ONLINE METHODS

Strains, plasmids and antibodies are detailed in supplementary information. Also contained in supplementary information are protocols for Northern blotting, electrophoretic mobility shift assays and mass spectrometry.

Protein Purification: All GST protein purifications were performed in cold lysis buffer A (50mM Tris-HCl, pH 7.5, 250mM NH_4SO_4, 250mM NaCl, 1mM EDTA, 5mM DTT and 1mM PMSF supplemented with Roche Complete Protease Inhibitor Cocktail). Cells were lysed, and cleared lysate was incubated with glutathione-Sepharose (GE Healthcare). Protein was eluted in lysis buffer A with 10mM reduced glutathione. When necessary, GST tags were removed with 1U Prescission Protease (GE Healthcare).

6xHis-CSR-1 was purified as above with minor modifications. Rather than lysis buffer A, lysis buffer B (50mM NaH_2PO_4, pH 7.4, 10% glycerol, 250mM NH_4SO_4, 250mM NaCl, 1mM DTT and 1mM PMSF supplemented with Roche Complete Protease Inhibitor Cocktail) was used. Samples were selected with Ni^{2+}–NTA Agarose (Qiagen) and eluted in lysis buffer B with 250mM imidazole.

Immunoprecipitation and Transient Transfection: Lysates from transgenic lines expressing GFP::FBF-1 and GFP::tubulin were prepared as above. Cleared lysates were subjected to anti-GFP immunoprecipitation with or without RNase A. Co-immunoprecipitating proteins were analyzed by western blotting.

HEK293T cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were then treated with 0.3% formaldehyde and quenched with 0.125M glycine. RIPA buffer was added (50mM Tris-HCl, pH 8.0, 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 2mM EDTA, 0.5μg RNase A). Samples were immunoprecipitated with anti-HA antibody, washed in lysis buffer and analyzed by western blotting.

Immunofluorescence and Fluorescence Microscopy: Adult hermaphrodite gonads were extruded in M9 buffer (with 0.1% Tween-20 and 0.25mM levamisole). Gonads

were washed in PBSTw (PBS with 0.1% Tween-20) and fixed in 3% paraformaldehyde dissolved in 100mM K_2HPO_4 (pH 7.2). Samples were washed in PBSTw, and ice-cold methanol was added. Samples were then blocked for 30 min at room temperature in PBSTw + 0.5% BSA. Primary antibodies (anti-CSR-1 at 1:100, anti-GLD-1 at 1:100 or anti-CYE-1 at 1:300) were added. Samples were washed with PBSTw + 0.5% BSA and incubated with the appropriate secondary antibodies (1:500 dilution) with DAPI. Samples were washed in PBSTw + 0.5% BSA and mounted with Vectashield (Vector Laboratories). Visualization was on a Zeiss LSM 510 Meta confocal microscope. GFP::H2B was visualized as above, but without antibody treatments. DAPI staining was performed. Samples were examined on an Axio Imager D.1.

RNAi: To perform RNA interference, L4 hermaphrodite feeding was performed. Briefly, the *unc-119(ed3); axIs1723[pie-1* prom:GFP::H2B:*gld-1* 3'UTR*]* and *unc-119(ed3); axIs1775[pie-1* prom:GFP::H2B:*gld-1* mutant 3'UTR*]* strains were picked to RNAi plates. Control plates contained the *E. coli* strain HT115. Only sterile animals (since *csr-1, drh-3, ego-1* and *ekl-1* all result in sterility) were stained with DAPI and scored.

Evolutionary Trace and Sequence Alignment: Conserved sites were identified using evolutionary trace analysis[49]. Protein sequences were aligned to human Pum1 with ClustalW. Heatmaps depicting normalized conservation Z-scores were generated using Matlab. Conserved residues were visualized based on the structure of human Pum1 (ref. 50).

Protein binding assays: Either 500ng each of GST-FBF-1, GST-FBF-1(F344R), pETDuet-expressed GST-FBF-1/CSR-1 complex or GST-FBF-1(F344R)/CSR-1 complex were added to 500ng purified EFT-3 in 20μL of PBSTw supplemented with 1mM DTT. Reactions were incubated with or without 0.5mM GTP. Reactions were then added to glutathione-Sepharose, incubated and washed. Equivalent amounts of input proteins and pellets were analyzed. GST-PUM2 binding assays were performed by adding 50ng of purified, recombinant protein to 20μL of reticulocyte lysate. Reactions were incubated with RNase A (10μg ml^{-1}), diluted with PBSTw, and added to glutathione-Sepharose.

Samples were washed with PBSTw, and bound proteins were western blotted as indicated.

GTPase Assay: 50ng each of EFT-3, EFT-3(H95L), GST-FBF-1/EFT-3, GST-FBF-1/CSR-1/EFT-3, or GST-FBF-1(F344R)/CSR-1 with EFT-3 were incubated with 5μCi [^{32}P]-γ-GTP/50fmol GTP. Reactions were performed in GTPase buffer (50mM MES/KOH, pH 7.5, 150mM NH_4Cl, 50mM KCl and 20mM $MgCl_2$). Three 5μL aliquots were quantitated. To these aliquots were added 40μL of 1M perchloric acid, 20μL of 0.25M imidazole (pH 5 with HCl) and 100μL of 1.5% $NaMoO_4$. Phosphomolybdic acid was extracted in butyl acetate and scintillation-counted.

***In vitro* Translation:** *In vitro* transcription reactions were performed with T7 RNA polymerase with mRNA cap analog, m^{7}G(5′)ppp(5′)G (New England Biolabs). For ribosomal footprinting analysis, *in vitro* transcriptions were performed with [^{32}P]-α-UTP.

250ng GST, GST-PUM2, GST-PUM2(ABD), GST-PUM2(EBD) and GST-PUM2(RBD) were added to 20μL nuclease-treated, rabbit reticulocyte lysate (Promega). 1μg nonadenylated or 1μg polyadenylated firefly luciferase 3xPBEs mRNA was added, as was an amino acid mixture (Promega) and 100ng control *Renilla* luciferase mRNA. After reaction, three 5μL aliquots were quantitated using the Dual Luciferase Assay System (Promega). Samples were averaged, and standard deviations are from three biological replicates.

Polyribosome Analysis: *In vitro* translation reactions were performed as above, and reactions were quenched with either cycloheximide or puromycin. Reactions were then separated over a 10-50% linear sucrose gradient. Fractions were collected in 500μL aliquots and used for Northern blotting.

Pulse-chase Analysis: *In vitro* translation reactions were performed as above with modification. Reactions were pre-incubated, and then m^{7}G(5′)ppp(5′)G (New England Biolabs) was added to 2mM. Immediately, aliquots were removed over the time course.

Ribosome Footprinting: *in vitro* translation reactions were prepared as above; however, only radiolabeled firefly luciferase mRNA was included. Reactions were quenched with cycloheximide. Samples were then treated with RNase One (Ambion). 2.5μL were removed (Input RNA) before the remaining samples were separated over a sucrose gradient. RNA was extracted from monoribosome fractions and hybridized to a DNA oligonucleotide array. Samples were compared to input RNA. Hybridization was overnight at 45°C with a wash in 2x and 0.5x SSC for 15 min at room temperature. Error bars indicate standard deviations between three biological replicates.

49. Rajagopalan, L., Pereira, F.A., Lichtarge, O. & Brownell, W.E. Identification of functionally important residues/domains in membrane proteins using an evolutionary approach coupled with systematic mutational analysis. *Methods Mol. Biol.* **493**, 287-97 (2009).
50. Gupta, Y.K., Nair, D.T., Wharton, R.P. & Aggarwal, A.K. Structures of human Pumilio with noncognate RNAs reveal molecular mechanisms for binding promiscuity. *Structure* **16**, 549-57 (2008).

Full methods and any associated references are available in the online version of the paper at http://www.nature.com/nsmb/.