The adipogenic transcriptional cofactor ZNF638 interacts with splicing regulators and influences alternative splicing

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Abstract Increasing evidence indicates that transcription and alternative splicing are coordinated processes; however, our knowledge of specific factors implicated in both functions during the process of adipocyte differentiation is limited. We have previously demonstrated that the zinc finger protein ZNF638 plays a role as a transcriptional coregulator of adipocyte differentiation via induction of PPARy in cooperation with CCAAT/enhancer binding proteins (C/EBPs). Here we provide new evidence that ZNF638 is localized in nuclear bodies enriched with splicing factors, and through biochemical purification of ZNF638's interacting proteins in adipocytes and mass spectrometry analysis, we show that ZNF638 interacts with splicing regulators. Functional analysis of the effects of ectopic ZNF638 expression on a minigene reporter demonstrated that ZNF638 is sufficient to promote alternative splicing, a function enhanced through its recruitment to the minigene promoter at C/EBP responsive elements via C/EBP proteins. Structure-function analysis revealed that the arginine/serine-rich motif and the C-terminal zinc finger domain required for speckle localization are necessary for the adipocyte differentiation function of ZNF638 and for the regulation of the levels of alternatively spliced isoforms of lipin1 and nuclear receptor co-repressor 1. Overall, our data demonstrate that ZNF638 participates in splicing decisions and that it may control adipogenesis through regulation of the relative amounts of differentiation-specific isoforms.—Du, C., X. Ma, S. Meruvu, L. Hugendubler, and E. Mueller. The adipogenic transcriptional cofactor ZNF638 interacts with splicing regulators and influences alternative splicing. J. Lipid Res. 2014. 55: 1886-1896.

Supplementary key words transcriptional coactivator • minigene reporter • nuclear speckles • adipocyte differentiation

The process of adipocyte differentiation is initiated and controlled by a number of transcription factors, including

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CCAAT/enhancer binding protein (C/EBP) β and C/EBP δ , and by cofactors (1), such as the recently characterized zinc finger protein ZNF638, which regulates the expression of the master controller of adipogenesis, PPAR γ , in conjunction with C/EBP proteins (2). The gene expression program occurring during differentiation includes the engagement and cooperation of these tissue-selective factors and cofactors with components of the RNA polymerase II machinery at active transcription sites, initiation of transcription followed by mRNA processing, through RNA capping, polyadenylation, and removal of noncoding intronic sequences, prior to protein translation (3). The process of splicing gives rise to the mature mRNA though the sequential succession of several complexes, starting from the precomplex in which sites of splicing are chosen, to the catalytic removal of introns executed by complex C components (4). During differentiation and development, tissue-specific enrichment of splicing factors ensures that alternative splicing is achieved to generate tissue-specific, temporally and developmentally regulated isoforms required to confer the specific phenotype (4-6). It has been shown that the relative abundance or activity of splicing regulators that have opposing effects determines the use of competing splice sites, ultimately controlling the exon composition of tissue-specific isoforms (4, 5). In adipocytes, alternatively spliced isoforms such as those of the insulin receptor (7), lipin (8), mitochondrial oxodicarboxylate carrier (9), nuclear receptor co-repressor 1 (NCoR) (10), preadipocyte factor 1 (11), and mechanistic target of rapamycin (12) have been shown to play a role in the differentiation process.

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Abbreviations: AR, acidic repeat; C/EBP, CCAAT/enhancer binding protein; CTD, C-terminal domain; DBD, DNA binding domain; EI, exon inclusion; ES, exon skipping; GFP, green fluorescent protein; GST, Glutathione S-transferase; HNRNP, heterogeneous nuclear ribonucleoprotein; NCoR, nuclear receptor co-repressor 1; NONO, non-POU domain-containing octamer binding protein; PABP1, polyadenylate binding protein 1; PGC1α, PPAR gamma coactivator 1-alpha; RRM, RNA recognition motif; RS, arginine/serine rich; ASF/SF2, pre-mRNAsplicing factor 2/alternative splicing factor; SR, serine/arginine rich; ZF, zinc finger; ZNF638, zinc finger protein 638.

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Serine/arginine-rich (SR) proteins are a major class of splicing regulators. These proteins contain RNA recognition motifs (RRMs) at the amino terminus and arginine/ serine-rich (RS) repeats at the carboxy terminus (13). SR proteins reside in discrete subnuclear regions, which represent storage areas for splicing factors serving nearby active transcription sites (14, 15). Furthermore, SR proteins bind to exonic splicing enhancer sequences and recruit spliceosomal ribonucleoproteins (RNPs) and non-RNPs to the pre-mRNA (16), thereby affecting the selection of splice sites already during the process of transcription. In addition to SR proteins, heterogeneous nuclear ribonucleoproteins (HNRNPs) bind to pre-mRNA transcripts and influence alternative splicing (17-19). It has been shown that pre-mRNA splicing is coupled to transcription both by the function of the C-terminal domain (CTD) of RNA polymerase II (3) and by a number of cofactors involved in both processes (20-23), as demonstrated for the metabolic coactivator PPAR gamma coactivator 1-alpha $(PGC1\alpha)$, which exerts a dual function when recruited at promoters containing direct repeat-1 responsive elements (23).

We have recently identified ZNF638 as a novel coactivator with domain homology to PGC1a and shown that ZNF638 functions as a transcriptional cofactor regulating the early stages of adipocyte differentiation through protein-protein interaction with C/EBP family members at the PPARy promoter (2). ZNF638 is composed of RS domains and RRMs, in addition to a homology region to the matrix-bound protein matrin 3 (MH1), two C₂H₂ zinc finger (ZF) motifs (ZF1 and ZF2), a putative DNA binding domain (DBD), and a region with acidic repeats (ARs) (24, 25). This characteristic domain composition of ZNF638, reminiscent of RNA binding proteins and of splicing regulators, and its dotted nuclear localization pattern in adipocytes (2), led us to hypothesize that ZNF638 may also participate in alternative splicing, as previously demonstrated for the coactivator PGC1 α (23). In this study, we tested this hypothesis using several experimental approaches, including confocal microscopy, mutagenesis, mass spectrometry analysis, and functional studies.

MATERIALS AND METHODS

Plasmids

Full-length murine ZNF638.pCR3.1 plasmid (2) was used as a template to generate eight deletion mutants by whole vector mutagenesis (Genewiz). ZNF638- Δ ZF1 contains a deletion from amino acid 424 to amino acid 456, eliminating the N-terminal ZF motif, the Δ RS construct lacks the RS motif present from amino acids 470 to 573, the Δ RRM1 has a deletion of the RRM1 motif from amino acids 676 to 751, Δ RRM2 has a deletion of the RRM2 motif from amino acids 901 to 981, and Δ RRM1-3 has a deletion encompassing all three RRM motifs, from amino acids 676 to 1,077. The ZNF638- Δ DBD construct has a deletion of the DBD, encompassing amino acids 1,353 to 1,482, the Δ AR contains a deletion of the ARs from amino acids 1,538 to 1,770, and Δ ZF2 lacks amino acids 1,876 to 1,908, eliminating the C-terminal ZF domain. The Δ RS plasmid served as a template to generate the

 $\Delta RS/ZF2$ mutant lacking both the RS and ZF2 motifs, with two deletions, one from amino acids 470 to 573 and one from 1,876 to 1,908. The primer sequences to generate the previous mutants are listed in Table 1. The empty vector backbone pCR3.1 served as control. glutathione S-transferase (GST)-ZNF638_{ZF2} and green fluorescent protein (GFP)-ZNF638_{ZF2} constructs expressing only the carboxy-terminal region of ZNF638, from amino acids 1,773 to 1,926, containing the C-terminal ZF domain were previously described (2). The fibronectin minigene reporter 7iBi89 plasmid (26) was obtained from Addgene (14065). This minigene construct contains exons 24 through 28 of the rat fibronectin gene including introns 24-25 and 25-26 flanking the alternatively spliced exon 25. The gene cassette is under the control of a human β -actin promoter and contains a human growth hormone polyadenylation signal. To generate a fibronectin minigene reporter containing C/EBP responsive elements, a 46 bp sequence of the PPARy2 promoter containing C/EBP responsive elements (5'-TTTTACTGCAATTTTAAAAAGCAATCAATATTGAACAATCTCT-GCT-3') (27) was inserted between the $\beta\text{-actin}$ promoter and the beginning of the fibronectin minigene cassette. The C/EBP responsive element sequence was synthesized including XhoI and BsrGI restriction sites and cloned into the minigene plasmid at those sites (Genewiz). C/EBPB and C/EBPb plasmids were obtained from Addgene, and the C/EBPa construct was a gift of Kai Ge (National Institutes of Health).

Antibodies

Rabbit anti-ZNF638 antibody (Bethyl, A301-548A) was used to detect ZNF638. Mouse anti-pre-mRNA-splicing factor 2 (SF2)/ alternative splicing factor (ASF) antibody (Invitrogen, 32-4600), Alexa Fluor 488 goat anti-rabbit antibody (Invitrogen, A-11008), and Alexa Fluor 594 goat anti-mouse antibody (Invitrogen, A-11005) were used for immunofluorescence staining. Mouse anti-β-actin antibody (Sigma, A5316), anti-GFP antibody (Invitrogen, A-11122), anti-HNRNPA1 antibody (Abcam, ab5832), anti-HNRNPA2B1 antibody (Abcam, ab31645), anti-HNRNPLL antibody (Santa Cruz, sc-132712), anti-non-POU domain-containing octamer binding protein (NONO) antibody (Santa Cruz, sc-136296), anti-polyadenylate binding protein 1 (PABP1) antibody (Cell Signaling, 4992), anti-C/EBPa antibody (Santa Cruz, sc-61), anti-C/EBPβ antibody (Santa Cruz, sc-7962), anti-C/EBPδ antibody (Santa Cruz, sc-636), anti-mouse-HRP antibody (Santa Cruz, sc-2055), anti-rabbit-HRP antibody (Santa Cruz, sc-2054), and anti-goat-HRP antibody (Santa Cruz, sc-2020) were used for Western blotting.

TABLE 1. Primers used to generate ZNF638 deletion mutants

ZNF638 Mutants	s Primer Sequences $(5' \rightarrow 3')$				
$\Delta ZF1$	Forward: GAATATTTCCACAACAATACCCTGATTG				
	Reverse: GGTATTGTTGTGGAAATATTCTTGGAG				
ΔRS	Forward: CCTCCCATCCGATAGGAAAAAGGCATTAG				
	Reverse: CTTTTTCCTATCGGATGGGAGGATCTC				
$\Delta RRM1$	Forward: AAAAAGCCACAGAACAAAGAAATGAAG				
	Reverse: GTTGGAACTCAAAGACTGATCCTTC				
$\Delta RRM2$	Forward: GAATCGGAGGAAGATGAGGAAGCTCTC				
	Reverse: CTTCCTCATCTTCCTCCGATTCCTTGTTC				
∆RRM1-3	Forward: CCAAAGACTGACTCAGAGGTTCAAAG				
	Reverse: GTTGGAACTCAAAGACTGATCCTTCTG				
ΔDBD	Forward: GAGAAAAGCCAATAACAAAACAGTCTC				
	Reverse: GTTTTGTTATTGGCTTTTCTCTTATTG				
ΔAR	Forward: GACAATGATTCAAAAGTTGAGTTAG				
	Reverse: TTCATCCAAATTAAATGTAAATAATG				
$\Delta ZF2$	Forward: GCCAAGCAAAGAAAGGAAAAGGAAGC				
	Reverse: TCCAGCCTTCGGAACAAGAAGTCC				

Cell culture and transfections

The 3T3-L1, 10T1/2, U2OS, and HEK-293 cells (ATCC), were cultured in high-glucose DMEM medium (Invitrogen), supplemented with 10% fetal bovine serum (HyClone) and 1% penicil-lin/streptomycin (Mediatech) at 37°C and 5% CO₂. The 3T3-L1 cells were cultured and differentiated as previously described (2). For minigene assays and transient transfections, cells were transfected with Xtreme Gene HP (Roche) and analyzed 24 h later.

Differentiation assays

The 10T1/2 cells were electroporated (Amaxa) with either control, full-length ZNF638, or mutant plasmids, induced to differentiate in culture medium supplemented with 5 µg/ml insulin, 0.5 mM 3-isobutyl-1-methylxanthine, 10 µM dexamethasone, and 10 µM troglitazone for 2 days and subsequently maintained in culture medium supplemented with 5 μ g/ml insulin (28). Gene expression was assayed by real-time PCR 3 days after induction of differentiation, following RNA extraction (TRIzol) and reverse transcription (Roche). The sequences of the primers used were the following: for 36B4, forward 5'-GCTTCATTGTG-GGAGCAGAC-3', reverse 5'-ATGGTGTTCTTGCCCATCAG-3'; for CD36, forward 5'-TTTGGAGTGGTAGTAAAAAGGGC-3', reverse 5'-TGACATCAGGGACTCAGAGTAG-3'; for aP2, forward 5'-ACACCGAGATTTCCTTCAAACTG-3', reverse 5'-CCATCTA-GGGTTATGATGCTCTTC-3'; for Ppary2, forward 5'-GATGCA-CTGCCTATGAGCACTT-3', reverse 5'-AGAGGTCCACAGAGCT-GATTC-3'; for ZNF638, forward 5'-TCCCAGTTGAGAGTGGA-ACC-3', reverse 5'-TGTGAGATCCGCTCTTGTTG-3'; for Lipin1a, forward 5'-GGTCCCCAGCCCCAGTCCTT-3', reverse 5'-GCA-GCCTGTGGCAATTCA-3'; for Lipin1β, forward 5'-CAGCCTGG-TAGATTGCCAGA-3', reverse 5'-GCAGCCTGTGGCAATTCA-3'; for NCoR, forward 5'-CTGACAGGCCTCAAGAAAGG-3', reverse 5'-AACCTGTTCCAGACGTGGTC-3'; and for NCoR ω , forward 5'-CTGGCTGCTCTTGTGGATGCT-3', reverse 5'-CTGTCCCATT-CCCTCTGACTG-3'. To quantify the extent of lipid accumulation, cells were stained with Oil Red O at day 6 of differentiation, and the dye was extracted with isopropanol and absorbance measured at 520 nm, as described previously (29, 30).

Immunofluorescence

U2OS cells were plated on chamber slides (Labtek) and transfected with either vector, full-length ZNF638, or one of the eight ZNF638 deletion mutants. Twenty-four hours after transfection, cells were fixed in 4% paraformaldehyde, permeabilized with 0.1% (w/v) Triton X-100 in PBS, blocked with 1% nonfat dry milk, and incubated with primary antibody for 1 h at room temperature or overnight at 4°C and with secondary antibodies for 1 h at room temperature. DAPI (4',6-diamidino-2-phenylindole, Invitrogen, D1306) was used for nuclear counterstaining. Confocal imaging was performed with an LSM 5 live DuoScan (Zeiss) laser scanning microscope with a 63× oil objective, using ZEN 2009 acquisition software (Zeiss).

Biochemical purification of ZNF638 interacting proteins present in adipocytes and mass spectrometry analysis

Purification of the GST-ZNF638_{ZF2} fusion protein from BL21 bacterial cells and preparation of nuclear extracts from differentiating 3T3-L1 cells 2 days after induction with 0.5 mM 3-isobutyl-1-methylxanthine (Sigma), 1 μ M dexamethasone (Fluka), and 5 μ g/ml insulin (Sigma) were performed as previously described (2). ZNF638 interacting proteins purified from nuclear lysates obtained from differentiating 3T3-L1 adipocytes were separated by SDS-PAGE and stained with Coomassie blue. Prominent bands were excised and subjected to mass spectrometry analysis (Taplin Biological Mass Spectrometry Facility, Harvard Medical School, Boston, MA).

Immunoprecipitation assays

HEK-293 cells were transfected with GFP-ZNF638_{ZF2}.pAcGFP or pAcGFP vector. Immunoprecipitation assays were performed using GFP-trap agarose beads (Chromotek, Allele), according to the manufacturer's instructions. Protein lysates were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore). After blocking in TBS with Tween containing 2% nonfat dry milk, membranes were incubated with primary antibodies for 1 h at room temperature or overnight at 4°C and subsequently exposed to secondary HRP-conjugated antibodies for 1 h. Blots were incubated with ECL substrate (Pierce) and exposed to film (Kodak).

Minigene splicing assays

HEK-293 cells were plated on 12-well plates and transfected at 70–80% confluency with 1 µg of fibronectin minigene reporter plasmid and either 5 µg of ZNF638.pCR3.1 or pCR3.1 vector. For C/EBP coexpression studies, cells were cotransfected with 1 µg fibronectin minigene plasmid containing C/EBP responsive elements and with 1 µg of C/EBP α , C/EBP β , or C/EBP δ plasmids or vector, and 3 µg of vector control or ZNF638. Twenty-four hours after transfection, cells were harvested, and the extracted mRNA (RNeasy, Qiagen) was reverse transcribed using anchored oligo dT primer (Transcriptor First Strand cDNA Synthesis Kit, Roche). Ratio of exon inclusion (EI) to exon skipping (ES) was assessed by real-time PCR. The EI primers specifically amplified the longer isoform including the alternatively spliced exon 25,



Fig. 1. ZNF638 colocalizes with the splicing factor ASF/SF2 in nuclear speckles. Subcellular localization of transiently expressed ZNF638 in U2OS cells detected by indirect immunofluorescence and by overlay with DAPI as nuclear counterstaining (A). Double immunofluorescence staining of transiently expressed ZNF638 in U2OS cells and overlay with endogenous ASF/SF2, a marker for nuclear speckles (B). A, B: Confocal imaging; scale bars: 10 µm.



Fig. 2. The RS domain and the C-terminal ZF motif are required for ZNF638 localization to nuclear speckles. Schematic illustration of the domains present in full-length ZNF638 and schematic representation of ZNF638 deletion mutants analyzed in this study (A). NLS, nuclear localization signal. Subcellular localization of ectopically expressed full-length ZNF638 and ZNF638 deletion mutants in U2OS cells, detected by indirect immunofluorescence staining and confocal imaging (B). Scale bar, 10 µm.

and the ES primers amplified the short isoform, skipping exon 25, as previously described (31). The EI and ES primers utilized were the following: EI forward 5'-CCGTCATCCCAGAGGTGCCCCA-3', EI reverse 5'-GGAGGGACGGCCGTTTGCTGTG-3'; and ES forward 5'-CCCCTATCTCTGATACCGTCATCCC-3', ES reverse 5'-GTTCGTACACGCTGGAGACACTGAC-3'. The following real-time PCR conditions were used: 10 min 95°C, 35 cycles of 10 s at 95°C, 30 s at 67°C, and 30 s at 72°C. PCR products corresponding to EI or ES were further confirmed by sequencing (Genewiz). Statistical analysis was performed by the two-sided Student's *t*-test with unequal variance on three replicates as indicated. A *P* value < 0.05 was considered statistically significant.

RESULTS

The adipogenic cofactor ZNF638 localizes in nuclear bodies enriched in splicing factors

We previously identified ZNF638 as a transcriptional cofactor implicated in the regulation of PPAR_Y expression and adipocyte differentiation through interaction with the C/EBP family of transcription factors. In addition, we showed that ZNF638 is localized in the nucleus of differentiating 3T3-L1 cells in a punctate pattern (2). To better characterize the identity of the nuclear bodies in which ZNF638 resides, we performed immuofluorescence staining using antibodies against ZNF638 and the splicing factor ASF/SF2, which served as a marker for nuclear speckles (32). Confocal imaging analysis of U2OS cells transiently expressing ZNF638 revealed that ZNF638 colocalizes with endogenous ASF/SF2 in nuclear speckles (**Fig. 1A, B**).

The RS and the C-terminal ZF domains are required for ZNF638's localization to nuclear speckles

To investigate the contribution of the domains present in ZNF638 to the speckled localization observed, we generated deletion mutants, as shown in the schematic representation in **Fig. 2A**. Indirect immunofluorescence staining of the ectopically expressed full-length ZNF638 and mutants followed by confocal imaging demonstrated that the ablation of either the RS domain (ZNF638- Δ RS) or the C-terminal ZF domain (ZNF638- Δ ZF2), or of both domains (ZNF638- Δ RS/ZF2), abolished ZNF638's localization to speckles (Fig. 2B). These findings indicate that the RS domain and the C-terminal ZF domain are required for ZNF638's localization to nuclear speckles.

ZNF638 interacts with regulators of pre-mRNA splicing present in adipocytes

Given the punctate nuclear localization of ZNF638 and the evidence that speckles are nuclear bodies enriched in pre-mRNA splicing factors (14), we hypothesized that ZNF638 may complex with spliceosomal proteins. To assess this, we performed biochemical purification of ZNF638 interacting proteins from differentiating 3T3-L1 adipocytes using a GST fusion protein expressing the region containing the ZF domain present at the carboxy terminus (GST-ZNF638_{ZF2}) required for speckled localization. Mass spectrometry analysis identified 172 novel ZNF638 interactors (Fig. 3A), and their clustering according to function revealed that 38% of these interacting proteins have been previously shown to be either associated with constitutive and alternative splicing, reported in interchromatin granule clusters or present in early or late spliceosomal complexes (33-36) (Fig. 3A, Table 2). Mass spectrometry analysis also revealed that the novel ZNF638 interacting proteins identified are involved in transcription, translation, nucleic acid binding, and metabolism, according to their annotation in the Uniprot database (Fig. 3A). Furthermore, we detected ZNF638 peptide sequences, which were not part of the ZNF638-GST fusion protein used for affinity purification (data not shown). To validate the results obtained via mass spectrometry analysis, we performed coimmunoprecipitation assays. As shown in Fig. 3B, ectopically expressed GFP-ZNF638_{ZF2} was able to immunoprecipitate endogenous HNRNPA1, HNRNPA2B1, HNRNPLL, NONO, and PABP1. Taken together, our data



Fig. 3. ZNF638 interacts with factors involved in pre-mRNA splicing present in differentiating adipocytes. Graphical representation of the clustering of the 172 novel ZNF638 interacting proteins present in differentiating adipocytes identified by mass spectrometry analysis (A). Interacting proteins purified from nuclear extracts obtained from differentiating 3T3-L1 cells using a GST-ZNF638_{ZF2} fusion protein were resolved by SDS-PAGE and subjected to mass spectrometry analysis. Coimmunoprecipitation assays were performed to validate mass spectrometry data (B). GFP-ZNF638_{ZF2} fusion protein was expressed in HEK-293 cells, and endogenous interacting proteins were detected by Western blot after GFP immunoprecipitation. Asterisk indicates nonspecific band.

indicate that ZNF638 can complex with factors implicated in alternative splicing expressed in differentiating adipocytes.

ZNF638 modulates alternative splicing decisions on a minigene reporter

In order to test the hypothesis that ZNF638 may participate in the regulation of pre-mRNA splicing, we investigated the effects of coexpressing ZNF638 with a minigene reporter containing a genomic exon cassette derived from the rat fibronectin gene. This reporter system is used for the quantification of alternative splicing through measurements of an alternatively spliced exon (**Fig. 4A**) (26). When ZNF638 was coexpressed with the minigene reporter, we observed an increase in the ratio of EI to ES, as compared with the ratio observed in control cells (Fig. 4B, C). Exon inclusion and exclusion were further confirmed by direct sequencing of PCR products (data not shown).

Given our previously published data demonstrating a function for ZNF638 as a coactivator of C/EBP proteins at C/EBP responsive elements present in the PPARy promoter,

we assessed the effect of ZNF638 on splicing of the fibronectin minigene when a C/EBP responsive element cassette was introduced in the fibronectin minigene reporter (Fig. 4D). As shown in Fig. 4E, F, low amounts of ectopically expressed ZNF638 were able to increase the ratio of EI to ES only when C/EBP α , C/EBP β , or C/EBP δ were coexpressed, suggesting that loading of ZNF638 on promoters enhances its action on splicing. These data indicate that ZNF638 is able to influence alternative splicing of a minigene reporter and that this process is facilitated in the presence of C/EBP responsive elements and C/EBP proteins.

The RS motif and the C-terminal ZF domain are necessary for the proadipogenic function of ZNF638

Given our previous characterization of ZNF638 as a coregulator of adipocyte differentiation, we assessed the requirement of each domain of ZNF638 for this process. We therefore ectopically expressed either vector, full-length ZNF638, or each deletion mutant in the mesenchymal cell line 10T1/2 and induced their differentiation. As shown in **Fig. 5A**, **B**, while 10T1/2 cells expressing full-length ZNF638 showed increased lipid accumulation and induced classic adipocyte markers, cells expressing ZNF638 mutants lacking either the RS, the C-terminal ZF, or both domains showed a decreased ability to induce adipocyte differentiation compared with full-length ZNF638, even though these mutants were expressed at the same levels as full-length ZNF638.

Given that alternatively spliced isoforms of lipin1 and NCoR have been previously shown to be differentially regulated during the adipogenic process (8, 10), we determined whether their levels were altered in 10T1/2 cells expressing ZNF638 or its deletion mutants. As shown in Fig. 5C, we observed an altered ratio of lipin1 β /lipin1 α and of NcoR ω /NcoR in cells expressing the mutants lacking the RS or the C-terminal ZF domains compared with cells expressing full-length ZNF638. Overall, these data demonstrate that the RS and the C-terminal ZF domains are necessary for the proadipogenic function of ZNF638 and for the regulation of alternatively spliced isoforms present in adipocytes.

DISCUSSION

The ZF protein ZNF638 is a multidomain protein initially cloned in the mid-1990s whose function has remained unknown for more than a decade. We have recently characterized ZNF638 as a transcriptional cofactor involved in adipocyte differentiation acting in cooperation with C/EBP β and C/EBP δ to regulate the expression of the nuclear receptor PPAR γ in adipocytes (2). In the present study, we have provided novel evidence that ZNF638 localizes in nuclear regions enriched in splicing factors, that it interacts with splicing regulatory proteins present in adipocytes, and that it participates in the modulation of alternative splicing of a pre-mRNA transcript derived from a minigene reporter.

TABLE 9	- ZNF638 interacting proteins implicated in pre-mRNA splicing identified by mass spectrometry analysis
monn 4.	214 050 interacting proteins implicated in pre-interve splicing identified by mass speed officity analysis

Gene Symbol Postein Name Postein Name Number of Papelie Marken Reterence Capif Cleavage and polyadersplation specificity factor subunit 6 Q61VE5			Uniprot		
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Dats/a Probable ATP-dependent RNA helicase DDX46 Q59925 3 (33, 34, 36, 53) Dats/a Probable ATP-dependent RNA helicase DDX50 Q50165 Q5017	Ddx3×	ATP-dependent RNA helicase DDX3×	Q62167	4	(33, 36)
Dids Probable ATP-dependent RNA helicase DIX5 Q61666 20 (33-35, 54) Dix30 Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DIX50 Q99P(15) 4 (35, 35, 55) Dix30 Putative ATP-dependent RNA helicase DIX50 Q99P(15) 4 (35, 35, 55) Dix30 Putative ATP-dependent RNA helicase DIX50 Q99P(15) 4 (35, 35) Earl LAWisk protein 1 Q99P(15) Q (33, 35) Earl LAWisk protein 1 Q9175 Q (33, 56) File RNA 47-0methyttransferase thotHarin P56959 18 (33, 34, 36) File RNA 47-0methyttransferase thotHarin Q9100 14 (35, 56) Harmpad Heterogeneous nuclear ribonucleoprotein A Q9000 15 (35, 56) Humpad Heterogeneous nuclear ribonucleoprotein A Q9000 8 (66) Humpad Heterogeneous nuclear ribonucleoprotein A Q9000 8 (65) Humpad Heterogeneous nuclear ribonucleoprotein A Q9001 4 (33, 35, 56, 57)	Ddx46	Probable ATP-dependent RNA helicase DDX46	Q569Z5	3	(33, 34, 36, 53)
Dixb Particle predmet RNA helicase DDX30 Q99MJ9 3 (36) Dixb Parative ATP-dependent RNA helicase DIX30 Q397U3 4 (36) Dix50 Parative ATP-dependent RNA helicase DIX30 Q997U3 4 (36) Dix50 Parative ATP-dependent RNA helicase DIX30 Q997U3 2 (34, 36) Eladi LLV-like protein 1 P10072 2 (34, 36) Fils RNA 2-0-methylitemsferase fibrillarin P35505 2 (35) Fils Gatch domain-containing protein 1 Q97D8M1 18 (33, 44, 36) Immpadb Heterogeneous nuclear ribonucleoprotein A0 QUCX86 2 (33) Humpadb Heterogeneous nuclear ribonucleoprotein A2 Q98020 15 (33, 35, 35, 65) Humpadb Heterogeneous nuclear ribonucleoprotein A3 Q98020 15 (33, 35, 35, 56) Humpadb Heterogeneous nuclear ribonucleoprotein A2 Q98020 16 (33, 35, 35, 56) Humpadb Heterogeneous nuclear ribonucleoprotein A2 Q98020 16 (33, 35, 35, 56)	Ddx5	Probable ATP-dependent RNA helicase DDX5	Q61656	20	(33-36, 54)
Dhx15 Putative PremRNAsplicing factor ATP-dependent RNA helicase DHX15 O33286 6 (33, 6, 55) Dhx30 ATP-dependent RNA helicase DHX30 QPPUV8 4 (36) Dhx1 LLW-like protein I P10372 2 (34, 36) Exort RNA binding protein BWS Q1145 10 (33, 36) Fast RNA V-Conchytramserrs fibrillarian P10564 1 (33, 36) Fast Gpatch domain-containing protein 1 P10564 1 (33, 36) Filsh IbC Fastore F1.2 P15664 1 (33, 36) Hurnpa0 Heterogeneous nuclear ribonucleoprotein A1 P49512 14 (33, 36) Hurnpa1 Heterogeneous nuclear ribonucleoprotein A2/14 Q88065 15 (33, 35, 36) Hurnpa1 Heterogeneous nuclear ribonucleoprotein A Q88061 16 (33, 35, 36) Hurnpa1 Heterogeneous nuclear ribonucleoprotein L Q88081 6 (33, 35, 36) Hurnpa2 Heterogeneous nuclear ribonucleoprotein L Q88081 6 (33, 35, 36) Hurnpa4	Ddx50	ATP-dependent RNA helicase DDX50	Q99MJ9	3	(36)
Dhx50 Pramixe XP-dependent RNA helicase DHX50 Q29PU8 4 (35) Elavit FIA-dependent RNA helicase A O70133 5 (33, 44, 36) Elavit FIA-Khinding protein LWS Q11454 10 (33, 56) Fol rRNA 2: Omethyltransferse fibrillarin P35550 2 (33) Fos RXA 2: Omethyltransferse fibrillarin P35550 2 (33) Fras RXA 2: Omethyltransferse fibrillarin P35550 2 (33) Fras RXA 2: Omethyltransferse fibrillarin P35550 2 (33) (33) Immpa0 Interrogeneous nuclear ribonucleoprotein A0 Q90044 2 (33, 35, 36) Humrpa2 Heterogeneous nuclear ribonucleoprotein A3 Q86055 15 (33, 35, 35, 57) Humrpa4 Heterogeneous nuclear ribonucleoprotein M Q10041 4 (33, 35, 35, 57) Humrpa4 Heterogeneous nuclear ribonucleoprotein U Q3747 3 (36) Humrpa4 Heterogeneous nuclear ribonucleoprotein U Q3747 3 (33, 35, 35, 57)	Dhx15	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	O35286	6	(33, 36, 55)
Dats All Pedependent RNA helicase A O'10133 5 (33, 43, 45) Easel RNA binding protein LWS Qi1545 10 (33, 36) Fas RNA 2-Genehylinzanderse fimillarin P35550 2 (33) Fas RNA binding protein TUS P36699 18 (33, 44, 46) Fas RNA binding protein TUS P36699 18 (33, 44, 46) Fas RNA binding protein TUS P36699 18 (33, 36) Hampad Heterogeneous nuclear ribonucleoprotein A P36695 15 (33, 36) Hampad Heterogeneous nuclear ribonucleoprotein A/B Q98020 8 (56) Hampad Heterogeneous nuclear ribonucleoprotein L Q88069 15 (33, 35, 35, 56) Hampad Heterogeneous nuclear ribonucleoprotein L QWR23 49 (33, 35, 35, 56) Hampad Heterogeneous nuclear ribonucleoprotein L QWR23 49 (33, 35, 35, 56) Hampad Heterogeneous nuclear ribonucleoprotein U QWV23 49 (33, 34, 36) Hampad Heterogene	Dhx30	Putative ATP-dependent RNA helicase DHX30	Q99PU8	4	(36)
Elavil ELAVike protein 1 P70372 2 (44, 56) Evarl RXN binding protein FUS P56569 18 (33, 56) Fal RXN binding protein FUS P56569 18 (33, 54) Fal RXN binding protein FUS P56569 18 (33, 54) Fals RXN binding protein FUS P56569 18 (33, 56) Fals RXN binding protein FUS P56569 18 (33, 56) Hampapal Heterogeneous nuclear ribonucleoprotein A P40312 (33, 56) Humpapal Heterogeneous nuclear ribonucleoprotein AZ/B1 Q88509 (35, 35, 56) Humpapal Heterogeneous nuclear ribonucleoprotein AZ Q81605 (33, 35, 56) Humpap Heterogeneous nuclear ribonucleoprotein M Q8001 (33, 35, 56) Humpap Heterogeneous nuclear ribonucleoprotein U Q804684 (33, 35, 56) Humpap Heterogeneous nuclear ribonucleoprotein J (34, 35) (35, 56) Humpap Heterogeneous nuclear ribonucleoprotein J (24, 24) (34, 35) Harpap <t< td=""><td>Dhx9</td><td>ATP-dependent RNA helicase A</td><td>O70133</td><td>5</td><td>(33, 34, 36)</td></t<>	Dhx9	ATP-dependent RNA helicase A	O70133	5	(33, 34, 36)
Ever RNA binding protein EWS Q61545 10 ($33, 36$) Ful RNA 2 ⁻ Omethyltransferase fibrillarin P35550 2 ($33, 34, 36$) Fus RNA binding protein FUS P36059 18 ($33, 34, 36$) Instal lic Fistone II.2 P15864 2 ($33, 36$) Immpal Iterrogeneous nuclear ribonucleoprotein A/ P49012 14 ($33-38$) Immpal Iterrogeneous nuclear ribonucleoprotein A/B1 Q80506 25 ($33-36, 39, 40$) Immpal Heterogeneous nuclear ribonucleoprotein A/B1 Q80506 8 ($33, 36$) Immpal Heterogeneous nuclear ribonucleoprotein D Q60568 4 ($33, 36$) Harnph Heterogeneous nuclear ribonucleoprotein D Q80568 16 ($33, 36$) Harnph Heterogeneous nuclear ribonucleoprotein D Q80758 4 ($33, 35, 55, 57$) Harnph Heterogeneous nuclear ribonucleoprotein U Q87453 49 ($33, 36, 55, 57$) Harnph Heterogeneous nuclear ribonucleoprotein U Q87454 10 ($33, 34, 36$)	Elavl1	ELAV-like protein 1	P70372	2	(34, 36)
Fb1 rRNA 2-Omerbyltransferase fibrillarin P35550 2 (33) Gpatch1 G patch domain-containing protein 1 Q90BM1 (35) Histh Lc P15864 2 (35) Harnpa0 Heterogeneous nuclear ribonucleoprotein A0 Q90X86 1 (33, 36) Harnpa0 Heterogeneous nuclear ribonucleoprotein A1 P1912 14 (33, 36) Harnpa1 Heterogeneous nuclear ribonucleoprotein A3 Q80C05 15 (33, 36, 56) Harnpa4 Heterogeneous nuclear ribonucleoprotein A1 Q9020 8 (36) Harnpa4 Heterogeneous nuclear ribonucleoprotein D0 Q60668 4 (33, 55, 56, 57) Harnpa4 Heterogeneous nuclear ribonucleoprotein U Q9020 8 (36) Harnpa4 Heterogeneous nuclear ribonucleoprotein U Q9020 4 (33, 55, 56, 57) Harnpa5 78 kDa glacose-regulard protein Q9020 8 (33, 43, 60) Harnpa5 Tearregeneous nuclear ribonucleoprotein Like Q9144 4 (41, 42) Hapa8 Heterogeneous nuclear ribonucleoprotein 1 Q9020 8 (33, 34, 36)	Ewsr1	RNA binding protein EWS	Q61545	10	(33, 36)
Fus RNA binding protein FUS P56959 18 ($35, 34, 36$) Gpatch1 Gpatch dominic-containing protein 1 QP15864 2 (33) Harnpad Heterogeneous nuclear ribonucleoprotein A0 QP2(X86) 1 ($33, 36$) Harnpad1 Heterogeneous nuclear ribonucleoprotein A2/B1 Q85609 25 ($33, 36$), 9, 40) Harnpad3 Heterogeneous nuclear ribonucleoprotein A2/B1 Q85609 25 ($33, 35, 6, 99, 40$) Harnpad1 Heterogeneous nuclear ribonucleoprotein A1 Q80645 8 ($36, 69, 69, 69, 69, 69, 69, 69, 69, 69, 6$	Fbl	rRNA 2'-O-methyltransferase fibrillarin	P35550	2	(33)
Gpatch G patch Gamma function Constraints CP CP Harnpa0 Heterogeneous nuclear ribonucleoprotein A1 P49312 14 (33.36) Harnpa0 Heterogeneous nuclear ribonucleoprotein A1 P49312 (33.36) Harnpa2 Heterogeneous nuclear ribonucleoprotein A3 Q88003 15 (33-36) Harnpa4 Heterogeneous nuclear ribonucleoprotein A3 Q88003 16 (33.36,56,56) Harnpa4 Heterogeneous nuclear ribonucleoprotein M Q90021 4 (33.53,56,57) Harnpa Heterogeneous nuclear ribonucleoprotein M Q91021 4 (33.56,56) Harnpa Heterogeneous nuclear ribonucleoprotein Like Q9214 4 (35.46,72) Harnpa Heterogeneous nuclear ribonucleoprotein Like Q9214 4 (35.66,72) Harnpa Heterogeneous nuclear ribonucleoprotein Like Q9214 4 (35.66,82) Harnpa Heterogeneous nuclear ribonucleoprotein S Q9212 4 (33.46,50) Harnpa Heterogeneous nuclear ribonucleoprotein S Q9212 (33)	Fus	RNA binding protein FUS	P56959	18	(33, 34, 36)
Hist Inle Histone H1.2 (33) Hurmpal Heterogeneous nuclear ribonucleoprotein A0 Q2CX86 1 (33, 36) Hurmpal Heterogeneous nuclear ribonucleoprotein A2 Q8569 25 (33-36, 99, 40) Hurmpal Heterogeneous nuclear ribonucleoprotein A7B Q89020 8 (36) Hurmpal Heterogeneous nuclear ribonucleoprotein A7B Q99020 8 (36) Hurmpal Heterogeneous nuclear ribonucleoprotein A7B Q90021 4 (33, 35, 95, 56) Hurmpal Heterogeneous nuclear ribonucleoprotein L Q80081 6 (33, 35, 95, 56) Hurmpal Heterogeneous nuclear ribonucleoprotein L Q80121 4 (33, 35, 95, 56) Hurmpal Heterogeneous nuclear ribonucleoprotein Like Q92124 1 (41, 42) Hapal Heterogeneous nuclear ribonucleoprotein 3 Q92713 (35) (36) Hurpal Heterogeneous nuclear ribonucleoprotein 1 Q80171 3 (35) Hapal Heterogeneous nuclear ribonucleoprotein 2 Q92713 (35) (36) Hurpal Heterogeneous nuclear ribonucleoprotein 3 Q92713 (35, 36, 57) <td>Gpatch1</td> <td>G patch domain-containing protein 1</td> <td>Q9DBM1</td> <td>1</td> <td>(35)</td>	Gpatch1	G patch domain-containing protein 1	Q9DBM1	1	(35)
Hmmpal Heterogeneous nuclear ribonucleoprotein A1 Q40X12 ($33, 36$) Hmmpal Heterogeneous nuclear ribonucleoprotein A3 Q81C05 ($33, 36$) Hmmpab Heterogeneous nuclear ribonucleoprotein A3 Q81C05 ($33, 36$) Hmmpab Heterogeneous nuclear ribonucleoprotein A Q99020 8 ($35, 36, 9, 9$) Hmmpab Heterogeneous nuclear ribonucleoprotein L Q81C05 ($33, 36, 56, 56$) Hmmpat Heterogeneous nuclear ribonucleoprotein U Q8VEK3 ($33, 36, 56, 56$) Hmmpat Heterogeneous nuclear ribonucleoprotein U Q8VEK3 ($33, 36, 56, 56$) Hmmpat Heterogeneous nuclear ribonucleoprotein U Q8VEK3 ($33, 36, 56, 56$) Hmmpat Heterogeneous nuclear ribonucleoprotein J Q802F1 ($33, 36, 56, 56$) Harpat Heterogeneous nuclear ribonucleoprotein J Q802F1 ($33, 36, 56, 56$) Harpat Heterogeneous nuclear ribonucleoprotein J Q802F1 ($33, 36, 56, 56$) Harpat Heterogeneous nuclear ribonucleoprotein J Q902F1 ($33, 36, 56, 56$) Marc Harpat Q812F2 ($33, 36, 56$	Histl h1c	Histone H1.2	P15864	2	(33)
Hampal Heterogeneous nuclear ribonucleoprotein AJ P49312 14 ($33-36$) ($33-36$) Hampa2h Heterogeneous nuclear ribonucleoprotein AJ Q88009 15 ($33-36$) Hampa2h Heterogeneous nuclear ribonucleoprotein AJB Q90920 8 (36) Hampal Heterogeneous nuclear ribonucleoprotein AJB Q9020 8 (35 , 35 , 35 , 56) Hampal Heterogeneous nuclear ribonucleoprotein L Q8081 (33 , 35 , 35 , 57) Hampan Heterogeneous nuclear ribonucleoprotein L Q81720 (33 , 35 , 35 , 35) Hampal Heterogeneous nuclear ribonucleoprotein Like Q92174 1 ($14, 42$) Hapal Heterogeneous nuclear ribonucleoprotein JLike Q92184 1 ($33, 36$, 36) Harpal Heterogeneous nuclear ribonucleoprotein JLike Q92184 3 ($33, 45$) Harpal Heterogeneous nuclear ribonucleoprotein JLike Q92184 1 ($33, 36$) Harpal Heterogeneous nuclear ribonucleoprotein JLike Q92184 1 ($33, 36$) Harpal Meteroseregulave Q830	Hnrnpa0	Heterogeneous nuclear ribonucleoprotein A0	Q9CX86	1	(33, 36)
Humpa2 Heterogeneous nuclear ribonucleoprotein A3 Q88C05 25 $(33-36, 39, 40)$ Humpab Heterogeneous nuclear ribonucleoprotein A/B Q90020 8 (36) Humpab Heterogeneous nuclear ribonucleoprotein D0 Q60668 4 $(33, 36, 56, 57)$ Humpi Heterogeneous nuclear ribonucleoprotein L Q800E1 4 $(33, 35, 56, 57)$ Humpi Heterogeneous nuclear ribonucleoprotein U Q8VEK3 49 $(33-36)$ Humpi Heterogeneous nuclear ribonucleoprotein Like Q21F4 1 $(41, 42)$ Hspa5 78 kD afuco-sergulated protein P20029 19 (33) Hspa5 78 kD afuco-sergulated protein P20029 19 (33) Hspa5 Test back guco-sergulated protein P20029 19 (33) Hspa5 Test back guco-sergulated protein P20029 19 (33) Hspa5 Test back guco-sergulated protein Q20717 3 (36) Hspa5 Insterokeneous nuclear ribonucleoprotein Q20718 10 $(33, 36)$	Hnrnpal	Heterogeneous nuclear ribonucleoprotein Al	P49312	14	(33–38)
Humpab Heterogeneous nuclear ribonucleoprotein A/B QB9020 8 (36) Humpab Heterogeneous nuclear ribonucleoprotein D0 Q60668 4 (33, 35, 36, 56) Humpab Heterogeneous nuclear ribonucleoprotein L Q8R081 6 (33, 35, 36, 56) Humpan Heterogeneous nuclear ribonucleoprotein M Q9D021 4 (33, 35, 36, 57) Humpan Heterogeneous nuclear ribonucleoprotein U Q8VEX3 49 (33-36) Humpan Heterogeneous nuclear ribonucleoprotein Like Q921F4 1 (41, 42) Hspaas Heterogeneous nuclear ribonucleoprotein S Q921 × 4 3 (33, 34, 36) Hayas Heat shock cognate 71 kDa protein P80301 1 (33, 34, 36) Hayas Matrias Matrias Q810 1 (33, 36, 57, 59, 60) Nata Matrias Q810 1 (33, 36, 57, 59, 60) Nop56 Nucleolar protein Sd Q9057 2 (36) (33, 36, 57, 59, 60) Nop56 Nucleolar protein 58 Q9057 1 (33, 36, 57, 59, 60) Nop56 Nucleolar prot	Hnrnpa2b1	Heterogeneous nuclear ribonucleoproteins A2/B1	O88569	25	(33-36, 39, 40)
Hurrpd Hererogeneous nuclear ribonucleoprotein D0 Q60068 4 (35, 36, 56) Hurrpd Heterogeneous nuclear ribonucleoprotein L Q80081 6 (33, 35, 36, 56) Hurrpn Heterogeneous nuclear ribonucleoprotein M Q9D0E1 4 (33, 35, 36, 56) Hurrpn Putetoyeneous nuclear ribonucleoprotein U Q8VEK3 49 (33-36) Hurrpl Heterogeneous nuclear ribonucleoprotein L-like Q921F4 1 (41, 42) Hspab F8 kD aglucose-regulated protein P30029 19 (33) Hspab Heat shock cognate 71 kDa protein P6017 3 (36) Hg2bp3 Instain-like growth factor 3 Q9174 3 (33, 36) Nards Matrin-3 Q8174 3 (33, 36, 58) Nol Nucleolin P09405 23 (36) Nop56 Nucleolar protein 55 Q91571 3 (35) (36) Nop55 Nucleolar protein 58 Q91571 3 (35, 36) (35) Nop55 Nucleolar protein 58 <	Hnrnpa3	Heterogeneous nuclear ribonucleoprotein A3	Q8BG05	15	(33–36)
Hampd Heterogeneous nuclear ribonucleoprotein L0 Q6068 4 (33, 35) Hamph Heterogeneous nuclear ribonucleoprotein M Q9DEL 4 (33, 35, 36, 57) Hamph Heterogeneous nuclear ribonucleoprotein U Q8VER3 49 (33-36) Hampu Heterogeneous nuclear ribonucleoprotein Likke Q921F4 1 (41, 42) Hspab 78 kDa glucose-regulated protein P63017 3 (36) Hgr2bp3 Insulin-like growth factor 2 mRNA binding protein 3 Q921F4 3 (33, 34, 65) Mar3 Matrin-3 Q81184 3 (33, 36) (38, 36) Mar4 Nuclear cap binding protein subunit 1 Q93184 3 (33, 36) Nobp1 Nuclear ap binding protein subunit 1 Q945184 (33, 36), 57, 59, 60) Nop56 Nucleolar protein 56 Q90671 3 (33) Nop56 Nucleolar protein 56 Q90671 3 (33, 56) Nucleolar protein 58 Q90187 2 (36) 1 Nucleolar protein 58 Q90187 2 <td>Hnrnpab</td> <td>Heterogeneous nuclear ribonucleoprotein A/B</td> <td>Q99020</td> <td>8</td> <td>(36)</td>	Hnrnpab	Heterogeneous nuclear ribonucleoprotein A/B	Q99020	8	(36)
Hamph Heterogeneous nuclear ribonucleoprotein L Q8081 6 (33, 35, 36, 56) Hamph Putative uncharacterized protein Q31021 10 (36) Hamph Putative uncharacterized protein Q31210 10 (36) Hamph Heterogeneous nuclear ribonucleoprotein Like Q2114 1 (41, 42) Hspab 78 kb a glucoseregulated protein P30029 19 (33) Hspab Heat shock cognate 71 kb a protein P60017 3 (36) Il23 Interleukin enhancer binding protein 3 Q9121 x 4 3 (33, 34, 36) Maritin-3 Q8110 (33, 36, 57) 90405 23 (36) Nucleoin P09405 23 (36) (33) (35, 61) Nucleoin Q90451 3 (33) (35) (36) Nop5 Nucleoar protein 56 Q40513 (33) (35, 61) Nop5 Nucleoar protein 57 Q40517 2 (36, 61) Nati Nucleoar protein 58 Q4051744 (35, 61)	Hnrnpd	Heterogeneous nuclear ribonucleoprotein D0	Q60668	4	(33, 36)
Hmmpm Heterogeneous nuclear ribonucleoprotein M Q90El 4 (33, 35, 35, 57) Hmmpu Heterogeneous nuclear ribonucleoprotein U Q87LZ10 10 (36) Hmmpu Heterogeneous nuclear ribonucleoprotein Like Q921F4 1 (41, 42) Hspa5 78 kDa glucoseregulated protein P2009 19 (33) Hg2bp3 Insulin-like growth factor 2 mRNA binding protein 3 Q9CPN8 10 (36) Hg2bp3 Insulin-like growth factor 2 mRNA binding protein 3 Q9CI × 4 3 (33, 36) Natrin-3 Q8K310 1 (33, 36) (33, 36) Nucleoin Nucleoin containing octamer binding protein Q9HX × 4 3 (35, 56) Non Non-POU domain-containing octamer binding protein Q9HX 1 (33, 36) Nop56 Nucleolar protein 58 Q6DFW4 1 (33, 76) Nph1 Nucleolar protein 58 Q6DFW4 1 (35) Nph1 Nucleolar protein 58 Q6DFW4 1 (35) Nph1 Nucleolar protein 58 <td< td=""><td>Hnrnpl</td><td>Heterogeneous nuclear ribonucleoprotein L</td><td>Q8R081</td><td>6</td><td>(33, 35, 36, 56)</td></td<>	Hnrnpl	Heterogeneous nuclear ribonucleoprotein L	Q8R081	6	(33, 35, 36, 56)
Hnmp Putative uncharacterized protein Q3V20 10 (36) Hnmpl Heterogeneous nuclear ribonucleoprotein U Q8VEK3 49 (33-36) Hanpl Heterogeneous nuclear ribonucleoprotein Like Q9UF4 1 (41, 42) Hspa8 Hat shock cognate 71 kDa protein P30029 19 (33) Igf2bp3 Insulin-like growth factor 2 mRNA binding protein 3 Q9T1 x 4 3 (35, 61) Idf3 Interleukin enhancer binding factor 3 Q9T1 x 4 3 (35, 65) Matr3 Matrin-3 Q8K310 1 (35, 36, 58) Nchel Nucleat cap binding protein subunit 1 Q5UVY9 1 (33, 36, 57, 59, 60) Nop56 Nucleolar protein 56 Q6DFW4 1 (33, 36, 61) Npp1 Nucleophosmin Q6H37 2 (36) Nxfl Nuclear RNA export factor 1 Q99K17 1 (33, 36) Paplca Polyadenylate binding protein, cytoplasmic 1 P20341 4 (35, 61) Paplca Serine, threonine-protein phosphatase 1 regulatory su	Hnrnpm	Heterogeneous nuclear ribonucleoprotein M	Q9D0E1	4	(33, 35, 36, 57)
Hnrpu Heterogeneous nuclear ribonucleoprotein Like Q921F4 1 (41, 42) Hspa5 78 kDa glucose-regulated protein P63017 3 (36) Hspa5 78 kDa glucose-regulated protein P63017 3 (36) Ig2bp3 Insulin-like growth factor 2 mRNA binding protein 3 Q921X4 3 (33, 34, 36) Il3 Interlevikin enhancer binding factor 3 Q921X4 3 (33, 34, 36) Narf Matrin-3 Ref Nather Construction of the Constructin the Construction of the Construction of the Constr	Hnrnpr	Putative uncharacterized protein	Q3UZI0	10	(36)
Hnrpl Heterogeneous nuclear ribonucleoprotein Likke Q202F4 1 (41, 42) Hspaå Tä kba glucose-regulated protein P20029 19 (33) Hspaå Heat shock cognate 71 kDa protein P3071 3 (36) Ild3 Interleukin enhancer binding factor 3 Q9CPN8 10 (35, 36) Matr3 Matrin-3 Q9TV + 4 3 (33, 36) Matr3 Matrin-3 Q9TV + 4 3 (33, 36) Nuclear cap binding protein subunit 1 Q3UVV9 1 (33, 36) Nono Non-POU domain-containing octamer binding protein Q99K4 1 (33) Nop56 Nucleolar protein 55 Q6DFW4 1 (33) Npn1 Nucleophosmin Q99K7 1 (33, 36) Nafl Nuclear RNA export factor 1 Q99H7 1 (33, 36) Paplca Serine/threonine-protein phosphatase 1 regulatory subunit 10 Q80W00 12 (33) Rbm59 RvA binding protein 72 (35) (36) (34)	Hnrnpu	Heterogeneous nuclear ribonucleoprotein U	Q8VEK3	49	(33–36)
Hspa5 78 KDa glucose-regulated protein P20029 19 (33) Hspa5 Insulin-like growth factor 2 mRNA binding protein 3 Q9CPN8 10 (36) Il8 Interleukin enhancer binding factor 3 Q9Z1X 4 3 (33, 34, 36) Mar3 Matrin-3 Q9K810 1 (33, 36) Nch1 Nucleoin P09405 23 (36) Nono Non-POU domain-containing octamer binding protein Q99K48 1 (33, 36, 57, 59, 60) Nop56 Nucleolar protein 56 QD67V4 1 (33, 36) Nop58 Nucleolar protein 58 Q6DFV4 1 (33, 61) Papc1 Polyadenylate binding protein, cytoplasmic 1 P0937 2 (36) Nxf1 Nucleoar protein 58 Q6DFV4 1 (33, 61) Papc1 Polyadenylate binding protein, cytoplasmic 1 P29341 4 (35, 61) Pp1ca Serine//threonine-protein phosphatase 1 regulatory subunit 10 Q50FW4 1 (36) Rbm39 RNA binding protein 39 Q81451 2 (21, 33, 35, 36, 62) Rp171 G85 ribosomal protein 17a<	Hnrpll	Heterogeneous nuclear ribonucleoprotein L-like	Q921F4	1	(41, 42)
Hspask Heat shock cognate 71 kDa proteinPF30173(36)Hg2bp3 III3 Interleukin enhancer binding factor 3QCIN810(35, 31, 36)Maria Matrin-3QSK3101(33, 36)Ncbp1Nuclear cap binding protein subunit 1Q3UVV91(33-36, 58)NchNuclear cap binding protein subunit 1Q3UVV91(33-36, 57, 59, 60)NonoNon-POU domain-containing octamer binding proteinQ994881(33, 36, 57, 59, 60)Nop56Nucleolar protein 56QED6213(33)Nop58Nucleolar protein 58QEDFW41(33, 36)Nph5Nucleolar protein 58QEDFW41(33, 36)Nph1NucleophosminQ991X71(33, 36)Pabpc1Polyadenylate binding protein, cytoplasmic 1P293414(35, 61)Pplp1rlaSerine/threonine-protein phosphatase 1 regulatory subunit 10Q80W0012(33)Rbm39RNA binding protein 99QSTHK41(36)Rbm39RNA binding protein 99QSTHK41(36)Rpf2060S arditer inbosonal protein G0354791(33, 36, 62)Rpf2060S arditer inbosonal protein S3P254442(33)Rpf2060S arditer inbosonal protein S4P254442(33)Rpf2060S arditer inbosonal protein S4P254442(33, 36, 63)Sf1Splicing factor 3Q80F151(33, 36, 65)Sf1Splicing factor 1Q90NS9 </td <td>Hspa5</td> <td>78 kDa glucose-regulated protein</td> <td>P20029</td> <td>19</td> <td>(33)</td>	Hspa5	78 kDa glucose-regulated protein	P20029	19	(33)
Igf2bp3Insulin-like growth factor 2 mRNA binding protein 3Q9CPN810(36)III3Interleukin enhancer binding factor 3Q9L1 × 43(33, 34, 36)Matr3Matrin-3Q8K3101(33-36, 58)Ncbp1Nuclear cap binding protein subunit 1Q3UVV91(33-36, 58)NclNucleolinP0940523(36)NonoNon-POU domain-containing octamer binding proteinQ906Z13(33)Nop56Nucleolar protein 56Q906Z13(33)Nop58Nucleolar protein 56Q60FW41(33)Nop51Nucleolar protein 56Q60FW41(33)Npm1Nucleor RNA export factor 1Q99X71(33, 36)Pabpc1Polyadenylate binding protein, cytoplasmic 1P293414(35, 61)Ppp1caSerine/threonine-protein phosphatase 1 regulatory subunit 10Q80W0012(33)Rbm15Putative uncharacterized proteinQ81HK41(36)Rbm39RNA binding protein 39Q81HK41(36)Rbm39RNA binding protein 52P254442(33)Rplp0608 acidic ribosomal protein 52P254442(33)Rplp0608 acidic ribosomal protein 52Q80R557(36)Sflp1Splicing factor 1Q90R53(35, 63)Sflp4Splicing factor 18Q80R551(33, 36, 63)Sflp4Splicing factor 38Q81H1Q90R551 <trr>Sflp4</trr>	Hspa8	Heat shock cognate 71 kDa protein	P63017	3	(36)
III3 Interleukin enhancer binding factor 3 Q921 $\times 4$ 3 (33, 34, 36) Matr3 Q8K310 1 (33, 36) Ncbp1 Nuclear cap binding protein subunit 1 P09405 23 (36) Nono Non-POU domain-containing octamer binding protein Q99K48 1 (33, 36, 57, 59, 60) Nop56 Nucleolar protein 55 Q6DFW4 1 (33) Nop58 Nucleolar protein 58 Q6DFW4 1 (33, 36) Nsfl Nucleor RNA export factor 1 Q99K7 1 (33, 36) Pabpc1 Polyadenylate binding protein, cytoplasmic 1 P29341 4 (35, 61) Ppp1r10 Serine / threonine-protein phosphatase 1 regulatory subunit 10 Q80W00 12 (33) Rbm39 RNA binding protein 39 Q87H51 2 (36) Rbm39 RNA binding protein 39 Q87H51 2 (33, 36, 62) Rpl7a 60S ribosomal protein 17a P12970 4 (33) Rpb10 60S acidic ribosomal protein S2 P25444 2 (33) Rps2 40S ribosomal protein S3 P07551 3 <td>Igf2bp3</td> <td>Insulin-like growth factor 2 mRNA binding protein 3</td> <td>Q9CPN8</td> <td>10</td> <td>(36)</td>	Igf2bp3	Insulin-like growth factor 2 mRNA binding protein 3	Q9CPN8	10	(36)
Matrix Matrin-3 Matrin-3 Q8K310 1 (33, 36) Nchp1 Nucleolar cap binding protein subunit 1 Q3UVY9 1 (33, 36, 57, 59, 60) Nono Non-POU domain-containing octamer binding protein Q99K48 1 (33, 36, 57, 59, 60) Nop56 Nucleolar protein 56 Q9DK21 3 (33) Nop58 Nucleolar protein 56 Q6DFW4 1 (33, 36) Nym1 Nucleorphosmin Q61937 2 (36) Nxf1 Nuclear RNA export factor 1 Q99JK7 1 (33, 36) Pabpc1 Polyadenylate binding protein, cytoplasmic 1 P29341 4 (35, 61) Ppp1ra Serine/ threonine-protein phosphatase PP1- α catalytic subunit PG2137 2 (21, 33, 34) Rbm15 Putative uncharacterized protein Q3 Q3THK4 1 (36, 6) Rbm16 Relify and the protein 127 Patative uncharacterized protein G Q3THK4 1 (33, 35, 36, 62) Rpl7a 60S ribosomal protein D2 P245444 2 (33) <tr< td=""><td>Ilf3</td><td>Interleukin enhancer binding factor 3</td><td>$Q9Z1 \times 4$</td><td>3</td><td>(33, 34, 36)</td></tr<>	Ilf3	Interleukin enhancer binding factor 3	$Q9Z1 \times 4$	3	(33, 34, 36)
Ncbpl Nuclear cap binding protein subunit 1 Q3UV9 1 (33-36, 58) Nol Nucleolin P09405 23 (36) Nono Non-POU domain-containing octamer binding protein Q99K48 1 (33, 36, 57, 59, 60) Nop56 Nucleolar protein 56 Q6DFW4 1 (33) Nop58 Nucleophosmin Q61937 2 (36) Nsrl1 Nucleophosmin Q991X7 1 (33, 36) Pabpc1 Polyadenylate binding protein, cytoplasmic 1 P29341 4 (35, 61) Ppp1ca Serine/threonine-protein phosphatase P1- α catalytic subunit 10 Q80W00 12 (33) Rbm15 Putative uncharacterized protein G Q35H151 2 (21, 33, 34) Rbm39 RAN binding protein 1/2 Q31 (33, 35, 66, 62) Rpl7a (36) Rbm39 RAN binding protein 1/2 P13 (33, 35, 36, 62) Rpl7a (36) (33) Rbm39 RAN binding protein S2 P25444 2 (33) Rpl2 408 ribosomal protein S3	Matr3	Matrin-3	Q8K310	1	(33, 36)
Ncl Nucleoin P09405 23 (36) Nonno Non-POU domain-containing octamer binding protein Q996X4 1 (33) Nop56 Nucleolar protein 56 Q9D6Z1 3 (33) Nop58 Nucleolar protein 58 Q6DFW4 1 (33) Npm1 Nucleophosmin Q61937 2 (36) Nxf1 Nuclear RNA export factor 1 Q991X1 1 (33, 36) 72 Pabpc1 Polyadenylate binding protein, cytoplasmic 1 P29341 4 (35, 61) Ppp1ca Serine/threonine-protein phosphatase 1 regulatory subunit 10 Q80W00 12 (33) Rbm15 Putative uncharacterized protein G Q5THK4 1 (36) Rbm39 RNA binding protein 39 Q8VH51 2 (21, 33, 34) Rpb7a 608 ribosomal protein L7a P12970 4 (33) Rps2 405 ribosomal protein S2 P25444 2 (33) Safb2 Scafold attachment factor B2 Q80NtS 7 (36)	Ncbpl	Nuclear cap binding protein subunit 1	Q3UYV9	1	(33–36, 58)
Nono Non-POU domain-containing octamer binding protein Q9948 1 (33, 36, 57, 59, 60) Nop56 Nucleolar protein 56 QBD6Z1 3 (33) Nop58 Nucleolar protein 58 QBDFW4 1 (33) Npm1 Nucleoar RNA export factor 1 Q991X7 1 (33, 36) Pabpc1 Polyadenylate binding protein, cytoplasmic 1 P29341 4 (35, 61) Ppp1ca Serine/threonine-protein phosphatase P1- α catalytic subunit P62137 2 (36) Rbm15 Putative uncharacterized protein Q3THK4 1 (36) (37) Rbm39 RNA binding protein 39 Q8TH51 2 (21, 33, 34) (21, 33, 34) Rbm49 60S ribosomal protein L7a P12970 4 (33) (33) Rplp0 60S ribosomal protein S2 P25444 2 (33, 36, 63) Safb2 Saffold attachment factor B2 Q80WR5 7 (36) Safb1 Splicing factor 1 Q64213 2 (33, 36, 65) Sifsb1 Splicin	Ncl	Nucleolin	P09405	23	(36)
Nop56 Nucleolar protein 56 Q9D6Z1 3 (33) Nop58 Nucleophar protein 58 Q6DFW4 1 (33) Npm1 Nucleophosmin Q61937 2 (36) Nxf1 Nuclear RNA export factor 1 Q99JX7 1 (33, 36) Pabpc1 Polyadenylate binding protein, cytoplasmic 1 P29341 4 (35, 61) Ppp1r10 Serine/threonine-protein phosphatase PP1- α catalytic subunit P62137 2 (36) Ppp1r10 Serine/threonine-protein phosphatase 1 regulatory subunit 10 Q80W00 12 (33, 35, 36, 62) Rbm39 RNA binding protein 39 Q8VH51 2 (21, 33, 34) Rbm39 RNA binding protein 17a P12970 4 (33) Rplp0 608 acidic ribosomal protein S2 P25444 2 (33) Rps2 408 ribosomal protein S3 P37351 3 (33) Safb2 Scaffold attachment factor B2 Q80WR5 7 (36) Sfl Splicing factor 1 Q9114 34 (33, 36, 63) <td>Nono</td> <td>Non-POU domain-containing octamer binding protein</td> <td>Q99K48</td> <td>1</td> <td>(33, 36, 57, 59, 60)</td>	Nono	Non-POU domain-containing octamer binding protein	Q99K48	1	(33, 36, 57, 59, 60)
Nop58 Nucleolar protein 58 Q61PW4 1 (33) Npm1 Nucleophosmin Q61937 2 (36) Nxf1 Nuclear RNA export factor 1 Q99JX7 1 (33, 36) Pabpc1 Polyadenylate binding protein, cytoplasmic 1 P29341 4 (35, 61) Ppp1ca Serine / threonine-protein phosphatase P1- α catalytic subunit P62137 2 (36) Rbm15 Putative uncharacterized protein Q3THK4 1 (36) Rbm39 RNA binding protein 39 Q8VH51 2 (21, 33, 34) Rbm3 Heterogeneous nuclear ribonucleoprotein G Q35479 1 (33, 35, 36, 62) Rpl7a 605 ribosomal protein S2 P25444 2 (33) Rps2 408 ribosomal protein S3 2 (36) (33) Safb2 Scaffold attachment factor B2 Q80YR5 7 (36) Sfls Splicing factor 1 Q464213 2 (33, 36, 65) Sflp Splicing factor proline and glutamine rich Q8VIJ6 34 (33, 36,	Nop56	Nucleolar protein 56	Q9D6Z1	3	(33)
Npm1 Nucleophosmin Qb1937 2 (36) NxrII Nuclear RNA export factor 1 Q991X7 1 (33, 36) Pabpc1 Polyadenylate binding protein, cytoplasmic 1 P29341 4 (35, 61) Ppp1r10 Serine/threonine-protein phosphatase PP1-a catalytic subunit P62137 2 (36) Ppp1r10 Serine/threonine-protein phosphatase 1 regulatory subunit 10 Q80W00 12 (33) Rbm15 Putative uncharacterized protein Q3THK4 1 (36) Rbm39 RNA binding protein 39 Q8VH51 2 (21, 33, 34) Rbm4 Heterogeneous nuclear ribonucleoprotein G O35479 1 (33, 35, 662) Rp17a 60S ribosomal protein P0 P14869 4 (33) Rps2 40S ribosomal protein S3a P97351 3 (33) Safb2 Scaffold attachment factor B2 Q80VR5 7 (36) Sfl Splicing factor 3B subunit 1 Q99NB9 1 (33-36, 63) Stp4 Splicing factor, poline and glutamine rich Q8	Nop58	Nucleolar protein 58	Q6DFW4	1	(33)
Nx11Nuclear KNA export factor 1Q99/X71(35, 36)Pabpc1Polyadenylate binding protein, cytoplasmic 1P293414(35, 61)Ppp1caSerine/threonine-protein phosphatase 1 regulatory subunit 10Q80W0012(33)Ppp110Serine/threonine-protein phosphatase 1 regulatory subunit 10Q80W0012(33)Rbm15Putative uncharacterized proteinQ3THK41(36)Rbm39RNA binding protein 39Q8VH512(21, 33, 34)Rbm3Rheterogeneous nuclear ribonucleoprotein GO354791(33, 25, 36, 62)Rpl7a60S ribosomal protein 17aP129704(33)Rps240S ribosomal protein S2P254442(33)Rps3a40S ribosomal protein S3aP973513(33)Safb2Scaffold attachment factor B2Q80WR57(36)SflSplicing factor 1Q642132(33, 36, 63)SfshSplicing factor 2 proline and glutamine richQ8VIJ634(33, 36, 65)StmSAFB-like transcription modulatorQ8CH251(33)Srst4Serine/arginine-rich splicing factor 7Q8BL971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923(35, 36, 69)Stafd3Tudor domain-containing protein 3Q91W182(34)Tr23, 36, 65)S1mSAFB-like transcription factor 7Q8BL971(33, 34, 66)SyncripHeterogeneous nuclear	Npm1	Nucleophosmin	Q61937	2	(36)
Pabpc1Polyadenylate binding protein, cytoplasmic 1P293414(35, 61)Ppplc1Serine/threonine-protein phosphatase PP1-a catalytic subunit 10Q80W0012(33)Ppp1r10Serine/threonine-protein phosphatase 1 regulatory subunit 10Q80W0012(33)Rbm39RNA binding protein 39Q8VH512(21, 33, 34)Rbm39RNA binding protein 17aQ8VH512(21, 33, 34)Rbm3Heterogeneous nuclear ribonucleoprotein GO354791(33, 35, 36, 62)Rpl7a60S ribosomal protein L7aP129704(33)Rps240S ribosomal protein S2P254442(33)Rps3a40S ribosomal protein S3P973513(33)Safb2Scaffold attachment factor B2Q80NFS7(36)Sf1Splicing factor 1Q642132(33, 36, 63)Sf3b1Splicing factor, proline and glutamine richQ8VIJ634(33, 36, 65)Strf4Serine/Arginine-rich splicing factor 7Q8EH251(33)Srsf4Serine/Arginine-rich splicing factor 7Q8USF51(33, 34, 36, 66)SyncripHeterogeneous nuclear ribonucleoprotein QQTTMK923(35, 36, 69)Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQBVE971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQTTMK923(35, 36, 69)Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQBU91 <t< td=""><td>Nxfl</td><td>Nuclear RNA export factor 1</td><td>Q99JX7</td><td>1</td><td>(33, 36)</td></t<>	Nxfl	Nuclear RNA export factor 1	Q99JX7	1	(33, 36)
Prp1caSerine/threonine-protein phosphatase PP1-a catalytic subunitP021372(3b)Ppp1r10Serine/threonine-protein phosphatase 1 regulatory subunit 10Q80W0012(33)Rbm15Putative uncharacterized proteinQ8THK41(36)Rbm39RNA binding protein 39Q8VH512(21, 33, 34)RbmxHeterogeneous nuclear ribonucleoprotein GO354791(33, 35, 36, 62)Rp17a60S ribosomal protein L7aP129704(33)Rpb240S ribosomal protein S2P254442(33)Rps3a40S ribosomal protein S3P973513(33)Safb2Scaffold attachment factor B2Q80YR57(36)Sf1Splicing factor 1Q99NB91(33-36, 64)SfpqSplicing factor 3B subunit 1Q99NB91(33-36, 64)SfpqSplicing factor, proline and glutamine richQ8VH263(33, 36, 65)Strk1Serine/arginine-rich splicing factor 4Q8VE971(33, 36, 67)Srsk1Serine/arginine-rich splicing factor 7Q8H2971(33, 36, 66)Srsk7Serine/arginine-rich splicing factor 7Q8B2463(36)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK92(34)SyncripLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331(33, 34, 36, 68)SyncripUzbandamine-rich splicing factor 7Q8B2463(36)Tarlot domain-containing protein 3Q91W18 </td <td>Pabpel</td> <td>Polyadenylate binding protein, cytoplasmic 1</td> <td>P29341</td> <td>4</td> <td>(35, 61)</td>	Pabpel	Polyadenylate binding protein, cytoplasmic 1	P29341	4	(35, 61)
Pripr10Serine/threeonine-protein phosphatase 1 regulatory subunit 10Q80W0012(33)Rbm15Putative uncharacterized proteinQ3THK41(36)Rbm39RNA binding protein 39Q8VH512(21, 33, 34)RbmxHeterogeneous nuclear ribonucleoprotein GQ3THK41(38, 35, 36, 62)Rpl7a60S ribosomal protein I.7aP129704(33)Rplp060S acidic ribosomal protein P0P148694(33)Rps240S ribosomal protein S2P254442(33)Safb2Scaffold attachment factor B2Q80YR57(36)Sf1Splicing factor 1Q642132(33, 36, 63)Sf3b1Splicing factor proline and glutamine richQ8VIJ634(33, 36, 65)SfpqSplicing factor, proline and glutamine richQ8VIJ634(33, 36, 67)Srpk1Serine/threonine-protein kinase SRPKIO705511(66)Srsf4Serine/arginine-rich splicing factor 4Q8VE971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923(35, 36, 69)Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463(36)ThrpoLamina-associated polypeptide 2, isoform $\alpha/zeta$ Q610331(33)U2surpU2 snRNP-associated SUP motif-containing proteinQ610331(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xrn25'3'	PppIca	Serine/threonine-protein phosphatase PP1- α catalytic subunit	P62137	2	(36)
Rbm15Putative uncharacterized proteinQ3 1Hk41(30)Rbm39RNA binding protein 39Q8VH512(21, 33, 34)RbmxHeterogeneous nuclear ribonucleoprotein GO354791(33, 35, 36, 62)Rpl7a60S ribosomal protein L7aP129704(33)Rplp060S acidic ribosomal protein P0P148694(33)Rps240S ribosomal protein S3P254442(33)Rps3a40S ribosomal protein S3aP973513(33)Sab2Scaffold attachment factor B2Q80VR57(36)Sf1Splicing factor 1Q642132(33, 36, 63)Sf2Splicing factor 3B subunit 1Q99NB91(33-36, 64)SfpqSplicing factor, proline and glutamine richQ8V1J634(33, 36, 65)SlmSAFB-like transcription modulatorQ8VE1251(33)Srpk1Serine/arginine-rich splicing factor 4Q8VE971(33, 36, 67)Srsf4Serine/arginine-rich splicing factor 7Q8BL971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923(35, 36, 69)Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463(36)TmpoLamina-associated Olypeptide 2, isoforms $\alpha/zeta$ Q610331(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xrn25'-3' exoRNase 2Q9DBRI2(36) <td< td=""><td>Ppp1r10</td><td>Serine/threonine-protein phosphatase 1 regulatory subunit 10</td><td>Q80W00</td><td>12</td><td>(33)</td></td<>	Ppp1r10	Serine/threonine-protein phosphatase 1 regulatory subunit 10	Q80W00	12	(33)
Rbm39RNA binding protein 39Q8VH512 $(21, 35, 34)$ RbmxHeterogeneous nuclear ribonucleoprotein G $O35479$ 1 $(33, 35, 366)$ Rpl7a60S ribosomal protein L7aP129704 (33) Rpl9060S acidic ribosomal protein P0P148694 (33) Rps240S ribosomal protein S2P254442 (33) Rps3a40S ribosomal protein S3aP973513 (33) Safb2Scaffold attachment factor B2Q80VR57 (36) Sf1Splicing factor 1Q642132 $(33, 36, 63)$ Sf3b1Splicing factor 3B subunit 1Q99NB91 $(33-36, 64)$ SfpqSplicing factor, proline and glutamine richQ8VIJ634 $(33, 36, 67)$ Strpk1Serine/Arginine-rich splicing factor 4Q8VE971 $(33, 34, 36, 68)$ SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923 $(35, 35, 36, 69)$ Tdr15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463 (36) Tdr43Tudor domain-containing protein 3Q91W182 (34) TmpoLamina-associated polypeptide 2, isoforms α /zetaQ610331 $(33, 34)$ Wbp11WW domain binding protein 11Q923D51 $(36, 70)$ Xrn25'.3' exoRNase 2Q9DBR12 $(35, 36)$ ZfrZinc finger RNA binding protein 11Q923D51 $(36, 70)$ Xrn25'.3' exoRNase 2Q6146427 <td< td=""><td>Kbm15</td><td>Putative uncharacterized protein</td><td>Q31HK4</td><td>1</td><td>(30)</td></td<>	Kbm15	Putative uncharacterized protein	Q31HK4	1	(30)
RomxHeterogeneous nuclear information of C $O_{53}(47)^{\circ}$ 1 $(55, 55, 56, 62)^{\circ}$ Rpl7a60S ribosomal protein L7aP129704 (33) Rplp060S acidic ribosomal protein P0P148694 (33) Rps340S ribosomal protein S2P254442 (33) Rps340S ribosomal protein S3aP973513 (33) Safb2Scaffold attachment factor B2Q80YR57 (36) Sf1Splicing factor 1Q642132 $(33, 36, 63)$ Sf3b1Splicing factor, proline and glutamine richQ8VIJ634 $(33, 36, 65)$ SfpqSplicing factor, proline and glutamine richQ8VEJ51 (33) Srpk1Serine/threonine-protein kinase SRPK1O705511 (66) Srsf4Serine/threonine-protein kinase SRPK1O705511 $(33, 36, 67)$ Srsf7Serine/arginine-rich splicing factor 7Q8BL971 $(33, 34, 36, 68)$ SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923 $(35, 36, 69)$ Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463 (36) ThrpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331 $(33, 34)$ U2 snRNP-associated SURP motif-containing proteinQ9DBR12 (36) Tuch fuger RNA binding protein 11Q923D51 $(36, 70)$ Xrn25'-3' exoRNase 2Q9DBR12 (36) Zinc finger RNA binding proteinQ88	Rbm39	KNA binding protein 39	Q8VH51	2	(21, 33, 34)
Rp17aOUS RIDOSOMAL protein L7aP129704(33)Rp1p060S acidic ribosomal protein P0P148694(33)Rps240S ribosomal protein S2P254442(33)Rps3a40S ribosomal protein S3aP973513(33)Safb2Scaffold attachment factor B2Q80YR57(36)Sf1Splicing factor 1Q642132(33, 36, 63)Sf2b1Splicing factor 3B subunit 1Q99NB91(33-36, 64)SfpqSplicing factor, proline and glutamine richQ8VIJ634(33, 36, 65)SImSAFB-like transcription modulatorQ8CH251(33)Srpk1Serine/threonine-protein kinase SRPK1O705511(66)Srsf4Serine/arginine-rich splicing factor 7Q8BL971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923(35, 36, 69)Tal15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463(36)Thrd3Tudor domain-containing protein 3Q91W182(34)U2surpU2 snRNP-associated SURP motif-containing proteinQ6NV831(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xrn25'-3' exoRNase 2QQ9DBR12(36)Zinc finger RNA binding proteinQ85326(33, 36)Zhr638Zinc finger protein 638Q6146427(33)	KDMX D = 17-	Gos eiteren and and an tribonucleoprotein G	D35479	1	(33, 35, 30, 62)
Rpp0005 action protein F0P145094(35)Rps240S ribosomal protein S2P254442(33)Rps3a40S ribosomal protein S3aP973513(33)Safb2Scaffold attachment factor B2Q80YR57(36)Sf1Splicing factor 1Q642132(33, 36, 63)Sfb1Splicing factor, proline and glutamine richQ8VIJ634(33, 36, 65)SfpqSplicing factor, proline and glutamine richQ8VIJ634(33, 36, 65)SltmSAFB-like transcription modulatorQ8CH251(33)Srpk1Serine/threonine-protein kinase SRPK1O705511(66)Srsf4Serine/arginine-rich splicing factor 7Q8BL971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923(35, 36, 69)Tdrd3Tudor domain-containing protein 3Q91W182(34)TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331(33, 34)U2surpU2 snRN-associated SURP motif-containing proteinQ6010331(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xrm25'-3' exoRNase 2Q9DBR12(36)Zinc finger protein 638Q6146427(33)	Kp1/a Bralm0	608 ridosomai protein L/a	P12970 D14960	4	(33)
Rps2405 H050mlar protein 527234442(35)Rps3a40S ribosomal protein S3a9973513(33)Safb2Scaffold attachment factor B2Q80VR57(36)Sf1Splicing factor 1Q642132(33, 36, 63)Sf3b1Splicing factor 3B subunit 1Q99NB91(33–36, 64)SfqSplicing factor, proline and glutamine richQ8VIJ634(33, 36, 65)SltmSAFB-like transcription modulatorQ8CH251(33)Srpk1Serine/threonine-protein kinase SRPK1O705511(66)Srsf4Serine/arginine-rich splicing factor 7Q8BL971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923(35, 36, 69)Tdrd3Tudor domain-containing protein 3Q91W182(34)TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331(33, 34, 36, 70)U2surpU2 snRNP-associated SURP motif-containing proteinQ6NV831(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xm25'-3' exoRNase 2QQ9DBR12(36)ZhrZinc finger RNA binding proteinQ885326(33, 36)ZhrZinc finger protein 638Q6146427(33)	Rpipu	405 vib accord protein F0	P14609	4	(33)
RpsaF93315(35)Safb2Scaffold attachment factor B2Q80YR57(36)Sf1Splicing factor 1Q642132(33, 36, 63)Sf2b1Splicing factor 3B subunit 1Q99NB91(33-36, 64)SfqSplicing factor, proline and glutamine richQ8VIJ634(33, 36, 65)SlmSAFB-like transcription modulatorQ8CH251(33)Srpk1Serine/threonine-protein kinase SRPK1O705511(66)Srsf4Serine/arginine-rich splicing factor 7Q8BL971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923(35, 36, 69)Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463(36)Tudor domain-containing protein 3Q91W182(34)TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xrn25'-3' exoRNase 2Q9DBR12(36)ZhrZinc finger RNA binding proteinO885326(33, 36)ZhrZinc finger protein 638Q6146427(33)	Rps2	405 ribosomal protein 52	P07251	2	(33)
SalabScalableScalableGoorks7(30)Sf1Splicing factor 1Q642132(33, 36, 63)Sf3b1Splicing factor 3B subunit 1Q99NB91(33–36, 64)SfpqSplicing factor, proline and glutamine richQ8VIJ634(33, 36, 65)SltmSAFB-like transcription modulatorQ8CH251(33)Srpk1Serine/threonine-protein kinase SRPK1O705511(66)Srsf4Serine/arginine-rich splicing factor 7Q8BL971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923(35, 36, 69)Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463(36)Tudor domain-containing protein 3Q91W182(34)TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331(33, 34)U2 surpU2 snRNP-associated SURP motif-containing proteinQ6NV831(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xrn25'-3' exoRNase 2Q9DBR12(36)ZhrZinc finger RNA binding proteinQ85326(33, 36)ZhrZinc finger protein 638Q6146427(33)	Kps5a Safb9	Scaffold attachment factor B9	O80VP5	3	(35)
SiltSplicing factor 1 $(20, 51, 60, 60)$ Sf3b1Splicing factor 3B subunit 1Q99NB91 $(33, -36, 64)$ SfpqSplicing factor, proline and glutamine richQ8VIJ634 $(33, 36, 65)$ SltmSAFB-like transcription modulatorQ8CH251 (33) Srpk1Serine/threonine-protein kinase SRPK1O705511 (66) Srsf4Serine/arginine-rich splicing factor 4Q8VE971 $(33, 34, 36, 68)$ SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923 $(35, 36, 69)$ Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463 (36) Tdrd3Tudor domain-containing protein 3Q91W182 (34) TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q61NV831 $(33, 34)$ U2surpU2 snRNP-associated SURP motif-containing proteinQ923D51 $(36, 70)$ Wbp11WW domain binding protein 11Q923D51 $(36, 70)$ Xrn25'-3' exoRNase 2Q9DBR12 (36) ZfrZinc finger RNA binding proteinO885326 $(33, 36)$ Znf638Zinc finger protein 638Q6146427 (33)	Sell Sell	Scalioli attachment factor b2	Q601K5	9	(30)
ShofSplicing factor of statistical $(25-36, 67)$ SfpqSplicing factor, proline and glutamine richQ8VIJ634 $(33, 36, 65)$ SltmSAFB-like transcription modulatorQ8CH251 (33) Srpk1Serine/threonine-protein kinase SRPK1O705511 (66) Srsf4Serine/arginine-rich splicing factor 4Q8VE971 $(33, 34, 36, 68)$ SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923 $(35, 36, 69)$ Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463 (36) Tdrd3Tudor domain-containing protein 3Q91W182 (34) TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331 $(33, 34, 36, 67)$ U2surpU2 snRNP-associated SURP motif-containing proteinQ6NV831 $(33, 34, 36)$ Wbp11WW domain binding protein 11Q923D51 $(36, 70)$ Xrn25'-3' exoRNase 2Q9DBR12 (36) ZfrZinc finger RNA binding proteinO885326 $(33, 36)$ Znf638Zinc finger protein 638Q6146427 (33)	Sf3b1	Splicing factor 3B subunit 1	Q04215 Q09NB0	1	(33, 30, 03) (33-36, 64)
ShpicSplitting factor, promite and gritamine rich $Qevryb$ Sr $(55, 56)$ SltmSAFB-like transcription modulatorQ8CH251 (33) Srpk1Serine/threonine-protein kinase SRPK1O705511 (66) Srsf4Serine/arginine-rich splicing factor 4Q8VE971 $(33, 36, 67)$ Srsf7Serine/arginine-rich splicing factor 7Q8BL971 $(33, 34, 36, 68)$ SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923 $(35, 36, 69)$ Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463 (36) Tdrd3Tudor domain-containing protein 3Q91W182 (34) TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331 $(33, 34)$ U2surpU2 snRNP-associated SURP motif-containing proteinQ6NV831 $(33, 34)$ Wbp11WW domain binding protein 11Q923D51 $(36, 70)$ Xrn25'-3' exoRNase 2Q9DBR12 (36) ZfrZinc finger RNA binding proteinO885326 $(33, 36)$ Znf638Zinc finger protein 638Q6146427 (33)	Sfpg	Splicing factor proline and glutamine rich	O8VII6	24	(33 - 36, 65)
SinkSink Internation protein inductionQuerter(35)SinkSerine/threonine-protein inducatorQuerter(35)SinkSerine/threonine-protein kinase SRPK1Qit(33, 36, 67)SinkSerine/arginine-rich splicing factor 4Qit(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQit(35, 36, 69)Staf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQit(36)Tdrd3Tudor domain-containing protein 3Qit(33)TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Qit(33, 34)U2surpU2 snRNP-associated SURP motif-containing proteinQit(33, 34)Wbp11WW domain binding protein 11Qit(36, 70)Xrn25'-3' exoRNase 2Qit(36)ZfrZinc finger RNA binding proteinQit(33, 36)Znf638Zinc finger protein 638Qit(33, 34)	Sltm	SAFB-like transcription modulator	08CH25	1	(33)
StraftSerine/arginine-rich splicing factor 7Q8VE971(33, 36, 67)Srsf4Serine/arginine-rich splicing factor 7Q8BL971(33, 34, 36, 68)SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923(35, 36, 69)Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463(36)Tdrd3Tudor domain-containing protein 3Q91W182(34)TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331(33, 34)U2 surpU2 snRNP-associated SURP motif-containing proteinQ6NV831(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xrn25'-3' exoRNase 2Q9DBR12(36)ZfrZinc finger RNA binding proteinO885326(33, 36)Znf638Zinc finger protein 638Q6146427(33)	Srok1	Serine /threenine-protein kinase SRPK1	070551	1	(66)
SharSerine/arginine-rich splicing factor 7Q8BL971 $(33, 34, 36, 68)$ SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923 $(35, 36, 69)$ Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463 (36) Tdrd3Tudor domain-containing protein 3Q91W182 (34) TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331 $(33, 34, 36, 68)$ U2surpU2 snRNP-associated SURP motif-containing proteinQ61N831 $(33, 34)$ Wbp11WW domain binding protein 11Q923D51 $(36, 70)$ Xrn25'-3' exoRNase 2Q9DBRI2 (36) ZfrZinc finger RNA binding proteinO885326 $(33, 36)$ Znf638Zinc finger protein 638Q6146427 (33)	Srsf4	Serine/arginine-rich splicing factor 4	O8VE97	1	$(33 \ 36 \ 67)$
SyncripHeterogeneous nuclear ribonucleoprotein QQ7TMK923 $(36, 69)$ Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463 (36) Tdrd3Tudor domain-containing protein 3Q91W182 (34) TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331 (33) U2surpU2 snRNP-associated SURP motif-containing proteinQ6NV831 $(33, 34)$ Wbp11WW domain binding protein 11Q923D51 $(36, 70)$ Xrn25'.3' exoRNase 2Q9DBRI2 (36) ZfrZinc finger RNA binding proteinQ885326 $(33, 36)$ Znf638Zinc finger protein 638Q6146427 (33)	Srsf7	Serine / arginine-rich splicing factor 7	08BL97	1	(33, 34, 36, 68)
SyntheticTAF15 Evaluation interval information protein 2 2 3 (36) Taf15TAF15 RNA polymerase II, TATA box binding protein-associated factorQ8BQ463 (36) Tdrd3Tudor domain-containing protein 3Q91W182 (34) TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331 (33) U2surpU2 snRNP-associated SURP motif-containing proteinQ6NV831 $(33, 34)$ Wbp11WW domain binding protein 11Q923D51 $(36, 70)$ Xm25'-3' exoRNase 2Q9DBR12 (36) ZfrZinc finger RNA binding proteinO885326 $(33, 36)$ Znf638Zinc finger protein 638Q6146427 (33)	Syncrip	Heterogeneous nuclear ribonucleoprotein O	07TMK9	23	(35, 36, 69)
Tdrd3Tudor domain-containing protein 3Q91W182(34)TmpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331(33)U2surpU2 snRNP-associated SURP motif-containing proteinQ6NV831(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xm25'-3' exoRNase 2Q9DBR12(36)ZfrZinc finger RNA binding proteinO885326(33, 36)Znf638Zinc finger protein 638Q6146427(33)	Taf15	TAF15 RNA polymerase II. TATA box binding protein-associated factor	08BO46	3	(36)
TimpoLamina-associated polypeptide 2, isoforms $\alpha/zeta$ Q610331(33)U2surpU2 snRNP-associated SURP motif-containing proteinQ6NV831(33, 34)Wbp11WW domain binding protein 11Q923D51(36, 70)Xrn25'-3' exoRNase 2Q9DBR12(36)ZfrZinc finger RNA binding proteinO885326(33, 36)Znf638Zinc finger protein 638Q6146427(33)	Tdrd3	Tudor domain-containing protein 3	Õ91W18	2	(34)
U2surRNP-associated SURP motif-containing protein Q6NV83 1 (33, 34) Wbp11 WW domain binding protein 11 Q923D5 1 (36, 70) Xm2 5'-3' exoRNase 2 Q9DBR1 2 (36) Zfr Zinc finger RNA binding protein O88532 6 (33, 36) Znf638 Zinc finger protein 638 Q61464 27 (33)	Tmpo	Lamina-associated polypeptide 2, isoforms $\alpha/zeta$	Õ61033	1	(33)
Wbp11 WW domain binding protein 11 QP 1 (36, 70) Xrn2 5'-3' exoRNase 2 Q9DBR1 2 (36) Zfr Zinc finger RNA binding protein O88532 6 (33, 36) Znf638 Zinc finger protein 638 Q61464 27 (33)	U2surp	U2 snRNP-associated SURP motif-containing protein	Õ6NV83	1	(33, 34)
Xm2 5'-3' exoRNase 2 Q9DBR1 2 (36) Zfr Zinc finger RNA binding protein O88532 6 (33, 36) Znf638 Zinc finger protein 638 Q61464 27 (33)	Wbp11	WW domain binding protein 11	O923D5	1	(36, 70)
Zfr Zinc finger RNA binding protein O88532 6 (33, 36) Znf638 Zinc finger protein 638 Q61464 27 (33)	Xrn2	5'-3' exoRNase 2	Õ9DBR1	2	(36)
Znf638 Zinc finger protein 638 Q61464 27 (33)	Zfr	Zinc finger RNA binding protein	Õ88532	6	(33, 36)
	Znf638	Zinc finger protein 638	Q61464	27	(33)



Fig. 4. ZNF638 modifies splicing of minigene reporter transcripts. Schematic representation of the fibronectin minigene reporter and of the assay performed (A). EI-specific primers (filled arrows) and ES primers (empty arrows) were used to quantify the mRNA isoforms retaining the alternative spliced exon 25 (black boxes) and the short isoform skipping the alternatively spliced exon. Gray boxes indicate constitutively retained exons; thin lines, introns; and thick lines, spliced isoforms. Quantification of the ratio of EI to ES in HEK-293 cells transiently expressing the minigene plasmid with ZNF638 or vector control (B). Mean ± SEM. Statistical analysis was performed on three independent experiments. ** *P* < 0.01. Western blot of ZNF638 expression levels from a representative experiment (C). β-actin was used as loading control. Schematic representation of the fibronectin minigene containing C/EBP responsive elements in the promoter (D). Ratio of EI over ES in HEK-293 cells transiently expressing the minigene of ZNF638 and C/EBP factors (E). Experiments were repeated at least three times. A representative experiment is shown. Mean ± SEM; n.s., not statistically significant; # *P* < 0.05, ## *P* < 0.01, ### *P* < 0.001. Western blot analysis to assess protein levels of transiently expressed ZNF638, C/EBPα, C/EBPδ in a representative experiment. β-actin was used as loading control (F).

Our systematic analysis of ZNF638 interactors in adipocytes revealed that ZNF638 complexes with 16 factors shown to be part of the early prespliceosomal complex, including U2SURP, SRSF7, TDRD3, SF3B1, FUS, DDX46, DDX5, DHX9, ELAVL1, ILF3, NCBP1, RBM39, and the HNRNP proteins A1, A2/B1, A3, and U (34). These interactions suggest an early association of ZNF638 with the splicing machinery and its participation in splice site selection during prespliceosomal assembly (4). Particularly noteworthy is the interaction of ZNF638 with HNRNPA1, HNRNPA2B1, and HNRNPLL, which have been critically implicated in alternative splicing (37-42). Given that HNRNPA1 functions by antagonizing the splicing factor ASF/SF2 on splice site selection (37), it is possible that ZNF638 may affect the relative local abundance of HNRNPA1 through binding and sequestration, shifting the cellular balance in favor of the splicing factor SF2, as proposed in Fig. 6A.

Our mass spectrometry analysis has also revealed that the C-terminal portion of ZNF638 interacts with other ZNF638 proteins present in adipocytes, suggesting that either multiple ZNF638 molecules may be recruited to protein complexes formed by ZNF638 or that ZNF638 may be able to di- or multimerize. Direct verification of the functional significance of these findings in adipocyte differentiation will require further investigation.

The present data indicating that ZNF638 affects alternative splicing of a minigene containing C/EBP binding sites combined with the previous reported function of ZNF638 in transcription through interaction with C/EBP proteins at C/EBP responsive elements (2) suggest that ZNF638 may act as a dual function regulator. This double role is consistent with emerging evidence indicating that cofactors can participate in both transcription and splicing, as described for p52, RNA binding motif protein 39, RNA binding motif protein 14, and PGC1 α (20–23). It is plausible that when loaded on a promoter through C/EBP responsive elements, ZNF638 could facilitate the recruitment of the splicing factor(s) required for splice choice to the CTD of the largest subunit of RNA polymerase II (3, 43) and to the proximity of the nascent pre-mRNA (Fig. 6B).



Fig. 5. Requirements of ZNF638 domains in adipocyte differentiation. Quantification of lipid accumulation in 10T1/2 cells expressing vector, full-length ZNF638, or deletion mutants at day 6 after induction of differentiation measured through quantification of Oil Red O extracted from stained cells (A). mRNA levels of adipocyte markers in 10T1/2 cells expressing either vector, full-length ZNF638, or deletion mutants, after 3 days of differentiation (B). Ratio of alternatively spliced isoforms during adipocyte differentiation in 10T1/2 cells expressing either vector, full-length ZNF638, or deletion mutants, after 3 days of induction of differentiation (C). A, B, C: Mean ± SEM. Full-length ZNF638 compared with vector control: # P < 0.05, ## P < 0.01. Mutants compared with full-length ZNF638: * P < 0.05, ** P < 0.01.

This model is supported by the identification through our mass spectrometry analysis of SRSF4, a splicing factor previously shown to be involved in alternative splice site selection during pre-mRNA processing (44, 45), as a novel ZNF638 interactor. This putative dual function of ZNF638 is reminiscent of the splicing role played by the coactivator PGC1 α when loaded on the promoter of its target genes (23).



Fig. 6. Proposed models of ZNF638's mode of action on alternative splicing. Sequestration model (A). ZNF638 may affect splicing decisions by altering the balance between available inhibitory and activating splicing factors competing for their binding to the pre-mRNA. Via sequestration of HNRNP proteins like HNRPA1, ZNF638 may prevent their binding to splice sites on the pre-mRNA, increasing the availability of activating splicing factors, such as SF2. Facilitator model (B). The loading of ZNF638 on the promoters of its target genes via C/EBP responsive elements may facilitate the recruitment of SR proteins to the CTD of the polymerase II and to the nearby nascent pre-mRNA. A, B: Purple exon is an alternatively spliced exon. Pol II, RNA polymerase II.

Through mutagenesis and confocal microscopy analysis, we showed that the RS domain and the C-terminal ZF motif are required for ZNF638's localization in nuclear bodies enriched in splicing factors. While it has been previously recognized that RS domains can confer speckle targeting (46–48), our data indicate that also the U1/matrin-like ZF (C-X₂-C-X_(12,16)-H-X₅-H) (49) present at the C terminus of ZNF638 is required for speckle localization. Interestingly, this type of ZF motif has been previously identified in other proteins present in nuclear speckles and shown to be required for their localization, as in the case of SF3A2 and SF3A3 (50). These observations suggest a possible contribution of matrin-like ZFs in targeting proteins to nuclear speckles.

Our structure-function analysis has revealed that the RS and the C-terminal ZF domains required for speckle localization are also necessary for the differentiation function of ZNF638. While it is well established that RS domains play a role in splicing (51), the requirement of SR domains in adipocyte differentiation has been only recently identified through the analysis of the RS domain in the function of the CDC-like kinase 1) kinase in adipocyte differentiation (52). Further studies will determine whether the RS and C-terminal ZF domains present in ZNF638 are necessary for the generation of adipocyte-specific isoforms through their function as splicing activation domains via interactions with spliceosomal components or whether their role in adipocyte differentiation is through the recruitment of cofactors involved in transcription.

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