Impact of Essential Genes on the Success of Genome Editing Experiments Generating 3,313 New Genetically Engineered Mouse Lines

Elrick et al.

Supplementary Figure 1. Schematic of null allele mouse line production. a. Allele Design. A critical region (CR) was identified that contains one or more exons present in all annotated full-
length protein-coding transcripts and when deleted would introduce a frameshift and premature stop codon in the first half of the open reading frame but at least 33 amino acids downstream of the translation start codon. In most cases, these transcripts are predicted to be targeted for nonsense-mediated decay. Intron sequences flanking the CR were examined for specific Cas9 protospacer sequences. Cas9 guide RNA specificity was gauged by the absence of predicted off-target sites with fewer than three mismatches and/or a specificity score >65. At some centres, specificity parameters included that off-target sites must have at least one mismatch in the seed region (11-bp immediately 5’ to the protospacer adjacent motif) of the guide RNA.

b. Mouse Production. Cas9 reagents were introduced into groups of zygotes by electroporation (EP) or pronuclear (PN) or cytoplasmic (CY) microinjection. Treated zygotes were transferred to pseudopregnant recipient for gestation and birth. Born pups were genotyped for the presence of the deletion allele (red asterisk), either by real-time PCR or droplet digital PCR to quantify the copies of the deleted region or by end-point PCR to detect an amplicon consistent with the deletion of the critical region. Some putative founders had undesired changes after Cas9 treatment (blue asterisk) and were not used for breeding. Founders were bred to wild-type C57BL/6N mice and N1 pups were screened for the presence of the desired deletion. Sanger sequencing of a PCR amplicon spanning the deletion confirmed the sequence of the deletion junction and at least 100-bp of DNA flanking either side of the deletion. N1 pups with the same allele sequence were used to establish the putative null mutant mouse line.
Supplementary Figure 2. Violin plot for birth rates from experiments generating null alleles in (a) non-essential and essential genes as well as (b) non-lethal and lethal genes. Reported are the p-values (p) of pairwise comparisons using the Wilcoxon rank sum test, the number of genes (N), and corresponding medians in boxplots.
Supplementary Figure 3. Line graph showing cumulative GLT rates for at each subsequent production attempt as a percentage of total number of all unique genes attempted at least once.
**Supplementary Table 1.** Experiments included in meta-analysis by production centre

|                      | BCM | CCP | HAR | ICS | JAX | TCP | UCD | WTSI | Total |
|----------------------|-----|-----|-----|-----|-----|-----|-----|------|-------|
| Number of experiments| 679 | 339 | 413 | 60  | 1430| 386 | 701 | 468  | 4473  |
| Number of genes      | 585 | 278 | 305 | 60  | 1340| 341 | 683 | 383  | 3973  |
| %Repeated experiments | 25.8| 32.7| 41.0| 0.0 | 12.4| 22.5| 5.1 | 31.2 | 20.1  |
| %Repeated genes      | 13.8| 18.0| 20.7| 0.0 | 6.6 | 12.3| 2.6 | 15.9 | 10.1  |

BCM, Baylor College of Medicine; CCP, Czech Centre for Phenogenomics; HAR, MRC Harwell; ICS, Institut Clinique de la Souris; JAX, The Jackson Laboratory; TCP, The Centre for Phenogenomics; UCD, University of California, Davis, Mouse Biology Program; WTSI, Wellcome Trust Sanger Institute.
**Supplementary Table 2.** Unique gene null allele production attempts.

Filename: Elrick-Nutter_ST2_UniqueGeneCas9Attempts.xlsx

Legend for Supplementary Table 2.

| Column Name | Column Description |
|-------------|--------------------|
| MI Attempt ID | database unique identifier |
| Gene Marker Symbol | mouse gene symbol |
| Gene MGI Accession ID | Mouse Genome Informatics accession identifier |
| Production Centre | mouse production centre at which attempt was made |
| Status Name | latest production status for knockout allele generation attempt |
| Zygote | strain in which allele was made |
| gRNA Concentrations (ng/µL) | ng/µL of guide RNA in Cas9 mix |
| gRNA Concentrations Individually Set? | toggle TRUE if gRNA concentrations are different for each guide sequence of first gRNA on the positive strand of chromosome |
| gRNA Sequence (+ strand) | chromosome on which first gRNA is located |
| Chromosome (+ strand) | start coordinate of first gRNA entered into database |
| Start co-ordinate | end coordinate of first gRNA entered into database |
| End co-ordinate | sequence of second gRNA on the positive strand of chromosome |
| gRNA Sequence (+ strand).1 | chromosome on which second gRNA is located |
| Chromosome (+ strand).1 | start coordinate of second gRNA entered into database |
| Start co-ordinate 1 | end coordinate of second gRNA entered into database |
| End co-ordinate 1 | sequence of third gRNA on the positive strand of chromosome |
| gRNA Sequence (+ strand).2 | chromosome on which third gRNA is located |
| Chromosome (+ strand).2 | start coordinate of third gRNA entered into database |
| Start co-ordinate 2 | end coordinate of third gRNA entered into database |
| End co-ordinate 2 | sequence of fourth gRNA on the positive strand of chromosome |
| gRNA Sequence (+ strand).3 | chromosome on which fourth gRNA is located |
| Chromosome (+ strand).3 | start coordinate of fourth gRNA entered into database |
| Start co-ordinate 3 | end coordinate of fourth gRNA entered into database |
| End co-ordinate 3 | concentration of Cas9 mRNA in Cas9 mix |
| mRNA Concentration (ng/µL) | concentration of Cas9 protein in Cas9 mix |
| Protein Concentration (ng/µL) | method used to deliver Cas9 reagents to embryos |
| Delivery Method | date of microinjection or electroporation of embryos |
| Mi Date | number of embryos treated (microinjected or electroporated) |
| #Embryos Injected | number of embryos that survived treatment |
| #Embryos Survived | number of treated embryos transferred into pseudopregnant recipients |
| #Embryos Transferred | number of pups born from treated and transferred embryos |
| #Founder Pups Born | number of pups assayed for the desired allele |
| #Founders Assayed | number of founders selected for breeding |
| #Founders Selected For Breeding | number of deletion founders identified among pups assayed |
| #G0 deletion event detected | type of allele planned to be generated |
Allele Subtype: subtype of allele generated
Mutant Fasta Sequence: sequence of knockout allele
Reason GLT Failed: inferred reason germline transmission of allele failed
Cas9 Type: Type of Cas9, mRNA or protein
Sorted Cut-Sites: coordinates of cut sites for gRNAs in order of chromosome location
Num Guides: number of guide RNAs used in experiment
Max Cut-size: maximum size of predicted deletion based on gRNA cut sites
Viability Consensus: IMPC viability phenotype
Cellular Essential: gene score as essential or non-essential from Cacheiro, et al, 2020.
Ratio Embryos Survived to Transfer: % of embryos that survived treatment and were transferred
Ratio of #G0 with Mutation Selected for Breeding: % of founders with desired mutation that were bred for germline transmission
Birth Rate: % of pups born from embryos transferred
Founder Rate (per Embryos Transferred): % of founders born from embryos transferred
GLT: germline transmission of desired allele
RepeatedGene: indicated whether a second (or more) attempt to generate founders was performed for a gene
Length: gene length in bp
GCcontent: %GC across a gene
CpGsites: number of CpG islands within a gene
PercentageCpG: % of gene represented by CpG islands
Human Ortholog: gene symbol of human ortholog
Human ENSID: Ensembl ID of human ortholog
Ortholog Relationship (human-to-mouse, mouse-to-human): type of ortholog relationship between mouse gene and human gene
pLI of Orthologs: probability of being loss of function intolerant for human ortholog
oe of Orthologs: Observed/expected is a continuous measure of how tolerant a gene is to a certain class of variation, in this case loss-of-function, for human ortholog
ExperimentRepeated: true (t) if experiment is the second or more attempt for a gene
SuccessfulAttemptExists: true (t) if a knockout allele was successfully made in any attempt

Supplementary Table 3. Repeat attempt sets for production of null alleles.
Filename: Elrick-Nutter_ST3_RepeatedGeneCas9Attempts.xlsx
Legend for Supplementary Table 3.

| Column Name              | Column Description                                                      |
|--------------------------|--------------------------------------------------------------------------|
| Mi Attempt ID            | database unique identifier                                              |
| Gene Marker Symbol       | mouse gene symbol                                                        |
| Gene MGI Accession ID    | Mouse Genome Informatics accession indentifier                          |
| Production Centre        | mouse production centre at which attempt was made                        |
Status Name
Zygote

**gRNA Concentrations (ng/µL)**
gRNA Concentrations Individually Set?

**gRNA Sequence (+ strand)**
Chromosome (+ strand)
Start Co-od
End Co-od
gRNA Concentrations Individually Set?

**gRNA Sequence (+ strand).1**
Chromosome (+ strand).1
Start Co-od.1
End Co-od.1
gRNA Sequence (+ strand).2
Chromosome (+ strand).2
Start Co-od.2
End Co-od.2
gRNA Sequence (+ strand).3
Chromosome (+ strand).3
Start Co-od.3
End Co-od.3
mRNA Concentration (ng/µL)
Protein Concentration (ng/µL)

**Delivery Method**
Mi Date

#Embryos Injected
#Embryos Survived
#Embryos Transfered

#Founder Pups Born
#Founders Assayed
#Founders Selected For Breeding
#G0 deletion event detected

**Allele Type**
Allele Subtype
Mutant Fasta Sequence
Reason GLT Failed
Cas9 Type
Sorted Cut-Sites

Num Guides
Max Cut-size
Viability Consensus

Latest production status for knockout allele generation attempt
strain in which allele was made
ng/µL of guide RNA in Cas9 mix
toggle TRUE if gRNA concentrations are different for eachguide sequence of first gRNA on the positive strand of chromosome chromosome on which first gRNA is located
start coordinate of first gRNA entered into database
end coordinate of first gRNA entered into database
sequence of second gRNA on the positive strand of chromosome chromosome on which second gRNA is located
start coordinate of second gRNA entered into database
end coordinate of second gRNA entered into database
sequence of third gRNA on the positive strand of chromosome chromosome on which third gRNA is located
start coordinate of third gRNA entered into database
end coordinate of third gRNA entered into database
sequence of fourth gRNA on the positive strand of chromosome chromosome on which fourth gRNA is located
start coordinate of fourth gRNA entered into database
end coordinate of fourth gRNA entered into database
concentration of Cas9 mRNA in Cas9 mix
concentration of Cas9 protein in Cas9 mix
method used to deliver Cas9 reagents to embryos
date of microinjection or electroporation of embryos
number of embryos treated (microinjected or electroporated)
number of embryos that survived treatment
number of treated embryos transferred into pseudopregnant recipients
number of pups born from treated and transferred embryos
number of pups assayed for the desired allele
number of founders selected for breeding
number of deletion founders identified among pups assayed
type of allele planned to be generated
subtype of allele generated
sequence of knockout allele
inferred reason germline transmission of allele failed
Type of Cas9, mRNA or protein
coordinates of cut sites for gRNAs in order of chromosome location
number of guide RNAs used in experiment
maximum size of predicted deletion based on gRNA cut sites
IMPC viability phenotype
| **Cellular Essential** | gene score as essential or non-essential from Cacheiro, et al, 2020. |
|------------------------|---------------------------------------------------------------------|
| **Ratio Embryos Survived to Transfer** | % of embryos that survived treatment and were transferred |
| **Ratio of #G0 with Mutation Selected for Breeding** | % of founders with desired deletion that were bred |
| **Birth Rate** | % of pups born from embryos transferred |
| **Founder Rate (per Embryos Transferred)** | % of founders born from embryos transferred |
| **GLT** | germline transmission of desired allele |
| **Length** | gene length in bp |
| **GC content** | %GC across a gene |
| **CpG sites** | number of CpG islands within a gene |
| **Percentage CpG** | % of gene represented by CpG islands |
| **Human Ortholog** | gene symbol of human ortholog |
| **Human ENSID** | Ensembl ID of human ortholog |
| **Ortholog Relationship (human-to-mouse, mouse-to-human)** | type of ortholog relationship between mouse gene and human gene |
| **pLI of Orthologs** | probability of being loss of function intolerant for human ortholog |
| **oe of Orthologs** | Observed/expected is a continuous measure of how tolerant a gene is to a certain class of variation, in this case loss-of-function, for human ortholog |
| **GeneSymbol_ProductionCentre** | gene symbol used by production centre |
| **Unique Set** | indicated whether first (unique) or repeated (repeat) attempt |
| **Δ Delivery Method** | Change in delivery method from the previous attempt; CY–>EP; EP–>CY; CY–>PN; EP–>PN; PN–>EP; or PN–>CY |
| **Δ mRNA Concentration** | Change in mRNA concentration from the previous attempt; marked as change when Cas9 type changed and mRNA concentration went to zero which is often accompanied by change from injection to electroporation |
| **Δ Protein Concentration** | Change in protein concentration from the previous attempt; marked as change when Cas9 type changed and protein concentration went to zero which is often accompanied by change from electroporation to injection |
| **Δ Cas9 Type** | Change between mRNA and protein from the previous attempt |
| **Δ Sorted Cut-Sites** | Change in location of Cas9 target cut sites from the previous attempt; occurred when number of guides changed or guide sequence changed |
| **Δ Num Guides** | Change in number of guides used from the previous attempt; could be an increase or decrease in number of guides |
| **Δ Guide Seq** | The sequence of at least one guide is different from the previous attempt; when a subset of guides are used, but all appear in the previous attempt set, Guide Seq did not change. |
| **Δ Exon** | Change in the exon targeted |
| **Any change** | indicates whether any parameter was changed between repeated attempts |
**Supplementary Table 4.** Gene annotation for various biological parameters  
Filename: Elrick-Nutter_ST4_Cas9_GeneAnnotations.xlsx  
Legend for Supplementary Table 4.

| Column Name | Column Description |
|-------------|--------------------|
| Gene Marker Symbol | mouse gene symbol |
| Gene MGI Accession ID | Mouse Genome Informatics accession identifier |
| ENSID | mouse gene Ensembl ID |
| Entrez_ID | mouse gene entrez ID |
| Viability Consensus | IMPC viability phenotype |
| Cellular Essential | gene score as essential or non-essential from Cacheiro, et al, 2020. |
| GLT | true (t) if germline transmission was obtained |
| Length | gene length in bp |
| GCcontent | %GC across a gene |
| CpGsites | number of CpG islands within a gene |
| PercentageCpG | % of gene represented by CpG islands |
| Human Ortholog | gene symbol of human ortholog |
| Human ENSID | Ensembl ID of human ortholog |
| Ortholog Relationship (human-to-mouse_mouse-to-human) | type of ortholog relationship between mouse gene and human gene |
| pLI of Orthologs | probability of being loss of function intolerant for human ortholog |
| oe of Orthologs | Observed/expected is a continuous measure of how tolerant a gene is to a certain class of variation, in this case loss-of-function, for human ortholog |
| Has Omim Annotation | true (t) if gene annotated in OMIM |
| Relative Chromosomal Position | gene location over total chromosome length |
| Staining Overlap | indicates whether gene overlaps with Giesma positive chromatin |
| AverageH3K27me3_Intron_PeakScore | average H3K27 methylation in introns of gene |
| AverageH3K27ac_PeakScore | average H3K27 acetylation along gene |
| all_stages | gene transcripts per million for all embryonic stages |
| all_stages_PercentileRank | percentile rank for gene transcripts per million for all embryonic stages |
| Placenta e8.5 | gene transcripts per million for E8.5 (embryonic day 8.5) placenta |
| Placenta e8.5_PercentileRank | percentile rank for gene transcripts per million for E8.5 placenta |
| Placenta e9.0 | gene transcripts per million for E9.0 placenta |
| Placenta e9.0_PercentileRank | percentile rank for gene transcripts per million for E9.0 placenta |
| Placenta e10.5 | gene transcripts per million for E10.5 placenta |
| Placenta e10.5_PercentileRank | percentile rank for gene transcripts per million for E10.5 placenta |
| Placenta e12.0 | gene transcripts per million for E12.0 placenta |
| Sample Type                  | Description                                                                 |
|-----------------------------|-----------------------------------------------------------------------------|
| Placenta e12.0_PercentileRank | percentile rank for gene transcripts per million for E12.0 placenta         |
| Placenta e13.5              | gene transcripts per million for E13.5 placenta                             |
| Placenta e13.5_PercentileRank | percentile rank for gene transcripts per million for E13.5 placenta         |
| Placenta e15.0              | gene transcripts per million for E15.0 placenta                             |
| Placenta e15.0_PercentileRank | percentile rank for gene transcripts per million for E15.0 placenta         |
| Placenta e17.0              | gene transcripts per million for E17.0 placenta                             |
| Placenta e17.0_PercentileRank | percentile rank for gene transcripts per million for E17.0 placenta         |
| Placenta e19.0              | gene transcripts per million for E19.0 placenta                             |
| Placenta e19.0_PercentileRank | percentile rank for gene transcripts per million for E19.0 placenta         |
| Placenta P0                 | gene transcripts per million for P0 (post natal day 0) placenta             |
| Placenta P0_PercentileRank  | percentile rank for gene transcripts per million for P0 (post natal day 0) placenta |
| Decidua e8.5                | gene transcripts per million for E8.5 (embryonic day 8.5) decidua (embryos) |
| Decidua e8.5_PercentileRank  | percentile rank for gene transcripts per million for E8.5 decidua (embryos) |
| Decidua e9.0                | gene transcripts per million for E9.0 decidua (embryos)                     |
| Decidua e9.0_PercentileRank  | percentile rank for gene transcripts per million for E9.0 decidua (embryos) |
| Decidua e10.5               | gene transcripts per million for E10.5 decidua (embryos)                    |
| Decidua e10.5_PercentileRank | percentile rank for gene transcripts per million for E10.5 decidua (embryos) |
| Decidua e12.0               | gene transcripts per million for E12.0 decidua (embryos)                    |
| Decidua e12.0_PercentileRank | percentile rank for gene transcripts per million for E12.0 decidua (embryos) |
| Decidua e15.0               | gene transcripts per million for E15.0 decidua (embryos)                    |
| Decidua e15.0_PercentileRank | percentile rank for gene transcripts per million for E15.0 decidua (embryos) |
| Decidua e17.0               | gene transcripts per million for E17.0 decidua (embryos)                    |
| Decidua e17.0_PercentileRank | percentile rank for gene transcripts per million for E17.0 decidua (embryos) |
| Decidua e19.0               | gene transcripts per million for E19.0 decidua (embryos)                    |
| Decidua e19.0_PercentileRank | percentile rank for gene transcripts per million for E19.0 decidua (embryos) |
| Decidua P0                  | gene transcripts per million for P0 (post natal day 0) decidua (embryos)    |
| Decidua P0_PercentileRank   | percentile rank for gene transcripts per million for P0 (post natal day 0) decidua (embryos) |
| Placenta and Decidua e17.0  | gene transcripts per million for E17.0 placenta and decidua (embryos)      |
Placenta and Decidua e17.0_PercentileRank percentile rank for gene transcripts per million for E17.0 placenta and decidua (embryos)
### Supplementary Table 5. Logistic regression output

Observations: 3209  
Dependent Variable: Founders  
Type: Logistic regression

#### GLM without Essentiality

|                     | Odds Ratio | Standard Error | z-value | p-value   | adjusted p-value |
|---------------------|------------|----------------|---------|-----------|-----------------|
| (Intercept)         | 11.975     | 0.269          | 9.219   | 2.0x10^{-16} | 2.7x10^{-19}    |
| Embryonic Expression| 0.5675     | 0.221          | -2.570  | 0.010     | 0.081           |
| pLI                 | 0.7375     | 0.199          | -1.529  | 0.126     | 0.866           |
| oe                  | 1.000      | 0.262          | -0.010  | 0.992     | 1.00            |
| Chromosomal Location| 1.100      | 0.204          | 0.467   | 0.640     | 1.00            |
| Acetylated chromatin| 0.8495     | 0.128          | -1.203  | 0.229     | 1.00            |
| Methylated chromatin| 1.3705     | 0.203          | 1.539   | 0.124     | 0.866           |
| Giemsa Positive Stain| 1.0105 | 0.117          | 0.092   | 0.926     | 1.00            |
| OMIM Annotation     | 0.8705     | 0.163          | -0.847  | 0.397     | 1.00            |

#### GLM with Essentiality

|                     | Odds Ratio | Standard Error | z-value | p-value   | adjusted p-value |
|---------------------|------------|----------------|---------|-----------|-----------------|
| (Intercept)         | 12.095     | 0.272          | 9.158   | 2.2x10^{-16} | 5.3x10^{-19}    |
| Essential           | 0.410      | 0.147          | -6.076  | 1.2x10^{-9}   | 1.1x10^{-8}     |
| Embryonic Expression| 0.7495     | 0.229          | -1.284  | 0.199     | 1.00            |
| pLI                 | 0.7795     | 0.202          | -1.230  | 0.219     | 1.00            |
| oe                  | 1.969      | 0.265          | -0.113  | 0.910     | 1.00            |
| Chromosomal Location| 1.100      | 0.206          | 0.442   | 0.659     | 1.00            |
| Acetylated chromatin| 0.9095     | 0.130          | -0.719  | 0.472     | 1.00            |
| Methylated chromatin| 1.2195     | 0.205          | 0.951   | 0.341     | 1.00            |
| Giemsa Positive Stain| 1.1495 | 0.117          | 0.124   | 0.901     | 1.00            |
| OMIM Annotation     | 0.8105     | 0.165          | -1.273  | 0.203     | 1.00            |

**MODEL FIT: Without Essentiality**  
Null deviance: 2211.4, 3208 degrees of freedom  
Residual deviance 2185.3, 3200 degrees of freedom  
AIC = 2203.30

**MODEL FIT: With Essentiality**  
Null deviance: 2211.4, 3208 degrees of freedom  
Residual deviance 2150.9, 3199 degrees of freedom  
AIC = 2170.91

pLI, probability of being loss of function intolerant; oe, Observed/expected is a continuous measure of how tolerant a gene is to a certain class of variation, in this case loss-of-function;
Supplementary Table 6. The number of genes across chromosomes and their distribution based on essentiality

| Chromosome | No. Genes | Essential | Non-essential | Unknown | %known* |
|------------|-----------|-----------|---------------|---------|---------|
| 1          | 251       | 24        | 205           | 22      | 10.5%   |
| 2          | 277       | 30        | 240           | 7       | 11.1%   |
| 3          | 212       | 21        | 173           | 18      | 10.8%   |
| 4          | 212       | 23        | 180           | 9       | 11.3%   |
| 5          | 254       | 38        | 200           | 16      | 16.0%   |
| 6          | 192       | 15        | 155           | 22      | 8.8%    |
| 7          | 311       | 36        | 237           | 38      | 13.2%   |
| 8          | 208       | 28        | 173           | 7       | 13.9%   |
| 9          | 251       | 26        | 206           | 19      | 11.2%   |
| 10         | 170       | 24        | 132           | 14      | 15.4%   |
| 11         | 262       | 31        | 216           | 15      | 12.6%   |
| 12         | 129       | 17        | 99            | 13      | 14.7%   |
| 13         | 145       | 19        | 106           | 20      | 15.2%   |
| 14         | 144       | 12        | 125           | 7       | 8.8%    |
| 15         | 150       | 19        | 120           | 11      | 13.7%   |
| 16         | 126       | 15        | 106           | 5       | 12.4%   |
| 17         | 172       | 24        | 131           | 17      | 15.5%   |
| 18         | 112       | 15        | 85            | 12      | 15.0%   |
| 19         | 131       | 14        | 108           | 9       | 11.5%   |
| X          | 159       | 0         | 0             | 159     | nd      |
| Y          | 1         | 0         | 0             | 159     | nd      |
| **TOTAL**  | **3869**  | **431**   | **2997**      | **441** | **12.6%** |

*known = genes for which essentiality of human ortholog has been reported
**Supplementary Table 7.** Pairwise comparisons of the proportion of essential genes by experimental parameter

| Experimental Parameter | Comparison (left vs. right)* | % left | % right | p-value* |
|------------------------|------------------------------|--------|---------|----------|
| Delivery method        | Cytoplasmic injection vs. electroporation | 11.0%  | 14.2%   | 0.025    |
|                        | Cytoplasmic vs. pronuclear injection | 11.0%  | 7.2%    | 0.077    |
|                        | Electroporation vs. pronuclear injection | 14.2%  | 7.2%    | 0.004    |
| No. guides             | 2 guides vs. 4 guides          | 12.8%  | 12.7%   | 0.986    |
|                        | [35,360] vs. [360,490]         | 13.8%  | 11.1%   | 1.000    |
|                        | [35,360] vs. [490,640]         | 13.8%  | 12.6%   | 1.000    |
|                        | [35,360] vs. [640,880]         | 13.8%  | 13.6%   | 1.000    |
|                        | [35,360] vs. [880,1400]        | 13.8%  | 8.6%    | 0.095    |
|                        | [35,360] vs. [1400+]           | 13.8%  | 15.9%   | 1.000    |
|                        | [360,490] vs. [490,640]        | 11.1%  | 12.6%   | 1.000    |
|                        | [360,490] vs. [640,880]        | 11.1%  | 13.6%   | 1.000    |
| Deletion size          | [360,490] vs. [880,1400]       | 11.1%  | 8.6%    | 1.000    |
|                        | [360,490] vs. [1400+]          | 11.1%  | 15.9%   | 0.274    |
|                        | [490,640] vs. [640,880]        | 12.6%  | 13.6%   | 1.000    |
|                        | [490,640] vs. [880,1400]       | 12.6%  | 8.6%    | 0.395    |
|                        | [490,640] vs. [1400+]          | 12.6%  | 15.9%   | 1.000    |
|                        | [640,880] vs. [880,1400]       | 13.6%  | 8.6%    | 0.124    |
|                        | [640,880] vs. [1400+]          | 13.6%  | 15.9%   | 1.000    |
|                        | [880,1400] vs. [1400+]         | 8.6%   | 15.9%   | 0.004    |
| No. founders bred      | 1 vs. 2                       | 10.1%  | 13.7%   | 0.132    |
|                        | 1 vs. 3                       | 10.1%  | 9.4%    | 1.000    |
|                        | 1 vs. 4                       | 10.1%  | 9.6%    | 1.000    |
|                        | 2 vs. 3                       | 13.7%  | 9.4%    | 0.132    |
|                        | 2 vs. 4+                      | 13.7%  | 9.6%    | 0.132    |
|                        | 3 vs. 4+                      | 9.4%   | 9.6%    | 1.000    |

*left indicates the variable before “vs.” and right indicates the variable after “vs.”
*Chi square test for proportions

**Supplementary Information 2, Table 8A:** All IMPC mouse line production attempts
Filename: Elrick-Nutter_ST8_Cas9_AllAttempts.xlsx
Legend for Supplementary Table 8A.

| Column Name     | Column Description |
|-----------------|--------------------|
| MGI Accession ID| mouse gene symbol  |
Marker Symbol
MI External Ref
Consortium
Production Centre
Pipeline
Delivery Method
Injection Date
Injection Status
GLT Date
Clone Name / MF External Ref
mRNA Nuclease
mRNA Nuclease Concentration (ng/µL)
Protein Nuclease
Protein Nuclease Concentration (ng/µL)
gRNAs
Electroporation Voltage
Electroporation # Pulses
Embryo / Blastocyst Strain
# Embryos / Blastocysts Injected
# Embryos Survived
# Embryos / Blastocysts Transferred
Transfer Day
2 Cell
# Pups Born
# Founders Selected For Breeding
# Founders Assayed
Assay Used
Colony Name
Background Strain
Test Cross Strain
Experimental?
Report Micro Injection Progress

To Public  whether attempt can be reported publicly
Active?  whether attempt is active (t)
Haplo_essential  whether attempt was part of the haplo-essential screen
URL  URL for the gene page at the IMPC web portal
Comments  comments entered by centre

Supplementary Information 2, Table 8B. Unique gene IMPC null protein-coding gene mouse lines produced as of Oct 11, 2020.
Filename: Elrick-Nutter_ST8_Cas9_AllAttempts.xlsx
Legend for Supplementary Table 8B.

| Column Name       | Column Description                                                                 |
|-------------------|-------------------------------------------------------------------------------------|
| MGI Accession ID  | mouse gene symbol                                                                   |
| Marker Symbol     | Mouse Genome Informatics accession identifier                                       |
| MI External Ref   | production-centre assigned experiment identifier                                    |
| Consortium        | consortium for which allele was produced                                           |
| Production Centre | mouse production centre at which line was made                                       |
| Unique            | "Yes" indicates unique attempt                                                     |
| Ortholog          | "Yes" indicates existence of human ortholog                                         |
| Protein Coding    | specifies protein coding or other type of gene                                      |
| Pipeline          | pipeline in which line was phenotyped                                               |
| Delivery Method   | method used to deliver Cas9 reagents to embryos                                     |
| Injection Date    | date of microinjection or electroporation of embryos                                |
| Injection Status  | final status of production attempt                                                  |
| GLT Date          | date germline transmission was reported                                             |
| URL               | URL for the gene page at the IMPC web portal                                        |

Supplementary Information 3.
Filename: Elrick-Nutter_SupplementaryMethodsTable1

Supplementary Methods Table 1A. Animals used for mouse line production.
Legend for Supplementary Methods Table 1A.

| Column Name              | Column Description                                                                 |
|--------------------------|-------------------------------------------------------------------------------------|
| Institution              | Institution hosting the mouse line production centre                              |
| ILAR Labcode             | Labcode used to designate alleles produced at a given institution in Mouse Genome Informatics |
| Production background strain | Mouse inbred strain used to produce embryos for genome editing                    |
| Strain #                 | Strain number used by commercial vendor for the production strain                 |
| Background strain source | Commercial source(s) of mice used for embryo production                            |
| Pseudopregnant recipient strain | Strain used to produce pseudopregnant recipients for embryo transfer |
|--------------------------------|-------------------------------------------------------------|
| Strain #                      | Strain number used by commercial vendor for the pseudopregnant strain |
| Pseudopregnant strain source  | Commercial source(s) of mice used as pseudopregnant recipients |
| Ethics statement              | Statement about ethical oversight for mouse studies at each centre |

**Supplementary Methods Table 1B.** Reagents used for mouse line production.

Legend for Supplementary Methods Table 1B.

| Column Name                  | Column Description |
|-----------------------------|--------------------|
| Institution                 | Institution hosting the mouse line production centre |
| sgRNA Design                | Software used to design guide RNAs |
| Oligonucleotide source(s)   | Commercial source of oligonucleotides used for guide RNA synthesis |
| sgRNA synthesis             | Protocol(s) used to synthesize sgRNAs |
| sgRNA commercial source(s)  | Commercial source(s) of sgRNAs |
| Cas9 type(s)                | Molecular composition of Cas9 delivered to mouse embryos |
| Cas9 source(s)              | Commercial source(s) of Cas9 mRNA or protein |
| RNA injection buffer        | Buffer used to suspend genome editing reagents when Cas9 was delivered as mRNA by microinjection |
| RNP buffer (injection)      | Buffer used to suspend genome editing reagents when Cas9 was delivered as RNP by microinjection |
| RNA buffer (electroporation)| Buffer used to suspend genome editing reagents when Cas9 was delivered as mRNA by electroporation |
| RNP buffer (electroporation)| Buffer used to suspend genome editing reagents when Cas9 was delivered as RNP by electroporation |
| Embryo generation           | Method to produce embryos for genome editing |

**Supplementary Methods Table 1C.** Reagents used for genotyping animals during mouse line production.

Legend for Supplementary Methods Table 1C.

| Column Name                  | Column Description |
|-----------------------------|--------------------|
| Institution                 | Institution hosting the mouse line production centre |
| DNA isolation               | Method(s) used to isolate DNA from founders and N1 mouse tissue biopsies for genotyping |
| PCR reagents                | Reagents used for genotyping PCR |
| Analysis                    | Methods used to analyze PCR amplicons |