

# Pi16 Cas9-CKO Strategy

Designer: Yunpeng Zhang

Reviewer: Xiaojing Li

Design Date: 2025-2-14

# Overview

## Target Gene Name

- Pi16

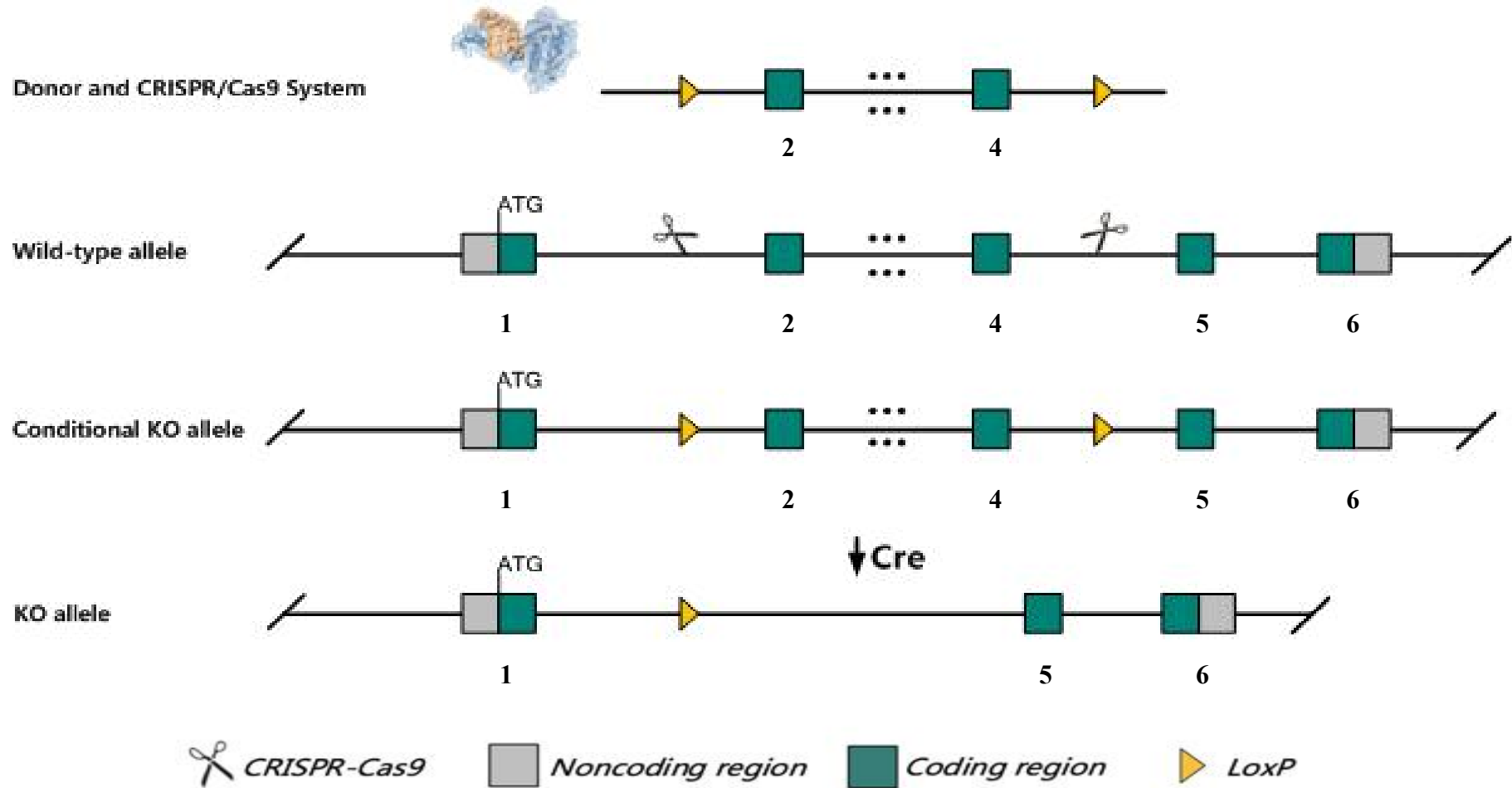
## Project Type

- Cas9-CKO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Pil6* gene.

# Technical Information

- The *Pil6* gene has 6 transcripts. According to the structure of *Pil6* gene, exon2-exon4 of *Pil6*-201 (ENSMUST00000114699.9) transcript is recommended as the knockout region. The region contains 421bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Pil6* 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

## Pi16 peptidase inhibitor 16 [ *Mus musculus* (house mouse) ]

[Download Datasets](#)

Gene ID: 74116, updated on 8-Feb-2025

### Summary

<b>Official Symbol</b>	Pi16 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	peptidase inhibitor 16 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1921366</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000024011</a> <a href="#">AllianceGenome:MGI:1921366</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>	Crip1; PI-16; 1200009H11Rik
<b>Summary</b>	Predicted to enable peptidase inhibitor activity. Acts upstream of or within negative regulation of cell growth involved in cardiac muscle cell development. Located in extracellular space. Is expressed in cranial ganglion; dorsal root ganglion; medulla oblongata basal plate mantle layer; and neural retina. Orthologous to human PI16 (peptidase inhibitor 16). [provided by Alliance of Genome Resources, Feb 2025]
<b>Expression</b>	Biased expression in bladder adult (RPKM 160.4), mammary gland adult (RPKM 72.5) and 6 other tissues <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>

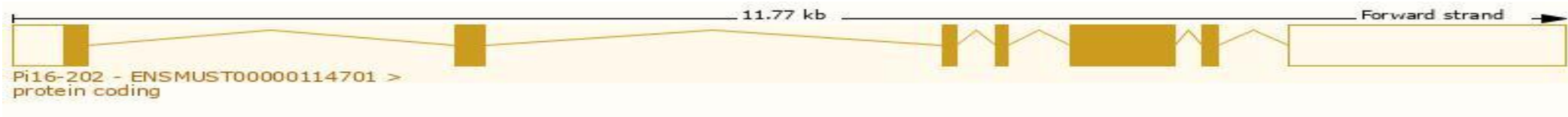
Source: <https://www.ncbi.nlm.nih.gov/>

# Transcript Information

The gene has 6 transcripts, all transcripts are shown below:

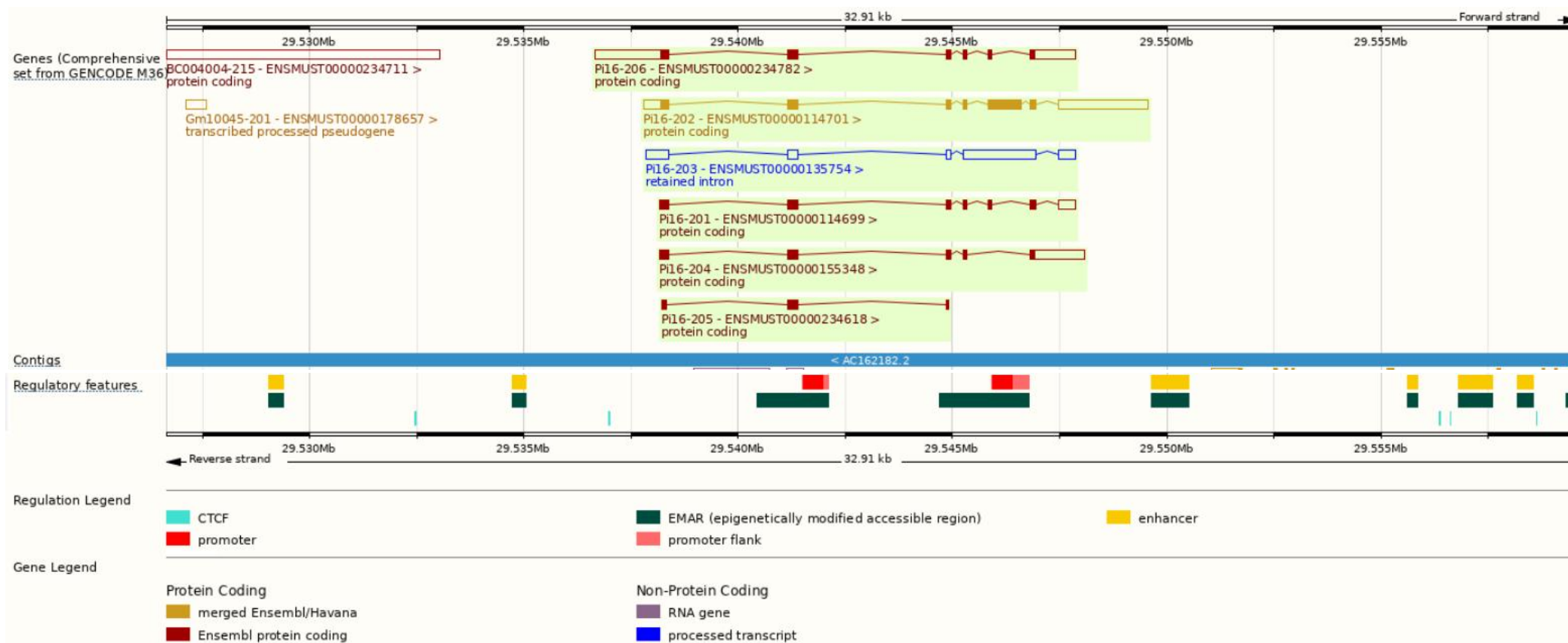
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
<a href="#">ENSMUST00000234782.2</a>	Pi16-206	3288	<a href="#">269aa</a>	Protein coding		<a href="#">D3Z6T6</a>	GENCODE Basic APPRIS ALT2
<a href="#">ENSMUST00000234618.2</a>	Pi16-205	397	<a href="#">132aa</a>	Protein coding		<a href="#">A0A3Q4EC30</a>	CDS 5' and 3' incomplete
<a href="#">ENSMUST00000155348.3</a>	Pi16-204	1875	<a href="#">236aa</a>	Protein coding		<a href="#">Q9ET66-2</a>	GENCODE Basic APPRIS ALT2 TSL:1
<a href="#">ENSMUST00000135754.2</a>	Pi16-203	2932	No protein	Retained intron		-	TSL:1
<a href="#">ENSMUST00000114701.10</a>	Pi16-202	3994	<a href="#">498aa</a>	Protein coding	<a href="#">CCDS28595</a>	<a href="#">Q9ET66-1</a>	Ensembl Canonical GENCODE Basic APPRIS P3 TSL:1
<a href="#">ENSMUST00000114699.9</a>	Pi16-201	1244	<a href="#">269aa</a>	Protein coding		<a href="#">D3Z6T6</a>	GENCODE Basic APPRIS ALT2 TSL:1

The strategy is based on the design of *Pi16-202* transcript, the transcription is shown below:

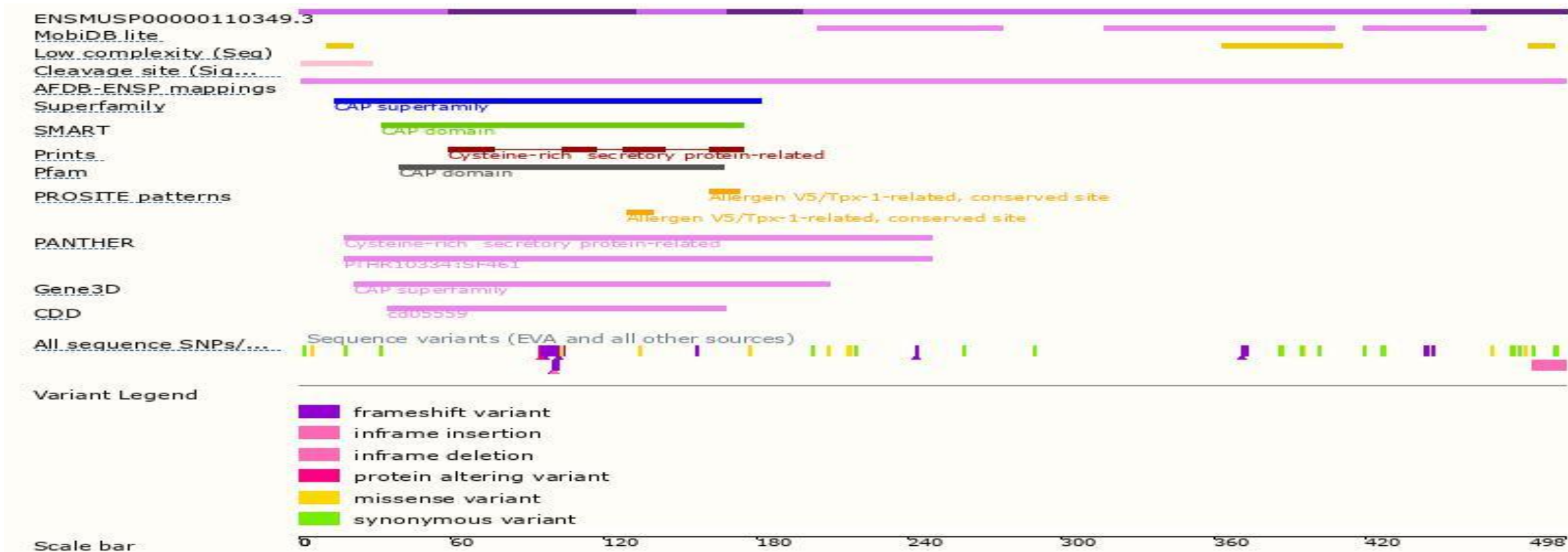


Source: <https://www.ensembl.org>

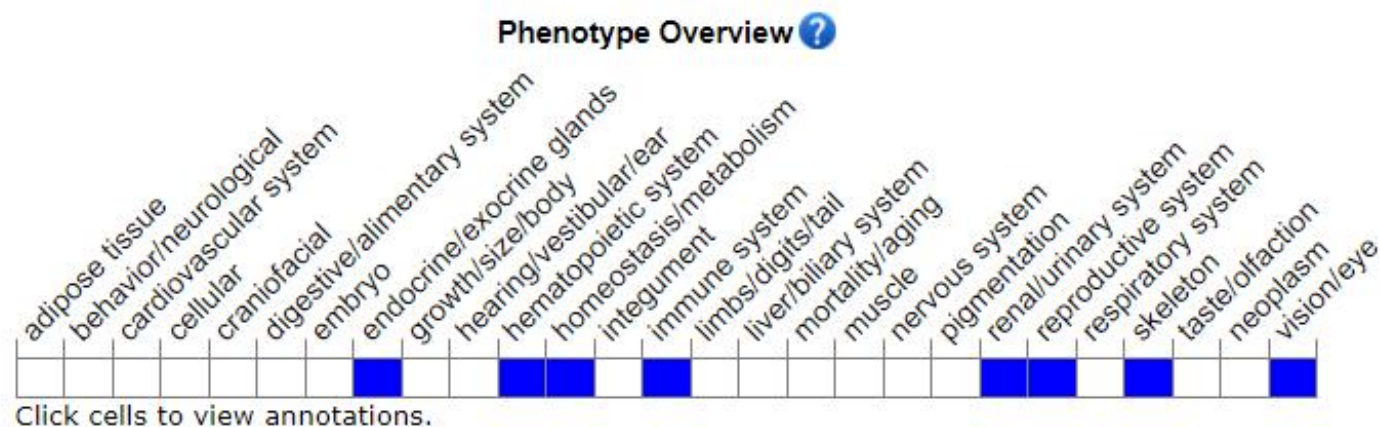
# Genomic Information



# Protein Information



# Mouse Phenotype Information (MGI)



- Homozygous mutant mice exhibited numerous immunological abnormalities during tissue-specific FACS analyses including an increased percentage of CD25+ cells in lymph node and B cell compartment differences in bone marrow spleen and lymph node.

# Important Information

- *Pil6* is located on Chr17. 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.
- The flox region overlap with *Gm46603* gene and the function is unknown.
- The intron 4-5 is short and the loxP site may affect the RNA splicing.
- 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.