

# *Spink5* Cas9-CKO Strategy

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# Overview

## Target Gene Name

- *Spink5*

## Project Type

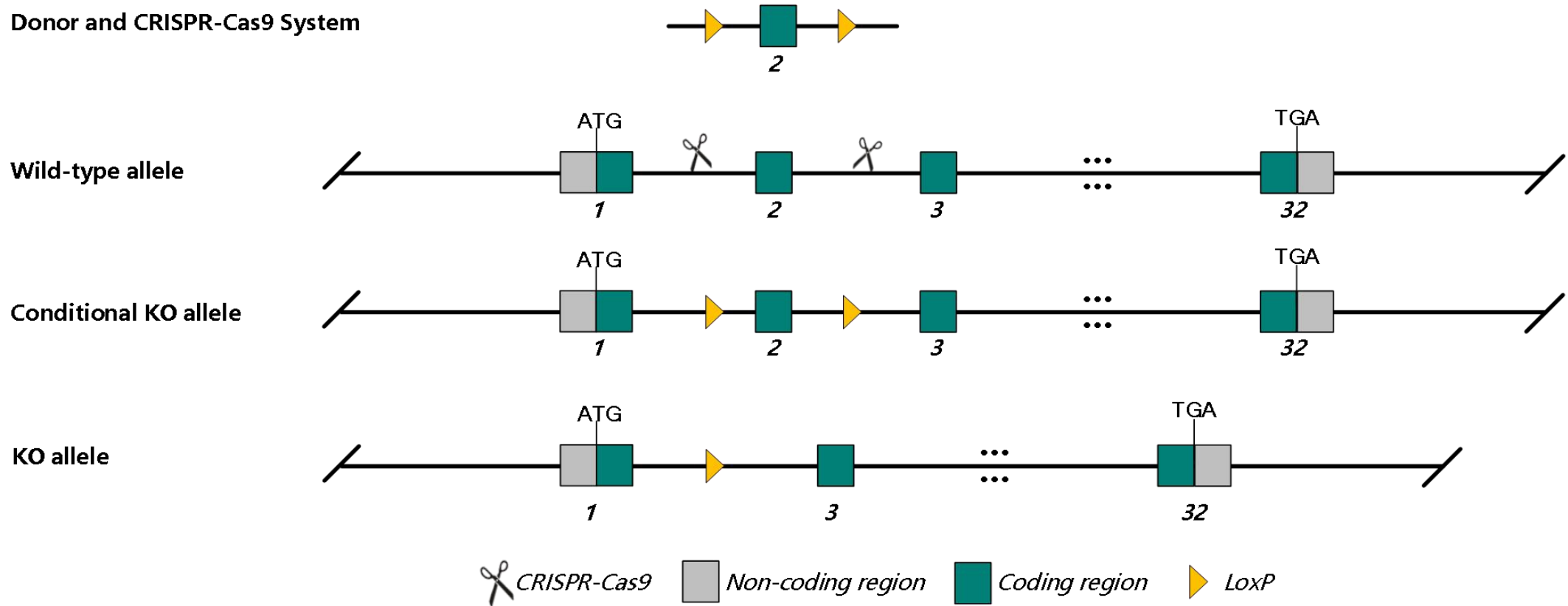
- Cas9-CKO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy

Donor and CRISPR-Cas9 System



Schematic representation of CRISPR-Cas9 engineering used to edit the *Spink5* gene.

# Technical Information

- The *Spink5* gene has 1 transcript. According to the structure of *Spink5* gene, exon 2 of *Spink5*-201 (ENSMUST00000069245.8) is recommended as the knockout region. The region contains 26 bp of coding sequence. Knocking out the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Spink5* gene. The brief process is as follows: CRISPR-Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

# Gene Information

**Spink5** serine peptidase inhibitor, Kazal type 5 [ *Mus musculus* (house mouse) ]

[Download Datasets](#)

Gene ID: 72432, updated on 16-May-2023

## Summary

<b>Official Symbol</b>	Spink5 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	serine peptidase inhibitor, Kazal type 5 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1919682</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000055561</a> <a href="#">AllianceGenome:MGI:1919682</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	LEKT1; LETK1; VAKT1; 2310065D10Rik
<b>Summary</b>	Enables peptidase inhibitor activity. Involved in negative regulation of antibacterial peptide production and negative regulation of serine-type endopeptidase activity. Acts upstream of or within negative regulation of serine-type peptidase activity and regulation of cell adhesion. Predicted to be located in several cellular components, including endoplasmic reticulum membrane; epidermal lamellar body; and perinuclear region of cytoplasm. Is expressed in back skin. Used to study Netherton syndrome. Human ortholog(s) of this gene implicated in Netherton syndrome. Orthologous to human SPINK5 (serine peptidase inhibitor Kazal type 5). [provided by Alliance of Genome Resources, Apr 2022]
<b>Expression</b>	Restricted expression toward genital fat pad adult (RPKM 9.9) <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>
<b>NEW</b>	Try the new <a href="#">Gene table</a> Try the new <a href="#">Transcript table</a>

## Genomic context

**Location:** 18 B3; 18 23.74 cM

See Spink5 in [Genome Data Viewer](#)

**Exon count:** 34

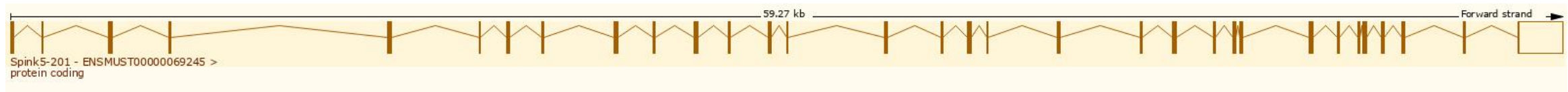
<https://www.ncbi.nlm.nih.gov/gene/72432>

# Transcript Information

The gene only has 1 transcript, which is shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
<a href="#">ENSMUST00000069245.8</a>	Spink5-201	4785	<a href="#">1017aa</a>	Protein coding	<a href="#">CCDS37805</a>	<a href="#">Q148R4</a>	Ensembl Canonical Gencode basic APPRIS P1 TSL:1

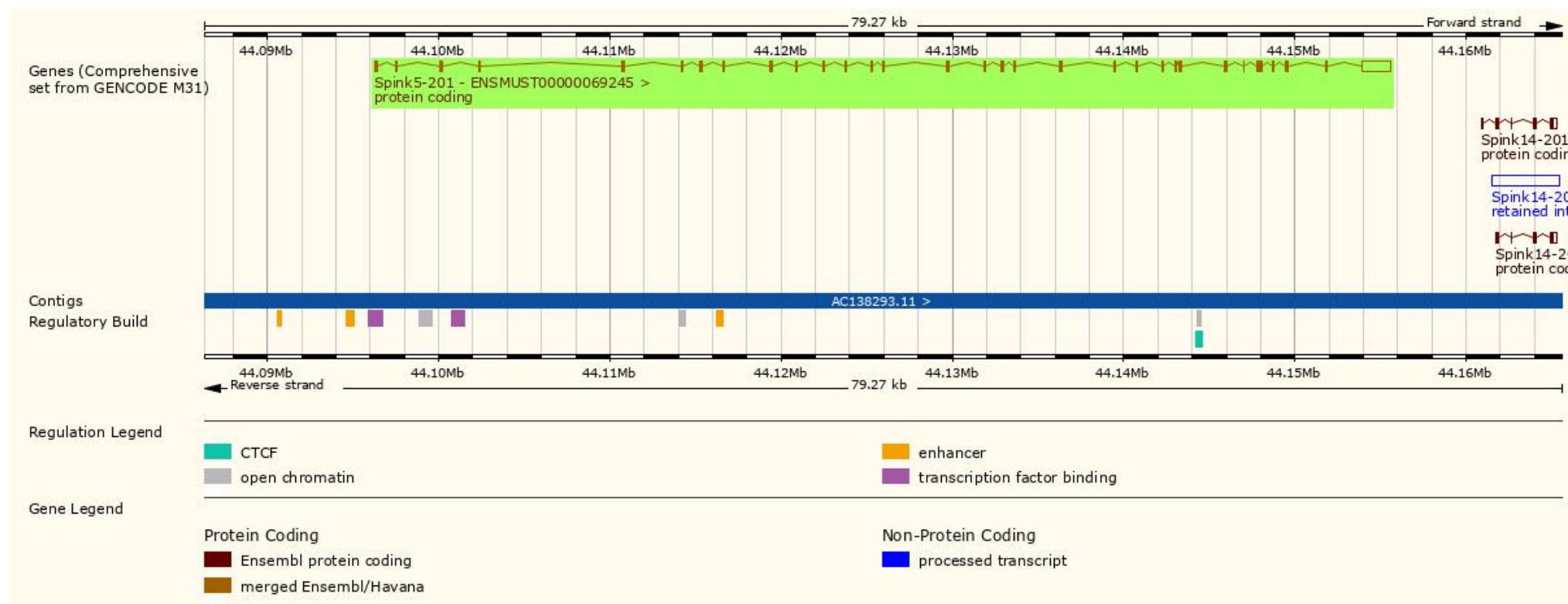
The strategy is based on the design of *Spink5*-201 transcript, the transcription is shown below:



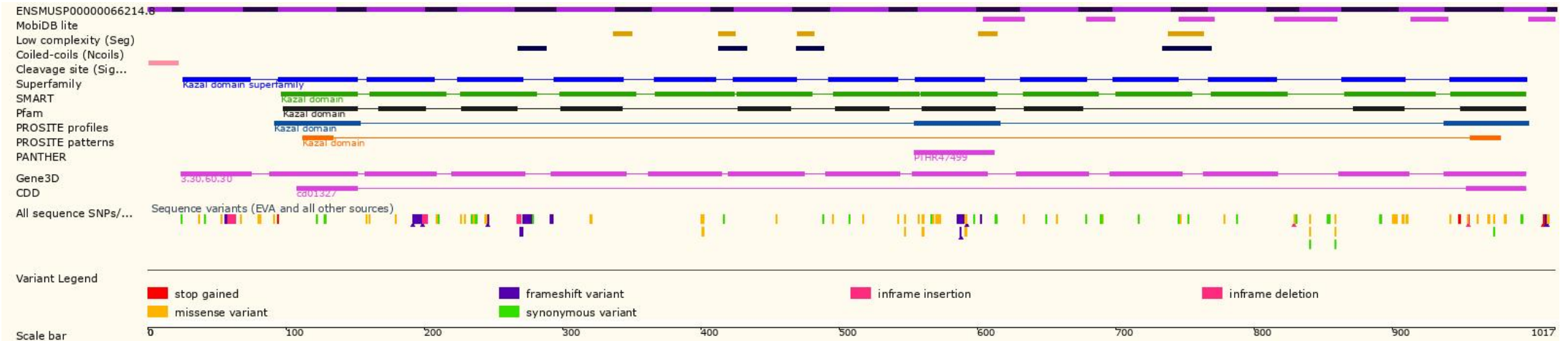
Source: <http://asia.ensembl.org/>



# Genomic Information

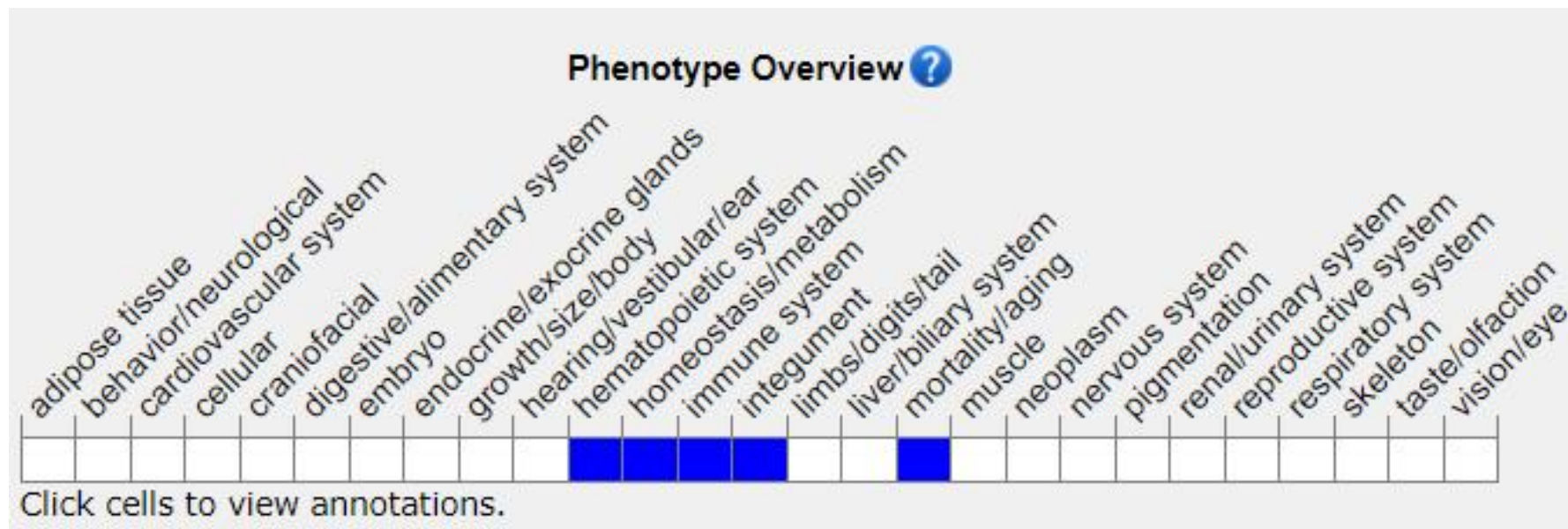


# Protein Information





# Mouse Phenotype Information (MGI)



Homozygous mutant mice display neonatal lethality, exfoliative erythroderma, and severe dehydration.

# Important Information

- According to the existing MGI data, homozygous mutant mice display neonatal lethality, exfoliative erythroderma, and severe dehydration.
- *Spink5* is located on Chr 18. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Reference

## Mice and *SPINK5* conditional KO generation

Homologous recombination and mouse embryonic stem cell (ES) technology<sup>E16,E17</sup> were used to generate a genetically modified mouse strain with a *SPINK5* conditional KO. A gene targeting the vector was constructed with an 1832 bp arm of 5' homology corresponding to GRCm38/mm10 chr18: 43,962,233-43,964,064 and a 2300 bp arm of 3' homology corresponding to chr18: 43,964,753-43,967,052. The 688 bp region flanked by loxP sites (exon 2) corresponds to chr18: 43,964,065-43,964,752.

[1] Jackman JK, Stockwell A, Choy DF, Xie MM, Lu P, Jia G, Li H, Abbas AR, Bronson PG, Lin WY, Chiu CPC, Maun HR, Roose-Girma M, Tam L, Zhang J, Modrusan Z, Graham RR, Behrens TW, White SR, Naureckas T, Ober C, Ferreira M, Sedlacek R, Wu J, Lee WP, Lazarus RA, Koerber JT, Arron JR, Yaspan BL, Yi T. Genome-wide association study identifies kallikrein 5 in type 2 inflammation-low asthma. *J Allergy Clin Immunol*. 2022 Oct;150(4):972-978.e7. doi: 10.1016/j.jaci.2022.03.033. Epub 2022 Apr 26. PMID: 35487308.