Supplementary Information

Biallelic editing of a lamprey genome using the CRISPR/Cas9 system

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Figure S1.

>LCas9-1

Globin 5’ UTR NLS

taatacgactcactatagggagagcttgttctttttgcagaagctcagaataaacgctcaactttggcagatctcgagccacc atggcttctccccctaagaag

Lamprey codon optimized Cas9-1

aagcgaaaggtgggctccATGGACAAGAAATACTCAATTGGGCTGGATATTGGAACTAACTCAGTCGGATGGGCTGTGATTACCGACGACTACAAAGTGCCCAG
CAAGAAATTCAAAGTCCTCGGAAACACCGATCGACACAGCATTAAGAAGAACCTCATCGGTGCCCTGCTCTTTGGCTCTGGAGAGACAGCAGAGGCGACACGCC
TCAAACGAACCGCGCGCCGACGATACACAAGGCGCAAGAACAGGATCTGCTATCTCCAAGAGATATTCTCTAATGAGATGGCGAAAGTGGACGACTCATTCTTT
CACCGACTGGAGGAGAGCTTTCTGGTGGAGGAGGACAAGAAACATGAGAGGCATCCGATCTTTGGCAATATTGTGGATGAGGTCGCATACCACGAGAAATACCC
CACAATCTATCATCTCCGTAAGAAACTGGCCGACTCTACCGACAAAGCCGACCTCCGACTGATCTATCTGGCACTGGCCCACATGATTAAGTTTAGGGGGCACT
TTCTGATTGAAGGTGACCTCAATCCTGACAATTCCGATGTCGATAAACTCTTTATCCAGCTGGTGCAGATATATAACCAACTGTTTGAAGAGAATCCTATTAAC
GCATCAAGGGTGGATGCTAAAGCTATCCTCTCGGCTAGGCTCTCGAAATCCCGACGACTCGAAAACCTCATTGCTCAACTCCCCGGCGAGAAACGAAATGGACT
GTTTGGGAATCTCATCGCCCTGTCCCTGGGGCTCACCCCCAACTTCAAATCAAATTTCGATCTCGCTGAGGACGCTAAACTCCAGCTCAGCAAAGATACATATG
ATGACGATCTGGACAATCTCCTGGCCCAAATCGGTGACCAATATGCTGATCTGTTTCTCGCAGCCAAGAACCTCAGCGACGCAATCCTGCTCTCAGACATTCTC
CGTGTTAATTCGGAAATTACTAAAGCACCGCTCAGCGCTAGCATGATTAAGCGCTACGACGAGCATCATCAAGACCTCACCCTGCTCAAAGCGCTGGTCCGTCA
ACAGCTGCCGGAGAAATATAAGGAAATCTTCTTTGATCAATCGAAGAATGGTTACGCGGGGTACATTGATGGTGGAGCGTCGCAAGAGGAGTTCTACAAATTCA
TTAAGCCAATTCTCGAAAAGATGGACGGAACGGAGGAACTCCTCGTCAAACTGAACCGCGAAGACTTGCTGCGGAAACAACGTACGTTTGACAACGGCTCAATT
CCCCATCAGATCCACTTGGGTGAGCTGCATGCGATCCTGCGCCGCCAGGAGGACTTCTATCCTTTCTTGAAAGACAATAGGGAGAAGATTGAGAAGATCCTCAC
TTTCAGGATTCCGTATTACGTGGGACCGTTGGCGCGTGGTAACAGCCGTTTCGCGTGGATGACCCGCAAATCCGAAGAAACGATTACTCCTTGGAACTTCGAGG
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TATGAGTACTTTACCGTGTATAATGAACTGACGAAAGTCAAGTACGTGACAGAAGGGATGCGCAAACCCGCGTTTCTCTCCGGAGAACAGAAGAAAGCAATCGT
GGACCTCTTGTTTAAGACGAACCGCAAAGTTACCGTGAAGCAGCTGAAAGAGGATTACTTTAAGAAGATAGAGTGTTTCGATTCAGTCGAGATTAGCGGGGTTG
AGGACAGGTTTAACGCGTCGCTGGGAGCATACCACGACCTGTTGAAGATAATTAAGGACAAGGATTTCCTGGACAACGAGGAGAATGAGGATATATTGGAGGAT
ATCGTGCTGACGTTGACATTGTTTGAGGACCGGGGCATGATCGAGGAGCGGTTGAAGACTTACGCCCACCTGTTTGACGATAAGGTTATGAAGCAGCTCAAACG
CCGGCGGTACACTGGTTGGGGCCGGCTGAGCCGCAAGCTGATTAACGGTATACGGGATAAACAGTCTGGAAAGACAATCCTCGACTTCCTGAAATCGGATGGGT
TCGCCAACAGGAATTTCATGCAGCTGATACACGATGATAGCCTGACTTTCAAGGAGGACATACAGAAAGCGCAGGTGTCTGGACAGGGGCACAGCCTCCACGAG
CAGATCGCAAATCTGGCCGGCTCTCCGGCAATCAAGAAAGGCATTCTCCAGACTGTGAAGATCGTCGATGAGCTGGTCAAAGTTATGGGGCACAAACCCGAGAA
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CGCAGATCCTGAAGGAGCACCCCGTCGAGAACACGCAGTTGCAGAACGAGAAGTTGTACCTCTACTACTTGCAGAATGGCCGGGACATGTACGTGGATCAGGAA
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GAATCGCGGGAAGTCCGACAACGTGCCCTCTGAGGAAGTTGTTAAGAAGATGAAGAACTACTGGCGCCAGCTCCTGAACGCGAAGTTGATAACGCAGCGGAAGT
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CCGCAAGGACTTCCAGTTCTACAAGGTCAGGGAGATCAACAACTACCACCACGCTCACGACGCGTACCTGAACGCCGTGGTGGGCACGGCGCTCATCAAGAAGT
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TACTTCTTCTACTCGAACATCATGAACTTCTTCAAGACCGAGATCACACTCGCGAACGGGGAGATCAGGAAGCGCCCACTGATCGAGACGAACGGCGAGACCGG
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TCAGCAAGGAGTCCATCTTGCCAAAGCGGAACAGCGACAAGCTCATCGCCCGTAAGAAGGACTGGGACCCAAAGAAGTACGGGGGCTTCGACAGCCCGACCGTG
GCGTACTCCGTGCTCGTCGTCGCCAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAGTCGGTGAAGGAGTTGCTCGGCATAACGATCATGGAGCGTAGCAGCTT
CGAGAAGAACCCGATCGACTTCTTGGAAGCCAAGGGCTACAAAGAAGTTAAGAAGGACCTGATCATCAAGCTCCCAAAGTACTCCCTGTTCGAATTGGAGAACG
GGCGGAAGCGCATGCTGGCCAGCGCCGGCGAATTGCAGAAGGGCAACGAATTGGCCCTGCCATCGAAGTACGTCAACTTCCTGTACCTCGCGAGCCACTACGAG
AAGCTGAAGGGCTCCCCGGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAAATATCGGAGTTCAGCAA
GCGCGTCATCCTGGCCGACGCCAACCTGGACAAGGTCCTGTCCGCGTACAACAAGCACAGGGACAAGCCAATCCGCGAGCAGGCCGAGAACATCATCCACCTGT
TCACCCTGACCAACCTCGGCGCCCCGGCCGCCTTCAAGTACTTCGACACGACCATCGACCGCAAGCGCTACACCTCCACCAAAGAAGTTCTGGACGCGACCCTG
ATCCACCAGAGCATCACGGGCCTGTACGAGACGCGCATCGACCTGTCCCAGCTCGGCGGCGACTccccagtgcggtcccccaagaagaaacggaaggtgtgatg
a

NLS

>LCas9-2

Globin 5’ UTR Flag

taatacgactcactatagggagacaagcttgcttgttcttttgcagaagctcagaataaacgctcaactttggcagatctcgagccaccatggactacaagga

NLS

ccacgatggagactataaggaccacgacattgactacaaggatgacgacgataag atggccccaagaagaaacgcaaggtgggcatccacggagtgcccgccg

Lamprey codon optimized Cas9-2

cgATGGACAAGAAATATTCTATTGGACTGGATATTGGGACCAACTCTGTGGGATGGGCGGTCATCACAGACGAGTACAAAGTCCCTTCCAAGAAATTCAAAGTG
CTCGGGAACACTGATAGGCACTCCATTAAGAAGAACCTCATAGGAGCACTGCTCTTTGACAGCGGCGAGACCGCGGAGGCGACTCGGCTGAAGAGGACCGCCAG
GCGGCGCTACACACGACGAAAGAACCGAATTTGCTATCTGCAAGAAATCTTCTCTAACGAGATGGCCAAAGTGGATGATTCATTCTTTCATCGCCTGGAGGAGA
GCTTTCTGGTGGAGGAGGACAAGAAACACGAGCGCCACCCAATCTTTGGCAACATTGTCGATGAAGTCGCCTATCATGAGAAATATCCTACCATCTATCATCTG
CGTAAGAAACTGGTGGATAGCACAGACAAAGCTGACCTCCGGCTGATCTATCTGGCCCTGGCCCATATGATCAAGTTTCGCGGGCATTTCCTGATTGAGGGTGA
CCTCAACCCTGACAACTCCGATGTGGATAAACTGTTTATCCAACTCGTGCAGACATACAACCAGCTCTTTGAGGAGAACCCCATCAATGCTTCCGGCGTGGACG
CGAAAGCTATCCTCTCCGCTCGTCTGTCCAAATCGCGTAGGCTGGAGAACCTCATTGCGCAGCTGCCGGGTGAGAAGAAGAATGGTCTGTTTGGGAACCTGATC
GCTCTGTCCCTCGGTCTGACTCCTAACTTTAAGAGCAATTTCGATCTGGCCGAGGATGCAAAGCTGCAACTCTCCAAAGATACTTATGATGACGATCTGGATAA
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TTACCAAAGCGCCGCTCAGCGCGTCCATGATCAAACGTTACGACGAGCATCATCAGGATCTGACTCTGCTGAAAGCTCTCGTGCGGCAACAACTCCCTGAGAAA
TATAAGGAAATATTCTTTGACCAGTCAAAGAACGGTTATGCAGGATATATAGATGGTGGAGCGAGCCAGGAGGAGTTCTACAAGTTTATCAAACCAATTCTCGA
AAAGATGGATGGCACCGAAGAACTGCTGGTGAAACTCAACAGGGAAGACCTGCTCCGTAAACAGCGCACCTTTGATAATGGCTCTATTCCGCATCAAATTCACC
TGGGAGAGTTGCACGCAATTCTCCGGCGCCAAGAGGATTTCTACCCATTCCTGAAAGACAATCGAGAGAAGATCGAGAAGATTCTGACGTTTCGTATTCCCTAC
TACGTCGGTCCACTGGCCCGCGGTAATTCTCGTTTCGCCTGGATGACGCGCAAATCCGAGGAAACTATAACCCCTTGGAATTTCGAGGAAGTGGTCGATAAAGG
CGCGTCTGCCCAGTCCTTCATCGAACGCATGACGAATTTCGACAAGAATCTCCCCAACGAGAAAGTGCTGCCGAAACACTCGCTCCTCTACGAGTACTTCACTG
TGTACAACGAGCTGACTAAAGTGAAATACGTCACGGAAGGAATGAGGAAACCAGCTTTCCTGTCAGGCGAACAGAAGAAAGCAATCGTGGATCTCTTGTTTAAG
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ATCACTCGGGACCTACCACGATCTCTTGAAGATAATCAAGGACAAAGACTTCCTGGATAACGAGGAGAACGAGGATATCCTGGAAGACATTGTGTTGACCCTCA
CACTGTTTGAGGACCGCGAGATGATCGAGGAACGACTGAAGACGTACGCCCACCTGTTTGACGACAAAGTCATGAAACAGCTGAAGCGACGACGGTACACGGGC
TGGGGCCGCCTCAGCCGTAAACTGATCAACGGCATCCGGGACAAGCAGAGCGGGAAGACGATATTGGACTTCTTGAAATCCGACGGCTTTGCGAATCGAAACTT
CATGCAGCTGATCCACGACGACAGCCTCACGTTCAAGGAGGACATCCAGAAAGCTCAGGTGAGCGGCCAGGGCGACTCGCTGCACGAGCACATTGCAAACCTGG
CTGGAAGCCCCGCCATCAAGAAGGGCATTCTGCAAACGGTTAAGGTCGTTGATGAACTGGTGAAGGTTATGGGGCGGCACAAGCCCGAGAACATCGTGATTGAG
ATGGCTCGCGAGAATCAAACCACGCAGAAGGGACAGAAGAACTCACGCGAGCGGATGAAGCGCATCGAGGAGGGGATCAAGGAGCTGGGATCACAGATTCTCAA
GGAGCACCCAGTCGAGAATACCCAGTTGCAGAATGAGAAGTTGTACTTGTACTACCTCCAGAACGGGCGCGACATGTACGTGGACCAGGAGCTGGACATTAACC
GTCTGTCGGACTACGACGTTGACCACATCGTGCCCCAGAGCTTCCTCAAGGACGACTCCATCGACAATAAGGTGTTGACGCGGTCTGACAAGAATCGCGGAAAG
AGCGACAATGTCCCGTCCGAAGAAGTTGTTAAGAAGATGAAGAATTACTGGCGGCAGCTCCTGAACGCCAAACTCATCACACAGAGGAAGTTCGACAACCTCAC
AAAGGCCGAGCGCGGCGGGCTCTCTGAATTGGACAAGGCGGGCTTCATCAAGCGCCAGTTGGTGGAGACGCGGCAGATCACAAAGCACGTGGCGCAGATCCTCG
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CAGTTCTACAAGGTGAGGGAGATCAACAACTACCACCACGCACACGACGCATACCTCAACGCGGTTGTTGGGACCGCCCTGATCAAGAAGTACCCCAAGCTGGA
GAGCGAGTTCGTGTACGGGGACTACAAGGTGTACGACGTGCGCAAGATGATCGCAAAGAGCGAGCAGGAGATCGGGAAGGCCACAGCCAAGTACTTCTTCTACT
CGAACATCATGAACTTCTTCAAGACGGAGATCACACTGGCGAATGGTGAAATACGGAAGAGGCCGCTCATCGAGACCAACGGCGAGACGGGGGAGATCGTGTGG
GACAAGGGGAGGGACTTCGCCACAGTGCGTAAAGTTCTCTCGATGCCCCAGGTGAACATCGTGAAGAAGACGGAGGTGCAGACCGGCGGCTTCTCTAAGGAGTC
CATCCTGCCGAAGCGGAACAGCGACAAGTTGATCGCACGCAAGAAGGACTGGGACCCGAAGAAGTACGGCGGCTTCGACTCACCGACCGTCGCCTACAGCGTGC
TGGTCGTGGCCAAGGTGGAGAAGGGGAAGTCGAAGAAGCTCAAGTCGGTCAAGGAATTGCTCGGCATCACGATCATGGAGCGCAGCTCGTTCGAGAAGAACCCG
ATCGACTTCTTGGAAGCGAAGGGCTACAAAGAAGTTAAGAAGGACCTGATCATCAAGCTGCCGAAGTACTCGCTGTTCGAGCTGGAGAACGGGAGGAAGCGCAT
GCTGGCCTCGGCAGGCGAGTTGCAGAAAGGCAACGAGCTGGCGCTCCCCTCGAAGTACGTGAACTTCTTGTACTTGGCCTCGCACTACGAGAAGCTCAAGGGCA
GCCCAGAGGACAACGAGCAGAAGCAGCTCTTCGTGGAGCAGCACAAGCACTACTTGGACGAGATCATCGAGCAAATATCGGAGTTCAGCAAGCGCGTGATCTTG
GCGGACGCCAACCTGGACAAGGTCCTGTCGGCCTACAACAAGCACCGCGACAAGCCCATCCGAGAGCAGGCCGAGAACATCATCCACCTCTTCACCCTCACGAA
CCTGGGCGCACCAGCGGCCTTCAAGTACTTCGACACGACCATCGACCGCAAGAGGTACACGAGCACCAAAGAAGTCTTGGACGCCACGCTGATCCACCAGAGCA
TCACGGGCTTGTACGAGACGCGCATCGACCTGTCCCAGCTGGGCGGCGACaagaggcccgcggccaccaagaaggcgggccaggccaagaagaagaaatga

NLS

Figure S1. The DNA sequences of LCas9-1 and LCas9-2.

Figure S2.

wee1

CCTCACGGTGGCAAAAGTACAACGTGGGCGAATTTTAAACTTTTTTTTACTTTAAAAAGTTTCAACAACAAAAACATTGGGATAAGAAGAC
GACGATAAGAACGAGAAAACGTCGCCCGATTTTTAAAACAACAATGAGCCGATTCTCGACATTTACGAGAGTTTCCGCCAAGCTGGATTACCT
CAGCGACGACGAAGAAGGCCCAGGTACACCTCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCTTGACACCTCCGCCGGGCGCCTCGCAC
CGAAGCCGGCGGGTCGTCCAGGCCACGGCGGGCTTCTTGGGCGGGAGTCGCGAGGCGCTGTCGTGTTGGGACG**CGTCGATGGAGTCTCCG**
TCGCCCGTGAAG

soxe2

TGTCGGATATCAATCACGAGATGTCGCGCACTCCGAGCCCGCACTGCTCCTCGGACGCCGGGTCTGGACTCGGATCCCCTGGCCGCAGCGA
TCCCGAGCCGAGCTCCGTCGAGCACGGAGGAGGCGGCGGCGGCGGCGGAGGAGGAGGAGGCTCGGGTGGCTGCAGCTCATCGGGCTCGGA
GCCGTCGAGGGGAGGAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTGAGTCAAGTGCTGGACGGCTACGACTGGTCGCTG
TTGCCCGTGCCCGTGCGTGGAGCCGGTGGGCCGGGGGGATGCAAGCCGGGCGAGAAGCCCCACGTGAAGAGACCCATGAACGCGTTCATGG
TGTGGGCGCAAGCGGCTCGCAGG**AAGCTGTCCGACCAATACCC**

kctd10

ACATCCAGACTCGTGTTCCTTCTGCGCACCGCCAAGATTGCCCG-TCAGCCACGTGCCGCTTCACGCAACGAGCCGCCTTTGTCGGGTACCC
CCGTGTTCACGCGAGCGCACCGCGCCTCTCTCCCACTCTCACCGCGACGCCGGCGAGCAGGCTGGATCCTGATCGACCGCTGCGGAAAGCAC
TTTGGCACGATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTA
CTACCTGGTGCAAGGCCTGGGACAGCAGTGCCACGCCGCCCT**GCAGGTATCATCGCGTATTTC**

wnt7b

TTATCGCCGTTTCCACTCCACCGACCAGGTGCTGGAGGAGAAAATGAAGCTGGAGTGTAAGTGCCACGGCGTGTCGGGCTCGTGCACCAC
CAAGACGTGCTGGACCACGCTGCCCAAGTTCCGCGAGGTGGGCTACGCGCTCAAGGACAAGTACGAGCACACGGTGCACGTGGAGCCGGTG
AGCGGCAGCCGTTACCTGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTACCGCAAGCCGCTCGACACGGAGCTCGTGTTCATCGA
GCGCTCGCCCAACTACTGCGAGGAGGACCCGGCTACGGGCAGCGCGGGGACACGCGGTCGCCTGTGTAACCGCACGTCGCCGCACGCTGAC
GGCTGCGACCTTCTGTGCTGCGGCCGC**GGCTACAACACGCACCAGTA**

golden

CTCTCGCTCGTGTCCGTCTACGCTGCCTACGTGGTGGCTCTGCACTTCGACGCGCGCCTCCGCCGCGCCTTCACACGCGGCTGCTTGTGCCT
CCGCACGGCCGGGCAGCACCGCTGCCATCAGCCGGGTCACGACCCCGAGACGCAGCCGCTCGTCGGCGTCGACGCGGCCGGCGAGGAACA
CAGT-GGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGCACGGGCGCGCCAGGTCGGACAGCGGCGTCATCCTCAAGGAC
GGCTCGGACTACT**CGCAGTTGACGCTCAGCCTGCACGG**

Figure S2. The sequences of the target regions of five genes of lamprey. The target sites of each gene are labeled with box, and the primer pairs for amplifying each target region are underlined and in bold.

Figure S3.

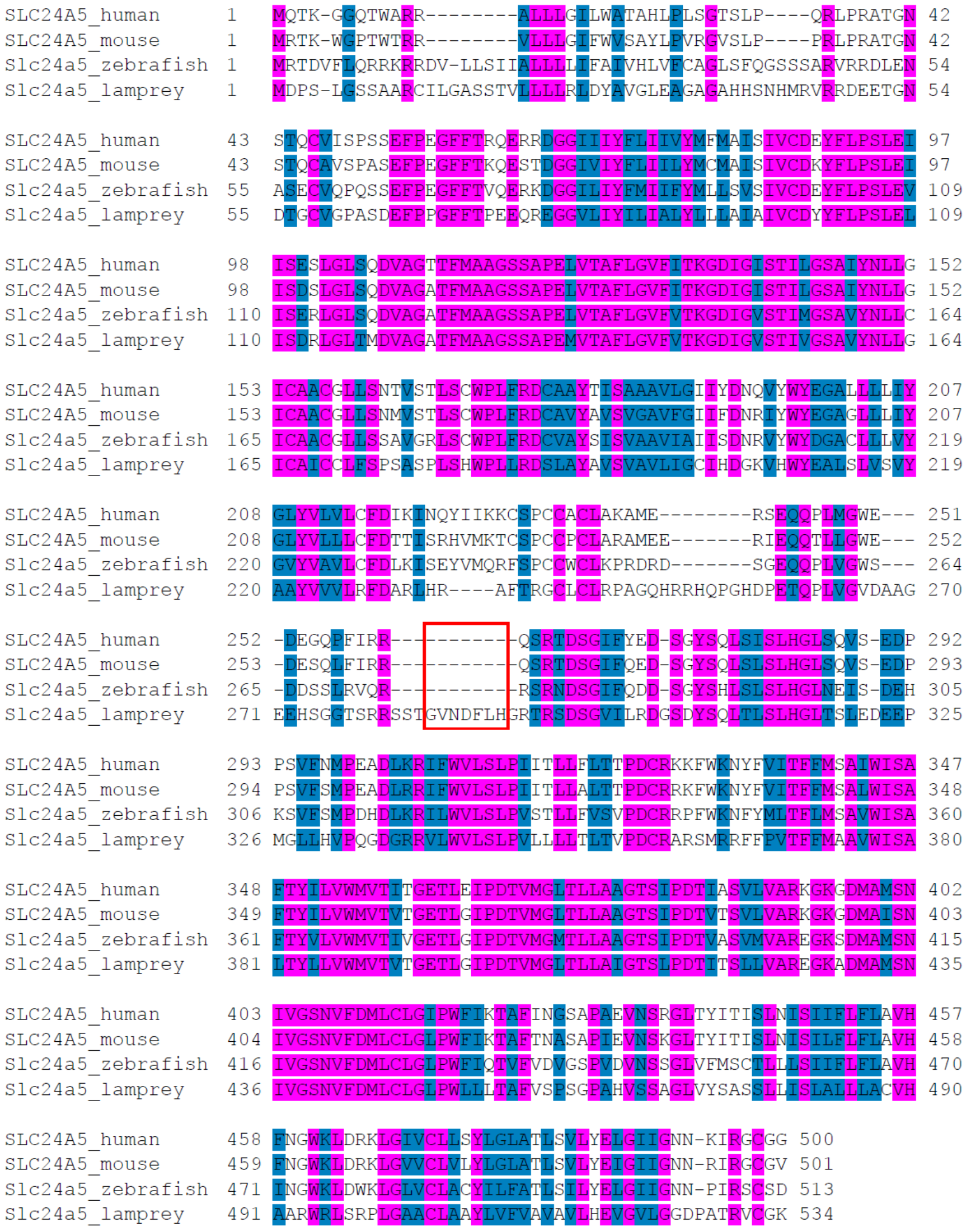

Figure S3. The alignment of the sequences of Slc24a5 (encoded by the gene of *golden*) among human, mouse, zebrafish and lamprey. The target site of Cas9-gRNA is labeled with the red box.

Figure S4.

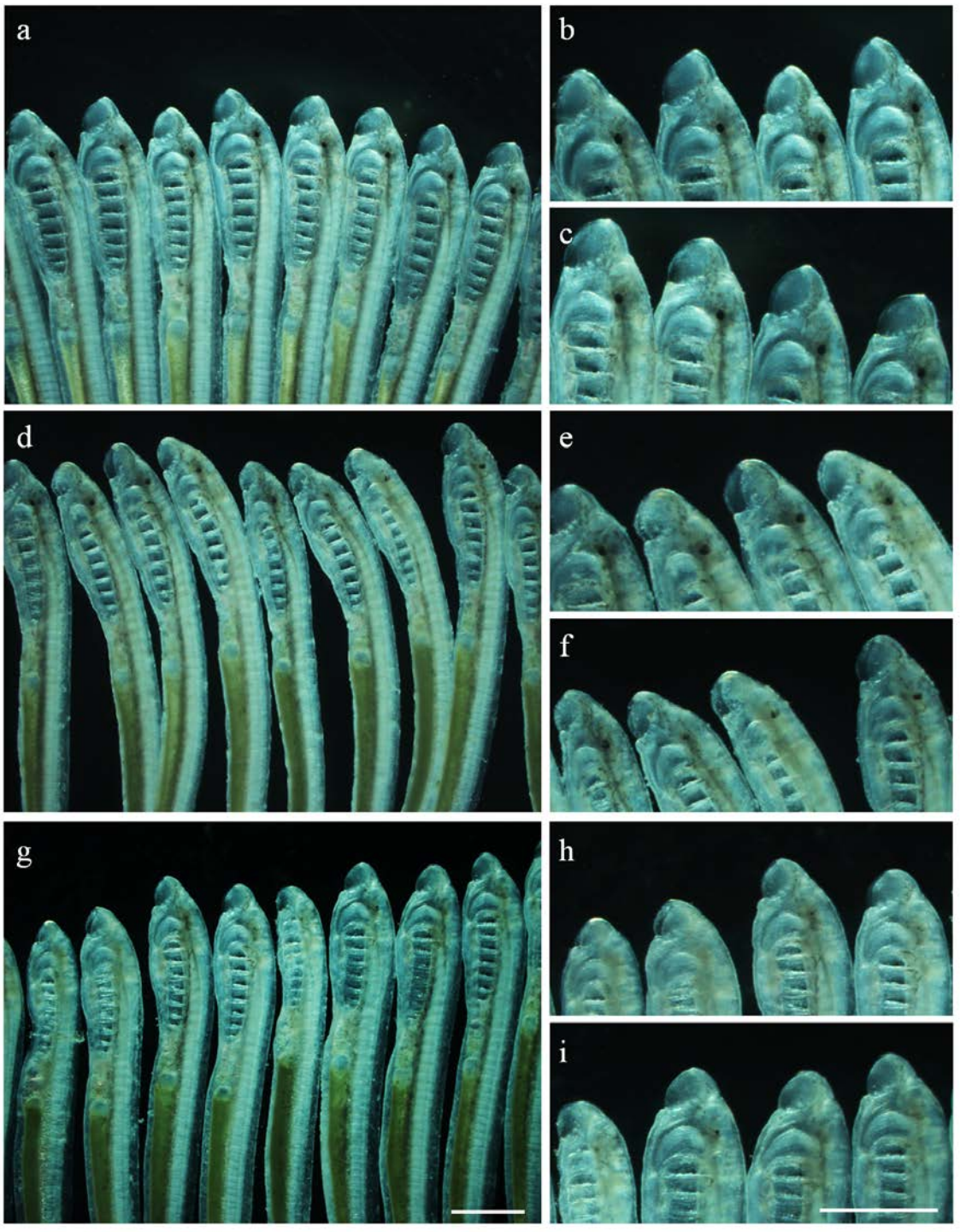

Figure S4. Injection of Cas9 and gRNA against *golden* resulting in mosaic and null pigmentation in the retinas caused by biallelic disruption of this gene. Lateral views of control lamprey larvae (**a-c**) and *gol* gRNA injected larvae (**d-i**) at 19 dpf. Some larvae injected with *Cas9* mRNA and *gol* gRNA showed mosaic hypopigmentation (**d-f**), some larvae were almost unpigmented in their retinas (**g-i**). (Scale bars: 1mm)

Figure S5.

A

N2

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Reference   GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGCACGGGCGCGCCAGGTCGGACAGCGGCGTCATC
Seq1:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC---------GGGCGCGCCAGGTCGGACAGCGGCGTCATC（-9bp）
Seq2:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC---------GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-9bp)
Seq3:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC---------GGGCGCGCCAGGTCGGACAGCGGCGTCATC（-9bp）
Seq4:       GGAACACAGTGGCGGCACCTCGGGTCGCTCCTCGACGG----------------------GCGCCAGGTCGGACAGCGGCGTCATC(-24bp)
Seq5:       ------------------------------------------------------------------------------TC(-111bp)
Seq6:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGT-CGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-2, +1bp)
Seq7:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGT-CGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-2, +1bp)
Seq8:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-----GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq9:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-----GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq10:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-----GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq11:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-----GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq12:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-----GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq13:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-----GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq14:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-----GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq15:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-----GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq16:      GGAACACAGTGGCGGCACCTCGCGCCGCTCCTCGACGGGGGTCAACGACTTCCTGC--------GCCAGGTCGGACAGCGGCGTCATC(-8bp)
Seq17:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGG------------------------------TCGGACAGCGGCGTCATC(-32bp)
Seq18:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGG------------------------------TCGGACAGCGGCGCCATC(-32bp)
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N5

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Reference   GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGCACGGGCGCGCCAGGTCGGACAGCGGCGTCATC
Seq1:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC---------GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-9bp)
Seq2:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC---------GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-9bp)
Seq3:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACG-----------------CCAGGTCGGACAGCGGCGTCATC(-18bp)
Seq4:       GGAACACAG-------------------------------------------------GCGCGCCAGGTCGGACAGCGGCGTCATC(-51bp)
Seq5:       GGAACACAGCGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTG----GGCGCGCCAGGTCGGACAGCGGCGTCATC(-4bp)
Seq6:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTG----GGCGCGCCAGGTCGGACAGCGGCGTCATC(-4bp)
Seq7:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC----CGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-4bp)
Seq8:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGC-----GGCGTCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq9:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC------------GCGCCAGGTCGGACAGCGGCGTCATC(-13bp)
Seq10:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC------------GCGCCAGGTCGGACAGCGGCGTCATC(-13bp)
Seq11:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC------------GCGCCAGGTCGGACAGCGGCGTCATC(-13bp)
Seq12:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC------------GCGCCAGGTCGGACAGCGGCGTCATC(-13bp)
Seq13:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGG------------------CGCGCCAGGTCGGACAGCGGCGTCATC(-20bp)
Seq14:      GGAACACAGTGGCGGCACCTCG----------------------------------GGCGCGCCAGGTCGGACAGCGGCGTCATC(-37bp)
Seq15:      GGAACACAGTGGCGGCACCTCGCGTC-------------------------------------------------------ATC（-59bp）
Seq16:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGCGTCACCGACTTCCGTCCTTGACCCCCGGGCGCGCCAGGTCGGACAGCG(-4,+12bp)
Seq17:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGCACGGGCGCGCCAGGTCGGACAGCGGCGTCATC(wt)
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B

M2

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Reference   GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGCACGGGCGCGCCAGGTCGGACAGCGGCGTCATC
Seq1:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC---------GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-9bp)
Seq2:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGATCAACG---------ACGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-9bp)
Seq3:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC--------------GCCAGGTCGGACAGCGGCGTCATC(-15bp)
Seq4:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC--------------GCCAGGTCGGACAGCGGCGTCATC(-15bp)
Seq5:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC--------------GCCAGGTCGGACAGCGGCGTCATC(-15bp)
Seq6:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC--------------GCCAGGTCGGACAGCGGCGTCATC(-15bp)
Seq7:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC--------------GCCAGGTCGGACAGCGGCGTCATC(-15bp)
Seq8:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACGGCC--------------GGTCGGACAGCGGCGTCATC(-19,+4bp)
Seq9:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCA----------------------GGTCGGACAGCGGCGTCATC(-24bp)
Seq10:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCT------------------------------（-36bp）
Seq11:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-----GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-5bp)
Seq12:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC----------GGCGCGCCAGGTCGGACAGCGGCGTCATC(-10bp)
Seq13:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC----------GGCGCGCCAGGTCGGACAGCGGCGTCATC(-10bp)
Seq14:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCA------------CGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-13bp)
Seq15:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGG--------------------CGCGCCAGGTCGGACAGCGGCGTCATC(-22bp)
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Seq16:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTC----------------------------GGACAGCGGCGTCATC(-29bp)
Seq17:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACG-------------------------------------(-56bp)
Seq18:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACG-------------------------------------(-56bp)
```

M5

```
Reference   GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGCACGGGCGCGCCAGGTCGGACAGCGGCGTCATC
Seq1:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC---ACGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-3bp)
Seq2:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC---ACGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-3bp)
Seq3:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC---ACGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-3bp)
Seq4:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGTTCAACGACTTCCTGCA---------CAGGTCGGACAGCGGCGTCATC(-9bp)
Seq5:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACG-----------GGCGCGCCAGGTCGGACAGCGGCGTCATC(-12bp)
Seq6:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACT-----------------GGTCGGACAGCGGCGTCATC(-18bp)
Seq7:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACG-----------------CCAGGTCGGACAGCGGCGTCATC(-18bp)
Seq8:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTT----CACGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-4bp)
Seq9:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTC----ACGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-4bp)
Seq10:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTG----GGCGCGCCAGGTCGGACAGCGGCGTCATC(-4bp)
Seq11:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTG----GGCGCGCCAGGTCGGACAGCGGCGTCATC(-4bp)
Seq12:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGC--------GCCAGGTCGGACAGCGGCGTCATC（-8bp）
Seq13:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCG----------------------------CCAGGTCGGACAGCGGCGTCATC(-31bp)
Seq14:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCG----------------------------CCAGGTCGGACAGCGGCGTCATC(-31bp)
Seq15:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCG----------------------------CCAGGTCGGACAGCGGCGTCATC(-31bp)
Seq16:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCG----------------------------CCAGGTCGGACAGCGGCGTCATC(-31bp)
```

C

W5

```
Reference   GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTGCACGGGCGCGCCAGGTCGGACAGCGGCGTCATC
Seq1:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTC------GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-6bp)
Seq2:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC---------GGGCGCGCCAGGTCGGACAGCGGCGTCATC(-9bp)
Seq3:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGAGGGTCAACGA-----------------CAGGTCGGACAGCGGCGTCATC(-18bp)
Seq4:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAAC--------------------AGGTCGGACAGCGGCGTCATC(-21bp)
Seq5:       GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGAC-------------------------AGCGGCGTCATC(-27bp)
Seq6:       GGAACACAGTGGCGGCACCTCGCGTCGC---------------------------------GCCAGGTCGGACAGCGGCGTCATC(-36bp)
Seq7:       GGAACACAGTGGCGGCACCTCGCGTCGC---------------------------------GCCAGGTCGGACAGCGGCGTCATC(-36bp)
Seq8:       ----------------------------------------------------------CGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-87bp)
Seq9:       ----------------------------------------------------------CGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-87bp)
Seq10:      ----------------------------------------------------------CGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-87bp)
Seq11:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTCGTGGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-4,+4bp)
Seq12:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTCGTGGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-4,+4bp)
Seq13:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCCTCGTGGGGCGCGCCAGGTCGGACAGCGGCGTCATC(-4,+4bp)
Seq14:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACGACTTCC-------------AGGTCGGACAGCGGCGTCATC(-14bp)
Seq15:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGG--------------------CGCGCCAGGTCGGACAGCGGCGTCATC(-22bp)
Seq16:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGGGGTCAACG------------------------GACAGCGGCGTCATC(-26bp)
Seq17:      GGAACACAGTGGCGGCACCTCGCGTCGCTCCTCGACGGG----------------------------TCGGACAGCGGCGTCATC(-31bp)
```

Figure S5. The sequences of the target region of *gol* in the lamprey larvae injected with Cas9 and *gol* gRNA. **A**, The sequences of the target region of *gol* in the injected lamprey larvae with no pigment in the retinas; **B**, The sequences of the target region of *gol* in the injected lamprey larvae with mosaic pigment in the retinas; **C**, The sequences of the target region of *gol* in the injected lamprey larvae with normal pigment in the retinas. The target sites are labeled with box, the PAM sequences are highlighted with yellow and insertions are highlighted with red.

Figure S6.

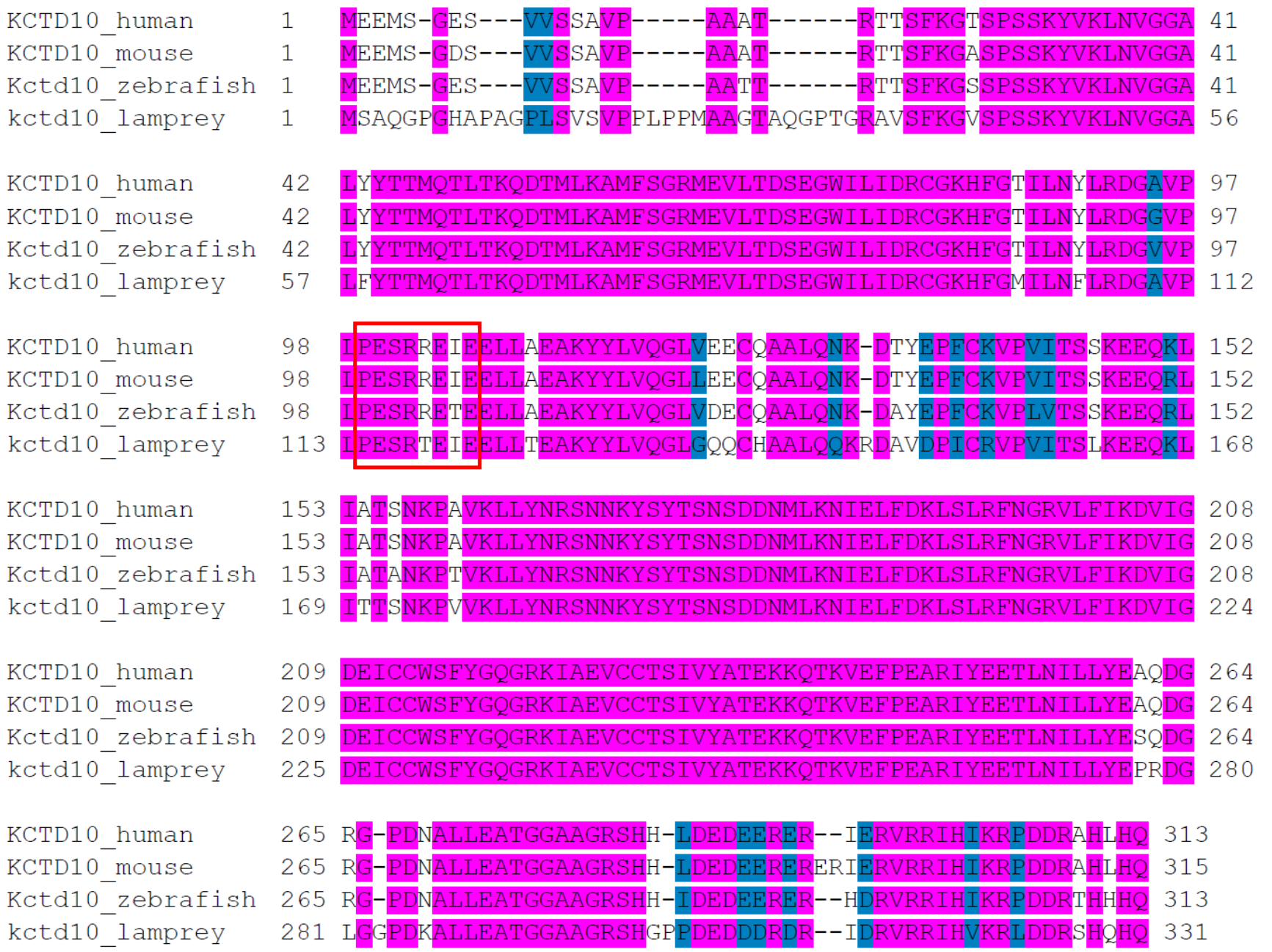

Figure S6. The alignment of the sequences of Kctd10 (encoded by the gene of *kctd10*) among human, mouse, zebrafish and lamprey. The target site of Cas9-gRNA is labeled with the red box.

Figure S7.

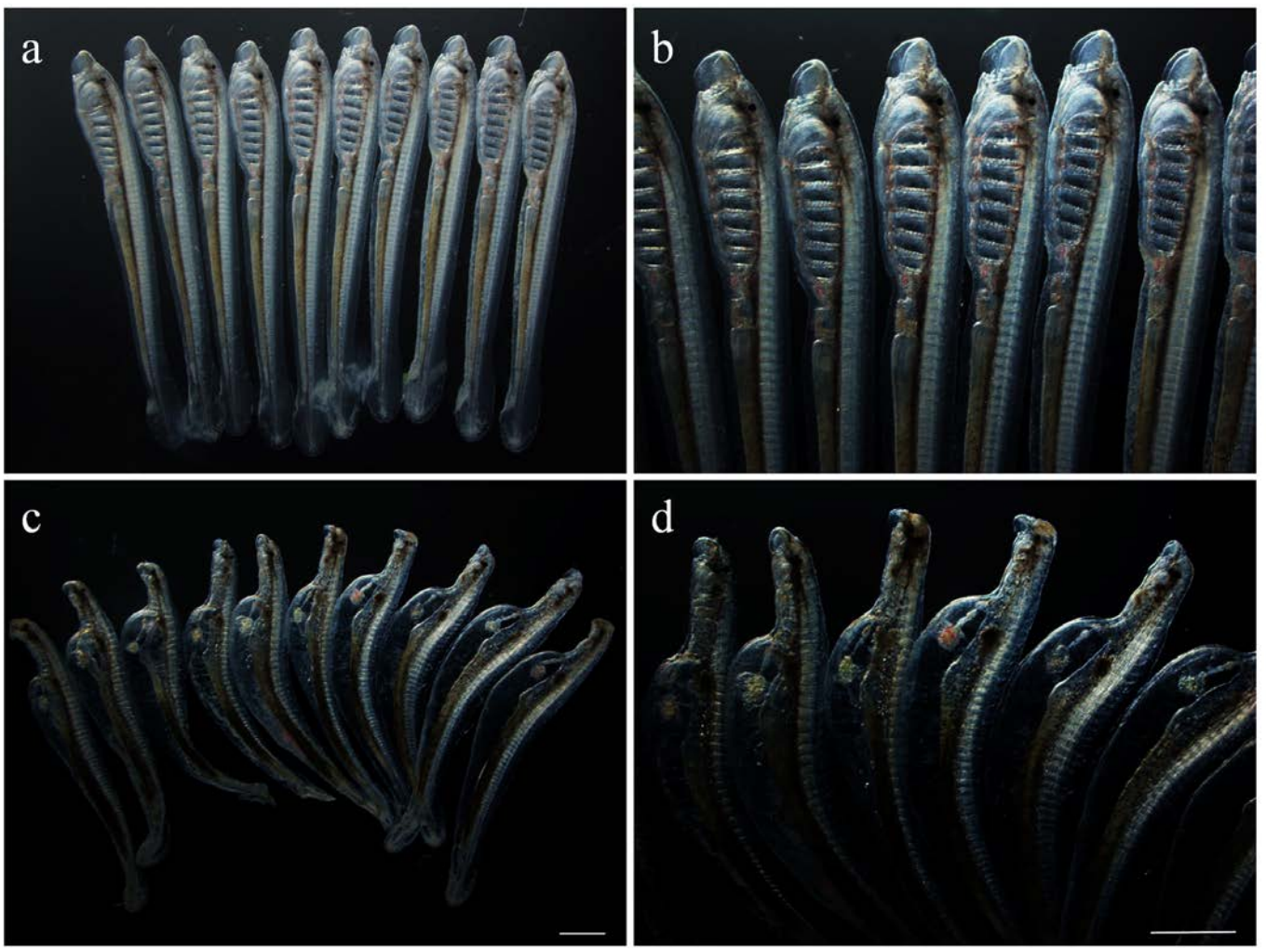

Figure S7. Injection of Cas9 and gRNA against *kctd10* resulting in heart malformations in lamprey larvae caused by biallelic disruption. Lateral views of control lamprey larvae (**a**, **b**) and *kctd10* gRNA injected larvae (**c**, **d**) at 21 dpf. The injected larvae showed severe pericardial edema. **d**, Enlarged view of **c** showed the marked heart malformations in the injected larvae. (Scale bars: 1mm).

Figure S8.

A

H1

```
Reference   ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC
Seq1:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC（-6bp）
Seq2:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGA------AGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq3:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq4:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG---------CTGCTCACAGAGGCCAAGTACTACC(-9bp)
Seq5:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG---------CTGCTCACAGAGGCCAAGTACTACC(-9bp)
Seq6:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTC-----------------TGCTCACAGAGGCCAAGTACTACC(-18bp)
Seq7:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGC-----------------------------------TCACAGAGGCCAAGTACTACC(-36bp)
Seq8:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCAC--------------------AGAGGCCAAGTACTACC(-21bp)
Seq9:       ATCCTCAA------G-----------------------------------------GAGGAGCTGCTCACAGAGGCCAAGTACTACC(-48bp)
Seq10:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTC----------------------ACAGAGGCCAAGTACTACC(-23bp)
Seq11:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTC----------------------ACAGAGGCCAAGTACTACC(-23bp)
Seq12:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTC----------------------ACAGAGGCCAAGTACTACC(-23bp)
Seq13:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGC-------------------------------------------------------TACC(-53bp)
Seq14:      ATCCTCAACTTC-----------------------------------------------------TCACAGAGGCCAAGTACTACC(-55bp)
Seq15:      ----------------------------------------------------------AGGAGCTGCTCACAGAGGCCAAGTACTACC(-149bp)
Seq16:      ----------------------------------------------------------GAGGAGCTGCTCACAGAGGCCAAGTACTACC(-187bp)
Seq17:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq18:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq19:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq20:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
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H2

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Reference   ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC
Seq1:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq2:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq3:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq4:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCA------TCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq5:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq6:       ATCCTCAACTTCATGCGCGACGGCGCCATCCCCCAGCCGGAGTCTGGCAC------CGAGGAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq7:       ATCCTCAACTTCCTGCACGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq8:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGT--------------------TGCTCACAGAGGCCAAGTACTACC(-21bp)
Seq9:       ATCCTC-----------------------------------------------GAGGAGCTGCTCACAGAGGCCAAGTACTACC(-51bp)
Seq10:      ATCCTCAA-------------------------------------------------------------GGCCAAGTACTACC(-66bp)
Seq11:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGA--GAGGAGCTGCTCACAGAGGCCAAGTACTACC(-2bp)
Seq12:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCAC-----T-GAGGAGCTGCTCACAGAGGCCAAGTACTACC(-5bp)
Seq13:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCG----------AGGAGCTGCTCACAGAGGCCAAGTACTACC(-11bp)
Seq14:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCG----------AGGAGCTGCTCACAGAGGCCAAGTACTACC(-11bp)
Seq15:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCG-----------------AGGAGCTGCTCACAGAGGCCAAGTACTACC（-19bp）
Seq16:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCT-------------------GCTCACAGAGGCCAAGTACTACC(-20bp)
Seq17:      ---------------------------------------------------------------TGCTCACAGAGGCCAAGTACTACC(-67bp)
Seq18:      ----------------------------------------------------------GAGGAGCTGCTCACAGAGGCCAAGTACTACC(-227bp)
Seq19:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
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H3

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Reference   ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC
Seq1:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC（-6bp）
Seq2:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC（-6bp）
Seq3:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC（-6bp）
Seq4:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC（-6bp）
Seq5:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC（-6bp）
Seq6:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq7:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq8:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGCAGCTCCTCGAGGAGCTGCTCACAGAGGCCAAGTA（+7，-1bp）
Seq9:       ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCT----------CGAGGAGCTGCTCACAGAGGCCAAGTACTACC(-11bp)
Seq10:      ATCCTCAACTTCCTGCGCGACGGCGCC----------------------------------------------------------(-97bp)
Seq11:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGAATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC（+1bp）
Seq12:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGAATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC（+1bp）
Seq13:      ATCCTCAACTTCCTGCGCGACGGCGCCGCGCCCCTGCCGGAGTCTCGCACCGAGAGTCGAGGAGCTGCTCACAGAGGCCAAGTACTACC（+1bp）
Seq14:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCCAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
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Seq15: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq16: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq17: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)

B

W1

Reference ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC

Seq1: ATCCTCAACTTCGTGCGCGCCGGCGCCGTGCCCCAGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAGGTACTACC(-6bp)
Seq2: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq3: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq4: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTC------GCCAAGTACTACC(-6bp)
Seq5: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGAT---------GCTCACAGAGGCCAAGTACTACC(-9bp)
Seq6: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAG----------------GAGCTGCTCACAGAGGCCAAGTACTACC(-18bp)
Seq7: ATCCTCAACTTCCTGCGC---ACAGAGGCCAAGTACTACC(-51bp)
Seq8: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTC------------------(-19bp)
Seq9: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGC------------------TCACAGAGGCCAAGTACTACC(-19bp)
Seq10: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCAC--------------------------------TACC(-34bp)
Seq11: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq12: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq13: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq14: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTAGTACC(wt)
Seq15: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACc(wt)
Seq16: ATCCTCAACTTCCTGC**A**CGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq17: ATCCTCAACTTCATGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq18: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)

W2

Reference ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC

Seq1: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCAC------CGAGGAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq2: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq3: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq4: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCA------TCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(-6bp)
Seq5: ----------GAGGAGCTGCTCACAGAGGCCAAGTACTACC(-81bp)
Seq6: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCA----------GGAGCTGCTCACAGAGGCCAAGTACTACC(-10bp)
Seq7: ATCCTCAACTTCGTGCGGGACGGCGCC----------CGAGGAGCTGCTCACAGAGGCCAAGTACTACC(-29bp)
Seq8: ATCCTCAACTTCCTGCGCGACGGCGCCGT----------ACTACC(-53bp)
Seq9: ----------GGAGCTGCTCACAGAGGCCAAGTACTACC(-71,+1bp)
Seq10: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGAGACTCCGGCAGAGGAGCTGCTCACAGAGGCCAA(-2,+10bp)
Seq11: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGAGACTCCGGCAGAGGAGCTGCTCACAGAGGCCAA(-2,+10bp)
Seq12: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq13: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq14: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq15: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq16: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq17: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq18: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq19: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGA**C**CGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq20: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)

W3

Reference ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC

Seq1: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG------GAGCTGCTCACTGAGGCCAAGTACTACC(-6bp)
Seq2: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAG---------CTGCTCACAGAGGCCAAGTACTACC(-9bp)
Seq3: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGT-----------TCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(-12bp)
Seq4: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCT--------------------CGAGGAGCTGCTCACAGAGGCCAAGTACTACC（-21bp）
Seq5: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCG----------AGGAGCTGCTCACAGAGGCCAAGTACTACC(-11bp)
Seq6: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGAGTCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(+1bp)
Seq7: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq8: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq9: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq10: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq11: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq12: ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)

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Seq13:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq14:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq15:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq16:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq17:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq18:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
Seq19:      ATCCTCAACTTCCTGCGCGACGGCGCCGTGCCCCTGCCGGAGTCTCGCACCGAGATCGAGGAGCTGCTCACAGAGGCCAAGTACTACC(wt)
```

Figure S8. The sequences of the target region of *kctd10* in the lamprey larvae injected with Cas9 and *kctd10* gRNA. **A**, The sequences of the target region of *kctd10* in the injected lamprey larvae with heart malformations; **B**, The sequences of the target region of *kctd10* in the injected lamprey larvae with normal phenotypes. The target sites are labeled with box, the PAM sequences are highlighted with yellow and insertions are highlighted with red.

Figure S9.

A

wee1 No.1

```
Reference   TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCTTGACACCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG
Seq1:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCT-----------CGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-12bp)
Seq2:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCT--------------------CGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-21bp)
Seq3:       TCCCGACCGAGAGGCCTCAAGCCTCGGCC-------------------------------------------------AGGCCACG (-51bp)
Seq4:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCC-----ACCTCCGCCGGGCGCCTCGCACCGAGGCCGGCGGGTCGTCCAGGCCACG (-5bp)
Seq5:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTC--------ACCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-8bp)
Seq6:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCC-------------GGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-14bp)
Seq7:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCC-------------GGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-14bp)
Seq8:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCC-------------GGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-14bp)
Seq9:       TCCCGACCGAGAGGCCTC------------------------CGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-26bp)
Seq10:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCTTGA-------G------------------GCCGGCGGGTCGTCCAGGCCACG (-26bp)
Seq11:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGC------------------------ACCGAAGCCGGTGGGTCGTCCAGGCCACG (-26bp)
Seq12:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGC------------------------ACCGAAGCCGGCGGGTCGTCCAGGCCACG (-26bp)
Seq13:      TCCCGACCGAGAGGCCTC------------------------CGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-26bp)
Seq14:      TCCCGACCGAGAGGCCTCAAGCCTCG-------------------------------CACCGAAGCCGGCGGGTCGTCCAGGCCACG (-32bp)
Seq15:      TCCCGACC---------------------------------TCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-34bp)
Seq16:      TCCCGACC---------------------------------TCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-34bp)
Seq17:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGGCCT---CACCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCAC (+1, -3bp)
Seq18:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGGCCT---CACCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCAC (+1, -3bp)
Seq19:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCTTGAGGCGAG-GCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (+6, -7bp)
Seq20:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCTTGACACCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (wt)
```

wee1 No.2

```
Reference   TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCTTGACACCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG
Seq1:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCT---------ACCTCCGCCGGGCGCCTTGCTCGGTAGCCGGCTGGTCGTCCAGGCCCCG (-9bp)
Seq2:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCT--------------------CGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-21bp)
Seq3:       TCCCGACCGAGAGGCCTC-------------------------------------GCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-39bp)
Seq4:       TCCCGACCGAGAGGCCTC-------------------------------------GCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-39bp)
Seq5:       TCCCGGCCGAGAGGCCTCAAGCCTCGGCCTCGCC-----ACCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-5bp)
Seq6:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCC-----ACCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-5bp)
Seq7:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCC-----ACCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-5bp)
Seq8:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCT--------CCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-8bp)
Seq9:       TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCT--------CCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-8bp)
Seq10:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCT--------CCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-8bp)
Seq11:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCT--------CCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-8bp)
Seq12:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCC-------------GGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-14bp)
Seq13:      TCCCGACCGAGGGGCCTCAAG-----------------CCTCCGCCGGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-19bp)
Seq14:      TCCCGACCGAGAGGCCTCAAGCC------------------------GGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-25bp)
Seq15:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGC------------------------ACCGAAGCCGGGGGGTCGTCCAGGCCACG (-26bp)
Seq16:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGCCTTGA------------------------AGCCGGCGGGTCGTCCAGGCCACG (-26bp)
Seq17:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGC------------------------ACCGAAGCCGGCGGGTCGTCCAGGCCACG (-26bp)
Seq18:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGC------------------------ACCGAAGCCGGCGGGTCGTCCAGGCCACG (-26bp)
Seq19:      TCCCGACCGAGAGGCC-----------------------------GGGCGCCTCGCACCGAAGCCGGCGGGTCGTCCAGGCCACG (-32bp)
Seq20:      TCCCGACCGAGAGGCCTCAAGCCTCGGCCTCGC---------------------------------------------------- (-61bp)
```

B

soxe2 No.1

```
Reference   GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTGAGTCAAGTGCTGGACGGCTACGACTGGTCGCTGTTGCCCGTGCCC
Seq1:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTGAGTCA----C-GGCCGCCTG-GACTGGTCGCTGTTGCCCGTGCCC (-6bp)
Seq2:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGT-------------------------------------------(-60bp)
Seq3:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTGAG----------GACGGCTACGACTGGTCGCTGTTGCCCGTGCCC (-10bp)
Seq4:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------------------TACGACTGGTCGCTGTTGCCCGTGCCC (-22bp)
Seq5:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------------------TACGACTGGTCGCTGTTGCCCGTGCCC (-22bp)
Seq6:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------------------TACGACTGGTCGCTGTTGCCCGTGCCC (-22bp)
Seq7:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------------------TACGACTGGTCGCTGTTGCCCGTGCCC (-22bp)
Seq8:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------------------TACGACTGGTCGCTGTTGCCCGTGCCC (-22bp)
Seq9:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------------------TACGACTGGTCGCTGTTGCCCGTGCCC (-22bp)
Seq10:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------------------TACGACTGGTCGCTGTTGCCCGTGCCC (-22bp)
```

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Seq11:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------TACGACTGGTCGCTGTTGCCCGTGCCC（-22bp）
Seq12:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------TACGACTGGTCGCTGTTGCCCGTGCCC（-22bp）
Seq13:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------TACGACTGGTCGCTGTTGCCCGTGCCC（-22bp）
Seq14:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------TACGACTGGTCGCTGTTGCCCGTGCCC（-22bp）
Seq15:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGC----------TGGTCGCTGTTGCCCGTGCCC（-28bp）
Seq16:      GAGGCACCGACGA----------CTGGACGGCTACGACTGGTCGCTGTTGCCCGTGCCC（-40bp）
Seq17:      GAGGCACCGACGA----------CTGGACGGCTACGACTGGTCGCTGTTGCCCGTGCCC（-40bp）
Seq18:      GAGGCACCGACGACCCCTTCTCG----------TGCCC（-61bp）
Seq19:      GAGGCACCGACGACCCCTTCTCG----------TGCCC（-61bp）
```

soxe2 No.2

```
Reference   GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTGAGTCAAGTGCTGGACGGCTACGACTGGTCGCTGTTGCCCGTGC
Seq1:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTG---------CTGGACGGCTACGACTGGTCGCTGTTGCCCGTGC（-9bp）
Seq2:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTG-GACGGCTAC--GACGGCTACGACTGGTCGCTGTTGCCCGTGC(-12,+9bp)
Seq3:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTG-GACGGCTAC--GACGGCTACGACTGGTCGCTGTTGCCCGTGC(-12,+9)
Seq4:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTG-GACGGCTAC--GACGGCTACGACTGGTCGCTGTTGCCCGTGC(-12,+9)
Seq5:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTG-GACGGCTAC--GACGGCTACGACTGGTCGCTGTTGCCCGTGC(-12,+9)
Seq6:       GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTG-----------GACGGCTACGACTGGTCGCTGTTGCCCGTGC（-12bp）
Seq7:       GAGGCACCGACGACCCCTTCTCGGAGAGCA----------ACTGGTCGCTGTTGCCCGTGC（-36bp）
Seq8:       GAGGCACCGACGACCCCTTCTCGGAGAGCA----------ACTGGTCGCTGTTGCCCGTGC（-36bp）
Seq9:       ----------GCTGGACGGCTACGACTGGTCGCTGTTGCCCGTGC(-66bp)
Seq10:      ----------GCTGGACGGCTACGACTGGTCGCTGC（-66bp）
Seq11:      ----------GCTGGACGGCTACGACTGGTCGCTGC（-66bp）
Seq12:      ----------GCTGGACGGCTACGACTGGTCGCTGC(-66bp)
Seq13:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTGA----------CGGCTACGACTGGTCGCTGTTGCCCGTGC（-13bp）
Seq14:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGG----------TCGCTGTTGCCCGTGC（-35bp）
Seq15:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGG----------TCGCTGTTGCCCGTGC（-35bp）
Seq16:      GAGGCACCGACGACCCCTTCTCGGAGAGCATCCAGG----------TCGCTGTTGCCCGTGC（-35bp）
Seq17:      GAGGCACCGACGACCCCCTCTCGGAGAGCATCCAGG----------TCGCTGTTGCCCGTGC（-35bp）
Seq18:      GAGGCGCCGACGACCCCTTCTCGGAGAGCATCCAGGCGGCCGTGAGTCAAGTGCTGGACGGCTACGACTGGTCGCTGTTGCCCGTGC
```

C

wnt7b No.1

```
Reference   GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTTACATGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC
Seq1:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCC------TGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-6bp）
Seq2:       GTACGAGCACACGGT----------CCTCAAGGTGAAGAAGTCGCGCTCGTAC（-45bp）
Seq3:       GTACGAGCACACGGTGCACGTG----------AAGAAGTCGCGCTCGTAC（-48bp）
Seq4:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGC----------GGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-10bp）
Seq5:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG----------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq6:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG----------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq7:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGC----------GGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq8:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGC----------GGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq9:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG----------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq10:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG----------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq11:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG----------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq12:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTT----------CCTCAAGGTGAAGAAGTCGCGCTCGTAC（-16bp）
Seq13:      GTACGAGCACACGGTGCACGTGGAGCCGGCGAGCGGCAGCC----------GTTCCTCAAGGTGAAGAAGCCGCGCTCGTAC（-16bp）
Seq14:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAG---------GC-------GTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-16bp）
Seq15:      GTACGAGCACACGGTGCACGTGGAGCC----------TCAAGGTGAAGAAGTCGCGCTCGTAC（-35bp）
Seq16:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTTACCTGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC
Seq17:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTTACCTGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC
Seq18:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTTACCTGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC
Seq19:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTTACCTGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC
Seq20:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTTACCTGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC
Seq21:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTTACCTGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC
```

wnt7b No.2

```
Reference   GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTTACATGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC
Seq1:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCC------TGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-6bp）
Seq2:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG---------GCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-9bp）
Seq3:       GTACGAGCACACGGT----------CCTCAAGGTGAAGAAGTCGCGCTCGTAC（-45bp）
Seq4:       GTACGAGCACACGGTGCACGTGGAGCCG----------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-27bp）
```

```
Seq5:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG------------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq6:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG------------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq7:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG------------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq8:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG------------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq9:       GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG------------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq10:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG------------ATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-13bp）
Seq11:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGC---------------CGATGTTCCTCAAGGTGAAGAAGCCGCGCTCGTAC（-16bp）
Seq12:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGC---------------CGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-16bp）
Seq13:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTT---------------CCTCAAGGTGAAGAAGTCGCGCTCGTAC（-16bp）
Seq14:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCGTT---------------CCTCAAGGTGAAGAAGTCGCGCTCGTAC（-16bp）
Seq15:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGC---------------CGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-16bp）
Seq16:      GTACGAGCACACGGTGCACGTGGAGCC-------------------TGCGGCCGATGTTCCTCAAGGTGAAGAAGTCGCGCTCGTAC（-20bp）
Seq17:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG-----------------------GTGAAGAAGTCGCGCTCGTAC（-25bp）
Seq18:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG-----------------------GTGAAGAAGTCGCGCTCGTAC（-25bp）
Seq19:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGCCG-----------------------GTGAAGAAGTCGCGCTCGTAC（-25bp）
Seq20:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAGCGGCAGC----A--------------------GTGAAGAAGTCGCGCTCGTAC（-26bp）
Seq21:      GTACGAGCACACGGTGCACGTGGAGCCGGTGAG-------------------------------------AAGTCGCGCTCGTAC（-40bp）
```

Figure S9. The sequences of the target region of three genes in the lamprey larvae injected with *Cas9* mRNA and the according gRNA. **A**, The sequences of the target region of *wee1* in two injected lamprey larvae; **B**, The sequences of the target region of *soxe2* in two injected lamprey larvae; **C**, The sequences of the target region of *wnt7b* in two injected lamprey larvae. The target sites are labeled with box, the PAM sequences are highlighted with yellow and insertions are highlighted with red.

Figure S10.

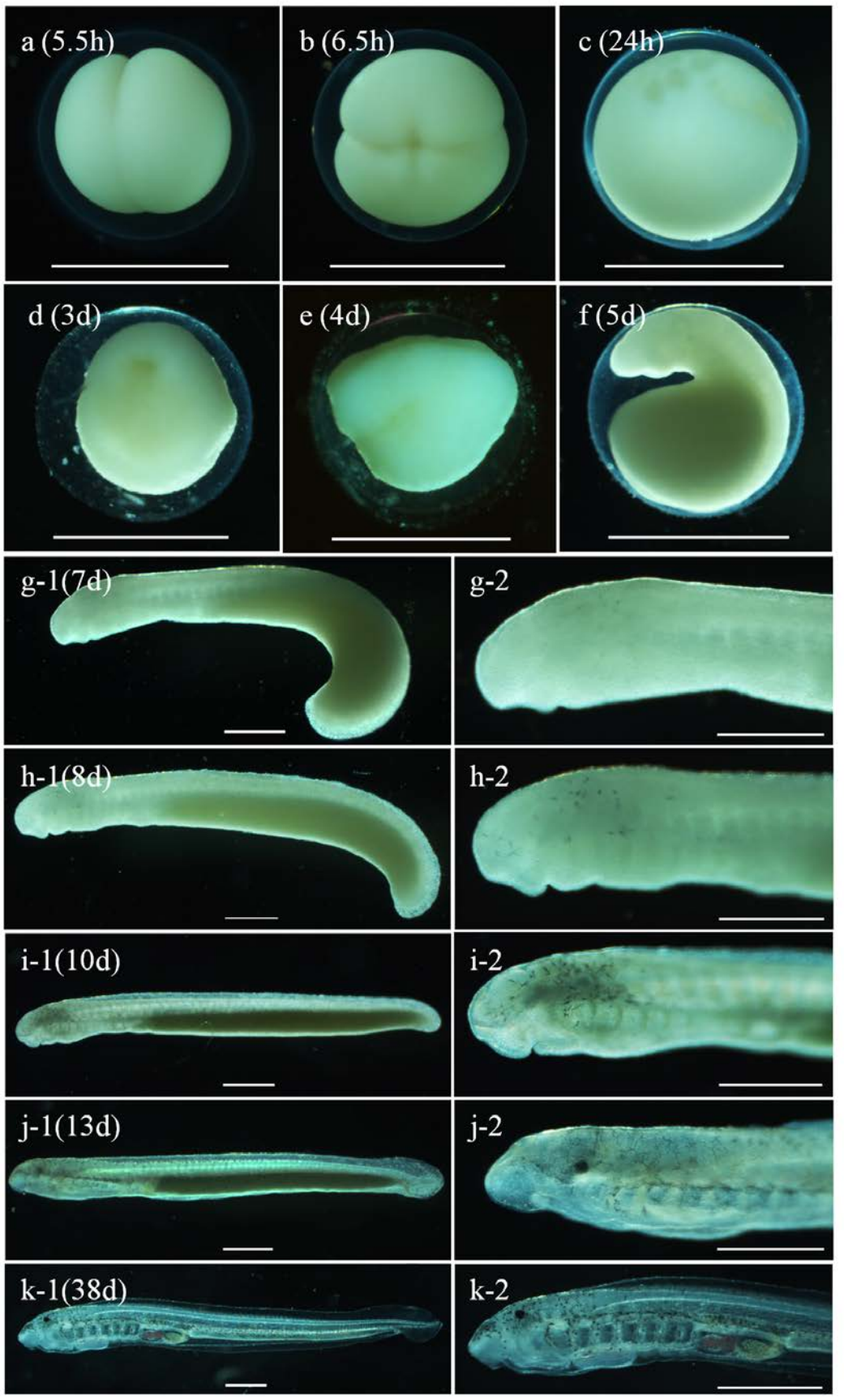

Figure S10. The development of lamprey embryos. **a**, two-cell stage, **b**, four-cell stage, **c**, blastula, **d**, gastula, **e**, neurula, **f**, lateral view of head protrusion, **g-1**, hatching, **g-2**, enlargement of g-1, **h-1**, gill pore, **h-2**, enlargement of h-1, **i-1**, eye spots, **i-2**, enlargement of i-1, **j-1**, oral hood, **j-2** enlargement of j-1. **k-1**, completion of digestive tract, **k-2**, enlargement of k-1.a, blastopore groove; b, neural fold; c, head protruded; d, melanophore; e, gill pore; f, eye spots ; g, dorsal fin; h, digestive tract. (Scale bars: 1 mm)

Table S1. The fraction of usage of each codon in the sea lamprey

Amino acid	Codon	Fraction	Amino acid	Codon	Fraction	Amino acid	Codon	Fraction	Amino acid	Codon	Fraction
Phe	TTT	0.31	Ser	TCT	0.11	Tyr	TAT	0.2	Cys	TGT	0.27
	TTC	0.69		TCC	0.22		TAC	0.8		TGC	0.73
Leu	TTA	0.03		TCA	0.1	*	UAA	-	*	UGA	-
	TTG	0.11		TCG	0.19		UAG	-	Trp	TGG	1
	CTT	0.09	Pro	CCT	0.16	His	CAT	0.23	Arg	CGT	0.11
	CTC	0.29		CCC	0.35		CAC	0.77		CGA	0.1
	CTA	0.04		CCA	0.21	Gln	CAA	0.21		CGC	0.34
	CTG	0.45		CCG	0.29		CAG	0.79		CGG	0.2
Ile	ATT	0.24	Thr	ACT	0.13	Asn	AAT	0.28	Ser	AGT	0.09
	ATC	0.64		ACC	0.33		AAC	0.72		AGC	0.3
	ATA	0.12		ACA	0.18	Lys	AAA	0.28	Arg	AGA	0.09
Met	ATG	1		ACG	0.36		AAG	0.72		AGG	0.16
Val	GTT	0.12	Ala	GCT	0.16	Asp	GAT	0.3	Gly	GGT	0.14
	GTC	0.26		GCC	0.39		GAC	0.7		GGC	0.44
	GTA	0.07		GCA	0.17	Glu	GAA	0.24		GGA	0.19
	GTG	0.55		GCG	0.29		GAG	0.76		GGG	0.23

Table S2. The target sites of five endogenous genes of lamprey

target gene	Target site*	PCR primers for amplification of the target region
wee1	CCTTGACACCTCCGCCGGGCGCC	F: CCTCACGGTGGCAAAAGTACAACGTGGG R: CTTCACGGGCGACGGAGACTCCATCGACG
soxe2	GGCGGCCGTGAGTCAAGTGCTGG	F: TGTCGGATATCAATCACGAG R: GGGTATTGGTCGGACAGCTT
kctd10	GGAGTCTCGCACCGAGATCGAGG	F: ACATCCAGACTCGTGTTCCTT R: GAAATACGCGATGATACCTGC
wnt7b	CCGTTACCTGCGGCCGATGTTCC	F: TTATCGCCGTTTCCACTCCA R: TACTGGTGCGTGTTGTAGCC
golden	GGGTCAACGACTTCCTGCACGGG	F: CTCTCGCTCGTGTCCGTCT R: CCGTGCAGGCTGAGCGTCAACTGCG

* The sequence underlined is the PAM region. The sequence highlighted is the sequence required for T7 RNA polymerase starting site

Table S3. The ratio of the larvae with hypopigmentation in the retinas when injected with Cas9 and *gol* gRNA in zebrafish

injection	Total injected larvae	Normal pigment	No pigment	Mosaic pigment	Mutants
control	100	100	0 (0%)	0 (0%)	0
ZCas9 + *gol* gRNA	46	4	4 (8.7%)	38 (82.6%)	91.3%