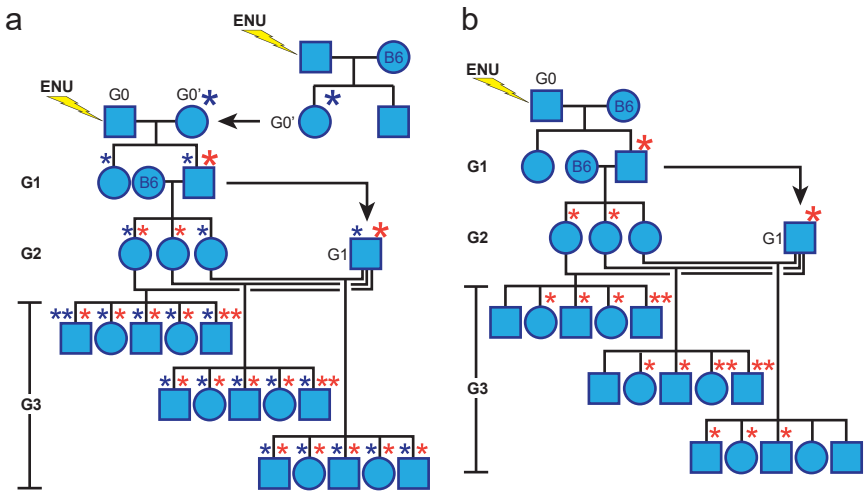
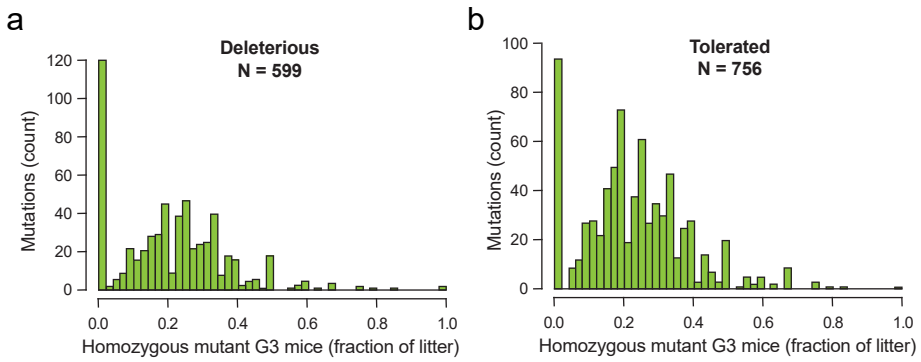
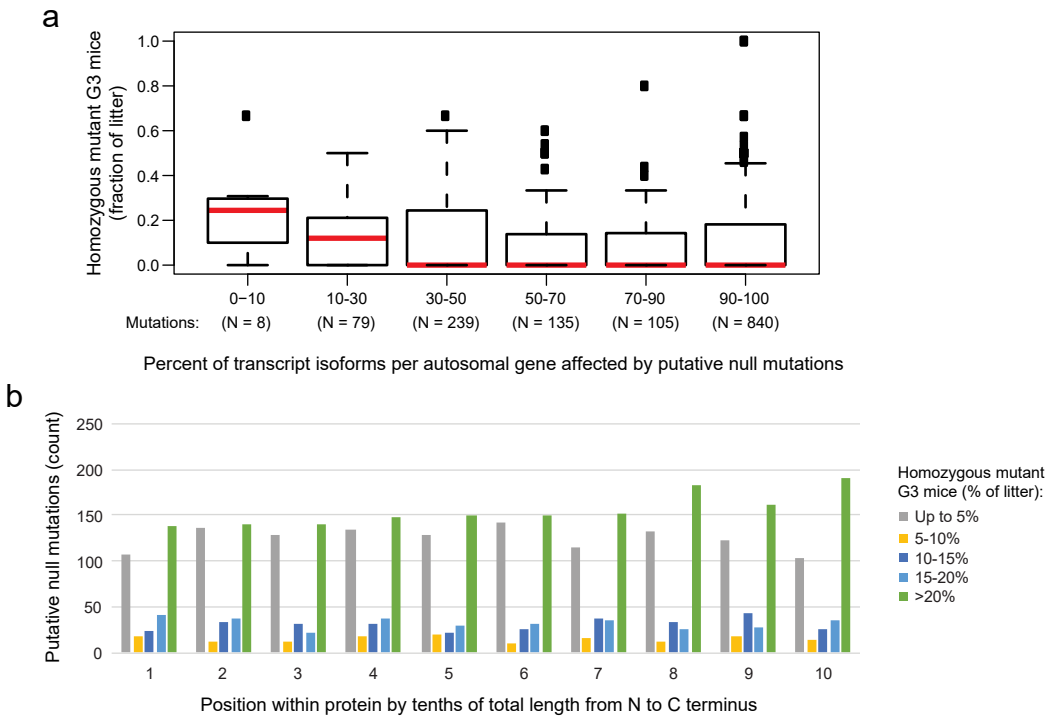


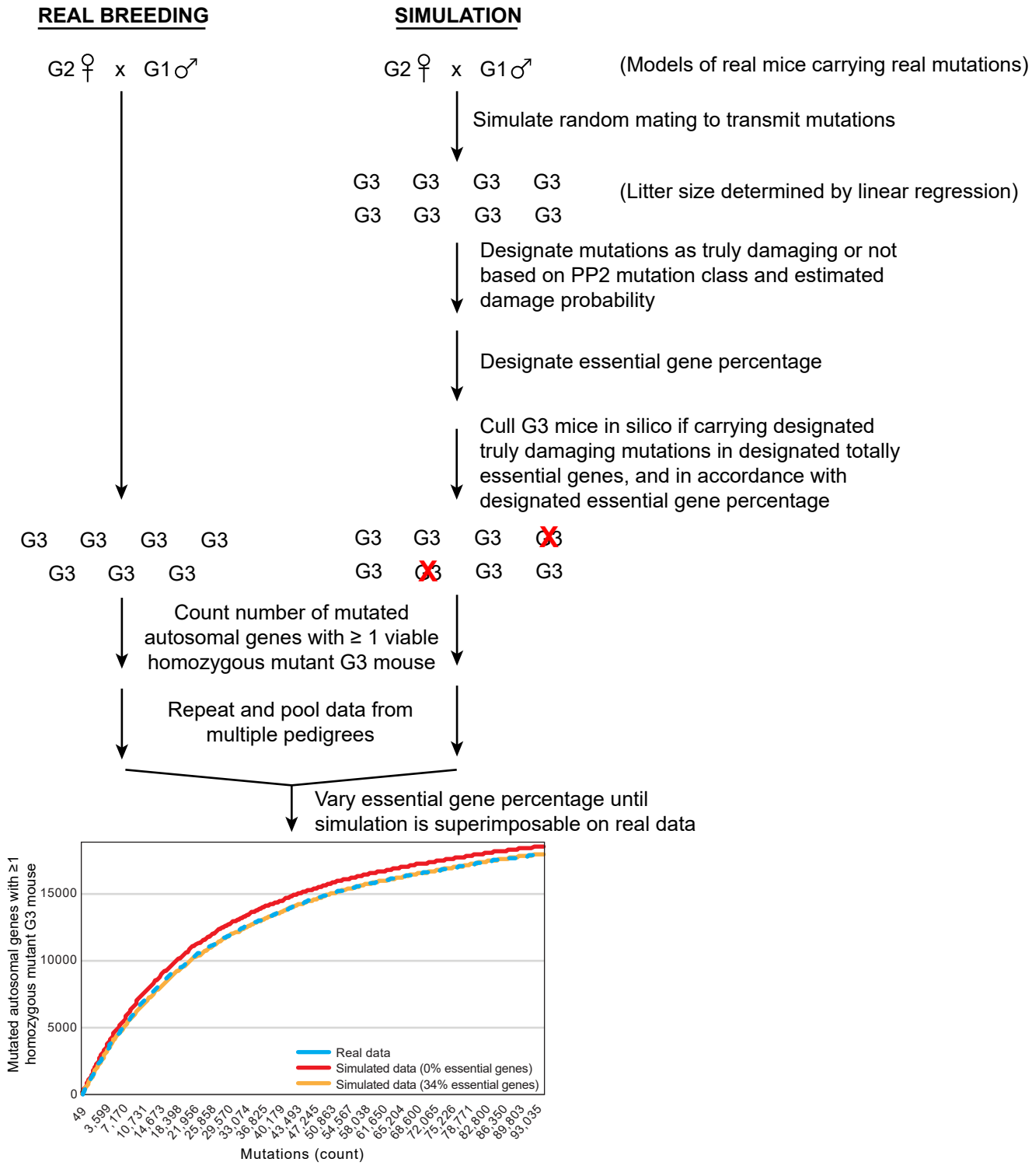

**Supplementary Figure 1. Breeding scheme to produce G3 mice carrying heterozygous and homozygous ENU induced mutations.** Mutagenized G0 males were bred to (a) G0' females carrying mutations derived from other mutagenized males, or (b) C57BL/6J (B6) females. The resulting G1 males were crossed to B6 females to produce G2 mice. G2 females were backcrossed to their G1 sires to yield G3 mice. Every G1 male founder was subjected to whole exome sequencing and its G2 and G3 descendants were genotyped at the identified mutation sites. Asterisks indicate mutations originating from the G0 male (red) or G0' female (blue), with larger asterisks representing initial germline transmission.



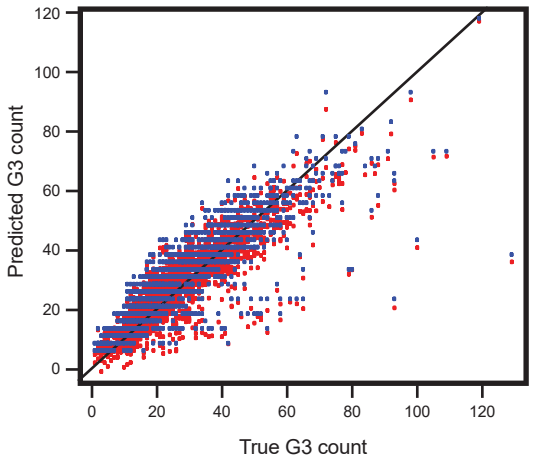

**Supplementary Figure 2. Distribution of homozygous mutant mouse frequencies among G3 mice produced by heterozygous G2 matings for SIFT-classified mutations.** (a-b) For mutations classified (a) deleterious or (b) tolerated by SIFT, the proportions of homozygous mutant G3 mice resulting from heterozygous G2 matings were plotted.



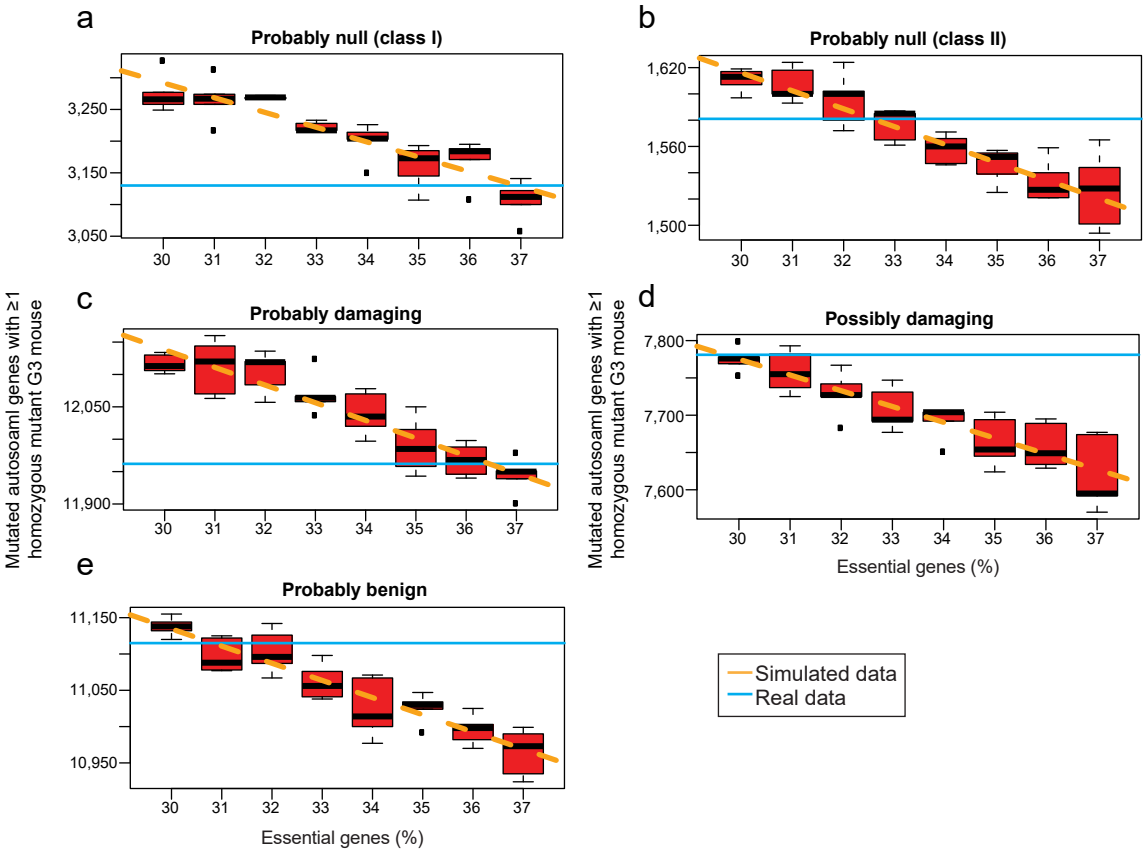

**Supplementary Figure 3. Effect of position of putative null mutations within the linear amino acid sequence of a protein or fraction of transcript isoforms affected on the propensity to cause functional damage.** (a-b) Essential genes were analyzed. (a) The proportions of homozygous mutant G3 mice resulting from heterozygous G2 matings plotted versus the percentage of transcript isoforms of a gene affected by putative null mutations. Boxplots generated as in Fig. 3. (b) The number of mutations positioned within each protein segment (by tenths of total amino acid length) and that were present in homozygous state in ≤5%, 5-10%, 10-15%, 15-20%, or >20% of G3 mice produced by heterozygous G2 matings. The position within the protein was obtained by dividing the number of amino acids N-terminal to the mutation by the full length of the non-mutated protein.



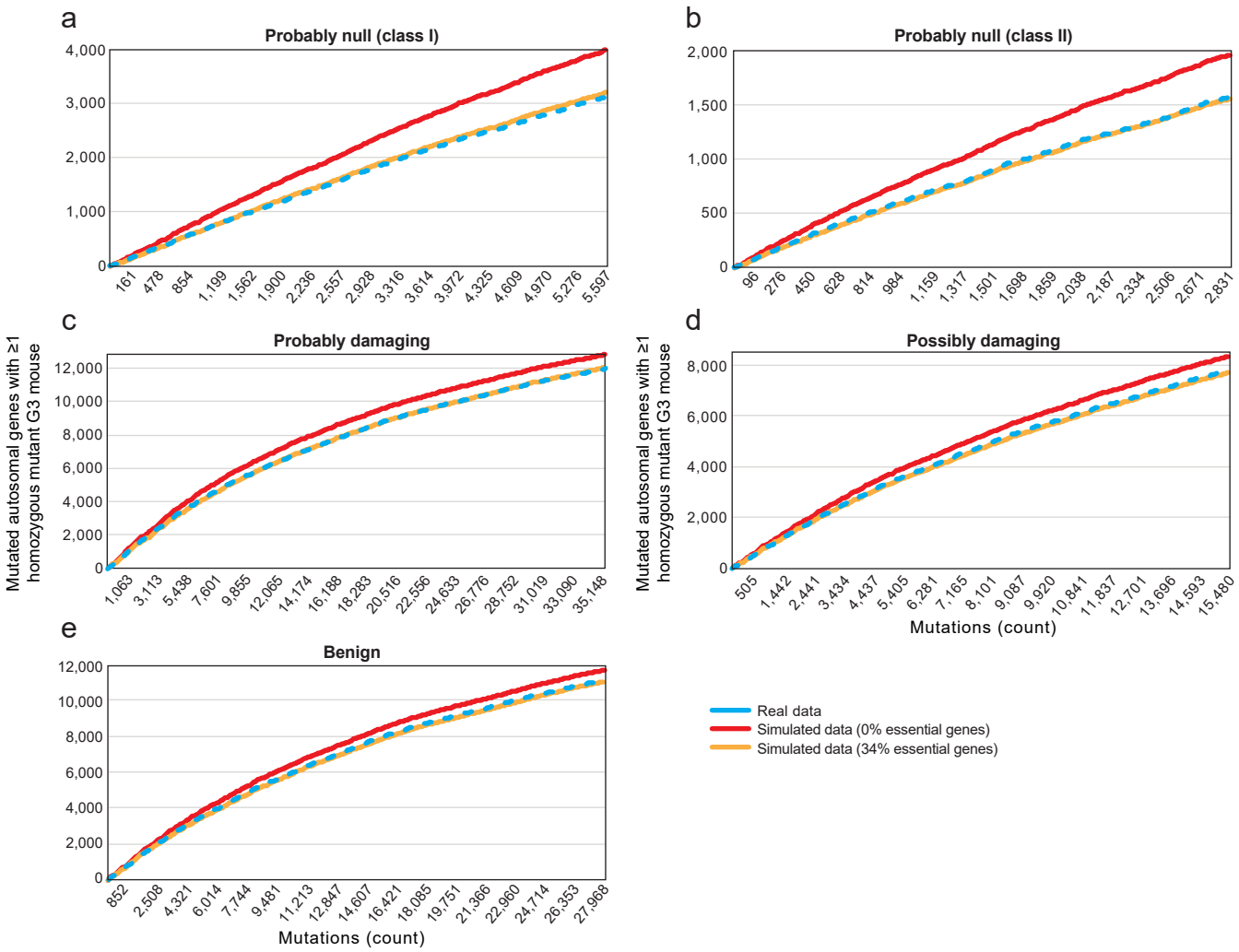

**Supplementary Figure 4. Schematic of simulation to determine the percentage of essential genes in the mouse genome.**



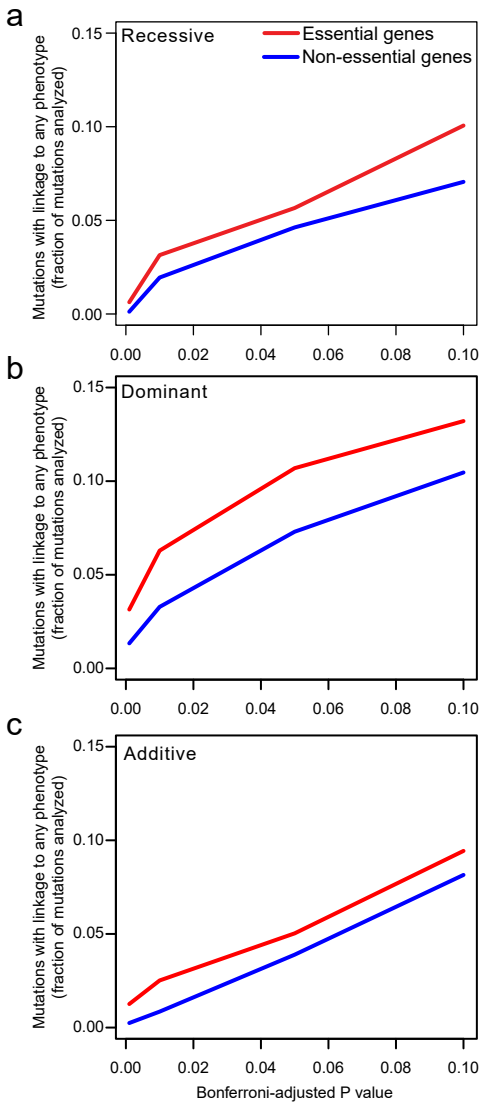

**Supplementary Figure 5. Predicted pedigree sizes assuming no genes are essential.** Pedigree size refers to the number of G3 mice in a litter. Linear regression model of true pedigree size (red) regressed by pedigree type, number of litters produced, and number of each type of mutations. A total of 2,005 pedigrees were used in the prediction. All mutations counts were then assumed to be zero, which is equal to assuming no mouse genes are essential, and the regression model predicted the hypothetical pedigree sizes (blue).



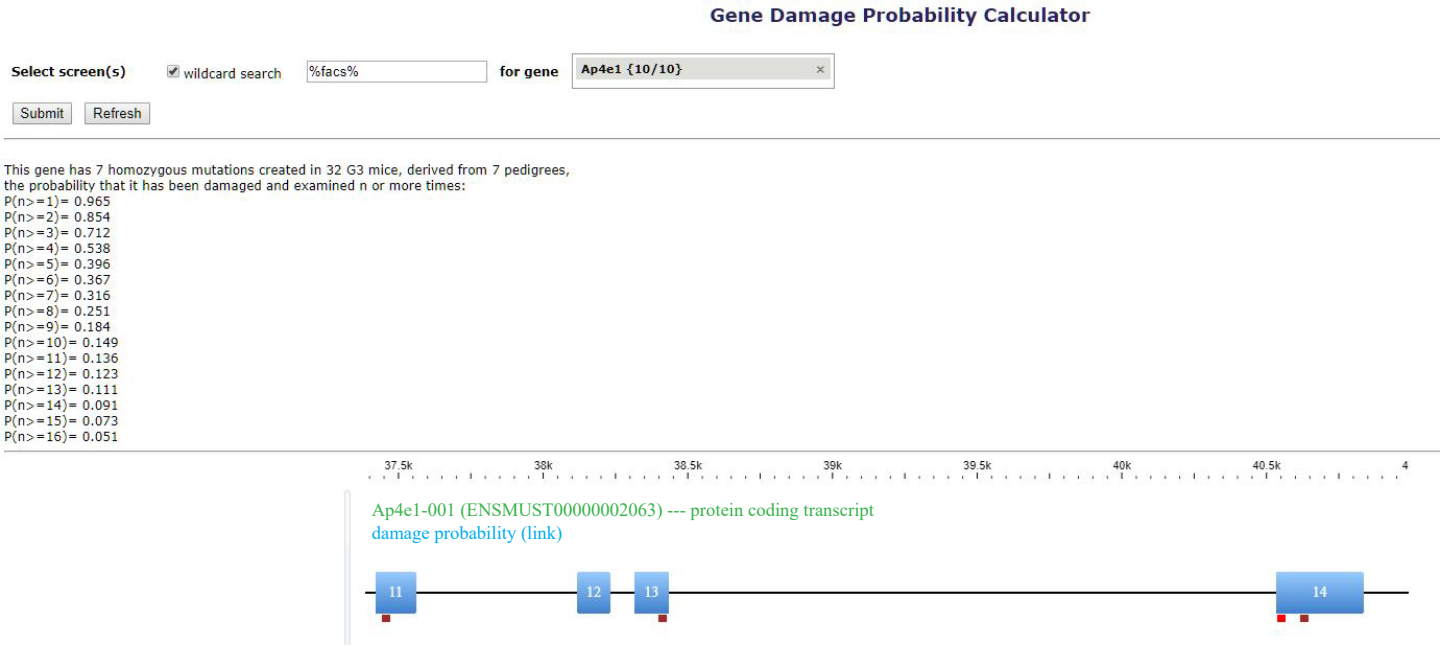

**Supplementary Figure 6. Determination of the percentage of essential genes by comparison of real and simulated lethality data.** Simulated data are plotted showing the number of genes carrying (a) probably null class I, (b) probably null class II, (c) probably damaging, (d) possibly damaging, or (e) probably benign mutations for which at least one homozygous mutant G3 mouse existed, for varying percentages of essential genes. For each essential gene percentage, sampling was performed five times and linear regression was used to fit all the sampled data as a function of essential gene percentage. Boxplots generated as in Fig. 3. Blue line indicates the true number of genes carrying mutations of the indicated classification for which at least one HOM mouse existed. N = 1,105,575 mutations analyzed.




**Supplementary Figure 7. Determination of the percentage of essential genes by comparison of real and simulated lethality data.** Cumulative plot of the number of genes carrying (a) probably null class I, (b) probably null class II, (c) probably damaging, (d) possibly damaging, or (e) probably benign mutations for which at least one homozygous mutant G3 mouse existed versus number of mutations using real data (blue) and simulated data, assuming essential gene percentages of 0% (red) or 34% (yellow). Each curve is the average of five simulations.




**Supplementary Figure 8. Greater frequency of viable phenotypes from mutations in essential genes than from mutations in non-essential genes.** The fraction of mutations in essential (red) or non-essential genes (blue) that showed linkage to any phenotype at the indicated Bonferroni-adjusted P values, using (a) recessive, (b) dominant, or (c) additive transmission models. N = 882 non-essential genes, 159 essential genes. Mutations were non-synonymous coding and potential splicing changes of all genes.




**Supplementary Figure 9. Damage probability calculation tool for individual genes.** The tool calculates the probability that a gene has been tested for function in at least a specified number of homozygous G3 mice in a particular phenotype screen (among those under surveillance in the lab). The web-based tool is available at https://mutagenetix.utsouthwestern.edu/report/gene_damage/damage_prob.cfm. After submitting the gene and screen(s) in the query form, the program displays two types of results: 1) the number of mutations and mice carrying them used for the probability calculation, along with the list of calculated damage probabilities for specified minimum numbers of G3 mice. 2) Diagrams of all known transcripts: these models can be magnified using the slider on the left. Exons (light blue); 5' and 3' UTRs (dark blue). Mutations are shown as color-coded squares below transcript (green, probably benign; orange, possibly damaging; maroon, probably damaging; red, probably null). Mousing over each mutation mark will display its detailed information. Clicking "damage probability" link will open a new window to summarize the damage probabilities for the designated transcript.

**Supplementary Table 1. Lethal annotations reported by MGI.**

| MGI LETHALITY MP TERM | MGI MP ID | ANNOTATIONS |
|---|---|---|
| Embryonic lethality | MP:0008762 | 408 |
| Embryonic lethality at implantation | MP:0008527 | 29 |
| Embryonic lethality before implantation | MP:0006204 | 153 |
| Embryonic lethality between implantation and placentation | MP:0009850 | 53 |
| Embryonic lethality between implantation and somite formation | MP:0006205 | 269 |
| Embryonic lethality between somite formation and embryo turning | MP:0006206 | 88 |
| Embryonic lethality during organogenesis | MP:0006207 | 748 |
| Embryonic lethality prior to organogenesis | MP:0013292 | 125 |
| Embryonic lethality prior to tooth bud stage | MP:0013293 | 161 |
| Lethality* | --- | 64 |
| Lethality at weaning | MP:0008569 | 89 |
| Lethality during fetal growth through weaning | MP:0010832 | 19 |
| Lethality throughout fetal growth and development | MP:0006208 | 378 |
| Neonatal lethality | MP:0002058 | 761 |
| Perinatal lethality | MP:0002081 | 483 |
| Postnatal lethality | MP:0002082 | 1078 |
| Prenatal lethality | MP:0002080 | 601 |
| Prenatal lethality prior to heart atrial septation | MP:0013294 | 51 |
| Preweaning lethality | MP:0010770 | 1160 |
| **Total** | | **6718** |

A single gene may have more than one lethality annotation.

Abbreviations: MGI: Mouse Genome Informatics; MP ID: mammalian phenotype identification number.

*Category does not have an associated mammalian phenotype ID on MGI 6.06 (accessed October 2016).

**Supplementary Table 2. Estimated damage probabilities for mutations in six PP2 score ranges.**

| PP2 score range | Mutations | Damage probability | 95% CI |
|---|---|---|---|
| 0-0.01 | 205 | 0.039 | 0-0.146 |
| 0.01-0.4 | 258 | 0.058 | 0-0.142 |
| 0.4-0.9 | 231 | 0.103 | 0.012-0.197 |
| 0.9-0.99 | 181 | 0.111 | 0.006-0.216 |
| 0.99-0.999 | 189 | 0.119 | 0.012-0.227 |
| 0.999-1 | 389 | 0.199 | 0.126-0.271 |

**Supplementary Table 3. PP2 and SIFT classifications of missense mutations.**

| | | PP2 classifications | | |
|---|---|---|---|---|
| | | Benign | Possibly damaging | Probably damaging |
| **SIFT classifications** | Deleterious | 3513 (11.6%)* | 5350 (17.6%)* | 21474 (70.8%)* |
| | Deleterious (Low confidence) | 681 (18.7%)* | 892 (24.4%)* | 2074 (56.9%)* |
| | Tolerated | 20925 (57.4%)* | 7639 (21.0%)* | 7856 (21.6%)* |

*Within each SIFT classification, percentages that were classified by PP2 as benign, possibly damaging, or probably damaging.

**Supplementary Table 4. Estimated damage probabilities for mutations classified by several mutation effect prediction algorithms**

| Classification | Mutations* | Damage probability | 95% CI |
|---|---|---|---|
| **PP2 (HumVar)** | | | |
| Benign | 481 | 0.05 | 0-0.115 |
| Possibly damaging | 187 | 0.165 | 0.062-0.267 |
| Probably damaging | 343 | 0.226 | 0.146-0.305 |
| **PP2 (HumDiv)** | | | |
| Benign | 367 | 0.047 | 0-0.123 |
| Possibly damging | 200 | 0.133 | 0.033-0.229 |
| Probably damaging | 444 | 0.199 | 0.129-0.268 |
| **LRT** | | | |
| Unknown | 51 | 0.076 | 0-0.272 |
| Neutral | 323 | 0.06 | 0-0.138 |
| Deleterious | 614 | 0.199 | 0.14-0.257 |
| **MutationAssessor** | | | |
| Neutral | 165 | 0.024 | 0-0.142 |
| Low | 342 | 0.068 | 0-0.148 |
| Medium | 415 | 0.185 | 0.115-0.251 |
| High | 73 | 0.423 | 0.209-0.607 |
| **FATHMM** | | | |
| Tolerated | 722 | 0.106 | 0.05-0.158 |
| Damaging | 236 | 0.2 | 0.104-0.292 |
| **PROVEAN** | | | |
| Neutral | 501 | 0.057 | 0-0.121 |
| Damaging | 488 | 0.215 | 0.15-0.278 |
| **MetaSVM** | | | |
| Tolerated | 753 | 0.069 | 0.015-0.122 |
| Damaging | 258 | 0.315 | 0.228-0.4 |
| **MetaLR** | | | |
| Tolerated | 738 | 0.08 | 0.025-0.134 |
| Damaging | 273 | 0.271 | 0.187-0.353 |
| **M-CAP** | | | |
| Tolerated | 372 | 0.065 | 0-0.138 |
| Damaging | 632 | 0.169 | 0.111-0.227 |
| **fathmm-MKL_coding** | | | |
| Neutral | 149 | 0.073 | 0-0.176 |
| Damaging | 900 | 0.157 | 0.107-0.206 |

*All mutations represent mouse mutations transferred to the human gene sequences. Only mutations that conferred the identical nucleotide and amino acid change in the mouse and human genes were analyzed.

**Supplementary Table 5. Designations of genes as essential or non-essential by MGI and IMPC.**

| | | MGI designations | | |
|---|---|---|---|---|
| | | Lethal | Nonlethal | Concordance (%)* |
| **IMPC designations** | Lethal | 635 | 15 | 97.7% |
| | Subviable | 250 | 8 | 96.9% |
| | Viable | 98 | 1685 | 94.5% |

*Percentage of genes classified similarly by MGI and IMPC.