Online Supplementary Material:

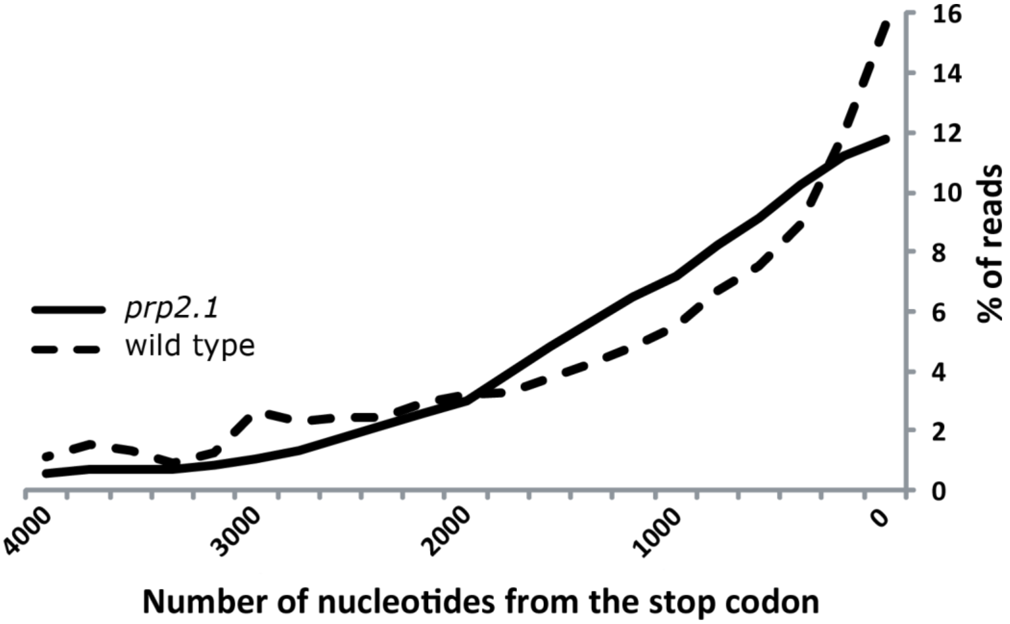

Figure S1. **A 3′ bias is observed in the Solexa sequence reads.** The percentage of sequence reads mapped to the coding region of all genes, with respect to the position from the stop codon, in *prp2.1* mutant and the published wild type (1).

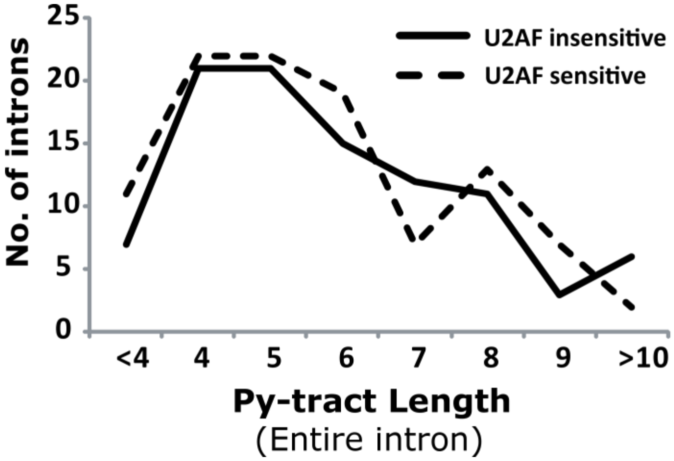

Figure S2. **Intron Py-tract strength did not correlate with U2AF insensitivity.** A histogram comparing the Py-tract length throughout the intron between U2AF-sensitive and -insensitive introns, (t-test *P*-value of 0.03).

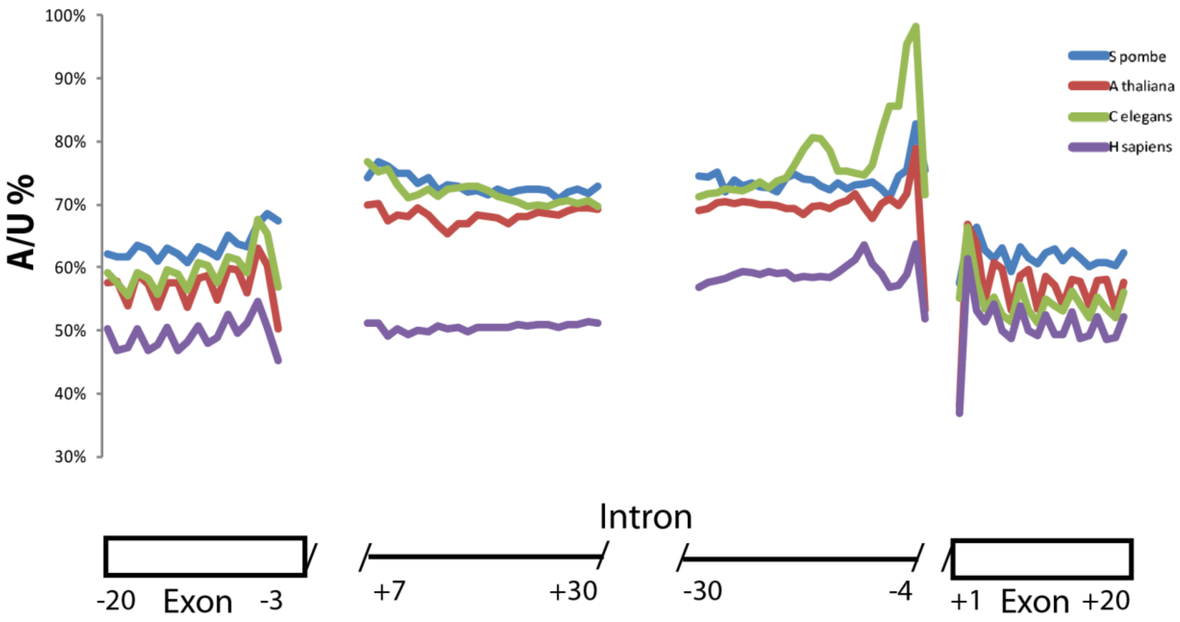

Figure S3. Single-nucleotide distribution of A/U % in different organisms. Single-nucleotide distribution of A/U% (Y-axis) in the intron and flanking exon sequences, indicated by the coordinates in relation to the splice sites. Rectangles represent exons and lines represent intron along the X-axis. Introns from four organisms (*S. pombe*, *A. thaliana*, *C. elegans*, and *H. sapiens*) were analyzed. All annotated introns from Sanger GeneDB at least 33 nts in length were used for *S. pombe*. NCBI annotated cis-spliced GT-AG introns were used for other organisms.

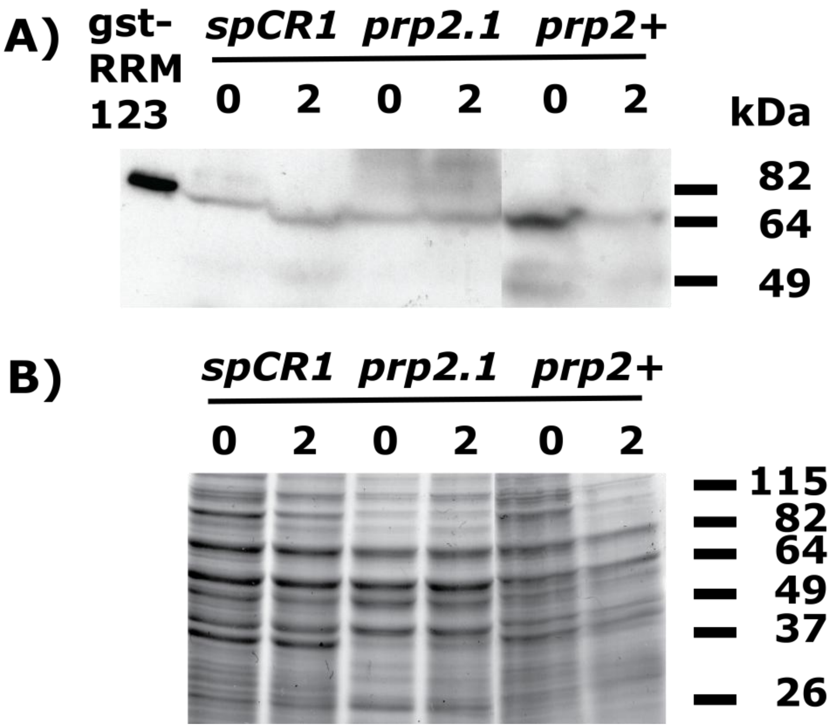

Figure S4. **The U2AF59 levels apparently remain stable upon heat inactivation in *prp2.1*.** **A)** Western Analysis. Comparison of U2AF59 protein levels between wild type and *prp2.1* mutants. U2AF levels from crude lysates of *S. pombe* strains *spCR1* (endogenous U2AF), *prp2+* (plasmid expressed U2AF) and *prp2.1* grown for 0 and 2 hrs non permissive (37^{o} C) temperatures were determined with anti-prp2 antibodies. The plasmid-expressed gst-RRM123 served as a control is shown. The anti-prp2 delta-RS polyclonal antibodies were used at a 1:500 dilution. **B)** Protein staining. A fraction (1/10th) of the lysates used for Western analysis was stained for comparison of the protein levels.

Supplementary Table 1. Oligonucleotides used for the study.

Primer	SEQUENCE
atp3_a F	AACGCTAACTCAGGCGTCTC
atp3_a R	TCTTTTAGCGTTGCCTCACA
Bp cn inv F	GCAAATACTAACAACAAATGTAGGAAAAGAGAG
Bp cn inv R	ACTAATAATCTCTTAAAACACTTACCTTCAGAAGC
Bp15cd5p3p invF	TTCTAACCCTTTTTTAAGACTTTTGG
Bp15cd5p3p invR	TTCCCATGCATCCGTTCTTAAAACACTTACCGAACACAATTTGATCGATTATTC
Bp30 Cn inv R	ACTAATAATCTCTTAAAACACTTACCTTCAGAAGCATTAGGTAACGACTCAAAAATC
Bpb1 F Nde	GCGCATATGGCTGAATTCAAGGTCAGTAGGG
Bpb1 R Bam	GCAGGATCCCTCATTCCATTTGCATCAGG
bpcd inv F	CAAATTCTAACCCTTTTTTAAGACTTTTGG
bpcd inv F cdc	CAAATTCTAACCCTTTTTTAAGACTTTTGG
bpcd inv R	CACTAATAATCTCTTAAAACACTTACCGAACACAATTTGATCGATTATTC
bpcd inv R cdc	CACTAATAATCTCTTAAAACACTTACCGAACACAATTTGATCGATTATTC
Bpcd invF	TTCTAACCCTTTTTTAAGGTTCGAATAATACTCCCTTAGG
Bpcd invF bpb	TTCTAACCCTTTTTTAAGGTTCGAATAATACTCCCTTAGG
Bpcd invR	TTTGCACTAATAATCTCTTAAAACACTTAC
Bpcd invR bpb	TTTGCACTAATAATCTCTTAAAACACTTAC
Cd15 Bp15 Cd inv R	CACTAATAATCCAAGTAAAAACTTACCGAACACAATTTGATCGATTATTC
Cd5p inv R	CCATGCATCCGTCAAGTAAAAACTTAC
Cd7 Bp23 Cd inv R	CACTAATAATCTCTTAAAAAACTTACCGAACACAATTTGATCGATTATTC
Cdbp inv F	GCAAATTATTAATATTTTTAGACTTTTGGATATTTTACATGCTGAATC
cdbp invR	CCCATGCATCCGTCAAGTAAAAACTTACCAGTAGACCCTACTGACCTTG
cdmidR	CAAAGTTTTATCAATGTAAACATTTCC
Cn7 Bp23 Cn inv R	ACTAATAATCTCTTAAAATCCCCACCTTCAGAAGCATTAGGTAAC
Cnbp clone F	CATTGCAAATTATTAATATTTTTAGGAAAAGAGAGAAAATTTATGATATCCTTATTGG
cnbp inv F	CATTGCAAATTATTAATATTTTTAGGAAAAGAGAG
cnbp inv R	TCGTTTCTTGTATTTAATCCCCACCTTC
Cox13_c F	GGTGGACCAGCATTGATATTG
Cox13_c R	CCAGGGTCCGTATCTTCAAC
Cwf25 F Nde	GCGCATATGTGGGTGGTGGAGATCTAAATATG
Cwf25 R Bam	GCAGGATCCGCTCGACTCGCTTCATCTCT
Cyp4 F Nde	GCGCATATGGGACACCATGTTGTTTTTGG
Cyp4 R Bam	GCAGGATCCTCACGGTTGTCAGTCTCAGC
Dim1_a F	TTTTTACCTCATTTACATTCTGGA
Dim1_a R	GATAAAATTGCTTGATCAACGTG
Dim1_b F	GCACGTTGATCAAGCAATTTT
Dim1_b R	CCAAATCGAATGACAACCAA

Dim1_c F	AAGAGCGCTTGGTTGTCATT
Dim1_c R	AGCCATGTTGACGACCTTTT
Dim1_d F	AGTGGACATTGACGAAGTTCC
Dim1_d R	TTGTAGTTCTGTCATAAAGCTCGT
Fim1_b F	TGATTACAATCTTGTTACCGATGG
Fim1_b R	GATTACGGCACCCAACTTTC
gar1_b	TGGTTCATAGGGCCAAAGAC
gar1_b F	CATGCACGATTGTGAAGGAG
grx1_c F	GAACAACGGTGATGAGATTCAA
grx1_c R	ACCAACATGCTTTTGGTGAA
mfm2_a F	TGGACTCCATTGCAACTAACA
mfm2_a R	CCTTGGCAAAAAGATTCCAG
nmt polyA R	AAACCCTAGCAGTACTGGCAAG
Paa1_f F	AAGCCATCCAAATTATTTATACCG
Paa1_f R	TCCAAAGTTGGAAGAATTTGTTT
Pgp2_a F	GGTTTGTCCAATGCACCTTC
Pgp2_a R	CGGAAAATGAACAATCCATACC
Png2 F Nde	GCGCATATGTTTGAAAGAGATCGATGCACA
Png2 R Bam	GCAGGATCCTCGCTCACATCTTTCTTCTCC
Prep1F	CACTTTCTGACTTATAGTCGCTTTGTTAAATCATATG
ptp4_a F	TGAAATTCCCCGTAAAGCAC
ptp4_a R	AAGCTGGCCAGTTAAAACGA
Rhb1_a F	GAATTGCTGTTCTTGGCAGTC
Rhb1_a R	GTTGGATAATACGATTCAACGAAA
Rhb1_b F	TTCGTGACAAGATATTGAATCACA
Rhb1_b R	CCAATGCTTTTCCCTCTTCA
rpl31_a F Nde	GCGCATATGGCCATCAAGGAAATTGTTGC
rpl31_a R Bam	GCAGGATCCTACCACGCTTCCAAACTTCC
rpl35a_a F	ATTTGGGTAAGCGTGTTTGC
rpl35a_a R	ACCGGAGTTTCCATGAGGAC
Sar1_a F	TAGATAACGCCGGAAAGACC
Sar1_a R	ACGTTGCCAATAGCCAGTTC
sla1_a	GGGTAAGTTGGTGTCCGAAA
sla1_a F	CCGAGGAAGCTGGAAAAGTA
Spac6f6.05_a F	GGCTTTAACTCAACAGGAATTACAA
Spac6f6.05_a R	TGGAGAAGCAAAACTCGAGAA
Spbc32H F Nde	GCGCATATGCAAAGAATGGAGAATTAGTTCGAGA
Spbc32H R Bam	GCAGGATCCCAATGATAACATGGTAGGGAGGT
spi1_b F	TGGCCTTCGTGATGGTTACT
spi1_b R	TCACAAACACGGACAAGATCA
srp2_9 F	TGACGAGCCTTAATGGTGAA
srp2_9 R	GTGGAGAACGTGAACGGAAG
syb1_a F Nde	GCGCATATGGTGGGGATTATGCGTGAAAA
syb1_a R Bam	GCAGGATCCTCTTCTTGCGAACACGATTG
vma3_c F	CCAAAGCTGGTGTCGGTATT
vma3_c R	CAGCCATAACAACAGGAATGG

Supplementary Table 2. 5′ splice site bias in U2AF-insensitive versus U2AF-sensitive introns. The two best 5′ splice site sequences in terms of complementarity to the U1 snRNA (2) and the consensus (3) is GUAAGU and GUAUGU. Also shown are the versions including the consensus guanosine from the upstream exon.

5′ splice site motif	U2AF-sensitive introns %	U2AF-insensitive introns %
GUAAGU	18.9	46.2
GUAUGU	9.4	22.6
G | GUAAGU	2.8	24.5
G | GUAUGU	3.7	20.8

1. **Wilhelm, B. T., S. Marguerat, S. Watt, F. Schubert, V. Wood, I. Goodhead, C. J. Penkett, J. Rogers, and J. Bahler.** 2008. Dynamic repertoire of a eukaryotic transcriptome surveyed at single-nucleotide resolution. Nature **453:**1239-43.
2. **Alvarez CJ, Romfo CM, Vanhoy RW, Porter GL, Wise JA.** 1996. Mutational analysis of U1 function in Schizosaccharomyces pombe: pre-mRNAs differ in the extent and nature of their requirements for this snRNA in vivo. RNA **2:**404-18.
3. **Kupfer DM, Drabenstot SD, Buchanan KL, Lai H, Zhu H, Dyer DW, Roe BA, Murphy JW.** 2004. Introns and splicing elements of five diverse fungi. Eukaryot Cell. **3:**1088-100.