

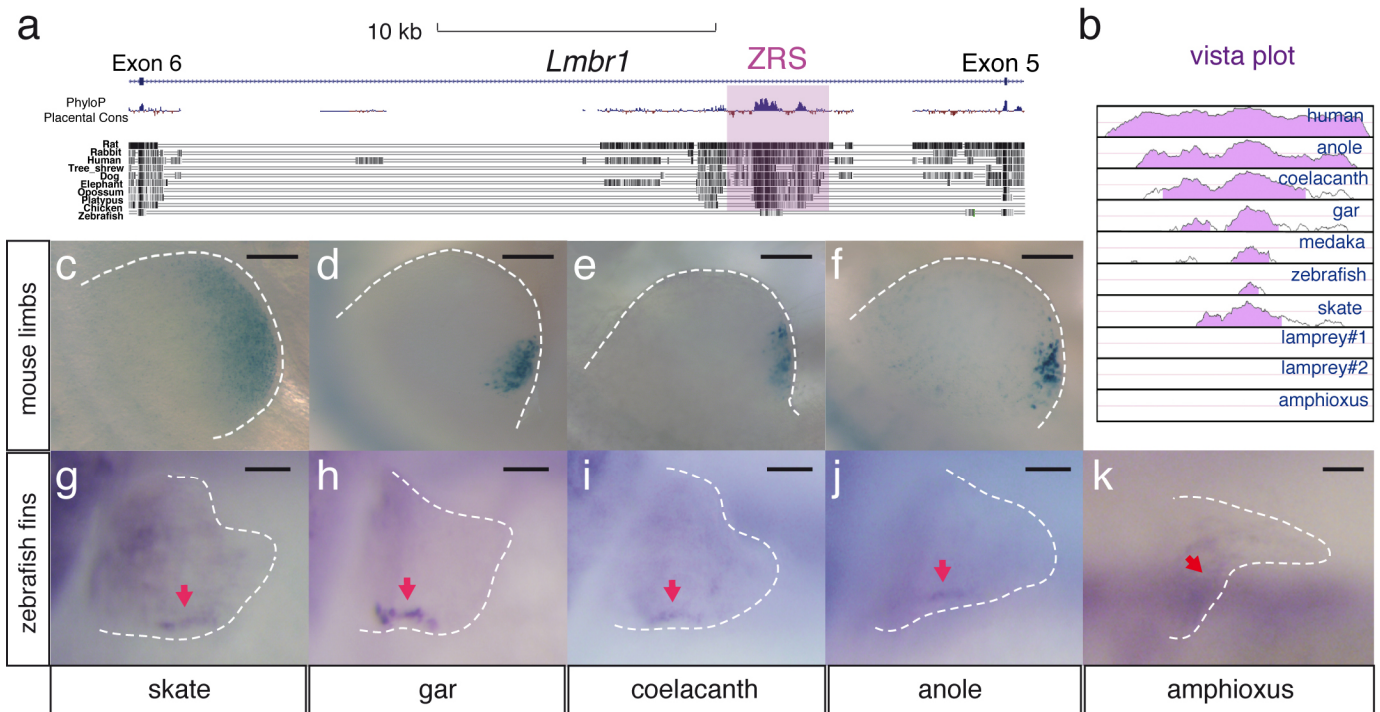
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A conserved *Shh* cis-regulatory module highlights a common developmental origin of unpaired and paired fins

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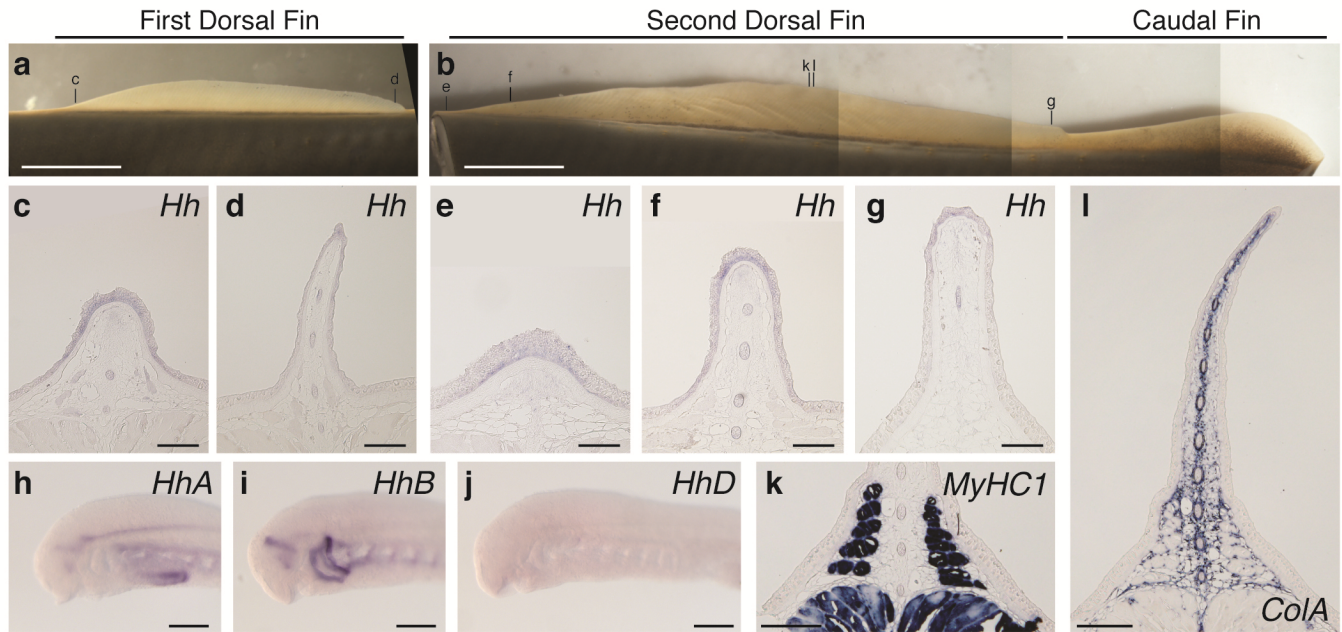
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Supplementary Figure 1

Sequence conservation and enhancer activity of the ZRS from different species and the corresponding amphioxus *Lmbr1* intron in zebrafish fins and mouse limbs.

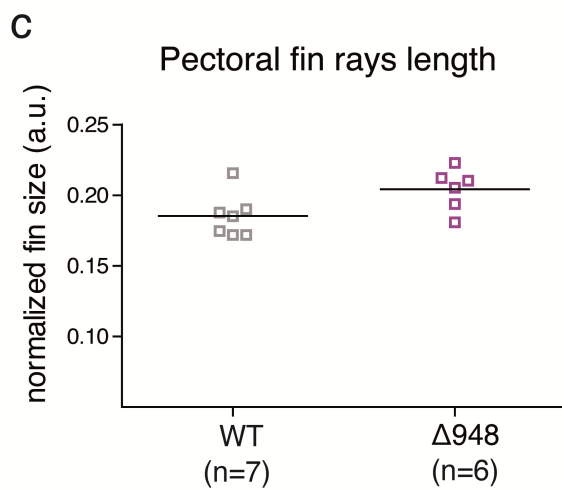
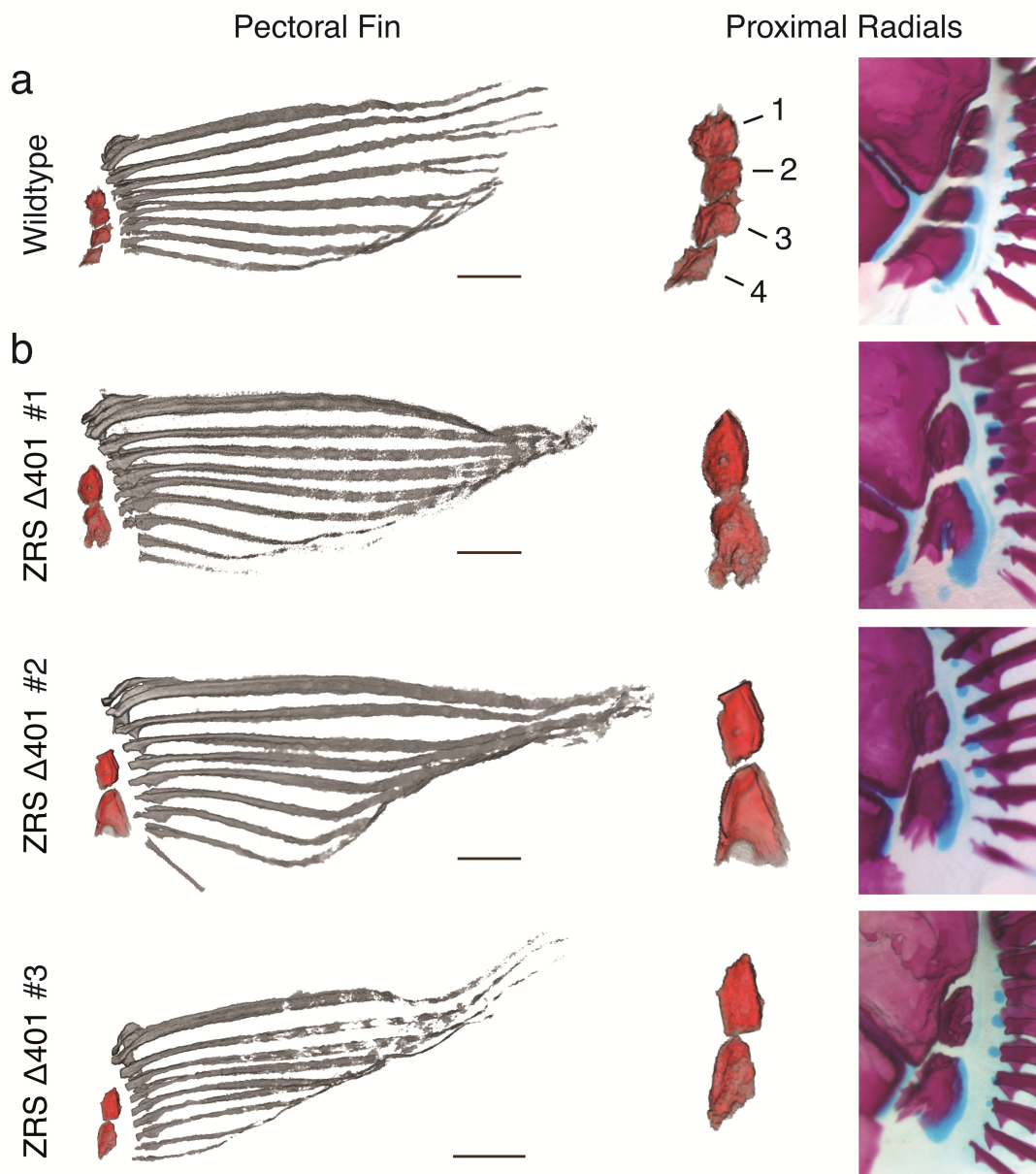
a, the ZRS enhancer is located between Exon 5 and 6 of the *Lmbr1* gene. **b**, vista plot showing the conservation degree of the ZRS enhancer in different species. **c-f**, transgenic mouse embryos showing the activity of the ZRS region from different species in the E10.5 developing limbs. Scale bars, 200µm. **g-k**, stable transgenic zebrafish larvae showing the activity of the ZRS region from different species at 72hpf pectoral fins. Arrows point to the ZPA domain. Scale bars, 50µm. For each construct shown in **c-k**, three or more independent transgenic lines were generated.



Supplementary Figure 2

Hh expression pattern in the dorsal fins of the lamprey ammocoete larva during metamorphosis.

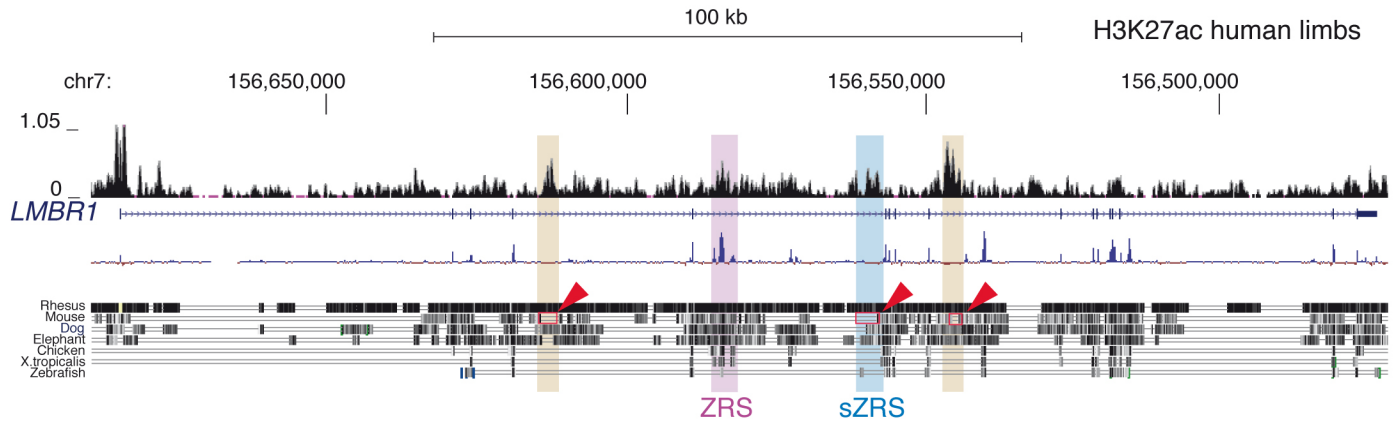
a, b, first dorsal (**a**) and second dorsal and caudal fins (**b**) of an ammocoete larva of the lamprey *L. reissneri*. Scale bars, 5 mm. **c-g**, *in situ* hybridizations of *L. reissneri* first (**c, d**) and second (**e-g**) dorsal fins in sections at different anterior-posterior levels (as shown in **a** and **b**), assayed with an equimolecular mix of digoxigenin-labeled riboprobes of the lamprey *L. camtschaticum* *HhA*, *HhB* and *HhD* genes. No expression of *Hh* genes in the underlying mesenchyme of developing dorsal fins was detected. Scale bars, 100 μ m. **h-j**, *L. camtschaticum* whole embryos at stage 27 hybridized with individual riboprobes of *HhA* (**h**), *HhB* (**i**) and *HhD* (**j**) used as positive controls for the *Hh* genes' probes. *HhD* expression pattern has not been hitherto described (see Sugahara et al., 2016). Scale bars, 200 μ m. **k, l**, section *in situ* hybridizations of *L. reissneri* second dorsal fin, at the levels shown in **b**, with digoxigenin-labeled riboprobes of the *L. camtschaticum* *MyHC1* (**k**) and *ColA* (**l**) genes (used as positive control). Expression in the skeletal muscle (*MyHC1*) and cartilage (*ColA*), was observed. Scale bars, 200 μ m. Lamprey expression experiments were repeated two times independently with similar results.



Supplementary Figure 3

μCT scanning of adult pectoral fins in wild-type and •401 mutant fish.

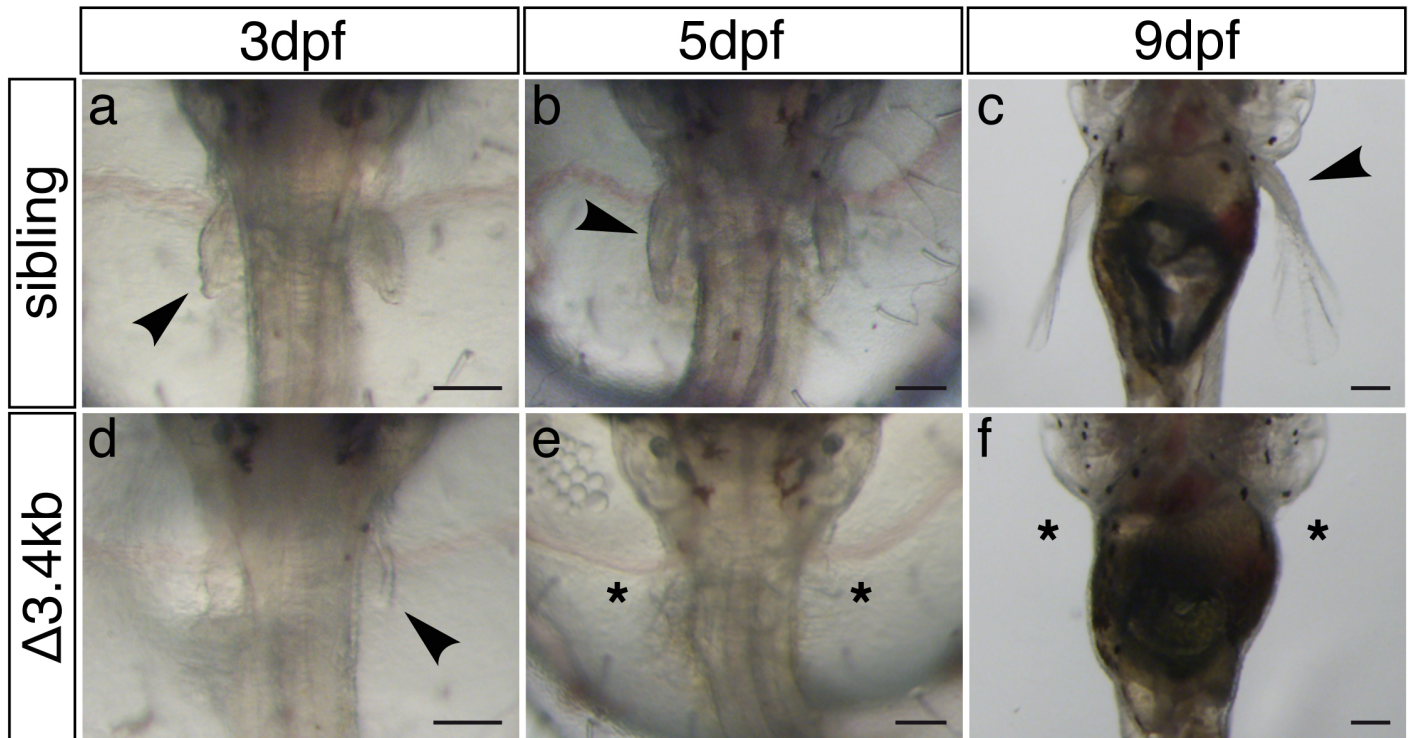
a, b the architecture of fin rays and proximal radials was revealed after scanning for three-month-old wild type and medaka mutants. Note the reduced number of endoskeletal elements in the mutants. μCT scanning experiments were performed for three WT and five •401 ZRS adult fish. Scale bars, 500μm **c**, in contrast to the endoskeletal defects, measurement of fin ray length did not show any significant differences (p -value=0.122) between wild type (mean=0.185) and •948 mutant fish (mean=0.204). A t-test was used for analysis of pectoral fin length measurements. Each point in the graph represent pectoral fin length measurements from independent animals.



Supplementary Figure 4

Potential fin and limb enhancers in the introns of the zebrafish and human *lmbr1* and *LMBR1* genes.

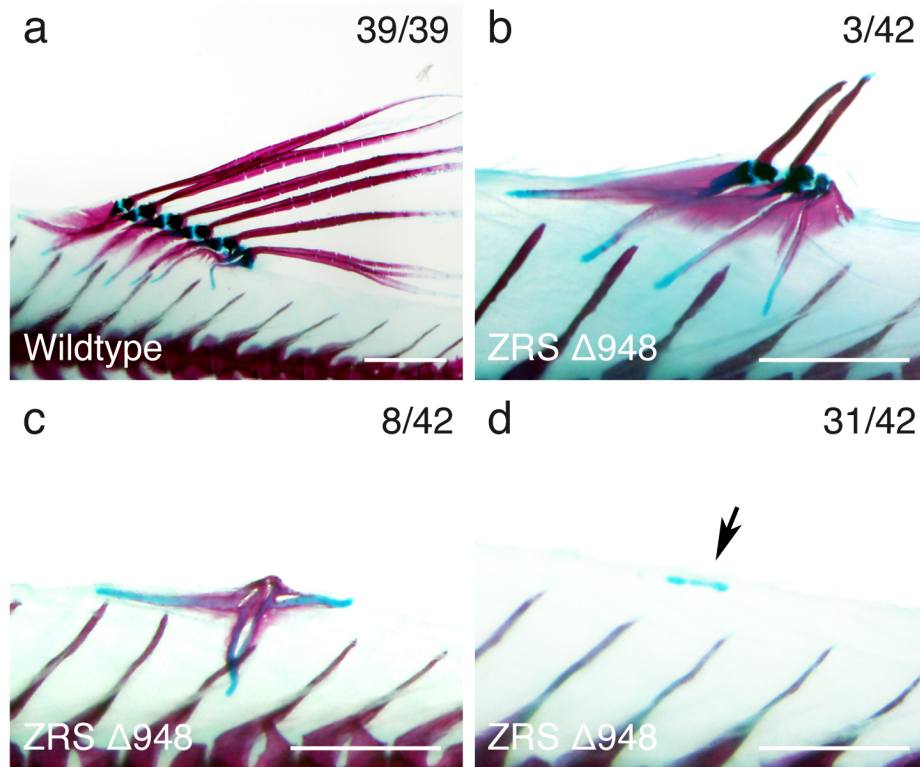
Genome coordinates are shown in the x axis and reads counts in the y axis. Several potential fin/limb enhancers, as predicted by H3K27ac signature are found in different introns of the zebrafish and human *lmbr1*/*LMBR1* genes. Although conserved with other mammals, these enhancers are not conserved in the mouse genome (red arrowheads). We search for putative ETS transcription factor binding sites in the sZRS of the three different species tested in the transgenesis assay. We found 3 ETS transcription-binding sites in the sZRS from zebrafish, 7 sites in medaka sZRS and 9 in human sZRS.



Supplementary Figure 5

Progression of pectoral fin development is truncated in $\Delta 3.4\text{-kb}$ ZRS mutants.

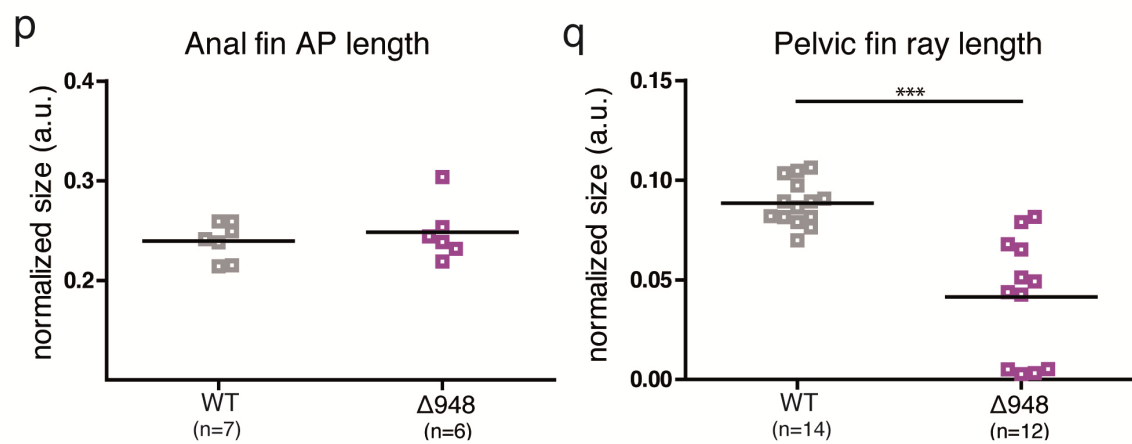
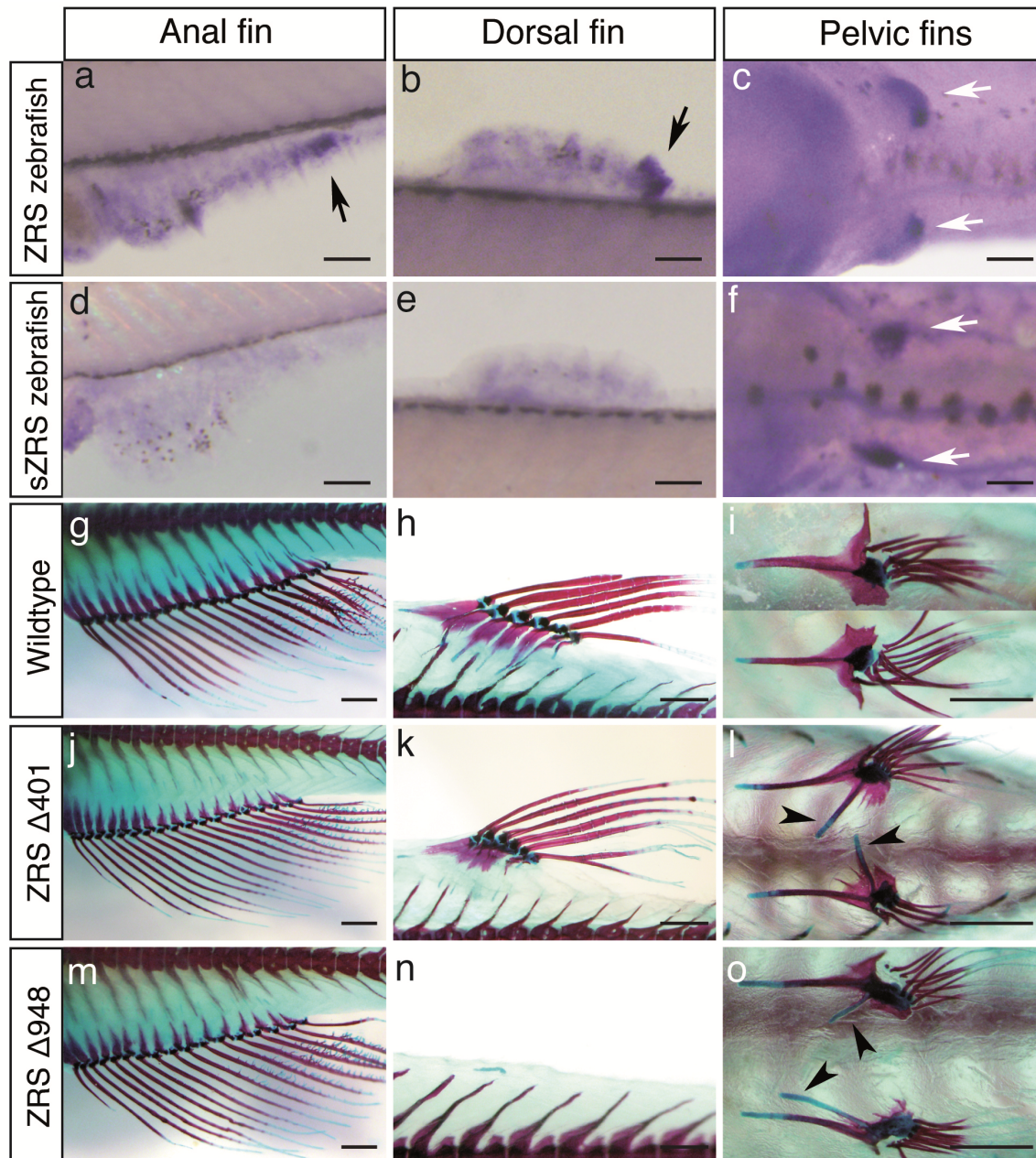
a-c, temporal series showing normal development of pectoral fins in wild type embryos (black arrowheads). **d-f**, at 3dpf pectoral fin buds appear reduced (black arrowhead) in mutant embryos and are absent during later developmental stages (asterisks). We could not analyse in detail the mature histology of other fins in the double mutant (ZRS-sZRS) due to fish lethality before the onset of pelvic and anal fins maturation (from 3-4 weeks post fertilization on). Nevertheless, the lack of activity of the sZRS in the anal fin (Extended data Figure 7d) and our observations of mutant hatchlings indicate that the ZRS-sZRS deletion does not affect significantly the development of the anal fin. For each stage represented in **a-f**, five or more individuals were analyzed. Scale bars, 100 μm .



Supplementary Figure 6

Detailed analyses of the dorsal fin phenotype in the ZRS $\Delta 948$ mutant.

Bone and cartilage staining showing the dorsal fin from wild type (a) or $\Delta 948$ (b-d) 4 month old fish. 74% (31/42) of the mutants show complete ablation (black arrow) of the dorsal fin (d) while in 19% (8/42) no fin rays are observed and endoskeletal elements are highly reduced (c). Only 7% (3/42) of the mutants analysed show fin rays although severely affected (b). Bone and cartilage staining protocol was performed in three independent experiments. Scale bars, 1mm.



Supplementary Figure 7

ZRS and sZRS enhancer expression and skeletal architecture for anal, dorsal and pelvic fins in wild type and •401 and •948 ZRS mutants.

a-c, whole mount ISH showing ZRS expression in the posterior region of the anal, dorsal (black arrows) and pelvic fin (white arrows) buds in zebrafish. **d-f**, whole mount ISH showing sZRS expression in pelvic fins (white arrows), but not in anal or dorsal zebrafish fin buds. ZRS and sZRS expression assessment in **a-f** was performed in three independent experiments with similar results. Scale bars, 100µm. **g-o**, Comparative analysis of skeletal elements, as revealed by alcian blue/alizarin red staining, for each of the fins in wild type and mutant adult fish. Ectopic elements are observed in pelvic mutant fins (black arrowheads). Bone staining was performed in three or more independent experiments for wildtype, •401 and •948 ZRS mutant animals with similar results. Scale bars, 1mm. **p-q**, Quantitative analysis of pelvic fins length illustrate the phenotypic defects observed in these appendages in the •948 mutants (WT mean= 0.088, •948 mean=0.041), p -value= 1.433×10^{-5} (***) . In contrast, no significant defects in structure and length are observed for the anal fin of •948 mutants (WT mean=0.239, •948 mean=0.248, p -value=0.512). Each point in **p** and **q** graphs represents measurements from single adult fins. Differences in fin length in **p** and **q** were analysed using a t-test.

Organism	ZRS Forward (5') Primer	ZRS Reverse (3') Primer
<i>M. musculus</i>	AGTTCAAGAAACCGCACACC	GACAGCAACATCCTGACCAA
<i>A. carolinensis</i>	GGCAGAGACAGACGTGATGA	TGGCATGCTTTTCTAGCTT
<i>L. menadoensis</i>	CAGCAAAGACCCCAAAATAG	GGAAAAAGACATTTTGTGTCTCC
<i>O. latipes</i>	ACTTTCTTCTGGTGTGCGTC	TCACCGCAGAAAGCTCATTA
<i>D. rerio</i>	AACACGGGACAATTTTGAGC	TTTCTGCTGCTGCATACAAAT
<i>L. oculatus</i>	GGTGCTTTCCTCATCTCTGC	AGTGAAACAGGTGCCGTCTT
<i>L. erinacea</i>	ATGGATCATCAATGGGGAGA	TTTGAGTTGACCATGCAAGC
<i>P. marinus</i> (1)	GTGATGTTGGTTGTCGTTGTG	CTAAGTCACGTGGGAAAGCTG
<i>P. marinus</i> (2)	ACTTGGTCTTCTTGTGCTCCA	CGAGGTCAAGTAGTGCCTCAC
<i>B. lanceolatum</i>	ATCGTGCTGAGGAAGGTGAC	TGTGCCTCTACGCATTCAAG

	sZRS Forward (5') Primer	sZRS Reverse (3') Primer
<i>D. rerio</i>	TTTTCATCAGCCTTCTGAACC	CAAACCTGCAACCAACTG
<i>H. sapiens</i>	TATGGTTTGCCTGTGTCCCC	CCCCTTGGCCTCCTAAAGTG
<i>O. latipes</i>	AGACAAGGTCGCTAGAACTGC	GTGTTTCAGCAGCACATCCAG

	Other regions tested	
	Forward (5') Primer	Reverse (3') Primer
<i>O. latipes</i>	TGTTTTACCCGCGAGATGCAG	TCACATTTTCGCGAGGATTGG
<i>O. latipes</i>	GGACATCACAGGTAGGGCTG	ATGCTGTCAGCCTGTACACC

	4C-seq primers	
	Enzyme: DpnII	Enzyme: Csp6I
<i>M. musculus</i>	<i>Shh</i> CCAGTCTGGGTGGGGATC	<i>Shh</i> CCTTATTTACACCCCATCCCC
<i>O. latipes</i>	<i>Shh</i> CATCAAGACTTCAGGACTGGATC	<i>Shh</i> TTTTGCCTTCGTATCTGCCGC
<i>O. latipes</i>	ZRS CTGGGAAAGGAGAGGAGGATC	ZRS ACAATCCTTGAAACGGCGAG

	CRISPR target sites	
<i>O. latipes</i> Δ401	GGTGAGTAATCAATTAAACG AGG	GTCCAGCTGTATTACCATGC AGG
<i>O. latipes</i> Δ948	GGCTTCAACATTAGCCCAG GAGG	AGCTGTATTACCATGCAG GAGG
<i>O. latipes</i> Δ3.4kb	GTCCAGCTGTATTACCATGC AGG	TGGCCTCTAACGAGCCACAC GGG

	Deletion screening primers	
	Forward (5') Primer	Reverse (3') Primer
<i>O. latipes</i> Δ401/Δ948	ATTTTAAAAAGCATCCACATTTCC	AAATGAAGTGTCTGGGTGTATCCT
<i>O. latipes</i> Δ3.4kb	CACATCATCTGTGATTGGAATTTTA	TTAAAAGCATGAATGAGTGTGAAAC

	Lamprey cloning primers	
	Forward (5') Primer	Reverse (3') Primer
<i>L. camtschaticum</i> MyHC1	ACGTGCAGGGACAGATCAAG	TCTCCAGCCTTGACAACACC
<i>L. camtschaticum</i> CoLA	CGATTCTACCGTGGCGATCA	CCGAACCTCTGATCCACACC

Supplementary Table 1

Oligos used for PCR amplification of the ZRS and sZRS enhancer from different species. Also listed are primers used for the 4C-seq analyses, genomic deletion target sites (PAM sequence in bold), genomic deletions screening and lamprey cloning.