Ager Cas9-CKO Strategy

Designer:Xueting Zhang
Reviewer:Yanhua Shen
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Project Overview

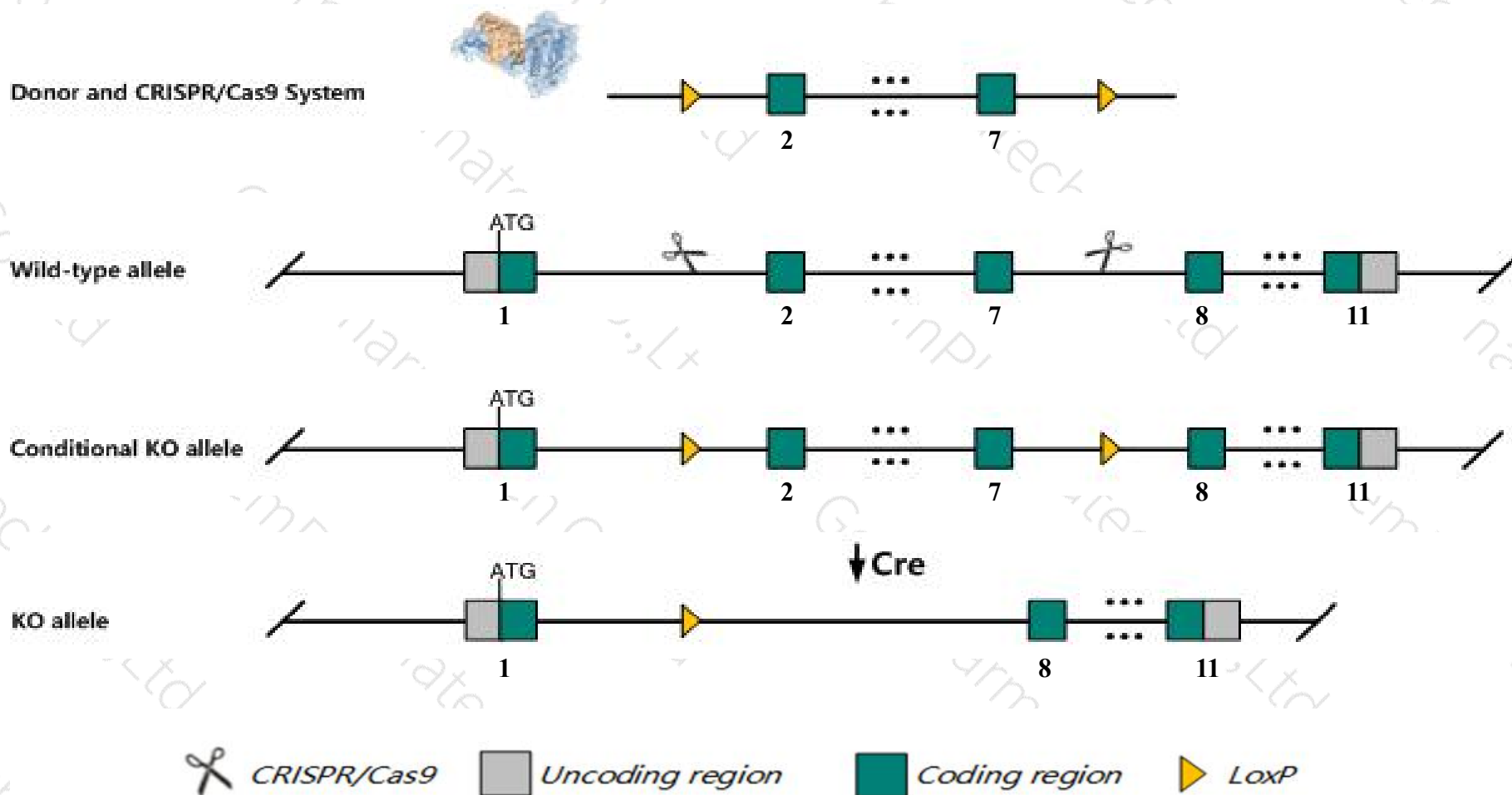|	
Project Name	*Ager*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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

Technical routes

➤ The *Ager* gene has 13 transcripts. According to the structure of *Ager* gene, exon2-exon7 of *Ager-201* (ENSMUST00000015596.9) transcript is recommended as the knockout region.The region contains Ens 764bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Ager* 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.

➢ According to the existing MGI data,Homozygotes for a null allele show increased bone mass and strength, reduced osteoclast number, abnormal blood vessel healing, and altered development of nephropathy and pain perception in induced diabetes. Homozygotes for another null allele show restored diabetes-induced angiogenic responses.

➢ The floxed region is near to the N-terminal of *Gm20463* gene and *Agpat1* gene,this strategy may influence the regulatory function of the N-terminal of these genes.

➢ The floxed region is near to the C-terminal of *Rnf5* gene and *Pbx2* gene,this strategy may influence the regulatory function of the C-terminal of these genes.

➢ This strategy may destroy directly the transcript *Ager*-203&204&205&206&211&212.

➢ The *Ager* 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）

Ager advanced glycosylation end product-specific receptor [*Mus musculus* (house mouse)]

Gene ID: 11596, updated on 29-Oct-2019

▲ Summary

Official Symbol	Ager provided by MGI
Official Full Name	advanced glycosylation end product-specific receptor provided by MGI
Primary source	MGI:MGI:893592
See related	Ensembl:ENSMUSG00000015452
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	RAGE
Expression	Restricted expression toward lung adult (RPKM 481.7) See more
Orthologs	human all

▲ Genomic context

**Location:**   17; 17 B1

See Ager in Genome Data Viewer

Exon count: 10

| Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	17	NC_000083.6 (34597460..34600937)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	17	NC_000083.5 (34734807..34737877)

Transcript information（Ensembl）

The gene has 13 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ager-201	ENSMUST00000015596.9	1370	402aa	Protein coding	CCDS28649	Q62151	TSL:1 GENCODE basic APPRIS P3
Ager-209	ENSMUST00000174496.8	1347	393aa	Protein coding	CCDS70795	Q62151	TSL:1 GENCODE basic APPRIS ALT2
Ager-207	ENSMUST00000173992.7	1248	384aa	Protein coding	CCDS70796	Q62151	TSL:1 GENCODE basic
Ager-208	ENSMUST00000174069.7	1301	333aa	Nonsense mediated decay	-	Q62151	TSL:1
Ager-203	ENSMUST00000172757.7	1876	No protein	Retained intron	-	-	TSL:2
Ager-205	ENSMUST00000173551.7	1787	No protein	Retained intron	-	-	TSL:2
Ager-211	ENSMUST00000174640.7	1663	No protein	Retained intron	-	-	TSL:2
Ager-210	ENSMUST00000174554.7	1625	No protein	Retained intron	-	-	TSL:2
Ager-204	ENSMUST00000172932.1	1470	No protein	Retained intron	-	-	TSL:1
Ager-202	ENSMUST00000170161.7	1468	No protein	Retained intron	-	-	TSL:1
Ager-206	ENSMUST00000173589.7	1461	No protein	Retained intron	-	-	TSL:1
Ager-212	ENSMUST00000184805.7	1400	No protein	Retained intron	-	-	TSL:1
Ager-213	ENSMUST00000184846.7	1252	No protein	lncRNA	-	-	TSL:5

The strategy is based on the design of *Ager-201* transcript,The transcription is shown below
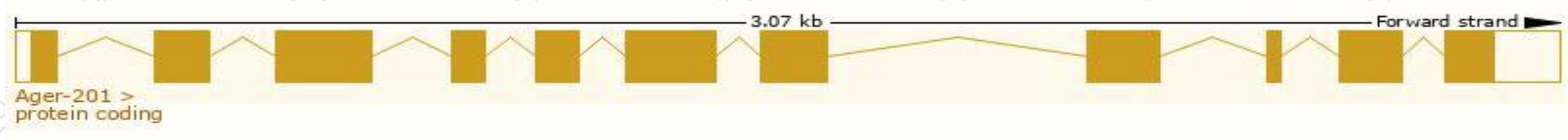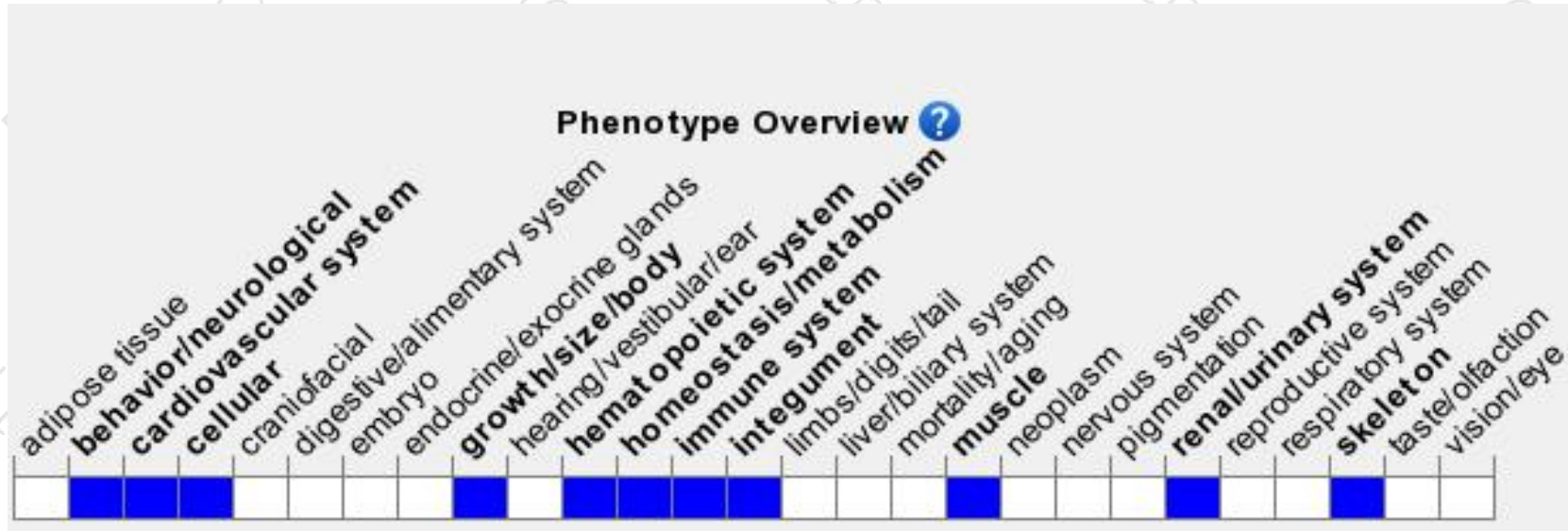
Genomic location distribution
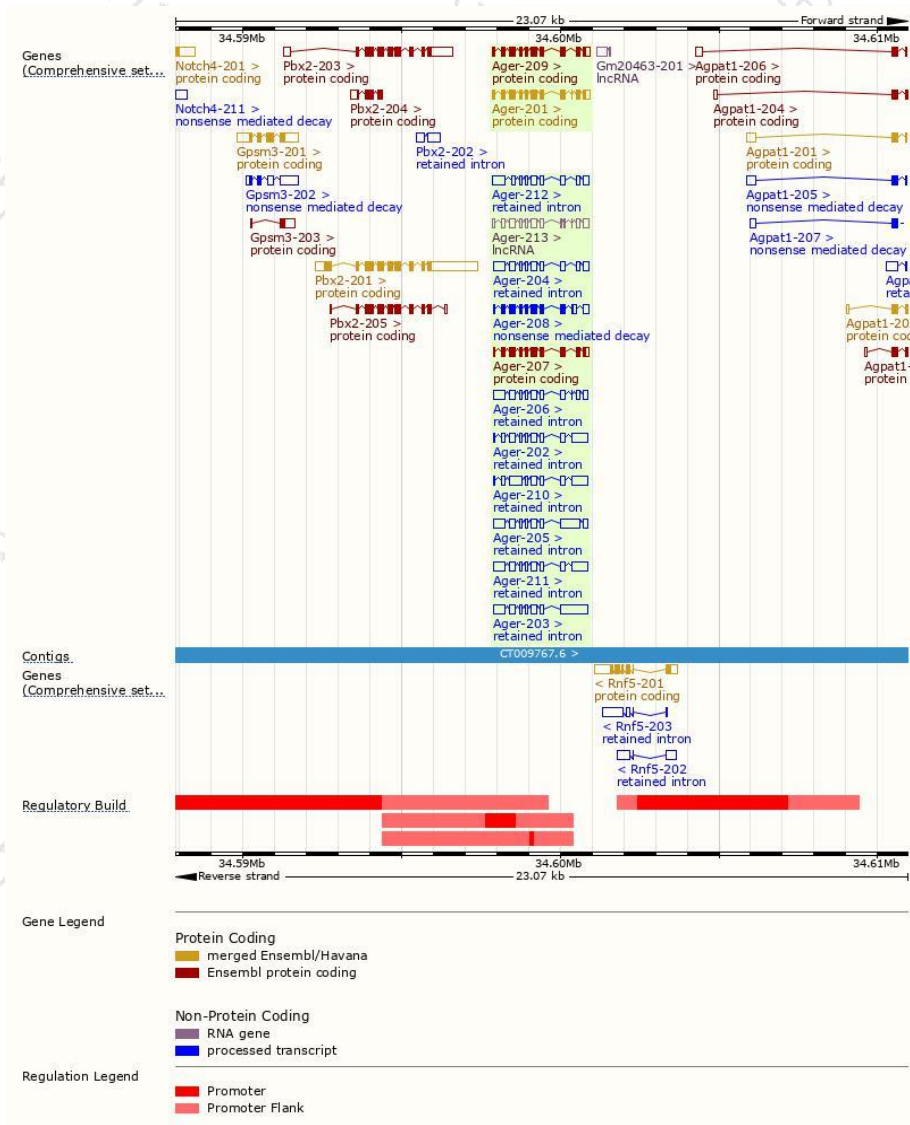
Protein domain
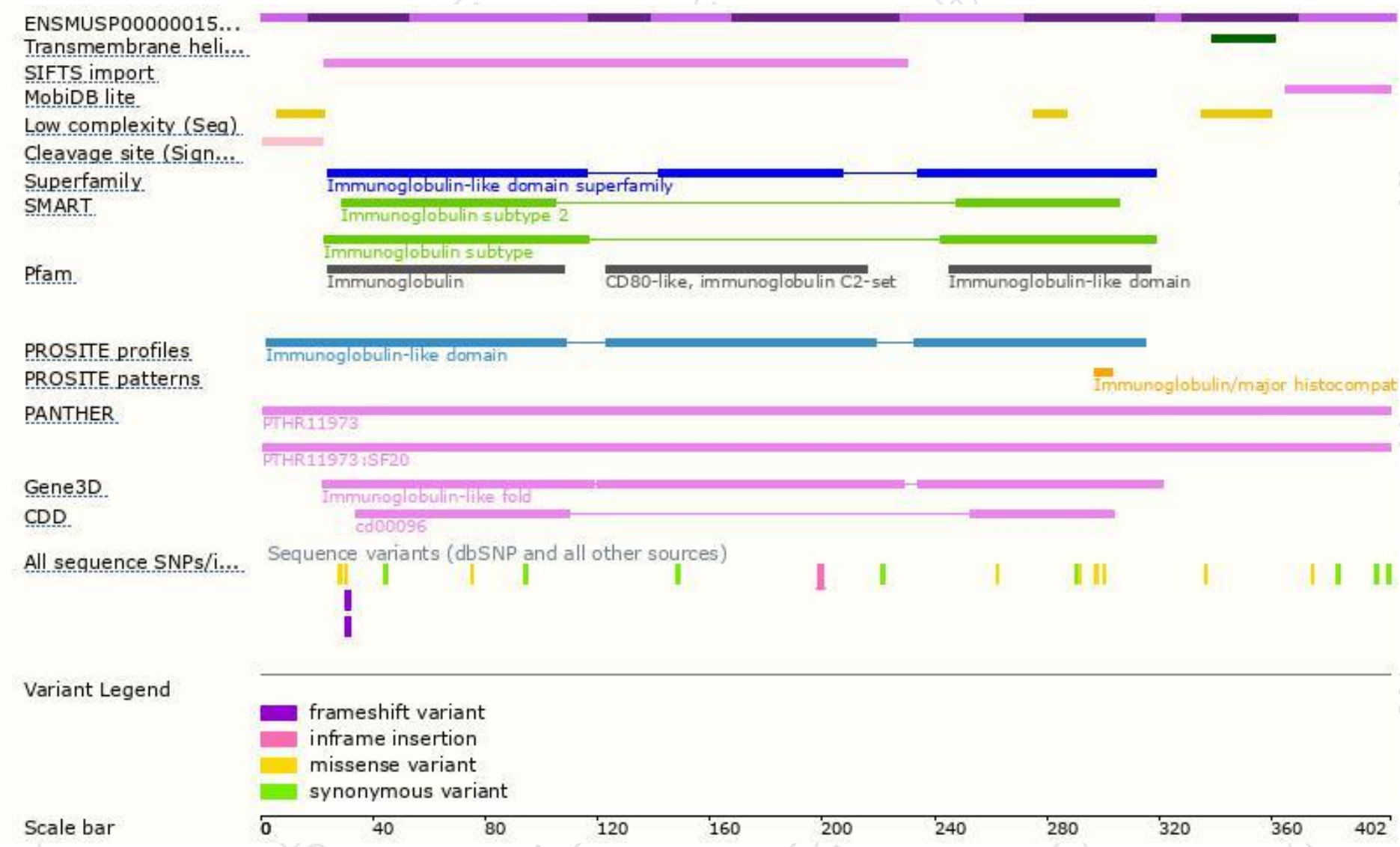
Mouse phenotype description(MGI)

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,Homozygotes for a null allele show increased bone mass and strength, reduced osteoclast number, abnormal blood vessel healing, and altered development of nephropathy and pain perception in induced diabetes. Homozygotes for another null allele show restored diabetes-induced angiogenic responses.

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

集萃药康生物科技
GemPharmatech Co.,Ltd