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Date: Jul 9, 2017

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work we publish. This form is published with all life science papers and is intended to promote consistency and transparency in reporting. All life sciences submissions use this form; while some list items might not apply to an individual manuscript, all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   No statistical methods were used to predetermine sample size. We chose to use 4 NSG mice per experimental group so that technical replicate tumours could be available for barcode data analysis.

2. Data exclusions

   Describe any data exclusions.

   Mice which did not harbour tumours at the pre-determined endpoint (6 months post-injection) were not used for immunohistochemistry or barcode sequencing analysis.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   Attempts to replicate our drug assays have been successful (n = 3 independent experiments). We did not attempt to replicate in vivo barcoding experiments due to limiting numbers for uncultured GBM cells.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   NSG mice from separate litters were randomized to vehicle control or TMZ treatment groups in order to control for age.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   For xenograft studies, investigators were not blinded during group allocation and data analysis. Investigators were blinded during data collection for in vitro limiting dilution analyses (LDA).

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *p* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Custom code used in this study are available upon reasonable request from B.D.S. Other software are listed within the relevant methods descriptions.

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	The lentiviral barcode library used in this study is available upon reasonable request from C.J.E. The primary GBM cells are unavailable due to limiting quantities and cell viability. The primary GBM cultures are available upon reasonable request from P.B.D. All other materials are commercially available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	All antibodies in this study are described in the methods section under "histopathology and immunohistochemistry".

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Primary cell lines were all derived from GBM tumours.
b. Describe the method of cell line authentication used.	Cell lines were confirmed to match their parental primary GBM tumour tissue by microsatellite genotyping (The Centre for Applied Genomics, Hospital for Sick Children).
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell lines were randomly and intermittently tested for mycoplasma to confirm lack of contamination.
d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used in this study.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Female NSG mice (Mus musculus) of age 1-3 months were used for all xenograft studies.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	GBM cells were processed following surgical resection from patients that were either untreated (Primary GBMs) or Temozolomide treated (Secondary GBM sample GBM-742).