Human cancer-associated mutations of SF3B1 lead to a splicing modification of its own RNA

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[4] Génétique, CHRU de Brest, France

Supplementary Materials and Methods

Immunofluorescence

After 48h of doxycycline induction, 300 000 cells were cultured on a coverslip coated with poly-L-lysine and incubated overnight in RPMI 1640 medium (Gibco), supplemented with 10% fetal bovine serum, 2 mM of L-glutamine and with 2 μg/mL of doxycycline. Cells were fixed with 4% para-formaldehyde and permeabilized with a PBS, 0,2% triton solution. Following incubation with primary anti-FLAG antibodies (1:1000, #F3165, Sigma) and secondary antibodies (1:1000, A-21121, Invitrogen), assembly between slide and cover-slide was done with the liquid mountant SlowFade™ Gold Antifade Mountant with DAPI (Invitrogen).

Supplementary figure legends

Table S1. Features of the MDS patients included in this study.

Table S2. Primers used in this study.

Figure S1. Complementary results obtained in K562 and UT-7 cells upon inducible expression of SF3B1^{K700E} or upon transitory expression of other important SF3B1 variants. (**A**) - Detection of SF3B1 proteins in K562 cells following inducible expression of *SF3B1*^{WT} or *SF3B1*^{K700E}. The immunoblot shows three independent K562 cell populations expressing *SF3B1*^{WT} (left) or *SF3B1*^{K700E} (right) (I: induced, NI: non- induced). (**B**) Detection of SF3B1 proteins in K562 expressing distinct *SF3B1* variants. K562 cells were transiently transfected by plasmids expressing *SF3B1*^{WT}, *SF3B1*^{K700E}, *SF3B1*^{E622D}, *SF3B1*^{H662Q} and *SF3B1*^{K666E}. (**C**) Detection of SF3B1 proteins in UT-7 cells following inducible expression of *SF3B1*^{WT} or *SF3B1*^{K700E}. The immunoblot shows two independent UT-7 cell populations expressing *SF3B1*^{WT} (left) or *SF3B1*^{K700E} (right) (I: induced, NI: non-induced). In (A-C), total SF3B1 proteins (endogenous and exogenous) were detected using anti-SF3B1 antibody. Plasmid-encoded SF3B1 was detected using anti-FLAG antibody.

Figure S2. SF3B1ins$_{FLAG}$ proteins are correctly addressed to the nucleus. Immunofluorescence staining of SF3B1-FLAG proteins in inducible K562 cell lines expressing various SF3B1 constructs. Nuclei were stained with DAPI.

Figure S3. Splicing analysis of RBM5, DUSP11, CCNA2 and STK6 in inducible K562 cell lines expressing shSF3B1 alone or in combination with various *SF3B1* constructs. (**A**) Analysis of splicing events known to be specifically altered upon SF3B1 loss of function (RBM5, DUSP11) or *SF3B1*^{mut} background (ENOSF1, TMEM14C, DPH5). (**B**) Quantification of CCNA2 or STK6 exon skipping was done by RT-qPCR, using primers allowing specific quantification of exon skipping events in CCNA2 (exon 5) and STK6 (exons 4, 5 and 6) transcripts, normalized to GAPDH.

Figure S4. Expression of the main disease-related *SF3B1* mutations does not affect growth of the *Schizosaccharomyces pombe prp10-1* strain. (**A**) Multiple alignment of the predominantly affected SF3B1 protein region in MDS (ClustalW software). The mouse (MOUSE), human (HUMAN), *Schizosaccharomyces pombe* (SCHPO) and *Saccharomyces cerevisiae* (YEAST) sequences are presented. The amino acids substitutions E622, R625, K666 and K700 found specifically in myelodysplastic syndromes are indicated in color. The lysine K700 residue, the most commonly affected in MDS, is preserved in *S. pombe* but not in *S. cerevisiae*. (**B**) *prp10-1* mutant cells were transformed with the empty vector pREP41, or a pREP41-HA expressing the wild type or mutated alleles of Sp*SAP155*, namely the counterparts of E622N, H662Q, K666N (R666N) and K700E. Streaks from independent transformants were made on EMM 2% glucose, replica-plated on EMM 2% glucose and cultured at 26°C or 37°C. (**C**) RT-PCR splicing analysis of TFIID and RPL7 transcripts in the *prp10-1* strain expressing different versions of Sp*SAP155*. Transformants were cultured at 26°C and subjected to a temperature switch at 37°C for 2 hours. RNA was extracted and analyzed by RT-PCR using specific primers allowing the detection of both spliced and un-spliced mRNA.

Figure S5. Uncropped blots corresponding to Figure 2A and Figure 2C. Densitometric ratios of SF3B1 or SF3B1-FLAG to GAPDH or β-actin are indicated for each lane.

Figure S6. Uncropped blots corresponding to Figure S1A, Figure S1B and Figure S1C. Densitometric ratios of SF3B1 or SF3B1-FLAG to GAPDH or β-actin are indicated for each lane.

Supplementary figures
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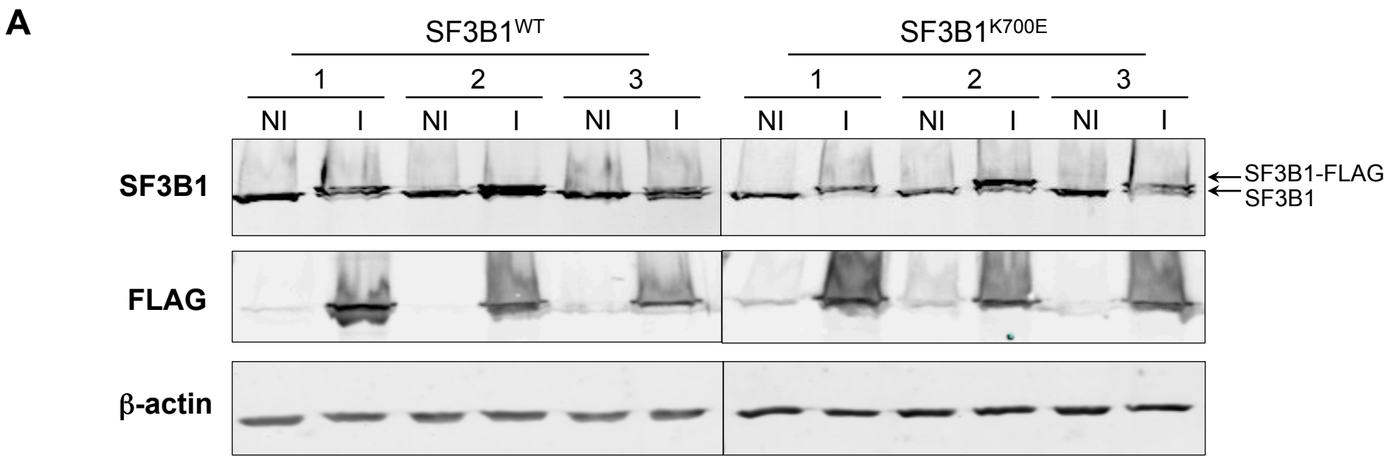
Figure S1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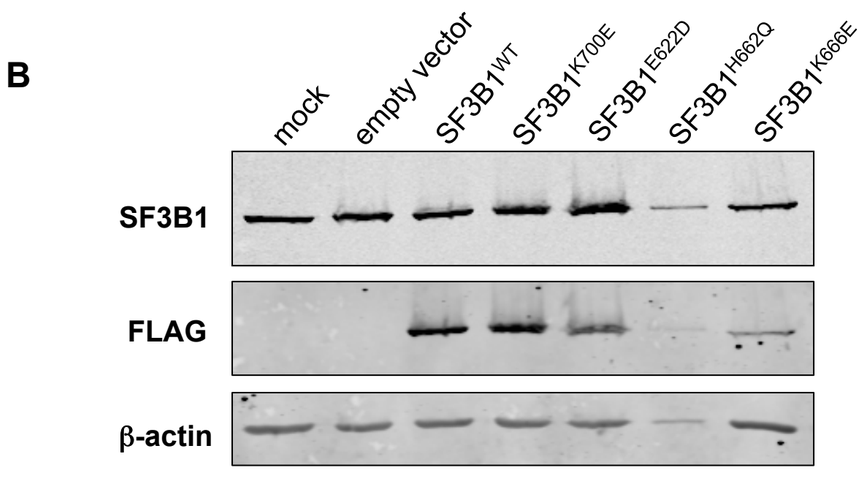
SF3B1
FLAG
β-actin
SF3B1-FLAG
SF3B1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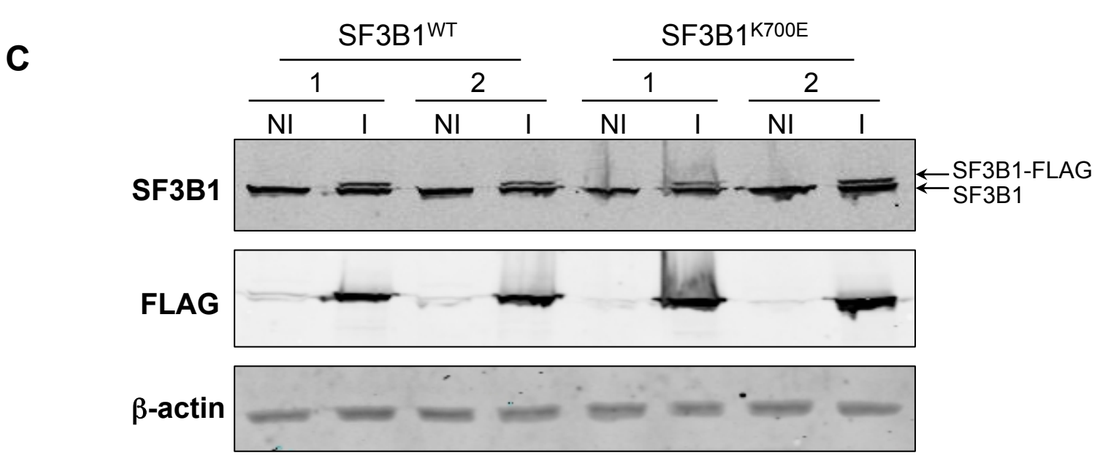
FLAG
β-actin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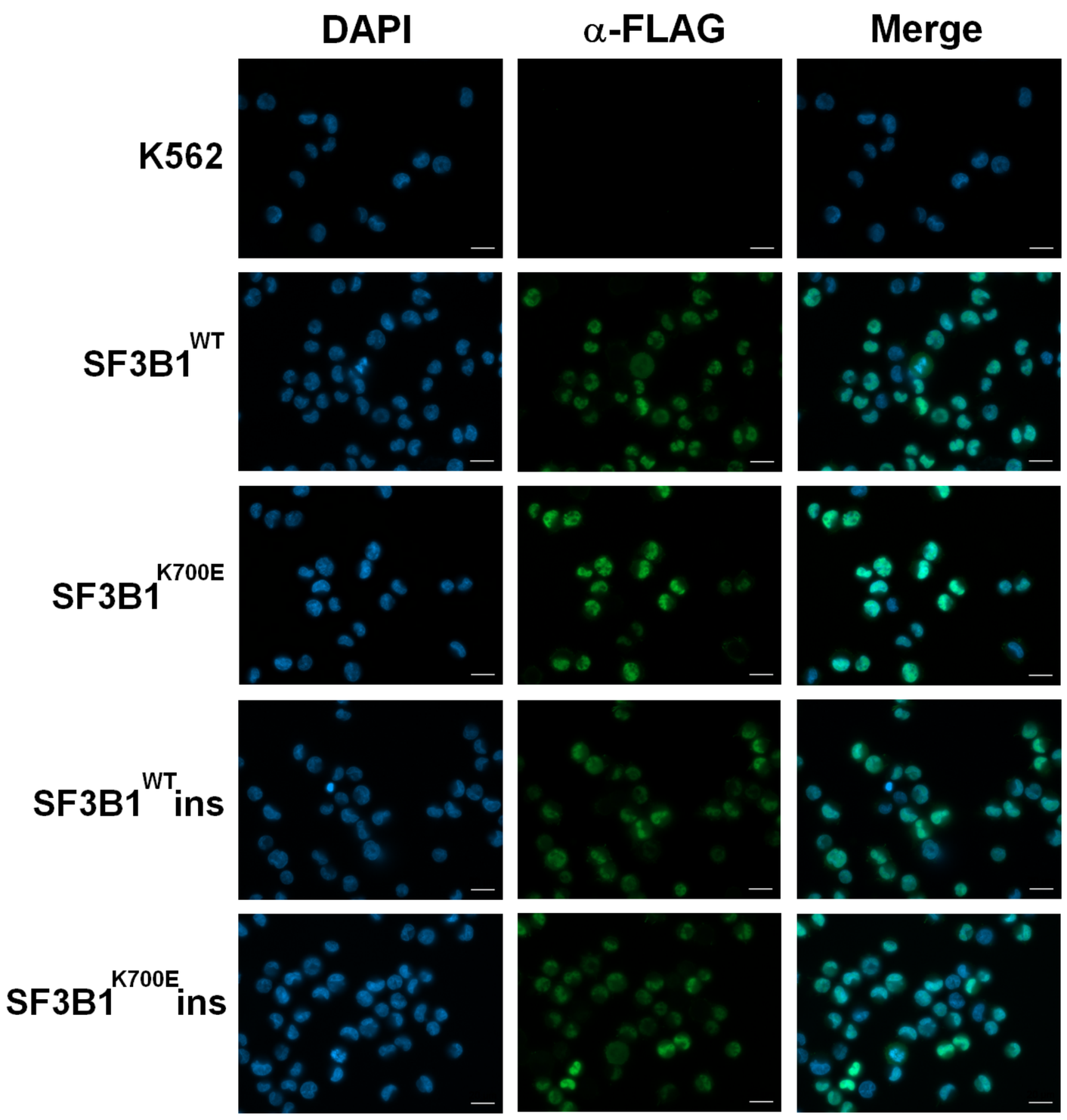
β-actin
SF3B1-FLAG
SF3B1

Figure S2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K562
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SF3B1K700E
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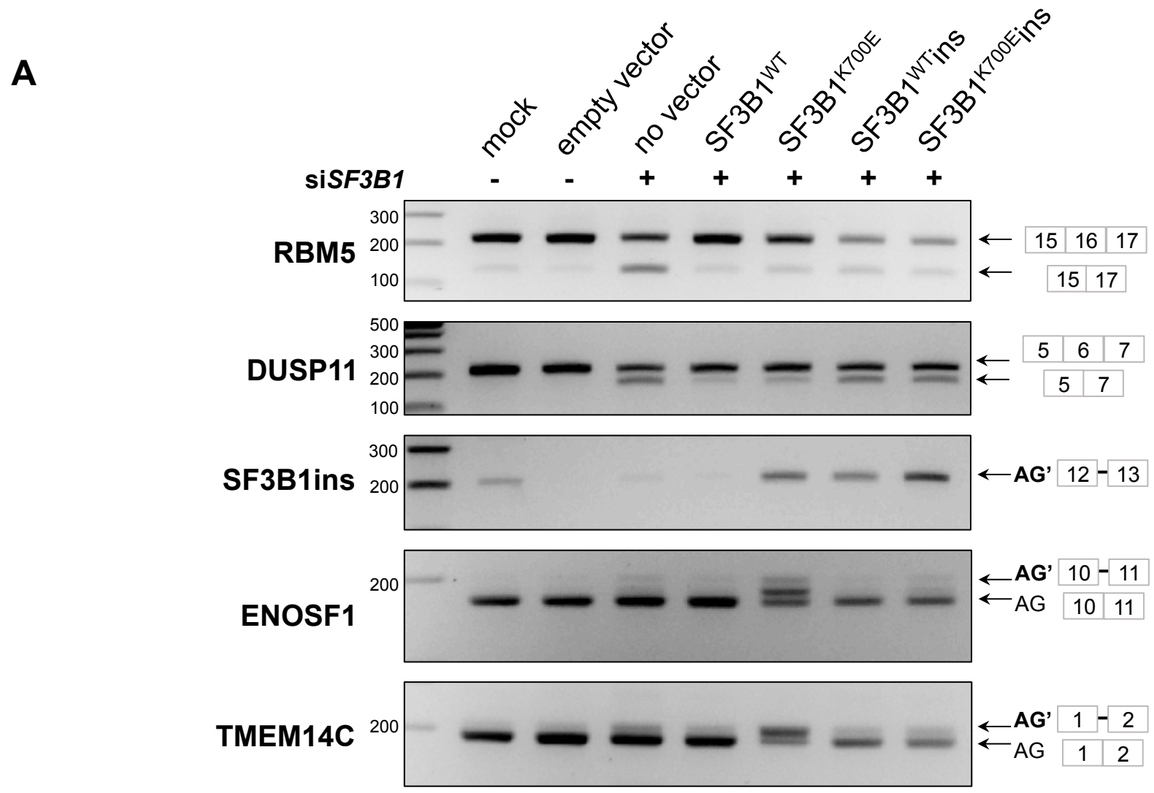
SF3B1K700Eins

Figure S3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RBM5
300
200
100
15 16 17
15 17
DUSP11
500
300
200
100
5 6 7
5 7
SF3B1ins
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200
AG' 12 13
ENOSF1
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AG' 10 11
AG 10 11
TMEM14C
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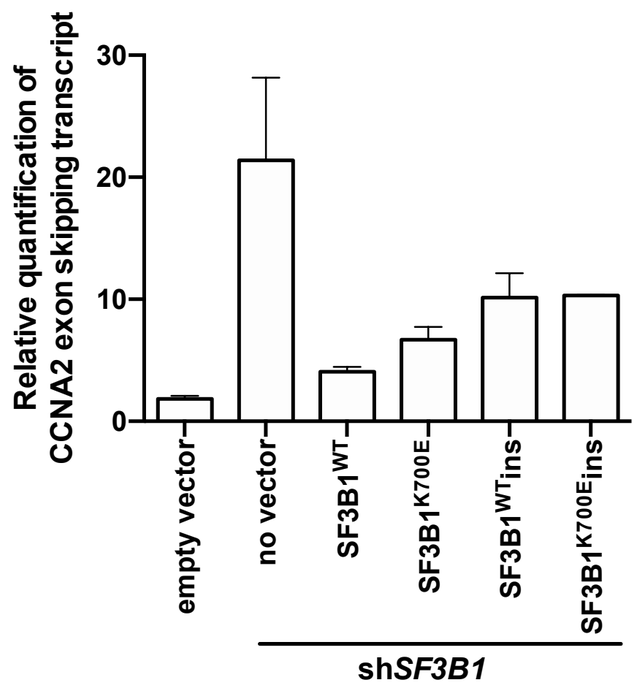
AG' 1 2
AG 1 2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shSF3B1

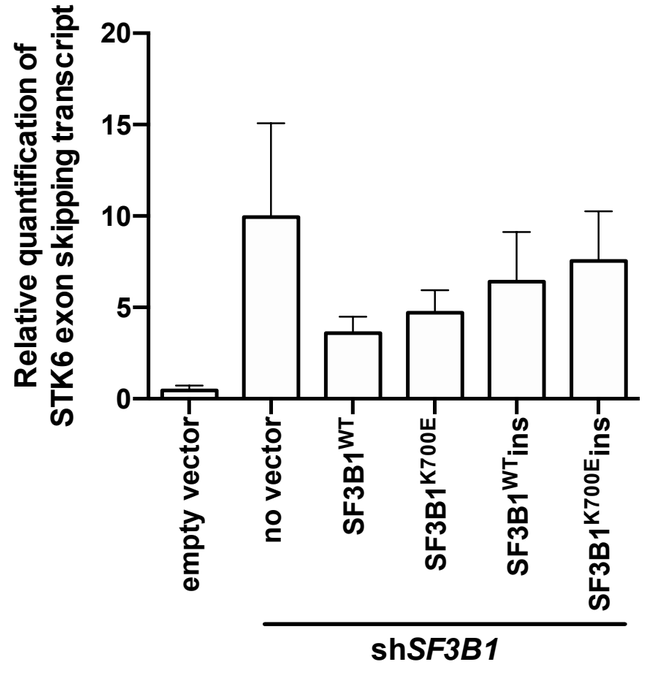
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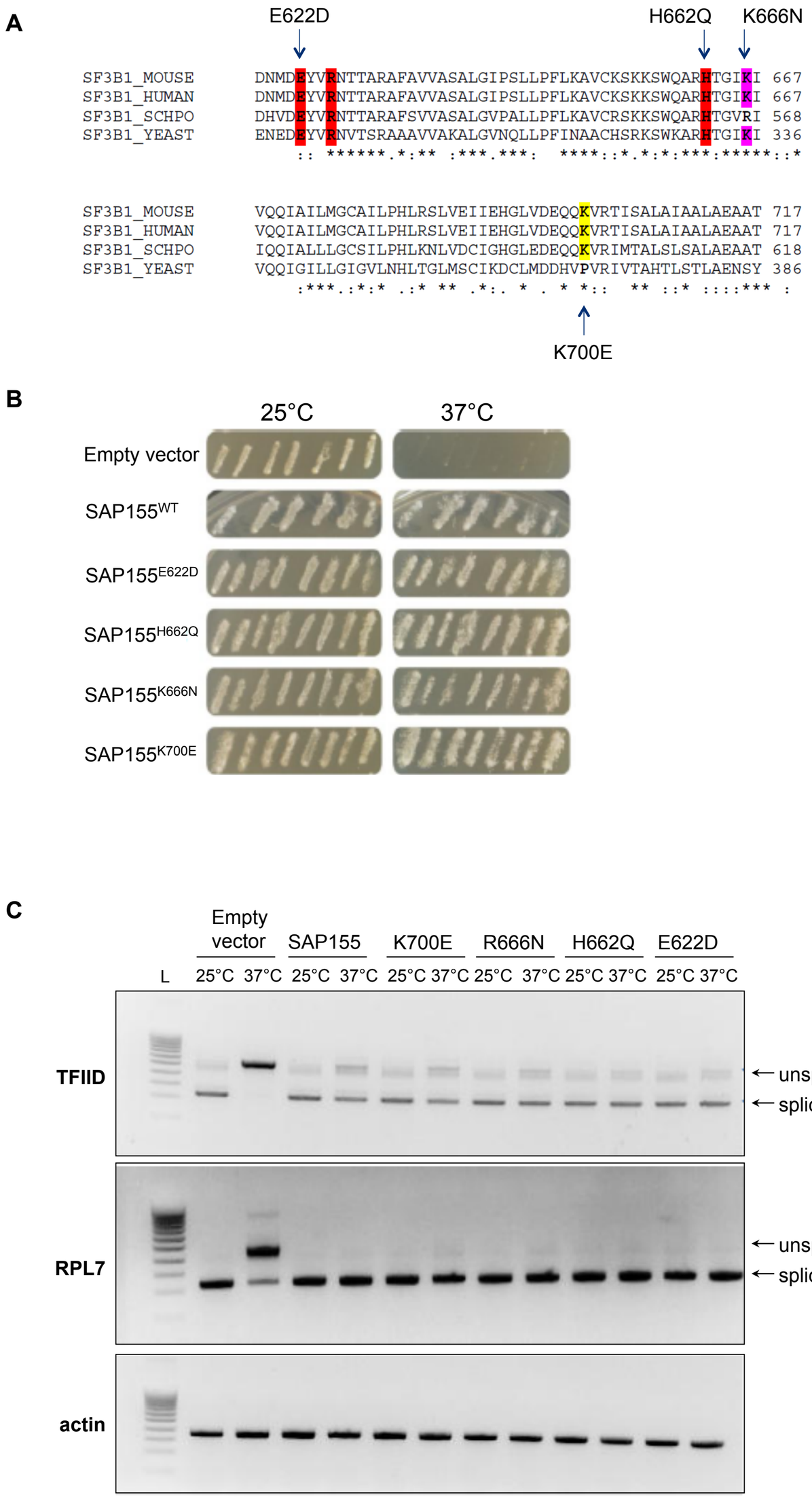
Figure S4


A
E622D
H662Q
K666N
SF3B1_MOUSE DNMDEYVRNTTARAFAVVASALGIPSLLPFLKAVCKSKKSWQARHTGIKI 667
SF3B1_HUMAN DNMDEYVRNTTARAFAVVASALGIPSLLPFLKAVCKSKKSWQARHTGIKI 667
SF3B1_SCHPO DHVDEYVRNTTARAFSVVASALGVPALLPFLKAVCRSKKSWQARHTGVRI 568
SF3B1_YEAST ENEDEYVRNVTSRAAAVVAKALGVNQLLPFINAACHSRKSWKARHTGIKI 336
SF3B1_MOUSE VQQIAILMGCAILPHLRSLVEIIEHGLVDEQQKVRTISALAIAALAEAAT 717
SF3B1_HUMAN VQQIAILMGCAILPHLRSLVEIIEHGLVDEQQKVRTISALAIAALAEAAT 717
SF3B1_SCHPO IQQIALLLGCSILPHLKNLVDCIGHGLEDEQQKVRIMTALSLSALAEAAT 618
SF3B1_YEAST VQQIGILLGIGVLNHLTGLMSCIKDCLMDDHVPVRIVTAHTLSTLAENSY 386
K700E
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37°C
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SAP155E622D
SAP155H662Q
SAP155K666N
SAP155K700E
C
Empty vector
SAP155
K700E
R666N
H662Q
E622D
L
25°C 37°C 25°C 37°C 25°C 37°C 25°C 37°C 25°C 37°C 25°C 37°C
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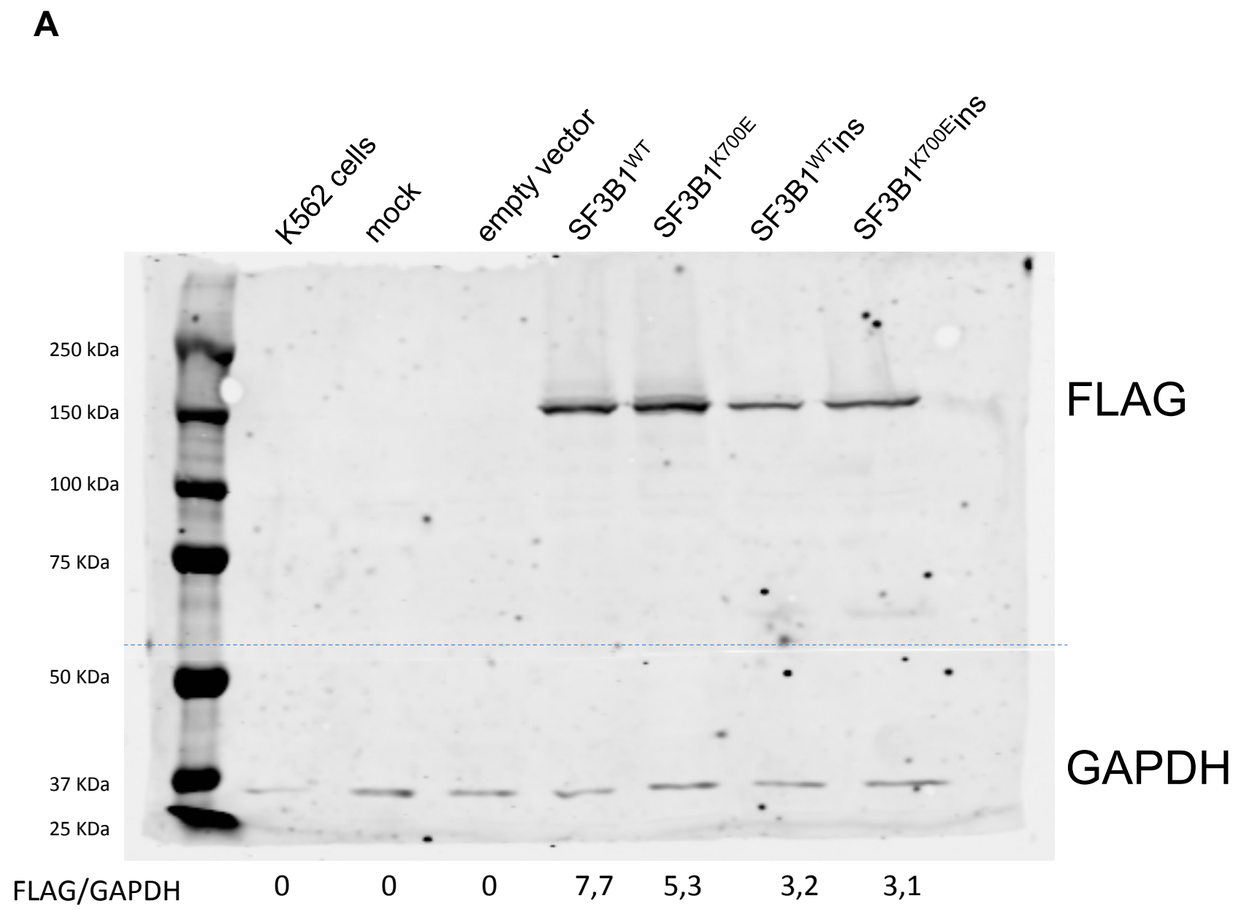
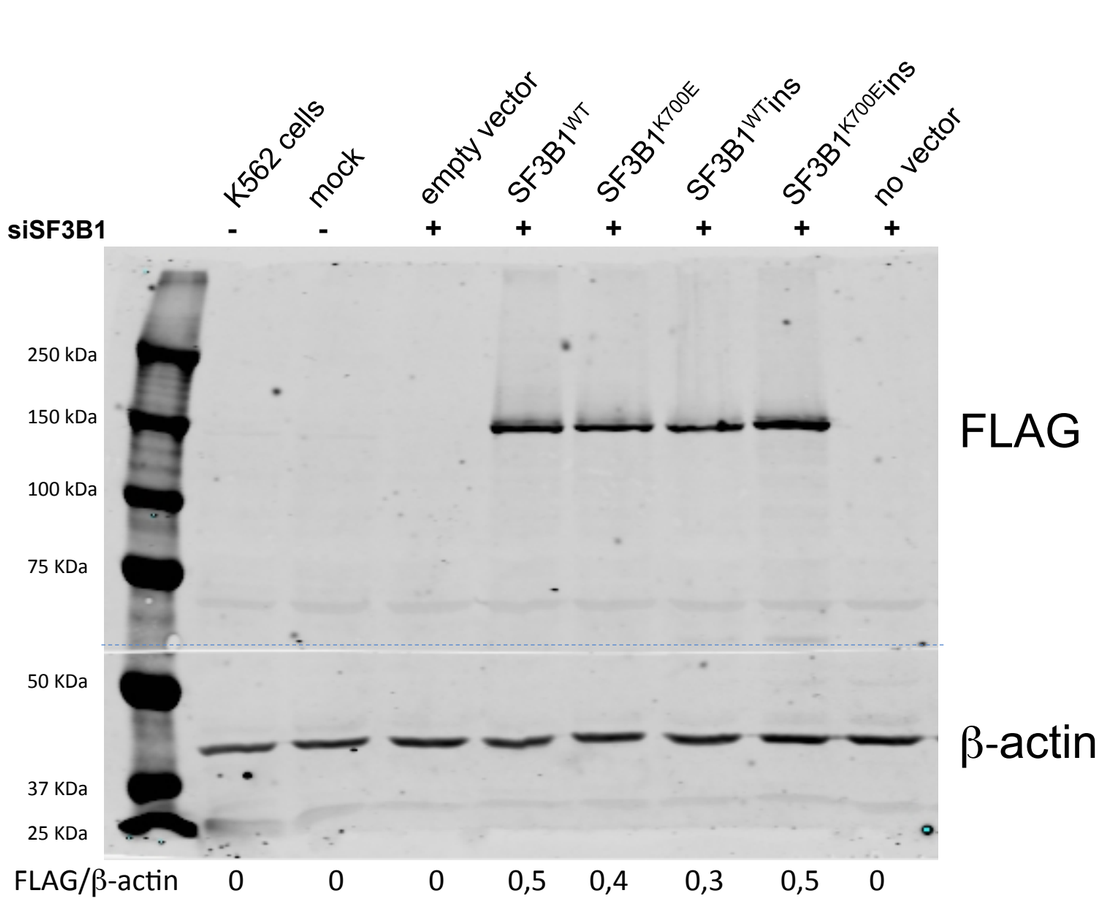
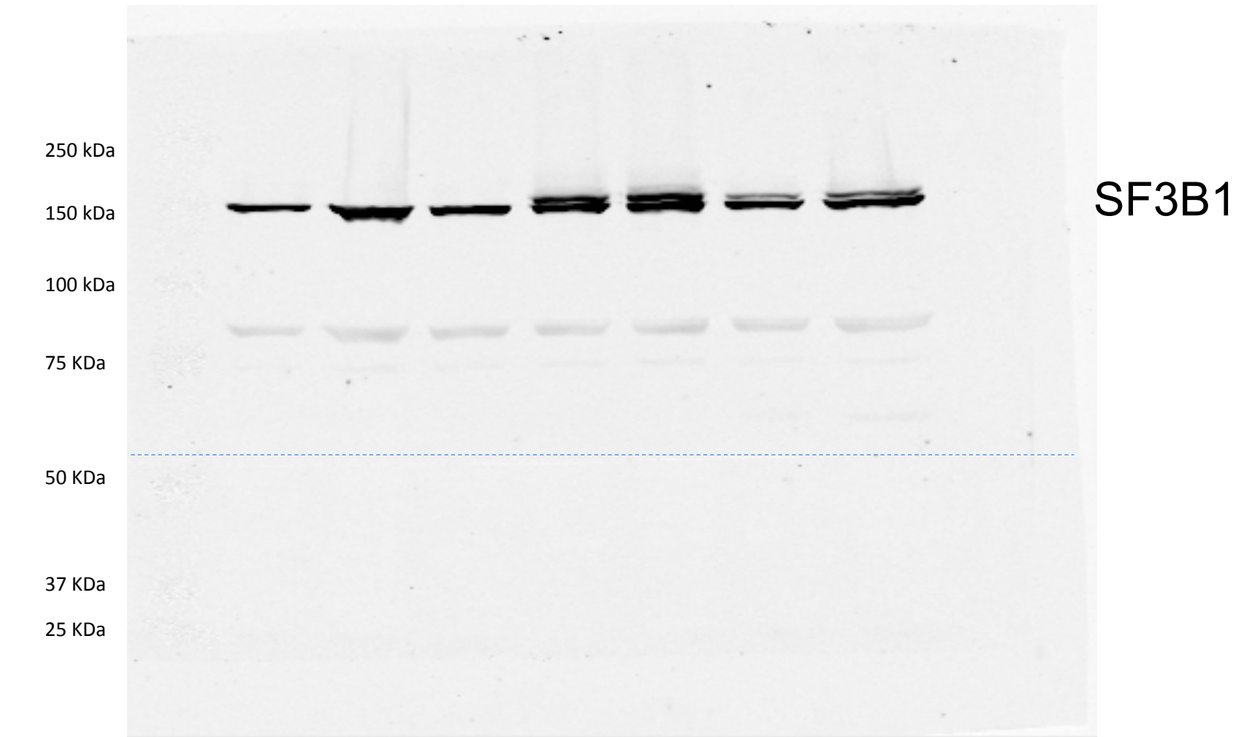
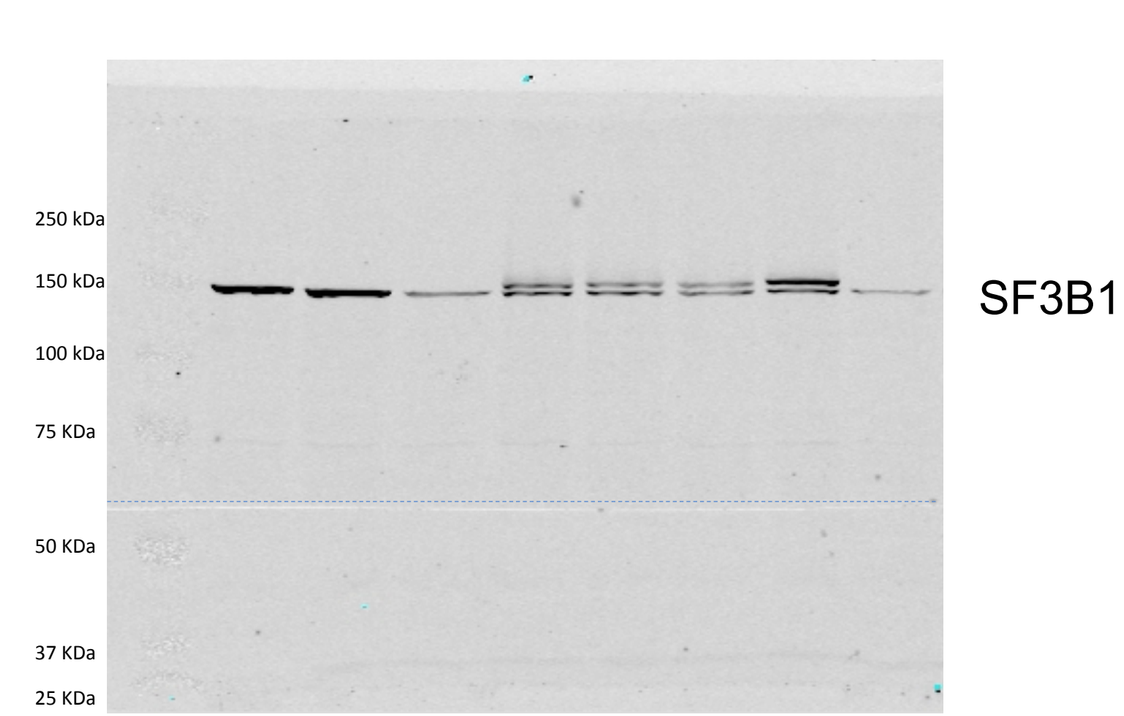
RPL7
actin
unspliced
spliced
unspliced
spliced

Figure S5



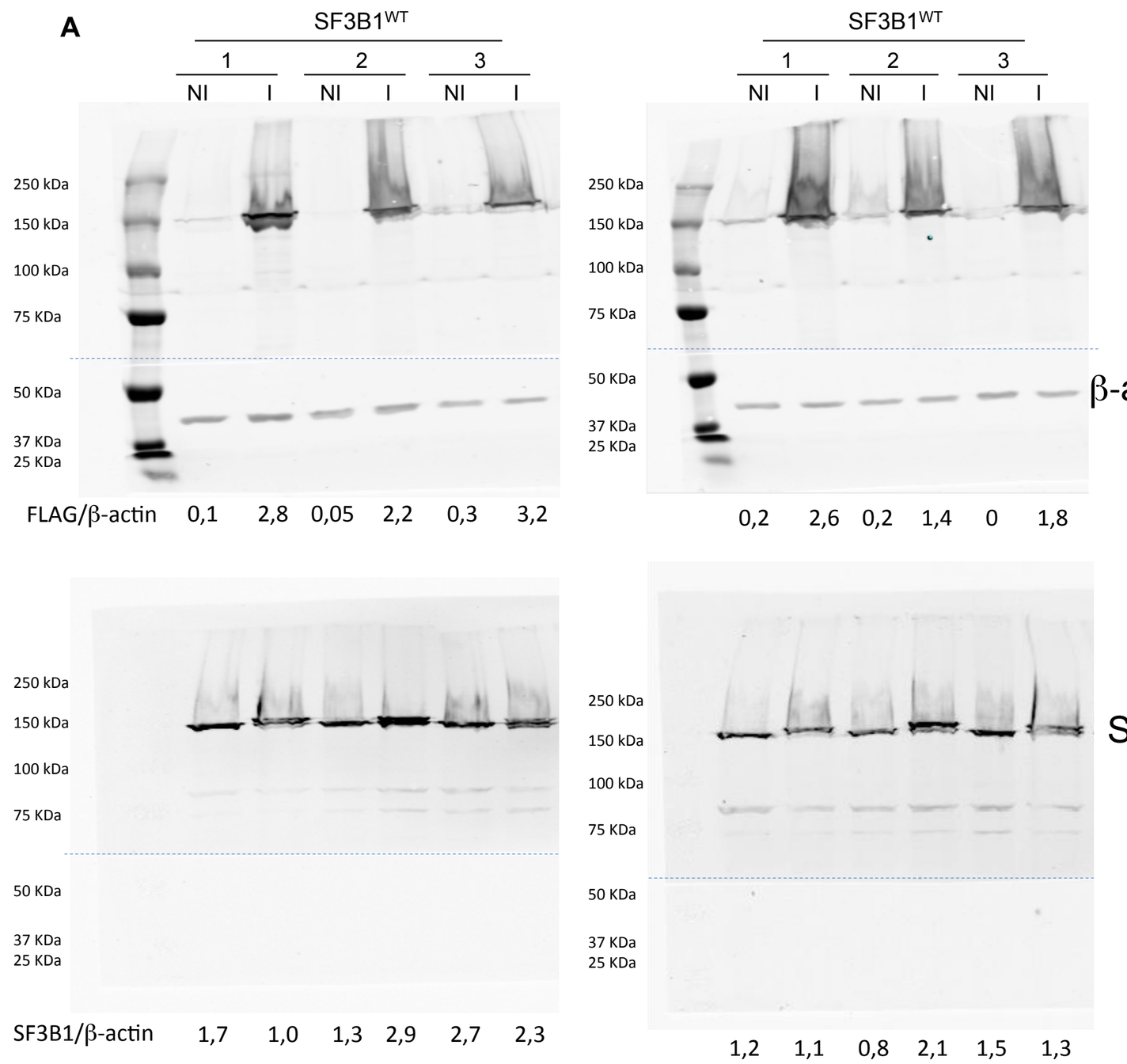


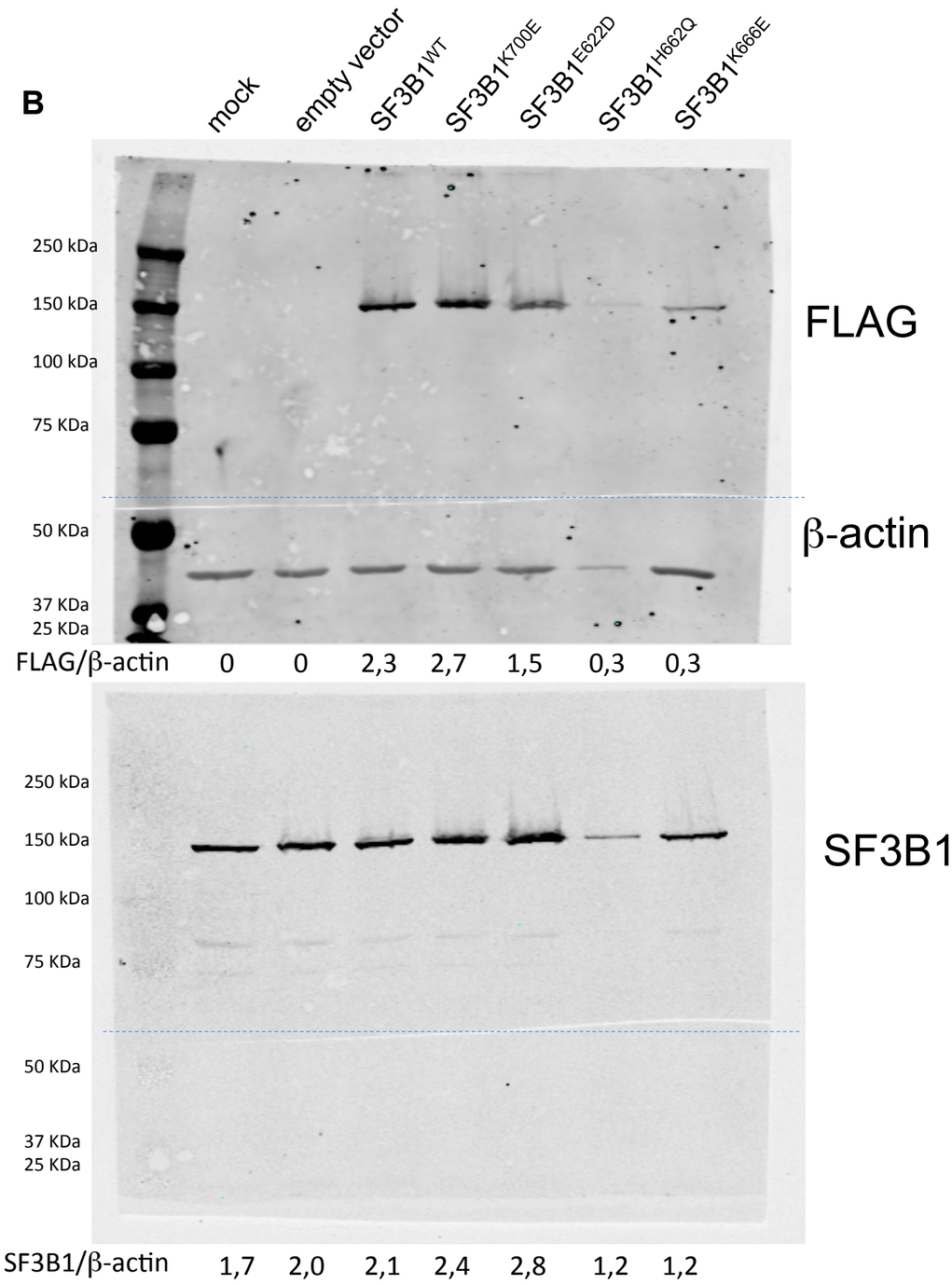


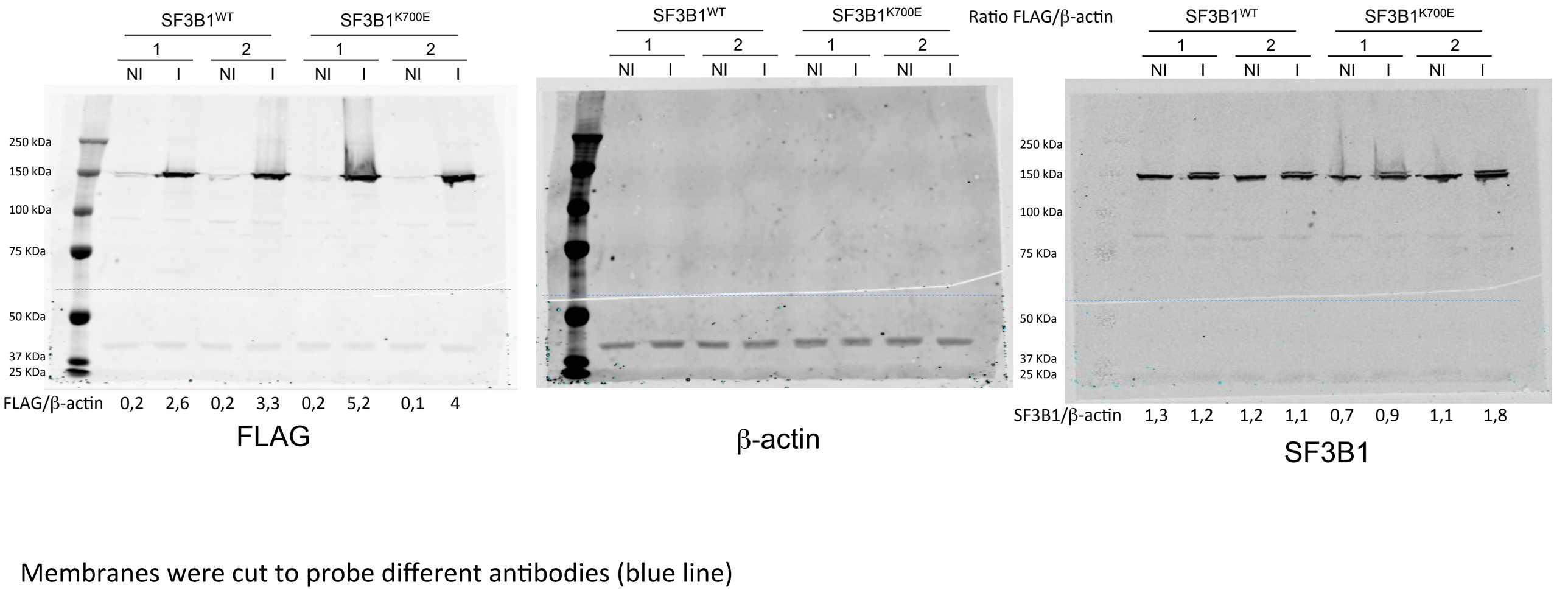


Membranes were cut to probe different antibodies (blue line)

Figure S6







Membranes were cut to probe different antibodies (blue line)

Supplemental Table S1

N°	Blood features	Bone Marrow features	MDS classification according 2016 revision WHO classification	% RS	medullar karyotype	MDS Cytogenetic scoring system
1	cytopenia	No dysmyelopoiesis	ICUS	U	normal	
2	cytopenia	No dysmyelopoiesis	ICUS	U	normal	
3	cytopenia	No dysmyelopoiesis	ICUS	0%	normal	
4	cytopenia	No dysmyelopoiesis	ICUS	0%	normal	
5	anemia	Dysmyelopoiesis	MDS-SLD	0%	normal	good prognosis
6	anemia	Dysmyelopoiesis	MDS-MLD	0%	normal	good prognosis
7	anemia	Dysmyelopoiesis	MDS-MLD	0%	normal	good prognosis
8	anemia	Dysmyelopoiesis	MDS-MLD	0%	normal	good prognosis
9	anemia	Dysmyelopoiesis	MDS-MLD	0%	normal	good prognosis
10	thrombopenia, neutropenia	Dysmyelopoiesis	MDS-MLD	0%	normal	good prognosis

N°	Blood features	Bone Marrow features	MDS classification according 2016 revision WHO classification	% RS	medullar karyotype	MDS Cytogenetic scoring system	**SF3B1** status (allele burden)	other mutated genes (NGS)
1	pancytopenia	Dysmyelopoiesis	MDS-EB1	44%	48,XY,+8,+12[5]/46,XY[38]	intermediate prognosis	**wild type**	TET2, SRSF2, RUNX1
2	thrombopenia	Dysmyelopoiesis	MDS-RS-MLD	18%	normal	good prognosis	**wild type**	TET2, ZRSR2
3	anemia	Dysmyelopoiesis	MDS-RS-MLD	53%	46,XY,del(20)(q11q13)[8]/45,X,-Y[8]/46,XY[7]	good prognosis	**R625C** (36,5%) R625L (7,3%)	TET2
4	anemia	Dysmyelopoiesis	MDS-RS-MLD	70%	normal	good prognosis	**K700E** (37%)	
5	anemia	Dysmyelopoiesis	MDS-RS-MLD	15%	normal	good prognosis	**E622D** (41%)	
6	anemia, thrombopenia	Dysmyelopoiesis	MDS-RS-MLD	62%	normal	good prognosis	**del gly740** (46%)	TET2, U2AF1, SH2B3
7	anemia	Dysmyelopoiesis	MDS-RS-SLD	12%	normal	good prognosis	**R625C** (33%)	TET2, EZH2, ASXL1
8	anema	Dysmyelopoiesis	MDS-RS-SLD	61%	normal	good prognosis	**K700E** (28,8%)	
9	anemia	Dysmyelopoiesis	MDS-RS-SLD	53%	45,X,-Y[12]/46,XY[9]	very good prognosis	**K666N** (12,3%) H662D (4,4%)	TP53
10	anemia	Dysmyelopoiesis	MDS-RS-SLD	70%	normal	good prognosis	**E622D** (40,35%)	
11	anemia	Dysmyelopoiesis	MDS-RS-SLD	80%	normal	good prognosis	**K666N** (39%)	
12	anemia, thrombocytosis	Dysmyelopoiesis	MDS/MPN-RS- with Thrombocytosis	90%	normal	good prognosis	**K700E** (48%)	MPL, DNMT3A, JAK2, ETNK1

ICUS : Idiopathic Cytopenia of Undetermined Significance
MDS-SLD : MDS wth single lineage dysplasia
MDS-MLD : MDS wth multilineage dysplasia
MDS-EB1: MDS with excess blasts-1
MPN: myeloproliferative neoplasms

Supplemental Table S2

		Name gene	Primers sense	exon number	Sequences
PCR	*Homo sapiens*	SF3B1	forward	exon 12	GCTCGTGAATTTGGAGCTGG
			reverse	exon 13	GCGGTTCAATGACCACGAGG
		SF3B1ins	forward	exon 12	GCATTGCGTCAGATTACTGA
			reverse	intron 12	CTGAAAAAGAGAAAAGAGAAGAAG
		DPH5	forward	exon 4	CTGTAGGCTGCTGTGGTTTAC
			reverse	exon 7-intron 7	CCTTCCCCTATAGGCAGAAA
		TMEM14C*	forward	exon 1	GACACCTCGCAGTCATTCCT
			reverse	exon 3	TGATCCCACCAGAAGCAACC
		ENOSF1*	forward	exon 10	GGGTGCTGATCTCCAGGATG
			reverse	exon 11	GGAGGTTGGCTCCTCAATCC
		RBM5	forward	exon 15	GGCTGTAGTGTCCCAGAGTC
			reverse	exon 17	GCCCTGTTGTCGGATCATAG
		DUSP11	forward	exon 5	GGACTGGCTACCTCATTTGC
			reverse	exon 8	GACTGGTTGCATGAGATGTGC
	S.pombe	TFIID	forward	exon 1	TTCCCTGTTCTCCCCAATGC
			reverse	exon 2	ACTTGGGTTCACGGATACGC
		NDA3	forward	exon 5	CGAGCAAATTCGCTCTGTTC
			reverse	exon 6	CATTGCAGAAAACTGATCACC
		RPL7	forward	exon 1	GTGTTGAGCAGGCTATTGCC
			reverse	exon 2	GCGTAATAAGCGCAGAACTTTGCGAATTT
		Actin	forward	/	GATTGTCGGTAGACCCCGTC
			reverse	/	CAGTCAACAAGCAAGGGTGC
qRT-PCR	*Homo sapiens*	CCNA2	forward	exon 4-exon 6	TGCTATGCTGTTAGCCTCTTTTT
			reverse	exon 6	CCCGTGACTGTGTAGAGTGC
		STK6	forward	exon 3-exon 7	CTGCCATCGGCACCTGTATAT
			reverse	exon 7	GCATGTACTGACCACCCAAA

* Primers from Dolatshad et al., 2016.

		Name	Primers sense	Application	Sequences
Cloning	*Homo sapies*	SF3B1 E622D	forward	mutagenesis	CCAGATATTGACAACATGGACGATTACGTGAGGAATACCAC
			reverse		GTGGTATTCCTCACGTAATCGTCCATGTTGTCAATATCTGG
		SF3B1 H662Q	forward		AAAGTTGGCAGGCCCGCCAGACCGGCATCAAGATTGTGC
			reverse		GCACAATCTTGATGCCGGTCTGGCGGGCCTGCCAACTAA
		SF3B1 K666E	forward		GCCCGCCACACCGGCATCGAGATTGTGCAGCAGATCGCA
			reverse		TGCGATCTGCTGCACAATCTCGATGCCGGTGTGGCGGGC
		pCW57.1	forward	Cloning	GTACAAACTTGTTGATGCTAGC
			reverse		GGTTTAGTAATGAACCGGTC
		SF3B1	forward		GCTAGCATCAACAAGTTTGTACCGCCACCATGGATTACAAG
			reverse		ACCGGTTCATTACTAAACCCTACAGGATGTAGTCCAGTTCGTAAC
		SF3B1ins	forward		CTTCTTCTCTTTTCTCTTTTTCAGATCCTGGTGGTCATTGAGCC
			reverse		CTTATGGACGTAGGGACGCAC
	S.pombe	SAP155 K700E	forward	mutagenesis	GAAGATGAACAACAAGAAGTACGAATTATGAC
			reverse		GTACATAATTCGTACTTCTTGTTGTTCATCTTC
		SAP155 R666N	forward		CCAGGCATACTGGCGTTAACATTATTCAACAGATTGC
			reverse		GCAATCTGTTGAATAATGTTAACGCCAGTATGCCTGG
		SAP155 H662Q	forward		CATGGCAAGCCAGGCAAACTGGCGTTCGGATTATTC
			reverse		GAATAATCCGAACGCCAGTTTGCCTGGCTTGCCATC
		SAP155 E662D	forward		GATCATGTCGACGACTATGTCCGAAACACC
			reverse		GGTGTTTCGGACATAGTCGTCGACATGATC