

Name of Mouse model or mutation:

ACVR2B-FLOX-EM2-B6N

Description:

Floxed allele made by CRISPR/Cas9 gene editing.

Type of mutation:

Floxing of exons ENSMUSE00000582984 and ENSMUSE00000582983.

Delivery method:

Cytoplasmic injection into 1-cell stage embryos generated by IVF (Het ACVR2B-FLOX-INTER-EM1-B6N male x WT females).

Genetic Background:

C57BL/6NTac

Nuclease:

Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
ATCTCTCATGCTTGCCTGGC	AGG
ATCTCCTCGGTCCCTGAGTT	TGG

ssODN donor sequence (5'-3'):

CACCTAACTACATGGCTGCCTGGTGTGTCAGAAGCCTCTGAGTATACACAATGCCAAAAACCAA
 CAGATAACTTCGTATAATGTATGCTATACGAAGTTATGCGATCGCctcgacgcggtacccccggatCTGC
 CAGGCAAGCATGAGAGATTCCCAGCCCCTGGACACAGTGGAGTGTAACCCCAGTGCTGGAAGC
 AG

Cytoplasmic 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 50 ng/μl, 6.5 ng/μl each and 100 ng/μl, respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F₀ progeny.

Sequence details

Acvr2b WT

TAGGGTCAGACGCCATGTTGGTTTTGGGTTCTGCCATTACTTCCGTGTTGTGTATGCAGTTTGCATGT
TTTGTGTACGCACAGGTCTGTGTGTGGAGGCCAGAGAATGAATCCCTTTATTGGACTCCTTTTTATTT
TTTTGGAGACAGGATTTTCAAGTATGTAATCCAGACTGGCCTAGAACTTGTATATAGACCAGGCTGGC
CTTGAACCAAAAAGTCTACCTGCCTCCACCTCCCAGATGCTAGAAATTAAGGTGTGCACCACCAAACT
GGCTCTCTACTTTTTACAGTTCTTTATTTTTATTCCTTTTTAATGAAAAAAAAAATTTTTTTTTTTTTCT
GTTTTTTCGAGACAGCCCTGTGTAGCCCTGCTGTCCTGGAACCTCACTTTGTAGATCAGGCTAGCCTCG
AACTCAGAAATCCACCTGCCTCTGTTTCCCAAGTGCTGGGATTAAAGGCGTGCGCCACCACCGCCTG
GCCCTCAGTGAAAATATTTTTATGTGTGCAGGTGCTTTTTTGGCCGCATGTATCTATGTGTCCTGCAT
GTGTGCCTGGTGCCTGAAGAAGCCAGAAGGGAGTGTTGGGTCCTTGAGACTGGAGTTATAGACAGT
TGTGAGCCACCATACATGTGCTGGGAATTGAACCCGGGTGCTCAGGGAGAGTGGCAGTGCTCTTAA
CCTCAGAGCCACTTCTCTAGCCTCTACGTTTTAAGATTTTTTGTAGACAAGGCCTCTCTTAGCCTGGAA
CTCACCGGTTTTGGTTGACGAGTTGGCAAACAAGTCCCAGGAACTCCACCTCTTCCTGCTTCCAGCACT
GGGGTTACACTCCACTGTGTCCAGGGGCTGGGAATCTCTCATGCTTGCCTGGCAGGCACCTCCGGA
GCATCTCCTCGGTCCCTGAGTTTGGTTTTTGGCATTGTGTATACTCAGAGGCTTCTGACACCAGGCAG
CCATGTAGTTAGGTGCTCAGGGTACTTAGGAAGACAAGCATTGTGCTGGCTGATACTGGCTGGTCACC
TCACTCAGCATTGTAAATCAGGGCGCTGTTGTGCTAGCTACCCAGAGATCGCTATTGGCCTGGTCTTT
GGATGCTGAGGCTAGGAAATCGGGGTGGGGATGGAGTAGGGTGTGTTGGGGTGGCTATGAGCAT
GGCAGGGCTGAGCTGCCTCCCTGCCTCAG**GACAAGCAGTCGTGGCAGAGTGAACGGGAAATCTTC**
AGCACACCCGGCATGAAGCACGAAAACCTGTTGCAGTTCATTGCTGCCGAGAAACGAGGCTCCAA
CCTGGAGGTGGAGCTGTGGCTCATCACAGCCTTCCACGACAAGGTGAGCCACACCCAGACAGATC
CCACGGTGAGATAGATGGGTCCCAGTGATGGTTTTGTGATGAGCTGACCCCGTGTGAGCCGCACTTC
CCACCTGGATCACGTCCGCCATTCTGCGCATTGCACTGAGACGTTCTGCTGGGCCTGGGTTGGTT
GCATCCTGTTCTGCATTGGAGCCTTGGCCACAGTCTGCTGCCTTCTCTTAG**GGCTCCCTACGGAT**
TACCTCAAGGGGAACATCATCACGTGGAACGAACTGTGCCACGTGGCGGAGACGATGTCACGAG
GCCTCTCATACCTGCATGAGGATGTGCCGTGGTGTGCTGGTGGAGGGCCACAAGCCTTCTATTGCCC
ACAGGTACCTGATTAAGGAGCCTCTCTGCAGGTGGGGTGCAGTTGCAGAGTTGCCTCTGCTTGCTTG
AATGTGCCTGTGGTGTGTGTCAGAGCAAACCTGGCCTTCTGTGTGCTGGAGATAGCTAGCTTCCAGGC
CAGCGTTCTGGTGCCCTCTGCTGGACCCAGGAGAGAGTGTGGACTCTGTTACTCTGGAGAGCTTCAC
ACGCTCAGACTGTGGTCAGAACCTTTGTATCCTTGAGAGGACTCTACTTACCTAGTCTGTGTCGGTT
ATGTTTGCTGATGCTTGCCGCTACAGAGCTCATGTGTGTTTATGTGTCATCATGATAACATTAGCTGT
CCATTGTCGGGGGTGGGGGTGGGGGTGGGGGTGGGGGGGGTGGGGGGGGAGGAGAGGCAGAT
GATAGCTGATTACCTCCCATAGCACTGATTAGGATCTTAGGATTTCTGAGCCACGCCTTTG

ACVR2B-FLOX-EM2-B6N

TAGGGTCAGACGCCATGTTGGTTTTGGGTTCTGCCATTACTTCCGTGTTGTGTATGCAGTTTGCATGT
TTTGTGTACGCACAGGTCTGTGTGTGGAGGCCAGAGAATGAATCCCTTTATTGGACTCCTTTTTATTT
TTTTGGAGACAGGATTTTCAAGTATGTAATCCAGACTGGCCTAGAACTTGTATATAGACCAGGCTGGC
CTTGAACCAAAAAGTCTACCTGCCTCCACCTCCCAGATGCTAGAAATTAAGGTGTGCACCACCAAACT
GGCTCTCTACTTTTTACAGTTCTTTATTTTTATTCCTTTTTAATGAAAAAAAAAATTTTTTTTTTTTTCT

GTTTTTTCGAGACAGCCCTGTGTAGCCCTGCTGTCCTGGAACCTCACTTTGTAGATCAGGCTAGCCTCG
 AACTCAGAAATCCACCTGCCTCTGTTTCCCAAGTGCTGGGATTAAAGGCGTGCGCCACCACCGCCTG
 GCCCTCAGTGAAAATATTTTTATGTGTGCAGGTGCTTTTTTTGCCCGCATGTATCTATGTGTCTCGCAT
 GTGTGCCTGGTGCCTGAAGAAGCCAGAAGGGAGTGTTGGGTCCTTGAGACTGGAGTTATAGACAGT
 TGTGAGCCACCATACATGTGCTGGGAATTGAACCCGGGTGCTCAGGGAGAGTGGCAGTGCTCTTAA
 CCTCAGAGCCACTTCTCTAGCCTCTACGTTTTAAGATTTTTTGAGACAAGGCCTCTCTAGCCTGGAA
 CTCACCGGTTTGGTTGACGAGTTGGCAAACAAGTCCCAGGAACTCCACCTCTTCCTGCTTCCAGCACT
 GGGGTTACACTCCACTGTGTCCAGGGGCTGGGAATCTCTCATGCTTGCCTGGCAG~~accgggggtaccgc~~
~~gtcgagGCGATCGCATAACTTCGTATAGCATACATTATACGAAGTTAT~~CTGAGTTTGGTTTTTGGCATT
 GTGTATACTCAGAGGCTTCTGACACCAGGCAGCCATGTAGTTAGGTGCTCAGGGTACTTAGGAAGA
 CAAGCATTTGCTGGCTGATACTGGCTGGTCACCTCACTCAGCATTTGTAAATCAGGGCGCTGTTGTC
 AGCTACCCAGAGATCGCTATTGGCCTGGTCTTTGGATGCTGAGGCTAGGAAATCGGGGTTGGGGA
 TGGAGTAGGGTGTGTTGGGGTGGCTATGAGCATGGCAGGGCTGAGCTGCCTCCCTGCCTCAG**GAC**
AAGCAGTCGTGGCAGAGTGAACGGGAAATCTTCAGCACACCCGGCATGAAGCACGAAACTTGT
GCAGTTCATTGCTGCCGAGAAACGAGGCTCCAACCTGGAGGTGGAGCTGTGGCTCATCACAGCCT
TCCACGACAAGGTGAGCCACACCCAGACAGATCCACGGTGAGATAGATGGGTCCCAGTGATGGT
 TTTGTGATGAGCTGACCCCGTGTGAGCCGCACTTCCACCTGGATCACGTCCGCCATTCTGCGCATT
 TGCATGAGACGTTCTGCTGGGCCTGGGTTGGTTGCATCCTGTTCTGCATTTGGAGCCTTTGCCAC
 AGTCTGCTGCCTTCTCTTAG**GGCTCCCTCACGGATTACCTCAAGGGGAACATCATCACGTGGAACG**
AACTGTGCCACGTGGCGGAGACGATGTACGAGGCCTCTCATACCTGCATGAGGATGTGCCGTGG
TGTCGTGGTGAGGGCCACAAGCCTTCTATTGCCACAGGTACCTGATTAAGGAGCCTCTCTGCAGG
 TGGGGTGCAGTTGCAGAGTTGCCTCTGCTTGAATGTGCCTGTGGTGTGTCAGAGCAAACCTGG
 CCTTCTGTGTGCTGGAGATAGCTAGCTTCCAGGCCAGCGTTCTGGTGCCTCTGCTGGACCCAGG**A**
~~TAACTTCGTATAGCATACATTATACGAAGTTAT~~~~CGCCGGCG~~~~ggtctgagctcgccatcagt~~CTCTGGAGAG
 CTTACACGCTCAGACTGTGGTCAGAACCTTTGTATCCTTGAGAGGACTCTACTTACCTAGTCTGTG
 TCGGTTATGTTTGCTGATGCTTGCCGCTACAGAGCTCATGTGTGTTTATGTGTCATCATGATAACATT
 AGCTGTCCATTGTCGGGGGTGGGGGGTGGGGGTGGGGTGGGGGGGGTGGGGGGGGAGGAGAG
 GCAGATGATAGCTGATTACCTCCCATAGCACTGATTAGGATCTTAGGATTTCTGAGCCACGCCTTTG

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 Alignment:

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      *      20      *      40      *      60      *      80      *      100     *      120
Acvr2b_WT : TAGGGTCAGACGCCATGTTGGTTTTGGGTTCTGCCATTACTTCCGTGTTGTGTATGCAGTTTGCATGTTTTGTGTACGCACAGGTCTGTGTGTGGAGGCCAGAGAAATGAATCCCTTTATT : 120
Acvr2b_EM2 : TAGGGTCAGACGCCATGTTGGTTTTGGGTTCTGCCATTACTTCCGTGTTGTGTATGCAGTTTGCATGTTTTGTGTACGCACAGGTCTGTGTGTGGAGGCCAGAGAAATGAATCCCTTTATT : 120

      *      140     *      160     *      180     *      200     *      220     *      240
Acvr2b_WT : GGACTCCTTTTTATTTTTTTGGAGACAGGATTTTCAGTATGTAATCCAGACTGGCCTAGAACTTGTTCATATAGACCAGGCTGGCCTTGAACCAAAAGTCTACCTGCCTCCACCTCCAGAT : 240
Acvr2b_EM2 : GGACTCCTTTTTATTTTTTTGGAGACAGGATTTTCAGTATGTAATCCAGACTGGCCTAGAACTTGTTCATATAGACCAGGCTGGCCTTGAACCAAAAGTCTACCTGCCTCCACCTCCAGAT : 240

      *      260     *      280     *      300     *      320     *      340     *      360
Acvr2b_WT : GCTAGAATTAAAGGTGTGCACCACCAAACTGGCTCTCTACTTTTACAGTTCTTTATTTTTATTCCTTTTAAATGAAAAAAAAAATTTTTTTTTTTTCTGTTTTTTCGAGACAGCCC : 360
Acvr2b_EM2 : GCTAGAATTAAAGGTGTGCACCACCAAACTGGCTCTCTACTTTTACAGTTCTTTATTTTTATTCCTTTTAAATGAAAAAAAAAATTTTTTTTTTTTCTGTTTTTTCGAGACAGCCC : 360

      *      380     *      400     *      420     *      440     *      460     *      480
Acvr2b_WT : TGTGTAGCCCTGCTGTCTGGAACCTCACTTTGTAGATCAGGCTAGCCCTCGAACTCAGAAATCCACCTGCCTCTGTTTCCCAAGTGCTGGGATTAAAGGCGTGCGCCACCACCGCCTGGCC : 480
Acvr2b_EM2 : TGTGTAGCCCTGCTGTCTGGAACCTCACTTTGTAGATCAGGCTAGCCCTCGAACTCAGAAATCCACCTGCCTCTGTTTCCCAAGTGCTGGGATTAAAGGCGTGCGCCACCACCGCCTGGCC : 480

      *      500     *      520     *      540     *      560     *      580     *      600
Acvr2b_WT : CTCAGTGAAAATATTTTTATGTGTGCAGGTGCTTTTTTTGCCCGCATGTATCTATGTGTCTGTCATGTGTGCCTGGTGCCTGAAGAAGCCAGAAGGGAGTGTGGGTCTTTGAGACTGGA : 600
Acvr2b_EM2 : CTCAGTGAAAATATTTTTATGTGTGCAGGTGCTTTTTTTGCCCGCATGTATCTATGTGTCTGTCATGTGTGCCTGGTGCCTGAAGAAGCCAGAAGGGAGTGTGGGTCTTTGAGACTGGA : 600

      *      620     *      640     *      660     *      680     *      700     *      720
Acvr2b_WT : GTTATAGACAGTTGTGAGCCACCATACATGTGCTGGGAATTGAACCCGGGTGCTCAGGGAGAGTGGCAGTGCTCTTAACCTCAGAGCCACTTCTCTAGCCTCTACGTTTTAAGATTTTTTT : 720
Acvr2b_EM2 : GTTATAGACAGTTGTGAGCCACCATACATGTGCTGGGAATTGAACCCGGGTGCTCAGGGAGAGTGGCAGTGCTCTTAACCTCAGAGCCACTTCTCTAGCCTCTACGTTTTAAGATTTTTTT : 720

      *      740     *      760     *      780     *      800     *      820     *      840
Acvr2b_WT : GAGACAAGGCCTCTCTTAGCCTGGAACCTACCGGTTTGGTTGACGAGTTGGCAAACAAGTCCCAGGAACCTCCACCTCTTCCCTGCTTCCAGCACTGGGGTTACACTCCACTGTGTCCAGGG : 840
Acvr2b_EM2 : GAGACAAGGCCTCTCTTAGCCTGGAACCTACCGGTTTGGTTGACGAGTTGGCAAACAAGTCCCAGGAACCTCCACCTCTTCCCTGCTTCCAGCACTGGGGTTACACTCCACTGTGTCCAGGG : 840

      *      860     *      880     *      900     *      920     *      940     *      960
Acvr2b_WT : GCTGGGAATCTCTCATGCTTGCTGGCAG-----GCACCTCCGGAGCATCTCCTCGGTCC-----CTGAGTTTGTTTTTTGGCATTGTGTATAC : 924
Acvr2b_EM2 : GCTGGGAATCTCTCATGCTTGCTGGCAGaccgggggtaccgqtcgagGCGATCGCATAAAGTTCTGATAGCATACATTATACGAAGTTATCTGAGTTTGTTTTTTGGCATTGTGTATAC : 960

      *      980     *      1000    *      1020    *      1040    *      1060    *      1080
Acvr2b_WT : TCAGAGGCTTCTGACACCAGGCAGCCATGTAGTTAGGTGCTCAGGGTACTTAGGAAGACAAGCATTGCTGGCTGATACTGGCTGGTCACCTCACTCAGCATTGTGAAATCAGGGCGCTG : 1044
Acvr2b_EM2 : TCAGAGGCTTCTGACACCAGGCAGCCATGTAGTTAGGTGCTCAGGGTACTTAGGAAGACAAGCATTGCTGGCTGATACTGGCTGGTCACCTCACTCAGCATTGTGAAATCAGGGCGCTG : 1080
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		*	1100	*	1120	*	1140	*	1160	*	1180	*	1200	
Acvr2b_WT :	TTGTCAGCTACCC	CAGAGATCGCTATTGGCCTGGTCTTTGGATGCTGAGGCTAGGAAATCGGGGTTGGGGATGGAGTAGGGTGTGTTGGGGTGGCTATGAGCATGGCAGGGCTGAGCTGC	:	1164										
Acvr2b_EM2 :	TTGTCAGCTACCC	CAGAGATCGCTATTGGCCTGGTCTTTGGATGCTGAGGCTAGGAAATCGGGGTTGGGGATGGAGTAGGGTGTGTTGGGGTGGCTATGAGCATGGCAGGGCTGAGCTGC	:	1200										

		*	1220	*	1240	*	1260	*	1280	*	1300	*	1320	
Acvr2b_WT :	CTCCCTGCCTCAG	GACAAGCAGTCGTGGCAGAGTGAACGGGAAATCTTCAGCACACCCGGCATGAAGCACGAAAACCTGTTGCAGTTCATTGCTGCCGAGAAAACGAGGCTCCAACCTGGA	:	1284										
Acvr2b_EM2 :	CTCCCTGCCTCAG	GACAAGCAGTCGTGGCAGAGTGAACGGGAAATCTTCAGCACACCCGGCATGAAGCACGAAAACCTGTTGCAGTTCATTGCTGCCGAGAAAACGAGGCTCCAACCTGGA	:	1320										

		*	1340	*	1360	*	1380	*	1400	*	1420	*	1440	
Acvr2b_WT :	GGTGGAGCTGTGGCTCATCACAGCCTTCCACGACAAG	GTGAGCCACACCCAGACAGATCCCACGGTGAGATAGATGGGTCCCAGTGATGGTTTTGTGATGAGCTGACCCCGTGTGAGCC	:	1404										
Acvr2b_EM2 :	GGTGGAGCTGTGGCTCATCACAGCCTTCCACGACAAG	GTGAGCCACACCCAGACAGATCCCACGGTGAGATAGATGGGTCCCAGTGATGGTTTTGTGATGAGCTGACCCCGTGTGAGCC	:	1440										

		*	1460	*	1480	*	1500	*	1520	*	1540	*	1560	
Acvr2b_WT :	GCACTTCCCACCTGGATCACGTCCGCCATTCTGCGCATTTGCACTGAGACGTTCTGCTGGGCCCTGGGTTGGTTGCATCCTGTTCTGCATTTGGAGCCTTTGCCACAGTCTGCTGCCT	:	1524											
Acvr2b_EM2 :	GCACTTCCCACCTGGATCACGTCCGCCATTCTGCGCATTTGCACTGAGACGTTCTGCTGGGCCCTGGGTTGGTTGCATCCTGTTCTGCATTTGGAGCCTTTGCCACAGTCTGCTGCCT	:	1560											

		*	1580	*	1600	*	1620	*	1640	*	1660	*	1680	
Acvr2b_WT :	TCTCTTAGGGCTCCCTCACGGATTACCTCAAGGGGAACATCATCAGTGGAACGAACTGTGCCACGTGGCGGAGACGATGTACAGAGGCCTCTCATACCTGCATGAGGATGTGCCGTGGT	:	1644											
Acvr2b_EM2 :	TCTCTTAGGGCTCCCTCACGGATTACCTCAAGGGGAACATCATCAGTGGAACGAACTGTGCCACGTGGCGGAGACGATGTACAGAGGCCTCTCATACCTGCATGAGGATGTGCCGTGGT	:	1680											

		*	1700	*	1720	*	1740	*	1760	*	1780	*	1800	
Acvr2b_WT :	GTCGTGGTGAGGGCCACAAGCCTTCTATTGCCACAG	GTACCTGATTAAGGAGCCTCTCTGCAGGTGGGGTGCGAGTTGCAGAGTTGCCCTCTGCTTGCTTGAATGTGCCTGTGGTGTGTCA	:	1764										
Acvr2b_EM2 :	GTCGTGGTGAGGGCCACAAGCCTTCTATTGCCACAG	GTACCTGATTAAGGAGCCTCTCTGCAGGTGGGGTGCGAGTTGCAGAGTTGCCCTCTGCTTGCTTGAATGTGCCTGTGGTGTGTCA	:	1800										

		*	1820	*	1840	*	1860	*	1880	*	1900	*	1920	
Acvr2b_WT :	GAGCAAACCTGGCCTTCTGTGTGCTGGAGATAGCTAGCTTCCAGGCCAGCGTTCTGGTGCCCTCTGCTGGACCCAGC	-----AGAGAGTGTGGACTCTGTTA-----	:	1862										
Acvr2b_EM2 :	GAGCAAACCTGGCCTTCTGTGTGCTGGAGATAGCTAGCTTCCAGGCCAGCGTTCTGGTGCCCTCTGCTGGACCCAGC	ATAACTTCGTATAGCATACATTATACGAAGTTATG	GCCGGCG	:	1920									

		*	1940	*	1960	*	1980	*	2000	*	2020	*	2040	
Acvr2b_WT :	-----	CTCTGGAGAGCTTCACACGCTCAGACTGTGGTCAGAACCTTTTGTATCCTTGAGAGGACTCTACTTACCTAGTCTGTGTCGGTTATGTTTGTCTGATGCTT	:	1962										
Acvr2b_EM2 :	ggtctgagctcgccatcagt	CTCTGGAGAGCTTCACACGCTCAGACTGTGGTCAGAACCTTTTGTATCCTTGAGAGGACTCTACTTACCTAGTCTGTGTCGGTTATGTTTGTCTGATGCTT	:	2040										

		*	2060	*	2080	*	2100	*	2120	*	2140	*	2160	
Acvr2b_WT :	GCCGCTACAGAGCTCATGTGTGTTTATGTGTCATCATGATAACATTAGCTGTCCATTGTCTGGGGGTGGGGGTGGGGGTGGGGGTGGGGGGGGAGGAGAGGCAGATGATAGC	:	2082											
Acvr2b_EM2 :	GCCGCTACAGAGCTCATGTGTGTTTATGTGTCATCATGATAACATTAGCTGTCCATTGTCTGGGGGTGGGGGTGGGGGTGGGGGTGGGGGGGGAGGAGAGGCAGATGATAGC	:	2160											

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          *      2180      *      2200      *
Acvr2b_WT : TGATTACCTCCCATAGCACTGATTAGGATCTTAGGATTCTGAGCCACGCCTTTG : 2137
Acvr2b_EM2 : TGATTACCTCCCATAGCACTGATTAGGATCTTAGGATTCTGAGCCACGCCTTTG : 2215

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LoxP sites are highlighted in red and genotyping handles (restriction enzyme site plus primer unique to each LoxP site) in yellow. Floxed exon highlighted in blue.

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_Acvr2b_F5 (5'-3')	TAGGGTCAGACGCCATGTTG
Geno_Acvr2b_R5 (5'-3')	CAAAGGCGTGGCTCAGAAAT
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1.25
WT product size (bp)	2137
Mutant product size (bp)	2215
Notes	Sequenced with the following primers (5' – 3'): Geno_Acvr2b_F9: TTTCGAGACAGCCCTGTG, Geno_Acvr2b_F6: CGGCATGAAGCACGAAAAC Geno_Acvr2b_R5: CAAAGGCGTGGCTCAGAAAT LoxPF: atccgggggtaccgctcgag LoxPR: actgatggcgagctcagacc

LoxPF (5'-3')	atccgggggtaccgctcgag
LoxPR (5'-3')	actgatggcgagctcagacc
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1.25
WT product size (bp)	N/A
Mutant product size (bp)	1090
Notes	PCR to identify the floxed allele.

Geno_Acvr2b_F5 (5'-3')	TAGGGTCAGACGCCATGTTG
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)	1940
Notes	Sequenced with the following primers (5' – 3'): Geno_Acvr2b_F9: TTTCGAGACAGCCCTGTG, Geno_Acvr2b_F6: CGGCATGAAGCACGAAAAC Geno_Acvr2b_R9: TTTCGAGACAGCCCTGTG LoxPF_R: ctcgacgcggtacccccggat LoxPF: atccgggggtaccgcgtcgag LoxPR: actgatggcgagctcagacc

LoxPF (5'-3')	atccgggggtaccgcgtcgag
Geno_Acvr2b_R5 (5'-3')	CAAAGGCGTGGCTCAGAAAT
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1.25
WT product size (bp)	N/A
Mutant product size (bp)	1346
Notes	PCR to check for floxed allele on target.

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 ≤ 2 mismatches for guide(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
9:104367414-104367436	AACCA ^C CATGCTTGCCTGGC TGG	Intergenic	Geno_Acvr2b_OT1F1: CCTTGCCTGGAAGAGCCTAAA Geno_Acvr2b_OT1R1: CTGGGACCTCAGGACTTGGGA Sequenced using primer Geno_Acvr2b_OT1SeqF: TAGAACCTTGTTATCCCAAAGA
9:83222654-83222676	TCTTCTC ^C TGCTTGCCTGGC GGG	Intergenic	Geno_Acvr2b_OT2F1: TCTGTGTGTACTGGTCCCTGA Geno_Acvr2b_OT2R1: CCTCAGCAGATGGCTCTAACAA Sequenced using primer Geno_Acvr2b_OT2F2: CTCAGTTTCATTTCCTACCTGTCC

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	Acvr2b-5'LoxP-MUT3
Forward Primer (5'-3')	TGGGAATCTCTCATGCTTGC
Reverse Primer (5'-3')	CCTCTGAGTATACACAATGCCAA
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	Acvr2b_FLOX_3'_MUT1
Forward Primer (5'-3')	CTGCTGGACCCAGGATAAC
Reverse Primer (5'-3')	GCGTGTGAAGCTCTCCAGAG
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.

Assay name	Acvr2b_UPL16_F2
Forward Primer (5'-3')	gtgtgttggggtggctatg
Reverse Primer (5'-3')	actctgccacgactgcttg
Probe (5'-3')	CTGCCTCC
Label	FAM

This ddPCR assay is specific to the WT allele and only WT alleles are expected to be 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 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.