

# Card11 Cas9-CKO Strategy

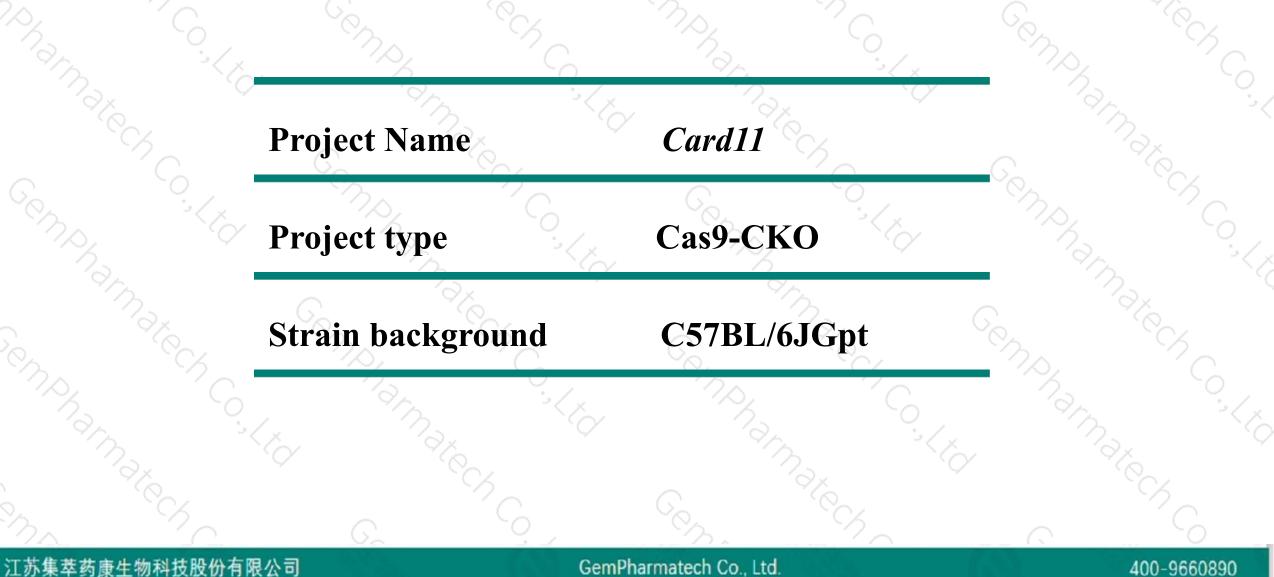
Designer: Reviewer:

**Design Date:** 

Huan Wang Huan Fan 2020-5-26

### **Project Overview**





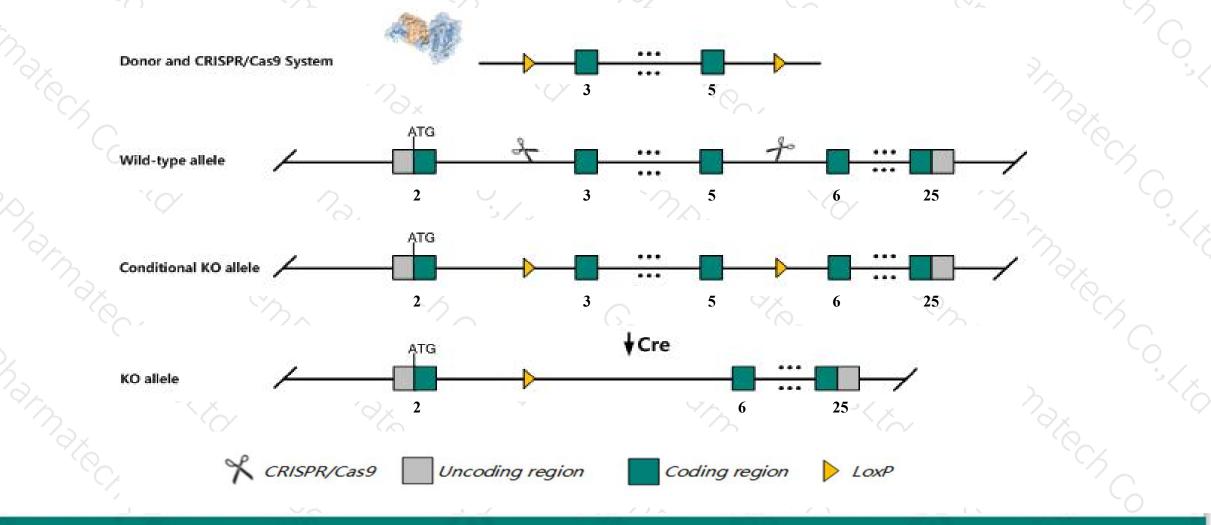
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### **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Card11 gene. The schematic diagram is as follows:



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The Card11 gene has 3 transcripts. According to the structure of Card11 gene, exon3-exon5 of Card11-201 (ENSMUST00000085786.6) transcript is recommended as the knockout region. The region contains 677bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Card11* 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 and cell types.



- According to the existing MGI data, mice homozygous for a targeted null mutation exhibit defects in antigen receptor signalling in both t and b lymphocytes.
- The *Card11* gene is located on the Chr5. 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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#### Card11 caspase recruitment domain family, member 11 [Mus musculus (house mouse)]

Gene ID: 108723, updated on 13-Mar-2020

#### Summary

Official Symbol	Card11 provided by MGI
<b>Official Full Name</b>	caspase recruitment domain family, member 11 provided by MGI
<b>Primary source</b>	MGI:MGI:1916978
See related	Ensembl:ENSMUSG0000036526
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	0610008L17Rik, 2410011D02Rik, BIMP3, CARMA1
Expression	Biased expression in spleen adult (RPKM 26.7), thymus adult (RPKM 15.8) and 6 other tissues See more
Orthologs	human all

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## **Transcript information (Ensembl)**



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Card11-201	ENSMUST0000085786.6	4107	<u>1154aa</u>	Protein coding	CCDS51686	Q8CIS0	TSL:1 GENCODE basic APPRIS P1
Card11-202	ENSMUST00000196169.1	1299	No protein	Retained intron		-	TSL:1
Card11-203	ENSMUST00000199091.1	1067	No protein	Retained intron		1	TSL:1

The strategy is based on the design of Card11-201 transcript, the transcription is shown below:

< Card11-201 protein coding

Reverse strand

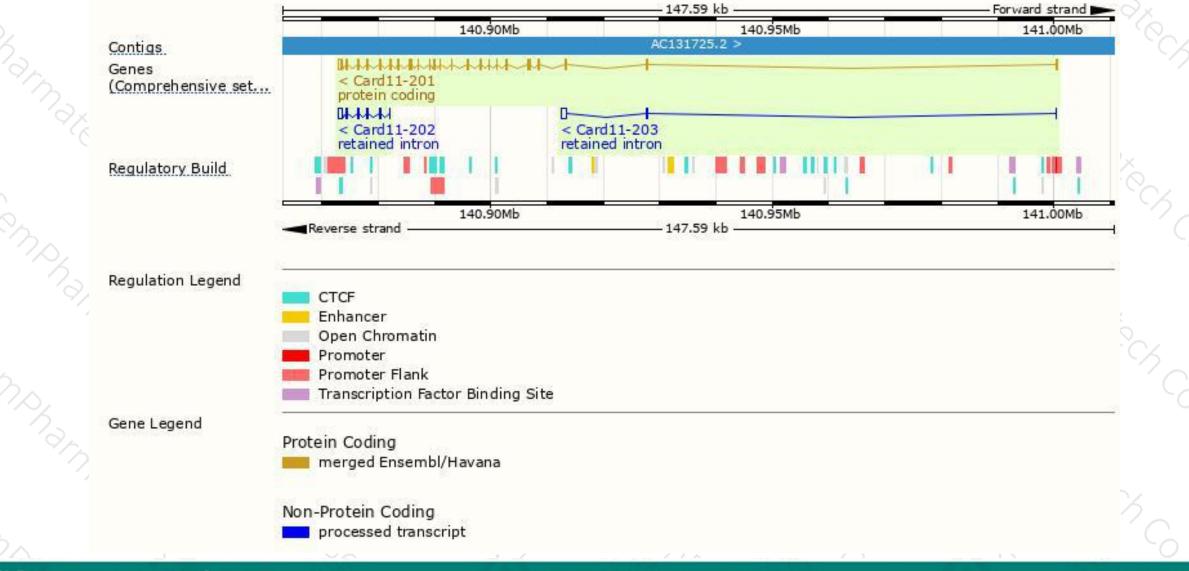
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127.59 kb

### **Genomic location distribution**





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# **Protein domain**

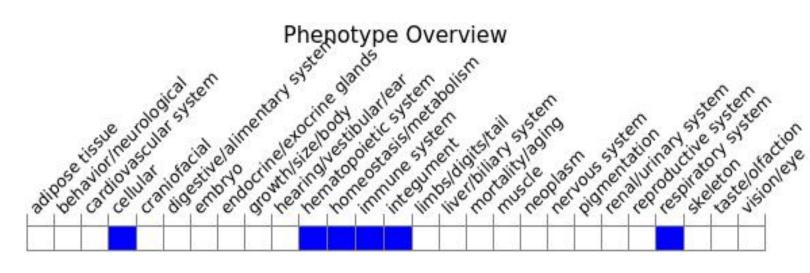
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3 rm	ENSMUSP0000082 MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils) Superfamily	Death-like domain superfamily	PDZ superfamily	P-loop containing
	Pfam.	CARD domain		
	PROSITE profiles	CARD domain		
	PANTHER	PTHR14559		
20	Gene3D	Caspase recruitment domain-containing protein 11	2,30,42,10 2,30,30,40	3.40.50.300
	CDD	CARD11, CARD domain	cd00992	
25.	All sequence SNPs/i	Sequence variants (dbSNP and all other sources)	apone re ricens	
· 9	Variant Legend	missense variant splice region variant synonymous variant		
	Scale bar	0 100 200 300 400 5	00 600 700 800 900	1000 1154
主茨苏	康生物科技股份有限公司	a GemPharma	tech Co., Ltd.	400-9660890
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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, mice homozygous for a targeted null mutation exhibit defects in antigen receptor signalling in both T and B lymphocytes.



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



