GemPharmatech™

Vstm4 Cas9-CKO Strategy

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Reviewer: Xingkai Xiao

Design Date: 2023-4-6

Overview

Target Gene Name

- *Vstm4*

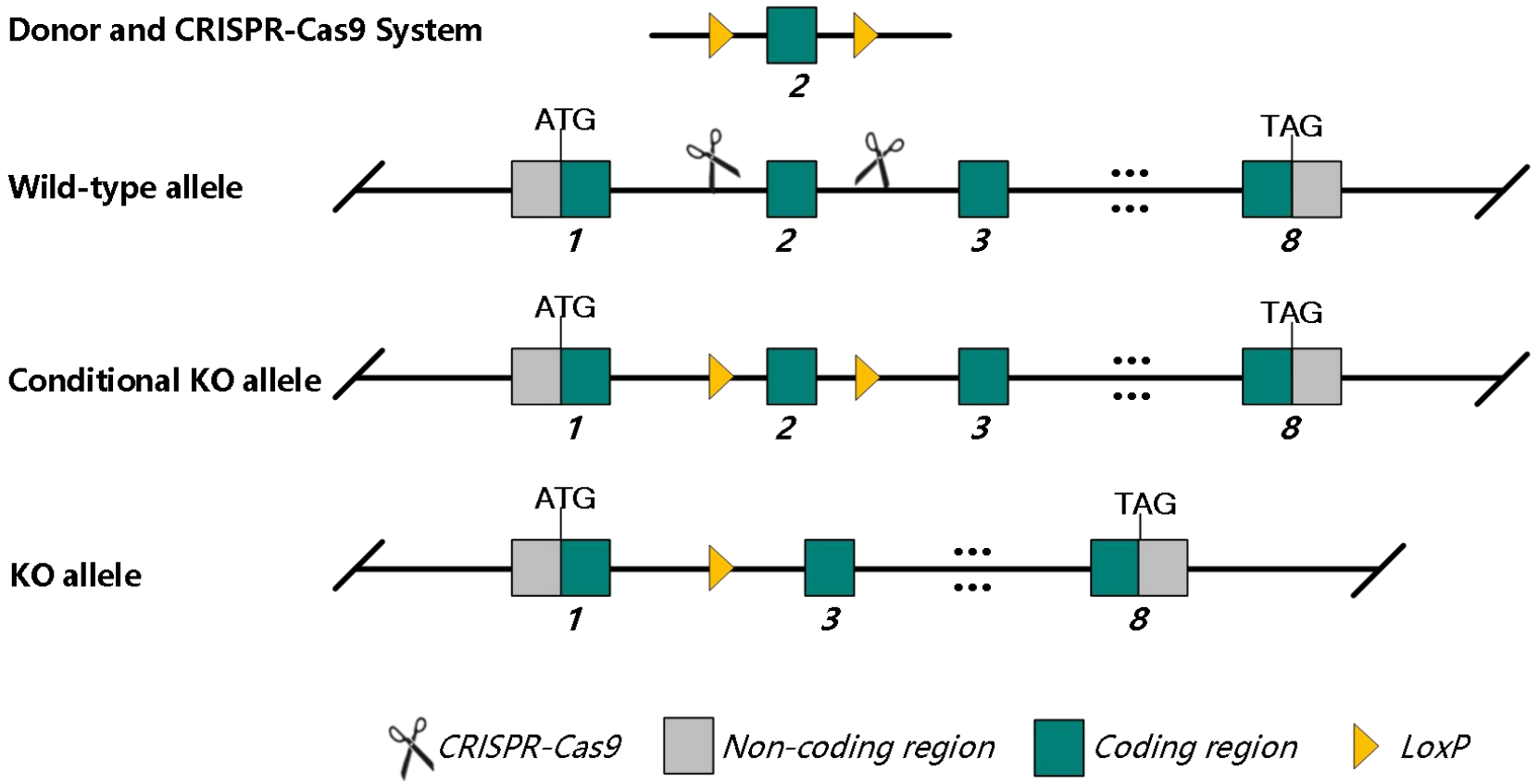
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Vstm4* gene.

Technical Information

- The *Vstm4* gene only has 1 transcript. According to the structure of *Vstm4* gene, exon 2 of *Vstm4*-201 (ENSMUST00000053175.13) is recommended as the knockout region. The region contains 399 bp of coding sequence. Knockout the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Vstm4* 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 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.
- 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.

Gene Information

Vstm4 V-set and transmembrane domain containing 4 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 320736, updated on 9-Mar-2023

Summary

Official Symbol	Vstm4 provided by MGI
Official Full Name	V-set and transmembrane domain containing 4 provided by MGI
Primary source	MGI:MGI:2444633
See related	Ensembl:ENSMUSG00000050666 AllianceGenome:MGI:2444633
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	E130203B14Rik
Summary	Acts upstream of or within several processes, including endothelial cell migration; retina blood vessel maintenance; and vasculature development. Predicted to be located in extracellular region and plasma membrane. Predicted to be integral component of membrane. Orthologous to human VSTM4 (V-set and transmembrane domain containing 4). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Broad expression in placenta adult (RPKM 15.0), bladder adult (RPKM 6.8) and 25 other tissues See more
Orthologs	human all

NEW **Try the new Gene table**
Try the new Transcript table

Genomic context

Location: 14; 14 B

See Vstm4 in Genome Data Viewer

Exon count: 8

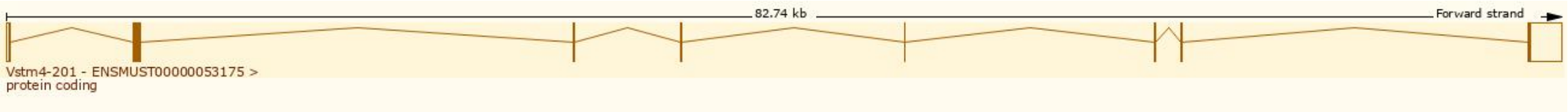
https://www.ncbi.nlm.nih.gov/gene/320736

Transcript Information

The gene only has 1 transcript, which is shown below:

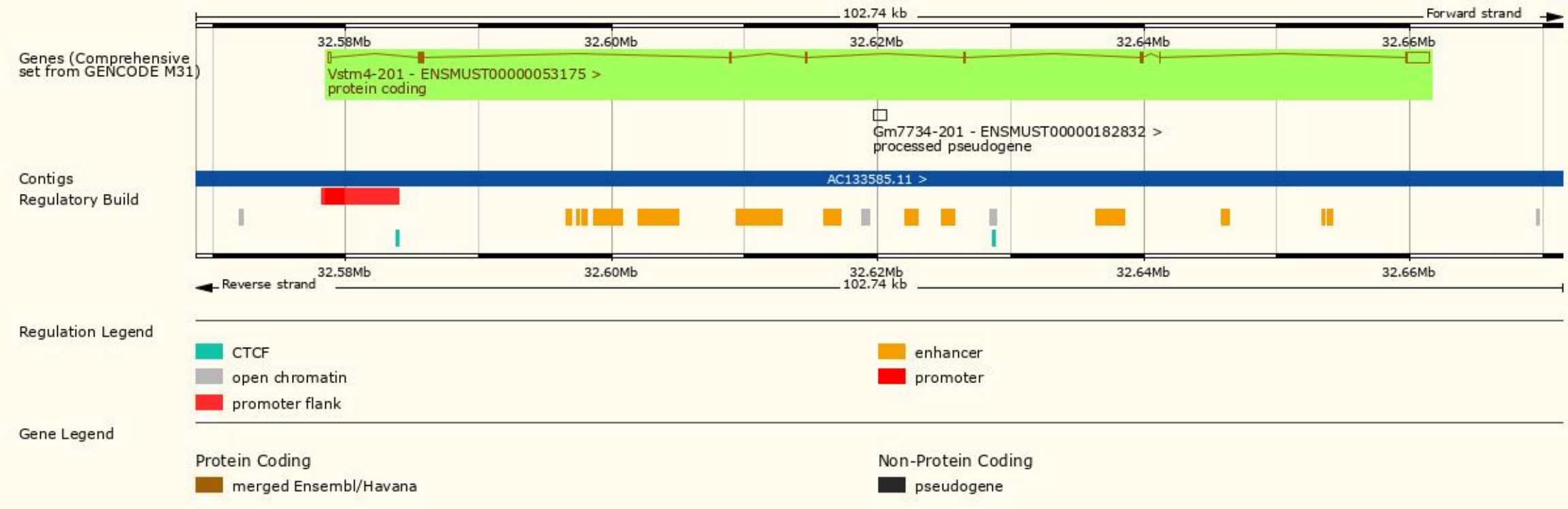
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000053175.13	Vstm4-201	2745	319aa	Protein coding	CCDS26923	T1NXB5	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1

The strategy is based on the design of *Vstm4*-201 transcript, the transcription is shown below:



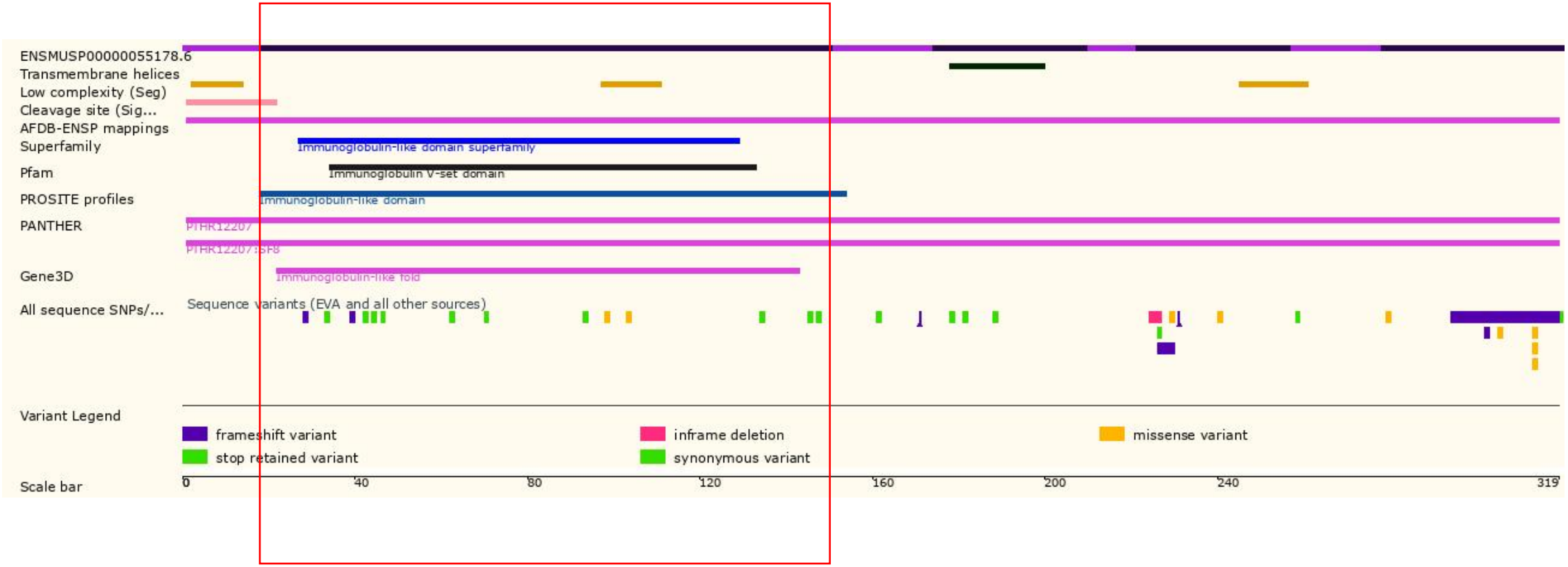
Source: http://asia.ensembl.org/

Genomic Information



Source:http://asia.ensembl.org/

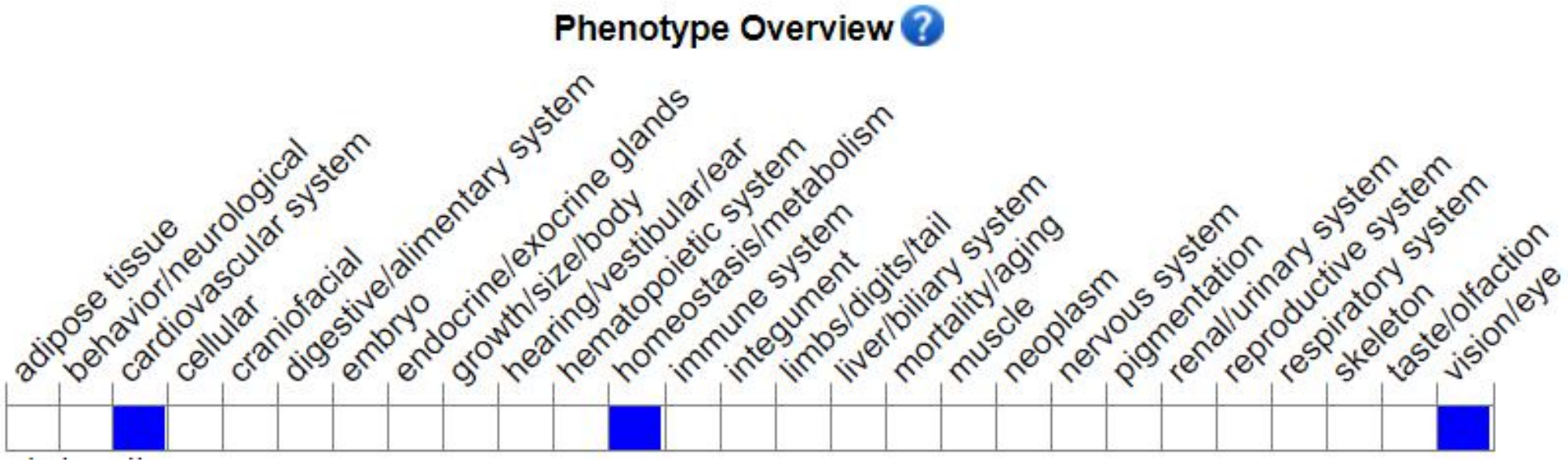
Protein Information



Knockout region

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

Mouse Phenotype Information (MGI)



Source: https://www.informatics.jax.org/marker/MGI:2444633

Important Information

- The deletion of exon 2 did not produce frameshift mutation, and the remaining part would continue to express.
- *Vstm4* is located on Chr 14. 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.
- 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 the existing technology level.

Reference

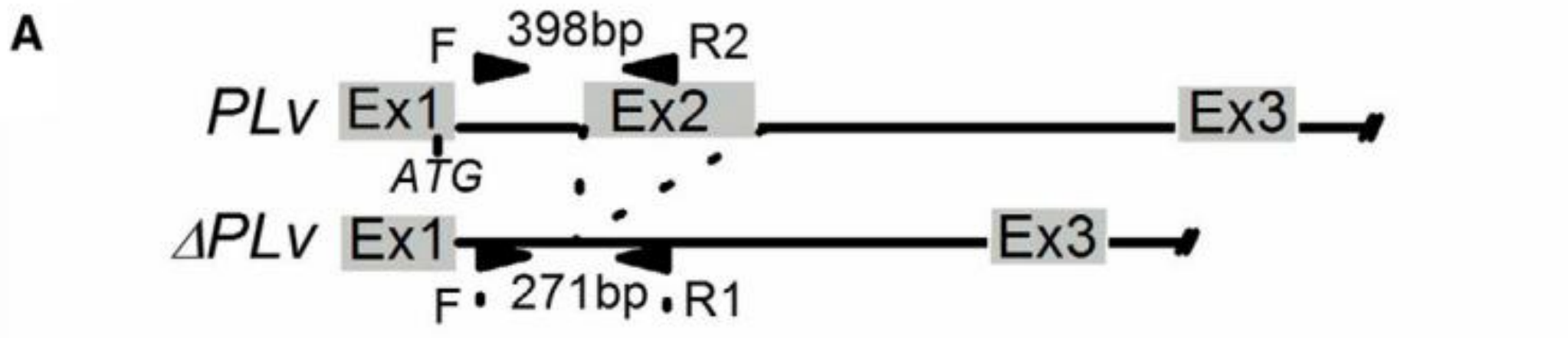


Figure 6. Genetic deletion of peptide Lv decreases retinal vasculature in aged mice. **A**, The CRISPR-Cas9 genetic editing method was used to genetically delete the peptide Lv precursor gene (*Vstm4*). The single-guide RNAs were designed to target the second exon (Ex2) of *Vstm4*. The polymerase chain reaction fragment was sequenced to confirm the successful deletion of exon 2. Western blotting was used to detect the expression of peptide Lv precursor in spleen samples from wild-type (WT), $PLv^{+/-}$ (heterozygeous; +/−), and $PLv^{-/-}$ (null; −/−) littermates. **B**,

[1] Shi L, Zhao M, Abbey C A, et al. Newly identified peptide, peptide Lv, promotes pathological angiogenesis[J]. Journal of the American Heart Association, 2019, 8(22): e013673.