Supplementary Table 1 - Reagents		
Reagents	**Supplier**	**Ref**
Fibronectin, Human	R&D Systems	1918-FN
Fibonectin, Bovine	Sigma-Aldrich	F1141
Carbonyl cyanide 3-chlorophenylhydrazone (CCCP)	Sigma-Aldrich	C2759
Concanavalin A (ConA)	Sigma-Aldrich	L7647
Aphidicolin	Sigma-Aldrich	A4487
Matrigel Basement Membrane Matrix	Corning	354234
Collagen I, Rat tail	Corning	354249
Phosphocreatine disodium salt hydrate	Sigma-Aldrich	P7936
2-Imino-1-imidazolidineacetic acid (Cyclocreatine)	Sigma-Aldrich	377627
1-Carboxymethyl-2-iminoimidazolidine (Cyclocreatine)	AstaTech	57312
U-13C6-D-Glucose, 99%	Cambridge Isotope Laboratories	CLM-1396-PK
Sigmacote	Sigma-Aldrich	SL2
sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino)hexanoate (sulfo-SANPAH)	Thermo Fisher Scientific	22589
3-(Acryloyloxy)propyltrimethoxysilane	Alfa Aesar	L16400
Acrylamide 40% Solution	Sigma-Aldrich	A4058
N,N'-methylene-bis-acrylamide 2% solution	Sigma-Aldrich	M1533
N,N,N',N'-Tetramethylethylenediamine (TEMED)	Sigma-Aldrich	T9281
Ammonium Persulfate	Sigma-Aldrich	A3678
N-Acetyl-L-cysteine	Sigma-Aldrich	A7250
Ammonium hydroxide	Sigma-Aldrich	221228
Hydrogen peroxide (30%w/w)	Sigma-Aldrich	31642
tert-Butyl hydroperoxide solution	Sigma-Aldrich	416665
L-Arginine:HCL (13C6, 99%; 15N4, 99%)	Cambridge Isotope Laboratories	CNML-539-H-0.1
Saponin	Sigma-Aldrich	47036
Gelatin from cold water fish skin	Sigma-Aldrich	G7041
Oligomycin A	Sigma-Aldrich	75351-5MG
Jasplakinolide	TOCRIS Bioscience	2792
Lipofectamine RNAiMAX	Thermo Fisher Scientific	13778150
Lipofectamine 2000	Thermo Fisher Scientific	11668019
Lullaby	OZ Biosciences	FLL73000
Amaxa Cell Line Nucleofector Kit V	Lonza	VCA-1003
Dialysed FBS	Gibco	16400-044
SILAC DMEM Flex Media, no glucose, no phenol red	Thermo Fisher Scientific	A2493901
Fluospheres Carboxylate-modified microspheres, 0.2 µm, red fluorescent (580/605), 2% solids	Thermo Fisher Scientific	F8810
Blebbistatin	Sigma-Aldrich	B0560
Precision Red Advanced Protein Assay	Cytoskeleton	ADV02-A
Puromycin dihydrochloride	Thermo Fisher Scientific	A1113803

Supplementary Table 2 - DNA constructs		
Name	**Backbone**	**Source**
pSpCas9(BB)-2A-Puro (PX459) V2.0		Addgene #62988
pEGFPC1 empty vector	pEGFPC1	Prof. L. M. Machesky
pEGFPC1/GgVcl1-258 (VD1)	pEGFPC1	Addgene #46270
pEGFPC3-hYAP1	pEGFPC3	Addgene #17843
pCMV-flag YAP2 5SA	pCMV	Addgene #27371
pWZL Neo Myr Flag CKB	pWZL-Neo-Myr-Flag-DEST	Addgene #20466
pEGFP-C1 CKB	pEGFPC1	This study
pEGFP-C1 YAP5SA	pEGFPC1	This study
LifeAct-mTagRFP	LifeAct -mTagRFP-T	Addgene #54586
PA-GFP Actin	mPA-GFP-C1	Addgene - #57121

Supplementary Table 3 - Oligos					
Target Name	**Species**	**Target sequence (5'->3')**	**Supplier**	**Cat. No.**	**Application**
AllStars Negative Control			Qiagen	SI03650318	siRNA
CKB siRNA #01	*Mus musculus*	CTGCCGCTTCCTAACTTATTA	Qiagen	SI00951867	siRNA
CKB siRNA #02	*Mus musculus*	AAGCTGTTGCTGACACTGAAA	Qiagen	SI00951888	siRNA
YAP siRNA #01	*Mus musculus*	CTGGTCAAAGATACTTCTTAA	Qiagen	SI00185584	siRNA
YAP siRNA #02	*Mus musculus*	ATGGAGAAGTTTACTACATAA	Qiagen	SI02669191	siRNA
CKB CRISPR #01	*Mus musculus*	AAAGCCGCTCGGCGTGCACT	Thermo Fisher Scientific		CRISPR
CKB CRISPR #02	*Mus musculus*	ACGAGCATCCGGCCAGTCCC	Thermo Fisher Scientific		CRISPR

Supplementary Table 4 - Hydrogels Composition		
Stiffness	**Acrylamide (%)**	**BisAcrylamide (%)**
0.7 kPa	3	0.06
7 kPa	5	0.3
38 kPa	10	0.3

Supplementary Table 5 - Antibodies/Dyes

Antibody/Dye	Species	Clone	Supplier	Ref	LOT	Dilution	Application
YAP	Rabbit	Monoclonal, D8H1X	CST	14074	4	1:1000 / 1:100	Western Blot / Immunofluorescence
α-Tubulin	Mouse	Monoclonal, DM1A	Sigma	T6199	029M4842V	1:3000	Western Blot
CKB	Rabbit	Polyclonal	Abcam	ab151579	GR143284-16	1:1000	Western Blot
GFP	Mouse	Monoclonal, 4B10	CST	2955	2	1:1000	Western Blot
ERK1/2	Mouse	Polyclonal	CST	9102	26	1:1000	Western Blot
AMPK-α	Rabbit	Polyclonal	CST	2532S	19	1:1000	Western Blot
phospho-AMPK α1/α2 (T183/T172)	Rabbit	Polyclonal	Abcam	ab23875	GR3244770-4	1:1000 / 1:100	Western Blot / Immunofluorescence
GAPDH	Mouse	Monoclonal, 6C5	Millipore	MAB374	3249425	1:1000	Western Blot
Anti-Rabbit 800nm	Goat	Polyclonal	Thermo Fisher Scientific	SA5-35571	TL277458	1:10000	Western Blot
Anti-Mouse 800nm	Goat	Polyclonal	Thermo Fisher Scientific	SA5-3552	TC263804	1:10000	Western Blot
Anti-Rabbit 680nm	Donkey	Polyclonal	Invitrogen	A21076	1874787	1:10000	Western Blot
Anti-Mouse 680nm	Donkey	Polyclonal	Invitrogen	A10038	2017408	1:10000	Western Blot
phosphoPaxillin (Tyr118)	Rabbit	Polyclonal	CST	2541	6	1:400	Immunofluorescence
Vinculin	Mouse	Monoclonal, hVIN-1	Sigma	V9131	036M4797V	1:400	Immunofluorescence
Cortactin	Mouse	Monoclonal, 4F11	Millipore	05-180	3153615	1:200	Immunofluorescence
GFP	Chicken	Polyclonal	Abcam	ab13970	GR236651-22	1:1000	Immunofluorescence
Anti-Rabbit 488nm	Donkey	Polyclonal	Invitogen	A21206	2045215	1:500	Immunofluorescence
Anti-Mouse 594nm	Donkey	Polyclonal	Invitrogen	A21203	1918277	1:500	Immunofluorescence
Anti-Chicken 488nm	Goat	Polyclonal	Invitrogen	A11039	1812246	1:500	Immunofluorescence
Phalloidin 647nm	n/a	n/a	Invitrogen	A22287	1941485	1:100	Immunofluorescence
DAPI	n/a	n/a	Invitrogen	D1306	1942280	0.5 µg mL-1	Immunofluorescence
CKB	Rabbit	Monoclonal, EPR3926	Abcam	ab108388	GR42673-13	1:200	IHC
YAP	Rabbit	Polyclonal	CST	4912	7	1:50	IHC
Fibronectin	Rabbit	Polyclonal	Dako	A0245	00062151	1:600	IHC
MitoTracker Green	n/a	n/a	Thermo Fisher Scientific	M7514	1861616	200nM	Live Imaging / FACS
TMRE	n/a	n/a	Abcam	ab113852		100nM	FACS
NucBlue Live reagent	n/a	n/a	Thermo Fisher Scientific	R37605	1825105		Live Imaging
Cell Tracker Red (CMTPX)	n/a	n/a	Thermo Fisher Scientific	C34552	1861191	1µM	Live Imaging
CellROX Deep Red	n/a	n/a	Thermo Fisher Scientific	C10422	1990333	1µM	FACS

Supplementary Table 6 - qRT-PCR primers			
Target Name	**Species**	**Sequence (5'->3')**	**Supplier**
Ckb	*Mus musculus*	Fw - CGGCCTCACTCAGATCGAAA	Thermo Fisher Scientific
Ckb	*Mus musculus*	Rv - TGAGGATGTAGCCCAGGTGA	Thermo Fisher Scientific
Ckm	*Mus musculus*	Fw - AACCTCAAGGGTGGAGACGA	Thermo Fisher Scientific
Ckm	*Mus musculus*	Rv - TGCGGAGGCAGAGTGTAAC	Thermo Fisher Scientific
Ckmt1	*Mus musculus*	Fw - CGAGGGATCTGGCACAACAA	Thermo Fisher Scientific
Ckmt1	*Mus musculus*	Rv - TCTCTTCATGTTGCCGCCTT	Thermo Fisher Scientific
CKmt2	*Mus musculus*	Fw - GTCGTGGACTAAAGGAAGTGGA	Thermo Fisher Scientific
CKmt2	*Mus musculus*	Rv - CGCAATCCAGTTCCGAGGTT	Thermo Fisher Scientific
Cdk2	*Mus musculus*	Fw - TGAAATGCACCTAGTGTGTACC	Thermo Fisher Scientific
Cdk2	*Mus musculus*	Rv - TCCTTGTGATGCAGCCACTT	Thermo Fisher Scientific
Gapdh	*Mus musculus*	Fw - CATGGCCTACATGGCCTCCA	Thermo Fisher Scientific
Gapdh	*Mus musculus*	Rv - TGGGATAGGGCCTCTCTTGC	Thermo Fisher Scientific
Yap	*Mus musculus*	Fw - TTTCGGCAGGCAATACGGAA	Thermo Fisher Scientific
Yap	*Mus musculus*	Rv - GCATTCGGAGTCCCTCCATC	Thermo Fisher Scientific

Supplementary Table 7 - Mice

Mouse ID	Strain	Genotype	Sex	Genetic Background	Age (days)	Application	Source
KPC endpoint PDAC							
FPZPR 103996	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Male	C57BL/6J	118	IHC	CRUK Beatson Institute
BALF 162992	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Male	C57BL/6J	123	IHC	CRUK Beatson Institute
BALF 192959	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Male	C57BL/6J	233	IHC	CRUK Beatson Institute
BALF 192970	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	114	IHC	CRUK Beatson Institute
BALA 27356	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Male	C57BL/6J	140	IHC	CRUK Beatson Institute
BALA 32316	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	152	IHC	CRUK Beatson Institute
BALA 32329	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	94	IHC	CRUK Beatson Institute
BALF 6.1b	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Male	C57BL/6J	170	IHC	CRUK Beatson Institute
BALF 6.1e	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	166	IHC	CRUK Beatson Institute
BALA 22160	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	99	IHC	CRUK Beatson Institute
BALA 24900	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	94	IHC	CRUK Beatson Institute
BALA 32310	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Male	C57BL/6J	139	IHC	CRUK Beatson Institute
BALA 47000	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Male	C57BL/6J	101	IHC	CRUK Beatson Institute
Normal Pancreas							
BALF 170396	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras +/+;LSL-p53 +/+	Male	C57BL/6J	204	IHC	CRUK Beatson Institute
BALF 171667	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras +/+;LSL-p53 +/+	Female	C57BL/6J	202	IHC	CRUK Beatson Institute
BALF 80410	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras +/+;LSL-p53 +/+	Male	C57BL/6J	46	IHC	CRUK Beatson Institute
BALF 80418	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras +/+;LSL-p53 +/+	Female	C57BL/6J	46	IHC	CRUK Beatson Institute
BALF 80415	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras +/+;LSL-p53 +/+	Male	C57BL/6J	46	IHC	CRUK Beatson Institute
KPC 10-week PanIN							
BAJD 67.3c	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Male	C57BL/6J	68	IHC	CRUK Beatson Institute
BAJD 91.1a	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	69	IHC	CRUK Beatson Institute
BAJD 91.1d	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	69	IHC	CRUK Beatson Institute
BAJD 91.1f	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	69	IHC	CRUK Beatson Institute
BAJD 91.2b	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	69	IHC	CRUK Beatson Institute
KPC 15-week PanIN							
BALF 11.2g	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	104	IHC	CRUK Beatson Institute
BALF 18.2b	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	103	IHC	CRUK Beatson Institute
BALF 18.2d	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	103	IHC	CRUK Beatson Institute
BALF 18.2e	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Female	C57BL/6J	103	IHC	CRUK Beatson Institute
BALF 11.2a	Pdx-1::Cre;LSL-Kras G12D;LSL-p53 R172H (KPC)	Pdx-1::Cre+;LSL-Kras G12D/+;LSL-p53 R172H/+ (KPC)	Male	C57BL/6J	104	IHC	CRUK Beatson Institute
Intrasplenic Transplantation Model							
BVCD5.1a-x	Foxn1-nu:Hom (CD1-nu)	Foxn1-nu:Hom (CD1-nu)	Female	CD1	62	Intrasplenic	Charles River Laboratories