nature portfolio

Corresponding author(s): Amyn A. Habib

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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	Luciferase/protein concentration/ELISA assay: software installed in POLARstar Omega Microplate Reader (BMG LABTECH) Real-time PCR: software installed in ViiA 7 Real-Time PCR System MRI imaging: software installed in a 7T small animal MRI scanner WB: ChemiDoc MP Imaging System Migrated cells in invasion assay: Tissue culture microscope & EVOS FL color imaging system Images of H&E staining and immunostaining: LECA DM6000B microscope In vivo time-lapse images of migrating tumor cells: Zeiss LSM780 confocal/multiphoton microscope Time lapse single cell/neurospheres images: Andor spinning disk confocal microscope RNA-sequencing, Whole exome sequencing: NextSeq500
Data analysis	Real-time PCR: software installed in ViiA 7 Real-Time PCR System (Applied Biosystems) and GraphPad Prism 8.0.0. Gene Set Enrichment Analysis (GSEA v4.2.1) and ssGSEA v.10.0.1 at http://software.broadinstitute.org/gsea/index.jsp. MRI, WB images: ImageJ bundled with Java (v 1.8.0_112). Sequence alignment and annotation: BWA-MEM, Picardtools (v2.25.5), bedtools (v2.30.0), GATK (v3.8),Samtools(v1.12), WGSA5, RNA-seq analysis: StringTie(v1.3.5),RseQC(v3.0.0),DESeq2(v1.32.0). Statistical Analysis: GraphPad Prism 8.0.0. Tracking of in vivo cell movements: Imaris 9.0. Tracking of single cell migration movements: ImageJ manual track Plugin (v 1.8.0_112). The invasion distance of cells out of the neurospheres: ImageJ manual track Plugin (v 1.8.0_112). Flow cytometry: FlowJo 7.6.

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

RNA–seq and WES data that support the findings of this study have been deposited in the NCBI Sequence Read Archive (SRA) with accession number PRJNA812870 and PRJNA827815. Publicly available WES data were from NCBI SRA with accession number PRJNA543854 (SRX5870263).
Previously published microarray data are available in Supplementary Table 1 of Ramnarain et al.1
Mass spectrometry data have been deposited in https://massive.ucsd.edu/ with Dataset Identifier: MSV000089272.
The human GBM data were derived from the TCGA Research Network: http://cancergenome.nih.gov/.
All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

Ramnarain, D. B., Park, S., Lee, D. Y., Hatanpaa, K. J., Scoggin, S. O., Otu, H., Libermann, T. A., Raisanen, J. M., Ashfaq, R., Wong, E. T., Wu, J., Elliott, R., and Habib, A. A. (2006) Differential gene expression analysis reveals generation of an autocrine loop by a mutant epidermal growth factor receptor in glioma cells, Cancer Res 66, 867-874.

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

Sample sizes of animal experiments were based on power analysis. 1. effect size of 1.67 was assumed to display a reduction of 50% tumor size with a standard deviation of 30% 32 days after treatment; 2. 85% power and 5% type I error; 3.Two-sample two-tailed t-test for two independent means.

For other experiments, no statistical methods were used to predetermine the sample size. Sample sizes were chosen based on our previous publications and similar studies in this field. (Gong, K. et al. EGFR inhibition triggers an adaptive response by co-opting antiviral signaling pathways in lung cancer, Nature Cancer 1, 394-409(2022); Guo G. et al. A TNF-JNK-Axl-ERK signaling axis mediates primary resistance to EGFR inhibition in glioblastoma, Nat. Neurosci. 20, 1074-1084 (2017).)

Data exclusions

No collected data were excluded.

Replication

Data of the following assays are the results of 3 independent experiments.
Matrigel invasion assay, 3D culture and spheroid invasion assay, Scratch wound assay, Brdu incorporation assay, Annexin V/PI positive staining assay, luciferasie assay, qPCR, ELISA.
The Western blot images are representative of three independent biological replicates.
Images of H&E staining and immunostaining, MRI images are representative of indicated number of mouse in animal study.
In vivo cell migration images are representative of three mice.
Data of single cell migration analysis are representative of two independent experiments.
RNA-sequencing was performed by triplicate.
Mass spectrum analysis and whole exome sequencing were done once.
All replications above have similar results and are reproducible.

Randomization

For in vivo experiments, the mice were randomly divided into control and different treatment groups. All female 4-6 weeks old nude mice were used in the same experiment, surgery, treatment were performed for the same period. For randomization, all mice in one experiment were put together and assigned into different groups randomly.
No randomization of samples were involved for other experiments, since randomization is not relevant.

Blinding

For quantitative analysis, a subset of data has been analyzed in a double-blind approach, resulting in similar results.

Patient data: The person preparing samples and running western blot were unaware of the sample identity, western blot results and survival analysis of the patient were analyzed by another person.

For TCGA data analysis, the investigators were not blinded to group allocation during data collection and/or analysis, since no subjective rating of data was involved, all patients meeting the selective critiques described in manuscript would be included in the study.

For all other experiments the investigators were blinded to group allocation during data collection and/or 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
☐	☒ 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

Antibodies

Antibodies used

Antibody Supplier Cat# Lot# Clone # Dilution (WB)
EGFR EDM Millipore 06-847 3524841 Polyclonal 1:1000-2000
EGFR EDM Millipore 04-338 VR1358129 monoclonal IHC 1:200
TGFalpha EDM Millipore GF06 Polyclonal 134A-2B3 1:200
pEGFR(Tyr1068) Cell Signaling Technology 2236 19 1H12 1:1000
pEGFR(Tyr845) Cell Signaling Technology 6963 1 D63B4 1:1000
pEGFR(Tyr1173) Cell Signaling Technology 4407 9 53A5 1:1000
ERK Cell Signaling Technology 4695 28 137F5 1:1000
pERK Cell Signaling Technology 4370 24 D13.14.4E 1:1000
TAK1 Cell Signaling Technology 5206 7 D94D7 1:1000
pTAK1 Cell Signaling Technology 4508 7 90C7 1:1000
EGR1 Cell Signaling Technology 4153 5 15F7 1:1000
FLAG Cell Signaling Technology 2368 4 Polyclonal 1:1000; IP 1:50
STAT1 Cell Signaling Technology 9172 8 Polyclonal 1:1000
Ki67 Cell Signaling Technology 9027 6 D2H10 IHC 1:200
Met Cell Signaling Technology 8198 4 D1C2 1:1000
pMet Cell Signaling Technology 3077 9 D26 1:1000
p65 Cell Signaling Technology 8242 16 D14E12 1:1000
pp65 Cell Signaling Technology 3033 17 93H1 1:1000
pShc Cell Signaling Technology 2431 6 Polyclonal 1:1000
SP1 Cell Signaling Technology 9389 6 D4C3 1:1000; IF,IHC 1:200
pSTAT1 Cell Signaling Technology 9167 15 58D6 1:1000
Stat3 Cell Signaling Technology 12640 4 D3Z2G 1:1000
pStat3 Cell Signaling Technology 9145 43 D3A7 1:1000
CDC42 Cell Signaling Technology 2466 6 11A11 1:1000
RhoA Cell Signaling Technology 2117 5 67B9 1:1000
HA-Tag Cell Signaling Technology 2367 5 6E2 1:1000
FAK Cell Signaling Technology 71433 1 D5O7U 1:1000
pFAK Cell Signaling Technology 8556 5 D20B1 1:1000
Myc-Tag Cell Signaling Technology 2276 2 9B11 1:1000
BIN3 Santa Cruz Biotechnology sc-514396 B2415 C-10 1:1000; IP 1:50
p65 Santa Cruz Biotechnology sc-109 D2407 A 1:1000
TAB1 Santa Cruz Biotechnology sc-166138 K2320 B-3 1:1000
Shc Santa Cruz Biotechnology sc-967 B1420 PG-797 1:1000
B-Actin Santa Cruz Biotechnology sc-47778 H3121 C4 1:1000
pTyr Santa Cruz Biotechnology sc-508 K1616 PY20 1:1000
EMP-1 Abcam ab191181 GR3331257-2 235-1 1:1000
DOCK7 Proteintech 13000-1-AP 15247 Polyclonal 1:1000; IP:1:50
pDOCK7 IBL America 28079 OC-912 NA 1:1000
EGFR (sepharoze bead conjugate) Cell Signaling Technology 5735 5 D38B1 1:20
HB-EGF Santa Cruz Biotechnology sc-365182 H0618 H-1 IHC, IF: 1:500
SMI-31 Biolegend 801601 B222936 SMI 31 IHC: 1:500

Validation

EGFR(06-847) https://www.emdmillipore.com/US/en/product/Anti-EGFR-Antibody,MM_NF-06-847
EGFR(04-338) https://www.citeab.com/antibodies/220389-04-338-anti-egfr-antibody-rabbit-monoclonal
TGFalpha(GF06) https://www.emdmillipore.com/US/en/product/Anti-TGF-Ab-1-Mouse-mAb-134A-2B3,EMD_BIO-GF06
pEGFR(Tyr1068)(2236) https://www.cellsignal.com/products/primary-antibodies/phospho-egf-receptor-tyr1068-1h12-mouse-mab/2236
pEGFR(Tyr845)(6963) https://www.cellsignal.com/products/primary-antibodies/phospho-egf-receptor-tyr845-d63b4-rabbit-mab/6963

pEGFR(Tyr1173)(4407) https://www.cellsignal.com/products/primary-antibodies/phospho-egf-receptor-tyr1173-53a5-rabbit-mab/4407
ERK(4695) https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-137f5-rabbit-mab/4695
pERK(4370) https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370
TAK1(5206) https://www.cellsignal.com/products/primary-antibodies/tak1-d94d7-rabbit-mab/5206
pTAK1(4508) https://www.cellsignal.com/products/primary-antibodies/phospho-tak1-thr184-187-90c7-rabbit-mab/4508
EGR1(4153) https://www.cellsignal.com/products/primary-antibodies/egr1-15f7-rabbit-mab/4153
FLAG(2368) https://www.cellsignal.com/products/primary-antibodies/dykddddk-tag-antibody-binds-to-same-epitope-as-sigma-s-anti-flag-m2-antibody/2368
STAT1(9172) https://www.cellsignal.com/products/primary-antibodies/stat1-antibody/9172
Ki67(9027) https://www.cellsignal.com/products/primary-antibodies/ki-67-d2h10-rabbit-mab-ihc-specific/9027
Met(8198) https://www.cellsignal.com/products/primary-antibodies/met-d1c2-xp-rabbit-mab/8198
pMet(3077) https://www.cellsignal.com/products/primary-antibodies/phospho-met-tyr1234-1235-d26-xp-rabbit-mab/3077
p65(8242) https://www.cellsignal.com/products/primary-antibodies/nf-kb-p65-d14e12-xp-rabbit-mab/8242
pp65(3033) https://www.cellsignal.com/products/primary-antibodies/phospho-nf-kb-p65-ser536-93h1-rabbit-mab/3033
pShc(2431) https://www.cellsignal.com/products/primary-antibodies/phospho-shc-tyr317-antibody/2431
SP1(9389) https://www.cellsignal.com/products/primary-antibodies/sp1-d4c3-rabbit-mab/9389
pSTAT1(9167) https://www.cellsignal.com/products/primary-antibodies/phospho-stat1-tyr701-58d6-rabbit-mab/9167
Stat3(12640) https://www.cellsignal.com/products/primary-antibodies/stat3-d3z2g-rabbit-mab/12640
pStat3(9145) https://www.cellsignal.com/products/primary-antibodies/phospho-stat3-tyr705-d3a7-xp-rabbit-mab/9145
CDC42(2466) https://www.cellsignal.com/products/primary-antibodies/cdc42-11a11-rabbit-mab/2466
RhoA(2117) https://www.cellsignal.com/products/primary-antibodies/rhoa-67b9-rabbit-mab/2117
HA-Tag(2367) https://www.cellsignal.com/products/primary-antibodies/ha-tag-6e2-mouse-mab/2367
FAK(71433) https://www.cellsignal.com/products/primary-antibodies/fak-d5o7u-xp-rabbit-mab/71433
pFAK(8556) https://www.cellsignal.com/products/primary-antibodies/phospho-fak-tyr397-d20b1-rabbit-mab/8556
Myc-Tag(2276) https://www.cellsignal.com/products/primary-antibodies/myc-tag-9b11-mouse-mab/2276
BIN3(sc-514396) https://www.scbt.com/p/bin3-antibody-c-10?requestFrom=search
p65(sc-109) https://www.scbt.com/p/nfkappab-p65-antibody-a?requestFrom=search
TAB1(sc-166138) https://www.scbt.com/p/tab1-antibody-b-3?requestFrom=search
Shc(sc-967) https://www.scbt.com/p/shc-antibody-pg-797?requestFrom=search
B-Actin(sc-47778) https://www.scbt.com/p/beta-actin-antibody-c4?requestFrom=search
pTyr(sc-508) https://www.scbt.com/p/p-tyr-antibody-py20?requestFrom=search
EMP-1(ab191181) https://www.abcam.com/human-nuclear-antigen-antibody-235-1-ab191181.html
DOCK7(13000-1-AP) https://www.ptglab.com/products/DOCK7-Antibody-13000-1-AP.htm
pDOCK7(28079) https://www.ibl-america.com/dock7-y1118-phosphorylated-anti-human-rabbit-igg-affinity-purify/
EGFR (sepharoze bead conjugate)(5735) https://www.cellsignal.com/products/wb-ip-reagents/egf-receptor-d38b1-xp-rabbit-mab-sepharose-bead-conjugate/5735
HB-EGF(sc-365182) https://www.scbt.com/p/hb-egf-antibody-h-1
SMI-31(801601) https://www.biolegend.com/en-us/products/purified-anti-neurofilament-h-nf-h-phosphorylated-antibody-11476

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

All PDXs were obtained from Mayo Clinic Brain Tumor Patient-Derived Xenograft National Resource.
Human astrocyte and A431 were purchased from ATCC. U251 was purchased from MillorePore Sigma.
GBM9 was provided by Dr. James Van Brocklyn.
GSC11 was provided by Dr. Shi-Yuan Cheng (Northwestern University).
GS622 was provided by Dr. Brent Cochran (Tufts University).
U343 GBM was provided by Dr. Luiz Penalva (University of Texas Health Science Center).

Authentication

All PDXs were authenticated using short-tandem repeat profiling by the Mayo Clinic Brain Tumor Patient-Derived Xenograft National Resource.
GBM9, GSC11, GS622 and U251 were authenticated by DNA fingerprints for cell-line individualization using Promega StemElite ID system, a short tandem repeat (STR)–based assay, at UT Southwestern genomics core.
No authentication was conducted for A431 and U343.

Mycoplasma contamination

Cells were tested for mycoplasma contamination using an e-Myco kit (Boca Scientific). All cell lines are negative for mycoplasma contaminatoin

Commonly misidentified lines
(See ICLAC register)

ICLAC cell lines were not used in this study.

Animals and other organisms

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

Laboratory animals

Nude mice (088), female, 4-6 weeks old, from Charles River Laboratories (Wilmington, MA).

Wild animals

No wild animals were used in this study

Field-collected samples

No field-collected samples were used in this study

Ethics oversight	All animal studies were done under Institutional Animal Care and Use Committee (IACUC)-approved protocols at UT Southwestern and North Texas VA Medical Center (Dallas, Texas, USA).

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

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 in 6 well plates and treated with drugs or vehicle alone. After 72 hours cells were trypsinized and washed 2 times with 1× PBS. The cells were incubated for 15 minutes at room temperature with Propidium Iodide and Annexin –V-FLUOS labeling solution in incubation buffer. Annexin and/or PI positive cells were detected by Flow Cytometry.
Instrument	BD FACSCalibur™ Flow Cytometer
Software	Data were analysed with FlowJo software
Cell population abundance	Cell sorting not employed
Gating strategy	Tumor cell population was gated on FSC/SCC plot by removing debris.

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