**Supplemental Figure Legends**

**Figure S1. Generation of the *sno1-nonsense* point mutant using negative selection.**

1. Schematic representation of generating the *sno1-nonsense* point mutant. The *SNO1* ORF was replaced with a *URA3* selectable marker PCR amplified from the plasmid pRS316 containing 80 base pair homology up and downstream of the *SNO1* ORF (shown in red and blue, respectively). Following selection on Ura- dropout plates and subsequent PCR genotyping, we performed a transformation of the *sno1∆::URA3* mutant using a PCR product containing the *sno1-nonsense* ORF from plasmid MG010 using the same 80 base pair homology sequence. Schematic adapted from (Gray *et al.* 2005).
2. Sanger sequencing results from PCR products amplifying a portion of *SNO1* genomic DNA extracted from two independent isolates of the *sno1-nonsense* point mutation (Colony 1 and 3) aligned using Benchling software to the MG010 plasmid sequence. The DNA sequencing primer used was MRG420. The sequencing window was cropped to an area including the site of the nonsense mutation (Cys 84, shown as a yellow codon arrow marked with a “\*” and highlighted in the template sequence), as well as 15 baepairs of sequencing reads up and downstream of this TGA stop codon.
3. qCTF assay CIN rate measurements of the *sno1∆::Kan* and the *sno1∆::URA3*strain. P-values are generated from a Tukey’s post-hoc test.

**Figure S2. KanMX gene orientation affects CIN at the *SNO1* locus, but not at the *CSM3* locus.**

1. qCTF assay CIN rate measurements of the *sno1∆6* and two independent isolates of the *sno1∆6-Watson* strain (“*sno1∆6W*”). P-values are generated from a Tukey’s post-hoc test.
2. qCTF assay CIN rate measurements of the *csm3∆-Watson* (“*csm3∆W*”) and the *csm3∆-Crick* strain (“*csm3∆C*”). P-values are generated from a Tukey’s post-hoc test.

**Figure S3. Analysis of *CTF13* mRNA abundance and length in KanMX insertion mutants in the presence and absence of NMD.**

1. qPCR analysis of *CTF13* mRNA levels expressed as fold changes performed on controland *sno1∆* strains using a *CTF13* ORF primer set (set #1, see Supplemental Table S3). Six independent replicates were performed each including technical triplicates. Each values is normalized to the level of *ACT1* mRNA. Technical triplicates are averaged and pooled together and standard deviation is calculated for means of the independent strains. P-values are generated from a Tukey’s post-hoc test.
2. qPCR analysis (as described in section A) of *CTF13* mRNA levels expressed as fold changes performed on a wild-typestrain and a *sno1∆::URA3* strain using a *CTF13* ORF primer set (set #3).
3. qPCR analysis (as described in section A) of *CTF13* mRNA levels expressed as fold changes performed on wild-type*, sno1∆6*, and *sno1∆6/upf1∆* strains using a *CTF13* ORF primer set (set #3).
4. qPCR analysis of the relative abundance of *CTF13* mRNA in a *upf1∆* single mutant compared to a control strain. Only one independent isolate for each strain was used in this experiment and the standard deviation is measured from the technical triplicates. This result was validated using the same cDNA samples with two *CTF13* primer sets (#1 and #3).
5. qCTF assay CIN rate measurements of control, *sno1∆*, *sno1∆2,* *sno1∆3,* and *sno1∆6* strains in the presence and absence of functional NMD. P-values are generated from a Tukey’s post-hoc test.

**Figure S4. Poly-adenylated *CTF13* mRNA suppresses CIN in the *sno1∆6* mutant.**

1. Graphical representation of the genotype of the *CTF13-TADH1*strain generated using Benchling software. TADH1 (grey box) is inserted at genomic coodinate chrXIII:457284 within the intergenic region between the stop codons of *SNO1* ORF (Purple box shows last 5 codons) and *CTF13* ORF (Blue box shows last 6 codons). DNA sequence alignment is from a single colony generated via negative selection. The DNA sequencing primer used was MRG316.
2. Sanger sequencing of a 3’ RACE product for the *CTF13* mRNA in the *CTF13-TADH1* strain trimmed to a 103 base pair window to confirm the presence of a polyA tail. The putative polyA tail signal is shown underlined in black.
3. qPCR analysis of *CTF13* mRNA levels expressed as fold changes performed on wild-type (WT), *CTF13-TADH1*, and *CTF13-TADH1/sno1∆6* strains using a *CTF13* ORF primer set (set #3). Each values is normalized to the level of *ACT1* mRNA.
4. qCTF assay measuring CIN rates of the WT, *CTF13-TADH1*, and *CTF13-TADH1/sno1∆6* strains. P-values are generated from a Tukey’s post-hoc test.

**Figure S5. Sanger sequence read for the complete *CTF13* 3’ RACE PCR product in the *sno1∆*strain**

1. Full length *CTF13* mRNA 3’ RACE Sanger sequencing read from the *sno1∆* strain. Highlighted TAA denotes the *CTF13* ORF stop codon. Purple “N” denote poor quality base calls.

**Figure S6. Sanger sequence read for the complete *CTF13* 3’ RACE PCR product in the *sno1∆::Kan-TADH1*strain**

1. Full length *CTF13* mRNA 3’ RACE Sanger sequencing read from the *sno1∆::Kan-TADH1*strain. Underlined TAA denotes the *CTF13* ORF stop codon as well as the final A residues corresponding to a putative polyA signal.

**Figure S7. Determining the contribution of TTEF1 to increased CIN in *SNO1* null backgrounds.**

1. Graphical representation of cassettes used to delete *SNO1*, each cassette utilizing PTEF1 and TTEF1 from *Ashbya gossypii*. KanMX (KanR), NatMX (NsrR) and HphMX (HphR) confer resistance to the drugs geneticin, nourseothricin, and hygromycin, respectively.
2. Sequence alignment of 3’ RACE transcripts to the TTEF1 sequence to confirm each strain (*sno1∆::Kan*, *sno1∆::Nat*, and *sno1∆::Hph*) contain the same genetic terminator sequences.
3. Nested reverse transcriptase (RT)-PCR products from a 3’ RACE assay using a *CTF13* gene specific primer (MRG577) and QI primer (see Supplemental Table S2) run on a 1% agarose gel stained with ethidium bromide. Lane 1: 8 μL 1 kb ladder (NEB # N3232L). Lane 2: Control strain, wild-type SNO1-CTF13 locus. Lane 3: *sno1∆::Kan*, Lane 4: *sno1∆::URA3*, Lane 5: *sno1∆::Nat*, Lane 3: *sno1∆::Hph*. All strains were generated in the qCTF background. This image is a representative example of three biological replicates.
4. qCTF assay measuring CIN rates of the *sno1∆::Kan, sno1∆::Nat*, and *sno1∆::Hph* strains compared to a wild-type (WT) strain. P-values are generated from a Tukey’s post-hoc test.
5. qPCR analysis of *CTF13* mRNA levels expressed as fold changes performed on a wild-typestrain and a *sno1∆::Kan, sno1∆::Nat*, and *sno1∆::Hph* strains using a *CTF13* ORF primer set (set #3).
6. Sanger sequencing of the 3’ RACE product for the *CTF13* mRNA in the *sno1∆::Nat* strain trimmed to a 61 base pair window within the *CTF13* 3’ UTR containing the final sequencing read. 3/3 biological replicates contained the same 3’ UTR sequence.
7. Sanger sequencing of the 3’ RACE product for the *CTF13* mRNA in the *sno1∆::Hph* strain trimmed to a 54 base pair window within the *CTF13* 3’ UTR containing the final sequencing read. 2/3 biological replicates contained the same 3’ UTR sequence, which matched that of the *sno1∆::Nat* strain.
8. Sanger sequencing of the 3’ RACE product for the *CTF13* mRNA in the *sno1∆::Hph* strain trimmed to a 77 base pair window within the *CTF13* 3’ UTR containing the final sequencing read. 1/3 bioloical replicates contained the a more heterogenous 3’ UTR sequence.

**Figure S8. Genotype and growth characterization of the *3xHA-CTF13* strain.**

1. Schematic representation of the N-terminal *3xHA-CTF13* tag. HA moieties and colored in pink and the final linker sequence is denoted in blue. The following proline residue is the first proline in the *CTF13* amino acid sequence. Sequence alignment of genomic DNA from a *3xHA-CTF13* isolate (colony 15) to the MG014 plasmid template sequence to confirm the in-frame 3xHA tag. Sanger sequencing was performed with primer MRG551.
2. An example YPD + 200 mg/L G418 single colony isolation plate showing the failed colony growth of the *3xHA-CTF13/sno1∆* strain. These colonies were isolated from a transformation plate in which small single colonies formed after more than 72 hours.
3. Spot dilution assay on a YPD plate showing ten-fold dilution series of a *CTF13* wild-type background strain (BY4741) and three *3xHA-CTF13* mutants with the following *SNO1* alleles: *SNO1* (WT allele), *sno1∆*, and *sno1∆::Kan-TADH1*.
4. Spot dilution assay on a YPD + 200 mg/L G418 plate showing ten-fold dilution series of a the *sno1∆* and *sno1∆::Kan-TADH1* *3xHA-CTF13* background strains. A single spot of the BY4741 control strain was also added to confirm lack of growth for strains without the KanMX construct.
5. Full size images of western blot membranes corresponding to Figure 4H and 4I. Samples are the same as in Figure 4, except that the molecular weight ladder, Precision Plus Protein™ Dual Color Standards (Bio-Rad, Cat #: 1610374) and a control samples (“C”) are now visible. The control samples lacks any HA tag and confirms that the signal observed is specific to the 3xHA-Ctf13p.