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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 our Editorial Policies and the Editorial Policy Checklist.

Statistics

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. *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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Detailed in the manuscript
Data analysis	Detailed in the manuscript

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 and 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 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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD030963 and processed data are provided in Supplementary Table 1.

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Mass spectrometry experiments were performed in triplicates, which is required for detecting statistically enriched proteins.
Data exclusions	No data was excluded.
Replication	Targeting of the ProtA-TurboID fusion enzyme to the primary antibody was validated independently using immunofluorescence, IP-western blot and mass spectrometry. All experiments for mass spectrometry were performed in biological triplicates.
Randomization	Not applicable as this is a protocols paper which serves mainly to demonstrate the use of proximity labeling without transfection or transduction.
Blinding	Not applicable as this is a protocols paper which serves mainly to demonstrate the use of proximity labeling without transfection or transduction.

Reporting for specific materials, systems and methods

We require information from authors 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

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Positive control primary antibody (for example H3K9me3 (Abcam Cat# ab8898, RRID: AB_306848, https://scicrunch.org/resolver/RRID: AB_306848), CENPC (MBL International Cat# PD030, RRID: AB_10693556, https://scicrunch.org/resolver/RRID: AB_10693556) INCENP (Abcam Cat# ab12183, RRID: AB_298914, https://scicrunch.org/resolver/RRID:AB_298914) Negative control primary antibody (for example normal rabbit IgG, Millipore Cat# 12-370, RRID: AB_145841, https://scicrunch.org/resolver/RRID:AB_145841) Secondary antibody for immunofluorescence (Alexa 568, Thermo Fisher Scientific Cat# A-11011, RRID: AB_143157, https://scicrunch.org/resolver/RRID: AB_143157) HRP-conjugated secondary antibody for western blot (Agilent Cat# P0399, RRID: AB_2617141, https://scicrunch.org/resolver/RRID:AB_2617141 for rabbit antibodies)
Validation	Commercial antibodies were validated by the vendor.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HeLa cells (source: ATCC)
Authentication	None of the cell lines were authenticated.

Mycoplasma contamination	All cells were routinely tested for mycoplasma.
Commonly misidentified lines (See ICLAC register)	Not applicable.