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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	The drug concentration data was collected using the Tecan SPARKCONTROL v2.1 Microscopic imaging of panitumumab-IRDye800 was acquired using Olympus slide scanner (VS200 ASW 3.3, Build 24382) Spatial proteomics was acquired using the CODEX-Keyence system (BZ-X710 fluorescence microscope). Nanostring DSP data was collected from GeoMx DSP v2.5.1145 Xenium data was collected from Xenium In Situ instrument with Xenium Onboard Analysis software (version: xenium-3.3.1.1)
Data analysis	Code for processing and analyzing CODEX multiplexed imaging data (https://github.com/nolanlab/RAPID, https://github.com/GuolanLu/ECM_neighborhoods, https://github.com/nolanlab/TissueSchematics) Data analysis was performed using Python (3.7.16) with key libraries including scanpy 1.9.3, matplotlib 3.5.3, scipy 1.7.3, scikit-learn 1.0.2, seaborn 0.11.2 Image analysis: Matlab R2024a.

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

Data supporting the findings of this study are available in the paper, its Supplementary Information, the accompanying Source Data files, and public repositories. Source Data files are provided with this paper for Figures 1–5 and Extended Data Figures 1, 3, 4, 6. The external HNSCC scRNA-seq dataset used for validation is available in the Gene Expression Omnibus (GEO) under accession GSE103322. NanoString GeoMx digital spatial profiling (DSP) data generated in this study are available in GEO under accession GSE249198.

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	Human tumor tissues analyzed in this study were obtained from previously conducted clinical trials under institutional review board (IRB) approval and informed patient consent. Patient sex (male or female) was recorded as part of the clinical metadata; gender identity information was not collected. Both male and female patients were included in the study cohorts where available. The study was not designed or powered to evaluate sex- or gender-based biological differences, and therefore no sex- or gender-stratified analyses were performed. Individual-level patient information is de-identified in accordance with IRB policies, and disaggregated patient-level metadata are not shared in the source data to protect patient privacy. The overall number of patients included in the study is reported in the manuscript.
Reporting on race, ethnicity, or other socially relevant groupings	Race and ethnicity information were not collected as part of the de-identified clinical metadata associated with the human tumor tissue samples analyzed in this study. Therefore, race- or ethnicity-based analyses were not performed. This study was designed to investigate spatial drug delivery and tumor microenvironment interactions at the tissue level and was not intended to evaluate differences across socially constructed demographic categories. No variables related to race or ethnicity were used in the study design, analysis, or interpretation.
Population characteristics	Human tumor tissues analyzed in this study were obtained from patients enrolled in previously conducted clinical trials (NCT02415881, NCT03384238, and NCT03582124) under IRB approval. Patient demographic information available to the investigators was limited to de-identified clinical metadata accompanying the tissue samples and were provided in Supplementary Table 1 and Table 4. The number of patients and tissue samples analyzed is reported in the manuscript.
Recruitment	No participants were recruited specifically for this study. Human tumor tissues were obtained from previously completed clinical trials under IRB-approved protocols.
Ethics oversight	Stanford IRB

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 formal prospective power calculation was performed. Sample sizes were constrained by the availability of clinically annotated human specimens, the rarity of suitable samples, and the requirement that each sample pass predefined technical quality-control criteria for staining, imaging, and downstream analysis. The final cohort sizes are comparable to those used in prior spatial omics and translational imaging studies and were sufficient for the descriptive, comparative, and correlative analyses presented here.
Data exclusions	None.
Replication	The major findings were supported by reproducible patterns observed across tissue regions and patients. Experimental procedures were performed using standardized staining, imaging, and analysis pipelines with quality-control filtering applied before downstream analysis. The major findings were also confirmed using orthogonal assays to demonstrate robustness. Where appropriate, complementary analyses were performed at both the region level and the patient level to ensure that conclusions were not driven by a small number of samples or tissue regions.

Randomization

This was an observational study using human tumor specimens, and random allocation of samples to experimental groups was not applicable. Samples and/or tissue regions were assigned to comparison groups based on predefined biological, spatial, and clinical criteria (e.g., markers, cell types, tissue compartments, and tumor types). All samples were processed using standardized experimental protocols and analyzed using consistent quality-control criteria and computational pipelines to minimize potential confounding.

Blinding

Investigators were not formally blinded during sample processing and data collection because samples were derived from archived or prospectively collected clinical specimens with predefined annotations, and the experimental workflows were standardized and not subject to real-time operator-dependent outcome assessment. For computational analyses, quantitative image processing, cell segmentation, and downstream statistical analyses were performed using predefined pipelines and objective computational criteria, which reduced the potential for observer bias.

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

See Supplementary Table S2 and details below:
Marker Oligo_Barcode Clone Vendor Cat# Lot_number Dilution RRID Validation
a-SMA 48 1A4 ThermoFisher MA1-06110 2/3/2020 1:100 AB_557419 Validated for IHC/IF by manufacturer and/or in-house control tissues
CAIX 53 poly R&D AF2188 VNQ0319011 1:50 AB_416562 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD11b 28 EPR1344 Abcam ab216445 11/17/2020 1:100 AB_2915959 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD11c 49 EP1347Y Abcam ab216655 GR3357092-9 1:200 AB_2864379 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD14 7 EPR3653 Abcam ab226121 GR3345113-1 1:200 AB_11188733 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD15 14 MMA BD Biosciences 559045 11/18/2020 1:100 AB_397181 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD16 26 D1N9L Cell signaling 24326S 12/21/2020 1:200 AB_2798877 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD163 45 EDHu-1 Novus Biologicals NB110-40686 1/6/2020 1:200 AB_714951 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD20 81 rlGEL/773 NovusBio NBP2-54591 8/7/2020 1:50 AB_2864380 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD206 55 poly Abcam ab64693 11/17/2020 1:200 AB_1523910 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD25 15 4C9 Cell Marque Custom 2022601 1:200 AB_1157926 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD271 2 NTR/912 Abcam ab212684 GR3354312-1 1:25 AB_3741743 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD3 77 MRQ-39 Cell Marque Custom 1925205 1:200 AB_2864399 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD31 68 C31.3 + C31.7 + C31.10 Novus Biologicals NBP2-47785 7/12/2021 1:200 AB_2864381 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD34 38 QBEnd/10 Novus Biologicals NBP2-34713 11/18/2020 1:100 AB_10005700 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD4 76 EPR6855 Abcam ab181724 GR3285644 1:100 AB_2864377 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD45 56 2B11+PD7/263 NovusBio NBP2-34528 8/28/2020 1:100 AB_960384 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD56 29 MRQ-42 Cell Marque Custom 2028901 1:200 AB_2941091 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD57 30 HNK-1 Biolegend 359602 11/18/2020 1:200 AB_2563757 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD68 70 KP-1 Biolegend 916104 B283618 1:200 AB_2616797 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD73 75 poly R&D AF5795 7/29/2020 1:100 AB_1964487 Validated for IHC/IF by manufacturer and/or in-house control tissues

CD74 24 LN2 BioLegend 326802 7/29/2020 1:50 AB_893401 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD79a 46 JCB117 Cell Marque Custom 1934030 1:100 AB_3741744 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD8 8 C8/144B Cell Marque Custom 20280902 1:100 AB_11000353 Validated for IHC/IF by manufacturer and/or in-house control tissues
CD90 51 EPR3231 Abcam ab221607 6/10/2021 1:200 AB_3095757 Validated for IHC/IF by manufacturer and/or in-house control tissues
CK19 72 RCK108 Abcam ab9221 GR3257988-1 1:400 AB_307088 Validated for IHC/IF by manufacturer and/or in-house control tissues
Collagen I 43 Poly Southern Biotech 1310-01 4/26/2021 1:500 AB_2753206 Validated for IHC/IF by manufacturer and/or in-house control tissues
Collagen IV 33 poly(ab6586) Abcam ab6586 GR3350938-7/GR3350938-9 1:200 AB_305584 Validated for IHC/IF by manufacturer and/or in-house control tissues
CXCL12 41 79018 ThermoFisher MA5-23759 8/6/2021 1:25 AB_2608711 Validated for IHC/IF by manufacturer and/or in-house control tissues
EGFR 58 EP38Y Abcam ab174481 GR273813-4 / GR273813-4 1:50 AB_3741745 Validated for IHC/IF by manufacturer and/or in-house control tissues
FAP 79 Poly R&D systems AF3715 ZKW0619101 1:100 AB_2102369 Validated for IHC/IF by manufacturer and/or in-house control tissues
Fibronectin 36 E5H6X Cell signaling 26836 2 1:50 AB_2924220 Validated for IHC/IF by manufacturer and/or in-house control tissues
FoxP3 20 236A/E7 Invitrogen 14-4777-80 6/17/2021 1:200 AB_467556 Validated for IHC/IF by manufacturer and/or in-house control tissues
GATA6 50 Poly R&D Systems AF1700 KWT0419121 10/11/2020 AB_2108901 Validated for IHC/IF by manufacturer and/or in-house control tissues
Granzyme B 3 EPR20129-217 Abcam ab219803 GR3349314 1:400 AB_2910576 Validated for IHC/IF by manufacturer and/or in-house control tissues
HLA-DR 65 EPR3692 Abcam ab215985 11/2/2020 1:100 AB_2864390 Validated for IHC/IF by manufacturer and/or in-house control tissues
Ki-67 6 B56 BD Biosciences 556003 7/12/2024 1:200 AB_396287 Validated for IHC/IF by manufacturer and/or in-house control tissues
Maspin 21 G167-70 BD Bioscience 554292 4/12/2021 1:100 AB_395347 Validated for IHC/IF by manufacturer and/or in-house control tissues
NaKATPase 5 EP1845Y Abcam ab167390 GR3257524-15 1:100 AB_2890241 Validated for IHC/IF by manufacturer and/or in-house control tissues
P53 52 D07 Cell Marque Custom 11/17/2020 1:200 AB_2864403 Validated for IHC/IF by manufacturer and/or in-house control tissues
Pan-CK 67 C-11 Biolegend 628602 4/12/2021 1:200 AB_439774 Validated for IHC/IF by manufacturer and/or in-house control tissues
PD-1 23 D4W2J Cell signaling Custom 7 1:50 AB_2728833 Validated for IHC/IF by manufacturer and/or in-house control tissues
PDGFRβ 44 Y92 abcam ab215978 GR296584-5 1:100 AB_2894841 Validated for IHC/IF by manufacturer and/or in-house control tissues
PDPN 32 D2-40 Biolegend 916606 6/1/2021 1:100 AB_2565820 Validated for IHC/IF by manufacturer and/or in-house control tissues
Periostin 60 EPR20806 Abcam ab215199 GR3281155-1 1:100 AB_2924310 Validated for IHC/IF by manufacturer and/or in-house control tissues
SPARC 11 SP205 Abcam ab245733 6/1/2021 1:50 AB_3741747 Validated for IHC/IF by manufacturer and/or in-house control tissues
Synaptophysin 69 7H12 Novus NBP1-47483 6/1/2021 1:50 AB_10010435 Validated for IHC/IF by manufacturer and/or in-house control tissues
Tenascin C 66 SPM319 Abcam ab212463 GR3311416-1 1:50 AB_3741748 Validated for IHC/IF by manufacturer and/or in-house control tissues
Tryptase 59 AA1 Abcam ab2378 GR3364935-8 1:1000 AB_303023 Validated for IHC/IF by manufacturer and/or in-house control tissues
Vimentin 62 RV202 BD Biosciences 550513 4/26/2021 1:100 AB_2915945 Validated for IHC/IF by manufacturer and/or in-house control tissues

Validation

All antibodies used are commercially validated by the vendors. CODEX antibodies were conjugated, titrated, and validated using control tissues, and Opal antibodies were screened in uniplex IF and orthogonally validated by DAB-IHC.

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.