



A resource of targeted mutant mouse lines for 5,061 genes

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A resource of targeted mutant mouse lines for 5,061 genes

A full list of authors and affiliations appears at the end of the article.

Summary

The International Mouse Phenotyping Consortium reports the generation of new mouse mutant strains for over 5,000 genes, including 2,850 novel null, 2,987 novel conditional-ready, and 4,433 novel reporter alleles.

Despite thirty years of mouse targeted mutagenesis, *in vivo* function of the majority of genes in the mouse genome are still unknown. This reflects the observation that a small number of genes have been the object of intensive study including the development of multiple mouse models, while a significant proportion of the coding genome remains

⁴⁰ Correspondence: l.teboul@har.mrc.ac.uk. ⁴¹ Correspondence: steve.murav@jax.org.

* A list of authors and their affiliations appears at the end of the paper.

Complete list of additional contributors available in the Supplemental Material

Author Contributions

M.-C. B., A.Y., S.A., J.B., A.Bu., W.B., F.C., S.C., G.F.C., F.J.D., B.D., M.D.F., A.G., M.G., A.G.-S., L.O.G., P.K. R.E.K., R.K., H.L., Y.J.L., I.L., A.-M.M., T.F.M., V.M.F., S.N., L.M.J.N., G.T.O., G.P., R.R.-S., B.R., E.J.R., L.A.S., J.S., J.R.S., C.S., M.T., H.W.-J., M.W.-D., M.V.W., B.J.W., J.A.W., L.T., and S.A.M. generated data, developed data tools and databases, and/or carried out data and statistical analyses; A.Y., D.J.A., A.L.B., A.B., S.D.M.B., H.-J.G.C., M.E.D., L.R.D., X.G., J.D.H., Y.H., M.H.A., S.-T.J., M.J.J., Z.L., K.C.K.L., C.M., A.-M.M., T.F.M., L.M.J.N., R.S., J.K.S., W.C.S., T.S., K.P.S., G.P.T.-V., C.-K.L.W., S.W., W.W., Y.X., L.T., and S.A.M. directed research at their respective institutions; M.-C. B., L.T., and S.A.M. wrote the paper.

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* IMPC Consortium Authors

Juan J Gallegos⁴, Jennie R Green⁴, Ritu Bohar⁴, Katie Zimmer⁴, Monica Pereira¹², Suzanne MacMaster¹², Sandra Tondat¹², Linda Wei¹², Tracy Carroll¹², Jorge Cabezas¹², Qing Fan-Lan¹², Elsa Jacob¹², Amie Creighton¹², Patricia Castellanos-Penton¹², Ozge Danisment¹², Shannon Clarke¹², Joanna Joeng¹², Deborah Kelly¹², Christine To¹², Rebekah van Bruggen¹², Valerie Gailus-Durner¹⁵, Helmut Fuchs¹⁵, Susan Marschall¹⁵, Stefanie Dunst¹⁵, Markus Romberger¹⁵, Bernhard Rey¹⁵, Sabine Fessele⁴², Philipp Gormanns⁴², Roland Friedel⁶, Cornelia Kaloff⁶, Andreas Hörlein⁶, Sandy Teichmann⁶, Adriane Tasdemir⁶, Heidi Krause⁶, Dorota German⁶, Anne Köntzer⁶, Sarah Weber⁶, Joachim Beig⁶, Matthew McKay¹⁰, Richard Bedigian¹⁰, Stephanie Dion¹⁰, Peter Kutny¹⁰, Jennifer Kelmenson¹⁰, Emily Perry¹⁰, Dong Nguyen-Bresinsky¹⁰, Audrie Seluke¹⁰, Timothy Leach¹⁰, Sara Perkins¹⁰, Amanda Slater¹⁰, Michaela Petit¹⁰, Rachel Urban¹⁰, Susan Kales¹⁰, Michael DaCosta¹⁰, Michael McFarland¹⁰, Rick Palazola¹⁰, Kevin A. Peterson¹⁰, Karen Svenson¹⁰, Robert E. Braun¹⁰, Robert Taft¹⁰, Mark Rhue²², Jose Garay²², Dave Clary²², Renee Araiza²², Kristin Grimsrud²², Lynette Bower²², Nicole L. Anchell²², Kayla M Jager²², Diana L Young²², Phuong T Dao²², Wendy Gardiner⁹, Toni Bell⁹, Janet Kenyon⁹, Michelle E Stewart⁹, Denise Lynch⁹, Jorik Loeffler⁹, Adam Caulder⁹, Rosie Hillier⁹, Mohamed M Quwailid⁵, Rumana Zaman⁵, Luis Santos⁵, Yuichi Obata², Mizuho Iwama², Hatsumi Nakata², Tomomi Hashimoto², Masayo Kadota², Hiroshi Masuya², Nobuhiko Tanaka², Ikuo Miura², Ikuko Yamada², Tamio Furuse², Mohammed Selloum¹, Sylvie Jacquot¹, Abdel Ayadi¹, Dalila Ali-Hadji¹, Philippe Charles¹, Elise Le Marchand¹, Amal El Amri¹, Christelle Kujath¹, Jean-Victor Fougerolle¹, Peggy Mellul¹, Sandrine Legeay¹, Laurent Vasseur¹, Anne-Isabelle Moro¹, Romain Lorentz¹, Laurence Schaeffer¹, Dominique Dreyer¹, Valérie Erbs¹, Benjamin Eisenmann¹, Giovanni Rossi¹, Laurence Luppi¹, Annelyse Mertz¹, Amélie Jeanblanc¹, Evelyn Grau³, Caroline Sinclair³, Ellen Brown³, Helen Kundt³, Alla Madich³, Mike Woods³, Laila Pearson³, Danielle Mayhew³, Nicola Griggs³, Richard Houghton³, James Bussell³, Catherine Ingle³, Sara Valentini³, Diane Gleeson³, Debarati Sethi³, Tanya Bayzatinova³, Jonathan Burvill³, Bishoy Habib³, Lauren Weavers³, Ryea Maswood³, Evelina Miklejewska³, Ross Cook³, Radka Platte³, Stacey Price³, Sapna Vyas³, Adam Collinson³, Matt Hardy³, Priya Dalvi³, Vivek Iyer³, Tony West³, Mark Thomas³, Alejandro Mujica³, Elodie Sins³, Daniel Barrett³, Michael Dobbie⁴³, Anne Grobler⁴⁴, Glaudina Loots⁴⁴, Rose Hayeshi⁴⁴, Liezl-Marie Scholtz⁴⁴, Cor Bester⁴⁴, Wiham Pheiffer⁴⁴, Kobus Venter⁴⁴, Fatima Bosch⁴⁵

⁴² INFRAFRONTIER GmbH

⁴³ Phenomics Australia, John Curtin School of Medical Research, The Australian National University, Acton, Canberra, Australia

⁴⁴ PCDDP, North-West University, Potchefstroom, South Africa

⁴⁵ Universitat Autònoma de Barcelona

A full list of members and their affiliations appears in the Supplementary Information.

entirely unexplored¹ The completion of the sequencing of the mouse genome, coupled with the use of mouse embryonic stem (ES) cells for gene targeting to create complex mutant alleles, presented an opportunity to functionally analyze all the protein coding genes of a mammalian species^{2,3} Taking advantage of comprehensive manual annotation of the genome⁴, the International Knockout Mouse Consortium (IKMC) systematically generated single-gene, reporter-tagged null alleles for protein-coding genes by homologous recombination in mouse ES cells^{5,6} Subsequently, large-scale mouse production and phenotyping programs deployed these unique resources, establishing the feasibility of genome-scale mouse production and phenotyping⁷⁻⁹ Building upon these successes, the International Mouse Phenotyping Consortium (IMPC) was established to coordinate a network of programs around the globe, assuring uniformity and reproducibility of these efforts, including standardization of phenotyping protocols and the use of a single inbred mouse strain background, C57BL/6N, with the ultimate goal of generating and phenotyping a single-gene knockout (KO) mouse line for every protein-coding gene in the genome.

Production of KO mice began in concert with the expansion of the ES cell library, but rapidly accelerated after 2011 with the funding of multiple IMPC programs. To date, more than 17,500 individual production attempts (microinjection or aggregation) have resulted in the germline transmission of KO alleles for 5,061 unique genes (Figure 1a; Supplementary Table 1). These lines have been expanded for phenotyping, providing key insights into mammalian biology and disease¹⁰⁻¹⁵; www.mousephenotype.org). To date, phenotype data for these lines shows that overall 72% of lines display at least one phenotype, revealing extensive pleiotropy (Supplementary Figure 1). This includes the 35.8% of lines that show partial or complete lethality, consistent with our earlier finding¹¹. The IMPC contribution extends the total number of genes with targeted KO alleles produced by the scientific community from the 8,391 reported and curated by Mouse Genome Informatics (MGI;¹⁶) (Figure 1b; Supplementary Table 1), to 11,241, or more than half of the genome. Much of the overlap (2,211 genes) reflects specific community requests for the production of novel complex alleles (see below), targeting on an inbred C57BL/6N background, or for mutant mouse lines unavailable through public repositories. The growing use of CRISPR/Cas9 editing to produce null alleles for the IMPC led to the decrease in ES cell-based production beginning in 2015.

While the primary goal of the IKMC and IMPC was to generate and phenotype a null allele for every protein-coding gene, the mutant alleles included additional functional features. All alleles included a *lacZ* reporter cassette to facilitate analysis of gene transcription *in situ* (Supplementary Figure 2; ^{5,6}). A large proportion of the alleles have conditional potential, providing future users with a useful tool for detailed, mechanistic analyses (Supplementary Figure 2a). The multifunctional utility of the alleles produced by the IMPC has greatly expanded the repertoire of genetic resources available to the scientific community. Of the 3,674 unique gene, conditional-ready mouse models generated and validated, 2,987 were novel alleles for genes without an existing conditional allele (81.3%). These nearly double the total number of genes with conditional KO alleles produced by the scientific community as a whole (2,987 IMPC conditional alleles added to the 3,295 conditional alleles reported in MGI as mouse lines; Figure 1c). The impact is even more significant for reporter alleles. The IMPC has produced reporter alleles for 5,059 unique genes, of which 4,433 are novel

(87.6%), complementing the 2,733 produced by the scientific community (Figure 1d). This has nearly tripled the total number of genes with reporter alleles available to the community as mouse lines.

The generation of mouse lines was underpinned by comprehensive quality control strategies for both ES cell karyotype and targeted allele, which ensured efficient production and integrity of the targeting event (Supplementary Table 1; Supplementary Figures 3, 4). Further quality control (QC) analysis also showed that part of the ES cell collection contained an additional insertion of a wild-type nonagouti (*A*) gene on chromosome 8, likely introduced with the targeted reversion event in these cell lines (subclone JM8A3¹⁷). However, as the insertion of the wild-type nonagouti gene results in an agouti coat color, this allele can be easily segregated from the mutant allele in most cases (Supplementary Figure 5). High-throughput allele validation of ES cells was performed using either a suite of quantitative and endpoint PCR-based tests or a combination of Southern blot¹⁸ and PCR-based analysis¹⁹, depending on production center (Supplementary Figures 3, 4 and 6; Supplementary Tables 2, 3; Supplementary Note). Despite these efforts, we found that additional quality control (QC) on the mouse lines themselves was required to ensure all IMPC lines resulted from the transmission of the correctly targeted allele (Supplementary Figure 4; ¹⁹). This additional QC at the mouse level identified a small but significant proportion of incorrect alleles that transmitted through the germline of chimera mice derived from clones that had passed initial and secondary validation QC testing in the ES cell. Our experience highlights the importance of careful allele validation before and after mouse production.

As a result of this effort, mouse lines with targeted alleles for more than 5,000 genes on a C57BL/6N genetic background with extensive and documented genetic validation of the targeted locus are now available to the biomedical research community, supporting high standards of reproducibility for future investigations. This resource nearly triples the number of genes with reporter alleles and almost doubles the number of conditional alleles available to the scientific community. When combined with more than 30 years of community effort, the total mutant allele mouse resource covers more than half of the genome. The IMPC resource has shown its usefulness through the continued and robust uptake of mutant mouse lines by investigators around the world. This includes both KO and conditional alleles with mouse lines distributed as live mice and cryopreserved stocks. To date, over 5,000 orders for mutant mice for 3,301 unique genes have been processed and shipped to more than 4,000 investigators around the world (Figure 1e). To date, more than 1,900 publications acknowledge the use of EUComm/KOMP alleles (for example ^{20–30}). This demonstrates the utility of these resources, the cumulative use of which continues to grow over time, and complements the systematic phenotyping efforts of IMPC centers. In the new era of genome editing, this ES cell-derived collection remains of unique value as it offers particularly sophisticated and quality-controlled alleles representing a cornerstone of the collective development of a null allele resource for the complete mammalian genome ².

All data are freely available from the IMPC database hosted at EMBL-EBI via a web portal (mousephenotype.org), ftp (<ftp://ftp.ebi.ac.uk/pub/databases/impc>) and automatic programmatic interfaces. An archived version of the database will be maintained after

cessation of funding (exp. 2021) for an additional 5 years. Information on alleles, together with phenotype summaries, are additionally archived with Mouse Genome Informatics at the Jackson Laboratory via direct data submissions (J: 136110, J:148605, J:157064, J:157065, J:188991, J:211773).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Marie-Christine Birling¹, Atsushi Yoshiki², David J Adams³, Shinya Ayabe², Arthur L Beaudet^{4,27}, Joanna Bottomley³, Allan Bradley^{3,28}, Steve DM Brown⁵, Antje Bürger⁶, Wendy Bushell^{3,29}, Francesco Chiani⁷, Hsian-Jean Genie Chin⁸, Skevoulla Christou⁹, Gemma F Codner⁹, Francesco J DeMayo^{4,30}, Mary E Dickinson⁴, Brendan Doe³, Leah Rae Donahue¹⁰, Martin D Fray⁹, Alessia Gambadoro⁷, Xiang Gao¹¹, Marina Gertsenstein¹², Alba Gomez-Segura¹⁴, Leslie O Goodwin¹⁰, Jason D Heaney⁴, Yann Hérault¹, Martin Hrabe de Angelis^{15,16,17}, Si-Tse Jiang⁸, Monica J Justice^{4,12,13}, Petr Kasperek¹⁸, Ruairidh E King⁵, Ralf Kühn^{6,31,32}, Ho Lee¹⁹, Young Jae Lee²⁰, Zhiwei Liu²¹, K C Kent Lloyd²², Isabel Lorenzo⁴, Ann-Marie Mallon⁵, Colin McKerlie^{12,13}, Terrence F Meehan^{14,33}, Violeta Munoz Fuentes¹⁴, Stuart Newman^{3,34}, Lauryl MJ Nutter^{12,13}, Goo Taeg Oh²³, Guillaume Pavlovic¹, Ramiro Ramirez-Solis³, Barry Rosen^{3,35}, Edward J Ryder^{3,36}, Luis A Santos⁵, Joel Schick^{6,37}, John R Seavitt⁴, Radislav Sedlacek¹⁸, Claudia Seisenberger⁶, Je Kyung Seong²⁴, William C Skarnes^{3,38}, Tania Sorg¹, Karen P Steel^{3,39}, Masaru Tamura², Glauco P Tocchini-Valentini⁷, Chi-Kuang Leo Wang⁸, Hannah Wardle-Jones³, Marie Wattenhofer-Donzé¹, Sara Wells⁹, Michael V. Wiles¹⁰, Brandon J Willis²², Joshua A Wood²², Wolfgang Wurst^{6,25,26}, Ying Xu²¹, IMPC Consortium*, Lydia Teboul^{9,40}, Stephen A Murray^{10,41}

Affiliations

¹ Université de Strasbourg, CNRS, INSERM, PHENOMIN-ICS, IGBMC, Illkirch, France

² RIKEN BioResource Research Center, Tsukuba, Ibaraki, Japan

³ Wellcome Sanger Institute, Hinxton, UK

⁴ Baylor College of Medicine, Houston, Texas, USA

⁵ MRC Harwell Institute, Mammalian Genetics Unit, Didcot, UK

⁶ Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Neuherberg, Germany

⁷ Monterotondo Mouse Clinic, Italian National Research Council (CNR), Institute of Cell Biology and Neurobiology, Monterotondo Scalo, Italy

⁸ National Laboratory Animal Center, National Applied Research Laboratories (NARLabs), Taipei, Taiwan

- ⁹ MRC Harwell Institute, The Mary Lyon Centre, Didcot, UK
- ¹⁰ The Jackson Laboratory, Bar Harbor, Maine, USA
- ¹¹ SKL of Pharmaceutical Biotechnology and Model Animal Research Center, Collaborative Innovation Center for Genetics and Development, Nanjing Biomedical Research Institute, Nanjing University, Nanjing, China.
- ¹² The Centre for Phenogenomics, Toronto, Ontario, Canada
- ¹³ The Hospital for Sick Children, Toronto, Ontario, Canada
- ¹⁴ European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL- EBI), Hinxton, UK
- ¹⁵ German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health GmbH, Neuherberg, Germany
- ¹⁶ Experimental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Freising-Weihenstephan, Germany
- ¹⁷ German Center for Diabetes Research, Neuherberg, Germany
- ¹⁸ Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Vestec, Czech Republic
- ¹⁹ Korea Mouse Phenotyping Center (KMPC) and Graduate School of Cancer Science and Policy, National Cancer Center, Gyeonggi, Republic of Korea
- ²⁰ Korea Mouse Phenotyping Center (KMPC) and Lee Gil Ya Cancer and Diabetes Institute, Gachon University, Incheon, Republic of Korea
- ²¹ CAM-SU Genomic Resource Center, Soochow University, Suzhou, China
- ²² Mouse Biology Program, University of California, Davis, California, USA
- ²³ Immune and Vascular Cell Network Research Center, National Creative Initiatives and Department of Life Sciences, Ewha Womans University, Seoul, Republic of Korea
- ²⁴ Korea Mouse Phenotyping Center (KMPC) and BK21 Program for Veterinary Science, Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea
- ²⁵ Developmental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Freising-Weihenstephan, Germany
- ²⁶ German Center for Neurodegenerative Diseases, Munich, Germany
- ²⁷ Current affiliation: Luna Genetics, Houston, Texas, USA
- ²⁸ Current affiliation: Cambridge Institute of Therapeutic Immunology & Infectious Disease (CITIID), Jeffrey Cheah Biomedical Centre, University of Cambridge, Cambridge, UK

- ²⁹ Current affiliation: IONTAS Ltd, London Road, Cambridge UK
- ³⁰ Current affiliation: National Institute for Environmental Health Science Research, North Carolina, USA
- ³¹ Current affiliation: Max Delbrueck Center for Molecular Medicine, Berlin, Germany
- ³² Current affiliation: Berlin Institute of Health, Berlin, Germany
- ³³ Current affiliation: Kymab Group Ltd, Cambridge, UK
- ³⁴ Current affiliation: PetMedix Ltd, Cambridge, UK
- ³⁵ Current affiliation: AstraZeneca, Discovery Sciences, Cambridge, UK
- ³⁶ Current affiliation: LGC, Sport and Specialised Analytical Services, Fordham, UK
- ³⁷ Current affiliation: Genetics and Cellular Engineering Group, Institute of Molecular Toxicology and Pharmacology, Helmholtz Zentrum Munich, Neuherberg, Germany
- ³⁸ Current affiliation: The Jackson Laboratory for Genomic Medicine, Farmington, Connecticut, USA
- ³⁹ Current affiliation: Wolfson Centre for Age-Related Diseases, King's College London, London, UK

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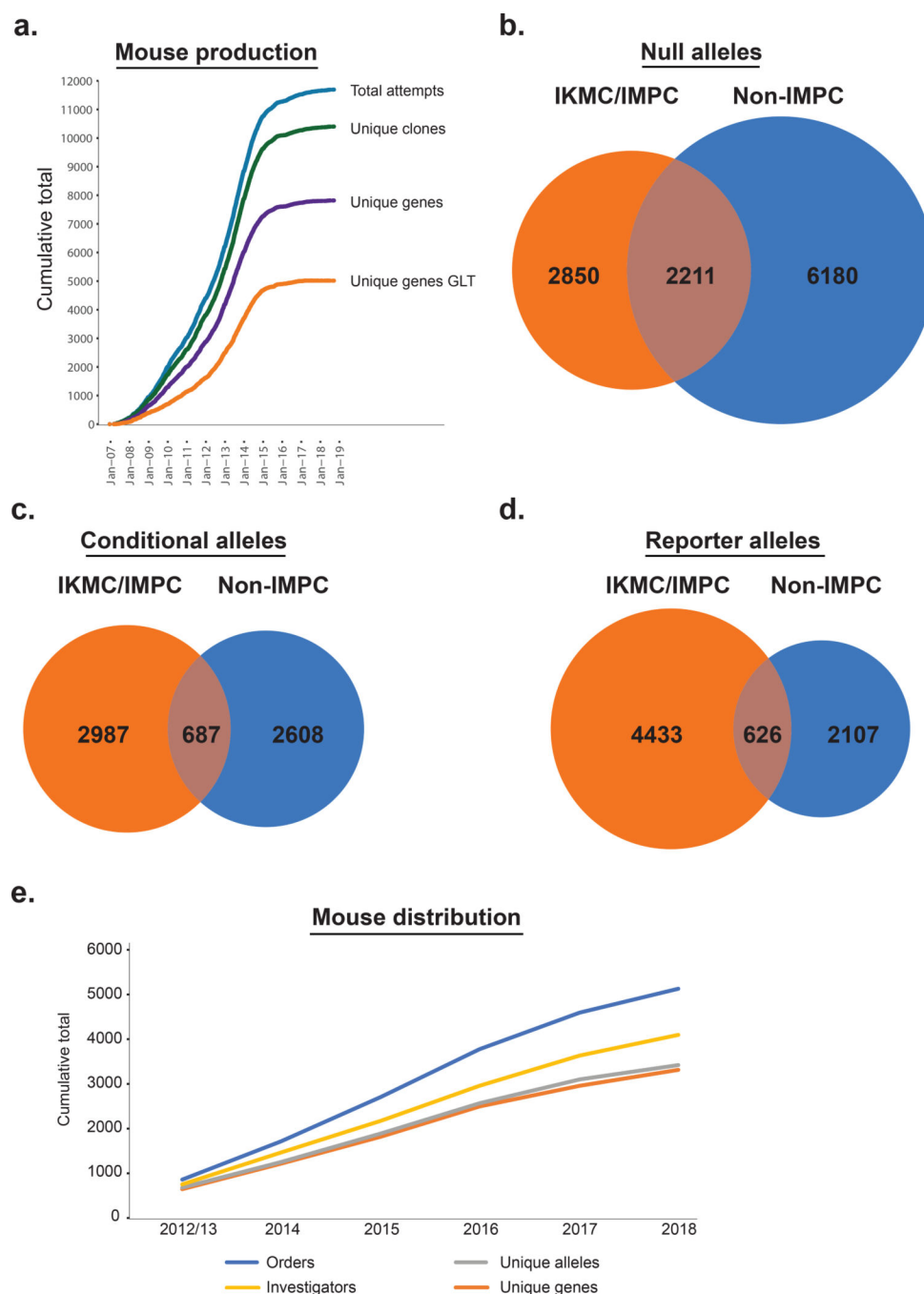
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**Figure 1:**

Generation and impact of targeted alleles for 5,061 unique mouse genes. (a) Cumulative production progress, including all attempts (microinjection or aggregation (black), unique ES cell clones injected (red), unique genes attempted (yellow), and unique genes that achieved germline transmission (GLT; blue). For GLT, the date reflects the date of microinjection, and only reports the first instance of transmission for the small number of duplicate mutations produced. (b) Venn representation of unique gene null alleles produced by the IMPC (orange) and by the rest of the scientific community as reported in MGI ("Non-

IMPC”; blue). (c) Unique gene conditional-ready alleles produced by the IMPC (orange) and by the rest of the scientific community (blue). (d) Unique gene reporter alleles produced by the IMPC (orange) and by the rest of the scientific community (blue). (e) Cumulative mouse orders of IMPC lines processed by production centres and mouse model Repositories from 2012–2018 (blue line). The cumulative number of ordering investigators, unique alleles ordered, and unique genes ordered are shown in yellow, grey, and orange, respectively.

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