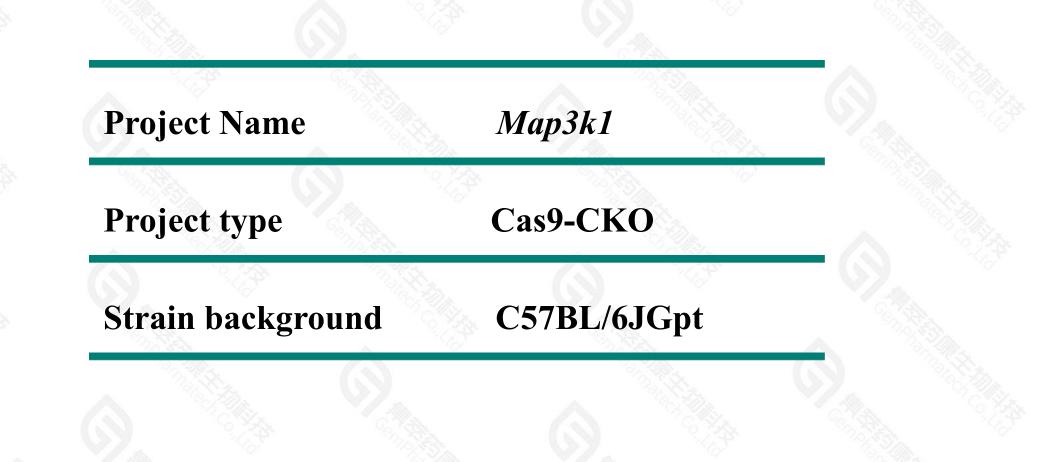


# Map3k1 Cas9-CKO Strategy

**Designer: Qiong Zhou** 

### **Project Overview**





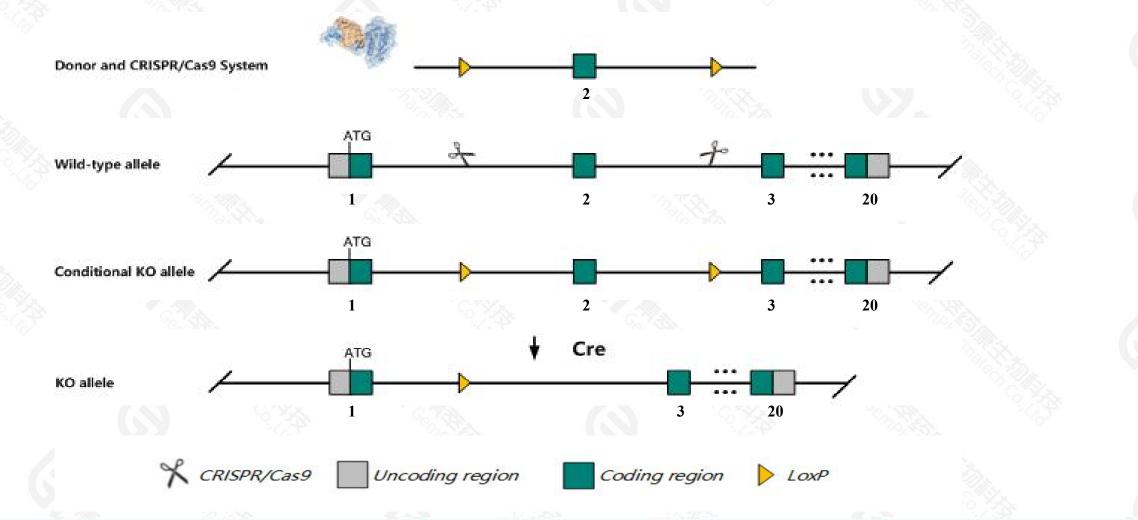
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### **Conditional Knockout strategy**

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This model will use CRISPR/Cas9 technology to edit the Map3k1 gene. The schematic diagram is as follows:



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### **Technical routes**



The Map3k1 gene has 5 transcripts. According to the structure of Map3k1 gene, exon2 of Map3k1-201(ENSMUST00000109267.9) transcript is recommended as the knockout region. The region contains 151bp coding sequence. Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify Map3k1 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 spontaneous allele are born with one or both eyes open, defects in eye morphology, and defects in ear morphology and function. Mice homozygous for a knock-out allele are born with open eyes and exhibit increased response to aortic banding and blood vessel healing.
- ➤ Transcript *Map3k1-202*, *Map3k1-203*, *Map3k1-205* may not be affected.
- > The *Map3k1* gene is located on the Chr13. 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)

#### Map3k1 mitogen-activated protein kinase kinase kinase 1 [Mus musculus (house mouse)]

Gene ID: 26401, updated on 17-Dec-2020

#### Summary

<b>Official Symbol</b>	Map3k1 provided by MGI
<b>Official Full Name</b>	mitogen-activated protein kinase kinase kinase 1 provided by MGI
<b>Primary source</b>	MGI:MGI:1346872
See related	Ensembl:ENSMUSG0000021754
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	MAPKKK1, MEKK1, Mekk
Expression	Ubiquitous expression in spleen adult (RPKM 7.6), thymus adult (RPKM 6.2) and 28 other tissuesSee more
Orthologs	human all

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## **Transcript information (Ensembl)**

### The gene has 5 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Map3k1-201	ENSMUST00000109267.9	6978	<u>1493aa</u>	Protein coding	CCDS26771		TSL:2 , GENCODE basic , APPRIS P1 ,
Map3k1-204	ENSMUST00000145055.2	867	<u>263aa</u>	Protein coding	<u>.</u>		CDS 3' incomplete , TSL:3 ,
Map3k1-203	ENSMUST00000133364.2	443	No protein	Processed transcript	2		TSL:3,
Map3k1-205	ENSMUST00000175936.2	544	No protein	Retained intron	-		TSL:3,
Map3k1-202	ENSMUST00000130594.2	396	No protein	Retained intron			TSL:5,

The strategy is based on the design of *Map3k1-201* transcript, the transcription is shown below:



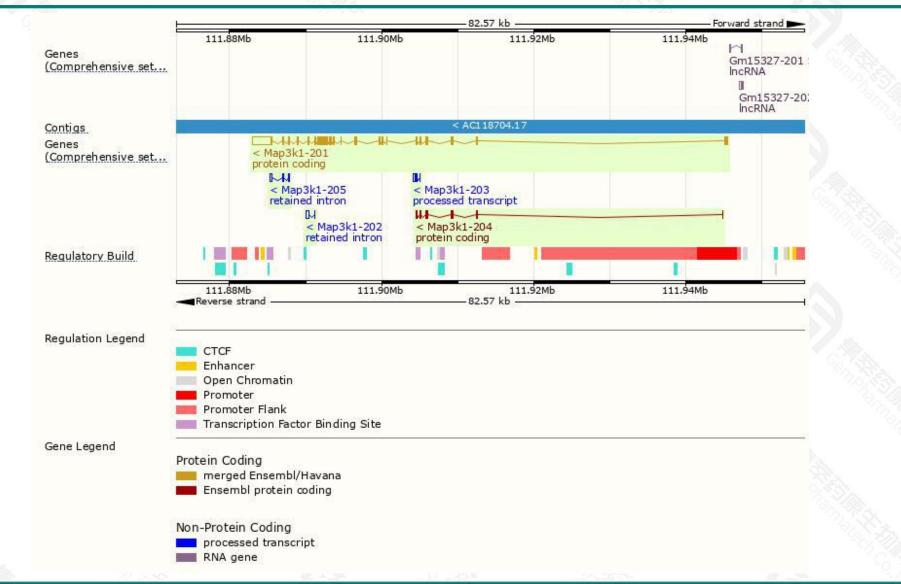
-62.57 kb

Reverse strand

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### **Genomic location distribution**



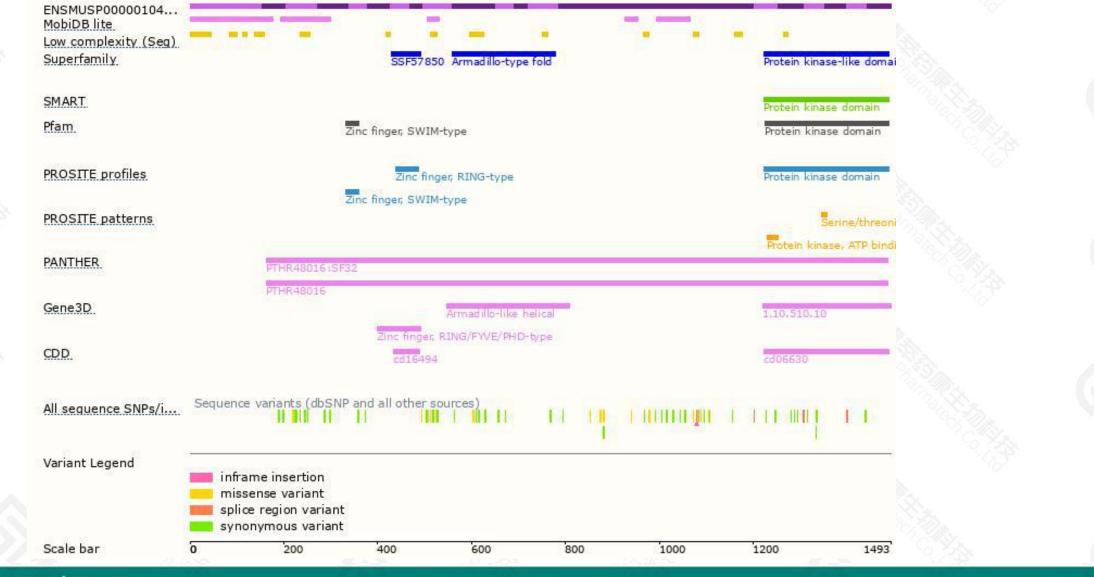


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### **Protein domain**

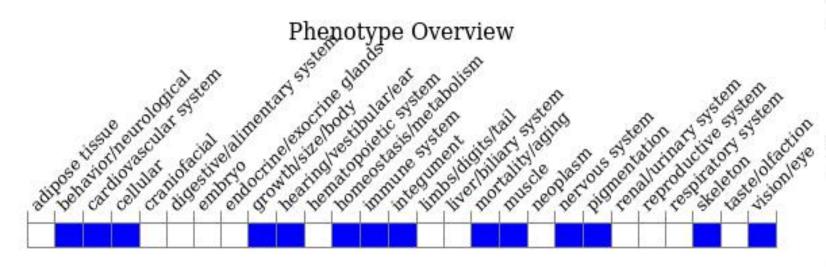




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### Mouse phenotype description(MGI)



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 spontaneous allele are born with one or both eyes open, defects in eye morphology, and defects in ear morphology and function. Mice homozygous for a knock-out allele are born with open eyes and exhibit increased response to aortic banding and blood vessel healing.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



