

SUPPLEMENTAL INFORMATION

Supplementary Table

Table S1: morpholino sequences, qRT-PCR primers and plasmids cloning strategies. Related to STAR Methods

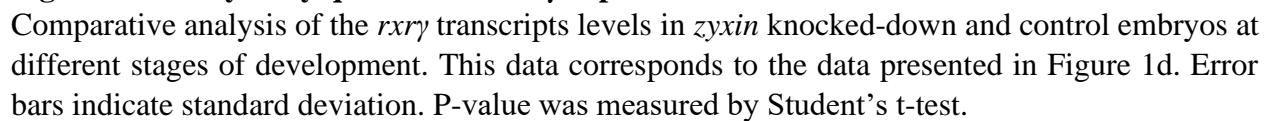
Name	Description and sequences of the morpholino oligonucleotides
<i>zyxin X MO1</i>	A common MO to positions +32—+57 of Zyxin mRNA of both <i>Xenopus laevis</i> zyxin pseudoalleles: TGAAATGTTGATGGTGAAGGAGGAC
<i>zyxin X MO2</i>	A common MO to positions 0—+25 of Zyxin mRNA of both <i>Xenopus laevis</i> Zyxin pseudoalleles: GGGTGGCAGGAGCCGCTGGGTCCAT
<i>zyxin splice X MO</i>	A common MO to positions +953—+972 of Zyxin mRNA of both <i>Xenopus laevis</i> Zyxin pseudoalleles: ctcccactactcacaactcaCCTGATG (intron) (exon)
control MO	A mismatched variant of <i>zyxin</i> MO TTAACTGTTTAATGTTGAATGAGAAC as a negative controls.
<i>zyxin Danio MO1</i>	MO to positions -23—+3 of <i>Danio</i> Zyxin mRNA : CATCTTGATTGTTTCGTTTTCTTCGT
qRT-PCR primers:	
Name	Description and sequences of the oligonucleotides
<i>Xenopus laevis</i>	
<i>ornithine decarboxylase 1 (odc1)</i>	Forward primer: 5'-GCCAGTTCTAACAAGAAACCCA Reverse primer: 5'-TCTACGATACGATCCAGCCCA Product length: 93
<i>eukaryotic translation elongation factor 1 alpha 1 (eef1a1)</i>	Forward primer: 5'-GTTCATTTACCGCACAGGTTATCA Reverse primer: 5'-ACACAGGGGCATATCCAGCA Product length: 70
<i>retinoic acid receptor gamma (rarg)</i>	Forward primer: 5'-GTCGACGAAGGCCTGACAAA Reverse primer: 5'-GAATCAGCGGAGGCATAGGT Product length: 141
<i>retinoid X receptor gamma (rxrg)</i>	Forward primer: 5'-CTTCCTTCTCGCACCGCTC Reverse primer: 5'-GCCTACTCCCGCATTGTGT Product length: 98

Danio rerio	
<i>ornithine decarboxylase 1 (odc1)</i>	Forward primer: 5'-CTCCACCTTCAATGGCTTCCAG Reverse primer: 5'-AGTGGGATGGCACGTTTCCAG Product length: 87
<i>elongation factor 1-alpha (ef1a)</i>	Forward primer: 5'- AAGAACGTGTCAGTCAAGGACAT Reverse primer: 5'- CGTAACCCTGAGAGATCTGACCA Product length: 76
<i>retinoid X receptor gamma (rxrg)</i>	Forward primer: 5'-AGCAATCGTCCTCTTCAACCC Reverse primer: 5'-TTGTGTTTGGTGTAGCCCTCC Product length: 108
DNA constructs	PCR primers and cloning strategy used to prepare reporter plasmids.
<i>pGl3pv -3xRARE</i>	1.The insert was obtained by annealing two primers: with forward primer 5'- CGGGTAGGGTTCACCGAAAGTTCACTCGGGGTA GGGTTACCGAAAGTTCACTCGA and reverse primer 5'- GATCTCGAGTGAACCTTTCGGTGAACCCTACCCC GAGTGAACCTTTCGGTGAACCCTACCCGGTAC 2.Cloning by KpnI and BglII into KpnI and BglII of <i>pGl3 promoter</i> vector (Promega); checking by sequencing.
<i>pGl3pv -6xRARE, pGl3pv -12xRARE</i>	1.PCR from <i>pGl3pv -3xRARE</i> with forward primer 5'- aatt gtcgacCTAGCAAATAGGCTGTCCC and reverse primer 5'- aatt ctcgagCTAAATGAGATGCAGATCGC 2.Cloning by SalI and XhoI into XhoI of <i>pGl3 promoter vector</i> (Promega), preprocessed by CIAP. 3. Checking by sequencing.
<i>pGl3pv - RXRE</i>	1. The insert was obtained by annealing two primers: with forward primer 5'- gtaccTGGGGTTGAAAGGTCAGATGGATGGGGTT GAAAGGTCAGATGGAAa and reverse primer 5'- gatctTCCATCTGACCTTTCAACCCCATCCATCTGA CCTTTCAACCCCAg 2.Cloning by Acc65I and BglII into Acc65I and BglII of <i>pGl3 promoter vector</i> (Promega); checking by sequencing.

Synthetic mRNA	PCR primers and cloning strategy used to prepare DNA templates for generation of synthetic mRNA.
<i>rxrg-3xmyc</i>	<p>1. PCR from the total <i>X. laevis</i> cDNA stage 8 with forward primer 5'- ATGCATCTTGCTACTGAGACA and reverse primer 5'- TCATGAGATCTGGTGAGGTG</p> <p>2. The obtained cDNA fragment was cloned into pAL2-T Vector (Evrogen) and verified by sequencing. Construct: pAL2T-rxrg</p> <p>3. PCR from pAL2T-rxrg with forward primer 5'- aatt aggcctGCCGCCACCATGCATCTTGCTACTGAGACA and reverse primer 5'- aatt ctcgagTGAGATCTGGTGAGGTGTTT</p> <p>4. Cloning by Eco147I and XhoI into EcoRI-Klenow and XhoI of pCS2-3xmyc; checking by sequencing. Final construct: pCS2-rxrg-3xmyc.</p>
<i>Zyxin;</i> <i>Myc-Ybx-1</i> ;	<i>p35T-Zyxin;</i> <i>pCS2MT-Ybx1</i> plasmids were obtained as described (Martynova et al, 2008; Parshina et al., 2020)

a

b



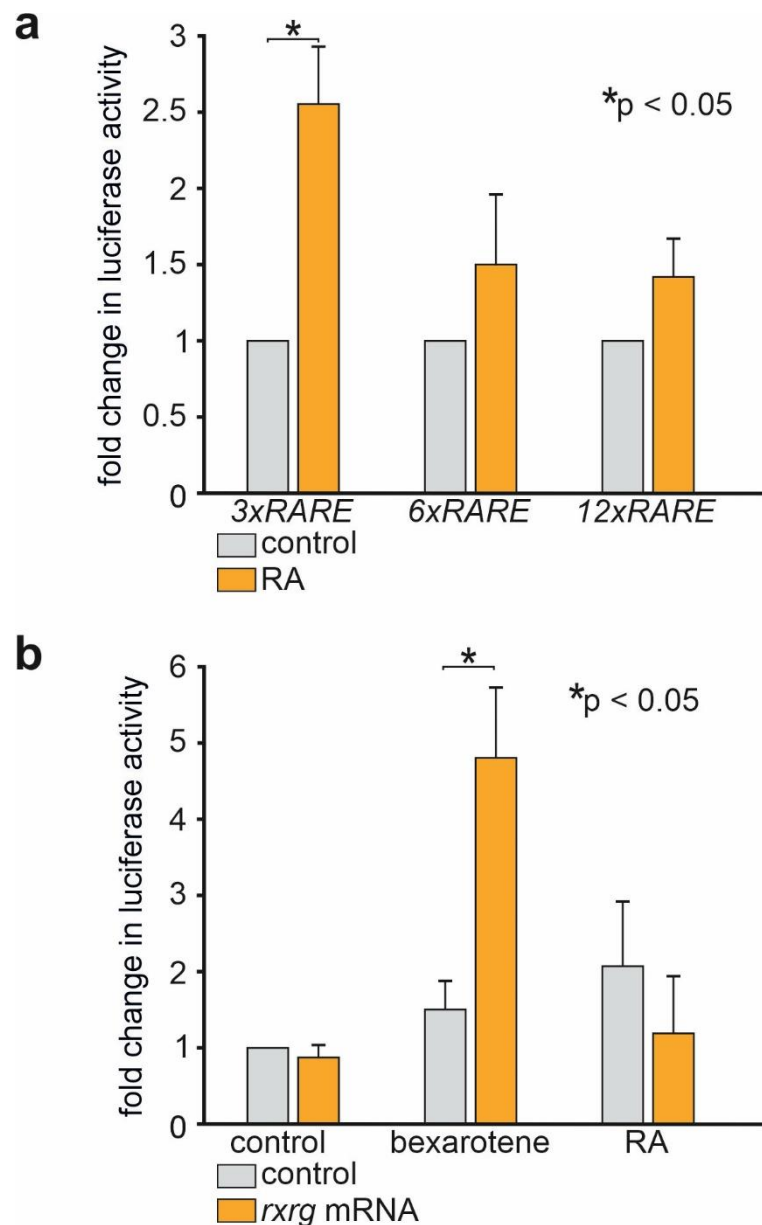


Figure S3. Testing of RARE and RXRE reporters on the AC explants

(a) Activation of *pGL3-Promoter Vector-3xRARE/6xRARE/12xRARE* in response to retinoic acid.

(b) *pGL3-Promoter Vector-gRXRE* reporter activity was compared between experimental samples with *rxrg* overexpression and control samples. ACs were incubated in solutions with the RXRs agonist bexarotene or all-trans RA.

The data presented are representative of at least three independent experiments.

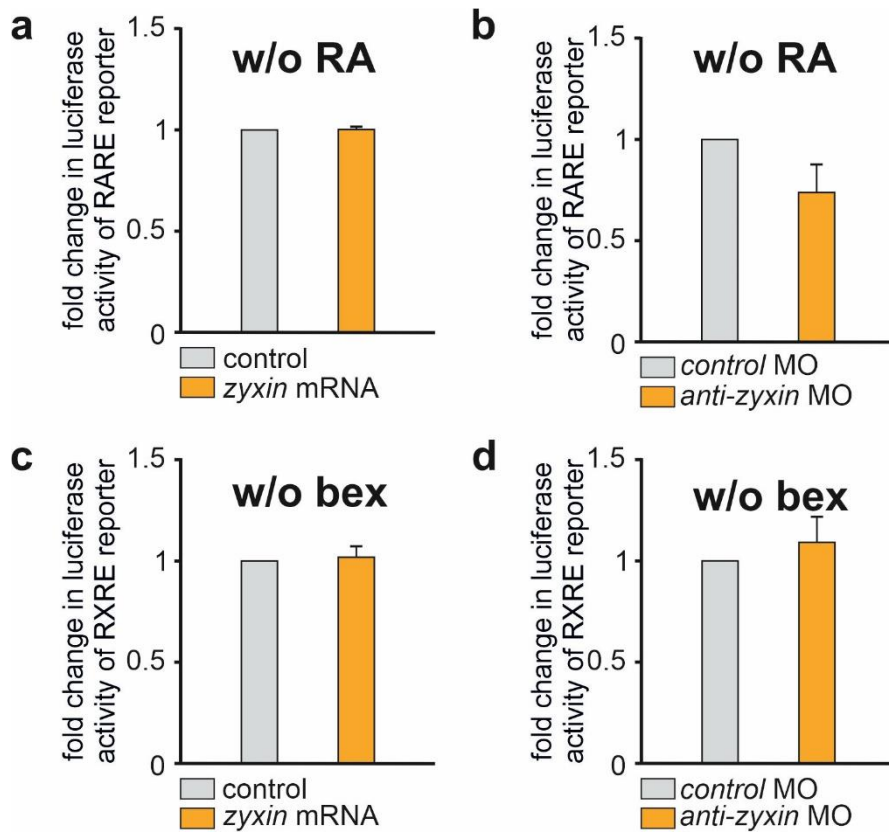


Figure S4. Effects of loss- and gain-of-function of zyxin on γ RARE and RARE cis-regulatory elements in the luciferase reporter assay in the absence of RA or bexarotene.

- (a) Zyxin overexpression does not result in the activation of *pGL3-3xRARE* reporter in the absence of RA.
- (b) Zyxin knockdown does not result in the activation of *pGL3-3xRARE* reporter in the absence of RA.
- (c) Zyxin overexpression does not result in the activation of *pGL3-2x γ RARE* reporter in the absence of RA.
- (d) Zyxin knockdown does not result in the activation of *pGL3-2x γ RARE* reporter in the absence of RA.

The data presented are averaged from at least three independent experiments.

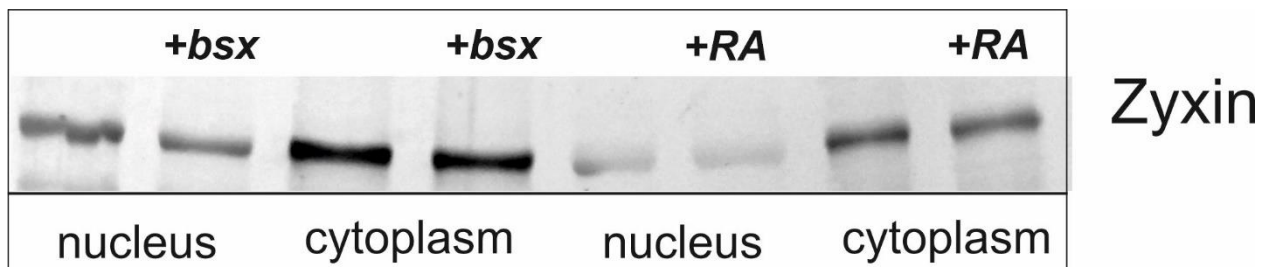


Figure S5. RA and bexarotene do not affect the distribution of Zyxin between the nucleus and the cytoplasm

AC explants were incubated in 1x MMR with 10^{-5} M bexarotene (lanes 1-4) or 10^{-5} M RA (lanes 5-8). Explants were lysed at stage 18 to separate the nuclei from the cytoplasm, and the resulting fractions were analyzed by Western blotting. The data presented are representative of at least three independent experiments.