

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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 The intensity-based NIR-II imaging results were acquired with LightField 6 (Princeton Instruments, USA). The time-resolved NIR-II imaging results were obtained by LabVIEW (2018) program.

Data analysis We applied imageJ 1.52p (NIH, USA), Office Excel 2019 (Microsoft, USA), Origin 9 (Origin Lab) and customized MATLAB (2014B) programs to analyze the collected data.

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 main data supporting the results in this study are available within the paper and its Supplementary Information. Source data for Figs. 2, 3, 5 and 6 are available as Supplementary Information. The raw and analysed datasets are available in figshare with the identifier <https://doi.org/10.6084/m9.figshare.14633316.v1> (ref. 29).

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](https://www.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 the in vivo imaging, experiment performed with 5 independent mice with similar results. The sample size (n) of each experiment was provided in the corresponding figure captions in the main text and supplementary information. Analyzed with two-tailed Student's t test, the advantages of NIR-II phosphorescence over NIR-II fluorescence were significant. The sample sizes were believed to support meaningful conclusions.
Data exclusions	No data were excluded in this study.
Replication	For intensity-based NIR-II imaging and time-resolved NIR-II imaging in vitro, at least 3 repetitive images within 3 min were collected in each imaging acquisition. In animal experiments, at selected imaging intervals, experiments performed with 5 independent mice within 30 min to verify the effectiveness of the proposed imaging technique. Observation of the same sample was performed from different views.
Randomization	The mice from different cage, but within the same experimental group, were selected to enable randomization. The nanoprobe from different synthesis batches, but with the same photophysical properties, were allocated into experimental groups randomly for optical studies. In cell-based assays, subjects were grouped into treatments by proximity.
Blinding	The investigators were blinded to group allocation during data collection and analysis.

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

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	The cell lines including normal 3T3, MCF-7 and 143B (human osteosarcoma) cells were purchased from American Type Culture Collection (Manassas, VA).
Authentication	All cell lines were authenticated by Short Tandem Repeat test.
Mycoplasma contamination	Cells used in this research were all mycoplasma-free.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Seven-week-old female C57BL/6 and nude mice were purchased from Charles River (USA).
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Wild animals	The study did not involve the use of wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experiment protocols were reviewed and approved by Stanford University’s administrative panel on Laboratory Animal Care and complied with all relevant ethical regulations.

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