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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	PET data were collected through the Inveon Acquisition Workstation, Version 2.2.2.1050. Siemens Biograph mCT data were acquired through the server command line in 64-bit mode. Bioluminescence images were acquired with a Perkin-Elmer IVIS Living Image V.4.3.1
Data analysis	PET listmode data processed using the custom mPET algorithm in conjunction with Monte Carlo, MCGPU-PET, can be accessed on Github at https://github.com/DIDSR/MCGPU-PET. Double-isotope and triple-isotope separation code can be accessed on Github at https://github.com/jlherraiz/GFIRST. Separated and calibrated PET images were viewed and exported in Inveon Research Workstation V4, Launcher 4.0.1.1. Data analysis was performed on Prism 9 for MacOS, V 9.4.0 (453).

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 main data supporting the results in this study are available within the paper and its Supplementary Information. All data generated in this study, including source data for the figures, are available from figshare with the identifier https://doi.org/10.6084/m9.figshare.21816069.

Human research participants

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

Reporting on sex and gender	The study did not involve human research participants.
Population characteristics	—
Recruitment	—
Ethics oversight	—

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	No sample-size calculated was performed. For in vivo imaging, a group size of n = 4 was used for Fig. 4, as up to 4 mice can be loaded into the PET scanner at one time. For Fig. 6, n = 5 mice were used for imaging, as the cage could house up to 5 mice. For Fig. 6, n = 4 mice were scanned for session one, and n = 1 for session two. Injections were staggered for mouse 5 to allow the same radiotracer distribution time at the start of imaging for each session. For Fig. 5, n = 1 one mouse was scanned, as the mouse holder had not been fabricated. For Supplementary Fig. 8, n = 1 mouse was scanned, as only one mouse was available, and availability of 86Y-DOTA-PSMA was limiting. For Supplementary Fig. 9, n = 3 mice were injected with a combined radiotracer-activity limit for the scanner (~1.2mCi). n = 3 mice were maintained for both weeks of imaging to ensure similar radiotracer-specific activity and total activity amount.
Data exclusions	No mice or phantoms were excluded from acquisition or from analysis.
Replication	Replication is shown in Supplementary Fig. 5e, with similar biodistributions of both isotopes one week after first radiotracer injection. All other experiments shown included a cohort of 4–5 mice (except for Fig. 5, where n = 1) per group to show inter-mouse variation in biodistribution. In general, replicates were not done by default, as imaging cohorts covered several time points and the experiments are expensive to repeat outright, given the cost of the radiotracer and of instrument reservation. However, as stated, multiple mice were used for each study group.
Randomization	Randomization was only performed for Extended Data Fig. 4, by S-rank by lung BLI intensity, so each weekly cohort had low, mid or high BLI. Mice in Fig. 4 were randomly sorted into one of four cages, after which the mice were put onto trametinib, vemurafenib, both or saline before imaging. Regardless, all mice received the same radiotracer combination. In all other studies, all mice were injected with identical amounts of the given radiotracer pair without any randomization.
Blinding	No blinding was done during any experiment, as the majority of radiotracers selected for this study have been tested in similar models and are known to target the epitope present in the tumour tissue. The dual CD39 Ly108 antibody imaging was performed on the basis of flow-cytometry data reported in another model. For mPET separation and analysis, the studies were partially blinded, whereby listmode PET data was sent for mPET separation alongside only the amount of each radiotracer injected per mouse for calibration.

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	Details of the antibodies used for in vivo imaging are provided in Supplementary Table 2.
Validation	The antibodies CD39 and Ly108 were validated for use in flow cytometry by Biolegend, but subsequently lightly modified with the chelator DFO for 89Zr or directly iodinated with 124I, and used in vivo.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The cell lines used in this study, with source and gender, are described in Supplementary Table 3.
Authentication	The cell lines were authenticated by STR profiling, with the subsequent reporter-gene modification characterized and managed by each collaborator. CAR-T-cell characterization can be found in Extended Data Fig. 3.
Mycoplasma contamination	The cell lines were tested, and found to be free of mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cells lines were used.

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	6–8-week-old (for Extended Data Fig. 4) and 8–10-week-old C57/bl6J female mice (For Figs. 4 and 5), and 12–14-week-old male NOD-SCID Gamma mice (for Fig. 6 and Extended Data Fig. 3).
Wild animals	The study did not involve wild animals.
Reporting on sex	Sex was selected on the basis of the animal model used; male mice for prostate cancer, and female mice otherwise.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The animal studies were approved by the MSKCC IACUC, under protocol 08-07-014.

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	1x10^5 PC3 human prostatic adenocarcinoma cells previously engineered to express either PSMA+/mTurquoise2+/Click Beatle Green+ or PSMA-/Td-Tomato+/Renilla Luciferase+, as well as anti-PSMA (P28z) CAR-T cells expressing GFP fluorescent protein were washed twice with PBS and resuspended in FACS buffer (PBS pH 7.4 with 10% (v/v) FCS). Cancer cells were stained with a mouse anti-human PSMA monoclonal antibody (10 ug/mL; MBL #K0142-3, mouse IgG1 clone 107-1A4) and a polyclonal goat anti-mouse Ig secondary antibody conjugated to APC (2 ug/mL; BD Pharmigen, #550826). P28z CAR-T cells were stained using a goat anti-human IgG-biotinylated polyclonal antibody (0.5 ug/mL; SouthernBiotech, #2040-08) and APC Streptavidin secondary antibody (0.2 ug/mL; BD Pharmigen, #554067). All cells were stained for 1 hour at 4 °C in FACS buffer. Untransduced cell samples unstained, stained with primary and secondary antibodies and only with indicated secondary antibodies, were used as controls.
Instrument	LSRFortessa II (BD Bioscience, USA)
Software	The LSRFortessa II (BD Bioscience, USA) was equipped with a FACSDiva analysis software (BD Bioscience, version 8.0.1). Data analysis was performed using FCS Express (De Novo Software, version 7.14.0020).
Cell population abundance	Targeted cancer cells retrovirally engineered to express the desired transgenes were subjected to fluorescence-activated cell sorting (FACS) to select for positively transduced cells. Post-sort fraction of the relevant population within the sorting gate was >95%, as determined by flow cytometric analysis post-sort.
Gating strategy	Gating single steps for Supplementary Figure 13: 1) Forward (FSC) and side scatter (SSC) gating were used to identify the cell population of interest on the basis of size and granularity, respectively; 2) Side scatter width (SSC-W) versus side scatter height (SSC-H) density plots were used for doublets exclusion; 3) Two-parameter density plots were used to represent the stained population of interest: each axis represented a specific marker/fluorochrome. 4) A quadrant tool was used to generate gates. Negative versus positive populations were selected on the basis of controls ((i) unstained, (ii) stained with primary and secondary antibodies and (iii) only with indicated secondary antibodies).

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