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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         | Cor         | firmed  |
|-------------|-------------|---|
|             | $\square$   | The exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement   |
|             | $\square$   | An indication of 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<br>Only common tests should be described solely by name; describe more complex techniques in the Methods section.   |
| $\boxtimes$ |             | A description of all covariates tested  |
|             | $\square$   | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons   |
|             | $\boxtimes$ | A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (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  |
|             | $\square$   | Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated  |
|             | $\boxtimes$ | Clearly defined error bars<br>State explicitly what error bars represent (e.g. SD, SE, Cl)  |

Our web collection on statistics for biologists may be useful.

#### Software and code

 Policy information about availability of computer code

 Data collection
 Axon GenePix 4000B array scanner and GenePix Pro (v6.0) was used to scan microarrays. NimbleScan (v2.5) was used to extract

 microarray data for ChIP experiments. A Typhoon FLA 7000 scanned phosphorimage screens of Northern Blots. ImageQuant LAS 4000

 Data analysis
 Data in this manuscript were analyzed with ClustalW, MEGA6 (v10.0.5), Hypertree (v1.2.2), PyMol (v1.8.2), Autodock (v4.2.6), STAR

 (v2.5.1a), HTSeq (v0.6.1), DEseq (1.32.0), DeepTools (2.4.0), R (v3.3.0), ImageJ (v1.50e, and MultiExperiment Viewer (v4.8.1). Custom R scripts (previously reported in Tietjen et al. NSMB. 2010) are available upon request.

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.

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

ChIP and RNA seq data are available with the following accession number: GSE107166

## Field-specific reporting

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K 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.

| Sample size     | No statistical methods were used to predefine sample size. ENCODE guidelines were used for ChIP and RNA-seq experiments. Western blots and Northern blots were performed in biological triplicates according to standards in the field. |  |  |
|-----------------|---|--|--|
| Data exclusions | No data were excluded   |  |  |
|                 |   |  |  |
| Replication     | All experiments were reliably repeated in independent replicates, as indicated in the figure legends. The Pearson correlation between biological ChIP and RNA-seq replicates were greater than 0.8.                                     |  |  |
|                 |   |  |  |
| Randomization   | The order of addition of kinase inhibitor to each sample was randomized both for RNA-seq, ChIP, and Western blot experiments.   |  |  |
|                 |   |  |  |
| Blinding        | No binding applied to these experiments. No animal or human research participants were utilized. All samples were processed in parallel.  |  |  |

## Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods                   |  |
|----------------------------------|---------------------------|--|
| n/a Involved in the study        | n/a Involved in the study |  |
| Unique biological materials      | ChIP-seq                  |  |
| Antibodies                       | Flow cytometry            |  |
| Eukaryotic cell lines            | MRI-based neuroimaging    |  |
| Palaeontology                    |                           |  |
| Animals and other organisms      |                           |  |
| Human research participants      |                           |  |
|                                  |                           |  |

#### Antibodies

| Antibodies used | anti-pThr4: 1G7 and 6D7, provided by Dirk Eick, used at 1/1000 dilution for Western blots and 1/100 for ChIP<br>anti-Rpb3: 1Y26, Biolegend, formerly Neoclone, used at 1/1000 for Western blots, 3/1000 for ChIP<br>anti HA: ab9110, Abcam, used at 1/1000 for ChIP<br>anti-TAP: CAB1001, ThermoFisher, used at 1/1000 for ChIP  |
|-----------------|--|
| Validation      | 1G7 and 6D7 were validated in Hintermair et al. EMBO J. 2012 using ELISAs. anti-Rpb3 was originally validated by Svetlov et al.<br>JBC. 1998, and is now commercially available from BioLegend.<br>anti-HA (abcam ab9110) has been validated by IHC, ChIP, ELISA, Co-IPs, and other experiments, and extensively used in over 380<br>publications. We observed no cross reactivity with any other protein when extract was probed via Western blot.<br>The anti-TAP antibody (Thermo CAB1001) has been validated by ChIP, Co-IPs, and other experiments, and extensively used in<br>over 70 publications. We observed no cross reactivity with any other protein when extract was probed via Western blot. |

#### Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

 Laboratory animals
 No animals were used in this study

 Wild animals
 No wild animals were used in this study.

 Field-collected samples
 No samples were collected from the field.