nature portfolio

Corresponding author(s): *Double-anonymous peer review submissions: write DAPR and your manuscript number here instead of author names.*

Last updated by author(s): *YYYY-MM-DD*

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	PerkinElmer VICTOR Nivo Control Software (version 4.0.7), LI-COR Image Studio (version 1.0.20), PerkinElmer Harmony (version 4.9), SpectroFlo (version 2.2.0.4), Bitplane Imaris imaging software
Data analysis	PerkinElmer Harmony (version 4.9), FCS Express (version 7), FIJI (version Madison), Microsoft Excel (version 16.66.1), GraphPad Prism (version 9.4.1), Molecular Operating Environment (MOE) software, Version 2022.02 (Chemical Computing Group ULC, 2022), R (version 4.1.2), R libraries - ggpubr (version 0.4), dplyr (version 1.0), Python (version 3.9.5) using pandas (version 1.3.4), numpy (version 1.20.3), seaborn (version 0.11.2), scipy (version 1.7.1), statsmodels (version 0.12.2), matplotlib (version 3.4.3).

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 authors declare that the data supporting the findings of this study are available within the article and its supplementary information files. The locations of

publicly available datasets can be found in the article. The versions of DEPMAP used in this study are the 2020 Q2 and the 2022 Q2 releases (https://depmap.org/portal/download/), as indicated in the Methods section of the manuscript. The published human KIF18A crystal structure (3LRE [http://doi.org/10.2210/pdb3lre/pdb]) was used for molecular docking.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 in vitro immunofluorescence studies, greater than 100 cells were analyzed in each experiment to permit statistical comparisons. In in vivo tumor efficacy studies, at least 4 mice were included in each group. For all other studies, no statistical method was used to predetermine sample size
Data exclusions	No data was excluded from the analyses in this study.
Replication	Experiments were conducted with technical and biological replicates as indicated in the Figure Legends. Reported findings were successfully replicated.
Randomization	Randomization was performed in grouping tumor-bearing mice into vehicle and treatment groups.
Blinding	Blinding was not performed as no human or patient information was analyzed in this study. Blinding was not necessary as all samples in the same experiment were collected and analyzed under the same conditions.

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	Antibodies for western blotting: rabbit anti-KIF18A (1:500, Fortis Lifesciences (A301-080A)), rabbit anti-GAPDH (1:2500, Abcam (ab9485)), rabbit anti-caspase-3 (1:1000, Cell Signaling Technology (9662)), rabbit anti-vinculin (1:3000, Abcam ab129002) goat anti-rabbit IgG (H+L) antibody conjugated to IRDye 680RD (1:20000, LI-COR (926-68071)) Antibodies used for immunofluorescence: anti-α-tubulin clone DM1A (1:2000, Millipore Sigma(T6199)), rat anti-phospho Histone H3 (Ser10) clone 6G8B7 (1:2000, Millipore Sigma (05-636-I)), rabbit anti-KIF18A (1:500, Fortis Lifesciences (A301-080A)). goat anti-mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (1:2000 (IF), Thermo Fisher Scientific (A11001)), Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 (1:2000 (IF), Thermo Fisher Scientific (A11077)), Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (1:2000 (IF), Thermo Fisher Scientific (A21244)). Antibodies used for tissue staining: pHH3 primary antibody dilution of 1:3200 (Millipore (06-750), KIF18A (Sigma-Aldrich, HPA039484, 1:250) and α-tubulin (Cell Signaling Technologies, 3873S, clone: [DM1A], 1:1000), Alexa Fluor-568 goat anti-rabbit IgG (Invitrogen, A11011) and Alexa Fluor-488 goat anti-mouse (Invitrogen, A11001) at 1:400.
Validation	Rabbit anti-Kif18A was validated by the manufacturer using whole cell lysates (50 µg) from HeLa, HEK293T, Jurkat, and MCF-7 cells prepared using NETN lysis buffer. Rabbit anti-GAPDH was validated by the manufacturer using whole cell lysates (20 µg) from HeLa, A431, and A549 cells. Rabbit anti-Caspase-3 was validated by the manufacturer using whole cell lysates from HCT116 wild-type and Caspase-3 knockout cells. Goat anti-rabbit IgG (H+L) antibody conjugated to IRDye 680RD was validated by the manufacturer using a dot blot and/or solid-phase adsorbed for minimal cross-reactivity with human, mouse, rat, sheep, and chicken serum proteins. Rat anti-phospho Histone H3 (Ser10) clone 6G8B7 was validated by the manufacturers using 10 µg of nocodazole treated HeLa cell lysates. Secondary antibodies from Thermo Fisher Scientific were validated as described on their website (https://www.thermofisher.com/us/en/home/life-science/antibodies/invitrogen-antibody-validation.html).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The following cell lines were purchased from American Type Culture Collection (ATCC): MDA-MB-231 (HTB-26), hTERT RPE-1 (CRL-4000), MCF10A (CRL-10317), HCC1806 (CRL-2335), and NIH:OVCAR-3 (HTB-161). The following cell lines were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ): CAL51 (ACC-302) and HCC-15 (ACC-496). JIMT-1 cells (C0006005) were purchased from AddexBio. HT-55 cells (85061105) were purchased from the European Collection of Authenticated Cell Cultures (ECACC).
Authentication	Information on cell line authentication by STR was provided by the commercial sources for each cell line.
Mycoplasma contamination	Mycoplasma testing was assessed with the MycoAlert Mycoplasma Detection Kit from Lonza (catalog number LT07-318)
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the 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	SCID Beige mice (Jihui Laboratory Animal Co. Ltd. and Beijing Vital River Laboratory Animal Technology Co., Ltd.), BALB/c nude mice (Beijing Vital River Laboratory Animal Technology Co., Ltd.), and CD-1 mice (Jihui Laboratory Animal Co. Ltd.) were used in this study, all at 6-8 week age.
Wild animals	The study did not involved wild animals
Reporting on sex	To avoid additional variables from mixed sex populations, all female mice (SCID Beige and Balb/c nude) or all male (CD-1 mice) were used in this study, as indicated in the Methods
Field-collected samples	The study did not involve samples collected in the field
Ethics oversight	Tumor studies involving animals were approved by Volastra Therapeutics and the Institutional Animal Care and Use Committee (IACUC) of WuXi AppTec following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	JIMT-1 cells were harvested from cell culture plates and fixed in 4% paraformaldehyde solution for 15 minutes. Cell pellets were washed before treatment with the FxCycle PI/RNase Staining Solution (Thermo Fisher (F10797)) according to the manufacturer's directions.
Instrument	Data was collected using a Cytek NL-2000.
Software	Data was collected using SpectroFlo (version 2.2.0.4) and analyzed with FCS Express (version 7).
Cell population abundance	No cell sorting was conducted for these experiments. 20,000 events were collected and the singlet population was selected for analysis. The singlet population represented greater than 60% of the collected events in each sample.
Gating strategy	Forward and side scattering was used to identify the singlet population and omit debris and clumped cells for PI intensity measurements. The entire singlet population was analyzed in subsequent analyses with a gate that was created to capture the population of cells positively stained with the FxCycle PI/RNase Staining Solution.

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