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A novel *Ifnar1* knockout mouse model generated by CRISPR/Cas9 genome editing

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Abstract

The type I interferon receptor gene (*Ifnar1*) encodes a subunit of the heterodimeric receptor complex responsible for mediating type I interferon (IFN-α/β) signaling, a critical pathway in antiviral defense and immune regulation. *Ifnar1* knockout (KO) mice are widely used in immunology and virology research to study host-pathogen interactions, immune signaling, and inflammatory processes. Although a conventional *Ifnar1* KO model was generated decades ago, advances in genome engineering technologies now allow for more efficient and precise generation of genetically modified animals. We employed CRISPR/Cas9 genome editing to generate a novel *Ifnar1* knockout mouse line. Single-guide RNAs targeting the third exon of mouse *Ifnar1* gene were electroporated into fertilized C57BL/6J zygotes along with Cas9 protein. The newborn founder mice were screened by PCR and Sanger sequencing to identify mutations at the target site. We successfully established a mouse line harboring a 14-nucleotide deletion in the third exon of *Ifnar1*. This deletion causes a frameshift mutation, introducing a premature stop codon that is predicted to produce a truncated, non-functional protein. The mutation was confirmed by direct sequencing of the targeted locus. Homozygous mutant mice are viable and fertile. This newly generated *Ifnar1* knockout mouse model provides a CRISPR-engineered alternative to the original targeted deletion model described by Müller et al. (1994). The frameshift mutation is expected to ablate IFNAR1 protein function. The model will serve as a valuable resource for immunology and virology research, particularly in studies focused on interferon signaling, antiviral responses, and host-pathogen interactions.

Keywords: *Ifnar1*, CRISPR/Cas9, genome editing, knockout mouse, interferon signaling, antiviral response

Introduction

The human *IFNAR1* gene encodes the interferon alpha and beta receptor subunit 1 (Uniprot P17181, 557 amino acids), which pairs with IFNAR2 to form a heterodimeric receptor complex responsible for mediating the effects of type I interferons (IFN-α/β). Upon ligand binding, this receptor activates the JAK-STAT signaling pathway, which in turn induces the expression of interferon-stimulated genes (ISGs) involved in antiviral, antiproliferative, and immunomodulatory responses (Platanias, 2005). Structurally, IFNAR1 contains an extracellular fibronectin type III-like domain, a single transmembrane segment, and a cytoplasmic region that interacts with TYK2 kinase to initiate downstream STAT1/STAT2 activation. The *IFNAR1* gene is broadly expressed in most human tissues but particularly enriched in immune-related tissues such as spleen, lymph nodes, and peripheral blood mononuclear cells (de Weerd & Nguyen, 2012). It is evolutionarily conserved across vertebrates and plays a central role in innate immunity, particularly in mounting effective responses against viral infections (Schreiber, 2017).

The functional relevance of IFNAR1 in host antiviral defense was first demonstrated by Müller and colleagues, who generated *Ifnar1* knockout (KO) mice using homologous recombination in embryonic stem cells. In this model, exon 3 of the mouse *Ifnar1* gene was replaced with a Neomycin 3 resistance cassette using a targeting vector. Deletion of exon 3, which is 179 nt. in length, results in frameshift and loss of IFNAR1 protein (Müller et al., 1994). These mice were completely unresponsive to type I interferons and

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showed heightened susceptibility to a broad spectrum of viral pathogens, including vesicular stomatitis virus and lymphocytic choriomeningitis virus. Since its development, the *Ifnar1* KO mouse has become a widely used model in immunology, virology, and inflammation research (Teijaro, 2016; Kim et al., 2024). Studies using this model have elucidated the role of type I interferons in antiviral defense, immunopathology, and cytokine regulation. Alternative models, including *Ifnar2* KO mice and conditional *Ifnar1* KOs, have also been developed to explore cell-specific functions of the interferon receptor (Shepardson et al., 2018; Mizutani et al., 2012), but the original constitutive *Ifnar1* KO remains the most broadly adopted model in the field.

In humans, autosomal recessive *IFNAR1* deficiency is a rare inborn disorder of immunity associated with severe vulnerability to viral infections. Affected individuals harbor biallelic loss-of-function mutations in *IFNAR1* gene, leading to truncated or non-functional receptor proteins which results in defective downstream STAT signaling (Hernandez et al., 2019; Bastard et al., 2021; Bastard et al., 2022). Clinically, patients often present with life-threatening complications following exposure to live-attenuated vaccines or natural viral infections such as SARS-CoV-2 (Khanmohammadi et al., 2022). These findings provide compelling evidence for the non-redundant, essential role of IFNAR1 in human antiviral defense and further validate the biological relevance of *Ifnar1* KO mice for translational research.

The advent of CRISPR/Cas9 genome editing has revolutionized functional genomics by enabling precise, efficient modifications of genomic DNA in a broad range of organisms (Doudna and Charpentier, 2014). The first CRISPR-edited mice was created by microinjecting Cas9 mRNA and guide RNAs into mouse zygotes, establishing a new standard for rapid, flexible genome engineering (Wang et al., 2013). Since then, CRISPR has been widely utilized for generating genetically modified mouse models via electroporation or microinjection of CRISPR components into fertilized embryos (Hashimoto et al., 2016; Modzelewski et al., 2018). These approaches significantly reduce production time and cost compared to traditional embryonic stem cell–based methods.
In this study, we aimed to generate a novel *Ifnar1* knockout mouse using CRISPR technology. Our goal was to produce a reproducible and cleanly defined allele using a modern technique that is both efficient and accessible. We designed single-guide RNAs targeting exon 3 of *Ifnar1* and successfully introduced a 14-nucleotide long deletion that causes a frameshift and premature stop codon. The original Ifnar1 knockout was produced by targeting and replacement of the exon 3 of the mouse *Ifnar1* gene (Müller et al., 1994), causing a loss of IFNAR1 protein function. Therefore the mutant allele developed in this study is predicted to result in loss of function by producing a truncated, non-functional protein. This model serves as a CRISPR-based alternative to the original *Ifnar1* knockout and will be a useful tool for future research into interferon signaling and antiviral immunity.

Materials and Methods

Superovulation, embryo collection, and culture

All procedures involving animals were conducted in accordance with protocols approved by the Izmir Biomedicine and Genome Center Animal Ethics Committee.
Female C57BL/6J mice (4–6 weeks old) were superovulated via intraperitoneal injection of 5 IU pregnant mare serum gonadotropin (Folligon, MSD Animal Health), followed 48 hours later by 5 IU human chorionic gonadotropin (Chorulon, MSD Animal Health). Each hormonally primed female was housed with a single C57BL/6J male (8–12 weeks old) overnight for mating. The following morning (8–9 a.m.), females were sacrificed for zygote (0.5 dpc) collection. Oviducts were dissected and transferred into 60 mm dishes, and the ampullae were opened under a stereomicroscope to release fertilized embryos.

Embryos were kept in Global-Hepes medium (Global, LGTH-100) containing 300 µg/ml hyaluronidase (Sigma, H3884) at room temperature, until cumulus cells dispersed. Embryos were subsequently washed through several droplets of Global-Hepes medium (supplemented with 4 mg/ml BSA) using a mouth pipette to ensure complete removal of remaining cumulus cells. The cleaned zygotes were then cultured in Global medium (Global, LGGG-050) supplemented with 4 mg/ml BSA (Sigma, A9647) in a CO_2 incubator for 1–2 hours prior to electroporation.

In vitro transcription of sgRNAs, RNP assembly and electroporation of zygotes

Templates for sgRNAs targeting mouse *Ifnar1* locus (5'CAGTAAAGAGAATTCACACT) were generated via template-fusion PCR using Q5 High-Fidelity DNA Polymerase (NEB, M0491) as described before (Diril et al., 2025). Templates were transcribed using the HiScribe T7 High Yield RNA Synthesis Kit (NEB, E2040S), and the resulting sgRNAs were purified with the Monarch RNA Cleanup Kit (NEB, T2040L).
Ribonucleoprotein (RNP) complexes were prepared by combining 80 pmol of Cas9 nuclease (IDT, 1081059) with 100 pmol of *Ifnar1* sgRNA in 20 µl OPTI-MEM and incubating for 10 minutes at room temperature. Zygotes were washed through multiple OPTI-MEM droplets and transferred to the RNP mixture in OPTI-MEM.

The 20 µl final electroporation mixture was transferred into a Bio-Rad electroporation cuvette (1652089) and electroporated as described before (Diril et al., 2025). Briefly, six pulses (30 V, 3 ms pulse length and 100 ms interval) were applied using a Bio Rad Gene Pulser XCell device. Embryos were recovered by flushing with Global-Hepes medium and transferred to recipient females.

Embryo transfer into pseudopregnant hosts

Embryo transfers were performed using CD-1 females that had been mated the previous night with vasectomized males to induce pseudopregnancy. Recipient females displaying copulation plugs were anesthetized with ketamine (100 mg/kg)

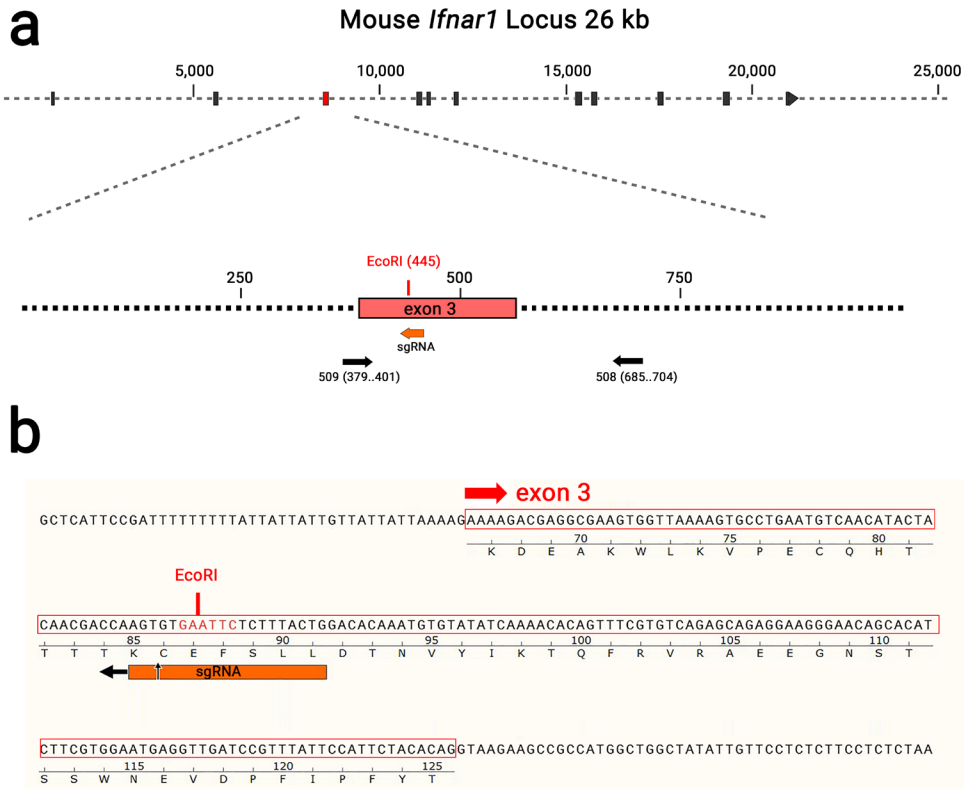

Figure 1. Mouse *Ifnar1* genomic locus and CRISPR targeting strategy.
(A) Map of mouse *Ifnar1* genomic locus showing all exons (black boxes) and the targeted exon (red box). Below, a 1000-nucleotide region including exon 3 is detailed. The genotyping primers (black arrows) and the sgRNA target site containing the EcoRI restriction site (orange arrow) are indicated. (B) Nucleotide sequence surrounding exon 3 and the wild-type coded amino acid sequence. The sgRNA target sequence (orange arrow) and the location of the Cas9-generated DNA double-strand break (perpendicular black arrow) are shown.

and xylazine (10 mg/kg) via intraperitoneal injection. Embryos were surgically implanted into the oviducts by accessing the infundibulum through a small incision of ovarian bursa.

Genotyping PCR, Sanger sequence analysis and RT-PCR

Genomic DNA was prepared from tail biopsies using the HotSHOT technique (Truett et al., 2000). PCR amplification was carried out using primers 508 (5'GATAACCACACGGGGAAAGC) and 509 (5'AAAAGAAAAGACGAGGCGAAGTG) that generate a 326 bp. long fragment from WT *Ifnar1* allele.

Products were first analyzed by agarose gel electrophoresis and subsequently reanalyzed after digestion with EcoRI to detect presence of indel mutations.

RNA samples were isolated from spleen tissue using Qiagen RNeasy mini kit (74104). RevertAid First Strand cDNA Synthesis Kit, (Thermo, K1621) was used to synthesize cDNAs used as template in PCR reactions. PCR amplification was carried out with primers 701 (5'AGCCACGGAGAGTCAATGG) and 702 (5'GCTCTGACACGAAACTGTGTTTT) that generate a 167 bp. long fragment from WT *Ifnar1* cDNA.

Sanger sequencing was performed by Eurofins Genomics on a Capillary Electrophoresis platform (ABI).

Results

Targeting mouse *Ifnar1* locus and genotyping strategy

The mouse *Ifnar1* gene (NCBI Gene ID: 15975, MGI:107658) is located on chromosome 16qB1, spans approximately 26 kilobases. It is comprised of 12 exons encoding the mouse IFNAR1 protein (Uniprot P33896) which is 590 aa. in length including signal sequence (Figure 1A).

We have used an sgRNA that targets the third exon of the mouse *Ifnar1* gene, introducing a DSB in the vicinity of an EcoRI restriction site. This exon codes for the 67-125 amino acids of the IFNAR1 protein corresponding to the C-terminal half of the first fibronectin type III like domain that it is critical for IFNAR1 interactions. Introduction of a DNA double strand break (DSB) at this site (codon 87) by Cas9 nuclease and subsequent repair by NHEJ (non-homologous end joining) potentially introduces indel (insertion/deletion) mutations and abrogates the EcoRI restriction enzyme target sequence

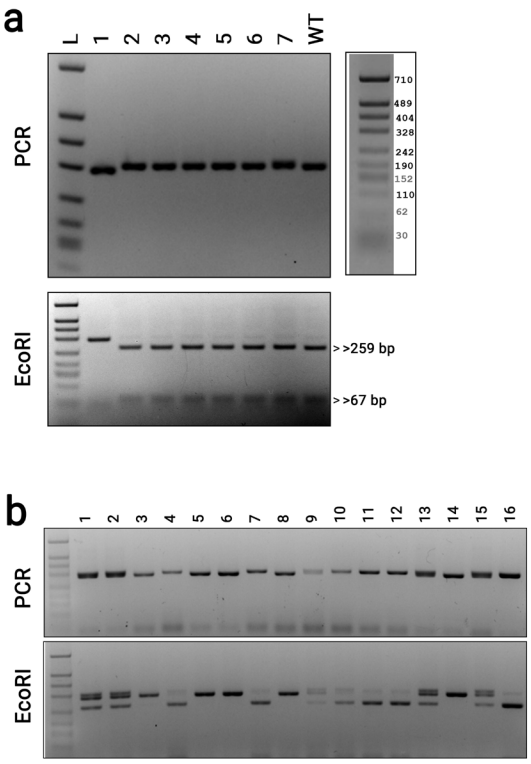

Figure 2. Genotyping and germline transmission of the *Ifnar1* knockout allele.
(A) Restriction Fragment Length Polymorphism (RFLP) analysis of founder mice. Genomic DNA from seven founder pups (#1 to #7), generated via Cas9/sgRNA RNP electroporation and embryo transfer, was PCR-amplified. PCR products were digested with EcoRI and analyzed by agarose gel electrophoresis. WT alleles are cut into two fragments (259 bp and 67 bp). The PCR product from founder #1 was uncut, confirming the loss of the EcoRI site due to an indel mutation. L indicates the DNA ladder (sizes were indicated on the right). (B) Genotype analysis of F3 generation pups. Pups born from a heterozygous x heterozygous cross were genotyped by EcoRI RFLP. Genotype distribution was consistent with Mendelian inheritance (5 WT, 6 Het, 5 KO out of 16 pups).

(GAATTC). Frameshift mutations would be generated roughly in two-thirds of the indel alleles that are generated after repair (Modzelewski et al. 2018). We reasoned that, screening for alleles where EcoRI site has been deleted is an efficient strategy to identify potential knockout alleles (Figure 1B).

The presence of mutant alleles can be screened and differentiated by restriction analysis of a PCR amplification product. The sequence flanking the sgRNA target site and EcoRI recognition site was amplified by PCR wherein primer pair 508-509 was used (326 bp amplicon in WT allele). Of note, mutations that do not change the EcoRI restriction site cannot be identified based on this screening strategy.

Embryo electroporation, transfer, and development of founder mice

sgRNAs targeting the EcoRI site were synthesized by *in vitro* transcription and zygote stage embryos isolated from super ovulated C57BL/6J female mice were electroporated with Cas9/sgRNA RNP complexes as described before (Diril et al. 2025). The embryos were surgically transferred to the oviducts of pseudopregnant CD1 female mice. Three weeks after the embryo transfer, seven pups were born. To genetically characterize the alleles, present in the founder mice, tail biopsies were taken, and genomic DNA was prepared. *Ifnar1* locus target site was amplified by PCR.

Analysis of the PCR products on agarose gel showed that, one of the founder mice (#1) had a mutant allele slightly shorter than the WT amplicon (326 bp). Absence of a second band corresponding to the WT allele suggests that, either there are deletions of similar sizes in both chromosomes, or one of the deletion alleles cannot be amplified as it is large enough to span the binding region of one of the primers. PCR products were digested using EcoRI enzyme and analyzed by agarose gel electrophoresis. PCR product from the founder #1 could not be cut by EcoRI. The rest of the PCR products (2-7) were cut yielding two fragments with sizes similar to the WT allele restriction products; 259 and 67 bp. (Figure 2A).

Development of *Ifnar1* knockout colony

To establish an *Ifnar1* knockout mouse colony, we used pup

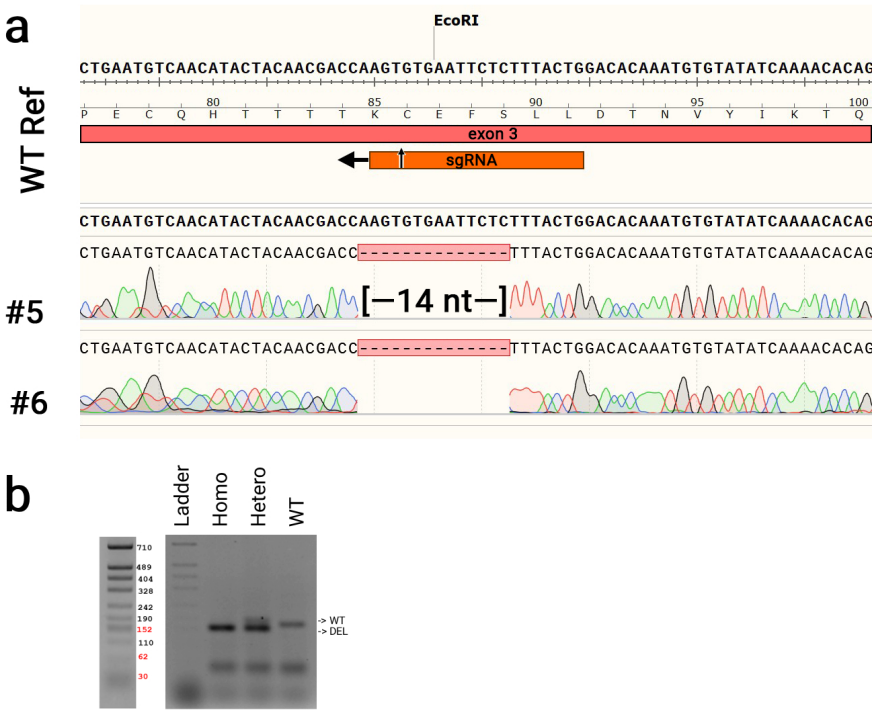

Figure 3. Molecular characterization of the *Ifnar1* KO allele.
(A) Sanger sequencing confirmation of the 14-nt deletion. Densitograms from PCR products of two homozygous knockout mice (lanes 5 and 6 in Figure 2B) were aligned against the WT reference sequence. The 14-nucleotide long deletion generated by CRISPR targeting is clearly displayed. (B) Expression analysis of the *Ifnar1* KO allele by RT-PCR. RNA samples isolated from wild-type (WT), heterozygous (Hetero), and homozygous (homo) mouse spleens were analyzed. Primers binding to exon 2 and exon 3 amplify a 167 bp fragment from the WT *Ifnar1* transcript and a shorter 153 bp fragment from the deletion allele.

#1 (female) as the colony founder (F0). After reaching sexual maturity, we crossed this founder female with a WT male of C57BL/6J genetic background, to obtain heterozygous pups (F1). One of the heterozygous male pups was chosen and backcrossed with WT females to generate more heterozygous mice (F2). By using a single heterozygous mouse and crossing it with WT females to establish the colony, we have avoided the risk of genetic mosaicism in the progeny (Marx, 2019). Breeding of these heterozygous mice with each other yielded pups with WT, heterozygous and homozygous knockout genotypes (Figure 2B). Analysis of the genotypes of three-week-old pups from three different litters showed a roughly Mendelian distribution of genotypes (25% WT, 50% heterozygous, 25% homozygous) expected of an allele that does not affect viability. *Ifnar1* knockout mice were reported to be viable and fertile without any overt anomalies (Müller et al., 1994).

Sanger analysis of the PCR products from two of the homozygous mice showed a 14 nt long deletion in the vicinity of the expected DNA DSB. The mutant allele cannot be cut with EcoRI due to the deletion of the recognition site (Figure 3A). The deletion allele is expressed in RNA samples isolated from the spleens of homozygous and heterozygous mice but not WT animals (Figure 3B).

The 14 nt long deletion causes a frameshift and alteration of the peptide sequence after T84. After 26 amino acids, a premature stop codon comes truncating the protein (Figure 4A). The mutant protein has 110 amino acids whereas WT mouse IFNAR1 has 590 amino acids (including the signal peptide). Knockout allele codes for a peptide that ends in the middle of the first Fibronectin Type III domain. Most of the extracellular domain, cytosolic domain and the transmembrane domain between 430-449 aa. are not expressed. Therefore, this truncated protein product encoded by the mutant *Ifnar1* gene is predicted to be nonfunctional (Figure 4B).

Discussion

In this study, we describe the successful generation of a novel *Ifnar1* knockout mouse model using CRISPR/Cas9-mediated genome editing. By targeting exon 3 of the *Ifnar1* gene, we introduced a 14-nucleotide deletion that disrupts an endogenous EcoRI restriction site and causes a frameshift mutation leading to a premature stop codon. The resulting truncated IFNAR1 protein lacks all known functional domains, including the extracellular fibronectin type III domains, transmembrane domain, and intracellular signaling motifs. Based on this molecular characterization, we predict that the allele generated is a true loss-of-function mutation.

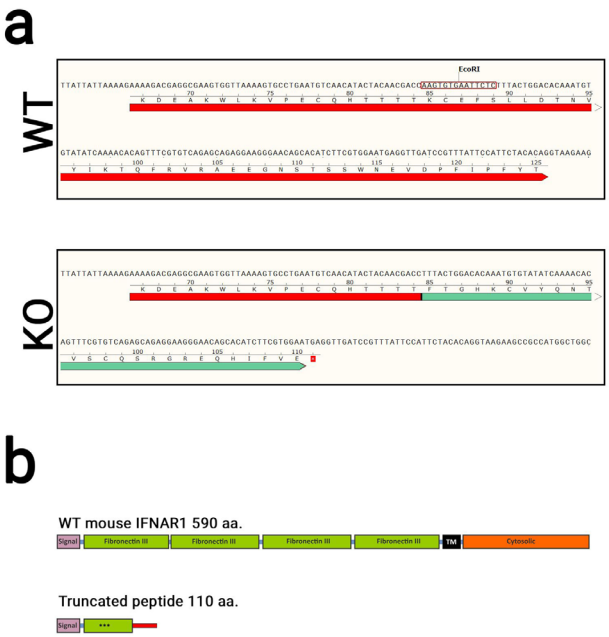

Figure 4. Predicted non-functional nature of the truncated IFNAR1 protein.
(A) Sequence comparison of WT and KO alleles. The DNA and resulting peptide sequences are displayed. The 14-nucleotide deletion encompassing the EcoRI site is shown (red rectangle). The frameshift mutation alters the peptide sequence after Threonine 84 (T84, green box) and introduces a premature stop codon, which truncates the IFNAR1 protein. (B) Domain organization of WT and truncated IFNAR1. Above: Domains of the wild-type mouse IFNAR1 protein (590 aa) are shown: Fibronectin Type III domains (green), transmembrane domain (black), and cytosolic domain (orange). Below: A diagram of the truncated peptide (110 aa) encoded by the *Ifnar1* knockout allele. The peptide is truncated in the middle of the first Fibronectin Type III domain and lacks all domains required for IFNAR1 function. The red line denotes the altered amino acid sequence resulting from the frameshift.

The *Ifnar1* gene encodes the type I interferon receptor subunit 1, which is essential for the cellular response to type I interferons (IFN-α/β). These cytokines play a central role in innate immunity, particularly antiviral defense, as well as modulation of adaptive immune responses and regulation of cell proliferation and survival. The frameshift-induced truncation of IFNAR1 after amino acid 110 is predicted to abolish receptor function entirely, since the remaining peptide contains none of the domains required for ligand binding or downstream signal transduction via the JAK-STAT pathway. This is consistent with previously reported IFNAR1-deficient mouse models, in which disruption of receptor expression abrogates cellular responsiveness to type I interferons and renders mice highly susceptible to viral infections (Müller et al., 1994; Sheehan et al., 2006).

Our genotyping strategy employed both restriction fragment length polymorphism (RFLP) analysis and Sanger sequencing to clearly distinguish wild-type, heterozygous, and homozygous mutant mice. The loss of the EcoRI site within the PCR amplicon provides a rapid screening method, while sequencing confirmed the precise nature of the deletion. This approach offers a reliable genotype determination for colony management.

Compared to conventional gene-targeting approaches that require isolation and genetic characterization targeted embryonic stem cells and long timelines, our use of zygote electroporation with Cas9/sgRNA ribonucleoproteins (RNPs) enabled rapid generation of founder mice without the need for selection markers or cloning steps. This strategy not only accelerated model development but also minimized potential off-target integrations. The successful generation of homozygous mutants in the F1 generation highlights the efficiency of this approach (Chen et al., 2016; Qin et al., 2015; Marx, 2019)

The availability of this *Ifnar1*-null model makes a variety of experimental applications possible. Type I IFN signaling has been implicated in diverse physiological and pathological processes, including viral immunity, tumor surveillance, autoimmune diseases, and immunotherapy response. As IFNAR1 deficiency is a known inborn error of human immunity, this model provides essential translational relevance. The complete ablation of IFNAR1 signaling provided by this model creates a robust platform for testing novel antivirals that target viral dependence on Type I IFN or for evaluating immunotherapies where IFNAR signaling is known to cause dose-limiting toxicities.

Conclusions

In conclusion, we have developed a genetically validated *Ifnar1* knockout mouse line via CRISPR-mediated genome editing. The mutation leads to early truncation of the protein and removal of all functional domains. Although no functional validation has been carried out, the mutation is predicted to result in complete loss function. Functional validation of the knockout allele, for instance by examining expression of interferon-stimulated genes (ISGs) in response to recombinant IFN-α, viral challenge models, or tumor xenografts, would further confirm the immunological phenotype and utility of this model. This model will be a valuable resource for investigating the roles of type I interferon signaling in health and disease and may serve as a platform for preclinical evaluation of IFN-targeted therapies.

Author Contributions

M.K.D. conceived and designed the project, and acquired the funding. M.K.D. and K.E., performed the experiments and acquired the primary data. M.K.D. analyzed and interpreted the data. M.K.D. wrote the main manuscript text. Both authors reviewed the manuscript.

Conflict of Interest

The authors declare no competing financial interest.

Ethical Approval

The research project involving experimental animals was approved by Izmir Biomedicine and Genome Center animal experimentation local ethics committee (IBG-HADYEK, Protocol No.2020-017).

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Abbreviations

CRISPR, clustered regularly interspaced short palindromic repeats; **Cas9**, CRISPR associated protein 9; **DSB**, double strand break; **indel**, insertion/deletion; **HDR**, homology directed repair; **KO**, knockout; **RNP**, ribonucleoprotein; **sgRNA**, small guide RNA; 10

References

1. Bastard P, Goffard JC, Emile JF, et al. A loss-of-function IFNAR1 allele in Polynesia underlies severe viral diseases in homozygotes. *J Exp Med.* 2022;219(6):e20220028.
2. Bastard P, Orlova E, Soheili-Nezhad S, et al. Autosomal recessive IFNAR1 deficiency with severe COVID-19 pneumonia. *J Clin Immunol.* 2021;42(1):19–24.
3. Chen S, Lee B, Lee AY, Modzelewski AJ, He L. Highly efficient mouse genome editing by CRISPR ribonucleoprotein electroporation of zygotes. *J Biol Chem*. 2016 Nov 25;291(51):14457–14467.
4. Diril MK, Esmen K, Sehitogullari T, Öztürk G. Generation of albino C57BL/6J mice by CRISPR embryo editing of the mouse tyrosinase locus. *J Genet*. 2025;104:7.
5. de Weerd NA, Nguyen T. The interferons and their receptors—distribution and regulation. *Immunol Cell Biol.* 2012;90(5):483–91.
6. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science.* 2014;346(6213):1258096.
7. Hashimoto M, Yamashita Y, Takemoto T. Electroporation of Cas9 protein/sgRNA into early pronuclear zygotes generates non-mosaic mutants in the mouse. *Dev Biol.* 2016;418(1):1–9.
8. Hernandez N, Megchelenbrink W, Campbell A, et al. Life-threatening MMR vaccine–related infection in an infant with complete IFNAR1 deficiency. *J Exp Med.* 2019;216(6):1321–1333.
9. Khanmohammadi S, Rezaei N, Khazaei M, Shirkani A. A Case of Autosomal Recessive Interferon Alpha/Beta Receptor Alpha Chain (IFNAR1) Deficiency with Severe COVID-19. J Clin Immunol. 2022 Jan;42(1):19-24.
10. Kim SR, Lee JM, Kang HJ, Ryou J, Shim SM. Antiviral activity of pimecrolimus against dengue virus type 2 infection in vitro and in vivo. Sci Rep. 2024 Jun 10;14(1):13303.
11. Marx V. A rocky road for the maturation of embryo-editing methods. Nat Methods. 2019;16(2):147-150.
12. Mizutani T, Neugebauer N, Putz EM, Moritz N, Simma O, Zebedin-Brandl E, Gotthardt D, Warsch W, Eckelhart E, Kantner HP, Kalinke U, Lienenklaus S, Weiss S, Strobl B, Müller M, Sexl V, Stoiber D. Conditional IFNAR1 ablation reveals distinct requirements of Type I IFN signaling for NK cell maturation and tumor surveillance. Oncoimmunology. 2012 Oct 1;1(7):1027-1037.
13. Modzelewski AJ, Chen S, Willis BJ, Lloyd KCK, Wood JA, He L. Efficient mouse genome engineering by CRISPR-EZ technology. *Nat Protoc.* 2018;13(9):1857–76.
14. Müller U, Steinhoff U, Reis LF, et al. Functional role of type I and type II interferons in antiviral defense. *Science.* 1994;264(5167):1918–21.

15. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol.* 2005;5(5):375–86.
16. Qin W, Dion SL, Kutny PM, Zhang Y, Cheng AW, Jillette NL, Malagon-Lopez J, Leonetti MD, Corn JE. Efficient CRISPR/Cas9-mediated genome editing in mice by zygote electroporation of nuclease. Genetics. 2015 Sep;200(2):423–30.
17. Sheehan KC, Lai KS, Dunn GP, Bruce AT, Diamond MS, Schreiber RD. Blocking monoclonal antibodies specific for mouse IFN-α/β receptor subunit 1 (IFNAR-1) from mice immunized by in vivo hydrodynamic transfection. *J Interferon Cytokine Res*. 2006 Nov;26(11):804–19.
18. Shepardson KM, Larson K, Johns LL, Stanek K, Cho H, Wellham J, Henderson H, Rynda-Apple A. IFNAR2 Is Required for Anti-influenza Immunity and Alters Susceptibility to Post-influenza Bacterial Superinfections. Front Immunol. 2018 Nov 9;9:2589.
19. Schreiber G. The molecular basis for differential type I interferon signaling. J Biol Chem. 2017;292(46):18261–8.
20. Teijaro JR. Type I interferons in viral control and immune regulation. Curr Opin Virol. 2016;16:31–40.
21. Wang H, Yang H, Shivalila CS, et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell. 2013;153(4):910–8.