

# Vav1 Cas9-CKO Strategy Rohalana Koch College

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# **Project Overview**



**Project Name** 

**Project type** 

Cas9-CKO

Vav1

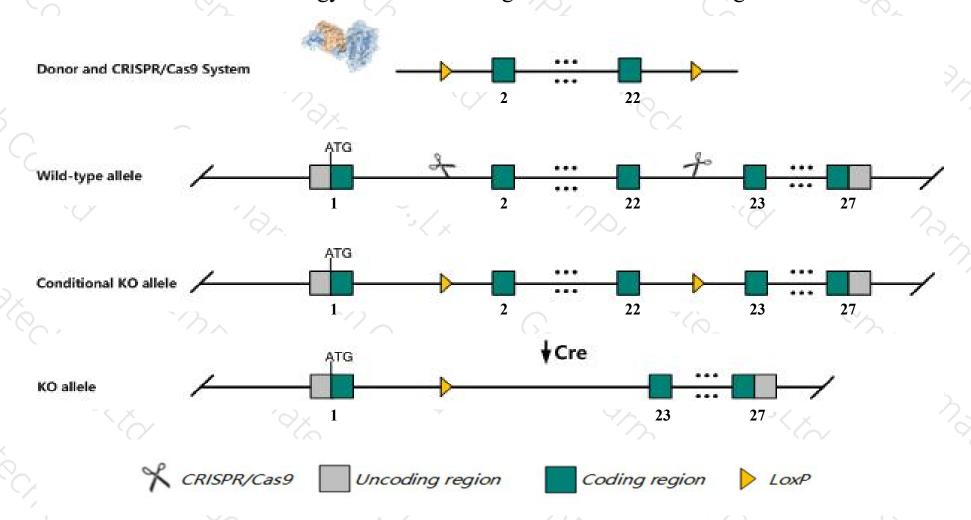
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Vav1 gene. The schematic diagram is as follows:



## Technical routes



- ➤ The *Vav1* gene has 5 transcripts. According to the structure of *Vav1* gene, exon2-exon22 of *Vav1-201*(ENSMUST0000005889.15) transcript is recommended as the knockout region. The region contains 1808bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Vav1* 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.

## **Notice**



- ➤ According to the existing MGI data, Homozygous null mutants exhibit defective T cell maturation, interleukin-2 production, and cell cycle progression. Immunoglobulin class switching is also impaired and attributed to defective T cell help.
- > Transcript *Vav1*-202 may not be affected.
- The *Vav1* gene is located on the Chr17. 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)



#### Vav1 vav 1 oncogene [Mus musculus (house mouse)]

Gene ID: 22324, updated on 3-Mar-2019

#### Summary

☆ ?

Official Symbol Vav1 provided by MGI

Official Full Name vav 1 oncogene provided by MGI

Primary source MGI:MGI:98923

See related Ensembl:ENSMUSG00000034116

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 Vav, vav-T

Expression Biased expression in thymus adult (RPKM 27.9), spleen adult (RPKM 15.9) and 7 other tissuesSee more

Orthologs <u>human</u> all

# Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

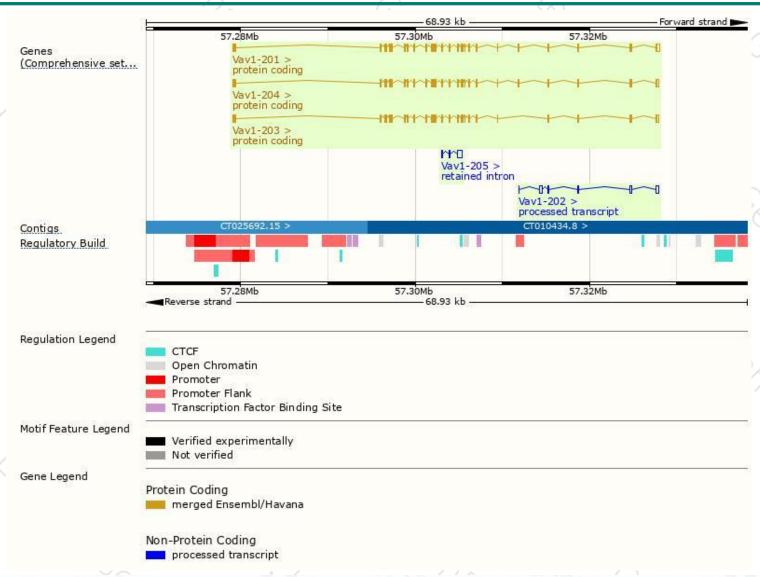
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Vav1-201	ENSMUST00000005889.15	2963	845aa	Protein coding	CCDS28931	P27870 Q3U9E2	TSL:1 GENCODE basic APPRIS P1
Vav1-204	ENSMUST00000169220.8	2810	<u>821aa</u>	Protein coding	CCDS50158	E9PXI0	TSL:1 GENCODE basic
Vav1-203	ENSMUST00000112870.4	2743	806aa	Protein coding	CCDS50159	Q8VDU4	TSL:1 GENCODE basic
Vav1-202	ENSMUST00000008847.6	1034	No protein	Processed transcript	20	20	TSL:1
Vav1-205	ENSMUST00000174878.1	717	No protein	Retained intron	14	T.	TSL:2

The strategy is based on the design of *Vav1-201* transcript, The transcription is shown below



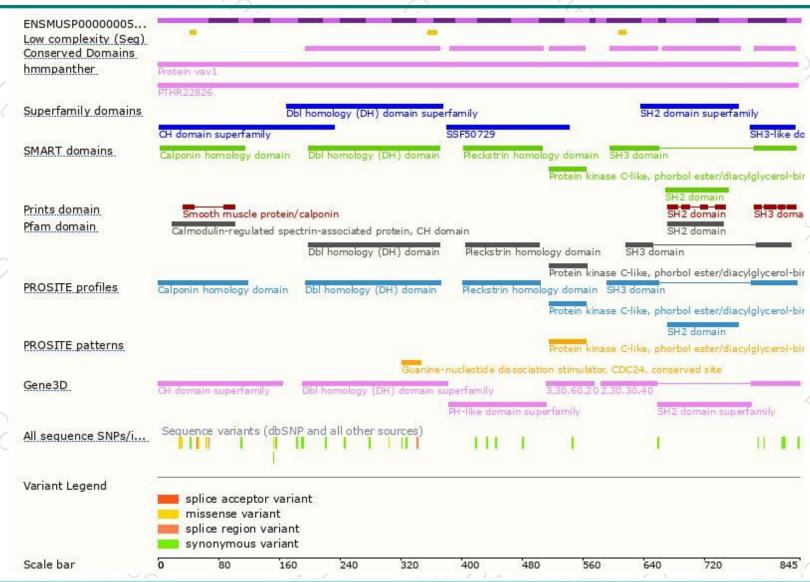
## Genomic location distribution





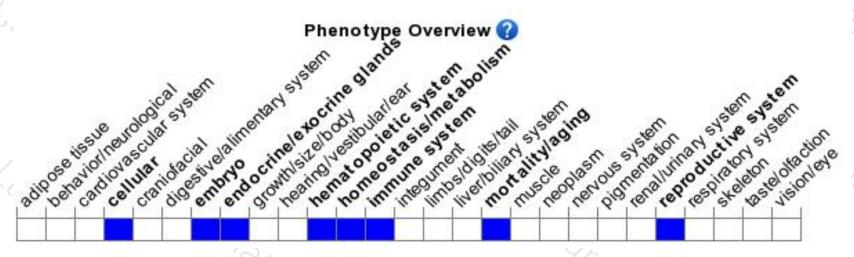
### Protein domain





# 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, Homozygous null mutants exhibit defective T cell maturation, interleukin-2 production, and cell cycle progression. Immunoglobulin class switching is also impaired and attributed to defective T cell help



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





