

# *Atg4b* Cas9-CKO Strategy

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# Project Overview

**Project Name**

*Atg4b*

**Project type**

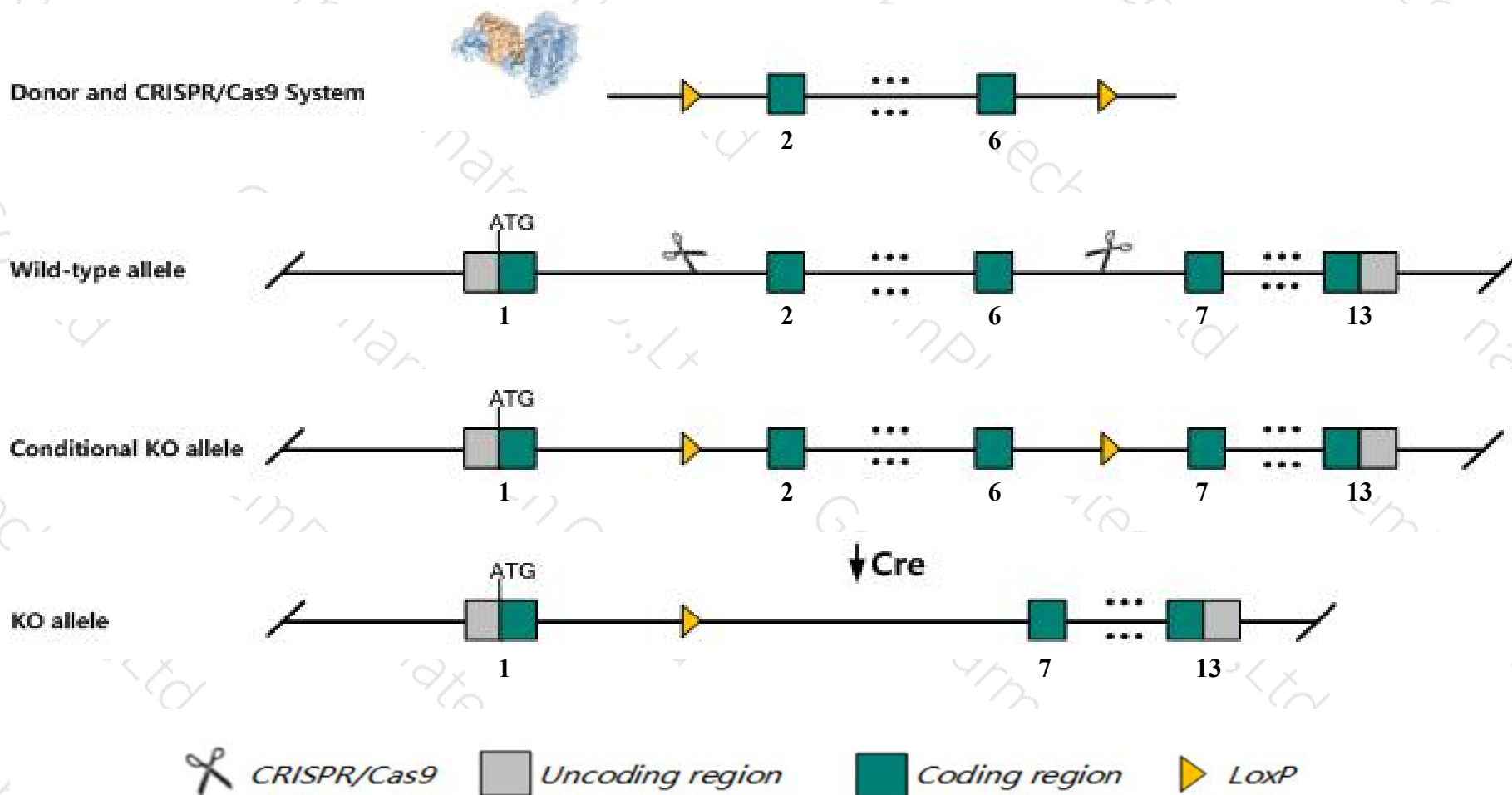
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

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



- The *Atg4b* gene has 11 transcripts. According to the structure of *Atg4b* gene, exon2-exon6 of *Atg4b-201* (ENSMUST00000027502.15) transcript is recommended as the knockout region. The region contains 448bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Atg4b* 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 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.



# Gene information (NCBI)

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

Gene ID: 66615, updated on 9-Mar-2019

### Summary



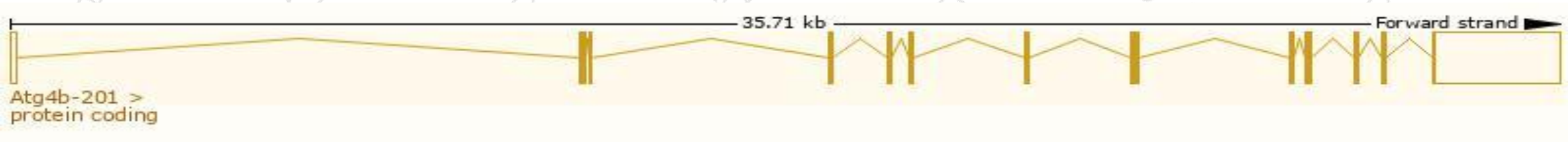
<b>Official Symbol</b>	Atg4b provided by <a href="#">MGI</a>
<b>Official Full Name</b>	autophagy related 4B, cysteine peptidase provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1913865</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000026280</a>
<b>Gene type</b>	protein 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>	2510009N07Rik, AW048066, Apg4b, Atg4bl, Autl1
<b>Expression</b>	Ubiquitous expression in CNS E14 (RPKM 14.9), whole brain E14.5 (RPKM 14.0) and 28 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

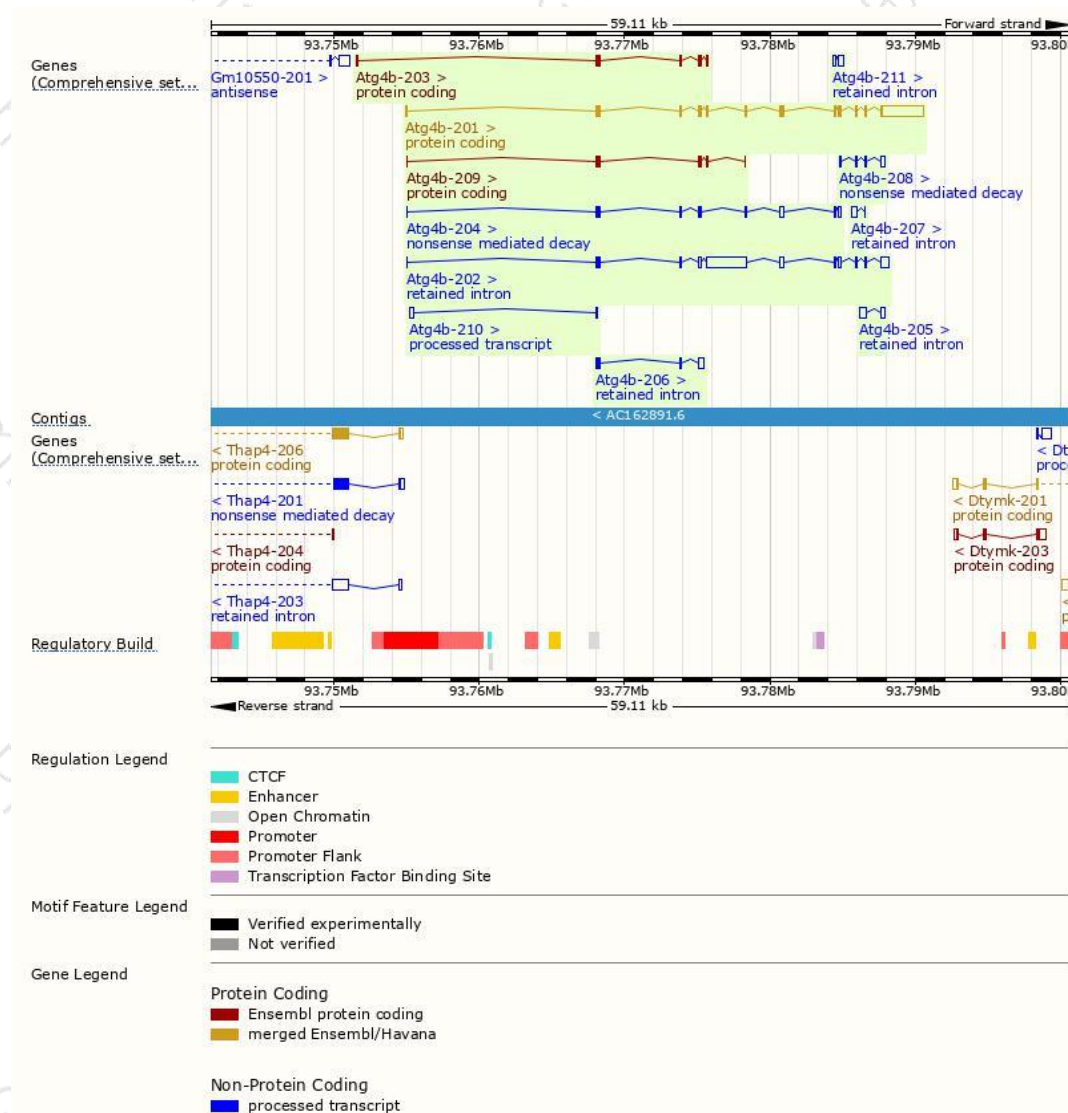
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Atg4b-201	<a href="#">ENSMUST00000027502.15</a>	4173	<a href="#">393aa</a>	Protein coding	<a href="#">CCDS15195</a>	<a href="#">A0A0R4J065</a>	TSL:1 GENCODE basic APPRIS P1
Atg4b-203	<a href="#">ENSMUST00000149436.7</a>	513	<a href="#">150aa</a>	Protein coding	-	<a href="#">D3YZP6</a>	CDS 3' incomplete TSL:3
Atg4b-209	<a href="#">ENSMUST00000187824.6</a>	420	<a href="#">121aa</a>	Protein coding	-	<a href="#">A0A087WNY2</a>	CDS 3' incomplete TSL:5
Atg4b-204	<a href="#">ENSMUST00000185482.6</a>	919	<a href="#">143aa</a>	Nonsense mediated decay	-	<a href="#">A0A087WRT0</a>	TSL:5
Atg4b-208	<a href="#">ENSMUST00000186811.1</a>	503	<a href="#">63aa</a>	Nonsense mediated decay	-	<a href="#">A0A087WNR6</a>	CDS 5' incomplete TSL:5
Atg4b-210	<a href="#">ENSMUST00000189152.1</a>	343	No protein	Processed transcript	-	-	TSL:2
Atg4b-202	<a href="#">ENSMUST00000135762.7</a>	4186	No protein	Retained intron	-	-	TSL:5
Atg4b-205	<a href="#">ENSMUST00000185754.1</a>	601	No protein	Retained intron	-	-	TSL:2
Atg4b-206	<a href="#">ENSMUST00000186001.1</a>	565	No protein	Retained intron	-	-	TSL:2
Atg4b-211	<a href="#">ENSMUST00000189872.1</a>	477	No protein	Retained intron	-	-	TSL:3
Atg4b-207	<a href="#">ENSMUST00000186124.1</a>	454	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Atg4b-201* transcript,The transcription is shown below

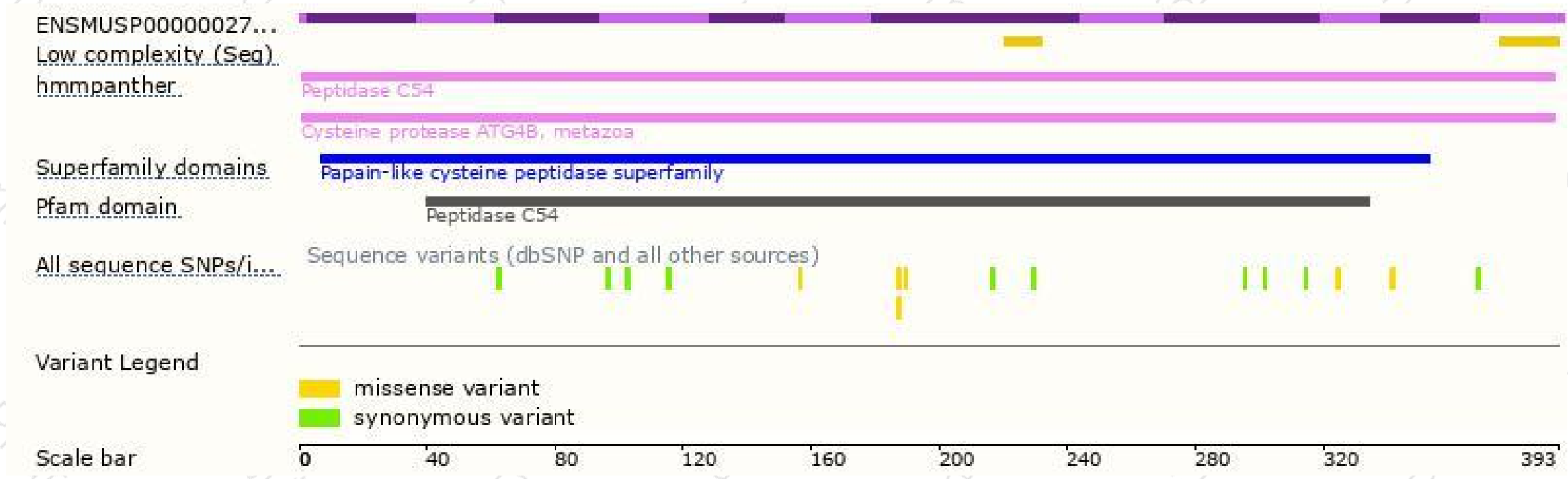


# Genomic location distribution





# 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 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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