

# *Map4k5* Cas9-KO Strategy

**Designer:**

**Yang Zeng**

**Reviewer:**

**Jia Yu**

**Design Date:**

**2019-12-16**

# Project Overview

**Project Name**

*Map4k5*

**Project type**

**Cas9-KO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

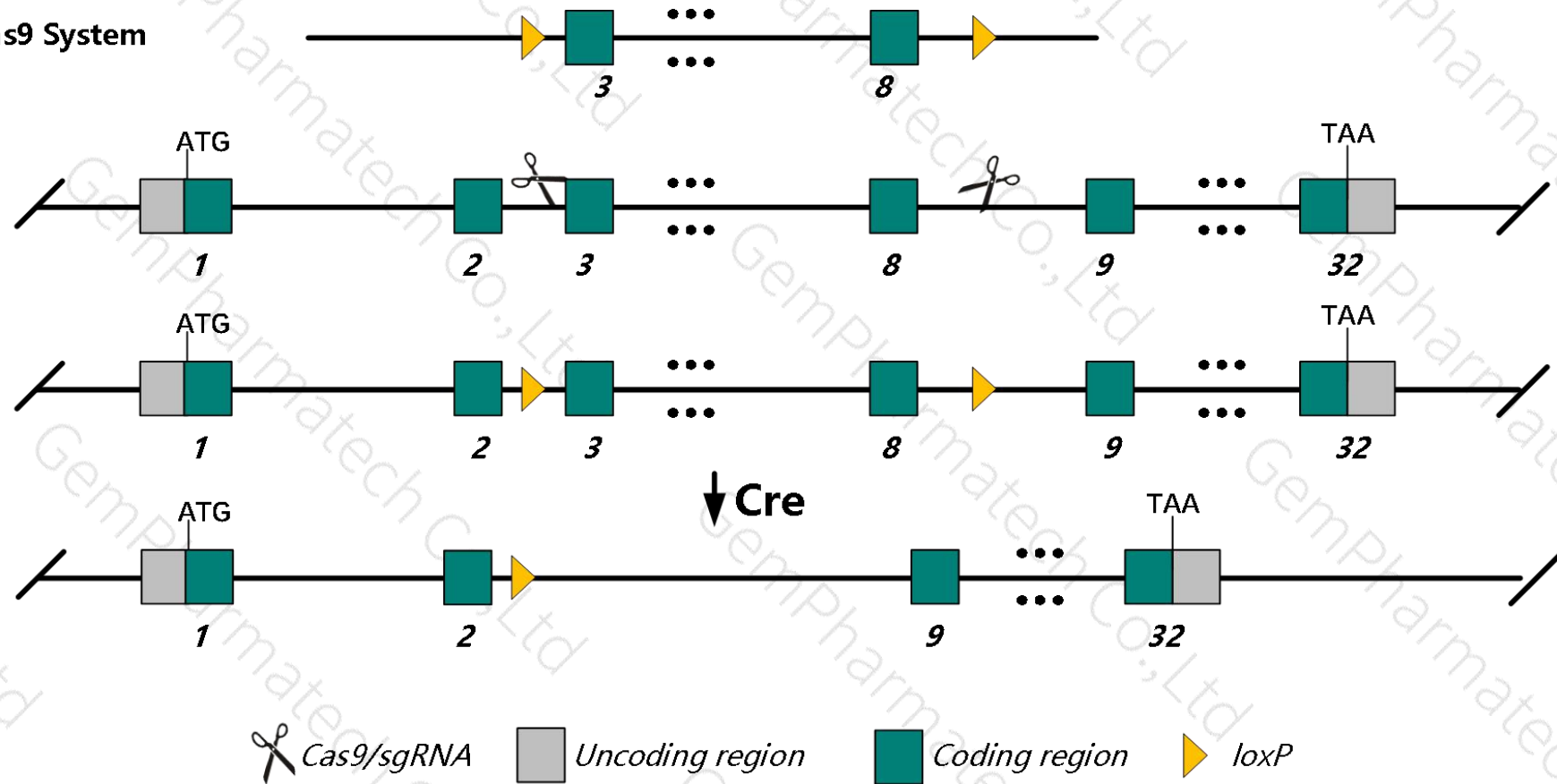
This model will use CRISPR/Cas9 technology to edit the *Map4k5* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Floxed allele

KO allele



# Technical routes

- The *Map4k5* gene has 5 transcripts. According to the structure of *Map4k5* gene, exon3-exon8 of *Map4k5-203* (ENSMUST00000110570.7) transcript is recommended as the knockout region. The region contains 376bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Map4k5* gene. The brief process is as follows: CRISPR/Cas9 system transcribed in vitro. Cas9 and gRNA 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the

- According to the existing MGI data, Mice homozygous for a null allele are viable and phenotypically normal but show impaired canonical and noncanonical Wnt signaling in progenitor B lymphocytes. Mice homozygous for a gene trap exhibit hypoalgesia, increased serum IgG1 and an increased percentage of peripheral blood CD4<sup>+</sup> cells.
- Transcript *Map4k5-208* lncRNA may not be affected.
- The *Map4k5* gene is located on the Chr12. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.



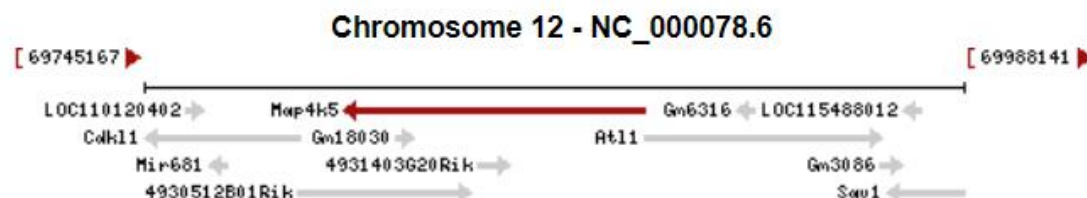
# Gene information (NCBI)

## Map4k5 mitogen-activated protein kinase kinase kinase kinase 5 [ *Mus musculus* (house mouse) ]

Gene ID: 399510, updated on 10-Oct-2019

### Summary

<b>Official Symbol</b>	Map4k5 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	mitogen-activated protein kinase kinase kinase kinase 5 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1925503</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000034761</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>	KHS; GCKR; MAPKKKK5; 4432415E19Rik
<b>Expression</b>	Ubiquitous expression in limb E14.5 (RPKM 9.4), bladder adult (RPKM 8.2) and 28 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

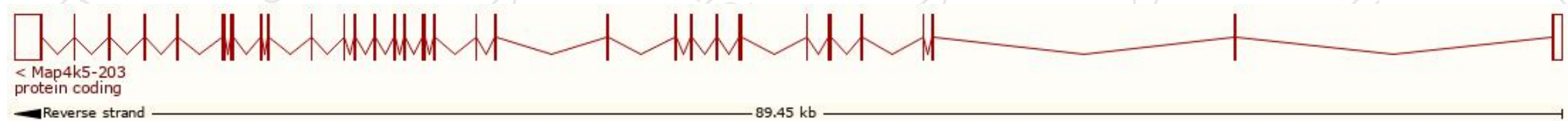


# Transcript information (Ensembl)

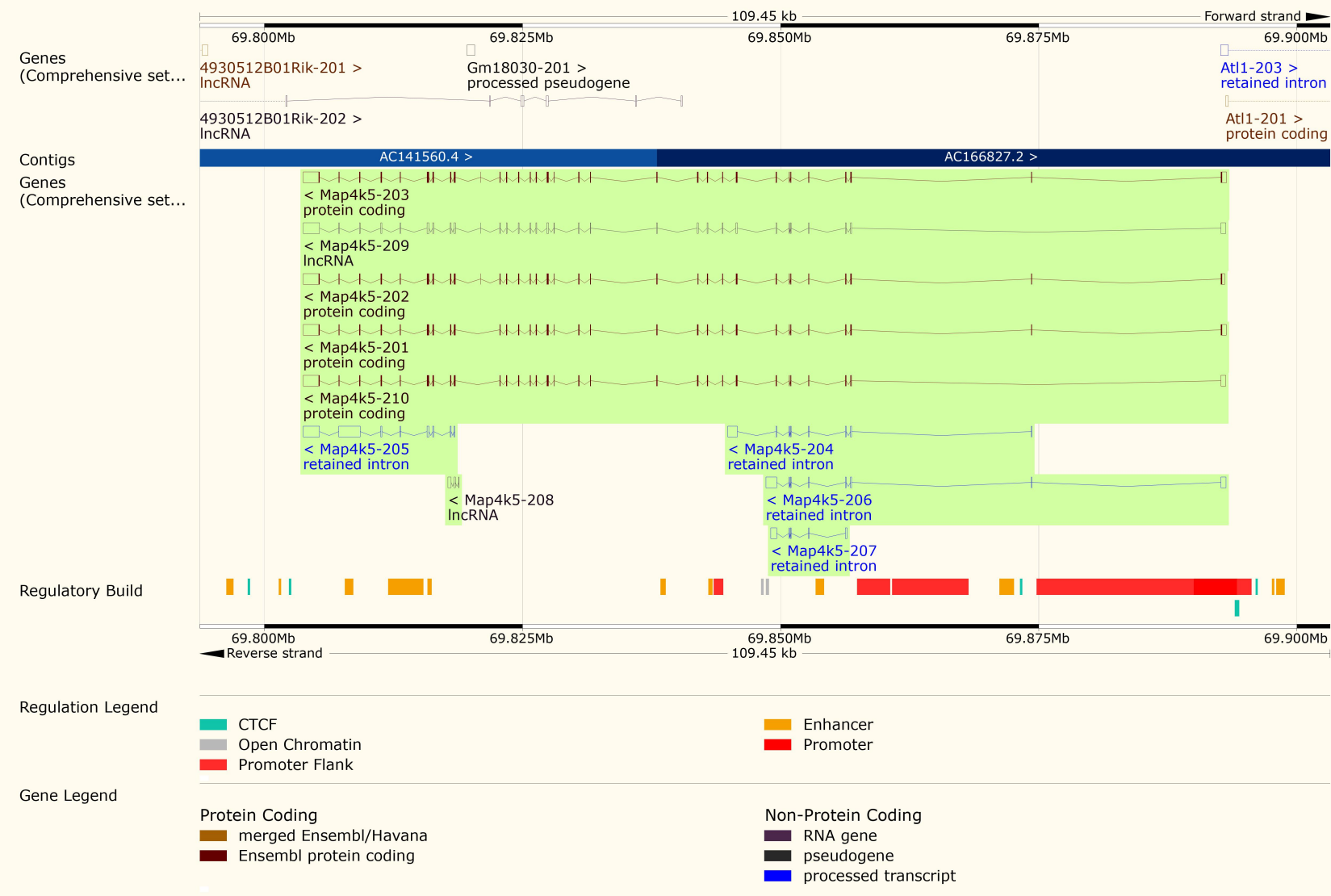
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Map4k5-203	<a href="#">ENSMUST00000110570.7</a>	4498	<a href="#">847aa</a>	<a href="#">ENSMUSP00000106199.1</a>	Protein coding	-	<a href="#">Q8BPM2</a>	TSL:5 GENCODE basic APPRIS ALT1
Map4k5-201	<a href="#">ENSMUST00000049239.7</a>	4452	<a href="#">847aa</a>	<a href="#">ENSMUSP00000047812.7</a>	Protein coding	-	<a href="#">E9PX30</a>	TSL:1 GENCODE basic APPRIS P5
Map4k5-210	<a href="#">ENSMUST00000171211.7</a>	4334	<a href="#">780aa</a>	<a href="#">ENSMUSP00000126006.1</a>	Protein coding	-	<a href="#">E9Q1T3</a>	TSL:2 GENCODE basic
Map4k5-202	<a href="#">ENSMUST00000110567.7</a>	4250	<a href="#">828aa</a>	<a href="#">ENSMUSP00000106196.1</a>	Protein coding	-	<a href="#">Q8BPM2</a>	TSL:5 GENCODE basic
Map4k5-205	<a href="#">ENSMUST00000126584.1</a>	4249	No protein	-	Retained intron	-	-	TSL:1
Map4k5-206	<a href="#">ENSMUST00000133688.7</a>	1885	No protein	-	Retained intron	-	-	TSL:1
Map4k5-204	<a href="#">ENSMUST00000124715.7</a>	1373	No protein	-	Retained intron	-	-	TSL:2
Map4k5-207	<a href="#">ENSMUST00000147451.1</a>	874	No protein	-	Retained intron	-	-	TSL:3
Map4k5-209	<a href="#">ENSMUST00000153712.7</a>	4343	No protein	-	lncRNA	-	-	TSL:5
Map4k5-208	<a href="#">ENSMUST00000153550.1</a>	463	No protein	-	lncRNA	-	-	TSL:3

The strategy is based on the design of *Map4k5-203* transcript,The transcription is shown below

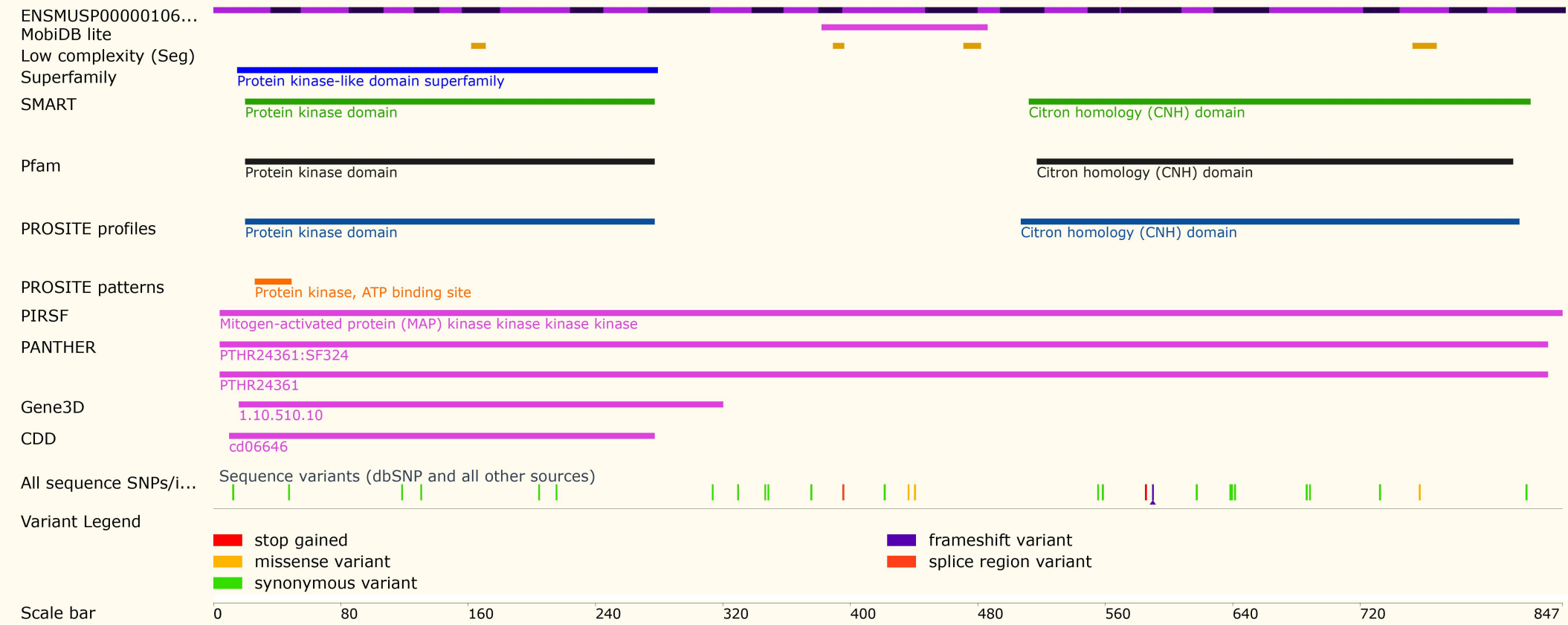


# Genomic location distribution



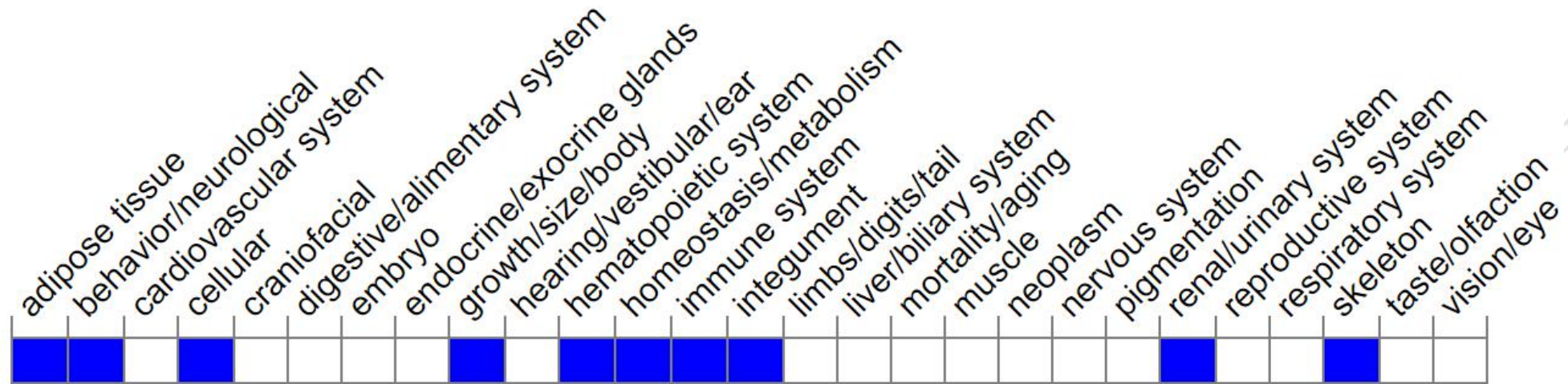


# Protein domain



# Mouse phenotype description(MGI)

## Phenotype Overview ?



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).

According to the existing MGI data, Mice homozygous for a knock-out allele exhibit decreased response of heart to stress following transverse aortic constriction.

If you have any questions, you are welcome to inquire.

Tel: 400-9660890

