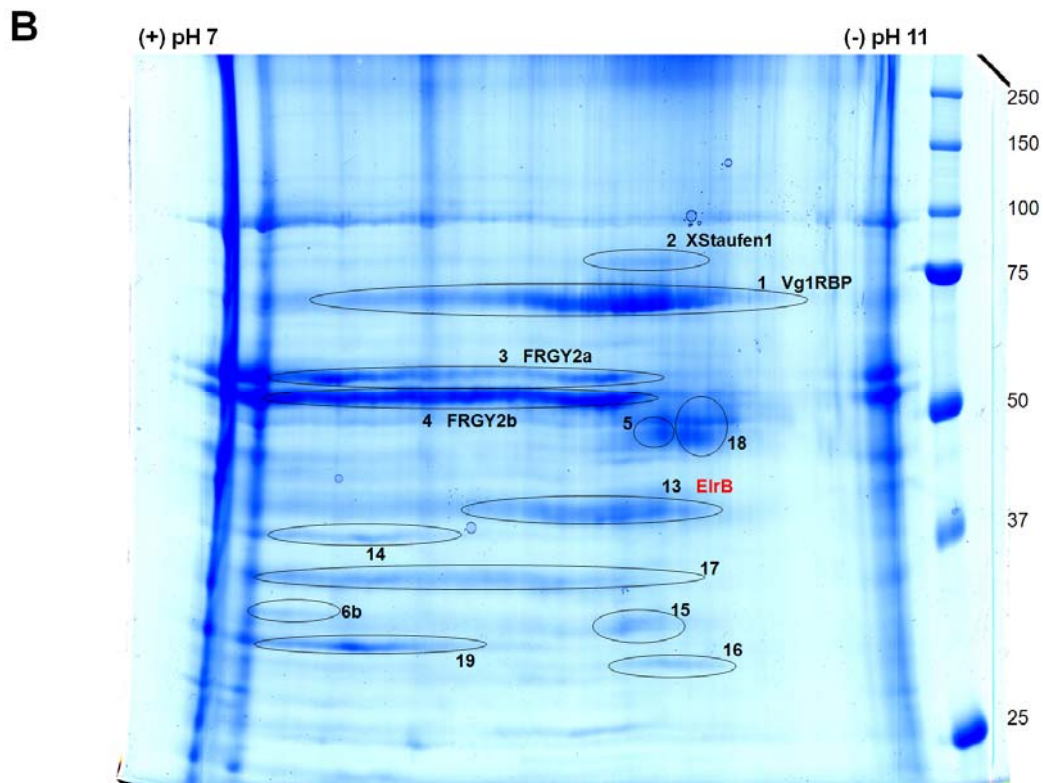
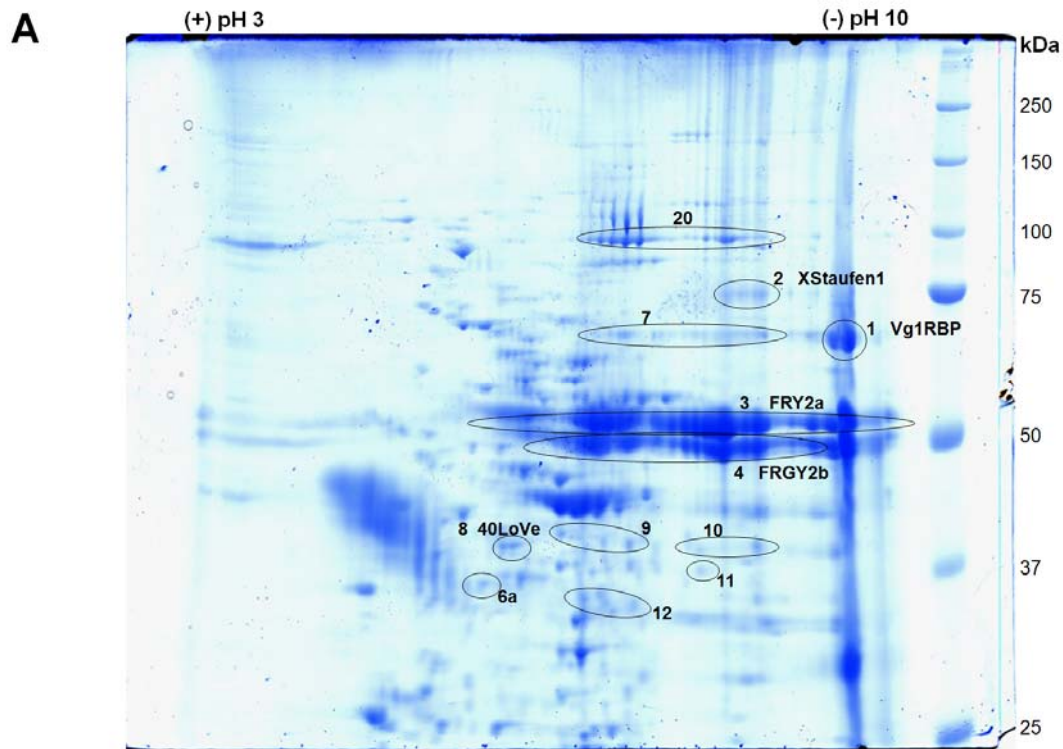
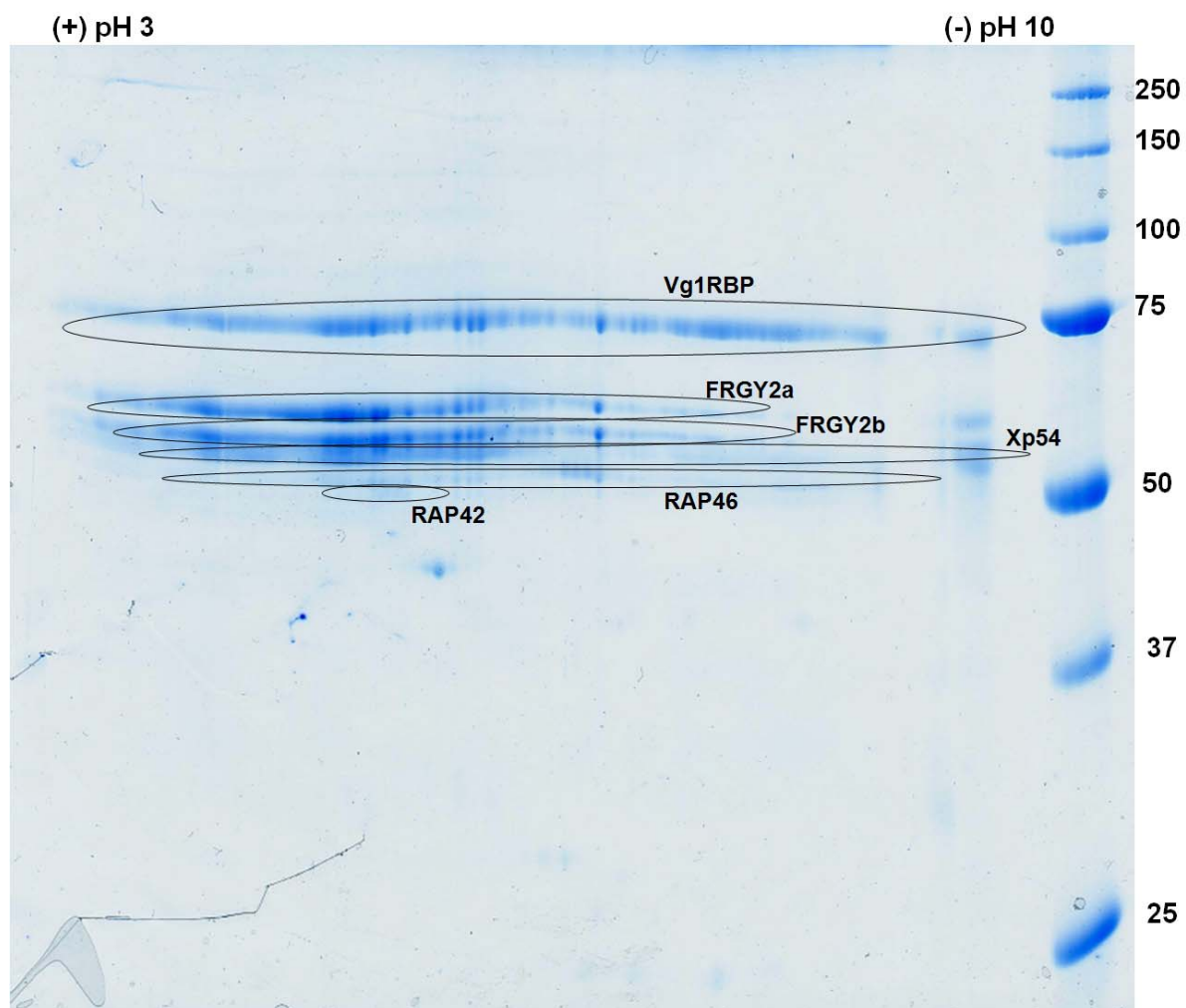


Supplementary Figure 1. Vegetal localization proteins and Elr-type proteins are part of large RNPs. Glycerol gradient fractionation of stage III-IV oocyte extracts plus/minus RNase A. In, input; lane 1-11, gradient fractions from top to bottom; P, pellet. Western blot analysis was performed using antibodies 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).

Lsm domain

xRAP55	1	MSGGTPYIGSKISLISKAEIRYEGILYITIDTENSTVALAKVRSFGTEDRP
xRAP46	1	..S.....Q.....
xRAP42	1	..S.....Q.....
xRAP55	51	TDRPIPPRDEIFEYIIFRGSDIKDLTVCEPPKQCQLPQDPAIVQSSLGS
xRAP46	51A...E.VY.....I.....ASHA.....
xRAP42	51A...E.VY.....I.....ASHA.S.....-
xRAP55	101	SSASSFQSVSSYGPFGRMPAYSQFNTGPLVGPQFGA-VGV---GSSLTSF
xRAP46	101	AP.A.Y.PSVP.S.RG..T...LAATS.LSQ.YA.SL.LEKL..PTA.A
xRAP42	100	-.A.Y.PSVP.S.RG..T...LAATS.LSQ.YA.-----
xRAP55	147	GAETTSSTSLPPSSAVGTSFTQEARLKTQSSQGQSSSPLDSLKSPNIE
xRAP46	151	..--S..C.S.SPQP.APEPDVP.EPPQLSQNA.YP.I.---V....MV.
xRAP42	135	-----L.LAGF.SIPV....MV.
xRAP55	197	QAVQTAAAPH-APSTATVGRRRSPVLSRPVPSIIQKTAESPEQRKGLHKM
xRAP46	196GPLENQ.QKKVQQAQGA..GQ.G.RQ.-----G.QSQPAP.---
xRAP42	154GPLENQ.QKKVQQAQGA..GL.G.RQ.-----G.QSQPAP.N--
xRAP55	246	QRPDIDQLKNDKNDPSKROPVLSAL-----QPRRGRGGNRGGRRGRF-GVR
xRAP46	237	-----V.PPAA...GTINDENRR.P.R.S...RT.N.SR.QN
xRAP42	196	VP.PAAPVLGTV..ENR.P.-----R.S...RT.N.SR.QN
xRAP55	290	R-----DGPMPKFEKDFDFESANAQFNKEEIDREFHNKLIKIKDDKPEKPVN
xRAP46	275	.PTTVKEN.I...G.....T.....R..L.K..KD..NF.EE....--E
xRAP42	233	.PTTVKENAI...G.....R..L.K..KD..NF.....A--
xRAP55	335	GEDKTDSVVDTONSEGNAEEEEVLGGVCYYDKTKSFFDNISCDDNRRR
xRAP46	323	..E....G.E...D..P..DPL--.PNT...RS.....-SEMKS..
xRAP42	281	..E....G.E...D..P..DPL--.PNT...RS.....-SEMKS..
xRAP55	385	QTWAEERRINVEITGLPLRSNRGRGGFRGRGGMGFRGGRGRGGERRGAP
xRAP46	370	T.....KL.T...VSG.FL...S-.....-.....SAAP..N--
xRAP42	328	T.....KL.T...VSG.FL...S-.....-.....SAAP..N--
xRAP55	435	GGGGFGPARGFRGGFRGGRGGREFADYEYRKDNKVAA
xRAP46	412	-----QTTQ.A.T.-----R.--
xRAP42	370	-----QTTQ.A.T.-----R.--

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.

```

XDE LE          1 CUGCCCUUGCAUCCUACAUUUUAAAAGGGAGAUUUUUUUUUUUUGCAUUGGU
XDE LE mut1    1 .....A..A..A.....
XDE LE mut2    1 .....G.....GA..A.GA.....

XDE LE          51 GUAAAAGCUAAAUUUUUGUUUCACUUUAUUUAUUUUUUUCACUUGUUAUUGC
XDE LE mut1    51 .....A..A..A.....A...A..A.....
XDE LE mut2    51 .....GA..AG..A....G...A...GA.GA.....G.....

XDE LE          101 ACUUUUUUUGUAUGUGUGUAUCUUGCACUUAAGAUCGGAAAAUAAGUGUUG
XDE LE mut1    101 ...A..A.....
XDE LE mut2    101 ...GA.GA.....

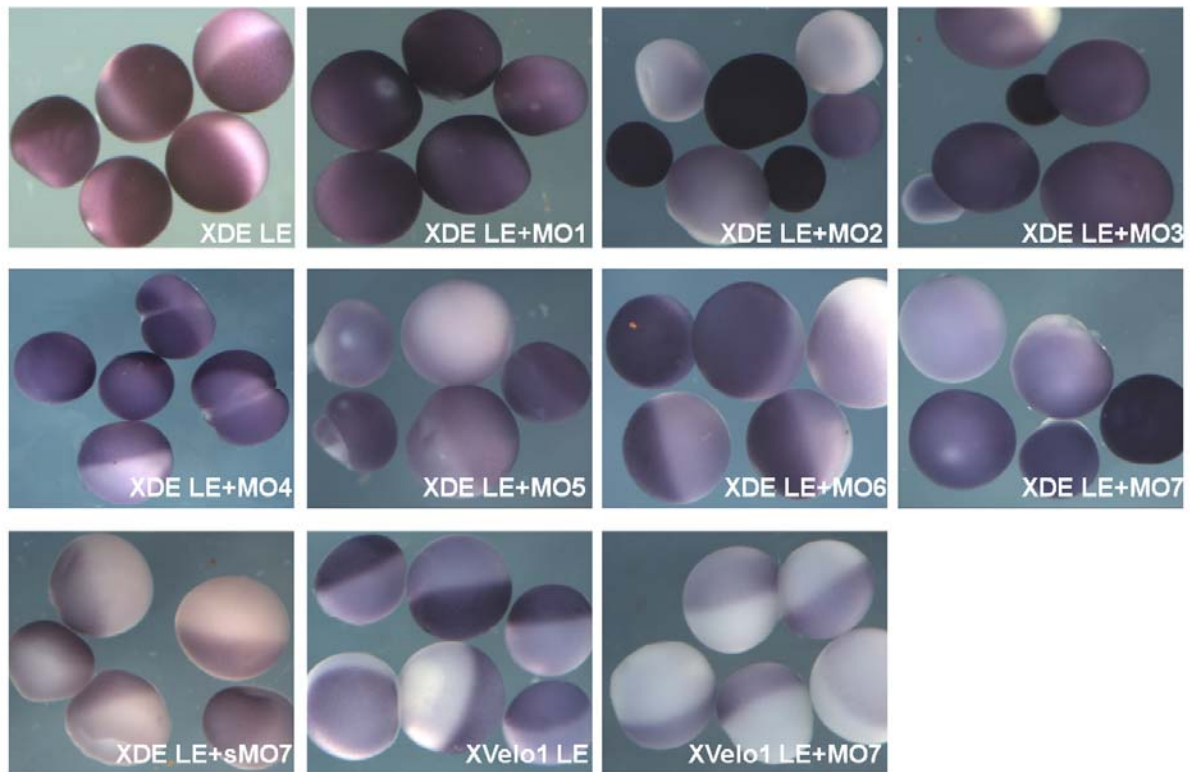
XDE LE          151 CCACCUGUCUUGAUUUUCACCUGGAAAGGCAGUUUUUCAUAAAAGGGCUUU
XDE LE mut1    151 .....A.....A..A.....
XDE LE mut2    151 .....A.....A..A.....

XDE LE          201 CCUGGUGAAAACUGUCUGUCCAGAACUUUGAAGUGAGGCGAUGAGCUGAA
XDE LE mut1    201 .....
XDE LE mut2    201 .....

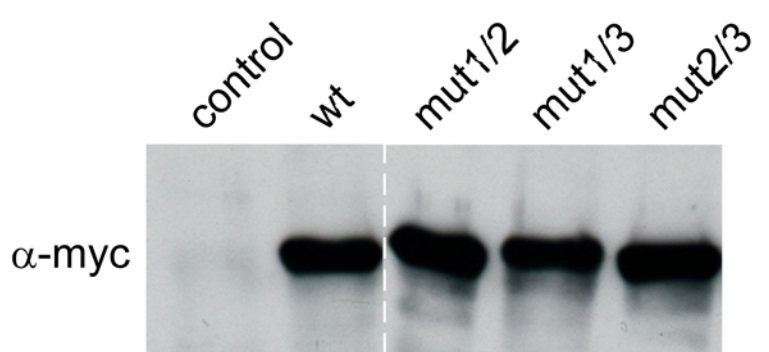
XDE LE          251 ACGUCAUCUGCCUGCUCUCCUGC
XDE LE mut1    251 .....
XDE LE mut2    251 .....

```

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 oligonucleotide 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	EF1 α	gi 416929	50.9	9.7
19	Guanine nucleotide BP β 2-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 AATAAATAAAGTGTAAATAATAATTAGCTTTACACCAATGC

XDE P3a f TTTTGTTCACCTTTATATATTATTATCACTTGTTATTGCAC

XDE P3a r GTGCAATAACAAGTGATAATAATATATAAAGTGAAACAAAA

XDE P3b f TATTATTACACTTTATATATTATTATCACTTGTTATTGCAC

XDE P3b r GTGCAATAACAAGTGATAATAATATATAAAGTGTAAATAATA

XDE P4f CACTTGTTATTGCACCTTATTATGTATGTGTGTATCTTGCAC

XDE P4r GTGCAAGATACACACATACATAATAAGTGTGCAATAACAAGTG

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 GTGCAATAGCAAGTGATCATATATACAGTGTACTAATC

XDE m2d F CACTTGGTATTGCACCTGATGATGTATGTGTGTATCTTGCAC

XDE m2d R GTGCAAGATACACACATACATCATCAGTGTGCAATAACAAGTG

ElrB site-directed mutagenesis primers

B1 mut1 F GAAGGACAAAGCTTGGGCGCTGGCGATGTGAACTACATTGACC

B1 mut1 R AGGGTCAATGTAGTTTCACATCGCCAGCGCCCAAGCTTTGTCCCT

B1 mut2 F ACTGGTGTGTCAAGAGGGGCTGGCGATATACGCTTTGACAAGA

B1 mut2 R CCTCTTGTCAAAGCGTATATCGCCAGCCCCTCTTGACACACCA

B1 mut3 F ACCAACAAGTGCAAGGGCGCTGGTGTATGTGACTATGACAAACTA

B1 mut3 R ATAGTTTGTATAGTACATCACCAGCGCCCTTGCACTTGTGGT

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 TACAAAAAAGTGCAATAACAAGTG

sMO7 CACTTGTTATTGCACCTTTTTTTGTAT

Quantitative RT-PCR primers

GapDH-F CTCCGCCCCCTCAGCAGATG
GapDH-R GCAGGCGGCAGGTCAGAT
ODC-F GCCATTGTGAAGACTCTCTCCATTC
ODC-R TTCGGGTGATTCCCTTGCCAC
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 TAATACGACTCACTATAGGGGTGTAAAGTGTATATATCTCTC
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).