

## Gene Knockout Research Literatures

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**Abstract:** A gene knockout is a genetic technique in which one of an organism's genes is made inoperative. Also known as knockout organisms or simply knockouts, they are used in learning about a gene that has been sequenced, but which has an unknown or incompletely known function. Researchers draw inferences from the difference between the knockout organism and normal individuals. The term also refers to the process of creating such an organism, as in "knocking out" a gene. The technique is essentially the opposite of a gene knockin. Knocking out two genes simultaneously in an organism is known as a double knockout. Similarly the terms triple knockout and quadruple knockouts are used to describe three or four knocked out genes, respectively. This article introduces recent research reports as references in the related studies.

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### Introduction

A gene knockout is a genetic technique in which one of an organism's genes is made inoperative. Also known as knockout organisms or simply knockouts, they are used in learning about a gene that has been sequenced, but which has an unknown or incompletely known function. Researchers draw inferences from the difference between the knockout organism and normal individuals. The term also refers to the process of creating such an organism, as in "knocking out" a gene. The technique is essentially the opposite of a gene knockin. Knocking out two genes simultaneously in an organism is known as a double knockout. Similarly the terms triple knockout and quadruple knockouts are used to describe three or four knocked out genes, respectively. The gene approach to the pathogenesis of male infertility may bring about some strategies for the diagnosis and manage of the condition. Gene knockout technology is the mainstream method currently used in the study of gene function. NANOG expression in prostate cancer is highly correlated with cancer stem cell characteristics and resistance to androgen deprivation.

The following introduces recent reports as references in the related studies.

Agrawal, A. F. and M. C. Whitlock "Inferences about the distribution of dominance drawn from yeast gene knockout data." *Genetics*. 2011 Feb;187(2):553-66. doi: 10.1534/genetics.110.124560. Epub 2010 Nov 23.

Data from several thousand knockout mutations in yeast (*Saccharomyces cerevisiae*) were used to estimate the distribution of dominance coefficients. We propose a new unbiased likelihood approach to measuring dominance coefficients. On average, deleterious mutations are partially recessive,

with a mean dominance coefficient ~0.2. Alleles with large homozygous effects are more likely to be more recessive than are alleles of weaker effect. Our approach allows us to quantify, for the first time, the substantial variance and skew in the distribution of dominance coefficients. This heterogeneity is so great that many population genetic processes analyses based on the mean dominance coefficient alone will be in substantial error. These results are applied to the debate about various mechanisms for the evolution of dominance, and we conclude that they are most consistent with models that depend on indirect selection on homeostatic gene expression or on the ability to perform well under periods of high demand for a protein.

Aragona, M. and M. T. Valente "Genetic transformation of the tomato pathogen *Pyrenochaeta lycopersici* allowed gene knockout using a split-marker approach." *Curr Genet*. 2015 May;61(2):211-20. doi: 10.1007/s00294-014-0461-y. Epub 2014 Nov 21.

*Pyrenochaeta lycopersici*, as other soil-transmitted fungal pathogens, generally received little attention compared to the pathogens affecting the aerial parts of the plants, although causing stunt and important fruit yield reduction of agronomic relevant crops. The scope of this study was to develop a system allowing to investigate the functional role of *P. lycopersici* genes putatively involved in the corky root rot of tomato. A genetic transformation system based on a split-marker approach was developed and tested to knock out a *P. lycopersici* gene encoding for a lytic polysaccharide monoxygenase (Pleg11) induced during the disease development. The regions flanking Pleg11 gene were fused with the overlapping parts of

hygromycin marker gene, to favour homologous recombination. We were able to obtain four mutants not expressing the *Pleg1* gene though, when tested on a susceptible tomato cultivar, *Pleg1* mutants showed unaltered virulence, compared with the wild-type strain. The strategy illustrated in the present work demonstrated for the first time that homologous recombination occurs in *P. lycopersici*. Moreover, a transformation system mediated by *Agrobacterium tumefaciens* was established and stable genetic transformants have been obtained. The transformation systems developed represent important tools for investigating both the role of genes putatively involved in *P. lycopersici* interaction with host plant and the function of other physiological traits which emerged to be genetically expanded from the recent genome sequencing of this fungus.

Banerjee, T., D. K. Jaijyan, et al. "Apicoplast triose phosphate transporter (TPT) gene knockout is lethal for *Plasmodium*." *Mol Biochem Parasitol.* 2012 Nov;186(1):44-50. doi: [10.1016/j.molbiopara.2012.09.008](https://doi.org/10.1016/j.molbiopara.2012.09.008). Epub 2012 Oct 3.

The C3, C5, C6 type sugar phosphate transporters bring sugars inside apicoplast, thus providing energy, reducing power and elements like carbon to apicoplast. *Plasmodium berghei* has two C3 type sugar phosphate transporters in the membrane of apicoplast: triose phosphate transporter (TPT) and phosphoenolpyruvate transporter (PPT). Here we report that *P. berghei* TPT knockout parasites failed to survive. However, PPT knockout parasite behaved similar to the wild type in the blood stages. The absence of PPT in other life stages, leads to defects in the development of parasite and was required at both mosquito as well as liver stages. This study also underlines the essentiality of triose transporters for apicoplast and its downstream pathways.

Boini, K. M., M. Xia, et al. "Acid sphingomyelinase gene knockout ameliorates hyperhomocysteinemic glomerular injury in mice lacking cystathionine-beta-synthase." *PLoS One.* 2012;7(9):e45020. Epub 2012 Sep 14.

Acid sphingomyelinase (ASM) has been implicated in the development of hyperhomocysteinemia (hHcys)-induced glomerular oxidative stress and injury. However, it remains unknown whether genetically engineering of ASM gene produces beneficial or detrimental action on hHcys-induced glomerular injury. The present study generated and characterized the mice lacking cystathionine beta-synthase (Cbs) and Asm mouse gene by cross breeding Cbs(+/-) and Asm(+/-) mice. Given that the homozygotes of Cbs(-/-)Asm(-/-) mice could not survive for 3 weeks. Cbs(+/-)Asm(+/+),

Cbs(+/-)Asm(+/-) and Cbs(+/-)Asm(-/-) as well as their Cbs wild type littermates were used to study the role of Asm(-/-) under a background of Cbs(+/-) with hHcys. HPLC analysis revealed that plasma Hcys level was significantly elevated in Cbs heterozygous (Cbs(+/-)) mice with different copies of Asm gene compared to Cbs(+/+) mice with different Asm gene copies. Cbs(+/-)Asm(+/+) mice had significantly increased renal Asm activity, ceramide production and O(2.)(-) level compared to Cbs(+/+)/Asm(+/+), while Cbs(+/-)Asm(-/-) mice showed significantly reduced renal Asm activity, ceramide production and O(2.)(-) level due to increased plasma Hcys levels. Confocal microscopy demonstrated that colocalization of podocin with ceramide was much lower in Cbs(+/-)Asm(-/-) mice compared to Cbs(+/-)Asm(+/+) mice, which was accompanied by a reduced glomerular damage index, albuminuria and proteinuria in Cbs(+/-)Asm(-/-) mice. Immunofluorescent analyses of the podocin, nephrin and desmin expression also illustrated less podocyte damages in the glomeruli from Cbs(+/-)Asm(-/-) mice compared to Cbs(+/-)Asm(+/+) mice. In in vitro studies of podocytes, hHcys-enhanced O(2.)(-) production, desmin expression, and ceramide production as well as decreases in VEGF level and podocin expression in podocytes were substantially attenuated by prior treatment with amitriptyline, an Asm inhibitor. In conclusion, Asm gene knockout or corresponding enzyme inhibition protects the podocytes and glomeruli from hHcys-induced oxidative stress and injury.

Boshra, H., J. Cao, et al. "Generation of Recombinant Capripoxvirus Vectors for Vaccines and Gene Knockout Function Studies." *Methods Mol Biol.* 2016;1349:151-61. doi: [10.1007/978-1-4939-3008-1\\_10](https://doi.org/10.1007/978-1-4939-3008-1_10).

The ability to manipulate capripoxvirus through gene knockouts and gene insertions has become an increasingly valuable research tool in elucidating the function of individual genes of capripoxvirus, as well as in the development of capripoxvirus-based recombinant vaccines. The homologous recombination technique is used to generate capripoxvirus knockout viruses (KO), and is based on the targeting a particular viral gene of interest. This technique can also be used to insert a gene of interest. A protocol for the generation of a viral gene knockout is described. This technique involves the use of a plasmid which encodes the flanking sequences of the regions where the homologous recombination will occur, and will result in the insertion of an EGFP reporter gene for visualization of recombinant virus, as well as the *E. coli* gpt gene as a positive selection marker. If an

additional gene is to be incorporated, this can be achieved by inserting a gene of interest for expression under a poxvirus promoter into the plasmid between the flanking regions for insertion. This chapter describes a protocol for generating such recombinant capripoxviruses.

Brown, A. J., D. A. Fisher, et al. "Whole-rat conditional gene knockout via genome editing." Nat Methods. 2013 Jul;10(7):638-40. doi: [10.1038/nmeth.2516](https://doi.org/10.1038/nmeth.2516). Epub 2013 Jun 9.

Animal models with genetic modifications under temporal and/or spatial control are invaluable to functional genomics and medical research. Here we report the generation of tissue-specific knockout rats via microinjection of zinc-finger nucleases (ZFNs) into fertilized eggs. We generated rats with loxP-flanked (floxed) alleles and a tyrosine hydroxylase promoter-driven cre allele and demonstrated Cre-dependent gene disruption in vivo. Pronuclear microinjection of ZFNs, shown by our data to be an efficient and rapid method for creating conditional knockout rats, should also be applicable in other species.

Carlson, D. F., W. Tan, et al. "Efficient TALEN-mediated gene knockout in livestock." Proc Natl Acad Sci U S A. 2012 Oct 23;109(43):17382-7. doi: [10.1073/pnas.1211446109](https://doi.org/10.1073/pnas.1211446109). Epub 2012 Oct 1.

Transcription activator-like effector nucleases (TALENs) are programmable nucleases that join FokI endonuclease with the modular DNA-binding domain of TALEs. Although zinc-finger nucleases enable a variety of genome modifications, their application to genetic engineering of livestock has been slowed by technical limitations of embryo-injection, culture of primary cells, and difficulty in producing reliable reagents with a limited budget. In contrast, we found that TALENs could easily be manufactured and that over half (23/36, 64%) demonstrate high activity in primary cells. Cytoplasmic injections of TALEN mRNAs into livestock zygotes were capable of inducing gene KO in up to 75% of embryos analyzed, a portion of which harbored biallelic modification. We also developed a simple transposon coselection strategy for TALEN-mediated gene modification in primary fibroblasts that enabled both enrichment for modified cells and efficient isolation of modified colonies. Coselection after treatment with a single TALEN-pair enabled isolation of colonies with mono- and biallelic modification in up to 54% and 17% of colonies, respectively. Coselection after treatment with two TALEN-pairs directed against the same chromosome enabled the isolation of colonies harboring large chromosomal deletions and inversions (10% and 4% of colonies, respectively). TALEN-

modified Ossabaw swine fetal fibroblasts were effective nuclear donors for cloning, resulting in the creation of miniature swine containing mono- and biallelic mutations of the LDL receptor gene as models of familial hypercholesterolemia. TALENs thus appear to represent a highly facile platform for the modification of livestock genomes for both biomedical and agricultural applications.

Chalfin, L., M. Dayan, et al. "Mapping ecologically relevant social behaviours by gene knockout in wild mice." Nat Commun. 2014 Aug 5;5:4569. doi: [10.1038/ncomms5569](https://doi.org/10.1038/ncomms5569).

The laboratory mouse serves as an important model system for studying gene, brain and behavioural interactions. Powerful methods of gene targeting have helped to decipher gene-function associations in human diseases. Yet, the laboratory mouse, obtained after decades of human-driven artificial selection, inbreeding, and adaptation to captivity, is of limited use for the study of fitness-driven behavioural responses that characterize the ancestral wild house mouse. Here, we demonstrate that the backcrossing of wild mice with knockout mutant laboratory mice retrieves behavioural traits exhibited exclusively by the wild house mouse, thereby unmasking gene functions inaccessible in the domesticated mutant model. Furthermore, we show that domestication had a much greater impact on females than on males, erasing many behavioural traits of the ancestral wild female. Hence, compared with laboratory mice, wild-derived mutant mice constitute an improved model system to gain insights into neuronal mechanisms underlying normal and pathological sexually dimorphic social behaviours.

Clasen, B. M., T. J. Stoddard, et al. "Improving cold storage and processing traits in potato through targeted gene knockout." Plant Biotechnol J. 2015 Apr 7. doi: [10.1111/pbi.12370](https://doi.org/10.1111/pbi.12370).

Cold storage of potato tubers is commonly used to reduce sprouting and extend postharvest shelf life. However, cold temperature stimulates the accumulation of reducing sugars in potato tubers. Upon high-temperature processing, these reducing sugars react with free amino acids, resulting in brown, bitter-tasting products and elevated levels of acrylamide—a potential carcinogen. To minimize the accumulation of reducing sugars, RNA interference (RNAi) technology was used to silence the vacuolar invertase gene (VInv), which encodes a protein that breaks down sucrose to glucose and fructose. Because RNAi often results in incomplete gene silencing and requires the plant to be transgenic, here we used transcription activator-like effector nucleases (TALENs) to knockout VInv within the commercial

potato variety, Ranger Russet. We isolated 18 plants containing mutations in at least one VInv allele, and five of these plants had mutations in all VInv alleles. Tubers from full VInv-knockout plants had undetectable levels of reducing sugars, and processed chips contained reduced levels of acrylamide and were lightly coloured. Furthermore, seven of the 18 modified plant lines appeared to contain no TALEN DNA insertions in the potato genome. These results provide a framework for using TALENs to quickly improve traits in commercially relevant autotetraploid potato lines.

Conover, C. A., H. B. Boldt, et al. "Pregnancy-associated plasma protein-A2 (PAPP-A2): tissue expression and biological consequences of gene knockout in mice." Endocrinology. 2011 Jul;152(7):2837-44. doi: 10.1210/en.2011-0036. Epub 2011 May 17.

Pregnancy-associated plasma protein-A2 (PAPP-A2) is a novel homolog of PAPP-A in the metzincin superfamily. However, compared with the accumulating data on PAPP-A, very little is known about PAPP-A2. In this study, we determined the tissue expression pattern of PAPP-A2 mRNA in wild-type (WT) mice and characterized the phenotype of mice with global PAPP-A2 deficiency. Tissues expressing PAPP-A2 in WT mice were more limited than those expressing PAPP-A. The highest PAPP-A2 mRNA expression was found in the placenta, with abundant expression in fetal, skeletal, and reproductive tissues. Heterozygous breeding produced the expected Mendelian distribution for the *pappa2* gene and viable homozygous PAPP-A2 knockout (KO) mice that were normal size at birth. The most striking phenotype of the PAPP-A2 KO mouse was postnatal growth retardation. Male and female PAPP-A2 KO mice had 10 and 25-30% lower body weight, respectively, than WT littermates. Adult femur and body length were also reduced in PAPP-A2 KO mice, but without significant effects on bone mineral density. PAPP-A2 KO mice were fertile, but with compromised fecundity. PAPP-A expression was not altered to compensate for the loss of PAPP-A2 expression, and proteolysis of PAPP-A2's primary substrate, IGF-binding protein-5, was not altered in fibroblasts from PAPP-A2 KO embryos. In conclusion, tissue expression patterns and biological consequences of gene KO indicate distinct physiological roles for PAPP-A2 and PAPP-A in mice.

Crouthamel, M. H., E. J. Kelly, et al. "Development and characterization of transgenic mouse models for conditional gene knockout in the blood-brain and blood-CSF barriers." Transgenic Res. 2012 Feb;21(1):113-30. doi: 10.1007/s11248-011-9512-z.

Epub 2011 May 3.

For many CNS acting drugs, penetration into the central nervous system (CNS) is limited by the blood-CNS-barriers. In an effort to quantitate the role of the protein components that make up the blood-CNS-barriers, we created transgenic mice that allow conditional gene knockout using Cre/loxP technology. We targeted the expression of Cre-recombinase to the choroid plexus (the blood-cerebral spinal fluid barrier) using the lymphotropic papovavirus control region (LPVcr) and to brain endothelium (the blood-brain-barrier) using the proximal promoter region of the human von Willebrand Factor gene (*hVWF-f*). We verified that LPVcr restricts expression to the choroid plexus in adult mice by using the LPVcr to drive *n-LacZ* expression in transgenic mice. The LPV-Cre and *hVWF-Cre* plasmids were then constructed and tested for Cre-recombinase function *in vitro*, and subsequently used to create transgenic mice. The resulting transgenic mice were characterized for cell-type specific Cre-mediated endonuclease activity by crossing them with transgenic mice containing a loxP-flanked-LacZ/EGFP dual reporter gene *Z/EG*. The dual Cre-*Z/EG* transgenic offspring were evaluated for the location of EGFP mRNA expression by reverse transcriptase PCR and for protein expression by immunohistochemistry. Immunohistochemistry for EGFP verified expression in the target cells, and no ectopic expression outside of the expected cell types. The LPV-Cre.0607 transgenic line expressed functional Cre only in the choroid plexus and *hVWF-Cre.1304* line in brain endothelium.

Das, S., R. Periyasamy, et al. "Activation of IKK/NF-kappaB provokes renal inflammatory responses in guanylyl cyclase/natriuretic peptide receptor-A gene-knockout mice." Physiol Genomics. 2012 Apr 2;44(7):430-42. doi: 10.1152/physiolgenomics.00147.2011. Epub 2012 Feb 7.

The present study was aimed at determining the consequences of the disruption of guanylyl cyclase/natriuretic peptide receptor-A (*GC-A/NPRA*) gene (*Npr1*) on proinflammatory responses of nuclear factor kappa B, inhibitory kappa B kinase, and inhibitory kappa B alpha (*NF-kappaB*, *IKK*, *IkappaBalpha*) in the kidneys of mutant mice. The results showed that the disruption of *Npr1* enhanced the renal *NF-kappaB* binding activity by 3.8-fold in 0-copy (-/-) mice compared with 2-copy (+/+) mice. In parallel, *IKK* activity and *IkappaBalpha* protein phosphorylation were increased by 8- and 11-fold, respectively, in the kidneys of 0-copy mice compared with wild-type mice. Interestingly, *IkappaBalpha* was reduced by 80% and the expression of proinflammatory cytokines and renal fibrosis were

significantly enhanced in 0-copy mice than 2-copy mice. Treatment of 0-copy mice with NF-kappaB inhibitors andrographolide, pyrrolidine dithiocarbamate, and etanercept showed a substantial reduction in renal fibrosis, attenuation of proinflammatory cytokines gene expression, and significantly reduced IKK activity and Ikbalpha phosphorylation. These findings indicate that the systemic disruption of Npr1 activates the renal NF-kappaB pathways in 0-copy mice, which transactivates the expression of various proinflammatory cytokines to initiate renal remodeling; however, inhibition of NF-kappaB pathway repairs the abnormal renal pathology in mutant mice.

Dave, K. D., S. De Silva, et al. "Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease." *Neurobiol Dis.* 2014 Oct;70:190-203. doi: 10.1016/j.nbd.2014.06.009. Epub 2014 Jun 24.

Recessively inherited loss-of-function mutations in the PTEN-induced putative kinase 1 (Pink1), DJ-1 (Park7) and Parkin (Park2) genes are linked to familial cases of early-onset Parkinson's disease (PD). As part of its strategy to provide more tools for the research community, The Michael J. Fox Foundation for Parkinson's Research (MJFF) funded the generation of novel rat models with targeted disruption of Pink1, DJ-1 or Parkin genes and determined if the loss of these proteins would result in a progressive PD-like phenotype. Pathological, neurochemical and behavioral outcome measures were collected at 4, 6 and 8 months of age in homozygous KO rats and compared to wild-type (WT) rats. Both Pink1 and DJ-1 KO rats showed progressive nigral neurodegeneration with about 50% dopaminergic cell loss observed at 8 months of age. The Pink1 KO and DJ-1 KO rats also showed a two to three fold increase in striatal dopamine and serotonin content at 8 months of age. Both Pink1 KO and DJ-1 KO rats exhibited significant motor deficits starting at 4 months of age. However, Parkin KO rats displayed normal behaviors with no neurochemical or pathological changes. These results demonstrate that inactivation of the Pink1 or DJ-1 genes in the rat produces progressive neurodegeneration and early behavioral deficits, suggesting that these recessive genes may be essential for the survival of dopaminergic neurons in the substantia nigra (SN). These MJFF-generated novel rat models will assist the research community to elucidate the mechanisms by which these recessive genes produce PD pathology and potentially aid in therapeutic development.

Desrumaux, C., A. Pisoni, et al. "Increased amyloid-

beta peptide-induced memory deficits in phospholipid transfer protein (PLTP) gene knockout mice." *Neuropsychopharmacology.* 2013 Apr;38(5):817-25. doi: 10.1038/npp.2012.247. Epub 2012 Dec 3.

Oxidative stress is recognized as one of the earliest and most intense pathological processes in Alzheimer's disease (AD), and the antioxidant vitamin E has been shown to efficiently prevent amyloid plaque formation and neurodegeneration. Plasma phospholipid transfer protein (PLTP) has a major role in vitamin E transfers in vivo, and PLTP deficiency in mice is associated with reduced brain vitamin E levels. To determine the impact of PLTP on amyloid pathology in vivo, we analyzed the vulnerability of PLTP-deficient (PLTP-KO) mice to the toxic effects induced by intracerebroventricular injection of oligomeric amyloid-beta 25-35 (Abeta 25-35) peptide, a non-transgenic model of AD. Under basal conditions, PLTP-KO mice showed increased cerebral oxidative stress, increased brain Abeta 1-42 levels, and a lower expression of the synaptic function marker synaptophysin, as compared with wild-type mice. This PLTP-KO phenotype was associated with increased memory impairment 1 week after Abeta25-35 peptide injection. Restoration of brain vitamin E levels in PLTP-KO mice through a chronic dietary supplementation prevented Abeta 25-35-induced memory deficits and reduced cerebral oxidative stress and toxicity. We conclude that PLTP, through its ability to deliver vitamin E to the brain, constitutes an endogenous neuroprotective agent. Increasing PLTP activity may offer a new way to develop neuroprotective therapies.

Dey, R., P. K. Dagur, et al. "Live attenuated Leishmania donovani p27 gene knockout parasites are nonpathogenic and elicit long-term protective immunity in BALB/c mice." *J Immunol.* 2013 Mar 1;190(5):2138-49. doi: 10.4049/jimmunol.1202801. Epub 2013 Jan 21.

Leishmaniasis causes significant morbidity and mortality worldwide, and no vaccines against this disease are available. Previously, we had shown that the amastigote-specific protein p27 (Ldp27) is a component of an active cytochrome c oxidase complex in Leishmania donovani and that upon deletion of its gene the parasite had reduced virulence in vivo. In this study, we have shown that Ldp27(-/-) parasites do not survive beyond 20 wk in BALB/c mice and hence are safe as an immunogen. Upon virulent challenge, mice 12 wk postimmunization showed significantly lower parasite burden in the liver and spleen. When mice were challenged 20 wk postimmunization, a significant reduction in parasite burden was still noted, suggesting long-term

protection by Ldp27(-/-) immunization. Immunization with Ldp27(-/-) induced both pro- and anti-inflammatory cytokine responses and activated splenocytes for enhanced leishmanicidal activity in association with NO production. Protection in both short- and long-term immunized mice after challenge with the wild-type parasite correlated with the stimulation of multifunctional Th1-type CD4 and CD8 T cells. Adoptive transfer of T cells from long-term immunized mice conferred protection against virulent challenge in naive recipient mice, suggesting involvement of memory T cell response in protection against *Leishmania* infection. Immunization of mice with Ldp27(-/-) also demonstrated cross-protection against *Leishmania* major and *Leishmania braziliensis* infection. Our data show that genetically modified live attenuated Ldp27(-/-) parasites are safe, induce protective immunity even in the absence of parasites, and can provide protection against homologous and heterologous *Leishmania* species.

Ding, X. J., C. L. Wang, et al. "[Application of gene knockout technology in spermatogenesis-associated gene study]." Zhonghua Nan Ke Xue. 2014 Sep;20(9):850-3.

The gene approach to the pathogenesis of male infertility may bring about some strategies for the diagnosis and manage of the condition. Gene knockout technology is the mainstream method currently used in the study of gene function. Screening and identification of testis-specific genes and insights into their features and functions in spermatogenesis are significant for a further understanding of testicular functions and searching for new therapeutic targets for male reproductive disorders. This review focuses on the application of gene knockout technology in the study of spermatogenesis-associated genes.

Doyle, A., M. P. McGarry, et al. "The construction of transgenic and gene knockout/knockin mouse models of human disease." Transgenic Res. 2012 Apr;21(2):327-49. doi: 10.1007/s11248-011-9537-3. Epub 2011 Jul 29.

The genetic and physiological similarities between mice and humans have focused considerable attention on rodents as potential models of human health and disease. Together with the wealth of resources, knowledge, and technologies surrounding the mouse as a model system, these similarities have propelled this species to the forefront of biomedical research. The advent of genomic manipulation has quickly led to the creation and use of genetically engineered mice as powerful tools for cutting edge studies of human disease research including the discovery, refinement, and utility of many currently available therapeutic regimes. In particular, the

creation of genetically modified mice as models of human disease has remarkably changed our ability to understand the molecular mechanisms and cellular pathways underlying disease states. Moreover, the mouse models resulting from gene transfer technologies have been important components correlating an individual's gene expression profile to the development of disease pathologies. The objective of this review is to provide physician-scientists with an expansive historical and logistical overview of the creation of mouse models of human disease through gene transfer technologies. Our expectation is that this will facilitate on-going disease research studies and may initiate new areas of translational research leading to enhanced patient care.

Ekser, B., J. Bianchi, et al. "Comparison of hematologic, biochemical, and coagulation parameters in alpha1,3-galactosyltransferase gene-knockout pigs, wild-type pigs, and four primate species." Xenotransplantation. 2012 Nov-Dec;19(6):342-54. doi: 10.1111/xen.12007. Epub 2012 Nov 12.

BACKGROUND: The increasing availability of genetically engineered pigs is steadily improving the results of pig organ and cell transplantation in non-human primates (NHPs). Current techniques offer knockout of pig genes and/or knockin of human genes. Knowledge of normal values of hematologic, biochemical, coagulation, and other parameters in healthy genetically engineered pigs and NHPs is important, particularly following pig organ transplantation in NHPs. Furthermore, information on parameters in various NHP species may prove important in selecting the optimal NHP model for specific studies. METHODS: We have collected hematologic, biochemical, and coagulation data on 71 alpha1,3-galactosyltransferase gene-knockout (GTKO) pigs, 18 GTKO pigs additionally transgenic for human CD46 (GTKO.hCD46), four GTKO.hCD46 pigs additionally transgenic for human CD55 (GTKO.hCD46.hCD55), and two GTKO.hCD46 pigs additionally transgenic for human thrombomodulin (GTKO.hCD46.hTBM). RESULTS: We report these data and compare them with similar data from wild-type pigs and the three major NHP species commonly used in biomedical research (baboons, cynomolgus, and rhesus monkeys) and humans, largely from previously published reports. CONCLUSIONS: Genetic modification of the pig (e.g., deletion of the Gal antigen and/or the addition of a human transgene) (i) does not result in abnormalities in hematologic, biochemical, or coagulation parameters that might impact animal welfare, (ii) seems not to alter metabolic function of vital organs, although this needs to be confirmed after their xenotransplantation, and (iii) possibly (though, by no means certainly) modifies

the hematologic, biochemical, and coagulation parameters closer to human values. This study may provide a good reference for those working with genetically engineered pigs in xenotransplantation research and eventually in clinical xenotransplantation.

Fujiwara, T., M. Snada, et al. "HPC-1/syntaxin 1A gene knockout mice show abnormal behavior possibly related to a disruption in 5-HTergic systems." *Eur J Neurosci*. 2010 Jul;32(1):99-107. doi: 10.1111/j.1460-9568.2010.07269.x. Epub 2010 Jun 22.

HPC-1/syntaxin 1A (STX1A) is thought to regulate the exocytosis of synaptic vesicles in neurons. In recent human genetic studies, STX1A has been implicated in neuropsychological disorders. To examine whether STX1A gene ablation is responsible for abnormal neuropsychological profiles observed in human psychiatric patients, we analysed the behavioral phenotype of STX1A knockout mice. Abnormal behavior was observed in both homozygotes (STX1A(-/-)) and heterozygotes (STX1A(+/-)) in a social interaction test, a novel object exploring test and a latent inhibition (LI) test, but not in a pre-pulse inhibition test. Interestingly, attenuation of LI, which is closely related to human schizotypic symptoms, was restored by administration of the selective serotonin reuptake inhibitor, fluoxetine, but not by the dopamine reuptake inhibitor, GBR12935, or the noradrenalin reuptake inhibitor, desipramine. We also observed that LI attenuation was restored by DOI (a 5-HT<sub>2A</sub>) receptor agonist, but not by 8-OH-DPAT (a 5-HT<sub>1A</sub>) receptor agonist, mCPP (a 5-HT<sub>2C</sub>) receptor agonist, SKF 38393 (a D(1) receptor agonist), quinpirole (a D(2)/D(3) receptor agonist) or haloperidol (a D(2)/D(3) receptor antagonist). Thus, attenuation of LI is mainly caused by disruption of 5-HT-ergic systems via 5-HT<sub>2A</sub> receptors. In addition, 5-HT release from hippocampal and hypothalamic slices was significantly reduced. Therefore, ablation of STX1A may cause disruption of 5-HT-ergic transmission and induce abnormal behavior.

Garcia, R. A., D. J. Search, et al. "11beta-hydroxysteroid dehydrogenase type 1 gene knockout attenuates atherosclerosis and in vivo foam cell formation in hyperlipidemic apoE(-/-) mice." *PLoS One*. 2013;8(2):e53192. doi: 10.1371/journal.pone.0053192. Epub 2013 Feb 1.

**BACKGROUND:** Chronic glucocorticoid excess has been linked to increased atherosclerosis and general cardiovascular risk in humans. The enzyme 11beta-hydroxysteroid dehydrogenase type 1 (11betaHSD1) increases active glucocorticoid levels within tissues by catalyzing the conversion of cortisone to cortisol. Pharmacological inhibition of

11betaHSD1 has been shown to reduce atherosclerosis in murine models. However, the cellular and molecular details for this effect have not been elucidated.

**METHODOLOGY/PRINCIPAL FINDINGS:** To examine the role of 11betaHSD1 in atherogenesis, 11betaHSD1 knockout mice were created on the pro-atherogenic apoE(-/-) background. Following 14 weeks of Western diet, aortic cholesterol levels were reduced 50% in 11betaHSD1(-/-)/apoE(-/-) mice vs. 11betaHSD1(+)/(+)/apoE(-/-) mice without changes in plasma cholesterol. Aortic 7-ketocholesterol content was reduced 40% in 11betaHSD1(-/-)/apoE(-/-) mice vs. control. In the aortic root, plaque size, necrotic core area and macrophage content were reduced approximately 30% in 11betaHSD1(-/-)/apoE(-/-) mice. Bone marrow transplantation from 11betaHSD1(-/-)/apoE(-/-) mice into apoE(-/-) recipients reduced plaque area 39-46% in the thoracic aorta. In vivo foam cell formation was evaluated in thioglycollate-elicited peritoneal macrophages from 11betaHSD1(+)/(+)/apoE(-/-) and 11betaHSD1(-/-)/apoE(-/-) mice fed a Western diet for approximately 5 weeks. Foam cell cholesterol levels were reduced 48% in 11betaHSD1(-/-)/apoE(-/-) mice vs. control. Microarray profiling of peritoneal macrophages revealed differential expression of genes involved in inflammation, stress response and energy metabolism. Several toll-like receptors (TLRs) were downregulated in 11betaHSD1(-/-)/apoE(-/-) mice including TLR 1, 3 and 4. Cytokine release from 11betaHSD1(-/-)/apoE(-/-)-derived peritoneal foam cells was attenuated following challenge with oxidized LDL. **CONCLUSIONS:** These findings suggest that 11betaHSD1 inhibition may have the potential to limit plaque development at the vessel wall and regulate foam cell formation independent of changes in plasma lipids. The diminished cytokine response to oxidized LDL stimulation is consistent with the reduction in TLR expression and suggests involvement of 11betaHSD1 in modulating binding of pro-atherogenic TLR ligands.

Gopinath, K., D. F. Warner, et al. "Targeted gene knockout and essentiality testing by homologous recombination." *Methods Mol Biol*. 2015;1285:131-49. doi: 10.1007/978-1-4939-2450-9\_8.

This chapter provides an updated experimental protocol for generating allelic exchange mutants of mycobacteria by two-step selection using the p2NIL/pGOAL system. The types of mutants that can be generated using this approach are targeted gene knockouts marked with a drug resistance gene, unmarked deletion mutants, or strains in which a point mutation/s has been introduced into the target gene. A method for assessing the essentiality of a gene for

mycobacterial growth by means of allelic exchange is also described. This method, which utilizes a merodiploid strain carrying a second copy of the gene of interest on an integration vector, allows the exploration by means of complement switching of structure-function relationships in proteins that are essential for mycobacterial growth.

Hauschild-Quintern, J., B. Petersen, et al. "Gene knockout and knockin by zinc-finger nucleases: current status and perspectives." Cell Mol Life Sci. 2013 Aug;70(16):2969-83. doi: 10.1007/s00018-012-1204-1. Epub 2012 Nov 17.

Zinc-finger nucleases (ZFNs) are engineered site-specific DNA cleavage enzymes that may be designed to recognize long target sites and thus cut DNA with high specificity. ZFNs mediate permanent and targeted genetic alteration via induction of a double-strand break at a specific genomic site. Compared to conventional homology-based gene targeting, ZFNs can increase the targeting rate by up to 100,000-fold; gene disruption via mutagenic DNA repair is similarly efficient. The utility of ZFNs has been shown in many organisms, including insects, amphibians, plants, nematodes, and several mammals, including humans. This broad range of tractable species renders ZFNs a useful tool for improving the understanding of complex physiological systems, to produce transgenic animals, cell lines, and plants, and to treat human disease.

Hickey, R. D., J. B. Lillegard, et al. "Efficient production of Fah-null heterozygote pigs by chimeric adeno-associated virus-mediated gene knockout and somatic cell nuclear transfer." Hepatology. 2011 Oct;54(4):1351-9. doi: 10.1002/hep.24490. Epub 2011 Aug 9.

Hereditary tyrosinemia type I (HT1) results in hepatic failure, cirrhosis, and hepatocellular carcinoma (HCC) early in childhood and is caused by a deficiency in the enzyme fumarylacetoacetate hydrolase (FAH). In a novel approach we used the chimeric adeno-associated virus DJ serotype (AAV-DJ) and homologous recombination to target and disrupt the porcine Fah gene. AAV-DJ is an artificial chimeric AAV vector containing hybrid capsid sequences from three naturally occurring serotypes (AAV2, 8, and 9). The AAV-DJ vector was used to deliver the knockout construct to fetal pig fibroblasts with an average knockout targeting frequency of 5.4%. Targeted Fah-null heterozygote fibroblasts were used as nuclear donors for somatic cell nuclear transfer (SCNT) to porcine oocytes and multiple viable Fah-null heterozygote pigs were generated. Fah-null heterozygotes were phenotypically normal, but had decreased Fah transcriptional and enzymatic activity

compared to wildtype animals. CONCLUSION: This study is the first to use a recombinant chimeric AAV vector to knockout a gene in porcine fibroblasts for the purpose of SCNT. In using the AAV-DJ vector we observed targeting frequencies that were higher than previously reported with other naturally occurring serotypes. We expect that the subsequent generation of FAH-null homozygote pigs will serve as a significant advancement for translational research in the areas of metabolic liver disease, cirrhosis, and HCC.

Higashida, H., O. Lopatina, et al. "Oxytocin signal and social behaviour: comparison among adult and infant oxytocin, oxytocin receptor and CD38 gene knockout mice." J Neuroendocrinol. 2010 May;22(5):373-9. doi: 10.1111/j.1365-2826.2010.01976.x. Epub 2010 Jan 5.

Oxytocin in the hypothalamus is the biological basis of social recognition, trust, love and bonding. Previously, we showed that CD38, a proliferation marker in leukaemia cells, plays an important role in the hypothalamus in the process of oxytocin release in adult mice. Disruption of Cd38 (Cd38 (-/-)) elicited impairment of maternal behaviour and male social recognition in adult mice, similar to the behaviour observed in Oxt and oxytocin receptor (Oxtr) gene knockout (Oxt (-/-) and Oxtr (-/-), respectively) mice. Locomotor activity induced by separation from the dam was higher and the number of ultrasonic vocalisation calls was lower in Cd38 (-/-) than Cd38 (+/+) pups. However, these behavioural changes were much milder than those observed in Oxt (-/-) and Oxtr (-/-) mice, indicating less impairment of social behaviour in Cd38 (-/-) pups. These phenotypes appeared to be caused by the high plasma oxytocin levels during development from the neonatal period to 3-week-old juvenile mice. ADP-ribosyl cyclase activity was markedly lower in the knockout mice from birth, suggesting that weaning for mice is a critical time window of plasma oxytocin differentiation. Breastfeeding was an important exogenous source of plasma oxytocin regulation before weaning as a result of the presence of oxytocin in milk and the dam's mammary glands. The dissimilarity between Cd38 (-/-) infant behaviour and those of Oxt (-/-) or Oxtr (-/-) mice can be explained partly by this exogenous source of oxytocin. These results suggest that secretion of oxytocin into the brain in a CD38-dependent manner may play an important role in the development of social behaviour.

Horiguchi, N., F. Lafdil, et al. "Dissociation between liver inflammation and hepatocellular damage induced by carbon tetrachloride in myeloid cell-specific signal transducer and activator of transcription 3 gene knockout mice." Hepatology. 2010 May;51(5):1724-34. doi: 10.1002/hep.23532.



Liver injury is associated with inflammation, which is generally believed to accelerate the progression of liver diseases; however, clinical data show that inflammation does not always correlate with hepatocellular damage in some patients. Investigating the cellular mechanisms underlying these events using an experimental animal model, we show that inflammation may attenuate liver necrosis induced by carbon tetrachloride (CCl<sub>4</sub>) in myeloid-specific signal transducer and activator of transcription 3 (STAT3) knockout mice. As an important anti-inflammatory signal, conditional deletion of STAT3 in myeloid cells results in markedly enhanced liver inflammation after CCl<sub>4</sub> injection. However, these effects are also accompanied by reduced liver necrosis, correlating with elevated serum interleukin-6 (IL-6) and hepatic STAT3 activation. An additional deletion of STAT3 in hepatocytes in myeloid-specific STAT3 knockout mice restored hepatic necrosis but decreased liver inflammation. **CONCLUSION:** Inflammation-mediated STAT3 activation attenuates hepatocellular injury induced by CCl<sub>4</sub> in myeloid-specific STAT3 knockout mice, suggesting that inflammation associated with a predominance of hepatoprotective cytokines that activate hepatic STAT3 may reduce rather than accelerate hepatocellular damage in patients with chronic liver diseases.

Hosken, I. T., S. W. Sutton, et al. "Relaxin-3 receptor (Rxfp3) gene knockout mice display reduced running wheel activity: implications for role of relaxin-3/RXFP3 signalling in sustained arousal." *Behav Brain Res.* 2015 Feb 1;278:167-75. doi: [10.1016/j.bbr.2014.09.028](https://doi.org/10.1016/j.bbr.2014.09.028). Epub 2014 Sep 22.

Anatomical and pharmacological evidence suggests the neuropeptide, relaxin-3, is the preferred endogenous ligand for the relaxin family peptide-3 receptor (RXFP3) and suggests a number of putative stress- and arousal-related roles for RXFP3 signalling. However, in vitro and in vivo evidence demonstrates exogenous relaxin-3 can activate other relaxin peptide family receptors, and the role of relaxin-3/RXFP3 signalling in specific brain circuits and associated behaviours in mice is not well described. In this study, we characterised the behaviour of cohorts of male and female Rxfp3 gene knockout (KO) mice (C57/B6J(RXFP3<sup>TM1</sup>/DGen)), relative to wild-type (WT) littermates to determine if this receptor KO strain has a similar phenotype to its ligand KO equivalent. Rxfp3 KO mice displayed similar performance to WT littermates in several acute behavioural paradigms designed to gauge motor coordination (rotarod test), spatial memory (Y-maze), depressive-like behaviour (repeat forced-swim test) and sensorimotor gating (prepulse inhibition of acoustic startle). Notably however, male and female

Rxfp3 KO mice displayed robust and consistent (dark phase) hypoactivity on voluntary home-cage running wheels (approximately 20-60% less activity/h), and a small but significant decrease in anxiety-like behavioural traits in the elevated plus maze and light/dark box paradigms. Importantly, this phenotype is near identical to that observed in two independent lines of relaxin-3 KO mice, suggesting these phenotypes are due to the elimination of ligand or receptor and RXFP3-linked signalling. Furthermore, this behavioural characterisation of Rxfp3 KO mice identifies them as a useful experimental model for studying RXFP3-linked signalling and assessing the selectivity and/or potential off-target actions of RXFP3 agonists and antagonists, which could lead to an improved understanding of dysfunctional arousal in mental health disorders, including depression, anxiety, insomnia and neurodegenerative diseases.

Jornayvaz, F. R., H. Y. Lee, et al. "Thyroid hormone receptor-alpha gene knockout mice are protected from diet-induced hepatic insulin resistance." *Endocrinology.* 2012 Feb;153(2):583-91. doi: [10.1210/en.2011-1793](https://doi.org/10.1210/en.2011-1793). Epub 2011 Dec 6.

Nonalcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease in the United States and is strongly associated with hepatic insulin resistance. We examined whether the thyroid hormone receptor-alpha (Thra) would be a potential therapeutic target to prevent diet-induced NAFLD and insulin resistance. For that purpose, we assessed insulin action in high-fat diet-fed Thra gene knockout (Thra<sup>0/0</sup>) and wild-type mice using hyperinsulinemic-euglycemic clamps combined with (3)H/(14)C-labeled glucose to assess basal and insulin-stimulated rates of glucose and fat metabolism. Body composition was assessed by (1)H magnetic resonance spectroscopy and energy expenditure by indirect calorimetry. Relative rates of hepatic glucose and fat oxidation were assessed in vivo using a novel proton-observed carbon-edited nuclear magnetic resonance technique. Thra<sup>0/0</sup> were lighter, leaner, and manifested greater whole-body insulin sensitivity than wild-type mice during the clamp, which could be attributed to increased insulin sensitivity both in liver and peripheral tissues. Increased hepatic insulin sensitivity could be attributed to decreased hepatic diacylglycerol content, resulting in decreased activation of protein kinase Cepsilon and increased insulin signaling. In conclusion, loss of Thra protects mice from high-fat diet-induced hepatic steatosis and hepatic and peripheral insulin resistance. Therefore, thyroid receptor-alpha inhibition represents a novel pharmacologic target for the treatment of NAFLD, obesity, and type 2 diabetes.

Juarez, P., M. G. Valdovinos, et al. "Serotonin(2A/C) receptors mediate the aggressive phenotype of TLX gene knockout mice." Behav Brain Res. 2013 Nov 1;256:354-61. doi: 10.1016/j.bbr.2013.07.044. Epub 2013 Aug 6.

Deleting the tailless (TLX) gene in mice produces a highly aggressive phenotype yet to be characterized in terms of heterozygous animals or neurotransmitter mechanisms. We sought to establish pharmacological control over aggression and study the role of serotonin (5-HT)(2A/C) receptors in mediating changes in aggression. We analyzed aggression in mice heterozygous (+/-) or homozygous (-/-) for the TLX gene and wild-types (++) using a resident-intruder paradigm. No ++ mice were aggressive, 36% of +/- TLX and 100% of -/- TLX mice showed aggression. Dose-effect functions were established for clozapine (0.1-1.5mg/kg, ip), ketanserin (0.3-1.25 mg/kg, ip), and (+/-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane [(+/-)DOI] (0.5-2.0 mg/kg, ip). Injecting clozapine decreased the frequency and duration of attacks for +/- TLX and -/- TLX mice. Clozapine did not decrease grooming in either +/- TLX or -/- TLX mice but may have increased locomotion for -/- TLX mice. Injecting ketanserin, a 5-HT(2A/C) receptor antagonist, produced differential decreases in frequency and latency to aggression between genotypes and corresponding increases in locomotor behavior. Injecting (+/-)DOI, a 5-HT(2A/C) receptor agonist, increased the frequency and duration of attacks, decreased the latency to attacks, and decreased locomotion in +/- and -/- TLX mice. Results of the current study suggest aggression displayed by TLX null and heterozygous mice involves 5-HT(2A/C) receptors.

Kadir, T. A., A. A. Mannan, et al. "Modeling and simulation of the main metabolism in Escherichia coli and its several single-gene knockout mutants with experimental verification." Microb Cell Fact. 2010 Nov 19;9:88. doi: 10.1186/1475-2859-9-88.

**BACKGROUND:** It is quite important to simulate the metabolic changes of a cell in response to the change in culture environment and/or specific gene knockouts particularly for the purpose of application in industry. If this could be done, the cell design can be made without conducting exhaustive experiments, and one can screen out the promising candidates, proceeded by experimental verification of a select few of particular interest. Although several models have so far been proposed, most of them focus on the specific metabolic pathways. It is preferred to model the whole of the main metabolic pathways in Escherichia coli, allowing for the estimation of energy generation and cell synthesis, based on intracellular fluxes and that may be used to characterize phenotypic

growth. **RESULTS:** In the present study, we considered the simulation of the main metabolic pathways such as glycolysis, TCA cycle, pentose phosphate (PP) pathway, and the anapleurotic pathways using enzymatic reaction models of E. coli. Once intracellular fluxes were computed by this model, the specific ATP production rate, the specific CO<sub>2</sub> production rate, and the specific NADPH production rate could be estimated. The specific ATP production rate thus computed was used for the estimation of the specific growth rate. The CO<sub>2</sub> production rate could be used to estimate cell yield, and the specific NADPH production rate could be used to determine the flux of the oxidative PP pathway. The batch and continuous cultivations were simulated where the changing patterns of extracellular and intracellular metabolite concentrations were compared with experimental data. Moreover, the effects of the knockout of such pathways as Ppc, Pck and Pyk on the metabolism were simulated. It was shown to be difficult for the cell to grow in Ppc mutant due to low concentration of OAA, while Pck mutant does not necessarily show this phenomenon. The slower growth rate of the Ppc mutant was properly estimated by taking into account the lower specific ATP production rate. In the case of Pyk mutant, the enzyme level regulation was made clear such that Pyk knockout caused PEP concentration to be up-regulated and activated Ppc, which caused the increase in MAL concentration and backed up reduced PYR through Mez, resulting in the phenotypic growth characteristics similar to the wild type. **CONCLUSIONS:** It was shown to be useful to simulate the main metabolism of E. coli for understanding metabolic changes inside the cell in response to specific pathway gene knockouts, considering the whole main metabolic pathways. The comparison of the simulation result with the experimental data indicates that the present model could simulate the effect of the specific gene knockouts to the changes in the metabolisms to some extent.

Kaniuka, O. P., Z. Filiak Ie, et al. "[Quantitative changes of main components of erythrocyte membranes which define architectonics of cells under pttg gene knockout]." Ukr Biochem J. 2014 Mar-Apr;86(2):41-9.

A pttg gene knockout affects the functional state of erythron in mice which could be associated with structural changes in the structure of erythrocyte membranes. The pttg gene knockout causes a significant modification of fatty acids composition of erythrocyte membrane lipids by reducing the content of palmitic acid and increasing of polyunsaturated fatty acids amount by 18%. Analyzing the erythrocyte

surface architectonics of mice under pttg gene knockout, it was found that on the background of reduction of the functionally complete biconcave discs population one could observe an increase of the number of transformed cells at different degeneration stages. Researches have shown that in mice with a pttg gene knockout compared with a control group of animals cytoskeletal protein--beta-spectrin was reduced by 17.03%. However, there is a reduction of membrane protein band 3 by 33.04%, simultaneously the content of anion transport protein band 4.5 increases by 35.2% and protein band 4.2 by 32.1%. The lectin blot analysis has helped to reveal changes in the structure of the carbohydrate determinants of erythrocyte membrane glycoproteins under conditions of directed pttg gene inactivation, accompanied by changes in the type of communication, which joins the terminal residue in carbohydrate determinant of glycoproteins. Thus, a significant redistribution of protein and fatty acids contents in erythrocyte membranes that manifested in the increase of the deformed shape of red blood cells is observed under pttg gene knockout.

Kato, Y., Y. Kubo, et al. "Gene knockout and metabolome analysis of carnitine/organic cation transporter OCTN1." Pharm Res. 2010 May;27(5):832-40. doi: 10.1007/s11095-010-0076-z. Epub 2010 Mar 12.

**PURPOSE:** Solute carrier OCTN1 (SLC22A4) is an orphan transporter, the physiologically important substrate of which is still unidentified. The aim of the present study was to examine physiological roles of OCTN1. **METHODS:** We first constructed octn1 gene knockout (octn1 (-/-)) mice. Metabolome analysis was then performed to identify substrates in vivo. The possible association of the substrate identified with diseased conditions was further examined. **RESULTS:** The metabolome analysis of blood and several organs indicated complete deficiency of a naturally occurring potent antioxidant ergothioneine in octn1 (-/-) mice among 112 metabolites examined. Pharmacokinetic analyses after oral administration revealed the highest distribution to small intestines and extensive renal reabsorption of [(3)H]ergothioneine, both of which were much reduced in octn1 (-/-) mice. The octn1 (-/-) mice exhibited greater susceptibility to intestinal inflammation under the ischemia and reperfusion model. The blood ergothioneine concentration was also much reduced in Japanese patients with Crohn's disease, compared with healthy volunteers and patients with another inflammatory bowel disease, ulcerative colitis. **CONCLUSIONS:** These results indicate that OCTN1 plays a pivotal role for maintenance of systemic and intestinal exposure of

ergothioneine, which could be important for protective effects against intestinal tissue injuries, providing a possible diagnostic tool to distinguish the inflammatory bowel diseases.

Kawamura, N., K. Nimura, et al. "CRISPR/Cas9-mediated gene knockout of NANOG and NANOGP8 decreases the malignant potential of prostate cancer cells." Oncotarget. 2015 Sep 8;6(26):22361-74.

NANOG expression in prostate cancer is highly correlated with cancer stem cell characteristics and resistance to androgen deprivation. However, it is not clear whether NANOG or its pseudogenes contribute to the malignant potential of cancer. We established NANOG- and NANOGP8-knockout DU145 prostate cancer cell lines using the CRISPR/Cas9 system. Knockouts of NANOG and NANOGP8 significantly attenuated malignant potential, including sphere formation, anchorage-independent growth, migration capability, and drug resistance, compared to parental DU145 cells. NANOG and NANOGP8 knockout did not inhibit in vitro cell proliferation, but in vivo tumorigenic potential decreased significantly. These phenotypes were recovered in NANOG- and NANOGP8-rescued cell lines. These results indicate that NANOG and NANOGP8 proteins are expressed in prostate cancer cell lines, and NANOG and NANOGP8 equally contribute to the high malignant potential of prostate cancer.

Kleigrewe, K., E. M. Niehaus, et al. "New approach via gene knockout and single-step chemical reaction for the synthesis of isotopically labeled fusarin c as an internal standard for the analysis of this fusarium mycotoxin in food and feed samples." J Agric Food Chem. 2012 Aug 29;60(34):8350-5. doi: 10.1021/jf302534x. Epub 2012 Aug 16.

The gold standard for quantitation of contaminants with high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) is the use of isotopically labeled standards. Herein, we report a new strategy for the synthesis of isotopically labeled 21-d3-fusarin C via a genetically modified Fusarium strain, followed by a one-step derivatization reaction. Fusarin C is a Fusarium mycotoxin, which is mutagenic after metabolic activation. Its occurrence has been demonstrated recently in corn-based samples, but up to now, little is known about the contamination of other grain samples. To collect further data, the quantitation method was enhanced by application of the 21-d3-fusarin C and the use of a QTRAP 5500 mass spectrometer. This new method has a limit of detection (LOD) of 1 mug/kg, a limit of quantitation (LOQ) of 4 mug/kg, and a recovery rate of 99%. A total of 21 corn samples

and 13 grain samples were analyzed, with resulting fusarin C levels varying from not detectable to 24.7 mug/kg.

Komine, H., L. Kuhn, et al. "Examination of MARCO activity on dendritic cell phenotype and function using a gene knockout mouse." *PLoS One*. 2013 Jul 1;8(7):e67795. doi: 10.1371/journal.pone.0067795. Print 2013.

We have reported the upregulation of MARCO, a member of the class A scavenger receptor family, on the surface of murine and human dendritic cells (DCs) pulsed with tumor lysates. Exposure of murine tumor lysate-pulsed DCs to an anti-MARCO antibody led to loss of dendritic-like processes and enhanced migratory capacity. In this study, we have further examined the biological and therapeutic implications of MARCO expression by DCs. DCs generated from the bone marrow (bm) of MARCO knockout (MARCO(-)/(-)) mice were phenotypically similar to DCs generated from the bm of wild-type mice and produced normal levels of IL-12 and TNF-alpha when exposed to LPS. MARCO(-)/(-) DCs demonstrated enhanced migratory capacity in response to CCL-21 in vitro. After subcutaneous injection into mice, MARCO(-)/(-) TP-DCs migrated more efficiently to the draining lymph node leading to enhanced generation of tumor-specific IFN-gamma producing T cells and improved tumor regression and survival in B16 melanoma-bearing mice. These results support targeting MARCO on the surface of DCs to improve trafficking and induction of anti-tumor immunity.

Krigel, A., M. P. Felder-Schmittbuhl, et al. "Circadian-clock driven cone-like photoreceptor phagocytosis in the neural retina leucine zipper gene knockout mouse." *Mol Vis*. 2010 Dec 28;16:2873-81.

**PURPOSE:** Whereas much information is available on rod outer segment phagocytosis by the retinal pigmented epithelium (RPE), corresponding data for cones are quite limited, especially in laboratory models of normal rats and mice with very low cone numbers. To characterize the light and circadian control of cone photoreceptor phagocytosis in mice, we capitalized on the blue cone-like phenotype of neural retina leucine zipper gene (Nrl) null mice (Nrl(-/-)). **METHODS:** Nrl(-/-) mice were maintained under standard cyclic light (12h:12h light-dark [LD] cycle; light=300 lux) for one month, then divided into two groups: 1) continued maintenance in LD (36 mice); or 2) transferred to constant darkness (DD; 21 mice) for 36 h. Animals were sacrificed every 3 h over 24 h, and their eyes were rapidly enucleated and fixed. Cryosections were stained using specific cone short-wavelength opsin antibodies. Phagosome

numbers in the RPE were quantified with a morphometric system. We monitored the expression of c-mer proto-oncogene tyrosine kinase (MerTK) in wild-type and knockout mice using a specific MerTK antibody. **RESULTS:** In LD, cone phagocytosis showed a statistically significant peak of activity 1 h after light onset, 2-3 fold higher than at other times. In constant darkness, the temporal phagocytic profile resembled that of LD (significant peak at 1 h of subjective day), but the number of phagosomes was decreased at all time points. Immunostaining of MerTK in wild-type and Nrl(-/-) mice showed expression at the apical surface of the RPE. **CONCLUSIONS:** Cone-like outer segment phagocytosis in Nrl(-/-) mice shows a similar profile to that of rods in normal mice and other species. These data are the first to quantify blue cone-like photoreceptor phagocytosis under different lighting conditions in mice, and suggest this model may constitute a valuable system for investigating circadian regulation of cone function.

Kumar, R. and K. Shimizu "Transcriptional regulation of main metabolic pathways of cyoA, cydB, fnr, and fur gene knockout Escherichia coli in C-limited and N-limited aerobic continuous cultures." *Microb Cell Fact*. 2011 Jan 27;10:3. doi: 10.1186/1475-2859-10-3.

**BACKGROUND:** It is important to understand the cellular responses emanating from environmental perturbations to redesign the networks for practical applications. In particular, the carbon (C) metabolism, nitrogen (N) assimilation, and energy generation are by far important, where those are interconnected and integrated to maintain cellular integrity. In our previous study, we investigated the effect of C/N ratio on the metabolic regulation of gdhA, glnL, glt B,D mutants as well as wild type Escherichia coli (Kumar and Shimizu, MCF, 1-17, 9:8,2010), where it was shown that the transcript levels of cyoA and cydB which encode the terminal oxidases, fnr and fur which encode global regulators were significantly up-regulated under N-limited condition as compared to C-limited condition. In the present study, therefore, the effects of such single-gene knockout on the metabolic regulation were investigated to clarify the roles of those genes in the aerobic continuous culture at the dilution rate of 0.2 h(-1). **RESULTS:** The specific glucose consumption rates and the specific CO2 production rates of cyoA, cydB, fnr, and fur mutants were all increased as compared to the wild type under both C-limited and N-limited conditions. The former phenomenon was consistent with the up-regulations of the transcript levels of ptsG and ptsH, which are consistent with down-regulations of crp and mlc genes. Moreover, the increase in the specific glucose consumption rate was

also caused by up-regulations of the transcript levels of *pfkA*, *pykF* and possibly *zwf*, where those are consistent with the down regulations of *cra*, *crp* and *mlc* genes. Moreover, the transcript levels of *rpoN* together with *glnK*, *glnB*, *glnE* were up-regulated, and thus the transcript levels of *glnA,L,G*, and *gltB,D* as well as *nac* were up-regulated, while *gdhA* was down-regulated. This implies the interconnection between cAMP-Crp and PII-Ntr systems. Moreover, *cyoA*, *cydB*, *fnr* and *fur* gene deletions up-regulated the transcript levels of respiration (*nuoA*, *ndh*, *cyoA*, *cydB*, and *atpA*) and the oxidative stress related genes such as *soxR*, *S* and *sodA*, where this was further enhanced under N-limitation. In the cases of *cyoA* and *cydB* mutants, *arcA*, *fnr*, *fur*, *cydB* (for *cyoA* mutant), and *cyoA* (for *cydB* mutant) genes were up-regulated, which may be due to incomplete oxidation of quinol. It was also shown that *fur* gene transcript level was up-regulated in accordance with the activation of respiratory chain genes. It was shown that the deletion of *fur* gene activated the enterobactin pathway. CONCLUSION: The present result demonstrated how the fermentation characteristics could be explained by the transcript levels of metabolic pathway genes as well as global regulators in relation to the knockout of such single genes as *cyoA*, *cydB*, *fnr*, and *fur*, and clarified the complex gene network regulation in relation to glycolysis, TCA cycle, respiration, and N-regulated pathways. The present result is quite important in understanding the metabolic regulation for metabolic engineering. Moreover, the present result may be useful in improving the specific glucose consumption rate and activation of the TCA cycle by modulating the respiratory chain genes and the related global regulators. The result obtained under N-limited condition may be useful for the heterologous protein production under N-limitation.

Lawson, V. A., H. M. Klemm, et al. "Gene knockout of tau expression does not contribute to the pathogenesis of prion disease." *J Neuropathol Exp Neurol.* 2011 Nov;70(11):1036-45. doi: [10.1097/NEN.0b013e318235b471](https://doi.org/10.1097/NEN.0b013e318235b471).

Prion diseases or transmissible spongiform encephalopathies are a group of fatal and transmissible disorders affecting the central nervous system of humans and animals. The principal agent of prion disease transmission and pathogenesis is proposed to be an abnormal protease-resistant isoform of the normal cellular prion protein. The microtubule-associated protein tau is elevated in patients with Creutzfeldt-Jakob disease. To determine whether tau expression contributes to prion disease pathogenesis, tau knockout and control wild-type mice were infected with the M1000 strain of mouse-adapted human prions. Immunohistochemical analysis for total tau

expression in prion-infected wild-type mice indicated tau aggregation in the cytoplasm of a subpopulation of neurons in regions associated with spongiform change. Western immunoblot analysis of brain homogenates revealed a decrease in total tau immunoreactivity and epitope-specific changes in tau phosphorylation. No significant difference in incubation period or other disease features were observed between tau knockout and wild-type mice with clinical prion disease. These results demonstrate that, in this model of prion disease, tau does not contribute to the pathogenesis of prion disease and that changes in the tau protein profile observed in mice with clinical prion disease occurs as a consequence of the prion-induced pathogenesis.

Martin, L. J. "An approach to experimental synaptic pathology using green fluorescent protein-transgenic mice and gene knockout mice to show mitochondrial permeability transition pore-driven excitotoxicity in interneurons and motoneurons." *Toxicol Pathol.* 2011 Jan;39(1):220-33. doi: [10.1177/0192623310389475](https://doi.org/10.1177/0192623310389475). Epub 2010 Dec 14.

Researchers used transgenic mice expressing enhanced-green fluorescent protein (eGFP) driven by either the glycine transporter-2 gene promoter to specifically visualize glycinergic interneurons or the homeobox-9 (Hb9) gene promoter to visualize motoneurons for assessing their vulnerabilities to excitotoxins in vivo. Stereotaxic excitotoxic lesions were made in adult male and female mouse lumbar spinal cord with the specific N-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid (QA) and the non-NMDA ion channel glutamate receptor agonist kainic acid (KA). QA and KA induced large-scale degeneration of glycinergic interneurons in spinal cord. Glycinergic interneurons were more sensitive than motoneurons to NMDA receptor-mediated and non-NMDA glutamate receptor-mediated excitotoxicity. Outcome after spinal cord excitotoxicity was gender-dependent, with males showing greater sensitivity than females. Excitotoxic degeneration of spinal interneurons resembled apoptosis, while motoneuron degeneration appeared non-apoptotic. Perikaryal mitochondrial accumulation was antecedent to both NMDA and non-NMDA receptor-mediated excitotoxic stimulation of interneurons and motoneurons. Genetic ablation of cyclophilin D, a regulator of the mitochondrial permeability transition pore (mPTP), protected both interneurons and motoneurons from excitotoxicity. The results demonstrate in adult mouse spinal cord that glycinergic interneurons are more sensitive than motoneurons to excitotoxicity that stimulates mitochondrial accumulation, and that the mPTP has pro-death functions mediating apoptotic and non-apoptotic neuronal degeneration in vivo.

Marzan, L. W., K. A. Siddiquee, et al. "Metabolic regulation of an *fir* gene knockout Escherichia coli under oxygen limitation." Bioeng Bugs. 2011 Nov-Dec;2(6):331-7. doi: 10.4161/bbug.2.6.16350. Epub 2011 Nov 1.

In addition to our previous study on the effect of *fir* gene knockout on the metabolism in Escherichia coli under aerobic conditions (Kumar and Shimizu, *Microb Cell Fact* 2011), here we further investigated the effect of *fir* gene knockout on the metabolism under micro-aerobic condition based on gene expressions, enzyme activities and intracellular metabolic fluxes. The objective of the present research is to clarify the metabolic regulation mechanism on how the culture environment, such as oxygen level, affects the cell metabolism in relation to gene expressions, enzyme activities and fluxes via global regulators such as Fnr and ArcA/B systems. Under micro-aerobic condition, the flux through Pfl and Frd were reduced for the mutant, which are due to *fir* gene knockout. The decreased flux through Pfl may have caused accumulation of PYR, which increased the flux through LDH. The *fir* gene knockout caused *arcA* to be downregulated, and thus the TCA cycle was activated, and *cyoA* and *cydB* genes were upregulated. The downregulation of *arcA* caused *lpdA* and *aceE, F* to be upregulated where the flux through PDHc increased. The *fir* gene knockout indirectly caused *cra* gene transcript level to be decreased, which in turn caused the glycolysis genes to be upregulated, which correspond to the increase in the specific glucose consumption rate. The *fir* gene knockout also caused *crp* transcript level to be increased, where there might be some relationship between the two due to similar structure and gene sequence. It may be quite important to understand the metabolic regulation mechanism based on different levels of information for the efficient metabolic engineering and control of the culture environment for process optimization.

Matsunaga, T. and J. K. Yamashita "Single-step generation of gene knockout-rescue system in pluripotent stem cells by promoter insertion with CRISPR/Cas9." Biochem Biophys Res Commun. 2014 Feb 7;444(2):158-63. doi: 10.1016/j.bbrc.2014.01.037. Epub 2014 Jan 22.

Specific gene knockout and rescue experiments are powerful tools in developmental and stem cell biology. Nevertheless, the experiments require multiple steps of molecular manipulation for gene knockout and subsequent rescue procedures. Here we report an efficient and single step strategy to generate gene knockout-rescue system in pluripotent stem cells by promoter insertion with CRISPR/Cas9 genome editing technology. We inserted a

tetracycline-regulated inducible gene promoter (tet-OFF/TRE-CMV) upstream of the endogenous promoter region of vascular endothelial growth factor receptor 2 (VEGFR2/Flk1) gene, an essential gene for endothelial cell (EC) differentiation, in mouse embryonic stem cells (ESCs) with homologous recombination. Both homo- and hetero-inserted clones were efficiently obtained through a simple selection with a drug-resistant gene. The insertion of TRE-CMV promoter disrupted endogenous Flk1 expression, resulting in null mutation in homo-inserted clones. When the inserted TRE-CMV promoter was activated with doxycycline (Dox) depletion, Flk1 expression was sufficiently recovered from the downstream genomic Flk1 gene. Whereas EC differentiation was almost completely perturbed in homo-inserted clones, Flk1 rescue with TRE-CMV promoter activation restored EC appearance, indicating that phenotypic changes in EC differentiation can be successfully reproduced with this knockout-rescue system. Thus, this promoter insertion strategy with CRISPR/Cas9 would be a novel attractive method for knockout-rescue experiments.

Middleton, R. J., G. J. Liu, et al. "Guwiyang Wurra-'Fire Mouse': a global gene knockout model for TSPO/PBR drug development, loss-of-function and mechanisms of compensation studies." Biochem Soc Trans. 2015 Aug 1;43(4):553-8. doi: 10.1042/BST20150039. Epub 2015 Aug 3.

The highly conserved 18-kDa translocator protein (TSPO) or peripheral benzodiazepine receptor (PBR), is being investigated as a diagnostic and therapeutic target for disease conditions ranging from inflammation to neurodegeneration and behavioural illnesses. Many functions have been attributed to TSPO/PBR including a role in the mitochondrial permeability transition pore (MPTP), steroidogenesis and energy metabolism. In this review, we detail the recent developments in determining the physiological role of TSPO/PBR, specifically based on data obtained from the recently generated *Tspo* knockout mouse models. In addition to defining the role of TSPO/PBR, we also describe the value of *Tspo* knockout mice in determining the selectivity, specificity and presence of any off-target effects of TSPO/PBR ligands.

Mitchell, K., E. E. Lebovitz, et al. "Nociception and inflammatory hyperalgesia evaluated in rodents using infrared laser stimulation after *Trpv1* gene knockout or resiniferatoxin lesion." Pain. 2014 Apr;155(4):733-45. doi: 10.1016/j.pain.2014.01.007. Epub 2014 Jan 13.

TRPV1 is expressed in a subpopulation of myelinated Adelta and unmyelinated C-fibers.

TRPV1+ fibers are essential for the transmission of nociceptive thermal stimuli and for the establishment and maintenance of inflammatory hyperalgesia. We have previously shown that high-power, short-duration pulses from an infrared diode laser are capable of predominantly activating cutaneous TRPV1+ Adelta-fibers. Here we show that stimulating either subtype of TRPV1+ fiber in the paw during carrageenan-induced inflammation or following hind-paw incision elicits pronounced hyperalgesic responses, including prolonged paw guarding. The ultrapotent TRPV1 agonist resiniferatoxin (RTX) dose-dependently deactivates TRPV1+ fibers and blocks thermal nociceptive responses in baseline or inflamed conditions. Injecting sufficient doses of RTX peripherally renders animals unresponsive to laser stimulation even at the point of acute thermal skin damage. In contrast, *Trpv1*<sup>-/-</sup> mice, which are generally unresponsive to noxious thermal stimuli at lower power settings, exhibit withdrawal responses and inflammation-induced sensitization using high-power, short duration Adelta stimuli. In rats, systemic morphine suppresses paw withdrawal, inflammatory guarding, and hyperalgesia in a dose-dependent fashion using the same Adelta stimuli. The qualitative intensity of Adelta responses, the leftward shift of the stimulus-response curve, the increased guarding behaviors during carrageenan inflammation or after incision, and the reduction of Adelta responses with morphine suggest multiple roles for TRPV1+ Adelta fibers in nociceptive processes and their modulation of pathological pain conditions.

Mitoma, J. and M. Fukuda "Core O-glycans required for lymphocyte homing gene knockout mice of core 1 beta1,3-N-acetylglucosaminyltransferase and core 2 N-acetylglucosaminyltransferase." Methods Enzymol. 2010;479:257-70. doi: [10.1016/S0076-6879\(10\)79015-7](https://doi.org/10.1016/S0076-6879(10)79015-7).

Mucin-type O-glycans are synthesized by sequential reaction of glycosyltransferases that have different substrate specificities. To know the significance of specific O-glycan structures, many researchers have been making mice deficient in corresponding enzymes for the synthesis of the O-glycan structures. Here we describe the analysis of gene knockout mice of core 2 branching enzyme (core 2 N-acetylglucosaminyltransferase, Core2GlcNAcT) and core 1 extension enzyme (core 1 beta1,3-N-acetylglucosaminyltransferase, Core1-beta3GlcNAcT). Because mucin-type O-glycans present sialyl Lewis X (sLeX) and sulfated version of the glycans, which are L-selectin ligands, at the reducing end, the amounts of the ligands of these knockout mice would be reduced. The methods described here are to analyze the interaction between L-selectin and its ligand 6-sulfo

sLeX such as lymphocyte homing assay, staining of frozen section, and blotting using L- and E-selectin-IgM chimeric proteins.

Miyasaka, K., T. Fujimoto, et al. "Pancreatic hypertrophy in Ki-ras-induced actin-interacting protein gene knockout mice." Pancreas. 2011 Jan;40(1):79-83. doi: [10.1097/MPA.0b013e3181f66c22](https://doi.org/10.1097/MPA.0b013e3181f66c22).

**OBJECTIVES:** Pancreatic functions were determined in a Ki-ras-induced actin-interacting protein (KRAP)-deficient (-/-) mouse mutant. **METHODS:** Pancreatic enzyme, protein, and DNA contents were measured, and histological examinations were conducted. The mixture of bile-pancreatic juice was collected, and amylase and bile acid outputs were determined. Oral glucose tolerance test was determined. Moreover, the gene expression of KRAP was determined in cholecystokinin (CCK)-A(1) receptor (-/-) mice. **RESULTS:** The body weight was smaller, and the ratio of pancreatic wet weight/body weight was higher in KRAP(-/-) mice compared with wild-type mice. The enzyme contents, but not DNA content, in the pancreas of KRAP(-/-) mice were higher than those of wild-type mice. Histological examination revealed the increase in the number of zymogen granules in the pancreatic acinar cells of KRAP(-/-) mice. Amylase secretions in response to CCK-octapeptide sulfate were significantly higher in KRAP(-/-) than wild-type mice, whereas the basal secretion did not differ between the 2 genotypes. A normal glucose tolerance was observed in KRAP(-/-) mice. The gene expression of KRAP in CCK-A(1) receptor (-/-) mice was significantly lower than in wild-type mice. **CONCLUSIONS:** The lack and/or decrease in KRAP level in the pancreas may promote the pancreatic growth and hypertrophy.

Nagahori, N., T. Yamashita, et al. "Effect of ganglioside GM3 synthase gene knockout on the glycoprotein N-glycan profile of mouse embryonic fibroblast." Chembiochem. 2013 Jan 2;14(1):73-82. doi: [10.1002/cbic.201200641](https://doi.org/10.1002/cbic.201200641). Epub 2012 Dec 7.

The structural and clinical significance of cellular glycoproteins and glycosphingolipids (GSLs) are often separately discussed. Considering the biosynthetic pathway of glycoconjugates, glycans of cell-surface glycoproteins and GSLs might partially share functions in maintaining cellular homeostasis. The purpose of this study is to establish a general and comprehensive glycomics protocol for cellular GSLs and N-glycans of glycoproteins. To test the feasibility of a glycoblotting-based protocol, whole glycans released both from GSLs and glycoproteins were profiled concurrently by using GM3 synthase-deficient mouse embryonic fibroblast GM3(-/-).

GM3(-/-) cells did not synthesize GM3 or any downstream product of GM3 synthase. Instead, expression levels of o-series gangliosides involving GM1-b and GD1-alpha increased dramatically, whereas a-/b-series gangliosides were predominantly detected in wild-type (WT) cells. We also discovered that glycoprotein N-glycan profiles of GM3(-/-) cells are significantly altered as compared to WT cells, although GM3 synthase is responsible only for GSLs synthesis and is not associated with glycoprotein N-glycan biosynthesis. The present approach allows for high-throughput profiling of cellular glycomes enriched by different classes of glycoconjugates, and our results demonstrated that gene knockout of the enzymes responsible for GSL biosynthesis significantly influences the N-glycans of glycoproteins.

Nazierbieke, W., Y. Zhang, et al. "[Construction and characterization of ompH gene knockout mutant of avian *Pasteurella multocida* C48-3]." Wei Sheng Wu Xue Bao. 2013 Jan 4;53(1):66-73.

**OBJECTIVE:** To study the role of the outer membrane protein H (OmpH) in pathogenicity of avian *Pasteurella multocida*. **METHODS:** The ompH knock-out mutant of avian *P. multocida* C48-3 was constructed by homologous recombination. The DNA replacement was confirmed by PCR, RT-PCR and Western blot. We compared the differences of biological characteristics such as growth rate, capsular structure, adhesion ability and virulence between the ompH knockout mutant of C48-3 Delta ompH and parent strain C48-3, as well as the complemented strain C48-3C. **RESULTS:** C48-3 Delta ompH was successfully constructed. Electron microscopy examination of C48-3 Delta ompH shows the absence of capsular material compared to the parent strain C48-3 and complemented strain C48-3C. The adhesion assay shows that the number of C48-3 Delta ompH adhered to CEF cells was significantly lower than that of C48-3 and C48-3C. C48-3 Delta ompH was relatively attenuated in mice by intraperitoneal injection. **CONCLUSION:** The construction of C48-3 Delta ompH would facilitate further study on pathogenesis of avian *Pasteurella multocida*.

Nguyen, P. T., T. Nakamura, et al. "Cognitive and socio-emotional deficits in platelet-derived growth factor receptor-beta gene knockout mice." PLoS One. 2011 Mar 18;6(3):e18004. doi: 10.1371/journal.pone.0018004.

Platelet-derived growth factor (PDGF) is a potent mitogen. Extensive in vivo studies of PDGF and its receptor (PDGFR) genes have reported that PDGF plays an important role in embryogenesis and development of the central nervous system (CNS).

Furthermore, PDGF and the beta subunit of the PDGF receptor (PDGFR-beta) have been reported to be associated with schizophrenia and autism. However, no study has reported on the effects of PDGF deletion on mice behavior. Here we generated novel mutant mice (PDGFR-beta KO) in which PDGFR-beta was conditionally deleted in CNS neurons using the Cre/loxP system. Mice without the Cre transgene but with floxed PDGFR-beta were used as controls. Both groups of mice reached adulthood without any apparent anatomical defects. These mice were further examined by conducting several behavioral tests for spatial memory, social interaction, conditioning, prepulse inhibition, and forced swimming. The test results indicated that the PDGFR-beta KO mice show deficits in all of these areas. Furthermore, an immunohistochemical study of the PDGFR-beta KO mice brain indicated that the number of parvalbumin (calcium-binding protein)-positive (i.e., putatively gamma-aminobutyric acid-ergic) neurons was low in the amygdala, hippocampus, and medial prefrontal cortex. Neurophysiological studies indicated that sensory-evoked gamma oscillation was low in the PDGFR-beta KO mice, consistent with the observed reduction in the number of parvalbumin-positive neurons. These results suggest that PDGFR-beta plays an important role in cognitive and socioemotional functions, and that deficits in this receptor may partly underlie the cognitive and socioemotional deficits observed in schizophrenic and autistic patients.

Osiak, A., F. Radecke, et al. "Selection-independent generation of gene knockout mouse embryonic stem cells using zinc-finger nucleases." PLoS One. 2011;6(12):e28911. doi: 10.1371/journal.pone.0028911. Epub 2011 Dec 14.

Gene knockout in murine embryonic stem cells (ESCs) has been an invaluable tool to study gene function in vitro or to generate animal models with altered phenotypes. Gene targeting using standard techniques, however, is rather inefficient and typically does not exceed frequencies of 10<sup>-6</sup>. In consequence, the usage of complex positive/negative selection strategies to isolate targeted clones has been necessary. Here, we present a rapid single-step approach to generate a gene knockout in mouse ESCs using engineered zinc-finger nucleases (ZFNs). Upon transient expression of ZFNs, the target gene is cleaved by the designer nucleases and then repaired by non-homologous end-joining, an error-prone DNA repair process that introduces insertions/deletions at the break site and therefore leads to functional null mutations. To explore and quantify the potential of ZFNs to generate a gene knockout in pluripotent stem cells, we generated a mouse ESC line containing an X-chromosomally integrated EGFP marker gene.



Applying optimized conditions, the EGFP locus was disrupted in up to 8% of ESCs after transfection of the ZFN expression vectors, thus obviating the need of selection markers to identify targeted cells, which may impede or complicate downstream applications. Both activity and ZFN-associated cytotoxicity was dependent on vector dose and the architecture of the nuclease domain. Importantly, teratoma formation assays of selected ESC clones confirmed that ZFN-treated ESCs maintained pluripotency. In conclusion, the described ZFN-based approach represents a fast strategy for generating gene knockouts in ESCs in a selection-independent fashion that should be easily transferrable to other pluripotent stem cells.

Park, T. S., H. J. Lee, et al. "Targeted gene knockout in chickens mediated by TALENs." Proc Natl Acad Sci U S A. 2014 Sep 2;111(35):12716-21. doi: [10.1073/pnas.1410555111](https://doi.org/10.1073/pnas.1410555111). Epub 2014 Aug 19.

Genetically modified animals are used for industrial applications as well as scientific research, and studies on these animals contribute to a better understanding of biological mechanisms. Gene targeting techniques have been developed to edit specific gene loci in the genome, but the conventional strategy of homologous recombination with a gene-targeted vector has low efficiency and many technical complications. Here, we generated specific gene knockout chickens through the use of transcription activator-like effector nuclease (TALEN)-mediated gene targeting. In this study, we accomplished targeted knockout of the ovalbumin (OV) gene in the chicken primordial germ cells, and OV gene mutant offspring were generated through test-cross analysis. TALENs successfully induced nucleotide deletion mutations of ORF shifts, resulting in loss of chicken OV gene function. Our results demonstrate that the TALEN technique used in the chicken primordial germ cell line is a powerful strategy to create specific genome-edited chickens safely for practical applications.

Pelascini, L. P., I. Maggio, et al. "Histone deacetylase inhibition rescues gene knockout levels achieved with integrase-defective lentiviral vectors encoding zinc-finger nucleases." Hum Gene Ther Methods. 2013 Dec;24(6):399-411. doi: [10.1089/hgtb.2013.107](https://doi.org/10.1089/hgtb.2013.107). Epub 2013 Oct 29.

Zinc-finger nucleases (ZFNs) work as dimers to induce double-stranded DNA breaks (DSBs) at predefined chromosomal positions. In doing so, they constitute powerful triggers to edit and to interrogate the function of genomic sequences in higher eukaryotes. A preferred route to introduce ZFNs into somatic cells relies on their cotransduction with two integrase-defective lentiviral vectors (IDLVs) each encoding a monomer of a functional heterodimeric

pair. The episomal nature of IDLVs diminishes the risk of genotoxicity and ensures the strict transient expression profile necessary to minimize deleterious effects associated with long-term ZFN activity. However, by deploying IDLVs and conventional lentiviral vectors encoding HPRT1- or eGFP-specific ZFNs, we report that DSB formation at target alleles is limited after IDLV-mediated ZFN transfer. This IDLV-specific underperformance stems, to a great extent, from the activity of chromatin-remodeling histone deacetylases (HDACs). Importantly, the prototypic and U.S. Food and Drug Administration-approved inhibitors of metal-dependent HDACs, trichostatin A and vorinostat, respectively, did not hinder illegitimate recombination-mediated repair of targeted chromosomal DSBs. This allowed rescuing IDLV-mediated site-directed mutagenesis to levels approaching those achieved by using their isogenic chromosomally integrating counterparts. Hence, HDAC inhibition constitutes an efficacious expedient to incorporate in genome-editing strategies based on transient IDLV-mediated ZFN expression. Finally, we compared two of the most commonly used readout systems to measure targeted gene knockout activities based on restriction and mismatch-sensitive endonucleases. These experiments indicate that these enzymatic assays display a similar performance.

Pileczki, V., C. Braicu, et al. "TNF-alpha gene knockout in triple negative breast cancer cell line induces apoptosis." Int J Mol Sci. 2012 Dec 24;14(1):411-20. doi: [10.3390/ijms14010411](https://doi.org/10.3390/ijms14010411).

Tumor necrosis factor alpha (TNF-alpha) is a pro-inflammatory cytokine involved in the promotion and progression of cancer, including triple negative breast cancer cells. Thus, there is significant interest in understanding the molecular signaling pathways that connect TNF-alpha with the survival of tumor cells. In our experiments, we used as an in vitro model for triple negative breast cancer the cell line Hs578T. The purpose of this study is to determine the gene expression profiling of apoptotic signaling networks after blocking TNF-alpha formation by using specially designed siRNA molecules to target TNF-alpha messenger RNA. Knockdown of TNF-alpha gene was associated with cell proliferation inhibition and apoptosis, as observed by monitoring the cell index using the xCELLigence RTCA System and flow cytometry. PCR array technology was used to examine the transcript levels of 84 genes involved in apoptosis. 15 genes were found to be relevant after comparing the treated group with the untreated one of which 3 were down-regulated and 12 up-regulated. The down-regulated genes are all involved in cell survival, whereas the up-regulated ones are involved in and interact with pro-apoptotic pathways. The results

described here indicate that the direct target of TNF-alpha in the Hs578T breast cancer cell line increases the level of certain pro-apoptotic factors that modulate different cellular networks that direct the cells towards death.

Sakaguchi, K., J. He, et al. "A targeted gene knockout method using a newly constructed temperature-sensitive plasmid mediated homologous recombination in *Bifidobacterium longum*." Appl Microbiol Biotechnol. 2012 Jul;95(2):499-509. doi: 10.1007/s00253-012-4090-4. Epub 2012 May 27.

Bifidobacteria are the main component of the human microflora. We constructed a temperature-sensitive (Ts) plasmid by random mutagenesis of the *Bifidobacterium-Escherichia coli* shuttle vector pKKT427 using error-prone PCR. Mutant plasmids were introduced into *Bifidobacterium longum* 105-A and, after screening approximately 3,000 colonies, candidate clones that grew at 30 degrees C but not at 42 degrees C were selected. According to DNA sequence analysis of the Ts plasmid, five silent and one missense mutations were found in the repB region. The site-directed mutagenesis showed only the missense mutation to be relevant to the Ts phenotype. We designated this plasmid pKO403. The Ts phenotype was also observed in *B. longum* NCC2705 and *Bifidobacterium adolescentis* ATCC15703. Single-crossover homologous-recombination experiments were carried out to determine the relationship between the length of homologous sequences encoded on the plasmid and recombination frequency: fragments greater than 1 kb gave an efficiency of more than 10(3) integrations per cell. We performed gene knockout experiments using this Ts plasmid. We obtained gene knockout mutants of the pyrE region of *B. longum* 105-A, and determined that double-crossover homologous recombination occurred at an efficiency of 1.8 %. This knockout method also worked for the BL0033 gene in *B. longum* NCC2705.

Sakudo, A. and T. Onodera "Prion protein (PrP) gene-knockout cell lines: insight into functions of the PrP." Front Cell Dev Biol. 2015 Jan 15;2:75. doi: 10.3389/fcell.2014.00075. eCollection 2014.

Elucidation of prion protein (PrP) functions is crucial to fully understand prion diseases. A major approach to studying PrP functions is the use of PrP gene-knockout (Prnp (-/-)) mice. So far, six types of Prnp (-/-) mice have been generated, demonstrating the promiscuous functions of PrP. Recently, other PrP family members, such as Doppel and Shadoo, have been found. However, information obtained from comparative studies of structural and functional analyses of these PrP family proteins do not fully reveal PrP functions. Recently, varieties of Prnp (-/-)

cell lines established from Prnp (-/-) mice have contributed to the analysis of PrP functions. In this mini-review, we focus on Prnp (-/-) cell lines and summarize currently available Prnp (-/-) cell lines and their characterizations. In addition, we introduce the recent advances in the methodology of cell line generation with knockout or knockdown of the PrP gene. We also discuss how these cell lines have provided valuable insights into PrP functions and show future perspectives.

Sasaki, K., J. Hamazaki, et al. "PAC1 gene knockout reveals an essential role of chaperone-mediated 20S proteasome biogenesis and latent 20S proteasomes in cellular homeostasis." Mol Cell Biol. 2010 Aug;30(15):3864-74. doi: 10.1128/MCB.00216-10. Epub 2010 May 24.

The 26S proteasome, a central enzyme for ubiquitin-dependent proteolysis, is a highly complex structure comprising 33 distinct subunits. Recent studies have revealed multiple dedicated chaperones involved in proteasome assembly both in yeast and in mammals. However, none of these chaperones is essential for yeast viability. PAC1 is a mammalian proteasome assembly chaperone that plays a role in the initial assembly of the 20S proteasome, the catalytic core of the 26S proteasome, but does not cause a complete loss of the 20S proteasome when knocked down. Thus, both chaperone-dependent and -independent assembly pathways exist in cells, but the contribution of the chaperone-dependent pathway remains unclear. To elucidate its biological significance in mammals, we generated PAC1 conditional knockout mice. PAC1-null mice exhibited early embryonic lethality, demonstrating that PAC1 is essential for mammalian development, especially for explosive cell proliferation. In quiescent adult hepatocytes, PAC1 is responsible for producing the majority of the 20S proteasome. PAC1-deficient hepatocytes contained normal amounts of the 26S proteasome, but they completely lost the free latent 20S proteasome. They also accumulated ubiquitinated proteins and exhibited premature senescence. Our results demonstrate the importance of the PAC1-dependent assembly pathway and of the latent 20S proteasomes for maintaining cellular integrity.

Soares, E. A., W. R. Nakagaki, et al. "Effect of hyperlipidemia on femoral biomechanics and morphology in low-density lipoprotein receptor gene knockout mice." J Bone Miner Metab. 2012 Jul;30(4):419-25. doi: 10.1007/s00774-011-0345-x. Epub 2012 Jan 14.

The objective of this study was to evaluate the effect of hyperlipidemia on the biomechanical and morphological properties of the femur of low-density

lipoprotein receptor gene knockout mice (LDLr<sup>-/-</sup>) mice. Ten wild-type mice (C57BL6) and 10 LDLr<sup>-/-</sup> mice generated on a C57BL6 background were used. Male 3-month-old animals were divided into four groups (n = 5): group W (wild type) and group L (LDLr<sup>-/-</sup>) receiving low-fat commercial ration, and group WH (wild type) and group LH (LDLr<sup>-/-</sup>) receiving a high-fat diet. After 60 days, blood samples were collected for laboratory analysis of calcium, triglycerides, and cholesterol. The femur was excised for mechanical testing and morphometric analysis. LDLr<sup>-/-</sup> mice receiving the high-fat diet presented more marked alterations in the mechanical and morphological properties of femoral cortical and trabecular bone. Changes in the plasma levels of calcium, triglycerides, cholesterol, and fractions were also more pronounced in this group. The present results demonstrate that hyperlipidemia causes alterations in the structure and mechanical properties of the femur of LDLr<sup>-/-</sup> mice. These effects were more pronounced when associated with a high-fat diet.

Stroud, D. A., L. E. Formosa, et al. "Gene knockout using transcription activator-like effector nucleases (TALENs) reveals that human NDUFA9 protein is essential for stabilizing the junction between membrane and matrix arms of complex I." J Biol Chem. 2013 Jan 18;288(3):1685-90. doi: 10.1074/jbc.C112.436766. Epub 2012 Dec 5.

Transcription activator-like effector nucleases (TALENs) represent a promising approach for targeted knock-out of genes in cultured human cells. We used TALEN-technology to knock out the nuclear gene encoding NDUFA9, a subunit of mitochondrial respiratory chain complex I in HEK293T cells. Screening for the knock-out revealed a mixture of NDUFA9 cell clones that harbored partial deletions of the mitochondrial N-terminal targeting signal but were still capable of import. A cell line lacking functional copies of both NDUFA9 alleles resulted in a loss of NDUFA9 protein expression, impaired assembly of complex I, and cells incapable of growth in galactose medium. Cells lacking NDUFA9 contained a complex I subcomplex consisting of membrane arm subunits but not marker subunits of the matrix arm. Re-expression of NDUFA9 restored the defects in complex I assembly. We conclude that NDUFA9 is involved in stabilizing the junction between membrane and matrix arms of complex I, a late assembly step critical for complex I biogenesis and activity.

Tajima, N., A. Kouzuma, et al. "Selection of *Shewanella oneidensis* MR-1 gene-knockout mutants that adapt to an electrode-respiring condition." Biosci Biotechnol Biochem. 2011;75(11):2229-33. Epub

2011 Nov 7.

Mutants of *Shewanella oneidensis* MR-1 that adapted to an electrode-respiring condition were selected from a random transposon-insertion mutant library to obtain active current-generating mutants and identify relevant cellular components. The mutants were selected in the presence of an electrode (poised at +0.2 V vs. an Ag/AgCl reference electrode) as the sole electron acceptor, and they were isolated on agar plates. Transposon-insertion sites in the isolated mutants were identified by inverse PCR coupled to sequence analyses. Southern blotting using a transposon probe was also performed to detect mutants that grew abundantly on the electrode. These analyses revealed that in many isolated mutants transposons were inserted in genes relevant to the synthesis of cell-surface structures, including SO\_3350 (pilus synthesis), SO\_3171 (polysaccharide synthesis), SO\_3174 (polysaccharide synthesis), and SO\_0165 (general secretion pathway). In microbial fuel cells, some of these (the SO\_3350 and SO\_4704 mutants) generated higher electrical outputs than wild-type MR-1, while the others generated lower outputs. The results suggest that cell-surface structures have a large influence on microbial current generation.

Tiwari, A. K., R. Zhang, et al. "Overlapping functions of ABC transporters in topotecan disposition as determined in gene knockout mouse models." Mol Cancer Ther. 2013 Jul;12(7):1343-55. doi: 10.1158/1535-7163.MCT-13-0100. Epub 2013 May 1.

It is established that efflux transporters of the ATP-binding cassette (ABC) superfamily can affect the pharmacokinetics of drugs through mechanisms pertaining to drug absorption, elimination, and distribution. To characterize the role of multiple transporters in topotecan's pharmacokinetics, total (lactone+carboxylate) and lactone forms were measured by liquid chromatography/tandem mass spectrometry (LC/MS-MS) in plasma, bile, urine, and feces following intravenous administration at doses of 1 and 4 mg/kg to eight mouse strains: C57BL/6 [wild-type (WT)], Abcb1(-/-), Abcc2(-/-), Abcc4(-/-), Abcg2(-/-), Abcc2;Abcb1(-/-), Abcc2;Abcg2(-/-), and Abcc4;Abcg2(-/-). Compared with WT mice and at both dose levels, the plasma areas under the curve for topotecan lactone were not significantly different in the Abcc2(-/-), Abcc4(-/-), and Abcb1(-/-) strains, whereas significant differences were found in Abcg2(-/-), Abcc2;Abcb1(-/-) (only at the high dose), Abcc4;Abcg2(-/-), and Abcc2;Abcg2(-/-) mice and ranged from 2.1- to 3.3-fold higher. Consistent with these changes, the fecal and biliary excretion of topotecan was reduced, whereas renal elimination was elevated in Abcg2(-/-)-based strains. Similarly, the Abcc2;Abcb1(-/-) strain also had elevated renal

elimination and reduced fecal excretion of topotecan lactone. This was more pronounced at the 4 mg/kg dose level, suggesting possible saturation of Abcg2. The Abcc4 transporter was found not to be a major determinant of topotecan pharmacokinetics. It is concluded that Abcg2 has the most significant effect on topotecan elimination, whereas both Abcb1 and Abcc2 have overlapping functions with Abcg2. As such it is relevant to examine how polymorphisms in these transporters influence topotecan activity in patients and whether coadministration of transport modulators could positively affect efficacy without increasing toxicity.

van Maanen, M. A., S. P. Stoof, et al. "Role of the cholinergic nervous system in rheumatoid arthritis: aggravation of arthritis in nicotinic acetylcholine receptor alpha7 subunit gene knockout mice." *Ann Rheum Dis.* 2010 Sep;69(9):1717-23. doi: 10.1136/ard.2009.118554. Epub 2010 May 28.

**BACKGROUND:** The alpha7 subunit of nicotinic acetylcholine receptors (alpha7nAChR) can negatively regulate the synthesis and release of proinflammatory cytokines by macrophages and fibroblast-like synoviocytes in vitro. In addition, stimulation of the alpha7nAChR can reduce the severity of arthritis in murine collagen-induced arthritis (CIA). **OBJECTIVE:** To provide more insight into the role of the alpha7nAChR in the pathogenesis of arthritis by investigating the effect of the absence of alpha7nAChR in CIA in alpha7-deficient (alpha7nAChR(-/-)) compared with wild-type (WT) mice. **METHODS:** CIA was induced in alpha7nAChR(-/-) and WT littermate mice at day 0 by immunisation with chicken collagen type II (cCII) followed by a booster injection with cCII on day 20. Mice were killed on day 44 or day 63 and arthritis activity as well as radiological and histological damage were scored. The effects on the immune response were evaluated by measurement of antigen-specific antibodies and cytokines, and evaluation of the effects on antigen-specific stimulated spleen cells. **RESULTS:** In alpha7nAChR(-/-) mice a significant increase in the incidence and severity of arthritis as well as increased synovial inflammation and joint destruction were seen. Exacerbation of CIA was associated with elevated systemic proinflammatory cytokines and enhanced T-helper cell 1 (Th1)-cytokine and tumour necrosis factor alpha production by spleen cells. Moreover, a specific decrease in the collagen-specific 'Th1-associated' IgG2a response was seen, whereas IgG1 titres were unaffected. **CONCLUSIONS:** The results presented here indicate that immune cell function in a model of rheumatoid arthritis is regulated by the cholinergic system and, at least in part, mediated by the alpha7nAChR.

Watanabe, M., K. Nakano, et al. "Generation of interleukin-2 receptor gamma gene knockout pigs from somatic cells genetically modified by zinc finger nuclease-encoding mRNA." *PLoS One.* 2013 Oct 9;8(10):e76478. doi: 10.1371/journal.pone.0076478. eCollection 2013.

Zinc finger nuclease (ZFN) is a powerful tool for genome editing. ZFN-encoding plasmid DNA expression systems have been recently employed for the generation of gene knockout (KO) pigs, although one major limitation of this technology is the use of potentially harmful genome-integrating plasmid DNAs. Here we describe a simple, non-integrating strategy for generating KO pigs using ZFN-encoding mRNA. The interleukin-2 receptor gamma (IL2RG) gene was knocked out in porcine fetal fibroblasts using ZFN-encoding mRNAs, and IL2RG KO pigs were subsequently generated using these KO cells through somatic cell nuclear transfer (SCNT). The resulting IL2RG KO pigs completely lacked a thymus and were deficient in T and NK cells, similar to human X-linked SCID patients. Our findings demonstrate that the combination of ZFN-encoding mRNAs and SCNT provides a simple robust method for producing KO pigs without genomic integration.

Werfel, S., A. Jungmann, et al. "Rapid and highly efficient inducible cardiac gene knockout in adult mice using AAV-mediated expression of Cre recombinase." *Cardiovasc Res.* 2014 Oct 1;104(1):15-23. doi: 10.1093/cvr/cvu174. Epub 2014 Jul 31.

**AIMS:** Inducible gene targeting in mice using the Cre/LoxP system has become a valuable tool to analyse the roles of specific genes in the adult heart. However, the commonly used Myh6-MerCreMer system requires time-consuming breeding schedules and is potentially associated with cardiac side effects, which may result in transient cardiac dysfunction. The aim of our study was to establish a rapid and simple system for cardiac gene inactivation in conditional knockout mice by gene transfer of a Cre recombinase gene using adeno-associated viral vectors of serotype 9 (AAV9). **METHODS AND RESULTS:** AAV9 vectors expressing Cre under the control of a human cardiac troponin T promoter (AAV-TnT-Cre) enabled a highly efficient Cre/LoxP switching in cardiomyocytes 2 weeks after injection into 5- to 6-week-old ROSA26-LacZ reporter mice. Recombination efficiency was at least as high as observed with the Myh6-MerCreMer system. No adverse side effects were detected upon application of AAV-TnT-Cre. As proof of principle, we studied AAV-TnT-Cre in a conditional knockout model (Srf-flex1 mice) to deplete the myocardium of the transcription factor serum response factor (SRF). Four weeks after

AAV-TnT-Cre injection, a strong decrease in the cardiac expression of SRF mRNA and protein was observed. Furthermore, mice developed a severe cardiac dysfunction with increased interstitial fibrosis in accordance with the central role of SRF for the expression of contractile and calcium trafficking proteins in the heart. **CONCLUSIONS:** AAV9-mediated expression of Cre is a promising approach for rapid and efficient conditional cardiac gene knockout in adult mice.

Wilson, G. A., P. Dhami, et al. "Resources for methylome analysis suitable for gene knockout studies of potential epigenome modifiers." *Gigascience*. 2012 Jul 12;1(1):3. doi: 10.1186/2047-217X-1-3.

**BACKGROUND:** Methylated DNA immunoprecipitation (MeDIP) is a popular enrichment based method and can be combined with sequencing (termed MeDIP-seq) to interrogate the methylation status of cytosines across entire genomes. However, quality control and analysis of MeDIP-seq data have remained to be a challenge. **RESULTS:** We report genome-wide DNA methylation profiles of wild type (wt) and mutant mouse cells, comprising 3 biological replicates of Thymine DNA glycosylase (Tdg) knockout (KO) embryonic stem cells (ESCs), in vitro differentiated neural precursor cells (NPCs) and embryonic fibroblasts (MEFs). The resulting 18 methylomes were analysed with MeDUSA (Methylated DNA Utility for Sequence Analysis), a novel MeDIP-seq computational analysis pipeline for the identification of differentially methylated regions (DMRs). The observed increase of hypermethylation in MEF promoter-associated CpG islands supports a previously proposed role for Tdg in the protection of regulatory regions from epigenetic silencing. Further analysis of genes and regions associated with the DMRs by gene ontology, pathway, and ChIP analyses revealed further insights into Tdg function, including an association of TDG with low-methylated distal regulatory regions. **CONCLUSIONS:** We demonstrate that MeDUSA is able to detect both large-scale changes between cells from different stages of differentiation and also small but significant changes between the methylomes of cells that only differ in the KO of a single gene. These changes were validated utilising publicly available datasets and confirm TDG's function in the protection of regulatory regions from epigenetic silencing.

Yokoyama, T., A. Yoshizaki, et al. "Age-Dependent Defects of Regulatory B Cells in Wiskott-Aldrich Syndrome Gene Knockout Mice." *PLoS One*. 2015 Oct 8;10(10):e0139729. doi: 10.1371/journal.pone.0139729. eCollection 2015.

The Wiskott-Aldrich syndrome (WAS) is a

rare X-linked primary immunodeficiency characterized by recurrent infections, thrombocytopenia, eczema, and high incidence of malignancy and autoimmunity. The cellular mechanisms underlying autoimmune complications in WAS have been extensively studied; however, they remain incompletely defined. We investigated the characteristics of IL-10-producing CD19+CD1dhighCD5+ B cells (CD1dhighCD5+ Breg) obtained from Was gene knockout (WKO) mice and found that their numbers were significantly lower in these mice compared to wild type (WT) controls. Moreover, we found a significant age-dependent reduction of the percentage of IL-10-expressing cells in WKO CD1dhighCD5+ Breg cells as compared to age-matched WT control mice. CD1dhighCD5+ Breg cells from older WKO mice did not suppress the in vitro production of inflammatory cytokines from activated CD4+ T cells. Interestingly, CD1dhighCD5+ Breg cells from older WKO mice displayed a basal activated phenotype which may prevent normal cellular responses, among which is the expression of IL-10. These defects may contribute to the susceptibility to autoimmunity with age in patients with WAS.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

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