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Corresponding author(s): Sangeeta N. Bhatia

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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	G8 Acquisition Engine (Version 2.1.1.0) was used to collect data on G8 PET/CT preclinical imaging system (PerkinElmer Inc.). Tecan icontrol software (Version 3.7.3.0) was used to collect data on Tecan Infinite 200pro microplate reader (Tecan Group Ltd.). ImageStudio (Version 5.2, Li-Cor Inc.) was used to collect data on Odyssey CLx imaging system (Li-Cor Inc.). Living Image (Version 4.5.5, PerkinElmer Inc.) was used to collect data on IVIS spectrum optical imaging system (PerkinElmer Inc.). Panoramic Scanner (Version 1.22) was used to collect data on 3D Histech P250 High Capacity Slide Scanner (PerkinElmer Inc.). ChemStation for LC 3D (Rev. B.03.01, Agilent Technologies Inc.) was used to control the Agilent Model 1100 HPLC system. The code of the pharmacokinetic model was run on MATLAB_R2018b. Details are specified in Methods, Figure Legends and Supplementary Information.
Data analysis	VivoQuant software (Version 4.0 patch3, InviCRO Inc.) was used for PET/CT image analysis. ZEN Software (Carl Zeiss, Jena, Germany) was used for confocal microscopy image analysis. ImageStudio (Version 5.2, Li-Cor Inc.) was used for near-infrared image analysis. Living Image (Version 4.7.3, Perkin Elmer Inc.) was used for IVIS in vivo optical imaging analysis. CaseViewer (Version 2.2, 3DHISTECH Ltd.) was used for visualization of images collected on the 3D Histech P250 High Capacity Slide Scanner. Zen software (Version 2.2.58, Carl Zeiss Microscopy, LLC.), ImageJ (1.48v, NIH) and inForm software (Version 2.3, PerkinElmer Inc.) were used for all other image analyses. FlowJo (TreeStar Software Version 9.9.6) was used for Flow Cytometry data analysis. Differential expression analyses were carried out by DESeq2 1.10.1. GraphPad Prism (Version 9) was used for data analysis and statistics. Details are specified in Methods, Figure Legends and Supplementary Information.

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

The Cancer Genome Atlas (TCGA) Research Network (http://cancergenome.nih.gov) and FireBrowse (http://firebrowse.org) are open access resources with enriched cancer genomics data. All data that support the findings of this study are available within the article and Supplementary Information or from the corresponding author (sbhatia@mit.edu) upon reasonable request.

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	Sample size is indicated in the figure legend for each experiment whenever possible. No sample-size calculations were performed. Sample size was determined to be adequate based on the magnitude and consistency of measurable differences between groups. Samples were allowed to have at least three biological replicates to derive statistical significance. For animal studies, experimental group sizes were practically associated according to the number of mice housed per cage (n=5 mice per cage).
Data exclusions	No data was excluded for in vitro experiments. Pre-established exclusion criteria were set based on failed injection or urine production within defined time course of the animal study. Animals were excluded solely on the basis of the pre-established exclusion criteria.
Replication	All experiments were repeated independently with similar results. In vitro and in vivo investigation of nanosensors were typically performed for three times, except for the human tissue microarray analysis, in vivo treatment monitoring experiments were completed twice independently. For immunofluorescence staining, tissue sections were independently stained by three investigators.
Randomization	In vitro samples were prepared, processed and analyzed in a random order. Grouping criteria of animals was included in methods and figure legends. All animals analyzed in this study were sex- and age-matched. For drug treatment experiments, animals were chosen to balance a similar range of tumor burden between the groups based on tumor signals quantified by IVIS to perform studies on. Animals receiving PRISM or control sensor were randomly selected.
Blinding	For immunohistochemistry analysis of human tissue microarrays, specimens were analyzed by a veterinary pathologist who was blinded to the treatment groups. In all other cases, investigators were not blinded to the groups and treatments during experiments, because the investigators who set up the experiments carried out the analyses. Data reported for these experiments are not subjective but based on the quantitative assays described.

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

Primary antibodies: anti-MMP9 antibody (Abcam ab38898, Lot# 573144), anti-biotin antibody (Abcam ab201341, Lot# GR3232475-1), anti-CAIX antibody (Abcam ab15086, Lot# GR290257-1), anti-FITC antibody (GeneTx GTX19224, Lot# 821300832, for IF); mouse anti-FITC monoclonal antibody Clone 2A3 (GeneTex GTX10257, Lot# 821504362, for ELISA).
Secondary antibodies: Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (ThermoFisher A-31573, Lot# 1322326); Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 (ThermoFisher A-31570, Lot# 412442); Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 633 (ThermoFisher A-21082, Lot# 1889311); Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 (ThermoFisher A-31572, Lot# 400466).
NeutrAvidin-HRP (ThermoFisher 31030, Lot# RA228784).

Validation

All commercially available antibodies have been thoroughly tested and validated by the manufacture. Validation statements are available on manufacture's website. Specifically:
The Anti-MMP9 antibody (ab38898, https://www.abcam.com/mmp9-antibody-ab38898.html) was shown to bind to the Recombinant Human MMP9, His tagged (ab82955) and also to the Recombinant Mouse MMP9 protein (ab39309) indicating that it is specific for the MMP9 target. ab38898 detects recombinant Human MMP9 running at ~85 kDa, and endogenous full-length MMP9 in LPS-stimulated cells at ~100 kDa. This antibody also detects a band at 90 kDa in U937 PMA-treated cells. Tested applications of the antibody include IHC-Fr, WB. This antibody has been referenced in 602 publications. Selected recent citations:
Qin T et al. J Ethnopharmacol 246:112128 (2020);
Chen X et al. J Exp Clin Cancer Res 39:65 (2020);
Hu L et al. J Gastric Cancer 20:95-105 (2020).

The Anti-Biotin antibody [Hyb-8] (ab201341, https://www.abcam.com/biotin-antibody-hyb-8-ab201341.html) has not been KO tested or tested for specificity using a blocking with immunizing peptide. It is suitable for Flow Cyt, In situ hybridization, WB, ICC/IF, IHC analysis and has been cited in 3 publications:
Shaw A et al. Nat Nanotechnol 14:184-190 (2019);
Powell ML et al. Anal Biochem 543:108-115 (2018);
Roussis IM et al. PLoS One 11:e0147967 (2016).

The Anti-Carbonic Anhydrase 9/CA9 antibody (ab15086, https://www.abcam.com/carbonic-anhydrase-9ca9-antibody-ab15086.html) has been used successfully in 99 publications. Western Blot analysis of the Anti-Carbonic Anhydrase 9/CA9 antibody (ab15086) in HeLa, MDA-MB-231, and A549 whole cell lysates showed a specific band detected for Carbonic Anhydrase IX/CA9 at a molecular weight of 50 kDa. Selected recent citations:
Lorenzo-Pouso AI et al. J Enzyme Inhib Med Chem 35:1258-1266 (2020);
Hart PC et al. Cell Rep 29:4086-4098.e6 (2019);
Dillon MT et al. Clin Cancer Res 25:3392-3403 (2019).

Fluorescein antibody (GTX19224) was generated from monospecific antiserum by immunoaffinity chromatography using Fluorescein conjugated Goat IgG coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Goat Serum and Fluorescein conjugated Bovine Serum Albumin. Tested applications for the antibody included WB, ICC/IF, ELISA and IHC. Specificity of the anti-FAM antibody was further validated in house using ELISA with a short synthetic peptide labeled with FAM and biotin ligands.
Citation: Lin KY et al. ACS Nano. 7,10, 9001-9009 (2013).

Mouse anti-fluorescein antibody (GTX10257) was tested to react with both free and conjugated FTIC. Immunogen: fluorescein conjugated Bovine Serum Albumin. Antibody was Protein A purified and tested applications for the antibody included ELISA and IHC.
Citations:
Mac, QD et al., Nat Biomed Eng 3, 281-291 (2019);
Holt BA et al., J Vis Exp. (137):57937 (2018);
Warren AD et al., Proc. Natl. Acad. Sci. U.S.A. 111 (10) 3671-3676 (2014).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Mouse cell lines MC26-LucF (Kenneth K. Tanabe Laboratory, Massachusetts General Hospital), D8-175 KPC-derived pancreatic cancer line (Tyler Jacks Laboratory, Massachusetts Institute of Technology) and 4T1 (ATCC CRL-2539); human cell lines MDA-MB-435 (NCI-60), U937 (ATCC CRL-1593.2), OVCAR8 (NCI-60) and LS174T (ATCC CL-188)

Authentication

The commercially available cell lines from ATCC have been thoroughly tested and authenticated using short tandem repeat profiling by the vendor. Statements are available on vendor's website (https://www.atcc.org/Products/Cells_and_Microorganisms/Testing_and_Characterization/STR_Profiling_Analysis.aspx). Cell line received from other research lab was previously validated upon receipt, references of the cell line were provided.
Selected citations for the MC26-LucF line:
Danino T et al., Sci Transl Med 7, 289, pp. 289ra84 (2015)
Kwon E et al., Nat Biomed Eng 1, 0054 (2017).

Selected citations for the D8-175 line:
Maud-Emmanuelle G et al., Clin Cancer Res 24(7):1734-1747 (2018)
Lo JH et al., Mol Cancer Ther 17(11): 2377–2388 (2018)

Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	MDA-MB-435 line was previously described as a metastatic breast cancer cell line, and is now considered a melanoma line. Cell lines were utilized to demonstrate the broad application of the low pH insertion peptide in solid tumors, especially ones with high invasive potential.

Animals and other organisms

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

Laboratory animals	Female BALB/c mice (Taconic, 6-8 weeks at time of tumor induction) and female NCR Nude mice (Taconic, 4-5 weeks at time of tumor induction) were used in this study. All animals were maintained in the Koch Cancer Institute animal facility, with a 12-h light/12-h dark cycle, at ~18-23°C and ~50% humidity. Complete descriptions can be found in relevant methods and figure legends.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All animal studies were approved by the Massachusetts Institute of Technology (MIT) committee on animal care (MIT protocol 0417-025-20 & 0217-014-20). All animals received humane care, and all experiments were conducted in compliance with institutional and national guidelines and supervised by Division of Comparative Medicine (DCM) of MIT staff.

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	Cells cultured in 6-well tissue culture plates were collected directly from the adherent surface by pipetting with and resuspending in PBS (pH 7.4 or 6.4) and analyzed on a BD LSRII flow cytometer immediately.
Instrument	BD LSR II HTS Flow Cytometer (BD Biosciences)
Software	Data acquisition was performed with FACS Diva Version 8.0 on an LSR II Flow Cytometer. Data were analyzed in FlowJo (TreeStar Software, Version 9.9.6).
Cell population abundance	The signal from 10,000 cells after post-sort fractions were collected and used for further analyses.
Gating strategy	The sub-population of viable cells after standard FSC/SSC gating for uniform population

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