Supplemental Material

Figure S1. Alignment of Hsh155p/SF3B1 from human to budding yeast *S. cerevisiae*

The sequences of Hsh155p/SF3B1 from 8 different organisms including *H. sapiens* and *S. cerevisiae* were aligned using Clustal W method within MegAlign software (DNASTAR). Residues that exactly match the sequence of *H. sapiens* SF3B1, are colored in blue. Seven conserved SF3B1 residues that are frequently mutated in human diseases (MDS & CLL) are indicated in red.

Figure S2. *hsh155* alleles tested in this study show no detectable grow defects

(A) Selected *hsh155* alleles from yeast genetic screens with increased splicing activity of BS mutant reporters

(B) Selected *hsh155* alleles from disease-mutant residues with inhibited splicing activity of BS mutant reporters.

Figure S3. *In vivo* interactions between Prp5p and Hsh155p & U4/5/6 snRNAs.

(A) All of the tested Hsh155p mutants that enhanced *in vitro* Prp5p-interaction show decreased *in vivo* affinity with Prp5p at various levels.

(B) Mutant Hsh155p proteins exhibit unaffected interaction within the SF3B complex, indicated by a SF3B subunit, Cus1p/SF3B2.

(C) Prp5p's association of U4, U5 and U6 snRNAs are not affected by *hsh155* mutants.

(D) In the presence of *hsh155*-L313S or -H331R allele, Prp5p co-immunoprecipitated much

less pre-mRNA of WT reporter, whereas Prp5's association with pre-mRNA of BS-U257C mutant reporter is partly restored. However, in the presence of *hsh155*-H331D or -K335N allele, Prp5's association with pre-mRNA, either WT or BS-U257C mutant reporter, is not obvious changed.

*: $p < 0.05$, **: $p < 0.01$.

Figure S4. Localization of *hsh155* mutant residues in the structure of spliceosomal B^{act} complex

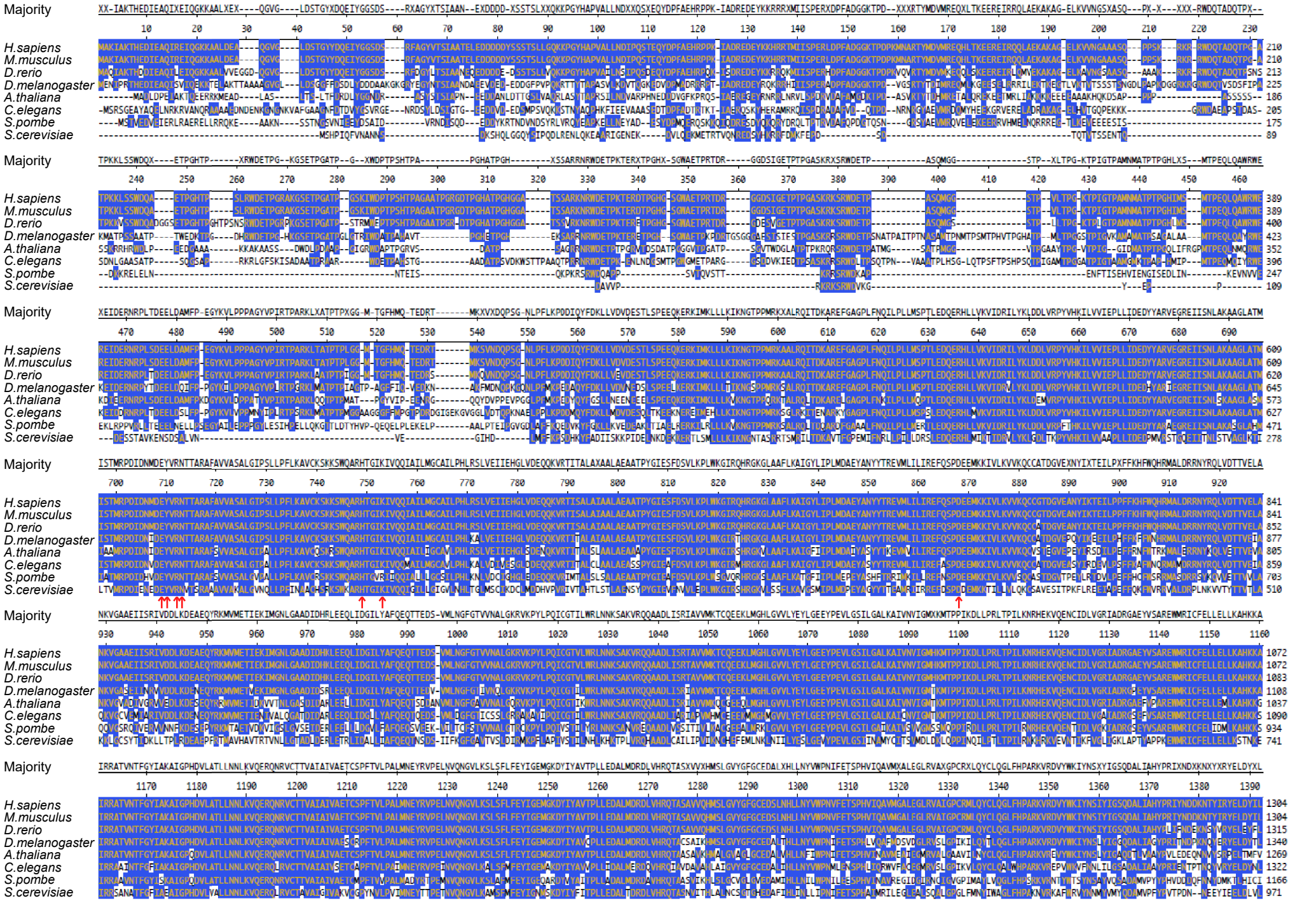
Locations of identified mutated residues of Hsh155p/SF3B1 in this study are indicated in pink and shown in the recently solved cryo-EM B^{act} structure (from Yan et al. 2016). Prp5p-direct-interacting HEAT repeats 4-9 of Hsh155p are labeled, which shows a tight holding of ribonucleotides close to downstream of branch site region.

Table S1. Recombinant proteins used in this study

Table S2. Yeast strains used in this study

Table S3. Primers used in RT-qPCR

Table S4. *hsh155* mutants derived from our yeast genetic screens that are also found in human diseases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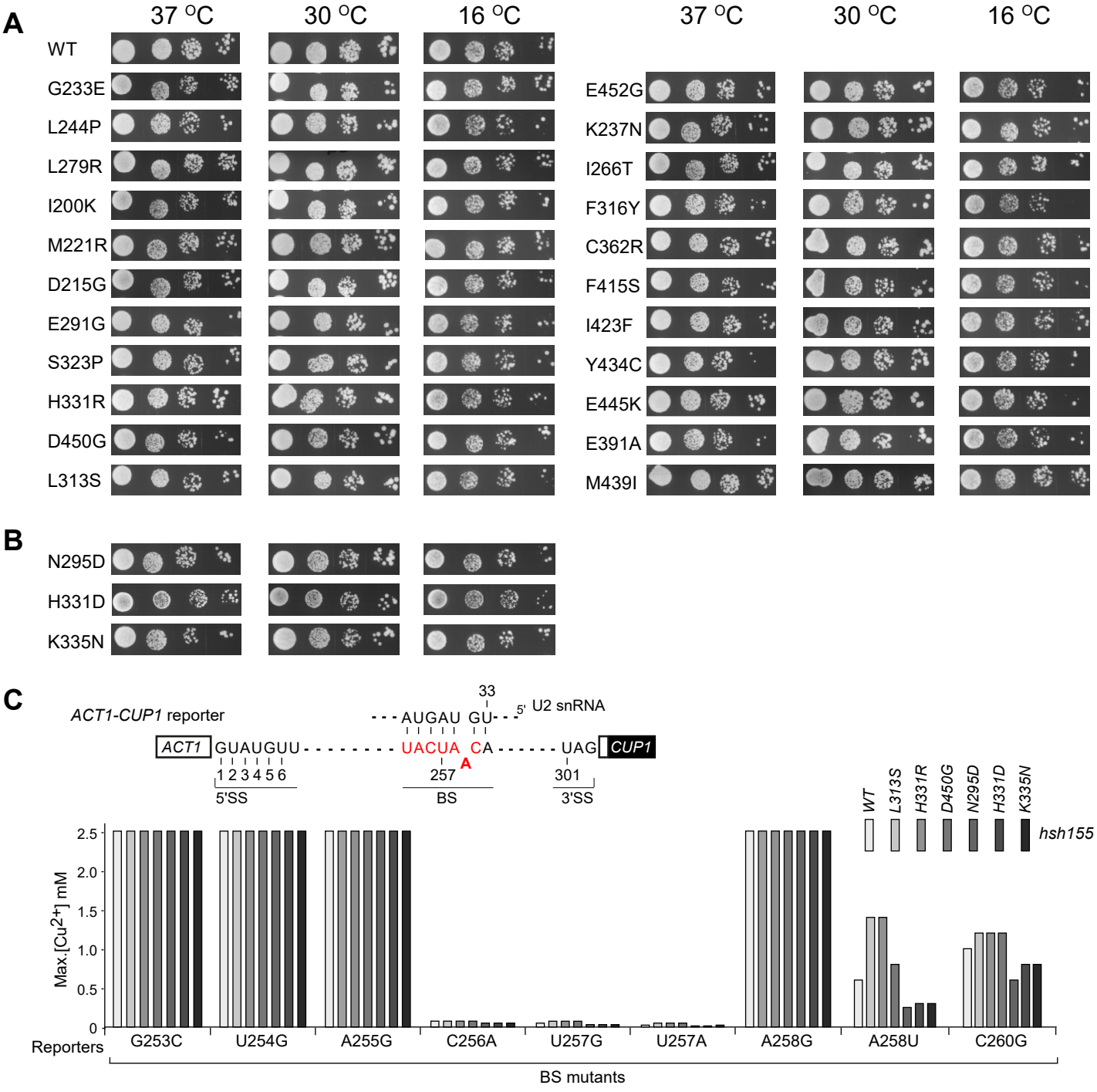
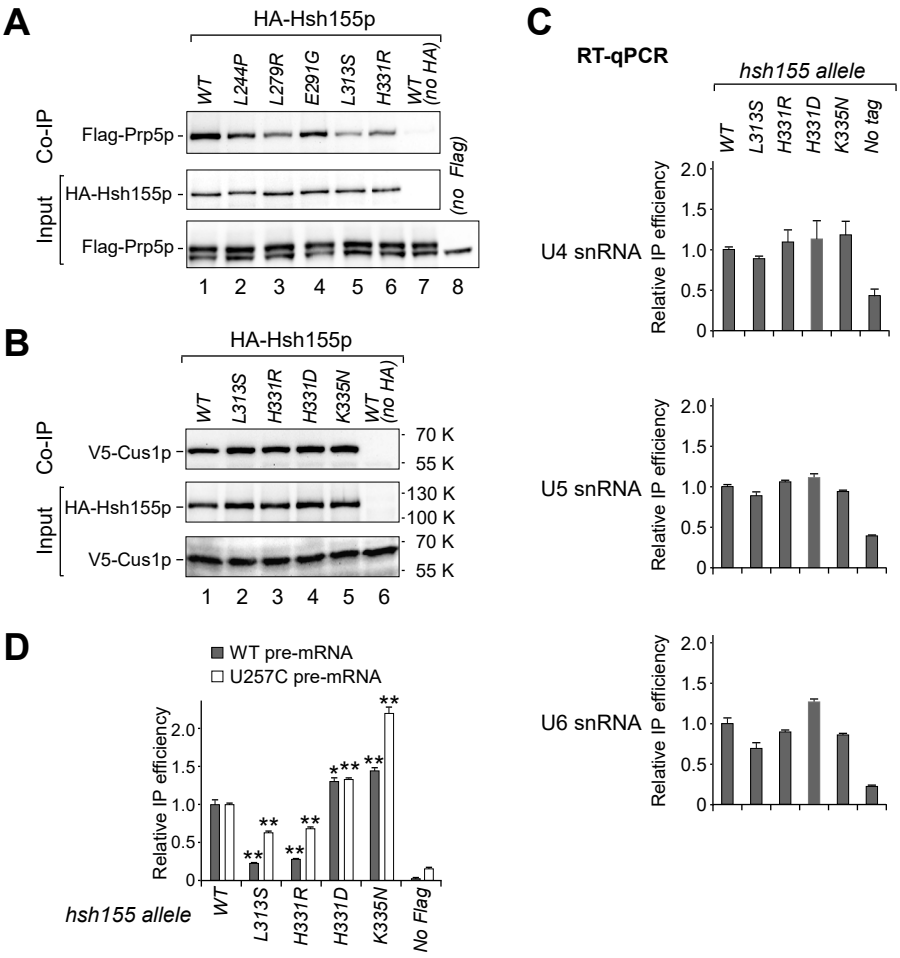
D.rerio
D.melanogaster
A.thaliana
C.elegans
S.pombe
S.cerevisiae


A
37 °C
30 °C
16 °C
WT
G233E
L244P
L279R
I200K
M221R
D215G
E291G
S323P
H331R
D450G
L313S
E452G
K237N
I266T
F316Y
C362R
F415S
I423F
Y434C
E445K
E391A
M439I
B
N295D
H331D
K335N
C
ACT1-CUP1 reporter
ACT1
GUAUGUU
1 2 3 4 5 6
5'SS
AUGAU GU
5' U2 snRNA
33
UACUA CA
A
257
BS
UAG
CUP1
301
3'SS
WT
L313S
H331R
D450G
N295D
H331D
K335N
hsh155
Max.[Cu2+] mM
2.5
2.0
1.5
1.0
0.5
0
Reporters
G253C
U254G
A255G
C256A
U257G
U257A
A258G
A258U
C260G
BS mutants

Tang_Fig.S3


A
HA-Hsh155p
WT
L244P
L279R
E291G
L313S
H331R
WT (no HA)
Co-IP
Flag-Prp5p
Input
HA-Hsh155p
Flag-Prp5p
(no Flag)
1 2 3 4 5 6 7 8
B
HA-Hsh155p
WT
L313S
H331R
H331D
K335N
WT (no HA)
Co-IP
V5-Cus1p
70 K
55 K
Input
HA-Hsh155p
130 K
100 K
V5-Cus1p
70 K
55 K
1 2 3 4 5 6
C
RT-qPCR
hsh155 allele
WT
L313S
H331R
H331D
K335N
No tag
U4 snRNA
U5 snRNA
U6 snRNA
Relative IP efficiency
2.0
1.5
1.0
0.5
0
D
WT pre-mRNA
U257C pre-mRNA
Relative IP efficiency
**
hsh155 allele
WT
L313S
H331R
H331D
K335N
No Flag

Tang_Fig.S4

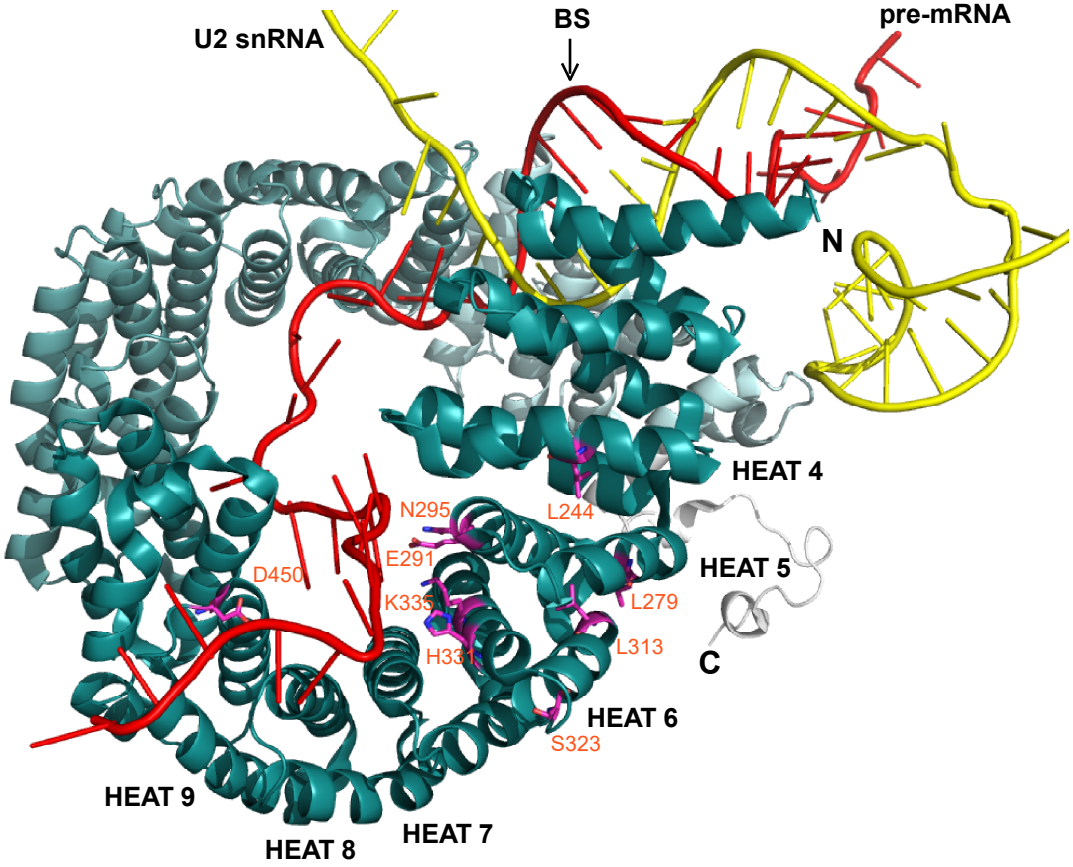

U2 snRNA
BS
pre-mRNA
N
HEAT 4
N295
L244
E291
D450
HEAT 5
K335
L279
H331
L313
C
HEAT 6
S323
HEAT 9
HEAT 8
HEAT 7

Table S1: Recombinant proteins used in this study

Proteins	Coverage of amino acids
Hsh155p-N	1-127 aa
Hsh155p-HEATs 1-8	122-434 aa
Hsh155p-HEATs 5-12	271-592 aa
Hsh155p-HEATs 9-16	425-761 aa
Hsh155p-HEATs 13-20	589-911 aa
Hsh155p-HEATs 17-22	755-971 aa
Hsh155p-HEATs 1-4	122-276 aa
Hsh155p-HEATs 5-8	271-434 aa
Hsh155p-HEATs 9-12	425-592 aa
Hsh155p-HEATs 13-16	589-761 aa
Hsh155p-HEATs 3-6	196-343 aa
Hsh155p-HEATs 1-5	122-308 aa
Hsh155p-HEATs 1-6	122-343 aa
Hsh155p-HEATs 1-12	122-592 aa
Prp5p-FL	1-849 aa
Prp5p-ΔN	206-849 aa
Prp5p-ΔC	1-698 aa
Prp5p-ΔN&ΔC	206-698 aa
Prp5p-APLD	1-849 aa (D137A)
Prp5p-AAAA	1-849 aa ($_{137}$DPLD$_{140}$ to AAAA)

Notes: All the Hsh155p recombinant proteins are GST tagged at their N-terminus; all the Prp5p recombinant proteins are 6xHis tagged at their N-terminus.

Table S2: Yeast strains used in this study

***S. cerevisiae* strains**	**Genotype**
yTQ01	*MATa ade2 cup1⊿:: ura3 his3 hsh155⊿:: loxP leu2 lys2 prp5⊿:: loxP trp1* pRS314-Prp5[*PRP5 TRP1* CEN ARS] pRS316-Hsh155[*HSH155 URA2* CEN ARS]
hsh155 mutants	Constructed from yTQ01; *hsh155* alleles were cloned into pRS317-LYS vector, and the WT pRS316-Hsh155 plasmid was removed using 5-FOA
prp5 mutants	Constructed from yTQ01; *prp5* alleles were cloned into pRS314-Trp, and introduced into the yeast strain by two-steps plasmid shuffling
V5-Cus1	Constructed from *hsh155* mutant strains, in which a pRS316-V5-Cus1 was introduced

Notes: Derived from the yTQ01 strain, other yeast strains were generated either by plasmid shuffling to lose WT sequence-containing plasmids, such as *hsh155* and *prp5* mutants (with/without tag), or by direct transformation of extract copy of gene, such as V5-Cus1.

Table S3: Primers used in the RT-qPCR

Primers	Sequences	Genes
TQ368-S	5'-TCTTTTATTTGCTACTGTGTCTCATGT	ACT1-CUP1
TQ369-AS	5'-TTAATTCGCTGAACCCGGTA	
TQ387-S	5'-GGAGATCAAGAAGTCCTACTG	U1 snRNA
TQ388-AS	5'-GTGTGTGACCAAGGAGTTTGC	
TQ434-S	5'-AGCCATGACTGCATCTGTTGTT	U2 snRNA
TQ435-AS	5'-CAACCATCAAGTCCGTTTCTTG	
TQ438-S	5'-ACGGGAAATACGCATATCAGTG	U4 snRNA
TQ421-AS	5'-GAACACCGAATTGACCATGAG	
TQ389-S	5'-GCAGCTTTACAGATCAATGG	U5 snRNA
TQ390-AS	5'-GGACAGCTTTACCTGTTTCTATGG	
TQ393-S	5'-GTTCGCGAAGTAACCCTTCG	U6 snRNA
TQ394-AS	5'-AACGAAATAAATCTCTTTGTAAAACGG	
TQ410-S	5'-GGTGGTCTCCTCTGACTTCAACA	GAPDH
TQ411-AS	5'-GTTGCTGTAGCCAAATTCGTTGT	

Table S4. *hsh155* mutants derived from genetic screens that are also found in human diseases

	hsh155 alleles	Positions in human SF3B1	In MDS or CLL	In other cancers (TCGA database)	In HEAT motifs	Max [Cu^{2+}] mM
From UV screening	WT					0.25
	L735S	*L1066*			16-helixA	0.8
	L732S	L1063			16-helixA	0.8
	E468K	D799			9-helixB	0.7
	L229P	L560			3-helixB	0.5
From error-prone PCR screening	WT					0.25
	G233E	D564			3-helixB	0.8
	L244P	L575			4-helixA	0.8
	L279R	I610			5-helixA	0.8
	I200K	L531			3-helixA	0.6
	M221R	L552			3-helixB	0.6
	D215G	D546			3-turn	0.6
	E291G	E622	E622D	Breast cancer (E622Q)	5-turn	0.6
	S323P	S654			6-turn	0.6
	H331R	H662	H662Q/D/Y	Uveal melanoma (H662R); Prostate cancer (H662Q)	6-helixB	0.6
	D450G	D781	D781G	Breast cancer (D781E)	9-turn	0.6
	L313S	L644			6-helixA	0.5
	E452G	E783			9-turn	0.5
	K237N	R568		Colorectal cancer (R568C)	3-linker	0.4
	I266T	I597			4-helixB	0.4
	F316Y	F647			6-helixA	0.4
	C362R	G693			7-turn	0.4
	F415S	F764			8-helixB	0.4
	I423F	I754			8-helixB	0.4
	Y434C	Y765		Breast cancer (Y765C); Breast cancer (Y765H)	8-linker	0.4
	E445K	E776			9-helixA	0.4
	E391A	E722		NCI-60 (E722K)	7-linker	0.3
	M439I	M770			9-helixA	0.3