

# ***Rasa4* Cas9-CKO Strategy**

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**Reviewer: Huimin Su**

**Design Date: 2020-4-26**

# Project Overview

**Project Name**

***Rasa4***

**Project type**

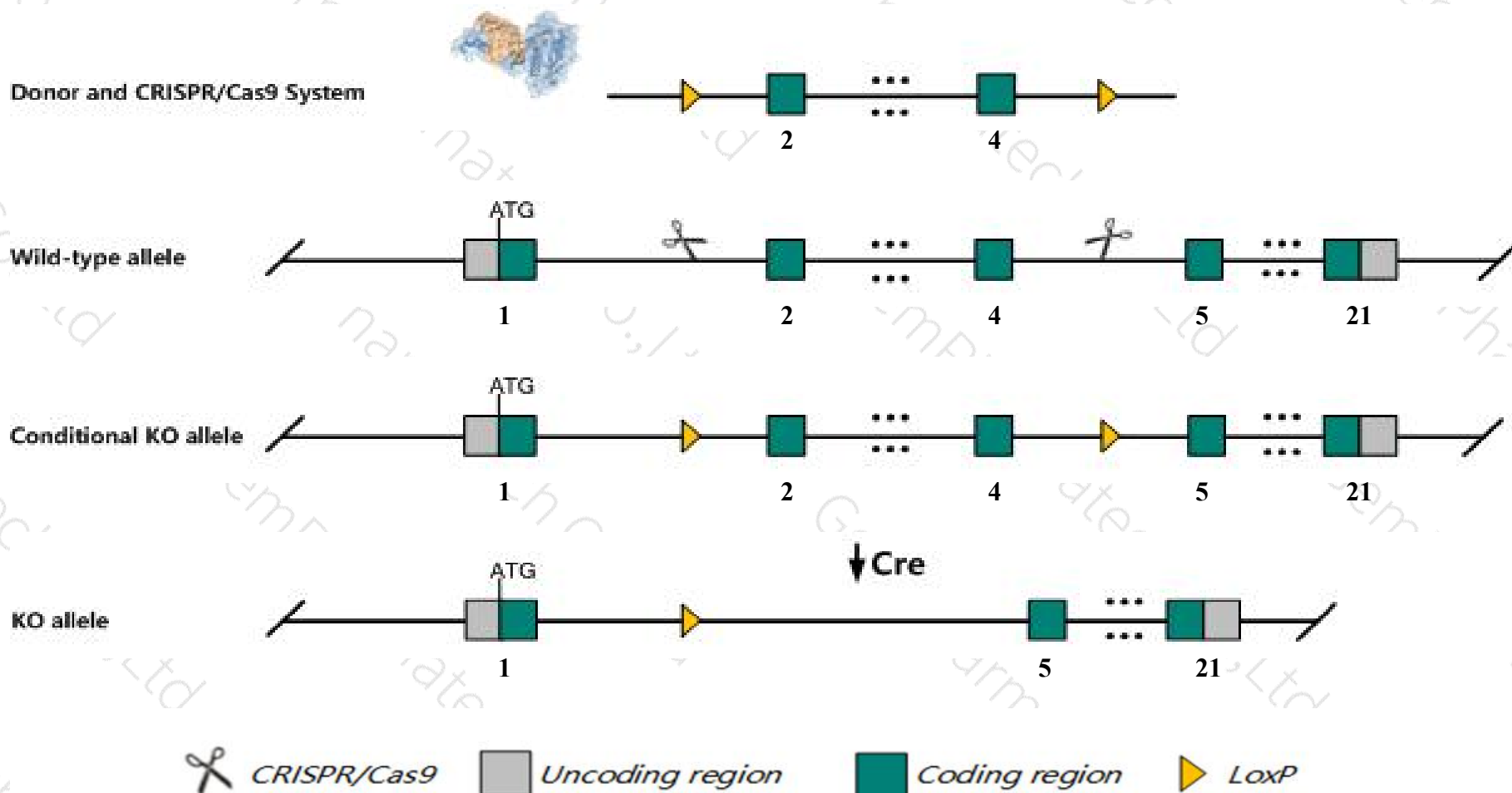
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rasa4* gene. The schematic diagram is as follows:



# Technical routes

- The *Rasa4* gene has 13 transcripts. According to the structure of *Rasa4* gene, exon2-exon4 of *Rasa4-201* (ENSMUST00000042135.13) transcript is recommended as the knockout region. The region contains 233bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rasa4* 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 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 target gene in specific tissues and cell types.

- According to the existing MGI data, mice homozygous for disruptions in this gene display an increased sensitivity to bacterial infections which involves reduced phagocyte function.
- Transcript *Rasa4-203* may not be affected.
- The *Rasa4* gene is located on the Chr5. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.



# Gene information (NCBI)

## Rasa4 RAS p21 protein activator 4 [Mus musculus (house mouse)]

Gene ID: 54153, updated on 20-Mar-2020

### Summary



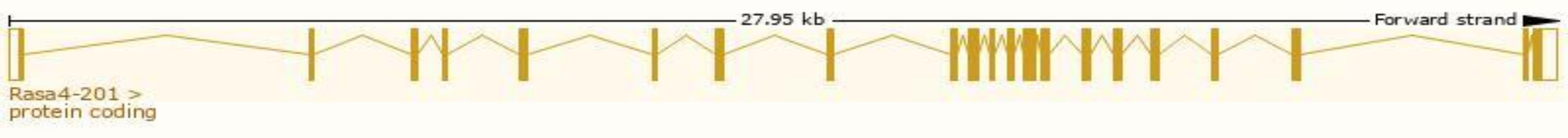
<b>Official Symbol</b>	Rasa4 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	RAS p21 protein activator 4 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1858600</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG000000004952</a>
<b>Gene type</b>	protein coding
<b>RefSeq 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>	AA793972, AW112107, BB079060, CAPRI, GAPL
<b>Expression</b>	Ubiquitous expression in colon adult (RPKM 17.6), spleen adult (RPKM 15.3) and 23 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

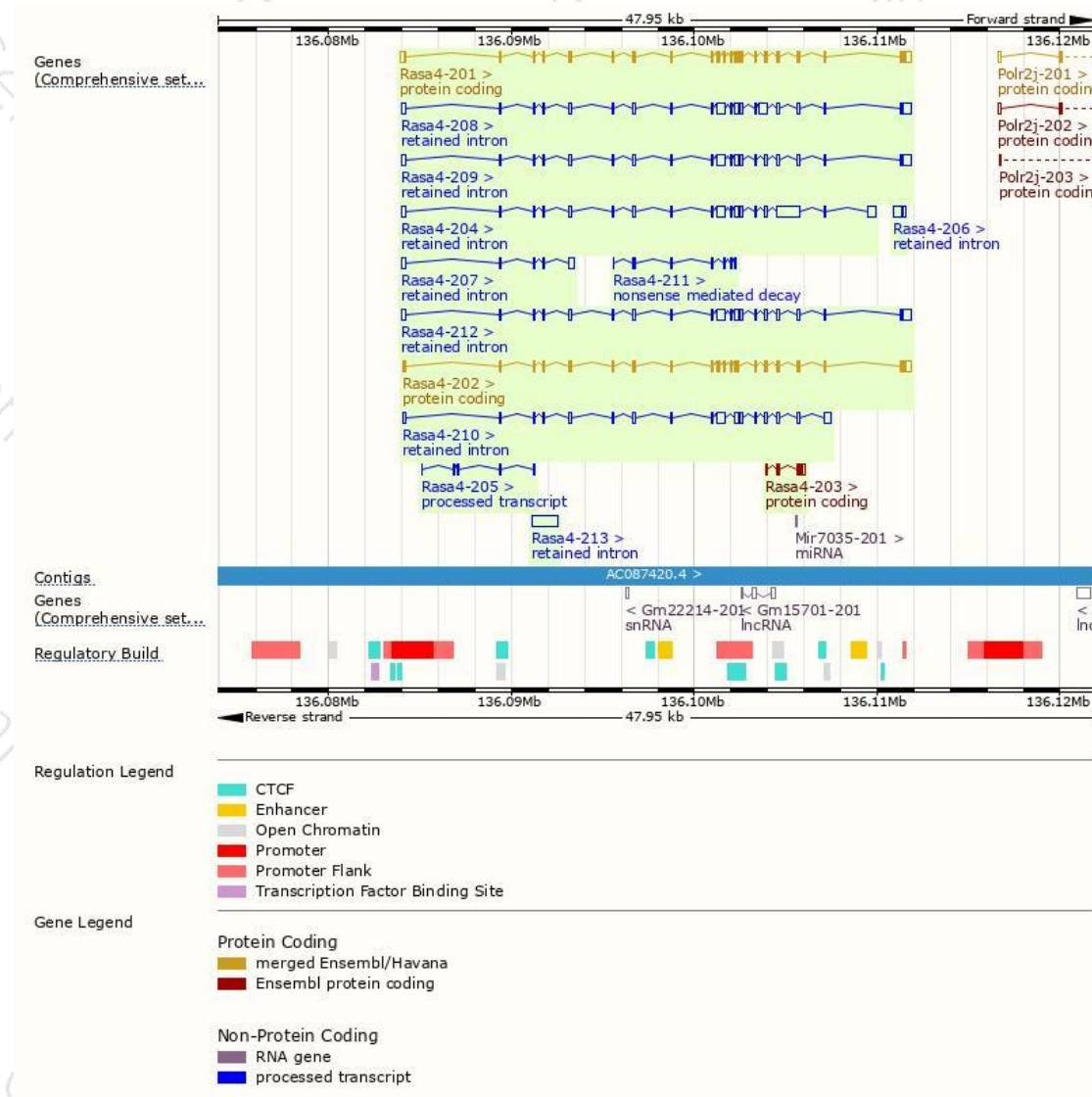
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rasa4-201	<a href="#">ENSMUST00000042135.13</a>	2914	<a href="#">802aa</a>	Protein coding	<a href="#">CCDS39324</a>	<a href="#">Q6PFQ7</a>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rasa4-202	<a href="#">ENSMUST00000100570.9</a>	2660	<a href="#">756aa</a>	Protein coding	<a href="#">CCDS39325</a>	<a href="#">Q6PFQ7</a>	TSL:1 GENCODE basic
Rasa4-203	<a href="#">ENSMUST00000122887.1</a>	681	<a href="#">178aa</a>	Protein coding	-	<a href="#">F6W8M5</a>	CDS 5' incomplete TSL:2
Rasa4-211	<a href="#">ENSMUST00000145294.1</a>	600	<a href="#">141aa</a>	Nonsense mediated decay	-	<a href="#">F6WTE9</a>	CDS 5' incomplete TSL:5
Rasa4-205	<a href="#">ENSMUST00000125247.1</a>	371	No protein	Processed transcript	-	-	TSL:2
Rasa4-204	<a href="#">ENSMUST00000125048.7</a>	3967	No protein	Retained intron	-	-	TSL:2
Rasa4-208	<a href="#">ENSMUST00000134509.7</a>	3396	No protein	Retained intron	-	-	TSL:2
Rasa4-209	<a href="#">ENSMUST00000135344.7</a>	3057	No protein	Retained intron	-	-	TSL:2
Rasa4-212	<a href="#">ENSMUST00000152723.7</a>	3030	No protein	Retained intron	-	-	TSL:2
Rasa4-210	<a href="#">ENSMUST00000140972.7</a>	2683	No protein	Retained intron	-	-	TSL:2
Rasa4-213	<a href="#">ENSMUST00000199502.1</a>	1470	No protein	Retained intron	-	-	TSL:NA
Rasa4-207	<a href="#">ENSMUST00000131353.7</a>	722	No protein	Retained intron	-	-	TSL:2
Rasa4-206	<a href="#">ENSMUST00000130598.1</a>	485	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Rasa4-201* transcript,the transcription is shown below:

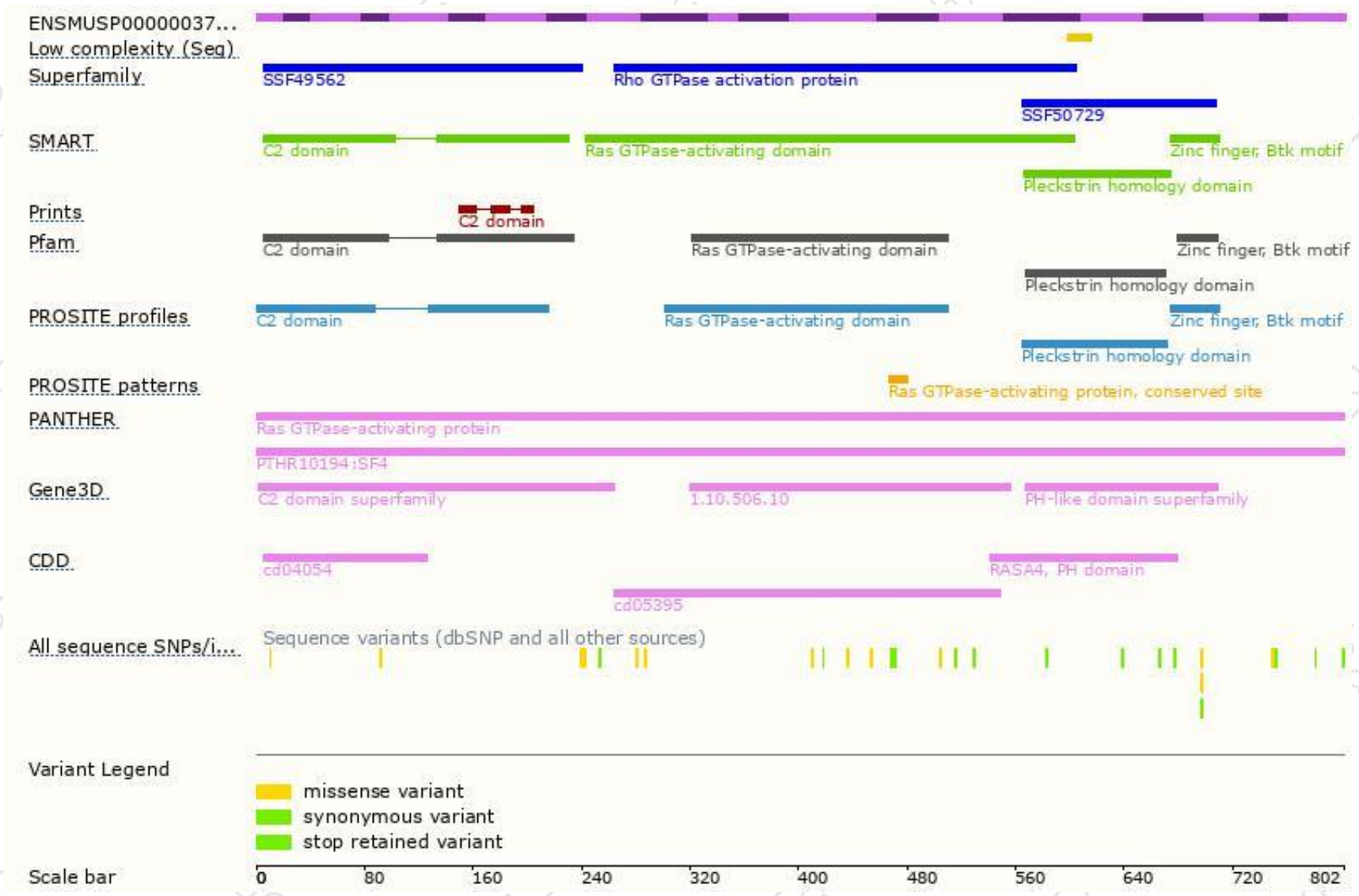


# Genomic location distribution



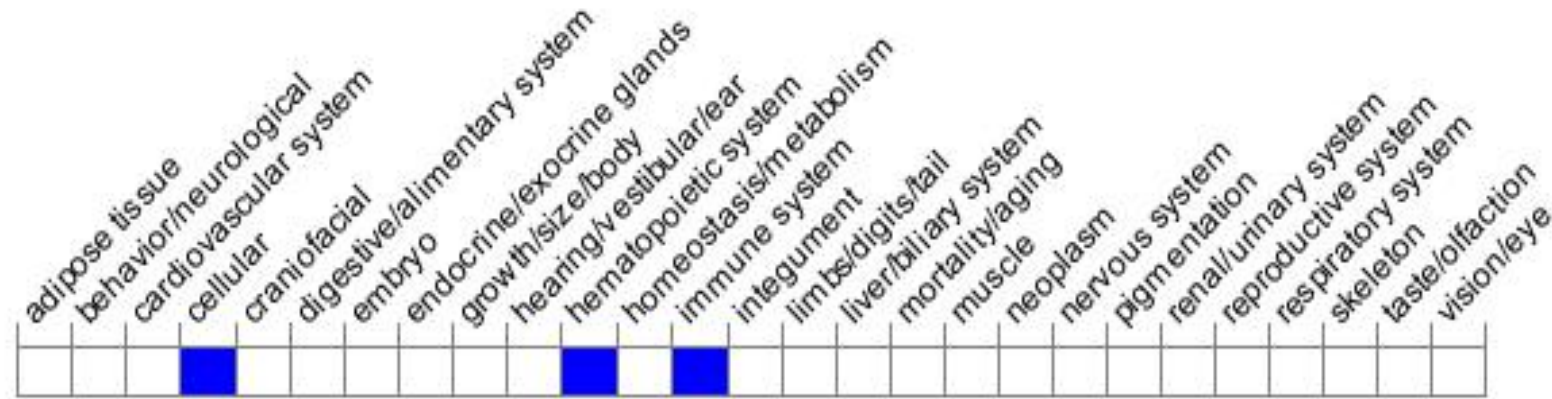


# Protein domain



# 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 disruptions in this gene display an increased sensitivity to bacterial infections which involves reduced phagocyte function.

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

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