# A suppressor locus for MODY3-diabetes

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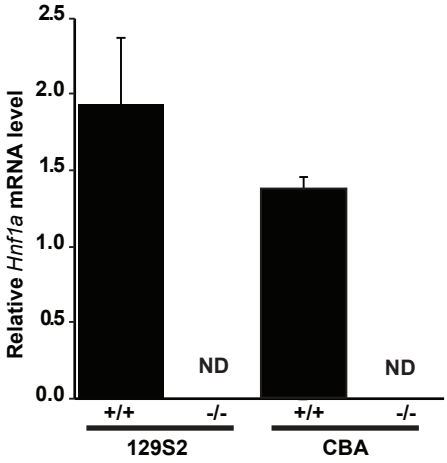
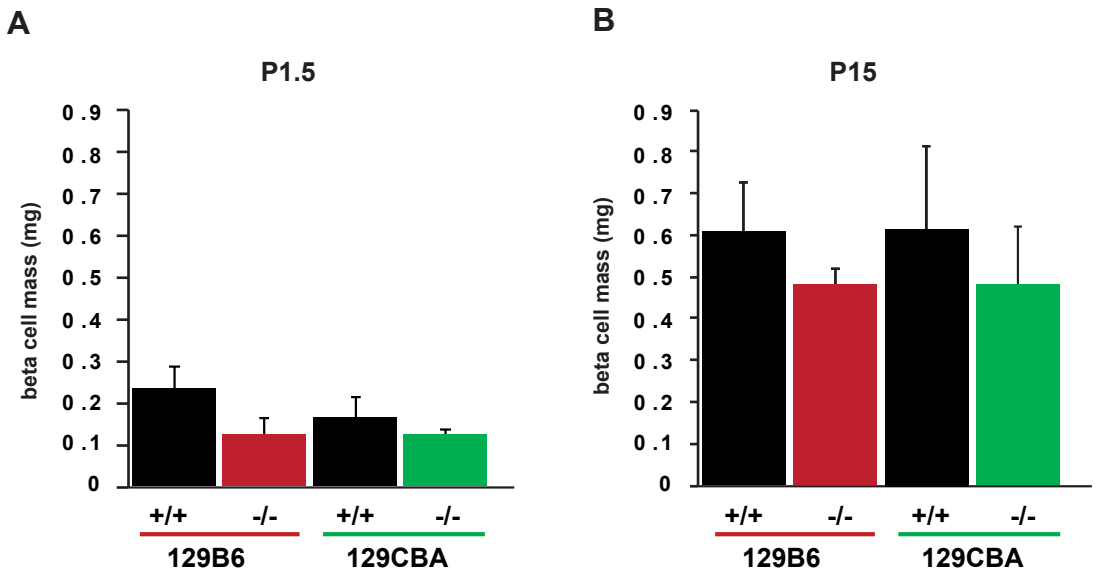
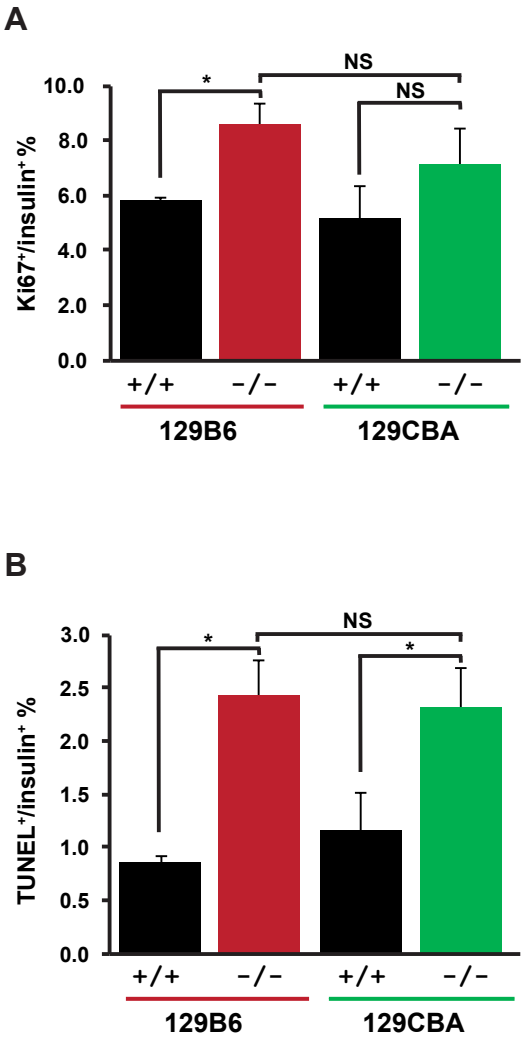
**Supplementary Figure S1: Absence of *Hnf1a* transcript in both resistant and sensitive mutant strains**
*Hnf1a* expression in P15 pancreas of wild-type 129S2 and CBA, and *Hnf1a*$^{-/-}$ 129S2 and CBA mice was analyzed by RT-qPCR. Number of animals in each group=3; ND=not detected



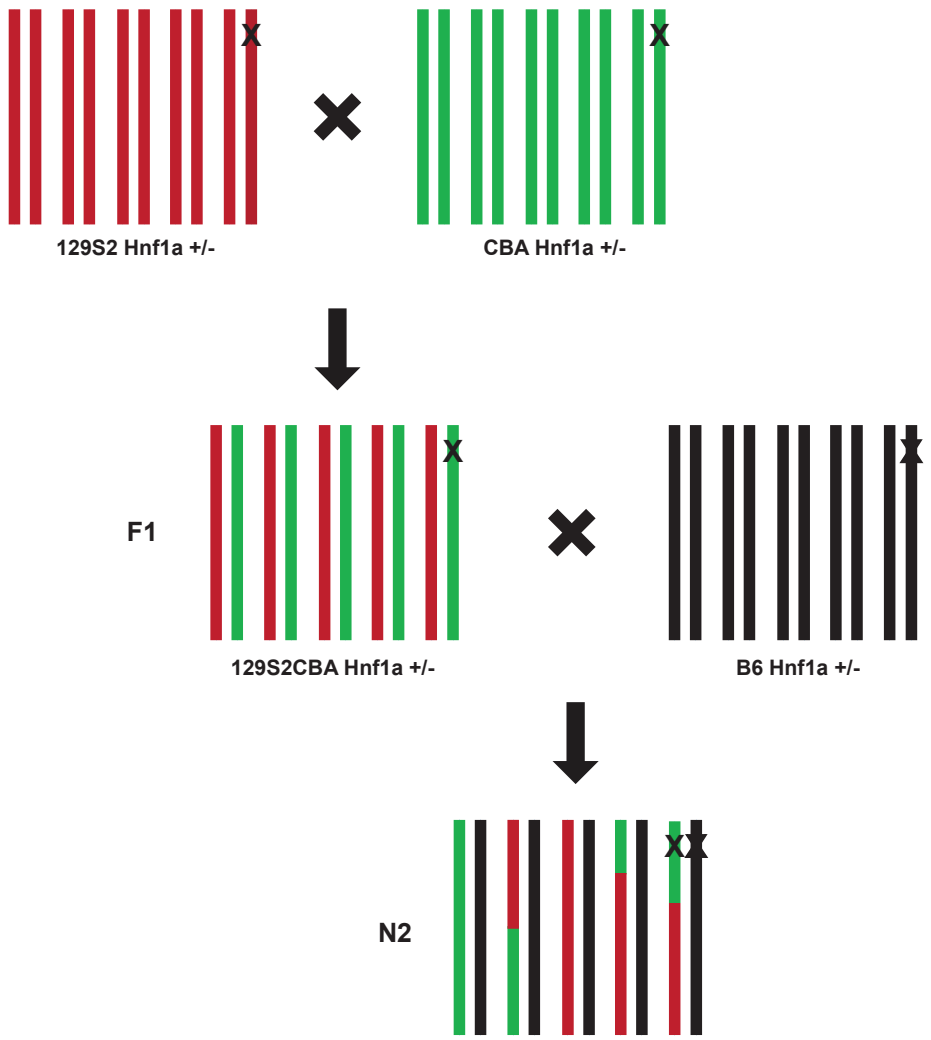
**Supplementary Figure S2: No difference in beta cell mass between *Hnf1a* mutant 129B6 and 129CBA mice at P1.5 and P15**. Beta cell mass was estimated at **(A)** P1.5 and **(B)** P15 by counting positive cells for insulin labeling and the total tissue area was determined with DAPI staining (3 animals per genotype). Results are expressed as mean ± SD



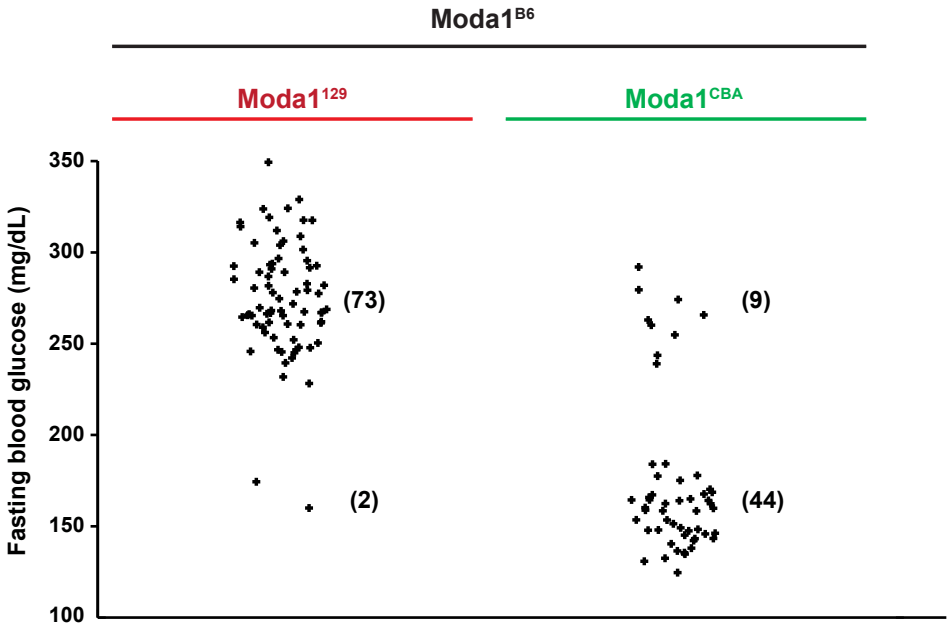
**Supplementary Figure S3: Proliferation and apoptosis in beta cells at P15. (A)** Proliferation and **(B)** apoptosis were determined by counting the proportion of insulin positive cells labeled with Ki67 antibody or TUNEL assay, respectively**.** No significant differences were observed between the two mutant strains. NS=non-significant; *p-value<0.05. Results are expressed as mean ± SD. Number of animals per genotype=3.



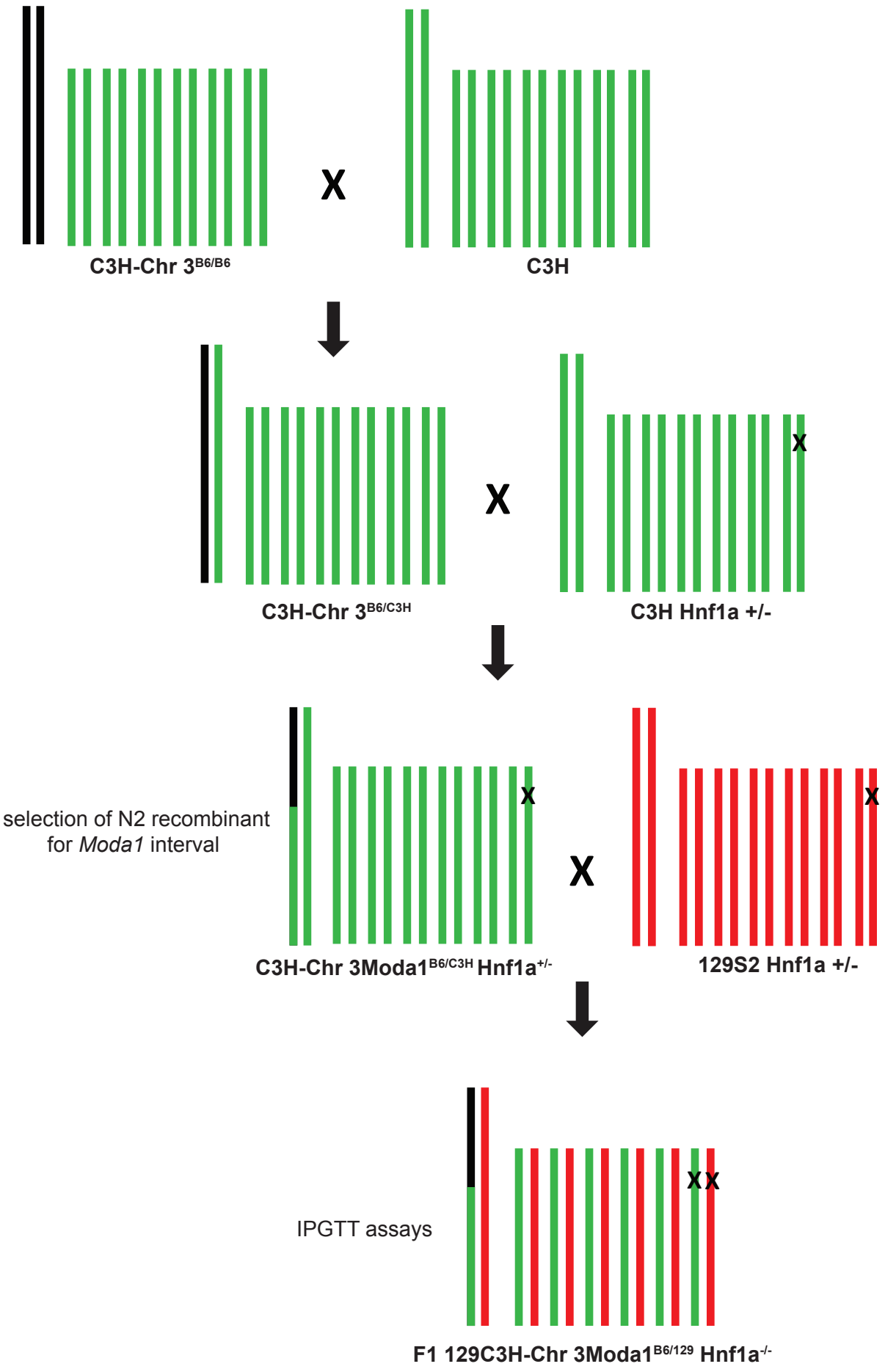
**Supplementary Figure S4: Mouse breeding strategy adopted to identify the resistant locus to diabetes.** Pure sensitive (129S2) and resistant (CBA) strains heterozygous for Hnf1a mutation were crossed. The resulting F1 progeny was outcrossed with a sensitive strain (B6) distinct from the parental one (129S2) to increase as much as possible the hybrid vigor. N2 Hnf1a null mice derived from this crossing were analyzed for their phenotype.



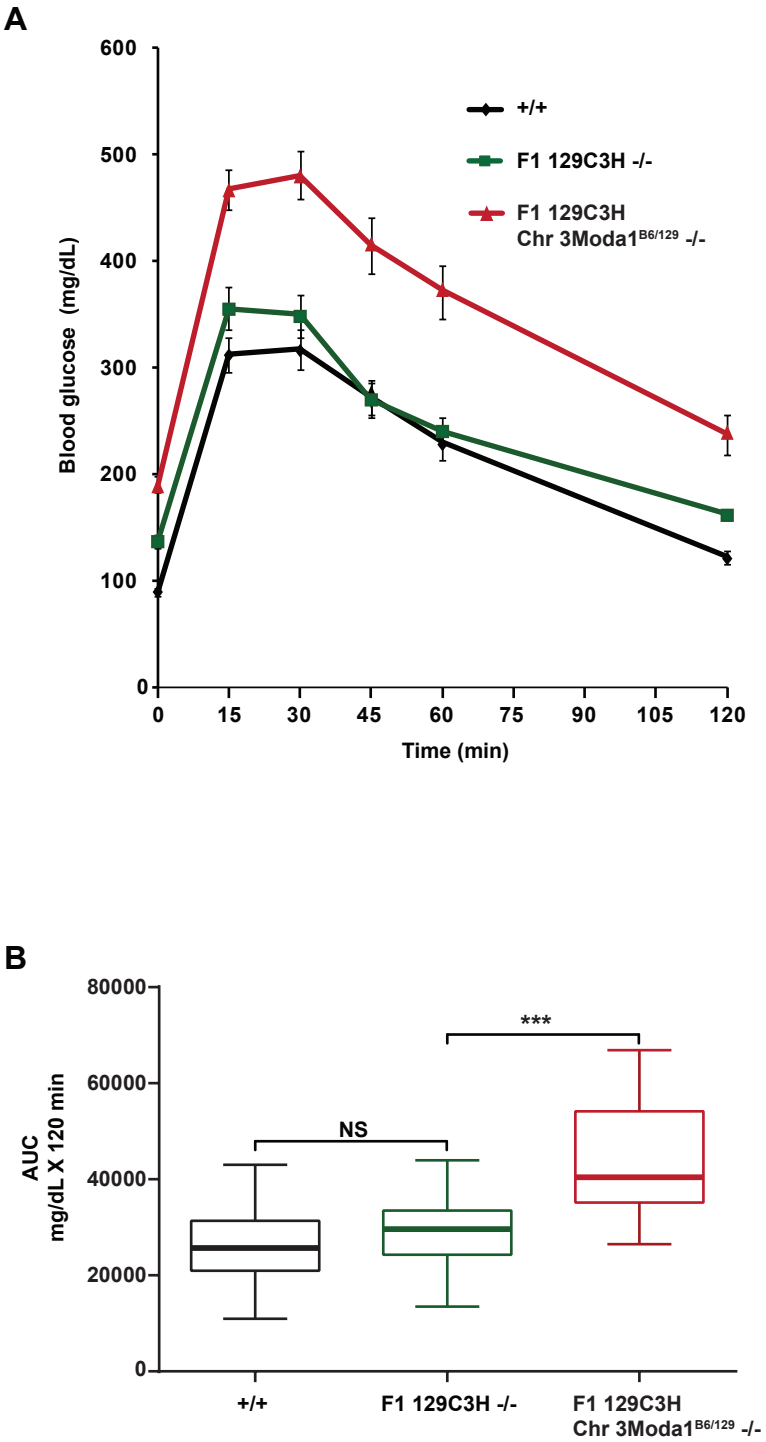
**Supplementary Figure S5: Impact of Moda1 allele on the fasting glucose levels in N2 mutant mice.** More than 97% of mice that inherited the *Moda1*$^{129}$ allele were hyperglycemic (left part of the chart) whereas 83% of mice carrying the *Moda1*$^{CBA}$ allele did not develop diabetes (right part of the chart). Numbers of mice are indicated in brackets.



**Supplementary Figure S6: Mouse breeding strategy adopted to generate recombinants around the *Moda1* locus.** A consomic strain (C3H/HeJ-Chr $3^{C57BL/6J}$/J or C3H-Chr $3^{B6/B6)}$) was crossed with a pure C3H strain. The progeny was mated with a C3H *Hnf1a*$^{+/-}$ strain and recombinant mice for the *Moda1* locus were selected. Three independent recombinant lines carrying *Moda1*$^{B6/C3H}$ were then crossed with 129S2 *Hnf1a+/-* strain and mice were challenged with glucose tolerant tests. Long bars denote chromosome 3 and short bars represent the rest of the genome.



**Supplementary Figure S7: The Moda1 interval confers resistance/sensitivity to diabetes**
**(A)** IPGTTs on wild type animals (black curve, n=32), F1 129C3H *Hnf1a*$^{-/-}$ resistant animals (green curve, n=31) and a pool of three distinct recombinant lines that carry *Moda1*$^{B6/129}$ on the chromosome 3 and 129C3H for the rest of the genome (red curve, n=25). Error bars represent SEM. **(B)** Boxplot representation of the area under the curve (AUC) determined from IPGTT presented in **(A)**. AUCs are not significantly different between wild type and F1 129C3H *Hnf1a*$^{-/-}$ mice (black box and green box, respectively). The AUC of recombinant *Moda1*$^{B6/129}$ *Hnf1a*$^{-/-}$ mice (red box) is significantly higher compared to F1 129C3H *Hnf1a* $^{-/-}$ (and wild type). NS=non significant, ***p-value < 0.0001.



**Supplementary Table S1: List of the genes in the *Moda1* locus carrying non-synonymous SNP changes between the CBA/C3H and 129S2 strains.** Non-synonymous SNPs, deletions/insertions and structural variants in the QTL interval were obtained from (http://www.sanger.ac.uk/sanger/Mouse_SnpViewer/rel-1303)

| Gene symbol | Gene name | Location | db SNP | CBA/C3H allele | Codon change | Amino acid change | Change description |
|---|---|---|---|---|---|---|---|
| ***Tmem212*** | Transmembrane protein 212 | 27,866,065 | rs29598656 | T | **C**ac/**T**ac | H/Y | Missense variant |
| ***Pld1*** | Phospholipase D1 | 28,048,083 | rs29602715 | G | **C**gg/**G**gg | R/G | Missense variant |
| ***Gm1527*** | Predicted gene 1527 | 28,895,732 | rs50586704 | A | c**G**t/c**A**t | R/H | Missense variant |
| ***Egfem1*** | EGF-like and EMI domain containing 1 | 29,648,265 | rs29790718 | G | a**C**t/a**G**t | T/S | Missense variant |
| | | 29,686,728 | rs30410185 | C | g**T**t/g**C**t | V/A | Missense variant |
| ***Mecom*** | MDS1 and EVI1 complex locus | 30,238,240 | rs47702462 | C | g**T**t/g**C**t | V/A | Missense variant |
| ***Gm10258*** | Predicted gene 10258 | 30,268,666 | rs29809455 | G | **A**tc/**G**tc | I/V | Missense variant |
| ***Lrrc31*** | Leucine rich repeat containing 31 | 30,689,871 | rs37203180 | A | **T**ca/**A**ca | S/T | Missense variant |
| ***Samd7*** | Sterile alpha motif domain containing 7 | 30,756,487 | rs31381373 | G | **A**ca/**G**ca | T/A | Missense variant |
| ***Usp13*** | Ubiquitin specific peptidase 13 | 32,902,049 | rs29854159 | T | a**C**g/a**T**g | T/M | Missense variant |
| ***Pex5l*** | Peroxisomal biogenesis factor 5-like | 32,956,647 | rs29869735 | A | a**G**a/a**A**a | R/K | Missense variant |
| ***Ttc14*** | Tetratricopeptide repeat domain 14 | 33,800,535 | rs49858437 | C | **A**tg/**C**tg | M/L | Missense variant |
| | | 33,801,070 | rs51789388 | G | ga**C**/ga**G** | D/E | Missense variant |

**Supplementary Table S2: Table: Primers used for PCR, RT-qPCR and pyrosequencing**

| **A. Primers used for PCR genotyping** | | |
|---|---|---|
| Gene symbol | FORWARD SEQUENCE (5'-3') | REVERSE SEQUENCE (5'-3') |
| *Hnf1alpha* | TACCTGATGGTTGGAGAGGGTC | TGAAGACCACATCTCCTAAGG |
| *LacZ* | TCAATCCGCCGTTTGTTCCC | GCATAACCACCACGCTCATC |
| **B. Primers used for PCR Genome scan** | | |
| Position | FORWARD SEQUENCE (5'-3') | REVERSE SEQUENCE (5'-3') |
| 3-del9488256 | TCTAACTCCAAACCACTCTGAGG | AGACCCTGGTGTCAAATTGC |
| 3-del13608521 | GTTAGCACTGCCCCAAAATG | GCTAACCCATGCTTTCTGGA |
| D3Mit62 | TTCATTTGTTTATGCTTGGGG | TTCATCCTGTCCTCCTCTGG |
| 3-del28725682 | TGGCTGACACCTGCATAGAC | CCAGAGATGTCTGCCTGTGA |
| 3-del32716930 | CTCGGGACATCGTAAATGCT | GCTATGACACCTGGGTGGAG |
| 3-del35074590 | CAGAGATTGAACCCCCTTGA | CTCCTTGAGGCTCTGGATGA |
| D3Mit224 | ATTGCTATACCAAGTAGACAAACACA | ACAAGGAGGCCGCACTAAC |
| D3Mit189 | GTTACCACCCAGAGAAAAGGC | TACTCCTCGCTGCTTCCCTA |
| D3Mit257 | CCTAGCGCAGGAATAGTTAACC | ACAAACAGAACAAAACAAAAAGTCC |
| D4Mit264 | TGAGTCAATAAGGCAGGTTGG | GCAGACCAAACCCCACAC |
| D4Mit108 | TCAGCCATCTCTATCAGGTGG | CCAATGTCAGATAATGCTTATGAG |
| D4Mit187 | AGGTCTTAAGCCCTTTCTCCC | GGAGGGGAATTCAGGAACAT |
| D4Mit336 | TTCATATATGTGTACCATGGCATG | CAGGAACCTACATAGGTGAGAGG |
| D4Mit158 | CCTCATGTGGAGGCCATC | TTCAATTCTCAGAATCCTTGATAGG |
| D4Mit126 | TGCACTTTTGAGATTGCCAG | GTCTTTCCCTCTCCCTCCC |
| D4Mit226 | CCCCCCAAAAAAAAGAGAAA | AGGATTAGTGAAGGCACTGAGG |
| D11Mit227 | CCAGCATTTGAACCCTGATT | AAACCCATAGCCTGCATCTG |
| D11Mit231 | TCTTTAAAATGTTTATGATCTGTGGC | GGCCTTGGATGTGCATAGAT |
| D11Mit173 | AAGTGACATATGGATTCCTGGG | TCAAAGTGGGTATGTGTCATCC |
| D11Mit242 | GAAGCCAGCAAGAAAAATGC | CTGTCTGGTAGTGCAGCCAA |
| D11Mit301 | AATAGTCTCATCGGGTTAAACAGC | AAGTAGACTGATGTGAGGCTAAGTACC |
| D11Mit214 | CATACAGCCTTCAACAATGACA | ACTGCATACATGTGCACTCATG |
| D18Mit94 | TCACCTAGGACCCCCCTC | AAGTAGTGAGAGGCCACCACA |
| D18Mit206 | GCAACCATTCACCATCAGG | TTTTTTTTTTTAAAGACACTAAATGCC |
| D18Mit153 | GCACTTCTGCTTACAAGGCC | CAGGAGTGCAAAGGTCATGA |
| D18Mit4 | ACTGTTGCTGGGGAATGG | CCAAGTTCAAAGCTGCTGG |
| **C. Primers used to discriminate C3H from B6 allele on chromosome 3** | | |
| Position | FORWARD SEQUENCE (5'-3') | REVERSE SEQUENCE (5'-3') |
| 3-del9488264 | CATGCTTGCAAGGACTCTCA | GCTCTATGCTCAGGGGTCAC |
| 3-del26404871 | GCAGAAGCAAGCAAACAAAA | ACCGGGCATTCTATGACAAA |
| 3-del27081831 | ACGAGCACCCTAAGGACAGA | TCAGCAAGAACAGTGGCATC |
| 3-del28725682 | TGGCTGACACCTGCATAGAC | CCAGAGATGTCTGCCTGTGA |
| 3-del30184839 | GACACACAGAGTTGGGCTGA | TTGCCATTGCTTTTCCTTTC |
| 3-del35988893 | ATCTAGAGACGCACCCCAGA | AGGACAGTGTGCTTGCTCCT |
| **D. Primers used for RT-qPCR** | | |
| Gene symbol | FORWARD SEQUENCE (5'-3') | REVERSE SEQUENCE (5'-3') |
| *Hprt* | AGTCCCAGCGTCGTGATTA | GGAATAAACACTTTTTCCAAATCC |
| *Slc2a2* | CGGAATGGTCGCCTCATT | ACATTGCTTTGATCCTTCCAAGTT |
| *Ghsr* | CAGACAGTGAAGATGCTTGCT | GGCTCGAAAGACTTGGAAAA |
| *Ghrelin* | AGCCCAGCAGAGAAAGGAATC | GGGAGCATTGAACCTGATCTC |
| **E. Primers used for pyrosequencing** | | |
| Gene symbol | FORWARD SEQUENCE (5'-3') | REVERSE SEQUENCE (5'-3') |
| *Ghsr* | TGGTAAAGCAGGCTCAGAATTTC | GAAGAGTGGCATGTGGGTAGA |
| | SEQUENCING PRIMER | GCATGTGGGTAGATCA |