nature research

Corresponding author(s): Swarnali Acharyya

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   Power analysis. Statistician (SLP) determined the sample size and performed the statistical tests.

2. Data exclusions

   Describe any data exclusions.

   RNA sequencing data analysis: From the differential gene lists of RNA seq of muscles from C26m2 and 4T1 models, a new list was created with common genes from both the models. To prevent spurious results from infinity values, genes with low expression on one condition and/or zero expression on another were excluded from the initial analysis using C26m2 as the base model. Details are described in the Methods.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   Experimental findings were reproduced and repeats are indicated in each figure.

4. Randomization

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

   Samples/mice were recorded by randomized cage numbers generated on Filemaker pro and treatment groups were assigned based on those numbers. Blinded analysis for the animal experimentalist who performed the injections/ animal data recordings.

5. Blinding

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

   Yes. Blinded immunohistochemical analysis was performed on ZIP14 on muscle sections by independent pathologists. Details in methods.

Note: all studies involving animals and/or human research participants must disclose 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 (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals 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

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.

Statistical analysis: GraphPad Prism 6
Image Quantification: Image J
Quantitative real-time PCR: Applied Biosystems software
RNA sequencing analysis: RTA (Illumina) for base calling and bcl2fastq (version 1.8.4), Tophat2. (version 2.0.11)
Gene expression: Gene Set Enrichment Analysis (GSEA), DAVID (Database for Annotation, Visualization and Integrated Discovery), Principal Component Analysis, cufflinks (version 2.2.1), R package of cummeRbund (version 2.20.0).
Gene pathways: Ingenuity Pathway Analysis (Qiagen).
LA-ICP-MS analysis:LSX-213 laser ablation system (LA, CETAC, Omaha, USA) coupled with ELEMENT 2™ inductively coupled plasma mass spectrometry (ICPMS, Thermo Fisher Scientific, Bremen, Germany).
Liver and kidney function tests:automated clinical chemistry analyzer (VetAce ® Clinical Chemistry System; Alfa Wasserman Diagnostic LLC West Caldwell, New Jersey)
Flow Cytometry: FlowJo (version 10)
Details are described in Methods.

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 for-profit company.

No restrictions on availability of any material, and can be obtained upon request to the corresponding author. Cell lines developed in this study will be available upon request with MTA.

9. Antibodies

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

CD31-PE (1:100, eBioscience, ref #12-0311-81)
CD45-PE (1:100, eBioscience, ref #12-0451-83)
Sca1-PE (1:100, eBioscience, ref #12-5981-81)
integrin-α7 antibody (1:10, Miltenyi Biotec, ref #130-103-774)
CD34-FITC (1:50, Miltenyi Biotec, ref #130-105-831)
integrin-α7-APC (1:100, Miltenyi Biotec, ref #130-103-356)
anti-mouse IgG magnetic beads (0.02-0.05ml, Miltenyi Biotec, ref #130-048-402)
anti-PE magnetic beads (0.04-0.1ml, Miltenyi Biotec, ref #130-105-639)

Antibodies used for immunofluorescence staining, immunohistochemical staining, or immunoblotting:
rabbit polyclonal antibody against mouse ZIP14 (1:2500, developed by our laboratory). Antibody validation by immunohistochemistry shown in Supplemental Data and described in Methods using knockout tissues, positive and negative controls for mouse and human tissues.

rabbit polyclonal antibody against human ZIP14 (1:500, developed by our laboratory) Antibody validation shown in Supplemental Data and described in Methods.

rabbit polyclonal antibody against Desmin (1:500, Sigma, ref #D8281)
mouse monoclonal antibody against fast MyHC (1:500, Sigma, clone MY-32, ref #M4276)
mouse monoclonal antibody against Tropomyosin (1:500, DSHB, clone CH1)
rabbit polyclonal antibody against Laminin (1:200, Sigma, L9393)
rat monoclonal antibody against CD31 (1:250, BD, 557355)
mouse monoclonal antibody against PAX7 (1:200, DSHB)

mouse monoclonal antibody against myosin heavy chain IIa (1:1, DSHB, SC-71)
mouse monoclonal antibody against myosin heavy chain IIb (1:1, DSHB, BF-F3)

rabbit polyclonal antibody against ZIP14 (1:250, Millipore, cat #06-1022, lot #NRG1830910)
rabbit polyclonal antibody against ZIP14 (1:1000, Sigma, ref #HPA016508)
rabbit polyclonal antibody against Cleaved-caspase 3 (1:1000, Cell signaling, ref #9661)
mouse monoclonal antibody against alpha tubulin (1:5000, Sigma, clone B-5-1-2, ref #T6074)
rabbit monoclonal antibody against p-p65 (S536) (1:1000, Cell signaling, ref #3033)
rabbit monoclonal antibody against p65 (1:1000, Cell signaling, ref #8242)
rabbit monoclonal antibody against pSmad2 (S465/467) (1:1000, Cell signaling, ref #3108)
rabbit monoclonal antibody against Smad2 (1:1000, Cell signaling, ref #5339)
rabbit polyclonal antibody against p-c-Jun (S63) (1:1000, Cell signaling, ref #9261)
rabbit monoclonal antibody against c-Jun (1:1000, Cell signaling, ref #9165)
mouse monoclonal antibody against skeletal actin (1:5000, Sigma, A2172)
mouse monoclonal antibody against troponin T (1:1000, DSHB, JLT12)
mouse monoclonal antibody against Myosin light chain (1:1000, DSHB, F310)
InVivoPlus anti-TGFβ (BioXCell, BP0057, Clone: 1D11.16.8)
InVivoPlus anti-TNFα (BioXCell, BP0058, Clone: XT3.11)
InVivoPlus Mouse IgG1 Isotype (BioXCell, BP0083, Clone: MOPC-21)
Goat anti-mouse IgG1 Alexa Fluor 488 (1:500, ThermoFisher, A21121)
Goat anti-mouse IgM Alexa Fluor 546 (1:500, ThermoFisher, A21045)
Goat anti-rat IgG Alexa Fluor 568 (1:500, ThermoFisher, A11077)
Goat anti-rabbit Alexa Fluor 488 (1:500, ThermoFisher, A11034)
Goat anti-rabbit Alexa Fluor 568 (1:500, ThermoFisher, A11011)
Goat anti-rabbit IgG-HRP (1:5000, Sigma, A0545)
Goat anti-mouse IgG-HRP (1:5000, Sigma, A9917)
Biotinylated goat anti-rabbit IgG (1:250, Vector, BA1000)
Biotinylated goat anti-mouse IgG (Vector, MOM kit)

10. Eukaryotic cell lines

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

KP1, C26 (parental), 4T1, PC9-BrM3 cells were kindly provided by Julien Sage (Stanford University), NCI, Yibin Kang (Princeton University) and Joan Massague (Memorial Sloan Kettering Cancer Center), respectively. C26m2 cells were derived from C26 parental cells by in vivo selection in this study. Human primary skeletal myoblasts were purchased from Lonza. C2C12 and 293T were purchased from ATCC. Mouse primary myoblasts were isolated from limb skeletal muscles from 1-2 week old mice. Muscle progenitor cells were isolated from gastrocnemius muscle from CD2F1 control or with C26m2 tumor burden mice, and from gastrocnemius muscle from Balb/C control or with 4T1 tumor burden mice. Detailed information in methods and Supplemental Data.

b. Describe the method of cell line authentication used.

Cell lines were authenticated by BioResearch IDEXX Laboratories (Cellcheck by STR authentication) and further confirmed by our laboratory using PCR analysis every 6 months.

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

All cell lines tested negative for mycoplasma contamination as determined by Lonza kit.

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.

Commonly misidentified cell lines were not used in our study.

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

All mouse studies were approved by IACUC and performed following ethical guidelines. Mice were housed in the animal facility at Columbia University Medical Center (CUMC) under conventional conditions with constant temperature and humidity and fed a standard diet (Labdiet 5053). Treatment of mice was in accordance with the institutional guidelines of CUMC's Institute of Comparative Medicine. Balb/c and C57Bl/6 mice were obtained from Jackson Laboratories. DBA/2 and 129P2/Ola mice were obtained from Envigo. Zip14 knockout (KO) mice generated by Hojyo and Fukada laboratory and were obtained on a congenic Balb/c background from the Knutson Laboratory (University of Florida). C57Bl/6 were crossed with 129P2/Ola to generate 129P2/Ola x C57Bl/6 mice; Balb/c were crossed with DBA/2 to generate CD2F1 mice, and Zip14 mice were crossed with DBA/2 to generate Zip14 knockout mice in CD2F1 background. K-ras(LSL-G12D/+), p53(fl/fl), Pten(fl/fl) and Lkb1(fl/fl) mice were obtained from the NCI Mouse Repository. K-ras(LSL-G12D/+) were crossed with p53(fl/fl) to generate K-ras(LSL-G12D/+)-p53(fl/fl) mice, Pten(fl/fl) were crossed with Lkb1(fl/fl) to generate Pten(fl/fl)-Lkb1(fl/fl), and K-ras(LSL-G12D/+) were crossed with Lkb1(fl/fl) to generate K-ras(LSL-G12D/+)-Lkb1(fl/fl). Genotyping for all the strains were performed using primers listed in Supplementary table 6. Mice were weighed weekly. Food and water intake was measured by weighing food and measuring water in a graduated cylinder weekly. Mice body condition as a measure of cachexia was assessed using a body condition scoring system reported before.

Adenoviral delivery of Cre-recombinase for tumorigenesis in genetic models of lung cancer following published literature outlined in Methods.

Metastasis assays in mice. Both male and female mice were used in these studies. Athymic mice aged 8-9 weeks were injected with 1x10^5 PC9-BrM3 cells by intracardiac route into arterial circulation for experimental metastasis assays. For C26m2, 4T1 and KP1 tumor studies, mice aged between 5-6 weeks for C26m2, 8-9 weeks for 4T1 and 4-5 weeks for KP1 injections were used. For each model, 1x10^6 tumor cells were subcutaneously injected in the right flank of syngeneic mice as previously described. Subcutaneous tumor was removed between 2-3 weeks to allow for metastasis formation following the tumor-resection-relapse approach. In brief, for tumor resection, mice were anesthetized with isoflurane (3-4%) administered with a precision vaporizer, and any large veins were cauterized. Buprenorphine (0.05 mg/kg) was given subcutaneously every 6-12 hours for 48 hours for pain relief after surgery. Zip14 WT or Zip14 KO mice in CD2F1 or Balb/c background at 4-5 weeks of age were subcutaneously injected with 1x10^6 C26m2 or 4T1 tumor cells, respectively. Tumors were not resected with survival-surgeries in the Zip14 WT and KO mice due to the phenotypic and behavioral abnormalities in the Zip14 KO mice. Spontaneous metastasis was monitored by bioluminescent imaging in the Zip14 WT and Zip14 KO groups at endpoint (5 weeks post-tumor cell injection with C26m2 and 4T1 injections, respectively).

12. Description of human research participants

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

Human Samples. Human tissues were obtained from autopsy and the studies were approved by IRB at New York Presbyterian/Columbia University Medical Center and Rapid Autopsy Pancreas Program at the University of Nebraska. All studies were performed following ethical guidelines. Human psoas muscles fixed in 10% buffered formalin were collected from cancer patients at New York Presbyterian/ Columbia University Medical Center upon autopsy, or obtained from the Rapid Autopsy Pancreas Program at the University of Nebraska, with approved IRB protocols. Description of cancer type and presence of cachexia relevant to this study are listed below and in Supplementary Table 3. All other deidentified clinical information are available upon request from the corresponding author.

Non-Cachectic Cancer Patient		Cachectic Cancer Patient	
13R2882M	Pancreatic	4R506	Pancreatic
33R6647M	Pancreatic	6R1030	Pancreatic
40R7635M	Pancreatic	7R1137	Pancreatic
42R7994M	Pancreatic	10R1911M	Pancreatic
49R8858M	Pancreatic	12R2486M	Pancreatic
52R9363M	Pancreatic	14R302M	Pancreatic
55R9939M	Pancreatic	17R3568M	Pancreatic
57R10316M	Pancreatic	23R4991M	Pancreatic
76RT1927M	Pancreatic	26R5572M	Pancreatic
80RT2023M	Pancreatic	28R5820M	Pancreatic
MP15-166	Hepatocellular carcinoma	32R6485M	Pancreatic
MP15-218	Lung adenocarcinoma	38R7419M	Pancreatic
MP15-222	Breast carcinoma	41R7922M	Pancreatic
MP15-108	Endometrioid uterine carcinoma	44R8210M	Pancreatic
MP15-63	Endometrial adenocarcinoma	53R9709M	Pancreatic
MP15-90	Lung squamous cell carcinoma	56R10118M	Pancreatic
MP16-114	Adenocarcinoma of colon	62R11144M	Pancreatic
MP16-148	Cholangiocarcinoma	66R11738M	Pancreatic
MP16-156	Breast cancer	71R12512M	Pancreatic
MP16-178	Renal cell carcinoma	72R12566M	Pancreatic
MP16-195	Squamous cell carcinoma lung	73R12693M	Pancreatic
MP16-203	Renal cell carcinoma and colon cancer	75R13034M	Pancreatic
MP16-223	Breast cancer	77R13304M	Pancreatic
MP15-251	Ovarian	78R13430M	Pancreatic
24R5148	Pancreatic	79R13538M	Pancreatic
25R5462	Pancreatic	83R14207M	Pancreatic
27R5690	Pancreatic	85R14385M	Pancreatic
29R6017	Pancreatic	86R14476M	Pancreatic
30R6158	Pancreatic	91R15141M	Pancreatic
31R6267	Pancreatic	93R15375	Pancreatic
34R6828	Pancreatic	96R15717M	Pancreatic
36R7244	Pancreatic	99R16059M	Pancreatic
39R7509	Pancreatic	100R16185M	Pancreatic
43R8120	Pancreatic	101R16293	Pancreatic
45R8426	Pancreatic		
48R8750	Pancreatic		
51R9236	Pancreatic		
54R9761	Pancreatic		
59R10731	Pancreatic		
60R10892	Pancreatic		
61R11036	Pancreatic		
63R13712	Pancreatic		
64R11470	Pancreatic		
70R12243	Pancreatic		
74R12854	Pancreatic		
81R13880	Pancreatic		
88R14781	Pancreatic		
89R14907	Pancreatic		
90R15069	Pancreatic		
94R15429	Pancreatic		
95R15609	Pancreatic		
97R15789	Pancreatic		
98R15987	Pancreatic		

14-212 A30	Desmoplastic small round cell tumors
MP15-174	Colon adenocarcinoma
MP15-220	Small cell lung cancer
MP15-230	Cholangiocarcinoma
MP15-253	Lymphoma and Lung carcinoma
MP16-38	Pancreatic ductal Adenocarcinoma
MP16-105	Colon cancer
MP16-115	Multiple myeloma and breast cancer
MP16-136	Adenocarcinoma of stomach