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Title
Substrains matter in phenotyping of C57BL/6 mice

Running Head
Use of C57BL/6 substrains

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Abstract:

The inbred mouse strain C57BL/6 has been widely used as a background strain for spontaneous and induced mutations. Developed in the 1930s, the C57BL/6 strain diverged into two major groups in the 1950s, namely, C57BL/6J and C57BL/6N, and more than 20 substrains have been established from them worldwide. We previously reported genetic differences among C57BL/6 substrains in 2009 and 2015. Since then, dozens of reports have been published on phenotypic differences in behavioral, neurological, cardiovascular, and metabolic traits. Substrains need to be chosen according to the purpose of the study because phenotypic differences might affect the experimental results. In this paper, we review recent reports of phenotypic and genetic differences among C57BL/6 substrains, focus our attention on the proper use of C57BL/6 and other inbred strains in the era of genome editing, and provide the life science research community wider knowledge about this subject.

Key words:

C57BL/6, genetic difference, phenotypic difference, SNP, substrain
Origin of the C57BL/6 mice

The inbred mouse strain C57BL/6 has a long history. It was derived from the C57BL line, which was established in 1921 by Dr. C.C. Little, who mated littermates female 57 and male 52, which were obtained from the stock of Ms. A.E. Lathrop. This line was separated into sublines 10 and 6 in 1937, thereby establishing the C57BL/10 and C57BL/6 strains, respectively [1]. The C57BL/6 strain was introduced to the Jackson Laboratory (Bar Harbor, ME, USA) in 1948, and in 1951, the mice were sent from the Jackson Laboratory to the National Institutes of Health (NIH; Bethesda, MD, USA); thereafter, the strain was separately maintained at both institutions [2, 3]. Subsequently, the Jackson Laboratory's C57BL/6J and NIH's N strains became the major lineages of C57BL/6 mice, with a large number of substrains having since been derived from each [4, 5]. At present, seven C57BL/6 substrains are available from domestic vendors in Japan, and 11 are offered by overseas vendors (Supplementary Table 1). Various substrains have been maintained for many years within individual laboratories in Japan, including the National Institute of Genetics (Ms; Mishima, Japan) and the National Institute of Radiological Sciences (Nrs; Chiba, Japan), all of which are substrains of C57BL/6J or C57BL/6N (Fig. 1). The C57BL/6J strain was the first line for which the mouse genome was sequenced [8], and it continues to be used as a representative inbred mouse strain in various life science fields. Through community effort, a highly germline-competent embryonic stem cell line was established from the C57BL/6NTac strain [9], and it has been used as a background strain in the International Knockout Mouse Project and the International Mouse Phenotyping Consortium [10–13].

We analyzed the substrain status of 2,939 genetically modified C57BL/6 mice deposited at the RIKEN BioResource Research Center (BRC) (Fig. 2). Each substrain was further subdivided into substrains supplied by several vendors. In addition, over 600 strains (21% of the total) had a mixed genetic background of C57BL6/J and C57BL6/N or a genetic background
of uncertain C57BL/6 substrain. We interviewed several scientists who either developed or
deposited these strains and found that they had purchased and used the mice without knowing
about the substrain distinctions or had not kept detailed breeding records. Similar occurrences
have been reported elsewhere. Over 70% of the genetically engineered strains generated at
German and Austrian institutions had a mixed genetic background [14]. A survey of Finnish
institutions revealed that 39.5% of researchers did not consider the importance of differences
among substrains and that 26% did not know which C57BL/6 substrain they had used [15].
Furthermore, 58% of the studies that were published in Diabetes during 2010–2014 and
involved genetically modified mice provided incomplete descriptions of the background strains
[16]. Despite the reported non-negligible phenotypic differences among C57BL/6 substrains,
there seems to be little awareness of the importance of substrain selection within the research
community.

Phenotypic differences among C57BL/6 substrains caused by identified genetic variants
To date, dozens of papers have reported various phenotypic differences among C57BL/6
substrains (Table 1). This information is classified according to the phenotype terms defined in
the Mammalian Phenotype Ontology [116] and includes descriptions of phenotypes or
phenotyping tests, the names of tested C57BL/6 substrains, and relevant references. Moreover,
several of these phenotypic differences have been analyzed with the aim of identifying
causative genes or genomic regions, as described below (summarized in Table 2).

The C57BL/6J strain is missing the exon that encodes the nicotinamide nucleotide
transhydrogenase (Nnt) gene, which plays an important role in glucose homeostasis and insulin
secretion, and therefore has abnormal glucose tolerance and impaired insulin secretion [117,
118, 126]. A study using mitogen-activated protein kinase 9 (Mapk9) knockout mice
demonstrated that susceptibility to acetaminophen-induced liver injury was influenced by the
presence or absence of the Nnt gene defect carried by C57BL/6 mice [127]. The Nnt gene mutation in the C57BL/6J strain is a prominent phenotype-associated mutation in C57BL/6 substrains that affects protein expression in the mitochondria of many tissues and consequently many different metabolic traits [62, 72, 78, 82, 88, 106, 128]. The Nnt gene deletion status differs among C57BL/6J substrains, and the Nnt gene defect likely originated in C57BL/6J mice at the Jackson Laboratory after 1976 (Fig. 3).

C57BL/6JOlaHsd mice are known to lack a 365 kb genomic region that includes the synuclein, alpha (Snca) and the nearby multimerin 1 (Mmnrn1) gene [119, 120]. SNCA aggregates in the nervous system of individuals with Parkinson’s disease [129]. Loss of the Snca gene in C57BL/6JOlaHsd mice does not appear to contribute to prion disease-mediated synaptotoxicity or neurodegeneration but may affect motor neuron degeneration [130–133]. In addition, the C57BL/6JOlaHsd strain has a low trabecular bone mass, which has been shown to be associated with the lack of Snca and Mmnrn1 genes, suggesting a role for these genes in bone metabolism [110]. Deletion of this genomic region is specific to the C57BL/6JOlaHsd strain; the mutation has not been identified in C57BL/6JRccHsd, C57BL/6J, C57BL/6NCrl, and C57BL/6NHsd strains [119]. Notably, we confirmed that the genotype is normal in other C57BL/6J and C57BL/6N substrains available in Japan (Supplementary Fig. 1). Furthermore, partial deletion of a chromosomal region was recently observed in C57BL/6JBomTac mice. In this strain, approximately 40 Mb of the long arm of the Y chromosome is deleted, and an increased rate of sperm morphological abnormalities, unbalanced sex ratio, and deregulation of several transcripts expressed in the testes have also been observed [70, 125].

Similarly, genetic mutations affecting physiological functions have been reported in C57BL/6N substrains. A nonsense mutation (retinal degeneration 8, rd8) that converts the amino acid codon of the crumbs family member 1, photoreceptor morphogenesis associated (Crb1) gene into a termination codon occurs in C57BL/6N substrains [121, 122]. Affected mice
present with the typical lesions of rd8, which are detected by fundoscopy and histopathology at
as early as 6 weeks of age. Although this mutation is seen in C57BL/6N substrains, C57BL/6J
substrains have the wild-type genotype [121].

C57BL/6NHsd mice have a copy-number variant in the dedicator of cytokinesis 2 (Dock2) gene, which has been shown to affect B cell signaling and immune tolerance, but this is not found in other C57BL/6 substrains [123, 134]. The dysfunction of this gene has likely contributed to the negative outcomes of experiments using the sialic acid acetylerase (Siae) knockout mice with an unclear C57BL/6 origin. Although the Siae gene was initially thought to be involved in B cell development and signal transduction, it was found that congenic mice generated by backcrossing to the C57BL6J strain did not reproduce the same negative outcomes. After much research, the cause was identified as a mutation in the Dock2 gene of the C57BL/6NHsd strain [134].

Other phenotypic differences among C57BL/6 substrains

In addition to the abovementioned cases in which the causative genes were identified, phenotypic differences have been reported among the substrains used to generate the C57BL/6 congenic strain. Greater improvement in terms of seizure severity and viability was achieved in Dravet syndrome model mice with knockout of the sodium channel, voltage-gated, type I, alpha (Scn1a) gene generated on a C57BL/6NJ versus C57BL/6J background [135]. Obvious phenotypic differences have been demonstrated between C57BL/6J and C57BL/6N substrains, as shown in studies on knockout of the coagulation factor VIII (F8) and fatty acid binding protein 1, liver (Fabpl) genes, suggesting the presence of genetic factors that differentially modify phenotypes between the substrains [136, 137]. It has also been demonstrated that tumor cell rejection in Eμ-TCL1 transgenic mice, a model of chronic lymphocytic leukemia, is associated with differences between the C57BL/6 substrains, suggesting that acute immune
rejection may be mediated by antigens that differ between C57BL/6J and C57BL/6N mice [138].

In addition, C57BL/6J-XY^POS congeneric mice, which have a Y chromosome derived from *Mus musculus poschiavinus* on a C57BL/6J genetic background, form ovotestes or ovaries [139, 140]. Changing from a C57BL/6J to C57BL/6N background increases the likelihood of testis-forming individuals, suggesting that genetic differences involved in the regulation of the sex determining region of Chr Y (*Sry*) and the SRY (sex determining region Y)-box 9 (*Sox9*) gene expression, which play an important role in testicular differentiation, exist between the C57BL/6J and N substrains [141, 142].

Differences in prevalence of sexual dimorphism among C57BL/6 substrains should also be considered. Significant variation in brain injuries has been reported among C57BL/6 substrains [69, 143]. In mouse stroke, male and female C57BL/6N mice show similar infarct sizes, although female C57BL/6J mice tend to have smaller infarcts [100]. Such substrain-specific sexual dimorphisms have also been identified in analyses of behavioral characteristics, serum biochemistry, and immune function [36, 79, 115]. Therefore, in studies of the effects of genetic modifications on sex differences, heterogeneity in substrain backgrounds would likely complicate the interpretation of experimental results. Thus, the phenotypic differences among C57BL/6 substrains are quite numerous, and they should be reported more in the future.

**Genetic polymorphisms in C57BL/6J substrains**

Single nucleotide polymorphisms (SNPs) are the most widely used genetic markers in genomes and are useful for uncovering genetic differences between genetically similar lineages, including substrains. In laboratory mouse strains, SNP databases have been developed by mapping sequence data from other inbred lines using the Jackson Laboratory's C57BL/6J strain as a reference [144–149]. SNPs that distinguish each C57BL/6J substrain can be extracted from candidates specific to the C57BL/6J strain in SNP databases as well as by identifying the
the genotypes of candidate SNP loci for each C57BL/6J substrain [6, 150–152]. In Table 3, genotypes of C57BL/6J substrains and the C57BL/6N strain at 45 SNP loci are shown in chronological order with C57BL/6N at the bottom and eight C57BL/6J substrains and the current C57BL/6J strain at the top. This table clearly indicates that the ancestral-type SNPs (shaded in blue) of C57BL/6J and N (as represented by the C57BL/6N strain) have decreased and been replaced by the C57BL/6J-type SNPs at the 23 loci (shaded in red) since 1951. The SNPs are considered to have occurred and accumulated in the original C57BL/6J strain. Since the SNP genotype pattern of each substrain branched at different years is distinct, these SNP loci genotypes can be used to distinguish every C57BL/6J substrain available in the research community.

The lymphocyte antigen 5 (Ly5) congenic strain is a representative mouse strain derived from the C57BL/6J substrain. The lymphoid cell surface antigen produced by the protein tyrosine phosphatase, receptor type, C (Ptprc; Ly5/Cd45 gene) gene distinguishes between hematopoietic cells from donor and recipient mice; hence, this strain has been widely used as a cell marker for bone marrow transplantation studies [153, 154]. The C57BL/6-Ly5.1 congenic inbred strain (B6JBoy.SJL-Ptptc<sup>a</sup>; RBRC00144) was established by Boyes et al. at Sloan-Kettering Institute through introduction of the Ly5.1 of the SJL/J strain into the C57BL/6JBoy strain background (Ly5.2 allele) by 23 serial backcrosses [155, 156]. The exact point at which the C57BL/6JBoy strain diverged from the C57BL/6J strain is not clear, but the literature suggests that divergence occurred in the 1970s, or possibly even earlier [157]. According to genetic background testing using C57BL/6J-specific SNP markers, the genetic background should be similar to that of older C57BL/6J substrains, which have an earlier divergence date (Table 4). Although C57BL/6-Ly5 antigen congenic mice are currently available from several vendors, a spontaneous mutation in the natural cytotoxicity triggering receptor 1 (Ncr1) locus and residual chromosomal fragments of the donor SJL/J strain, both of which affect immune
response, have been reported [158, 159]. These effects must be considered to properly interpret the results of experiments involving these strains [158, 159].

**Genetic polymorphisms in C57BL/6N substrains**

In the C57BL/6N substrain, genotyping of the above C57BL/6J-specific SNP loci could not detect polymorphism as observed in C57BL/6J [6]. This is because the previous C57BL/6J-specific SNP information was obtained by mapping the abundant reference sequence data of C57BL/6J mice to the sequences of other strains. However, there was a lack of previous sequence data for C57BL/6N, and thus the detection of C57BL/6N strain-specific SNPs was infeasible. Later, in 2011, a re-sequencing analysis of the C57BL/6NJ strain was performed [160, 161], and highly accurate sequence information was published on the website of the Sanger Institute (Hinxton, United Kingdom; https://www.sanger.ac.uk/sanger/Mouse_SnpViewer/rel-1505). The results enabled a subsequent search for C57BL/6N strain SNPs and revealed SNP loci specific to the C57BL/6NJ strain and their genotypes in seventeen C57BL/6 substrains [7]. There were 86 polymorphic SNP loci at regular intervals on every chromosome, selected from approximately 1,400 C57BL6/NJ strain-specific SNP candidate loci. As results similar to those of the C57BL/6J group, the number of C57BL/6N-specific SNPs was correlated with the branching year and breeding history of each lineage (see Fig. 1 in Mekada et al. 2015 [7]). The previous information required for genotyping each SNP locus listed in Mekada et al. 2015 [7] was updated with additional primer information for allele-specific PCR, and it is listed in Supplementary Table 3.

Our research group continues to search for SNPs that can distinguish among various C57BL/6 substrains; such information is expected to improve our understanding of the genetic background of these strains. In addition to SNPs, DNA polymorphisms such as indels and short tandem repeats as well as structural variants, including C57BL/6-specific genetic variants found
in *Nnt* and *Snca* genes, can be used to distinguish C57BL/6 strains in terms of genetic background [14, 115, 162]. These DNA polymorphism markers may be useful for comparing substrains and can also be applied to gene mapping involving the crossing of C57BL/6 substrains. A genetic screening model based on these principles would enable the mapping of polymorphic loci that contribute to the variability of a trait among strains with a largely homogenous background; this could lead to improvements in mapping resolution and aid in the selection of candidate genes. To date, numerous quantitative trait loci have been identified, including genes associated with susceptibility to heat nociception [53], diet-induced obesity [84], dominant obesity [163], circadian activity [164], feeding disorder [37], response to cocaine [41], defective neutrophil recruitment [91], and non-ectopic endogenous retroviral dysregulation [92]. With advances in genome sequencing technology, genomic information for inbred mice, including substrains, is being continually updated [165, 166]. It is expected that many new strain-specific haplotypes will be identified in the future.

**Large-scale and complex resources**

C57BL/6J substrains have been the most widely used background for many mutant strains, including genetically-modified lines. Another major lineage, the C57BL/6N substrain, has been used as the genetic background for embryonic stem cells in large-scale generation of knockouts [12]. As a result, many mutant mice derived from the C57BL/6J and C57BL/6N substrains are used worldwide, including in Japan. However, given that the substrains are widely considered interchangeable, significant phenotypic differences among substrains are often overlooked. The phenotypic differences among C57BL/6 substrains reported to date should be considered by researchers. For example, certain substrains should be used with caution in conditional knockout models because conditional knockout mice should be crossed with *Cre* and/or *Flp* recombinase mice. Combining recombinase strains with different genetic backgrounds might
produce knockout animals with mixed genetic backgrounds, leading to unexpected phenotypic variations. When the genetic backgrounds of experimental mice are not identical, erroneous conclusions are likely to be drawn. If a suitable substrain cannot be used, wild-type littermates might serve as an appropriate control group. Recently, it has become clear that phenotypic differences among substrains are caused by a complex combination of genetic and microbial alterations [167, 168]. Use of wild-type littermates might standardize the microbiota, thereby facilitating investigation of the phenotype or function of specific genes that could be affected by the microbial community [169, 170].

**Genome-edited mice**

Today, the rise in prominence of genome editing technologies has made it possible to directly generate various types of genetically modified mice, including gene knockout, conditional knockout, and gene knock-in, by using the fertilized eggs of inbred mice without the need for embryonic stem cells. At present, more than 50% of the strains deposited at the RIKEN BRC have been generated by genome editing using fertilized eggs from inbred strains, although not necessarily C57BL/6 mice. Just as there are C57BL/6 substrains, there are also substrains of other inbred lines, and as is the case with the C57BL/6 strain, an accumulation of genetic and phenotypic differences is to be expected. More recently, many mouse models have been generated for coronavirus research; the BALB/c mouse strain is known to be popular in the immunology field and susceptible to SARS-CoV-2 [171, 172], and it is increasingly being used as a background strain for COVID-19 research [173–175]. The BALB/c strain has a long history in the form of the above B6 mice [2, 3]. At least 16 BALB/c substrains, including BALB/cJ, BALB/cBy, and BALB/cAnN, available to the research community can be found by performing a PubMed research with the search term “BALB/c”. Furthermore, several papers have reported phenotypic variations in infection and immune responses [176–178]. Therefore, we should pay
much more attention to the substrain status of BALB/c mice in research on COVID-19, which is increasing around the world.

Conclusion
Experimental data must be robust, reliable, and reproducible; only under these circumstances can science progress [179]. To ensure experimental reproducibility and minimize bias, researchers should recognize potential phenotypic differences among substrain mouse models, select appropriate substrains for their own research purposes, and share the full names of the substrains used with the scientific community. In the future, it is expected that journals will adopt a policy requiring a detailed description of the substrain and the breeding history of model mice used in research.

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Figures and Tables

Fig. 1. Genealogy of the C57BL/6 substrains. Dates and relationships are derived from the vendors’ websites and Mekada et al. (2009 and 2015) [6, 7]. Abbreviations: J, The Jackson Laboratory (JAX); Jcl, CLEA Japan, Inc.; Jms, Institute of Medical Science, Japan; Slc, Japan SLC, Inc.; Ms, National Institute of Genetics; Ei, Dr. Eva M. Eicher; Lac, Laboratory Animal Centre (MRC Carshalton Lab Animal Centre); Ola, Olac, Ltd.; Hsd, Harlan Sprague-Dawley, Inc.; CSAL, Centre de Sélection des Animaux de Laboratoire; Rj, Centre D’Elevage R. Janvier; Rcc, RCC Ltd.; Han, Zentralinstitut fur Versuchstierzucht, Hannover; M&B A/S, Taconic’s Bomholtgard, Denmark, facility; Tac, Taconic Biosciences, Inc.; Nrs, National Institute for Quantum and of Radiological Science and Technology; N, National Institutes of Health; Hla, Hilltop Lab Animals, Inc.; Jic, Central Institute for Experimental Animals, Japan; Cr, NCI, DCTD Animal Production Program; Sim, Simonsen Laboratories, Inc.; Crl, Charles River Laboratories; Crlj, Charles River Laboratories Japan; Seac, Seac Yoshitomi, Ltd. (Kyodo Co., Ltd.); By, Dr. Donald W. Bailey. *Derived from embryos frozen in 1984.

Fig. 2. Summary of a survey on the use of C57BL/6 substrains in Japan. According to information provided by the developer scientists of the 2,939 mouse strains deposited at RIKEN BRC, 49% were crossed with C57BL/6J substrains, including C57BL/6JJcl, C57BL/6JJmsSlc, and C57BL/6J, and 30% were crossed with C57BL/6N substrains, including C57BL/6NJcl, C57BL/6NCrSlc, C57BL/6NCrlCrlj (including C57BL/6NCrl in 35 strains), and C57BL/6NTac. Another 13% were crossed with C57BL/6 substrains of mixed background, and 8% were crossed with uncertain C57BL/6 substrains.

Fig. 3. The deletion of exons 7–11 in the Nnt gene of C57BL/6J substrains. Genomic
polymerase chain reaction (PCR) analysis of exons 6–12 in the Nnt gene showed the absence of PCR products from exons 7–11 in the C57BL/6J, C57BL/6Jc1, C57BL/6JmsSlc, and C57BL/6JMs substrains. The deletion of the Nnt gene occurred in the C57BL/6J colony of the Jackson Laboratory between 1976 and 1984. The branching years of each strain from the original C57BL/6J strain are given in parentheses. PCR and gel electrophoresis procedures were performed according to the protocol described in Mekada et al. (2009) [6]. PCR product size data are from Huang et al. (2006) [117].

Table 1. C57BL/6 substrains used for testing various phenotypes

Table 2. Genetic or genomic structural variations affecting phenotypes among C57BL/6 substrains

Table 3. Single nucleotide polymorphisms (SNPs) in C57BL/6J substrains

Table 4. Genetic background of C57BL/6JBoy congenic strains

Supplementary Fig. 1. Detection of the Snca gene locus in the C57BL/6 substrains available in Japan. Exons 4 and 6 of the Snca gene together with flanking SSLP markers, D6Mit122 and D6Mit299, were amplified by genomic PCR, and the amplified products were separated on an agarose gel. The D6Mit122 and D6Mit299 markers were mapped to about 1 cM upstream and downstream of the Snca locus, respectively. Exon-specific PCR primers for the Snca gene were designed according to a previous report [119].
Supplementary Table 1. Major C57BL/6 mouse strains in Japan and worldwide, maintained by vendors and individual laboratories

Supplementary Table 2. Profile of the 45 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6J substrains

Supplementary Table 3. Profile of the 100 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6N substrains
Figure 1. (Mekada & Yoshiki)
Figure 2. (Mekada & Yoshiki)

The numbers in parentheses indicate numbers of substrains.

**C57BL/6 J (1,436) 49%**
Subdivision:
- C57BL/6JJcl (1,019)
- C57BL/6JJmsSlc (232)
- C57BL/6J (158)
- Others (27)

**C57BL/6 N (880) 30%**
Subdivision:
- C57BL/6NJcl (337)
- C57BL/6NCrSlc (237)
- C57BL/6NCrlCrlj (186)
- C57BL/6NTac (111)
- Others (9)

**Uncertain C57BL/6 (239) 8%**

**Mixed C57BL/6 (384) 13%**

Total: 2,939 C57BL/6 substrains
| Nnt gene | C57BL/6J | C57BL/6Jcl (1989) | C57BL/6JmsSc (1987) | C57BL/6JEI (1976) | C57BL/6JOlaHsd (1974) | C57BL/6JRccHsd (1973) | C57BL/6JNomTac (1971) | C57BL/6JNrs (1971) | C57BL/6J (1951) |
|----------|----------|-------------------|---------------------|-------------------|----------------------|-----------------------|----------------------|------------------|-----------------|
| Exon 6   |          |                   |                     |                   |                      |                       |                      |                  |                 |
| Exon 7   |          |                   |                     |                   |                      |                       |                      |                  |                 |
| Exon 8   |          |                   |                     |                   |                      |                       |                      |                  |                 |
| Exon 9   |          |                   |                     |                   |                      |                       |                      |                  |                 |
| Exon 10  |          |                   |                     |                   |                      |                       |                      |                  |                 |
| Exon 11  |          |                   |                     |                   |                      |                       |                      |                  |                 |
| Exon 12  |          |                   |                     |                   |                      |                       |                      |                  |                 |

**Figure 3.** (Mekada & Yoshiki)
| Phenotype terms                        | Descriptions and/or tests                                                                 | C57BL/6 substrains tested                                                                 | Publication |
|----------------------------------------|-------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|-------------|
| Behavior/neurological phenotype        | Aggression, light-dark box, nest-building                                                  | C57BL/6JNmg (Nijmegen Univ.), C57BL/6JKun (Nijmegen Univ.)                              | [17]        |
| Alcohol preference                     |                                                                                           | C57BL/6J (JAX), C57BL/6NCrSim (Simonsen Lab)                                           | [18]        |
| Alcohol preference and innate immune response |                                                                                           | C57BL/6J (JAX), C57BL/6Ncr (CRL)                                                       | [19]        |
| Behavioral response to amphetamine     |                                                                                           | C57BL/6J (JAX), C57BL/6By (JAX)                                                        | [20]        |
| Conditioned place preference           |                                                                                           | C57BL/6J (JAX), C57BL/6NCrl (CRL)                                                      | [21]        |
| Corticosterone sensitivity             |                                                                                           | C57BL/6J (JAX), C57BL/6NCrl (CRL)                                                      | [22]        |
| Degree of cuprizone-induced binge-like eating reduction |                                                                                           | C57BL/6J (JAX), C57BL/6NJ (JAX)                                                       | [23]        |
| Degree of naloxone-induced conditioned place aversion |                                                                                           | C57BL/6J (JAX), C57BL/6NJ (JAX)                                                       | [24]        |
| Eight-arm radial maze, elevated plus maze, open field, rotarod, Porsolt forced swim, prepulse inhibition, wire hang |                                                                                           | C57BL/6J (JAX), C57BL/6NCrlCrlj (CRL Japan), C57BL/6NCrSlc (Japan SLC)              | [25]        |
| Elevated plus maze, fear conditioning, light-dark box, prepulse inhibition, open field, social approach, acoustic startle |                                                                                           | C57BL/6J (JAX), C57BL/6JRecHsd (Envigo), C57BL/6JRecRj (Janvier Labs), C57BL/6NCrl (CRL), C57BL/6NHsd (Envigo), C57BL/6NRj (Janvier Labs) | [26]        |
| Elevated plus maze, hole board, light-dark box Fear conditioning |                                                                                           | C57BL/6JOlaHsd (Envigo), C57BL/6NCrl (CRL)                                             | [27]        |
| Fear conditioning, hot plate, rotarod, tail withdrawal |                                                                                           | C57BL/6J (JAX), C57BL/6NCrl (CRL), C57BL/6NHsd (Envigo), C57BL/6NTac (TAC)            | [28]        |
| Fear conditioning, Morris water maze, open field, rotarod |                                                                                           | C57BL/6J (JAX), C57BL/6NJ (JAX)                                                       | [29]        |
| Food consumption in conditioned site preference |                                                                                           | C57BL/6J (JAX), C57BL/6NJ (JAX)                                                       | [30]        |
| High-fat diet-induced mechanical sensitivity |                                                                                           | C57BL/6J (JAX), C57BL/6NCrl (CRL), C57BL/6NJ (JAX)                                     | [31]        |
| Light-dark box                         |                                                                                           | C57BL/6J (JAX), C57BL/6NHsd (Envigo), C57BL/6NCrl (CRL)                                | [32]        |
| Locomotor activity and anxiety-like behavior during the postpartum period |                                                                                           | C57BL/6J (JAX), C57BL/6Jcl (CLEA Japan)                                                | [33]        |
| Locomotor response to cocaine         |                                                                                           | C57BL/6J (JAX), C57BL/6N (NCI)                                                        | [34]        |
| Metabolic and behavioral response to shortened 21-hour day and high-fat diet |                                                                                           | C57BL/6J (JAX), C57BL/6Ncr (CRL)                                                      | [35]        |
| Morris water maze                     |                                                                                           | C57BL/6J (JAX), C57BL/6NTac (TAC)                                                     | [36]        |
| Open field                             |                                                                                           | C57BL/6J (JAX), C57BL/6NTac (TAC)                                                     | [37]        |
| Open field, rotarod                    |                                                                                           | C57BL/6JHcomTac (TAC), C57BL/6NcrlOri (Orient Bio), C57BL/6NTacSam (Samtako Bio)       | [38]        |
| Prepulse inhibition                    |                                                                                           | C57BL/6J (JAX), C57BL/6NHsd (Envigo)                                                  | [39]        |
| Response to formalin-induced pain      |                                                                                           | C57BL/6J (JAX), C57BL/6Ncr (CRL)                                                      | [40]        |
| Response to circadian disruption and wheel-running access |                                                                                           | C57BL/6J (JAX), C57BL/6Ncr (CRL)                                                      | [41]        |
| Response to neuropathic pain           |                                                                                           | C57BL/6J (JAX), C57BL/6Ncr (CRL)                                                      | [42]        |
| Sensitivity to the convulsant pilocarpine |                                                                                           | C57BL/6J (JAX), C57BL/6NJ (JAX)                                                       | [43]        |
| Sensitivity to nicotine                |                                                                                           | C57BL/6J (JAX), C57BL/6Ncr (CRL)                                                      | [44]        |
| Sensitivity to thermal nociception      |                                                                                           | C57BL/6J (JAX), C57BL/6Ncr (CRL)                                                      | [45]        |
| Phenotype terms           | Descriptions and/or tests                                                                 | C57BL/6 substrains tested                                      | Publication |
|--------------------------|-------------------------------------------------------------------------------------------|--------------------------------------------------------------|-------------|
|                           | Susceptibility to cocaine-induced seizure                                                  | C57BL/6J (JAX), C57BL/6ByJ (JAX)                              | [54]        |
|                           | Susceptibility to pilocarpine-induced status epilepticus                                    | C57BL/6Jcl (CLEA Japan), C57BL/6NJcl (CLEA Japan)             | [55]        |
|                           | Tail suspension                                                                           | C57BL/6J (JAX), C57BL/6NHsd (Envigo)                         | [56]        |
| Cardiovascular system    | Cardiac effects of tribromoethanol and ketamine-midazolam                                 | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [57]        |
|                           | Cardiac functional and metabolic flux parameters                                           | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [58]        |
|                           | Echocardiographic and electrocardiographic values                                          | C57BL/6J (HzM), C57BL/6N (HzM)                                | [59]        |
|                           | Incidence of cardiac rupture after myocardial infarction                                   | C57BL/6J (JAX), C57BL/6(Hsd) (Envigo)                        | [60]        |
|                           | Incidence of dilated cardiomyopathy                                                       | C57BL/6J (JAX)                                                | [61]        |
|                           | High-fat diet-induced vascular superoxide                                                  | C57BL/6J (JAX), C57BL/6NJ (JAX)                               | [62]        |
|                           | Response to Ang II-dependent left ventricle remodeling                                      | C57BL/6J (JAX), C57BL/6NJ (JAX)                               | [63]        |
|                           | Response to left ventricular pressure overload                                            | C57BL/6J (JAX), C57BL/6NCrl (CRL), C57BL/6NTac (TAC)          | [64]        |
|                           | Response to neonatal hypoxia/ischemia                                                     | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [65]        |
|                           | Response to transverse aortic constriction stimulation                                       | C57BL/6J (JAX), C57BL/6NTac (TAC)                             | [66]        |
|                           | Salt sensitivity of blood pressure                                                        | C57BL/6J (JAX), C57BL/6NTac (TAC)                             | [67]        |
|                           | Systolic blood pressure level                                                             | C57BL/6J (JAX), C57BL/6NJ (JAX)                               | [68]        |
|                           | Vulnerability to neonatal hypoxia-ischemia                                                | C57BL/6J (Saarland Univ.), C57BL/6N (Saarland Univ.)         | [69]        |
| Cellular phenotype       | Abnormal sperm head morphology                                                            | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [70]        |
|                           | Hydrogen peroxide-producing capacities of liver mitochondria                              | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [71]        |
|                           | Mitochondrial redox abnormalities                                                         | C57BL/6J (JAX), C57BL/6Unib (CEMIB)                           | [72]        |
| Embryonic phenotype      | Incidence of fetal alcohol syndrome                                                       | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [73]        |
|                           | Patterns of alcohol-induced facial dysmorphology                                          | C57BL/6J (JAX), C57BL/6NHsd (Envigo)                         | [74]        |
|                           | Susceptibility to Porphyromonas gingivalis                                                | C57BL/6J (JAX), C57BL/6NJ (JAX)                               | [75]        |
| Endocrine/exocrine gland | Susceptibility to cerulein-induced chronic pancreatitis                                    | C57BL/6J (JAX), C57BL/6NHsd (Envigo)                         | [76]        |
| Hearing/vestibular/ear   | Susceptibility to aminoglycoside-induced ototoxicity                                      | C57BL/6J (JAX), C57BL/6NHsd (Envigo)                         | [77]        |
| Hematopoietic system     | Sensitivity of hematopoietic stem cells to oxidative stress                               | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [78]        |
| Homeostasis/metabolic    | Clinical chemistry parameters                                                             | C57BL/6J (JAX), C57BL/6NTac (TAC)                             | [79]        |
| phenotype                | Effects of oats on plasma cholesterol and lipoproteins                                    | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [80]        |
|                           | Hematological and iron-related parameters                                                 | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [81]        |
|                           | Insulin secretory response to high-fat diet                                                | C57BL/6J (JAX), C57BL/6NTac (TAC)                             | [82]        |
|                           | Response to intermittent hypoxia                                                         | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [83]        |
|                           | Response to diet-induced obesity                                                          | C57BL/6J (Janvier Labs), C57BL/6NTac (TAC)                    | [84]        |
|                           | Response to glucose-stimulated insulin secretion                                          | C57BL/6J (JAX), C57BL/6NJ (JAX)                               | [85]        |
|                           | Response to high-fat diet                                                                 | C57BL/6J (JAX), C57BL/6NJ (JAX)                               | [86]        |
|                           | Skeletal and metabolic responses to high-fat diet                                          | C57BL/6J (JAX), C57BL/6NJ (JAX)                               | [87]        |
| Immune system phenotype  | Neutrophil recruitment in response to inflammatory stimuli                                 | C57BL/6J (JAX), C57BL/6N (NCI)                                | [91]        |
|                           | Non-ecotropic ERV expression level                                                        | C57BL/6J (JAX), C57BL/6NJ (JAX)                               | [92]        |
|                           | Susceptibility to influenza A virus                                                       | C57BL/6J (JAX), C57BL/6NCrl (CRL)                             | [93]        |
| Phenotype terms                              | Descriptions and/or tests                                                                 | C57BL/6 substrains tested                                                                 | Publication |
|----------------------------------------------|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-------------|
| **Integument phenotype**                     | Susceptibility to *Listeria monocytogenes*                                                | C57BL/6J (JAX), C57BL/6ByJ (JAX)                                                         | [94]        |
|                                              | Sensitivity to Aldara™-induced psoriasiform dermatitis                                    | C57BL/6JRj (Janvier Labs), C57BL/6NRj (Janvier Labs)                                    | [95]        |
| Liver/biliary system phenotype               | Susceptibility to acetaminophen-induced liver injury                                      | C57BL/6J (JAX), C57BL/6NCr (NIH)                                                        | [96]        |
|                                              | Susceptibility to CC4- and high-fat diet–induced non-alcoholic steatohepatitis           | C57BL/6J (JAX), C57BL/6NCrSlc (Japan SLC)                                              | [97]        |
| Neoplasm phenotype                           | Incidence of 1,2-dimethylhydrazine–induced colorectal tumors                              | C57BL/6J (JAX), C57BL/6N (NIH), C57BL/6Ha (Health Research Inc.)                        | [98]        |
|                                              | Susceptibility to Ehrlich ascites carcinoma                                              | C57BL/6J (Andreevka), C57BL/6N (Pushchino)                                             | [99]        |
| Nervous system phenotype                     | Degree of stroke vulnerability                                                           | C57BL/6J (JAX), C57BL/6J (JAX), C57BL/6NJ (JAX), C57BL/6NTac (TAC), C57BL/6ByJ (JAX) | [100]       |
|                                              | Ectopic distribution of cerebrospinal fluid contacting neurons                          | C57BL/6J (JAX), C57BL/6NCr (CRL)                                                       | [101]       |
|                                              | Hippocampal mossy fiber morphology                                                       | C57BL/6JKun (Nijmegen Univ.), C57BL/6Nmg (Nijmegen Univ.)                              | [102]       |
|                                              | Hippocampal mossy fiber morphology, radial arm maze                                      | C57BL/6JKun (Nijmegen Univ.), C57BL/6Nmg (Nijmegen Univ.)                              | [103]       |
|                                              | Prevalence of molecular-layer heteropia                                                  | C57BL/6J (JAX), C57BL/6NCr (CRL), C57BL/6NCrSim (Simonsen Lab), C57BL/6NHla (Hilltop Lab.), C57BL/6NHsd (Envigo), C57BL/6NTac (TAC) | [104]       |
|                                              | Susceptibility to scopolamine-induced neuronal impairment                                 | C57BL/6J (Dae Han Bio Link), C57BL/6N (Dae Han Bio Link)                                | [105]       |
| Renal/urinary system phenotype               | Degree of glyoxylate-induced kidney crystal deposition                                    | C57BL/6J (JAX), C57BL/6NCr (CRL)                                                       | [106]       |
| Respiratory system phenotype                 | Airway responsiveness                                                                     | C57BL/6J (JAX), C57BL/6NCr (CRL), C57BL/6NTac (TAC), C57BL/6NHsd (Envigo), C57BL/6Ncr (NIH) | [107]       |
|                                              | Response to neonatal hyperoxia-induced lung injury                                        | C57BL/6J, C57BL/6N                                                                          | [108]       |
| Skeleton phenotype                           | Bone structure and unloading-induced bone loss in adolescents                            | C57BL/6J (HJAX), C57BL/6NJ (JAX)                                                        | [109]       |
|                                              | Trabecular bone mass                                                                     | C57BL/6J (JAX), C57BL/6J0laHsd (Envigo), C57BL/6ReclHsd (Envigo)                       | [110]       |
| Vision/eye phenotype                         | Constriction during pupillary light response with aging                                   | C57BL/6J, C57BL/6N                                                                         | [111]       |
|                                              | Ocular dominance plasticity                                                              | C57BL/6J (JAX), C57BL/6J0laHsd (Envigo)                                                | [112]       |
|                                              | Susceptibility to laser-induced choroidal neovascularization                            | C57BL/6J (JAX), C57BL/6NTac (TAC)                                                      | [113]       |
|                                              | Susceptibility to photoreceptor oxidative stress                                        | C57BL/6J (JAX), C57BL/6NCr (CRL)                                                      | [114]       |
| Comprehensive phenotype*                     | EMPReSS.slim pipelines                                                                  | C57BL/6J (JAX), C57BL/6NTac (TAC)                                                     | [115]       |

The phenotype terms column basically follows the Mammalian Phenotype Ontology [116]. The parentheses to the right of the strain names indicate the vendor or facility where the mice were produced. JAX, The Jackson Laboratory; CRL, Charles River Laboratories; TAC, Taconic Biosciences; WEHI, Walter and Eliza Hall Institute; HZM, Helmholtz Zentrum München; CEMIB, Multidisciplinary Center for Biological Investigation on Laboratory Animal Science, University of Campus. Strains without parentheses are those for which information on the vendor or production facility was not included in the publication. * By comprehensive phenotype screening (substrain differences were identified in cardiovascular, hematopoietic, adipose tissue, behavioral/neurological, renal/urinary, skeletal, hematopoietic, vision/eye, and immune system phenotypes).
**Table 2.** Genetic or genomic structural variations affecting phenotypes among C57BL/6 substrains

| Strain name | Reported genetic or genomic variations | Affected phenotypes |
|-------------|----------------------------------------|---------------------|
| C57BL/6 (JAX), C57BL/6Jcl (CLEA Japan), C57BL/6JmsSlc (Japan SLC), C57BL/6JMs (National Institute of Genetics) | 17,814 bp deletion between exons 6 and 12, which corresponds to exons 7-11 of the *Nnt* gene [117, 118]. | Abnormal glucose tolerance and impaired insulin secretion |
| C57BL/6JolaHsd (Envigo) | 365 kb spontaneous deletion of chromosome 6, including the *Snca* and *Mmrn1* genes [119, 120]. | No significant effects on brain structure or composition, but may affect motor neuron degeneration and trabecular bone mass |
| All C57BL/6N substrains, including C57BL/6ByJ (JAX) and C57BL/6By (JAX) | Single base deletion of the *Crb1* gene, causing a frameshift and premature stop codon which truncates the transmembrane and cytoplasmic domain of the protein [121, 122]. | Typical lesions of rd8 retinal degeneration |
| C57BL/6NHsd (Envigo) | Genomic duplication of exons 28 and 29 and a frameshift mutation after exon 29 in the *Dock2* gene [123, 124]. | Affected B cell signaling and immune tolerance |
| C57BL/6BomTac (TAC) | 40 Mb-long deletion between 6.12/6.57 and 46.73/47.31 Mb on the Y chromosome long arm [125]. | Increased rate of sperm morphological abnormalities, unbalanced sex ratio, and dysregulation of several transcripts expressed in the testes |

The parentheses to the right of the strain names indicate the vendor or facility where the mice were produced. JAX, The Jackson Laboratory; TAC, Taconic Biosciences.
Table 3. Single nucleotide polymorphisms (SNPs) in C57BL/6J substrains

| Strain name | Branching year | No. of C57BL/6 ancestral-type SNPs | Locus No.† |
|-------------|----------------|-----------------------------------|------------|
|             |                | 1 2 3 4 5 6 7 8 9 10 11 12 13   |            |
| C57BL/6J    | 0              | T/T A/A G/G A/A C/C T/T G/G T/T T/T C/C A/A T/T T/T |
| C57BL/6JJcl | 1989           | 3  T/T A/A A/A A/A C/C A/A G/G T/T T/T C/C A/A T/T T/T |
| C57BL/6JMsSlc| 1987          | 5  T/T A/A A/A A/A C/C A/A G/G T/T T/T C/C A/A C/C T/T |
| C57BL/6JMs  | 1984           | 7  T/T A/A A/A G/G T/T T/T C/C A/A C/C T/T |
| C57BL/6JEt| 1976           | 13 T/T A/A A/A C/C C/C A/A G/G T/T T/T C/C A/A C/C T/T |
| C57BL/6JOlahsd | 1974    | 14 T/T A/A A/A C/C A/A C/A T/T T/T C/C A/A C/T T/T |
| C57BL/6JRsTc | 1973       | 14 T/T A/A A/A C/C C/C A/A A/A T/T T/T C/C A/A C/T T/T |
| C57BL/6JMs  | 1971           | 17 T/T T/T A/A C/C C/C A/A C/C T/T T/T C/C A/A C/T T/T |
| C57BL/6JNs  | 1965           | 23 T/T T/T A/A C/C C/C A/A C/C T/T T/T C/C A/A C/T T/T |

* The year in which each C57BL/6 substrain branched from the C57BL/6J substrain of the Jackson Laboratory.
† Corresponds to Locus No. in Supplementary Table 2. The genotypes of all SNP loci were identical among the C57BL/6 substrain.

Supplementary Table 2. The genotypes of all SNP loci were identical among the C57BL/6N substrain, including C57BL/6NTac, C57BL/6NHom, C57BL/6NJ, C57BL/6Seac, C57BL/6NJel, C57BL/6NChim, C57BL/6NChirj, C57BL/6NChr, C57BL/6NChim, C57BL/6ByJ, and C57BL/By.
| Strain name          | Branching year | Locus No.† |
|----------------------|----------------|------------|
| C57BL/6J            | 1951           |            |
| C57BL/6JJcl         | 1989           |            |
| C57BL/6JMs          | 1984           |            |
| C57BL/6JEiJ         | 1976           |            |
| C57BL/6JQaldsd      | 1974           |            |
| C57BL/6JdomTac      | 1971           |            |
| C57BL/6JNrs         | 1965           |            |
| B6JBoy.SJLPtprcaLy5.1|               |            |
| B6JBoy.RFCd8aCd8b1aLy2.1Ly3.1| | |
| C57BL/6JN substrains| 1951           |            |

The year in which each C57BL/6 substrain branched from the C57BL/6J strain of the Jackson Laboratory. †Corresponds to Locus No. in Supplementary Table 2.
| Gene         | C57BL/6J | C57BL/6Jcl | C57BL/6JmsSlc | C57BL/6JNrs | C57BL/6NCrl | C57BL/6NCrSlc | C57BL/6Seac | Size |
|--------------|----------|------------|---------------|-------------|-------------|---------------|-------------|------|
| *D6Mit122*   |          |            |               |             |             |               |             | 122 bp|
| *Scna_exon 4*|          |            |               |             |             |               |             | 130 bp|
| *Scna_exon 6*|          |            |               |             |             |               |             | 266 bp|
| *D6Mit299*   |          |            |               |             |             |               |             | 199 bp|

**Supplementary Figure 1.** (Mekada & Yoshiki)
**Supplementary Table 1.** Major C57BL/6 mouse strains in Japan and worldwide, maintained by vendors and individual laboratories

| Strain name | Source |
|-------------|--------|
| C57BL/6J substrains | |
| C57BL/6J | The Jackson Laboratory (Bar Harbor, MA, USA) |
| C57BL/6JJcl | CLEA Japan, Inc. (Tokyo, Japan) |
| C57BL/6JJmsSlc | Japan SLC, Inc. (Hamamatsu, Japan) |
| C57BL/6JBomTac | Taconic Biosciences, Inc. (Rensselaer, NY, USA) |
| C57BL/6JOlaHsd | Envigo RMS, Inc. (Indianapolis, IN, USA) |
| C57BL/6JRecHsd | Envigo RMS, Inc. (Indianapolis, IN, USA) |
| C57BL/6JRj | Janvier Labs (Saint Berthevin, France) |
| C57BL/6JM | National Institute of Genetics (Mishima, Japan) |
| C57BL/6JNrs | National Institutes for Quantum and Radiological Science and Technology (Chiba, Japan) |
| C57BL/6N substrains | |
| C57BL/6NJ | The Jackson Laboratory (Bar Harbor, MA, USA) |
| C57BL/6NCrl | Charles River Laboratories International, Inc. (Wilmington, MA, USA) |
| C57BL/6NCrlCrlj | Charles River Laboratories Japan, Inc. (Yokohama, Japan) (discontinued) |
| C57BL/6NJcl | CLEA Japan Inc. (Tokyo, Japan) |
| C57BL/6NCrlSlc | Japan SLC, Inc. (Hamamatsu, Japan) |
| C57BL/6NSea | Kyudo Co. Ltd. (Tosu, Japan) |
| C57BL/6NTac | Taconic Biosciences, Inc. (Rensselaer, NY, USA) |
| C57BL/6NHsd | Envigo RMS, Inc. (Indianapolis, IN, USA) |
| C57BL/6NRj | Janvier Labs (Saint Berthevin, France) |
| C57BL/6NHla | Hilltop Lab Animals, Inc. (Scottsdale, PA, USA) |
| C57BL/6NCrlSim | Simonsen Laboratories, Inc. (Gilroy, CA, USA) |
| C57BL/6ByJ | The Jackson Laboratory (Bar Harbor, MA, USA) |
| Locus No. | dbSNP ID | Chromosome | Position (bp) (GRCh38.p6) | Linked gene | SNP type | SNP allele in C57BL/6J allele | Allele flanking primer sequence (5’ to 3’) | Product size (bp) | Allele-specific primer sequence (5’ to 3’) | Product size (bp) |
|-----------|-----------|------------|---------------------------|-------------|----------|-----------------------------|------------------------------------------|-----------------|----------------------------------|-----------------|
| 1         | rs13475886 | 1          | 61,171,619 | TF | binding site variant | T/C | T/T | F: GCAGGTTGTTTGGTTTTGTTG | 503 | T: CAGAAAGAGAAAAGTTGGAGATTTTT | 331 |
| 2         | rs13476337 | 2          | 8,069,855 | A/T | A/A | G/C | A/G | R: GGGTAGGTAGTATGATTGCTTCG | 556 | R: GAGGAGAAGATCTTGGAGAGATAT | 286 |
| 3         | rs13476358 | 2          | 16,685,831 | Phlda2 | Intron variant | G/A | G/G | F: CATTGAGTGGATGAAATGG | 498 | G: CAGGAGAATCTTGGAGAGATATGAT | 217 |
| 4         | rs31342586 | 2          | 23,887,704 | Intergenic variant | A/C | A/A | F: GCAGGCAGTGGACTGCTTTCTT | 498 | A: F: CATGGGGATGAAATGG | 417 |
| 5         | rs13476956 | 3          | 5,370,727 | Zfx1d | Intron variant | C/T | C/C | F: TGATGAGTGGTCCAGCCCG | 500 | C: F: CATGGGGATGAAATGG | 308 |
| 6         | rs13477019 | 3          | 23,824,920 | Nualad2 | Intron variant | T/A | T/T | F: TGGAGAAGATCTTGGAGAGATAT | 534 | T: GAGGAGAAGATCTTGGAGAGATAT | 277 |
| 7         | rs13477101 | 3          | 50,109,942 | Gm40056 | Intergenic variant | G/C | G/G | F: GCAGGCAGTGGACTGCTTTCTT | 493 | A: F: CATGGGGATGAAATGG | 295 |
| 8         | rs13477622 | 3          | 28,322,410 | Intergenic variant | T/C | T/T | F: TTTGATTTGGTTGTGAATGAA | 499 | T: AGGAGAAGATCTTGGAGAGATAT | 191 |
| 9         | rs13477746 | 3          | 65,944,235 | Atsn2 | Intron variant | T/C | T/T | F: CCCCCGGTTCGCTGCTT | 516 | T: GAGGAGAAGATCTTGGAGAGATAT | 248 |
| 10        | rs13478320 | 5          | 70,742,061 | Intergenic variant | C/A | C/C | T: GCCTAAAGCCCCATCTTGGGA | 574 | C: F: CATGGGGATGAAATGG | 317 |
| 11        | rs3662161 | 5          | 117,459,347 | Kerp | Intron variant | A/G | A/A | A: TGTGCGGGTTTCTTGG | 487 | A: No allele-specific primers found | 318 |
| 12        | rs33208334 | 5          | 149,626,021 | Hoph1 | Intron variant | T/C | T/T | F: TGAGGCTCATCAACACCATCTT | 485 | T: CCAAGGAGCAGACTCTTAAAC | 317 |
| 13        | rs30764547 | 6          | 28,715,963 | Snul/Lroc4 | Intron variant | T/A | T/T | F: AACGAAACGACAGAAGAAGG | 495 | C: No allele-specific primers found | 318 |
| 14        | rs13478783 | 6          | 60,591,379 | Gm35386 | Downstream gene variant | A/G | A/A | A: TCGGCGGAAAGCGAATGGAT | 396 | A: No allele-specific primers found | 318 |
| 15        | rs6153367 | 6          | 67,287,180 | Serbp1 | 3 prime UTR variant | T/A | T/A | F: TGGTTGCTTATGTTGATGAA | 491 | T: GACATGAAATAGGGTTTCACTTA | 216 |
| 16        | rs13479523 | 7          | 129,035,694 | Intergenic variant | A/G | A/A | A: AGGGGAGTAATAGGAGAAGAAGG | 494 | A: No allele-specific primers found | 257 |
| 17        | rs13479540 | 7          | 133,996,942 | Adam12 | Intron variant | A/G | A/A | A: TCTAGGAGGATGAGAAGG | 504 | A: No allele-specific primers found | 257 |
| 18        | rs3706924 | 8          | 15,191,287 | rs3706924 | Intergenic variant | T/C | T/T | F: GGAGATTAGGGGGT | 500 | T: ACATGAAATAGGGTTTCACTTA | 315 |
| 19        | rs13480100 | 9          | 21,450,142 | Dm2 | Intron variant | T/C | T/T | F: GCCTATGATGGCTGCTT | 559 | T: No allele-specific primers found | 315 |
| 20        | rs13480109 | 9          | 25,889,972 | Intergenic variant | A/G | A/A | A: AAGGAGAATAGGAGAAGAAGG | 541 | A: No allele-specific primers found | 257 |
| 21        | rs13480212 | 9          | 31,516,626 | Apol | Intron variant | T/C | T/T | F: TGGCTGATGATTTCTTAAACAA | 564 | T: No allele-specific primers found | 315 |
| 22        | rs13480575 | 10         | 33,653,023 | Gm15939 | Intron variant | T/C | T/T | F: TGGTAGGAGGAGG | 490 | R: No allele-specific primers found | 325 |
| 23        | rs13480619 | 10         | 57,752,462 | Intergenic variant | T/C | T/T | F:TGCTGCAGGGATAGTACGCA | 558 | T: No allele-specific primers found | 296 |

Supplementary Table 2. Profile of the 45 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6J substrains.
### Supplementary Table 2: Profile of the 45 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6J substrains

| Locus No. | dbSNP ID | Chromosome | Position (bp) (GRCh38.96) | Linked gene | SNP type | Allele flanking primer sequence (5′ to 3′) | Allele-specific primer sequence (5′ to 3′) | Product size (bp) | Allele-specific primer sequence (5′ to 3′) | Product size (bp) |
|-----------|----------|------------|----------------------------|-------------|----------|------------------------------------------|------------------------------------------|-----------------|------------------------------------------|-----------------|
| 24        | rs29359333 | 10         | 58,401,504                 | *Lim1*      | Intron variant | G/A                                    | G/G                                      | 485 G          | No allele-specific primers found       | 226             |
| 25        | rs13480628 | 10         | 67,238,174                 | *JmjD1c*    | Missense variant | T/C                                    | T/T                                      | 491 T          | R: GCAAGGGAAATCAAGCAAGATTTA            | 302             |
| 26        | rs13459122 | 10         | 80,795,365                 | *Dot1l*     | 3 prime UTR variant | A/C                                    | T/T                                      | 521 G          | T: GCAAGGGAAATCAAGCAAGATTTA            | 241             |
| 27        | rs13480759 | 10         | 109,378,627                | Intergenic variant | C/T                                    | C/C                                      | 581 C          | A: GTCTGAGGGAAATCAAGCAAGATTTA          | 322             |
| 28        | rs3659787 | 11         | 4,508,730                  | *Mmr3*      | Intron variant | G/A                                    | G/G                                      | 589 G          | R: CTCTGAGGGAAATCAAGCAAGATTTA          | 266             |
| 29        | rs13481014 | 11         | 48,117,382                 | Intergenic variant | T/C                                    | T/T                                      | 512 T          | R: GCAAGGGAAATCAAGCAAGATTTA            | 240             |
| 30        | rs13481117 | 11         | 79,252,230                 | *Wsb1*      | Intron variant | G/T                                    | G/G                                      | 497 G          | T: GCAAGGGAAATCAAGCAAGATTTA            | 200             |
| 31        | rs13481403 | 12         | 39,488,993                 | Non-coding transcript exon variant | A/G                                    | A/A                                      | 282 G          | R: AATGGTTATCAGAGGTGCGGTTCA            | 191             |
| 32        | rs13481439 | 12         | 48,965,551                 | Intergenic variant | A/G                                    | A/A                                      | 488 G          | R: CTCTGAGGGAAATCAAGCAAGATTTA          | 200             |
| 33        | rs22393594 | 12         | 56,480,365                 | Intergenic variant | T/A                                    | T/T                                      | 499 G          | R: GCAAGGGAAATCAAGCAAGATTTA            | 271             |
| 34        | rs13481569 | 12         | 85,645,337                 | Intergenic variant | G/A                                    | G/G                                      | 399 G          | R: GCAAGGGAAATCAAGCAAGATTTA            | 162             |
| 35        | rs13481634 | 12         | 106,833,655                | Upstream gene variant | A/C                                    | A/A                                      | 300 A          | R: CTCTGAGGGAAATCAAGCAAGATTTA          | 158             |
| 36        | rs13481676 | 13         | 6,304,055                  | Intergenic variant | A/G                                    | A/A                                      | 497 A          | R: AATGGTTATCAGAGGTGCGGTTCA            | 157             |
| 37        | rs13481734 | 13         | 27,037,150                 | *Gm47888*    | Intergenic variant | A/G                                    | A/A                                      | 488 G          | R: CTCTGAGGGAAATCAAGCAAGATTTA          | 263             |
| 38        | rs3722331 | 13         | 41,442,786                 | *Nad9*       | Intergenic variant | T/C                                    | T/T                                      | 529 G          | R: GCAAGGGAAATCAAGCAAGATTTA            | 300             |
| 39        | rs31233932 | 14         | 124,108,797                | Fgf14        | Intergenic variant | C/T                                    | C/C                                      | 507 C          | R: AATGGTTATCAGAGGTGCGGTTCA            | 247             |
| 40        | rs3702158  | 15         | 57,160,486                 | Intergenic variant | A/G                                    | A/A                                      | 521 G          | R: AATGGTTATCAGAGGTGCGGTTCA            | 251             |
| 41        | rs4165065  | 16         | 17,412,079                 | Snap29       | Intergenic variant | T/C                                    | T/T                                      | 536 T          | No allele-specific primers found       | 289             |
| 42        | rs13483055 | 17         | 60,319,945                 | Intergenic variant | T/C                                    | T/T                                      | 536 T          | R: GCAAGGGAAATCAAGCAAGATTTA            | 292             |
| 43        | rs13483237 | 18         | 19,289,435                 | Intergenic variant | G/C                                    | G/G                                      | 473 G          | T: GCAAGGGAAATCAAGCAAGATTTA            | 233             |
| 44        | rs3724876  | 19         | 49,976,154                 | Intergenic variant | G/T                                    | G/G                                      | 598 G          | R: GCAAGGGAAATCAAGCAAGATTTA            | 319             |
| 45        | rs33867485 | 10         | 146,165,950                | A/T          | A/A                                    | F: TCAATTTTCTTTTCTGCAGGTG                | 447 A          | R: AATGGTTATCAGAGGTGCGGTTCA            | 319             |
Supplementary Table 2. Profile of the 45 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6J substrains

| Locus No. | dbSNP ID | Chromosome Position (bp) | Linked gene | SNP type | C57BL/6J allele | Allele flanking primer sequence (5′ to 3′) | Product size (bp) | Allele-specific primer sequence (5′ to 3′) | Product size (bp) |
|-----------|----------|--------------------------|-------------|----------|-----------------|------------------------------------------|-----------------|------------------------------------------|-----------------|

Information about the chromosomal position, linked genes, and SNP type (sequence variant type) was obtained from the Ensembl Mouse Genome Server (ensembl.org/Mus_musculus/Info/index). SNPs were amplified by PCR with allele flanking primers. PCR was performed using a QIAGEN Multiplex PCR kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. The PCR products were electrophoresed and separated on an E-Gel CloneWell 0.8% SYBR Safe gel using an E-Gel iBase Power system (both from Life Technologies, Carlsbad, CA, USA). Sequencing reactions were performed in a DNA Engine® and Dyad® PTC-220 Peltier Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using an ABI BigDye® Terminator v3.1 Cycle Sequencing Kit with AmpliTaq DNA polymerase (Life Technologies), following the protocols supplied by the manufacturers. Single-pass sequencing was performed on each template primer. The fluorescently labeled fragments were purified from the unincorporated terminators with an ethanol precipitation. The samples were resuspended in distilled water and then subjected to electrophoresis in an ABI 3730xl sequencer (Life Technologies). SNP detection was also performed using the allele-specific PCR method. Two PCR reactions were run in parallel, combining a common forward or reverse allele flanking primer and one of the two reverse or forward allele-specific primers. The PCR products were visualized with agarose gel electrophoresis. All PCR primers were designed using BatchPrimer3 v1.0 (robes.pw.usda.gov/batchprimer3/index.html).
| Locus No. | dbSNP ID | Chromosome (bp) (GRCm38.p6) | Linked gene | SNP type | Allele flanking primer sequence (5’ to 3’) | Product size (bp) | Allele-specific primer sequence (5’ to 3’) | Product size (bp) |
|----------|----------|-----------------------------|-------------|----------|------------------------------------------|------------------|-----------------------------------------|------------------|
| 1        | rs24623630 | 1 11,996,705                 | No allele-specific primers designed. | C/T      | F: ACCCCTGAACCTTAACTTC                  | 493              | T: No allele-specific primers found.    | 158              |
| 2        | rs246940354 | 1 14,344,561                 | No allele-specific primers designed. | T/A      | F: GGGAAGAGGGGGAAGTACTA                | 506              | A: No allele-specific primers found.    | 171              |
| 3        | rs212521754 | 1 16,968,405                 | No allele-specific primers designed. | C/A      | F: GCAAGAAGGAAATTTAGACG                 | 500              | T: No allele-specific primers found.    | 156              |
| 4        | rs27394848 | 1 19,544,960                 | No allele-specific primers designed. | A/T      | F: GGGCGAACTCTTCCTCT                  | 505              | T: No allele-specific primers found.    | 175              |
| 5        | rs232920323 | 1 21,639,642                 | Kcnq5       | C/G      | F: GAAATACGACAGGCCTCATAA               | 501              | G: F: ACCCTCTCATTTGATTTGTTTC            | 196              |
| 6        | rs213024334 | 1 30,167,141                 | No allele-specific primers designed. | C/T      | F: GGAATCCTCAACCTTACGAGCA              | 512              | T: No allele-specific primers designed. | 196              |
| 7        | rs247947800 | 1 40,107,883                 | Ilv2        | T/A      | F: TTCGATGCTCTCTCACAGT                | 500              | T: No allele-specific primers designed. | 196              |
| 8        | rs260670033 | 1 50,146,448                 | No allele-specific primers designed. | C/T      | F: AGCAGAATGGCCTTCTTCT                | 489              | T: No allele-specific primers designed. | 196              |
| 9        | rs249807793 | 1 61,950,812                 | Purd3b      | C/A      | F: CCATGGGTAATGTTGGAATT              | 494              | A: R: TAAATGGGACATGTGGAGGT             | 122              |
| 10       | rs265151779 | 1 83,182,637                 | Wdr69       | G/A      | F: ATTTGCACCTGCGGAGGG                | 496              | A: No allele-specific primers designed. | 196              |
| 11       | rs229124202 | 1 89,861,338                 | Agap1       | G/T      | F: GGAAAGCATCAGCAACACT              | 496              | G: No allele-specific primers designed. | 196              |
| 12       | rs276563339 | 1 99,547,673                 | No allele-specific primers designed. | G/A      | F: TGGCTCCTGACATCTTCT                  | 516              | A: F: TTTCTGGTTGTAATCTTAGGTAAGA         | 184              |
| 13       | rs223540754 | 1 110,024,886                | Cdh7        | C/C      | F: GACCAAAATGCTGGCAAAATGA            | 500              | C: No allele-specific primers designed. | 196              |
| 14       | rs259683636 | 1 119,116,297                | No allele-specific primers designed. | C/T      | F: CTCCCACTCCTTCTTCT                  | 501              | T: No allele-specific primers designed. | 196              |
| 15       | rs22991128 | 1 132,980,179                | No allele-specific primers designed. | T/A      | F: TTTTTGTTGTTGACAGAGGAT            | 499              | A: F: CTAAGGACGAGCGAGTGGTTTGGTGA       | 394              |
| 16       | rs215622703 | 1 142,008,378                | No allele-specific primers designed. | C/A      | F: TTTTTGTTGTTGACGAGGAT            | 502              | A: F: CTAAGAGTACGACGATGAGGTTTGGTGA     | 394              |
| 17       | rs239017398 | 1 154,474,620                | Cacna1e     | C/T      | F: CAGATCCGCCGCTCAATTTTA             | 501              | T: No allele-specific primers designed. | 196              |
| 18       | rs214254072 | 1 161,859,644                | Ahb4100     | C/T      | F: AGTGTACCGGCTCAGAGTG               | 494              | T: No allele-specific primers designed. | 196              |
| 19       | rs255914894 | 1 172,611,934                | No allele-specific primers designed. | G/A      | F: ATGGCGGCTGACAGGTCTCTCT            | 503              | A: F: GGATGCGCGACGACGAGTTGGTGA          | 166              |
| 20       | rs222303818 | 1 179,503,532                | Smyd3       | G/A      | F: CTGCGCGACATCTGCTCAG               | 501              | A: No allele-specific primers designed. | 196              |
| 21       | rs262282675 | 1 188,434,376                | Ush2a       | A/T      | F: AGGGCTGTGACAGGAGAACAA             | 496              | T: No allele-specific primers designed. | 196              |
| 22       | rs251976963 | 2 11,214,185                 | Prkg4       | C/T      | F: CTGCGCGACATCTGCTCAG               | 493              | T: No allele-specific primers designed. | 196              |
| 23       | rs230606069 | 2 21,681,174                 | Gpr158      | G/T      | F: GGGCTGCTGTCAGAGGCCTT             | 498              | T: No allele-specific primers designed. | 196              |
| Locus No | dbSNP ID | Chromosome | Position (bp) (GRCm38/p6) | Linked gene | SNP type | SNPs allele | C57BL/6NJ allele | Allele flanking primer sequence (5' to 3') | Product size (bp) | Allele-specific primer sequence (5' to 3') | Product size (bp) |
|----------|----------|------------|---------------------------|-------------|----------|-------------|------------------|-------------------------------------------|-----------------|------------------------------------------|-----------------|
| 24       | rs242780245 | 2         | 30,188,488                | Cebbl       | Intron variant | A/C          | C/C             | R: CACCTGTCACCCGACCATCTCCTGCGGAA         | 474             | C: CTAGGACCTGTCACCATAC              | 294             |
| 25       | rs228546410 | 2         | 41,205,764                | Lrp1b       | Intron variant | T/A          | A/A             | R: ATGGCCAATGCAACACATA                  | 504             | A: No allele-specific primers designed. |                   |
| 26       | rs254996546 | 2         | 51,969,852                | Nmi         | Intron variant | C/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 509             | T: No allele-specific primers designed. |                   |
| 27       | rs256541267 | 2         | 70,251,451                | Mysl        | Intron variant | C/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 512             | T: GCGTACGAAAGGCGACCATC              | 185             |
| 28       | rs248280077 | 2         | 80,873,138                | Intergenic  | variant      | C/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 462             | T: No allele-specific primers designed. |                   |
| 29       | rs214356625 | 2         | 96,674,180                | Lrce4c      | Intron variant | G/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 469             | T: No allele-specific primers designed. |                   |
| 30       | rs224344563 | 2         | 102,710,505               | Slc1a2      | Intron variant | A/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 502             | T: F: CACCTGTCACCCGACCATC            | 159             |
| 31       | rs251933504 | 2         | 112,966,408               | Rps1        | 3' UTR variant | T/C          | C/C             | R: CACCTGTCACCCGACCATCTGCGGAA          | 502             | C: No allele-specific primers designed. |                   |
| 32       | rs258508221 | 2         | 122,708,738               | 4930417H01Rk | Intron variant | T/A          | A/A             | R: CACCTGTCACCCGACCATCTGCGGAA          | 499             | A: No allele-specific primers designed. |                   |
| 33       | rs255014110 | 2         | 132,432,999               | 492508D12Rk | Intron variant | C/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 505             | T: No allele-specific primers designed. |                   |
| 34       | rs242413924 | 2         | 140,793,056               | Macro2      | Intron variant | T/G          | G/G             | R: CACCTGTCACCCGACCATCTGCGGAA          | 499             | G: R: GATGACGATGGCTGACGAC           | 398             |
| 35       | rs217443774 | 2         | 152,781,403               | Bcl2l1      | 3' UTR variant | A/G          | G/G             | R: CACCTGTCACCCGACCATCTGCGGAA          | 498             | G: No allele-specific primers designed. |                   |
| 36       | rs253212197 | 2         | 164,813,748               | Intergenic  | variant      | C/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 501             | T: No allele-specific primers designed. |                   |
| 37       | rs213376233 | 2         | 170,240,434               | Intergenic  | variant      | G/A          | A/A             | R: CACCTGTCACCCGACCATCTGCGGAA          | 502             | A: No allele-specific primers designed. |                   |
| 38       | rs264719247 | 2         | 180,149,012               | Otxbp2      | Intergenic variant | A/G          | G/G             | R: CACCTGTCACCCGACCATCTGCGGAA          | 502             | G: R: GATGACGATGGCTGACGAC           | 285             |
| 39       | rs222512392 | 2         | 181,868,891               | Pole3       | 3' UTR variant | C/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 502             | T: No allele-specific primers found.   | 285             |
| 40       | rs265520809 | 3         | 8,498,163                 | Intergenic  | variant      | G/A          | A/A             | R: CACCTGTCACCCGACCATCTGCGGAA          | 505             | A: No allele-specific primers designed. |                   |
| 41       | rs214801792 | 3         | 32,773,111                | Intergenic  | variant      | G/C          | C/C             | R: CACCTGTCACCCGACCATCTGCGGAA          | 502             | C: F: GACATGTCACCCGACCATC            | 402             |
| 42       | rs222821429 | 3         | 66,305,330                | Intergenic  | variant      | C/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 499             | T: F: GACATGTCACCCGACCATC            | 219             |
| 43       | rs243666799 | 3         | 72,616,062                | Intergenic  | variant      | G/A          | A/A             | R: CACCTGTCACCCGACCATCTGCGGAA          | 488             | A: No allele-specific primers designed. |                   |
| 44       | rs262827930 | 3         | 109,597,274               | Fav3        | Intron variant | A/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 482             | T: F: GACATGTCACCCGACCATC            | 395             |
| 45       | rs254145219 | 3         | 147,657,254               | Intergenic  | variant      | C/T          | T/T             | R: CACCTGTCACCCGACCATCTGCGGAA          | 487             | T: No allele-specific primers found.   | 136             |
| 46       | rs219227155 | 4         | 19,328,298                | Cngb3       | Intron variant | T/C          | C/C             | R: CACCTGTCACCCGACCATCTGCGGAA          | 501             | C: No allele-specific primers found.   | 236             |

**Profile of the 100 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6N substrains**

**Supplementary Table 3.**
Supplementary Table 3. Profile of the 100 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6N substrains

| Locus No. | dbSNP ID | Chromosome (bp) (GRCm38.p6) | Linked gene | SNP type | SNP allele C57BL/6J allele | Allele flanking primer sequence (5’ to 3’) | Product size (bp) | Alleles-specific primer sequence (5’ to 3’) | Product size (bp) |
|-----------|----------|-----------------------------|-------------|----------|---------------------------|-------------------------------------------|-----------------|--------------------------------------------|-----------------|
| 47        | rs235104023 | 4                            | 56,463,984  | Intergenic variant | C/T                           | T/F                                        | 501             | T                                          | No allele-specific primers found. |
| 48        | rs261879287 | 4                            | 104,973,294 | Intergenic variant | T/G                           | G/G                                        | 499             | G                                          | T                                          | 274             |
| 49        | rs256724446 | 5                            | 35,701,259  | Intron variant    | A/G                           | A/A                                        | 498             | A                                          | R                                          | 274             |
| 50        | rs260260328 | 5                            | 80,026,465  | Intergenic variant | A/G                           | A/A                                        | 493             | A                                          | R                                          | 153             |
| 51        | rs217297994 | 5                            | 117,118,668 | Intron variant    | A/G                           | G/G                                        | 499             | A                                          | F                                          | 279             |
| 52        | rs221990668 | 5                            | 150,224,989 | Intergenic variant | G/T                           | T/T                                        | 501             | T                                          | C                                          | 367             |
| 53        | rs257294810 | 6                            | 39971164    | Intergenic variant | G/A                           | A/A                                        | 505             | A                                          | C                                          | 146             |
| 54        | rs224069095 | 6                            | 74169211    | Intergenic variant | C/A                           | A/A                                        | 509             | A                                          | A                                          | 405             |
| 55        | rs37540455  | 6                            | 113159679   | Intergenic variant | G/A                           | A/A                                        | 502             | A                                          | C                                          | 223             |
| 56        | rs217544076 | 6                            | 144513005   | Intergenic variant | G/C                           | C/C                                        | 495             | C                                          | R                                          | 367             |
| 57        | rs212452109 | 7                            | 16,595,985  | Intergenic variant | A/T                           | T/T                                        | 501             | T                                          | F                                          | 367             |
| 58        | rs224103578 | 7                            | 53390545    | Intergenic variant | C/T                           | A/A                                        | 510             | R                                          | C                                          | 197             |
| 59        | rs243575509 | 7                            | 102,973,309 | Missense variant  | T/C                           | T/F                                        | 491             | T                                          | C                                          | 410             |
| 60        | rs229340185 | 7                            | 140,821,590 | Intergenic variant | A/T                           | T/F                                        | 500             | T                                          | G                                          | 378             |
| 61        | rs263791105 | 8                            | 22,903,742  | Intergenic variant | G/A                           | A/A                                        | 509             | A                                          | C                                          | 378             |
| 62        | rs225341040 | 8                            | 58,790,625  | Intergenic variant | G/A                           | A/A                                        | 500             | A                                          | C                                          | 400             |
| 63        | rs239219835 | 8                            | 79,117,401  | Intergenic variant | G/A                           | A/A                                        | 500             | A                                          | C                                          | 410             |
| 64        | rs256624163 | 8                            | 94,046,068  | Intergenic variant | G/A                           | A/A                                        | 529             | A                                          | F                                          | 400             |
| 65        | rs211750147 | 8                            | 118,442,679 | Intergenic variant | G/A                           | A/A                                        | 511             | F                                          | A                                          | 421             |
| 66        | rs52003732  | 9                            | 10,125,214  | Intergenic variant | T/C                           | C/C                                        | 464             | C                                          | A                                          | 378             |
| 67        | rs214490504 | 9                            | 60,662,109  | Intergenic variant | G/A                           | A/A                                        | 499             | A                                          | C                                          | 178             |
| 68        | rs243500146 | 9                            | 116,160,235 | Intergenic variant | C/T                           | T/T                                        | 489             | G                                          | F                                          | 166             |
| 69        | rs51123066  | 10                           | 11,070,460  | Intergenic variant | G/T                           | G/T                                        | 493             | T                                          | No allele-specific primers found. |
|           |           |                             |             |           |                           |                                            |                 |                                            |                 | 338             |
| Locus No. | dbsNP ID | Chromosome position (bp) (GRCh38.p6) | Linked gene | SNP type | SNP allele | C57BL/6NJ allele | Allele flanking primer sequence (5' to 3') | Product size (bp) | Allele-specific primer sequence (5' to 3') | Product size (bp) |
|----------|----------|---------------------------------------|-------------|----------|------------|-----------------|------------------------------------------|----------------|------------------------------------------|----------------|
| 70       | rs219489973 | 41,344,745                             | Armc2       | Intron variant | G/A       | A/A           | F: CTCCTGGCGACACTCAACAGGA<br>R: GCTTGGCTGCTTGCAGTTGTTGAGGA<br>T/G: GCTTGGCTGCTTGCAGTTGTTGAGGA | 492         | A: CGTCTGGCGACACTCAACAGGA<br>G: GCTTGGCTGCTTGCAGTTGTTGAGGA | 365           |
| 71       | rs213583872 | 49,357,252                             | Grik2       | Intron variant | G/T       | T/T           | F: GTGTGCACAGCGTGAAGATAGG<br>R: CAACTAGATGTTACAAAGAG<br>T/C: CAACTAGATGTTACAAAGAG | 540         | T: CGACAGCTACAAATACGAA<br>G: CGACAGCTACAAATACGAA | 365           |
| 72       | rs246274290 | 88,091,833                             | Pmch        | Missense variant | T/C       | C/C           | F: CACAGAACACAGCCTCAGAAA<br>R: GCCACATGCGTCTCGAGTAC<br>T/C: GCCACATGCGTCTCGAGTAC | 499         | F: TACACAGCGACAGCCTCAGAAA<br>C: GCCACATGCGTCTCGAGTAC | 371           |
| 73       | rs235870779 | 12,253,003                             | Cdh8        | Intron variant | A/T       | T/T           | F: TTGGTGGCTGCTCAGTCA<br>R: AAAAGCCGCGAACGAGTTGAAT | 498         | A: ACTGACGCAGCCTGCTCAGAAA<br>F: TTGGTGGCTGCTCAGTCA | 119           |
| 74       | rs240617401 | 46,222,615                             | Cyp1b2      | Missense variant | G/A       | A/A           | F: TGACCCCCAATCACACATAGT<br>R: GCCAGCTTACCTACGTGAC<br>A/A: GCCAGCTTACCTACGTGAC | 505         | A: TGCATACCCACCACCTGTTGAA<br>F: TGACCCCCAATCACACATAGT | 137           |
| 75       | rs231656457 | 29886047                              | Myst1       | Intron variant | T/G       | G/G           | F: TCTTGGTAAATGTTGGCAAGG<br>R: ATTCAGAAATGTCGAGCTCA<br>T/C: ATTCAGAAATGTCGAGCTCA | 500         | G: CAGTCTGGTATGCTCCTTGGAG<br>F: TCTTGGTAAATGTTGGCAAGG | 353           |
| 76       | rs217422777 | 70772479                              | Intergenic variant | C/T       | T/T           | F: CGGGAAATAAACACTACAC<br>R: ACCTGCTCTTCTCGGA<br>A/A: ACCTGCTCTTCTCGGA | 497         | C: GAGGCTGACCTACGAGTT<br>F: CGGGAAATAAACACTACAC | 366           |
| 77       | rs221345442 | 97702669                              | Intergenic variant | A/T       | T/T           | F: TTCTCTACCGTGATCTGCTG<br>C/T: TTCTCTACCGTGATCTGCTG | 489         | T: TTTGCTGTTGTTGTTGTTGGA<br>F: TTCTCTACCGTGATCTGCTG | 334           |
| 78       | rs226310424 | 41494375                              | Intergenic variant | G/C       | C/C           | F: CATCCTCAATGGTCTGATA<br>R: TCCACAGTTCAGCCAAAGG<br>G/C: TCCACAGTTCAGCCAAAGG | 500         | C: CACATCCATCGTCCGC<br>F: CATCCTCAATGGTCTGATA | 219           |
| 79       | rs230596409 | 64921972                              | Span3l      | Stop gained | C/T       | T/T           | F: GGGTTGACACTAGTGCTGAT<br>R: CTGGGGTGTAGCATTGGTGTC<br>C/T: GGGTTGACACTAGTGCTGAT | 511         | T: GGGCAAGAAAACACTCAGG<br>F: CTGGGGTGTAGCATTGGTGTC | 152           |
| 80       | rs251507217 | 101112155                             | Intergenic variant | A/T       | T/T           | F: CCCCCAGCCGACATC<br>R: TCCACAGTTCAGCCAAAGG<br>A/T: TCCACAGTTCAGCCAAAGG | 500         | C: GTACAGAGAAGGAACG<br>F: CCCCCAGCCGACATC | 212           |
| 81       | rs242991609 | 119477808                             | Misseense variant | C/A       | A/A           | F: TTGGTGGTGCATCCTG<br>R: CACACAGAGAAGGCTTAT<br>A/A: CACACAGAGAAGGCTTAT | 517         | T: TCTTGGTGAAGGGTGAC<br>F: TTGGTGGTGCATCCTG | 247           |
| 82       | rs265139270 | 39164780                              | Nrg3        | Intron variant | C/T       | T/T           | F: GGCATCTCATCATGAGT<br>R: AGGCTACAGATGTTGAGGA<br>C/T: GGCATCTCATCATGAGT | 502         | T: CCACCTCAATCGGTTGTT<br>F: GGCATCTCATCATGAGT | 249           |
| 83       | rs235426868 | 75727272                              | Cog1        | Synonymous variant | G/A       | A/A           | F: ATACATCAGCTTACGAC<br>R: CGGAGGCGAATGAAAGGA<br>G/A: CGGAGGCGAATGAAAGGA | 492         | A: CATACATCAGCTTACGAC<br>F: ATACATCAGCTTACGAC | 252           |
| 84       | rs222697275 | 117850332                             | Gp6         | Intron variant | G/A       | A/A           | F: GTTGTTGGTTCAGGAGGA<br>R: GCGCAAATCTTGTGAGTT<br>G/A: GTTGTTGGTTCAGGAGGA | 497         | A: GCGCAAATCTTGTGAGTT<br>F: GTTGTTGGTTCAGGAGGA | 285           |
| 85       | rs243245803 | 22748238                              | Cdh18       | Intron variant | T/G       | G/G           | F: TTGGTGGTTCAGGAGGA<br>R: TTGGTGGTTCAGGAGGA<br>T/G: TTGGTGGTTCAGGAGGA | 492         | G: No allele-specific primers designed. | 249           |
| 86       | rs243400512 | 55816925                              | Sntb1       | Intron variant | C/T       | T/T           | F: TCAAGGCGTAAGTGAC<br>R: GGGCGACGTCTGCTGAGG<br>T/G: TCAAGGCGTAAGTGAC | 512         | T: No allele-specific primers designed. | 125           |
| 87       | rs231321125 | 97760563                              | Raggy5      | Intron variant | C/G       | G/G           | F: CTCTACAGACCAGAGG<br>R: GGCCCTCAGGAA<br>C/G: GGCCCTCAGGAA | 507         | A: No allele-specific primers designed. | 176           |
| 88       | rs230243864 | 20462996                              | Intergenic variant | C/T       | T/T           | F: TTGGTGGTTCATCTG<br>R: ATCTAAAAGCAACCCCCAG<br>C/T: TTGGTGGTTCATCTG | 500         | T: No allele-specific primers designed. | 125           |
| 89       | rs240948896 | 61450798                              | Intergenic variant | G/A       | A/A           | F: TCGAGCATGACAGGAAGT<br>R: TTGGCAAAAATCGTGAATG<br>G/A: TCGAGCATGACAGGAAGT | 491         | A: No allele-specific primers designed. | 212           |
| 90       | rs240667957 | 40854409                              | Intergenic variant | C/T       | T/T           | F: TGCTCAGGTAATCTG<br>R: TGGTTGTTGTTCTG<br>C/T: TGCTCAGGTAATCTG | 515         | T: No allele-specific primers designed. | 152           |
| 91       | rs259144033 | 69131609                              | Intergenic variant | C/T       | T/T           | F: CAGGTTGAGAGAGA<br>R: CAGGTTGAGAGAGA<br>C/T: CAGGTTGAGAGAGA | 506         | C: No allele-specific primers designed. | 176           |
| 92       | rs225963780 | 22550101                              | Axl3        | 3' UTR variant | G/T       | T/T           | F: AGGAGTTGACTGTTG<br>R: ACAAGGCGGAAAAATTTG<br>G/T: AGGAGTTGACTGTTG | 485         | G: No allele-specific primers designed. | 212           |
| Locus No. | dbsNP ID     | Chromosome | Position (bp) (GRCm38.p6) | Linked gene        | SNP type  | Allele flanking primer sequence (5’ to 3’)                                      | Product size (bp) | Allele-specific primer sequence (5’ to 3’)                                      | Product size (bp) |
|----------|--------------|------------|-----------------------------|--------------------|-----------|----------------------------------------------------------------------------------|------------------|----------------------------------------------------------------------------------|------------------|
| 93       | rs214638331  | 18         | 41344093                    |                    | Intergenic variant | T/C                                                                                 | 495              | C                                                                                  | No allele-specific primers designed. |
| 94       | rs255789242  | 18         | 59519801                    |                    | Intergenic variant | C/T                                                                                 | 481              | T                                                                                  | No allele-specific primers designed. |
| 95       | rs265387961  | 18         | 90448757                    |                    | Intergenic variant | A/G                                                                                 | 507              | G                                                                                  | No allele-specific primers designed. |
| 96       | rs232414357  | 19         | 23329888                    | Mme12              | Intron variant    | C/T                                                                                 | 492              | T                                                                                  | No allele-specific primers designed. |
| 97       | rs230656170  | 19         | 40364531                    |                    | Intergenic variant | G/A                                                                                 | 500              | A                                                                                  | No allele-specific primers designed. |
| 98       | rs246037535  | X          | 84805631                    | Dmd                | Intron variant    | C/T                                                                                 | 493              | T                                                                                  | No allele-specific primers designed. |
| 99       | rs266019057  | X          | 112095948                   |                    | Intergenic variant | A/G                                                                                 | 559              | G                                                                                  | No allele-specific primers designed. |
| 100      | rs212226666  | X          | 157445480                   |                    | 3' UTR variant    | T/A                                                                                 | 500              | A                                                                                  | No allele-specific primers designed. |

Information about the chromosomal position, linked genes, and SNP type (sequence variant type) was obtained from the Ensembl Mouse Genome Server (ensembl.org/Mus_musculus/Info/Index). SNPs were amplified by PCR with allele flanking primers. PCR was performed using a QIAGEN Multiplex PCR kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. The PCR products were electrophoresed and separated on an E-Gel CloneWell 0.8% SYBR Safe gel using an E-Gel iBase Power system (both from Life Technologies, Carlsbad, CA, USA) using an ABI BigDye® Terminator v3.1 Cycle Sequencing Kit with AmpliTaq DNA polymerase (Life Technologies), following the protocols supplied by the manufacturers. Single-pass sequencing was performed on each template primer. The fluorescently labeled fragments were purified from the unincorporated terminators with an ethanol precipitation. The samples were resuspended in distilled water and then subjected to electrophoresis in an ABI 3730xl sequencer (Life Technologies). SNP detection was also performed using the allele-specific PCR method. Two PCR reactions were run in parallel, combining a common forward or reverse allele flanking primer and one of the two reverse or forward allele-specific primers. The PCR products were visualized with agarose gel electrophoresis. All PCR primers were designed using BatchPrimer3 v1.0 (robes.pw.usda.gov/batchprimer3/index.html).