nature portfolio

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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	Data collection was performed using the R programming language (v.4.0.5). The list of cancer genes was retrieved from the Network of Cancer Genes v.7 (http://ncg.kcl.ac.uk/). The list of genes with actionable alteration was collected from the PrecisionTrialDrawer' R package (https://gmelloni.github.io/ptd/). The list of genes with oncogenic mutations that are therapeutically targeted with drugs were collected from the Oncology Knowledge Base (OncoKB, https://www.oncokb.org/) data repository
Data analysis	Software employed in the analyses: GraphPad Prism 8; SynergyFinder 3.0; Novoalign V4.03.04 (http://www.novocraft.com/); STAR v.2.4.2a; RSEM v1.3.1; Picard v.1.9; GATK v.3.6; MuTect v.1.1.17 and v.1.1.4; HatSPiL (https://github.com/dodomorandi/hatspil); Strelka v.1.0.15 ; Varscan2 v.2.3.6; Sequenza v.3.0.0; EXCAVATOR2 v.1.1.2; ANNOVAR frozen at 2016-02-01; CancerVa v.1.1r; PyClone v.0.13.1 ; Clonevol R package v. .99.11; EaCoN R package (https://github.com/gustaveroussy/EaCoN) ; QuantaSoft software (1864011, Bio-Rad); CyQUANT (Fisher Scientific). The R code used for the analysis of microarray and WES data generated in the study is publicly available on Code Ocean at https://codeocean.com/capsule/7375761/tree.

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

Sequencing data of SD and PD biopsies, PDOX and PDOX-derived cell lines generated in this study have been deposited in NCBI Sequence Read Archive database under the accession code PRJNA1020204 [https://www.ncbi.nlm.nih.gov/sra/PRJNA1020204] and are publicly available. Processed OncoScan SNP-array data are provided in the Code Ocean capsule, doi:10.24433/CO.7375761.v1. The data generated in this study are provided in the Source Data file. Source data are provided with this paper. All remaining information can be found in the Article, Supplementary and source Data files.

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	There is no individual level patient data presented. Sex (biological attribute) was taken directly from the clinical chart review. All data was de-identified Sex was used as a covariate in analysis.
Reporting on race, ethnicity, or other socially relevant groupings	There is no individual level patient data presented.
Population characteristics	Clinical, pathologic, and outcomes data were abstracted via chart review. These included age, sex, smoking history, ECOG PS, histology. Genomic data were abstracted from tumor DNA sequencing data performed for clinical care.
Recruitment	Consecutive patients with advanced/metastatic KRAS G12C positive non-small lung cancer were included. Patients were treated as per standard of care. Patients were not recruited for participation.
Ethics oversight	The studies involving patient were conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS) and approved by Institut Bergonié review board. All procedures and animal housing conformed to the regulatory standards and were approved by the Italian Health Minister (authorization n° 1227/2020-PR). Similarly, the animal experimental design involving orthotopic implantation was approved by the IDIBELL animal facility committee (AAALAC Unit1155) and received the procedure reference# 9111. All experiments were performed in accordance with the guideline for Ethical Conduct in the Care and Use of Animals as stated in The International Guiding Principles for Biomedical Research Involving Animals, developed by the Council for International Organizations of Medical Sciences. Patient studies were conducted according to the ethical guidelines of the Declaration of Helsinki. De-identified patient data were used from patients who consented to IRB approved protocols Dana-Farber/Harvard Cancer Center 02-180, and/or 17-000.

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	No statistical methods were used to pre-determine sample sizes. Sample size was adequeate to receive significant results. Data distribution was assumed to be normal, however, this was not formally tested.
Data exclusions	No data were excluded from the analysis
Replication	All in vitro experiments were repeated at least three times. All replication attempts were successful.
Randomization	For in vivo experiments where cells were implanted subcutaneously, mice were randomized into treatment groups when tumours reached 50-100 mm3.
Blinding	Investigators were not blinded to mice allocation after treatment administration and during outcome 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
[ ]	[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

WB: Antibodies against TGFBR2 (#79424, 1:1000), P-SMAD2 Ser465/467 (#3108, 1:1000), SMAD2 (#5339, 1:1000), P-SMAD3 Ser423/425 (#9520, 1:1000), SMAD3 (#9523, 1:1000), KEAP1 (#8047, 1:1000), P-ERK1/2 (#9101, 1:1000), ERK (#9102, 1:1000), RAS (#8832, 1:1000), BIM (#2933, 1:1000), HSP90 (#4877, 1:2000), V5-Tag (#13202, 1:1000) were purchased from Cell Signaling Technology. The KRAS clone F234 (sc-30, 1:1000, Santa Cruz Biotechnology) was also used for western blot analysis.

RAS activation assay : (Cell Signaling Technology, #11871).

IHC: pERK1/2 (Cell Signaling Technology; #9101), phosphorylated Histone 3 (06-570, Millipore), CK7 (OV-TL 12/30, Agilent-Dako), CK20 (Ks20.8, Agilent-Dako), Napsin A (TMU-Ad02, Biocare Medical), TTF1 (8G7G3/1, Agilent-Dako), p40 (BC28, Gennova), Chromogranin (Agilent-Dako) and Synaptophysin (SY38, Agilent-Dako).

Validation

TGFBR2 (#79424) was validated by the manufacturer as follows: Western blot analysis of extracts from Hep G2 cells, untreated or treated with PNGase F.

P-SMAD2 Ser465/467 (#3108) was validated by the manufacturer as follows: Western blot analysis of extracts from untreated or TGF-beta treated HeLa and NIH/3T3 cells.

SMAD2 (#5339) was validated by the manufacturer as follows: Western blot analysis of extracts from HeLa cells or SMAD2 knock-out cells.

P-SMAD3 Ser423/425 (#9520) was validated by the manufacturer as follows: Western blot analysis of extracts from HT-1080, C2C12, or KNRK cells, untreated or treated with TGF-β (10 ng/ml, 30 min).

SMAD3 (#9523) was validated by the manufacturer as follows: Western blot analysis of extracts from control HeLa cells or HeLa cells with an apparent in-frame truncation mutation in the gene encoding SMAD3.

KEAP1 (#8047) was validated by the manufacturer as follows: Western blot analysis of extracts from various cell lines.

P-ERK1/2 (#9101) was validated by the manufacturer as follows: The antibody reacts specifically with as little as 0.25 ng of phosphorylated p42 MAP kinase and does not cross-react with up to 4 µg of nonphosphorylated p42 MAP kinase. The antibody does not cross-react with the corresponding phosphorylated residues of either JNK/SAPK or p38 MAP Kinase.

ERK (#9102) was validated by the manufacturer as follows: Western blot analysis of extracts from HeLa cells transfected with 100 nM control siRNA #6201 or p44 MAPK (Erk1) siRNA. The antibody does not recognize either JNK/SAPK or p38 MAP kinase.

RAS (#8832) & was validated by the manufacturer as follows: NIH/3T3 cell lysates were treated in vitro with GTPγS or GDP to activate or inactivate Ras. The lysates were then incubated with glutathione resin and GST-Raf1-RBD. GTPγS-treated lysate was also incubated without GST-Raf1-RBD in the presence of glutathione resin as a negative control.

BIM (#2933) was validated by the manufacturer as follows: Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Fluorescein Conjugate) #6201 or SignalSilence® Bim siRNA I #6461 or SignalSilence® Bim siRNA II.

HSP90 (#4877) was validated by the manufacturer as follows: Western blot analysis of extracts from HeLa, NIH/3T3, PC12 and COS cells.

KRAS clone F234 (sc-30) was validated by the manufacturer as follows: Western blot analysis of K-Ras expression in non-transfected: sc-110760 (A) and human K-Ras transfected: sc-111225 (B) 293 who le cell extracts.

Phosphorylated Histone 3 (06-570) was validated by the manufacturer as follows: Immunocytochemistry Analysis: 1:500 dilution from a representative lot detected Histone H3 in HeLa and A431 cells.

CK7 (OV-TL 12/30) was validated by the manufacturer as follows: SDS-PAGE analysis of immunoprecipitates formed between Triton X-100-extracted cytoskeleton preparations of several cell cultures and tissues and the antibody a 54 kDa band is labeled corresponding to cytokeratin 7. Labels several types of normal and neoplastic epithelia, including many ductal and glandular epithelia. The staining performance of all FLEX RTU antibodies has been defined, tested and approved through collaboration with leading, international pathology experts.

CK20 (Ks20.8) was validated by the manufacturer as follows: Labels a 46 kDa polypeptide corresponding to CK 20 in Western immunoblotting of SDS-PAGE-separated cytoskeletal proteins from human duodenal mucosa. For classification of most most adenocarcinomas including lung.

Napsin A (TMU-Ad02) was validated by the manufacturer as follows: Raised against a synthetic peptide of a part of the N-terminus of

human Napsin A. The reactivity of the antibody showed immunohistochemically in type II pneumocytes, alveolar macrophages, renal tubules and exocrine glands and ducts in pancreas.

TTF1 (8G7G3/1) was validated by the manufacturer as follows: Identifies the 40 kDa TTF-1 band in nuclear extracts or whole cell lysates of TTF-1-positive cell lines of rat, mouse and man.

p40 (BC28) was validated by the manufacturer as follows: The testing of p40 (BC28) was conducted in 115 laboratories with 93% reporting sufficient marking and 107 technicians reporting optimal-to-good staining results under the test conditions. The pass rate in this run improved from 74% (2016) to 86% (2020) and can be attributed to the use of 'highly sensitive and robust mAb (monoclonal) clone BC28 both as concentrate and Ready-To-Use (RTU) format.

Chromogranin was validated by the manufacturer as follows: Reacts with an epitope on the C-terminal half of the chromogranin A molecule and exclusively labels cells of neuroendocrine origin.

Synaptophysin (SY38) was validated by the manufacturer as follows : Raised against a recombinant immunogen corresponding to the C-terminal cytoplasmic domain of human synaptophysin. In Western blot analysis of human neuroblastoma cell lysate, the antibody labels a major band at 40 kDa corresponding to the expected molecular weight of synaptophysin.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human NSCLC cell line H358 (CRL-5807), H23 (CRL-5800), H1792 (CRL-5895), H2030 (CRL-5914) and H2122 (CRL-5985) were purchased from ATCC. Patient derived cell lines DSF3B & 3C were generated in the study from biopsy material, generation protocol and experimental validations are described in the manuscript. HEK-293T cells were used for lentiviral production and purchased from ATCC (HEK 293T/17, CRL-11268™).
Authentication	Cells were authenticated using the Promega GenePrint 10 System (RTSF Genomics Core at Michigan State University) as well as by Thermo Fisher, AmpFlSTR® Identifiler® Plus PCR Amplification Kit, (Eurofins Genomics Europe Applied Genomics GmbH).
Mycoplasma contamination	Cells were tested negative for mycoplasma contamination by Mycoplasma check (qPCR with ISO ISO17025 accreditation, Eurofins Genomics Europe Applied Genomics GmbH) as well as by monthly PCR in the laboratory.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Crl:NU-Foxn1nu mice (Envigo) were used for engraftment of patient derived samples. NSG mice (Charles RIver) were used for subcutaneous implantation experiments and drug treatments.
Wild animals	No wild animals were involved in this study
Reporting on sex	Not applicable
Field-collected samples	Not applicable
Ethics oversight	All procedures and animal housing conformed to the regulatory standards and were approved by the Italian Health Minister (authorization n° 1227/2020-PR). Similarly, the animal experimental design involving orthotopic implantation was approved by the IDIBELL animal facility committee (AAALAC Unit1155) and received the procedure reference# 9111. All experiments were performed in accordance with the guideline for Ethical Conduct in the Care and Use of Animals as stated in The International Guiding Principles for Biomedical Research Involving Animals, developed by the Council for International Organizations of Medical Sciences.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completedCONSORT checklist must be included with all submissions.

Clinical trial registration	N/A Patients were treated with standard of care therapies
Study protocol	IRB approved protocols Dana-Farber/Harvard Cancer Center 02-180, 11-104,13-364, and/or 17-000
Data collection	Clinical chart review was used for data collection. Consecutive patients from 2018 to 2023 were included.
Outcomes	Endpoint included changes in gene expression profile in post- vs pre-KRAS G12i samples.

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

Cells were plated at 250,000 cells/well on 6-well plates. The following day, the cells were subjected to treatment protocols with 10uM Sotorasib for the indicated times. Cells were trypsinized, washed with PBS and with annexin binding buffer. The cell pellets were then suspended in 100µl annexin binding buffer containing 5µl of annexin V Alexa Flour 488 conjugate (Life Technologies) and PI solution at the final concentration of 10µg/ml, followed by incubation for 15minutes in the dark. 400µl of annexin binding buffer was added before the samples were analyzed by FACS.

Instrument

BD Accuri™ C6 Flow Cytometer

Software

FlowJo™ Software

Cell population abundance

n/a

Gating strategy

Cells were gated according to their SSC/FSC features. Cell doublets were discriminated and excluded from the analysis. PI and Annexin-V positive cells were gated to identify apoptotic cells. Unstained cells were used to set up the gating strategy for FL-1 (Annexin-V) and and FL-3 channels (PI).

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