

1 **CPSF3 inhibition blocks pancreatic cancer cell proliferation through**
2 **disruption of core histone processing**

3
4
5
6
7 Abdulrahman. A. Alahmari^{1,2}, Aditi H. Chaubey¹, Arwen A. Tisdale¹, Carla D. Schwarz¹,
8 Abigail C. Cornwell¹, Kathryn E. Maraszek¹, Emily J. Paterson¹, Minsuh Kim¹, Swati
9 Venkat¹, Eduardo Cortes Gomez³, Jianmin Wang³, Katerina V. Gurova⁴, and Michael E.
10 Feigin¹

11
12
13
14 ¹Department of Pharmacology and Therapeutics, Roswell Park Comprehensive Cancer Center, Buffalo,
15 NY 14203

16 ²Department of Medical Laboratory Sciences, Prince Sattam Bin Abdulaziz University, Alkharj 16278,
17 Saudi Arabia

18 ³Department of Biostatistics and Bioinformatics, Roswell Park Comprehensive Cancer Center, Buffalo,
19 NY 14203

20 ⁴Department of Cell Stress Biology, Roswell Park Comprehensive Cancer Center, Buffalo, NY 14203

21
22 Correspondence may be addressed to: MEF (michael.feigin@roswellpark.org), Twitter:
23 @TheFeiginLab

24
25
26 The authors declare no potential conflicts of interest.

27
28 Running title: Targeting CPSF3 in pancreatic ductal adenocarcinoma

29 **Abstract**

30 Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with limited effective treatment
31 options. This potentiates the importance of uncovering novel drug targets. We have discovered
32 global dysregulation of the gene regulatory process alternative polyadenylation (APA) in PDAC.
33 APA is a pre-mRNA processing mechanism that generates mRNAs with distinct 3' ends,
34 impacting gene expression and protein function. We revealed that APA dysregulation in PDAC
35 drives oncogenic signatures and predicts poor patient outcome. As APA directs widespread gene
36 expression dysregulation across the PDAC patient population, we hypothesized that inhibition of
37 APA has therapeutic potential. APA is controlled by a complex of proteins, including cleavage and
38 polyadenylation specificity factor 3 (CPSF3). CPSF3 is the endonuclease catalyzing mRNA
39 cleavage, and a potentially druggable target. We now find that *CPSF3* is highly expressed and
40 associated with poor prognosis in PDAC patients. CPSF3 knockdown decreases PDAC
41 proliferation and clonogenicity *in vitro* and tumor growth *in vivo*. We demonstrate that *CPSF3*
42 knockdown induces widespread APA alterations of oncogenes and tumor suppressors, and
43 determine the contribution of one of these events to CPSF3-induced cell proliferation phenotype.
44 Furthermore, we find that PDAC, but not non-transformed pancreatic cells, are sensitive to the
45 CPSF3 small molecule inhibitor JTE-607. Mechanistically, JTE-607 impairs replication-dependent
46 histone processing, disrupting nucleosome assembly and destabilizing chromatin structure.
47 Finally, we determine that JTE-607 attenuates cell proliferation by arresting cells in early S-phase
48 of the cell cycle. Altogether, we identify CPSF3 as a druggable target in PDAC and reveal novel
49 mechanisms by which CPSF3 controls cancer cell growth.

50

51 **Significance:** This work identifies CPSF3 as a potential drug target in pancreatic ductal
52 adenocarcinoma and reveals new mechanisms by which CPSF3 inhibition attenuates PDAC cell
53 proliferation through modulating alternative polyadenylation and histone processing.

54 Introduction

55 Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer deaths with a five-
56 year survival rate of 11%, due in part to the lack of effective treatment options (1). PDAC is
57 primarily driven by mutations in the oncogene *KRAS* and several tumor suppressors, including
58 *TP53*, *CDKN2A* and *SMAD4* (2). However, as clinically effective modulators of activity of these
59 proteins are not currently available, identification of novel targets amenable to small molecule
60 inhibition is a critical undertaking. Recently, large-scale RNA sequencing efforts of PDAC tumors
61 have revealed widespread dysregulation of oncogenic gene expression, allowing the
62 characterization of several PDAC subtypes and phenotypic states (3–6). These gene expression
63 changes are critical for driving tumor phenotypes, including metastatic progression (7–11). While
64 these gene expression changes have been extensively catalogued, the mechanisms underlying
65 this transcriptional heterogeneity remain largely unknown (12). We propose that targeting these
66 drivers of dysregulated gene expression represents an opportunity to reverse widespread
67 oncogenic activity in transformed cells.

68
69 One such gene regulatory process that has been implicated in cancer is alternative
70 polyadenylation, or APA (13–15). APA is a co-transcriptional mRNA processing mechanism that
71 generates distinct transcript isoforms with different 3' untranslated region (UTR) lengths,
72 ultimately affecting mRNA stability, localization and translation (13). Recently, we identified
73 widespread APA alterations in PDAC patients that are associated with functional changes in both
74 gene and protein expression of growth-promoting genes (16). We revealed APA as a new
75 mechanism that regulates PDAC gene expression, identified patterns of APA associated with
76 poor patient outcome, and uncovered novel APA-regulated therapeutic targets. As APA is widely
77 dysregulated across the PDAC patient population, and drives known pro-tumorigenic pathways,
78 we propose that APA inhibition represents a promising therapeutic approach.

79
80 Cleavage and polyadenylation specificity factor 3 (CPSF3) is the endonuclease responsible for
81 mRNA cleavage and is part of two distinct RNA processing complexes (17). One is the APA
82 complex where CPSF3 cooperates with other APA factors to cleave the mRNA prior to the
83 addition of the poly(A) tail. The second is the histone cleavage complex (HCC) where pre-mRNAs
84 of replication-dependent core histones are cleaved by CPSF3 but not polyadenylated. Recently,
85 CPSF3 was identified as the target of the small molecule JTE-607 (18,19). JTE-607 is hydrolyzed
86 into an active compound that directly interacts with the CPSF3 interfacial cavity (19). This
87 interaction inhibits CPSF3 catalytic activity leading to accumulation of unprocessed newly

88 synthesized pre-mRNAs. JTE-607 induces apoptosis of human acute myeloid leukemia (AML)
89 and Ewing sarcoma cells *in vitro* and prolongs survival of tumor-bearing mice in xenograft models
90 *in vivo* (20,21). Notably, administration of JTE-607 in healthy volunteers demonstrated the safety
91 of this compound in humans, with no severe adverse events reported (22). However, the role of
92 CPSF3 and the effect of JTE-607 in epithelial cancers remains largely unknown.

93
94 Here, we show that knockdown and/or inhibition of CPSF3 attenuates PDAC cell proliferation *in*
95 *vitro* and *in vivo*. We find that CPSF3 is highly expressed in PDAC patients and is a predictor of
96 poor outcome. We conduct the first global analysis of CPSF3 loss in cancer, uncovering APA
97 events correlating with expression alterations in tumor suppressors and oncogenes. Additionally,
98 we find that small molecule inhibition of CPSF3 by JTE-607 selectively attenuates proliferation of
99 PDAC cells but not non-transformed cells. Finally, we uncover a new mechanism by which JTE-
100 607 attenuates cell proliferation, through disruption of replication-dependent histone mRNA
101 processing, thus altering chromatin stability and dysregulating the cell cycle.

102
103 To our knowledge, our study is the first to pharmacologically target CPSF3 activity in epithelial
104 cancers in general, and in PDAC specifically. We reveal that CPSF3 disruption blocks cell
105 proliferation through multiple mechanisms, including APA-mediated gene expression alterations
106 and disruption of proliferation-dependent histone mRNA processing. Furthermore, we provide the
107 first connection between CPSF3 inhibition and chromatin stability. Overall, our findings uncover
108 new functions of CPSF3 in cancer and nominate CPSF3 as a novel therapeutic target in PDAC.

109

110 **Materials and Methods**

111 **Cell lines and *in vitro* culture**

112 HEK293T, MiaPaCa2 and Panc1 were purchased from ATCC. Suit2 cells were obtained from Dr.
113 David Tuveson (Cold Spring Harbor Laboratory). Human immortalized C7 CAFs and PancPat
114 CAFs cells were obtained from Dr. Edna Cukierman (Fox Chase Cancer Center). Cells were
115 cultured in DMEM (Corning, DMEM [+] 4.5 g/L glucose, L-glutamine, sodium pyruvate)
116 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Non-
117 transformed pancreatic cell lines HPNE and HPDE were obtained from Dr. Ethan Abel (Roswell
118 Park Comprehensive Cancer Center). HPNE cells were cultured in 75% DMEM+25% Medium M3
119 Base supplemented with 2mM L-glut, 1.5g/L sodium bicarbonate, 5% FBS, 10ng/mL hEGF and
120 5.5mM D-glucose. HPDE cells were cultured in Keratinocyte SFM (serum-free media)
121 supplemented with 25mg BPE, 2.5µg EGF, 1X anti-anti and 50µg/mL Gentamicin. HeLa-TI cells

122 were obtained from Dr. Katerina Gurova (Roswell Park Comprehensive Cancer Center) and were
123 cultured in phenol red-free FluoroBrite DMEM complete Media (ThermoFisher). All cell lines were
124 cultured at 37°C with 5% CO₂ and tested negative for Mycoplasma.

125

126 **Generation of stable CPSF3 knockdown cells**

127 Vectors expressing short-hairpin RNA (shRNA) targeting CPSF3 (sh1 Target sequence:
128 GCTGAGATTGATCTCCTATTA; Clone ID: NM_016207.2-219s1c1, sh2 Target sequence:
129 CCAGTGAATTTATTCGTGCTT; Clone ID: NM_016207.2-1240s1c1) were purchased from
130 Sigma-Aldrich. Cells were infected with lentivirus harboring pLKO.1-shNTC (non-targeting
131 control) and pLKO.1-shCPSF3. Polybrene was used to increase the efficacy of infection. After 72
132 hours, cells were selected with 2.5µg/ml puromycin. Knockdown was confirmed by qPCR and
133 immunoblotting.

134

135 **RNA isolation and quantitative PCR**

136 Cells were lysed with TRIzol reagent (Thermofisher Scientific; Cat # 15596026). RNA was isolated
137 using Direct-zol RNA Miniprep Kit (Zymo Research; Cat # R2050). cDNA was synthesized using
138 iScript cDNA Synthesis Kit (Bio-Rad; Cat# 1708891). qPCR was conducted with SYBR Green
139 PCR primers (CPSF3; Unique Assay ID: qHsaCID0007422, ACTB; Unique Assay ID
140 qHsaCED0036269, HIST1H3B; Unique Assay ID: qHsaCED0007746, HIST1H2BC; Unique
141 Assay ID: qHsaCED0007746, FHL1; Unique Assay ID: qHsaCED0038537) mixed with iTaq
142 Universal SYBR Green Supermix (Bio-Rad; Cat# 1725120) and run on CFX connect systems
143 (Bio-Rad). Data were analyzed in Microsoft Excel and graphed using GraphPad Prism (v 9.3.0).

144

145 **RNA-sequencing and APA analysis**

146 Cells were trypsinized, washed with 1X PBS and sent frozen (-80°C) for RNA sequencing (RNA-
147 seq). 500ng total RNA was used to prepare the sequencing libraries using KAPA RNA HyperPrep
148 Kit with RiboErase (HMR) (Roche Sequencing Solutions) following manufacturer's protocol.
149 Briefly, ribosomal RNA (rRNA) was depleted from total RNA and DNase-digested to remove
150 gDNA contamination. RNA was purified, fragmented and first strand cDNA was synthesized using
151 random primers. cDNA:RNA hybrids were converted into double-stranded cDNA (dscDNA) using
152 dUTP incorporation. Adapters were added to the 3' ends, ligated to library insert fragments and
153 the library amplified in a strand-specific manner. Data were analyzed by the Bioinformatics Shared
154 Resource (Roswell Park Comprehensive Cancer Center). APA analysis was done using Dynamic

155 Analysis of Alternative Polyadenylation from RNA-seq (DaPars) algorithm (23). Exact steps
156 followed for this analysis were previously described in (16).

157

158 **Immunoblotting**

159 Samples were lysed using RIPA lysis buffer (50mM Tris. HCl pH 7.5, 150mM NaCl, 5mM EDTA
160 pH 8, 1% Triton X-100, 0.5% NP-40) in the presence of 10ug/ml protease inhibitors (Aprotinin,
161 Leupeptin, PMSF), boiled at 95°C for 5min and resolved by SDS-PAGE. Proteins were transferred
162 to nitrocellulose membranes (0.2 μ m, Bio-Rad, Cat. # 1620112) at a constant voltage of 100V for
163 70 minutes at 4°C using Mini Trans-Blot® Cell (Bio-Rad). Membranes were blocked in TBST (Tris-
164 buffered saline (TBS) with 0.1% v/v TWEEN-20; Sigma Aldrich) and 5% w/v nonfat dry milk
165 (Blotting-Grade Blocker #1706404, Bio-Rad). Primary antibodies were diluted in 3% BSA in TBST
166 and incubated overnight at 4°C (mouse monoclonal CPSF3 antibody, Abcepta, AT1610a; rabbit
167 polyclonal Histone H3 antibody, Cell Signaling Technology, 9715S; rabbit polyclonal Histone H2B
168 antibody, Cell Signaling Technology, 8135S; mouse monoclonal GAPDH antibody, Proteintech,
169 60004-1-Ig; rabbit polyclonal FHL1 antibody, Proteintech, 10991-1-AP). Membranes were
170 incubated with horseradish peroxidase-conjugated secondary antibodies at 1:2,000 dilution
171 (Donkey anti-rabbit; Fisher Scientific; Catalog number: 45-000-682, or Goat anti-mouse; Sigma-
172 Aldrich; Catalog number: A4416) for 1 hour at room temperature. Pierce ECL Western Blotting
173 Substrate (Thermo Scientific, Catalog number: 32106) and Supersignal West Femto Maximum
174 Sensitivity substrate (Thermo Scientific, Catalog number: 34094) were used for chemiluminescent
175 detection. Signals were visualized and imaged using the ChemiDoc XRS+ System and Image
176 Lab Software (Bio-Rad).

177

178 **Proliferation and clonogenicity assays**

179 For proliferation experiments, cells were seeded at a density of 250 cells/well (MiaPaCa2 and
180 Panc1 cells) or 1000 cells/well (HPNE cells) into a white 96-well plate in triplicate. Cell proliferation
181 was measured using CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega) at days 0, 2, 4
182 and 6. For clonogenicity assays, cells were seeded at a density of 500 cells/well into a 6-well plate
183 in triplicate. After 11 days, cells were fixed with 4% formalin, stained with 0.2% Crystal Violet and
184 images were obtained for analysis. Colony area was measured using ImageJ software. Data were
185 normalized to control data points.

186

187 **Cell cycle analysis**

188 Cells were trypsinized and resuspended in 1X PBS, then fixed with ice-cold 70% ethanol for 1
189 hour at -20°C. Cells were then washed with cold 1X PBS and incubated with RNaseA (200µg/ml)
190 at 37°C for 1 hour. Propidium iodide (40µg/ml) was then added, incubated for 1 hour in the dark
191 and analyzed by FACS at 488nm. Data were analyzed by FCS express software (v7.06.0015).

192

193 **BrdU incorporation assay**

194 Cells were cultured under optimum conditions and incubated with 50µM BrdU (5-Bromo-2'-
195 deoxyuridine; Sigma-Aldrich; B5002) for 4 hours. Cells were then rinsed with 1X PBS, trypsinized,
196 permeabilized in 70% ice cold ethanol with gentle vortexing and stored at -20°C overnight. Next,
197 cells were pelleted and DNA was hydrolyzed by incubating with 500µl of 2N HCl, 0.5% Triton X-
198 100 in 1X PBS, incubated for 30 minutes at room temperature and then neutralized by adding
199 1.5ml of 0.1 M sodium tetraborate (pH 8.5) for 2 minutes. Cells were then pelleted, washed once
200 with 1% BSA in 1X PBS and resuspended in 50µl 0.5% Tween 20, 1% BSA in 1X PBS. Next, 10⁶
201 cells were incubated with 1µg Anti-BrdU-FITC (FITC anti-BrdU Antibody; BioLegend;
202 Cat#364104) for 1 hour at room temperature. Cell pellets were washed once with 150µl 1% BSA
203 in 1X PBS, resuspended in 500µL 1X PBS with RNaseA (200 µg/ml) and PI (40 µg/ml) and
204 incubated at room temperature for 30 minutes in the dark. Cells were analyzed by flow cytometry
205 immediately and a compensation step was performed. Data were analyzed by FCS Express
206 software (v7.06.0015).

207

208 **3' RACE**

209 3' RACE was performed as previously described (16). Briefly, first strand cDNA was synthesized
210 from the poly(A) tail using an adapter primer (5'-
211 GACTCGAGTCGACATCGATTTTTTTTTTTTTTTTTTTT-3'). Gene specific PCR amplification was
212 then performed using a gene specific primer spanning the stop codon (FHL1: 5'-
213 TCCACTGCAAAAATGCTCCGTGA-3') and an adapter-targeting primer (5'-
214 GACTCGAGTCGACATCG-3'). The PCR products were run on a 1.2% agarose gel and visualized
215 and imaged using the ChemiDoc XRS+ System and Image Lab Software (Bio-Rad).

216

217 **Xenograft experiments**

218 Animal experiments were approved by the Roswell Park Institutional Animal Care and Use
219 Committee. MiaPaCa2 cells infected with shNTC and sh1 CPSF3 constructs were trypsinized,
220 washed with 1X PBS and counted. 5x10⁵ cells were resuspended in 50µl of 1X PBS/Matrigel
221 (Corning Life Sciences, 356231) in a 1:1 ratio and injected subcutaneously into the flanks of 8-

222 week old NOD/SCID/IL2R $\gamma^{-/-}$ (NSG) mice. When palpable, tumor volume was determined by
223 caliper measurements obtained in 2 dimensions and calculated as width² x length/2 twice a
224 week. Mice were euthanized when the first tumor reached 1400 mm³, tumors were dissected,
225 and tumor volumes were measured.

226

227 **JTE-607 studies**

228 For dose-response measurements, cells were seeded at a density of 1000 cells per well in a 96-
229 well white plate. The next day, JTE-607 was titrated over a range of concentrations using the
230 Tecan D300e Digital Dispenser and cell viability was measured 72 hours post drug titration using
231 a CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega). For cell proliferation experiments,
232 cells were seeded at a density of 250 cells per well in a 96-well white plate. DMSO control or JTE-
233 607 was dispensed at varying concentrations and proliferation was measured using CellTiter-Glo
234 Assay at days 0, 2, 4 and 6. For clonogenicity experiments, cells were seeded at a concentration
235 of 500 cells per well and treated with different concentrations of JTE-607. Cells were allowed to
236 grow over a period of 11-14 days after which they were fixed in 4% formalin, stained with 0.2%
237 Crystal Violet and images were obtained for analysis. Colony area was measured using ImageJ
238 software. Data were normalized to DMSO control data points.

239

240 **Micrococcal digestion**

241 Micrococcal Nuclease (MNase) was performed as previously described (24). Briefly, cells were
242 trypsinized, washed with 1X RSB buffer (10mM Tris HCL, pH7.6; 15mM NaCl; 1.5mM MgCl₂)
243 and pelleted at 1000rpm for 4 minutes at room temperature. Cell pellets were resuspended in 1X
244 RSB buffer with 1% TritonX-100, homogenized with a loose pestle (5 strokes) and centrifuged for
245 5 minutes at 2000rpm at 4°C. Pellets were washed two times with 1ml of buffer A (10mM Tris
246 HCL, pH7.6; 15mM NaCl; 60mM KCl; 0.34M Sucrose; 0.1% B-mercaptoethanol; 0.15mM
247 Spermine; 0.5 mM Spermidine; 0.25mM PMSF) and nuclei were pelleted at 160g for 10 minutes
248 at 4°C. Nuclei were resuspended in 1.5ml of buffer A supplemented with 1mM of CaCl₂. Nuclear
249 suspensions (500 μ l) were digested with 200U/ml Micrococcal nuclease (NEB, cat#MO247S) at
250 37°C at different time points. Digestion was inactivated by 15mM EDTA. 10%SDS and 1M NaCl
251 were added to extract genomic DNA. DNA was run and visualized using TapeStation 4200 system
252 (Genomic Shared Recourse, Roswell Park Comprehensive Cancer Center).

253

254 **Statistical analyses**

255 Experimental findings were obtained from three independent experiments unless stated
256 otherwise. Statistics were performed in GraphPad Prism 9. In general, $P < 0.05$ was considered
257 statistically significant. All statistical methods and P-values are provided in the figure legends.
258 Asterisks in graphs denote statistically significant differences as described in figure legends.

259

260 **Code availability**

261 The code used to analyze the data is available at (https://github.com/feiginlab/CPSF3_PDAC).

262

263

264 **Results**

265 ***CPSF3* is upregulated in human PDAC and predicts poor patient outcome**

266 To determine the clinical significance of *CPSF3* expression in PDAC, we first analyzed gene
267 expression data from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) (25). *CPSF3*
268 expression was significantly higher in PDAC tumors (n=135), as compared with non-tumor
269 adjacent tissues (n=18) and normal pancreata (n=7) (Fig. 1A). Consistent with this finding, *CPSF3*
270 expression was also significantly higher in the Pancreatic Adenocarcinoma (PAAD) dataset from
271 The Cancer Genome Atlas (TCGA) (n=147) as compared to normal pancreata (n=165) from The
272 Genotype-Tissue Expression (GTEx) project (Fig. 1B). Next, we sought to assess the relationship
273 between *CPSF3* expression and PDAC patient outcome. Patients with high *CPSF3* expression
274 had significantly worse overall survival than patients with low *CPSF3* expression (p=0.00164,
275 hazard ratio 5.047 (1.842-13.827)). Specifically, patients in the top quartile of *CPSF3* expression
276 had a median survival of 14.2 months, while those in the bottom quartile of *CPSF3* expression
277 had a median survival of 33.5 months (Fig. 1C). We then sought to assess *CPSF3* expression
278 status in our cell line models. In agreement with the clinical data, we found that *CPSF3* is
279 upregulated in PDAC cell lines (MiaPaCa2, Suit2, Panc1) as compared to non-transformed
280 pancreatic epithelial cells (HPNE, HPDE) by RT-qPCR and western blot (WB) (Fig 1D,E).
281 Therefore, *CPSF3* is highly expressed in PDAC, high expression correlates with poor patient
282 outcome, and our cell models are appropriate for mechanistic studies.

283

284 ***CPSF3* is required for PDAC cell proliferation and tumor growth**

285 To define the functional role of *CPSF3* in PDAC we first took a genetic approach and generated
286 stable *CPSF3* knockdown MiaPaCa2 and Panc1 cells. We used two different short hairpin RNAs
287 (sh1 and sh2) targeting *CPSF3*, and a non-targeting control (shNTC). Successful knockdown of
288 *CPSF3* was confirmed at the RNA and protein level by RT-qPCR and WB, respectively, with sh1
289 cells having the highest level of knockdown in both cell lines (Fig. 2A,B). We then examined the
290 effect of *CPSF3* knockdown on cell proliferation and colony formation capability. *CPSF3*
291 knockdown significantly attenuated proliferation as compared with shNTC controls in both
292 MiaPaCa2 and Panc1 cells (Fig. 2C). *CPSF3* knockdown also significantly decreased colony
293 formation (Fig. 2D,E). In both the proliferation and colony formation assays, and in both PDAC
294 cell lines, sh1 *CPSF3* had the strongest phenotype, consistent with higher levels of *CPSF3*
295 knockdown.

296

297 Next, we sought to determine the requirement for CPSF3 in PDAC tumor growth *in vivo*. We
298 implanted MiaPaCa2 cells (either shNTC or sh1 CPSF3, 5×10^5 per mouse) subcutaneously into
299 the flanks of NOD/SCID/IL2R $\gamma^{-/-}$ (NSG) mice. *CPSF3* knockdown tumors grew significantly slower,
300 and weighed significantly less at endpoint, than shNTC tumors (Fig. 2F-H). No changes in tumor
301 histopathology were noted by Hematoxylin and Eosin (H&E) staining (Fig. 2I).
302 Immunohistochemical (IHC) analysis revealed that CPSF3 knockdown was maintained *in vivo*
303 (Fig. 2J). Finally, IHC for Ki67 revealed a significant decrease in proliferation in *CPSF3*
304 knockdown tumors as compared with shNTC controls (Fig. 2K). Overall, these data support the
305 requirement for CPSF3 in PDAC cell proliferation and tumor growth.

306

307 **CPSF3 knockdown dysregulates global gene expression in PDAC cells**

308 CPSF3 is an integral component of the polyadenylation complex; therefore, we hypothesized that
309 *CPSF3* knockdown would dysregulate APA, leading to global changes in mRNA expression. To
310 test this hypothesis, we subjected sh1 CPSF3 and shNTC Panc1 cells to RNA-sequencing (RNA-
311 seq). Differential expression analysis revealed 376 genes significantly upregulated and 98 genes
312 significantly downregulated (FDR<0.05; fold change >1.5) upon *CPSF3* knockdown. To uncover
313 significantly altered changes in 3'-UTR length upon *CPSF3* knockdown, we applied the DaPars
314 algorithm (23). DaPars identifies APA changes from standard RNA-seq data by generating a
315 Percentage Distal Usage Index (PDUI) score for each gene based on the relative abundances of
316 3'-UTR long and short forms. A negative PDUI indicates a shortening event, while a positive PDUI
317 indicates 3'-UTR lengthening. In accordance with our hypothesis, loss of *CPSF3* resulted in global
318 APA dysregulation, with 402 genes having significantly shorter 3'-UTRs, and 292 genes having
319 significantly longer 3'-UTRs in *CPSF3* knockdown cells ($-0.1 > \text{PDUI} > 0.1$; $P < 0.05$) (Fig. 3A). APA-
320 mediated alterations in 3'-UTR length impact mRNA stability and thus, gene expression (13,26–
321 28). To determine which CPSF3-mediated APA events might be driving individual changes in
322 gene expression, we looked for genes that were significantly altered in both the DaPars and gene
323 expression analyses (Fig. 3B). Intriguingly, this list included multiple downregulated oncogenes,
324 including SMAD Family Member 6 (*SMAD6*) and Mitogen-Activated Protein Kinase Kinase 6
325 (*MAP2K6*), and upregulated tumor suppressors, including Four And A Half LIM Domains 1 (*FHL1*)
326 and CKLF-Like MARVEL Transmembrane Domain Containing 3 (*CMTM3*). Therefore, these data
327 support a role for *CPSF3* in controlling cell proliferation via APA-mediated dysregulation of a suite
328 of cancer-associated genes.

329

330 We next sought to determine if any of the APA-regulated oncogenes or tumor suppressors were
331 directly responsible for *CPSF3* knockdown-mediated attenuation of cell proliferation. We focused
332 on *FHL1*, as it has been reported to possess tumor suppressor activity in non-PDAC cancers (29–
333 32), but has no known roles in PDAC biology. FHL1 interacts with transcription factors and
334 signaling proteins, thus modulating gene transcription and signaling pathways (29,33). FHL1
335 suppresses tumor growth through several mechanisms, including interaction with tumor-
336 regulating estrogen receptors and SMAD family proteins, and reduction of PI3K/AKT signaling
337 (29,34,35). The *FHL1* 3'-UTR was significantly lengthened ($P=0.029$), and *FHL1* gene expression
338 was significantly enriched, in *CPSF3* knockdown cells, as compared with shNTC controls (Fig.
339 3B,C). *FHL1* was also significantly overexpressed in *CPSF3* knockdown MiaPaCa2 cells, as
340 compared with shNTC controls (Fig. 3D). We validated the shift in APA patterns of *FHL1* by 3'
341 RACE (rapid amplification of 3' ends), revealing an increase in the long 3'-UTR form of *FHL1* in
342 *CPSF3* knockdown cells, and a concomitant decrease in the abundance of the short 3'-UTR form
343 (Fig. 3E). As APA can impact mRNA stability and translation, we determined FHL1 protein
344 expression in shNTC and *CPSF3* knockdown cells by WB (Fig. 3F). We found that FHL1 protein
345 levels were much higher in *CPSF3* knockdown cells. We then asked whether the *CPSF3*-
346 mediated upregulation of FHL1 was responsible for the effect of *CPSF3* knockdown on PDAC cell
347 growth. We transiently knocked down *FHL1* using siRNA in both shNTC and *CPSF3* knockdown
348 cells and assessed the effect on proliferation. Knockdown of *FHL1* was greater than 90% effective
349 (Fig. 3G). Knockdown of *FHL1* in shNTC cells had no effect on cell viability over the 7-day time
350 course of the experiment (Fig. 3H). However, knockdown of *FHL1* significantly rescued the
351 *CPSF3* knockdown-induced proliferation phenotype. Therefore, *CPSF3* knockdown reduces
352 PDAC cell viability at least in part through upregulation of *FHL1*. Finally, we sought to determine
353 if there was a correlation between *CPSF3* and *FHL1* gene expression levels in PDAC patients.
354 We observed a significant negative correlation ($R=-0.36$, $P<0.0001$) between *CPSF3* and *FHL1*
355 levels within the CPTAC database (Fig. 3I), supporting our conclusion that *FHL1* levels are
356 mediated by *CPSF3* expression in PDAC.

357

358 **PDAC cells are sensitive to chemical inhibition of CPSF3**

359 *CPSF3* was recently identified as the target for the small molecule JTE-607. JTE-607 is a prodrug
360 that, when metabolized by the ester hydrolyzing enzyme carboxylesterase 1 (CES1), binds to
361 *CPSF3* and inhibits its catalytic activity, impairing the processing of newly synthesized mRNAs
362 (19). As genetic depletion of *CPSF3* attenuated PDAC cell proliferation (Fig. 2), we hypothesized
363 that pharmacologic inhibition of *CPSF3* with JTE-607 could represent a novel therapeutic

364 approach in PDAC. We therefore examined the sensitivity of multiple human pancreatic cell lines,
365 both non-transformed and PDAC, to JTE-607 in a 72-hour dose-response cell viability assay.
366 Non-transformed pancreatic epithelial cells (HPNE, IC50=130.4 μ M; HPDE, IC50=60.11 μ M) and
367 human cancer associated fibroblast cell lines (C7 CAF, IC50=70.04 μ M; PancPat CAFs,
368 IC50=114.2 μ M) were not sensitive to JTE-607 (Fig. 4A-C). In contrast, human PDAC cell lines
369 displayed a range of sensitivities to JTE-607, with Panc1 cells being the most sensitive
370 (IC50=2.163 μ M) (Fig. 4A,C). Next, we determined the effect of JTE-607 on cell proliferation by
371 treating cells with increasing concentrations of JTE-607 and assessing cell viability in a time-
372 dependent fashion (Fig. 4D,E). JTE-607 had no effect on proliferation in non-transformed HPNE
373 cells (Fig. 4D). However, proliferation of MiaPaCa2 and Panc1 PDAC cells was significantly
374 attenuated by JTE-607, in a dose-dependent manner (Fig. 4E). Finally, we tested the effect of
375 JTE-607 on colony formation in PDAC cell lines. JTE-607 significantly decreased colony formation
376 in all PDAC cell lines tested (Fig. 4F,G). Therefore, JTE-607 selectively attenuates proliferation
377 of PDAC cells over non-transformed pancreatic cells.

378

379 **JTE-607 inhibits expression of replication-dependent histones**

380 To determine the gene regulatory alterations underlying the ability of JTE-607 to attenuate PDAC
381 cell proliferation, we performed RNA-seq on Panc1 cells treated with DMSO or JTE-607 for 24
382 hours. Differential gene expression and alterations in 3'-UTR length were analyzed as described
383 above. Differential expression analysis revealed 1270 genes significantly upregulated and 646
384 genes significantly downregulated (FDR<0.05; fold change >1.5) upon JTE-607 treatment. We
385 predicted that inhibition of CPSF3 would result in a global shift towards lengthened 3'-UTRs. In
386 accordance with our hypothesis, JTE-607 resulted in global APA dysregulation, with 1242 genes
387 having significantly longer 3'-UTRs, and 429 genes having significantly shorter 3'-UTRs in JTE-
388 607 treated cells (-0.1>PDUI>0.1; P<0.05) (Fig. 5A). To determine which JTE-607-mediated APA
389 events might be driving individual changes in gene expression, we looked for genes that were
390 significantly altered in both 3'-UTR length and gene expression (Fig. 5B). This revealed multiple
391 downregulated oncogenes, including N-Acetyltransferase 10 (*NAT10*) and Casein Kinase 1 Delta
392 (*CSNK1D*), and upregulated tumor suppressors, including Elongation Factor For RNA
393 Polymerase II 2 (*ELL2*) and Cyclin Dependent Kinase Inhibitor 1A (*CDKN1A*). Next, we compared
394 global APA alterations between the *CPSF3* knockdown and JTE-607 treatment conditions. This
395 analysis revealed an overlap in APA altered genes upon *CPSF3* knockdown and JTE-607
396 treatment (120 genes altered in both conditions; -0.1>PDUI>0.1; P<0.05), supporting the
397 contention that JTE-607 suppresses *CPSF3* activity (Fig. 5C). However, many APA events were

398 distinct between the two conditions, suggesting that long term CPSF3 knockdown may be
399 mechanistically distinct from short term CPSF3 inhibition.

400
401 Next, we sought to determine how short term JTE-607 treatment attenuates PDAC proliferation.
402 We were intrigued to find that numerous histone genes were significantly downregulated upon
403 JTE-607 treatment in Panc1 cells (Fig. 5D). Gene set enrichment analysis (GSEA) demonstrated
404 a dysregulation in many histone-related pathways, including histone methylation, acetylation and
405 deacetylation (Fig. 5E). Interestingly, the majority of the differentially expressed histones were
406 replication-dependent histones, including *HIST1H2AD*, *HIST1H2BJ*, *HIST1H3A* and *HIST1H4E*.
407 We validated the JTE-607-induced decrease in replication-dependent histones in MiaPaCa2 cells
408 (Fig 5F). Therefore, JTE-607 treatment decreases the expression of proliferation-dependent
409 histones.

410
411 Replication-dependent histone genes are not polyadenylated and undergo pre-mRNA processing
412 via the histone cleavage complex (HCC) (36). CPSF3 is the endonuclease component of the HCC
413 (17,37,38); while *CPSF3* knockdown studies have demonstrated a role for CPSF3 in histone
414 processing, the effect of inhibiting CPSF3 activity on histone mRNA processing has never been
415 determined. We reasoned that lack of CPSF3-mediated cleavage activity would result in
416 transcriptional read-through extending beyond the boundaries of the 3'-UTR. Indeed, we found
417 that replication-dependent histones underwent transcriptional read-through upon JTE-607
418 treatment (Fig. 6A). Improperly processed mRNAs fail to be exported into the cytoplasm for
419 translation, leading to decreased protein levels (17,39,40). Therefore, we examined replication-
420 dependent histone protein levels upon JTE-607 treatment and found that JTE-607 reduced both
421 H3 and H2B protein levels in a dose- and time-dependent fashion (Fig. 6B). In contrast,
422 replication-independent histones did not undergo transcriptional read-through upon JTE-607
423 treatment, and did not decrease in gene expression levels (Fig. 6C,D). Finally, we sought to
424 determine if there was a correlation between *CPSF3* and histone gene expression levels in PDAC
425 patients. We calculated the Spearman's correlation for *CPSF3* and 98 histone genes from the
426 CPTAC database (Spearman = $-0.15 > R > 0.15$, $P < 0.05$) (Fig. 6E-G). In accordance with our
427 experimental findings, there were significantly more positive correlations (43 genes) between
428 *CPSF3* and histone gene expression than negative correlations (3 genes) among replication-
429 dependent histones (Fig. 5F). In contrast, there were few significant correlations between *CPSF3*
430 and replication-independent histones (only 8 genes), and those significant alterations were

431 equally positive and negative (Fig. 5G). Collectively, these results indicate that JTE-607 inhibits
432 the function of CPSF3 in both the APA and histone mRNA processing complexes.

433

434 **JTE-607 destabilizes chromatin and blocks cell cycle progression**

435 As replication-dependent histones are required for nucleosome assembly (36,41–43), we
436 hypothesized that JTE-607 would dysregulate chromatin dynamics. First, we performed a
437 Micrococcal Nuclease (MNase) assay to assess relative chromatin condensation. In this assay,
438 protein-free DNA is digested by MNase, producing DNA fragmentation patterns that are indicators
439 of whether chromatin is in a condensed or relaxed state. The chromatin destabilizing agent
440 CBL0137 was used as a positive control (44). Panc1 cells treated with JTE-607 or CBL037
441 displayed rapid and complete chromatin digestion, as compared with DMSO-treated cells (Fig.
442 7A, quantification in 7B). To assess chromatin destabilization in a living cell, we utilized the HeLa-
443 TI cell line model that has a silenced GFP reporter within a heterochromatic region of the genome.
444 Treatment of these cells with chromatin destabilizing agents, including CBL0137, allows
445 derepression of GFP silencing. Therefore, we monitored GFP expression in HeLa-TI cells upon
446 JTE-607 treatment by both fluorescence microscopy and flow cytometry. Cells treated with JTE-
447 607 induced GFP expression to levels comparable with CBL0137 in a dose- and time-dependent
448 manner (Fig. 7C-E).

449

450 Finally, we sought to determine how JTE-607-mediated depletion of replication-dependent
451 histones led to defects in cell viability. As replication-dependent histones are required for cell cycle
452 progression, we assessed the effects of JTE-607 on cell cycle distribution. In non-transformed
453 HPNE cells, JTE-607 had no impact on cell cycle distribution (Fig. 8A). In contrast, JTE-607
454 arrested Panc1 and MiaPaCa2 PDAC cells in S-phase of the cell cycle within 24 hours (Fig. 8B).
455 To more specifically investigate the timing and extent of S-phase arrest, we examined BrdU
456 incorporation upon JTE-607 treatment in a time-dependent manner (Fig. 8C). We found that JTE-
457 607 arrests cells in early to mid S-phase of the cell cycle as early as 8 hours (Fig. 8C). Therefore,
458 JTE-607 destabilizes chromatin and attenuates PDAC cell viability through S-phase cell cycle
459 arrest.

460

461 **Discussion**

462 Dysregulation of gene expression is a fundamental driver of cancer (45). This dysregulation can
463 be driven by non-mutational epigenetic reprogramming, a mechanism that is now recognized as
464 a hallmark of cancer (46). Emerging evidence has implicated dysregulation of one such non-

465 mutational gene regulatory process, APA, in the pathogenesis of cancer (23,47–49). Pan-cancer
466 analyses have revealed global changes in APA across the cancer landscape, and mechanistic
467 studies have characterized how these alterations promote oncogenesis (23,50–52). Recently, we
468 reported the first large-scale, single cancer study of APA and discovered widespread alterations
469 in 3'-UTR length across the PDAC landscape. Importantly, many of these APA alterations were
470 associated with expression changes in growth-promoting genes, highlighting the importance of
471 APA in driving PDAC pathogenesis (16). Therefore, we hypothesized that therapeutically
472 targeting APA in PDAC would alleviate this global repatterning of 3'-UTR usage, normalizing the
473 expression of oncogenes and tumor suppressors, and attenuating tumor growth. To directly test
474 this hypothesis, we focused on CPSF3, an enzymatic component of the APA machinery that
475 catalyzes the endonucleolytic cleavage of the pre-mRNA. While CPSF3 has known roles in the
476 regulation of APA and histone mRNA processing, our study defines the first roles of CPSF3
477 activity in an epithelial cancer, with implications for therapeutic intervention in intractable
478 pancreatic cancer. We demonstrate that high *CPSF3* expression is a predictor of poor patient
479 outcome and uncover the requirement for *CPSF3* in PDAC cell proliferation *in vitro* and tumor
480 growth *in vivo*. We characterize the global APA alterations driven by loss of *CPSF3*, revealing the
481 dysregulation of numerous tumor suppressors and oncogenes. We then determine the direct
482 connection between a CPSF3-mediated APA event in the tumor suppressor *FHL1* and PDAC cell
483 proliferation. Finally, we reveal dysregulation of histone processing downstream of CPSF3
484 inhibition, regulating cell cycle progression. These results demonstrate the potential for targeting
485 CPSF3 as a novel therapeutic approach in PDAC.

486

487 Our study has several clinical implications. First, we show that *CPSF3* expression is dysregulated
488 in PDAC and high expression correlates with poor prognosis. This is consistent with similar
489 findings across the cancer landscape, where *CPSF3* has been reported to be a predictor of
490 unfavorable prognosis in lung and liver cancers (53,54). While several studies have
491 experimentally manipulated various APA factors and determined the phenotypic impacts, little is
492 known about the function of CPSF3 in disease, particularly cancer. This is noteworthy for several
493 reasons. First, CPSF3 is the enzymatic component of the APA machinery, and is thus a potentially
494 druggable target. Second, despite acting in the same complex, knockdown of specific APA factors
495 can have opposing impacts on APA and cellular phenotypes (50,51,55–58). Therefore,
496 understanding the role of CPSF3 specifically in PDAC cell proliferation is critical for elucidating its
497 potential as a novel therapeutic target. Recently, homozygosity in *CPSF3* missense variants was

498 found to cause intellectual disability and embryonic lethality in humans. However, these
499 phenotypes were completely absent in the heterozygous carriers (59). In cancer cell line models,
500 *CPSF3* is essential for cell proliferation when knocked out completely by CRISPR; however,
501 *CPSF3* is not an essential gene upon shRNA-mediated partial knockdown (www.depmap.org).
502 This suggests that pharmacological targeting of such an essential gene may be biologically
503 feasible. In support of this hypothesis, we show that knockdown of *CPSF3* blocks PDAC cell
504 proliferation and tumor growth, and that the efficiency of knockdown is a determinant of
505 phenotypic strength. Furthermore, *CPSF3* inhibition does not impair cell cycle progression or
506 proliferation of non-transformed pancreatic epithelial cells, and the *CPSF3* inhibitor JTE-607 is
507 non-toxic in humans. Therefore, inhibition of *CPSF3* may preferentially target transformed cells.

508

509 Genetic manipulation of APA factors has been shown to alter APA patterns, dysregulate gene
510 and protein expression and drive cancer phenotypes (50,51,63,64,52,53,55–57,60–62). While
511 several recent reports have linked *CPSF3* loss to defects in tumor cell growth, no study has
512 mechanistically connected *CPSF3* to global dysregulation of APA, gene expression and cell
513 phenotype. We and others have previously shown the direct effect of cancer-associated APA
514 alterations on gene expression and cancer cell proliferation and tumor growth (16,23,47,48).
515 Adding to that body of literature, we now demonstrate that *CPSF3* knockdown alters 3'-UTR
516 length of multiple tumor suppressors and oncogenes, including *FHL1*, *CMTM3*, *SMAD6* and
517 *MAP2K6*. To understand if these gene expression changes are responsible for driving *CPSF3*
518 knockdown-mediated phenotypes, we determined the requirement for FHL1 expression in
519 mediating cell proliferation. *FHL1* possesses tumor suppressing activity in different cancer types
520 but has not been investigated in the context of PDAC (29–32). We find that silencing of *FHL1*, in
521 the context of *CPSF3* knockdown, sufficiently rescues the defect in cell proliferation. Therefore,
522 we uncover a novel mechanism by which *CPSF3* loss attenuates PDAC cell growth. We also
523 provide the first global view of the contribution of *CPSF3* activity to PDAC gene expression,
524 revealing widespread control of genes implicated in cell growth. While dysregulation of FHL1 is
525 at least partially responsible for the *CPSF3* knockdown cell proliferation phenotype, we propose
526 that *CPSF3* loss dysregulates the expression of a suite of cell growth genes through altering 3'-
527 UTR length, thereby attenuating PDAC cell growth.

528

529 Recently, two groups independently demonstrated that *CPSF3* is the target of the small molecule
530 JTE-607 (18,19). JTE-607 was first identified over 20 years ago as a cytokine synthesis inhibitor;

531 however, the direct molecular target remained elusive. Despite the lack of a defined mechanism,
532 JTE-607 was tested in a Phase I dose-escalation trial in healthy human volunteers, with no serious
533 adverse effects (22). Therefore, despite inhibiting an essential enzyme responsible for processing
534 >70% of polyadenylated mRNAs, JTE-607 is not uniformly toxic in humans. This property, coupled
535 with our data demonstrating JTE-607's anti-proliferative effects on cancer cells, supports the
536 contention that targeting CPSF3 is a feasible prospect in PDAC. In humans, endotoxin-induced
537 production of C-reactive protein, IL-10 and IL-1ra was inhibited by JTE-607 (22). In animal
538 models, JTE-607 inhibited the production of proinflammatory cytokines, prevented endotoxin
539 shock and attenuated artificially induced lung and heart injury (65–67). JTE-607 has also been
540 used in models of acute myeloid leukemia (AML) and Ewing sarcoma and showed growth
541 inhibitory activity both *in vitro* and *in vivo* (xenograft models) (19–21). However, these studies
542 were limited to leukemia and sarcoma models, with no efficacy shown for epithelial-derived
543 tumors. Therefore, the potential for CPSF3 as a therapeutic target in adenocarcinoma was an
544 open question. Now, we show that JTE-607 preferentially blocks proliferation of PDAC cell lines,
545 sparing non-transformed cell lines, including epithelial cells and fibroblasts. The mechanisms
546 underlying this difference in sensitivity are currently unknown, but may relate to variability in basal
547 proliferation rate. Finally, even though JTE-607 was first described as an inhibitor of cytokine
548 synthesis, our RNA-seq analysis did not show an enrichment of such pathways. One possible
549 explanation is that JTE-607 action is cell type dependent. Many of the studies assessing cytokine
550 levels were performed using measurements from blood, and therefore the cell type responsible
551 for the changes in cytokine secretion is unknown. It is possible that the effect of JTE-607 on
552 proliferating epithelial cells is distinct from its effect on cells within the circulation, many of which
553 are non-proliferative when terminally differentiated. The effects of JTE-607 in different cellular
554 contexts and cell states warrants further investigation.

555

556 A previous report demonstrated that JTE-607 attenuates cell proliferation in AML and Ewing
557 sarcoma through increasing R-loop formation and downregulating the expression of DNA damage
558 response genes. R-loops are DNA:RNA hybrids that form as a result of aberrant transcription, a
559 characteristic of cancers with genetic rearrangements such as AML and Ewing sarcoma (68,69).
560 Of note, R-loops increase in models with mRNA cleavage and polyadenylation defects (70),
561 suggesting that sensitivity of AML and Ewing sarcoma to JTE-607 may be a consequence of high
562 basal levels of R-loops, which eventually accumulate leading to DNA damage and genomic
563 instability. In our study, gene set enrichment analysis did not reveal changes in DNA damage

564 response pathways upon *CPSF3* knockdown or inhibition in PDAC cells. Therefore, we propose
565 that *CPSF3* regulates cell proliferation through distinct mechanisms in AML and Ewing sarcoma
566 relative to PDAC. In PDAC cells, we find that JTE-607 impairs processing of proliferation-
567 dependent histone mRNAs. This is consistent with the role of *CPSF3* in the HCC (17,38,71–73).
568 Defects in the HCC have been shown to reduce the availability of replication-dependent histones
569 (17,40,74,75). However, prior to now, no studies have described the effect of *CPSF3* inhibition on
570 HCC activity. Depletion of many HCC genes led to an accumulation of histone read-through
571 transcripts in the nucleus (37,39). Similarly, we find extensive transcript read-through in
572 proliferation-dependent histone mRNAs, but not proliferation-independent histone mRNAs upon
573 JTE-607 treatment. Histone read-through transcripts accumulate in the nucleus, thus failing to be
574 exported into the cytoplasm and translated into protein. In accordance with this model, we find
575 that JTE-607 depletes core histone protein levels in PDAC cells. Limited histone supplies
576 destabilize chromatin integrity through disruption of nucleosome assembly (43). We find that JTE-
577 607 destabilizes chromatin stability, as demonstrated by increased sensitivity to MNase digestion,
578 and derepression of heterochromatin-mediated gene expression silencing. These findings reveal
579 a novel mechanism of JTE-607 activity: dysregulation of proliferation-dependent histone mRNA
580 processing.

581

582 Expression of replication-dependent histones increases ~30-50 fold during DNA synthesis
583 (76,77). The life cycle of these core histone genes starts late in G1 through mid S phase of the
584 cell cycle and degradation occurs at late S phase (36,78). Silencing of the HCC core component
585 *FLASH* induces S phase arrest in HeLa cells (79). We find that JTE-607 arrests cells in the S
586 phase of the cell cycle, with cells slowly cycling through early-mid S phase but failing to progress
587 through late S phase. This is consistent with a previous study where depletion of the histone
588 chaperone ASF1, an important gene for histone deposition during DNA replication, disrupts
589 progression through mid to late S-phase (80). In addition, knockdown of *CSTF2*, a gene with dual
590 functions in APA and histone pre-mRNA processing, delays progression through S phase, but its
591 expression is highly dependent on cell cycle stage (39). The same study showed that *CPSF3*
592 expression is not cell cycle regulated, suggesting that the histone phenotype we observe is driven
593 by *CPSF3* loss and not merely a consequence of cell cycle arrest. Our findings strongly suggest
594 that JTE-607 mediates its growth attenuating phenotype by reducing histone supplies during S
595 phase, thereby blocking cell cycle progression.

596

597 This newly discovered mechanism of JTE-607 represents a potential window for new combination
598 therapy. One possibility is that JTE-607-mediated cell cycle arrest may promote synergism with
599 cell cycle check-point inhibitors. For instance, the chromatin remodeling histone deacetylase
600 (HDAC) inhibitors have shown synergistic effect when combined with checkpoint kinase 1 (Chk1)
601 inhibitors in lung cancer models (81). Histone disruption by JTE-607 may also promote synergism
602 with chromatin modifying drugs. For example, CBL0137 has shown synergistic effect when
603 combined with HDAC inhibitors by exacerbating chromatin destabilization (44). These discoveries
604 may improve the efficacy of approved chromatin remodeling agents and suggest a path forward
605 for use of JTE-607 in the clinic.

606

607 The mechanistic differences underlying the CPSF3 knockdown and inhibition phenotypes raises
608 several important questions. As CPSF3 is an integral subunit of both the APA and histone
609 processing complexes, *CPSF3* knockdown may disrupt proper recruitment of other complex
610 components. This dysregulation of complex formation may alter complex function in different ways
611 than inhibition of CPSF3 activity, resulting in divergence in APA patterns and gene expression
612 alterations. Furthermore, the process of generating stable cells for long-term *CPSF3* knockdown
613 can result in upregulation of compensatory mechanisms, allowing cell growth in the absence of
614 an essential gene. These mechanisms will not be accounted for upon pharmacological CPSF3
615 inhibition, again resulting in differences in APA patterns and gene expression alterations.
616 However, it should be noted that both CPSF3 knockdown and inhibition led to decreased cell
617 proliferation and global dysregulation of APA, including that of known tumor suppressors and
618 oncogenes. Therefore, our results support the development of CPSF3 targeting agents, including
619 those that can specifically degrade CPSF3.

620

621 In conclusion, our study has revealed the role of CPSF3 in pancreatic cancer and uncovered new
622 mechanisms by which CPSF3 mediates cell proliferation. CPSF3 knockdown or inhibition induces
623 APA changes that alter the expression of known tumor suppressors and oncogenes. CPSF3
624 inhibition disrupts the processing of proliferation-dependent histones, destabilizing chromatin
625 structure and inhibiting cell cycle progression. Our findings reveal novel insight into how CPSF3
626 inhibition blocks cell proliferation and provides a new therapeutic target in pancreatic cancer.

627

628 **Authors' Disclosures**

629 The authors declare no potential conflicts of interest.

630

631 **Authors' Contributions**

632 **A.A. Alahmari:** Conceptualization, methodology, investigation, data analysis, writing—original
633 draft, writing—review and editing. **A.H. Chaubey:** Investigation. **A.A. Tisdale:** Investigation. **C.D.**
634 **Schwarz:** Investigation. **A.C. Cornwell:** Investigation. **K.E. Maraszek:** Investigation. **E.J.**
635 **Paterson:** Investigation. **M. Kim:** Investigation. **S. Venkat:** Investigation, bioinformatics analysis.
636 **E.C. Gomez:** Bioinformatics analysis. **J. Wang:** Bioinformatics analysis. **K.V. Gurova:**
637 Resources. **M.E. Feigin:** Conceptualization, supervision, funding acquisition, project
638 administration, writing—review and editing.

639

640 **Acknowledgements**

641 This work was supported by National Cancer Institute (National Institutes of Health) grants P30
642 CA016056 and R25 CA181003, an award from the Roswell Park Alliance Foundation to M.E.F.,
643 and a scholarship and support to A.A.A. from Prince Sattam bin Abdulaziz University in Saudi
644 Arabia, the Saudi Arabian Cultural Mission in the USA, and the Office of International
645 Collaborations in Oncology at Roswell Park Comprehensive Cancer Center. M.E.F. is supported
646 by a Research Scholar Grant, RSG-21-014-01 - RMC, from the American Cancer Society. We
647 thank the Roswell Park Genomics and Small Molecule Screening Shared Resources for their
648 assistance. We thank Safina Alfya for her help with the MNase experiment and Brian Buckley for
649 assistance in imaging HeLa-TI cells. We thank the members of the Abel Laboratory for their
650 insightful comments and support.

651

652 **References**

- 653 1. Rush JE. Cancer Statistics. *Lancet*. 1925;206(5333):1036–7.
- 654 2. Kleeff J, Korc M, Apte M, La Vecchia C, Johnson CD, Biankin A V., et al. Pancreatic
655 cancer. *Nat Rev Dis Prim*. 2016 Apr 21;2.
- 656 3. Collisson EA, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, et al. Subtypes of
657 pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat Med*.
658 2011;17(4):500–3.
- 659 4. Moffitt RA, Marayati R, Flate EL, Volmar KE, Loeza SGH, Hoadley KA, et al. Virtual
660 microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic
661 ductal adenocarcinoma. *Nat Genet*. 2015;47(10):1168–78.
- 662 5. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, et al. Genomic
663 analyses identify molecular subtypes of pancreatic cancer. *Nature*. 2016;531(7592):47–
664 52.
- 665 6. Peng J, Sun BF, Chen CY, Zhou JY, Chen YS, Chen H, et al. Single-cell RNA-seq
666 highlights intra-tumoral heterogeneity and malignant progression in pancreatic ductal
667 adenocarcinoma. *Cell Res [Internet]*. 2019;29(9):725–38. Available from:
668 <http://dx.doi.org/10.1038/s41422-019-0195-y>
- 669 7. Wang L, Yang H, Zamperone A, Diolaiti D, Palmboos PL, Abel E V., et al. ATDC is
670 required for the initiation of KRAS-induced pancreatic tumorigenesis. *Genes Dev*
671 *[Internet]*. 2019 Jun 1;33(11–12):641–55. Available from:
672 <http://genesdev.cshlp.org/lookup/doi/10.1101/gad.323303.118>
- 673 8. Abel E V., Goto M, Magnuson B, Abraham S, Ramanathan N, Hotaling E, et al. HNF1A is
674 a novel oncogene that regulates human pancreatic cancer stem cell properties. *Elife*.
675 2018;7:1–35.
- 676 9. Roe J-S, Hwang C-I, Somerville TDD, Milazzo JP, Lee EJ, Da Silva B, et al. Enhancer
677 Reprogramming Promotes Pancreatic Cancer Metastasis. *Cell [Internet]*. 2017
678 Aug;170(5):875-888.e20. Available from:
679 <https://linkinghub.elsevier.com/retrieve/pii/S0092867417308140>

- 680 10. Sodir NM, Kortlever RM, Barthet VJA, Campos T, Pellegrinet L, Kupczak S, et al. MYC
681 Instructs and Maintains Pancreatic Adenocarcinoma Phenotype. *Cancer Discov.*
682 2020;10(4):588–607.
- 683 11. Shankar S, Pitchiaya S, Malik R, Kothari V, Hosono Y, Yocum AK, et al. KRAS Engages
684 AGO2 to Enhance Cellular Transformation. *Cell Rep [Internet]*. 2016 Feb;14(6):1448–61.
685 Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2211124716000553>
- 686 12. Venkat S, Alahmari AA, Feigin ME. Drivers of Gene Expression Dysregulation in
687 Pancreatic Cancer. *Trends in Cancer [Internet]*. 2021 Apr 26;1–12. Available from:
688 <https://doi.org/10.1016/j.trecan.2021.01.008>
- 689 13. Gruber AJ, Zavolan M. Alternative cleavage and polyadenylation in health and disease.
690 *Nat Rev Genet [Internet]*. 2019; Available from: [http://dx.doi.org/10.1038/s41576-019-](http://dx.doi.org/10.1038/s41576-019-0145-z)
691 0145-z
- 692 14. Yuan F, Hankey W, Wagner EJ, Li W, Wang Q. Alternative polyadenylation of mRNA and
693 its role in cancer. *Genes and Diseases*. Chongqing University; 2019.
- 694 15. Masamha CP, Wagner EJ. The contribution of alternative polyadenylation to the cancer
695 phenotype. *Carcinogenesis*. 2018;39(1):2–10.
- 696 16. Venkat S, Tisdale AA, Schwarz JR, Alahmari AA, Maurer HC, Olive KP, et al. Alternative
697 polyadenylation drives oncogenic gene expression in pancreatic ductal adenocarcinoma.
698 *Genome Res*. 2020;1–14.
- 699 17. Sullivan KD, Steiniger M, Marzluff WF. A Core Complex of CPSF73, CPSF100, and
700 Symplekin May Form Two Different Cleavage Factors for Processing of Poly(A) and
701 Histone mRNAs. *Mol Cell [Internet]*. 2009;34(3):322–32. Available from:
702 <http://dx.doi.org/10.1016/j.molcel.2009.04.024>
- 703 18. Kakegawa J, Sakane N, Suzuki K, Yoshida T. JTE-607, a multiple cytokine production
704 inhibitor, targets CPSF3 and inhibits pre-mRNA processing. *Biochem Biophys Res*
705 *Commun [Internet]*. 2019;518(1):32–7. Available from:
706 <https://doi.org/10.1016/j.bbrc.2019.08.004>
- 707 19. Ross NT, Lohmann F, Carbonneau S, Fazal A, Weihofen WA, Gleim S, et al. CPSF3-

- 708 dependent pre-mRNA processing as a druggable node in AML and Ewing's sarcoma. *Nat*
709 *Chem Biol.* 2020 Jan 1;16(1):50–9.
- 710 20. Uesato N, Fukui K, Maruhashi J, Tojo A, Tajima N. JTE-607, a multiple cytokine
711 production inhibitor, ameliorates disease in a SCID mouse xenograft acute myeloid
712 leukemia model. *Exp Hematol.* 2006;34(10):1385–92.
- 713 21. Tajima N, Fukui K, Uesato N, Maruhashi J, Yoshida T, Watanabe Y, et al. JTE-607, a
714 multiple cytokine production inhibitor, induces apoptosis accompanied by an increase in
715 p21waf1/cip1 in acute myelogenous leukemia cells. *Cancer Sci.* 2010;101(3):774–81.
- 716 22. Borozdenkova S, Mant TGK, Allen E, Pu K, Hoshino S, Jurcevic S. Effects of a cytokine
717 inhibitor, JTE-607, on the response to endotoxin in healthy human volunteers. *Int*
718 *Immunopharmacol [Internet].* 2011;11(11):1837–43. Available from:
719 <http://dx.doi.org/10.1016/j.intimp.2011.07.013>
- 720 23. Xia Z, Donehower LA, Cooper TA, Neilson JR, Wheeler DA, Wagner EJ, et al. Dynamic
721 analyses of alternative polyadenylation from RNA-seq reveal a 3'-UTR landscape
722 across seven tumour types. *Nat Commun.* 2014;5.
- 723 24. Safina A, Cheney P, Pal M, Brodsky L, Ivanov A, Kirsanov K, et al. FACT is a sensor of
724 DNA torsional stress in eukaryotic cells. *Nucleic Acids Res.* 2017;45(4):1925–45.
- 725 25. Cao L, Huang C, Cui Zhou D, Hu Y, Lih TM, Savage SR, et al. Proteogenomic
726 characterization of pancreatic ductal adenocarcinoma. *Cell.* 2021;184(19):5031-
727 5052.e26.
- 728 26. Mayr C. What Are 3' UTRs Doing? *Cold Spring Harb Perspect Biol.* 2018;a034728.
- 729 27. Tian B, Manley JL. Alternative polyadenylation of mRNA precursors. *Nat Rev Mol Cell*
730 *Biol [Internet].* 2016;18(1):18–30. Available from: <http://dx.doi.org/10.1038/nrm.2016.116>
- 731 28. Elkon R, Ugalde AP, Agami R. Alternative cleavage and polyadenylation: Extent,
732 regulation and function. *Nat Rev Genet.* 2013;14(7):496–506.
- 733 29. Li SZ, Hu YY, Zhao JL, Zang J, Fei Z, Han H, et al. Downregulation of FHL1 protein in
734 glioma inhibits tumor growth through PI3K/AKT signaling. *Oncol Lett.* 2020;19(6):3781–8.

- 735 30. Wang J, Huang F, Huang J, Kong J, Liu S, Jin J. Epigenetic analysis of FHL1 tumor
736 suppressor gene in human liver cancer. *Oncol Lett.* 2017;14(5):6109–16.
- 737 31. Asada K, Ando T, Niwa T, Nanjo S, Watanabe N, Okochi-Takada E, et al. FHL1 on
738 chromosome X is a single-hit gastrointestinal tumor-suppressor gene and contributes to
739 the formation of an epigenetic field defect. *Oncogene.* 2013;32(17):2140–9.
- 740 32. Liu Y, Wang C, Cheng P, Zhang S, Zhou W, Xu Y, et al. FHL1 inhibits the progression of
741 colorectal cancer by regulating the Wnt/ β -catenin signaling pathway. *J Cancer.*
742 2021;12(17):5345–54.
- 743 33. Cowling BS, McGrath MJ, Nguyen M-A, Cottle DL, Kee AJ, Brown S, et al. Identification
744 of FHL1 as a regulator of skeletal muscle mass: implications for human myopathy. *J Cell*
745 *Biol* [Internet]. 2008 Dec 15;183(6):1033–48. Available from:
746 [https://rupress.org/jcb/article/183/6/1033/35367/Identification-of-FHL1-as-a-regulator-of-](https://rupress.org/jcb/article/183/6/1033/35367/Identification-of-FHL1-as-a-regulator-of-skeletal)
747 [skeletal](https://rupress.org/jcb/article/183/6/1033/35367/Identification-of-FHL1-as-a-regulator-of-skeletal)
- 748 34. Ding L, Wang Z, Yan J, Yang X, Liu A, Qiu W, et al. Human four-and-a-half LIM family
749 members suppress tumor cell growth through a TGF- β -like signaling pathway. *J Clin*
750 *Invest.* 2009;119(2):349–61.
- 751 35. Ding L, Niu C, Zheng Y, Xiong Z, Liu Y, Lin J, et al. FHL1 interacts with oestrogen
752 receptors and regulates breast cancer cell growth. *J Cell Mol Med.* 2011;15(1):72–85.
- 753 36. Marzluff WF, Wagner EJ, Duronio RJ. Metabolism and regulation of canonical histone
754 mRNAs: Life without a poly(A) tail. *Nat Rev Genet.* 2008;9(11):843–54.
- 755 37. Wagner EJ, Burch BD, Godfrey AC, Salzler HR, Duronio RJ, Marzluff WF. A Genome-
756 wide RNA Interference Screen Reveals that Variant Histones Are Necessary for
757 Replication-Dependent Histone Pre-mRNA Processing. *Mol Cell.* 2007;28(4):692–9.
- 758 38. Yang X, Sun Y, Aik WS, Marzluff WF, Tong L, Dominski Z. Studies with recombinant U7
759 snRNP demonstrate that CPSF73 is both an endonuclease and a 5'-3' exonuclease. *Rna.*
760 2020;rna.076273.120.
- 761 39. Romeo V, Griesbach E, Schümperli D. CstF64: Cell Cycle Regulation and Functional
762 Role in 3' End Processing of Replication-Dependent Histone mRNAs. *Mol Cell Biol.*

- 763 2014;34(23):4272–84.
- 764 40. Sullivan KD, Mullen TE, Marzluff WF, Wagner EJ. Knockdown of SLBP results in nuclear
765 retention of histone mRNA. *Rna*. 2009;15(3):459–72.
- 766 41. Gunjan A, Paik J, Verreault A. Regulation of histone synthesis and nucleosome
767 assembly. *Biochimie*. 2005;87(7):625–35.
- 768 42. Groth A, Corpet A, Cook AJL, Roche D, Bartek J, Lukas J, et al. Regulation of replication
769 fork progression through histone supply and demand. *Science* (80-).
770 2007;318(5858):1928–31.
- 771 43. Günesdogan U, Jäckle H, Herzig A. Histone supply regulates S phase timing and cell
772 cycle progression. *Elife*. 2014;3:e02443.
- 773 44. Xiao L, Somers K, Murray J, Pandher R, Karsa M, Ronca E, et al. Dual targeting of
774 chromatin stability by the curaxin CBL0137 and histone deacetylase inhibitor
775 panobinostat shows significant preclinical efficacy in neuroblastoma. *Clin Cancer Res*.
776 2021;27(15):4338–52.
- 777 45. Luo J, Solimini NL, Elledge SJ. Principles of Cancer Therapy: Oncogene and Non-
778 oncogene Addiction. *Cell*. 2009;136(5):823–37.
- 779 46. Hanahan D. Hallmarks of Cancer : New Dimensions. 2022;(January):31–47.
- 780 47. Mayr C, Bartel DP. Widespread Shortening of 3'UTRs by Alternative Cleavage and
781 Polyadenylation Activates Oncogenes in Cancer Cells. *Cell* [Internet]. 2009;138(4):673–
782 84. Available from: <http://dx.doi.org/10.1016/j.cell.2009.06.016>
- 783 48. Lee SH, Singh I, Tisdale S, Abdel-Wahab O, Leslie CS, Mayr C. Widespread intronic
784 polyadenylation inactivates tumour suppressor genes in leukaemia. Vol. 561, *Nature*.
785 Nature Publishing Group; 2018. p. 127–31.
- 786 49. Xiang Y, Ye Y, Lou Y, Yang Y, Cai C, Zhang Z, et al. Comprehensive characterization of
787 alternative polyadenylation in human cancer. *J Natl Cancer Inst*. 2018;110(4):379–89.
- 788 50. Zhang L, Zhang W. Knockdown of NUDT21 inhibits proliferation and promotes apoptosis
789 of human K562 leukemia cells through ERK pathway. *Cancer Manag Res*.

- 790 2018;10:4311–23.
- 791 51. Chen X, Zhang JX, Luo JH, Wu S, Yuan GJ, Ma NF, et al. CSTF2-induced shortening of
792 the RAC1 3'UTR promotes the pathogenesis of urothelial carcinoma of the bladder.
793 *Cancer Res.* 2018;78(20):5848–62.
- 794 52. Xiong M, Chen L, Zhou L, Ding Y, Kazobinka G, Chen Z, et al. NUDT21 promotes
795 bladder cancer progression through ANXA2 and LIMK2 by alternative polyadenylation.
796 *Theranostics.* 2019;9(24):7156–67.
- 797 53. Li N, Jiang S, Fu R, Lv J, Yao J, Mai J, et al. Cleavage and polyadenylation-specific
798 factor 3 induces cell cycle arrest via PI3K/Akt/GSK-3 β signaling pathways and predicts a
799 negative prognosis in hepatocellular carcinoma. *Biomark Med.* 2021;15(5):347–58.
- 800 54. Ning Y, Liu W, Guan X, Xie X, Zhang Y. CPSF3 is a promising prognostic biomarker and
801 predicts recurrence of non-small cell lung cancer. *Oncol Lett.* 2019;18(3):2835–44.
- 802 55. Park HJ, Ji P, Kim S, Xia Z, Rodriguez B, Li L, et al. 3' UTR shortening represses tumor-
803 suppressor genes in trans by disrupting ceRNA crosstalk. *Nat Genet.* 2018 Jun
804 1;50(6):783–9.
- 805 56. Li X, Ding J, Wang X, Cheng Z, Zhu Q. NUDT21 regulates circRNA cyclization and
806 ceRNA crosstalk in hepatocellular carcinoma. *Oncogene [Internet].* 2020;39(4):891–904.
807 Available from: <http://dx.doi.org/10.1038/s41388-019-1030-0>
- 808 57. Fang S, Zhang D, Weng W, Lv X, Zheng L, Chen M, et al. CPSF7 regulates liver cancer
809 growth and metastasis by facilitating WWP2-FL and targeting the WWP2/PTEN/AKT
810 signaling pathway. *Biochim Biophys Acta - Mol Cell Res.* 2020 Feb 1;1867(2).
- 811 58. Tan S, Ding K, Chong QY, Zhao J, Liu Y, Shao Y, et al. Post-transcriptional regulation of
812 ERBB2 by miR26a/b and HuR confers resistance to tamoxifen in estrogen receptor-
813 positive breast cancer cells. *J Biol Chem.* 2017;292(33):13551–64.
- 814 59. Arnadottir GA, Oddsson A, Jensson BO, Gisladdottir S, Simon MT, Arnthorsson AO, et al.
815 Population-level deficit of homozygosity unveils CPSF3 as an intellectual disability
816 syndrome gene. *Nat Commun.* 2022;13(1).
- 817 60. Zhang B, Liu Y, Liu D, Yang L. Targeting cleavage and polyadenylation specific factor 1

- 818 via shRNA inhibits cell proliferation in human ovarian cancer. *J Biosci*. 2017 Sep
819 1;42(3):417–25.
- 820 61. Brumbaugh J, Di Stefano B, Wang X, Borkent M, Forouzmand E, Clowers KJ, et al.
821 Nudt21 Controls Cell Fate by Connecting Alternative Polyadenylation to Chromatin
822 Signaling. *Cell* [Internet]. 2018;172(1–2):106–120.e21. Available from:
823 <https://doi.org/10.1016/j.cell.2017.11.023>
- 824 62. Tan S, Li H, Zhang W, Shao Y, Liu Y, Guan H, et al. NUDT21 negatively regulates
825 PSMB2 and CXXC5 by alternative polyadenylation and contributes to hepatocellular
826 carcinoma suppression. *Oncogene* [Internet]. 2018;37(35):4887–900. Available from:
827 <http://dx.doi.org/10.1038/s41388-018-0280-6>
- 828 63. Chu Y, Elrod N, Wang C, Li L, Chen T, Routh A, et al. Nudt21 regulates the alternative
829 polyadenylation of Pak1 and is predictive in the prognosis of glioblastoma patients.
830 *Oncogene* [Internet]. 2019;38(21):4154–68. Available from:
831 <http://dx.doi.org/10.1038/s41388-019-0714-9>
- 832 64. Masamha CP, Xia Z, Yang J, Albrecht TR, Li M, Shyu A Bin, et al. CFIm25 links
833 alternative polyadenylation to glioblastoma tumour suppression. *Nature*.
834 2014;510(7505):412–6.
- 835 65. Ryugo M, Sawa Y, Ono M, Miyamoto Y, Aleshin AN, Matsuda H. Pharmacologic
836 preconditioning of JTE-607, a novel cytokine inhibitor, attenuates ischemia-reperfusion
837 injury in the myocardium. *J Thorac Cardiovasc Surg*. 2004;127(6):1723–7.
- 838 66. Asaga T, Ueki M, Chujo K, Taie S. JTE-607, an inflammatory cytokine synthesis inhibitor,
839 attenuates ischemia/reperfusion-induced renal injury by reducing neutrophil activation in
840 rats. *J Biosci Bioeng*. 2008;106(1):22–6.
- 841 67. Kakutani M, Takeuchi K, Waga I, Iwamura H, Wakitani K. JTE-607, a novel inflammatory
842 cytokine synthesis inhibitor without immunosuppression, protects from endotoxin shock in
843 mice. *Inflamm Res* [Internet]. 1999 Aug 1;48(8):461–8. Available from:
844 <http://link.springer.com/10.1007/s000110050487>
- 845 68. Luo H, Zhu G, Eshelman MA, Fung TK, Lai Q, Wang F, et al. HOTTIP-dependent R-loop
846 formation regulates CTCF boundary activity and TAD integrity in leukemia. *Mol Cell*

- 847 [Internet]. 2022;82(4):833-851.e11. Available from:
848 <https://doi.org/10.1016/j.molcel.2022.01.014>
- 849 69. Gorthi A, Romero JC, Loranc E, Cao L, Lawrence LA, Goodale E, et al. EWS-FLI1
850 increases transcription to cause R-Loops and block BRCA1 repair in Ewing sarcoma.
851 Nature. 2018;555(7696):387–91.
- 852 70. Stirling PC, Chan YA, Minaker SW, Aristizabal MJ, Barrett I, Sipahimalani P, et al. R-
853 loop-mediated genome instability in mRNA cleavage and polyadenylation mutants.
854 Genes Dev. 2012;26(2):163–75.
- 855 71. Yang X-C, Sabath I, Debski J, Kaus-Drobek M, Dadlez M, Marzluff WF, et al. A Complex
856 Containing the CPSF73 Endonuclease and Other Polyadenylation Factors Associates
857 with U7 snRNP and Is Recruited to Histone Pre-mRNA for 3'-End Processing. Mol Cell
858 Biol. 2013;33(1):28–37.
- 859 72. Gutierrez PA, Baughman K, Sun Y, Tong L. A real-time fluorescence assay for CPSF73,
860 the nuclease for pre-mRNA 3'-end processing. Rna. 2021;27(10):1148–54.
- 861 73. Sun Y, Zhang Y, Aik WS, Yang XC, Marzluff WF, Walz T, et al. Structure of an active
862 human histone pre-mRNA 3'-end processing machinery. Science (80-).
863 2020;367(6478):700–3.
- 864 74. Armstrong C, Spencer SL. Replication-dependent histone biosynthesis is coupled to cell-
865 cycle commitment. Proc Natl Acad Sci U S A. 2021;118(31):1–8.
- 866 75. Zhao X, McKillop-Smith S, Müller B. The human histone gene expression regulator
867 HBP/SLBP is required for histone and DNA synthesis, cell cycle progression and cell
868 proliferation in mitotic cells. J Cell Sci. 2004;117(25):6043–51.
- 869 76. Marzluff WF, Pandey NB. Multiple regulatory steps control histone mRNA concentrations.
870 Trends Biochem Sci. 1988 Feb;13(2):49–52.
- 871 77. Osley MA. The regulation of histone synthesis in the cell cycle. Annu Rev Biochem.
872 1991;60:827–61.
- 873 78. Mendiratta S, Gatto A, Almouzni G. Histone supply: Multitiered regulation ensures
874 chromatin dynamics throughout the cell cycle. J Cell Biol. 2019;218(1):39–54.

- 875 79. Barcaroli D, Bongiorno-Borbone L, Terrinoni A, Hofmann TG, Rossi M, Knight RA, et al.
876 FLASH is required for histone transcription and S-phase progression. Proc Natl Acad Sci
877 U S A. 2006;103(40):14808–12.
- 878 80. Groth A, Ray-Gallet D, Quivy JP, Lukas J, Bartek J, Almouzni G. Human Asf1 regulates
879 the flow of S phase histones during replicational stress. Mol Cell. 2005;17(2):301–11.
- 880 81. Brazelle W, Krehling JM, Gemmer J, Ma Y, Douglas Cress W, Haura E, et al. Histone
881 deacetylase inhibitors downregulate checkpoint kinase 1 expression to induce cell death
882 in non-small cell lung cancer cells. PLoS One. 2010;5(12).

883

884

885

886

887 **Figure Legends**

888 **Figure 1. CPSF3 is highly expressed in PDAC patients and predicts poor prognosis. A,**
889 *CPSF3* expression from CPTAC PDAC patient data. Whiskers indicate minimum and maximum
890 data points. ***, $P < 0.0001$, Ordinary one-way ANOVA with Tukey multiple comparisons test. **B,**
891 *CPSF3* expression from PDAC patient data (TCGA) as compared to normal pancreas (GTEx).
892 Whiskers indicate minimum and maximum data points. ***, $P < 0.0001$, unpaired t test with Welch's
893 correction. **C,** Kaplan Meier survival curves of PDAC patients with high and low *CPSF3* mRNA
894 levels. Data were obtained from CPTAC database. **D,** Quantitative RT-PCR showing *CPSF3*
895 mRNA expression levels in non-transformed pancreatic epithelial and PDAC cells. Data are
896 shown as mean \pm SEM. *, $P < 0.05$, unpaired t test with Welch's correction. **E,** Immunoblotting of
897 *CPSF3* in non-transformed pancreatic epithelial cells and PDAC cells. *CPSF3* protein levels were
898 normalized to its corresponding GAPDH levels. Fold change compares normalized data of
899 different cell lines to HPNE.

900
901 **Figure 2. CPSF3 is required for PDAC cancer cell proliferation. A,** mRNA expression of
902 *CPSF3* in shNTC, sh1 *CPSF3* and sh2 *CPSF3* knockdown cells by qPCR. Graphs are
903 representative of at least two independent experiments. Data are shown as mean \pm SEM of
904 technical duplicates. **, $P < 0.01$, Ordinary one-way ANOVA with Dunnett's multiple comparisons
905 test. **B,** Immunoblotting of *CPSF3* in shNTC, sh1 and sh2 *CPSF3* knockdown cells. **C,**
906 Proliferation rates at days 0, 2, 4 and 6 of shNTC, sh1 and sh2 *CPSF3* knockdown cells. **, $P <$
907 0.01 ; ***, $P < 0.001$; 2way ANOVA with Dunnett's multiple comparisons test. **D,** Clonogenic growth
908 assay of shNTC, sh1 and sh2 *CPSF3* knockdown cells. **E,** Normalized colony area percentage of
909 shNTC, sh1 and sh2 *CPSF3* knockdown cells from **(D)**. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$;
910 Ordinary one-way ANOVA with Dunnett's multiple comparisons test. **F,** Volume of *CPSF3*-
911 knockdown and control MiaPaCa2 tumors. ***, $P < 0.001$, 2way ANOVA. **G,** Endpoint tumor
912 weight. *, $P < 0.05$, unpaired t test with Welch's correction. **H,** Gross images of shNTC (n=7) and
913 sh*CPSF3* (n=6) dissected tumors. **I,** Hematoxylin and Eosin (H&E) staining of xenograft tumors.
914 **J** and **K,** IHC for *CPSF3* and Ki67, respectively. Box and whisker plots indicate the percentage of
915 *CPSF3*- and Ki67-positive areas in the tumors. **, $P < 0.01$; ***, $P < 0.001$; unpaired t test.

916
917 **Figure 3. CPSF3 knockdown drives global APA dysregulation in PDAC. A,** PDUI score of
918 each gene in shNTC and sh*CPSF3* cells. Dashed lines represent 0.1 cutoffs. Blue dots represent
919 3'-UTR-lengthened genes, and red dots represent 3'-UTR-shortened genes. **B,** Genes showing
920 lengthening (right) or shortening (left) events ($-0.1 > \text{PDUI} > 0.1$; $P < 0.05$) and are differentially

921 expressed (FDR<0.05; fold change >1.5) as color coded. Up=upregulated gene expression,
922 Down=downregulated gene expression. **C**, *FHL1* DSeq2 normalized counts. ***, $P < 0.0001$. **D**,
923 *FHL1* mRNA levels in *CPSF3* knockdown MiaPaCa2 cells. *, $P < 0.05$, unpaired t test. **E**, 3'-
924 RACE of *FHL1* in shNTC, sh1 and sh2 *CPSF3* Panc1 cells. Approximate length of the 3'-UTR
925 form is denoted beside each band. **F**, Western blot of *FHL1* protein levels in shNTC, sh1 and sh2
926 *CPSF3* Panc1 cells. **G**, *FHL1* mRNA levels in sh2 *CPSF3* Panc1 cells transfected with siRNA
927 against *FHL1* (si*FHL1*). *, $P < 0.05$, unpaired t test. **H**, Proliferation assay of si*FHL1* in *CPSF3*
928 knockdown and control Panc1 cells. **, $P < 0.01$; 2way ANOVA with Tukey multiple comparisons
929 test. **I**, Correlation of *FHL1* with *CPSF3* mRNA levels from the CPTAC database. Spearman's
930 correlation= -0.36. $P < 0.0001$.

931
932 **Figure 4. PDAC cell lines are sensitive to CPSF3 inhibition by JTE-607.** **A**, IC50 of JTE-607
933 on non-transformed and PDAC cell lines. **B**, IC50 of JTE-607 on human fibroblast C7 and PancPat
934 CAFs. **C**, IC50 values. **D and E**, Proliferation rates at days 0, 2, 4 and 6 of non-transformed and
935 PDAC cell lines after treatment with escalating concentrations of JTE-607. *, $P < 0.05$; 2way
936 ANOVA with Dunnett's multiple comparisons test. Data are shown as mean±SEM. **F**, Clonogenic
937 growth assay of PDAC cell lines after treatment with increasing concentration of JTE-607. **G**,
938 Normalized colony area percentage of PDAC cell lines from (**F**). *, $P < 0.01$; **, $P < 0.001$; ***, P
939 < 0.0001 ; Ordinary one-way ANOVA with Dunnett's multiple comparisons test. Data are shown
940 as mean±SEM.

941
942 **Figure 5. JTE-607 induces global APA dysregulation and decreases histone expression in**
943 **PDAC cells.** **A**, Changes in PDUI score ($-0.1 > PDUI > 0.1$; $P < 0.05$) denoting 3'-UTR-shortened
944 (red) and lengthened (blue) genes. **B**, Genes showing lengthening (right) or shortening (left)
945 events ($-0.1 > PDUI > 0.1$; $P < 0.05$) and are differentially expressed (FDR<0.05; fold change >1.5)
946 as color coded. Up=upregulated gene expression, Down=downregulated gene expression. **C**,
947 Venn diagram showing overlapping genes with significant APA alterations between JTE-607-
948 treated and *CPSF3* knockdown cells. **D**, Heatmap of differentially expressed genes in Panc1 cells
949 treated with JTE-607. Expression is plotted as transformed expression value. Blue triangles
950 denote replication-dependent histone genes. **E**, Gene set enrichment analysis (GSEA) of RNA-
951 seq data from (**D**). **F**, mRNA expression of *H2B* and *H3* in MiaPaCa2 cells treated with JTE-607.
952 *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, Ordinary one-way ANOVA with Dunnett's multiple
953 comparisons test.

954

955 **Figure 6. JTE-607 induces replication-dependent histone transcription read-through. A,**
956 IGV-generated density plots of replication-dependent histones highlighting the differences of 3'-
957 UTR coverage between DMSO (red) and JTE-607 (blue) treated cells. **B,** Western blot of H2B
958 and H3 protein levels in Panc1 cells treated with 0-10 μ M JTE-607 for 24 and 48hrs. **C,** IGV-
959 generated density plots of replication-independent histones highlighting the differences of 3'-UTR
960 coverage between DMSO (red) and JTE-607 (blue) treated cells. **D,** DSeq2 normalized counts of
961 *H2AFZ* and *H3F3A* histone variants in Panc1 cells treated with JTE-607. **, $P < 0.001$. **E-G,**
962 Volcano plots of Spearman's correlation of CPSF3 and: **E,** all histone genes; **F,** replication-
963 dependent histone genes; **G,** replication-independent histone genes. Each dot represents a
964 histone gene. Blue and red dots denote positive and negative correlation, respectively.
965 (Spearman = $-0.15 > R > 0.15$, $P < 0.05$).

966
967 **Figure 7. JTE-607 induces chromatin instability. A,** Micrococcal Nuclease assay of Panc1 cells
968 treated with JTE-607 or CBL0137. **B,** Quantification of nucleosome fragments after 30 minutes of
969 MNase digestion. Data are shown as mean \pm SEM of two technical repeats. *, $P < 0.05$, unpaired
970 t test with Welch's correction. **C,** GFP+ HeLa-TI cells following JTE-607 or CBL0137 treatment.
971 **D,** Fold change of GFP+ HeLa-TI from **(C)**. ***, $P < 0.0001$; 2way ANOVA with Tukey's multiple
972 comparisons test. **E,** Flow cytometry analysis of GFP+ HeLa-TI cells following JTE-607 or
973 CBL0137 treatment. Fold change is shown as mean \pm SEM of two independent experiments. **, P
974 < 0.01 , ***, $P < 0.0001$, Ordinary one-way ANOVA with Tukey's multiple comparisons test.

975
976 **Figure 8. JTE-607 induces S-phase arrest. A and B,** Cell cycle distribution and quantification
977 of Panc1, MiaPaCa2 and HPNE cell lines. *, $P < 0.05$, **, $P < 0.001$, ***, $P < 0.0001$, 2way ANOVA
978 with Dunnett's multiple comparisons test. **C,** BrdU incorporation assay showing cell cycle
979 population upon JTE-607 treatment.

Figure 1

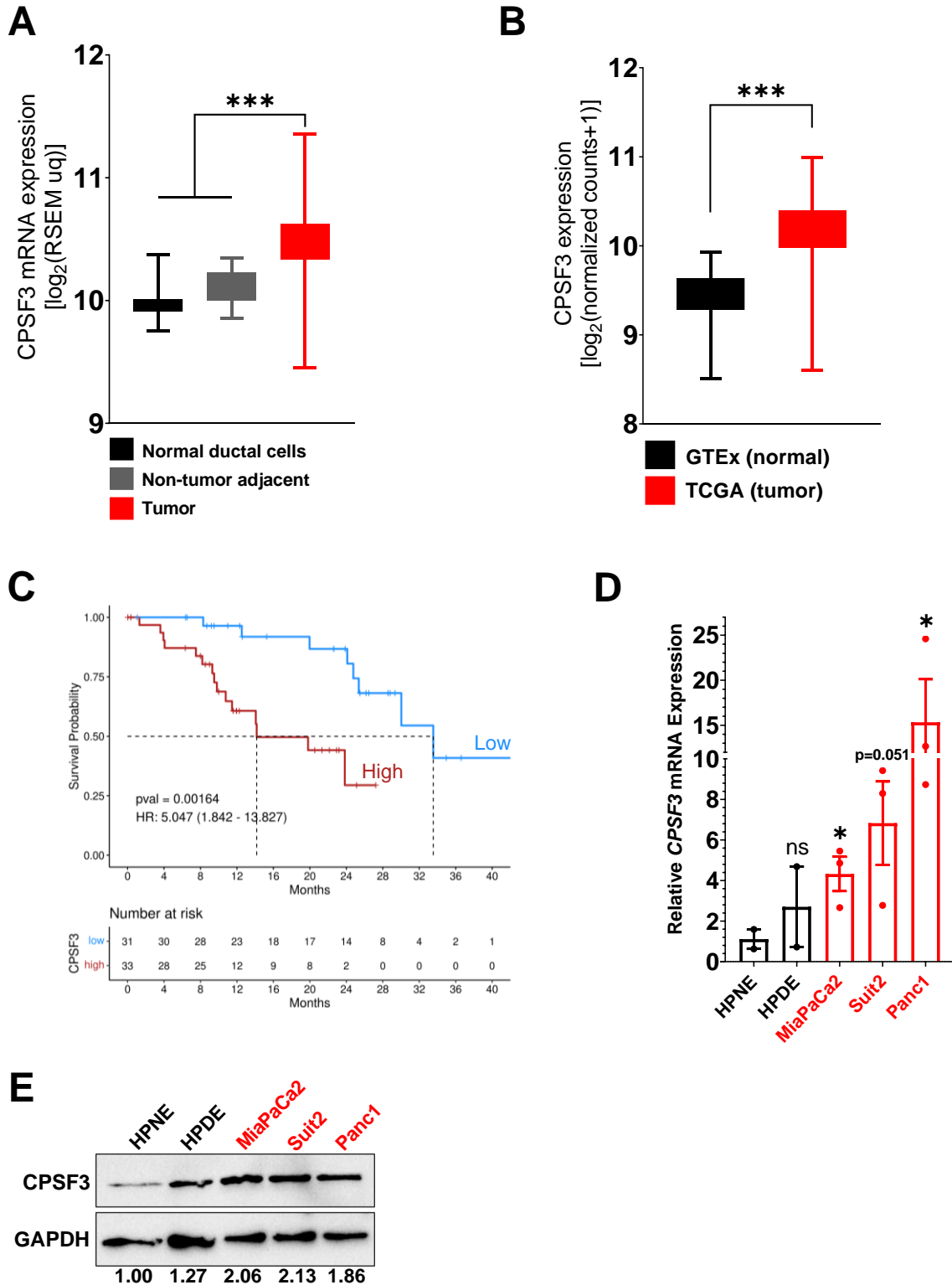


Figure 2

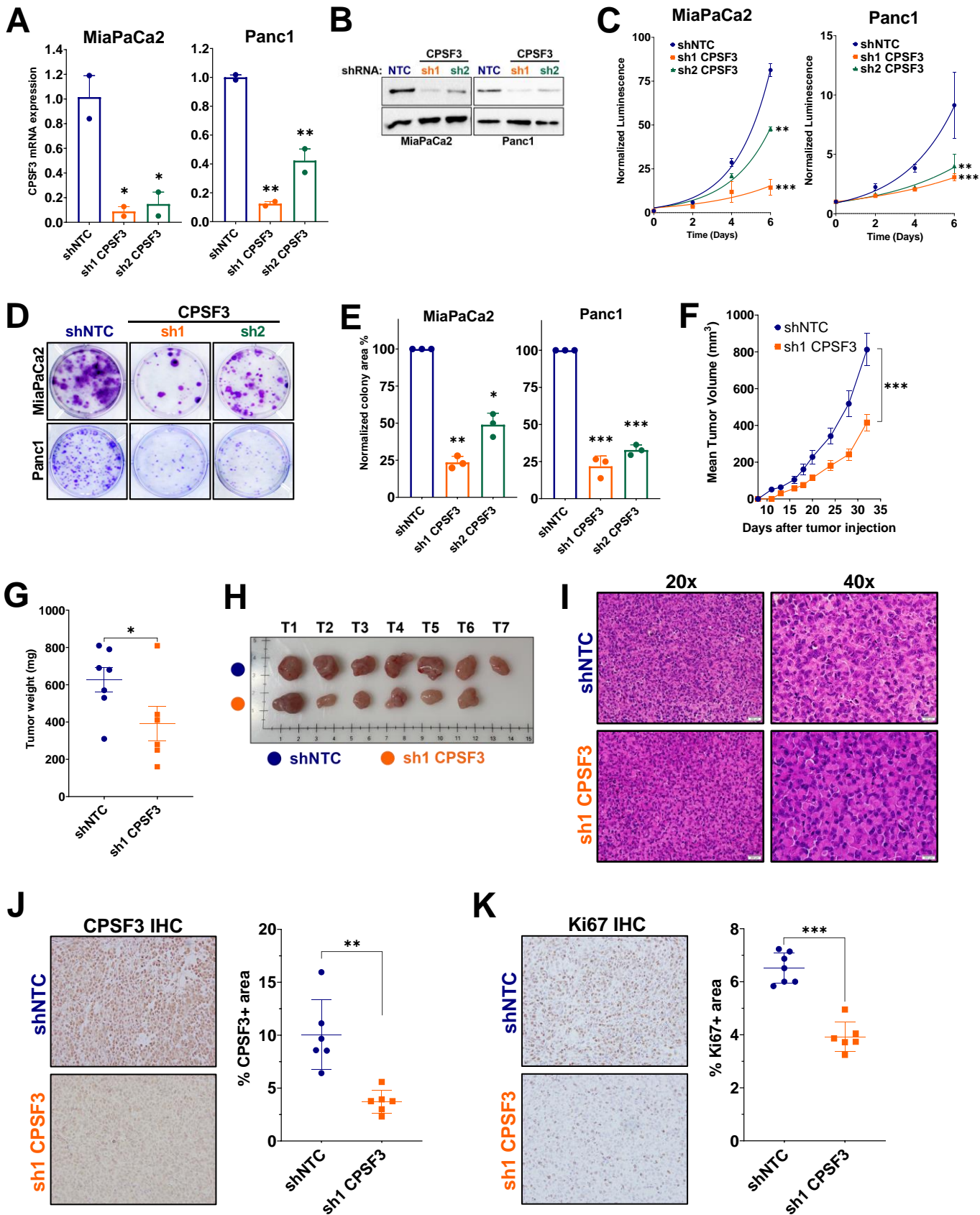


Figure 3

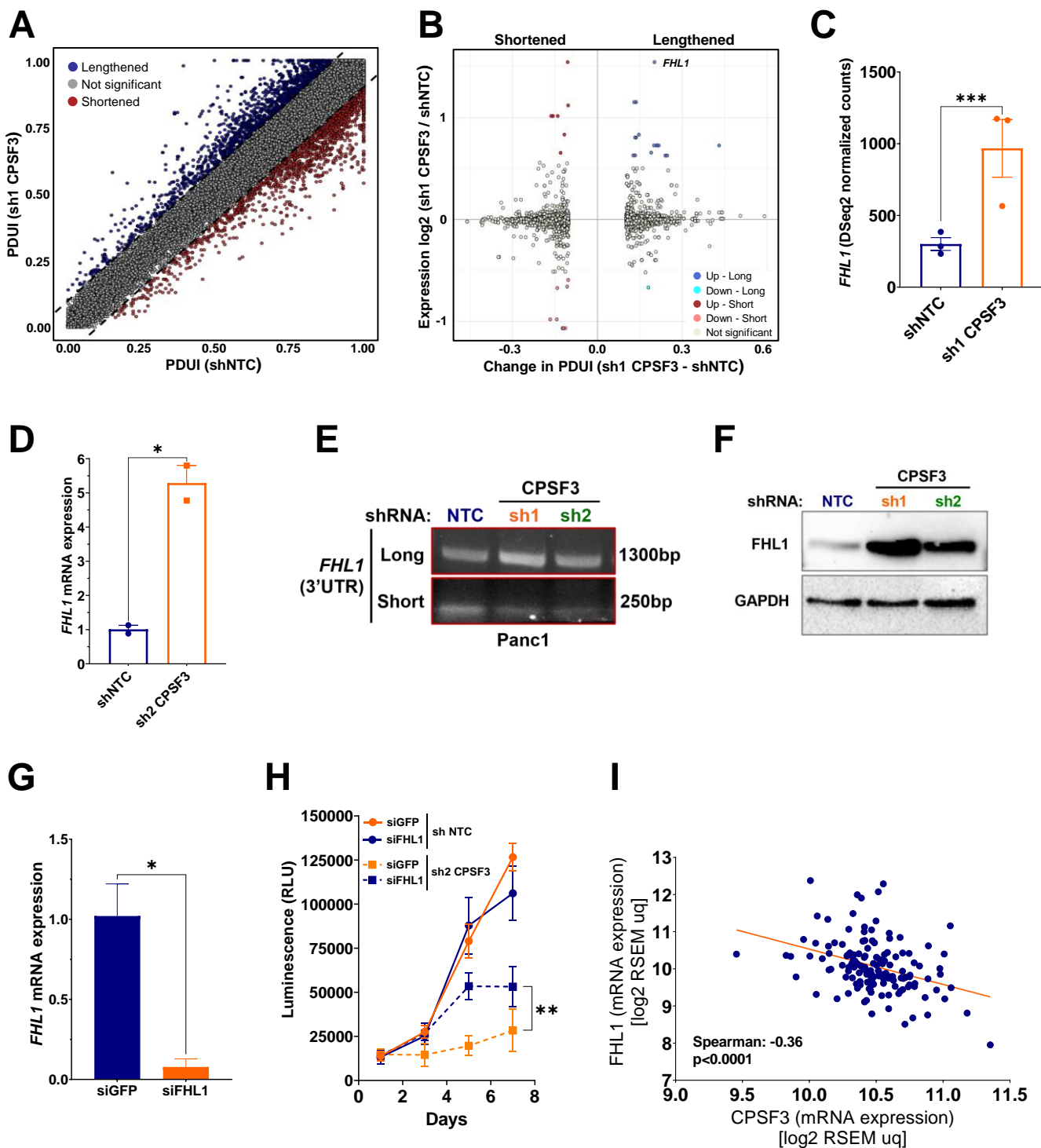


Figure 4

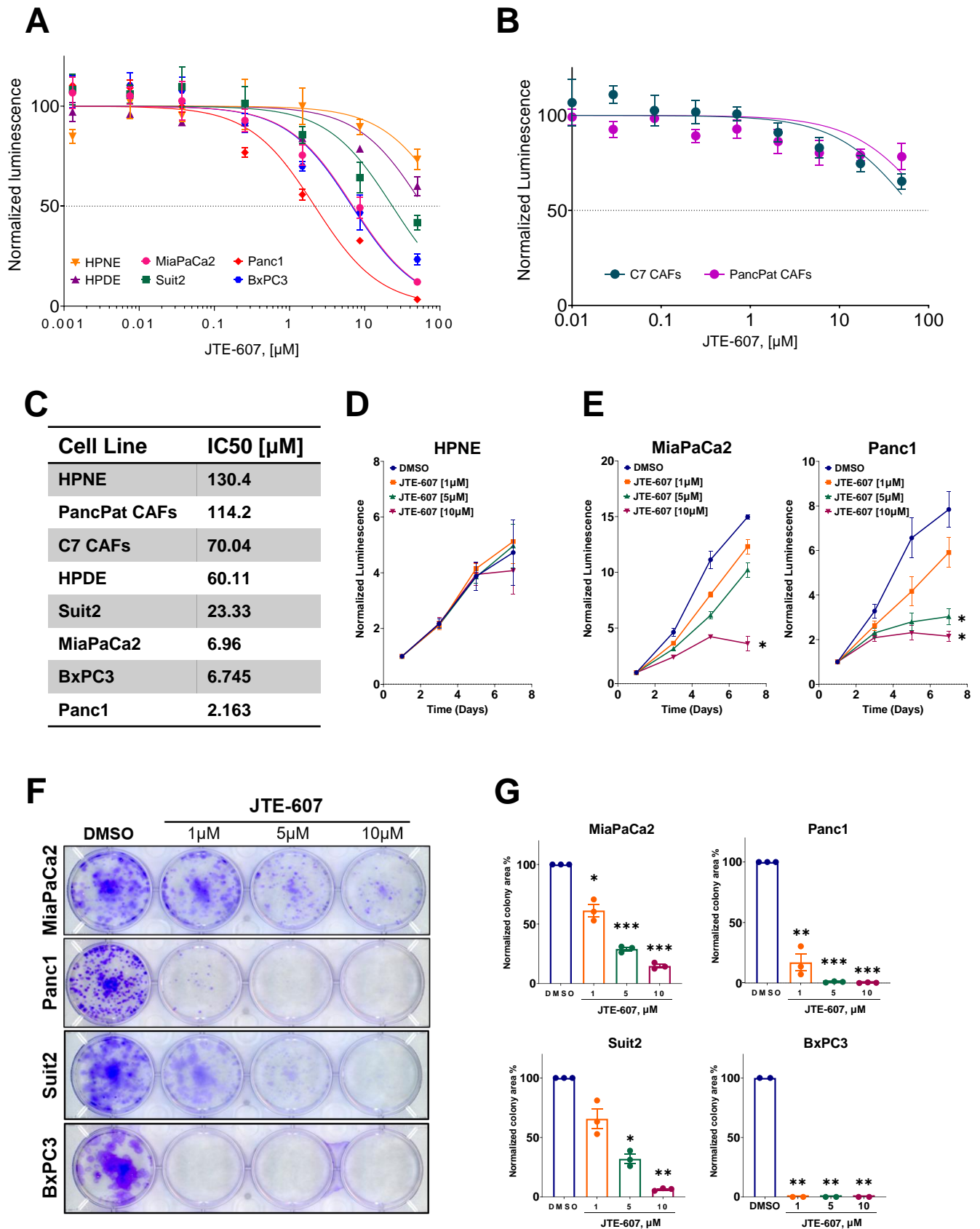


Figure 5

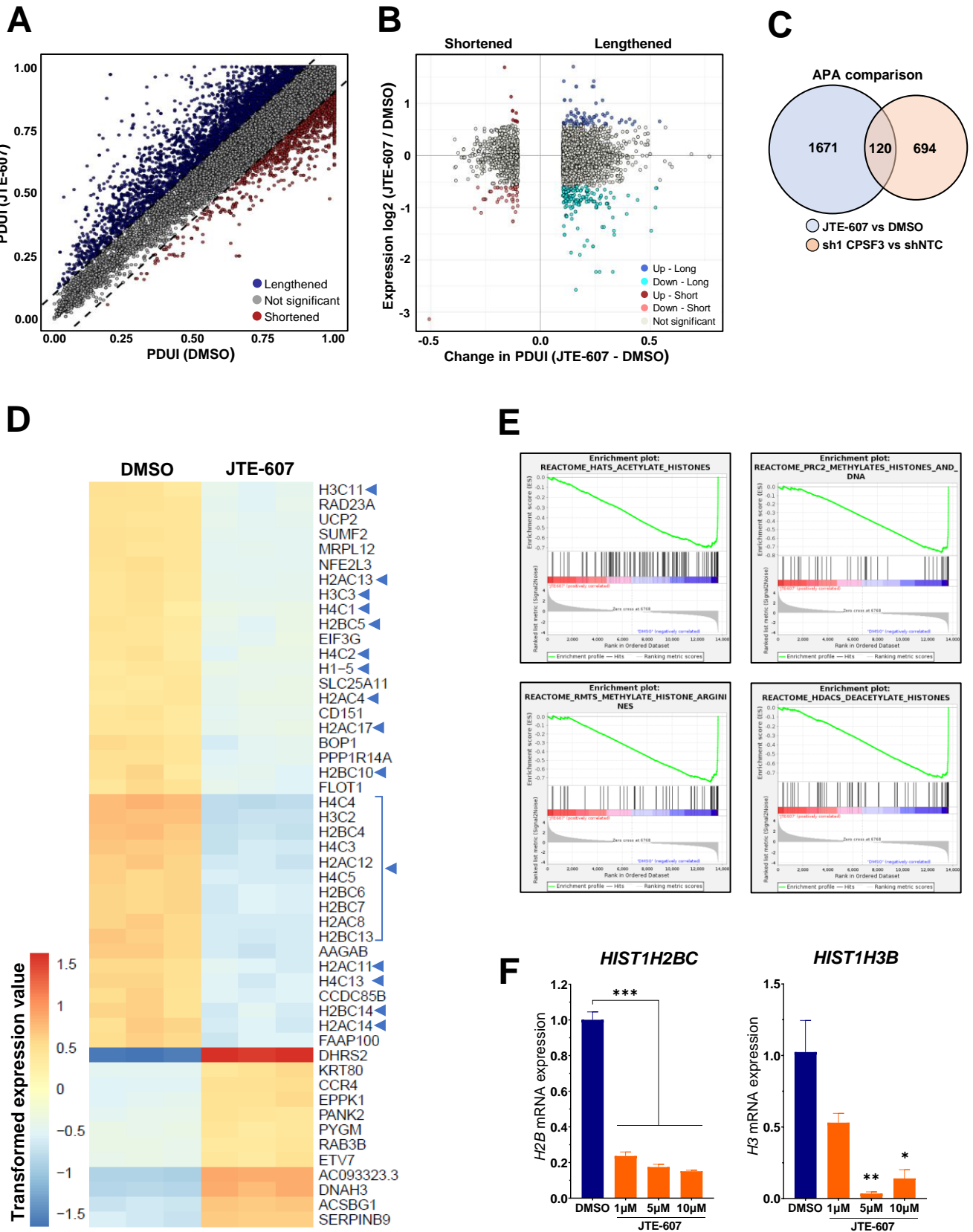


Figure 6

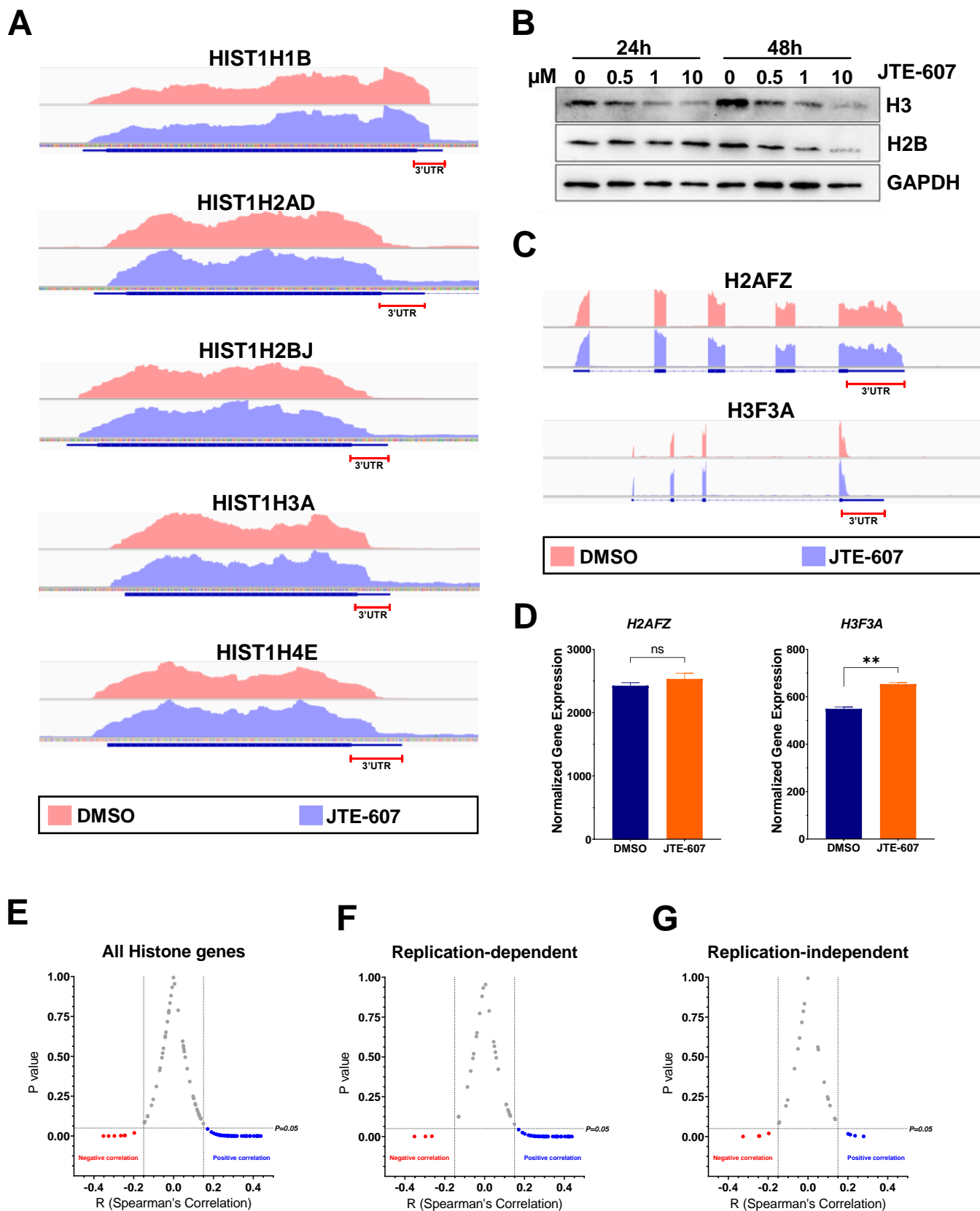
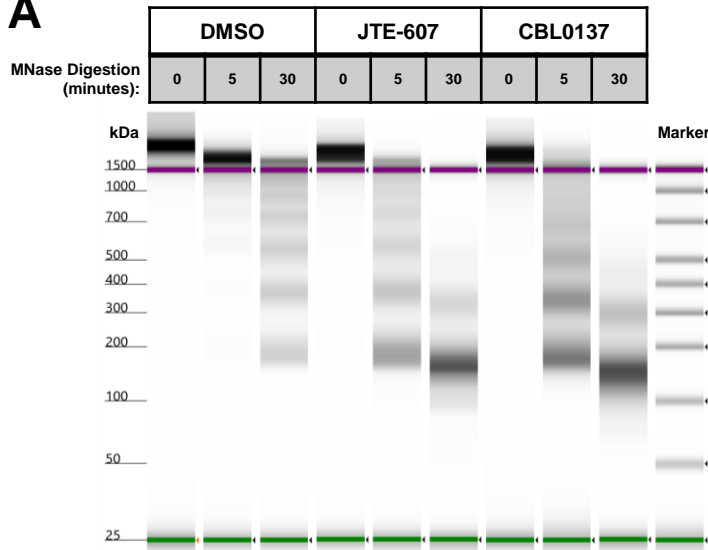
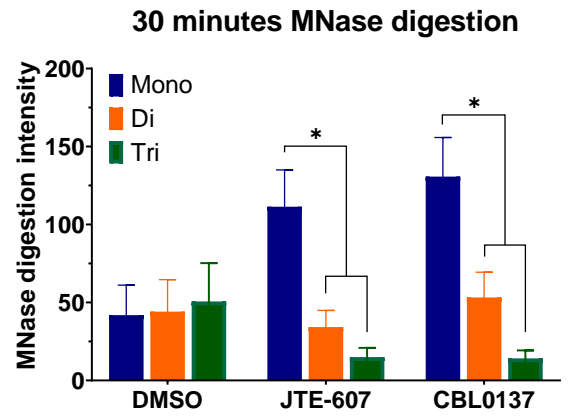


Figure 7

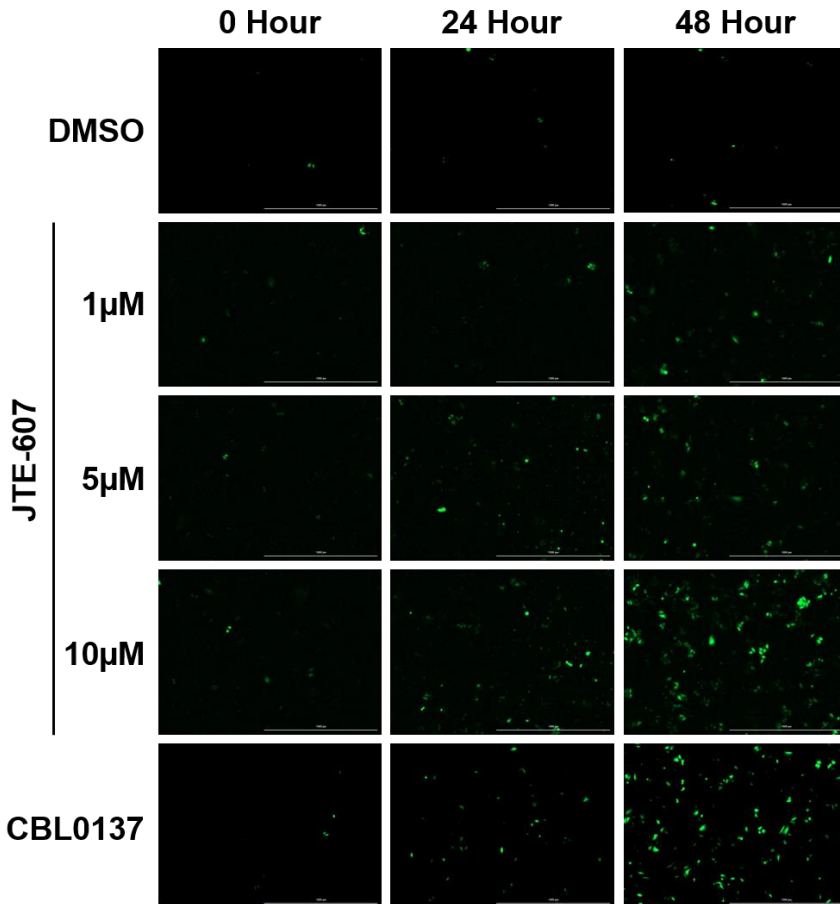
A



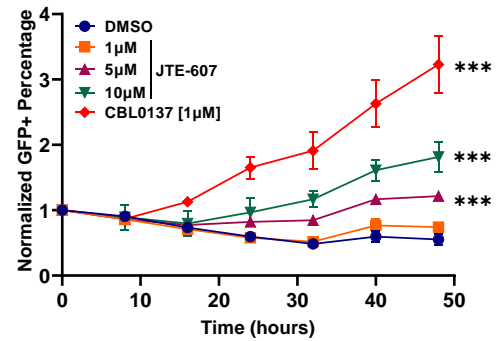
B



C



D



E

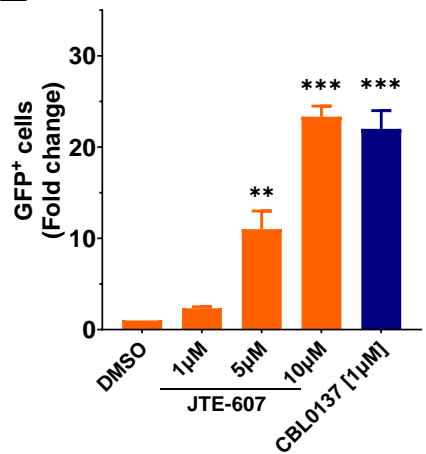


Figure 8

