SUPPLEMENTARY FIGURES

Figure S1.

(a) Relative expression of pro-fibrotic cytokines measured by real-time quantitative PCR in nodular versus infiltrative human BCC. GAPDH was used for normalization.

(b) *In vivo* experimental design.

(c) *In vivo* growth of UW_BCC_T2/MEF tumors injected intratumorally either with PBS (control) versus recombinant TGFβ (400ng/inj, 3x/week) and ASZ_001_ns/MEF versus ASZ_001_TGFβshRNA/MEF tumors.

(d) Ki67 and cleaved Caspase 3 (undetectable) immunostainings and quantification of Ki67 in UW_BCC_T2/MEF tumors injected either with PBS (control) or recombinant TGFβ.

(e) E-cadherin and N-cadherin (undetectable) immunostainings and quantification of E-cadherin in UW_BCC_T2/MEF tumors injected either with PBS (control) or recombinant TGFβ.

(f) Western blot and quantification of TGFβ in ASZ_001 infected with non-silencing (ns) or TGFβ1-targeting (TGFβshRNA) shRNA sequences.

Scale bars indicate 25μm. Horizontal bars or columns and error bars represent the mean ± SD for $n \geq 5$ per group. *$p < 0.01$ and **$p < 0.01$.

Figure S1

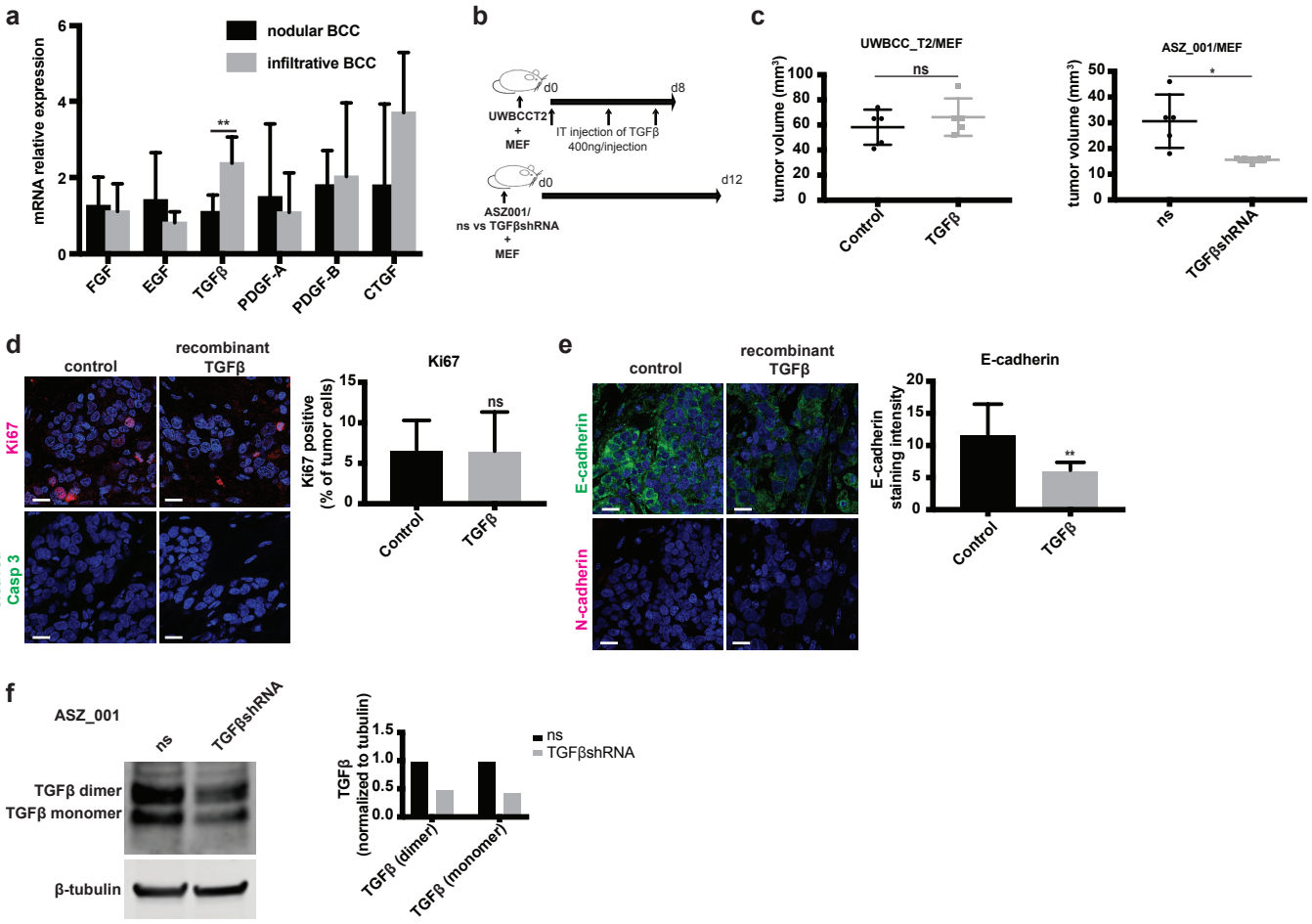

a
nodular BCC
infiltrative BCC
mRNA relative expression
FGF
EGF
TGFβ
PDGF-A
PDGF-B
CTGF
b
UWBCCT2
+
MEF
d0
d8
IT injection of TGFβ
400ng/injection
ASZ001/
ns vs TGFβshRNA
+
MEF
d0
d12
c
UWBCC_T2/MEF
tumor volume (mm³)
ns
Control
TGFβ
ASZ_001/MEF
tumor volume (mm³)
*
ns
TGFβshRNA
d
control
recombinant
TGFβ
Ki67
cleaved
Casp 3
Ki67
Ki67 positive
(% of tumor cells)
ns
Control
TGFβ
e
control
recombinant
TGFβ
E-cadherin
N-cadherin
E-cadherin
E-cadherin
staining intensity
**
Control
TGFβ
f
ASZ_001
ns
TGFβshRNA
TGFβ dimer
TGFβ monomer
β-tubulin
TGFβ
(normalized to tubulin)
ns
TGFβshRNA
TGFβ (dimer)
TGFβ (monomer)

Figure S2.

(a) Migration timecourse of UW_BCC_T2 on fibronectin when treated with either DMSO (control) or K34C (10μM and 20μM respectively).

(b) Migration timecourse of ASZ_001 on fibronectin when treated with either DMSO (control) or K34C (10μM and 20μM respectively).

(c) Migration timecourse of UW_BCC_T2 on fibronectin when treated either with isotype (control) or P1D6 (10μg/ml) antibodies.

(d) *In vitro* growth curve of UW_BCC_T2 on fibronectin when treated either with DMSO (control) or K34C inhibitor (10μM and 20μM respectively). Cell density was assessed using Chalkley score analysis.

(e) *In vitro* growth curve of ASZ_001 on fibronectin when treated either with DMSO (control) or K34C inhibitor (10μM and 20μM respectively). Cell density was assessed using Chalkley score analysis.

(f) *In vitro* growth curve of UW_BCC_T2 on fibronectin when treated either with isotype (control) or P1D6 (10μg/ml) antibodies. Cell density was assessed using Chalkley score analysis.

Figure S2

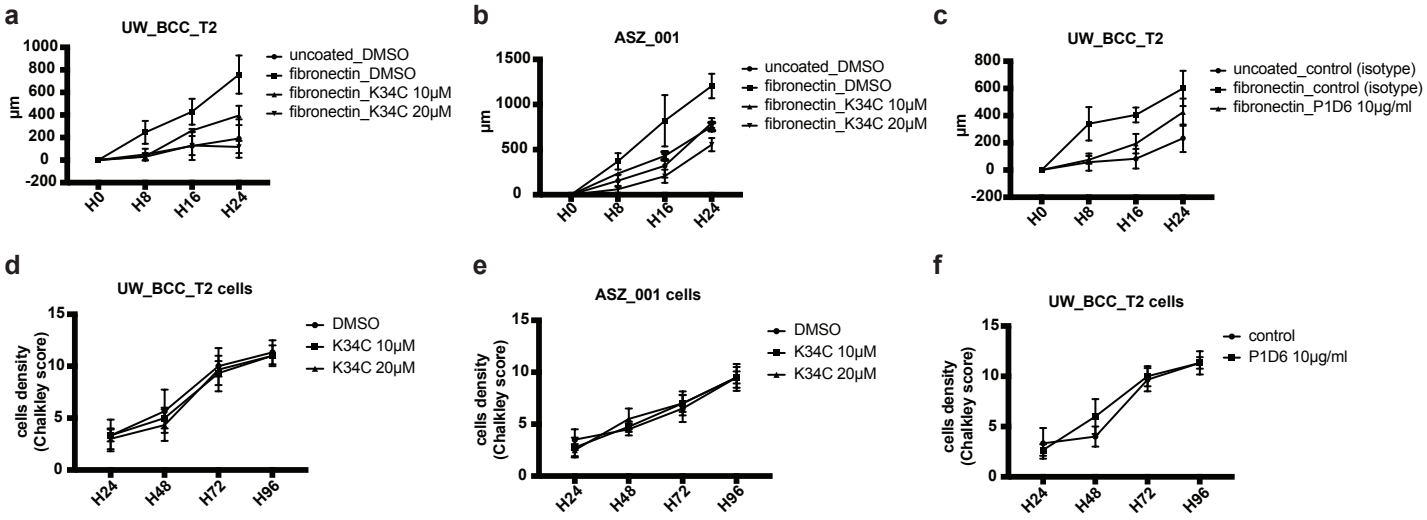

a
UW_BCC_T2
µm
1000
800
600
400
200
0
-200
H0
H8
H16
H24
uncoated_DMSO
fibronectin_DMSO
fibronectin_K34C 10µM
fibronectin_K34C 20µM
b
ASZ_001
µm
1500
1000
500
0
H0
H8
H16
H24
uncoated_DMSO
fibronectin_DMSO
fibronectin_K34C 10µM
fibronectin_K34C 20µM
c
UW_BCC_T2
µm
800
600
400
200
0
-200
H0
H8
H16
H24
uncoated_control (isotype)
fibronectin_control (isotype)
fibronectin_P1D6 10µg/ml
d
UW_BCC_T2 cells
cells density (Chalkley score)
15
10
5
0
H24
H48
H72
H96
DMSO
K34C 10µM
K34C 20µM
e
ASZ_001 cells
cells density (Chalkley score)
15
10
5
0
H24
H48
H72
H96
DMSO
K34C 10µM
K34C 20µM
f
UW_BCC_T2 cells
cells density (Chalkley score)
15
10
5
0
H24
H48
H72
H96
control
P1D6 10µg/ml

Figure S3.

(a) Quantification of p-FAK(Y397) normalized to total FAK (loading control) measured by Western blot in UW_BCC_T2 and ASZ_001 when plated on poly-L-lysine (control) or fibronectin in the presence of DMSO (control) or K34C inhibitor (20 μM).

(b) Quantification of p-FAK(Y397) normalized to total FAK (loading control) measured by Western blot in UW_BCC_T2 when plated on poly-L-lysine (control) or fibronectin in the presence of isotype (control) or P1D6 (10μg/ml) antibodies.

Figure S3

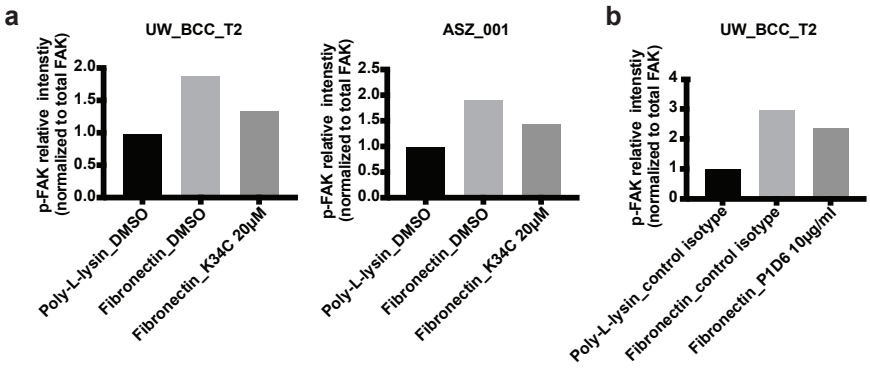

a
UW_BCC_T2
p-FAK relative intenstiy (normalized to total FAK)
2.0
1.5
1.0
0.5
0.0
Poly-L-lysin_DMSO
Fibronectin_DMSO
Fibronectin_K34C 20μM
ASZ_001
p-FAK relative intenstiy (normalized to total FAK)
2.5
2.0
1.5
1.0
0.5
0.0
Poly-L-lysin_DMSO
Fibronectin_DMSO
Fibronectin_K34C 20μM
b
UW_BCC_T2
p-FAK relative intenstiy (normalized to total FAK)
4
3
2
1
0
Poly-L-lysin_control isotype
Fibronectin_control isotype
Fibronectin_P1D6 10μg/ml

Figure S4.

(a) Western blot for total FAK and β-tubulin in UW_BCC_T2 and ASZ_001 infected with non-silencing (ns) or FAK-targeting (FAKshRNA) shRNA sequences.

(b) Quantification of total FAK normalized to β-tubulin (loading control) measured by Western blot in (a).

(c) Migration timecourse of UW_BCC_T2_ns versus UW_BCC_T2_FAKshRNA and ASZ_001_ns versus ASZ_001_FAKshRNA on uncoated versus fibronectin-coated dishes.

(d) *In vitro* growth curve of UW_BCC_T2_ns versus UW_BCC_T2_FAKshRNA and ASZ_001_ns versus ASZ_001_FAKshRNA on fibronectin. Cell density was assessed using Chalkley score analysis.

(e) *In vivo* experimental design.

(f) *In vivo* growth of UW_BCC_T2_ns/MEF versus UW_BCC_T2_FAKshRNA/MEF tumors injected either with PBS (control) or recombinant TGFβ (400ng/inj, 3x/week).

(g) *In vivo* growth of ASZ_001_ns/MEF versus ASZ_001_FAKshRNA/MEF tumors.

Horizontal bars represent the mean ± SD for n = 5 per group. *$p < 0.01$ and ***$p < 0.001$.

Figure S4

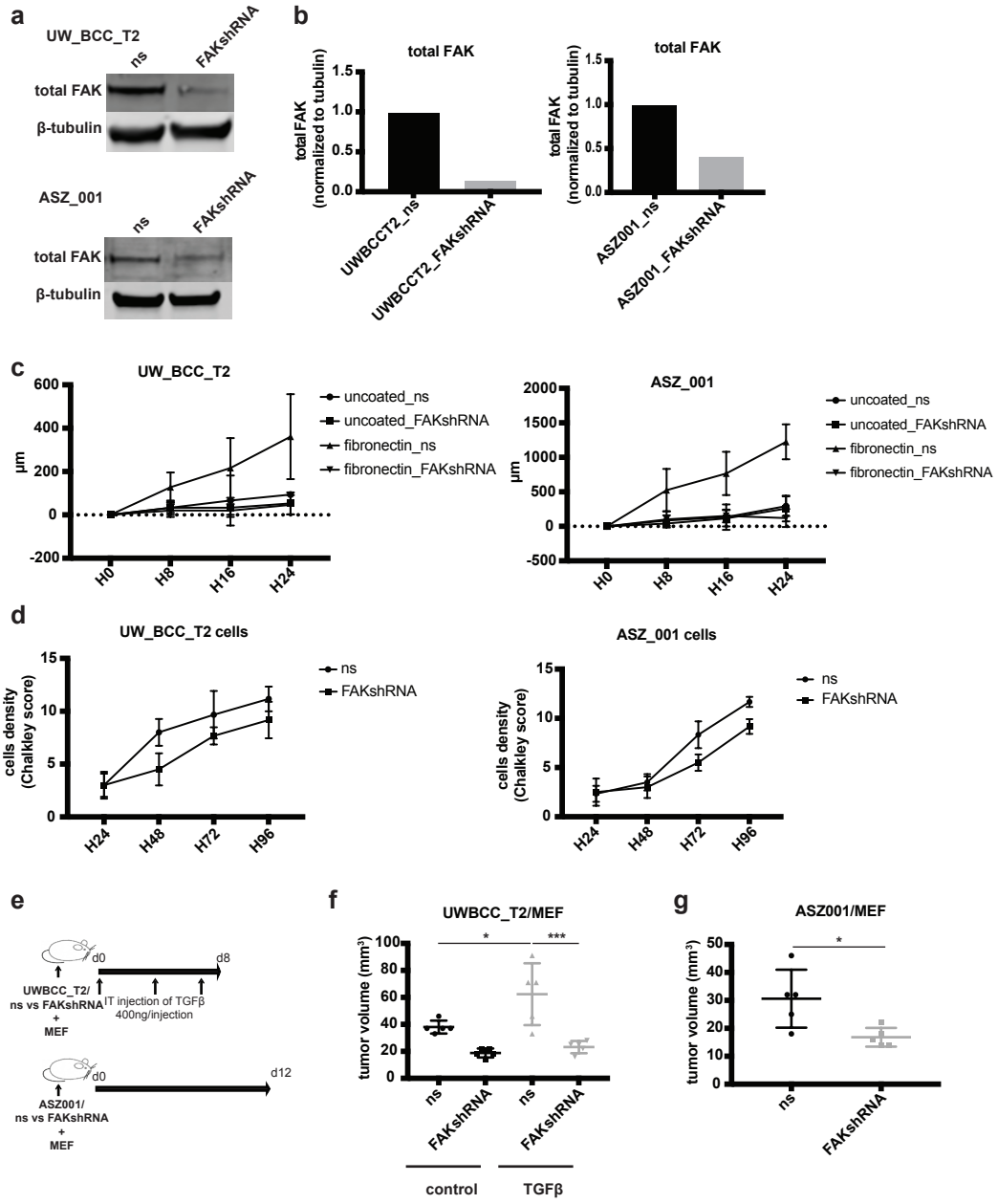

a
UW_BCC_T2
ns
FAKshRNA
total FAK
β-tubulin
ASZ_001
ns
FAKshRNA
total FAK
β-tubulin
b
total FAK
total FAK (normalized to tubulin)
1.5
1.0
0.5
0.0
UWBCCT2_ns
UWBCCT2_FAKshRNA
total FAK
total FAK (normalized to tubulin)
1.5
1.0
0.5
0.0
ASZ001_ns
ASZ001_FAKshRNA
c
UW_BCC_T2
µm
600
400
200
0
-200
H0
H8
H16
H24
uncoated_ns
uncoated_FAKshRNA
fibronectin_ns
fibronectin_FAKshRNA
ASZ_001
µm
2000
1500
1000
500
0
-500
H0
H8
H16
H24
uncoated_ns
uncoated_FAKshRNA
fibronectin_ns
fibronectin_FAKshRNA
d
UW_BCC_T2 cells
cells density (Chalkley score)
15
10
5
0
H24
H48
H72
H96
ns
FAKshRNA
ASZ_001 cells
cells density (Chalkley score)
15
10
5
0
H24
H48
H72
H96
ns
FAKshRNA
e
d0
d8
UWBCC_T2/ ns vs FAKshRNA + MEF
IT injection of TGFβ 400ng/injection
d0
d12
ASZ001/ ns vs FAKshRNA + MEF
f
UWBCC_T2/MEF
tumor volume (mm³)
100
80
60
40
20
0
*
***
ns
FAKshRNA
ns
FAKshRNA
control
TGFβ
g
ASZ001/MEF
tumor volume (mm³)
50
40
30
20
10
0
*
ns
FAKshRNA

Figure S5.

(a) Migration timecourse of UW_BCC_T2 and ASZ_001 on fibronectin when treated either with DMSO (control) or PF-562271 inhibitor (1μM and 10μM respectively).

(b) *In vitro* growth curve of UW_BCC_T2 and ASZ_001 on fibronectin when treated either with DMSO (control) or PF-562271 inhibitor (1μM and 10μM respectively). Cell density was assessed using Chalkley score analysis.

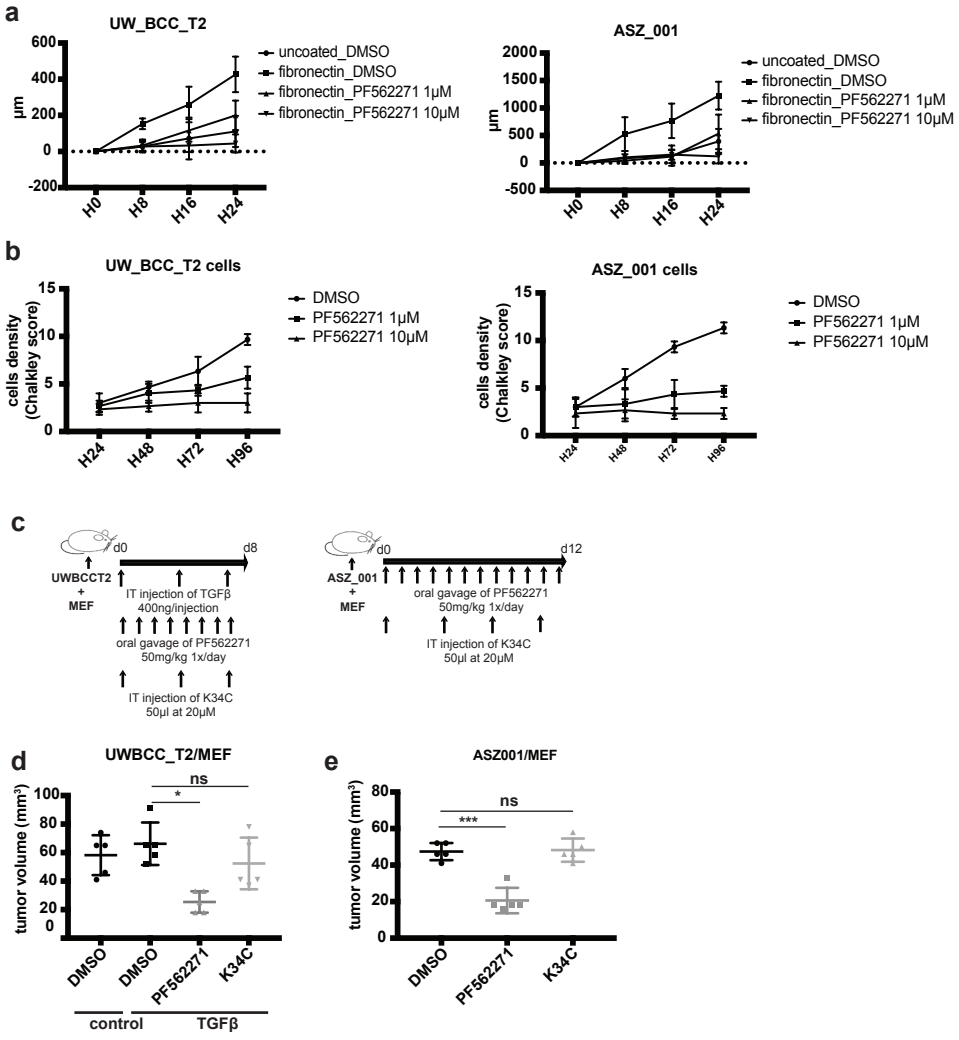
(c) *In vivo* experimental design.

(d) *In vivo* growth of UW_BCC_T2/MEF tumors injected either with PBS or recombinant TGFβ (400ng/injection, 3x/week) and simultaneously treated with either DMSO (control), PF-562271 inhibitor (50mg/kg, 1x/day) or K34C (50μl at 20μM).

(e) *In vivo* growth of ASZ_001/MEF tumors treated with either DMSO (control), PF-562271 inhibitor (50mg/kg, 1x/day) or K34C (50μl at 20μM).

Horizontal bars represent the mean ± SD for n = 5 per group. *$p < 0.01$ and ***$p < 0.001$. ns stands for non-significant.

Figure S5


a
UW_BCC_T2
µm
uncoated_DMSO
fibronectin_DMSO
fibronectin_PF562271 1µM
fibronectin_PF562271 10µM
H0
H8
H16
H24
ASZ_001
b
UW_BCC_T2 cells
cells density (Chalkley score)
DMSO
PF562271 1µM
PF562271 10µM
H24
H48
H72
H96
ASZ_001 cells
c
UWBCCT2 + MEF
d0
d8
IT injection of TGFβ 400ng/injection
oral gavage of PF562271 50mg/kg 1x/day
IT injection of K34C 50µl at 20µM
ASZ_001 + MEF
d12
d
UWBCC_T2/MEF
tumor volume (mm³)
ns
*
DMSO
PF562271
K34C
control
TGFβ
e
ASZ001/MEF
***

SUPPLEMENTARY MATERIALS AND METHODS

Antibodies. FITC-conjugated mouse monoclonal anti-integrin β1 (MEM-101A), mouse monoclonal IgG1 isotype, rabbit monoclonal anti-integrin α5 (EPR7854), rabbit monoclonal IgG isotype, mouse monoclonal anti-integrin α5 blocking antibody (P1D6), mouse monoclonal IgG3 isotype, rabbit polyclonal anti-phospho-FAK (EP2160Y), rabbit polyclonal IgG isotype, rabbit polyclonal anti-fibronectin (ab2413), mouse monoclonal anti-N-cadherin (5D5) and rabbit monoclonal anti-cleaved Caspase 3 (5A1E) antibodies were all purchased from Abcam (Cambridge, MA). Rabbit monoclonal anti-phospho-FAK (31H5L17) and rabbit monoclonal anti-Ki67 (SP6) were purchased from ThermoFisher Scientific (Waltham, MA). Rabbit polyclonal anti-E-cadherin (20874-1-AP) antibody was obtained from Proteintech (Rosemont, IL). Rabbit polyclonal anti-S100A4 (HPA007973) antibody was obtained from Sigma-Aldrich (Buchs, Switzerland). Rabbit polyclonal anti-TGFβ (3711), rabbit polyclonal anti-phospho-FAK (3283) and anti-total FAK (3285) antibodies were obtained from Cell Signaling Technology (Beverly, MA). Mouse anti-β-tubulin (E7) was purchased from Developmental Studies Hybridoma Bank (University of Iowa, IO). Goat anti-mouse AlexaFluor 546 and goat anti-rabbit Alexa 488 were purchased from BD Biosciences (Basel, Switzerland). Peroxidase anti-rabbit IgG was obtained from Vector Laboratories (Burlingame, CA).

Proliferation assay. For *in vitro* proliferation assay, cells were collected and seeded in 96-well-plates previously coated with fibronectin 0.5 mg/ml (Sigma-Aldrich, Buchs, Switzerland) at a concentration of 1000 cells/well in the absence or presence of anti-integrin α5 blocking antibody (P1D6), anti-integrin α5β1 inhibitor K34C (provided by Dr. M. Dontenwil (Ray et al., 2014)) or FAK-inhibitor PF-562271 (MedKoo Biosciences, Chapel Hill, NC) as indicated. Cells were then fixed with 4% formaldehyde and stained with 0.5% crystal violet 24, 48, 72 and 96 hours after the seeding. Cell density was assessed using a 12-

point Chalkley eyepiece under a DM2000 Leica Microsystems microscope (Wetzlar, Germany) as previously described (Kuonen et al., 2012). The number of cells hit by the graticule points was recorded. For each well, the Chalkley score was calculated as the mean value of 3 random fields.

Adhesion assay. 2,5 x10^4 cells were collected and seeded in 96-well-plates previously coated over-night with BSA 10mg/ml (Sigma-Aldrich, Buchs, Switzerland), collagen I 1 mg/ml (Sigma-Aldrich, Buchs, Switzerland), or fibronectin 0.5 mg/ml. Cells were let adhere in serum-free conditions medium for 2 hours at 37°C in the absence or presence of anti-integrin α5β1 blocking antibody P1D6, anti-integrin α5β1 inhibitor K34C or FAK-inhibitor PF-562271 as indicated. At the end of the experiment, cells were washed with PBS, fixed with 4% formaldehyde and stained with 0.5% crystal violet solution. Cell density was calculated under a DM2000 Leica Microsystems microscope (Wetzlar, Germany) as the mean of cell number counted over 3 different 100x random fields per well.

Wound healing assay. 5x10^4 cells were collected and seeded in tissue culture 96-well-plates previously coated over-night with fibronectin 0.5 mg/ml or not. At confluency, a wound was performed in each well of the plate and the healing monitored every 8 hours for 24 hours in the absence or presence of anti-integrin α5 blocking antibody P1D6, anti-integrin α5β1 inhibitor K34C or FAK-inhibitor PF-562271 as indicated. Healing progression was measured using a graduated eyepiece under a DM2000 Leica Microsystems microscope (Wetzlar, Germany). Cell migration velocity was calculated as the distance traveled over the 24 hours of monitoring (expressed in µm/hour).

Immunohistochemistry and immunofluorescence. Fibronectin (ab2413), S100A4 (HPA007973), phospho-FAK (31H5L17**)** and integrin α5 (EPR7854**)** were stained using a standard immunohistochemistry protocol. Quantification of immunostaining was performed by Chalkley counting procedure using a 12-point eyepiece graticule as described in detail

earlier (Kuonen et al., 2012). The Chalkley count for an individual tumor was taken as the mean value of the three graticule counts measuerd over a minimum of 3 different random fields. For phospho-FAK (EP2160Y**)** *in vitro* immunofluorescence, cells were cultured on coverslips previously coated with either poly-L-lysine (Invitrogen, Basel, Switzerland) or fibronectin 0.5mg/ml. DAPI-containing mounting medium (ThermoFisher Scientific, Waltham, MA) was used to stain nuclei. Phospho-FAK intensity was expressed as the percentage of cells showing positive membranous staining under Zeiss LSM700, Axio Observer Z.1 confocal fluorescence microscope (Carl Zeiss AG, Germany). For each slide, the mean value of 3 random fields was recorded.

Flow cytometry. For flow cytometry on BCC cell lines, tumor cells were collected using PBS containing 5mM EDTA and filtered to form single-cell suspensions. Staining and acquisition were done as described previously (Kuonen et al., 2012). All samples were acquired with a 4-color FACS Calibur flow cytometer from BD Biosciences (San Jose, CA) and data analyzed using FlowJo LLC software (Ashland, OR).

Real-time quantitative PCR. RNA samples were obtained from adherent cells using RNeasy kit from QIAGEN (Basel, Switzerland) according to manufacturer's instructions. For *ex vivo* RNA extraction, fresh-frozen tumors were mechanically disrupted. RNA extraction was then performed using RNeasy kit from QIAGEN (Basel, Switzerland) according to manufacturer's instructions. Quantitative real-time PCR was performed using TaqMan assay human-specific or mouse-specific primers obtained from ThermoFisher Scientific (Waltham, MA**)**. Each reaction was performed in triplicate and values were normalized to GAPDH.

Western blotting. Total cell lysates were resolved by SDS_PAGE and blotted onto a nitrocellulose membrane (Whatman, 0.45 µm, Sigma-Aldrich, Buchs, Switzerland). Membranes were blocked for 30 min at room temperature (RT) with 5% BSA blocking buffer, and incubated with a monoclonal anti-p-FAK (1:1000; 3283, Cell Signaling

Technology), anti-total FAK (1:1000; 3285, Cell Signaling Technology), anti-TGFβ (1 :1000 ; 3711, Cell Signaling Technology) and anti-β-tubulin (1 :2000 ; E7, DSHB) antibodies overnight at 4°C. Membranes were visualized using Li-Cor system (ImageStudioLite software, Lincoln, OR). Signal intensity was quantified using ImageJ software (NIH; Bethesda, MA).

Lentiviral constructs. Murine TGFβ1 silencing, murine and human FAK silencing and non-silencing shRNA sequences cloned into the pLKO.1-puro vector were purchased from Sigma-Aldrich (Buchs, Switzerland). All sequences are available online (www.sigmaaldrich.com). Lentiviruses were produced in 293T cells using Lipofectamine LTX and Plus protocol (ThermoFisher Scientific, Waltham, MA) by co-transfecting the pLKO.1-puro constructs with pMD2G and psPAX2 plasmids. Infection of targeted cells was done by 1 hour centrifugation in the presence of 8μg/ml polybrene. Selection was started 48 hours after infection using 5μg/ml puromycin (Sigma-Aldrich, Buchs, Switzerland).

TGFβ level in human samples. For the quantification of TGFβ in human tumors, fresh-frozen tumors were first mechanically disrupted in Tissue Protein Extraction reagent from ThermoFisher Scientific (Waltham, MA). After centrifugation, supernatants were collected and TGFβ levels quantified using a cytometric bead array kit for human TGFβ1 from BD Biosciences (San Jose, CA) following the manufacturer's instructions. TGFβ levels were normalized to the sample weight and expressed as pg/mg of sample.