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## SRSF1 governs progenitor-specific alternative splicing to maintain adult epithelial tissue homeostasis and renewal

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### SUMMARY

Alternative splicing generates distinct mRNA variants and is essential for development, homeostasis, and renewal. Proteins of the serine/arginine (SR)-rich splicing factor family are major splicing regulators that are broadly required for organ development as well as cell and organism viability. However, how these proteins support adult organ function remains largely

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#### AUTHOR CONTRIBUTIONS

Conceptualization, T.Y., O.C., and O.D.K.; Methodology, T.Y., O.C., and O.D.K.; Investigation, T.Y., O.C., H-Y.K., A.D.T., and T.W.; Writing—Original Draft, T.Y. and O.D.K.; Writing, Review & Editing, T.Y., O.C., H-Y.K., A.D.T., T.W., Q.L., M.H.B-H., S.N.F., H-S.J., A.N.B. and O.D.K.; Funding Acquisition, O.D.K.; Supervision, O.D.K.

#### DECLARATION OF INTERESTS

The authors declare no competing interests.

#### INCLUSION AND DIVERSITY

The sex balance of our samples was included as part of our experimental designs. Our laboratories take pride in fostering a diverse and inclusive environment, and we strive to make equitable opportunities for all our trainees. The authors of this paper identify as members of one or more of the following communities: AAPI, Black, Differently Able, Latinx, and LGBTQ+. Also, in this study, one or more of the authors received support from a program designed to increase minority representation.

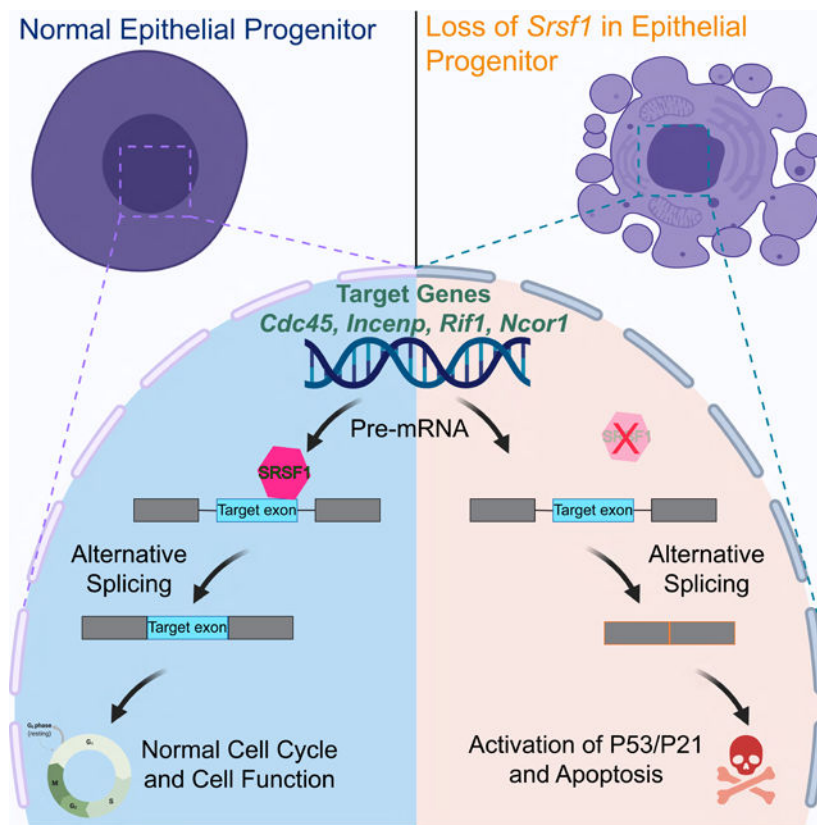
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unknown. Here, we used the continuously growing mouse incisor as a model to dissect the functions of the prototypical SR-family protein SRSF1 during tissue homeostasis and renewal. We identified an SRSF1-governed alternative splicing network that is specifically required for dental proliferation and survival of progenitors but dispensable for the viability of differentiated cells. We also observed a similar progenitor-specific role of SRSF1 in the small intestinal epithelium, indicating a conserved function of SRSF1 across adult epithelial tissues. Thus, our findings define a regulatory mechanism by which SRSF1 specifically controls progenitor-specific alternative splicing events to support adult tissue homeostasis and renewal.

### eTOC Blurp:

Cycling progenitors generate the terminally differentiated cells needed to maintain a functional organ. Yu et al. demonstrate in two self-renewing tissues, the ever-growing mouse incisor and the small intestine, that epithelial progenitors are maintained through proper splicing of several targets of the splice factor SRSF1.

### Graphical Abstract



### Keywords

Alternative splicing; splicing factor; progenitor; tissue homeostasis; tissue renewal; mouse; incisor; intestine

## INTRODUCTION

Alternative splicing generates mRNA variants through selective inclusion or exclusion of exons or introns during pre-mRNA processing. This process is the primary mechanism to increase the diversity of protein isoforms that are structurally and functionally specialized in different tissues to ensure normal development (reviewed in Jin et al., 2018). Over the past decade, transcriptomic analyses performed in individual organs or whole organisms have identified distinct clusters of alternative splicing events that are required in various cell populations as well as at different developmental stages to support cell viability and tissue development (Salomonis et al., 2010; Graveley et al., 2011; Zhang et al., 2014). However, the spatial and temporal regulation of alternative splicing events by splicing regulators remains relatively unexplored. One mechanism for precisely controlling alternative splicing is via specific expression of splicing regulators in certain cell types (Hayakawa-Yano et al., 2017) or at specific developmental stages (Kalsotra et al., 2008). However, because many splicing regulators are broadly expressed (Kalsotra et al., 2008; Yeo et al., 2008; Damianov and Black, 2010), additional mechanisms that confer specificity on these important RNA binding proteins must exist.

Among the principal regulators of alternative splicing are members of the serine/arginine (SR)-rich splicing factor family, which bind to splicing enhancer sequences to promote alternative splicing (reviewed in Shepard and Hertel, 2009). The SR splicing factor family has 12 members in humans that share a conserved SR domain (reviewed in Manley and Krainer, 2010). Many SR splicing factors have been reported to be essential for development. Germline deletion of these factors leads to embryonic lethality or perinatal death (Jumaa et al., 1999; Ding et al., 2004; Xu et al., 2005; Feng et al., 2009), and tissue-specific deletion of SR genes causes organ failure, resulting in growth retardation and often lethality (Xu et al., 2005; Cheng et al., 2016). Despite the indispensable role of SR splicing factors during early development, their functions in adult tissues are largely unknown. With more than 95% of genes undergoing alternative splicing in adult tissues (Pan et al., 2008; Wang et al., 2008), many important questions remain unanswered, including how SR splicing factors maintain tissue homeostasis and renewal, as well as their roles in various cell types, including stem cells, transit-amplifying progenitors and differentiated cells.

We have turned to the continuously growing adult mouse incisor as a model to study the cellular function of SR splicing factors. The constant wear on the incisor as the animal gnaws on food is balanced by rapid replacement of cells and mineralized tissue thanks to a pool of adult stem cells. Therefore, like other high turnover epithelial organs, such as the intestine, the balance between cell proliferation and differentiation must be maintained to ensure proper homeostasis and renewal. An epithelial structure located at the proximal end of the adult mouse incisor called the labial cervical loop (laCL) houses a group of proliferating progenitors that produce multiple cell populations to support the continuously growing incisors throughout the animal's lifetime (Fig. 1A) (reviewed in Yu and Klein, 2020). Dental progenitors reside in the inner enamel epithelium (IEE), giving rise to multiple cell lineages, including those in the stellate reticulum and the outer enamel epithelium (OEE) regions at the proximal end of laCL. IEE progenitors also contribute to

the differentiation and maturation of enamel-secreting ameloblasts and to the formation of a single layer of stratum intermedium cells immediately adjacent to the ameloblasts.

Here, we identify a regulatory mechanism by which the ubiquitously expressed SRSF1 protein specifically controls progenitor-specific alternative splicing events to maintain adult dental tissue homeostasis and renewal. Misregulation of the SRSF1-dependent splicing network activates the p53/p21 pathway, which arrests the cell cycle and impairs cell survival in the adult mouse laCL. Notably, a similar progenitor-specific function of SRSF1 is also observed in the small intestine epithelium, indicating a conserved role of SR splicing factors in regulating alternative splicing in adult epithelial tissues.

## RESULTS

### SRSF1 is required for maintenance of dental progenitors but dispensable in differentiated cell populations in the adult mouse incisor

We found that 11 SR genes were expressed in the adult mouse laCL (Fig. S1A). In addition, our previous analysis suggested that *Srsf3* is highly expressed in the proliferating IEE cells of the laCL (Seidel et al., 2017), pointing to a role for SR splicing factors in proliferative cells in this region. Furthermore, extensive autoregulation and cross-regulation have been reported among different SR splicing factors, especially between SRSF1 and SRSF3 (Ni et al., 2007; Gonçalves et al., 2009). Therefore, to better understand the role of SR splicing factors in adult tissue, we set out to study the cellular and regulatory mechanism of SRSF1 and SRSF3 in regulating adult mouse incisor homeostasis and renewal. We first performed immunostaining with amplification (Fig. 1B, C and Fig. S1B, C) and quantitative RNAscope *in situ* hybridization (Fig. S1D–E) to determine at high resolution the expression patterns of SRSF1 and SRSF3 in the adult mouse laCL. These analyses demonstrated that both SR splicing factors were ubiquitously expressed in the wild-type laCL.

To determine the cellular functions of SR splicing factors in the dental epithelium, we genetically deleted *Srsf1* or *Srsf3* in the adult incisor epithelium by crossing *Srsf1* (*Srsf1<sup>fl/fl</sup>*) (Xu et al., 2005) or *Srsf3* (*Srsf3<sup>fl/fl</sup>*) (Jumaa et al., 1999) conditional alleles with an inducible recombinase expressed in the epithelium, *Keratin 14<sup>CreER</sup>* (*K14<sup>CreER</sup>*) (Li et al., 2000). After two consecutive doses of tamoxifen injection (Fig. 1D), Cre recombinase was induced in the incisor epithelium of *K14<sup>CreER</sup>;*Srsf1<sup>fl/fl</sup>** and *K14<sup>CreER</sup>;*Srsf3<sup>fl/fl</sup>** conditional knockout mutants, and this resulted in a loss of SRSF1 or SRSF3 protein, respectively, shown by immunostaining (Fig. S2A–D) and Fig. S3A, B).

The impact upon deletion of SR genes was apparent two days after tamoxifen injection. Both proliferation and survival of dental progenitors in the *K14<sup>CreER</sup>;*Srsf1<sup>fl/fl</sup>** and *K14<sup>CreER</sup>;*Srsf3<sup>fl/fl</sup>** mutants were strongly disrupted. The number of 5-bromo-2'-deoxyuridine (BrdU<sup>+</sup>) cells was significantly reduced in the Survivin co-labeled proliferating IEE region (Fig. 1E, F, K and Fig. S3C, F). A decrease in BrdU<sup>+</sup> progenitors was also observed in the dental mesenchyme adjacent to IEE in both mutants (Fig. 1E, F, L and Fig. S3C, F), most likely resulting from mesenchymal-epithelial cross-talk. In addition, TUNEL<sup>+</sup> apoptotic cells were increased in the proliferating IEE region (Fig. 1G–J, M and Fig. S3D, G). In the *K14<sup>CreER</sup>;*Srsf3<sup>fl/fl</sup>** mutant laCL, cell death was also detected

in the differentiating and differentiated regions, including pre-ameloblasts and ameloblasts (Fig. 1N and Fig. S3D, G). In contrast, very low numbers of TUNEL<sup>+</sup> cells were observed in the mature region of *K14<sup>CreER</sup>;**Srsf1<sup>fl/fl</sup>* dental epithelium (Fig. 1I, J, N). Furthermore, whereas the expression of the mature ameloblast marker ameloblastin (AMBN) was almost undetectable in *K14<sup>CreER</sup>;**Srsf3<sup>fl/fl</sup>* mutant dental epithelium (Fig. S3E, H), the expression pattern of AMBN remained similar in both control and *K14<sup>CreER</sup>;**Srsf1<sup>fl/fl</sup>* mutant (Fig. 1I, J). Disruption of the ameloblast layer with *Srsf3* deletion resulted in lack of enamel formation (Fig. S3K), compared to the orderly aligned ameloblasts and enamel mineralization in control (Fig. S3I) and *K14<sup>CreER</sup>;**Srsf1<sup>fl/fl</sup>* mutant (Fig. S3J). These data suggest that SRSF1 and SRSF3 have intrinsically different roles in regulating ameloblast maturation.

Six days after tamoxifen injection, the *K14<sup>CreER</sup>;**Srsf3<sup>fl/fl</sup>* mutant epithelium exhibited a much more severe loss of dental epithelial tissue (Fig. 1P) compared to the *K14<sup>CreER</sup>;**Srsf1<sup>fl/fl</sup>* mutants (Fig. 1O), consistent with the larger number of affected cells in the *K14<sup>CreER</sup>;**Srsf3<sup>fl/fl</sup>* mutants. Because of the rapid tissue turnover, the IEE had almost fully recovered in both mutants over this time period. As a result, the regenerated IEE cells pushed the earlier affected tissue to a more distal region. (Fig. 1P, Q). Both *K14<sup>CreER</sup>;**Srsf1<sup>fl/fl</sup>* and *K14<sup>CreER</sup>;**Srsf3<sup>fl/fl</sup>* mutant animals ceased eating and became moribund seven days after Cre induction, potentially due to the requirement for SR proteins in other epithelial organs, which precluded assessment of longer-term consequences of *Srsf1* or *Srsf3* deletions.

These findings indicate that, despite both SR proteins showing ubiquitous expression patterns, they have distinct roles. SRSF3 is required in various cell types regardless of their cycling status. On the other hand, SRSF1 displays a progenitor-specific function, and it is dispensable in differentiated cells. Given the specific role of SRSF1 in regulating incisor progenitors that are required for continual tissue renewal, we next focused on studying the function of SRSF1 and understanding its progenitor-specific regulatory role.

### **SRSF1 regulates alternative splicing events related to maintaining the survival and proliferation of dental progenitors**

To detect alternative splicing events governed by the main splicing regulator SRSF1, we performed bulk RNA-Seq of dissected adult dental epithelium from 3 control and 4 *K14<sup>CreER</sup>;**Srsf1<sup>fl/fl</sup>* mutant mice. Using the JuncBASE (junction based analysis of splicing events) tool (Brooks et al., 2011), we evaluated and classified alternative splicing events with a false discovery rate lower than 0.1. We identified 170 alternative splicing events strongly associated with *Srsf1* deletion with the absolute “percent spliced in” value (PSI) higher than 10% (Fig. 2A, Table S1). Almost half of these SRSF1-regulated alternative splicing events corresponded to cassette exons (CA-exons), also known as exon skipping (Fig. 2A). The pervasive effect of *Srsf1* deletion on CA-exons splicing was further supported by a skew toward p-values that were lower than expected (Fig. 2B). A larger proportion of the CA-exon events identified had PSI lower than -10%, indicating that *Srsf1* mutation was more associated with the skipping of exons rather than inclusion (Fig. 2C). All 170 alternative splicing events were plotted in a heatmap (Fig. 2D), revealing a trend toward

a higher exon inclusion rate in control samples, or in other words, more exon skipping events in *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutants. Together, these data indicate that CA-exon events were highly prevalent with the deletion of *Srsf1*.

To identify direct binding targets of SRSF1, we compared published cross-linking immunoprecipitation (CLIP) tags (Sanford et al., 2008; Pandit et al., 2013; Anczuków et al., 2015) with the bulk RNA-Seq alternative spliced gene list (Table S1). As a result, 52 overlapping targets were found as putative alternatively spliced binding targets of SRSF1 (Table S2). To predict the biological impact of alternatively spliced targets upon *Srsf1* deletion, we analyzed the topological correlation between alternatively spliced SRSF1 binding targets (Table S2) and differentially expressed genes in *Srsf1* mutants (Table S3) using STRING plot (Fig. S4A). 10 highlighted putative SRSF1 targets were identified, grouping into different clusters (Fig. S4A). The rankings of |PSI| of these highlighted targets are shown in the heatmap (Fig. 2D).

Next, we assessed the accuracy of splicing changes from the RNA-Seq data. Then, we quantitatively visualized sequencing reads aligned to gene annotations in control and *Srsf1* mutants with Sashimi plots (Fig. 3). To further validate the PSI of 10 SRSF1 binding targets, we performed reverse transcriptase PCR (RT-PCR) in triplicate, using primers flanking the shaded CA-exons to amplify both isoforms that included and excluded the exon (Fig. 3). All 10 targets showed significant downregulation of their full-length transcripts and upregulation of their truncated transcripts upon *Srsf1* deletion (Fig. 3), consistent with the quantification of the Sashimi plots (Fig. S5).

To determine the biological functions altered by *Srsf1* deletion, the STRING analysis was cross-referenced with Gene Ontology (GO) clusters. GO analysis was performed on the list of SRSF1 translational genes (Table S3) using the DAVID database for functional annotation under the category of “Biological Process”. Genes clustered into two main terms: 1) cell death and DNA damage or 2) cell cycling (Fig. S4B). These assignments were consistent with our observation that cell death was increased, and proliferation was reduced, following *Srsf1* deletion. The biological functions of targets located within the main branch in the STRING plot (Fig. S4A, blue-labeled targets), namely *Cdc45*, *Incepn*, *Rif1*, and *Ncor1*, were closely related to cell survival and cycling, as indicated in the GO clusters. In contrast, the yellow-labeled SRSF1 targets, which were reported as genes functioning during cell adhesion and RNA splicing, were not directly associated with the two main biological GO clusters. In addition, they were located outside of the main topological branch in the STRING plot (Fig. S4A), suggesting they are less likely to be responsible for gene expression or histological changes after *Srsf1* deletion. Because we observed significant changes in cell proliferation and survival upon *Srsf1* deletion, we proceeded to analyze the roles of *Cdc45*, *Incepn*, *Rif1*, and *Ncor1* in maintaining dental tissue homeostasis and supporting tissue renewal.

To address how the ubiquitously expressed SRSF1 protein can regulate distinct functions in specific cell types, we analyzed the expression of its four putative binding targets related to cell survival and cycling in the adult IaCL. All four targets were expressed at significantly higher levels in the proliferating IEE (Fig. 4A–H) compared to the other regions of the

dental epithelium. Among them, CDC45 and INCENP were most strongly enriched in the IEE (Fig. 4A–B', E, F), whereas RIF1 and NCOR1 were mainly expressed in the IEE and in the laCL (Fig. 4C–D', G–H). Since we observed some background staining using the INCENP, RIF1, and NCOR1 antibodies (Fig. 4B–D'), we also performed *in situ* hybridization to confirm the IEE-enriched expression of *Incenp*, *Rif1*, and *Ncor1* in the adult laCL (Fig. S6A–C"). Together, our findings suggest that the progenitor-specific phenotype of SRSF1 arises because it controls alternative splicing of progenitor-enriched targets.

### **SRSF1 plays an essential role in maintaining progenitors but is dispensable in differentiated cells in the adult mouse small intestine**

Our data suggested that SRSF1 plays a specific role in splicing key targets required for dental progenitor function. Given that *in vivo* studies have shown that SRSF1 is required to maintain normal development of various organs at different stages (Xu et al., 2005; Kanadia et al., 2008; Katsuyama et al., 2019), we therefore considered whether the regulation of progenitor function by SRSF1 was conserved across different tissues. To address this question, we tested whether SRSF1 serves a similar progenitor-specific function in another highly regenerative epithelium, the adult mouse small intestine. In the intestinal crypts, lysozyme-secreting differentiated Paneth cells are intermingled between *Lgr5*<sup>+</sup> intestinal stem cells, which divide daily to give rise to transit-amplifying (T-A) progenitors (Barker et al., 2007). T-A cells proliferate and contribute to various secretory and absorptive cell lineages in the villi (reviewed in Zwick et al., 2019). To determine whether SRSF1 also has a distinct cellular function in different intestinal cell types, we deleted *Srsf1* in the adult mouse small intestine by crossing an *Srsf1* conditional allele (*Srsf1*<sup>fl/fl</sup>) (Xu et al., 2005) with the pan-intestinal epithelial *VillinCre*<sup>ER</sup> (Marjou et al., 2004).

As in the adult mouse incisor epithelium, SRSF1 was also expressed ubiquitously in the small intestinal epithelium throughout the crypt-villus axis, suggesting a potential role in intestinal epithelial cell dynamics (Fig. 5A). Three days after two consecutive tamoxifen injections, SRSF1 expression was reduced significantly in the epithelium of both crypts and villi (Fig. 5B). As determined by both TUNEL assay (Fig. 5C, F) and cleaved caspase 3 (CASP3) immunostaining (Fig. 5D, H), increased cell death was observed specifically in the intestinal crypts upon *Srsf1* deletion compared to controls (Fig. 5E, G). TUNEL<sup>+</sup> cells were found among the BrdU<sup>+</sup> progenitor-enriched region in the intestinal crypts in *VillinCre*<sup>ER</sup>;*Srsf1*<sup>fl/fl</sup> mutants, without overlapping with the Paneth cells at the crypt base (Fig. 5I, J). Correspondingly, the number of BrdU<sup>+</sup> cells was reduced (Fig. 5I–K). Neither mature Paneth cells in the crypt (Fig. 5I, J, L), nor the number of differentiated goblet cells in the villi (Fig. 5M) were obviously affected by the loss of SRSF1. Taken together, our results demonstrate that SRSF1 plays a critical role in maintaining the cycling population of T-A cells in intestinal crypts of adult mice, whereas it is not required for the survival of differentiated cells in either crypts or villi. These data indicate a similar progenitor-specific function of SRSF1 in another epithelial tissue distinct from the adult mouse incisor. Interestingly, the expression of the intestinal stem cell marker, OLFM4, was also decreased in the *VillinCre*<sup>ER</sup>;*Srsf1*<sup>fl/fl</sup> compared to control (Fig. 5O, N), suggesting that there might be feedback from progenitors to stem cells in adult mouse intestinal epithelium.



To determine whether these same alternatively spliced putative binding targets of SRSF1 can be identified in the intestinal epithelium, we used fluorescence activated cell sorting (FACS) to purify crypt cells and performed RT-PCR for validation. Similar changes in splicing events of all four genes were observed in *VillinCre<sup>ER</sup>;Srsf1<sup>fl/fl</sup>* mutants, showing a significant downregulation of their full-length transcripts and upregulation of their truncated transcripts upon *Srsf1* deletion (Fig. S6D, E). In addition, we also analyzed the expression of these four putative binding targets in the intestinal epithelium. CDC45, INCENP, RIF1, and NCOR1 were all expressed at significantly higher levels in crypt cells (Fig. 4I–P) compared to villus cells, providing another piece of evidence in a different epithelial system to support the hypothesis that the progenitor-specific phenotype of SRSF1 arises because it controls alternative splicing of progenitor-enriched targets.

### ***Srsf1* mutation activates the p53/p21 pathway, impairing cellular function of both dental and intestinal progenitors**

The functions of all four SRSF1 binding targets have been linked to the p53/p21 pathway via various *in vitro* studies (Battaglia et al., 2010; Datta et al., 2017; Sun et al., 2019; Eke et al., 2020). Given that defects in cell proliferation and cell survival were two main consequences of *Srsf1* deletion in the adult mouse laCL and small intestine, and changes in the p53 pathway are key to both cell cycling and apoptosis (reviewed in Bartek and Lukas, 2001; reviewed in Harris and Levine, 2005), we hypothesized that SRSF1 might control the activity of P53 and P21 through the regulation of the splice variants of *Cdc45*, *Incenp*, *Rif1*, and *Ncor1*. Therefore, we next analyzed the expression of P53 and P21 in the *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* and *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutants.

In both adult mouse laCL and small intestine crypts, P53 was not detectable in the controls (Fig. 6A, A', F), whereas its immunostaining signal was upregulated in IEE progenitors in *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutants (Fig. 6B, B', E) and in the intestinal crypt progenitors in *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutants (Fig. 6G, J). The immunostaining of the p53-dependent cell-cycle regulator P21 was also increased in the similar IEE region in laCLs (Fig. 6C–E), as well as in the intestinal crypts (Fig. 6H–J) upon *Srsf1* deletion. Together, these data suggest that misregulation of alternative splicing events due to loss of SRSF1 activates P53 to stabilize P21, which then inhibits the activity of cyclin-dependent kinase to arrest cell cycle progression. In turn, inappropriate activation of the p53/p21 pathway stimulates apoptosis.

### **AMO-mediated exon exclusion of targeted genes recapitulates loss of *Srsf1***

Although traditionally considered as a splicing regulator, SRSF1 has been shown to regulate other cellular functions outside of alternative splicing (Das et al., 2014). To understand whether the observed activation of p53/p21 is due to mis-splicing of the identified targets or other SRSF1-dependent functions, we reasoned that inducing the specific mis-splicing events without affecting *Srsf1* levels could help identify how SRSF1 is acting. We tested this approach using antisense morpholino oligonucleotides (AMOs), splice-switching molecules that bind to a reverse complementary sequence of a pre-mRNA target. Steric blocking of SRSF1's ability to bind and promote exon-inclusion results in an exon skipping event. This allows for the following possible splice site to be used, thus mimicking the exon skipping event observed in the *Srsf1* mutant (Figure 7A). Primary dental epithelial cells harvested

from the progenitor-rich cervical loop region (Chavez et al., 2014) and primary intestinal cells harvested from organoid crypts (Thorne et al., 2018) were cultured and transfected with a control AMO or an AMO targeting *Cdc45* exon (E)5, *Incenp1* E13, or *Rif1* E32 for 48 hours. Each target-specific AMO induced the respective exon skipping event observed *in vivo* without affecting the levels of *Srsf1*, as observed by RT-PCR (Figure 7B and 7F). Furthermore, immunostaining showed increased activation of both P53 and P21 with the target-specific AMO for both primary dental epithelial cells or intestinal epithelial cells (Figure 7C–E and G–I). These data suggest that a major role of SRSF1 in the incisor is as a splicing regulator of these target genes, which are essential for the survival of highly proliferative progenitor cells.

## DISCUSSION

To maintain tissue homeostasis and regenerative capability, proliferative progenitors must generate various cell populations. Among the mechanisms contributing to the regulation of self-renewal, proliferation, and differentiation of progenitor cells, alternative splicing can play a significant role in producing the complex repertoire of protein isoforms required from embryonic stages to adulthood, as well as under health and disease conditions. In this study, using the adult mouse incisor and small intestine as two fast-cycling *in vivo* model systems, we identified a mechanism by which the ubiquitously expressed SRSF1 splicing factor governs alternative splicing in a progenitor-specific manner. Loss of SRSF1 function resulted in misregulation of alternative splicing events, thus activating p53/p21 signaling and leading to cell cycle arrest and apoptosis of progenitors. Our findings thus reveal an intricate alternative splicing regulatory system in rapidly renewing tissues.

### Progenitor-specific alternative splicing regulation

Previously reported progenitor-specific function of splicing regulators mainly focused on identifying pro-pluripotent splicing events using pre-enriched human/mouse embryonic stem cells or mouse neural stem cells (Atlasi et al., 2008; Hayakawa-Yano et al., 2017). However, many splicing regulators exhibit broad expression patterns (Kalsotra et al., 2008; Yeo et al., 2008; Damianov and Black. 2010), pointing to a need to look beyond stem cells to reveal distinct cellular functions of splicing regulators at the tissue or organ level. In this study, by interrogating cells at various stages of differentiation, our findings identified a key role of SRSF1 in specifically maintaining progenitor proliferation and survival during homeostatic turnover in adult epithelial tissues. Interestingly, instead of having a regionally restricted expression pattern, the master splicing regulator SRSF1 was expressed ubiquitously and yet specifically governed the splicing of genes enriched in the progenitors in the adult mouse laCL. Even though SRSF1 was also expressed in other more differentiated cell types, it was dispensable for their function and survival. To rule out the possibility that the dental progenitor-specific defects observed in *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutant laCL were indirectly introduced from potential disruption in other cell types in which *K14<sup>CreER</sup>* was activated, we further included *Shh<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* to allow more restricted deletion of *Srsf1* in lower IEE in the incisor cervical loops (Fig. S7). In this knockout system, we observed similar disrupted function specifically in dental progenitors (Fig. S7E–L), as well as similar changes in alternative splicing of 4 SRSF1 targets (Fig. S7M). These data support the notion

that the splicing defects were caused by a specific impact on dental progenitors upon *Srsf1* deletion.

In adult organs that require rapid cell turnover to counterbalance constant tissue loss, stem cells and progenitors play an essential role in ensuring these systems' normal function. In recent years, numerous studies have identified cell plasticity in committed cell types. When high levels of cell death occur in the progenitor pool upon acute tissue injury, differentiated cells can undergo dedifferentiation or transdifferentiation to replenish the loss in the progenitor niche to repair the tissue (reviewed in Varga and Greten, 2017; reviewed in McKinley et al., 2020). For example, in the small intestine, cellular plasticity has been observed in numerous immature and committed cell types, which can dedifferentiate to restore intestinal homeostasis upon ablation of *Lgr5+* intestinal stem cells (Jadhav et al., 2017; Yan et al., 2017). Therefore, maintaining ubiquitous expression of SRSF1 might be essential for the dedifferentiation or transdifferentiation of cells to quickly restore progenitor cells and support tissue injury repair.

### ***In vivo* analysis of the cellular mechanism of alternative splicing regulation during adult tissue homeostasis and renewal**

Studies performed in human or mouse embryonic stem cells, as well as in cancer cells, have provided important information about how alternative splicing events change in response to different stages or under disease conditions (Salomonis et al., 2010; Xu et al., 2018; reviewed in Qi et al., 2020). However, whether all *in vitro* data are representative or able to recapitulate *in vivo* conditions remains debatable. For example, cross-regulation and antagonism between SRSF1 and SRSF3 have been reported through *in vitro* studies (Jumaa et al., 1997; Gonçalves et al., 2009; Sun et al., 2010). In this study, we did not observe a strong inter-regulatory function between these two SR proteins in the adult mouse laCL. Although 60% of alternative splicing events regulated by SRSF3 corresponded to CA-exons (Fig. S3L), similar to SRSF1 (Fig. 2A), only three overlapping genes were governed by SRSF1 and SRSF3, and no antagonistic regulation was identified (data not shown). A slight down-regulation of RNA expression of *Srsf3* was detected in the *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutant laCL (Fig. S3M). However, due to cell heterogeneity in the dental tissue, no solid conclusion can be drawn regarding whether *Srsf1* and *Srsf3* can compensate for each other in distinct cell types. Compared to the relatively simpler *in vitro* culture systems that have one or a small number of cell types, single cell RNA Seq will be needed to answer this question in the incisor laCL. It will be important to consider tissue-specific differences or potential variations between *in vitro* and *in vivo* studies in future work.

Furthermore, cellular responses were observed *in vivo* upon *Srsf1* deletion in laCLs that could not be detected through *in vitro* analysis. Consistent with mGFP expression reporting Cre-mediated recombination throughout the *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutant laCL, the expression of SRSF1 in IEE cells, SI cells, pre-ameloblasts, and ameloblasts was almost undetectable one to two days after tamoxifen injection (1–2 dpi), whereas SRSF1 was still highly expressed in cells in the stellate reticulum and OEE regions (Fig. S2B–B", F, G). SRSF1 expression started to decrease in the stellate reticulum and OEE at 3 dpi, and the reduction became evident at 4 dpi (Fig. S2H, I). Recovery of SRSF1 expression in the IEE initiated at

around 4 dpi (Fig. S2I). By 5 dpi, a more pronounced expression of SRSF1 was observed in the IEE (Fig. S2J). These data together suggest that the half-life of SRSF1 varies in different cell types in the adult mouse laCL, which could inform further understanding of the cellular regulation of alternative splicing during injury repair.

Together, our findings define a mechanism by which SRSF1-dependent alternative splicing events regulate homeostasis in adult tissues, revealing a paradigm for cell-type specific splicing factor function. We identified a conserved mechanism of SRSF1 function in both the incisor and small intestine, suggesting a fundamental role of this splicing factor during development and posing new questions for future research into the regulation of alternative splicing in tissue regeneration and injury repair.

## LIMITATIONS OF THE STUDY

A major limitation of conducting *in vivo* analysis on alternative splicing regulation is the difficulty in obtaining sufficient protein. For example, more than 250 mice (500 laCLs) of relatively similar age would be needed to obtain enough SRSF1 protein to perform RNA immunoprecipitation, such as CLIP. Therefore, we cross-referenced to published CLIP data from *in vitro* culture of HEK293T cells, mouse embryo fibroblasts, MCF-10A cells, and HeLa cells (Sanford et al., 2008; Pandit et al., 2013; Anczuków et al., 2015). Next, the alternatively spliced transcripts of all 10 putative binding targets (Table S2) were validated through RT-PCR, strongly suggesting that they were directly regulated by SRSF1. Thus, immediate splicing responses were recorded one day after tamoxifen injection. Using AMOs, we validated the effects of the particular exon exclusion for *Cdc45*, *Incenp1*, and *Rif1* in the activation of p53/p21. However, inducing an exon inclusion event as seen in *Ncor1* is much more challenging, given the frequency of sequences involved in splicing found within the large intronic regions. Nevertheless, the robust activation of P53 and P21 upon the exon skipping events in *Cdc45*, *Incenp1*, and *Rif1* gives us confidence to suggest important roles for these targets in the proper maintenance of adult epithelial progenitors.

## STAR Methods

### Resource availability

**Lead Contact and Materials Availability**—Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr. Ophir Klein (Ophir.Klein@ucsf.edu). All unique/stable reagents generated in this study are available by contacting the Lead Contact, but we may require a completed Materials Transfer Agreement if there is potential for commercial application.

### Experimental models and subject details

**Animals**—*K14Cre<sup>ER</sup>* (Li et al., 2000), *R26<sup>mT/mG</sup>* (Muzumdar et al., 2007), *VillinCre<sup>ER</sup>* (Marjou et al., 2004) and *ShhCre<sup>ER</sup>* (Harfe et al., 2004), as well as conditional alleles of *Srsf1<sup>fl/fl</sup>* (Xu et al., 2005) and *Srsf3<sup>fl/fl</sup>* (Jumaa et al., 1999) mice were housed and genotyped as previously published. Both male and female adult mutant and Cre-negative littermate control mice at 8–10 weeks of age were used for experiments. All experimental procedures involving mice were approved by the Institutional Animal Care and Use Committee

(IACUC) and Laboratory Animal Resource Center (LARC) at the University of California, San Francisco, and the mice were handled in accordance with the principles and procedures of the Guide for the Care and Use of Laboratory Animals under the approved protocol AN180876.

## Method Details

**Tamoxifen injection**—Tamoxifen (Sigma T5648) was prepared in corn oil at the concentration of 25 mg/ml. Both control and mutant mice, were injected intraperitoneally with 2.5 mg tamoxifen per day per 20 g body weight for two successive days to induce Cre-mediated gene deletion. Animals were euthanized two to six days after tamoxifen injection.

**BrdU injection**—5-bromo-2'-deoxyuridine (BrdU) (Sigma B9285) was prepared in D-PBS (w/o calcium and magnesium salts) at the concentration of 10 mg/ml. Both control and mutant mice were injected intraperitoneally with a single dose (1 mg) per 20g body weight. Mice were euthanized 30 minutes after BrdU injection. The number of BrdU positive cells was counted for quantitative analyses.

## Tissue preparation

**Lower mandible:** Adult mice were euthanized and perfused with 4% paraformaldehyde (PFA) in PBS. Lower mandibles were dissected from control,  $K14^{CreER};Srsf1^{fl/fl}$  and  $K14^{CreER};Srsf3^{fl/fl}$  mutants, and post-fixed in 4% PFA overnight at 4°C. Mandibles were decalcified with 0.5M EDTA for 2 weeks, dehydrated in 70% EtOH, embedded in paraffin and sectioned at 6  $\mu$ m.

**Small intestine:** Adult control and  $Villin^{CreER};Srsf1^{fl/fl}$  mutants were euthanized and perfused with 4% PFA in PBS. The first 10 cm of small intestinal tissues were perfusion-fixed with 4% PFA and post-fixed in 4% PFA overnight at 4°C. Tissues were then dehydrated in 70% EtOH, embedded in paraffin and sectioned at 6  $\mu$ m.

**Immunofluorescence assays**—Immunofluorescence staining was performed as previously described (Hu et al., 2017). Additionally, for staining of CASP3, CDC45, NCOR1, NICD, OLFM4, P53, P21, RIF1, SRSF1 and SRSF3, primary antibodies were detected by biotinylated secondary antibodies, and then sequentially amplified using VECTASTAIN Elite ABC HRP Kit (Vector Laboratories) and Tyramide Signal Amplification (Perkin Elmer). All images were acquired with a Leica TCS SP8 X confocal microscope.

**TUNEL assay**—TUNEL assay was performed using the *In Situ* Cell Death Detection Kit, TMR red (Roche), according to the manufacturer's instructions. The number of TUNEL positive nuclei was counted for quantitative analyses.

**Hematoxylin & Eosin (H&E) staining**—Adult mouse mandibles were dissected and fixed in 4% PFA overnight at 4°C. Samples were washed, decalcified for 2 weeks and then

dehydrated in 70% EtOH. Mandibles were embedded in paraffin and sectioned at 6  $\mu\text{m}$ . H&E staining was performed as previously described (Hu et al., 2017).

**Bulk RNA-Seq and analysis**—3 control and 4 *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutant mice were used for RNA extraction. Each RNA sample was isolated from 2 laCLs using the RNeasy mini kit (QIAGEN 74104) according to the manufacturer's instructions. RNA quality was assessed using a Pico Chip on an Agilent 2100 Bioanalyzer (Agilent Technologies). Samples with RNA-integrity (RIN) scores > 8.0 were used by the UCSF Functional Genomics Core. Bulk RNA-Seq was performed on Illumina HiSeq4000 system according to the manufacturer's instructions with paired-end 100bp sequencing type. For each condition, RNA-Seq libraries were prepared with the TruSeq mRNA Kit. RNA-Seq reads were mapped to the reference mouse genome (GRCm38.78). Kallisto (0.44.0) was used to count the number of reads aligned to each transcript. Differential expression analysis was performed using the DESeq2 (v1.16.1) to identify genes that were significantly up- or down-regulated in the *Srsf1* mutant laCL (n=4), relative to controls (n=3). The p-values were adjusted to account for multiple testing with the false discovery rate (FDR) < 0.1. The bulk RNA-Seq data reported in this study will be uploaded upon acceptance of the paper.

**Bulk RNA-Seq alternative splicing analysis.**—JuncBASE (Brooks et al., 2011) was used to identify and quantify AS events. The length-normalized counts for each AS inclusion or exclusion event in each of the sample was obtained from the last step of JuncBASE. The counts were adapted to a format amenable for DRIMSeq (Nowicka and Robinson, 2016), a statistical testing framework. DRIMSeq was used to assign p-values to each AS event. Q-Q plots and volcano plots were output from JuncBASE. The heatmap was plotted using a custom script written with the matplotlib package in python. Sashimi plots for quantitative visualization of sequencing reads aligned to gene annotations were generated using ggsashimi (Garrido-Martin et al., 2018). To identify the potential binding targets of SRSF1, the output list of alternative spliced events from JuncBASE was overlapped with published CLIP tags from 4 papers (Sanford et al., 2008; Pandit et al., 2013; Anczuków et al., 2015), respectively using Venn diagram. 52 overlapping targets were found as putative alternatively spliced binding targets of SRSF1, shown in Table S2. Venn diagrams were made using the matplotlib venn package in python.

**Reverse transcription PCR (RT-PCR)**—LaCL samples were isolated as previously described (Chavez et al., 2014). DAPI<sup>low</sup>EpCAM<sup>+</sup>CD44<sup>+</sup> small intestine crypt epithelial cells were obtained through flow cytometry as previously described (Nusse et al., 2018). RNA samples obtained from control and *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutant laCL 1 day after tamoxifen injection, as well as control and *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* mutant small intestine crypt epithelial cells 2 days after tamoxifen injection were isolated using GenCatch™ Total RNA miniprep kit (Epoch Life Science 1660050). RNA samples were then reverse-transcribed to cDNA using SensiFAST™ cDNA Synthesis Kit (BioLine BIO-65053). Both steps were performed according to the manufacturer's instructions. RT-PCR were performed using iTAQ CYBR green (Biorad) with the following conditions: 5 minutes at 95°C and 35 cycles of amplification (30 seconds at 95°C, 50 seconds at 60°C and 1 minute at 72°C). The relative changes of transcript expression between controls and *Srsf1* mutants were

analyzed by ImageJ as previously described (Yu et al., 2017). In brief, band intensities were first converted into histograms, from which the area under the curve can be measured using the Wand tool and the relative expression between control and mutant samples were calculated. Percent spliced in changes (PSI) were calculated using the band intensity of the inclusion event divided by the band intensity of the inclusion event plus band intensity of the exclusion (i.e., inclusion event/(inclusion + exclusion event)). The primer sets used are listed in the key resources table. All measurements were normalized to GAPDH.

### Quantification and statistical analysis

**Statistical analysis**—All of the data points are biological replicates and were replicated at least three times. Bar charts indicate the mean of samples and error bars represent mean  $\pm$  SD (standard deviation). *P* values were derived from unpaired two tail Student's *t* tests, assuming equal variance (\**P* < 0.05, \*\* *P* < 0.001, \*\*\* *P* < 0.001 and n.s. not significant).

**ImageJ image analysis**—Colocalization of SRSF1, SRSF3, CDC45, RIF1, NCOR1 with DAPI was measured using the ImageJ plugin “Colocalization”. Percentage of the immunostained area was detected as previously described in Hu et al., 2017.

**Data and Code Availability**—RNA-seq data have been deposited at GEO and are publicly available as of the date of publication. Accession numbers are listed in the key resources table.

This manuscript did not generate new code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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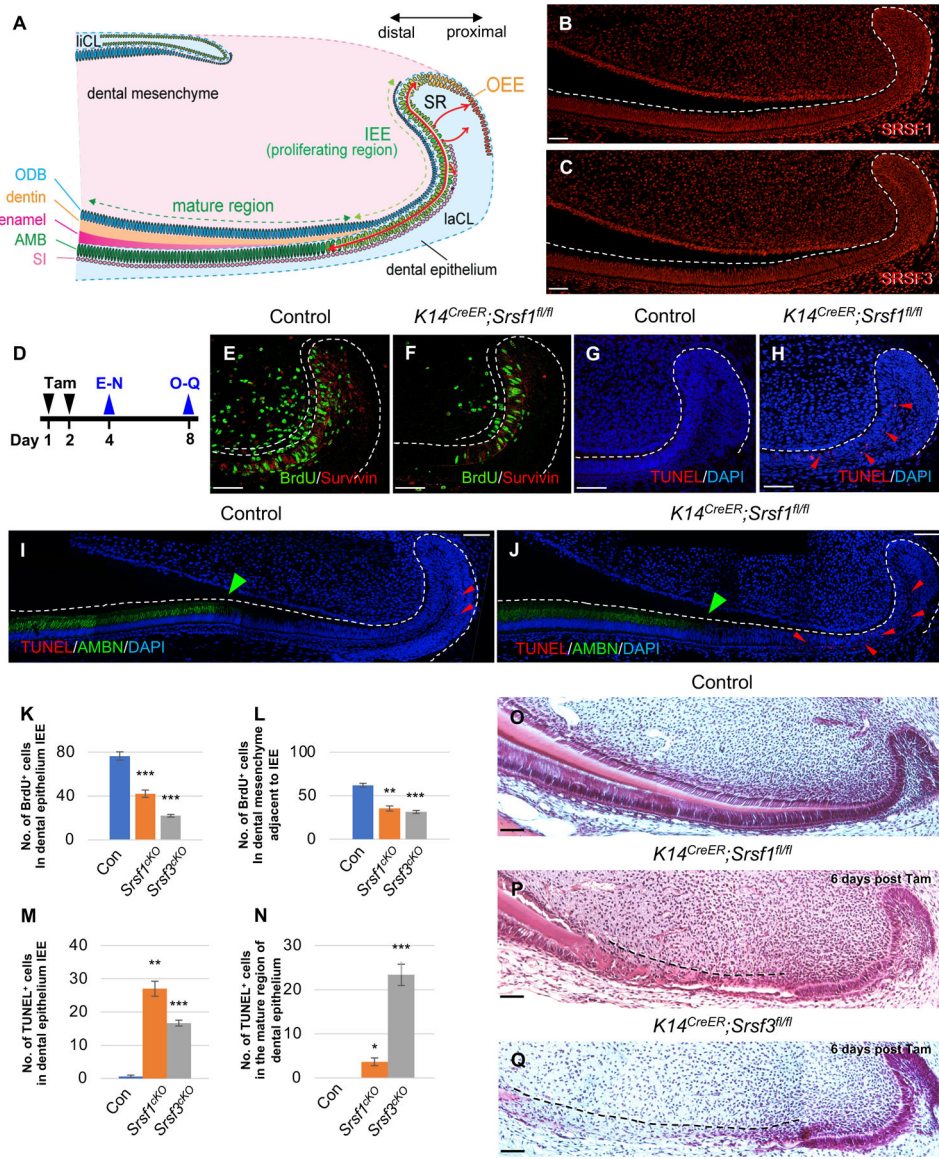


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**Highlights:**

- SRSF1 governs epithelial progenitor homeostasis.
- Mis-splicing of SRSF1 targets leads to epithelial progenitor cell death.
- *Cdc45*, *Incep*, *Rif1*, and *Ncor1* are important targets of SRSF1.



**Figure 1. Deleting *Srsf1* specifically disrupts the function of dental progenitors in the adult mouse laCL.**

(A) Schematic diagram of the labial cervical loop (laCL), located at the proximal mouse incisor. Dental progenitors residing in the proliferating inner enamel epithelium (IEE) drive the formation and differentiation of a variety of cell types (red arrow lines), including the enamel-secreting ameloblasts (AMB), stellate intermedium (SI) cells and other cell populations that reside in outer enamel epithelium (OEE) and stellate reticulum (SR). Dental mesenchyme-derived odontoblasts (ODB) produce dentin at the lingual surface of enamel. liCL, lingual cervical loop. Light green dashed line with arrowheads marks the IEE region. Dark green dashed line with arrowheads marks the mature region.

(B and C) Immunostaining of SRSF1 (B) and SRSF3 (C) in the laCL.

(D) Timeline depicting tamoxifen (Tam)-induced Cre activation (black arrowheads) and sample collection (blue arrowheads).

(E and F) BrdU/Survivin co-labelling in the control (E) and *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (F) laCL. White dashed lines with arrowheads outline the reduced region of proliferation in *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (F), compared to control (E).

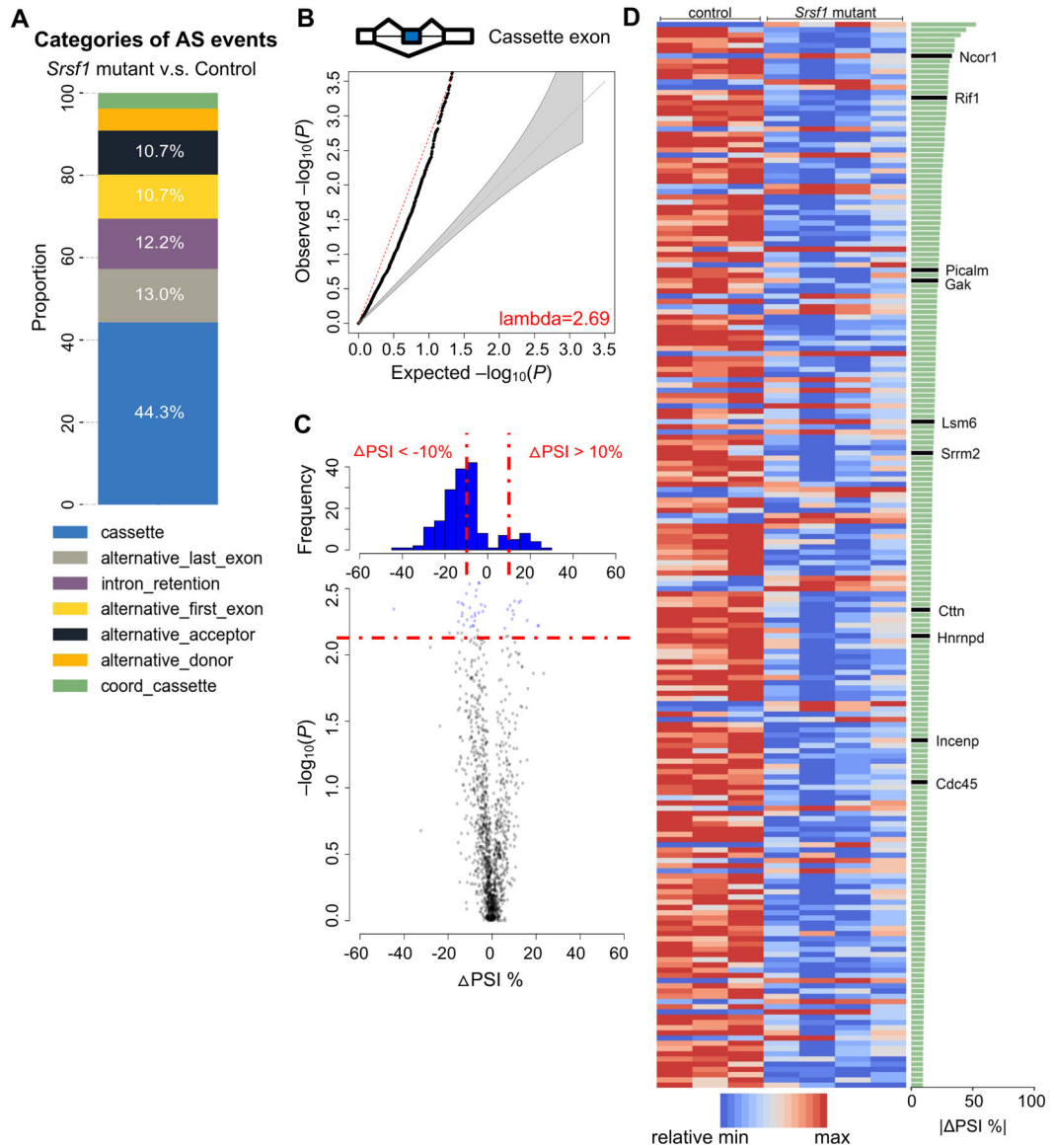
(G and H) TUNEL staining in the control (G) and *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (H) laCL. Increased cell death (red arrowheads) can be detected specifically in the proliferating IEE upon *Srsf1* deletion (H).

(I and J) Co-labelling of TUNEL and immunostaining of AMBN in control (I) and *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (J) laCL. Normal initiation and pattern of AMBN expression (green arrowheads) in both control (I) and *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (J). Increased cell death (red arrowheads) detected specifically in the proliferating IEE and no TUNEL<sup>+</sup> cells detected in the mature region with AMBN expression upon *Srsf1* deletion (J). Images were stitched manually, selecting a common feature appearing in the overlapping region. A black background was applied to the top edges of the image to make up the gaps due to image stitching.

(K-N) Quantification of the number of BrdU<sup>+</sup> cells in dental epithelium (K) and mesenchyme adjacent to IEE (L), TUNEL<sup>+</sup> cells in the proliferating IEE region (M) and mature region (N) in the control (*Con*), *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (*Srsf1<sup>cKO</sup>*) and *K14<sup>CreER</sup>;Srsf3<sup>fl/fl</sup>* (*Srsf3<sup>cKO</sup>*) laCL. All quantitative data are shown as mean ± SD (\**P* < 0.05, \*\* *P* < 0.001 and \*\*\* *P* < 0.001).

(O-Q) H&E staining of the control (O), *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (P) and *K14<sup>CreER</sup>;Srsf3<sup>fl/fl</sup>* (Q) laCL 6 days after Tamoxifen treatment. Black dashed lines with arrowheads outline the region of tissue damage in (P) and (Q) upon *Srsf1* (P) and *Srsf3* (Q) deletion, respectively. Images were stitched manually, selecting a common feature appearing in the overlapping region.

Dashed lines outline laCL. Representative images and quantitative data are shown. Scale bar: 50 μm. See also Figures S1, S2, S3, and S7.



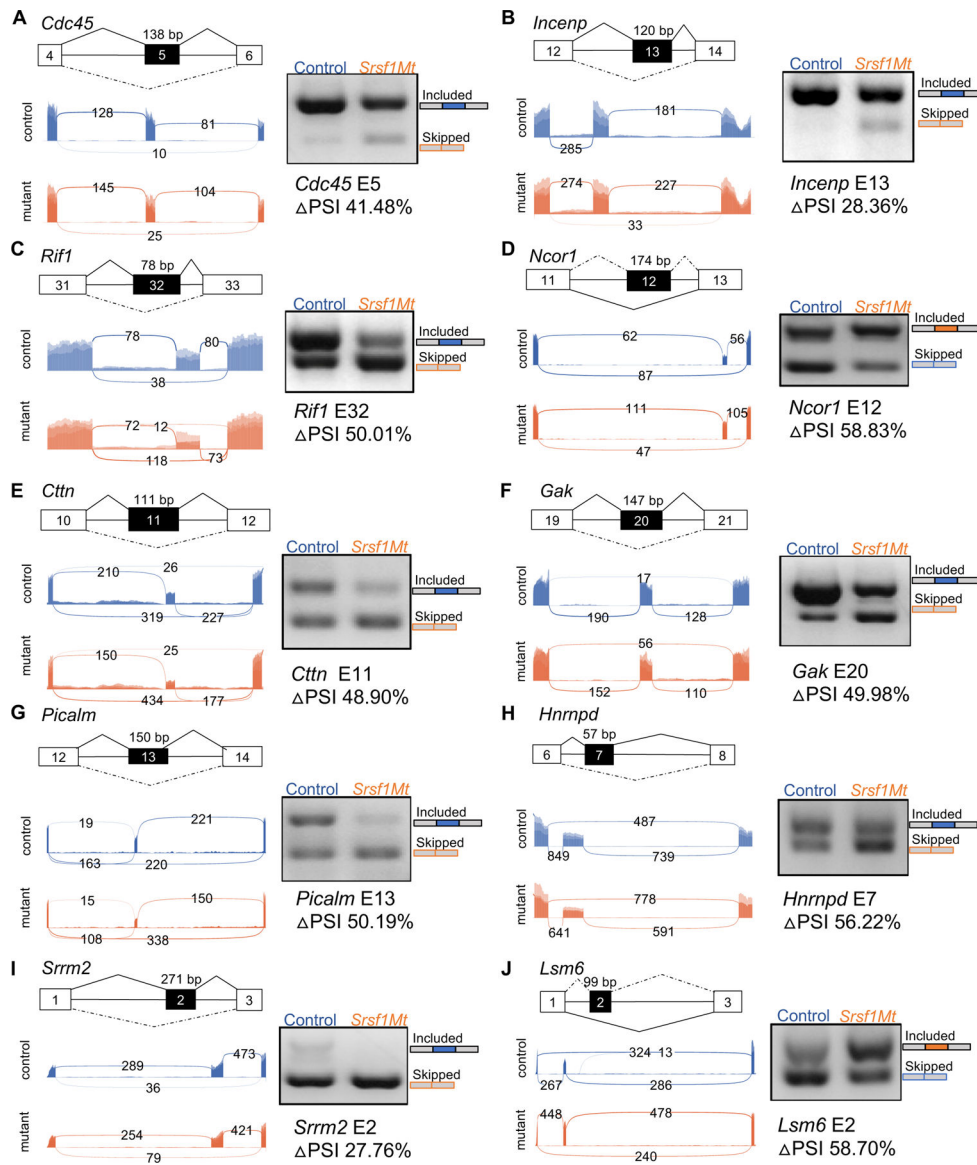
**Figure 2. SRSF1 governs a large number of alternative splicing events in the adult mouse laCL.**

(A) Categories of alternative splicing events significantly associated with *Srsf1* deletion versus control adult mouse laCL from bulk RNA-Seq.

(B) Q-Q plot analysis of cassette exon (CA-exon) splicing events.

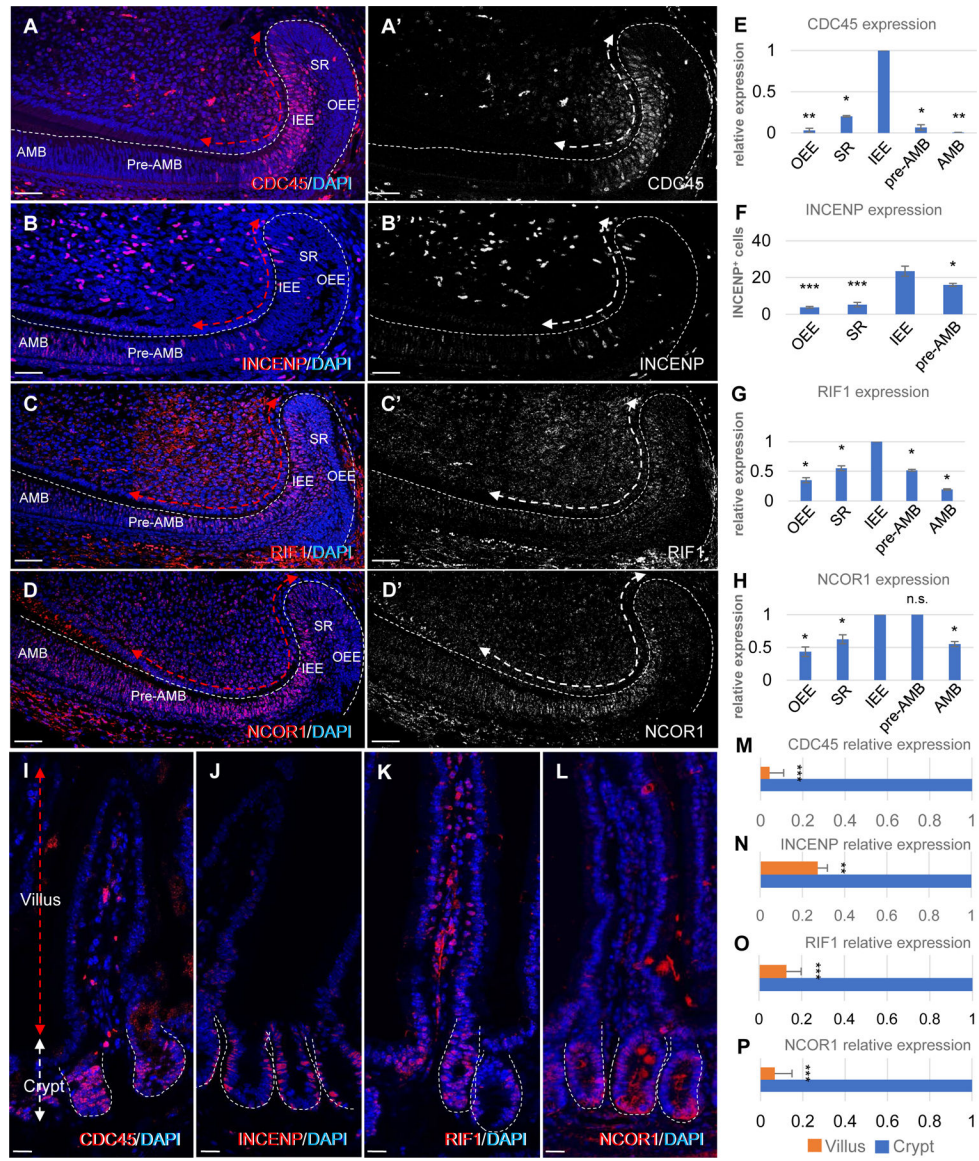
(C) Volcano plot analysis of alternative splicing events upon deletion of *Srsf1*. Red dashed/dotted lines indicate a large magnitude of splicing change percent spliced in  $|\text{PSI}| \geq 10\%$  (top) or a significant p-value (bottom).

(D) Heatmap of up- and downregulated alternative splicing events between the control and *K14<sup>CreER</sup>, Srsf1<sup>f/f</sup>* (*Srsf1* mutant) laCL. The genes that contain splicing events that are highly likely to be directly regulated by SRSF1 are labeled and highlighted in black. Red or blue color indicates up or downregulation of each splicing event, respectively. See also Figure S4.



**Figure 3. Deletion of *Srsf1* alters alternative splicing of its binding targets.**

(A–J) SRSF1-regulated CA-exons events of *Cdc45* (A), *Incenp* (B), *Rif1* (C), *Ncor1* (D), *Ctnn* (E), *Gak* (F), *Picalm* (G), *Hnrnpd* (H), *Srm2* (I) and *Lsm6* (J) in the mouse incisor laCL. For each gene, exons and introns are indicated in scaled diagrams. CA-exons are shaded. Included exons transcripts are spliced with solid lines and skipped transcripts are spliced with dashed lines. Exon number and the size of CA-exons are labeled in and on top of the diagram, respectively. Sashimi plots (below the scaled diagrams) were used to quantitatively visualize splice junctions in control and *Srsf1* mutants (*Srsf1* Mt). Sashimi plots are depicted under the gene structure with each peak representing read coverage of each exon. RT-PCR validation of each gene is shown at the right side of the panel, with representative gel images and quantification of the PSI expression in *Srsf1* mutants. The representative cropped gel images are shown from 3 biological replicates. All quantitative data are shown as mean  $\pm$  SD (\* $P < 0.05$  and \*\* $P < 0.001$ ). See also Figure S5.



**Figure 4. SRSF1 binding targets are highly expressed in proliferating IEE and crypts in the laCL and the small intestine, respectively.**

(A, B, C, and D) Immunostaining of CDC45 (A), INCENP (B), RIF1 (C) and NCOR1 (D) in the laCL. Red dashed lines with arrowheads mark higher expression regions of the proteins. INCENP antibody staining showed strong background in ameloblasts, making it hard to quantify in AMB region.

(A', B', C', and D') Greyscales of protein expression of CDC45 (A'), INCENP (B'), RIF1 (C') and NCOR1 (D') in the laCL. White dashed lines with arrowheads mark higher expression regions of the proteins.

(E-H) Quantification of regional protein expression of CDC45 (E), INCENP (F), RIF1 (G) and NCOR1 (H).

OEE, outer enamel epithelium. SR, stellate reticulum. IEE, inner enamel epithelium. pre-AMB, pre-ameloblast. AMB, ameloblast.

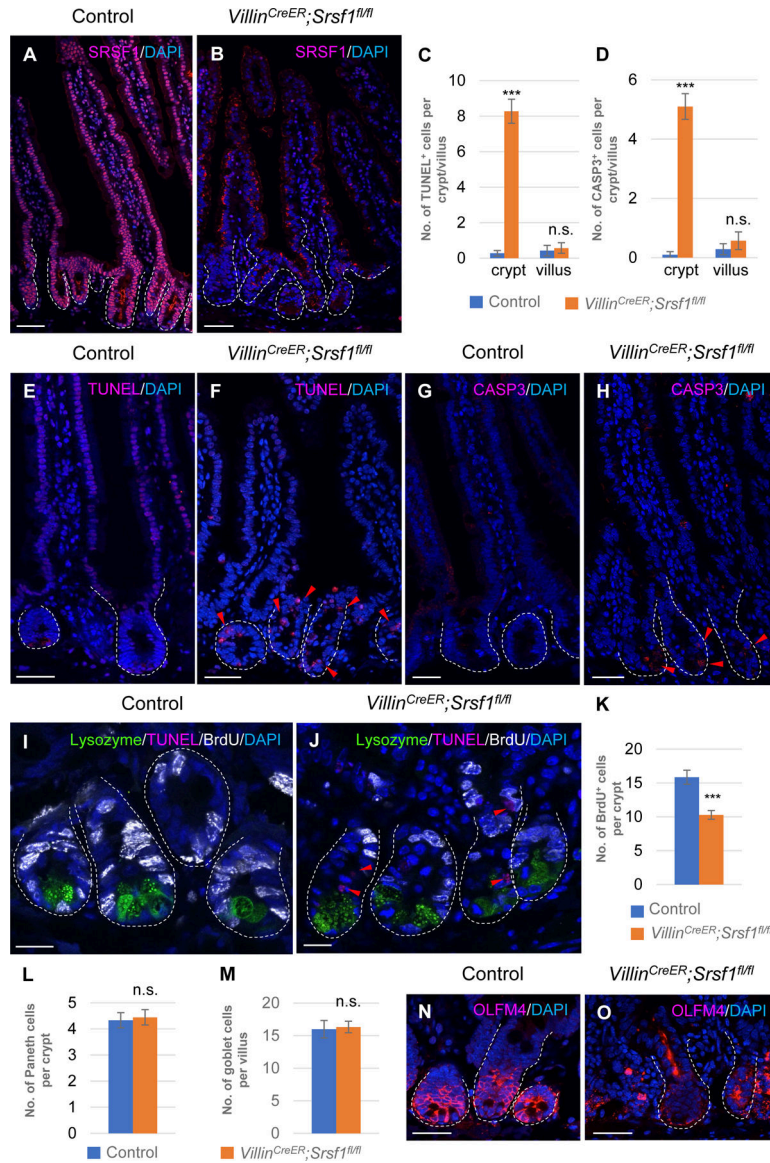


Dashed lines outline laCL. Images were stitched manually, selecting a common feature appearing in the overlapping region.

**(I-L)** Immunostaining of CDC45 (I), INCENP (J), RIF1 (K) and NCOR1 (L) in the small intestine. Red dashed lines with arrowheads mark the villus region. White dashed lines with arrowheads mark the crypt region. Dashed lines outline small intestine crypts. INCENP antibody showed strong background, therefore in situ hybridization data was added in Figure S6 (A-A’’).

**(M-P)** Quantification of regional protein expression of CDC45 (M), INCENP (N), RIF1 (O), and NCOR1 (P).

Representative images and quantitative data are shown. All quantitative data are shown as mean  $\pm$  SD (\* $P < 0.05$ , \*\* $P < 0.001$ , \*\*\* $P < 0.001$  and n.s. not significant). Scale bar represents 50  $\mu\text{m}$  in A-D’; 20  $\mu\text{m}$  in I-L. See also Figure S6.



**Figure 5. SRSF1 is required for maintaining the survival of intestinal progenitors, while it is dispensable for the differentiated cell populations.**

(A and B) Immunostaining of SRSF1 in control (A) and *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (B) the mouse small intestine. Significantly reduced expression of SRSF1 upon intestinal epithelial deletion of *Srsf1* (B). Images were stitched manually, selecting a common feature appearing in the overlapping region.

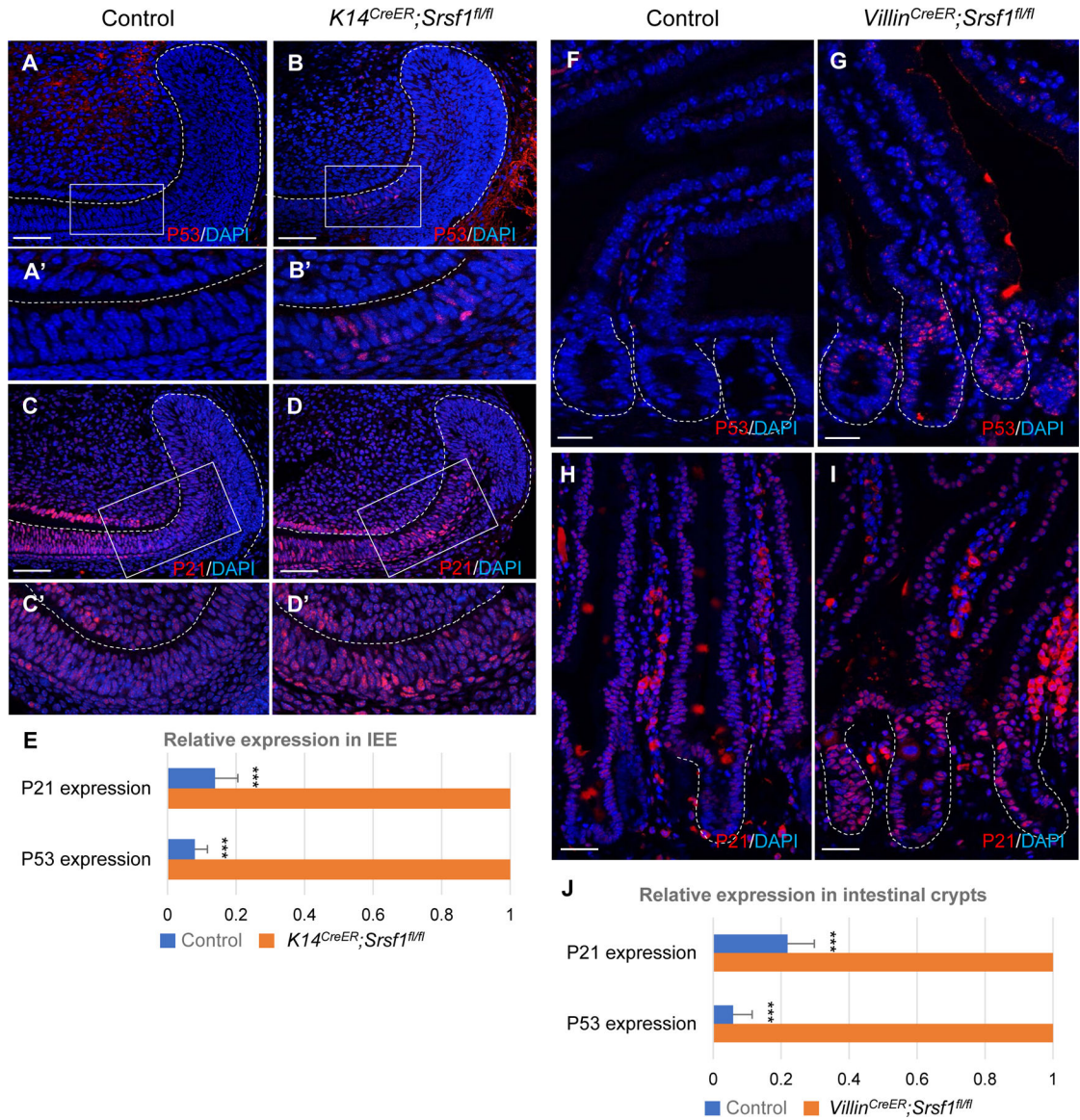
(C and D) Quantification of the number of TUNEL<sup>+</sup> cells (C) and CASP3<sup>+</sup> cells (D) in the crypts and villi, respectively.

(E-H) Detection of cell apoptosis using TUNEL assay (E-F) and immunostaining of CASP3 (G-H) in control (E and G) and *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (F and H) small intestine. Increased apoptotic cells (red arrowheads) can be detected in *Srsf1* mutants (F and H).

(I and J) Triple-labelling TUNEL (red) with immunostaining of Paneth cell marker, Lysozyme (green) and BrdU (grey) in control (I) and *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (J) adult mouse small intestine. Increased cell death (red arrowheads) can be detected specifically in the

proliferating region enriched in BrdU<sup>+</sup> (grey) cells (J). The number of Paneth cells (green) shows no difference upon *Srsf1* deletion in the small intestine (J), compared to control (I). **(K-M)** Number of BrdU<sup>+</sup> cells (K), Paneth cells (L) in the crypt, and goblet cells (M) per crypt-villus unit in control and *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* small intestine. All quantitative data are shown as mean  $\pm$  SD (\*\**P* < 0.001 and n.s. not significant).

**(N and O)** Immunostaining of OLFM4 in control (N) and *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (O) the mouse small intestine. Significantly reduced expression of OLFM4 upon *Srsf1* deletion (O). Dashed lines outline small intestine crypts. Representative images and quantitative data are shown. Scale bar represents 50  $\mu$ m in A and B, E-H, N and O; 20  $\mu$ m in I and J.



**Figure 6. Deletion of *Srsf1* activates p53/p21 pathway, leading to impaired function of both dental and intestinal progenitors.**

(A-B') Immunostaining of P53 in the control (A) and *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (B) laCL. Enlarged images of boxed regions are displayed in (A') and (B'), respectively.

(C-D') Immunostaining of P21 in the control (C) and *K14<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (D) laCL. Enlarged images of boxed regions are displayed in (C') and (D'), respectively.

(E) Quantification of regional P21 and P53 expression in the IEE.

(F and G) Immunostaining of P53 in the control (F) and *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (G) small intestine.

(H and I) Immunostaining of P21 in the control (H) and *Villin<sup>CreER</sup>;Srsf1<sup>fl/fl</sup>* (I) small intestine.

(J) Quantification of regional P21 and P53 expression in the small intestine.

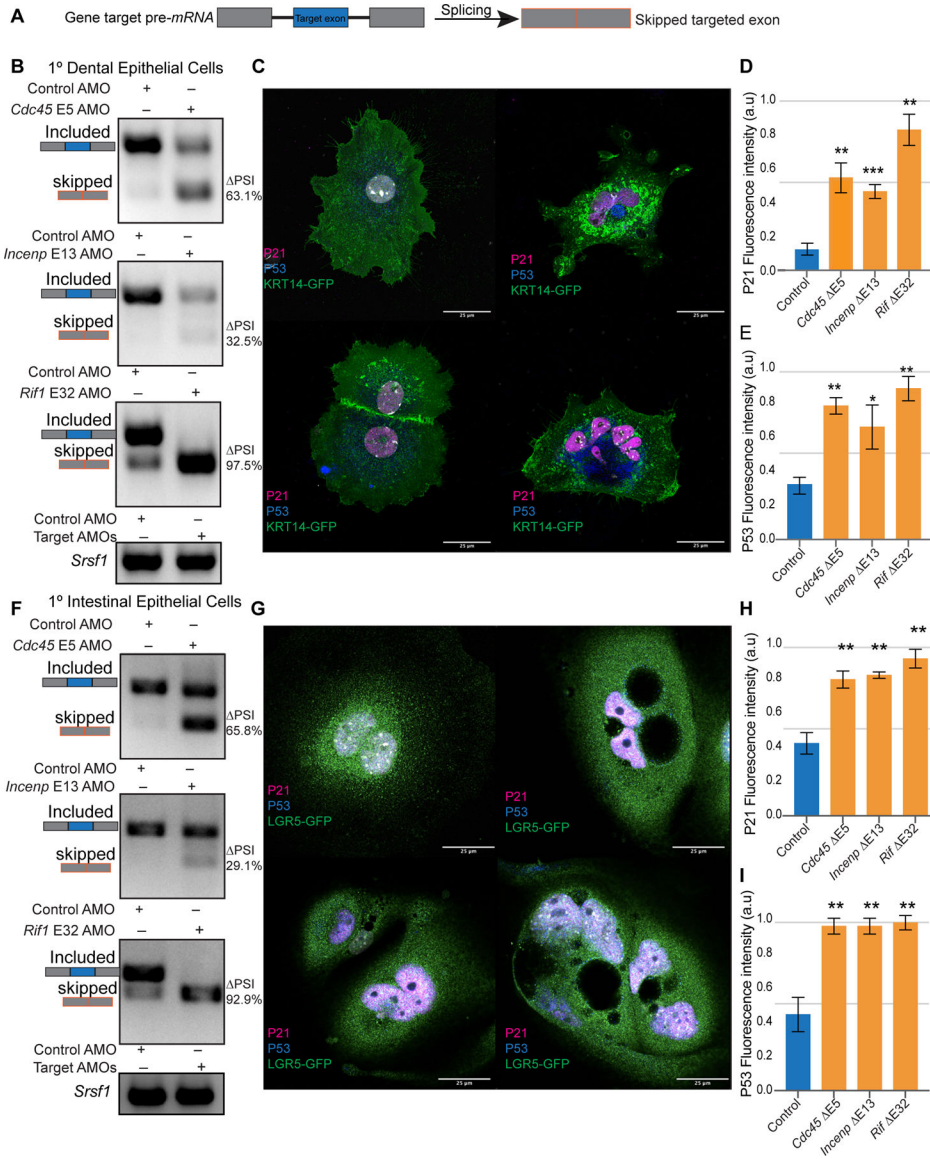
Dashed lines outline laCL and small intestine crypts. Representative images and quantitative data are shown. All quantitative data are shown as mean  $\pm$  SD (\*\*\*)  $P < 0.001$ ). Scale bar: 50  $\mu\text{m}$ .

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**Figure 7. AMO-mediated exon exclusion of targeted genes recapitulates loss of Srsf1 phenotype**  
**(A)** Schematic of AMO-mediated exon skipping event.  
**(B)** Representative RT-PCR of control and target gene AMO transfections in primary dental epithelial cells and resulting PSI upon transfection.  
**(C)** Representative immunocytochemistry staining for P21 (magenta) and P53 (blue) in control AMO and individual target gene AMOs treated primary dental epithelial cells as marked by KRT14-mGFP (green). E denotes the exon skipped upon AMO transfection.  
**(D-E)** Quantification of regional P21 and P53 expression, respectively, in primary dental epithelial cells.  
**(F)** Representative RT-PCR of control AMO and target gene AMO transfections in primary intestinal epithelial cells and resulting PSI upon transfection.

(G) Representative immunocytochemistry staining for P21(magenta) and P53 (blue) in control AMO and individual target gene AMOs treated primary intestinal epithelial cells as marked by LGR5-mGFP (green). E denotes the exon skipped upon AMO transfection. (H-I) Quantification of regional P21 and P53 expression, respectively, in primary intestinal epithelial cells.

All quantitative data are shown as mean  $\pm$  SD (\*\*\*)  $P < 0.001$ . Scale bar: 25  $\mu$ m.

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit Anti-Ameloblastin (M-300) antibody	Santa Cruz Biotechnology	Cat# sc-50534, RRID:AB_2226393
Rat Anti-BrdU antibody	Abcam	Cat# ab6326, RRID:AB_305426
Rabbit Anti-Caspase-3, phospho (Cleaved Asp175) antibody	Cell Signaling Technology	Cat# 8202, RRID:AB_1658166
Rabbit Anti-Survivin antibody	Cell Signaling Technology	Cat# 2808, RRID:AB_2063948
Rabbit Anti-Phospho-Histone H3 antibody	Cell Signaling Technology	Cat# 9706, RRID:AB_331748
Rabbit Anti-Cleaved Notch1 (Val1744) (D3B8) antibody	Cell Signaling Technology	Cat# 4147, RRID:AB_2153348
Rabbit Anti-Olfm4 (D6Y5A) XP® antibody	Cell Signaling Technology	Cat# 39141, RRID:AB_2650511
Rabbit Anti-Cdc45 (D7G6) antibody	Cell Signaling Technology	Cat# 11881, RRID:AB_2715569
Mouse Anti-P21 antibody	BD Biosciences	Cat# 556431, RRID:AB_396415
Mouse Anti-Incnp antibody (B-4)	Santa Cruz Biotechnology	Cat# sc-376514, RRID:AB_11149761
Mouse Anti-Rif1 antibody (B-4)	Santa Cruz Biotechnology	Cat# Sc-515573
Rabbit Anti-P53 antibody	Proteintech	Cat# 10442-1-AP, RRID:AB_2206609
Rabbit Anti-SRSF1 antibody	Abcam	Cat# ab38017, RRID:AB_882519
Rabbit Anti-SRSF3 antibody	ProSci	Cat# 8179
Chicken Anti- Green Fluorescent Protein (GFP) Antibody	Aves Lab	Cat# GFP-1020, RRID:AB_10000240
Chemicals, peptides, and recombinant proteins		
Control AMO	GeneTools Inc.	Stand. Control Oligo Cat# (PCO-100)
Oligo: <i>mCdc45</i> E5 ACTGAAAAACAGGACGAGGCACTGT	GeneTools Inc.	Custom Oligo Cat# CO-300
Oligo: <i>Incnp</i> E13 TGTCCCCACCTAGTCGCACCTGTTG	GeneTools Inc.	Custom Oligo Cat# CO-300
Oligo: <i>Rif1</i> E32 GGTATTATGCTAGACAGAAGTAGA	GeneTools Inc.	Custom Oligo Cat# CO-300
Endo-Porter (PEG)	GeneTools Inc.	Cat# OT-EP-PEG-1
Critical commercial assays		
RNAscope 2.5 HD Red detection kit	ACD	Cat#322350
<i>In Situ</i> Cell Death Detection Kit, TMR red	Roche	Cat# 12156792910
TSA Cyanine 3 Tyramide Reagent Pack	Perkin Elmer	Cat# AT704B001EA
VECTASTAIN Elite ABC HRP Kit (Peroxidase, Standard)	Vector Laboratories	Cat # PK-6100
Deposited Data		
Raw and processed RNAseq data	This paper	GEO: GSE193492
Oligonucleotides		
Primer: <i>Srsf1<sup>fl/fl</sup></i> Forward GGGACTAATGTGGGAAGAATG Primer: <i>Srsf1<sup>fl/fl</sup></i> Reverse AACCTAACTATTGCTCCCATCTG	IDT	Xu et al., 2005
Primer: <i>Srsr31<sup>fl/fl</sup></i> Forward GCGCAGGTACTTGAGAGA Primer: <i>Srsr3<sup>fl/fl</sup></i> Reverse CCCTTTTATTGGTCACTGA	IDT	Jumaa et al., 1999
Primer: <i>Cre<sup>ER</sup></i> Forward TGGAGATCTTCGACATGCTG Primer: <i>Cre<sup>ER</sup></i> Reverse CACGTTCTTCGACATTCATGC	IDT	JAX



REAGENT or RESOURCE	SOURCE	IDENTIFIER
Primer: <i>Cdc45</i> Forward TGTGTGCTTGCAAGATCCTC Primer: <i>Cdc45</i> Reverse ACCCATCACCGTCACTGTCT	IDT	This Paper
Primer: <i>Ctnn</i> Forward AGAGAAGCACGAATCCCAGA Primer: <i>Ctnn</i> Reverse TCTTGTCATCCGATCC	IDT	This Paper
Primer: <i>Gak</i> Forward GGAAGAGCAGCAGGACATTC Primer: <i>Gak</i> Reverse GTAGCCCCAGGAGATCAACA	IDT	This Paper
Primer: <i>GAPDH</i> Forward CATGGCCTCCGTGTTCTTA Primer: <i>GAPDH</i> Reverse CCTGCTCACACCTTCTTGAT	IDT	This Paper
Primer: <i>Hnmpd</i> Forward GACGCCAGTAAGAACGAGGA Primer: <i>Hnmpd</i> Reverse TCAGGTGTGTCTGGAGAAAGG	IDT	This Paper
Primer: <i>Incnp</i> Forward CGTGAGAGGGTGGAACAGAT Primer: <i>Incnp</i> Reverse CTGCTCCTCCGTTCCTGTT	IDT	This Paper
Primer: <i>Lsm6</i> Forward TGTGAGCGTTCTGGATCCG Primer: <i>Lsm6</i> Reverse GGATGAACGCATCTCCGACT	IDT	This Paper
Primer: <i>Ncor1</i> Forward GCTCTTTACCAACGGCACAT Primer: <i>Ncor1</i> Reverse GTTGGTCGTGTTGTTGGAAG	IDT	This Paper
Primer: <i>Picalm</i> Forward CTCAGCAGGGGGAATAATGA Primer: <i>Picalm</i> Reverse AGAATGTGGCTGTGCAACTG	IDT	This Paper
Primer: <i>Rif1</i> Forward GTGTCTCGTTTGACAGATCCA Primer: <i>Rif1</i> Reverse GCGTACTCAAATCCCAATG	IDT	This Paper
Primer: <i>Srm2</i> Forward ACCTCCCTGTTTGACAGTCG Primer: <i>Srm2</i> Reverse GGGAGGCTCAGGAGCTATTT	IDT	This Paper
Software and algorithms		
ImageJ	Schneider et al., 2012	<a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a>
ImageJ Colocalization plugin	Image J	<a href="https://imagej.nih.gov/ij/plugins/colocalization.html">https://imagej.nih.gov/ij/plugins/colocalization.html</a>
SPRING Viewer	Klein Lab	<a href="https://github.com/AllonKleinLab/SPRING_de">https://github.com/AllonKleinLab/SPRING_de</a>
Prism 9	Graphpad software, Inc	N/A
Adobe illustrator 2020	Adobe Computer software company	N/A