Supplemental Figure Legends

Figure S1: CRISPR verification of *prp-8* alleles. The genomic sequence and amino acid substitutions for *prp-8* alleles: G654E (left) and T524S (right). Amino acid changes are bold, uppercase, underlined, and nucleic acid substitutions are lower-case, bold.

Figure S2*:* Schematic of the N-terminal domain of Prp8. Isolated Prp8 N-terminal domains (amino acids 1-860) from structures of *S. cerevisiae* tri-snRNP(1), B complex (2), Bact complex (3), C complex (4), Cstar complex (5), and P complex (6) were aligned using pymol. The lower halves of all structures align well, while the upper portions are rotated in Bact, C, Cstar, and P complexes (blue colors) relative to their position in the tri-snRNP and B complex (white, gray). The Prp8 740 loop (indicated), of which Prp8-G736 is a component, only structures at Bact.

Figure S3*:* Analysis of relative abundances of pre-mRNA, splicing intermediates, and mRNA of Act-Cup reporters amplified from *PRP8* and *prp8-G736E*. The 5'exon-intron primer set spans the 5' splice-site providing an estimate of pre-mRNA level. The Intron-3'exon primer set spans the 3'splice-site reflecting the relative abundances of pre-mRNA and splicing intermediates. The junction primer set spans the 5'3' exon junction and is indicative of total mRNA level, while the 3'exon primer set provides a measure of all populations of Act-Cup reporter RNA. A primer set that amplifies 18S rRNA was included as a control. Data are normalized relative to *PRP8* carrying a WT Act-Cup reporter. Compared to *PRP8*, *prp8-G736E* exhibits a slight decrease in G5A reporter mRNA abundance, consistent with copper growth data. Error bars represent SEM of three biological replicates.

Figure S4*:* Genetic interactions between *prp8-G736E* and *snu114* alleles implicated in GTP binding and hydrolysis. Double mutant strains carrying either prp8-G736E or PRP8 and a snu114 allele were serially diluted and then spotted onto rich growth media and then allowed to grow at 37°C, 30°C, 25°C, and 17°C. The only snu114 allele that genetically interacted with prp8-G736E was snu114-12.

Figure S5*:* CRISPR mutagenesis to identify viable PRP-8 G654 and T524 substitutions. A) CRISPR strategy to randomize G654. The top shows the genomic sequence and codons of the PRP-8. The location of the CRISPR-cas9 crRNA-guided cut site is indicated. The central region of the 100mer repair oligonucleotide is shown. The 3 nts that define the G654 codon were

www.pnas.org/cgi/doi/10.1073/pnas.1819020116

randomized to produce a range of possible amino acid substitutions at this position. B) Alleles of *prp-8* recovered from this experiment. The allele name, new 654 codon triplet sequence and amino acid are indicated. *az83* inserts 8 amino acids between codons 652 and 653. C) CRISPR strategy to randomize T524. The top shows the genomic sequence and codons of PRP-8. The location of the CRISPR-cas9 crRNA-guided cut site is indicated. The center of the 113mer repair oligonucleotide is shown. The 3 nucleotides that define the T524 codon have been randomized during oligonucleotide synthesis to produce a range of possible amino acid substitutions at this position. D) Viable alleles of *prp-8* recovered from this experiment. The allele name, new 524 codon triplet sequence and amino acid are indicated. Several of these substitution alleles were isolated independently, and the number of multiple incidents indicated.

Figures S6*:* Alternative splicing events predicted by RNASeq analysis confirmed by ^{32}P RT-PCR. The gene measured, along with whether it is an alternative 5' or 3' splicing event is indicated. Each panel shows the sequence of the gene at the alternative splicing region. Upper case are sequences that can be exonic while lower case letters are always intronic. The triangles (filled or open) correspond to the splice-sites detected by RT-PCR in the storage phosphor image below. Sequences of all RT-PCR primers are found in Table S4. Tables 4 and 5 show quantitation of the relative usage of the alternative 5' (Table 4) or 3' (Table 5) splice-sites. Two of the panels (B and G) show strong dimers for each PCR band, likely due to Taq polymerase slippage on A/T rich runs. We combined both bands in the doublet for relative splicing quantitation.

References:

1. Nguyen THD, et al. (2016) Cryo-EM structure of the yeast U4/U6.U5 tri-snRNP at 3.7 Å resolution. *Nature* 530(7590):298–302.
2. Plaschka C, Lin P-C, Nagai K (2017) Structure of a pre-catalytic spliceosome. *Nature* 546(7660):617–621.
3. Yan C, Wan R, Bai R, Huang G, Shi Y (2016) Structure of a yeast activated spliceosome at 3.5 Å resolution. *Science* 353(6302):904–911.
4. Galej WP, et al. (2016) Cryo-EM structure of the spliceosome immediately after branching. *Nature* 537(7619):197–201.
5. Fica SM, et al. (2017) Structure of a spliceosome remodelled for exon ligation. *Nature* 542(7641):377–380.
6. Bai R, Yan C, Wan R, Lei J, Shi Y (2017) Structure of the Post-catalytic Spliceosome from Saccharomyces cerevisiae. *Cell* 171(7):1589-1598.e8.

Supplemental Tables

Table S1: Summary of high throughput sequencing library data

Strain - Lib #	Genotype	Unique Mapped Reads x 10^6	Junction Reads for each *unc-73* cryptic 5'SS -1	wt	+23
May 2016 PRP-8 G654E analysis					
CB936-1	*unc-73(e936); prp-8(+)*	40.0	1	0	9
CB936-2	*unc-73(e936); prp-8(+)*	30.6	3	3	13
SZ116-1	*unc-73(e936); prp-8(az24)*	14.9	0	2	3
SZ116-2	*unc-73(e936); prp-8(az24)*	34.0	2	6	11
SZ155-1	*unc-73(e936); prp-8(az29)*	36.8	3	6	8
SZ155-2	*unc-73(e936); prp-8(az29)*	33.7	1	8	8
SZ118-1	*unc-73(e936) snrp-27(az26)*	29.4	0	4	8
SZ118-2	*unc-73(e936) snrp-27(az26)*	41.3	2	9	12
Sept 2017 PRP-8 T524S analysis					
SZ181-1	*unc-73(e936); prp-8(+)*	16.1	2	0	3
SZ181-2	*unc-73(e936); prp-8(+)*	16.0	1	0	14
SZ179-1	*unc-73(e936); prp-8(az43)*	9.7	0	1	2
SZ179-2	*unc-73(e936); prp-8(az43)*	30.9	0	3	7
SZ195-1	*unc-73(e936); prp-8(az43)*	9.0	1	1	4
SZ195-2	*unc-73(e936); prp-8(az43)*	10.6	0	1	2
SZ197-1	*unc-73(e936); prp-8(az50)*	13.2	2	2	3
SZ197-2	*unc-73(e936); prp-8(az50)*	14.1	2	3	1

Table S2: Additional alternative 3' splice-sites predicted to change in the *prp-8* T524S mutant strains. These changes suggested by the RNASeq analysis were not confirmed by RT-PCR. The alternative 3' splice-site promoted in the T524S mutant strain are indicated by a filled triangle ▲, while the partner 3' splice-site is indicated by an open triangle △.

Gene	Sequence
hda-6	tgagaataaag▲TTTCAG△AACTCGATGAA
smrc-1	caaaaagtttag▲AATTTCCAG△CCAATGAAGCC
F52H2.6	tgattaaaattgcag△GCGGCAAAGACGGAGCAGGCGAAACAG▲GATAATTCTTT
ppfr-1	agctccgacctag▲AATTTTTTGCAG△GCTCATGCGGCAGA
B0001.7	atgatgtctcatttacag△CCTCTAATTCAG▲GAACTATTA

Table S3: CRISPR oligos used

CRISPR crRNA sequences	
prp-8 G654 crRNA	CUGUUGUCUCGACAAUUCGA
prp-8 T524 crRNA	AGUUAAAACAUUGACAACAA
CRISPR Repair Oligos	
G654E	GAATCACACCACTGCTCGAGAGATGGCTTGGAAATCTGTTGTCTCGACAATTtGAAGaAAGACATTCAAAGGGAGTGGCCAAGACAGTCACCAAGCAAAG
The t is a point mutation that eliminates a BstBI site. It is translationally silent. The a changes codon G654 to E. It also disrupts the PAM sequence.	
T524S	CTCAACTATCTTCATCTTGATTACAACTTCAATTTGAAACCAGTTAAAACgTTGACAtCAAAaGAAAGAAAGAAATCTCGTTTCGGAAATGCATTCCATTTGTGCAGAGAAAT
The g is a silent mutation that generates a new AclI restriction site. The t changes the T524 codon ACA to TCA (Serine). The a is a silent mutation that disrupts the PAM sequence.	
G654 random	GAATCACACCACTGCTCGAGAGATGGCTTGGAAATCTGTTGTCTCGACAATTtGAAnnnAGACATTCAAAGGGAGTGGCCAAGACAGTCACCAAGCAAAG
The t is a point mutation that eliminates a BstBI site. It is translationally silent. The nnn changes codon G654 to a random codon. It also disrupts the PAM sequence.	
T524 random	CTCAACTATCTTCATCTTGATTACAACTTCAATTTGAAACCAGTTAAAACgTTGACAnnnAAaGAAAGAAAGAAATCTCGTTTCGGAAATGCATTCCATTTGTGCAGAGAAAT
The g is a silent mutation that generates a new AclI restriction site. The nnn randomizes the T524 codon. The a is a silent mutation that disrupts the PAM sequence.	

Table S4: PCR primers used in ^{32}P-RT-qPCR splicing analysis and *C. elegans* genotyping

Target	Forward Primer	Reverse Primer
unc-73(e936) splicing	GCAGTGTGCCTAGAAAAGTG	TCGTCCCTTAAAGTAGGCTC
T12C9.7 splicing	GAAACGGAGCTCAGATTACG	CGAAGTGCTACGTAGTTTCG
stip-1 splicing	GTTCGAGATCAACGACATGG	GGCTTGTTCCTTGTTCATCG
spat-2 splicing	GGCTTGTTCCTTGTTCATCG	TTCCACCCTGTGTTCCAATC
Y46H3C.5 splicing	ATCAAGTCGACGAAGGATGC	CTCCCGATCCAACCATTCTG
faah-4 splicing	TCTCTGTTTCTTGGCTAATTTGC	GTTATCGTCAAGAGCTTCTGC
cdt-2 splicing	GCCTCACTTGAATCAAGAGC	CGTCCATAATTGCACCATCG
T10E9.14 splicing	GATAATGGAGAAACAGATGGAAGAG	ATTCAGCGAATTGGCGTGTG
F48D6.4 splicing	TCACGATGAATCGACACACTTG	AGAAAAGCGCATTGTCGGTC
W09G3.7 splicing	GTCGAAAAATCGCCTCTACG	CGGACGAAATCTCGAGAATC
dct-1 splicing	TCATCGGCTCAACAGACAAC	CTACTGCACAAACTGGTACG
prp-8 T524 region	AATGCCCGTGAAAGTTCGTG	TCCACGCAAGAAGAACAACC
prp-8 G654 region	GTACCAACTTGCAGATGGTC	GTTTTGTCTACTGTCGCTCC

Figure S1

PRP-8 G654E Alleles

	651	652	653	**654**	655
prp-8(+)	caa	tt**c**	gaa	g**g**a	aga
	Q	F	E	**G**	R
Suppressor allele					
prp-8(az24)				**a**	
				E	
CRISPR allele					
prp-8(az29)		**t**		**a**	
				E	

PRP-8 T524S Alleles

	521	522	523	**524**	525
prp-8(+)	ac**a**	ttg	aca	**a**ca	aag
	T	L	T	**T**	K
Suppressor allele					
prp-8(az43)				**t**	
				S	
CRISPR allele					
prp-8(az50)	**g**			**t**	
				S	

Figure S2

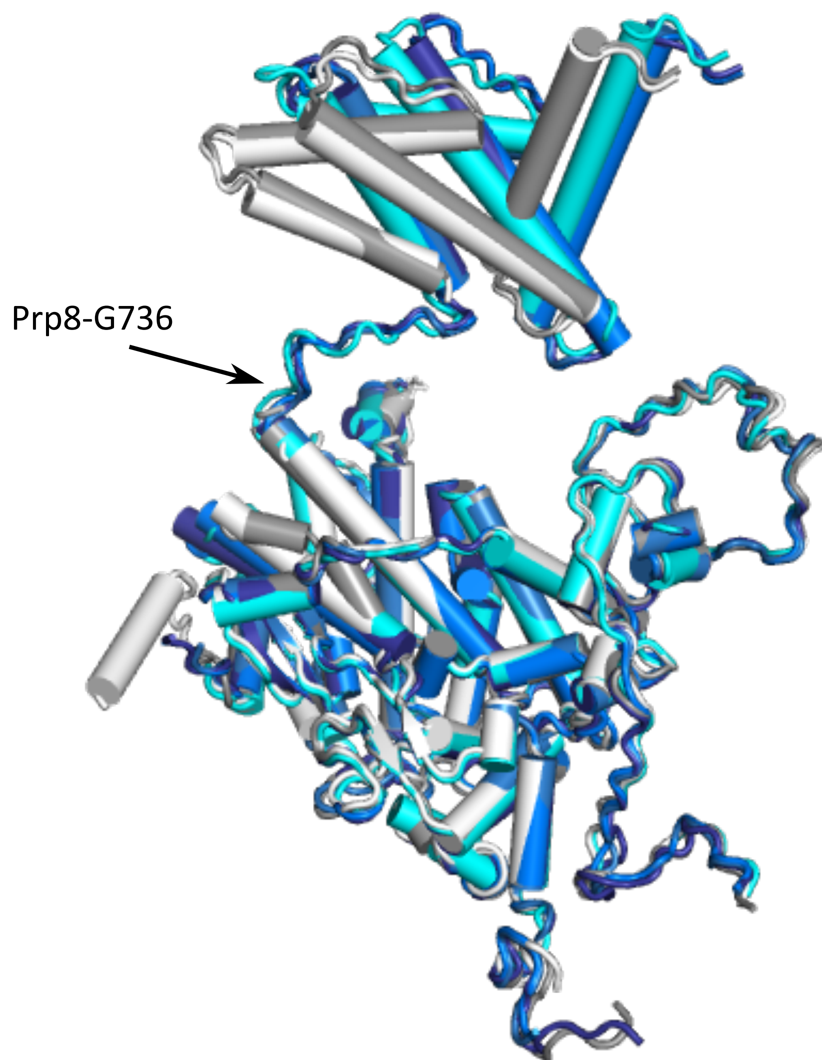


Figure S3

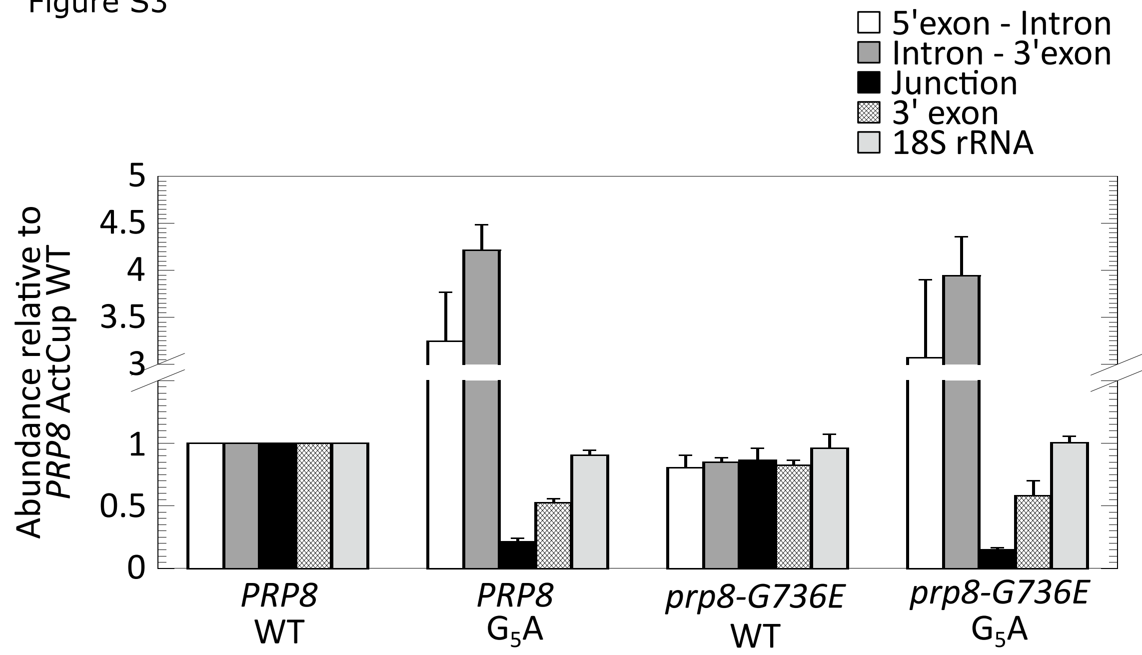


Figure S4

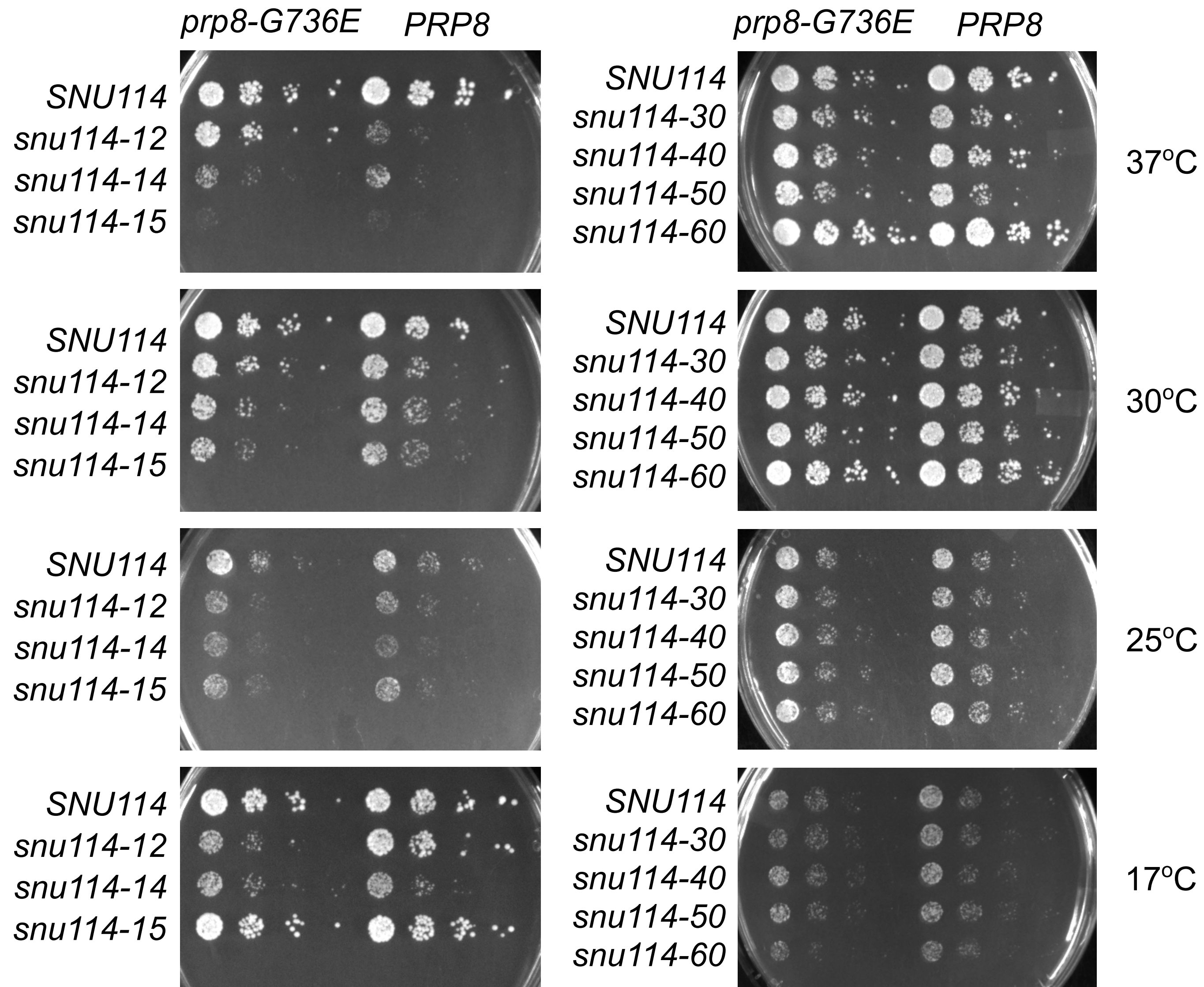


Figure S5

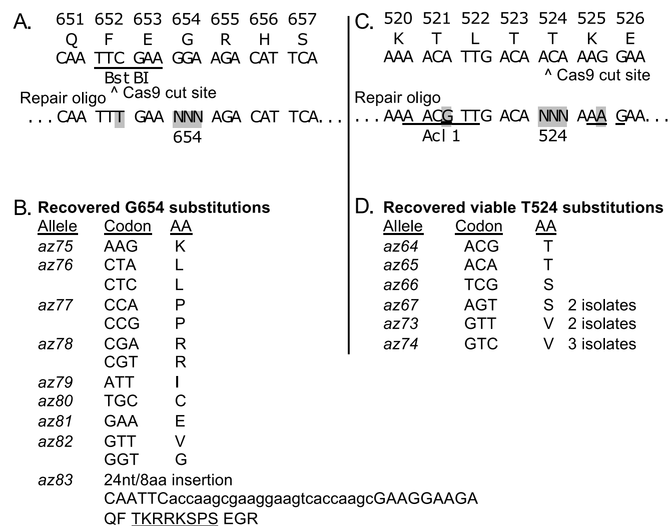
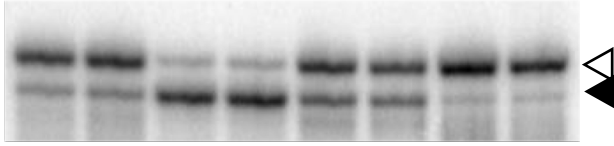


Figure S6

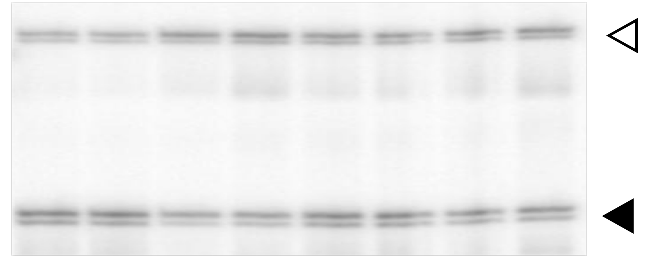
Alternative 5'SS

A. T12C9.7 – identified in G654E sequencing analysis – top scorer for snrp-27 M141T. TTTATTTCTG▲GTT△gtgagaatttgttttatagttt



prp-8 + + | + + | G654E | T524S
snrp-27 + + | M141T | + + | + +

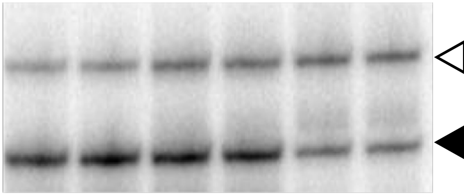
B. spat-2 identified in T524S sequencing analysis
GCGAACAGCGTT▲GTGAGGATGGCCTTGCAAGAAGGG△gtgcgggctta



prp-8 + + | + + | G654E | T524S
snrp-27 + + | M141T | + + | + +

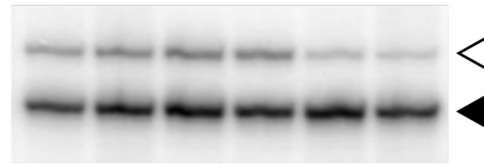
Alternative 3'SS

C. cdt-2 – alternative 3'ss identified in the T524S analysis
tcaaaaag△ATTTCAG▲TTTTTCC



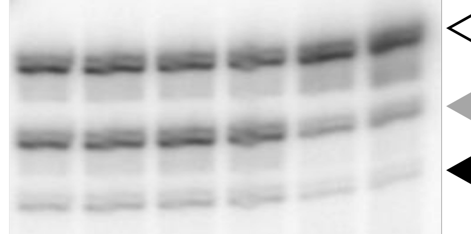
prp-8 + + | G654E | T524S

D. T10E9.14 – identified in T524S alternative splicing analysis
tattttgcag△TTTCAG▲GAAACTATC



prp-8 + + | G654E | T524S

E. F48D6.4 – alternative 3'ss from T524S analysis. Note 3 sites
tttatcaaaaatccctaggttattcag△TGTTCCAG▲AAACAG▲TGACGACTTCATTC

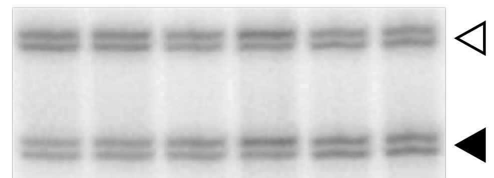


prp-8 + + | G654E | T524S

F. W09G3.7 – alternative 3'ss identified in T524S analysis
Atcacgaatttag△CTTCCAG▲ATCGGTGGTC

prp-8 + + | G654E | T524S

G. dct-1 – identified in T524S alternative splicing analysis
Gttcttgaacgcttcag△GTATGTCAG▲AATCGTGGGTGGAA



prp-8 + + | G654E | T524S