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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	Product formation of pseudo-first order reactions was monitored by a Agilent Technologies 1220 Infinity Liquid Chromatography system using the OpenLAB CDS vA.03.02.024 software. UV-Vis absorption spectra were recorded in a Varian Cary 300 Bio UV-Visible Spectrophotometer using the Cary WinUV v4.20 software. Fluorescence spectra was recorded in an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer using the Cary Eclipse v1.2 software. Fluorescence live-cell imaging was recorded in a Olympus FluoView IX81 confocal microscope using the FV10-ASW 3.0 software. Fluorescence measurements in 96-well plates were performed in a Molecular Devices SpectraMax M5 plate reader using the SoftMax Pro v5.4 software. LC-MS/MS Raw data files for proteomics studies were processed with the pFind studio (version 3.1.2, http://pfind.ict.ac.cn/software/pFind3/index.html) for peptide identification and quantification. Electronspray Ionization Mass Spectrometry (ESI-MS) of Gpx3 was measured on a Thermo Scientific LTQ XL linear ion trap mass spectrometer (Thermo Scientific) using the Tune Plus v2.5.0 software. Flow cytometry was performed in a Beckman Coulter Gallios Flow Cytometer using the Kaluza for Gallios v1.1 software.
Data analysis	ImageJ v1.52a (https://imagej.nih.gov/ij/download.html) and its Fiji extension (v1.52p) were used to image and analyze live cell imaging (Fig. 3b, 3d, S12, S13, and S14). The deconvolution program MagTran was used to obtain the mass spectra of Gpx3 samples (Fig. S20C-D). Dot plots (Fig. 4a, 4d, 4f, 5b, 5c, S3-S4, and S7-S8), bar plots (Fig. 2b, and S21B-C), Line plots (Fig. 2c-d, S2-S10, and S20A), and box plots (Fig. 3c, 3e, 4b, 5d, S11-S13, and S15-S17) were generated and analyzed by GraphPad Prism v7.0. Product formation traces of pseudo-first order reactions were generated and analyzed using Kaleidagraph v4.1.1 software (Fig. S1).

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 datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable 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	No statistical method was used to determine sample sizes. Sample sizes for imaging and cell-based assays were chosen to be between 5 and 10 measurements per condition, based on previous experience in similar types of experiments.
Data exclusions	Data points were not excluded.
Replication	In general, experiments were replicated at least twice to guarantee reproducibility. The only exception was the preliminary kinase inhibitor screening, which was performed only once to identify potential hits for further evaluation.
Randomization	No randomization was used because cells used in the different experimental groups were derived from the same cell population.
Blinding	No blinding group allocation was judged necessary because cells were derived from the same cell population in the comparison of different experimental groups.

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)	HeLa cells were obtained from frozen stocks kept in a -130 C freezer, maintained by our lab. Jurkat, clone E6-1 cells were sourced from ATCC (Cat. No. TIB-152).
Authentication	HeLa cells were authenticated by morphology. Cell line authentication for Jurkat, clone E6-1 cells were peformed by the vendor, ATCC.
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell line was used.

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

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	Cell suspensions were centrifuged, resuspended with FACS buffer ((PBS buffer containing 1 mM EDTA, 25 mM HEPES and 1% FBS, 0.2 µM filter-sterilized)) and kept on ice until analysis
Instrument	Beckman Coulter Gallios Flow Cytometer.
Software	Data acquisition and analysis was performed using the Kaluza Analysis Software. GraphPad Prism 7.0 was used to plot the

	ntensities of the FL3 signal for the different conditions.
Cell population abundance	Gated populations that were included in the analysis represented 72.3-86.8% of the total events in the samples analyzed.
Gating strategy	A plot of SS(area) vs. FS(area) was used to select single cells from cell debris and cell aggregates. The selected population was further gated using a sequence of FS(width) vs. FS(height) and SS(width) vs. SS(height) plots.

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