**Supplementary Materials for Brow 2019**

**Figure S1**

Workflow for analysis of raw sequencing reads from the yeast spliceosome targeted sequencing panel.

**Figure S2**

Alignment of *Saccharomyces cerevisiae* and human Prp8 showing the identity and location of suppressor substitutions isolated in the Brow lab. (See also Table S6.)

**Table S1**

112 genes for known or possible splicing factors included in the yeast spliceosome custom targeted sequencing panel.

**Table S2**

PCR primer pairs present in each well of the yeast spliceosome custom targeted sequencing panel. Kindly provided by Illumina, Inc.

**Table S3**

Reference genome sequence for each amplicon generated by the yeast spliceosome targeted sequencing panel. Kindly provided by Illumina, Inc.

**Table S4**

Candidate U4-cs1-suppressor mutations identified by targeted sequencing of 62 cold-resistant yeast strains. The sequenced strain containing a suppressor mutation in the U4 gene (DAB122) was omitted from this table due to an absence of reads over *SNR14*. “Pick #” indicates the plate (B-T) from which a colony was picked and the order in which it was picked from that plate (1-6). Plates A, E, H, M, N, and S yielded no suppressors. “Days” indicates the number of days after the start of the 18° incubation that the colony grew to a diameter of ~2 mm and was picked. Both the nucleotide substitution (“HGVS\_C”, measured from the first nucleotide of the start codon in cDNA) and the amino acid substitution (“HGVS\_P”) are indicated. The highlighted substitutions in Prp8 display the temporal clustering that is likely indicative of suppressor strength. Note that strain DAB123 was identified to have two insertions, of 18 and three nucleotides. Direct inspection of the DNA sequence resulted in annotation of a single insertion/duplication of 21 nucleotides followed by a single nucleotide substitution (Table 1).

**Table S5**

Days from the start of the 18° incubation at which U4-cs1-suppressor colonies reached ~2 mm in diameter and were picked. Strains are grouped by the gene in which the suppressor mutation was identified; “U4” indicates the U4 snRNA gene *SNR14* and “none” indicates no mutation found. See Table S4 for time picked for individual strains.

**Table S6**

Suppressor mutations isolated in *PRP8* by the Brow lab in the indicated gene-targeted (*PRP8*) and whole-genome (genome) selections. The selections for *brr2-1-* and *prp28-1*-suppressors targeted only a portion of the *PRP8* gene.

**Table S7**

Sequence variants judged to be sequencing errors or polymorphisms. Listed by gene are variants that appeared in less than 80% of reads of the relevant base pair(s). The one exception is *SYF2* n.213T>A, which appeared in 56 of 63 strains (89%) and was just below the 90% cutoff used to screen out polymorphisms.

**File 1**

This document

**File 2**

PyMOL file for Figure 3.

**File 3**

PyMOL file for Figure 4.

**File 4**

PyMOL file for Figure 5.

**File 5**

PyMOL file for human tri-snRNP in Figure 6.

**File 6**

PyMOL file for yeast B complex in Figure 6.

**File 7**

PyMOL file for Figure 7.

**File 8**

PyMOL file for Figure 8.

**File 9**

PyMOL file for Figure 9.