**Supplemental Figures.**

**Supplemental Figure 1. Hierarchical clustering of samples based on high impact variants.** A hierarchical clustering of high impact variant data revealed several significant clusters, defined by inbred strain background. A clear separation between wild-derived laboratory strains and classical laboratory strains was observed, with the outlying clusters comprised of the wild-derived PWK, CAST, and WSB respectively. Colors denote different strains as shown in the figure legend. Asterisks indicate significant clusters with AU p-values greater or equal to 90%. Samples 107:S10, 18:S06, 23:S02, 76:S02, 78:S02 and 102:S00 appeared in unexpected clusters. Samples denoted #:S11 (green) were samples with previously unknown strain background. This analysis allows approximate strain background to be assigned to these samples.

**Supplemental Figure 2. Alignments of whole exome or whole genome sequencing data spanning ‘exome recalcitrant’ mutations.** BWA alignment of whole genome or exome sequencing data from strains for which our exome pipeline failed to yield candidate mutations. In reference-based alignments duplications, deletions and insertions resulted in diagnostic mapping signatures. The nature of these signatures depended on the size, location (with respect to exons), mapping algorithm and sequencing approach employed (exome or whole genome, paired end or single end, short reads or long reads). Alignment of whole genome sequencing (WGS) data across *4732456N10Rik* (A), *Rorb* (B) and *Samd4* (C) demonstrate an insertion, duplication, and deletion respectively. These structural mutations were successfully called using Pindel. In the absence of whole genome sequencing data, some mutations could be identified through manual analysis of exome alignments across strong candidate genes. For example, alignments of across *Grid2* (D), *Atp8a2* (E) and *Slc45a2* (F) demonstrate deletions within exons or spanning multiple exons. The general location of deletion breakpoints in *Grid2* were inferred by lack of coverage in exons 3 and 4, however, precise breakpoints in introns 2 and 5 could not be determined from exome data. A second allele of *Grid2* did not impact exon coverage but did alter splicing as evidenced via RT-PCR (data not shown). Two alleles of *Atp8a2 (wl-vmd* and *wl-3J)* are shown in (E). An alignment of the same region in a control sample is shown for comparison. The *wl-vmd* allele is ~9kb deletion spanning exon 32 (middle panel, E), while the *wl-3J* allele is a 641 bp deletion spanning exons 30 and 31, combined with a 10bp duplication in exon 32. The more complex *wl-3J* lesion results in zero coverage for exons 30 and 31 (not shown), as well as split reads in exon 32 (E, bottom panel, arrow). The *uw-6J* allele is a large deletion spanning the last 4 exons of Slc45a2 (F). The *jal* allele of *Gata3* is an insertion that was evidenced by read pairs that mapped to different chromosomes and were therefore, flagged by BWA (G, colored reads, arrows). The *bs* allele in *Tbc1d20* is a 12 bp deletion that, depending on the position of the mapped read, resulted in split reads, with clusters of SNP calls, or properly aligned reads with the deletion correctly called, along with a missense mutation in exon 6 (H). All exon annotations are from RefSeq and alignments are to NCBI37/mm9).

**Supplemental Tables.**

**Supplemental Table 1. Mouse strains with Mendelian disorders included in this study.** For each strain, mode of inheritance and Mammalian Phenotype terms are provided. In cases where causative mutations were identified in this study or where spontaneous phenotypic alleles were previously published, official allele nomenclature is provided. \*Mutations discovered that were also published by other groups during the course of our study ([Yang et al. 2011](#_ENREF_50); [Zhu et al. 2012](#_ENREF_52); [Akiyama et al. 2013](#_ENREF_1); [Liegel et al. 2013](#_ENREF_30); [Ramirez et al. 2013](#_ENREF_41); [Bisaillon et al. 2014](#_ENREF_6)). \*\*Causative gene proven by allele test. \*\*\*ENU mutant strain with two closely linked mutations.

**Supplemental Table 2. Comparison of MMR control exome variant data for seven inbred strains with published whole genome variant data from the Sanger Mouse Genomes Project.** Homozygous variant (SNP/indel) calls within the regions captured by the exome probe pools were compared across datasets. Total homozygous variant calls from each project (JAX MMR exome and Sanger whole genome) are provided, as well as the number of variant calls unique to and shared between each dataset (MMR exome vs. Sanger whole genome, unique and overlap). Columns “All unique to JAX SNPs, PASS” and “All unique to Sanger SNPs, PASS” provide the number of unique SNPs (or indel) passing GATK soft filters (see materials and methods) and a commonality filter, i.e. if the variant was present in more than 75% of the samples in our study it was filtered. Approximately 10% of the variants found to be unique to one dataset or the other were found in other samples in our study and were therefore filtered out. However, for C57BL/6NJ ~90% of what appeared to be unique variants in each data set were found in 75% or more of the samples in our study, which explains the apparently low % overlap in C57BL/6NJ. Data the origin of each sequenced sample, including sex, pedigree and birthdate are also provided.

**Supplemental Table 3. All pathogenic mutations discovered by whole exome and whole genome sequencing of mouse strains with Mendelian disorders.** For each confirmed mutant allele, the mouse gene symbol, the human ortholog, associated human disease where known, inheritance, allele annotation (Annovar) as predicted by our pipeline and the primary clinical phenotype by Mammalian Phenotype terms (system or tissue level) are provided. If the same mutation was found in more than one strain, that allele is represented only once this table, as is only one associated human disease per allele.

\*Novel mouse genes for which we report the first allele and phenotype.

\*\*Mutations that were reported in our pilot study and used here to validate our pipeline (Fairfield et al. 2011).

\*\*\*Mutation published during the course of our study (Akiyama et al. 2013; ([Zhu et al. 2012](#_ENREF_52); [Liegel et al. 2013](#_ENREF_30)).

**Supplemental Table 4. Genomic position and molecular consequences of new mutations discovered by WES and WGS of strains with Mendelian disorders.** The physical position(GRCm38, mm10) and molecular consequences (SnpEff and ANNOVAR) of causative mutations discovered in our study.

**Supplemental Table 5.** **False discovery rates (FDR) and false negative rates (FNR) calculated for 6 inbred strain control data sets.**

**Supplemental Table 6. Validation primer sequences listed by gene and allele.**

**References:**

Akiyama K, Noguchi J, Hirose M, Kajita S, Katayama K, Khalaj M, Tsuji T, Fairfield H, Byers C, Reinholdt L, et al. 2013. A mutation in the nuclear pore complex gene Tmem48 causes gametogenesis defects in skeletal fusions with sterility (sks) mice. *J Biol Chem* **288:** 31830–31841.

Bisaillon JJ, Radden LA II, Szabo ET, Hughes SR, Feliciano AM, Nesta AV, Petrovic B, Palanza KM, Lancinskas D, Szmurlo TA, et al. 2014. The retarded hair growth (rhg) mutation in mice is an allele of ornithine aminotransferase (Oat). *Mol Genet Metab Rep* **1:** 378–390.

Liegel RP, Handley MT, Ronchetti A, Brown S, Langemeyer L, Linford A, Chang B, Morris-Rosendahl DJ, Carpanini S, Posmyk R, et al. 2013. Loss-of-function mutations in TBC1D20 cause cataracts and male infertility in blind sterile mice and Warburg micro syndrome in humans. *Am J Hum Genet* **93:** 1001–1014

Ramirez F, Feliciano AM, Adkins EB, Child KM, Radden LA II, Salas A, Vila-Santana N, Horak JM, Hughes SR, Spacek DV, et al. 2013. The juvenile alopecia mutation (jal) maps to mouse Chromosome 2, and is an allele of GATA binding protein 3 (Gata3). *BMC Genet* **14:** 40.

Yang B, Tian C, Zhang ZG, Han FC, Azem R, Yu H, Zheng Y, Jin G, Arnold JE, Zheng QY. 2011. Sh3pxd2b mice are a model for craniofacial dysmorphology and otitis media. *PLoS One* **6:** e22622.

Zhu X, Libby RT, de Vries WN, Smith RS, Wright DL, Bronson RT, Seburn KL, John SW. 2012. Mutations in a P-type ATPase gene cause axonal degeneration. *PLoS Genet* **8:** e1002853.