

**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' oligouridylate 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  $2mF_o$ -D $F_c$  and is contoured at 1.5 rmsd. Positive and negative density (colored green and red, respectively) is of form  $mF_o$ -D $F_c$  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 <sup>32</sup>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.



Lsm2 disruption with pRS414-Lsm2 Lsm2 disruption with pRS414-Lsm2 Lsm8 disruption with pRS414-Lsm8 SD-Trp 5FOA @ 30 °C SD-Trp 5FOA @ 30 °C SD-Trp 5FOA @ 30 °C Biological replicate #1 Biological replicate #2 Biological replicates #1 and 2 empty empty WT empty WT wт plasmig plasmig ∆intron ∆intron empt WΤ ∆intron ∆intron K20E K20E #1 #2 Lsm8 ∆42C Aintron Aintror Aintron ∆intron 6Asub #1 K20A K20A 5Asub1 5Asub1 #2 Lsm8 ∆42C ∆intror ∆intron ∆intror Aintron 6Asub #2 5Asub2 5Asub1 5Asub1 5Asub2 #1 5Asub2 5Asub2

С

**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.

b





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 Hfg 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.

а



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

top panel	bottom panel				

## Figure 3e

	1	head	hand	-	-	-	-	-				m	m	H	ht	-	-
-					-				-	-	-			-			
	4	6									1						

#### Figure 3f





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 6	0	70	80	90	100		
	1	1	1						1		1		
H.sapiens	MLFYSFFK	SLVGKDVVV	ELKNDLSICG	TLHSVDQYLN	IKLTDISVTDPE	KYPHMLSVKNC	FIRGSVVR	YVQLPADEVDI	QLLQDAARK	EALQQKQ	-		
B.taurus	MLFYSFFK	SLVGKDVVV	ELKNDLSICG	TLHSVDOYLN	IKLTDISVTDPE	KYPHMLSVKNC	FIRGSVVR	YVOLPADEVDI	OLLODAARK	EALOOKO	-		
S.scrofa	MLFYSFFK	SLVGKDVVV	ELKNDLSICG	TLHSVDOYLN	IKLTDISVTDPE	KYPHMLSVKNC	FIRGSVVR	YVÕLPADEVDI	OLLODAARK	EALÕÕKÕ	-		
F.catus	MLFYSFFK	SLVGKDVVV	ELKNDLSICG	TLHSVDOYLN	TKLTDISVTDPE	KYPHMLSVKNC	FIRGSVVR	~ YVOLPADEVD1	OLTODAARK	EALOOKO			
C.familiaris	MLFYSFFK	SLVGKDVVV	ELKNDLSICG	TLHSVDOYLN	TKLTDISVTDPE	KYPHMLSVKNC	FIRGSVVH	YVOLPADEVDI	OLTODAARK	EALOOKO			
D rerio	MLFYSFFK	SLVGKDVVV	ELKNDLSICG	TLHSVDOYLN	TKLTDISVTDPE	KYPHMLSVKNC	FIRGSVVR	YVOL PADEVD1	OTTODAARK.	EATOOKO	-		
V laovie	MIEVGEEK	SIVCKDWW	EI KNDI SICC	T LIGVDOVI N	TKITDIGVTDIE	KADAWI GAKNU	FIRCENT			ENVOOKO	_		
A thaliana	MLEESVER	DLVGOEVTV	ETKNDT 7 LBC	TLHSVDOVI.N	TKLENTRVVDOD	KYPHMLSVRNC	FIRGSVVR	VVOL PKDGVDI	UITTHDZZBS.	EARGG	_		
∩ estiva	MIEEGVER	FIVCKEVTV	EI KNDI VIBC	T LIGVDOVI N	TKI ENTRVVDOD	KADAWI GADNU	FIDCONVD	VVI I PODCVDI	ססייגמי דדמי.	EARCC	_		
C. nombo	MIEVOEEN		ELKNDLAIRG	TIRGVDQILN	TUTENTUAND	VX DIMA AVVDI	FINGSVVN	TATIMO CA ATO		LANGG			
S.pombe	MLTECEEV		ELKNDMSIKG	TTURS ADOLE N	VALENISVVDAS		FIRGSVVR	UTINA A DOUDE	ILLADACKK	DLANNKKQ	-		
C. HEOLOLIMANS	MLIFSFFR		ELKNDLSIIG	T T K 2 A D O D I N	IKLDNISVEDPE	KRPRMAVKINC	FIRGSVVR		ILLEDAIRE	EAREGRA			
N.Crassa	MLEESEEK	TLIDHEVIV	ELKNDIQIKG	VLKSVDQFLN	IKLDNIQVVEEL	KIPHLSAVKNV	FIRGSVVR	IVHLPQESVDI	QLLEDATRR.	EAANQATKAKÇ	ĮG		
C.glabrata	MLFFSFFK	TLVDQEVVV	QLKNDIEIKG	TLQSVDQFLN	LKLDNISCNDDK	KIPHLSSVRNI	FIRGSTVR	IVILNKNMVD'I	NLLQDATRR.	EHLSDRK			
S.cerevisiae	MLF'F'SF'F'K	TLVDQEVVV	EL <mark>K</mark> NDIEIKG	TLQSVDQFLN	LKLDNISCTDEK	KYPHLGSVRNI	FIRGSTVR	YVYLNKNMVD'I	'N <b>L</b> LQ <b>D</b> ATR <b>R</b>	EVMTERK	-		
	**::*:**	* • ••*	***** * *	*:****	::* : :	::**: :*::	*****	** : **	:* ** *:	:			
Lsm3	1	10	20	30	40	50 6	50	70	80	90	100		
									1				
H.sapiens	MADDVDQQ	QTTNTVEEP	LDLIRLSLDE	RIYVKMRNDR	ELRGRLHAYDQH	LNMILGDVEEI	VTTIEIDE	ETYEEIYKSTŀ	KRNIPMLFVR	GDGVVLVAPPI	RVG		
B.taurus	MADDVDQQ	QTTNTVEEP	LDLIRLSLDE	RIYVKMRNDR	ELRGRLHAYDQH	LNMILGDVEEI	VTTIEIDE	ETYEEIYKSTŀ	KRNIPMLFVR	GDGVVLVAPPI	RVG		
S.scrofa	MADDVDQQ	QTTNTVEEP	LDLIRLSLDE	RIYVKMRNDR	ELRGRLHAYDQH	LNMILGDVEEI	VTTIEIDE	ETYEEIYKSTF	KRNIPMLFVR	GDGVVLVAPPI	RVG		
F.catus	MADDVDQQ	QTTNTVEEP	LDLIRLSLDE	RIYVKMRNDR	ELRGRLHAYDQH	LNMILGDVEET	VTTIEIDE	ETYEEIYKSTF	KRNIPMLFVR	GDGVVLVAPPI	RVG		
C.familiaris	MADDVDQQ	QTTNTVEEP	LDLIRLSLDE	RIYVKMRNDR	ELRGRLHAYDQH	LNMILGDVEET	VTTIEIDE	ETYEEIYKSTM	KRNIPMLFVR	GDGVVLVAPPI	RVG		
G.gallus	MADEVDQQ	QTTNTVEEP	LDLIRLSLDE	RIYVKMRNDR	ELRGRLHAYDQH	LNMILGDVEEI	VTTIEIDE	ETYEEIYKSTF	RNIPMLFVR	GDGVVLVAPPI	RVG		
D.rerio	MADDAEQQ	QTTNTVEEP	LDLIRLSLDE	RIYVKMRNDR	ELRGRLHAYDQH	LNMILGDVEEI	VTTVEIDE	ETYEEIYKSTF	RNIPMLFVR	GDGVVLVAPPI	RVG		
X.laevis	MADDGEOO	OTTNTVEEP	LDLIRLSLDE	RIYVKMRNDR	ELRGRLNAYDOH	LNMILGDVEET	VTTIEIDE	ETYEEIYKSTM	RNIPMLFVR	GDGVVLVAPPI	RVG		
A.thaliana	MSG	EEEATVREP	LDLIRLSLDE	RIYVKLRSDR	ELRGKLHAFDOH	LNMILGDVEET	ITTVEIDD	ETYEEIVRTTM	RTIEFLFVR	GDGVILVSPPI	RTAA		
0.sativa	(8aa)-AA	EEEIAVKEP	LDLIRLSLDE	RIYVKLRSDR	ELRGKLHAYDOH	LNMILGDVEEI	VTTVEIDD	ETYEEIVRTTF	RTIPFLFVR	GDGVILVSPPL	RTA		
S.pombe	M	ESAOAVAEP	LDLVRLSLDE	IVYVKLRGDR	ELNGRLHAYDEH	LNMVLGDAEEI	VTIFDDEE	FDKDKALKTIF	REALEVR	GDSVILIAPPR	N		
C.neoformans	MDA	VVNŠOIOEP	LDLVKLALGE	RVLIKLRGDR	IVTGVLHAYDAH	MNVVISOAEES	IHVVDVTE	EGOPLPPRIEF	RTAEMLFVR	GDGVILLSPAE	0		
N.crassa	MADAV	EDAGSVSEP	MDLVRLLLDE	VVCVKLRGDR	ELKGRLHAYDSH	CNLVLGEVEET	TYVVDDED		RKSEMLEVR	GDSVVLTSPFT	~ 'RS		
C.glabrata		MSLSTP	I'DI'I'RI'NI'DE	RVYVKLRGAR	AMEGVLOAFDSH	CNIVLSDAVET	TYELVDG-	ELKSOF	RASEMIEVR	GDSVTLTTAVS	EEE		
S.cerevisiae		METP	T'DI'I'RI'NI'DE	RVYTKLRGAR	TLVGTLOAFDSH	CNIVLSDAVET	TYOLNNE-	ELSESE	RRCEMVETR	GDTVTLISTPS	EDDDGAVET		
0.0010.10140		• *	• * * • • * * . *	• • * • * . *	• * *•*•* *	* • • • • • • *	•	220202	• • • • • • •	** * * • •			
							• •				•		
Lsm4	1	10	20	30 4	0 50	60	70	80	90	100	110	120	130
	1	1			1		1	1	1	1	1	1	
H.sapiens	MLPLSLLKT	AQNHPMLVEL	KNGETYNGHL	SCDNWMNINL	REVICTSRDGDKF	WRMPECYIRGS	TIKYLRIPDE	IIDMVKEEVVA	KGRGRGGLQQ	QKQQKGRGMGG.	AGRGVFGGRGRG	GIPGTGRGQP	EKKPGRQAGKQ
B.taurus	MLPLSLLKT	AQNHPMLVEL	KNGETYNGHL	SCDNWMNINL	REVICTSRDGDKF	WRMPECYIRGS	TIKYLRIPDE	IIDMVKEEVVA	KGRGRGGLQQ	QKQQKGRGMGG.	AGRGVFGGRGRG	GIPGTGRGQP	EKKPGRQAGKQ
F.catus	MLPLSLLKT	AQNHPMLVEL	KNGETYNGHLV	SCDNWMNINL	REVICTSRDGDKF	WRMPECYIRGS	CIKYLRIPDE	IIDMVKEEVVA	KGRGRGGLQQ	QKQQKGRGMGG.	AGRGVFGGRGRG	GIPGTGRGQP	EKKPGRQAGKQ
C.familiaris	MLPLSLLKT	AQNHPMLVEL	KNGETYNGHLV	SCDNWMNINL	REVICTSRDGDKF	WRMPECYIRGS	TIKYLRIPDE	IIDMVKEEVVA	KGRGRGGLQQ	QKQQKGRGMGG.	AGRGVFGGRGRG	GIPGTGRGQP	EKKPGRQAGKQ
G.gallus	MLPLSLLKT	AQNHPMLVEL	KNGETYNGHLV	SCDNWMNINL	REVICTSRDGDKF	WRMPECYIRGS	TIKYLRIPDE	IIDMVKEEVVS	KGRGRGGMQQ	QKQQKGRGVGG.	AGRGVFGGRGR-	GIPGSGRGQQ	EKKPGRQSAKQ
D.rerio	MLPLSLLKT	AQNHPMLVEL	KNGETYNGHLV	/SCDNWMNINL	REVICTSRDGDKF	WRMPECYIRGS	TIKYLRIPDE	IIDMVKEEVVS	KGRGRGGMQQ	NKPQ-(14aa)	-GRGVFGGRGRG	MQGSGRGQQQ	DKKPGKQQV
X.laevis	MLPLSLLKT	AQNHPMLVEL	KNGETYNGHLV	/SCDNWMNINL	REVICTSRDGDKF	WRMPECYIRGS	TIKYLRIPDE	IIDMVKEEVMS	KGRGRGGMQQ	QKQMKGRGAGG	GGRGVFGGRGR-	GAPGGGRGQQ	DKKPGRQSGKQ
A.thaliana	MLPLSLLKT	AQGHPMLVEL	KNGETYNGHLV	VNCDTWMNIHL	REVICTSKDGDRF	WRMPECYIRGN	TIKYLRVPDE	VIDKVQEEK- (	(46aa)				
O.sativa	MLPLSLLKT	AQGHPMLVEL	KNGETYNGHLV	VNCDTWMNIHL	REVICTSKDGDKF	WRMPECYIRGN	TIKYLRVPDE	CVIDKVQEET-(	(64aa)				
S.pombe	MLPLTLLNA	FQGRPILVEL	KNGETFNGHL	ENCONYMNLTL	REVIRTMPDGDKF	FRLPECYIRGNN	NIKYLRIQDE	VLSQVAKQQ- (	(38aa)				
C.neoformans	MLPLSLLTA	AQGKPMLVEL	KNGVTFNGHLV	VECDTFMNVTL	REVYQTSADGERF	WKMKEMFIKGNI	IKYFRIADN	IILEQAAEEQ-(	64aa)				
N.crassa	MLPLGILTA	AQGHPMLVEL	KNGETLNGHL	IQCDTWMNLTL	REVVQTSPEGDKF	VRLPEVYVKGNN	IIKYLRVPDI	VIDIAREQQ- (	47aa)				
C.glabrata	MLPLYLLTN	AKGQQMRIEL	KNGDIVEGEL	INVDNWMNLTL.	ANV- (15aa) -KV	VKSKEIYVRGVY	YIKYITLQDI	IIEKVKQQI- (	(80aa)				
S.cerevisiae	MLPLYLLTN	AKGQQMQIEL	KNGEIIQGIL	FNVDNWMNLTL:	SNV-(18aa)-KA	VKLNEIYIRGTH	FIKFIKLQDN	IIDKVKQQI-(	(94aa)				
	**** :*.	::.: : :**	*** :* *	. *.:**: *	:* :	: * :::*	**:: : *:	:: ::					

Lsm5	1	10	20	30	40	50	60	70	80	90
	1		1		1	1		1	I	
H.sapiens	MAANATTN	IPSQLLPLELV	/DKCIGSRIHI	VMKSDKEIVG	TLLGFDDFVNN	AVLEDVTEFE:	ITPEGRRITK	LDQILLNGNN	ITMLVPGGEO	GPEV
B.taurus S.scrofa	MAANATTN MAANATTN	IPSQLLPLELV IPSQLLPLELV	/DKCIGSRIHI /DKCIGSRIHI	VMKSDKEIVG VMKSDKEIVG	TLLGFDDFVNN TLLGFDDFVNN	IVLEDVTEFE: IVLEDVTEFE:	ITPEGRRITK ITPEGRRITK	LDQILLNGNN LDQILLNGNN	ITMLVPGGE0	GPEV GPEV
F.catus C.familiaris	MAANATTN MAANATTN	IPSQLLPLEL\ IPSQLLPLEL\	/DKCIGSRIHI /DKCIGSRIHI	VMKSDKEIVG VMKSDKEIVG	TLLGFDDFVNN TLLGFDDFVNN	AVLEDVTEFE: AVLEDVTEFE:	ITPEGRRITK: ITPEGRRITK:	LDQILLNGNN LDQILLNGNN	ITMLVPGGE0	GPEV GPEV
G.gallus	MAANASTN	IPSQLLPLELV	/DKCIGSRIHI	VMKSDKEIVG	TLLGFDDFVNN	AVLEDVTEFE:	ITPEGRRITK:	LDQILLNGNN	ITMLVPGGEO	GPEV
D.rerio	MAAITATN	IPSOLLPLELV	/DKCIGSRIHI	VMKNDKEIVG	TLLGFDDFVNN	AVLEDVTEFE:	ITPEGRRITK:	LDOILLNGNN	ITMLIPGGEO	GPEV
X.laevis	MAATVPPN	ISSQLLPLELV	/DKCIGSRIHI	VMKSDKEIVG	TLLGFDDFVNN	AVLEDVTEFE:	ITPEGRRITK:	LDQILLNGNN	ITMLVPGGEO	GPEV
A.thaliana	MANN	IPSQLLPSELI	DRCIGSKIWV	IMKGDKELVG	ILKGFDVYVNN	AVLEDVTEYE:	ITAEGRRVTK	LDQILLNGNN	IAILVPGGSI	PEDGE
O.sativa	MSQNN	IPSQLLPSELI	DRCIGSKIWV	IMKGDKELVG	TLCGFDVYVNN	4VLEDVTEYE	YTAEGRRITK	LDQILLNGNN	IAILVPGGSB	PPDVA
S.pombe	M	ISMTILPLELI	DKCIGSNLWV	IMKSEREFAG	TLVGFDDYVNI	UVLKDVTEYD:	TVTGVTEK	HSEMLLNGNG	MCMLIPGGKE	PE
C.neoformans	M	ASTILPLELV	/DRCIGSPIWV	LMKNEREFTG	TLMGFDDYVNN	AVLKDVKEYEV	/TASGITETD	LGDTLLNGNN	IAMLIPGGKO	GPKA
N.crassa	M	IASQLLPLELI	DKCVGSRIWV	VMKGDKEFAG	TLVGFDDYVNN	4VLEDVTEFD	YSGNHTK	LKKILLNGNN	ICMLIPGGE	GPIAASA
C.glabrata	MS	SLDVLPLEVI	IDKTIDQQVHI	ILQSNREFSG	KLVGFDEFVNV	/ILEDPIEY-	(15aa)-MEH	HGRMLLSGNN	ITMLVPGGK	(L
S.cerevisiae	MS	SLPEILPLEVI	IDKTINQKVLI	VLQSNREFEG	TLVGFDDFVNV	/ILEDAVEW-	(13aa)-MQH	HGRMLLSGNN	IAILVPGGK	TPTEAL
		:** *::	*: : : :	:::.:*: *	* *** :**:	:*:* *:		**•**•	: :*:***.	
Lsm6	1	10	20	30	40	50	60	70	80	
	1							I	I	
H.sapiens	MSLRKQTP	SDFLKQIIGF	RPVVVKLNSGV	DYRGVLACLD	GYMNIALEQTH	EEYVNGQLKNI	KYGDAFIRGN	NVLYISTQKR	.RM	
B.taurus	MSLRKQTP	SDFLKQIIGF	RPVVVKLNSGV	DYRGVLACLD	GYMNIALEQTH	EEYVNGQLKNI	KYGDAFIRGN	NVLYISTQKR	.RM	
S.scrofa	MSLRKQTP	SDFLKQIIGF	RPVVVKLNSGV	DYRGVLACLD	GYMNIALEQTH	EEYVNGQLKNI	KYGDAFIRGNI	NVLYISTQKR	.RM	
F.catus	MSLRKQTP	SDFLKQIIGF	RPVVVKLNSGV	DYRGVLACLD	GYMNIALEQTH	SEYVNGQLKNI	KYGDAF'IRGNI	NVLYISTQKR	.RM	
C.familiaris	MSLRKQTP	SDFLKQIIGF	RPVVVKLNSGV	DYRGVLACLD	GYMNIALEQTH	SEYVNGQLKNI	KYGDAFIRGN	NVLYISTQKR	.RM	
G.gallus	MSLRKQTP	SDFLKQIIGF	RPVVVKLNSGV	DYRGVLACLDO	GYMNIALEQTH	SEYVNGQLKNI	KYGDAF IRGN	NVLYISTQKR	.RM	
D.rerio	MSLRKQTP	SDFLKQIIGF	RPVVVKLNSGV	DYRGVLACLD	JYMNIAVEQTE CYMNIAL DODI	SEY VNGQLKNI	XYGDAF LRGN	NVLIISTQKR	KM	
A.Idevis	MSLRKQTP (14aa) D	SDFLKQIIGF	Y V V KLINSGV		GYMNIALEQTI CYMNIAMEOUT	SEI VNGQLKNI FEVUNCOLKNI	XIGDAFIRGN XXCDAFIRGN	NVLILSTQKK		
A. UIIdilialia	(14aa) - P	ADFLKSIKGF	VEVVVKLINGGV	DIRGILICLD	GIMNIAMEQII CVMNIAMEOUI	SE I VNGQLANI	XIGDAF IRGN	NVLIISIVNM WUVVICHCKD	II VADGA	
S nombe	(20dd) -P	NEEINKVICK SDEFUSIKGE	KVI TRI SCV	DIRGILACLD	CYMNIAMEQII	SEI VNGQLANI	VCDAFIRGN	NVL VVGAT DD	ILIDDA	
C peoformane	(16aa) - P	SEFIDNIVCK	CRUKUPTCSCU		CVMNUALEAL	SEI VNGRRIN FEWACEVKTAJ	VGDGEI RGNI	WIVISALDD	т	
N crassa	(13aa) - P	SGFLSEIIGN	JPVTVKI.NSGV	VYKGELOSVD	GYMNTALEVTI	CEFINGVERR	SYGDA FVRGNI	WMYISAD		
C glabrata	-MAEESVS	TOFLSNIIG	VIVIVILINGOV	U.YSGVLESTD	GEMNVALSET:	RE- (12aa) - 1	YASDVFLRGT	NVMYISEA		
S.cerevisiae	(11aa) - T	TEFLSDIIG	TVNVKLASGI	LYSGRLESID	GEMNVALSSA	СЕ (12аа) -1 ГЕ-(12аа) -1	NNDVFLRGT	OVMYTSEOKT		
0.0010110140	(1100) 1	** .: *.	* ::: **:	* * * * :	* : * * : * : . :	* .	.* * • * *	* * * * *		
Lsm7	1	10	20	30	40	50	60	70	80	90 100
	1				1	1		1	I	
H.sapiens	MADKEKKK	KESILDLSKY	IDKTIRVKFQ	GGREASGILK	GFDPLLNLVLI	OGTIEYMRDPI	DDQYKLTEDT	RQLGLVVCRG	TSVVLICPQI	GMEAIPNPFIQQQDA-
B.taurus	MADKEKKK	KESILDLSKY	IDKTIRVKFQ	GGREASGILK	GFDPLLNLVLI	OGTIEYMRDPI	DDQYKLTEDT	RQLGLVVCRG	TSVVLICPQI	OGMEAIPNPFIQQQDA-
S.scrofa	MADKEKKK	KESILDLSKY	IDKTIRVKFQ	GGREASGILK	GFDPLLNLVLI	OGTIEYMRDPI	DDQYKLTEDT	RQLGLVVCRG	TSVVLICPQI	OGMEAIPNPFIQQQDA-
F.catus	MADKEKKK	KESILDLSKY	IDKTIRVKFQ	GGREASGILK	GFDPLLNLVLI	DGTVEYMRDPI	DDQYKLTEDT	RQLGLVVCRG	TSVVLICPQI	DGMEAIPNPFIQQQDA-
C.familiaris	MADKEKKK	KESILDLSKY	IDKTIRVKFQ	GGREASGILK	GFDPLLNLVLI	DGTVEYMRDPI	DDQYKLTEDT	RQLGLVVCRG	TSVVLICPQI	DGMEAIPNPFIQQQDA-
G.gallus	(20aa)-K	KESILDLSKY	IGKTIRVKFQ	GGREASGVLK	GFDPLLNLVLI	DGTIEYMRDPI	DDQYKLTEDT	RQLGLVVCRG	TSVVLICPQI	OGMEAIPNPLIQQQDG-
D.rerio	MADKDKKK	KESIFDLSKY	IDKHIRVKFQ	GGREASGVLK	GFDPLLNLVLI	DGTIEYMRDPI	DDQYKLTEDT	RQLGLVVCRG	TSVVLICPQI	DGMEAIPNPFIQQQDG-
X.laevis	MADKEKKK	KESILDLSKY	ZIDKTIRVKFQ	GGREASGVLK	GFDPLLNLVLI	DGTIEYMRDPI	DDQYKLTEDT	RQLGLVVCRG	TSVVLICPQI	DGMEAIPNPFVQQQEG-
A.thaliana	MSGR	RETVLDLAKE	VDKGVQVKLT	GGRQVTGTLK	GYDQLLNLVLI	DEAVEFVRDHI	DDPLKTTDQT	RRLGLIVCRG	TAVMLVSPTI	DGTEEIANPFVTAEAV-
0.sativa	MSGR	KETVLDLAKE	"VDKGVQVKL'I	GGRQVTGTLK	GYDQLLNLVLI	DEAVEFEREQI	DDPLKLSGKT	RQLGLIVCRG	TAVMLVSPTI	DGTDEIANPFQSDGA
S.pombe	(20aa)-R	RESILDLSRY	QDQRIQATET	GGRQITGILK	GFDQLMNLVLI	DVEEQLRNPI	SDG-KLTGAI	RKLGLVVVRG	TTLVLIAPMI	DGSEEIPNPFVQAE
C.neoIormans	(ZIAA) -K	KESILNLAQE	VDKSIKVKFM	IGGKEA'I'GI LK(	JIDQLMNLVMI	JUVVEEYEDG-	KPT	KALGLVVLRG	FNIVLVSPT	JGSSETENFFQQ
N.Crassa	(JUAA) -K	NENILULKKI	MUQKITVKEN	IGGKEVTGTLK(	JIUALMNLVLI CVDOIMNIIVII	JUVQEAVKDEI	JENCAROOJ		TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	JGSEVIANFFAQQEEEA
C. YIADIALA	(20aa) = K	REATI DI AVA	KUCKID//KIM	ICCKI VICVI V	ЗАРОТ WMI MI 1 ЗТ ОЙТЫШТ ОТ 1	JULAET PET PET PET PET PET PET PET PET PET P	-(11aa)-NN	SKI CI WALBO	TTT.Vet een	CODVI YMOR
S.CETEATPIGE	(22aa) = N	•* • •* •		**• * ***	717714117777 7177141177777	· *	(IIIaa) -NA	* *** * **		• * •
	:			•	• • • • • • • • • • • •	•••				

Lsm8	1	10	20	30	40	50	60	70	80	90	
	1		1	1		I.					
H.sapiens	MTSALENYIN	RTVAVITSD	GRMIVGTLKO	FDQTINLILD	ESHERVFSSS	QGVEQVVLO	GLYIVRGDNVAV	IGEIDEETDS	ALDLGNIRAE	PLNSVAH	
B.taurus	MTSALENYIN	RTVAVITSD	GRMIVGTLKO	FDQTINLILD	ESHERVFSSS	QGVEQVVLO	GLYIVRGDNVAV	IGEIDEETDS	ALDLGNIRAE	PLNSVAH	
S.scrofa	MTSALENYIN	RTVAVITSD	GRMIVGTLKO	FDQTINLILD	ESHERVFSSS	QGVEQVVLG	GLYIVRGDNVAV	IGEIDEETDS	ALDLGNIRAE	PLNSVAH	
F.catus	MTSALENYIN	RTVAVITSD	GRMIVGTLKO	FDQTINLILD	ESHERVFSSS	QGVEQVVLO	GLYIVRGDNVAV	IGEIDEETDS	ALDLGNIRAE	PLNSVAH	
C.familiaris	MTSALENYIN	RTVAVITSD	GRMIVGTLKO	FDQTINLILD	ESHERVFSSS	QGVEQVVLO	GLYIVRGDNVAV	IGEIDEETDS	ALDLGNIRAE	PLNSVAH	
G.gallus	MTSALENYIN	RTVAVITSD	GRMIVGTLKO	FDQTINLILD	ESHERVFSSS	QGVEQVVLO	GLYIVRGDNVAV	IGEIDEETDS	ALDLGNIRAE	PLNSVVH	
D.rerio	MSTALESYIH	RTVAIVTSD	GRMIVGTLKG	FDQAINLILD	ESHERVFSSS	QGVEQVVLO	GLYIVRGDNVAV	IGEIDEETDS	ALDLGNIRAEI	PLNSVVH	
X.laevis	MASALENYIN	RTVAVITAD	GRMIVGTLKO	FDQTINLILD	ESHERVFSSS	QGVEQVVLO	GLYIVRGDNVAV	IGEIDEETDS	SLDLGNIRAE	PLNSVVN	
A.thaliana	(13aa)-LVDQ	QIISVITND	GRNIVGVLKO	FDQATNIILD	ESHERVFSTK	EGVQQHVLO	GLYIIRGDNIGV	IGELDEELDA	SLDFSKLRAHI	PLKPVVH	
0.sativa	(9aa)-SLVD	QIISVITND	GRNIVGTLRG	FDQATNIILD	ESHERVYSTR	EGVQQLVLO	GLYIIRGDNISV	VGEVDEELDA	RLDLSNLRAHI	PLKPVIH	
S.pombe	MSLADFME	QRVQVITND	GRVVLGSLKO	FDHTTNLILS	DSFERIISMD	QDMETIPLO	GVYLLRGENVAM	VGLVNEELDS	EIEWTKIRGEA	AIPDVVH	
C.neoformans	-MASIESYVD	HTVQVILQD	GRVIVGKLKO	GYDPRTNLILS	DSVEREFSMD	QGVEMIPLO	GLYVIKGDNVAV	VAELDEEKDS	TINYNDIRAEI	PLAELRY	
N.crassa	(7aa)-SYLNI	KKVCIITVD	GRTLVGTLIS	SVDMSTNVFLQ	RAVERVI-(1	laa)-IELG	GTHMIRGDTVCI	VGLVDEPLDE	SIDWTKVKGAT	FIGTTKH	
C.glabrata	MSPLLKQYLN	KDIVVVTTA	GEVMHVILDO	GYDKYTNLVVK	EG	DK	KIRLLRGSEIVV	CGLLEDAKAL	EGLPMDSH	HVYKDTKNVIKDEYLIW	EAVNKKHQSTHKKRKLK
S.cerevisiae	MSATLKDYLNI	KRVVIIKVD	GECLIASLNO	FDKNTNLFIT	NVFNRISKE-	FICK	KAQLLRGSEIAI	VGLI <mark>D</mark> AENDD	SLAPIDEKKVI	PML <b>KDTKNK</b> IENEHVIW	EKVYESKTK
	: :.	: : ::	*.: *.	* *:.:			:* .:	: ::			

	U6 snRNP	U6 snRNP
	nt. 30-112	nt. 30-113
	with 2' PO <sub>3</sub>	with 3' $PO_3$
	5750	6450
Data Collection		
Wavelength (A)	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
		β = 105.9°
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/σ(I)*	13.0 (1.2)	8.7 (0.5)
Wilson B-factor (Å <sup>2</sup> )	129.7	79.1
anisotropic ∆B (Ų)	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)
Refinement		
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

Supplementary Table 1 Diffraction data collection and structure refinement statistics

\*Values shown in parentheses are for the highest resolution shell. Isotropic data statistics are only shown to 2.8 Å for the anisotropic 3'  $PO_3$  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

doubling	time i	in YEPD	(minutes)
----------	--------	---------	-----------

strain	30 °C	37 °C
PRP24	94 ± 3	88 ± 4
prp24-8Asub	112 ± 5	150 ± 9
prp24-∆SNFFL	106 ± 1	114 ± 1
prp24-8Asub-∆SNFFL	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.

			<i>K</i> d ratio	<i>K</i> <sub>d</sub> ratio
	diol	PO <sub>3</sub>	diol/PO3	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  $K_d$  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**

- 1. Didychuk, A.L., Montemayor, E.J., Brow, D.A. & Butcher, S.E. Structural requirements for protein-catalyzed annealing of U4 and U6 RNAs during di-snRNP assembly. *Nucleic Acids Res* **44**, 1398-410 (2016).
- 2. Rader, S.D. & Guthrie, C. A conserved Lsm-interaction motif in Prp24 required for efficient U4/U6 di-snRNP formation. *RNA* **8**, 1378-92 (2002).
- 3. Achsel, T. et al. A doughnut-shaped heteromer of human Sm-like proteins binds to the 3'-end of U6 snRNA, thereby facilitating U4/U6 duplex formation in vitro. *EMBO J* **18**, 5789-802 (1999).
- 4. Montemayor, E.J. et al. Core structure of the U6 small nuclear ribonucleoprotein at 1.7-A resolution. *Nat Struct Mol Biol* **21**, 544-51 (2014).
- 5. Zhou, L. et al. Crystal structures of the Lsm complex bound to the 3' end sequence of U6 small nuclear RNA. *Nature* **506**, 116-20 (2014).
- 6. Li, J., Leung, A.K., Kondo, Y., Oubridge, C. & Nagai, K. Re-refinement of the spliceosomal U4 snRNP core-domain structure. *Acta Crystallogr D Struct Biol* **72**, 131-46 (2016).
- 7. Li, X. et al. CryoEM structure of Saccharomyces cerevisiae U1 snRNP offers insight into alternative splicing. *Nat Commun* **8**, 1035 (2017).
- 8. Wilusz, C.J. & Wilusz, J. Lsm proteins and Hfq: Life at the 3' end. *RNA Biol* **10**, 592-601 (2013).
- 9. Dimastrogiovanni, D. et al. Recognition of the small regulatory RNA RydC by the bacterial Hfq protein. *Elife* **3**(2014).
- 10. Someya, T. et al. Crystal structure of Hfq from Bacillus subtilis in complex with SELEXderived RNA aptamer: insight into RNA-binding properties of bacterial Hfq. *Nucleic Acids Res* **40**, 1856-67 (2012).
- 11. Updegrove, T.B., Zhang, A. & Storz, G. Hfq: the flexible RNA matchmaker. *Curr Opin Microbiol* **30**, 133-138 (2016).
- 12. Sharif, H. & Conti, E. Architecture of the Lsm1-7-Pat1 complex: a conserved assembly in eukaryotic mRNA turnover. *Cell Rep* **5**, 283-91 (2013).
- 13. Spiller, M.P., Reijns, M.A. & Beggs, J.D. Requirements for nuclear localization of the Lsm2-8p complex and competition between nuclear and cytoplasmic Lsm complexes. *J Cell Sci* **120**, 4310-20 (2007).
- 14. Ashkenazy, H. et al. ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. *Nucleic Acids Res* **44**, W344-50 (2016).