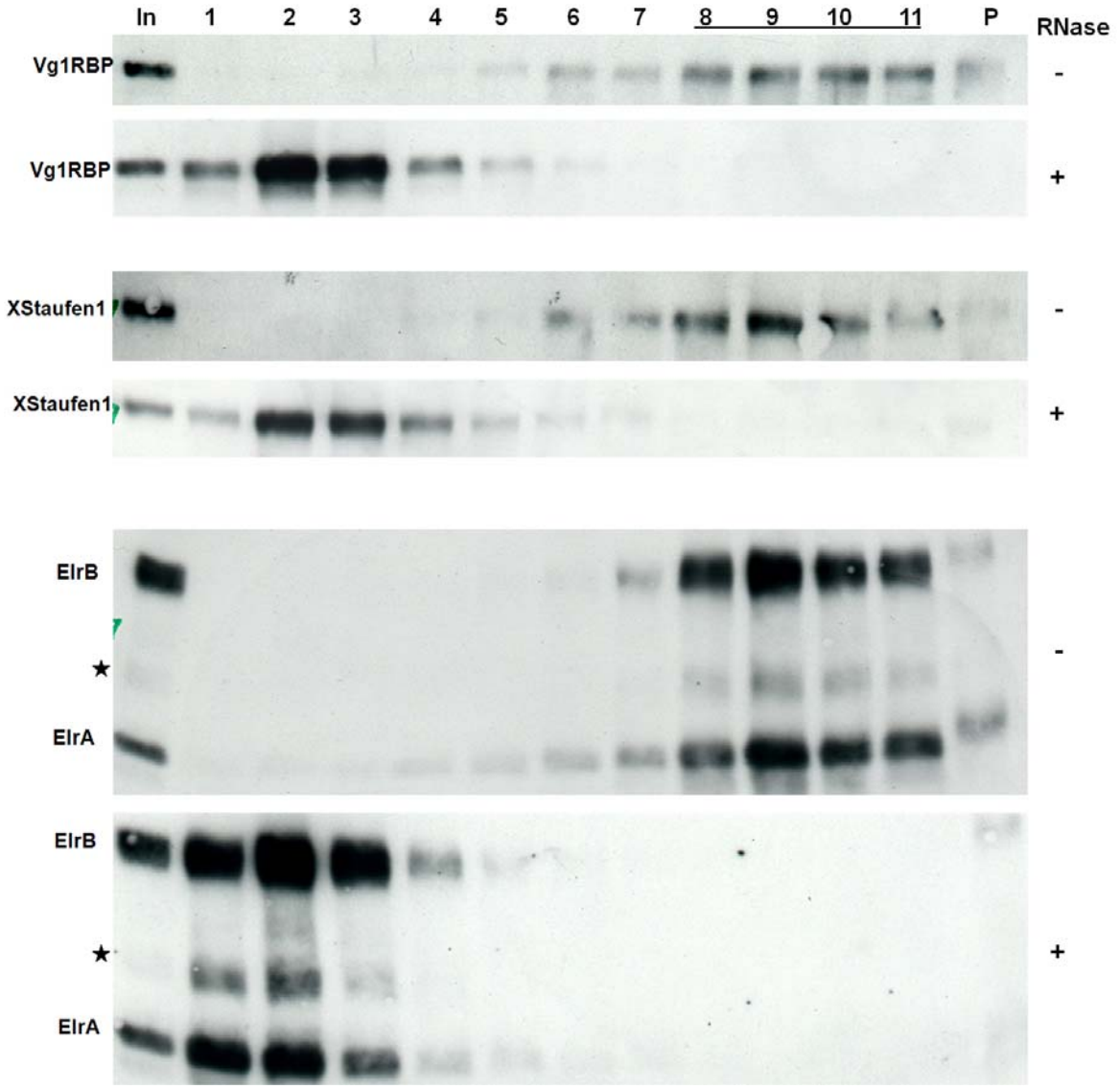
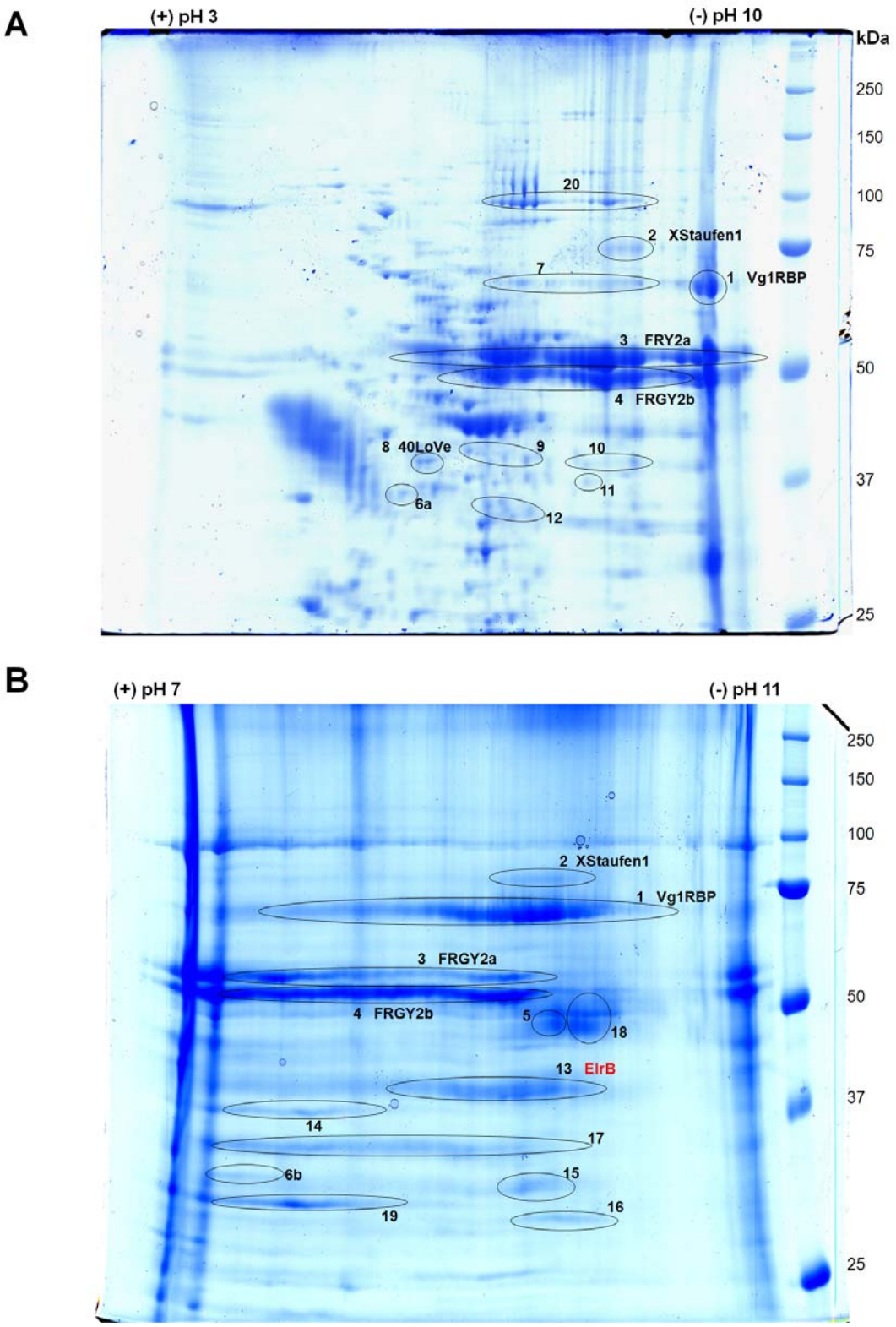
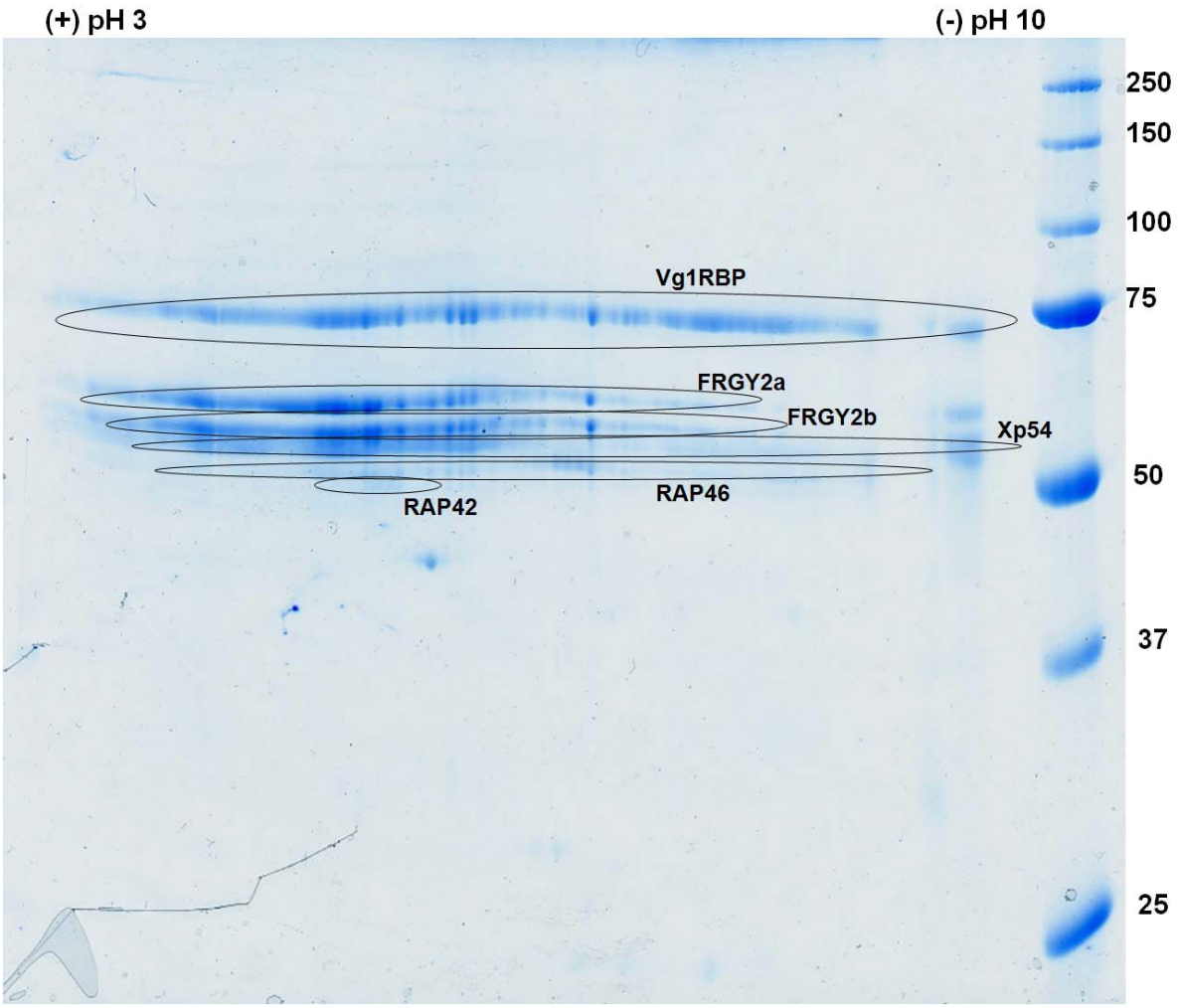


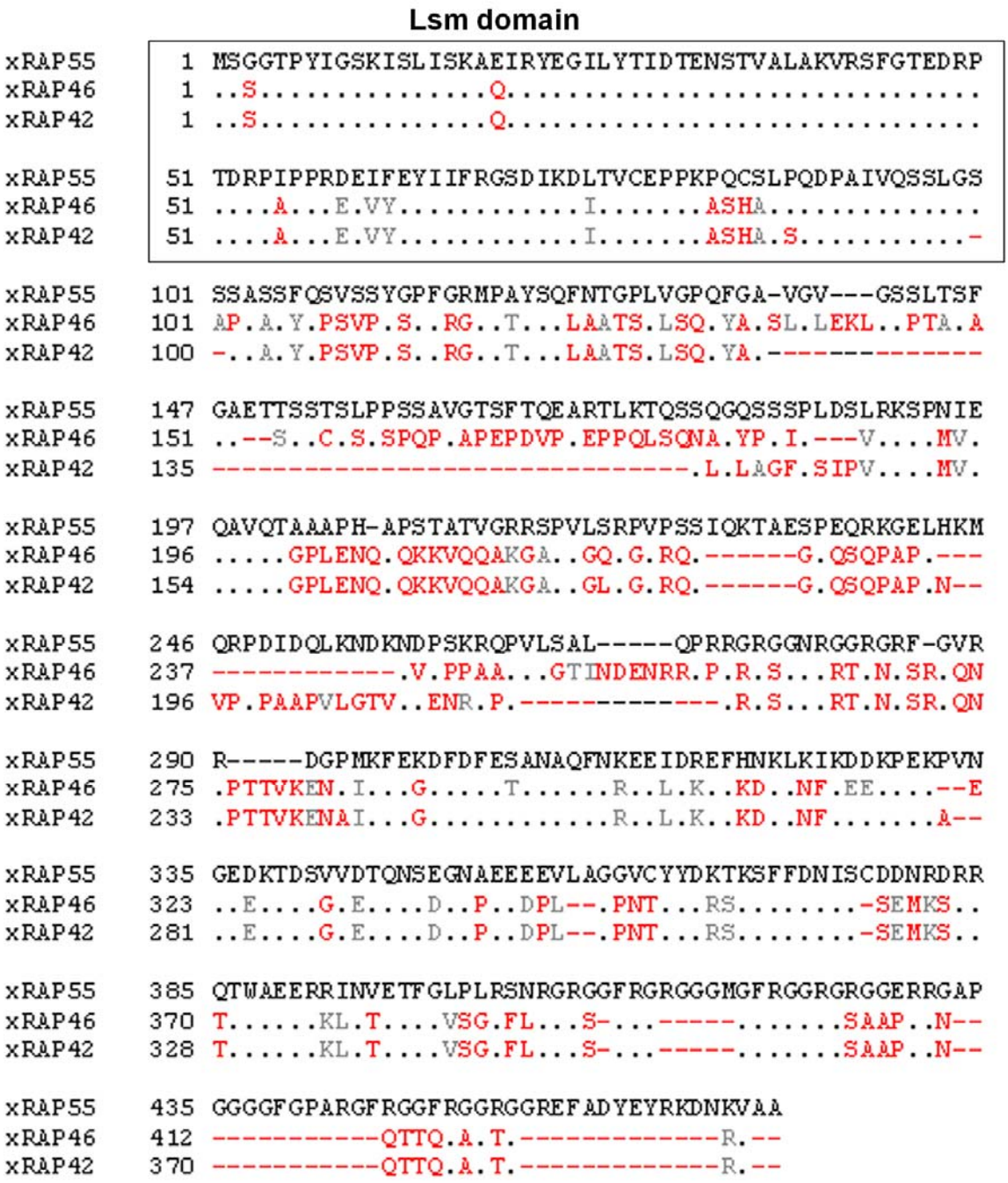
Supplementary Figure 1. Vegetal localization proteins and Elr-type proteins are part of large RNPs**.** Glycerol gradient fractionation of stage III-IV oocyte extracts plus/minus RNase A. In, input; lane 1-11, gradient fractions from top to bottom; P, pellet. Western blot analysis was performed using antibodies directed against XStaufen1, Vg1RBP and ElrA/B. Underlined fractions represent the peak localization RNP fractions (RNP S16 fraction, see also Fig 1A). A signal corresponding to a putative ElrB-isoform is marked by an asterisk.



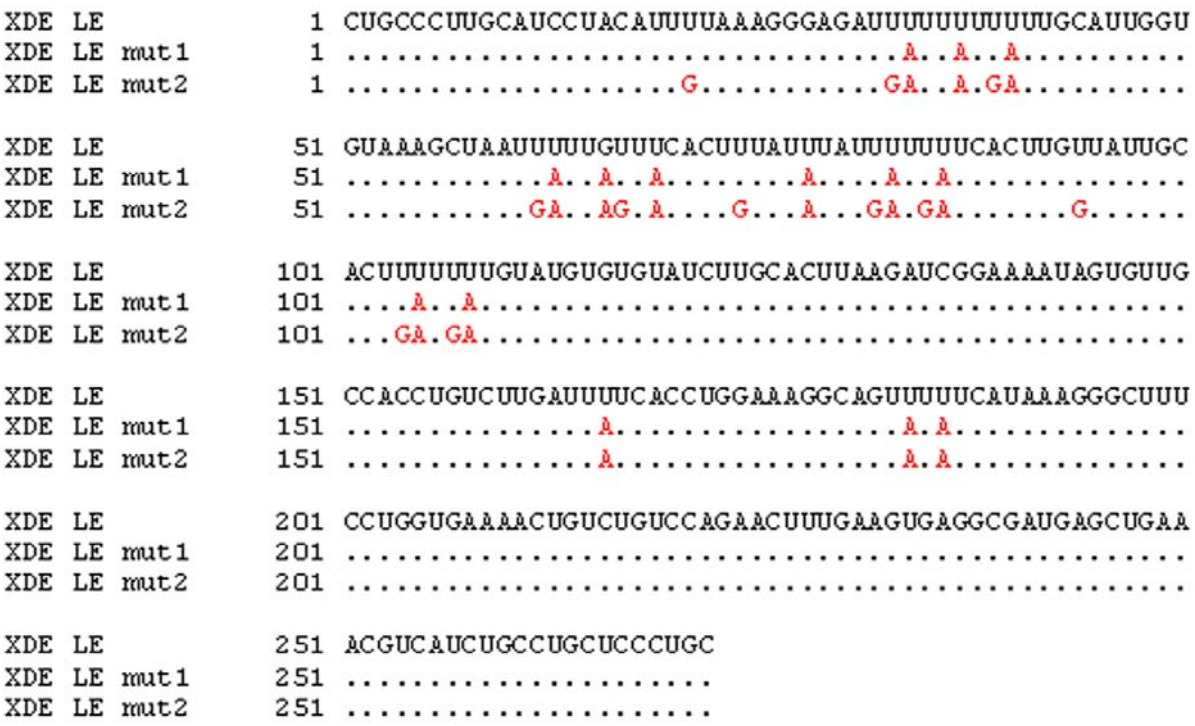
Supplementary Figure 2. 2D gel electrophoretic separation of RNP S200 proteins. Proteins of the RNP S200 preparation were resolved by 2D gel electrophoresis using either pH 3-10 (Bio-Rad) **(A)** or pH 7-10 (GE healthcare) **(B)** immobilised pH gradient (IPG) strips for the isoelectric focussing. Protein spots were analyzed by mass spectrometry. Proteins with a functional link to RNA metabolism are indicated by circles (for protein identities see Table S1).



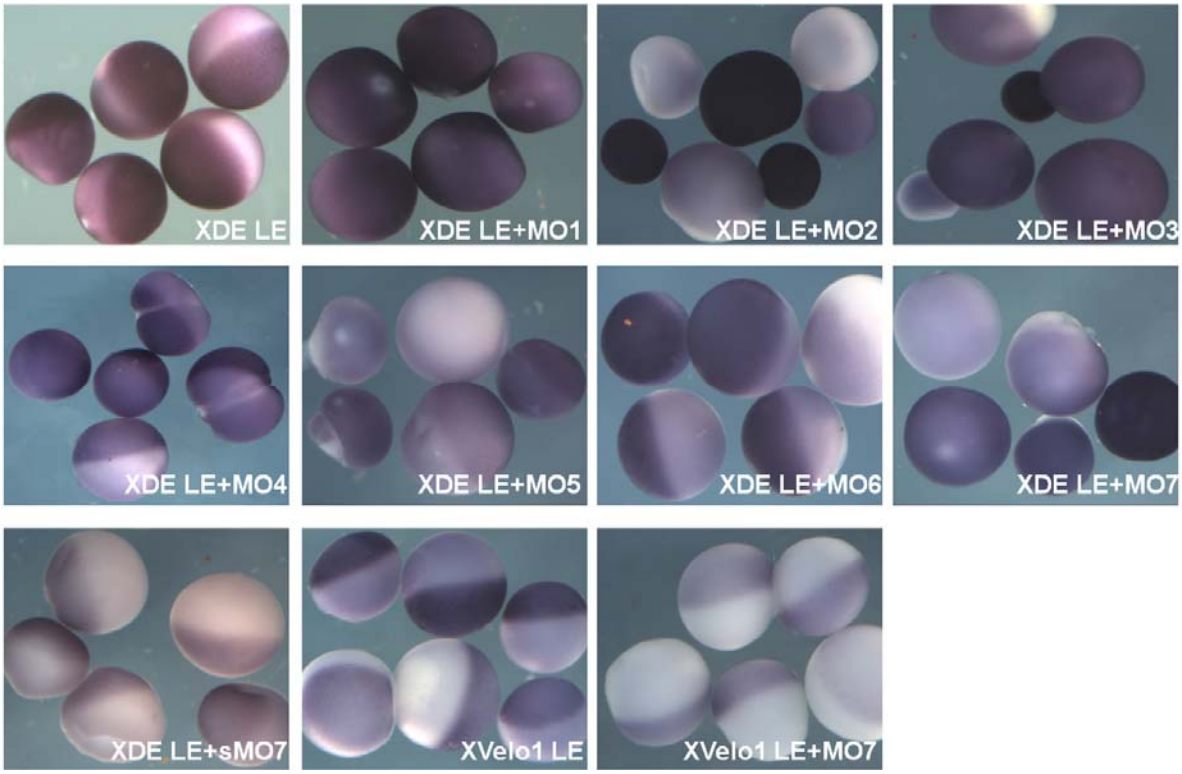
Supplementary Figure 3. 2D gel electrophoretic separation of ElrA/B interacting proteins**.** Proteins co-immunoprecipitating with ElrA/B in an RNA-dependent manner (fraction "d" depicted in figure 2) were separated by 2D gelelectrophoresis and analyzed by mass spectrometry. Spots picked from the circled protein bands were identified as indicated (for protein identities see also Table S2).



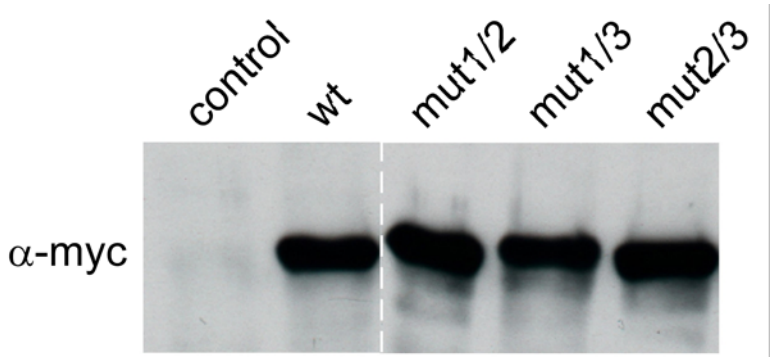
Supplementary Figure 4. Protein alignment of ElrA/B interacting Rap55 homologs. Amino acid sequence comparison between *Xenopus* RAP55 (accession number BAF36055) and the two Rap55-related proteins RAP46 (accession number BC079811) and RAP42 (accession number BC100174) was performed using the Clone Manager software (Sci-Ed software). The following protein sequence identities in comparison to XRap55 were observed: xRAP46 (48%), xRAP42 (47%). The conserved N-terminal Lsm-domain is marked by a box.



Supplementary Figure 5. Mutagenesis of putative ElrA/B binding sites in the XDE-LE. Sequence comparison of XDE-LE wt and XDE-LE point mutants 1 and 2. Single U residues of the A/U-rich putative Elr-binding sites were replaced by A or G residues by site-directed mutagenesis.



Supplementary Figure 6. Co-injection of MOs blocking ElrB binding interferes with localization of XDE-LE RNA. 0.3 fmol lacZ tagged XDE-LE and 50 fmol morpholino ologonucleotide were co-injected into stage III-IV oocytes. Co-injection with lacZ-tagged Xvelo1-LE lacking binding sites for MO7 served as a control. The distribution of the XDE-LE RNA was visualised by whole mount *in situ* hybridization using an antisense lacZ probe.



Supplementary Figure 7. Overexpression of wt and mutant ElrB proteins. Overexpression of myc-tagged wt and mutant versions of ElrB in stage III/IV oocytes prior to injection of XDE-LE and Velo1-LE RNAs (see also figure 3G). Proteins from one oocyte equivalent were detected by western blotting using a α-myc antibody.

Spot #	Protein Name	Accession #	kDa	pI
1	KH domain-containing transcription factor B3 (Vg1RBP)	gi|148229563	65.6	9.08
2	XStaufen1	gi|49256219	78.1	9.6
3	FRGY2a	gi|1175535	37.2	10.0
4	FRGY2b	gi|1175534	35.9	9.4
5	Hypothetical protein (RAP42)	gi|71679779	42.0	9.7
6a	p37 AUF1/hnRNP D	gi|52082714	33.1	5.7
6b	hnRNP D-like	gi|46249880	33.1	8.9
7	Similar to G3BP 2	gi|148236557	54.4	5.4
8	hnRNP A/B (40LoVe)	gi|49257602	35.9	5.7
9	hnRNP A3b	gi|147905111	39.4	6.8
10	hnRNP A3a	gi|148230657	38.6	6.8
11	hnRNP A1	gi|148237229	40.5	9.3
12	hnRNP A1a	gi|147900728	37.9	9.1
13	ElrB	gi|608539	42.7	9.7
14	hnRNP E2	gi|5459450	37.5	7.6
15	dsRNA Binding Protein A - XIRBPA	gi|3334381	33.3	8.5
16	ePABP2 (embryonic nuclear type)	gi|28273596	24.5	8.8
17	GAPDH	gi|27882192	36.1	8.2
18	EF1α	gi|416929	50.9	9.7
19	Guanine nucleotide BP ß2-like 1	gi|27371211	35.5	7.6
20	Eukaryotic translation elongation factor 2,	gi|27882475	95,42	6,45

Supplementary Table 1. Proteins identified from RNP S200 with a functional link to RNA metabolism**.** Partial list of proteins identified from the 2D gels in Fig. S2, selected on the basis of their functional link to RNA metabolism. The nominal molecular mass and the putative isoelectric point (pI) were estimated from the amino acid sequences using the Mascot Software 2.0 (Matrix Science). Spot numbers correspond to numbered circles indicated on the gels in figure S2.

Protein Name	Accession #	Size (kDa)	pI	Remarks
KH domain-containing transcription factor B3 (Vg1RBP)	gi|2801766	65.6	9.1	Vg1RBP
FRGY2a	gi|1175535	37.2	9.6	
FRGY2b	gi|1175534	36.0	7.9	
Xp54	gi|1709533	54.5	8.9	
RAP46	gi|51258579	46,2	9.4	Unknown proteins which are related to RAP55,
RAP42	gi|71679779	42.0	9.7	

Supplementary Table 2. ElrA/B associated proteins. List of proteins identified from the 2D gel shown in figure S3. The nominal molecular mass and the putative isoelectric point (pI) were estimated from the amino acid sequences using the Mascot Software 2.0 (Matrix Science).

Supplementary materials

XDE-LE site-directed mutagenesis primers

External primers:

T7	TAATACGACTCACTATAGGG
SP6	GATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCA

XDE-LE mutagenesis primers:

XDE P1f	ACATTTTAAAGGGAGATTATTATTATTGCATTGGTGTAAAGC
XDE P1r	GCTTTACACCAATGCAATAATAATAATCTCCCTTTAAAATGT
XDE P2f	GCATTGGTGTAAAGCTAATTATTATTACACTTTATTTATT
XDE P2r	AATAAATAAAGTGTAATAATAATTAGCTTTACACCAATGC
XDE P3a f	TTTTGTTTCACTTTATATATTATTATCACTTGTTATTGCAC
XDE P3a r	GTGCAATAACAAGTGATAATAATATATAAAGTGAAACAAAA
XDE P3b f	TATTATTACACTTTATATATTATTATCACTTGTTATTGCAC
XDE P3b r	GTGCAATAACAAGTGATAATAATATATAAAGTGTAATAATA
XDE P4f	CACTTGTTATTGCACTTATTATGTATGTGTGTATCTTGCAC
XDE P4r	GTGCAAGATACACACATACATAATAAGTGCAATAACAAGTG
XDE P5 f	CTGTCTTGATTATCACCTGGAAAGGCAGTATATCATAAAGGGC
XDE P5 r	GCCCTTTATGATATACTGCCTTTCCAGGTGATAATCAAGACAG
XDE m2a F	ACATTGTAAAGGGAGATGATTATGATTGCATTGGTGTAAAGC
XDE m2a R	GCTTTACACCAATGCAATCATAATCATCTCCCTTTACAATGT
XDE m2b F	GCATTGGTGTAAAGCTAATGATTAGTACACTGTATATATT
XDE m2b R	AATATATACAGTGTACTAATCATTAGCTTTACACCAATGC
XDE m2c F	GATTAGTACACTGTATATATGATGATCACTTGCTATTGCAC
XDE m2c R	GTGCAATAGCAAGTGATCATCATATATACAGTGTACTAATC
XDE m2d F	CACTTGGTATTGCACTGATGATGTATGTGTGTATCTTGCAC
XDE m2d R	GTGCAAGATACACACATACATCATCAGTGCAATACCAAGTG

ElrB site-directed mutagenesis primers

B1 mut1 F	GAAGGACAAAGCTTGGGCGCTGGCGATGTGAACTACATTGACC
B1 mut1 R	AGGGTCAATGTAGTTCACATCGCCAGCGCCCAAGCTTTGTCCTT
B1 mut2 F	ACTGGTGTGTCAAGAGGGGCTGGCGATATACGCTTTGACAAGA
B1 mut2 R	CCTCTTGTCAAAGCGTATATCGCCAGCCCCTCTTGACACACCA
B1 mut3 F	ACCAACAAGTGCAAGGGCGCTGGTGATGTGACTATGACAAACTA
B1 mut3 R	ATAGTTTGTCATAGTCACATCACCAGCGCCCTTGCACTTGTTGGT

Morpholino oligonucleotides

Antisense and sense morpholino oligonucleotides were purchased from Gene Tools:

MO1	TAAATAAAGTGAAACAAAAATTAGC
MO2	AAAGTGCAATAACAAGTGAAAAAAA
MO3	AAGTGCAAGATACACACATACAAAA
MO4	GGTGGCAACACTATTTTCCGATCTT
MO5	ACTGCCTTTCCAGGTGAAAATCAAG
MO6	GTTTTCACCAGGAAAGCCCTTTATG
MO7	TACAAAAAAAGTGCAATAACAAGTG
sMO7	CACTTGTTATTGCACTTTTTTTGTAT

Quantitative RT-PCR primers

GapDH-F	CTCCGCCCCCTCAGCAGATG
GapDH-R	GCAGGCGGCAGGTCAGAT
ODC-F	GCCATTGTGAAGACTCTCTCCATTC
ODC-R	TTCGGGTGATTCCTTGCCAC
LaminB1-F	CAACTAAAGGCAAAAGAAAGAGAA
LaminB1-R	ACGGTTGTGCGCTGTGCTACT
Vg1-primers were as in (Kress et al., 2004)	
VegT-F	CAAGTAAATGTGAGAAACCG
VegT-R	CAAATACACACACATTTCCC
XDE-primers were as in (Horvay et al., 2006)	
XNIF-F	CTGCTAGACCGGTGGGGAGTGT
XNIF-R	AGGGTGGAGAAGCGAAGAGTCAA

Primers for the generation of T7 promoter containing templates for in vitro transcription

T7_XDeadsouth2 LE F	TAATACGACTCACTATAGGGCGGGATCCATTCTATTAATAA
XDeadsouth2 LE_R	CCGCTCGAGTGGGCGATTCTACACAATTTG
T7_Vg1TE_F	TAATACGACTCACTATAGGGATGCCATTGACATACAAGTG
Vg1TE_R	ATGCATTTTTAGGTATGTAAAAGCACGTTAA
T7_VegT-LE_F	TAATACGACTCACTATAGGGGTGGTGGTACAGCCATCTAA
VegT-LE_R	CATTGCCAGAGTTATGCTAATGTGAAAATAT
T7_Xvelo_3'UTR_F	TAATACGACTCACTATAGGGTGTAAAGTGTATATATCTCTC
Xvelo_3'UTR_R	CCGCTCGAGTAAGTACATTATTTGCAG

Xvelo1 LE, XNIF and Vg1LE were transcribed from pBluescript constructs produced with BamHI/XhoI site containing primers as used previously (Claussen and Pieler, 2004; Claussen et al., 2004).