

Fig. S1. *Hox13* paralog expression in the *P. waltil* forelimb development and regeneration

RT-PCR analysis of the developing forelimb, tail, and regenerating blastema of the forelimb. *Gapdh* was used as an internal control. The tail at st40 was used as a positive control for *Hoxb13*.

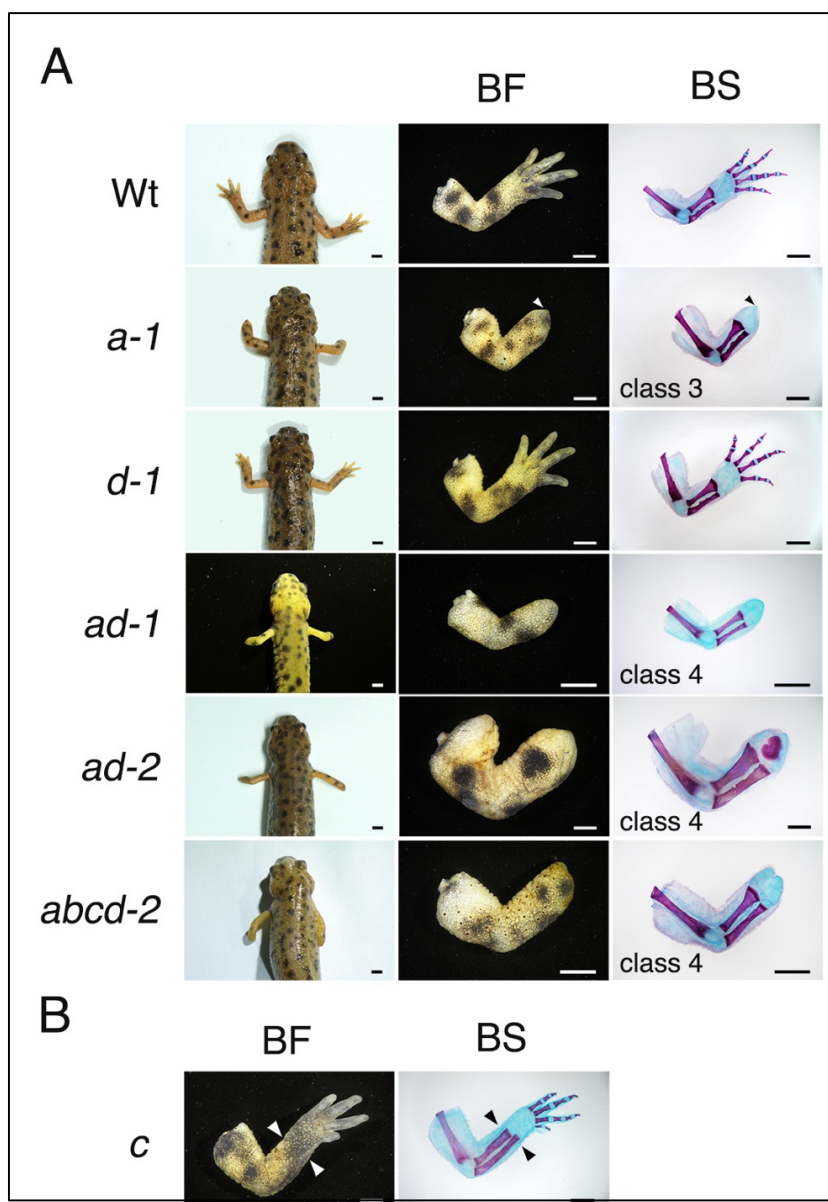


Fig. S2. Development of forelimbs in other *Hox13* crispants

(A) BF and BS: dorsal views of bright field (BF) and bone staining (BS) patterns of representative developed forelimbs. Representative phenotypes are shown. Arrowheads in *a-1* crispants represent digital phalange-like projections. For classification, see Fig. 4A. Anterior, up. Bar, 2 mm. The photos of Wt are the same as the ones used in Fig. 3A. (B) Dorsal BF and BS views of a forelimb of a *c* crispant. Because the limb was amputated first at the most distal region in the zeugopod (arrowheads), the autopod region has been regenerated. The remaining proximal part is the developed region.

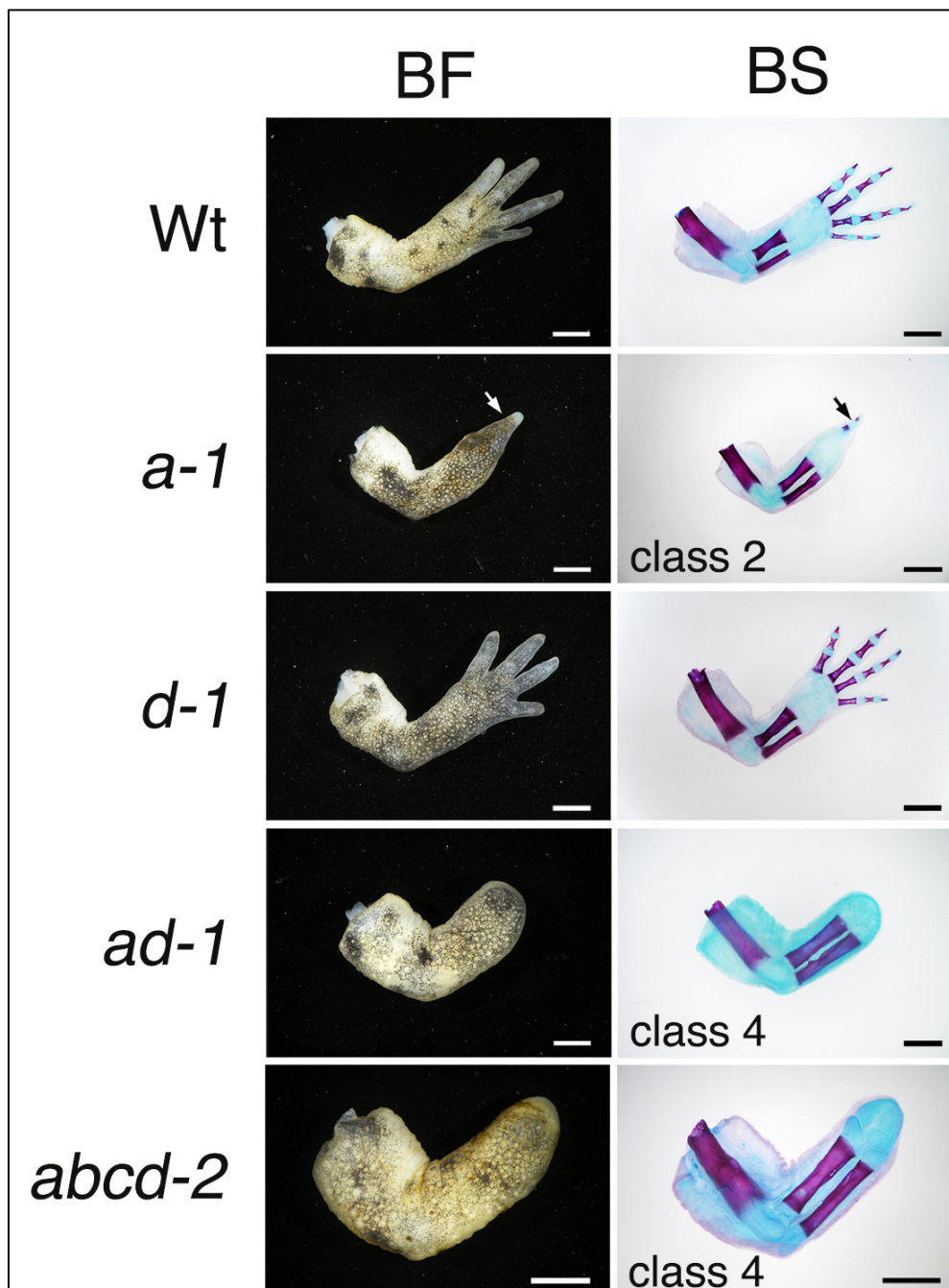


Fig. S3. Regeneration of forelimbs in other *Hox13* crispants

BF and BS: Dorsal views of bright field (BF) and bone staining (BS) patterns of representative regenerated forelimbs. Arrows in *a-1* crispants represent phalange bones. For classification, see Fig. 4A. The photos of Wt are the same as the ones used in Fig. 7A. Anterior, up. Bar, 2 mm.

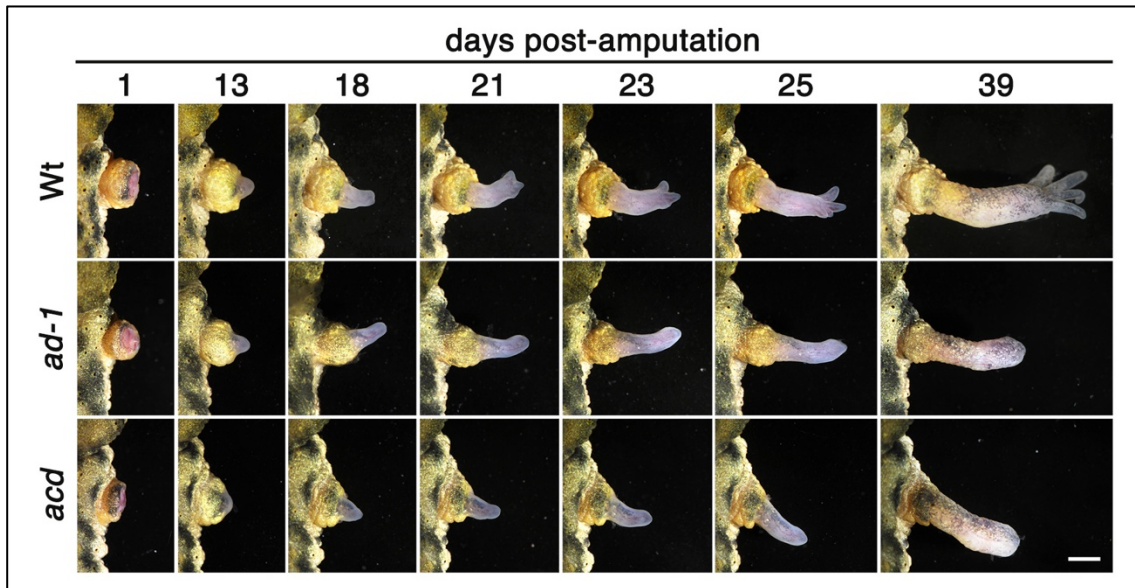


Fig. S4. Limb regeneration patterns of *Hox13* crispants

Dorsal views of representative regenerates of wild-type (Wt), *ad*, and *acd* animals. Limbs were amputated at 25 weeks post-fertilization (over 5 mpf) at the most proximal region in the stylopod. Days post-amputation are shown above. Anterior, up. Bar, 2 mm. See also Movies 1 and 2.

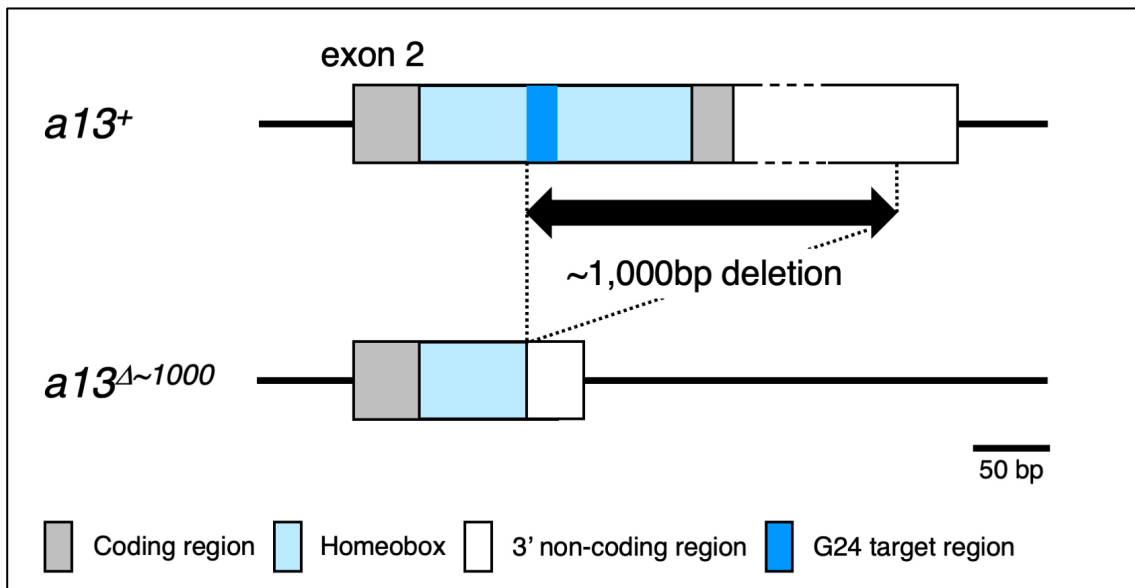


Fig. S5. The structure of *a13*^{Δ~1000} allele

A two-headed arrow shows the position of the deletion, although the exact location is unknown.

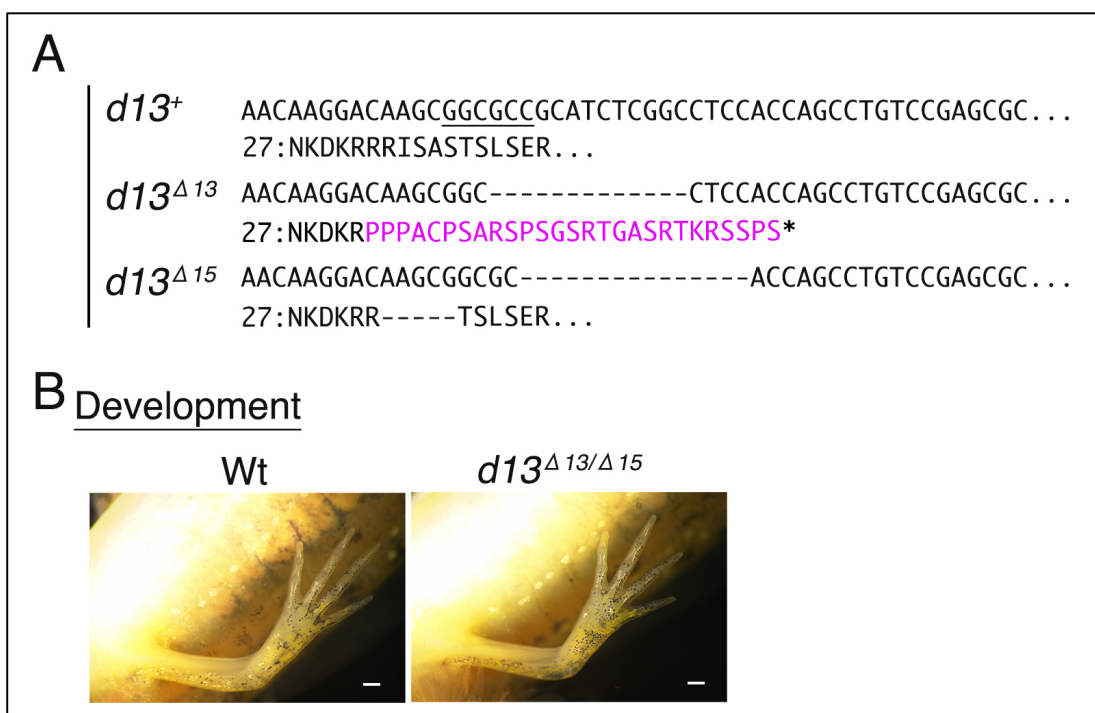


Fig. S6. Digit formation was normal in *Hoxd13* F2 germline mutants

(A) Nucleotide and amino acid sequences of *Hoxd13* wild-type and mutant alleles. SfoI recognition sites used for genotyping are underlined. The numbers to the left of the amino acid sequences indicate the position of the first residue in the homeodomain. Magenta characters, * and - indicate sequences different from the wild type, “stop”, and deleted sequences, respectively. (B) Dorsal views of bright field patterns of representative developed right forelimbs at 40 pdf. Anterior, left. Bar, 0.5 mm.

Table S1. Mutation rate of each gRNA

gRNA	target gene	analyzed crispants	mean of mutation rate \pm SD	total reads	average reads
G22	<i>a13</i>	16	97.7 \pm 5.2	64686	4042.9
G22	<i>c13</i>	16	91.3 \pm 6.8	130127	8132.9
G22	<i>d13</i>	16	97.3 \pm 4.8	37965	2372.8
G24	<i>a13</i>	14	99.2 \pm 1.1	114353	8168.1
G25	<i>d13</i>	2	100.0 \pm 0.0	6860	3430.0
G26	<i>d13</i>	10	94.6 \pm 7.5	153767	15376.7
G27	<i>a13</i>	2	98.0 \pm 0.1	12526	6263.0
G27	<i>b13</i>	2	97.5 \pm 0.4	25965	12982.5
G27	<i>c13</i>	2	96.9 \pm 0.6	16034	8017.0
G27	<i>d13</i>	2	98.8 \pm 0.8	14101	7050.5
G34	<i>b13</i>	4	100.0 \pm 0.1	78193	19548.3
G36	<i>c13</i>	6	95.2 \pm 3.6	76452	12742.0
G41	<i>d13</i>	7	99.6 \pm 0.5	14240	2034.3
G43	<i>a13</i>	8	97.8 \pm 5.3	56464	7058.0
mean \pm SD			97.4 + 2.3		

Table S2. Mutation rates, major alleles and phenotypes in individual crispants in which a single Hox13 paralog was mutated

[Click here to download Table S2](#)

Table S3. Mutation rates, major alleles and phenotypes in individual crispants in which two Hox13 paralogs were mutated

[Click here to download Table S3](#)

Table S4. Mutation rates, major alleles and phenotypes in individual crispants in which three Hox13 paralogs were mutated

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Table S5. Mutation rates, major alleles and phenotypes in individual crispants in which all four Hox13 paralogs were mutated

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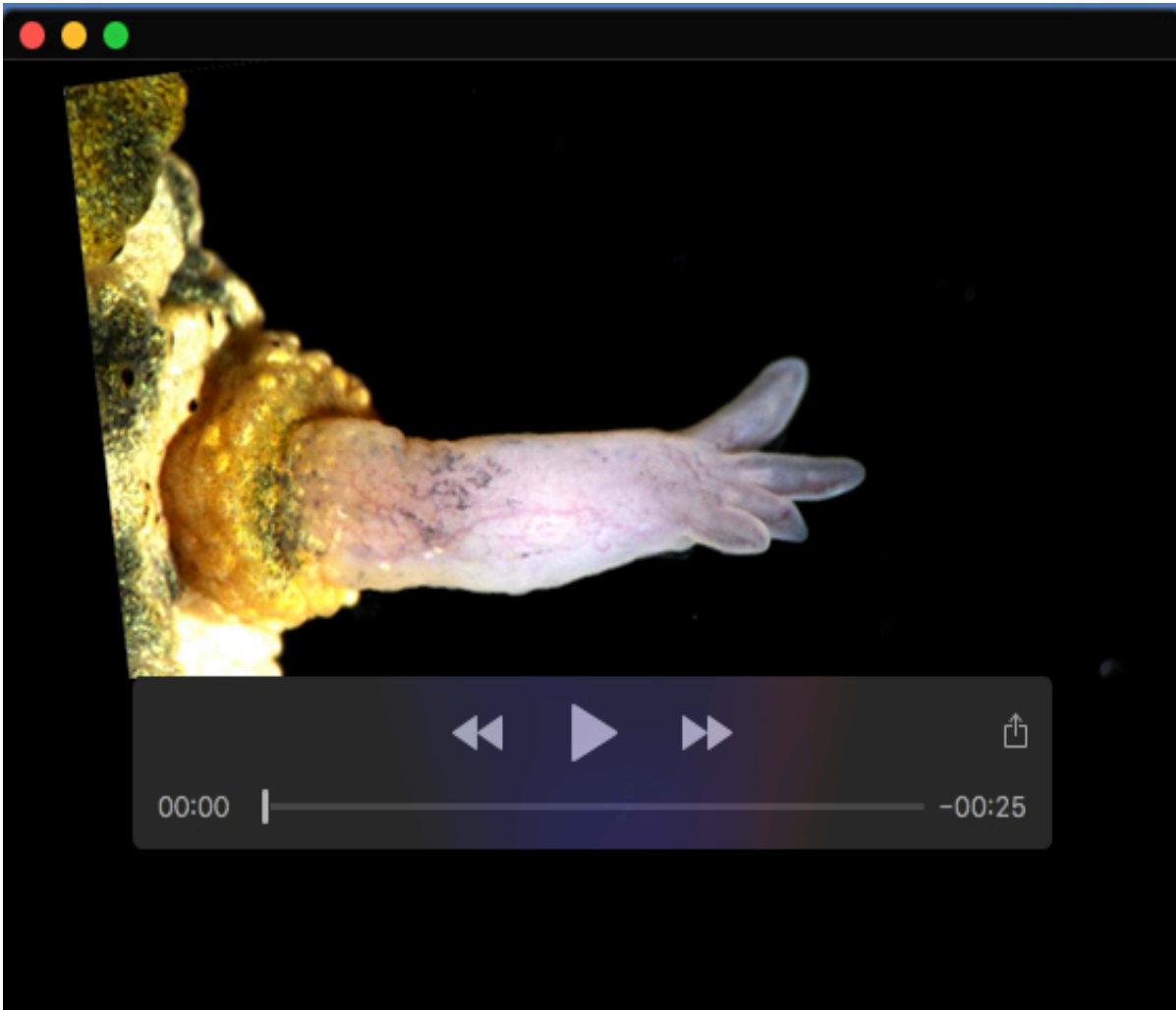
Table S6. gRNAs

gRNA	target gene	target sequence
G22	<i>a13, c13, d13</i>	CTACACCAAGGTGCAGCTGAAGG
G24	<i>a13</i>	TCATCACCAAAGACAAGCGGAGG
G25	<i>d13</i>	CCCTTTTGTCTTTGCCGCAGGGG
G26	<i>d13</i>	GGACAAGCGGCGCCGCATCTCGG
G27	<i>a13, b13, c13, d13</i>	TCACCATCTGGTTCCAGAACAGG
G34	<i>b13</i>	CAAAGCCAGCTGCGAGAGCTGG
G36	<i>c13</i>	GATGCGCCGGCGCTTCTCTTTGG
G41	<i>d13</i>	CCATCTGGTTCCAGAACAGGCGC
G43	<i>a13</i>	CGGAGAGGCAGGTCACCATCTGG

Restriction enzyme (G22, PvuII; G26, SfoI) sites and PAM sites are double underlined and underlined, respectively.

Table S7. DNA sequences of PCR primers

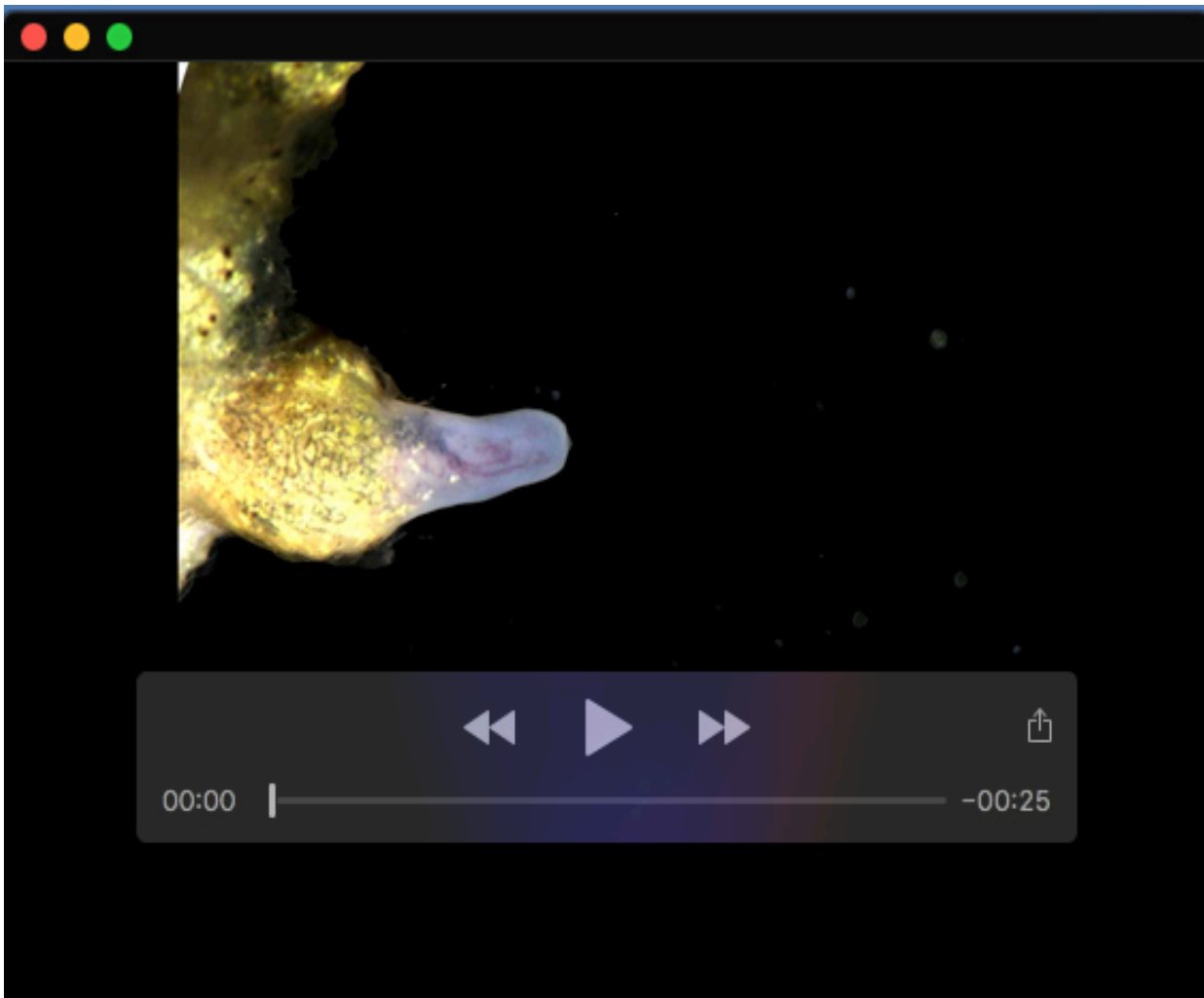
[Click here to download Table S7](#)



Movie 1. Regeneration of the right forelimb of wild-type (Movie 1) and *ad-2* (Movie 2) animals

Dorsal views of representative regenerates of wild-type and *ad-2* animals. Limbs were amputated at 25 weeks post-fertilization (over 5 mpf) at the most proximal region in the stylopod. Movies were produced by using photos of 1–46 days post-amputation.

Anterior, up. See also Fig. S5.



Movie 2. Regeneration of the right forelimb of wild-type (Movie 1) and *ad-2* (Movie 2) animals

Dorsal views of representative regenerates of wild-type and *ad-2* animals. Limbs were amputated at 25 weeks post-fertilization (over 5 mpf) at the most proximal region in the stylopod. Movies were produced by using photos of 1–46 days post-amputation.

Anterior, up. See also Fig. S5.