

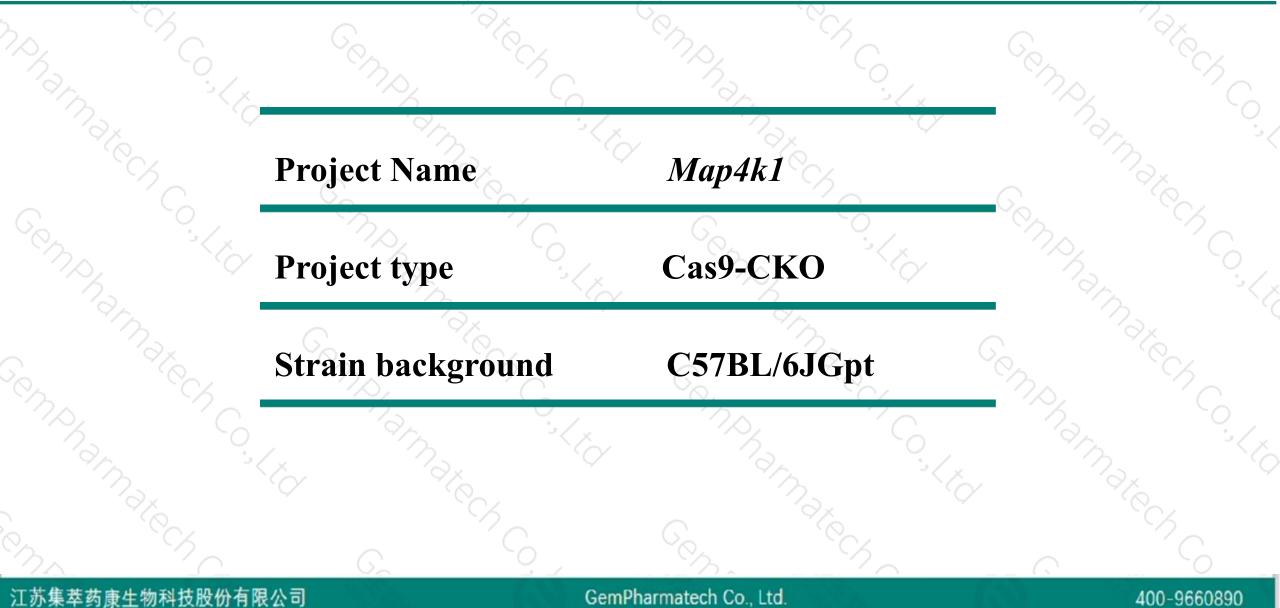
Map4k1 Cas9-CKO Strategy

Designer: Reviewer: Design Date:

Yang Zeng Xueting Zhang 2019-10-28

Project Overview

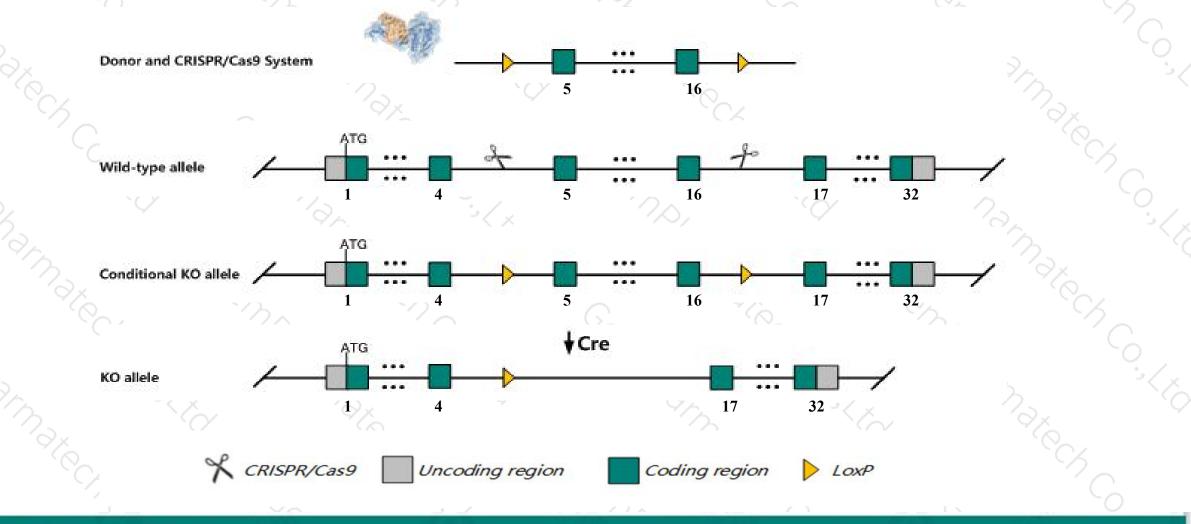




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Map4k1 gene. The schematic diagram is as follows:



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 The Map4k1 gene has 4 transcripts. According to the structure of Map4k1 gene, exon5-exon16 of Map4k1-201 (ENSMUST00000085835.7) transcript is recommended as the knockout region. The region contains 838bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Map4k1* 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, Homozygous null mice have increased responses of B and T cells. Dendritic cells are also hyperresponsive to stimulation.
- > The Map4k1 gene is located on the Chr7. 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)



Map4k1 mitogen-activated protein kinase kinase kinase kinase 1 [Mus musculus (house mouse)] Gene ID: 26411, updated on 24-Oct-2019 Summary ☆ ? Official Symbol Map4k1 provided by MGI Official Full Name mitogen-activated protein kinase kinase kinase kinase 1 provided by MGI Primary source MGI:MGI:1346882 See related Ensembl:ENSMUSG00000037337 Gene type protein coding RefSeg 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 Hpk1; mHPK1 Biased expression in spleen adult (RPKM 40.1), thymus adult (RPKM 25.1) and 10 other tissues See more Expression Orthologs human all Chromosome 7 - NC_000073.6 28893248 29125179 Actn4

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Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

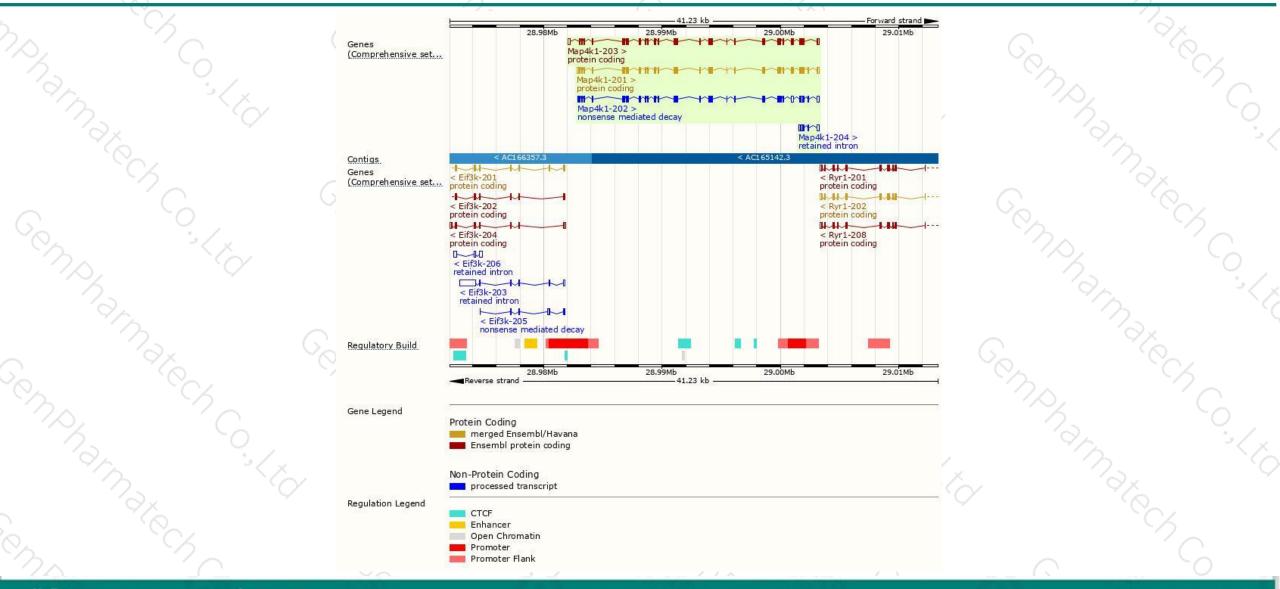
Name 🖕	Transcript ID 🖕	bp 🖕	Protein 🖕	Translation ID	Biotype 🖕	CCDS 🖕	UniProt 🖕	Flags 🔶	
Map4k1-201	ENSMUST0000085835.7	2724	<u>827aa</u>	ENSMUSP0000082995.6	Protein coding	<u>CCDS39865</u> 67	<u>P70218</u> 교	TSL:1 GENCODE basic APPRIS P1	
Map4k1-203	ENSMUST00000208227.1	2720	<u>750aa</u>	ENSMUSP00000146807.1	Protein coding	i n	<u>Q3UPT4</u> &	TSL:1 GENCODE basic	
Map4k1-202	ENSMUST00000207185.1	2764	<u>641aa</u>	ENSMUSP00000147189.1	Nonsense mediated decay	-	<u>A0A140LJF0</u> &	TSL:1	
Map4k1-204	ENSMUST00000208784.1	523	No protein	-	Retained intron	4 . 8	1.0	TSL:1	

The strategy is based on the design of Map4k1-201 transcript, The transcription is shown below



Genomic location distribution





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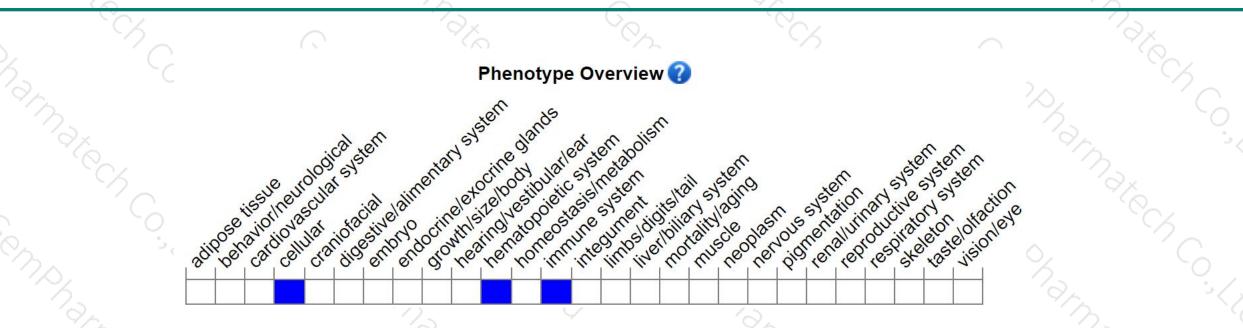
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Protein domain



nonarmar	ENSMUSP0000082 SIFTS import MobiDB lite Low complexity (Seg) Superfamily SMART	Protein kinase-like domain superfamily Protein kinase domain Citron homology (CNH) domain	
`°°	Pfam.	Protein kinase domain Gitron homology (CNH) domain	
G	PROSITE profiles	Protein kinase domain Citron homology (CNH) domain	~~~~
	PROSITE patterns PIRSF PANTHER	Protein kinase, ATP binding site Mitogen-activated protein (MAP) kinase kinase kinase PTHR24361:SF88	
Senphan 1	Gene3D CDD All sequence SNPs/i	PTHR24361 1.10.510.10 cd06613 Sequence variants (dbSNP and all other sources)	
CU US	Variant Legend Scale bar	missense variant splice region variant synonymous variant 0 80 160 240 320 400 480 560 640 720 8	27
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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, Homozygous null mice have increased responses of B and T cells. Dendritic cells are also hyperresponsive to stimulation.



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



