Appl2 Cas9-KO Strategy

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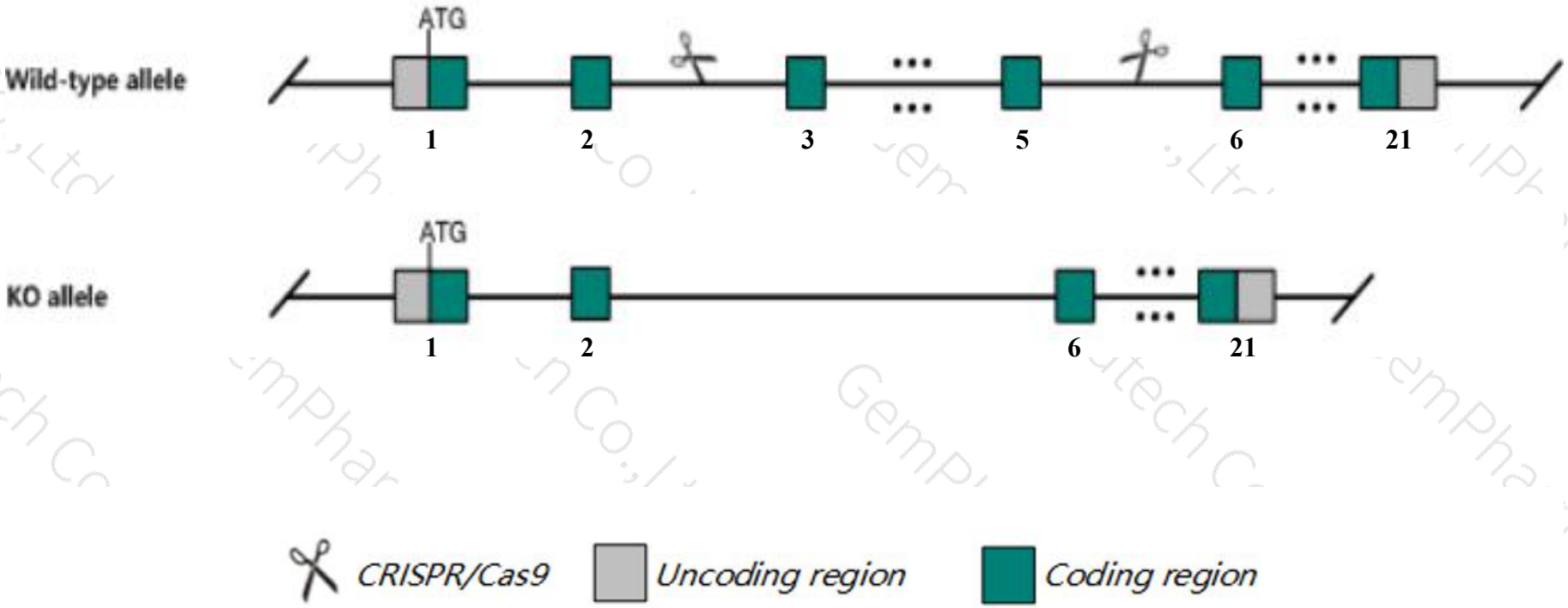
Design Date: 2018/12/7

Project Overview

Project Name	***Appl2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Appl2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Appl2* gene has 14 transcripts. According to the structure of *Appl2* gene, exon3-exon5 of *Appl2-201*(ENSMUST00000020500.13) transcript is recommended as the knockout region.The region contains 220bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Appl2* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null allele display altered red blood cell physiology. Mutant MEFs exhibit defects in HGF-induced Akt activation, migration, and invasion.

➢ The *Appl2* gene is located on the Chr10. 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.

Gene information（NCBI）

Appl2 adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 2 [Mus musculus (house mouse)]

Gene ID: 216190, updated on 13-Mar-2020

Summary

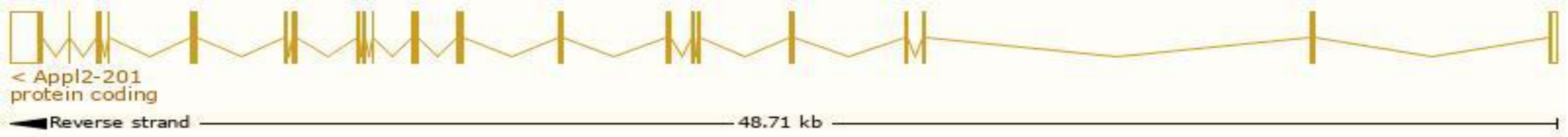
Official Symbol	Appl2 provided by MGI
Official Full Name	adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 2 provided byMGI
Primary source	MGI:MGI:2384914
See related	Ensembl:ENSMUSG00000020263
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	DIP13 beta, Dip3 beta, Dip3b
Expression	Broad expression in cerebellum adult (RPKM 26.5), adrenal adult (RPKM 22.2) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

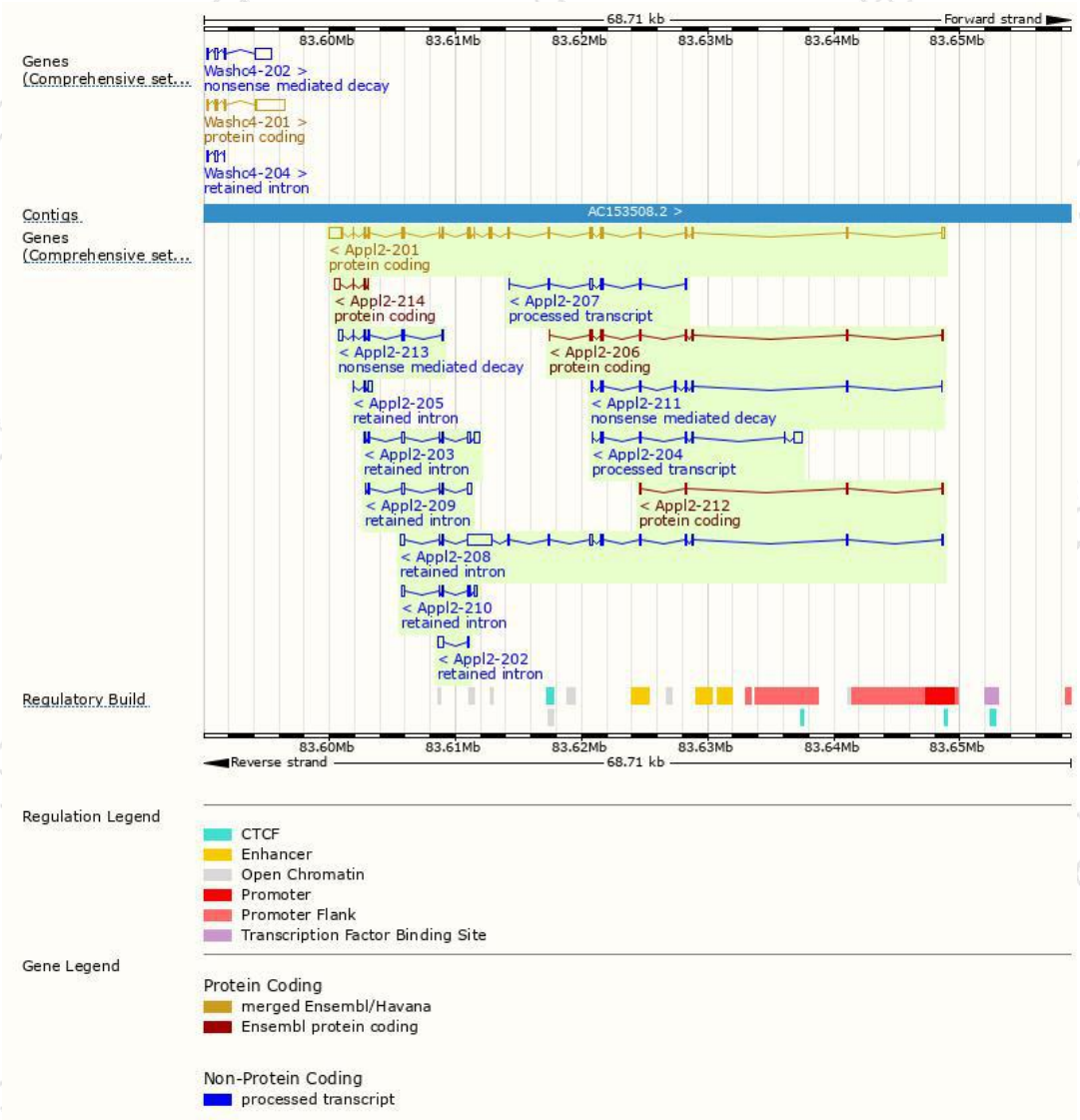
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Appl2-201	ENSMUST00000020500.13	3020	662aa	Protein coding	CCDS24078	Q3TVI6 Q8K3G9	TSL:1 GENCODE basic APPRIS P1
Appl2-206	ENSMUST00000146876.8	713	209aa	Protein coding	-	D3Z340	CDS 3' incomplete TSL:5
Appl2-214	ENSMUST00000177187.1	625	67aa	Protein coding	-	H3BJX0	CDS 5' incomplete TSL:5
Appl2-212	ENSMUST00000176294.1	380	95aa	Protein coding	-	H3BL43	CDS 3' incomplete TSL:3
Appl2-211	ENSMUST00000150685.7	774	117aa	Nonsense mediated decay	-	D6RFR7	TSL:3
Appl2-213	ENSMUST00000176675.7	773	42aa	Nonsense mediated decay	-	H3BL67	CDS 5' incomplete TSL:5
Appl2-204	ENSMUST00000133719.2	961	No protein	Processed transcript	-	-	TSL:5
Appl2-207	ENSMUST00000147118.7	530	No protein	Processed transcript	-	-	TSL:5
Appl2-208	ENSMUST00000147582.7	3377	No protein	Retained intron	-	-	TSL:1
Appl2-203	ENSMUST00000130285.8	1210	No protein	Retained intron	-	-	TSL:3
Appl2-210	ENSMUST00000150351.1	807	No protein	Retained intron	-	-	TSL:2
Appl2-209	ENSMUST00000148096.7	743	No protein	Retained intron	-	-	TSL:3
Appl2-202	ENSMUST00000127788.1	614	No protein	Retained intron	-	-	TSL:2
Appl2-205	ENSMUST00000141048.1	427	No protein	Retained intron	-	-	TSL:5

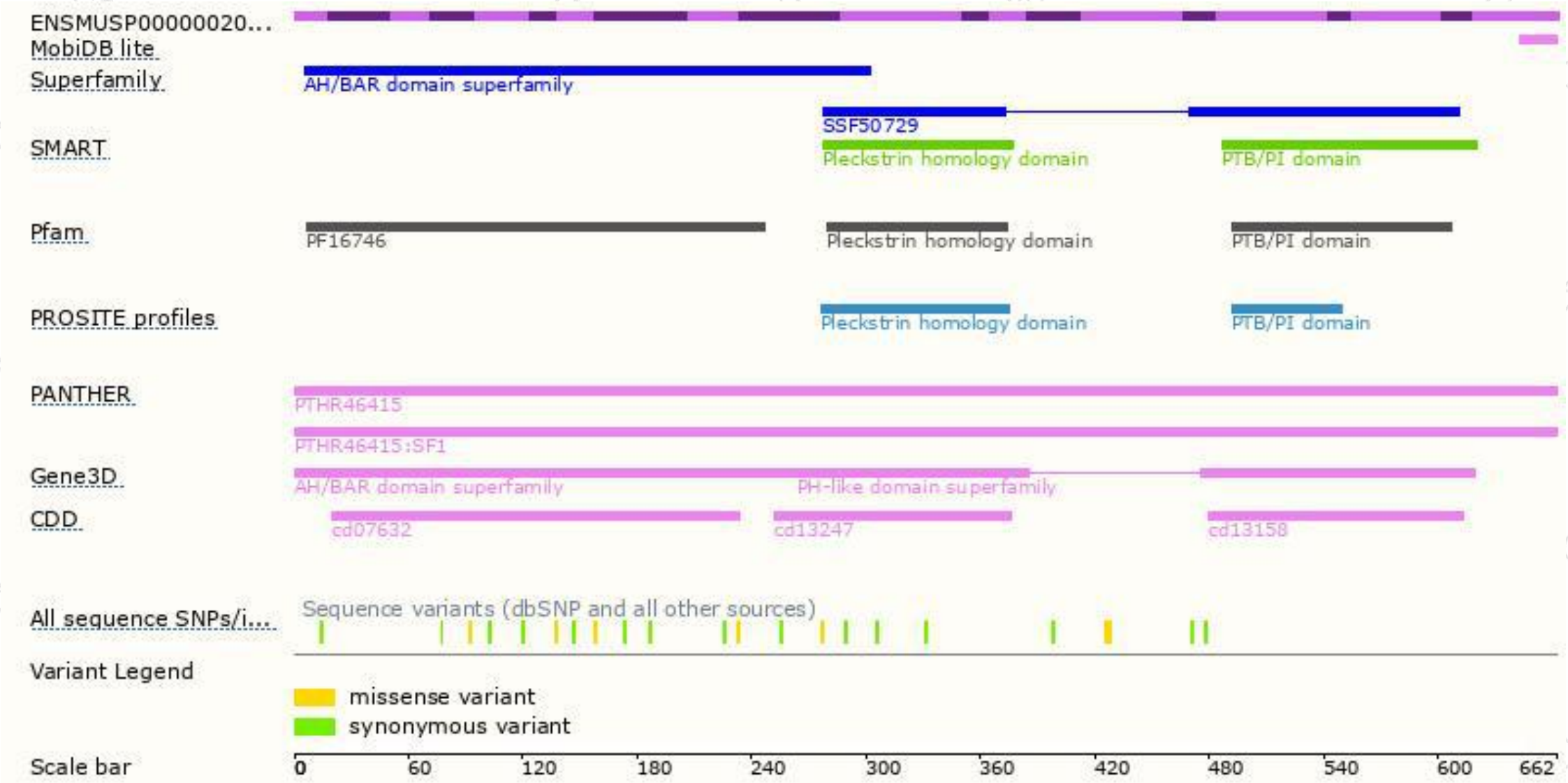
The strategy is based on the design of *Appl2-201* transcript,the transcription is shown below:



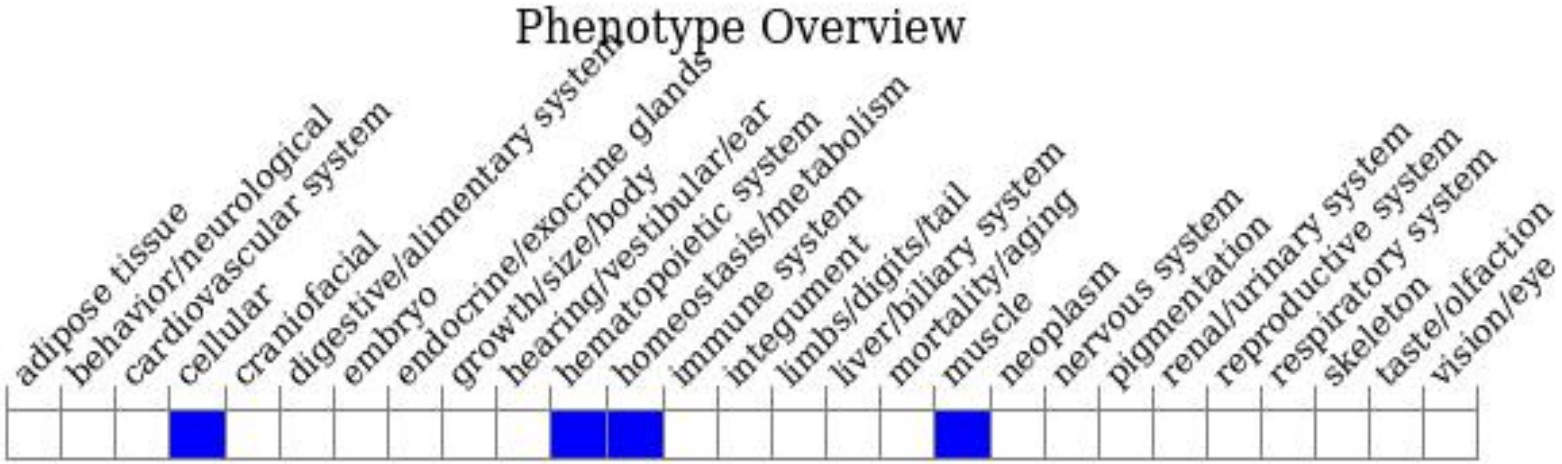
Genomic location distribution



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,mice homozygous for a null allele display altered red blood cell physiology.

Mutant MEFs exhibit defects in HGF-induced Akt activation, migration, and invasion.

If you have any questions, you are welcome to inquire.

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