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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	Image acquisition for the determination of GI50 by Celigo Imaging Cytometer (Nexcelom Bioscience, Lawrence, MA, USA); the measurement of Caspase-3/7 activity by EnSpire Multimode Plate Reader (PerkinElmer, Waltham, MA, USA); the mRNA expression levels by Applied Biosystems 7900HT Fast RealTime PCR System (Applied Biosystems, Waltham, MA, USA); light microscopy imaging was performed by Olympus Digital Camera XC50 (Olympus Corporation, Shinjuku-ku, Tokyo, Japan); flow cytometry experiments were performed on FACS CantoII equipped with a FACSDiva 6.1 CellQuestTM software (Becton Dickinson Instrument, San Josè, CA, USA); B-galactosidase staining assay images were acquired with Leica DMi8 microscope (Leica microsystems, Mannhein, Germany); fluorescence images were acquired with Leica DMi8 microscope (Leica microsystems, Mannhein, Germany); Proximity Ligation Assay images were acquired by Olympus FV3000 confocal microscopy with Olympus FV315S-SW image 26 acquisition software; the light microscopy immunohistochemistry imaging was performed on a Nikon 28 E600 light microscope equipped with NIS Elements BR software, using 20x objective.
Data analysis	Poly-A selected RNA libraries were prepared and sequenced on an Illumina HiSeq2000. Reads were aligned to hg38 using STAR version 2.6.0a, and gene expression was calculated as Fragments Per Kilobase of transcript per Million mapped reads (FPKM) and Transcripts per Million (TPM) using edgeR v3.7. GO enrichment output was created using GSEA running filtered up-regulated gene lists (> 1.5 log2 fold change). z score normalization has been performed to visualize differentially expressed genes as heatmap. ChIP-seq enrichment peaks of public datasets were visualized with IGV. AQuA HiChIP data of public dataset was visualized in Juicebox. Software used for statistical tests include Graphpad Prism(8.4.3) and Microsoft Excel (16.0.10392). Other used softwares were ImageJ(2.0.0), Adobe Photoshop (2022), Adobe Illustrator (2022).

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

Previously published GEO datasets used in the study were: Affymetrix profiling data (GSE66533, GSE14333, GSE108474, GSE111678, GSE14827, GSE16011, GSE26673, GSE31684, GSE32676, GSE32701, GSE34620, GSE39671, GSE42743, GSE43580, GSE64019, GSE64415, GSE7553, GSE7696, GSE87371, GSE9843, GSE2658, GSE16476, GSE9891, GSE9103, GSE2109, GSE7307 and (Northcott PA. et al., Nature 2017).
ChIP-seq data (GSE83728, GSE137168, GSE29611).
RNA-seq data (GSE52529).
AQuA HiChIP data (GSE120770).
The remaining data are available within the article, supplementary Information or available from the authors upon request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	No sex or gender information associated with the tissue samples were requested because the study was only aimed at evaluating the expression of the SKP2 protein in primary tissues sections.
Population characteristics	Formalin-fixed paraffin embedded (FFPE) tissue blocks from cases of fusion negative or fusion positive rhabdomyosarcoma were obtained at the diagnosis in patients aged from 1 to 19 years from department archives per Institutional Review Board approval. Biopsy of adjacent normal muscle tissues were done when ethically possible. All tissue sections were de-identified prior to their use in immunohistochemical stains for the manuscript. No clinical information associated with the tissue samples was requested because the study was only aimed at evaluating the expression of the SKP2 protein in primary tissues sections and, thus, no clinical correlations were needed or done.
Recruitment	All FFPE tissue samples were obtained retrospectively. No patients were recruited for this study.
Ethics oversight	Sections of FFPE human samples were obtained from the Pathology Unit of Bambino Gesù Children's Hospital (Rome, Italy) and the approval of the study was obtained from the Institutional Ethical Committee of the Research Center (Authorization 120 LB, 02/10/2015).

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	In vivo studies were designed to obtain statistically significant results while maintaining minimum animal sacrifice according to 3Rs principle (Arfin & Zahiruddin 2017). No sample-size calculation was performed for the in vitro experiments. Each condition was analyzed with three biological replicates, a standard for the experiments performed to account for reasonable range of variability among samples.
Data exclusions	No data was excluded from the study.
Replication	Experiments were repeated in three biological replicates unless stated otherwise.
Randomization	Cell lines and mice used in the study were isogenic and maintained at minimally perturbative conditions so randomization is not relevant to the study.
Blinding	Blinding approach was not possible as the tumor phenotype was visually prominent in the shSKP2 knock down injected tumors and in the MLN4924 treated tumors. For cell line experiments blinding was either not necessary as the collection and analysis were performed by software/equipment uniformly post treatment and not possible in cases were the phenotype was prominent during data collection.

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

Methods

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

Antibodies

Antibodies used

SKP2 (H-435) Santa Cruz Biotechnology Cat# sc-7164, RRID:AB_ 2187650
MyoD (M-318) Santa Cruz Biotechnology Cat# sc-760, RRID:AB_2148870
pMYOD (Ser-200) Santa Cruz Biotechnology Cat# sc-101741, RRID:AB_ 2148764
p27 Kip1 (C-19) Santa Cruz Biotechnology Cat# sc-1641, RRID:AB_ 628074
Myosin Heavy Chain DSHB Cat# MF 20, RRID:AB_2147781
p21 Waf1/Cip1 (12D1) Cell Signaling Technology Cat# 2947, RRID:AB_823586
Nedd8 19E3 Cell Signaling Technology Cat# 2754, RRID:AB_659972
MYOG DSHB Cat# F5D, RRID:AB_2146602
p57 Kip2 BD Biosciences Cat# 556346, RRID:AB_396375
GAPDH (B16H11) Cell Signaling Technology Cat# 5174, RRID:AB_10622025
β- ACTIN (I-19) Santa Cruz Biotechnology Cat# sc-1616, RRID:AB_ 630836
aTubulin (DM1A) Novus Biologicals Cat# NB100-92249, RRID:AB_ 1218281
Vinculin (hVIN-1) Sigma Cat# V9131, RRID:AB_477629
HRP (Horseradish peroxidase) anti-rabbit Cell Signaling Technology Cat# 7074, RRID:AB_2099233
HRP anti-mouse Cell Signaling Technology Cat# 7076, RRID:AB_330924
SKP2 Thermofisher Scientific Cat# 32-3300, RRID:AB_2533074
p27 Kip1 (SX53G8.5) Santa Cruz Biotechnology Cat# sc-53871, RRID:AB_ 785029
p57 Kip2 (KP39) Santa Cruz Biotechnology Cat# sc-56341, RRID:AB_ 785045
Cleaved Caspase 3 Cell Signaling Technology Cat# 9661, RRID:AB_2341188

Validation

All antibodies were validated in RMS cell line samples by cross comparing to vendor provided molecular size information using western blotting. SKP2 antibody was validated by western blotting in SKP2 knock down in RD, JR1, RD18 and RH36 cells and by immunostaining in negative control muscle tissue.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

RD and RH30 were obtained from ATCC (Rockville, MD, USA), RD18 cells were a gift of C. Ponzetto (Department of Oncology, University of Turin, Turin, Italy). RH2, JR1, RH36 and RH4 cells were provided by P. Houghton. HSMM were purchased from Lonza (Walkersville, MD, USA). C2C12 and C3H/10T1/2 were purchased from ATCC (Rockville, MD, USA). Orthotopic patient-derived xenograft (o-PDX) cells SJRHB011_YC, SJRHB012_YC and SJRHB012_ZC were obtained through the Childhood Solid Tumor Network (CSTN) at St. Jude Children's Hospital (Memphis, TN, USA).

Authentication

The cell lines used were authenticated by STR profiling.

Mycoplasma contamination

Cell lines are tested for mycoplasma contamination by PCR every month. All lines to date tested negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

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

NSG female mice aged 6–8 weeks were used for xenografts experiments. Animals were maintained in sterile conditions, 12h light/12h dark cycle, ambient temperature 18–23°C with 40–60% humidity.

Wild animals	Study did not involve wild animals.
Reporting on sex	The xenograft studies involved female mice as in our previous published work (Pomella S et al., Nature Communication 2021). Female mice allow the housing of maximum five mice per cage.
Field-collected samples	Study did not involve field collected samples.
Ethics oversight	All animal experiments were performed in accordance with the Guidelines for Animal Care and Use of the National Institutes of Health and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas Health Science Center, San Antonio, Texas (protocol number 20150015AR) and in accordance with the European Communities Council Directive N. 2010/63/EU, the Italian Ministry of Health guidelines (DL 26/2014) and approved by the Italian Ministry of Health for the Children's Hospital Bambino Gesù/Plaisant animal facility of Castel Romano in Rome, Italy (protocol number 88/2016-PR).

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

Flow Cytometry

Plots

Confirm that:

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

☒ 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).

☐ All plots are contour plots with outliers or pseudocolor plots.

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

Methodology

Sample preparation	RD, JR1, RD18 and RH36 human fusion negative rhabdomyosarcoma cells were harvested by trypsinization, washed in cold phosphate buffered saline (PBS), fixed in cold 50% PBS and 50% acetone/methanol (1:4 v/v) for at least 1h. Fixed cells were pelleted 5 minutes 1500 rpm and alcoholic fixative removed. Pellet was stained in the dark with a solution of 50 µg/ml propidium iodide (PI) (ThermoFisher Scientific, Rockford, USA) and 50 µg/ml RNase (Sigma-Aldrich, St Louis, MO, USA) for 30 min at room temperature.
Instrument	BD FACS CantoII (Becton Dickinson Instrument, San Josè, CA, USA).
Software	BD FACSDiva 6.1 CellQuestTM software (Becton Dickinson Instrument, San Josè, CA, USA).
Cell population abundance	10.000 events were analyzed for each experimental condition. No sorting experiments were performed.
Gating strategy	All the acquired events were gated, based on SSC and FSC, to analyze only the singlets population to avoid possible bias due to doublets.

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