**Supplementary materials and methods**

**Isolation of pancreatic cancer cells.** Pancreatic tumors were resected from tumor-bearing KPC mice and mechanically and chemically dissociated into single cell suspensions as previously described(37). Red blood cell lysis buffer (Sigma) was used to remove red blood cells. Serial passes through a 40µM filter were used to remove and disrupt cell clusters. Cells were centrifuged and resuspended in PBS and subsequently underwent fluorescence activated cell sorting (FACS) with a FACSAria flow cytometer (BD). Cell doublets/clusters were gated and excluded through forward scatter/side scatter profiles and dead cells gated and excluded based on DAPI exclusion. Immune cells were gated and excluded using a hematopoietic lineage cocktail (Miltenyi Biotec) containing antibodies directly labeled with APC. tdTomato(+)/DAPI(-)/APC(-) cells were sorted and single tdTomato(+) cells were visually confirmed using a fluorescent microscope (EVOS). tdTomato(+) cell suspensions were then centrifuged and resuspended for RNA purification using a RNeasy micro kit (Qiagen).

**Generation of patient-derived xenograft (PDX) tumors and tissue microarray (TMA).** Patient tumors were heterotopically engrafted into immunodeficient mice as previously published(38). PDX tumors were established from patients with written, informed consent in accordance with IRB-approved laboratory research protocol (LAB07-0854). Once grown to sizes no larger than 1.5cm, host mice were euthanized and PDX tumors were harvested, fixed in 10% formalin and embedded in paraffin. Tissue cores were taken from paraffin blocks and arrayed to generate a PDAC PDX tissue microarray.

**Wound healing cell migration assay.** 5×103 KPC cells were resuspended in 70μl of DMEM and incubated overnight with a two well silicone insert (ibidi) in a 6-well plate. The insert was removed after 12 hours using a stopper tool to expose a 500μm ±100μm migration zone. Each well was washed with PBS and 2 ml of fresh media was added and images at 0 hour were captured using brightfield microscopy (Olympus IX71). Cells were incubated for 24 hours, washed with PBS and images again captured for analysis. Migration was calculated as the average area of the cell-free gap zone as measured by ImageJ software (NIH).

**Clonogenic assay of cells in vitro**

Soft Agar Colony Formation Assay for anchorage independent cell growth: PDAC cells ( 5000 per well) were mixed with 0.3% agarose in growth medium, plated on top of a solidified layer of 0.6% agarose in growth medium, in a 6-well plate, and refreshed every 3 days with growth medium. After 3 weeks, the colonies were washed with PBS, imaged by a digital camera (Olympus IX71) and counted using ImageJ software (NIH). Colony Formation Assay for adherent cell growth: PDAC cells (500 per well) were seeded in a 6-well plate to form colonies in 3 weeks. Colonies were stained with crystal violet (0.5% w/v) and counted using ImageJ software (NIH).

**Multiplex fluorescent Immunohistochemistry (mIHC)**

Formalin-fixed, paraffin-embedded tissue sections were deparaffinized in xylene, rehydrated, and subjected to antigen retrieval in 10X AR6 buffer (pH 6.0, Opal™ 7 Immunology Discovery Kit). Prior to blocking, slides were incubated in 3% hydrogen peroxide for 15 minutes at room temperature. Sections were then blocked using 1X Antibody Diluent solution (Opal™ 7 Immunology Discovery Kit) for 1 hour at room temperature. After blocking, the primary antibody was added to tissue sections and incubated overnight at 4° C. Slides were then washed with TBS-T (.05% Tween-20) and incubated in Opal Polymer HRP Ms + Rb (Opal™ 7 Immunology Discovery Kit) for 10 minutes at room temperature and washed with TBS-T (.05% Tween-20) for 2 minutes, 3 times. Afterwards, tissue sections were incubated with OPAL 540 fluorophore (Opal™ 7 Immunology Discovery Kit) diluted in the 1x Amplification Diluent solution (1:50) and washed with TBS-T (.05% Tween-20) 3 times for 2 minutes. Slides were then placed in 10X AR6 buffer (pH 6.0, Opal™ 7 Immunology Discovery Kit) and subjected to antigen retrieval via microwave treatment (MWT). Following completion of initial antibody staining, slides were blocked again using 1X Antibody Diluent solution (Opal™ 7 Immunology Discovery Kit) for 10 minutes at room temperature and the process of antibody incubation, secondary antibody washing via OPAL Polymer HRP, application of OPAL fluorophore, and MWT were repeated for additional antibodies. Once complete, each slide was incubated in spectral DAPI (Opal™ 7 Immunology Discovery Kit, 2 drops in 1ml of TBS-T) for 5 minutes at room temperature. Slides were then washed twice with TBS-T (.05% Tween-20) for 2 minutes, mounting media (Vectashield Hardset without DAPI) was applied, and coverslips mounted.

**Chromatin immunoprecipitation (ChIP) assay**

Pancreatic cancer cells were crosslinked in 1% formaldehyde for 10 minutes, followed by glycine to stop the crosslinking. Cells were collected, washed with PBS and lysed with lysis buffer (5mM Hepes pH 8.0, 85mM KCl, 0.5% NP40, and protease inhibitors). The nuclear pellet was lysed in nuclear lysis buffer (50mM Tris-HCl pH 7.5, 10mM EDTA, 1% SDS and protease inhibitors). Lysates with glass beads (Sigma) were fragmented with a Bioruptor (Diagenode) to obtain DNA fragments with an average length under 300bp. Lysates were precleared with Protein A beads (GE Healthcare) and IgG (Fisher) for 2 hours and incubated with 4ug of both p53 (CM5, Leica) or IgG (Fisher) overnight at 4C. Immunocomplexes were collected on magnetic Protein A beads (Dynabeads), washed, treated with RNaseA and Proteinase K, reverse-crosslinked overnight followed by phenol:chloroform extraction. The DNA region of interest was detected by SYBR real-time quantitative PCR using the following primers: FOXA1 promoter forward primer 5’-CACTTTGCTCGGCTGACT and reverse primer 5’-TGGTCCACTTGGCTCCTT and FOXA1 exon forward primer 5’-TGTAGGTGCGAGCGTCTT and reverse primer 5’-GCCTGTGCGAAGCGAGTG. Percent bound IgG was subtracted from percent bound p53. Graphs represent the fold-enrichment of adjusted p53 percent bound at the promoter over the exon.

**Gene Set Enrichment Analysis**

Gene Set Enrichment Analysis (GSEA) was performed using GSEA software (version 4.0.3) which can be downloaded via GSEA website (https://www.gsea-msigdb.org/gsea/index.jsp). Raw read counts were normalized via DESeq2 package and gene set enrichment analysis was performed following this pipeline (https://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Using\_RNA-seq\_Datasets\_with\_GSEA) and using the 50 hallmark gene sets provided by the Molecular Signatures Database (<https://www.gsea-msigdb.org/gsea/msigdb/collections.jsp>).

**Reagents and inhibitors.**

|  |  |  |
| --- | --- | --- |
| critical commercial assays |  Source |  Identifier |
| MISSION® siRNA Transfection Reagent | sigma | S1452 |
| Lipofectamine 2000 | Invitrogen | 11668019 |
| Recombinant Human EGF Protein | R&D systems | 236-EG |
| Recombinant Human PDGF-BB Protein | Novus | NBP2-35203 |
| Dual-Luciferase® Reporter Assay System | promega | E1910 |
| Pierce gaussia luciferase glow assay kit  | ThermoFisher | 16160 |
| Duo-link In Situ-Fluorescence kits  | Sigma-Aldrich | DUO92101 |
| Red blood cell lysis buffer | ThermoFisher | 00-4333-57 |
| RNeasy micro kit | Qiagen | 74004 |
| DNA-gel extraction kit | Qiagen | 51304 |

|  |  |  |
| --- | --- | --- |
| inhibitors |  Source |  Identifier |
| 666-15  | Tocris | 5661 |
| trametinib | Selleckchem | S2673 |
| H89 | Selleckchem | S1582 |
| Pictilisib | Selleckchem | S1065 |
| AZD6244 | Selleckchem | S1008 |
| U0126 | sigma | U120 |

|  |  |  |
| --- | --- | --- |
| siRNA | Source | ID  |
| mouse Trp53 | Sigma | NM\_001127233 |
| human Tp53  | Sigma | NM\_000546 |
| mouse FOXA1 | Sigma | NM\_008259 |
| human FOXA1  | Sigma | NM\_004496 |
| mouse KRAS | Sigma | NM\_021284 |
| human KRAS | Sigma | NM\_004985 |
| mouse CREB1 | Sigma | NM\_001037726 |

**siRNA**

**shRNA**

|  |  |  |  |
| --- | --- | --- | --- |
| Name |  Sequence |  Source |  Clone ID |
| pGIPZ-GFP-shRNA-Trp53  | 5’-CACTACAAGTACATGTGTA-3’ | Dharmacon | V3LHS 646511 |
| pGIPZ-GFP-shRNA-hFOXA1 | 5'-CTCCGTATATTTACATAAC-3' | Dharmacon | V2LHS 16780  |
| pGIPZ-GFP-shRNA-hFOXA1 | 5'-GCAATACTCTTAACCATAA-3' |  Dharmacon | V2LHS 16813 |

**Plasmids**

|  |  |  |
| --- | --- | --- |
| Name |  Source |  Identifier |
| pBABE-mouse Trp53R172H-puro | Our group (dio: 10.1073/pnas. 1404139111) |
| pLenti-C-mGFP-P2A-Puro-FOXA1 | Origene | MR225487 |
| pCMV-CREB1 | Clontech | 631925 |
| pCMV-CREB1-133  | Clontech | 631925 |
| pLC-Flag-Puro-CSNK1A1 | Addgene | #123319 |
| pLC-Flag-Puro-CSNK1A1G40N  | Addgene | #12332 |
| pcDH-EF1-MCS-RFP  | CSB1 system bioscience | CD530A-2 |

**Reporter plasmids**

|  |  |  |
| --- | --- | --- |
| Name |  Source |  Identifier |
| pEZX-LvPG02-FOXA1 reporter | GeneCopoeia | LPP-MPRM39684-LvPG02-A00 |
| M50 Super 8X TOPFlash | Addgene | #12456 |
| M51 Super 8X FOPFlash (TOPFlash mutant) | Addgene  | #12457 |

**Antibodies**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antibodies | Target species | Host species |  Dilution | Source | Identifier |
| p53 (clone DO-1) | Human, Mouse | mouse | 1:1000 | Santa Cruz | sc-126 |
| β-catenin (E-5)  | Human, Mouse | mouse | 1:1000 | Santa Cruz | sc-7963 |
| Phospho-β-Catenin (Ser33/37/Thr41)  | Human, mouse | rabbit | 1:1000 | cell signaling |  #9561 |
| CREB-1 (E306) | Human, Mouse | rabbit | 1:1000 | abcam | ab32515 |
| Phospho-CREB (Ser133) | Human, Mouse | rabbit | 1:1000 | cell signaling | #9198 |
| FOXA1  | Human, Mouse | mouse | 1:1000 | abcam | ab55178 |
| Cyclin D1  | Human, Mouse | rabbit | 1:1000 | cell signaling | #2922 |
| CK1α | Human, mouse | mouse | 1:1000 | Santa Cruz | sc-74582 |
| p44/42 MAPK (Erk1/2)  | Human, Mouse | rabbit | 1:1000 | cell signaling | #4695 |
| Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) | Human, Mouse | rabbit | 1:1000 | cell signaling | #9101 |
| AKT | Human, Mouse | rabbit | 1:1000 | cell signaling | #9272 |
| phosphor-AKT (Ser 473) | Human, Mouse | rabbit | 1:1000 | cell signaling | #9271 |
| K-Ras  | Human, Mouse | mouse | 1:500 | Santa Cruz | sc-30 |
| β-Actin  | Human, Mouse | mouse | 1:2000 | Santa Cruz | sc-69879 |
| Vinculin | Human, Mouse | rabbit | 1:1000 | cell signaling | #13901 |
| PARP | Human, Mouse | rabbit | 1:1000 | cell signaling | #9542 |
| Anti-rabbit IgG secondary antibody |  | goat | 1:5000 | ThermoFisher | #31460     |
| Anti-mouse IgG secondary  antibody      |  | goat | 1:5000 | ThermoFisher | #31430      |

**Immunoprecipitation (IP) antibodies**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antibodies | Target species | Host species |  Dilution | Source | Identifier |
| p53 (clone DO-1) | Human, Mouse | mouse | 1:1000 | Santa Cruz | sc-126 |
| CREB-1 | Human, Mouse | mouse | 1:1000 | Santa Cruz | sc-374227 |

**Immunohistochemistry (IHC) antibodies**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antibodies | Target species | Host species | Dilution | Source | Identifier |
| p53 (clone CM-5) | Mouse | rabbit | 1:250 | Leica biosystems | P53-CM5P-L |
| p53 (clone DO-1) | Human | mouse | 1:150 | Santa Cruz | sc-126 |
| β-catenin (E-5) | Mouse | mouse | 1:150 | Santa Cruz | sc-7963 |
| FOXA1 | Mouse | mouse | 1:150 | abcam | ab55178 |
| CK1α | Mouse | mouse | 1:100 | Santa Cruz | sc-74582 |

**Immunofluorescence (IF) antibodies**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Antibodies | Target species | Host species |  Dilution | Secondary antibody | Source | Identifier |
| p53 (clone CM-5) | Mouse | Rabbit | 1:250 | Alex Fluor 555 | Leica biosystems | P53-CM5P-L |
| FOXA1 | Mouse | Mouse | 1:150 | Alex Fluor 488 | abcam | ab55178 |
| phosphor-CREB1 (ser133) conjugated with Alexa Fluor 647 | Mouse | Rabbit | 1:50 | - | Sigma-Aldrich | 06-519-AF647 |
| Anti-rabbit IgG with Alex Fluor 555 | Rabbit | Donkey | 1:250 |  | Thermo Fisher  | #A32794 |
| Anti-mouse IgG with Alex Fluor 488 | Mouse | Goat | 1:250 |  | Thermo Fisher | #A32723 |

**Proximity Ligation Assay (PLA) antibodies**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antibodies | Target species | Host species |  Dilution | Source | Identifier |
| p53 (clone CM-5) | Human, mouse | rabbit | 1:250 | Leica biosystems | P53-CM5P-L |
| CREB-1 | Human, mouse | mouse | 1:200 | Santa Cruz | sc-374227 |

**Multiplex fluorescent Immunohistochemistry (mIHC) antibodies**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antibodies | Target species | Host species |  Dilution | Source | Identifier |
| p53 (clone CM-5) | Mouse | Rabbit | 1:250 | Leica biosystems | P53-CM5P-L |
| α-smooth muscle actin (1A4) | Mouse | Mouse | 1:200 | Abcam | Ab7817 |
| RFP | Mouse | Rabbit | 1:300 | Rockland | 600-401-379 |

|  |  |
| --- | --- |
| Name  | Sequences |
| Human |   |
| FOXA1 | Forward : 5’–CAAGGATGCCTCTCCACACTT–3’ |
|   | Reverse: 5’–TGACCATGATGGCTCTCTGAA–3’ |
| GAPDH | Forward: 5’–GAGTCAACGGATTTGGTCGT–3’ |
|   | Reverse: 5’–TTGATTTTGGAGGGATCTCG–3’ |
| CK1α | Forward: 5’– CTTCTTGTCTGTAAGCCAGC–3’ |
|   | Reverse: 5’– TCTTATGTCTTCACAGGTAAGC–3’  |
| CREB1 | Forward: 5′–CATTAACCATGACCAATGCAG–3′ |
|   | Reverse: 5′–CTGTGCGAATCTGGTATGTTT–3′ |
| FZD1  | Forward: 5′-CCAAGAGAGGAGCCGAGA-3′ |
|  | Reverse: 5′-CGGCACAAAGTTCCCAG-3′ |
| FZD2 | Forward: 5’-CGACTGCGCTTCCACCTTCTTCA-3’ |
|  | Reverse: 5’-CGTAATGATAGGCCGCTCTGGGTA-3’ |
| Axin2 | Forward: 5’-CAAGGGCCAGGTCACCAA-3’ |
|  | Reverse: 5’-CCCCCAACCCATCTTCGT-3’ |
| HES-1 | Forward: 5′-AGTGAAGCACCTCCGGAAC-3′ |
|  | Reverse: 5′-TCACCTCGTTCATGCACTC-3′ |
| MMP-7 | Forward: 5’-TCGGCGGAGATGCTCACT-3’ |
|  | Reverse: 5’-TGGCAACAA ACAGGAAGTTCA C-3’ |
| Wnt3a | Forward: 5’-TGTTGGGCCACAGTATTCCT-3’ |
|  | Reverse: 5’-GGGCATGATCTCCACGTAGT-3’ |
| Wnt2 | Forward: 5’-AAAGAAGATGGGAAGCGCCA-3’ |
|  | Reverse: 5’-TTCATCAGGGCTCTGGCATC-3’ |
| LEF-1 | Forward: 5’-ATGTCAACTCCAAACAAGGCA-3’ |
|  | Reverse: 5’-CCCGGAGACAAGGGATAAAAAGT-3’ |
| DKK1 | Forward: 5’-CGACGGGCGGGAATAAGTACCA-3’ |
|  | Reverse: 5’-CGTGGACTAGCGCAGTACTCATCA-3’ |
| cFOS | Forward: 5’-GGCCCACGAGACCTCTGAGACA-3’ |
|  | Reverse: 5’-GCCTTGGCGCGTGTCCTAATCT-3’ |
| IRF-4 | Forward:5’-ACAGCAGTTCTTGTCAGAG-3’ |
|  | Reverse:5’-GAGGTTCTACGTGAGCTG-3’ |
| ZBTB16 | Forward:5’-CACTTACTGGCTCATTCAGCGG-3’ |
|  | Reverse:5’-CTTACACTCAAAGGGCTTCTCACC-3’ |
| RUNX1 | Forward:5’-GCACCGACAGCCCCAACTT-3’ |
|  | Reverse:5’-GTCTTGTTGCAGCGCCAGTG-3’ |
| GAS7 | Forward: 5’-AGACACGATGCCGGAACAG-3’ |
|  | Reverse: 5’-TTATCCTTTCCCGGATGAATTCT-3’ |

**RT-qPCR primers**

|  |  |
| --- | --- |
| Name |  Sequence |
| Mouse |  |
| Foxa1 | Forward : 5’–CAAGGATGCCTCTCCACACTT–3’ |
|   | Reverse: 5’–TGACCATGATGGCTCTCTGAA–3’ |
| Rplpo | Forward: 5’–CCCTGAAGTGCTGACATCA–3’ |
|   | Reverse: 5’–TGCGGACACCCTCCAGAA–3’ |
| Ck1α | Forward: 5’–ATGGGTATTGGGCGTCACTG–3’ |
|   | Reverse: 5’–TCGGCGACTCTGCTCAATAC–3’ |
| Creb1 | Forward: 5’–CTGATTCCCAAAAACGAAGG–3’ |
|   | Reverse: 5’–CTGCCCACTGCTAGTTTGGT–3’ |
| Fzd1  | Forward: 5’-AAACAGCACAGGTTCTGCAAAA-3’ |
|  | Reverse: 5’-TGGGCCCTCTCGTTCCTT-3’ |
| Fzd2 | Forward: 5’-TCCATCTGGTGGGTGATTCTG-3’ |
|  | Reverse: 5’-CTCGTGGCCCCACTTCATT-3’ |
| Axin2 | Forward: 5’-GCCAATGGCCAAGTGTCTCT-3’ |
|  | Reverse: 5’-GCGTCATCTCCTTGGGCA-3’ |
| Hes-1 | Forward: 5’-CCAGCCAGTGTCAACACGA-3’ |
|  | Reverse: 5’-AATGCCGGGAGCTATCTTTCT-3’ |
| Ihh | Forward: 5’-CACGTGCATTGCTCTGTCAA-3’ |
|  | Reverse: 5’-AGGAAAGCAGCCACCTGTCTT-3’ |
| Mmp-7 | Forward: 5’-CACTCACTGGGTCCTCCATT-3’ |
|  | Reverse: 5’-GAAGAGGGAAACAGGTGCAG-3’ |
| Wnt3a | Forward: 5’-CCCAACTTCTGCGAACCTAA-3’ |
|  | Reverse: 5’-TCTCCGCCCTCAAGTAAGAA-3’ |
| Wnt2 | Forward: 5’-ATCTCTTCAGCTGGCGTTGT-3’ |
|  | Reverse: 5’-AGCCAGCATGTCCTCAGAGT-3’ |
| Lef-1 | Forward: 5’-AGTGCAGCTATCAACCAGATCCT-3’ |
|  | Reverse: 5’-TTTCCGTGCTAGTTCATAGTATTTGG-3’ |
| Dkk1 | Forward: 5’-CCGGGAACTACTGCAAAAAT-3’ |
|  | Reverse: 5’-CCAAGGTTTTCAATGATGCTT-3’ |
| c-Fos | Forward:5’-AGAATCCGAAGGGAAAGGAA-3’ |
|  | Reverse:5’-ATTGAGAAGAGGCAGGGTGA-3’ |
| Irf-4 | Forward:5’-CAGCTCATGTGGAACCTC-3’ |
|  | Reverse: 5’-GGAAGAATGACGGAGGGA-3’ |
| Zbrb16 | Forward: 5’-CCCAGTTCTCAAAGGAGGATG-3’ |
|  | Reverse: 5’-TTCCCACACAGCAGACAGAAG-3’ |
| Runx1 | Forward: 5′-CTCGGCAGAACTGAGAAATG-3′ |
|  | Reverse: 5′-GGTGATGGTCAGAGTGA-3′ |
| Gas7 | Forward: 5’-GAGCCAGACGGTCATCCTTC-3’ |
|  | Reverse: 5’-CGTGGTATCCATTCACTGTTGT-3’ |