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Corresponding author(s): E. Sally Ward
Raimund J. Ober

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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Biacore T200 Control Software was used to acquire the surface plasmon resonance data. The flow cytometry data was acquired using the BD Accuri C6 software (version 1.0.264.2). Immunoblot data was acquired with the LiCor Image Studio Digits Ver 3.1. Microscopy data was obtained with custom written software. Both size exclusion and hydrophobic interaction column data were acquired with the LabSolutions Lite software provided by Shimadzu Corporation. ELISA, MTS assay, and ALT assay readings were taken by the KinetiCalc (Version #3.4 Rev #21) program. Mass spectrometry data was acquired with TraceFinder.

Data analysis

Custom written software in MATLAB (available at www.wardoberlab.com/software/sprtool) was used to process the acquired surface plasmon resonance data. Half-life estimations for pharmacokinetic analyses in mice were determined by fitting exponentially decaying models in MATLAB (software is available upon request). All flow cytometry data was analyzed using FlowJo. Flow cytometry histograms were produced and analyzed in FlowJo and mean fluorescent intensity values were exported to Excel to be processed. Liquid chromatography data was acquired with the LabSolutions Lite software. Pymol was used to analyze the Pertuzumab:HER2 crystal structure (Protein Data Bank: 1S78). Graphpad was used for both plotting and statistical analyses of tumor size, ALT activity, body weight, ELISA, and dose response curves. Mass spectrometry data was analyzed with TraceFinder. Acquired imaging data was analyzed with custom written software in MATLAB (available at www.wardoberlab.com/software/miatool). A combination of PROPKA v.3.1, CHARMM v36, and NAMD2 v.2.10 were used for modeling analysis.

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/reviewers upon request. 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 data that supports the findings in this study are available upon request from the corresponding author.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	We determined the number of mice used per group using the resource equation method mentioned in Charan J., & Kantharia, N.D. (2013) Journal of Pharmacology & Pharmacotherapeutics.
Data exclusions	There were no data exclusions from the data provided.
Replication	The replication number is indicated in the legend of corresponding figures where applicable. Replication of experiments generated similar results as indicated in the Figure legends. Crystal structure analysis (Supplementary Fig. 2) was not replicated. Modeling simulation (Supplementary Fig. 11) was not replicated as the algorithm iCFN is exact.
Randomization	No specific randomization was done for in vitro experiments or assays shown in Figs. 1, 2b, supplementary Figs. 8 and 10c. Samples in Fig. 2a were randomly positioned in the 96-well plates to ensure that there was no positional bias on the plate. For pharmacokinetic experiments using mice, mice were randomly assigned into treatment groups, and mice in different treatment groups were caged together to avoid cage effects. For therapy experiments using mice, tumor-bearing mice were assigned into treatment groups so that the mean tumor size for each group was similar at the start of treatment, and mice in different treatment groups were caged together to avoid cage effects.
Blinding	No blinding was carried out during allocation of mice to treatment groups. For tumor sizes and weight measurements following the start of therapy, there were two scorers (one blinded, one non-blinded), and the blinded one took measurements at a lower frequency with similar results.

Behavioural & social sciences study design

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

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*

Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

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

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☒ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access and import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study	
☒	☐	Unique biological materials
☐	☒	Antibodies
☐	☒	Eukaryotic cell lines
☒	☐	Palaeontology
☐	☒	Animals and other organisms
☒	☐	Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials: Pertuzumab mutants and all other unique materials are available from authors upon request.

Antibodies

Antibodies used: Anti-Alexa Fluor 488 polyclonal antibody (Thermo Fisher Scientific; A-11094; lot#1214711 and 1314344; 5 ug/ml) was used for quenching Alexa 488 fluorescence. Clinical grade pertuzumab and trastuzumab were acquired from the University of Texas Southwestern Medical Center Pharmacy. Goat anti-human IgG (H + L) polyclonal antibody conjugated with HRP (Jackson ImmunoResearch; #109-035-003; 1:10,000 dilution) was used to detect human IgG antibodies in immunoblotting analyses for the serum stability assay. Anti-M13 monoclonal antibody conjugated with HRP (GE Healthcare; 27-9421-01; lot#9645987; 1: 5,000 dilution) was used to detect bound phage in ELISAs. Anti-human IgG (Fab-specific) polyclonal antibody conjugated with HRP (Sigma-Aldrich; A-0293; lot#083K4874; 1:10,000 dilution) was used to detect human IgG antibodies in ELISAs.

Validation: All commercially available antibodies were validated by the vendor. Anti-Alexa Fluor 488 antibody was validated for surface quenching for every experiment. HRP-conjugated antibodies were tested for specific binding using appropriate control experiments. Links for each antibody used are:
Anti-Alexa Fluor 488 polyclonal antibody (https://www.thermofisher.com/antibody/product/Alexa-Fluor-488-Antibody-Polyclonal/A-11094)
Anti-human IgG (Fab-specific) polyclonal antibody conjugated with HRP (https://www.sigmaaldrich.com/catalog/product/sigma/a0293?lang=en®ion=US)
Goat anti-human IgG (H + L) polyclonal antibody conjugated with HRP (https://www.jacksonimmuno.com/catalog/products/109-035-088)
Anti-M13 monoclonal antibody conjugated with HRP (https://www.sigmaaldrich.com/catalog/product/sigma/ge27942101?lang=en®ion=US)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s): MDA-MB-453, MDA-MB-468, SK-OV-3, and SK-BR-3 were purchased from the ATCC. JIMT-1 was from AddexBio and HCC1954 was a gift from Drs. Adi Gazdar, John Minna, and Kenneth Huffman (Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center at Dallas).

Authentication: Cell lines were authenticated by short tandem repeat analysis (University of Arizona Genetics Core).

Mycoplasma contamination: Cells lines were tested regularly for mycoplasma contamination and were negative.

Commonly misidentified lines (See ICLAC register): No commonly misidentified cell line was used.

Palaeontology

Specimen provenance: *Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).*

Specimen deposition: *Indicate where the specimens have been deposited to permit free access by other researchers.*

Dating methods: *If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals: Female BALB/c SCID mice of 6-8 weeks of age were used for therapy experiments. Female BALB/c SCID mice of 7-10 weeks of age were used for pharmacokinetic experiments. BALB/c SCID mice were purchased from Jackson Laboratories and bred in-house for use in experiments. All breeding and experimental procedures were approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Wild animals: This study did not involve wild animals.

Field-collected samples: This study did not include samples collected from the field.

Human research participants

Policy information about studies involving human research participants

Population characteristics: *Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "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.*

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.: *For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*

Files in database submission: *Provide a list of all files available in the database submission.*

Genome browser session
(e.g. UCSC): *Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates: *Describe the experimental replicates, specifying number, type and replicate agreement.*

Sequencing depth: *Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*

Antibodies: *Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*

Peak calling parameters: *Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*

Data quality: *Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*

Software: *Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

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

Cancer cells were treated in 48 well plates as described in the Methods. Cells were then harvested by trypsinization and washed with phosphate buffered saline.

Instrument

BD Accuri C6 flow cytometer.

Software

BD Accuri C6 software (version 1.0.264.2) was used to acquire the data on the flow cytometer. FlowJo was used to determine mean fluorescence intensities. Exported mean fluorescence intensity values were analyzed in Excel. Histograms were produced in FlowJo.

Cell population abundance

We used homogenous cancer cell lines and did not sort our cells.

Gating strategy

We gated upon the population of single cells based on FSC and SSC values.

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

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference
(See Eklund et al. 2016)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte

Correction | *Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity | *Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis | *Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis | *Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*