A novel inducible mutagenesis screen enables to isolate and clone both embryonic and adult zebrafish mutants

Zhipeng Ma¹a, Peipei Zhu¹a, Meijun Pang³a, Liwei Guo¹, Nannan Chang³, Jiyuan Zheng³, Xiaojun Zhu¹, Ce Gao², Honghui Huang⁵, Zongbin Cui⁴, Jing-Wei Xiong³*, Jinrong Peng²*, Jun Chen¹*

¹Key laboratory for Molecular Animal Nutrition, Ministry of Education, Innovation Center for Signaling Network, College of Life Sciences, Zhejiang University, 866 Yu Hang Tang Road, Hangzhou, China 310058; ²College of Animal Sciences, Zhejiang University, 866 Yu Hang Tang Road, Hangzhou, China 310058; ³Institute of Molecular Medicine, Beijing Key Laboratory of Cardiometabolic Molecular Medicine, and State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, China 100871; ⁴Key Laboratory of Aquatic Biodiversity and Conservation, Institute of Hydrobiology, Chinese Academy of Sciences, 8 Dong Hu Nan Road, Wuhan, Hubei, P. R. China 430072; ⁵Key Laboratory of Freshwater Fish Reproduction and Development, Ministry of Education, State Key Laboratory Breeding Base of Eco-Environments and Bio-Resources of the Three Gorges Reservoir Region, School of Life Sciences, Southwest University, 2 Tiansheng Road, Beibei, Chongqing, China 400715.

These authors contributed equally to this work.

Author Information: The authors declare no competing financial interest.

Correspondence and requests for materials should be addressed to J.C (chenjun2009@zju.edu.cn) or J.R.P (pengjr@zju.edu.cn) or J.W.X (jingwei_xiong@pku.edu.cn).
Supplementary information
Supplementary Figures
Supplementary figure 1
The first generation of pIDM

|                          | Total No. of F0 | No. of F0 with fluorescent progenies | Frequency of F0 with fluorescent progenies |
|--------------------------|----------------|--------------------------------------|--------------------------------------------|
| pIDM without insulators  | 331            | 84                                   | 25.4%                                      |
| pIDM with insulators     | 700            | 300                                  | 43%                                        |

C

pIDM line without insulators

pIDM line with insulators

D

E

Type 1 without insulators

Type 2 with insulators

Supplementary figure 2
Supplementary figure 3
A

$p\text{IDM-A11 line 3.5 dpf}$

|        | Ctr | Dox |
|--------|-----|-----|
| 72/72  | 164/350 |

B

$faf1$

| WT Ctr at 2 dpf | pIDM-A11 Dox at 2 dpf |
|-----------------|-----------------------|
| 78/78           | 25/49                 |

| WT Dox at 2 dpf | pIDM-A11 Dox at 2 dpf |
|-----------------|-----------------------|
| 62/62           | 18/49                 |

| pIDM-A11 Ctr at 2 dpf | pIDM-A11 Dox at 2 dpf |
|-----------------------|-----------------------|
| 75/75                 | 6/49                  |

C

|        | WT       | pIDM-A11 |
|--------|----------|----------|
|        | Ctr      | Dox      |
|        | DNA fragment amplified from anti-sense $faf1$ transcript | $eta$-actin |

$Supplementary figure 4$
A. pIDM-A200/δ-sgcd X1/sgcd

B. Relative expression of sgcd and X1 at 36 hpf (fold).

C. pIDM-A200 line

D. DNA fragment amplified from anti-sense sgcd transcript

Supplementary figure 5
A

pIDM-E17/ ap2b1

B

Relative expression of ap2b1 at 48 hpf (Fold)

WT Ctr  
plDM-E17 Ctr  
plDM-E17 Dox

C

Ctr 36 hpf  
Dox 36 hpf

pIDM-E17 line

37/37

D

WT  
pIDM-E17

Ctr  
Dox  
Ctr  
Dox

DNA fragment amplified from anti-sense ap2b1 transcript

β-actin

Supplementary figure 6
**A**

WT Ctr 24 hpf

WT Dox 24 hpf

E10 Ctr 24 hpf

E10 Dox 24 hpf

*grb14*

36/36

28/28

28/31

3/31

**B**

|       | WT Ctr | Dox Ctr | pMDM-E10 Ctr | pMDM-E10 Dox |
|-------|--------|---------|--------------|--------------|
| 1     | 2      | 3       | 4            | 5            |
| 6     | 7      | 8       | 9            | 10           |
| 11    | 12     |         |              |              |

DNA fragment amplified from anti-sense *grb14* transcript

|       | WT Ctr | Dox Ctr | pMDM-E10 Ctr | pMDM-E10 Dox |
|-------|--------|---------|--------------|--------------|
| 1     | 2      | 3       | 4            | 5            |
| 6     | 7      | 8       | 9            | 10           |
| 11    | 12     |         |              |              |

β-actin

**C**

Supplementary figure 7
Supplementary figure 8

|          | WT       | plDM-A1   |
|----------|----------|-----------|
|          | Ctr  | Dox | Ctr | Dox |
| 1        | 2    | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |

DNA fragment amplified from anti-sense *nid2a* transcript

|          | WT Ctr | WT SC-MO | WT *nid2a*-MO |
|----------|--------|----------|---------------|
|          | 1      | 2        | 3             | 4   | 5   | 6   | 7   | 8   | 9   |

*nid2a*

|          | 1      | 2        | 3             | 4   | 5   | 6   | 7   | 8   | 9   |

β-actin
**A**

pIDM-E3/pcyt1aa

![Diagram showing ~1.0 kb region with Ctr and Dox conditions]

---

**B**

Relative expression of pcyt1aa at 48 hpf (fold)

| Condition       | Relative Expression |
|-----------------|---------------------|
| WT Ctr          | 1                   |
| WT Dox          | 0.8                 |
| pIDM-E3 Ctr     | 1.2                 |
| pIDM-E3 Dox     | 1.4                 |

N.S, **p < 0.01**

---

**C**

Images showing Ctr 3 dpf and Dox 3 dpf conditions with measurements 319 μm, 239 μm, 238 μm.

---

**D**

Images showing WT Ctr, WT Dox, pIDM-E3 Ctr, and pIDM-E3 Dox conditions at 24 hpf. Relative sizes: 48/48, 28/46, 50/50, 15/46, 46/46, 3/46.

---

**E**

Images showing WT, pIDM-E3, and pcyt1aa-MO conditions at 2.5 dpf. Relative sizes: 85/85, 54/78, 64/105.

---

**F**

Gel image showingMarker, WT Ctr, SC-MO, pcyt1aa-MO. 373 bp abnormal transcript.

---

**Supplementary figure 9**
Injection Ctr

WT at 24 hpf

A

B

Injection with normal development

C

D

10 pg/PE dsRNA-injection with moderate phenotypes

E

F

10 pg/PE dsRNA-injection with severe phenotypes

10 pg/PE dsRNA-injection

G

Supplementary figure 10
Supplementary figure 11
A

\( nid2a \)

\[ \text{exon1} \rightarrow \text{exon2} \rightarrow \text{exon3} \rightarrow \text{exon24} \]

WT: GGAAAAGATCCATCTACTACCGG
Mutation: GGAAAAGATCCATCTACTACCGG

B

4 dpf

WT

Nid2a\(^{-/-}\)

C

Body length (cm)

Genotype: \( nid2a^{+/-} \), \( nid2a^{-/-} \), \( nid2a^{-/-} \)

Supplementary figure 12
Supplementary figure 13
Supplementary figure 14-1
Supplementary figure 14-2
Supplementary figure 15
A pair of galnt2 and insertion primers

A

800 bp

8k

6k

plDM-A28

plDM-A28

A pair of ggt71 and insertion primers

B

plDM-A3

plDM-A3

A pair of cry61 and insertion primers

C

plDM-E7

plDM-E7

WT

1 dpa

2 dpa

3 dpa

4 dpa

5 dpa

6 dpa

galnt2

ggt71

cry61

Supplementary figure 17
Supplemental Figure Legends

**Supplementary Figure 1** The expression of HA-DsRed was induced by Dox treatment in the transgenic embryonic and adult fish harboring the modified *pIDM:HA-DsRed* transgene. (A) Transgenic zebrafish were generated as described in Figure 1B. Transgenic embryos were treated with Dox at 12 hpf. The treated and untreated embryos at 2 dpf were fixed with 4% PFA for 1 hour and then subjected to cryo-sections. The photo was taken with a fluorescent microscope. (B) Three-month-old transgenic fish were treated with Dox. The picture was taken under a confocal microscope at 15 dpt. Tg\(^{+/−}\), heterozygous transgenic fish; Ctr, untreated control.

**Supplementary Figure 2** Improvement of the pIDM system by including the combination of a pair of insulators. (A) Diagram of the first generation pIDM vector. The abbreviations for each item in the first generation pIDM construct are the same as those in the improved pIDM construct (Figure 1A), except where it lacks the pair of insulators. (B) Frequencies of F\(_0\) founder fish with visible fluorescent F1 progenies. F\(_0\) transgenic founder fish were generated with either the first generation or improved pIDM constructs. F\(_1\) population containing visible green fluorescent embryos was considered to be positive transgenic lines. (C) The line on the left presented most transgenic lines generated with the first generation pIDM and the line on the right presented most transgenic lines generated with the improved pIDM. The picture was taken at 36 hpf. Red arrow, embryo without visible fluorescence. (D) Identification of transgene by PCR using *rtTA*-transgenic-fish-ID-for and *rtTA*-transgenic-fish-ID-rev primer pairs in F1 transgenic embryos generated with the first generation pIDM. Genomic DNA was extracted from WT embryos and F1 embryos either presence or absence of fluorescence. The plasmid DNA of pIDM (plasmid) was used as a positive control. (E) The inclusion of the insulator in pIDM sharply decreased the leakage of the Tet-on promoter. Upper panel: Diagram showing two types of vectors under analysis, either without or with a pair of insulators. In this construct, we used DsRed as the reporter gene and *Egfp* as the inducible
Lower panels: Western blot analysis of EGFP. The plasmid was injected into WT embryos at the one-cell stage. The injected embryos were treated with Dox at 12 hpf. Total protein was extracted at 8, 18 or 24 hpt. An EGFP antibody was used to detect EGFP protein. β-actin was used as the protein loading control.

Supplementary Figure 3 Analysis of transgenic lines harboring the improved pIDM. (B) Southern blot analysis of insertion copy numbers in transgenic lines. Genomic DNA was extracted from pooled F₁ embryos of each line (a total of eleven lines examined, lane 1-11) and digested with the restriction enzyme EcoRV, which is located at the β-actin promoter. Number in the diagram, the position of EcoRV at the β-actin promoter. A digoxin (DIG) labeled Egfp fragment was used as the probe for the southern blot assay.

Supplementary Figure 4 Supplementary information for line pIDM-A11. (A) The F₁ pIDM-A11 embryos were treated with Dox at 12 hpf. The picture was taken at 3.5 dpf. Red arrow: pericardial edema. Number, type of represented embryos /total embryos in F₁ population. (B) Reduction of the faf1 expression in the pIDM-A11 embryos treated with Dox. The treated embryos were sampled at 2 dpf and subjected to whole mount in situ hybridization (WISH). Anti-sense RNA of faf1 was used to perform WISH. Black arrow, pharyngeal arch. Number, type of represented embryos/total embryos in F₂ population. (C) Induction of anti-sense transcript of faf1 in the pIDM-A11 embryos treated with Dox. Total RNA was sampled at 2 dpf. The anti-sense faf1 transcript was analyzed with quantitative reverse transcription PCR (qRT-PCR). The qRT-PCR in each treatment was repeated three times. β-actin was used as the qRT-PCR positive control. Red arrow, DNA fragment was amplified from anti-sense faf1 transcript.

Supplementary Figure 5 Supplementary information for line pIDM-A200. (A) Diagram showing the position and orientation of pIDM in the δ-sgcd genomic DNA (including two isoforms, δ-sgcd and X1). (B) The relative expression levels of total sgcd and X1 transcripts
were analyzed with specific primers at 48 hpf. β-actin was used to normalize the total RNA. (C) Pictures of pIDM-A200 embryos treated or untreated with Dox at 36 hpf. The enlarged pictures showed the abnormal muscular development phenotype (red oval). Number, type of represented embryos/total embryos in F2 population. (D) The induction of the anti-sense transcript of sged in the pIDM-A200 embryos treated with Dox was analyzed with qRT-PCR using primer pairs sgcd-intron2-F and sgcd-intron2-R. For all analysis on the relative expression of the target genes the statistically significant differences between samples were assessed using independent-sample T-tests (*P<0.05, **P<0.01, ***P<0.001).

**Supplementary Figure 6** Supplementary information for line pIDM-E17. (A) Diagram showing the position and orientation of pIDM in the ap2b1 genomic DNA. (B) The relative expression level of the ap2b1 transcripts was analyzed with specific primers ap2b1-F and ap2b1-R at 48 hpf. β-actin was used to normalize the total RNA. (C) Pictures of pIDM-E17 embryos treated or untreated with Dox at 36 hpf. Red arrow, extruded-yolk. Number, type of represented embryos/total embryos in F2 population. (D) The induction of the anti-sense transcript of ap2b1 in the pIDM-E17 embryos treated with Dox was analyzed with qRT-PCR using primer pairs ap2b1-intron21-F and ap2b1-intron21-R.

**Supplementary Figure 7** Supplementary information for line pIDM-E10. (A) Reduction of the grb14 expression in the pIDM-E10 embryos treated with Dox. The treated embryos were sampled at 24 hpf and subjected to WISH. Anti-sense RNA of grb14 was used to perform WISH. Number, type of represented embryos/total embryos in F2 population. (B) Induction of the anti-sense transcript of grb14 in the pIDM-E10 embryos treated with Dox was analyzed with qRT-PCR using the primer pairs grb14-intron2-F and grb14-intron2-R. (C) RT-PCR analysis showed that, due to abnormal splicing, the grb14 morpholino (grb14-MO) had created an aberrant transcript with the extra 386 nt. WT embryos injected with the standard control morpholino (SC-MO) were used as the control.
**Supplementary Figure 8** Supplementary information for line pIDM-A1. (A) The induction of the anti-sense transcript of *nidogen2a* in the pIDM-A1 embryos treated with Dox was analyzed with qRT-PCR using primer pairs *nidogen2*-intron9-F and *nidogen2*-intron9-R. (B) qRT-PCR analysis showed that *nid2a*-MO had decreased the expression of the normal transcript of *nidogen2a*, due to abnormal splicing. WT embryos and WT embryos injected with SC-MO were used as the control.

**Supplementary Figure 9** Supplementary information for line pIDM-E3. (A) Diagram showing the position and orientation of pIDM in the *pcyt1aa* genomic DNA. (B) The relative expression levels of the *pcyt1aa* transcript were analyzed with specific primers *pcyt1aa*-F and *pcyt1aa*-R at 48 hpf. β-actin was used to normalize the total RNA. (C) Pictures of pIDM-E3 embryos treated or untreated with Dox at 3 dpf. The diameter of the eyes in the enlarged pictures is shown as a yellow number. Red arrow, less pigmentation in eyes. (D) Reduction of the *pcyt1aa* expression in the pIDM-E3 embryos treated with Dox. The treated embryos were sampled at 24 hpf and subjected to WISH. Anti-sense RNA of *pcyt1aa* was used to perform WISH. (E) Pictures of WT and pIDM-E3 embryos with different treatments at 2.5 dpf as indicated. *pcyt1aa*-MO was designed to specifically block *pcyt1aa* transcript splicing and was injected into WT embryos at the one-cell stage. Red arrow, less pigmentation in eyes. (F) RT-PCR analysis showed that *pcyt1aa*-MO had created an aberrant transcript of *pcyt1aa*, due to abnormal splicing. WT embryos and WT embryos injected with SC-MO were used as the control.

Numbers in C-E, type of represented embryos/total embryos in F₂ population.

**Supplementary Figure 10** Embryonic abnormalities caused by dsRNA injection. (A-F) WT embryos were injected with 10 pg/per embryo (pg/PE) *Egfp* dsRNA or *nid2a*, *grb14*, *faf1*, *sgcd*, *ap2b1* dsRNA at the one-cell stage. Embryos injected phenol-red buffer as the injection
control (A). Most of embryos injected with all of the 6 dsRNAs showed similar abnormal development. The similar abnormal development can be divided into three categories: Normal development with no obvious phenotypes (B), Moderate phenotypes (C, E) and Severe phenotypes (D, F). (G) Statistics of different types of embryos in each treatment with dsRNA injection. Approximately 300 injected embryos were counted in each treatment.

**Supplementary Figure 11** Dox treated transgenic embryos from either pIDM-anti-faf1 or pIDM-anti-grb14 developed normally and did not display similar phenotypes as Dox treated mutant embryos from either pIDM-A11 or pIDM-E10 did. (A) Diagram of pIDM-anti-faf1 and pIDM-anti-grb14 vectors. The abbreviations for each item in the vectors are the same as those in the pIDM:HA-DsRed construct (Figure 1B), except where HA-DsRed was replaced with either the anti-sense of faf1 or grb14 coding region cDNA. (B) Induction of the anti-sense transcript of faf1 or grb14 in the pIDM-anti-faf1 or pIDM-anti-grb14 embryos treated with Dox was analyzed with qRT-PCR using the primer pairs anti-faf1-qRT-For and –Rev or anti-grb14-qRT-For and -Rev. (C) The relative expression level of the faf1 or grb14 transcripts was analyzed with specific primers faf1-F and faf1-R or grb14-F and grb14-R in different samples as indicated at 2.5 dpf. β-actin was used to normalize the total RNA. (D) The pIDM-anti-faf1 embryos were treated with Dox at 12 hpf. The pictures were taken at 4 dpf (1st and 3rd panels). The embryos treated and untreated with Dox (Ctr) were sampled at 5 dpf and subsequently subjected to alcian-blue-staining for pharyngeal cartilage (2nd and 4th panels). (E) The pIDM-anti-grb14 embryos were treated with Dox at 12 hpf. The pictures were taken at 4 dpf.
Supplementary Figure 12 nid2a knockout mutants display similar phenotypes to those in pIDM-A1 embryos upon Dox treatment. (A) Upper panel: Diagram showing the genome structure of nid2a and the gRNA target site. Bottom panel: Comparison of genomic DNA among WT and nid2a<sup>-/-</sup> mutant (with a 7 bp-deletion and a 27 bp-insertion in the 2<sup>nd</sup> exon ). ATG: translation start codon. The mutation will lead to an early stop codon. (B) Picture of WT and nid2a homozygous mutant (nid2a<sup>-/-</sup>) embryos at 4 dpf. (C) Statistics of body length (centimeter cm) of different genotype embryos in a F<sub>2</sub> population at 4 dpf as indicated. Numbers: embryos with the genotype versus total embryos in the F<sub>2</sub> population examined.

Supplementary Figure 13 Characterization of line pIDM-E8. Pictures of WT and mutant embryos with differing treatments at 36 hpf as indicated. For mRNA injection, WT embryos were injected with plcd1a mRNA, or plcd1aX1 mRNA, or plcd1a together with plcd1aX1 mRNAs (plcd1a+X1 mRNAs), or both of the mutant mRNAs carrying an early stop codon (plcd1a+X1 mRNA<sup>M</sup>s) at the one cell stage. Enlarged pictures to show delayed eye’s development. Numbers: embryos showing the displayed phenotype versus total embryos examined are provided in the corresponding pictures.

Supplementary Figure 14 Morphology of Dox treated pIDM mutants with multiple insertions. (A) Line pIDM-A20. Early embryonic lethality for Dox treated pIDM-A20 embryos at 36 hpf. Red arrow, dead embryos; white arrow, WT embryos. In untreated pIDM-A20 embryos, 58 embryos were EGFP positive and 34 embryos were EGFP negative. None of these embryos had died. In Dox treated pIDM-A20 embryos (total 85), 41 embryos were dead at 36 hpf, while among 34 living embryos, only one embryo (with clearly retarded
development) was EGFP positive with 33 embryos EGFP negative. (B) Line pIDM-A33. Epidermal blisters in Dox treated pIDM-A33 embryos at 3 dpf. Red arrow, lesions. (C) Line pIDM-E46. Less pigmentation in Dox treated pIDM-E46 embryos at 56 hpf. (D) Line pIDM-E22. Less pigmentation in Dox treated pIDM-E22 embryos at 36 hpf. (E) Line pIDM-E14. Short stature and no pericardium in Dox treated pIDM-E14 embryos at 60 hpf. Red arrow, pericardium. (F) Line pIDM-E54. Shorter and thicker yolk extension in Dox treated pIDM-E54 embryos at 56 hpf. Red ellipse, yolk extension. (G) Line pIDM-E19. Small head, curved body and unabsorbed yolk in Dox treated pIDM-E19 embryos at 36 hpf. (H) Line pIDM-A199. Curved body with severe cell death in Dox treated pIDM-A199 embryos at 36 hpf. (I) Line pIDM-E256. Arrested development in Dox treated pIDM-E256 embryos at 30 hpf. (J) Line pIDM-A96. Arrested development in Dox treated pIDM-A96 embryos at 30 hpf. (K) Line pIDM-A28. Short stature in Dox treated pIDM-A28 embryos at 60 hpf. (L) Line pIDM-E1. Pericardial edema and deformed head in Dox treated pIDM-E1 embryos at 36 hpf. Red arrow, pericardial edema; (*), deformed head.

Number in B-L, type of represented embryos/total embryos in F_2 population.

Details of insertion positions, orientation of pIDM and expression changes of tagged genes in each line are provided in Table 1.

**Supplementary Figure 15** Non-specific phenotypes in Dox treated pIDM mutants with multiple insertions. No-midbrain-hindbrain boundaries, lack of normal brain ventricles, defects in eye development and pericardial edema were observed in Dox treated embryos of pIDM-E6 (A), pIDM-E43 (B), pIDM-A31 (C) and pIDM-E55 (D) lines. Black arrow, brain-boundaries; purple arrow, heart edema.

Number in A-D, type of represented embryos/total embryos in F_1 population.

**Supplementary Figure 16.** Morphological abnormalities of ubi-pIDM-transgenic embryos after Dox induction. (A-B) Nine mutant lines had Dox-induced cardiovascular defects (A) and five lines had abnormal blood circulation and tail defects (B). Either wild-type (WT; left) or
ubi-pIDM transgenic embryos (such as #6Tg; right) were treated with DMSO (control) or Dox (Dox-induction), and cardiovascular, tail and circulation phenotypes were documented at 48 hpf. The numbers on the lower right indicate phenotypical numbers out of the total embryos examined. The Scale bar: 25 μm. (C) RNA in situ hybridization revealed that Dnajb5, proza, St6galnac1.2, and sps2 were expressed in the heart on the left column (lateral view), and the ventral view of the heart area is enlarged on the right column. Arrows point to the heart. Staged embryos are noted for dnajb5 at 36 hpf, for proza and st6galnac1.2 at 48 hpf, and for sps2 at 72 hpf.

**Supplementary Figure 17.** Segregation analysis of causative mutation from the multiple-inserted lines with Dox-dependent fin regeneration defects. (A) Segregation of galnt2<sup>BM</sup> from line pIDM-A28. To segregate the causative mutation, pIDM-A28 F<sub>1</sub> mutants with Dox-dependent fin regeneration defects were crossed with WT. The cross containing about 50% EGFP positive offspring was selected to produce F<sub>2</sub> population. Left panel: Southern blot analysis of insertion copy numbers in the pool of embryos as indicated. Pool of EGFP positive F<sub>2</sub>: The F<sub>2</sub> EGFP positive embryos were from the cross between the selected F<sub>1</sub> fish and WT; Pool of EGFP positive F<sub>3</sub>: The F<sub>3</sub> EGFP positive embryos were from the cross between one of F<sub>2</sub> adult fish and WT. Southern blot was performed as described in Supplementary Figure 3. Right panel: PCR analysis to identify the insertion site. A pair of galnt2 intron 1 and pIDM specific primers were designed to amplify the insertion site. The DNA lysed from caudal fin of individual F<sub>2</sub> fish was used to do PCR analysis. The DNA from EGFP positive F<sub>1</sub> embryo pool was used as the positive control. The DNA from EGFP negative F<sub>2</sub> fish was used as the negative control. (B) Induction of the listed genes in WT zebrafish at different time points during fin regeneration as indicated. The WT caudal fin was sampled at different times after amputation and subjected to a WISH assay with the corresponding anti-sense RNA probes as indicated. (C) Segregation of ggt7l<sup>BM</sup> from line pIDM-A3. To segregate the causative mutation, pIDM-A3 F<sub>2</sub> mutants with Dox-dependent fin regeneration defects were crossed with WT. The cross containing about 50% EGFP positive
offspring were selected to produce $F_3$ population. Left panel: Southern blot analysis of insertion copy numbers in the pool of embryos as indicated. Pool of EGFP positive $F_3$: The $F_3$ EGFP positive embryos were from the cross between the selected $F_2$ fish and WT; Pool of EGFP positive $F_4$: The $F_4$ EGFP positive embryos were from the cross between one of $F_3$ adult fish and WT. Southern blot was performed as described in Supplementary Figure 3. Right panel: PCR analysis to identify the insertion site. A primer from $ggt7l$ exon 5 was used with pIDM specific primer to amplify the insertion site. The DNA lysed from caudal fin of individual $F_3$ fish was used to do PCR analysis. The DNA from EGFP positive $F_1$ embryo pool was used as the positive control. The DNA from EGFP negative $F_3$ fish was used as the negative control. (D) Segregation of $cry61^{IM}$ from line pIDM-E7. To segregate the causative mutation, pIDM-E7 $F_2$ mutants with Dox-dependent fin regeneration defects were crossed with WT. The cross containing about 50% EGFP positive offspring were selected to set up $F_3$ population. Left panel: Southern blot analysis of insertion copy numbers in the pool of embryos as indicated. Pool of EGFP positive $F_3$: The $F_3$ EGFP positive embryos were from the cross between the selected $F_2$ fish and WT; Pool of EGFP positive $F_4$: The $F_4$ EGFP positive embryos were from the cross between one of $F_3$ adult fish and WT. Southern blot was performed as described in Supplementary Figure 3. Right panel: PCR analysis to identify the insertion site. A primer from $cry61$ intron 1 was used with pIDM specific primer to amplify the insertion site. The DNA lysed from caudal fin of individual $F_3$ fish was used to do PCR analysis. The DNA from EGFP positive $F_1$ embryo pool was used as the positive control. The DNA from EGFP negative $F_3$ fish was used as the negative control.

Detailed protocol

Plasmid construction
Construction of the first generation pIDM vector:

The first step: The rtTA DNA fragment was amplified with the primer pair rtTA-BamHI-F and -R from the pT2HB-PUHrT62 plasmid. The amplified rtTA DNA fragment was then digested with BamHI and cloned into a pIRES2-EGFP plasmid (Clontech) to generate the prtTA-IRES2-EGFP construct. The prtTA-IRES2-EGFP construct was then used to amplify the rtTA-IRES-Egfp-polyA fragment with the primer pair rtTA-IRES-Egfp-SV40-NcoI-F and -R.

The second step: TRE3G promoter was synthesized by the Biosune company and ligated into the pT2-β-actin plasmid to generate the pT2-β-actin-TRE3G vector. For the pT2-elf1a-TRE3G vector, an elf1a element was amplified from the T2KXIG plasmid with elf1a-HindIII-F and elf1a-NcoI-R primers to replace β-actin in the pT2-β-actin plasmid, and then TRE3G ligated to produce the pT2-elf1a-TRE3G vector.

The third step: The NcoI digested rtTA-IRES-Egfp-polyA fragment from the first step was ligated into the pT2-β-actin/elf1a-TRE3G vector from the second step. The TRE3G-β-actin-rtTA-IRES-Egfp-polyA fragment was then amplified with TRE3G-SwaI-F and polyA-HindIII-R primers and digested with SwaI and HindIII. The restriction enzyme digested fragment was cloned into a pTol2mini plasmid to generate the first generation pIDM vector.

Construction of the second generation pIDM vector:

A homologous recombination ligation method was used in the construction of the second generation pIDM vector.

The first step: the rtTA-IRES-Egfp-polyA fragment was amplified with a primer pair rtTA-IRES-Egfp-polyA-SpeI-F and rtTA-IRES-Egfp-polyA-EcoRV-R from the prtTA-IRES2-EGFP construct.

The second step: SwaI digested TRE3G promoter was ligated into pTol2mini plasmid to produce a pTol2mini-TRE3G vector. β-actin promoter was then amplified with a primer pair, β-actin-BglII-F and β-actin-NotI-R, from pT2-β-actin. The elf1a promoter was amplified from the T2KXIG plasmid with the primer pair elf1a-BglII-F and elf1a-NotI-R. The
β-actin/elf1a promoter was then ligated into a pTol2mini-TRE3G vector that was subjected to digestion with BglII and NotI to generate pTol2mini-β-actin/elf1a-TRE3G vector.

The third step: The amplified rtTA-IRES-Egfp-polyA fragment was ligated into the pTol2mini-β-actin/elf1a-TRE3G vector digested with SpeI and EcoRV to generate the pTol2mini-β-actin/elf1a-rtTA-IRES-EGFP-TRE3G vector. This vector had single-restriction enzyme site for SwaI between the TRE3G and β-actin/elf1a promoters, and for EcoRI between the left border of Tol2 and the SV40 terminator.

The fourth step: The core element of the chicken β-globin insulator was synthesized by the Biosune Company and cloned in a PUC57 vector. The insulator-SwaI and insulator-EcoRI fragments were respectively amplified with two pairs of primers: insulator-SwaI-F/R and insulator-EcoRI-F/R from the PUC57-insulator vector. The insulator-SwaI and insulator-EcoRI fragments were ligated into pTol2mini-β-actin/elf1a-rtTA-IRES-EGFP-TRE3G vectors by a homologous recombination ligation method using two separate steps.

Construction of pIDM:HA-DsRed vector:

An HA-tagged DsRed fragment was amplified from the pIRES2-DsRed vector (Clontech) with the primer pair HA-DsRed-EcoRI-F and DsRed-EcoRI-R and then ligated into a pCS2+ plasmid to obtain the pCS2+HA-DsRed vector. The HA-DsRed-polyA fragment was amplified with a primer pair HA-DsRed-polyA-Ascl-F and HA-DsRed-polyA-Ascl-R from a pCS2+HA-DsRed vector. The sequence of GGCGAATT downstream of the Tol2 right border in the pIDM vector was mutated to GGCGCGCC, an Ascl restriction enzyme site, by site-directed mutation with Ascl-site-mutation-F/R primers. Ascl digested HA-DsRed-polyA fragment was then ligated into the modified pIDM vector to generate the pIDM-HA-DsRed vector.
**Linker-mediated PCR for identification of pIDM insertion sites**

Linker annealing was performed according to the protocol described in a previous study. A cocktail containing 50 μl of 25 μM AluI or BfaI linker+, 50 μl of 25 μM AluI or BfaI linker- and 2 μl of 5 M NaCl was incubated at 95 °C for 5 minutes and then slowly cooled down to room temperature to allow the linkers to anneal. Annealed linkers were stored at -20°C.

Linker-mediated PCR was performed as described previously. Briefly, 3 μg genomic DNA was digested with AluI or BfaI overnight. The digested DNA was purified and dissolved in 30 μl H2O. The Linker DNA together with the digested genomic DNA fragments was ligated with T4 DNA ligase (NEB). The ligation solution was used as the template for the first-round PCR with a pair of Linker primer and Tol2 5’ N1 primer. Then the first-round PCR product was used as the template for the second-round PCR with another pair of primers: Linker AluI (BfaI) nested primer and Tol2 5’ N2 primer.

After the second-round of PCR, the PCR products were purified using a PCR clean kit (Axygen) according the manufacturer’s instructions and then ligated to a pGEMT-Easy vector (Promega). The Tol2 5’ S1 primer was used to sequence the PCR products. The sequence of the PCR product was blasted with the database GRCz10. The sequence of genomic DNA with a high identity (>95%) to the PCR product was considered to be the insertion site. If the similarity between the PCR product and genomic sequences was less than 95%, the insertion site was considered as non-identified (N.D).

**Segregation of mutation responsible for fin regeneration defect from multiple-inserted lines**

From line pIDM-A28, LM-PCR amplified two insertion sites. The first insertion located in the 1st intron of galnt2 gene with the Tet-On promoter oriented in the opposite direction (Fig. 6A). The second insertion was unable to be identified due to that the sequence of second LM-PCR amplified DNA fragment has high similarity to a number of genomic DNAs at different locations. We crossed fin regeneration defective F1 mutants with WT to set up F2
population. About 50% EGFP positive embryos was found in the offspring of one of these crosses, which suggested that this F1 mutant fish may contain single insertion. Then we raised the EGFP-positive embryos from this cross to generate F2 generation. Using a pair of galnt2 and insertion specific primers, the insertion within the galnt2 gene was identified in all of the F2 fish (Fig. S17A). Southern blot showed that only one insertion site was detected in the F2 offspring from this F1 mutant as well as the F3 offspring from one of the F2 mutant adult fish (Fig. S17A). The results demonstrated that all of these F2 mutants had only one insertion within the galnt2 gene.

There were three insertion sites in both pIDM-A3 and E7. Single-inserted mutant was segregated from F2 individuals with fin regeneration defects in both lines and the EGFP positive embryos from the F2 individuals were used to generate F3 population. The single insertion was confirmed by southern blot with the F3 offspring from the F2 mutant as well as the F4 offspring from the F3 population in both lines (Fig. S17C,D). The insertion in segregated pIDM-A3 and E7 located in the 4th intron of the γ-glutamyltransferase7-like (ggt7l) gene and the 1st intron of cry61 gene with the Tet-on promoter oriented in the opposite direction (Fig. 6A), respectively. Using a pair of gene and insertion specific primers, the insertion site was identified in all of the F3 mutant fish from both of lines (Fig. S17C,D).

Dox-dependent fin regeneration defective phenotype was also observed in the F3 population of both lines (54% in pIDM-A3 and 66.7% in pIDM-E7), whereas F4 EGFP positive embryos from these F3 mutants of both lines did not show Dox-dependent developmental defects (Fig. 6C), which was consistent with those in respective F1 population.

References
1. Loew, R., Heinz, N., Hampf, M., Bujard, H. & Gossen, M. Improved Tet-responsive promoters with minimized background expression. BMC Biotechnol 10, 81 (2010).
2. Kotani, T., Nagayoshi, S., Urasaki, A. & Kawakami, K. Transposon-mediated gene trapping in
zebrafish. *Methods* **39**, 199-206 (2006).

Yergeau, D. A., Kuliyev, E. & Mead, P. E. Injection-mediated transposon transgenesis in Xenopus tropicalis and the identification of integration sites by modified extension primer tag selection (EPTS) linker-mediated PCR. *Nat Protoc* **2**, 2975-2986 (2007).
### Supplementary Table S1: PCR primers

#### Primers for construction of the first generation pIDM

| Primer                  | Sequence                                      |
|-------------------------|-----------------------------------------------|
| rtTA-BamHI-F            | CGAGCTCAAGCTTCGCCGCCACCATGTCTAGACTGGACAAGAGA |
| rtTA-BamHI-R            | TACCGTGAAAGCTTTTACCCGGGGAGCATGTCA             |
| rtTA-IRES-Egfp-SV40-NcoI-F | CCTCGACCATGGGCCGCCACCATGTCTAGACTGGAGA       |
| rtTA-IRES-Egfp-SV40-NcoI-R | CCTCGACCATGGCAGTGAAAAAAATGCT              |
| elf1a-HindIII-F         | CGAGCTAAGCTTCAGGGGATCATCTCTAT               |
| elf1a-NcoI-R            | CCTCGACCATGGCAGCTAGAACTCGCCGC              |
| TRE3G-Swal-F            | CGAGCTCATTTAATTATTACAGGGGTAGGAGA            |
| polyA-HindIII-R         | CGAGCTAAGCTTCAGTTGAAAAATGCT                |

#### Primers for construction of the secondary generation pIDM

| Primer                  | Sequence                                      |
|-------------------------|-----------------------------------------------|
| rtTA-IRES-EGFP-polyA-Spel-F | TCCTCGACCGGATCCACCGGTGCAGCCACCATGTCTAGACTGGA |
| rtTA-IRES-EGFP-polyA-EcoRV-R   | TTGTTCAGCTTGAATCATACATTGATGAGTTTG           |
| elf1a-BglIII-F          | AGCTCCTCGAAATACAGGGGATCATCTAAT              |
| elf1a-NotI-R            | TTCATGAGTGCAGGCGCCACCATGTAGAATG             |
| β-actin-BglIII-F        | AGCTCCTCGAAAAATATAGACCTTCTTACTT             |
| β-actin-NotI-R          | TTCATGAGTGCAGGCGCCACCATGTAGAATG             |
| insulator-Swal-F        | TGCAGTTTTAATTTCACACTGGGGGAGCT               |
| insulator-Swal-R        | GCAGGCGCTAGATTCTTCAGCTTCTTACTA              |
| insulator-EcoRI-F       | ACTCATCAATGTGATGACACTGGGGAGAGCT            |
| insulator-EcoRI-R       | TGTATTTAAGCTTGAGTTCTGGCTTCTTACTA           |

#### Primers for construction of pIDM:HA-DsRed
| Primers for the southern blot probe |  |
|------------------------------------|--|
| EGFP 226 probe-F                   | CCCGACCACATGAAGCAGCAGCAC |
| EGFP 646 probe-R                   | GCTTCTCGTTGGGTTTGGT |

| Primers for the identification of transgenic fish |  |
|-----------------------------------------------|--|
| rtTA transgenic fish ID for                   | ATGTCTAGACTGGACAAAGGAC |
| rtTA transgenic fish ID rev                   | TTACCGGGGAGCATGTCAAGG |

| Primers for Linker-mediated PCR |  |
|----------------------------------|--|
| Linker AluI-H                    | CTCCCTTAAGCGGAGC |
| Linker AluI+H                    | GTAATACCGACTCATAGGCTCCTGCTTAAGGG |
| Linker BfaI-H                    | TAGTCCCTAAAGCGGAG |
| Linker BfaI+H                    | GTAATACGACTCATAGGCTCCTGCTTAAGGG |
| Linker primer                    | GTAATACGACTCATAGGAC |
| Linker AluI nested primer        | AGGGCTCCGCTTAAGGGGAG |
| Linker BfaI nested primer        | AGGGCTCCGCTTAAGGGAC |
| Tol2 5’ N1                       | CGCAATTCAATGGTTGG |
| Tol2 5’ N2                       | GCAAGGAAAAATAGAATGAG |

| HA-DsRed-EcoRI-F                  | GATCGAATTCGCCACCATGTACCCATACGGATGGTC |
| DsRed-EcoRI-R                     | GATCGAATTTCCTACAGGAAACAGGTGGTGG |
| HA-DsRed-polyA-AcI-F              | CGACTCCTATAGGGCCGCGGCAATTTAAAAAACC |
| HA-DsRed-polyA-AcI-R              | CACCTCTGGGCCCGCAGCCACATGTCACCATACG |
| AscI site mutation-F              | ACTATAGGGCCGCGCCGCCAGGCTGTAAGG |
| AscI site mutation-R              | ACCCTCTGGGCCCGCAGCCACATGTCAGT |
| Primers for the southern blot probe|  |
| EGFP 226 probe-F                  | CCCGACCACATGAAGCAGCAGCAC |
| EGFP 646 probe-R                  | GCTTCTCGTTGGGTTTGGT |

| Primers for the identification of transgenic fish |  |
|-----------------------------------------------|--|
| rtTA transgenic fish ID for                   | ATGTCTAGACTGGACAAAGGAC |
| rtTA transgenic fish ID rev                   | TTACCGGGGAGCATGTCAAGG |

| Primers for Linker-mediated PCR |  |
|----------------------------------|--|
| Linker AluI-H                    | CTCCCTTAAGCGGAGC |
| Linker AluI+H                    | GTAATACCGACTCATAGGCTCCTGCTTAAGGG |
| Linker BfaI-H                    | TAGTCCCTAAAGCGGAG |
| Linker BfaI+H                    | GTAATACGACTCATAGGCTCCTGCTTAAGGG |
| Linker primer                    | GTAATACGACTCATAGGAC |
| Linker AluI nested primer        | AGGGCTCCGCTTAAGGGGAG |
| Linker BfaI nested primer        | AGGGCTCCGCTTAAGGGAC |
| Tol2 5’ N1                       | CGCAATTCAATGGTTGG |
| Tol2 5’ N2                       | GCAAGGAAAAATAGAATGAG |
| Gene      | Forward Primer                          | Reverse Primer                          |
|-----------|----------------------------------------|----------------------------------------|
| Tol2 5' S1| AGTATTGATTTTTAATTTGTA                 |                                        |
| Primers for WISH |                                        |                                        |
| faf1-F    | AACGCGATCTAGAAACAGCAA                  |                                        |
| faf1-R    | CATAGGGCCCGTGTGGAGAT                  |                                        |
| pcyt1aa-F | TGAGATCACGAATATCGAGA                   |                                        |
| pcyt1aa-R | CGTCTTCGTTTATGAGA                     |                                        |
| galnt2-F  | GGAGGAATGCAAGATGGTTTTA                |                                        |
| galnt2-R  | AGCAAAATGACTTTGGACACTG                |                                        |
| nidogen2-F| TTCAGATGTTGCTTGGGTA                    |                                        |
| nidogen2-R| CCGGAATGTCGGTGCGAT                   |                                        |
| grb14-F   | TGCATGTGGTGGTGTTGTCTT                 |                                        |
| grb14-R   | GTTTGTTCGCGAGGATAACAG                |                                        |
| ttyh3b-F  | GGCTTCACCTGGGCTTGAG                   |                                        |
| ttyh3b-R  | GAGGTGTACCTCTCTGCTGCC                 |                                        |
| sema5ba-F | CCGCTTGAGTGCCAGTGAAT                  |                                        |
| sema5ba-R | TGCGTGTGGTGTTGAGTGT                   |                                        |
| Primers for qRT-PCR to analyze the relative levels of sense mRNA |                                        |
| β-actin-F | ACCACCGGATTTGTGCTGGATGC               |                                        |
| β-actin-R | ACAGATCCCTACGATGCTCAATGTC            |                                        |
| elf1a-F   | CTTCTCAGGGCTGAATGTGC                 |                                        |
| elf1a-R   | CCGCTACCATACCACTCC                    |                                        |
| 18sRNA-F  | CGCCACTGTCCTCCCTAAAGAA               |                                        |
| 18sRNA-R  | GTAGTTCGACCGTAAACGAT                 |                                        |
| faf1-F    | TGACAACATTGCTGGCGA                   |                                        |
| faf1-R    | CATAGGGCCGTTGAGAT                    |                                        |
| grb14-F   | TGTAGCTGGGCTCCATGAGTG                |                                        |
| grb14-R   | GTGAGCGCAAATGGGATGAAG                |                                        |
| grb14 X3-F| CGTGTACGGAAGAGGACG                   |                                        |
| grb14 X3-R| GGCTCGGCTGGGTTATCTT                  |                                        |
| Gene    | Primer 1 | Primer 2 |
|---------|----------|----------|
| pcyt1aa | AGCAGTATCAGCGTGTACACC | TGTCGAGATCCCAATCAGCG |
| ap2b    | TCAACAAGCTTCTGACGGCT | TTAGCAGTGAGAGCAGTGG |
| loc556929 | CGTGGCTCGGCTCTAAAAGGTGT | ACCACGAATCTCTGATTCAAGACA |
| ap2b    | TCAACAAGCTTCTGACGGCT | TTAGCAGTGAGAGCAGTGG |
| loc556929 | CGTGGCTCGGCTCTAAAAGGTGT | ACCACGAATCTCTGATTCAAGACA |
| plcd1a X1 | ACGGGCTTTGCTGAGGATTC | AGAAGACAGGCAAGGGCAAAAC |
| plcd1a X1 | ACGGGCTTTGCTGAGGATTC | AGAAGACAGGCAAGGGCAAAAC |
| plcd1a | ACGGGCTTTGCTGAGGATTC | AGAAGACAGGCAAGGGCAAAAC |
| nido2en | ATCAACCTGTGTAGTTTCTCCAA | GTTGAACCTACCGCAGGA |
| sgcd    | CCAGGAACAGTGCCCTCATA | GTAAGGCAACGTTTCCGCC |
| galnt2  | CTTCCTCTAGCCACCGCTG | ATGCGGTGATACAGTGCTC |
| si dkey | ATGCTCTGTTGTCACTCGCA | ACAATTGAGAGACATCCCAGCA |
| ms4a17a.8 | CAGAGCGAAGACGCAAAGC | GTTGGGGTTGCTGGCGCTGG |
| f2rl1.1 | CCAGAGATCCTGAGCTTGGAT | TTGGTGACCTTCACGGTCTG |
| hes6    | GAACTGCAGCAATATGGGCCC | GCGGTCTCTCTTTTCTCCAC |
| mty1l X1/X2 | CCCAGAACCACCCACGTTTTTA | CAGTCCCCGACTCTTTTGAT |
| mty1l X3  | CAGTCCCCGACTCTTTTGAT | CTGTACACATGCCCAGCTGA |
| mty1l X3  | CAGTCCCCGACTCTTTTGAT | CTGTACACATGCCCAGCTGA |
| dner    | TTAGGACGATCTGACCCCG | TTAGGACGATCTGACCCCG |
| dner-R                  | ACGTCAGAGGGGTCCCCAAA |
|------------------------|-----------------------|
| loc103909182-F         | CACACTGACGGTCGGTTATGCG |
| loc103909182-R         | ACGCTGGCAGTCAATCGGTA  |
| syt10-F                | GCTCGCCCTTTTGTTAGTCT  |
| syt10-R                | CATTGGACGTGAACGGCTTGT |
| grik4-F                | TGACAACATTCGTAGGCGGA  |
| grik4-R                | CATAGGGGCGGTGGTGAAT   |
| loc103908836-F         | TGCGGAGGTGGAATCCCTTC |
| loc103908836-R         | GACGCGGTTCCTTCCTCTCT |
| grik4-002(402)-F       | GATCAAGGCAAGGTTTTCCC |
| grik4-002(402)-R       | TCCCCCTCCTTTCCCTTGTT |
| grik4-003(532)-F       | CCGCAGAAGTGCTGCTGCTA |
| grik4-003(532)-R       | TGAACTGATAAAGGCGGCA  |
| loc103910266-F         | TGACACGCAAGACAGTCAGT |
| loc103910266-R         | AGTGCTTCTGCAAACCTGGA |
| syt17-F                | CCGATCCACCTTCAGCTTGG |
| syt17-R                | GTGTAGGGTGCTTTGCAGA  |
| sich211-15e22.3-F      | CACATCGGAAAAACATCGCCC |
| sich211-15e22.3-R      | GCGACTTCTCGTACCCCAA  |
| acbd5a-F               | ACTTCTGGGCTTGGCTTGAC |
| acbd5a-R               | ACCGCTTCAAGGAAGATCCGAA |
| abi1a-F                | GGTCTAAAAGAAGACTCCGA |
| abi1a-R                | AGCTTCTACTTCCTCGCTTG |
| TANCI-like-F           | GGAGAAGGGGTGCTGATCG  |
| TANCI-like-R           | AGGAGACGTCTGTAGCCTAGT |
| Loc103911696-F         | CACAGGGCTACTCCAGAACG |
| Loc103911696-R         | CTTCTCAAGACTCGACCAG |
| evi5a-F                | TTGGAGAGGAGGTGCTCTGA |
| evi5a-R                | AGCTGATTAGCTCTGTCGCC |
| Gene          | Primer 1 | Primer 2 |
|--------------|----------|----------|
| Loc101886424-F | TGTCTTGTTATTAAGCTATGGGC |                    |
| Loc101886424-R | GCCGGAAGATGGATGTGG |                    |
| wu:fc21g02-F  | TGTTGGGCGCATCGAAAGACA |                    |
| wu:fc21g02-R  | GCCATGGCTATAGCTTTGCG |                    |
| tcrig1-F      | TGTCGGCGCAAGGCTATGT |                    |
| tcrig1-R      | TCCCTCCATAACGACAGGA |                    |
| Loc101882854-001-F | ACACCGTATGGTTGATAACTG |                    |
| Loc101882854-001-R | ATCCAAAGCAACCCACACTT |                    |
| Loc101882854-002-F | ACACCGTATGGTTGATAACTGC |                    |
| Loc101882854-002-R | ATCCAAAGCAACCCACACTT |                    |
| scn8aa-F     | AACCCGGTGCTACCTATGGGC |                    |
| scn8aa-R     | TGGCGGATGTCGCCATCTTG |                    |
| tmprss12-F   | CATCACTGGATGCGGCGT |                    |
| tmprss12-R   | ACATGGCCCATATGCCCACCA |                    |
| NLRC3-like-F | GCTGTGATCGACCAAGCCT |                    |
| NLRC3-like-R | GAAGCCACGAACTCCATGA |                    |
| snx17-F      | TTTTCCGTAGCGTTCGCTT |                    |
| snx17-R      | AGGAGTGAAGACGTTTTGCC |                    |
| prpf18-F     | TGTGAGCACAAGATGGACAAACCT |                |
| prpf18-R     | CGTCTGATCACCTCCTGTCG |                    |
| ogdhh-F      | GCACTGCTTAAAGACTTGCG |                    |
| ogdhh-R      | AGTGGGAGTCTCGATCCATTT |                |
| gpr128-F     | CTGCAAGCCGGTTTAATGGTTC |                |
| gpr128-R     | GCCAGCCAGCAAACCTTTC |                    |
| picalmb-F    | CACGAGCTTGGTCTCGGTCT |                    |
| picalmb-R    | TAAATGCTCTGCCCAGC |                    |
| shisa-7-like-F | TGCCACAACCTACCAATCCCC |                |
| shisa-7-like-R | TCCACCAATGAGCTACAGG |                    |
| efnb1 A-F    | CAAGAGAACCAGGCAAGC |                    |
| Gene   | Primer Set | Sequence      |
|--------|------------|---------------|
| efnb1  | A-R        | ATTCTCTGTTGTCGCCAGGG |
| efnb1  | B-F        | GAAGTGACGGCTCGTGGA |
| efnb1  | B-R        | ACCGACTCAGTGATTGGC |
| loxl2a | F          | ACGCAAGCATACGAGGGT |
| loxl2a | R          | GTGGACATTGCTGAGCCAG |
| tbx1   | F          | CAAGCTCAACGCAGAACAA |
| tbx1   | R          | TCTGCCATTGGGTCCATTCC |
| abl1m3 | F          | CAGGCTTTGTTTACCCAG |
| abl1m3 | R          | TGATAATGGGAAACGGGAG |
| dapk1  | A-F        | ACAAGCATGGGAACCTCCCT |
| dapk1  | A-R        | CTCAAGTCCCCACATGACC |
| dapk1  | B-F        | ATCACCCTGCAAGATGTTT |
| dapk1  | B-R        | GGCTTCAGTCGAAAGGGG |
| ebag9  | F          | CAGATCACCCTCCCCAAACCAC |
| ebag9  | R          | CCTGATGGTTGGAGCCATGT |
| matn4  | F          | GGACTGTCACTCCATGCCT |
| matn4  | R          | GACGCAGGAATGCTTCACAG |
| prdm5  | F          | TCCACAGTGAGGACAAACCG |
| prdm5  | R          | CGCTGTAAGAGAAACGGGGGT |
| ube2nb | F          | TCAACCTCAACGCAGGAAC |
| ube2nb | R          | ATAATCTGCGCGGCAATCC |
| tbc1d8 | F          | AGGTTTGAATTGCGGAGAGG |
| tbc1d8 | R          | AGGAGTAATTGCACCACCAG |
| ifnphi3| F          | GTGCAAGGGTTGTCATTGCC |
| ifnphi3| R          | ATAGAACCAGGGTGTCACAC |
| sema5ba| F          | CACTCAGGAATGTGGCAAAGG |
| sema5ba| R          | AGCAGAGTGAACCTGGACA |
| plekha5| F          | GTCCAGGCCTCAGTTCAACA |
| plekha5| R          | AGCAACAGATGAAGGCACAA |
| Gene                        | Forward Primer                  | Reverse Primer                  |
|-----------------------------|---------------------------------|---------------------------------|
| solute carrier family 45 member 4-like-F | ATGATCCACGAGACGAGCC             |                                  |
| solute carrier family 45 member 4-like-R | TGGCTAAAACGGAGTCGCTT            |                                  |
| lhfp4-R                      | TGGCTAAAACGGAGTCGCTT            |                                  |
| nsmecl-R                     | CACAATCAGATCCATCAAACAAAA        |                                  |
| sfswap-R                     | TAATCTTTGCTTTTCCGCC             |                                  |
| itcha-R                      | CTTCGACTGTCCGTCCACAA            |                                  |
| anti-faf1 qRT-For            | tttagctccaccagattcc             |                                  |
| anti-faf1 qRT-Rev            | gcagagtttaaatgctcac             |                                  |
| anti-grb14 qRT-For           | ctgaaactctctctagc               |                                  |
| anti-grb14 qRT-Rev           | gcacggtgatacaagga               |                                  |
| Gene specific primer for in vitro reversely transcribing sense-cDNA  |                                  |                                  |
| β-actin sp primer            | TGTAAGGCTGAGAGACTGAG            |                                  |
| faf1 sp primer               | TTAGGGAGGGTTAGGGGACTG           |                                  |
| nidogen2a sp primer          | AAACAGACGGCTTCATCC              |                                  |
| grb14 sp primer              | TTAGGTCGAGCGACGAGAAGT           |                                  |
| sgcd sp primer               | GGGGCCCAGAGGATAAAAA             |                                  |
| ap2b1 sp primer              | GCTTTGAATGTCAGGAGGCA            |                                  |
| Gene specific primers for qRT-PCR to analyze antisense transcripts |                                  |                                  |
| faf1-intron6-F               | ACAAATGGCACAACACCTTTACA         |                                  |
| faf1-intron6-R               | TGCTCAGGCTCTTTACTTTGT           |                                  |
| grb14-intron2-F              | AGGACATCAGGGAGGTCAGG            |                                  |
| grb14-intron2-R              | TAAAAGCGTCTTGCCCCGAA            |                                  |
| galnt2-intron1-F             | GTAGGTGGCAGCTGGGATGTT           |                                  |
| gene          | primers                                      |
|--------------|----------------------------------------------|
| galnt2-intron1-R | TACACCCCTCTCCAGCAGT                          |
| nidogen2-intron9-F | TGACTGCCCCTTGCTATTCC                      |
| nidogen2-intron9-R | GTTGGGCTGAGTAGCTGGTT                          |
| sgc-d-intron2-F | GTGTCCAGAGGGTTTACGGG                          |
| sgc-d-intron2-R | CTGTAGCTGCCGACAAATTC                          |
| ap2b1-intron21-F | GTATTTCAGGGTGCTCGGC                          |
| ap2b1-intron21-R | CGCGATCAGAAACCCCTACCT                          |

**Primers for analyzing morpholino function**

| gene          | primers                                      |
|--------------|----------------------------------------------|
| nidogen2a-MO detection for | CAGCTCTAGCCCATCCCATC |
| nidogen2a-MO detection rev | GGTTCTTGACGGACTGCTCA |
| grb14-MO detection for | TCACACTGCTGCCCTGACTT |
| grb14-MO detection rev | TGGATATCGACACATGTGCT |
| pcyt1aa-MO detection for | AAATCGCGTACACTTCCGA |
| pcyt1aa-MO detection rev | GTCGAAGATCCCATCAGCGT |

**Primers for analyzing insertion sites**

| gene          | primers                                      |
|--------------|----------------------------------------------|
| galnt2 ID for | GCAAGGGAAAATAGAATGAAG                          |
| galnt2 ID rev | GTTCGCTTTGGTGGGCA                            |
| ggt7l ID for | GCGATGCCACCTACGGCA                          |
| ggt7l ID rev | TGCTAAACCAGAGGACAGGG                          |
| cyr61 ID for | GGACGACGGCAACTACAAGA                        |
| cyr61 ID rev | ATACCTCAAGCACTGTCCCA                        |
| ttyh3bID for | GCAAGGGAAAATAGAATGAAG                        |
| ttyh3b ID rev | GTTGTGAAGCGTGCTTA                           |
| si:dkey-26i13.8 ID for | GCAAGGGAAAATAGAATGAAG |
| si:dkey-26i13.8 ID rev | GCTCTGAGGGATGGCTTG |

**Primers for identification of nid2a cas9-mutant**

| gene          | primers                                      |
|--------------|----------------------------------------------|
| nid2a cas9 ID for | GCAGGTGACCACCAATGGAA                          |
| nid2a cas9 ID rev | CCTCAAGCTGTTTCTTACCCG                        |

**Primers for constructing ubi-pIDM vector**
| Primer | Sequence |
|--------|----------|
| **TRE3G-F** | CTGATGCCCATTTTATTTGCG |
| **TRE3G-F** | gatgatcaactgagCAAATTCGAGGAGC TT |
| 4XIns-F | aagctctcgaatctttctcgagATTGATCATCAGACATCAG |
| 4XIns-R | tgggtCTTGTTTATTTGGCCCATAGAT |
| Ubi-F | tagtgggcccataaaacagACCAGCAAAGTTCTAGAAT |
| Ubi-R | ttaatggaaatggtgtgtgtgaatatcttaacttttaatgctata |
| rtTA-F | atcttaactttgaatggtttacagacccgtgCCACCATGTCTAGA |
| rtTA-R | ACCGCAATGTAGCAGACTTCTGTGCCCCTTCC |
| T2A-RFP-F1 | TAACATGCGGCGAGCTCAGAGAGATCTCGG |
| T2A-RFP-F2 | gagggcagaggaagtctgtaatgcctcgggctcgagcgtgaggaatctgg |
| T2A-RFP-R | cttaatgctgatcatcagctgctgctgctgctgCAGGAGGAT |

**Primers for Tail-PCR**

| Primer | Sequence |
|--------|----------|
| R1 | GGCCCCAATAAAACAAGAATCTCTAGTTTTTTTC |
| R2 | ACGATGGACTCCAGTCGGCCTAATACTCAAG |
| R3 | TTTCCCTAAGTGATTTGTACTTTCCATTTGAGTAA |
| L1 | CGCGCGGAAATTAAAATTGCAGATCATCAGGCA |
| L2 | aagatgactccgTCCGGCCTAGAATGGAATGATCT |
| L2 | CCAADAAAAATAAGTA |
| L3 | ACTTAAGTACAGTAAATCAAGTAAAATTACTCAAGTAC |
| --- | --- |
| LAD1-1 | acgatggactccagagcggccgcvnvnnngaa |
| LAD1-2 | acgatggactccagagcggccgcbnnnnggtt |
| LAD1-3 | acgatggactccagagcggccgcvnnnnccaa |
| LAD1-4 | acgatggactccagagcggccgcbdnbnncnggt |
| AC1 | acgatggactccagag |

**Primers for cloning transgenic insertions**

| LP3 | AAATAGAATGAAGTGATCTCC |
| --- | --- |
| RP3 | CTCAAGTAAGATTCTAGCCAG |
| 6-GR1 | AAGTTGCGTGAAATGCACAA |
| 6-GL1 | ATGCTGGTTAGTTTACAGA |
| 6-GL2 | AAAACCTTGAGGTGTGCGTTA |
| 6-GR2 | ATACACTACCACAACACTA |
| 6-GL3 | TGCCACAGAAGAAATGAACCG |
| 6-GR3 | TATTTAGGTGGTCCGCTGG |
| 8-GL1 | GTCAGCAAAAGAACCTCGCAC |
| 8-GR1 | ATGAGGTCATTCGTCTGCGAT |
| 8-GL2 | GCATTAAGCTGTGTCGTCAGC |
| 8-GR2 | GTAGACATGACAGCTGTTAAT |
| 8-GL3 | ACACCAAAAACCAACACACAA |
| 8-GR3 | ATAAATTGCTTGTAAAAACG |
| 13-GL1 | CATGGTCTGCTGAGAGCACGAC |
| 13-GR1 | AGGAGAGGCTGCTGCAACATC |
| 13-GR2 | GCGTATTCCGAAAGTGACAT |
| 13-GL2 | CACAAAAATGTTAGACGTTCTGGA |
| 23GR1 | TTTTCTTCAATGACCTATT |
| Primer | Sequence |
|--------|----------|
| 23GL1  | TGGGCCAGATTACCAACCAG |
| 23-GR2 | TTCGATGACTGATACAGATTGTGT |
| 23-GL2 | ACCCAGTTAGGATAGCTGATT |
| 37-GL1 | GTCCAAAACGAGTCAGCGATGG |
| 37-GR1 | ACGTTAGTAAGGTGCTA |
| 37-GR2 | GAGGGAGGTAGAGCTGCCCT |
| 37-GL2 | ACTGTGACAAGGCTCAGCT |
| 37-GL3 | GAAACATGCCAAGTGACAG |
| 37-GR3 | TACATGACATTATTGGCCAT |
| 37-GL4 | ATCCCCATAGACAAAGAAAA |
| 37-GR4 | CTCTCTCAACCTGTAAAATT |
| 43-GL1 | CTGAATGCCCTGCAAGCCCTGACA |
| 43-GR1 | GTGTGGACGGGAGATATTTCC |
| 43-GL2 | CTGAATGCCCTGCAAGCCCTGACA |
| 43-GR2 | GTGTGGACGGGAGATATTTCC |
| 43-GL3 | ATTTTTGACAAGCACCATAAA |
| 43-GR3 | CTTGAATCTGTGTATTATGCA |
| 84GR1  | TATTTGACCATGTGTGTTATA |
| 84GL1  | GGCTGGTGGCTGAGTGAAAT |
| 84-GL2 | CAATGCTAGGCAGACATGTC |
| 84-GR2 | AAAGGTCTAGTTGTTCTTCAAT |
| 84-GL3 | CCGTCTCCGCTCCTCCAGGTGAGTT |
| 84-GR3 | TAAAACCTAACCCTCATTGGGTGATG |
| 84-GL4 | AGCAACTCTGCGTAAAACACGCT |
| 84-GR4 | CTAGTAGACTTACGTGAGAA |

**Primers for generating RNA probes**

| Primer     | Sequence         |
|------------|------------------|
| dnajbF1057Spe | TGGCGAAAACCGGCATCATT |
| dnajbR1057spe | GGAAAGGCGAGGCCTTCTCCA |
|                   |                  |
|-------------------|------------------|
| proza-F           | TCCTCCGTCAATCATGGCTTG |
| proza-R           | ACACCTCTCTAGGTTTTCCATCAAA |
| pE3-ST6-F1130     | GACTTAATTACACAGCCATT |
| pe3-ST6-R1130     | GTGAAATAACTGACTTAGCTGT |
| SPS2 F-1111spe    | CAAATGTCCTGAGTGATCTG |
| SPS2 R-1111spe    | ATTTTTGTCTGTTGCTCTC |