

# ***Creb1* Cas9-CKO Strategy**

**Designer:**

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**Design Date:**

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# Project Overview

**Project Name**

*Creb1*

**Project type**

**Cas9-CKO**

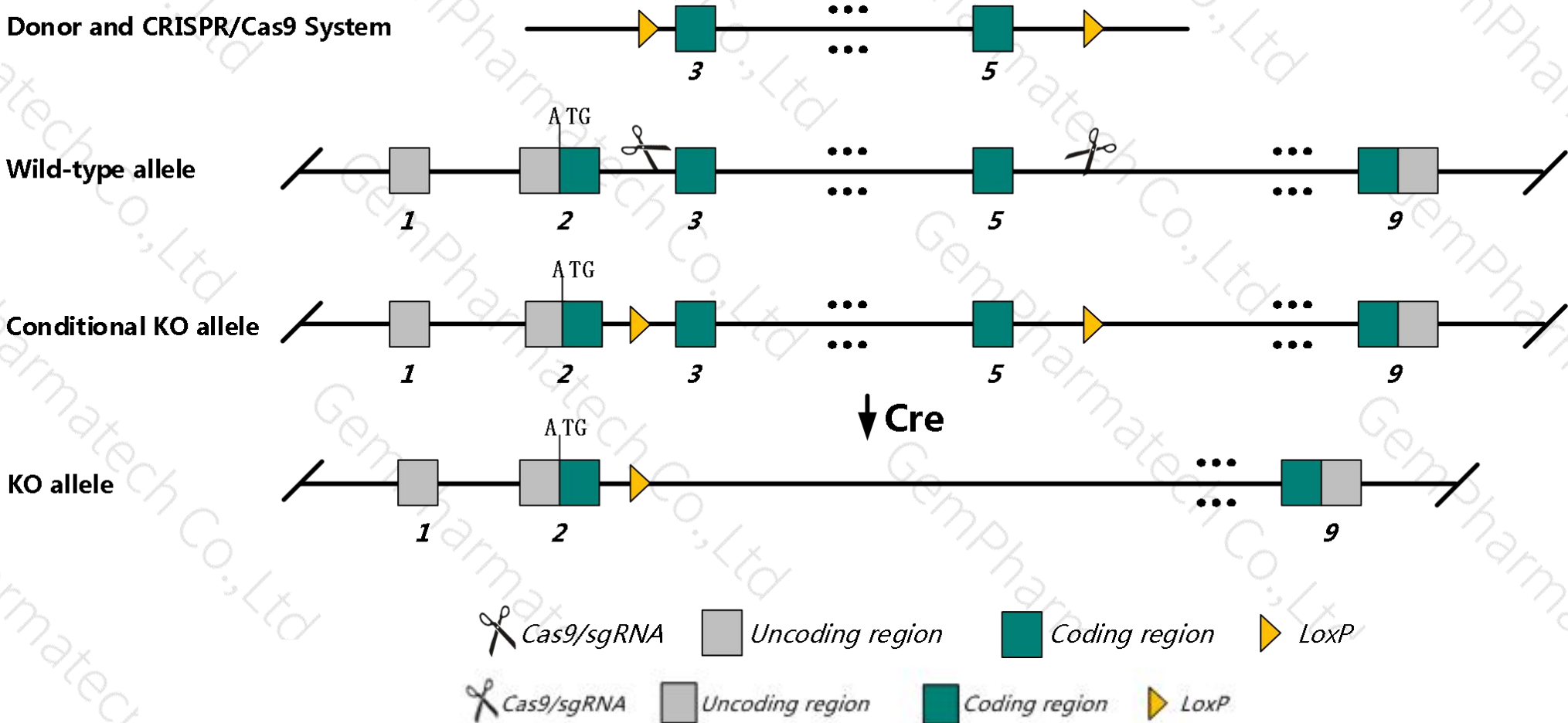
**Strain background**

**C57BL/6JGpt**



# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Creb1* gene. The schematic diagram is as follows:





# Technical routes

- The *Creb1* gene has 11 transcripts. According to the structure of *Creb1* gene, exon3-exon5 of *Creb1-201* (ENSMUST00000049932.11) transcript is recommended as the knockout region. The region contains 248bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Creb1* 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 alleles lacking some or all isotypes exhibit a range of defects involving circadian rhythms, axonal growth, sensory neuron survival, long-term memory, fear conditioning, body size, respiration, and neonatal viability.
- The *Creb1* gene is located on the Chr1. 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)

## Creb1 cAMP responsive element binding protein 1 [Mus musculus (house mouse)]

Gene ID: 12912, updated on 7-Apr-2019

### Summary



|                           |   |
|---------------------------|---|
| <b>Official Symbol</b>    | Creb1 provided by <a href="#">MGI</a>   |
| <b>Official Full Name</b> | cAMP responsive element binding protein 1 provided by <a href="#">MGI</a>   |
| <b>Primary source</b>     | <a href="#">MGI:MGI:88494</a>   |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG000000025958</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>      | 2310001E10Rik, 3526402H21Rik, AV083133, Creb, Creb-1  |
| <b>Expression</b>         | Ubiquitous expression in CNS E11.5 (RPKM 5.1), thymus adult (RPKM 4.9) and 28 other tissues <a href="#">See more</a>  |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>   |

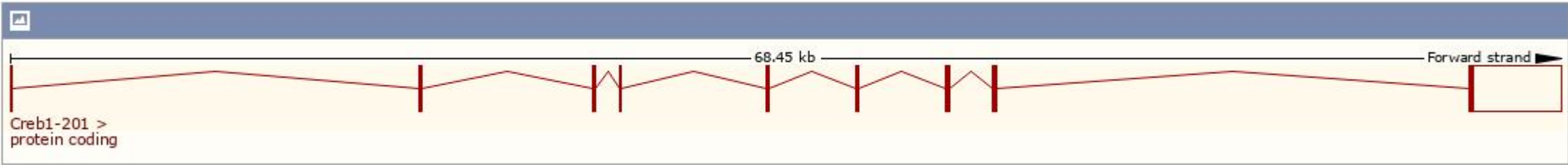


# Transcript information (Ensembl)

The gene has 11 transcripts,all transcripts are shown below:

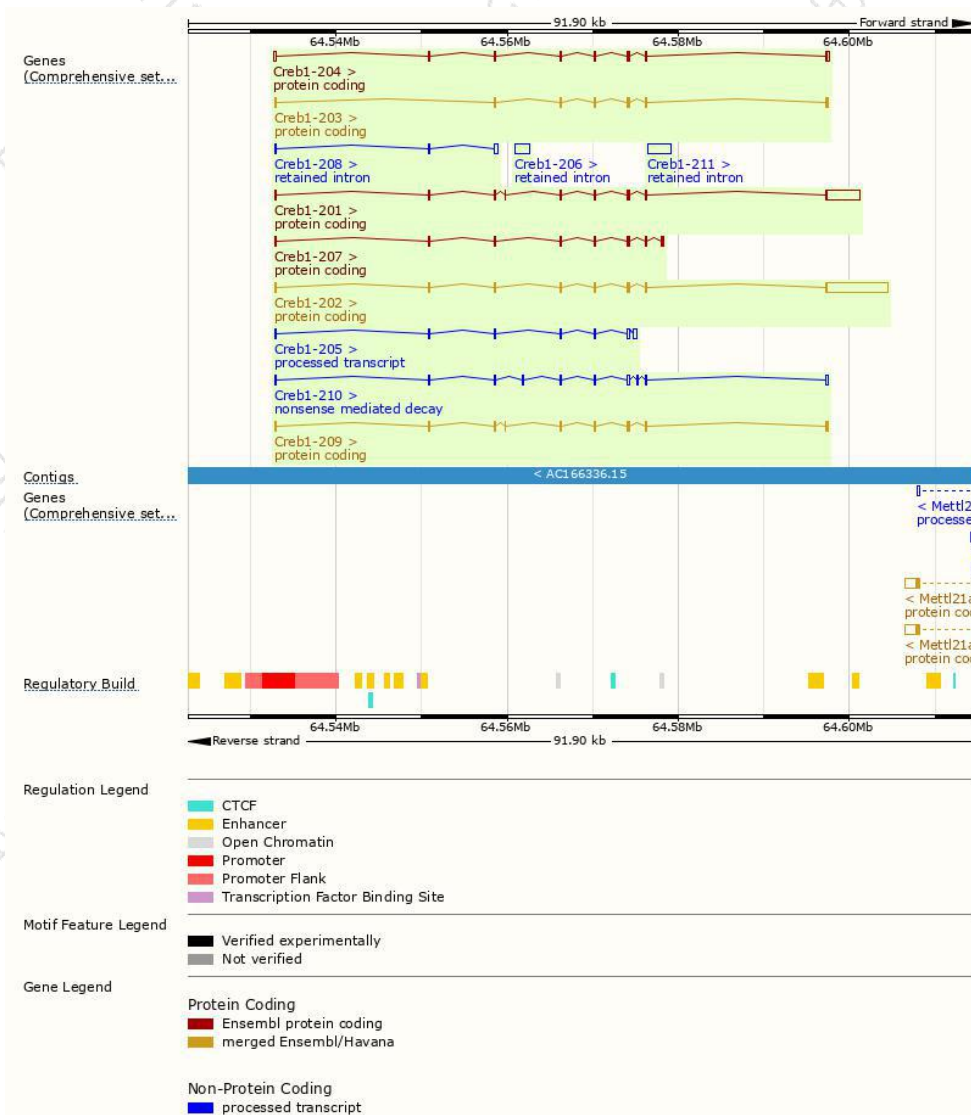
| Name      | Transcript ID                         | bp   | Protein               | Biotype                 | CCDS                      | UniProt                       | Flags                           |
|-----------|---------------------------------------|------|-----------------------|-------------------------|---------------------------|-------------------------------|---------------------------------|
| Creb1-202 | <a href="#">ENSMUST00000087366.10</a> | 8364 | <a href="#">327aa</a> | Protein coding          | <a href="#">CCDS15005</a> | <a href="#">Q01147 Q543W0</a> | TSL:1 GENCODE basic APPRIS ALT1 |
| Creb1-201 | <a href="#">ENSMUST00000049932.11</a> | 5009 | <a href="#">341aa</a> | Protein coding          | <a href="#">CCDS15004</a> | <a href="#">Q01147 Q547S9</a> | TSL:5 GENCODE basic APPRIS P4   |
| Creb1-204 | <a href="#">ENSMUST00000185594.6</a>  | 1569 | <a href="#">327aa</a> | Protein coding          | <a href="#">CCDS15005</a> | <a href="#">Q01147 Q543W0</a> | TSL:5 GENCODE basic APPRIS ALT1 |
| Creb1-203 | <a href="#">ENSMUST00000171164.7</a>  | 1287 | <a href="#">287aa</a> | Protein coding          | <a href="#">CCDS48280</a> | <a href="#">Q62347</a>        | TSL:1 GENCODE basic             |
| Creb1-209 | <a href="#">ENSMUST00000190348.1</a>  | 1260 | <a href="#">341aa</a> | Protein coding          | <a href="#">CCDS15004</a> | <a href="#">Q01147 Q547S9</a> | TSL:1 GENCODE basic APPRIS P4   |
| Creb1-207 | <a href="#">ENSMUST00000187811.6</a>  | 1255 | <a href="#">317aa</a> | Protein coding          | -                         | <a href="#">A0A087WRI6</a>    | TSL:1 GENCODE basic             |
| Creb1-210 | <a href="#">ENSMUST00000190876.6</a>  | 1420 | <a href="#">90aa</a>  | Nonsense mediated decay | -                         | <a href="#">Q61441</a>        | TSL:5                           |
| Creb1-205 | <a href="#">ENSMUST00000186335.6</a>  | 1288 | No protein            | Processed transcript    | -                         | -                             | TSL:1                           |
| Creb1-211 | <a href="#">ENSMUST00000190979.1</a>  | 2763 | No protein            | Retained intron         | -                         | -                             | TSL:NA                          |
| Creb1-206 | <a href="#">ENSMUST00000187035.1</a>  | 1642 | No protein            | Retained intron         | -                         | -                             | TSL:NA                          |
| Creb1-208 | <a href="#">ENSMUST00000188855.6</a>  | 660  | No protein            | Retained intron         | -                         | -                             | TSL:3                           |

The strategy is based on the design of *Creb1-201* transcript,The transcription is shown below



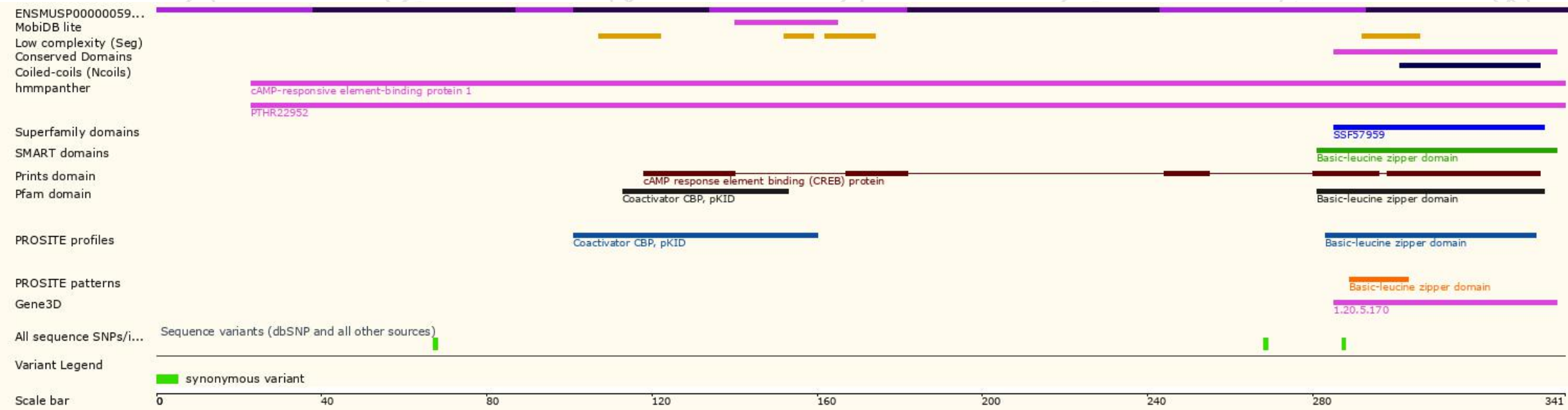


# Genomic location distribution



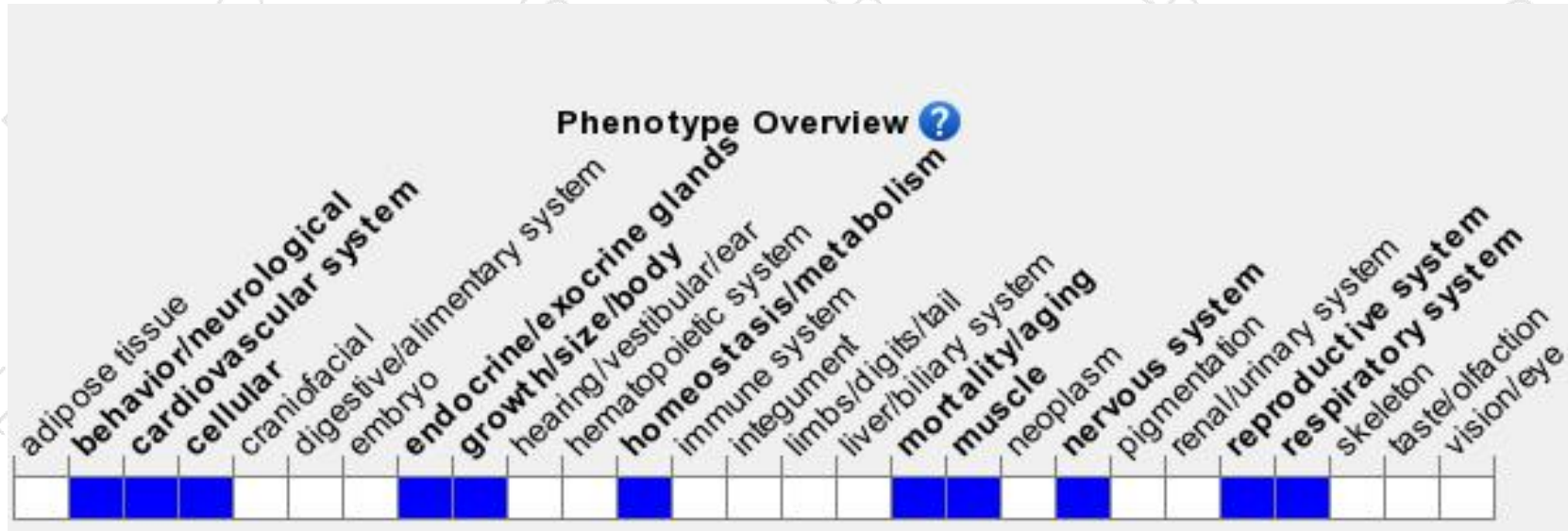


# Protein domain





# Mouse phenotype description(MGI)



*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 alleles lacking some or all isotypes exhibit a range of defects involving circadian rhythms, axonal growth, sensory neuron survival, long-term memory, fear conditioning, body size respiration, and neonatal viability.



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

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