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Last updated by author(s): 2022-FEB-19

Reporting Summary

Nature Research 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 Research 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

1) Image acquisition for PDOs (Fig. 1b-e; Extended data 2a,b- 2a-e; 7e)

- Molecular Devices MetaXpress (v5.3) (ImageXpress Micro XLS)

Image acquisition for PDOs (Fig 7b)
- Molecular Devices MetaXpress (v6.2.3) (ImageXpress Micro Confocal)

Image acquisition for PDOs (Supp Fig 6a,b)
- Nikon NIS-Elements (Nikon A1 confocal)

2) Images of the organoid formation experiments (Fig. 4d-i; extended data Fig. 3c-d):
- ScanR Acquisition Software v2.3 (Olympus Soft Imaging Solutions)

3) Flow cytometry and cell sorting:
- BD FACS DIVA v8.0.1

4) Cell cycle experiments:
- Gallios Cytometry List Mode Data Acquisition & Analysis Software Gallios Cytometer v1.2

Data analysis

1) PDO image analyses, antibody screening and calculation of the multiparametric score:

- Crown Bioscience Ominer®
- KNIME Analytics Platform v2.11.3 (http://www.knime.com)

2) Flow cytometry:
- FlowJo (v10.4)

3) Image analysis of PDO formation experiments:
- FIJI/ImageJ (v1.51d) – Scripts are available upon request.

4) Statistical tests and graphs generation
- GraphPad Prism (v7.03) - For estimation of Kd values (GraphPad v6.0)
- R programming package (v4.0.5)

5) Image processing:
- FIJI/ImageJ (v1.51d)

6) PDO genomic data
- Sequence read alignment - BWA (v0.5.9)
- Somatic substitutions - CaVEMan (v1.14.0)
- Indel calling - Pindel (v2.0)

8) RNA seq analysis:
- Read alignment - STAR (version 2.5.2b).
- Conversion to bam files- Sambamba (v. 0.6.7)
- Count matrices generation - Rsubread package (v. 2.4.3) using the GENCODE homo sapiens v34.GRCh38.p13 version.
- Complementary genes annotations - biomaRt version GRCh38.p12.
- Normalization and contrasts - DESeq2 R package (v. 1.30.1).
- R programming package (v4.0.5)

9) Geneset enrichment analyses
- ROAST algorithm as implemented in the R package limma (v. 3.46.0).

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Organoid exome sequencing has been deposited at European Genome-Phenome Archive (study number EGAS00001004584). RNA sequencing data of P18T and C55T organoids treated with antibodies is deposited at Gene expression Omnibus (GSE186531). Microarray data of Fab072+ versus Fab072- tumor cells are deposited at Gene expression Omnibus (GSE190543). Further information and requests for resources and reagents should be directed to the corresponding authors. All requests for raw and analyzed data and materials will be reviewed promptly by the corresponding author to verify whether the request is subject to any intellectual property or confidentiality obligations. Any data and materials that can be shared will be released via a material transfer agreement. Source data are provided with this paper.
The following figures have associated source data: Fig 1c,d,f; Fig 2a,b,c,d,e ; Fig 3e,g,i; Fig. 4a,b,c,d,f,g,h,i ; Fig. 5a,b,c,d,e,f,h,i,j,k ; Fig.6a-h; Fig. 7a, d, e; Extended data Fig. 1a-e; Extended data Fig. 2c,d,f,g; Extended data Fig. 3c,d; Extended data Fig. 4a-g; Extended data Fig. 5; Extended data Fig. 6.

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	No statistical method was used to predetermine sample size. Except for some experiments with PDXs in fig. 5e, we used at least 5 mice per group, which is sufficient to detect meaningful differences based on historical record with similar experiments. We reproduced therapeutic results of antibody treatments using multiple different in vivo models. For the majority of in vitro experiments, we used n=>3 for according to historical experience with similar experiments. The sample size typically results in standard error <25% of the mean.

Data exclusions	No data from in vitro experiments were excluded. For in vivo experiments, animals were excluded only if they died or had to be killed according to protocols approved by the animal experimental committees.
Replication	As reported in figure legends and methods, the findings were reliably reproduced with similar results. Therapeutic outcomes upon antibody treatments were reproduced using multiple different in vitro and in vivo models. Representative experiments included in the manuscript were repeated the indicated number of times with similar results as follows; Fig.3c; 3 times, Fig. 7b; 4 times, Fig 7c; 3 times, Fig. 7d; 2 times, Fig. 7e; 3 times, Extended data Fig. 6a-b; 4 times; Extended data Fig. 6c; 3 times for P18 and 1 time for C55T and C82N. For the rest of experiments, only data that we were able to replicate at least two times with equivalent results were included in the manuscript.
Randomization	For in vivo experiments, animals were randomized, with each group receiving mice with similar tumor sizes. For in vitro experiments, all samples were analyzed equally with no subsampling; therefore, there was no requirement for randomization.
Blinding	Data collection and analyses were not performed blinded to the conditions of the experiments. For in vivo experiments, blinding was not performed because identification of mice was required for treatment administration and husbandry. For in vitro studies, experiments were not performed in a blinded manner because investigator need it to identify samples to perform treatments.

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

For characterization of Fab arms:
ICR10 (Abcam cat#ab231; RRID: AB_2293306) - tested in excess concentration for anti-EGFR competitions assays.
EGFR.1 (Thermo scientific cat# MS-311-P, RRID: AB_10982394) - tested in excess concentration for for anti-EGFR competitions assays.
Matuzumab (generated as described for monoclonal antibodies)- tested in excess concentration for for anti-EGFR competitions assays.
Goat anti-human IgG-Fc (Bethyl Laboratories cat# #A80-104A: 5 µg/mL for coating in anit-EGFR competition assays.
OMP88R20 (generated as described for monoclonal antibodies) - 50ng/mL in RSPO blocking assays

Therapeutic antibodies:
Monoclonal antibodies against WNT, RTK and control targets (generated as part of the article as described in the materials and methods and used as indicated in different experiments)
Bispecific antibodies against WNT, RTK and control targets (generated as part of the article as described in the materials and methods and used as indicated in different experiments)
cetuximab (Merck KGaA, purchased from hospital pharmacy) -different concentration as indicated in the text

EGFR western-blot
EGF Receptor [(D38B1) XP® Rabbit mAb Cell Signaling Technology cat# 4267 (RRID: AB_2246311)] - 1:25
HRP Anti-beta Actin (ab20272, Abcam) - 1:1000

Organoid IF
Mouse anti-EGFR. Cat# MA5-13319 (Thermo Fisher Scientific - cat#MA5-13319) - 1:40 for IF
goat-anti-mouse-Alexa488 secondary antibody (Thermo Fisher Scientific)- 1:2000 for IF
Goat-anti-human-Cy5 secondary antibody (Thermo Fisher Scientific) - 1:2000 (antibody localization studies)

FACS
EPCAM-PeCy7 (human, eBioScience, 25-9326-42) -1:100
donkey anti-human IgG Alexa Fluor-647 secondary antibody (Jackson Immunoresearch, 709-605-149) - 1:400

IHC of organoid sections
Rabbit polyclonal anti-Ki67 (ab15580, Abcam) - 1:100
BrightVision Poly-HRP-Anti Rabbit IgG Biotin-free (Immunologic, DPVR-110HRP) - undiluted

Validation	All antibodies generated in this study were validated for specificity and binding by FACS and binding antigen positive/negative cell lines. Antibodies used for IF, IHC, flow cytometry and western blot have been previously validated on supplier's website and used in multiple studies previously.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	A431 cells [DSMZ cat# ACC 91 (RRID:CVCL_0037)] MCF-7 [DSMZ cat# ACC 115 (RRID:CVCL_0031)] CHO-K1 [DSMZ [cat# ACC 110 (RRID:CVCL_0214)] CHO-K1 cell clones expressing EGFR variants [generated from CHO-K1 [DSMZ [cat# ACC 110 (RRID:CVCL_0214)] Freestyle 293-F cells [Invitrogen cat# P/N 51-0029 (RRID:CVCL_D603)] DLD-1 [DSMZ cat# ACC 278]
Authentication	CHO-K1 cell clones expressing EGFR variants were authenticated by sequencing. No other authentication was carried out for the other cell lines. HUB collects tissue and organoid pellet when generating Master Cell Bank (or Working Cell Bank) and performing assays, isolate gDNA and run TaqMan® OpenArray® Genotyping Barcode Panel 64 (Thermo Fisher) for fingerprint analysis.
Mycoplasma contamination	Every four weeks all above cell lines are tested for mycoplasma and confirmed negative. Mycoplasma test on PDOs was done before freezing any master or working cell bank at the HUB. Also after running any experiment performed with the organoid cultures (i.e. collecting for RNA and DNA analysis). At IRB, mycoplasm test was run weekly for all PDO cultures.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines according to the ICLAC database were used in the study

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	For experiments described in Fig 4j-k: female NOD.CB17/AlhnRj-Prkdcscid/Rj mice (Janvier Labs) aged between 6-8 weeks were used. For experiment described in Fig 5j-k: M001 & M005:: NOD-SCID (NOD. CB17-Prkdcscid/NcrCrl) 8-10 females purchased from Charles River Laboratories. For LM-CRCX3: five-week-old male nu/nu Swiss mice (Envigo). MeMo mice (C57BL/6 background): 12-16 weeks of age males and females Animal housing rooms temperature; 22 +/- 2 Celsius; humidity:30-70%; 12 hours cycles of light/darkness
Wild animals	No wild animals were used in the study.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	Experiments in Fig 4j-k and extended data Fig. 4a-c were approved by the Animal Care and Use Committee of Barcelona Science Park (CEEA-PCB-14-000053). Experiments in Fig. 5e and Extended data Fig 5 by the Committee of the Ethics of Animal Experiments of Crown Bioscience (Crown Bioscience IACUC Committee). Experiments described in Fig 5j-k were approved by the Ethical Committee of Animal Experimentation of the VHIR–the Vall d'Hebron Research Institute (ID: 17/15 CEEA and 18/15 CEEA) and by the IDIBELL animal facility committee (AAALAC Unit1155) under approved procedure 9111.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	All relevant patient population co-variates from which PDOs were derived are reported in Supplementary Table 3
Recruitment	We derived and stocked PDOs from 61 primary CRCs and 11 CRC liver metastases obtained from 68 patients treated at two different hospitals (University Medical Center Utrecht (UMCU) and Meander). Patients were identified by their treating physician/specialist in the hospitals. Patients were selected from those who are undergoing resection. The patients who will be included were diagnosed with colorectal cancer. Patients received a letter with the information at least 2 days prior to their visit to the hospital. Furthermore, biobank study coordinator in the hospital visited the patient and explained the research project and answered any questions related to the project. In addition, the patients were informed that they can retract the permission at all times, without having to provide a reason for doing so.
Ethics oversight	The collection of patient data and tissue for the generation and distribution of organoids has been performed according to the guidelines of the European Network of Research Ethics Committees (EUREC) following European, national, and local laws.

The Biobank Research Ethics Committee of the UMC Utrecht (TCBio) approved the biobanking protocol: 12-093 HUB-Cancer under which this research was performed. All donors participating in this study signed informed consent.

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

Fab072 antibody staining of dissociated PDOS (Extended data Fig 2e-f):
The day prior to analysis, the organoids were disaggregated into single cells. After 12 hours cells were isolated from the BME using cell recovery solution. After 1 hour on ice, the cells were centrifuged, and washed once in 10 mL of PBS containing 0.5% BSA and 0.5 mM EDTA (staining buffer). A maximum of 100,000 cells/100 µL of antibody was used, and labelled using a primary antibody provided by Merus diluted to 10 µg/mL. The cells were incubated on ice for 45 minutes, with regular inversion of the tubes to ensure homogeneous staining. The cells were washed and incubated with anti-human IgG antibody conjugated to R-PE (Invitrogen, H10104) diluted 1:400. The cells were incubated for 20 minutes on ice before washing and re-suspending them in staining buffer containing 0.1 µM DAPI (Sigma-Aldrich) and were then immediately analysed.

Fab072antibody staining of dissociated xenografts (Fig 3h):
P18T-derived subcutaneous tumour xenografts were minced into small pieces using a blade and incubated 30' at 37ºC with HBSS and 166U/mL of Collagenase IV (Sigma ref: C5138-1G). After incubation samples were pipetted up and down and subsequently filtered through 70um and 40um mesh filters. Cells were spun down (5' at 1500 rpm) and the pellet was resuspended with 2 mL of Ammonium Chloride and incubated at RT for 3' to lysate the red blood cells. After the lysis, HBSS was added and the cells were spun down again. Finally, the cell pellet was resuspended in 5mL of staining buffer. Cells were Fc blocked with CD16/32 (mouse, Tonbo Biosciences, 70-0161-U500) for 10 min at 4ºC, stained with EPCAM-PeCy7 (human, eBioScience, 25-9326-42) and 5 µg/ml Fab072 monospecific antibody or negative control TT primary antibodies for 40 min at 4ºC and then with 1:400 donkey anti-human IgG Alexa Fluor-647 secondary antibody (Jackson Immunoresearch, 709-605-149) for 30 min at 4ºC. Samples were resuspended in 1 µg/ml DAPI in FACS buffer (HBSS- 0.5% BSA- 0.25mM EDTA).

Cell Cycle analysis (extended data Fig 3b): PDOs were harvested and a single cell suspension using the following protocol: organoids were first liberated from the BME by removing the culturing media, and re-suspending the BME in cell recovery solution (BD Biosciences), and incubating for 1 hour on ice. Subsequently, the organoids were centrifuged (all centrifuge steps were for 5 mins, 200 g at 4°C). The pellet was re-suspended in 1 mL of 50% Trypsin/EDTA Solution (TE); 50% PBS, and pipetted up and down, and regularly visually assessed until a single cell suspension was achieved. The TE was diluted in 10 mL of PBS and centrifuged. Click-iT® EdU Alexa Fluor® 647 Flow Cytometry Assay Kit was the followed according to the manufacturer's protocol.

TT x TT and TT x Lgr5 staining of dissociated PDOs (Fig 5g-i): C55N and C55T PDOs were cultured in 60% growth factor reduced matrigel drops (Corning, ref 356231) and normal mucosa medium consisting of AdvancedDMEM/F-12- 10 mM Hepes - Glutamax - B27 - 10 mM Nicotinamide - 1.25 mM nAcetylcystein - 50 ng/ml EGF - 100 ng/ml Noggin - 250 ng/ml Rspondin-3, 10uM SB202190 - 10 nM gastrin - 500 nM A83-01 - 0.5 nM Wnt surrogate fusion protein (U-protein Express BV, N001) - normocin. 1-week grown PDOs were harvested and dissagregated into single cells with TryplE Express (Life Technologies, 12604-13). Cells were counted and resuspended in FACS buffer (HBSS - 10% FBS - 0.5% BSA - 10uM rock inhibitor Y27632 (Sigma Y0503)) and 5 ug/ml negative control antibody (TT x TT) (C55T) or TT x Lgr5 antibody (0.1M cells in 100 ul) (C55N and C55T) for 30 min on ice. Samples were washed twice and then incubated with 10µg/ml secondary Alexa Fluor 647 Goat anti Human IgG (Life Technologies, A21445) for 30 min on ice. Samples were washed twice and resuspended in 1µg/ml DAPI in FACS buffer and analyzed in BD Diva FACS Aria Fusion. Centrifugation steps were performed at 200g for 3 min at 8ºC.

TT x TT and MCLA-158 staining of dissociated PDOs (Extended data Fig 3e): Non-infected, shControl (Mission Sigma SHC002) and shLgr5 (TRCN0000011586) P18T PDOs were grown in 70% BME and tumor organoid medium consisting of AdvancedDMEM/F-12- 10 mM Hepes - Glutamax - B27 - 10 mM Nicotinamide - 1.25 mM nAcetylcystein - 50 ng/ml EGF - 100 ng/ml Noggin - 1 ug/ml Rspondin-1, 10uM SB202190 - 10 nM gastrin - 500 nM A83-01. Infected cells were maintained in 0.5 ug/ml puromycin (invivogen, ant-pr). 1-week grown PDOs were single-cell dissociated in TryplE Express and plated in 70% BME and tumor organoid medium overnight. The following day, PDOs were harvested in cold PBS. Cells were counted and stained with 5 µg/ml MCLA-158 or TT x TT control antibody in stain buffer (AdvancedDMEM/F12-Hepes-Glutamax, 10% FBS, 0.5% BSA) for 30 min on ice, washed three times and stained with 5µg/ml of secondary antibody Alexa Fluor 647 Goat anti Human igG (Life Technologies, A21445) for 30 min on ice. Samples were washed and resuspended in 1µg/ml DAPI in HBSS-0.5% BSA and analyzed in BD Diva FACS Aria Fusion. Centrifugation steps were performed at 450g for 5 min at 4ºC.

Instrument	- FACS-Aria for Fig 3h and Extended data Fig 2e - Gallios flow cytometry machine for Extended data Fig 3b - BD FACSCanto for Extended data Fig 2g
Software	For analyses: FlowJo (v10.4) For cell sorting: BD FACS DIVA v8.0.1
Cell population abundance	The enrichment in LGR5+ cells in the sorted samples was determined by expression analysis of marker genes as shown in Fig 3i and Extended data Fig 2f
Gating strategy	- Fab072 antibody staining of dissociated PDOs (Extended data Fig 2c-d): Gates were set according to antibody control (TT) staining. We collected cells that stained positive or negative above and below this threshold as indicated in the figure. - Fab072 antibody staining of dissociated xenografts (Fig 3h): We sorted alive (DAPI negative) epithelial tumor cells (Epcam+). Gates were set according to antibody control staining. We collected the brightest 10% of the cells. Negative cell fraction corresponded to center of the major pick as indicated in the figure. - For cell cycle analysis, G1/S/G2-M gates were manually set on the control samples according to distribution of DNA content and Edu incorparation as shown in Extended data Fig 3b.

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