GemPharmatech™

Tmc8 Cas9-KO Strategy

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Overview

Target Gene Name

- Tmc8

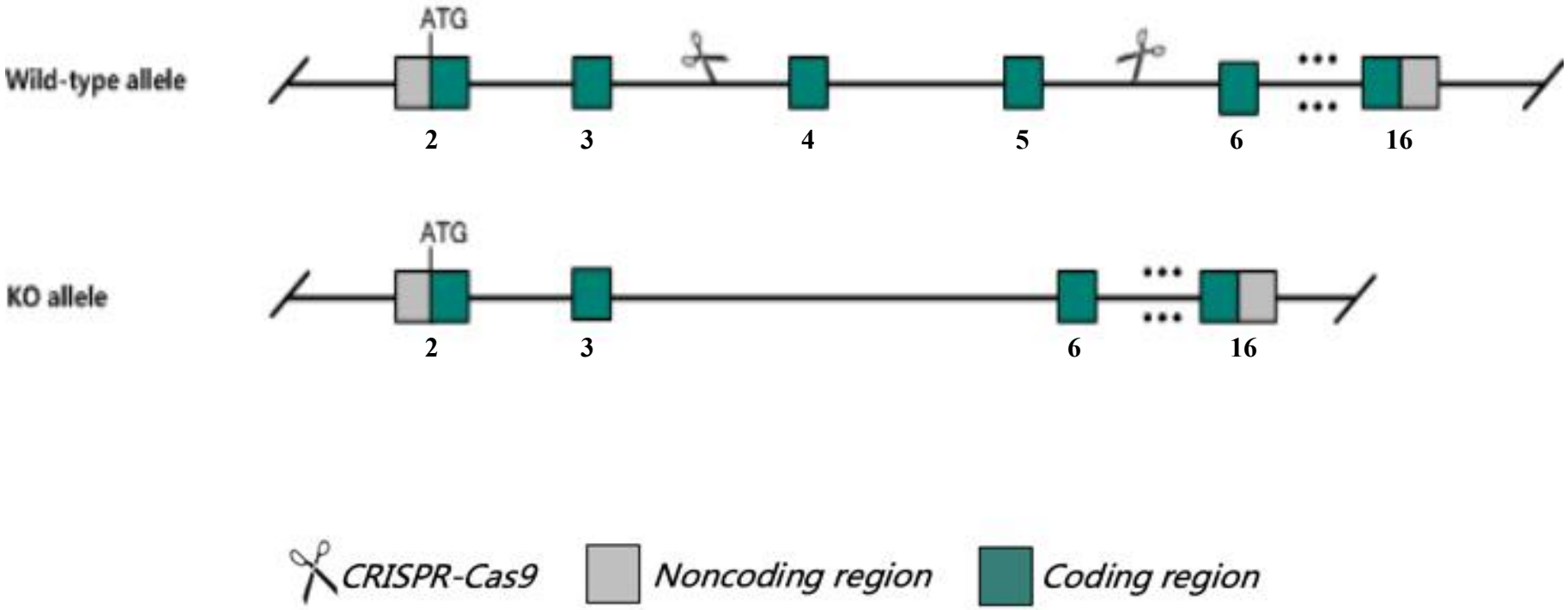
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Technical Information

- The *Tmc8* gene has 6 transcripts. According to the structure of *Tmc8* gene, exon4-exon5 of *Tmc8*-202 (ENSMUST00000106334.9) transcript is recommended as the knockout region. The region contains 236bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Tmc8* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

Gene Information

Tmc8 transmembrane channel-like gene family 8 [Mus musculus (house mouse)]

Gene ID: 217356, updated on 12-Apr-2023

Summary

Official Symbol	Tmc8 provided by MGI
Official Full Name	transmembrane channel-like gene family 8 provided byMGI
Primary source	MGI:MGI:2669037
See related	Ensembl:ENSMUSG00000050106
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	EVIN2, Ever2, mFLJ00400
Summary	Predicted to enable mechanosensitive ion channel activity; protein sequestering activity; and tumor necrosis factor binding activity. Predicted to be involved in several processes, including negative regulation of protein binding activity; regulation of extrinsic apoptotic signaling pathway via death domain receptors; and zinc ion homeostasis. Predicted to act upstream of or within ion transport. Predicted to be located in Golgi apparatus; endoplasmic reticulum; and nuclear membrane. Human ortholog(s) of this gene implicated in epidermodysplasia verruciformis. Orthologous to human TMC8 (transmembrane channel like 8). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Biased expression in thymus adult (RPKM 68.4), spleen adult (RPKM 27.6) and 9 other tissuesSee more
Orthologs	human all

Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

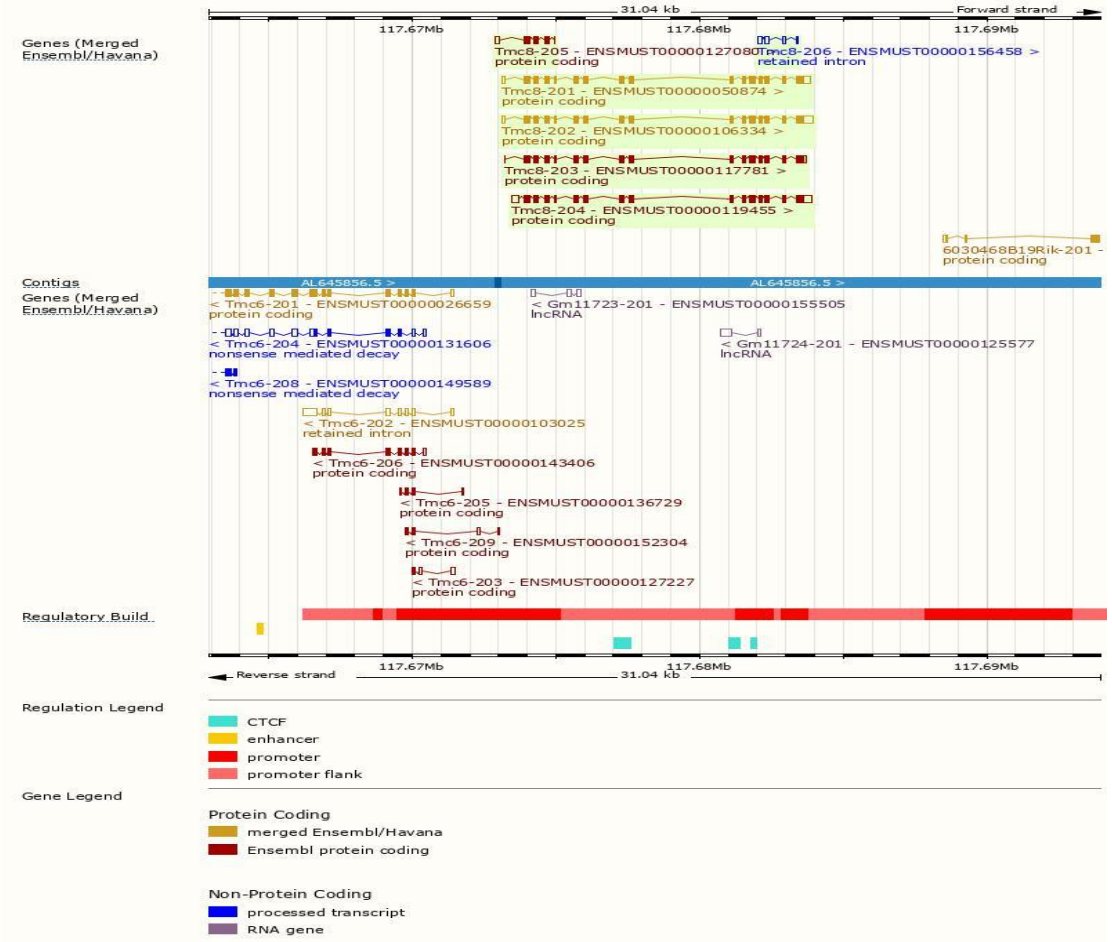
The gene has 6 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000119455.2	Tmc8-204	2736	723aa	Protein coding	CCDS56824	Q7TN58-2	Ensembl Canonical GENCODE basic APPRIS ALT2 TSL:1
ENSMUST00000106334.9	Tmc8-202	2644	723aa	Protein coding	CCDS56824	Q7TN58-2	GENCODE basic APPRIS ALT2 TSL:1
ENSMUST00000050874.14	Tmc8-201	2577	722aa	Protein coding	CCDS25689	Q7TN58-1	GENCODE basic APPRIS P5 TSL:1
ENSMUST00000117781.8	Tmc8-203	2344	722aa	Protein coding	CCDS56825	B0QZP7	GENCODE basic APPRIS ALT2 TSL:1
ENSMUST00000127080.9	Tmc8-205	693	166aa	Protein coding		B1ATB9	TSL:3 CDS 3' incomplete
ENSMUST00000156458.2	Tmc8-206	485	No protein	Retained intron		-	TSL:3

The strategy is based on the design of *Tmc8*-202 transcript, the transcription is shown below:

Source: https://www.ensembl.org

Genomic Information



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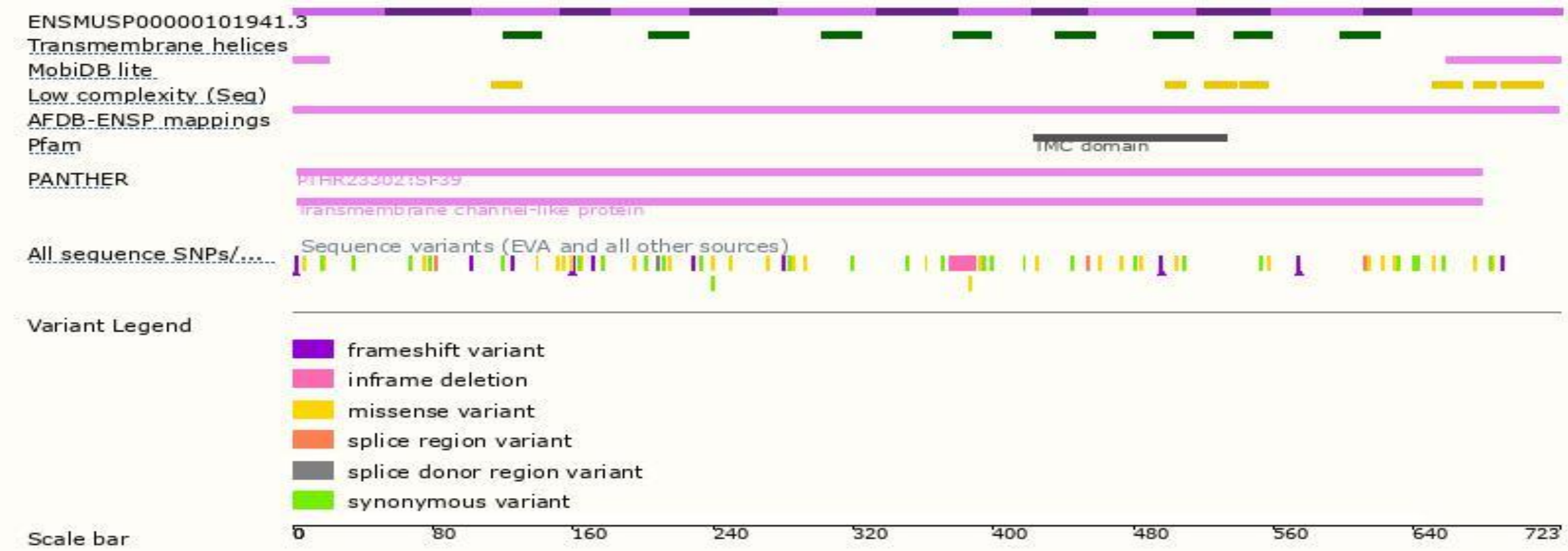
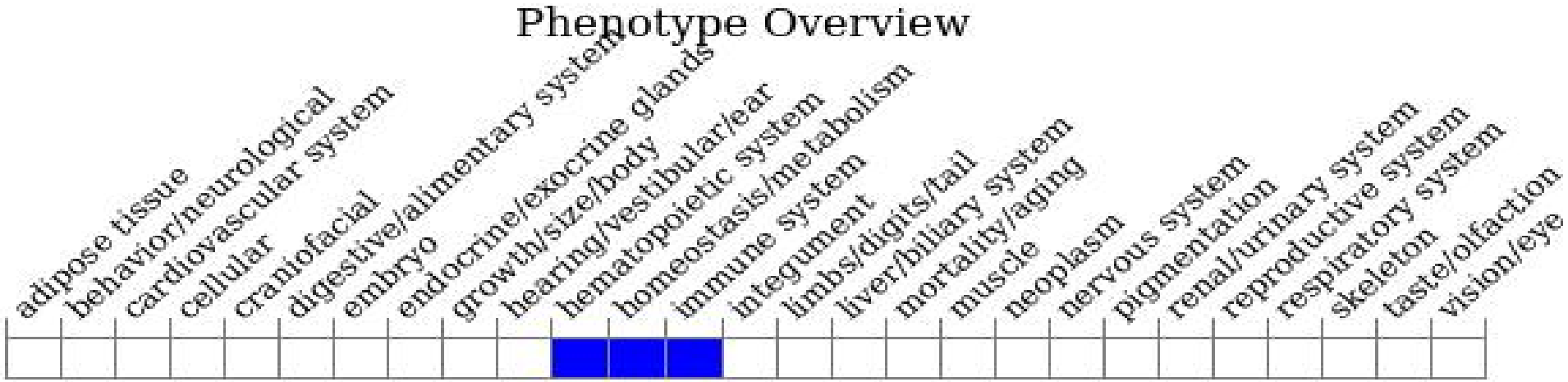
Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Mice homozygous for a knock-out allele are viable, fertile and overtly normal with no major defects in T cell subset numbers or function.

Source: https://www.informatics.jax.org

Important Information

- *Tmc8* is located on Chr11. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- The KO region contains functional region of the *Gm11723*.Knockout the region may af fect the function of *Gm11723* gene.
- The KO region is about ~1.5kb away to the N-terminal of *Tmc6* gene, this strategy may influence the regulatory function of the N-terminal of *Tmc6* gene.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.