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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	UNICORN 7.7 (Cytiva), Chromeleon 7 (Thermo Fisher), LabChip GX 5.3 (PerkinElmer), BioRad Image Lab 6.0, BD FACSDiva, Tecan iControl 2.0, Microsoft Excel 2016, Bruker Compass 6.2, Uncle Analysis Software v3.2, Biacore 8K Control Software 4.0 (Cytiva)
Data analysis	UNICORN 7.7 (Cytiva), Chromeleon 7 (Thermo Fisher), LabChip GX 5.3 (PerkinElmer), Microsoft Excel 2016, GraphPad Prism 9, FlowJo v10, in-house mass spectrometry analysis software, Uncle Analysis Software v3.2, Biacore Insight Evaluation Software 4.0 (Cytiva)

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

Data availability statement: The ADC cytotoxicity data generated in this study and source data for all quantitative figures are provided in the Source Data file. All other data are available from the corresponding author(s) upon request.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, 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.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	Replicate experiments for platform design, set-up and validation were performed. Sample sizes are provided in the figure legends and Methods section. No sample size calculations were performed. For the main in vitro cell-based ADC cytotoxicity assays, n=3 was chosen because the ADCs were tested on pure homogeneous cell lines in vitro, which have limited variability. For a screening approach like pair-FORCE, we believe this sample size is sufficient to draw conclusions about the ADCs being tested. For the in vitro ADC cytotoxicity assays on various HER2-expressing cell lines, n=2 was chosen because the differences between the different groups were sufficient to show clear trends. All flow cytometry experiments were performed on pure, well-characterized cell lines under controlled conditions. Therefore, we expect very minimal biological and technical variability. Because the experiments were performed with the appropriate controls and the data were conclusive, they were performed with a sample size of n=1 (with the exception of Fig. S3, which was performed twice with similar results). The same rationale applies to the immunoprecipitation and Western Blot experiments, which were performed with n=1. Mass spectrometry (MS) measurements were performed on purified antibody derivatives with a sample size of n=1. Because we analyzed very pure molecules with appropriate internal controls for calibration and data validation, we believe that single measurements are justified for this resource-intensive method. Other antibody quality control (QC) experiments including SEC, CE-SDS, HIC, and SPR were performed with a sample size of n=1, We believe this is justified due to the clear and unambiguous results, and the nature of pair-FORCE as a screening method. The same rationale applies to thermal stability experiments, which were performed with a sample size of n=2 technical replicates (yielding very similar results).
Data exclusions	No data were excluded.
Replication	Information about the number of replicates is provided in the figure legends or Methods section, and all replication of experiments yielded similar results.
Randomization	No randomization was performed. Randomization is not applicable for this study because it covers rational structure-guided protein design and in vitro testing.
Blinding	No blinding was performed. Blinding is not applicable for this study because it covers rational structure-guided protein design and in vitro testing.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used: This study primarily utilized recombinant antibody derivatives based on the HER2-binding antibodies Trastuzumab and Pertuzumab, and the EGFR-binding antibodies Cetuximab, Imgatuzumab, P1X, P2X, and mab806. The sequences from which these recombinant antibodies are derived are referenced in the Methods section. This study also utilized the following commercial antibodies: 1. Mouse anti-EGFR antibody [EMab-134] (Abcam ab264540, 1:1000 dilution) 2. Goat Anti-Mouse Immunoglobulins/HRP (Agilent P044701-2, 1:1500 dilution) 3. anti-BrdU POD (Roche, clone BMG 6H8, 11647229001, 1:100 dilution) 4. Mouse anti-HER2 (BioLegend, clone 24D2, 324402, 3.75 µg) 5. Mouse IgG1, κ Isotype Ctrl Antibody (BioLegend, clone MOPC-21, 400102, 3.75 µg).

Validation: The commercial antibodies used in this study were validated by their respective manufacturers and yielded the expected results, as indicated on the manufacturer's websites. The specificities of recombinant in-house antibody derivatives are demonstrated in this study.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): Human embryonic kidney 293 cells (Expi293 expression system, Thermo Fisher), SK-BR-3 (ATCC, HTB-30), MCF-7 (ATCC, HTB-22), A431 (ATCC, CRL-1555), SK-OV-3 (ATCC, HTB-77), MDA-MB-453 (ATCC, HTB-131), NCI-H1650 (ATCC, CRL-5883), MDA-MB-468 (ATCC, HTB-132)

Authentication: HEK293 Expi cells were commercially obtained (Thermo Fisher) and used without further authentication, except for confirmation of expected functionality as expression hosts for secretion of recombinant antibodies. All other cell lines were characterized by measurement/quantification of target antigen expression (HER2 or EGFR). Absolute quantification of HER2 copy numbers on various cell lines resulted in similar values as reported in the literature. All cell lines showed the expected morphology.

Mycoplasma contamination: Negative (in-house tested)

Commonly misidentified lines (See ICLAC register): No commonly misidentified cell lines were used in this study.

Plants

Seed stocks: *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*

Novel plant genotypes: *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*

Authentication: *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	Sample preparation and the source of cells are described in the Methods section.
Instrument	FACS Canto II, BD Biosciences
Software	FACSDiva (BD) for data collection, FlowJo v10 for data processing and analysis
Cell population abundance	No sorting was performed, only homogeneous cell lines were used
Gating strategy	Gating of cell lines was performed by FSC/SSC to analyze single cells, see figure in supplementary information

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