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Life Sciences Reporting Summary

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For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   Sample size was determined using G*Power software.

2. Data exclusions

   Describe any data exclusions.

   No data excluded

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   We have identified and characterized Doublecortin-expressing (DCX+) neural cells in the tumour microenvironment of 3 different models :
   -human cancer specimens.
   -orthotopic xenogeneic (luciferase-expressing PC-3 cancer cells) mouse cancer model.
   -transgenic (Hi-Myc, Pten, PyMT) mouse cancer models.

   To track and isolate DCX+ cells, we generated triple-transgenic cancer mice (DCX-CreERT2/ loxp-EYFP/Hi-Myc mice, called DCX-EYFP/Hi-Myc) and used flow cytometry and confocal image analyses as well as quantitative RT-PCR to characterize DCX+ cells in a reproducible manner.

   We have designed gain-and-loss of function studies using genetic (DCX-CreERT2/loxp-HBEGF/ Hi-Myc or nu/nu mice) and chemical (Tamoxifen/ Diphteria toxin) approaches combined with surgical transplantation of DCX+ cells.

   We have performed tdTomato lentiviral vector injections by stereotaxy in the SVZ/or DG of the 4 different mouse cancer models described above.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   The effect of covariates on statistical comparisons was verified using regression analyses, as indicated on the method section.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Human data collection:
   Quantification of DCX+ cells was conducted blind, without knowledge of clinical data, in prostate tumour or hyperplastic areas and in remaining normal prostate tissues surrounding cancer areas.

   Mouse data collection:
   Flow cytometry and bioluminescence data collection, and PIN quantifications were done blind.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ 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 any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Live imaging software, version4.4 (Perkin Elmer, Waltham, MA) for bioluminescence analysis.
Flow Jo software (Tree Star, Ashland, OR).
Microsoft Excel 2011 (Microsoft, Redmond, WA).
GraphPad Prism7 software (GraphPad software, San Diego, CA).
LAS X 2.0.1.14392 software (Leica, Wetzlar, Germany).
Volocity 6.3.1 software (Perkin Elmer, Waltham, MA).
Zen microscope software (Zeiss MicroImaging, Thornwood, NY).
R software (https://www.r-project.org/, R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria, version 3.3)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No unique materials were used

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Flow cytometry
CD45 (clone 30F11), (Miltenyi biotec, Bergisch Gladbach, Germany).
TER119 (clone Ter-119), (Miltenyi biotec, Bergisch Gladbach, Germany).
CD31 (clone 390), (Miltenyi biotec, Bergisch Gladbach, Germany).
CD326 (clone caa7-9G8), (Miltenyi biotec, Bergisch Gladbach, Germany).
CD49f (clone REA518), (Miltenyi biotec, Bergisch Gladbach, Germany).
Sca-1 (clone REA422), (Miltenyi biotec, Bergisch Gladbach, Germany).
PSA-NCAM (clone 2-2B), (Miltenyi biotec, Bergisch Gladbach, Germany).
CD24 (clone M1/69), (Miltenyi biotec, Bergisch Gladbach, Germany).
Biotinylated EGF complexed with BV785-streptavidin (Invitrogen by Thermo Fischer Scientific).

Immunofluoresecnce
DCX (clone 2G5, lot VP1309040), (Millipore, Billerica, MA).
PSA-NCAM (clone 2-2B, cat: ABC-AbC0019), (ABC scientific, Los Angeles, CA).
βIII-tubulin (polyclonal,cat: PRB-435P, lot D13GF02055), (Covance, Princeton, NJ).
α-Internexin (polyclonal, AB5354, Lot 2208747) (Millipore, Billerica, MA).
EYFP (IgY fraction, cat: GFP-1020, Lot GFP697986) (Aves labs, Tigard, OR).
NF-H (Polyclonal, Cat : AB5539, Lot: 2219329) (Millipore, Billerica, MA).
Pan-cytokeratine (Monoclonal C-11, PCK-26, CY-90, KS-1A3, M20, A53-B/A2, Cat: C2562, Lot 033M4760V)(Sigma-Aldrich).
MAP-2 (Polyclona, Cat: AB5622) (Millipore, Billerica, MA).
CD31 (clone MEC13.3) (BioLegend, San Diego, CA)
Albumin (Polyclonal, Cat: GTX102419) (Genetex, Irvine, CA)
Alexafluor 647-, 568-, 488-conjugated goat antibodies to mouse, rabbit or chicken IgG respectively (Life technologies, Carlsbad, CA).
Primary antibodies were validated on mouse and human tissues.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

PC-3 cells (Human prostate adenocarcinoma) stably transfected with the luciferase 2 gene under the control of human ubiquitin C promoter have been provided by Perkin Elmer, Waltham, MA.

b. Describe the method of cell line authentication used.

The authentification of PC-3 cells was made by Perkin Elmer (Source of parental line ATCC CRL-1435TM)

c. Report whether the cell lines were tested for mycoplasma contamination.

All cell lines provided by Perkin Elmer (Caliper life sciences/Perkin Elmer) are confirmed to be pathogen free by the IMPACT profile I (PCR) at the university of Missouri research animal diagnostic and investigative laboratory.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Balb/c nu/nu (B6.Cg-Foxn1nu) and cMyc mice (FVB-Tg(ARR2/Pbsn-MYC)7Key21, called Hi-Myc hereafter) were obtained from Charles River laboratories and the National Cancer Institute, respectively.
Hi-Myc mice were intercrossed with C57BL/6-Gt(ROSA)26Sortm1(EYFP)Cos or C57BL/6-Gt(ROSA)26Sortm1(HBEGF)Awai/J mice which were previously crossed with Tg(DCX-cre/ERT2)1Mul mice to generate CreERT2-inducible expression of the enhanced yellow fluorescent protein (EYFP) or simian Diphtheria Toxin Receptor (DTR; from simian Hbegf) under the control of a doublecortin (DCX) promoter (all obtained from the Jackson laboratory). The resulting offsprings DCX-CreERT2/loxp-EYFP/Hi-Myc or DCX-CreERT2/loxp-HBEGF/Hi-Myc express EYFP or DTR, respectively, in DCX-expressing cells after administration of tamoxifen to the animals. Cells expressing DTR can be ablated following diphtheria toxin administration. Respective controls were also generated by intercrossing the three strains. Immunodeficient B6.Cg-Foxn1nu+/- heterozygous nude mice were also intercrossed with Tg(DCX-cre/ERT2)1Mul bred with Gt(ROSA)26Sortm1(HBEGF)Awai/J to deplete cells that express DCX in nu/nu mice.

Males (Hi-Myc or Pten lines) were used for all the experiments described in the manuscript (between week3 to week24 after birth).
Females (PyMT line) were used for experiments described in ED figure 10.
Males and females were used as breeders (between week6 and week16 after birth).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

All patient and tumour characteristics were provided in Table 1 (age, gender, PSA levels, date of surgery, Gleason score, pathological stage, number of invaded zones, recurrence).
All patient had histologically confirmed and clinically localized prostate cancer or benign hyperplasia, and did not received prior treatment at the institution.