	√
Functional gene annotations																
Gene Ontology	√	√	√	√	√	√	√	√	√	√	√	√				
KEGG	√	√	√	√	√	√	√	√	√	√	√	√				
Reactome	√	√	√	√	√	√										
Disease Ontology	√	→	→	→	→	→										
Network of Cancer Gene	√	→	→	→	→	→										
DisGeNET disease genes	√	→	→	→	→	→										
MSigDB	√															

The label "→" indicates that the data can be orthologically transferred to the relevant species from human.

Table S2. The feature comparison among integrated tools for ribosome profiling data analysis

Features	RiboToolkit	RiboTools	riboSeqR	plastid	RiboProfiling	RiboGalaxy	Proteoformer	systemPipeR	RiboVIEW	riboStreamR	RiboFlow/Ribo R/RiboPy
Software install type											
Web upload	√	√				√				√	
Batch uploading	√										
Local install	√		√	√	√	√	√	√	√		√
Input types											
FASTA	√										
FASTQ							√	√			√
SAM/BAM		√	√	√	√	√			√	√	
Table											
Link	√										
Quality control											
Contamination checking	√			√			√	√	√	√	√
Quality checking	√	√	√	√	√	√	√	√	√	√	√
RPF alignment											
RPF alignment	√						√	√			√
Codon related analyses											
Codon occupancy	√	√		√	√	√	√		√		
Codon pause events	√				√				√		
Codon frequency	√										
Meta-codon plots	√								√		

Active ORF translation											
Active ORF calling	√		√				√	√			
Translation read through		√				√					
Proteomic analysis							√				
RPF expression and translational analyses											
mRNA expression	√		√	√		√		√		√	
RPF expression	√		√	√	√	√	√	√		√	
Translation efficiency analysis	√		√	√		√		√		√	
Differential translation analysis	√		√			√		√		√	
Functional analyses of differentially translated genes											
GO & KEGG	√							√			
Other pathways	√										
Gene Set Enrichment Analysis	√										
Other functionalities											
RPF distribution browse	√	√		√		√		√	√	√	√
IGV mapping viewer	√					√					
RPF metagene plots	√	√	√	√	√	√		√	√	√	√
Reproducibility between replicates									√		

Table S3. Upload and analysis speed for different Ribo-seq datasets on the servers

Data source	Sample IDs	Species	Number of Samples	Upload file format	Total file Size	Sever #1			Sever #2		
						Upload in the USA (Boston)	Upload in China (Chengdu)	Run time	Upload in The USA (Boston)	Upload in China (Chengdu)	Run time
GSE103667	GSM2778762 GSM2778763 GSM2778764 GSM2778765	Mouse	4	.fa.gz	97 M	1m36s	1m57s	25m	3m3s	2m45s	13m
GSE67387*	GSM1646015 GSM1646016 GSM1646017 GSM1646024 GSM1646025 GSM1646026	Yeast	2	.fa.gz	289M	5m12s	5m42s	49m	8m23s	6m57s	26m
GSE52968	GSM1279568 GSM1279579	Yeast	2	.fa.gz	67M	1m5s	1m12s	16m	1m58s	1m36s	8m
GSE112670	GSM3076129 GSM3076131	Mouse	2	.fa.gz	44M	42s	50s	21m	1m15s	1m6s	11m
GSE32060	GSM794854 GSM794855 GSM794856	Mouse	3	.fa.gz	78M	1m6s	1m18s	18m	2m36s	2m12s	8m

"m" and "s" represent minute and second, respectively. "*" indicates three replicates are merged into one FASTA file to be uploaded to the server.

Server #1: https://rnabioinfor.tch.harvard.edu/RiboToolkit. Server #2: https://bioinformatics.sc.cn/RiboToolkit