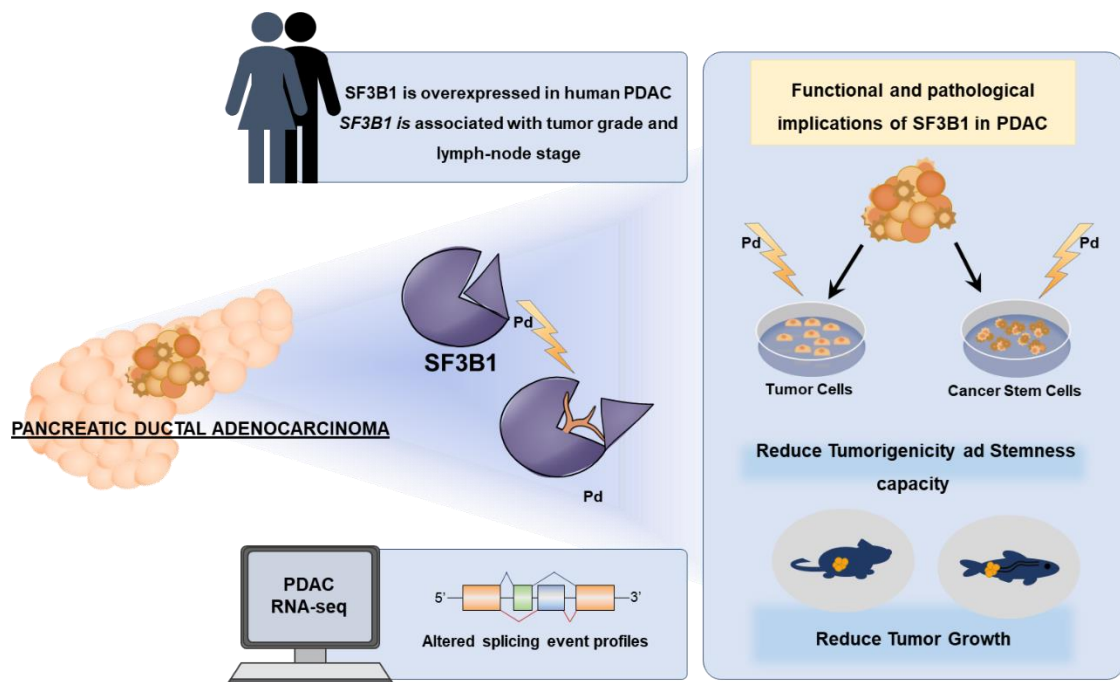


Graphical Abstract



The process that matures mRNA, splicing, is altered in pancreatic cancer, enhancing its malignancy. By targeting the splicing-factor SF3B1, Pladienolide-B inhibits pancreatic cancer cells and cancer stem cells, unveiling a novel approach to treat pancreatic cancer.

Supplemental Table 1. Additional clinical characteristics of the patients

Characteristic		Samples n=75 (100%)
Smoker	Yes	28 (37.3)
	Former smoker	11 (14.7)
Drinker		50 (66.7)
Body Mass Index	< 20	10 (13.04)
	20 – 25	45 (60.87)
	25 – 30	16 (21.74)
	> 30	3 (4.35)
Diabetes	Type 1 Diabetes Mellitus	3 (4.2)
	Type 2 Diabetes Mellitus	23 (30.6)
Arterial Hypertension		44 (58.6)
Hypercholesterolemia		44 (58)
Prior Acute Pancreatitis		7 (9.3)
Family Background	Pancreatic cancer	1 (1.3)
	Other cancers	2 (2.7)
Perineural Invasion		56 (74.7)
Linfovascular Invasion	Small blood vessel	22 (29.3)
	Big blood vessel	20 (26.7)
Grading	Grade X	2 (2.7)
	Grade 1	15 (20)
	Grade 2	43 (57.3)
	Grade 3	8 (10.7)
	Grade 4	1 (1.3)

Supplemental Table 2. Clinicopathological characteristics of 94 pancreatic adenocarcinoma patients profiled by RNASeq

Characteristic	Samples n=94 (100%)
Age	Median 67 (range 37-86)
Sex	
Female	46 (48.9)
Male	48 (51.1)
T stage	
T1	1 (1.1)
T2	2 (2.1)
T3	91 (96.8)
T4	0 (0)
N stage	
N0	12 (12.8)
N1	82 (87.2)
M stage	
M0	91 (96.8)
M1	3 (3.2)
Grading	
G1	4 (4.3)
G2	60 (63.8)
G3	30 (31.9)
Vascular invasion	
Yes	10 (10.6)
No	84 (89.4)
Perineural invasion	
Yes	89 (94.7)
No	5 (5.3)
Fat invasion	
Yes	85 (90.4)
No	9 (9.6)

Supplemental Table 3. Specific primers for human transcripts used in this study.

	Sense Primer Sequence	Antisense Primer Sequence
<i>ACTB</i>	ACTCTTCCAGCCTTCCTTCCT	CAGTGATCTCCTTCTGCATCCT
<i>GAPDH</i>	AATCCCATCACCATCTTCCA	AAATGAGCCCCAGCCTTC
<i>HPRT1</i>	CTGAGGATTTGGAAAGGGTGT	TAATCCAGCAGGTCAGCAAAG
<i>SF3B1</i>	CAGTTCCGTCTGTGTGTTTCG	GCTGCCTTCTTGCCTTGA
<i>KRAS</i>	CTTGATATTCTCGACACAGCA	AAAGAAAGCCCTCCCCAGT
<i>KRAS4A</i>	ACAGAGAGTGGAGGATGCTTTTT	AGCCAGGAGTCTTTTCTTCTTTG
<i>BCL-XL</i>	GATGGCCACTTACCTGAATGA	TGCTGCATTGTTCCCATAGA
<i>BCL-XS</i>	GAGCTTTGAACAGGATACTTTTGTG	GAAGAGTGAGCCCAGCAGAA
<i>TP53</i>	AAGGAAATTTGCGTGTGGAG	CCAGTGTGATGATGGTGAGG
<i>TP53Δ133</i>	ACTCTGTCTCCTTCCTTACAG	GTGTGGAATCAACCCACAGCT
<i>NFKB1</i>	CCTGTCCTTTCTCATCCCATC	TGCCAGAGTTTCGGTTCACT
<i>CASP3</i>	GTCTCAATGCCACAGTCCAGT	TTTTTCAGAGGGGATCGTTG
<i>MIK67</i>	GACATCCGTATCCAGCTTCCT	GCCGTACAGGCTCATCAATAAC
<i>ERB2</i>	CTGTGTTCCATCCTCTGCTG	TGCCTGTCCCTACAACCTT
<i>KLF4</i>	ACCCACACAGGTGAGAAACC	ATGTGTAAGGCGAGGTGGTC
<i>NANOG</i>	TGAACCTCAGCTACAAACAGGTG	AACTGCATGCAGGACTGCAGAG
<i>NODAL</i>	AGCATGGTTTTGGAGGTGAC	CCTGCGAGAGGTTGGAGTAG
<i>OSCT3/4</i>	CTTGCTGCAGAAGTGGGTGGAGGAA	CTGCAGTGTGGGTTTCGGGCA
<i>SOX2</i>	AGAACCCCAAGATGCACAAC	CGGGGCCGGTATTTATAATC

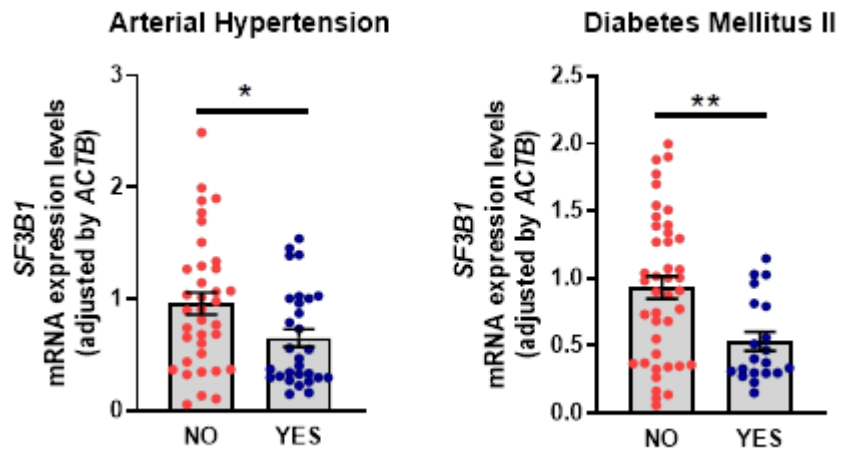
Supplemental Table 4. Specific data for human transcript variants primers used in this study.

	Ensembl id	Gene name	SUPPA_name	dPSI	p- Value	Forward	Reverse
1_AF	ENSG00000153187	<i>HNRNPU</i>	ENSG00000153187,20; AF:chr1:244862730- 244863617:244863653: 244862730- 244863674:244864091: -	0,484 372	0,0224 8	AGCTAGGAGAG GAGAACGGG	CGAGCTCATCTTCCC CTTCC
2_AF	ENSG00000172071	<i>EIF2AK3</i>	ENSG00000172071,14; AF:chr2:88613853- 88624746:88624929:88 613853- 88626967:88627464:-	0,484 871	0,0119 9	GCTCCACCTCA GCGAC	GTCTCATCGTCTGGTT CCGG
3_A3	ENSG00000164039	<i>BDH2</i>	ENSG00000164039,15; A3:chr4:103092718- 103095203:103092696- 103095203:-	0,488 784	0,0209 8	ACCCGGTGCCT CTTGTTTTA	AACTTCATTGGCAAAC TGATCAA
4_A5	ENSG00000224032	<i>NA</i>	ENSG00000224032,7; A5:chr5:112161295- 112161703:112160991- 112161703:+	0,492 791	0,0339 7	CATCGACTATGC CAGGGAGT	GCAGGGCAAGCATAA AGTCA
5_AF	ENSG00000102554	<i>KLF5</i>	ENSG00000102554,14; AF:chr13:73054976:73 055158- 73061861:73059005:73 059588-73061861:+	0,493 420	0,0050 0	CGCTTGGCCTAT AACTTGGT	TGGAGAGACTGGGAT TGCTT
6_MX	ENSG00000140391	<i>TSPAN3</i>	ENSG00000140391,15; MX:chr15:77054279- 77055789:77055863- 77070892:77054279- 77056064:77056255- 77070892:-	0,493 740	0,0269 7	CCGTGCTGGTC TTTCTCAAC	TCCCCAAACCACTACA ACAAC
7_RI	ENSG00000103363	<i>ELOB</i>	ENSG00000103363,15; RI:chr16:2771414:2771 604-2771994:2772102:-	0,506 647	0,0037 5	GAGACCCTGGC TGAGAACTT	GTGTCTCTCCCAGTC CTTCC
8_AF	ENSG00000171729	<i>TMEM51</i>	ENSG00000171729,14; AF:chr1:15152532:151 52784- 15214895:15153733:15 153954-15214895:+	0,518 320	0,0269 7	GAACCTCAAAGG GCTGGACG	GCTCCCATTCCCTCTC TGAG
9_AF	ENSG00000127022	<i>CANX</i>	ENSG00000127022,15; AF:chr5:179698417:17 9698603- 179705679:179698988: 179699102- 179705679:+	0,519 071	0,0158 2	TCGGACTCCTAC CCCTTTTG	GTTCTTGAGCCGAG ACTT
10_A5	ENSG00000106399	<i>RPA3</i>	ENSG00000106399,11; A5:chr7:7639144- 7640320:7639144- 7640723:-	0,521 667	0,0124 9	TTTCAGAGACAG CGCGATTG	ATTTCTCGGCACCAAT CAGC
11_A5	ENSG00000203760	<i>CENPW</i>	ENSG00000203760,8; A5:chr6:126340444- 126346205:126340399- 126346205:+	0,526 509	0,0094 9	GAGATTCCATCC CTTCTCGG	GCCAGTACATGCTCC TTGTT
12_SE	ENSG00000140848	<i>CPNE2</i>	ENSG00000140848,17; SE:chr16:57092790- 57093950:57094091- 57110708:+	0,528 395	0,0494 5	TGAAGGCGTGG TTTTATGGC	GTAGGTTCTGGCCAC TCACT
13_AF	ENSG00000164713	<i>BRI3</i>	ENSG00000164713,10; AF:chr7:98252379:982 52528- 98282351:98281686:98 281937-98282351:+	0,530 920	0,0199 8	AGTTGCCGCGTT CTCTCT	TATCCCCAGGTACCC TCTCC
14_AF	ENSG00000160179	<i>ABCG1</i>	ENSG00000160179,18; AF:chr21:42216077:42 216188- 42225671:42219118:42 219304-42225671:+	0,533 905	0,0149 9	TTCACCTTCCGC CATGATCA	ATTCAGCAGGTCCGT CTCAG

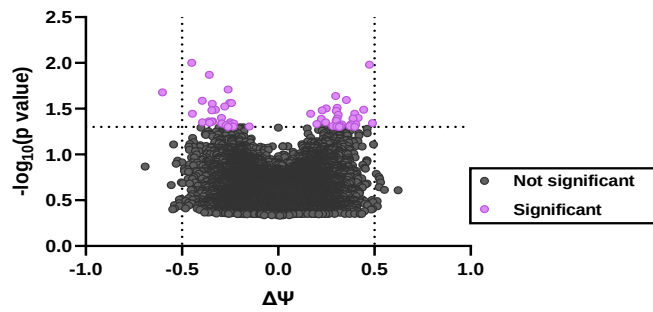
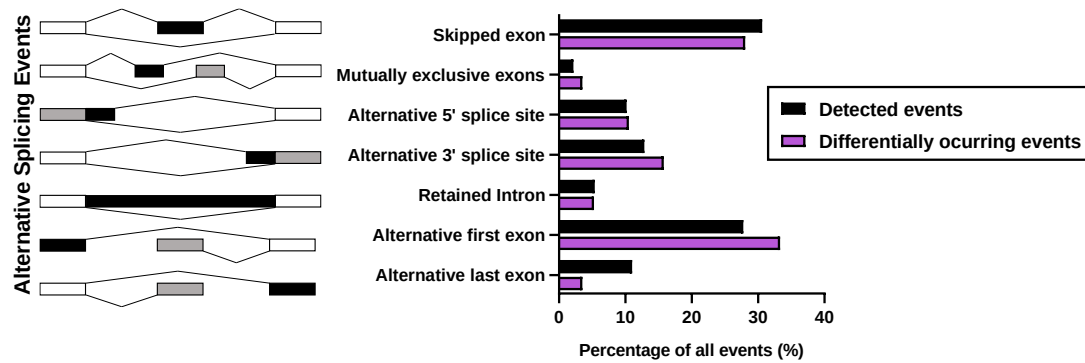
15_AL	ENSG00000197756	RPL37A	ENSG00000197756,10; AL:chr2:216500031-216501341:216501462:216500031-216529270:216529454:+	0,541604	0,01399	ATGAAGAGACG AGCTGTGGG	AGCGTTGCATTTGGT CCATT
16_A3	ENSG00000245910	NA	ENSG00000245910,8; A3:chr8:66922725-66925437:66922392-66925437:-	0,542885	0,02248	CGAAGAGCCGT TAGTCATGC	ATACATGCCGCGTGA TCCTA
17_AF	ENSG00000123562	MORF4L2	ENSG00000123562,17; AF:chrX:103685260-103686631:103686705:103685260-103687989:103688064:-	0,547206	0,01399	TGCTTGCTTGGA GATCAGGA	CAGGGAAGGTTCTGC AATCA
18_SE	ENSG00000135678	CPM	ENSG00000135678,12; SE:chr12:68871956-68885792:68885885-68932678:-	0,551704	0,04196	GCTGCGCTGGA TTTCAACTA	CCCACAACAAGAACC CACAG
19_A3	ENSG00000170584	NUDCD2	ENSG00000170584,11; A3:chr5:163457080-163457562:163457005-163457562:-	0,556731	0,02398	AGCTGATGAGG GAACATGGA	GAAGTCCAACAATTTG CTGCA
20_A5	ENSG00000141750	STAC2	ENSG00000141750,7; A5:chr17:39217173-39217867:39217173-39218069:-	0,557282	0,03247	CTCCCCATGCC CAGTCC	CATGTTCTGGAAGC TGTGC
21_SE	ENSG00000136895	GARNL3	ENSG00000136895,19; SE:chr9:127385145-127387193:127387331-127388904:+	0,557902	0,02797	CTGCAGCTGTG AATGAGGTC	GTCTGAGGAGAATTT CGGGC
22_A3	ENSG00000162704	ARPC5	ENSG00000162704,16; A3:chr1:183627594-183630461:183623471-183630461:-	0,560535	0,00599	AGGGTCCATTGT TCGTGTCT	TCCCGAGGCAGATAA TCCAC
23_AF	ENSG00000198242	RPL23A	ENSG00000198242,14; AF:chr17:28719985:28720030-28720707:28720299:28720328-28720707:+	0,568048	0,00450	ACCCTTTTCACA AGATGGCG	GTGACGTGCGGATCT TCTTC
24_AF	ENSG00000075426	FOSL2	ENSG00000075426,12; AF:chr2:28392802:28392836-28404107:28395511:28395776-28404107:+	0,568878	0,02331	CAGGGCTGGAG AATAAAGAGT	AGGGTATGGGTTGGA CATGG
25_SE	ENSG00000229833	PET100	ENSG00000229833,10; SE:chr19:7630846-7631232:7631321-7631473:+	0,570591	0,00400	TAAGGAACCGA GAGCAGAGG	TGGGATCCGCTTCAC TCTTC
26_AF	ENSG00000240065	PSMB9	ENSG00000240065,8; AF:chr6:32844136:32844274-32856138:32854148:32854289-32856138:+	0,570656	0,04570	GCTTCTCTGCTC TCCCGTTA	ATCAGAACCCATCACA ACGC
27_AF	ENSG00000115666	MAP4K3	ENSG00000115666,15; AF:chr2:39378123-39379752:39379809:39378123-39436892:39437301:-	0,577151	0,01399	CAGGAGGACTT CGAGCTGAT	CATTCCGTGCCTTGTA GACG
28_SE	ENSG00000116209	TMEM59	ENSG00000116209,12; SE:chr1:54047372-54048650:54048682-54053000:-	0,583466	0,01049	AGAAGAGGAGT TGTACGCATG	TCCATCATCCACAAAC TGACA
29_SE	ENSG00000156381	ANKRD9	ENSG00000156381,9; SE:chr14:102508549-102508641:102508846-102509529:-	0,588917	0,02198	ACCCTCCTATCT CCTCCAG	GTTCCGGGGATGTGG GTATA
30_AF	ENSG00000153250	RBMS1	ENSG00000153250,20; AF:chr2:160367391-160416138:160416178:160367391-160493289:160493807:-	0,591632	0,01499	AGCTTCATGGG CAAAGTGTG	AGGTTCTTTTGCTGA GCTG

31_AL	ENSG00000105750	ZNF85	ENSG00000105750,15; AL:chr19:20935047-20942801:20943977:20935047-20945499:20945709:+	0,598403	0,04795	TCTGGAGCAAG GGAAAGAGG	GGAGAATTGCTTGAA CCCAGG
32_AF	ENSG00000113387	SUB1	ENSG00000113387,12; AF:chr5:32531633:32531916-32588512:32585559:32585625-32588512:+	0,604633	0,01049	ACAGTCCTCTTA GTGCACCA	CTCCACCATCTCTGA GCTGT
33_SE	ENSG00000170906	NDUFA3	ENSG00000170906,16; SE:chr19:54106011-54106353:54106446-54106811:+	0,605968	0,00400	CCGTCATGATCA ACAAGGCC	ACAATCCTGGCTAACA CGGT
34_AF	ENSG00000091490	SEL1L3	ENSG00000091490,11; AF:chr4:25847864-25860601:25860631:25847864-25863434:25863537:-	0,608459	0,03497	CGCAAAAGGGA ATGTTGTACC	GCTCAGCTTTGGGTA TCACTG
35_AL	ENSG00000127022	CANX	ENSG00000127022,15; AL:chr5:179726759-179728591:179728907:179726759-179729360:179729649:+	0,619790	0,01424	AGAAGATGGTG GCACTGTCA	TCTATTCCGGAGCTCA CGTG
36_A3	ENSG00000230989	HSBP1	ENSG00000230989,7; A3:chr16:83809425-83811421:83809425-83817786:+	0,638175	0,00799	AAACAAGATACC TGCCACGC	TCGATCTTCTCTTGGC TTGGA
37_AF	ENSG00000241343	RPL36A	ENSG00000241343,10; AF:chrX:101391016:101391046-101391459:101391202:101391235-101391459:+	0,643030	0,00749	GCAAGCATGGTT AACGTCCC	AATCCTTGCCCTTCTT GTACTG
38_SE	ENSG00000241343	RPL36A	ENSG00000241343,10; SE:chrX:101391822-101392050:101392095-101395335:+	0,655676	0,00599	GAAAGCGGCGT TATGACAGG	TAGGAAAGCAGGGCA CATTC
39_A5	ENSG00000182899	RPL35A	ENSG00000182899,17; A5:chr3:197951328-197954003:197951311-197954003:+	0,664481	0,01199	GTAAGTTTATGA CACTGCAACACA	CATGGGCCCCGAGTTA CTTTT
40_AF	ENSG00000160014	CALM3	ENSG00000160014,17; AF:chr19:46601074:46601176-46605827:46601276:46601437-46605827:+	0,671171	0,02797	GCGGCGAGGGA AAGTAGT	GAATCTCTCGTCCCC ACCC
41_AF	ENSG00000166741	NNMT	ENSG00000166741,7; AF:chr11:114295825:114296710-114297951:114297363:114297398-114297951:+	0,676463	0,00300	CCTAGACGGTG TGAAGGGAG	GTCAGTGACGACGAT CTCCT
42_A3	ENSG00000223865	HLA-DPB1	ENSG00000223865,11; A3:chr6:33080534-33080672:33080534-33080689:+	0,684339	0,00000	TTCTCTCTCTGC GTGGTGAG	TTCTCGAGTTCTGTGG TCCT
43_AF	ENSG00000185088	RPS27L	ENSG00000185088,14; AF:chr15:63156521-63157400:63157477:63156521-63157916:63158021:-	0,697218	0,00000	AAGCAAACCTAA GGCACAGC	TGGACTTTGTACTAGG CGTTTC
44_A5	ENSG00000112695	COX7A2	ENSG00000112695,11; A5:chr6:75237988-75240277:75237988-75240301:-	0,702383	0,00500	AAAGGGTGGGG TAGCTGATG	CTTCAAACATTCCAAA GGCCTT
45_AL	ENSG00000197756	RPL37A	ENSG00000197756,10; AL:chr2:216500031-216501341:216504086:216500031-216529270:216529454:+	0,755854	0,01399	ATGAAGAGACG AGCTGTGGG	AGCGTTGCATTTGGT CCATT

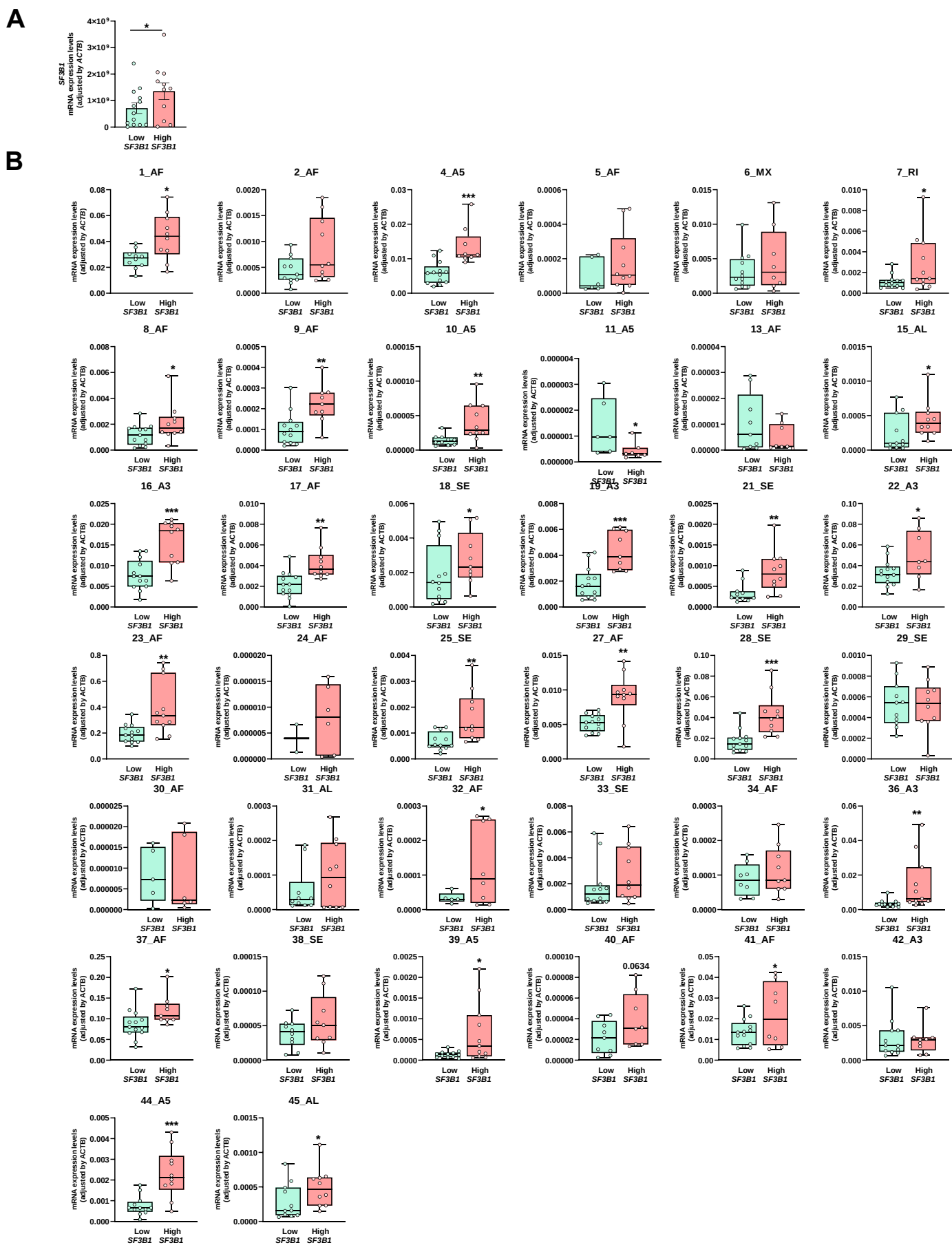
46_SE	ENSG00000197756	RPL37A	ENSG00000197756,10; SE:chr2:216499398-216499832:216499866-216499949:+	0,773962	0,01399	ACGTACCAAGAA AGTCGGGA	TCCCTTTCCTTTGC CACA
-------	-----------------	--------	--	----------	---------	--------------------------	------------------------



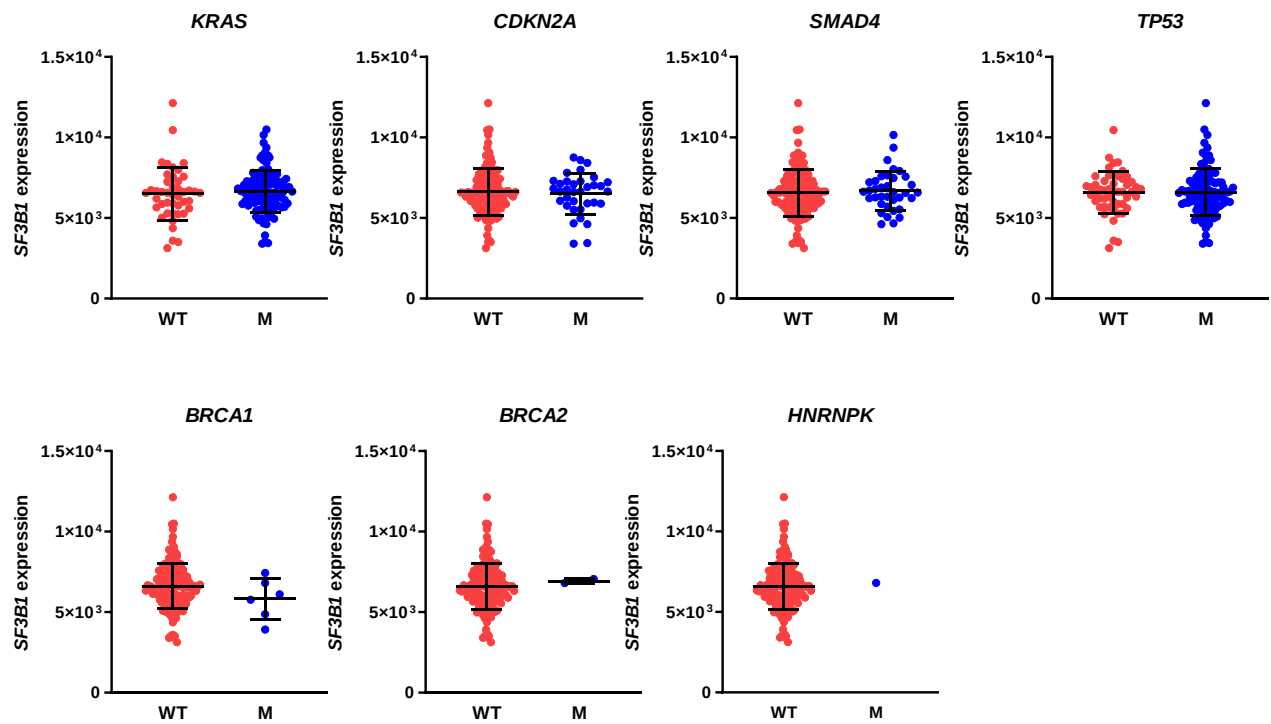
Supplemental Figure 1) *SF3B1* expression level correlation in patient without/with AHT or T2DM in PDAC FFPE samples compared with non-tumoral adjacent tissue (NTAT).

A**B**

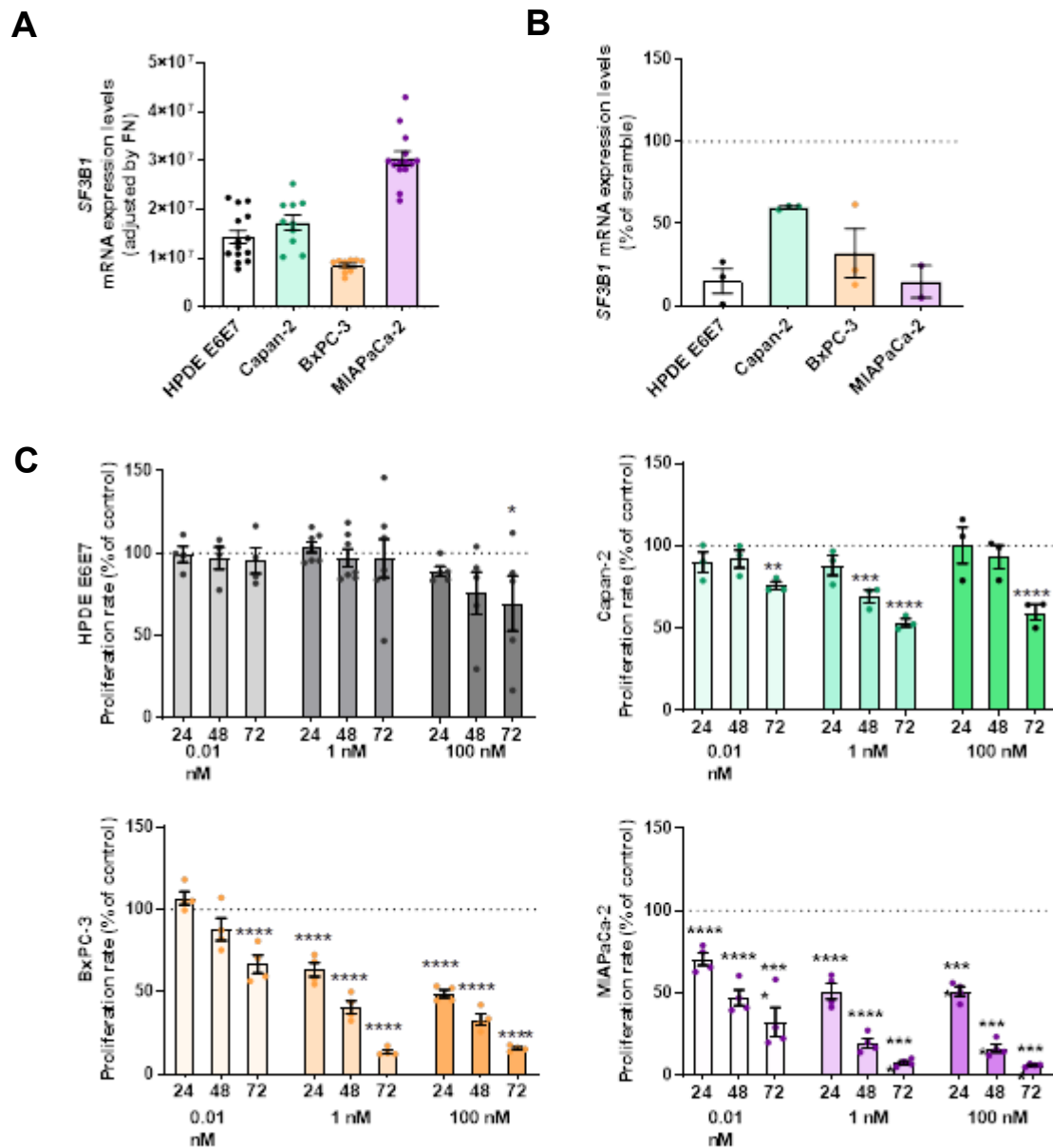
Supplemental Figure 2) A) Relationship of *SF3B1* expression levels with splicing event patterns of key genes in PDAC. **B)** Volcano-plot where $\Delta\Psi$ of total events calculated is plotted against the $-\log_{10}$ p-value of the Fisher's Exact Test to assay differential splicing events between high and low *SF3B1* expression groups of samples, showing that *SF3B1* tumor expression may influence alternative splicing patterns.



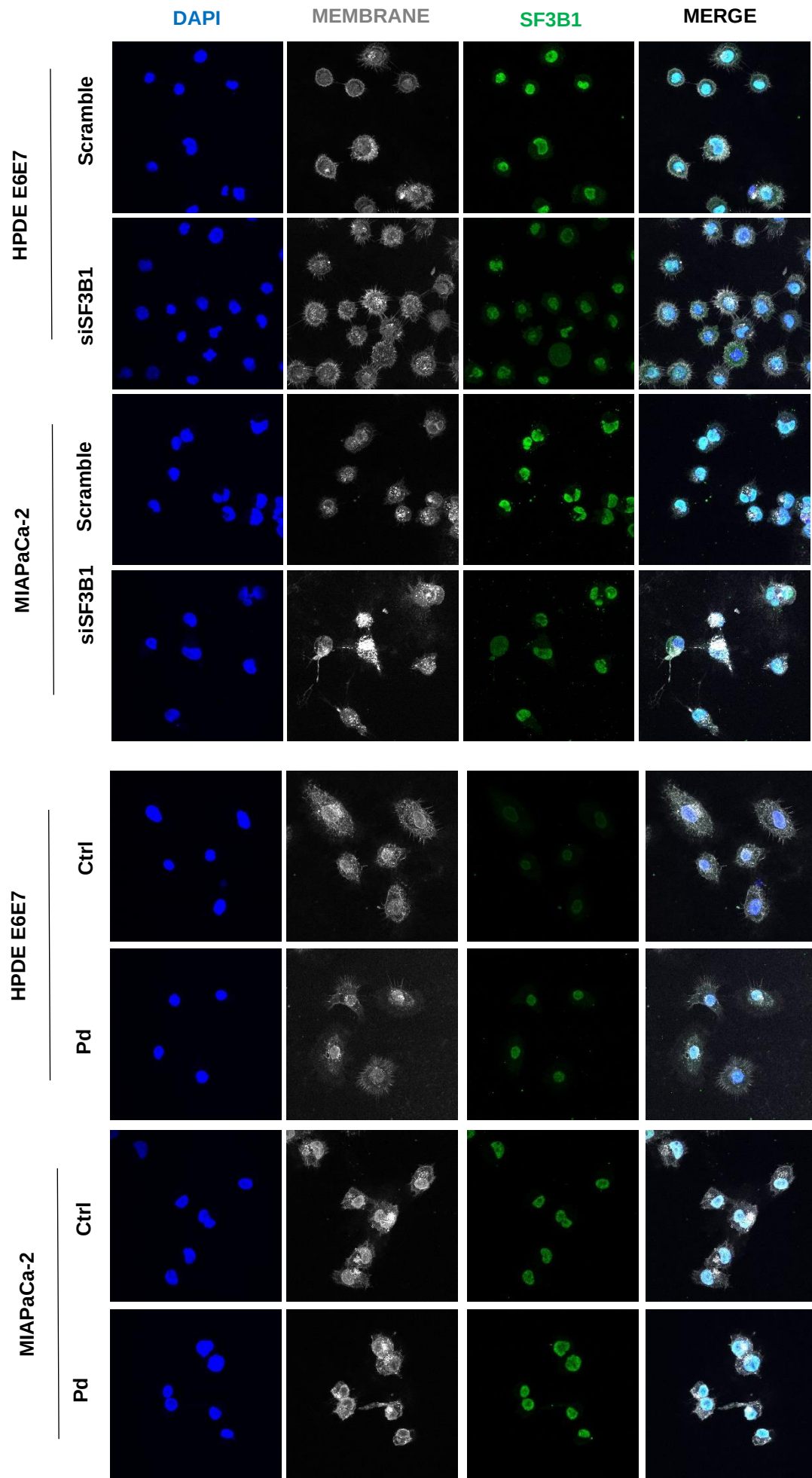
Supplemental Figure 3) A) Levels of *SF3B1* expression in PDAC samples from patients of the fresh tissue cohort in low and high *SF3B1* expression groups. **B)** Levels of expression of significantly different alternative splicing events transcripts (listed in Suppl Table 4) between High (n=11) and Low (n=13) *SF3B1* expression groups. Gene expression was normalized to *ACTB* expression. Asterisks indicate significant differences (*p < 0.05; **p < 0.01; ***p < 0.001).

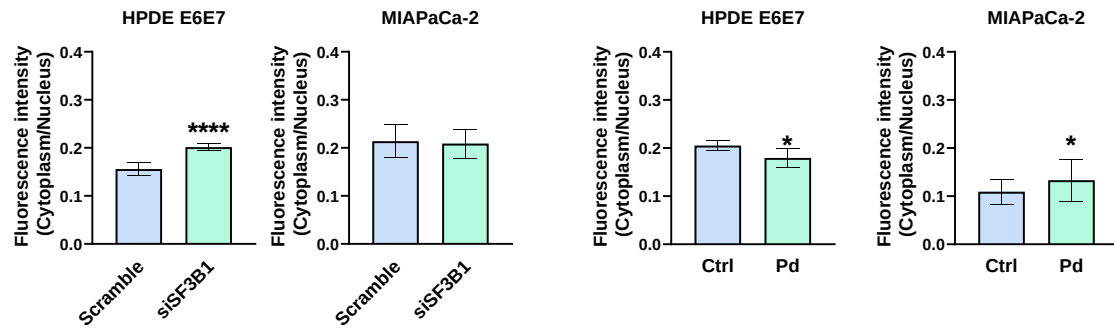


Supplemental Figure 4) Levels of *SF3B1* expression in PDAC samples from patients of the PanCancer cohort with (M) or without (WT) mutations in *KRAS*, *CDKN2A*, *SMAD4*, *TP53*, *BRCA1*, *BRCA2* and *HNRNPK*.

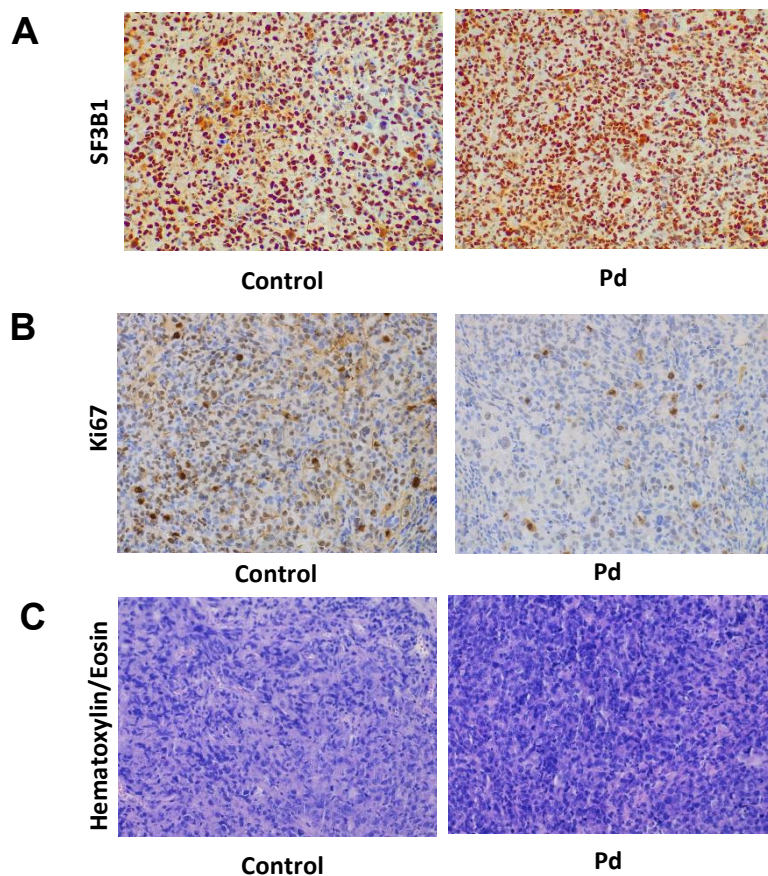


Supplemental Figure 5. Validation of SF3B1 expression silencing and optimization of Pladienolide-B dosage. **A)** SF3B1 basal expression levels in normal pancreatics HPDEE6E7 cells and Capan-2, BxPC-3 and MIAPaCa-2 PDAC cell lines adjusted by a normalization factor calculated from the expression levels of HPRT1, GAPDH and ACTB (n=5-7). **2B)** qPCR validation of the silencing efficiency achieved with SF3B1 specific siRNAs in PDAC cell lines. mRNA expression levels were normalized to ACTB expression levels. Data are expressed as a percentage of control (Scramble; set at 100%) (n=2-3). **2C)** Proliferation rates of HPDEE6E7, Capan-2, BxPC-3 and MIAPaCa-2 cell lines following treatment with different doses of Pladienolide-B compared to vehicle-treated control cells, used as a control (set as 100%, represented as a dotted line; n=3-5). Asterisks indicate significant differences (*p<0.05; **p<0.01; ***p<0.001).

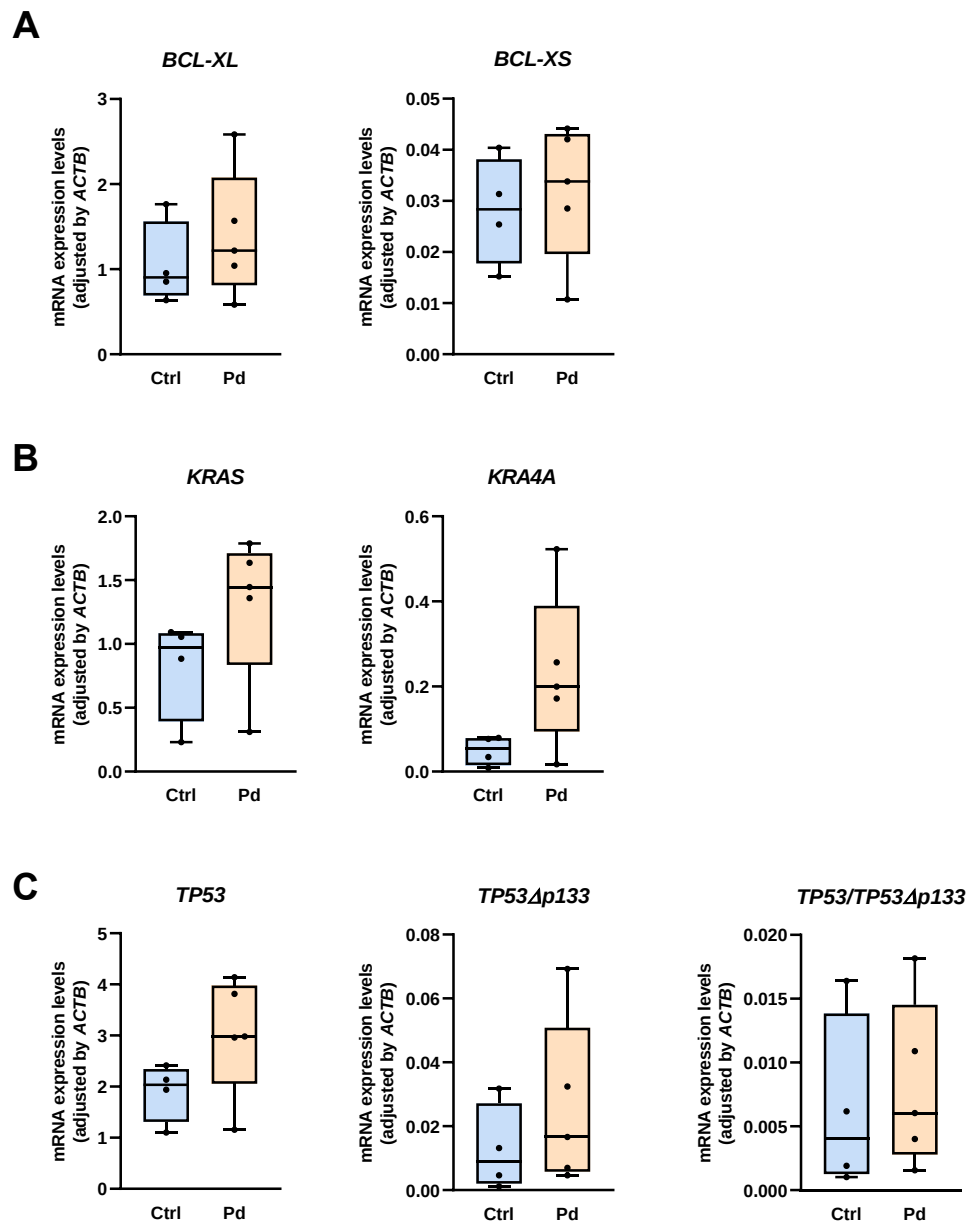




Supplemental Figure 6. SF3B1 localization in HPDE E6E7 and MIAPaCa-2 cell lines. A) Confocal images of Control and Pladienolide-B treated HPDE E6E7 and MIAPaCa-2 cell lines. **B)** Confocal images of Scramble and siSF3B1 in HPDE E6E7 and MIAPaCa-2 cell lines. **C)** Quantification of SF3B1 subcellular distribution in each set of samples. Asterisks indicate significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).



Supplemental Figure 7. Pladienolide-B effect in mice tumor xenografts. A) SF3B1 IHC analysis in vehicle (Control) and Pladienolide (Pd)-treated mice. Representative IHC 20X-images showing the general SF3B1 nuclear immunostaining in Control- and Pd-treated tumors. **B)** Ki-67 staining in representative tissue sections from tumor xenografts treated with vehicle (Control) and Pd. **C)** Hematoxylin-eosin-stained sections from vehicle (Control) and Pd-treated tumors illustrating the similarly scarce necrosis foci found in these tissues.



Supplemental Figure 8. Molecular profile in Pladienolide-treated mice. mRNA levels in malignancy-implicated genes. Values represent the $\log_{10}$ of expression compared with vehicle-treated (n=4). **A)** mRNA levels of *BCL-XL* and *BCL-XS* in HPDE E6E7 and MIAPaCa-2 cells treated 24h with or without (vehicle, control) Pladienolide-B (n=4). **B)** mRNA expressions of *KRAS* and *KRAS4a* in HPDE E6E7 and MIAPaCa-2 cells treated 24h with or without (vehicle, control) Pladienolide-B (n=4). **C)** Ratio of $\Delta 133TP53/TP53$ mRNA levels in HPDE E6E7 and MIAPaCa-2 cells treated 24h with or without (vehicle, control) Pladienolide-B (n=4). Gene expression was normalized to *ACTB* expression. Asterisks indicate significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).