Ample evidence indicates that codon usage bias regulates gene expression. How viruses, such as the emerging mosquito-borne Chikungunya virus (CHIKV), express their genomes at high levels despite an enrichment in rare codons remains a puzzling question. Using ribosome footprinting, we analyze translational changes that occur upon CHIKV infection. We show that CHIKV infection induces codon-specific reprogramming of the host translation machinery to favor the translation of viral RNA genomes over host mRNAs with an otherwise optimal codon usage. This reprogramming was mostly apparent at the endoplasmic reticulum, where CHIKV RNAs show high ribosome occupancy. Mechanistically, it involves CHIKV-induced overexpression of KIAA1456, an enzyme that modifies the wobble U34 position in the anticodon of tRNAs, which is required for proper decoding of codons that are highly enriched in CHIKV RNAs. Our findings demonstrate an unprecedented interplay of viruses with the host tRNA epitranscriptome to adapt the host translation machinery to viral production.
Viruses completely depend on the host translation machinery to express their proteins. This is particularly apparent for positive-strand RNA (\(+\)RNA) viruses that, upon release of their genomes into the cytoplasm, have first to translate their replicases to establish an infection because they do not carry viral replicases within their virions. Paradoxically, however, genomes of diverse \(+\)RNA viruses are enriched in rare codons and consequently should be translated with poor efficiency. Redundancy of the genetic code permits amino acids to be encoded by more than one codon. The frequency of these synonymous codons is universally biased and organism-specific. As tRNA concentrations in a given organism are adapted to the codon usage, rare codons slow down the elongation rate and thus decrease translation efficiency because their cognate tRNAs are lowly abundant in comparison to those tRNAs used for optimal codons\(^1\)–\(^3\).

Given the dependence of \(+\)RNA viruses on the host translation machinery, one would predict a strong selection pressure for viral genes to adapt to the host codon usage, at least for viruses that highly express their genomes. Indeed, this seems to be the case for viruses that infect bacteria\(^4\), however, it is not a general trait for viruses that infect mammals\(^5\)–\(^8\). The genes of highly expressed human host mRNAs are enriched in G/C-ending codons whereas the genomes of diverse \(+\)RNA viruses such as coronaviruses or the emerging Dengue virus (DENV) and Chikungunya virus (CHIKV) are enriched in A/U ending codons\(^9\)–\(^10\). How these viruses efficiently translate their genomes in spite of their unfavorable codon usage remains a fundamental question in virology. To address this question, we use CHIKV as a model virus because its \(+\)RNA genome is translated and replicated at extremely high levels\(^11\).

In this work we show how CHIKV infection induces a codon-specific reprogramming of the host translation machinery to favor viral protein expression via alterations of the host tRNA epitranscriptome.

**Results**

CHIKV infection specifically alters the translational landscape at the endoplasmic reticulum. The CHIKV \(+\)RNA genome consists of a 5’ capped and 3’ poly(A)-tailed single-stranded RNA that contains two open reading frames (ORFs). The first ORF encodes four non-structural proteins required for RNA replication. The second ORF is expressed from a subgenomic (SG) RNA transcribed during infection and encodes five structural proteins present in the virion (Fig. 1a)\(^12\). To investigate how CHIKV efficiently translates its genomic and SG RNAs, we performed global translational profiling of both endoplasmic reticulum (ER) and cytosol fractions from non-infected and CHIKV-infected human HEK 293T cells. Cells were infected at a multiplicity of infection (MOI) of 4 and samples collected at twelve hours post-infection, a time of high viral production and no cytopathic effect (Supplementary Fig. 1a–c). We separately analyzed effects at the ER and the cytosol, two distinct biological environments for translation whose contribution to viral RNA translation is mainly unexplored. Proper subcellular fractionation was confirmed (Supplementary Fig. 1d) and corresponding libraries for RNA-seq and ribosome profiling (Ribo-seq) were generated. After initial quality assessment of both sequencing techniques and removal of low-quality samples (see Methods), our RNA-seq analysis showed that CHIKV infection caused a massive decrease of cellular mRNA levels in both translation compartments (Fig. 1b) while solely a small group of genes enriched for immune response functions were up-regulated (Supplementary Data 1). This drastic decrease of cellular mRNA levels was not surprising as CHIKV nsP2 mediates the degradation of Rpb1, a subunit of the RNA Polymerase II (RNAPII)\(^13\). A virus-induced host RNA decay might also contribute to the effect as Sindbis, a closely related virus, upregulates host mRNA decay\(^14\). Of note, some mRNAs exhibited an opposing behavior in the two compartments suggesting that CHIKV infection might induce specific mRNA shuttling. The mRNAs up-regulated in the cytosol and down-regulated at the ER were functionally enriched in gene ontology (GO) terms related to membranes, exocytosis, lysosomes, protein maturation and ER stress. By contrast, the mRNAs that were up-regulated at the ER and down-regulated in the cytosol were enriched in GO terms related to metabolism, mitochondrial organization and translational elongation (Supplementary Data 2). Expression levels of a selected set of mRNAs was validated by qPCR in an independent experiment (Supplementary Fig. 2).

A comparison of viral and host reads in the RNA-seq and Ribo-seq libraries indicated that CHIKV RNAs exhibited a far higher ribosome occupancy at the ER than at the cytosol (Fig. 2a, Supplementary Fig. 3). The percentages of viral ribosome protected fragments (RPFs) and RNA-seq reads in the cytosol were similar whereas the percentage of viral RPFs at the ER was much higher than expected when compared to that of viral RNA-seq reads. Here, one might argue that an important fraction of the RNA-seq reads of CHIKV RNAs might correspond to viral RNAs
destined for replication and encapsidation, thereby confounding our analyses. However, in such a case we would expect to see substantial differences between the two CHIKV ORFs, since the NSP ORF is translated from the genomic CHIKV RNA, that acts as an mRNA and as a template for replication and encapsidation, whereas the SP ORF is translated from the subgenomic CHIKV RNA that mainly acts as an mRNA. As both CHIKV ORFs showed very similar behavior in our analysis, any potential biases seem negligible for our conclusions although we cannot eliminate an underestimation of the ribosome occupancy values for the NSP ORF. Regarding the cellular host mRNAs, when inspecting ribosome occupancy before and after normalization
to the total observed RPFs, the occupancy profiles did not exhibit any marked differences across the CDS (Supplementary Fig. 3a, b), while no evidence for ribosome stalling could be found at either translation initiation or translation termination site (Supplementary Fig. 3c–f). These observations indicate that ribosomes are not stalled and that translation of host mRNAs still occurs, albeit at lower levels than in non-infected cells. Such a decrease in total RPF levels would correlate with the decrease of mRNA levels, reflecting the previously described shutoff occurring in alphaviruses15–18. However, we did note an increased occupancy in the 3′UTR of ER samples after infection (Supplementary Fig. 3b), which may indicate non-RPF contaminants in the library that could lead to inflated RPF counts. We therefore further assessed the quality of our libraries in terms of RNA integrity by calculating the transcript integrity number (TIN)19 for all transcripts across all RNA-seq samples (Supplementary Fig. 4a) and noted markedly lower TIN scores for infected ER samples. Since RNA decay rates can vary between functional groups20,21, it might be that ER-associated transcripts exhibit higher decay rates than cytosolic RNAs upon CHIKV infection. However, this would not explain an increased ribosome occupancy specific to the 3′UTR. Thus, to investigate the possibility of non-RPF contaminants that may be introduced by co-sedimentation or binding by viral RNA-binding proteins22, we calculated the normalized ribosomal footprints for long non-coding RNAs (lncRNAs) before and after infection (Supplementary Fig. 4b). In the case of random binding or co-sedimentation of contaminants, we would expect to see a significant increase of nRFs for lncRNAs in infected ER samples, which was not the case. Instead, we observed this behavior for lncRNAs in the cytosol, where we in turn did not observe a similar 3′UTR anomaly (Supplementary Fig. 3b). As additional validation, we visualized the RPF length distributions of host and viral RPFs across the CDS and both UTRs for all three reading frames (Supplementary Fig. 5–8). While host RPFs in the CDS showed a preference for frame 0 in both cytosol and ER, viral RPFs did not, indicating that these RPFs may indeed be contaminated by non-RPF fragments which could lead to an overestimation of viral RPFs in our Ribo-seq libraries. We therefore focused on the host RPFs and compared their length distributions of CDS and 3′UTR in each compartment. Should non-RPF fragments co-sediment with ribosomes, we would expect to see marked differences in these distributions. Remarkably, this was not the case, indicating that the fragments mapping to 3′UTRs may indeed mostly originate from ribosomes. We did not observe any dominant reading frame in either of the UTRs when comparing across all samples, suggesting that any translation in this region would occur both in- and out-of-frame if at all. Based on this combined evidence, we concluded that the 3′UTR-specific occupancy is likely not caused by contaminants, but may be the result of an impaired post-termination recycling of 80S ribosomes, as has been described previously23–25. In line with this, ABCE1, a key component of the ribosome recycling machinery, was down-regulated after CHIKV infection in both the ER and cytosol translation compartments (ER: RNA log2FC = −6.56, RPF log2FC = −3.21; cytosol: RNA log2FC = −1.81, RPF log2FC = −1.35).

We therefore continued our analyses by subsequently calculating the normalized ribosomal footprints (nRF = mean RPF counts / mean RNA-seq counts) of host mRNAs and viral genomic and SG RNAs (Fig. 2b). In the cytosol, CHIKV infection caused a mild nRF increase of host mRNAs, with viral RNAs exhibiting comparable nRF levels. In contrast, at the ER, CHIKV infection caused a bimodal distribution of nRF values of host mRNAs. A subset of mRNAs exhibited very high nRF values, in the range of those of viral RNAs, while another subset of mRNAs displayed low ones. Differential ribosome occupancy was validated in an independent experiment for a selected set of mRNAs (Supplementary Fig. 9).

We then analyzed differential translation in both compartments using an interaction term in limma-voom26,27 to estimate significant differences between RPF and RNA-seq read counts. This type of analysis assesses whether changes in ribosome occupancy can be solely explained by changes in the corresponding mRNA levels while simultaneously accounting for variance between samples and controlling the false discovery rate (FDR). Genes were plotted according to their log, fold changes in mRNA abundance and ribosome occupancy (as measured by RPF) in the cytosol and ER translation compartments. After CHIKV infection, few significant translational changes were observed in the cytosol (Fig. 2c), which may be attributable to a limited statistical power in our dataset. Thus, we focused our attention on the ER, where we observed dramatic changes (Fig. 2d). Enrichment analysis with Gene ontology (GO), Kyoto Encyclopedia of genes and genomes (KEGG) and REACTOME pathway databases showed that translationally activated mRNAs were enriched for genes related to cell cycle, RNA transport and DNA damage response, whereas translationally repressed mRNAs were enriched for genes related to mitochondria, oxidative phosphorylation, ribosomes and RNA translation (Supplementary Data 3). Collectively, these results indicate that CHIKV infection induces profound alterations of the translation landscape at the ER, the compartment at which CHIKV RNAs exhibit very high ribosome occupancy and therefore likely efficiently translates.

CHIKV infection reprograms codon optimality. To investigate the mechanism by which CHIKV achieves this high ribosome occupancy at the ER, we examined whether the CHIKV RNAs and the translationally-activated host mRNAs share any common features such as length of codon sequence (CDS) and...
activated host genes and the CHIKV RNAs we observed that
translationally-activated and translationally-repressed (Supple-
cementary Fig. 13), as well as of the CHIKV RNAs and
translated lines indicate CAI of CHIKV ORFs: continuous line - NSP, dashed line - SP. Upper and lower limits of the box correspond to the 1st and 3rd quartile
score distribution with whiskers extending to 1.5 times the range from top/ bottom of the box. The line represents the median. Translationally activated
mRNAs n = 4726, translationally repressed mRNAs n = 3875, not significantly changed mRNAs n = 3619.

untranslated regions (UTRs), GC content, RNA secondary
structure or codon usage bias. Overall, translationally activated
host mRNAs tended to be similar to the CHIKV RNAs, featuring
a longer CDS with a lower GC content when compared to host
mRNAs that were either translationally repressed or not signif-
ificantly altered (Supplementary Fig. 10). Likewise, these
mRNAs were generally also less structured (Supplementary
Fig. 11), although a comparison with CHIKV RNAs was not
possible since the utilized Parallel Analysis of RNA Structure
(PARS) scores were not available for the viral RNA. Codon usage
bias was measured via the codon adaptation index (CAI) which
measures the synonymous codon bias with respect to a set of
reference genes. It is used as a quantitative method to predict the
level of protein expression of a gene based on its codon sequence
under basal conditions. For instance, a viral ORF sequence with a
CAI value of 1 would exclusively contain the most commonly
used codons of the host. Remarkably, the CHIKV RNA and the
host translationally-activated mRNAs featured low CAI values,
whereas the translationally-repressed host mRNAs featured high
CAI values (Fig. 3), indicating a virus-induced reprogramming of
codon optimality. Codon usage comparisons revealed a clustering
of viral RNAs with translationally activated host mRNAs (Sup-
plementary Fig. 12). To further characterize this behavior, we
calculated the mean difference between the codon frequencies of
translationally-activated and translationally-repressed (Supple-
mentary Fig. 13), as well as of the CHIKV RNAs and
translationally-repressed mRNAs. In both the translationally
activated host genes and the CHIKV RNAs we observed that
GAA (Glu), AAA (Lys), CAA (Gln) and AGA (Arg) were among
the most enriched codons (Fig. 4). Interestingly, their decoding is
linked to two modifications at the wobble U34 position of the
cognate tRNAs, mcm5 (5-methoxy-carbonyl-methyl) and
mcm5s2 (5-methoxy-carbonyl-methyl-2-thio) (reviewed in 29).

Moreover, we also observed an enrichment of GGA (Gly) codons
whose decoding in humans is linked to the modifications mcm5
and mcm5 (5-methoxy-carbonyl-hydroxymethyl)29.

CHIKV, as all mosquito-borne viruses, replicates alternatively in
vertebrates and mosquitoes, two hosts separated by one billion
years in evolution that harbor different codon usage biases. Thus, the
suboptimal coding usage of CHIKV RNA in human cells could be
driven by a competing selection to adapt to the mosquito codon
usage. However, this does not seem to be the case, as the CAI of
CHIKV RNA relative to the codon usage of human (CAI = 0.7123)
and Aedes albopictus (CAI = 0.7113), a known CHIKV vector, are
quite similar when considering all annotated protein-coding genes.
Moreover, as shown in Fig. 4, the CHIKV codon usage does not show
a preference for the same rare codons when compared to A.
albopictus genes, however, this comparison does not account for any
possible virus-induced changes in gene expression or differences in the
codon optimality. Since the CAI of CHIKV relative to human
translationally activated genes slightly increased to 0.7288 (NSP =
0.7224; SP = 0.7416), global suboptimality of the CHIKV genome
might be determined by alternating hosts, whereas preference for
some specific codons would be determined by stress-related changes
in tRNA modification status.

KIAA1456 mediates virus-induced reprogramming of codon
optimality. In Saccharomyces cerevisiae, Trm9 converts the cm5-
modified wobble uridine addition into mcm5 in a subgroup of
tRNAs30. Further thiolation of mcm5 to mcm5s2 involves the
activity of Urm1, CTU1/2 and NFS131. While we did not detect
ALKBH8 mRNA in HEK293T cells, TRM9b/TRM9L/KIAA1456 mRNA was amongst the most
translationally activated mRNAs in CHIKV-infected cells at the
ER (Supplementary Data 1). From here on, we will refer to this
mRNA as KIAA1456. During infection, KIAA1456 mRNA levels
were downregulated akin to the majority of mRNAs. However,
this effect was buffered by a dramatic translational activation
(12-fold) that resulted in an absolute increase of RPFs and
corresponded to a 1.4-fold increase in KIAA1456 protein
expression levels (Supplementary Data 1, Fig. 5a and Supple-
mentary Fig. 14). Liquid chromatography with tandem mass
spectrometry (LC–MS/MS) analyses of tRNA isolated from non-
infected and CHIKV-infected cells, as well as from non-infected
cells overexpressing a myc-DDK-tagged-KIAA1456 protein
(Fig. 5b), showed that both CHIKV infection and KIAA1456
overexpression induced a significant increase in the levels of
mcm5 modification (CHIKV-infection: 1.3-fold change, p = 0.011, two-tailed t-test; KIAA1456: 1.4-fold change, p = 0.016, two-tailed t-test) (Fig. 5c). This suggests that
KIAA1456, like ALKBH8, catalyzes the required methylation to
generate mcm5. In agreement with the observed CHIKV-induced
translational repression of the four enzymes directing mcm5
thiolation (Supplementary Data 4), we did not observe an

Fig. 3 CHIKV RNA and translationally activated mRNAs feature low CAI
values. Distribution of codon adaptation index (CAI) among different
translational groups at the ER. Each dot represents one gene. Horizontal
lines indicate CAI of CHIKV ORFs: continuous line - NSP, dashed line - SP. Upper and lower limits of the box correspond to the 1st and 3rd quartile
lines indicate CAI of CHIKV ORFs: continuous line - NSP, dashed line - SP.
increase in mcm\textsuperscript{5} modification levels (Fig. 5c). KIAA1456 overexpression and CHIKV infection caused a similar increase in the levels of tRNA mcm\textsuperscript{5} modification in spite of that KIAA1456 protein expression levels achieved by overexpression were 12-fold higher than those achieved via CHIKV infection (Fig. 5b). This suggests that steps prior to the KIAA1456 catalyzed methylation might become limiting. One possible limiting step is the acetylation step directly prior to the methylation which is catalyzed by the acetyltransferase complex Elongator ELP1-6, a complex consisting of six subunits. In line with this, our ribosome profiling data show that the absolute RPF levels of all ELP subunits are downregulated after CHIKV infection (Supplementary Data 1 and 4). As predicted for a positive role of KIAA1456 in CHIKV infection, overexpression of KIAA1456 protein favored CHIKV RNA replication (fold change 1.2, p-value = 0.0045, two-tailed t-test) (Fig. 5d), and CHIKV protein expression (Fig. 5e). However, we did not detect any effect in virus titers (Supplementary Fig. 15a).
might be explained by the lower sensitivity of the plaque assay method in comparison with Western blot and qPCR methods. Alternatively, the amount of CHIKV RNA and protein produced in the infected cell might be in excess in relation to the production of infectious viral particles. Next, we analyzed the effect of the depletion of KIAA1456 in CHIKV infection. Consistently with a role of KIAA1456 in mcm5 modification and thereby viral RNA translation, siRNA-mediated silencing of KIAA1456 (Fig. 5f) decreased mcm5 levels (Fig. 5g), inhibited CHIKV RNA and protein levels (Fig. 5h, i) and at a low MOI hindered infectious viral particle production (Supplementary Fig. 15b).
To investigate whether other RNA viruses might also use a KIAA1456-mediated tRNA modification reprogramming as a mechanism to enhance viral RNA translation, we analyzed viral sequences from diverse viruses for GAA, AAA, CAA, AGA and GGA codon enrichment. We observed that multiple (+)RNA viruses, such as DENV, showed an enrichment in these codon subsets, whereas other viruses such as HCV did not (Supplementary Data 5). In agreement with these observations, infection by DENV but not with HCV induced a statistically significant increase ($p = 0.097$ vs. $p = 0.69$) in KIAA1456 protein expression (Fig. 6a). Moreover, this upregulation also coincided with an increase in mcm5 modification levels of tRNAs in DENV-infected (fold change 1.8, $p$-value $= 0.035$, two-tailed $t$-test) but not HCV-infected cells (Fig. 6b). Collectively, these results indicate a mechanistic link between viral RNA enrichment in GAA, AAA, CAA, AGA and GGA codons, virus-induced increase in KIAA1456 levels and elevation of the tRNA mcm5 modification at the wobble U34 position.

**Discussion**

Our study identifies an unanticipated adaptation of the viral codon usage to the host translation environment under infection conditions. This adaptation occurs through a reprogramming of the host tRNA epitranscriptome that involves the tRNA modifying enzyme KIAA1456. Interestingly, the KIAA1456 mRNA, one of the most translationally activated mRNAs in CHIKV-infected cells, is itself enriched in the GAA, AAA, CAA, AGA and GGA codons. This suggests a positive feedback loop that might be triggered by the massive CHIKV-induced decrease of mRNA levels and the subsequent imbalance between mRNA and tRNA levels. We hypothesize that this imbalance especially affects the ER translation compartment favoring translation of KIAA1456 mRNA and the observed codon usage changes. Two main observations support this idea. First, our RNA-seq data show higher decreases of mRNA levels at the ER translation compartment than at the cytosol one (Fig. 2c, d). Second, our Riboseq data show that KIAA1456 mRNA preferably translates at the ER (Fig. 2c, d and Supplementary Data 1). This would result in a higher protein concentration of KIAA1456 protein around the ER that ultimately would favor translation of viral and host mRNAs enriched in GAA, AAA, CAA, AGA and GGA.

CHIKV-infection caused an increase in the levels of tRNA mcm5 but not of mcm5s2 modifications. Indeed, a similar observation was obtained by overexpressing KIAA1456 under translational stress conditions in the highly tumorigenic cells SW62037. Our interpretation is that overexpression of KIAA1456 results in mcm5 modification of previously unmodified tRNAs. If the Urm1, CTU1/2 and NFS1 enzymes required for thiolation would be limiting, the percentage of mcm5s2-modified tRNAs would be conserved. Moreover, the fact that these enzymes are downregulated after CHIKV infection and that we do not observe an increase of mcm5s2 suggests that in humans the mcm5 modification is sufficient to favor translation of these A-ending codons and that mcm3 and mcm5s2 modifications act cooperatively. This has been previously proposed and goes in line with the fact that *Saccharomyces cerevisiae* cells lacking either mcm5 or s2 are viable, whereas their simultaneous loss is lethal. However, further studies dissecting the roles of the different U34 modifications on decoding in human cells will be required to understand their impact on the human translatome and to complete the picture of the tRNA epitranscriptome function in infections and disease.

Coordinated interplays between the tRNA epitranscriptome and biased codon usage have been previously described in response to stress conditions to favor expression of codon-biased transcripts expressing stress proteins. For example, in yeast, alkylation stress increases the dependence on Trm9 for the expression of GAA- and AGA-enriched DNA damage and cell cycle control genes which are essential for surviving alkylation stress. These two codons are among the most enriched in CHIKV RNAs as well as in CHIKV-induced translationally activated host mRNAs whose GO terms include “cell cycle” and “DNA damage response” (Supplementary Data 3). Together, this is in agreement with KIAA1456 being a TRM9 functional homolog and would suggest that viruses might have hijacked components of these highly conserved cell-defense mechanisms to their benefit. A stress response triggered by viral infections would result in a host reprogramming of codon optimality that ultimately would favor translation of viral RNA genomes adapted to this stress environment. Indeed, the use of TRM9-related enzymes by viruses seems to be evolutionary conserved beyond human viruses. In *Escherichia coli* the tRNA Lys U34 modification is essential for the lambda phage to express two crucial proteins...
for viral replication, gpG and gpGT. However, in this case the tRNA modification influences translation not by globally reprogramming the codon usage but by affecting a specific ribosomal frameshift required to express the proper gpG:gpGT ratio. Thus, it seems likely that other, still unknown tRNA modifying enzymes contribute to the observed virus-induced translational reprogramming.

Further advances in our understanding of the human tRNA modifying enzymes and of the effect of the corresponding modifications in decoding will be essential to identify them.

In summary, we propose that codon-specific reprogramming of translation via tRNA modification represents a general mechanism used by some viruses to enhance viral protein expression. Besides shedding light on a novel layer of virus–host cell interactions, our results provide a rationale to consider the regulation of the tRNA epitranscriptome as a promising therapeutic target.

As neurodegenerative diseases and cancer show alterations in tRNA modification enzymes, the therapeutic interests reach well beyond virology.

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0.1 mg/ml CHX) and centrifuged for 3 h at 209,678 × g at 4 °C in a Beckman SW41 rotor. Monosomes peaks were collected and subjected to hot phenol RNA extraction in the presence of 1% SDS. Preparation of Ribo-seq and RNA-seq libraries. Ribosomes profiling (Ribo-seq) was carried out as previously described. In brief, RNA extracted from monosomes was size-selected on a 1% polyacrylamide gel, dephosphorylated, ligated to a universal miRNA cloning linker (Biolabs) and subjected to RNA fragmentation using the Ribo-Zero Gold RNA Removal kit (Epicenter). The purified adapter ligated RNA was reverse transcribed using SuperScript III (Thermo- Scientific) with a second-strand A-tailed cDNA generated in the presence of 1% SDS. 20 μg of DNase-treated total RNA were incubated with RNase-free DNase for 2 min at 37 °C. 3 μl of RNase-free water were added and the sample was incubated for 5 min at 30 °C to stop the enzyme activity. The samples were kept at −80 °C until analysis.

Quantitation of tRNA modifications by LC-MS/MS. HEK 293T and Hub cells were seeded in 100 mm dishes at a density of 5 × 10⁵ and 2 × 10⁵ cells per plate, respectively. 24 h after seeding, cells were transfected using lipofectamine with the corresponding plasmid or infected with CHIKV, DENV or HCV as described in the manufacturer’s instructions. 1 μg of the obtained tRNA was digested using the Nuclease Digestion Mix (New England Bio Labs) according to the manufacturer’s instructions and further desalted using HyperSep SpinTip Microscale (Thermo Fisher Scientific). Samples were run in the LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) coupled to an EASY-nLC 1000 (Thermo Fisher Scientific Progress). Online reversed phase liquid chromatography (RPLC) conditions were a 30 min run at a flow rate of 300 nl/min with a gradient of 5–90% ACN/5% 20 mM Ammonium Acetate pH 4.5 at 150 °C. Chromatographic gradients started at 95% buffer A and 5% buffer B with a flow rate of 300 nl/min and gradually increased to 20% buffer B and 80% buffer A in 40 min. After each analysis, the column was washed for 10 min with 20% buffer A and 80% buffer B. Buffer A: 20 mM Ammonium Acetate pH 4.5. Buffer B: 95% ACN/5% 20 mM Ammonium Acetate pH 4.5. The mass spectrometer was operated in positive ionization mode with a normalized collision energy of 35 and source temperature at 200 °C. Full MS scans were set to 1 microscan at a resolution of 60,000 and a mass range of m/z 100-700 in the Orbitrap mass analyzer. A list of masses was defined for further fragmentation (Supplementary Table 1). Fragment ion spectra were produced via collision-induced dissociation (CID) at normalized collision energy of 35% and they were acquired in the MS2 and MS3 mass analyzer. iTRAQ and the MS/MS spectra were extracted and searched using the Proteome Discoverer (version 2.2, Thermo Fisher Scientific). The search parameters were adjusted according to the following criteria: 1. Precursor ions were specified as monoisotopic, 2. Tolerance for precursor ions was set to 20 ppm, 3. Tolerance for fragment ions was set to 0.6 Da, 4. Mass accuracy was set to 2 Da, 5. SEQUEST algorithm was used for searching the database.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Source data are provided as Source Data file. The Ribo-seq and RNA-seq data from this study have been submitted to the NCBI Gene Expression Omnibus (GEO) under accession number GSE143390. The raw proteomics data have been deposited to the MetaboLights study repository with the dataset identifier MTBLS2443. Data resources...
used in this study was hg38 genome FASTA ([https://hgdownload.soe.ucsc.edu/ goldenPath/hg38/gold/hg38.fasta](https://hgdownload.soe.ucsc.edu/goldenPath/hg38/gold/hg38.fasta)). CHIKV genome FASTA & annotation ([https://www.ncbi.nlm.nih.gov/nuccore/KT449801.1](https://www.ncbi.nlm.nih.gov/nuccore/KT449801.1)), codon usage tables for different species ([https://dnaheve.fda.gov/dna?cmd=codon_usage&genid=537&mode=coccpnts]) and Appris principal isoforms ([https://appris.bioinfo.cnio.es/#/downloads]). Source data are provided with this paper.

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Author contributions

J.J., R.B. and J.D. conceived the project. A.M., established the CHIKV system. J.J., M.T. and G.P.V. designed and executed the experiments. R.B. carried out the NGS data processing and analyses. R.B. and E.M.N. carried out the codon data analysis. J.J., R.B. and J.D. evaluated the results. J.J., R.B. and J.D. wrote the manuscript. All authors revised and commented on the manuscript.

Competing interests

The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to René Böttcher or Juana Diez.

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