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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	All metabolomics data was collected and analyzed using Agilent MassHunter software. qRT-PCR data was gathered using 7500 Software v2.3. Cell number for cell viability experiments were collected using the Countess III cell counter (Thermo Fisher Scientific). All in vivo bioluminescence data was acquired by IVIS Spectrum (Perkin Elmer) instrument. Immunohistochemistry images were visualized using the Aperio Vista scanning system (AperioScanscope Scanner) and Aperio ImageScope software program was used to view each slide at 40x magnification.
Data analysis	All immunoblot images were quantified using Image J 1.53k. Graphs were generated and statistical analyses were performed using Prism software (version 9.5.1, Graph Pad, La Jolla, CA). Quantification of Immunohistochemistry images was performed using on MATLAB using an automated analysis program previously published by our lab (Panwalkar et al, Acta Neuropathologica, 2017 PMID:28733933). For 13C labelled samples, isotopic correction of raw GC-MS peaks for all reported metabolites was performed using the IsoCorrectoR package (v 1.5.1) available as part of the Bioconductor library (BioC 3.8) and implemented in R (CRAN 3.6.1). DEseq2 was used to identify differentially upregulated and downregulated genes from the RNA seq experiments and the differentially enriched pathways were identified using Enrichr (https://maayanlab.cloud/Enrichr/).

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

The ChIP seq and ATAC seq data generated in this study are accessible through the GEO series accession numbers GSE294954 and GSE294955. Gene expression data shown in (Extended Data Fig 7F, 8J) were derived from Pajtler et al, Cancer Cell, 2015 and were downloaded from the NCBI GEO database with the accession number GSE64415. Single cell RNA sequencing data shown in (Extended Data Fig 7G, 8K) are from Gojo et al, Cancer Cell, 2020 and were downloaded using the accession number GSE141460. Data in (Fig 4G, Extended Data Fig 8G, 8L) derived from Pediatric cbioportal ependymoma GSE50385 dataset (https://pedcbioportal.kidsfirstdrc.org/).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

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	For in vivo animal experiments sample size was calculated based on tumor variance to use at least n= 5 -20 mice to detect differences in overall survival with 80% power (0.05 significance). All metabolomics, isotope tracing, and qPCR experiments, utilized sample sizes of 4-5 biological replicates and the number of replicates were chosen based on the variability observed in pilot studies. For RNA sequencing, ChIP sequencing, and ATAC sequencing, n=2 replicates were utilized for each sample shown. For cell viability and cell count experiments a minimum of n=3 replicates were used per experiment and each experiment was repeated at least twice with the exact number indicated in the Figure legends and in the Statistics and Reproducibility section of the methods.
Data exclusions	No data was excluded from the analyses.
Replication	All experiments were reproduced atleast twice with n=3 independent experimental replicates unless noted. The exact number of independent

Replication	replicates or samples is indicated in the Figure legends and in the Statistics and Reproducibility section of the methods.
Randomization	All in vivo experiments were performed after randomizing the mice to avoid any bias. The cells used in the in vitro experiments were not randomized as they were collected from a homogeneous, clonally identical population.
Blinding	Quantification of immunohistochemical staining was performed in a blinded manner where an experimenter unaware of the study design captured JPEG images from three randomly selected areas of stained sections for every sample. For in vivo experiments blinding was not possible since Vehicle/drug treatments needed to be conducted in all tumor bearing mice.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	The following antibodies were used in the immunoblotting experiments, RELA (Cell Signaling Technology #8242, 1:1000), GAPDH (Cell Signaling Technology, #2118, 1:10,000), Vinculin (Sigma Aldrich, #V9264, 1:40,000), ACOD1-human (Abcam #ab222411, 1:1000 and Novus Biologicals #NBP3-06244, 1:1000), ACOD1-mouse (Cell Signaling Technology #17805, 1:1000), ZFTA (C11orf95) (VWR #89379-010, Supplier number #AP11349B, 1:1000), RFP (Abcam #Ab124754, 1:1000), MAML3 (Invitrogen #PA5-13678, 1:1000), SLC1A5 (Cell Signaling Technology #5345, 1:1000), Glutaminase-human (Cell Signaling Technology #49363, 1:1000), Glutaminase-mouse (Invitrogen #PA5-35365, 1:1000), c-MYC (Abcam #32072, 1:1000), PTEN (Cell Signaling Technology #9559, 1:1000), p-AKT (S473) (Cell Signaling Technology #9271, 1:1000), AKT (Cell Signaling Technology #4056, 1:1000), p-S6RP (S235/236) (Cell Signaling Technology #4858, 1:1000), S6RP (Cell Signaling Technology #2217, 1:1000), p-GSK3α/β (Cell Signaling Technology #9331, 1:1000), GSK3α/β (Cell Signaling Technology #5676, 1:1000), H3K4me3 (Cell Signaling Technology #9751, 1:1000), H3K9me3 (Cell Signaling Technology #13969, 1:1000), H3K27Ac (Cell Signaling Technology #8173, 1:1000), H3K27me3 (EMD Millipore #07-449, 1:1000), and Total H3 (Cell Signaling Technology #3638, 1:5000). The following secondary antibodies were used Goat-anti-mouse (BIORAD, #1706516), Goat-anti-rabbit (BIORAD, #1706515). For immunohistochemistry, a rabbit monoclonal anti-ACOD1 antibody (1:200, Abcam, #ab238580) or anti-PTEN (1:200, Abcam, #ab170941), H3K27me3 (EMD Millipore #07-449, 1:150), Ki-67 (Invitrogen #MA5-14520, 1:400), and SLC1A5 (Sigma Aldrich #HPA035240, 0.1ug/mL) were used.
Validation	RELA (Cell Signaling Technology #8242): Figs.1E, 1G, Figs. 2A, 2C, 2H, 2J Figs. 3B, 3H, 3L, Figs. 5C, 5G Extended Data Figs. 1H, 3A, 3B, 3D, 3E, 3F, 3I, 3M, 3N, 7B, 11B, 11D. ACOD1-mouse (Cell Signaling Technology #17805): Figs. 1E, Extended Data Figs. 1I, 1L, 2A, 5N ACOD1-human (Abcam #ab222411): Fig 1H, Extended Data Figs. 1E, 2A, 3C, ACOD1-human (Novus Biologicals #NBP3-06244): Extended Data Fig. 1E ZFTA (C11orf95) (VWR #89379-010, Supplier number #AP11349B): Figs. 3B, 3C, Extended Data Figs. 5C, 5D, 5E, 5F RFP (Abcam #Ab124754):Fig. 3H MAML3 (Invitrogen #PA5-13678): Fig 5F SLC1A5 (Cell Signaling Technology #5345): Extended Data Fig 7E Glutaminase-human (Cell Signaling Technology #49363): Fig 4C, Extended Data Figs. 7B, 7D, 9J Glutaminase-mouse (Invitrogen #PA5-35365): Figs. 4C, 4H, Extended Data Figs. 7C, 8M, 9K c-MYC (Abcam #32072): Fig 4H, Extended Data Figs. 8C, 8M PTEN (Cell Signaling Technology #9559): Figs. 4E, 4K, Extended Data Figs. 8H, 9D, 9I, 9J p-AKT (S473) (Cell Signaling Technology #9271): Fig 4E, 4H, Extended Data Figs. 8C, 8D, 8M, 9K AKT (Cell Signaling Technology #4056): Fig 4E, 4H, Extended Data Figs. 8C, 8D, 8M, 9K p-S6RP (S235/236) (Cell Signaling Technology #4858): Fig 4E S6RP (Cell Signaling Technology #2217): Fig 4E p-GSK3α/β (Cell Signaling Technology #9331): Fig 4E, 4H, Extended Data Figs. 8M, 9K GSK3α/β (Cell Signaling Technology #5676): Fig 4E, 4H, Extended Data Figs. 8M, 9K H3K4me3 (Cell Signaling Technology #9751): Figs. 2F, 2G, 2H, Extended Data Figs. 3H, 3I, 3J, 3K, 3M, 3O, 5L, 10B H3K9me3 (Cell Signaling Technology #13969): Fig 4I, Extended Data Figs. 3H, 3L, 10B H3K27Ac (Cell Signaling Technology #8173): Fig 4I, Extended Fig 3L H3K27me3 (EMD Millipore #07-449): Fig 4I, 4K, Extended Data Fig. 9D Total H3 (Cell Signaling Technology #3638): Figs. 2F, 2G, 2H, 4I, 4K, Extended Data Figs. 3H, 3I, 3J, 3K, 3M, 3O, 5L, 9D, 10B GAPDH (Cell Signaling Technology, #2118): Figs. 1E, 2A, 2C, 3E, 3J, 3N, 4C, 4E, 5C, 5G, Extended Data Figs. 1E, 1H, 1I, 3B, 3C, 3D, 3E, 3F, 3K, 3J, 5C, 5D, 5F, 5N, 7B, 7C, 7D, 7E, 8C, 8D, 9I, 11B, 11D Vinculin (Sigma Aldrich, #V9264): Figs. 1G, 1H, 2A, 2H, 2J, 3B, 3C, 4C, 4H, 4K Extended Data Figs. 1E, 1L, 2A, 3A, 5E, 7E, 8M, 9D, 9J, 9K

Immunohistochemistry:
anti-ACOD1 antibody (Abcam, #ab238580): Fig. 1F, Extended Data Fig. 1F
anti-PTEN (Abcam, #ab170941): Fig 4F, Extended Data Fig 8I, 12M
H3K27me3 (EMD Millipore #07-449): Fig. 4J, Extended Data Fig 9C
Ki-67 (Invitrogen #MA5-14520, 1:400): Extended Data Fig 12K
SLC1A5 (Sigma Aldrich #HPA035240): Extended Data Fig 7H

All antibodies utilized in the study were validated by the manufacturer.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Cell lines from collaborators were obtained under MTA. Mouse neural stem cells (mNSCs) with p16 Ink4a-/p14Arf background and EP1NS (human ZFTA-RELA) cell line were provided by Dr. Richard Gilbertson (University of Cambridge, UK). RCAS-TV control (NS-1, NS-2, and NS-3) and RCAS-TV ZFTA-RELA (H-57, H-41, and H-59) cell lines were provided by Dr. Eric Holland (Fred Hutchinson Cancer Research Center). ST-1 (ZFTA-NEAT1, ZFTA-MAML3) , ST-2 (ZFTA-RELA) , ST-4 (ZFTA-RELA) were provided by Dr. Kulandaimanuvel Antony Michaelraj (University of Pittsburgh School of Medicine). CPITT-1 (ZFTA-RELA) cell line was provided by Dr. Sameer Agnihotri (University of Pittsburgh School of Medicine). MAF-1329 (ZFTA-RELA) and MAF-811 (PF-A) cell lines were provided by Dr. Andrea Griesinger and Dr. Nicholas Foreman (University of Colorado Anschutz Medical Campus). CPITT-1 (ZFTA-RELA) cell line was provided by Dr. Sameer Agnihotri (University of Pittsburgh School of Medicine). MAF-1329 (ZFTA-RELA) and MAF-811 (PF-A) cell lines were provided by Dr. Andrea Griesinger and Dr. Nicholas Foreman (University of Colorado Anschutz Medical Campus). HEK293 cells were procured from ATCC (Cat #CRL-1573)
Authentication	All cell lines were authenticated by STR testing.
Mycoplasma contamination	Cell lines were routinely screened for Mycoplasma using the MycoAlert kit (Lonza) and tested negative.
Commonly misidentified lines (See ICLAC register)	None used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	4- 6 weeks old, NOD-SCID-IL2R gamma chain deficient (NSG) mice (NOD.Cg-Prkdcscidll2rgtml Wjl/SzJ, #005557) were used for all experiments involving subcutaneous and orthotopic injections of the ZFTA-RELA fusion mNSCs as well as for the subcutaneous MAF-1329 patient-derived xenograft (PDX) model. For the in-utero electroporation (IUE) model we utilized pregnant 6 week old CD-1 (Charles River, Crl:CDl(ICR), #022), Acod1 WT C57BL6 animals (Jax laboratories, #003771). Similarly 6-8 week old Acod1 -/- or Nestin Cre, and Acod1 floxed mice were used for breeding before in-utero electroporation of the ZFTA-RELA plasmid into the embryos. Electroporation was performed on E15 for the embryos.
Wild animals	No wild animals were used in the study.
Reporting on sex	All in vivo experiments were performed on an equal proportion of male and female animals and all findings were reported in both sexes.
Field-collected samples	Field-collected samples were not used in this study.
Ethics oversight	All animal procedures were approved by the University of Michigan Committee on the Use and Care of Animals (PRO00010599 and PRO00011114).

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	All files are deposited in GEO under the following accession numbers, GSE294954 and GSE294955
Files in database submission	SI_39603_MNSC_DMSO_50K_Repl_R2.fq.gz SI_39604_MNSC_DMSO_50K_Rep2_R2.fq.gz SI_39606_MNSC_Citraconate_50K_Repl_R2.fq.gz SI_39607 _MNSC_Citraconate_50K_Rep2_R2.fq.gz SI_33920_mNSC_EmptyVector_Repl_R2.fq.gz SI_33921_mNSC_EmptyVector_Rep2_R2.fq.gz SI_33922_mNSC_ZFTA_RELA_Repl_R2.fq.gz SI_33923_mNSC_ZFTA_RELA_Rep2_R2.fq.gz SI_33924_mNSC_ZFTA_RELA_DMSO_Repl_R2.fq.gz SI_33925_mNSC_ZFTA_RELA_DMSO_Rep2_R2.fq.gz SI_33926_mNSC_ZFTA_RELA_GSK_Repl_R2.fq.gz SI_33927 _mNSC_ZFTA_RELA_GSK_Rep2_R2.fq.gz SI_33928_mNSC_ZFTA_RELA_dmCitra_Repl_R2.fq.gz SI_33929_mNSC_ZFTA_RELA_dmCitra_Rep2_R2.fq.gz SI_39405_EPN1425_DMSO_H3K4me3_R2.fq.gz SI_39406_EPN1425_DMSO_H3K4me3_R2.fq.gz SI_39407 _EPN 1425 _lOm M_ Citraconate_H3K4me3_R2. fq.gz SI_39408_EPN1425_10mM_Citraconate_H3K4me3_R2.fq.gz SI_39409_EPN1425_DMSO_H3K27me3_Rl_R2.fq.gz SI_39410_EPN1425_DMSO_H3K27me3_R2.fq.gz SI_39411_EPN1425_10mM_Citraconate_H3K27me3_R2.fq.gz SI_39412_EPN1425_10mM_Citraconate_H3K27me3_R2.fq.gz SI_39263_MNSC_DMSO_Rl_H3K4me3_R2.fq.gz SI_39264_MNSC_DMSO_R2_H3K4me3_R2.fq.gz SI_39265_MNSC_10mM_Citraconate_Rl_H3K4me3_R2.fq.gz SI_39266_MNSC_10mM_Citraconate_R2_H3K4me3_R2.fq.gz SI_39267 _M NSC_DMSO _Rl_H3K27me3 _R2.fq.gz SI_39268_MNSC_DMSO_R2_H3K27me3_R2.fq.gz SI_39269_MNSC_10mM_Citraconate_Rl_H3K27me3_R2.fq.gz SI_39270_MNSC_10mM_Citraconate_R2_H3K27me3_R2.fq.gz SI_34243_EPN1425_DMSO_H3K4me3_R2.fq.gz SI_ 34245 _EPN 1425 _ Citraconate _24h _H 3 K4me3 _R2. fq.gz
Genome browser session (e.g. UCSC)	No longer applicable

Methodology

Replicates	n=2 independent replicates were used for all ChIP-seq and ATAC-seq experiments [except Extended Data Fig. 5H (n=l each)]
Sequencing depth	Sequence depth of 40-45M
Antibodies	H3K4me3 (CST, Cat# 97515), H3K27me3 (Millipore,Cat# 07-449)
Peak calling parameters	MACS2 was used to call peaks, filtered using bedtools, and converted to bigwigs with UCSC wigtoBigwigl. Cistrome overlap analysiswas performed in R (v3.6.0) using ChipSeekAnno (v3.0.0) and ChipSeeker (vl.29.1). Enrichment heatmaps were generated using Deeptools. ATAC-seq libraries were sequenced on the lllumina HiSeq 2500 platform, utilizing a 2x50-nucleotide paired-end read length with a sequence depth of 40-45M. Sequencing of ATAC-seq libraries generated fastq files, which were initially processed using Trimmomatic (version 0.39) for trimming. These files were then aligned to the mmlO (GRCm38) mouse genome reference or hg38 (GRCh38) reference using bwa mem (version 0.7.17-r1198-dirty), and the alignments were converted to binary format with SAMtools (version 1.9). Next, we eliminated mitochondrial and duplicated reads using SAMtools and PICARD MarkDuplicates (version 2.26.0-1-gbaf4d27-SNAPSHOT). Peaks in the ATAC-seq data were identified using MACS2 (version 2.1.1.20160309). Finally, the conversion of data to bigwig format was accomplished using the UCSC tool wigtoBigwig.
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	MACS2 was used to call peaks, filtered using bedtools, and converted to bigwigs with UCSC wigtoBigwigl. Cistrome overlap analysiswas performed in R (v3.6.0) using ChipSeekAnno (v3.0.0) and ChipSeeker (vl.29.1). Enrichment heatmaps were generated using Deeptools. ATAC-seq libraries were sequenced on the lllumina HiSeq 2500 platform, utilizing a 2x50-nucleotide paired-end read length with a sequence depth of 40-45M. Sequencing of ATAC-seq libraries generated fastq files, which were initially processed using Trimmomatic (version 0.39) for trimming. These files were then aligned to the mmlO (GRCm38) mouse genome reference or hg38

(GRCh38) reference using bwa mem (version 0.7.17-r1198-dirty), and the alignments were converted to binary format with SAMtools (version 1.9). Next, we eliminated mitochondrial and duplicated reads using SAMtools and PICARD MarkDuplicates (version 2.26.0-1-gbaf4d27-SNAPSHOT). Peaks in the ATAC-seq data were identified using MACS2 (version 2.1.1.20160309). Finally, the conversion of data to bigwig format was accomplished using the UCSC tool wigtoBigwig.