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Life Sciences Reporting Summary

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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.

Based on the variance of xenograft growth in control mice, power calculations indicated use of at least 3 mice per genotype to give 80% power to detect an effect size of 20% with a significance level of 0.05. For all animal studies, 2 or more independent cohorts were included with n=3 or more animals per treatment group

2. Data exclusions

Describe any data exclusions.

(Fig 4e) A single CD19-CAR control animal with tumor flux much larger than matched animals of the cohort (identified as an outlier, GraphPad Prism ROUT method, Q=1%), was excluded from statistical analysis.

3. Replication

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

All experiments were reproduced a minimum of 2 times, and attempts at replication were successful.

4. Randomization

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

For animal studies utilizing luciferase-expressing cell lines, animals were rank-ordered by initial tumor flux and sequentially randomized to GD2 or CD19 CAR T cell treatment groups. For survival cohorts lacking luciferase expression, animals were randomized into GD2 or CD19 CAR T cell treatment groups.

5. Blinding

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

Investigators were initially blinded to treatment in initial cohorts, but the dramatic nature of GD2 response renders blinding ineffective upon tumor clearance. For all microscopy analysis, investigators were blinded to treatment group during cell counting.

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 tests were conducted using Prism (GraphPad) software for most analyses. For confirmation of Cas9-mediated deletion of GD2 synthase, the TIDE webtool was utilized (version 2.0.1). Microscopy images were processed in Stereoinvestigator (MBF Biosciences) and ImageJ.

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.	Materials including patient-derived cell cultures are freely available with a standard MTA from the corresponding authors.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Primary antibodies used were: rabbit anti-H3K27M (Abcam [EPR18340], 1:1000), rabbit polyclonal anti-cleaved caspase-3 (Cell Signaling Technology, 9661, 1:250), mouse anti-NeuN (Millipore, MAB377, 1:500), and rabbit polyclonal Iba1 (Wako, 019-19741, 1:500). Secondary antibodies raised in donkey and conjugated with AlexaFluor 594 or 647 were used at 4C overnight to detect primary labeling (Jackson ImmunoResearch, 1:500). All antibodies have been validated in the literature and/or in Antibodypedia for use in mouse immunohistochemistry or human cell immunohistochemistry or FACS. To further validate the antibodies on our hands, we confirmed that each antibody stained in the expected cellular patterns and brain-wide distributions.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

patient-derived DMG cultures were derived as indicated in the methods and references from primary tumor samples.

b. Describe the method of cell line authentication used.

cell cultures were authenticated by STR fingerprinting

c. Report whether the cell lines were tested for mycoplasma contamination.

Cell cultures are routinely tested for mycoplasma, and all tested negative for mycoplasma

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.

NSG mice were used for all animal studies in the manuscript as indicated in methods section and were housed according to institutional guidelines. Mice of both sexes were used throughout all studies. SU-DIPG6 and SU-DIPG13FL xenografts were established at P0-2 and treated 7-8 weeks later. SU-DIPG13P* were established at P35 and treated 14 days later. SU-pSCG1, and QCTB-R059 xenografts were established at P35 and treated 7-8 weeks later.

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.

This study did not involve human research participants. Covariate characteristics of cell cultures including histone mutation status and sex are provided in Supplementary Table 2.

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☐ Initial submission ☐ Revised version ☒ Final submission

Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Cultured cell lines as indicated were harvested and stained with relevant fluorochome-conjugated antibodies.
6. Identify the instrument used for data collection.	Data were collected on a BD LSR Fortessa analyzer and BD FacsAria II.
7. Describe the software used to collect and analyze the flow cytometry data.	Data were collected in BD FacsDiva and analyzed with FlowJo.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Surface marker intensity was determined within singlet, live cell fractions as assessed by forward/side scatter and ability to exclude DNA intercalating stain (DAPI). Live cell fractions were routinely >70%.
9. Describe the gating strategy used.	Cell singlets were identified by FSC/SSC gating, and live cells further identified by exclusion of DAPI compared to unstained controls. For cell-surface screens, a fixable live/dead violet stain was used to exclude dead cells. Positive stain intensity was then defined as the median fluorescence intensity (MFI) of the stained population above matched isotype controls as indicated in the methods.

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