Name of Mouse model or mutation:
TLR2-FLOX-EM2-B6N

Description:
Floxed mutant made (in stages) by CRISPR/Cas9 gene editing of existing line (TLR2-5LOXP-EM1-B6N).

Type of mutation:
Floxed allele: ENSMUSE00000441912 of Tlr2.

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

Genetic Background:
C57BL/6NTac

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
GAGTCACAGTATGGGCATCC	AGG

ssODN donor sequence (5'-3'):
GCTAATCTACTCACCTGCCATCCCTCTGTGTAGCTGATCCCAAGGGCAAGGAGTCACAGTATGGGAT
AACTTCGTATAGCATACATTATACGAAGTTATCGCCGGCGgtctgagctcgccatcagtAAATCTGTTGCT
CTTCCTAGACAGGGGGTTGTGTCCCACCATCCCACCAGATCTTCCAATATAAATGACACAG

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

TLR2-5LOXP-EM1-B6N

TGGGTAGTAGCGCACGTATGCCATCCCCCAACCATTACAGAAGCATAAAACATATTTTGGGAAATTG
TTGCGTTATTTCACTGAGCAAATATGACCACAGCACGTGTGGTTGCATGGAACAGCTACAGGGTATA
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TCAGCAAACGCTGTTCTGCTCAGGAGTCTCTGTCATGTGATGCTTCTGGGGTGTGTGATGGCCGCTC
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TAATGGGAAATCCTTACCAGACACTGGGGGTAACATCGCTTTTTCCCAATCTCACAAATTTACAAACC
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CGTAGATGAAGTCAGCTCACCGATGAAGAAGCTGGCATTCCGAGGCTCGGTTCTCACTGATGAAAG
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TCTGTGTAGCTGATCCCAAGGGCAAGGAGTCACAGTAT**[3nt_del]**GGGCATCCAGGCAAATCTGTT
GCTCTTCCTAGACAGGGGGTTGTGTCCCACCATCCCACCAGATCTTCCAATATAAATGACACAGGCT
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TLR2-FLOX-EM2-B6N

TGGGTAGTAGCGCACGTATGCCATCCCCCAACCATTACAGAAGCATAAAACATATTTTGGGAAATTG
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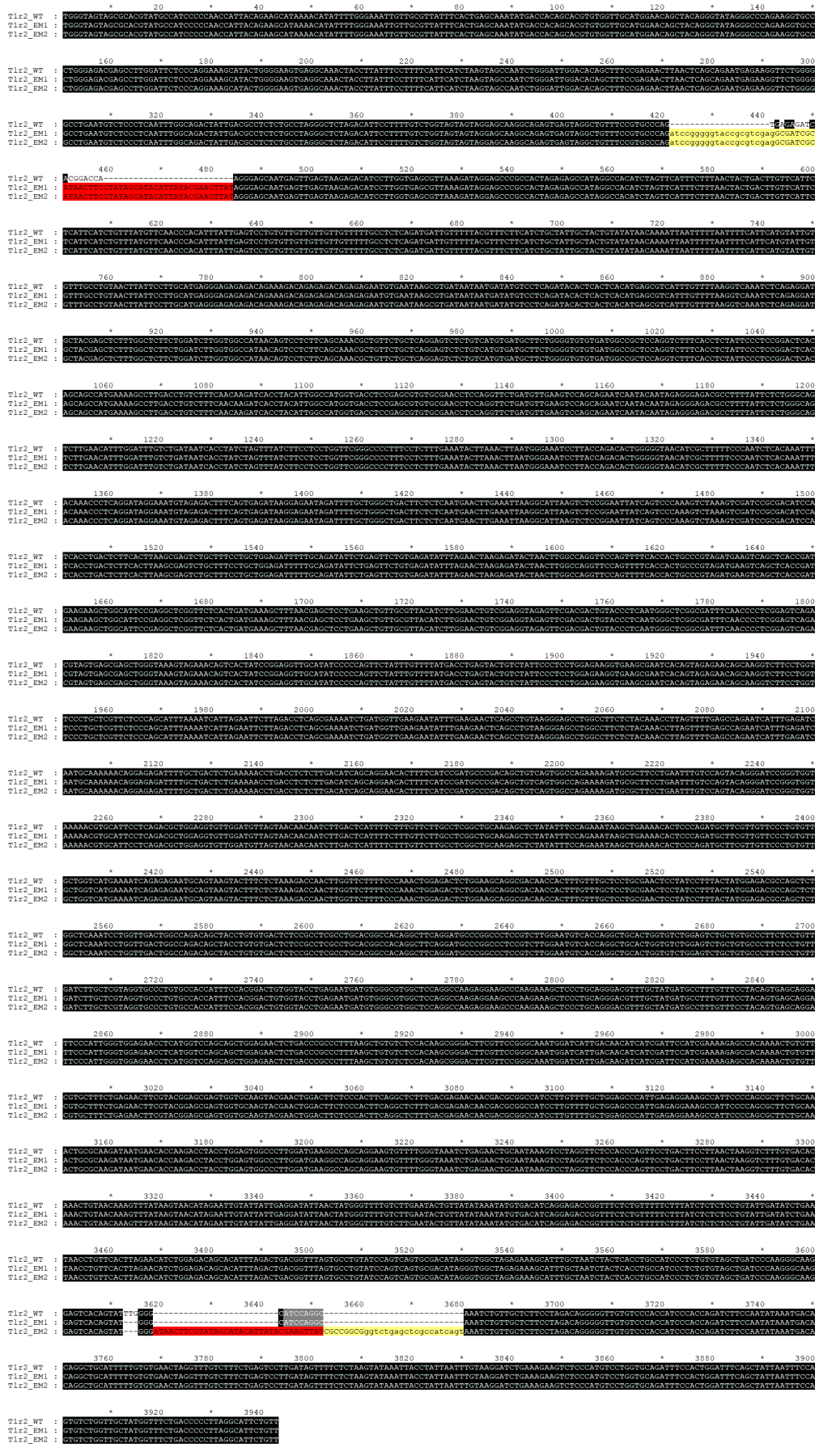
AAGTCTCCCATGTCCTGGTGCAGATTTCCACTGGATTTCAGCTATTAATTTCCAGTGTCTGGTTGCTAT
GGTTTCTGACCCCCTTAGGCATTCTGTT

Grey highlight denotes floxed exon ENSMUSE00000441912, yellow denotes genotyping handles, red denotes LoxP site. Please note that the 3 nt deletion from EM1 is still present in the EM2 allele.

Nucleotide 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_Tlr2_F1 primer (5'-3')	TGGGTAGTAGCGCACGTATG
Geno_Tlr2_R3 primer (5'-3')	AACAGAATGCCTAAGGGGGT
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	2
WT product size (bp)	3844
Mutant product size (bp)	3915
Notes	PCR external to the LoxP donor insertions. Sequenced with the following primers (5'-3'): Geno_Tlr2_F1: TGGGTAGTAGCGCACGTATG Geno_Tlr2_R3: AACAGAATGCCTAAGGGGGT LoxPF: ATCCGGGGGTACCGCGTCGAG LoxPR: ACTGATGGCGAGCTCAGACC

LoxP_F primer (5'-3')	ATCCGGGGGTACCGCGTCGAG
LoxP_R primer (5'-3')	ACTGATGGCGAGCTCAGACC
Taq Polymerase used	ThermoFisher SuperFi PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	2
WT product size (bp)	N/A
Mutant product size (bp)	3252
Notes	PCR to screen for floxed allele i.e., both LoxP sites. Sequenced with the following primers (5'-3'): LoxPF: atccgggggtaccgcgtcgag LoxPR: ACTGATGGCGAGCTCAGACC Tlr2_PW1: AACCCACATTTATTGAGTCC Tlr2_PW2: GGGTAACATCGCTTTTTCCC Tlr2_PW3: AACTCAGCCTGTAAGGGAGC Tlr2_PW4: CCTTCTCCTGTTGATCTTGC

Geno_Tlr2_F1 primer (5’-3’)	TGGGTAGTAGCGCACGTATG
LoxP_R primer (5’-3’)	ACTGATGGCGAGCTCAGACC
Taq Polymerase used	ThermoFisher SuperFi PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	2
WT product size (bp)	N/A
Mutant product size (bp)	3677
Notes	PCR to interrogate 5’ end of the allele. Sequenced with the following primers (5’-3’): Tlr2_R1: GACGCTCATGTGAGTGAGTGTA Tlr2_R4: GGATAGGAGTTCGCAGGAGC Tlr2_PW2: GGGTAACATCGCTTTTTCCC Tlr2_PW3: AACTCAGCCTGTAAGGGAGC Tlr2_PW4: CCTTCTCCTGTTGATCTTGC

LoxP_F primer (5’-3’)	ATCCGGGGGTACCGCGTCGAG
Geno_Tlr2_R3 primer (5’-3’)	AACAGAATGCCTAAGGGGGT
Taq Polymerase used	ThermoFisher SuperFi PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	2
WT product size (bp)	N/A
Mutant product size (bp)	3519
Notes	PCR to interrogate 3’ end of the allele. Sequenced with the following primers (5’-3’): LoxPF: atccgggggtaccgcgtcgag Tlr2_PW1: AACCCACATTTATTGAGTCC Tlr2_PW2: GGGTAACATCGCTTTTTCCC Tlr2_R4: GGATAGGAGTTCGCAGGAGC Tlr2_PW3: AACTCAGCCTGTAAGGGAGC Tlr2_R3: AACAGAATGCCTAAGGGGGT

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.

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	TLR2_FLOX-DON-Mut5
Forward Primer (5'-3')	GGAGCAAGGCAGAGTGAGTAG
Reverse Primer (5'-3')	GCTCACCAAGGATGTCTCTTAC
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	Tlr2-Flox-3.6-MUT2
Forward Primer (5'-3')	GGAGTCACAGTATGGGATAAC
Reverse Primer (5'-3')	GTCTAGGAAGAGCAACAGATT
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	TLR2-CR-LOA-WT
Forward Primer (5'-3')	TGTGCCCTTCTCCTGTTGA
Reverse Primer (5'-3')	AGCCACGCCCACATCATTC

Probe (5’-3’)	CTTGCTCGTAGGTGCCCTGTGC
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.