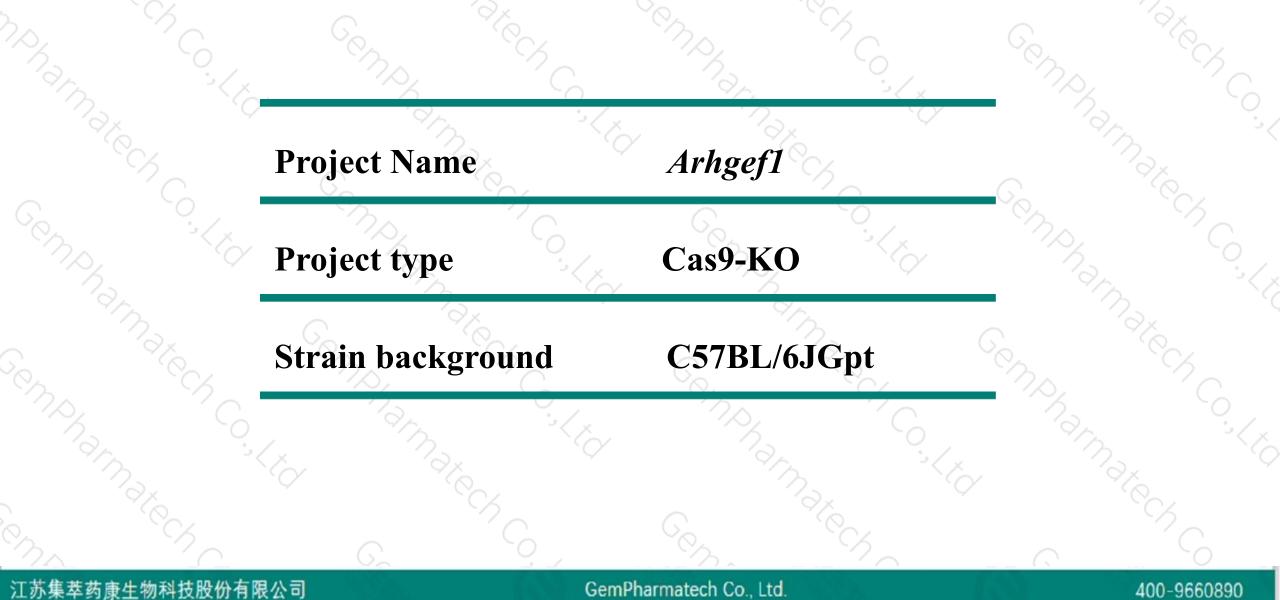


Arhgef1 Cas9-KO Strategy

Designer: Reviewer: Design Date: Ruirui Zhang Huimin Su 2020-2-24

Project Overview

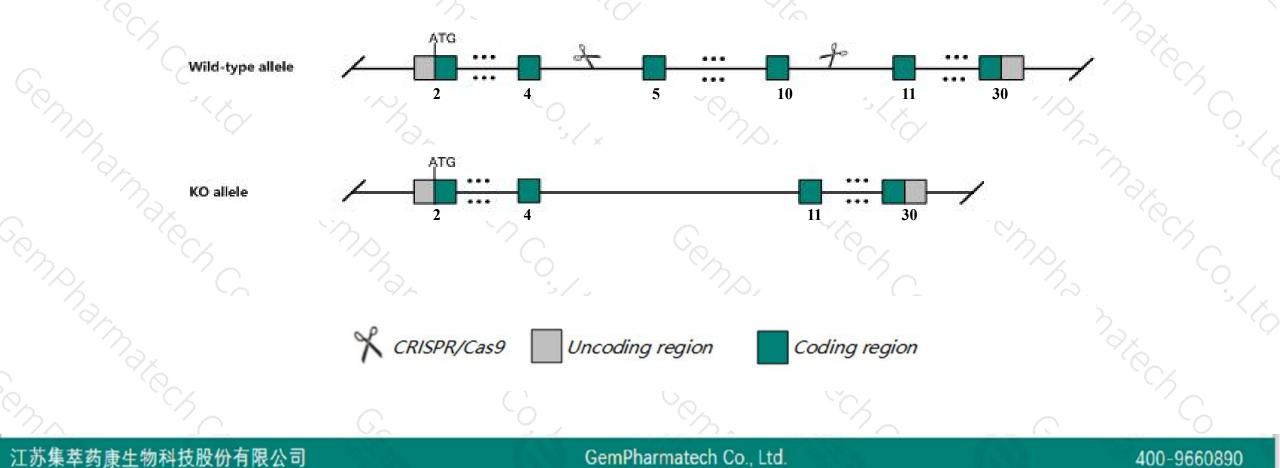




Knockout strategy



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





- The Arhgef1 gene has 19 transcripts. According to the structure of Arhgef1 gene, exon5-exon10 of Arhgef1-204 (ENSMUST00000117796.7) transcript is recommended as the knockout region. The region contains 616bp 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 syste



- > 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, part of null homozygous die before weaning.
- ➢ Transcript Arhgef1-210 and Arhgef1-217 may not be affected.
- 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

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Gene information (NCBI)



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

Gene ID: 16801, updated on 5-Jan-2020

Summary

Official Symbol	Arhgef1 provided by MGI							
Official Full Name	tho guanine nucleotide exchange factor (GEF) 1 provided by MGI							
Primary source	<u>//GI:///GI:1353510</u>							
See related	Ensembl:ENSMUSG0000040940							
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	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							
and from the	$\mathcal{A}_{\mathbf{X}} = \mathcal{A}_{\mathbf{X}} = \mathcal{A}_{\mathbf{X}} = \mathcal{A}_{\mathbf{X}} = \mathcal{A}_{\mathbf{X}} = \mathcal{A}_{\mathbf{X}} = \mathcal{A}_{\mathbf{X}} = \mathcal{A}_{\mathbf{X}}$							

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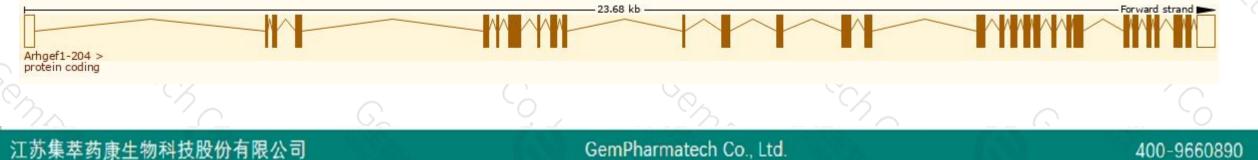
Transcript information (Ensembl)



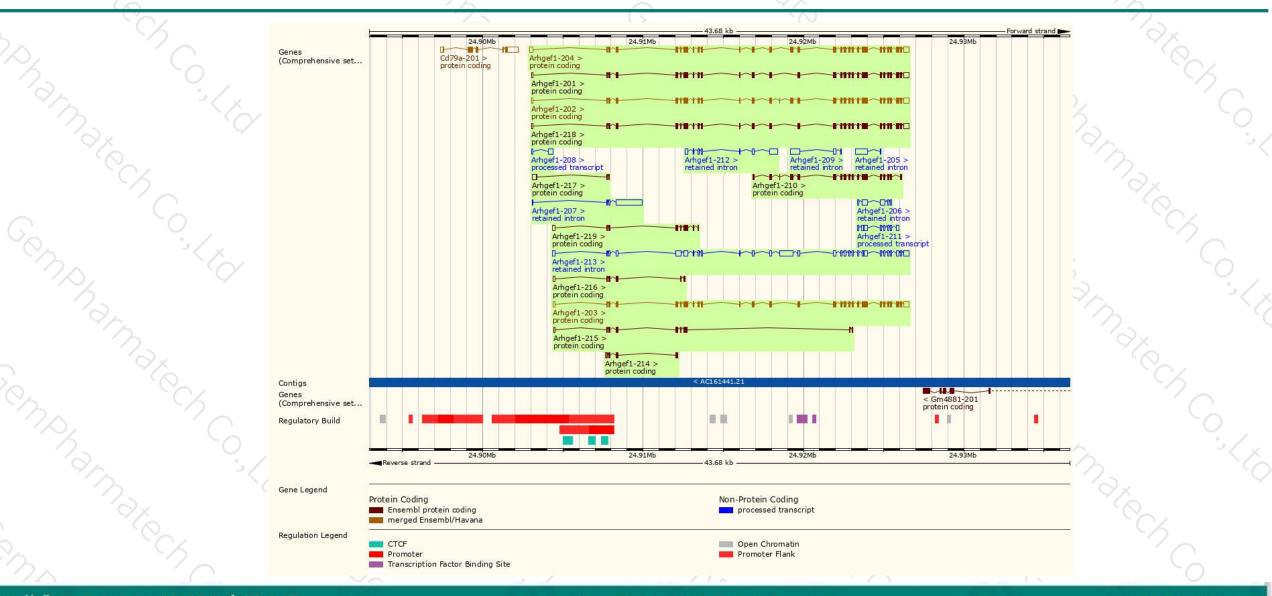
The gene has 19 transcripts, all transcripts are shown below:

1.2		- E	1		The second		· · /)			
Name 🍦	🛔 Transcript ID 💧		Protein 🖕	Biotype 💧	CCDS	UniProt 💧	Flags			
Arhgef1-204	ENSMUST00000117796.7	3508	<u>976aa</u>	Protein coding	CCDS52140 P	E9PUF7	TSL:1 GENCODE basic APPRIS ALT2			
Arhgef1-202	ENSMUST0000098683.10	3375	<u>979aa</u>	Protein coding	CCDS52142 @	<u>Q61210</u> 🗗	TSL:1 GENCODE basic APPRIS P4			
Arhgef1-203	ENSMUST00000117419.7	3214	<u>920aa</u>	Protein coding	CCDS52141	<u>Q61210</u> 🗗	TSL:1 GENCODE basic APPRIS ALT2			
Arhgef1-201	ENSMUST00000047873.15	3212	<u>920aa</u>	Protein coding	CCDS52141	Q61210 &	TSL:1 GENCODE basic APPRIS ALT2			
Arhgef1-218	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			
Arhgef1-215	ENSMUST00000205295.1	777	<u>233aa</u>	Protein coding	-	A0A0U1RPN7 🗗	CDS 3' incomplete TSL:5			
Arhgef1-219	ENSMUST00000206906.1	737	<u>192aa</u>	Protein coding	12	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	12	<u>D3YVJ9</u> &	CDS 3' incomplete TSL:3			
Arhgef1-217	ENSMUST00000206028.1	374	<u>30aa</u>	Protein coding	-	<u>A0A0U1RNQ2</u> &	CDS 3' incomplete TSL:3			
Arhgef1-211	ENSMUST00000132786.1	832	No protein	Processed transcript	12	2	TSL:5			
Arhgef1-208	ENSMUST00000129383.1	351	No protein	Processed transcript	-	-	TSL:2			
Arhgef1-213	ENSMUST00000145783.7	4243	No protein	Retained intron	121	22	TSL:5			
Arhgef1-207	ENSMUST00000127761.7	1827	No protein	Retained intron	-	-	TSL:2			
Arhgef1-212	ENSMUST00000144714.1	1087	No protein	Retained intron	121	22	TSL:3			
Arhgef1-206	ENSMUST00000126918.7	821	No protein	Retained intron	-	-	TSL:5			
Arhgef1-209	ENSMUST00000129928.1	777	No protein	Retained intron	121	22	TSL:2			
Arhgef1-205	ENSMUST00000126484.1	775	No protein	Retained intron	-	-	TSL:1			

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



Genomic location distribution



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Protein domain



	~								
	ENSMUSP00000113 MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils) Superfamily	RGS domain superfamily		DbI ho	mology (DH) domain superfamily	SSF50729			0
	SMART			t	Dbl homology (DH) domain	Pleckstrin ho	mology domain		~
	Pfam	Regulator of G protein signalling-like domai	n	Ē	Dbl homology (DH) domain	ARHGEF1-like, P	PH domain		
~	PROSITE profiles			D	bl homology (DH) domain	Pleckstrin ho	mology domain		
	PANTHER	PTHR45872							
	Gene3D	PTHR45872:SF4 1.20.58.1850		Db	l homology (DH) domain superfami	ily PH-like domain su	perfamily		
	CDD	p115RhoGEF, RGS domain		D	bl homology (DH) domain	cd14679			
	All sequence SNPs/i	Sequence variants (dbSNP and all other source	es)		i ii ana i	an a	1		
	Variant Legend	missense variant synonymous variant	splice region variant						
	Scale bar	0 100 200	300	400	500 600	700	800	9	976
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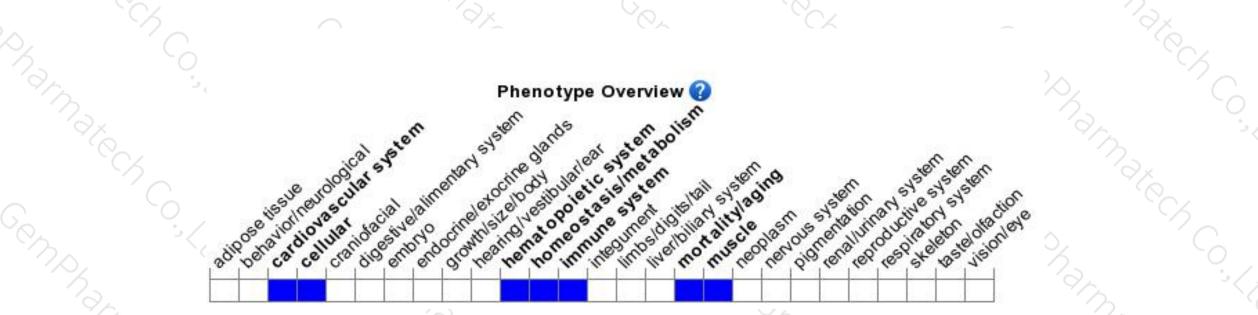
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Mouse phenotype description(MGI)





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, 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, part of null homozygous die before weaning.

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



