Cav2 Cas9-CKO Strategy

Designer: Jinling Wang

Design Date: 2019-7-26

Project Overview



Project Name

Cav2

Project type

Cas9-CKO

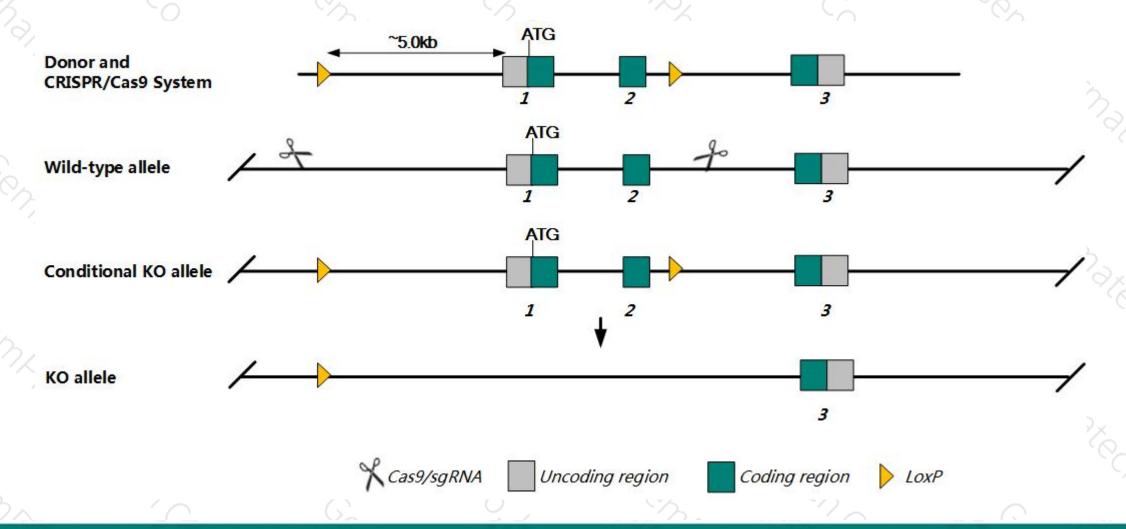
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Cav2 gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Cav2* gene has 3 transcripts. According to the structure of *Cav2* gene, the predicted promoter region and exon1-2 of *Cav2*-201 (ENSMUST0000000058.6) transcript is recommended as the knockout region. The region contains the predicted promoter sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Cav2* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 or cell types.

Notice



- According to the existing MGI data: Mice homozygous for a null allele show lung parenchyma hypercellularity, alveolar constriction and septal thickening, impaired exercise endurance, and skeletal muscle defects. Mice homozygous for a different null allele show reduced tumor vascularization and growth of subcutaneously implanted tumors.
- ➤ The *Cav2* gene is located on the Chr6. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



Cav2 caveolin 2 [Mus musculus (house mouse)]

Gene ID: 12390, updated on 2-Oct-2018

Summary

☆ ?

Official Symbol Cav2 provided by MGI

Official Full Name caveolin 2 provided by MGI

Primary source MGI:MGI:107571

See related Ensembl:ENSMUSG00000000058 Vega:OTTMUSG00000024026

Gene type protein coding
RefSeq status REVIEWED
Organism Mus musculus

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

Murinae; Mus; Mus

Also known as Al447843

Summary This gene belongs to the caveolin family whose members encode the major protein components of caveolae, which are invaginations of plasma membrane. This

gene is located adjacent to caveolin-1 and the proteins coexpressed by the two genes localize together in caveolae, where they form hetero-oligomers. The encoded protein may be involved in diverse cellular functions including proliferation, differentiation, endocytosis and trafficking. Alternative splicing of this gene

results in transcript variants encoding different isoforms. [provided by RefSeq, Apr 2013]

Expression Biased expression in subcutaneous fat pad adult (RPKM 86.2), genital fat pad adult (RPKM 79.9) and 10 other tissues See more

Orthologs human all

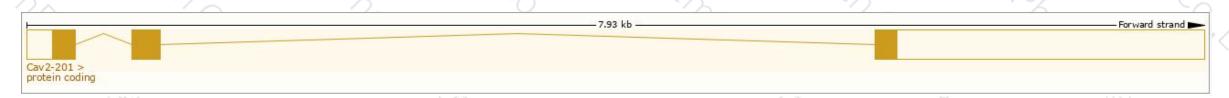
Transcript information (Ensembl)



The gene has 3 transcripts, and all transcripts are shown below:

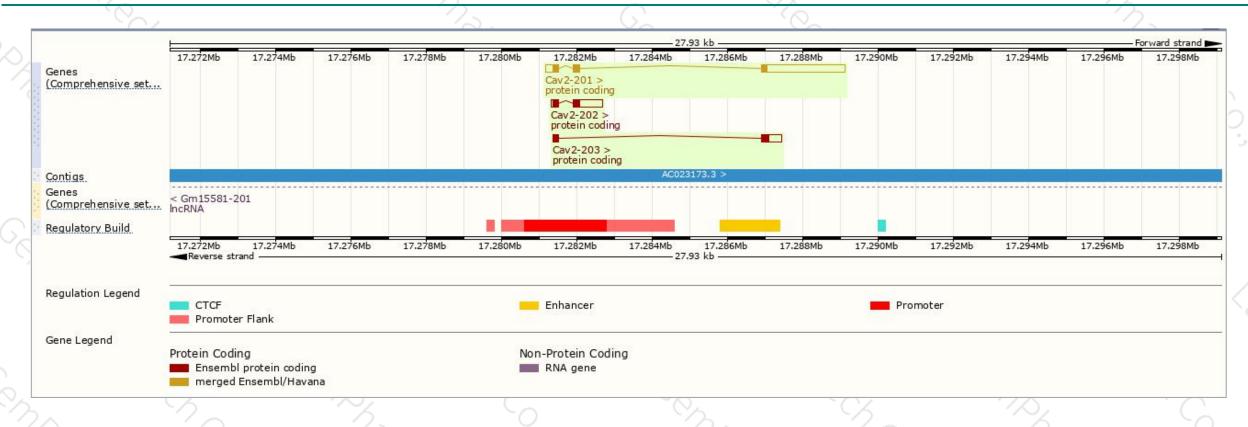
Name ▲	Transcript ID	bp 🍦	Protein	Biotype	CCDS	UniProt	Flags
Cav2-201	ENSMUST00000000058.6	2733	162aa	Protein coding	CCDS19923 ₽	Q9WVC3₽	TSL:1 GENCODE basic APPRIS P1
Cav2-202	ENSMUST00000115459.1	974	<u>113aa</u>	Protein coding	CCDS71727 ₺	Q924U4 &	TSL:1 GENCODE basic
Cav2-203	ENSMUST00000115462.1	694	<u>117aa</u>	Protein coding	-	D3Z147 ₺	TSL:5 GENCODE basic

The strategy is based on the design of *Cav2*-201 transcript, The transcription is shown below



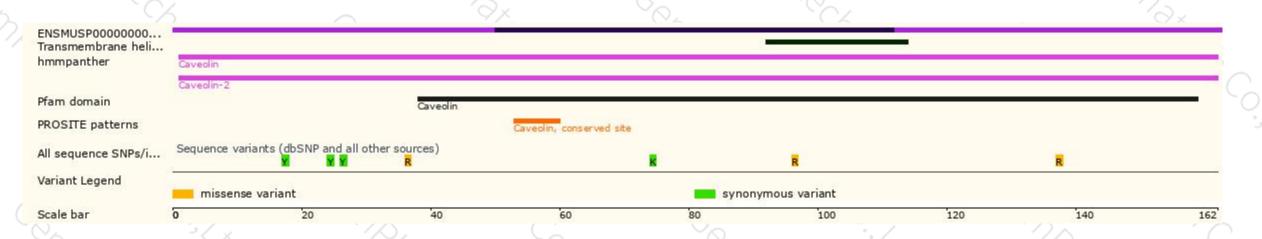
Genomic location distribution





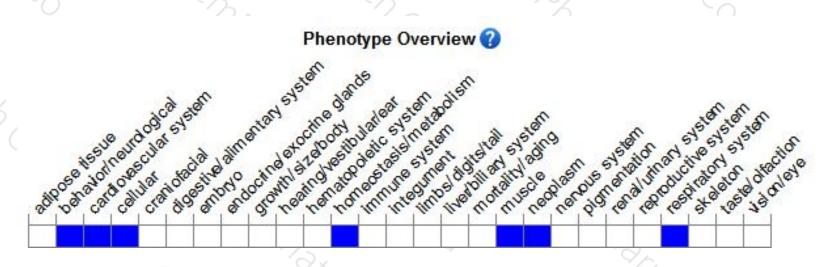
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

Mice homozygous for a null allele show lung parenchyma hypercellularity, alveolar constriction and septal thickening, impaired exercise endurance, and skeletal muscle defects. Mice homozygous for a different null allele show reduced tumor vascularization and growth of subcutaneously implanted tumors.

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





