SUPPLEMENTAL INFORMATION FOR

Yeast U6 snRNA made by RNA polymerase II is less stable but functional

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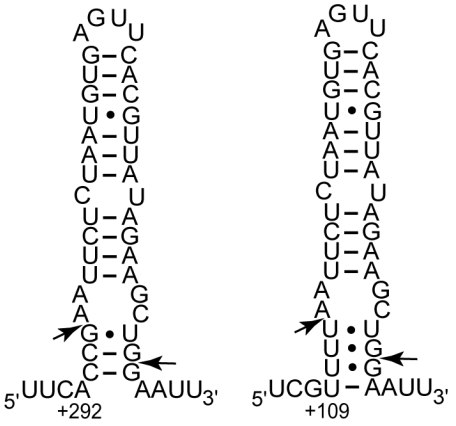

Figure S1. Predicted positions of Rnt1 cleavage sites (arrows) in the primary transcript of *SNR6-II* (right) compared to the previously mapped cleavage sites in pre-U4 snRNA (left; Allmang et al. 1999). The first 135 base pairs downstream of U6 position +112 were deleted from the SNR14-6-14 allele to align the mature 3' end of the shortest version of yeast U6 snRNA with the upstream Rnt1 cleavage site (see Fig. 1A).

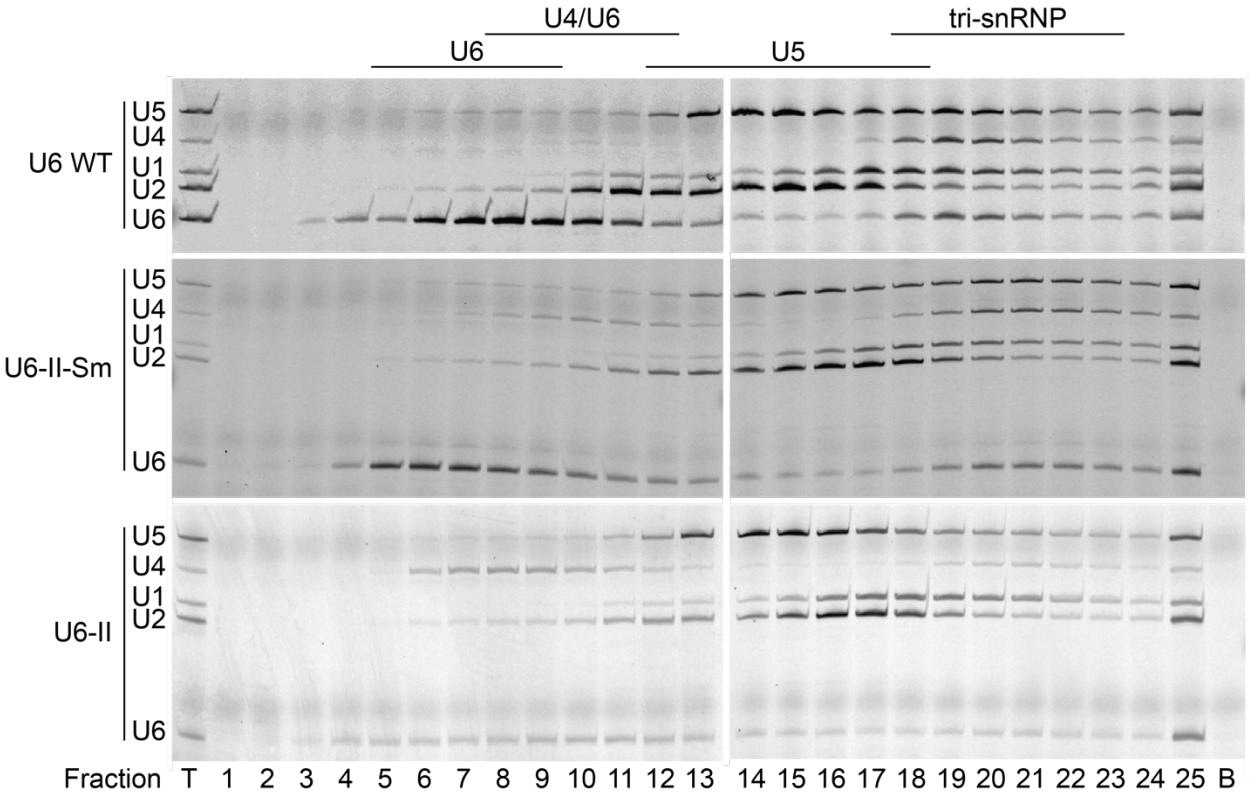

Figure S2. Primer extension of snRNAs from glycerol gradient fractions. RNAs were isolated from fractions taken from glycerol gradient sedimentation. Levels of primer extension products were quantitated as seen in Figure 5.

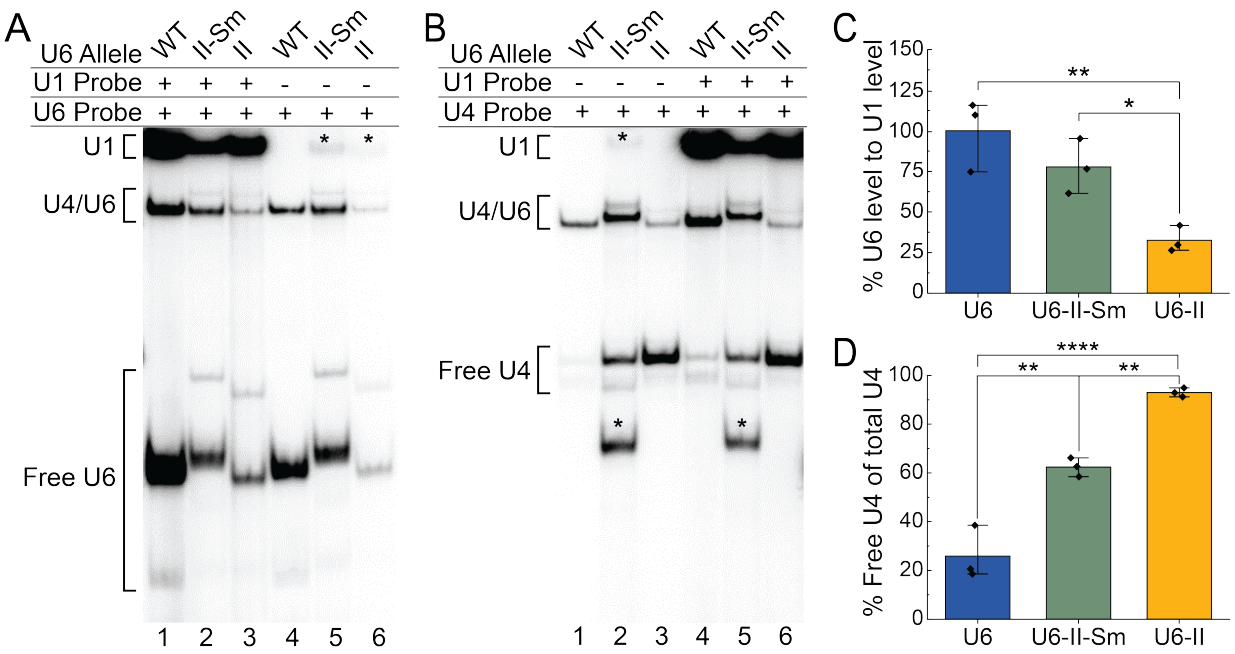

Figure S3. Free U4 snRNA accumulates in yeast cells producing U6-II. A, B) Non-denaturing gel electrophoresis of cold-extracted whole-cell RNA from strains bearing the indicated U6 allele hybridized to probes against the indicated snRNAs. Positions of U4/U6 di-snRNA and free U1, U4, and U6 snRNAs are indicated. The fast-migrating bands in lanes 2 and 5 indicated with asterisks are believed to be U6-II-Sm snRNA hybridized with the U4-14B probe due to inclusion of the U4 Sm binding site. **C, D)** Quantitation of U6 snRNA abundance and fraction of U4 snRNA not paired with U6, respectively, from bands present in solution hybridization assays. Values were calculated from three biological replicates. Signal for U1 was adjusted to exclude contributions from a faint band in U6-II-Sm lanes running at the same position, also indicated with asterisks. Sample means were compared with one-way ANOVA followed by a post hoc Tukey multi-pairwise analysis. (p = 0.05*, 0.01**, 0.001***, 0.0001****)

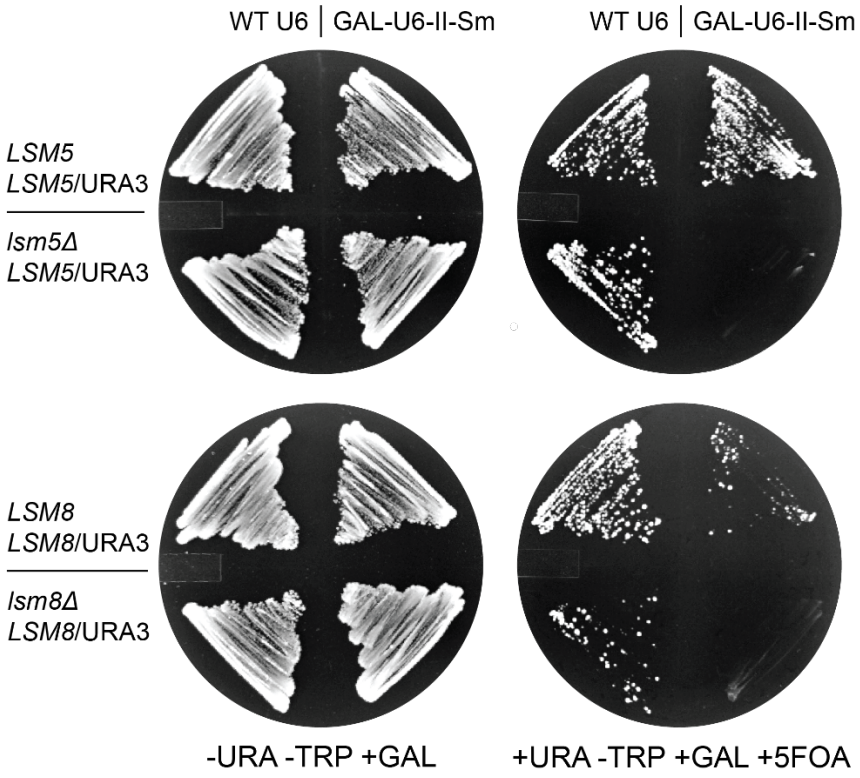

Figure S4. Genetic interaction between U6-II-Sm and Lsm Proteins. *lsm5* or *lsm8* genes were deleted from strains expressing either WT U6 or U6-II-Sm RNAs and complemented with Lsm5 or Lsm8 expression from a URA3/CEN-marked plasmid. These deletion strains or their corresponding controls were then grown on medium selecting for (-URA -TRP +GAL) or against (+URA -TRP +GAL +5FOA) the *URA3*-marked plasmid bearing the WT *LSM* gene. Deletion of the *lsm5* or *lsm8* genes and loss of the corresponding URA3-marked plasmid is lethal in the presence of U6-II-Sm but not WT U6.

51 **Supplementary Table 1: Yeast strains**

Strain	Parent	Genotype	Reference
MWK027	PJ43-2b	MATα ura3-52 trp1-1 his3-11,15 leu2-3,112 ade2-1 lys2Δ2 met2Δ1 can1-101 snr6::LEU2 [YCp50-ΨWT]	Kaiser & Brow 1995
CJM000	ANK640	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, [pRS316-U4wt-U6mini]	McManus et al. 2007
DAB101	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, [pRS313-U4wt + pRS314-U6wt]	This work
yAAH1361	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, [pRS313-U4wt + pRS314-U6-II-Sm]	This work
yAAH1442	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, [pRS313-U4wt + pRS314-U6-II]	This work
yAAH2687	BY4743	MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0 LSM5::kanMX [pRS316-LSM5]	Roth et al. 2018
yAAH2690	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, SMD1::3xFLAG-HygR [pRS316-U4wt-U6mini]	This work
yAAH2719	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, LSM8::2xV5-HygR, [pRS316-U4wt-U6mini]	This work
yAAH2720	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, [pRS313-U4wt + pRS314-U6wt + pRS416-LSM5]	This work
yAAH2721	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, [pRS313-U4wt + pRS314-U6wt + pRS416-LSM8]	This work

yAAH2722	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, [pRS313-U4wt + pRS314-U6-II-Sm + pRS416-LSM5]	This work
yAAH2723	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, [pRS313-U4wt + pRS314-U6-II-Sm + pRS416-LSM8]	This work
yAAH2736	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, LSM5Δ::KanMX [pRS313-U4wt + pRS314-U6wt + pRS416-LSM5]	This work
yAAH2737	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, LSM8Δ:: HygR [pRS313-U4wt + pRS314-U6wt + pRS416-LSM8]	This work
yAAH2750	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, LSM5Δ::KanMX [pRS313-U4wt + pRS314-U6-II-Sm + pRS416-LSM5]	This work
yAAH2751	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, LSM5Δ:: HygR [pRS313-U4wt + pRS314-U6-II-Sm + pRS416-LSM5]	This work
yAAH2773	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, LSM8::2xV5 HygR, [pRS313-U4wt + pRS314-U6WT]	This work
yAAH2774	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, LSM8::2xV5 HygR, [pRS313-U4wt + pRS314-U6-II-Sm]	This work
yAAH2775	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, LSM8::2xV5 HygR, [pRS313-U4wt + pRS314-U6-II]	This work
yAAH2853	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, SMD1::3xFLAG HygR, [pRS313-U4wt + pRS314-U6wt]	This work

yAAH2854	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, SMD1::3xFLAG HygR, [pRS313-U4wt + pRS314-U6-II-Sm]	This work
yAAH2855	CJM000	MATa, snr6::LEU2, snr14::trp1::ADE2, trp1, ura3, lys2, his3, ade2, SMD1::3xFLAG HygR, [pRS313-U4wt + pRS314-U6-II]	This work

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Supplementary Table 2: Oligonucleotides

Oligo	Sequence (5' to 3')
PsnR14(223)-Xho-F	CCGCTCGAGTAAGTAACCTCTGCATTGTC
U4-U6-R	CCCTCGCGAACGGAGTATTAAGGAAGGAAGTG
U4(12)-U6(24)-R	TCGGTAATGAAAAAAACGAAATAAATCTCTTTGTAA
U6(12)-U4(23)F	TTATTTCGTTTTTTTCATTACCGATATTCATTCTT
snR14-BamHI-701R	CGGGATCCTTCCTCTCTGCTGTTTTAG
Rnt1(12)-U6(24)R	CATTAGAGAATTAAAACGAAATAAATCTCTTTGTAA
U6(12)-Rnt(23)F	TTATTTCGTTTTAATTCTCTAATGTGAGTTCACGT
GAL1-XhoI-608F	CCGCTCGAGATCATATTACATGGCATTACCA
U4(14)-GAL1(24)R	CTGTTCCTTTTATATCTGTTAATAGATCAAAAATCATC
GAL1(11)-U4(25)F	CTATTAACAGATATAAAAGGAACAGAATATTAGTTA
snR14-Kpn1-701R	CGGGGTACCTTCCTCTCTGCTGTTTTAG
Sew-U6::U4-R	AAAAGGTATTCCAAAAATTCTTTGTAAAACGGTTCATCCTT
Sew-U6::U4-F	CAAAGAATTTTTGGAATACCTTTTAATTCTCTAATGTGAGTTCA
U1-SH	CCGTATGTGTGTGTGACC
U4-14B	AGGTATTCCAAAAATTCCC
U6-SH	ATTGTTTCAAATTGACCAAAT
U1RT136	GAC CAA GGA GTT TGC ATC AAT GAC
U2RTALL124	TTT GGG TGC CAA AAA ATG TGT ATT GTA
U4RTALL	GGT ATT CCA AAA ATT CCC TAC ATA GTC
U5	AAG TTC CAA AAA ATA TGG CAA GC
U6B	TCATCCTTATGCAGGG
U6D	AAA ACG AAA TAA ATC TCT TTG
U3 21-mer	CCAAGTTGGATTCAGTGGCTC

57 **Supplementary Table 3: Plasmids**

Plasmid	Description	Reference
pRS314	*TRP1*-marked, low-copy yeast shuttle plasmid	Sikorski and Hieter 1989
pRS424	*TRP1*-marked, high-copy yeast shuttle plasmid	Christianson et al. 1992
pRS313-SNR14	*SNR14* -224 to +701 cloned into the BamHI site	Kuehner and Brow 2006
pRS314-14-6-14	See Materials and Methods	This work
pRS424-14-6-14	See Materials and Methods	This work
pRS314-SNR6-II	See Materials and Methods	This work
pRS424-SNR6-II	See Materials and Methods	This work
pRS314-GAL-SNR6-II	See Materials and Methods	This work
pRS314-GAL-SNR6-II-Sm	See Materials and Methods	This work
pAAH0229	pRS314-GAL-SNR6-SM (U6-II-Sm)/TRP/AMP	This work
pAAH0412	pRS314 WT U6/TRP/AMP	McManus et al. 2007
pAAH0413	pRS313 WT U4/HIS/AMP	McManus et al. 2007
pAAH0667	pRS314-GAL-SNR6-II (U6-II)/TRP/AMP	This work
pAAH1285	pRS416 LSM5/URA/CEN	Roth et al. 2018
pAAH1286	pRS416 LSM8/URA/CEN	Roth et al. 2018

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SUPPLEMENTAL REFERENCES

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