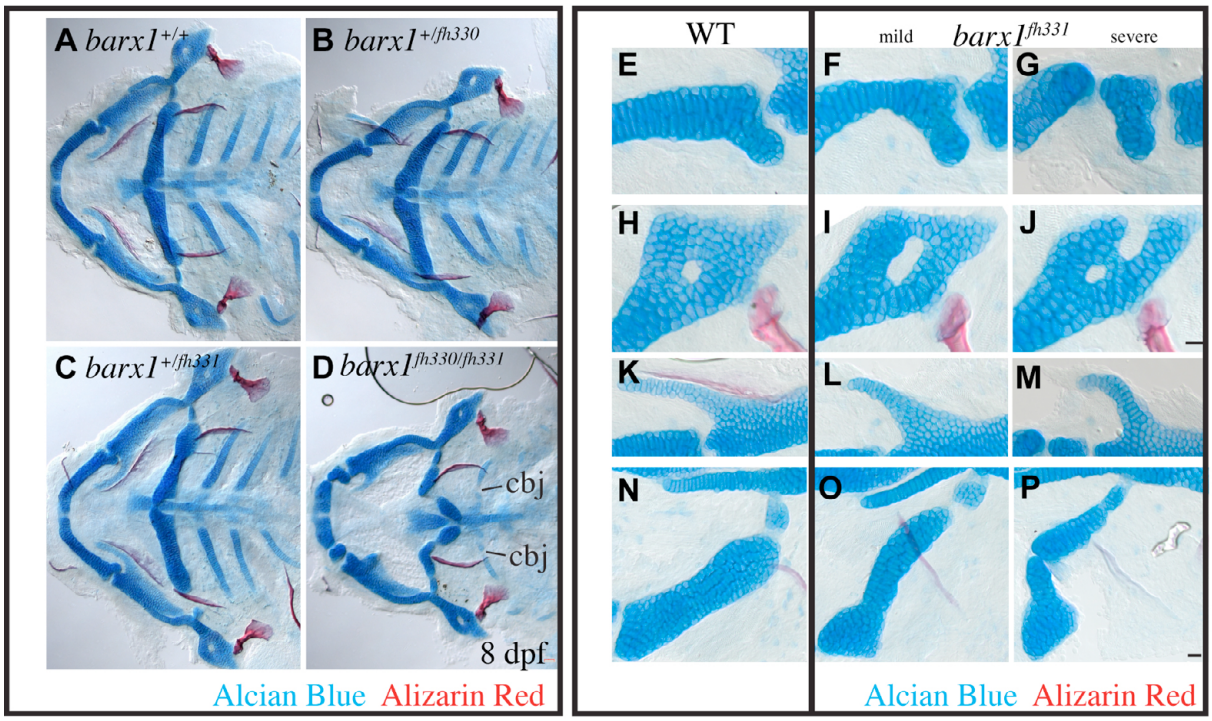
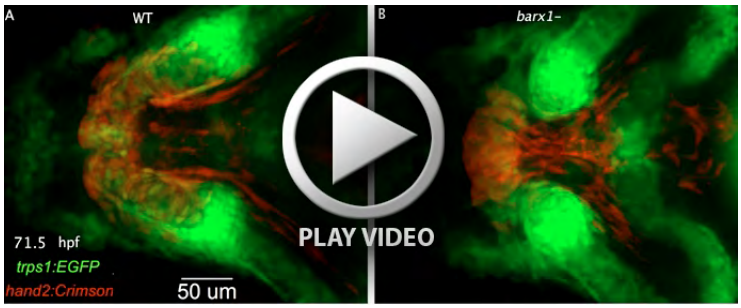
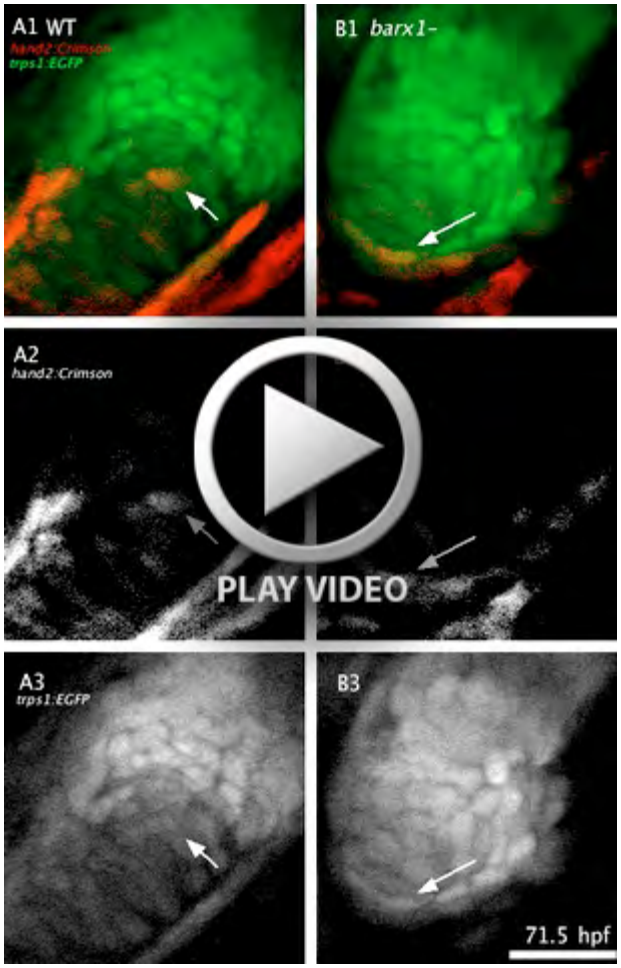


Fig. S1. Skeletal phenotype variability in *barx1* mutant zebrafish. (**A-D**) Zebrafish heterozygous for *barx1*^{fh330} and *barx1*^{fh331} alleles were crossed and offspring were stained with Alcian Blue and Alizarin Red, dissected and imaged with transmtted light. Genotyped trans-heterozygotes displayed *barx1*-associated phenotypes revealing that these two alleles fail to complement. Anterior is towards the left; left is upwards. (**E-P**) Alcian Blue- and Alizarin Red-stained larvae were dissected and flat mounts imaged to reveal the range of phenotypic severity found in *barx1* mutants. Anterior is towards the left; dorsal is upwards. Scale bar: 10 μm.

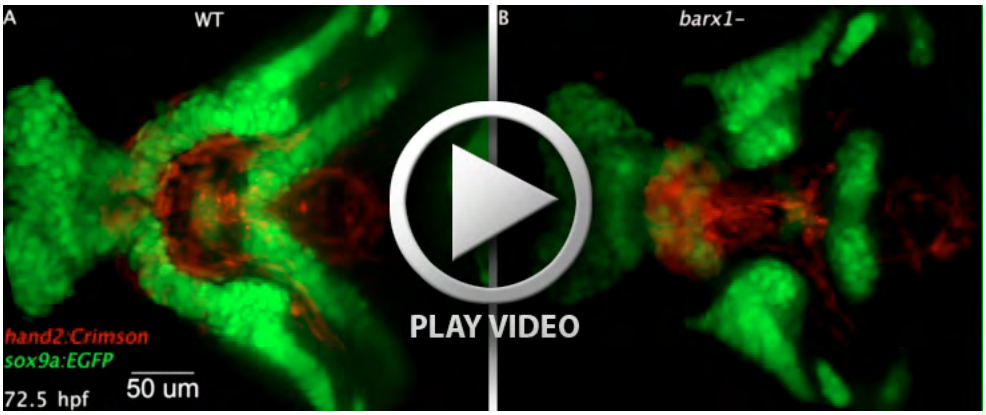
Movie 1. Joints afford the zebrafish cartilage skeleton flexibility. 4 dpf *sox9a:EGFP* transgenic zebrafish larvae were anesthetized and laterally mounted in 1.5% agarose in a glass-bottomed dish. Following removal of agarose from just the head, anesthetic was removed and larvae were allowed to recover for several minutes. Non-anesthetized larvae were imaged at six scans/second. In wild-type larvae, skeletal flexibility is evident at the jaw joint between Meckel's cartilage and the palatoquadrate. In *barx1* mutants, skeletal flexibility can be seen at the Meckel's joint, as well as the primary jaw joint. Anterior is towards the left; dorsal is upwards.



Movie 2. Subintermediate domain precursors give rise to cartilage in wild type and to joints in *barx1* mutants. *trps1:EGFP;hand2:Crimson* double-transgenic embryos were ventrally mounted and imaged by time lapse microscopy from 50 to 71.5 hpf. Movies are maximum confocal projections. Anterior is towards the left; left is upwards.



Movie 3. Cell rearrangement does not contribute to the *barx1* mutant phenotype. Individual confocal sections were compiled from Movie S2, allowing the tracking of isolated cells (arrows). Cells from similar locations differentiate into chondrocytes in wild types and *trps1:EGFP*-expressing joint cells in *barx1* mutants. *hand2:Crimson* fluorescence was imaged with high gain to image perduring protein even after reporter expression had probably been downregulated. Anterior is towards the left; left is upwards.



Movie 4. *barx1* mutant zebrafish fail to downregulate *hand2*, and develop cartilage gaps in the lower jaw. *sox9a:EGFP;hand2:Crimson* double transgenic embryos were ventrally mounted and imaged by time-lapse microscopy from 50 to 72.5 hpf. Movies are maximum confocal projections. Anterior is towards the left; left is upwards. Wild-type (WT) cells differentiating into chondrocytes of the lower jaw initiate expression of *sox9a:EGFP* (green), while downregulating expression of *hand2:Crimson* (red). By contrast, *barx1* mutants fail to downregulate *hand2:Crimson* and ectopic joints develop in the skeleton.

Table S1. Arch 1 and 2 skeletal gaps in *barx1* mutant zebrafish

barx1 allele	% hm notch	% reduced ptp	% mj (complete gap)	% chj (complete gap	*n*
fh331	46	95	100 (33)	100(62)	176
fh330	56	98	100 (29)	100 (37)	52

Fixed 4 dpf Alcian Blue/Alizarin red-stained skeletons were scored on both sides for phenotypes. An obviously thin mc or ch was scored as an ectopic joint, the subset of these with a complete gap is shown in parentheses.

n, number of sides.

Table S2. Summary of arch 1 and 2 joints in *barx1;furina* compound mutant zebrafish

Genotype	% hm notch	% reduced ptp	% mj (complete gap)	% chj (complete gap	% jaw joint loss	% ih joint loss	*n*
barx1$^{+/+}$ *furina*$^{+/+}$	0	0	0 (0)	0 (0)	**0**	0	10
barx1$^{+/-}$ *furina*$^{+/+}$	0	0	0 (0)	0 (0)	**0**	0	30
barx1$^{-/-}$ *furina*$^{+/+}$	50	96	100 (60)	100(88)	**0**	0	48
barx1$^{+/+}$ *furina*$^{+/-}$	0	0	0 (0)	0 (0)	**0**	0	36
barx1$^{+/-}$ *furina*$^{+/-}$	0	2	0 (0)	0 (0)	**2**	2	54
barx1$^{-/-}$ *furina*$^{+/-}$	37	95	100 (48)	100 (87)	**0**	0	60
barx1$^{+/+}$ *furina*$^{-/-}$	2	7	0 (0)	0 (0)	**72**	69	54
barx1$^{+/-}$ *furina*$^{-/-}$	8	2	0 (0)	10 (6)	**63**	60	120
barx1$^{-/-}$ *furina*$^{-/-}$	91	98	93 (38)	47 (22) *	**33**	3	58

Fixed 4 dpf Alcian Blue/Alizarin Red-stained skeletons were scored on both sides for phenotypes. An obviously thin mc or ch was scored as an ectopic joint, the subset of these with a complete gap is shown in parentheses.

First arch joint-loss phenotype typical of *furina* mutants shown in bold.

n, number of sides.

*Severe loss of ventral arch two cartilage in some individuals precludes the detection of ch joint.

Table S3. Summary of arch 1 and 2 joints in *barx1;hand2* compound mutant zebrafish

Genotype	% hm notch	% reduced ptp	% mj (complete gap)	% jaw joint loss	*n*
barx1$^{+/+}$ *hand2*$^{+/?}$	0	0	0 (0)	**0**	32
barx1$^{+/-}$ *hand2*$^{+/?}$	0	3	3 (0)	**0**	68
barx1$^{-/-}$ *hand2*$^{+/?}$	52	100	100 (24)	**0**	58
barx1$^{+/+}$ *hand2*$^{-/-}$	3	0	0 (0)	**96**	26
barx1$^{+/-}$ *hand2*$^{-/-}$	6	2	0 (0)	**97**	118
barx1$^{-/-}$ *hand2*$^{-/-}$	95	100	0 (0)	**100**	44

Fixed 4 dpf Alcian Blue/Alizarin Red-stained skeletons were scored on both sides for phenotypes. An obviously thin mc or ch was scored as an ectopic joint, the subset of these with a complete gap is shown in parentheses.

n, number of sides.

The first arch joint-loss phenotype typical of *hand2* mutants is shown in bold.