

# Acsl1 Cas9-CKO Strategy

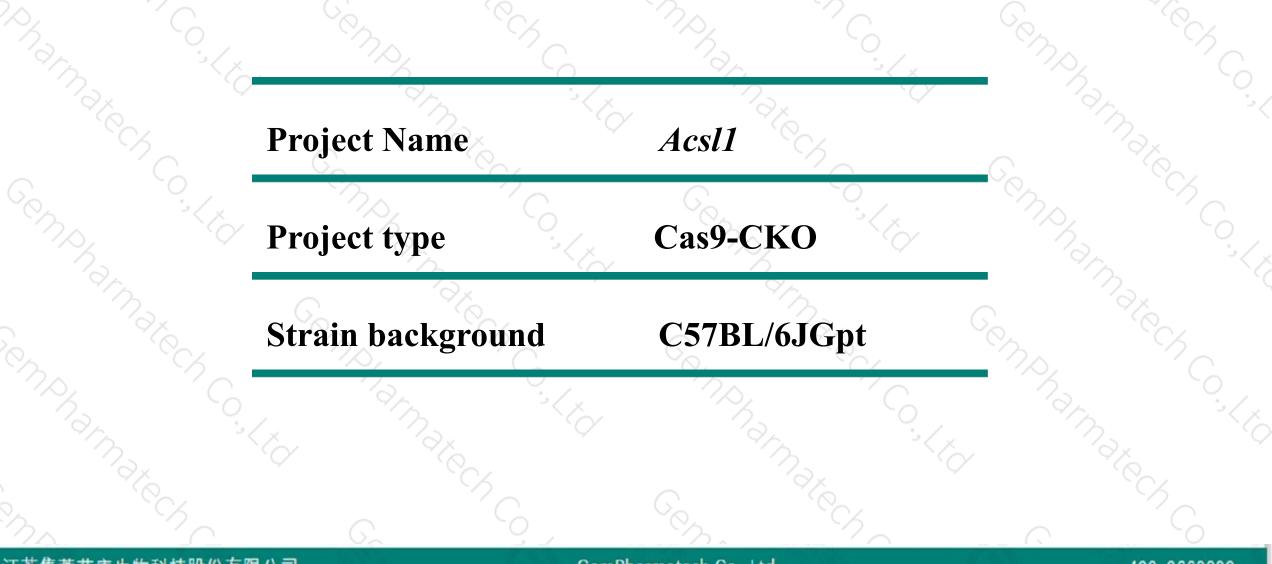
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Daohua Xu 2019-8-5

## **Project Overview**





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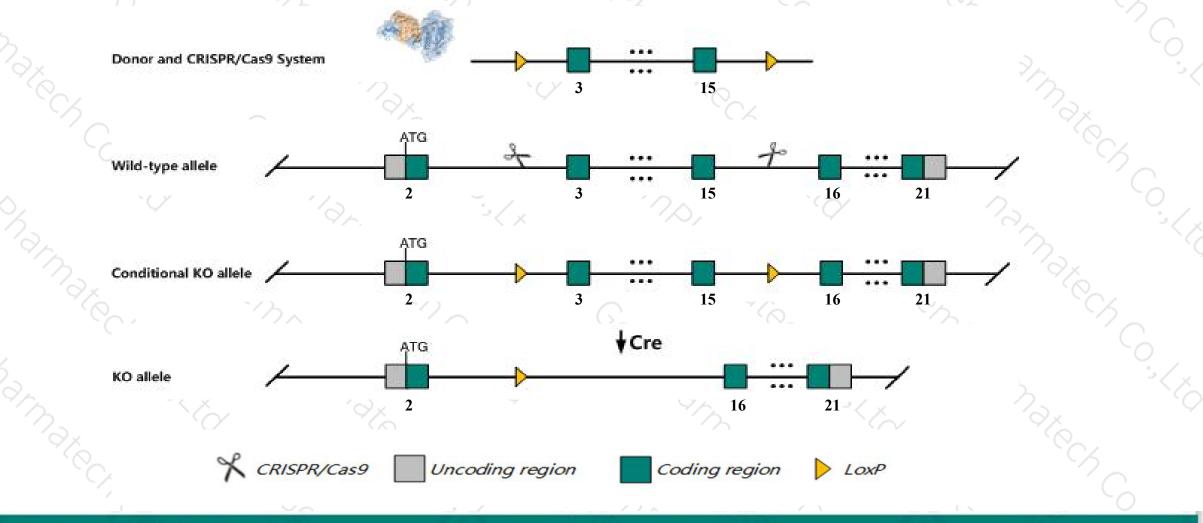
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## **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the Acsl1 gene. The schematic diagram is as follows:



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The Acsl1 gene has 11 transcripts. According to the structure of Acsl1 gene, exon3-exon15 of Acsl1-201 (ENSMUST00000034046.12) transcript is recommended as the knockout region. The region contains 1240bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Acsl1* 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,Liver acyl-CoA levels are reduced when this gene is conditionally knocked out in the liver. Impaired adaptive thermogenesis when this gene is conditionally knocked out in adipose tissue.
- The Acsl1 gene is located on the Chr8. 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)



400-9660890

#### Acsl1 acyl-CoA synthetase long-chain family member 1 [Mus musculus (house mouse)]

Gene ID: 14081, updated on 31-Jan-2019

#### Summary

Official Symbol Acsl1 provided by MGI Official Full Name acyl-CoA synthetase long-chain family member 1 provided by MGI Primary source MGI:MGI:102797 See related Ensembl:ENSMUSG0000018796 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 Acas, Acas1, Acs, FACS, Facl2, LACS 1, LACS1 Summary The protein encoded by this gene belongs to a family of acyl coenzyme A synthetase proteins, which convert long chain fatty acids to acyl CoA products via an ATP-dependent pathway. This enzyme is enriched in heart, liver and adipose tissue, where it functions in lipid synthesis and mitochondrial and peroxisomal beta-oxidation. In addition, it is expressed in monocytes and macrophages where it appears to have a functionally distinct role in mediating inflammatory and innate immune responses. A pseudogene of this gene is found on chromosome 5. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Oct 2014] Expression Biased expression in liver E18 (RPKM 94.6), subcutaneous fat pad adult (RPKM 79.5) and 13 other tissues See more Orthologs human all

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## **Transcript information (Ensembl)**



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

Name 🚦	Transcript ID	bp 🖕	Protein 🝦	Biotype	CCDS 🛊	UniProt	RefSeq	Flags
Acsl1-201	ENSMUST0000034046.12	3897	<u>699aa</u>	Protein coding	<u>CCDS22291</u> @	P41216	<u>NM 007981</u> മ NP 032007മ	TSL:1 GENCODE basic APPRIS P2
Acsl1-203	ENSMUST00000110372.8	3429	<u>699aa</u>	Protein coding	<u>CCDS22291</u> @	P41216@	NM 001302163@ NP 001289092@	TSL:1 GENCODE basic APPRIS P2
Acsl1-202	ENSMUST00000110371.7	2100	<u>699aa</u>	Protein coding	2	D3Z041@		TSL:5 GENCODE basic APPRIS ALT
Acsl1-207	ENSMUST00000135955.7	673	<u>136aa</u>	Protein coding	-	D3Z457@	-	CDS 3' incomplete TSL:3
Acsl1-208	ENSMUST00000152423.1	441	<u>95aa</u>	Protein coding	-	F6WNZ2@	-	CDS 5' incomplete TSL:5
Acsl1-205	ENSMUST00000130563.1	368	<u>86aa</u>	Protein coding		D3YVF6@	-	CDS 3' incomplete TSL:2
Acsi1-211	ENSMUST00000211644.1	343	<u>58aa</u>	Protein coding	2	<u>A0A1B0GRS8</u> മ	2	CDS 3' incomplete TSL:2
Acsl1-209	ENSMUST00000210929.1	474	No protein	Processed transcript	-	-	-	TSL:5
Acsl1-204	ENSMUST00000128746.2	5676	No protein	Retained intron	-	1. 1.	-	TSL:2
Acsi1-210	ENSMUST00000211289.1	1253	No protein	Retained intron		1.052		TSL:NA
Acsl1-206	ENSMUST00000133161.1	573	No protein	Retained intron	2	147	2	TSL:3

The strategy is based on the design of Acsl1-201 transcript, The transcription is shown below

Acsl1-201 > protein coding

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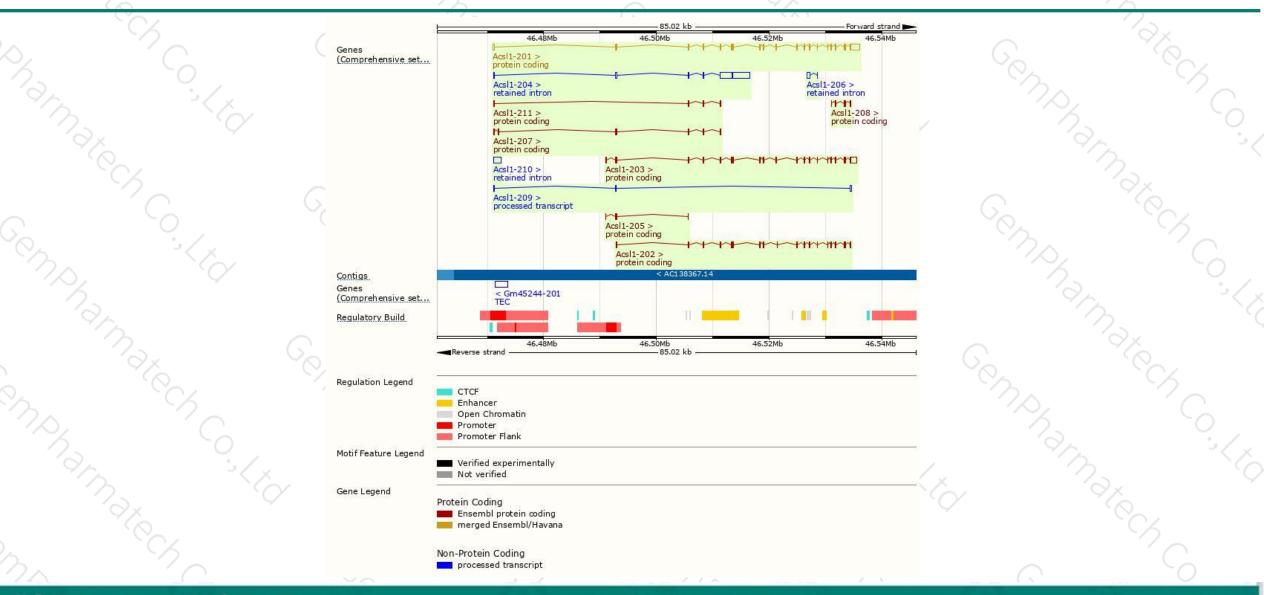
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65.02 kb

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Forward strand

## **Genomic location distribution**



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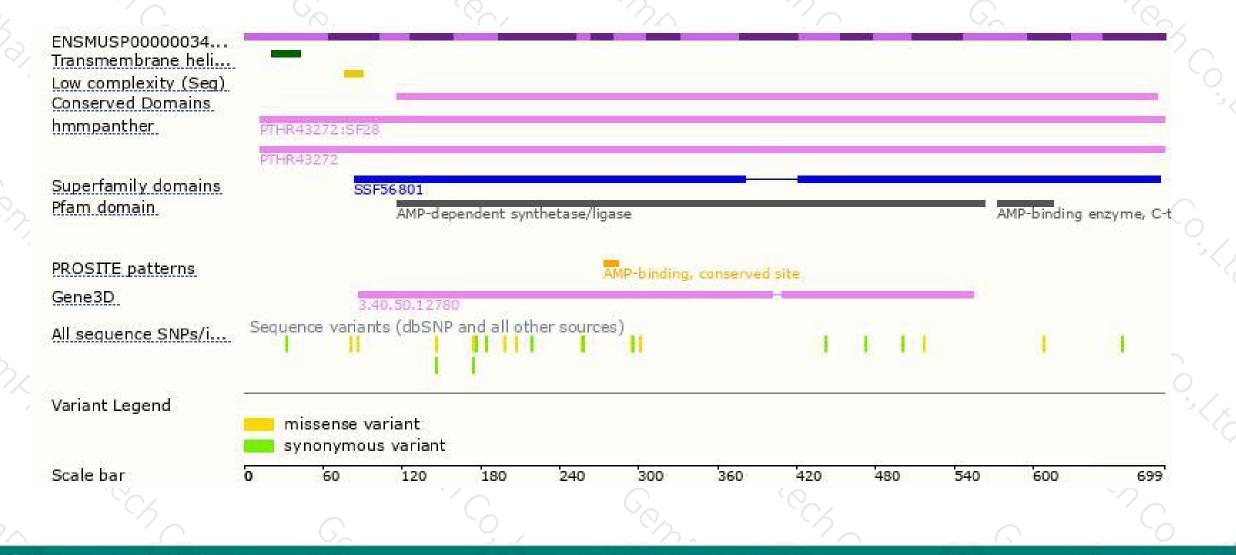
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### **Protein domain**





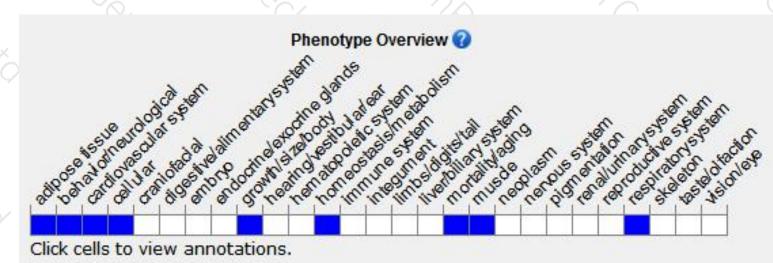
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## Mouse phenotype description(MGI)





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,Liver acyl-CoA levels are reduced when this gene is conditionally knocked out in the liver. Impaired adaptive thermogenesis when this gene is conditionally knocked out in adipose tissue.



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



