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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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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.

Three independent replicates were performed for most in vitro experiments, based on experience with similar previous studies. For in vivo work, where variance is increased, we used 5 mice per experimental group. This provides the power (0.7) to detect an effect size of 2 (2x increase or decrease), at a probability level of 0.05.

2. Data exclusions

Describe any data exclusions.

No data were excluded

3. Replication

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

Experiments were performed on three independent occasions. For in vitro, transduction-based experiment that make up the majority of this manuscript, this means three independent transduction / selection and target sequencing. All attempts at replication were successful

4. Randomization

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

No randomization was performed

5. Blinding

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

Investigators were not blinded during collection or analysis

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.	Statistical calculations were performed in Prism. Sequence analysis was performed on MiSeq control software, and independently validated using Geneious R11. Additional software packages used for the analysis of recurrent cancer-associated mutations: R (version 3.2.1), Bioconductor (version 2.30.0), Bsgenome (version 1.38.0), Biostrings (version 2.38.4). Each of these is open-access.

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.	All vectors described in the manuscript have been deposited at Addgene for ease of distribution

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	The specificity of the Cas9 antibody used was confirmed by including non-Cas9 expressing cells as a negative control (Supplementary Figure 5). The specificity of the Apc antibody used has previous been confirmed using shRNA-mediated knockdown of Apc in mouse tissue (Dow et al, Cell, 2015). Specificity of the Glutamine Synthetase antibody is demonstrated in Figure 3, where it marks pericentral hepatocytes. Specificity of the Alexa594 secondary antibody was confirmed by performing a 'secondary only' staining control - no staining was observed.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	293T, NIH3T3, H23, PC9, and DLD1 cells were purchased from ATCC. KH2 mESCs were derived by the Jaenisch laboratory in 2006 (Beard et al, 2006) and have been maintained by us since then. Irradiated MEF feeders were derived from DR4 embryos. Primary organoids were generated directly from C57Bl/6 mice.
b. Describe the method of cell line authentication used.	All lines were purchased directly from ATCC and frozen at early passage (P2), thus they did not require additional authentication
c. Report whether the cell lines were tested for mycoplasma contamination.	Yes. All lines tested negative for mycoplasma by PCR
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.	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.	For hydrodynamic liver transfections, 8 week old, female C57Bl/6N mice (Charles River) were used, and sacrificed 4 weeks following injection.

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.	The study did not involve human subjects