nature portfolio

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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

- H&E and IHC images were collected with Aperio ScanScope (v12.2.2 Leica Biosystem).
- Immuno Blotting acquisitions were performed by films and/or with Image Lab software (v5.2.1 Bio-Rad Laboratories).
- Immunofluorescence data were collected with LAS X (v3.5 Leica) or NZAcquire (v2.1.6.55, Hamamatsu Photonics).
- Fluorescence Images of transwell assays were collected with NIS-Elements software (v5.41.01, Nikon).
- Phase-contrast images of organoids grown in Matrigel were acquired with EVOS Software (Revision 17625, Thermo Fisher Scientific).
- NGS data were sequenced with Illumina NovaSeq 6000.
- RT-qPCR data were collected with 7500 Software (v2.3 Applied Biosisyems) or with LightCycler480 (Roche).

Data analysis	- H&E and IHC images were analyzed with Aperio ScanScope (v12.2.2. Leica) and/or Fiji (v2.14.0, https://fiji.sc/). - Immunofluorescence images were analyzed with Fiji (v2.14.0, https://fiji.sc/) or QuPath (v0.4.3, https://qupath.github.io/). - Phase-contrast images of organoids grown in Matrigel were analyzed in Fiji (v2.14.0, https://fiji.sc/). - Fuorescence Images of transwell assays were analyzed with Fiji (v2.14.0, https://fiji.sc/). - NGS data were processed with NF-CORE RNASeq pipeline (v3.8.1, https://github.com/nf-core/rnaseq) using the Salmon pseudo-aligner to quantify transcript abundances. - Differential expression analysis of RNA sequencing experiments was performed using the DESeq2 R package (v1.34). - Gene Set Enrichment Analysis (GSEA) was performed with ClusterProfiler R package (v4.2.2). - Gene Set Variation Analysis (GSVA) was performed with GSVA R package (v1.51.17). - Upstream Regulator Analysis was performed with Ingenuity Pathway Analysis (IPA, Qiagen). - All Statistical analyses were performed in R (v4.1.2).

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 raw and processed RNA sequencing data generated in this study have been deposited in the GEO database under accession code GSE256272 (https://urlsand.esvalabs.com/?u=https%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fgeo%2Fquery%2Facc.cgi%3Facc%3DGSE256272&e=8c00e339&h=a349627d&f=y&p=n); Private access token: kjyvygyctheblab

Human and mouse gene set annotations for pathway analysis were retrieved from MsigDB [www.gsea-msigdb.org/gsea/msigdb/] with the msigdbr package (version 7.4.1) in R. Human GRCh38 and mouse GRCm39 reference genomes (primary genome assembly) used for the alignment of RNA sequencing data are available on GENCODE [www.gencodegenes.org/].
The clinicopathological data from the UROMOL cohort used in this study are available in the source data of the referenced publication. Raw gene abundances were kindly provided by the authors.
Expression intensity data of CK5 and NUMB across diverse tissues were retrieved from the Human Protein Atlas [www.proteinatlas.org].
All data are available in the main text or the source data provided with this paper.

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	Sex, assigned at birth, was recorded for all the patients in our retrospective cohorts. The post-cystectomy cohort included 287 males (80.6%) and 69 females (19.4%), while the NMIBC cohort comprised 65 males (84.4%) and 12 females (15.6%) reflecting the natural prevalence of bladder cancer. In both cohorts, sex was included as a covariable in multivariable survival analyses. We evaluated the association between NUMB protein expression by IHC and patient sex, however, no significant association was observed. Within the limits imposed by the smaller number of females in our cohorts, we did not observe sex-specific outcomes. Consent was obtained to share de-identified individual patient-level data. Gender was not considered in our study.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity or other socially relevant variables were not considered in our study.
Population characteristics	- The post-cystectomy cohort included patients with a mean age of 68.1 years who underwent radical cystectomy for Bladder Cancer. - The NMIBC cohort included patients with a mean age of 73.6 years who underwent transurethral resection (TUR) for High-Grade Non Muscle Invasive Bladder Cancer.
Recruitment	- Post cystectomy cohort: archival FFPE tissue specimens were collected from a retrospective consecutive cohort of 383 patients who underwent cystectomy for bladder cancer at the European Institute of Oncology (IEO, Milan) between 1999 and 2016. - NMIBC cohort: Archival FFPE transurethral resection (TUR) specimens were collected from a longitudinal series of patients diagnosed with NMIBC at the European Institute of Oncology (Milan, Italy) and Policlinico Ospedali Riuniti di Foggia (Foggia, Italy). Patients with the first available high-grade NMIBC lesion with a 4-month minimum follow-up period were selected for the analyses, excluding those patients with a synchronous muscle-invasive disease.
Ethics oversight	Ethical Boards of the the European Institute of Oncology (IEO, Milan, Italy) and Policlinico Ospedali Riuniti di Foggia (Foggia, Italy)

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 calculations were performed; sample sizes were determined to be adequate based on the magnitude and consistency of measurable differences between groups. The number of experiments (n) or samples/tumors analyzed (n) is indicated in each figure legend.
Data exclusions	Post-cystectomy cohort: a total of 383 patients who underwent radical cystectomy were initially considered for this study. Of these, 356 had available material for immunohistochemical (IHC) analysis of NUMB protein expression. Follow-up data were available for 338 patients. Eighty patients with T4 stage disease (locally advanced) were excluded from the survival analysis to minimize the confounding effects linked to their generally very poor 5-year survival rate. This resulted in a final cohort of 258 patients for the survival analysis. NMIBC cohort: patients with no available follow-up or with a synchronous muscle-invasive disease were excluded from the cohort.
Replication	The number of experiments replicated (n) is indicated in each figure legend.
Randomization	Samples were randomly assigned.
Blinding	Investigators were blinded to patients prognosis during IHC analysis of specimens. Investigators were blinded to mice genotype during histopathological evaluation of the tissues. For the other experiments, the investigators were not blinded to experimental groups during data collection and analysis. Data reported in this latter case is not subjective.

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

Primary Abs:
- anti-Numb mouse monoclonal antibody (clone Ab21, 1:1000 for IB) (#MABN2311 from Sigma Aldrich)
- anti-Numb rabbit monoclonal (clone C29G11) (#2756 from Cell Signaling Technologies, 1:500 for IF, 1:150 for IHC)
- anti-phospho MST1 (Thr183)/MST2(Thr180) (#49332 from Cell Signaling Technology, 1:1000 for IB)
- anti-MST1 (#3682 from Cell Signaling Technology, 1:1000 for IB)
- anti-phospho LATS1 (Thr1079) (#PA5-105442 from Invitrogen, 1:1000 for IB)
- anti-LATS1 (#3477 from Cell Signaling Technology, 1:1000 for IB)
- anti-phospho YAP (Ser127) (#4911 from Cell Signaling Technology, 1:1000 for IB)
- anti-YAP (63.7) (#sc-101199 from Santa Cruz, 1:1000 for IB, 1:200 for IF)
- anti-YAP (D8H1X) (#14074 from Cell Signaling Technology, 1:200 for IF)
- anti-active YAP (EPR19812) (#ab205270 from Abcam, 1:500 for IF)
- anti-phospho Myosin Light Chain 2 (Ser19) (pMLC2) (#3671 from Cell Signaling Technology, 1:1000 for IB; 1:200 for IF)
- anti-phospho Cofilin-1 (Ser3) (F-11) (#sc-365882 from Santa Cruz, 1:1000 for IB, 1:300 for IF)
- anti-Cofilin (D3F9) (#5175 from Cell Signaling Technology, 1:1000 for IB)

- anti-RAC1 (clone 102/Rac1 (RUO) from BD Biosciences, 1:350 for IF)
- anti-RhoA (26C4) (#sc-418 from Santa Cruz, 1:400 for IF)
- anti-RhoA (#ARH03 from Cytoskeleton, 1:2000 for IB)
- anti-Actin (NB600-503 from Bio-techne Srl, 1:4000 for IB)
- anti-Vinculin (#V9131 from Sigma Aldrich, 1:10000 for IB)
- anti-CK5 (EP1601Y) (ab52635 from Abcam, 1:2000 for mice IHC, 1:200 for human IHC)
- anti-CK14 (LL002) (ab7800 from Abcam, 1:1000 for IF)
- anti-CK7 (RCK105) (ab9021 from Abcam, 1:500 for IF)
- anti-CK20 (ab118574 from Abcam, 1:200 for IF and IHC)
- anti-CYR61 (NB100-356 from Bio-techne Srl, 1:100 for IF)

Secondary Abs:
- goat anti-rabbit IgG (H+L)-HRP conjugate (#1706515 Bio-Rad, 1:2500 for IB)
- goat anti-mouse (H+L)-HRP conjugate (#1706516 Bio-Rad, 1:2500 for IB)
- Alexa Cy3- and 488-conjugated secondary antibodies from Jackson ImmunoResearch (1:500 for IF)
- Alexa Fluor 555 donkey anti-mouse IgG (1:400 for IF) from ThermoFisher
- Alexa Fluor 488 donkey anti-rabbit IgG (1:500 for IF) from ThermoFisher

Validation

Validation statements available from manufacturers:
Primary Abs:
- anti-Numb mouse monoclonal antibody (clone Ab21): https://www.sigmaaldrich.com/IT/it/product/mm/mabn2311
- anti-Numb rabbit monoclonal (clone C29G11): https://www.cellsignal.com/products/primary-antibodies/numb-c29g11-rabbit-mab/2756
- anti-phospho MST1 (Thr183)/MST2(Thr180): https://www.cellsignal.com/products/primary-antibodies/phospho-mst1-thr183-mst2-thr180-e7u1d-rabbit-mab/49332
- anti-MST1: https://www.cellsignal.com/products/primary-antibodies/mst1-antibody/3682
- anti-phospho LATS1 (Thr1079): https://www.thermofisher.com/antibody/product/Phospho-LATS1-Thr1079-Antibody-Polyclonal/PA5-105442
- anti-LATS1: https://www.cellsignal.com/products/primary-antibodies/lats1-c66b5-rabbit-mab/3477
- anti-phospho YAP (Ser127): https://www.cellsignal.com/products/primary-antibodies/phospho-yap-ser127-antibody/4911
- anti-YAP (63.7): https://www.scbt.com/p/yap-antibody-63-7
- anti-YAP (D8H1X: https://www.cellsignal.com/products/primary-antibodies/yap-d8h1x-xp-174-rabbit-mab/14074
- anti-active YAP (EPR19812): https://www.abcam.com/products/primary-antibodies/active-yap1-antibody-epr19812-ab205270.html
- anti-phospho Myosin Light Chain 2 (Ser19) (pMLC2): https://www.cellsignal.com/products/primary-antibodies/phospho-myosin-light-chain-2-ser19-antibody/3671
- anti-phospho Cofilin-1 (Ser3) (F-11): https://www.scbt.com/p/p-cofilin-1-antibody-f-11
- anti-Cofilin (D3F9): https://www.cellsignal.com/products/primary-antibodies/cofilin-d3f9-xp-174-rabbit-mab/5175
- anti-RAC1 (clone 102/Rac1 (RUO)): https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-rac1.610651
- anti-RhoA (26C4): https://www.scbt.com/p/rho-a-antibody-26c4
- anti-RhoA: https://www.cytoskeleton.com/arh03
- anti-Actin: https://www.bio-techne.com/p/antibodies/beta-actin-antibody_nb600-503
- anti-Vinculin: https://www.sigmaaldrich.com/IT/it/product/sigma/v9131
- anti-KRT5 (EP1601Y): https://www.abcam.com/products/primary-antibodies/cytokeratin-5-antibody-ep1601y-cytoskeleton-marker-ab52635.html
- anti-CK14 (LL002): https://www.abcam.com/products/primary-antibodies/cytokeratin-14-antibody-ll002-ab7800.html
- anti-CK7 (RCK105): https://www.abcam.com/products/primary-antibodies/cytokeratin-7-antibody-rck105-cytoskeleton-marker-ab9021.html
- anti-CK20: https://www.abcam.com/ab118574.pdf
- anti-CYR61: https://www.bio-techne.com/p/antibodies/cyr61-ccn1-antibody_nb100-356

Secondary Abs:
- goat anti-rabbit IgG (H+L)-HRP conjugate: https://www.bio-rad.com/it-it/sku/1706515-goat-anti-rabbit-igg-h-l-hrp-conjugate?ID=1706515
- goat anti-mouse (H+L)-HRP conjugate: https://www.bio-rad.com/it-it/sku/1706516-goat-anti-mouse-igg-h-l-hrp-conjugate?ID=1706516
- Alexa Cy3-conjugated secondary antibody: https://www.jacksonimmuno.com/technical/products/conjugate-selection/cyanine/cy3
- Alexa 488-conjugated secondary antibody: https://www.jacksonimmuno.com/technical/products/conjugate-selection/alexa-fluor/488
- Alexa Fluor 555 donkey anti-mouse IgG: https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31570
- Alexa Fluor 488 donkey anti-rabbit IgG: https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

- RT4, HT1376, cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, Virginia).
- RT112 cell lines were acquired from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures

	GmbH (Braunschweig, Germany). - CLS439 cell lines were obtained from the CLS-Cell Lines Service GmbH (Eppelheim, Germany). - 5637 and KK47 cell lines were obtained from the European Institute of Oncology (IEO) cell lines collection. - BBN WT and Numb-KO tumor cells were derived in our lab from male mice.
Authentication	All human cell lines were authenticated at each batch freezing by STR profiling (StemElite ID System, Promega)
Mycoplasma contamination	All cell lines were tested for mycoplasma by PCR (Uphoff and Drexler, 2002) and biochemical assay (MycoAlert, Lonza) and the results were negative.
Commonly misidentified lines (See ICLAC register)	No

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	Mice strains used were: FVB; NUMB-KO mice in FVB background; NOD/SCID IL2R gamma-chain null (NSG) mice. Bladders from untreated mice were collected from aged-matched 4 to 12-month FVB WT and NUMB-KO mice. Aged-matched 8- to 16-week-old FVB WT and NUMB-KO mice were used for the experiments involving the administration of BBN.
Wild animals	This study did not involve wild animals.
Reporting on sex	Only male animals were used for the experiments.
Field-collected samples	This study did not involve animals collected from the field.
Ethics oversight	All animal studies were conducted with the approval of Italian Minister of Health (AUT. N. 125/2019-PR) and were performed in accordance with the Italian law (D.lgs. 26/2014), which enforces Dir. 2010/63/EU (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes) and EU 86/609 directive and under the control of the institutional organism for animal welfare and ethical approach to animals in experimental procedures (Cogentech OPBA).

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

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.*

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.*