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, see Authors & Referees and the Editorial Policy Checklist.

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).

- 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
- 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
The data collected for this study was done using our custom-made algorithms available at http://bioimaging.usc.edu

Data analysis
The data analysis for this study was done using our custom-made algorithms available at http://bioimaging.usc.edu

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 software (and an example of dataset analysed during this study), available at http://bioimaging.usc.edu extracts brightness (...
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 ☐ Ecological, evolutionary & environmental 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.

<table>
<thead>
<tr>
<th>Sample size</th>
<th>This paper reports a protocol on how to perform the method described in (Ojosnegros et al. PNAS 2017). The sample size was determined by technical reasons, such as cell availability (non-migrating cells, cells on focus etc.) and image acquisition times. We have analyzed more than 350 cells in total, which provide enough statistical power to detect differences between ligand stimulated and non-stimulated cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data exclusions</td>
<td>Cells that were either: too bright (saturated signal), too dim, migrating out of focus, moving inside the focal plane, pre-stimulated before the induction, where excluded from the analysis.</td>
</tr>
<tr>
<td>Replication</td>
<td>The conclusions presented in this paper derive from the individual analysis of more than 350 cells. The consistency of the results was previously reported (Ojosnegros et al. PNAS 2017) (Hortigüela V. et al. Nano Lett, 2018).</td>
</tr>
<tr>
<td>Randomization</td>
<td>Samples were randomly assigned to different experiments</td>
</tr>
<tr>
<td>Blinding</td>
<td>The analysis was not blinded. The complete analysis is done by an algorithm.</td>
</tr>
</tbody>
</table>

Reporting for specific materials, systems and methods

Materials & experimental systems

<table>
<thead>
<tr>
<th>n/a</th>
<th>Involved in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>Unique biological materials</td>
</tr>
<tr>
<td>☑️</td>
<td>Antibodies</td>
</tr>
<tr>
<td>☐</td>
<td>Eukaryotic cell lines</td>
</tr>
<tr>
<td>☐</td>
<td>Palaeontology</td>
</tr>
<tr>
<td>☐</td>
<td>Animals and other organisms</td>
</tr>
<tr>
<td>☑️</td>
<td>Human research participants</td>
</tr>
</tbody>
</table>

Methods

<table>
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<tr>
<td>☑️</td>
<td>ChIP-seq</td>
</tr>
<tr>
<td>☑️</td>
<td>Flow cytometry</td>
</tr>
<tr>
<td>☑️</td>
<td>MRI-based neuroimaging</td>
</tr>
</tbody>
</table>

Unique biological materials

Policy information about availability of materials

Obtaining unique materials | All biological materials described in the materials sections are either commercially available of available from the authors. |

Antibodies

Antibodies used | • Goat Anti-Human IgG (Jackson ImmunoResearch 109-005-088), • Donkey-anti-rabbit (Jackson Immuno, cat. no. 711-005-152) |
Validation | Both are secondary antibodies |

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) | • HEK293T cells were purchased directly to the distributor to avoid misidentification or cross-contamination (Sigma-Aldrich, cat. no. 85120502, ATCC® CRL-3216™) |
Authentication | The cells were purchased directly from distributor and no further validation was performed |
Mycoplasma contamination

All the cells used in this study tested negative (routinely) for ELISA and PCR.

Commonly misidentified lines
(See ICLAC register)

HEK293T cells