Progress towards completing the mutant mouse null resource

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Abstract
The generation of a comprehensive catalog of null alleles covering all protein-coding genes is the goal of the International Mouse Phenotyping Consortium. Over the past 20 years, significant progress has been made towards achieving this goal through the combined efforts of many large-scale programs that built an embryonic stem cell resource to generate knockout mice and more recently employed CRISPR/Cas9-based mutagenesis to delete critical regions predicted to result in frameshift mutations, thus, ablating gene function. The IMPC initiative builds on prior and ongoing work by individual research groups creating gene knockouts in the mouse. Here, we analyze the collective efforts focusing on the combined null allele resource resulting from strains developed by the research community and large-scale production programs. Based upon this pooled analysis, we examine the remaining fraction of protein-coding genes focusing on clearly defined mouse–human orthologs as the highest priority for completing the mutant mouse null resource. In summary, we find that there are less than 3400 mouse–human orthologs remaining in the genome without a targeted null allele that can be further prioritized to achieve our overall goal of the complete functional annotation of the protein-coding portion of a mammalian genome.

Introduction
Animal models, including mouse knockouts, play an instrumental role in advancing our understanding of how disruption of normal gene function relates to human disease. Traditionally, much of this work focuses on a relatively small number of conserved genes and pathways, reflecting a common tendency for investigators to incrementally build upon existing knowledge. Often these advancements reflect the critical importance of specific genes to human disease (Dolgin 2017); however, the “lamppost effect” greatly limits the opportunity for novel discoveries of gene function (Stoeger et al. 2018), and ultimately restricts the development of new therapeutic avenues (Oprea et al. 2018). This effect is strongly driven by the availability of tools to interrogate gene/pathway functions (Edwards et al. 2011), including mouse mutants (Stoeger et al. 2018). Further, this problem is compounded by poor public availability and/or limited phenotypic characterization of many of these mutant mouse lines. Herein, we summarize our progress towards completion of the mutant mouse null resource that promises to advance our understanding of novel genes and pathways, while reducing the research barriers to entry for individual investigators.

Over the past 20 years, tremendous progress has been made in generating a complete mutant mouse resource covering the roughly 23,000 protein-coding genes in the mouse genome. These efforts, spurred by the publication of the draft sequence of the mouse genome in 2002 (Mouse Genome Sequencing et al. 2002), reflect the consensus view that the mouse is the premier model of mammalian biology and the accumulation of technological innovations in mouse genetics presented a viable path towards functional annotation of the entire mouse genome (Fig. 1; Austin et al. 2004; Auwerx et al. 2004; Birling et al. 2021; Capecchi 1989; de Angelis et al. 2015; International Mouse Knockout et al. 2007; Jinek et al. 2012; Wang et al. 2013). This technological convergence includes the widespread adoption of mouse embryonic stem cells (mESCs) as a platform for gene targeting and innovations in molecular cloning that allowed for high-throughput generation of complex targeting constructs (Angrand et al. 1999; Copeland et al. 2001; Lee et al. 2001; Valenzuela et al. 2003). Early proposals for a more systematic strategy advocated for a hybrid approach of chemical mutagenesis (e.g., ENU), gene trapping, and gene targeting for complete genome coverage and emphasized the need for...
adopting systematic phenotyping protocols, the development of databases to provide easy access to functional data, and animal repositories to ensure broad access to models and other key resources (Battey et al. 1999; Hrabe de Angelis et al. 2000; Nadeau et al. 2001; Nolan et al. 2000; Skarnes et al. 2004).

The Banbury meeting in 2003 further refined this concept to set forth a goal of creating a null, reporter allele for every gene as a foundational starting point for functional annotation (Austin et al. 2004). Despite deliberately flexible in the exact approach, the group adopted several key principles, including the use of a single inbred genetic background, and the open availability of cell, animal, and data resources to the scientific community. Generation of mice from these resources would follow at a pace that reflected both the community demand and capacity for centralized production, ultimately supporting individual investigator research programs. The concept aligned with European efforts, which emphasized the value of generating a conditional version of each allele (Auwerx et al. 2004). Despite deliberately flexible in the exact approach, the group adopted several key principles, including the use of a single inbred genetic background, and the open availability of cell, animal, and data resources to the scientific community. Generation of mice from these resources would follow at a pace that reflected both the community demand and capacity for centralized production, ultimately supporting individual investigator research programs.

The bold IKMC mission of generating a null or conditional null allele for every gene in the mouse genome was complemented by the more modest goal of turning a small subset of these alleles into mouse strains for systematic characterization (Bradley et al. 2012; Skarnes et al. 2011). Further refinement of standard protocols for mouse phenotyping and the demonstration of the feasibility of large-scale mouse generation and phenotyping (de Angelis et al. 2015; White et al. 2013) underpin the current efforts of the International Mouse Phenotyping Consortium, which are now expanding on the initial vision first proposed at Banbury (Lloyd et al. 2020). The IMPC has generated over 5000 knockout lines from IKMC ES resources (Birling et al. 2021), and the advent of CRISPR/Cas9 technology has further augmented capacity for the generation of mutant mice. To date, the IMPC has generated knockout lines for 7590 genes (Data Release 14, May 7, 2021) and established a robust infrastructure sufficient for completing the draft functional annotation of a mammalian genome. This effort has had a enormous impact on our understanding mammalian biology and human disease (Brown et al. 2018; Meehan et al. 2017). The IMPC has continued to expand the catalog of mammalian essential genes, which are highly enriched in human disease (Cachero et al. 2020; Dickinson et al. 2016), while providing novel insights into developmental mechanisms through detailed embryonic phenotyping (Dickinson et al. 2016). The IMPC pipeline has revealed numerous novel

**Fig. 1** Timeline highlighting major milestones enabling complete functional annotation of the protein-coding fraction of the mouse genome. Technological advances that made possible the large-scale generation of mutant mouse resources are shown in black. Multi-institutional collaborative programs (e.g., European Mouse Disease Clinic; EUMODIC, and Knockout Mouse Phenotyping Program; KOMP2) implemented these advancements to perform high-throughput animal model production and systematic broad-based phenotyping. This collective work has grown to include additional international sites that have been centralized under the International Mouse Phenotyping Consortium (IMPC) to coordinate animal production, phenotyping, and data dissemination.
gene associations with disease-relevant traits (Bowl et al. 2017; Rozman et al. 2018; Swan et al. 2020), and systematic phenotyping of both sexes reveals widespread sexual dimorphism (Karp et al. 2017).

What was once a long-term aspirational goal of the mouse genetics community is now an achievable milestone? This raises several interesting and important questions: How many more genes remain to be knocked out? How should these genes be prioritized for systematic mutagenesis? Are there other features in the genome (e.g., noncoding RNAs) that merit further consideration as mutagenesis targets? Here, we explore the collective catalog of mutant mouse null alleles generated by the IMPC and the broader scientific community to address these questions and chart a course towards finalizing a blueprint for understanding the activity of the protein-coding fraction of the mammalian genome.

Results

Current state of the mutant mouse null allele catalog to assess protein-coding gene function

To focus on defining the community-wide effort to mutagenize the mouse genome, we include all null alleles, irrespective of their relationship to systematic production efforts or public availability. We define “IMPC” as all mouse model generation activities of the IKMC or IMPC, and “community” for all other alleles (individual investigator, non-IMPC programs, etc.). For curating null alleles, we used the MouseMine query tool to obtain annotation information detailing the target gene, allele symbol, methodology, and associated publications (Motenko et al. 2015). These queries identified 29,341 unique null alleles corresponding to 13,973 protein-coding genes with the majority of community-derived alleles generated using an ES-based resource and IMPC alleles a mixture of both ES and Cas9 derived. In addition to these targeted null mutations, we identified an additional 265 unique genes as having an annotated null allele resulting from random mutagenesis (spontaneous, ENU or gene trap). While our emphasis here is on the null resource, there are currently over 67,000 mutant mouse alleles documented by Mouse Genome Informatics (http://www.informatics.jax.org) that represent a diverse set of alleles ranging from hypomorphs to dominant negative, and humanized regions as well as others. This set of curated null alleles covers ~62% (14,238/23,000) of the protein-coding genes currently cataloged in the mouse genome. A detailed look at the time course for animal model generation highlights community production of null alleles rapidly accelerating in the mid-1990s as the technology spread and was implemented in core facilities to provide stable production of 400–500 new alleles published each year peaking in 2011 (Fig. 2a). About this time, IMPC production of null alleles ramped up significantly (Birling et al. 2021), which has been accompanied by a decline in community production of ESC-based alleles. This reduction may reflect the uptake of IKMC- and IMPC-generated resources by individual investigators, obviating the need to produce their own knockout. In 2015, the IMPC pivoted to the use of CRISPR/Cas9 to generate knockout alleles, which quickly replaced ESC-based animal production resulting in the establishment of ~4000 new lines within this 5-year window from 2015 to 2020 (Fig. 2a). Concurrently, there is a lower, but growing rate of community produced null alleles utilizing CRISPR/Cas9. Reflecting challenges in public availability of resources and parallel research aims, multiple null alleles are frequently generated for genes (Fig. 2b), including 150 genes with more than 10 null alleles. Most of community-generated null alleles are ESC based, while the vast majority of Cas9-generated null alleles reported in MGI were generated by the IMPC, due to rapid adoption of the technology as a core mutagenesis method to reduce cost and increase throughput (Fig. 2c).

Given the current efforts for complete functional annotation of the mammalian genome, with an overarching goal to understand human biology and disease, we examined how many mouse knockout alleles corresponded to protein-coding genes with high-confidence human orthologs defined by having multiple independent lines of supporting evidence from different resources (Munoz-Fuentes et al. 2018), and how many of these genes remain to be targeted. Of the 14,238 mouse genes that have a null allele, 94% (13,466) have a human ortholog while the remaining 772 lack a clearly identifiable ortholog or are mouse specific (Fig. 2d). Therefore, of the total 16,847 mouse genes with a high-confidence human ortholog, 79.9% have a reported null allele, leaving 3381 genes to complete the mutant mouse null resource.

To further develop a prioritization framework, we analyzed the remaining 3381 genes to determine if there were any gene families that were over-represented or if there was evidence of functional constraint on these genes lacking null alleles. Within this set of non-targeted genes, olfactory receptors were the most highly represented class of genes followed by zinc finger domain containing genes and RIKEN cDNA clones (Fig. 3a). This is consistent with large size of these gene families and highlights the barriers to research on genes for which there is little existing functional data (Stoeger et al. 2018). In addition, it is important to consider whether the information generated from knockout mice for a class of genes such as olfactory receptors is merited given the phenotyping tests included in the IMPC, and the expected impact of single gene mutation. Conversely, transmembrane proteins and solute carriers are more likely to be druggable and, thus, could be prioritized as gene families warranting completion. There are also many
ribosome-related genes left to be characterized, and ribosomopathies are an emerging disease class that displays a wide range of phenotypes (Kampen et al. 2020). Additionally, genes essential for life are highly enriched for human disease genes (Bartha et al. 2018; Cacheiro et al. 2020; Dickinson et al. 2016; Georgi et al. 2013). Of the 3381 remaining genes, 15% (507/3381) are classified as cell essential based upon CRISPR/Cas9 screens in human cell lines (Cacheiro et al. 2020; Tsherniak et al. 2017). To determine if these genes were under constraint against mutation in the human population, we used the human orthologs to obtain the probability of loss-of-intolerance (pLI) score from the gnomAD database (Karczewski et al. 2020). pLI scores range from 0 to 1 with values closer to 1 indicative of significant constraint in the human genome (Lek et al. 2016). Of the cell essential genes, 144/507 have a human ortholog associated with a pLI score greater than 0.8 further supporting the idea that this set of cell essential genes is under functional constraint. In total, 11% (378/3381) of the genes without a null allele have a pLI score greater than 0.8 (Fig. 3b). Genes that are nonessential in cell lines but have high pLI scores have previously been shown to be enriched for critical developmental regulators that are also associated with human disease (Cacheiro et al. 2020).

To determine the extent that genes without knockout alleles are related to human disease genes, we examined the overlap with ORPHANET (http://www.orpha.net) and Tier 1 (solved cases) and Tier 2 (unsolved cases) gene lists from the Centers for Mendelian Genomics (CMG; http://mendelian.org/phenotypes-genes) (Posey et al. 2019). This analysis highlighted 358 genes with support from ORPHANET-only or had additional evidence from the CMG (Fig. 4a). While these genes were identified as lacking a knockout mouse, the ongoing production efforts of the IMPC have made progress
Progress towards completing the mutant mouse null resource

Fig. 3 Analysis of gene family representation and constraint for the 3381 mouse–human orthologs that currently lack a null allele in mouse. a The set of remaining genes is specifically enriched for large gene families and the RIKEN cDNA collections. Olfactory receptors and zinc finger proteins are the most highly represented gene families. b Classification of the remaining genes using human orthologs to assess cell essentiality based upon CRISPR/Cas9 screens in cancer cell lines and functional constraint using probability of loss-of-intolerance (pLI) scores that range from 0 to 1 with values closer to 1 associated with higher level of constraint.

Towards establishing knockouts for 54 genes and begun phenotyping on another 15 genes (Fig. 4b). Production attempts have failed for 134 genes with the remaining 106 yet to be targeted by the IMPC. It will be important to emphasize this set of human disease related genes for generation of mouse knockouts.
Next, we performed Gene Ontology (GO) term enrichment (Fig. 5a–c) and KEGG pathway analysis (Fig. 5d) on the previously defined cell essential and nonessential gene sets using WebGestalt (Liao et al. 2019). Strikingly, these analyses revealed a stark distinction in the biological processes and pathways associated with these two groups of genes. Cell essential genes were significantly enriched (FDR < 0.05) for terms associated with pathways for mRNA splicing (spliceosome), translation (ribosome), and protein degradation (proteasome; Fig. 5a–d). In contrast, nonessential genes showed enrichment for olfactory transduction and metabolic pathways (Fig. 5d). These findings indicate a clear distinction between core biological functions required for cell survival versus potentially tissue-specific differences in energy requirements or activity of certain cell types. In support of these differences, we used the human orthologs for the remaining genes to identify potential human disease associations within OMIM and observed an enrichment for Mitochondrial Complex I Deficiency (P-value = 2.0673e−11), Alcohol Dependence (P-value = 6.5255e−4), and Mitochondrial Complex IV Deficiency (P-value = 4.4197e−5).

Overview of noncoding transcripts annotated in the mouse genome

Despite the intense focus on the protein-coding portion of the genome, there has been a long-standing appreciation for the role of noncoding transcripts for regulating diverse cellular events such as modulating chromatin structure (e.g., Xist), targeting genes to regulate expression (e.g., miRNA), and building the translational machinery (e.g., rRNA and tRNA). While these functions are known, the number of annotated noncoding transcripts continues to grow and now exceeds the number of protein-coding genes with the vast majority generically referred to as long noncoding RNA’s (lncRNAs) with unknown function (Fig. 6). To gain a greater understanding of how noncoding transcripts impact biology, we will need to invest and explore in alternative targeting strategies to determine if the noncoding transcript itself is functional or if it is merely the act of transcription that is required. Functional testing of the transcripts can be achieved using whole gene ablation strategies with CRISPR/Cas9; however, while large deletions (> 10 kb) are feasible with CRISPR/Cas9 these are often accompanied with other...
Progress towards completing the mutant mouse null resource

structural variants such as inversions and duplications which would require additional screening (Birling et al. 2017). Additionally, the removal of large genomic regions has the potential to impact the expression of neighboring genes by altering the cis-regulatory landscape. An alternative approach is to introduce a poly-adenylation sequence to terminate transcription (Engreitz et al. 2016) or knockdown the target the transcript by introducing a degradation sequence or using Cas13a (Gao et al. 2020). These approaches would keep the locus intact but prevent the transcript from accumulating; and thereby, determine if the transcript itself is functional or is it merely the engagement of RNA polymerase II that is required. There is also a pre-existing set of ESC-based resources available for studying noncoding RNA function (Hansen et al. 2021). Our current animal production methodologies are well suited to be scaled to address these questions and can readily implement all of these approaches.

Conclusions

In summary, extraordinary progress has been made towards generating the first complete functional annotation of the protein-coding fraction of the mammalian genome. This

Fig. 5 GO term enrichment and pathway analysis on the set of 3381 genes classified into cell essential and nonessential categories. GO terms for a Biological process, b Cellular component, and c Molecular function highlight a role for essential genes in core biological processes including transcription, splicing, and translation, and a role for nonessential genes in metabolic processes and mitochondrial function. d KEGG pathway analysis supports the distinction between cell essential and nonessential genes and associated biological function. Enrichment values were determined using WebGestalt with FDR < 0.5
Fig. 6 Top 10 noncoding RNA sequence features annotated in the mouse genome. The number of known long noncoding RNA’s (lncRNAs) currently exceeds the number of protein-coding genes and the vast majority remain to be studied in depth.

Noncoding RNA Type
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Declarations

Conflict of interest  On behalf of all authors, the corresponding author states that there is no conflict of interest.

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References

Angrand PO, Daigle N, van der Hoeven F, Scholer HR, Stewart AF (1999) Simplified generation of targeting constructs using ET recombination. Nucleic Acids Res 27:e16

Austin CP, Battey JF, Bradley A, Bucan M, Capecchi M, Collins FS, Dove WF, Duyk G, Dymecki S, Eppig JT, Grieder FB, Heintz N, Hicks G, Insel TR, Joyner A, Koller BH, Lloyd KC, Magnuson T, Moore MW, Nagy A, Pollock JD, Roses AD, Sands AT, Seed B, Skarnes WC, Snoddy J, Soriano P, Stewart DJ, Stewart F, Stillman B, Varmus H, Vitricovici L, Verma IM, Vogt TF, von Melchner H, Witkowski J, Wooythic RP, Wurst W, Yancopoulos GD, Young SG, Zambrowicz B (2004) The knockout mouse project. Nat Genet 36:921–924

Auwerx J, Avner P, Baldock R, Ballabio A, Balling R, Barbacid M, Berns A, Bradley A, Brown S, Carmelit P, Chambon P, Cox R, Davidson D, Davies K, Duboule D, Forejt J, Granucci F, Hastie N, de Angelis MH, Jackson I, Kioussis D, Kollias G, Lathrop M, Lendahl U, Malumbres M, von Melchner H, Muller W, Parpavan J, Ricciardi-Castagnoli P, Rigby P, Rosen B, Rosenthal N, deangelis MH, Hurleybroeck D, Iyer V, de Jong PK, Kadin JA, Kallof C, Kennedy K, Koutsourakis M, Lloyd KC, Marshall S, Mason J, McKerlie C, McLeod MP, von Melchner H, Moore M, Mujica AO, Nagy A, Nefedov M, Nutter LM, Pavlovic G, Peterson JL, Pollock J, Ramirez-Solis R, Bancourt DE, Raspa M, Remacle JE, Ringwald M, Rosen B, Rosenthal N, Rossant J, Ruiz Noppening P, Ryder E, Schick JZ, Schnutgen F, Schofield P, Seelenberger C, Selloum M, Simpson EM, Skarnes WC, Smedley D, Stanford WL, Stewart AF, Stone K, Swan K, Tadepally H, Teboul L, Todcin-valenti GP, Vanzenzuela D, West AP, Yamamura K, Yoshinaga Y, Yust W (2012) The mammalian gene function resource: the International Knockout Mouse Consortium. Mamm Genome 23:580–586

Brown SD, Holmes CC, Mallon AM, Meehan TF, Smedley D, Wells S (2018) High-throughput mouse phenomics for characterizing mammalian gene function. Nat Rev Genet 19:357–370

Cachero P, Munoz-Fuentes V, Murray SA, Dickinson ME, Bucan M, Nutter LMJ, Peterson KA, Haselmaleshadi H, Flenniken AM, Morgan H, Westerberg H, Konopka T, Hsu CW, Christiansen A, Lanza DG, Beaudet AL, Heaney JD, Fuchs H, Gaulius-Durner V, Sorg T, Prochazka J, Nosovadova V, Lelliot CJ, Wardle-Jones H, Wells S, Teboul L, Ceter H, Stewart M, Hough T, Wurst W, Sedlacek R, Adams DJ, Seavit JR, Todcin-valenti GP, Mammamo F, Braun RE, McKerlie C, Hurley Y, de Angelis MH, Mallon AM, Lloyd KCK, Brown SDM, Parkinson H, Meehan TF, Smedley D, Genomics England Research Consortium, International Mouse Phenotyping Consortium (2020) Human and mouse essentiality screens as a resource for disease gene discovery. Nat Commun 11:655

Capecchi MR (1989) Altering the genome by homologous recombination. Science 244:1288–1292

Copeland NG, Jenkins NA, Court DL (2001) Recombineering: a powerful new tool for mouse functional genomics. Nat Rev Genet 2:769–779
de Angelis MH, Nicholson G, Selloum M, White J, Morgan H, Ramírez-Solis R, Sorg T, Wells S, Fuchs H, Fray M, Adams DJ, Adams NC, Adler T, Aguilar-Pimentel A, Ali-Hadji D, Amann G, Andre P, Atkins S, Auburtin A, Ayadi A, Becker J, Becker L, Bedu E, Bekeredjian R, Birling MC, Blake A, Bottomley J, Bowl M, Braut V, Busch DH, Bussell JN, Calzada-Wack J, Cater H, Champy MF, Charles P, Chevalier C, Chiania F, Codner GF, Combe R, Cox R, Dalloneau E, Dierich A, Di Fenza A, Doe B, Duchen A, Eckelberg O, Esapa CT, El Fertak L, Feigel T, Emelyanova I, Estabel J, Favor J, Flenniken A, Gambadoro A, Garrett L, Gates H, Gerdin AK, Gkoutos G, Greeneway S, Glasd L, Goetz P, Da Cruz IG, Goz A, Graw J, Guimond A, Hans W, Hicks G, Holter SM, Holler H, Hancock JM, Hoehndorf R, Hough T, Houghton R, Hurt A, Ivan- dic B, Jacobs H, Jacquot S, Jones N, Karp NA, Katus HA, Kitchen S, Klein-Rodelwed T, Klingenempor S, Klopstock T, Lalanne V, Leblanc S, Lenger C, le Marchand E, Ludwig T, Lux A, McKerlie C, Maier H, Mandel JL, Marschall S, Mark M, Melvin DG, Meziane H, Mikklich K, Mittelhauser C, Monassier L, Moularet D, Muller S, Naton B, Neff F, Nolan PM, Nutter LM, Ollert M, Pavlovic G, Pellegata NS, Peter E, Petit-Demouliere B, Pickard A, Podrini C, Potter P, Pouilly L, Puik O, Richardson D, Rouss- seau S, Quintana-Fendell L, Quivalid MM, Racc I, Rathkobol B, Riet F, Rossant J, Roux M, Rozman J, Ryder E, Salisi J, San- tos L, Schable KH, Schiller E, Schrewie A, Schulz H, Steininkamp R, Simon M, Stewart M, Stoger C, Stoger T, Sun S, Sunner D, Teboul L, Tilly I, Tocchini-Valentini GP, Tost M, Treise I, Vasseur L, Velot E, Vogt-Weisenhorn D, Wagner C, Walling A, Weber B, Wendling O, Westerberg H, Willershauser M, Wolf E, Wolter A, Wood I, Wurst W, Yildirim AO, Zeh R, Zimmer A, Zimprich A, Consortium E, Holmes C, Steel KP, Herault Y, Gailus-Durner V, Mallon AM, Brown SD (2015) Analysis of mammalian gene function through broad-based phenotypic screens across a consortium of mouse clinics. Nat Genet 47:969–978

Dickinson ME, Flenniken AM, Ji X, Teboul B, Wong MD, White JK, Meehan TF, Weninger WJ, Westerberg H, Adissu H, Baker CN, Bower L, Brown JM, Caddie LB, Chiani F, Clary D, Cleak J, Daly MJ, Denegre JM, Doe B, Dolan ME, Edie SM, Fuchs H, Gailus-Durner V, Galli A, Gambadoro A, Gallegos J, Guo S, Horner NR, Hsu CW, Johnson SJ, Kalaga S, Keith LC, Lanoue L, Law -tos L, Lek M, Mark M, Marschall S, Mason J, McElwee ML, Newbigging S, Nutter LM, Peterson KA, Ramirez-Solis R, Row-lland DJ, Ryder E, Samocha KE, Scavitt JR, Selloum M, Szoke-Kovacs Z, Tamura M, Trainor AG, Tudose I, Wakana S, Warren J, Wendling O, West DB, Wong L, Yoshiki A, International Mouse Phenotyping Consortium, Jackson Laboratory, Infrastructure Nationale Phenomin Institut Clinique de la Souris, Charles River Laboratories, Harwell MRC, Toronto Centre for Phenogenomics, Wellcome Trust Sanger Institute, Riken BioResource Center, MacArthur DG, Tocchini-Valentini GP, Gao X, Flice P, Bradley A, Skarnes WC, Justice MJ, Parkinson HE, Moore M, Wells S, Braun RE, Svenson KL, de Angelis MH, Herault Y, Mohun T, Mallon AM, Henkelman RM, Brown SD, Adams DJ, Lloyd KC, McKerlie C, Beaudet AL, Bucan M, Murray SA (2016) High-throughput discovery of novel developmental phenotypes. Nature 537:508–514

Dolgin E (2017) The most popular genes in the human genome. Nature 551:427–431

Edwards AM, Isserlin R, Bader GD, Frye SV, Willson TM, Yu FH (2011) Too many roads not taken. Nature 470:163–165

Engreitz JM, Haines JE, Perez EM, Munson G, Chen J, Kane M, McDonel PE, Guttman M, Lander ES (2016) Local regulation of gene expression by IncRNA promoters, transcription and splicing. Nature 539:452–455

Gao F, Cai Y, Kapranov P, Xu D (2020) Reverse-genetics studies of IncRNAs—what we have learnt and paths forward. Genome Biol 21:93

Georgi B, Voight BF, Bucan M (2013) From mouse to human: evolutionary genomics analysis of human orthologs of essential genes. PLoS Genet 9:e1003484

Gondo Y (2008) Trends in large-scale mouse mutagenesis: from geneticsto functional genomics. Nat Rev Genet 9:803–810

Hansen J, von Melchner H, Wurst W (2021) Mutant non-coding RNA resource in mouse embryonic stem cells. Dis Model Mech. https://doi.org/10.1242/dmm.047803

Hrabe de Angelis MH, Flaswinkel H, Fuchs H, Rathkobol B, Soewarto D, Marschall S, Heffner S, Pargent W, Wiuschen K, Jung M, Reis A, Richter T, Alessandri F, Jakob T, Fuchs E, Kolb H, Kremmer E, Schaeble K, Rollinski B, Roscher A, Peters C, Meitinger T, Strom T, Steckler T, Hoisboer F, Klopstock T, Gekeler F, Schindewolf C, Jung T, Avraham K, Behrendt H, Ring J, Zimmer A, Schughart K, Pfefker K, Wolf E, Balling R (2000) Genome-wide, large-scale production of mutant mice by ENU mutagenesis. Nat Genet 25:444–447

International Mouse Knockout Consortium, Collins FS, Rossant J, Wurst W (2007) A mouse for all reasons. Cell 128:9–13

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna J, Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821

Kampen KR, Sulima SO, Vereecke S, De Keersmaecker K (2020) Hallmarks of ribosomopathies. Nucleic Acids Res 48:1013–1028

Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfolid J, Wang Q, Collins RL, Larichia KM, Ganna A, Birnbaum DP, Gauthier LD, Brand H, Solomonson M, Watts NA, Rhodes D, Singer-Berk M, England EM, Seaby EG, Kosmickia JA, Walters RK, Tashman K, Farjoun Y, Banks E, Poterba T, Wang A, Seed C, Whif-fin N, Chong JX, Samocha KE, Pierce-Hoffman E, Zappala Z, O’Donnell-Luria AH, Minikel EV, Weisburd B, Lek M, Ware JS, Vittal C, Armein JM, Bergelson L, Cibulsikis K, Connolly KM, Covarrubias M, Donnelly S, Ferriera S, Gabriel S, Gentry J, Gupta A, Neandet T, Kaplan D, Lianwarne C, Munshi R, Novod V, Petirillo N, Roazen D, Ruano-Rubio V, Saltzman A, Schleicher M, Soto J, Tibbetsk B, Tolonen C, Wade G, Talkowski ME, Genome Aggregation Database Consortium, Neale BM, Daly MJ, Mac-Arthur DG (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581:434–443

Karp NA, Mason J, Beaudet AL, Benjamini Y, Bower L, Braun RE, Brown SDM, Chesler EJ, Dickinson ME, Flenniken AM, Fuchs H, Angelis MH, Gao X, Guo S, Greeneway S, Heller R, Herault Y, Justice MJ, Kurbatova N, Lelliott CJ, Lloyd KC, Mallon AM, Mannie J, Masuya H, McKerlie C, Meehan TF, Mott RF, Murray SA, Parkinson H, Ramirez-Solis R, Santos L, Seavitt JR, Smedley D, Sorg T, Speak AO, Steel KP, Svenson KL, International Mouse Phenotyping Consortium, Jackson Laboratory, Infrastructure Nationale Phenomin Institut Clinique de la Souris, Charles River Laboratories, Harwell MRC, Toronto Centre for Phenogenomics, Wellcome Trust Sanger Institute, Riken BioResource Center, MacArthur DG, Tocchini-Valentini GP, Gao X, Flice P, Bradley A, Skarnes WC, Justice MJ, Parkinson HE, Moore M, Wells S, Braun RE, Svenson KL, de Angelis MH, Herault Y, Mohun T, Mallon AM, Henkelman RM, Brown SD, Adams DJ, Lloyd KC, McKerlie C, Beaudet AL, Bucan M, Murray SA (2016) High-throughput discovery of novel developmental phenotypes. Nature 537:508–514

Lee EC, Yu D, Martínez de Velasco J, Tesselarolo L, Swing DA, Court DL, Jenkins NA, Copeland NG (2001) A highly efficient Escherichia coli-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. Genomics 73:56–65

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O’Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukianen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Delaux N, Depristo M, Do R, Flannick J, Froemer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezan A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DL, Ardissino D, Boehnke M, Danesh J, Donnelly S, Eloua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCaffrey S, McCarthy
for Mendelian Genomics (2019) Insights into genetics, human biology and disease gleaned from family based genomic studies. Genet Med 21:798–812

Rozman J, Rathkolb B, Oestereicher MA, Schutt C, Ravindranath AC, Leuchtenberger S, Sharma S, Kistler M, Willershauer M, Brommage R, Meehan TF, Mason J, Haselimanahshi H, Consortium I, Hough T, Mallon AM, Wells S, Santos L, Lelliott CJ, White JK, Sorg T, Champy MF, Bower LR, Reynolds CL, Flenniken AM, Murray SA, Nutter LMJ, Svensson KL, West D, Tochichi-Valentini GP, Beaudet AL, Bosch F, Braun RB, Dobbie MS, Gao X, Herault Y, Moshiri A, Moore BA, Kent Llloyd KC, McKeffer C, Masuya H, Tanaka N, Flice P, Parkinson HE, Sedlacek R, Seong JK, Wang CL, Moore M, Brown SD, Tschop MH, Wurst W, Klingenspor M, Wolf E, Beckers J, Machicoa F, Peter A, Staiger H, Haring Hu, Grallert H, Campillos M, Maier H, Fuchs H, Gailus-Durner V, Werner T, Hrabe de Angelis M (2018) Identification of genetic elements in metabolism by high-throughput mouse phenotyping. Nat Commun 9:288

Skarnes WC, von Melchner H, Wurst W, Hicks G, Nord AS, Cox T, Young SG, Ruiz P, Soriano P, Tessier-Lavigne M, Conklin BR, Stanford WL, Rossant J, International Gene Trap Consortium (2004) A public gene trap resource for mouse functional genomics. Nat Genet 36:543–544

Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W, Iyer V, Mujica AO, Thomas M, Harrow J, Cox T, Jackson D, Severin J, Biggs P, Fu J, Nefedov M, de Jong PJ, Stewart AF, Bradley A (2011) A conditional knockout resource for the genome-wide study of mouse gene function. Nature 474:337–342

Stoeger T, Gerlach M, Morimoto RI, Nunes Amaral LA (2018) Large-scale investigation of the reasons why potentially important genes are ignored. PLoS Biol 16:e2006643

Swan AL, Schutt C, Rozman J, Del Mar Muniz Moreno M, Brandmaier S, Simon M, Leuchtenberger S, Griffiths M, Brommage R, Keskiival-Bond P, Grallert H, Werner T, Teperino R, Becker L, Miller G, Moshiri A, Seavitt JR, Cissell DD, Meehan TF, Acar EF, Lelliott CJ, Flenniken AM, Champy MF, Sorg T, Ayadi A, Braun RE, Cater H, Dickinson ME, Flice P, Gallegos J, Ghirardello EJ, Heaney JD, Jacquot S, Lally C, Logan JG, Teboul L, Mason J, Spielmann N, McKeffer C, Murray SA, Nutter LJM, Odfeldt KP, Parkinson H, Prochazka J, Reynolds CL, Selloum M, Spoutil F, Svensson KL, Vales TS, Wells SE, White JK, Sedlacek R, Wurst W, Lloyd KCK, Croucher PI, Fuchs H, Williams GR, Bassett JHD, Gailus-Durner V, Herault Y, Mallon AM, Brown SDM, Mayer-Kuckuk P, Hrabe de Angelis M, Consortium I (2020) Mouse mutant phenotyping at scale reveals novel genes controlling bone mineral density. PLoS Genet 16:e1009190

Tsherniak A, Vazquez F, Montgomery PG, Weir BA, Kryukov G, Cowley GS, Gill S, Harrington WF, Pantel S, Krill-Burger JM, Meyers RM, Ali L, Goodale A, Lee Y, Jiang G, Hsiao J, Gerath WFJ, Hollow S, Merkel E, Ghandi M, Garraya LA, Root DE, Golub TR, Boehm JS, Hahn WC (2017) Defining a cancer dependency map. Cell 170:564–576 e516

Valenzuela DM, Murphy AJ, Freadewey D, Gale NW, Economides AN, Auerbach W, Poureymouri WT, Adams NC, Rojas J, Yasenchak J, Chernomorsky R, Boucher M, Elsasser AL, Essau L, Zheng J, Griffiths JA, Wang X, Su H, Xue Y, Domínguez MG, Noguera I, Torres R, Macdonald LE, Stewart AF, DeChiara TM, Yancopoulos GD (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat Biotechnol 21:652–659

Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, Jaenisch R (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153:910–918

White JK, Gerdin AK, Karp NA, Ryder E, Buljan M, Bussell JN, Salisbury J, Clare S, Ingham NJ, Podrini C, Houghton R, Estabel J, Bottomley JR, Melvin DG, Sunter D, Adams NC, Sanger Institute Mouse Genetics P, Tannahill D, Logan DW, Macarthur DG, Flint J, Mahajan VB, Tsang SH, Smyth I, Watt FM, Skarnes WC, Dougan G, Adams DJ, Ramirez-Solis R, Bradley A, Steel KP (2013) Genome-wide generation and systematic phenotyping of knockout mice reveals new roles for many genes. Cell 154:452–464

Yesbolatova A, Saito Y, Kitamoto N, Makino-Ito H, Ajima R, Nakano R, Nakaoka H, Fukushima K, Gamo K, Tominari Y, Takeuchi H, Saga Y, Hayashi KI, Kanemaki MT (2020) The auxin-inducible degron 2 technology provides sharp degradation control in yeast, mammalian cells, and mice. Nat Commun 11:5701

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