

**Name of Mouse model or mutation:****STAT2-FLOX-EM1-B6N****Description:**

Floxed allele made by CRISPR/Cas9 gene editing.

**Type of mutation:**

Floxed allele made by CRISPR/Cas9 gene editing.

**Delivery method:**

Pronuclear injection into 1-cell stage embryo.

**Genetic Background:**

C57BL/6NTac

**Nuclease:**

Cas9 mRNA

**sgRNAs:**

Protospacer sequence	PAM sequence
ACCCTCTGCGGTAAAATTC	<b>AGG</b>
AATTTTCAGGCCCTGATTTTA	<b>AGG</b>
AATAGTTAGCAAATGACGGG	<b>GGG</b>
ATAGTTAGCAAATGACGGG	<b>GGG</b>

**IssDNA long donor sequence (5'-3'):**

LOCUS Tm1c 1252 bp DNA linear 17-SEP-2020

FEATURES Location/Qualifiers

misc\_feature 229..262

/note="loxP"

misc\_feature 991..1024

/note="loxP"

misc\_feature 221..228

/note="AsiSI (SfaAI)"

misc\_feature 1025..1032

/note="Mrel"

PCR\_primer 200..220

/note="LoxPF"

PCR\_primer complement(1033..1052)

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        /note="LoxPR"
misc_feature 1..96
        /note="Stat2-201 transcript Exon 4: ENSMUSE00001063374"
misc_feature 1..199
        /note="5'HA "
misc_feature 562..651
        /note="Stat2- 201 transcript Exon 5: ENSMUSE00000969764"
misc_feature 263..990
        /note="Critical Region"
misc_feature 740..815
        /note="Stat2-201 transcript Exon 6: ENSMUSE00001035174"
misc_feature 1053..1252
        /note="3'HA "
misc_feature 1242..1252
        /note="Stat2-201 transcript Exon 7: ENSMUSE00001039638"
source      1..1252
        /dnas_title="Tm1c gBlock Stat2 Flox2"

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ORIGIN

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1 ACCTTTCCCA ATGGCCCTAC CCAGTTGGCT GAGATGATCT TTAATCTTCT TCTGGAAGAG
61 CAAAGGATTC TGAATCAGGC TCAAAGAGCT CAAGAGGTAA GGTTAGTGTG ATGTTGACAT
121 TCAAAGCCCC CTGGGACAGC AAGGTGCCTG GAGGAGAGTC AGGGAGCTTG TCTCAGACCA
181 GGATCTCCTC CACCCTCTGa tccgggggta ccgcgctcgag GCGATCGCAT AACTTCGTAT
241 AGCATACATT ATACGAAGTT ATTTAAGGAT GAAAACCTCTA GAGTTCATGT GGGAGTGAGG
301 GGATGTAGCC CACACCGTTA ATCCTAGCAT TCTGGGCTGC AGAGGCAGGT GGATCTCTGT
361 GAGTTTGAGC CCTGCTTGGT CTATGTAGTG AGTTCAGAA CATCCAGAAC ATACATAGAG
421 AAACCCTGTC TTGAAAACAA ACAAAACAAAC AAATAAACAA AACCACACAA TAACAAAAAA
481 ACAACAAAA AAACCCAAAA CCTGACAAAC AAAAAACAA GTCAGAGTTC AAACACACTC
541 GGAACCTCTT ACCTCATTCA GGTGCAGCCC CCACCAGCCC CCGAAGCAGT TGTGGAGAGC
601 CAGCAGCTTG AGATTGAAAA TCGAATCCAG GGTTTACATG TGGACATTGA GGTTGGCGGG
661 TGTGGCAAGT GCTGGGTGGG GAGGACTCGG TTTTCCTGCC CTCAGAAGCA GCCTCACTGG
721 GGTCCCATCT CCTCTACAGT TCTTGGTGAG ATCCATCAGG CAGCTGAAGG ACGAACAGGA
781 TGTCTTCAGC TTCAGATACA CAGTTTTAG TCTGAGTAGG TCCTCTCAAT GTGGGAGGAG
841 GACAGGGGCC ATTTTTGGGG TCTAGGGGAA GGACGGACAA CAGAAGCTAA ATGATCCAGA
901 GAGCAGTGGT CACATATGGC CGGTGGTAGT ACAGCATCAG AGGTGTCGGG TGTGTTGTGG
961 AGGGTGGACC TGAAGAATAG TTAGCAAATG ATAACCTCGT ATAGCATACA TTATACGAAG
1021 TTATCGCCGG CGggtctgag ctcgcatca gtCTGTGTAG AAAACTGCCT TAGCAGATTG
1081 AGTGGGCTTC TGGTCTGTCC AAAATTTGCC TGTGGGATTC CATGTACCTG TACTTTATCT
1141 GGTCTCCCT TCCTCCCCAT CCAACATTTT CGGTTTCTGA GACCCTGAGG CAGAAAAGAG
1201 CAGAAGGACC CTGTGGTCCC AGTTTTGGTC TTGCCTTCA GAGAAGACGT CG

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**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/μl, 50 ng/μl each and 50 ng/μl, 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

### Stat2 WT

```
CTTGCGAAAATTCAGCCGGGATATTCAGGTAAGGACTTGGAAAGAGCCGGGAATAGTGGCATGCAGCTGGC
CTTTAAGTATAGGGTAAGAGCTGGGGTACAGAGTCTGGACGTGGAGTGTCCCTAGCAGAGTAAAG
GACTGAATTTAGATTCAGGGACTGTGAAGGAGGAAAATACTAAAGACACTGAAGGCAGGGTCTGG
ATTGGCAGGTCACCTAAGGTAGTTAAATGGGAGCTTGCCTCTGTTTTCTACTTAGACCTTTCCCAAT
GGCCCTACCCAGTTGGCTGAGATGATCTTTAATCTTCTTCTGGAAGAGCAAAGGATTCTGAATCAGG
CTCAAAGAGCTCAAGAGGTAAGGTTAGTGTGATGTTGACATTCAAAGCCCCTGGGACAGCAAGGT
GCCTGGAGGAGAGTCAAGGAGCTTGTCTCAGACCAGGATCTCCTCCACCCTCTGCGGTAAAATTTCA
GGCCCTGATTTTAAGGATGAAAACCTAGAGTTCATGTGGGAGTGAGGGGATGTAGCCACACCGT
TAATCCTAGCATTCTGGGCTGCAGAGGCAGGTGGATCTCTGTGAGTTTGAGCCCTGCTTGGTCTATG
TAGTGAGTTCAGAACATCCAGAACATACATAGAGAAACCCTGTCTTGAAAACAAACAAACAAACAA
ATAAACAAAACCACACAATAACAAAAAACAACAAAAAACCACAAACCTGACAAACAAAAAAC
AAGTCAGAGTTCAAACACACTCGGAACCTTTACCTCATTGAGGTGCAGCCCCCACCAGCCCCGAA
GCAGTTGTGGAGAGCCAGCAGCTTGAGATTGAAAATCGAATCCAGGGTTTACATGTGGACATTGAG
GTTGGCGGGTGTGGCAAGTGCTGGGTGGGGAGGACTCGGTTTTCTGCCCTCAGAAGCAGCCTCAC
TGGGGTCCCATCTCCTCTACAGTTCTTGGTGGAGATCCATCAGGCAGCTGAAGGACGAACAGGATGTC
TTCAGCTTCAGATACACAGTTTTTCTGAGTAGGTCTCTCAATGTGGGAGGAGGACAGGGGCC
ATTTTTGGGGTCTAGGGGAAGGACGGACAACAGAAGCTAAATGATCCAGAGAGCAGTGGTCACAT
ATGGCCGGTGGTAGTACAGCATCAGAGGTGTGGGTGTGTTGTGGAGGGTGGACCTGAAGAATAG
TTAGCAAATGACGGGGGGGGGGGGGGGGGGTACTGTGTAGAAAACCTGCTTAGCAGATTGAGT
GGGCTTCTGGTCTGTCCAAAATTTGCCTGTGGGATTCCATGTACCTGTACTTTATCTGGTTCTCCCTC
CTCCCCATCCAACATTTTCGTTTTCTGAGACCCTGAGGCAGAAAAGAGCAGAAGGACCCTGTGGTCC
CAGTTTTGGTCTTGCCTTTCAGAGAAGACGTCGTCTTACAGACCCCATCAGAGCCAACAGGGCGCAGC
TTGTGCAGGCAACAGCCAACAAAGTCGACAGAATGAGAAAGGTGGGGGCAGGGCACAGAGGGGG
CAGTGGGGAGCTGGGGGGAGGGGGGAGAGGGCAGTAGGAAACTGGGAGCCAGGACAGGGAGG
GTCTGAGGGCAGCAGTGGGAGGTCATGGATGGGGGGCGCAGCAGGGAACCTATGGGAGAGGGGG
ATGGGGGCAGCAGGAAACTGGGAGACACCAGAGGGGGTTCAGGGCACAGCAGGGAACCGGGGG
GGGGGCAGCAGAGGGGGTTCATGGGGGCAGCTGGATGACAGGAGTGGTATGAGATACTTAGCCC
TCAGGAGCTCTTTCTAGGAGGTGCTGGACATCTCAAAGGACTGGTTGGCCGATTAACCACCT
GGTCGACCTATTGCTGCCCAAGCT
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### STAT2-FLOX-EM1-B6N

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CTTGCGAAAATTCAGCCGGGATATTCAGGTAAGGACTTGGAAAGAGCCGGGAATAGTGGCATGCAGCTGGC
CTTTAAGTATAGGGTAAGAGCTGGGGTACAGAGTCTGGACGTGGAGTGTCCCTAGCAGAGTAAAG
GACTGAATTTAGATTCAGGGACTGTGAAGGAGGAAAATACTAAAGACACTGAAGGCAGGGTCTGG
ATTGGCAGGTCACCTAAGGTAGTTAAATGGGAGCTTGCCTCTGTTTTCTACTTAGACCTTTCCCAAT
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GGCCCTACCCAGTTGGCTGAGATGATCTTTAATCTTCTTCTGGAAGAGCAAAGGATTCTGAATCAGG  
CTCAAAGAGCTCAAGAGGTAAGGTTAGTGTGATGTTGACATTCAAAGCCCCTGGGACAGCAAGGT  
GCCTGGAGGAGAGTCAGGGAGCTTGTCTCAGACCAGGATCTCCTCCACCCTCTGatccgggggtaccg  
tcgagGCGATCGCATAACTTCGTATAGCATACTTATACGAAGTTATTTAAGGATGAAAACCTAGAG  
TTCATGTGGGAGTGAGGGGATGTAGCCCACACCGTTAATCCTAGCATTCTGGGCTGCAGAGGCAGG  
TGGATCTCTGTGAGTTTGTAGCCCTGCTTGGTCTATGTAGTGAGTTCCAGAACATCCAGAACATACAT  
AGAGAAACCCTGTCTTGAAAACAAACAAACAAATAAACAAAACACACAATAACAAAAAAC  
AAACAAAAAACCCAAACCTGACAAACAAAAAACAAAGTCAGAGTTCAAACACACTCGGAACCTC  
TTACCTCATTGAGGTGCAGCCCCACCAGCCCCGAAGCAGTTGTGGAGAGCCAGCAGCTTGAGAT  
TGAAAATCGAATCCAGGGTTTACATGTGGACATTGAGGTTGGCGGGTGTGGCAAGTGCTGGGTGG  
GGAGGACTCGTTTTCTGCCCTCAGAAGCAGCCTCACTGGGGTCCCATCTCCTCTACAGTTCTTGGT  
GAGATCCATCAGGCAGCTGAAGGACGAACAGGATGTCTTCAGCTTCAGATACACAGTTTTCAGTCT  
GAGTAGGTCCTCTCAATGTGGGAGGAGGACAGGGGCCATTTTTGGGGTCTAGGGGAAGGACGGAC  
AACAGAAGCTAAATGATCCAGAGAGCAGTGGTACATATGGCCGGTGGTAGTACAGCATCAGAGGT  
GTCGGGTGTGTTGTGGAGGGTGGACCTGAAGAATAGTTAGCAAATGATAACTTCGTATAGCATA  
TTATACGAAGTTATCGCCGGCGggtctgagctcgccatcagtCTGTGTAGAAAACCTGCTTAGCAGATTGAG  
TGGGCTTCTGGTCTGTCCAAAATTTGCCTGTGGGATTCCATGTACCTGTACTTTATCTGGTTCTCCCT  
CCTCCCATCCAACATTTTCGGTTTCTGAGACCCTGAGGCAGAAAAGAGCAGAAGGACCCTGTGGTC  
CCAGTTTTGGTCTTGCCTTTCAGAGAAGACGTCGTCTTCAGACCCCATCAGAGCCAACAGGCGCAG  
CTTGTGCAGGCAACAGCCAACAAAGTCGACAGAATGAGAAAGGTGGGGGCAGGGCACAGAGGGG  
GCAGTGGGGAGCTGGGGGGAGGGGGGAGAGGGCAGTAGGAACTGGGAGCCAGGACAGGGAG  
GGTCTGAGGGCAGCAGTGGGAGGTCATGGATGGGGGGCGCAGCAGGGAACATGGGAGAGGGG  
GATGGGGGCAGCAGGAACTGGGAGACACCAGAGGGGGTTCAGGGCACAGCAGGGAACCGGGG  
GGGGGGCAGCAGAGGGGGTTCATGGGGGCAGCTGGATGACAGGAGTGGTATGAGATACTTAGCC  
CTCAGGAGCTCTTTCCTAGGAGGTGCTGGACATCTCAAAGGACTGGTTGGCCGATTAACCAACC  
TGGTCGACCTATTGCTGCCCAAGCT

LoxP sites are highlighted in red and genotyping handles (restriction enzyme site plus primer unique to each LoxP site) in yellow. Exons are highlighted in grey, with floxed exons in bold also.

# Nucleotide Alignment:

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                *      20      *      40      *      60      *      80      *      100     *      120     *      140
Stat2_WT : CTTGCGAAAATTCAGCCGGGATATTCAGTACTTGGAAAGAGCCGGGAATAGTGGCATGCAGCTGGCCCTTAAGTATAGGGTAAGAGCTGGGGTACAGAGTCTGGACGTGGAGTCCCTAGCAGAGTAAGGACTGAAT
Stat2_EM1 : CTTGCGAAAATTCAGCCGGGATATTCAGTACTTGGAAAGAGCCGGGAATAGTGGCATGCAGCTGGCCCTTAAGTATAGGGTAAGAGCTGGGGTACAGAGTCTGGACGTGGAGTCCCTAGCAGAGTAAGGACTGAAT

                *      160     *      180     *      200     *      220     *      240     *      260     *      280
Stat2_WT : TAGATTCAGGGACTGTGAAGGAGGAAAATACTAAAGACACTGAAGCAGGGTCTGGATTGGCAGGTCACTAAGGTAGTTAAATGGGAGCTGCCTCTGTTTTCTACTTAGACCTTTCCCAATGGCCCTACCCAGTTGG
Stat2_EM1 : TAGATTCAGGGACTGTGAAGGAGGAAAATACTAAAGACACTGAAGCAGGGTCTGGATTGGCAGGTCACTAAGGTAGTTAAATGGGAGCTGCCTCTGTTTTCTACTTAGACCTTTCCCAATGGCCCTACCCAGTTGG

                *      300     *      320     *      340     *      360     *      380     *      400     *      420
Stat2_WT : CTGAGATGATCTTTAATCTTCTTCTGGAAAGACAAAGGATTCGAATCAGGCTCAAAGAGCTCAAGAGTAAGGTTAGTGTGATTTGACATTCAAAAGCCCTGGGACAGCAAGTCCCTGGAGGAGTCAAGGAGCT
Stat2_EM1 : CTGAGATGATCTTTAATCTTCTTCTGGAAAGACAAAGGATTCGAATCAGGCTCAAAGAGCTCAAGAGTAAGGTTAGTGTGATTTGACATTCAAAAGCCCTGGGACAGCAAGTCCCTGGAGGAGTCAAGGAGCT

                *      440     *      460     *      480     *      500     *      520     *      540     *      560
Stat2_WT : TGTCTCAGACCAGGATCTCCCTCACCCCTCTG-----GGTTAAATTTTCAAGTCCCTGATGTTAAGGATGAAAACCTAGAGTTCATGTGGGAGTGGGGATCTAG
Stat2_EM1 : TGTCTCAGACCAGGATCTCCCTCACCCCTCTGatccgggggtaccgctcgagGCGATCGCTAAATTCGATATACACACATATACGAAGTAAATTAAGGATGAAAACCTAGAGTTCATGTGGGAGTGGGGATCTAG

                *      580     *      600     *      620     *      640     *      660     *      680     *      700
Stat2_WT : CCCACACCGTTAATCTTAGCATTTCTGGGCTGCAGAGGCAGTGGATCTCTGTGAGTTTGGCCCTGCTTGGTCTATGTAGTGTAGTCCAGAACATCCAGAACATACATAGAGAAACCTGTCTTGAAAACAAACAAACA
Stat2_EM1 : CCCACACCGTTAATCTTAGCATTTCTGGGCTGCAGAGGCAGTGGATCTCTGTGAGTTTGGCCCTGCTTGGTCTATGTAGTGTAGTCCAGAACATCCAGAACATACATAGAGAAACCTGTCTTGAAAACAAACAAACA

                *      720     *      740     *      760     *      780     *      800     *      820     *      840
Stat2_WT : ACAATAAAACAAACCACACATAACAAAAAACAAACAAAAACCCAAAACCTGCAGAAAACAAAAAACAGTCAAGGTTCAAACACACTCGGAACCTCTTACCTCATTCAGTGCAGCCCCCAGCCCCGAAGCA
Stat2_EM1 : ACAATAAAACAAACCACACATAACAAAAAACAAACAAAAACCCAAAACCTGCAGAAAACAAAAAACAGTCAAGGTTCAAACACACTCGGAACCTCTTACCTCATTCAGTGCAGCCCCCAGCCCCGAAGCA

                *      860     *      880     *      900     *      920     *      940     *      960     *      980
Stat2_WT : GTTGTGGAGAGCCAGCAGCTTGAGATTGAAATCGAATCCAGGGTTTACATGTGGACATTTGAGTTGGGGGGTGGGAAAGTCTGGGTGGGGAGGACTCGGTTTTCTGCCTCAGAAAGCAGCTCAGTGGGTCCCA
Stat2_EM1 : GTTGTGGAGAGCCAGCAGCTTGAGATTGAAATCGAATCCAGGGTTTACATGTGGACATTTGAGTTGGGGGGTGGGAAAGTCTGGGTGGGGAGGACTCGGTTTTCTGCCTCAGAAAGCAGCTCAGTGGGTCCCA

                *      1000    *      1020    *      1040    *      1060    *      1080    *      1100    *      1120
Stat2_WT : CTCCTCTACAGTTCTTGGTGGATCCATCAGGCAGCTGAAGGACGAAACAGGATGCTTCAGCTCAGATACACAGTTTCTAGTCTGTAAGTCCCTCAATCTGGGAGGAGCAGGGGCCATTTTTGGGGTCTAGGG
Stat2_EM1 : CTCCTCTACAGTTCTTGGTGGATCCATCAGGCAGCTGAAGGACGAAACAGGATGCTTCAGCTCAGATACACAGTTTCTAGTCTGTAAGTCCCTCAATCTGGGAGGAGCAGGGGCCATTTTTGGGGTCTAGGG

                *      1140    *      1160    *      1180    *      1200    *      1220    *      1240    *      1260
Stat2_WT : AAGGACGGACAACAGAGTAAATGATCCAGAGAGCAGTGGTTCACATATGGCCGGTGGTAGTACAGCATCAGAGGTGTCGGGTGTTGTGGAGGGTGGACCTGAAGAATAGTTAGCAAATG-----
Stat2_EM1 : AAGGACGGACAACAGAGTAAATGATCCAGAGAGCAGTGGTTCACATATGGCCGGTGGTAGTACAGCATCAGAGGTGTCGGGTGTTGTGGAGGGTGGACCTGAAGAATAGTTAGCAAATGATACTGGTATAGCA

                *      1280    *      1300    *      1320    *      1340    *      1360    *      1380    *      1400
Stat2_WT : ---ACGGGGCCGGGGCCGGCCCTA-----CTGTGTAGAAAACCTGGCTTAGCAGATTGAGTGGGCTTCTGGTCTGTCCAAAATTTGCCGTGGGATTCATGTACCTGTACTTTAICTGGTCTCC
Stat2_EM1 : AATTAAGAAATTAAGGCGCCGGCGggtctgagctgcccacagtCTGTGTAGAAAACCTGGCTTAGCAGATTGAGTGGGCTTCTGGTCTGTCCAAAATTTGCCGTGGGATTCATGTACCTGTACTTTAICTGGTCTCC

                *      1420    *      1440    *      1460    *      1480    *      1500    *      1520    *      1540
Stat2_WT : CTTCTCCCATCCCAAGATTTTCGGTTTCTGAGACCTGAGGCAGAAAAGAGCAGAAGGACCTGTGGTCCAGTTTGGTCTTGCCTTTCAGAGAAGACGTGCTTTAGACCCCCATCAGAGCCAAACAGGGCAGCT
Stat2_EM1 : CTTCTCCCATCCCAAGATTTTCGGTTTCTGAGACCTGAGGCAGAAAAGAGCAGAAGGACCTGTGGTCCAGTTTGGTCTTGCCTTTCAGAGAAGACGTGCTTTAGACCCCCATCAGAGCCAAACAGGGCAGCT

                *      1560    *      1580    *      1600    *      1620    *      1640    *      1660    *      1680
Stat2_WT : GTGCAGGCAACAGCCAAACAAAGTCGACAGAATGAGAAACGTGGGGCAGGGCACAGAGGGGGCAGTGGGGAGCTGGGGGGAGGGGGAGAGGGCAGTGGAAACTGGGAGCCAGGACAGGGAGGGTCTGAGGGCAGCACT
Stat2_EM1 : GTGCAGGCAACAGCCAAACAAAGTCGACAGAATGAGAAACGTGGGGCAGGGCACAGAGGGGGCAGTGGGGAGCTGGGGGGAGGGGGAGAGGGCAGTGGAAACTGGGAGCCAGGACAGGGAGGGTCTGAGGGCAGCACT

                *      1700    *      1720    *      1740    *      1760    *      1780    *      1800    *      1820
Stat2_WT : GGGAGGTCATGGATGGGGGGCCGACAGGGAACCTATGGGAGAGGGGGATGGGGGCAGCGAAACTGGGAGACACAGAGGGGGTTGAGGGCACAGAGGGAACCGGGGGGGGGCAGCAGAGGGGGTCTAGGGGGCAG
Stat2_EM1 : GGGAGGTCATGGATGGGGGGCCGACAGGGAACCTATGGGAGAGGGGGATGGGGGCAGCGAAACTGGGAGACACAGAGGGGGTTGAGGGCACAGAGGGAACCGGGGGGGGGCAGCAGAGGGGGTCTAGGGGGCAG

                *      1840    *      1860    *      1880    *      1900    *      1920    *      1940
Stat2_WT : CTGGATGACAGGACTGGTATGAGTACTTAGCCCTCAGGAGCTCTTTCCCTAGGAGGTGCTGGACATCTCCAAAGGACTGGTGGCCGATTAAACCCCTGGTGGACCTATTGCTGCCCAAGCT
Stat2_EM1 : CTGGATGACAGGACTGGTATGAGTACTTAGCCCTCAGGAGCTCTTTCCCTAGGAGGTGCTGGACATCTCCAAAGGACTGGTGGCCGATTAAACCCCTGGTGGACCTATTGCTGCCCAAGCT
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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:

LoxP_F	ATCCGGGGGTACCGCGTCGAG
LoxP_R	ACTGATGGCGAGCTCAGACC
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	60
Elongation time (min)	1.5
WT product size (bp)	N/A
Mutant product size (bp)	853
Notes	Screening PCR to identify floxed allele.

Geno_Stat2_F1 primer (5'-3')	CTTGCGAAAATTCAGCCGGG
Geno_Stat2_R1 primer (5'-3')	AGCTTGGGCAGCAATAGGTC
Taq Polymerase used	ThermoFisher SuperFi PCR kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	1866
Mutant product size (bp)	1945
Notes	Sequenced using the following primers (5'-3'): Geno_Stat2_F2 primer: TTGGAAGAGCCGGGAATAGTG Geno_Stat2_R1 primer: TTAATCGGCCAACCAGTCCT LoxPF: ATCCGGGGGTACCGCGTCGAG LoxPR: ACTGATGGCGAGCTCAGACC

Geno_Stat2_F1 primer (5'-3')	CTTGCGAAAATTCAGCCGGG
Geno_Stat2_R4 primer (5'-3')	CCGCCAACCTCAATGTCCA
Taq Polymerase used	ThermoFisher SuperFi II PCR kit
Annealing Temperature (°C)	65

Elongation time (min)	0.75
WT product size (bp)	871
Mutant product size (bp)	911
Notes	PCR carried out to better assess the 5' end of the allele. Sequenced using the amplification primers and the following primer: LoxPF_R: ctcgacgcggtacccccgat

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 in the seed sequence for guide(s) used were checked with the following primers:**

Off-target site	Sequence	Type	Primers used (5'-3')
<a href="#">10:116181714-116181736</a>	CCCTCTACGGTAAAATTTA AGG	Intronic	Stat2_OT1_F1: TTTTGGGACCAAGGCCAACT Stat2_OT1_R1: ATCGAAGTTGCCACACACT Sequenced also with Stat2_OT1_F2: GCATGGCTGGGA ACTCTGTA

All amplicons were sent for Sanger sequencing. No evidence of any off-target activity was detected.

#### **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	STAT2-FLOX-5'-MUT1
Forward Primer (5'-3')	GATCTCCTCCACCCTCTGAT
Reverse Primer (5'-3')	CCTCACTCCCACATGAACTCTA
Probe (5'-3')	TCGAGGCGATCGCATAACTTCG
Label	FAM

This ddPCR assay is specific to the Stat2 Flox donor and only floxed 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	STAT2-CR-LOA-WT1
Forward Primer (5'-3')	ATGTGGACATTGAGGTTGG
Reverse Primer (5'-3')	AGAACTGTAGAGGAGATGGG
Probe (5'-3')	TTTCCTGCCCTCAGAAGCAGC
Label	FAM

This ddPCR assay is universal to Stat2 - both WT and floxed 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	STAT2-FLOX-3'-MUT1
Forward Primer (5'-3')	GGAGGGTGGACCTGAAGAATA
Reverse Primer (5'-3')	GAAGCCCACTCAATCTGCTAAG
Probe (5'-3')	AAGTTATCGCCGGCGGGTCTGA
Label	FAM

This ddPCR assay is specific to the Stat2 Flox donor and only floxed 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 evidence of random donor integrations were detected in the animals selected for breeding.





## Allele Description

This is a CRISPR/Cas9 induced mutation creating a conditional knock-out by floxing critical exon, ENSMUSE00000969764 and ENSMUSE00001035174 of *Stat2*. The stock was generated at MRC Harwell via pronuclear injection of CRISPR/Cas9 reagents into 1-cell stage embryos.

## qPCR Copy Counting Genotyping Strategy

The genotyping strategy presented here has been optimized for reagents and conditions used by the Genotyping Core at MRC Harwell. To genotype animals, we recommend researchers validate the assay independently. PCR cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Samples are genotyped using qPCR copy counting with both a wildtype loss of allele (WT-LOA) and a mutant assay against a known reference assay (*Dot1l* on chromosome 10; 2 copies present). Samples for this line are genotyped using the following primers and probe:

- Universal probe and Universal primer designed 5' of the deleted region.
- Wildtype specific primer situated within the deleted region.
- Mutant specific primer that binds to the inserted LoxP sequence

For autosomal genes that have been targeted, the following results would be expected:

Genotype of the Modified allele	WT Assay	Mutant Assay
Wildtype	2	0
Heterozygous	1	1
Homozygous mutant	0	2



## Stat2-Flox-3'-WT1 assay (FAM labelled)

ATTTTTGGGGTCTAGGGGAAGGACGGACAACAGAAGCTAAATGATCCAGAGAGCAGTGGTCACAT  
 ATGGCCGGTGGTAGTACAGCATCAGAGGTGTCGGGTGTGTTGTGGAGGGTGGACCTGAAGAATA  
**GTTAGCAAATGACGGGGGGGGGGGGGGGGGGTACTGTGTAGAAAAGTGCCTTAGCAGATTGAG**  
**TGGGCTTCTGGTCTGTCCAAATTTGCCTGTGGGATTCCATGTACCTGTACTTTATCTGGTTCTCCCT**

Probe sequence is in bold and shaded grey  
 Primer sequences are in bold and underlined

Oligo Stat2	5' label	Sequence 5' → 3'	3' label	Oligo Type
Stat2-WT_F	n/a	<u>GTAGTACAGCATCAGAGGTGTC</u>	n/a	WT Forward
Stat2-WT_PROBE	FAM	<b>ACCTGAAGAATAGTTAGCAAATGACGGGG</b>	ZEN-IBFQ	WT Probe
Stat2-WT_R	n/a	<u>AGCCCACTCAATCTGCTAAG</u>	n/a	WT Reverse

## Stat2-Flox-3'-MUT1 assay (FAM labelled)

AACAGAAGCTAAATGATCCAGAGAGCAGTGGTCACATATGGCCGGTGGTAGTACAGCATCAGAGGT  
 GTCGGGTGTGTTGTGGAGGGTGGACCTGAAGAATAGTTAGCAAATGataacttcgtatagcatatatac  
**gaagttatCGCCGGCGGGTCTGA**GCTCGCCATCAGTCTGTGTAGAAAAGTGCCTTAGCAGATTGAGTG  
**GGCTTC**TGGTCTGTCCAAATTTGCCTGTGGGATTCCATGTACCTGTACTTTATCTGGTTCTCCCT

Lower case letters denote the inserted LoxP sequence  
 Probe sequence is in bold and shaded grey  
 Primer sequences are in bold and underlined

Oligo Stat2	5' label	Sequence 5' → 3'	3' label	Oligo Type
Stat2-MUT_F	n/a	<u>GGAGGGTGGACCTGAAGAATA</u>	n/a	Mutant Forward
Stat2-MUT_PROBE	FAM	<b>AAGTTATCGCCGGCGGGTCTGA</b>	ZEN-IBFQ	Mutant Probe
Stat2-MUT_R	n/a	<u>GAAGCCCACTCAATCTGCTAAG</u>	n/a	Mutant Reverse

## Dot1l internal control (VIC labelled)

CTGATGGGTGTGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTTGCC  
**CCAGCAGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAAGATGAAGCATAAGGATGCCAACTACTAACA  
 GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT  
 CACCCTATGA

Oligo Stat2	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	<u>GCCCCAGCAGACCATT</u>	n/a	WT Forward
Dot1l_Probe	VIC	<b>CCAGCTCTCAAGTCG</b>	BHQ	WT Probe
Dot1l_Reverse	n/a	<u>TAGTTGGCATCCTTATGCTTCATC</u>	n/a	WT Reverse

Probe sequence is in bold and shaded grey  
 Primer sequences are in bold and underlined



## DNA extraction method

DNA is extracted from ear clips using Applied Biosystems Taqman Sample-to-SNP Kit and qPCR run using 1:10 dilution from the crude preparation.

## qPCR master mix 1X

Applied Biosystems GTX Taqman master mix	5 $\mu$ l
Dot1l_Forward (20 $\mu$ M)	0.225 $\mu$ l
Dot1l_Reverse (20 $\mu$ M)	0.225 $\mu$ l
Dot1l_Probe (5 $\mu$ M)	0.2 $\mu$ l
FAM Assay (probe 5 $\mu$ M & primers 15 $\mu$ M each)	0.3 $\mu$ l
ddH <sub>2</sub> O	1.55 $\mu$ l
DNA (1:10 dilution of ABI Sample-to-SNP prep)	2.5 $\mu$ l

Each sample is ran in technical duplicate. Seven WT and/or mutant controls are also included in duplicate along with non-template controls.

## qPCR cycling conditions

qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

95°C for 20 sec  
Then 40 cycles of;  
95°C for 3 sec  
60°C for 30 sec

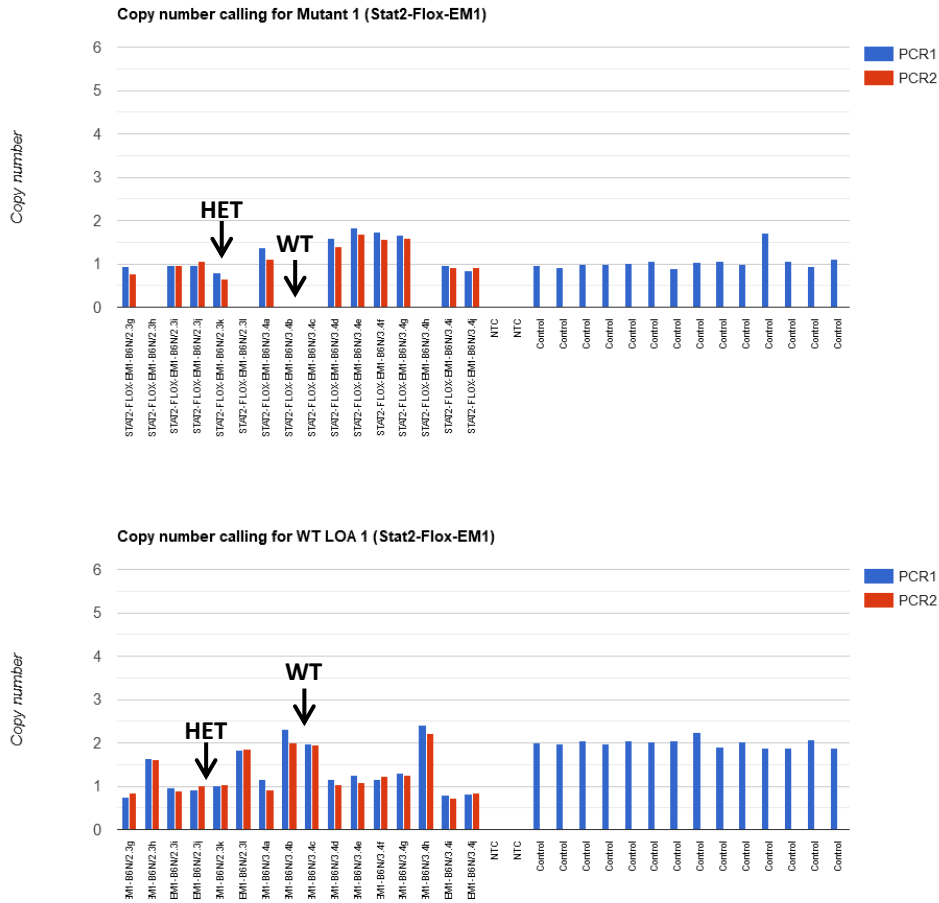
## Analysis

The results are analysed using CopyCaller software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.



# STAT2-Flox

Stat2'-WT1 and Stat2-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 323514 results)



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