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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, seeAuthors & Referees and theEditorial 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	Gen5 2.05 (Fluorescence data collection)
Data analysis	GraphPad Prism 8.4.1 (curve fitting), PyMol 1.7.4.3 (structure rendering), COOT 0.8.9.1 (X-ray structure model building), PHENIX 1.14-3260 (X-ray structure model refinement), XDS 20170923 (X-ray diffraction data processing), PHASER 2.5.2 (molecular replacement), Elbow (ligand coordinate and restraint generation), MaxQuant version 1.6.2.10 (Mass spectrometry quantification), Chem3D v15.1 (Spacer-length calculation), Zen Microscope Imaging v14.0.18.201 (Image acquisition), ImageJ 1.52u (Image processing), FastQC v0.11.5 (Data quality inspection), multiQC v1.8.dev0 (Data quality inspection), Tophat version 1.4.1 (RNAseq data mapping), bowtie version 0.12.7 (RNAseq data mapping), R version 3.6.1 (RNAseq data processing), samtools version 0.1.18 (File conversion), heatmaply v1.0.0 (RNAseq data correlation), DESeq2 v1.24.0 (RNA seq data comparison), Bio-Rad Image Lab software v6.0.1 (Image quantification)

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. 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

Coordinates and structure factors (6UUO) are available at the Protein Data Bank, www.rcsb.org.
"The kinase inhibitor competition data used for the protein kinase-biased analysis was also submitted as an incomplete submission to the MassIVE respository (https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp) and assigned the accession number MSV000085271. The dataset is available at ftp://massive.ucsd.edu/MSV000085271/ "

"Data for the proteome-wide protein level analysis has been deposited as an incomplete submission to the MassIVE repository (https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp) and assigned the accession number MSV000084551. The dataset is available at ftp://massive.ucsd.edu/MSV000084551/"

The RNAseq data reported in this paper are available at the Gene Expression Omnibus database (GEO) with Accession number GSE148500.
All data supporting the findings are available in the paper and Supplementary Information files.

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	Statistical methods were not used to determine sample sizes. However, for quantification purposes, a sample size of n = 2 unless otherwise stated (reproduced two or more independent times) was used to assess reproducibility and robustness of each experiment performed. Sample sizes were based on prior experience in the field.
Data exclusions	Mass spectrometric data analysis was performed using the MaxQuant software package. Peptides (minimum 8 aa in length) and proteins passing the 1% FDR threshold were deemed confident identifications. The relative abundance of these proteins across the concentration range were calculated using LFQ (label-free quantification) and additional filtering was performed to remove contaminants and to ensure sufficient data points to generate a curve (see methods section). Non protein kinases were excluded from further inspection. All curves were manually reviewed and a threshold of quantitative variation of ≥40% below the DMSO control at the highest concentration (30 uM) was selected to identify kinases with strong binding to the inhibitor being tested.
Replication	To ensure reproducibility of experimental findings, all biochemical or cellular assays were repeated independently at least two times. One representative result for each experiment is presented in the main Figures or the Extended Data Figures. For the RNA-seq analysis, each condition was processed and analyzed in triplicate. We confirm that all attempts of replication were successful.
Randomization	Groups of mice used for this study were randomized before treatment. Randomization for experiments other than mice study was not relevant because all cells and biological samples used for analysis were from the same initial stocks.
Blinding	Sample collection and data analysis for the pharmacodynamic analyses using a mouse A375 xenograft could not be done in a blinded fashion as it involved following the progression of time points or because we only dosed vehicle and compound for MTD study. Blinding for experiments other than mice study was not relevant because all data collection and analysis were quantitative and not qualitative in nature.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Antibodies against BRAF (sc-5284; clone F-7; lot C0166), ARAF (A-5, sc-166771), phospho-MEK1/2 (7E10, sc-81503), phospho-ERK (E-4, sc-7383), SRMS (E5, sc-376223), KRAS (sc-30; clone F-234; lot F2713), HRAS (sc-53958; clone M3; lot C1008), NRAS (sc-31; clone F155; lot D1712), were purchased from Santa Cruz; CRAF (9422), phospho-MEK1/2 (9121; lot 56), MEK1/2 (9122; lot 14), ERK1/2 (9102 or 4695), HSP90 (4877; clone C45G5; lot 5) from Cell Signaling; CRAF (610152; clone 53/c-Raf-1; lot 7208706), p27 (610242) from BD Biosciences; KSR1 (EPR2421Y), panRAS (ab108602; clone EPR3255; lot GR11071-26), BRAF(V600E) (RM8 clone) (ab200535) from Abcam; Anti-Flag M2 HRP conjugated (A8592), Anti-Flag M2 magnetic beads (M8823), phospho-ERK (M9692;

clone MAPK-YT) and alfa-tubulin (T6199; clone DM1A; lot 078M4796V) from Sigma-Aldrich. MG132 (S2619) was purchased from Selleckchem, MLN4924 from EMD Millipore.

Validation

All used antibodies are accompanied with their respective catalogue numbers or with a relevant reference to confirm that they have been proven validated or have been used in other publications.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	A375, SK-MEL-28, Colo-205, HT-29, MeWo, NCI-H1666, NCI-H508, SK-MEL-2, MDA-MB-231, NCI-H2087, HCT116, HEK293T, RKO, WM-266-4 and NCI-H1755 cell lines were purchased from ATCC and maintained as instructed.
Authentication	Cell lines were not authenticated
Mycoplasma contamination	All cell lines used were regularly tested negative for mycoplasma contamination as assessed by DAPI staining. Proliferation rates and proper cell morphology of all the cell lines were continuously monitored
Commonly misidentified lines (See ICLAC register)	none

Animals and other organisms

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

Laboratory animals	NU(NCr)-Foxn1nu female mice, 6–8 weeks old, weighing approximately 18–22 g, were purchased from Charles River. Animals were maintained at 22°C, 50% humidity with dark/light cycles of 12h/12h with gradual 'sunrise' and 'sunset'.
Wild animals	The study did not involve wild animals
Field-collected samples	The study did not involved sample collected from the field
Ethics oversight	All procedures were in accordance with the regulations of the Canadian Council on Animal Care, the University of Montreal and the University Health Network

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