Figures and figure supplements
The translational landscape of the splicing factor SRSF1 and its role in mitosis

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Figure 1. Identification of SRSF1 mRNA translational targets. (A) Experimental approach to identify SRSF1 mRNA translational targets. A characteristic fractionation profile of empty vector (pCG) and SRSF1 transfected-HEK 293T cells (Figure 1—figure supplement 1) is depicted. Absorbance at 254 nm was monitored. (B) A plot showing the distribution of mRNAs from RNA-seq analysis according to the polysome shift ratio (PSR). The null distribution (comparing two control subsamples) is symmetric and sharply centered at 0. The PSR of SRSF1 versus empty vector shows an enrichment over the null distribution. The mRNAs with a p<0.01 (PSR>0.889) were considered SRSF1 translational targets (Supplementary file 1).
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Figure 1—figure supplement 1. Optimization of SRSF1 transient transfection for polysomal shift analysis.
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Figure 1—figure supplement 2. Luciferase reporter containing an SRSF1 binding site.
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Figure 2. SRSF1 translational targets. (A) Motif identified on putative direct translational targets using 5-mers enrichment in combination with MEME algorithm (width = 10, sites = 508/508, E value = 1.5E-308, IC = 7.9 bits). Over-represented k-mers were obtained by double comparison CLIP+ versus CLIP− translational targets and CLIP+ translational targets versus null CLIP+. (B) Box plot showing the density of consensus motif (translational targets CLIP+ > translational targets CLIP− > null CLIP+ > null CLIP+). (C) Venn diagram showing the overlap (505 mRNAs) between translational targets (1576 mRNAs with p<0.01) and CLIP-tag mRNAs containing the consensus motif.
(CLIP+ CM) (6065) (Fisher’s exact test: OR 1.1686; p<2.2E-16. (D) The most representative classes of Gene Ontology terms enriched in direct translational targets (with CLIP-tag and consensus motif [CLIP+ CM]) relative to all the mRNAs detected in HEK 293T by RNA-seq. The number of genes observed in each category is indicated in the pie chart. Modified Fisher’s exact p value, EASE score is given for each category. In all cases, the Benjamini–Hochberg-corrected EASE score was <0.1. (E) Table giving the gene names of SRSF1 translational targets related to cell cycle and RNA processing pathways.

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Figure 2. Continued

Figure 2—figure supplement 1. Analysis of SRSF1 translational targets.

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Figure 2—figure supplement 2. The positional bias of the SRSF1 consensus motif in the 5'UTR, protein coding sequence (CDS), and 3'UTR of SRSF1 translational mRNA targets (red) was compared to the null population (blue, PSR=0).

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Figure 3. Validation of SRSF1 translational targets. (A) RT-qPCR validation confirms an increased polysome to subpolysome ratio for selected SRSF1 translational targets upon SRSF1 overexpression (CLIP+: green; CLIP+ and harboring an SRSF1 consensus motif (CLIP+ CM+: purple). The polysome to subpolysome ratio is relative to cells transfected with empty vector (pCG) and normalized to actin. Plotted data are the average of three biological replicates. (B) Dose-dependent effect of SRSF1 overexpression on polysome to subpolysome ratio. (C) SILAC ratio analysis of SRSF1 and SRSF1-NRS target sets. (D) Effect of PP242 on polysome to subpolysome ratio for SRSF1 targets.
replicates. The asterisks indicate statistical significance (p<0.05) Error bars, Gene Ontology terms and cancer relationship are indicated. (B) RT-qPCR validation of SRSF1 translational targets involved in RNA metabolic processes. The polysome to subpolysome ratio as measured by RT-qPCR in empty vector-transfected cells compared to cells overexpressing SRSF1 is indicated. Two different concentrations of pCGT7-SRSF1 plasmid were used. A mutant version of SRSF1 that is constitutively nuclear and does not activate translation was also included (SRSF1-NRS). (C) Box plot showing the values of the stable isotope labeling by amino acids in cell culture (SILAC) index, defined as log₂ SRSF1 ratio/empty vector ratio. ‘All’ refers to mRNAs encoding for all the proteins found by SILAC (2471 mRNAs); ‘CLIP+’ refers to the SRSF1 translational targets (PSR>0.889536; p<0.01) harboring CLIP-tag (125 mRNAs); ‘CLIP+CM’ is for direct translational targets (105 mRNAs); ‘PSR≥1, CLIP+CM’ refers to direct translational targets with a PSR≥1 (72 mRNAs) (p = 0.002487). (D) PP242-mediated mTOR inhibition suppresses SRSF1-dependent activation of translation of a subset of mRNA targets. Control and SRSF1-overexpressing cells were treated with PP242 for 90 min. The polysome to subpolysome ratio was measured by RT-qPCR in empty vector-transfected cells compared to cells overexpressing SRSF1 treated with or without PP242. mTOR inhibition was validated by Western blotting (data not shown). DOI: 10.7554/eLife.02028.009
Figure 4. Coupling of alternative splicing and translational regulation. (A) Correlation between SRSF1-induced changes in alternative splicing with polysomal distribution of those isoforms. Changes in alternative splicing induced by SRSF1 overexpression were determined by an exon-junction array. PSR: polysome shift ratio. (B) RT-qPCR analysis of the effect of SRSF1 on CLK1 alternative splicing and preferential polysomal association. The exon-intron structure of both isoforms is indicated (not to scale) and the CLK1 isoform that is an SRSF1 direct translational target is underlined. SRSF1-induced changes in CLK1 alternative splicing were determined and normalized to exon 7 (constitutive exon) levels (left panel). Two different concentrations of pCGT7-SRSF1 plasmid were used. Polysomal distribution of CLK1 mRNA isoforms upon SRSF1 overexpression normalized to exon 7 (constitutive exon) levels (right panel).

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Figure 5. SRSF1 translational targets involved in cell division. (A) List of cell cycle proteins regulated by SRSF1 at the translational level (left panel). The cartoon depicting their involvement in chromosome segregation during mitosis was adapted from Kitagawa and Hieter (2001). APC stands for (Anaphase-promoting complex). (B) Validation of cell cycle translational targets. The polysome to subpolysome ratio for a subset of cell cycle-related mRNAs was measured by RT-qPCR in empty vector-transfected cells compared to cells overexpressing SRSF1 or depleted of SRSF1. Two different concentrations of pCGT7-SRSF1 plasmids were used. A mutant version of SRSF1 that is constitutively nuclear and does not activate translation was also included (SRSF1-NRS). The asterisks indicate statistical significance (p<0.05). (C) Western

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blot validation of selected cell cycle SRSF1 translational targets in empty vector or SRSF1-transfected cells. β-Actin was used as a loading control. (D) Western blot validation of selected cell cycle SRSF1 translational targets in control or SRSF1-depleted cells in an asynchronous (A) or mitotic population (M). Tubulin was used as a loading control.

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Figure 5—figure supplement 1

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Figure 6. SRSF1 is required for cell cycle progression. (A) and (C) Schematic representation of the protocols used to assess mitotic progression. (B) HeLa cells were treated as in (A) and the number of cells at different stages of mitosis was determined by classification of images of fixed cells stained for DNA, tubulin, and pericentrin. (D) HeLa cells were treated as in (C) and time-lapse imaging of mCherry-H2B was performed. Images were captured every 15 min over 24 hr at three different positions. Representative images of cells transfected with control siRNA and SRSF1 siRNA are shown. (E) The graph indicates the elapsed time (minutes) from nuclear envelope breakdown (NEBD) or chromatin condensation to the onset of anaphase/telophase or to mitotic cell death. (F) HT1080 cells stably expressing GFP-CENPA were transfected with control or SRSF1-specific siRNA, and 24 hr later cells were retransfected with either empty vector or SRSF1. The next day cells were seeded in six-well plates and time-lapse imaging of GFP-CENPA was performed as in (C). The graph indicates the elapsed time (minutes) from nuclear envelope breakdown (NEBD) or chromatin condensation to the onset of anaphase/telophase or to mitotic cell death. Scale bar is 10 μm. The asterisks indicate statistical significance (*p<0.05, **p<0.01, ***p<0.001).

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Figure 7. SRSF1 is required for bipolar spindle formation. (A and B) HeLa cells were transfected with control or SRSF1-specific siRNA and 48 hr later stained for DNA (blue), tubulin (green), and pericentrin (red). Representative images for cells transfected with control or SRSF1-siRNAs (A and B, respectively) are shown. (B) Images show the formation of multipolar spindles upon SRSF1 depletion. (C) Quantitation of the multipolar spindle phenotype observed in (B) upon SRSF1 depletion. (D) HeLa cells were treated as in (A and B) and 24 hr later transfected with either wild-type SRSF1 or its
nuclear-retained version (SRSF1-NRS). The next day cells were fixed and stained for DNA, tubulin, and pericentrin and the appearance of multipolar spindle was quantified. Scale bar is 5 μm.

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Figure 7—figure supplement 1. SRSF1 is required for appropriate chromosome alignment.

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