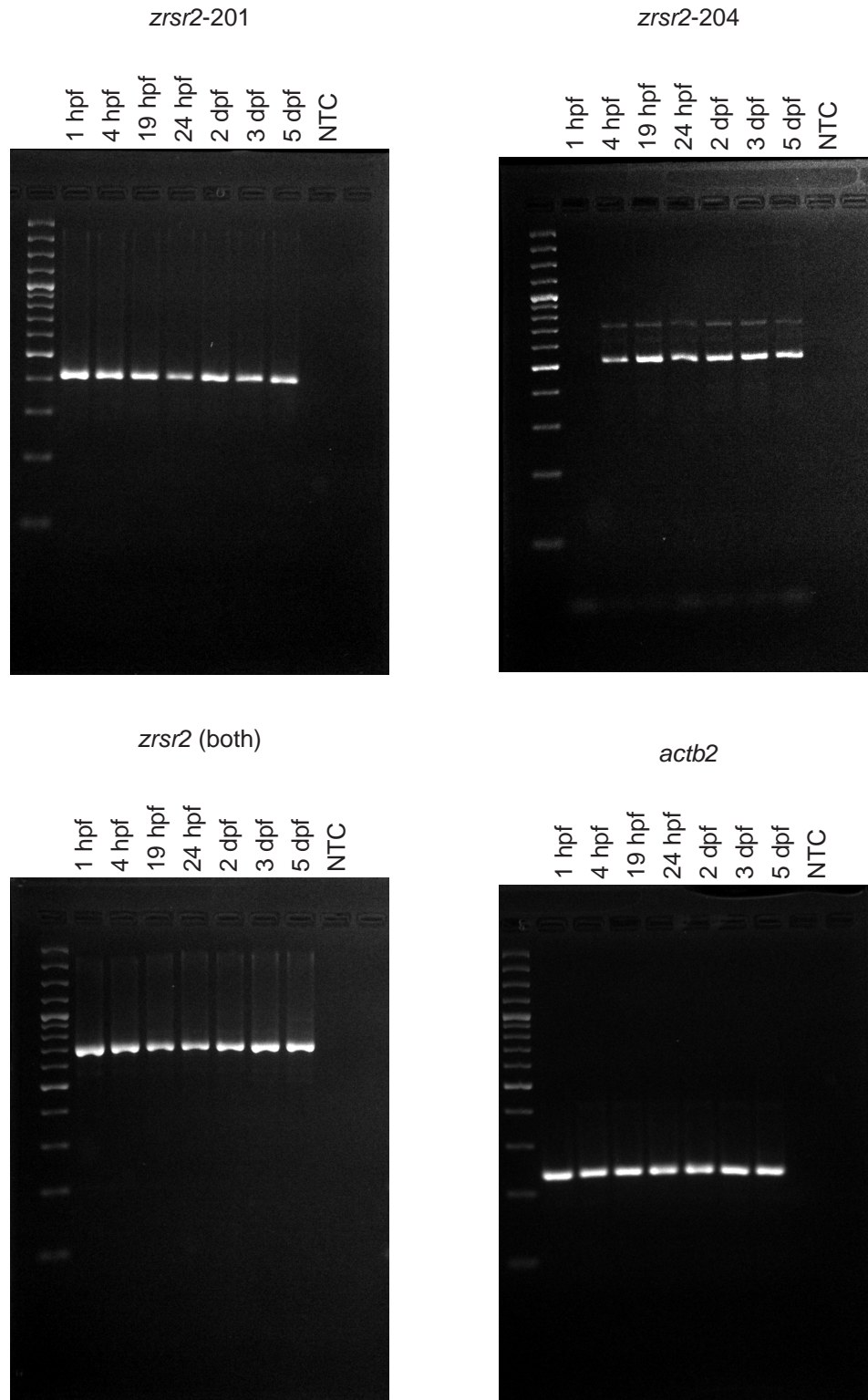


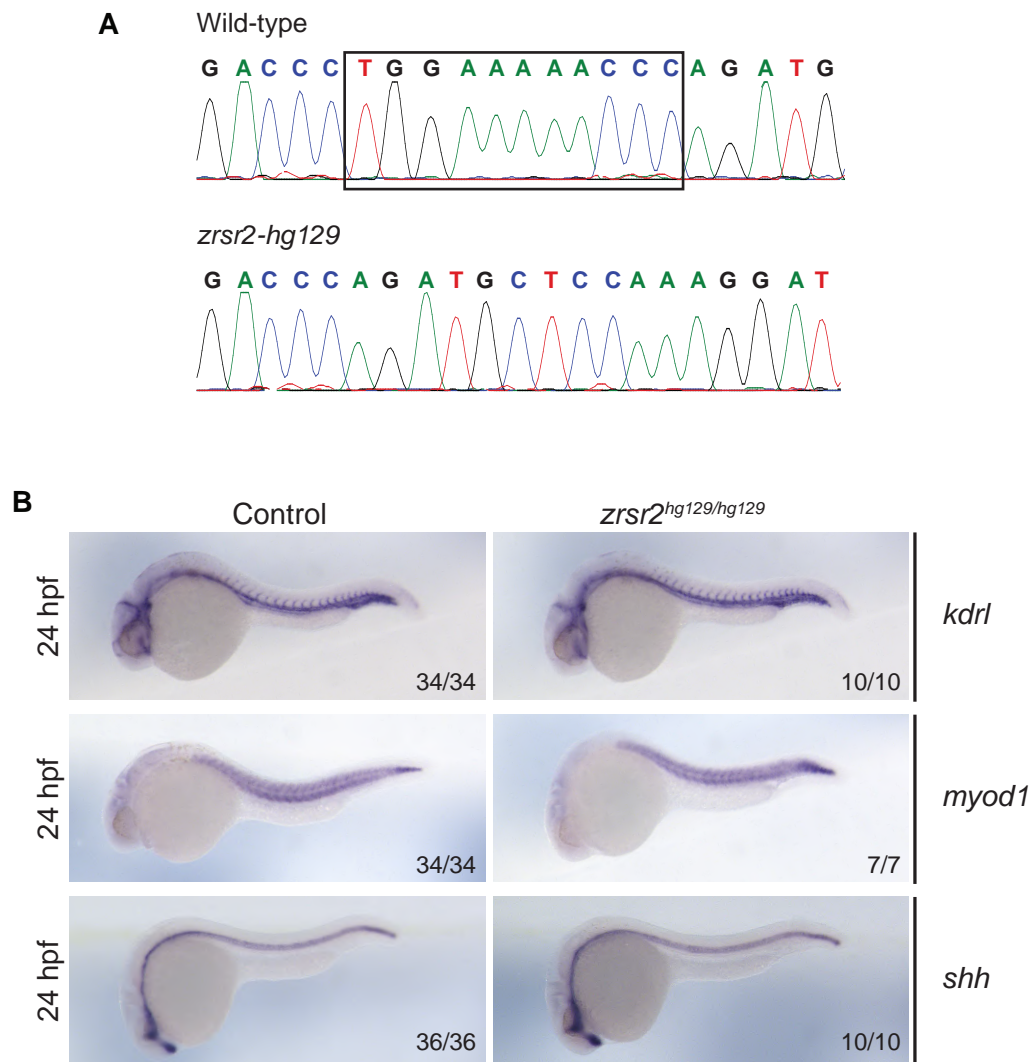
Supplementary Figure S1. Alignment of human ZRSR2 with both isoforms of zebrafish Zrsr2. Sequence alignment was performed using CLUSTALW in the MacVector software. Identical amino acids are shaded dark grey and similar amino acids are shaded light grey. Numbers on the left and right sides of each row represent the amino acid numbers in the protein sequence. The green boxes indicate the zinc finger domains, and the purple box indicates the RNA recognition motif.

ZRSR2_Human	1	MAA	PEKMT	FPEKP	SHKKY	RAALK	KEKRR	KRRQE	LAR	LD	SGLS	43																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
Zrsr2_Zebrafish_201	1	MTEKGCCS	IQTETQDSE	NRL	NMCET	TPSVVL	SHKKR	RAALKR	ERRRKR	RQALAK	IKKEAD	60																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
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ZRSR2_Human	44	QKE	EEEE	DTFIE	EQL	EE	EKL	LER	ER	QRL	HE	EWLL	RE	QK	AQEE	FR	IKKE	KEE	AA	KKR	QEE	Q	103																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
Zrsr2_Zebrafish_201	61	- - -	LQNE	EELQS	EAE	D	E	DKL	KVDE	ES	ER	LHQ	EWME	RER	I	AQEE	FR	LKKER	ED	AA	QQR	KEE	177																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
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ZRSR2_Human	104	ERK	LKE	QWEE	QQR	KERE	EE	EQ	KR	QEKKE	KEE	AL	QKMLD	QAE	NE	LE	NGT	T	W	QNP	EP	PVDF	FR	163																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
Zrsr2_Zebrafish_201	118	ERK	IKE	EWEE	QQR	KDK	EER	EH	KE	QEKRE	REE	AV	QKMLD	QAE	S	Q	LE	NGG	P	W	KNP	DAP	KDYG	177																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
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ZRSR2_Human	164	VME	CDR	ANCP	FYS	KTG	ACR	FGD	RCS	RKH	NFPT	SSP	TTLL	IKS	MF	TT	F	GME	Q	CR	DDYD	P	DA	223																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
Zrsr2_Zebrafish_201	178	-TE	CDK	ANCP	FFLKT	GTAC	RFGD	RCS	RKH	CH	PASS	CT	LMV	RG	MFVS	F	GME	Q	S	RR	DDYD	T	DA	236																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
Zrsr2_Zebrafish_204	26	-TE	CDK	ANCP	FFLKT	GTAC	RFGD	RCS	RKH	CH	PASS	CT	LMV	RG	MFVS	F	GME	Q	S	RR	DDYD	T	DA	84																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
ZRSR2_Human	224	SLEY	SEEE	TY	QQ	FLD	FYED	V	L	P	E	F	K	N	V	G	K	V	I	Q	F	K	V	S	C	N	L	E	P	H	L	R	G	N	V	Y	V	Q	Y	Q	S	E	E	E	C	Q	A	A	283																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
Zrsr2_Zebrafish_201	237	SLEY	SEEE	LH	QQ	FLD	FYED	A	L	P	E	F	K	N	A	G	R	V	V	Q	F	K	V	S	C	N	F	E	P	H	L	R	G	N	V	Y	V	Q	Y	E	T	E	E	Q	C	K	E	A	296																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
Zrsr2_Zebrafish_204	85	SLEY	SEEE	LH	QQ	FLD	FYED	A	L	P	E	F	K	N	A	G	R	V	V	Q	F	K	V	S	C	N	F	E	P	H	L	R	G	N	V	Y	V	Q	Y	E	T	E	E	Q	C	K	E	A	144																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
ZRSR2_Human	284	L	S	L	F	N	G	R	W	Y	A	G	R	L	Q	C	E	F	C	P	V	T	R	W	K	T	A	I	C	G	L	F	D	R	R	K	C	P	K	G	K	H	C	N	F	L	H	V	F	R	N	P	N	N	E	F	W	E	A	N	343																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
Zrsr2_Zebrafish_201	297	F	V	M	F	N	G	R	W	Y	A	G	R	L	Q	C	E	F	S	P	V	T	R	W	K	T	A	I	C	G	L	F	D	R	R	K	C	P	K	G	K	H	C	N	F	L	H	V	F	R	N	P	D	N	E	F	W	E	A	D	356																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
Zrsr2_Zebrafish_204	145	F	V	M	F	N	G	R	W	Y	A	G	R	L	Q	C	E	F	S	P	V	T	R	W	K	T	A	I	C	G	L	F	D	R	R	K	C	P	K	G	K	H	C	N	F	L	H	V	F	R	N	P	D	N	E	F	W	E	A	D	204																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
ZRSR2_Human	344	R	D	I	Y	L	S	P	D	R	T	G	S	S	F	G	K	N	S	E	R	R	E	- - -	R	M	G	H	H	D	Y	Y	S	R	L	R	G	-	R	R	N	P	S	P	D	-	H	S	Y	K	-	-	R	N	G	E	S	395																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
Zrsr2_Zebrafish_201	357	R	D	L	H	M	S	P	D	R	G	G	G	F	S	A	R	H	M	S	G	R	D	G	M	W	S	Q	R	G	N	S	S	E	R	F	Q	R	R	R	T	D	R	R	S	H	S	R	E	R	Y	S	Y	R	G	R	R	S	R	416																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
Zrsr2_Zebrafish_204	205	R	D	L	H	M	S	P	D	R	G	G	G	F	S	A	R	H	M	S	G	R	D	G	M	W	S	Q	R	G	N	S	S	E	R	F	Q	R	R	R	T	D	R	R	S	H	S	R	E	R	Y	S	Y	R	G	R	R	S	R	264																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
ZRSR2_Human	396	E	R	K	S	-	S	R	H	R	G	K	S	H	K	R	T	S	K	S	R	E	R	H	N	S	R	S	R	G	N	R	D	R	S	R	D	R	S	R	G	R	G	S	R	S	R	S	R	S	R	R	S	R	S	R	S	R	454																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
Zrsr2_Zebrafish_201	417	E	R	R	S	R	S	R	E	R	R	S	R	S	R	E	R	R	S	R	E	R	Q	N	R	N	R	E	R	Q	S	R	S	R	S	R	S	R	E	K	R	K	S	E	N	S	I	R	R	S	T	S	Q	R	E	Q	S	R	S	R	476																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
Zrsr2_Zebrafish_204	265	E	R	R	S	R	S	R	E	R	R	S	R	S	R	E	R	Q	N	R	N	R	E	R	Q	S	R	S	R	S	R	S	R	S	R	E	K	R	K	S	E	N	S	I	R	R	S	T	S	Q	R	E	Q	S	R	S	R	324																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
ZRSR2_Human	455	S	Q	S	S	S	R	S	R	- - - - -	G	R	R	S	G	N	R	- - - - -	- - - - -	- - - - -	- - - - -	R	T	V	Q	S	P	- - - - -	479																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
Zrsr2_Zebrafish_201	477	S	R	S	Q	S	R	D	R	L	K	Q	S	D	S	A	D	S	G	K	K	M	S	K	S	N	S	P	E	H	V	S	K	N	G	S	V	K	R	K	S	H	R	K	E	S	Q	S	P	E	N	T	D	R	M	D	A	H	H	H	K	536																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
Zrsr2_Zebrafish_204	325	S	R	S	Q	S	R	D	R	L	K	Q	S	D	S	A	D	S	G	K	K	M	S	K	S	N	S	P	E	H	V	S	K	N	G	S	V	K	R	K	S	H	R	K	E	S	Q	S	P	E	N	T	D	R	M	D	A	H	H	H	K	384																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
ZRSR2_Human	480	- - -	K	S	K	*																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												

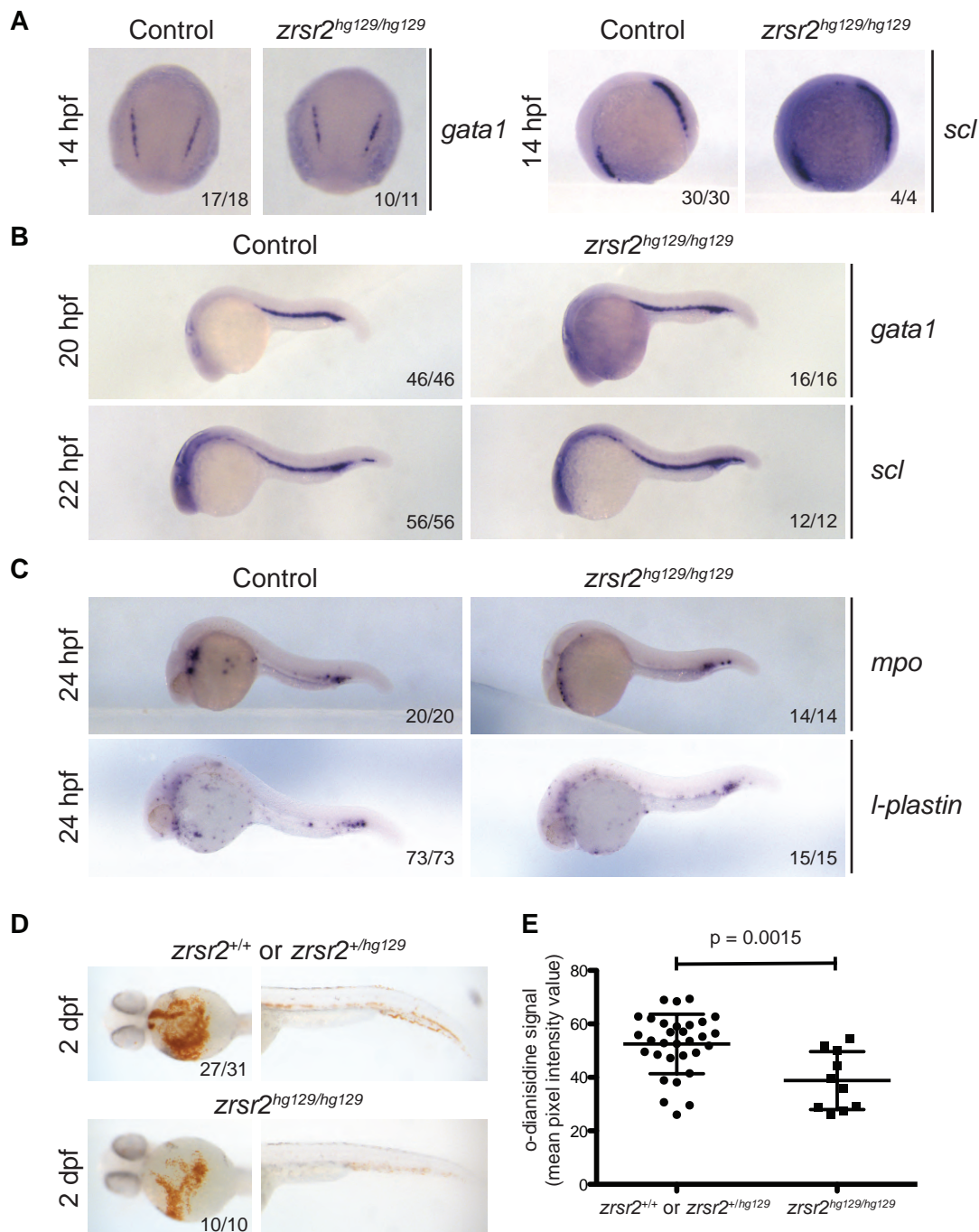
Supplementary Figure S2. RT-PCR data showing *zrsr2* expression at various stages of development. Agarose gel images showing RT-PCR products with primers unique to isoform-201 (418 bp product), unique to isoform 204 (514 bp product), common to both isoforms (697 bp product) and loading control *actb2* (232 bp product) in WT embryos at various stages of development as marked across the top. Water was used as a no template control (NTC).



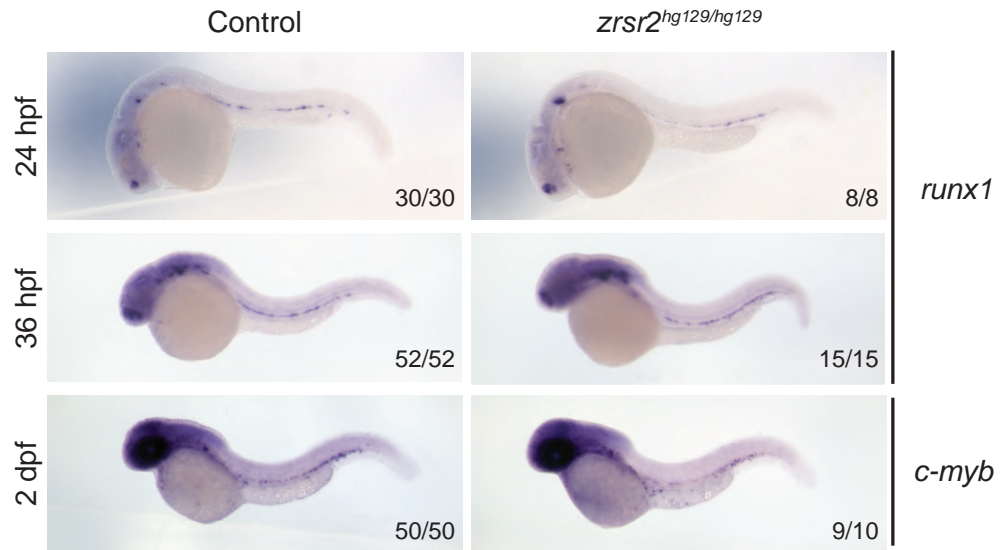
Supplementary Figure S3. Expression of mutant allele and normal expression of markers of early embryonic development in *zrsr2*^{hg129/hg129} embryos. A) Sequence chromatograms showing the expression of the 11 bp deletion in cDNA from 5 dpf mutant embryos compared to the WT embryos. The black rectangle in WT sequence marks the nucleotides that are deleted in the mutant fish. B) WISH of *zrsr2*^{hg129/hg129} (right panel) and control embryos (left panel) with *kdr1*, *myod1*, and *shh* at 24 hpf. Lateral views of embryos are shown in all images.



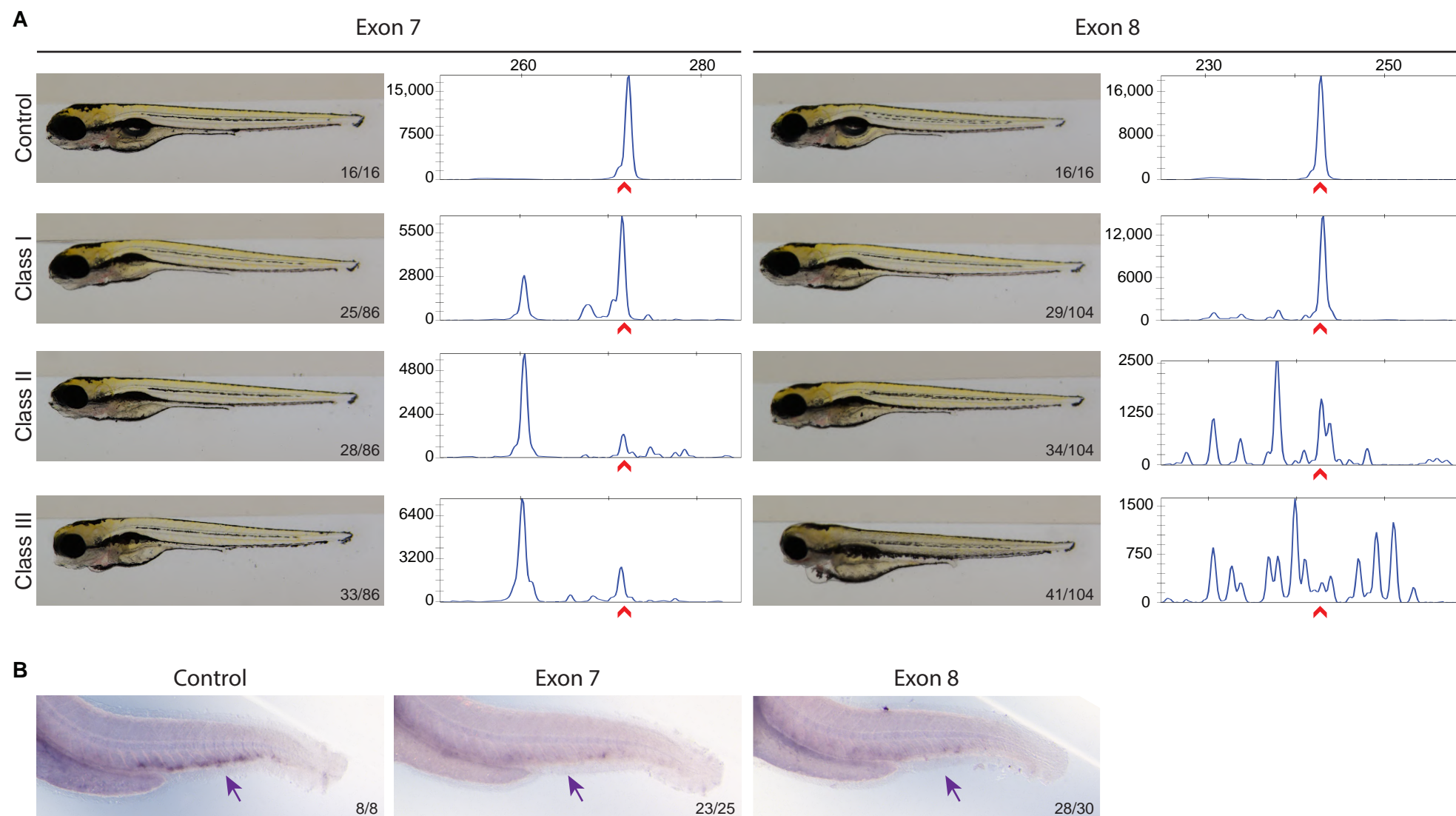
Supplementary Figure S4. *zrsr2*^{hg129/hg129} embryos display normal primitive hematopoiesis and mild anemia. A-C) WISH of *zrsr2*^{hg129/hg129} (right panel) and control embryos (left panel) with primitive hematopoietic markers, *gata1*, *scl*, *mpo*, and *i-plastin* at 14 hpf and 20 hpf (*gata1*), 14 hpf and 22 hpf (*scl*), and 24 hpf (*mpo* and *i-plastin*). D) Ventral views of the yolk sac region and lateral views of the tail of 2 dpf *zrsr2*^{hg129/hg129} embryos (bottom panel) compared to their siblings (top panel) stained with o-dianisidine showing reduced red blood cells in the mutant embryos. E) Quantitative image analysis of all stained embryos, measured in mean pixel intensity, showing the significantly reduced o-dianisidine staining ($p = 0.0015$) in *zrsr2*^{hg129/hg129} embryos compared to their siblings. Horizontal bars indicate the mean and standard deviation of mean pixel intensity.



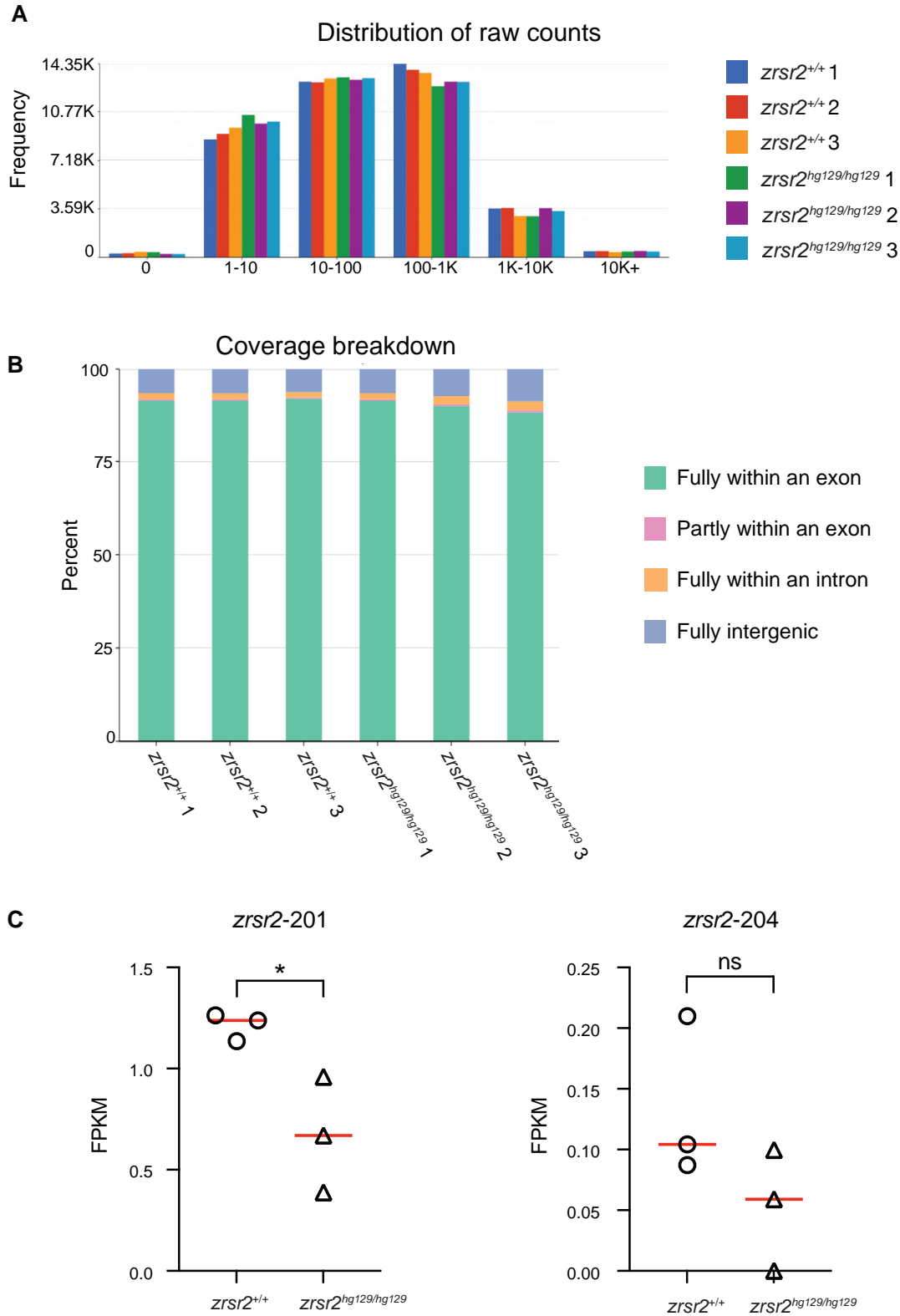
Supplementary Figure S5. *zrsr2*^{hg129/hg129} embryos display normal specification of HSCs and early definitive hematopoiesis. WISH of *zrsr2*^{hg129/hg129} (right panel) and control embryos (left panel) with HSC markers, *runx1* at 24 hpf and 36 hpf and *c-myb* at 2 dpf. Lateral views of embryos are shown in all images.



Supplementary Figure S6: *zrsr2* crispants phenocopy the *zrsr2*^{hg129/hg129} genetic mutant. A) 5 dpf embryos injected with the sgRNAs targeting exon 7 (left panel) and exon 8 (right panel) depicting 3 classes of the morphological phenotypes based on the sgRNA activity. Class I: Lack of an inflated swim bladder; Class II: lack of an inflated swim bladder and defective jaw; Class III: lack of an inflated swim bladder, defective jaw and cardiac edema. Representative lateral view images of embryos for each class are shown with their corresponding CRISPR-STAT plots. Class I to III progressively presented an increased degree of sgRNA activity, shown by a reduction of the WT allele (red arrow) and presence of additional alleles with different indels. B) WISH with *c-myb* probe of exon 7 and exon 8 crispants at 3 dpf compared to uninjected control embryos. Lateral views of embryos are shown with purple arrows marking the CHT region showing reduced *c-myb* expression in the majority of crispant embryos.



Supplementary Figure S7. Analysis of RNA-Seq data for quality and expression of *zrsr2* isoforms. A) Histogram of the distribution of raw read counts for the 3 WT and 3 *zrsr2*^{hg129/hg129} samples. B) Percentages of genomic regions covered by the reads for each sample showing that majority of the reads lie within an exon. C) Expression of *zrsr2*-201 (left) and *zrsr2*-204 shown as fragments per kilobase of transcript per million mapped fragments (FPKM) on the Y-axis for each sample in WT (circle) and *zrsr2*^{hg129/hg129} groups (triangle) on the X-axis. Red lines mark the average FPKM values within each group. (*: pval< 0.05; ns: not significant)



Supplementary Figure S8. Comparison of sequence features between retained versus not-retained U12-type introns in *zrsr2* mutants. Sequence features of 20 nucleotides from both ends of the intron sequences are shown as 5' splice site and 3' splice site in retained introns on the left and not-retained introns on the right side.

