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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	All the data analysed in this study was produced in this study. The sequencing reads of each sample (two inputs and six outputs) were processed and filtered independently. Each sequencing read covered the entire tRNA. The 5' and 3' constant regions of the read (primers annealing sites) were removed with the 'cutadapt' software. The forward and reverse reads were merged using 'PEAR' and sequences that were either not assembled due to low quality or unexpected length were discarded. Unique genotypes were called and quantified with custom python scripts. Genotypes with less than nine input reads in any input replicate, unexpected nucleotide substitutions (sequencing or PCR errors) or 0 reads in the outputs were discarded.
Data analysis	All data analysis were performed in R (version 3.3.3). We used the software 'MAGELLAN' to generate theoretical fitness landscapes and calculate the gamma statistic to compare the tRNA landscape to theoretical models.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The complete dataset is available as Supplementary Table 1. Custom code used in this study is available upon request. Raw sequencing data has been submitted to GEO (accession number GSE99418).

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined by the number substitutions to co-occur in the evolution of the Arginine tRNA which results in a total library size of of 5,184 (=2^6 x 3^4) possible mutation combinations.
Data exclusions	Sequencing data was filtered with the following criteria before being analyzed: Sequences that were either not assembled, due to low quality or unexpected length, were discarded. Variants with less than 10 input reads, unexpected nucleotide substitutions (sequencing or PCR errors) or 0 reads in the output were discarded. After filtering, we ended up with a total of 4,176 sequence variants quantified in all input and outputs.
Replication	The study included in total 6 replicates. 2 independent transformations (inputs) with each split into 3 independent selection experiments (outputs). All attempts of replications were successful.
Randomization	Samples were grouped by replicates and no other grouping or randomization of samples were done.
Blinding	There was no blinded data in this study. The only group of data during the analysis were the replicates.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Research animals
☒	☐ Human research participants

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging