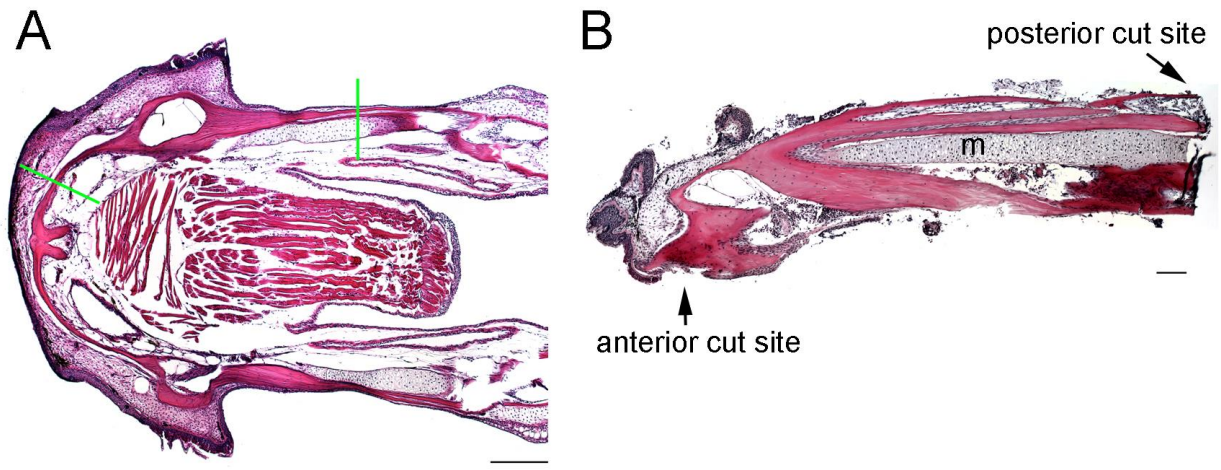


Fig. S1. Extent of lower jaw resections.

(A) Coronal section of un-resected adult lower jaw stained with H&E. Green lines show where resection cuts are made. Anterior is to the left. (B) Histological section through the tissue that was removed showing the extent of bone removed and complete removal of the distal end of Meckel's cartilage (m). Scale bars: A, 1 mm; B, 100 microns.

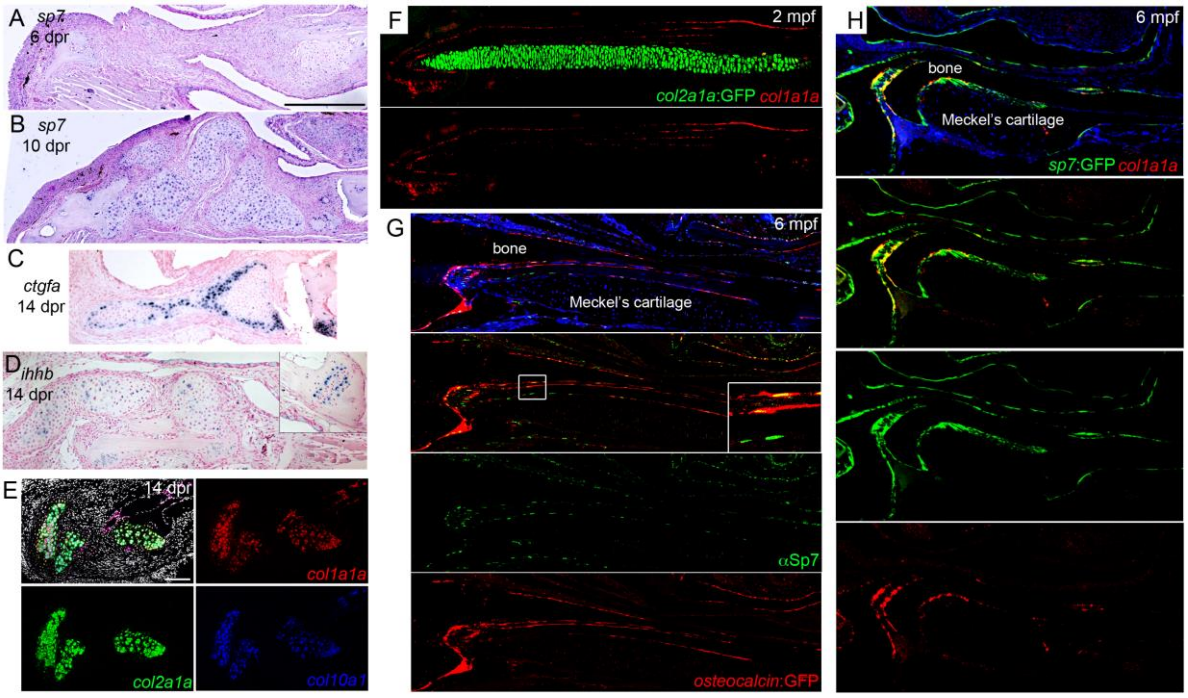


Fig. S2. Gene expression in the cartilage callus and adult jaw.

(A-D) Colorimetric RNA in situ hybridization shows gene expression in the cartilage callus. The expression of *sp7* is not yet visible in the mesenchyme at 6 dpr and evident in the cartilage callus by 10 dpr. At 14 dpr, *ctgfa* is expressed in a subset of chondrocytes within the callus. Also at 14 dpr, *ihhb* is expressed weakly in some repair chondrocytes; inset shows stronger expression in the remnant growth plate of the ceratohyal cartilage. (E) Three-color fluorescent in situ hybridization shows co-expression of *col1a1a* (red), *col2a1a* (green), and *col10a1* (blue) within repair chondrocytes at 10 dpr. Note the similar level of expression of *col1a1a* in the bone (top right). Nuclei are detected with Hoechst (white). (F) Transgenic *col2a1a*^{BAC}:GFP fish have distinct and non-overlapping expression of GFP in Meckel's cartilage and *col1a1a* in periosteum at 2 mpf. (G) A subset of Sp7+ cells (green, detected by anti-

Sp7 antibody) also express an *osteocalcin:*GFP transgene (red, detected by anti-GFP antibody). Inset corresponds to boxed region and shows both Sp7-only cells and Sp7+/*osteocalcin*:GFP+ cells. Note nuclear Sp7 localization compared to cytoplasmic and nuclear GFP. (H) A subset of *sp7:*GFP+ cells express *col1a1a*. Scale bars = 100 microns.

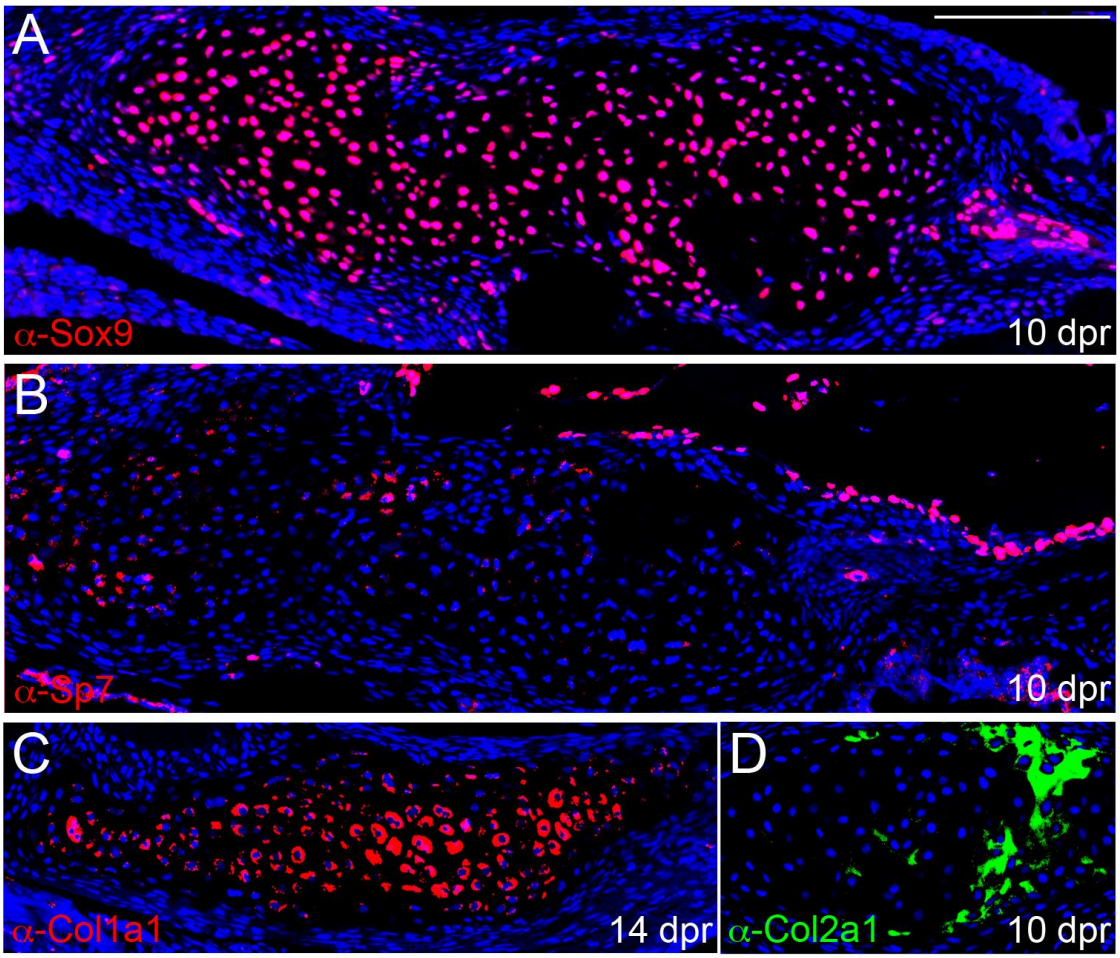


Fig. S3. Protein expression in the cartilage callus.

(A-D) Immunofluorescent assays on sections show protein expression of Sox9, Sp7, Col1a1, and Col2a1 within the cartilage callus. Nuclei are labeled with Hoechst (blue). Scale bar = 100 microns.

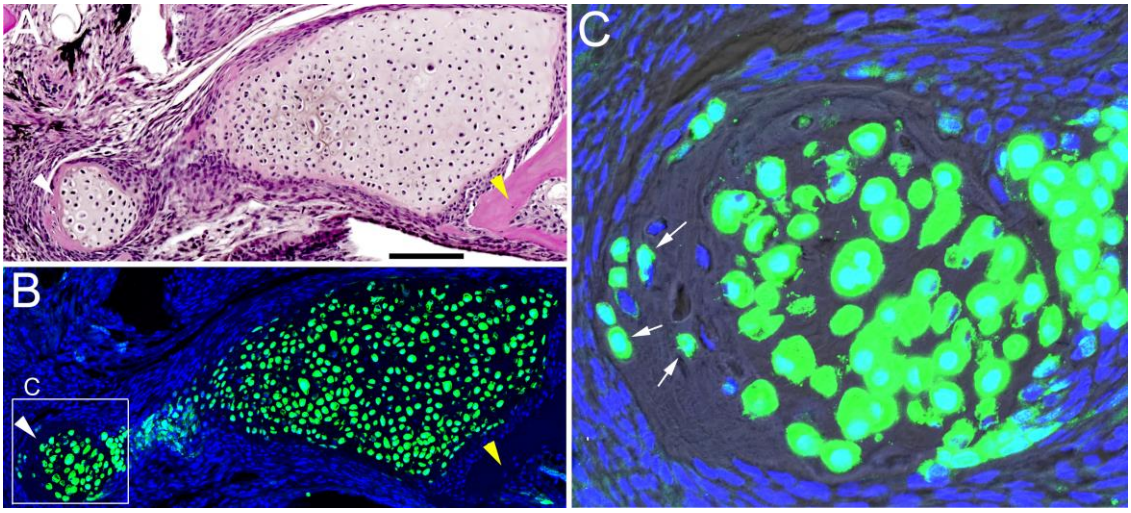


Fig. S4. Contribution of *col2a1a*^{BAC}:GFP-derived cells to bone.

(A) H&E staining at 30 dpr shows remnant cartilage surrounded by bone (white arrowhead) which has a similar appearance to bone on the right (yellow arrowhead). (B) An adjacent section from this *col2a1a*^{BAC}:GFP animal was processed for anti-GFP staining (green). Hoescht labels nuclei in blue. (C) Magnification shows that GFP+ cells are embedded in bone (arrows). A Normarski channel is included to show bone matrix. Scale bar = 100 microns.

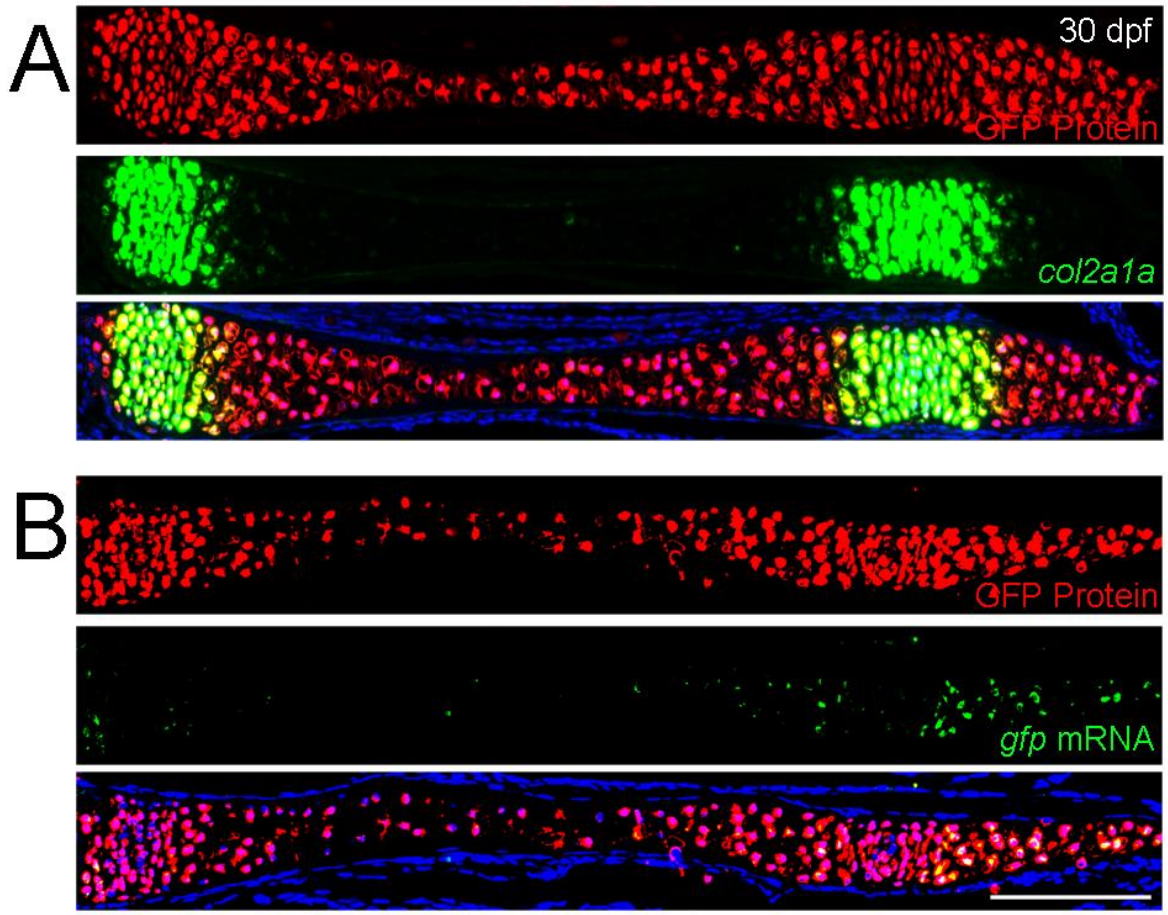
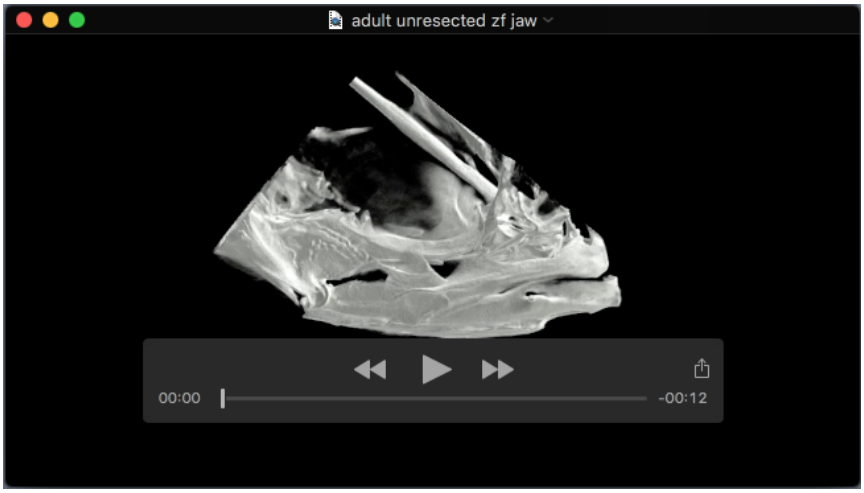
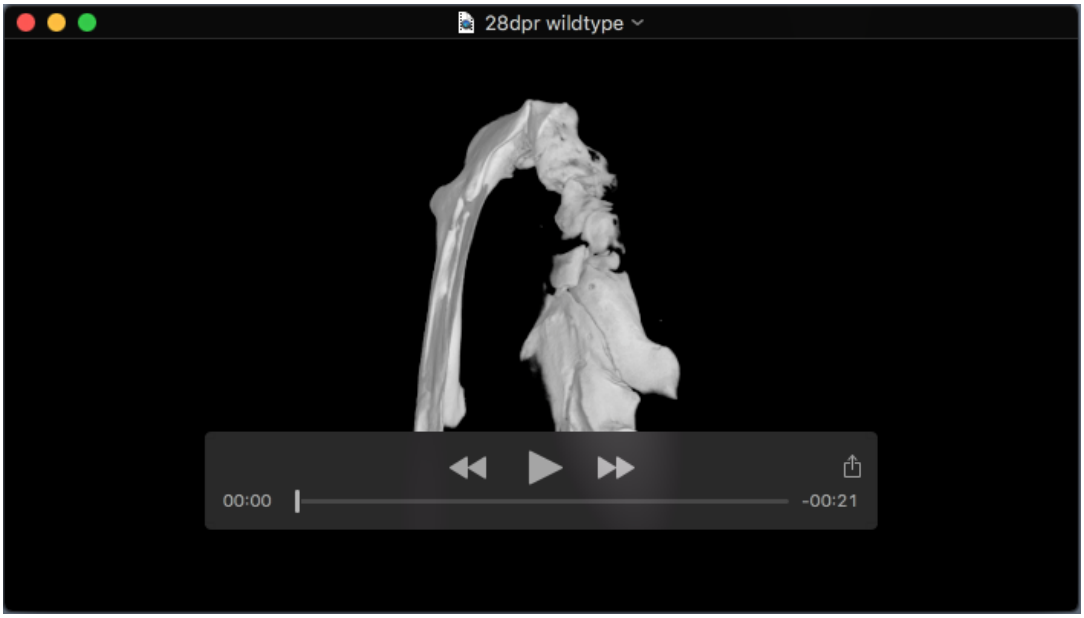


Fig. S5. Perdurance of GFP in developing cartilage.

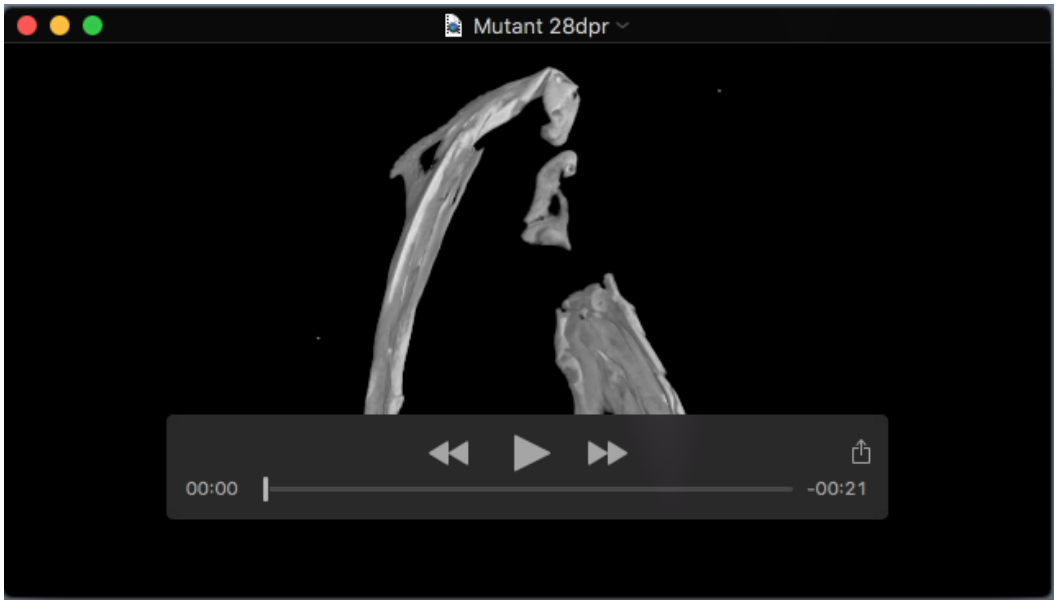
(A and B) Sections of the ceratohyal cartilage in 30 dpf *col2a1a*^{BAC}:GFP juvenile zebrafish. Anti-GFP staining (red) compared to endogenous *col2a1a* RNA (green, A) or GFP RNA (green, B) shows that cells retain GFP protein after they shut down expression of *col2a1a* or GFP message. Scale bar = 100 microns.



Movie 1. Volume reconstruction of µCT scans showing anatomy of an uninjured adult zebrafish lower jawbone (highlighted to discern from other structures).



Movie 2. Volume reconstruction of µCT scans of 28 dpr wild-type sibling of an *ihha* mutant. Note the near complete bridging and thickness of the bone.



Movie 3. Volume reconstruction of µCT scans of 28 dpr *ihha* mutant shows incomplete bridging and hollow bone.

Table S1. Primers used for riboprobe synthesis.

Gene	Fwd Primer	Rev Primer	Probe Length	
col1a1a	GTATTGTAGGTCTCCCTGGACAAA	TGTTCTTGCAGTGGTATGTAATGTT	1239bp	HindIII, T7
col2a1a	GTAAAGATGGAGAGACTGGACCTTC	ATTCTCTCCTCTGTCTCCCTGTTT	1376bp	NotI, SP6
col10a1	GTCTAAAAGGTGACAGAGGAGTACCT	GTAGACACTGATCAGTAACAAGGAAACA	1421bp	HindIII, T7
spp1	AACGGCCACCTCCTATTCTT	CACTGCCGTCTGTCGTCTAA	1399bp	HindIII, T7
runx2b	GATGTACTTTTCCTGATAACTGGAGTG	CACAGCTATTTCGCTTTATACTGTAGG	1657bp	HindIII, T7
sp7	GTCAATACTTATTTAGACATGACGCATCCTTAC	GCAAGTTTTGAGAAAAACTTTGTATTCACTCTA	1993bp	NotI, SP6
bglap	CTCTGAGCTGACAATATCAACTAAACA	GGTTCTAGAAGGGAATGGGCCCATA	342bp	NotI, SP6
ctgfa	AGAGTCTTTCCAGAGCAGTTGTAAATA	CTCTGAGCTGACAATATCAACTAAACA	1216bp	HindIII, T7
ihha	AACCGCTGAGCAACAGGTTTAAT	GACAAATGGGTTCAAAGGATATGGTATAA	1625bp	SpeI, T7
ihhb	GTTATCTTCACCGTCTTTGACACTC	TGGAAGAGTTCTGATTCTAGCAGTAGT	1657bp	NotI, SP6
gli1	ATATGGAAACTCTCCCCTAAAACACAACTTTAC	GAATTTGCTTTAGTTTGTCGATCTTCAGGTT	2115bp	HindIII, T7
ptc1 (ENSDARG00000055026)	GACTTTGGCTCAAGGAAAGACTAGAGAATA	GTACCATAGAGGCTGAGGCTTAAAAGAG	2428bp	NotI, SP6
ptc2 (ENSDARG00000016404)	GCCCAGTTCCGTTATTTTTCATTCTAC	GTCTCCTGAAGTCTGATAGCTGTCATTG	1857bp	NotI, SP6

Table S2. Experimental numbers.

Figure	Experimental numbers
1	**Regeneration of the lower jawbone in adult zebrafish**
1A,B	Skeletal staining of un-resected animal (n = 9)
	Skeletal staining of 0 dpr animal (n = 4)
	Skeletal staining of 7 dpr animals. 5/9 displayed more than 50% bridging. 3/9 displayed up to 50% bridging and 1/9 did not show any cartilage response. Additionally 5/9 animals showed about 20% deposition of bone at the cut surface
	Skeletal staining of 14 dpr animals. 7/11 animals displayed more than 50% cartilage across the resected region and variable degrees of mineralized matrix. 8/11 animals displayed about 80% mineralization across the lesion,

	and 3/7 animals displayed about 50% mineralization.
	Skeletal staining of 35 dpr animals. 6/7 animals displayed complete bridging of the gap by mineralized matrix. 1/7 animal showed about 80% bridging of mineralized matrix. 5/7 animals had more than 50% bridging of cartilage (intermingled with bone) while 2/7 animals had about 20% cartilage left.
1C	H&E for un-resected animals (n = 5)
	H&E for 10 dpr (n = 5/5 animals showed chondrocytes embedded in regenerating bone edges while extensive cartilage was present within the resected region).
	H&E for 30 dpr (n = 4/4 animals showed increased matrix deposition around chondrocytes in regeneration and the edges of cartilage callus showed transition to bone histology).
	H&E for 60 dpr (n = 3/3 animals showed extensive bone formation and very few cells with chondrocyte morphology entrapped in the bone matrix).
1D	Representative example from 1B for 35dpr and unresected control.
2	**Co-expression of chondrocyte and osteoblast programs in repair cartilage.**
2A	*sox9a* ISH at 6 dpr (n = 3/6 had expression in regenerating cartilage, n = 4/6 in mesenchyme), 8 dpr (n = 6/6 in regenerating cartilage as well as mesenchyme), 10 dpr (n = 5 in regenerating cartilage),
	col2a1a ISH at 6 dpr (n = 5/6 in regenerating cartilage, n = 6/6 in mesenchyme), 8 dpr (n = 7/7 strong expression in regenerating cartilage), 10 dpr (n = 8/8 strong expression in regenerating cartilage).
	col10a1 ISH at 6 dpr (n = 0/6 show expression in regenerating cartilage), 8 dpr (n = 5/5 in regenerating cartilage), 10 dpr (n = 8/8 in regenerating cartilage)
	runx2b ISH at 6 dpr (n = 6/6 in mesenchyme, 2/6 in regenerating cartilage), 8 dpr (n = 4/4 in regenerating cartilage), 10 dpr (n = 5/5 in regenerating cartilage),
	col1a1a ISH at 6 dpr (n = 3/3 show strong expression in injured periosteum, n = 3/3 in mesenchyme surrounding the cut bone, n = 3/3 in regenerating cartilage in salt and pepper manner), 8 dpr (n = 6/6 show strong expression in periosteum, n = 6/6 in mesenchyme spanning the lesion, n = 6/6 strong expression in regenerating cartilage), 10 dpr (n = 4/4 strong expression in regenerating cartilage)
	spp1 ISH at 6 dpr (n = 3/3 very weak expression in periosteum), 8 dpr (n = 3/3 weak expression in periosteum), 10 dpr (n = 3/3 in regenerating cartilage)
2B	*col2a1a/col1a1a* two color ISH at 10 dpr (n = 3/3 show co-expression in cells of the regenerating cartilage)
	col2a1a/runx2b two color ISH at 10 dpr (n = 4/4 show co-expression in cells in the regenerating cartilage)
3	**Mineralization and osteocyte maturation of repair chondrocytes.**
3A	Trichrome staining at 8dpr (n = 4)
3B	*col2a1a*^{BAC}:GFP/Alizarin red at 30 dpr (n = 13/13 animals showed chondrocytes embedded in calcified matrix.
3C	a-GFP/*spp1* on 16 dpr *col2a1a*^{BAC}:GFP (n = 2/2 with expression in cartilage)

3D	a-GFP/*col2a1a* on 30 dpr *col2a1a*^{BAC}:GFP (n = 5/5 with expression in cartilage)
	a-GFP/*bglap* on 30 dpr *col2a1a*^{BAC}:GFP (n = 3/3 with expression in cartilage)
4	**Development of growth plate cartilage in juvenile zebrafish**
4A	Trichrome staining at 14 dpf (n = 2)
4B,C	*col10a*/a-BrdU/a-GFP on sp7:GFP 28 dpf (n = 2)
4C	*sox9a/col1a1a* in ceratohyal at 14 dpf (n = 2)
	col2a1a/col1a1a in ceratohyal at 14 dpf (n = 3)
	col2a1a/spp1 in ceratohyal at 14 dpf (n = 3)
	col2a1a/col1a1a in ceratohyal at 21 dpf (n = 3)
	col2a1a/bglap in ceratohyal at 28 dpf (n=4)
	col2a1a/col10a1 in ceratohyal at 28 dpf (n=2)
4D	Perichondral mineralization of *col2a1a*^{BAC}:GFP (n=3 at each stage)
5	**Mobilization of the periosteal cells in response to jaw resection.**
5A, B	H&E staining of re-sected jawbone in un-resected WT (n = 3) and at 0 dpr (n = 3), 2 dpr (n = 3), 4 dpr (n = 3), and 6 dpr (n = 3)
5C	Anti-BrdU/*col1a1a* in un-resected adult (n = 2), 4 dpr (n = 2)
5D, E	Anti-GFP/anti-mCherry in uninjured RUNX2:GFP;sp7:mCherry adult animals (n = 4/4)
5F	Anti-GFP in runx2:GFP line at 4 dpr (n = 3), and 7dpr (n = 3)
5F	Anti-GFP in sp7:GFP line at 4 dpr (n = 3), and 7dpr (n = 4)
6	**Requirement of ihha in the generation of repair cartilage.**
6A	*ptc2* expression at 6dpr (n=2/2 animals show ptc2 expression in nascent cartilage and pre cartilaginous mesenchyme).
	sox9a/ptc1 at 8dpr (n = 2/2 animals show *ptc1* co-expressing with *sox9a*+ cells.
	ihha colorimetric ISH at 10 dpr (n = 6/6 in regenerating cartilage)
	gli1 colorimetric ISH at 10 dpr (n = 6/6 in regenerating cartilage)
6B	Alcian blue-alizarin red labeling of cartilage and bone at 14 dpr in *ihha-/-* (n = 3) & WT siblings (n = 4)
	Alcian blue-alizarin red labeling of cartilage and bone at 28 dpr in *ihha-/-* (n =9) & WT siblings (n = 10).
6C	µCT analysis of same animals depicted in alcian-alizarin images in 6B
6D	Quantification of BrdU+ nuclei in *ihha-/-* vs WT (2 sections from 2 animals each).
6E	BrdU incorporation in 4 dpr WT (n = 2) and *ihha-/-* (n = 2) animals.
6F	BrdU+ nuclei in Col2:GFP+ cells n=3/3 wildtype showed extensive cartilage while n=3/3 *ihha-/-* mutants showed severe reduction of chondrocytes
6G	Quantification of BrdU+ nuclei/100 chondrocytes. n = 3/3 animals show no significant difference in chondrocyte proliferation in *ihha-/-* vs wild types.
6H	Anti-Sox9a/anti-GFP staining on *ihha-/-*, *col2a1a*^{BAC}:GFP (n = 2/3 animals showed small nodules of cartilage with very few chondrocytes expressing Sox9a/GFP while 1/3 did not show any cartilage); and WT siblings (n = 3/3 animals showed large areas of Sox9a/GFP+ chondrocytes)

S1A	Un-resected adult zebrafish jaw (n = 4)
S1B	Bone fragment taken out after resection (n = 6)
S2A	*sp7* expression at 6 dpr (n = 3/3 weak expression in periosteum)
S2B	*sp7* expression at 10 dpr (n = 3/3 in regenerating cartilage)
S2C	*ctgfa* expression at 14 dpr (n = 3/3 animals showed expression in subsets of chondrocytes)
S2D	*ihhb* expression in 14 dpr animals. (n = 2/2 animals showed very weak expression in regenerating cartilage versus strong expression in the growth plates of the ceratohyal
S2E	Triple fluoresecnt ISH for *col1a1a, col2a1a* and *col10a1* at 14 dpr (n = 2/2 animals showed co-expression of all three genes in regenerating chondrocytes)
S2F	*col1a1a* ISH/ anti-GFP in Col2:GFP line n=5/5 animals show well segregated *col1a1a* expression in periosteum and GFP expression in Meckel's cartilage.
S2G	Anti-sp7/anti-GFP immunofluorescence in osteocalcin:GFP line, n = 4/4 animals show cells co-expressing as well as individually expressing these markers.
S2H	*col1a1a* expressing cells are a subset of sp7:GFP+ cells (n = 3/3)
S3A	anti-Sox9a immunohistochemistry (n = 8/8 animals showed Sox9a immunoreactivity in regenerating cartilage)
S3B	anti-Sp7 immunohistochemistry (n = 3/3 animals showed Sp7 immunoreactivity in a minority of regenerating chondrocytes)
S3C	anti-Col1a1a immunohistochemistry (n = 4/4 animals showed Col1a1a immunoreactivity in regenerating cartilage)
S3D	anti-Col2a1 immunohistochemistry (n = 3/3 animals showed Col2a1 immunoreactivity in regenerating cartilage, but more so at edges of cartilage).
S4A	H&E staining of 30 dpr *col2a1a*^{BAC}:GFP animals (n = 3/3 animals showed cells embedded in newly formed bone at the edge of regenerating cartilage)
S4B, C	anti-GFP staining on adjacent sections from Fig S4A (n = 3/3 animals showed GFP+ cells embedded in bone at the edge of the regenerating cartilage)
S5A	*col2a1a* mRNA/anti-GFP in 30 dpf *col2a1a*^{BAC}:GFP fish (n = 3/3 animals showed that *col2a1a* mRNA is restricted to growth plates in ceratohyal, while GFP protein persists in the entire ceratohyal).
S5B	*gfp* mRNA/anti-GFP in 30 dpf *col2a1a*^{BAC}:GFP fish (n= 3/3 animals showed *gfp* mRNA restricted to growth plates and GFP protein throughout the ceratohyal).