Extended Data Figure 1. The mutation patterns in *gli3* and *gli2b* knockout zebrafish and adult pigmentation phenotype.

Frame shift mutations in *gli3* exon5, *gli3* exon14, and *gli2b* knockout fish, used in this paper. Green- and magenta-colored nucleotides are gRNA target sites and genetic mutations, respectively.

Extended Data Figure 2. The survival ratio of wildtype and *gli3*$^{\mathbf{14ins/14ins}}$ juveniles.

One clutch of fertilized eggs from a mating pair of wildtype fish and two clutches of fertilized eggs from two mating pairs of *gli3*$^{14ins/14ins}$ fish were obtained. They were raised up until 49 days and the number of living juveniles was counted at each time point. The mortality of wildtype and *gli3*$^{14ins/14ins}$ juveniles did not show significant difference.

Extended Data Figure 3. HCR of *sp7* and *col2a1a* in wildtype and BMS-833923 treated embryos.

a and **b**) confocal-microscopy imaging of embryos stained by HCR with *sp7* (osteoblasts in the cleithrum) and *col2a1a* (chondrocytes in the scapulocoracoid) probes in wildtype (**a**) and BMS-833923-treated embryos (**b**) at 72 hpf. Images are maximum intensity projections of the lateral views of the embryos. In BMS-833923 treated embryos, the *sp7* staining is weaker in the dorsal extremity and middle portion of the cleithrum (arrows) than those of wildtype embryos. **c**) The number of chondrocytes in the scapulocoracoid of *gli3*$^{+/+}$, *gli3*$^{14ins/14ins}$, and BMS-833923 treated embryos. The number of chondrocytes increases in *gli3*$^{14ins/14ins}$ and BMS-833923 embryos (N= 19, 18, and 18 for *gli3*$^{+/+}$, *gli3*$^{14ins/14ins}$, and BMS-833923 embryos, respectively). The scale bar is 300 μm. ** and * indicate $p<0.01$ and $p<0.05$ in one tailed t-test, respectively. $p= 0.0011$ between *gli3*$^{+/+}$ and *gli3*$^{14ins/14ins}$ embryos and $p=0.0329$ between *gli3*$^{+/+}$ and BMS-833923 treated embryos.

Extended Data Figure 4. **Evolution of *activin receptor 1* genes in vertebrates**. A) Maximum likelihood protein phylogeny from representative jawed vertebrates, rooted with the sequence of lancelet as non-vertebrate outgroup. Bootstrap support values (100 replicates) >50 are shown. See Extended Data Table 4 for accession number. B) Conserved syntenies obtained from the Genomicus Browser for *Acvr1l* (B.1-3), *Acvr1* (B.5), and *Acvrl1* (B.5) gene regions. Synteny conservation of the alligator and chondrichthyan *Acvr1l* loci establishes their orthology (B.1). Microsynteny of the alligator *Acvr1l* locus is conserved with chicken and spotted gar (B.2) despite the absence of this gene due to gene loss in birds and translocation to another chromosome in ray-finned fishes (B.3), respectively. C) Phylogenetic distribution of *Acvr1l* across jawed vertebrates. In tetrapods, retention of *Acvr1l* in crocodilians only suggests multiple independent gene losses. Species silhouettes obtained from www.phylopic.org.

Extended Data Figure 5. The expression pattern of *activin receptor type 1* in the pectoral fin of skates (*L.erinacea*) and spotted gar (*Lepisosteus oculatus*)

a and **b**) *Acvr1l* is expressed in the pectoral fin and the body trunk of skate embryos at stage 30 (**a**; ISH with sense probe and **b**; with antisense probe). Lateral view. The anterior is towards left in **a** and right in **b**. The scale bar length is 1 mm. *n*=3 for each staining. **c-e**) Expression of *acvr1l* is also observed in the pectoral fin and the body trunk of spotted gar embryos at embryonic stage 30-31[5]. The pectoral fin position is indicated by white dotted lines. **c**) ISH with no probe, **d**) with antisense probe, and **e**) a dorsal view of a side-by-side comparison of the *acvr1l* staining with sense (left) and antisense (right) probes. *n*=3 for ISH with no probe and *n*=6 with antisense probe. The scale bar length is 0.5 mm in **c** and **d**, and 1 mm in **e**.

Extended Data Figure 6. Bone staining of *gli3*$^{\mathbf{14ins/14ins}}$ fish and pectoral girdle phenotype.

a-d, g, and **h**) Alcian blue and alizarin red staining of wildtype (**a**,**c**, and **g**) and *gli3*$^{14ins/14ins}$ fish (**b**, **d**, and **h**). In *gli3*$^{14ins/14ins}$, ectopic ossification is observed in the eye lens (5/6 individuals), indicated by white arrows in **b**). The skull roof bones of *gli3*$^{14ins/14ins}$ fish are more mineralized than those of wildtype fish (**a** and **b**). The number of pectoral fin rays do not show significant difference between wildtype and *gli3*$^{14ins/14ins}$ fish (**c**, **d**). The pectoral fin on one random side of the body shifts dorsally due to the cleithrum defect in severely affected *gli3*$^{14ins/14ins}$ fish (**e**, **f**, **g**, **h**). On one side, the pectoral fin position is normal (**e**, black arrow), and the fin on the other side attaches dorsally to the body (**f**, white arrow). All scale bars are 1.25 mm.

Extended Data Figure 7. Quantitative analysis of the pectoral girdle by 3D reconstruction.

Red dots indicate landmarks used to quantify pectoral girdle bone morphology. The method to set the landmarks and quantify the line length that connects them is described in Method. **a**) the lateral view of the wildtype left pectoral girdle. **b**) the posterior view of the wildtype left pectoral girdle bones. A; the dorsal extremity of the cleithrum. B and C; the anterior and posterior ends of the dorsal shaft of the cleithrum. D; the attachment of the scapula to the cleithrum. E and F; the anterior and posterior ends of the scapula. G; the anterior extremity of the cleithrum. H; the anterior extremity of the coracoid. I; the posterior extremity of the coracoid, J and K; the dorsal and ventral ends of the widest point of the cleithrum. L; the ventral extremity of the scapula. In this paper, the following distance were measured to compare pectoral girdle morphology among different genotypes. A-D; the cleithrum height, B-C; the cleithrum width, G-D; the cleithrum length, D-L; the scapula length, E-F; the scapula width, H-I; the coracoid length, J-K; the coracoid width. M, N; the dorsal and ventral edges of the glenoid fossa. O, P; the ends of the line orthogonal to the M-N line. R, Q; the dorsal and ventral extremities of the mesocoracoid. S,T; the left and right ends of the line orthogonal to the R-Q line. All measurement is summarized in Extended Data Table 3.

Extended Data Figure 8. The modest phenotype of the cleithrum in *gli3*$^{\mathbf{14ins/14ins}}$ fish. While the pectoral fin on one side in approximately 20 % of *gli3*$^{14ins/14ins}$ fish shows the dorsal shift, the pectoral fins on the other side of these fish and pectoral fins on both sides in the remaining 80% fish show the modest cleithrum phenotype. The modestly affected cleithrum in *gli3*$^{14ins/14ins}$ fish is dorsoventrally shorter (black arrows) and anteroposteriorly wider (white arrows) than that of wildtype fish.

Extended Data Figure 9. Skeletal preparation of adult spotted gar (*Lepisosteus oculatus*) pectoral girdle.

The lateral view (**a**) and medial view (**b**) of the pectoral girdle. cl; cleithrum, clmw; medial wing of cleithrum, r; fin rays, s; scapula, scl; supracleithrum, scldp; dorsoanterior process of supracleithrum, sclvp; ventroanterior process of supracleithrum. Note that the supracleithrum is detached from the cleithrum. Scale bar = 1 cm.

Extended Data Table 1. PCR primers used to create gRNAs, conduct T7 assay, and clone genes.

Extended Data Table 2. Upregulated and Downregulated gene lists identified by RNA-seq in *gli3*$^{14ins/14ins}$ pectoral fin.

Extended Data Table 3. The list of wildtype ATAC-seq peaks annotated to genes. ACRs called by MACS[6] were annotated to genes depending on the proximity to transcription start sites (see Method).

Extended Data Table 4. Gene IDs of *acvr* genes analyzed in the phylogeny and synteny analyses. The IDs of *acvr* genes used to synthesize the data in Extended Data Fig. 4 are listed.

Extended Data Table 5. Quantification data of pectoral girdle morphology. The distance between landmarks of 3D-reconstructed pectoral girdle bones in wildtype, *gli3*$^{14ins/14ins}$, and *gli3*$^{14ins/14ins}$; *shha*$^{30\%}$ fish were measured in Amira. The landmark points for each measurement were defined in Extended Data Fig. 8.