

Vcam1 Cas9-CKO Strategy

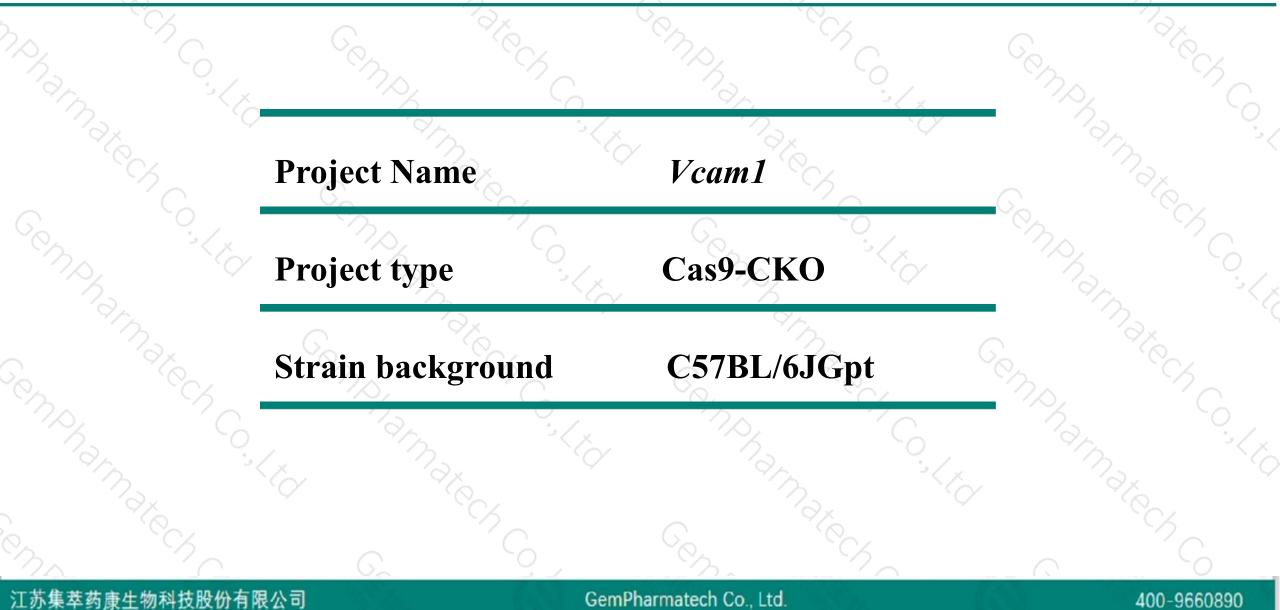
Designer: Reviewer:

Design Date:

Huan Fan Huan Wang 2019-12-11

Project Overview



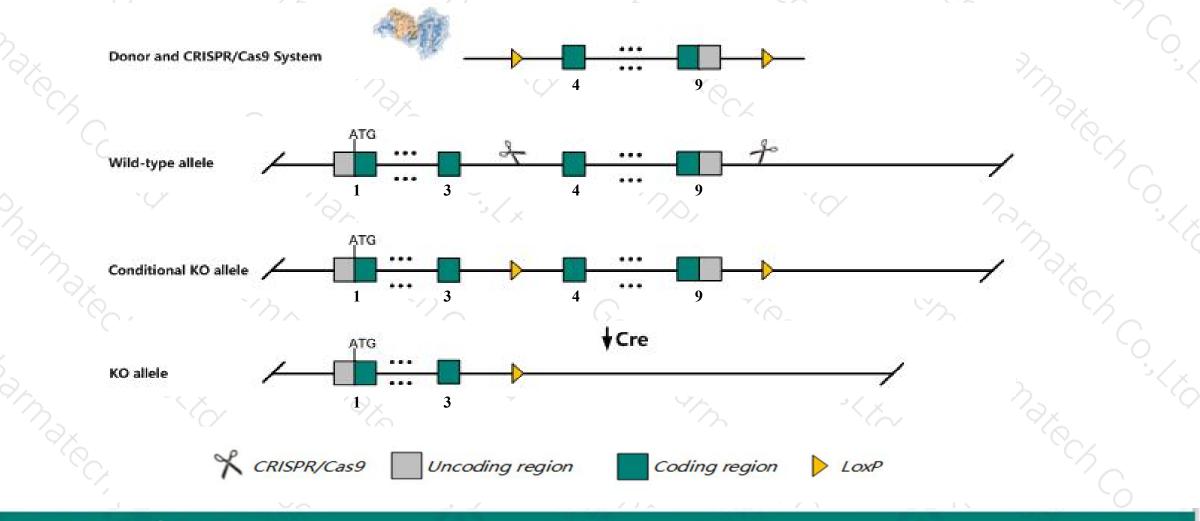


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Vcam1* gene. The schematic diagram is as follows:



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The Vcam1 gene has 4 transcripts. According to the structure of Vcam1 gene, exon4-exon9 of Vcam1-201 (ENSMUST00000029574.12) transcript is recommended as the knockout region. The region contains 1559bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Vcam1* 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, Most homozygous null mutants die by embryonic day 12.5 due to defective placenta and failure of chorion/allantois fusion, and heart developmental anomalies. Survivors are generally normal, but have high numbers of circulating blood mononuclear leukocytes.
- The Vcam1 gene is located on the Chr3. 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)



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Vcam1 vascular cell adhesion molecule 1 [Mus musculus (house mouse)]

Gene ID: 22329, updated on 2-Apr-2019

Summary

Official Symbol	Vcam1 provided by MGI
Official Full Name	vascular cell adhesion molecule 1 provided by MGI
Primary source	MGI:MGI:98926
See related	Ensembl:ENSMUSG0000027962
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	CD106, Vcam-1
Expression	Broad expression in liver E18 (RPKM 27.1), limb E14.5 (RPKM 20.2) and 18 other tissues See more
Orthologs	human all

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Transcript information (Ensembl)



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

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Vcam1-201	ENSMUST00000029574.12	3469	<u>739aa</u>	Protein coding	CCDS17785	Q3UPN1	TSL:1 GENCODE basic APPRIS P1	
Vcam1-203	ENSMUST00000196449.4	2532	<u>345aa</u>	Protein coding		P29533 Q544V4	TSL:1 GENCODE basic	
Vcam1-202	ENSMUST00000196309.1	749	<u>237aa</u>	Protein coding	120	A0A0G2JFP9	CDS 3' incomplete TSL:3	
Vcam1-204	ENSMUST00000197031.1	4088	No protein	Retained intron	10 <u>1</u> 0	24	TSL:NA	

The strategy is based on the design of Vcam1-201 transcript, The transcription is shown below

< Vcam1-201 protein coding

Reverse strand

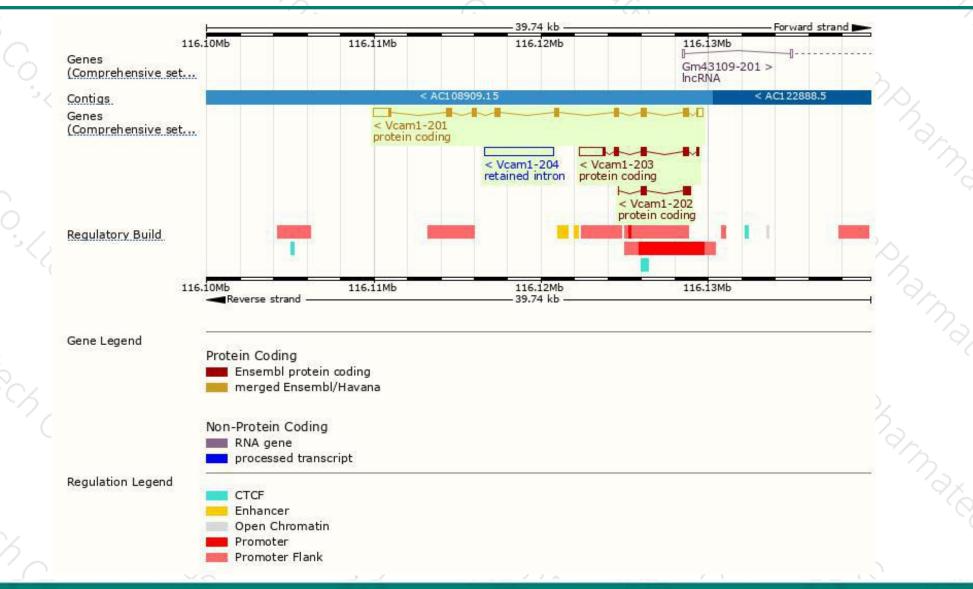
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19.74 kb

Genomic location distribution



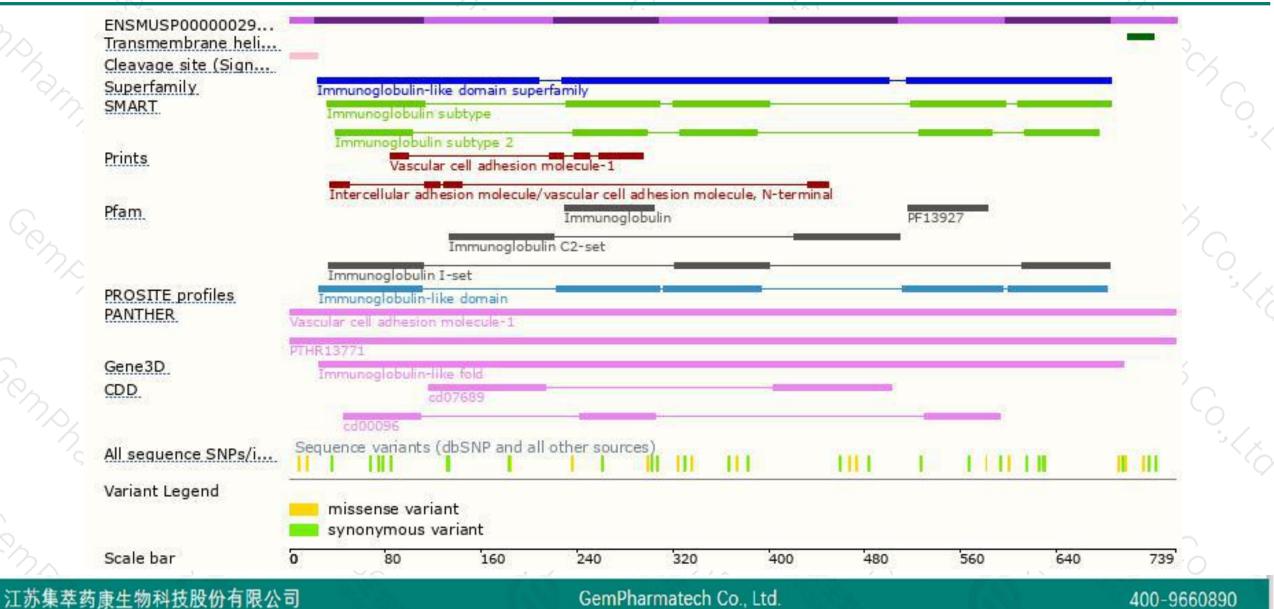


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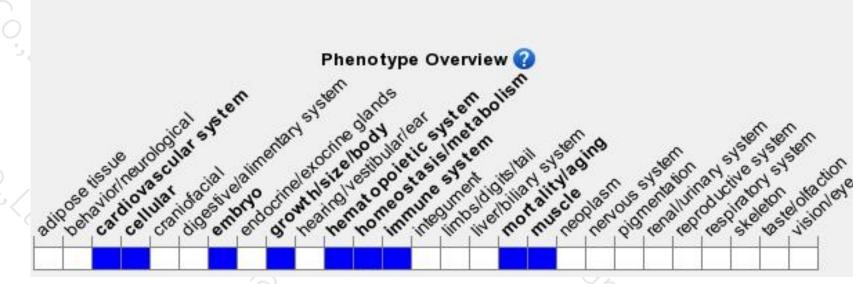
Protein domain





Mouse phenotype description(MGI)





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

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



