Supplemental Figure 1. Longitudinal orientation of human PanIN and PDG. PDG (arrowheads) are found closely associated with overlying PanIN (arrows). Asterisk shows normal ductal epithelium. Modest TFF2 expression and Ki-67 positive proliferating cells can be found only in PDG but not in overlying PanIN. Scale Bars: 100μm.

Supplemental Figure 2. PDG in KC/TFF2KO mice. Compared to KC mice, KC/TFF2KO show enlarged PDG with micro-papillary changes. BrdU incorporation is high in KC/TFF2KO. Scale Bar: 50μm.

Supplemental Figure 3. RNA expression of TFF2 in HPDE and cancer cell lines (RT-PCR).

Supplemental Figure 4. TFF2 inhibit proliferation of PANC-1 cells. (A) Enforced expression of TFF2 result in the decrease of BrdU incorporation in PANC-1. (B) TFF2-transfected PANC-1 cells show almost a 3-fold decrease of BrdU incorporation.

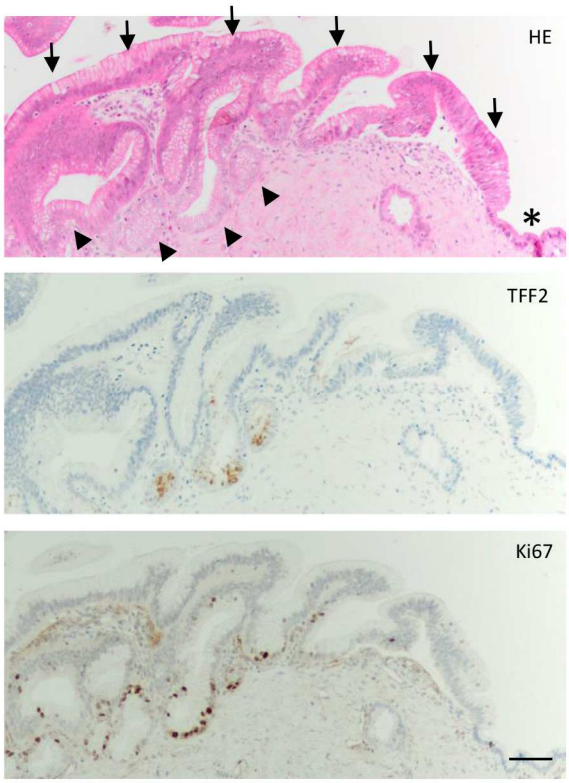
Supplemental Figure 5. (A) Western blot of other pancreatic cancer cell lines (MiaPAca-2 and colo-357) also shows TFF2 induced upregulation. (B) The upregulation of SMAD4 by TFF2 is abrogated by the co-transfection of sh-SMAD4.

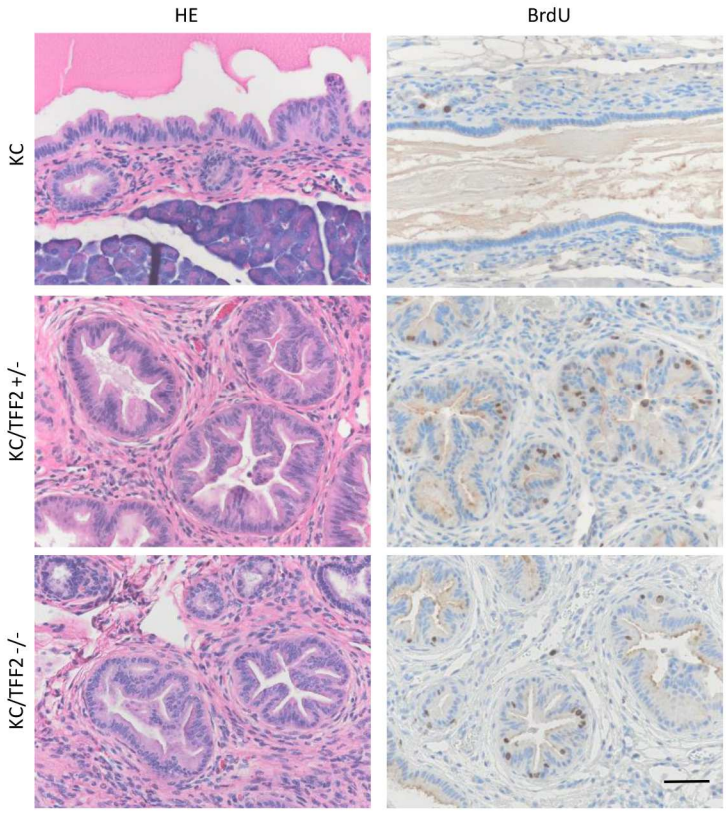
Supplemental Figure 6. Treatment of (A) PANC-1 and (B) AsPC-1 cell lines with TFF2 peptide (600nM) for 24 hours showing an upregulation of SMAD4 protein and mRNA.

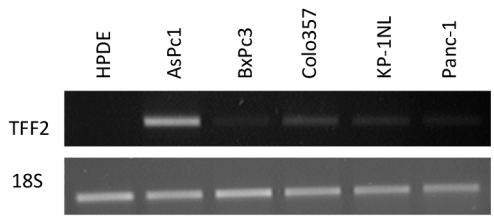
Supplemental Figure 7. Effect of TFF2 overexpression (TFF2 ox) on cell proliferation in

SMAD4 null (BxPC3, Cfpac-1) and mutated (Capan-1) PDAC cell lines.

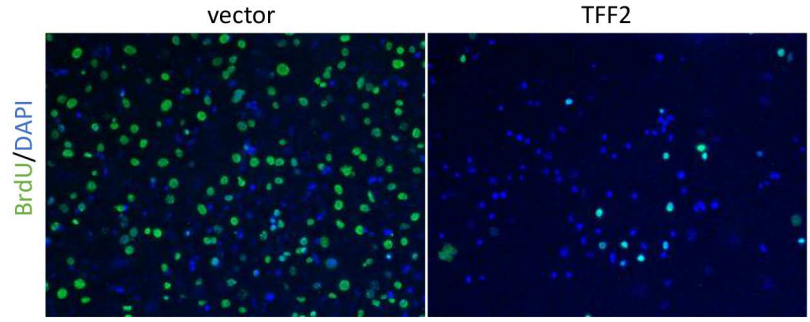
Supplemental Figure 8. TFF2 promoter methylation and SMAD4 regulation in HPDE cells. (A) TFF2 promoter DNA methylation profile of HPDE cells by pyrosequencing. Lymphocyte DNA and Sss1 methylated DNA are used as controls. (B) TFF2 overexpression in HPDE cells for 48 hours results in SMAD4 upregulation.


HE
TFF2
Ki67

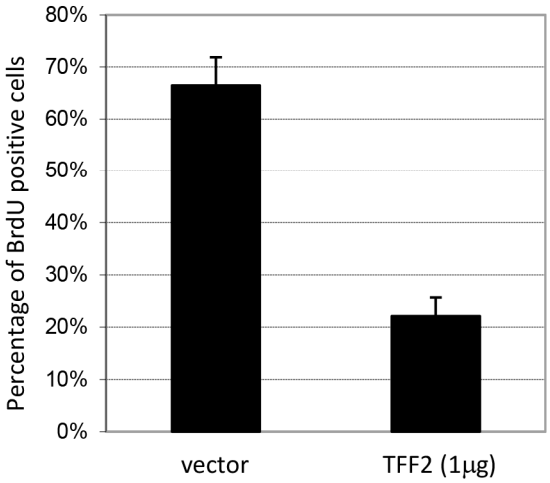

HE
BrdU
KC
KC/TFF2 +/-
KC/TFF2 -/-


HPDE
AsPc1
BxPc3
Colo357
KP-1NL
Panc-1
TFF2
18S

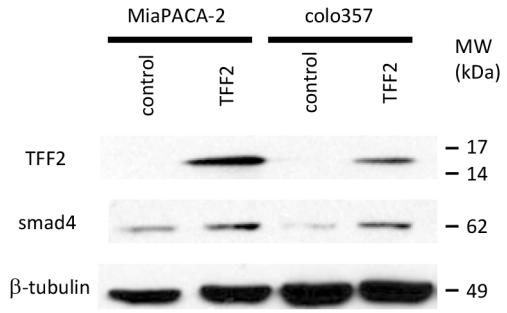
A


vector
TFF2
BrdU/DAPI

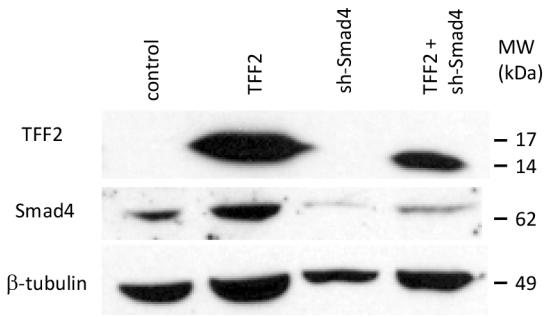
B


80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of BrdU positive cells
vector
TFF2 (1μg)

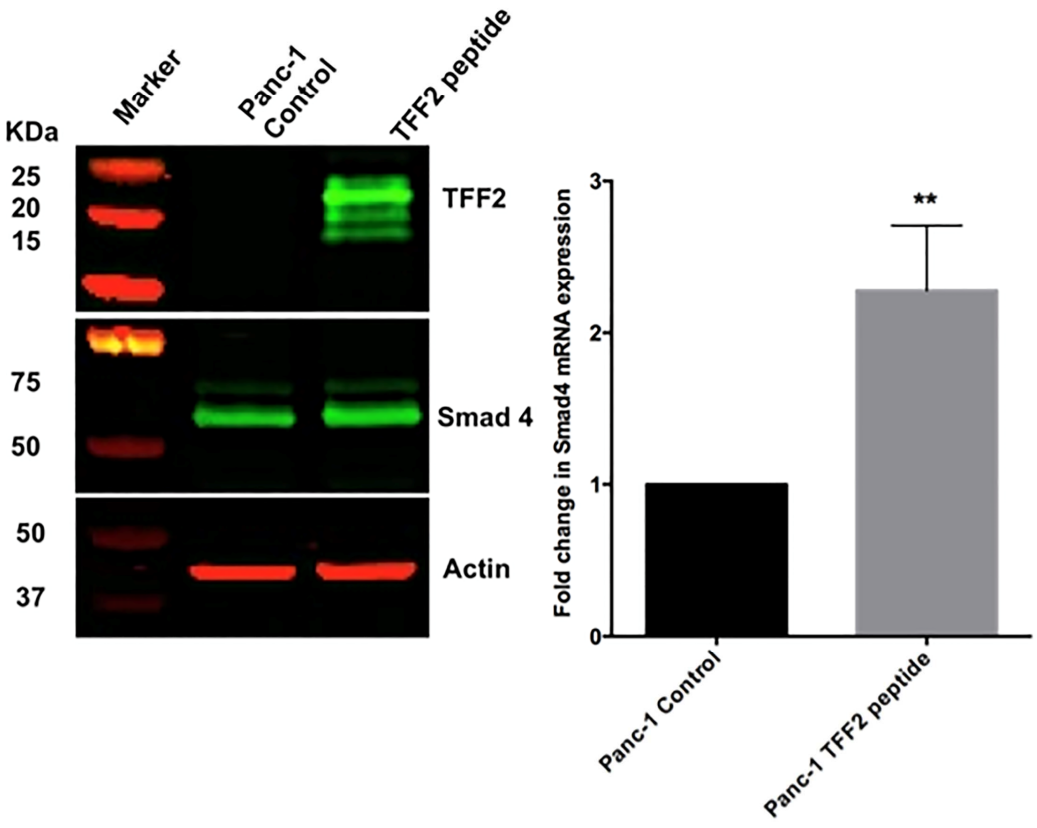
A


MiaPACA-2
colo357
control
TFF2
control
TFF2
MW
(kDa)
TFF2
17
14
smad4
62
β-tubulin
49

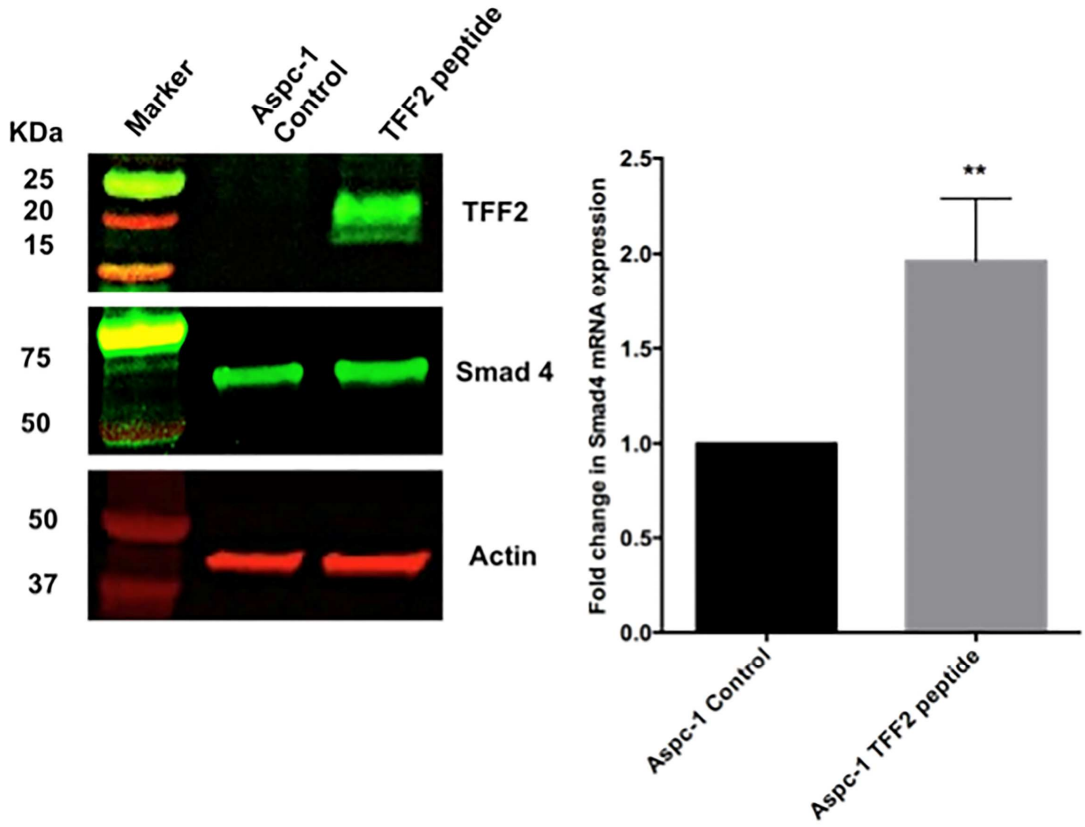
B

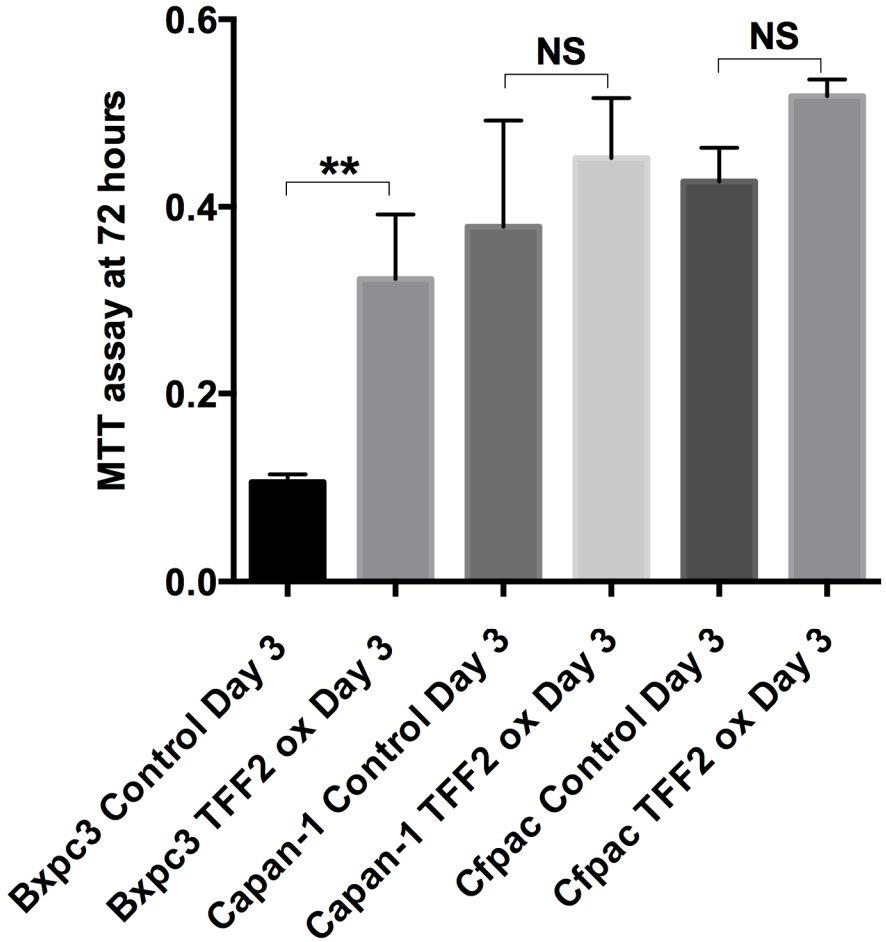

control
TFF2
sh-Smad4
TFF2 +
sh-Smad4
MW
(kDa)
TFF2
17
14
Smad4
62
β-tubulin
49

A


Marker
Panc-1 Control
TFF2 peptide
KDa
25
20
15
TFF2
75
50
Smad 4
50
37
Actin
Fold change in Smad4 mRNA expression
3
2
1
0
**
Panc-1 Control
Panc-1 TFF2 peptide

B

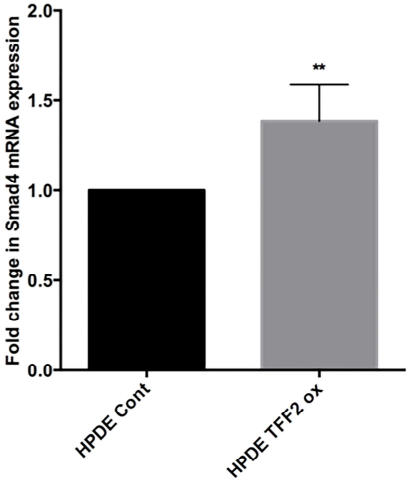

Marker
Aspc-1 Control
TFF2 peptide
KDa
25
20
15
TFF2
75
50
Smad 4
50
37
Actin
Fold change in Smad4 mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
**
Aspc-1 Control
Aspc-1 TFF2 peptide


0.6
0.4
0.2
0.0
MTT assay at 72 hours
**
NS
NS
Bxpc3 Control Day 3
Bxpc3 TFF2 ox Day 3
Capan-1 Control Day 3
Capan-1 TFF2 ox Day 3
Cfpac Control Day 3
Cfpac TFF2 ox Day 3

A

chr21:423….. (hg38)	50951	50948	50943	50926	50919
Relative to Transcription Start Site	149	152	157	174	181
Sample	% methylation				
HDPE cells	98	88	71	77	71
Lymphocyte DNA	96	96	100	92	89
Sss1 methylated DNA	98	98	100	94	93

B



Supplemental Table 1. Conditions and primary antibodies for immunohistochemistry.

Protein of Interest	Species	Antibody by	Clone	Concentration
TFF1	human	Invitrogen	BC04	1:50
TFF1	human and mouse	Abcam	ab50806	1:200
TFF2	human	Novocastra	GE16C	1:50
TFF2	human and mouse	Protein Tech		1:500
MUC2	mouse	Santa Cruz	H-300	1:400
MUC5AC	human	Novocastra	CLH2	1:200
MUC5AC	mouse	Santa Cruz	H-160	1:200
MUC6	human	Novocastra	CLH5	1:100
MUC6 (deep gastric mucin)	mouse	Dr. Ho		1:3000
Ki67	human	Dako	MIB-1	1:100
Ki67	mouse	Dako	TEC3	1:25
BrdU	mouse	Abcam	ab6326	1:100

Supplemental Table 2:　The sequences of primer sets for D-loop of mitochondrial DNA

	forward (5'-3')	reverse　(5'-3')
primer set 1	ACTCCACCATTAGCACCCAAAGC	GGGGTCATCCATGGGGACGAGA
primer set 2	AGCCACCCCTCACCCACTAGG	ATGGAGAGCTCCCGTGAGTGGT
primer set 3	TCGCTCCGGGCCCATAACACT	TGTCTGTGTGGAAAGTGGCTGTGC
primer set 4	GCCGGAGCACCCTATGTCGC	GGGCGGGGGTTGTATTGATGAGA
primer set 5	GCTTCTGGCCACAGCACTTAAACA	TGGGGTGATGTGAGCCCGTCT
primer set 6	TCTCATCAATACAACCCCCGCCCA	GAGCTGCATTGCTGCGTGCT

Supplemental Table 3: Sequencing of the primer pairs

Primer sequences for RT-PCR

Gene	TFF2
Sense Primer (5’ to 3’)	AAACCCTCCCCCTGCCAGTGC
Anti-sense Primer (5’ to 3’)	CCGAGAGGCGCATTCCTCGG
Anneal. °C	60

Gene	SMAD4
Sense Primer (5’ to 3’)	TTGCATTCCAGCCTCCCATT
Anti-sense Primer (5’ to 3’)	AAGACCGCGTGGTCACTAAG
Anneal. °C	60

Pyrosequencing primer sequences

Gene	TFF2
Sense Primer (5’ to 3’)	TGTTTGTTGGATAAATAGAGGGGAGATT
Anti-sense Primer (5’ to 3’)	TCCTTCACTTACAAAATTTCTCACTCCC
Sequencing Primer (5’ to 3’)	GTGTAGTTGAGTTAGATATGG
Anneal. °C	60

Supplemental Table 4. Summary of mutational mapping of PDG and IPMN.

Of 195 pairs of PDG and IPMN, only 7 pairs were found to be mismatched (p=0.0012).

	site	72	73	195	263	489	16223	16278	16294	16298	16311	16320	16355	16362	16519	16558
Case 1	PDG 1	T	A	C	G	T	C	C	C	C	C	C	C	T	T	G
	IPMN 1	T	A	C	G	T	C	C	C	C	C	C	T	T	C	G
	PDG 2	T	G	C	G	T	T	C	C	C	C	C	C	C	T	G
	IPMN 2	T	G	C	G	T	T	C	C	C	C	C	C	C	T	G
	PDG 3	T	G	T	G	C	T	T	C	T	T	C	C	C	T	G
	IPMN 3	T	G	T	G	C	T	T	C	T	T	C	C	C	T	G
Case 2	PDG 4	T	A	T	G	T	T	T	C	T	T	C	C	C	C	G
	IPMN 4	T	A	T	G	C	T	T	C	T	T	C	C	C	C	G
	PDG 5	T	G	T	G	T	T	T	C	T	T	C	C	C	C	G
	IPMN 5	T	G	T	G	T	T	T	C	T	T	C	C	C	C	G
	PDG 6	T	A	T	G	C	T	T	T	T	T	C	C	T	C	A
	IPMN 6	T	A	T	G	C	T	T	T	T	T	C	C	T	C	A
Case 3	PDG 7	T	G	T	G	C	C	C	C	T	T	C	C	T	T	G
	IPMN 7	T	G	T	G	C	C	C	C	T	T	C	C	T	T	G
	PDG 8	T	G	T	G	C	C	C	C	T	T	C	C	T	T	G
	IPMN 8	T	G	T	G	C	C	C	C	T	T	C	C	T	T	G
	PDG 9	T	G	T	G	C	C	C	C	T	T	C	C	T	T	G
	IPMN 9	T	A	T	A	T	C	C	C	T	T	C	C	T	T	G

Case 4	PDG 10	C	G	T	G	T	C	T	C	T	T	C	C	C	T	G
	IPMN 10	C	G	T	G	T	C	T	C	T	T	C	C	C	T	G
	PDG 11	T	G	T	G	T	C	T	C	T	T	C	C	C	T	G
	IPMN 11	T	G	T	G	T	C	T	C	T	T	C	C	C	T	G
	PDG 12	C	A	T	G	T	C	T	C	T	T	C	C	C	T	G
	IPMN 12	C	A	T	G	T	C	T	C	T	T	C	C	C	T	G
	PDG 13	C	G	T	G	T	C	C	C	T	C	C	C	C	C	G
	IPMN 13	C	G	T	G	T	C	C	C	T	T	C	C	C	C	G

Supplemental Table 5. IPMN development found in each mice

	$KRAS^{G12D}$; TFF2 +/+		$KRAS^{G12D}$; TFF2 +/-		$KRAS^{G12D}$; TFF2 -/-	
	low grade IPMN	high grade IPMN	low grade IPMN	high grade IPMN	low grade IPMN	high grade IPMN
2 m/o	0/6 (0%)	0/6 (0%)	7/10 (70.0%)	1/10 (10.0%)	7/9 (77.8%)	2/9 (22.2%)
4 m/o	2/6 (33.3%)	0/6 (0%)	9/10 (90%)	1/10 (10.0%)	3/8 (37.5%)	5/8 (62.5%)
6 m/o	3/7 (42.9%)	0/7 (0%)	4/6 (66.6%)	1/6 (16.7%)	3/6 (50.0%)	3/6 (50.0%)