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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	Cell viability data were collected using Tecan M200 Pro plate reader (i-control™). Single cell RNA sequencing data were obtained with the NovaSeq platform. Whole exome sequencing were obtained with Illumina NovaSeq 6000
Data analysis	All software used was either open source or commercially available. Brightfield images of tissue sections were acquired with slide scanner (3DHistech Pannoramic 250 Flash II). Immunofluorescence staining was imaged using a confocal microscope (ZEISS LSM 710 with Airyscan). Image analysis was performed with QuPath (v.0.2.3 Queen's University, Belfast, Northern Ireland) and Fiji (v 2.1.0103). Statistical analyses were performed using GraphPad Prism (v 9.2.0) and R (v 4.0.3) single cells RNA sequencing: Raw reads were mapped to the GRCh38 reference genome using 10x Genomics Cell Ranger (v 6.1.1) to infer read counts per gene per cell. Since samples were sequenced on the NovaSeq platform, we performed index-hopping removal using a method developed by Griffiths et al.. After the mapping, the resulting UMI counts were quality controlled, and cells and genes filtered to remove known contaminants and potential doublets. Doublet detection was performed using the tool scDblFInder. Cells were removed if more than 50% of their reads mapped to mitochondrial genes or if they expressed fewer than 400 different genes. Genes were removed if they are not protein-coding or if they were expressed in less than 20 cells. Subsequently, counts were normalized and corrected for cell-cycle effects and library size using

sctransform. Similar cells were grouped based on unsupervised clustering using Phenograph. An automated cell type classification was performed independently for each cell (manuscript in preparation). Cell type annotation was performed using lists of cell type defining, highly expressed marker genes. Marker genes have been selected from previous publications as well as based on in-house knowledge on expected tumor markers. Literature-based marker lists have been curated to only select genes that are actually expressed in the cohort. Results were visualized using Uniform Manifold Approximation and Projection (UMAP).

Whole exome sequencing:
FASTA files were trimmed using Trimmomatic, quality checked performed with FastQC, and reads were aligned with BWA algorithm on hg19. Deduplication, realignment around indels and base recalibration were then performed using GATK4. Mutation, copy-number data and samples level statistics were obtained through the recently established SPICE analysis pipeline. Briefly, it includes quality control step to assess the similarity between matched samples by running SPIA, allele-specific copy number assessment upon data segmentation by running CLONET v2 and mutation and annotation calling via MuTect2 and VEP

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

Whole exome sequencing data generated during this study are available under the accession number (to be uploaded)

Single cells RNAseq data generated during this study are available under the accession number (to be uploaded)

Human research participants

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

Reporting on sex and gender	Clinical details of the patients included in this study are reported in Table 1. The patient cohort comprised 36 males and 4 females. Sex- and gender-based analyses were not performed in this study.
Population characteristics	Clinical details of the patients included in this study are reported in Table 1. At the time of the sampling patients were at 40 to 91 years of age (median of 69 years).
Recruitment	Participants who underwent TUR-B, cystectomy or nephroureterectomy at the Inselspital, University Hospital in Bern were recruited in this study after providing written informed consent.
Ethics oversight	All analyses were carried out in accordance with protocol approved by the Ethical Committee Bern (Cantonal Ethical approval KEK 06/03 and 2017-02295)

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	We included 51 samples from 40 patients. From a subset of samples we performed a deeper characterization (WES, drug screening, proliferation, morphology evaluation). The sample size was determined basing on the recruitment capacity of the study as well as on biological material availability. Multiple independent measurements were taken for each patient-derived sample (drug screening, genetic sequencing, organoid formation assay). Sample size for the performed assays was optimized based on expected mean differences and according to assay manufacturer's indications (where applicable) and on previous experiments.
Data exclusions	From cell viability data, outliers data were identified with GraphPad Prism (v 9.2.0, ROUT method, FDR or Q = 1%) and excluded.

Replication	For cell viability experiments an average of 7 technical replicates for untreated and vehicles, and an average of 3 replicates for each drug condition were seeded
Randomization	For the in vitro organoid drug screening, blanks and functional controls were consistently included to reduce the effect of confounders and covariates. Randomization was not relevant for this study, which was a direct comparison of organoids derived from bladder cancer and primary tumor.
Blinding	Bliding was not relevant for this study, which was a direct comparison of organoids derived from bladder cancer and primary tumor. For molecular analyses, data collection and analysis were performed by different investigators to minimize 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

Methods

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

Antibodies

Antibodies used	Antibodies for immunofluroescence: Mouse monoclonal Anti-CD44 BD Pharmingen 550988, Rabbit polyclonal Anti-CK5/6 BioLegend 905501, Mouse monoclonal Anti-CK8 Thermo Fisher Scientific MA1-06318, Mouse monoclonal Anti-CK14 Abcam ab9220, Mouse monoclonal Anti-CK20 Abcam Ab854, Rabbit monoclonal Anti-GATA3 Cell Signalling 5852, Rabbit monoclonal Anti-Ki67 Gene Tex GTX16667, Rabbit monoclonal Anti-p63 Abcam ab124762-100, Rabbit monoclonal Anti-UPKII Abcam ab213655, Donkey anti-mouse IgG, Alexa Fluor 488 Thermo Fisher Scientific A21202, Donkey anti-rabbit IgG, Alexa Fluor 555 Thermo Fisher Scientific A21434, DAPI Thermo Fisher Scientific 62248. Antibodies for immunohistochemistry: Mouse monoclonal Anti-CD44 BD Pharmingen 550988, Mouse monoclonal Anti-CK5/6 Merck & Cie MAB1620, Mouse monoclonal Anti-CK8 BD Bioscience 345779, Mouse monoclonal Anti-CK14 Biosystems NCL-L-LL002, Mouse monoclonal Anti-CK20 Biosystems 320M-16, Mouse monoclonal Anti-GATA3 Biosystems 390M-14,Rabbit monoclonal Anti-Ki67 Biosystems RM-9106-S1, Mouse monoclonal Anti-p63 Biosystems NCL-L-p63, Rabbit monoclonal Anti-UPKII Abcam ab213655.
Validation	The concentration and method specifications (e.g. antigen retrieval) were used based on the protocols of the antibody manufacturer`s. Immunohistochemistry stainings were performed by the Translational Research Unit (TRU) at the Institute of Pathology, University of Bern where all antibodies were previously validated for human diagnostic purposes. For IF applications, isotype controls matching the primary antibodies were used as negative controls. Ki67: validated on human cervical carcinoma and by Murai et al., PMID: 26965827 CD44: human specific, validate by Kansas et al., PMID: 1702327 p63: validated on prostate tissue, https://doi.org/10.1073/pnas.0510652103 Ck5/6: human specific, validated on breast cancer tissue by Kittrell et al. 2011, PMID:21466693 CK14: validated on bladder cancer tissue by Li et al. 2019, PMID: 29350066 Ck20: human specific, validated on intestinal epithelium by Wang et al. 2017, PMID: 27935584 GATA3: validated on trophoblast cells by Okae et al. 2018, https://doi.org/10.1016/j.stem.2017.11.004 UPKII: human specific, validated on human urothelial cells by Rashidbenam et al. 2021, PMID: 33805910 CK8: validated by knockdown as per reports available at the manufacturer's website. Positive control used: frozen sections human colon

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*