Organism	ZRS Forward (5') Primer	ZRS Reverse (3') Primer
M. musculus	AGTTCAAGAAACCGCACACC	GACAGCAACATCCTGACCAA
A. carolinensis	GGCAGAGACAGACGTGATGA	TGGCATGCTTTTCCTAGCTT
L. menadoensis	CAGCAAAGACCCCCAAATAG	GGAAAAAGACATTTTTAGTGTCTCC
O. latipes	ACTTTCTTCTGGTGTCGGTC	TCACCGCAGAAAGCTCATTA
D. rerio	AACACGGGACAATTTTGAGC	TTTCTGCTGCTGCATACAAAT
L. oculatus	GGTGCTTTCCTCATCTCTGC	AGTGAAACAGGTGCCGTCTT
L. erinacea	ATGGATCATCAATGGGGAGA	TTTGAGTTGACCATGCAAGC
P. marinus (1)	GTGATGTTGGTTGTCGTTGTG	CTAAGTCACGTGGGAAAGCTG
P. marinus (2)	ACTTGGTCTTCTTGTGCTCCA	CGAGGTCAAGTAGTGCCTCAC
B. lanceolatum	ATCGTGCTGAGGAAGGTGAC	TGTGCCTCTACGCATTCAAG

	sZRS Forward (5') Primer	sZRS Reverse (3') Primer
D. rerio	TTTTCATCAGCCTTCTGAACC	CAAACCTGCAACCAACACTG
H. sapiens	TATGGTTTGCCTGTGTCCCC	CCCCTTGGCCTCCTAAAGTG
O. latipes	AGACAAGGTCGCTAGAACTGC	GTGTTCAGCAGCACATCCAG

	Other regions tested	
	Forward (5') Primer	Reverse (3') Primer
O. latipes	TGTTTTACCCGCAGATGCAG	TCACATTTCGCAGCGATTGG
O. latipes	GGACATCACAGGTAGGGCTG	ATGCTGTCAGCCTGTACACC

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

	CRISPR target sites	
O. latipes Δ401	GGTGAGTAATCAATTAAACG**AGG**	GTCCAGCTGTATTACCATGC**AGG**
O. latipes Δ948	GGCTTCAACATTAGCCCAGG**AGG**	AGCTGTATTACCATGCAGGA**GGG**
O. latipes Δ3.4kb	GTCCAGCTGTATTACCATGC**AGG**	TGGCCTCTAACGAGCCACAC**GGG**

	Deletion screening primers	
	Forward (5') Primer	Reverse (3') Primer
O. latipes Δ401/Δ948	ATTTTAAAAAGCATCCACATTTCC3	AAATGAAGTGTCTGGGTGTATCCT3
O. latipes Δ3.4kb	CACATCATCTGTGATTGGAATTTTA	TTAAAAGCATGAATGAGTGTGAAAC

	Lamprey cloning primers	
	Forward (5') Primer	Reverse (3') Primer
L. camtschaticum MyHC1	ACGTGCAGGGACAGATCAAG	TCTCCAGCCTTGACAACACC
L. camtschaticum ColA	CGATTCTACCGTGGCGATCA	CCGAACTCCTGATCCACACC

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.