# Cav1 Cas9-CKO Strategy

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**Design Date:** 2019-7-26

# **Project Overview**



**Project Name** 

Cav1

**Project type** 

Cas9-CKO

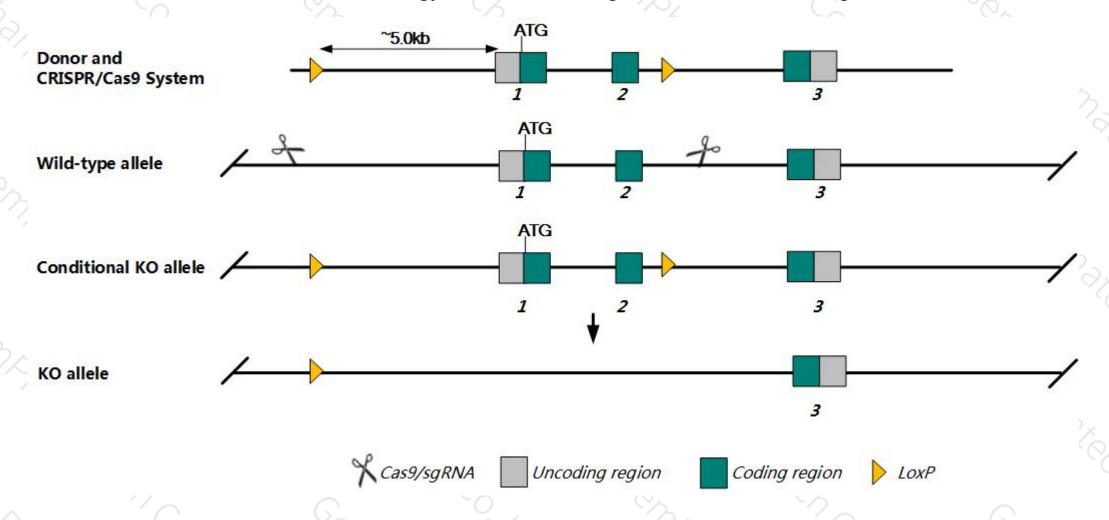
Strain background

C57BL/6JGpt

### Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Cav1 gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Cav1* gene has 10 transcripts. According to the structure of *Cav1* gene, the predicted promoter region and exon1-2 of *Cav1*-201 (ENSMUST00000007799.12) 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 *Cav1* 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: Homozygous targeted mutants displayed vascular system dysfunctions and thickening of lung aveloar septa from hyperproliferation and fibrosis, ultimately causing the mice physical limitations. Mice also display increased incidence of calcium calculi, kidney stones, and decreased adiposity.
- ➤ The *Cav1* 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)



#### Cav1 caveolin 1, caveolae protein [ Mus musculus (house mouse) ]

Gene ID: 12389, updated on 23-Oct-2018

#### Summary

^ ?

Official Symbol Cav1 provided by MGI

Official Full Name caveolin 1, caveolae protein provided by MGI

Primary source MGI:MGI:102709

See related Ensembl:ENSMUSG00000007655 Vega:OTTMUSG00000024023

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 Cav; Cav-1

Expression Biased expression in subcutaneous fat pad adult (RPKM 331.4), genital fat pad adult (RPKM 281.9) and 8 other tissues See more

Orthologs human all

# Transcript information (Ensembl)



The gene has 10 transcripts, and all transcripts are shown below:

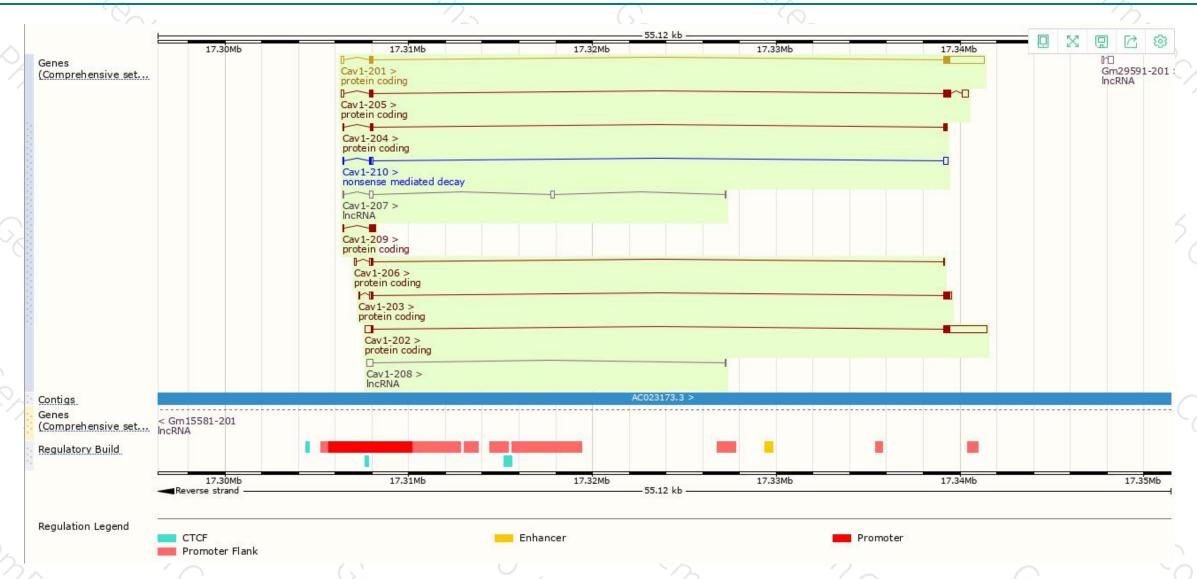
Name A	Transcript ID	bp 🌲	Protein	Biotype	CCDS 🍦	UniProt	Flags
Cav1-201	ENSMUST00000007799.12	2522	<u>178aa</u>	Protein coding	CCDS19924 ₽	P49817@	TSL:1 GENCODE basic APPRIS P3
Cav1-202	ENSMUST00000115453.1	2746	<u>147aa</u>	Protein coding	CCDS57410 ₽	P49817₽	TSL:1 GENCODE basic APPRIS ALT1
Cav1-203	ENSMUST00000115454.1	613	<u>147aa</u>	Protein coding	CCDS57410 ₽	P49817@	TSL:2 GENCODE basic APPRIS ALT1
Cav1-204	ENSMUST00000115455.2	386	<u>115aa</u>	Protein coding	-	<u>D3Z148</u> ₽	CDS 3' incomplete TSL:3
Cav1-205	ENSMUST00000115456.5	995	178aa	Protein coding	CCDS19924 ₺	<u>P49817</u> 굡	TSL:2 GENCODE basic APPRIS P3
Cav1-206	ENSMUST00000123439.7	323	<u>47aa</u>	Protein coding		D3Z0J2₽	CDS 3' incomplete TSL:3
Cav1-207	ENSMUST00000130505.2	461	No protein	IncRNA	32	=	TSL:3
Cav1-208	ENSMUST00000133065.1	345	No protein	IncRNA	725	a i	TSL:5
Cav1-209	ENSMUST00000150901.1	380	<u>93aa</u>	Protein coding	150	H3BKG0₽	TSL:2 GENCODE basic
Cav1-210	ENSMUST00000177234.1	483	40aa	Nonsense mediated decay	80.	H3BLQ4₽	TSL:3
							7.3

The strategy is based on the design of Cav1-201 transcript, The transcription is shown below



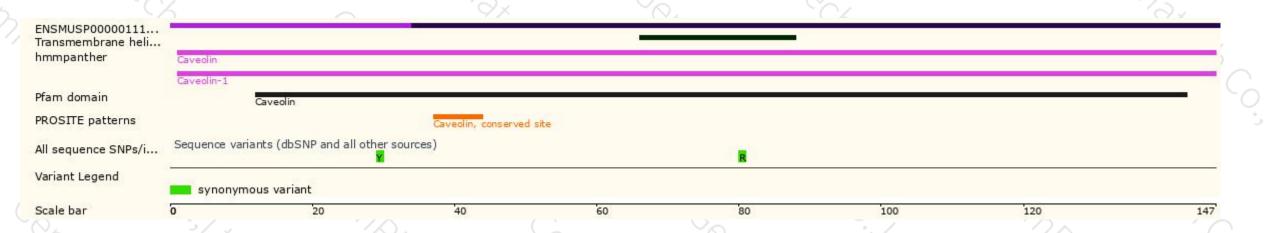
### Genomic location distribution





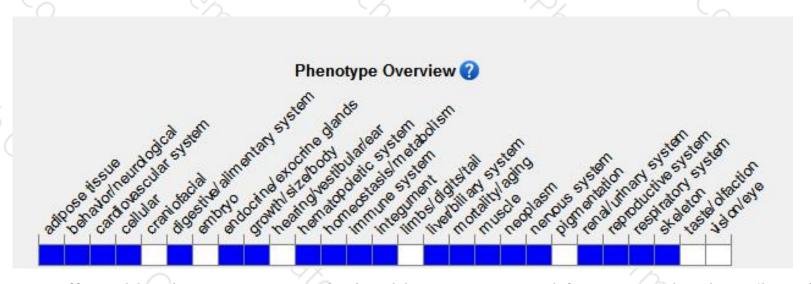
### Protein domain





## Mouse phenotype description(MGI)





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

Homozygous targeted mutants displayed vascular system dysfunctions and thickening of lung aveloar septa from hyperproliferation and fibrosis, ultimately causing the mice physical limitations. Mice also display increased incidence of calcium calculi, kidney stones, and decreased adiposity.

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





