Name of Mouse model or mutation:
STAC3-W280S-EM1-B6J

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

Type of mutation:
SNP: W280S

Delivery method:
Electroporation into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
TTGATGACTCCAATGAGGAG	**TGG**

ssODN donor sequence (5'-3'):
GATTTTCCCCTAGAGAAGGAAGGCAGTGGCTACGCTGGAGATCTCAGGTTCTAGACTGCCCATCTCCCACCTTAGCGGTGGCAACGTCTTACCCGCCAC**g**ACTCCTCATTGGAGTCATCAATGACTGTGATCTTCTCCCCTGGCCTGGGATAGAAAGGAGACCCTCTCTGTGATTTACAGTCACATGCCCGTGCCTGGCT

Electroporation mixes:
Cas9 protein, sgRNAs and ssODNs were diluted and mixed in Electroporation buffer (EB; Gibco Opti-MEM I Reduced Serum Media – (Thermo Fisher Scientific)) to the working concentrations of 650 ng/µl, 130 ng/µl each and 400 ng/µl, respectively. Embryos were electroporated using the following conditions: 30 V, 3 ms pulse length, 100 ms pulse interval, 12 pulses. Electroporated embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

WT

GAGGGAAGTGATGGATGCCCTCCTGGAGGCAGAAGCAATTTGTCTATTTTTCCCATCCAAGGAAAC
AATGCAGAAAAGGACAAGAAGGCCGAGAAGAAAACTCCTGATGACAAAGTAAGTCCTTCCTCCCTG
TAGAACCCTGGTCCTCGTCCCAGCCAACGTCTGACATCACTCACAGCTCTTCCCATCACCCCTGACCC
ACCCTCCACACTGAGACAGACACACAAACCGATGCAGCCCTCTCATCCCGGGAGAGGTAAATCGGA
GTCTGGTAACTCATCGCTCTCTTGCACCTCCAGAACAAGCAGCCTGGCTTCCAGCAGTCTCATTACTT
TGTGGCTCTCTATCGGTTCAAAGCCCTGGAGAAGGACGACCTGGATTTCCCGTGAGGAGCCTTCAAG
GGAGGCGGGAGTGTGGGGAGCATCGAGGAGTAAGAGATGAGCTCTCTGTGGGAATGGAGGAGGG
CAGAAGGGCGGGTACCCCGCAGCACAGCCAGGCACGGGCATGTGACTGTAAATCACAGAGAGGGT
CTCCTTTCTATCCCAGGCCAGGGGAGAAGATCACAGTCATTGATGACTCCAATGAGGAGTGGTGGC
GGGTAAGACGTTGCCACCGCTAAGGTGGGAGATGGGCAGTCTAGAACCTGAGATCTCCAGCGTAG
CCACTGCCTTCCTTCTCTAGGGGAAAATCGGAGAGAAAGTCGGATTCTTCCCTCCAAACTTCATCATT
CGGGTGCGGGCTGGAGAACGTGTGCACCGCGTGACCAGATCGTTTGTGGGGAACCGCGAGATTGG
ACAGATCACTCTCAAGAAGGACCAGGTATCTCGGGAACCTTAACCCAATGCCAGGCCTGGTCGTACC
TCTCCATCCATGCACCTCAGAGGGAGGCCCTAACCCATCAGTATGTACGACCCTCCCCAACGTCCCTG
CGCTCCTCTTCTAATTTTTTTTTCCTGTTCCTCAATCCTTTAAACCCCTAGATCGTAGTGCAGAAAGGA
GATGAAGCTGGTGGTTACGTCAAGGTCTACACCGGCCGCAAGGTGGGGCTGTTTCCCACCGACTTC
CTGGAGGAGATTTAAGAGTACTGGTGCCTGCTGGCGGGAGACACCCACTCCTCGTTCTGGGCGGGG
CCCAGTGCGAGCTGGGGAGGGAAGGGGCAAGGGGAGGGAGTACTGGA

STAC3-W280S-EM1-B6J

GAGGGAAGTGATGGATGCCCTCCTGGAGGCAGAAGCAATTTGTCTATTTTTCCCATCCAAGGAAAC
AATGCAGAAAAGGACAAGAAGGCCGAGAAGAAAACTCCTGATGACAAAGTAAGTCCTTCCTCCCTG
TAGAACCCTGGTCCTCGTCCCAGCCAACGTCTGACATCACTCACAGCTCTTCCCATCACCCCTGACCC
ACCCTCCACACTGAGACAGACACACAAACCGATGCAGCCCTCTCATCCCGGGAGAGGTAAATCGGA
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GGGTAAGACGTTGCCACCGCTAAGGTGGGAGATGGGCAGTCTAGAACCTGAGATCTCCAGCGTAG
CCACTGCCTTCCTTCTCTAGGGGAAAATCGGAGAGAAAGTCGGATTCTTCCCTCCAAACTTCATCATT
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CGCTCCTCTTCTAATTTTTTTTTCCTGTTCCTCAATCCTTTAAACCCCTAGATCGTAGTGCAGAAAGGA
GATGAAGCTGGTGGTTACGTCAAGGTCTACACCGGCCGCAAGGTGGGGCTGTTTCCCACCGACTTC
CTGGAGGAGATTTAAGAGTACTGGTGCCTGCTGGCGGGAGACACCCACTCCTCGTTCTGGGCGGGG
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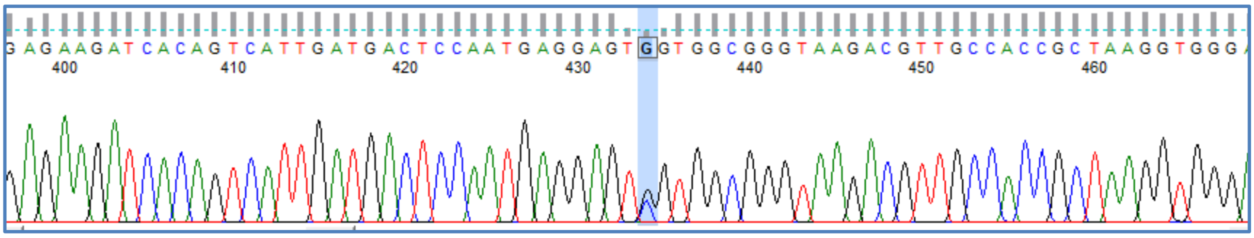
Heterozygous F1 animal sequence trace:



Nucleotide Alignment:

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                      *        20         *        40         *        60         *        80         *       100         *       120
Stac3_WT   : GAGGGAAGTGATGGATGCCCTCCTGGAGGCAGAAGCAATTTGTCTATTTTTCCCATCCAAGGAAACAATGCAGAAAAGGACAAGAAGGCCGAGAAGAAAACTCCTGATGACAAAGTAAGT
Stac3_EM1  : GAGGGAAGTGATGGATGCCCTCCTGGAGGCAGAAGCAATTTGTCTATTTTTCCCATCCAAGGAAACAATGCAGAAAAGGACAAGAAGGCCGAGAAGAAAACTCCTGATGACAAAGTAAGT

                      *       140         *       160         *       180         *       200         *       220         *       240
Stac3_WT   : CCTTCCTCCCTGTAGAACCCTGGTCCTCGTCCCAGCCAACGTCTGACATCACTCACAGCTCTTCCCATCACCCCTGACCCACCCTCCACACTGAGACAGACACACAAACCGATGCAGCCC
Stac3_EM1  : CCTTCCTCCCTGTAGAACCCTGGTCCTCGTCCCAGCCAACGTCTGACATCACTCACAGCTCTTCCCATCACCCCTGACCCACCCTCCACACTGAGACAGACACACAAACCGATGCAGCCC

                      *       260         *       280         *       300         *       320         *       340         *       360
Stac3_WT   : TCTCATCCCGGGAGAGGTAAATCGGAGTCTGGTAACTCATCGCTCTCTTGCACCTCCAGAACAAGCAGCCTGGCTTCCAGCAGTCTCATTACTTTGTGGCTCTCTATCGGTTCAAAGCCC
Stac3_EM1  : TCTCATCCCGGGAGAGGTAAATCGGAGTCTGGTAACTCATCGCTCTCTTGCACCTCCAGAACAAGCAGCCTGGCTTCCAGCAGTCTCATTACTTTGTGGCTCTCTATCGGTTCAAAGCCC

                      *       380         *       400         *       420         *       440         *       460         *       480
Stac3_WT   : TGGAGAAGGACGACCTGGATTTCCCGTGAGGAGCCTTCAAGGGAGGCGGGAGTGTGGGGAGCATCGAGGAGTAAGAGATGAGCTCTCTGTGGGAATGGAGGAGGGCAGAAGGGCGGGTAC
Stac3_EM1  : TGGAGAAGGACGACCTGGATTTCCCGTGAGGAGCCTTCAAGGGAGGCGGGAGTGTGGGGAGCATCGAGGAGTAAGAGATGAGCTCTCTGTGGGAATGGAGGAGGGCAGAAGGGCGGGTAC

                      *       500         *       520         *       540         *       560         *       580         *       600
Stac3_WT   : CCCGCAGCACAGCCAGGCACGGGCATGTGACTGTAAATCACAGAGAGGGTCTCCTTTCTATCCCAGGCCAGGGGAGAAGATCACAGTCATTGATGACTCCAATGAGGAGTGGTGGCGGGT
Stac3_EM1  : CCCGCAGCACAGCCAGGCACGGGCATGTGACTGTAAATCACAGAGAGGGTCTCCTTTCTATCCCAGGCCAGGGGAGAAGATCACAGTCATTGATGACTCCAATGAGGAGTCGTGGCGGGT

                      *       620         *       640         *       660         *       680         *       700         *       720
Stac3_WT   : AAGACGTTGCCACCGCTAAGGTGGGAGATGGGCAGTCTAGAACCTGAGATCTCCAGCGTAGCCACTGCCTTCCTTCTCTAGGGGAAAATCGGAGAGAAAGTCGGATTCTTCCCTCCAAAC
Stac3_EM1  : AAGACGTTGCCACCGCTAAGGTGGGAGATGGGCAGTCTAGAACCTGAGATCTCCAGCGTAGCCACTGCCTTCCTTCTCTAGGGGAAAATCGGAGAGAAAGTCGGATTCTTCCCTCCAAAC

                      *       740         *       760         *       780         *       800         *       820         *       840
Stac3_WT   : TTCATCATTCGGGTGCGGGCTGGAGAACGTGTGCACCGCGTGACCAGATCGTTTGTGGGGAACCGCGAGATTGGACAGATCACTCTCAAGAAGGACCAGGTATCTCGGGAACCTTAACCC
Stac3_EM1  : TTCATCATTCGGGTGCGGGCTGGAGAACGTGTGCACCGCGTGACCAGATCGTTTGTGGGGAACCGCGAGATTGGACAGATCACTCTCAAGAAGGACCAGGTATCTCGGGAACCTTAACCC

                      *       860         *       880         *       900         *       920         *       940         *       960
Stac3_WT   : AATGCCAGGCCTGGTCGTACCTCTCCATCCATGCACCTCAGAGGGAGGCCCTAACCCATCAGTATGTACGACCCTCCCCAACGTCCCTGCGCTCCTCTTCTAATTTTTTTTTCCTGTTCC
Stac3_EM1  : AATGCCAGGCCTGGTCGTACCTCTCCATCCATGCACCTCAGAGGGAGGCCCTAACCCATCAGTATGTACGACCCTCCCCAACGTCCCTGCGCTCCTCTTCTAATTTTTTTTTCCTGTTCC

                      *       980         *      1000         *      1020         *      1040         *      1060         *      1080
Stac3_WT   : TCAATCCTTTAAACCCCTAGATCGTAGTGCAGAAAGGAGATGAAGCTGGTGGTTACGTCAAGGTCTACACCGGCCGCAAGGTGGGGCTGTTTCCCACCGACTTCCTGGAGGAGATTTAAG
Stac3_EM1  : TCAATCCTTTAAACCCCTAGATCGTAGTGCAGAAAGGAGATGAAGCTGGTGGTTACGTCAAGGTCTACACCGGCCGCAAGGTGGGGCTGTTTCCCACCGACTTCCTGGAGGAGATTTAAG

                      *      1100         *      1120         *      1140         *      1160         *
Stac3_WT   : AGTACTGGTGCCTGCTGGCGGGAGACACCCACTCCTCGTTCTGGGCGGGGCCCAGTGCGAGCTGGGGAGGGAAGGGGCAAGGGGAGGGAGTACTGGA
Stac3_EM1  : AGTACTGGTGCCTGCTGGCGGGAGACACCCACTCCTCGTTCTGGGCGGGGCCCAGTGCGAGCTGGGGAGGGAAGGGGCAAGGGGAGGGAGTACTGGA
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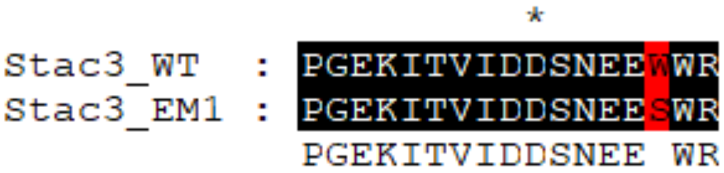
Predicted Protein Alignment:

Alignment of protein predicted from exon 10 (ENSMUSE00000276947)



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                     *
Stac3_WT  : PGEKITVIDDSNEEWWR
Stac3_EM1 : PGEKITVIDDSNEESWR
            PGEKITVIDDSNEE WR
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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_Stac3_W280S_F1 (5’-3’)	GAGGGAAGTGATGGATGCCC
Geno_Stac3_W280S_R1 (5’-3’)	TCCAGTACTCCCTCCCCTTG
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	1177
Mutant product size (bp)	1177
Notes	Amplicons sequenced with Geno_Stac3_W280S_F2 (TCCTCCCTGTAGAACCCTGG) and Geno_Stac3_W280S_R2 (CAGGAAGTCGGTGGGAAACA)

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 ≤ mismatches in the seed sequence and on the same chromosome for guide(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5’-3’)
10:75936505-75936527	**C**T**T**A**A**GA**G**TCCAATGAGGAG TGG	Intronic	Geno_Stac3_OT1F1 (ATGACCTTGGAGTCTGCTGC) & Geno_Stac3_OT1R1 (CCACACGGATTCTCCTGACC)

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	Stac3_W280S_UNI1
Forward Primer (5’-3’)	ACGGGCATGTGACTGTAAAT
Reverse Primer (5’-3’)	GCGGTGGCAACGTCTTA
Probe (5’-3’)	AGAGGGTCTCCTTTCTATCCCAGGC
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.

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.