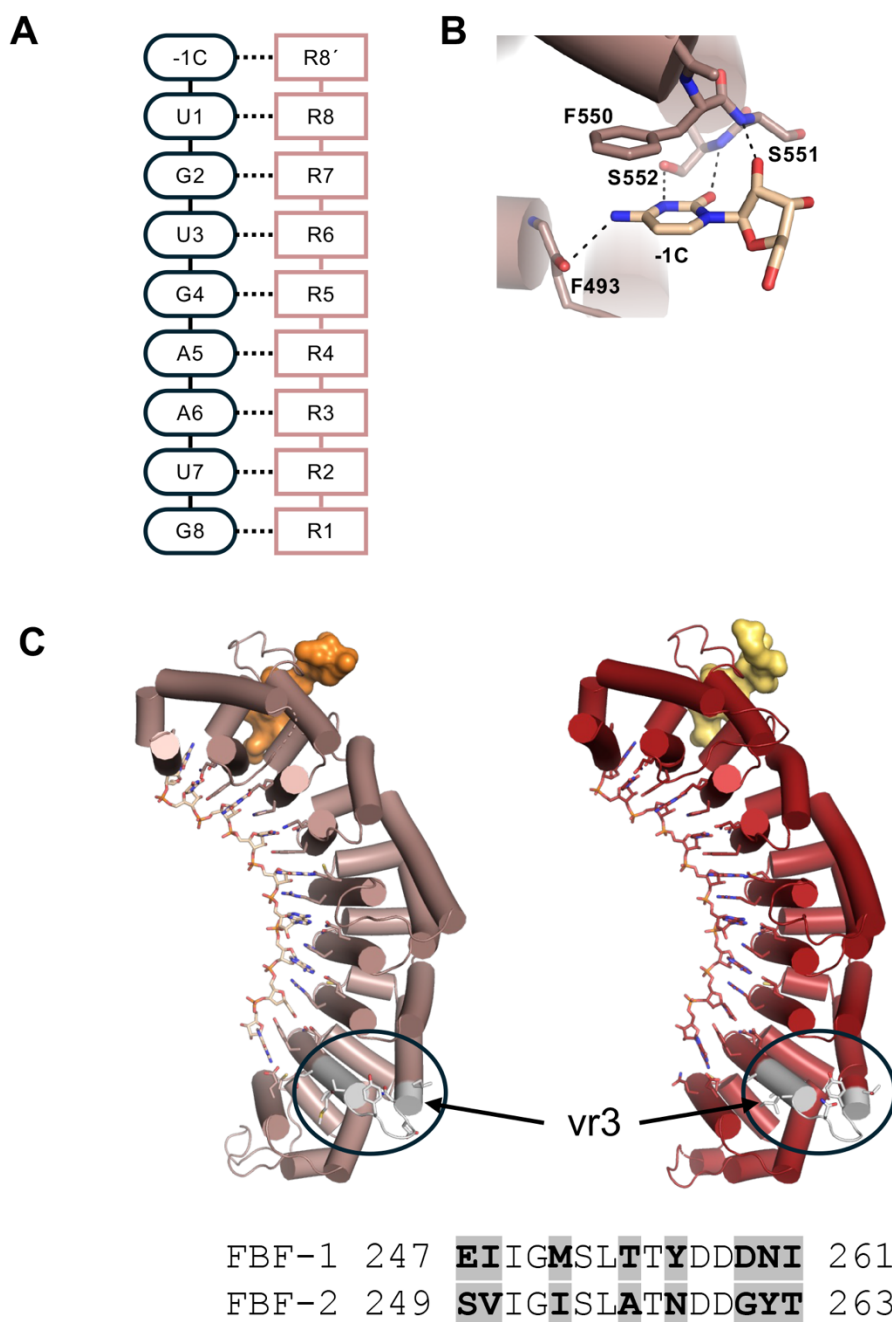


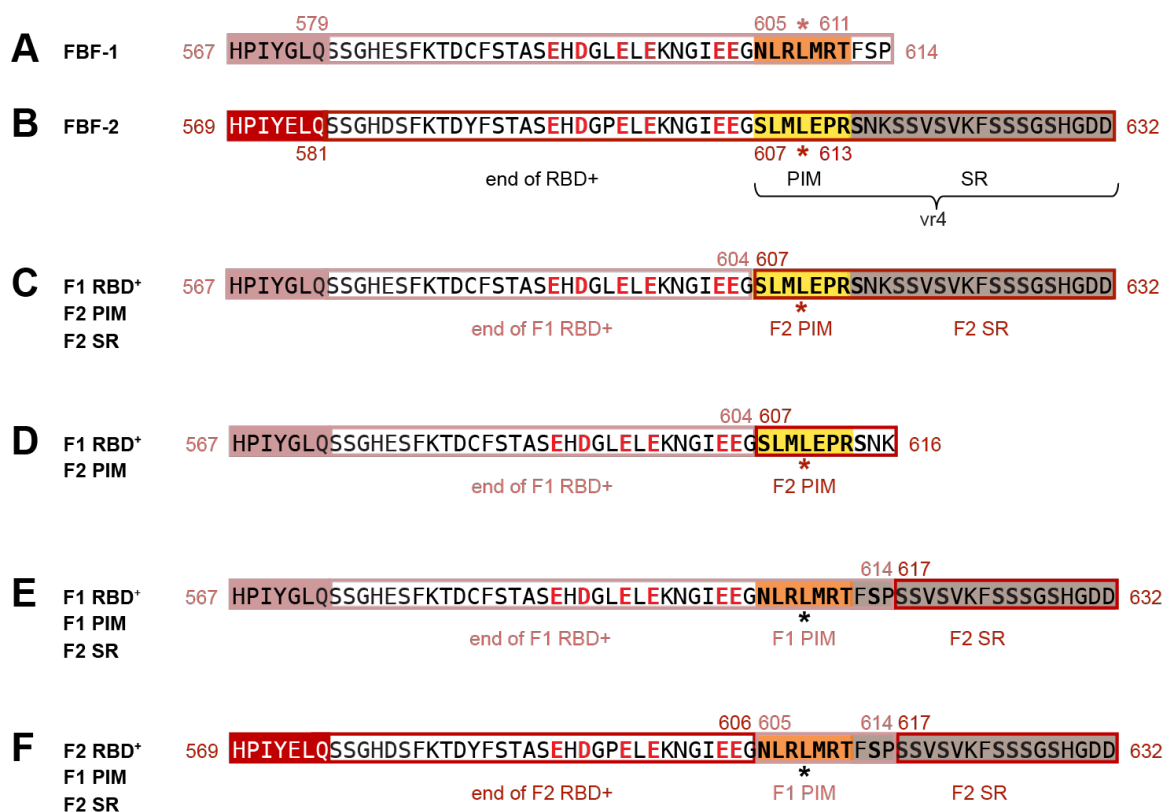
Supplemental Material

Supplemental Table S1. ITC was used to measure protein interaction affinities, stoichiometries, and other thermodynamic parameters between LST-1 B and FBF-1 proteins.

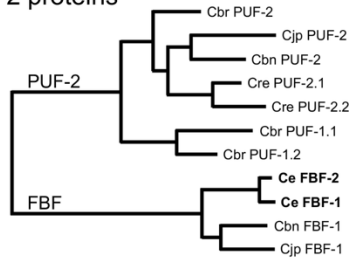
Line	FBF-1	Peptide	K_d (μ M)	N (sites)	ΔH (kJ/mol)	ΔG (kJ/mol)	$-T\Delta S$ (kJ/mol)
1	RBD	LST-1 B	0.21 ± 0.03	0.94 ± 0.01	-64.0 ± 1.1	-37.5	26.5
2			0.18 ± 0.08	0.90 ± 0.02	-60.2 ± 2.8	-37.8	22.3
3	RBD+CT	LST-1 B	0.41 ± 0.14	0.87 ± 0.02	-53.6 ± 2.5	-35.9	17.7
4			0.41 ± 0.10	0.88 ± 0.02	-57.1 ± 2.2	-35.9	21.2



SUPPLEMENTAL FIGURE S1. Crystal structure of FBF-1 RBD+CT reveals RNA recognition features. (A) Schematic representation of interaction between *C. elegans* FBF-1 repeats R1-R8' (pink) and cFBE RNA nucleotides -1C to G8 (black). (B) Recognition of upstream -1C in cFBE RNA by FBF-1. Dashed lines indicate hydrogen bonds. (C) Location of variable region 3 (vr3, grey) in *C. elegans* FBF-1 (left) and FBF-2 (right). The vr3 amino acid sequences are below and variable residues are highlighted grey.



SUPPLEMENTAL FIGURE S2. Amino acid sequences of the C-terminal regions of (A) FBF-1, (B) FBF-2, and (C-F) chimeric proteins used in these studies.

A *Caenorhabditis* FBF and PUF-2 proteins**B**

PUF protein	PIM binding residues	CT PIM	Acidic cluster	Serine rich
Ce FBF-1	yes	yes	yes	no
Ce FBF-2	yes	yes	yes	yes
Cbn FBF-1	yes	likely	yes	yes
Cjp FBF-1	yes	likely	yes	no
Cbr PUF-1.1	yes	no	no	yes
Cbr PUF-1.2	yes	no	no	yes
Cbn PUF-2	yes	no	no	yes
Cjp PUF-2	yes	no ¹	no ¹	no ¹
Cre PUF-2.1	yes	no	no	yes
Cre PUF-2.2	yes	no	no	yes
Cbr PUF-2	yes	no	no	yes

¹The protein sequence for Cjp PUF-2 appears to be truncated after its repeat 8 and therefore may yet possess a CT PIM, acidic cluster, and/or serine-rich region.

C C-terminal tail

		RBD
Ce FBF-1	569	IYGLQSSGHESEFKTDCFSTASEHDGLELEKNGIEEENLRLMRTFSP-----
Ce FBF-2	571	IYELQSSGHDSEFKTDYFSTASEHDGPELEKNGIEEESIMLEPRSNKSSVSVKFSSSSGSHGDD-----
Cbn FBF-1	575	YLGFQQTGNPAFSEFDFASASEDE--DYDKNVIEEENLMLQARSEKSTGSIKISSSDSHVHDGDEAEVFSS
Cjp FBF-1	585	FFGLHPTGLSEFQFECFSTASEDE--DAEKSEIEEERMLLAPRG-----SPGSHAHGDDDEAGTYS
Cbr PUF-1.1	509	FPYGD-----SPS-----ASTNGDRISFGSYEFDFKDSNFPVL
Cbr PUF-1.2	532	EMPPY-----DDSSPSTRQSSRRSSISSEPHFEFDVNNSNYHVF
Cbn PUF-2	546	FTPIPTYDCAPFSPLPQDVLSLCP-----SALFSPSNTTS-IDWPSRTNSFSSEQHVVDYEFNFNNTKF
Cjp PUF-2	-	-----
Cre PUF-2.1	529	FNPGYDRLSPYS--PTHDLVLAALCP-----SSFFSPPGTSS-VDWPSRTTSLSSEHPIDGFEYTFPNNYHQKF
Cre PUF-2.2	533	-----YDRRASPY--PTHDLVLSALCP-----SSFFSPPGTSS-VDWPSRASVTSEHPNEY-----
Cbr PUF-2	512	YVPTATSFDLHSPFPQVDVLAALFCP-----SSLFSPSATTSTNDWTSRTTSSISSEPHFDYDFNFNNTKF

D PIM binding residues

Ce FBF-1	431	CIIEKCLMRNLLSLSQEKFASHVVEKAFLHAPMELLAEMMDEIFDGYMBEP-----GTGKDALDLMFHQFGNYVVQ
Ce FBF-2	433	CIIEKCLMRNLLSLSQEKFASHVVEKAFLHAPLELLAEMMDEIFDGYIIEP-----DTGKDALDLMFHQFGNYVVQ
Cbn FBF-1	435	CIIEKCLMRNLLSLSQEKFASHVVEKAFLHAPLDLLAEMMDEIFDGYVIEP-----DTGKDALDLMFHQFGNYVVQ
Cjp FBF-1	445	VIIEKCLMRNLLSLSQEKFASHVVEKAFLKAPLDLLAEMMDEIFDGYVIEP-----DTGKDALDLMFHQFGNYVVQ
Cbr PUF-1.1	372	TIIECTLLRNLLSMSQEKYASHVIERAFVYAPLSLIAEMMDEIFDGYIEP-----ETGKDALDILFHQFGNYVVQ
Cbr PUF-1.2	394	TIIEQCLLRNLLSMSQEKYASHVIERAFVYAPLSLMAEMMDEIFDGYVIEP-----DTGKDALDILFHQFGNYVVQ
Cbn PUF-2	408	AIIECTLLRNLLSMSQEKYASHVVERAFCFAPPAHVAEMMEEIFDGYVIEP-----DTGKDALDILFHQFGNYVVQ
Cjp PUF-2	395	AIIECTLLRNLLSMSQEKYASHVVERAFVYAPLAHIAEMMEEIFDGYVIEP-----DTGKDALDILFHQFGNYVVQ
Cre PUF-2.1	399	AIIECTLLRSLLSMSQEKYASHVIERAFVYAPLPHIAEMMEEIFDGYVIEP-----ETGKDALDILFHQFGNYVVQ
Cre PUF-2.2	387	LVIECTLLRNLLSMSQEKYASHVIERAFELAPLPYIAEMMEEIFDGYVIEPYGFRIDFQETGKDALDILMFHFGNYVVQ
Cbr PUF-2	377	AIIECTLLRNLLSMSQEKYASHVIERAFVYAPLSHIIEMMEEIFDGYVIEP-----ETGKDALDILMFHFGNYVVQ

SUPPLEMENTAL FIGURE S3. Conservation of FBF and PUF-2 proteins in *Caenorhabditis*. (A)

Phylogram of amino acid sequences of FBF and PUF-2 proteins from *C. elegans* (Ce), *C. brenneri* (Cbr),

C. japonica (Cjp), *C. briggsae* (Cbr), and *C. remanei* (Cre). Phylogram and sequence alignments were generated with CLUSTALW (<https://www.genome.jp/tools-bin/clustalw>). (B) Table summarizing conservation of PUF protein features. (C) Amino acid sequence alignment of the C-terminal tails of FBF and PUF-2 proteins. The end of the RBD is indicated with a grey box, acidic residues are red with a box around an acidic cluster, CT PIMs are yellow, and serine residues are blue. (D) Amino acid sequence alignment of the regions of FBF and PUF-2 proteins containing PIM binding residues. Residues that form a hydrophobic pocket around the critical leucine residues in PIMs are highlighted red with white letters. Residues that contact PIMs in FBF-1, FBF-2, LST-1 PIM-A and PIM-B (light red), both LST-1 PIM-A and PIM-B (cyan), or specific to LST-1 PIM-A (blue) are also highlighted.