Supplemental Data

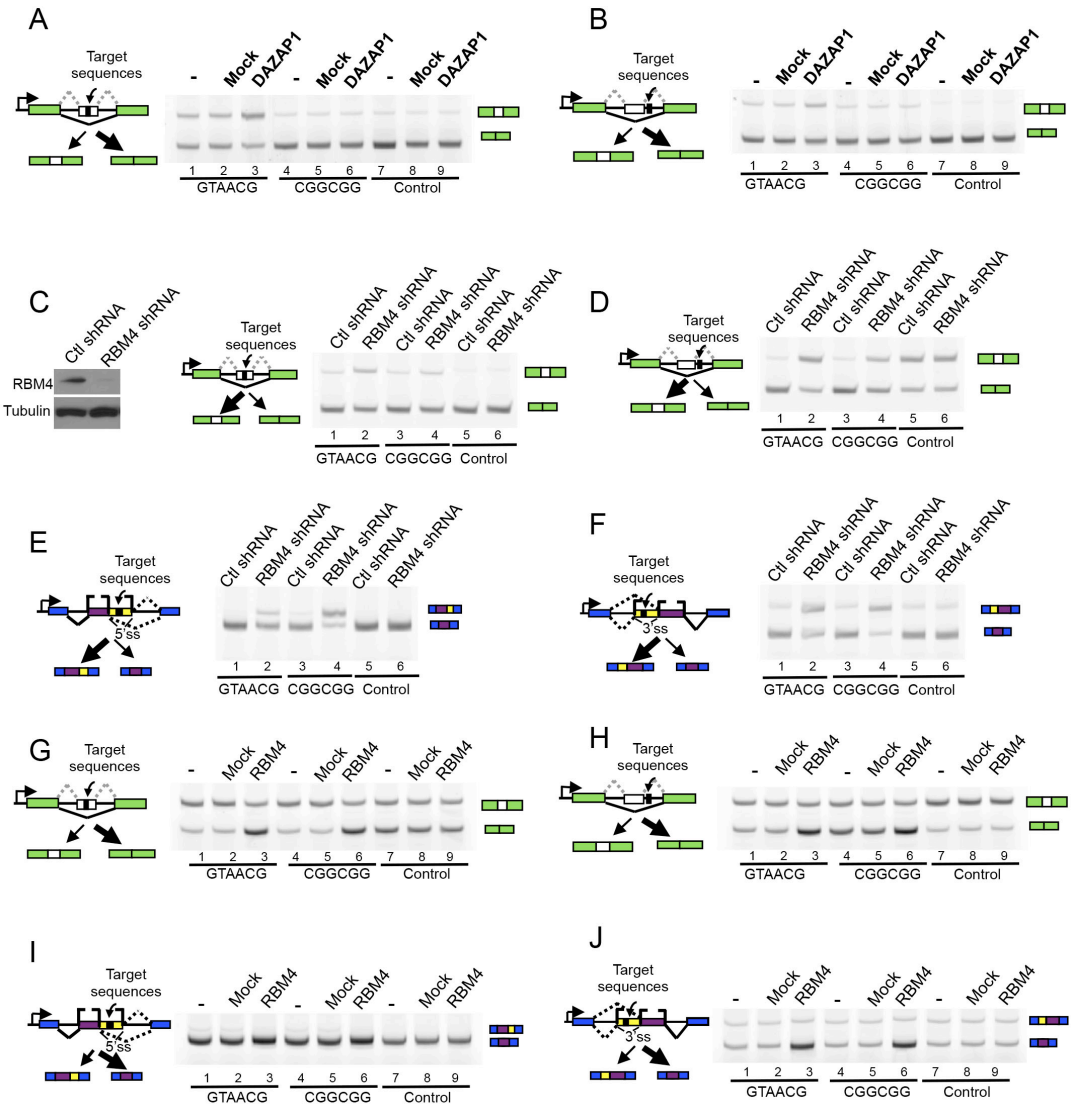

Figure S1, related to Figure 1. Regulation of various AS events by RBM4 or DAZAP1. (**A-B**) The binding sequences (GTAACG or CGGCGG) of RBM4 and a neutral control sequence (GAATTG) were inserted inside a cassette exon of pGZ3 (**A**) or at the downstream intron of a cassette exon of pZW2C (**B**). The resulting reporters and DAZAP1 expressing vector were co-transfected into 293T cells, and splicing changes were examined with RT-PCR one day after transfection. Mock transfections with vector only were used as control. **(C-D)** The same set of RBM4 binding sequences and a neutral control were inserted inside (C) or at the downstream (D) of the alternative exon of pGZ3 reporter. The resulting reporters were transfected into 293T cells that were stably

transfected with RBM4-targeting shRNA or control shRNA. Splicing changes were examined with RT-PCR 24 hours after transfection. **(E-F)** The same set of binding sequences of RBM4 and a control were inserted between the alternative 5′ ss of pEZ1B reporter (**E**) or the alternative 3′ ss of pEZ2F reporter (**F**), and the splicing assays were carried out as described in panel C. **(G-H)** The same set of RBM4 binding sites and control sequence were inserted inside (G) or at the downstream (H) of the alternative exon of pGZ3 reporter. The resulting reporters and RBM4 expressing vector were co-transfected into HeLa cells, and splicing changes were examined with RT-PCR one day after transfection. Mock transfections with vector only were used as control. **(I-J)** The same binding sequences of RBM4 and a control sequence were inserted between the alternative 5′ ss of pEZ1B reporter (**I**) or the alternative 3′ ss of pEZ2F reporter (**J**). All experiments were repeated at least twice and a representative gel was shown for each reporter.

Table S1, related to Figure 2 Lists of each RBM4-regulated alternative splicing event.

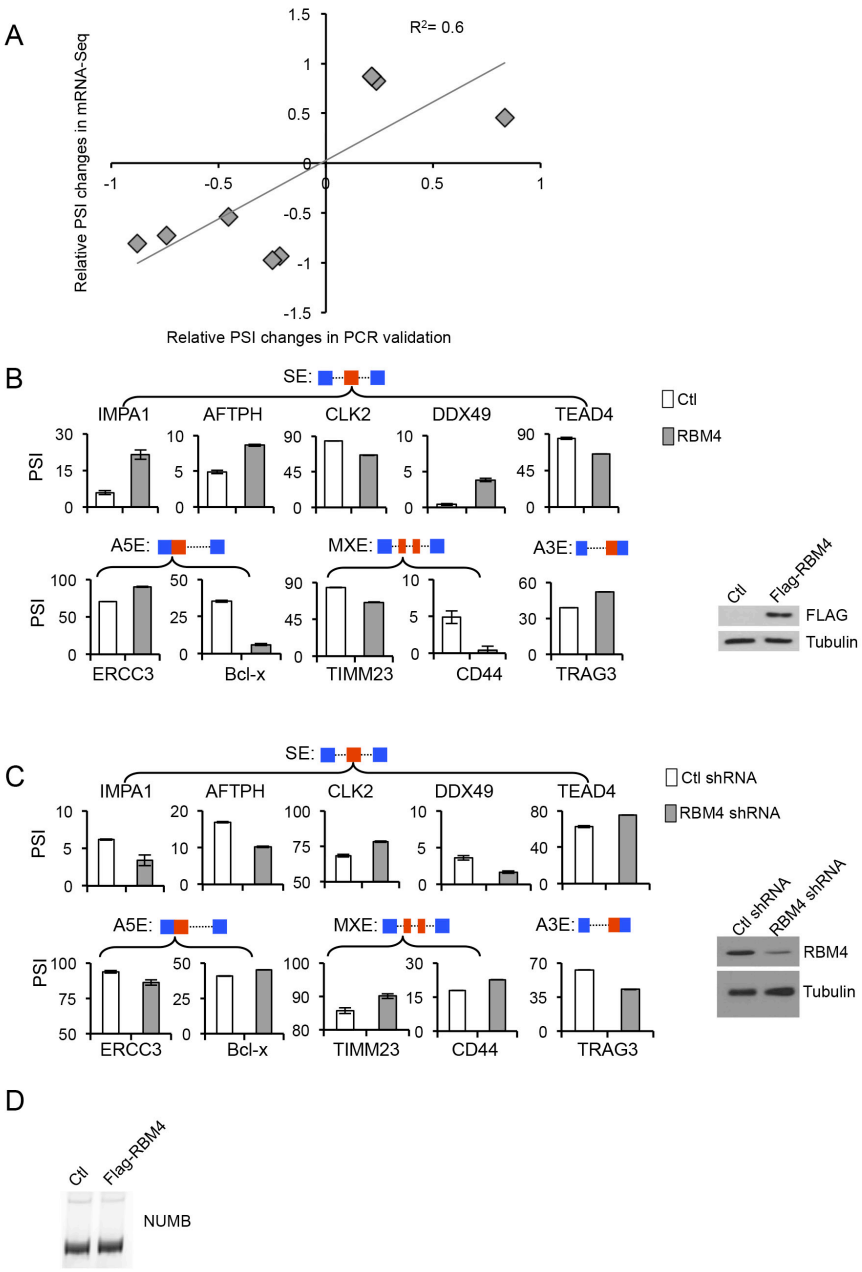

Figure S2, related to Figure 2. Validation of different RBM4-regulated AS events. (**A**) Correlation between the relative changes in PSI values observed by RNA-seq vs RT-PCR confirmation. (**B**) Total RNAs from HeLa cells transfected with expression vectors of RBM4 or control were analyzed by semi-quantitative RT-PCR. The means +/- SD of PSI values from triplicate experiments were plotted. The over-expression of RBM4 was confirmed by western blot at right panel. (**C**) Validation of RBM4-regulated AS events

using RNAi of RBM4. Total RNAs from A549 cells stably transfected with shRNA vector for RBM4 or control were analyzed by semi-quantitative RT-PCR. The means +/- SD of PSI values from triplicate experiments were plotted. The knock-down of RBM4 was confirmed by western blot at right panel. (**D**) The splicing of exon 9 of NUMB gene was examined in H157 cells stably transfected with RBM4 expression vector or empty vector. Total RNAs were isolated from the transfected cells and the splicing of NUMB was examined with RT-PCR. The primer sequences are as follows: NUMB-fwd: CTC CCT GTG CTC ACA GAT CA; NUMB-rev: CGG ACG CTC TTA GAC ACC TC.

Table S2, related to Figure 2. Lists of RBM4-regulated expression level changes of target genes (see supplementary excel file).

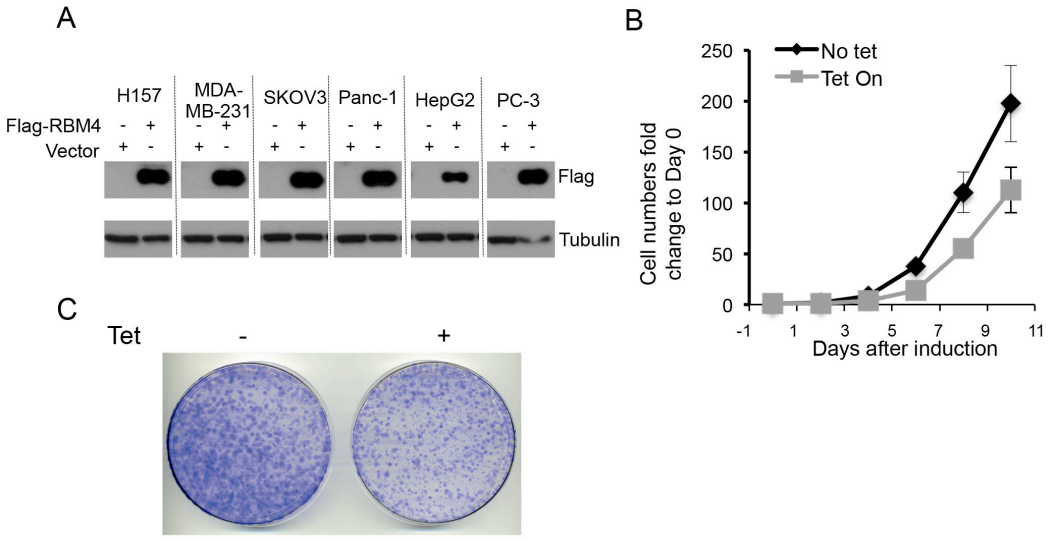

Figure S3, related to Figure 3. RBM4 inhibits cell growth and colony formation in various cells. (**A**) The over-expression of RBM4 in multiple cancer cell lines is confirmed by with western blot. (**B**) The 293-FlpIn T-REx cells stably transfected with RBM4 expression vector were induced by tetracycline, and the effects of RBM4 on cell growth was measured by counting cell numbers. The means +/- SD of relative cell numbers from triplicate experiments were plotted. (**C**) The same 293-FlpIn T-REx cells stably expressing RBM4 are analyzed by colony formation assay.

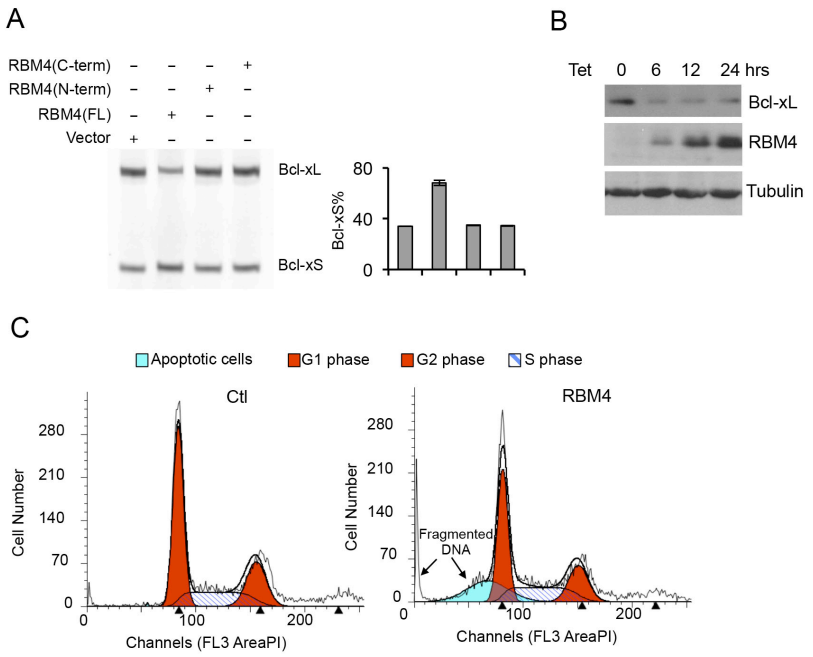

Figure S4, related to Figure 4. RBM4 regulates Bcl-x splicing and induces apoptosis. (**A**) The effects of different fragments of RBM4 on Bcl-x splicing were measured with RT-PCR. H157 cells stably transfected with expression vectors of full-length RBM4, N-terminal or C-terminal fragments of RBM4 were used for RNA purification and RT-PCR. The means +/- SD of percentage of Bcl-xS from triplicate experiments were plotted. **(B)** The expression of RBM4 in 293 FlpIn T-REx cells was induced by tetracycline, and the cells were collected at 6, 12, and 24 hours after induction. The expressions of RBM4 and Bcl-x were examined with western blot. **(C)** The apoptotic cells were examined with flow cytometry to count 10,000 cells that satisfied gating criteria. Left: H157 cells transfected with control vector; Right: H157 cells transfected with RBM4 vector. Different cell populations were quantified based on DNA contents using ModFit software to discern cells with 2N (G1), S-phase, and 4N (G2 and M), and fragmented DNA (apoptotic cells, indicated by arrow).

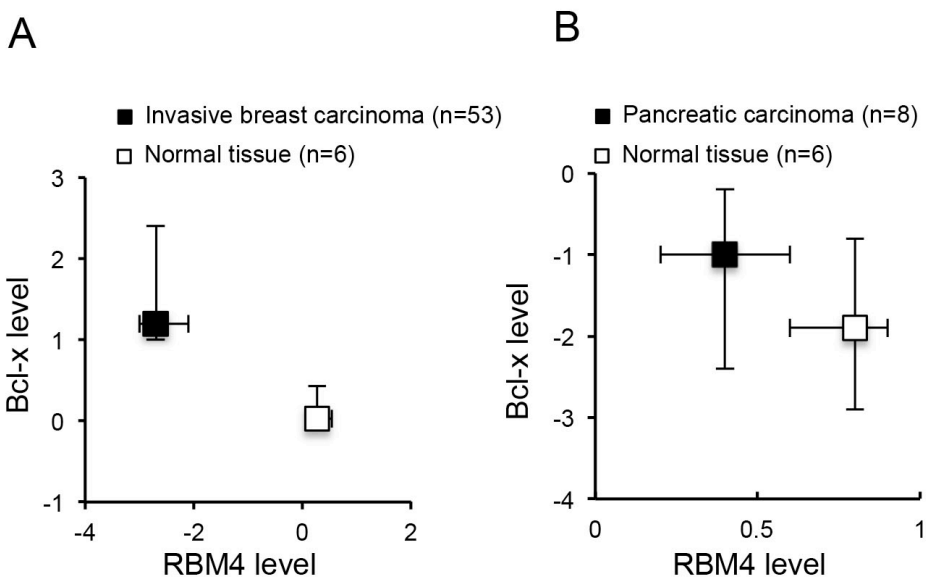

Figure S5, related to Figure 5. The inverse correlation of RBM4 and Bcl-xL in different cancers. The expression levels of RBM4 and Bcl-xL were obtained from ONCOMINE data, including breast cancer **(A)** and pancreatic cancer dataset (**B**), and the data were plotted as the log2(median-centered intensity). Error bars indicate upper and lower quartile.

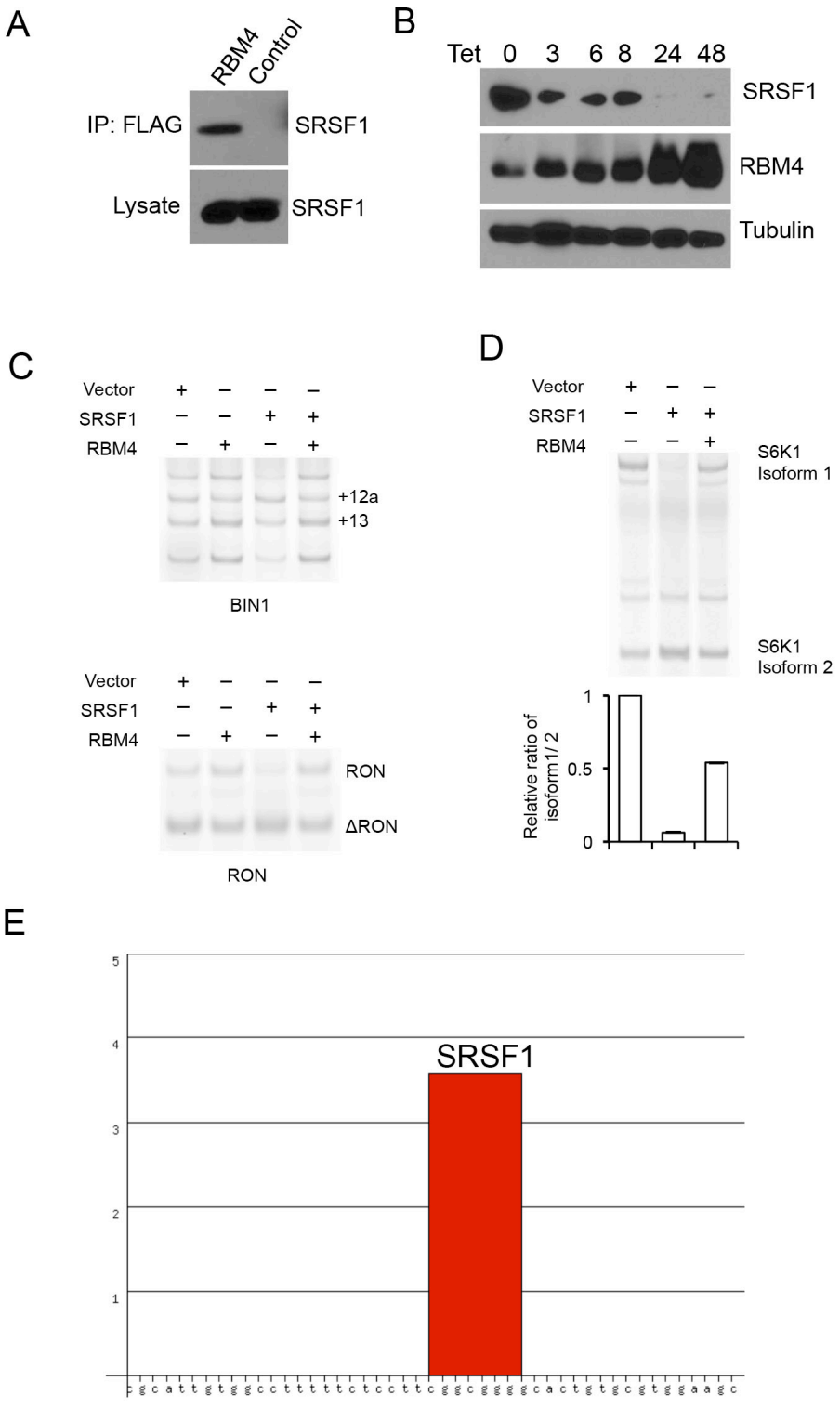

Figure S6, related to Figure 6. RBM4 antagonizes the activity of SRSF1. (A) The Flag tagged RBM4 was expressed and pulled down using anti-Flag beads. The

endogenous SRSF1 was examined. The input and the precipitates were probed with western blots. (**B**) The expression of RBM4 was induced by tetracycline in 293-FlpIn T-REx cells, and cells were collected at 0, 3, 6, 8, 24 and 48 hours after induction. The expressions of RBM4 and SRSF1 were examined with western blot. (**C**) H157 cells were stably transfected with RBM4, SRSF1, both, or a control. Total RNAs were purified from these cells and the splicing of BIN1 and RON was measured by RT-PCR. (**D**) Total RNAs were purified from these cells and the splicing of S6K1 was measured by RT-PCR. (**E**) The binding of SRSF1 to the same CGGCGG site in Bcl-x pre-mRNA were analyzed by ESFfinder tool (Cartegni et al., 2003).

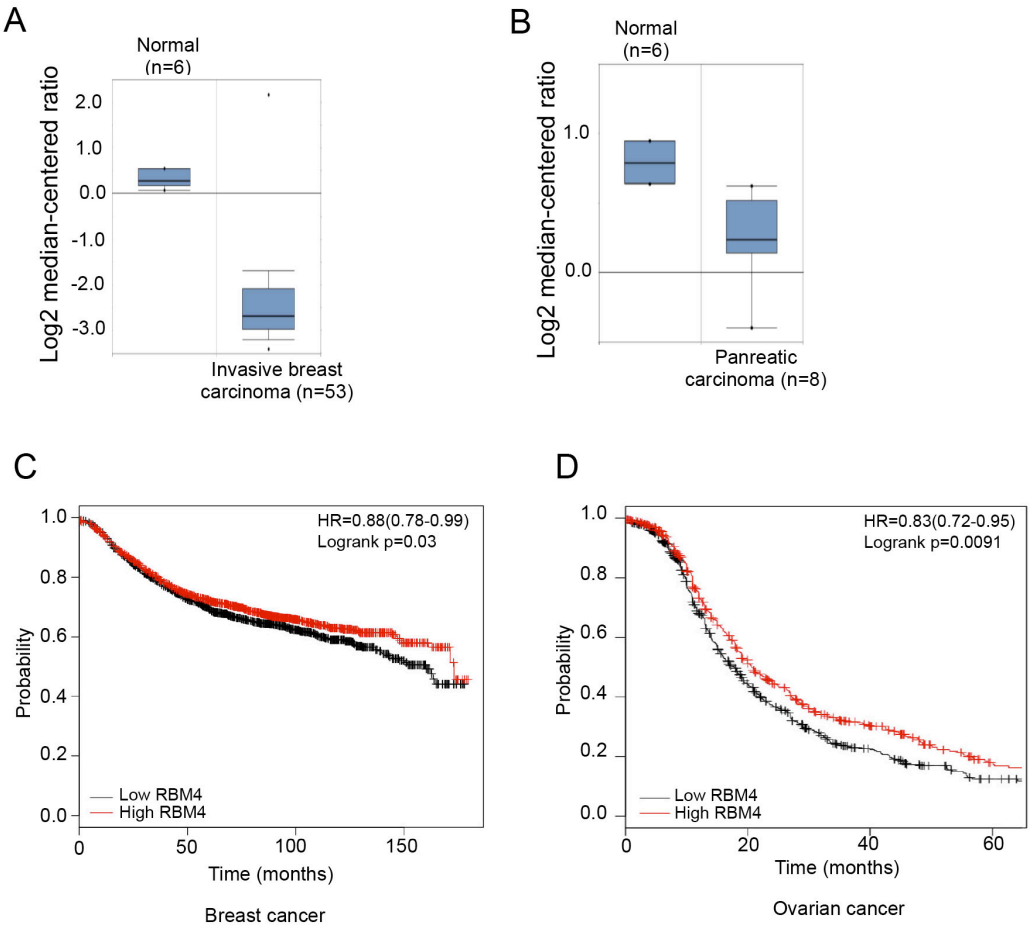

Figure S7, related to Figure 7. The mRNA level of RBM4 is decreased in other human cancers. **(A)** RBM4 mRNA level is analyzed in breast cancer, and **(B)** Pancreatic cancer. In all panels, Oncomine was used to analyze the previously collected data. The median, upper and lower quartiles were plotted, and the whiskers that extend from each box indicate the range values that are outside of the intra-quartile range. **(C-D)** The overall survival of breast cancers (**C**) and ovarian cancers (**D**) with high or low RBM4 expression were analyzed with Kaplan-Meier curve.

Supplemental Experimental Procedures

Splicing reporter constructs

All splicing reporters were modified from the backbone construct, pZW1 that included multi-cloning sites between two GFP exons (Wang et al., 2004). To construct the reporter pZW2C, we modified pZW2 that was originally used in the FAS-ESS screen and contained an *Xho*I/*Apa*I restriction site inside the test exon 2 (Wang et al., 2004). The pZW2 was digested with XhoI/ApaI and filled in to destroy the exonic restriction sites. We then introduced a new *Xho*I/*Apa*I restriction site at 18 nt downstream of the exon 2 by three consecutive PCR reactions. To increase the detection sensitivity, the pZW2C was further generated by weakening the 3′SS of exon 2 with site-directed mutagenesis so that the exon 2 was included in ~50% of mRNA. The RBM4 binding sequences and control sequence were inserted between *Xho*I/*Apa*I sites.

The reporters with competing 5′SS and 3′SS were described previously (Wang et al., 2006), and we inserted RBM4 binding sequences and control sequence by annealing primers containing target sequence and cognate restriction sites. To test if RBM4 can affect splicing when bound to a skipped exon, we used a modular splicing reporter pGZ3. The RBM4 binding sequences were inserted into this vector using *Xho*I/*Apa*I sites located inside the test exon.

To determine the functional modules of RBM4, we employed the pCI-new vector (Promega) to express fusion protein as described before. Briefly, we started with an expression construct that encodes from N- to C-terminals, Flag epitope, different fragments of RBM4, and the MS2 coat protein (gift of Dr. R. Breathnach form Institut de Biologie-CHR). The fragment encoding the MS2 coat protein fragment was removed

using *BamH*I/*Sal*I digestion and replaced with a fragment encoding a NLS (PPKKKRKV) and the PUF domain of human Pumilio1, resulting the PUF-RBM4 (full length, N-, and C-terminal fragments). To generate splicing reporters containing target sequences of modified PUF domain, PUF(3-2) (Wang et al., 2009), we synthesized and annealed oligonucleotides containing UGUAUGUA or UUGAUAUA sequence flanked by *Xho*I and *Apa*I sites, digested with *Xho*I/*Apa*I, and inserted into the exon of splicing reporter pGZ3 or the intron of splicing reporter pZW2C. All primers used in this study were listed in table S1.

Cell culture and transfection

To generate stable cell line expressing RBM4 upon tetracycline induction, we used pCDNA5 FRT/TO vector and 293 FlpIn/T-Rex cells (Invitrogen). The FLAG-tagged full length RBM4 was cloned into the vector, and transfected with pOG44 in 1:9 ratio. The stably integrated cells were selected with 100 μg/ml hygromycin at 2 days after transfection for ~2 weeks to obtain individual colonies. One day before the induction, the cells were transferred to hygromycin-free medium. The inductions were carried out by adding tetracycline to a final concentration of 2 μg/ml. The induced cells were collected 24 hours after induction to extract RNA and protein for further analysis.

To overexpress RBM4, cells were plated into 24-well plates 1 day before transfection. To determine the effect of over-expression of RBM4 on splicing changes, 0.2 μg of mini-gene reporters were co-tranfected with 0.4 μg of RBM4 using lipofectamine 2000 according to the manual. After 48 hours, cells were collected for further analysis of RNA and protein levels.

To stably express RBM4 (or other proteins) in H157 cells, we use lentivial vectors. We transfected 293 cells with pCDH-flag-RBM4 or pCDH-flag-empty vectors as per manufactures protocols. The supernatant media containing virus was collected by centrifugation to remove any cellular contaminant. The resulting viral particles were used to infect H157 cells, and stably integrated cells were selected by 5 μg/ml puromycin for one week. The expression of transgenes was confirmed by western blots before further analysis.

To inhibit the activity of Bcl-xL, H157 cells expressing RBM4 or vector control were treated with a specific Bcl-xL inhibitor, WEHI-539, at the final concentration of 10 μM. The treated cells were applied to colony formation assay and soft agar assay in the medium containing Z-VAD or DMSO control. To eliminate apoptosis effects by RBM4, H157 cells expressing RBM4 were treated with a pan-caspase inhibitor, Z-VAD, at the final concentration of 20 μM. The treated cells were applied to colony formation assay, soft agar assay and wound healing assay in the medium containing Z-VAD or DMSO control.

Assay of splicing reporters with semi-quantitative RT-PCR

The total RNA were isolated from transfected cells with TRIzol reagent (Invitrogen) according to the manufacturer's instructions, followed by 1 h DNase I (Invitrogen) treatment at 37 °C and then heat inactivation of DNase I. Total RNA (2 μg) was then reverse-transcribed with SuperScript III (Invitrogen) with poly T primer or gene specific primer (for GFP based splicing reporter), and one-tenth of the RT product was used as the template for PCR amplification (25 cycles of amplification, with trace amount

of Cy5-dCTP in addition to non-fluorescent dNTPs). RT-PCR products were separated on 10% PAGE gels, and scanned with a Typhoon 9400 scanner (Amersham Biosciences). The amount of each splicing isoform was measured with ImageQuant 5.2. The primers used to amplify GFP based minigene reporters were AGTGCTTCAGCCGCTACCC for GFP exon 1 and GTTGTACTCCAGCTTGTGCC for exon 3.

Western blot

Cells were lysed in lysis buffer containing 50 mM HEPES, 150 mN NaCl (4.38g), 1mM EDTA, 1% (w/v) CHAPS and Sigma protease inhibitor cocktail. Subsequently the cell lysates were boiled in 2X SDS-PAGE loading buffer for 10 min and then resolved by 10% SDS-PAGE and transferred to nitrocellulose membrane. The following antibodies were used in this study: cleaved Caspase-3 (#9664), PARP (#9542), hnRNP A1 (#5380), p70 S6 kinase (#2708), phosphor-p70 S6 kinase (#9234), phosphor-4E-BP1 (#9455), phosphor-Erk (#4370), Erk1/2 (#4695), phosphor-Akt (#2965), Akt (#4685) antibodies were purchased from Cell Signaling Technology, Bcl-x antibody (610211) was purchased from BD Bioscience, and antibodies against actin (A5441), alpha-tubulin (T5168) and FLAG M2 were purchased from Sigma-Aldrich. RBM4 antibody was purchased from Proteintech. SRSF1 and DAZAP1 antibodies was purchased from SCBT. Bound antibodies were visualized with the ECL kit (GE Healthcare).

Co-immunoprecipitation of RBM4

FLAG-tagged RBM4 were transfected into 293T cells. After 48 hours, the transfected cells were lysed in lysis buffer containing 50 mM HEPES, 150 mN NaCl

(4.38g), 1mM EDTA, 1% (w/v) CHAPS and Sigma protease inhibitor cocktail (with or without 50 μg/ml of RnaseA) at room temperature for 10 minutes. The M2 FLAG agarose resin (40 μl) was prepared as described by manufacturer instruction, and incubated with 1000 μl of cell lysate supernatant with gentle agitation at 4°C for overnight. The IP samples were span down at 8000rpm for 30 seconds and washed with wash buffer (50 mM HEPES, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol, pH to 7.5) for 3 times. Then the proteins were eluted with FLAG peptide (200ng/μl) and transferred into a new tube for further analysis.

High throughput mRNA-sequence and data analysis

RNAs from the H157 line with RBM4 expression or a mock transfected line were purified using Trizol^{TM} method and subsequently cleaned using RNAeasy Kit (Qiagen). The RNAs were digested in column with RNAse free DNAse as per manufacturers instruction. Total RNA not exceeding 3 μg was further used to purify polyadenylated RNA using Illumina TruSeq Total RNA Sample Prep kits. We used the Ribo-Zero Human to remove cytoplasmic rRNA. The mRNA purified was further analyzed using Bioanalyzer (Agilent Technologies) prior to generation of cDNA library with bar coded ends. RNA-seq libraries were robotically prepared with Illumina TruSeq Total RNA Sample Prep kits according to the manufacturer's protocol. The cluster generation and sequencing were carried out by standard procedures in HiSeq 2000 Illumina platform, and we used paired end sequencing protocol to generate 100 nt read at each end.

The paired-end sequences were mapped to human genome (hg18) using MapSplice 2.0.1.6 (default parameters) to discover splicing junctions. The mapped reads

were further analyzed with Cufflinks to calculate the level of gene expression. The change of splicing isoforms were analyzed using MISO package with the annotation of all known alternative splicing events (Katz et al., 2010), and we filtered the results based on the PSI (percent spliced in) values.

The gene ontology analysis was performed using DAVID GO analysis software to search for enriched pathways. The functional association of RBM4 targets were analyzed using protein interaction data from STRING database, generating a set of functional interaction networks. The sub-network containing more than five nodes were demonstrated.

Immunohistochemical analyses and semiquantitative evaluation

The tissue samples of human lung cancer were surgically collected from patients in the First Affiliated Hospital of Dalian Medical University. All human tumor tissues were obtained with written informed consent from patients or their guardians at Dalian Medical University prior to participation in the study. Tissue sections were treated as described previously (Shao et al., 2010). Briefly, the sections were incubated with primary antibody against RBM4 (1:100, ProteinTech Group) at 4°C overnight, and then were washed with PBS for three times and incubated with biotinylated secondary antibody (Abcam) at 1:200 for 30 min. Subsequently the sections were incubated with Vectastain ABC reagent for 30 min, and then developed with 3,3-Diaminobenzidine (DAB). Sections were counterstained with hematoxylin and mounted on coverslip. For evaluation, the visible brown granules were determined to indicate positive staining. Specimens were reviewed with staining intensity and staining extent. The relationship

between RBM4 expression and pathological parameters was analyzed using the $\chi 2$ test with SPSS software (SPSS Inc.). Statistical significance was reached at $P < 0.05$.

Soft agar assay

Equal volumes of 1.2% agar and 2× DMEM medium were mixed and placed onto 6-well dishes to generate 0.6% base agar. RBM4 expressing H157 and control H157 cells were seeded in 0.3% top agar (10^4 cells per plate) and incubated at 37°C in humidified atmosphere for three weeks. Colonies were stained with crystal violet and counted.

Colony formation assay

The cancer cells expression RBM4 or vector control (5000 cells per dish) were seeded in the 10-cm dishes and incubated at 37°C in humidified incubator for two weeks. Colonies were fixed and stained with crystal violet and the number of colonies was counted.

Wound-healing assay

The cancer cells expressing H157 or control vectors were implanted into 6-well culture dishes. When the cells grew to 90% confluence, a sterilized tip was used to draw a line with the same width on the bottom of the dishes. Images were captured at 0 and 16 hours after the wounding. Data shown were representative of three independent repeats.

Xenograft Assays

Female BALB/c nude mice, 4 weeks old, were purchased from Vital River Laboratories (VRL) for *in vivo* tumorigenicity study. The Institutional Animal Care and Use Committee of the Dalian Medical University approved use of animal models in this study. Mice were injected subcutaneously with 1×10^6 H157-luc-RBM4 (Left flank) and H157-luc-ctl (right flank) cells and raised in the following five weeks. The mice were then monitored for tumor volume and overall health. The size of the tumor was determined by caliper measurement of the subcutaneous tumor mass every three days. Tumor volume was calculated according to the formula $(4/3)\pi r1^2 r2$, $(r1 < r2)$. Each experimental group contained 7 mice. At the end of five weeks, all mice were sacrificed, and tumors were removed for further analysis. For all data points, three independent measurements were performed and means were used for calculation.

RNA immunoprecipitation

293T cells (1×10^6) expressing RBM4 or control vector are collected and washed twice with 10ml PBS, and then resuspended in 10ml PBS. Formaldehyde (37% stock) is added to the above solution to a final concentration of 1% and incubated at room temperature for 10 min with slow rotating. Crosslinking reactions are quenched by the addition of glycine solution (pH7.0) to a final concentration of 0.25M followed by incubation at RT for 5 min. The cells are collected by centrifugation at 3000 rpm for 4 min at 4 °C followed by two washes with ice-cold PBS. Fixed cells are resuspended in 2ml of RIPA buffer (50mM Tris-Cl, pH 7.5, 1% NP-40, 0.5% sodium deoxycholate, 0.05% SDS, 1mM EDTA, 150 mM NaCl) containing protease inhibitors. The cells are subsequently lysed by three rounds of sonication. Insoluble material is removed by

microcentrifugation at 14,000 rpm (16,000g) for 10min at 4 °C. An aliquot of solubilized cell lysate is mixed with protein A–Sepharose beads along with nonspecific competitor tRNA. This mixture is rotated for 1 h at 4 °C, followed by microcentrifugation at 4000 rpm for 5 min. The supernatant is removed and used for immunoprecipitation.

Protein A or protein G–Sepharose beads are coated with the Flag antibody for 2 h at 4 °C followed by extensive washing with RIPA buffer containing protease inhibitors. Before immunoprecipitation, the beads are incubated for 10 min RNasin. The precleared lysate is diluted with RIPA buffer, mixed with the antibody–coated beads, and incubated with rotation for 60–90 min. The beads are collected using a minicentrifuge at 6000 rpm for 45 s. The beads are washed five or six times with 1 ml of highstringency RIPA buffer (50mM Tris-Cl, pH 7.5, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 1mM EDTA, 1M NaCl, 1M Urea, 0.2mM PMSF) by 10 min rotation at room temperature. The beads containing the immunoprecipitated samples are collected and resuspended in 100 μl of 50 mM Tris–Cl, pH 7.0, 5 mM EDTA, 10 mM dithiothreitol (DTT) and 1% SDS. Samples (resuspended beads) are incubated at 70 °C for 45 min to reverse the crosslinks. The RNA is extracted from these samples using Trizol according to the manufacturer's protocol, and reverse transcribed into cDNA for PCR detection.

Statistics

Statistical analyses of colony formation, soft agar, wound healing, and splicing changes were performed by using Student's t test.

List of oligonucleotide sequences used in this study

Primer #	Name	Sequence	Notes
1	RBM4_BamHF1	CAC GGATCC ACC ATG GAC TAC AAG GAC GAC GAT	The primer pair used for cloning RBM4 coding region into pCDH vector with FLAG epitope
2	RBM4_NotR1	CAC GCGGCCGCTTAAAAGGCTGAGTACCGCG	
3	RBM4_RRMNotR	CACGCGGCCGCTTATATCGGACACTCTTTGGA CCA	Reverse primer used for cloning N-ternimal RRM domain into pCDH vector with FLAG epitope
4	RBM4_AlaXbaF	CAC TCTAGA ATG GAC TAC AAG GAC GAC GAT GAC AAG GGT GCTAGC G ATCGTTCAGG CCGC	The forward primer for Alanine-rich stretch amplification
5	RBM4_ESFfwd	CAC CTCGAG AT GGTGAAGCTG TTCATCGG	The forward and reverse primer pairs used to clone full length RBM4 for tethering assay
6	RBM4_ESFrev	CAC GGATCC C AAAGGCTGAGTACCGCG	
7	RBM4_ESFRRMrev	CAC GGATCC C TATCGGACACTCTTTGGACCA	Reverse primer used to clone RRM of RBM4 for tethering assay
8	RBM4_ESFAlafwd	CAC CTCGAG G ATCGTTCAGG CCGCGTGGC	Reverse primer used to clone Alanine rich stretch for tethering experiment
9	ISE-F-fwd	TCGAG GTAACGC GGGCC	The primer pair used for cloning ISE group F sequence into pGZ3, pZW2C, pEZ2F
10	ISE-F-rev	CGCGTTACC	
11	ISE-F-1B-fwd	TCGAG GTAACGC G	The primer pair used for cloning ISE group F sequence into pEZ1B
12	ISE-F-1B-rev	AATTC GCGTTACC	
13	CGGCGG-fwd	TCGAG CGGCGG GGGCC	The primer pair used for cloning CGGCGG sequence into pGZ3, pZW2C, pEZ2F
14	CGGCGG-rev	CCCGCCGC	
15	CGGCGG-1B-fwd	TCGAGCGGCGGG	The primer pair used for cloning CGGCGG sequence into pEZ1B
16	CGGCGG-1B-rev	AATTCCCGCCGC	
17	Contro-fwd	TCGAGGAATTGTTTGGGCC	The primer pair used for cloning control sequence into pGZ3, pZW2C, pEZ2F
18	Control-rev	CAAACAATTCC	
19	Control-1B-fwd	TCGAG GAATTGTTT G	The primer pair used for cloning control sequence into pEZ1B
20	Control-1B-rev	AATTC AAACAATTC C	
21	RBM4-test-fwd	GAGCGCTCGATGCCTACTAC	The primer pair used for testing RBM4
22	RBM4-test-rev	GGTCGGCAACAGGTGTCTAT	
23	TRAG3-F	ACGAATAAGGCCATCACCAG	The primer pair used for testing TRAG3
24	TRAG3-R	TCCTGGAACTTCCTTGATGG	

25	IMPA1-F	ACTTCCTGTTGCGAGTAGCC	The primer pair used for testing IMPA1
26	IMPA1-R	CCAAGGATCAGCCATCTTCT	
27	AFTPH-F	TCTCTACCACCCGTCCAGTT	The primer pair used for testing AFTPH
28	AFTPH-R	CTTTGAGGCTTGAGGTGGAG	
29	ERCC3-F	AAGCAGGTGGATGAGTCAGG	The primer pair used for testing ERCC3
30	ERCC-R	TTGTGCTTCAAGACCAGCTT	
31	DDX49-F	GTGGCTCGTGGAACAATGTC	The primer pair used for testing DDX49
32	DDX49-R	GAACTGCTCTGCGATCTGGT	
33	AP2M1-F	GCAAGATCAGCGAGGAAAAC	The primer pair used for testing AP2M1
34	AP2M1-R	CATCCCAAACTTGCATTCAG	
35	TEAD4-F	GGACTCCTTGGAACTGGCTTA	The primer pair used for testing TEAD4
36	TEAD4-R	TTTAGCTTGGCCTGGATCTC	
37	TIMM23-F	CTGCGATACCATGGAAGGAG	The primer pair used for testing TIMM23
38	TIMM23-R	CCCCTTGCCTAGTCACCATA	
39	CD44-F	ATCACCGACAGCACAGACAG	The primer pair used for testing CD44
40	CD44-R	CATCAATGCCTGATCCAGAA	
41	CLK2-F	GGACATTTAGCCGCTCATCT	Mutation oligos used for mutating Thr^{315} to Ala
42	CLK2-R	CTTGTTGTCAGGGTCTTTCTCA	

Supplemental References

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