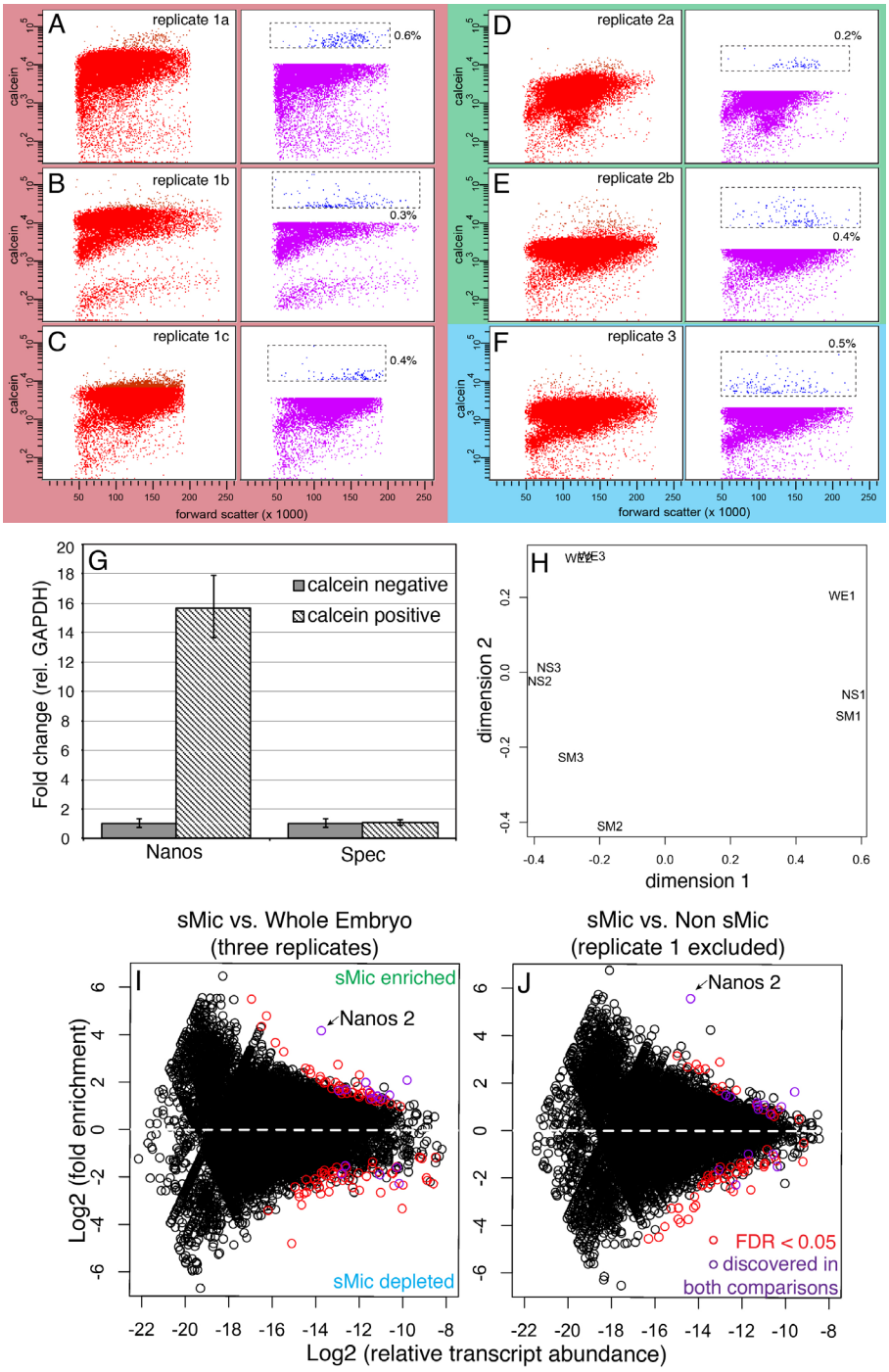

Figure S1, related to Figures 1. FACS isolation of sMics and transcriptomic analysis. (A-F) Scatter plot representations of FACS sorts for three biological replicates. Replicate 1 (A-C, red background) consisted of three sorts of embryos derived from distinct parental crosses. Replicate 2 (D-E, green background) consisted of two sorts from two parental crosses. Replicate 3 (F, blue background) was sorted from a single parental cross. Red points indicate the total cell population, blue points indicate the collected calcein+ cells, and purple points indicate collected calcein— cells. Percentages represent fraction of collected calcein+ cells relative to the whole population. (G) qPCR for Nanos indicates enrichment of sMics in the calcein+ fraction, as opposed for Spec, an ectodermal negative control. (H) Multidimensional scaling (MDS) analysis of replicate transcriptomes for isolated sMics (SM1-3), non-sMics (NS1-3), and whole embryo (WE1-3). Replicate 2 and 3 samples were highly related with whole embryo, sMic, and non-sMic transcriptomes clustering separately. Replicate 1 samples are outliers, showing low relatedness to the others in both dimensions, and poor separation between SM and NS samples. This variation likely points to issues with sample handling (RNA collection, or RNA-seq sample preparation) or less efficient calcein labeling. In subsequent differential expression analysis, we therefore made comparisons both with and without including Replicate 1 samples. (I,J) Smear plot depiction of differentially expressed genes. (I) sMic vs. Whole Embryo with replicate 1 included, and (J) sMic vs. non-sMic with replicate 1 excuded are considered here. Transcripts are represented by open circles, with fold enrichment in the sMics on the y-axis and relative transcript abundance on the x-axis. Transcripts meeting a significance threshold of 0.05 (false discovery rate, FDR) are in red; transcripts identified in both comparisons are in purple. Arrows indicate the previously identified sMic factor Nanos2.

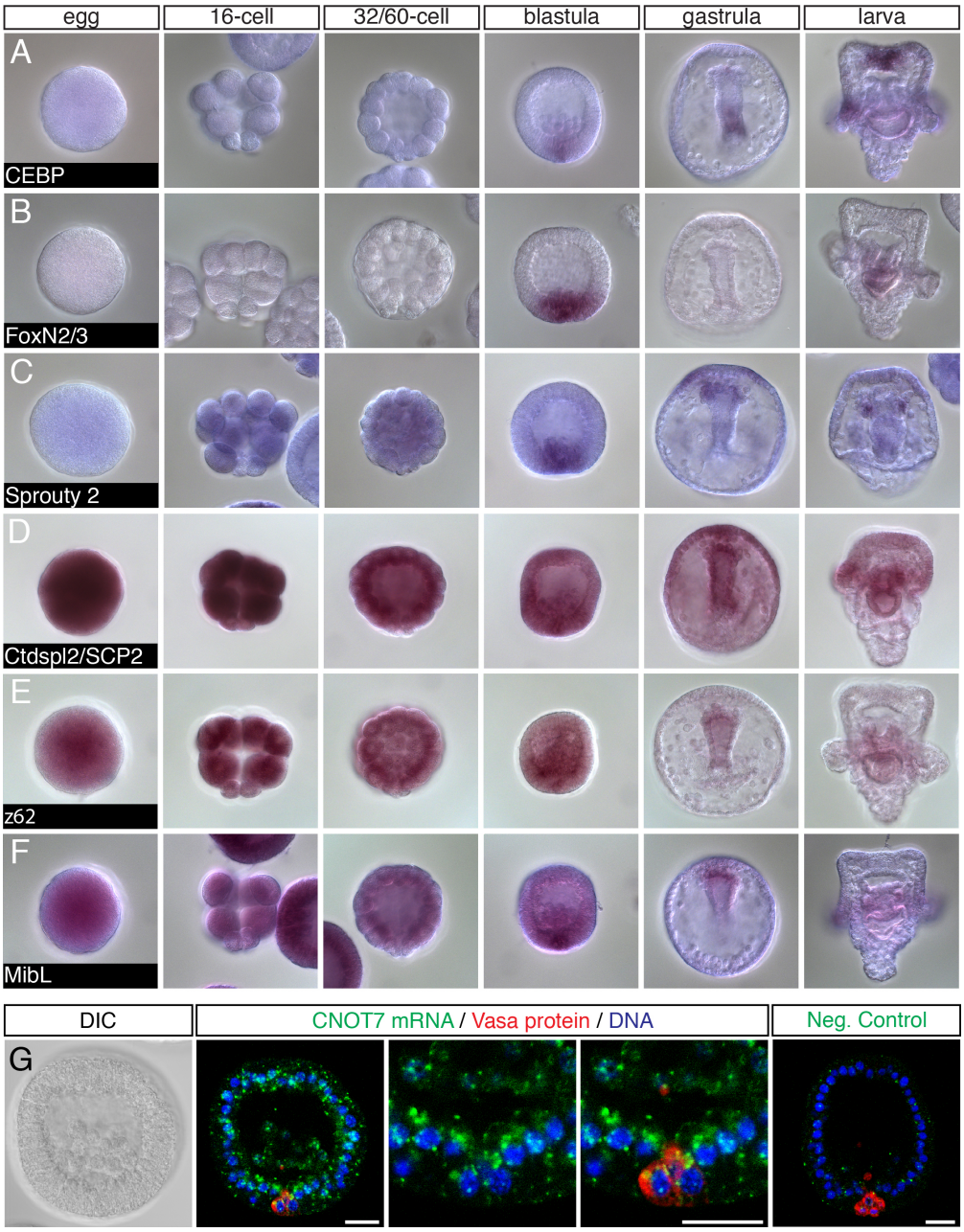

Figure S2, related to Figures 1 and 3. WMISH for selected transcripts. (A-F) WMISH for sMic-enriched transcripts identified through differential expression analysis. (G) The S. purpuratus genome contains two CNOT-related nucleases: CNOT6 and CNOT7. Unlike CNOT6, which is specifically depleted in the sMics, CNOT7 transcript (green) is ubiquitously distributed. sMics are labeled in red with vasa antibody.

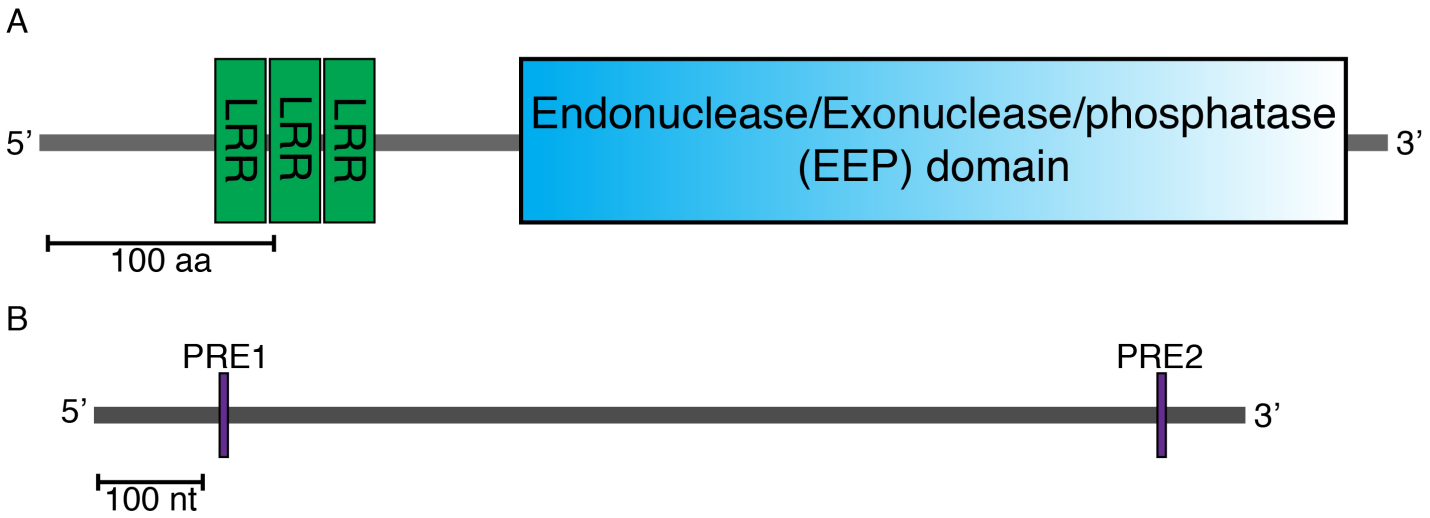

Figure S3, related to Figure 4. Organization of the CNOT6 open reading frame and 3'UTR. (A) Domain organization of CNOT6 predicted by PFAM (Punta et al., 2012). (B) Schematic of PRE motifs within the CNOT6 3'UTR and full-length sequence.

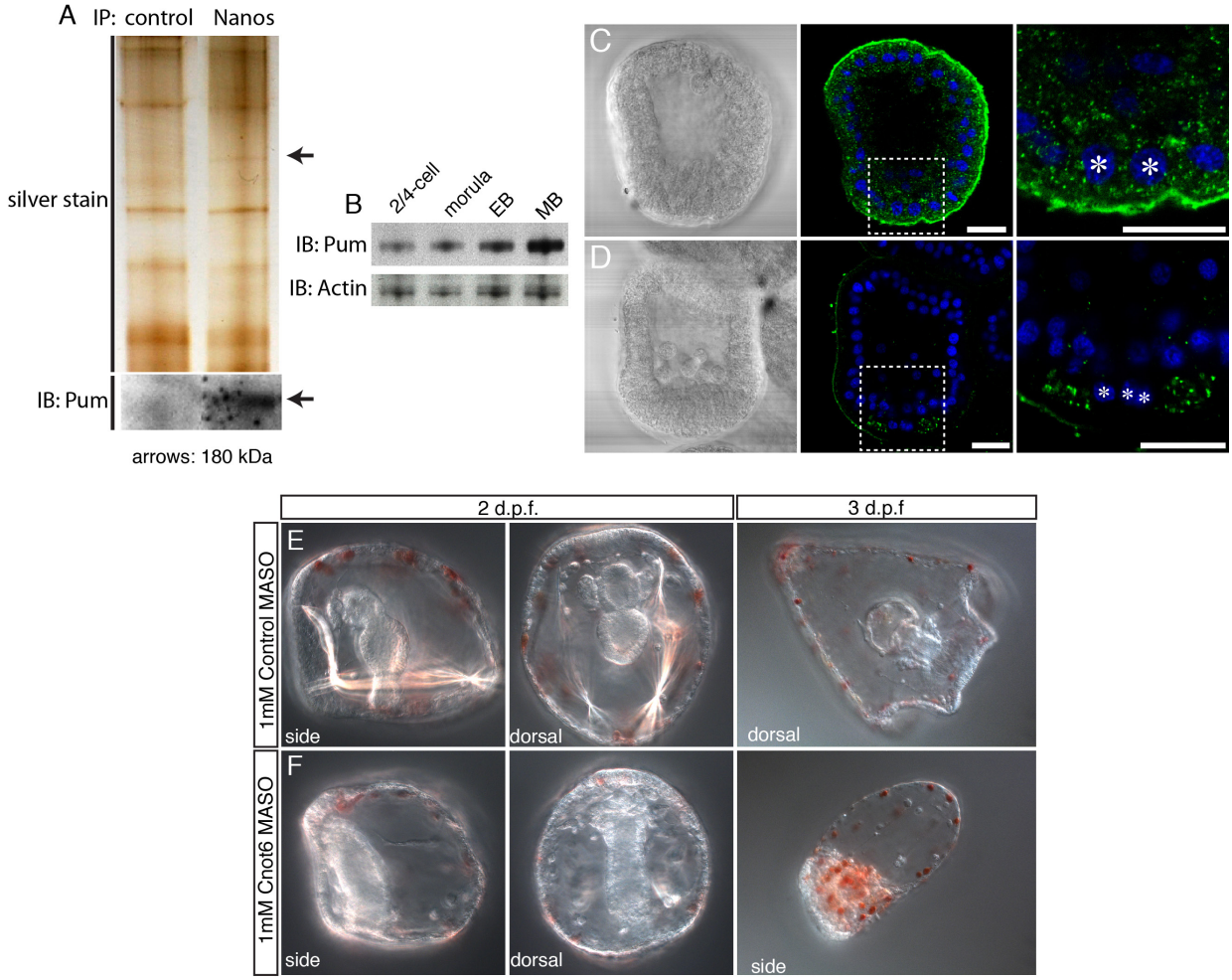

Figure S4, related to Figure 3 and 4. (A) Association of Nanos and Pumilio. Compared to Vasa antibody control, Nanos antibody specifically pulls down a 180 kDa band recognized by silver stain and Pumilio immunoblot. (B) Temporal expression of Pumilio. Pumilio is detectable in early embryos, and increases in abundance through early blastulae (EB) and mesenchyme blastulae (MB) stages. (C,D) Spatial localization of Pumilio protein. Pumilio is detectable in granule structures in all cells, including the sMics, of early blastula (C), but becomes highly enriched in the Veg2 mesodermal precursors after primary mesenchyme ingression (D). Asterisks indicate sMic nuclei. (E,F) Injection of 1mM CNOT6 MASO 1 results in failure to produce skeletons or a tripartite gut. By 3 days, the endoderm degenerates into mesenchyme-like cells.

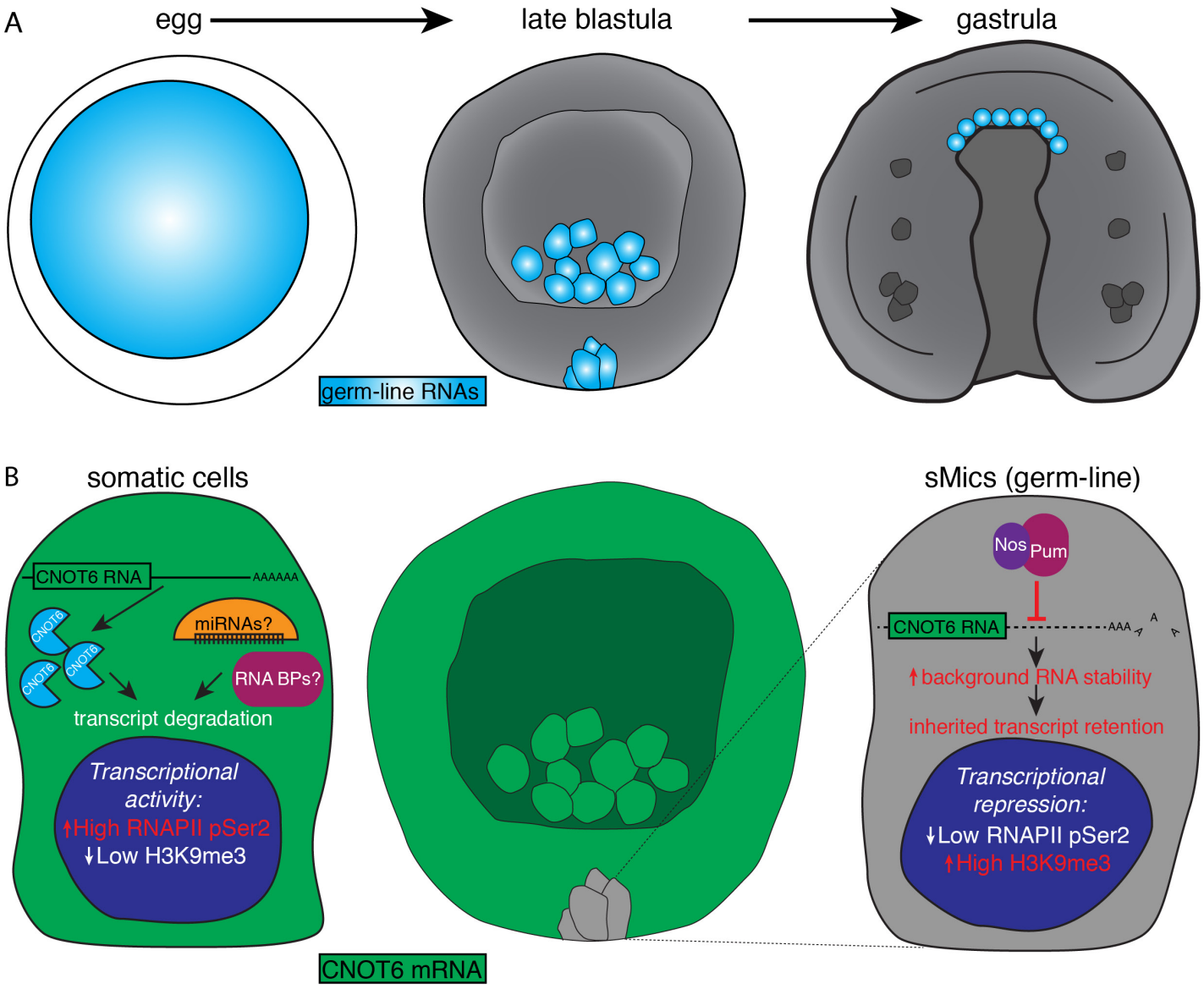

Figure S5. Model. Time capsule model for germ line development. (A) Maternally deposited mRNAs are coordinately degraded in somatic cells at blastula/gastrula stage but stabilized in the sMics. (B) Mechanism for sMic segregation. CNOT6 transcript is in green, and is present in all cells except sMics (gray), where Nanos/Pumilio (Nos/Pum) represses it. This repression creates a stable environment for inherited RNA. Conversely, CNOT6 protein accumulates in somatic cells, where it may act in parallel with and/or downstream of miRNA and RNA binding protein (BP) mediated degradation.

Supplementary References

Punta, M., Coggill, P. C., Eberhardt, R. Y., Mistry, J., Tate, J., Boursnell, C., Pang, N., Forslund, K., Ceric, G., Clements, J., et al. (2012). The Pfam protein families database. *Nucleic Acids Res* **40**, D290–301.

Table S1, related to Figure 1. Gene set enrichment analysis of the union sets of 78 sMic enriched transcripts, and 152 sMic-depleted transcripts. Analysis was performed using the topGO Bioconductor package. *P*-values are by Fisher's exact test.

sMic-enriched categories			sMic-depleted categories		
GO ID	**Term**	**p-val**	**GO ID**	**Term**	**p-val**
0003676	nucleic acid binding	4.40E-05	0005198	structural molecule activity	4.50E-08
0000988	protein binding transcription factor act...	0.0015	0032561	guanyl ribonucleotide binding	0.0042
0000989	transcription factor binding transcripti...	0.0015	0019001	guanyl nucleotide binding	0.0045
0003712	transcription cofactor activity	0.0015	0005201	extracellular matrix structural constitu...	0.0053
0043565	sequence-specific DNA binding	0.0049	0015399	primary active transmembrane transporter...	0.01
0008134	transcription factor binding	0.006	0015405	P-P-bond-hydrolysis-driven transmembrane...	0.01
0008349	MAP kinase kinase kinase kinase activity	0.0067	0004683	calmodulin-dependent protein kinase acti...	0.016
0043425	bHLH transcription factor binding	0.0067	0004197	cysteine-type endopeptidase activity	0.0212
0043426	MRF binding	0.0067	0022804	active transmembrane transporter activit...	0.0228
0005488	binding	0.0086	0004674	protein serine/threonine kinase activity	0.0237
0035258	steroid hormone receptor binding	0.0178	0003723	RNA binding	0.0287
0003729	mRNA binding	0.0189	0016289	CoA hydrolase activity	0.0368
0035257	nuclear hormone receptor binding	0.0224	0050839	cell adhesion molecule binding	0.0368
0051427	hormone receptor binding	0.0275			
0090079	translation regulator activity, nucleic ...	0.0328			
0003677	DNA binding	0.0389			
0042974	retinoic acid receptor binding	0.0393			
0045182	translation regulator activity	0.0393			
0008092	cytoskeletal protein binding	0.0433			
0032561	guanyl ribonucleotide binding	0.0446			
0046332	SMAD binding	0.0457			
0019001	guanyl nucleotide binding	0.0467			

Table S2. Summary of FACS replicates and RNA extraction yields.

	sMic	non-sMic	whole embryo
Replicate 1 *(3 pooled parental crosses)*	700 ng RNA 113,191 cells	1800 ng RNA 618,511 cells	3800 ng RNA
Replicate 2 *(2 pooled parental crosses)*	1080 ng RNA 213,174 cells	1820 ng RNA 892,329 cells	6700 ng RNA
Replicate 3 *(1 parental cross)*	350 ng RNA 55,571 cells	1278 ng RNA 438,938 cells	1508 ng RNA

Table S3. Helicos run statistics.

Sample Name	Total Filtered Reads	Mean Read Length (nt)	Percent aligned reads	Sequencing Error Rate
WE1	27,035,746	31.21	49.08%	6.95%
SMM1	25,331,121	31.15	44.29%	6.86%
NSM1	30,889,594	31.27	42.25%	7.02%
WE2	14,670,195	33.75	60.12%	5.44%
SMM2	15,096,355	33.28	60.84%	5.49%
NSM2	12,043,418	34.24	57.30%	5.63%
WE3	14,450,706	33.84	54.68%	5.89%
SMM3	8,300,956	33.66	56.06%	5.62%
NSM3	10,630,234	34.15	58.47%	5.69%

Table S4. Primers used for generation of template DNA for ISH probe transcription.

Primer Name	Sequence
Baf250	**F:** TCGACAACCACCACTACCAA **R:** taatacgactcactatagggTGTTGTTCACTCCACCCGTA
CEBP	**F:** AGGGCTGAGGTACAGCAGAA **R:** taatacgactcactatagggCCCTCGACACGTTTCTTTA
FoxN2/3	**F:** CGAATGGACAAAGGACCACT **R:** taatacgactcactatagggCTTGGTGATGGGGTACACT
Sprouty2	**F:** GCTCTGTTCCCTTGAGCAAC **R:** taatacgactcactatagggGCAGGGATCATCCGTACAGT
Ctdspl2/SCP2	**F:** AAGCCACCAATCCTGTGTTC **R:** taatacgactcactatagggCAGAAAGGCACAAGCAATCA
z62	**F:** AGTCTAGCAAATGGCGTCGT **R:** taatacgactcactatagggATGTAACCACATTCGCAGCA
MibL	**F:** CTGGACAGACACCAGAGCAA **R:** taatacgactcactatagggCATCTGCTCCGTGCATAAGA
CNOT6	**F:** GCAGGTGCTAGGTCTGAAGG **R:** taatacgactcactatagggCGAGTTGGAGGAGAAGTTGC
CNOT7	**F:** TGCCAACTCAAACCAATGAA **R:** taatacgactcactatagggGCACCCTGGTTAAAAGGTCA

Table S5. Primers used for CNOT6 reporter constructions.

Primer Name (description)	Sequence
CNOT6 3'UTR (reporter construct)	**F:** GGGACTCAGGGTGGTGTTC **R:** ACAGAGAATTGCACATTGGTTGG
PRE1 (site-directed mutagenesis)	**F:** TTCGTACTTTGTACAGTGaaaAAATTGATGCTATTTTGC **R:** GCAAAATAGCATCAATTTtttCACTGTACAAAGTACGAA
PRE2 (site-directed mutagenesis)	**F:** ATTTGAGACCATGGGTTGaaaAAATGAGGATTTGAACCA **R:** TGGTTCAAATCCTCATTTtttCAACCCATGGTCTCAAAT

Table S6. Custom morpholino sequences used.

MASO Name	Sequence	Concentration of injection solution
Nanos	GTGACTAAAGTGCGTGGAAACTCGA	500 µM
Pumilio	TTTCCTTCTATGATACAGACCCGCT	100 µM
CNOT6 1	ATTTATCTTTGGGCATCCTGGTGGC	500 µM
CNOT6 2	GTCGGTTTTCACCAGTTCAGGAGGC	500 µM
PRE1	AATAGCATCAATTTACACACTGTAC	1000 µM
PRE2	GTTCAAATCCTCATTTACACAACCC	1000 µM

Table S7.

Download Table S7

Table S8.

Download Table S8

Table S9.

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