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Mutant SF3B1 promotes PDAC malignancy through TGF- β resistance

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1 **ABSTRACT**

2 The splicing factor SF3B1 is recurrently mutated in various tumors, including pancreatic ductal
3 adenocarcinoma (PDAC). The impact of the hotspot mutation SF3B1^{K700E} on the PDAC
4 pathogenesis, however, remains elusive. Here, we demonstrate that Sf3b1^{K700E} alone is
5 insufficient to induce malignant transformation of the murine pancreas, but increases
6 aggressiveness of PDAC if it co-occurs together with mutated KRAS and p53. We further
7 demonstrate that SF3B1^{K700E} reduces epithelial–mesenchymal transition (EMT) and confers
8 resistance to TGF- β 1-induced cell death, and provide evidence that this phenotype is in part
9 mediated through aberrant splicing of *Map3k7*. Taken together, our work suggests that
10 SF3B1^{K700E} acts as an oncogenic driver in PDAC through enhancing resistance to the tumor
11 suppressive effects of TGF- β .

12 **Keywords:** SF3B1, K700E mutation, splicing, pancreatic cancer, TGF- β , apoptosis, MAP3K7

13 INTRODUCTION

14 Genes involved in RNA splicing are frequently mutated in various cancer types (Yoshida et
15 al., 2011). The splicing factor subunit 3b 1 (SF3B1) is amongst the most commonly mutated
16 components of the splicing machinery, with high incidence in myelodysplastic syndromes
17 (MDS) (Je et al., 2013) and chronic lymphocytic leukemia (CLL) (Miao et al., 2019). However,
18 also in various solid tumors SF3B1 is recurrently mutated, including uveal melanoma (UVM)
19 (Furney et al., 2013), breast cancer (BRCA) (Fu et al., 2017; Maguire et al., 2015; Sun et al.,
20 2020), prolactinomas (Li et al., 2020) hepatocellular carcinoma (HCC) (Zhao et al., 2021) and
21 pancreatic adenocarcinoma (PDAC) (Bailey et al., 2016; Yang et al., 2021). As part of the U2
22 small nuclear ribonucleoprotein (U2 snRNP) SF3B1 exerts an essential function in RNA
23 splicing by recognizing the branchpoint sequence (BPS) of nascent RNA transcripts (Wahl et
24 al., 2009; Zhang et al., 2020). This process is crucial for the definition of the 3' splice site (3'
25 ss) of the upstream exon-intron boundary, a prerequisite for the accurate removal of introns
26 (Wahl et al., 2009). It is well understood that hotspot mutations in SF3B1 at HEAT repeats 5-
27 9 allow the recognition of an alternative BPS, resulting in the inclusion of a short intronic
28 region into the mature messenger RNA (mRNA) (Alsafadi et al., 2016; Canbezdi et al., 2021;
29 Darman et al., 2015; DeBoever et al., 2015; Kesarwani et al., 2017). These alternatively spliced
30 transcripts are prone to degradation through nonsense mediated RNA decay (NMD) (Darman
31 et al., 2015). Several recent studies have evaluated the mechanistic contribution of genes mis-
32 spliced by oncogenic SF3B1 to tumor progression. So far, missplicing of *PPP2R5A* was found
33 to increase malignancy through stabilizing c-Myc (Liu et al., 2020a; Yang et al., 2021), and
34 aberrant *MAP3K7* splicing was reported to promote NF- κ B-driven tumorigenesis (Liu et al.,
35 2021).

36 Despite compelling evidence on the oncogenic role of mutated SF3B1 in hematologic
37 malignancies, its contribution to the formation and/or progression of solid tumors is less well
38 understood. Since splicing deregulation has been reported as a hallmark of PDAC, with SF3B1
39 being recurrently mutated (Bailey et al., 2016), we aimed at elucidating the impact of the
40 frequently occurring SF3B1^{K700E} mutation to the pathogenesis of this tumor type. We
41 demonstrate that *Sf3b1*^{K700E} increases malignancy in a mouse model for PDAC by decreasing
42 the sensitivity of tumor cells to TGF- β -induced cell-death. We further provide evidence that
43 TGF- β -resistance is mediated through missplicing of *Map3k7*. Together, our work suggests

44 that SF3B1^{K700E} exerts its oncogenic role in PDAC by dampening the tumor-suppressive effect
45 of TGF- β .

46 RESULTS

47 SF3B1^{K700E} is a tumor driver in PDAC

48 RNA processing has been previously identified as a hallmark of pancreatic cancer in the
49 PACA-AU cohort (Bailey et al., 2016). Validating these findings, we found that genes
50 encoding for the splice factors RBM10, SF3B1 and U2AF1 are also frequently mutated in the
51 PACA-CA cohort (Suppl. Fig. 1A). In accordance with its described function as a tumor
52 suppressor (Hernández et al., 2016), 56% of the mutations found in RBM10 lead to a truncated
53 protein. Conversely, in SF3B1 and U2AF1 the majority of mutations were missense mutations
54 that occurred at hotspot sites, indicating a neomorphic function of the mutated proteins. Like
55 in other cancer types, also in PDAC the most frequently found mutation in SF3B1 led to a
56 lysine (K) to glutamic acid (E) amino acid change at position 700 (SF3B1^{K700E}) (Suppl. Fig.
57 1B). Therefore, we experimentally tested if the SF3B1^{K700E} mutation contributes to PDAC
58 malignancy by generating a mouse model where the *Sf3b1*^{K700E} mutation is specifically
59 activated in the pancreas using *Ptf1a*-Cre (Fig. 1A, Suppl. Fig. 1C). First, we tested if
60 *Sf3b1*^{K700E/+} alone could already induce PDAC formation. However, heterozygous activation
61 of the *Sf3b1*^{K700E} allele did not have any effect on survival of mice or weight of the pancreas
62 after 300 days (Fig. 1B, C). In addition, no difference in tissue architecture and cell
63 proliferation was observed (Suppl. Fig. 1D, E). Next, we assessed if the K700E mutation could
64 enhance aggressiveness of PDAC, and crossed the *Sf3b1*^{K700E} allele into the *Ptf1a*-Cre;
65 *Kras*^{G12D/+}; *Trp53*^{fl/fl} (KPC) mouse model (Fig. 1D), which is known to induce PDAC within
66 2 to 3 months (Bardeesy et al., 2006; Hingorani et al., 2005; Marino et al., 2000). Importantly,
67 KPC-Sf3b1^{K700E/+} animals displayed a significantly shorter survival and an increased tumor
68 size compared to KPC mice, although no obvious differences in tissue architecture were found
69 (Fig. 1E-G, Suppl. Fig. 1F-H). Likewise, we also observed an effect on tumor malignancy when
70 we introduced the *Sf3b1*^{K700E/+} mutation into *Kras*^{G12D/+} mice, which is a model for pre-
71 cancerous pancreatic neoplasms with sporadic PDAC formation after a prolonged latency
72 period (Hingorani et al., 2005). Introducing the *Sf3b1*^{K700E/+} mutation in *Kras*^{G12D/+} mice
73 resulted in increased pancreas weight, a larger area of neoplastic pancreas tissue (Fig. 1I - K),

74 and in 64% of mice compared to 10% of *Kras*^{G12D/+} control mice pancreatic lesions developed
75 into PDAC at an age of 43 weeks (Fig 1L).

76 **SF3B1^{K700E} reduces expression of EMT genes in pancreatic tumors**

77 In order to elucidate the functional impact of SF3B1^{K700E} on the transcriptome, we isolated
78 cancer cells of mouse tumors by fluorescence-activated cell sorting (FACS) of Epithelial cell
79 adhesion molecule (EpCAM) positive cells and performed RNA-sequencing (RNA-seq) (Table
80 1). High purity of isolated tumor cells was confirmed by the absence of sequencing reads for
81 *Trp53* exons 2–10, which are excised via Cre-recombination specifically in tumor cells (Suppl.
82 Fig. 2A), and by the presence of the *Sf3b1*^{K700E} mutation in 38% of the transcripts (Fig. 1D).
83 Principal component analysis separated the sequenced replicates (3 KPC and 4 KPC-
84 *Sf3b1*^{K700E/+} tumors) according to the genotype, indicating a major impact of the K700E
85 mutation on the transcriptome (Suppl. Fig. 2B).

86 We next performed gene set enrichment analysis (GSEA), which revealed IFN- α -response as
87 the most significantly enriched pathway in KPC-Sf3b1^{K700E/+} tumor cells (Suppl. Fig. 2C). This
88 result is in line with a previous study, which found that aberrant splicing caused by SF3B1
89 inhibition or oncogenic SF3B1 mutations induces an IFN- α -response through retinoic acid-
90 inducible gene I (RIG-I) mediated recognition of cytosolic aberrant RNA-species (Chang et
91 al., 2021). Interestingly, the most significantly depleted gene set in KPC-Sf3b1^{K700E/+} cells was
92 epithelial-mesenchymal transition (EMT) (Fig. 2A, B, Suppl. Fig. 2C). We first confirmed
93 downregulation of the most significantly depleted gene of the EMT gene set, the glycoprotein
94 Tenascin-C (*Tnc*), by qPCR on additional KPC-Sf3b1^{K700E/+} tumor samples (Fig. 2C) and by
95 histology in KPC-Sf3b1^{K700E/+}PDAC sections (Fig. 2D, E). Next, we assessed if the reduction
96 of EMT genes was induced cell-autonomously by the *Sf3b1*^{K700E/+} mutation, or if it was an
97 indirect consequence of the altered micro-environment in *Sf3b1*^{K700E} KPC tumors. We therefore
98 compared the expression of the 15 most significantly depleted EMT genes (Fig. 2B) *in vitro* in
99 *Sf3b1*^{K700E} vs. *Sf3b1* WT KPC pancreatic organoids. Importantly, 71% of the analysed genes
100 were significantly reduced in KPC-Sf3b1^{K700E/+} vs. KPC organoids, with none of the genes
101 showing a trend towards elevated expression (Fig. 2F). Finally, we analysed whether
102 differences in EMT gene expression could be a consequence of differences in the tumor stage
103 between *Sf3b1*^{K700E} versus *Sf3b1* WT KPC tumors. We therefore established non-cancerous
104 pancreatic organoids from LSL-*Kras*^{G12D/+}; *Trp53*^{fl/fl}; *Sf3b1*^{fl^{K700E}/+} and LSL-*Kras*^{G12D/+};

105 *Trp53^{fl/f}; Sf3b1^{+/+}* mice, and induced recombination *in vitro* through lentiviral Cre transduction
106 (Suppl. Fig. 2D). However, also in this experimental setup, 67% of the analysed EMT genes
107 were significantly downregulated in *Sf3b1^{K700E}* vs. *Sf3b1* WT organoids, with only one of the
108 analysed genes showing a minor trend for elevated expression (Suppl. Fig. 2E). Together, these
109 data indicate that *Sf3b1^{K700E}* mediates downregulation of EMT genes in a cell autonomous
110 manner independently of the PDAC microenvironment or stage.

111 **SF3B1^{K700E} confers resistance to TGF- β 1-induced cell death**

112 The two major EMT-promoting cytokines are TNF- α and TGF- β (Bulle and Lim, 2020). We
113 therefore determined if the EMT genes that are most significantly downregulated by *Sf3b1^{K700E}*
114 in KPC tumours are induced by TNF- α or TGF- β . Importantly, we found that 80% of the
115 analysed genes were strongly induced by TGF- β in tumour derived KPC cells (Fig 3A),
116 whereas TNF- α significantly upregulated only 20% of these genes (Suppl. Fig. 2F).
117 Furthermore, we observed that *in vitro* induced *Sf3b1^{K700E}* KPC organoids show a 6-fold
118 reduced invasion through matrigel when stimulated with TGF- β , linking the repression of TGF-
119 β responsive EMT genes with a functional migratory impairment (Figure 3B, C).

120 In pancreatic lesions TGF- β induces EMT, followed by apoptosis of the affected cells in a
121 process termed lethal EMT (David et al., 2016). This prompted us to speculate that SF3B1^{K700E}
122 could drive PDAC progression by reducing sensitivity of epithelial cells to TGF- β -mediated
123 lethal EMT. Performing immunofluorescence staining for cleaved caspase 3 in KPC tumors,
124 we first confirmed that the majority of apoptotic cells reside in the lumen of PanINs (Suppl.
125 Fig. 2G) and that these cells are negative for the epithelial markers E-cadherin and high
126 Fibronectin-1 (Fig. 3D, Suppl. Fig. 3A) (Hruban et al., 2006). We then analysed *Sf3b1^{K700E}*
127 tumors by immunofluorescence staining. In line with our hypothesis, we observed a reduction
128 in luminally extruded cells and a reduction in cleaved caspase 3-positive cells (Fig. 3D, E,
129 Suppl. Fig. 3B). To further analyse the impact of *Sf3b1^{K700E}* on the tumor suppressive effect of
130 TGF- β , we exposed *Sf3b1* WT and *Sf3b1^{K700E}* KPC tumor organoids to TGF- β 1. We again
131 observed reduced caspase 3 and 7 activity in *Sf3b1* mutant organoids, and a greatly increased
132 survival rate (72% vs. 17% surviving organoids, Fig. 3F-H). Since the tumor-suppressing effect
133 of TGF- β is most prominent on pre-cancerous epithelial cells (Massagué, 2008), we
134 additionally established organoid lines with- and without *Sf3b1^{K700E}* from non-cancerous
135 mouse pancreata. While *Sf3b1^{K700E}* reduced proliferation without supplementation of TGF- β 1

136 (Suppl. Fig. 3C), treatment with TGF- β 1 led to significantly lower caspase 3 and 7 activity in
137 *Sf3b1*^{K700E/+} organoids, and to significantly enhanced survival (77% vs. 3%) (Fig. 3I-K).
138 Likewise, also in *Kras*^{G12D/+} organoids SF3B1^{K700E} led to increased survival in the presence of
139 TGF- β 1 (Suppl. Fig. 3D). Finally, exposure to low levels (1 ng/ml and 2 ng/ml) of TGF- β 1
140 also allowed long-term expansion of *Sf3b1*^{K700E/+} organoids (analysed for over 120 days), while
141 the number of *Sf3b1* WT organoids rapidly declined within the first 15 days (Fig. 3L, Suppl.
142 Fig. 3E, F).

143 To assess if reduced sensitivity to TGF- β is also observed in human pancreas cells containing
144 the SF3B1^{K700E} mutation, we stably overexpressed either wildtype or mutated SF3B1 in the
145 pancreatic duct cell line H6c7. This cell line is derived from healthy pancreatic tissue, which
146 unlike all tested PDAC-derived cell lines (BxPC-3, Mia PaCa-2, PANC-1 and PSN-1) is still
147 partially responsive to the suppressive effect of TGF- β signalling (Fig. 3M, Suppl. Fig. 3G-K).
148 In line with our results from murine PDAC, overexpression of mutant SF3B1^{K700E} resulted in
149 an increased viability upon TGF- β 1 exposure compared to overexpressing wildtype SF3B1
150 (Fig. 3M), while no effect of SF3B1^{K700E} on proliferation was observed in absence of TGF- β 1
151 treatment in human pancreatic duct cells and PDAC cell lines (Suppl. Fig. 3L).

152 **SF3B1^{K700E} reduces TGF- β sensitivity through *Map3k7* missplicing**

153 To identify how SF3B1^{K700E} could mediate TGF- β resistance, we next assessed the impact of
154 the mutation on RNA-splicing. By analysing RNA-seq data from sorted KPC vs. KPC-
155 *Sf3b1*^{K700E/+} tumor cells we predominantly identified alternative 3' splice events (Fig. 4A,
156 Table 2), with cryptic 3' ss showing an upstream adenosine enrichment and a less pronounced
157 polypyrimidine tract most often located 8-14 bases upstream of the canonical 3' ss (Fig. 4B,
158 C). These findings are in accordance with previous splice-analyses performed in various
159 murine SF3B1 mutant tissues (Liu et al., 2021, 2020a; Mupo et al., 2017; Obeng et al., 2016;
160 Yin et al., 2019). Next, we sought to determine which of the identified K700E-dependent
161 alternative splice events are conserved between mice and humans. Due to limited publicly
162 available RNA-seq datasets in human PDAC, we analysed a pan-cancer dataset containing
163 samples of 32 different cancer types. In agreement with previous studies, we found that also in
164 human cancers the SF3B1^{K700E} hotspot mutation leads to a predominant use of cryptic 3' ss
165 (Suppl. Fig. 4A, B, Table 2) (Alsafadi et al., 2016; DeBoever et al., 2015; Kesarwani et al.,
166 2017; Tang et al., 2020; Wang et al., 2016). Importantly, we further identified 11 genes that
167 contained an alternative 3' splice-event linked to SF3B1^{K700E} in human tumors and KPC mice
168 (Fig. 4D). Of those genes, *MAP3K7* (formerly known as TGF- β activated kinase 1 / *TAK1*) in

169 particular raised our attention. It is a well-described effector of cytokine-signalling that
170 mediates non-canonical TGF- β signalling (Kim and Choi, 2012), and it was recently shown to
171 induce EMT and apoptosis in TGF- β stimulated human mammary cells (Tripathi et al., 2019).
172 Using targeted RNA-seq, we found that one third of *Map3k7* transcripts were misspliced in
173 pancreata of *Sf3b1*^{K700E/+} and KPC-*Sf3b1*^{K700E/+} mice (Fig. 4E, F, Suppl. Fig. 4C). Confirming
174 inter-species conservation of this alternative splice-event, we also found *MAP3K7* missplicing
175 in H6c7 cells and human PDAC cell lines overexpressing *SF3B1*^{K700E} (Fig. 4F, Suppl. Fig.
176 4D). Notably, we also assessed *SF3B1*^{K700E} dependent alternative splicing of *Ppp2r5a*, which
177 was reported to impair apoptosis via post-translational modification of BCL2 in leukaemia (Liu
178 et al., 2020a). However, we did not observe significant alternative 3'ss usage or mRNA
179 expression of *Ppp2r5a* in *Sf3b1*^{K700E} mutant KPC tumors (Suppl. Fig. 4E, F), indicating tissue-
180 specificity of this splice-event.

181 Since *Sf3b1*^{K700E} dependent missplicing in *MAP3K7* was shown to result in reduced RNA and
182 protein levels of MAP3K7 in leukaemia (North et al., 2022), we hypothesized that the reduced
183 responsiveness to TGF- β signalling in *SF3B1*^{K700E} mutant pancreas cells is caused by lower
184 MAP3K7 levels. First, we confirmed a reduction in *Map3k7* levels in vitro and in vivo in
185 *Sf3b1*^{K700E} mutant pancreatic cells by RT-qPCR and western blotting (Fig. 5A-D). Next, we
186 tested whether this reduction could explain the observed resistance to TGF- β in *Sf3b1*-mutant
187 PDAC, and assessed the expression of EMT genes in TGF- β treated KPC cells with a stable
188 knock-down of MAP3K7 (Fig. 5E, Suppl. Fig. 4G). A decrease of *Map3k7* mRNA levels to
189 35% (SD \pm 10%) led to a reduced expression in 7 out of 10 EMT genes (Suppl. Fig. 4H).
190 Knocking down *Map3k7* in pancreatic organoids, moreover, led to increased viability upon
191 TGF- β 1-treatment (Fig. 5F, Suppl. Fig. 4I), and chemical inhibition of p38, one of the major
192 effectors of MAP3K7, partially protected organoids against TGF- β 1 induced cell death (Fig.
193 5G). Further supporting our hypothesis that *Sf3b1*^{K700E} mediates resistance to TGF- β 1 via
194 MAP3K7, overexpression of the full-length isoform of MAP3K7 in TGF- β 1-treated *Sf3b1*^{K700E}
195 mutant organoids significantly decreased their viability (Fig. 5H, I).

196 To finally assess conservation of this mechanism between mice and humans, we treated human
197 pancreatic H6c7 cells as well as human pancreatic organoids exposed to TGF- β 1 with Takinib,
198 a chemical inhibitor for MAP3K7. In line with our results in murine cells, also in the human
199 pancreatic cells inhibition of MAP3K7 led to a significant increase in survival (Fig. 5J, K).
200 Taken together, our results suggest that *Sf3b1*^{K700E} mediates resistance of pancreatic epithelial

201 cells to TGF- β 1 via MAP3K7, providing a potential mechanism for its role of in PDAC
202 progression.

203 **DISCUSSION**

204 The frequent occurrence of SF3B1 hotspot mutations in various tumor types implies a
205 contribution to tumorigenesis. While the molecular function of oncogenic SF3B1 on RNA-
206 splicing is well described, how deregulation of misspliced genes contribute to malignancy in
207 different cancer entities is less understood. Previous studies have shown that in chronic
208 lymphocytic leukaemia (CLL) SF3B1^{K700E} leads to missplicing of *PPP2R5A*, which in turn
209 stabilizes c-Myc and thereby promotes aggressiveness of tumor cells (Liu et al., 2020a; Yang
210 et al., 2021). Furthermore, in breast cancer SF3B1^{K700E} causes a tumor-promoting effect
211 through missplicing in *MAP3K7* and downstream activation of NF- κ B-signalling (Liu et al.,
212 2021). In our study we analysed the oncogenic function of SF3B1^{K700E} in the context of PDAC.
213 Using a mouse model, we provide the first experimental evidence that the mutation indeed
214 promotes PDAC progression. We show that SF3B1^{K700E} reduces TGF- β induced EMT and cell-
215 death, thereby providing a potential explanation for the oncogenicity of SF3B1^{K700E} in PDAC.
216 In line with our hypothesis, pancreatic epithelial cells have previously been found to undergo
217 lethal EMT when stimulated with TGF- β (David et al., 2016), and acquiring TGF- β resistance
218 is considered to be essential in early stages of PDAC tumorigenesis (Hezel et al., 2012).
219 A PDAC-promoting effect of reduced TGF- β signalling was previously demonstrated in a
220 conditional mouse model for *Smad4*, a core-component of TGF- β -signalling. In line with our
221 observations, *Smad4* deletion was not sufficient to induce malignant transformation, but was
222 found to be oncogenic only in combination with concomitant activation of Kras^{G12D} (Bardeesy
223 et al., 2006b). SF3B1^{K700E}, nevertheless, only partially suppressed non-canonical TGF- β -
224 signalling, and the inactivation of canonical TGF- β -signalling via SMAD4 inactivation or both
225 branches via TGF- β receptor inactivation is likely to confer additional tumor promoting effects
226 (Hezel et al., 2012). Therefore, we speculate that the SF3B1^{K700E} mutation does not eliminate
227 the selective pressure in PDAC for additional inactivating mutations in TGF- β -signalling, and
228 in case additional mutations impede both branches of TGF- β signalling, SF3B1^{K700E} might lose
229 its beneficial role for the tumor. Like in myeloid cancers, targeting SF3B1^{K700E} via small

230 molecules might therefore only benefit a subset of patients carrying this mutation (Steensma et
231 al., 2021).

232 Reducing *Map3k7* levels by shRNA did not impair TGF- β -mediated EMT and cell death to
233 the same extent as *Sf3b1*^{K700E}. It is therefore likely that the aberrant splicing of other genes is
234 also contributing to the observed resistance to TGF- β . Nevertheless, our hypothesis that TGF-
235 β resistance in SF3B1^{K700E} mutant PDAC is at least partly mediated via MAP3K7 is also
236 supported by previous studies. These already demonstrated that SF3B1 mutations induce 3'
237 missplicing of *MAP3K7* in various tumor entities (Bondu et al., 2019; Li et al., 2021; Lieu et
238 al., 2022; Liu et al., 2020b; Wang et al., 2016; Zhang et al., 2019), that aberrant splicing by
239 SF3B1^{K700E} reduces MAP3K7 protein levels (North et al., 2022) and that MAP3K7 mediates
240 TGF- β -induced EMT and apoptosis in mammary epithelial cells (David et al., 2016).

241 While the most common oncogenic drivers in PDAC are well described, oncogenes occurring
242 at lower frequency are understudied (Hudson et al., 2010). This study provides a first
243 demonstration that oncogenic SF3B1^{K700E} promotes tumor progression *in vivo* in a mouse
244 model for PDAC, and sheds mechanistic insights on the oncogenicity of SF3B1^{K700E} by
245 reducing the responsiveness to tumor-suppressive TGF- β .

246 **AUTHOR CONTRIBUTIONS**

247 P.S. and G.S. conceived the study. G.S., M.S. and G.R. supervised the study. P.S. designed,
248 executed and analyzed most of the *in vitro* and *in vivo* experiments. T.M. performed *in vitro*
249 experiments. TT. and AK. developed and ran the RNA-seq pipeline, K.L. performed the *in*-
250 silico statistical analysis of RNA-splicing in human and murine samples. P.S. and G.S. wrote
251 the manuscript with input from all authors.

252 **COMPETING INTERESTS**

253 The authors declare no competing interests.

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262 **METHODS**

263 **Animal models**

264 *Sf3b1*^{K700E/+} mice were a gift from E. A. Obeng (Dana-Farber Cancer Institute, Boston, USA)
265 and B. L. Ebert (Brigham and Women's Hospital, Harvard Medical School, Boston, USA).
266 *LSL-Kras*^{G12D/+}, *LSL-Trp53*^{R172H/+} and *Ptfla-Cre* mice were purchased from the Jackson
267 Laboratory (Bar Harbor, Maine, USA). All *Sf3b1*^{K700E/+} and KPC-*Sf3b1*^{K700E/+} mice were bred
268 in a C57BL/6J background, *Sf3b1*^{K700E/+}; *Kras*^{G12D/+} mice were a C57BL/6J-BALB/c strain.
269 Female and male mice were used for all experiments. Animals displaying dwarfism were
270 excluded from analysis. The minimum of animals needed for the study was estimated by
271 Fisher-Yates analysis. Due to the observed variance of the mouse model, more mice than
272 initially estimated were used for the study. Mice were held in a specific-pathogen-free (SPF)
273 animal facility at the ETH Phenomics Center EPIC (ETH Zurich, Switzerland). All animal
274 experiments were conducted in accordance with the Swiss Federal Veterinary Office (BVET)
275 guidelines (license no. ZH055/17).

276 **Cell lines**

277 The human cell lines AsPC-1 (CRL-1682), BxPC-3 (CRL-1687), MIA-PaCa-2 (CRM-
278 CRL275 1420), PANC-1 (CRL-1469), PSN-1 (CRL-3211) and HEK293T (CRL-1573) cells
279 were purchased from ATCC. H6c7 cells (ECA001-FP) were purchased from Kerablast. MIA-
280 PaCa277 2, PANC-1 and HEK293T cells were maintained in DMEM with 4.5 g/l D-Glucose
281 and GlutaMAX (Gibco), supplemented with 10% fetal calf serum (FCS, Sigma-Aldrich) and
282 1% Penicillin- streptomycin (P/S, Invitrogen). AsPC-1, BxPC-3 and PSN-1 were cultured in
283 RPMI1640 (Thermo Fisher), supplemented with 10% FCS and 1% P/S. H6c7 cells were

284 cultured in Keratinocyte serum-free medium (Thermo Fisher), supplemented with recombinant
285 EGF and bovine pituitary extract according to the manufacturer's instructions (Thermo Fisher),
286 as well as 100 µg/ml Primocin (InvivoGen). Cell lines were regularly checked for mycoplasma
287 infections by Mycoplasma PCR-detection test (Thermo-Fisher).

288 **Murine organoids**

289 Murine organoid lines from WT and *Sf3b1*^{K700E/+} animals were established as previously
290 described (Boj et al., 2015). Briefly, 43-week-old animals were euthanized and their pancreata
291 excised. The organs were dissected to thin pieces and digested in 4 mg/ml collagenase IV for
292 7 minutes at 37°C. Then, pancreatic ducts were manually picked under a light microscope and
293 seeded in drops of growth factor reduced matrigel. In vitro activated (pre-) cancer organoid
294 lines (*Kras*^{G12D/+}, *Kras*^{G12D/+}; *Sf3b1*^{K700E/+}, *Kras*^{G12D/+}; *Trp53*^{R172H/+} and *Kras*^{G12D/+};
295 *Trp53*^{R172H/+}; *Sf3b1*^{K700E/+}) were established from 8-week-old animals using the same protocol,
296 except that recombination was achieved by delivering Cre-GFP by lentiviral transduction,
297 followed by FACS sorting for GFP positive cells. Tumor-derived KPC and KPC-*Sf3b1*^{K700E/+}
298 organoid lines were established from solid tumors of KPC or KPC-*Sf3b1*^{K700E/+} mice. Tumor
299 tissue was digested for 2–3 hours in 4 mg/ml collagenase IV at 37°C, pelleted and seeded in
300 drops of matrigel. The presence of the K700E mutation was validated with Sanger-sequencing
301 on RNA level for each organoid line. Each organoid line was isolated from an individual
302 mouse. Tumor-derived KPC and KPC-*Sf3b1*^{K700E/+} organoid lines were additionally plated in
303 regular cell culture dishes and grown as monolayer cell culture. Organoids were cultured in
304 organoid medium (OM) composed of AdDMEM/F12 (Gibco) supplemented with GlutaMAX
305 (Gibco), HEPES (Gibco), Penicillin-Streptomycin (Invitrogen), B27 (Gibco), 1.25 mM N-
306 Acetyl-L-cysteine (Sigma), 10 nM Gastrin I (Sigma) and the growth factors: 100 ng/ml FGF10
307 (Peprotech), 50 ng/ml EGF (Peprotech), 100 ng/ml Noggin, 100 ng/ml RSPO-1 (Peprotech),
308 and 10 mM Nicotinamide (Sigma). For the first week after duct isolation the culture medium
309 was supplemented with 100 µg/ml Primocin (InvivoGen).

310 **Immunohistochemistry**

311 Murine tissue specimens were dissected and fixed in 10% neutral buffered formalin for 48 - 72
312 hours. Thereafter, formalin was replaced with 70% ethanol before paraffin-embedding and
313 sectioning at a thickness of 4 µm. Hematoxylin and eosin stainings were performed according
314 to the manufacturer's instructions. Masson-Goldner-Trichome staining was performed as

315 previously described (Goldner, 1938). Quantification of the collagenous tissue area was
316 performed with QuPath-0.2.3. The pixel classifier was trained to separate tissue areas stained
317 red from tissue areas stained green and from areas without tissue. Anti-Ki-67 (Antigen Clone
318 TEC-3) antibody (Dako), anti-Cleaved Caspase 3 (Asp175) antibody (Cell Signaling
319 Technology), anti-E-Cadherin (Clone 36) antibody (BD Biosciences), anti-FN-1 antibody
320 (Chemicon) and anti-TNC (Clone 578) antibody was used according to the manufacturer's
321 recommendations. TNC staining was quantified by calculating the average of Raw Int Density
322 of 3 randomly chosen fields per specimen using ImageJ. Luminal necrotic cells were defined
323 as shed cells residing within the lumen of PanIN-lesions.

324 **Organoid growth assay**

325 Growth of organoids was assessed with CellTiter-Glo 3D (Promega). For absolute
326 quantification of ATP levels, standard curves with defined concentrations of ATP were used
327 for every measurement according to the manufacturer's instructions. As approximation of
328 proliferation rate, the ratio of ATP concentrations at the indicated time points was calculated.

329 **Crystal violet assay**

330 To measure proliferation of cell lines, 5000 cells were seeded per 96 wells. At the indicated
331 time points, cells were stained with 0.5% (w/v) crystal violet (Sigma-Aldrich) dissolved in an
332 aqueous solution with 20% Methanol (v/v). After washing, plates were allowed to air-dry and
333 the crystal violet was dissolved in 10% acetic acid. Optical density was measured at 595 nm in
334 an Infinite 200 plate reader (Tecan).

335 **Organoid viability assay**

336 For short-term treatment, organoids were seeded as fragments in 10 μ l of Matrigel and allowed
337 to form spheres for 24 hours in regular organoid medium. Organoids were thereafter exposed
338 to the indicated concentration of TGF- β 1 (Thermo Fisher). To assess viability of the organoids,
339 intact organoids were counted and compared to untreated organoids after 48h of TGF- β 1-
340 exposure. This method of quantification was validated by correlating counts of intact organoids
341 with ATP levels as described above (data not shown). For long-term treatment, organoids were
342 seeded as fragments in 40 μ l of Matrigel. After allowing to form spheres for 24 hours, the
343 indicated concentration of TGF- β 1 was added. After 4 days of TGF- β 1-treatment, matrigel-
344 drops were imaged and the number of intact organoids was counted. Then, organoids were

345 reseeded as fragments in normal organoid medium and TGF- β 1 was added after 24 hours.
346 Every 4th passage, organoids were split in a 1:1 ratio in the 1ng/ml TGF- β 1 condition.
347 Commercial cell lines were seeded and TGF- β 1 was added at the indicated concentrations after
348 12h after plating.

349 **Organoid invasion assay**

350 Organoids were seeded at equal density in 40 μ l of matrigel in 24-well plates. 24 hours after
351 seeding, organoid growth medium was supplemented with 10 ng/ml TGF- β 1 (Thermo Fisher).
352 96 hours after seeding, matrigel domes were detached by rinsing and the migrated organoids
353 (i.e. cells attached to the cell culture dish) were stained by crystal violet. The fraction of
354 attached organoids was calculated by dividing the number of attached organoids by the number
355 of attached organoids plus the number of non-attached organoids (i.e. organoids residing in the
356 matrigel dome).

357 **Cleaved-caspase 3/7 assay**

358 Organoids were seeded 10 μ l of Matrigel and allowed to form spheres for 24 hours in regular
359 organoid medium. Organoids were thereafter exposed to 10 ng/ml TGF- β 1 (Thermo Fisher)
360 overnight. Cleavage of Caspase 3 and 7 was quantified by using Caspase-Glo 3/7 Assay System
361 (Promega) according to the manufacturer's instructions.

362 **Chemical inhibitors**

363 The following chemical inhibitors targeting different effectors of the TGF- β -pathway were
364 used: TGF β R-inhibitor A83-01 [50 nM] (Tocris Bioscience), p38-inhibitors SB202190 [10
365 μ M] (Sigma-Aldrich) and SB203580 [10 μ M] (Selleckchem), JNK-inhibitor SP600125 [25
366 μ M] (Sigma-Aldrich), SMAD3-inhibitor SIS3 [10 μ M] (Sigma-Aldrich) and MAP3K7-
367 inhibitor Takinib [5 μ M] (Sigma-Aldrich). The inhibitors were added to the organoid medium
368 directly after seeding.

369 **shRNA-mediated *Map3k7* knockdown**

370 shRNA targeting murine *Map3k7* was purchased from Sigma-Aldrich (TRCN0000022563). A
371 pLKO.1-puro Non-Target shRNA was used as control. Lentivirus was produced by PEI-based
372 transfection of HEK293T cells. Briefly, HEK293T cells were seeded at 70% confluency in 6-
373 well plates, and the following plasmids were transfected: PAX2 plasmid (1100 ng), VSV-G
374 plasmid (400 ng), cargo plasmid (1500 ng). Medium was changed 12 hours after transfection
375 and the virus-containing supernatant collected after 36 hours. Organoids were dissociated into

376 single cells by Tryp-LE treatment for 5 minutes at 37°C and consecutive mechanical disruption.
377 After centrifugation, 10% Lentivirus-containing supernatant in organoid medium (v/v) was
378 added to the cell suspension. After a 4–6h incubation at 37°C, cells were seeded in matrigel as
379 described above. Organoids were selected in 2ng/ml Puromycin after the first passage for at
380 least 5 days.

381 **Overexpression of *Map3k7***

382 Murine *Map3k7* (full-length isoform) was amplified from cDNA of murine WT duct organoids
383 and cloned into a Lenti-backbone. Production of lentivirus and transduction of organoids was
384 performed as described above. Organoids stably overexpressing GFP (addgene #17488) were
385 used as experimental control.

386 **Overexpression of SF3B1-K700E**

387 Codon-optimized human SF3B1-WT and SF3B1-K700E was derived from the plasmids
388 pCDNA3.1-FLAG-SF3B1-WT (addgene #82576) and pCDNA3.1-FLAG-hSF3B1-K700E
389 (addgene #82577) and cloned into a Lenti-backbone. The lentiviruses were produced as
390 described above and used to transduce various cell lines. Puromycin-selection was used to
391 select for transduced cells for at least two passages.

392 **RNA sequencing**

393 **Cell sorting and RNA extraction**

394 Murine tumors were excised and digested for 2–3 hours in collagenase (4mg/ml) at 37°C. After
395 addition of fetal calf serum (FCS) to stop the digestion, cells were strained through a 100 µm
396 and a 70 µm cell strainer. Then, cells were washed twice in PBS + 2% FCS + 2 mM EDTA
397 and incubated with mouse FcBlock (BD Biosciences), Epcam-APC (CD326 Monoclonal
398 Antibody (G8.8), APC, eBioscience™, Thermo Fisher) and CD45-BV785 (Clone 30-F11,
399 Biologened) antibodies for 30 minutes at 4°C. After washing, Epcam-positive-CD45-negative
400 cells were sorted into lysis buffer with a BD FACSAriaIII Cell Sorter (BD Biosciences).
401 Finally, RNA was extracted using NucleoSpin RNA XS kit (Macherey Nagel) according to the
402 manufacturer's instructions.

403 **Library preparation**

404 The quantity and quality of the isolated RNA was determined with a Qubit® (1.0) Fluorometer
405 (Life Technologies, California, USA) and a TapeStation (Agilent, Waldbronn, Germany). The
406 SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian (Clontech Laboratories, Inc.,

407 A Takara Bio Company, California, USA) was used in the succeeding steps. Briefly, total RNA
408 samples (0.25–10 ng) were reverse-transcribed using random priming into double-stranded
409 cDNA in the presence of a template switch oligo (TSO). When the reverse transcriptase reaches
410 the 5' end of the RNA fragment, the enzyme's terminal transferase activity adds non-templated
411 nucleotides to the 3' end of the cDNA. The TSO pairs with the added non-templated
412 nucleotide, enabling the reverse transcriptase to continue replicating to the end of the
413 oligonucleotide. This results in a cDNA fragment that contains sequences derived from the
414 random priming oligo and the TSO. PCR amplification using primers binding to these
415 sequences can now be performed. The PCR adds full-length Illumina adapters, including the
416 index for multiplexing. Ribosomal cDNA is cleaved by ZapR in the presence of the
417 mammalian-specific R-Probes. Remaining fragments are enriched with a second round of PCR
418 amplification using primers designed to match Illumina adapters. The quality and quantity of
419 the enriched libraries were validated using Qubit® (1.0) Fluorometer and the TapeStation
420 (Agilent, Waldbronn, Germany). The product is a smear with an average fragment size of
421 approximately 360 bp. The libraries were normalized to 10nM in Tris-Cl 10 mM, pH8.5 with
422 0.1% Tween 20.

423 **Cluster Generation and Sequencing**

424 The TruSeq SR Cluster Kit HS4000 or TruSeq PE Cluster Kit HS4000 (Illumina, Inc,
425 California, USA) was used for cluster generation using 8 pM of pooled normalized libraries on
426 the cBOT. Sequencing was performed on the Illumina HiSeq 4000 paired end at 2 X126 bp or
427 single end 126 bp using the TruSeq SBS Kit v4-HS (Illumina, Inc, California, USA).

428 **RNAseq data analysis**

429 Adapters have been trimmed with trimmomatic (v0.35). Pairs for which both reads passed the
430 trimming have been mapped to the murine genome using STAR (v2.7.0a) and indexed BAM
431 files obtained with samtools (v1.9). Reads were counted with featureCounts from subread
432 package (v1.5.0). The read counts have been processed in a statistical analysis using edgeR
433 (v3.24.3), obtaining a list of genes ranked for differential expression by p-value and Benjamini-
434 Hochberg adjusted p-value as the estimate of the false discovery rate. All data is summarized
435 in Table 1.

436 **Gene set enrichment analysis**

437 Gene set enrichment analysis (v.4.1.0, Broad Institute, MIT) was used to determine enriched
438 gene sets in KPC or KPC-Sf3b1^{K700E/+} tumor cells. Standard parameters of the software were
439 used to perform the analysis. Molecular Signatures Database v7.4, Hallmark Gene Sets (H)
440 was used to query enriched gene sets. The input gene expression matrix contained read-count
441 information (count per million) of 21,633 genes.

442 **Alternative splicing analysis**

443 We ran a 2-pass alignment of the fastq files using STAR v2.7 (Dobin et al., 2013) using the
444 GRCm38.p6 genome as reference. The gene annotation used was GENCODE v.m25. For gene
445 expression quantification we used a custom script, available at github:

446 <https://github.com/ratschlab/tools-omicstools/tree/master/gromics/counting>; commit hash
447 d074114f1d0a9f518c9cd039f68de0cdf8d583ff.

448 SplAdder v.2.2 (Kahles et al., 2016) was run to build splicing graphs and determine splice
449 events. Differential splicing events were determined by calculating a log(psi+x) transformation
450 of the percent spliced in (calculated as ratio of reads supporting the splice event over the
451 number of reads supporting the alternate event). Splice events that did not show any variability
452 over the samples were removed and missing values were mean imputed. After standardization
453 a two-sided t-test was used to calculate p-values of splice events differences between KPC and
454 KPC-Sf3b1^{K700E} mice. All data is summarized in Table 2.

455 **Motif analysis**

456 Consensus 3' ss motif in proximity of the canonical and the cryptic 3' ss in sorted KPC-
457 Sf3b1^{K700E/+} tumor cells was assessed by query 30-40 bases spanning the respective 3'ss of the
458 7 main splice events for a motif using weblogo-sequence creator
459 (<https://weblogo.berkeley.edu/logo.cgi>).

460 **RT-PCR and quantitative RT-PCR (qPCR)**

461 RNA-extraction was performed with QIAGEN RNeasy Mini Kit, and cDNA was generated
462 with GoScript Reverse Transcriptase kit (Promega) according to the manufacturers'
463 instructions. RT-PCR was performed with GoTaq G2 Green Master Mix (Promega) and gene
464 specific primers. Amplicons were fractionated on 2% TBE gel (Life Technologies)
465 supplemented with 0.01% GelRed (Biotium). For qPCR, 2 µL of 1:10-diluted cDNA was added
466 to 8 µL of 5x HOT FIREPol Evagreen qPCR Supermix (SolisBiodyne). RT-qPCR was
467 performed with a LightCycler480 II (Roche). Relative gene expression was determined with

468 the comparative CT method. Genes with a median CT value of more than 33 cycles and a
469 difference of less than 3.3 cycles to the template control (H₂O) were defined as not detectable.
470 Sequences of all primers used in this study are listed in Supplementary Table 1.

471 **NGS-based isoform quantification of *Map3k7***

472 Primers generating an amplicon including the exon 4 and exon 5 junction of Map3k7 cDNA
473 were used. Briefly, a gene-specific amplicon was generated in a 20 µL reaction for 35 cycles
474 with GoTaq G2 Green Master Mix (Promega). The PCR product was purified using the
475 NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel). Thereafter, the isolated product was
476 amplified for 8 cycles using primers with sequencing adapters. After column-based isolation
477 of the amplicon and quantification of DNA-yield using a Qubit 3.0 fluorometer and the dsDNA
478 HS assay kit 392 (Thermo Fisher), paired-end sequencing was performed on an Illumina Miseq.
479 The sequencing data was subsequently analyzed with CRISPResso2 (Clement et al., 2019).

480 **Western blotting**

481 Cells were lysed in RIPA buffer, supplemented with Protease Inhibitor (Cell Signaling
482 Technologies) and PhosStop (Sigma-Aldrich) and centrifuged for 10 min at 21'000 g. The
483 protein concentration of the supernatant was determined using Pierce BCA assay
484 (ThermoFisher) and a standard curve of albumin. Then, samples were heated for 5' at 95°C in
485 Lämmli buffer and protein lysates were resolved on polyacrylamide Mini-PROTEAN TGX
486 gels (BioRad) and transferred onto nitrocellulose membrane by wet-transfer. The following
487 antibodies were used for immunoblotting: Recombinant anti-GADPH (EPR16891, Abcam)
488 and rabbit monoclonal anti-MAP3K7 (anti-TAK1, D94D7, Cell Signaling Technology)
489 IRDye-conjugated secondary antibodies (donkey anti-goat: LI-COR cat. no. 926-32214; anti-
490 rabbit: LI-COR cat. no. 926-68073) were used for signal detection by an Odyssey Imager (LI-
491 COR) imaging system.

492 **Data availability**

493 The RNA sequencing raw data were deposited in the NCBI Gene Expression Omnibus (GEO)
494 under accession number GSE203339. Splice analysis of human cancers was performed on a
495 previously published dataset, accessible at [https://gdc.cancer.gov/about-](https://gdc.cancer.gov/about-data/publications/PanCanAtlas-Splicing-2018)
496 [data/publications/PanCanAtlas-Splicing-2018](https://gdc.cancer.gov/about-data/publications/PanCanAtlas-Splicing-2018) (Kahles et al., 2018). Material created in this
497 study (i.e. primary cell lines, plasmids) are provided upon request and shall be directed at the

498 corresponding author of this study (Prof. G. Schwank). Source data of the blots shown in Fig.
499 4E,F, Fig. 5D and Suppl. Fig. 4D,E,G can be found in source file zip in this manuscript.

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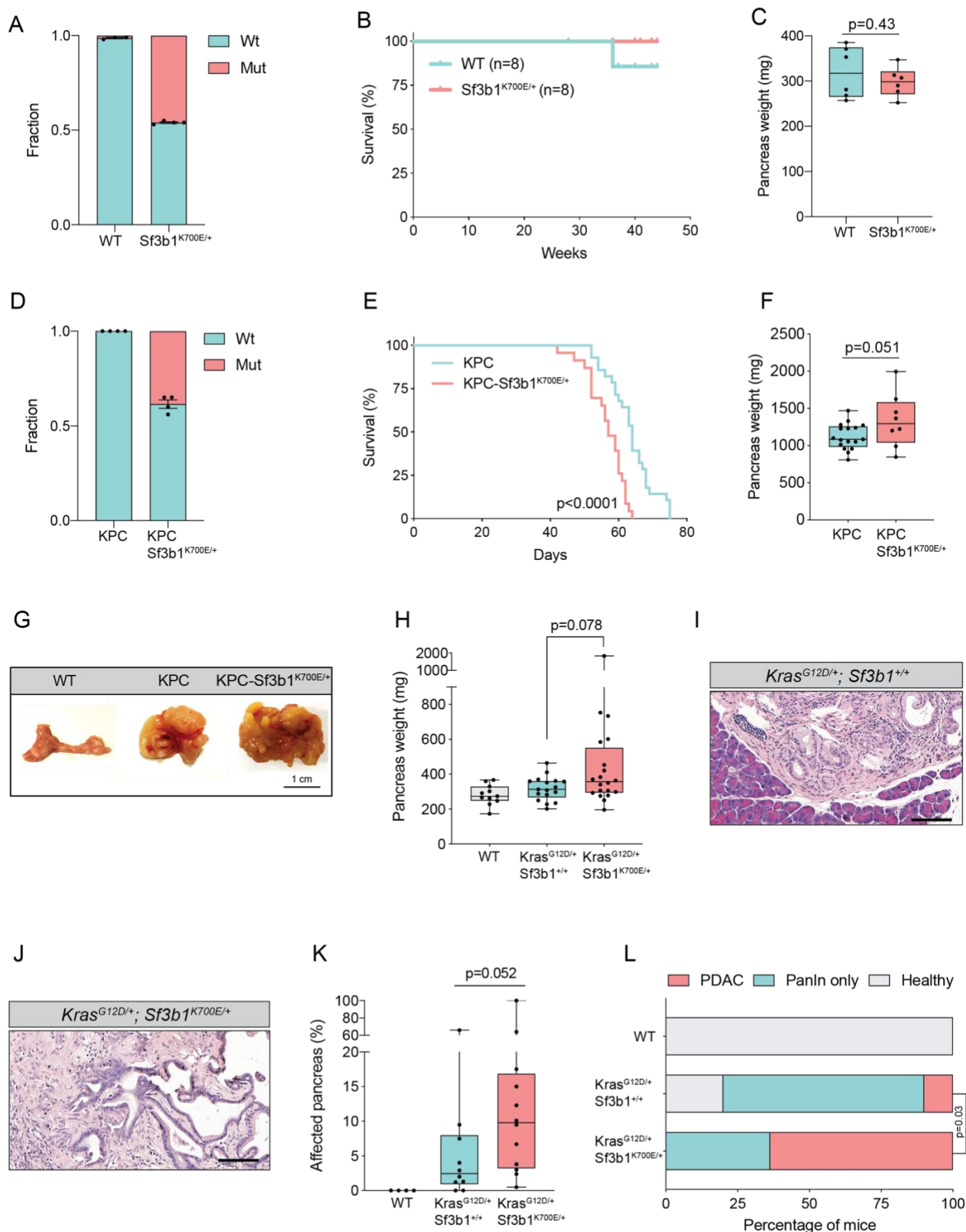
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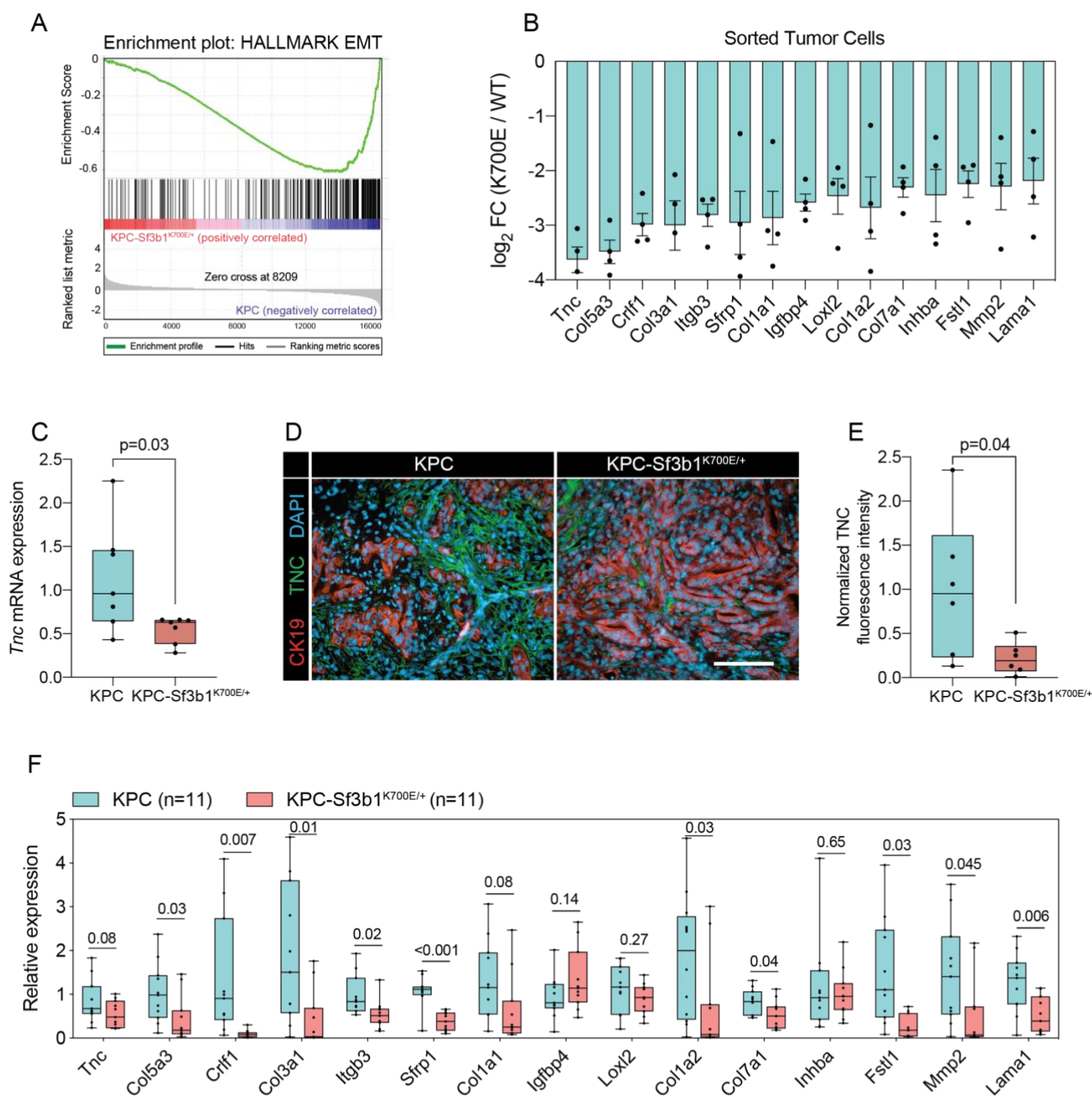
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Figure 1



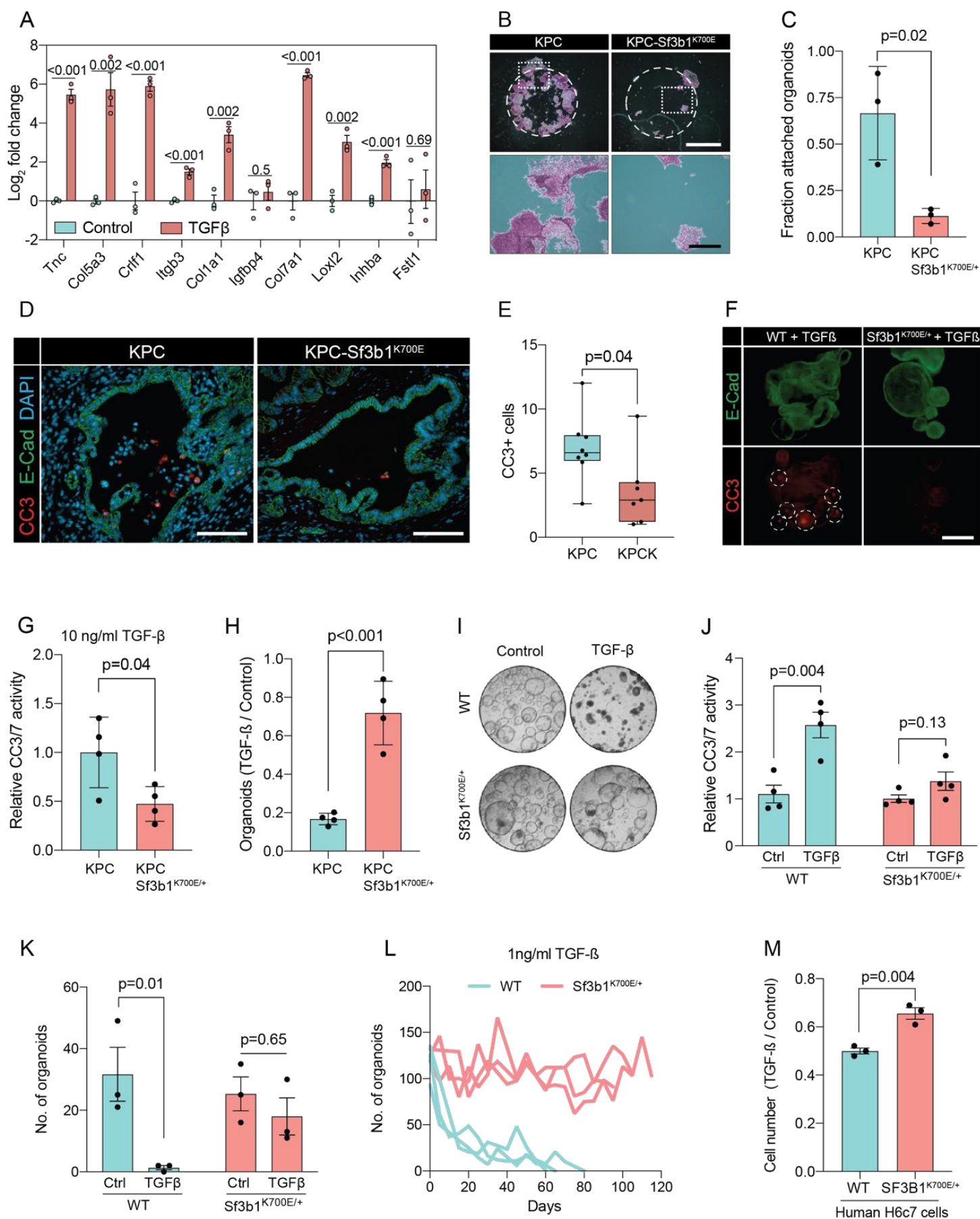
658 **Figure 1. *Sf3b1*^{K700E} increases aggressiveness of murine PDAC** (A) Fraction of the K700E mutation
659 (T>C) of cDNA isolated from pancreata of *Ptf1a-Cre* (WT) (n=3) or *Ptf1a-Cre; Sf3b1*^{K700E/+} mice (n=4),
660 assessed by Sanger-sequencing. (B) Survival of WT and *Sf3b1*^{K700E/+} mice followed over 300 days
661 (n=8). (C) Pancreatic weight of WT and *Sf3b1*^{K700E/+} mice at 300 days of age (3 males and 3 females
662 for each genotype). Two-tailed unpaired t-test was used to compute the indicated p-value. (D) Fraction
663 of K700E mutation (T>C) of RNA isolated from sorted KPC (n=3) and KPC-*Sf3b1*^{K700E/+} cells (n=4),
664 assessed by RNA-seq. (E) Survival of KPC and KPC-*Sf3b1*^{K700E/+} mice. P-value was determined by
665 Log-rank (Mantel-Cox) testing. (F) Pancreatic weight of KPC and KPC-*Sf3b1*^{K700E/+} mice at 9 weeks of
666 age. Two-tailed unpaired t-test was used to compute the indicated p-value (* p<0.05). (G)
667 Representative photographs of WT, KPC and KPC-*Sf3b1*^{K700E/+} pancreata. (H) Pancreatic weight of
668 WT, *Kras*^{G12D/+} and *Kras*^{G12D/+}; *Sf3b1*^{K700E/+} mice at 43 weeks of age. Welch's unequal variances t-test
669 was used to compute the indicated p-value. (I-J) Representative micrograph images of *Kras*^{G12D/+} (I)
670 and *Kras*^{G12D/+}; *Sf3b1*^{K700E/+} (J) pancreata at 43 weeks of age stained with H&E, scale bar is 100 μ m.
671 (K) Affected area (including PanINs and PDAC) of WT, *Kras*^{G12D/+} and *Kras*^{G12D/+}; *Sf3b1*^{K700E/+} pancreata
672 at 43 weeks of age. Mann-Whitney test was used to compute the indicated p-value. (L) Percentage of
673 mice at 43 weeks of the indicated genotypes showing PanINs (blue) or PanINs and PDAC formation
674 (red). P-value indicates significance of the difference in PDAC formation, computed by Chi-square test.

Figure 2



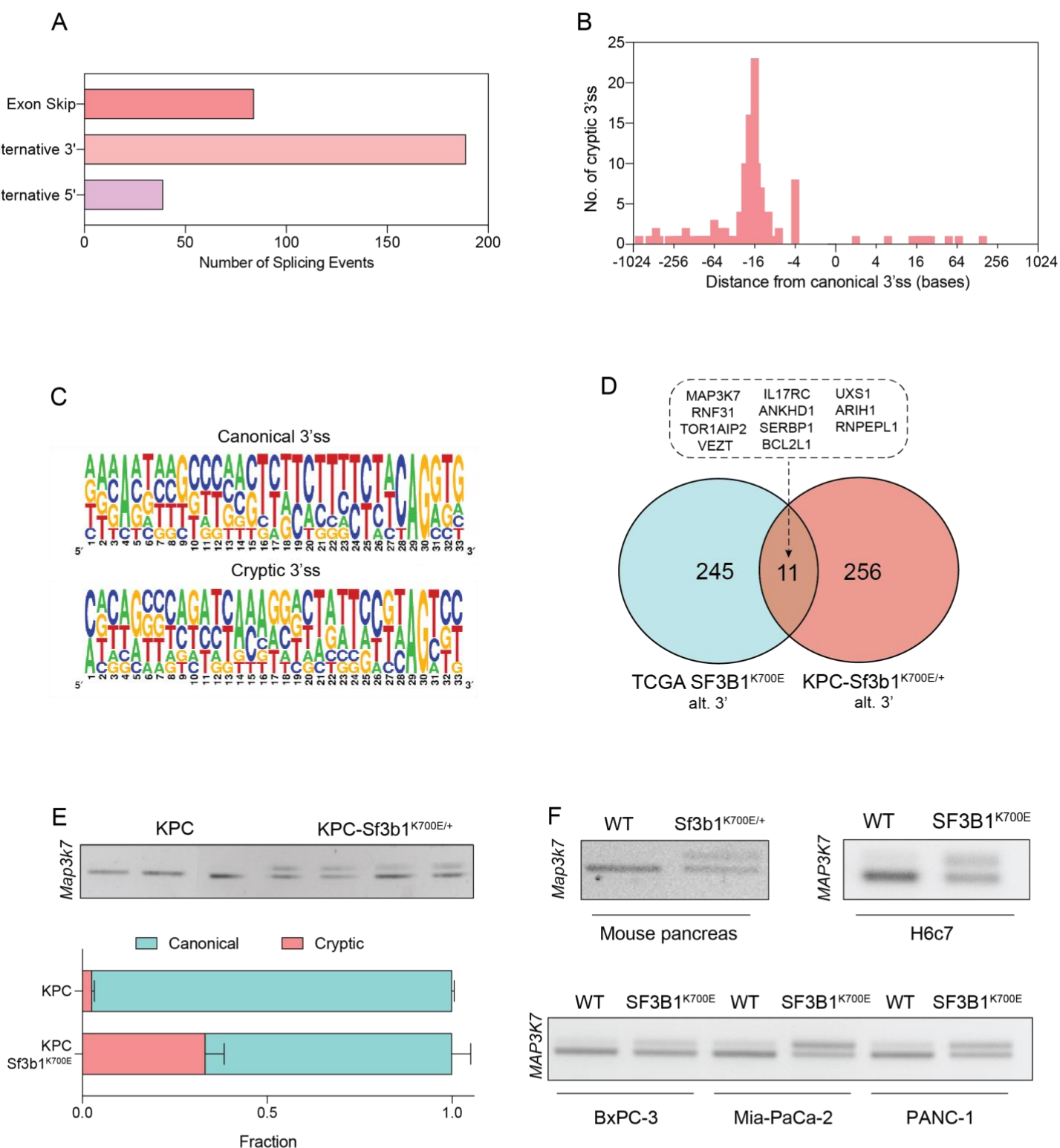
675 **Figure 2. *Sf3b1*^{K700E} induces downregulation of EMT. (A)** Gene-set enrichment analysis (GSEA)
676 enrichment plot of epithelial-mesenchymal transition (EMT), representing the most deregulated
677 pathway of the GSEA-Hallmark pathways when comparing KPC (n=3) and KPC-Sf3b1^{K700E/+} (n=4)
678 sorted tumor cells. **(B)** Top 15 of downregulated genes of the GSEA-EMT gene list in sorted KPC-
679 Sf3b1^{K700E/+} cells (FDR < 0.05, logCPM > 1). **(C)** *Tnc* expression in KPC (n=7) and KPC-Sf3b1^{K700E/+}
680 (n=7) tumors, assessed by RT-qPCR. Two-tailed unpaired t-test was used to compute the indicated p-
681 value. **(D)** Representative Immunofluorescence staining of CK19 (red) and TNC (green) in murine
682 PDAC samples, counterstained with DAPI (blue). Scale bar is 50 μ m. **(E)** Quantification of TNC staining
683 in KPC (n=6) and KPC-Sf3b1^{K700E/+} (n=6) tumors. The averaged area of TNC staining in 3 randomly
684 chosen fields per tumor specimen was compared by a two-tailed unpaired t-test. **(F)** The expression of
685 the EMT genes displayed in (B) was assessed by RT-qPCR in tumor-derived cancer cell lines (KPC,
686 n=11 and KPC-Sf3b1^{K700E/+} n=11) after one passage of ex-vivo culture. For analysis, Ct-values of the
687 indicated genes were normalized to *Actb* and a two-tailed unpaired t-test was used to compute the
688 indicated p-values.

Figure 3



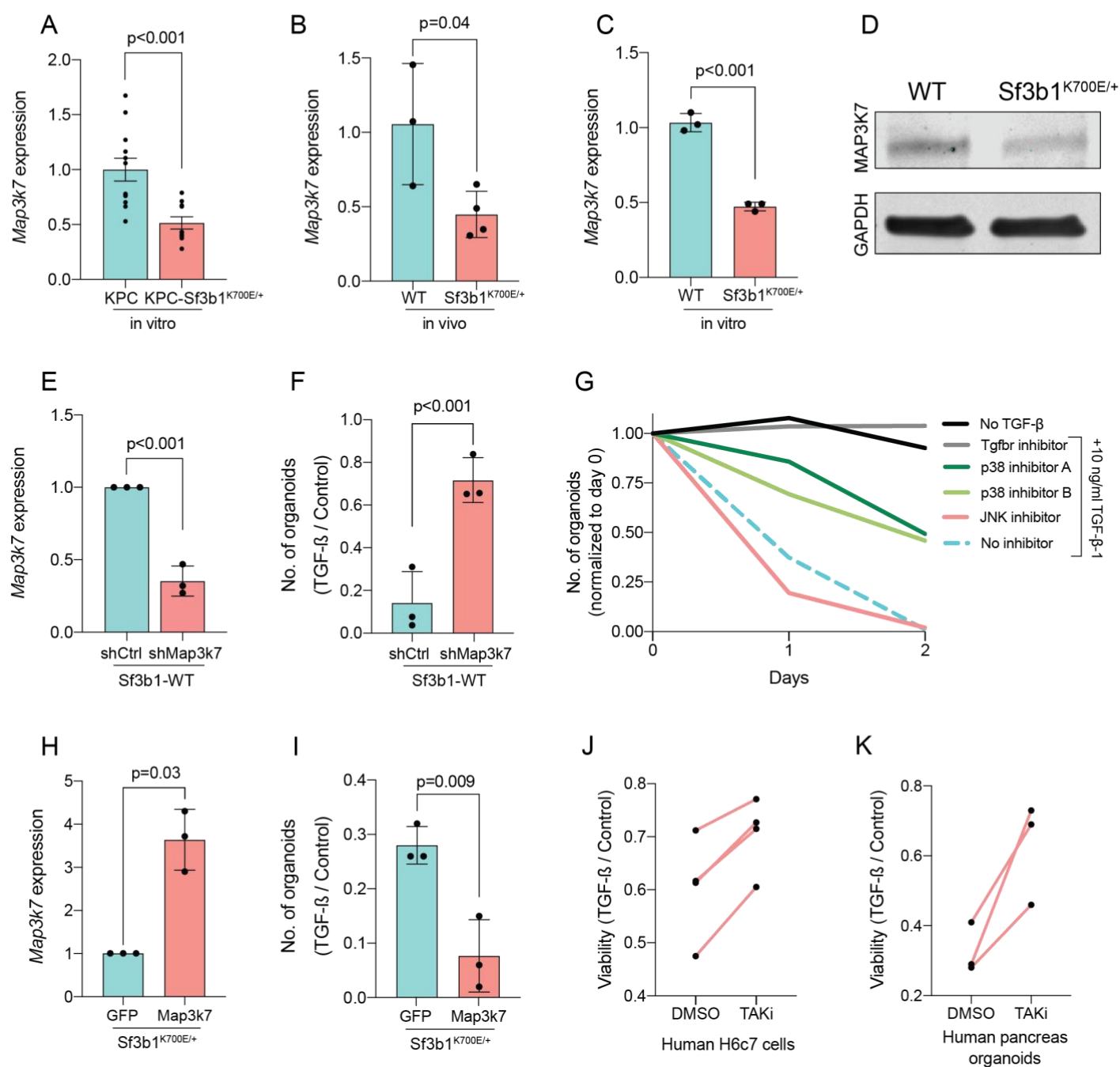
689 **Figure 3. *Sf3b1*^{K700E} reduces TGF- β -induced apoptosis. (A)** RT-qPCR analysis of EMT genes
690 displayed in Fig. 2B in 3 different KPC cell lines treated with 10 ng/ml TGF- β 1 for 24 hours. The
691 experiment was performed independently 3 times for every cell line. *Col3a1*, *Sfrp1*, *Col1a2*, *Mmp2* and
692 *Lama1* were not detected and therefore excluded from analysis (see methods for details). **(B)**
693 Representative micrographs of KPC (n=3) and KPC-Sf3b1^{K700E/+} (n=3) cancer organoid lines treated
694 with TGF- β 1 (10 ng/ml) for 48 hours. Matrigel was detached prior to staining with crystal violet. Scale
695 bar is 1 mm (panel above) or 100 μ m (panel below). **(C)** Quantification of (B). The fraction of attached
696 organoids was calculated by dividing the number of attached organoids by the number of total
697 organoids. The experiment was performed independently 3 times for every cell line, the average of all
698 replicates is shown. Two-tailed unpaired t-test was used to compute the indicated p-value. **(D)**
699 Representative microscopy images of E-Cadherin (green) and CC3 (red) in murine PDAC samples.
700 Scale bar is 50 μ m. **(E)** Quantification of CC3 positive cells in KPC (n=8) and KPC-Sf3b1^{K700E/+} (n=7)
701 tumor samples. The average number of CC3 positive cells of 5 microscopic fields is plotted, two-tailed
702 unpaired t-test was used to compute the indicated p-value. **(F)** Immunofluorescence staining of E-
703 Cadherin (green) and Cleaved Caspase 3 (CC3, red) in WT and Sf3b1^{K700E/+} organoids exposed to
704 TGF- β 1 (10 ng/ml) for 12 hours. CC3 positive cells are highlighted by white dashed lines. Scale bar is
705 100 μ m. **(G)** Quantification of Cleaved Caspase 3 and 7 (CC3/7) activity measured by Caspase-Glo
706 assay of KPC (n=3) and KPC-Sf3b1^{K700E/+} (n=4) in-vitro activated cancer cell lines treated with TGF- β 1
707 (10 ng/ml) for 24 hours. The experiment was repeated independently twice for every cell line, the
708 average of the replicates is shown. Two-tailed unpaired t-test was used to compute the indicated p-
709 value. **(H)** Quantification of viable organoids of the indicated genotype exposed to 10 ng/ml TGF- β 1 for
710 48 hours, normalized to organoid numbers of untreated control samples. Each data point shows a
711 different organoid line. For each organoid line, the experiment was independently performed three
712 times, the average of replicates is plotted. Two-tailed unpaired t-test was used to compute the indicated
713 p-value. **(I)** Representative microscopy images of WT and Sf3b1^{K700E/+} organoids exposed to 10 ng/ml
714 TGF- β 1 for 48 hours. **(J)** Quantification of CC3/7 in WT and Sf3b1^{K700E/+} organoids exposed to 10 ng/ml
715 TGF- β 1 for 48 hours. The experiment was repeated independently four times. Two-tailed unpaired t-
716 test was used to compute the indicated p-values. **(K)** Quantification of viable organoids of the indicated
717 genotype exposed to 10 ng/ml TGF- β 1 for 48 hours, normalized to organoid numbers of untreated
718 control samples. Each data point shows a different organoid line. For each organoid line, the experiment
719 was independently performed three times, the average of replicates is plotted. Two-tailed unpaired t-
720 test was used to compute the indicated p-values. **(L)** Organoid count of organoids cultured in medium
721 containing 1 ng/ml TGF- β 1 for up to 120 days. One organoid line per genotype was used, the
722 experiment was repeated three times independently. **(M)** Viability of the human pancreatic duct cell line
723 H6c7 overexpressing wildtype or mutated SF3B1 after 72 hours of exposure to 10 ng/ml TGF- β 1
724 assessed by crystal violet staining. The optical density of TGF- β 1 treated cells was normalized to
725 untreated control cells. The experiment was independently performed three times, two-tailed unpaired
726 t-test was used to compute the indicated p-value.

Figure 4



727 **Figure 4. SF3B1-K700E predominantly induces aberrant 3' splice site selection.**
728 **(A)** Summary of alternative splice events detected in KPC-Sf3b1^{K700E/+} sorted tumor cells (PSI > 0.1, p
729 < 0.01). **(B)** Histogram displaying the distance of cryptic 3'ss from the adjacent canonical 3'ss in sorted
730 KPC-Sf3b1^{K700E/+} tumor cells on a logarithmic scale. **(C)** Consensus 3' ss motif in proximity of the
731 canonical (top) and the cryptic (bottom) 3' ss for 7 alternative 3' splicing events identified in sorted KPC-
732 Sf3b1^{K700E/+} tumor cells. **(D)** Venn-diagram depicting alternative 3' splice events in the pan-cancer data-
733 set (PSI>0.05 and p<1⁻¹⁰) and sorted KPC-Sf3b1^{K700E/+} tumor cells (PSI>0.1, p<0.01). **(E)**
734 Representative gel image (top) and NGS-results (bottom) of *Map3k7* cDNA isolated from sorted KPC
735 and KPC-Sf3b1^{K700E/+} tumor cells (n=3) (A). The amplicon includes the 3' splice site of exon 4 and 5,
736 the upper band of the gel image represents the non-canonical transcript variant. **(F)** Representative gel
737 image of RT-PCR amplicon of *Map3k7* cDNA isolated from WT and *Sf3b1*^{K700E/+} pancreata and from
738 the indicated human cell lines.

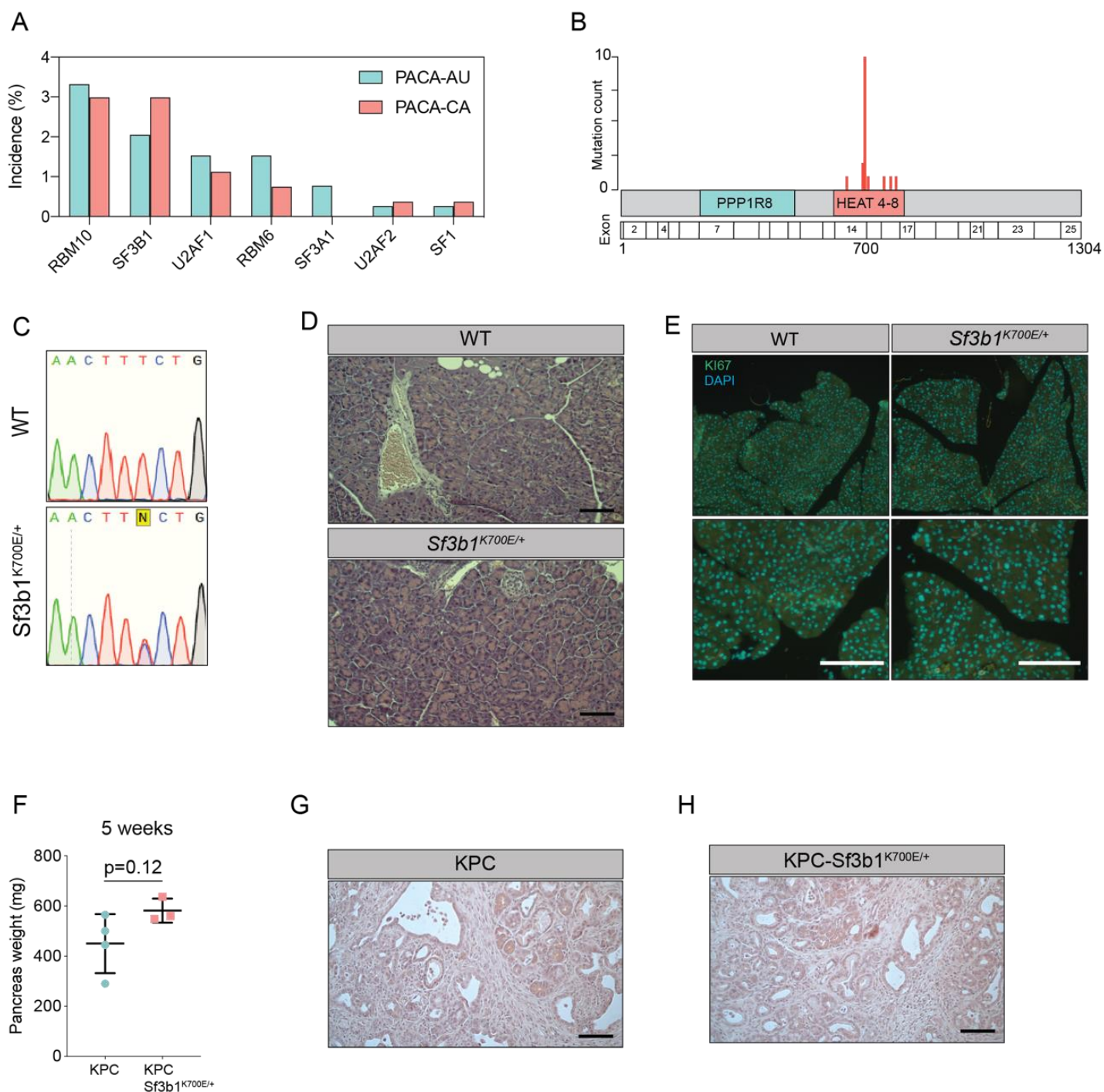
Figure 5



739 **Figure 5. Reduction in *Map3k7* lowers sensitivity to TGF- β 1. (A-C)** RT-qPCR data showing *Map3k7*
740 expression in KPC (n=13) and KPC-Sf3b1^{K700E/+} (n=12) cancer-derived organoid lines (A), as well as
741 WT (n=3) and Sf3b1^{K700E/+} (n=4) pancreata (B) and organoid lines (C). For analysis, Ct-values of
742 *Map3k7* were normalized to *Actb* and a two-tailed unpaired t-test was used to compute the indicated p-
743 values. Data show mean and standard error of the mean in A and B. **(D)** Representative Western blot
744 gel-image of MAP3K7 and GAPDH in WT and Sf3b1^{K700E/+} organoids. **(E)** RT-qPCR analysis of *Map3k7*
745 in cells transduced with a shMap3k7 compared to a control shRNA, a two-tailed unpaired t-test was
746 used to compute the indicated p-values. **(F)** Quantification of viable WT and Sf3b1^{K700E/+} murine
747 pancreatic duct organoids or WT transduced with control shRNA (shCtrl) or shRNA targeting *Map3k7*
748 (shMap3k7). The organoids were exposed to 10 ng/ml TGF- β 1 for 24 hours prior to analysis. The
749 experiment was independently performed three times. Two-tailed unpaired t-test was used to compute
750 the indicated p-value. **(G)** Viability of wildtype (WT) organoids cultured in medium containing 10 ng/ml
751 TGF- β 1, supplemented with chemical inhibitors targeting the indicated effectors of TGF- β 1-signalling.
752 Two independent experiments are summarized. Further details are provided in the methods section.
753 **(H)** RT-qPCR analysis of *Map3k7* in cells transduced by lentivirus with an overexpression construct of
754 *Map3k7*, compared to overexpression of GFP, a two-tailed unpaired t-test was used to compute the
755 indicated p-values. **(I)** Quantification of viable murine pancreatic duct organoids with stable
756 overexpression of *Map3k7*, exposed to 10 ng/ml TGF- β 1 for 96 hours. Data represents one organoid
757 line per condition, the experiment was independently performed three times. Two-tailed unpaired t-test
758 was used to compute the indicated p-value. **(J-K)** Viability of human pancreatic duct H6c7 cells (J) or
759 human pancreatic duct organoids (K) exposed to 10 ng/ml TGF- β 1 with addition of the MAP3K7 inhibitor
760 Takinib (TAKi, 5 μ M) or DMSO. The viability was assessed after 48 hours of TGF- β 1 treatment and
761 normalized to cells grown in absence of TGF- β 1. The experiment was independently performed four (J)
762 or three (K) times.

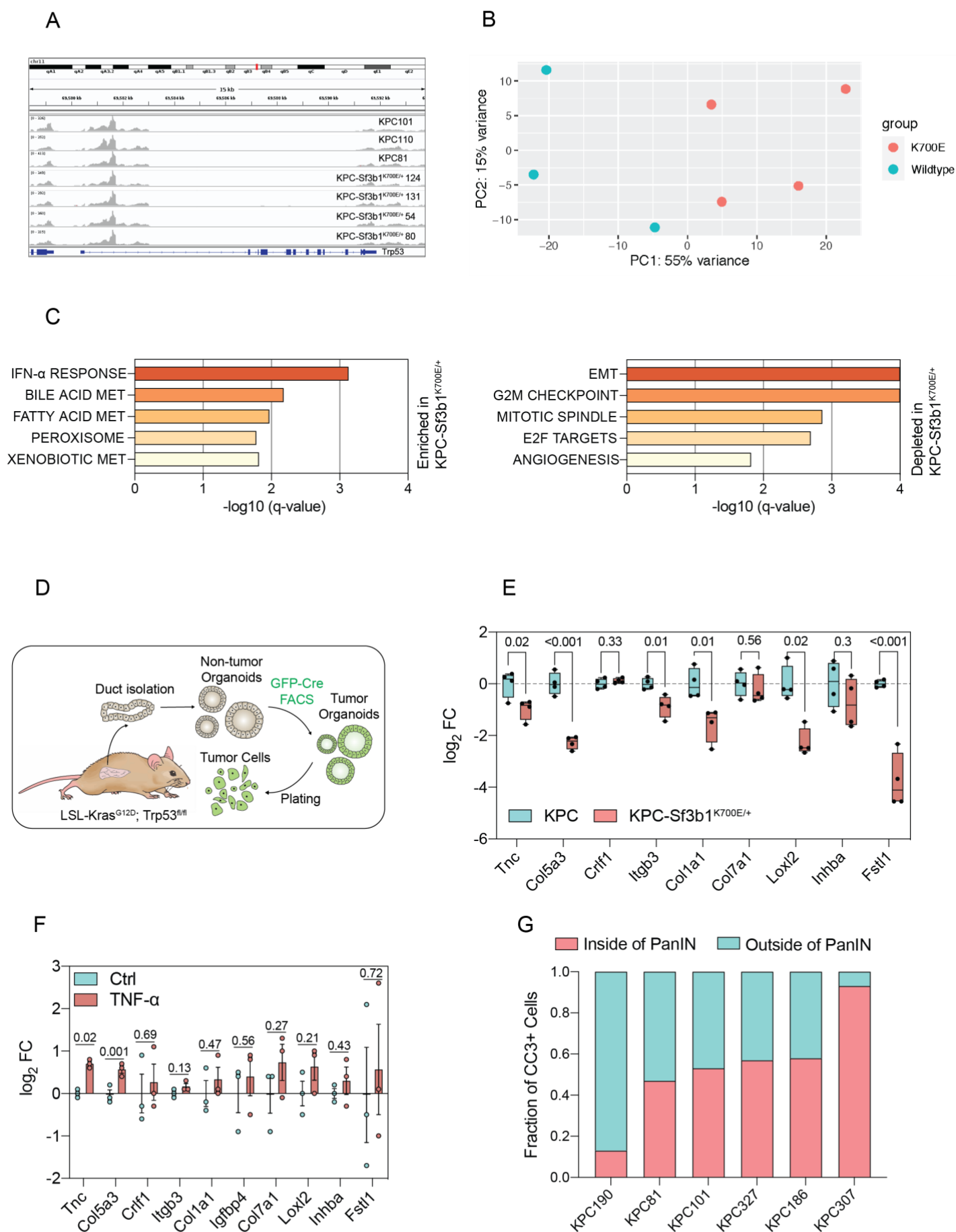
763 SUPPLEMENTAL INFORMATION

Supplementary Figure 1



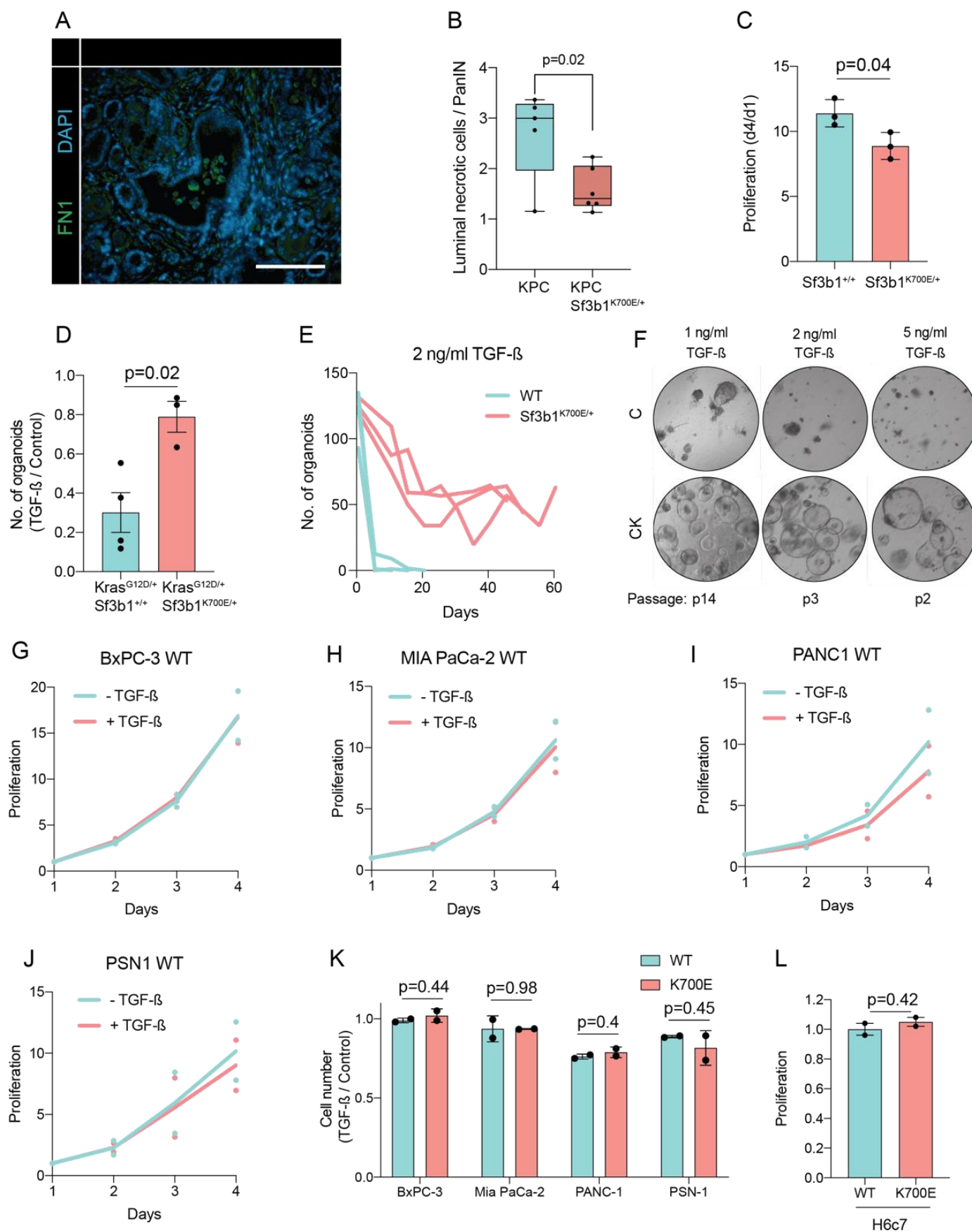
764 **Supplementary Figure 1. (A)** Incidence of mutations in splicing factors in PDAC patients derived from
765 the ICGC database (PACA-AU, n=391 and PACA-CA, n=268). **(B)** Incidence of *SF3B1* missense
766 mutations in PDAC patients derived from PACA-AU and PACA-CA. **(C)** Representative Sanger-
767 sequencing results of the *Sf3b1*^{K700E/+} mutation (T>C) of cDNA isolated from pancreata at 43 weeks of
768 age of *Ptf1a-Cre* (WT) or *Ptf1a-Cre; Sf3b1*^{K700E/+} (*Sf3b1*^{K700E/+}) mice. **(D-E)** WT and *Sf3b1*^{K700E/+}
769 pancreata at 43 weeks of age stained with H&E (D) and Ki67 (E) Scale bar is 50 μ m. **(F)** Pancreatic
770 weight of KPC and KPC-*Sf3b1*^{K700E/+} mice, at 5 weeks of age. Two-tailed unpaired t-test was used to
771 compute the indicated p-value. **(G-H)** Representative micrograph images of KPC (G) and KPC-
772 *Sf3b1*^{K700E/+} (H) pancreata at 9 weeks of age stained with H&E, scale bar is 100 μ M.

Supplementary Figure 2



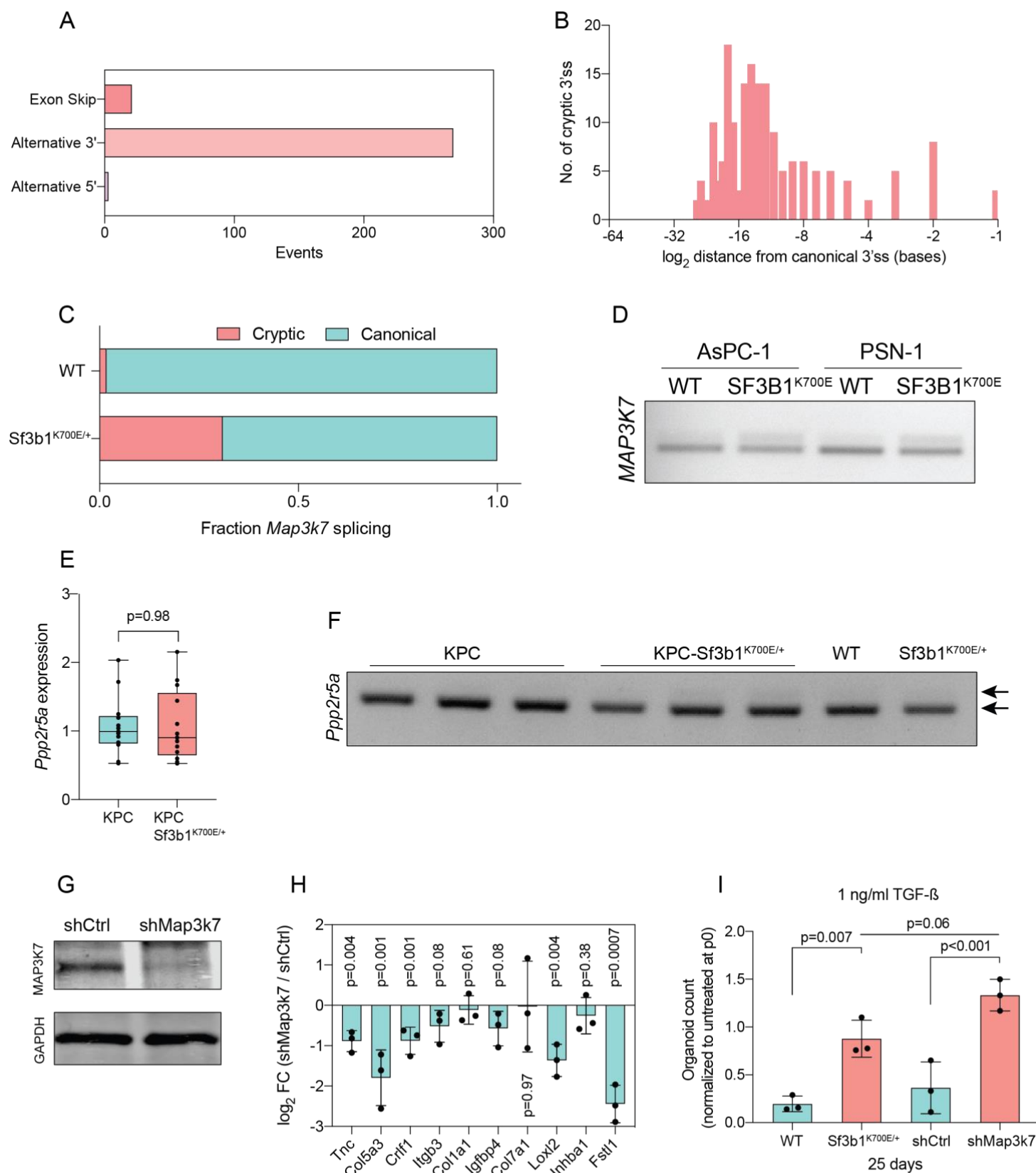
773 **Supplementary Figure 2. (A)** Integrated genome viewer (IGV) displaying RNA-seq reads of *Trp53* of
774 sorted KPC and KPC-Sf3b1^{K700E/+} cells. **(B)** Principal component analysis showing the variance in two
775 dimensions in relation to the genotypes of the sorted tumor cells (Wildtype = KPC, K700E = KPC-
776 Sf3b1^{K700E/+}). **(C)** Results of GSEA analysis, displaying most enriched (top) and depleted (bottom)
777 GSEA-Hallmark pathways in KPC-Sf3b1^{K700E/+} animals. **(D)** Schematical overview of generation of in-
778 vitro activated tumor cells: Pancreatic ducts of LSL-*Kras*^{G12D/+}; *Trp53*^{fl/fl} or LSL-*Kras*^{G12D/+}; *Trp53*^{fl/fl};
779 *Sf3b1*^{fl/K700E/+} were isolated and expanded as organoids. Cells were transduced with GFP-Cre and
780 selected by FACS. After expansion of sorted cells in a 3D culture, organoids were plated in cell culture
781 dishes and grown as monolayer culture. **(E)** RT-qPCR analysis of EMT genes displayed in (G) of KPC
782 (n=3) and KPC-Sf3b1^{K700E/+} (n=3) in-vitro activated cancer cell lines treated with TGF-β1 (10 ng/ml) for
783 24 hours. The experiment was performed independently 4 times for every cell line, the average of all
784 replicates is shown. *Col3a1*, *Sfrp1*, *Igfbp4*, *Col1a2*, *Mmp2* and *Lama1* were not detected and therefore
785 excluded from analysis (see methods for details). Two-tailed unpaired t-test was used to compute the
786 indicated p-values. **(F)** RT-qPCR analysis of EMT genes displayed in Fig 2B in 3 different KPC cell lines
787 treated with TNF-α (100 ng/ml) for 24 hours. The experiment was performed independently 3 times for
788 every cell line. *Col3a1*, *Sfrp1*, *Igfbp4*, *Col1a2*, *Mmp2* and *Lama1* were not detected and therefore
789 excluded from analysis (see methods for details). **(G)** Quantification of CC3 positive (CC3+) cells
790 residing within PanIN lesions in 6 different KPC tumor specimen.

Supplementary Figure 3



791 **Supplementary Figure 3. (A)** Representative microscopy images of FN1 (green) in murine PDAC.
792 Scale bar is 50 μ m. **(B)** Blinded quantification of luminal necrosis in KPC (n=5) and KPC-Sf3b1^{K700E/+}
793 (n=6) tumor samples. The average number of necrotic cells per PanIN lesion is plotted, two-tailed
794 unpaired t-test was used to compute the indicated p-value. **(C)** Proliferation of pancreatic ductal
795 organoids derived from WT and *Sf3b1*^{K700E/+} mice without TGF- β 1 supplementation. Two-tailed
796 unpaired t-test was used to compute the indicated p-value. **(D)** Organoid count of organoids of the
797 indicated genotypes exposed to 10 ng/ml TGF- β 1 for 48h. Two-tailed unpaired t-test was used to
798 compute the indicated p-value. **(E)** Organoid count of organoids cultured in medium containing 2 ng/ml
799 TGF- β 1 for the indicated period of time. One organoid line for each genotype was used, the experiment
800 was independently performed twice. **(F)** Representative microscopy images of WT and *Sf3b1*^{K700E/+}
801 organoids exposed to 1, 2 or 5 ng/ml TGF- β 1 at the indicated number of passages. **(G-J)** Proliferation
802 of the indicated PDAC cell lines overexpressing SF3B1 exposed to 10 ng/ml TGF- β 1 compared to
803 normal growth medium. The experiment was independently performed twice. **(K)** Viability of indicated
804 cell lines overexpressing wildtype or mutated SF3B1 after 72 hours of exposure to 10 ng/ml TGF- β 1.
805 The experiment was independently performed twice, two-tailed unpaired t-test was used to compute
806 indicated p-values **(L)** Normalized growth of the pancreatic duct cell line H6c7 overexpressing SF3B1-
807 WT or SF3B1-K700E after 4 days of culture in normal growth medium. The experiment was
808 independently performed twice, two-tailed unpaired t-test was used to compute indicated p-value.

Supplementary Figure 4



809 **Supplementary Figure 4. (A)** Pan-cancer analysis of alternative splice-events identified in solid tumors
810 carrying the *SF3B1*^{K700E} mutation (PSI>0.05, FDR<1⁻¹⁰). **(B)** Histogram displaying the distance of cryptic
811 3'ss from the adjacent canonical 3'ss on a logarithmic scale in solid tumors carrying the *SF3B1*^{K700E}
812 mutation. **(C)** NGS-results of *Map3k7* cDNA isolated from WT and *Sf3b1*^{K700E/+} pancreas organoids
813 (n=1). **(D)** RT-PCR amplicon of *MAP3K7* cDNA isolated from four human PDAC cell lines
814 overexpressing wildtype SF3B1 (OE WT) or K700E-mutated SF3B1 (OE K700E). The amplicon
815 includes the 3' splice site of exon 4 and 5, the upper band of the gel image represents the non-canonical
816 transcripts. **(E)** RT-qPCR of *Ppp2r5a* expression in KPC (n=13) and KPC-Sf3b1^{K700E/+} (n=12) cancer-
817 derived organoid lines. Two-tailed unpaired t-test was used to compute the indicated p-value. **(F)** RT-
818 PCR amplicon of *Ppp2r5a* cDNA isolated from sorted KPC (n=3) and KPC-Sf3b1^{K700E/+} (n=4), as well
819 as WT and *Sf3b1*^{K700E/+} pancreas organoids. The amplicon includes the 3' splice site of exon 4 and 5,
820 the upper arrowhead represents the predicted size of non-canonical transcripts. **(G)** Western blot gel-
821 image of MAP3K7 and GAPDH in a KPC cell line transduced with a control shRNA (shCtrl) or a shRNA
822 targeting Map3k7 (shMap3k7). **(H)** RT-qPCR analysis of EMT genes displayed in Fig. 2G in a KPC cell
823 line transduced with a control shRNA (shCtrl) or a shRNA targeting Map3k7 (shMap3k7) treated with
824 TGF-β1 (10 ng/ml) for 24 hours. The experiment was performed independently 3 times. **(I)** Organoid
825 count normalized to untreated organoids of respective genotypes at passage 0. Duct organoids of
826 indicated genotypes / treated with shRNA were exposed to 1 ng/ml TGF-β1 for 25 days. Data represents
827 one organoid line per condition, the experiment was independently performed three times. One-way
828 ANOVA was used to compute the indicated p-values.