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Last updated by author(s):	Apr 5, 2021	

Reporting Summary

Nature Research 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 Research policies, seeAuthors & Referees and theEditorial Policy Checklist.

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For all statistical analyses, 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
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 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)
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 Give <i>P</i> 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 <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.
Software and code
Policy information about availability of computer code

Data collection

Flow cytometry data were collected using BD FACSDiva v.8.0.1 (BD Biosciences) software. Fluorescence in neurons was measured on a microscope controlled by Micro-Manager 1.3 (Vale Lab, UCSF) and Matlab R2018b (MathWorks), images were acquired by HCImage (Hamamatsu). Transmembrane current and voltage were measured with CLAMP interface software v.1.4.0 (Intan Technologies).

Data analysis

The microscopy data were analyzed using ImageJ v.1.50b (NIH). The Flow cytometry data were analyzed using FCS Express V3 (De Novo Software). Images of fluorescent neurons and traces of current and voltage were processed in Matlab R2018b (MathWorks). Bioluminescence in animals was analyzed using Living Image v.3.0 software (Perkin Elmer/Caliper Life Sciences).

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. 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 <u>data availability statement</u>. 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 main data supporting the findings of this study are available within the article and its Supplementary information. The additional data are available from the corresponding author on reasonable request. The major plasmids constructed during this research, their maps and sequences are deposited to Addgene depository. The iLight nucleotide sequence is available at GenBank.

Field-spe	ecific re	porting		
Please select the or	ne below that i	s the best fit for your research. If you are not sure, read the appropriate sections before making your selection.		
x Life sciences	В	Behavioural & social sciences		
For a reference copy of t	the document with	all sections, see nature.com/documents/nr-reporting-summary-flat.pdf		
Life scier	nces stu	udy design		
All studies must dis	sclose on these	points even when the disclosure is negative.		
Sample size	'	ize calculations were performed. Unless noted in the figure legend, standard n>3 independent experiments were performed for es to allow statistical analysis.		
Data exclusions	No data were e	excluded from analysis.		
Replication	All attempts at	pts at replication were successful. Usually three independent replications of the experiments were performed.		
Randomization	The experimen	experiments were not randomized because of the nature of optogenetic experiments in the article.		
Blinding	The investigato	ors were not blinded because because it was not possible for optogenetic experiments in the article.		
Reportin	g for sp	pecific materials, systems and methods		
		about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material,		
system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.				
Materials & experimental systems Methods				
n/a Involved in th	•	n/a Involved in the study ChIP-seq		
Eukaryotic		Flow cytometry		
Palaeontol		MRI-based neuroimaging		
Animals an	d other organisn	ns		
Human res	earch participan	ts		
Clinical dat	a			
Eukaryotic c	all lines			
Policy information				
Cell line source(s		HeLa (CCL-2) cell line were obtained from ATCC. AAV-293 cells were obtained from Agilent Technologies.		
Authentication		No additional authentication was performed for cells purchased in ATCC. ATCC authenticates cell lines using STR analysis,		
		according to the product specification.		
Mycoplasma con	tamination	Cell lines were not tested for mycoplasma.		
Commonly miside (See <u>ICLAC</u> register)		No commonly misidentified cell lines were used.		
Animals and other organisms				
Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research				
Laboratory anima		ne Swiss Webster 2- to 3-month-old female mice (National Cancer Institute, NIH) with body weights of 22–25 g and newborn wiss-Webster pups of either sex were used.		

Laboratory animals

The Swiss Webster 2- to 3-month-old female mice (National Cancer Institute, NIH) with body weights of 22–25 g and newborn Swiss-Webster pups of either sex were used.

Wild animals

No wild animals were used in the study.

No field-collected samples

No field-collected samples were used in the study.

Ethics oversight

All animal experiments were performed in an AAALAC-approved facility using protocols approved by the Albert Einstein College of Medicine Animal Usage Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- | All plots are contour plots with outliers or pseudocolor plots.
- 🗶 A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Prior to analysis or sorting, bacterial TOP10 cell were washed with cold phosphate-buffered saline (PBS) and diluted in PBS to Sample preparation OD600 of 0.005.

BD LSRII flow cytometer and BD FACSAria cell sorter. Instrument

BD FACSDiva v.8.0.1 (BD Biosciences) and FCS Express v.3 (De Novo Software). Software

Collected during molecular evolution fractions of bacterial cells were less than 1%. The fraction purity was further analyzed by Cell population abundance

plating bacterial cells on Petri dishes followed by fluorescence imaging. Gating strategy Initial gates - FSC-A/SSC-A to discriminate cells from debris; then cells were gated in FSC-W/FSC-A to discriminate single cells;

then cells were gated in SSC-W/SSC-A to discriminate live cells. Resulted population were analyzed on SSC-A/GFP and SSC-A/ mCherry plots to find cells expressing msfGFP and mCherry or msfGFP only. Untransformed bacterial TOP10 cells were used for

selecting negative population.

x Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.