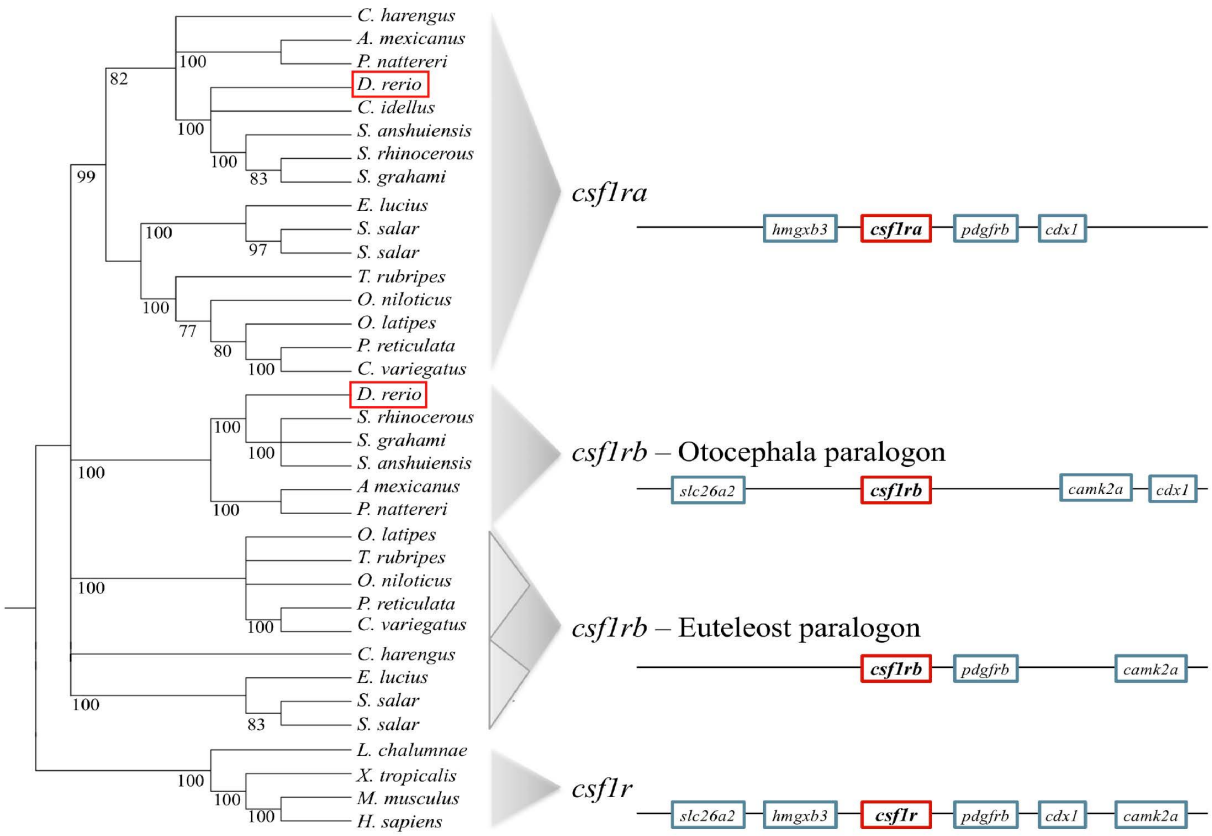

Figure S1. Phylogenic analysis of *csf1r* paralogues in vertebrates. Phylogenetic relationships between zebrafish *csf1ra* and *csf1rb* paralogs and vertebrate orthologs. Amino acid divergence based on maximum likelihood algorithm (500 replicates; ≥70 bootstrap shown as unique branch). In support of homology assessments, synteny between *csf1ra* and *csf1rb* was analyzed across multiple vertebrate species. Data supports *csf1rb* being an ohnologue of *csf1ra*, stemming from a teleost whole genome duplication of a common homologue.

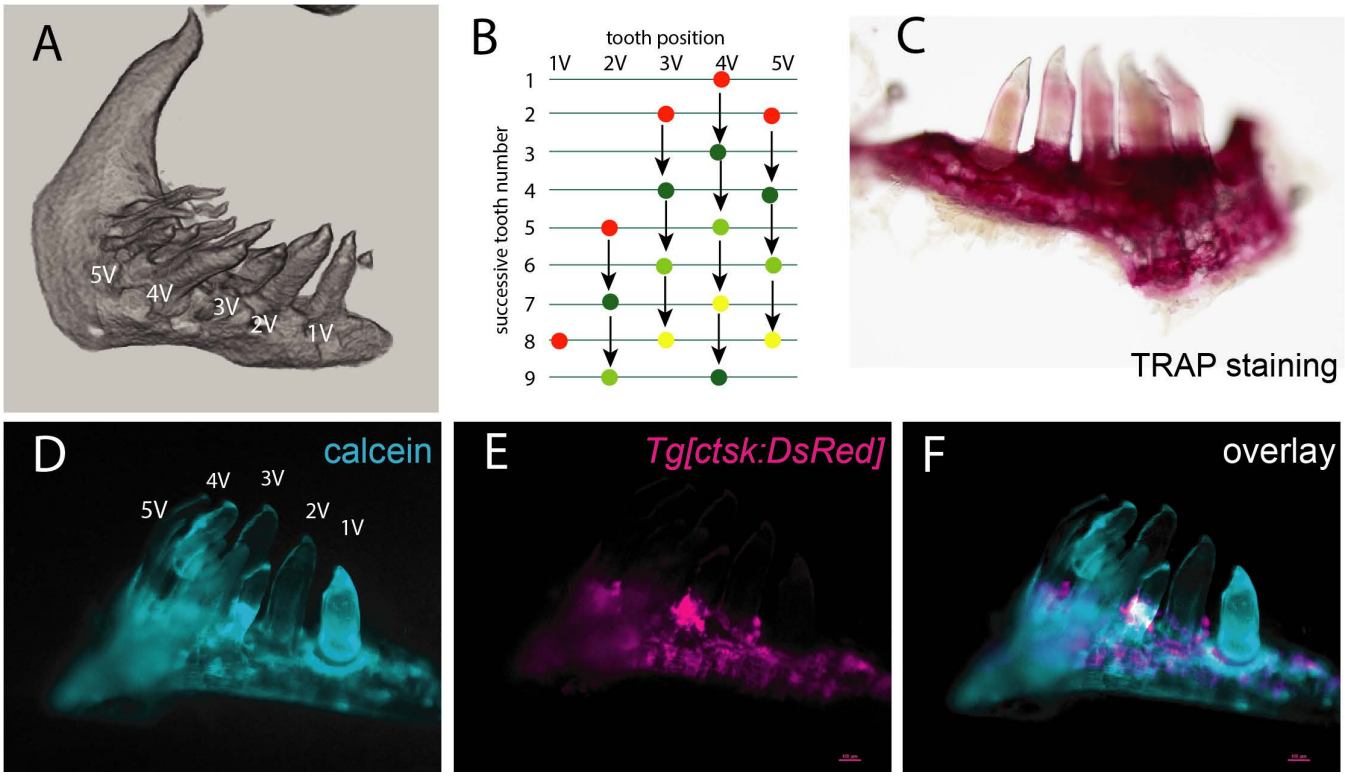

Figure S2. Generation of mutants in *csf1r* paralogues in the zebrafish. (**A**) Clustering of zebrafish *csf1r* genes with closely related receptor tyrosine kinases supports paralogous relationship between *csf1ra* and *csf1rb*. (**B-C**) Schematic of the gene and protein structure of *csf1r* genes indicating the position and consequence of the mutation. (**B**) The *csf1ra*^{mh5} allele of *csf1ra* is a G>T substitution at bp 1466 of the coding sequence predicted to result in a premature termination of the protein (E454X). **C**) The *csf1rb*^{mh108} mutant zebrafish allele is a combination of an 8bp insertion and a 4bp deletion within exon 3 due to targeted editing of the *csf1rb* locus by CRISPR/Cas9. This change is predicted to cause a frameshift at amino acid 160 leading to a premature termination of the Csf1rb protein at amino acid 233 (P160L;fs73X). The *csf1rb*^{mh112} mutant allele is a 13bp deletion within exon 3 resulting in a frameshift and premature termination of the Csf1rb protein at amino acid 161 (D159A;fs2X).

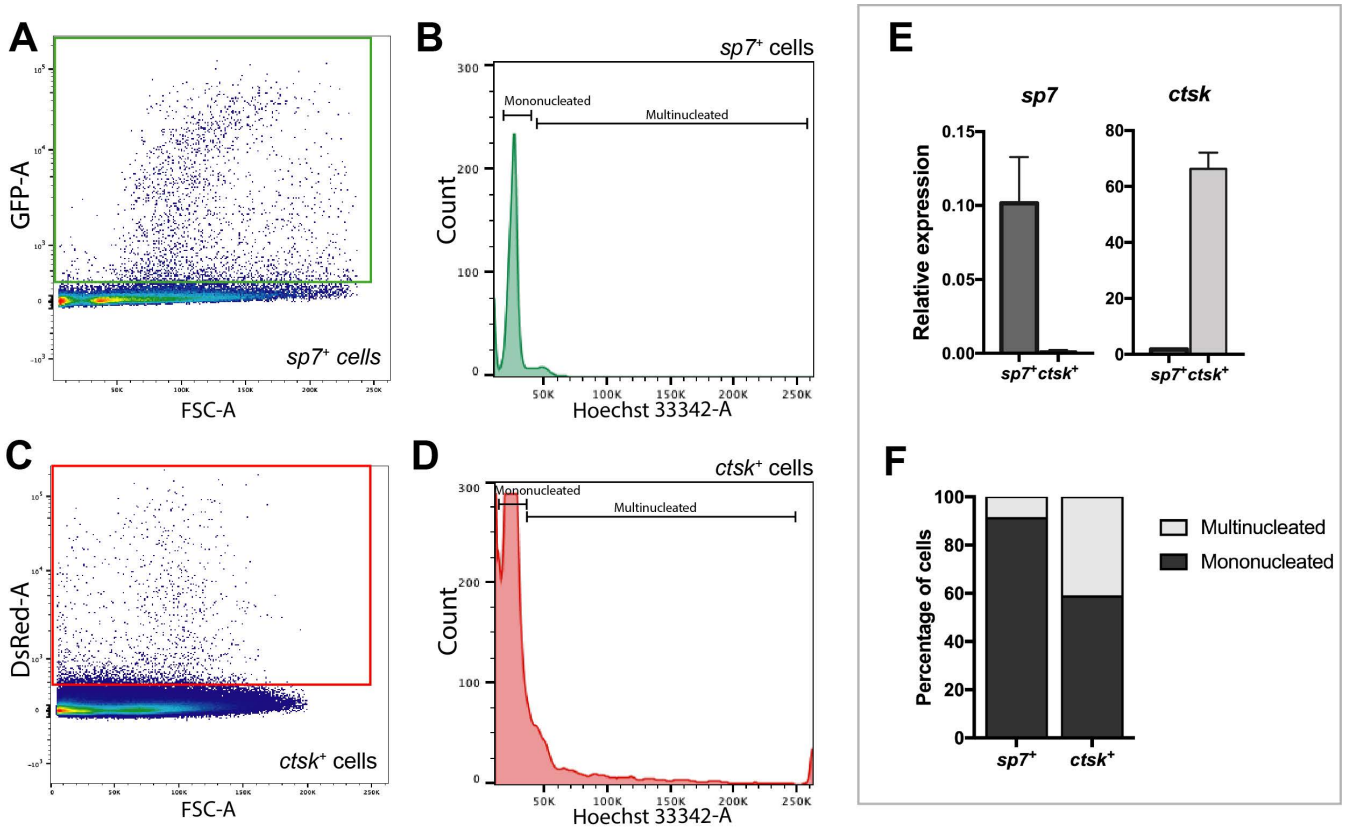

Figure S3. *CathepsinK* transgenic line specifically marks osteoclasts during late skeletal development in zebrafish. (**A**) Schematic detailing the construct used to generate an osteoclast reporter line expressing *DsRed* under the control of the medaka *cathepsin K* promoter. This construct has the capability to drive *Cre* recombinase expression by flippase-mediated recombination. (**B-C**) Expression of *Tg[ctsk;DsRed]* in opercula (B) and in the spine (C) of 2wpf zebrafish in comparison with sites of calcified bone as detected by calcein staining (cyan). (**D**) Co-localization of *DsRed* expression with TRAP staining in a 10wpf wild-type scale.

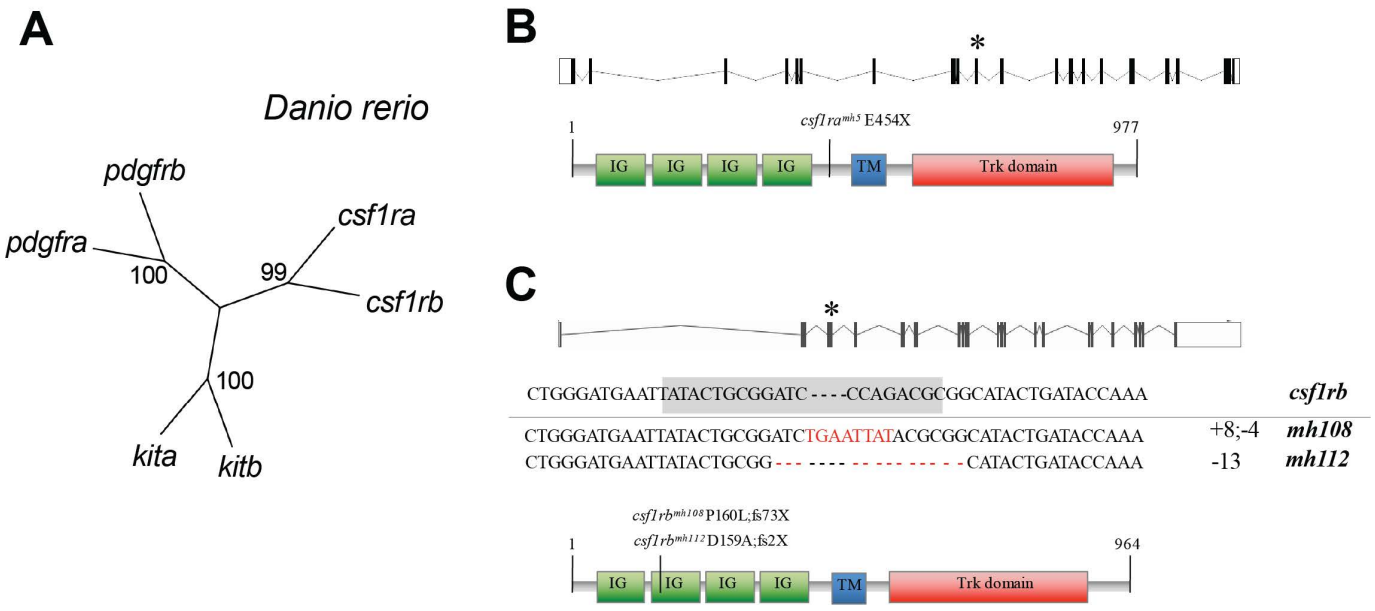

Figure S4. FACS isolation of osteoclasts and osteoblasts from adult zebrafish. (A, C) *sp7*$^{+}$ and *ctsk*$^{+}$ cells were isolated from adult *Tg[sp7:EGFP]* and *Tg[ctsk:DsRed]* fish, respectively, and identified by their expression of *EGFP* (A) and *DsRed* (C). (**E**) *EGFP*$^{+}$ and *DsRed*$^{+}$ cells were sorted and analyzed regarding their expression of *sp7* and *ctsk*. (**B, D**) Cells were gated and analyzed for Hoechst 33342 expression. A Hoechst 33342 histogram was plotted and *sp7:EGFP*$^{+}$ cells were used to set the gating strategy for identification of mononucleated cells (B). Osteoblasts are traditionally mononucleated cells, so the small population that falls inside the multinucleated gate is due to higher DNA content during cell division. By applying the same gates to the *ctsk:DsRed*$^{+}$ cells we identified the multinucleated cell population (D). (**F**) The percentage of mononucleated and multinucleated cells was quantified for both fish lines.

Figure S5. Pattern of osteoclast distribution on juvenile and adult vertebrae. (**A-C**) Representative fluorescent photomicrographs of the spine of individual *csf1r* mutants and wild-type siblings at 2wpf carrying the osteoclast reporter *Tg[ctsk:DsRed]* and counterstained with calcein (cyan). (**D-F**) At 4 months of age, spines of wt and individual *csf1r* mutants exhibit a wide range of *ctsk:DsRed*$^{+}$ cell numbers. Shown here are representative fluorescent photomicrographs of spines with high and low number of cells.

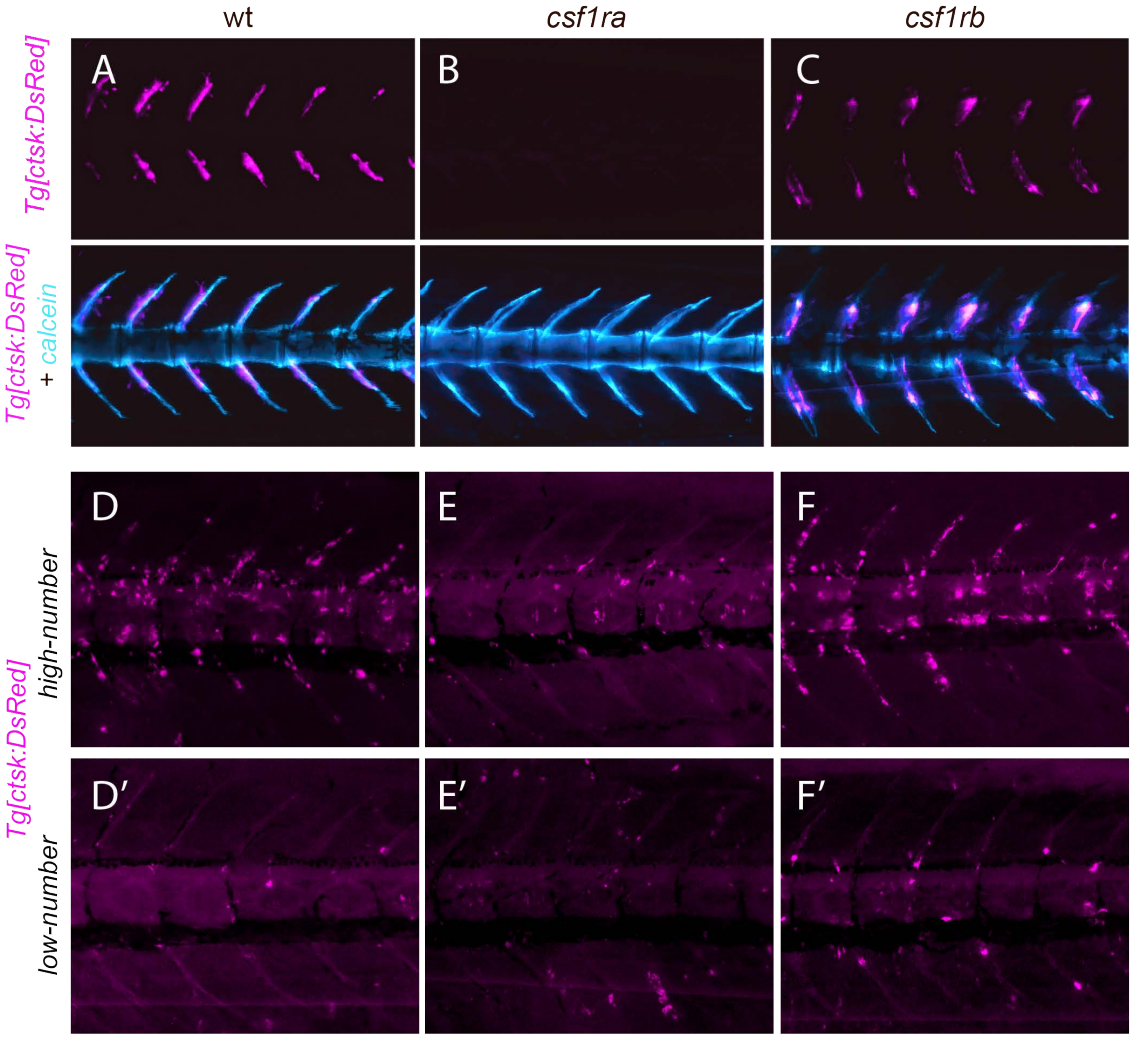

Figure S6. Tooth development and expression of osteoclast markers.

(**A**) MicroCT of the teeth bearing fifth ceratobranchial of the adult zebrafish. Tooth labeled 1V-5V in order of position. More medial teeth, 4M or 1D and 2D are not noted. (**B**) Diagram of timing of tooth formation and pattern of replacement (after (Huysseune and Witten, 2006)). Changes in color indicate sequential teeth replaced in a series. (**C**) Whole mount TRAP staining of ceratobranchial 5 of an adult zebrafish showing presence of active osteoclasts covering the extent of the arch. (**D-F**) Representative pictures of ceratobranchial 5 from *Tg[ctsk:DsRed]* transgenic adult fish, marking osteoclasts surrounding the dentition; (**F**) overlay of *ctsk*-marked cells (**E**) and mineralized tissue labeled with calcein (**D**).

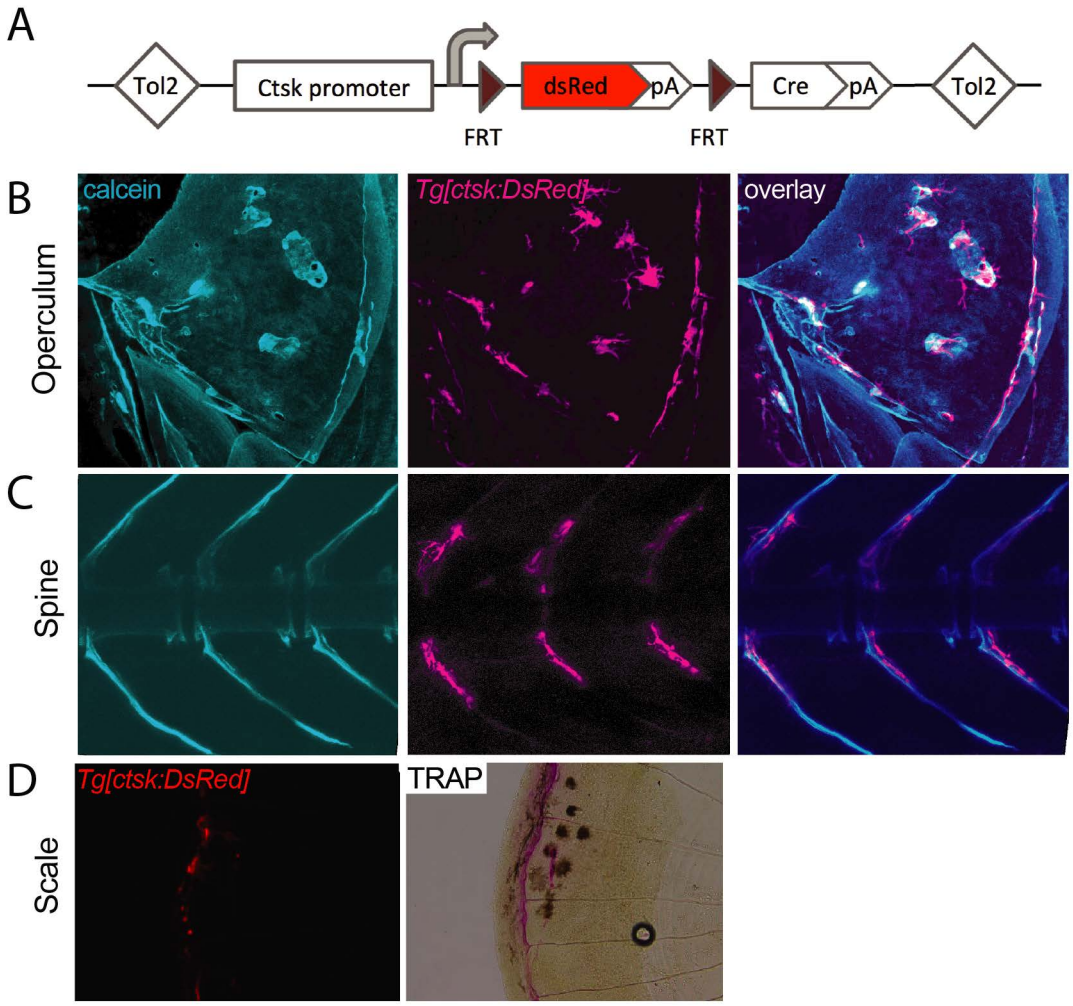

Figure S7. Multispecies alignment of *csf1ra* conserved non-coding element.

Yellow, conserved core regions common to all fishes; other colors are specific to the different teleost lineages. Sites of FOXO and CREB/AP1 binding site shown.

Table S1- Accession numbers of protein sequences used in the phylogenetic analysis.

Species	gene	Accession number
Danio rerio	*csf1ra*	NP_571747
	csf1rb	F1QPE2
	pdgfrα	F1QNY7
	pdgfrβ	A0A0G2L2B8
	kita	Q8JFR5
	kitb	B8A5K6
Oryzias latipes	*csf1ra*	H2LJC3
	csf1rb	H2MBW3
Takifugu rubripes	*csf1ra*	H2SUK2
	csf1rb	I6L6X5
Astyanax mexicanus	*csf1ra*	W5LCP3
	csf1rb	W5KLJ6
Oreochromis niloticus	*csf1ra*	I3K5N3
	csf1rb	I3KMS8
Poecilia reticulata	*csf1ra*	XP_008400061.1
	csf1rb	XP_017158513.1
Sinocyclocheilus grahami	*csf1ra*	XP_016131860
	csf1rb	XP_016102236
Sinocyclocheilus rhinocerous	*csf1ra*	XP_016426425
	csf1rb	XP_016377545
Sinocyclocheilus anshuiensis	*csf1ra*	XP_016344784
	csf1rb	XP_016361100
Clupea harengus	*csf1ra*	XP_012687948
	csf1rb	XP_012669788
Pygocentrus nattereri	*csf1ra*	XP_017559280
	csf1rb	XP_017575061
Ctenopharyngodon idellus	*csf1ra*	AKM12662
Esox lucius	*csf1ra*	XP_010895130
	csf1rb	XP_01901945
Salmo salar	*csf1ra*	XP_014067972
	csf1ra	XP_014055375
	csf1rb	XP_013985018
	csf1rb	XP_014051429
Cyprinodon variegatus	*csf1ra*	XP_015246436
	csf1rb	XP_015257023
Latimeria chalumnae	*csf1r*	M3XGP0
Xenopus tropicalis	*csf1r*	F6Z3F8
Mus musculus	*csf1r*	P09581
Homo sapiens	*csf1r*	NP_001275634

Table S2- Accession numbers of *csf1ra* genomic sequences used to retrieve CNE sequences.

Species		Accession number
Danio	*rerio*	ENSDARG00000102986
	aesculapii	D.Parichy (*unpublished*)
	erythromicron	
	margaritatus	
	nigrofasciatus	
	choprae	
	abolineatus	
Takifugu rubripes		ENSTRUG00000006553
Tetraodon nigroviridis		ENSTNIG00000014226
Oreochromis niloticus		ENSONIG00000013065
Gasterosteus aculeatus		ENSGACG00000018007
Oryzias latipes		ENSORLG00000004849
Poecilia formosa		ENSPFOG00000003496
Xiphophorus maculatus		ENSXMAG00000008588
Astyanax mexicanus		ENSAMXG00000017088