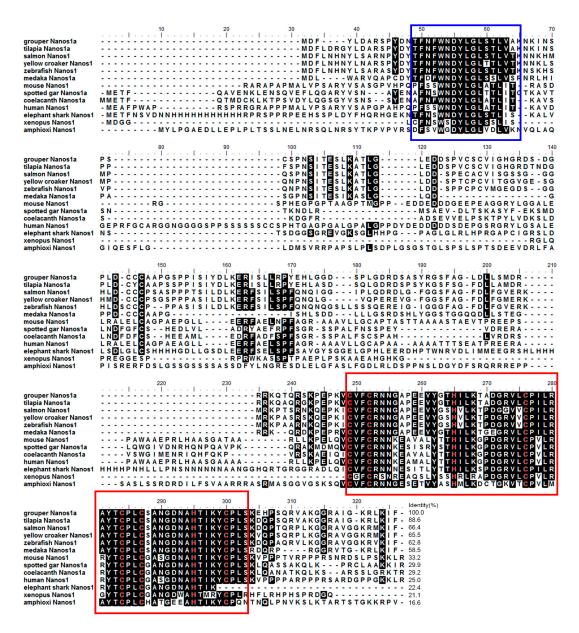
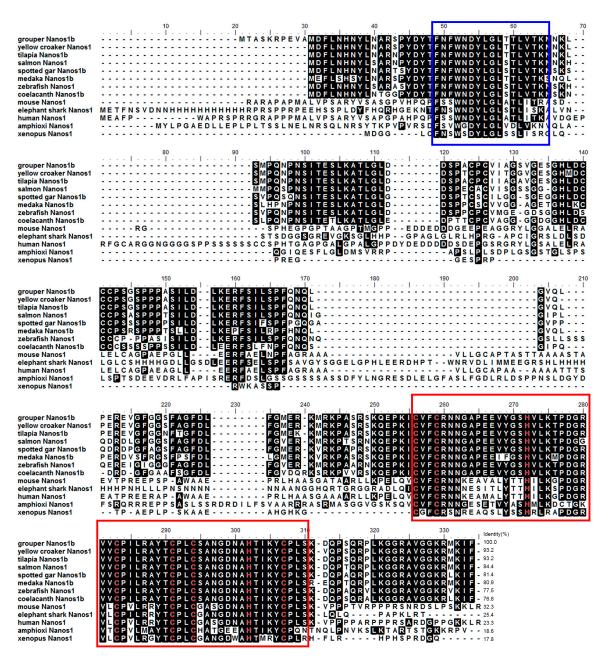
## Supplementary Materials

Primer Name	Purpose	Sequence(5' to 3')
nanos1a-5' RACE-R1	RACE	AGCAGTCCAGGTGCCCGCTCTCTCCAA
nanos1a-5'RACE-R2	RACE	ACGCCGGGGAATCGTCCAAGCCCAGG
nanos1a-5'RACE-R3	RACE	TCGGTGATGGAGTTGGGGTTCTGGGG
nanos1a-3' RACE-F1	RACE	TAATCGCGGGCAGCGTTGGAGA
nanos1a-3' RACE-F2	RACE	GTTTCTCGATACTGAGCCCCTTC
nanos1b-5' RACE-R1	RACE	GTCCATGGAGAGCAGGTCGAG
nanos1b-5' RACE-R2	RACE	GTGCTCGTACGGCCTGAGAAGAGA
nanos1b-3' RACE-F1	RACE	TAATTGGGCACGGCAGGGACA
nanos1b-3' RACE-F2	RACE	ACCCTTGGACTGCTGCTGCGC
nanos1b-3' RACE-F3	RACE	CTCTCTTCTCAGGCCGTACGAGCAC
nanos2-5' RACE-R1	RACE	GTCCGAGAGGCTGGAGGGTGA
nanos2-5' RACE-R2	RACE	TGGGTGACATAGTCCCGGAGA
nanos2-3' RACE-F1	RACE	GGGACGAGTACACCTTTGACAGTG
nanos2-3' RACE-F2	RACE	CCTGTTAGGGACTAGTTCACCC
nanos3-5' RACE-R1	RACE	TCAGCACGGAAGGGGTCTGTAGCG
nanos3-5' RACE-R2	RACE	CGAGGTCGCCGCTTTGGCACA
nanos3-5' RACE-R3	RACE	GACAGTCCTATGTAGTCTCTCC
nanos3-3' RACE-F1	RACE	CGCTACAGACCCCTTCCGTGCTGA
nanos3-3' RACE-F2	RACE	AACCAGGCCGGGGATGTTCTG
nanos1a-F	RT-PCR	GACTTGAAGGAGCGTTTCTCGAT
nanos1a-R	RT-PCR	CTTTAATGGTCGCTGGGATGGC
nanos1b-F	RT-PCR	CGGTTTGCTCCTGCGTAATTGG
nanos1b-R	RT-PCR	CTTCAGGATGTGGGTGCCGTAA
nanos2-F	RT-PCR	ACTACCCTCTCCGGGACTATGT
nanos2-R	RT-PCR	GCGTCATCAGTCATTTCACTTTCCC
nanos3-F	RT-PCR	TGTGCCAAAGCGGCGACCTCG
nanos3-R	RT-PCR	CAGAACATCCCCGGCCTGGTT
β-actin-F	RT-PCR	GAAATCGCCGCACTGAGTTGTTG
β-actin-R	RT-PCR	ACCATCACTCCCTGATGCCT
nanos1a-probe-F	ISH	GCCAGGAGCTGATGGAGTTTGAGT
nanos1a-probe-R	ISH	TAATACGACTCACTATAGGAGACTTTGGAATG
		TCAGGCTTTTCAT
nanos1b-probe-F	ISH	GCCATCCCAGCGACCATTAAAG
nanos1b-probe-R	ISH	TAATACGACTCACTATAGGTCATCCCCTTTGC
		CAATGTCAG
nanos2-probe-F	ISH	GACGGGGGAGTGCTTCGACATGT
nanos2-probe-R	ISH	TAATACGACTCACTATAGGGCGTCATCAGTCA TTTCACTTTCCC
nanos2 proho E	тсп	CTGGAGAGACTACATAGGACTGTC
nanos3-probe-F	ISH	TAATACGACTCACTATAGGTGGCCCCCGTGGC
nanos3-probe-R	ISH	TCCGCACA

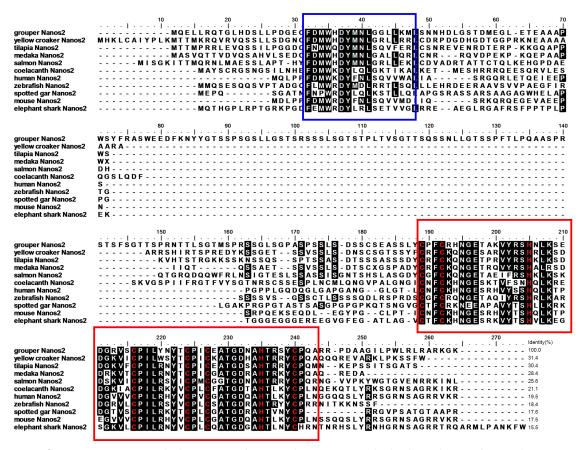
Table S1. Sequences of the primers used for PCR.



**Figure S1.** Amino acid alignments of *E. coioides* Nanos1A with the homologues from other vertebrates. The identities relative to *E. coioides* Nanos1A are exhibited at the end of each sequence. The blue frame indicates the conserved position of CCR4-NOT deadenylase interaction domain. The red frame indicates the conserved position of zf-domain. The 8 invariant cysteine and histidine residues in zf-domain were marked with red color.

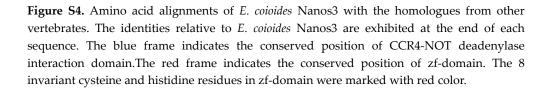


**Figure S2.** Amino acid alignments of *E. coioides* Nanos1B with the homologues from other vertebrates. The identities relative to *E. coioides* Nanos1B are exhibited at the end of each sequence. The blue frame indicates the conserved position of CCR4-NOT deadenylase interaction domain. The red frame indicates the conserved position of zf-domain. The 8 invariant cysteine and histidine residues in zf-domain were marked with red color.



**Figure S3.** Amino acid alignments of *E. coioides* Nanos2 with the homologues from other vertebrates. The identities relative to *E. coioides* Nanos2 are exhibited at the end of each sequence. The blue frame indicates the conserved position of CCR4-NOT deadenylase interaction domain. The red frame indicates the conserved position of zf-domain. The 8 invariant cysteine and histidine residues in zf-domain were marked with red color.

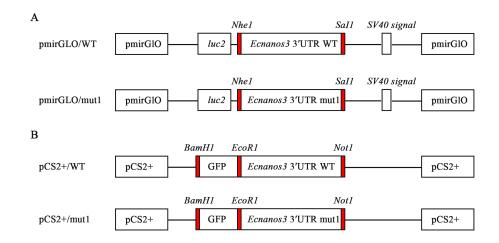
grouper Nanos3 yellow croaker Nanos3 tilapia Nanos3 medaka Nanos3 salmon Nanos3b salmon Nanos3a zebrafish Nanos3 coelacanth Nanos3 elephant shark Nanos3 xenopus Nanos3 spotted gar Nanos3 mouse Nanos3	10  20  30  40  50  60  70    M VWG L FD HLP RF MESD L KS GO P WR DY IGUS DT I KE    M NG M VWG L FD HLP RF MESD J KS GO P WR DY IGUS DT I KE    M NG M VWG L HHLP R FM ESD KS GO P WR DY MG L CD T I RD    M SO V SG LL HL I P R FM ESD KS GO P WR DY MG L CD T I RD    M SO V SG LL HL I P MESS KE HL WR DY MG L CD T I RD    M SS SK HL WK DY MG L CD T I RD    M SS SK HL WK DY MG L CD T I RD    M SS SK HL WK DY MG L CD T I RD    M SS SK HL WK DY MG L CD T I RD    M SS SK HL WK DY MG L CD T I RD    M SS SK HL WK DY MG L CD T I RD    M SS SK HL WK DY MG L CD T I RD    M SS SK HL WK DY MG L AD WR B A WR B A A VRE A A    M SS ME T R NQ D G P WK DY MG L AD M I RG A K
grouper Nanos3 yellow croaker Nanos3 tilapia Nanos3 medaka Nanos3 salmon Nanos3b salmon Nanos3a zebrafish Nanos3 coelacanth Nanos3 celephant shark Nanos3 kenopus Nanos3 spotted gar Nanos3 mouse Nanos3	40  90  100
grouper Nanos3 yellow croaker Nanos3 tilapia Nanos3 medaka Nanos3 salmon Nanos3b salmon Nanos3b coelacanth Nanos3 coelacanth Nanos3 coelacanth Nanos3 xenopus Nanos3 human Nanos3 spotted gar Nanos3 mouse Nanos3	GG TE TE VPMHDRQQE VPWRGRKMPRGGTE TE VPMHARQQE VPWRGREMPRGGTE KE VPMHARQQE VPWRG
grouper Nanos3 yellow croaker Nanos3 tilapia Nanos3 salmon Nanos3b salmon Nanos3a zebrafish Nanos3 coelacanth Nanos3 elephant shark Nanos3 xenopus Nanos3 human Nanos3 spotted gar Nanos3 mouse Nanos3	220  230  240  250  260  270  280    P VAHQRP D G LWY AT D F RAD P D AVDMK P VP R REPKDRKKSTRFK K I PES - A    P SSLACPP ESLRYT P D SLP AD T P NN VD LK LVP R T KS QG P KERKKSTRFK KAP D AP A    REPKDRKKSTRFK KAP D AP A    REPKDRKKTSRFKTPE A V L    REPKDRKKTSRFKTPE AV L    REPKDRKKTSRFKTPE - V    REPKDRKKTSRFKTPE E - V    REPKDRKKTSRFKTPE E - V    P LDSE I VP VRKDAAL RPSSTEG PEPT TAASRPS - AGT RGQRERKK AT RSKTPE E - V
grouper Nanos3 yellow croaker Nanos3 tilapia Nanos3 salmon Nanos3 salmon Nanos3a zebrafish Nanos3 coelacanth Nanos3 celephant shark Nanos3 xenopus Nanos3 human Nanos3 spotted gar Nanos3 mouse Nanos3	290 30 310 320 330 340 350 LS PASP EPTF USFCKHNGES DLVYCRSTRUKNOAG DVLBPYLR QYVCPLCGATGAKATTKR LT PSS ERMF DSFCKHNGES DLVYCRSTRUKNOAG DVLBPYLR QYVCPLCGATGAKATTKR LT PSS ERMF DSFCKHNGES ELVYGSTWLKNOAG DVLPYLR QYVCPLCGATGAKATTKR SSPDG
grouper Nanos3 yeliow croaker Nanos3 tilapia Nanos3 medaka Nanos3 salmon Nanos3a zebrafish Nanos3 coelacanth Nanos3 coelacanth Nanos3 elephant shark Nanos3 xenopus Nanos3 human Nanos3 spotted gar Nanos3 mouse Nanos3	360  370  380  390    FC PK VD S AV S S VV AK S R R  1  1  1  100.0    FC PK VD S AV S S VV AK S R R  1  1  100.0    FC PK VD S AV S S VV AK S R R  1  1  100.0    FC PK VD S AV S S VV AK S R R  1  100.0    FC PK VD S AV S S VV AK S R R  1  100.0    FC PK VD R T V S S VV V K  1  100.0    FC PR VE T T N S S VV V K  100.0  38.0    FC PR VE T T V S S VV V K  100.0  38.0    FC PR VD K NY C S VV VK  100.0  38.0    FC PR VD K NY C S VV AK S T W  100.0  38.0    FC PR VD K NY C S VV AK S T W  100.0  38.0    FC PR VE T T S S VV AK S T W  100.0  38.0    FC PR VE T T S S VV AK S T W  100.0  39.0    FC PR VE T T S S S VV AK S T W  100.0  30.2    FC PR VE T K S S VY AK S R R  100.0  30.2    FC P N T E K G S S Y S L T K A A G K K N N K K D  19.4    FC P L T G A G M T S VY S H T T R N S A G K K N N K K D  10.7    FC P L T S A G M T S VY C Y T T R N S A G K K L T R P D K A K T Q D A G H R L G  16.4



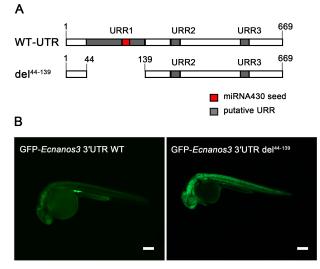
## Ecnanos3 3'UTR



**Figure S5.** The sequence of *Ecnanos3 3'*-UTR. U-rich motifs are marked with gray shadow and the putative miR-430 binding site is indicated with frame where the different nucleotide against canonical seed sequence of miR-430 is marked by red.



**Figure S6.** Schematic of the luciferase constructs and GFP constructs. (**A**) Schematic of the luciferase constructs. (**B**) Schematic of the GFP constructs.



**Figure S7**. Schematic representation of *Ecnanos3* 3'-UTR del44-139 and function of URR1 in *Ecnanos3* 3'-UTR. (**A**) Schematic representation of *Ecnanos3* 3'-UTR del44-139; (**B**) PGCs visualization at 26 hpf by using GFP fused *Ecnanos3* 3'-UTR WT and *Ecnanos3* 3'-UTR del44-139, respectively. Bar: 250 µm.