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Supplemental information

A seven-transmembrane protein-TM7SF3, resides in nuclear speckles and regulates alternative splicing

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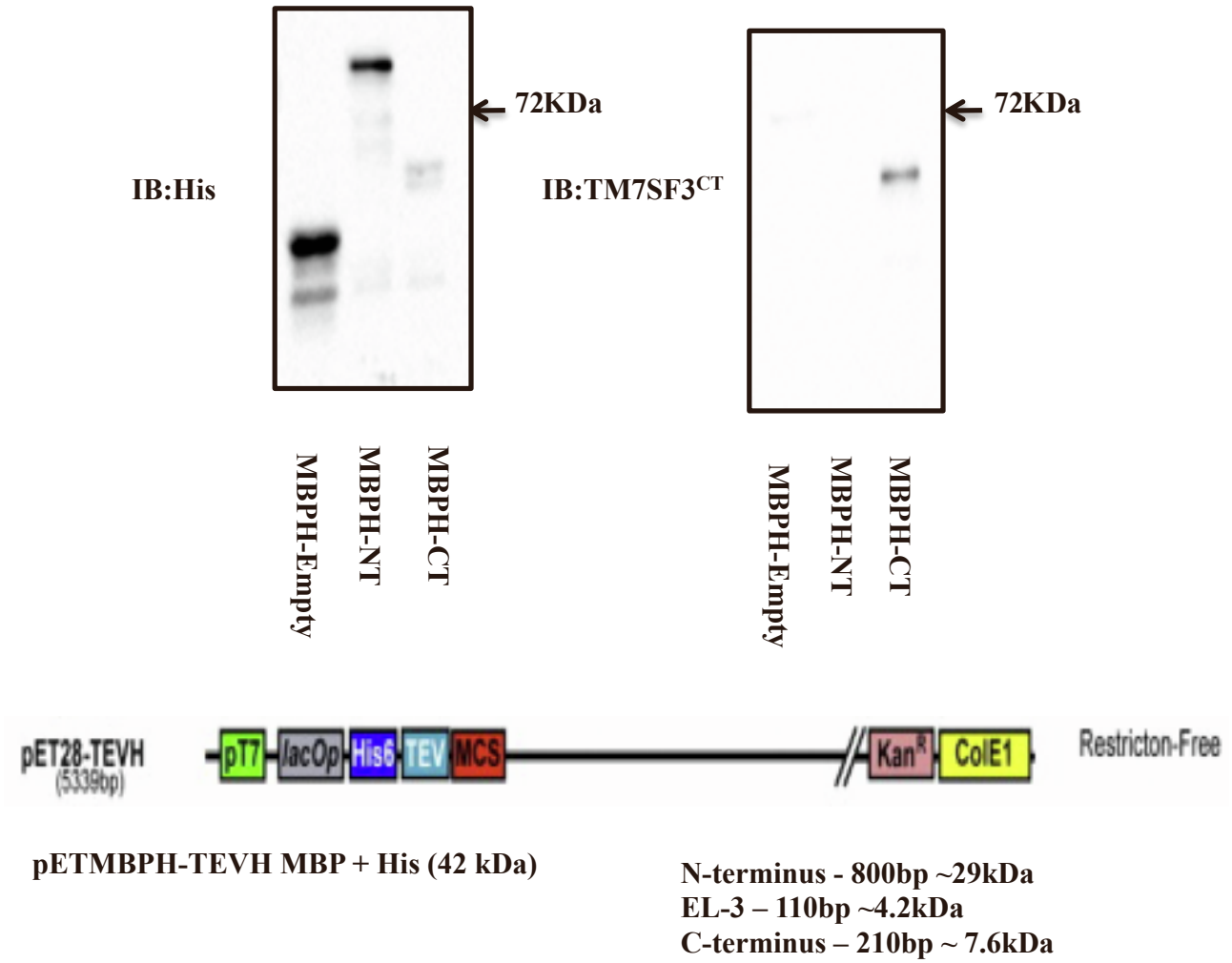
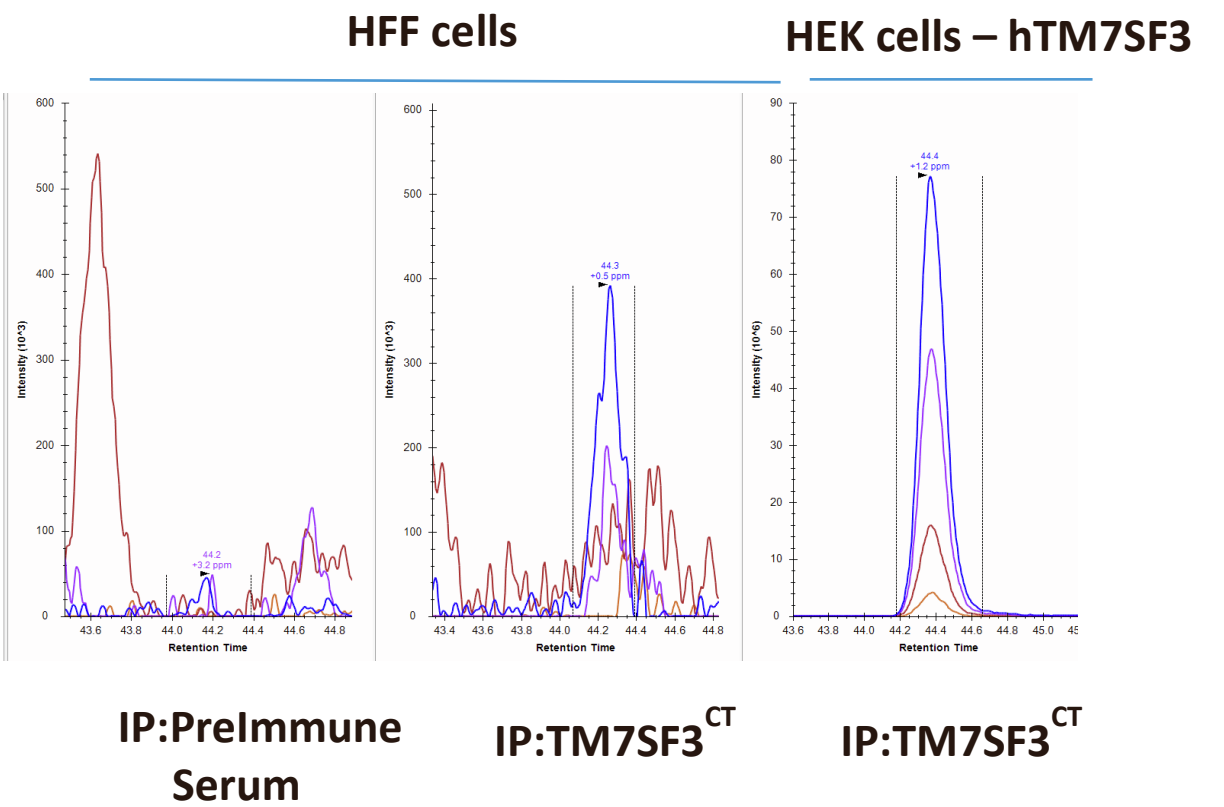
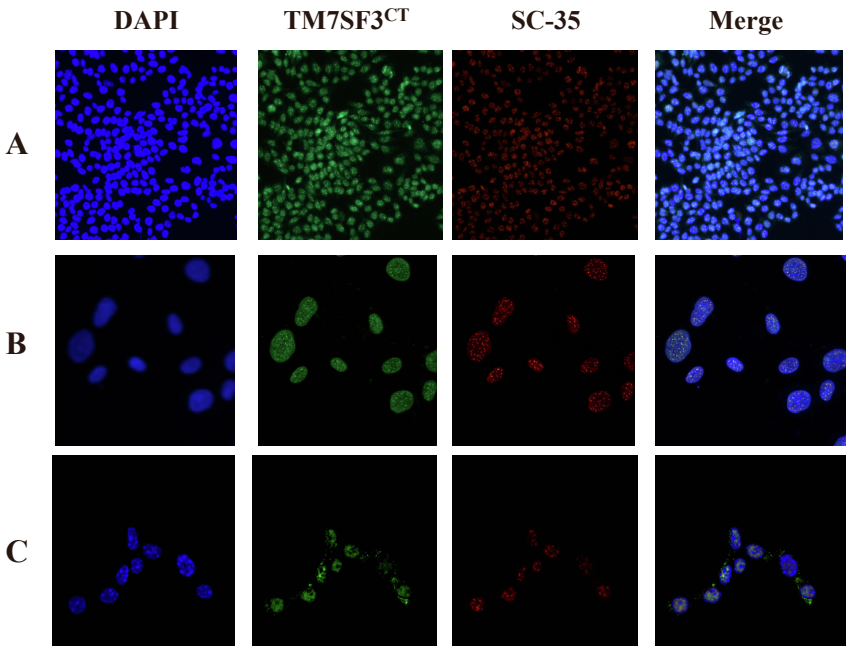


Fig S1. TM7SF3^{CT} antibodies interact with the C terminal end of the TM7SF3 protein. Related to Figure 1. Proteins were extracted from DH5alpha cells expressing maltose binding protein (MBP) with 6XHIS tag alone, or cells expressing recombinant TM7SF3 protein fragments. An N-terminus fragment (aa 24-290) and a C-terminus fragment (aa 496-564), both tagged at their amino terminus with 6xHis and MBP (Maltose binding protein)-TEVH. Samples were immunoblotted with antibodies against His or TM7SF3^{CT} as indicated.



https://youtu.be/lmrtvv3DNJQ

Fig. S2 Examples of Unique peptides identified by mass spectrometry. Related to Figure 1. Unique peptide intensity (FAPANLGYAR; blue peek) among 3 unique TM7SF3 peptides that were identified in experiment described in Table S4 B-C.



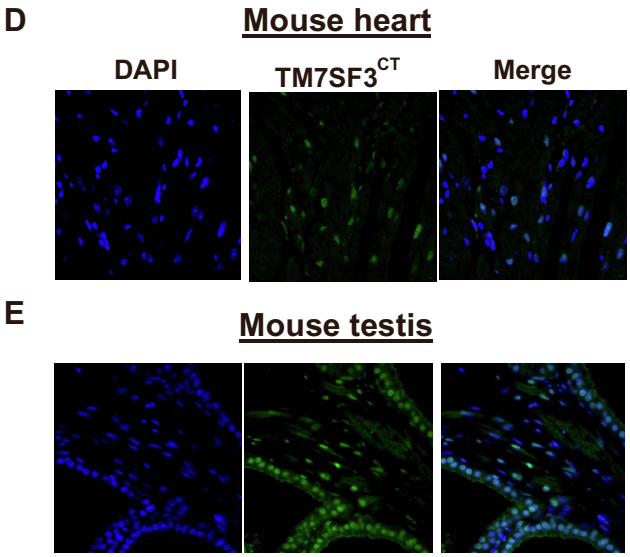


Fig S3. TM7SF3 is localizes to nuclear speckles. Related to Figure 2. A-C. HCT (**A**), HFF (**B**) and MIN6 (**C**) cells were fixed by MeOH for 40 min. and stained with DAPI (blue) and immunostained with anti-TM7SF3^{CT} (Green) and anti-SC-35 (Red) antibodies. Paraffin sections of mouse Heart (**E**) and Testis (**F**) were stained with DAPI (blue) and immunostained with anti-TM7SF3^{CT} (Green) antibodies. n=2-3.

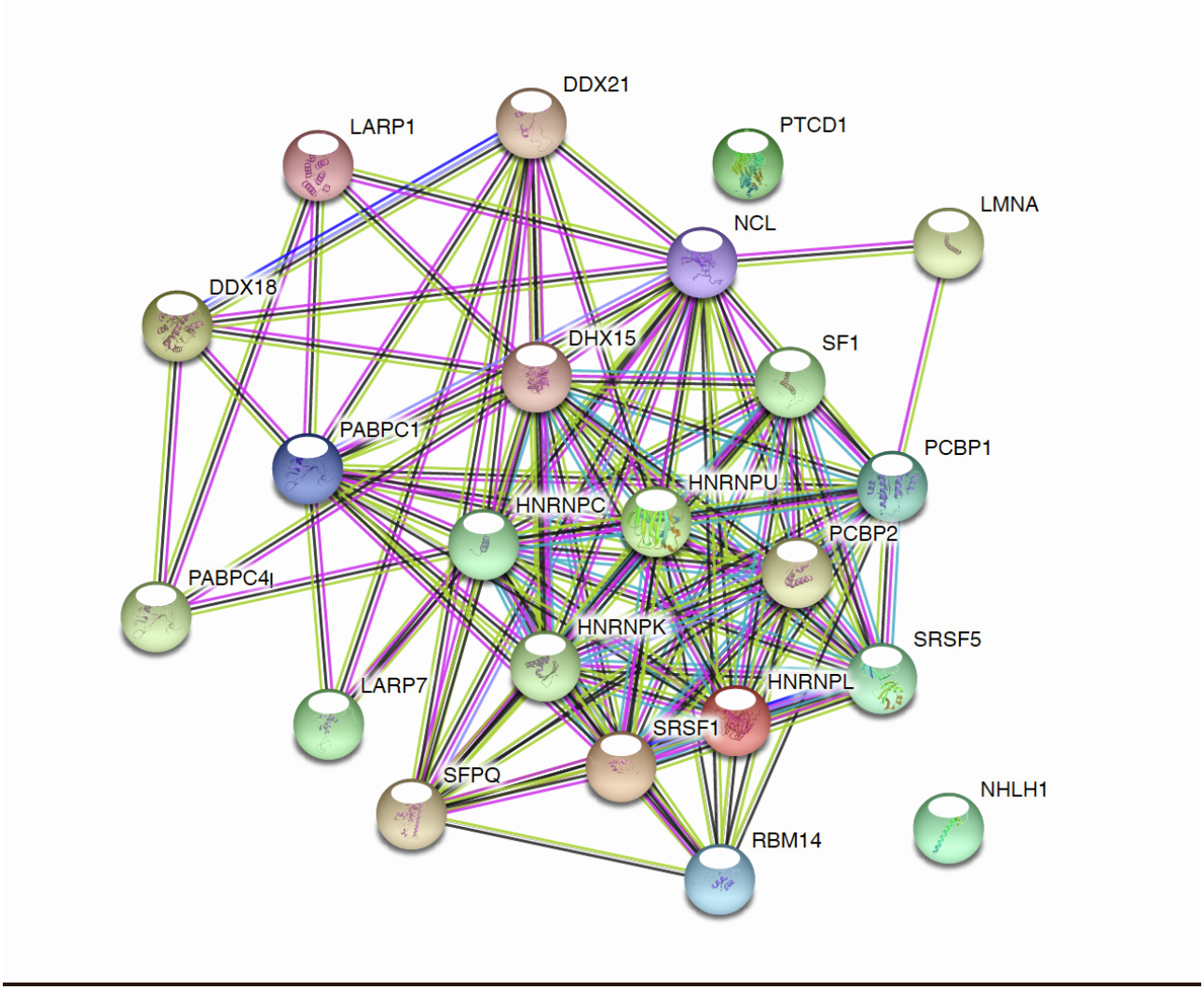
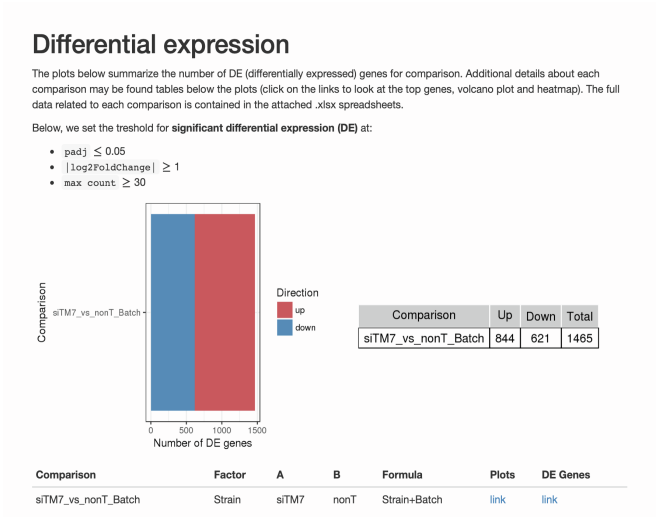
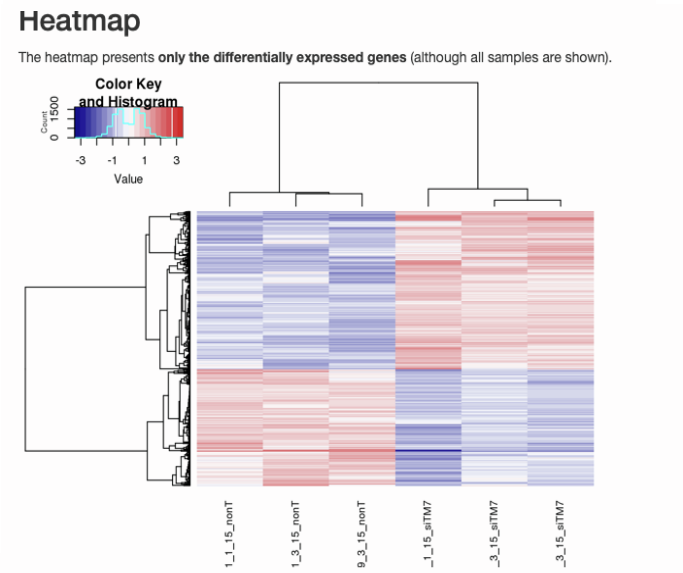


Fig S4. STRING analysis of RNA binding proteins that co-precipitated with TM7SF3 and the splicing regulators that interact with them. Related to Figure 4 (DHX15, DDX21, NCL, LMNA , HNRNPK, PABPC1 , LARP7, HNRNPU ,HNRNPL, PABPC4 , PTCD1, DDX18, RBM14, LARP1) and the splicing regulators (SRSF1, HNRNPC, PCBP1, PCBP2, HEN1, SRSF5, SFPQ, and SF1, Table S6) that bind the motif at the start/end of introns, having the highest confidence of enrichment in siTM7SF3 (PPI enrichment p-value: $< 1.0^{e-16}$).

A



B



C

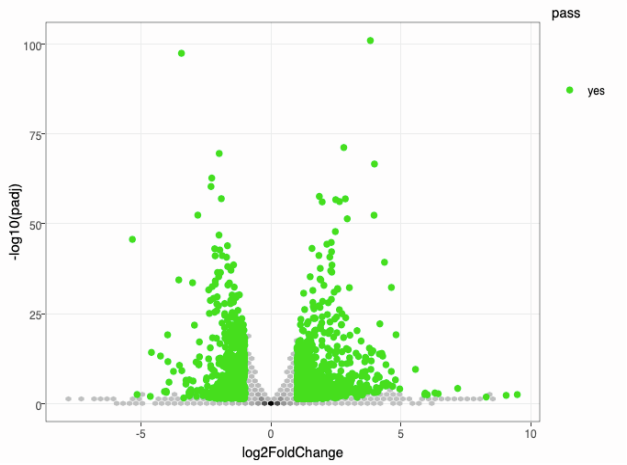


Fig S5 Differential expression genes in U2-OS cells that were transfected with siRNA TM7SF3. Related to Figure 4. RNAseq was performed as described in methods. The threshold set for significant differential expression (DE) genes is shown in (A). The genes that were differentially expressed (844 upregulated and 621 downregulated) is shown in the heatmap (B). The Volcano plot the genes whose expression was significantly affected is shown in C.

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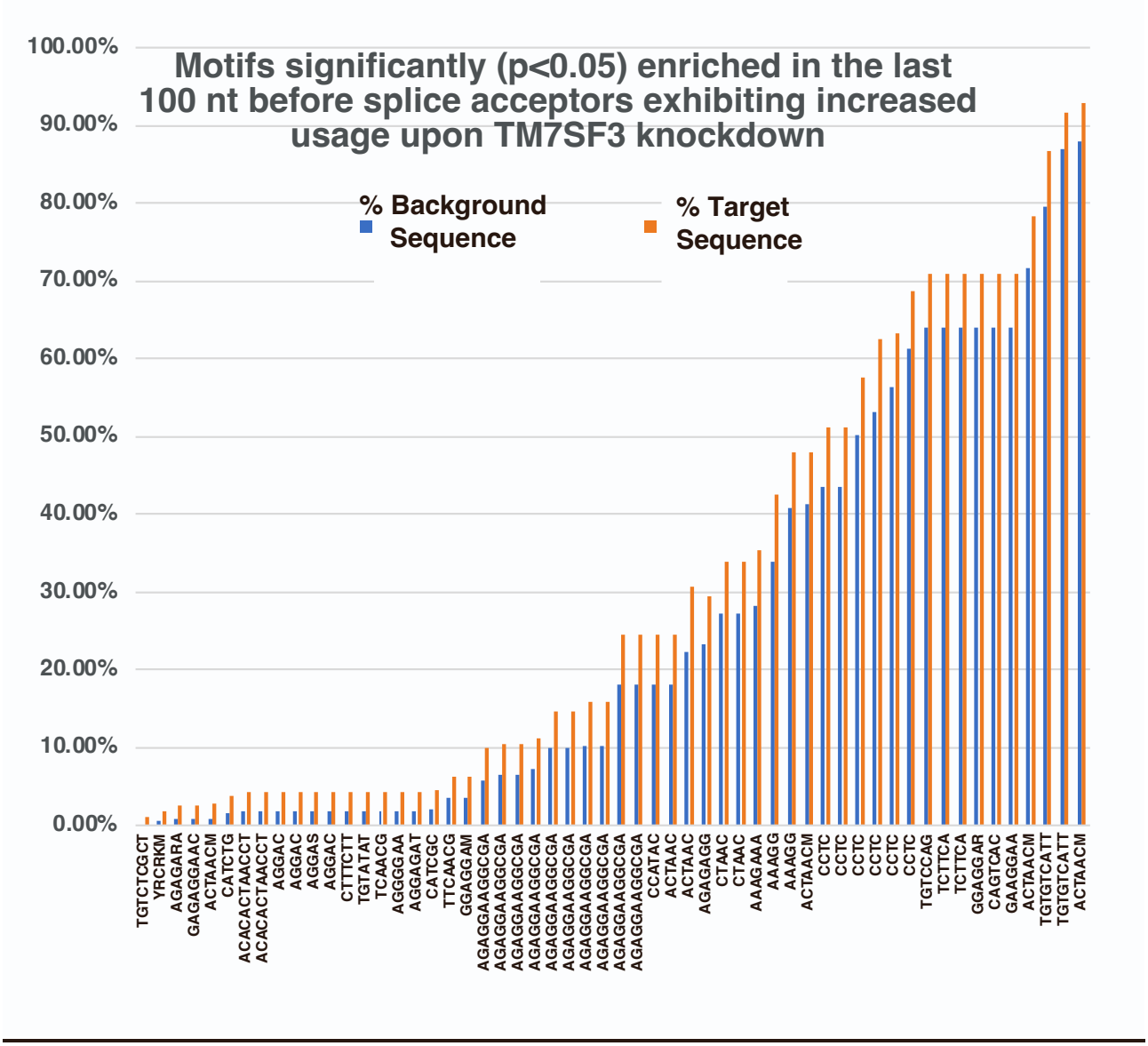

Fig S6 RNA binding protein motifs enriched around the last 100 nt before splice acceptor sites, exhibiting increased usage upon TM7SF3 silencing. Related to Figure 4. The motifs with significant enrichment after multiple testing correction (FDR adjusted p-value <0.05; Benjamini-Hochberg) is shown for increased spliced site usage upon knock-down of TM7SF3. The % background sequences containing the motif in untreated cells and the % target sequences containing the motif in cells expressing siTM7SF3, are shown. Only motifs enriched around the last 100 nt before splice acceptor sites, exhibiting increased usage upon TM7SF3 silencing are reported. The full data list is provided in the accompanying Excel file of Table S6.

Supplemental Tables

Table S1. Growth medium of the cell-lines used. Related to STAR Methods

Cell line	Growth medium	Comments
HFF	MEM-Eagle (01-040-01) 15% FBS	
U2-OS	McCoy's 5A 10% FBS	
HEK 293, HepG2, HCT	DMEM (4.5 g glucose/L), 10% FBS	
MIN6	DMEM 10% FBS, 11.1mM glucose	70 μM β-mercaptoethanol
Human Islets	CMRL 1066 medium containing 10% (FBS), 1% L-glutamine	40 μg/ml Gentamycin

Table S2. List of primers used for real time PCR analysis in this study. Related to STAR Methods

Primer Name	**Sequence (5'-3')**
hPIG3ex1 f	AGGACCGGAAAACCTCTACG
hPIG3ex1 r	GCCACCTTCAGGAGGACTTC
hPIG3ex35 f	CTGAAATTCACCAAAGTACAAGCA
hPIG3ex35 r	CTGGATTTCGGTCACTGGGT

Table S3. **List of primers used for PCR analysis of genes whose alternative splicing is affected by TM7SF3. Related to STAR Methods**.

Oligo Name	Sequence (5'-3')
EZH2 F	GTGAGCTCATTGGCGGGACTAGGGAG
EZH2 R	ATTCAGAGTCTTTAATGGGATGACT
SRSF7 F	GGGAATGAAGTGAGGCCAGTGG
SRSF7 R	AATACCGCCAGAATCCATGGG
XBP1 F	GCGATCGGAGGCTCAGAGAAC
XBP1 R	GCCCTCGAACACACTGTAGGG
EIF6 F	GCGATCGGAGGCTCAGAGAAC
EIF6 R	GCCCTCGAACACACTGTAGGG
VEGFB F	AGGGAGACGTTTGCAGTGAG
VEGFB R	TGCCATGCTTGTAATCCAGCG
MRPL52 F	AGGGAGACGTTTGCAGTGAG
MRPL52 R	TGCCATGCTTGTAATCCAGCG
PIG3 F	TTACGACGGGTTGGGGATGG
PIG3 R	TGCCAAACCAGCTCCTACTC
MCL1 F	TTACGACGGGTTGGGGATGG
MCL1 R	TGCCAAACCAGCTCCTACTC
ATP5C1 F	ACCACTAGTGAGCAGAGTGC
ATP5C1 R	TTCTTCGGACAAAGGCAGCA
TM7SF3 F	AGTGCTCTCAAGGTGGTTAC
TM7SF3 R	GAAACGGGTCCCAAACAATG
GAPDH F	CCACTCCTCCACCTTTGAC
GAPDH R	ACCCTGTTGCTGTAGCCA
CLSTN1 F	TTCTGCAGCTCACAGTCCAG
CLSTN1 R	CCACCTACCACGGCATAGTC
ADD3 F	ACCAGCTCCTCCTAACCCAT
ADD3 R	TCACTCGCTTAGCAAGCTCAT
VLDLR F	CCTGCCAGCACCACAGATTA
VLDLR R	CCCTTTTGGGGGAACACTGA
ECHDC1 F	ACCCAATGCCCAACCTTGAA
ECHDC1 R	GGAAAGGCCTCATTGTCCGT
PLEKHM2 F	GGTCCACGGCTCAGACAG
PLEKHM2 R	CCTCGTTGAAGGGGTTCTGG

Table S4 TM7SF3 protein identified by Mass spectrometry in samples immunoprecipitated by TM7SF3^{CT} antibodies. **Related to Figure 1**. Proteins were extracted from U2-OS (**A**), HFF or HEK (**B**) cells overexpressing the human TM7SF3 protein. Samples were immunoprecipitated with Pre-immune serum or TM7SF3^{CT} antibody and gel bands (above and below 72KDa) were subjected to Mass spectrometry analysis.

A

Accession	Description							
Q9NS93	Transmembrane 7 superfamily member 3 OS=Homo sapiens GN=TM7SF3 PE=2 SV=1							
	ΣCoverage	Σ# Proteins	# Unique Peptide	Σ# Peptides	Σ# PSMs			
	8.77	8	4	4	16			
	IP:PreImmune serum							
	Mascot				Sequest			
	Score	Coverage	# Peptides	# PSM	Score	Coverage	# Peptides	# PSM
Gel Band #1						0.00		
Gel Band #2						0.00		
	IP:TM7SF3-CT antibody							
	Mascot				Sequest			
	Score	Coverage	# Peptides	# PSM	Score	Coverage	# Peptides	# PSM
Gel Band #1	109.51	7.02	3	3	5.91	4.21	2	2
Gel Band #2	59.48	5.44	2	2	5.79	4.21	2	2
Gel Band #3	138.15	6.14	3	3	10.81	8.77	4	4

B.

Accession	Description							
Q9NS93	Transmembrane 7 superfamily member 3 OS=Homo sapiens GN=TM7SF3 PE=2 SV=1							
	ΣCoverage	Σ# Proteins	Unique Pept	Σ# Peptides	Σ# PSMs			
	10.18	6	3	5	15			
	HFF cells - IP:PreImmune Serum							
	Mascot				Sequest			
	Score	Coverage	# Peptides	# PSM	Score	Coverage	# Peptides	# PSM
Gel Band #1						0.00		
Gel Band #2						0.00		
	HFF cells - IP:TM7SF3 CT Antibody							
Gel Band #1		0.00				0.00		
Gel Band #2		0.00				0.00		
	HEK cells overexpressing hTM7SF3 - IP:TM7SF3 CT Antibody							
Gel Band #1	125.98	6.14	3	3	6.10	4.39	2	2
Gel Band #2	174.67	7.02	3	4	17.31	8.42	4	6

Table-S9- Gene set enrichment analysis. Related to Figure 4. Analysis was carried out as described under "STAR Methods". Briefly, DESeq2 was used on the raw count values for each sample to identify differential expressed genes between the two conditions. The Wald test was used within DESeq2 to rank the genes according to their Wald test statistic. Gene set enrichment analysis (GSEA) was employed using the fgsea package along with the MSigDB 7.0 collections: KEGG and REACTOME curated gene sets, and Gene Ontology (GO) gene sets. The default two-sided enrichment p-value with Benjamini–Hochberg correction from the fgsea package was utilized.

pathway	pval	padj
REACTOME_ACTIVATED_PKN1_STIMULATES_TRANSCRI	0.0002	0.003617
REACTOME_TELOMERE_MAINTENANCE	0.000202	0.003617
REACTOME_ERCC6_CSB_AND_EHMT2_G9A_POSITIVELY	0.000201	0.003617
REACTOME_CONDENSATION_OF_PROPHASE_CHROMOS	0.000199	0.003617
REACTOME_MEIOTIC_RECOMBINATION	0.000203	0.003617
REACTOME_HDACS_DEACETYLATE_HISTONES	0.000202	0.003617
REACTOME_SIRT1_NEGATIVELY_REGULATES_RRNA_EX	0.0002	0.003617
REACTOME_DEPURINATION	0.0002	0.003617
REACTOME_BASE_EXCISION_REPAIR_AP_SITE_FORMAT	0.000199	0.003617
REACTOME_DNA_METHYLATION	0.0002	0.003617
REACTOME_RUNX1_REGULATES_GENES_INVOLVED_IN_	0.000202	0.003617
REACTOME_RHO_GTPASES_ACTIVATE_PKNS	0.000204	0.003617
REACTOME_NUCLEOSOME_ASSEMBLY	0.000203	0.003617
REACTOME_NONHOMOLOGOUS_END_JOINING_NHEJ	0.000203	0.003617
REACTOME_CHROMOSOME_MAINTENANCE	0.000203	0.003617
REACTOME_PRC2_METHYLATES_HISTONES_AND_DNA	0.000199	0.003617
REACTOME_BASE_EXCISION_REPAIR	0.000203	0.003617
REACTOME_RNA_POLYMERASE_I_PROMOTER_ESCAPE	0.000203	0.003617
REACTOME_MEIOTIC_SYNAPSIS	0.000203	0.003617

pathway	pval	padj
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	0.000203	0.003044
KEGG_PYRUVATE_METABOLISM	0.000202	0.003044
KEGG_PATHWAYS_IN_CANCER	0.00019	0.003044
KEGG_FOCAL_ADHESION	0.000192	0.003044
KEGG_ECM_RECEPTOR_INTERACTION	0.000198	0.003044
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	0.000192	0.003044
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	0.0002	0.003044
KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	0.000198	0.003044
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	0.000199	0.003044
KEGG_AUTOIMMUNE_THYROID_DISEASE	0.000201	0.003044
KEGG_LYSOSOME	0.000195	0.003044
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	0.000405	0.004774
KEGG_P53_SIGNALING_PATHWAY	0.000396	0.004774
KEGG_ALLOGRAFT_REJECTION	0.000403	0.004774
KEGG_CELL_ADHESION_MOLECULES_CAMS	0.000585	0.00643
KEGG_TYPE_I_DIABETES_MELLITUS	0.000996	0.010271
KEGG_VEGF_SIGNALING_PATHWAY	0.001385	0.013446
KEGG_GRAFT_VERSUS_HOST_DISEASE	0.001615	0.014803
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	0.002193	0.019041

pathway	pval	padj
GO_DNA_PACKAGING_COMPLEX	0.000204	0.00786
GO_PROTEIN_HETEROTETRAMERIZATION	0.000202	0.00786
GO_ORGAN_OR_TISSUE_SPECIFIC_IMMUNE_RESPONSE	0.000203	0.00786
GO_NUCLEAR_NUCLEOSOME	0.000405	0.012087
GO_LIGASE_ACTIVITY_FORMING_CARBON_OXYGEN_BONDS	0.001012	0.020075
GO_NUCLEOSIDE_BISPHOSPHATE_METABOLIC_PROCESS	0.001638	0.025561
GO_REGULATION_OF_LIPID_CATABOLIC_PROCESS	0.001411	0.023512
GO_DNA_REPLICATION_DEPENDENT_NUCLEOSOME_ORGANIZATION	0.002239	0.029806
GO_HIPPO_SIGNALING	0.001206	0.021638
GO_REGULATION_OF_DENDRITE_EXTENSION	0.003234	0.03737
GO_CALCIUM_ION_REGULATED_EXOCYTOSIS_OF_NEUROTRANSMITTER	0.002233	0.029806
GO_AMINO_ACID_ACTIVATION	0.002045	0.028165
GO_DENDRITE_EXTENSION	0.002032	0.028128
GO_MITOCHONDRIAL_RNA_METABOLIC_PROCESS	0.004093	0.042049
GO_REGULATION_OF_MICROTUBULE_DEPOLYMERIZATION	0.002435	0.031649
GO_THIOESTER_METABOLIC_PROCESS	0.003838	0.040887
GO_CELLULAR_RESPONSE_TO_CHOLESTEROL	0.006915	0.057
GO_PROTEIN_TRANSPORT_ALONG_MICROTUBULE	0.002046	0.028165
GO_ANTIBACTERIAL_HUMORAL_RESPONSE	0.007484	0.05869