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Corresponding author(s): Monte M. Winslow

Life Sciences Reporting Summary

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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.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   The sample size of mice and of tumors are reported. The number of mice in each group was based on our previous studies of KTC mice. Tumor numbers were determined using the Tuba-seq pipeline as described in the Methods and provided on-line: github.com/petrov-lab/tuba-seq.

2. Data exclusions

   Describe any data exclusions.

   No Data was excluded.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   The Lenti-sgPool tumor size distributions within KT;Cas9 mice were highly-reproducible (R2 = 0.96 of LN Mean estimates) between mice harboring tumors for 12 and 15 weeks. The RB1-TP53 and SETD2-STK11 interactions were tested using traditional methodologies and reproduced our Tuba-seq finding.

4. Randomization

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

   Mice cohorts were selected to be approximately half males and half females. The selection of mice for this study was based on availability.

5. Blinding

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

   Our Tuba-seq methodology naturally blinds researchers to the attributes of tumors, as they are only identifiable by DNA barcodes. Investigators were by necessity not blind to mouse genotypes, but the analysis closely followed our previous pipeline.

Note: all in vivo studies must report how sample size was determined and 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 in 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

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.	The analysis software is provided here: github.com/petrov-lab/tuba-seq, and was described in a previous paper: DOI:10.1038/NMETH.4297.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

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 third party.	The mouse models are publicly-accessible via the Jackson Laboratory or directly from our lab. Plasmid libraries are accessible via addgene.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No cell lines were used.
b. Describe the method of cell line authentication used.	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used have been authenticated OR state that no eukaryotic cell lines were used.*
c. Report whether the cell lines were tested for mycoplasma contamination.	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination OR state that no eukaryotic cell lines were used.*
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	*Provide a rationale for the use of commonly misidentified cell lines OR state that no commonly misidentified cell lines were used.*

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 all relevant details on animals and/or animal-derived materials used in the study.

Mouse models were described previously or detailed in the Methods. The mice were on a mixed BL6/129 background. All experiments were performed in accordance with Stanford University Institutional Animal Care and Use Committee guidelines.

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.

No human participants were used.