Supplementary Table 1. X-ray data collection and refinement statistics

Protein:RNA complex	FBF-2:*gld-1* FBEa13	FBF-2:mutant FBEa13	PUF-6:5BE13
Data collection			
Space group	$P6_1$	$P6_1$	$P2_12_12_1$
Unit cell dimensions a, b, c (Å)	98.5, 98.5, 108.0	99.8, 99.8, 105.6	67.8, 87.8, 129.8
α, β, γ (°)	90.0, 90.0, 120.0	90.0, 90.0, 120.0	90.0, 90.0, 90.0
Resolution (Å)	50-2.30 (2.34-2.30)	50-2.50 (2.59-2.50)	50-2.90 (3.00-2.90)
R_{sym} or R_{merge}	0.094 (0.65)	0.099 (0.68)	0.073 (0.54)
$I / \sigma I$	17.7 (2.3)	26.1 (3.8)	16.6 (2.4)
Completeness (%)	99.9 (100)	99.8 (100)	99.1 (98.5)
Redundancy	5.5 (4.5)	11.2 (11.2)	4.0 (3.9)
Refinement			
Resolution (Å)	24.01-2.30	31.20-2.50	36.47-2.90
No. reflections	26,381	19,320	16,837
R_{work} / R_{free}	0.177/0.227	0.190 / 0.249	0.206 / 0.241
No. atoms			
Protein	3140	3130	2930
RNA	232	214	145
Water	318	89	0
B-factors			
Protein	38.9	44.6	69.20
RNA	43.7	48.2	79.37
Water	44.9	40.4	N/A
R.m.s deviations			
Bond lengths (Å)	0.007	0.008	0.006
Bond angles (°)	0.80	1.10	0.88

*Values in parentheses are for highest-resolution shell.

Supplementary Table 2. RNA-binding analyses of wild-type and mutant FBF-2. Upstream cytosines, when present, are indicated in boldface. N.D. indicates not determined.

RNA	FBF-2 WT K_d (nM)	FBF-2 S554A K_d (nM)
UGUGCCAUA (*gld-1* FBEa9)	27.7 ± 0.4	26.6 ± 0.8
U**C**AUGUGCCAUAC (*gld-1* FBEa13)	3.01 ± 0.09	9.9 ± 0.3
UAA	57.3 ± 2.0	20.9 ± 0.2
UA**C**	3.9 ± 1.2	7.8 ± 0.8
CAA	42.8 ± 3.3	19.5 ± 1.1
AAA	70.1 ± 3.5	20.7 ± 1.9
GAG	64.6 ± 4.4	16.7 ± 1.1
UUA	39.5 ± 3.0	17.7 ± 0.3
U**C**UUGUGUCAUUC (*fem-3* PME13)	3.4 ± 0.5	N.D.
U**C**UUGUAAAAUCU (*fog-1* FBEa13)	5.8 ± 0.4	N.D.
UU**C**UGUGUUAUCU (*gld-1* FBEb13)	4.1 ± 0.4	N.D.

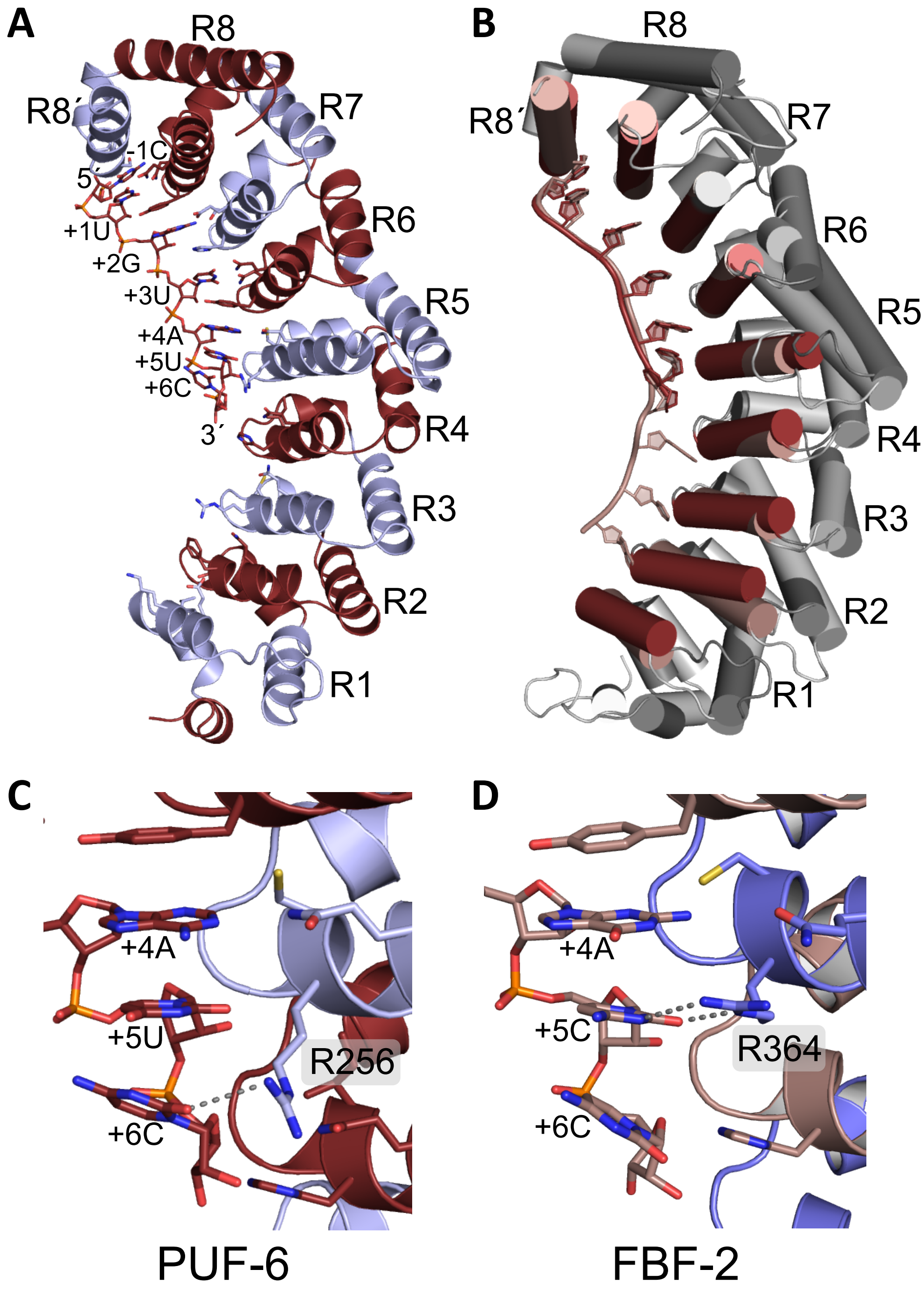
Supplementary Table 3. RNA-binding analyses of wild-type and mutant PUF-6. Upstream cytosines, when present, are indicated in boldface.

RNA	PUF-6 WT K_d (nM)	PUF-6 S441A K_d (nM)
UGUAUCUUGU (5BE10)	73.8 ± 8.5	38.5 ± 2.2
CU**C**UGUAUCUUGU (5BE13)	7.4 ± 1.5	27.3 ± 6.5
CUU	27.9 ± 4.7	39.5 ± 1.5
C**C**U	23.8 ± 2.7	42.5 ± 3.4

Supplementary Table 4. RNA-binding analyses of wild-type and mutant PUF-11. Upstream cytosines, when present, are indicated in boldface.

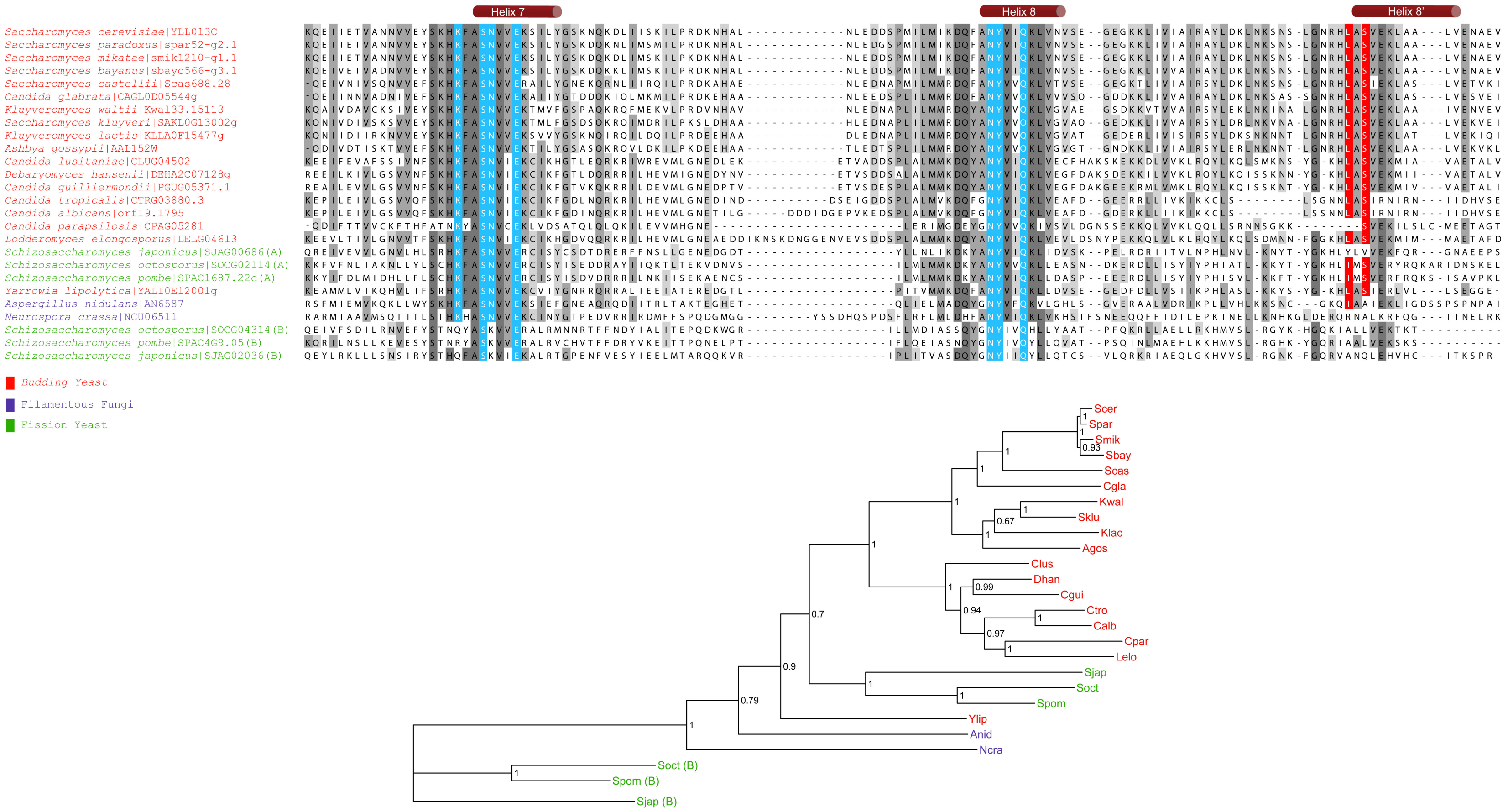
RNA	PUF-11 WT K_d (nM)	PUF-11 S491A K_d (nM)
UA**C**UGUGAAUAGG (11BE I-1)	0.05 ± 0.003	3.2 ± 0.2
UAA	2.9 ± 0.3	2.9 ± 0.3
U**C**A	0.11 ± 0.03	4.9 ± 0.2
CAA	1.3 ± 0.3	5.5 ± 0.6

Supplementary Figure 1



SUPPLEMENTARY FIGURE 1. Crystal structure of *C. elegans* PUF-6 RNA-binding domain. A. Ribbon diagram of PUF-6 in complex with 5BE RNA. B. Superposition of PUF-6 (maroon) and FBF-2 (mauve) RNA-binding domains. RNA-interacting helices are colored as indicated. C. Interaction of PUF-6 with bases +4 to +6. D. Interaction of FBF-2 with bases +4 to +6 of FBEa RNA. In panels C and D, dashed lines indicate hydrogen bond interactions with R256 and R364, respectively.

Supplementary Figure 2



SUPPLEMENTARY FIGURE 2. Alignment of Puf3p homologue amino acid sequences. Top, Shown in the figure are the regions from R7 to R8. Dark and light gray denote 80% identical and 50% similar amino acid residues, respectively; dark blue, residues that make edge-on contacts with the RNA; light blue, residues that form stacking interactions with the RNA bases; red, leucine and serine residues predicted to form the upstream C-binding pocket. Species and gene designations are given to the left. Bottom, a gene tree derived from the full-length sequences of the Puf3p proteins. Numbers indicate posterior clade possibilities. MUSCLE was used for the alignments (Edgar, 2004) and MrBayes to define the tree (Clamp, et al., 2004). Display is based on Ronquist, et al. 2003.

Edgar, RC., MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Research* **32**, 1792-1797 (2004).
Clamp M, Cuff J, Searle SM, Barton GJ. The Jalview Java alignment editor. *Bioinformatics* **20**, 426-427 (2004).
Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574 (2003).