Supplementary materials

Truncated forms of U2 snRNA (U2-tfs) are shunted toward a novel uridylylation pathway that differs from the degradation pathway for U1-tfs

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MATERIAL AND METHODS

Reagents and antibodies—Antibodies used are listed in Supplementary Table S5. All reagents used in this study were purchased from Wako pure chemical (Japan), Kanto Chemical (Japan) or Nakalai Tesque (Japan).

Cell culture and transfection—HEK293T and Flp-In T-REx-293 cells were maintained according to standard methods in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum in a humidified atmosphere of 95% air and 5% CO_2[1]. Transfection was performed by lipofection method[1]. Lipofection method was performed with Lipofectamine 2000 or Lipofectamine RNAiMAX reagent according to manufacturer's protocol (Thermo Fisher Scientific).

Construction of RAT RNA tag only expression vector—Annealed primer set of HIW394 and HIW395 (Supplementary Table S4) that contained pol III terminator sequence was cut at *Cla* I / *Eco*R I sites and ligated into the same sites as those in pRcU6PT7SK[2]; 7SK sequence present at the downstream of the RAT RNA tag sequence was replaced by terminator sequence and produces pRcU6PT vector that encodes only RAT tag RNA.

RNA interference—Stealth siRNA or Silencer Select siRNA (siRNA/scRNA/control RNA; Thermo Fisher Scientific) (Supplementary Table S6) transfection was performed using Lipofectamine RNAiMAX according to manufacture's protocol (Life Technologies)[3].

Construction of vectors expressing HEF/HF (HEF: FLAG-TEV-HA, HF: FLAG-HA) tagged proteins and cell lines expressing HEF/HF-proteins inducibly with doxycycline—Constructions of all vectors expressing HEF/HF-tagged proteins except enzyme-dead Dis3L2 are described previously[1]. To construct pcDNA5-FRT/TO vector expressing C-terminal epitope tagged enzyme-dead Dis3L2, we started first cutting pcDNA3.1(+)-C-terminal-HEF plasmid [1] with *Hin*d III / *Xho* I to obtain epitope tag coding fragment and ligated the digest into *Hin*d III / *Xho* I sites of pcDNA5-FRT/TO vector and named this vector as pcDNA5-FRT/TO-HEFc. A cDNA fragment encoding Dis3L2 (NM_152383), on the other hand, was obtained by PCR using primer set HIW561 and HIW562 (Supplementary Table S4) using the first strand DNAs as templates that were prepared by RT reaction of total RNA extracted from 293T cells. The PCR product was cut with *Bam*H I /*Sma* I, ligated into *Bam*H I / *Eco*R V sites of pcDNA5-FRT/TO-HEFc vector prepared above, verified by DNA sequencing and used for further mutant construction. The vector expressing a Dis3L2 enzyme-dead mutant, in which a conserved catalytic residue, aspartic acid (D391) was replaced by asparagine (Dis3L2-D391N) by site directed PCR mutagenesis using primer set HIW591 and HIW592 (Supplementary Table S4)[4,5]. The PCR product was subjected to *Dpn* I (methylation specific restriction endonuclease) digestion for removing template, and used in *E. coli* transformation to obtain clonal plasmid (pcDNA5-FRT/TO-Dis3L2-D391N-HEFc). The doxycycline inducible cells expressing Dis3L2-D391N-HEF were obtained by co-transfection of pcDNA5-FRT/TO-Dis3L2-D391N-HEFc with pOG44 vector into Flp-In T-REx-293 cells and selected as described[1].

General procedures for RNA and protein analyses—Total RNA extraction was performed with TRIzol reagent (Thermo Fisher Scientific) according to manufacturer's protocol. Other RNA experiments including RNA extraction from Pull-downed ribonucleoprotein (RNP) complex, Urea-PAGE followed by northern blotting, RNA-MS analyses were performed as described by Ishikawa et al.[1]. Protein analysis of SDS-PAGE followed by western blotting was also performed with modification of the methods described by Ishikawa et al.[1]; briefly, combination of alkaline phosphatase-NBT/BCIP detection method was changed to HRP based detection method; accordingly, HRP-conjugated antibodies were used for secondary antibodies in western blotting (Supplementary Table S5), and Clarity™ Western ECL substrate or Clarity Max™ Western ECL substrate for detection. Chemiluminescence was detected with LAS4000 luminescent image analyzer (FUJI FILM) and stained band was quantified with ImageJ (NIH: open source, public domain) software.

1. Ishikawa H, Nobe Y, Izumikawa K, Yoshikawa H, Miyazawa N, Terukina G, Kurokawa N, Taoka M, Yamauchi Y, Nakayama H, et al. Identification of truncated forms of U1 snRNA reveals a novel RNA degradation pathway during snRNP biogenesis. Nucleic Acids Res 2014; 42:2708-24; PMID: 24311566; https://doi.org/10.1093/nar/gkt1271
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4. Lubas M, Damgaard CK, Tomecki R, Cysewski D, Jensen TH, Dziembowski A. Exonuclease hDIS3L2 specifies an exosome-independent 3'-5' degradation pathway of human cytoplasmic mRNA. EMBO J 2013; 32:1855-68; PMID: 23756462; https://doi.org/10.1038/emboj.2013.135
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Supplementary Figure Legends

Supplementary Figure S1 Reverse-phase LC separation of the RNase T1 digest of U2-tfsL or U2-tfs

(A) Gemin5-HEF (hemagglutinin/TEV protease/FLAG tag)- or HEF-associated RNAs pulled down with anti-FLAG antibody-fixed beads were stained with SYBR Gold after Urea-PAGE separation. Sizes of RNA markers (Base) are shown at left. Boxed areas were excised and analyzed by LC/MS. (B) Effluent was monitored at m/z=917.09 (m^7G-capped AUCGp + one methyl residue, MMG-1m; top) or m/z=924.10 (m^7G-capped AUCGp + two methyl residues, MMG-2m; bottom). MMG-1m and MMG-2m are present in both the U2-tfsL and U2-tfs digests.

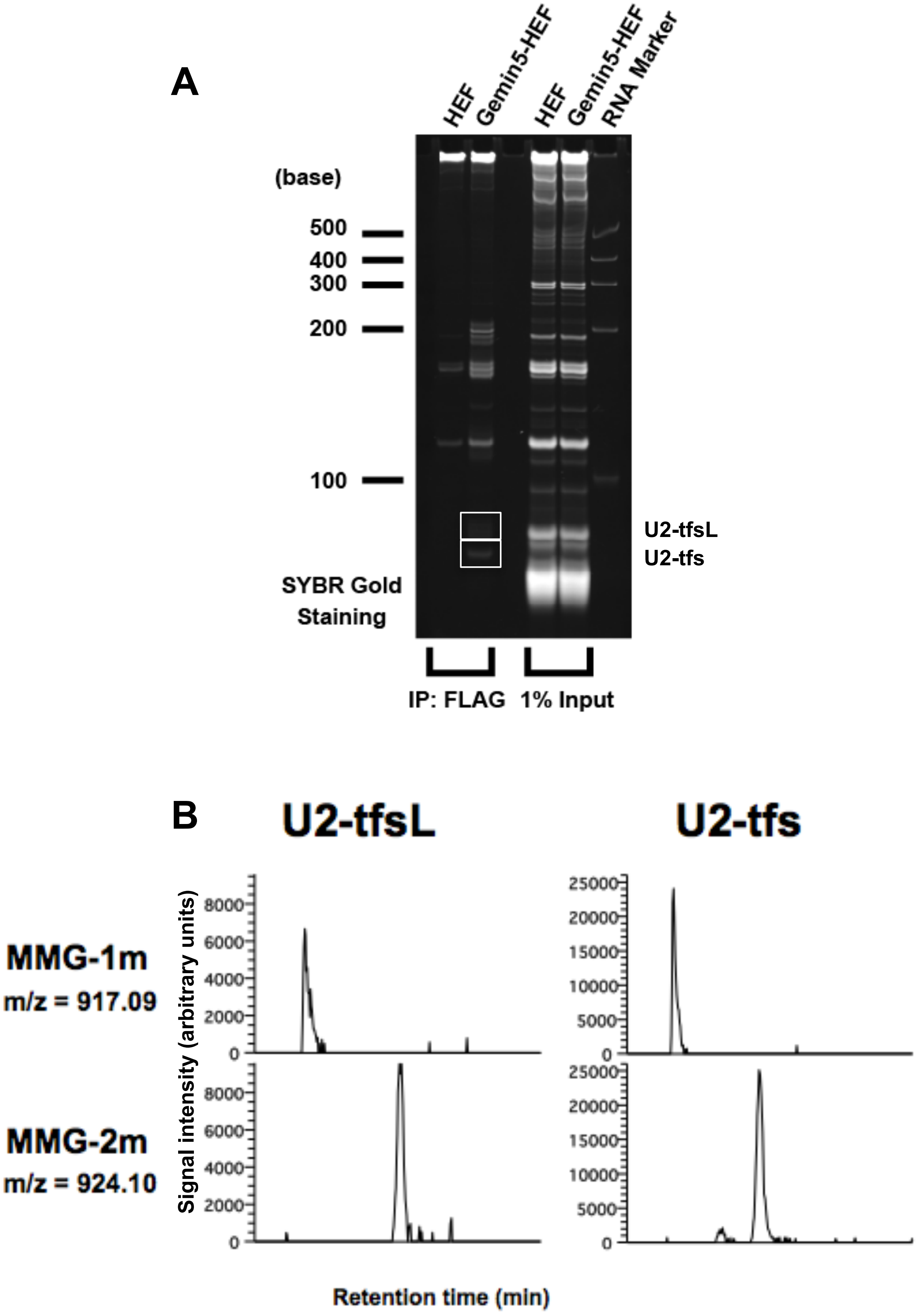
Supplementary Figure S2 Construction of vectors expressing y18Sn-tagged U2

(A) Experimental scheme for the U2 gene construct expressing y18Sn-tagged U2 snRNA. The cleavage sites for the restriction enzymes used and the regions amplified by PCR, are shown. (B) Nucleotide sequence of the U2 gene construct (based on Homo sapiens chromosome 17, GRCh38.p7 Primary Assembly, NC_000017.11, Range: 43232907 to 43234977). Red text shows matured U2 sequence, magenta a sequence of Oct, light green a sequence of PSE and blue a sequence of 3' box**.** An extra sequence corresponding to the first 11 nucleotides at the 5' end of U2 (ATCGCTTCTCG, U2-5' in (A), the y18Sn tag sequence (blue background CGAGGATTCAGGCTTTGG), Bgl II recognition sequence (AGATCT) and Cla I recognition sequence (ATCGAT) are underlined. (C) Total RNA extracted from cells expressing y18Sn-U2 or y18Sn-U1 was analyzed by northern blotting using the probe for y18Sn. (D) Schematic secondary structure for the U2 mutants expected to be produced from the short U2 gene constructs (with or without the 3' box) lacking the SmSL3SL4 region (U2-ΔSmSL3SL4), Sm (U2-ΔSm), SL3 (U2-ΔSL3), SL4 (U2-ΔSL4), or SL3SL4 (U2-ΔSLSL4). Those constructs contain the region containing the 190-nt sequence upstream of the Oct and the 229-nt sequence downstream of the 3' box in the sequence shown in Supplementary Fig. S2B. (E) RNA extracted from cells expressing U2 gene [y18Sn-U2 (2086 bp)] and short U2 gene [y18Sn-U2 (908 bp)] constructs shown was analyzed by northern blotting using the probes shown to the left of the blot. A long-exposure image is shown for probe y18Sn. The U2 gene construct lacking a cis-element (y18Sn-U2-ΔOct, y18Sn-U2-ΔPSE , y18Sn-U2-Δ3' box) were prepared from the short U2 gene construct. The RAT tag was used for expression control.

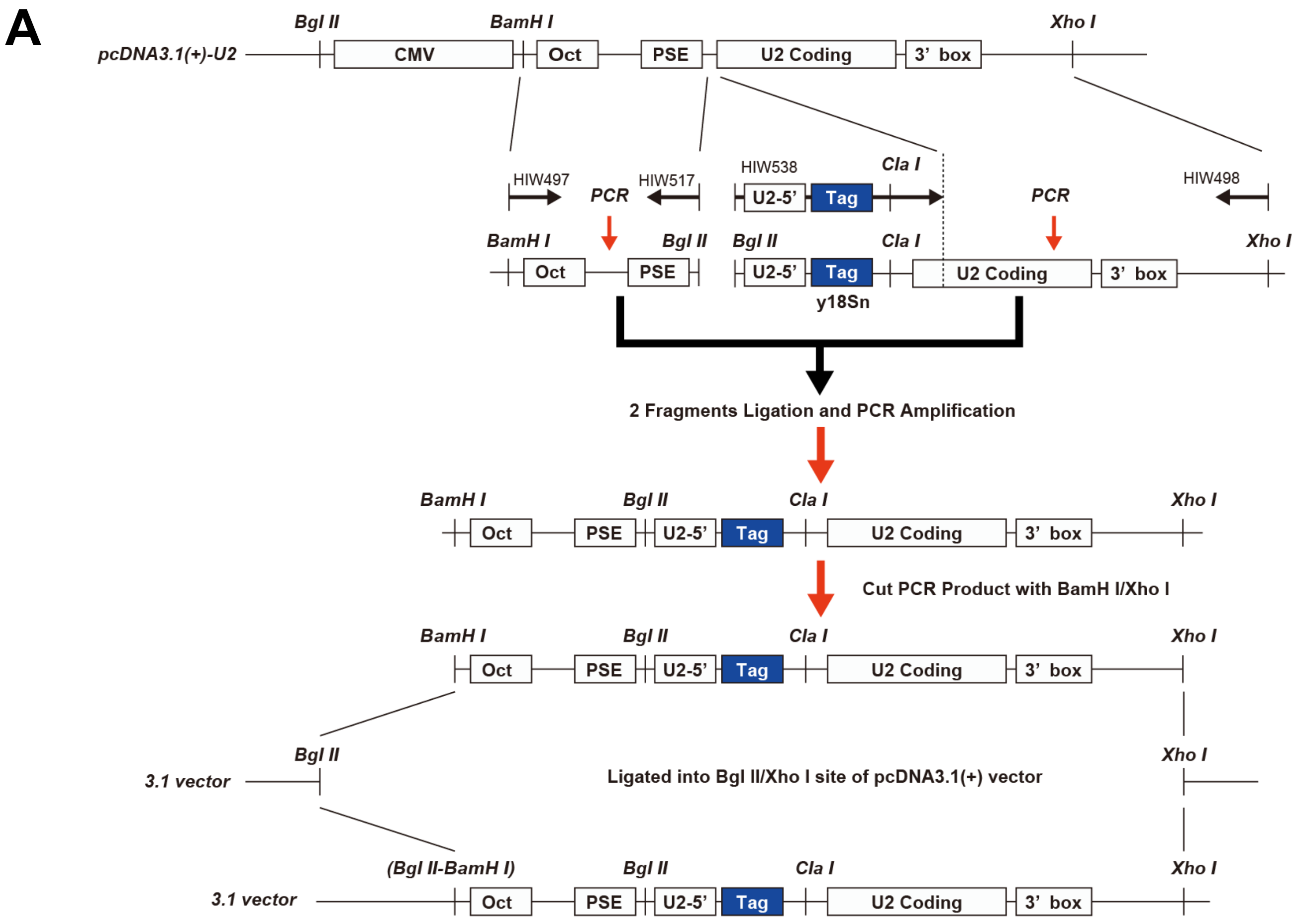
Supplementary Figure S3 Reciprocal effects of SmB/B' and TUT7 on the cellular level of U2-tfs/U2-tfs-polyU

(A) Deficiency of TUT4 does not affect the level of U2-tfs/U2-tfs-polyU. RNA extracted from cells at 48 h and 72 h post-transfection with Silencer Select siRNA (+) or Silencer Select Negative Control siRNA (-) for knockdown of SmB/B', Dis3L2, or TUT4, or a combination of these siRNAs was analyzed by northern blotting using probes shown to the left (lower panel). Protein was analyzed by immunoblotting (IB) with antibodies against the proteins shown to the left (upper panel). (B) Deficiency of TUT7 increases the level of U2-tfs. RNA extracted from cells at 72 h post-transfection with Silencer Select siRNA (+) or Silencer Select Negative Control siRNA (-) for knockdown of SmB/B’, Dis3L2, or TUT7, or a combination of these siRNAs was analyzed by northern blotting using probes shown to the left (lower panel). Stained bands corresponding to U2-tfs and U2-tfs-polyU are shown to the right. Proteins were analyzed by immunoblotting with antibodies against the proteins shown to the left (upper panel). (C) HEF-SmB/B’ or snurportin (SPN1)-HF was pulled down and analyzed by immunoblotting (IB) with the antibody against the corresponding protein indicated at left (upper left), or by northern blotting with the probe indicated at left (right). SYBR gold staining of RNAs pulled down by HEF-SmB/B’ or SPN1-HF are shown (lower left).

Supplementary Figure S1



Supplementary Figure S2



B

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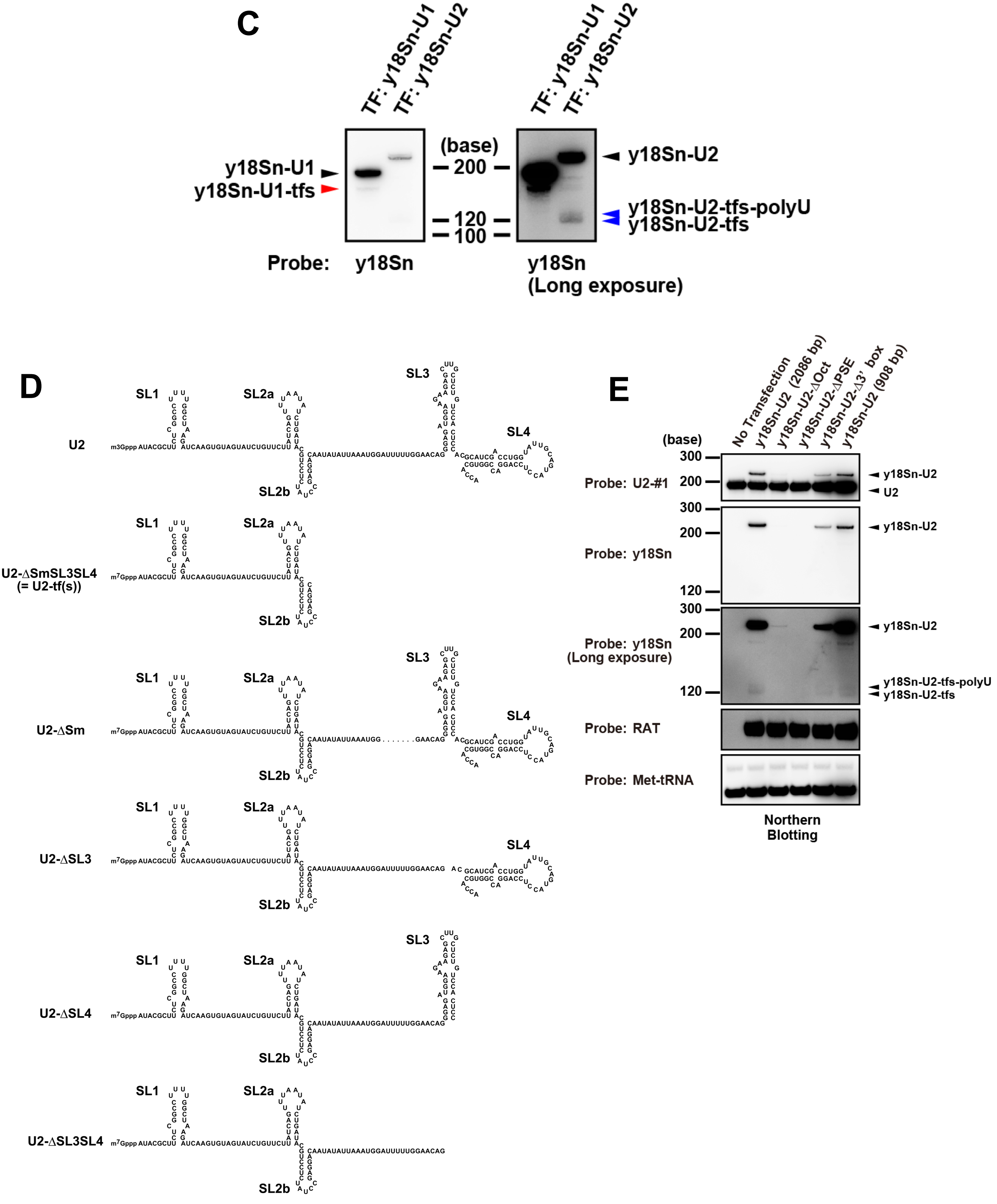
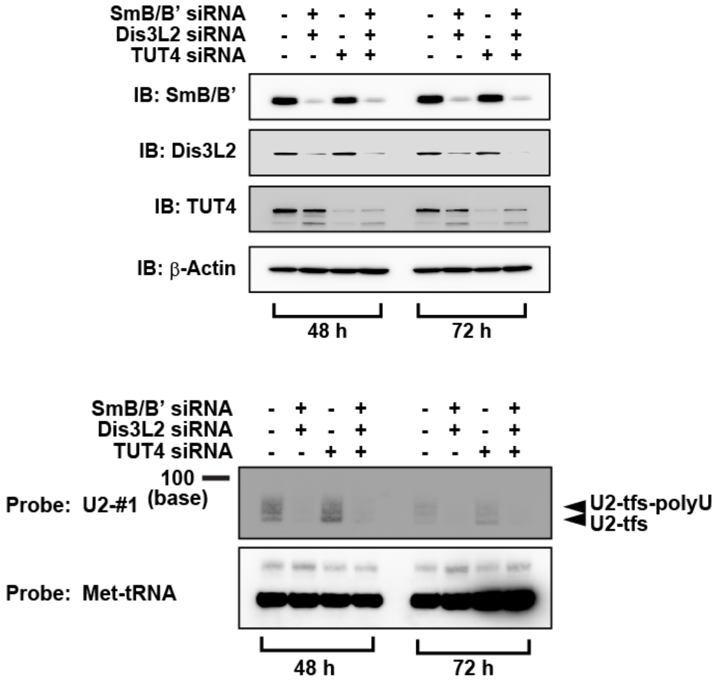
Supplementary Figure S2



Supplementary Figure S3

A



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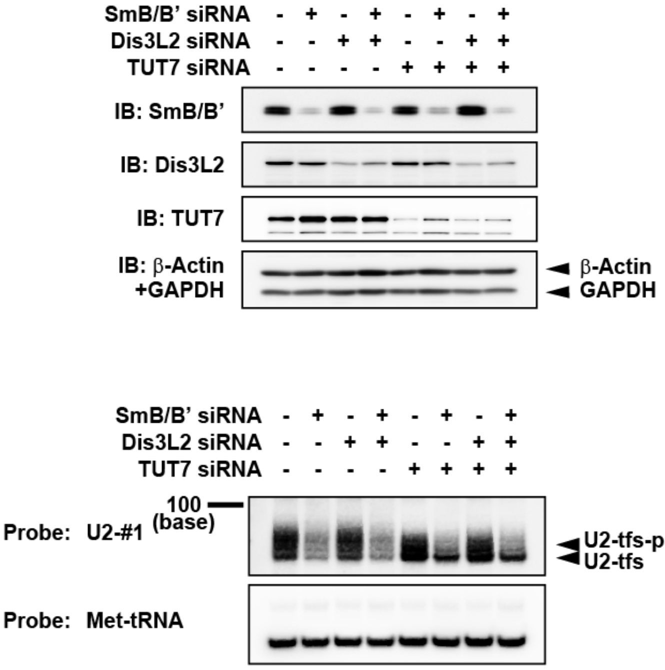

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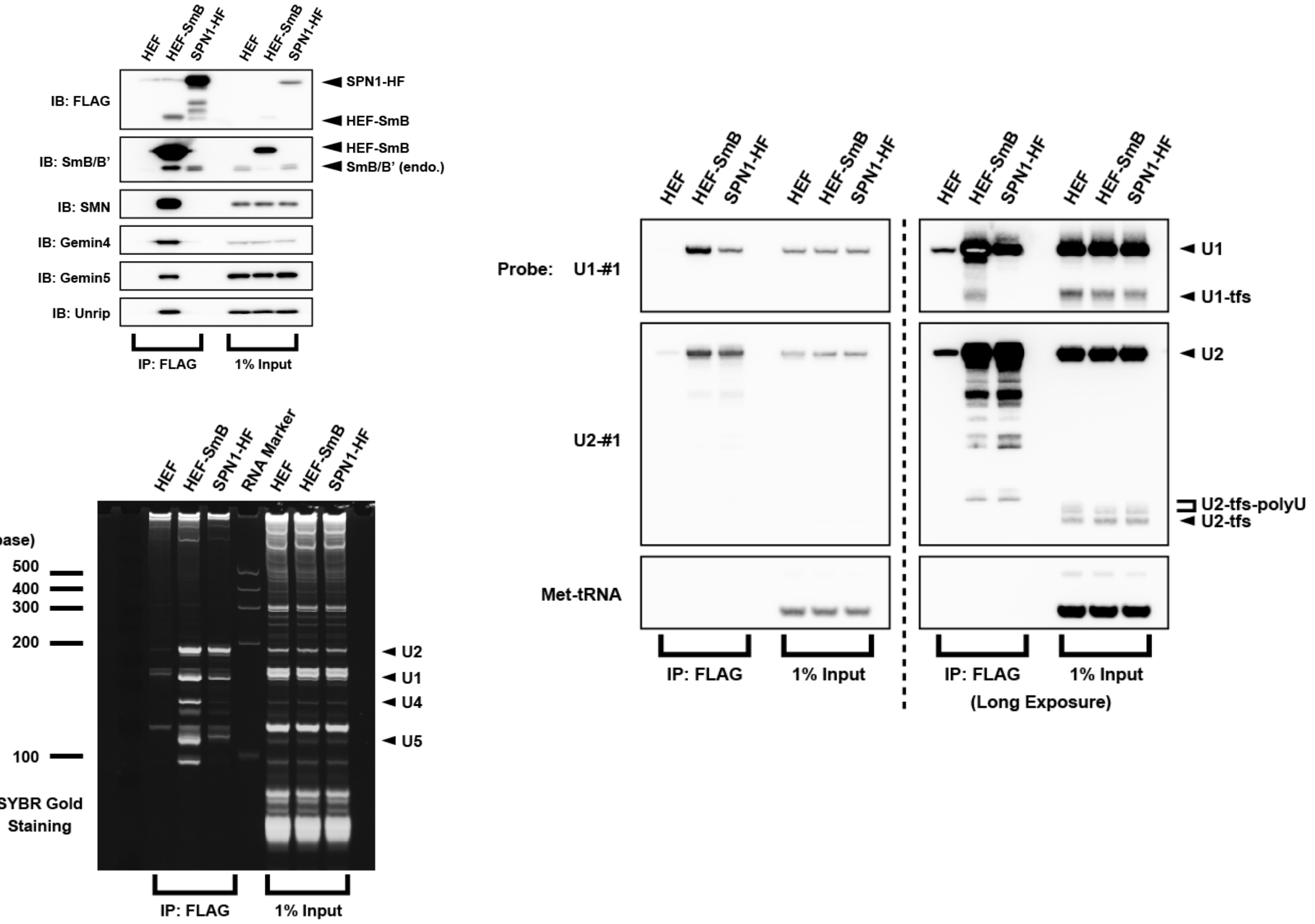

Supplementary Table S1 Oligonucleotide for northern blotting probe

Name	Sequence (5' -> 3')	Application	Note
y18Sn	CCAAAGCCTGAATCCTCG[Biotin]	Probe for y18Sn Tagged RNA detection	3' end biotin labeled
Met-tRNA	[Biotin]TAGCAGAGGATGGTTTCGATCCATCGA	Probe for Met-tRNA detection	5' end biotin labeled
U2-#1	GATCTTAGCCAAAAGGCCGAGAAGCGAT[Biotin]	Probe for U2 RNA detection as #1	3' end biotin labeled
U2-#2	TTGTCCTCGGATAGAGGACGTATCAGAT[Biotin]	Probe for U2 RNA detection as #2	3' end biotin labeled
U2-#3	TTCCATCTCCCTGCTCCAAAAATCCAT[Biotin]	Probe for U2 RNA detection as #3	3' end biotin labeled
U2-#4	TACTGCAATACCAGGTCGATGCGT[Biotin]	Probe for U2 RNA detection as #4	3' end biotin labeled
U1-#1	[Biotin]GTATCTCCCCTGCCAGGTAAGTAT	Probe for U1 and U1-tfs detection	5' end biotin labeled
RAT	[Biotin]ACGTCTAAGGGTTTCCATATAAACTCCTT	Probe for RAT Tagged RNA detection	5' end biotin labeled

Supplementary Table S2 Identification of oligonucleotides originating from U2-tfs by LC-MS-Ariadne

_mod	Positions	label	5'-ter	Sequence	3'-ter	MW theo	m/z theo	MWobs	error(ppm)	m/z	z	Apex RT	Start RT	End RT	Area	Height
eac	'1-4	non	m7Gp	(mAm)(Um)CG	p	1864.22	931.103	1864.22	-1.4	931.101	2	41.9	41.5	42.5	4.86E+05	3.77E+04
eac	'1-4	non	m7Gp	(mAm)(Um)CG	>p	1846.21	922.097	1846.21	-1.19	922.096	2	42.5	41.8	42.9	4.35E+05	3.96E+04
eac	'1-4	non	m7Gp	(Am)(Um)CG	p	1850.2	924.095	1850.2	-1.54	924.093	2	40.9	38.5	41.6	1.86E+06	7.19E+04
eac	'1-4	non	m7Gp	(Am)(Um)CG	>p	1832.19	915.09	1832.19	-1.06	915.089	2	41.7	41	41.9	2.14E+06	2.22E+05
eac	'1-4	non	m7Gp	(Am)(U)CG	p	1836.19	917.087	1836.18	-2.07	917.085	2	33.7	30.7	37.7	1.67E+06	1.40E+04
eac	'1-4	non	m7Gp	(Am)(U)CG	>p	1818.18	908.082	1818.18	-1.51	908.08	2	35.7	34.9	36.7	2.26E+06	8.21E+04
eac	'5-11	non	OH-	CUUCUCG	p	2196.26	1097.122	2196.26	-0.35	1097.12	2	43.7	43.1	44.1	1.63E+06	1.30E+05
eac	'5-11	non	OH-	CUUCUCG	>p	2178.25	1088.116	2178.25	-0.56	1088.12	2	45.1	44.6	45.5	6.89E+06	2.98E+05
eac	'12,20,82,98,106,113,119,	non	OH-	G		345.047	#DIV/0!	0	-1000000							
eac	'13-19	non	OH-	CCUUUUG	p	2197.24	1097.614	2197.24	-0.52	1097.61	2	41.5	41.1	41.6	3.38E+06	3.84E+05
eac	'13-19	non	OH-	CCUUUUG	>p	2179.23	1088.608	2179.23	-0.61	1088.61	2	42	41.7	42.3	2.80E+06	3.65E+05
eac	'21-25	non	OH-	CUAAG	p	1632.23	815.108	1632.23	-0.89	815.107	2	42.7	42.4	43.1	5.07E+06	4.15E+05
eac	'21-25	non	OH-	CUAAG	>p	1614.22	806.102	1614.22	-1.54	806.101	2	44	43.7	44.4	9.87E+05	7.46E+04
eac	'26-31	non	OH-	AUCAAG	p	1961.28	979.634	1961.28	-1.01	979.633	2	50.5	50.1	51.1	1.17E+07	6.89E+05
eac	'26-31	non	OH-	AUCAAG	>p	1943.27	970.629	1943.27	-0.04	970.628	2	51.8	51.4	52.4	1.56E+06	7.79E+04
eac	'32-33,182-183	non	OH-	UG		651.073	650.065	1.00728	-998452.9		1					
eac	'34-36	non	OH-	UAG	p	998.136	997.129	998.135	-0.44	997.128	1	41.9	41.5	42.1	1.68E+06	1.36E+05
eac	'34-36	non	OH-	UAG	>p	980.125	979.118	1.00728	-998972.3		1					
eac	'37-42	non	OH-	UAUCUG	p	1915.23	956.607	1915.23	-0.65	956.606	2	43	42.6	43.3	6.39E+06	5.22E+05
eac	'37-42	non	OH-	UAUCUG	>p	1897.22	947.601	1897.22	-1.07	947.6	2	44.4	44	44.9	2.30E+06	1.36E+05
eac	'43-52	non	OH-	UUCUUAUCAG	p	3161.37	1052.783	3161.38	1.4	1052.78	3	48.4	48.1	48.9	7.06E+06	4.53E+05
eac	'43-52	non	OH-	UUCUUAUCAG	>p	3143.36	1046.78	3143.37	1.39	1046.78	3	48.7	48.2	48.9	4.19E+05	2.69E+04
eac	'53-63	non	OH-	UUUAAUAUCUG	p	3491.41	1162.796	3491.41	-0.38	1162.8	3	49.2	48.7	49.4	6.02E+06	5.53E+05
eac	'53-63	non	OH-	UUUAAUAUCUG	>p	3473.4	1156.792	3473.41	2.24	1156.79	3	49.8	49.4	50.1	3.09E+06	2.14E+05
eac	'64-68	non	OH-	AUACG	p	1632.23	815.108	1632.23	-1.49	815.106	2	45.3	44.9	45.7	1.28E+06	5.84E+04
eac	'64-68	non	OH-	AUACG	>p	1614.22	806.102	1614.22	-0.94	806.102	2	48.5	47.9	48.9	2.54E+06	1.64E+05
eac	'69-79	non	OH-	UCCUCUAUCCG	p	3441.42	1146.132	3441.41	-1.2	1146.13	3	47.3	46.8	47.7	3.42E+05	1.98E+04
eac	'69-79	non	OH-	UCCUCUAUCCG	>p	3423.41	1140.129	3423.41	0.28	1140.13	3	47.7	47.3	48.2	1.64E+06	1.14E+05
eac	'80-81,114-115,126-127	non	OH-	AG	p	692.111	691.103	692.109	-2.35	691.102	1	42.7	42.2	43	7.95E+05	5.79E+04
eac	'80-81,114-115,126-127	non	OH-	AG	>p	674.1	673.093	674.099	-1.85	673.091	1	47.3	46.7	47.7	2.09E+05	9.99E+03
eac	'83-97	non	OH-	ACAAUAUAUUAAAUG	p	4830.65	1609.208	3.02183	-999374.45		3				0.00E+00	0.00E+00

eac	'83-97	non	OH-	ACAAUAUAUUAAAUG	>p	4812.64	1603.205	3.02183	-999372.11		3				0.00E+00	0.00E+00
eac	u-tail	non	OH-	ACA	-	901.191	900.183	901.189	-1.48	900.182	1	53.9	53.5	54.4	6.22E+05	2.72E+04
eac	u-tail	non	OH-	ACAA	-	1230.24	614.114	1230.24	-2.98	614.112	2	53.9	53.3	54.4	3.60E+05	1.54E+04
eac	u-tail	non	OH-	ACAAU	-	1536.27	767.127	1536.27	-1.39	767.126	2	51.1	50.7	51.4	2.92E+05	1.88E+04
eac	u-tail	non	OH-	ACAAUU	-	1842.29	920.14	2.01455	-998906.5		2					
eac	u-tail	non	OH-	ACAAUUU	-	2148.32	1073.152	2.01455	-999062.27		2					
eac	u-tail	non	OH-	ACAAUUUU	-	2454.34	1226.165	2.01455	-999179.19		2					
eac	u-tail	non	OH-	ACAAUUUUU	-	2760.37	1379.178	2.01455	-999270.19		2					
eac	u-tail	non	OH-	ACAAUUUUUU	-	3066.39	1021.124	3.02183	-999014.53		3					
eac	u-tail	non	OH-	ACAAUUUUUUU	-	3372.42	1123.133	3.02183	-999103.96		3					
sum	'1-4	non	OH-	AUCG	p	1303.18	1302.17	1.00728	-999227.06		1				8.87E+06	
sum	'5-11	non	OH-	CUUCUCG	p	2196.26	2195.25	1.00728	-999541.37		1				8.51E+06	
sum	'12,20,82,98,106,113,119,	non	OH-	G	p	363.058	362.051	1.00728	-997225.58		1				0.00E+00	
sum	'13-19	non	OH-	CCUUUUG	p	2197.24	2196.235	1.00728	-999541.57		1				6.18E+06	
sum	'21-25	non	OH-	CUAAG	p	1632.23	1631.222	1.00728	-999382.88		1				6.06E+06	
sum	'26-31	non	OH-	AUCAAG	p	1961.28	1960.275	1.00728	-999486.42		1				1.32E+07	
sum	'32-33,182-183	non	OH-	UG	p	669.083	668.076	1.00728	-998494.54		1				0.00E+00	
sum	'34-36	non	OH-	UAG	p	998.136	997.129	1.00728	-998990.84		1				1.68E+06	
sum	'37-42	non	OH-	UAUCUG	p	1915.23	1914.22	1.00728	-999474.07		1				8.69E+06	
sum	'43-52	non	OH-	UUCUUAUCAG	p	3161.37	3160.365	1.00728	-999681.38		1				7.48E+06	
sum	'53-63	non	OH-	UUUAAUAUCUG	p	3491.41	3490.401	1.00728	-999711.5		1				9.10E+06	
sum	'64-68	non	OH-	AUACG	p	1632.23	1631.222	1.00728	-999382.88		1				3.82E+06	
sum	'69-79	non	OH-	UCCUCUAUCCG	p	3441.42	3440.411	1.00728	-999707.31		1				1.98E+06	
sum	'80-81,114-115,126-127	non	OH-	AG	p	692.111	691.103	1.00728	-998544.63		1				1.00E+06	
sum	'83-97	non	OH-	ACAAUAUAUUAAAUG	p	4830.65	4829.639	1.00728	-999791.48		1				0.00E+00	
sum	u-tail	non	OH-	ACAA(U)n	p	1616.23	1615.227	1.00728	-999376.78		1				1.27E+06	

Supplementary Table S3 Identification of oligonucleotides originating from U2-tfsL by LC-MS-Ariadne

tag	Positions	label	5'-ter	Sequence	3'-ter	MW theo	m/z theo	MWobs	error(ppm)	m/z	z	Apex RT	Start RT	End RT	Area	Height
eac	'1-4	non	m7Gp	(mAm)(Um)CG	P	1864.22	931.103	1864.22	-1.08	931.102	2	33.9	33.5	35.6	5.14E+05	2.21E+04
eac	'1-4	non	m7Gp	(mAm)(Um)CG	>p	1846.21	922.097	1846.21	-0.39	922.097	2	35.3	34.9	36.7	6.29E+05	2.54E+04
eac	'1-4	non	m7Gp	(Am)(Um)CG	p	1850.2	924.095	1850.2	-1.47	924.093	2	32.9	27.6	37.6	2.01E+06	7.72E+03
eac	'1-4	non	m7Gp	(Am)(Um)CG	>p	1832.19	915.09	1832.19	-1.18	915.088	2	32.9	30.2	34.3	2.94E+06	1.42E+05
eac	'1-4	non	m7Gp	(Am)(U)CG	p	1836.19	917.087	1836.19	-0.53	917.086	2	26	24.5	34.3	1.30E+06	5.80E+03
eac	'1-4	non	m7Gp	(Am)(U)CG	>p	1818.18	908.082	1818.18	-1.3	908.081	2	26.6	25.6	29.3	1.97E+06	6.56E+04
eac	'5-11	non	OH-	CUUCUCG	p	2196.26	1097.122	2196.26	-0.35	1097.12	2	41	40.6	42.4	1.46E+06	8.76E+04
eac	'5-11	non	OH-	CUUCUCG	>p	2178.25	1088.116	2178.25	0.45	1088.12	2	42	41.5	42.6	4.13E+06	3.46E+05
eac	'12,20,82,98,106,113,119,125,1	non	OH-	G		345.047	#DIV/0!	0	-1000000							
eac	'13-19	non	OH-	CCUUUUG	p	2197.24	1097.614	2197.24	0.36	1097.61	2	33.3	32.7	34.9	3.29E+06	1.71E+05
eac	'13-19	non	OH-	CCUUUUG	>p	2179.23	1088.608	2179.23	-0.5	1088.61	2	34.7	34.3	35.6	4.81E+06	3.40E+05
eac	'21-25	non	OH-	CUAAG	p	1632.23	815.108	1632.23	-1.86	815.106	2	36.8	35.9	41.5	4.59E+06	7.99E+04
eac	'21-25	non	OH-	CUAAG	>p	1614.22	806.102	1614.22	-1.23	806.101	2	40.8	40.2	41.8	1.13E+06	5.02E+04
eac	'26-31	non	OH-	AUCAAG	p	1961.28	979.634	1961.28	0.11	979.634	2	48.5	48	50.9	8.40E+06	2.82E+05
eac	'26-31	non	OH-	AUCAAG	>p	1943.27	970.629	1943.27	-0.04	970.628	2	50	49.5	50.6	1.70E+06	9.61E+04
eac	'32-33,182-183	non	OH-	UG		651.073	650.065	1.00728	-998453		1					
eac	'34-36	non	OH-	UAG	p	998.136	997.129	998.135	-0.38	997.128	1	32.7	32.1	34.1	2.28E+06	8.64E+04
eac	'34-36	non	OH-	UAG	>p	980.125	979.118	1.00728	-998972		1				0.00E+00	0.00E+00
eac	'37-42	non	OH-	UAUCUG	p	1915.23	956.607	1915.23	-1.22	956.605	2	38.7	37.8	41.5	7.83E+06	1.82E+05
eac	'37-42	non	OH-	UAUCUG	>p	1897.22	947.601	1897.22	-0.04	947.601	2	41.5	41	41.9	2.56E+06	2.02E+05
eac	'43-52	non	OH-	UUCUUAUCAG	p	3161.37	1052.783	3161.37	0.82	1052.78	3	44.9	43.9	47.4	8.79E+06	1.79E+05
eac	'43-52	non	OH-	UUCUUAUCAG	>p	3143.36	1046.78	3143.36	-0.35	1046.78	3	45.2	44.5	45.9	9.31E+05	3.49E+04
eac	'53-63	non	OH-	UUUAAUAUCUG	p	3491.41	1162.796	3491.41	0.25	1162.8	3	47	46.2	49.4	5.28E+06	1.42E+05
eac	'53-63	non	OH-	UUUAAUAUCUG	>p	3473.4	1156.792	3473.4	1.5	1156.79	3	48.4	47.9	48.8	3.74E+06	2.62E+05
eac	'64-68	non	OH-	AUACG	p	1632.23	815.108	1632.23	-0.37	815.107	2	41.9	41.5	45.1	6.47E+05	1.23E+04
eac	'64-68	non	OH-	AUACG	>p	1614.22	806.102	1614.22	-1.38	806.101	2	43.9	43.3	44.6	2.26E+06	1.06E+05
eac	'69-79	non	OH-	UCCUCUAUCCG	p	3441.42	1146.132	3441.42	1.79	1146.13	3	42.4	42.2	42.9	1.74E+05	8.00E+03
eac	'69-79	non	OH-	UCCUCUAUCCG	>p	3423.41	1140.129	3423.41	0.49	1140.13	3	43.2	42.6	43.7	1.29E+06	5.79E+04
eac	'80-81,114-115,126-127	non	OH-	AG	p	692.111	691.103	692.11	-0.93	691.103	1	34.5	33.7	35.7	6.35E+05	2.42E+04
eac	'80-81,114-115,126-127	non	OH-	AG	>p	674.1	673.093	674.1	-0.31	673.092	1	42.3	41.5	42.9	1.57E+05	7.09E+03
eac	'83-97	non	OH-	ACAAUAUAUUAAA	p	4830.65	1609.208	3.02183	-999374		3				0.00E+00	0.00E+00

eac	'83-97	non	OH-	ACAAUAUAUUAAA	>p	4812.64	1603.205	3.02183	-999372		3				0.00E+00	0.00E+00
eac	u-tail	non	OH-	ACA	-	901.191	900.183	1.00728	-998882		1				0.00E+00	0.00E+00
eac	u-tail	non	OH-	ACAA	-	1230.24	614.114	2.01455	-998362		2				0.00E+00	
eac	u-tail	non	OH-	ACAAU	-	1536.27	767.127	2.01455	-998689		2				0.00E+00	
eac	u-tail	non	OH-	ACAAUU	-	1842.29	920.14	2.01455	-998907		2				0.00E+00	
eac	u-tail	non	OH-	ACAAUUU	-	2148.32	1073.152	2148.32	0.11	1073.15	2	48.9	48.6	49.2	5.22E+05	2.98E+04
eac	u-tail	non	OH-	ACAAUUUU	-	2454.34	1226.165	2454.35	0.54	1226.17	2	49.1	48.8	49.4	6.42E+05	5.31E+04
eac	u-tail	non	OH-	ACAAUUUUU	-	2760.37	1379.178	2760.37	-0.64	1379.18	2	49.4	49	49.8	3.18E+05	1.73E+04
eac	u-tail	non	OH-	ACAAUUUUUU	-	3066.39	1021.124	3066.4	0.57	1021.12	3	49.7	49.3	50	1.45E+05	8.71E+03
eac	u-tail	non	OH-	ACAAUUUUUUU	-	3372.42	1123.133	3.02183	-999104		3				0.00E+00	0.00E+00
sum	'1-4	non	OH-	AUCG	p	1303.18	1302.17	1.00728	-999227		1				9.36E+06	
sum	'5-11	non	OH-	CUUCUCG	p	2196.26	2195.25	1.00728	-999541		1				5.59E+06	
sum	'12,20,82,98,106,113,119,125,1	non	OH-	G	p	363.058	362.051	1.00728	-997226		1				0.00E+00	
sum	'13-19	non	OH-	CCUUUUG	p	2197.24	2196.235	1.00728	-999542		1				8.11E+06	
sum	'21-25	non	OH-	CUAAG	p	1632.23	1631.222	1.00728	-999383		1				5.72E+06	
sum	'26-31	non	OH-	AUCAAG	p	1961.28	1960.275	1.00728	-999486		1				1.01E+07	
sum	'32-33,182-183	non	OH-	UG	p	669.083	668.076	1.00728	-998495		1				0.00E+00	
sum	'34-36	non	OH-	UAG	p	998.136	997.129	1.00728	-998991		1				2.28E+06	
sum	'37-42	non	OH-	UAUCUG	p	1915.23	1914.22	1.00728	-999474		1				1.04E+07	
sum	'43-52	non	OH-	UUCUUAUCAG	p	3161.37	3160.365	1.00728	-999681		1				9.72E+06	
sum	'53-63	non	OH-	UUUAAUAUCUG	p	3491.41	3490.401	1.00728	-999712		1				9.02E+06	
sum	'64-68	non	OH-	AUACG	p	1632.23	1631.222	1.00728	-999383		1				2.91E+06	
sum	'69-79	non	OH-	UCCUCUAUCCG	p	3441.42	3440.411	1.00728	-999707		1				1.46E+06	
sum	'80-81,114-115,126-127	non	OH-	AG	p	692.111	691.103	1.00728	-998545		1				7.93E+05	
sum	'83-97	non	OH-	ACAAUAUAUUAAA	p	4830.65	4829.639	1.00728	-999791		1				0.00E+00	
sum	u-tail	non	OH-	ACAA(U)n	p	1616.23	1615.227	1.00728	-999377		1				1.63E+06	

Supplementary Table S4 Oligonucleotides for construction

	Sequence (5' -> 3')
HIW497	GAAGGATCCCCCAGCCCTCAGCGATGGGATTTCAG
HIW498	CAACTCGAGGAATTACTACGCAGCCATAAAAAAGGATG
HIW517	TCCAGATCTGCCTTCGCGCCCGCCGTCAC
HIW538	GGAAGATCTATCGCTTCTCGCGAGGATTCAGGCTTTGGATCGATATCGCTTCTCGGCCTTTTGG
HIW553	GCCCCGCCCTTTCACAGAGG
HIW554	TCGAAATGAAAGCCCGGGAAC
HIW555	CCCCAGCCTCGCTCCTTGC
HIW556	GATGAGAGTGGGACGGTGACG
HIW557	ACGTTGTATCCCCGGAGGGGGTG
HIW558	GGGAGGTGAGAGACGGTAGCAC
HIW559	GAAGGATCCGCTCTCCGCCGACAGGTCTCTTC
HIW560	CAACTCGAGGGTCGCCACCCACTCGACTG
HIW602	GTCCTCGGATAGAGGACGTATCAG
HIW603	CCTCCGGGGATACAACGTGTTTCC
HIW604	CCATTTAATATATTGTCCTCGGATAGAG
HIW605	GAGCAGGGAGATGGAATAGGAG
HIW606	CTGCTCCAAAAATCCATTTAATATATTGTCCTC
HIW607	ACGCATCGACCTGGTATTGCAG
HIW608	GGAGTGGACGGAGCAAGCTC
HIW394	CGATTTTTTTG
HIW395	AATTCAAAAAAAT
HIW561	GAAGGATCCATGAGCCATCCTGACTACAG
HIW562	CAACCCGGGCCGCTGGTGCTTGAGTCCTC
HIW591	CAACCGCCCGAGACCTCAATGATGCCCTCTCCTG
HIW592	CAGGAGAGGGCATCATTGAGGTCTCGGGCGGTTG

Supplementary Table S5 Antibodies

Name (clone name)	Host / Class	Dilution	Supplier	Product code
Anti SmB/B' (clone Y12)	mouse / monoclonal	1:1000	Thermo Fisher Scientific	MS-450-P
Anti Dis3L2	rabbit / polyclonal	1:1000	Novus Biologicals	NBP1-84740
Anti b-Actin (clone AC-15)	mouse / monoclonal	1:2000	Santa Cruz Biotechnology	sc-69879
Anti GAPDH (clone 6C5)	mouse / monoclonal	1:10000	Thermo Fisher Scientific	AM4300
Anti TUT7	rabbit / polyclonal	1:1000	Sigma-Aldrich	HPA020620
Anti TUT4	rabbit / polyclonal	1:1000	Proteintech	18980-1-AP
Anti FLAG (clone M2)	mouse / monoclonal	1:10000	Sigma-Aldrich	F3165
Anti SMN (clone 2B1)	mouse / monoclonal	1:1000	Santa Cruz Biotechnology	sc-32313
Anti-mouse IgG, HRP-linked Antibody	horse / polyclonal	1:2000	Cell Signaling Technology	#7076
Anti-rabbit IgG, HRP-linked Antibody	goat / polyclonal	1:2000	Cell Signaling Technology	#7074

Supplementary Table S6 siRNA information

Name	Product Type	Sequence (anti-sense)	Sequence (sense)	Target RNA	Note
SmB/B' (sc)	Stealth siRNA	AUAAGCCAGUAUCUUUGGGAGGGUC	GACCCUCCCAAAGAUACUGGCUUAU	－	Used in our previous report / main figure
SmB/B' (si)	Stealth siRNA	AUACCAGUAUCUUUGGGAGGAGGUC	GACCUCCUCCCAAAGAUACUGGUAU	NM_003091	Used in our previous report / main figure
Dis3L2 (sc)	Stealth siRNA	UUAUCACAUCCUUCUAACCUCGCGG	CCGCGAGGUUAGAAGGAUGUGAUAA	－	Used in main figure
Dis3L2 (si)	Stealth siRNA	UUAACUACCUUCCAAUGCUCCUCGG	CCGAGGAGCAUUGGAAGGUAGUUAA	NM_152383	Used in main figure
TUT7 (sc)	Stealth siRNA	UGGAUACGUAACCUCAACGUUUCUG	CAGAAACGUUGAGGUUACGUAUCCA	－	Used in main figure
TUT7 (si)	Stealth siRNA	UGGCAAUGCCAACUGCAUUUAUCUG	CAGAUAAAUGCAGUUGGCAUUGCCA	NM_024617	Used in main figure
Silencer Select Negative Control No.1	Silencer Select siRNA	not specified (Thermo : product code #4390843)		－	Used in supplementary figure
SmB/B' siRNA	Silencer Select siRNA	UCGAGCAAUACCAGUAUCUtt	AGAUACUGGUAUUGCUCGAtt	NM_003091	Used in supplementary figure
Dis3L2 siRNA	Silencer Select siRNA	UAUCUGAUUCAUACGCAGCtt	GCUGCGUAUGAAUCAGAUAtt	NM_152383	Used in supplementary figure
TUT7 siRNA	Silencer Select siRNA	AGUCGUCUUACAGUUCUGCag	GCAGAACUGUAAGACGACUtt	NM_024617	Used in supplementary figure
TUT4 siRNA	Silencer Select siRNA	UAUCACGAAAUCCAAAUCCat	GGAUUUGGAUUUCGUGAUAtt	NM_015269	Used in supplementary figure

＊ lowercase in silencer select sequence means deoxyribonucleotide