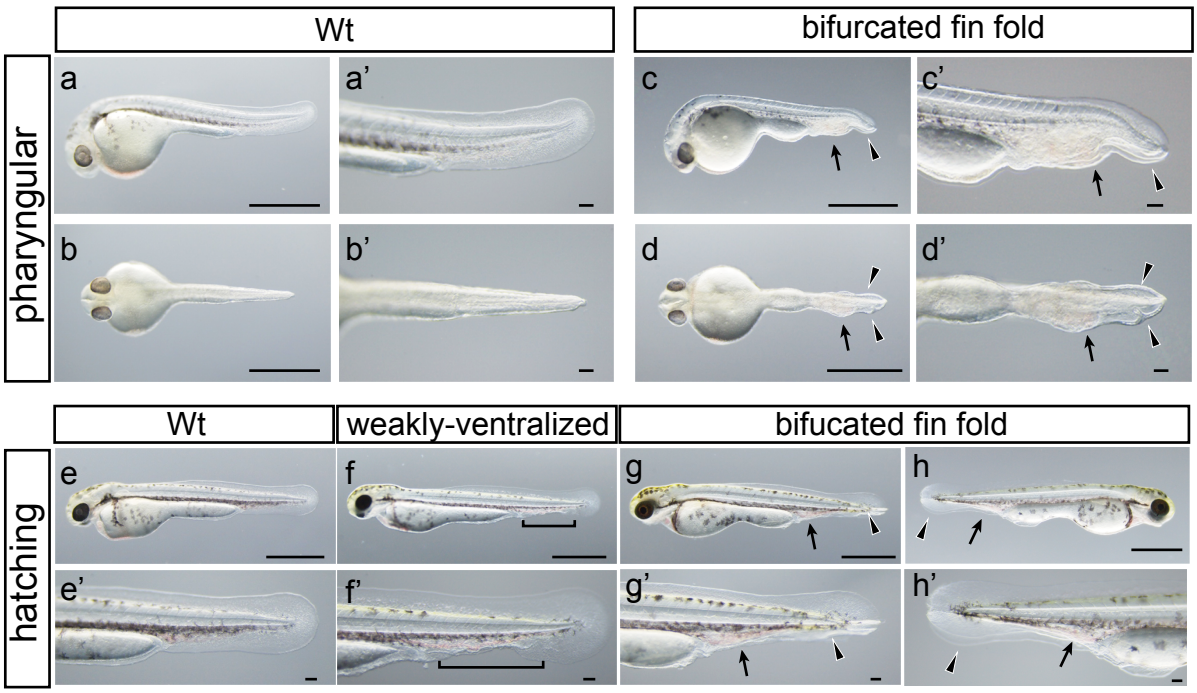
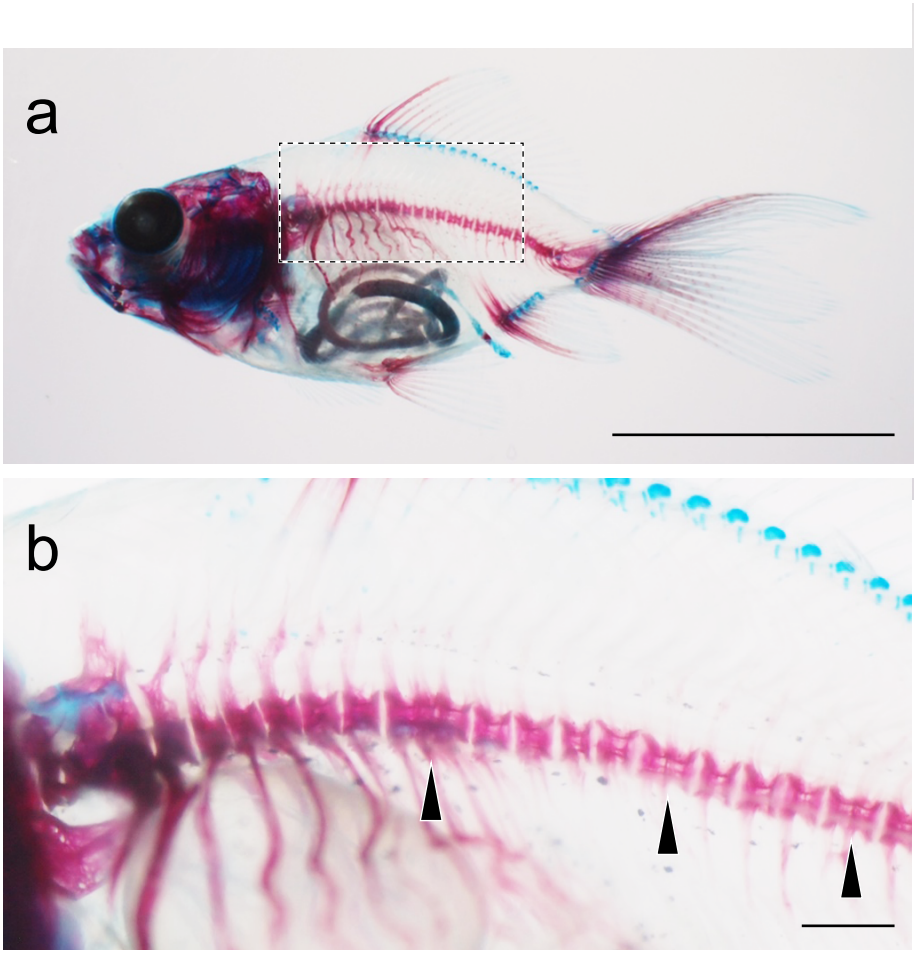
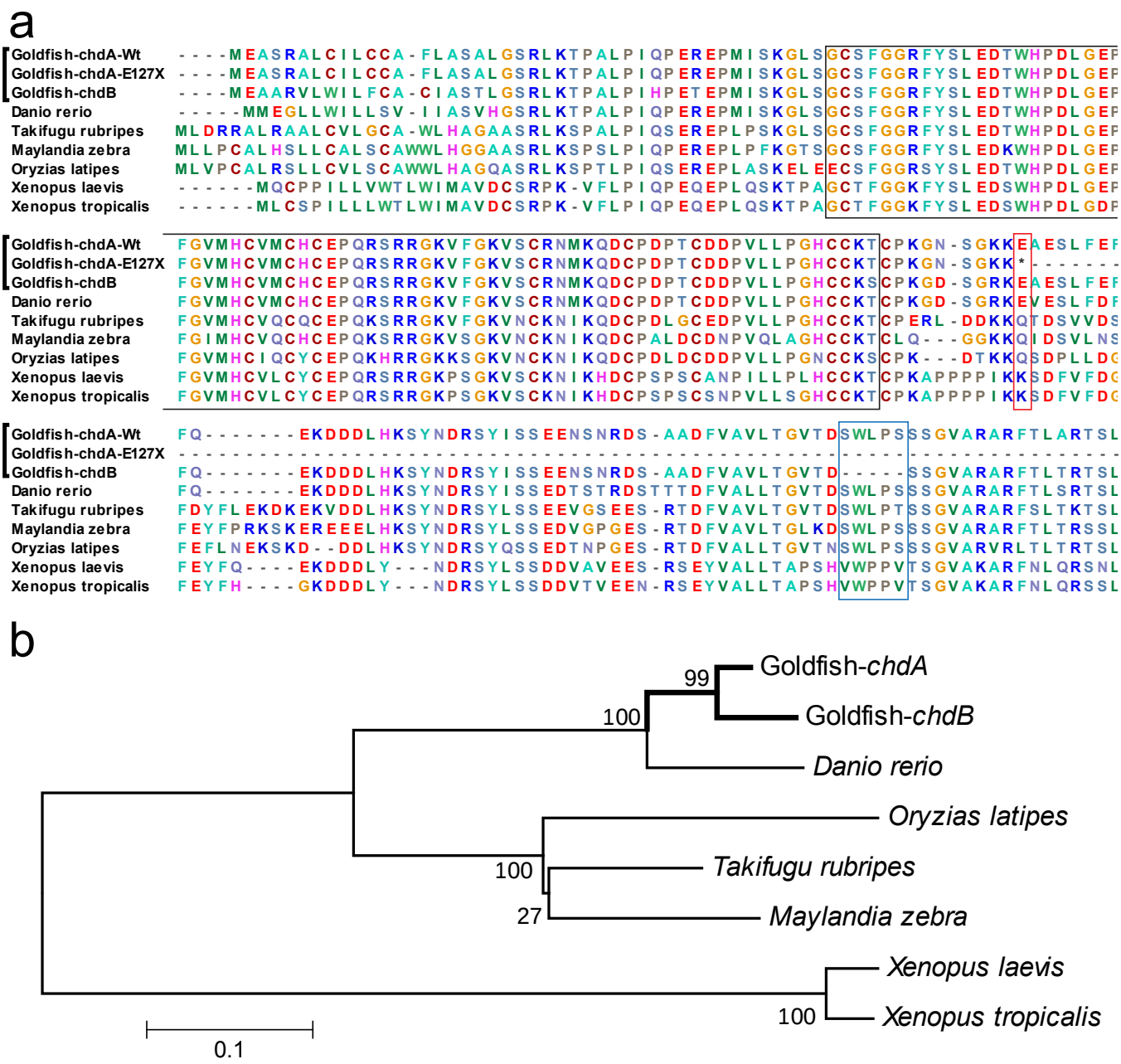


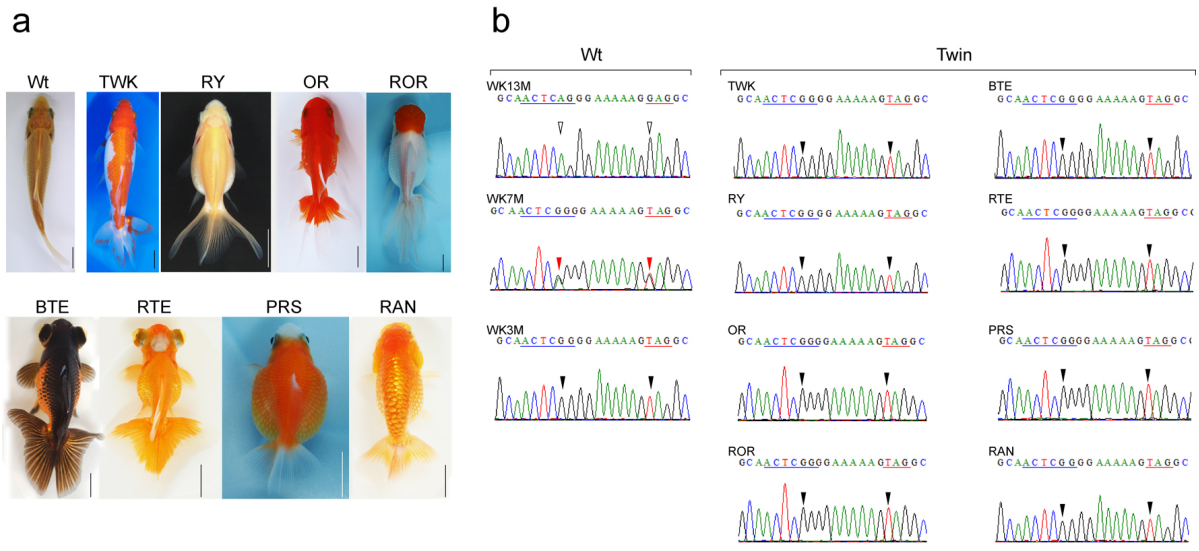
Supplementary Figure 1: Embryonic and larval phenotypes of goldfish. a-d', Pharyngular stage embryos. **e-h'**, Hatching stage larvae. **a-b'**, **e**, **e'**, Wild type specimens. **c-d'**, **g-h'**, Twin-tail specimens. **f, f'**, Weakly-ventralised embryos. Embryos with weak-ventralisation do not exhibit bifurcated fin folds (as shown in panels **g** and **h'**), but do exhibit fin fold disruptions and blood cell accumulation. **g-h'**, Typical bifurcated fin fold phenotype during the hatching stage. **g**, **g'**, Left side views. **h**, **h'**, Right side views. Left and right side views are derived from different embryos. Arrows and arrowheads indicate accumulated blood cells and bifurcated fin folds, respectively. Brackets indicate disrupted fin folds and accumulated blood cells (**f**, **f'**). Scale bars = 1mm (**a**, **b**, **c**, **d**, **e**, **f**, **g**), 0.1mm (**a'**, **b'**, **c'**, **d'**, **e'**, **f'**, **g'**).



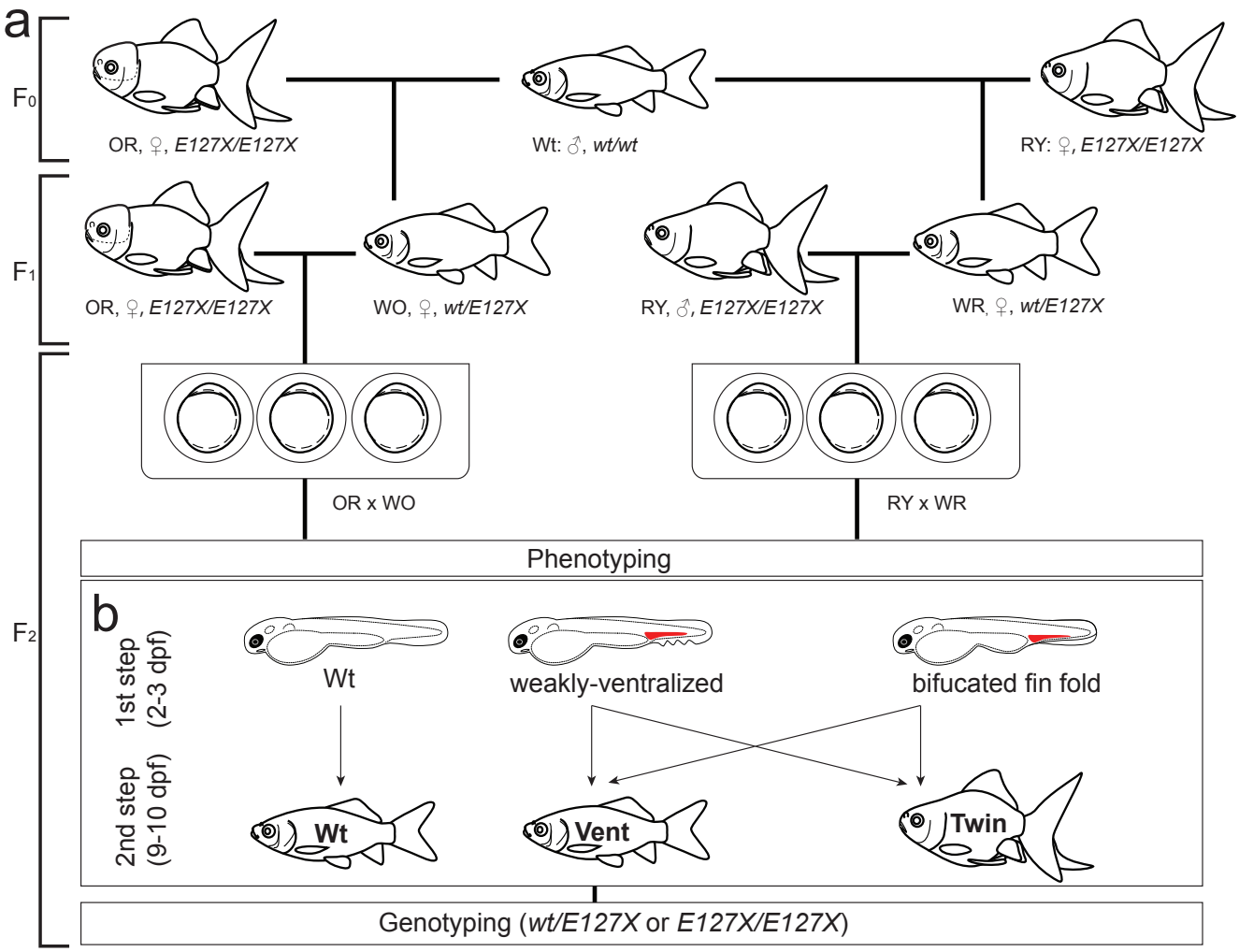
Supplementary Figure 2: Axial skeletal morphology of twin-tail goldfish. a, Lateral view of the skeleton of twin-tail goldfish. **b**, Magnified view of the boxed area in **a**. Black arrowheads indicate fused vertebrae. Scale bars = 1cm (**a**) and 1mm (**b**).



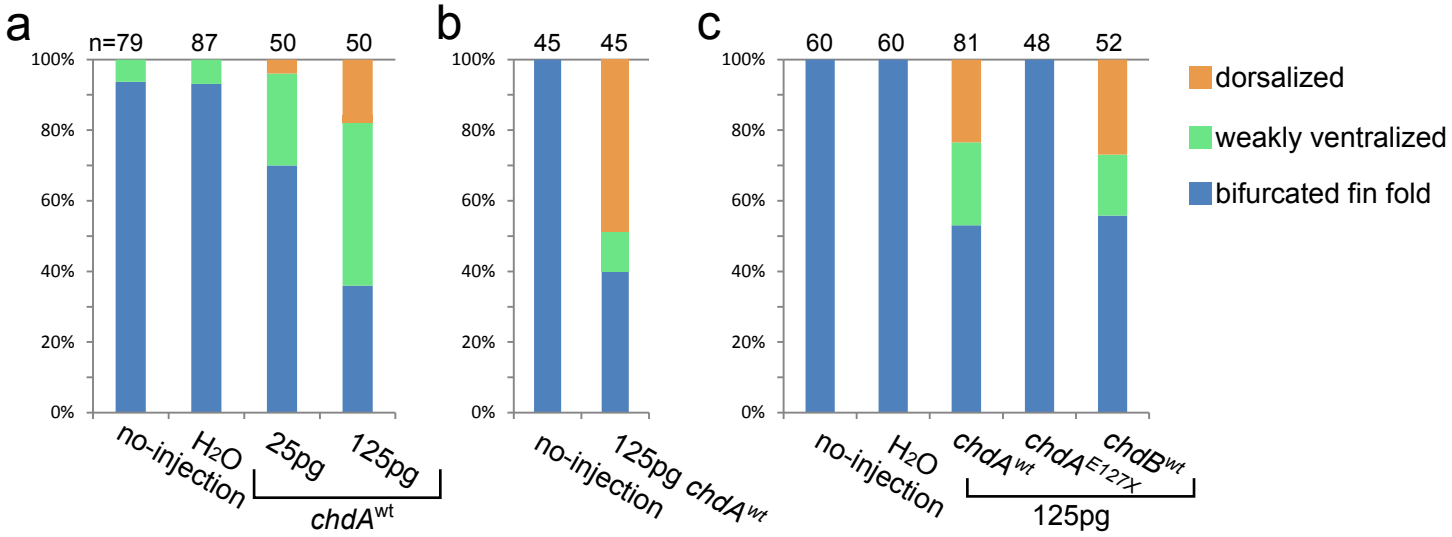
Supplementary Figure 3: Comparison of *chordin* amino sequences. a, Multiple amino acid sequence alignment of the first cysteine-rich (CR) domains and adjacent regions of *chordin* genes. Brackets to the left indicate goldfish sequences. Black, red, and blue boxes enclose the first CR domain, the stop codon site in twin-tail goldfish *chdA*, and the goldfish-specific *chdB* deletion site, respectively. The goldfish *chdA* and *chdB* amino acid sequences exhibit 83% and 85% similarity to that of *Danio rerio chordin*, respectively. Goldfish *chdA* and *chdB* exhibit 92.6% similarity at the amino acid and DNA sequence levels. **b**, Phylogenetic tree of the indicated *chordin* genes. The phylogenetic tree was reconstructed by the maximum likelihood method, using the MEGA5 program. The goldfish lineage is indicated by bold lines. Goldfish *chdA* and *chdB* clustered into the same clade with high support values (over 99%). The multiple alignment and phylogenetic trees were generated using genes from four teleost and two amphibian species. The accession numbers of these *chordin* sequences are as follows: Goldfish-chdA-Wt, AB874473; Goldfish –chdA-E127X, AB874474; Goldfish- *chdB*, AB874475; *Danio rerio*, NP_571048.1; *Takifugu rubripes*, XP_003968786.1; *Maylandia zebra*, XP_004562901.1; *Oryzias latipes*, XP_004075770.1; *Xenopus laevis*, NP_001081778.1; *Xenopus tropicalis*, NP_001136129.1.



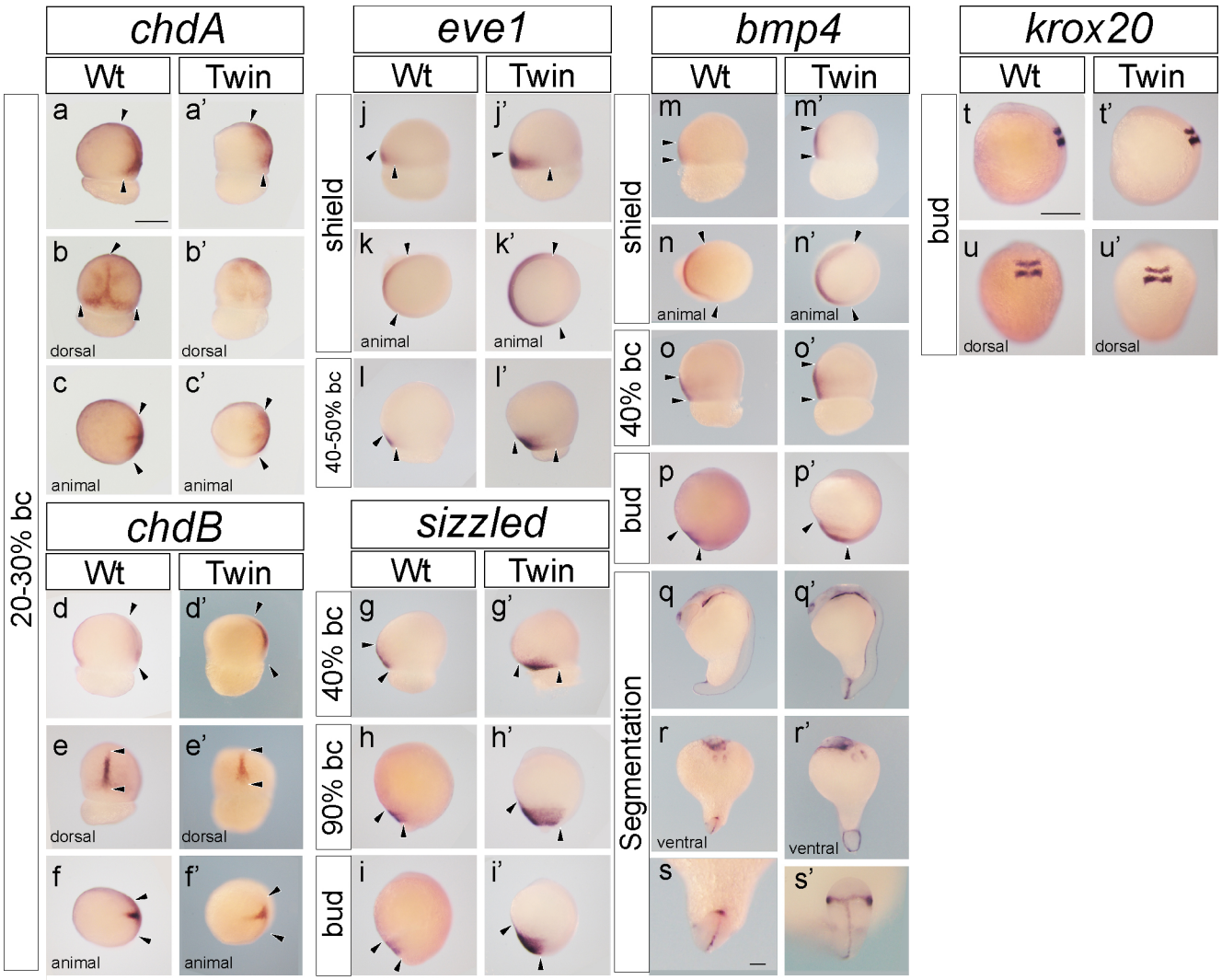
Supplementary Figure 4: Phenotypes and genotypes of goldfish strains. a, Dorsal views of the following goldfish strains: single fin Wakin (Wt), duplicated caudal fin Wakin (TWK), Ryukin (RY), Oranda (OR), Redcap Oranda (ROR), Black telescope (BTE), Red telescope (RTE), Perl scale (PRS), and Ranchu (RAN). **b**, Band patterns for genotyping of the *chdA* stop codon loci. All twin-tail goldfish strains were homozygous for the *Ava*I site in the *chdA* gene (this site is closely associated with the stop codon in $chdA^{E127X}$; Figure 2). WK13M, WK7M, and WK3M indicate individual Wt specimens. In total, 14 of Wt specimens were genotyped (*wt/wt*: *wt/E127X*: *E127X/E127X* = 3: 9 :2). **c**, Chromatographs from direct sequencing of PCR amplicons. White, red, and black arrowheads indicate $chdA^{wt/wt}$, $chdA^{wt/E127X}$, and $chdA^{E127X/E127X}$ loci, respectively. Blue and red bars indicate *Ava*I- and stop codon sites, respectively. Scale bars = approximately 1cm (**a**).



Supplementary Figure 5: Procedures for backcrossing, phenotyping, and genotyping. **a**, Pedigree diagram showing the strain, genotype and sex of goldfish used for backcross analysis. The F_2 strains are named as OR x WO and RY x WR. **b**, Schematic depicting the process of goldfish phenotyping. Based on their morphology at the post-cloacal level (Supplementary Fig. 1), 2-3 day-post-fertilization (dpf) embryos were first divided into the following three categories: (i) wild type; (ii) weakly-ventralized; and (iii) bifurcated fin folds. At 9-10 dpf, these segregants were further divided into the following three types: (i) wild type (Wt) larvae from wild type embryos; (ii) single caudal fin larvae (Vent) from weakly-ventralized or bifurcated fin fold embryos; and (iii) bifurcated caudal fin larvae (Twin) from weakly ventralized or bifurcated fin fold embryos. The embryonic phenotypes were determined based on the descriptions of zebrafish *dino* mutants[1, 2, 3, 4]. Larval phenotypes were described based on the presence or absence of the duplicated caudal skeletons. A total of 1016 segregants were obtained, of which 670 were phenotyped at the larval and juvenile stages. Of those phenotyped segregants, 296 exhibited a twin-tail phenotype, and these were all homozygous for $chdA^{E127X}$. The results of genotyping are shown in Supplementary Table 1



Supplementary Figure 6: Rescue of the twin-tail phenotype by microinjection of embryos with *chdA*^{wt} mRNA. a, Dose-dependent effect of microinjection of *chdA*^{wt} mRNA on phenotype. **b**, Repeat of the *chdA*^{wt} microinjection experiment. **c**, Effect of microinjection of *chdA*^{wt}, *chdA*^{E127X} or *chdB* mRNA on phenotype. The embryos for each experiment were derived from different clutches. The twin-tail goldfish embryos were categorized at 2 dpf. The larval phenotypes were divided into the following three categories: i) dorsalized; ii) weakly-ventralized; and iii) bifurcated fin fold. The dorsalized embryo criteria were based on previous phenotypic descriptions[5].



Supplementary Figure 7: Expression patterns of *chdA*, *chdB*, *eve1*, *sizzled*, *bmp4* and *krox20*. Unless otherwise noted, panels show lateral views of embryos. Black arrowheads indicate areas of gene expression. The expression area of *chdA* (**a-c'**) is larger than that of *chdB* (**d-f'**). The *eve1*, *sizzled* and *bmp4* genes exhibit ventralized expression patterns (**g-s'**). **s s'**, Magnified view of r and r', respectively. During the segmentation stages, *bmp4* showed bifurcated expression patterns in the tail bud (**s, s'**). **t-u'**, Expression patterns of *krox20* in wild type and twin-tail embryos at the bud stage. Scale bars= 500µm (**a**, **t**), 100µm (**s**).

Supplementary Table 1: Survival rate of the backcross progenies

	3dpf embryos	9dpf juvenlies	Survival rate (%)
Wild type	506	385	76.0
Vent+Bif	510	426	83.5

Vent; weakly-ventralized; Bif, bifurcated caudal fin fold

Supplementary Table 2: Primers list

gene	primer names	sequence (5' -> 3')
chdA and *-B*	chd-f1	GARAAYAAYCTNCAYTTYAT
	chd-r1	TCRTCCATYTCNCCDATYTC
	chd-r2	ATYTCRTARTGNAGRTGRCA
	chd-r3	GCCAGGACTTCAGCCAAGTCGGAG
	chd-r4	GTATTTCTCTTAGCAGATGCTGTCGG
chdA	chda-f1	GCGTCAGGAGCACGACTCACACTC
	chda-r1	TTAGTGTCTCCAGTTTTTCTTTTTCTCCACCA
	chda-f2	GCTGTCCTGAATGCATAGAGGACTTCATG
	chda-r2	GTCACAACATGTGATTGTACTCACATTTATTTACAA
	chda-f3	GGAAAAAGAAGAAATGGCAAAAATGGT
	chda-f4	GAACTGGTGTTTGTCCGTGACCCGG
chdB	chdb-f1	CACTGATATTTCTTTGCTTTCTAAAGG
	chdb-r1	AGACGACCTGATCTCAAGGCAGGAGG
	chdb-f2	CTGCGCGGACAGATACAAATGCTGCC
	chdb-r2	CTGTGATACAAGCATATGATTGTACTCGCAG
	chdb-f3	CGTCGAAACAAGCTTCCAGTTCCTC
	chdb-f4	GATCTCATACTCGTCCGTGACCCGA
genotyping PCR of *chdA* allele	chda-f5	TAACGCACAGATGCAGACGTGTG
	chd-r5	TGCTGTTCTCCTCAGAGCTGATGTAGG
sizzled	szl-f1	TACWCRGAGATGCGKYTRCC
	szl-r1	GTCVAGRCANACNGGRGCRA
	szl-f2	ATGCGTYTRCCHAACYTNYT
	szl-r1	GTCVAGRCANACNGGRGCRA
	szl-r2	TGGGTGACAGCCGGTGTGGAGCAG
	szl-r3	GAACCGCTTCCTCCAGACTGCTGTGG
	szl-f3	ACGCTGCTCCACACCGGCTGTCACC
	szl-f4	GCCCGGGCCTTCGTCTGCTCGCTC
	szl-f5	CCTCAGTCAGGATCATGCATCTGTCTCA
	szl-r4	CTCTATGTACAACAGTCGGAGTGCATCTCA
	szl-f6	TGCATCTGTCTCACCTGCTGCTCCT
	szl-r5	TCGGAGTGCATCTCAAGAGCTGTGT
eve1	eve1-f1	ATGCTCGCAGAGGGCAGGGAG
	eve1-r1	TCCTGAAGCACTGCCAAAGGTTTTGG
bmp4	bmp4-f1	CCTGGTAATCGAATGCCGATGGT
	bmp4-r1	GGCAGCCACATCCCTCCACC
krox20	krox20-f1	ATGACAGCTAAAACTTTGGAG
	krox20-r1	GGGTTTGTGGCCGGTGTGATGC

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