

# Arhgef1 Cas9-CKO Strategy

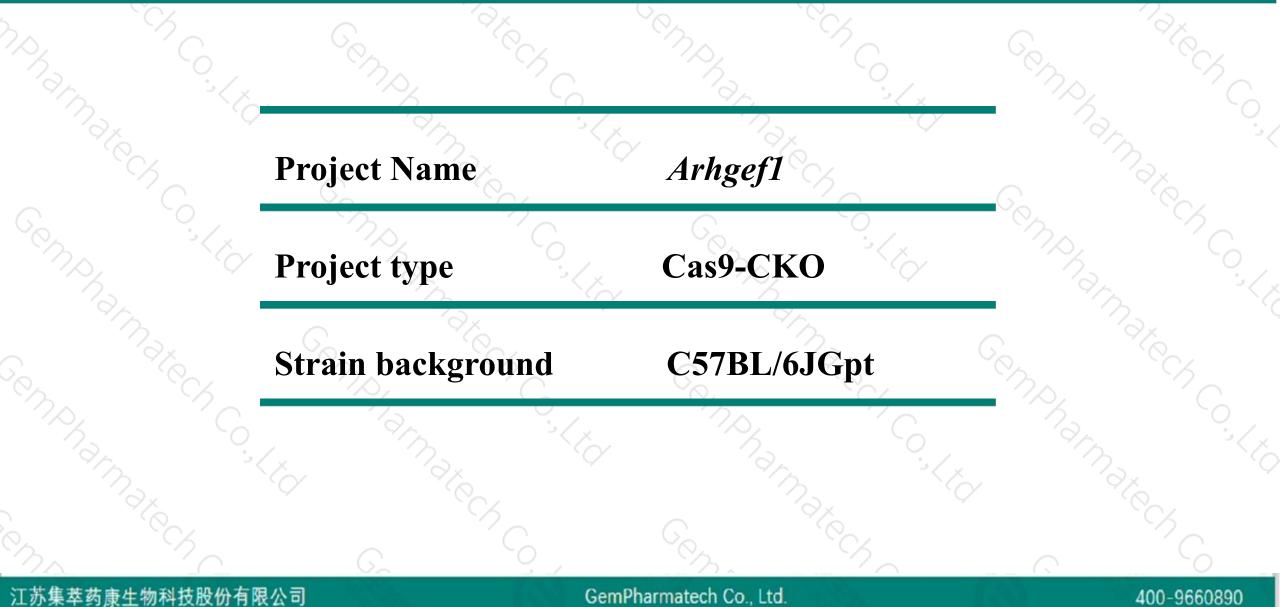
Designer: Ruirui Zhang

**Reviewer: Yanhua Shen** 

Design Date: 2020/10/13

## **Project Overview**

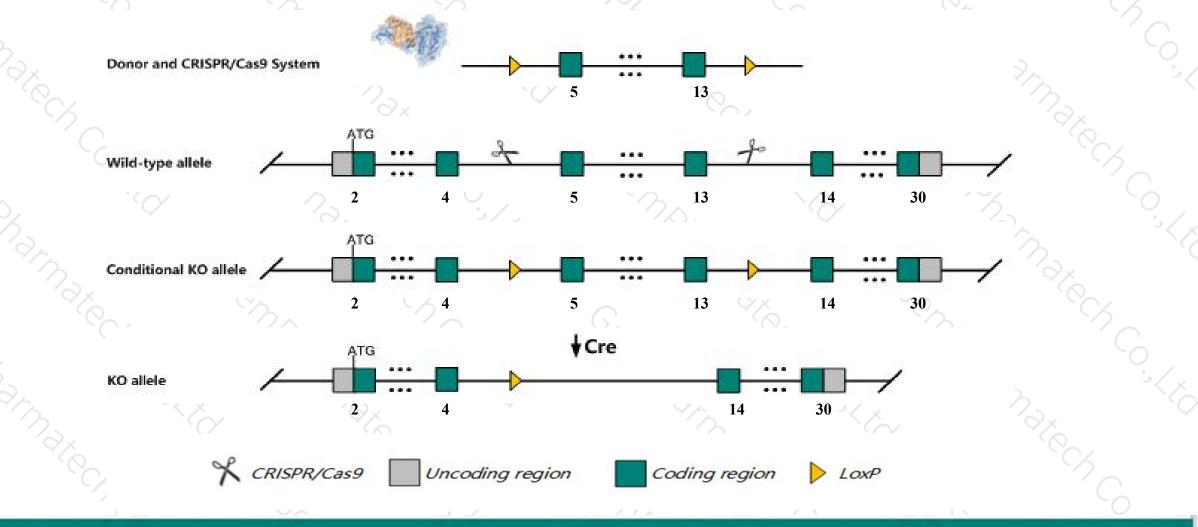




## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Arhgef1* gene. The schematic diagram is as follows:



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The Arhgef1 gene has 19 transcripts. According to the structure of Arhgef1 gene, exon5-exon13 of Arhgef1-204(ENSMUST00000117796.7) transcript is recommended as the knockout region. The region contains 899bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Arhgef1* 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, homozygous mutation of this gene results in impaired humeral immunity, reduced numbers of marginal zone B (MZB) cells, decreased basal T cell proliferation, and reduced basal motility of lymphocytes but enhanced migration of MZB cells after serum activation.

> The *Arhgef1* gene is located on the Chr7. 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)



Arhgef1 Rho guanine nucleotide exchange factor (GEF) 1 [ Mus musculus (house mouse) ]

Gene ID: 16801, updated on 3-Oct-2020

Summary

 Official Symbol
 Arhgef1 provided by MGi

 Official Full Name
 Rho guanine nucleotide exchange factor (GEF) 1 provided by MGi

 Primary source
 MGI:MGI:1353510

 See related
 Ensembl:ENSMUSG00000040940

 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; Muridae; Murinae; Mus; Mus

 Also known as
 L; Lbc; Lsc; Lbcl2

 Expression
 Broad expression in thymus adult (RPKM 202.0), spleen adult (RPKM 157.1) and 26 other tissues See more

 Orthologs
 human all

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## **Transcript information (Ensembl)**

Name 🍦	Transcript ID	bp 🖕	Protein 🖕	Biotype 🔺	CCDS 🍦	UniProt 🝦		Flags	\$
Arhgef1-213	ENSMUST00000145783.7	4243	No protein	Retained intron	-	-		TSL:5	
Arhgef1-207	ENSMUST00000127761.7	1827	No protein	Retained intron	100	1. <u>2</u> )		TSL:2	
Arhgef1-212	ENSMUST00000144714.1	1087	No protein	Retained intron	-	-		TSL:3	
rhgef1-206	ENSMUST00000126918.7	821	No protein	Retained intron		2		TSL:5	
rhgef1-209	ENSMUST00000129928.1	777	No protein	Retained intron	-	-		TSL:2	
rhgef1-205	ENSMUST00000126484.1	775	No protein	Retained intron		20		TSL:1	
Arhgef1-204	ENSMUST00000117796.7	3508	<u>976aa</u>	Protein coding	CCDS52140	E9PUF7 P	TSL:1	GENCODE basic	APPRIS ALT2
rhgef1-202	ENSMUST0000098683.10	3375	<u>979aa</u>	Protein coding	CCDS52142	<u>Q61210</u> &	TSL:1	GENCODE basic	APPRIS P4
rhgef1-203	ENSMUST00000117419.7	3214	<u>920aa</u>	Protein coding	CCDS52141	<u>Q61210</u> &	TSL:1	GENCODE basic	APPRIS ALT2
Arhgef1-201	ENSMUST0000047873.15	3212	<u>920aa</u>	Protein coding	CCDS52141	<u>Q61210</u> &	TSL:1	GENCODE basic	APPRIS ALT2
rhgef1-218	ENSMUST00000206508.1	3195	<u>919aa</u>	Protein coding	CCDS85239	<u>Q61210</u> &	TSL:1	GENCODE basic	APPRIS ALT2
Arhgef1-210	ENSMUST00000132751.1	1851	<u>616aa</u>	Protein coding		F6ZN61		CDS 5' incomplete	TSL:5
rhgef1-215	ENSMUST00000205295.1	777	<u>233aa</u>	Protein coding	-	A0A0U1RPN7		CDS 3' incomplete	TSL:5
rhgef1-219	ENSMUST00000206906.1	737	<u>192aa</u>	Protein coding		A0A0U1RPP2		CDS 3' incomplete	TSL:5
Arhgef1-216	ENSMUST00000206011.1	419	<u>102aa</u>	Protein coding	-	A0A0U1RPH2		CDS 3' incomplete	TSL:2
Arhgef1-214	ENSMUST00000151121.1	390	<u>101aa</u>	Protein coding		<u>D3YVJ9</u> &		CDS 3' incomplete	TSL:3
Arhgef1-217	ENSMUST00000206028.1	374	<u>30aa</u>	Protein coding	-	A0A0U1RNQ2	1	CDS 3' incomplete	TSL:3
Arhgef1-211	ENSMUST00000132786.1	832	No protein	Processed transcript	-	-		TSL:5	
rhgef1-208	ENSMUST00000129383.1	351	No protein	Processed transcript		-		TSL:2	

The strategy is based on the design of *Arhgef1-204* transcript, the transcription is shown below:

Arhgef1-204 > protein coding

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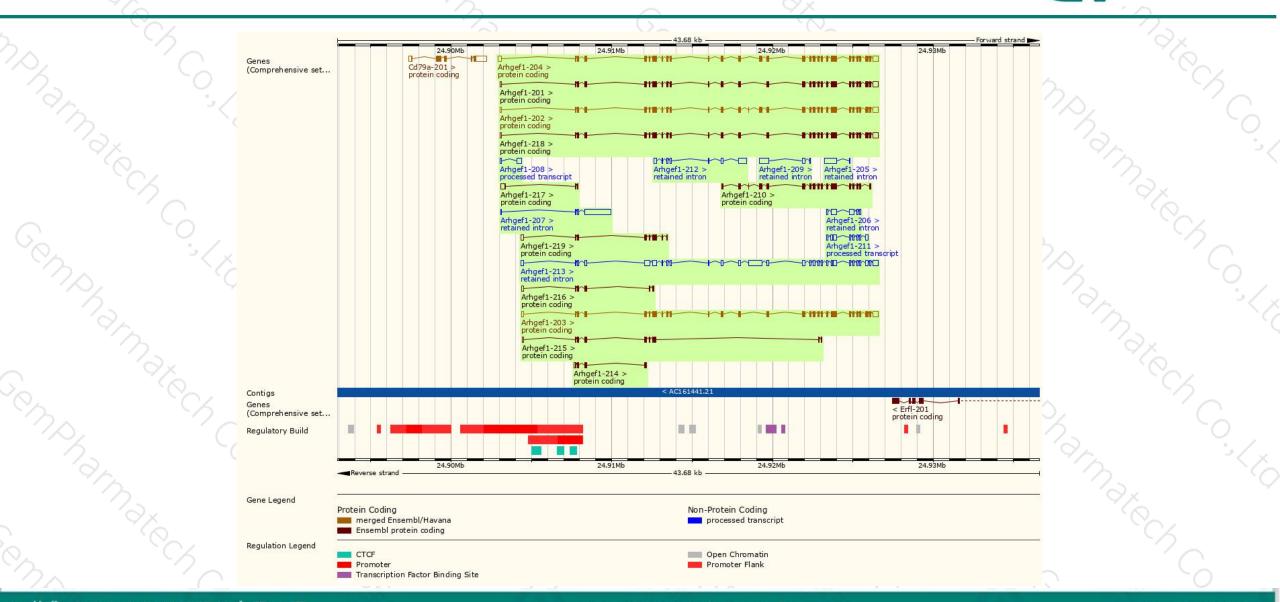
23.68 kb

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Forward strand



## **Genomic location distribution**



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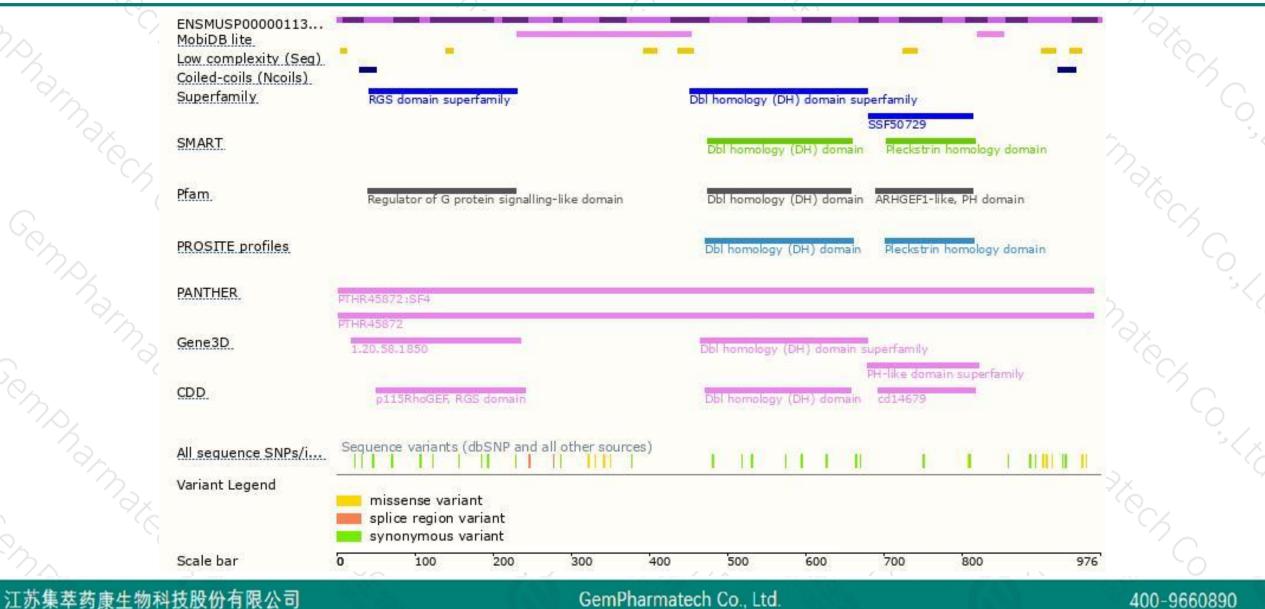
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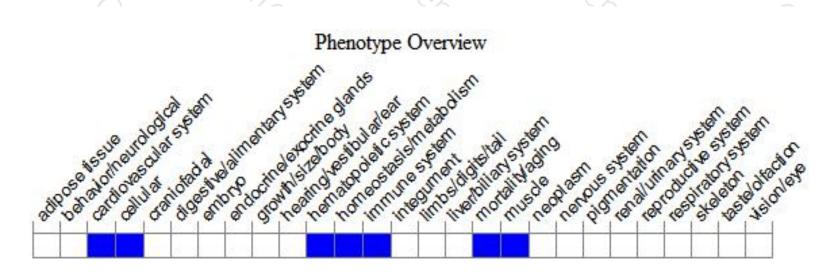
## **Protein domain**





## Mouse phenotype description(MGI)





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

According to the existing MGI data, homozygous mutation of this gene results in impaired humeral immunity, reduced numbers of marginal zone B (MZB) cells, decreased basal T cell proliferation, and reduced basal motility of lymphocytes but enhanced migration of MZB cells after serum activation.

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



