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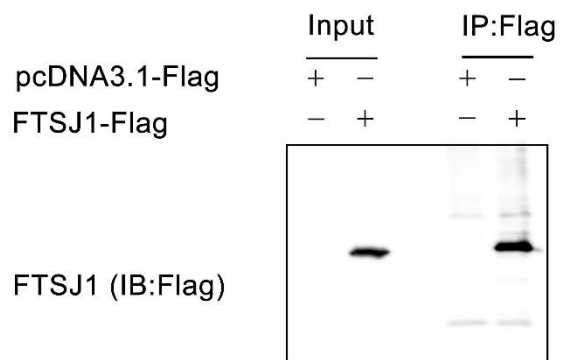
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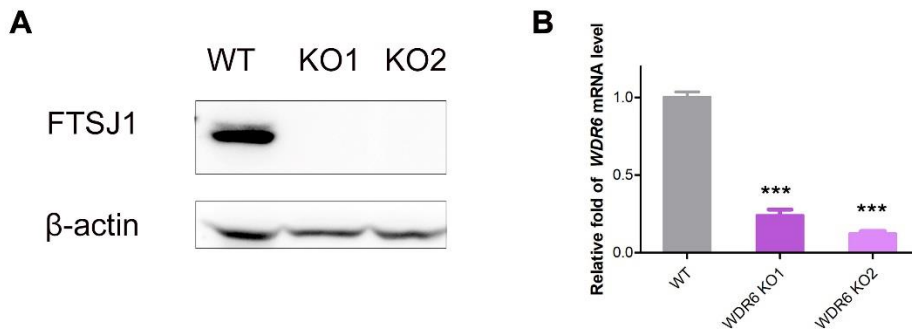
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Appendix Figure S1. IP assays of FTSJ1-Flag. The empty vector was transfected as a control.

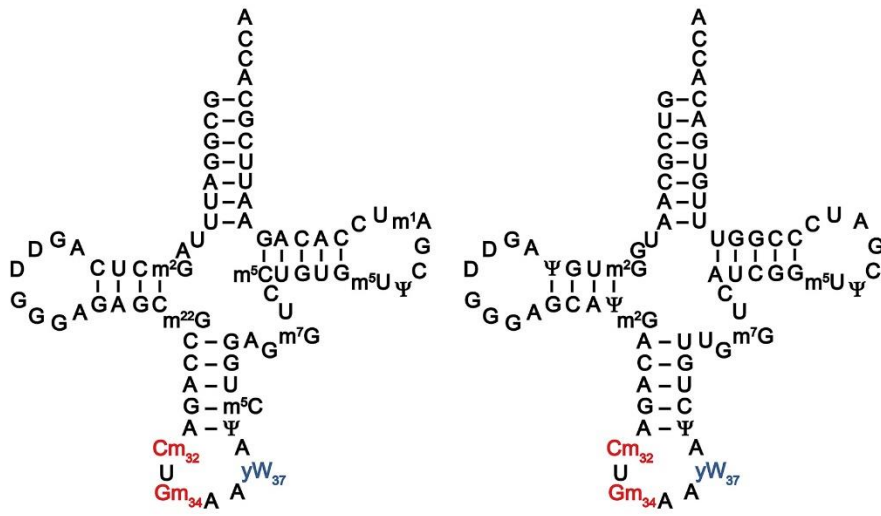


Appendix Figure S2. The knockout efficiency of *ftsj1* and *wdr6*.

(A) The western blotting to show *ftsj1* knockout efficiency. (B) QRT-PCR to show *wdr6* knockout efficiency. Error bars represent the standard deviation of three independent experiments. p values were determined using two-tailed Student's t-test for paired samples. *p < 0.05, **p < 0.01, ***p < 0.001. n.s., no significance.

S. cerevisiae tRNA^{Phe}(GAA)

S. pombe tRNA^{Phe}(GAA)



Appendix Figure S3. Secondary structures of *S. cerevisiae* and *S. pombe* tRNA^{Phe}(GAA).

Appendix Table S1. List of potential FTSJ1-interacting proteins identified by IP-MS analysis.

	Swiss-Prot ID	Gene Symbol	Description
1	P0DMV9	HSP1A	Heat shock 70 kDa protein 1B
2	B4DNT8		cDNA FLJ54370, highly similar to Heat shock 70 kDa protein 1
3	Q00610	CLTC	Clathrin heavy chain 1
4	P23246	SFPQ	Splicing factor, proline- and glutamine-rich
5	B4DI39		cDNA FLJ54328, highly similar to Heat shock 70 kDa protein 1
6	Q86VG2		Splicing factor proline/glutamine-rich (Polypyrimidine tract binding protein associated)
7	G8JLB6	HNRNPH1	Heterogeneous nuclear ribonucleoprotein H
8	Q15233	NONO	Non-POU domain-containing octamer-binding protein
9	V9HW22	HEL-S-72p	Epididymis luminal protein 33
10	P49750	YLPM1	YLP motif-containing protein 1
11	P78371	CCT2	T-complex protein 1 subunit beta containing TCP1, subunit 2 (Beta), isoform CRA_b
12	Q9UET6	FTSJ1	Putative tRNA (cytidine(32)/guanosine(34)-2'-O)-methyltransferase
13	Q8NF45	FLJ00353	FLJ00353 protein
14	P38646	HSPA9	Stress-70 protein, mitochondrial
15	O00571	DDX3X	ATP-dependent RNA helicase DDX3X
16	V9HW37	HEL-S-69	Epididymis secretory protein Li 69
17	P50990	CCT8	T-complex protein 1 subunit theta
18	P40227	CCT6A	T-complex protein 1 subunit zeta
19	P11021	HSPA5	Endoplasmic reticulum chaperone BiP
20	P22626	HNRNPA2B1	Heterogeneous nuclear ribonucleoproteins A2/B1
21	P35658	NUP214	Nuclear pore complex protein Nup214
22	Q5JP53	TUBB	Tubulin beta chain
23	Q53HV2		T-complex protein 1 subunit eta
24	P17987	TCP1	T-complex protein 1 subunit alpha
25	P49368	CCT3	T-complex protein 1 subunit gamma
26	F8VU51	YLPM1	YLP motif-containing protein 1
27	P50991	CCT4	T-complex protein 1 subunit delta
28	P68371	TUBB4B	Tubulin beta-4B chain
29	Q53FA3	HSPA1L	Heat shock 70 kDa protein 1-like (Fragment)
30	B7Z9L0		T-complex protein 1 subunit delta
31	Q9BVA1	TUBB2B	Tubulin beta-2B chain
32	P55795	HNRNPH2	Heterogeneous nuclear ribonucleoprotein H2
33	H0Y9K7	SFPQ	Splicing factor, proline- and glutamine-rich (Fragment)

34	Q92499	DDX1	ATP-dependent RNA helicase DDX1
35	O15042	U2SURP	U2 snRNP-associated SURP motif-containing protein
36	Q92841	DDX17	Probable ATP-dependent RNA helicase DDX17
37	Q7Z6Z7	HUWE1	E3 ubiquitin-protein ligase HUWE1
38	Q9Y3I0	RTCB	tRNA-splicing ligase RtcB homolog
39	J3KTA4	DDX5	Probable ATP-dependent RNA helicase DDX5
40	F8VPD4	CAD	CAD protein
41	P09651	HNRPA1	Heterogeneous nuclear ribonucleoprotein A1
42	Q02809	PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1
43	P09651	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1
44	P04350;	TUBB4A	Tubulin beta-4A chain
45	O75533;	SF3B1	Splicing factor 3B subunit 1
46	P68363	TUBA1B	Tubulin alpha-1B chain 1A
47	Q9BQE3	TUBA1C	Tubulin alpha-1C chain
48	P14866	HNRNPL	Heterogeneous nuclear ribonucleoprotein L
49	Q15393;	SF3B3	Splicing factor 3B subunit 3
50	H0YBG7	HNRNPH1	Heterogeneous nuclear ribonucleoprotein H (Fragment)
51	Q01844;	EWSR1	RNA-binding protein EWS
52	P33993;	MCM7	DNA replication licensing factor MCM7
53	P61978	HNRNPK	Heterogeneous nuclear ribonucleoprotein K
54	Q92734	TFG	Protein TFG
55	Q9BTE3;	MCMBP	Mini-chromosome maintenance complex-binding protein
56	P33992	MCM5	DNA replication licensing factor MCM5
57	Q13509	TUBB3	Tubulin beta-3 chain
58	Q13435	SF3B2	Splicing factor 3B subunit 2
59	Q2NKY5	TUBB6	TUBB6 protein
60	Q8IXB1;	DNAJC10	DnaJ homolog subfamily C member 10
61	Q9H0D6	XRN2	5'-3' exoribonuclease 2
62	P52597	HNRNPF	Heterogeneous nuclear ribonucleoprotein F
63	P15924;	DSP	Desmoplakin
64	B2R802		cDNA, FLJ93681, highly similar to Homo sapiens small nuclear ribonucleoprotein polypeptide A (SNRPA), mRNA
65	A0A024QZD5	SNRP70	Small nuclear ribonucleoprotein 70kDa polypeptide (RNP antigen), isoform CRA_b
66	P04637	TP53	Cellular tumor antigen p53
67	Q4VCS5;	AMOT	Angiomotin
68	Q9C0J8;	WDR33	pre-mRNA 3' end processing protein WDR33
69	Q8WXF1	PSPC1	Paraspeckle component 1
70	P35637	FUS	RNA-binding protein FUS

71	P24928	POLR2A	DNA-directed RNA polymerase II subunit RPB1
72	B3KUD7	MCM7	NA replication licensing factor MCM7
73	Q9NNW5	WDR6	WD repeat-containing protein 6
74	P19338	NCL	Nucleolin
75	Q10570	CPSF1	Cleavage and polyadenylation specificity factor subunit 1
76	B4E3A4		cDNA FLJ57283, highly similar to Actin, cytoplasmic 2
77	Q86X55;	CARM1	Histone-arginine methyltransferase CARM1
78	Q99567;	NUP88	Nuclear pore complex protein Nup88
79	Q5RLJ0		CLE
80	P11940	PABPC1	Polyadenylate-binding protein 1
81	D3DWL9	CPSF1	Cleavage and polyadenylation specific factor 1, 160kDa, isoform CRA_a
82	P05387	RPLP2	60S acidic ribosomal protein P2
83	P63104	YWHAZ	14-3-3 protein zeta/delta
84	B4DZW4		Polyadenylate-binding protein
85	O14654;	IRS4	Insulin receptor substrate 4
86	P17066;	HSPA6	Heat shock 70 kDa protein 6
87	A0A024RDP4	PSPC1	Paraspeckle component 1, isoform CRA_b
88	P30876	POLR2B	DNA-directed RNA polymerase II subunit RPB2
89	Q4LE79	DSP	DSP variant protein (Fragment)
90	Q86YZ3;	HRNR	Hornerin
91	P49411;	TUFM	Elongation factor Tu, mitochondrial
92	Q8NCA5;	FAM98A	Protein FAM98A
93	P52272	HNRNPM	Heterogeneous nuclear ribonucleoprotein M
94	Q8IWX8	CHERP	Calcium homeostasis endoplasmic reticulum protein
95	P25705	ATP5A1	ATP synthase subunit alpha, mitochondrial
96	Q9BUF5	TUBB6	Tubulin beta-6 chain
97	Q99700;	ATXN2	Ataxin-2
98	P21333	FLNA	Filamin-A
99	O43143	DHX15	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15
100	Q08211;	DHX9	ATP-dependent RNA helicase A
101	Q15149;	PLEC	Plectin
102	Q96I25	RBM17	Splicing factor 45
103	O43823;	AKAP8	A-kinase anchor protein 8
104	Q14444;	CAPRIN1	Caprin-1
105	Q00839	HNRNPU	Heterogeneous nuclear ribonucleoprotein U
106	Q52LJ0;	FAM98B	Protein FAM98B
107	P51991;	HNRNPA3	Heterogeneous nuclear ribonucleoprotein A3

Appendix Table S2. List of potential FTSJ1-interacting proteins identified by unnatural amino acid photocrosslinking-IP-MS analysis.

	Swiss-Prot ID	Gene Symbol	Description
1	Q9NNW5	WDR6	WD repeat-containing protein 6
2	O14654	IRS4	Insulin receptor substrate 4
3	P51532	SMARCA4	Transcription activator BRG1
4	Q9UET6	FTSJ1	Putative tRNA (cytidine(32)/guanosine(34)-2'-O)-methyltransferase
5	Q99729-3	HNRNPAB	Isoform 3 of Heterogeneous nuclear ribonucleoprotein A/B
6	O14979-2	HNRNPDL	Isoform 2 of Heterogeneous nuclear ribonucleoprotein D-like
7	P16403	HIST1H1C	Histone H1.2
8	P31483	TIA1	Nucleolysin TIA-1 isoform p40
9	Q01085-2	TIAL1	Isoform 2 of Nucleolysin
10	Q04837	SSBP1	Single-stranded DNA-binding protein, mitochondrial
11	Q00325-2	SLC25A3	Isoform B of Phosphate carrier protein, mitochondrial
12	Q9BTE3	MCMBP	Mini-chromosome maintenance complex-binding protein
13	P04350	TUBB4A	Tubulin beta-4A chain
14	Q9BUF5	TUBB6	Tubulin beta-6 chain
15	Q15366-2	PCBP2	Isoform 2 of Poly(rC)-binding protein 2
16	Q13247	SRSF6	Serine/arginine-rich splicing factor 6
17	P01605	IGKV1D-33	Immunoglobulin kappa variable 1D-33
18	Q01650	SLC7A5	Large neutral amino acids transporter small subunit 1
19	Q7Z2W4	ZC3HAV1	Zinc finger CCCH-type antiviral protein 1
20	P10809	HSPD1	60 kDa heat shock protein, mitochondrial
21	P07900-2	HSP90AA1	Isoform 2 of Heat shock protein HSP 90-alpha OS
22	Q71U36	TUBA1A	Tubulin alpha-1A chain
23	P12236	SLC25A6	ADP/ATP translocase 3
24	Q9BQ39	DDX50	ATP-dependent RNA helicase DDX50
25	Q13243	SRSF5	Serine/arginine-rich splicing factor 5
26	O00264	PGRMC1	Membrane-associated progesterone receptor component 1
27	P20674	COX5A	Cytochrome c oxidase subunit 5A, mitochondrial
28	P63173	RPL38	60S ribosomal protein L38
29	P30954	OR10J1	Olfactory receptor 10J1
30	O60783	MRPS14	28S ribosomal protein S14, mitochondrial
31	Q9GZQ3	COMMD5	COMM domain-containing protein 5
32	P51843	NR0B1	Nuclear receptor subfamily 0 group B member 1
33	Q9NX58	LYAR	Cell growth-regulating nucleolar protein

34	P82932	MRPS6	28S ribosomal protein S6, mitochondrial
35	O75908-3	SOAT2	Isoform 3 of Sterol O-acyltransferase 2
36	Q9UNA1	ARHGAP26	Rho GTPase-activating protein 26
37	Q8N5I4	DHR SX	Dehydrogenase/reductase SDR family member on chromosome X
38	Q99832	CCT7	T-complex protein 1 subunit eta
39	Q9UKF6	CPSF3	Cleavage and polyadenylation specificity factor subunit 3
40	P02745	C1QA	Complement C1q subcomponent subunit A
41	Q14246	ADGRE1	Adhesion G protein-coupled receptor E1
42	P52292	KPNA2	Importin subunit alpha-1
43	Q8WUZ0-2	BCL7C	Isoform 2 of B-cell CLL/lymphoma 7 protein family member C
44	P40227	CCT6A	T-complex protein 1 subunit zeta
45	P35613	BSG	Basigin
46	Q6PK04	CCDC137	Coiled-coil domain-containing protein 137
47	Q9BU76	MMTAG2	Multiple myeloma tumor-associated protein 2
48	P10243	MYBL1	Myb-related protein A
49	Q9Y5S1	TRPV2	Transient receptor potential cation channel subfamily V member 2
50	Q08999	RBL2	Retinoblastoma-like protein 2
51	O94979-8	SEC31A	Isoform 8 of Protein transport protein
52	Q9Y383	LUC7L2	Putative RNA-binding protein Luc7-like 2
53	P13639	EEF2	Elongation factor 2
54	P33993	MCM7	DNA replication licensing factor MCM7
55	Q9Y4P3	TBL2	Transducin beta-like protein 2
56	P61619	SEC61A1	Protein transport protein Sec61 subunit alpha isoform 1
57	Q68CZ2	TNS3	Tensin-3
58	O14980	XPO1	Exportin-1
59	Q9Y2X0	MED16	Mediator of RNA polymerase II transcription subunit 16
60	Q6P158	DHX57	Putative ATP-dependent RNA helicase DHX57
61	O14917	PCDH17	Protocadherin-17
62	P25705	ATP5A1	ATP synthase subunit alpha, mitochondrial
63	Q5T9A4	ATAD3B	ATPase family AAA domain-containing protein 3B
64	Q9Y2I1	NISCH	Nischarin
65	P57678	GEMIN4	Gem-associated protein 4

Appendix Table S3. Information about the 17 human cytoplasmic G37-tRNA species.

17 G37-tRNAs		Site 32	Site 34
tRNA ^{Arg}	tRNA ^{Arg} (ACG)	C32	A34
	-(CCG)	U32	C34
	-(UCG)	C32	U34

tRNA ^{Cys} (GCA)	tRNA ^{Cys} (GCA)	C32	G34
tRNA ^{His} (GUG)	tRNA ^{His} (GUG)	U32	G34
tRNA ^{Leu}	tRNA ^{Leu} (AAG)	U32	A34
	-(CAA)	C32	C34
	-(CAG)	U32	C34
	-(UAA)	C32	U34
	-(UAG)	U32	U34
tRNA ^{Phe} (GAA)	tRNA ^{Phe} (GAA)	C32	G34
tRNA ^{Pro}	tRNA ^{Pro} (AGG)	U32	A34
	-(CGG)	U32	C34
	-(UGG)	U32	U34
tRNA ^{Trp} (CCA)	tRNA ^{Trp} (CCA)	C32	C34
tRNA ^{Tyr}	tRNA ^{Tyr} (AUA)	C32	A34
	-(GUA)	C32	G34

Appendix Table S4. Quantification of the Nm/A ratios in 16 G37-tRNAs after incubation with FTSJ1-WDR6 by UPLC-MS/MS analysis.

tRNAs	Am/A%	Cm/A%	Gm/A%	Um/A%	m ¹ G/A%
m ¹ G37_tRNA ^{Arg} (<u>A</u> CG)	n.d.				5.21±0.24
m ¹ G37_tRNA ^{Arg} (<u>C</u> CG)		n.d.			0.34±0.01
m ¹ G37_tRNA ^{Arg} (<u>U</u> CG)				n.d.	0.92±0.08
m ¹ G37_tRNA ^{Cys} (<u>G</u> CA)			n.d.		1.38±0.06
m ¹ G37_tRNA ^{His} (<u>G</u> UG)			n.d.		6.67±0.15
m ¹ G37_tRNA ^{Leu} (<u>A</u> AG)	n.d.				6.89±0.67
m ¹ G37_tRNA ^{Leu} (<u>U</u> AG)				n.d.	7.05±0.28
m ¹ G37_tRNA ^{Leu} (<u>C</u> AG)		n.d.			3.01±0.45
m ¹ G37_tRNA ^{Leu} (<u>U</u> AA)				n.d.	4.99±0.28
m ¹ G37_tRNA ^{Pro} (<u>A</u> GG)	n.d.				8.53±0.32
m ¹ G37_tRNA ^{Pro} (<u>C</u> GG)		n.d.			8.37±0.22
m ¹ G37_tRNA ^{Pro} (<u>U</u> GG)				n.d.	2.70±0.10
m ¹ G37_tRNA ^{Phe} (<u>G</u> AA)			0.75±0.04		2.26±0.20
m ¹ G37_tRNA ^{Trp} (<u>C</u> CA)		n.d.			5.73±0.11
m ¹ G37_tRNA ^{Tyr} (<u>G</u> UA)			n.d.		5.47±0.50
m ¹ G37_tRNA ^{Tyr} (<u>A</u> UA)	n.d.				3.35±0.12

The results are the average of three independent repeats with standard deviations indicated. n.d., not detected.

Appendix Table S5. Constructs used in this study.

Proteins	Tag	Position	Vector
For expression in insect cells			
FTSJ1	His ₆	C-terminus	pFastbac1
WDR6	GST	N-terminus	pFastbac1

TrmD	His ₆	N-terminus	pET28a
For expression in HEK293T cells			
FTSJ1	Flag	C-terminus	pcDNA3.1
FTSJ1- Δ N19, - Δ C39, -220, -241, -256 and -Asn221DIZPK			
WDR6	HA	C-terminus	pcDNA3.1
For immunofluorescence analysis			
FTSJ1	Flag	C-terminus	pcDNA3.1

Appendix Table S6. The biotinylated DNA probes for fishing tRNAs.

tRNAs	Probes
tRNA ^{Arg} (ACG)	5'biotin-CAGACGCGTTATCCATTGCGCCACTGGCCC
tRNA ^{Arg} (CCG)	5'biotin-TGGCGACCACGAAGGGACTCGAACCCCTCAA
tRNA ^{Arg} (UCG)	5'biotin-TGGCGAGCCAGCCAGGAGTCGAACCTGGAA
tRNA ^{Cys} (GCA)	5'biotin-TCAAATGCTCTACCACTGAGCTATGCCCCC
tRNA ^{His} (GUG)	5'biotin-GCAGAGTACTAACCACTATAACGATCACGGC
tRNA ^{Leu} (UAA)	5'biotin-CCAACGCCTTAACCACTCGGCCATCCCGGT
tRNA ^{Leu} (AAG) and -(UAG)	5'biotin-CAGCGCCTTAGACCGCTCGGCCACGCTACC
tRNA ^{Leu} (CAG)	5'biotin-CAGCGCCTTAGACCGCTCGGCCATCCTGAC
tRNA ^{Pro} (AGG), -(CGG) and -(UGG)	5'biotin-GCGAGAATCATAACCCCTAGACCAACGAGCC
tRNA ^{Trp} (CCA)	5'biotin-TCAGACGCGCTACCATTGCGCCACGAGGTC
tRNA ^{Phe} (GAA)	5'biotin-CTAACGCTCTCCCAACTGAGCTATTTGCGC
tRNA ^{Tyr} (AUA)	5'biotin-CCTCTGCTCTACCAGCTGAACTATTGAAGG
tRNA ^{Tyr} (GUA)	5'biotin-CCTCCGCTCTACCAACTGAGCTATCGAAGG
elongator tRNA ^{Met} (CAU)	5'biotin-CTGACGCGCTACCTACTGCGCTAACGAGGC
tRNA ^{Ala} (AGC)	5'biotin-GCACGCGCTCTACCACTGAGCTATACCCCC
tRNA ^{Gly} (GCC)	5'biotin-GCGAGAATTCTACCACTGAACCACCCATGC
tRNA ^{Gly} (CCC)	5'biotin-TCTTGCATGATAACCACTACACCAGCGGCGC
tRNA ^{Gln} (UUG)	5'biotin-CCAGAGTGCTAACCATTACACCATGGGGCC