Supplementary Information

Supplementary Methods

Whole–mount immunostaining and *in situ* hybridization in Chondrichthyans

For *Pax3* and *Lbx1* expression, 12 replicates for each gene were investigated. For *Wnt3*, *Cyp26a1*, *Fgf8*, and *Hox* genes, 3 embryos for each gene were investigated. The nerve staining method was modified from a previous study (50). Briefly, skate and shark embryos were transferred from salt water to 500 mg/L Tricaine-S and then fixed in 4% PFA overnight. The following day, embryos were washed in phosphate buffered saline (PBS) containing 1% Triton (PBTriton) for 3 hours. Embryos were incubated in 0.25% trypsin/PBS for 5 minutes and immersed in pre-cooled acetone at –20ºC for 10 minutes. After brief rinsing with PBTriton, specimens were placed in blocking solution (PBTriton containing 10% goat serum, 1% dimethyl sulfoxide, and 5% H2O2) at 4ºC overnight. The following day, the solution was replaced with blocking solution containing 1:50 3A10 antibody for nerve staining (Developmental Studies Hybridoma Bank) or 1:70 myosin heavy chain for muscle staining (Developmental Studies Hybridoma Bank). Embryos were incubated in antibody solution at 4ºC for 72 hours. Embryos were washed with PBTriton for 5 hours and then incubated with blocking solution containing 1:1000 peroxidase-conjugated secondary antibody (Jackson Laboratory, catalogue number 115-035-003). The next day, embryos were washed with PBTriton for 5 hours and subjected to DAB color development. At each stage, we stained nerves of 6 embryos and muscles of 4 embryos.

Supplementary Figure 1 | The dual contribution of Pax3-positive muscle precursor cells into the pectoral fin and body wall muscles.

(**A**) *Pax3*-positive cells migrated into the pectoral fin (arrowheads) and body wall muscles (arrows) in skate embryos at stage 29. Scale bar is 1 mm.

Supplementary Figure 2 | The development of hypaxial muscles in shark embryos at stage 30.

(**A**) Ventral view of shark muscles stained by myosin heavy chain antibody at stage 30. Note that pectoral fin muscles (arrow) and body wall muscles (arrowheads) develop at the same axial level. Scale bar is 1 mm.

Supplementary Figure 3 | The pectoral and pelvic fin domains in wildtype and RA-treated embryos.

(**A**) The pectoral fin (dark blue) develops in the lateral plate mesoderm adjacent to somites 1-36 (or 37) and pelvic fins (light blue) from 36 (or 37) to 50. We counted observable somites under the microscope. In RA-treated embryos, the pectoral fins were narrower than wildtype (somites 1-28). The severity of phenotypes varies, however, all embryos showed the same phenotype.

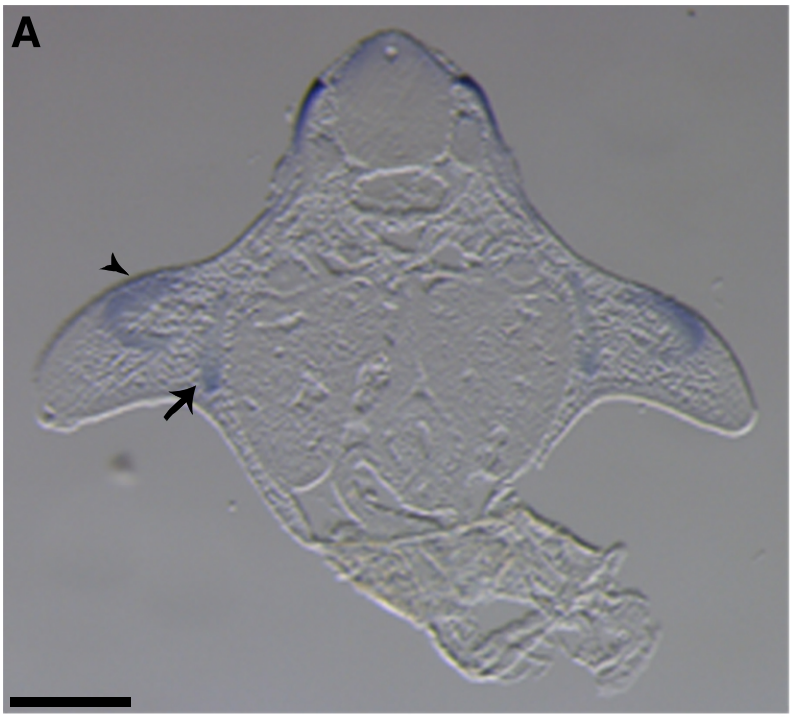
Supplementary Figure 4 | Hoxa9 expression patterns of embryos treated by retinoic acid from stage 23 to 24.

Whole-mount *in situ* hybridization of *L. erinacea* embryos at stage 23 to 24. (**A**) *Hoxa9* expression in a wild type embryo. LPM expression starts from somite 26 (arrowhead). (**B**) *Hoxa9* expression in an embryo treated by RA. LPM expression starts from somite 23 (arrowhead). Note that *Hoxa9* expression is shifted anteriorly in the RA+ embryo by 3 somites. Scale bars are 1 mm.

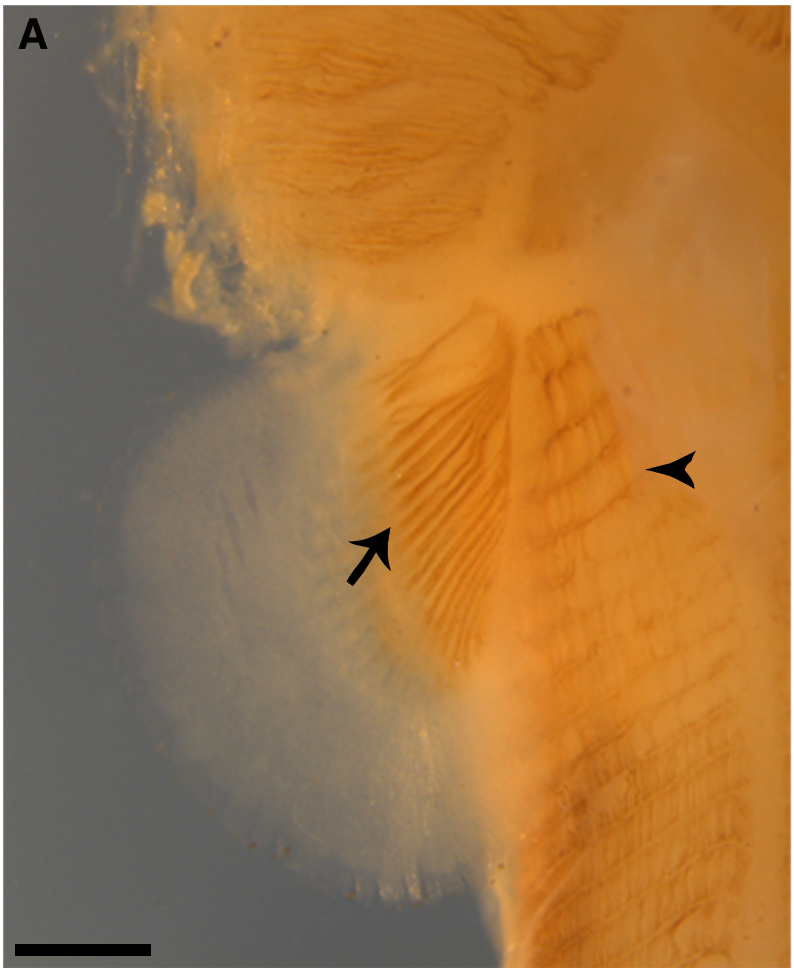
Supplementary Figure 5 |. Neurofilament staining of RA-treated embryos

(**A**) The brachial plexus did not develop in embryos treated by RA, although nerves innervate hypobranchial and pectoral muscles (arrows). A scale bar is 1 mm.

Supplementary Table 1 | Cloning primers for *Cdx2, Cyp26a1, Lbx1, Pax3,* and *Hox* genes of skate

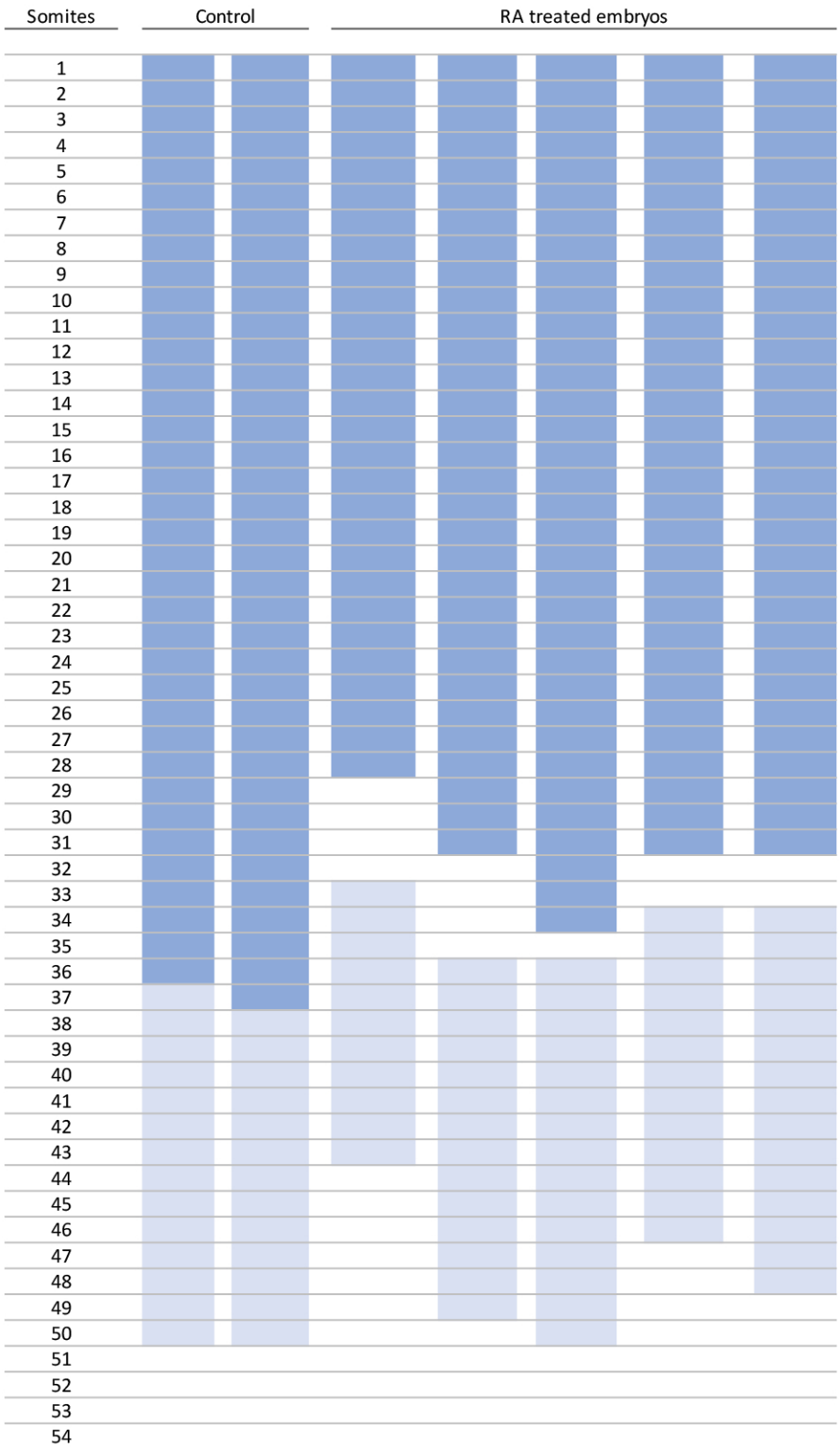


Supplementary Figure 1

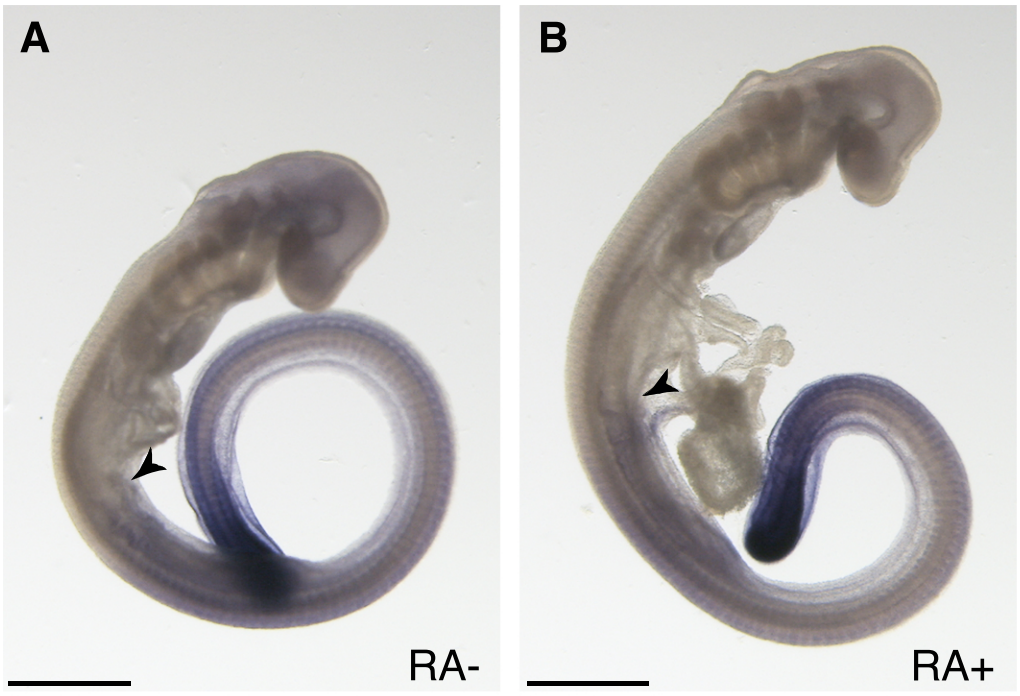


Supplementary Figure 2

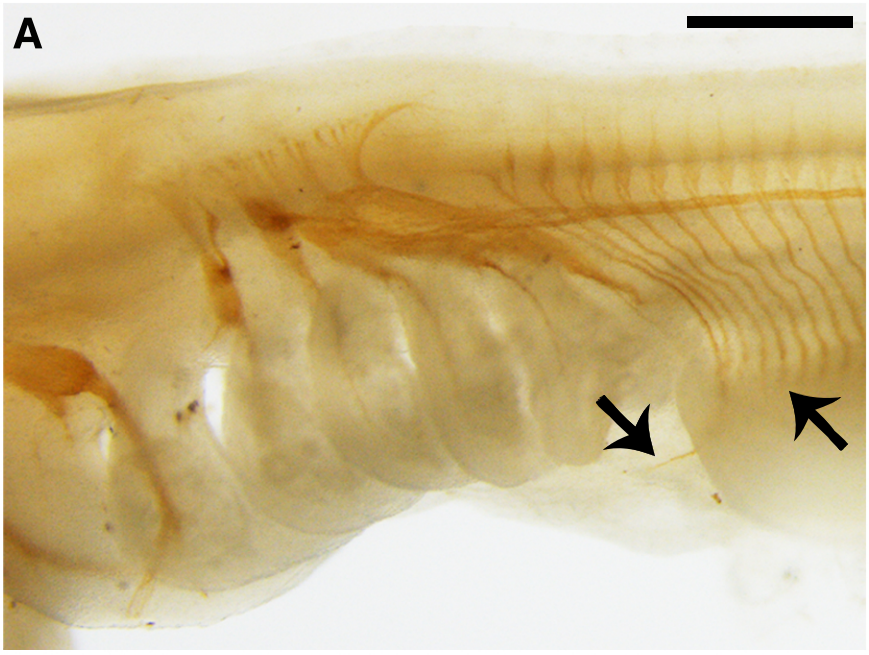
A



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

Gene	Forward Sequence	Reverse Sequence	Length
Hoxa2	CAAACCATCCCTAGCCTGAA	TGGACCCATGTATTGCTGAA	1133bp
Hoxa3	CTCAATCCAGCACAAGCAAA	GGCTGGAGTCCATGAAAAGA	1104bp
Hoxa4	CCCCAATTACACTGGAGGAG	GGCAGTTTGTGATCTTTCTTCC	209bp
Hoxa5	TAGACGCACAAACGACCAAG	CAGGCAACAGTACGACCAGA	1053bp
Hoxa9	GCTGCCCTTACACCAAACAT	TTTCTTTGCACCACAACACC	975bp
Hoxa10	ACCTTATTGCCGTTCTGGTG	TTTTCTCCCCAATTCACTCG	803bp
Hoxa11	GTTTAGCTGTGCGAATGCAA	TGCGCTTCTTATCGCCTTAT	1391bp
Hoxa13	GAACTTCACCGCAAACCAAT	CGTGCAGGCAGAAATGTAAA	917bp
Hoxd1	GTCACCAGGGCTGTGACTTT	AGCGTTCGCTATTTCCACAC	469bp
Hoxd3	TCAGACATCCAGTGCCAGAG	TCCCTGAGCTGTGTGATGAG	1002bp
Hoxd4	ATCCCAAATTTCCTCCTTGC	GGGTTCCCCTCCAGTGTAAT	400bp
Hoxd8	AAGCCAGGAGAAGCCCTAAG	TTCTATCCGGCGGTTGATAC	929bp
Cdx2	TTTGTCAGCTTTCGCTCCTT	ATGTACGTGAGYTAYCYT	623bp
Cyp26a1	CAATGCTCGACATGGTCTTG	CTTCACAGTGGAGCTGGTCA	552bp
Lbx1	CCGTTCAGCATCGAGGATA	GCCTTCATCTCTTCCAGGTC	473bp
Pax3	ATGAAGGGTGGGGTGGATA	ATCTGGGACTGAACGTGTCC	1505bp

Supplementary Table 1