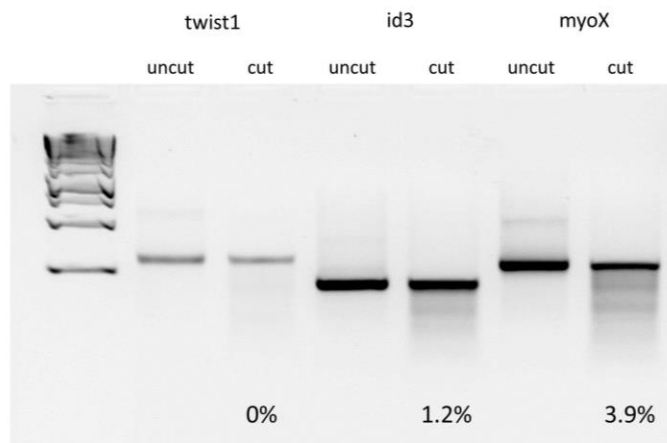


Supplementary Fig. 2



MyoX [%indel:3.9]

AATCATCTTGTAAGGGTTGACAGATGCCAGGATGGAGCCAATATATGTCTG

AATCATCTTGTAAGGGTTGACAGATGCCAGGATGGAGCCAATATATGTCTG

[x9]

Supplementary Fig. 3

AP2α-963 (0/10)
AGTATAGGATCTGCTGGATAGCGCACCAGGTCTCAGGGTCCCACTAAGGGT WT
AGTATAGGATCTGCTGGATAGCGCACCAGGTCTCAGGGTCCCACTAAGGGT [x10]

zic1-645 (0/11)
CCCCACCCCTTCATACATGGGTCTGGGCTTCTCTGTGGTGCCTGCACGAGGT WT
CCCCACCCCTTCATACATGGGTCTGGGCTTCTCTGTGGTGCCTGCACGAGGT [x11]

ets1-302 uninjected
CCATCCCTTTCAATGGCATCTCCAGGGCTGAGTGAGGGTGCCTCACA CT C
CCATCCCTTTCAATGGCATCTCCAGGGCTG---GAGGGTGCCTCACA CT C (-3)
CCATCCCTTTCAATGGCATCTCCAGGGCTGAGTcGAGGGTGCCTCACAa (+3) [x2]
CCATCCCTTTCAATGGCATCTCCAGGaCTaGAGTcGAGGGTGCCTCACAa (+3)

ets1-302 (5/5)
CCATCCCTTTCAATGGCATCTCCAGGGCTGAGTGAGGGTGCCTCACA CT C WT
CCATCCCTTTCAATGGCATCTCCAGGGCTG---GAGGGTGCCTCACA CT C (-3) [x2]
CCATCCCTTTCAATGGCATCTCCAGGGCTGAGTcGAGGGTGCCTCACA CT C (+2) [x2]
CCATCCCTTTCAATGGCATCTCCAGGaCTaGAGTcGAGGGTGCCTCACA CT C (-1, +3)

Irig3-E306 (3/5)
TTGTTTGGGAAAAATAAGAGGATTTCATGGAAATGGGCATTTAAAAAATGA WT
GAGGATTCATGGAAatgggca...//...ttcatggaAATGGGCATTTAAAAA (+135) [x2]
GAGGATTCATGGAAatgggca...//...ttcatggaAATGGGCATTTAAAAA (+134)
TTGTTTGGGAAAAATAAGAGGATTTCATGGAAATGGGCATTTAAAAAATGA [x2]

sox9-826 (11/11)
AAAAACATGGATCTGGAAGTGAATAAGGAGACAGGGGTGTCCCCTCTAACC WT
AAAAACATGGATCTGG-----AACC (-31)
AAAAACATGGTtaG-----GGGGTGTCCCCTCTAACC (-21, +2)
AAAAACATGGATCTGGAAGTGAATAAGG-----AGGGGTGTCCCCTCTAACC (-4)
AAAAACATGGATCTGGAAGTGAATAAGGAGA-----GGTGTCCCCTCTAACC (-4)
AAAAACATGGATCTGGAAGTGAATAAGGAGAGAGGGGTGTCCCCTCTAACC (-1, +1) [x7]

sox9-579 (2/5)
GCAACAGTGGAGAGAGAGGTGAAAAAGGAGACAGAGGCGCAAAAAGGAGAAC WT
GCAACAGTGGAGAGAGAGGTGAAAAAGGAGAG-----aCGCAAAAAGGAGAAC (-6, +1) [x2]
GCAACAGTGGAGAGAGAGGTGAAAAAGGAGACAGAGGCGCAAAAAGGAGAAC [x3]

sox9-600 (5/5)

AAACCGCAGAGCCTGAAGCTGAAGAAGGAACAGGGGGGACTGGTTCTCCG WT
AAACCGAAATGCAT-----//-----GCCAGCAGTCTCCG (-66)
AAACCGTGCTAGCA-----//-----AGGGGGGACTGGTTCTCCG (-34)
AAACCGCAGAGCCTGAAGCTGAAGAA-----GGACTGGTTCTCCG (-11)
AAACCGCAGAGCCTGAAGCTGAAGAAGGA-----GACTGGTTCTCCG (-9)
AAACCGCAGAGCCTGAAGCTGAAGAAGGA-----GGACTGGTTCTCCG (-7)

sox9-766 (4/4)

AAACCGCAGAGCCTGAAGCTGAAGAAGGATACAGGGGGGACTGGTTCTCCG WT
AAACCGCAGAGCCTGAAGCTGAAGAAGGA-----GACTGGTTCTCCG (-9) [x2]
AAACCGCAGAGCCTGAAGCTGAAGAAGGA-----GGGACTGGTTCTCCG (-6)
AAACCGCAGAGCCTGAAGCTGAAGAAGGAACAGGGGGGACTGGTTCTCCG (-1, +1)

sox9-794 (4/4)

AAACTGCAGAGCCTGAAGCTGAAGAAGGACAGGGGGGACTGGTTCTCCG WT
AAACTGCAGAGCCTGAAGCTGAAG-----GGGGGACTGGTTCTCCG (-9)
AAACTGCAGAGCCTGAAGCTGAAGAAGGAG-----CAGGGGGACTGGTTCTCCG (-1)
AAACTGCAGAGCCTGAAGCTGAAGAAGGATACAGGGGGGACTGGTTCTCCG (-1, +1) [x2]

sox9-807 (5/5)

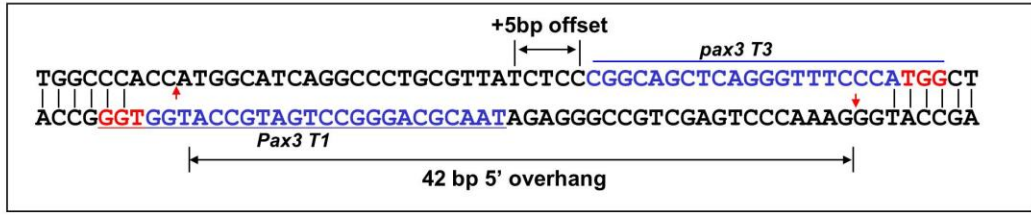
ACGTTTTTTTTTGCAGCAGCTAAAGAAGGAGACAGAGGCAGCTTGCTGCAGA WT
ACGTTTTTTTTT-----GAGGCAGCTTGCTGCAGA (-22)
ACGTTTTTTTTTGCAGCAGCTAAAaAaAaAaAa-----//---AGAGTGCAGA (-20, +5)
ACGTTTTTTTTTGCAGCAGCTAAAGAAGG---gGAGGCAGCTTGCTGCAGA (-5, +1)
ACGTTTTTTTTTGCAGCAGCTAAAGAAGG---AGAGGCAGCTTGCTGCAGA (-4)
ACGTTTTTTTTTGCAGCAGCTAAAGAAGGAGaCAGAGGCgGCTTGCTGCAGA (-1, +2)

sox9-815 (5/5)

GGGGCCTCTACTGGGGAGGTGAAGAAGGAGACAGGGGCCATTGGGGGGGTG WT
GGGGCCTCTACTG-----GGGGCCATTGGGGGGGTG (-19)
GGGGCCTCaACTGGtAAGGtAAGAAGG-GACcctGGGcctAGGGGGGTG (-10, +9)
GGGGCCTCTAGTttAAGGtAAGAAGGAGtCAGGGGCCATTGGGGGGGTG (-6, +6)
GGGGCCTCTACTGGGGAGGTGAAGAAGGA-----GGCCagtctgATGGGG (-5, +5)
GGGGCCTCTACTGGGGAGGtAAGAAGGAGAtgCAttGGGgggatGCCAGG (-1, +10)

Supplementary Fig. 4

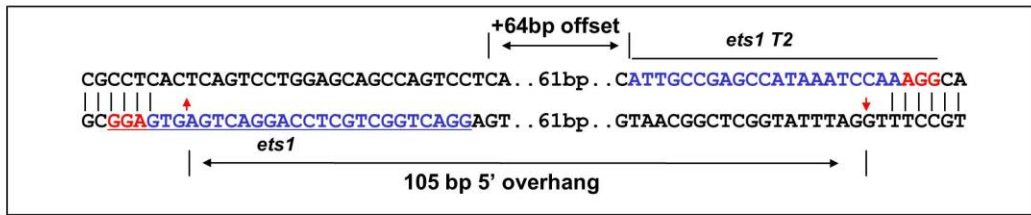
A



B



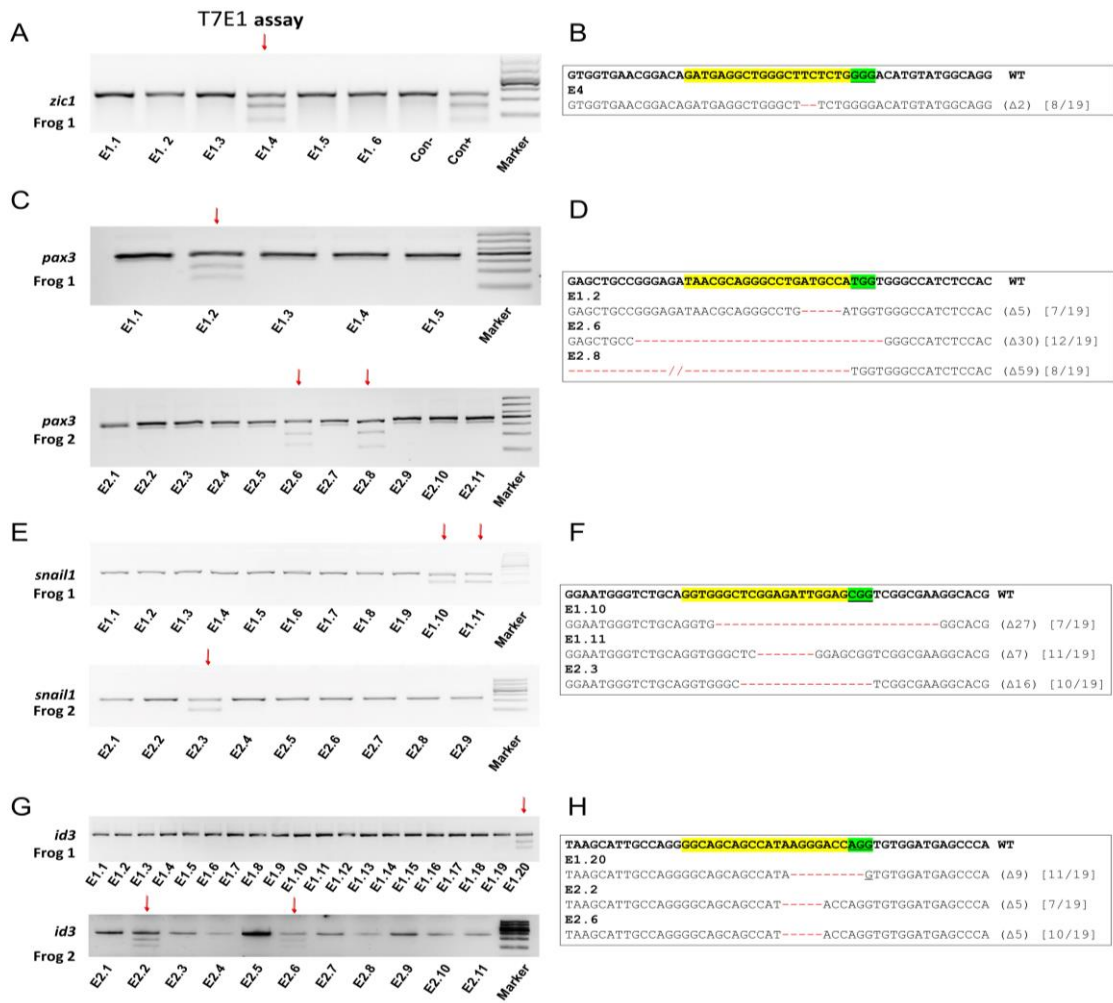
C



D



Supplementary Fig. 5



Supplementary Fig. 6

Gene	Target sequence (5'-3')	PAM	Oligonucleotide 1 (5'-3')	Oligonucleotide 2 (5'-3')
<i>pax3</i>	TACGCAGGGCCTGATGCCA	TGG	TAGGTAACGCAGGGCCTGATGCCA	AAACTGGCATCAGGCCCTGCCTTA
<i>pax3 T2</i>	GGGCTAATTCAGGGCCAGAC	TGG	TAGGGCTAATTCAGGGCCAGAC	AAACGTCTGGCCCTGAATTAGC
<i>pax3 T3</i>	CGGCAGCTCAGGGTTTCCCA	TGG	TAGGCGGCAGCTCAGGGTTTCCCA	AAACTGGGAAACCCCTGAGCTGCCG
<i>ctnnb</i>	GGAAAGCAGCAGTCAGTCAC	TGG	TAGGAAAGCAGCAGTCAGTCAC	AAACGTGACTGACTGCTGCTTT
<i>zic1</i>	GATGAGGCTGGGCTTCTCTG	GGG	TAGGATGAGGCTGGGCTTCTCTG	AAACCAGAGAAGCCCAGCCTCAT
<i>ap2a</i>	GGGATACCGCACCAGCTCTC	TGG	TAGGGATACCGCACCAGCTCTC	AAACGAGAGCTGGTGGCGTATC
<i>c-myc</i>	GGAGATGGTGACCGAATTCC	TGG	TAGGAGATGGTGACCGAATTCC	AAACGGAATTCGGTCACCATCT
<i>snail1</i>	GGTGGGCTCGGAGATTGGAG	CGG	TAGGTGGGCTCGGAGATTGGAG	AAACCTCCAATCTCCGAGCCCA
<i>snail1 T2</i>	GGGGGTGGGATTGGGGTGGG	AGG	TAGGGGTGGGATTGGGGTGGG	AAACCCACCCCAATCCACCCCC
<i>snail2</i>	GGAGAGCCCCATCAGCGATG	AGG	TAGGAGAGCCCCATCAGCGATG	AAACCATCGCTGATGGGGCTCT
<i>tyr</i>	GGAAGTGGCCCTGCAAACA	TGG	TAGGAAGTGGCCCTGCAAACA	AAACTGTTTGAGGGGCCAGTT
<i>foxD3</i>	GGACATCGATGTGGTGGGCG	AGG	TAGGACATCGATGTGGTGGGCG	AAACCGCCACCACATCGATGT
<i>sox9</i>	GGAGCTGAAGAAGGAGACAG	AGG	TAGGAGCTGAAGAAGGAGACAG	AAACCTGTCTCCTTCTTACGCT
<i>sox9 T2</i>	TTGGGGAAAGTGTCTCTTG	GGG	TAGGTTGGGGAAAGTGTCTCTTG	AAACCAAGAGAACACTTTCCCCAA
<i>ets1</i>	GGCTGCTCCAGGACTGAGTG	AGG	TAGGCTGCTCCAGGACTGAGTG	AAACCACTCAGTCTGGAGCAG
<i>ets1 T2</i>	TTGCCGAGCCATAAATCCAA	AGG	TAGGTTGCCGAGCCATAAATCCAA	AAACTTGATTTATGGCTCGGCAA
<i>ets2</i>	GGCCGCCAAGGAATTCTCCT	TGG	TAGCCGCCAAGGAATTCTCCT	AAACAGGAGAATTCTTGGCGG
<i>id3</i>	GGCAGCAGCCATAAGGGACC	AGG	TAGGCAGCAGCCATAAGGGACC	AAACGGTCCCTTATGGCTGCTG
<i>inca</i>	GGGTTCCTCCAGTCACGACA	AGG	TAGGGTTCCTCCAGTCACGACA	AAACTGTCGTGACTGGAGGAAC
<i>lrig3</i>	TGAAGATGATTCATGGAAC	GGG	TAGGTGAAGATGATTCATGGAAC	AAACAGTTCATGAATCATCTTCA
<i>lrig3 3'UTR</i>	GGGATTTTCTATTAAAATA	AGG	TAGGTATTCATCTTGAATGTACA	AAACTGTACATCCAAGATGAATA
<i>myoX</i>	GGTTGACAGATGCCAGGATG	GAG	TAGGTTGACAGATGCCAGGATG	AAACCATCCTGGCATCTGTCAA
<i>id3</i>	GGCAATGCTTAGACTCTGCT	CAG	TAGGCAATGCTTAGACTCTGCT	AAACAGCAGAGTCTAAGCATTG
<i>twist1</i>	GGGATCCTCAGGGCTTTTCC	TAG	TAGGGATCCTCAGGGCTTTTCC	AAACGGAAAAGCCCTGAGGATC

Supplementary Table 1

primers for T7E1 assay and colony PCR at the target sites with PAM -NGG			
Target gene	Primer 1	Primer 2	Primer 3
<i>pax3</i>	GCTGACAGGACCCAGGACTAA	CAGGGCCTGATGCCATG	CGCAGGAGAGTGTGAGAGCTT
<i>ctnnb</i>	AATGGAGCTGGACATGGCC	GGAAAGCAGCAGTCAGTCAC	CTGAGAGAAACCTTGCTCCCA
<i>zic1</i>	TGATTTTCCCAGGTCTCCA	GAGGCTGGGCTTCTCTGG	GGCTGCCTCATGTAACGAA
<i>ap-2a</i>	CGCCCTACCAGCCAATAT	CGGGGGATACCGCACCAGC	GTATGGAGTGTAAAGGCAGGT
<i>c-myc</i>	CCAGCAAAGCCGACTGCA	CGAGAAGCCGCTCCACATA	CAGCTGGAGATGGTGACCG
<i>snail</i>	GAAATTGCCGACTGTCCCC	TGGGCTCGGAGATTGGAG	TGCACCTGTTCCTCGT
<i>snai2</i>	AGCGATCTCTCCCCCTCT	AGTCTTTCCTCCTCATCGCT	TGTGCTTAGCCAACCCAGA
<i>tyr</i>	ATGGTCCCTCTGGCATTCT	CCTCCATGTTTGCAGGGGCC	CATTGGCACGTCTGTGTGA
<i>foxD3</i>	GCCCTGCCGAGCCTATTTT	ACCACATCGATGTCCGCA	CCGAGTTCGTCCGCTTCCT
<i>sox9</i>	CCAGCCGCTCAACTCTCT	ACCCGGAGCTGAAGAAGGA	GGCTTGCTCTTGCTGGAT
<i>ets1</i>	TTGGTGGGCAGGAATCATT	CCTCACTCAGTCTGGAGC	GTGTAGCCAGCTAGAGCAGC
<i>ets2</i>	CTGATCCCTTGCAGTGTGG	AATTCACGTTTTCCAAGGAGA	GGAAGACTGGCTCGTCCA
<i>id3</i>	AGCCATCAGCCAGTGAG	AGGGCAGCAGCCATAAG	TTCCCCAGGACTATCTGC
<i>inca</i>	TGGCATTGTGGAGGAGGA	GTGGGTTCTCCAGTCAC	AGACAGGCTGACAAGGAAGC
<i>lrig3</i>	CAATCTTTACATCCCCTTCC	ACTGTCTTCAATCAATCCCAG	CTGGTACAATTTGCAGTTTCA
<i>lrig3 3'utr</i>	GCTGGATACATAGCCGCATT	GTATATTCATCTTGAATGTACATG	TGCAAGTGTACAAGGCTTACA
primers for long fragment deletion and inversion assay			
<i>pax3 ts1F</i>	ATGTGCTTTTGGTTGGCATT		
<i>pax3 ts1R</i>	CTGTGCGACTTGCAACAAC		
<i>pax3 ts2F</i>	GACTTTCAGGGGTGGATTTG		
<i>pax3 ts2R</i>	TGGGCTCTGTGTACTAGGG		
<i>snail ts1f</i>	GAGCAGAGAGGGCATGTAGG		
<i>snail ts1r</i>	GATTTTGGATGGTGCCTCTG		
<i>snail ts2f</i>	TTGTGCAAGTCCATTGTGGT		
<i>snail ts2r</i>	CTGTTTCTCGTGCTTGTGA		
primers for colony PCR at the target sites with PAM -NAG			
Target gene	Primer 1	Primer 2	Primer 3
<i>myoX</i>	GCTGTCATACGCTACTTATCTGCT	GACAGATGCCAGGATGGAG	ATTGTCATGACGCTCCACA
<i>id3</i>	CCCTGGCACCAACTCTTTTA	AGCCATAAGGGACCAGGTG	AAGCCATCAGCCAGTGA
<i>twist1</i>	CCCAGTCTCCCCAGTAGACA	GATCCTCAGGGCTTTTCTAGT	CTGCGAAGGCTTCATTTAGG

Supplementary Table 2

On-target/ Off-target	Reference	Position	Coding/ Non-coding	Sequence
Irig3 - exon2	gi 288985368 ref NW_003163937.1 	608368	C	TGAAGATGATTCATGGA<u>ACTGGG</u>
Irig3-E306	gi 288974306 ref NW_003174987.1	7525	N	TAAAGAGGATTCATGGAAATGGG
Irig3-E306	gi 288974306 ref NW_003174987.1	7390	N	TAAAGAGGATTCATGGAAATGGG
Irig3 - 3'UTR	gi 288985368 ref NW_003163937.1 	620127	N	GGGATTTTTCTATTAA<u>ATAAGG</u>
Irig3-U931	gi 288985931 ref NW_003163374.1	2112551	N	GGCATTTTTCTATTAAACAGGG
Irig3-U890	gi 288985890 ref NW_003163415.1	1856747	N	TGAATTTTTCTATTAAATGGG
Irig3-U827	gi 288985827 ref NW_003163478.1	1830431	N	GAGATTTCTCTATCAAATATGG
Irig3-U571	gi 288985571 ref NW_003163734.1	752484	N	TAGATTTTTCTATTAGAATAGGG
Irig3-U967	gi 288985967 ref NW_003163338.1	6705	N	TGTATTATTCTATTAAATATGG
Irig3-U967	gi 288985967 ref NW_003163338.1	28014	N	TGTATTATTCTATTAAATATGG
sox9	gi 288985803 ref NW_003163502.1 	1305840	C	GGAGCTGAAGAAGGAGACAG<u>AGG</u>
sox9-815	gi 288985815 ref NW_003163490.1	629385	N	GGAGCTGAAGAAGGAGACAGGG
sox9-807	gi 288984807 ref NW_003164498.1	90668	N	GCAGCTAAAGAAGGAGACAGAGG
sox9-794a	gi 288985794 ref NW_003163511.1	655986	N	GAGCTGAAGAAGGAGACAGGG
sox9-794b	gi 288985794 ref NW_003163511.1	1172360	N	GAGCTGAAGAAGGAGACAGGG

sox9-826	gi 288985826 ref NW_003163479.1	329806	N	GGA <u>ACTGAAT</u> AAGGAGACAG <u>GG</u>
sox9-766	gi 288985766 ref NW_003163539.1	1033563	N	GAAGCTGAAGAAGGATACAG <u>GG</u>
sox9-600	gi 288985600 ref NW_003163705.1	294512	N	GAAGCTGAAGAAGGAAACAG <u>GG</u>
sox9-863	gi 288985863 ref NW_003163442.1	1884871	N	TGAGATGAAGCAGGAGACAG <u>AGG</u>
sox9-579	gi 288985579 ref NW_003163726.1	865328	C	AGAGCTGAAAAGGAGACAG <u>AGG</u>
foxd3	gi 288985975 ref NW_003163330.1 	5300676	C	GGACATCGATGTGGTGGGCG<u>AGG</u>
foxd3-745	gi 288985745 ref NW_003163560.1	849099	N	GGACA <u>GG</u> GATGTGGTGGG <u>GGGG</u>
pax3	gi 288985755 ref NW_003163550.1 	892370	C	TAACGCAGGGCCTGATGCCAT<u>GG</u>
pax3-534	gi 288985534 ref NW_003163771.1	524010	C	TAAGGCATGTCTCTGATGCCA <u>CGG</u>
zic1	gi 288985695 ref NW_003163610.1 	1003587	C	GATGAGGCTGGGCTTCTCTG<u>GGG</u>
zic1-871	gi 288985871 ref NW_003163434.1	1242807	N	GATCAAGCTGGGCTTCTCTGT <u>GG</u>
zic1-905	gi 288984905 ref NW_003164400.1	47502	N	CATTAGGCTGGGCTTCTCTG <u>GGG</u>
zic1-645	gi 288985645 ref NW_003163660.1	463191	N	CATGGGCTCTGGGCTTCTCTGT <u>GG</u>
AP2α	gi 288985946 ref NW_003163359.1 	1109930	C	GGGATACCGCACCAGCTCTCT<u>GG</u>
AP2α-963	gi 288985963 ref NW_003163342.1	590383	N	TGGATAGCGCACCAGGTCTC <u>AGG</u>
inca	gi 288985764 ref NW_003163541.1 	1385923	C	GGGTTCTCCAGTCACGACA<u>AGG</u>

inca-409	gi 288985409 ref NW_003163896.1	408790	N	GTCTTCCTCCAGTCAC <u>CACAAGG</u>
tyr	gi 288985760 ref NW_003163545.1 	1213668	C	GGAAGTGGCCCCTGCAAACAT<u>G</u>G
tyr-813	gi 288985813 ref NW_003163492.1	59728	N	GG <u>G</u> ACTGGCCCCTG <u>G</u> AAACA <u>A</u> GG
tyr-951	gi 288985951 ref NW_003163354.1	442151	N	GGAGATGGCCCCTTCAAACAG <u>G</u> GG
ctnnb	gi 288985314 ref NW_003163991.1 	133962	C	GGAAAGCAGCAGTCAGTCACT<u>G</u>G
ctnnb-835	gi 288977835 ref NW_003171470.1	15844	N	GAAAGTAGCAGT <u>G</u> AGTCACT <u>G</u> GG
ets1	gi 288985863 ref NW_003163442.1 	1450968	C	GGCTGCTCCAGGACTGAGTGAG<u>G</u>
ets1-302	gi 288984302 ref NW_003165003.1	3417	N	GGC <u>A</u> TCTCCAGG <u>G</u> CTGAGTGAG <u>G</u>
ets2	gi 288985577 ref NW_003163728.1 	296233	C	GGCCGCCAAGGAATTCTCCT<u>T</u>G
c-myc	gi 288985584 ref NW_003163721.1 	1036894	C	GGAGATGGTGACCGAATTCTCCT<u>G</u>
id3	gi 288985873 ref NW_003163432.1 	2578641	C	GGCAGCAGCCATAAGGGACC<u>A</u>G
snail1	gi 288985931 ref NW_003163374.1 	1055144	C	GGTGGGCTCGGAGATTGGAG<u>C</u>GG
snail2	gi 288985896 ref NW_003163409.1 	625775	C	GGAGAGCCCCATCAGCGATGAG<u>G</u>

Supplementary Table 3

Off-target sites	Forward Primer	Reverse Primer
pax3-534	GTTTGCTATCATTTCGATTGGAAG	CAAGACACTGGCTCTGAATGTGAC
zic1-871	AAGCTGACTGTATTATATCCCTTGAG	GGAGTTCAGTGGTGCCAGATG
zic1-905	TGGAACCATTAAGCAAGTGCC	ACAAATATCAGGCATCAGATACGG
lrig3-E306	GGTCCTTCAGGAAATTAAGGTCC	CGAATTTATGATGAACAGTTGCAG
lrig3-U931	AGTACTCCAGTTCTAGAGCATGCC	AACCACTGCCATTATAAATCTTGC
lrig3-U890	TTCTGGATAACGGGTCCTATGC	CAGCTATTTTGTGACAGAAATAGG
lrig3-U827	TTACATAACTGGCTACTGCTCATGG	GTTGTGCTTCTTATAGCAGCTGAAC
lrig3-U571	TTATTGGTTGCTATGGGTTACTGC	AACCAACAAAACGTACACCATTTG
lrig3-U967	CACAACAGCAGGTCTTTGACTACC	TGTTCCAGGAGACCATAGACC
ctn nb-835	TAGCTAAATAGCTGCACATACGCTC	AATCATGTTACTGACTTGTGCTC
ets1-302	CCAGTTTTACCTGTACCCTGATCTC	ATCCCATGTGTTTGGACAGCTAC
inca-409	CTGTATGCCCAATGTATGTAACCC	AGAAATTCAGTCTAATCCATAGCC
ap2a-963	AGCACTAAGTATTTGCTGAGTGCTG	TACAGGGATCTTGTTACCATTTGTG
foxD3-745	ATTTCTCTCCAAATTATTCTAGGCC	TATTGCCAATGGATTTGCTACC
tyr-813	CAAACTTACTCTTGCCAATACCAC	AGCACCTTGATGGCTTTTAATATC
tyr-951	GTCCAATGCTCTTCATATCATTGG	CATCTCATCATGAAACGTAGAGACC
sox9-815	TGCCCTAACTTACTCAGCAGGTAC	GATGCTAGAAAGTAAAGGTGGGC
sox9-807	AGAAAATGCAGGCTTGTAACCTTAC	ATATGAGCTATACATACAGGGCAGC
sox9-794	GGATTTGGAAGAAGATGGCAAC	GAATGTCGGGGGTGTGTTAATC
sox9-826	TTACCCACACCTTGAGATAAGG	CTCTTCCCATCCCATCTGAC
sox9-766	ATGTTCTTGATTTGGAAGAAG	AAGCTGGTTAAGAAGAAAATGTTTC
sox9-600	TCCGAATTCAGTGGAAGAGAGAG	TTCTGCAGTTTGCCTAAAAAGAAG
sox9-863	ATTGTCTGCTCAGCAATTTAAAATG	TTCGCTTGGTGCTACTTCTACTG
sox9-579	TGAAACTACCTGAAATAATGTTGGC	CTGTTTGCTATGGGTTGAAGACC

Supplementary Table 4

Supplementary Fig. 1. Sequences of somatic mutations induced by Cas9 and corresponding sgRNAs in *X. tropicalis* G0 embryos. The 2NLS indicates the sgRNAs were injected together with *Cas9-2NLS*. The sequences in bold are the wild type sequences, and the mutated regions are shaded in gray. The letters highlighted in yellow are the target sites, the letters in green are the PAMs. Red dashes indicated deletions (Δ), while low letters in blue represent insertions (+). The numbers in parentheses showed the number of deleted or inserted base pairs, while numbers in square brackets show the frequencies of the mutations in the sequenced samples.

Supplementary Fig. 2. CRISPR/Cas9 mediated mosaic gene knockout G0 *Xenopus*. (A-D) Pigmentary defects (A, B) or paralyzed upper limb (C, D) induced by coinjection of *Cas9* mRNA and *pax3* sgRNA. (E, F) loss of pigments caused by coinjection of *Cas9* mRNA and *tyrosinase (tyr)* sgRNA.

Supplementary Fig. 3. NAG is not an effective PAM sequence in *Xenopus* embryos. T7E1 result showing the mutation efficiency induced by sgRNA (with NAG as PAM) and *Cas9* at *twist1*, *id3* and *myosin X* genes. Genomic sequence of targeted *myosin X* locus is highlighted in yellow, PAM was in green/purple and underlined. PCR fragments of *myosin X* of 3.9% mutation rate were subcloned into the T-vector. However, none of the sequenced colonies harboured mutations at the *myosin X* locus (myoX, myosin X).

Supplementary Fig. 4. DNA sequences of off-target mutations induced by CRISPR/Cas9. For all the panels, wild types of off-target sites are shown on the top. sgRNA-DNA interface sequences are highlighted in yellow, while PAMs are in yellow and underlined. Red dashes indicate deletions (-) and lowercase letters in red indicate insertions (+) or substitutions. All the indels are highlighted in gray. The numbers in parentheses show the number of indels in all sequence number, whereas numbers in square brackets show the frequencies of the mutations in all the sequenced samples.

Supplementary Fig. 5. Double nicking induced genome editing in the loci of *pax3* and *ets1*. (A, C) sgRNA pairs targeting sites in the loci of *pax3* (A) or *ets1*(C). Letters in blue are the target sites and the PAMs are highlighted in red. The offset indicates

the base pairs between the 5' ends of two target sites. The 5' overhang indicates the base pairs between the cutting sites of Cas9 induced by two sgRNAs. (B, D) Sequencing data of the target sites in the loci of *pax3* (B) or *ets1* (D) induced by D10A with double nicking. Red dashes indicate the deletions. Lowercase letters indicate the insertions.

Supplementary Fig. 6. Germline transmission of mutant G0 *Xenopus*. The G0 frogs harboring disrupted genes as indicated were crossed with wild type frog, respectively. The genomic DNA extracted from individual embryos was PCR amplified, and then analyzed by T7E1 assay (A, C, E, G). The positive PCR amplicons were TA-cloned into pMD-18T and followed by sequencing to confirm germ line transmission of mutations (B, D, F, H). Letters in bold indicate wild type sequence, and PAM sequences were underlined. Red dashes indicate deletions. For each heterozygous embryo, 19 clones were selected randomly for sequencing. Red arrows indicated the DNA samples from embryos that harbor mutations. Con- or Con+, PCR products harboring mutations without or with T7E1 treatment.

Supplementary Table 1. The target sites, PAM and oligos of each locus. The DNA sequences with the NGG PAM targeted in this study and oligonucleotides used to subclone the spacer sequences into the sgRNA expression vector. In some cases, extra nucleotides GG were added to the oligo1 to complement the BsaI cloning site on the sgRNA expression vector. NAG PAMs were shown in bold.

Supplementary Table 2. Primers used in this study for identifying target site mutations in T7E1 assay and colony PCR. All of the primer sequences are written in 5'- 3', from left to right.

Supplementary Table 3. The potential off-target sites of the 16 loci knocked out in this study. The sequences in bold font is the target sites. The PAMs are underlined. The mismatched nucleotides in off-target sites are highlighted in red.

Supplementary Table 4. Primers used in this study for identifying off-target effect by T7 Endonuclease 1 and colony PCR in T7E1 and colony PCR. All of the primer sequences are written in 5'- 3', from left to right.

