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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. <u>For final submission</u>: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1.	Sample size		
	Describe how sample size was determined.	Sample size was not pre-determined. Critical data was repeated three times independently to acquire sufficient statistical power for calculation of standard error of the mean.	
2.	Data exclusions		
	Describe any data exclusions.	No data was excluded.	
3.	Replication		
	Describe the measures taken to verify the reproducibility of the experimental findings.	All main findings have been repeated at least three times and can be reproducible. Wild type sequencing data has been repeated at least twice and results are consistent.	
4.	Randomization		
	Describe how samples/organisms/participants were allocated into experimental groups.	No randomization is used as the strain of E.coli is identical to begin with.	
5.	Blinding		
	Describe whether the investigators were blinded to group allocation during data collection and/or analysis.	The investigators were not blinded to the group allocation during data collection and analysis. All the data was processed by the same analysis code.	
	Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.		
6.	Statistical parameters		

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
	\boxtimes	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	A statement indicating how many times each experiment was replicated
\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 any assumptions or corrections, such as an adjustment for multiple comparisons
\boxtimes		Test values indicating whether an effect is present Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.
	\boxtimes	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
	\boxtimes	Clearly defined error bars in <u>all</u> relevant figure captions (with explicit mention of central tendency and variation)
		See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

R (3.4.2), Python (3.5.3 |Anaconda 4.4.0 (x86_64)|), dplyr (0.7.4), tidyr (0.7.2), ggplot2 (2.2.1), pysam (0.10.0), plastid (0.4.7), Galaxy tools: Barcode splitter (1.2), Cutadapt (1.6 with Python 2.7.10), BWA (0.7.15.1), 2100 Expert (Prokaryote Total RNA Nano v 2.5), Tecan i-control (1.12.4.0), Gen5 (2.00.18), Gradient Profiler (2.07), MATLAB (R2016b), peakfit (2.0)

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used have been authenticated OR state that no eukaryotic cell lines were used.

Confirm that all cell lines tested negative for mycoplasma contamination OR describe the

results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination OR state that no eukaryotic cell lines were used.

Provide a rationale for the use of commonly misidentified cell lines OR state that no commonly

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used in the study.

No unique materials were used

No eukaryotic cells were used.

- 10. Eukaryotic cell lines
 - a. State the source of each eukaryotic cell line used.
 - b. Describe the method of cell line authentication used.
 - c. Report whether the cell lines were tested for mycoplasma contamination.
 - d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

> Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

No animals were used in the study.

misidentified cell lines were used.

Policy information about studies involving human research participants

12. Description of human research participants Describe the covariate-relevant population

Describe the covariate-relevant population characteristics of the human research participants.

No human participants were used in the study.