nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all statistical an	alyses, confirm that the following items are present in the 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			
	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly			
\boxtimes	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.			
\boxtimes	A description of all covariates tested			
\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons			
	A full description of the statistical parameters 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)			
\boxtimes	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>			
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings			
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes			
\boxtimes	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated			
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.		
So	ftware an	d code		
Poli	cy information a	about <u>availability of computer code</u>		
Da	ata collection	Agilent Biotek Gen 5 software (plate reader data), Metamorph (microscopy), Fiji (Microscopy)		
Da	ata analysis	Kaleidagraph (Synergy), elementary Python codes for sequence analysis and Galaxy software for NGS analysis.		
For m	nanuscripts utilizing	custom algorithms or software that are central to the research but not vet described in published literature, software must be made available to editors and		

Data

Policy information about availability of data

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

reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Source Data are provided with this paper. The data set used in the main Figures are presented as a Source data excel document. All Source Data necessary to reproduce analysis and plots of this paper are also available on the GitHub repository: https://github.com/Noireauxlab-TXTL/PHEIGES. Supplementary Source Data containing Source Microscopy Data are provided on the University of Minnesota Digital Conservancy (https://conservancy.umn.edu/) with the permanent URL: https://hdl.handle.net/11299/260237, and under public available license. The engineered T7 genome sequences generated in this study are available in the

GenBank database under consecutive accession codes PP384393 to PP384410 (correspondence table provided in Supplementary Data 6). The mutated tip of tail fiber sequences generated in this study are available in the GenBank database under consecutive accession codes PP379475 to PP379532 (correspondence table provided in Supplementary Data 6). The raw genome data generated in this study are available in the SRA database under accession code PRJNA1077253 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1077253). The raw NGS tail fiber data used in this study are available in the SRA database under accession code PRJNA1077490 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1077490).

Research involving huma	n participants,	their data,	or biological	material
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Policy information a and sexual orientation		vith <u>human participants or human data</u> . See also policy information about <u>sex, gender (identity/presentation),</u> thnicity and racism.	
Reporting on sex a	ex and gender Our work does not contain any human participants.		
Reporting on race other socially release groupings	•	Our work does not contain any human participants.	
Population charac	teristics	Our work does not contain any human participants.	
Recruitment		Our work does not contain any human participants.	
Ethics oversight		Our work does not contain any human participants.	
Note that full informat	ion on the appr	oval of the study protocol must also be provided in the manuscript.	
Field-spe	cific re	porting	
Please select the on	e below that is	s the best fit for your research. If you are not sure, read the appropriate sections before making your selection.	
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For a reference copy of th	ne document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>	
<u>Lite scien</u>	ces stu	udy design	
All studies must disc	close on these	points even when the disclosure is negative.	
	Around 50 tail fiber gene sequences were sequenced to assess the mutation landscape. For NGS analysis, we used a large sample size (600 000 reads) to evaluate the mutation rate. For mutation landscape, a small subset (50 tail fibers) were sequenced to verify the mutations.		
Data exclusions	No data points were excluded.		
Replication	At least triplicates for cell-free gene expression reactions. All attempts at replication were successful.		
	Our research aimed at exploring new engineering ideas and proving concepts. Randomization was not prioritized as this small-scale study focused on generating preliminary data statistically valid.		
0	We prioritized concept validations. Blinding would be used at later stages for broader validation, especially considering the added complexity and resource implications.		
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Reporting	g tor sp	pecific materials, systems and methods	
		about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.	
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Seed stocks	NA
Novel plant genotypes	NA NA
Authentication	NA