Supplementary Information

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Supplementary Figures:

Supplementary Figure 1

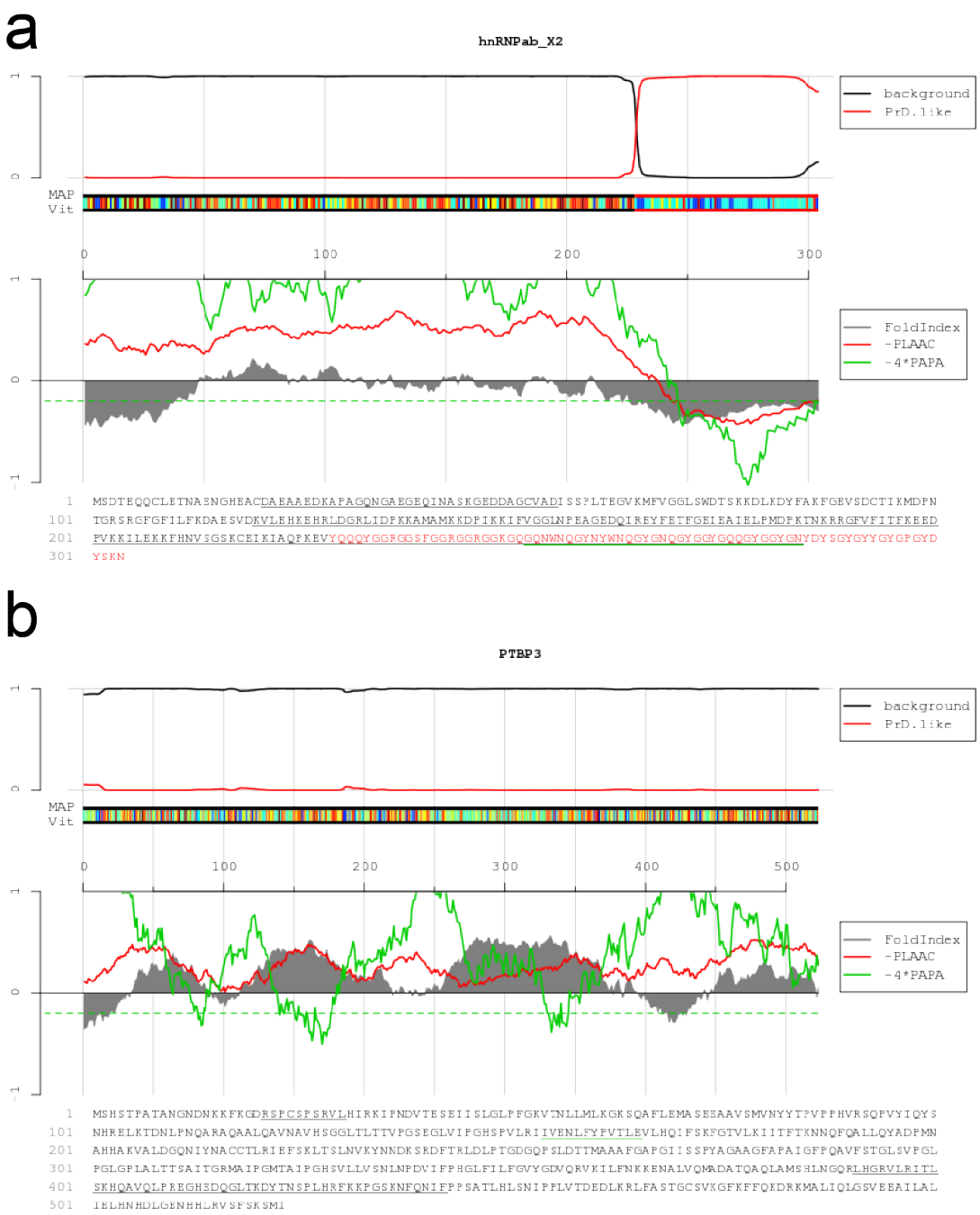


Figure S1: PLAAC Analysis of proteins. The protein sequences of **(a)** hnRNPAB X2 and **(b)** PTBP3 were analyzed using Prion-Like Amino Acid Composition (PLAAC)[1] to identify prion-

like domains. The program identified a region at the C-terminus of hnRNPAB X2 as having a high degree of prion-like character (highlighted in red). PTBP3 was not found to have significant prion-like character.

Supplementary Figure 2

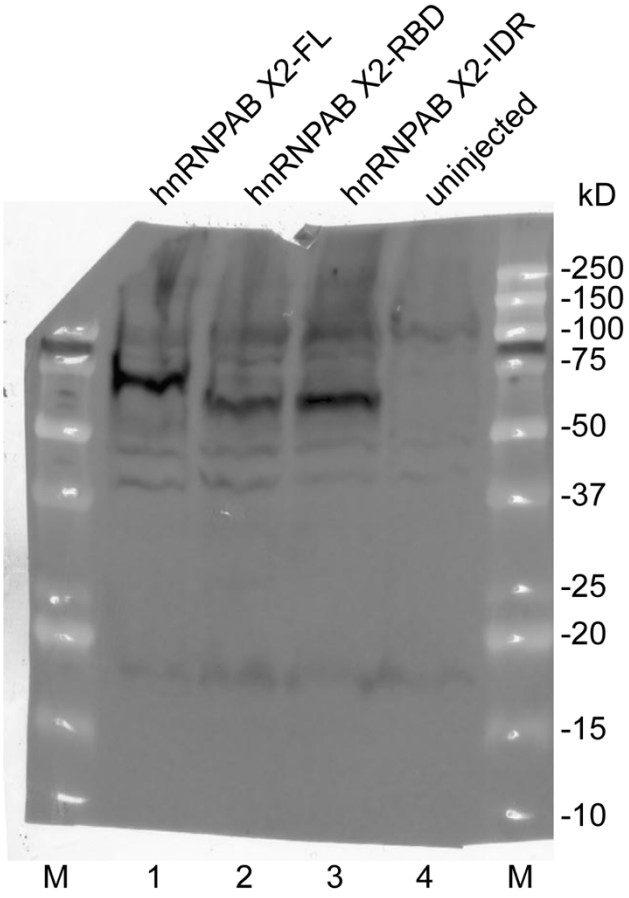
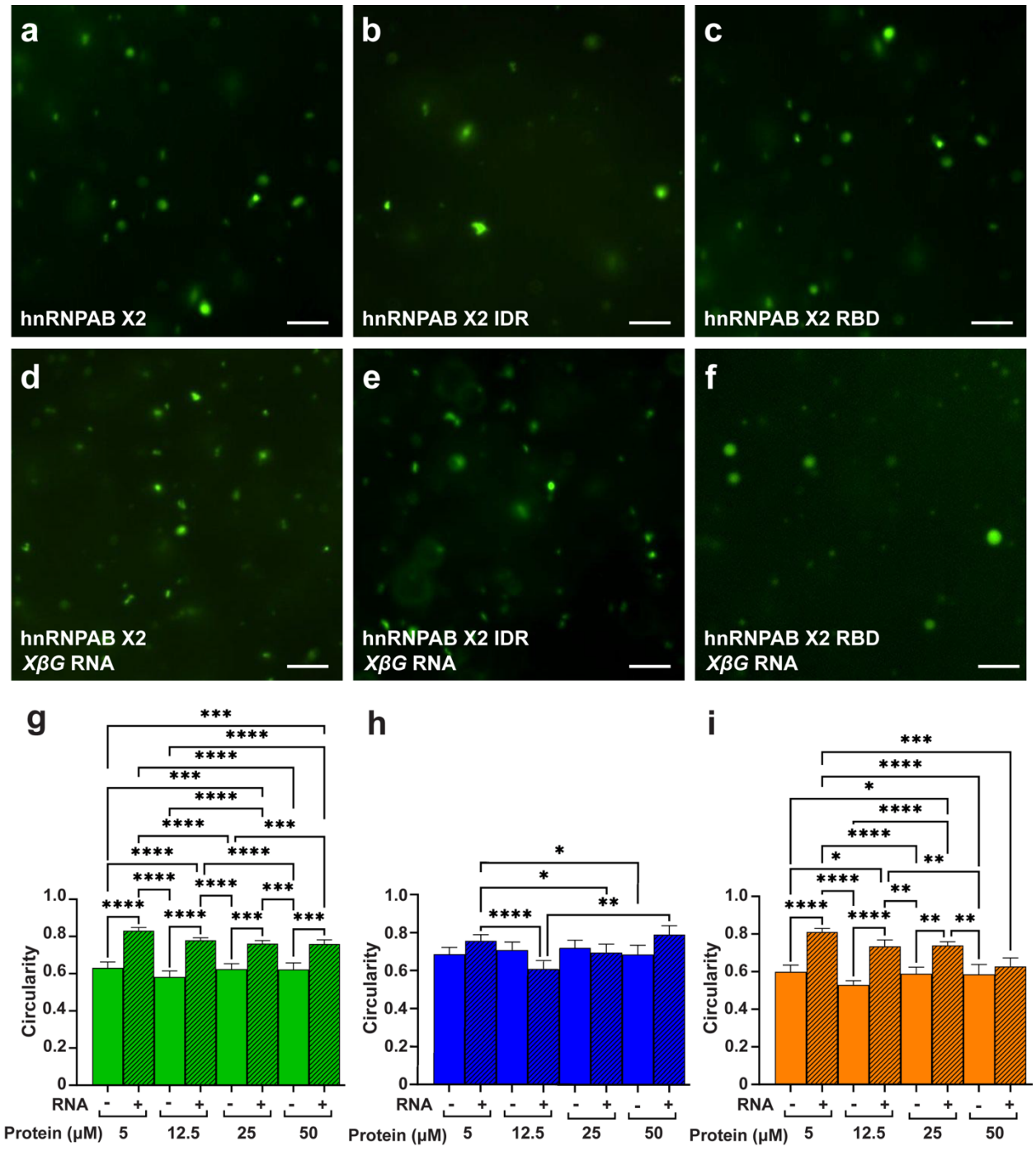


Figure S2: Expression of hnRNPAB X2 domain constructs in oocytes. Lysates were prepared from stage II oocytes injected with RNA transcribed from mCh-hnRNPAB X2 full-length (FL, lane 1), mCh-hnRNPAB X2-RBD (lane 2), mCh-hnRNPAB X2-IDR constructs, and uninjected control oocytes (lane 4). The constructs are diagrammed in Figure 2a. Immunoblot analysis was carried out using anti-RFP to detect the expressed proteins. The sizes (in kD) of molecular weight standards (lanes M) are indicated at the right.

Supplementary Figure 3



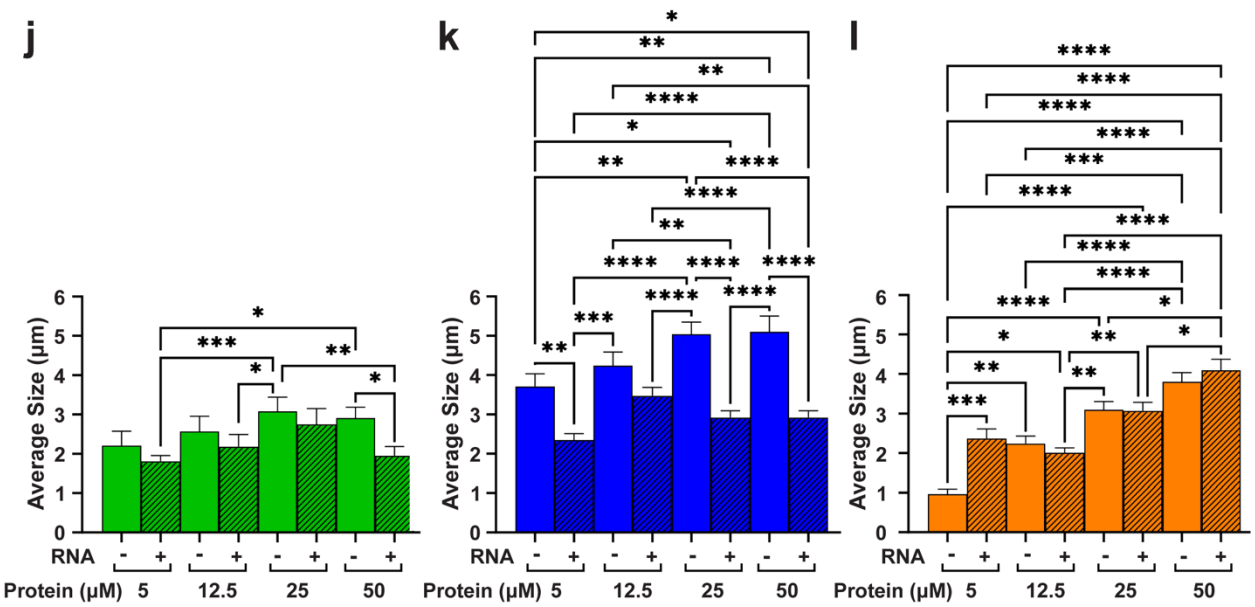


Figure S3: Quantitation of hnRNPAB X2 condensates formed *in vitro*. (a-f) Fluorescent micrographs of (a, d) full-length hnRNPA2 X2, **(**b. e**)** hnRNPAB X2 IDR, and (c, f) hnRNPAB X2 RBD proteins at 12.5 µM in 20 mM NaPi, pH 7.4, 150 mM NaCl, 10% PEG, in the absence (a-c) or the presence (d-f) of 0.25 mg/mL *Xenopus* β-globin (*XβG*) RNA. After cleavage of the MBP tags with TEV protease, phase separation was carried out the presence of 25 µM Thioflavin T for fluorescent labeling. Images are representative from three or more biological replicates (with independently expressed and purified protein) and three or more technical replicates. Scale bars=10 µm. **(g-i)** Circularity of the condensates was analyzed using the ImageJ Analyze Particles plugin on a scale of 0-1.0 arbitrary units, with 1.0 representing a perfect circle, for (g) full-length hnRNPA2 X2 (green), (h) hnRNPAB X2 IDR (blue), and (i) hnRNPAB X2 RBD (orange) at 5, 12.5, 25, and 50 µM protein concentrations in the presence (+RNA, hatched bars) and absence (-RNA, open bars) of 0.25 mg/mL *Xenopus* β-globin RNA. **(j-l)** Average size (in µm) of the condensates was determined using the ImageJ Analyze Particles plugin for (j) full-length hnRNPA2 X2 (green), (k) hnRNPAB X2 IDR (blue), and (l) hnRNPAB X2 RBD (orange) at 5, 12.5, 25, and 50 µM protein concentrations in the presence (+RNA, hatched bars) and absence (-RNA, open bars) of 0.25 mg/mL *Xenopus* β-globin RNA. (g-l) Error bars represent standard error of the mean, **** indicates $p<0.0001$, *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$, and all brackets not shown are not significant ($p>0.05$). Statistics shown are an ordinary two-way ANOVA followed by Tukey's multiple comparisons.

Supplementary Video Legends:

Videos S1: Time-lapse video of hnRNPAB X2 RBD condensates. Condensates were formed at room temperature by incubation of 50 μM hnRNPAB X2 RBD in 20 mM NaPi, pH 7.4, 150 mM NaCl, followed by treatment with 0.03 mg/ml TEV protease. Condensates were imaged on a Nikon Ti2-E Fluorescence microscope, using a 20× objective, with frames collected every 0.6 sec. Shown are videos of hnRNPAB X2 RDB condensates **(a)** fusing and **(b)** wetting the slide, beginning 20 min. after TEV addition; the display rate is 7 frames per sec.

Videos S2: Time-lapse video of hnRNPAB X2 RBD condensates in the presence of RNA. Condensates were formed at room temperature by incubation of 50 μM hnRNPAB X2 RBD with 0.25 mg/mL *Xenopus* β-globin RNA in 20 mM NaPi, pH 7.4, 150 mM NaCl, followed by treatment with 0.03 mg/ml TEV protease. Condensates were imaged on a Nikon Ti2-E Fluorescence microscope, using a 20× objective, with frames collected every 0.6 sec. Shown are videos of hnRNPAB X2 RDB condensates **(a)** fusing and **(b)** wetting the slide, beginning 20 min. after TEV addition; the display rate is 7 frames per sec.

Video S3: Time-lapse video of hnRNPAB X2 condensates. Condensates were formed at room temperature by incubation of 50 μM hnRNPAB X2 in 20 mM NaPi, pH 7.4, 150 mM NaCl, followed by treatment with 0.03 mg/ml TEV protease. Condensates were imaged on a Nikon Ti2-E Fluorescence microscope, using a 20× objective, with frames collected every 0.6 sec. Shown are videos of hnRNPAB X2 condensates, beginning 20 min. after TEV addition; the display rate is 7 frames per sec.

Video S4: Time-lapse video of hnRNPAB X2 condensates in the presence of RNA. Condensates were formed at room temperature by incubation of 50 μM hnRNPAB X2 with 0.25 mg/mL *Xenopus* β-globin RNA in 20 mM NaPi, pH 7.4, 150 mM NaCl, followed by treatment with 0.03 mg/ml TEV protease. Condensates were imaged on a Nikon Ti2-E Fluorescence microscope, using a 20× objective, with frames collected every 0.6 sec. Shown are videos of hnRNPAB X2 condensates, beginning 20 min. after TEV addition; the display rate is 7 frames per sec.

Video S4: Time-lapse video of hnRNPAB X2 IDR condensates. Condensates were formed at room temperature by incubation of 50 μM hnRNPAB X2 IDR in 20 mM NaPi, pH 7.4, 150 mM NaCl, followed by treatment with 0.03 mg/ml TEV protease. Condensates were imaged on a Nikon Ti2-E Fluorescence microscope, using a 20× objective, with frames collected every 0.6 sec. Shown are videos of hnRNPAB X2 IDR condensates, beginning 20 min. after TEV addition; the display rate is 7 frames per sec.

Video S6: Time-lapse video of hnRNPAB IDR condensates in the presence of RNA. Condensates were formed at room temperature by incubation of 50 μM hnRNPAB IDR with 0.25 mg/mL *Xenopus* β-globin RNA in 20 mM NaPi, pH 7.4, 150 mM NaCl, followed by treatment with 0.03 mg/ml TEV protease. Condensates were imaged on a Nikon Ti2-E Fluorescence microscope, using a 20× objective, with frames collected every 0.6 sec. Shown are videos of hnRNPAB X2 IDR condensates, beginning 20 min. after TEV addition; the display rate is 7 frames per sec.

Supplementary Tables:

Supplementary Table 1

Protein Classification	% of L-body proteome	% of L-body IDR-containing proteins
No IDR	53%	n/a
Non-Prion-Like IDR	17%	37%
Prion-Like IDR	29%	63%

Table S1: Survey of L-body proteome for prion-like character. Using the L-body proteome[1] (*n*=86 proteins), L-body protein sequences available in Xenbase[2] were analyzed using PLAAC[1] to identify predicted IDRs and prion-like character. Proteins were rated as either having no IDR, a prion-like IDR, or a non-prion-like IDR, and percentages of the L-body proteome as a whole were generated based on these counts.

Supplementary Table 2

Antibody	Source
Rabbit polyclonal anti-RFP	Abcam (ab62341)
Rabbit polyclonal anti-40LoVe	K. Czaplinski[3]
Goat anti-rabbit AF546	Thermofisher (11010)

Table S2: Antibodies used in this study. The name of the antibody and the antigen are specified on the left, and the source is shown on the right.

Supplementary Table 3

Primer	Sequence
X2AB_FL_Fwd_GibsonCherry	GTACAAGAGATCTGATCATGCCATGGGGCCCATG TCCGACACCGAGCAGC
X2AB_RBD_Fwd_GibsonCherry	GTACAAGAGATCTGATCATGCCATGGGGCCCAAA ATGTTTGTTGGTGGCTTGAGCTG
X2AB_IDR_Fwd_GibsonCherry	GTACAAGAGATCTGATCATGCCATGGGGCCCAAA GAAGTGTATCAGCAACAGTATGGCG

X2AB_FL_Rev_GibsonCherrySpeI	GTTTAGTGGTAACCAGATCCTAGTCAGTCATCAGTTTTTACTGTAGTCATAGCCTGGTCC
X2AB_RBD_Rev_GibsonCherrySpeI	GTTTAGTGGTAACCAGATCCTAGTCAGTCATGGTTGTGCAATCTTTATCTCACACTTGC
hnRNPAB_PYNull_Cherry_Rev	GTTTAGTGGTAACCAGATCCTAGTCAGTCATCACTTGTAGTTATTCTGGTGGCTCCCAC
X2AB_FL_Fwd	TCCGACACCGAGCAGCAGTGTCTAGAA
X2AB_FL_Rev	GTTCTTACTGTAGTCATAGCCTGGTCCATATCCATAATAG
X2AB_RBD_Fwd	AAAATGTTTGTTGGTGGCTTGAGCTGG
X2AB_RBD_Rev	TGGTTGTGCAATCTTTATCTCACACTTG
X2AB_IDR_Fwd	AAAGAAGTGTATCAGCAACAGTATGGCGG
P3_IDR_Fwd	GTACAAGAGATCTGATCATGCCATGGGGCCCATGAGCCATTCCACTCCAGCTACAG
P3_IDR_Rev	CAGAGACAGAGACAGAGACAGAGAGATCATCGATTTGGAGAAAGAGACACG
XBM_Fwd	GAAATTAATACGACTCACTATAGGGAGAGTTGAACTTGTAGCATCCAGCTCAGAATAAACGCTCAACTTTG
XBM_Rev	GGATCCACATGTAGGGTCTCT
Vg1_qPCR_Fwd	GGTATCTCCTCCTCCTGTCCCT
Vg1_qPCR_Rev	TGGGTGGATGTCATCGGAGT
CanAB_qPCR_Fwd	GGCTATTATGGATATGGACCAG
X2AB_qPCR_Fwd	ACAATTACTGGAACCAGGGCT
CanAB_qPCR_Rev	GGCTATTATGGATATGGACCAG
X2AB_qPCR_Rev	GTTTTTACTGTAGTCATAGCCTG

Table S3: Primers used in this study. All Forward (Fwd) and Reverse (Rev) primers are listed from 5′ to 3′. The following abbreviations are used: X2AB (hnRNPAB X2), FL (full-length), P3 (PTBP3), XBM (*Xenopus* β globin), and CanAB (canonical hnRNPAB). XBM_Fwd contains a T7 promoter sequence (underlined).

Supplementary References:

1. Lancaster, A. K., Nutter-Upham, A., Lindquist, S. & King, O. D. PLAAC: a web and command-line application to identify proteins with prion-like amino acid composition. *Bioinformatics* **30**, 2501–2502 (2014).

2. Fisher, M. *et al.* Xenbase: key features and resources of the *Xenopus* model organism knowledgebase. *Genetics* **244**, iyad018 (2023).

3. Czaplinski, K. & Mattaj, I. W. 40LoVe interacts with Vg1RBP/Vera and hnRNP I in binding the Vg1-localization element. *RNA (New York, N.Y.)* **12**, 213–222 (2006).