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Corresponding author(s): Elena Ethel Vidal-Calvo
Mads Daugaard
Ali Salanti

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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	ELISA data were collected on Microsoft Excel (Microsoft 365) Flow cytometry data were collected on DIVA (BD FACS v.8.0) Attana data were collected using Attester software. When stated, tissue slides were scanned on Zeiss Axio Z1 automated slide scanner on ZEISS Zen Blue software. Cell microscopy slides were scanned on Cytation 5 Cell Imaging Multi-Mode Reader (Agilent Technologies) HPLC disaccharide data were collected on Waters Acquity UPLC system Mass spectrometry data were collected on Evosep ONE HPLC (Evosep) Mass photometry data were collected on Refeyn instrument and Refeyn AcquireMP software (v.2023 R1.1) Structural data were collected on a TITAN Krios Electrob Microscope (FEI) and generated by Alphafold software (v2.3.1)
Data analysis	Attana data were analyzed on Evaluation software (V.3.5.07) and fitted in TraceDrawer software (Ridgeview Instruments AB) Flow cytometry data were analyzed on FlowJo software (Becton Dickinson) Microscopy images were analyzed on the Gen5 software (BioTeK, version 3.10), the Zen blue software (Zeiss, version 3.5). Mean pixel intensities were calculated on Fiji software (image J, v. 2.1.0/1.53c) HPLC disaccharide data were analyzed on Waters Acquity UPLC system Mass spectrometry data were analyzed with MaxQuant software (version 1.6.15.0), statistics on python package scipy (v1.11.2) and statsmodels (v0.14.0), heat-map visualiation (matplotlib package v3.7.1), python (v.3.9.0) Mass photometry data were analyzed on Refeyn DiscoverMP software (v203 R1.2) RNA seq data analysis and graphical visualization was performed with scanpy v.1.9.1, anndata v.0.8.0, umap v.0.5.3, numpy v.1.23.5, scipy v.1.10.1, pandas v.2.0.0, scikit-learn v.1.2.2, statsmodels v.0.13.2, pynndescent v.0.5.8, and python-igraph v.0.10.2.

IVIS data were analyzed on Living Image software (Perkin Elmer)
All data were plotted on GraphPad (Prism, version 9.5.0) and edited on Illustrator (Adobe, version 27.6.1)

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

All data relating to the findings in the article are contained in the manuscript and Supporting information. Source data are provided with this paper.
The atomic coordinates and electron-microscopy data have been deposited in the RCSB Protein Data Bank (https://www.rcsb.org) and in Electron Microscopy Data Bank (https://www.ebi.ac.uk/emdb/) under the entry PDB: 8P2E and EMDB: 17362.
The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE82 partner repository with the data set identifier PXD044455 (https://www.ebi.ac.uk/pride/archive/projects/PXD34723319).

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	We have performed experiments on animal and cell lines, with gender not being a factor in our study as the expression of ofCS in cancer is unrelated to gender. The sex of the mice was only taken into account when assessing antibody localization in reproductive organs (testis or ovary), as specified in the text
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	The tissues for immunofluorescence staining were obtained from commercialized tissue micro-array (US BioMax, Inc). Approval of the local ethical committees was given, and informed consent was obtained from all patients prior to sample acquisition and experimentation. Their tissue samples were excised by licensed medical doctors, received from certified hospitals, collected with informed consent from the donors and relatives, and diagnosed and identified by at least two evaluators. All patient data were used in an anonymized fashion according to the ethical guidelines. The in vitro use of colorectal tumor biopsies obtained from Saelland University hospital (Køge) was approved by the Danish Regional Ethical Committee (De Videnskabsetiske Komiteers) (SJ-826).

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	The number of mice used in each study was chosen using the principles of the three Rs (Replacement, Reduction and Refinement) according to previous experiment to ensure sufficient sample size resulting in meaningful differences between groups.

Data exclusions	In the mean pixel intensities quantification (Fig. 2d and Supplementary Fig. 7c), regions without DAPI and damages cores were excluded from the analysis. In the serum half-life study (Supplementary Fig. 9g), one data point was excluded of the standard curve as it was not fitting the trend of the curve. In the cytotoxicity experiments (Supplementary Fig. 10g) with CT26 and MiaPaca3 and flow on tumor suspensions (Fig. 4i and Fig. 4l), some outliers were excluded from the analysis when their value differed from more than 30% from the other replicates, or when values were not following the trend suggesting an experimental error (less reactive reagents added for instance, inducing lower signals). Otherwise, no data were excluded from the rest of the study
Replication	When possible, data were performed in replicates or repeated. However when this was not possible, for example in animal studies, the most stringent and appropiate controls were used to assess the quality of the experiment. The SDS gels on dimerized and labeled antibody fragments were repeated at least 5 times, all showed similar results demonstrating robust quality of produced proteins. Flow binding experiments were tested multiple times, at least three times on white blood cells and five time on some cancer cells lines (Karpas299, 4T1, CT26, MiaPaca2). IF staining of cancer cell in full blood is run regularly and always shows similar results. IF staining of large TMA were only run once to minimize the use of precious patient derived material. Our IF protocol has been tested extensively previously and always showed robust results.
Randomization	Randomization was performed in animal models to ensure homogeneity in treatment groups. CDX models, mice were randomized to be grouped based on average tumor size of 100-150 mm3 In PDX models, mice were randomized to reach a starting volume of 100mm3.
Blinding	Blinding was used to avoid possible human bias during experiments: both at the time of treatment injections and at each tumor measurements performed single-blinded.

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	Anti-V5-HRP antibody (Abcam, Cat:ab1325, lot:GR3289189-3) Anti-V5-HRP antibody (Invitrogen, Cat:R961-25, lot: 2525622) Anti-V5-Alexa647 antibody (Cat:45-1098, lot:2449689 (Fig. 2a and 2c + Supplementary Fig. 7a-c), lot:2498301 (Fig. 2d-e and Supplementary Figs.3-5), lot: 2356825 (Supplementary Fig. 7e+Supplementary Fig.8a+Supplementary Fig. 9i) Anti-V5-FITC antibody (Invitrogen, Cat: R963-25, lot:2331791 (Fig. 1c and Supplementary Fig. 11c) + lot: 2290072 + lot:2184511 (Fig. 1d and Supplementary Fig 2c), lot: 2498435 (Supplementary Fig 2d), lot:2614613 (Supplementary Fig. 6b + Supplementary Fig. 9g) Anti-FLAG antibody (Sigma Aldrich, Cat: F3165, lot: SLCC44005) Anti-FLAG-HRP antibody (Sigma Aldrich, Cat: A8592, lot: SLCH6307) Anti-FLAG-Alexa647 antibody (Abcam, Cat: ab245893, lot: GR3392177-9) Anti-Mouse-FITC antibody (Vector Laboratories, #FI-2000, lot: 2H0416) Anti-CD45-Alexa647 antibody (Invitrogen, clone: HI30, Cat: 17-0459-42, lot:2301141 (Fig. 1e), lot:2464430 (Supplementary Fig. 2d) Anti-CD45-FITC antibody (Miltenyi Biotec clone 5B1, lot: 5180910064 (compensation control for experiment Supplementary Fig. 2d) Anti-His-FITC antibody (Qiagen, Cat:1019199, lot:166027019) Anti-CD45-PE antibody (Biolegend, clone 30-F11, Cat:103106, lot:B399691 (Fig. 4i)) Anti-CD3-PerCP/Cy5 antibody (BioLegend, Cat:100218, lot: lot:B260625 (Fig. 4m) Anti-CD8-APC antibody (Invitrogen, Cat:48-0441-82, lot: lot:B244173 (Fig. 4lm) Anti-CD8-APC antibody (Invitrogen, clone 53-6.7, Cat:17-0081-82, lot:2023410 (Fig. 4i). Anti-CD4-APC/Cy7 antibody (BioLegend, Cat: 100414, lot:B326190) (Fig. 4i and 4m) Anti-PDL-1 antibody (anti-CD279, BioXCell, lot:842922M2) Capture anti-TNF-alpha antibody (BioLegend, Cat:79094, lot:8355708) Detection anti-TNF-alpha antibody (BioLegend, Cat:78335, lot:B332699) Anti-penta His-Alexa488 antibody (Qiagen, Cat:35310, lot: 1019199) Anti-C-term-His-HRP antibody (Miltenyi Biotec, lot: 5230203142) Anti-V5 antibody (Invitrogen, Cat: 46-0705, lot:2524682)

Anti-alphaSMA antibody (Abcam, Cat: AB5694, lot:1038192-2)
Anti-Rabbit-Alexa647 antibody (Invitrogen, Cat:A27040, lot:2531831)

Validation

Antibodies for ELISA and flow cytometry experiments were titrated internally in previous experiment on rVAR2 protein binding to positive cancer cells. The lower saturating concentration was selected for further experiments. Isotype controls were used to see the background binding of each antibody.
Antibody concentrations for IF staining were selected the same way, after performing a titration and selecting the concentration giving the highest signals with lowest background to a control slide (slide which has not been treated with primary antibody).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

A375 WT (ID:CRL-1619) and B4GALT7 KO: were kindly gifted by Charlotte Spliid from University of California, San Diego, USA, who generated the B4GALT7 knock out cell line from wild type cell.
from Peter Holst at University of Copenhagen, Denmark: A549 (ID:CCL185), CT26 (ID: CRL-2638)
from Bioneer, Hillerød, Denmark: Colo205 (ID:CCL-222), Sw480 (ID; CCL-228)
from Andreas Kjær at University of Copenhagen, Denmark: HT29 (ID: HTB-38),
from Mads Daugaard at Vancouver Prostate Center, Canada: MiaPaca2 (ID: CRL-1420), PC-3M-Luc-C6 (ID: CRL-1435),
from Chris Madsen at Lund University, Sweden: 4T1 (ID: CRL-2539), mCAFs and vCAFs
from Karen Dybkaer at Aalborg University: KG-1 (ID: CCL-246), NAML-6 (ID: CRL-3273), 697 (ID: CVCL_0079), originally from DSMZ, Leibmiz Institute, Germany.
from Bonkolab at Rigshopitalet, Denmark: REH (ID: CRL-8286),
from Poul Sorensen at British Columbia Cancer Research Center, Canada: MG63 (ID: CRL-1427), MNNG (ID: CRL-1547), U2OS (ID: HTB-96), TC-71 (ID: CVCL_2213)
from Lara at BRIC: U87mg (ID: HTB-14)
Purchased from Sigma Aldrich: Karpas299 (Cat: 06072604)
from Niels Ødum at University of Copenhagen, Denmark: Myla2059 (ID: CVCL_5342)
from RICC: SkBR3 (ID: HTB-30)
generated by Yen-Hsi Chen from University of Copenhagen: CHO KI/KI library

Authentication

The genetic background was not essential to draw conclusions so commercialized cancer cells were not authenticated. CHO cell lines were obtained from Copenhagen Center for Glycomics, Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, and were previously generated and authenticated as described in https://doi.org/10.1038/s41592-018-0086-z.
The CAFs cell lines were kindly shared by Chris Madsen. Human CAFS (vCAFs) were collected from patients an immortalized using hTERT lentivirus, as described in https://www.nature.com/articles/ncb1658, and murine CAFs (mCAFs) were harvested in the mammary fat pads of MMTV-PyMT mammary carcinoma, as described in https://www.nature.com/articles/ncb2756

Mycoplasma contamination

The cell lines were tested regularly (approx. every 3-4 weeks) and have at all times tested negative for mycoplasma. The following commercialized mycoplasma kit was used every time: BioDordika, Cat: LZ-LT07-318.

Commonly misidentified lines
(See ICLAC register)

There is no misidentified eukaryotic cell lines.

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

Female BALB/cAnNRJ mice (CT26, 4T1), female CB-17/lcr-Prkdc scid/scid/Rj (Karpas299) and female BALB/cAnNRJ-Foxn1nu/nu mice (A549, MiaPaca2) were purchased from Janvier at age of 6-8 weeks, Sprague Dawley male rats were purchased from Janvier at the age of 8-9 weeks. All animals were acclimatized for at least 1 week before performing the first cell inoculation, and were kept in a 12h light/12h dark cycle period at 40-60 % humidity with ambient temperature of 20°C in accordance with the FELASA Rodent Health Surveillance program.
The LTL370 prostate tumor model was established following the method described in doi:10.1158/0008-5472.CAN-13-2921-T, in male NOD/SCID mice, following the same light cycle as for the CDX models, humidity levels between 50-60% with ambient temperature of 21-22°C.

Wild animals

No wild animal were used in this study

Reporting on sex

Both male and female mice were used to assess the in vivo tumor localization or our antibodies demonstrating a non existing bias in our results regarding the sex. We performed IVIS localization in female and male mice to assess possible unwanted antibody accumulation in reproductive organs, in particular in testis where we saw in vitro IF staining on human tissues.

Field-collected samples

No Field-collected samples were used in this study

Ethics oversight

Animal studies in Copenhagen were approved by the Animal Experiments Inspectorate (P23-118, P23-103, P23-071 and P21-P19). Ethical approvals were obtained from Dyreforsøgstilsynet Danmark.
Experiments on LTL370 Prostate Patient-Derived-Xenograft (PDX) models were approved by the Animal Care Committee at the University of British Columbia (A22-0206, A19-0324).
Experiments on PDAC and Sarcoma PDX models were done at a contract research organization (CRO EPO Berlin), in accordance with

the United Kingdom Coordinating Committee on Cancer Research regulations for the Welfare of Animals and of the German Animal Protection Law and approved by the local responsible authorities.

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.

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

In white blood cell (WBC) flow experiment, fresh blood was drawn from healthy donor, collected in K2EDTA-tubes and processed within 1h of collection. Red blood cells were lysed in a lysis buffer containing NH4Cl, KHCO3 and EDTA.
Adherent cancer cells were cultured to reach 80% confluency and detached with a non-enzymatic CellStripper solution. Following dissociation, cells were counted on a Nucleocounter® (NC-200, ChemoMetec), spun down at 400xg for 5min and reconstituted in 1xPBS with 2% Fetal Bovine Serum (GibcoTM, #A4766801) (PBS2). Suspension cells were directly counted, spun down, and resuspended in PBS2. For the Cell-based GAG assay, the CHO cells were treated with PBS with 1% BSA.

Instrument

Binding to cancer cells, CHO and murine splenocytes : BD Fortessa 3-laser instrument (Becton Dickinson)
Binding to tumor cell suspensions: BD Fortessa 5-laser instrument (Becton Dickinson)
Binding to white blood cells: CytoFLEX S (Bec) instrument

Software

FlowJo software (BD Biosciences, v10)

Cell population abundance

Cancer cell and CHO KO/KI lines are permanent established clonal cell lines and are therefore are homogeneous population. WBC preparation contain about 85-90% of WBC with some contaminants from other blood compartments. All analysis was made on CD45 positive cells preventing any possible bias in the results.
Binding to murine T-cells from harvested splenocytes was analyzed on the CD3+ population representing around 10-15% of the total cells.

Gating strategy

Homogeneous cell populations were selected on the Side-Forward scatter plot (SSC/FSC) by discarding dead cells and debris based on to their size. Single cells were picked on the FSC-H/FSC-A plot for further analysis.
In WBC experiments, cells were first gated as described above, then gated on live cells and subsequently gated on CD45 positive population (compared to unstained control) before further analysis.
The binding to murine splenocytes was assessed on total cells which were gated as described above. (for the GeoMFI, Supp. Fig- 11 a). Subsequently, the cells which bound to the different constructs were plotted back on the SSC-A/FSC-A plot and compared to the CD3+ population (defined with a CD3+ positive control)
The immune cell population from the tumor suspension flow experiment were gated as above (total cells and single cells). Next, each gate was defined thanks to an FMO control, and a positive control of splenocytes cells stained with the immune cell markers or live dead marker.

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