nature portfolio

Corresponding author(s): Petr Beier

Last updated by author(s): Jan 9, 2026

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	No software was used.
Data analysis	No software was used.

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

The data supporting this article are included as part of the Supplementary Information. The mass spectrometry proteomic data have been deposited to the ProteomeXchange Con-sortium via the PRIDE partner depository with the dataset identifier PXD066546.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Our experiments only include labeling of proteins and experiments on cell cultures. So gender is irrelevant.
Reporting on race, ethnicity, or other socially relevant groupings	Our experiments only include labeling of proteins and experiments on cell cultures. So race, ethnicity is irrelevant.
Population characteristics	See above.
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	https://www.uochb.cz/en/code-of-ethics

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

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	We did not perform sample size calculations. The samples were selected based on available materials and included experimental conditions, as well as positive and negative controls where applicable.
Data exclusions	We have excluded some data (experiments) that were used to optimize the labeling system. Preliminary/pilot experiments. All other data are shown in the manuscript main text or supporting information.
Replication	All experiments were replicated to some degree. The optimized labeling conditions used in the study are described in the manuscript.
Randomization	Our samples were not randomized because the experiments required the knowledge of which sample was which. For example, which protein was labeled with what reagent. Using sample randomization, we would lose control over the experiments.
Blinding	As mentioned in the previous reply, we have not randomized or grouped our samples because that would not allow us to draw relevant conclusions. In our labeling experiments, it was necessary to know what we mix with what.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Herceptin (Trastuzumab, 150 mg, Roche) monoclonal antibody was purchased from local pharmacy for scientific purposes (Všeobecná Fakultní Nemocnice in Prague).
Validation	The antibody is being used in patients. We have confirmed its specificity through control experiments, where cells lacking the antigen were not labeled.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	U2OS (purchased from Sigma Aldrich; UNSPSC Code: 41106514) and SKBR3 (was a kind gift from Dr. Karel Souček (Masaryk University, Brno) in 2024).
Authentication	Both cell lines were authenticated using AmpFLSTR Identifiler PCR Amplification Kit.
Mycoplasma contamination	Cell lines were not tested for micoplasma contamination.
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Plants

Seed stocks	We have not used plants in our study.
Novel plant genotypes	We have not used plants in our study.
Authentication	We have not used plants in our study.

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

Sample preparation	After the confocal microscopy experiment, all the cellular samples were washed 3 times with PBS. The cells were detached (after ca 10 min of incubation) from the 96-well plate using 50 µl of Accutase (StemPro® Gibco A11105-01) solution. Each well was additionally washed 2 times with 50 µl of PBS (+ 1 mg/ml BSA) and centrifuged at 300 ×g for 3 min. Supernatant was removed and samples were resuspended to 150 µl of PBS (+ 1 mg/ml BSA). Then the cellular samples were measured by a flow cytometer (LSR Fortessa (BD Biosciences)) and the results are represented as histograms or median fluorescence intensity (Supplementary Figure 23 and 24).
Instrument	LSR Fortessa (BD Biosciences)
Software	FlowJo (version 10.10.0)
Cell population abundance	We did not sort the cells we only analyzed them.
Gating strategy	Cells were first gated based on forward scatter (FSC) and side scatter (SSC) to exclude debris and to select the main cell population. Doublets were excluded using FSC-A versus FSC-H gating. Subsequent gates were applied sequentially based on specific fluorescence markers to identify the population of interest.

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