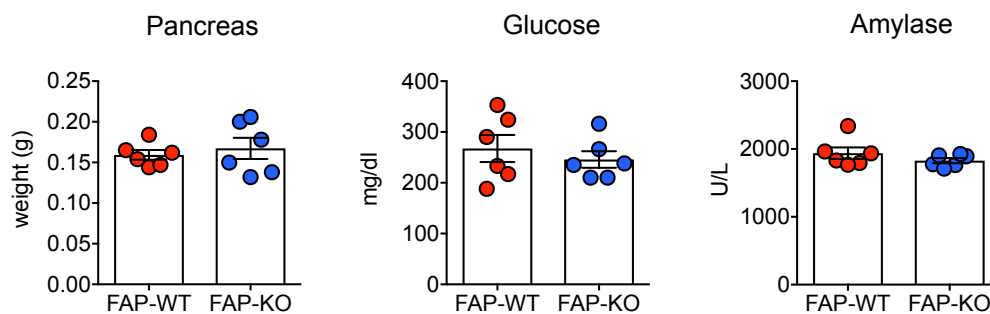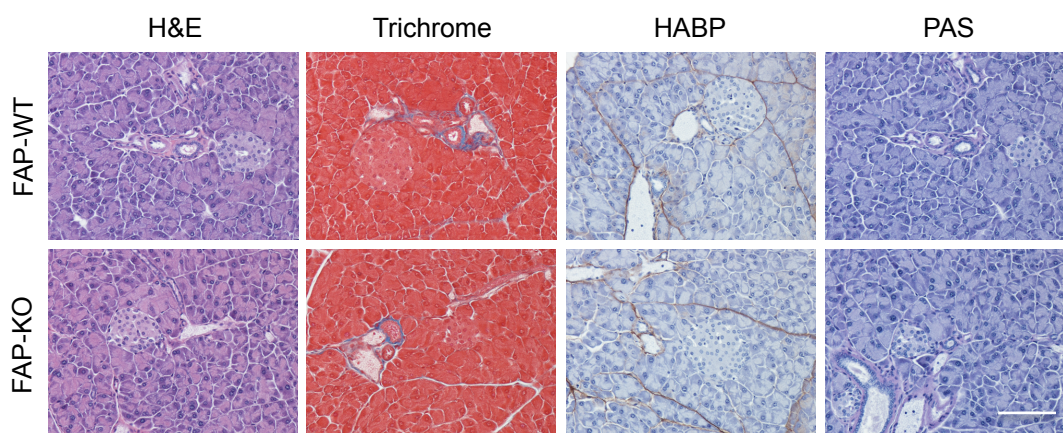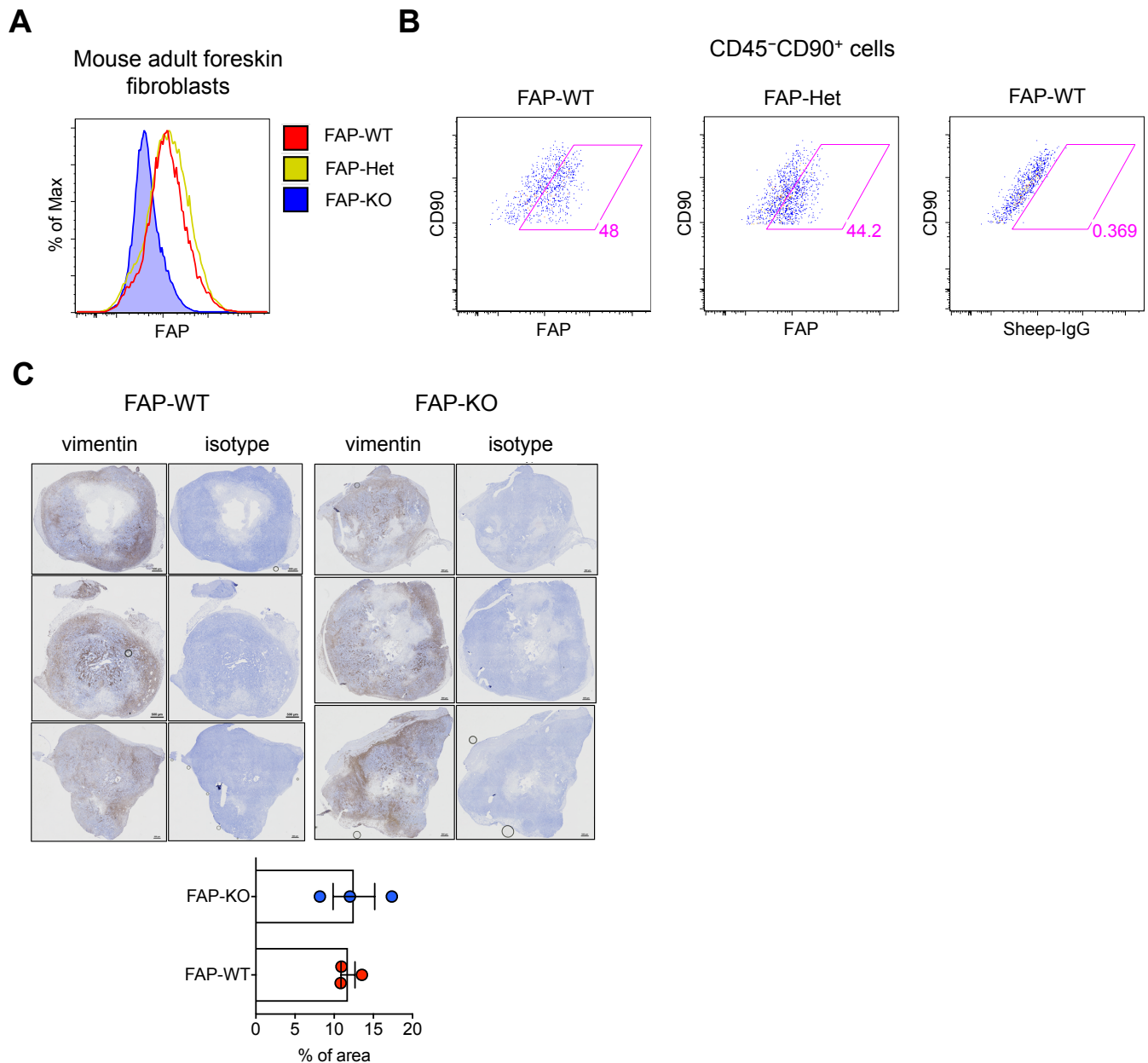# Supplemental Figures

A

B

Supplemental Figure 1. FAP is dispensable for pancreatic development.

(A) The pancreas was harvested from FAP-WT and FAP-KO C57BL/6 mice and weighed. Serum glucose and amylase were measured. Results are shown as mean ± SEM (n=6).

(B) Representative hematoxylin and eosin (H&E), trichrome, hyaluronan-binding peptide (HABP) and periodic acid–Schiff (PAS)-stained pancreas from FAP-WT and FAP-KO C57BL/ 6 mice; scale: 100 μm. No significant difference was found.

A Mouse adult foreskin fibroblasts

% of Max — FAP

FAP-WT
FAP-Het
FAP-KO

B CD45⁻CD90⁺ cells

FAP-WT — CD90 / FAP — 48

FAP-Het — CD90 / FAP — 44.2

FAP-WT — CD90 / Sheep-IgG — 0.369

C FAP-WT — vimentin / isotype FAP-KO — vimentin / isotype

FAP-KO
FAP-WT

0 5 10 15 20
% of area

Supplemental Figure 2. Expression of FAP is haplosufficient and its genetic deletion does not result in stromal cell ablation in pancreatic tumors.

(A) Analysis of FAP expression in mouse adult fibroblasts isolated from FAP-wild type (FAP-WT), FAP-heterozygous (FAP-Het) or FAP-knockout (FAP-KO) C57BL/6 mice. FAP-WT and FAP-Het fibroblasts exhibited similar FAP expression.

(B) Analysis of FAP expression in orthotopically implanted PanO2 PDA cells in FAP-WT or FAP-Het C57BL/6 mice. Intratumoral CD45⁻CD90⁺ cells from FAP-WT and FAP-Het mice showed equivalent FAP expression.

(C) Syngeneic KPC tumor cells were implanted subcutaneously in FAP-WT and FAP-KO mice and harvested for vimentin staining to evaluate stromal cell content. scale: 500 μm. Results are shown as mean ± SEM (n=3). No significant difference was found.
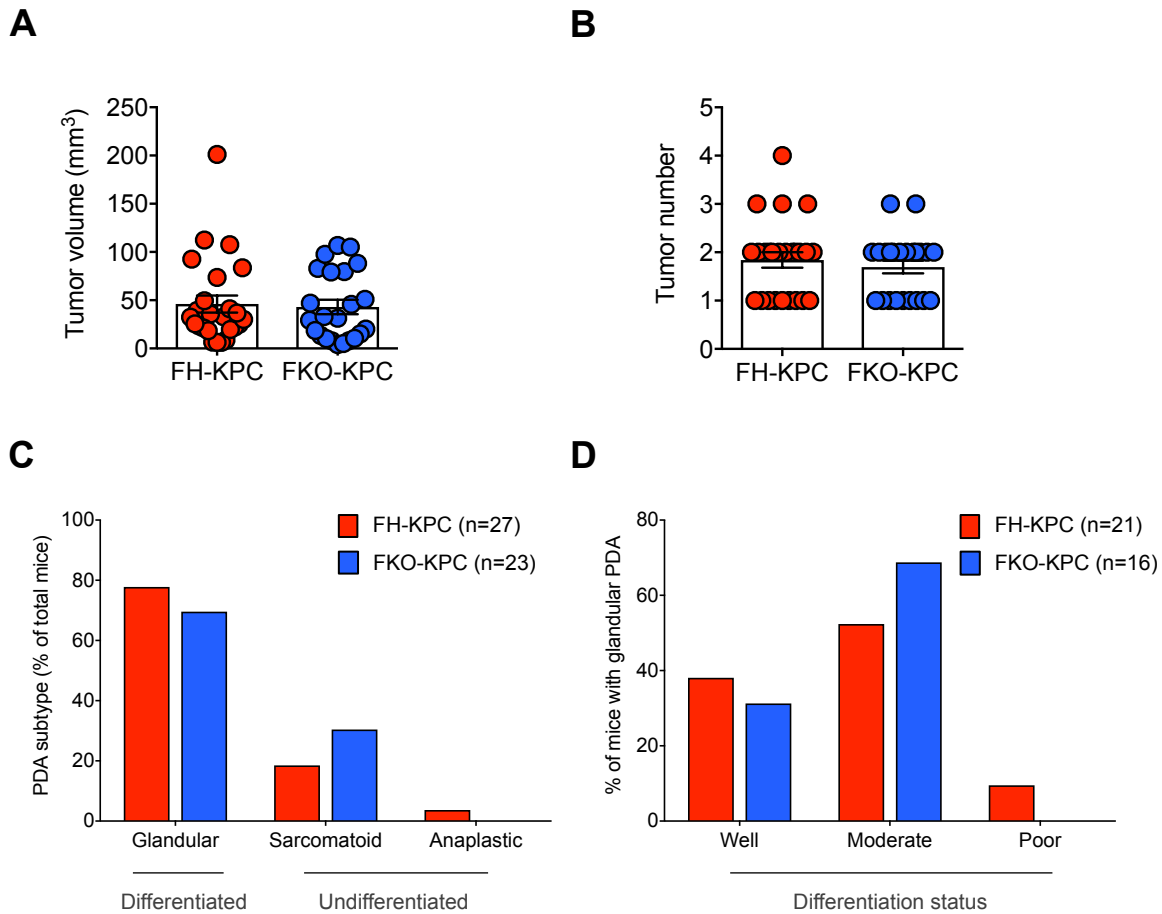
Supplemental Figure 3. Targeting FAP does not impact the size, number, subtypes or differentiation of pancreatic tumors in the KPC mouse model.

(A) FH-KPC and FKO-KPC mice with PDAs were digitally visualized and reconstructed to determine tumor volumes using the Integrated Vevo Workstation software package.

(B) The number of PDAs from FH-KPC and FKO-KPC mice was assessed during ultrasound scanning. Data shown in (A) and (B) represent mean ± SEM (FH-KPC, n=25; FKO-KPC, n=23). No statistical significance was found in tumor volume or tumor number by Student's t-test.

(C) PDAs derived from FH-KPC and FKO-KPC mice exhibited a mix of histological phenotypes (subtypes): glandular type, sarcomatoid type and anaplastic type. The predominant histology of each tumor was defined by the presence of more than 50% of a subtype within each sample as determined by a board-certified pathologist (ELB).

(D) The differentiation status of the glandular PDA was further evaluated and scored. No statistical significance was found by chi-square test in tumor subtypes or differentiation.

Supplemental Table 1. Summary of clinicopathological features of primary pancreatic ductal carcinoma from 121 patients analyzed for FAP expression.

Characteristics		Patient number	(%)
Age (years)			
	<60	29	24
	>60	92	76
Sex			
	Male	82	67.8
	Female	39	32.2
Alcohol			
	No	86	71.1
	Yes	35	28.9
Smoking			
	No	82	67.8
	Yes	39	32.2
pT(primary tumor)			
	pT1	3	2.5
	pT2	24	19.8
	pT3	87	71.9
	pT4	7	5.8
pN(regional lymph node metastasis)			
	pN0	52	43
	pN1	69	57
pM(distant lymph node metastasis)			
	pM0	120	99.2
	pM1	1	0.8
Recurrence			
	No	0	0
	Yes	121	100
Microvessel invasion			
	No	111	91.7
	Yes	10	8.3

Supplemental Table 2. Relationship between FAP expression and clinicopathological factors in primary pancreatic ductal carcinomas.

| Characteristics | FAP expression | | *P*-value |
	Low (0, 1) n=28	High (2, 3) n=93	
Age			0.7305[a]
Years (mean ± SD)	67.3 ± 13.4	66.4 ± 12.2	
Sex			1.000[b]
Male	19	63	
Female	9	30	
Alcohol status			0.6013[b]
No	21	65	
Yes	7	28	
Smoking status			0.0634[b]
No	23	59	
Yes	5	34	
Tumor size			0.7979[a]
Centimeter (mean ± SD)	3.04 ± 1.06	3.1 ± 1.43	
Stage			0.1189[b]
I	4	9	
II	23	77	
III	0	7	
IV	1	0	
			0.4602[b]
I + II	27	86	
III + IV	1	7	
Tumor status			0.4278[b]
T1	1	2	
T2	7	17	
T3	20	67	
T4	0	7	
			0.3643[b]
T1 + T2	8	19	
T3 + T4	20	74	
Lymph node status			1.000[b]
N0	12	40	
N1	16	53	
Distal metastasis status			0.2314[b]
M0	27	93	
M1	1	0	
Recurrence status			-
No	0	0	
Yes	28	93	
Microvessel invasion status			0.5913[b]
No	25	86	
Yes	3	7	

Student's t test and Pearson chi-square test were performed for continuous variables and categorical variables, respectively. The tumor stage, tumor, lymph node, and distal metastasis status were classified according to the international system for staging pancreatic cancer. SD represents standard deviation. *P value < 0.05 was considered statistically significant.

[a]denotes Student's t test
[b]Chi-square test

Supplemental Table 3. COX univariate regression and COX multiple regression analysis of prognostic factors for overall and disease-free survival in 121 patients with pancreatic ductal carcinoma.

Cox univariate regression (Overall survival)			
Variables	Comparison	Hazard Ratio (95% CI)	P-value
T	T2-4; T1	1.555 (0.493-4.902)	0.451
N	N1; N0	1.088 (0.757-1.536)	0.649
M	M1; M0	29.412 (3.300-250.00)	0.002[*]
FAP	High (2,3); Low (0,1)	1.818 (1.181-2.801)	0.007[*]
Cox multiple regression (Overall survival)			
Variables	Comparison	HR (95% CI)	P-value
T	T2-4; T1	1.395 (0.439-4.444)	0.573
N	N1; N0	1.013 (0.702-1.462)	0.943
M	M1; M0	50.000 (5.236-500.00)	0.001[*]
FAP	High (2,3); Low (0,1)	1.876 (1.211-2.915)	0.005[*]
Cox univariate regression (Disease-free survival)			
Variables	Comparison	HR (95% CI)	P-value
T	T2-4; T1	2.058 (0.650-6.536)	0.220
N	N1; N0	1.148 (0.794-1.658)	0.464
M	M1; M0	4.695 (0.635-34.483)	0.130
FAP	High (2,3); Low (0,1)	3.021 (1.818-5.025)	<0.001[*]
Cox multiple regression (Disease-free survival)			
Variables	Comparison	HR (95% CI)	P-value
T	T2-4; T1	1.873 (0.584-5.988)	0.291
N	N1; N0	1.058 (0.729-1.536)	0.766
M	M1; M0	12.048 (1.524-90.909)	0.018[*]
FAP	High (2,3); Low (0,1)	3.165 (1.883-5.319)	<0.001[*]