GemPharmatech™

Srcap Cas9-CKO Strategy

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Reviewer: Xueting Zhang

Design Date: 2023-6-21

Overview

Target Gene Name

- *Srcap*

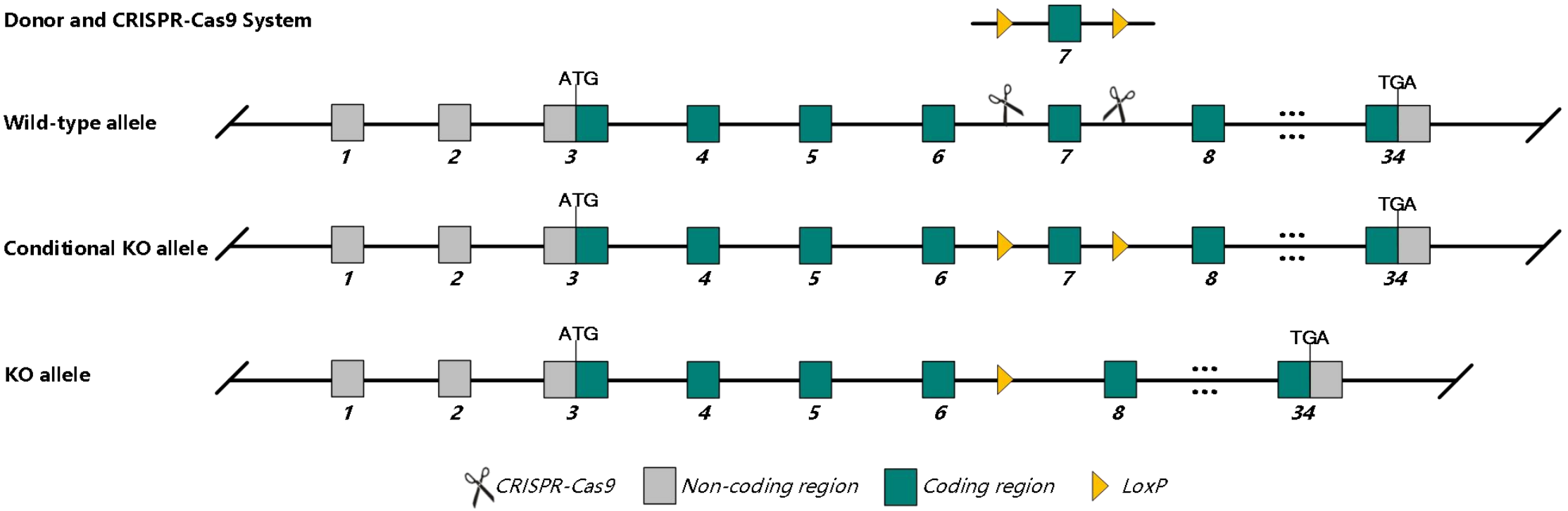
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Srcap* gene.

Technical Information

- The *Srcap* gene has 14 transcripts. According to the structure of *Srcap* gene, exon 7 of *Srcap*-207 (ENSMUST00000187040.7) is recommended as the knockout region. The region contains 223 bp of coding sequence. Knockout the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Srcap* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

Gene Information

Srcap Snf2-related CREBBP activator protein [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 100043597, updated on 12-Apr-2023

Summary

Official Symbol	Srcap provided by MGI
Official Full Name	Snf2-related CREBBP activator protein provided by MGI
Primary source	MGI:MGI:2444036
See related	Ensembl:ENSMUSG00000053877 AllianceGenome:MGI:2444036
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	B930091H02Rik; D030022P06Rik; F630004O05Rik
Summary	Predicted to enable ATP hydrolysis activity and histone binding activity. Predicted to be involved in histone exchange. Predicted to be located in Golgi apparatus; nuclear body; and perinuclear region of cytoplasm. Predicted to be part of Swr1 complex. Is expressed in early conceptus; inner cell mass; and oocyte. Human ortholog(s) of this gene implicated in Floating-Harbor syndrome. Orthologous to human SRCAP (Snf2 related CREBBP activator protein). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in thymus adult (RPKM 33.8), spleen adult (RPKM 21.1) and 28 other tissues See more
Orthologs	human all

NEW Try the new Gene table

Try the new Transcript table

Genomic context

Location: 7; 7 F3

See Srcap in Genome Data Viewer

Exon count: 34

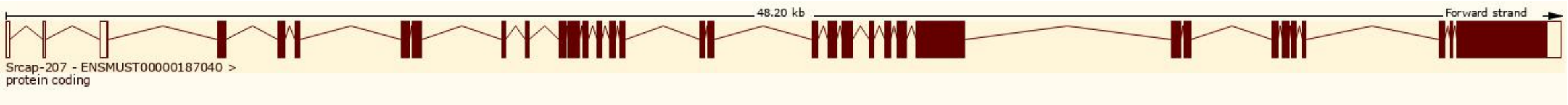
https://www.ncbi.nlm.nih.gov/gene/100043597

Transcript Information

The gene has 14 transcripts, all transcripts are shown below:

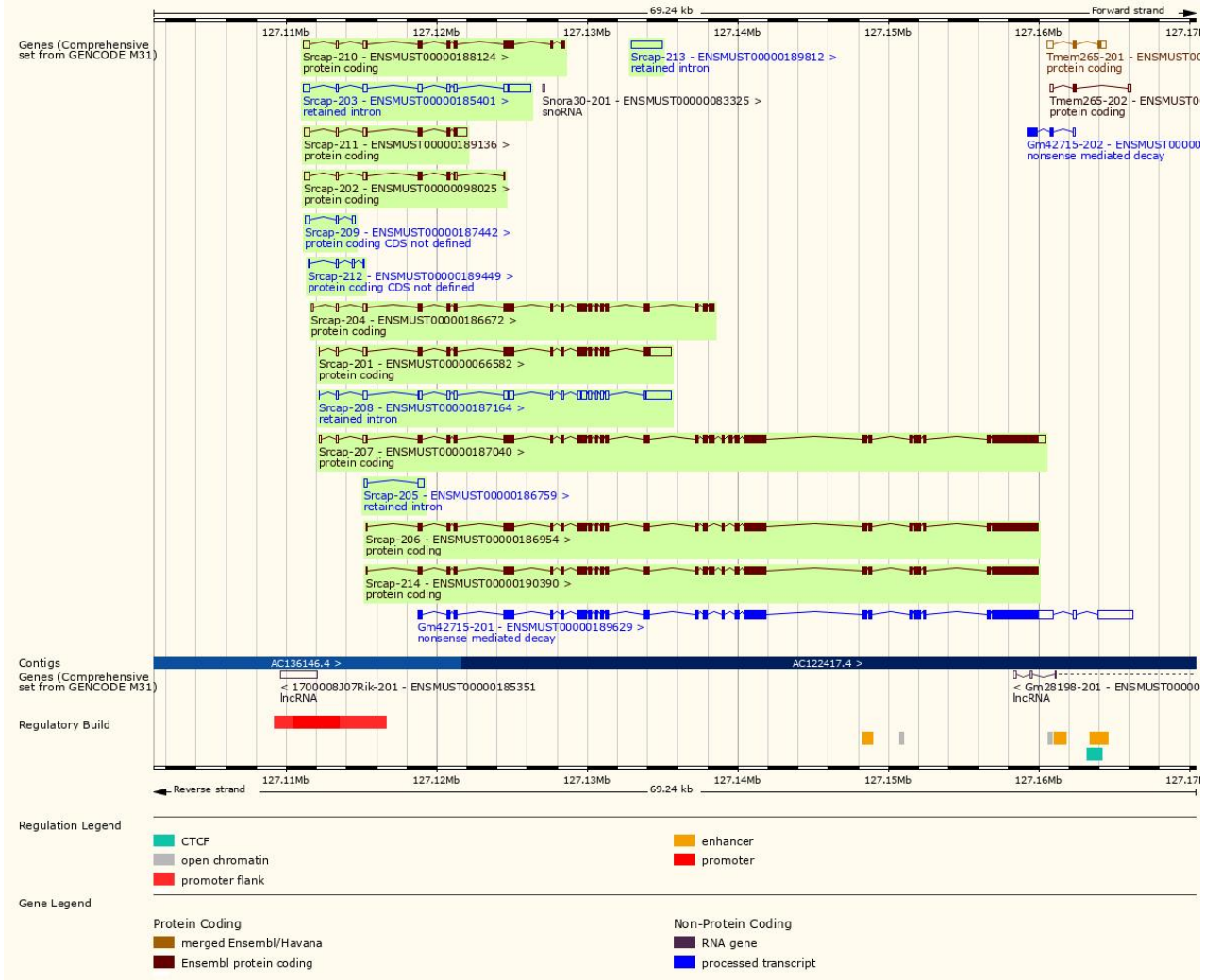
Transcript ID	Name ▲	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000066582.12	Srcap-201	4545	936aa	Protein coding		E9Q9V7	GENCODE basic TSL:1
ENSMUST00000098025.11	Srcap-202	1257	168aa	Protein coding		Q8BQA6	GENCODE basic TSL:5
ENSMUST00000185401.7	Srcap-203	2904	No protein	Retained intron		-	TSL:1
ENSMUST00000186672.7	Srcap-204	4025	1192aa	Protein coding		A0A087WQY5	TSL:5 CDS 3' incomplete
ENSMUST00000186759.2	Srcap-205	598	No protein	Retained intron		-	TSL:3
ENSMUST00000186954.5	Srcap-206	9345	3114aa	Protein coding		A0A087WNX7	GENCODE basic APPRIS ALT2 TSL:5
ENSMUST00000187040.7	Srcap-207	10654	3271aa	Protein coding	CCDS80806	A0A087WQ44	Ensembl Canonical GENCODE basic APPRIS ALT2 TSL:5
ENSMUST00000187164.7	Srcap-208	4548	No protein	Retained intron		-	TSL:5
ENSMUST00000187442.7	Srcap-209	513	No protein	Protein coding CDS not defined		-	TSL:1
ENSMUST00000188124.7	Srcap-210	2048	445aa	Protein coding		A0A087WS36	GENCODE basic TSL:1
ENSMUST00000189136.7	Srcap-211	1960	225aa	Protein coding		A0A087WSL6	GENCODE basic TSL:1
ENSMUST00000189449.7	Srcap-212	276	No protein	Protein coding CDS not defined		-	TSL:1
ENSMUST00000189812.2	Srcap-213	2033	No protein	Retained intron		-	TSL:NA
ENSMUST00000190390.7	Srcap-214	9633	3210aa	Protein coding		A0A087WNL7	GENCODE basic APPRIS P4 TSL:5

The strategy is based on the design of *Srcap*-207 transcript, the transcription is shown below:



Source: http://asia.ensembl.org/

Genomic Information



Source:http://asia.ensembl.org/

Protein Information

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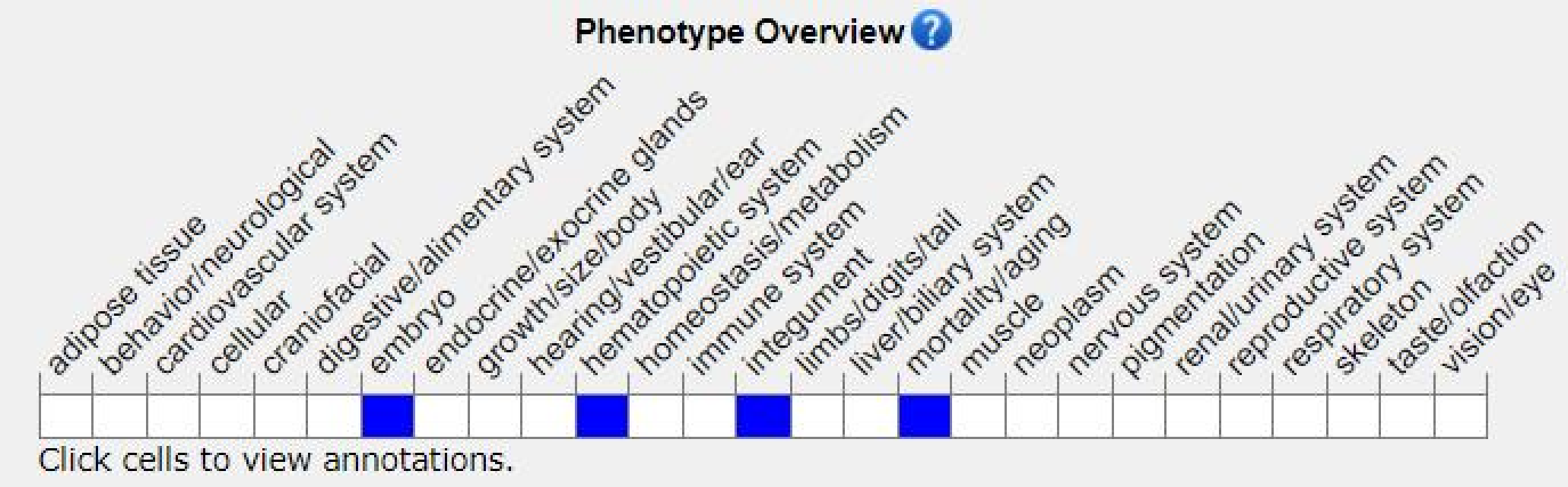
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



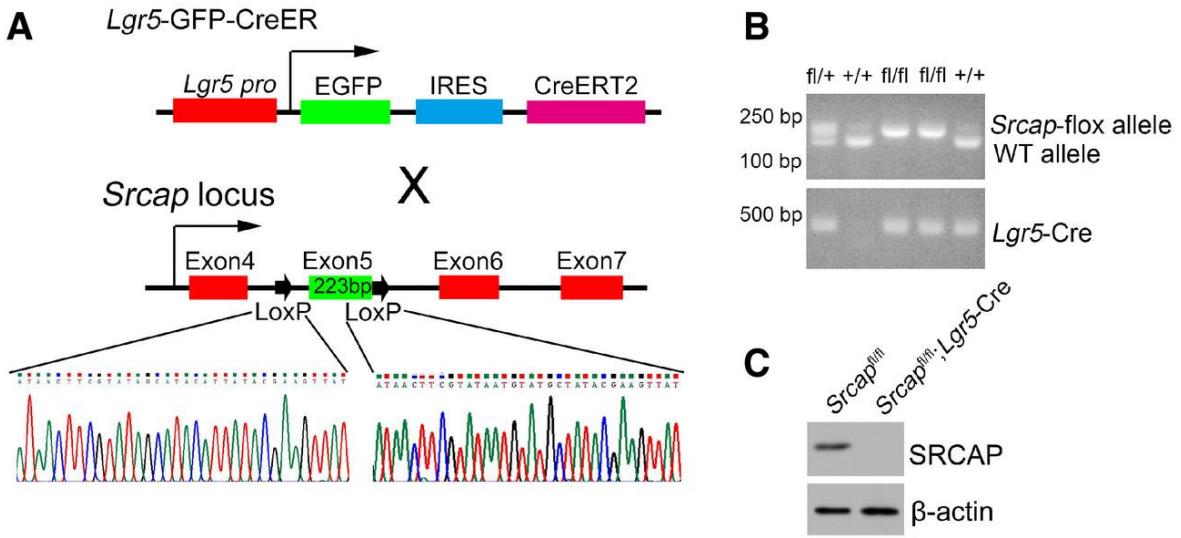
Mice homozygous for a null allele show impaired blastocyst development at E3.5 and early embryonic lethality.

Source: https://www.informatics.jax.org

Important Information

- According to the existing MGI data, mice homozygous for a null allele show impaired blastocyst development at E3.5 and early embryonic lethality.
- The intron 7-8 is 115 bp, the loxp insertion may affect the regulation of this gene.
- This stratergy may not affect *Srcap*-205, *Srcap*-209, *Srcap*-211, *Srcap*-212 and *Srcap*-213 transcript.
- The knockout region overlaps with *Gm42715*-201 transcript, which may affect the function of this gene.
- A part of amino acid sequence will still remain at the N-terminal of the *Srcap* gene.
- The knockout region is about 2.1 kb away from the 5' of the *Snora30* gene, which may affect the regulation of this gene.
- *Srcap* is located on Chr 7. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

Reference



We next generated *Srcap* knockout (KO) mice through a CRISPR/Cas9 approach (Fig EV1D). *Srcap* deficiency impaired blastocyst development at E3.5 and caused early embryonic lethality (Fig EV1E–G). We then generated $Srcap^{flox/flox}$ mice through insertion of *loxP* sequences flanking at the exon5 of *Srcap* gene locus (Fig EV2A). We established $Srcap^{flox/flox}$;$Lgr5^{GFP\text{-}CreERT2}$ mice through crossing $Srcap^{flox/flox}$ mice with $Lgr5^{GFP\text{-}CreERT2}$ mice (Fig EV2A). With administration of tamoxifen (TAM), *Srcap* was

[1] Ye B, Yang L, Qian G, Liu B, Zhu X, Zhu P, Ma J, Xie W, Li H, Lu T, Wang Y, Wang S, Du Y, Wang Z, Jiang J, Li J, Fan D, Meng S, Wu J, Tian Y, Fan Z. The chromatin remodeler SRCAP promotes self-renewal of intestinal stem cells. EMBO J. 2020 Jul 1;39(13):e103786. doi: 10.15252/embj.2019103786. Epub 2020 May 25. PMID: 32449550; PMCID: PMC7327502.