

**Name of Mouse model or mutation:**

**ZFHX3-FLOX-EM1-B6N**

**Description:**

Floxed allele made by CRISPR/Cas9 gene editing.

**Type of mutation:**

Floxing of exon ENSMUSE00000326565.

**Delivery method:**

Pronuclear injection into 1-cell stage embryo.

**Genetic Background:**

C57BL/6NTac

**Nuclease:**

Cas9 mRNA

**sgRNAs:**

Protospacer sequence	PAM sequence
ACGAGCTCTCGTCCCCTCCC	AGG
GTTAGGCGGTTCTGACAGAC	AGG
ACGCATGAATGGCGCACTCT	GGG
GTGTTTAGCATACAGCGAGA	TGG

**IssDNA donor sequence template (5'-3', NOTE donor delivered to embryo will be reverse complement (Codner et al., 2018: Figure 1)):**

LOCUS Zfhx3 1638 bp DNA linear 28-JUN-2021

FEATURES Location/Qualifiers

misc\_feature 130..163

/note="loxP"

misc\_feature 122..129

/note="AsiSI (SfaAI)"

PCR\_primer 101..121

/note="LoxPF"

misc\_feature 1..100

/note="5'HA"

misc\_feature 164..1478  
     /note="CR"  
 PCR\_primer 8..28  
     /note="lss\_Zfhx3\_Flox\_F1"  
 misc\_feature 680..1176  
     /note="Zfhx3-204 Exon9: ENSMUSE00000326565"  
 misc\_feature 1177..1478  
     /note="intron 9-10"  
 misc\_feature complement(1474..1478)  
     /note="Zfhx3\_Flox\_3\_3 ID: 536830681"  
 misc\_feature 1479..1512  
     /note="loxP"  
 misc\_feature 1513..1520  
     /note="Mrel"  
 PCR\_primer complement(1521..1540)  
     /note="LoxPR"  
 misc\_feature 1541..1638  
     /note="3'HA"  
 PCR\_primer complement(1619..1638)  
     /note="Zfhx3\_RNR\_R"  
     /note=""  
 PCR\_primer complement(1603..1627)  
     /note="lss\_Zfhx3\_Flox\_R1"  
 source 1..1638  
     /dnas\_title="Zfhx3 lssDNA donor"

ORIGIN

1 gtggagggct agctatgacc caggattcca tgtgatacag ggtcttgaa aaggaagatg  
 61 ttctagaaa gggtaagagg tgggttaggc ggttctgaca atccgggggt accgcgtcga  
 121 gGCGATCGCA TAACTTCGTA TAGCATACAT TATACGAAGT TATaggggac gagagctcgt  
 181 tactgaaatc tcttacagta tagttagctg tgctgcagtg tggccattga tattaacatt  
 241 aatagcaatg aaaaattcag tttcttgctg tntagacagc aaggtacccc atagcccact  
 301 tcttaccatg gcaggaagtt ttgctccgag atcatgtagg tgaatacttg gccacttta  
 361 ggtttctacc agaggcctgg tattatggac atttccgatt ctggcagtag gttgggtct  
 421 ggactttcag atcctgtgtg atacaaaggg tgatgtggac ttagctcatt tgttgatcc  
 481 tgaagagggg ggcgcattga gatcattcac ggggccacag tccataaatg tacctagctg  
 541 ttcttattcc accatcacag actatcaaaa cacagcagga ggattttgtt tcaccagcca

601 ctagcacctt gcagggttg ttctctgtac catggcttcg aggctgactt ctgactgtct  
661 tcctctacct tcctacagT GGGCGGTGAG ATCCCCCTCG ACATGCGCCT CGGGGGCGGG  
721 CAGCTGGTGT CGGAGGAGCT GATGAACCTG GGCGAGAGCT TCATCCAGAC CAACGACCCA  
781 TCGCTGAAGC TCTCCAATG TGCCGTCTGC AACAAGTTCA CGACGGACAA CCTGGACATG  
841 CTGGGGCTGC ACATGAACGT GGAGCGCAGC CTCTCGGAGG ACGAGTGGAA GGCCGTGATG  
901 GGGGACTCTT ACCAGTGCAA GCTCTGCCGC TACAACACGC AGCTCAAGGC CAACTTCCAG  
961 CTGCACTGCA AGACGGACAA GCACGTGCAG AAGTACCAGC TGGTGGCCCA CATCAAGGAG  
1021 GGTGGCAAGG CCAATGAGTG GAGGCTCAAG TGTGTGGCCA TCGGCAACCC TGTGCACCTG  
1081 AAGTGCAACG CTTGTGACTA CTACACCAAT AGCCTGGAGA AGCTGCGGCT GCACACAGTC  
1141 AACTCCAGGC ACGAGGCCAG CCTGAAGCTG TACAAGgtga ggtcgacctc atgagacca  
1201 gggacggtct ctccacaggg caaactaact ctaaaggttt tgcattccagt gcccagtga  
1261 gcctgagatt gtgtcgatgg cttcctctgg tggctgaagg ttgggggtgt ctcatctcag  
1321 ggggcagggtt aatttccagc agcccactac tgtgtggctc ctttctttgg tgctggtctc  
1381 tgcagtagtc tgttgaatct ccctcagctg ttgcggacca gcctcccttt ctatcctttg  
1441 gtctatcttt tatttctca ctctctgtg ctcccagaAT AACTTCGTAT AGCATACATT  
1501 ATACGAAGTT ATCGCCGGCG ggtctgagct cgccatcagt cgctgtatgc taaacacctt  
1561 atgtatactg accacttatt gttctggaag tatatcagtg cacataagat agccaatac  
1621 aaaccctgc tcacacac

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#### **Pronuclear Microinjection mixes:**

Microinjection buffer (MIB; 10 mM Tris-HCl, 0.1 mM EDTA, 100 mM NaCl, pH7.5) was prepared and filtered through a 2 nm filter and autoclaved. Cas9 mRNA, sgRNAs and ssODNs were diluted and mixed in MIB to the working concentrations of 100 ng/ $\mu$ l, 50 ng/ $\mu$ l each and 50 ng/ $\mu$ l, respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F<sub>0</sub> progeny.

## Sequence details

### Zfhx3 WT

GCCATTTAGAACAAGGCTGGACTCTGGCCCTAGAGGGCCCCACCTCAGTAGAGATGCAGCTGCGA  
TACTGTCTTGTGGAGGGCTAGCTATGACCCAGGATTCCATGTGATACAGGGTCTTGGAAAAGGAAG  
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GGGGCCTGGGAGGGGACGAGAGCTCGTTACTGAAATCTCTTACAGTATAGTTAGCTGTGCTGCAGT  
GTGGCCATTGATATTAACATTAATAGCAATGAAAAATTCAGTTTCTTGCTGTGTAGACAGCAAGGTA  
CCCCATAGCCCACTTCTACCATGGCAGGAAGTTTTGCTCCGAGATCATGTAGGTGAATACTTGGCC  
CACTTTAGGTTTCTACCAGAGGCCTGGTATTATGGACATTTCCGATTCTGGCAGTAGGTTGGGGTCT  
GGACTTTAGATCCTGTTGTATACAAAGGGTGATGTGGACTTAGCTCATTGTTGGATCCTGAAAGA  
GGGGGCGCATTGAGATCATTACGGGGCCACAGTCCATAAATGTACCTAGCTGTTCTTATTCCACCA  
TCACAGACTATCCAAACACAGCAGGAGGATTTTGTTCACCAGCCACTAGCACCTTGCAGGGTTTGT  
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GATCCCCCTCGACATGCGCCTCGGGGGCGGGCAGCTGGTGTGCGGAGGAGCTGATGAACCTGGGC  
GAGAGCTTCATCCAGACCAACGACCCATCGCTGAAGCTCTTCCAATGTGCCGTCTGCAACAAGTTC  
ACGACGGACAACCTGGACATGCTGGGGCTGCACATGAACGTGGAGCGCAGCCTCTCGGAGGACG  
AGTGGAAAGGCCGTGATGGGGACTCTTACCAGTGCAAGCTCTGCCGCTACAACACGCAGCTCAAG  
GCCAACTTCCAGCTGCACTGCAAGACGGACAAGCACGTGCAGAAGTACCAGCTGGTGGCCACAT  
CAAGGAGGGTGGCAAGGCCAATGAGTGGAGGCTCAAGTGTGTGGCCATCGGCAACCCTGTGCAC  
CTGAAGTGCAACGCTTGTGACTACTACCAATAGCCTGGAGAAGCTGCGGCTGCACACAGTCAA  
CTCCAGGCACGAGGCCAGCCTGAAGCTGTACAAGGTGAGGTCGACCTCATGAGACCCAGGGACGG  
TCTCTCCACAGGGCAAATACTCTAAAGTTTTGCATCCAGTGCCCAGTGAAGCCTGAGATTGTGT  
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GCCCACTACTGTGTGGCTCCCTTCTTGGTGCTGGTCTCTGCAGTAGTCTGTTGAATCTCCCTCAGCT  
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TGCGCCATTATGCGTGCATTTTGTATCCATCTCGCTGTATGCTAAACACCTTATGTATACTGACCA  
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ATACACATATATACATATATATGTATATACACACACACATACATATGTGTATATGCACATACATATAA  
ATACATGTATACATGTTTATACAGATATGCACACATGTATGTACTIONCACATCCAGATGCCTGCCCTCAC  
AGCGTTTTTATTCTTT

### ZFHX3-FLOX-EM1-B6N

GCCATTTAGAACAAGGCTGGACTCTGGCCCTAGAGGGCCCCACCTCAGTAGAGATGCAGCTGCGA  
TACTGTCTTGTGGAGGGCTAGCTATGACCCAGGATTCCATGTGATACAGGGTCTTGGAAAAGGAAG  
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GATCGCATAACTTCGTATAGCATAATTATACGAAGTTATAGGGGACGAGAGCTCGTTACTGAAATC  
TCTTACAGTATAGTTAGCTGTGCTGCAGTGTGGCCATTGATATTAACATTAATAGCAATGAAAAATTC  
AGTTTCTTGCTGTGTAGACAGCAAGGTACCCCATAGCCCACTTCTACCATGGCAGGAAGTTTTGCTC

CGAGATCATGTAGGTGAATACTTGGCCCACTTTAGGTTTCTACCAGAGGCCTGGTATTATGGACATT  
TCC**A**ATTCTGGCAGTAGGTTGGGGTCTGGACTTTTCAGATCCTGTTGTATACAAAGGGTGATGTGGAC  
TTAGCTCATTGTTGGATCCTGAAAGAGGGGGCGCATTGAGATCATTACGGGGCCACAGTCCATAA  
ATGTACCTAGCTGTTCTTATTCCACCATCACAGACTATCCAAACACAGCAGGAGGATTTTGTTCACC  
AGCCACTAGCACCTTGCAAGGTTTGT **[1 nt deletion]**  
TCTGTACCATGGCTTCGAGGCTGACTTCTGACTGTCTTCTCTACCTTCCCTACAGT**GGGCGGTGAGA**  
**TCCCCCTCGACATGCGCCTCGGGGGCGGGCAGCTGGTGTGCGGAGGAGCTGATGAACCTGGGCGA**  
**GAGCTTCATCCAGACCAACGACCCATCGCTGAAGCTCTTCCAATGTGCCGTCTGCAACAAGTTCAC**  
**GACGGACAACCTGGACATGCTGGGGCTGCACATGAACGTGGAGCGCAGCCTCTCGGAGGACGAG**  
**TGGAAGGCCGTGATGGGGGACTCTTACCAGTGCAAGCTCTGCCGCTACAACACGCAGCTCAAGGC**  
**CAACTCCAGCTGCACTGCAAGACGGACAAGCACGTGCAGAAGTACCAGCTGGTGGCCACATCA**  
**AGGAGGGTGGCAAGGCCAATGAGTGGAGGCTCAAGTGTGTGGCCATCGGCAACCCTGTGCACCT**  
**GAAGTGAACGCTTGTGACTACTACCAATAGCCTGGAGAAGCTGCGGCTGCACACAGTCAACT**  
**CCAGGCACGAGGCCAGCCTGAAGCTGTACAAGGTGAGGTCGACCTCATGAGACCCAGGGACGGTC**  
TCTCCACAGGGCAAATACTAAAGTTTTGCATCCAGTGCCCAGTGAAGCCTGAGATTGTGTGC  
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CCACTACTGTGTGGCTCCCTTCTTTGGTGTGGTCTCTGCAGTAGTCTGTTGAATCTCCCTCAGCTGTT  
GCGGACCAGCCTCCCTTCTATCCTTTGGTCTATCTTTTATTTCTTCACTCCTCTGTGCTCCAGA**AATAA**  
CTTCGTATAGCATAATTATACGAAGTTATCGCCGGC**A**GGTCTGAGCTCGCCATCAGTCGCTGTATGC  
TAAACACCTTATGTATACTGACCACTTATTGTTCTGGAAGTATATCAGTGACATAAGATAGCCCAAT  
ACAAACCCGTGCTCACACACACACATACATATATACATATATATGTATATACACACACACATACAT  
ATGTGTATATGCACATACATATAAATACATGTATACATGTTTATACAGATATGCACACATGTATGTAC  
TCACATCCAGATGCCTGCCCTCACAGCGTTTTTCATTCTTT

*LoxP sites are underlined and genotyping handles (restriction enzyme site plus primer unique to each LoxP site) in italics. Floxed exon highlighted in bold. Nucleotide highlighted in bold and red is an intronic point mutation. Nucleotide in bold, red and italic is a point mutation in the MreI site. Nucleotide highlighted in red, bold and underlined is deleted in the mutant intronic sequence.*

## Nucleotide Alignment:

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                *      20      *      40      *      60      *      80      *      100     *      120
Zfhx3_WT : GGCATTTAGAACAAAGGCTGGACTCTGGCCCTAGAGGGCCCCACCTCAGTAGAGATGCAGCTGCGATACTGTCTTGTGGAGGGCTAGCTATGACCCAGGATTCCATGTGATACAGGGTCT : 120
Zfhx3_EM1 : GGCATTTAGAACAAAGGCTGGACTCTGGCCCTAGAGGGCCCCACCTCAGTAGAGATGCAGCTGCGATACTGTCTTGTGGAGGGCTAGCTATGACCCAGGATTCCATGTGATACAGGGTCT : 120

                *      140     *      160     *      180     *      200     *      220     *      240
Zfhx3_WT : TGGAAAAGGAAGATGTTTCTAGAAAGGTAAGAGGTGGGTAGGCCGTTCTGACAGACAGGTTAGGATAGTGATAAGAGGGGCTGGG-----AG : 209
Zfhx3_EM1 : TGGAAAAGGAAGATGTTTCTAGAAAGGTAAGAGGTGGGTAGGCCGTTCTGACAGATCCGGGGTACC CGCTCGAGGCGATCGCATAA CTTCTGTATAGCATACATTATACGAAGTTAAG : 240

                *      260     *      280     *      300     *      320     *      340     *      360
Zfhx3_WT : GGGACGAGAGCTCGTTACTGAAATCTCTTACAGTATAGTTAGCTGTGCTGCAGTGTGCCATTGATATTAACATTAATAGCAATGAAAAATTCAGTTTCTTGCTGTGTAGACAGCAAGGT : 329
Zfhx3_EM1 : GGGACGAGAGCTCGTTACTGAAATCTCTTACAGTATAGTTAGCTGTGCTGCAGTGTGCCATTGATATTAACATTAATAGCAATGAAAAATTCAGTTTCTTGCTGTGTAGACAGCAAGGT : 360

                *      380     *      400     *      420     *      440     *      460     *      480
Zfhx3_WT : ACCCATAGCCCACTTCCCTACCATGGCAGGAAGTTTGTCTCCGAGATCATGTAGGTGAATACTTGGCCCACTTTAGGTTTCTACCAGAGGCCTGGTATTATGGACATTTCCATTCTGGC : 449
Zfhx3_EM1 : ACCCATAGCCCACTTCCCTACCATGGCAGGAAGTTTGTCTCCGAGATCATGTAGGTGAATACTTGGCCCACTTTAGGTTTCTACCAGAGGCCTGGTATTATGGACATTTCCATTCTGGC : 480

                *      500     *      520     *      540     *      560     *      580     *      600
Zfhx3_WT : AGTAGGTTGGGGTCTGGACTTTCAGATCCTGTTGTATACAAAGGGTGATGTGGACTTAGCTCATTTGTTGGATCCTGAAAGAGGGGGCGCATTGAGATCATTACGGGGCCACAGTCCAT : 569
Zfhx3_EM1 : AGTAGGTTGGGGTCTGGACTTTCAGATCCTGTTGTATACAAAGGGTGATGTGGACTTAGCTCATTTGTTGGATCCTGAAAGAGGGGGCGCATTGAGATCATTACGGGGCCACAGTCCAT : 600

                *      620     *      640     *      660     *      680     *      700     *      720
Zfhx3_WT : AAATGTACCTAGCTGTTCTTATTCACCATCACAGACTATCCAACACAGCAGGAGGATTTTGTTCACCAGCCACTAGCACCTTGCAGGGTTTGTCTCTGTACCATGGCTTCGAGGCT : 689
Zfhx3_EM1 : AAATGTACCTAGCTGTTCTTATTCACCATCACAGACTATCCAACACAGCAGGAGGATTTTGTTCACCAGCCACTAGCACCTTGCAGGGTTTGTCTCTGTACCATGGCTTCGAGGCT : 719

                *      740     *      760     *      780     *      800     *      820     *      840
Zfhx3_WT : GACTTCTGACTGTCTTCCCTACCTTCCCTACAGTGGGCGGTGAGATCCCCCTCGACATGCGCCTCGGGGGCGGGCAGCTGGTGTGCGAGGAGCTGATGAACCTGGGCGAGAGCTTCATC : 809
Zfhx3_EM1 : GACTTCTGACTGTCTTCCCTACCTTCCCTACAGTGGGCGGTGAGATCCCCCTCGACATGCGCCTCGGGGGCGGGCAGCTGGTGTGCGAGGAGCTGATGAACCTGGGCGAGAGCTTCATC : 839

                *      860     *      880     *      900     *      920     *      940     *      960
Zfhx3_WT : CAGACCAACGACCCATCGCTGAAGCTCTTCCAATGTGCCGCTGCAACAAGTTCACGACGGACAACCTGGACATGCTGGGGCTGCACATGAACCTGGAGCGCAGCTCTCGGAGGACGAG : 929
Zfhx3_EM1 : CAGACCAACGACCCATCGCTGAAGCTCTTCCAATGTGCCGCTGCAACAAGTTCACGACGGACAACCTGGACATGCTGGGGCTGCACATGAACCTGGAGCGCAGCTCTCGGAGGACGAG : 959

                *      980     *      1000    *      1020    *      1040    *      1060    *      1080
Zfhx3_WT : TGGAAGGCCGTGATGGGGACTCTTACCAGTGCAAGCTCTGCCGCTACAACACGCAGCTCAAGGCCAACTTCCAGCTGCACTGCAAGACGGACAAGCACGTGCAGAAGTACCAGCTGGTG : 1049
Zfhx3_EM1 : TGGAAGGCCGTGATGGGGACTCTTACCAGTGCAAGCTCTGCCGCTACAACACGCAGCTCAAGGCCAACTTCCAGCTGCACTGCAAGACGGACAAGCACGTGCAGAAGTACCAGCTGGTG : 1079

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*      1100      *      1120      *      1140      *      1160      *      1180      *      1200
Zfhx3_WT : GCCCACATCAAGGAGGGTGGCAAGGCCAATGAGTGGAGGCTCAAGTGTGTGGCCATCGGCAACCCCTGTGCACCTGAAGTGCAACGCTTGTGACTACTACACCAATAGCCTGGAGAAGCTG : 1169
Zfhx3_EM1 : GCCCACATCAAGGAGGGTGGCAAGGCCAATGAGTGGAGGCTCAAGTGTGTGGCCATCGGCAACCCCTGTGCACCTGAAGTGCAACGCTTGTGACTACTACACCAATAGCCTGGAGAAGCTG : 1199

*      1220      *      1240      *      1260      *      1280      *      1300      *      1320
Zfhx3_WT : CGGCTGCACACAGTCAACTCCAGGCACGAGGCCAGCCTGAAGCTGTACAAGGTGAGGTCGACCTCATGAGACCCAGGGACGGTCTCTCCACAGGGCAAATAACTCTAAAGGTTTTGCAT : 1289
Zfhx3_EM1 : CGGCTGCACACAGTCAACTCCAGGCACGAGGCCAGCCTGAAGCTGTACAAGGTGAGGTCGACCTCATGAGACCCAGGGACGGTCTCTCCACAGGGCAAATAACTCTAAAGGTTTTGCAT : 1319

*      1340      *      1360      *      1380      *      1400      *      1420      *      1440
Zfhx3_WT : CCAGTGCCAGTGAAGCCTGAGATTGTGTCGATGGCTTCCTCTGGTGGCTGAAGGTTTGGGGTGTCTCATTTTCAGGGGGCAGGTTAATTTCCAGCAGCCCACTACTGTGTGGCTCCCTTC : 1409
Zfhx3_EM1 : CCAGTGCCAGTGAAGCCTGAGATTGTGTCGATGGCTTCCTCTGGTGGCTGAAGGTTTGGGGTGTCTCATTTTCAGGGGGCAGGTTAATTTCCAGCAGCCCACTACTGTGTGGCTCCCTTC : 1439

*      1460      *      1480      *      1500      *      1520      *      1540      *      1560
Zfhx3_WT : TTTGGTGCCTGGTCTCTGCAGTAGTCTGTTGAATCTCCCTCAGCTGTTGCGGACCAGCCTCCCTTTCTATCCTTTGGTCTATCTTTTATTTCTTCACTCCCTCTGTGCTCCCAGAGTGGCGCC : 1529
Zfhx3_EM1 : TTTGGTGCCTGGTCTCTGCAGTAGTCTGTTGAATCTCCCTCAGCTGTTGCGGACCAGCCTCCCTTTCTATCCTTTGGTCTATCTTTTATTTCTTCACTCCCTCTGTGCTCCCAGAGTGGCGCC : 1559

*      1580      *      1600      *      1620      *      1640      *      1660      *      1680
Zfhx3_WT : ATTCATGCGTGCATTTTGTATCCATCT-----CGCTGTATGCTAAACACCTTATGTATACTGACCACTTATGTTCTGGAAGTATATCAGTGCACAT : 1623
Zfhx3_EM1 : CGTATAGCATACATTATACGAAGTTA|CGCCGGCA|GGTCTGAGCTCGCCATCAGT|CGCTGTATGCTAAACACCTTATGTATACTGACCACTTATGTTCTGGAAGTATATCAGTGCACAT : 1679

*      1700      *      1720      *      1740      *      1760      *      1780      *      1800
Zfhx3_WT : AAGATAGCCCAATACAAACCCGTGCTCACACACACACATACACATATATACATATATATGTATATACACACACACATACATATGTGTATATGCACATACATATAAATACATGTATACATG : 1743
Zfhx3_EM1 : AAGATAGCCCAATACAAACCCGTGCTCACACACACACATACACATATATACATATATATGTATATACACACACACATACATATGTGTATATGCACATACATATAAATACATGTATACATG : 1799

*      1820      *      1840      *      1860
Zfhx3_WT : TTTATACAGATATGCACACATGTATGTACTCACATCCAGATGCCCTGCCCTCACAGCGTTTTTCATTCTTT : 1812
Zfhx3_EM1 : TTTATACAGATATGCACACATGTATGTACTCACATCCAGATGCCCTGCCCTCACAGCGTTTTTCATTCTTT : 1868

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**QC strategy employed at Harwell to check the edited allele:**

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_Zfhx3_Flox_F1 (5'-3')	GCCATTTAGAACAAGGCTGGAC
Geno_Zfhx3_Flox_R1 (5'-3')	AAAGAATGAAAACGCTGTGAGGG
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	1812
Mutant product size (bp)	1869
Notes	This PCR sits externally to the floxed region

LoxPF (5'-3')	ATCCGGGGGTACCGCTCGAG
LoxPR (5'-3')	ACTGATGGCGAGCTCAGACC
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	N/A
Mutant product size (bp)	1440
Notes	This PCR is used to screen for floxed alleles.

Geno_Zfhx3_Flox_F1 (5'-3')	GCCATTTAGAACAAGGCTGGAC
LoxPR (5'-3')	ACTGATGGCGAGCTCAGACC
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	N/A
Mutant product size (bp)	1615

Notes	This PCR is used to screen for on-target floxed alleles.
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LoxPF (5'-3')	ATCCGGGGGTACCGCGTCGAG
Geno_Zfhx3_Flox_R1 (5'-3')	AAAGAATGAAAACGCTGTGAGGG
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	N/A
Mutant product size (bp)	1694
Notes	This PCR is used to screen for on-target floxed alleles.

All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences should be heterozygous unless on sex chromosome.

**Off-target site with  $\leq 2$  mismatches for guide(s) used were checked with the following primers:**

Off-target site	Sequence	Type	Primers used (5'-3')
<a href="#">8:119706019-119706041</a>	CCCAGCTCTAGTCCCAATCCC CGG	Intronic	Zfhx3_Flox_OT1F1: GAGTCCCTAGTTTTGTAATGTTTCC Zfhx3_Flox_OT1R1: CCCAACGTGCACCTGATAC
<a href="#">8:57600268-57600290</a>	CTGAGGTGGTTCTGACAGAC CGG	Intronic	Zfhx3_Flox_OT2F1: GCGGTCAGTAGGCATAACAAG Zfhx3_Flox_OT2R1: CTTGGCATCGCTGTAAATGCT

All amplicons were sent for Sanger sequencing.

No off-target activity was detected in the animals selected to establish the colony.

#### **Additional integrations of the donor sequence**

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to copy count the donor sequence compared against a VIC-labelled reference assay for Dot1l:

Assay name	Zfhx3-Flox-5'-MUT1
Forward Primer (5'-3')	AGGGTAAGAGGTGGGTTAG
Reverse Primer (5'-3')	GCACAGCTAACTATACTGTAAGA
Probe (5'-3')	TCGAGGCGATCGCATAACTTCG
Label	FAM

This ddPCR assay is specific to the donor used to create the engineered mutation and only mutant alleles are expected to be recognised by this assay. Therefore, WT controls are expected to call at 0 copies and a single integration for a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Assay name	Zfhx3-CR-LOA-WT1
Forward Primer (5'-3')	CAAGTTCACGACGGACAA
Reverse Primer (5'-3')	TTGAGCTGCGTGTGTAG
Probe (5'-3')	ACTCTTACCAGTGCAAGCTCTGCC
Label	FAM

This ddPCR assay is universal; both the WT and mutant alleles are recognised by this assay. Therefore, WT controls are expected to call at 2 copies and a single integration for a correct mutation is expected to call at 2 copies for F1 (HET) animals.

Assay name	Zfhx3-Flox-3'-MUT1
Forward Primer (5'-3')	GCTCCCAGAATAACTTCGTATAG
Reverse Primer (5'-3')	GCACTGATATACTCCAGAACA
Probe (5'-3')	AAGTTATCGCCGGCGGGTCTGA
Label	FAM

This ddPCR assay is specific to the donor used to create the engineered mutation and only mutant alleles are expected to be recognised by this assay. Therefore, WT controls are expected to call at 0 copies and a single integration for a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Reference Assay Name	Dot1l
Forward primer (5'-3')	GCCCCAGCACGACCATT

Reverse primer (5'-3')	TAGTTGGCATCCTTATGCTTCATC
Probe (5'-3')	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay for Dot1l gene.

No additional donor integrations were detected in the animals taken forward to establish the colony.