

Supplementary Material for

CSTF2 mediated mRNA N^6 -methyladenosine modification drives pancreatic ductal adenocarcinoma m^6A subtypes

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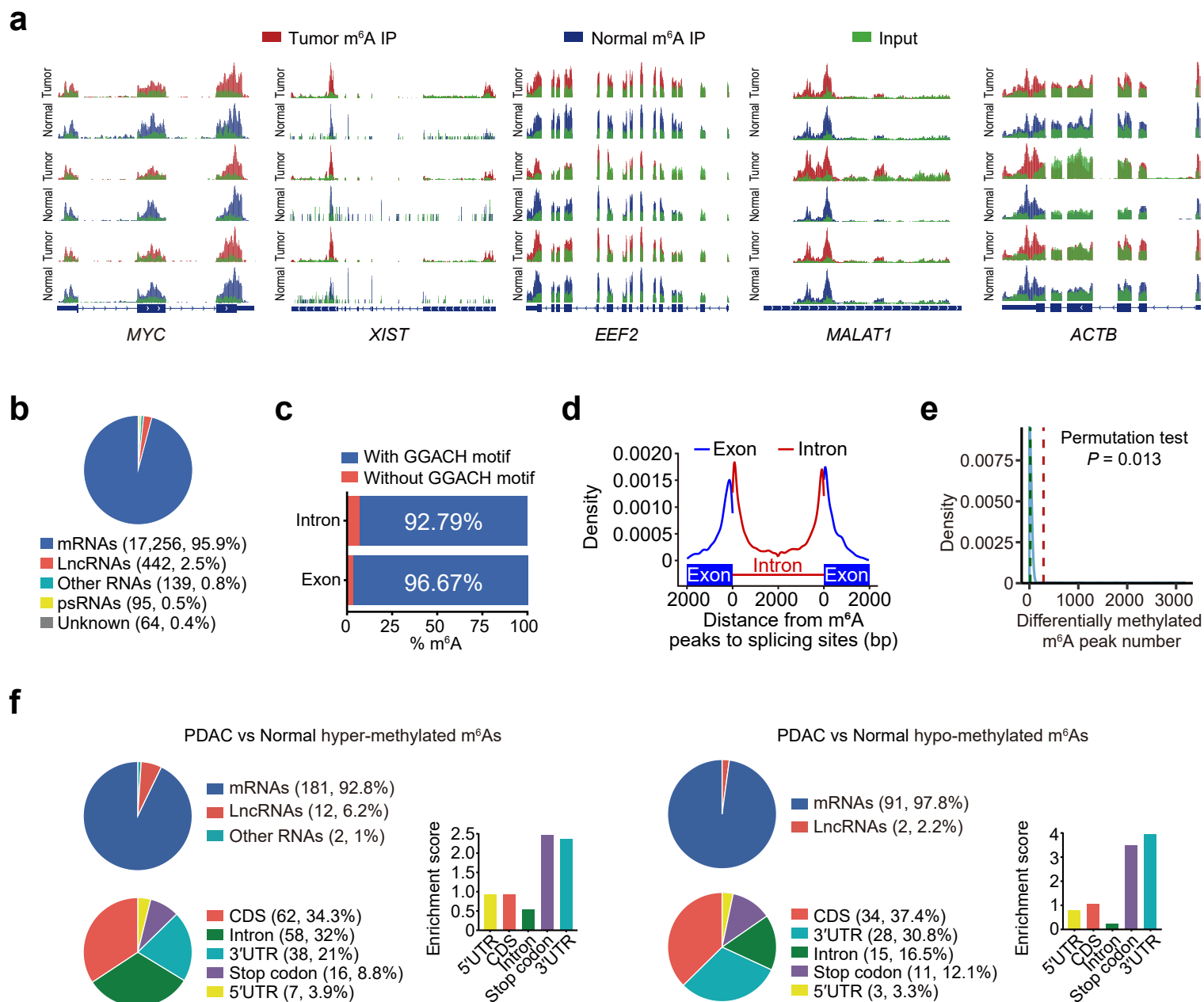
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Supplementary Table 1. Baseline demographic and clinical characteristics of PDAC patients in this study.

Supplementary Table 2. Validation results of selected differentially methylated m^6A s between PDAC and normal tissues by MeRIP qRT-PCR method.

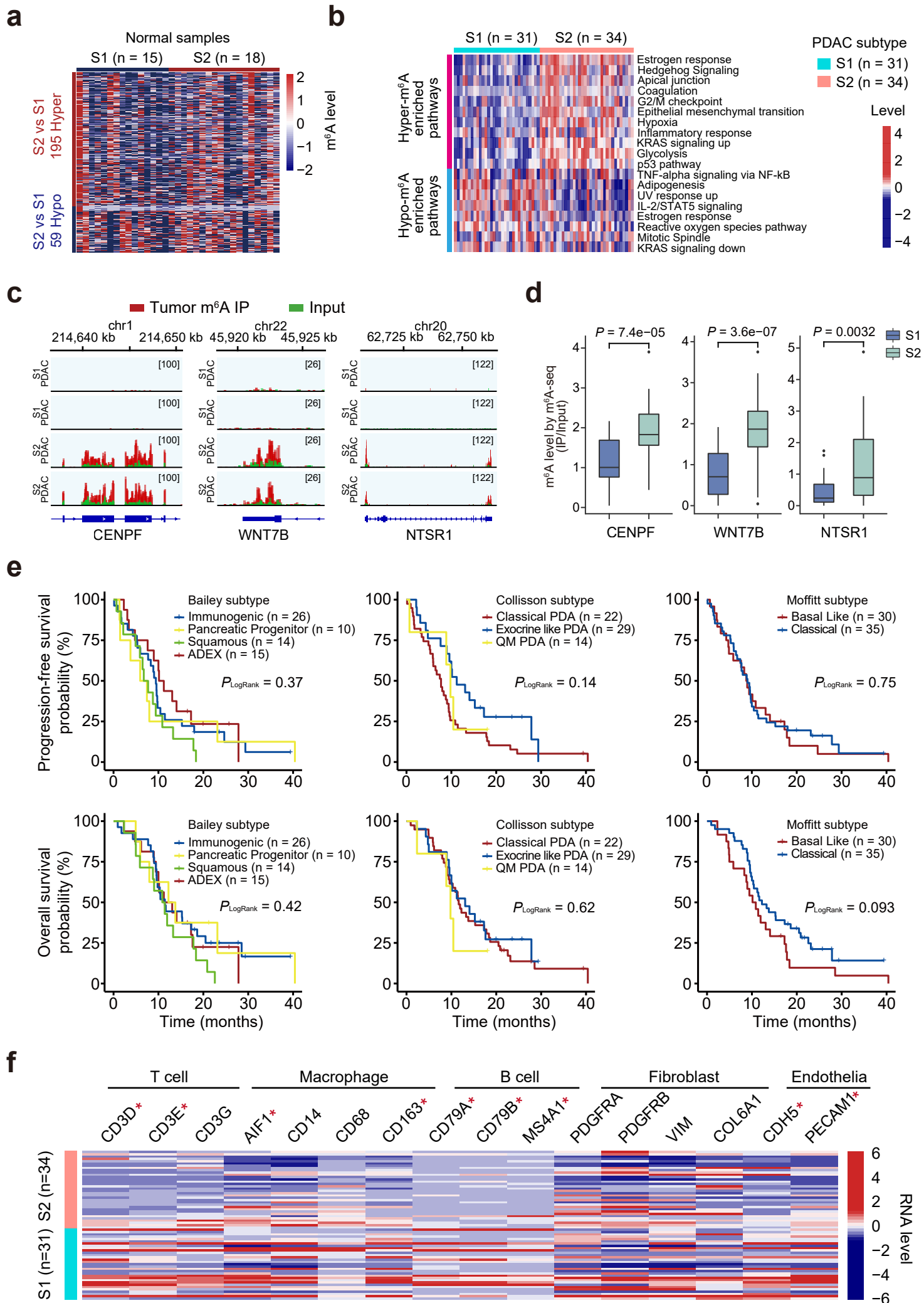
Supplementary Table 3. The primers sequences used in this study.

Supplementary Table 4. The RNA knockdown and gRNA sequences used in this study.



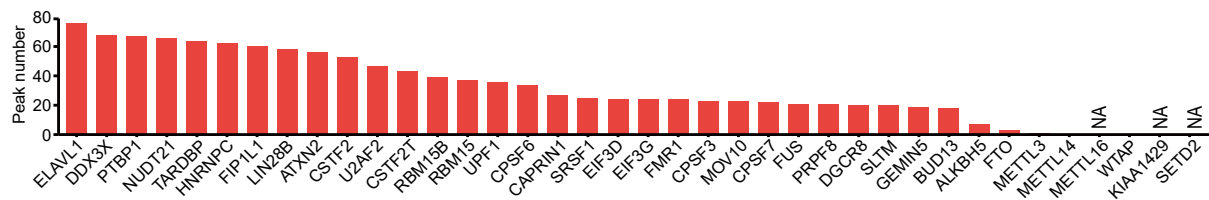
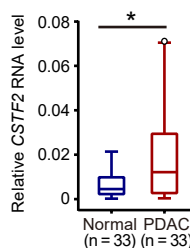
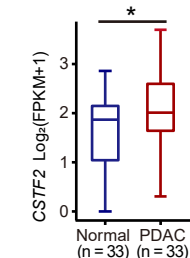
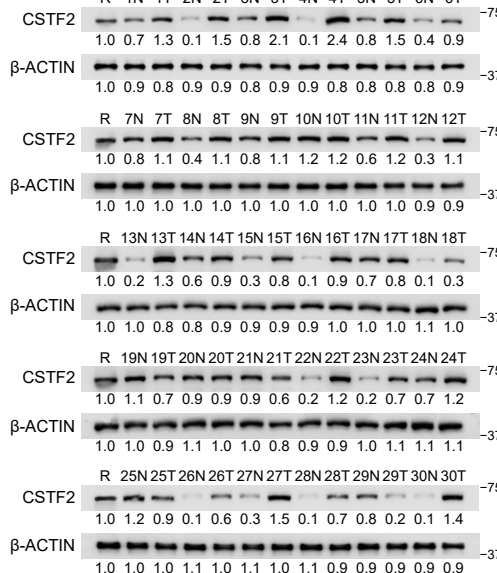
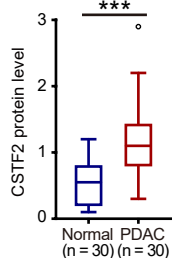
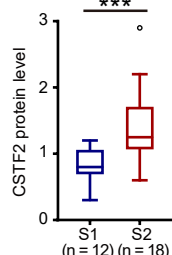
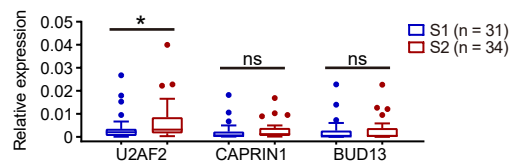
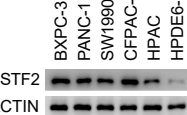
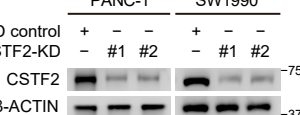
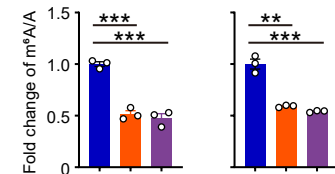
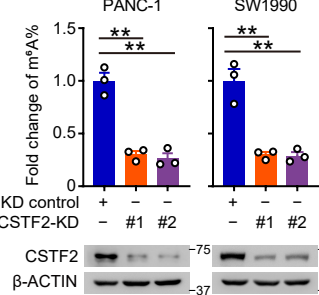
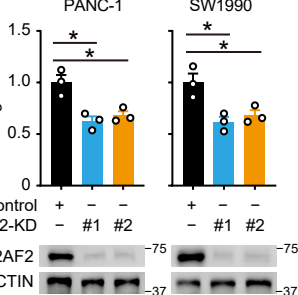
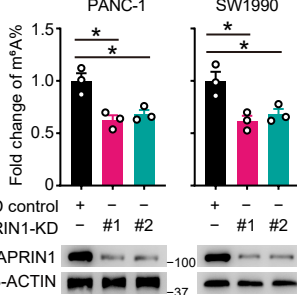
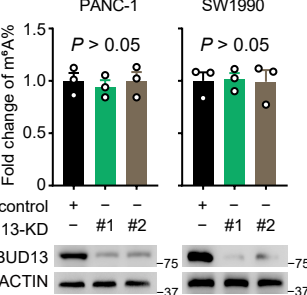
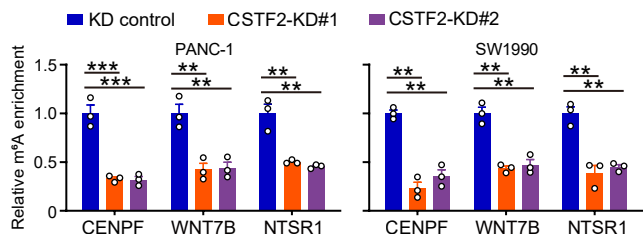
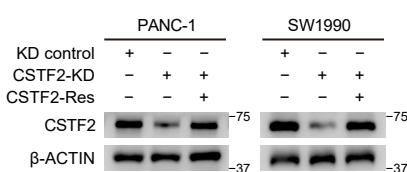
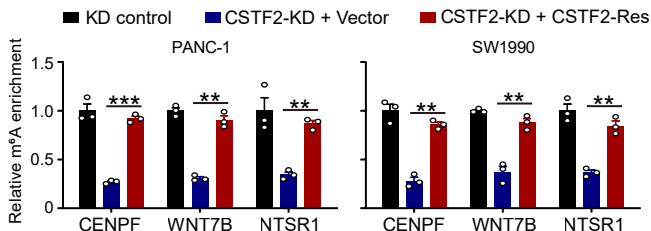
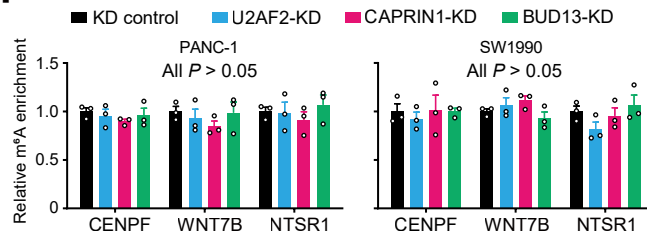
Supplementary Fig. 1. The distribution of m⁶As in PDAC and adjacent normal samples and annotation of the dysregulated m⁶As.

a The abundance of m⁶A in *MYC*, *XIST*, *EEF2*, *MALAT1* and *ACTB* mRNAs that are well-known to have m⁶A modifications. **b** Percentage of annotated mRNAs, long non-coding RNAs (lncRNAs), pseudogene RNAs (psRNAs), other type RNAs and unknown. **c** GGACH motif distribution in exon-derived or intron-derived m⁶As. **d** Distance between m⁶A peak and splicing site. **e** Density plot of the distribution of differentially methylated m⁶As number resulted from 1,000 permutation test. The red dash line represents observed number while the green dash line represents the average number of 1,000 time permutation. P value was calculated from permutation test. **f** The proportion of hyper-methylated m⁶As (*left panel*) and hypo-methylated m⁶As (*right panel*) in mRNA, lncRNA and other RNAs or in 5'UTR, CDS, 3'UTR, stop-codon of mRNAs and intron region.



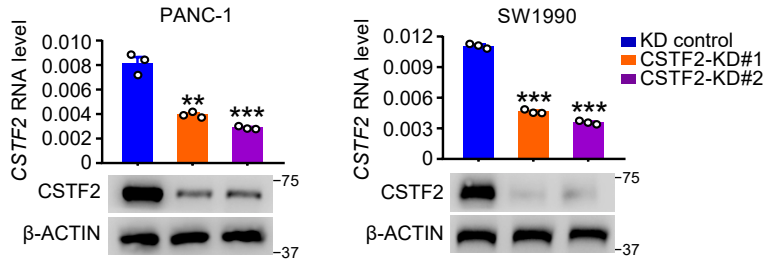
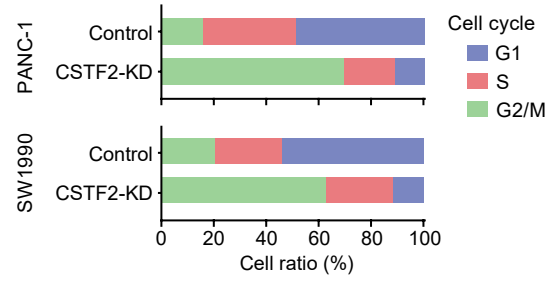
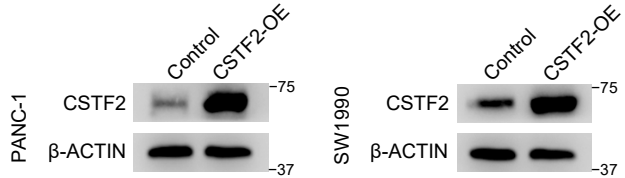
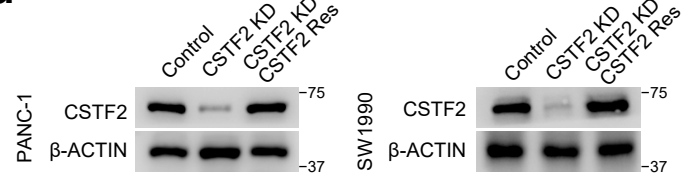
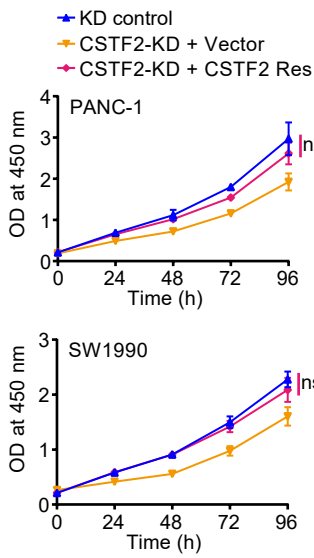
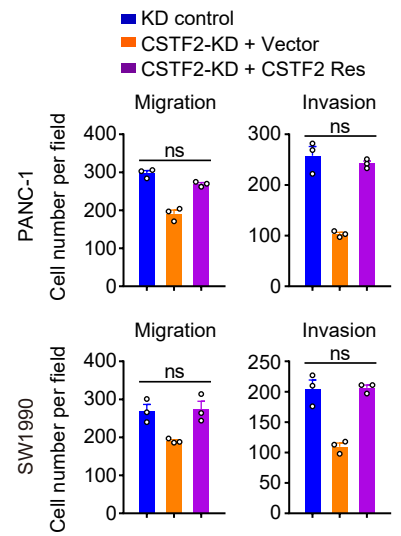
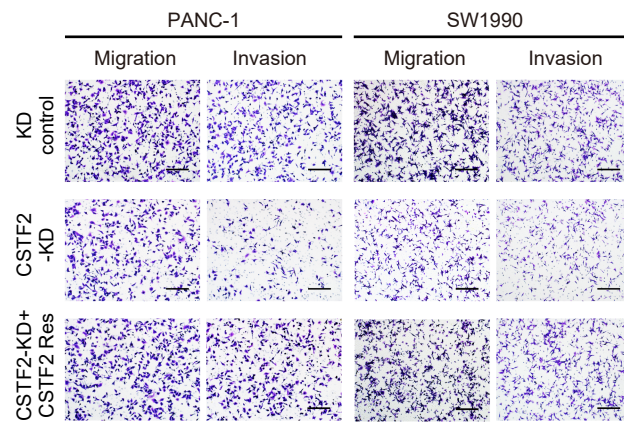
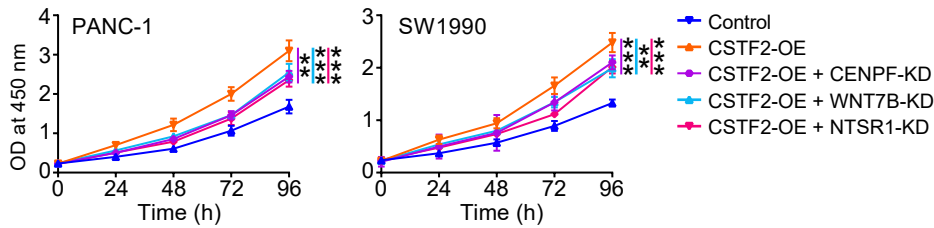
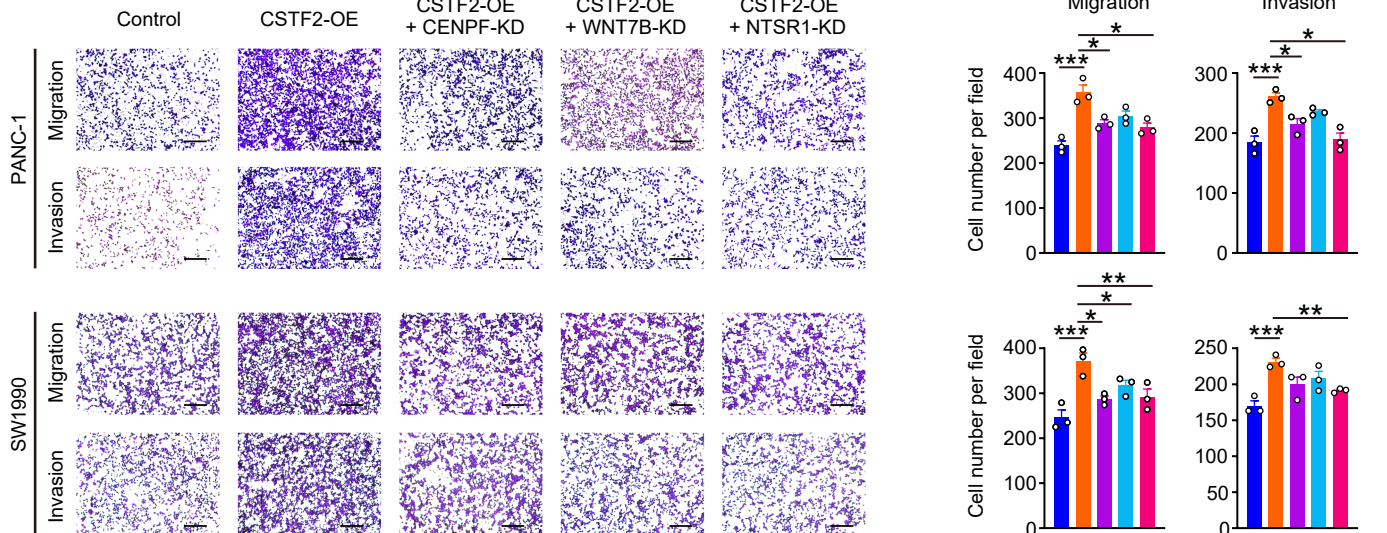
Supplementary Fig. 2. Characters of two PDAC subtypes and integrated analysis with reported PDAC subtype.

a Heatmap showing the expression level of different m⁶A (S2 vs S1 PDAC, FDR < 0.1 of Wilcoxon rank-sum test) in normal tissues. **b** Heatmap showing the differential m⁶A level of gene sets in indicated pathways in S1 and S2 PDAC. **c** The abundance of m⁶A in *CENPF*, *WNT7B*, and *NTSR1* mRNAs that are detected by m⁶A-seq in S1 and S2 PDAC. Number represents the range of the m⁶A signals. **d** The abundance of m⁶A in *CENPF*, *WNT7B*, and *NTSR1* mRNAs that are qualified by m⁶A-seq in S1 PDAC (n = 31) and S2 PDAC (n = 34). Boxplots indicate median (middle line), 25th, 75th percentile (box) and 5th and 95th percentile (whiskers). *P* values were from Wilcoxon rank-sum test. **e** Kaplan-Meier estimates of progression-free survival (*upper panel*) and overall survival (*lower panel*) in patients with PDAC by previously reported transcriptional subtypes in our patient set. **f** Heatmap showing the differences in mRNA levels of indicated microenvironmental cell markers in S1 and S2 PDAC subtypes. *, *P* < 0.05 of Wilcoxon rank-sum test.

a**b****c****d****e****f****g****h****i****j****k****l****m****n****o****p****q**

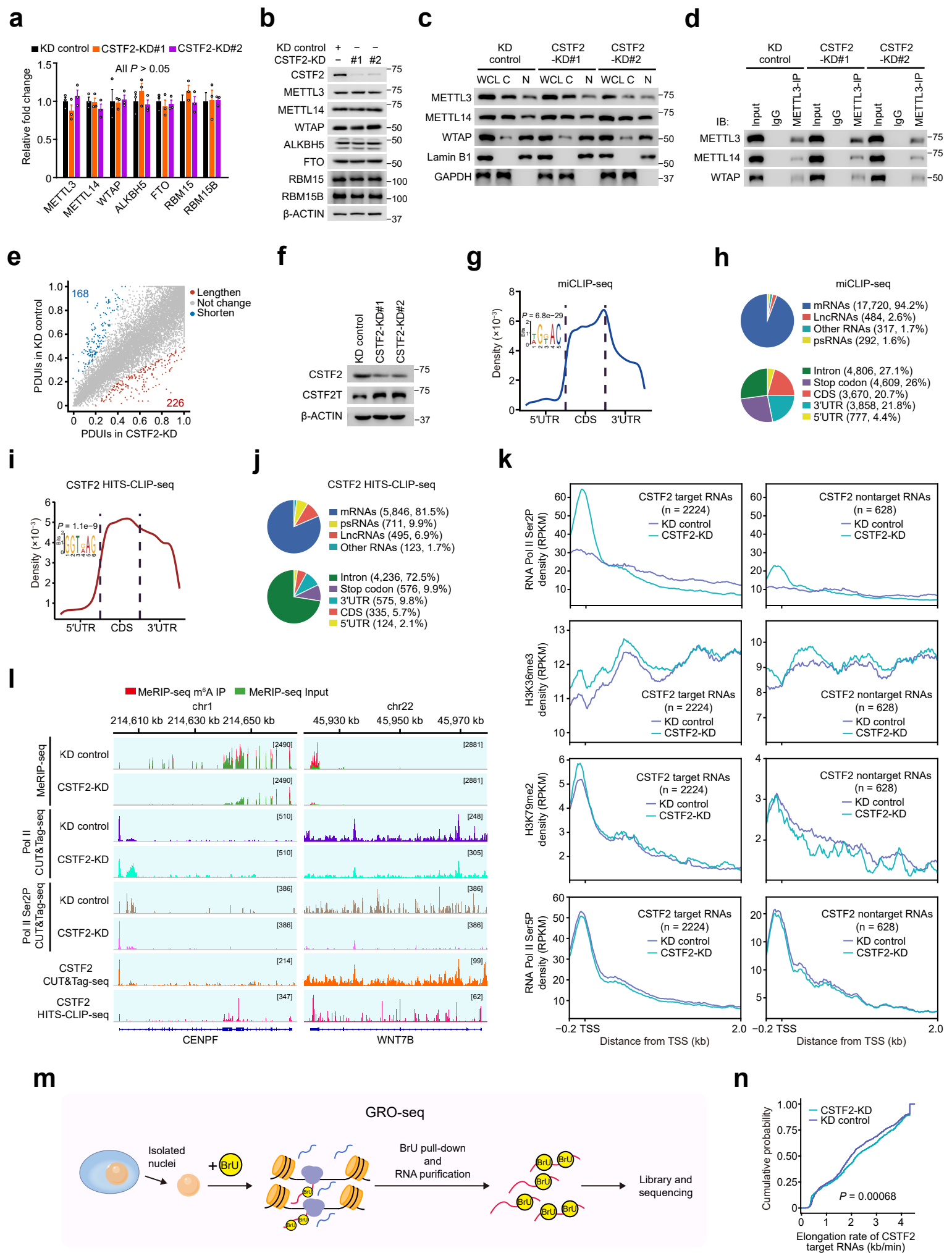
Supplementary Fig. 3. High expression of CSTF2 contributes to hyper-methylated m⁶A in PDAC.

a The identified hyper-methylated m⁶A peak number for different RNA binding proteins (RBPs). The peak number for each RBP was calculated based on whether the genomic regions of the peak and RNA binding site of the specific RBP have any overlap (1 bp). **b** *CSTF2* expression levels of paired PDAC tumor and normal samples in our data determined by RNA-sequencing (*upper panel*) or qRT-PCR (*lower panel*). *, $P < 0.05$ of Wilcoxon rank-sum test. **c** Western blot analysis of *CSTF2* levels in 30 paired PDAC tumor and normal samples. R was the same positive reference sample used for loading control on each gel. Each protein band was quantified by gray density and the value for each band is relative to density of both β -ACTIN and the corresponding R band. **d**, **e** *CSTF2* expression levels in PDAC tumor and normal samples (**d**) and S1 and S2 PDAC subtypes (**e**) determined by western blot data from (**c**). ***, $P < 0.001$ of Wilcoxon rank-sum test. **f** The expression levels of indicated transcript in S1 and S2 PDAC in our data determined by qRT-PCR. *BUD13* serves as negative control. *, $P < 0.05$; ns, not significant of Wilcoxon rank-sum test. **g** Western blot analysis of *CSTF2* levels in PDAC cells and normal pancreas cell line. **h** Knockdown efficiency was verified by western-blot. **i** The effect of *CSTF2* expression change on m⁶A level in PANC-1 and SW1990 cells determined by m⁶A-LC-MS. **, $P < 0.01$; ***, $P < 0.001$ of Student's *t*-test compared with KD control. **j–m** The effect of *CSTF2* (**j**), *U2AF2* (**k**), *CAPRIN1* (**l**), *BUD13* (**m**) knockdown on m⁶A level in PANC-1 and SW1990 cells determined by m⁶A-ELISA. *BUD13* serves as negative control. **n** The abundance of m⁶A in *CENPF*, *WNT7B*, and *NTSR1* mRNAs that are detected by MeRIP-qPCR in PANC-1 with or without *CSTF2* KD. **o** Western blot analysis of *CSTF2* levels in PDAC cells upon *CSTF2* KD with or without rescued with shRNA-resistant *CSTF2*. **p** The abundance of m⁶A in *CENPF*, *WNT7B*, and *NTSR1* mRNAs that are detected by MeRIP-qPCR in PDAC cells upon *CSTF2* KD with or without rescued with shRNA-resistant *CSTF2*. **q** The abundance of m⁶A in *CENPF*, *WNT7B*, and *NTSR1* mRNAs that are detected by MeRIP-qPCR in PDAC cell line upon *U2AF2* KD, *CAPRIN1* KD and *BUD13* KD. Boxplots in **b**, **d–f** indicate median (middle line), 25th, 75th percentile (box) and 5th and 95th percentile (whiskers). Data are the mean $\pm$ S.E.M. of three independent experiments in **i–n**, **p** and **q**. *, $P < 0.05$ and **, $P < 0.01$ of Student's *t*-test compared with each control.

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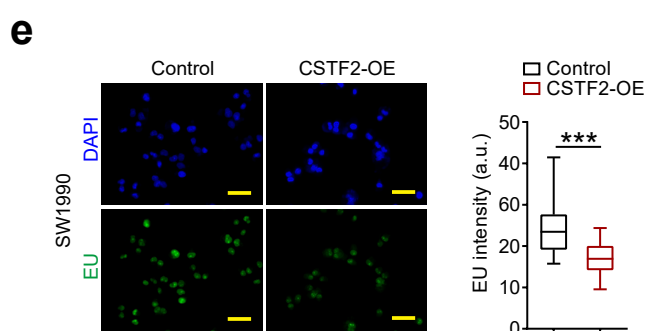
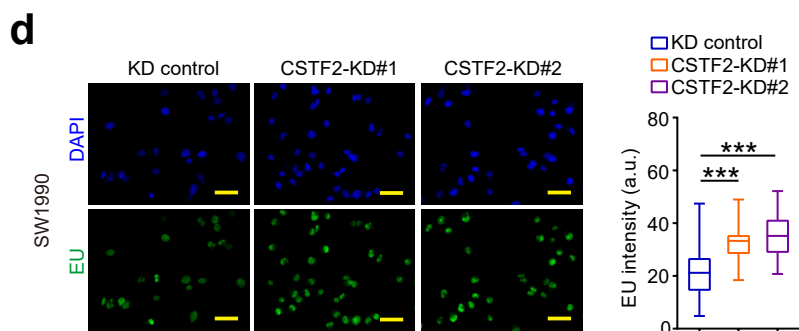
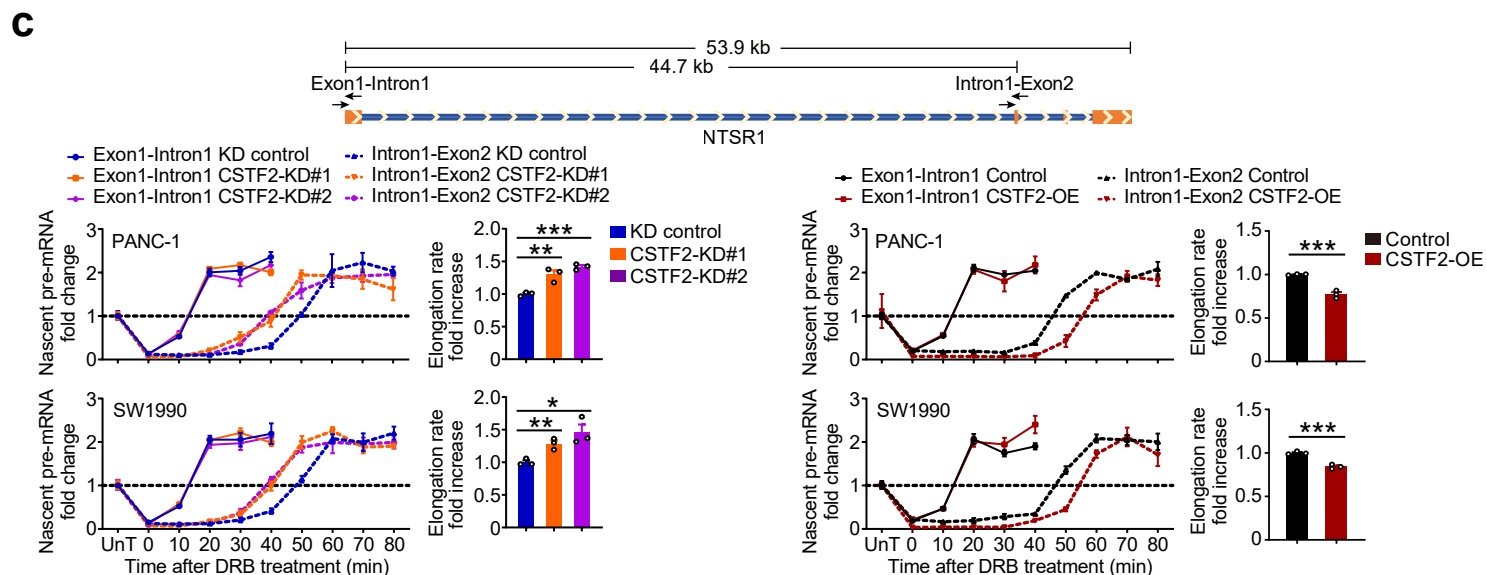
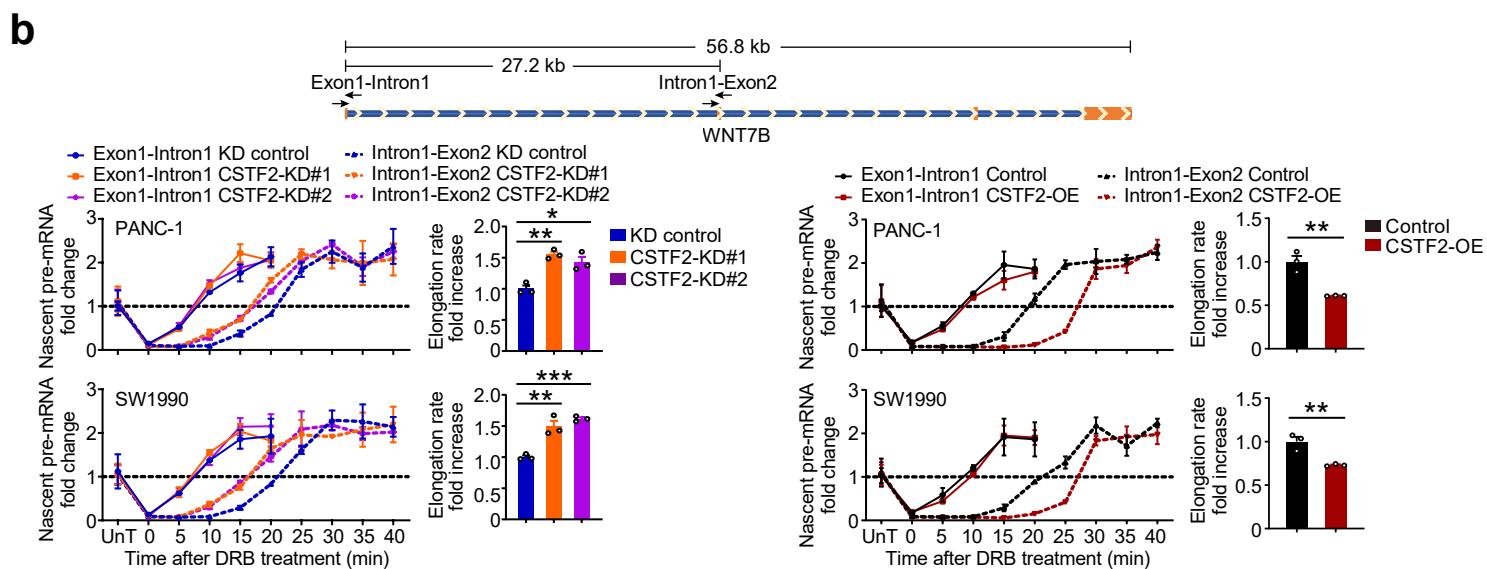
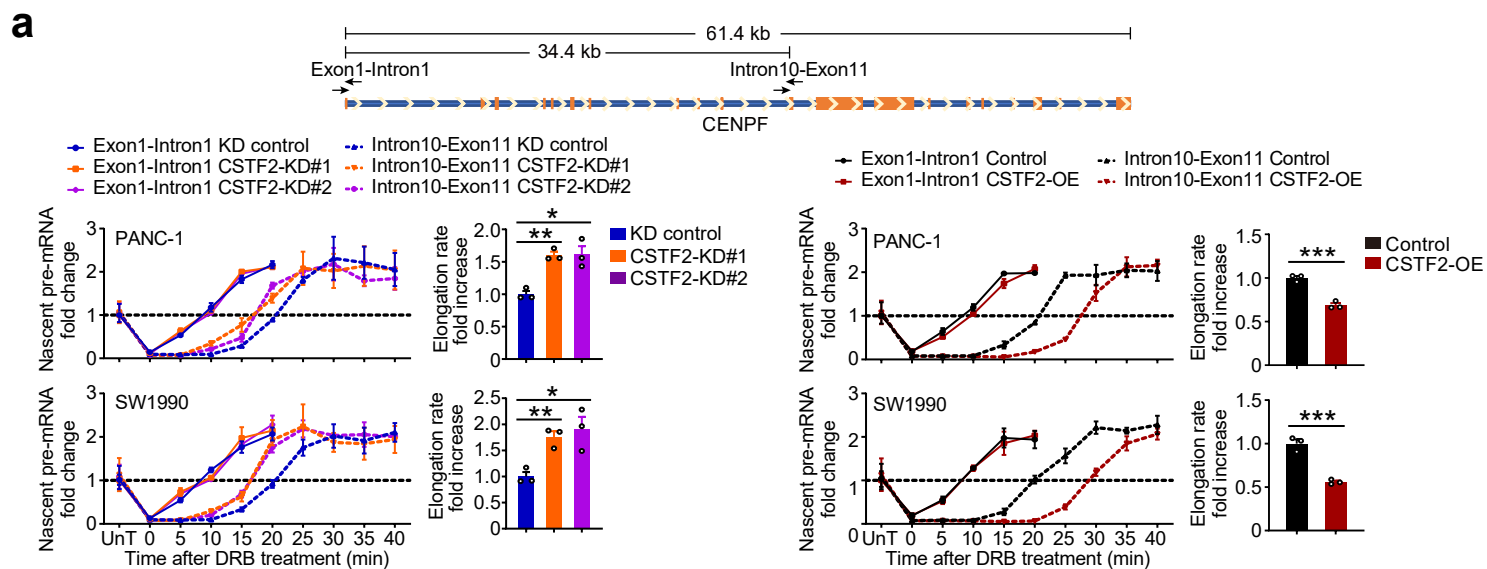
Supplementary Fig. 4. Knockdown of CSTF2 inhibits proliferation and metastasis of PDAC cells.

a *CSTF2* expression levels in PANC-1 and SW1990 cells with or without *CSTF2* KD. **b** Cell cycle profiling shows the effect of *CSTF2* KD on cell cycle distribution. **c** *CSTF2* expression levels in PANC-1 and SW1990 cells with or without forced *CSTF2* expression. **d** Western blot analysis of *CSTF2* in PDAC cells upon *CSTF2* KD with or without rescued with shRNA-resistant *CSTF2*. **e, f** Inhibition of proliferation (**e**), migration and invasion (**f**) upon *CSTF2* KD can be rescued by shRNA-resistant *CSTF2*. **g, h** Malignant phenotypes promoted by forced *CSTF2* expression can be partially rescued by knockdown of specific *CSTF2*-m⁶A target (*CENPF*, *WNT7B*, *NTSR1*) respectively. Data are the mean $\pm$ S.E.M. of three independent experiments. Scale bars, 200 μ m. *, $P < 0.05$; **, $P < 0.01$ and ***, $P < 0.001$ of Student's *t*-tests compared with each control.



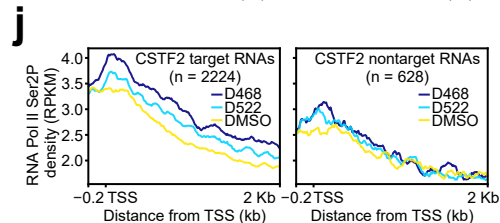
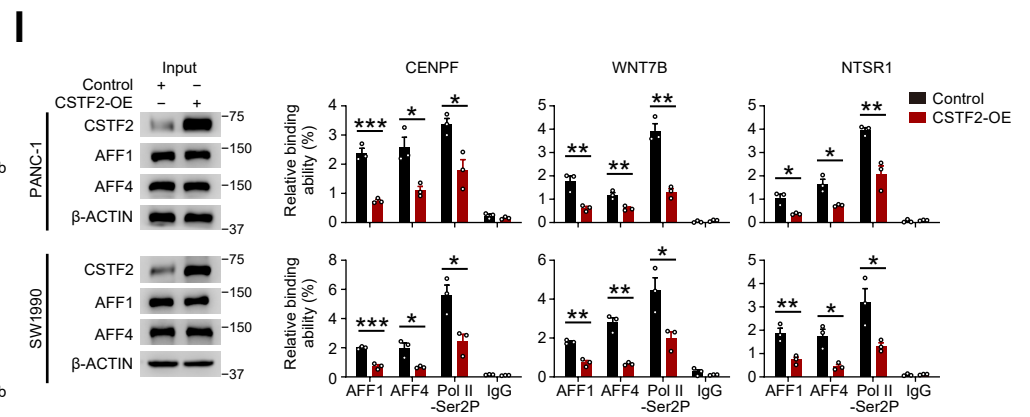
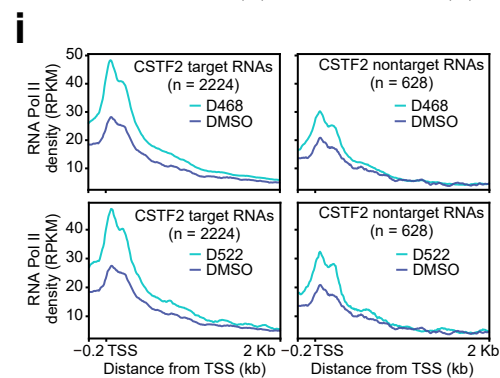
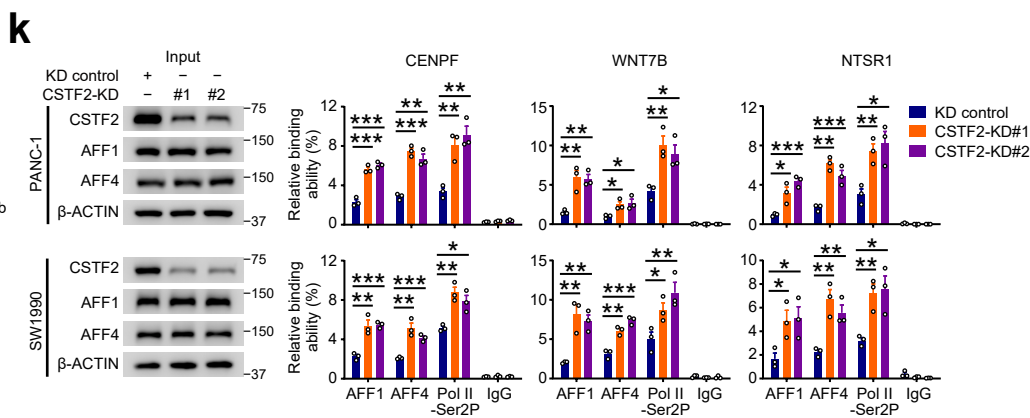
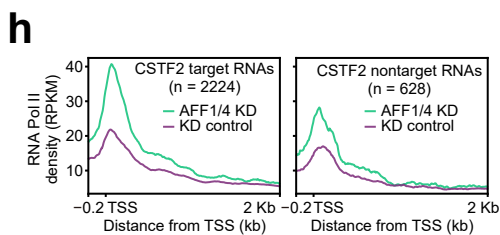
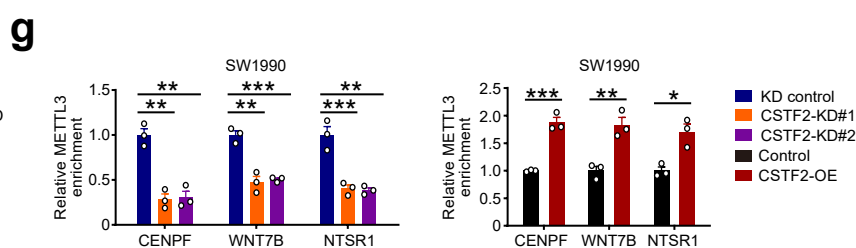
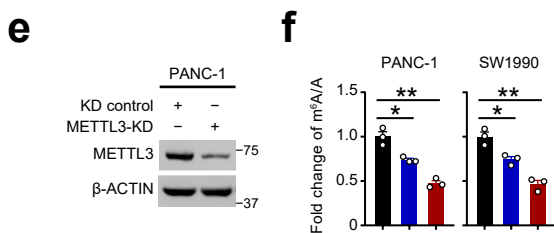
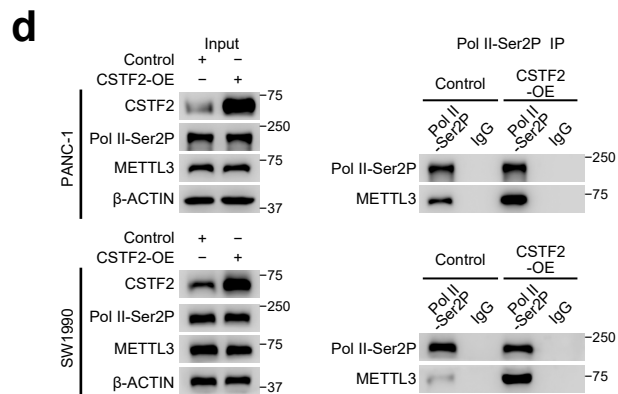
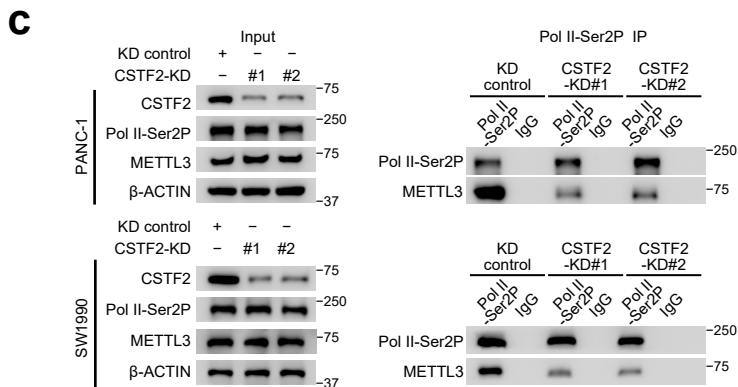
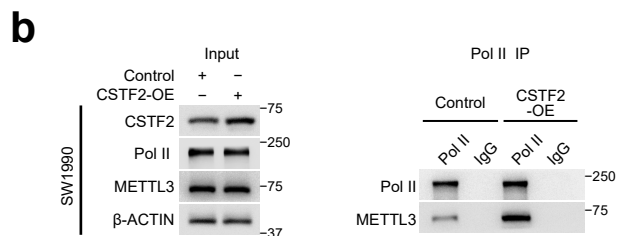
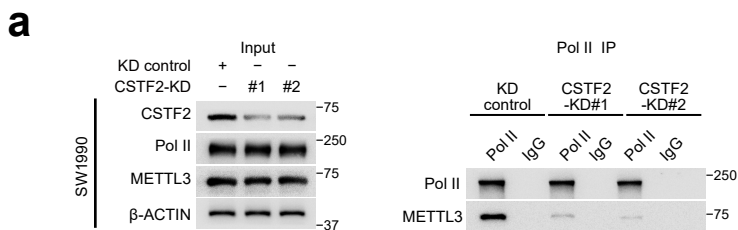
Supplementary Fig. 5. CSTF2 promotes mRNA-m⁶A modifications co-transcriptionally.

a and **b** Effects of *CSTF2* knockdown in PANC-1 cells on mRNA and protein levels of known writers (METTL3, METTL14, WTAP) and erasers (FTO, ALKBH5, RBM15, RBM15B) detected by qRT-PCR (**a**) or western blot (**b**). Values are mean $\pm$ S.E.M. in $n = 3$ independent experiments in **a**. **c** The subcellular distribution of methyltransferase complex upon *CSTF2* KD. **d** The intact methyltransferase complex was not affected upon *CSTF2* KD. **e** Global APA profiling in PANC-1 upon *CSTF2* KD. **f** Western blot analysis of CSTF2T in PANC-1 upon *CSTF2* KD. **g** Metagene analysis of the m⁶A sites identified in miCLIP sequencing in PANC-1 cells. **h** The proportions of m⁶A sites in mRNAs, lncRNAs, pseudogene RNAs and other type RNAs (*upper panel*) and in the 5'UTR, CDS, 3'UTR and stop-codon of mRNAs and intron (*lower panel*). **i** Metagene analysis of CSTF2-binding sites identified in CSTF2 HITS-CLIP sequencing in PANC-1 cells. **j** The proportion of CSTF2-binding sites in mRNAs, lncRNAs, pseudogene RNAs and other type RNAs (*upper panel*) and in the 5'UTR, CDS, 3'UTR and stop-codon of mRNAs and intron (*lower panel*). **k** Comparison of the RNA Pol II-Ser2P, H3K36me3, H3K79me2 and RNA Pol II-Ser5P density along the mRNA (*left*: CSTF2 target RNAs; *right*: CSTF2 nontarget RNAs) with hypo m⁶A upon *CSTF2*-KD in PANC-1 cells with or without *CSTF2*-KD. **l** Shown are examples of transcripts experiencing m⁶A level change and Pol II/Pol II-Ser2P binding densities change with or without *CSTF2* KD. CSTF2 binding in DNA and RNA were also revealed by CUT&Tag-seq and HITS-CLIP-seq, respectively. **m** Scheme of DRB/GRO-seq in this study. **n** Elongation rate of CSTF2 target RNAs was measured by DRB/GRO-seq in PANC-1 cells with or without *CSTF2* KD. *P* values were from two-sided student's *t*-tests in **a**, from DRMEM motif analysis in **g**, **i**, and from Kolmogorov-Smirnov test in **m**, respectively.



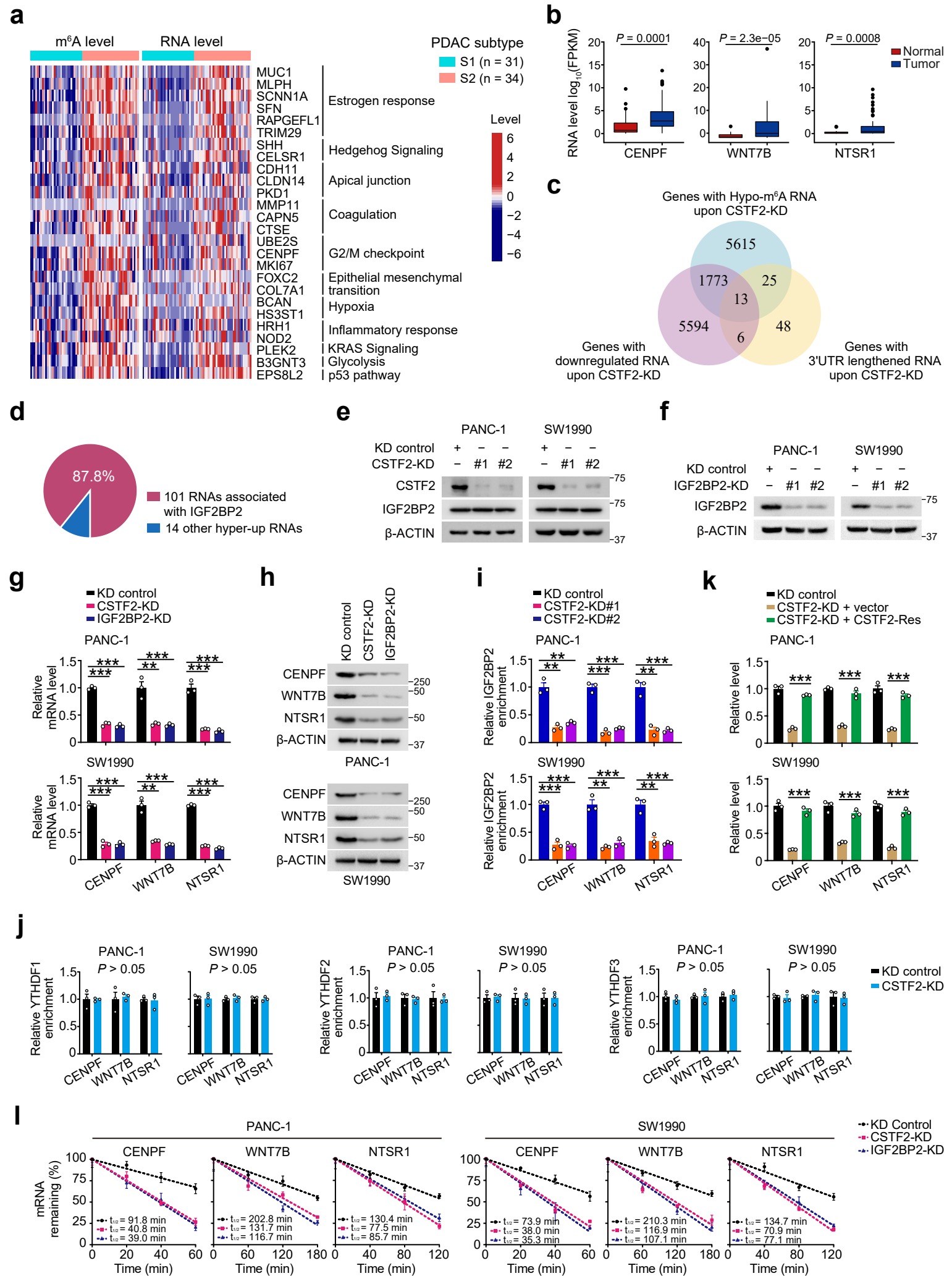
Supplementary Fig. 6. CSTF2 promotes mRNA-m⁶A via slowing elongation.

a–c Diagram demonstrating the positions of PCR products in investigating the DRB-treated RNA Pol II elongation rate. Nascent mRNA production in two different regions of *CENPF* (**a**), *WNT7B* (**b**), *NTSR1* (**c**) after release from DRB inhibition in PANC-1 (*upper panel*) and SW1990 (*lower panel*) cells with *CSTF2* KD (*left panel*) or overexpression (*right panel*). The histograms show fold changes of elongation rates. Data in this figure are means $\pm$ S.E.M. ($n = 3$) from three independent experiments. **d, e** 5-ethynyluridine (EU) labeling affected by *CSTF2* KD (**d**) or overexpression (**e**) in SW1990 cells. Shown are representative images (*left panel*) of three independent experiments and quantification analysis of EU signals from one representative experiment (*right panel*). Scale bar, 100 μ m. Boxplots indicate median (middle line), 25th, 75th percentile (box) and 5th and 95th percentile (whiskers) (Cell number: $n = 33, 40$ and 36 for KD control, *CSTF2*-KD#1 and *CSTF2*-KD#2; $n = 38$ and 27 for Control and *CSTF2* OE, respectively). *, $P < 0.05$; **, $P < 0.01$ and ***, $P < 0.001$ of Student's *t*-test.



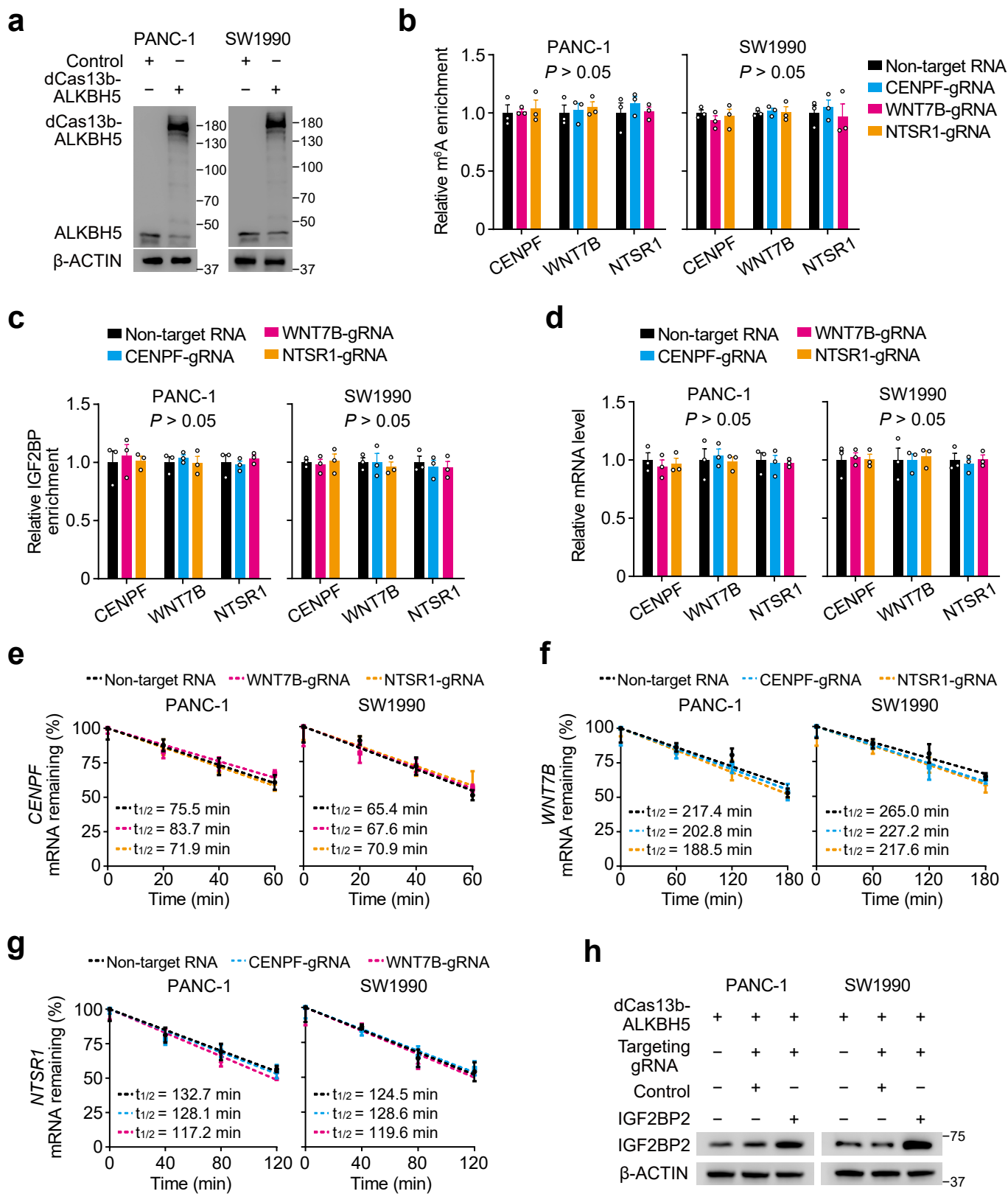
Supplementary Fig. 7. CSTF2-induced moderate elongation facilitates METTL3-catalyzed m⁶A modifications.

a, b Western blotting analysis of the immunoprecipitated product by antibody against Pol II showing effects of *CSTF2* KD (**a**) or *CSTF2* overexpression (**b**) on the interaction between Pol II and METTL3 in SW1990 cells. **c, d** Western blotting analysis of the immunoprecipitated product by antibody against Pol II Ser2P showing effects of *CSTF2* KD (**c**) or *CSTF2* overexpression (**d**) on the interaction between Pol II and METTL3 in PANC-1 (*upper*) and SW1990 (*lower*) cells. **e** Western blotting analysis of METTL3 in PANC-1 upon *METTL3* knockdown. **f** The effect of *CSTF2* or *METTL3* expression change on m⁶A level in PANC-1 and SW1990 cells determined by m⁶A-LC-MS. **g** CLIP-qPCR showed that *CSTF2* KD impaired (*left panel*) but *CSTF2* overexpression enhanced (*right panel*) the binding ability of METTL3 to target transcripts in SW1990. **h, i** Effects of AFF1/4 knockdown (**h**) or AFF inhibitor (D468 and D522) treatment (**i**) on RNA Pol II density along the mRNA (*left*: *CSTF2* target RNAs; *right*: *CSTF2* nontarget RNAs) in HEK293T cells. **j** Effects of AFF inhibitor (D468 and D522) treatment on RNA Pol II-Ser2P density along the mRNA (*left*: *CSTF2* target RNAs; *right*: *CSTF2* nontarget RNAs) in HEK293T cells. **k, l** ChIP-qPCR showed that *CSTF2* KD enhanced (**k**) but *CSTF2* overexpression impaired (**l**) the enrichment of AFF1, AFF, Pol II-Ser2P to target genes in PDAC cells. Data in **f, g, k, l** are means $\pm$ S.E.M. in (n = 3) independent experiments. *, $P < 0.05$; **, $P < 0.01$ ** and ***, $P < 0.001$ of Student's *t*-test compared with each control.



Supplementary Fig. 8. Hyper m⁶A methylation enhances mRNA stability.

a Heatmap showing the differential m⁶A and RNA level of indicated genes involved in distinct pathways between S2 (n = 34) and S1 (n = 31) PDAC tissues. **b** Box plot showing differential expression of indicated genes in PDAC tissues compared with matched normal tissues. Boxplots indicate median (middle line), 25th, 75th percentile (box) and 5th and 95th percentile (whiskers). *P* values were from Wilcoxon rank-sum test. **c** Venn plot of genes with hypo-methylated upon *CSTF2* KD, genes with lengthened 3'UTR upon *CSTF2* KD and genes with downregulation upon *CSTF2* KD. **d** Pie chart showing the percentage of IGF2BP2-positively correlated RNAs in all S2-hyper-up RNAs. **e** Western blotting analysis of *CSTF2* and IGF2BP2 in PDAC cells upon *CSTF2* KD. **f** Western blotting analysis of IGF2BP2 in PDAC cells upon *IGF2BP2* KD. **g, h** Effect of *CSTF2* KD or *IGF2BP2* KD on RNA (**g**) and protein (**h**) level of *CENPF*, *WNT7B*, *NTSR1* determined by qRT-PCR and western blotting, respectively. **i** Relative binding ability of IGF2BP2 to indicated RNAs upon *CSTF2* KD determined by CLIP-qPCR. **j** CLIP-qPCR showed the binding ability of YTHDF1, YTHDF2, YTHDF3 to target transcripts in cells with or without *CSTF2* KD. **k** Relative RNA level of *CENPF*, *WNT7B*, *NTSR1* upon *CSTF2* KD with or without rescued with shRNA-resistant *CSTF2* determined by qRT-PCR. **l** Half-lives of target transcript upon *CSTF2* KD or *IGF2BP2* KD determined by qRT-PCR. Data of **g, i-l** were mean ± S.E.M. in (n = 3) independent experiments. **, *P* < 0.01; ***, *P* < 0.001 of Student's *t*-test compared with each control.



Supplementary Fig. 9. Effects of m⁶A level alteration by using the dCas13 based m⁶A editing system.

a Western blot analysis of dCas13 based m⁶A editing system. **b** Relative m⁶A enrichment of indicated transcript detected by MeRIP-qPCR upon transfected with dCas13b-ALKBH5 and non-target gRNA or gRNA targeting m⁶A of specific RNA, respectively. **c** Relative IGF2BP2 enrichment of indicated transcript detected by CLIP-qPCR upon transfected with dCas13b-ALKBH5 and non-target gRNA or gRNA targeting m⁶A of specific RNA, respectively. **d-g** Relative mRNA level (**d**) and half-lives (**e-g**) of *CENPF*, *WNT7B*, *NTSR1* detected by qRT-PCR upon transfected with dCas13b-ALKBH5 and non-target gRNA or gRNA targeting m⁶A of indicated RNA, respectively. **h** Western blot analysis of dCas13 based m⁶A editing system with or without forced expression of IGF2BP2. Data were mean $\pm$ S.E.M. of three independent experiments in **b-g**. *P* value were from Student's *t*-test compared with each control.

Supplementary Table 1. Baseline demographic and clinical characteristics of PDAC patients in this study

	PDAC S1 (n = 31)	PDAC S2 (n = 34)	P value⁵
Age, mean (SEM¹)	59.1 (2.1)	62.7 (2.3)	0.266
Sex, N (%)			0.184
Male	15 (48.4)	22 (64.7)	
Female	16 (51.6)	12 (35.3)	
Smoking status², N (%)			> 0.5
Smoker	9 (29.0)	11 (32.4)	
Nonsmoker	22 (71.0)	23 (67.6)	
Drinking status³, N (%)			0.456
Drinker	5 (16.1)	8 (23.5)	
Nondrinker	26 (83.9)	26 (76.5)	
Tumor differentiation, N (%)			> 0.5
Well	6 (19.4)	7 (20.6)	
Moderate	20 (64.5)	20 (58.8)	
Poor	5 (16.1)	7 (20.6)	
Neural invasion, N (%)			0.039
Yes	7 (22.6)	16 (47.1)	
No	24 (77.4)	18 (52.9)	
Vascular invasion, N (%)			> 0.5
Yes	6 (19.4)	7 (20.6)	
No	25 (80.6)	27 (79.4)	
Lymph node metastasis, N (%)			0.375
Yes	13 (41.9)	18 (52.9)	
No	18 (58.1)	16 (47.1)	
Tumor stage⁴, N (%)			0.264
I+ II	23 (74.2)	29 (85.3)	
III+ IV	8 (25.8)	5 (14.7)	
Survival status, N (%)			0.039
Alive	8 (25.8)	2 (5.9)	
Deceased	23 (74.2)	32 (94.1)	
Stromal content, mean (SEM)	31.8 (1.4)	30.7 (1.6)	> 0.5

¹SEM, standard error of mean.

²Individuals who smoked an average of <1 cigarette/day and for <1 year in their lifetime were defined as nonsmokers; otherwise, they were defined as smokers.

³Individuals were classified as drinkers if they drank at least twice a week and continuously for at least 1 year during their lifetime; otherwise, they were defined as nondrinkers.

⁴Tumor TNM staging were reviewed by at least 3 pathologists and defined according to the American Joint Committee on Cancer (AJCC) 7th edition.

⁵P values were calculated by two-sided Student's *t*-tests (age and stromal content) or Fisher's exact test .

Supplementary Table 2. Validation results of selected differentially methylated m⁶As between PDAC and normal tissues by MeRIP qRT-PCR method

m ⁶ A ¹	Gene symbol	RNA type ²	Region ³	m ⁶ A specific qRT-PCR						
				Upstream Primer Sequence (5'→3')	Downstream Primer Sequence (5'→3')	Average level in tumor	Average level in normal	Fold change (T/N)	P value ⁴	Validated
chr1:156637890-156638313	RP11-284F21.9	LncRNA	exon	ATGAGCTGGTCAGACCGCGCTTTG	CCATAGGAGGCTGGGAGAGGCACT	0.81	0.15	5.33	3.88E-05	Yes
chr1:178865092-178865816	ANGPTL1	mRNA	CDS	AAGATACCCTCGTGCCACAGATGGT	TTCTGCCTGGAGAGCACATCCTT	1.26	2.33	0.54	8.34E-03	Yes
chr1:36319598-36321379	SH3D21	mRNA	3UTR	AGGTGATGCAGGGGACCCAGAA	TAGGCAGAGTTTACTTTCTCGCGTT	2.31	1.10	2.09	1.10E-03	Yes
chr11:618614-619205	CDHR5	mRNA	CDS	ACAACCTCTGAGGCCACCAACCTCGT	TGGTTGGTGGGAGGTGCTGGTT	1.74	1.03	1.69	7.03E-03	Yes
chr11:77123734-77124356	CAPN5	mRNA	3UTR	TCAACTGTCAACCACTGCTAAG	AGAACACCAGGTATTGCTGAT	1.72	1.15	1.50	5.57E-08	Yes
chr15:90919736-90920346	MAN2A2	mRNA	3UTR	TCTATTTGGAGCCCATTGGAGAT	ATGAGGGCAGAGCCCAGTGT	1.28	1.86	0.69	4.39E-02	Yes
chr16:23690323-23691518	ERN2	mRNA	Stop codon	AGAAGCACCACTACAGGGAGC	AGTCTGGCGGGTAGTAGGGCAGGA	1.48	0.66	2.26	7.08E-06	Yes
chr16:2831942-2832405	ZG16B	mRNA	intron	AGCAAGGACCGCTATTTCTATT	AGTTTGCTGAGTATGTGAGATTAA	1.47	0.55	2.69	1.50E-06	Yes
chr16:68698181-68698864	CDH3	mRNA	3UTR	TTCAAGAAGCTGGCAGACATGTA	ACTTCCTGACAAGCTCCGAAG	1.51	0.79	1.90	2.87E-09	Yes
chr16:86581265-86581619	FOX1	mRNA	3UTR	TGTTGGTCAATGTGTTGGAAGGAC	AGCCTGGATCTCAGCAATGGCTCCT	1.45	0.77	1.88	4.49E-07	Yes
chr17:43861966-43862983	CD300LG	mRNA	3UTR	ATTCTGGCTTCTCTTTGAACCA	AATGCAGACTCTAAGCTGGAG	0.56	1.01	0.55	6.91E-03	Yes
chr17:80964686-80965088	RPTOR	mRNA	3UTR	AAGAGCGAGCGAGAGGCGCT	GCTTCCTGCTCAGTGATGCT	1.39	2.36	0.59	7.61E-03	Yes
chr19:41206396-41207418	CYP2S1	mRNA	3UTR	ACTGACCTTCACTCCACCACGCA	TCTTAGCAGCATGAGTCAAGTGA	1.32	0.70	1.90	5.11E-04	Yes
chr19:48702801-48704688	FUT2	mRNA	CDS	ATCTTCAGAATCACCCGTGCCG	TGAACCTCTGGAGGATCTCCT	1.95	1.07	1.81	2.52E-10	Yes
chr19:49064299-49064759	CTB-60B18.23	mRNA	intron	TCTGAGGGAGAAGGGTGCTG	TCAGACCGAGTAGTGCAGAC	1.09	0.39	2.76	4.34E-03	Yes
chr2:5696221-5697163	SOX11	mRNA	3UTR	AAGGAGAGAAAGTGCTGGTGTCTGT	ACAGAGGCCATGCCATGTTTCCT	0.74	0.35	2.12	3.39E-12	Yes
chr20:54028271-54028882	BCAS1	mRNA	CDS	AGGGATCCCACGCTTCTCCACCT	ACTGCCCTGATAAGCCAGGAA	2.95	1.42	2.08	3.90E-16	Yes
chr20:63529193-63529722	PTK6	mRNA	3UTR	ATGTCCAACCATGAGGCCCTTCCTGA	ATGCCCGCTCCACAGCAGCTCAGGT	1.25	0.64	1.95	3.30E-04	Yes
chr22:45921180-45921758	WNT7B	mRNA	3UTR	TATCAAGCAAATCAAAATATCAC	AAGTGGGACAAACATCCCTGA	1.45	0.29	4.97	9.63E-13	Yes
chr3:124927664-124928084	MUC13	mRNA	CDS	AGCTGATACCACTGAACTAATT	AGGTAGCTAATGAATTTACAT	1.18	0.90	1.31	2.90E-02	Yes
chr3:195789583-195791565	MUC4	mRNA	CDS	AAGCTACAGTGTGACTCAGATGAT	TGAGTGTGACCCCTTTGGGAAA	1.13	0.47	2.41	2.75E-05	Yes
chr5:135031120-135031681	PITX1	mRNA	CDS	AGGGAAGACCGCCAGCGCTGGT	TGAAGTGCGTACGTTGCCGCCGCT	2.09	0.66	3.18	4.28E-13	Yes
chr5:160563837-160564139	ATP10B	mRNA	3UTR	TATCACTTGAGAACCTCCTCAGCAA	AGCTCCAGGACAGTGCTGCAAAAT	1.16	0.62	1.86	1.50E-04	Yes
chr5:41312412-41312820	PLCXD3	mRNA	intron	TACAAGTGTGTTAAATTGAA	AATTCCTTCCTTCCTTCTGTCT	1.97	2.84	0.69	8.70E-02	No
chr6:10397719-10398393	TFAP2A	mRNA	3UTR	GTGAAGCCAGCAGAGAAAAGTT	AACTGAAGTATGTAAGGGAAGGTGG	0.96	0.36	2.67	1.76E-07	Yes
chr6:30948675-30952367	DPCR1	mRNA	CDS	ATCTCTAGCAGAGCCTACAGAA	TCATTGGCTGTCTTTCTCTATT	1.78	0.62	2.86	1.84E-09	Yes
chr7:29566178-29567256	PRR15	mRNA	Stop codon	AGCCCGGACTGGACCAGCAGCT	TCACCAGGAAAGCCTGCCTCCT	1.77	0.80	2.21	8.36E-10	Yes
chr8:101688671-101689421	NCALD	mRNA	intron	AGCAGTGCCGCGCCAGTTCTGA	AGCTGAAGGCGTCACGGAGGAA	1.08	1.74	0.62	3.40E-04	Yes
chr8:143743217-143743756	FAM83H-AS1	mRNA	intron	TGCATCTTTGTAAACATTAGACAT	TTAAATATGCACTGTGAGGCAGATG	1.09	0.46	2.39	1.15E-07	Yes

¹ m⁶As are represented by human hg38 genomic positions.

² LncRNA, long non-coding RNA

³ The regions in mRNA structure.

⁴ Student's *t*-test.

Supplementary Table 3. The primers sequences used in this study

Primers for quantitative real time-PCR		
Gene symbol	Forward primer (5'→3')	Reverse primer (5'→3')
<i>CSTF2</i>	AGCACTTCGAGTGGACAATGC	AAGGTGACTCAATGACAGGGG
<i>METTL3</i>	TTGTCTCCAACCTTCCGTAGT	CCAGATCAGAGAGGTGGTGTAG
<i>METTL14</i>	GAGTGTGTTTACGAAAATGGGGT	CCGTCTGTGCTACGCTTCA
<i>WTAP</i>	TTGTAATGCGACTAGCAACCAA	GCTGGGTCTACCATTGTTGATCT
<i>FTO</i>	AACACCAGGCTCTTTACGGTC	TGTCCGTTGTAGGATGAACCC
<i>ALKBH5</i>	CGGCGAAGGCTACACTTACG	CCACCAGCTTTTGGATCACCA
<i>RBM15</i>	GTGAGGACTCGACTTCCCG	GCCGCTATCGGTCTTTCCG
<i>RBM15B</i>	TACACGGAGGCTACCAGTACA	GTCGTACAGCCCGTAGTAGTC
<i>IGF2BP2</i>	TCGAGACCCTCTCGGGTAAA	GTTGACAACGGCGGTTTCTG
<i>CENPF</i>	GGGAGAATGATTCACTTA	TCGGCATCAAGAATCACT
<i>WNT7B</i>	GAGGATTCTCGGCACTAACA	GGAAAGGAAACAGAGGGTGT
<i>NTSR1</i>	AGGACACCACATGGGAGGG	CACAGCCACAAGCAAAGCA
<i>U2AF2</i>	TCTTCAACGCCAGATGC	CGTTCAGGTAGTTGGGTAAGC
<i>CAPRIN1</i>	AACGGTTGAGGTGGTAAAT	GCAGATACAATGGCAGGAT
<i>BUD13</i>	TCTGAAGCGTTACTTGTCC	ATGTCTGTTTGAGGGTAGGT
<i>β-ACTIN</i>	CAGGGCGTGATGGTGGGCATG	GTAGAAGGTGTGGTGCCAGATT
Primers for MeRIP-qPCR, CLIP-qPCR and ChIP-qPCR		
<i>CENPF</i> -m ⁶ A	GGAGCTAGTGATTCTTGA	TCTGAAGCATCTCCACTG
<i>WNT7B</i> -m ⁶ A	CCAGGGGTCTTGGTGCTC	GCCTGGGAACTGGTCTGG
<i>NTSR1</i> -m ⁶ A	GTGCTTTGCTTGTGGCTGTG	CCTGGGACCTGGCATCTTAC
Primers for DRB-qPCR		
<i>CENPF</i> Exon1-Intron1	CGAGAGGTCGTTTTCCCGT	GGGGCGGATTCTCCAGTTC
<i>CENPF</i> Exon10-Intron11	ACTTGAGCATTTCGTTTTGGCT	CAGGTGGCAGACTTCTCTGG
<i>WNT7B</i> Exon1-Intron1	TTTCTCTGCTTTGGCGTCCT	CAGCTCCTTCGTGCTGTCTT
<i>WNT7B</i> Intron1-Exon2	GTGACTGAGAGGCTGAGTGG	GGCCAGGAATCTTGTTGCAG
<i>NTSR1</i> Exon1-Intron1	TATGCTGTTACCATGGGCG	TCCCACTCCCTACCCAGAAG
<i>NTSR1</i> Intron1-Exon2	CAGCTGAGGGTGCATGAGTC	AGCTTGTTGGCGATGATGGT

Supplementary Table 4. The RNA knockdown and gRNA sequences used in this study

	RNA knockdown sequences (5'→3')
KD Control	UUCUCCGAACGUGUCACGUTT
<i>CSTF2</i> -KD#1	GACCAAGAGACAGCACUUATT
<i>CSTF2</i> -KD#2	GGGAGGCACUUUACUUUCUTT
<i>U2AF2</i> -KD#1	GCAGUACAAGGCCAUGCAATT
<i>U2AF2</i> -KD#2	CCAACUACCUGAACGAUGATT
<i>CAPRIN1</i> -KD#1	GGAGCAGCUUAUGAGAGAATT
<i>CAPRIN1</i> -KD#2	CCAUGCAAACGGUGUUCAATT
<i>BUD13</i> -KD#1	CUGCCAGAAAGCAACUGAUTT
<i>BUD13</i> -KD#2	GCUCAAGGAACAGGAUCAATT
<i>CENPF</i> -KD#1	GACCCAGAAACUAGCUUAUTT
<i>CENPF</i> -KD#2	CCAGCUUCGUGGAGAAUUATT
<i>WNT7B</i> -KD#1	GCAGGGCUACUACAACCAATT
<i>WNT7B</i> -KD#2	CCACCUUCCUGCGCAUCAATT
<i>NTSR1</i> -KD#1	GCACAUUCAGCAUGGCCAUTT
<i>NTSR1</i> -KD#2	GCCUCAUGUUCUGCUACAUTT
<i>METTL3</i> -KD#1	GGAGATCCTAGAGCTATTA
<i>METTL3</i> -KD#2	GCACATCCTACTCTTGTA
<i>IGF2BP2</i> -KD#1	CAGUUUGAGAACUACUCCUTT
<i>IGF2BP2</i> -KD#2	GAAACAGGGACCAAGAUAATT
	gRNA sequence for dCas13b-ALKBH5 system (5'→3')
Non-Target-gRNA	GTAATGCCTGGCTTGTGACGCATAGTCTG
<i>CENPF</i> -m ⁶ A-gRNA	CAGACCTTAACGTGACAAGGTCTAATTCAAAAACCTTTCAG
<i>WNT7B</i> -m ⁶ A-gRNA	CCCAGAGAGAAGAAAGAGAACTTGCCGGGCCCCGTCTGGGG
<i>NTSR1</i> -m ⁶ A-gRNA	CTCCAGACCTCAGCCTGCACAGCCACAAGCAAAGCACACG