Supplementary Information

Host Mature tRNAome as a Decoding Switch Regulates Antiviral and Proviral Responses

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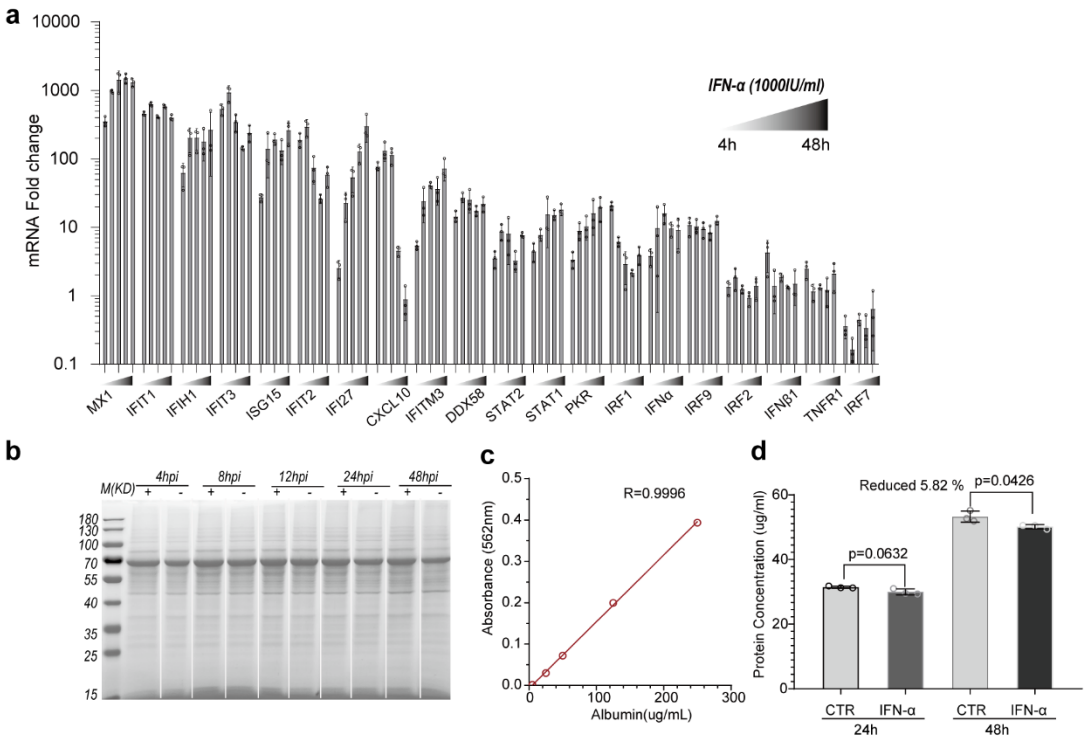
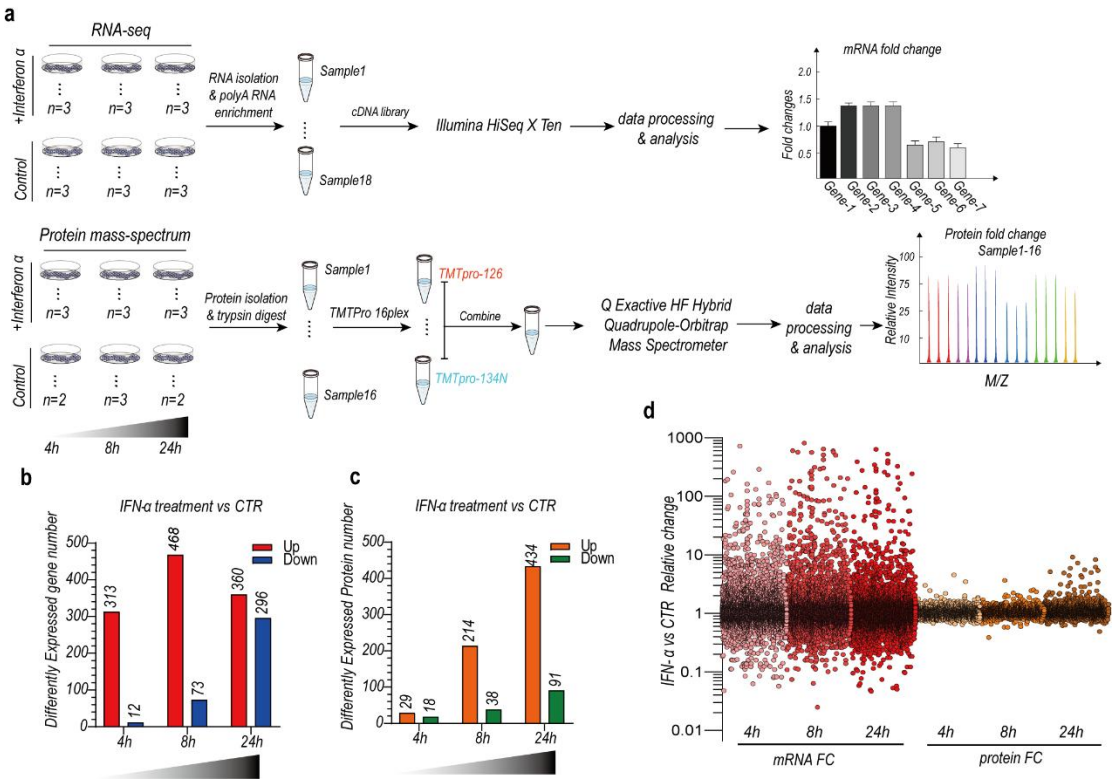
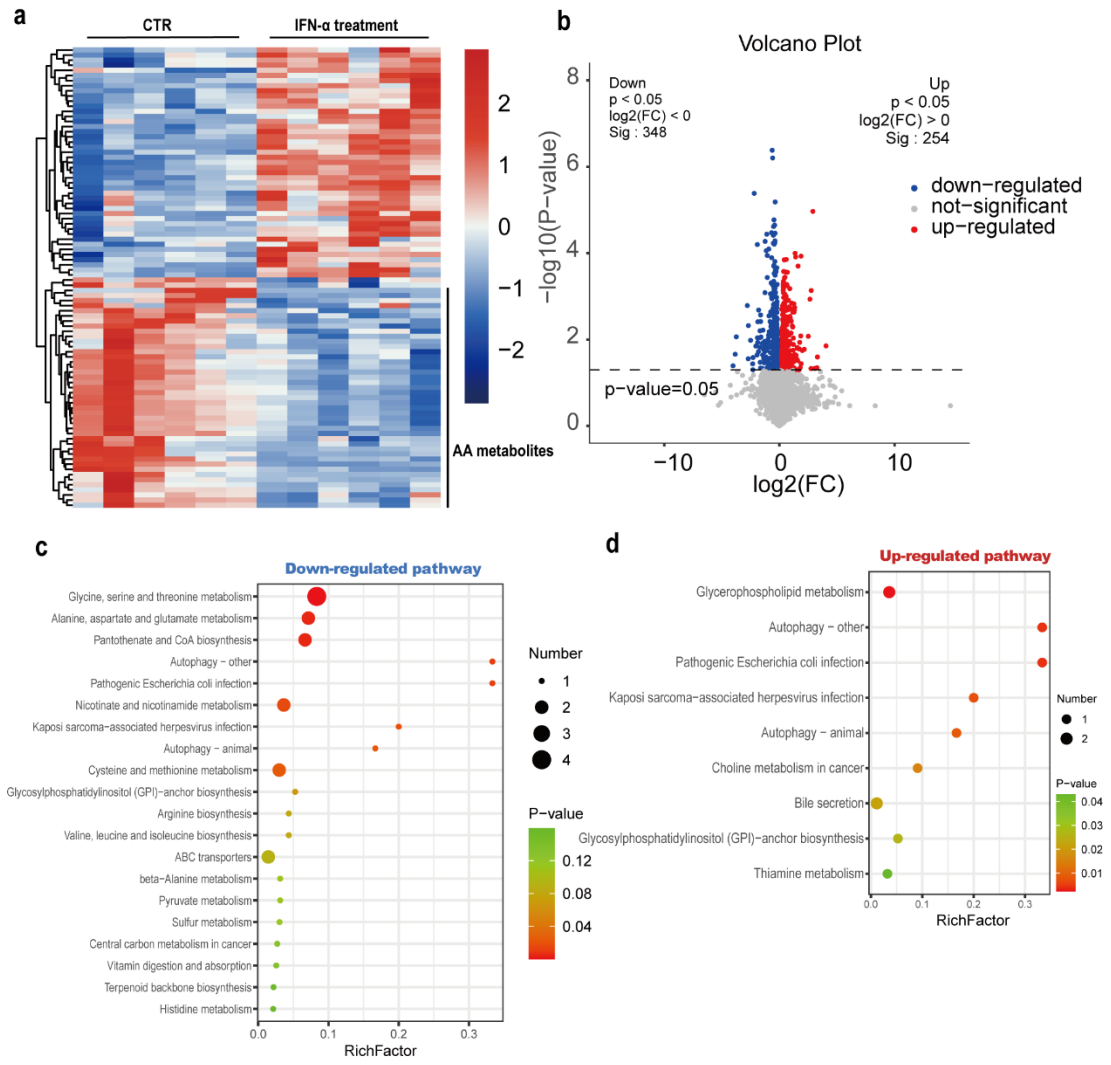


Supplementary Fig. 1 **Transcription of 20 ISGs and global protein translation upon IFN-α treatment. a** The transcriptional levels of human ISGs were quantified by reverse transcription quantitative polymerase chain reaction (RT-qPCR) following treatment with IFN-α (1000 IU/mL, n = 3 biological replicates). ISG levels were normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. **b** SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of crude extracts from cells treated or not treated with IFN-α (1000 IU/mL) at various time points was performed to assess protein changes. **c** Measurement of total protein abundance was conducted via a bicinchoninic acid (BCA) protein assay; the standard curve with defined protein concentrations is shown. **d** Total protein levels in cells treated with or without IFN-α were compared at 24 and 48 hours and presented in this panel. Error bars represent mean ± standard deviation (s.d.). Statistical significance was determined using a two-tailed student's t-test (n = 3 biological replicates). Source data are provided as a Source Data file.

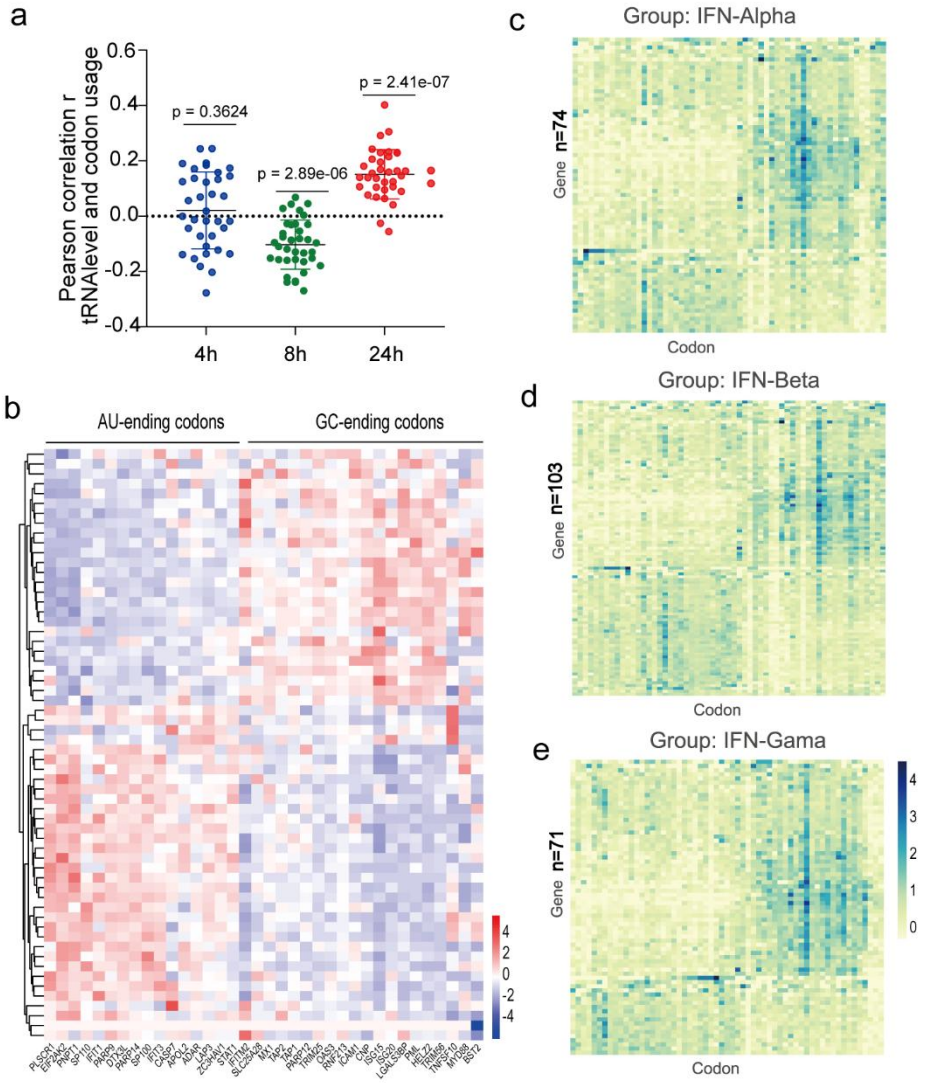


Supplementary Fig. 2 **Experimental design of high-throughput RNA-seq and quantitative proteome MS to study ISG transcriptome and proteome. a** Cells were subjected to high-throughput RNA-seq and quantitative proteome MS using tandem mass tag (TMT) 16-plex labeling to analyze the temporal expression of ISGs. Cells were treated with IFN-α (1000 IU/mL) for 4, 8, and 24 hours, with three biological replicates for RNA-seq. For quantitative proteome MS, the same treatment regimen was maintained, with two biological replicates for proteomic analysis at 4 hours and 24 hours for untreated cells, and three biological replicates for 8 hours for both treated and untreated conditions. **b** The number of differentially expressed genes at the mRNA level during treatment (n = 3 biological replicates) is shown, based on three biological replicates. Genes with a fold change (FC) above 2.0 were considered differentially expressed. **c** The number of differentially expressed genes at the protein level during treatment (n = 3 biological replicates) is presented, with genes exhibiting an FC above 1.2 identified. **d** Global ISG responses at the transcriptome and proteome levels are displayed. Source data are provided as a Source Data file.

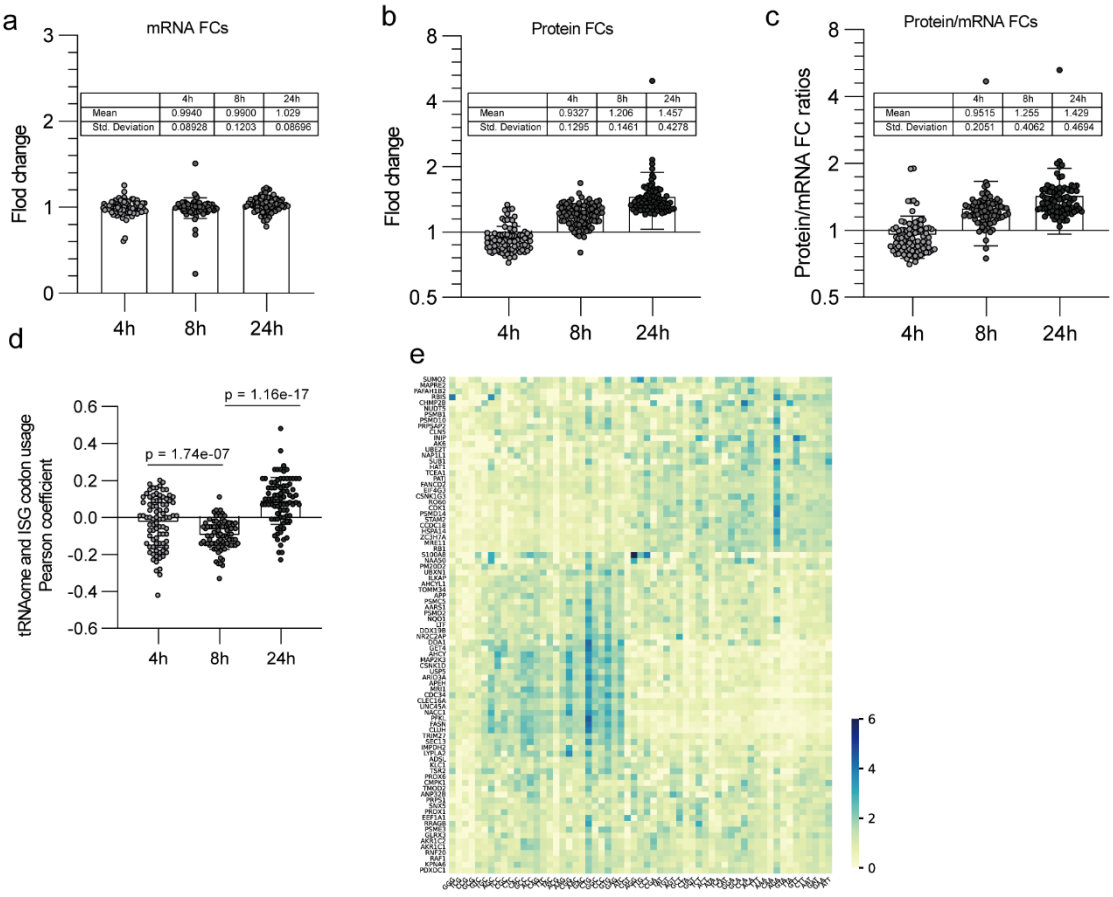


Supplementary Fig. 3 **Metabolomic changes after IFN-α treatment. a** A heatmap illustrating metabolic changes with or without IFN-α treatment is presented. The impact of IFN-α on cellular amino acid (AA) metabolism, which is closely linked to tRNA charging (a process that enables the twenty amino acids to be charged on the mature tRNAome for translational decoding), was investigated. Cells treated with 1000 IU/mL IFN-α for 8 hours (n = 6 biological replicates) were collected for metabolomic analysis, with normal cells serving as a control. **b** A volcano plot displaying metabolic changes with or without IFN-α treatment is shown. **c-d** KEGG enrichment analysis of downregulated pathways (c) and upregulated pathways (d) upon IFN-α treatment. Source data are provided as a Source Data file.

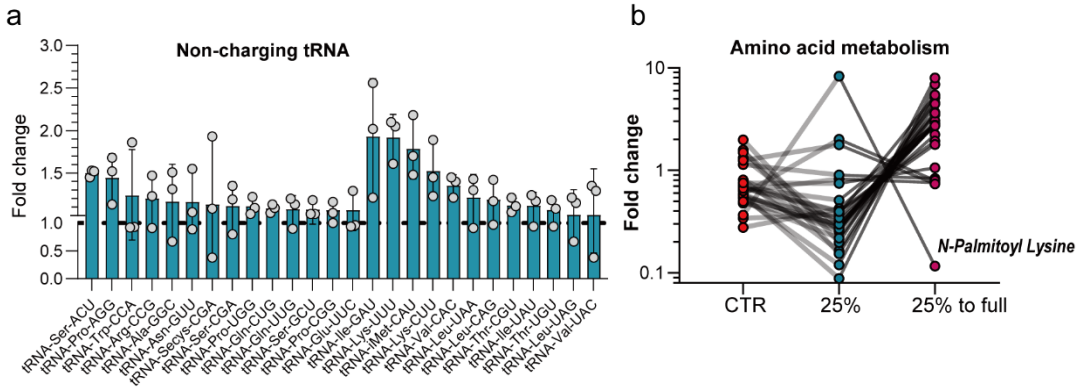
Supplementary Fig. 4 **Design of the tRNA transcription reporter.**
This system utilizes an upstream tRNA-His-GUG sequence containing the TATA box, a key element for binding type II RNA polymerase III (tRNA-specific RNA pol III). This sequence is followed by a tRNA-Met-CAU sequence and 44 nucleotides of random sequence. After transfection, cellular RNAs were isolated, reverse transcribed, and analyzed by quantitative polymerase chain reaction (qPCR) using the tRRep-F and tRRep-R primers as indicated.



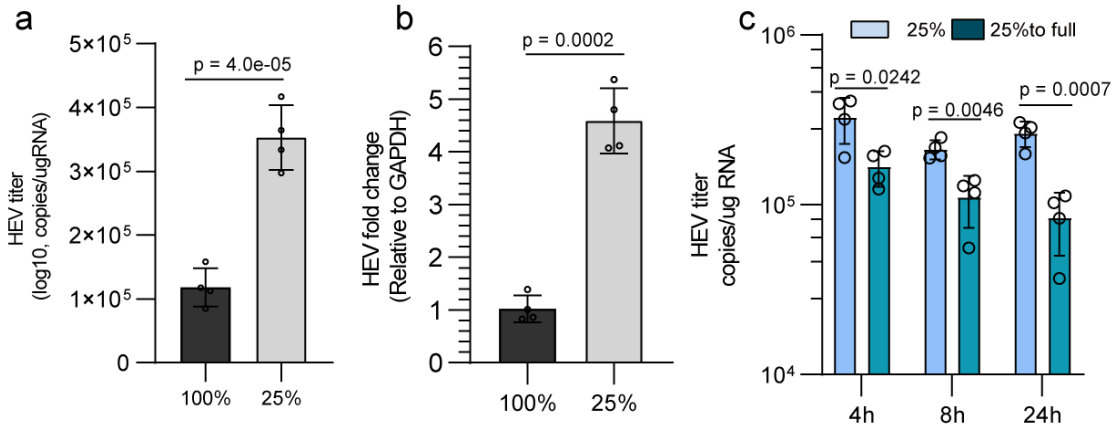
Supplementary Fig. 5 **Codon usage of human ISGs triggered by different interferon types is highly biased. a** The Pearson correlation coefficients (Rs) were calculated between the tRNAome abundance and the codon usage of 36 core ISG members. This analysis was performed by matching the tRNA anticodons with the codon usage data according to Watson-Crick base pairing rules. Each point represents the correlation value (R) for one ISG in relation to the tRNAome abundance at 4, 8, and 24 h. Statistical significance was determined using a two-tailed Wilcoxon signed-rank test (n= 36 gene-tRNAome pairs per group) to compare the median of each experimental group against zero. **b** Codon usage patterns of the 36 core ISG members identified through proteomic profiling are displayed. The relative synonymous codon usage (RSCU) of these 36 ISGs is presented in a heatmap. Gene sets enriched with either AU-ending or GC-ending codons are indicated in the top panel. **c-e** The same analysis was performed for human consensus-annotated ISGs triggered by human IFN-α (c), IFN-β (d), and IFN-γ (e). Raw ISG gene sets were obtained from the Human Hallmark Gene Set database (https://www.gsea-msigdb.org), the open reading frames of these genes were downloaded from NCBI and their RSCUs were analyzed. Source data are provided as a Source Data file.



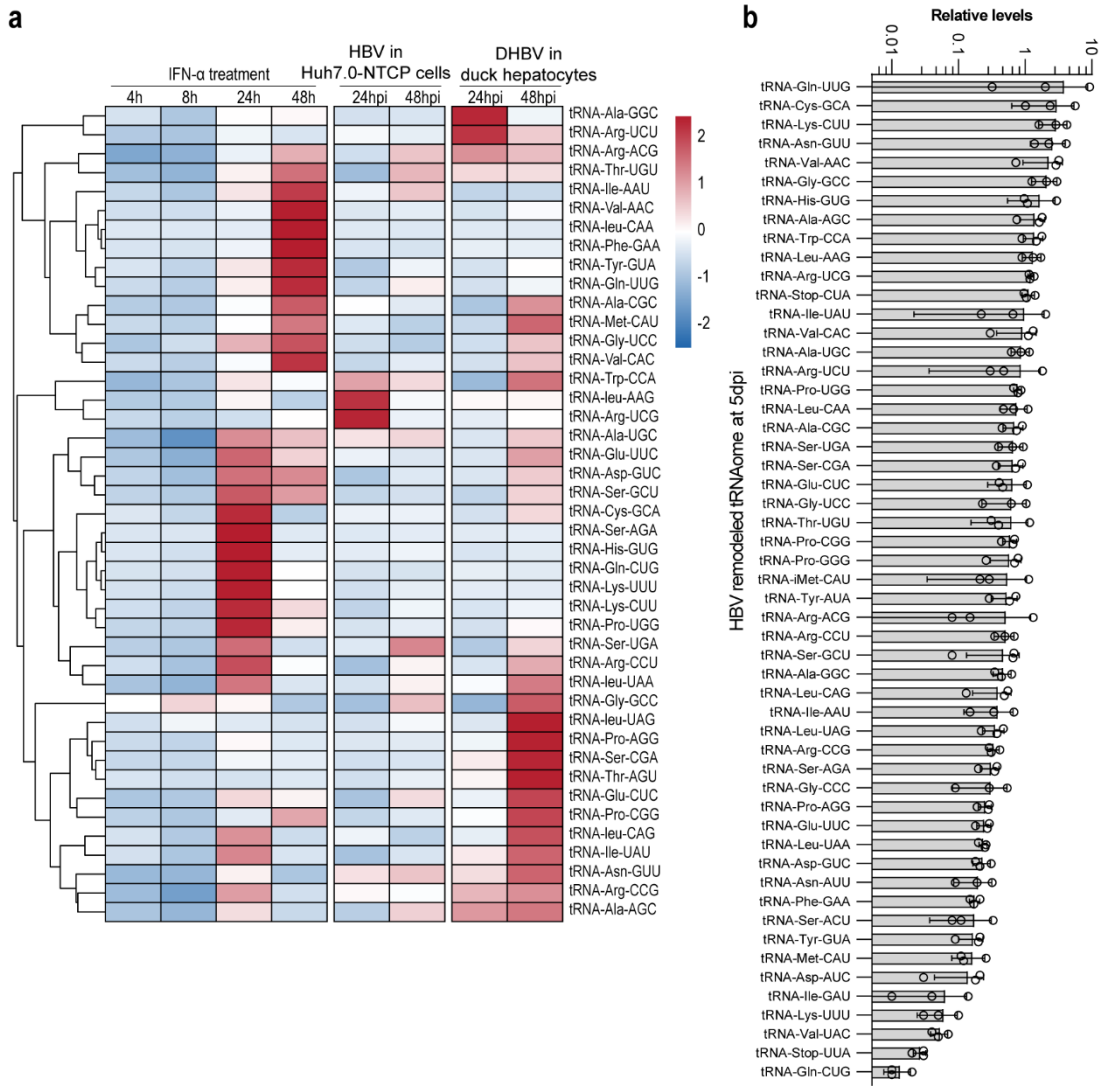
Supplementary Fig. 6 **The coordination between mature tRNAome remodeling and the translational decoding of ISG candidates exclusively identified by proteomic MS but not by RNA-seq. a** In addition to the highly transcribed ISG candidates, there are 85 ISG candidates that are not upregulated at the mRNA level **b**. However, these 85 ISG candidates were identified by proteomic MS, and reached statistical significance at the final time point 24h. The mean and standard deviation are displayed. **c** Dynamic TEs (protein/mRNA FC ratios) of these 85 ISG candidates. **d** The Pearson correlation coefficient (R) was calculated between the tRNAome abundance remodeled by IFN-α and the codon usage data of these 85 ISG candidates. Each point represents the correlation value (R) for one ISG in relation to the tRNAome abundance at 4, 8, and 24 h. A two-sided paired student's t-test was used to determine statistical significance (n = 85 pairs of R values). **e** Codon usage patterns of the 85 ISG candidates. Source data are provided as a Source Data file.



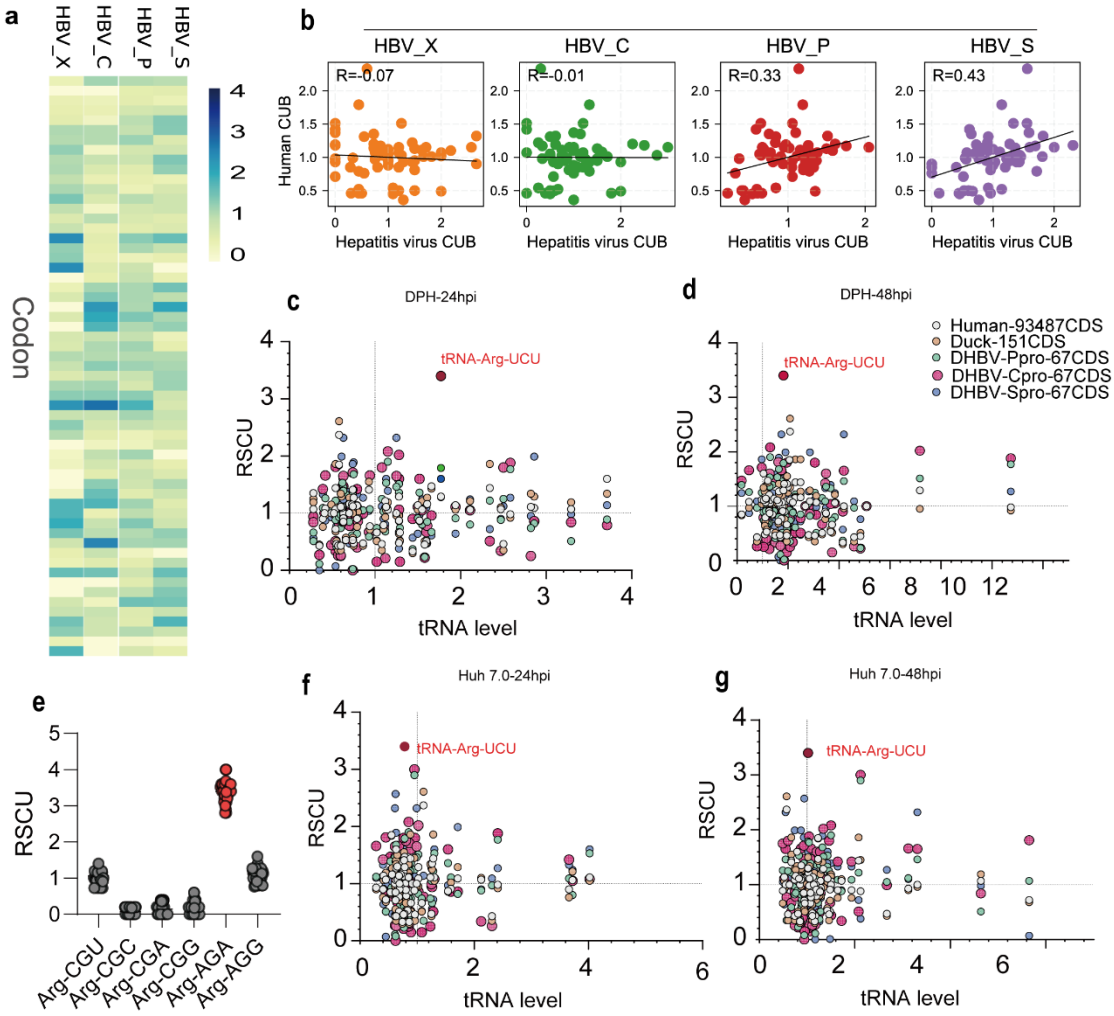
Supplementary Fig. 7 **AA limitation affects non-charging tRNA levels and intracellular AA metabolism. a** The cells were treated for 2 days with an optimized DMEM medium containing 25% of the AA concentration compared to normal DMEM medium. Through a deacylation reaction, we can remove the charged AAs; thus, not performing this reaction allows us to quantify only the levels of non-charging tRNAs (n = 3 biological replicates). **b** Comparison of AA-related metabolic changes in different culturing mediums triggered upon 8h of IFN-α: normal medium (group: CTR, i.e., 100%); AA poor medium (group: 25%); and AA poor-to-normal medium (group: 25% to full) (n = 6 biological replicates). Source data are provided as a Source Data file.



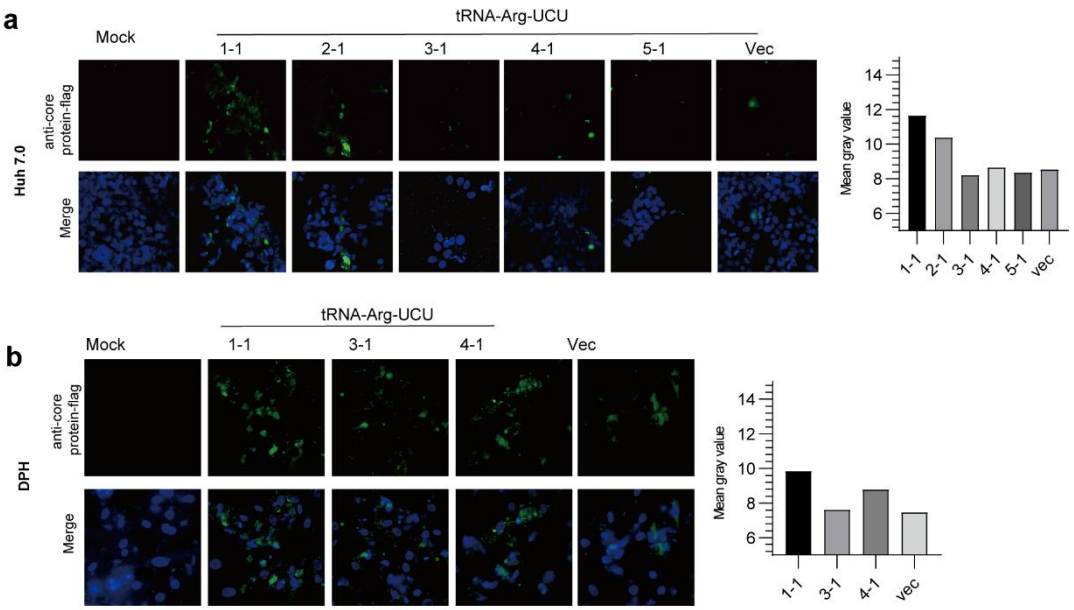
Supplementary Fig. 8 **The impact of AA limitation on HEV replication and the recovery of anti-HEV effects of IFN-α. a-b** The cells were treated for 2 days with an optimized DMEM medium containing 25% of the AA concentration compared to the normal DMEM medium. The cells were then infected with HEV. Viral titers in the AA-limited and unlimited cells were quantified by qRT-PCR and compared (n = 4 biological replicates). Absolute values are expressed in copies per μg RNA, while relative fold changes compared to the 100% group are displayed in panels a and b, respectively. **c** The AA concentration in the culture medium was restored from 25% to 100%. After 48 h of culture, cells were infected with HEV and treated with 1000 IU/mL of IFN-α for 4, 8, or 24 h. HEV RNA loads were quantified and compared with groups that were consistently cultured under AA-limited conditions with the same treatment duration. Statistical significance was determined using a two-tailed Student's t-test (n = 4 biological replicates, related to Fig. 3k). Source data are provided as a Source Data file.



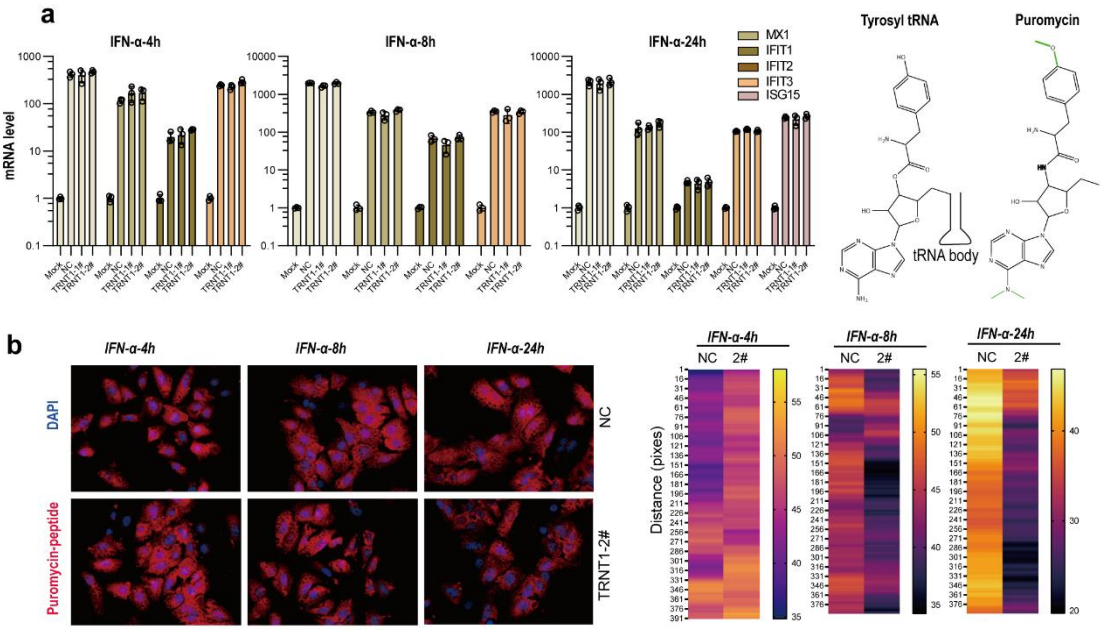
Supplementary Fig. 9 **Comparison of the mature tRNAome remodeled under different conditions. a** Heatmap comparison of the mature tRNAome remodeled by IFN-α and HBV or DHBV infections in human and duck hepatocytes at the indicated time points. **b** Plotting of the tRNAome remodeled in human HBV-infected NTCP-expressing hepatocytes at 5 dpi (n =3 biological replicates). Source data are provided as a Source Data file.



Supplementary Fig. 10 **The prominent role of tRNA-Arg-UCU in the translational decoding of DHBV. a** Heatmap depicting codon usage bias (CUBs) of the human HBV X, surface(S) protein, polymerase (P) and core (C) protein genes, utilizing data on relative synonymous codon usage (RSCU) for the four HBV genes. **b** Correlation of codon usage between the four human HBV protein genes and overall human CUB. For each codon, the RSCU values in the human genome (y-axis) are plotted against the corresponding RSCU values in the viral open reading frames (ORFs) X, C, P, and S (x-axis). The Pearson correlation coefficients (Rs) are indicated in the respective subplots. **c-d** Correlation of mature tRNA abundance in infected duck hepatocytes with the average RSCU values on average of 67 duck HBV-encoding genes, including P (green dots), C (red dots) and S (blue dots) genes, as well as duck and human protein genes derived from the Codon Usage Database: 93,487 human coding sequences (yellow dots) and 151 duck coding sequences (organelle dots). The average of mean RSCU values were used. Correlation analysis was performed at 24hpi (c) and 48hpi (d). **e** Codon usage patterns specifying arginine codons in core protein genes of 67 DHBV strains. **f-g** Correlation analysis of tRNAome remodeling in infected human hepatocytes at 24hpi (f) and 48hpi (g) with the RSCU of duck HBV-encoding genes, and duck and human protein genes as described in panels c and d. Source data are provided as a Source Data file.



Supplementary Fig. 11 **Immunofluorescent analysis of DHBV core protein transduction by different tRNA-Arg-TCT genes. a** The levels of flag-tagged core protein in human cells transducing different tRNA-Arg-TCT genes were analyzed by IFA assays after 48h of DHBV infection, displaying green fluorescence (an independent assay related to Fig. 5d). Uninfected cells and the cells transfected with scrambled vector were used as control groups. **b** The DPHs were pre-transfected with the plasmids expressing the three duck tRNAs and subsequently infected with DHBV; then, the levels of flag-tagged core protein were analyzed by IFA assays (an independent assay related to Fig. 5g).



Supplementary Fig. 12 **The impact of TRNT1 knockdown on ISG transcription and host protein translation. a** The cells were transfected with siRNA targeting TRNT1. The transcription levels of notable ISGs were then quantified after 4, 8, and 24 hours of IFN-α treatment (n = 3 biological replicates). **b** The cells were treated in the same manner as described in panel A. Prior to the indicated time points, the cells were pre-incubated with puromycin for 30 minutes to label the newly produced polypeptides (red fluorescence). The average intensity of the newly synthesized polypeptides along the cell's long axis was measured using Image J and plotted in heatmaps (right panel, n >= 22 cells). The chemical structures of puromycin and terminal tyrosyl tRNA are shown in the top right panel. Source data are provided as a Source Data file.

Supplementary Tables

Supplementary Table 1 **Summary of mRNA/protein ratios of ISGs.**

Groups		**RNA-seq**						**TMT-mass spectrum**					
		IFN-α(10^3IU/ml) (n=3)			**Control** (n=3)			**IFN-α(10^3IU/ml)** (n=3)			**Control** (n=2,3)		
#	**Time**	**4h**	**8h**	**24h**	**4h**	**8h**	**24h**	**4h**	**8h**	**24h**	**4h**	**8h**	**24h**
Gene No.		13999	13668	13644	13999	13668	13644	4937	4925	4928	4937	4925	4928
Abundance*	Mean	27.75	28.33	28.10	28.65	28.73	27.07	100.67	97.26	97.07	99.38	95.93	93.70
	Max	13078.2	14193.6	18586.8	12138.8	12521.8	14415.3	319.2	184.1	317.2	226.1	160.4	176.4
Fold change	Mean	1.48	2.06	1.66				1.03	1.03	1.07			
	Max	721.71	812.63	634.43	Normalized			4.53	3.65	9.13	Normalized		
	Min	0.048	0.025	0.046				0.642	0.386	0.489			
mRNA/protein ratio	Mean	**1.19**	**1.53**	**1.15**									
	Max	**196.10**	**565.00**	**184.30**	Normalized								
	Min	**0.13**	**0.19**	**0.11**									

*, represented by related RNA-seq reads or mass-spectrum abundance.

Supplementary Table 2 **siRNAs used for TRNT1 knockdown experiment**			
Gene symbol	**TRC ID**	**Sense (5'-3')**	**antisense (5'-3')**
Control siRNA		UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
siRNA-TRNT1-1#	TRCN0000035751	CCCUAUCAAGACUUCAUUAUACU	UAUAAUGAAGUCUUGAUAGGGUU
siRNA-TRNT1-2#	TRCN0000035752	CGCAGAGAUCUCACUAUAAAUCU	AUUUAUAGUGAGAUCUCUGCGUU
siRNA-SLFN11-1#	TRCN0000155578	CAGTCTTTGAGAGAGCTTATTCT	AATAAGCTCTCTCAAAGACTGTT
siRNA-SLFN11-2#	TRCN0000152057	CAGAGGACTAGAGGAGTTAATCT	ATTAACTCCTCTAGTCCTCTGTT
siRNA-SLFN11-3#	TRCN0000155564	CCGATAACCTTCACACTCAAACT	TTTGAGTGTGAAGGTTATCGGTT

Supplementary Table 3 **Inserted sequences of different tRNA vectors in PLko.1 plasmids.**

Gene	Forward (5'-3')
tRNA-Arg-TCT-1-1	accggtGGCTCTGTGGCGCAATGGATAGCGCATTGGACTTCTAATTCAAAGGTTGT GGGTTCGAGTCCCACCAGAGTCGttttttcggccggctgccgctggaggtgctcaaagagatgggagg gcctatttcccatgattccttcatatttgcatatacgatacaaggctgttagagagataattggaattaatttgactgtaaaca caaagatattagtacaaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagttttaaaattatgttttaaaatg gactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaaggacgaaacaccgGG CTCTGTGGCGCAATGGATAGCGCATTGGACTTCTAATTCAAAGGTTGTGGGTTC GAGTCCCACCAGAGTCGttttttacgcgtgatcctctagaactatagctagtcgaattc
tRNA-Arg-TCT-2-1	accggtGGCTCTGTGGCGCAATGGAtAGCGCATTGGACTTCTAATTCAAAGGtTGTG GGTTCGAATCCCACCAGAGTCGttttttcggccggctgccgctggaggtgctcaaagagatgggaggg cctatttcccatgattccttcatatttgcatatacgatacaaggctgttagagagataattggaattaatttgactgtaaacac aaagatattagtacaaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagttttaaaattatgttttaaaatgg actatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaaggacgaaacaccgGGC TCTGTGGCGCAATGGAtAGCGCATTGGACTTCTAATTCAAAGGtTGTGGGTTCGA ATCCCACCAGAGTCGttttttacgcgtgatcctctagaactatagctagtcgaattc
tRNA-Arg-TCT-3-1	accggtGGCTCTGTGGCGCAATGGAtAGCGCATTGGACTTCTAATTCAAAGGtTGTG GGTTCGAGTCCCACCAGAGTCGttttttcggccggctgccgctggaggtgctcaaagagatgggaggg cctatttcccatgattccttcatatttgcatatacgatacaaggctgttagagagataattggaattaatttgactgtaaacac aaagatattagtacaaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagttttaaaattatgttttaaaatgg actatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaaggacgaaacaccgGGC TCTGTGGCGCAATGGAtAGCGCATTGGACTTCTAATTCAAAGGtTGTGGGTTCGA GTCCCACCAGAGTCGttttttacgcgtgatcctctagaactatagctagtcgaattc
tRNA-Arg-TCT-4-1	accggtGTCTCTGTGGCGCAATGGAcgAGCGCGCTGGACTTCTAATCCAGAGGtTC CGGGTTCGAGTCCCGGCAGAGATGttttttcggccggctgccgctggaggtgctcaaagagatggga gggcctatttcccatgattccttcatatttgcatatacgatacaaggctgttagagagataattggaattaatttgactgtaa acacaaagatattagtacaaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagttttaaaattatgttttaaa atggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaaggacgaaacaccgG TCTCTGTGGCGCAATGGAcgAGCGCGCTGGACTTCTAATCCAGAGGtTCCGGGTT CGAGTCCCGGCAGAGATGttttttacgcgtgatcctctagaactatagctagtcgaattc
tRNA-Arg-TCT-5-1	accggtGGCTCTGTGGCGCAATGGAtAGCGCATTGGACTTCTAATTCAAAGGtTGCG GGTTCGAGTCCCTCCAGAGTCGttttttcggccggctgccgctggaggtgctcaaagagatgggaggg cctatttcccatgattccttcatatttgcatatacgatacaaggctgttagagagataattggaattaatttgactgtaaacac aaagatattagtacaaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagttttaaaattatgttttaaaatgg actatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaaggacgaaacaccgGGC TCTGTGGCGCAATGGAtAGCGCATTGGACTTCTAATTCAAAGGtTGCGGGTTCGA GTCCCTCCAGAGTCGttttttacgcgtgatcctctagaactatagctagtcgaattc
tRNAm-Ala-TCT	accgGTGGGGGTGTAGCTCAGTGGTAGAGCGCGTGCTTTCTATGTACGAGGTCC CGGGTTCAATCCCCGGCACCTCCAttttttcggccggctgccgctggaggtgctcaaagagatgggag ggcctatttcccatgattccttcatatttgcatatacgatacaaggctgttagagagataattggaattaatttgactgtaaac acaaagatattagtacaaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagttttaaaattatgttttaaaat ggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaaggacgaaacaccgGG GGGTGTAGCTCAGTGGTAGAGCGCGTGCTTTCTATGTACGAGGTCCCGGGTTC AATCCCCGGCACCTCCAttttttacgcgtgatcctctagaactatagctagtcgaattc
tRNA-Ser-UGA	accggtGCTGTGATGGCCGAGCGGTGAAGGCGTTGGACTTGAAATCCAATGGGGT TTCCCCACGCAGGTTCAAATCCTGCTCACAGCGttttttcggccggctgccgctggaggtgctca aagagatgggagggcctatttcccatgattccttcatatttgcatatacgatacaaggctgttagagagataattggaatt aatttgactgtaaacacaaagatattagtacaaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagttttaa aattatgttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaagga cgaaacaccgGCTGTGATGGCCGAGCGGTGAAGGCGTTGGACTTGAAATCCAATGG GGTTTCCCCACGCAGGTTCAAATCCTGCTCACAGCGttttttacgcgtgatcctctagaactata

	gctagtcgaattc

Supplementary Table 4 **Sequences of wild and mutant type of DHBV core protein expression vector (pcDNA 3.1).**

Gene	Forward (5'-3')
Cpro-WT-AGA	GCTAGCACC**ATG**GCTTGGAACTTAAGAATTACACCCCTCTCCTTCGGAGCTGCC TGCCAAGGTATCTTTACGTCTACATTGCTGTTGTCAGCCTTGACTGTACCTTTGG TATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGAGCC TTAGCCAATGTATATGATCTGCCAGATGATTTCTTTCCAAAAATTGATGATCTTGT AAGGGATGCTAAAGACGCTTTAGAACCTTACTGGAAATCTGATTCAATAAAGAAA CATGTTTTGATTGCAACTCACTTTGTGGATCTTATTGAAGACTTCTGGCAGACTA CTCAGGGTATGCATGAAATTGCTGAATCCTTAAGAGCAGTAATACCACCTACCAC TGCTCCTGTACCTACTGGATATCTCATTCAACACGAGGAGGCTGAAGAGATACC CTTAGGTGATTTATTTAAACATCAGGAAGAAAGAATAGTCAGTTTTCAACCAGAC TATCCTATTACAGCAAGAATTCATGCACACCTAAAAGCTTATGCAAAAATTAATGA GGAATCTTTGGATAGGGCTAGGAGATTGCTTTGGTGGCATTACAACTGTTTACTG TGGGGAGAAGCTAACGTTACTAATTACATTTCTCGGCTCCGTACTTGGTTGTCAA CTCCGGAAAAGTACCGAGGCCGTGATGCCCCAACCATTGAAGCAATCACTAGAC CAATCCAAGCGGCTCAGGGAGGCAGAAAAACATCTTCGGGAACTAGAAAACCTC GTGGACTCGAACCTAGAAGAAGAAAAGTTAAAACCACAGTTGTCTATGGGAGAA GACGTTCAAAGTCCAGGGAAAGGAGAGCCCCTTCACCCCAACGTGCGGGCTCC CCTCTCCCACGTAGTTCGAGCAGCCACCATAGATCTCCCTCGCCTAGGAAAGAC TACAAGGACCACGACGGTGACTACAAGGACCACGACATCGACTACAAGGACGA CGACGACAAGTGAGGTACC
Cpro-AGA>AGG	GCTAGCACC**ATG**GCTTGGAACTTAAGGATTACACCCCTCTCCTTCGGAGCTGCC TGCCAAGGTATCTTTACGTCTACATTGCTGTTGTCAGCCTTGACTGTACCTTTGG TATGTACCATTGTTTATGATTCTTGCTTATATATGGATATCAATGCTTCTAGGGCC TTAGCCAATGTATATGATCTGCCAGATGATTTCTTTCCAAAAATTGATGATCTTGT AAGGGATGCTAAAGACGCTTTAGAACCTTACTGGAAATCTGATTCAATAAAGAAA CATGTTTTGATTGCAACTCACTTTGTGGATCTTATTGAAGACTTCTGGCAGACTA CTCAGGGTATGCATGAAATTGCTGAATCCTTAAGGGCAGTAATACCACCTACCA CTGCTCCTGTACCTACTGGATATCTCATTCAACACGAGGAGGCTGAAGAGATAC CCTTAGGTGATTTATTTAAACATCAGGAAGAAAGGATAGTCAGTTTTCAACCAGA CTATCCTATTACAGCAAGGATTCATGCACACCTAAAAGCTTATGCAAAAATTAAT GAGGAATCTTTGGATAGGGCTAGGAGGTTGCTTTGGTGGCATTACAACTGTTTA CTGTGGGGAGAAGCTAACGTTACTAATTACATTTCTCGGCTCCGTACTTGGTTGT CAACTCCGGAAAAGTACCGAGGCCGTGATGCCCCAACCATTGAAGCAATCACTA GGCCAATCCAAGCGGCTCAGGGAGGCAGGAAAACATCTTCGGGAACTAGGAAA CCTCGTGGACTCGAACCTAGGAGGAGGAAAGTTAAAACCACAGTTGTCTATGGG AGGAGGCGTTCAAAGTCCAGGGAAAGGAGGGCCCCTTCACCCCAACGTGCGG GCTCCCCTCTCCCACGTAGTTCGAGCAGCCACCATAGGTCTCCCTCGCCTAGGA AAGACTACAAGGACCACGACGGTGACTACAAGGACCACGACATCGACTACAAG GACGACGACGACAAGTGATTCTAGA

Supplementary Table 5 **Primers were used for the quantification of 20 key human ISGs.**

#	Gene	Sequences 5' to 3'
1	DDX58-F	5'CACCTCAGTTGCTGATGAAGGC3'
	DDX58-R	5'GTCAGAAGGAAGCACTTGCTACC3'
2	ISG15-F	5'CTCTGAGCATCCTGGTGAGGAA3'
	ISG15-R	5'AAGGTCAGCCAGAACAGGTCGT3'
3	STAT2-F	5'CAGGTCACAGAGTTGCTACAGC3'
	STAT2-R	5'CGGTGAACTTGCTGCCAGTCTT3'
4	IFITM3-F	5'CTGGGCTTCATAGCATTCGCCT3'
	IFITM3-R	5'AGATGTTCAGGCACTTGGCGGT3'
5	STAT1-F	5'ATGGCAGTCTGGCGGCTGAATT3'
	STAT1-R	5'CCAAACCAGGCTGGCACAATTG3'
6	IFI27-F	5'CGTCCTCCATAGCAGCCAAGAT3'
	IFI27-R	5'ACCCAATGGAGCCCAGGATGAA3'
7	PKR-F	5'GAAGTGGACCTCTACGCTTTGG3'
	PKR-R	5'TGATGCCATCCCGTAGGTCTGT3'
8	IFIH1-F	5'GCTGAAGTAGGAGTCAAAGCCC3'
	IFIH1-R	5'CCACTGTGGTAGCGATAAGCAG3'
9	IFIT3-F	5'CCTGGAATGCTTACGGCAAGCT3'
	IFIT3-R	5'GAGCATCTGAGAGTCTGCCCAA3'
10	IRF1-F	5'GAGGAGGTGAAAGACCAGAGCA3'
	IRF1-R	5'TAGCATCTCGGCTGGACTTCGA3'
11	IFIT1-F	GCCTTGCTGAAGTGTGGAGGAA
	IFIT1-R	ATCCAGGCGATAGGCAGAGATC
12	IFIT2-F	5'GGAGCAGATTCTGAGGCTTTGC3'
	IFIT2-R	5'GGATGAGGCTTCCAGACTCCAA3'
13	CXCL10-F	5'GGTGAGAAGAGATGTCTGAATCC3'
	CXCL10-R	5'GTCCATCCTTGGAAGCACTGCA3'
14	MX1-F	GGCTGTTTACCAGACTCCGACA
	MX1-R	CACAAAGCCTGGCAGCTCTCTA
15	IRF2-F	5'TAGAGGTGACCACTGAGAGCGA3'
	IRF2-R	5'CTCTTCATCGCTGGGCACACTA3'
16	IRF7-F	5'CCACGCTATACCATCTACCTGG3'
	IRF7-R	5'GCTGCTATCCAGGGAAGACACA3'
17	IRF9-F	5'CCACCGAAGTTCCAGGTAACAC3'
	IRF9-R	5'AGTCTGCTCCAGCAAGTATCGG3'
18	IFN-α-F	5'GACTCCATCTTGGCTGTGA3'
	IFN-α-R	5'TGATTTCTGCTCTGACAACCT3'
19	IFNβ1-F	5'CTTGGATTCCTACAAAGAAGCAGC3'
	IFNβ1-R	5'TCCTCCTTCTGGAACTGCTGCA3'
20	TNFR1-F	5'CCGCTTCAGAAAACCACCTCAG3'
	TNFR1-R	5'ATGCCGGTACTGGTTCTTCCTG3'

Supplementary Table 6 **Primers used for duck ISGs and (D)HBV**

Gene	**Forward (5'-3')**	**Reverse (5'-3')**
IFN-α	CAGAACCACACCTACAGCCC	CCGGTCAGTTCTTGTCAGCG
IFN-λ	ACCAGGCTCTTCAATCGGAA	TTGGGTGAAGAAGGCCAGG
Mx	CTGCTGTCCTTCATGACTTCG G	CTGCTGTCCTTCATGACTTCGG
DHBV	GGATCGGGCTAGGAGATTGCT TTG	CCCTGAGCCACTTGGATTGGT
HBV	TATCGCTGGATGTGTCTGCG	GGTGCAATTTCCGTCCGAAG

Supplementary Table 7 **Primers used for human mature tRNAome quantification.**

tRNA isodecoder	Forward (5'-3')
tRNA-Ala-AGC	TGGTRGAGCGCGTGCTTAGC
tRNA-Arg-UCG	CCTAATGGATAAGGCGTCTGACTTCG
tRNA-Gly-UCC	AGTGGTDAGCATAGCTGCCTTCC
tRNA-His-GUG	ATAGTGGTTAGTACTCTGCGCTGTG
tRNA-Leu-AAG	CGAGCGGTCTAAGGCGCTGGATTAAG
tRNA-Ile-UAU	CGGTTAGCGGGCGGTACTTAT
tRNA-Ile- -AAU	AGTTGGTYAGAGCGTGGTGCTAAT
tRNA-Pro-CGG	GTCTAGGGGTATGATTCTCGCTTCGG
tRNA-Pro-UGG	GTCTAGGGGTATGATTCTCGCTTTGG
tRNA-Thr-CGU	TTKGTTAARGCGYCKGTCTCGT
tRNA-Glu-UUC	GTCTAGYGGYTAGGATTCSKSGYTTTC
tRNA-Ala-CGC	GGTAGAGCGCRYGCTTCGC
tRNA- Ala -UGC	GTGGTAGAGCGCATGCTTTGC
tRNA-Arg-ACG	GCAATGGATAACGCGTCTGACTACG
tRNA-Arg-CCG	CCTAATGGATAAGGCRTCWGMYTCCG
tRNA-Arg-CCU	CTAATGGATAAGGCAYTGGCCTCCT
tRNA-Asn-GUU	GGTTAGCGCRTTYGRCTGTT
tRNA-Asp-GUC	GGTGAGTATCCCCGCCTGTC
tRNA-Cys-GCA	CTCAGKGGTAGAGCATTTGACTGCA
tRNA-Gln-CUG	GTGTAATGGTDAGCACTCTGGACTCTG
tRNA-Gln-UUG	GGTGTAATGGTTAGCACTCTGGACTTTG
tRNA-Glu-CUC	TGGTTAGGATTCGGCGCTCTC
tRNA-Gly-CCC	TKYAGTGGTAKMATKCWMGMYTCCC
tRNA-Gly-GCC	TTCAGTGGTAGAATTCTYGCCTGCC
tRNA-Leu-CAA	GTGGTCTAAGGCGCCAGACTCAA
tRNA-Leu-CAG	GTCTAAGGCGCTGCGTTCAG
tRNA-Leu-UAA	GAGTGGTTAAGGCGTTGGACTTAAG
tRNA-Leu-UAG	AGYGGTCTAAGGCGCTGGATTTAG
tRNA-Lys-CUU'	CTCAGTCGGTAGAGCATGRGACTCTT
tRNA-Lys-UUU	CTCAGTYGGTAGAGCATCAGACTTTT
tRNA-Phe-GAA	CTCAGTTGGGAGAGCGTTAGACTGAA
tRNA-Pro-AGG	GGTCTAGGGGTATGATTCTCGCTTAGG
tRNA-Ser-AGA	TGGCCGAGTGGTTAAGGCGATGGACTAGA
tRNA-Ser-CGA	GAGTGGTTAAGGYGTTGGACTCGA
tRNA-Ser-GCU	GTGGTTAAGGCGATGGACTGCT
tRNA-Ser-UGA	CGAGTGGTTAAGGCGWTGGACTTGA
tRNA-Thr-UGU	AGKGGTTAGAGCRCTGGTCTTGT
tRNA-Trp-CCA	CGGTAGCGCGTCTGACTCCA
tRNA-Tyr-GUA	TCAGYTGGTAGAGCGGAGGACTGTA
tRNA-Val-AAC	TGTAGTGGTTATCACGTTCGCCTAAC
tRNA-Val-CAC	AGTGGTTATCACGTTCGCCTCAC
tRNA-Val-UAC	CCATAGTGTAGTGGTTATCACRTCTGCTTTAC
tRNA-Arg-UCU	AYGAGCGCRYTGGACTTCT
tRNA-Met-CAU	DGGCAGCGCGTCAGTYTCAT
tRNA-Thr-AGU	GCTGGTTAAAGCGCCTGTCTAGT

tRNA-Ala-GGC	CTTTGTGGGTTAAAGTACTTGTCTGGC
tRNA-Asn-AUU	CGGTCAGAGCGTTCGGCTATT
tRNA-Tyr-AUA	GGTAAAATGGCTGAGTAAGCATTAGACTATAA
tRNA-Stop-UUA	AGTTCAGTTGGTAGAGCATCAGACTTA
tRNA-Stop-CUA	ATGTGGAGGAAGGGAGCATGTACTCTA
tRNA-Ser-ACU	GTTGGTTAGAGCGTGCTGCTACT
tRNA-Secys-CGA	GTCTGGGGTGCAGGCTTCA
tRNA-Pro-GGG	TGGTTAGAAATGTGCGCTCTGGG
tRNA-iMet-CAU	GAAGCGTGCTGGGCCCAT
tRNA-Ile-GAU	GTTGGTAAGAGCGTGGTGCTGAT
tRNA-Cys-ACA	GGTAAATCAAAAGCAACTCTATAAGCTATGTAACAAAC
tRNA-Asp-AUC	ATAGCTCAGTGGTAGAGAGTGTACTTATCA
Common reverse primer: CTTCTCCCTTAGCCTACCGAAGT	

Supplementary Table 8 **Primers used for duck mature tRNAome quantification.**

tRNA isodecoder	Forward primer (5'-3')
tRNA-Ala-AGC	GGTAGAGCGCTCGCTTAGC
tRNA-Arg-UCG	CCTAATGGATAAGGCGTCTGACTTCG
tRNA-Gly-UCC	TGGTKAGCATAGCTGCCTTCC
tRNA-His-GUG	ATAGTGGTYAGTACTCTGCGTTGTG
tRNA-Leu-AAG	CGGTCTAAGGCGCTGGATTAAG
tRNA-Ile-UAU	GGTYAGCGCGCGGTACTTAT
tRNA-Ile-AAU	AGTTGGTTAGAGCGTGGTGCTAAT
tRNA-Pro-CGG	AGGGGTATGATTCTCGCTTCGG
tRNA-Pro-UGG	GTCTAGGGGTATGATTCTCGSTTTGG
tRNA-Val-GAC	GTGGTTATCACGTTCGCCTGAC
tRNA-Thr-CGU	GGTAAGGCGTCGGTCTCGT
tRNA-Glu-UUC	GYGGYTAGGATYCSKSGYTTTC
tRNA-Ala-CGC	GCTCAGTGGKAGAGCGC
tRNA-Ala-UGC	AGYGGKAGAGCRCHTGCTTTGC
tRNA-Arg-ACG	ATGGAYAACGCRYCTGACTACG
tRNA-Arg-CCG	TGGATAAGGCATCAGCCTCCG
tRNA-Arg-CCU	GGAYAAGGCAYTGGCCTCCT
tRNA-Asn-GUU	AGCGCRTTCGGCTGTT
tRNA-Asp-GUC	GGTRAGTATCCCCGCCTGTC
tRNA-Cys-GCA	CTCAGKGGYAGAGCATTTGACTGCA
tRNA-Gln-CUG	GTGTAATGGTKAGCACTCTGGACTCTG
tRNA-Gln-UUG	GGKYAGCACTCTGGACTTTG
tRNA-Glu-CUC	TGGKYAGGATTYGGCRCTCTC
tRNA-Gly-CCC	GTGTAGTGGTATCATGCAAGAYTCCC
tRNA-Gly-GCC	GTGGTAGAATTCTCGCCTGCC
tRNA-Leu-CAA	GGTCTAAGGCGCCAGACTCAA
tRNA-Leu-CAG	GTCYAAGGYGCTGCGTTCAG
tRNA-Leu-UAA	AGTGGTGAAGGCGTTGGACTTAA
tRNA-Leu-UAG	GTGGTCTAAGGCGCTGGATTTAG
tRNA-Lys-CUU	GTYGGTAGAGCRTKRGRCTCTT
tRNA-Lys-UUU	CAGTYGGKAGAGCATCAGACTTTT
tRNA-Phe-GAA	CAGYTGGGAGAGYGTTAGACTGAA
tRNA-Pro-AGG	GTCTAGGGGTATGATTCTCGCTTAGG
tRNA-Ser-AGA	CGAGTGGTKAAGGYGATGGACTAGA
tRNA-Ser-CGA	GGTGAAGGCGTTGGACTCGA
tRNA-Ser-GCU	TGGTKAAGGCGATGGACTGCT
tRNA-Ser-UGA	AGBGGTKAAGGCGTTGGACTTGA
tRNA-Thr-UGU	AGKGGTTAGAGCACTGGTCTTGT
tRNA-Trp-CCA	GGYAGCGCGTCTGACTCCA
tRNA-Tyr-GUA	GCTGGTAGAGCGGAGGACTGTA
tRNA-Val-AAC	TGTAGTGGTTATCACGTTYGCCTAAC
tRNA-Val-CAC	GYGGTTATCACRTTCGCCTCAC
tRNA-Val-UAC	TGTAGTGGTYATCACRTCTGCTTTAC
tRNA-Arg-UCU	AATGGATARSGCAYTGGMCTTCT
tRNA-Met-CAU	GCGYGYYRGKCYCAT
tRNA-Thr-AGU	BTGGTTARAGCRCYKGTCTAGT

Common reverse primer	CTTCTCCCTTAGCCTACCGAAGT