Supplementary Materials for:

Molecular basis for the distinct cellular functions of the Lsm1-7 and Lsm2-8 complexes

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Supplementary Table 1. Summary of *in vitro* binding affinity between the indicated RNAs and *S. pombe* Lsm rings. All values were determined by fluorescence polarization binding assays with 5' fluorescein-labeled RNA at 1 nM and a broad concentration range of Lsm proteins. Binding constants were determined by non-linear regression with a constrained Hill coefficient of 1. Raw polarization data and determined binding curves are available as Supplementary Data File 1.

Supplementary Figure 1. Production of recombinant *S. pombe* Lsm rings that can bind RNA and form macromolecular complexes. A) SDS-PAGE analysis of Lsm2-8 and Lsm1-7 proteins made by the pQLink system (36) in *E. coli* and purified with the use of multiple, cleavable affinity tags. Lsm4 appears as a very close doublet, either due to incomplete denaturation on SDS PAGE or partial proteolysis of its C-terminus which has no visible electron density in the crystal structures reported here and is predicted to be a region of low complexity. All masses for all Lsm proteins were confirmed by mass spectrometry and no proteolytic fragments of Lsm4 were detected by mass spectrometry. The Lsm7 subunit in Lsm2-8 (lane 1) retains a C-terminal oligohistidine tag that is not present in the final Lsm1-7 samples (lanes 2-4). B) *S. pombe* Lsm2-8 can form recombinant U6 snRNPs after addition of Prp24 and U6 snRNA.

Supplementary Figure 2. Structural details of *S. pombe* Lsm2-8 with RNA. A) Overview of unprocessed pentauridylate bound to Lsm2-8. Only the bridging phosphodiester between the last and penultimate uridine nucleotides is clearly visible in the electron density maps. B) Denaturing urea PAGE gels showing that prolonged incubation with Lsm2-8 does not result in cleavage and shortening of diol-terminated RNA, and as such the observed terminal phosphate in panel A is not likely to be a product of hydrolysis. C,D) Simulated annealing omit maps for RNA bound to Lsm2-8. Depicted maps are of form mF_o-DF_c, and are contoured at 1 r.m.s.d. E) Crystal packing of Lsm2-8 complexes in space group $P2_12_12$ is bridged by an unidentified buffer component that is putatively a contaminant within the pentaerythritol propoxylate (5/4 PO/OH) precipitant used for crystal growth. The depicted map is identical to that in panels C and D. F) Two Lsm2-8 complexes are present in the crystallographic asymmetric unit, which vary significantly in their local atomic displacement parameters (colored blue to red), especially around the Lsm5 subunits. Representative local $2mF_o$-DF_c maps at 1 r.m.s.d. are shown in the inserts. G) The poor density in one of the Lsm2-8 rings can be attributed to a lack of substantial crystal packing contacts in one copy of Lsm2-8. An asterisk denotes the same regions of poor main chain density in panels F and G.

Supplementary Figure 3. Comparison of how 3' processed U6 RNA is recognized by *S. cerevisiae* and *S. pombe* Lsm2-8. A) *S. pombe* Lsm2-8 binds the 3′ uridine cyclic phosphate in the middle of the ring through non-Sm contacts with Lsm8 and Lsm3. B) The C-terminal tail of Lsm8 in *S. cerevisiae* is elongated and extends past the terminal uridine-phosphate. In lieu of direct interaction with 3′ uridine-phosphate, the tail of Lsm8 in *S. cerevisiae* interacts with the phosphate through long range electrostatics. C,D) Detailed comparisons of how the cyclic and non-cyclic phosphates are coordinated by the *S. pombe* and *S. cerevisiae* Lsm2-8 rings, respectively. Lsm3-Arg27 directly coordinates the cyclic phosphate in *S. pombe*.

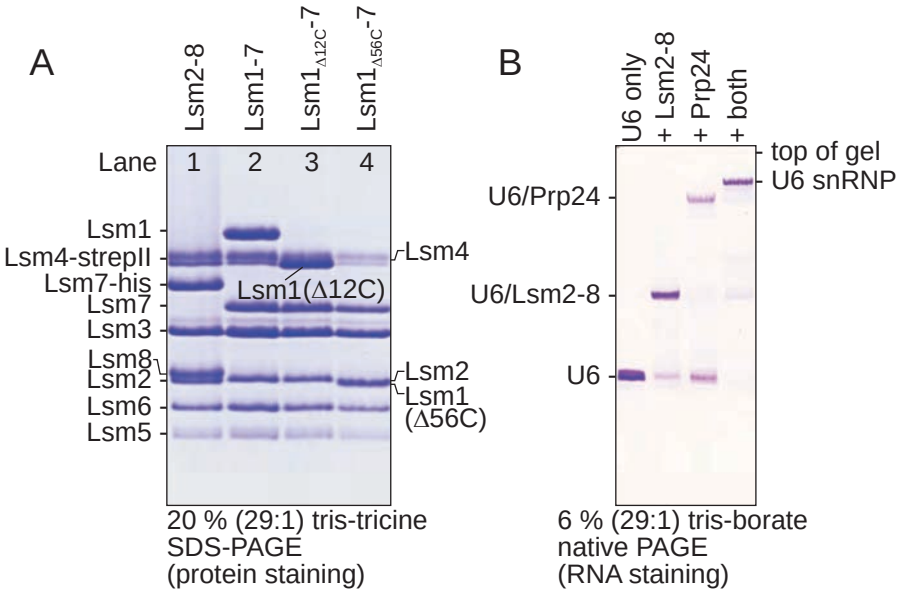
Supplementary Figure 4. Simulated annealing omit maps depicting density for the 3′ purine residues bound by Lsm1-7. A) Cross-eyed stereo electron density map for adenosine terminated RNA. B) Map for guanosine terminated RNA. All maps are calculated and depicted similar to those in Supplementary Figure 2.

Supplementary Figure 5. Surface properties of Lsm rings. A) Electrostatic surface analysis of *S. pombe* Lsm1$_{\Delta 56C}$-7 bound to AUUUUG (PDB 6PPV). RNA is depicted in green for clarity. B) Comparison of the distal RNA entry sites of Lsm rings and the homologous Sm ring. Conserved residues in the Lsm and Sm rings are observed to interact with "threaded" RNA in the human U4 snRNP.

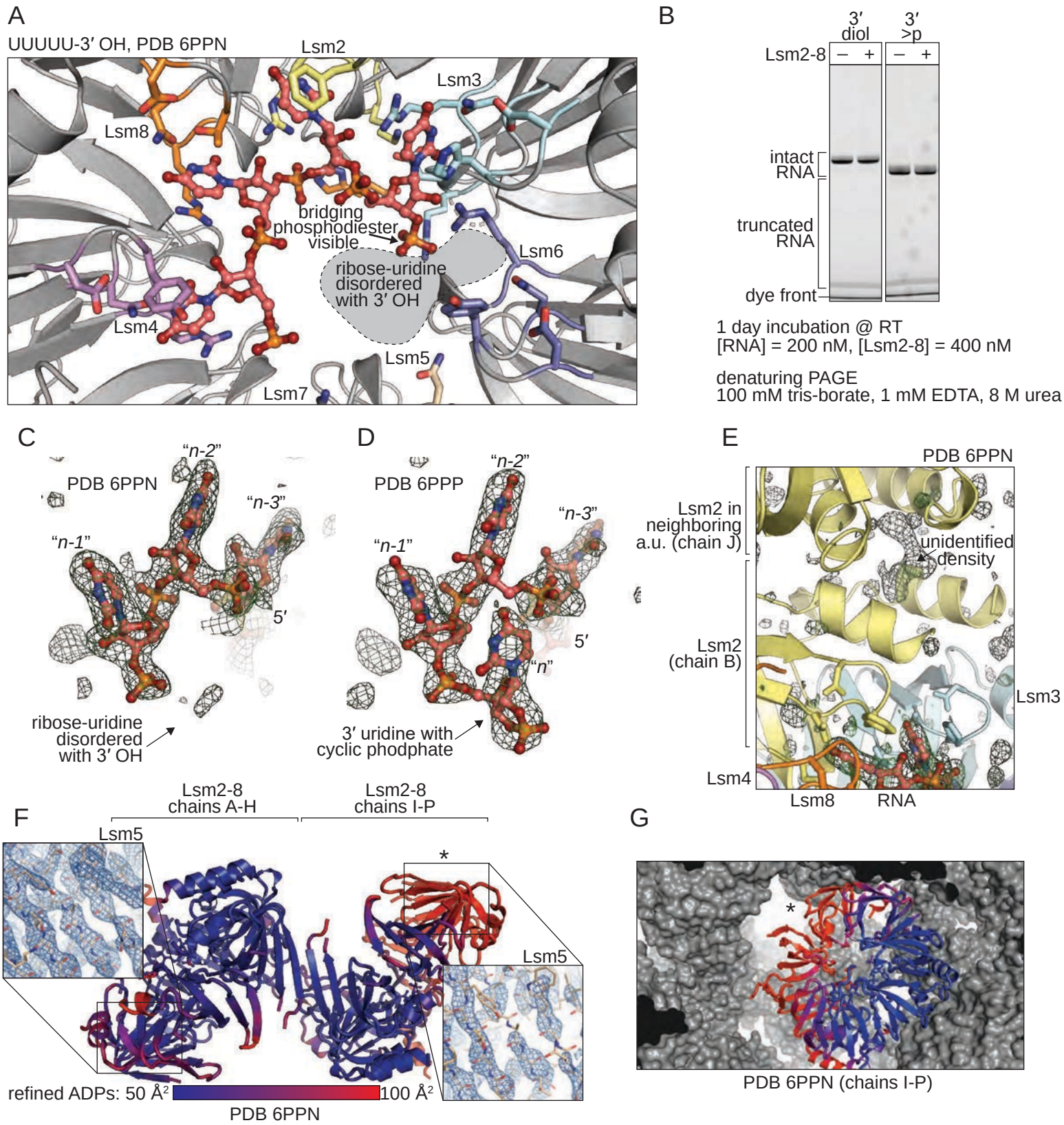
Supplementary Table 1

	RNA (5' FAM)	K_d (nM)
Lsm2-8	ACCCAUUUUU-3p	152 ± 7.4
	ACCCAUUUUU	100 ± 4.3
	ACCCAUUUUU>p	26.0 ± 3.3
	ACCCAUUUUUA	40.8 ± 3.1
Lsm1-7	ACCCAUUUUU>p	329 ± 13
	ACCCAUUUUUA	22.9 ± 1.1
	ACCCAUUUUU	161 ± 10
	ACCCAUUUUC	422 ± 20
	ACCCAUUUUG	173 ± 15
	ACCCAUUUUA	133 ± 11
	ACCCAUUUUAA	378 ± 16
	ACCCAUUUUAAA	879 ± 29
	CCCCCUUUUUA	29.7 ± 2.9
	UUUUUACCCCC	189 ± 14
	UUUUUUUUUUUUUUU	24.7 ± 6.8
	UUUUUUUUUUUUUUA	22.4 ± 5.5
	UUUUUUUUUUUUUAA	15.7 ± 2.8
	AAAAAAAAAAAAAAA	ND
	hairpin-oligoU	70.3 ± 9.1
	oligoU-hairpin	ND
	hairpin-oligoU-hairpin	ND
$Lsm1_{\Delta 56C}$-7	ACCCAUUUUU>p	95.1 ± 10
	ACCCAUUUUU	90.3 ± 5.5
	ACCCAUUUUC	169 ± 11
	ACCCAUUUUG	99.8 ± 8.7
	ACCCAUUUUA	61.8 ± 8.0
	ACCCAUUUUAA	48.4 ± 5.5
	ACCCAUUUUAAA	98.2 ± 16
	CCCCCUUUUUA	13.0 ± 1.3
	UUUUUACCCCC	34.3 ± 4.1
	UUUUUUUUUUUUUUU	8.70 ± 4.4
	UUUUUUUUUUUUUUA	16.0 ± 6.0
	UUUUUUUUUUUUUAA	8.90 ± 3.1
	AAAAAAAAAAAAAAA	ND
	hairpin-oligoU	31.6 ± 2.5
	oligoU-hairpin	ND
	hairpin-oligoU-hairpin	ND
$Lsm1_{\Delta 12C}$-7	ACCCAUUUUU>p	78.0 ± 13
	ACCCAUUUUUA	18.7 ± 2.7
Lsm1-7 (Lsm5-N66A/N68A)	ACCCAUUUUU>p	288 ± 35
	ACCCAUUUUUA	40.6 ± 4.0

Supplementary Figure 1



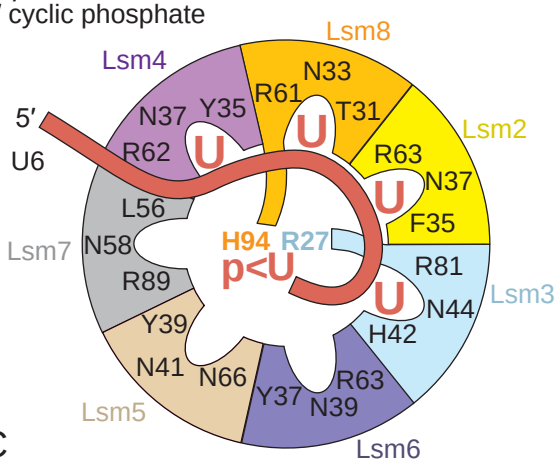
Supplementary Figure 2



Supplementary Figure 3

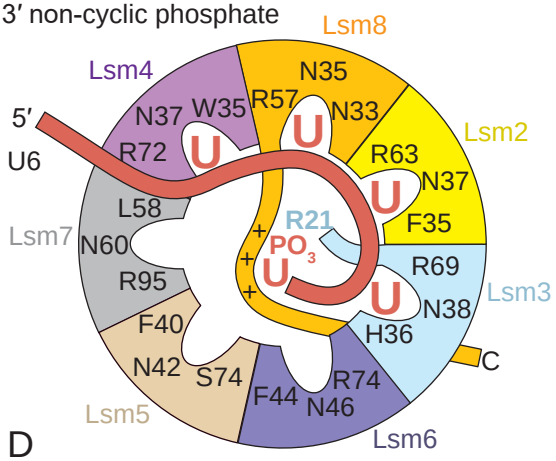
A

S. pombe Lsm2-8 with RNA:
3′ cyclic phosphate

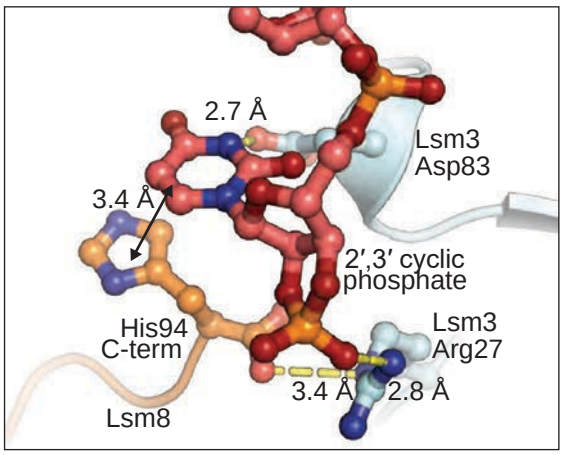


B

S. cerevisiae Lsm2-8 with RNA:
3′ non-cyclic phosphate

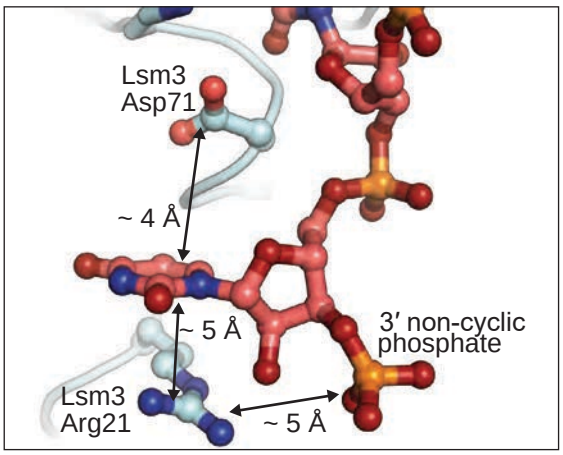


C



PDB 6PPP

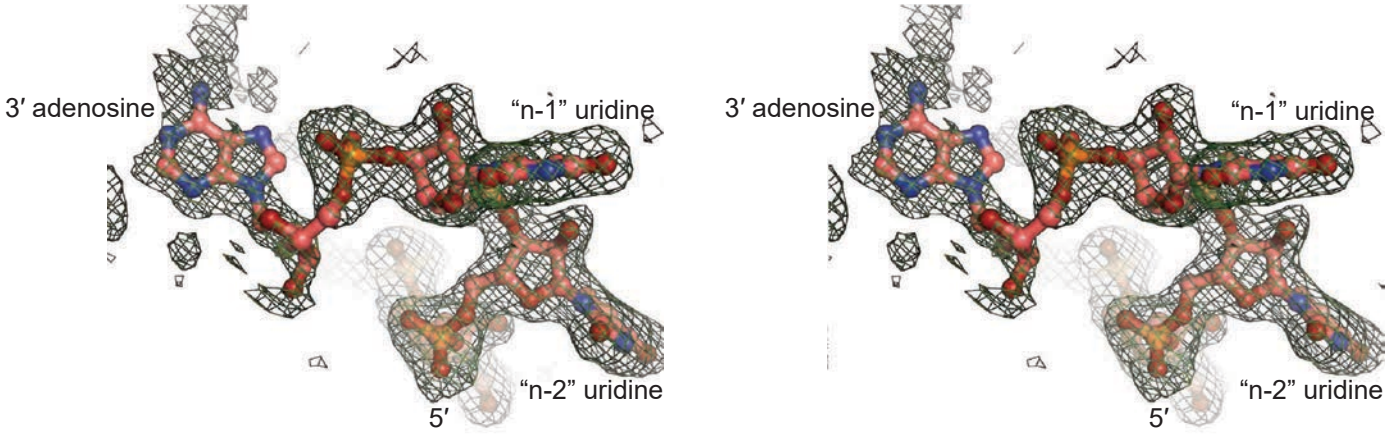
D



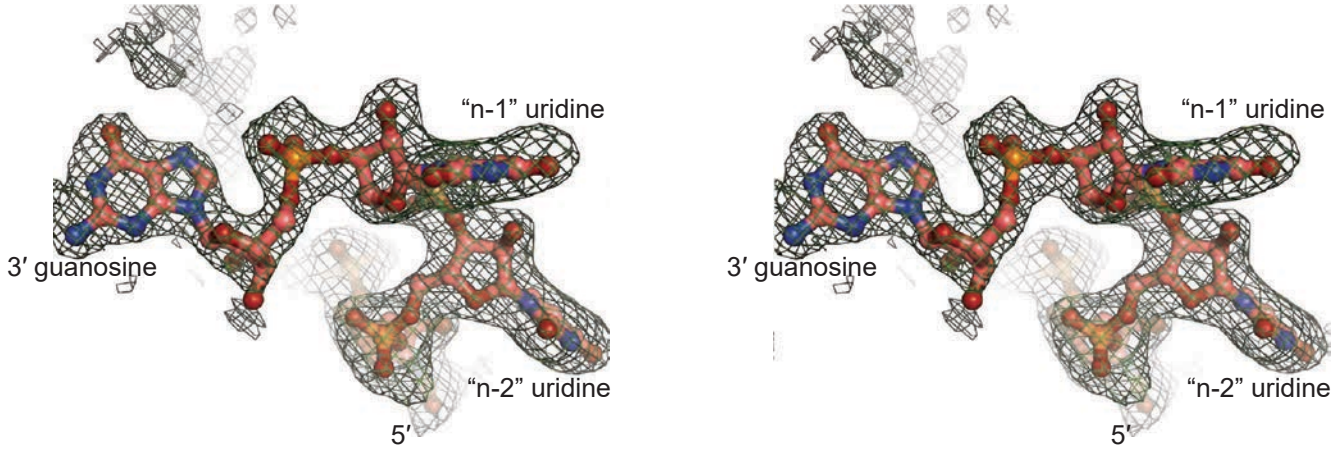
PDB 6ASO

Supplementary Figure 4

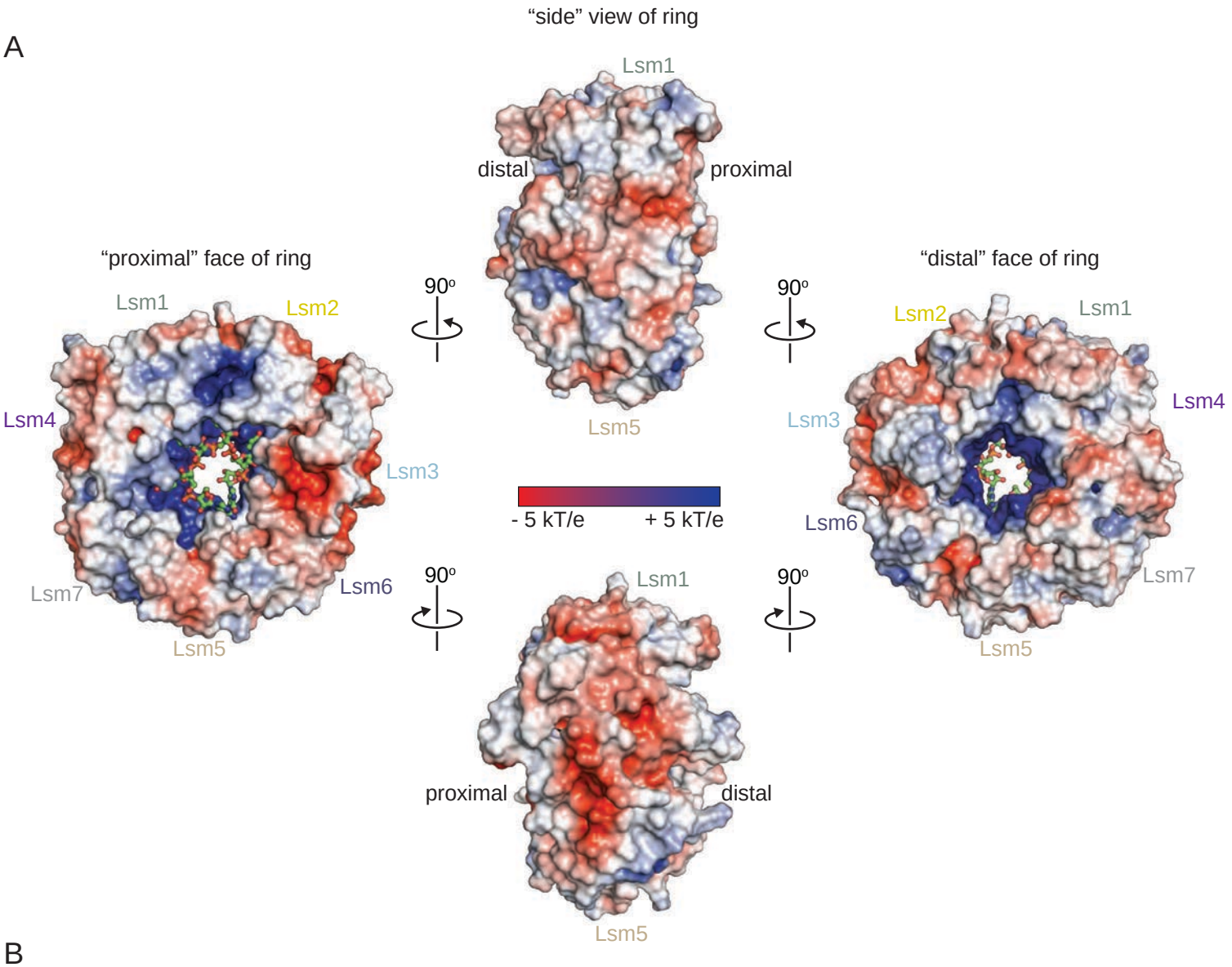
A



B

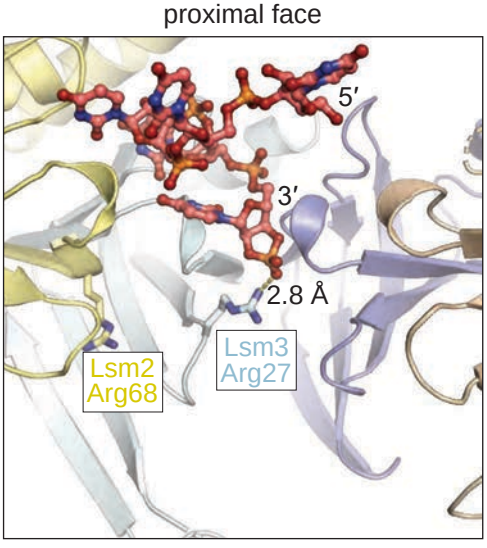


Supplementary Figure 5

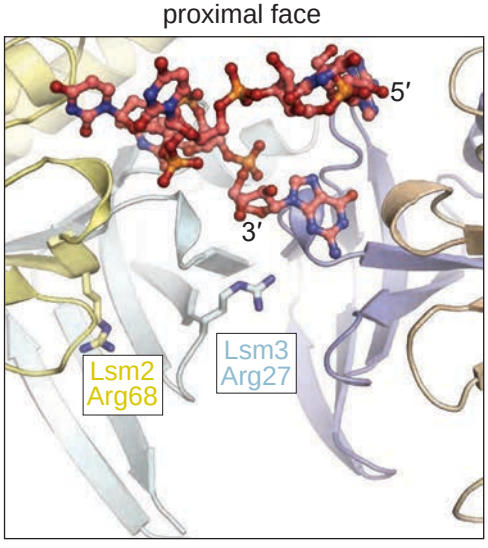


B

S. pombe Lsm2-8 with 3′ cyclic phosphate (PDB 6PPP)



S. pombe Lsm1-7 with 3′ purine (PDB 6PPV)



H. sapiens Sm rings with U4 snRNA (PDB 4WZJ)