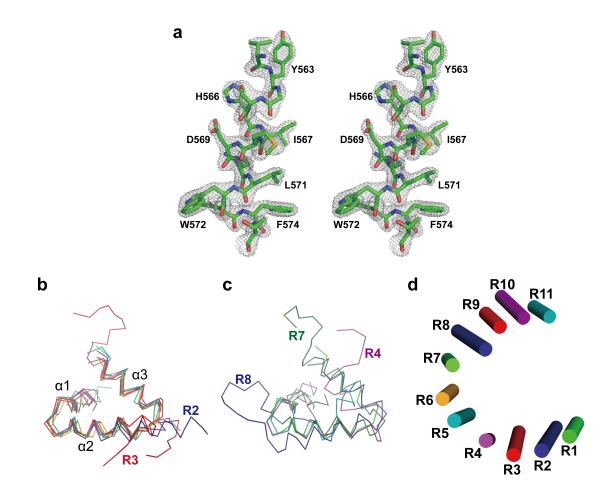
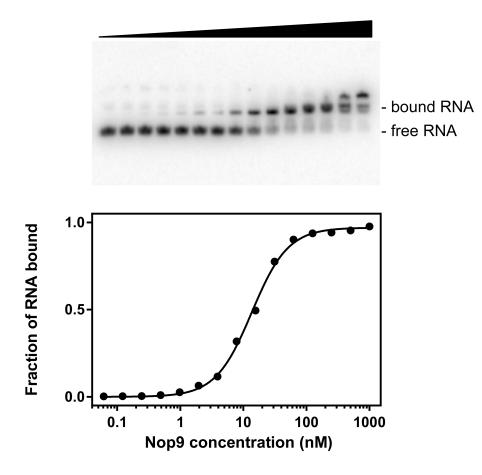
Supplementary Information



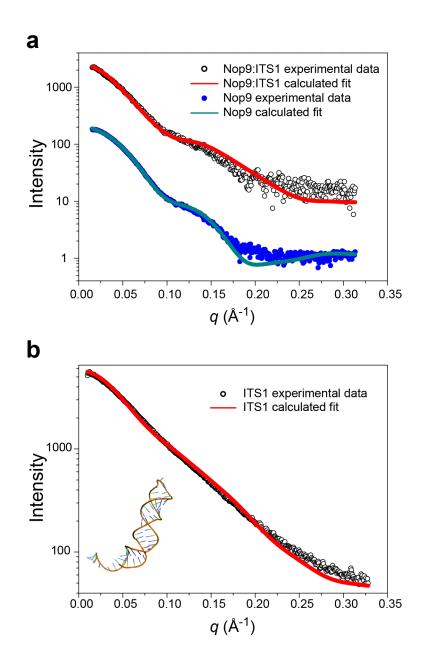
Supplementary Figure 1. Structure of the Nop9 PUM repeat protein family. (a) Stereo image of a representative portion of the Nop9 electron density map. Residues V562-T575 are shown superimposed with a $2F_0$ - F_c composite omit map contoured at 2.0 σ . (b) Superposition of Nop9 PUM repeats R1-R3, R5-R6, and R9-R10, which are structurally similar. (c) Superposition of Nop9 PUM repeats R4, R7, R8, and R11, which bear divergent loops that correspond to perturbations in repeat-to-repeat angles. (d) Nop9 PUM repeats form a twisted C shape. The α 2 helices lining the concave surface of Nop9 are shown as cylinders to allow visualization of the major repeat-to-repeat twists focused around repeats R4 and R7. Repeats are colored as in Fig. 1b. In the absence of twisting, the curved inner surface of Nop9 could be rendered with the axes of all α 2 helices orthogonal to the page.

		60 80 R1 ¹⁰⁰	
ScNop9	50	QMFFGVLDREELEYFKQAESTLQLDAFEAPEEKFQFVTSIIEEAKGKELKLVTSQI	105
HsNop9	50	APDSHPHLSPEALGYFRRALSALK-EAPETGEERDLMVHNIMKEVETQALALSTNRT	105
AtPUM23	78	EHQNQFVRKEIDPETSKYFSEIANLFD-SNEVELEERSVICGNALEETRGREYEIA T DYI	136
		$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
ScNop9	106	TSKLMERVILECDETQLKDIFQSFNGVFFGLSCHKYASHVLETLFVRSAALVERELLT	163
HsNop9	106	GSEMLQELLGFSPLKPLCRVWAALRSNLRTVACHRCGVHVLQSALLQLPRLLGSAAEE	163
AtPUM23	137	ISHVLQTLLEGCELDQLCSFIRNSASVFPAIAMDRSGSHVAESALKSLATHLEN	190
		$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
ScNop9	164	PSFDN-NEKEGPYVTMENMFLFMLNELKPHLKTMMNHQYASHVLRLLILISSKTLP	219
HsNop9	164	EEEEEEDGKDGPTETLEELVLGLAAEVCDDFLVYCGDTHGSFVVRTLLQVLGGTILE	220
AtPUM23	191	PDAYSVIEEALHSICKVIVDNPLDMMCNCYGSHVLRRLLCLCKGVSLD	238
		220 240 260	
ScNop9	220	+a.3 N-STKANSTLRSKKSKIARKMIDIKDNDDFNKVYQT P ESFKSELRDIITTLYKGFTNGAE	278
HsNop9	221	SERARPRGSQSSEAQKTPAQECKPADFEV P ETFLNRLQD	259
AtPUM23	239	SPELYGAKSSKALAKRLNLKMSQLDDNNLEIPHQGF P GMLTYLLSG	284
		$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
ScNop9	279	SRSDISQSTITKFREYSVDKVASPVIQLIIQVEGIFDRDRSFWRLVFNTA	328
HsNop9	260	LSSSFLKDIAVFITDKISSFCLQVALQVLHRKLPQFCAHLCNAVIGYLST	309
AtPUM23	285	LLSCSREDMKYLQVDQYSSLVLQTALRLMLKQDEQL-LEIIPLILRCNSTNK	335
		R5 $\frac{340}{a1}$ $\frac{360}{a2}$ R6 $\frac{380}{a1}$	
ScNop9	329	DEKDPKEESFLEYLLSDPVGSHFLENVIGSARLKYVERLYRLYMKDRIVKLAKRDTT	385
HsNop9	310	RGSSVDGSPLLLFLRDQTSSRLLEQVLLVLEPPRLQSLFEEHLQGQLQTLAAH-PI	364
AtPUM23	336	KVEGFHIETNVAKEILESMK <mark>D</mark> NSFSHLVEVILEVAPESLYNEMFNKVFKNSLFELSVD-RC	395
		400 R7 420 440	
		$\alpha 2$ $\alpha 3$ $\alpha 1$ $\alpha 2$ $\alpha 3$	
ScNop9	386	GAFVVRALLEHL-KEKDVKQILDAVVPEL-SMLLNSNMDFGTAIINASNKQGGYLRDDVI	443
HsNop9 AtPUM23	365 396	ANFPLORLLDAVTTPELLSPVF <mark>E</mark> ELS P VLEAVLAQGHPGVVIALVGA C RRVGA Y QAK-VL ANFVIQALISHARDQEOMGIMWEELAPRFKDLLEQGKSGVVASLIAV S ORLOS H ENK-CC	424 454
ATPUM23	390		454
-			
ScNop9	444	AQLIQKYYPEKSDAKNILESCLLLSASTLGNTRDDWPTAEERR	486
HsNop9 AtPUM23	425 455	QLLLEAFHCAEPSSRQVACVPLFATLMAYEVYYGLTEEEGAVPAEHQVAMAAARALGDVT EALVGAVCSTNESRISILPRLLFLDYYFGCRDKSTWEWAPGAKMH	483 499
ALPOM25	455		499
		$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
ScNop9	487	RSVFLEQLIDYDDKFLNITIDSMLALPEERLIQMCYHGVFSHVVEHVLQTTRVDIIKR	544
HsNop9	484	VLGSLLLQHLLH F STPGLVLR S LGALTGPQLLSLAQSPAGSHVLDAILTSPSVTRKLR	541
AtPUM23	500	VMG <mark>CLILQ</mark> GIFK F SSDHIQPYIT S LTSMKAEYITETAKDSSG <mark>ARVIE</mark> AFLASDA-ATKQ K	558
		R10 560 580 R11 600 g1 g2 g3 g1 g1 g2 g3 g1 g1 g2 g3 g1 g3 g3 g1 g3 g3 g3 g3 g3 g3 g3 g3	
ScNop9	545	KMLLNILSKESVNLACNVYGSHIMDKLWEFTAKLTLYKERIARALVLETEKVKNSIYG	602
HsNop9	542	RRVLQNLKGQYVALACSRHGSRVLDAIWSGAALRARKEIAAELGEQNQELIRDPFG	597
AtPUM23	559	RRLIIKLRGHFGELSLHTSG <mark>SFTVE</mark> KCFDACNLTLREAIASELLDVKVDLSKTKQG	614
		620 620	
ScNop9	603	RQVWKNWKLELYVRKMWDWKKLIKEQEFEIFPNSKPLQ	640
HsNop9	598	HHVARNVALTTFLKRREAWEQQQGAVAKRRRALNSILE	635
AtPUM23	615	PYLLRKLDIDGY ASRPDQW KSRQEAKQSTYNEFCSAFGSNKSNFPKNTFVSDASEDAA	672

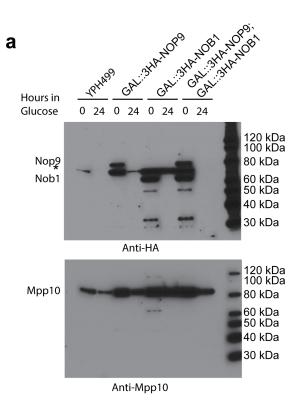
Supplementary Figure 2. Protein sequence alignment of Nop9 family members. Amino acid sequences are shown for *S. cerevisiae* Nop9, human NOP9 and *Arabidopsis thaliana* APUM23. α helices are indicated by rectangles and colored by repeat as in Fig. 1b. Classical PUF protein RNA recognition motif sequences are boxed. Conserved hydrophobic residues are indicated with gray boxes, and conserved acidic and basic residues are colored red and blue, respectively. Residues disordered in the Nop9 crystal structure are indicated by dashed lines above the sequences. The sequences were aligned using ClustalX 2.1¹. APUM23 is involved in 18S rRNA processing^{2,3}, but it is not an essential gene in *A. thaliana*. SELEX experiments identified a 10-nt RNA recognition sequence for APUM23, however, selection with a 20-nt randomized region did not identify a specific recognition sequence for yeast Nop9⁴.

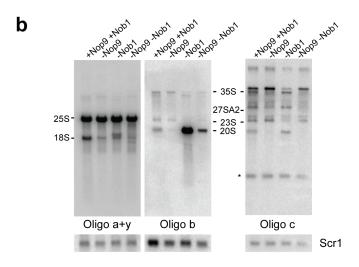


Supplementary Figure 3. Representative EMSA binding data for Nop9 binding to ITS1 Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$. A representative gel (top) with corresponding binding curve (bottom) is shown. Protein concentrations are serially diluted 2 fold from 1000 nM.

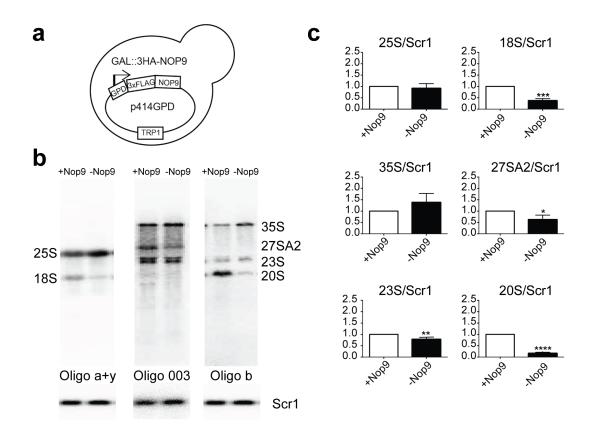


Supplementary Figure 4. SAXS experimental and calculated scattering curves. (a) Superimposed experimental and calculated scattering curves for Nop9 alone and a Nop9:ITS1 RNA complex calculated by rigid-body docking. The χ^2 for the calculated fit of Nop9 alone and its corresponding data is 2.9, and the χ^2 for the calculated fit of Nop9:ITS1 and its corresponding data is 2.0. (b) Superimposed experimental and calculated scattering curves for ITS1 rRNA (7-38_184-206). The χ^2 for the calculated fit of ITS1 rRNA alone and its corresponding data is 3.8. The predicted RNA model is shown in the inset. This figure was generated using the FoXS web server^{5,6}.

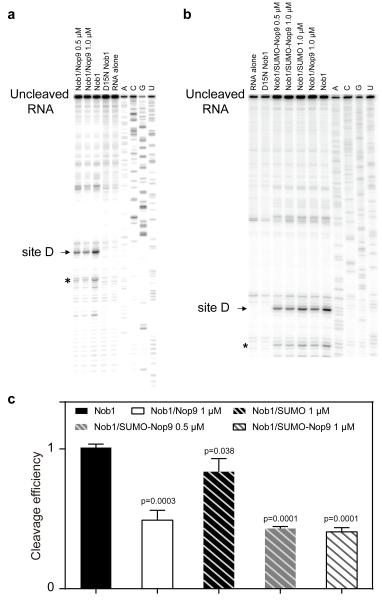




Supplementary Figure 5. *In vivo* effects of depletion of Nop9 and/or Nob1. (a) Western blot confirming depletion of HA-tagged Nop9 and/or Nob1 protein expression. The positions of HA-tagged Nop9 and Nob1 proteins are indicated in the top blot. * denotes a 50 kDa anti-HA tag cross-reactive band observed in all strains. The blot was stripped and probed with an anti-Mpp10 antibody as a loading control. Molecular weight markers are shown at the right of each blot. (b) Northern blot analysis of Nop9/Nob1 double depletion. Full representative northern blots detecting precursor and mature rRNA in total RNA as presented in Fig. 5c. An oligo complementary to Scr1 was used as a loading control (indicated with * in the full northern blot probed with oligo c).

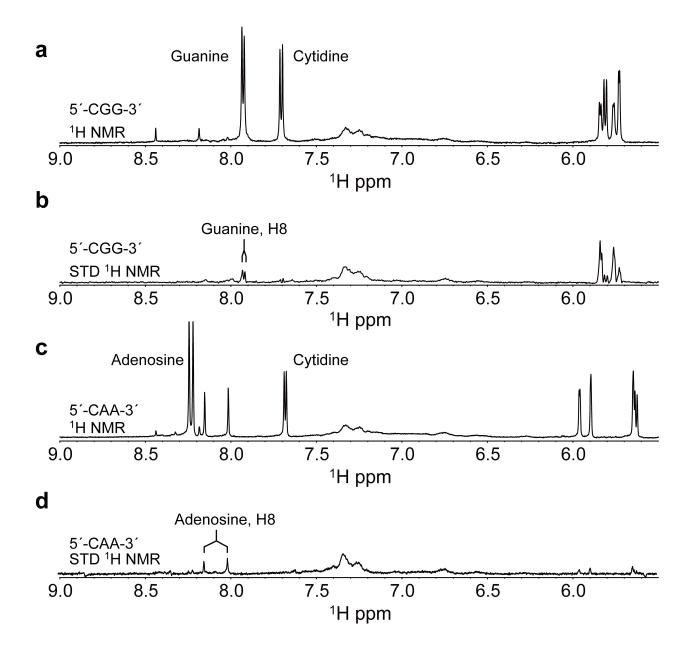


Supplementary Figure 6. Depletion of Nop9 reduces 20S pre-rRNA levels. (a) Schematic drawing of the yeast strain used for testing the effects of Nop9 depletion. Chromosomal Nop9 expression was placed under the control of the inducible GAL promoter and sequences encoding triple-HA tags were inserted. The GAL::3HA-NOP9 yeast strain was transformed with the empty vector (-Nop9) or Nop9 (+Nop9) in p414GPD-3xFLAG plasmid. (b) Representative northern blots detecting precursor and mature rRNA in yeast depleted of Nop9 or expressing wild type Nop9. Total RNA was extracted from yeast bearing a plasmid expressing wild type Nop9 (+Nop9) or empty vector (-Nop9) after depletion of endogenous Nop9 in glucose for 72 hours at 17 °C. The pre-rRNA intermediates and mature rRNAs were detected using a series of oligonucleotide probes, 003, a, b and y, that are indicated in Fig. 1a. Oligo 003 was used to detect 35S, 27SA2, and 23S pre-rRNAs, oligo b was used to detect 20S pre-rRNA, and oligos a + y were used to detect 25S and 18S rRNAs. (c) Plot of the intensities of the mature rRNAs and pre-rRNA intermediates detected in panel b relative to Scr1, the loading control. Bar graphs created in GraphPad PRISM plot the means calculated from three biological replicate experiments with error bars representing the standard error of the mean. The significance of the levels of mature and pre-rRNAs in Nop9-depleted yeast (-Nop9) compared with +Nop9 was assessed by an unpaired t-test, and p-values are indicated (* indicates $p \le 0.05$, ** indicates $p \le 0.01$, **** indicates $p \le 0.0001$, non-significant differences have pvalues > 0.05). The analysis of 35S rRNA and 23S pre-rRNA using oligo 003 is shown, but similar results are obtained when analyzing the blot probed with oligo b.



Incubation time (60 min)

Supplementary Figure 7. Primer extension analysis demonstrating Nob1 cleavage at site D and inhibition by Nop9. (a) Representative full-length gel of primer extension analysis of Nob1 cleavage products. (b) Inhibition of Nob1 cleavage by SUMO-Nop9 fusion protein or Nop9 protein. For both panels, an arrow indicates a major product with cleavage at site D. An asterisk indicates a minor product at an alternative cleavage site. Sequencing reactions are shown in the right lanes. Three technical replicates were conducted. (c) Nob1 cleavage efficiencies in the presence of Nop9 or SUMO-Nop9. Cleavage efficiencies were calculated as the ratio of site D cleavage product to total RNA. The efficiencies of cleavage by Nob1 or Nob1/SUMO were set to 1. Mean cleavage efficiencies \pm S.E.M. were 24.4 \pm 0.36% for Nob1 and 20.2 \pm 1.3% for Nob1/SUMO. Bar graphs, created in GraphPad PRISM, plot the means calculated from three technical replicate experiments with error bars representing the S.E.M. The significance of the Nob1 cleavage efficiencies in the presence of Nop9 relative to Nob1 cleavage alone or SUMO-Nop9 relative to SUMO alone was assessed by an unpaired, two-sided t-test, and p-values are indicated.



Supplementary Figure 8. Saturation-Transfer Difference (STD) NMR of short RNA fragments with Nop9. (a) Reference ¹H spectrum of 5'-CGG-3' with Nop9 and (c) STD spectrum. (c) Reference ¹H spectrum of 5'-CAA-3' with Nop9 and (d) STD spectrum.

RNA	$K_{\rm d} ({\rm nM})^{\rm a}$	<i>K</i> _{rel} ^b	<i>p</i> -value ^c
ITS1 Subdomain A	2.4 ± 0.3	1	N/A
ITS1 Subdomain B	105.0 ± 2.9	44	0.0001
ITS1 Subdomain C	74.4 ± 4.1	31	0.0001
Subdomain A $\Delta 5'_{1-16}$	>1000		N/A
Subdomain A 1-16	>1000		N/A
Subdomain A $\Delta 5'_{1-6}$	1.7 ± 0.4	0.7	0.23
Subdomain A $\Delta 3'$	15.7 ± 2.6	6.5	0.0071
Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	11.8 ± 0.4	4.9	0.0001

Supplementary Table 1. Nop9 binds to the ITS1 RNA stem loop

 ${}^{a}K_{d}$'s are mean \pm SEM for three technical replicates with the exception of binding to Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$, which was for four technical replicates. ${}^{b}K_{rel}$ was calculated relative to the K_{d} for Nop9 binding to ITS1 Subdomain A. ^cThe significance of Nop9 binding affinity to the indicated RNAs relative to Nop9 binding to ITS1 Subdomain A was assessed by an unpaired, two-sided t-test, and *p*-values are indicated.

Supplementary Table 2. Nop9 binding to ITS1 RNA is modestly sensitive to salt concentration

RNA	$K_{\rm d} ({\rm nM})^{\rm a}$	<i>K</i> _{rel} ^b	<i>p</i> -value ^c
Subdomain A $\Delta 5'_{1-6}$, 150 mM NaCl	1.7 ± 0.4	1	N/A
Subdomain A Δ5' ₁₋₆ , 250 mM NaCl	4.8 ± 1.0	2.8	0.083
Subdomain A $\Delta 5'_{1-6}$, 500 mM NaCl	15.6 ± 2.4	9.2	0.0055

 ${}^{a}K_{d}$'s are mean \pm SEM for three technical replicates. ${}^{b}K_{rel}$ was calculated relative to the K_{d} for Nop9 binding to ITS1 Subdomain A $\Delta 5'_{1-6}$ in buffer containing 150 mM NaCl. ^cThe significance of Nop9 binding affinity at the indicated NaCl concentration relative to Nop9 binding to ITS1 Subdomain A $\Delta 5'_{1-6}$ in buffer containing 150 mM NaCl was assessed by an unpaired, two-sided t-test, and *p*-values are indicated.

RNA	$K_{\rm d} ({\rm nM})^{\rm a}$	K _{rel} ^b	<i>p</i> -value ^c
Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	11.8 ± 0.4	1	N/A
Subdomain A $\Delta 5'_{1-12}$, $\Delta 3'$	18.2 ± 1.9	1.5	0.0119
Subdomain A $\Delta 5'_{1-15}$, $\Delta 3'$	346 ± 40	29.3	0.0002
UUU(7-9)GGG Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	6.6 ± 0.3	0.6	0.0002
AAU(10-12)CCC Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	8.5 ± 0.6	0.7	0.0050
AAU(13-15)CCC Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	27.6 ± 3.7	2.3	0.0039
UUU(16-18)GGC, AA(205-206)GC Subdomain A Δ5' ₁₋₆ , Δ3'	103 ± 12	8.7	0.0003
U16G Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	47.7 ± 1.8	4.0	0.0001
U16A Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	62.3 ± 4.9	5.3	0.0001
U16C Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	200.9 ± 27.3	17.0	0.0001
UU(17-18)GC, AA(205-206)GC Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	45.8 ± 5.9	3.9	0.0010
AA(205-206)GG Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	33.2 ± 1.5	2.8	0.0001
UUG(17-19)AAC, CAA(204-206)GUU Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	14.3 ± 1.4	1.2	0.1047
AAA(20-22)CCC, UUU(201-203)GGG Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	7.9 ± 0.4	0.7	0.0011
AUUU(27-30)GCGC, AAAA(194-197)GCGC Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$	20.0 ± 1.5	1.7	0.0017

Supplementary Table 3. Nop9 binds to the base of the ITS1 RNA stem loop

^a K_d 's are mean ± SEM for three technical replicates with the exception of binding to Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$, which was for four technical replicates. ^b K_{rel} is calculated relative to the K_d for Nop9 binding to ITS1 Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$. Shading corresponds to Figure 3: $K_{rel} > 3$, dark orange; $2 < K_{rel} < 3$, light orange; $1 < K_{rel} < 2$, dark gray; $K_{rel} \le 1$, light gray. ^cThe significance of Nop9 binding affinity to the indicated RNAs relative to Nop9 binding to Subdomain A $\Delta 5'_{1-6}$, $\Delta 3'$ was assessed by an unpaired, two-sided t-test, and *p*-values are indicated.

Nop9		ITS1 RNA		Nop9:ITS1 RNA	
Concentration (mg/ml)	$R_{\rm g}({ m \AA})^{ m a}$	Concentration (mg/ml)	$R_{\rm g}({ m \AA})$	Concentration (mg/ml)	$R_{\rm g}({\rm \AA})$
1.27	32.67 ± 0.01	0.67	28.64 ± 0.19	0.88	39.53 ± 0.64
2.88	32.66 ± 0.02	1.33	26.73 ± 0.01	1.43	40.51 ± 0.55
6.13	34.45 ± 0.03	2.67	26.33 ± 0.33	2.37	40.73 ± 0.20

Supplementary Table 4. Radius of gyration (R_g) values at different sample concentrations

^a R_g 's are mean \pm SD for three technical replicates.

Sequence description	Sequence $(5' \text{ to } 3')^a$
5' ITS1 1-212	GAAATTAATACGACTCACTATAGG
5 1151 1-212	AAGAAATTTAATAATTTTGAAAAATGGATTTTTTTG
3' ITS1 1-212	GTTGTATTGAAACGGTTTTAATTGTCCTATAACAAAAGCACAG
ITS1 77-140	GAAATTAATACGACTCACTATAGG
Subdomain C	AGAGATGGAGAGTCCAGCCGGGCCTGCGCTTAAGTGCGCGGTCT
sense strand	TGCTAGGCTTGTAAGTTTCT
ITS1 39-77_140-183	GAAATTAATACGACTCACTATAGG
Subdomain B	TGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACAA_
sense strand	TTTCTTGCTATTCCAAACGGTGAGAGATTTCTGTGCTTTTGTTA
ITS1 1-38_184-212	GAAATTAATACGACTCACTATAGG
Subdomain A	AAGAAATTTAATAATTTTGAAAAATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCGTTTCAATACAAC
ITS1 17 29 194 206	GAAATTAATACGACTCACTATAGG
ITS1 17-38_184-206	TTGAAAATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCGTTTCAA
ITC1 7 20 104 200	GAAATTAATACGACTCACTATAGG
ITS1 7-38_184-206	ΤΤΤΑΑΤΑΑΤΤΤΤΤGAAAATGGATTTTTTTGTTT <i>ΤΑ</i>
sense strand	TAGGACAATTAAAACCGTTTCAA
	GAAATTAATACGACTCACTATAGG
ITS1 10-38_184-206	AATAATTTTGAAAATGGATTTTTTTGTTT TA
sense strand	TAGGACAATTAAAACCGTTTCAA
	GAAATTAATACGACTCACTATAGG
ITS1 13-38_184-206	AATTTTGAAAATGGATTTTTTTTTTTTTTTTTTT
sense strand	TAGGACAATTAAAACCGTTTCAA
	GAAATTAATACGACTCACTATAGG
ITS1 16-38_184-206	TTTGAAAATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCGTTTCAA
ITS1 7-38_184-206	GAAATTAATACGACTCACTATAGG
UUU(7-9)GGG	<u>GGG</u> AATAATTTTGAAAATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCGTTTCAA
ITS1 7-38_184-206	GAAATTAATACGACTCACTATAGG
AAU(10-12)CCC	TTT <u>CCC</u> AATTTTGAAAATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCGTTTCAA
ITS1 7-38_184-206	GAAATTAATACGACTCACTATAGG
AAU(13-15)CCC	TTTAAT <u>CCC</u> TTTGAAAATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCGTTTCAA
ITS1 7-38 184-206	GAAATTAATACGACTCACTATAGG
UUU(16-18)GGC	TTTAATAAT <u>GGC</u> GAAAATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCGTTTC <u>GC</u>
ITC1 7 20 104 204 114C	GAAATTAATACGACTCACTATAGG
ITS1 7-38_184-206 U16G	TTTAATAAT <u>G</u> TTGAAAATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCGTTTCAA
ITS1 7-38_184-206	GAAATTAATACGACTCACTATAGG
UU(17-18)GC	TTTAATAATT_GC_TGAAAATGGATTTTTTTGTTT_TA_
sense strand	TAGGACAATTAAAACCGTTTC <u>GC</u>
sense strand ITS1 7-38_184-206 UU(17-18)GC	TAGGACAATTAAAACCGTTTCAA GAAATTAATACGACTCACTATAGG TTTAATAATT_ <i>GC</i> _TGAAAATGGATTTTTTGTTT_ <i>TA</i> _

Supplementary Table 5. Primers used in this study

ITS1 7-38_184-206	GAAATTAATACGACTCACTATAGG
UUG(17-19)AAC	TTTAATAATT <u>AAC</u> AAAATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCGTTT <u>GTT</u>
ITS1 7-38_184-206	GAAATTAATACGACTCACTATAGG
AAA(20-22)CCC	TTTAATAATTTTG <u>CCC</u> ATGGATTTTTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATTAAAACCG <u>GGG</u> CAA
ITS1 7-38_184-206	GAAATTAATACGACTCACTATAGG
AUUU(27-30)GCGC	TTTAATAATTTTGAAAATGG <u>GCGC</u> TTTTGTTT_ <i>TA</i> _
sense strand	TAGGACAATT <u>GCGC</u> CCGTTTCAA
5′ ITS1 -164	GAAATTAATACGACTCACTATAGG
sense strand	CCGCCCGTCGCTAGTACCGATTG
ITS1_62-84_R	CCATCTCTTGTCTTGCCCAG
Oligo 003	TGCTTACCTCTGGGCC
Oligo c	ATGAAAACTCCACAGTG
Oligo a	CATGGCTTAATCTTTGAGAC
Oligo y	GCCCGTTCCCTTGGCTGTG
Oligo b	GCTCTTTGCTCTTGCC
Oligo Scr1	CGTGTCTAGCCGCGAGGAAGGATTTGTTCC
Nop9 depletion strain	GTTACAACTAGTATACTGTGAACGACGCTGAAACCTTCACGGAA
Forward primer	AAACCACATTATTGTTATTGAATTCGAGCTCGTTTAAAC
Nop9 depletion strain	GGGTTCAAATTCGTCCTTTCTCTGTTTGTCTTGGTGTCTTCTGCCT
Reverse primer	CTTGTTTTAGTCTTTCCCATGCACTGAGCAGCGTAATCTG
Nob1 depletion strain	CGGCGATCATAGTTCAGTATTTTTCTAAAGTTTCTTTAAAGGAGC
Forward primer	ATAATGAATTCGAGCTCGTTTAAAC
Nob1 depletion strain	GGGGTGGCATCCAATATCAACGCCCTTACATGTGCGGTTTGGTTT
Reverse primer	TCGGTCATGCACTGAGCAGCGTAATCTG

^a For sense strands, the first line indicates the T7 promoter sequence. *TA* denotes the truncated ITS1 loop. Mutated bases are italicized and underlined.

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