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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	IF and FISH data were collected using LSM 800. HE data was collected by OlympusBx53. Two-photon fluorescence data was collected using LSM 780. Images of cleared tissue were acquired by Lattice Light sheet 7 microscope. Bioluminescence imaging data was obtained by IVIS Lumina LT system. MRI data was collected by 3.0T MR system. Laser speckle contrast images were obtained by moorFLPI2 system. Intravital imaging data was collected by IVM MS2. Hydrolysis rate detection was performed by AB SCIEXTriple Quad 4500 c-MS/MS System. MST data was collected by MonolithNT.115. ITC data was collected by MicroCalPEAQ-ITC. The signal intensity of blood flow was analyzed by Open CV.
Data analysis	Image J (v1.48) and GraphPad Prism (v8.0) were used for statistical analysis. The 10x Genomics Cell Ranger software suite (v.3.1.0) with human reference GRCh38 was used to process the scRNA-seq Fastq files. ScRNA-seq analysis was proformed using R package Seurat (v.3.1.2). SCENIC was used to identify the cluster-specific gene regulatory networks. Low-quality reads of ChIP-seq were filtered out using Trimmomatic (version 0.38) and the chromatin accessible peaks were called using MACS2. GO enrichment analysis of ChIP-seq was performed using EasyGO (http://bioinformatics.cau.edu.cn/easygo). StringTie and edgeR were used to assess the expression levels of all transcripts in RNA-seq. Radiomic analysis was performed by 3D Slicer (v5.6.2).Venn diagram was performed in https://bioinfogp.cnb.csic.es/tools/venny/.

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

scRNA-seq data of the TPCs acquired using the 10× Chromium protocol was downloaded from GEO accession GSE199726. ScRNA-data of tumors from colorectal cancer patients that are resistant (non-responders) and sensitive (responders) to PD-1 blockade was downloaded from GSE236581. ScRNA data of tumors from human colorectal cancer with MSI-H and MSS status was downloaded from GSE178341. RNA-seq and ChIP-seq data were deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession numbers GSE252596 and GSE248500. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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	This analysis was not based on sex or gender.
Reporting on race, ethnicity, or other socially relevant groupings	This analysis was not based on race, ethnicity, or other socially relevant groupings
Population characteristics	Chinese person, patient information were listed in supplementary Data 1-8.
Recruitment	Computed tomography (CT) and ultrasound doppler images of human colorectal cancer (CRC), tumor samples of human CRC, breast cancer and lung cancer patient, blood samples and adjacent normal tissues of human CRC were collected from the First Affiliated Hospital of Jinan University.
Ethics oversight	The human samples used in this study were approved by the Clinical Ethics Committee of the First Affiliated Hospital of Jinan University (Guangzhou, China), and written informed consent was received from participants prior to inclusion in the study. The approval ID was JNUKY-2023-0067.

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	In the article, it has been described that in vivo studies used 6 samples per group, while in vitro studies used 3 samples per group.
Data exclusions	No data were excluded.
Replication	All experimental replicates were reported in figure legends. In vitro experiments were performed at least three times (n = 3) showing reproducible patterns. Animal experiments included at least three biological replicates of each condition and repeated by two times.
Randomization	In animal experiments, samples can be randomly assigned to different experimental groups using a random number generator. This method ensures that each sample has an equal chance of being assigned to different groups, minimizing bias and ensuring the reliability of the results. For in vitro experiments, cells were seeded and wells were randomly assigned.
Blinding	The study described in the article is a single-blind study, meaning that the researchers were aware of the group assignments during the course of the study.

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

NKX2-3 Antibody, Cat.HPA047561, RRID:AB_2680088, Sigma-Aldrich; WB(1:1000), IF(1:200);
FAPα Antibody, Cat.AF3715, RRID:AB_2102369, R&D, WB(1:1000);
p-MLC2Ser15 Antibody, Cat.AF8618, RRID:AB_2846214, Affinity, WB(1:1000);
MLC2 Antibody, clone D18E2, Cat.8505, RRID:AB_2728760, Cell Signaling Technology, WB(1:1000);
Rab 27A Antibody, Cat.17817-1-AP, RRID:AB_2176728, Proteintech, WB(1:1000);
HSP70 Antibody, clone EPR17677, Cat.ab182844, RRID:AB_2925226, Abcam, WB(1:1000);
CD9 Antibody, clone D8O1A, Cat.13174S, RRID:AB_2798139, Cell Signaling Technology, WB(1:1000);
CD63 Antibody, clone EPR21151, Cat.ab217345, RRID:AB_2754982, Abcam, WB(1:1000);
TSG101 Antibody, clone EPR7130(B), Cat.ab125011, RRID:AB_10974262, Abcam, WB(1:1000);
GM130 Antibody, clone D6B1, Cat.12480, RRID:AB_2797933, Cell Signaling Technology, WB(1:1000);
PDE1C Antibody, Cat.13785-1-AP, RRID:AB_2268145, Proteintech, WB(1:1000);
GAPDH Antibody, Cat.GB15004, RRID:AB_2943040, Servicebio, WB(1:1000);
α-SMA Antibody, Cat.GB111364, RRID:AB_2910228, Servicebio, IF(1:300);
CD31 Antibody, Cat.GB113151, RRID:AB_2923131, Servicebio, IF(1:300);
NG2 Antibody, clone EPR20244, Cat.ab183929, RRID:AB_3095078, Abcam, IF(1:200);
VE-cadherin Antibody, clone D87F2, Cat.2500S, RRID:AB_10839118, Cell Signaling Technology, IF(1:200);
ZO-1 Antibody, Cat.21773-1-AP, RRID:AB_10733242, Proteintech, IF(1:400);
EpCAM Antibody, Cat.GB11274, RRID:AB_2941859, Servicebio, IF(1:300);
CD45 Antibody, clone 35-Z6, Cat.sc-1178, RRID:AB_627074, Santa Cruz Biotechnology, IF(1:200);
HIF1α Antibody, clone 35-Z6, Cat.36169, RRID:AB_2799095, Cell Signaling Technology, WB(1:1000);
Mouse Nkx2-3,Cat.SAB2105276,RRID:AB_10743069,Sigma,IF(1:200);
VWF,Cat.ab11713,RRID:AB_298501,Abcam,IF(1:400);
CA9,Cat.ab15086,RRID:AB_2066533,Abcam,IHC(1:300);
MAdCAM-1,Cat.GB111006,Servicebio,IF(1:500);
MECA79,Cat.sc-19602,RRID:AB_627143,Santa Cruz,Biotechnology,IF(1:100);
MYH11 Anitibody, Cat.21404-1-AP, RRID:AB_10732819, Proteintech; IF(1:500);
SM22α Anitibody, Cat.GB11366-100, Servicebio; IF(1:500);
PDGFRB Anitibody,Clone 28E1, Cat.3169s, RRID:AB_2162497, Cell Signaling Technology; IF(1:100);
Rabbit IgG,Cat.GB111738,Servicebio,IF(1:400),WB(1:1000);
HRP conjugated Goat Anti Rabbit IgG, Cat.GB23303, RRID:AB_2811189, Servicebio, WB(1:4000),IF(1:1000);
HRP conjugated Goat Anti Mouse IgG, Cat.GB23404, RRID:AB_2904020, Servicebio, WB(1:4000),IF(1:1000);
HRP conjugated Goat Anti Rat IgG,Cat.GB23302,RRID:AB_2928139,Servicebio,IF(1:1000);
HRP conjugated Rabbit Anti sheep,Cat.GB23302,RRID:AB_2928981,Servicebio WB(1:4000),IF(1:1000).

Validation

All antibodies were purchased from reputable commercial suppliers with detailed information provided;
NKX2-3 Antibody,validation:https://www.sigmaaldrich.com/HK/zh/product/sigma/hpa047561;
FAPα Antibody,validation:https://www.rndsystems.com/cn/products/human-fibroblast-activation-protein-alpha-fap-antibody_af3715;
p-MLC2Ser15 Antibody,validation:https://affbiotech.cn/goods-16570-AF8618-Phospho_MLC2_Ser15_Antibody.html;
MLC2 Antibody,validation:https://www.cellsignal.com/products/primary-antibodies/myosin-light-chain-2-d18e2-rabbit-mab/8505;
Rab 27A Antibody,validation:https://www.ptglab.com/products/RAB27A-Antibody-17817-1-AP.htm;
HSP70 Antibody,validation:https://www.abcam.cn/products/primary-antibodies/hsp70-antibody-epr17677-bsa-and-azide-free-ab250639.html;
CD9 Antibody,validation:https://www.cellsignal.com/products/primary-antibodies/cd9-d8o1a-rabbit-mab/13174;
CD63 Antibody,validation:https://www.abcam.cn/products/primary-antibodies/cd63-antibody-epr21151-ab217345.html;
TSG101 Antibody,validation:https://www.abcam.cn/products/primary-antibodies/tsg101-antibody-epr7130b-ab125011.html;
GM130 Antibody,validation:https://www.cellsignal.cn/products/primary-antibodies/gm130-d6b1-xp-rabbit-mab/12480;
PDE1C Antibody,validation:https://www.ptgcn.com/Products/PDE1C-Antibody-13785-1-AP.htm;
GAPDH Antibody,validation:https://www.servicebio.cn/goodsdetail?id=11279;
α-SMA Antibody,validation:https://www.servicebio.cn/goodsdetail?id=3743;
CD31 Antibody,validation:https://www.servicebio.cn/goodsdetail?id=6394;
NG2 Antibody,validation:https://www.abcam.cn/products/primary-antibodies/ng2-antibody-epr20244-ab183929.html;
VE-cadherin Antibody, validation:https://www.cellsignal.com/products/primary-antibodies/ve-cadherin-d87f2-xp-rabbit-mab/2500;

ZO-1 Antibody,validation:https://www.ptgcn.com/Products/ZO1-Antibody-21773-1-AP.htm;
EpCAM Antibody,validation:https://www.servicebio.cn/goodsdetail?id=1497;
CD45 Antibody,validation:https://www.scbt.com/p/cd45-antibody-35-z6;
HIF1α Antibody, validation:https://www.cellsignal.cn/products/primary-antibodies/hif-1a-d1s7w-xp-rabbit-mab/36169;
Mouse Nkx2-3 Antibody,validation:https://www.sigmaaldrich.cn/CN/zh/product/sigma/sab2105276;
VWF Antibody, validation:https://www.abcam.cn/products/primary-antibodies/von-willebrand-factor-antibody-ab11713.html;
MADCAM-1 Antibody, validation:https://www.servicebio.cn/goodsdetail?id=2658;
MECA79 Antibody, validation:https://www.scbt.com/zh/p/meca-79-antibody-meca-79;
MYH11 Anitibody validation:https://www.ptglab.co.jp/Products/MYH11-Antibody-21404-1-AP.htm;
SM22α Anitibody validation:https://www.servicebio.cn/goodsdetail?id=1189;
PDGFRB Anitibody validation:https://www.cellsignal.com/products/primary-antibodies/pdgf-receptor-b-28e1-rabbit-mab/3169;
Rabbit igG validation:https://www.servicebio.cn/goodsdetail?id=4756;
HRP conjugated Goat Anti Rabbit IgG, validation:https://www.servicebio.cn/goodsdetail?id=266;
HRP conjugated Goat Anti Mouse IgG, validation:https://www.servicebio.cn/goodsdetail?id=264;
HRP conjugated Goat Anti Rat IgG, validation:https://www.servicebio.cn/goodsdetail?id=265;
HRP conjugated Rabbit Anti Sheep IgG, validation:https://www.servicebio.cn/goodsdetail?id=263.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The mouse colorectal cancer cell line MC-38 (Cat.BNCC337716) was purchased from BeNa CultureCollection (Beijing, China); Human colorectal cancer cell lines HCT116 (Cat.CCL-247), DLD-1 (Cat.CCL-221), HT-29 (CVCL_0320), Caco2 (RRID: CVCL_0025) and human microvascular endothelial cell-1 (HMEC-1, Cat.CRL-3243) were acquired from American Type Culture Collection (Manassas, Virginia, USA). Lymphatic endothelial cell was obtained from iCell (Shanghai, China).Tumor pericyte and smooth muscle cell (SMC) were isolated by microdissection combined with pericyte medium-based approach (MPMA) and microdissection combined with SMC medium-based approach in our lab according to previous study (PMID: 37635201). Primary cancer-associated fibroblast (CAF) was isolated according to previous study (PMID: 38346978).
Authentication	All the cell lines were authenticated by short tandem repeat profiling prior to use.
Mycoplasma contamination	All the cell lines were tested for mycoplasma contamination by PCR every two months, only mycoplasma negative cells were used for experiments.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell 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	C57BL/6JGpt mice (5-6 weeks, 18-20 g), BALB/c nude mice (5-6 weeks, 20-22 g), Rosa26-CAG-LSL-Cas9-tdTomato mice (B6/JGpt-Rosa26tm1(CAG-LSL-Cas9-tdTomato)/Gpt; RRID: IMSR_GPT:T002249), Cspg4-CreERT2 mice (B6/JGpt-Cspg4em1Cin(CreERT2-P2A)/Gpt; RRID: IMSR_GPT:T006187), and Nkx2-3-flox mice (B6/JGpt-Nkx2-3em1Cflox/Gpt; Cat.016620) were acquired from GemPharmatech Co., Ltd. (Nanjing, Jiangsu, China). All mice were housed in a specific pathogen-free (SPF) facility at Jinan University Laboratory Animal Center under a 12-hour light/12-hour dark cycle , with controlled temperature (22-24 ℃) and humidity (40-60 %). Animals had ad libitum access to autoclaved food and water and were maintained in individually ventilated cages (IVCs) with corn cob bedding and environmental enrichment.
Wild animals	No wild animals were used in this study.
Reporting on sex	In this study, to control for experimental bias, we exclusively used male mice. As sex was not the focus our research, no sex-based analyses were performed. The use of single sex in the study design could minimize potential confounding factors and data disaggregation by sex was not applicable.
Field-collected samples	No Field-collected samples were used in this study.
Ethics oversight	All animal experiments were approved by the Institute of Experimental Animal Ethics Committee of Jinan University (Guangzhou, China). The approval ID for the use of animals were 00365755 and 00379905.

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

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	The data of ChIP-seq was deposited in NCBI's Gene Expression Omnibus and accessible through GEO Series accession GSE248500 (tokens: kpuvyoyubfsptyf).
Files in database submission	GSE248500_NKX_annotation.xls.gz; GSE248500_RAW.tar.
Genome browser session (e.g. UCSC)	No longer applicable.

Methodology

Replicates	Three biological replicates for NKX2-3 over-expressing pericytes.
Sequencing depth	35 million raw reads for each sample with the length of 150bp; uniquely mapped rate was 97% and they were paired-end.
Antibodies	NKX2-3, RRID: AB_2680088, Sigma-Aldrich, Rabbit IgG.
Peak calling parameters	Peaks calling was finished by MACS2 (v 2.1.1) with the default parameters-bandwidth 300 bp; model fold 5-50; q value 0.05.
Data quality	Numbers of peaks with FDR<0.05, Fold>5 were 65.
Software	Trimmomatic (version 0.38) was used to filter out low-quality read. MACS2 software (version 2.1.1.20160309) was used to call peaks by default parameters (bandwidth, 300 bp; model fold, 5, 50; q value, 0.05).