nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio 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	timsTOF Pro (Bruker Dalton, Bremen, Germany) was used to analyze the PKA (before and after PAL probe chemical modification). The complete molecular sizes of the modified PKAs were determined by deconvoluting the MS spectra. MALDI-TOF (New ultrafleXtreme, Bruker Dalton, Bremen, Germany) was used to analyze PKA tryptic fragments ms/ms sequencing. A monochrome sCMOS camera (SPOT) with built-in software SPOT Basic was used to acquire and quantify cell immunostaining images.
Data analysis	ESI mass spectrometry data were collected using timsTOF Pro (Bruker Dalton) and HyStarNT 5.0 commercial software. For MADLI mass spectrometry data, which were collected using New ultraflexXtreme (Bruker Dalton) and flexControl Application 3.4 commercial software. Cell immunostaining images were using a monochrome sCMOS camera (SPOT) with build-in software SPOT Basic.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All relevant characterization data from this study is included in this published article and supplementary information files, including mass of PAL probes, mass of protein (before and after PAL chemical modification), mass of photolytic fragmentation of PAL probes, protein fragments ms/ms sequencing, kinase activities before

and after chemical modifications, and Western blotting before and after protein modification. Additional cell images after PAL probes treatments before and after blue light irradiation and after subsequent UV irradiation are available from the corresponding author (Hsien-Ming Lee) upon request.

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	For protein chemistry section, we test four PAL probes on one protein (PKA). PKA chemical modifications and subsequent photochemical reactions, kinase activity before and after PAL probe treatment and subsequent light irradiations experiment were replicated at least three times to ensure the quantitative reproducibility. For cell immunostaining, the cell experiments were repeated three times. Each time there are at least 10-20 cells microinjected by PAL probes (for a total of 30-50 cells per irradiating conditions) to ensure the quantitative reproducibility.
Data exclusions	No data was excluded.
Replication	To demonstrate the PAL probes can selectively modify our protein-of-interest, PKA, experiments were performed multiple times, and most experiments were reproduced with at least three replicates with similar results for the methodology development, including protein-modification mass analysis, Western blotting, and kinase activities analysis before and after blue light and UV irradiations. Cellular signal transduction reproducibility was ensured using up to 30-50 microinjected cells for each photoirradiating conditions.
Randomization	Our negative control PAL probe design was based on a randomized peptide sequence to demonstrate that the randomized sequence is lack of protein specificity, as shown in three Western blotting experiments.
Blinding	Not relevant to the study. For protein chemical modification, blinding was not performed as the focus is to demonstrate the technical performance.

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	1. primary Mouse anti-PKAcat (Santa Cruz #sc-28315) 2. primary Anti-FLAG ® M2 (Sigma-Aldrich) 3. secondary Mouse IgGκ binding protein (m-IgGκ BP-HRP, 1:1000, Santa Cruz #sc-516102) 4. primary anti-VASP pS153 (Abcam, #ab47268) 5. secondary Alexa 647 goat anti-rabbit IgG (Invitrogen, #A32733)
Validation	Antibodies validation and citations were available on vendors websites.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	REF52 fibroblasts cell was obtained from Dr. David Lawrence's lab (School of Pharmacy, University of North Carolina at Chapel Hill)
Authentication	None of the cell lines were authenticated.
Mycoplasma contamination	The cell lines were tested negative for mycoplasm contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines are used in this study.

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents