

# Atg4b Cas9-KO Strategy

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Reviewer: Xiaojing Li

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## **Project Overview**



Project Name Atg4b

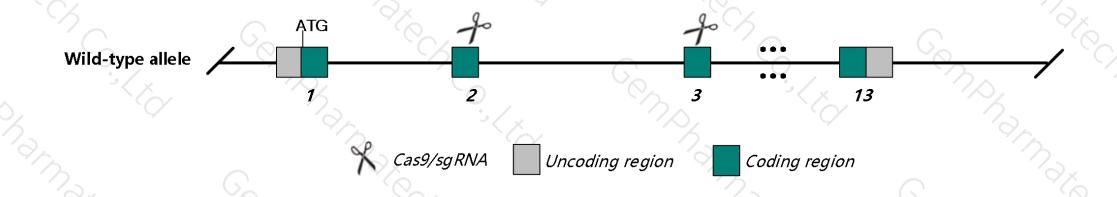
Project type Cas9-KO

Strain background C57BL/6N

# **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Atg4b gene. The schematic diagram is as follows:



### **Technical routes**



➤ In this project we use CRISPR/Cas9 technology to modify *Atg4b* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6N 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/6N mice.

### **Notice**



- > According to the existing MGI data, Mice homozygous for a gene trap allele exhibit decreased autophagy, impaired swimming, circling, head tilting, and abnormal utricle, saccular, and otolith morphology. Mice homozygous for another gene trap allele exhibit partial preweaning lethality and impaired motor coordination and learning.
- ➤ The Atg4b 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

### Gene information (NCBI)



#### Atg4b autophagy related 4B, cysteine peptidase [ Mus musculus (house mouse) ]

Gene ID: 66615, updated on 13-Aug-2019

#### Summary

2 7

Official Symbol Atg4b provided by MGI

Official Full Name autophagy related 4B, cysteine peptidase provided by MGI

Primary source MGI:MGI:1913865

See related Ensembl: ENSMUSG00000026280

Gene type protein coding
RefSeq status VALIDATED
Organism <u>Mus musculus</u>

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;

Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as Apg4b; Autl1; Atg4bl; AW048066; 2510009N07Rik

Expression Ubiquitous expression in CNS E14 (RPKM 14.9), whole brain E14.5 (RPKM 14.0) and 28 other tissues See more

Orthologs human all

# Transcript information (Ensembl)

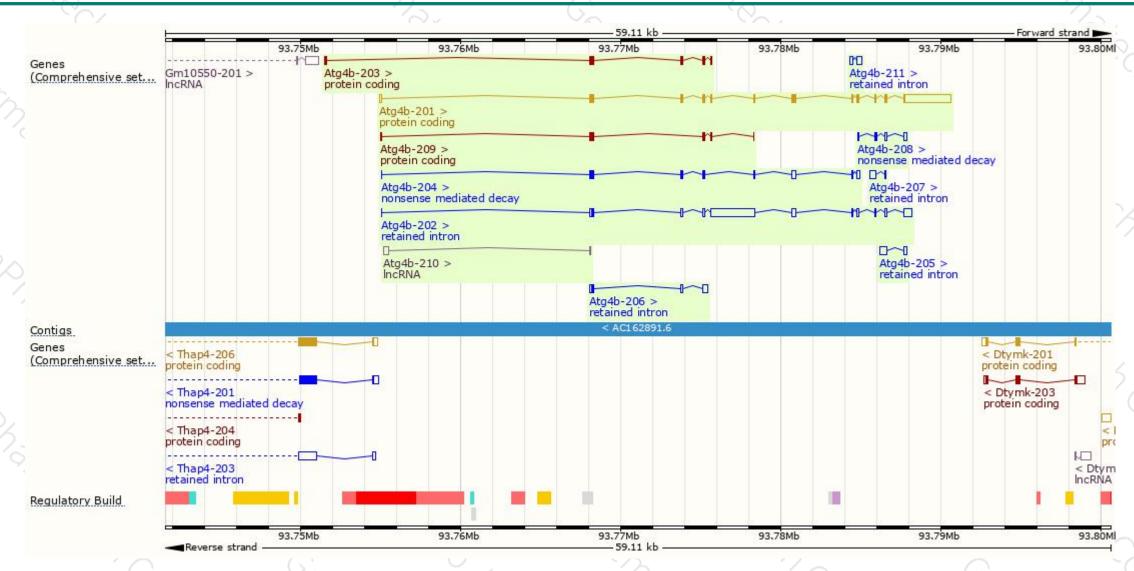


The gene has 11 transcripts, all transcripts are shown below:

| Name 4    | Transcript ID         | bp 🍦 | Protein 4    | Biotype                 | CCDS 🍦      | UniProt 🍦           | Flags                         |
|-----------|-----------------------|------|--------------|-------------------------|-------------|---------------------|-------------------------------|
| Atg4b-201 | ENSMUST00000027502.15 | 4173 | 393aa        | Protein coding          | CCDS15195 ₽ | A0A0R4J065 €        | TSL:1 GENCODE basic APPRIS P1 |
| Atg4b-203 | ENSMUST00000149436.7  | 513  | 150aa        | Protein coding          | 139         | D3YZP6函             | CDS 3' incomplete TSL:3       |
| Atg4b-209 | ENSMUST00000187824.6  | 420  | <u>121aa</u> | Protein coding          | 139         | <u>A0A087WNY2</u> ₽ | CDS 3' incomplete TSL:5       |
| Atg4b-204 | ENSMUST00000185482.6  | 919  | 143aa        | Nonsense mediated decay | 32          | A0A087WRT0₽         | TSL:5                         |
| Atg4b-208 | ENSMUST00000186811.1  | 503  | <u>63aa</u>  | Nonsense mediated decay | 139         | <u>A0A087WNR6</u> ₺ | CDS 5' incomplete TSL:5       |
| Atg4b-202 | ENSMUST00000135762.7  | 4186 | No protein   | Retained intron         | 139         | 35                  | TSL:5                         |
| Atg4b-205 | ENSMUST00000185754.1  | 601  | No protein   | Retained intron         | 39          | 35                  | TSL:2                         |
| Atg4b-206 | ENSMUST00000186001.1  | 565  | No protein   | Retained intron         | 139         | 35                  | TSL:2                         |
| Atg4b-211 | ENSMUST00000189872.1  | 477  | No protein   | Retained intron         | 139         | 35                  | TSL:3                         |
| Atg4b-207 | ENSMUST00000186124.1  | 454  | No protein   | Retained intron         | - 1         | 35                  | TSL:3                         |
| Atg4b-210 | ENSMUST00000189152.1  | 343  | No protein   | IncRNA                  | -           | 58                  | TSL:2                         |
| N. 1979   | 7 3 2 2               |      |              |                         | 13.1        |                     | / / /                         |

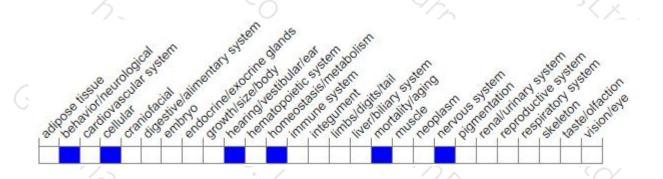
### Genomic location distribution





### 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 a gene trap allele exhibit decreased autophagy, impaired swimming, circling, head tilting, and abnormal utricle, saccular, and otolith morphology. Mice homozygous for another gene trap allele exhibit partial preweaning lethality and impaired motor coordination and learning.



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

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