Name of Mouse model or mutation:
ACACA-S1215A-EM1-B6N
ACACA-S1215A-EM2-B6N

Description:
Point mutation made by CRISPR/Cas9 gene editing.

Type of mutation:
SNP: S1215A

Delivery method:
Cytoplasmic injection into 1-cell stage embryo.

Genetic Background:
C57BL/6NTac

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
TAGTGTCAGCGATGTTCTGT	TGG

ssODN donor sequence (5'-3'):
GCCTTACCTGACAAAATCTTCAAAGGTCCGGAAAGAGACCATTCCGCCCATCCGCTGACAAGGTGGC
GTGAAGGCGTTGTCaAgCAGAACATCGCTGACAgcAGCTACATGAGTCATGCCATAGTGGTTGAGGT
TGGAGGCAAAGGACATTCTAAACAGTAATTCAATCAAGAGCATTAGAAAAGGATCTAGGACTGGGA

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 F_0 progeny.

Sequence details

WT

TCTGTCACTCAACCAACCAAAGGAAAAGGTAAATATCTCAATAAAGTTCCTTTTGAGGACTTTTGTTC
CATAAATGATAATTTGCTATCATTATTTACTAAACCACTTCATTCTTCTTTATTAGAATTATTCTTTGAT
ATTAATAAAACTGTATCTAGGGATGTTTAATCTATTCCTTCGGGGGGAAAACGTCTTAAATATTAAAT
AATTGCAACTCACTTGGCTTCCTTACTGTTTTGAAAGGAAGAAAGGAGACGGGGAAGATGCAGGAT
GGTATTTAACCCCTATGTACTGCTCATCCATCCAATATGTCATTATTATGTGGGTTAAAGACTACCAC
ACGATTCCTTGTGGTCTCTAGATTTCTATCTGTTCAAATTTTGGAGTGATGAAGCAATAGTGTTGGGA
AACTCCAGTGAAACATGAATCTCACCACAGTTTTATCTGTAGTGGGTTAAAAAAACCATATCTGCTCT
GCAACCTGACCCCACATGACCGTTTTCTCCACACTCCCTTCCTCCTTTGTTAATCTCCCAGTCCTAGAT
CCTTTTCTAATGCTCTTGATTGAATTACTGTTTAGAATGTCCTTTGCCTCCAACCTCAACCACTATGGC
ATGACTCATGTAGCTAGTGTCAGCGATGTTCTGTTGGACAACGCCTTCACGCCACCTTGTCAGCGGA
TGGGCGGAATGGTCTCTTTCCGGACCTTTGAAGATTTTGTCAGGTAAGGCATTATAGCACTGTCTTCC
CTGTCTGTACAAGGGTAGCCATGCATCTCTAGTTTTAGAGGCCTGACTTTTCTAAGAAAAAAAAAAA
AAAAAAAAAAAAAAGAGGAGGTCTGAAGAGCTATGAGGTAGCCAGAGAGATACAGCTTTCATTATAT
ATGCCCTCAGAGAGATAGATGTCTTCCGGTCTCTACTCCTCATTCAGACTCTCCTTCCAGTATAAA
GCCTAGTCCGGAGGAAAACAAAACCAAAAAAAAAAAAAAAAAAAGGGGGGGGTGGGGGGTGGAT
GGGAGAGAAACAAATAAAATCATCTTTACCTCAGGAGCTAACAGAAGAAGCTGTCTGCTCTGTGAT
GAGTGAAATGTCAGGAGAAATTAAACACAACACTAGAGTGAGCCCCACTAGAGACGATACATCTTT
TGATTAAATGCATTCAGCATCTATCTGTTGATGTATACTGATGCATTACCCTAGGGGGAGGGGTAAT
TACATTGGCCTGTCACTGC

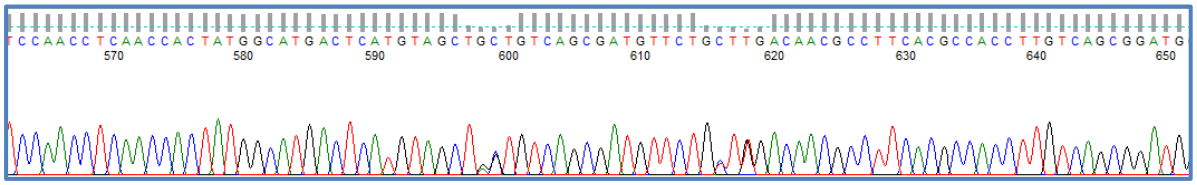
ACACA-S1215A-EM1-B6N and ACACA-S1215A-EM2-B6N

TCTGTCACTCAACCAACCAAAGGAAAAGGTAAATATCTCAATAAAGTTCCTTTTGAGGACTTTTGTTC
CATAAATGATAATTTGCTATCATTATTTACTAAACCACTTCATTCTTCTTTATTAGAATTATTCTTTGAT
ATTAATAAAACTGTATCTAGGGATGTTTAATCTATTCCTTCGGGGGGAAAACGTCTTAAATATTAAAT
AATTGCAACTCACTTGGCTTCCTTACTGTTTTGAAAGGAAGAAAGGAGACGGGGAAGATGCAGGAT
GGTATTTAACCCCTATGTACTGCTCATCCATCCAATATGTCATTATTATGTGGGTTAAAGACTACCAC
ACGATTCCTTGTGGTCTCTAGATTTCTATCTGTTCAAATTTTGGAGTGATGAAGCAATAGTGTTGGGA
AACTCCAGTGAAACATGAATCTCACCACAGTTTTATCTGTAGTGGGTTAAAAAAACCATATCTGCTCT
GCAACCTGACCCCACATGACCGTTTTCTCCACACTCCCTTCCTCCTTTGTTAATCTCCCAGTCCTAGAT
CCTTTTCTAATGCTCTTGATTGAATTACTGTTTAGAATGTCCTTTGCCTCCAACCTCAACCACTATGGC
ATGACTCATGTAGCT**GC**TGTCAGCGATGTTCTG**C**T**T**GACAACGCCTTCACGCCACCTTGTCAGCGGAT
GGGCGGAATGGTCTCTTTCCGGACCTTTGAAGATTTTGTCAGGTAAGGCATTATAGCACTGTCTTCCC
TGTCTGTACAAGGGTAGCCATGCATCTCTAGTTTTAGAGGCCTGACTTTTCTAAGAAAAAAAAAAAA
AAAAAAAAAAAAAGAGGAGGTCTGAAGAGCTATGAGGTAGCCAGAGAGATACAGCTTTCATTATAT
ATGCCCTCAGAGAGATAGATGTCTTCCGGTCTCTACTCCTCATTCAGACTCTCCTTCCAGTATAAAGC
CTAGTCCGGAGGAAAACAAAACCAAAAAAAAAAAAAAAAAAAGGGGGGGGTGGGGGGTGGATGG
GAGAGAAACAAATAAAATCATCTTTACCTCAGGAGCTAACAGAAGAAGCTGTCTGCTCTGTGATGA
GTGAAATGTCAGGAGAAATTAAACACAACACTAGAGTGAGCCCCACTAGAGACGATACATCTTTTG

ATTAAATGCATTCAGCATCTATCTGTTGATGTATACTGATGCATTACCCTAGGGGGAGGGGTAATTA
CATTGGCCTGTCACTGC

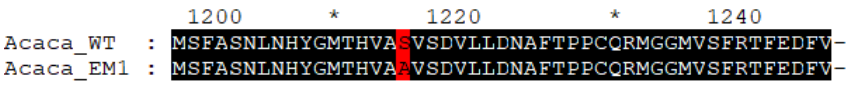
Red, bold and underlined = S1215A change
Red and bold = silent change to prevent re-processing by CRISPR/Cas9
Alleles EM1 and EM2 have the same sequence but were derived from different founders from the same microinjection session.

Heterozygous F1 animal sequence trace:



Nucleotide Alignment:

Predicted Protein Alignment:



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_Acaca_S1215A_F1 (5'-3')	TCTGTCACTCAACCAACCAAA
Geno_Acaca_S1215A_R1 (5'-3')	GCAGTGACAGGCCAATGTAA
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	1231
Mutant product size (bp)	1231
Notes	Amplicons sequenced in reverse with Acaca_S1215A_SeqR (CTTAGAAAAGTCAGGCCT) due to poly A region.

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')
12:10154839-10154861	TAGTGTCAGCGATGTTCTGT TGG	Intergenic	Acaca_OT1_F1 (AGCGACATGAACACTGTACTGA) Acaca_OT1_R1 (TCGGAATGTGAAAAATTTGGGGA)

All amplicons were sent for Sanger sequencing.
Off-target activity (5 nt deletion) at the off-target site was detected in the animals carrying the EM1 and EM2 alleles. These animals will be backcrossed to WT in order to segregate the off-target from the intended allele, and re-checked at the next generation.

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	Acaca_S1215A_MUT1
Forward Primer (5’-3’)	CTTCAAAGGTCCGGAAAGAGA
Reverse Primer (5’-3’)	TGGCATGACTCATGTAGCTG
Probe (5’-3’)	CAGCGATGTTCTGCTTGACAACGC
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.