Title Page

Title: Rescuing lethal phenotypes induced by disruption of genes in mice: a review of novel strategies

Nándor Lipták1*, Zoltán Gál1, Bálint Biró, László Hiripi and Orsolya Ivett Hoffmann

Short title: Homozygous KO mice lethality

NARIC-Agricultural Biotechnology Institute, Animal Biotechnology Department, Gödöllő, Hungary

1These authors contributed equally to this work.

*Corresponding author: Nándor Lipták, NARIC-Agricultural Biotechnology Institute, Animal Biotechnology Department

H-2100 Gödöllő, Szent-Györgyi Albert st. 4. Hungary

E-mail address: liptak.nandor@abc.naik.hu

Phone: +36-28-526-253

Fax: +36-28-526-151
Summary

Approximately 35% of the mouse genes are indispensable for life, thus, global knock-out (KO) of those genes may result in embryonic or early postnatal lethality due to developmental abnormalities. Several KO mouse lines are valuable human disease models, but viable homozygous mutant mice are frequently required to mirror most symptoms of a human disease. The site-specific gene editing systems, the transcription activator-like effector nucleases (TALENs), Zinc-finger nucleases (ZFNs) and the clustered regularly interspaced short palindrome repeat-associated Cas9 nuclease (CRISPR/Cas9) made the generation of KO mice more efficient than before, but the homozygous lethality is still an undesired side-effect in case of many genes. The literature search was conducted using PubMed and Web of Science databases until June 30th, 2020. The following terms were combined to find relevant studies: “lethality”, “mice”, “knock-out”, “deficient”, “embryonic”, “perinatal”, “rescue”. Additional manual search was also performed to find the related human diseases in the Online Mendelian Inheritance in Man (OMIM) database and to check the citations of the selected studies for rescuing methods. In this review, the possible solutions for rescuing human disease-relevant homozygous KO mice lethal phenotypes were summarized.

Keywords: knock-out mice, CRISPR/Cas9, lethality, knock-out rabbits
Introduction

Generating KO animals gives the opportunity to observe a whole organism if a gene is disrupted and it provides an answer to the origin and course of the appearance of various diseases. The production of these animal models is efficient enough nowadays although, a long journey led to the techniques of developing models that are now easy to produce.

The first two methods to generate KO mice were gene trapping (Gossler et al., 1989) and gene targeting (Mansour et al., 1988). Both methods required embryonic stem cells (ESCs), produced chimeric mice and were neither cost nor time effective. Transposon systems were also practical tools to disrupt genes in mice (Dupuy et al., 2001), however, transposon-based approaches proved to be very effective in creating transgenic animals later (Garrels et al., 2011; Katter et al., 2013). Site-specific endonucleases, TALENs, ZFNs and CRISPR/Cas9 are the latest members of the gene-editing toolbox. TALENs and ZFNs require engineered proteins, while CRISPR/Cas9 is RNA-guided. CRISPR/Cas9 gene editing requires the Cas9 mRNA or protein and the single guide RNA (sgRNA), which consists of the trans-activating RNA and CRISPR RNA. All of the aforementioned endonucleases induce site-specific double-strand breaks (DSBs) in the genome, which are usually repaired by either non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ is predominant during the G1 phase, while HDR is active in S and G2 phases (Zaboikin et al., 2017). Both HDR and NHEJ can evoke small indels and point mutations, but HDR may also generate large insertions in the targeted genes if homologous template DNA is available. CRISPR/Cas9 system became the most efficient and broad spread tool for creating KO laboratory animals, e.g. mice (Wang et al., 2013), rats (Yoshimi et al., 2014), rabbits (Yang et al., 2014), etc., and a less expensive alternative of the previously described TALEN and ZFN applications (Ceasar et al., 2016). The widely accepted method of disrupt genes in animals with site-specific endonucleases is the microinjection of those gene-editing constructs into once-cell stage embryos.
Systemic phenotyping data, which were provided by International Mouse Phenotyping Consortium revealed that approximately 35% of the mouse genes were essential for viability (Brown and Moore, 2012). Several reports, along with recent studies claimed that heterozygous mutant mice did not develop the symptoms of a human disease and the homozygous KO mice were not viable.

The novel strategies to overcome KO mouse embryonic and postnatal lethality are described in detail in the following sections and Table 1, along with the related human diseases.

**Mosaic inactivation of the target gene for rescuing the KO lethal phenotype**

The generation of chimeric mice with gene targeting using ESCs was successful to establish KO mice (Crosby *et al.*, 1998; Lindahl *et al.*, 1998), but this method was laborious and expensive.

Mutations in the serine protease inhibitor Kazal-type 5 (SPINK5) gene were associated with Netherton syndrome, an autosomal recessive disorder which caused dermatitis, severe dehydration due to the malfunctioning epidermal barrier (OMIM 256500) (Chavanas *et al.*, 2000). *Spink5* KO (*Spink5*−/−) mice were created by insertional mutagenesis (Yang *et al.*, 2004) for studying Netherton syndrome, but *Spink5*−/− mice died within few hours after birth. Mosaic inactivation of the *Spink5* gene using TALEN resulted in viable *Spink5*−/− KO mice, an appropriate animal model for human Netherton syndrome (Kasparek *et al.*, 2016). Mosaicism could occur normally if the gene-editing endonucleases activated after the one-cell embryonic stage. One-cell stage embryos were microinjected with different concentrations of TALEN mRNA. Mild skin phenotype was observed in 8% and 17% of the pups from the higher concentrations of TALEN mRNA-groups (Kasparek *et al.*, 2016).
Mutations of cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene were identified as potential basis of autoimmune lymphoproliferative syndrome 5 (ALPS 5, OMIM 616100 (Schubert et al., 2014)). Loss of Cita-4 caused premature lethality in case of gene-targeted Cita-4−/− KO mice (Waterhouse et al., 1995). This issue remained unsolved until the invention of two-cell microinjection (Wang et al., 2017). This method was further developed to create KO mosaic mice with lethal mutations by one-step microinjection of the CRISPR/Cas9 reagents into one blastomere of two-cell stage embryos. Among others, a premature lethal phenotype, which was caused by Cita-4−/− mutation was rescued and Cita-4−/− KO mice survived for more than five months (Wu et al., 2019).

**Disruption of other genes involved in the affected pathway**

In the first two reports, embryonic lethal phenotype caused by the deletion of *Mouse double minute 2 homolog* (*Mdm2*) was rescued by the disruption of *p53* (Jones et al., 1995; Luna et al., 1995). Mdm2−/−p53−/− double KO mice became a valuable model for studying human tumorigenesis (OMIM 614401, (Xiao et al., 1995)).

*SPINK5* gene encodes lympho-epithelial Kazal-type related inhibitor (LEKTI), an inhibitor of kallikrein-related peptidases 5, 7 (KLK5, 7) and other serine proteases in the epidermis (Chavanas et al., 2000). In newborn *Spink5−/−* mice, elevated activation of the pro-kallikrein-cascade in the epidermis and stratum corneum was observed earlier (Sales et al., 2010). Taking advantage of this pathway, *Klk5+/−* mouse line was generated, crossed with *Spink5+/−* mice to create *Klk5−/−Spink5−/−* double KO mice for modelling Netherton syndrome. The loss of *Klk5* rescued the neonatal lethal phenotype, which was evoked by *Spink5* deficiency but the life span of *Klk5−/−Spink5−/−* mice was not as long as wild type (wt) littermates (Furio et al., 2015). *Klk5*-7 were disrupted by gene-targeting and TALEN, respectively, then *Klk5−/−Klk7−/−* double KO
mice mated with Spink5+/− mice. Triple KO mice developed as normally as wt mice and fatal dehydration or severe defects of the epidermal barrier were not detected (Kasparek et al., 2017). In a very recent study, the deletion of Klk5 and Camp (Cathelicidin antimicrobial peptide) were also sufficient to alleviate the severe symptoms evoked by the disruption of Spink5 in mice (Zingkou et al., 2020).

Inducible KO models

Conditional KO technologies were developed for temporal gene expression in mice; e.g. the inducible KO (iKO) method, in which the target gene could be switch on and off with doxycycline treatment (Zeng et al., 2008); the mifeprisone-inducible GeneSwitch approach (Wang et al., 1994), etc.

Polymorphism in INHIBIN alpha (INHA) promoter was associated with premature ovarian failure (OMIM: 147380 (Harris et al., 2005)). Inha deficient mice (Inha−/−) died 12-17 weeks after birth due to gonadal tumors (Froguel et al., 1992). GeneSwitch approach was successfully applied to rescue the lethal phenotype which was evoked by the disruption of Inha in mice. Bigenic mice were produced by crossing of transgenic mice with liver-specific mifepristone-induced chimeric nuclear receptor (GLVP) and transgenic target mice containing a GVLP-responsive promoter upstream of polio-virus internal ribosome entry site-linked sequences coding of inhibin A (Pierson et al., 2000).

Overcome perinatal lethality by integration of the target gene into the X-chromosome

SPINK1 mutations may lead to various types of chronic pancreatitis (OMIM 167800, 608189) (Witt et al., 2000)). Spink3, the mouse homologue of human SPINK1 was disrupted by gene
targeting, but Spink3 KO (Spink3−/−) mice died within two weeks after birth due to the improper inactivation of intrapancreatic trypsinogen and Spink3+/− mice did not develop this disorder (Ohmuraya et al., 2005). For rescuing the lethal phenotype, human SPINK1 minigene was integrated into the X chromosome. Mosaic expression of the SPINK1 mRNA was achieved by the random inactivation of the X-chromosome (Sakata et al., 2016). Spink3+/− and XXSPINK1 knock-in mice were mated to generate Spink3−/−; XXSPINK1 mice. Spink3−/−; XXSPINK1 mice also showed the symptoms of chronic pancreatitis such as loss of acinar cells, intralobular fibrosis but reached sexual maturity and therefore proved to be a valuable model for the human disorder (Sakata et al., 2016).

Tetraploid complementation assay

Placental defects induced by gene deletions are frequently responsible for embryonic lethality in mice (for more details, see review (Rossant and Cross, 2001)). ETS proto-oncogene 2 (ETS2) repressor factor (ERF) mutations evoke craniosynostosis (Twigg et al., 2013) and Chitayat syndrome (OMIM 611888 (Chitayat et al., 1993)). Ets proto-oncogene 2 (Ets2) was disrupted by gene targeting, but Ets2+/− embryos were arrested at day 8 (Yamamoto et al., 1998). Tetraploid complementation assay could be a valuable method where a tetraploid embryo (morula or blastocyst stage) is aggregated with diploid ESCs. The aggregation results a normally developed fetus which is exclusively derived from the ESCs, while the extra-embryonic tissues are completely derived from the tetraploid cells. Ets2-deficient embryos were efficiently rescued by tetraploid complementation (Yamamoto et al., 1998).
Lethal phenotype rescuing by transgene-complementation

X-ray cross-complementing 1 (XRCC1) protein is an indispensable part of the DNA single-strand break repair system. (Whitehouse et al., 2001). Spinocerebellar ataxia was evoked by mutations in the XRCC1 in human patients (Hoch et al., 2017). \textit{Xrcc1}^{+/−} mice were developed by gene targeting earlier to study the functions of that gene, but \textit{Xrcc1}^{−/−} mouse embryos aborted between day 6 and 8, thus, the function of that protein in adult mice was not possible to assess (Tebbs et al., 1999).

\textit{Xrcc1} minigene was integrated into the mouse genome to rescue the phenotype. Although the \textit{Xrcc1} mRNA level was only 10% in transgenic mice compared with wt littermates, this reduced \textit{Xrcc1} mRNA level was enough the overcome embryonic lethality (Tebbs et al., 2003). Lentiviral gene transfer was also effective in rescuing the lethal phenotype evoked by \textit{Ets2}, \textit{Mitogen-activated protein kinase (Mapk)} 14 and \textit{Mapk1} deficiency in mice (Okada et al., 2007).

Tissue-specific deletion of the target gene

Mutations in the glucokinase gene (GCK) were associated with maturity-onset diabetes of the young 2 (MODY-2, OMIM 125851, (Froguel et al., 1992)). Heterozygous \textit{Glucokinase} (\textit{Gck}^{+/−}) mutant mice were generated by gene targeting as a MODY-2 animal model, but their plasma glucose and insulin levels were similar compared to \textit{Gck}^{+/+} mice. Unfortunately, the complete lack of the enzyme resulted in lethality at embryonic day 9.5 (Bali et al., 1995). Numerous mouse lines were created with liver or pancreatic β-cell-specific deletion of the \textit{Gck} gene to prevent embryonic lethality. Pancreatic-specific \textit{Gck}^{−/−} mice died seven days after birth due to glycosuria and severe dehydration, while \textit{Gck}^{+/−} mice showed only mild diabetes (Postic et al., 1999; Terauchi et al., 1995). Liver-specific \textit{Gck}^{−/−} mice were created using Cre/Lox technology. The lack of hepatic \textit{Gck} rescued the lethal phenotype, which was observed in global
and pancreatic $Gck^{-/-}$ mice. Hepatic $Gck^{-/-}$ mice showed mild hyperglycemia and impaired insulin secretion. (Postic et al., 1999) These data were confirmed by another research group later (Zhang et al., 2004).

**Biochemical approaches**

Several attempts were made to create adequate KO mouse models for different types of congenital disorder of glycosylation (CDG), e.g. CDG-Ia, OMIM 212065 (Dupre et al., 2001); CDG-It, OMIM 614921 (Ondruskova et al., 2014), CDG-Ib, OMIM 602579 (Niehues et al., 1998), etc. *Phosphomannose isomerase* KO ($Mpi^{-/-}$) mice died during embryonic development due to mannose 6-phosphate accumulation (DeRossi et al., 2006). *Phosphomannomutase 2*-deficient ($Pmm2^{-/-}$) mouse line, a promising model for human CDG-Ia could not be established due to embryonic lethality (Thiel et al., 2006). *Phosphoglucomutase 2* KO ($Pgm2^{-/-}$) newborn mice were not detected after ten $Pgm2^{+/+} \times Pgm2^{+/-}$ crossings, and the $Pgm2^{+/-}$ mice had different glycosylation pattern compared to human patients with CDG-It (Balakrishnan et al., 2019). Prenatal mannose supplementation was utilized to overcome embryonic lethality of $Pmm2^{-/-}$ mice (Schneider et al., 2011). $Pmm2^{R137H/F118I}$ compound heterozygous mouse line was created as an analog of the human CDG-Ia-associated $Pmm2^{R141H/F122L}$ genotype. 9 mg/mL mannose were added to the drinking water of female $Pmm2^{+/F118L}$ before mating with $Pmm2^{R137H/+}$ males and during pregnancy. This mannose-drinking protocol proved to be an efficient method to rescue lethality of $Pmm2^{R137H/F118L}$ (Schneider et al., 2011) and $Pmm2^{F115L/F115L}$ embryos (Chan et al., 2016), but not in case of $Pmm2^{R137H/F118L}$ mice, a model of the human CDG-Ia-related $Pmm2^{R141H/F119L}$ genotype (Chan et al., 2016). Strikingly, mannose treatment worsened embryonic lethality in the *Phosphomannose isomerase* KO ($Mpi^{-/-}$) (DeRossi et al., 2006) and $Mpi$ hypomorphic mouse line, models of human CDG-Ib (Sharma et al., 2014).
Unfortunately, drinking galactose (9 mg/mL) to pregnant Pgm2+/− mice, model of human CDG-
It was not effective to induce survival of Pgm2−/− mice beyond embryonic development
(Balakrishnan et al., 2019).

Disruption of the target gene in rabbits

If a KO mouse line is not able to develop the symptoms of a human disease, using other
laboratory animals could be a practical option.

GCK mutant rabbits were generated using the CRISPR/Cas9 system for modelling MODY-2
(Froguel et al., 1992). Both GCK+/− and GCK−/− rabbits with frameshift mutations (GCK-FS)
died before sexual maturity. Heterozygous GCK mutant rabbits with non-frameshift mutation
(GCK-NFS) were viable, fertile, hence homozygous GCK-NFS rabbit line could be established.
Homozygous GCK-NFS rabbits had similar symptoms as human MODY-2 patients (elevated
fasting serum glucose, decreased serum insulin, glycosuria), thus may serve as a valuable model
for human MODY-2 (Song et al., 2019).

Nuclear lamin A gene (LMNA) mutations were related to numerous human diseases, e.g.
lipodystrophy (OMIM 151660, (Shackleton et al., 2000)), Hutchinson-Gilford progeria
syndrome (HGPS, OMIM 176670, (Cao and Hegele, 2003)), Emery-Dreifuss muscular
dystrophy (EDMD, OMIM 181350, (Bonne et al., 1999)). Lmna−/− KO mice showed similar
symptoms as human EDMD and HPGS patients but died at 8 and 4 weeks of age, respectively.
Lmna+/− mutant mice developed as normal as wt mice, but human-EDMD or HPGS-like
symptoms were not observed (Mounkes et al., 2003; Sullivan et al., 1999). LMNA−/− KO
rabbits created by CRISPR/Cas9 system as a model for human LMNA mutations-related
disorders (Sui et al., 2019). LMNA−/− KO rabbits had dilated cardiomyopathy, lipodystrophy
and premature aging, reflecting human EDMD, lipodystrophy and HPGS. However, LMNA−/−
rabbits had shorter life span compared with $Lmna^{-/-}$ mice (Sui et al., 2019), indicating a limited application of the LMNA$^{-/-}$ rabbit line for studying human diseases.

**Conclusions**

Embryonic and postnatal lethality limited the translational value of many KO mouse lines in the past three decades. The most promising method to rescue a lethal phenotype is the creation of mosaic mice by TALEN or CRISPR/Cas9 system. This one-step technique can be performed on either one-cell or two-cell stage embryos, but two-cell microinjection is more efficient. The disruption of other genes is a convincing method as well but it requires the development or purchasing of further KO mouse lines and also needs several crossings to establish double or triple KO mice. Mannose supplementation was utilized in several KO mouse line, which were developed for studying various types of human CDG. The success of this approach was restricted to $Pmm2^{R137H/F118L}$ and $Pmm2^{F115L/F115L}$ genotypes, modelling human CDG-Ia. KO rabbits may provide an alternative of KO mice to overcome embryonic or postnatal lethality in the future, but the establishment and characterization of a KO rabbit line could be expensive and time consuming.

**Disclosure statement**

The authors have no conflicts of interest to declare.

**Funding**

This work was supported by the National Research, Development and Innovation Office (NKFIH) grant no. 124708. The project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.
References

BALAKRISHNAN, B, VERHEIJEN, J, LUPO, A, RAYMOND, K, TURGEON, C, YANG, Y, CARTER, KL, WHITEHEAD, KJ, KOZICZ, T, MORAVA, E, and LAI, K: A novel phosphoglucomutase-deficient mouse model reveals aberrant glycosylation and early embryonic lethality. *J Inherit Metab Dis* 42, 998-1007, 2019.

BALI, D, SVETLANOV, A, LEE, HW, FUSCO-DEMANGE, D, LEISER, M, LI, B, BARZILAI, N, SURANA, M, HOU, H, FLEISCHER, N, and ET AL.: Animal model for maturity-onset diabetes of the young generated by disruption of the mouse glucokinase gene. *J Biol Chem* 270, 21464-7, 1995.

BONNE, G, DI BARLETTA, MR, VARNOUS, S, BECANE, HM, HAMMOUDA, EH, MERLINI, L, MUNTONI, F, GREENBERG, CR, GARY, F, URTIZBEREA, JA, DUBOC, D, FARDEAU, M, TONIOLO, D, and SCHWARTZ, K: Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat Genet* 21, 285-8, 1999.

BROWN, SD, and MOORE, MW: The International Mouse Phenotyping Consortium: past and future perspectives on mouse phenotyping. *Mamm Genome* 23, 632-40, 2012.

CAO, H, and HEGELE, RA: LMNA is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). *J Hum Genet* 48, 271-274, 2003.

CEASAR, SA, RAJAN, V, PRYKHOZHIJ, SV, BERMAN, JN, and IGNACIMUTHU, S: Insert, remove or replace: A highly advanced genome editing system using CRISPR/Cas9. *Biochimica Et Biophysica Acta-Molecular Cell Research* 1863, 2333-2344, 2016.

CHAN, B, CLASQUIN, M, SMOLEN, GA, HISTEN, G, POWE, J, CHEN, Y, LIN, Z, LU, C, LIU, Y, CANG, Y, YAN, Z, XIA, Y, THOMPSON, R, SINGLETON, C, DORSCH, M, SILVERMAN, L, SU, SM, FREEZE, HH, and JIN, S: A mouse model of a human congenital disorder of glycosylation caused by loss of PMM2. *Hum Mol Genet* 25, 2182-2193, 2016.

CHAVANAS, S, BODEMER, C, ROCHAT, A, HAMEL-TEILLAC, D, ALI, M, IRVINE, AD, BONAFE, JL, WILKINSON, J, TAEIB, A, BARRANDON, Y, HARPER, JI, DE PROST, Y, and HOVNANIAN, A: Mutations in SPINKS, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 25, 141-2, 2000.

CHITAYAT, D, HAJ-CHAHINE, S, STALKER, HJ, AZOUZ, EM, COTE, A, and HALAL, F: Hyperphalangism, facial anomalies, hallux valgus, and bronchomalacia: a new syndrome? *Am J Med Genet* 45, 1-4, 1993.

CROSBY, JR, SEIFERT, RA, SORIANO, P, and BOWEN-POPE, DF: Chimaeric analysis reveals role of Pdgf receptors in all muscle lineages. *Nat Genet* 18, 385-8, 1998.

DEROSSI, C, BODE, L, EKLUND, EA, ZHANG, F, DAVIS, JA, WESTPHAL, V, WANG, L, BOROWSKY, AD, and FREEZE, HH: Ablation of mouse phosphomannose isomerase (Mpi) causes mannose 6-phosphate accumulation, toxicity, and embryonic lethality. *J Biol Chem* 281, 5916-27, 2006.

DUPRE, T, CUER, M, BARROT, S, BARNIER, A, CORMIER-DAIRE, V, MUNNICH, A, DURAND, G, and SETA, N: Congenital disorder of glycosylation Ia with deficient phosphomannomutase activity but normal plasma glycoprotein pattern. *Clin Chem* 47, 132-4, 2001.

DUPUY, AJ, FRITZ, S, and LARGAESPADA, DA: Transposition and gene disruption in the male germline of the mouse. *Genesis* 30, 82-8, 2001.

FROGUEL, P, VAXILLAIRE, M, SUN, F, HELPO, G, ZOULALI, H, BUTEL, MO, LESAGE, S, VIONNET, N, CLEMENT, K, FOUGEROUSSE, F, and ET AL.: Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. *Nature* 356, 162-4, 1992.

FURIO, L, PAMPALAKIS, G, MICHAEL, IP, NAGY, A, SOTIROPOULOU, G, and HOVNANIAN, A: KLK5 Inactivation Reverses Cutaneous Hallmarks of Netherton Syndrome. *PLoS Genet* 11, e1005389, 2015.
GARRELS, W, MATES, L, HOLLER, S, DALDA, A, TAYLOR, U, PETERSEN, B, NIEMANN, H, IZSVAK, Z, IVICS, Z, and KUES, WA: Germline Transgenic Pigs by Sleeping Beauty Transposition in Porcine Zygotes and Targeted Integration in the Pig Genome. *PLoS One* **6**, 2011.

GOSSLER, A, JOYNER, AL, ROSSANT, J, and SKARNES, WC: Mouse embryonic stem cells and reporter constructs to detect developmentally regulated genes. *Science* **244**, 463-5, 1989.

HARRIS, SE, CHAND, AL, WINSHIP, IM, GERSAK, K, NISHI, Y, YANASE, T, NAWATA, H, and SHELLING, AN: INHA promoter polymorphisms are associated with premature ovarian failure. *Mol Hum Reprod* **11**, 779-84, 2005.

HOCH, NC, HANZLIKOVA, H, RULTEN, SL, TETREAULT, M, KOMULAINEN, E, JU, LM, HORNYYAK, P, ZENG, ZH, GITTENS, W, REY, SA, STARAS, K, MANCINI, GMS, MCKINNON, PJ, WANG, ZQ, WAGNER, JD, YOON, G, CALDECOTT, KW, and CONSORTIUM, CRC: XRCC1 mutation is associated with PARP1 hyperactivation and cerebellar ataxia. *Nature* **541**, 87-+, 2017.

JONES, SN, ROE, AE, DONEhower, LA, and BRADLEY, A: Rescue of Embryonic Lethality in Mdm2-Deficient Mice by Absence of P53. *Nature* **378**, 206-208, 1995.

KASPAREK, P, ILENINOVA, Z, HANECKOVA, R, KANCHEV, I, JENICKOVA, I, and SEDLACEK, R: A viable mouse model for Netherton syndrome based on mosaic inactivation of the Spink5 gene. *Biol Chem* **397**, 1287-1292, 2016.

KASPAREK, P, ILENINOVA, Z, ZBODAKOVA, O, KANCHEV, I, BENADA, O, CHALUPSKY, K, BRATTSAND, M, BECK, IM, and SEDLACEK, R: KLK5 and KLK7 Ablation Fully Rescues Lethality of Netherton Syndrome-Like Phenotype. *PLoS Genet* **13**, e1006566, 2017.

KATTER, K, GEURTS, AM, HOFFMANN, O, MATES, L, LANDA, V, HIRIPI, L, MORENO, C, LAZAR, J, BASHIR, S, ZIDEK, V, POPOVA, E, JERCHOW, B, BECKER, K, DEVARAJ, A, WALTER, I, GRZYBOWKSI, M, CORBETT, M, FILHO, AR, HODGES, MR, BADER, M, IVICS, Z, JACOB, HJ, PRAVENEC, M, BOSZE, Z, RULICKE, T, and IZSVAK, Z: Transposon-mediated transgenesis, transgenic rescue, and tissue-specific gene expression in rodents and rabbits. *Faseb Journal* **27**, 930-41, 2013.

LINDAHL, P, HELLSTROM, M, KALEN, M, CARLSSON, L, PEKNY, M, PEKNA, M, SORIANO, P, and BETSHOLTZ, C: Paracrine PDGF-B/PDGF-Rbeta signaling controls mesangial cell development in kidney glomeruli. *Development* **125**, 3313-22, 1998.

LUNA, RMD, WAGNER, DS, and LOZANO, G: Rescue of Early Embryonic Lethality in Mdm2-Deficient Mice by Deletion of P53. *Nature* **378**, 203-206, 1995.

MANSOUR, SL, THOMAS, KR, and CAPECCHI, MR: Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. *Nature* **336**, 348-52, 1988.

MATZUK, MM, FINEGOLD, MJ, SU, JG, HSUEH, AJ, and BRADLEY, A: Alpha-inhibin is a tumour-suppressor gene with gonadal specificity in mice. *Nature* **360**, 313-9, 1992.

MOUNKES, LC, KOZLOV, S, HERNANDEZ, L, SULLIVAN, T, and STEWART, CL: A progeroid syndrome in mice is caused by defects in A-type lamins. *Nature* **423**, 298-301, 2003.

NIEHUES, R, HASILIK, M, ALTON, G, KORNER, C, SCHIEBE-SUKUMAR, M, KOCH, HG, ZIMMER, KP, WU, RR, HARMS, E, REITER, K, VON FIGURA, K, FREEZE, HH, HARMS, HK, and MARQUARDT, T: Carbohydrate-deficient glycoprotein syndrome type Ib - Phosphomannose isomerase deficiency and mannosé therapy. *Journal of Clinical Investigation* **101**, 1414-1420, 1998.

OHMURAYA, M, HIROTA, M, ARAKI, M, MIZUSHIMA, N, MATSUI, M, MIZUMOTO, T, HARUNA, K, KUME, S, TAKEYA, M, OGAWA, M, ARAKI, K, and YAMAMURA, K: Autophagic cell death of pancreatic acinar cells in serine protease inhibitor Kazal type 3-deficient mice. *Gastroenterology* **129**, 696-705, 2005.

OKADA, Y, UESHIN, Y, ISOTANI, A, SAITO-FUJITA, T, NAKASHIMA, H, KIMURA, K, MIZOGUCHI, A, OH-HORA, M, MORI, Y, OGATA, M, OSHIMA, RG, OKABE, M, and IKAWA, M: Complementation of placental defects and embryonic lethality by trophoblast-specific lentiviral gene transfer. *Nat Biotechnol* **25**, 233-7, 2007.
ONDROSKOVA, N, HONZIK, T, VONDRACKOVA, A, TESAROVA, M, ZEMAN, J, and HANSIKOVA, H: Glycogen storage disease-like phenotype with central nervous system involvement in a PGM1-CDG patient. *Neuro Endocrinol Lett* **35**, 137-41, 2014.

PIERSON, TM, WANG, Y, DEMAYO, FJ, MATZUK, MM, TSAI, SY, and OMALLEY, BW: Regulable expression of inhibin A in wild-type and inhibin alpha null mice. *Mol Endocrinol* **14**, 1075-85, 2000.

POSTIC, C, SHIOTA, M, NISWENDER, KD, JETTON, TL, CHEN, YJ, MOATES, JM, SHELTON, KD, LINDNER, J, CHERINGTON, AD, and MAGNUSON, MA: Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. *Journal of Biological Chemistry* **274**, 305-315, 1999.

ROSSANT, J, and CROSS, JC: Placental development: lessons from mouse mutants. *Nat Rev Genet* **2**, 538-48, 2001.

SAKATA, K, ARAKI, K, NAKANO, H, NISHINA, T, KOMAZAWA-SAKON, S, MURAI, S, LEE, GE, HASHIMOTO, D, SUZUKI, C, UCHIYAMA, Y, NOTOHARA, K, GUKOVSKAYA, AS, GUKOVSKY, I, YAMAMURA, K, BABA, H, and OHMURAYA, M: Novel method to rescue a lethal phenotype through integration of target gene onto the X-chromosome. *Scientific Reports* **6**, 2016.

SALES, KU, MASEDUNSKAS, A, BEY, AL, RASMUSSEN, AL, WRIGHT, R, LIST, K, SZABO, R, OVERBEEK, PA, and BUGGE, TH: Matriptase initiates activation of epidermal pro-kallikrein and disease onset in a mouse model of Netherton syndrome. *Nat Med* **18**, 71-3, 2011.

SCHUBERT, D, BODE, C, KENEFECK, R, HOU, TZ, WING, JB, KENNEDY, A, BULASHEVSKA, A, PETERSEN, BS, SCHaffer, AA, GRUNING, BA, UNGER, S, FREDE, N, BAUMANN, U, WITTE, T, SCHMIDT, RE, DUECKERS, G, NIEHUES, T, SENEVIRATNE, S, KANARIouI, M, SPECKMANN, C, EHL, S, RENSING-EHL, A, WARNATZ, K, RAKHMANOV, M, THIMME, R, HASSELBLATT, P, EMMERICH, F, CATHOMEN, T, BACKOFEN, R, FISCH, P, SEIDL, M, MAY, A, SCHMITT-GRAEFF, A, IKEMIZU, S, SALZER, U, FRANKE, A, SAKAGUCHI, S, WALKER, LSK, ANSOM, DM, and GRIMBACHER, B: Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat Med* **20**, 1410-1416, 2014.

SHACKLETON, S, LLOYD, DJ, JACKSON, SNJ, EVANS, R, NIERMEIJER, MF, SINGH, BM, SCHMIDT, H, BRABANT, G, KUMAR, S, DURRINGTON, PN, GREGORY, S, O’RAHILLY, S, and TREMBATH, RC: LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. *Nat Genet* **24**, 153-156, 2000.

SHARMA, V, NAYAK, J, DEROSII, C, CHARBONO, A, ICHIKAWA, M, NG, BG, GRAJALES-ESQUIVEL, E, SRIVASTAVA, A, WANG, L, HE, P, SCOTT, DA, RUSSELL, J, CONTRERAS, E, GUESS, CM, KRAJEWSKI, S, DEL RIO-TSONIS, K, and FREEZE, HH: Mannose supplements induce embryonic lethality and blindness in phosphomannose isomerase hypomorphic mice. *Faseb Journal* **28**, 1854-69, 2014.

SONG, Y, SUI, T, ZHANG, Y, WANG, Y, CHEN, M, DENG, J, CHAI, Z, LALI, L, and LI, Z: Genetic deletion of a short fragment of glucokinase in rabbit by CRISPR/Cas9 leading to hyperglycemia and other typical features seen in MODY-2. *Cell Mol Life Sci*, 2019.

SUI, T, LI, D, LIU, T, DENG, J, CHEN, M, XU, Y, SONG, Y, OUYANG, H, LALI, L, and LI, Z: LMNA-mutated Rabbits: A Model of Premature Aging Syndrome with Muscular Dystrophy and Dilated Cardiomyopathy. *Aging Dis* **10**, 102-115, 2019.

SULLIVAN, T, ESCALANTE-ALCALDE, D, BHATT, H, ANVER, M, BHAT, N, NAGASHIMA, K, STEWART, CL, and BURKE, B: Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J Cell Biol* **147**, 913-20, 1999.

TEBBS, RS, FLANNERY, ML, MENESES, JJ, HARTMANN, A, TUCKER, JD, THOMPSON, LH, CLEAVER, JE, and PEDERSEN, RA: Requirement for the Xrcc1 DNA base excision repair gene during early mouse development. *Developmental Biology* **208**, 513-529, 1999.

TEBBS, RS, THOMPSON, LH, and CLEAVER, JE: Rescue of Xrcc1 knockout mouse embryo lethality by transgene-complementation. *DNA Repair* **2**, 1405-1417, 2003.
TERAUCCI, Y, SAKURA, H, YASUDA, K, IWAMOTO, K, TAKAHASHI, N, ITO, K, KASAI, H, SUZUKI, H, UEDA, O, KAMADA, N, and ET AL.: Pancreatic beta-cell-specific targeted disruption of glucokinase gene. Diabetes mellitus due to defective insulin secretion to glucose. J Biol Chem 270, 30253-6, 1995.

THIEL, C, LUBEKE, T, MATTHIJS, G, VON FIGURA, K, and KORNER, C: Targeted disruption of the mouse phosphomannomutase 2 gene causes early embryonic lethality. Mol Cell Biol 26, 5615-20, 2006.

TWIGG, SRF, VORGIA, E, MCGOWAN, SJ, PERAKI, I, FENWICK, AL, SHARMA, VP, ALLEGRA, M, ZARAGKOUILAS, A, AKHA, ES, KNIGHT, SJL, LORD, H, LESTER, T, IZATT, L, LAMPE, AK, MOHAMMED, SN, STEWART, FJ, VERLOES, A, WILSON, LC, HEALY, C, SHARPE, PT, HAMMOND, P, HUGHES, J, TAYLOR, S, JOHNSON, D, WALL, SA, MAVROTHALASSITIS, G, and WILKIE, AOM: Reduced dosage of ERF causes complex craniosynostosis in humans and mice and links ERK1/2 signaling to regulation of osteogenesis. Nat Genet 45, 308-313, 2013.

WANG, H, YANG, H, SHIVALILA, CS, DAWLATY, MM, CHENG, AW, ZHANG, F, and JAENISCH, R: One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153, 910-8, 2013.

WANG, L, LI, MY, QU, C, MIAO, WY, YIN, Q, LIAO, J, CAO, HT, HUANG, M, WANG, K, ZUO, E, PENG, G, ZHANG, SX, CHEN, G, LI, Q, TANG, K, YU, Q, LI, Z, WONG, CC, XU, G, JING, N, YU, X, and LI, J: CRISPR-Cas9-mediated genome editing in one blastomere of two-cell embryos reveals a novel Tet3 function in regulating neocortical development. Cell Res 27, 815-829, 2017.

WANG, YL, O'MALLEY, BW, TSAIL, SY, and O'MALLEY, BW: A Regulatory System for Use in Gene-Transfer. Proceedings of the National Academy of Sciences of the United States of America 91, 8180-8184, 1994.

WATERHOUSE, P, PENNINGER, JM, TIMMS, E, WAKEHAM, A, SHAHINIAN, A, LEE, KP, THOMPSON, CB, GRIESSER, H, and MAK, TW: Lymphoproliferative disorders with early lethality in mice deficient in Ctl-a. Science 270, 985-8, 1995.

WHITEHOUSE, CJ, TAYLOR, RM, THISTLETHWAITE, A, ZHANG, H, KARIMI-BUSHERI, F, LASKO, DD, WEINFELD, M, and CALDECOTT, KW: XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. Cell 104, 107-17, 2001.

WITT, H, LUCK, W, HENNIES, HC, CLASSEN, M, KAGE, A, LASS, U, LANDT, O, and BECKER, M: Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. Nat Genet 25, 213-6, 2000.

WU, Y, ZHANG, J, PENG, B, TIAN, D, ZHANG, D, LI, Y, FENG, X, LIU, J, LI, J, ZHANG, T, LIU, X, LU, J, CHEN, B, and WANG, S: Generating viable mice with inheritable embryonically lethal mutations using the CRISPR-Cas9 system in two-cell embryos. Nature Communications 10, 2883, 2019.

XIAO, ZX, CHEN, JD, LEVINE, AJ, MODJTAHEDI, N, XING, J, SELLERS, WR, and LIVINGSTON, DM: Interaction between the Retinoblastoma Protein and the Oncoprotein Mdm2. Nature 375, 694-698, 1995.

YAMAMOTO, H, FLANNERY, ML, KUPIRIYANOVA, S, PEARCE, J, MCKERCHER, SR, HENKEL, GW, MAKI, RA, WERB, Z, and OSHIMA, RG: Defective trophoblast function in mice with a targeted mutation of Ets2. Genes & Development 12, 1315-1326, 1998.

YANG, DS, XU, J, ZHU, TQ, FAN, JL, LAI, LX, ZHANG, JF, and CHEN, YE: Effective gene targeting in rabbits using RNA-guided Cas9 nucleases. Journal of Molecular Cell Biology 6, 97-99, 2014.

YANG, T, LIANG, D, KOCH, PJ, HOHL, D, KHERADMAND, F, and OVERBEEK, PA: Epidermal detachment, desmosomal dissociation, and destabilization of corneodesmosin in Spink5-/- mice. Genes Dev 18, 2354-8, 2004.

YOSHIMI, K, KANEKO, T, VOIGT, B, and MASHIMO, T: Allele-specific genome editing and correction of disease-associated phenotypes in rats using the CRISPR-Cas platform. Nature Communications 5, 4240, 2014.
ZABOIKIN, M, ZABOIKINA, T, FRETER, C, and SRINIVASAKUMAR, N: Non-Homologous End Joining and Homology Directed DNA Repair Frequency of Double-Stranded Breaks Introduced by Genome Editing Reagents. *PLoS One* **12**, e0169931, 2017.

ZENG, H, HORIE, K, MADISEN, L, PAVLOVA, MN, GRAGEROVA, G, ROHDE, AD, SCHIMPF, BA, LIANG, Y, OJALA, E, KRAMER, F, ROTH, P, SLOBODSKAYA, O, DOLKA, I, SOUTHON, EA, TESSAROLLO, L, BORNFELDT, KE, GRAGEROV, A, PAVLAKIS, GN, and GAITANARIS, GA: An inducible and reversible mouse genetic rescue system. *PLoS Genet* **4**, e1000069, 2008.

ZHANG, YL, TAN, XH, XIAO, MF, LI, H, MAO, YQ, YANG, X, and TAN, HR: Establishment of liver specific glucokinase gene knockout mice: a new animal model for screening anti-diabetic drugs. *Acta Pharmacologica Sinica* **25**, 1659-1665, 2004.

ZINGKOU, E, PAMPALAKIS, G, and SOTIROPOULOU, G: Cathelicidin represents a new target for manipulation of skin inflammation in Netherton syndrome. *Biochim Biophys Acta Mol Basis Dis* **1866**, 165831, 2020.
| Human genetic disorders, OMIM entries | Affected human genes | KO mice with lethal phenotype | Methods for phenotype resucing |
|---------------------------------------|----------------------|-----------------------------|-------------------------------|
| ALPS 5, 616100                        | CTLA-4               | Premature lethality (Waterhouse et al., 1995). | Two-cell microinjection, highly effective, CRISPR/Cas9 (Wu et al., 2019). |
| Accelerated tumor formation, 614401   | MDM2                 | Embryonic lethality (Jones et al., 1995; Luna et al., 1995) | Disruption of p53, (Jones et al., 1995; Luna et al., 1995) |
| MODY-2, 125851                        | GCK                  | Embryonic lethality (Bali et al., 1995). | Tissue-specific KO mice, modest success (Postic et al., 1999; Terauchi et al., 1995; Zhang et al., 2004) Homozygous mutant rabbits, CRISPR/Cas9 (Song et al., 2019). |
| Lipodystrophy, 151660 
HGPS, 176670 
EDMD, 181350 | LMNA                 | Premature lethality (Mounkes et al., 2003; Sullivan et al., 1999). | KO rabbits, CRISPR/Cas9, modest success (Sui et al., 2019). |
| Netherton syndrome, 256500            | SPINK5               | Neonatal lethality (Yang et al., 2004). | Mosaic mice, TALEN (Kasparek et al., 2016); Disruption of Klk 5 (Furio et al., 2015); Klk5 and Klk7 (Kasparek et al., 2017); Klk5 and Camp (Zingkou et al., 2020) |
| Craniosynostosis, Chitayat syndrome, 611888 | ERF                 | Embryonic lethality (Yamamoto et al., 1998) | Tetraploid complementation (Yamamoto et al., 1998), lentiviral gene transfer (Okada et al., 2007) |
| SCAR26, 617633                        | XRCC1                | Embryonic lethality (Tebbs et al., 1999) | Transgene complementation (Tebbs et al., 2003) |
| Chronic pancreatitis, 167800, 608189  | SPINK1               | Perinatal lethality (Ohmuraya et al., 2005) | Spink3<sup>−/−</sup>; XX<sup>SPINK1</sup> knock-in mice (Sakata et al., 2016) |
| CDG-Ia, 212065                        | PMM2                 | Embryonic lethality (Thiel et al., 2006) | Mannose drinking, modest success (Schneider et al., 2011), (Chan et al., 2016). Hypomorphic mice (Sharma et al., 2014). No solutions yet, galactose drinking was inefficient (Balakrishnan et al., 2019) |
| CDG-Ib, 602579                        | MPI                  | (DeRossi et al., 2006) | |
| CDG-It, 614921                        | PGM1                 | (Balakrishnan et al., 2019) | |
| Premature ovarian failure, 147380     | INHA                 | (Matzuk et al., 1992) | Bigenic mice, GeneSwitch method (Pierson et al., 2000) |
Abbreviations: ALPS 5: autoimmune lymphoproliferative syndrome 5, CTLA-4: cytotoxic T-lymphocyte antigen-4, MDM2: mouse double minute 2 homolog, MODY-2: maturity-onset diabetes of the young 2, GCK: glu cokinase, Klk: Kallikrein-related peptidase, HPGS: Hutchinson-Gilford progeria syndrome, EDMD: Emery-Dreifuss muscular dystrophy, LMNA: nuclear lamin A, SPINK: serine protease inhibitor Kazal-type, Camp: Cathelicidin antimicrobial peptide, ERF: ETS proto-oncogene 2 (ETS2) repressor factor, SCAR26: spinocerebellar ataxia-26, XRCC1: x-ray cross-complementing 1, CDG: congenital disorder of glycosylation, PMM2: phosphomannomutase 2, MPI: phosphomannose isomerase, PGM1: phosphoglucomutase 1, INHA: inhibin alpha.