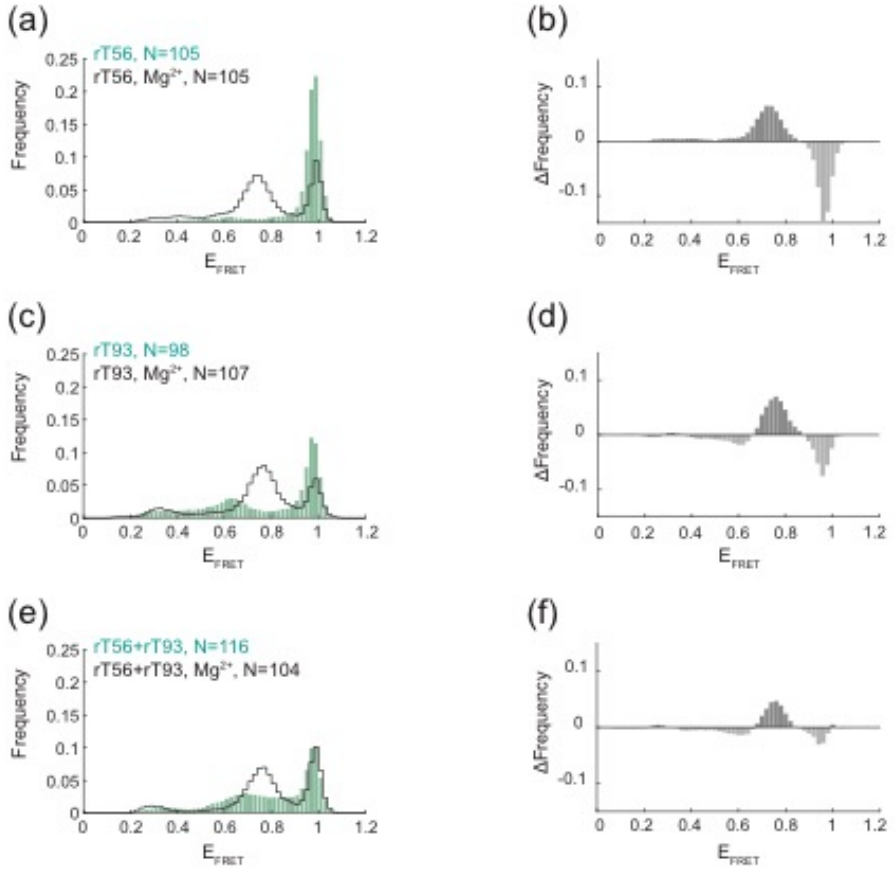
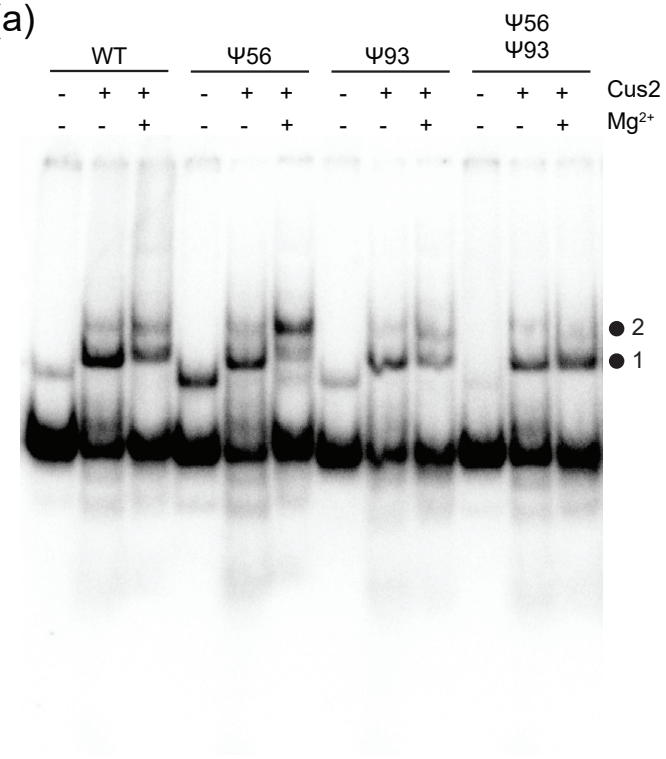
SUPPLEMENTAL INFORMATION

Supplemental Figures



Supplemental Figure 1. Influence of rT incorporation on stem II structure and Mg^{2+}-dependent conformational switching. (a, c, e) Histograms of E_{FRET} values obtained from the indicated number (*N*) of single molecules of rT-containing RNAs in the absence (filled, green bars) or presence of 10 mM Mg^{2+} (black lines). (b, d, f) Changes in E_{FRET} due to Mg^{2+} addition for each of the indicated RNAs. Each plot represents the result of subtraction of the histogram obtained in the absence of Mg^{2+} from the histogram obtained in the presence of Mg^{2+}.

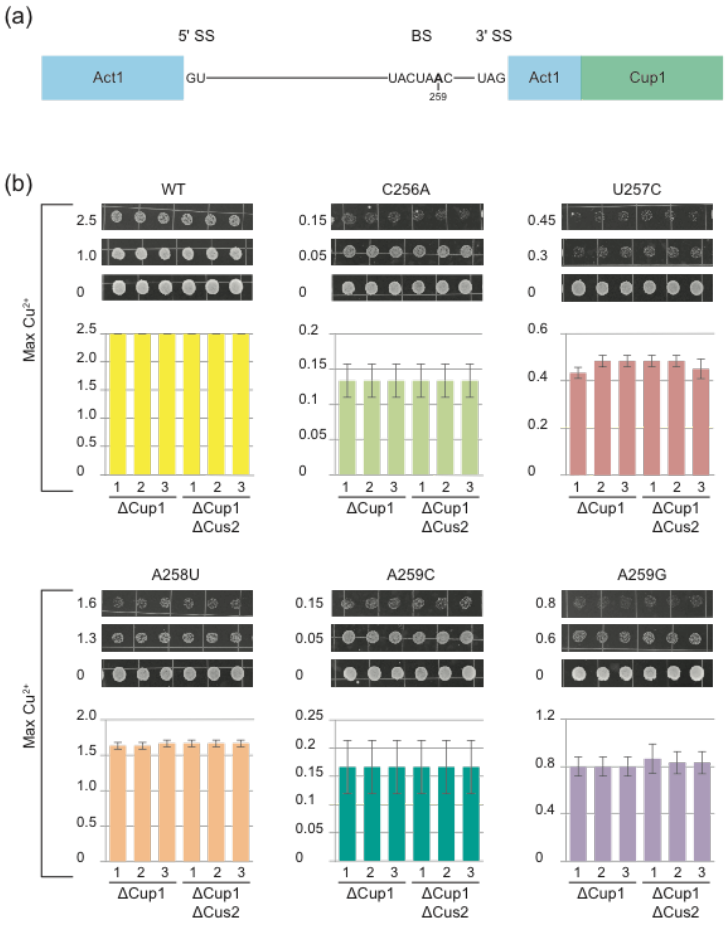
(a)



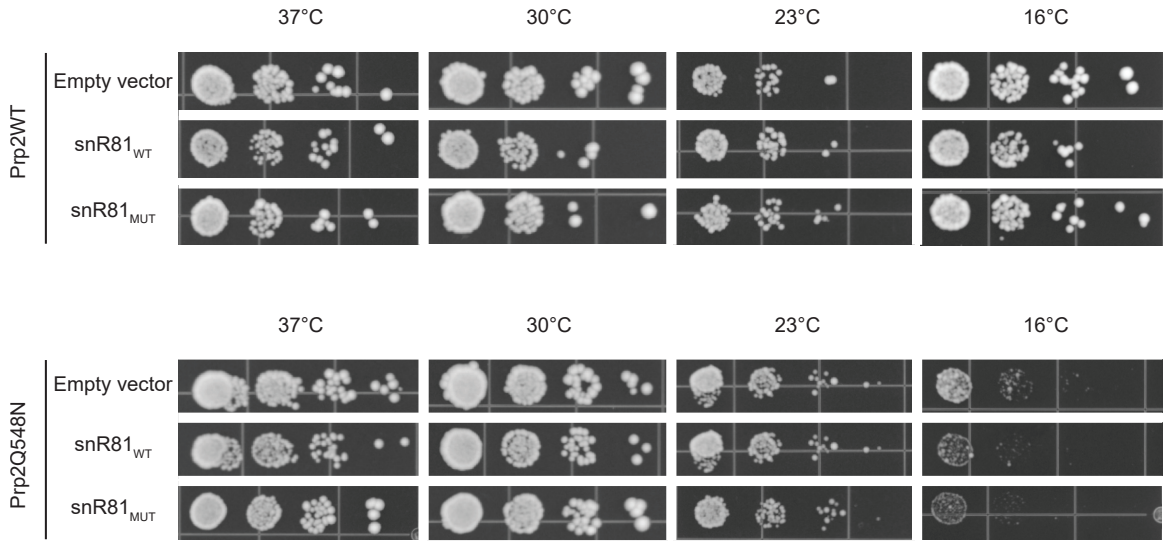
(b)

RNA with Cus2	Shift 1	Shift 2	Total
WT	34.8 ±4.0	7.5 ±2.5	42.3 ±3.8
WT-Mg^{2+}	29.6 ±8.1	12.8 ±1.6	42.4 ±6.7
Ψ56	28.0 ±5.1	10.8 ±3.8	38.8 ±4.5
Ψ56-Mg^{2+}	20.2 ±5.5	17.7 ±3.4	37.8 ±5.3
Ψ93	26.8 ±4.6	8.7 ±3.8	35.5 ±4.9
Ψ93-Mg^{2+}	16.4 ±2.4	12.3 ±7.6	28.7 ±6.5
Ψ56+Ψ93	24.4 ±1.7	6.5 ±1.8	30.7 ±1.7
Ψ56+Ψ93-Mg^{2+}	27.6 ±5.7	7.6 ±3.1	35.2 ±4.4

Supplemental Figure 2. EMSA analysis of Cus2 interaction with pseudouridine-containing stem II RNAs. (a) Representative EMSA results showing the migration of [^{32}P]-labeled stem II RNAs (WT or containing the indicated pseudouridines) in the presence or absence of Cus2 (2 μM) or Mg^{2+} (10 mM). The two Cus2-dependent band shifts are noted (shift 1 and 2). Note that each stem II RNA also migrates as a major and minor band in a non-denaturing gel, consistent with structural heterogeneity. (b) Quantification of EMSA results for each condition. Results reported are the average percent of shifted RNA from assays carried out in triplicate ±SD.



Supplemental Figure 3. ACT1-CUP1 assay for detecting changes in splicing of introns containing nonconsensus branchsites in the presence or absence of Cus2 and the $snR81_{MUT}$ snoRNA, which induces constitutive pseudouridylation at Ψ93. (a) Schematic of the ACT1-CUP1 reporter RNA. The branchpoint adenosine is noted (A259). (b) Results from ACT1-CUP1 assays using the noted WT or branchsite substitution reporters. All Cu^{2+} concentrations are in mM. Representative images of yeast are shown at the indicated [Cu^{2+}] and bar graphs represent the maximum [Cu^{2+}] at which growth was observed. Bar height represents the average from three replicates, while error bars represent SD. In all cases "1" denotes a strain transformed with an empty vector control, "2" denotes a strain transformed with a plasmid encoding the expression of the WT snR81 snoRNA ($snR81_{WT}$), and "3" denotes a strain transformed with a plasmid encoding expression of $snR81_{MUT}$. No significant changes in Cu^{2+} tolerance were observed due to the combination of the $snR81_{MUT}$ plasmid and Cus2Δ.



Supplemental Figure 4. Yeast growth assay for genetic interactions between a cold-sensitive allele of the Prp2 ATPase and the $snR81_{MUT}$ snoRNA, which induces constitutive pseudouridylation at Ψ93. Yeast containing combinations of Prp2 alleles and snR81 plasmids were spotted onto dropout (DO) plates and grown at the indicated temperatures. $snR81_{MUT}$ did not enhance or suppress the *cs* phenotype of the Prp2-Q548N allele or induce proliferative defects at other temperatures.

Supplemental Table 1. RNA Oligonucleotides

Oligo name	Oligo Sequence (5' to 3')	Notes
WT1-aa54	AGUGUAGUAUCUGUUCUUUUCAG[aaU]GUAAC AACUGAAAUGACCUCAAUG	5' portion of stem II, used for preparing WT or Ψ93 RNAs for smFRET
WT2-aa101	AGGCUCAUUACCUUUUAAUUUG[aaU]UACAAU ACACAUUUUUUGGCACCCA-Bio	3' portion of stem II, used for preparing WT or Ψ56 RNAs for smFRET
Ψ56-U54	AGUGUAGUAUCUGUUCUUUUCAG[aaU]G[Ψ]AA CAACUGAAAUGACCUCAAUG	5' portion of stem II, used for preparing Ψ56 or Ψ56+Ψ93-containing RNAs for smFRET
Ψ93-U101	AGGCUCAUUACCUU[Ψ]UAAUUUG[aaU]UACAA UACACAUUUUUUGGCACCCA-Bio	3' portion of stem II, used for preparing Ψ93 or Ψ56+Ψ93-containing RNAs for smFRET
rT56-U54	AGUGUAGUAUCUGUUCUUUUCAG[aaU]G[rT]AA CAACUGAAAUGACCUCAAUG	5' portion of stem II, used for preparing rT56 or rT56+rT93-containing RNAs for smFRET
rT93-U101	AGGCUCAUUACCUU[rT]UAAUUUG[aaU]UACAA UACACAUUUUUUGGCACCCA-Bio	3' portion of stem II, used for preparing rT93 or rT56+rT93-containing RNAs for smFRET
WT1	AGUGUAGUAUCUGUUCUUUUCAGUGUAACAA CUGAAAUGACCUCAAUG	5' portion of stem II, used for preparing WT or Ψ93 RNAs for RNase T1 probing
WT2	AGGCUCAUUACCUUUUAAUUUGUUACAAUACA CAUUUUUUGGCACCCA	3' portion of stem II, used for preparing WT or Ψ56 RNAs for RNase T1 probing
Ψ56	AGUGUAGUAUCUGUUCUUUUCAGUG [Ψ]AACAACUGAAAUGACCUCAAUG	5' portion of stem II, used for preparing Ψ56 or Ψ56+Ψ93 RNAs for RNase T1 probing
Ψ93	AGGCUCAUUACCUU[Ψ]UAAUUUGUUACAAUAC ACAUUUUUUGGCACCCA	3' portion of stem II, used for preparing Ψ93 or Ψ56+Ψ93 RNAs for RNase T1 probing

Abbreviations: [aaU], aminoallyluridine; Bio, 3' biotin; Ψ, pseudouridine; rT, ribothymidine