

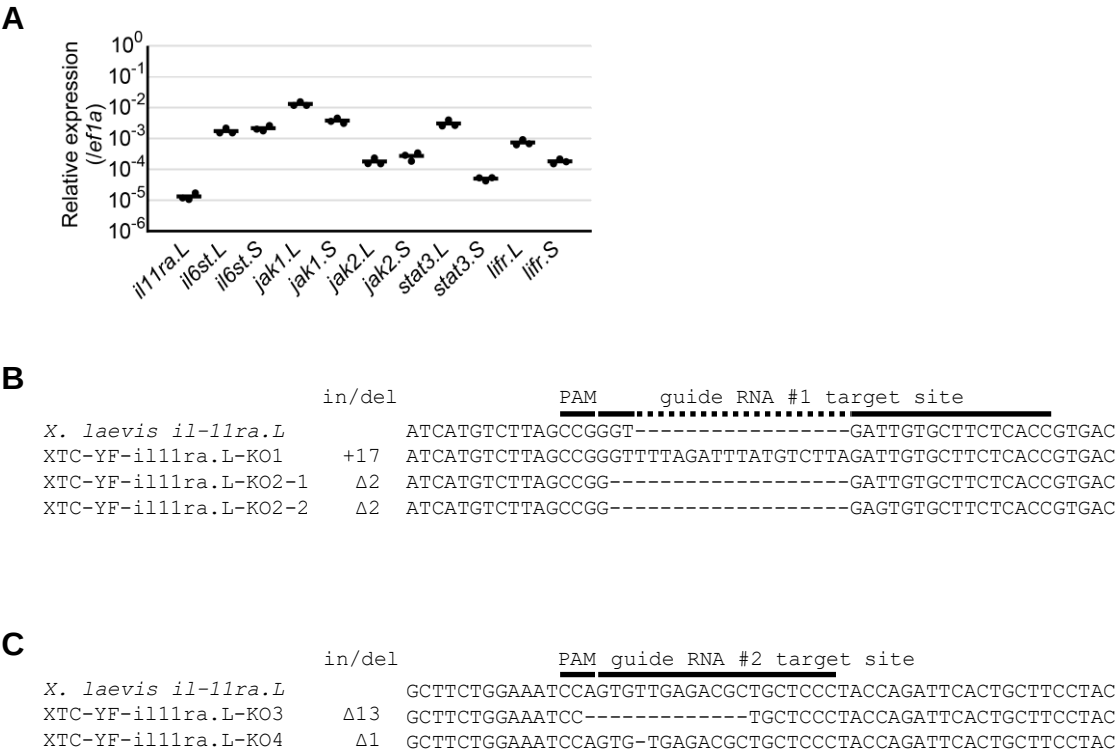
***Xenopus laevis il11ra.L* is an experimentally proven interleukin-11 receptor component that is required for tadpole tail regeneration.**

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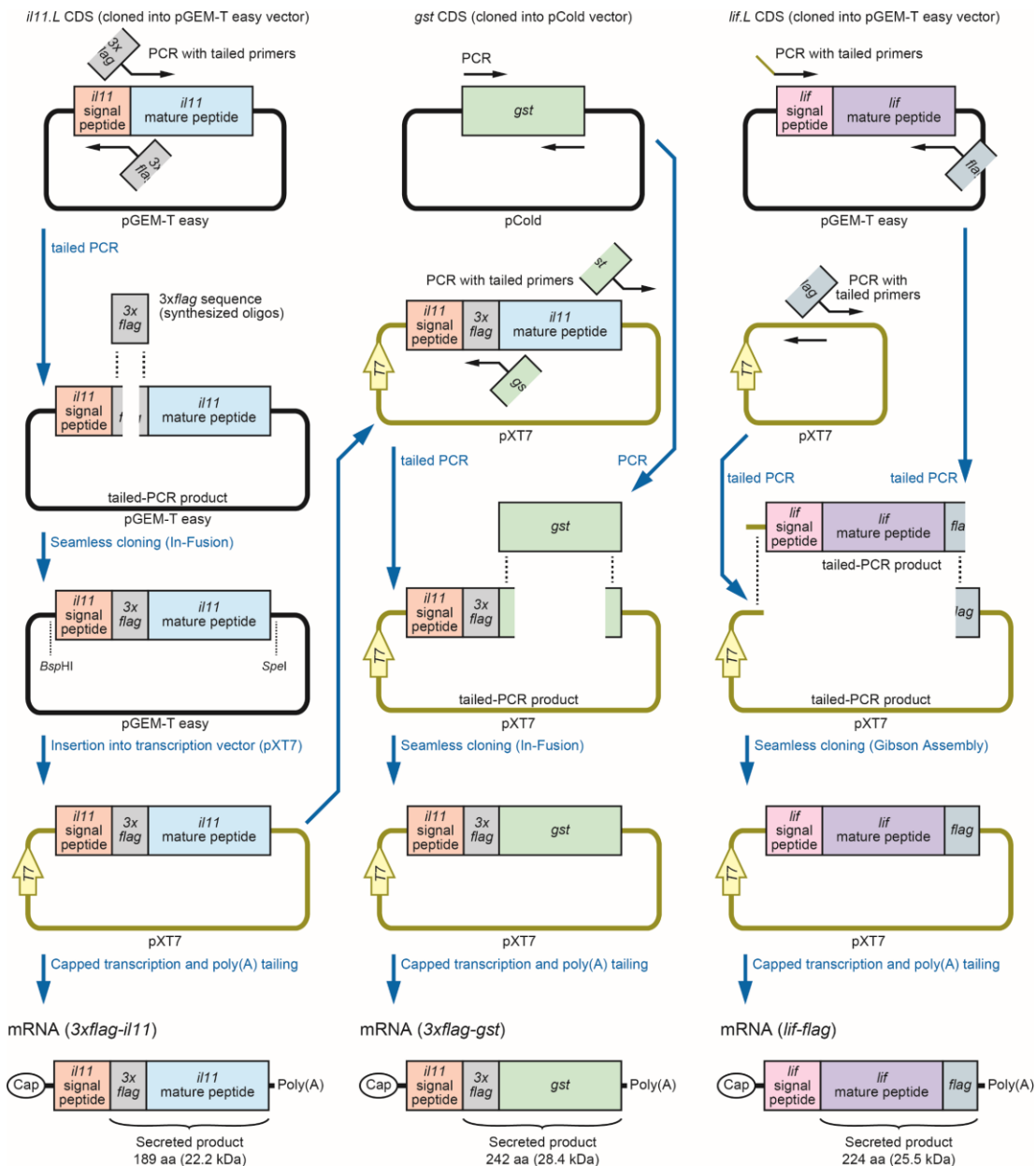
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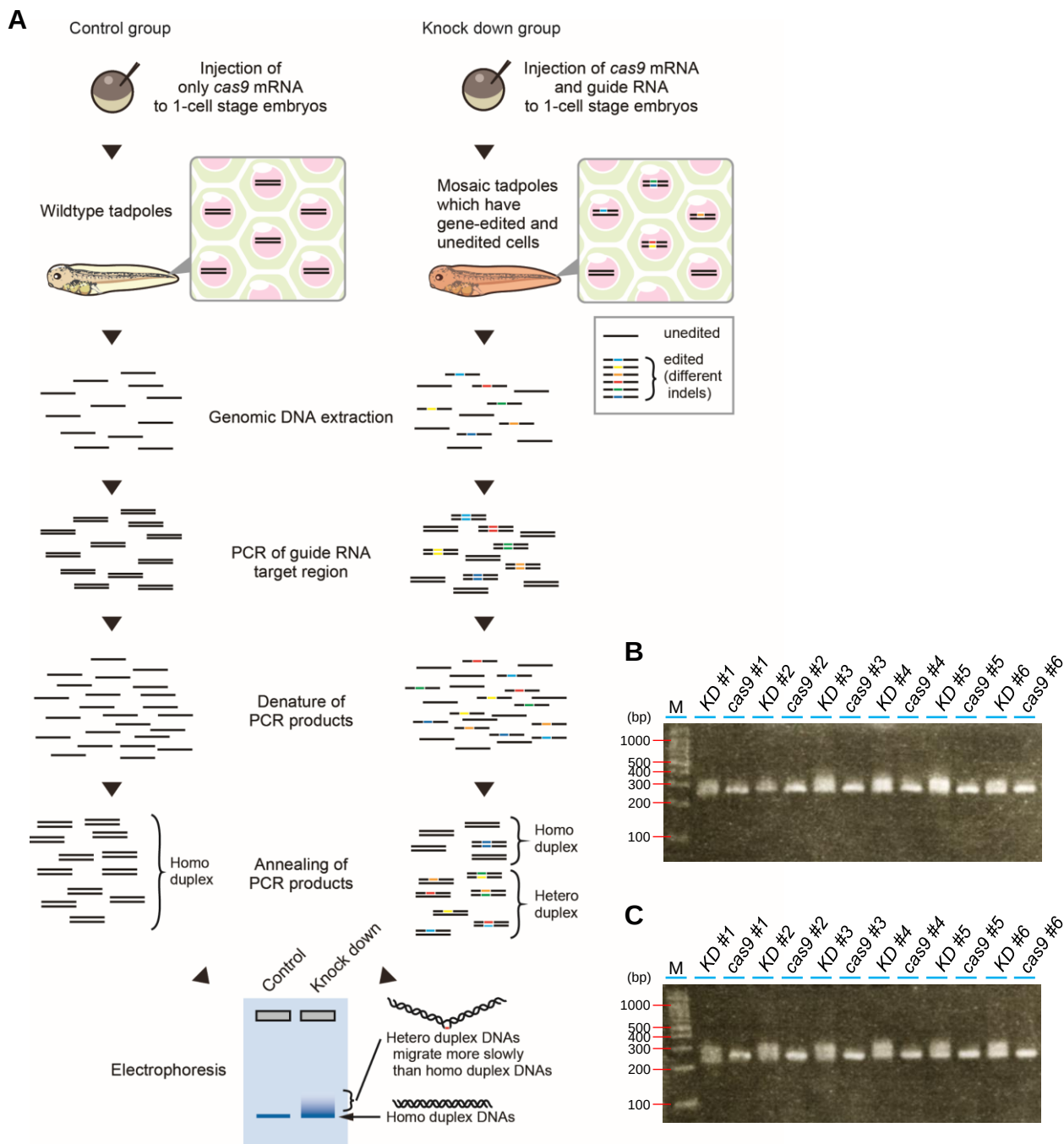


Supplementary Fig. S1
 Expression of Il11 signaling molecules in XTC-YF culture cell line and establishment of XTC-YF *il11ra.L* KO cell lines. (A) Expression levels of genes related to Il11 signaling measured by quantitative RT-PCR using 3 lots of RNA extracted from XTC-YF. Relative expression levels of the genes normalized by those of *ef1a* are shown in logarithmic scale. Bars indicate the mean value. (B and C) Genomic *il11ra.L* sequences of established XTC-YF *il11ra.L* KO cell lines. (B) KO1 and KO2 were established with guide RNA #1, (C) KO3 and KO4 were established with guide RNA #2. PAM, protospacer adjacent motif.



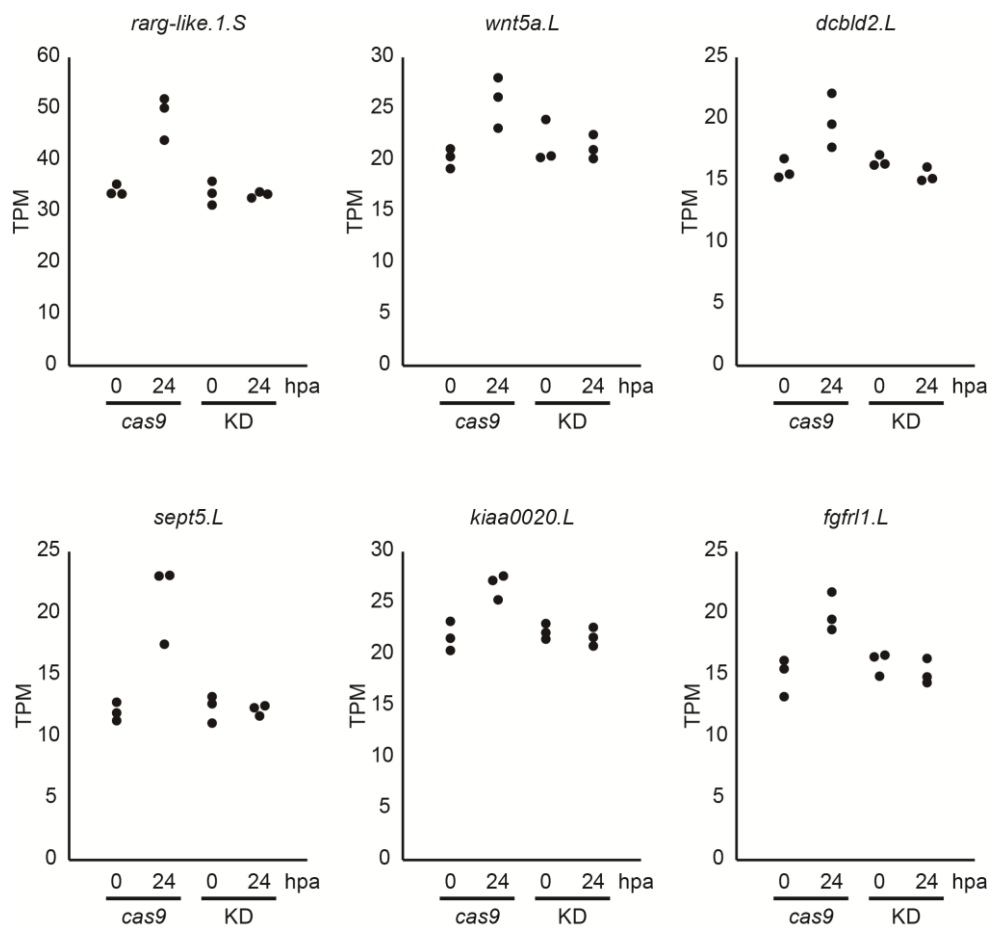
Supplementary Fig. S2

Construction of mRNAs of (left) N-terminal $3 \times \text{flag}$ -tagged *il11.L*, (center) N-terminal $3 \times \text{flag}$ -tagged *gst*, and (right) C-terminal *flag*-tagged *lif.L*.



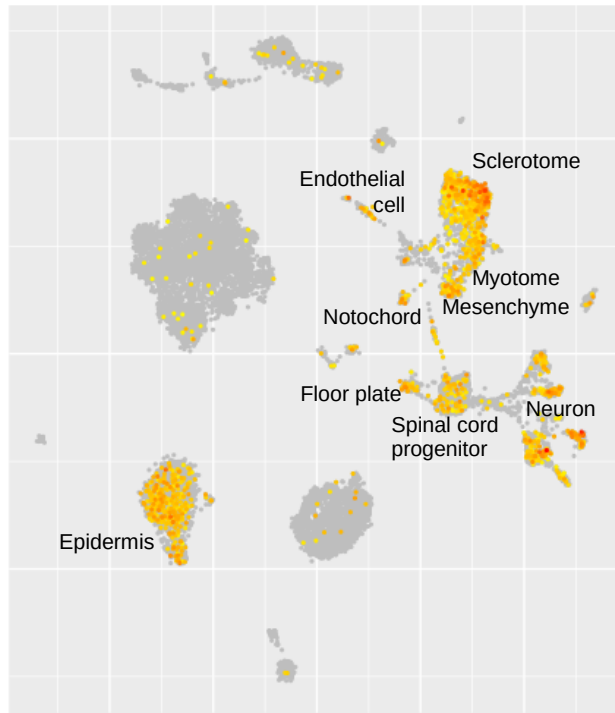
Supplementary Fig. S3

Heteroduplex mobility assay (HMA) for detection of gene editing. (A) Schematic figure of HMA. (B and C) HMA on amputated tails. Genomic loci including the target site of (B) guide RNA #1 or (C) #2 were amplified by PCR. Smear bands representing multiple heteroduplexes are shown from the PCR products derived from embryos injected with guide RNA and *cas9* mRNA (KD), whereas single bands are shown from control embryos injected with only *cas9* mRNA (*cas9*). Numbers represent the serial numbers of samples. M, Marker lane. Full-length gel images are presented in Fig. S9.



Supplementary Fig. S4

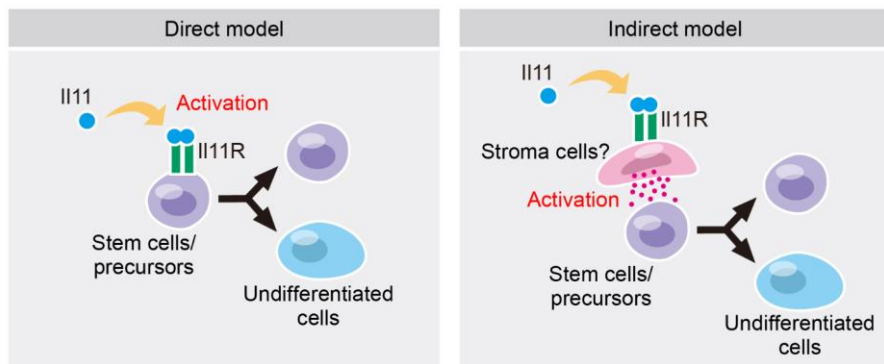
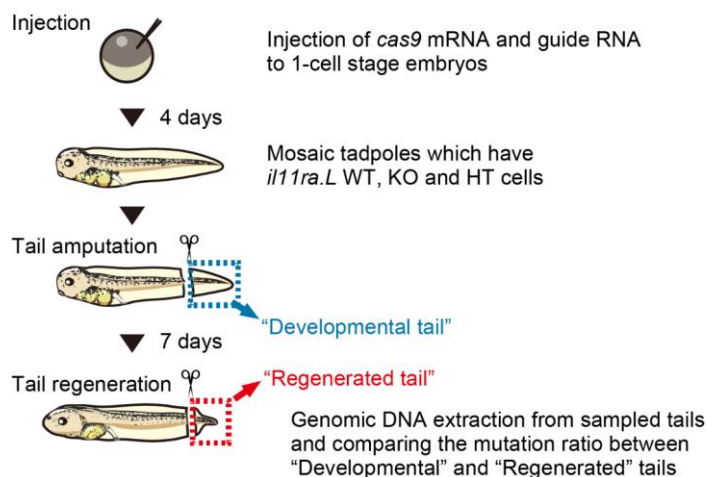
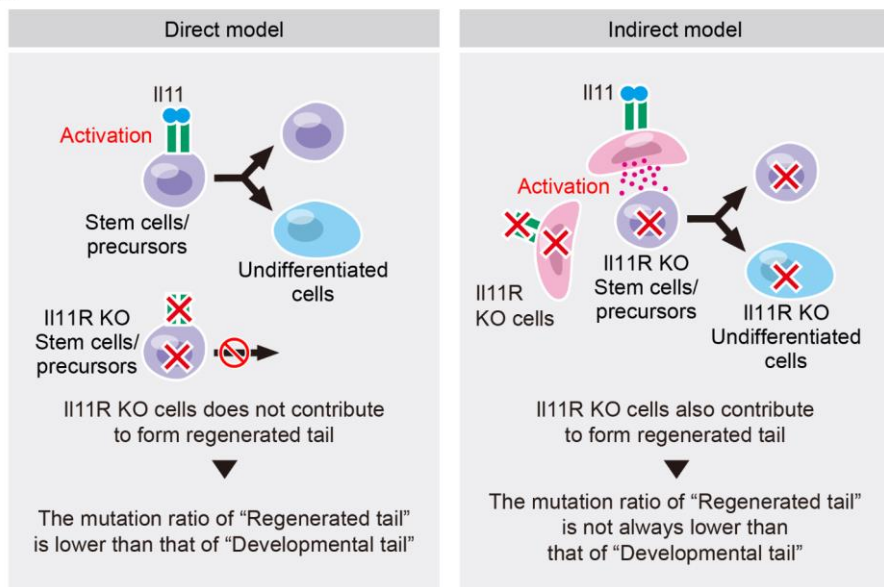
Expression of genes that are significantly upregulated in *cas9* 24 hpa tail stumps compared with *cas9* 0 hpa tail, and 0 and 24 hpa *il11ra.L* KD tail stumps. TPM, transcriptions per million.



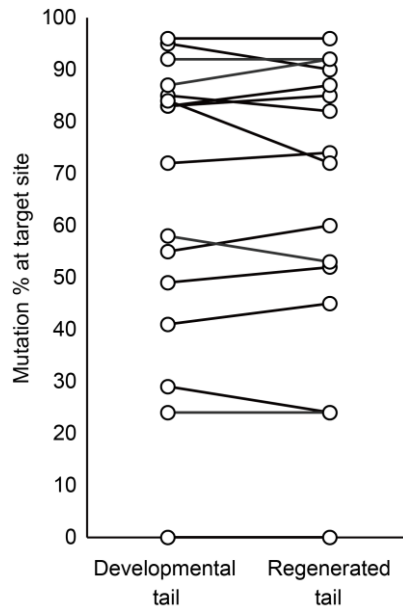
Supplementary Fig. S5

il11ra.L expression in single cell RNA-seq of regenerating tails reported by Aztekin *et al.*¹ is visualized with the web platform (Xenopus tail regeneration at single cell resolution; <https://marionilab.cruk.cam.ac.uk/XenopusRegeneration/>).

1. Aztekin, C. *et al.* Identification of a regeneration-organizing cell in the Xenopus tail. *Science* **364**, 653–658 (2019).

A**B****C****Supplementary Fig. S6**

Schematic of the putative mechanism of Il11 function. (A) Putative models of the mechanism of Il11 function. Direct model; Il11 directly activates stem cells or precursors of each tissue in the tail. Indirect model; Il11 triggers downstream events that activate stem cells or precursors of each tissue in the tail. Il11R, Il11 receptor complex. (B) Experimental procedure. (C) Putative behaviors of *il11ra.L* KO cells in both models and results of the mutation ratio comparison.



Supplementary Fig. S7

Second result of the mutation ratio estimation of developmental and regenerated tails. n=16. P=0.79, paired t-test.

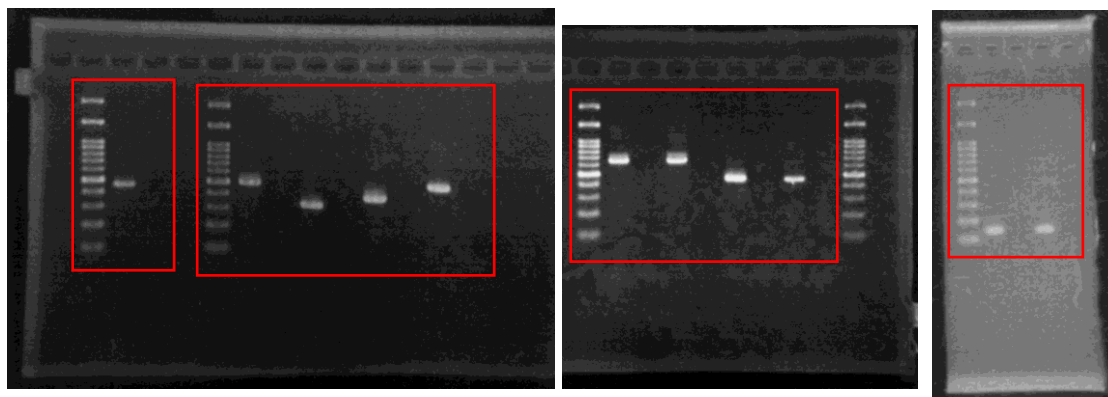
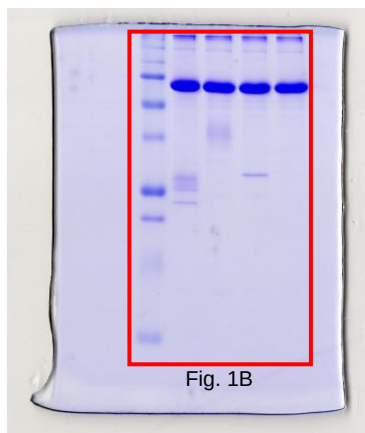
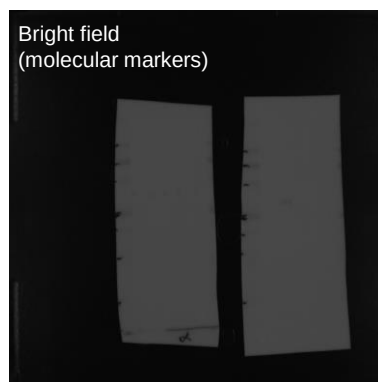


Fig. 1A



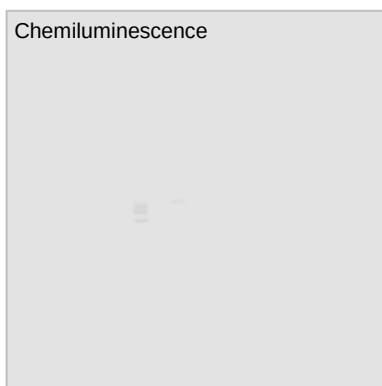
CBB staining

Fig. 1B



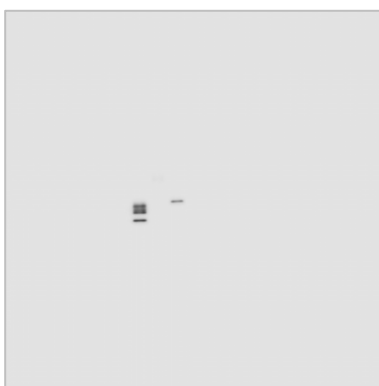
Bright field
(molecular markers)

Western blot



Chemiluminescence

Exposure 3 sec



Exposure 20 sec



anti-FLAG Mouse IgG2b

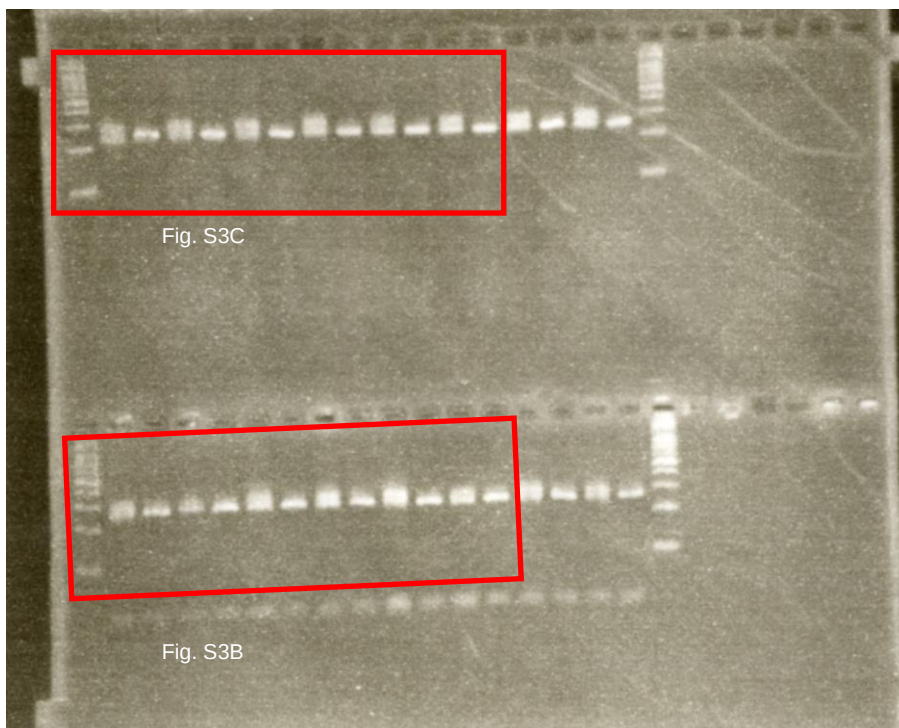
Fig. 1C

Fig. 1D

Exposure 2 min

Supplementary Fig. S8

Raw images of full-length gels and blots of Fig. 1.



Supplementary Fig. S9
Raw images of full-length gels of Fig. S3.

Supplementary Table S1.

The numbers and ratios of normally developed tadpoles, and tadpoles surviving after tail amputation in *il-11ra.L* KD experiments. Statistical significance was assessed by Fisher's exact test (for Exp.1, 2 and 3, the p values were corrected using the Benjamini-Hochberg method²). No significant differences were detected among the 3 (Exp. 1 to 3) or 2 (Exp. 4) groups.

Exp.	groups	Numbers of normally developed tadpoles / injected eggs, and ratios of normally developed tadpoles at 4 dpf	Numbers and ratio of tadpoles surviving after tail amputation
1	<i>cas9</i>	68/96 (71%)	68/68 (100%)
	#1 KD	59/96 (62%)	49/55 (89%)
	#1 KD	70/96 (73%)	69/70 (99%)
2	<i>cas9</i>	54/96 (56%)	52/54 (96%)
	#1 KD	48/95 (51%)	45/48 (94%)
	#1 KD	49/95 (52%)	49/49 (100%)
3	<i>cas9</i>	21/93 (23%)	21/21 (100%)
	#1 KD	23/89 (26%)	22/23 (96%)
	#1 KD	26/88 (30%)	25/26 (96%)
4	<i>cas9</i>	25/48 (52%)	25/25 (100%)
	#1 KD	32/48 (67%)	32/32(100%)

2. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B 57, 289–300 (1995).

Supplementary Table S2.

Genes that are significantly upregulated in *cas9* 24 hpa tail stumps but whose upregulation was abolished in tail stumps of the *illlra.L* KD group, in RNA-seq. FWER, familywise error rate.

Gene name	FWER
<i>rarg-like.1.S</i>	0.0099
<i>wnt5a.L</i>	0.0192
<i>dcbl2.L</i>	0.0307
<i>sept5.L</i>	0.0366
<i>kiaa0020.L</i>	0.0424
<i>fgfr11.L</i>	0.0487

Supplementary Table S3.

Primer sequences used for RT-PCR of XTC-YF.

genes	primers (5' to 3')	PCR products (bp)
<i>il-11ra.L</i>	AGGAGGCTTCGTGTCAGTT	461
	TCCATTCCTTCTTCCGC	
<i>il6st.L</i>	CTGTCCAGTGCTCGATCAG	504
	GTACAGTGTGTTTCCTTCTAGCTTG	
<i>il6st.S</i>	ACTGCCAAGTACTCCATCCA	335
	CTCCACTTGCTTTGTATGTATGC	
<i>jak1.L</i>	TGGACCTGAAACTCTCCTCA	390
	AATCTTTCAGCGAGTCAAAGC	
<i>jak1.S</i>	GAGCTGAAGCTTTCACG	494
	GCGACCAACAAGTTCGAG	
<i>jak2.L</i>	AATACTGCAGGGGAGACTTG	671
	TTCAGAGTAGAAAGCTGACTGTAGC	
<i>jak2.S</i>	TTACCATACTTCAGCAGAGACAGA	674
	CAGAGTAGAAAGCCGACTGTAAG	
<i>stat3.L</i>	GGATTAAACAGTTCTTGCAGAGTA	468
	CAAGCTCTTCATCAGTCAGTG	
<i>stat3.S</i>	TAAACTGTTTCCTGCAGAGCC	458
	CAAGCTCCTCGTCTGTCAGAT	
<i>lifr.L</i>	CGTGCAACTGACAAATGATGT	148
	CCTCAGCGCAGAGATGTTAG	
<i>lifr.S</i>	GCAGCTGCCAAATGAAGAT	147
	ATTAGCGCTGAGGTGTCCA	

Supplementary Table S4.

Primer sequences used for quantitative RT-PCR of XTC-YF.

genes	primers (5' to 3')	PCR products (bp)
<i>efla</i>	GGAACGGTGACAACATGC AGGCAGACGGAGAGGCTTA	161
<i>il-11ra.L</i>	CCAGATTCACTGCTTCCTACC GAACTCCGACTTCCTGACG	101
<i>il6st.L</i>	GGCTGTGAAAGTGTAAGAAGTGA CTGCAAGATGGCCCTGTA	108
<i>il6st.S</i>	TGGCTGTGAGTGTGTAAGAACTAC ACTGCAAGATGGCCCTATG	109
<i>jak1.L</i>	GAAAGCAAAAGTAAGAACTCTGTTG TCTGGTGAGGAGAGTTTCAGG	160
<i>jak1.S</i>	GAAAGCAAAAGTAAGAACTCAGACA GGCGTGGAAGCTTCAGC	157
<i>jak2.L</i>	GTCCCGTCCTTGATGACA TGTGATTGGTACAGAAATCCGA	89
<i>jak2.S</i>	CAGAGAGTCCTGTTCTTGATGATG GTGTGATTGGTACTGTTATTCGG	96
<i>stat3.L</i>	CTGAGCTGAATGGCAACAAT CCATGGCTGACAGGAGACTC	129
<i>stat3.S</i>	CTGAGATGAATGGCAACCAA CATGGCTGACAGGAGACTG	128
<i>lifr.L</i>	CGTGCAACTGACAAATGATGT CCTCAGCGCAGAGATGTTAG	148
<i>lifr.S</i>	GCAGCTGCCAAATGAAGAT ATTAGCGCTGAGGTGTCCA	147

For *lifr.L* and *lifr.S*, the same primers were used as for RT-PCR.

Supplementary Table S5.

Primer sequences used for genotyping the *il-1lra.L* KO XTC-YF and heteroduplex mobility assay of KD tadpoles. The same primer sets were used in both experiments.

target	direction	primers (5' to 3')	PCR products (bp)
guide RNA #1	forward	CCTGGTAAACTGCTCTGTGCT	250
	reverse	CTAGACTTGCAAAGGCTGCTTC	
guide RNA #2	forward	CCCTTTGGCACTCAGATCCC	262
	reverse	CCAACGCAGAAGCTAATGATCG	

Supplementary Table S6.

Primer sequences used for estimating mutation ratios of KD embryo tails.

target	direction	primers (5' to 3')	PCR product (bp)
guide RNA #1	forward	TGGTTGTACTCTTACCCAGCA	411
	reverse	TATGCTGCTATAGAGGGTCCGT	

Supplementary Table S7.

Primer sequences used for DIG-labeled probe synthesis.

gene	primers (5' to 3')	PCR product (bp)
<i>il-11ra.L</i>	CCGGGTGATTGTGCTTCTCA	1082
	TTCTTCCGCACTTTCACCCA	