Supplemental Materials

Molecular Biology of the Cell

Claußen et al.

Supplementary figures and tables

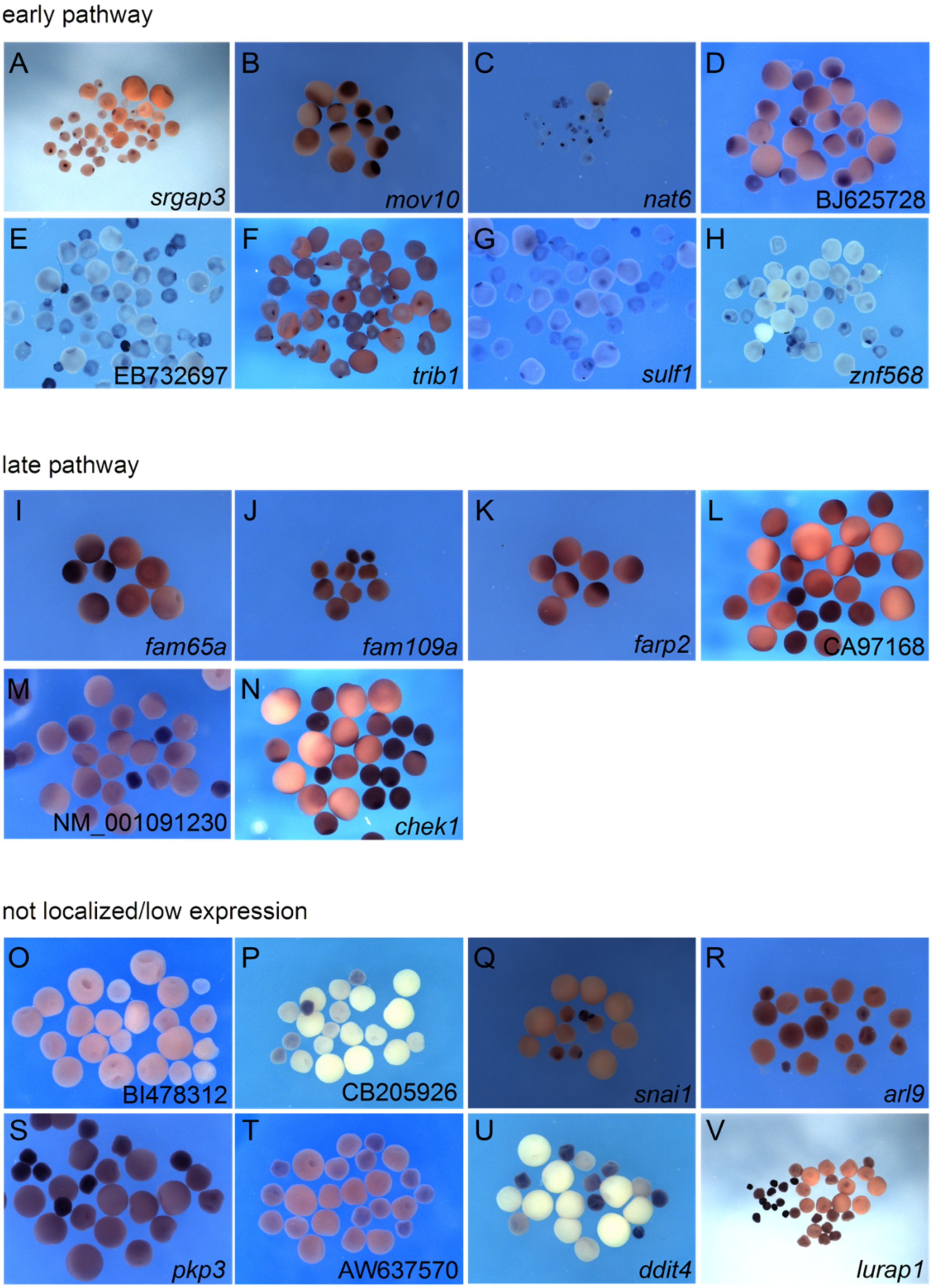


Figure S1. *In situ* hybridization analysis of novel vegetally enriched candidate RNAs in *X. laevis.* RNA distribution in *X. laevis* oocytes was detected by *in situ* hybridization. (A-H) Early pathway/mitochondrial cloud localization has been detected for *srgap*, *mov10*, *nat6*, BJ625728, EB732697, *trib1, sulf1 and znf568* in stage I-III oocytes. (I-N) Late pathway localization was observed for *fam65a, fam109a, farp2,* CA97168, NM_001091230 and *check1* transcripts. Stage II-IV oocytes are shown. (O-V) No vegetal enrichment or very weak expression levels were observed for BI478312, CB205926, *snai1*, *arl9*, *pkp3*, AW637570, *ddit4* and *lurap1*. Stage I-IV oocytes are shown.

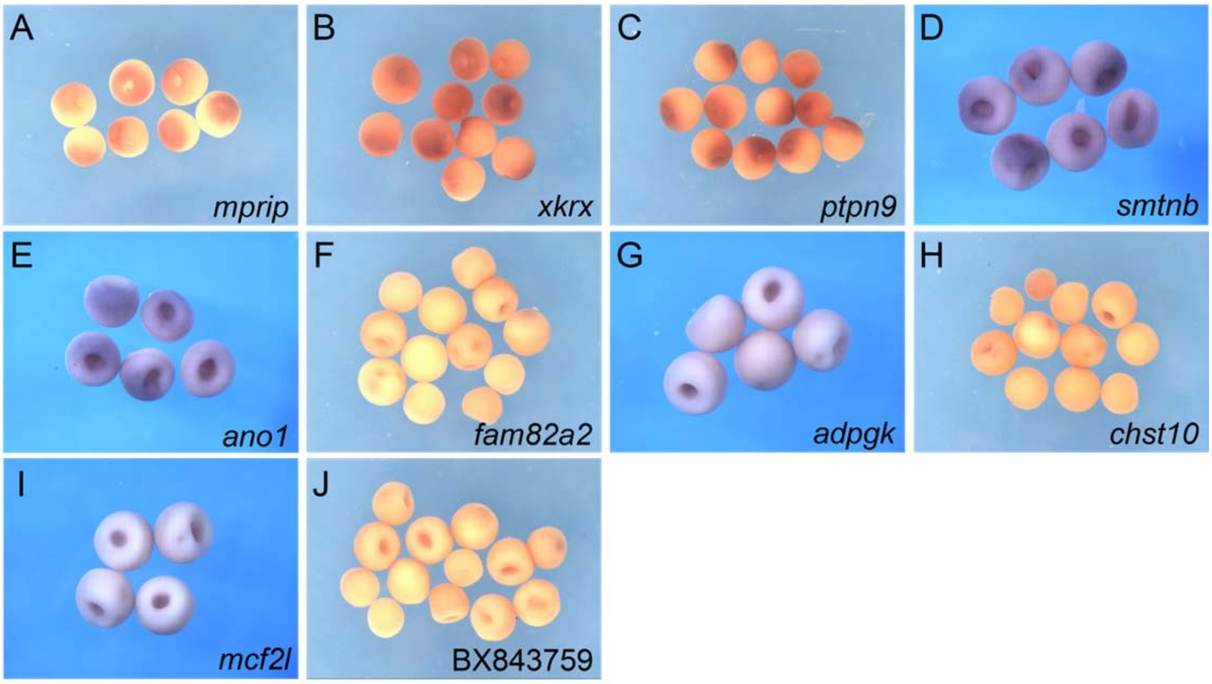


Figure S2. Expression and localization of novel animally enriched candidate RNAs in *X. laevis*. (A-E) *In situ* hybridization reveals enrichment of *mprip*, *xkrx*, *ptpn9*, *smtnb* and *ano1* transcripts in the animal hemisphere of stage VI *X. laevis* oocytes. (F-J) No animal enrichment or very weak expression levels were detected for *fam82a2*, *adpgk*, *chst10*, mcf2l and BX843759 transcripts.

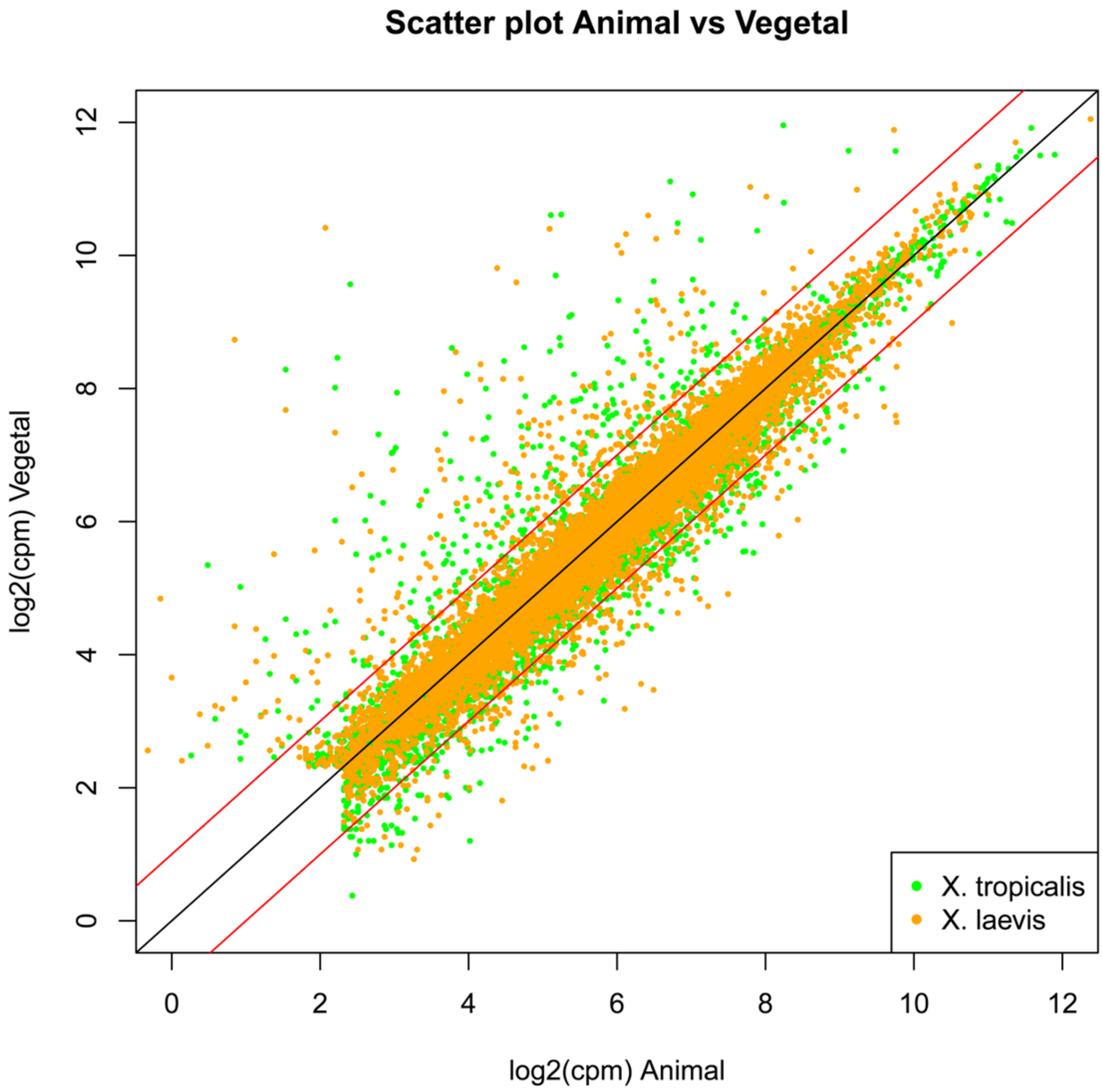


Figure S3. Comparison of transcript levels for vegetal and animal halves from *X. laevis* (orange) and *X. tropicalis* (green) oocytes. Expression values correspond to logarithmic, normalized average read counts (cpm) for each gene from animal and vegetal samples. The black line denotes identical values for animal and vegetal samples, the red lines denote 2-fold changes of RNA levels in either animal or vegetal samples. This comparative analysis was restricted to transcripts identified in both species.

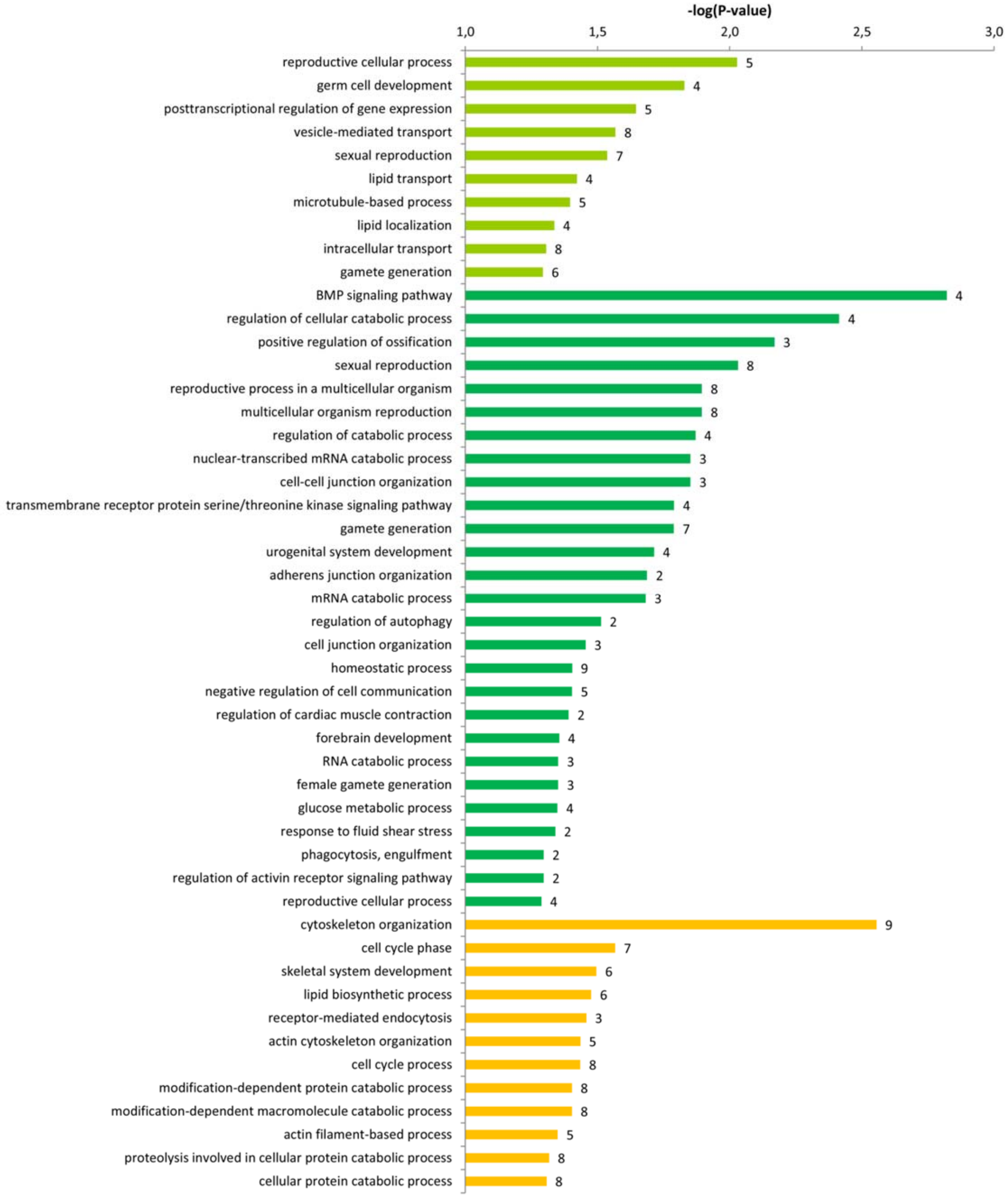


Figure S4. GO analysis of vegetally enriched transcripts. Enrichment of genes associated with various biological processes are expressed as -log (P-values) and shown for transcripts with conserved vegetal enrichment (light green) as well as species-specific vegetal enrichment in *X. laevis* (dark green) and *X. tropicalis* (orange). Biological process functional annotation terms (BP_FAT) with p-values ≤ 0,05 are listed. Numbers of genes associated with a given process are indicated. Only transcripts with expression in oocytes from both species were considered.

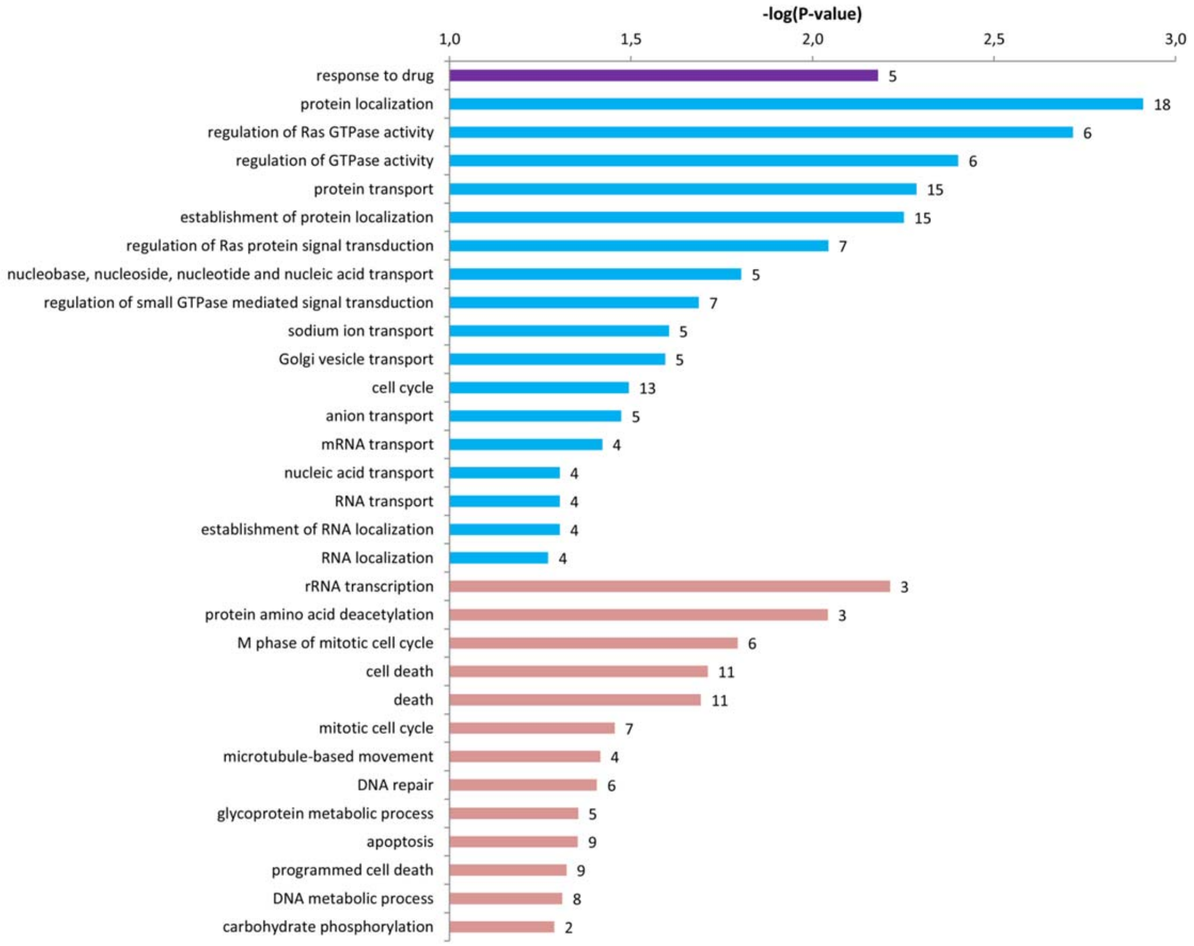


Figure S5. GO analysis of animally enriched transcripts. Enrichment of genes associated with various biological processes are expressed as -log (P-values) and shown for transcripts with conserved animal enrichment (purple) as well as species-specific animal enrichment in *X. laevis* (blue) and *X. tropicalis* (mauve). Biological process functional annotation terms (BP_FAT) with p-values ≤ 0,05 are listed. Numbers of genes associated with a given process are indicated. Only transcripts with expression in oocytes from both species were considered.

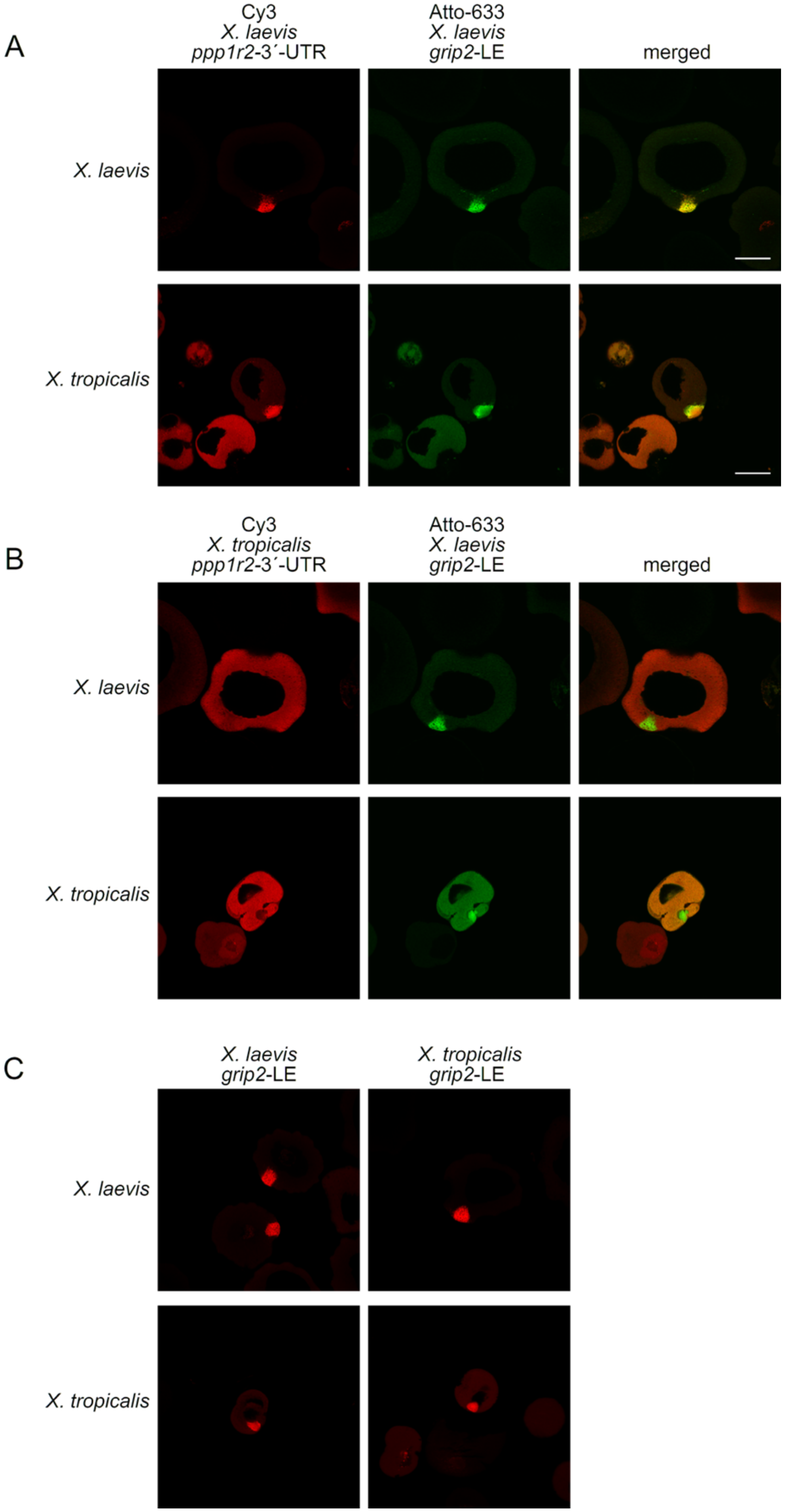
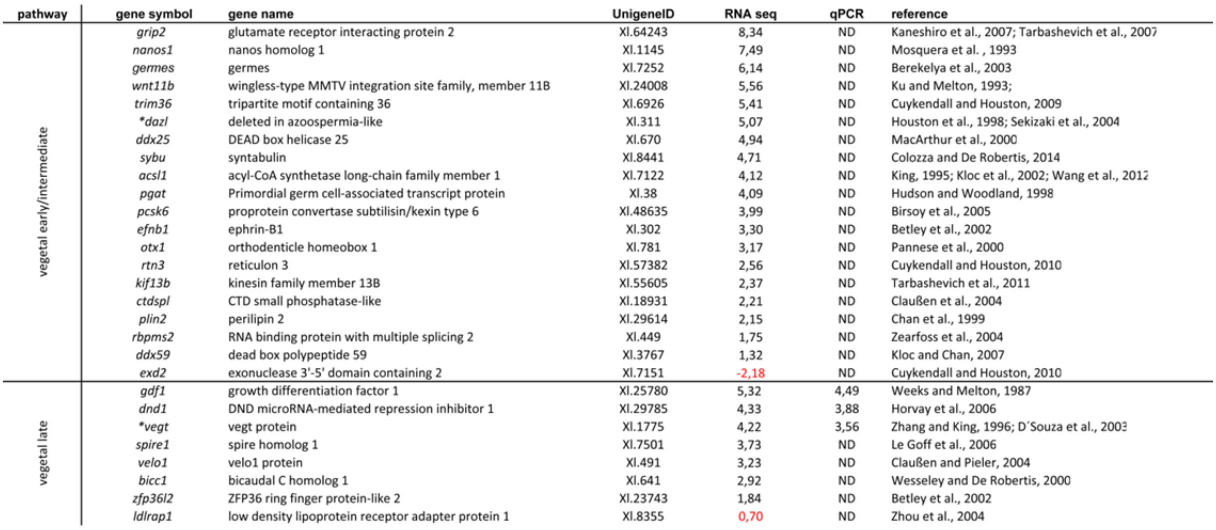

A
Cy3
X. laevis
ppp1r2-3´-UTR
Atto-633
X. laevis
grip2-LE
merged
X. laevis
X. tropicalis
B
Cy3
X. tropicalis
ppp1r2-3´-UTR
Atto-633
X. laevis
grip2-LE
merged
X. laevis
X. tropicalis
C
X. laevis
grip2-LE
X. tropicalis
grip2-LE
X. laevis
X. tropicalis

Figure S6. Early pathway localization of *ppp1r2* 3´-UTRs and grip2-LEs after injection into stage I oocytes from *X. laevis* and *X. tropicalis*. (A) Co-injection of Cy3-labelled *X. laevis ppp1r2*-3´-UTR and Atto633-labelled *X.laevis grip2*-LE into oocytes from *X. laevis* and *X. tropicalis*. (B) Co-injection of Cy3-labelled *X. tropicalis ppp1r2*-3´-UTR and Atto633-labelled *X.laevis grip2*-LE into oocytes from both species. (C) Injection of Cy3-labelled *X. laevis* and *X. tropicalis grip2*-LEs into stage I oocytes from *X. laevis* and *X. tropicalis*. Scale bars approximate 100 µm. Settings of the confocal imaging were adjusted to fit the requirements for imaging the oocyte in the center of the image. Additional oocytes present in the image may thus be over- or under exposed or do not represent the appropriate focal plane for imaging the mitochondrial cloud.

Table S1. Summary of RNA sequencing results for known vegetally localizing mRNAs.



pathway	gene symbol	gene name	UnigeneID	RNA seq	qPCR	reference
vegetal early/intermediate	*grip2*	glutamate receptor interacting protein 2	Xl.64243	8,34	ND	Kaneshiro et al., 2007; Tarbashevich et al., 2007
	nanos1	nanos homolog 1	Xl.1145	7,49	ND	Mosquera et al. , 1993
	germes	germes	Xl.7252	6,14	ND	Berekelya et al., 2003
	wnt11b	wingless-type MMTV integration site family, member 11B	Xl.24008	5,56	ND	Ku and Melton, 1993;
	trim36	tripartite motif containing 36	Xl.6926	5,41	ND	Cuykendall and Houston, 2009
	**dazl*	deleted in azoospermia-like	Xl.311	5,07	ND	Houston et al., 1998; Sekizaki et al., 2004
	ddx25	DEAD box helicase 25	Xl.670	4,94	ND	MacArthur et al., 2000
	sybu	syntabulin	Xl.8441	4,71	ND	Colozza and De Robertis, 2014
	acsl1	acyl-CoA synthetase long-chain family member 1	Xl.7122	4,12	ND	King, 1995; Kloc et al., 2002; Wang et al., 2012
	pgat	Primordial germ cell-associated transcript protein	Xl.38	4,09	ND	Hudson and Woodland, 1998
	pcsk6	proprotein convertase subtilisin/kexin type 6	Xl.48635	3,99	ND	Birsoy et al., 2005
	efnb1	ephrin-B1	Xl.302	3,30	ND	Betley et al., 2002
	otx1	orthodenticle homeobox 1	Xl.781	3,17	ND	Pannese et al., 2000
	rtn3	reticulon 3	Xl.57382	2,56	ND	Cuykendall and Houston, 2010
	kif13b	kinesin family member 13B	Xl.55605	2,37	ND	Tarbashevich et al., 2011
	ctdspl	CTD small phosphatase-like	Xl.18931	2,21	ND	Claußen et al., 2004
	plin2	perilipin 2	Xl.29614	2,15	ND	Chan et al., 1999
	rbpms2	RNA binding protein with multiple splicing 2	Xl.449	1,75	ND	Zearfoss et al., 2004
	ddx59	dead box polypeptide 59	Xl.3767	1,32	ND	Kloc and Chan, 2007
	exd2	exonuclease 3'-5' domain containing 2	Xl.7151	-2,18	ND	Cuykendall and Houston, 2010
vegetal late	*gdf1*	growth differentiation factor 1	Xl.25780	5,32	4,49	Weeks and Melton, 1987
	dnd1	DND microRNA-mediated repression inhibitor 1	Xl.29785	4,33	3,88	Horvay et al., 2006
	**vegt*	vegt protein	Xl.1775	4,22	3,56	Zhang and King, 1996; D´Souza et al., 2003
	spire1	spire homolog 1	Xl.7501	3,73	ND	Le Goff et al., 2006
	velo1	velo1 protein	Xl.491	3,23	ND	Claußen and Pieler, 2004
	bicc1	bicaudal C homolog 1	Xl.641	2,92	ND	Wesseley and De Robertis, 2000
	zfp36l2	ZFP36 ring finger protein-like 2	Xl.23743	1,84	ND	Betley et al., 2002
	ldlrap1	low density lipoprotein receptor adapter protein 1	Xl.8355	0,70	ND	Zhou et al., 2004

Relative vegetal enrichment as revealed by RNA sequencing analysis (expressed as log2FC) is indicated for previously reported vegetally localizing transcripts only. Vegetally enriched RNAs, for which vegetal cortex localization had been approved by *in situ* hybridization analysis, were considered. Documented vegetal enrichment in *X. tropicalis* oocytes is indicated by asterisks. Vegetally localizing RNAs are grouped into early or late pathway RNAs and sorted according to descending enrichment in the vegetal hemisphere of *X. laevis* oocytes. The two examples for vegetal enrichment below the threshold of log2FC ≥ 1 (*exd2* and *ldlrap1*) are marked in red.

Table S3. Summary of RNA sequencing results for published, animally enriched mRNAs.

gene symbol	gene name	UnigeneID	RNA seq	qPCR	reference	note
tnfaip1	tumor necrosis factor, alpha-induced protein 1	Xl.24189	-1,89	ND	Grant et al., 2014	#
srebf2	sterol regulatory element binding transcription factor 2	Xl.78417	-1,48	ND	Grant et al., 2014	#
nup93	nucleoporin 93kDa	Xl.98	-1,20	ND	Hudson et al. 1996	$
aurka	aurora kinase A	Xl.124	-1,17	ND	Grant et al., 2014	#
smcr7l	Smith-Magenis syndrome chrom. reg., cand. 7-like	Xl.78192	-1,12	ND	Grant et al., 2014	#
atxn7	ataxin 7	Xl.29665	-1,10	ND	Grant et al., 2014	#
c17orf63	chromosome 17 open reading frame 63	Xl.54800	-1,01	ND	Grant et al., 2014	#
mark3	MAP/microtubule affinity-regulating kinase 3	Xl.1967	-0,72	ND	Ossipova et al., 2002	#
mcm6	minichromosome maintenance complex component 6	Xl.3661	-0,45	ND	Kloc et al., 1991	$
wbp2nl	WBP2 N-terminal like	Xl.76974	-0,27	ND	Grant et al., 2014	#
ddx3x	DEAD box helicase 3	Xl.60716	-0,26	-0,30	Rebagliati et al., 1985; Gururajan et al., 1991	
oct60	POU class V protein oct-60	Xl.53382	-0,25	ND	Whitfield et al., 1993	#
phax	phosphorylated adaptor for RNA export	Xl.13915	-0,22	ND	Snedden et al., 2013	
gnb1	G-protein beta subunit	Xl.4840	-0,21	ND	Devic et al. 1996	
dnah9	dynein, axonemal, heavy chain 9	Xl.21651	-0,16	ND	Vick et al., 2009	#
abi2	abl-interactor 2	Xl.956	-0,06	ND	Reddy et al., 1992	$
atp5a1	mitochondrial ATP Synthase subunit	Xl.8876	-0,05	ND	Rebagliati et al., 1985; Weeks and Melton, 1987	$
trim33	tripartite motif containing 33	Xl.57890	-0,04	ND	Dupont et al., 2005	#
zfand4	zinc finger, AN1-type domain 4	Xl.865	0,01	0,00	Rebagliati et al., 1985; Linnen et al., 1993	$
pabpc1	poly(A) binding protein, cytoplasmic 1	Xl.35547	0,12	ND	Schroeder and Yost, 1996	
igf2bp3	insulin-like growth factor 2 mRNA binding protein 3	Xl.319	0,14	ND	Zhang et al., 1999	
tcf7	transcription factor 7 (T-cell specific, HMG-box)	Xl.3962	0,16	ND	Roel et al., 2003	#,*
ets1	avian erythroblastosis virus E26 oncogene homolog 1	Xl.1148	0,20	ND	Meyer et al., 1997	
csnk2a1	casein kinase 2, alpha 1 polypeptide	Xl.11560	0,24	ND	Imbrie et al., 2012	
fn1	fibronectin 1	Xl.2243	0,73	ND	Oberman and Yisraeli, 1995	

Relative animal enrichment as revealed by RNA sequencing or qPCR analysis is indicated for transcripts with animal enrichment published previously. Transcripts, for which differential localization has been described for the zygote or early embryo, but not for oocytes, are marked (#). Transcripts for which in situ hybridization data are missing are indicated ($).Documented animal enrichment in *X. tropicalis* is indicated by an asterisk (*). RNAs are sorted by descending enrichment in the animal hemisphere of *X. laevis* oocytes. Values above the threshold for animal enrichment of log2FC ≤- 1 are indicated in red.

Table S9. Quantification of vegetal localization of injected RNA

	***X. laevis* oocytes**				***X. tropicalis* oocytes**			
injected RNA	**ratio Veg/An**	**SDEV**	**N**	**SEM**	**ratio Veg/An**	**SDEV**	**N**	**SEM**
X.l. gdf1 -LE	8,21	3,50	17	0,85	5,40	3,07	24	0,63
X. t. gdf1 -LE	3,07	0,76	8	0,27	4,76	2,44	18	0,57
X.l. grip2 -LE	5,82	1,47	12	0,42	5,52	2,90	15	0,75
X.t. grip2 -LE	7,56	3,13	11	0,94	5,51	2,14	15	0,55
β-globin 3´-UTR	0,94	0,25	26	0,05	0,79	0,20	22	0,04
X.l.ppp1r2 3´-UTR	12,39	8,84	18	2,08	5,31	2,95	22	0,63
X.t.ppp1r2 3´-UTR	1,00	0,38	18	0,09	0,85	0,22	22	0,05
X.l. acp6 5´-UTR	6,95	1,85	18	0,44	4,27	1,42	8	0,50
X.t. acp6 5´-UTR	5,33	1,84	20	0,41	4,08	1,64	13	0,46

Mean pixel intensities at the vegetal cortex and in the animal cytoplasm were quantified and average Veg/An ratios are listed. SDEV, standard deviation; N, number of images quantified; SEM, standard error of the mean.

Table S10. Pairwise global alignment analysis of 3´-UTR sequences from selected transcripts with conserved and species-specific vegetal enrichment.

conserved localization					
3´-UTR	***X. laevis***	***X. tropicalis***	**length**	**% identity**	**% gaps**
bicc1	NM_001095090	CR855500	264	82,6	11,0
dazl	NM_001088303	NM_203748	1441	46,2	45,9
dnd1	BC078132	NM_001044434	824	49,4	38,7
gdf1	NM_001095591	NM_001016819	1373	59,4	29,3
grip2	NM_001097913	XM_002938230	2498	83,0	6,8
nanos1	NM_001088034	NM_203526	1001	29,3	65,0
pgat	NM_001087463	XM_002940115	3741	40,9	49,6
trim36	NM_001091117	NM_001045780	1717	70,9	19,0
vegt	NM_001088196	NM_203527	1284	81,8	7,9
velo1	NM_001089216	CU075535	386	75,9	17,9
average				**61,9**	**29,1**

species-specific localization					
3´-UTR	***X. laevis***	***X. tropicalis***	**length**	**% identity**	**% gaps**
arl9	NM_001096919	NM_001016174	994	46,3	43,2
tob2	NM_001090713	NM_001016213	1754	79,4	11,9
ppp1r2	NM_001097667	CU075815	1671	69,6	21,5
tmem82	NM_001094923	NM_001016278	731	54,7	33,2
e2f1	NM_001097139	XM_002932857	1276	38,1	53,5
krt8	NM_001087056	NM_001002797	581	80,4	10,5
pxk	NM_001096762	XM_002935048	2596	44,3	44,1
tmem54	BC058879	XM_002940024	1181	39,6	51,9
rarres1	NM_001089741	NM_001127974	2984	37,7	54,5
itm2a	NM_001086092	NM_001004833	1609	21,8	75,0
average				**51,2**	**39,9**

3´-UTRs for pairwise global alignments were deduced from the indicated nucleotide sequence accessions.

Supplementary References

Arthur, P.K., Claussen, M., Koch, S., Tarbashevich, K., Jahn, O., and Pieler, T. (2009). Participation of Xenopus Elr-type Proteins in Vegetal mRNA Localization during Oogenesis. J Biol Chem *284*, 19982-19992.

Berekelya, L.A., Ponomarev, M.B., Luchinskaya, N.N., and Belyavsky, A.V. (2003). Xenopus Germes encodes a novel germ plasm-associated transcript. Gene expression patterns : GEP *3*, 521-524.

Betley, J.N., Frith, M.C., Graber, J.H., Choo, S., and Deshler, J.O. (2002). A ubiquitous and conserved signal for RNA localization in chordates. Curr Biol *12*, 1756-1761.

Birsoy, B., Berg, L., Williams, P.H., Smith, J.C., Wylie, C.C., Christian, J.L., and Heasman, J. (2005). XPACE4 is a localized pro-protein convertase required for mesoderm induction and the cleavage of specific TGFbeta proteins in Xenopus development. Development *132*, 591-602.

Claussen, M., Horvay, K., and Pieler, T. (2004). Evidence for overlapping, but not identical, protein machineries operating in vegetal RNA localization along early and late pathways in Xenopus oocytes. Development *131*, 4263-4273.

Colozza, G., and De Robertis, E.M. (2014). Maternal syntabulin is required for dorsal axis formation and is a germ plasm component in Xenopus. Differentiation *88*, 17-26.

Cuykendall, T.N., and Houston, D.W. (2009). Vegetally localized Xenopus trim36 regulates cortical rotation and dorsal axis formation. Development *136*, 3057-3065.

D'Souza, A., Lee, M., Taverner, N., Mason, J., Carruthers, S., Smith, J.C., Amaya, E., Papalopulu, N., and Zorn, A.M. (2003). Molecular components of the endoderm specification pathway in Xenopus tropicalis. Dev Dyn *226*, 118-127.

Devic, E., Paquereau, L., Rizzoti, K., Monier, A., Knibiehler, B., and Audigier, Y. (1996). The mRNA encoding a beta subunit of heterotrimeric GTP-binding proteins is localized to the animal pole of Xenopus laevis oocyte and embryos. Mech Dev *59*, 141-151.

Houston, D.W., Zhang, J., Maines, J.Z., Wasserman, S.A., and King, M.L. (1998). A Xenopus DAZ-like gene encodes an RNA component of germ plasm and is a functional homologue of Drosophila boule. Development *125*, 171-180.

Hudson, C., and Woodland, H.R. (1998). Xpat, a gene expressed specifically in germ plasm and primordial germ cells of Xenopus laevis. Mech Dev *73*, 159-168.

Hudson, J.W., Alarcon, V.B., and Elinson, R.P. (1996). Identification of new localized RNAs in the Xenopus oocyte by differential display PCR. Dev Genet *19*, 190-198.

Kaneshiro, K., Miyauchi, M., Tanigawa, Y., Ikenishi, K., and Komiya, T. (2007). The mRNA coding for Xenopus glutamate receptor interacting protein 2 (XGRIP2) is maternally transcribed, transported through the late pathway and localized to the germ plasm. Biochem Biophys Res Commun *355*, 902-906.

King, M.L. (ed.) (1995). mRNA localization during frog oogenesis. Landes Publishing: Austin, TX.

Kloc, M., Dougherty, M.T., Bilinski, S., Chan, A.P., Brey, E., King, M.L., Patrick, C.W., Jr., and Etkin, L.D. (2002). Three-Dimensional Ultrastructural Analysis of RNA Distribution within Germinal Granules of Xenopus. Dev Biol *241*, 79-93.

Kloc, M., Reddy, B.A., Miller, M., Eastman, E., and Etkin, L.D. (1991). x121: a localized maternal transcript in Xenopus laevis. Mol Reprod Dev *28*, 341-345.

Kress, T.L., Yoon, Y.J., and Mowry, K.L. (2004). Nuclear RNP complex assembly initiates cytoplasmic RNA localization. J Cell Biol *165*, 203-211.

Ku, M., and Melton, D.A. (1993). Xwnt-11: a maternally expressed Xenopus wnt gene. Development *119*, 1161-1173.

Le Goff, C., Laurent, V., Le Bon, K., Tanguy, G., Couturier, A., Le Goff, X., and Le Guellec, R. (2006). pEg6, a spire family member, is a maternal gene encoding a vegetally localized mRNA in Xenopus embryos. Biol Cell *98*, 697-708.

MacArthur, H., Houston, D.W., Bubunenko, M., Mosquera, L., and King, M.L. (2000). DEADSouth is a germ plasm specific DEAD-box RNA helicase in Xenopus related to eIF4A. Mech Dev *95*, 291-295.

Meyer, D., Durliat, M., Senan, F., Wolff, M., Andre, M., Hourdry, J., and Remy, P. (1997). Ets-1 and Ets-2 proto-oncogenes exhibit differential and restricted expression patterns during Xenopus laevis oogenesis and embryogenesis. Int J Dev Biol *41*, 607-620.

Oberman, F., and Yisraeli, J.K. (1995). Two protocols for nonradioactive in situ hybridization to Xenopus oocytes. Trends in genetics : TIG *11*, 83-84.

Ossipova, O., He, X., and Green, J. (2002). Molecular cloning and developmental expression of Par-1/MARK homologues XPar-1A and XPar-1B from Xenopus laevis. Gene expression patterns : GEP *2*, 145-150.

Pannese, M., Cagliani, R., Pardini, C.L., and Boncinelli, E. (2000). Xotx1 maternal transcripts are vegetally localized in Xenopus laevis oocytes. Mech Dev *90*, 111-114.

Reddy, B.A., Kloc, M., and Etkin, L.D. (1992). The cloning and characterization of a localized maternal transcript in Xenopus laevis whose zygotic counterpart is detected in the CNS. Mech Dev *39*, 143-150.

Roel, G., van den Broek, O., Spieker, N., Peterson-Maduro, J., and Destree, O. (2003). Tcf-1 expression during Xenopus development. Gene expression patterns : GEP *3*, 123-126.

Schroeder, K.E., and Yost, H.J. (1996). Xenopus poly (A) binding protein maternal RNA is localized during oogenesis and associated with large complexes in blastula. Dev Genet *19*, 268-276.

Sekizaki, H., Takahashi, S., Tanegashima, K., Onuma, Y., Haramoto, Y., and Asashima, M. (2004). Tracing of Xenopus tropicalis germ plasm and presumptive primordial germ cells with the Xenopus tropicalis DAZ-like gene. Dev Dyn *229*, 367-372.

Tarbashevich, K., Dzementsei, A., and Pieler, T. (2011). A novel function for KIF13B in germ cell migration. Dev Biol *349*, 169-178.
Tarbashevich, K., Koebernick, K., and Pieler, T. (2007). XGRIP2.1 is encoded by a vegetally localizing, maternal mRNA and functions in germ cell development and anteroposterior PGC positioning in Xenopus laevis. Dev Biol *311*, 554-565.

Vick, P., Schweickert, A., Weber, T., Eberhardt, M., Mencl, S., Shcherbakov, D., Beyer, T., and Blum, M. (2009). Flow on the right side of the gastrocoel roof plate is dispensable for symmetry breakage in the frog Xenopus laevis. Dev Biol *331*, 281-291.

Wang, H.W., Fang, J.S., Kuang, X., Miao, L.Y., Wang, C., Xia, G.L., King, M.L., and Zhang, J. (2012). Activity of long-chain acyl-CoA synthetase is required for maintaining meiotic arrest in Xenopus laevis. Biology of reproduction *87*, 74.

Weeks, D.L., and Melton, D.A. (1987a). A maternal mRNA localized to the animal pole of Xenopus eggs encodes a subunit of mitochondrial ATPase. Proc Natl Acad Sci U S A *84*, 2798-2802.

Weeks, D.L., and Melton, D.A. (1987b). A maternal mRNA localized to the vegetal hemisphere in Xenopus eggs codes for a growth factor related to TGF-beta. Cell *51*, 861-867.

Wessely, O., and De Robertis, E.M. (2000). The Xenopus homologue of Bicaudal-C is a localized maternal mRNA that can induce endoderm formation. Development *127*, 2053-2062.

Whitfield, T., Heasman, J., and Wylie, C. (1993). XLPOU-60, a Xenopus POU-domain mRNA, is oocyte-specific from very early stages of oogenesis, and localised to presumptive mesoderm and ectoderm in the blastula. Dev Biol *155*, 361-370.

Zearfoss, N.R., Chan, A.P., Wu, C.F., Kloc, M., and Etkin, L.D. (2004). Hermes is a localized factor regulating cleavage of vegetal blastomeres in Xenopus laevis. Dev Biol *267*, 60-71.

Zhang, J., and King, M.L. (1996). Xenopus VegT RNA is localized to the vegetal cortex during oogenesis and encodes a novel T-box transcription factor involved in mesodermal patterning. Development *122*, 4119-4129.

Zhang, Q., Yaniv, K., Oberman, F., Wolke, U., Git, A., Fromer, M., Taylor, W.L., Meyer, D., Standart, N., Raz, E., and Yisraeli, J.K. (1999). Vg1 RBP intracellular distribution and evolutionarily conserved expression at multiple stages during development. Mech Dev *88*, 101-106.

Zhou, Y., Zhang, J., and King, M.L. (2004). Polarized distribution of mRNAs encoding a putative LDL receptor adaptor protein, xARH (autosomal recessive hypercholesterolemia) in Xenopus oocytes. Mech Dev *121*, 1249-1258.