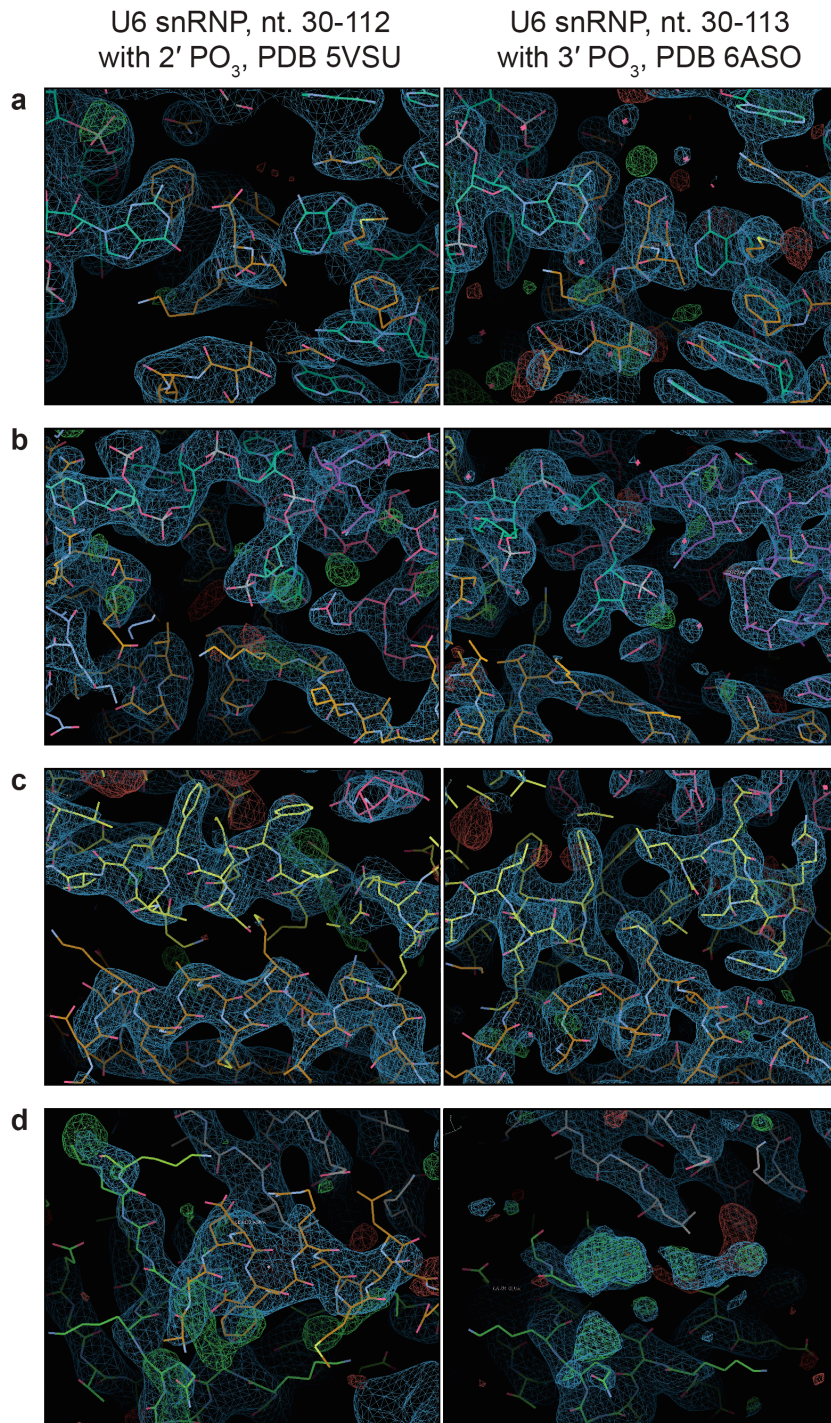
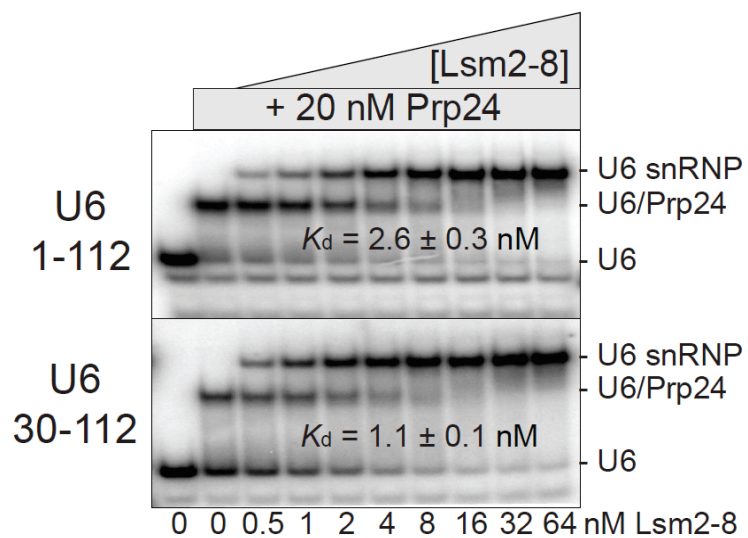


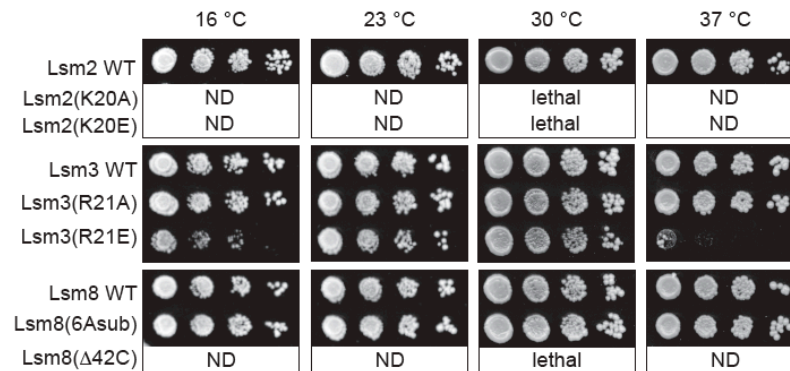
**Supplementary Fig 1** Overview of U6 snRNA structure and assembly into the spliceosome. **a** Simplified cartoon representation of the yeast U6 and U4/U6 snRNPs. For clarity, U4-associated proteins are not depicted. Prp24 binds U6 and catalyzes formation of the U4/U6 snRNP, which requires unwinding of the U6 internal stem loop (ISL). Lsm2-8 is known to bind the conserved C-terminal “SNFFL box” motif in Prp24 and the 3’ oligouridylylate tract in U6, and accelerates Prp24-mediated annealing of U4/U6 through an unknown mechanism<sup>1-3</sup>. **b** U6 snRNA undergoes extensive structural remodeling during pre-mRNA splicing. U6 is highly compact in the U6 snRNP due to extensive intramolecular base-pairing. In contrast, U6 is highly extended in the U4/U6.U5 tri-snRNP, in which Prp24 is absent but the Lsm2-8 ring is still bound to U6. In activated spliceosomes the Lsm2-8 ring is gone, the ISL is mostly reformed, and U6 makes numerous protein-RNA contacts, in addition to pairing with U2 snRNA.



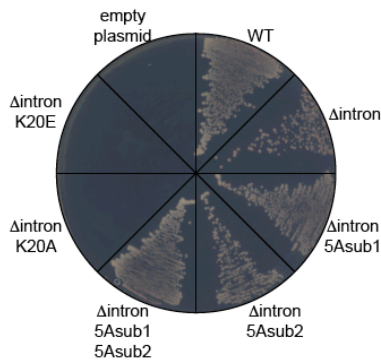
**Supplementary Fig 2** Representative electron density. Blue density is of form  $2mF_o-DF_c$  and is contoured at 1.5 rmsd. Positive and negative density (colored green and red, respectively) is of form  $mF_o-DF_c$  and is contoured at 3.0 rmsd. **a** Density for the core of the U6-Prp24 interaction, centered at Asp288 (the aspartate bridge<sup>4</sup>) and U6 nucleotides A42 and G55. **b** Density for the 3' tail of U6 RNA bound inside the Lsm2-8 ring. **c** Density for the Prp24-RRM4/Lsm2 interface. **d** Density for the Prp24-SNFFL box interaction with Lsm5 and Lsm7. Alpha helical density was modeled as the SNFFL box in PDB 5VSU in space group  $P2_12_12_1$ . Due to weak density, the SNFFL box was not modeled into PDB 6ASO in space group  $P2_1$ .



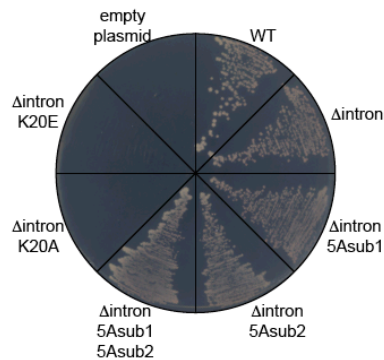
**Supplementary Fig 3** The 5' stem of U6 does not contribute to U6 snRNP stability *in vitro*. Electrophoretic mobility shift was used to monitor formation of U6 snRNPs in the presence of 20 nM Prp24 and varying concentrations of Lsm2-8, using RNA that had been 5' labeled with  $^{32}\text{P}$ .  $K_d$  is the dissociation constant for Lsm2-8 from the ternary complex (Prp24•U6•Lsm2-8). Note that the binary complex of Prp24•U6 appears to be less stable in the absence of the U6 5' stem.

**a****b**

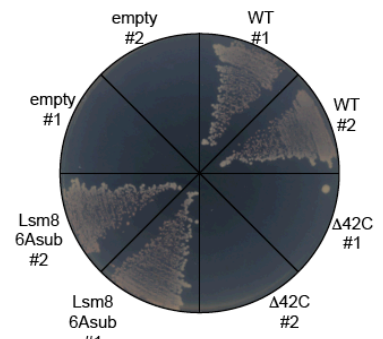
Lsm2 disruption with pRS414-Lsm2  
SD-Trp 5FOA @ 30 °C  
Biological replicate #1



Lsm2 disruption with pRS414-Lsm2  
SD-Trp 5FOA @ 30 °C  
Biological replicate #2

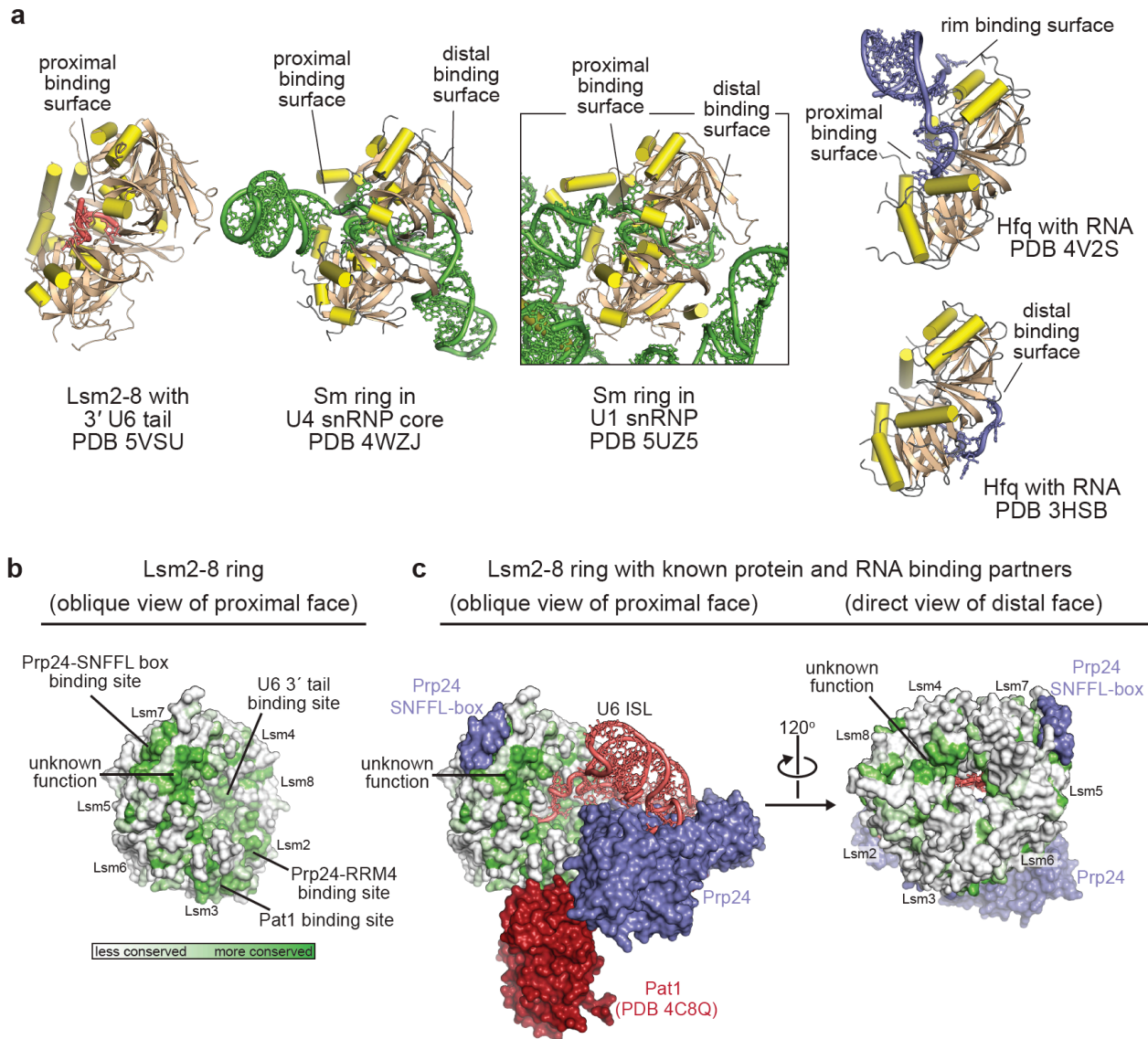
**c**

Lsm8 disruption with pRS414-Lsm8  
SD-Trp 5FOA @ 30 °C  
Biological replicates #1 and 2

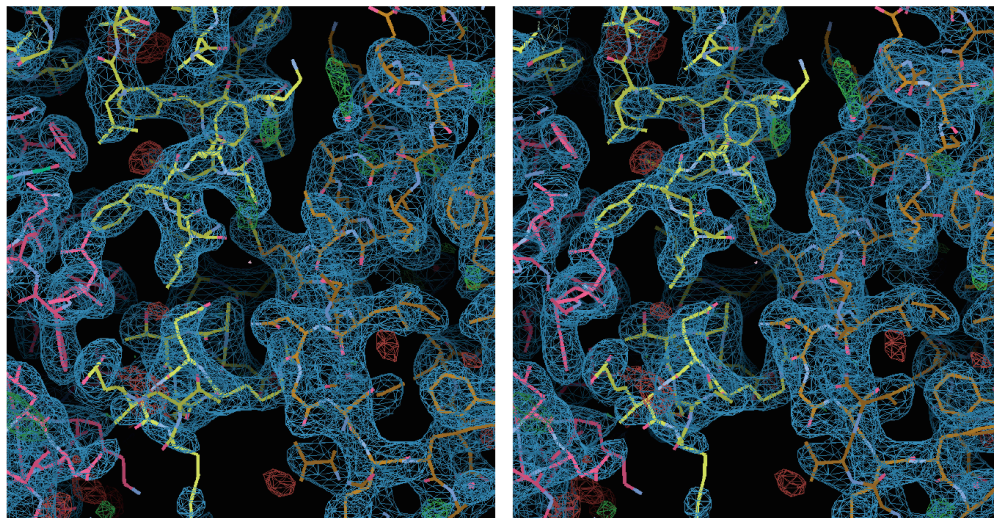


**Supplementary Fig 4** Mutation of 3' end binding residues in Lsm2-8. **a** Point mutant strains exhibit diverse growth phenotypes *in vivo*, which agree well with *in vitro* binding defects (Table 3). ND = not tested due to lethality during initial 5-FOA selection at 30 °C. **b** The intron in Lsm2 is not essential to viability, and mutation of Lsm2 residue K20 is lethal. The 5Asub1 and 5Asub2 alleles contain Lsm2 substitutions at the Prp24/Lsm2 interface (5Asub1 = S5A/K8A/T9A/V11A/D12A, 5Asub2 = L81A/D84A/R88A/E89A/T92A). **c** Removal of the C-terminal 42 residues of Lsm8 is also lethal. The Lsm8 6Asub allele contains K87A, D88A, T89A, K90A, N91A and K92A substitutions at the Lsm8 contact with the 3' end of U6 snRNA. Lethality was determined via 5-FOA counter-selection of the wild-type Lsm alleles on solid media lacking tryptophan at 30 °C. Two independent transformants were tested for each allele.





**Supplementary Fig 5** Homology and surface conservation in protein-protein and protein-RNA binding surfaces of the Lsm2-8 ring. **a** Comparison of known binding mechanisms between RNA and Lsm2-8 or homologous protein ring structures. Unlike the Lsm2-8 ring<sup>5</sup>, the U1 and U4 snRNAs are threaded through the homologous Sm ring<sup>6,7</sup> in the U1 and U4 snRNPs, respectively, and employ a mixture of proximal, rim, and distal RNA binding mechanisms like in the Hfq homologs of the Lsm2-8 ring<sup>8-11</sup>. **b** There are numerous patches of sequence conservation on the proximal and rim surfaces of the Lsm2-8 ring that can be attributed to binding of U6 snRNA and Prp24 in the U6 snRNP, or Pat1 in the Lsm1-7/Pat1 complex<sup>12</sup>. **c** Overlay of known binding partners in the U6 snRNP or Lsm1-7/Pat1 complex shows an additional patch of sequence conservation in the N-terminal region of Lsm5 (approximately residues 7-16 and 37) that is proximal to the bound C-terminal SNFFL-box motif of Prp24. There is an additional patch of sequence conservation along the distal face of the ring between Lsm4 (approximately residues 18-24) and Lsm8 (approximately residues 17-21) that does not interact with Prp24 or U6 in the U6 snRNP, or Pat1 in the Lsm1-7/Pat1 complex, that could provide a binding surface for protein and/or RNA during U4/U6 annealing, or partially comprise the nuclear localization sequence in the Lsm2-8 ring<sup>13</sup>. Conservation was determined with the program ConSurf<sup>14</sup> and the alignment in Supplementary Note 1.



**Supplementary Fig 6** Cross-eyed stereo view of electron density at the Lsm2/RRM4 interface. Blue density is of form  $2mF_o-DF_c$  and is contoured at 1 r.m.s.d. Positive and negative density (colored green and red, respectively) is of form  $mF_o-DF_c$  and is contoured at 3.0 r.m.s.d. Prp24 RRM4 is colored orange, Lsm2 is colored yellow, and Lsm3 is colored pink.

Figure 3d

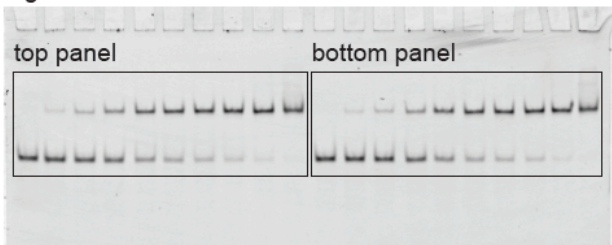


Figure 3e

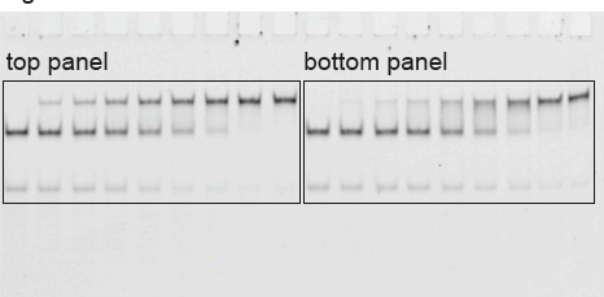
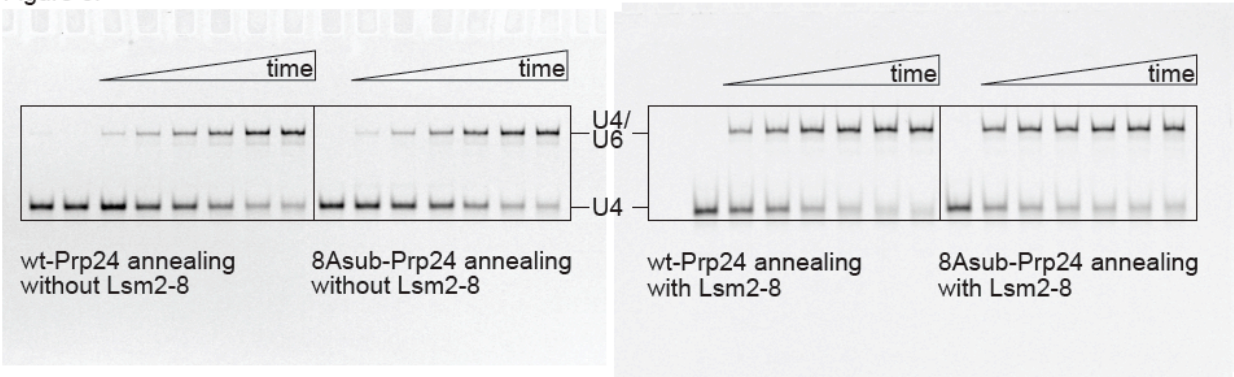
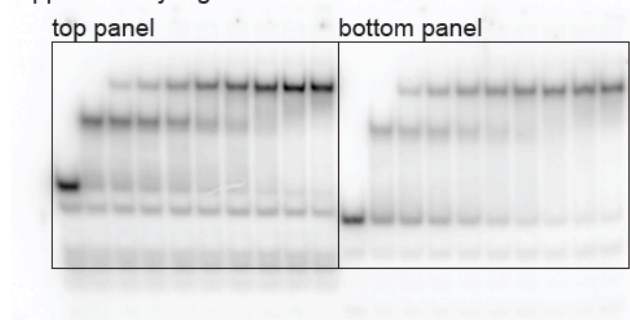


Figure 3f



Supplementary Figure 3



Supplementary Fig 7 Representative, uncropped gel images.

**Supplementary Note 1: Sequence alignment of Lsm2-8 proteins**

**red** = truncations used for crystallization, or substitutions tested in this study

**grey** = disordered in yeast U6 snRNP crystal structure

Lsm2	1	10	20	30	40	50	60	70	80	90	100
H.sapiens	MLFYSFFKSLVGKDVVVELKNDLSICGTLHSVDQYLNKLTDISVTDPEKYPHMLSVKNCFIRGSVVRYVQLPADEVDTQLLQDAARKEALQQKQ-----										
B.taurus	MLFYSFFKSLVGKDVVVELKNDLSICGTLHSVDQYLNKLTDISVTDPEKYPHMLSVKNCFIRGSVVRYVQLPADEVDTQLLQDAARKEALQQKQ-----										
S.scrofa	MLFYSFFKSLVGKDVVVELKNDLSICGTLHSVDQYLNKLTDISVTDPEKYPHMLSVKNCFIRGSVVRYVQLPADEVDTQLLQDAARKEALQQKQ-----										
F.catus	MLFYSFFKSLVGKDVVVELKNDLSICGTLHSVDQYLNKLTDISVTDPEKYPHMLSVKNCFIRGSVVRYVQLPADEVDTQLLQDAARKEALQQKQ-----										
C.familiaris	MLFYSFFKSLVGKDVVVELKNDLSICGTLHSVDQYLNKLTDISVTDPEKYPHMLSVKNCFIRGSVVRYVQLPADEVDTQLLQDAARKEALQQKQ-----										
D.rerio	MLFYSFFKSLVGKDVVVELKNDLSICGTLHSVDQYLNKLTDISVTDPEKYPHMLSVKNCFIRGSVVRYVQLPADEVDTQLLQDAARKEATQQKQ-----										
X.laevin	MLFYSFFKSLVGKDVVVELKNDLSICGTLHSVDQYLNKLTDISVTDPEKYPHMLSVKNCFIRGSVVRYVQLPADEVDTQLLQDAARKEAVQQKQ-----										
A.thaliana	MLFFSYFKDLVVGQEVTVLKNLDLAIRGTLHSVDQYLNKLTDISVTDPEKYPHMLSVKNCFIRGSVVRYVQLPKDGVDDVLLHDAARREARGG-----										
O.sativa	MLFFSYFKDLVVGQEVTVLKNLDLAIRGTLHSVDQYLNKLTDISVTDPEKYPHMLSVKNCFIRGSVVRYVQLPKDGVDDVLLHDAARREARGG-----										
S.pombe	MLFYSFFKTLIDTEVTVLKNLMSIRGILKSVLQFLNVKLENISSVDASKYPHMAAVKDLFIRGSVVRYVHMSSAYVDTILLADACRRDLANNKQ-----										
C.neoformans	MLIFSFKTLIDTEVTVLKNLMSIRGILKSVLQFLNVKLENISSVDASKYPHMAAVKDLFIRGSVVRYVHMSSAYVDTILLADACRRDLANNKQ-----										
N.crassa	MLFFSFKTLIDTEVTVLKNLMSIRGILKSVLQFLNVKLENISSVDASKYPHMAAVKDLFIRGSVVRYVHMSSAYVDTILLADACRRDLANNKQ-----										
C.glabrata	MLFFSFKTLIDTEVTVLKNLMSIRGILKSVLQFLNVKLENISSVDASKYPHMAAVKDLFIRGSVVRYVHMSSAYVDTILLADACRRDLANNKQ-----										
S.cerevisiae	MLFFS <b>FFKTLID</b> TEVTVLKNLMSIRGILKSVLQFLNVKLENISSVDASKYPHMAAVKDLFIRGSVVRYVHMSSAYVDTILLADACRRDLANNKQ-----										

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Lsm3	1	10	20	30	40	50	60	70	80	90	100
H.sapiens	MADDVDQQQTNTTVEEPLDLIRLSLDERIVYKMRNDRELGRRLHAYDQHLNMLIGDVEETVTTIEIDEETYEEIYKSTKRNI PMLFVRGDGVVAVPPLRVG-----										
B.taurus	MADDVDQQQTNTTVEEPLDLIRLSLDERIVYKMRNDRELGRRLHAYDQHLNMLIGDVEETVTTIEIDEETYEEIYKSTKRNI PMLFVRGDGVVAVPPLRVG-----										
S.scrofa	MADDVDQQQTNTTVEEPLDLIRLSLDERIVYKMRNDRELGRRLHAYDQHLNMLIGDVEETVTTIEIDEETYEEIYKSTKRNI PMLFVRGDGVVAVPPLRVG-----										
F.catus	MADDVDQQQTNTTVEEPLDLIRLSLDERIVYKMRNDRELGRRLHAYDQHLNMLIGDVEETVTTIEIDEETYEEIYKSTKRNI PMLFVRGDGVVAVPPLRVG-----										
C.familiaris	MADDVDQQQTNTTVEEPLDLIRLSLDERIVYKMRNDRELGRRLHAYDQHLNMLIGDVEETVTTIEIDEETYEEIYKSTKRNI PMLFVRGDGVVAVPPLRVG-----										
G.gallus	MADDVDQQQTNTTVEEPLDLIRLSLDERIVYKMRNDRELGRRLHAYDQHLNMLIGDVEETVTTIEIDEETYEEIYKSTKRNI PMLFVRGDGVVAVPPLRVG-----										
D.rerio	MADDVAEQQTNTTVEEPLDLIRLSLDERIVYKMRNDRELGRRLHAYDQHLNMLIGDVEETVTTIEIDEETYEEIYKSTKRNI PMLFVRGDGVVAVPPLRVG-----										
X.laevin	MADDGEQQQTNTTVEEPLDLIRLSLDERIVYKMRNDRELGRRLHAYDQHLNMLIGDVEETVTTIEIDEETYEEIYKSTKRNI PMLFVRGDGVVAVPPLRVG-----										
A.thaliana	-----MSGEEEATVREPLDLIRLSLDERIVYKLRSDRELGRKLHAYDQHLNMLIGDVEETITTTVEIDEETYEEIVRTTKRTIEFLFVRGDGVILVSPPLRTAA-----										
O.sativa	(8aa) -AAEEIIVKEPLDLIRLSLDERIVYKLRSDRELGRKLHAYDQHLNMLIGDVEEIVTTVEIDEETYEEIVRTTKRTIEFLFVRGDGVILVSPPLRTA-----										
S.pombe	-----MESAQAVAEPLDLVRLSLDEIVYKLRSDRELGRKLHAYDQHLNMLIGDVEEIVTTVEIDEETYEEIVRTTKRTIEFLFVRGDGVILVSPPLRTA-----										
C.neoformans	-----MDAVNSQIQEPLDLVLKALGERVLIKLRGDRIVTGVVHAYDAHMMNVVISQAEESIHVVDVTEEGQLPPIRRTAEMLFVRGDGVILVSPPLRTA-----										
N.crassa	---MADAVEDAGSVSEPMDLVRLLLDEVVCKLRGRELGRKLHAYDQHLNMLIGDVEEIVTTVEIDEETYEEIVRTTKRTIEFLFVRGDGVILVSPPLRTA-----										
C.glabrata	-----MSLSTPLDLLKLNLDREVYVKLRGAREMGLVQAFDHSCHNIVLSDAVETIYELVDG-----ELKSQERASEMIFVRGDGVILVSPPLRTA-----										
S.cerevisiae	-----METPLDLLKLNLDREVYVKLRGAREMGLVQAFDHSCHNIVLSDAVETIYELVDG-----ELSESERCEMVFIRGDTVTLIISTP <b>SEDDDGAVEI</b>										

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Lsm4	1	10	20	30	40	50	60	70	80	90	100	110	120	130
H.sapiens	MLPLSLLKTAQNHMPMLVELKNGETYNGHLVSCDNWMMNINLREVICTSRDGDKFWRMPECYIRGSTIKYLRIPDEIIDMVKEEVVAKGRGRGGLQQQKQKQKGRGMGGAGRVFVGGGRGGIPGTGRGQPEKKPGRQAGKQ													
B.taurus	MLPLSLLKTAQNHMPMLVELKNGETYNGHLVSCDNWMMNINLREVICTSRDGDKFWRMPECYIRGSTIKYLRIPDEIIDMVKEEVVAKGRGRGGLQQQKQKQKGRGMGGAGRVFVGGGRGGIPGTGRGQPEKKPGRQAGKQ													
F.catus	MLPLSLLKTAQNHMPMLVELKNGETYNGHLVSCDNWMMNINLREVICTSRDGDKFWRMPECYIRGSTIKYLRIPDEIIDMVKEEVVAKGRGRGGLQQQKQKQKGRGMGGAGRVFVGGGRGGIPGTGRGQPEKKPGRQAGKQ													
C.familiaris	MLPLSLLKTAQNHMPMLVELKNGETYNGHLVSCDNWMMNINLREVICTSRDGDKFWRMPECYIRGSTIKYLRIPDEIIDMVKEEVVAKGRGRGGLQQQKQKQKGRGMGGAGRVFVGGGRGGIPGTGRGQPEKKPGRQAGKQ													
G.gallus	MLPLSLLKTAQNHMPMLVELKNGETYNGHLVSCDNWMMNINLREVICTSRDGDKFWRMPECYIRGSTIKYLRIPDEIIDMVKEEVVAKGRGRGGLQQQKQKQKGRGMGGAGRVFVGGGRGGIPGTGRGQPEKKPGRQAGKQ													
D.rerio	MLPLSLLKTAQNHMPMLVELKNGETYNGHLVSCDNWMMNINLREVICTSRDGDKFWRMPECYIRGSTIKYLRIPDEIIDMVKEEVVAKGRGRGGLQQQKQKQKGRGMGGAGRVFVGGGRGGIPGTGRGQPEKKPGRQAGKQ													
X.laevin	MLPLSLLKTAQNHMPMLVELKNGETYNGHLVSCDNWMMNINLREVICTSRDGDKFWRMPECYIRGSTIKYLRIPDEIIDMVKEEVVAKGRGRGGLQQQKQKQKGRGMGGAGRVFVGGGRGGIPGTGRGQPEKKPGRQAGKQ													
A.thaliana	MLPLSLLKTAQGHMPMLVELKNGETYNGHLVNCNNTWMMNINLREVICTSKDGRFWRMPECYIRGNTIKYLRVPDEVIDKVQEET- (46aa)-----													
O.sativa	MLPLSLLKTAQGHMPMLVELKNGETYNGHLVNCNNTWMMNINLREVICTSKDGRFWRMPECYIRGNTIKYLRVPDEVIDKVQEET- (64aa)-----													
S.pombe	MLPLTLNATQGRPILVELKNGETYNGHLVNCNNTWMMNINLREVICTSKDGRFWRMPECYIRGNTIKYLRVPDEVIDKVQEET- (38aa)-----													
C.neoformans	MLPLSLLTAAQKMPMLVELKNGETYNGHLVNCNNTWMMNINLREVICTSKDGRFWRMPECYIRGNTIKYLRVPDEVIDKVQEET- (64aa)-----													
N.crassa	MLPLGLTAAQGHMPMLVELKNGETYNGHLVNCNNTWMMNINLREVICTSKDGRFWRMPECYIRGNTIKYLRVPDEVIDKVQEET- (47aa)-----													
C.glabrata	MLPLYLLTNAKQQMRIELKNGDIYVGLTQVNDWMMNINLREVICTSKDGRFWRMPECYIRGNTIKYLRVPDEVIDKVQEET- (80aa)-----													
S.cerevisiae	MLPLYLLTNAKQQMRIELKNGDIYVGLTQVNDWMMNINLREVICTSKDGRFWRMPECYIRGNTIKYLRVPDEVIDKVQEET- (18aa) -KAVKLNIEIYIRGTFIKFLQDN <b>IIDKVKKQI</b> - (94aa)													

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1	10	20	30	40	50	60	70	80	90
H.sapiens	MTSALENYINRTVAVITSDGRMIVGTLKGF	QDTINLILDESHERV	FSSSQGVEQVVLG	LYIVRGDNVAVIGE	IDEETDSALDLGN	IRAEPLNSVAH	-----		
B.taurus	MTSALENYINRTVAVITSDGRMIVGTLKGF	QDTINLILDESHERV	FSSSQGVEQVVLG	LYIVRGDNVAVIGE	IDEETDSALDLGN	IRAEPLNSVAH	-----		
S.scrofa	MTSALENYINRTVAVITSDGRMIVGTLKGF	QDTINLILDESHERV	FSSSQGVEQVVLG	LYIVRGDNVAVIGE	IDEETDSALDLGN	IRAEPLNSVAH	-----		
F.catus	MTSALENYINRTVAVITSDGRMIVGTLKGF	QDTINLILDESHERV	FSSSQGVEQVVLG	LYIVRGDNVAVIGE	IDEETDSALDLGN	IRAEPLNSVAH	-----		
C.familiaris	MTSALENYINRTVAVITSDGRMIVGTLKGF	QDTINLILDESHERV	FSSSQGVEQVVLG	LYIVRGDNVAVIGE	IDEETDSALDLGN	IRAEPLNSVAH	-----		
G.gallus	MTSALENYINRTVAVITSDGRMIVGTLKGF	QDTINLILDESHERV	FSSSQGVEQVVLG	LYIVRGDNVAVIGE	IDEETDSALDLGN	IRAEPLNSVH	-----		
D.rerio	MSTALESYIHR	TVAIVTSDGRMIVGTLKGF	QAINLILDESHERV	FSSSQGVEQVVLG	LYIVRGDNVAVIGE	IDEETDSALDLGN	IRAEPLNSVH	-----	
X.laevis	MASALENYINRTVAVITADGRMIVGTLKGF	QDTINLILDESHERV	FSSSQGVEQVVLG	LYIVRGDNVAVIGE	IDEETDSSLDLGN	IRAEPLNSVH	-----		
A.thaliana	(13aa) -LVDQIISVITNDGRNIVGTLKGF	QATNIILDESHERV	FSTKEGVQHV	LGLYIIRGDNIGVIGELDEEL	DASLDFSKLRAHPLKPVH	-----			
O.sativa	(9aa) -SLVDQIISVITNDGRNIVGTLR	GFQATNIILDESHERV	STREGVQQLV	LGLYIIRGDNISVVGVEVDEEL	DARLDLSNLR	AHPLKPVH	-----		
S.pombe	--MSLADFMEQRVQVITNDGRVVLGSLKGF	DHTTNLILSDS	FERIISMDQMETIPLGVYLLRGENVAMVGLVNEEL	DSEIEWTKIRGEAIPDVH	-----				
C.neoformans	-MASIESYVDHTVQVILQDGRVIVGKLG	YDPRTNLILSDS	VEREF	SMDQGVEMIPGLGYVIKGNVAVVAELDEEKD	STINYNDIRAEPLAELRY	-----			
N.crassa	(7aa) -SYLNKKVCIITVDGRTL	VGTLISVDMSTNVFLQRAVERVI	- (11aa) -IELGTHMIRGDTVCLVGLVDEPLDES	IDWTKVKGATIGTTKH	-----				
C.glabrata	MSPLLKQYLNKDIVVVT	TAGEVMHVILDGYDKYTNLVVKEG	-----DKIRLLRGSEIVVCGLLED	AKALEGLPMS	---HVYKDTKNVIKDEYLIWEAVNKKHQSTHKKRKLK	-----			
S.cerevisiae	MSATLKDYLNKRVVVIKVDGECLIASLNGFDKNTNLFITNVFNR	ISKE	-----FICKAQLLRGSEIALVGLID	AENDDSLAPIDEKK	VPLMKDTKNKIENEHVIWEKVYESKTK	-----			
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**Supplementary Table 1** Diffraction data collection and structure refinement statistics

	U6 snRNP nt. 30-112 with 2' PO <sub>3</sub>	U6 snRNP nt. 30-113 with 3' PO <sub>3</sub>
PDB ID	5VSU	6ASO
<b>Data Collection</b>		
Wavelength (Å)	0.9792	0.9786
Resolution range (Å)*	179.8-3.10 (3.29-3.10)	90.59-2.80 (2.91-2.80)
Space group	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub>
Unit cell dimensions (Å)	70.2, 114.7, 179.8	94.2, 77.4, 118.6 $\beta = 105.9^\circ$
Total reflections*	1,389,951 (206,204)	2,475,089 (267,471)
Unique reflections*	27,164 (4,321)	40,745 (4,574)
Multiplicity*	51.2 (47.7)	60.7 (58.5)
Completeness (%)*	100 (100)	100 (100)
Mean <i>I</i> / $\sigma$ ( <i>I</i> )*	13.0 (1.2)	8.7 (0.5)
Wilson B-factor (Å <sup>2</sup> )	129.7	79.1
anisotropic $\Delta$ B (Å <sup>2</sup> )	17.4	87.2
R <sub>merge</sub> *	0.25 (4.03)	0.45 (16.6)
R <sub>pim</sub> *	0.035 (0.59)	0.058 (2.18)
CC <sub>1/2</sub> *	1.00 (0.445)	0.997 (0.418)
<b>Refinement</b>		
Resolution	96.7-3.10	55.6-2.71
R <sub>work</sub> /R <sub>free</sub> *	0.234/0.298	0.195/0.247
Total number of atoms	9,240	8,863
macromolecules	9,240	8,724
ligands	0	5
water	0	134
RMS(bonds)	0.011	0.011
RMS(angles)	1.667	1.656
Ramachandran favored	93.1	94.8
Ramachandran outliers	1.81	1.02
Average B factor (Å <sup>2</sup> )	133.4	79.5
macromolecules	133.4	79.5
ligands/ions	NA	74.5
solvent	NA	45.6

\*Values shown in parentheses are for the highest resolution shell. Isotropic data statistics are only shown to 2.8 Å for the anisotropic 3' PO<sub>3</sub> dataset, where ellipsoidal truncation was used to generate the final dataset for structure refinement with resolution limits of 2.71 x 3.50 x 3.59 Å. Ellipsoidal truncation was performed on the StarAniso web server with the default *I*/ $\sigma$ (*I*) cutoff of 1.2.

**Supplementary Table 2** Growth rate of wild-type and *prp24* strains of *S. cerevisiae* in liquid media

strain	doubling time in YEPD (minutes)	
	30 °C	37 °C
<i>PRP24</i>	94 ± 3	88 ± 4
<i>prp24-8Asub</i>	112 ± 5	150 ± 9
<i>prp24-ΔSNFFL</i>	106 ± 1	114 ± 1
<i>prp24-8Asub-ΔSNFFL</i>	132 ± 0	188 ± 6



**Supplementary Table 3** Binding affinity of wild-type and mutant Lsm2-8 for the 3' region of *S. cerevisiae* U6 RNA (nucleotides 104-113) with either a 2',3'-*cis* diol or a 3' phosphate.

	diol	PO <sub>3</sub>	<i>K<sub>d</sub></i> ratio diol/PO <sub>3</sub>	<i>K<sub>d</sub></i> ratio PO <sub>3</sub> /diol
WT	37.4 ± 4.5	6.13 ± 0.87	6.11	0.16
Lsm2-K20A	522 ± 48	841 ± 89	0.62	1.61
Lsm2-K20E	1,870 ± 370	2,320 ± 450	0.81	1.24
Lsm3-R21A	40.8 ± 3.3	123 ± 12	0.33	3.01
Lsm3-R21E	195 ± 27	1,150 ± 190	0.17	5.89
Lsm8-6Asub	132 ± 16	301 ± 33	0.44	2.29
Lsm8-delC	277 ± 32	502 ± 47	0.55	1.81

Affinities are reported as *K<sub>d</sub>* values with units of nM. Binding data were obtained via fluorescence polarization with 5' FAM labeled oligonucleotides (Fig. 5) and are from two technical replicates.

## Supplementary References

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