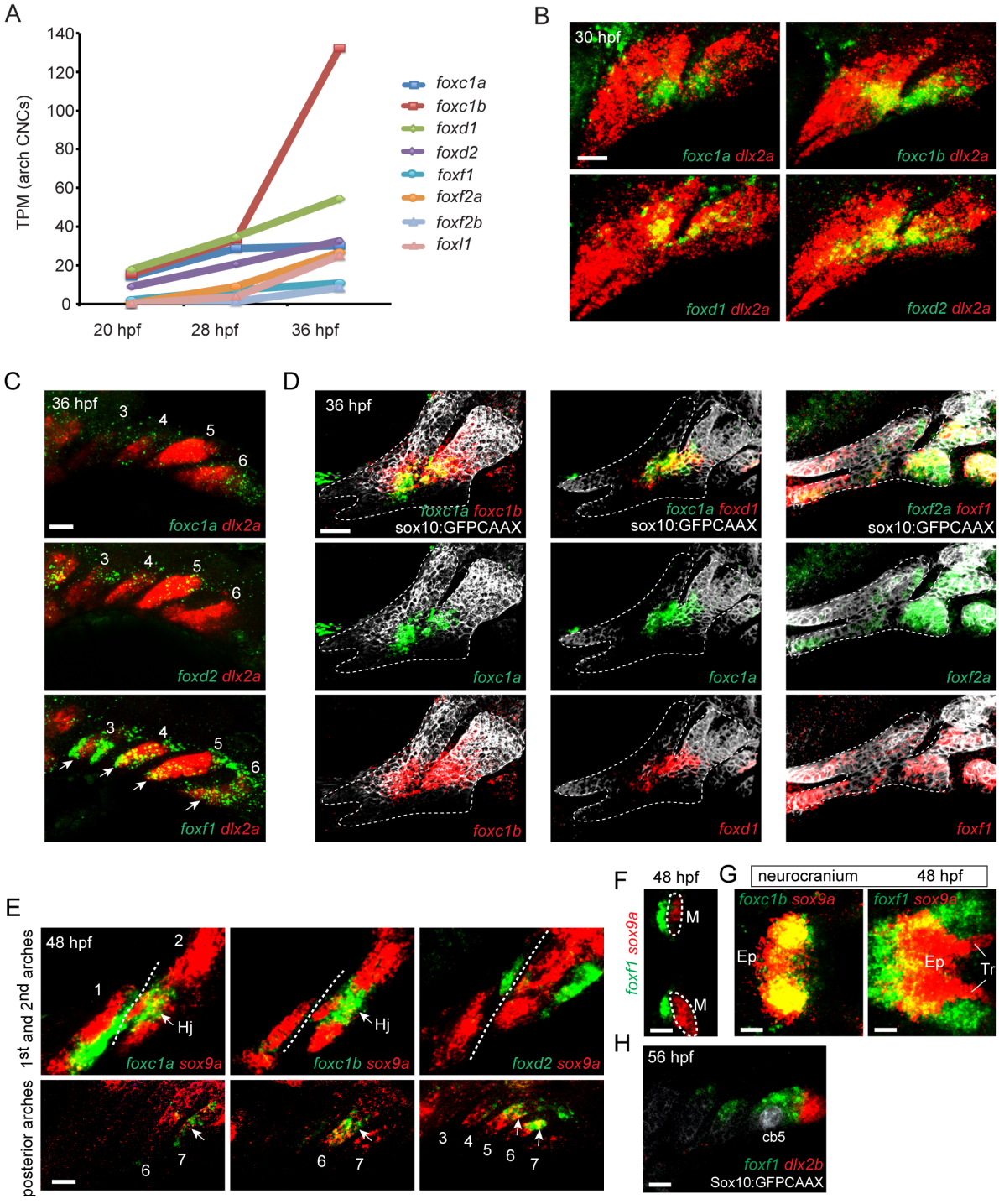

Figure S1. Arch expression of Fox genes

(A) Transcripts Per Million Reads (TPM) values for Fox genes in FACS-purified arch CNCs at 20, 28, and 36 hpf.

(B) Fluorescent in situ hybridizations at 30 hpf show similar expression of *foxc1a, foxc1b, foxd1*, and *foxd2* (green) in *dlx2a*+ CNCs (red) of the first two arches.

(C) Fluorescent in situ hybridizations at 36 hpf show expression of *foxf1* in the ventral domains (arrows) of the gill-bearing branchial arches (numbered 3-6), but only minimal expression of *foxc1a* and *foxd2*.

(**D**) Double fluorescent in situ hybridizations at 36 hpf show relative expression of *foxc1a* (green) to *foxc1b* (red) and *foxd1* (red), and *foxf1* (red) to *foxf2a* (green), in Sox10:GFPCAAX+ CNCs (white) of the first two arches (dotted lines). *foxc1b* is more broadly expressed than *foxc1a* and *foxd1*. *foxf1* and *foxf2a* are expressed in largely similar domains.

(E-F) Fluorescent in situ hybridizations show expression of *foxc1a*, *foxc1b*, *foxd2*, and *foxf1* (green) relative to *sox9a*+ chondrocytes (red) at 48 hpf. By this stage, Fox gene expression is generally excluded from early cartilage, including around the distal tip of Meckel's (M), although expression of *foxc1a* and *foxc1b* is observed in nascent *sox9a*+ chondrocytes of the hyoid joint (Hj, arrows). We also observe expression of *foxc1b* and *foxd2* in intermediate and dorsal domains (arrows), respectively, of the posterior arches, and weak expression of *foxc1a* in the seventh arch (arrow).

(G) In the developing neurocranium, *foxc1b* co-localizes with *sox9a*, while *foxf1* is seen in cells surrounding the trabeculae (Tr) and ethmoid plate (Ep).

(H) Confocal section shows expression of *foxf1* (green) in the dental mesenchyme adjacent to the *dlx2b*+ dental epithelium (red) and the *sox10*:GFPCAAX+ ceratobranchial cartilage 5 (cb5, labeled in white by anti-GFP antibody). Scale bars = 25 μm.

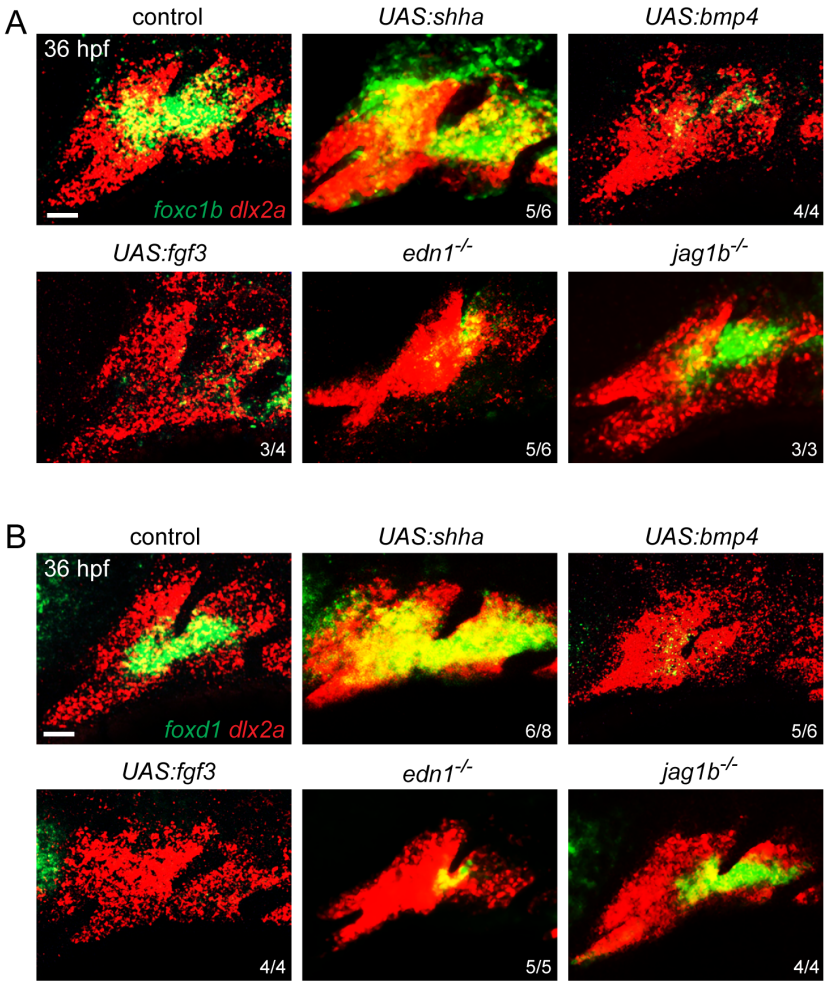

Figure S2. Regulation of *foxc1b and foxd1* arch expression

Expression of *foxc1b* **(A)** and *foxd1* **(B)** in green, relative to *dlx2a*+ CNCs (red) of the first two arches. Similar to *foxc1a* in Figure 1, *foxc1b* and *foxd1* expression is expanded by Shha misexpression, inhibited by Bmp4 and Fgf3 misexpression, and reduced in *edn1* but not *jag1b* mutants. Embryos doubly transgenic for *hsp70l*:Gal4 and *UAS*:*shha*, *UAS*:*bmp4*, or *UAS*:*fgf3* were subjected to a heat-shock from 20-24 hpf to induce ligand expression throughout embryos. Numbers indicate proportion of animals showing the displayed patterns. Scale bars = 25 μm.

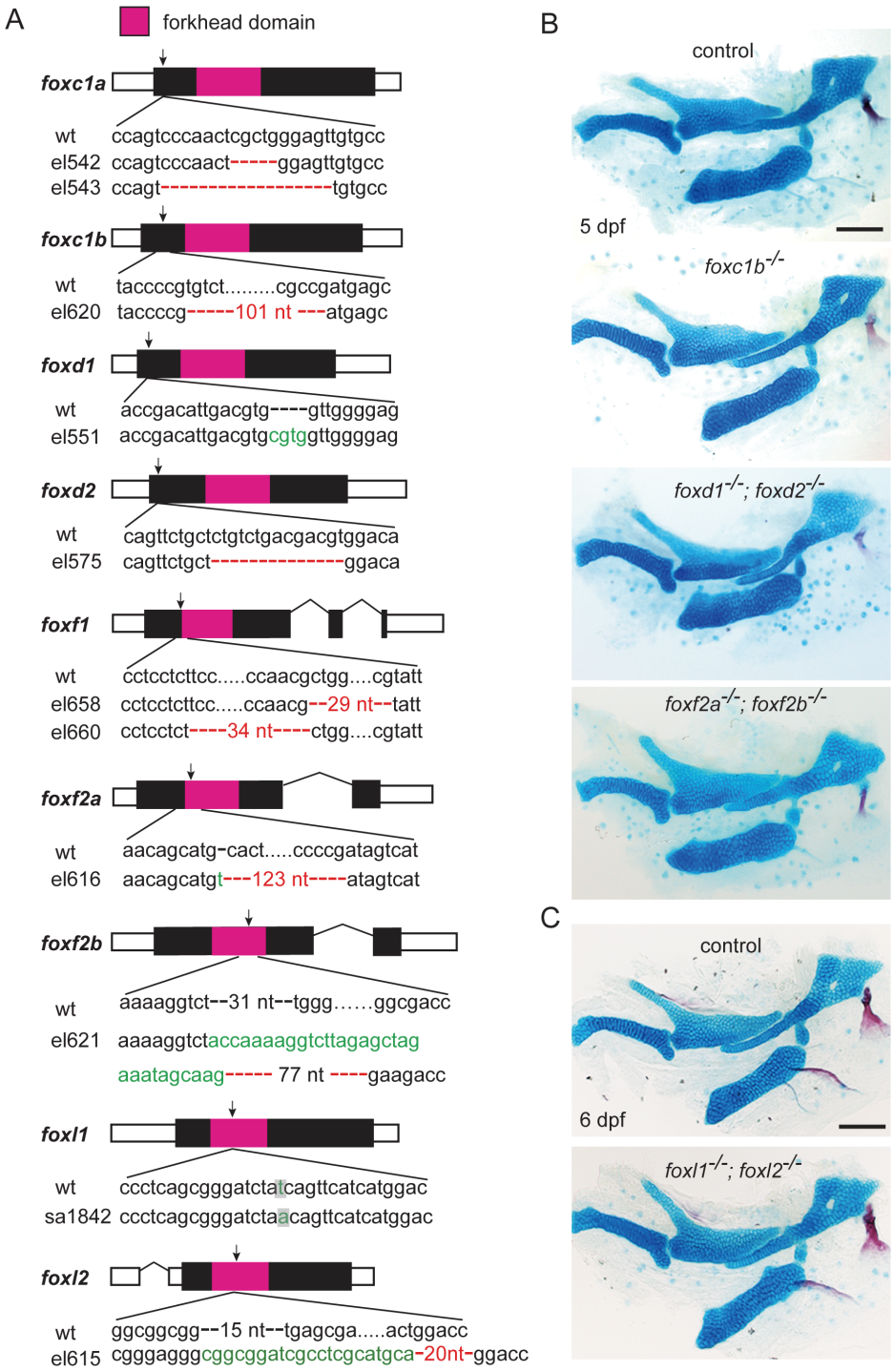

Figure S3. Fox mutant alleles

(A) Schematics of Fox alleles generated for this study. The predicted protein products are shown in black with the Forkhead DNA-binding domains in magenta. For the targeted DNA sequences, series of red dashes indicate deletions, and green letters indicate insertions. For the *foxl1*^{sa1842} generated by

the Sanger Center, the nonsense mutation (t > a) is indicated in green. Sites where the predicted proteins are truncated by mutations are indicated with arrows.

(B-C) Unilateral dissections of the skeletons of the first two arches stained with Alcian Blue (cartilage) and Alizarin Red (bone) at 5 dpf **(B)** or 6 dpf **(D)**. No defects were seen in at least 20 larvae examined for each genotype. Scale bars = 25 μm.

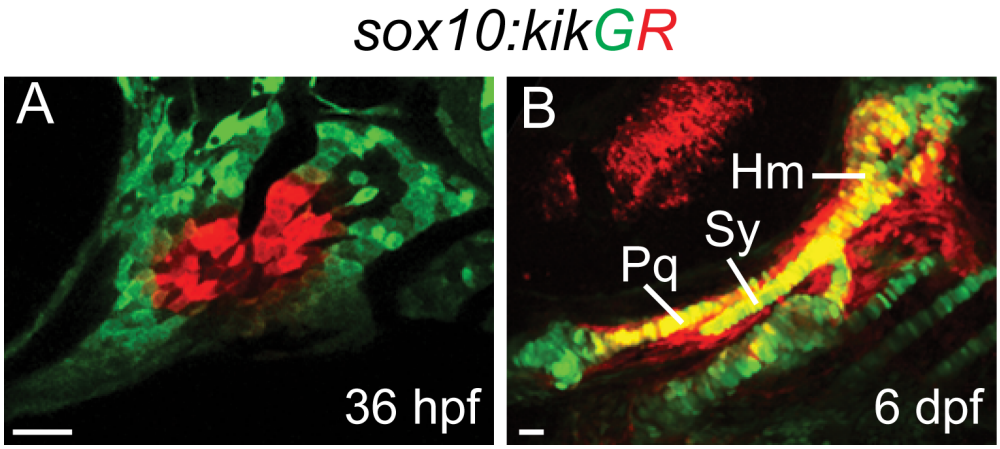

Figure S4. Fate mapping of the Fox-C expression domain

Images show the first two arches at 36 hpf **(A)** and the resultant skeleton at 6 dpf **(B)**. *sox10:kikGR+* arch CNCs (green) were photoconverted to red fluorescence with UV light using the ROI function on a Zeiss LSM800 confocal microscope. Photoconversion in a similar domain to where *foxc1a* and *foxd1* are expressed resulted in labeling of the palatoquadrate (Pq), symplectic (Sy), and hyosymplectic (Hm) cartilages. Scale bars = 25 μm.

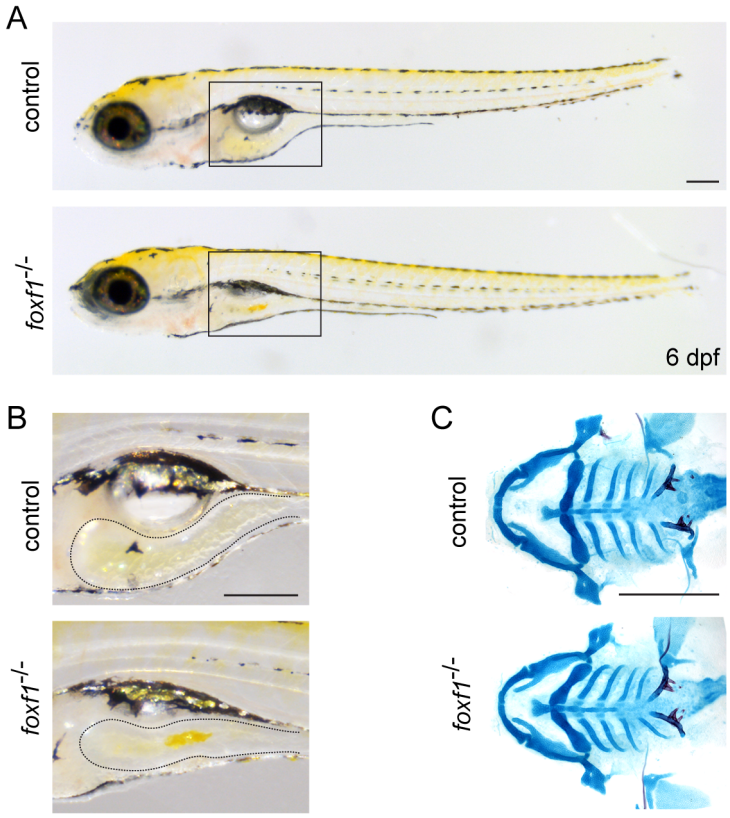

Figure S5. Phenotype of *foxf1* single mutants

(**A**) Brightfield images show morphology of control and *foxf1* single mutants in lateral view at 6 dpf. Swim bladders do not properly inflate in all *foxf1* mutants.

(**B**) Enlarged images of the boxed regions show reduced intestine (dotted areas) in *foxf1* mutants compared to controls.

(**C**) Ventral views of dissected facial skeletons stained by Alcian Blue (cartilage) and Alizarin Red (bones and teeth). No defects are observeded in *foxf1* mutants. Scale bars = 100 μm.

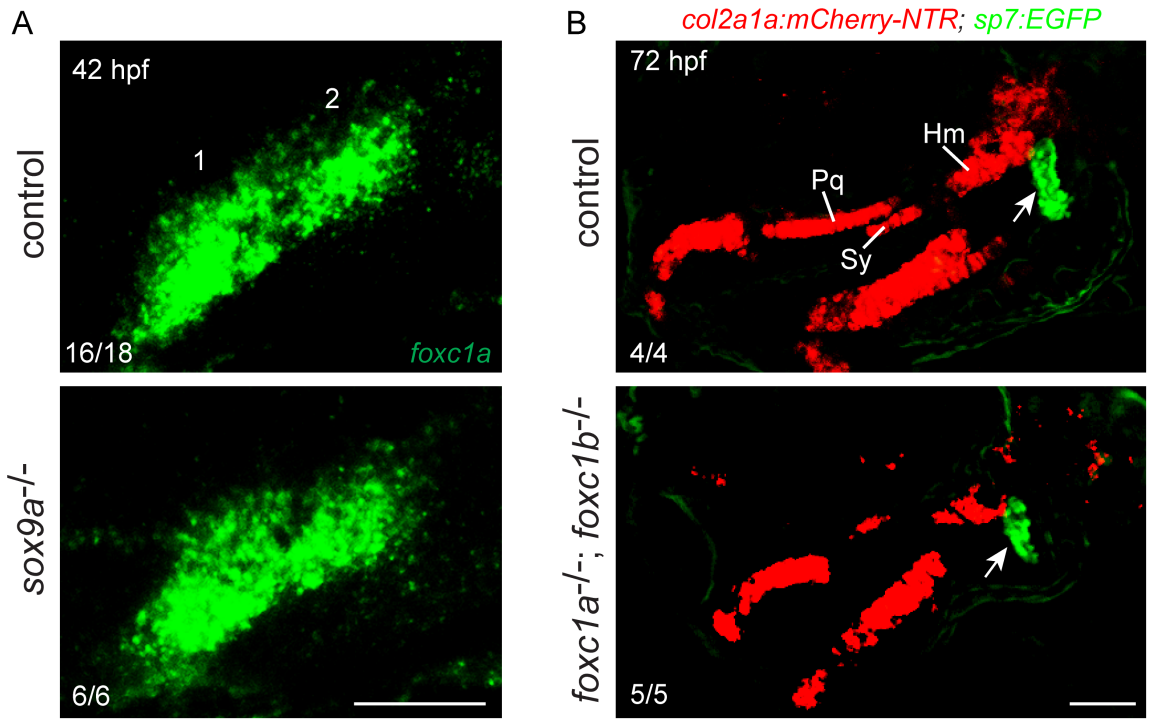

Figure S6. Expression of *foxc1a* in *sox9a* mutants and chondrocyte defects in Fox-C mutants

(**A**) Expression of *foxc1a* in the first two arches is unchanged in *sox9a* mutants at 42hpf.

(**B**) Confocal imaging of chondrocytes labeled by *col2a1*:*mCherry-NTR* (red) and osteoblasts labeled by *sp7*:*GFP* (green) at 72 hpf. In *foxc1a*; *foxc1b* mutants, *col2a1*:*mCherry-NTR* expression is weaker and/or reduced in the palatoquadrate (Pq), symplectic (Sy), and hyosymplectic (Hm) domains. *sp7*:*GFP*+ osteoblasts of the developing opercular bone (Op, arrows) are less affected. Numbers indicate proportion of animals showing the displayed patterns. Scale bars = 100 μm.

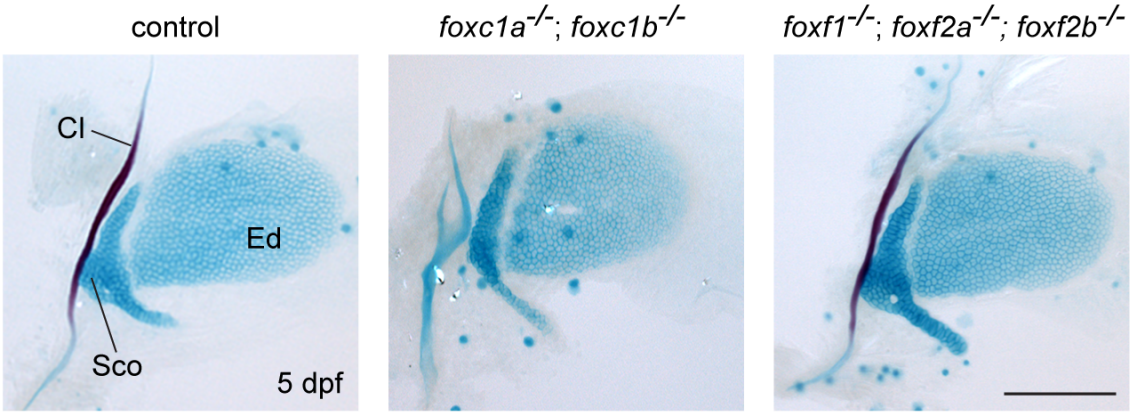

Figure S7. Normal pectoral fins in Fox-C and Fox-F mutants

Images show pectoral fin skeletons stained with Alcian Blue (cartilage) and Alizarin Red (bone) at 5 dpf. No defects were seen in either *foxc1a*; *foxc1b* or *foxf1*; *foxf2a*; *foxf2b* mutants. The lack of mineralization of the Cl bone in *foxc1a*; *foxc1b* mutants is likely an indirect consequence of cardiac edema. Cl, cleithrum; Ed, endoskeletal disc; Sco, scapulocoracoid. Scale bar = 100 μm.

Table S1. TALEN/CRISPR target sequences and genotyping conditions

Gene	TALEN/CRISPR target sequences	Mutation strategy	Mutation type	Genotyping primers (5'-3')		wt product size (bp)	Restriction enzyme
				Forward	Reverse		
foxc1a	L:ATTCCGTCTCC AGTC R:GCTGATGTAC GGCA	TALEN	el542: 5-bp deletion induces frameshift after aa 12 (of 476) el543: 17-bp deletion induces frameshift after aa 10 (of 476)	GCATTTCAA GCAGGATTG TG	CGCGTGAGA GTACATGGT CA	155	BseY1
foxc1b	GGCGTTGTGCCT TATATCCC	CRISPR	el620: 101-bp deletion induces frameshift after aa 7 (of 433)	ACCGAAGAA AGGGGTACG AT	TGTCGGATG AGTTCTGGA TG	472	–
foxd1	L:GCTCTCGGAG GAGACC R:CACCATCATC TCCCTC	TALEN	el551: 4-bp insertion induces frameshift after aa 12 (of 343)	AAACCCGAG AGAGCCATG A	ATCTCCCTCC CCAACCACG T	93	BmgB1
foxd2	L:CGGACAGTTC TGCTCT R:CGGACAGTTC TGCTCT	TALEN	el575: 14-bp deletion induces frameshift after aa 12 (of 369)	ACGGAACGT GAGAGAGGA AG	CGCGTTCTG GGATAGATT GT	198	Hpy166II
foxf1	GGGATATAAGG CACAACGCC	CRISPR	el658: 29-bp deletion induces frameshift after aa 45 (of 380) el660: 34-bp deletion induces frameshift after aa 33 (of 380)	GCGCAGTCC GTTTCTAATG A	TGGATGGCC ATGACAATA AG	281	–
foxf2a	GGCATCCAACA GCATGCACT	CRISPR	el616: 122-bp deletion induces frameshift after aa 25 (of 383)	TCCAGCATTT GCGATGACC A	GGGCAGCTT GATGAAACA CT	566	–
foxf2b	GGTCTTGGGCG ACCGGGTAA	CRISPR	el621: 46-bp deletion induces frameshift after aa 176 (of 429)	TACAAACAC GCTTCCCGTT T	CCGGTAGGC GATTGATAG TC	304	–
foxl1	–	TILLING	sa1842: premature stop codon is formed at aa 79 (of 363)	CAAAAACCC CCGTACAGC TA	GAGAGGTTA TGGCGGATT GA	164	HpyCH4III
foxl2	GGAGGGCGGCG GTGAGCGAA	CRISPR	el615: 5-bp deletion induces frameshift after aa 112 (of 306)	CATCCGACA CAACCTGTC AC	GTGGAGGCC TAAACGGTC TT	315	HphI

Table S2. In situ probes

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Enzyme for linearization	RNA polymerase
foxc1a	TCAGCGTGGACAACATCATG	AAATACTGGTTTGGTCAAAA	EcoRI	T7
foxc1b	GTTCATCATGGAGCGCTTTC	CGAGATAGAGGAGGCGTTTG	EcoRI	T7
foxd2	AACTCCATCCGTCACAACCT	AACGGACTGCTGCACTTTCT	EcoRI	T7
foxf2b	GGCTGGAAGAACTCTGTTCG	AGTCCTTCCGTTCTCCGACT	EcoRI	T7
foxl1	TATGTGTACGGTGGCGAAGT	GTGTCACTCTTTACGGGCAC	EcoRI	T7
dlx2b	GGAACGTATGGAGCCAGCTC	TCAAAAAGGCTACCCGTTTG	NotI	SP6
matn4	CTTCTTCTGTCGCTGCAATG	CCTCACTGCTGCTGTGTGTT	NotI	SP6

Table S3. Primers used for transgenic constructs.

Name	5'-3'
fgf3-B1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTATGGTTATAATTCTGCTCTT
fgf3-B2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTTAAATGTCAGCCCTTCTGT
foxc1a-B1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCATGCAGGCGCGCTATTCCGT
foxc1a-B2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTCAAAATTTGCTGCAGTCAT
foxf1-B1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCATGACGGCTGAAGTGCAGCA
foxf1-B2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTCACATCACACAAGGTTTGA