RNA splicing is a key mediator of tumour cell plasticity and a therapeutic vulnerability in colorectal cancer

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Tumour cell plasticity is a major barrier to the efficacy of targeted cancer therapies but the mechanisms that mediate it are poorly understood. Here, we identify dysregulated RNA splicing as a key driver of tumour cell dedifferentiation in colorectal cancer (CRC). We find that Apc-deficient CRC cells have dysregulated RNA splicing machinery and exhibit global rewiring of RNA splicing. We show that the splicing factor SRSF1 controls the plasticity of tumour cells by controlling Kras splicing and is required for CRC invasion in a mouse model of carcinogenesis. SRSF1 expression maintains stemness in human CRC organoids and correlates with cancer stem cell marker expression in human tumours. Crucially, partial genetic downregulation of Srsf1 does not detrimentally affect normal tissue homeostasis, demonstrating that tumour cell plasticity can be differentially targeted. Thus, our findings link dysregulation of the RNA splicing machinery and control of tumour cell plasticity.
Colon cancer (CRC) is a leading cause of cancer related mortality responsible for around 900,000 deaths annually. The emergence of novel targeted therapies for treatment has offered hope of improved outcome, but these are generally beneficial to only small subsets of patients and emergence of resistance is common, even in initially responsive patients. For example, resistance to the highly specific EGFR inhibitor cetuximab, used for the treatment of some KRAS wild-type tumours, routinely emerges and survival benefit is limited. In this case resistance emerges via multiple, distinct genetic and transcriptomic mechanisms that bypass the tumour’s requirement for signalling via EGFR, suggesting tumour heterogeneity may play a role in mediating therapy resistance. Indeed, intra- and inter-tumoural mutational diversity within patients has been shown to result in CRC subpopulations having variable responses to therapeutic agents, together suggesting that targeting single specific oncogenic driver genes may not deliver durable responses, even in highly stratified patient populations. Therefore, there is an urgent need to identify more general, clinically targetable biological mechanisms that exploit general cancer cell characteristics rather than the classically studied oncogenic driver genes that mediate them, such mutated APC, TP53 or RAS/MAPK.

Another key therapeutic challenge is overcoming the plasticity of stem cell fate, where stem cells can not only differentiate into more specialised cell types, but these differentiated cells have the ability to revert back to the stem cell phenotype following tissue damage. This is especially important when considering clinical treatment as this phenomenon has also been observed in tumours where differentiated tumour cells (non-Lgr5 +) can be reactivated following cancer stem cell depletion and subsequently dedifferentiate and fuel tumour growth. Alternative RNA splicing increases proteome diversity and can induce radical effects on cellular phenotype including promoting carcinogenesis. As RNA splicing activity is dysregulated in CRC, RNA splicing factors and global reprogramming of RNA splice isoforms in a Wnt-driven CRC driving efficient tumourigenesis. mRNA splicing is thought to be the rate-limiting step in generating functional transcripts and has been proposed as a potential therapeutic vulnerability in MYC driven breast cancer and lymphoma. Therefore, activity levels of the RNA spliceosome may be an important facilitator of cancer cell growth and its targeting may present a viable therapeutic strategy.

Here, we aimed to identify universal therapeutic targets for CRC by determining the mechanisms important for cancer cell growth. We identified widespread dysregulation of RNA splicing factors and global reprogramming of RNA splice isoforms in a Wnt-driven animal model of CRC. Using 3D organoid cultures, we performed a synthetic lethal screen of splicing factors altered in CRC, identifying the splicing factor SRSF1 as a therapeutic vulnerability in CRC organoids. Moreover, we determined that SRSF1, by modulating the pro-proliferative Kas 4B isoform, alters cancer stem cell plasticity, the invasive potential of advanced tumours and the viability of primary CRC organoids derived from patients. Together, these data suggest that RNA splicing, and SRSF1 in particular, control the plasticity of tumour cells and is a viable therapeutic target in CRC.

**Results**

RNA splicing is dysregulated following Wnt hyperactivation. To identify cellular processes activated at early stages of intestinal carcinogenesis, we deleted both copies of the CRC tumour suppressor gene Apc in mice using the intestinal specific villin-CreERT2 (villinCreERT2 Apcfl/fl). Five days after tamoxifen induced-gene recombination, small intestinal tissue was dissected and activation of the Wnt hyperproliferative phenotype confirmed by immunohistochemical staining of BrdU and Wnt/β-catenin target gene upregulation (Fig. S1a, S1b and S3a).

Wild-type and Apcfl/fl intestinal tissue was subjected to RNaseq (Supplementary Data 1) and biological pathway analysis (KEGG) used on differentially expressed transcripts to identify enriched biological processes. Compared to normal tissue, RNA processing events were the most significantly upregulated processes following Apc loss (Fig. 1a). Specifically, we found that 60 genes involved in RNA splicing were upregulated (Supplementary Data 2 and Fig. S1c). This suggests there may be an increased requirement for RNA splicing following CRC initiation.

To ascertain if splicing factor upregulation correlated with changes in RNA splicing events, we conducted differential splicing analysis using SUPPA2 and identified 1,661 alternative splicing events following Apc loss (p < 0.05) (Fig. 1b and Supplementary Data 3) indicating a global rewiring of alternative RNA splicing upon Wnt activation. The largest category of splicing event detected using this method was alternative first exon (46.4%) which could be an indication of alternative transcriptional regulation. To investigate this, we compared these events to differentially expressed genes identified in Supplementary Data 1. 140/631 alternative first exon events were also differentially expressed indicating the majority of these events are not linked to transcriptional changes and likely represent alternative use of first exons and/or differential promoter usage (Fig. S1d). SUPPA2 analysis detects annotated splicing events but we next analysed previously published RNA splicing analysis of The Cancer Genome Atlas (TCGA) data to determine whether the splicing events identified in our mouse model are found in human CRC samples. Of the top 100 identified skipped exon splicing events, 18 showed the same alteration in human CRC including KRAS, CD44 and EIF4A2 (Supplementary Data 5 and Fig. S1i). In summary, we identified widespread alterations of alternative splicing events following Apc loss, several of which are associated with cancer progression and a proportion of which are found in human CRC samples.

As RNA splicing activity is dysregulated in Apcfl/fl intestine, we investigated the effects of global splicing inhibition on oncogenic growth compared to wild-type. 3D intestinal organoids from normal or Apcfl/fl intestine were exposed to the highly potent splicing inhibitor pladienolide B (Fig. 1d) and a drop in cell viability (Fig. 1e and S1j), whereas wild-type organoids were more resilient. We found a significant difference in the IC50 of pladienolide B for wild-type and Apcfl/fl intestinal organoids (Fig. 1e). We extended this analysis to organoids derived from human patient material. We found that CRC tumour organoids exhibited significant loss of viability upon pladienolide B treatment but organoids derived from normal tissue did not (Fig. 1f, g). These results support a proof-of-concept that the spliceosome may be a therapeutic vulnerability in Apc-deficient cells.
The splicing factor SRSF1 is critical for Apc-deficient growth. To identify which splicing factors are required for Apc-deficient cell growth, we conducted a splicing factor-targeted, synthetic lethal CRISPR screen comparing wild-type to Apc<sup>fl/fl</sup> intestinal organoids. We generated a guide RNA (gRNA) library targeting the 60 splicing factor genes upregulated following Apc deletion (Supplementary Data 2 and 6) and delivered them by lentiviral transduction into either wild-type-Cas9 or Apc<sup>fl/fl</sup>-Cas9 intestinal organoids (Fig. 1h) at low multiplicity of infection (MOI). Surviving organoids were harvested after 7 days of antibiotic selection and gRNA sequences in surviving clones analysed via deep sequencing. A comparison of the gRNAs present in surviving clones showed a significant enrichment for SRSF1.
wild-type-Cas9 and Apcfl/fl-Cas9 organoids showed that guide RNAs targeting Ddx10 and Srsf1 were significantly under-represented in Apcfl/fl-Cas9 population (Fig. 1i and Supplementary Data 7). This implies deletion of these genes is less well tolerated in the context of Apc loss compared to normal growth conditions. To confirm these findings, we conducted CRISPR-targeted Ddx10 and Srsf1 individually in pooled gene-edited populations of both wild-type and Apcfl/fl organoids. Targeting Ddx10 or Srsf1 reduced ApcΔ/Δ organoid growth, but only Srsf1 showed a significant reduction in viability in ApcΔ/Δ organoids compared to wild-type organoids where Srsf1 is deleted (Fig. 2a, b and S2a, S2b). Therefore, we chose to investigate the function of Srsf1 in further detail.

Notably, despite showing significantly reduced viability in ApcΔ/Δ organoids, deletion of Srsf1 by CRISPR also reduced viability in wild-type organoids. However, previous reports have demonstrated that modest reductions in Srsf1 expression levels are well tolerated in mice suggesting a potential therapeutic window for targeting Srsf1. As therapeutic targeting does not lead to target inhibition equivalent to genetic knockout we aimed to phenocopy clinical intervention more accurately using doxycycline-inducible short hairpin RNA (shRNA) expression. This also allowed us to deplete Srsf1 levels in fully developed organoids of increasing genetic complexity and aggressiveness. Expression of the Srsf1 shRNA reduced SRSF1 levels in wild-type, Apc-deficient and AKP (ApcΔ/Δ; Kras; G12D/+; Trp53Δ/Δ) organoids (Figures S2c, S2d). Validating the findings with CRISPR deletion, knockdown of Srsf1 significantly reduced the growth of fully mature Apc-deficient and AKP organoids (Fig. 2e–h). However, we observed no impact of Srsf1 depletion on wild-type organoid growth (Fig. 2c, d). Together, these data suggest a potential therapeutic vulnerability of CRC cells to depletion of Srsf1.

SRSF1 is required for epithelial cell hyperproliferation. To investigate the effects of targeting Srsf1 in vivo, we combined Apc deletion in our mouse model, where SRSF1 was upregulated (Fig. S3a), with a deletion of a single copy of Srsf1 (villinCreERT2 ApcΔ/Δ Srsf1 Δ/Δ) (Figs. S3b–S3c and S3h). Homozygous deletion of Srsf1 led to gut toxicity with elevated levels of intestinal cell apoptosis (Fig. S3k) so heterozygous deletion of Srsf1 was selected to phenocopy a more clinically relevant scenario where impaired SRSF1 activity might be achieved rather than complete deletion. A 30–50% reduction in Srsf1 levels (Figure S3h) had no proliferative or morphological effect in wild-type intestinal tissue (Fig. 3a, b and S3d–S3e). However, we observed a significant reduction in the number of BrdU-labeled cells (Fig. 3a, b) and Ki-67-positive cells (Figs. S3d, S3e) upon deletion of one copy of Srsf1 in the context of Apc loss. Interestingly we found that the proliferative reduction seen in villinCreERT2 ApcΔ/Δ Srsf1 Δ/Δ was observed predominantly in the intestinal villous zone (Fig. 3a–b and S3f) and this was independent of variations in crypt-villus size or levels of apoptosis (Figures S3g and S3i–S3j). We investigated this proliferative reduction further by harvesting villi from either ApcΔ/Δ or ApcΔ/Δ Srsf1Δ/Δ tissue and generated villi-derived 3D organoids in vitro. The clonogenic capacity of these purified ApcΔ/Δ Srsf1Δ/Δ epithelial cells was strongly impaired (Fig. 3c–e) demonstrating a persistence of this phenotype in a stromal-independent context. To determine in more detail whether Srsf1 depletion affected normal intestinal stem cell homeostasis we generated inducible vil-Cre-ERT2 WT and Srsf1Δ/Δ mice carrying the Lgr5 GFP-CreER(T2) transgene. Following tamoxifen-induced gene deletion, we analysed the LGR5 + ISC population using GFP IHC to visualise LGR5-GFP expression and flow cytometry to determine the percentage of GFP positive cells. Both analyses showed that Srsf1 depletion had no impact on the LGR5 ISC population (Figure S3i–n). In addition, sorted single LGR5-GFP positive cells had the same capacity to form organoids when plated in vitro and showed no changes stem cell marker gene expression following Srsf1 deletion (Figure S3o–q). Together, these data indicate Srsf1 depletion does not affect normal LGR5 + intestinal stem cell homeostasis but significantly impairs the growth of Apc deficient cells.

Oncogenic SRSF1 levels affect cell-type plasticity. To investigate whether the reduced proliferative phenotype observed in the intestinal mucosa of ApcΔ/Δ Srsf1Δ/Δ was due to changes in intestinal cell identity, we performed RNAseq analysis of ApcΔ/Δ and ApcΔ/Δ Srsf1Δ/Δ intestines. We compared the ApcΔ/Δ Srsf1Δ/Δ transcriptome (Supplementary Data 8) with previously defined signatures of intestinal cell types using gene set enrichment analysis (GSEA). Interestingly, we found a significant overlap between genes associated with late transit-amplifying and differentiated enterocytes and gene overexpressed in ApcΔ/Δ Srsf1Δ/Δ intestinal cells (Figs. S3r–S3u) (Supplementary Data 9). Numerous differentiated cell marker genes were overexpressed in ApcΔ/Δ Srsf1Δ/Δ compared to ApcΔ/Δ, including Slc13a2 and Apoc2 (Fig. 3f). We also found that expression of the Wnt target gene and cancer stem cell marker Prox1 was decreased in ApcΔ/Δ Srsf1Δ/Δ compared to ApcΔ/Δ, including Slc13a2 and Apoc2 (Fig. 3g). Interestingly, the increase in the splicing isoform ratio of KrasAB/KrasA observed after Apc deletion (Fig. 1c and S1f) was reversed upon simultaneous deletion of Srsf1 (Fig. 3h). This was not due to alterations in nuclear localisation of β-catenin, a previously described function of Srsf1, as demonstrated by IHC analysis (Fig. S3v, w). These data suggest that Srsf1 promotes a less differentiated, stem-cell-like phenotype following Apc deletion. To investigate the generality of these findings we depleted Srsf1 using shRNA in a number of tumorigenic organoid models (Fig. S3x–z) and determined stem cell function using clonogenicity assays. Validating our results in vivo experiments, Srsf1 knockout led to a significant reduction in clonogenicity, growth rate and viability of ApcΔ/Δ organoids suggesting reduced stem cell function (Fig. 3i–k and S3x–z). Notably, the same results were observed following Srsf1 knockout in two mouse CRC organoid lines that model late-stage, metastatic disease (Fig. 3i–k and S3x–z). The expression of the stem cell marker Lgr5 was also reduced in Srsf1 depleted
organoid lines (Fig. S2d). Together, these data demonstrate that Srsf1 depletion reduces stem cell properties and results in colorectal cancer cells adopting a more differentiated cell transcriptional phenotype.

SRSF1 facilitates intestinal cell dedifferentiation. Due to these findings we hypothesised that SRSF1 is required for differentiated enterocytes to dedifferentiate and acquire stem cell properties. Activation of KRAS or IκB kinase (IKK) combined with Apc loss has been shown to induce dedifferentiation of villus enterocytes34. Therefore, we generated mice carrying villinCreERT2 Apcfl/fl KrasG12D and villinCreERT2 Apcfl/fl KrasG12D Srsf1fl/+ mice and found that depletion of Srsf1 led to a significant reduction in the proportion of organoids formed (Fig. 4a, b). The clonogenic capacity of the organoid lines derived from these dedifferentiated cells was also reduced in the Srsf1fl/+ genotype (Fig. 4c) suggesting that the acquisition of stem cell properties was impaired. Intriguingly, Kras isoform splicing was still modulated by SRSF1

Fig. 2 The splicing factor SRSF1 is critical for Apc-deficient growth. a Images of clonogenicity assays in wild-type and Apcfl/fl Cas9 organoids, treated with the indicated gRNA. Scale bar 250 µm. b Clonogenicity quantification of organoids treated with indicated gRNA. c Images of doxycycline (dox)-induced (Tet-On) shRNAs against Renilla or Srsf1 in wild-type intestinal organoids. Red fluorescent protein (RFP) is co-expressed with the shRNA following addition of dox. White arrows indicate a tracked organoid over the time-course following dox treatment. d Organoid size of tracked wild-type intestinal organoids. e Images of tracked Apcfl/fl organoids treated with the indicated Tet-On shRNA following addition of dox. f Size quantification of Apcfl/fl organoids with the indicated shRNA, +/- addition of dox. Statistical test indicated for shSrsf1 +/- dox. g AKP organoids with the indicated Tet-On shRNA following addition of dox. h Quantification of AKP organoid size. For (c, e and g) scale bar is 250 µm. All images shown are representative. Data shown as mean and error bars are SD (b) and SEM (d, f, h). All data are n = 3 independent experiments and analysed with two-tailed, unpaired t-tests, with (d, f, h) using Bonferroni post-hoc correction (alpha = 0.05), p values are indicated in figure panels. See also Fig. S2.
even in the context of constitutively active KRAS signalling (Figure S4b).

To confirm these observations, we employed a different dedifferentiation model driven by hyperactive NF-κB signalling \(^3\), \((\text{villin}^{\text{CreERT2}} \ Apc^{\text{fl}}/\text{fl} \ IKK2^{ca})\) and subjected these mice to the same experimental procedure. Again, we found a similar impairment in dedifferentiation capacity of villi cells and reduced clonogenicity in derived organoids following heterozygous \(Srsf1\) deletion (Fig. 4d–f).

To rule out the possibility that these clonogenic cells derived from crypt cells migrating into the villi we sorted differentiated villus cells based on expression of EPHB2. EPHB2 marks a
Fig. 3 SRSF1 is required for epithelial cell hyperproliferation and affects cell-type plasticity. a Representative images of BrdU-stained mouse small intestines with the indicated genotypes, 5-days after tamoxifen induction. Letters ‘c’ and ‘v’ indicate demarcated crypt and villus compartments respectively and the red line highlights the proliferative zone. Scale bar 250 μm. b Quantification of proliferative cells in the crypts and villi of the indicated genotypes, n = 4 (WT) vs 3 (Srsf1Δ/Δ) vs 5 (ApCfl/fl) vs 4 (ApCfl/fl Srsf1fl/fl) biologically independent mice. c Representative images of clonogenicity assays using villi-derived intestinal organoids of indicated genotypes. Scale bar 500 μm. d Quantification of villi-derived organoid clonogenicity, n = 10 vs 7 independent experiments. e qPCR gene expression of Srsf1 in organoids normalised to β-actin, n = 3 vs 4 independent experiments. f qPCR quantification of upregulated late transit-amplifying (differentiated) cell markers in ApCfl/fl Srsf1fl/+ mouse intestinal tissue, relative to ApCfl/fl intestinal tissue, using Gapdh loading control, n = 3 vs 3 biologically independent mice. g Prox1 stem cell marker qPCR quantification in mouse small intestines of indicated genotypes, normalised to Gapdh, n = 3 vs 3 biologically independent mice. h qPCR-derived ratio of Kras splicing isoforms in the indicated genotypes, n = 7 (WT) vs 3 (Srsf1Δ/Δ) vs 8 (ApCfl/fl) vs 4 (ApCfl/fl Srsf1fl/fl) biologically independent mice. i Images of organoids of the indicated genotype, treated with control or Srsf1 shRNA. AKP (ApCfl/fl; Δ/Δ; Thrs2/3Δ/Δ; KPN; Δ/Δ; Rosa26Nlxx/+) was used as a control for the shRNA transfection efficiency in organoid cultures. Scale bar 500 μm. j Relative clonogenicity and size of surviving organoids following control or Srsf1 shRNA treatment, n = 3 vs 3 independent experiments. Data in bar charts are represented as mean and error bars are SD with data analysed with two-tailed, unpaired t-tests, p values are indicated in figure panels. All biological replicates are shown as individual value plots. See also Fig. S3.

gradient of stem and progenitor crypt cells with expression absent in differentiated villus cells30. We sorted EPHB2 negative epithelial cells from wild-type crypts and confirmed that they did not have clonogenic capacity (Figure S4c–e). We then utilised this sorting strategy to obtain differentiated villus cells from induced villinCreERT2 ApCfl/fl KrasG12D and villinCreERT2 ApCfl/fl KrasG12D; Srsf1Δ/Δ mice (Fig. 4g). EPHB2 negative differentiated ApCfl/fl KrasG12D cells were able to form colonies, indicative of cellular dedifferentiation. This ability was significantly impaired following Srsf1 depletion, demonstrating the requirement of Srsf1 for the acquisition of stem cell properties (Fig. 4h, i). To confirm these findings we utilised an analogous model where doxycycline-inducible shRNA to Srsf1 was used to activate Wnt signalling in KrasG12D mutant colon cancer organoids. We observed40. In the absence of doxycycline, organoid growth is dependent on media supplementation with Wnt3a and R-spondin. In addition, EPHB2 negative cells have poor clonogenic capacity indicative of a differentiated cell phenotype (Figure S4f). We used shRNA to decrease Srsf1 in these organoids and following doxycycline induction FACTS sorted for EPHB2 negative, differentiated cells. Again, depletion of Srsf1 significantly impaired the ability of such cells to form colonies (Fig. 4j–l). In addition, organoids derived from Srsf1 depleted differentiated cells expressed significantly higher levels of the differentiated cell markers Mus21 and Krt20 (Figure S4g, S4h). Together, these data demonstrate a requirement for Srsf1 expression in mediating intestinal cell plasticity.

To determine whether Srsf1 also drives increased stem cell properties, we overexpressed wild-type Srsf1 (Srsf1WT) or Srsf1 with mutations in the second RNA recognition motif (RRM2) leading to impaired RNA binding37, which (Srsf1D136A,K138A) in ApCfl/fl organoids (Figure S4i). Consistent with our deletion studies, Srsf1WT overexpression led to increased organoid growth, clonogenicity and increased expression of the stem cell marker Lgr5 (Figures S4i–l). By contrast the Srsf1 mutant did not affect organoid growth, demonstrating that the RNA-binding ability of SRSF1 is necessary for its dedifferentiation activity. Based on these findings, we conclude that SRSF1 mediates intestinal cell dedifferentiation and the acquisition of stem cell properties.

Wnt-induced SRSF1 levels enforce splicing dysregulation. To ascertain the extent to which Srsf1 contributes to Wnt-driven splicing dysregulation and identify potential splicing changes that mediate oncogenesis, we conducted differential splicing analysis on ApCfl/fl and ApCfl/fl Srsf1fl/+ intestinal tissue, in which we observed reduced proliferation and stem cell activity, using SUPPA2. We identified 577 alternative splicing events (p < 0.05) (Fig. 5a and Supplementary Data 10). The majority of these were exon skipping alternative splicing events (30.5%), supporting previous work using in vitro models showing that SR proteins promote splice site selection through exon splicing enhancer recognition38. We then determined the proportion of unique dysregulated alternative splicing events in ApCfl/fl that were SRSF1-dependent. We compared the alternative splicing events identified from our wild-type/Apcfl/fl analysis (Fig. 1b and Supplementary Data 3) with the alternative splicing events found in ApCfl/fl/Apcfl/fl Srsf1fl/+ (Supplementary Data 10). There was a significant enrichment of alternative splicing events from ApCfl/fl/Apcfl/fl Srsf1fl/+ that were also found in our wild-type/Apcfl/fl dataset (p < 1e−5) (Fig. 5b). In addition, a significant proportion of the alternative splicing events occurring after Apc deletion were reverted upon deletion of Srsf1fl/+ (127 alternative splicing events with discordant dPSIs, p = 0.049) (Supplementary Data 11). Again, rMATS analysis identified a large proportion of potentially novel splicing events suggesting widespread changes in alternative splicing mediated by SRSF1 (Figure S5a and Supplementary Data 12).

We validated several of the alternative splicing events discovered from our differential splicing analysis (Fig. 5c, d). Additionally, isoform level changes in Kras splicing were detected and validated confirming that the Wnt-induced increase in the pro-proliferative Kras4b isoform over the pro-apoptotic Kras4a isoform was reversed upon Srsf1 deletion. SRSF1-dependent changes in Kras isoform splicing levels were also present in multiple other models (Figs. 3h, S4b, S6j and S6m). To determine whether these transcripts were direct targets of SRSF1 we carried out RNA immunoprecipitation experiments. By pulling down SRSF1 from the CMT93 mouse colorectal cancer cells line and carrying out qRT-PCR we identified significant binding of Srsf1 to several of the alternatively spliced transcripts identified by RNAseq (Fig. S5b, c). In addition, alternative splicing events (events identified via SUPPA2 analysis, not including AF events) in our ApCfl/fl/Apcfl/fl Srsf1fl/+ dataset, which were repressed following Srsf1 depletion, were enriched for SRSF1 binding motifs, together suggesting loss of direct, functional SRSF1 binding to these transcripts led to the splicing changes we observed (Fig. S5d). Besides the well-characterised role of SRSF1 in pre-mRNA splicing in the nucleus, this shuttling SR protein also has post-splicing functions, which include mRNA export, mRNA translation and nonsense-mediated decay12,39. We utilised a novel mouse model carrying a nuclear-cytoplasmic shuttling defective Srsf1 mutant to determine whether non-nuclear functions of SRSF1 could explain the phenotypes we observed80. Apc deficient organoids carrying a single allele of Srsf1-IRS (which is unable to shuttle to the cytoplasm) had no defect in clonogenic capacity (Fig. S5e–g). This is unlike organoids carrying heterozygous Srsf1 deletion (Fig. 3c, e) demonstrating that the cytoplasmic function of Srsf1 does not
explain the phenotypes we observe in this model. Together, these data suggest the primary cause of the effects we observed are due to nuclear (presumably splicing) functions of SRSF1.

To functionally determine whether Kras4b plays a role in colorectal tumourigenesis we treated Apc\(^{fl/fl}\) and metastatic KPN (Kras\(^{G12D}\); Trp53\(^{fl/fl}\); Rosa26\(^{N1icd}\); Apc\(^{fl/fl}\)Rosa26\(^{N1icd}\) + Kras\(^{G12D}\); Trp53\(^{fl/fl}\); Rosa26\(^{N1icd}\) + Kras\(^{G12D}\); Trp53\(^{fl/fl}\)Rosa26\(^{N1icd}\)) organoids with the KRAS4B specific inhibitor deltarasin (Fig. S5h)\(^{41,42}\). Deltarasin is a small molecule inhibitor of the KRAS4B -PDE\(\delta\) interaction, and PDE\(\delta\) has been shown to chaperone and recruit KRAS4B to the plasma membrane, but is not required for KRAS4A recruitment. Treatment with deltarasin led to a rapid organoid dissociation and a highly significant reduction in viability of both Apc\(^{fl/fl}\) and KPN organoid lines demonstrating an important role for KRAS4B in maintaining CRC organoid viability (Fig. 5e, f). Treatment of wild-type organoids led to a smaller reduction in viability (Fig. 5e, f). Treatment of human normal and CRC
organoids showed a similar effect, with organoids derived from normal colon being resistant but those derived from tumours being highly sensitive to deltarasin treatment (Fig. S5i, S5j). To validate these findings we designed an antisense morpholino that inhibits the splicing of *Kras4b*. Treatment of organoids with this morpholino proved highly effective at reducing the *Kras4b*/ *Kras4a* splice ratio (Fig. S5m, S5n) and significantly reduced the clonogenic capacity and viability of *Apc*Δ715Δ721 and KPN organoids (Fig. S5k–S5p).

To further this analysis, we next asked whether *Kras4b* expression is sufficient to rescue organoid viability in *Srsf1* depleted organoids. We generated organoid lines overexpressing GFP alone, or *Kras4b* (also marked with GFP expression) both carrying a DOX inducible *shSrsf1* RFP construct. In this model *Kras4b* expression (marked by GFP) can be maintained alongside *Srsf1* depletion (marked by RFP expression) (Fig. S5q). We allowed organoids to form, induced with DOX and tracked GFP/RFP double-positive organoids over 3 days. Similar to our previous results, depletion of *Srsf1* in GFP expressing controls led to a significant reduction in organoid growth (Fig. 5g, h). However, this growth reduction was significantly rescued to wild-type levels by ectopic expression of *Kras4b* indicative of a functional role for *Kras4b* in mediating the phenotypic effects of *SRSF1* expression in this model (Fig. 5g, h).

To infer the biological function of the two *Kras* splicing isoforms in the context of CRC, we employed proximity-dependent biotin identification (BioID) to uncover interacting proteins of *Kras4A* and *Kras4B* (Fig. S5r–S5u). We found 83 proteins that significantly (*p < 0.05*) interacted with both *Kras4A* and *Kras4B* (Fig. S5t and Supplementary Data 13). We also identified 23 proteins that monogamously interacted with *Kras4A* and 22 proteins that uniquely interacted with *Kras4B*. Some of these uniquely interacting proteins were associated with contrasting cell signalling pathways and associated with unique cellular compartments (Supplementary Data 13). For example, IGF1R and RALA interacted only with *Kras4A* whereas BRAF and RAP1A were exclusively associated with *Kras4B*. These data show that *Kras4A* and *Kras4B* have specific subsets of protein interactors indicating *SRSF1*-controlled splicing changes can result in significant changes in the oncogenic protein interactome.

**High *Srsf1* expression mediates tumour cell plasticity and colorectal cancer invasiveness.** To explore the role of *SRSF1* in advanced stage colonic carcinogenesis we utilised a previously described model of carcinogen-induced tumourigenesis. Cohorts of *villinCreERT2* p53−/− and *villinCreERT2* p53−/− *Srsf1Δl/Δl* mice were treated with repeated rounds of azoxymethane (AOM) and aged until signs of colonic tumourigenesis became apparent (Fig. S6a). Mice treated with AOM presented with colonic tumours, with tumours of the small intestine rarely occurring (Fig. S6b). Although we did not find evidence that targeting *Srsf1* provided a survival advantage or a change in tumour number or burden (Figs. S6c–S6e) we found that deletion of one copy of *Srsf1* (Fig. S6f) significantly reduced the proportion of mice presenting with invasive tumours in this model (Fig. 6a, b). Additionally, overall number and percentage of invasive tumours per mouse and the presence of extensive collagen deposition was significantly reduced in *Srf1* depleted mice (Figs. 6c and S6g–S6i).

Upon examination of the invasive tumours, we found evidence of cell plasticity changes. Immunohistochemical analysis of the cancer stem cell marker PROX1 revealed a significant decrease in PROX1 expression in invasive *p53Δl/Δl* *Srsf1Δl/Δl* tumour epithelial cells (Fig. 6d, e) corroborating our RNAseq results in short-term Cre-lox recombinated *Apc*Δ715Δ721 *Srsf1Δl/Δl* tissue (Fig. 3g). We also found a significant corresponding increase in expression in the differentiation marker, SLC13A2 (Figs. 6d, f), and altered *Kras* splicing in these tumours (Fig. S6i). These data suggest that impaired *Srf1* expression reduces the invasive and stem cell behaviour of colonic tumours in vivo. To determine whether *SRSF1* directly controls these phenotypes, we cultured 3D tumour organoids from invasive AOM *p53Δl/Δl* tumours and depleted *Srf1* expression using shRNA (Fig. S6k). We found that both PROX1 expression and *Kras4b*/ *Kras4a* splicing ratio were impaired upon *Srf1* knockdown confirming a direct role for *Srf1* in mediating these effects (Figs. S6l, S6m). We then carried out an organoid invasion assay and observed a significant reduction in organoid cell invasion through matrigel upon *Srf1* deletion (Fig. 6g–i).

Together, these data suggest that *SRSF1* directly controls tumour cell invasion and maintenance of stem cell properties in late-stage colon cancer.

**SRSF1 depletion inhibits growth and stemness of human colorectal cancer-derived organoids.** To ascertain how *SRSF1* levels correlate with human CRC progression, we analysed colon cancer tissue microarrays (TMAs) containing varying severities of the disease. First, we investigated if the cancer stem cell marker PROX1, which has been show to promote metastatic outgrowth of cells,44, correlated with *SRSF1* expression. We found a positive correlation of *SRSF1* and PROX1 expression (Fig. 7a, b and S7a) similar to that seen in our laboratory models (Figs. 3g and 6d, e). We next examined *SRSF1* expression in different tumour stages and found that more invasive and metastatic tumours had significantly higher expression of *SRSF1* (Fig. 7c) showing that *SRSF1* levels correlate with advanced-stage colorectal cancer.

To phenocopy clinical intervention by way of attenuating *SRSF1* levels in human CRC, we manipulated *SRSF1* in patient-
derived organoids (PDOs) using shRNA. Consistent with our animal experiments the clonogenicity, size and viability of the PDOs was impaired by depleting SRSF1 expression (Fig. 7d–g). Reduced SRSF1 expression was associated with a corresponding reduction in LGR5 suggesting that in human cancer, as in our mouse models, SRSF1 mediates stem cell function (Figs. 7h and S7b–S7d). We also observed an impairment in the ratio of KRAS4B/KRAS4A (Fig. 7h) which we had earlier found to be critical in facilitating oncogenic growth in mouse (Fig. 5e, f).

Taken together these data establish that, not only can SRSF1 attenuation mitigate the oncogenic potential of CRC in mouse models, but also in the human disease.
Discussion

Tumour growth has been shown to increase the transcriptional output of cells leading to an elevated burden of pre-mRNAs requiring processing, including an increased requirement for RNA splicing.45 The RNA addiction of hyperproliferative cells might therefore be exploited for clinical purposes. This idea was supported by earlier work showing that the spliceosome is a potential therapeutic vulnerability following MYC induction in 2D breast cancer cells and also during lymphomagenesis.18,19 Here we have shown that genes associated with RNA metabolic processing are upregulated in a mouse model of colorectal cancer initiation. These include genes involved in RNA splicing. 60 splicing related genes are upregulated following Apc loss, accounting for approximately a quarter of all known splicing genes. Based on this, we surmised that splicing impairment might effectively target rapidly proliferating intestinal organoids with hyperactive Wnt signalling. Indeed, treating organoids with pialdenolide B (which targets the SF3B complex) successfully targeted Apcfl/fl organoids without adversely affecting wild type organoids suggesting that RNA splicing generally may act as a therapeutic vulnerability in CRC. However, this drug has recently failed in clinical trials as it caused optic-nerve dysfunction and vision loss in patients.46 On the other hand, a novel drug targeting the splicing factor SRSF1, ABX300, has been shown to impair SRSF1 splicing activity to treat diet-induced obesity in mice without any observed toxicity.47 Furthermore, villinCreERT2 Srsf1fl/+ mice had normal intestinal morphology without any change in cell proliferation or normal stem cell function, and an inducible shRNA against Srsf1 had no observable phenotype in wild-type organoids. Thus, there is a clear therapeutic window for targeting SRSF1 in colorectal cancer.

In conjunction with an increased requirement for splicing factors, we identified dysregulation of splicing itself, as shown by the shift in RNA isoforms generated following Apc loss. We found that the Kras Tca and 4b isoforms were alternatively spliced depending on the level of Wnt activation, and this was mediated by SRSF1. SRSF1 has previously been proposed to be oncogenic and is a direct transcriptional target of Myc.48,49 In addition, SRSF1 expression has been shown to be dependent on Wnt signalling in colorectal cancer suggesting that Wnt driven dysregulation of RNA splicing is partially mediated by SRSF1 following Apc loss.50 The Krasa isoform has been shown to promote cell death24 and, when mutated, has significantly less oncogenic potential in mice than Krasb.51 Not only did we see a shift in splicing of these isoforms following Apc deletion, but we found that when we impaired the function of Apc-upregulated KrasA4B using deltarasin or an antisense morpholino, we could hamper oncogenic growth in our organoid models. Furthermore, the expression of Krasb could rescue the growth of cells with impaired Srsf1 levels. As Srsf1 depletion leads to alterations in numerous splicing events it is unlikely that this single splicing event fully explains the role of SRSF1 in CRC. It is likely that numerous SRSF1 targets contribute to its phenotypic effect, making SRSF1 an attractive therapeutic target, depletion of which could impact on multiple different pathways.

Targeting Srsf1 resulted in reduced proliferation and stem cell function consistent with similarly described functions of SRSF1 in breast cancer.50,52 Transcriptome analysis of Apcfl/fl Srsf1fl/+ intestinal tissue revealed a role for SRSF1 in modulating cellular plasticity with the gene expression signature becoming distinctly enterocyte-like. Dedifferentiation of enterocytes has been shown to support a ‘top-down’ model of colorectal tumour morphogenesis where adenomas originate at the top of intestinal crypts.43,53. Our data support a role for SRSF1-driven dedifferentiation in enterocytes. Following early activation of constitutive KRAS or NF-kB signalling with Apc loss, a reduction in dedifferentiation-driven clonal events arising from villi-derived differentiated cells was observed with impaired Srsf1 levels. Thus, SRSF1 can mediate cellular plasticity. As SRSF1 is a splicing factor, such plasticity changes might be brought about indirectly as a result of a general splicing repression response, or due to a change in the RNA isoform repertoire. As well as showing increased expression of differentiation marker genes, Srsf1 reduction resulted in a decrease in the stem cell marker Prox1. Prox1 is a Wnt-regulated transcription factor that has been shown to advance colon cancer progression by promoting dysplasia in colonic adenomas, as well as enhancing metastasis in Wnt-driven progenitor cells.44 Not only were Prox1 levels impaired in the intestines of Apcfl/fl Srsf1fl/+ mice following acute Apc deletion, but this reduced level was observed in the advanced tumours of AOM treated p53fl/fl Srsf1fl/+ mice. These tumours were also significantly less invasive and displayed evidence of the same cell differentiation phenotypes observed in our early-stage tumour model. Encouragingly, this demonstrates a potential to target SRSF1 mediated cellular plasticity even in the advanced stages of the disease.

In conclusion, our investigation using mouse models, ex-vivo organoid systems and patient-derived samples demonstrates that intestinal Wnt-driven cancers are addicted to the spliceosome. We have shown that targeting an individual oncogenic splicing factor, SRSF1, impairs cancer progression through a variety of mechanisms (Fig. 7). Modulation of cell-type plasticity in favour of a gene expression signature with reduced stemness, an impaired ability of cells to dedifferentiate, as well as tumours having a lower invasive potential, all may targeting SRSF1 a highly attractive option, and may complement current standard-of-care therapies.

Methods

Contact for reagent and resources sharing. Requests for further information, reagents and resources should be directed to and will be fulfilled by the Lead Contact, Kevin B. Myant: kevin.myant@igmm.ed.ac.uk.

Animals models. Species used: Mus musculus. All animal experiments were performed in accordance with a UK Home Office licence (Project License 70/8885), and were subject to review by the animal welfare and ethics board of the University of Edinburgh. Both genders of mice were used for all experiments at an age of
between 6 and 12 weeks once they had reached a minimum weight of 20 g. Mice were bred at the animal facilities of the University of Edinburgh and were kept in 12 h light–dark cycles and were given access to water and food ad libitum. Mice were maintained in a temperature- (20–26 °C) and humidity- (30–70%) controlled environment. Colonies had a mixed background (50% C57Bl6J, 50% S129). The genetic alleles used for this study were as follows: villinCreER\textsuperscript{55}, Apc (floxed)\textsuperscript{56}, ASF/SF2 (Srsf1 floxed)\textsuperscript{57}, Kras (G12D)\textsuperscript{57}, IKK2ca\textsuperscript{58}, P53 (floxed)\textsuperscript{59}. Mice were genotyped by Transnetyx (Cordoba, USA). At experiment endpoints, mice were humanely sacrificed by cervical dislocation (CD) in line with UK Home Office regulations.

**Tumour models and treatments.** For short-term Cre-Lox recombination where animals were taken at a specific time point of between 2 and 5 days postinduction
of gene recombination (depending on the experimental model), mice were induced with a single dose of tamoxifen (Sigma, Aldrich, T36468) by intraperitoneal injection of 120 µg/kg on the first day and then optionally with a further treatment of 80 µg/kg on the second and third days. For the long-term Cre-Lox recombination in tumour cohorts, mice were given a 300 µL, 10 mg/mL dose of tamoxifen on day 0 and a 200 µL, 10 mg/mL dose of tamoxifen dose on day 1. Mice were then treated via intraperitoneal injection with 10 µM Y-27632 dihydrochloride (ROCK inhibitor, Tocris, 1254), and 10 µM RGD634 (Advanced Drug Delivery Systems, Inc.), 10 µM Y-27632 dihydrochloride (ROCK inhibitor, Tocris, 1254), 10 µM Y-27632 dihydrochloride (ROCK inhibitor, Tocris, 1254), and 10 µM RGD634 (Advanced Drug Delivery Systems, Inc.).

Signs of intestinal tumourigenesis were monitored by weight loss, diarrhoea and/or pale feet. Mice were randomly distributed by sex and age and a total of 10 mice per sex were used. Mice were killed by CO2 inhalation 2 h before culling. The work was approved via the institutional animal ethics committee.

Clonogenicity analysis. Intestinal organoids were passaged as usual via mechanical disaggregation and DME/MEM/PBS washes. After the last wash step, organoid cells were treated with 1 mL StemPro Accutase cell dissociation Reagent ( Gibco, A11050) and incubated at 37 °C for 5–10 min in order to generate single cells. An equal volume of 1% BSA was added to stop the digestion reaction and then diluted in 10 mL DME/F12 media. Cells were then passed through a 40 µm cell strainer and cell counting was performed using a haemocytometer to get an estimate of the number of single cells. Cells were then resuspended in 100 µL Matrigel (BD) and 100 µL of PBS. The tube was gently inverted and left to settle for 30 s. After the larger aggregates settled down, the supernatant containing the villi was discarded and the remaining organoid medium was added to a fresh tube. The tube was then centrifuged at 100 g for 3 min and the collected villi pellet was resuspended in 100 µL DME/F12 medium.

For animals which were used for BrdU analysis, 200 µL of cell proliferation labelling reagent (GE Healthcare, RPN201) was administered via intraperitoneal injection 2 h before culling.

Splicing factor-targeted synthetic lethal CRISPR screen & organoid transductions. A targeted guide RNA (gRNA) library for the 60 APC-induced splicing factors was generated using sequences from Mouse GECIV2 library A (Zhang Lab/GECKO website), using 3 guide RNAs per gene and 9 non-targeting control guides (Supplementary Data 6). In order to avoid ligation bias, oligonucleotides (Sigma) corresponding to each guide RNA cloned individually into the lentivirus packaging plasmid (Addgene, 52963). Sanger sequenced, and then pooled at equal concentrations. LentiGuide-Puro was a gift from Feng Zhang (addgene, 69504). Genes were verified via next-generation sequencing (NGS). The guide RNA library was used to generate infectious lentiviral vector particles.

The workflow of the synthetic lethal screen was as follows: Day 0 – Wild type Cas9 and Apcfl/fl Cas9 expressing organoids (derived from tamoxifen-inducible villin-CreERT2 PSCs) were plated and grown (full 24-well plate each, 20 µL Matrigel/well) in organoid growth media supplemented with 6 µM CHIR-99021 (GSK-3 inhibitor, Abcam, ab120890). Day 1 – Organoids were further expanded to two full 24-well plates each and grown in ‘Organoid+ media’: ADF (Advanced DMEM/F12 + B2 + N2) (500 µL/well), Noggin, EGF, R-spondin (only for Wild type organoids), 10 µM Y-27632 dihydrochloride (ROCK inhibitor, Tocris, 1254), 6 µM CHIR99021, 1 µM Jagged 1 (Addgene, 47054), Notch Ligand, A2-Mac, 61298) and 1 mM valproic acid (Sigma, PHIR016). Day 5 – organoids were dissociated via mechanical digestion followed by treatment with StemPro Accutase Cell Dissociation Reagent ( Gibco, A11050) for 5 min at 37 °C and then neutralised in 1% BSA. Cells were counted and then passaged using the Countess II Automated Cell Counter (Invitrogen). Between 1×105 and 1×106 single cells were plated per 5 µL drop of Matrigel/BME and a minimum of 4 drops were plated for each genotype/condition. Organoid growth media was added and resuspended in 50 µL of organoid growth media, with the addition of 10 µM Y-27632 dihydrochloride (ROCK inhibitor, Tocris, 1254). Colonies/spheres resulting from clonally derived single cells were scored after 7 days.

RNAseq. For the wild type vs Apcfl/fl and Apcfl/fl vs Apcfl/fl Srsf1fl/fl RNAseq experiments, 3 mice from each genotype were given intraperitoneal injections of tamoxifen of 120 µg/kg on day 0 and 80 µg/kg tamoxifen on days 1 and 2. On day 3, mice were culled and the first 10 cm of the small intestine following the duodenum was dissected and washed twice with PBS. The intestine was opened longitudinally with small scissors and the opened intestine was rinsed in PBS. Villi were removed by scraping using a glass coverslip, and were collected in DMEM/F12 in a 50 mL centrifuge tube. The tube was then centrifuged at 100 g for 3 min and then resuspended in 3–5 mL TrypLE Express ( Gibco, 12605010) and incubated at 37 °C for 10 min in order to generate single cells. Cells were then resuspended in 10 mL DMEM/F12 and passed through a 40 µm cell strainer. Single cells were counted and then 12 droplets of Matrigel/BME of 10 µL containing 50,000 cells per droplet were plated in organoid growth media, with the addition of 10 µM Y-27632 dihydrochloride (ROCK inhibitor, Tocris, 1254). Colonies/spheres resulting from clonally derived single cells were scored after 7 days.
The following PDOs were generated by Dr Farhat Din and Prof Malcolm Dunlop, University of Edinburgh: MD175 is a 50 yr old female with familial adenomatous polyposis who previously underwent a colectomy and ileorectal anastomosis who then developed a stage 2 rectal cancer - individual polyp used in this study. MD20043 is an 81 yr old male with stage 4 rectal cancer, TNM (T3, 2, 1). MD19648 is a 45-year-old female with familial adenomatous polyposis complicated by a stage 3 rectal cancer - FAP rectum tumour, TNM (pT1, pN1a, 0). MD20853 is a 71 yr old male with a tubulovillous adenoma with low-grade dysplasia. MD20910 is a 60 yr old male with rectal cancer, TNM (pT2, N1b, 0). Ethical approval for human CRC organoid derivation was carried out under NHS Lothian Ethical Approval Scottish Colorectal Cancer Genetic Susceptibility Study 3 (SOCCS3) (REC reference: 11/SS/0109). All patients provided fully informed consent for the use of their tissues.
Biological pathway enrichment analysis. RNAseq data of the 2330 genes upregulated in ApdH
to wild type were analysed using gProfiler version e98_egg5_p14_ce5b097, accessed on 24/01/2020 using the following data sources: biological pathways-KEGG.

Organoid Culture. To generate organoids from wild type or genetically modified mice, the first 10 cm of small intestine following the duodenum was isolated, flushed with phosphate-buffered saline solution (PBS) and opened longitudinally using scissors. Villi were removed by scraping using a microscope coverslip. Remaining tissue was then washed several times with PBS and then incubated with 2 mM EDTA in PBS with gentle shaking at 4 °C for 30 min. Crypts were then removed from the tissue by vigorous pipetting and selecting through a 70 µm cell strainer. Crypts were scored and ~100 crypts were plated in 20 µL droplets of cold liquified Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-free (Corning #356231/734-1101) or Cultrex Reduced Growth Factor Basement Membrane Extract Matrix, Type 2 (BME) (2) (Trevigen #3533-010-02). A single 20 µl matrix droplet was grown in each well of a 24-well plate. Matrix droplets with suspended cells were placed on solidifying at 37 °C for 10 min prior to the addition of growth media. All organoids were grown in a humidified incubator at 37 °C supplemented with 5% CO2.

Organoids were grown in ‘organoid growth media’: Advanced DMEM/F-12 media (ADF, Gibco) supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin, 10 mM HEPES, 2 mM L-Glutamine and 10 mM HEPES (Life technologies), N2 (Gibco, 17502048), B27 (Gibco, 17504044), 50 mM EGf (Peprotech, 315-09-500) and 100 ng/ml Noggin (Peprotech, 250-38-500). Wild type organoids were grown with the addition of 500 ng/ml R-spondin-1 (Peprotech, 120-38-500). We later used Noggin and R-spondin via way of conditioned media by transformed HEK 293 cell lines. The transformed Noggin producing cell line was a gift from Hans Clevers’ group (Hubrecht Institute) and the R-spondin producing cell line was purchased from Trevigen (#3710-001-01). Organoids were passaged via mechanical disaggregation using a serological pipette, where several rounds of ADf or PBS washing and centrifugation were used to remove the residual matrix, before resuspending organoid fragments in the fresh matrix.

Tumour cell isolation and organoid culture. Tumours were dissected from the intestine and then cut into pieces. Tissue tumour fragments were then incubated in 2 ml organoid growth media supplemented with 1 mg/ml collagenase II (Sigma, C1764), 0.5 mg/ml hyaluronidase (Sigma, H3506) and 10 µM Y-27632 dihydrodrochloride (ROCK inhibitor, Tocris, 1254). Incubation was at 37 °C for 60–90 min with vigorous shaking until the tumour was completely digested. The digested reaction was then neutralised using 100 µl 1% BSA and then fragments were filtered through a 70 µm cell strainer. The filtered cells were then centrifuged at 600 g for 3 min and washed 2–3 times in DMEM/F12, before being re-suspended and plated in an 20 µl droplets of BME in 24-well plates and overlaid with organoid growth media.

Pladienolide B treatment. Pladienolide B stock (Calbiochem, 5.39169.0001) was made up to 1 mM in DMSO. Wild type and ApdH transgenic organoids were broken into fragments via mechanical disaggregation and were plated at a density of ~40 fragments/20 µl droplet of Matrigel in a 96-well plate, with at least 6 wells per Pladienolide B concentration for each of the three biological replicates. Organoids were allowed 48 h to develop prior to the addition of Pladienolide B, and were treated for 48 h with concentrations of Pladienolide B ranging from 0.5 nM–200 nM. For human organoid experiments the same protocol was used but a single dose of 1 nM Pladienolide B was used.

MTT/Resazurin cell viability analysis. For Thiazolyl Blue Tetrazolium Blue (MTT) cell viability assay, organoids were plated and treated in 96-well plates as already described. MTT (Sigma, M2128) solution was added to the organoid culture media at a final concentration of 500 µg/ml and incubated for 2–3 h at 37 °C, 5% CO2. The medium was then discarded and 20 µl of 2% SDS solution in H2O was added to solubilize the Matrigel (2 h, 37 °C). Then, 100 µl of DMSO were added for 1 h (37 °C) to solubilize the reduced MTT. The optical density was measured on a microplate absorbance reader (Wallac, 1420 Victor2 microplate reader) at 570 nm and a background read at 690 nm was subtracted from this value. Cell viability was normalised to either Wild type or ApdH DMSO control vehicle-treated organoids.

For Resazurin cell viability assay, Resazurin (R&D systems, AR002) was added at a volume equal to 10% of the cell culture media volume and incubated for 4 h at 37 °C. Fluorescence was read using 544 nm excitation and 590 nm emission wavelength. For CRISPR gene targeting assays, cell viability was assessed by firstly removing the fluorescence of puromycin treated, non-transduced (and therefore dead) organoids, followed by normalisation relative to either wild type or ApdH non-targeting guide RNA control transduced organoids.

Overexpression of SRSF1 wild-type/DK mutant. Sry19WT and Sry19DK were generated as above and cloned into the pUltra-GFP vector (Addgene, 24129). The pUltra was a gift from Malcolm Moore.25. Amplification by PCR was performed 5’-Xbal and 3’- BamHI restriction sites, with the addition of a C-terminal FLAG tag. Transduction was performed as already described and cells were expanded and subsequently subjected to cell sorting (FACS) for GFP + cells. For the clonogenicity assay – cells were subjected to EACS and seeded at 500 cells/10 µl BME drop in Y27632, and cells were counted 5 days following FACS sorting.

Real-time quantitative polymerase chain reaction (qPCR). Gene expression analysis was achieved via qPCR of mRNA. Total RNA was extracted using the RNeasy Mini Kit (Qagen, 74106) followed by DNase treatment (Invitrogen, AM1996) and first-strand cDNA synthesis using 500 ng -1 µg RNA (Quantitas, 90548 100). qPCR was performed on 1/10 diluted cDNA using SYBR Select Master Mix (Applied Biosystems, 4472920) according to the manufacturer’s instructions on the CFX Connect Real-Time System (Bio Rad). Gene-specific oligonucleotides used are listed in (Supplementary Data 14). Ct-values were normalized to β-Actin, GAPDH or 18sRNA Ct-values, or the geometric mean of a combination of these loading controls. The delta-delta Ct method was used in order to calculate the relative fold change in gene expression of samples.

Reverse transcription polymerase chain reaction (RT-PCR) analysis of splicing isoforms. RNA extraction and first-strand cDNA synthesis using 500 ng RNA were performed as previously described. A PCR reaction was then performed with gene-specific oligonucleotides (Supplementary Data 14) using Phusion High-Fidelity DNA Polymerase (NEB, M0530S) following the manufacturer’s instructions using 5 µl of 1/10 diluted cDNA. PCR products were then separated by electrophoresis on a 1% agarose gel and fragment sizes were analysed using ImageJ.26. GAPDH RT-PCR was performed for loading normalisation. Slicing isoform changes were calculated by generating a ratio of expression of each respective isoform in each condition.

CRISPR screen target validation experiments. For validation of individual genes arising from the CRISPR screen, the most effective guide RNA (based on Z-score) for each gene was used to transduce single cell organoids. Non-Targeting2 (5’-GCTTTCAGGCGTTGAC-3’), Srsf1 (5’-CGGCTCTGGAGAA CTCAGCA-3’), Sfb3 (5’-TTTTAGATGGCCAGGGTACG-3’) and Ddx10 (5’-AATATCACCCTAGCAGAC-3’) Guide RNAs were individually cloned into the lentiGuide-Puro vector (Addgene) and then transformed into Sib3 chemically competent E. coli (Invitrogen, C737703). Wild type-Cas9 and ApdH-Cas9 expressing organoids, as well as non-CAS9 expressing wild type and ApdH organoids, were transduced with lentivirus containing the specific guide RNA. This was done using 250,000 cells for each of the four cell genotypes. Two days after transduction, cells were selected with puromycin for 3 days. Surviving organs were then seeded to single cells using StemPro Accutase Cell Dissociation Reagent ( Gibco, A1105001) and a clonogenicity assay was performed by plating 10,000 cells per 5 µl drop of Matrigel, with at least 4 Matrigel droplets per condition. Organoid growth media was added, with the addition of R-spondin-1 and 10 µM Y-27632 dihydrodrochloride (ROCK inhibitor, Tocris, 1254) to the Wild type-Cas9 and Wild type-Cas9-null organoids only. Four days after seeding, organoids resulting from single cells were scored and the clonogenic capacity was calculated as a percentage based on the number of cells seeded. Normalised clonogenicity values were then
calculated by first normalising the Cas9-expressing cells with the Cas9-null cells for each genotype, followed by normalising these values to the non-targeting guide RNA control. For the cell viability assay in A431, organoids, 10,000 single cells of either A431

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sequence encoding the Birc4 enzyme with an N-terminal Myc tag (Myc-Birc4D2-MC, Addgene, 74223) was then subcloned into the pU6Rita vector (Addgene, 24129). Myc-Birc4D2-MC was a gift from Kyle Roux. Finally, primers containing the sequences of EcoRV and EcoRI restriction enzymes were used for Kras 4 A and Kras 4B amplification. Primer sequences were Kras 4 A/4B forward: 5'-CTCTCTTGATATCAGTATAAATTCTGTGTTGACAGTCGAGCTTGGTGCG-TAG-3' and reverse: 5'-CTGAGAAGATTCTTATATGAAAGGTATTATTATTTTTACCTA-GAGACCGCAGGCAATCC-3' and Kras 4B reverse: 5'-CTGAGAAGATTCTTATATGAAAGGTATTATTATTTTTACCTA-GAGACCGCAGGCAATCC-3'. For the purpose of a BioID interaction control protein, the gene encoding GFP was cloned into the BirA-pU6Rita vector.

The BioID strand affinity purification experiment was based on a previously published protocol66. In the first instance, 10 cm² dishes were seeded each with 2 x 10⁶ CMT-93 cells (mouse rectal carcinoma), 24 h prior to transfection. CMT-93 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) supplemented with 10% foetal bovine serum, 2 mM glutamine and 1% of penicillin-streptomycin. Four plates were used for each of the three conditions: BirA-GFP, BirA-Kras4A and BirA-Kras4B. The following day, 8 µg of plasmid DNA was used for transfection in each plate using Lipofectamine 2000 (Invitrogen, 11668030) and Opti-MEM (Gibco, 51985026) according to the manufacturer's instructions. 24 h later, growth media was replaced and supplemented with 10% FBS, 5% mouse biotin (Sigma, B4501). Cells were incubated for 24 h, washed in PBS, and then scraped into 500 µL RIPA buffer. Following protein clarification, 25 µL Strepavidin Sepharose slurry (GE Healthcare, 17-5113-01) was washed in RIPA buffer before being added to 1 mL of protein lysate, which was incubated for 6 h at 4°C with rotation. Beads were purified by centrifugation at 10,000 g for 5 min and washed four times with buffer (50 mM TrisCl and 8 M Urea, pH 7.4). On-bead digestion was done with trypsin as previously described68, followed by mass spectrometry using the Fusion Lumos mass spectrometer (Thermo Fisher Scientific). Proteins analysis was done using the MaxQuant-Peruse software36, and proteins were mapped to the mouse Uniprot database. Proteins enriched in the in vitro BioID BirA-Kras4B vs BirA-GFP were determined by first generating a Log2 of the LFQ value 15 and then normalising by Z-score. A student's t-test (p < 0.05) was performed versus the GFP control samples.

Significant hits were also filtered by two additional parameters: >1.5 fold higher levels in Kras 4A/4B expressing cells and at least 2 peptides detected in each of the three biological replicates. Cluster analysis was done using STRING7. Western blotting. Cells were lysed using RIPA buffer (Sigma, R0278) supplemented with 1% of phosphatase and protease inhibitors (Sigma, P0044 and P8340). Protein concentration was calculated using the BCA Protein Assay kit (Pierce). 10 µg of denatured protein lysate was separated by electrophoresis on NuPAGE 4–12% Bis-Tris precast polyacrylamide gels (Invitrogen) and blotted onto 4 µm nitrocellulose membrane (Amersham). Membranes were immersed in blocking solution (5% milk, 0.1% PBS-tween) for 1 h at room temperature, before being incubated in primary antibody at 4°C overnight. Following 3 washes in 0.1% PBS-tween, the membrane was incubated in secondary antibody for 1 h at room temperature. Following PBS-tween wash, Antibody signal was detected by using ECL Plus Western blotting substrate (Pierce, 32132) and visualised using the ImageQuant LAS 4000 (GE Healthcare). Primary antibodies used were: β-actin, 1/5000 (Cell Signalling Technology, 4970), SF2/SRSF1, 1/1000 (Abcam, ab133689), c-Myc, 1/1,000 (Cell Signalling Technology, 9402), Prox1, 1/5000 (Cell Signalling Technology, 4970), SF2/SRSF1, 1/1000 (Cell Signalling Technology, 4970), Myc-Tag (9B11), c-Myc, 1/1,000 (Cell Signalling Technology, 9402), Prox1, 1 µg/mL (R&D systems, AF2727), Ras (27H5), 1/1000 (Cell Signalling Technology, 3339), Myc-BioID2-MCS was a gift from Kyle Roux66,67. Finally, primers containing the Kras 4b morpholine treatment. Organoids were mechanically dissociated by vigorous pipetting. 20–100 fragments were seeded in 10 µL BME in a 24-well plate. 24 h after seeding, media was removed and replaced with fresh BME containing either 5 µM Kras4b vivo-morpholinom (Genetools - GTATAGAGAACTGCTGCA ACAACCT) or 5 µM control vivo-morpholinom (Genetools - CTCCTACTACGC TTAATATTTA). Cell proliferation was assessed after 6 days later by adding 10% Resazurin (R&D systems, AR002). Fluorescence was measured using Victor2 Multilabel Plate Reader (PerkinElmer) as already described.

Organoid invasion assay. The invasion assay was based on a previously described protocol71. Fluoroblok HTS 24 Multiwll Insert System with 8.0 µm Pore High Density PET Membrane (Scientific Laboratory Supplies Ltd., 351157) was used. Invasion was initiated with 50 µg/ml Matrigel solution diluted in cooled DMEM/F12 media containing EGF and Noggin. The organoids were dissociated with TrypLE (Gibco, 12605010), resuspended in DMEM/F12 media containing EGF, Noggin and 5 ng/ml TGFβ1 (PeproTech EC Ltd, 100-21-10) and 3 x 10⁶ cells were seeded in each apical chamber of the Fluoroblock insert, while DMEM/F12 media containing EGF and Noggin was added in the basal chambers. Following 72 h incubation at 37°C, 5% CO₂ atmosphere, cells were stained for 1 h with Calcein AM (Abcam, ab141420) and bottom and top fluorescence was read on a Victor2 Multilabel Plate Reader (PerkinElmer) at wavelengths of 485/535 nm (Ex/Em).
Images were taken at the indicated time points and analysed using ImageJ 2.0. For organoid size (area) determination for the constitutive shRNA model, the minimum number of organoids used to calculate organoid area per biological replicate were: Apo-ΔF 15; APo-20 and KPN: 40. For tracking and size determination in the Tet-On models, the number of organoids measured & tracked per biological repeat were: Wild-type organoids: 5–17; Apo-ΔF organoids: 8–20 and Apo SENS organoids: 5. For the shRNA SRF1/Kras 4B rescue experiments in Apo-ΔF organoids, 14–22 organoids were measured for size per biological replicate. For the human PDO organoids, the number of organoids measured were 33–250. All organoid size experiments had n = 3 biological replicates.

**Human shRNAs.** TRC lentiviral shRNAs (Human SRSF1 ENTREZGENE: 6426.) were purchased from Dharmacon. These were supplied in pLKO.1 vectors.

**shSRSF1-1 Clone ID:** TRC00000011094, Sequence: TTAACCCGGATGTAAG GCAGT.

**Quantification and statistical analysis.** Statistical analyses were performed using GraphPad Prism software (v8.3 GraphPad software, La Jolla, CA, USA) and Microsoft Excel (v2016, Redmond, WA, USA) performing the tests as indicated in the figure legends or main text. Significance levels were calculated according to p < 0.05 (*), p < 0.01(**) and p < 0.001 (***).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The RNAseq data generated in this study have been deposited in the GEO database under accession code GSE196623 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE196623) and are freely available. All data are provided within the article, Supplementary Information and source data.

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Author contributions
The project was conceived and experiments planned by A.E.H. and K.B.M. Experiments were conducted by A.E.H., S.O.P., M.R., P.C., A.B., P.P, N.T.Y and K.B.M. Bioinformatics analysis was conducted by S.A. C.V.B. helped with tissue histology and lentiviral preparation. A.B.C. and A.Y.K conducted the mass spectrometry and analysis. P.F., M.D. and F.D. generated and provided the patient-derived organoids. F.H., I.R.A and J.F.C generated the Srsf1-NRS mouse. All aspects of the study were supervised by K.B.M. The manuscript was prepared by A.E.H. and K.B.M, and all authors read and approved it.

Competing interests
The authors declare no competing interests.

Additional information

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