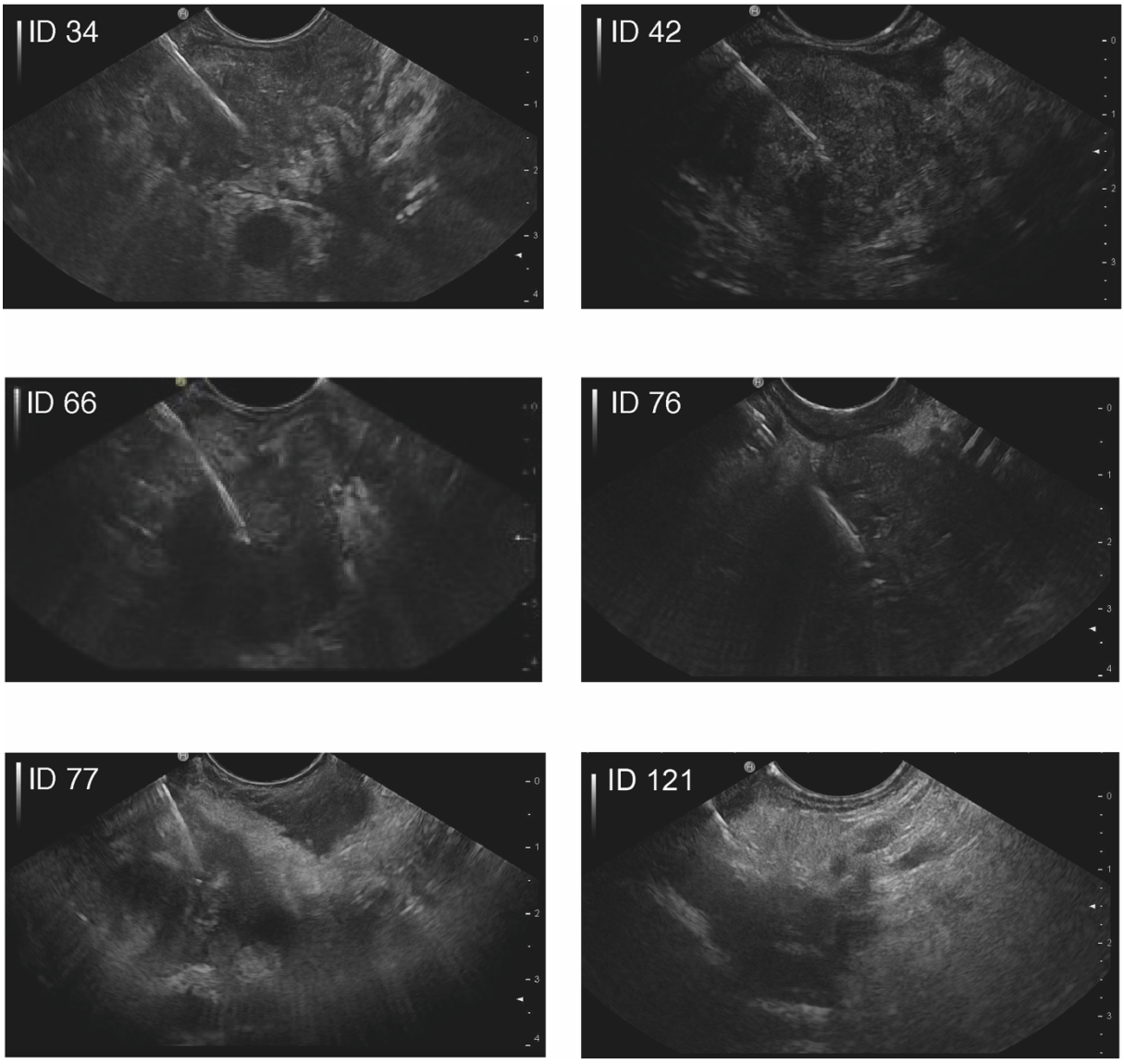
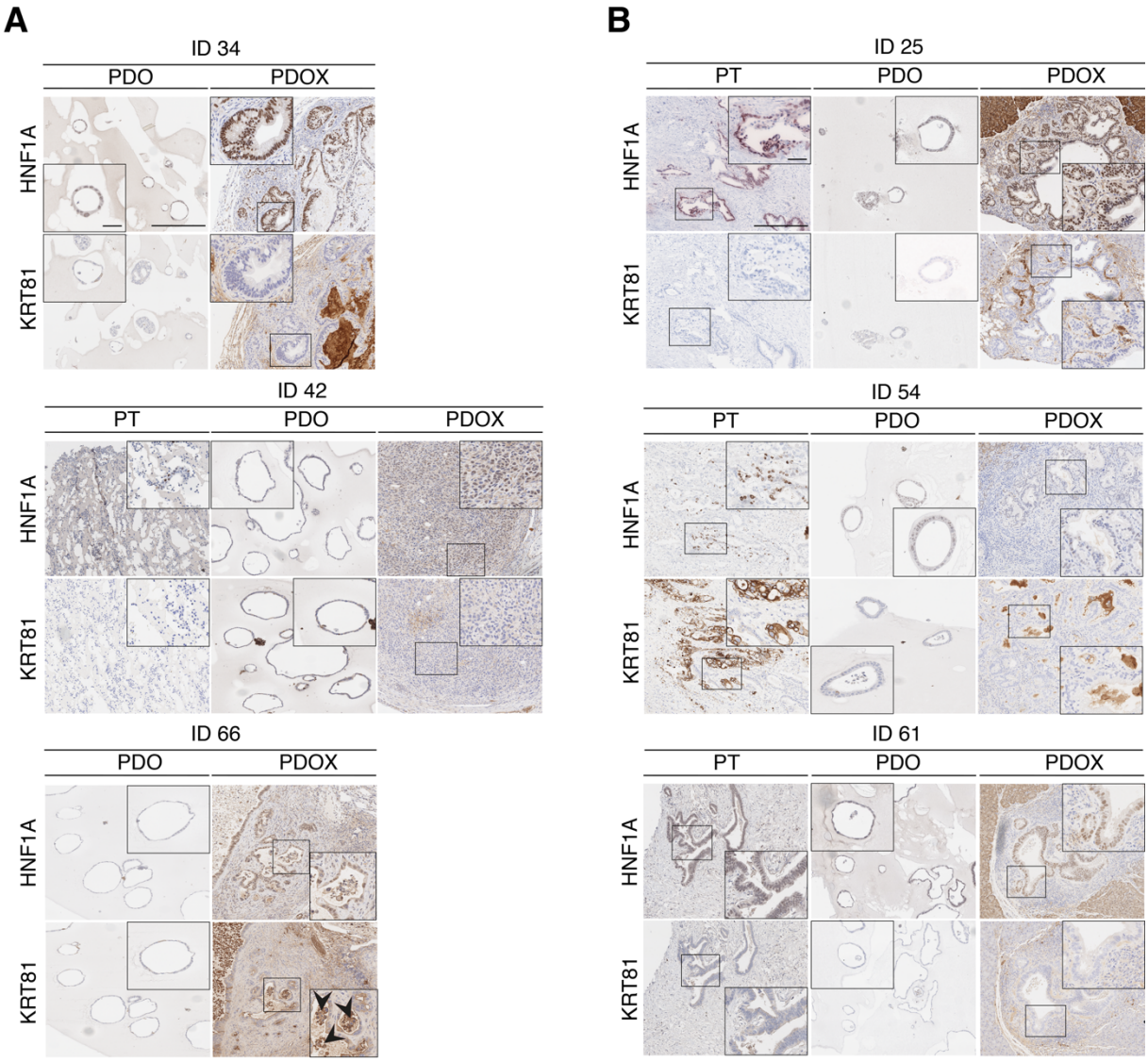
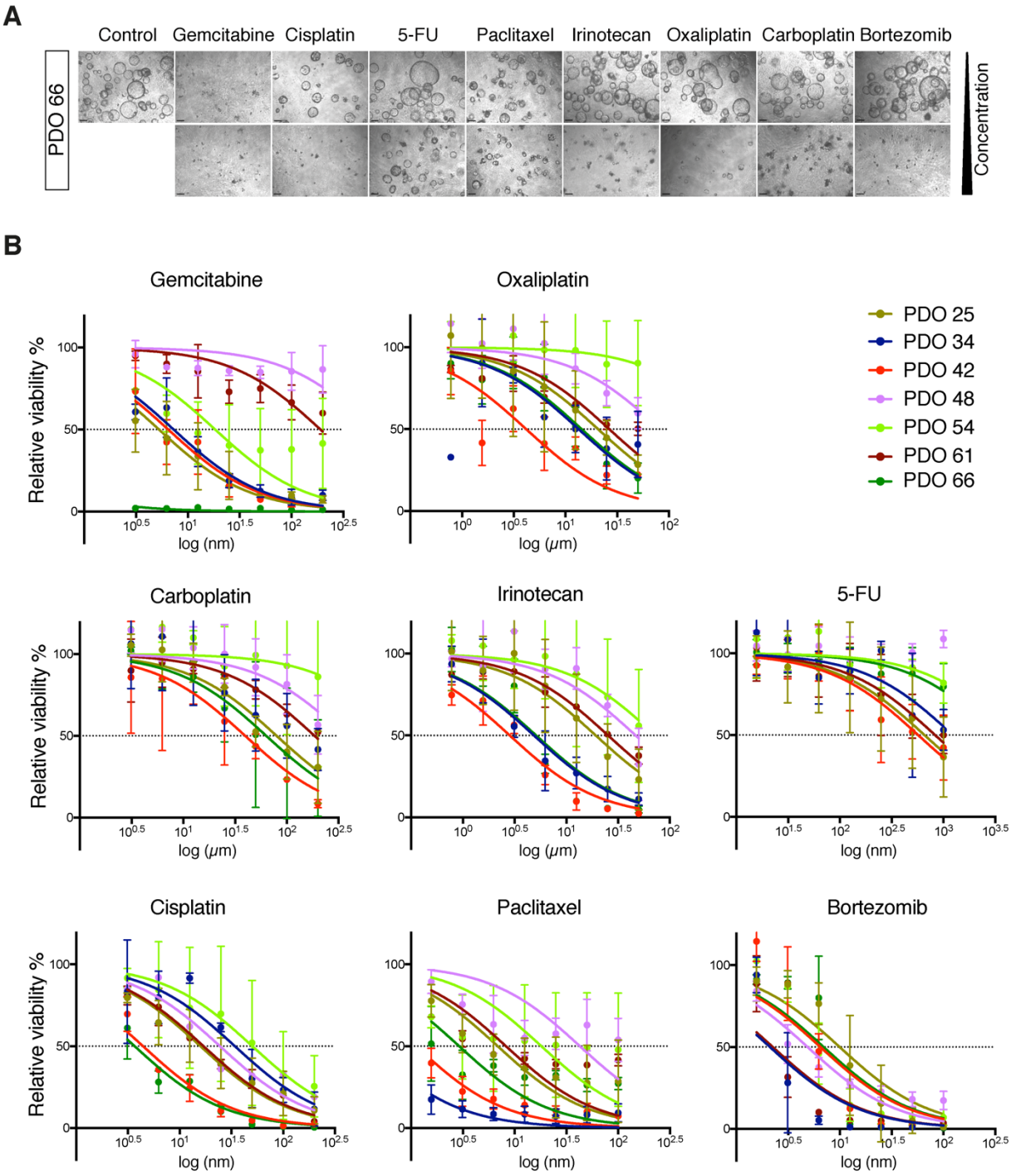


Supplemental Figure 1. Endoscopic ultrasound (EUS) images of pancreatic mass and biopsy needle of indicated patients.



Supplemental Figure 2. Representative images of Immunohistochemical subtyping of EUS-FNA PDOs (n = 3; ID 34, 42, 66) and (B) Surgical resection PDOs (n = 3; ID 25, 54 and 61 , PT and corresponding PDOX based on HNF1A+ (exocrine-like), KRT81+ (quasi-mesenchymal; QM-like) and double-negative (classical-like). Arrow indicates intraluminal cell debris. Scale bars represent 300 μm for the main image and 60 μm for the inset image.



Supplemental Figure 3. (A) Brightfield microscopy of untreated PDOs (ID 66) as well as PDOs treated with the lowest concentration (upper panel) and PDOs treated with the highest concentration (lower panel) of indicated drugs. Scale bars represent 50 µm. (B) Dose-response curves of PDO lines from both resected samples and FNAs (n = 7) with the indicated drugs. Data are represented as mean ± SEM.

Supplemental Table 1. Summary of patients with endoscopy ultrasound (EUS)-guided fine needle aspiration (FNA) for initial diagnosis and PDO generation, summary of PDO-derived xenograft (PDOXs) tumors and corresponding clinical data (n.a.: not available, Res.: Resection).

PDO-ID	EUS-FNA/Res.	Needle	Histopathology/ Cytology	Metastasis (Patient)	Therapy	Number of cells/ transplantation	Metastasis (PDOX)
34	EUS-FNA	22G	Negative	No	Gemcitabine	500000	No
42	EUS-FNA	22G	Positive (5% dysplastic cells)	V. linealis	Gemcitabine and nab-Paclitaxel	500000	Yes (liver)
66	EUS-FNA	22G	Negative	No	n.a.	500000	No
76	EUS-FNA	20G	Suspicious	No	Surgery/ Gemcitabine and nab-Paclitaxel	n.a.	n.a.
77	EUS-FNA	20G	Suspicious	Liver	Surgery/ Gemcitabine and nab-Paclitaxel	n.a.	n.a.
121	EUS-FNA	22G	Positive	Liver	Best Supportive Care	n.a.	n.a.
25	Res.	-	pT3, pN1 (4/27), L1, PN1, G2, R0	No	Surgery/ Gemcitabine	500000	No
48	Res.	-	pT2, pN2 (9/28), L1, V1, Pn1, G2, R0	No	Surgery/ Gemcitabine	n.a.	n.a.
54	Res.	-	pT3b, pN1 (2/38), Pn1, L1, G2, R0	No	Surgery/ Gemcitabine	300000	No
61	Res.	-	n.a.	n.a.	n.a.	500000	No

Supplemental Table 2. Criteria for PDAC subtyping by immunohistochemistry

Subtype	
double-negative subtype	HFN1A negative or slight; KRT81 ≤30% positive tumor cells
KRT81-positive subtype	HFN1A negative or slight; KRT81 >30% positive tumor cells
HNF1A-positive subtype	HFN1A moderate or strong; KRT81 ≤30% positive tumor cells
unclassifiable (u.c.)	HFN1A moderate or strong; KRT81 >30% positive tumor cells

Intensity(int)	
0	Negative
1	Slight
2	Moderate
3	Strong

Supplemental Table 3. Summary of PDO characterization (n.a.: not available, Pos.: positive, Neg,: negative, Sus.: suspicious).

PDO ID	Age	Gender	Resection/FNA	Pathology	PDO subtyping (IHC)	PDOX subtyping (IHC)	DNA sequencing	Normal tissue	Tumor tissue (TT)	Supernatant analyzed	*KRAS* mutational-status
25	63	F	Res.	Pos.	HNF1+	HNF1+	WXS	Muscle	n.a.	ddPCR/NGS	G12V
34	83	F	FNA	Neg.	HNF1+	HNF1+	WXS and panel-seq	n.a.	n.a.	ddPCR/NGS	G12D
42	81	F	FNA	Pos.	KRT81+	HNF1+	WXS and panel-seq	n.a.	Microdissection (LCM)	ddPCR/NGS	G12V
48	83	F	Res.	Pos.	n.a.	n.a.	WXS	Pancreas	TT	ddPCR/NGS	G12D
54	78	M	Res.	Pos.	Double -	Double -	WXS	Pancreas	TT	ddPCR/NGS	G12D
61	n.a	n.a.	Res.	Pos.	Double -	HNF1+	WXS	Blood	n.a.	ddPCR/NGS	G12D
66	75	M	FNA	Neg.	Double -	HNF1+	n.a.	n.a.	n.a.	n.a.	G12D
76	68	F	FNA	Sus.	n.a.	n.a.	panel-seq	n.a.	Macrodissection	n.a.	G12D
77	74	M	FNA	Sus.	n.a.	n.a.	panel-seq	n.a.	Macrodissection	ddPCR/NGS	G12V
121	76	M	FNA	Pos.	n.a.	n.a.	WXS	n.a.	n.a.	ddPCR/NGS	G12V

Supplemental Table 4. Summary of type of mutations in Patient-derived Organoids (PDO), Primary Tumor (PT), Patient-derived organoid xenografts (PDOX) by NGS. n.a.: not available.

PDO-Nr	Gene	Acc-Nr	PDO (WXS)	PDO (panel seq.)	PDO-SN (panel seq.)	PDO-SN (ddPCR)	Primary tumor (FNA/Res./tumor tissue)	PDOX (panel seq.)
PDO 25	*KRAS*	NM_004985	c.35G>T, p.G12V (40%)	not available	c.35G>T, p.G12V (54%)	*KRAS* G12V(52%)	not available	c.35G>T, p.G12V (14%)
	TP53	NM_000546	c.637C>T, p.R213* (94%)	not available	c.637C>T, p.R213* (86%)	not applicable	not available	c.637C>T, p.R213* (24%)
	ITGA9	NM_002207	not present	not available	not present	not applicable	not available	c.199G>A, p.A67T (24%)
	CDKN2A	NM_000077	Homozygous deletion	not available	Homozygous deletion	not applicable	not available	Homozygous deletion
PDO 34	*KRAS*	NM_004985	c.35G>A, p.G21D (100%)	c.35G>A, p.G12D (100%)	c.35G>A, p.G12D (93%)	*KRAS* G12D (96%)	not available	c.35G>A, p.G12D (99.7%)
	TP53	NM_000546	c.740A>T, p.N247I (99%)	c.740A>T, p.N247I(100%)	c.740A>T, p.N247I(95%)	not applicable	not available	c.740A>T, p.N247I (99.1%)
	SMAD4	NM_005359	c.1586delT, p.L529Yfs*8 (96%)	c.1586delT, p.L529Yfs*8 (100%)	c.1586delT, p.L529Yfs*8 (100%)	not applicable	not available	c.1586delT;p.L529Yfs*8 (99%)
	CDKN2A	NM_000077	c.132C>G, p.Y44* (99%)	c.132C>G, p.Y44* (100%)	not present	not applicable	not available	c.132C>G, p.Y44* (100%)
	TRRAP	NM_003496	c.5131A>G, p.M1711V (48%)	c.5131A>G, p.M1711V (40%)	c.5131A>G, p.M1711V (40%)	not applicable	not available	c.5131A>G, p.M1711V (40%)
PDO 42	*KRAS*	NM_004985	c.35G>T, p.G12V (60%)	c.35G>T, p.G12V (50%)	c.35G>T, p.G12V (44%)	*KRAS* G12V (39%)	c.35G>T, p.G12V (38%)	not available
	JAK1	NM_002227	c.2050G>C, p.D684H (28%)	c.2050G>C, p.D684H (36.4%)	c.2050G>C, p.D684H (44.4%)	not applicable	c.2050G>C, p.D684H (15.2%)	not available
	CDKN2A	NM_000077	c.47_50delTGGC, p.L16Pfs*9 (98%)	c.47_50delTGGC, p.L16Pfs*9 (100%)	c.47_50delTGGC, p.L16Pfs*9 (75%)	not applicable	not present	not available
	TP53	NM_000546	c.352G>T, p.V118F (98%)	c.469G>T, p.V157F (100%)	c.469G>T, p.V157F (98%)	not applicable	c.469G>T, p.V157F (40%)	not available
	ZNF521	NM_015461	c.1078G>C, p.D360H (82%)	c.1078G>C, p.D360H (56.7%)	c.1078G>C, p.D360H (81.8%)	not applicable	c.1078G>C, p.D360H (53.2%)	not available
	ZNF521	NM_015461	c.1992G>C, p.L664F (75%)	c.1992G>C, p.L664F (68.2%)	c.1992G>C, p.L664F (75.3%)	not applicable	c.1992G>C, p.L664F (53.9%)	not available
	KDM6A	NM_021140	c.2049delC, p.T684Pfs*7(97%)	c.2049delC, p.T684Pfs*7 (100%)	c.2049delC, p.T684Pfs*7 (not analyzable)	not applicable	c.2049delC, p.T684Pfs*7 (36.3%)	not available
	MYC	NM_002467	gene amplification (10 gene copies)	gene amplification (>20 gene copies)	gene amplification (>20 gene copies)	not applicable	not present	not available
PDO 48	*KRAS*	NM_004985	c.35G>A, p.G12D (47%)	not available	c.35G>A, p.G12D (52%)	*KRAS* G12D (43%)	c.35G>A, p.G12D (14%)	not available
	TP53	NM_000546	c.371A>G, p.Y124C (93%)	not available	c.488A>G, p.Y163C (100%)	not applicable	c.371A>G, p.Y124C (6%)	not available
	CDKN2A	NM_000077	Homozygous deletion	not available	Homozygous deletion	not applicable	not detectable	not available
	SMAD4	NM_005359	c.1324C>T, p.Q442* (97%)	not available	not detectable	not applicable	c.1324C>T, p.Q442*(6%)	not available
PDO 54	*KRAS*	NM_004985	c.35G>A, p.G12D (49%)	not available	c.35G>A, p.G12D (47%)	*KRAS* p.G12D (48%)	c.35G>A, p.G12D (17%)	c.35G>A, p.G12D (46%)
	TP53	NM_000546	c.774dupA, p.D259Rfs*5 (95%)	not available	c.774dupA, p.D259Rfs*5 (78%)	not applicable	c.774dupA;p.D259Rfs*5 (28%)	c.774dupA;p.D259Rfs*5 (100%)
	BCL9	NM_004326	c.1829G>A, p.G610D (33%)	not available	c.1829G>A, p.G610D (50%)	not applicable	c.1829G>A, p.G610D (4%)	c.1829G>A, p.G610D (35%)
	LRP1B	NM_018557	c.4910G>A, p.G1637E (50%)	not available	c.4910G>A, p.G1637E (69%)	not applicable	c.4910G>A, p.G1637E (20%)	c.4910G>A, p.G1637E (46%)
	CDKN2A	NM_000077	Homozygous deletion	not available	Homozygous deletion	not applicable	Homozygous deletion	Homozygous deletion
PDO 61	*KRAS*	NM_004985	c.35G>A, p.G12D (42%)	not available	c.35G>A, p.G12D (50%)	*KRAS* p.G12D (40%)	not available	c.35G>A, p.G12D (50%)
	TP53	NM_000546	c.662_665dup, p.P223Afs*3 (89%)	not available	c.662_665dup, p.P223Afs*3 (95%)	not applicable	not available	c.662_665dup, p.P223Afs*3 (98%)
	CDKN2A	NM_000077	Heterozygous deletion	not available	not deleted	not applicable	not available	Heterozygous deletion
	FLT4	NM_182925	c.247G>A, p.D83N (30%)	not available	c.247G>A, p.Asp83Asn (31%)	not applicable	not available	c.247G>A, p.D83N (27%)
PDO 76	*KRAS*	NM_004985	not available	c.35G>A, p.G12D (26%)	not available	not available	c.35G>A, p.G12D (21%)	not available
	TP53	NM_000546	not available	c.395A>G, p.K132R (25%)	not available	not available	c.395A>G, p.K132R (26%)	not available
	CDKN2A	NM_000077	not available	c.126T>A , p.N42K (26%)	not available	not available	c.126T>A , p.N42K (27%)	not available
	NLRP1	NM_033004	not available	c.658A>G, p.R220G (38%)	not available	not available	c.658A>G, p.R220G (38%)	not available
PDO 77	*KRAS*	NM_004985	not available	c.35G>T, p.G12V (44%)	c.35G>T, p.G12V (43%)	*KRAS* p.G12V (45%)	c.35G>T, p.G12V (42,4%)	not available
	TP53	NM_000546	not available	c.637C>T, p.R213* (100%)	c.637C>T, p.R213* (100%)	not applicable	c.637C>T, p.R213* (39,8%)	not available
	COL1A1	NM_000088	not available	c.2420C>A, p.P807H (48.4%)	c.2420C>A, p.P807H (54%)	not applicable	c.2420C>A, p.P807H (53.9%)	not available
	KDM6A	NM_021140	not available	c.1824_1827dup, p.Q611Pfs*11 (97.7%	c.1824_1827dup, p.Q611Pfs*11 (53%)	not applicable	c.1824_1827dup, p.Q611Pfs*11 (34,7%)	not available
	JAK3	NM_000215	not available	c.2398G>T, p.D800Y (40%)	c.2398G>T, p.D800Y (45.9%)	not applicable	c.2398G>T, p.D800Y (13%)	not available
	KAT6A	NM_006766	not available	gene amplification (~8-10 gene copies	gene amplification	not applicable	gene amplification (~4-8 gene copies)	not available
	CDKN2A	NM_000077	not available	Homozygous deletion	Full gene deletion (homozygous)	not applicable	not detectable	not available
	CDKN2B	NM_004936	not available	Homozygous deletion	Full gene deletion (homozygous)	not applicable	not detectable	not available
PDO 121	*KRAS*	NM_004985	c.35G>T, p.G12V (93%)	not available	c.35G>T, p.G12V (100%)	*KRAS* G12V(88%)	not available	not available
	TP53	NM_000546	c.856G>A, p.E286K (99%)	not available	c.856G>A, p.E286K (99%)	not applicable	not available	not available
	CDKN2A	NM_000077	Homozygous deletion	not available	Homozygous deletion	not applicable	not available	not available
	SMAD4	NM_005359	c.1482_1483insT, p.L495Sfs*32 (96%)	not available	c.1482_1483insT, p.L495Sfs*32 (100%)	not applicable	not available	not available

Supplemental Table 5. Antibodies used in the manuscript.

Name	Company	Order Number	Dilution for FNAs, Primary tumors and PDOs	Dilution for PDOX
HNF1A	Santa Cruz	8986	**1:200**	**1:50**
KRT81	Santa Cruz	100929	**1:250**	**1:200**