Supporting Information

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SI Materials and Methods

Protein Expression and Purification. MBP-MEX-3 (45–205) was expressed and purified from BL21 (DE3) Gold (Stratagene) *Escherichia coli*. A liquid culture grown at 37 °C was induced at midlog phase with 1 mM isopropyl 1-thio-β-D-galactopyranoside and grown for 3 h before harvesting cells. Cells were lysed and purified using an amylose (New England Biolabs) affinity column, followed by HiTrap Q and source 15Q (GE Healthcare) ion exchange columns at 4 °C. Purified MEX-3 was dialyzed into storage buffer (25 mM Tris, pH 8.0, 25 mM NaCl, 2 mM DTT) and stored at 4 °C.

In Vitro RNA Selection. The DNA library was amplified from the template 5′-GGGAAGATCTCGACCAGAAG-(N30)-TATGTGCGTCTACATGGATCCTCA with a forward (5′-CGGAATTCTAATACGACTCACTATAGGGAAGATCTCGACCAGAAG-3′) and reverse (5′-TGAGGATCCATGTAGACGCACATA-3′) primer pair using three cycles of PCR to make the initial double stranded DNA pool. RNA was transcribed from 10 pmol of starting DNA template and purified as described in ref. 1.

Binding reactions were carried out in 200 μl of selection buffer (25 mM Hepes, pH 7.4, 200 mM NaCL, 75 μg/ml tRNA, 0.01% Igepal CA-630). Between 1–800 nM of purified MBP-MEX-3 (45–205) was equilibrated with transcribed RNA in selection buffer for 1 h and then mixed with amylose resin (New England Biolabs). The amount of protein used was progressively decreased in later rounds to increase stringency. Unbound RNA was separated using a Zeba spin column (Pierce) and washed 2–8 times with 200 μl of selection buffer. MEX-3 bound to RNA was eluted from amylose resin with 10 mM maltose in selection buffer. All reactions were carried out at room temperature. Recovered RNA was phenol/chloroform extracted, ethanol precipitated, and resuspended in 10 μl of TE buffer. RNA was then reverse transcribed and amplified with 10–15 rounds of PCR using the SuperScript III One-Step RT-PCR kit with Platinum Taq (Invitrogen). The new DNA pool was in vitro transcribed to generate the next RNA pool to be used.

A total of 7 rounds of selection were performed. To increase the selection stringency, rounds 6 and 7 included two washes where the salt concentration was increased to 500 mM NaCl. The DNA from pool 7 was subcloned into pUC18 using EcoRI and BamHI restriction enzymes, and DNA from individual isolates was sequenced.

Electrophoretic Mobility Shift Assay. Varying concentrations of purified MEX-3 were equilibrated with 3 nM labeled RNA in equilibration buffer (0.01% IGEPAL, 0.01 mg/ml tRNA, 10 mM Tris, pH 8.0, 100 mM NaCl) for 3 h. Samples were loaded on a 5% slab polyacrylamide gel in 0.5X TBE buffer. Both the gel and running buffer were prechilled to 4 °C before running each sample. The gels were run for 60 min at 120 volts and immediately scanned using a fluor-imager (Fujifilm FLA-5000) with a blue laser at 473 nm.

Worm Strain Generation. The unspliced coding region and 3′UTR of *mex-3* was PCR amplified from genomic DNA containing NheI and NsiI restriction sites and subcloned into the plasmid pJH4.52 (a generous gift of Dr. Geraldine Seydoux, Johns Hopkins University) using the NsiI and SpeI sites to generate the construct pJMP001 (*Ppie-1*::GFP::MEX-3::*mex-3* 3′UTR). The cloned *mex-3* insert was sequenced confirming 794 bp from the 5′ end and 191 bp from the 3′ end. The transgenic worm was made using biolistic transformation with *unc-119* rescue (2, 3). An equal mixture of pJMP001 and pDEST-DD03, harboring the *unc-119* gene (a gift of Dr. Marian Walhout, University of Massachusetts Medical School), was cobombarded to generate the transgenic worm (*sprIs1*[*Ppie-1*::*GFP*::*MEX-3*::*mex-3 3′UTR*, *unc-119*(+)]).

The *nos-2* and *glp-1* 3′UTR reporter constructs were made using the Gateway system (4). The 3′UTR of *nos-2* was PCR amplified from genomic DNA containing *attB* sites and recombined into pDONRP2R-P3 with Gateway BP Clonase II Enzyme Mix (Invitrogen) to generate the 3′UTR entry clone pJMP015. Quick-change was performed on this plasmid to generate the *nos-2* MREmut 3′UTR entry clone pJMP044. The *glp-1* 3′UTR was amplified off of pCM5.40 (provided by the third generation Seydoux Lab Vector kit) with *attB* sites and used to generate pBMF3.1. Multisite Gateway reactions were performed using Gateway LR Clonase II Plus Enzyme Mix (Invitrogen) with the plasmids pCG142 or pCM1.111, pCM1.35 (provided by the third generation Seydoux Lab Vector kit), pCFJ150 (a generous gift from Dr. Erik Jorgensen, University of Utah), and either pJMP015 or pJMP044 to generate pJMP046 (*Ppie-1*::*GFP*::*H2B*::*nos-2 3′UTR*), pJMP049 (*Ppie-1*::*GFP*::H2*B*::*nos-2 MREmut 3′UTR*), and pBMF4.1 (*Pmex-5*::*GFP*::*H2B*::*glp-1 3′UTR*). The plasmids pJMP046, pJMP049, or pBMF4.1 were used along with pCFJ90, pCFJ104, and pJL43.1 and coinjected into the strain EG4322 to generate single copy gene insertions (*sprSi1*[*Ppie-1*::*GFP*::*histone H2B*::*nos-2 3′UTR cb-unc-119*(+)] *II*; *sprSi2*[*Ppie-1*::*GFP*::*histone H2B*::*nos-2 MREmut 3′UTR cb-unc-119*(+)] *II*; *sprSi3*[*Pmex-5*::*GFP*::*histone H2B*::*glp-1 3′UTR cb-unc-119*(+)] *II*) following the direct insertion protocol described (5). The integrated worm strain *sprSi3* was confirmed by PCR and strains *sprSi1* and *sprSi2* were confirmed by PCR and sequencing.

RNAi experiments were performed using *mex-3* RNAi food (a generous gift from Dr. Craig Mello, University of Massachusetts Medical School). The 3′UTR reporter strains (JH2436, JH2311, JH2236, JH2223, JH2261, JH2324, JH2377, JH2381, and JH2207) used in the *mex-3* RNAi screen were obtained from the Caenorhabditis Genetics Center (CGC). All strains were grown at 25 °C with the exception of *sprIS1*, *sprSI3*, and JH2261, which were grown at room temperature (≈20 °C). Room temperature incubation was done to decrease the stability of GFP::histone H2B reducing background fluorescence that does not recapitulate the expression of endogenous protein. DIC and GFP images were collected with live specimens using a Zeiss Axioskop microscope with 40× or 100× objectives. All GFP::H2B reporter images were taken with the same exposure time and contrasted equally for each strain.

1. Loughlin FE, et al. (2009) The zinc fingers of the SR-like protein ZRANB2 are single-stranded RNA-binding domains that recognize 5′ splice site-like sequences. *Proc Natl Acad Sci USA* 106:5581–5586.
2. Praitis V (2006) Creation of transgenic lines using microparticle bombardment methods. *Methods Mol Biol* 351:93–107.
3. Praitis V, Casey E, Collar D, Austin J (2001) Creation of low-copy integrated transgenic lines in *Caenorhabditis elegans*. *Genetics* 157:1217–1226.
4. Walhout AJ, et al. (2000) GATEWAY recombinational cloning: Application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol* 328:575–592.

5. Frokjaer-Jensen C, et al. (2008) Single-copy insertion of transgenes in *Caenorhabditis elegans*. *Nat Genet* 40:1375–1383.
6. Draper BW, Mello CC, Bowerman B, Hardin J, Priess JR (1996) MEX-3 is a KH domain protein that regulates blastomere identity in early *C. elegans* embryos. *Cell* 87:205–216.
7. D'Agostino I, Merritt C, Chen PL, Seydoux G, Subramaniam K (2006) Translational repression restricts expression of the *C. elegans* Nanos homolog NOS-2 to the embryonic germline. *Dev Biol* 292:244–252.

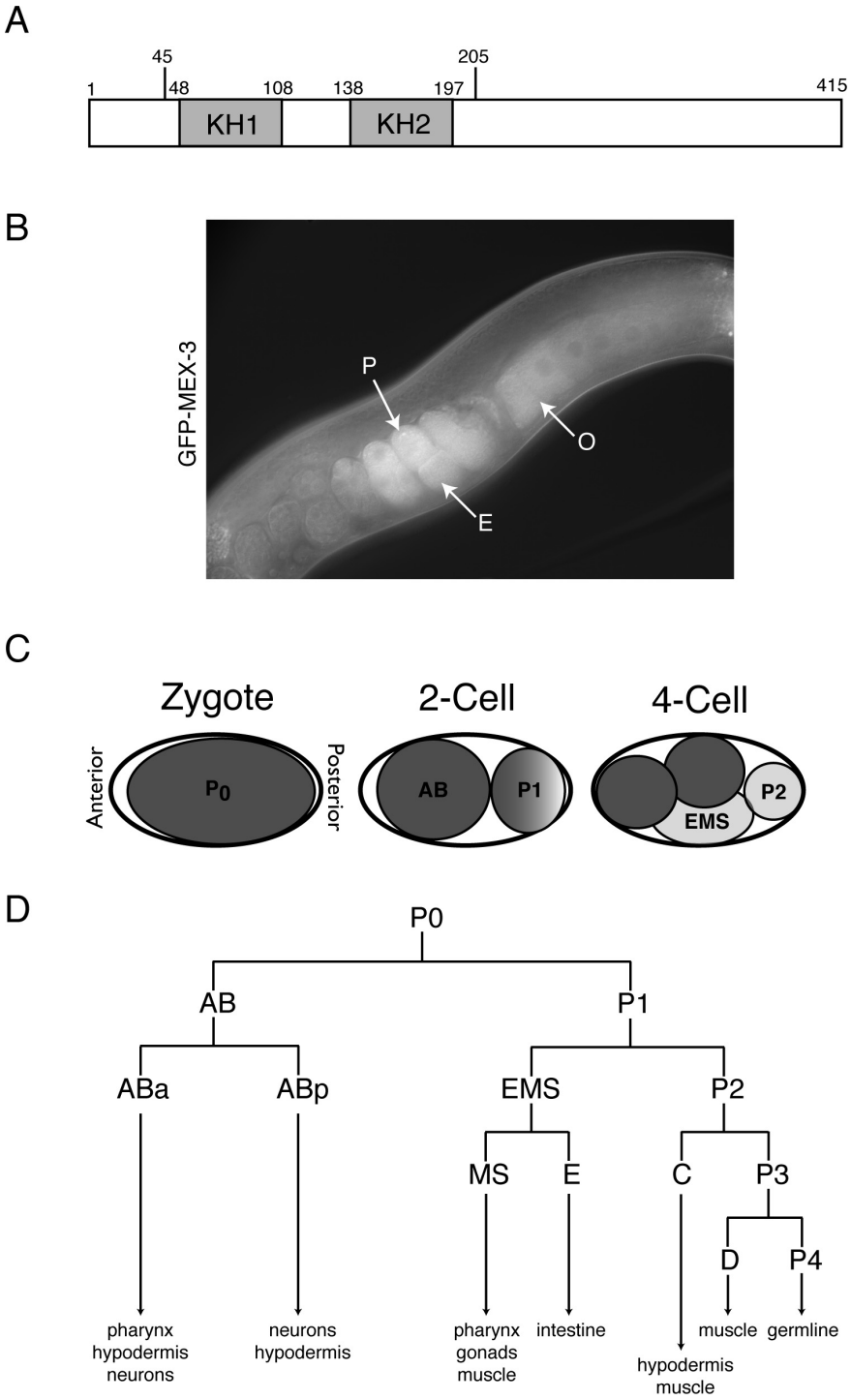
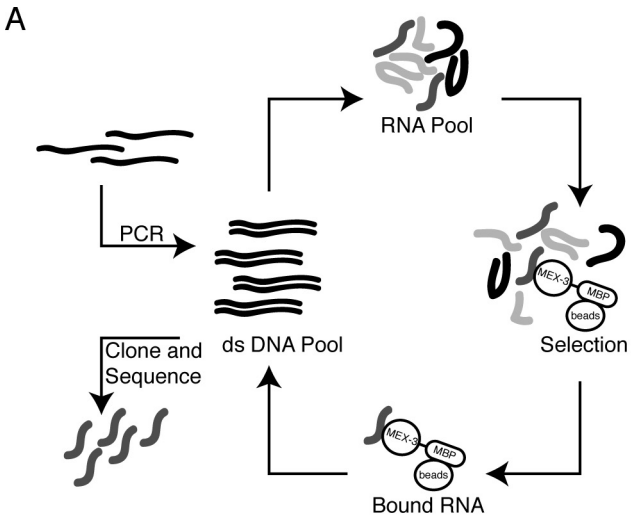
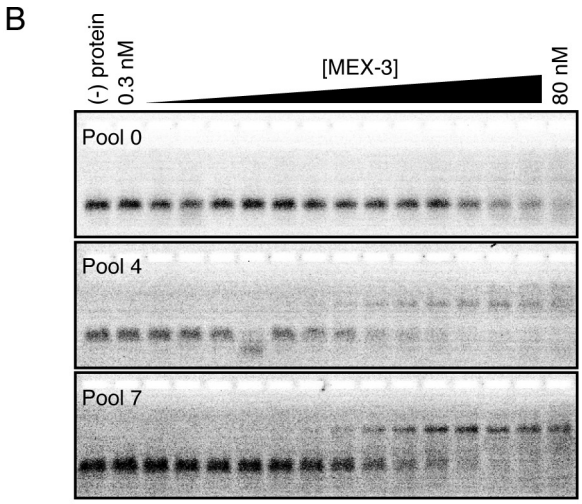


Fig. S1. MEX-3 in *C. elegans* development. (*A*) Domain structure of the RNA binding protein MEX-3. The two KH domains (KH1 and KH2) are shaded dark gray. The numbers represent the primary amino acid sequence. (*B*) A transgenic worm expressing GFP::MEX-3 (GFP and Nomarski overlay). This strain recapitulates most of the expression pattern of endogenous MEX-3 (6), being translated in oocytes (O), early embryos (E), and localized to *p*-granules (P). However, expression is not observed in the distal end of the germline, and polarized expression from the 2–4 cell stage is less pronounced than endogenous MEX-3. (*C*) A model representing the MEX-3 expression pattern in the 1–4 cell embryo. At the end of the 2-cell stage MEX-3 is restricted predominantly to the anterior blastomere (AB), shown in dark gray. The blastomeres AB, EMS, and P are labeled. (*D*) Cellular lineage of early embryogenesis.





C

	Sequence	Copy #
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	GTTGAGGGGTTGTGGAGGGAAAGGAGTCTA	1
	GTCGGTCACGTCGGATTAAATTCGTCGTTA	1
	GTAGGGATTATCACGGGCTTTGCTCCCGTTA	1
	GTAACGAGAGGGTTGTGGTGGGAAAGTTTT	1
	GCCCACATCTCTTCGGAGGTGGCGGTTGAC	1
seq.44	**CACAGTAATATCGCGGTCTTATTAGCATCCG**	1
seq.63	**ATTGGGGGTGGGGTAGGGTGGGTAAGTGGT**	1
	ATTGGGGGTGGGGTAGGGTGGGAAGTTGGA	1
	ATTGGGGGCGGTGGTGGGCGGGGATTGAACG	1
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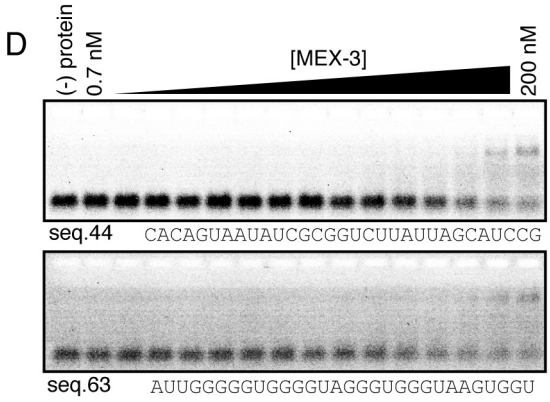


Fig. S2. In vitro selection of MEX-3 RNA aptamers. (*A*) A schematic of the affinity elution-based in vitro selection experiment. (*B*) An EMSA of MEX-3 bound to fluorescently labeled Pool 0, Pool 4, or Pool 7. (*C*) Thirteen sequences from the selection, which are unrelated to the two main classes of sequences. The sequences that are bold were used in EMSA experiments. (*D*) An EMSA experiment with fluorescently labeled *seq.44* and *seq.63*.

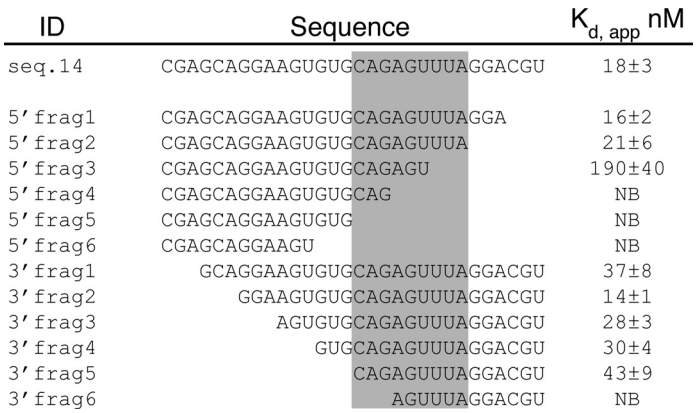
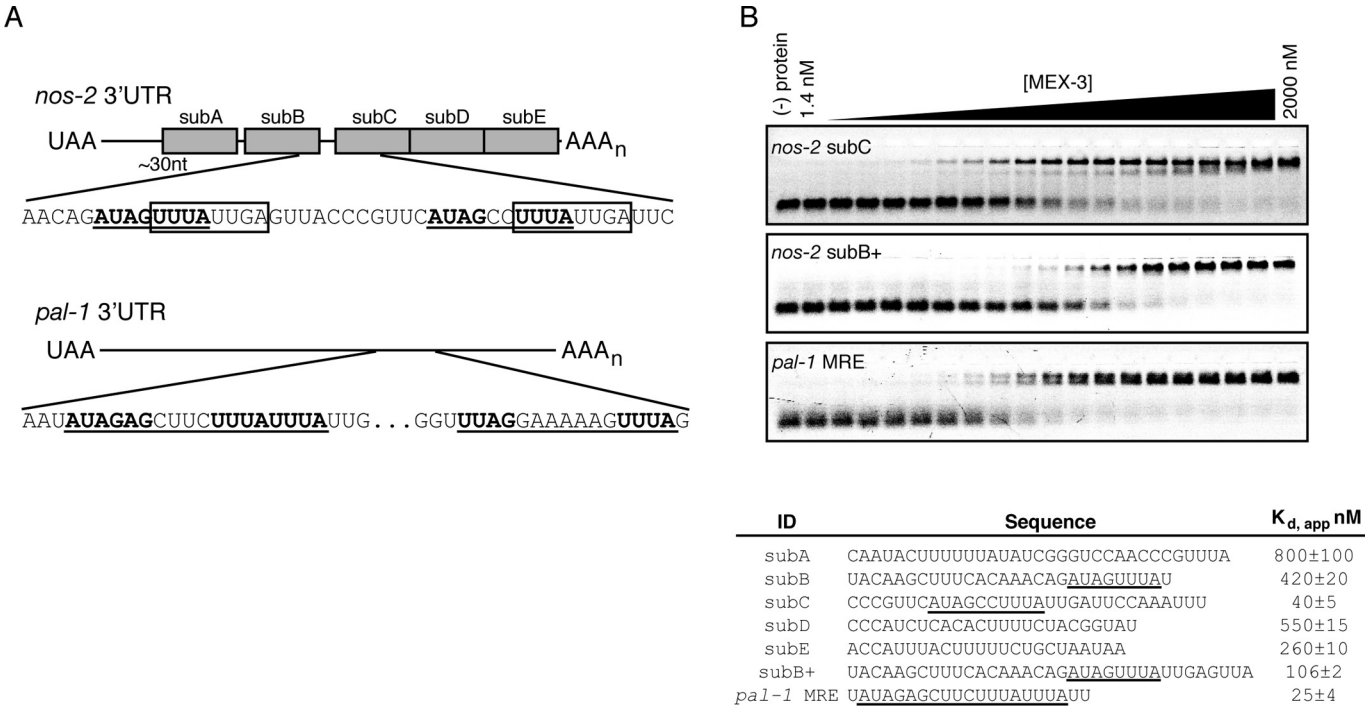


Fig. S3. Truncation analysis of *seq.14* RNA. Binding data are shown for 3′ and 5′ truncations of *seq.14* RNA. The $K_{d,app}$ for each sequence is given. NB stands for no binding observed. The shaded region represents the nucleotides that are critical for MEX-3 binding.



ID	Sequence	$K_{d,app}$ nM
subA	CAAUACUUUUUUAUAUCGGGUCCAACCCGUUUA	800±100
subB	UACAAGCUUUCACAAACAGAUAGUUUAU	420±20
subC	CCCGUUCAUAGCCUUUAUUGAUUCCAAAUUU	40±5
subD	CCCAUCUCACACUUUUCUACGGUAU	550±15
subE	ACCAUUUACUUUUUCUGCUAAUAA	260±10
subB+	UACAAGCUUUCACAAACAGAUAGUUUAUUGAGUUA	106±2
pal-1 MRE	UAUAGAGCUUCUUUAUUUAUU	25±4

Fig. S4. MEX-3 binds specifically to the MRE of *nos-2* and *pal-1*. (*A*) Analysis of the *nos-2* 3′UTR reveals two MRE sites (underlined) within the subB and subC regulatory elements. The half-sites are shown in bold. The conserved repeat element is boxed (7). The *pal-1* 3′UTR is shown with each MRE underlined. (*B Upper*) EMSA experiments for *nos-2* subC, *nos-2* subB+, and the *pal-1* MRE are shown. (*Lower*) Binding data of MEX-3 with each *nos-2* regulatory element (subA–subE and subB+) and the *pal-1*MRE site are shown. The MRE site within each sequence is underlined. The $K_{d,app}$ is given for each sequence.

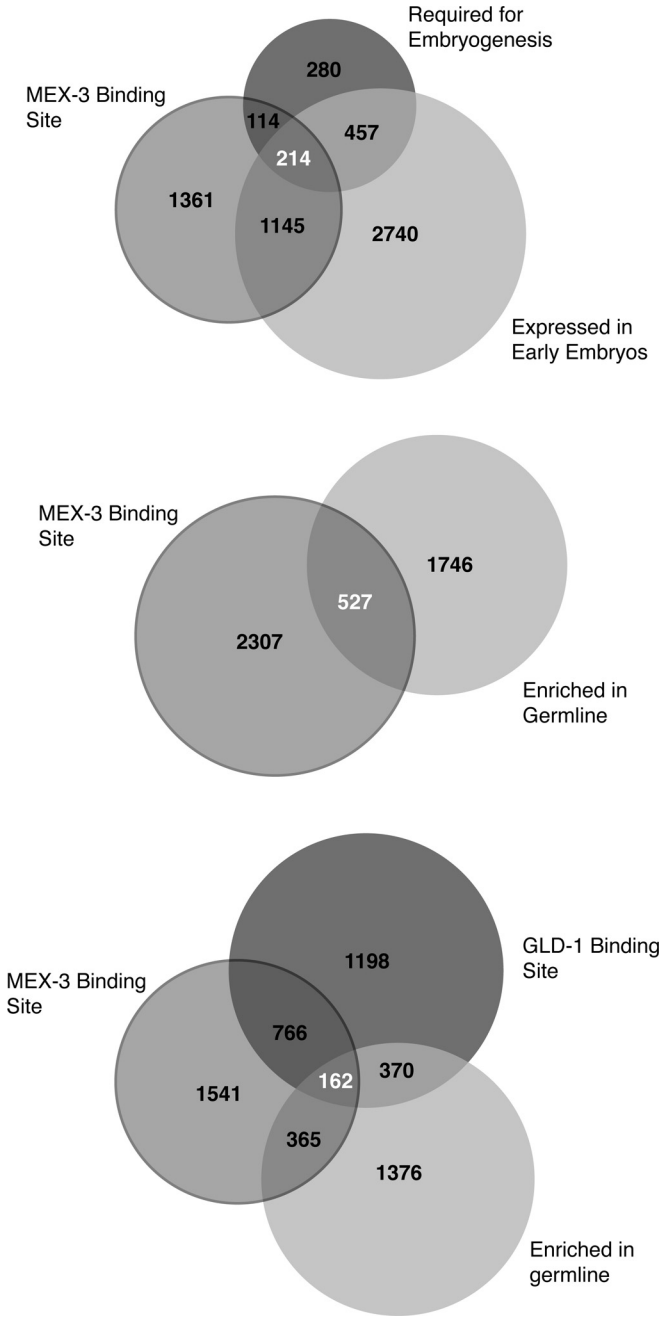


Fig. S5. Bioinformatic analysis of genes that contain an MRE within their 3′UTR. Genes that harbor a MEX-3 binding site were compared with transcripts that are expressed in early embryos, required for embryogenesis, enriched in the germline, and/or contain a GLD-1 binding site.

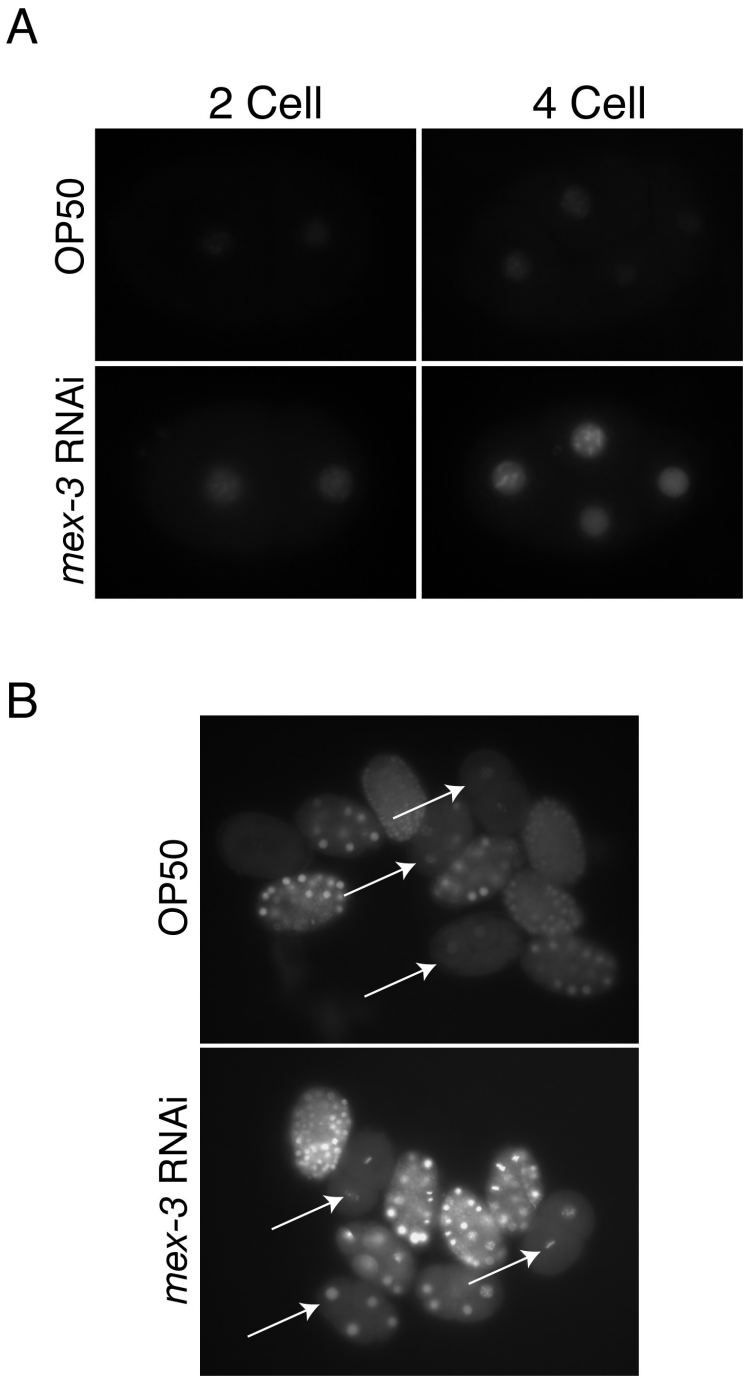


Fig. S6. A glp-1 3′UTR reporter is dependent upon MEX-3 for its expression pattern. (*A*) There is an increase in GFP expression in early embryos when *mex-3* mRNA is knocked down by RNAi. The image compares a 2- and 4-cell embryo when grown on OP50 food or *mex-3* RNAi food. (*B*) A group of embryos expressing the *glp-1* 3′UTR reporter are shown. Arrows are pointing to 2- and 4-cell embryos.

Other Supporting Information Files

Table S1 (XLS)
Table S2 (PDF)
Table S3 (PDF)