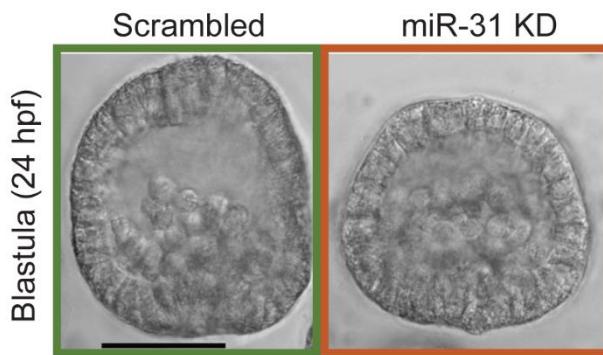


A.



B.

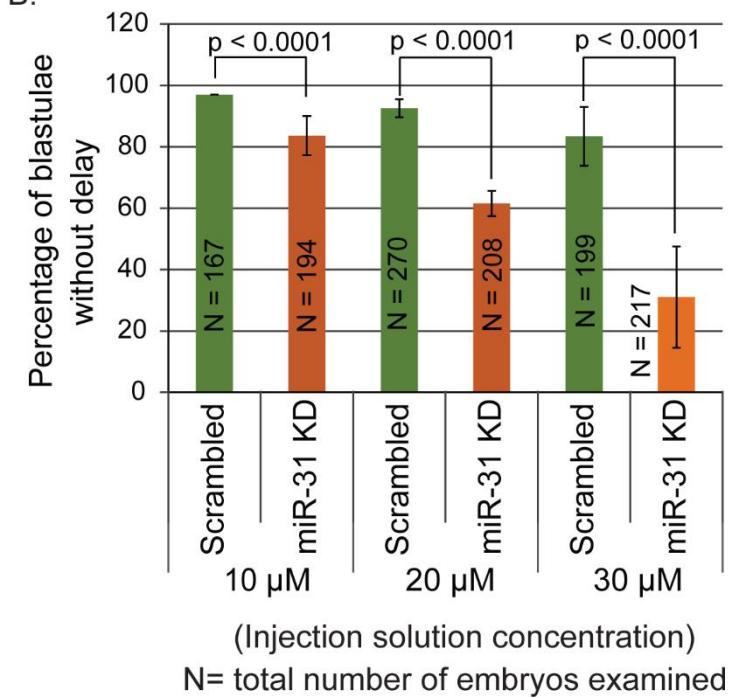
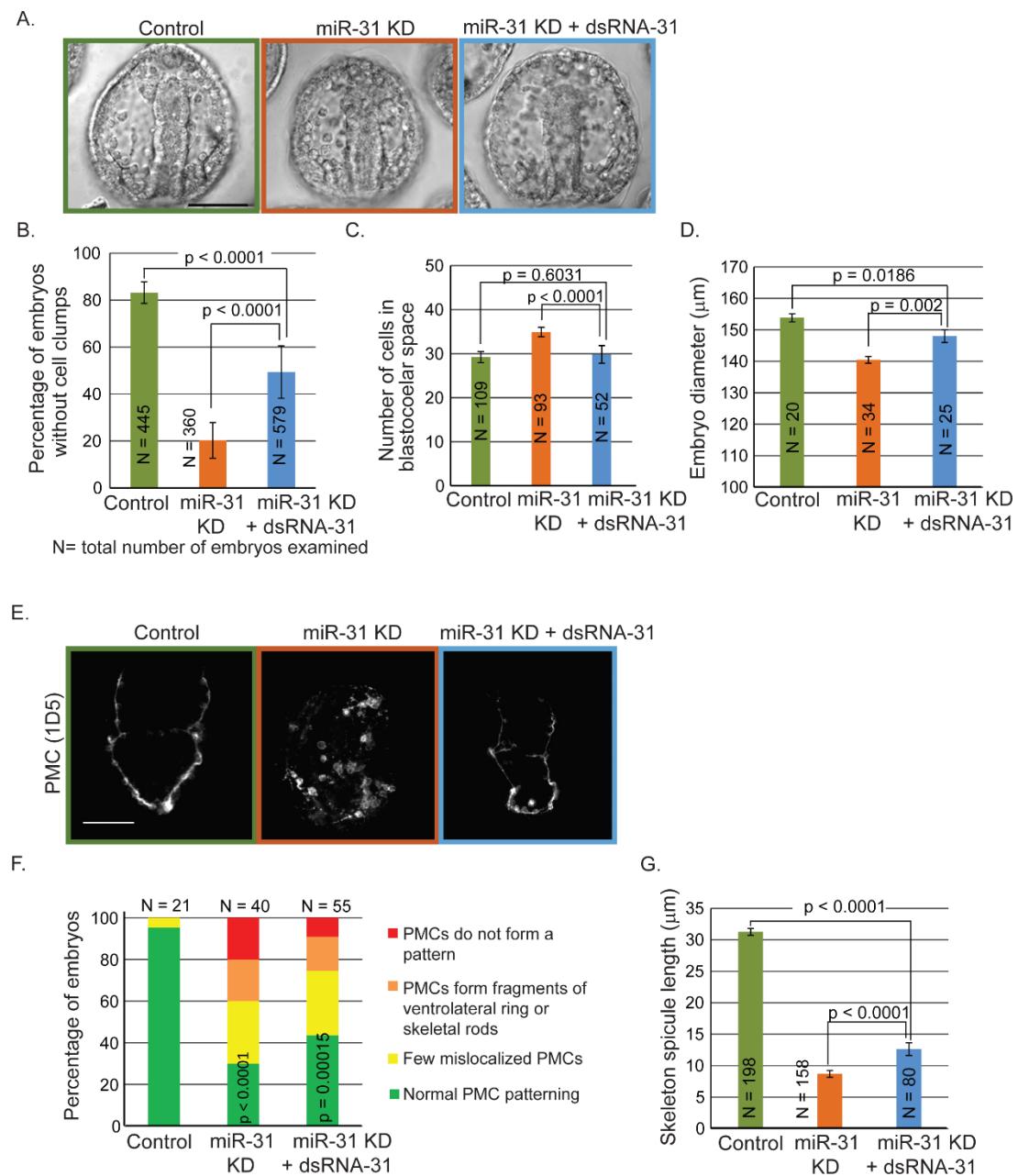


Fig. S1. miR-31 KD resulted in dose-dependent developmental delay at the mesenchyme blastula stage. (A) DIC images of control and miR-31 inhibitor injected blastulae. (B) Developmental delay was dose-dependent on the miR-31 inhibitor injected into the embryos. Scale bar is 50 µm.

**Fig. S2. miR-31 KD phenotypes were partially rescued by the addition of dsRNA-31.**

(A) miR-31 inhibitor (30 μM), cocktail of miR-31 inhibitor and dsRNA-31 (1:1 molar ratio, 30 μM each) or scrambled control (30 μM) were microinjected into newly fertilized eggs. (B) Percentage of cells without cell clumps in the blastocoel space was increased in the presence of miR-31 KD + dsRNA-31 compared to miR-31 KD embryos (5 biological replicates, Cochran-Mantel-Haenszel test). (C) The number of cells in the blastocoel space

of the miR-31 KD + dsRNA-31 injected embryos was similar to that of the control embryos, indicating a rescue of this miR-31 KD phenotype (Student's T-test). (D) The diameter of miR-31 KD embryo was restored by addition of dsRNA-31 (Student's T-test). (E-F) Immunostaining using PMC-specific antibody 1D5 indicated that PMC patterning was partially restored by the co-injection of dsRNA-31 with the miR-31 inhibitor. Fisher's Exact Test of Independence was used for statistical analysis. (G) The skeletal spicule length in miR-31 KD embryos supplemented with dsRNA-31 was partially rescued compared to the miR-31 KD embryos and control embryos (Student's T-test). S.e.m. bars plotted. Scale bar is 50 μ m.

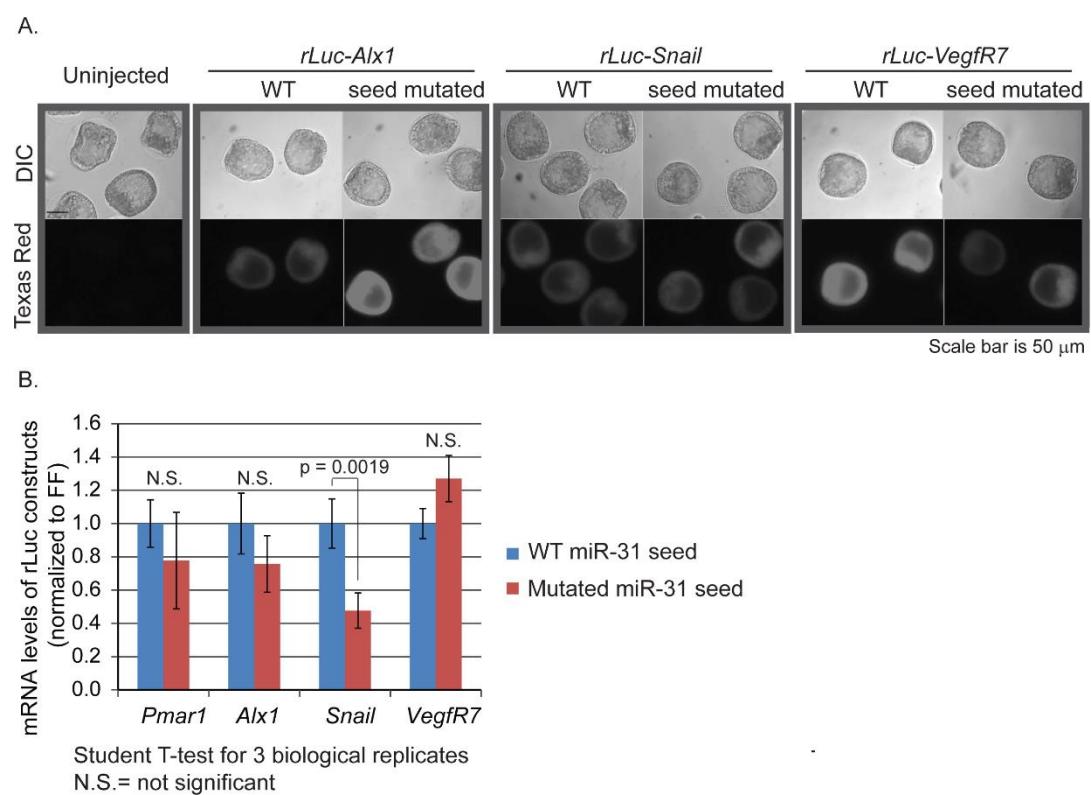


Fig. S3. The injected embryos contained similar transcript levels of luciferase reporter constructs. (A) Embryos injected with exogenous reporter construct transcripts have normal development. (B) Zygotes were injected with luciferase constructs with WT or mutated miR-31 seed sequences and the Firefly construct. The injected mRNA levels of *Renilla* (rLuc) and *Firefly* (FF) constructs were measured by qPCR. Rluc data were normalized first to internal Firefly readouts, followed by normalization to the rLuc with WT miR-31 seed sequences.

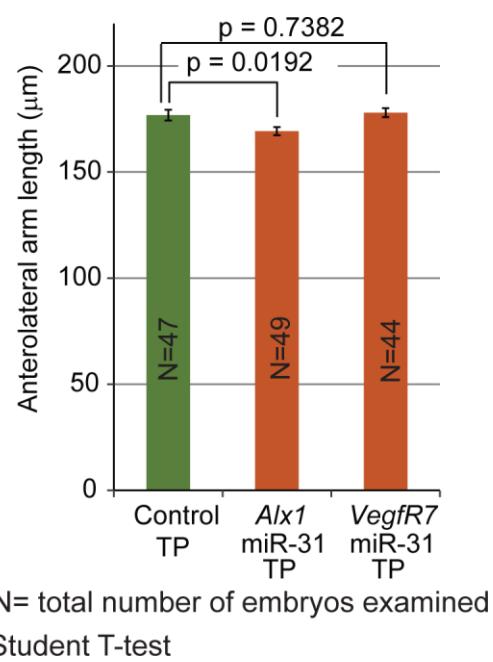


Fig. S4. A shorter skeletal length is maintained in *Alx1* miR-31 TP-injected embryos compared to the control larvae. The decrease in skeleton spicules in *Alx1* TP-treated embryos remained statistically shorter compared to the control, while the skeletal length of *VegfR7* miR31-TP injected embryos recovered by the larval stage. Student T-test. S.e.m bars graphed.

Table S1. Primers used for cloning and mutagenesis.

Name	Sequence (5' to 3') Restriction enzymes cut sites are underlined
Alx1_1F	TGACCAACATGACCTCAATGA
Alx1_2F_Xhol	GACG <u>CTCGAG</u> ATTGTTTAATAAAATACGTATATAT
Alx1_1R	ATCACGTTGTCAATGACATCAA
Alx1_2R_NotI	TGCT <u>GCGGCCG</u> CACAAGTATATCCATGCTCTTC
Pmar1_RACE_F1	CGCCAAAAGTCCATTGATGT
Pmar1_RACE_F2	CACCGTCTTCATTCTCCACA
Pmar1_Xhol	CG <u>CTCGAG</u> ATTGACTGCATCGACTCA
Pmar1_NotI	CT <u>GCGGCCG</u> CAATAGCGGCCGCAATTATTT
VegfR7_Xhol	CAGT <u>CTCGAG</u> TGAGATATGAACAAATTAAAAACC
VegfR7_R_NotI	TGCT <u>GCGGCCG</u> GTTGGACACTGATCTCATAAAT
Snail_F_Xhol	GACT <u>CTCGAG</u> AGCATTCTAAATCATAGAAC
Snail_R_NotI	CTAT <u>GCGGCCG</u> CTCCCCAACCTCGTAGTCA
Alx1_mut1_F	CTGAAAAAGCCATTGAGTCCTACCTGCGATGTGCAAACGTGA
Alx1_mut1_R	TCAGTTGCACATCGCAGGTAGGACTCAATGGCTTTTCAG
Alx1_mut2_F	TGCCAATTTGTTATTGTTATATTCATCCTACCAACGACATGA TCCTAACCTC
Alx1_mut2_R	GAGGTTAGGATCATGTCGTTGGTAGGATGAAAATATAACAATAA CAAAATTGGCA
Pmar_mut_F	CTGAAGGGATCTTCTTATTATTATATATTAAATCATCCCTTTCCAA AGATGCATTCTT
Pmar_mut_R	AAATGAATGCATCTTGAAAAGGGATGATTAATATATAATAATA AGAAGATCCCTTCAG
VegfR7_mut_F	TACAAGTCTACAAGTGAATCTCAAGGTAGGAATGCATTATAGT AGTTGAAAAATTG
VegfR7_mut_R	CAATTTCAACTACTATAATGCATTCTACCTTGAAGATTCACT

	TGTAGACTTGTA
Snail_mut_F	AGCCATTCTCTCCAACCTGGTAGGATAATAGAACATTCTT TTGCAAT
Snail_mut_R	ATTGCAAAAGAATTGTTCTATTATCCTACCAGAGTTGGAAGAG AAATGGCT
Vegf3_F_in situ	CGCGCAATTCACTGATGTAT
Vegf3_R_Sp6_in situ	GATTTAGGTGACACTATAGAGTCCTGTCCTGGCTGAGA
VegfR10_F_in situ	GACTTACGTTCACTCTATACGT
VegfR10_R_Sp6in situ	GATTTAGGTGACACTATAGTGAACCATCTCCTCCCA
VegfR7_F_in situ	GTCCTGTCTTGGCCATTGTT
VegfR7_R_in situ	GATGGTTGAAATGGGTTGG

Table S2. miRNA target protector morpholino antisense oligonucleotide sequences (miRNA TPs)

Name	Sequence (5' to 3')
Control MASO	GTGTAACACGTCTATACGCCCA
Alx1 miRNA TP for seed 1 (at 1686bp)	ACATCGCAGGCAAGACTCAATGGCT
Alx1 miRNA TP at seed 2 (at 1852 bp)	ATCATGTCGTTGGCAAGATGAAAAT
VegfR7 miRNA TP (at 2254 bp)	AAGGCAAGAATGCATTATAGTAGTT

Table S3. Primers used for qPCR

Name	Sequence (5' to 3')
Pmar1_qPCR_F	TCCAGCAAAAGACACCACGT
Pmar1_qPCR_R	TGAAGACGGTGTGCGGTAAA
Alx1_qPCR_F	CACCCGTAGAGGGCGCTATG
Alx1_qPCR_R	TGCTGGAGTCTTGCATTG
Snail_qPCR_F	CCACAGCATA CGACGCAGG
Snail_qPCR_R	CTCGGGTTGTGGATAACTGG
VegfR7_qPCR_F	GTGTGGGCCTATGCTGTTT
VegfR7_qPCR_R	TAGTCAGGTGCGTTCAATTG
FGF_qPCR_F	CGGCAGGTTATCAGACGAT
FGF_qPCR_R	GCGTCTCTGATTGTTGCTGA
Vegf3_qPCR_F	TGCAAATGTTCCCATTACGA
Vegf3_qPCR_F	GTTCGTTGGTTATGCGTCA