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Supplemental figure legends

Figure S1:

- **(A)** Typical morphological appearance of NIH3T3 cells cultured on soft or stiff hydrogels (phase-contrast images). Scale bar is 50 µm.
- **(B)** Protein levels of AMPKα and mTOR in different mesenchymal cell types of mouse and human origin. MEF = Mouse embryonic fibroblast; hPSC = Human pancreatic stellate cell.
- **(C)** Protein levels of AMPKα and mTOR in primary mouse cancer-associated fibroblasts (CAFs) derived from the KPC (*Pdx1-Cre; LSL-Kras$^{G12D/+}$; LSL-Trp53$^{R172H/+}$*) PDAC mouse model and cultured on soft or stiff hydrogels.
- **(D)** The partial Smoothened agonist Cyclopamine (10 µM, 48h) induces AMPKα activation in NIH3T3 cells cultured on soft/stiff hydrogels.
- **(E)** Stability of mTOR protein in NIH3T3 cells. Quantification of data depicted in figure 1 J. Shown is the mean ±SD of n=3 experiments.

Figure S2:

- **(A)** Knock-down efficiency (qPCR) upon transfection of the indicated siRNAs in MEFs cultured on plastic. Control siRNA (siCon) was set to 1.
- **(B)** *Integrin* mRNA expression in MEF cells as determined by qPCR (n=1).
- **(C)** Effect of the indicated siRNA transfections on *Prkaa1* (encoding AMPKα1) and *Prkaa2* (encoding AMPKα2) mRNA levels in MEF cells cultured on plastic. The samples with knock-down of *Itgav* are highlighted. Shown is the mean ±SD of n=2 independent experiments.
- **(D)** Immunofluorescence analysis of ITGAV (red) expression in myCAF (upper panel, αSMA, green) and iCAF (lower panel, PDGFRα, green) populations in human PDAC tissue. Nuclei are stained blue. Scale bar is 25 µm. Pictures are maximum intensity projections of 3D-deconvoluted images.
- **(E)** Examples of low/absent or high ITGAV expression (brown) in spindle-shaped stromal CAFs. Scale bar is 50 µm.
- **(F)** Quantification of ITGAV expression (ranked as absent (0), intermediate (1), strong (2)) in a set of 75 human PDAC cases.

Figure S3:

Kaplan-Meier curves (TCGA dataset) of PDAC patients depicting overall survival probability in relation to indicated gene expression (best cut-off by scan, minimum group size of n=40).

- **(A)** *ITGA1*
- **(B)** *ITGA3*
- **(C)** *ITGA5*
- **(D)** *ITGA7*
- **(E)** *ITGA9*
- **(F)** *ITGA11*
- **(G)** *ITGB3*
- **(H)** *ITGB5*
- **(I)** *ITGB8*
- **(J)** *PTK2*

Figure S4:

(A) Knock-down efficiency (qPCR) upon transfection of the indicated siRNAs in MEFs cultured on plastic. Control siRNA (siCon) was set to 1. Shown is the mean ±SD of n=2 independent experiments.

(B) Quantification of AMPKα and mTOR protein abundance as assessed by Western blotting in siRNA-transfected MEFs. Cells were cultured on plastic. Shown is the mean ±SD of n=3 independent experiments.

(C) Immunoblot depicting changes in phospho-AMPK levels upon transfection with the indicated pools of siRNAs and subsequent culture on soft/stiff hydrogels. Shown is one of n=2 independent experiments.

(D) Immunoblot depicting changes in phospho-AMPK levels upon treatment with PF-573228 (FAK inhibitor = FAKi (10 µM)) while culturing on soft/stiff hydrogels. Shown is one of n=2 independent experiments.

(G) Black-and-white 3D-deconvoluted wide-field fluorescence images (maximum intensity projections) of Phalloidin/DAPI-stained NIH3T3 cells cultured on plastic. Concentrations were: Cytochalasin D: 1 µM (8h); Blebbistatin: 10 µM (8h), Y-27632: 10 µM (8h). Scale bar is 10 µm.

(H) Quantification of NIH3T3 Western blot results (mean ±SD of n=3-4 independent experiments) from cells as depicted in (E). Cyt D=Cytochalasin D; Blebbi=Blebbistatin.

(I) Immunofluorescence analysis of transiently transfected EGFP-PRKAA1 (green) in NIH3T3 cells. Cells were cultured on glass slides and treated with DMSO, FAK inhibitor (FAKi: PF-573228, 10 µM) or ITGAV inhibitor (CWHM-12, 500 nM). Focal adhesions were marked by staining of endogenous phospho-Paxillin (P-Paxillin, red). Pictures are maximum intensity projections of 3D-deconvoluted images.

Figure S5:

(A) Quantification of LC3-I and LC3-II in NIH3T3 cells as depicted in Fig. 3A. Shown is the mean of n=2 experiments.

(B) NIH3T3 cells stably expressing LC3B-EGFP. Cells were cultured in full 10% FBS-containing medium or in serum-starvation medium (0.5% FBS).

(C) Quantification of the results depicted in panel (B). Shown is the mean diameter of LC3B-EGFP spots per cell. Each dot represents one cell.

(D) Quantification of LC3B-EGFP fluorescence in stably expressing NIH3T3 cells cultured on soft or stiff matrices. Shown is the mean EGFP intensity per cell. Each dot represents the mean fluorescence per cell calculated from one field of view each (encompassing 16-51 cells). In total, more than 200 cells were measured.

(E) Staining of endogenous LC3AB (green) in NIH3T3 cells cultured on soft/stiff matrices. Shown is a confocal image. Scale bar is 10 µm. Nuclei appear in blue.

(F) Quantification of endogenous LC3AB fluorescence intensity in NIH3T3 cells cultured on soft or stiff matrices. Shown is the mean fluorescence intensity per cell. Each dot represents the mean fluorescence per cell calculated from one field of view each (encompassing 37-72 cells). In total, more than 460 cells were measured.

(G) Quantification of phagosome diameter as determined by electron microscopy of NIH3T3 cells cultured on soft/stiff hydrogels.

Figure S6:

(A) ITGAV and phospho-ACC^{S79} (pACC) immunohistochemistry (brown) of human PDAC specimen. Scale bars are 50 µm.

(B) Correlative quantification of stromal ITGAV and stromal pACC staining in a set of n=75 human PDAC tissues. Each red dot represents one patient. Scores were ranked based on stromal IHC signals being absent (0), intermediate (1) or strong (2).

(C) Proposed stiffness-dependent signaling flow to autophagy and lipid droplets. FA=Fatty acid.

(D) Oil-Red O staining of matrigel-embedded mouse pancreatic stellate cells treated with DMSO or ACC inhibitor (PF-05175157, 10 µM, 48h).

(E) Quantification of Oil-Red O content in mPSCs embedded in matrigel and treated with either DMSO or the ACC inhibitor (ACCi) PF-05175157 (10µM) for 2d (as shown in (B)). Shown is the mean ±SD of two independent experiments.

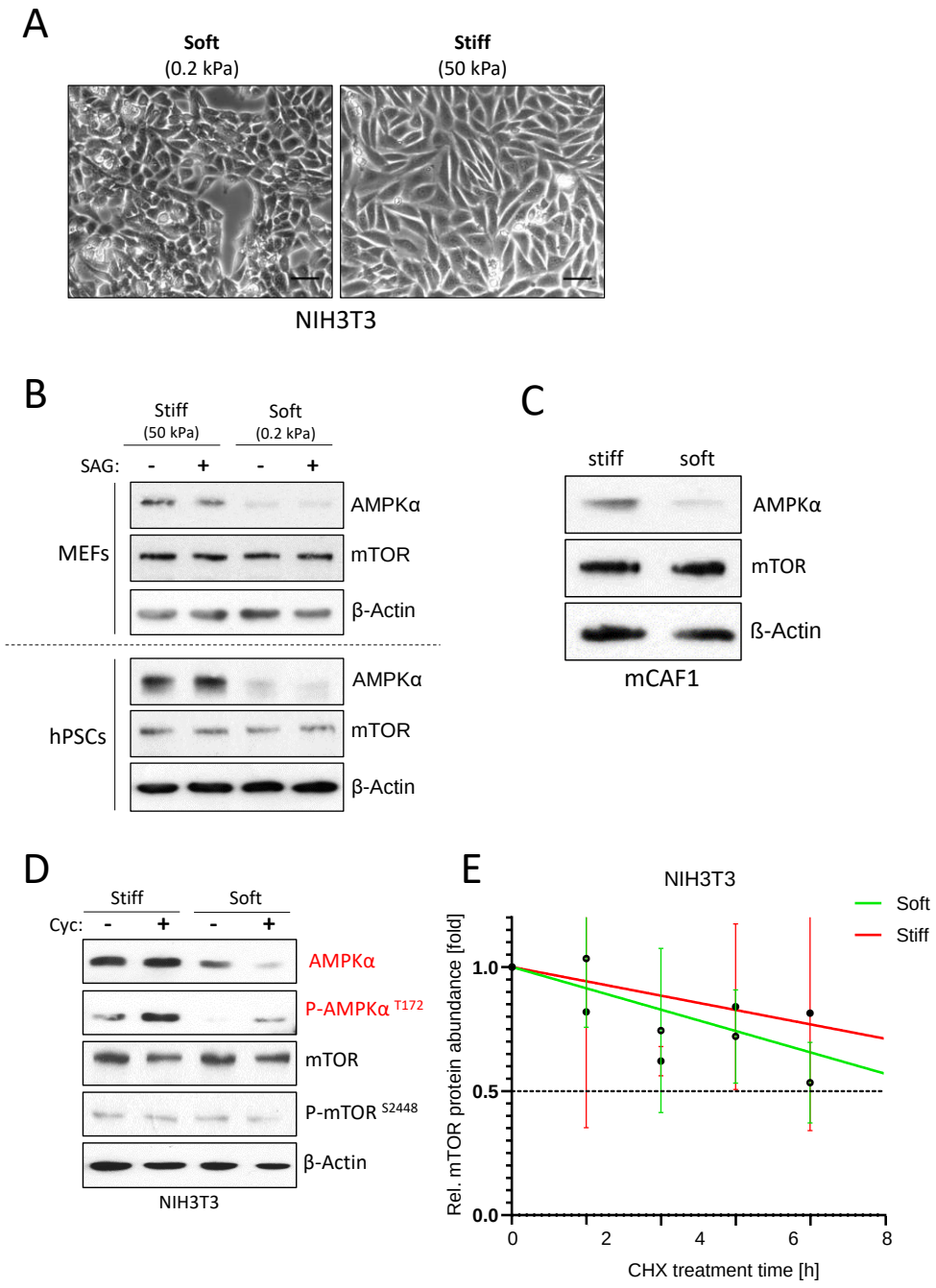
(F) *In vitro* myCAF polarization of mPSCs as determined by measuring the expression of four different myCAF marker genes. The ACC inhibitor (ACCi) PF-05175157 was used at a concentration of 10 µM (2d). Shown is the mean ±SD of n=4 independent experiments.

(G) *In vitro* iCAF polarization of mPSCs as determined by measuring the expression of four different myCAF marker genes. The ACC inhibitor (ACCi) PF-05175157 was used at a concentration of 10 µM (2d). Shown is the mean ±SD of n=3-4 independent experiments.

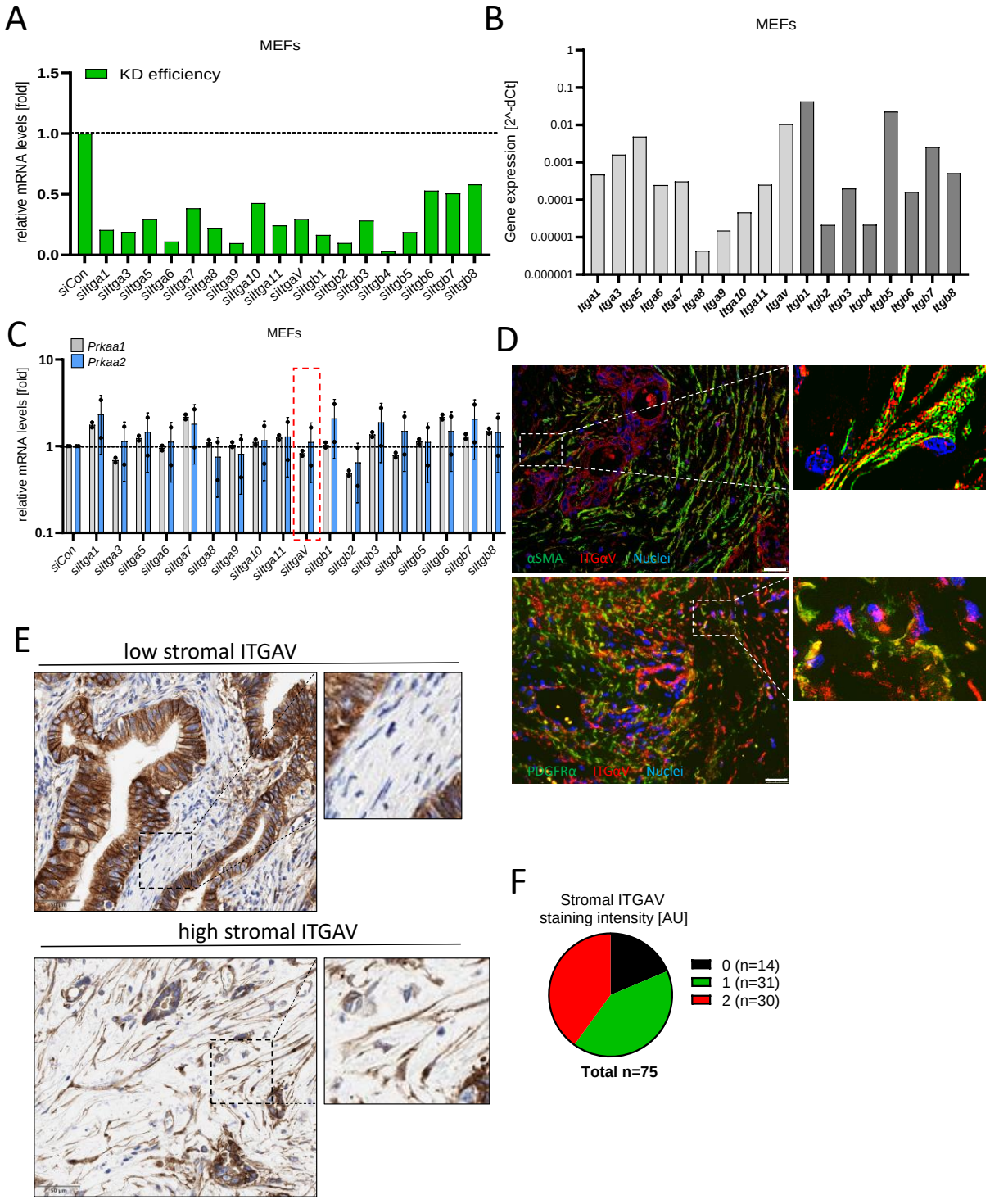
(H) Heatmap depicting the correlation between the expression of indicated *Integrin* genes and the gene set '*regulation of autophagy'* (related to Fig. 4B). The data set comprises microdissected epithelial and stromal material from n=66 human PDAC patients each.

(I) Heatmap depicting the correlation between the expression of indicated *focal adhesion* genes and the gene set '*regulation of autophagy'* (related to Fig. 4B). The data set comprises microdissected epithelial and stromal material from n=66 human PDAC patients each.

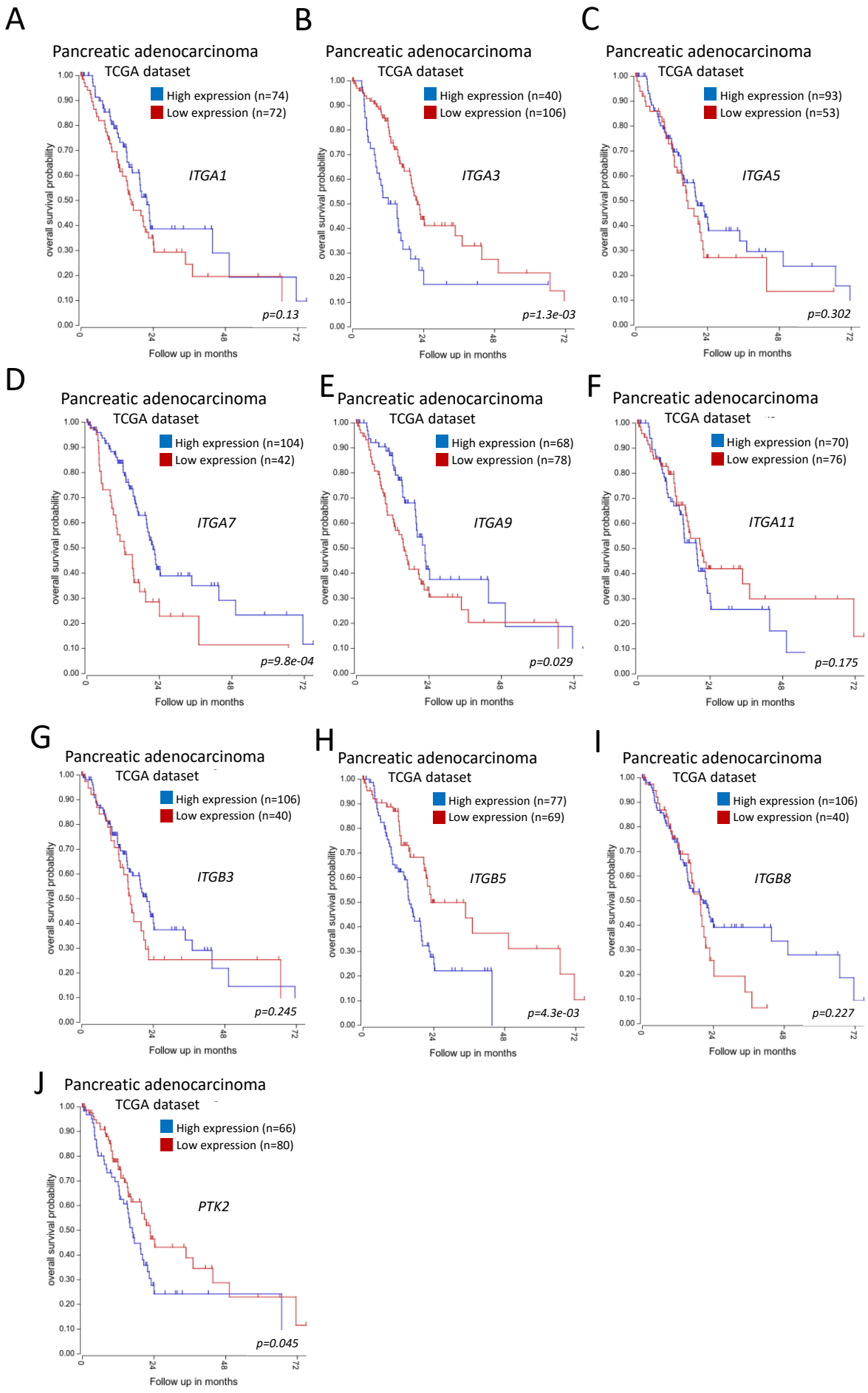
(J) Cell growth assay of luciferase-tagged human Patu8988T PDAC cells cultured alone or together with human PSCs on soft or stiff hydrogels (4d). Shown is the mean ±SD of one representative experiment measured in triplicate of a total of n=3 independent experiments.


A
Soft
(0.2 kPa)
Stiff
(50 kPa)
NIH3T3
B
Stiff
(50 kPa)
Soft
(0.2 kPa)
SAG: - + - +
MEFs
AMPKα
mTOR
β-Actin
hPSCs
AMPKα
mTOR
β-Actin
C
stiff
soft
AMPKα
mTOR
ß-Actin
mCAF1
D
Stiff
Soft
Cyc: - + - +
AMPKα
P-AMPKα T172
mTOR
P-mTOR S2448
β-Actin
NIH3T3
E
NIH3T3
Soft
Stiff
Rel. mTOR protein abundance [fold]
1.0
0.5
0.0
0
2
4
6
8
CHX treatment time [h]

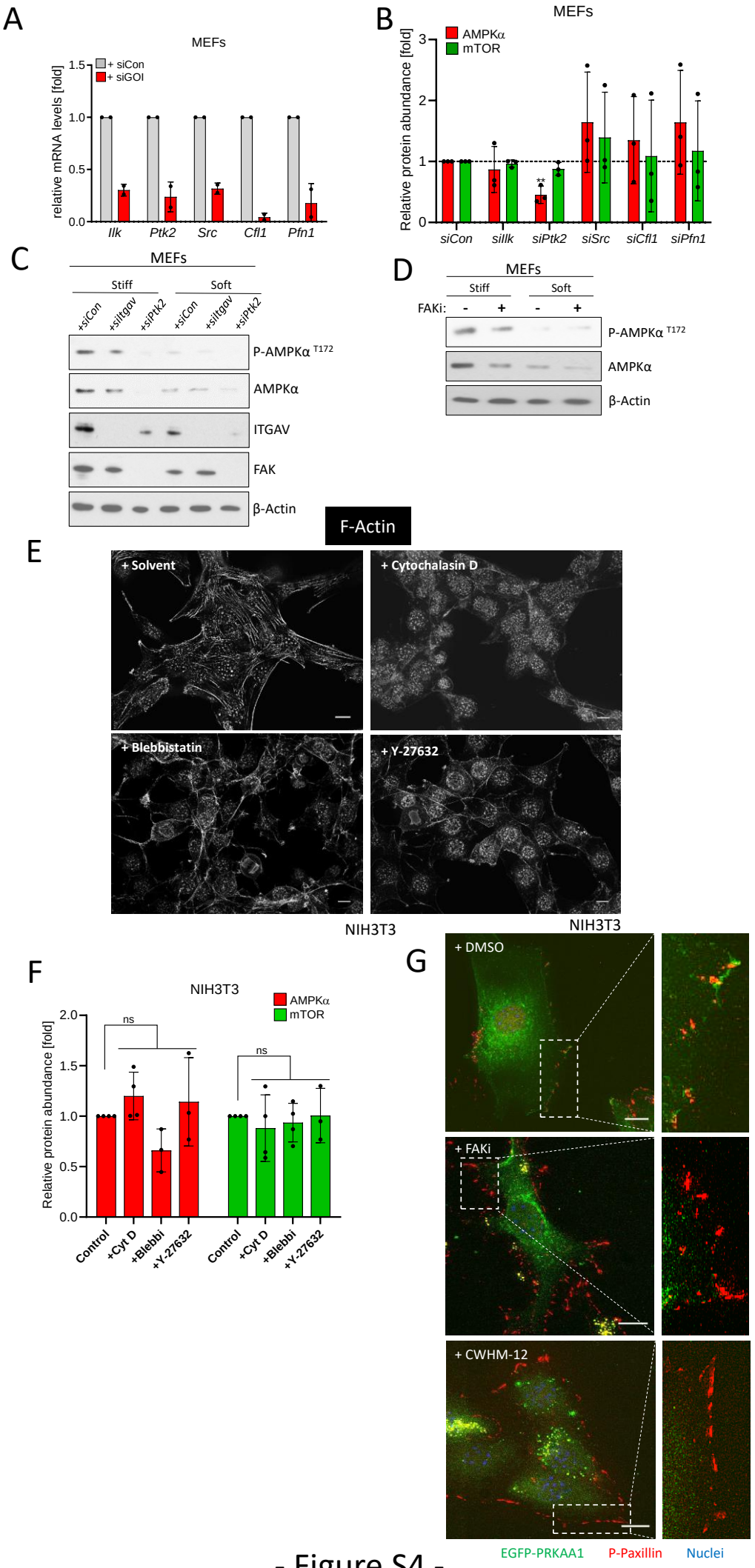
- Figure S1 -


A
MEFs
KD efficiency
relative mRNA levels [fold]
1.5
1.0
0.5
0.0
siCon
siItga1
siItga3
siItga5
siItga6
siItga7
siItga8
siItga9
siItga10
siItga11
siItgaV
siItgb1
siItgb2
siItgb3
siItgb4
siItgb5
siItgb6
siItgb7
siItgb8
B
MEFs
Gene expression [2^-dCt]
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
Itga1
Itga3
Itga5
Itga6
Itga7
Itga8
Itga9
Itga10
Itga11
Itgav
Itgb1
Itgb2
Itgb3
Itgb4
Itgb5
Itgb6
Itgb7
Itgb8
C
MEFs
Prkaa1
Prkaa2
relative mRNA levels [fold]
10
1
0.1
siCon
siItga1
siItga3
siItga5
siItga6
siItga7
siItga8
siItga9
siItga10
siItga11
siItgaV
siItgb1
siItgb2
siItgb3
siItgb4
siItgb5
siItgb6
siItgb7
siItgb8
D
αSMA
ITGαV
Nuclei
PDGFRα
ITGαV
Nuclei
E
low stromal ITGAV
high stromal ITGAV
F
Stromal ITGAV
staining intensity [AU]
0 (n=14)
1 (n=31)
2 (n=30)
Total n=75

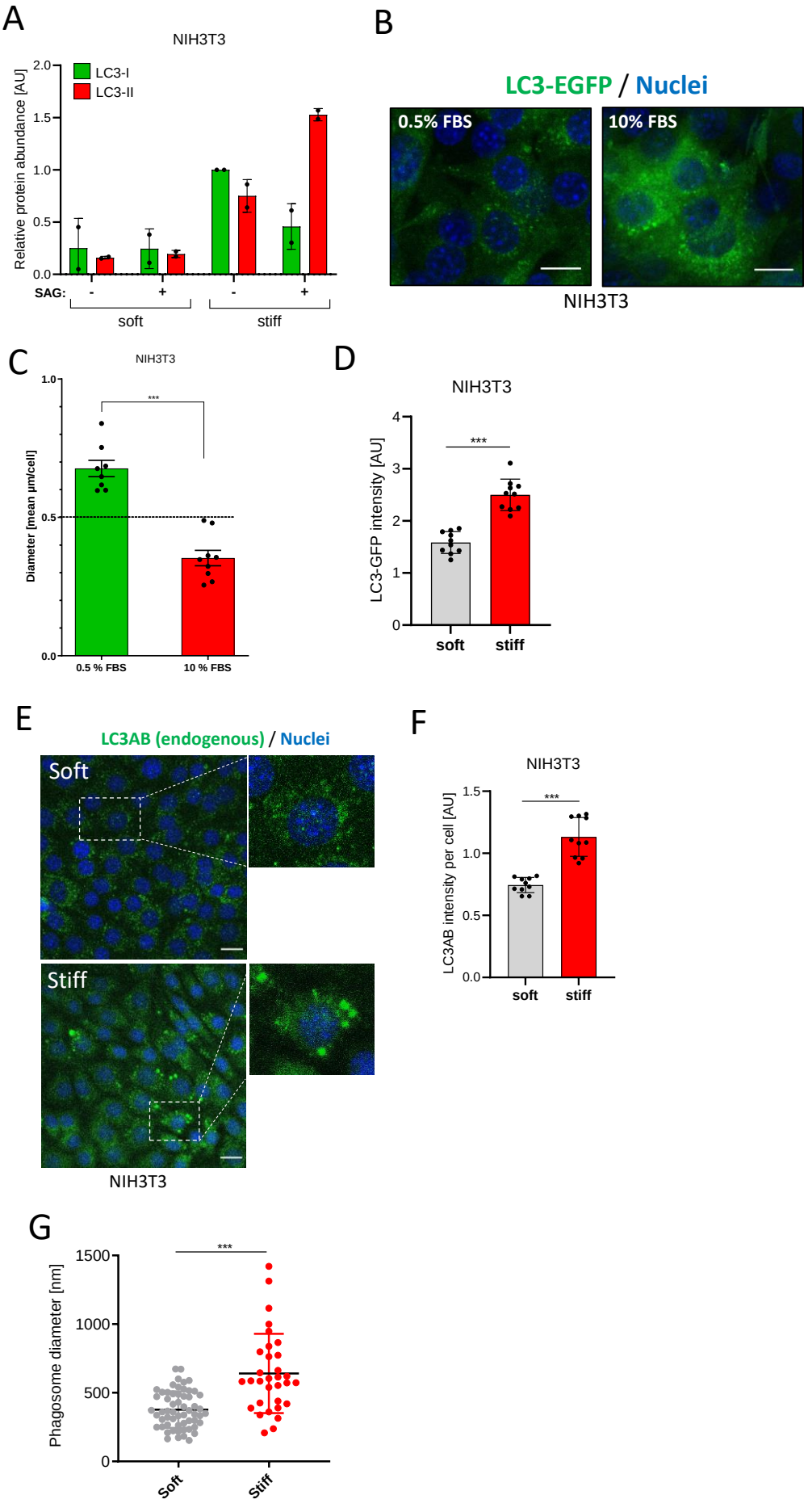
- Figure S2 -


A
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=74)
Low expression (n=72)
ITGA1
p=0.13
B
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=40)
Low expression (n=106)
ITGA3
p=1.3e-03
C
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=93)
Low expression (n=53)
ITGA5
p=0.302
D
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=104)
Low expression (n=42)
ITGA7
p=9.8e-04
E
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=68)
Low expression (n=78)
ITGA9
p=0.029
F
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=70)
Low expression (n=76)
ITGA11
p=0.175
G
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=106)
Low expression (n=40)
ITGB3
p=0.245
H
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=77)
Low expression (n=69)
ITGB5
p=4.3e-03
I
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=106)
Low expression (n=40)
ITGB8
p=0.227
J
Pancreatic adenocarcinoma
TCGA dataset
High expression (n=66)
Low expression (n=80)
PTK2
p=0.045
overall survival probability
Follow up in months

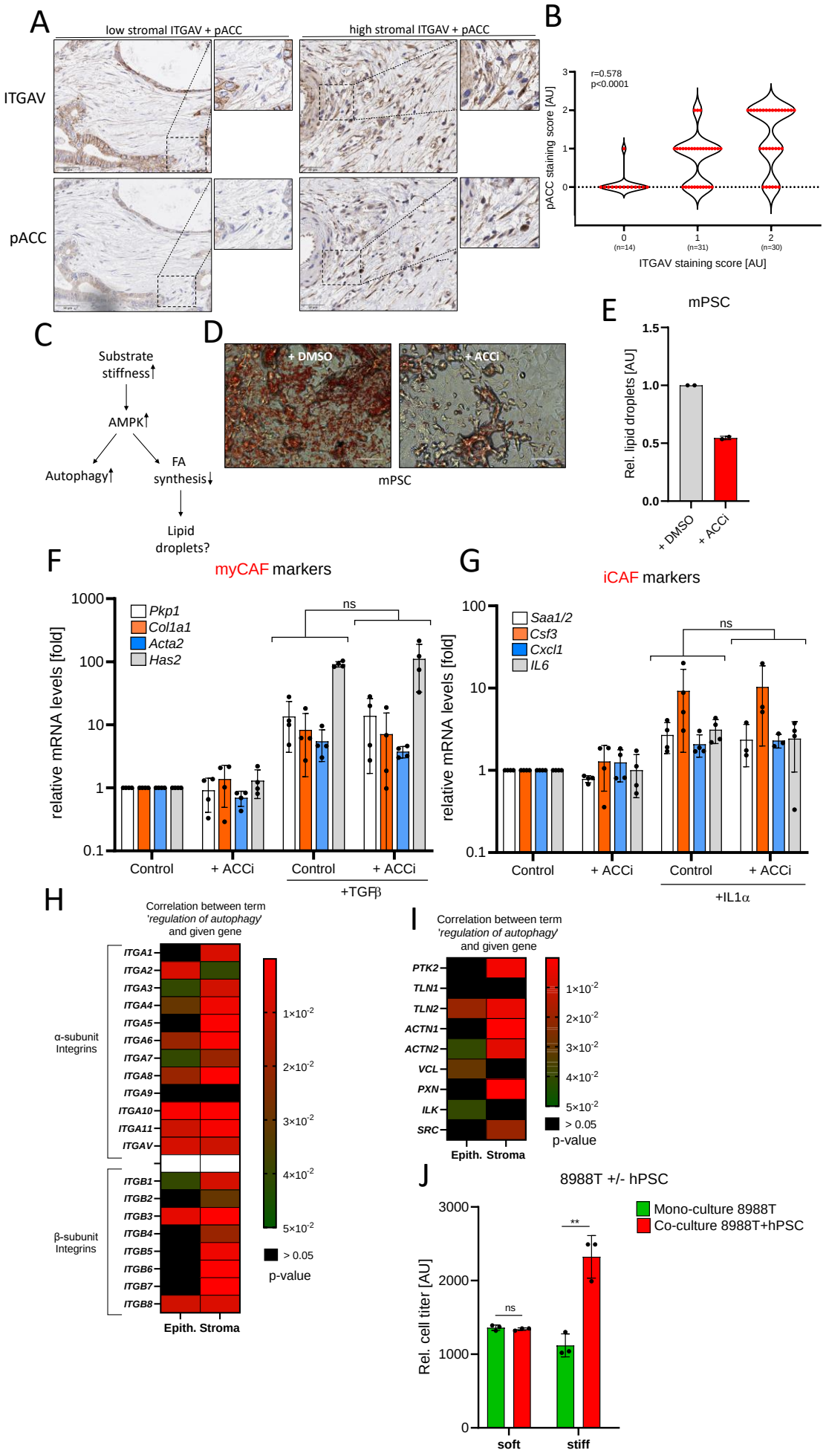
- Figure S3 -


A
MEFs
+ siCon
+ siGOI
relative mRNA levels [fold]
1.5
1.0
0.5
0.0
Ilk
Ptk2
Src
Cfl1
Pfn1
B
MEFs
AMPKα
mTOR
Relative protein abundance [fold]
3
2
1
0
**
siCon
siIlk
siPtk2
siSrc
siCfl1
siPfn1
C
MEFs
Stiff
Soft
+siCon
+siItgav
+siPtk2
+siCon
+siItgav
+siPtk2
P-AMPKα T172
AMPKα
ITGAV
FAK
β-Actin
D
MEFs
Stiff
Soft
FAKi: - + - +
P-AMPKα T172
AMPKα
β-Actin
F-Actin
E
+ Solvent
+ Cytochalasin D
+ Blebbistatin
+ Y-27632
NIH3T3
F
NIH3T3
AMPKα
mTOR
Relative protein abundance [fold]
2.0
1.5
1.0
0.5
0.0
ns
ns
Control
+Cyt D
+Blebbi
+Y-27632
Control
+Cyt D
+Blebbi
+Y-27632
G
NIH3T3
+ DMSO
+ FAKi
+ CWHM-12
EGFP-PRKAA1
P-Paxillin
Nuclei

- Figure S4 -


A
NIH3T3
LC3-I
LC3-II
Relative protein abundance [AU]
2.0
1.5
1.0
0.5
0.0
SAG: - + - +
soft
stiff
B
LC3-EGFP / Nuclei
0.5% FBS
10% FBS
NIH3T3
C
NIH3T3
Diameter [mean µm/cell]
1.0
0.5
0.0
***
0.5 % FBS
10 % FBS
D
NIH3T3
LC3-GFP intensity [AU]
4
3
2
1
0
***
soft
stiff
E
LC3AB (endogenous) / Nuclei
Soft
Stiff
NIH3T3
F
NIH3T3
LC3AB intensity per cell [AU]
1.5
1.0
0.5
0.0
***
soft
stiff
G
Phagosome diameter [nm]
1500
1000
500
0
***
Soft
Stiff

- Figure S5 -


A
low stromal ITGAV + pACC
high stromal ITGAV + pACC
ITGAV
pACC
B
r=0.578
p<0.0001
pACC staining score [AU]
ITGAV staining score [AU]
0 (n=14)
1 (n=31)
2 (n=30)
C
Substrate stiffness↑
AMPK↑
Autophagy↑
FA synthesis↓
Lipid droplets?
D
+ DMSO
+ ACCi
mPSC
E
mPSC
Rel. lipid droplets [AU]
+ DMSO
+ ACCi
F
myCAF markers
Pkp1
Col1a1
Acta2
Has2
relative mRNA levels [fold]
ns
Control
+ ACCi
+TGFβ
G
iCAF markers
Saa1/2
Csf3
Cxcl1
IL6
+IL1α
H
Correlation between term 'regulation of autophagy' and given gene
α-subunit Integrins
β-subunit Integrins
Epith. Stroma
p-value
I
Correlation between term 'regulation of autophagy' and given gene
PTK2
TLN1
TLN2
ACTN1
ACTN2
VCL
PXN
ILK
SRC
J
8988T +/- hPSC
Mono-culture 8988T
Co-culture 8988T+hPSC
Rel. cell titer [AU]
soft
stiff

- Figure S6 -

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Supplemental methods

Cell lines

NIH3T3 and Panc1 cell lines were purchased from CLS and Patu8988T cells from DSMZ. Immortalized human (hPSC1) and mouse PSCs (mPSC4) were kindly obtained from Matthias Löhr (1) and Albrecht Neese (2), respectively. MEF cells were kindly provided by Wade Bushman (3). All cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM (high Glucose plus Glutamine and Pyruvate), Invitrogen) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/ Streptomycin at 37°C with 5% CO_2. If not otherwise stated, serum concentrations were reduced to 0.5% during experiments for all cell types. All cells were regularly checked for mycoplasma contamination.

Reagents

Cells were cultured on Collagen I-coated polyacrylamide hydrogels of an elastic modulus of either 0.2 or 50 kPa (Matrigen Petrisoft/Softslip, Cell Guidance Systems). Inhibitors were purchased from the following companies: SelleckChem: PF-573228 (FAKi); Biomol/Cayman: PF-05175157 (ACCi), Y-27632, Bafilomycin A1, Cyclopamine, Cycloheximide; Merck/Millipore: SAG; Adipogen: MG132; Sigma: Cytochalasin D, Blebbistatin.

Generation of stable cell lines

PaTu8988T cells were transfected with PGK-Luc (Firefly luciferase driven by PGK promoter) and co-transfected with pEF-MCS (= empty pEF6/V5-His (Invitrogen)) using TransIT-2020 (Mirus Bio) according to the manufacturer's protocol. Cells were subsequently selected with Blasticidin (Capricorn Scientific GmbH) at a concentration of 5 µg/ml.

NIH3T3 cells were transfected with pMRX-IP-GFP-LC3-RFP (LC3B-EGFP; Addgene #84573) using TransIT-2020 (Mirus Bio) according to the manufacturer's protocol. Cells were selected with Puromycin (PAA Laboratories GmbH) at a concentration of 2 µg/ml.

***Itgav* knockout cell line via CRISPR/Cas9**

For generating *Itgav* knockout mPSC4 cells the CRISPR/Cas9 gene editing system was used (target sequence underlined): Primers mItgaV_Crispr1_s (5'-CACCG<u>TGGAGTTTAAGTCCCACCAG</u>) and mItgav_Crispr1_as (5'-AAAC<u>CTGGTGGGACTTAAACTCCA</u>C) were annealed and cloned into the BbsI site of pU6-(BbsI)-CBh-Cas9-T2A-mCherry vector (Addgene: #64324). The KO cells were generated according to a strategy described in (4). To this end, the mPSC4 cell line was transfected with the cloned plasmid (pU6-mItgav_Crispr1), pTia-2A-Hygro (kindly provided by Till Adhikary) and empty pU6-(BbsI)-CBh-Cas9-T2A-mCherry (ratio 1:1:0.5) using Helix-In transfection reagent (Oz Biosciences, OZB-HX10500) for 24h. Subsequently, cells were selected with Hygromycin (VWR International GmbH, J60681.MC) at a concentration of 400 µg/ml until resistant clones appeared. Single cell clones were picked and the knockout was confirmed via Western blot and RT-qPCR.

Western blotting

Separation of lysates by SDS-PAGE was followed by subsequent blotting on Immobilon-PVDF membranes (Millipore) and incubation with the respective primary antibody, followed by incubation with an HRP-coupled secondary antibody (Cell signaling Technology). Detection of the HRP signal was performed using Pierce ECL Western Blotting Substrate (Thermo Scientific) according to the manufacturer's protocol.

qPCR analyses

Total RNA was extracted using NucleoSpin RNA II kit (Macherey-Nagel) according to the manufacturer's protocol. cDNA synthesis of 1 µg total RNA was performed using iScript cDNA Synthesis Kit (Biorad) following the manufacturer's guidelines. Quantitative PCR reactions were performed using the Absolute QPCR SYBR Green Mix (ABGene). qPCR reactions were performed on 96 well qPCR plates (ABGene) using either the Mx3000P or Mx3005P qPCR systems (Agilent). Results were calculated as relative mRNA expression ($2_{\Delta\Delta Ct}$). Data was obtained from at least three independent experiments and is shown as the mean ± StDev.

Small interfering RNA (siRNA) transfections

Cells were transfected with 35 nM siRNA (Dharmacon SMARTpools and Qiagen control siRNA (All-Stars siRNA; siCon) using RNAiMax (Invitrogen). For siRNA sequences see enclosed table.

Microscopy

Transfections with pEGFP-PRKAA1 (Addgene #30305) were done on plastic plates using TransIT-2020 (Mirus Bio) according to the manual. 24h later, cells were transferred onto glass cover slips or hydrogels and incubated in 0.5% FBS-containing media for 1-2d. Cells were subsequently fixed with 4% formaldehyde/PBS for 10 min at RT. After washing twice with PBS and permeabilization with 0.5% Triton-X100/PBS at RT for 5 min, hydrogels were blocked with 10% serum/PBS for 1h at RT and washed once with PBS. Primary antibody was diluted in PBS containing 10% serum and 0.1% Saponin and incubated overnight at 4 °C. After washing twice with PBS at RT for 5 min, the hydrogels were incubated with fluorophore-coupled secondary antibodies diluted in PBS containing 10% serum and 0.1% Saponin at RT in the dark for 2 h. After washing twice with PBS for 5 min and rinsing with H2O, hydrogels were covered with mounting medium containing DAPI (Vectashield). Microscopy was performed on a Leica TCS SP8 confocal microscope (Leica Microsystems, Wetzlar, Germany).

***In vitro* PSC polarization**

PSCs (4 x10^5) were seeded in a 70µl matrigel drop (Growth Factor Reduced (GFR) Basement Membrane Matrix, Corning, 734-1101) mixed at a 1:1 ratio with DMEM (10 % FBS) on a 3.5 cm suspension plate (Sarstedt). The matrigel was covered with 0.5 % FBS-containing DMEM, treated with ACCi (PF-05175157, 10 µM) and incubated for 48 h. Afterwards, cells were treated with either recombinant human TGF-ß1 (Peprotech, #100-21) or recombinant human IL-1α (Peprotech, #200-01A) at a concentration of 2 ng/ml or 1 ng/ml, respectively, followed by an incubation for 48 h. Warm medium was removed and drops were collected in falcon tubes mixed with ice cold DMEM. After centrifugation (400 rpm, 4°C, 10 min) and removing of the medium, cells were resuspended in ice cold DMEM and incubated for 30 min at 4°C. Cells were centrifuged (400rpm, 4°C, 10 min), medium was removed and pellets were frozen at -80°C for later RNA preparation.

Oil-Red-O staining and quantification

Oil Red O staining was performed as described in (5). Briefly, stellate cells were fixed in 10% formaldehyde/DPBS for 10 min at RT and incubated in 60 % Isopropanol at RT for 5 min. The cells were air-dried and incubated with filtered Oil-Red-O solution (Sigma, O0625-25G) diluted in water at a 3:2 ratio (Stock Solution : Water) for 10 min at RT. Afterwards the cells were washed with water and microscopy was performed on a Leica DM3000 microscope (Leica Microsystems, Wetzlar, Germany). For quantification, cells were incubated in 100% isopropanol for 10 min on an Orbital Shaker.

Supernatant was taken and measurement was performed on a Spectra Max 340 microplate reader (Molecular Devices) at an absorbance wavelength of 510 nm.

Phalloidin staining

Cells were seeded on hydrogels and fixed with 4% formaldehyde/PBS for 10 min at RT. After washing twice with PBS, cells were permeabilized with 0.5% Triton-X100/PBS at RT for 5 min and washed twice with PBS. Phalloidin-California Red Conjugate (Biomol, #23103) was diluted 1:1000 in PBS containing 1% Albumin Fraction V (Roth, 8076.1). Cells were incubated with Phalloidin for 20 min at RT. After washing twice with PBS and rinsing with water, hydrogels were covered with mounting medium containing DAPI (Vectashield). Microscopy was performed on a Leica TCS SP8 con focal microscope (Leica Microsystems, Wetzlar, Germany).

Immunohistochemistry

For immunohistochemistry, heat-induced epitope retrieval was performed with EDTA. Staining was performed on a DAKO Autostainer-Plus. After blocking endogenous peroxidase, sections were incubated for 45 minutes with rabbit polyclonal Anti-Phospho-Acetyl-CoA Carboxylase Antibody (1:100; Cell Signaling #3661). Sections were washed and incubated with Dako REAL EnVision HRP Rabbit/Mouse polymer, which reacts with DAB-Chromogen, according to the manufacturer´s protocol. The use of patient material (in form of tissue microarrays (TMAs)) was approved by the local ethics committee (Ethics Board University Hospital Marburg). Material on the TMA was from patients suffering from PDAC eligible for surgery. Pathological diagnosis of patient from Fig. 4A: G2 ductal adenocarcinoma of the pancreas, pT2, pN0, L0, V0, Pn0.

Transmission electron microscopy (TEM)

Cells were seeded on hydrogels and incubated for 48 h in growth medium containing 0.5% FBS. Afterwards, cells were washed once with PBS and fixed with 2 % glutaraldehyde (in H_2O) for at least 1h at RT, followed by processing for TEM (EM core facility Philipps University Marburg).

Co-culture assays

Cells were seeded on hydrogels in a cellular ratio of tumor cells (1/5) and stellate cells (4/5) or as tumor cell monoculture. Cells were treated with Bafilomyin A1 (100 nM) or DMSO and incubated for 4 d in 0% FBS DMEM. Afterwards, cells were lysed in Passive Lysis Buffer (Promega, E1941) and Firefly Luciferase activity was measured using Beetle-Juice reagent (PJK, Kleinblittersdorf, Germany) on an Orion L microplate luminometer (Berthold Detection Systems).

Xenograft experiment

Female athymic nude mice were randomly divided into 3 groups. 0.5×10^6 PaTu8988T cells were suspended in a total volume of 200 µl of DMEM medium for group 1. 0.5×10^6 PaTu8988T cells + 1×10^6 $mPSC4^{WT}$ cells were suspended in a total volume of 200 µl of DMEM medium for group 2. 0.5×10^6 PaTu8988T cells + 1×10^6 $mPSC4^{KO}$ (*Itgav* KO) were suspended in a total volume of 200 µl of DMEM medium for group 3. The cell suspensions were injected s.c. at the posterior flank of the mice. Tumor volumes were calculated by the formula (length x $width^2$)/2. The length represents the longer axis and the width represents the shorter axis of the tumor. At the experimental endpoint mice were euthanized and tumors were removed. The study was approved by the regional agency on animal experimentation (Regierungspräsidium Giessen).

Statistics and data availability

Statistical comparisons were made of n≥3 experiments using a two-tailed Student's t-test unless otherwise stated. Significances were indicated as ns (not significant) p>0.05, *p<0.05, **p<0.01, ***p<0.001. Kaplan-Meier curves and gene correlations were done using the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl).

References

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Supplemental material section

Primary antibodies

Antibody	Species	Clone	Order #	Source
β-Actin	mouse	AC-15	A5441	Sigma
AMPKα	mouse	F6	#2793	Cell Signaling Technology
phospho-AMPKα (Thr172)	rabbit	40H9	#2535	Cell Signaling Technology
mTor	rabbit	7C10	#2983	Cell Signaling Technology
phospho-mTor (Ser2448)	rabbit	D9C2	#5536	Cell Signaling Technology
phospho-ACC (Ser79)	rabbit	D7D11	#11818	Cell Signaling Technology
FAK	rabbit		#3285	Cell Signaling Technology
ITGAV (WB)	rabbit		#4711	Cell Signaling Technology
ITGAV (IF+IHC)	rabbit		HPA004856	Sigma/Merck
αSMA; AF488-coupled	mouse		53-9760-82	eBioscience/Thermo
LC3A/B	rabbit	D3U4C	#12741	Cell Signaling Technology
PDGFRα	mouse		sc-398206	Santa Cruz Biotechnology
phospho-Paxillin (Tyr118)	rabbit		#2541	Cell Signaling Technology

qPCR primer sequences

Gene	Species	sequence (5'-->3')
Rplp0 (P0)	murine	for: TGCACTCTCGCTTTCTGGAGGGTGT rev: AATGCAGATGGATCAGCCAGGAAGG
Prkaa1	murine	for: GGATCCAAGAGCCGAGTTGCTCACA rev: TGGCTGACATGATAGCGTCTCTGTGC
Prkaa2	murine	for: GCCTCCACAGAGCACGGTCAAGTTT rev: TGCTGCCAGTCAAAGAGCCAGTGAG
Itga1	murine	for: AAAAGAGCTTCGTGTTCCGTGAAGCAG rev: TCACCCCATTCCCGTGTTGTCATTT
Itga3	murine	for: CCTACCCAGTCCTCAACCAGGCACA rev: CTTGTTGTCTGGCCCGCACTCTTTC
Itga5	murine	for: GCCACCCTTACCCAGACCCTGCTTA rev: TGAGGGCAATGTGAATTGGGGAGAG
Itga6	murine	for: GGAGCTGACCCTGAATCGGCAGAAG rev: GGATGGGACGCAGCTTGTCTCTGAT
Itga7	murine	for: GTGTGGCTGAAGCACCAACATGACC rev: CACCACAATGGCCCGTAGCTTGTCT
Itga8	murine	for: ATGAGGGAGAAGGGGCCTACGAAGC rev: GGTCACACACCACCATCCTGGTCAC

Itga9	murine	for: TTTTTCTGCTGCTGGCTGTGCTCCT rev: CTGGACCCAGTCCCAACCATCTTCA
Itga10	murine	for: GCTGCTCCTGCTTGCTCTCCTTGTC rev: AAGGCTGCCAAAGTGGGCTTCTGAG
Itga11	murine	for: ATCTGGATCATCGTGGGCAGCACTC rev: GTTCCCTCTTGCGCTTGGCACTTTT
ItgaV	murine	for: CAGGGCTGCTGCTACTGGCTGTGTT rev: GCTCTTCTTGAGGTGGTCGGACACG
Gene	**Species**	**sequence (5'-->3')**
Itgb1	murine	for: AGGAGAAAATGAATGCCAAGTGGGACA rev: TTGGTGTTGCAAAATCCGCCTGAGT
Itgb2	murine	for: CAGCCACCGATGTGTGAGGATTGTC rev: TGCAGCGTCATACCAGCACACTGAA
Itgb3	murine	for: TGAGGAAGAACGAGCCAGAGCCAAG rev: CCCCCGGTAGGTGATATTGGTGAAGG
Itgb4	murine	for: TGGACAATGGCAAGTCCCAGGTCTC rev: CTTCCAGGTCTCCCCAGGATGGAAC
Itgb5	murine	for: CCATCTGGAAGCTGCTCGTCACCAT rev: GGCGAAATCGACAGTGTGTGTGGAG
Itgb6	murine	for: GAACGCTCTAAGGCCAAGTGGCAAA rev: AGGCCTGCTTTGTGCTTCTCCCTGT
Itgb7	murine	for: AGGCTGCAAGTCACCATGTGAGCAG rev: AGGGGCCAAGGTCAAGGTCACATTC
Itgb8	murine	for: TGCCAGTGCTTTAGTGGCTGGGAAG rev: CTGCAAACTTGGCCCTTGGAGTTGA
Ilk	murine	for: TGAAGCCCTGCAGAAGAAGCCTGAA rev: TTCCAGTGCCACCTTCATCCCAATC
Fak	murine	for: CCTCCAAATTGTCCTCCCACCCTCT rev: TCTTGCTGCACCTTCTCCTCCTCCA
Src	murine	for: GGTTGGCCCGGCTCATAGAAGACAA rev: ACCTGCCGTACAGAGCAGCTTCAGG
Cfl1	murine	for: GGCCTCTGGTGTGGCTGTCTCTGAT rev: AAAGAGCACCGCCTTCTTGCGTTTC
Pfn1	murine	for: GATCCGGGACTCACTGCTGCAAGAC rev: GTGGACACCTTCTTTGCCCATCAGC
Pkp1	murine	for: TGCTTCAGCAACAGGGGTGACAAGA rev: CATGAGGTTCAGGTAGGTCCGGATGG
Col1a1	murine	for: TGGCAAAGACGGACTCAACGGTCTC rev: GAAGTCATAACCGCCACTGGGAGGTC
Acta2	murine	for: CCTGGAGAAGAGCTACGAACTGCCTGA rev: TTTCGTGGATGCCCGCTGACTC
Has2	murine	for: ACCCTATGGTTGGAGGTGTTGGAGGA rev: CACGCTGCTGAGGAAGGAGATCCAG
Saa1/2	murine	for: CATGCTCGGGGGAACTATGATGCTG rev: CTGTTGGCTTCCTGGTCAGCAATGG
Csf3	murine	for: TGGACTTGCTTCAGCTGGATGTTGC

		rev: AGGCAGAAGTGAAGGCTGGCATGG
Cxcl1	murine	for: GAGGCTTGCCTTGACCCTGAAGCTC
		rev: GTCAGAAGCCAGCGTTCACCAGACA
IL6	murine	for: TCCTTCCTACCCCAATTTCCAATGCTC
		rev: GGATGGTCTTGGTCCTTAGCCACTCCT

siRNA sequences (targeting mouse genes)

Name	Target sequence
siCon (Qiagen's All-Star; siAll)	AAUUCUCCGAACGUGUCACGU
siItga1_1	AGGGCAAGGUGUACGUGUA
siItga1_2	CCACAAUUGACAUCGACAA
siItga1_3	GCCUAUGACUGGAACGGAA
siItga1_4	CUUUAAUGACGUCGUGAUU
siItga3_1	GGACAAUGUUCGCGAUAAA
siItga3_2	GCUACAUGAUUCAGCGGAA
siItga3_3	AGACAUACCACAACGAGAU
siItga3_4	GCACACUGGUUCUAGGUCU
siItga5_1	CAAGAUGAGUUCAGCCGAU
siItga5_2	GCUUCAACCUAGACGCGGA
siItga5_3	UGUGAAGGACCCUCGUUUA
siItga5_4	CAGCAAAGGCUCCCGGAUU
siItga6_1	GUAUAUAUUUGACGGAGAA
siItga6_2	UGGCUCACCUAGCGGGAUA
siItga6_3	CGAGAGUUCAGUUUCGAAA
siItga6_4	GGACCAAAGACUCGAUGUU
siItga7_1	CGAUAUGAGUCUCGACAGA
siItga7_2	GAAGCAAGGCUGCGGUCAA
siItga7_3	GGGCAAGAUUGUUACGUGU
siItga7_4	GGGCGAAAGUGCCAUGCGA
siItga8_1	CCUAAAGGUCAGAUCGAGA
siItga8_2	GGUCAUAAUACUAGCGAUC
siItga8_3	GCGGUUAAGACGUCGGUUA
siItga8_4	UCAAGAAGAUGCCGUAUAA
siItga9_1	UGCUGAACCUCACGGACAA
siItga9_2	ACAUGGAGCCCUCGAGGAA
siItga9_3	UUUCCAGUGUUGACGAGAA
siItga9_4	GUUGAACGAUGAAGCGAUU
siItga10_1	GCACCGAGGUUUAGACAUC
siItga10_2	GCUCAUACUUUGGCAGCGA
siItga10_3	GGAUACAGAUAAGGACGGA
siItga10_4	GAGGAAAAGUUAUCGCCUU
siItga11_1	CCAGCGACCCUGACGACAA
siItga11_2	GCAUCGAGUGUGUGAACGA
siItga11_3	UCAGAAGACAGGAGACGUA
siItga11_4	AUGGAUGAGAGGCGGUAUA

siItgaV_1	GUGAACAGAUGGCUGCGUA
siItgaV_2	GUGAGGAACUGGUCGCCUA
siItgaV_3	CCAAUUAGCAACACGGACU
siItgaV_4	GCGCAAUCCUGUACGUGAA

Name	Target sequence
siItgb1_1	CAAUGAAGCUAUCGUGCAU
siItgb1_2	GUGAAGACAUGGACGCUUA
siItgb1_3	UUACAAGAGUGCCGUGACA
siItgb1_4	UGCCAAAUCUUGCGGAGAA
siItgb2_1	GGAAUGCACCAAGUACAAA
siItgb2_2	GUAGUUGCAAGCCCGGUUA
siItgb2_3	GGUAUGACGCUGCAGACUA
siItgb2_4	GGUCAUGGCUUCCGAGUGU
siItgb3_1	AAACAGAGCGUGUCCCGUA
siItgb3_2	AAACACGUGCUGACGCUAA
siItgb3_3	GAGCAGUCUUUCACUAUCA
siItgb3_4	GUGAAAGAGCUGACGGAUA
siItgb4_1	GCUACAACGUGGAGGGCGA
siItgb4_2	AAGUCAAGAUGGUAGACGA
siItgb4_3	GAUCAACUACUCUGCGAUA
siItgb4_4	GGAUAAUACAGCACACGGA
siItgb5_1	CCGCUUAGGUUUCGGGUCU
siItgb5_2	GCUAGGCACGCACGGAUAA
siItgb5_3	AGAAGAUCGGAUGGCGAAA
siItgb5_4	ACUGCUAAGGACUGCGUUA
siItgb6_1	GGACAUUCCUAUCCCGUAU
siItgb6_2	CGGCCAACUCAUUGAUAAA
siItgb6_3	GGAGUUGCAUCGAGUGCUA
siItgb6_4	GCUCAUCGGAGUUGUGCUA
siItgb7_1	GGACCUUCAGUGCGGGAUA
siItgb7_2	GGUCUGUGCUGACGUGAAU
siItgb7_3	ACUCAAUGAAGGACGACUU
siItgb7_4	AGAAAUUCCGGGZCCGCUU
siItgb8_1	GCGGAUGGUGUGUUCAAGA
siItgb8_2	GGCCAAAGUGAACACAAUA
siItgb8_3	CGUCAAGUGAGAAUGAAAU
siItgb8_4	GGAAGUGAACGUUGUGAUA
siPtk2_1	GUACAGCACUCGCGUAUCU
siPtk2_2	GCUCCAGAGUCAAUCAAUU
siPtk2_3	GGGCAUCAUUCAGAAGAUA
siPtk2_4	GAAGUUGGGUUGUUUGGAA
siSrc_1	GCUCGUGGCUUACUACUCC
siSrc_2	UCAGAUCGCUUCAGGCAUG

siSrc_3	GGGAGCGGCUGCAGAUUGU
siSrc_4	GCACGGGACAGACCGGUUA
siIlk_1	GAAGGCACCAAUUUCGUUG
siIlk_2	GUAAGCUCAUGAAGAUUUG
siIlk_3	CUCAAUAGCCGCAGUGUAA
siIlk_4	GAACAAACACUCCGGUAUU

Name	Target sequence
siCfl1_1	CCAGAAGAAGUGAAGAAAC
siCfl1_2	CAGACCUGCUCUUGGGUGU
siCfl1_3	CUAACUGCUACGAGGAGGU
siCfl1_4	GUGUCAUCAAGGUGUUCAA
siPfn1_1	GCAAAGACCGGUCAAGUUU
siPfn1_2	GGGAAUUUACAAUGGAUCU
siPfn1_3	UGGAACGCCUACAUCGACA
siPfn1_4	CUUCGUUAGCAUUACGCCA