# *Gzma* Cas9-KO Strategy

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**Reviewer: Huimin Su**

**Design Date: 2020-7-29**

# Project Overview

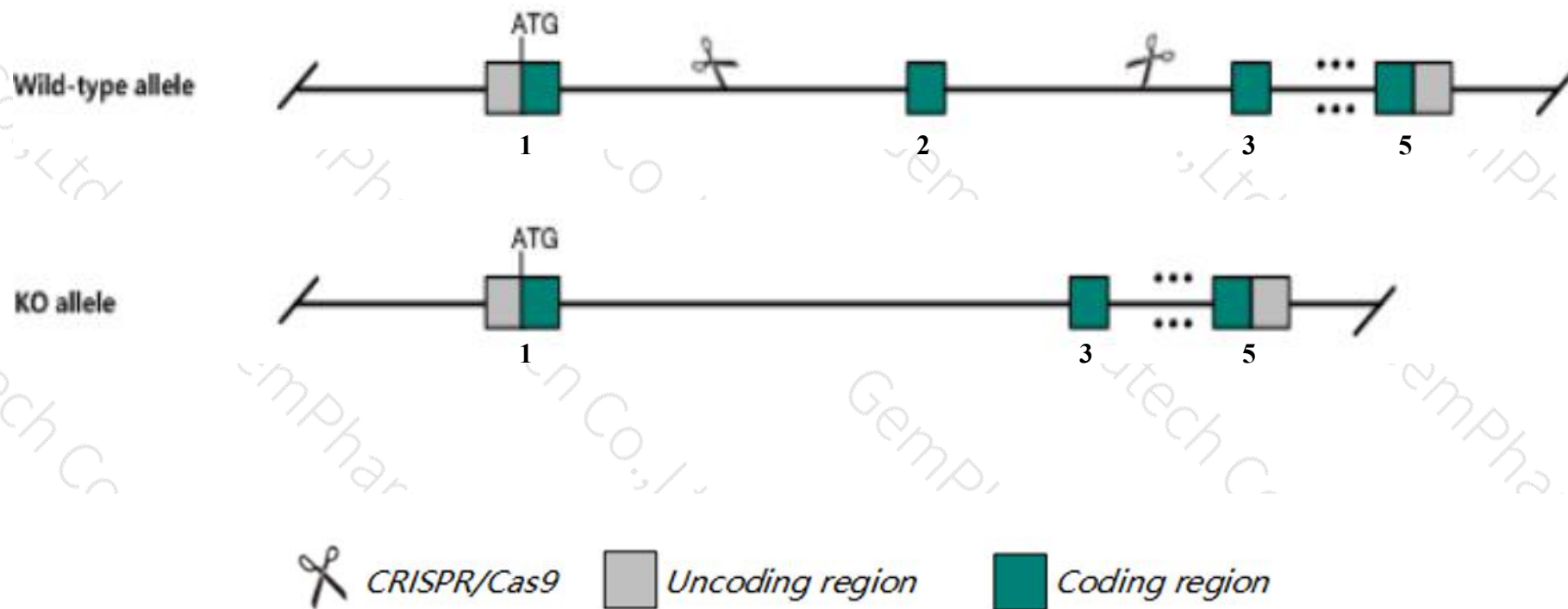| | |
|---|---|
| **Project Name** | *Gzma* |
| **Project type** | **Cas9-KO** |
| **Strain background** | **C57BL/6JGpt** |

# Knockout strategy

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

➢ The *Gzma* gene has 2 transcripts. According to the structure of *Gzma* gene, exon2 of *Gzma-201*(ENSMUST00000023897.6) transcript is recommended as the knockout region. The region contains 145bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Gzma* 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.

➢ According to the existing MGI data,homozygotes for a null allele show normal T/NK cell-mediated cytotoxicity, recovery from LCM virus or L. monocytogenes infection, and control of syngeneic tumor growth. Homozygotes for a different null allele show defective CTL cytolysis and increased tumor burden upon challenge with RMAS cells.

➢The KO region contains functional region of the *Cdc20b* gene.Knockout the region may affect the function of *Cdc20b* gene.

➢ The *Gzma* gene is located on the Chr13. 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）

## Gzma granzyme A [Mus musculus (house mouse)]

Gene ID: 14938, updated on 13-Mar-2020

### ▲ Summary

| | |
|---|---|
| **Official Symbol** | Gzma provided by MGI |
| **Official Full Name** | granzyme A provided by MGI |
| **Primary source** | MGI:MGI:109266 |
| **See related** | Ensembl:ENSMUSG00000023132 |
| **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** | AW494114, Ctla-3, Ctla3, Hf, Hf1, SE1, TSP-1, TSP1 |
| **Expression** | Biased expression in large intestine adult (RPKM 10.7), thymus adult (RPKM 4.7) and 9 other tissuesSee more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

The gene has 2 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|------|--------------|-----|---------|---------|------|---------|-------|
| Gzma-201 | ENSMUST00000023897.6 | 878 | 260aa | Protein coding | CCDS26782 | P11032 Q3U0N0 | TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2 |
| Gzma-202 | ENSMUST00000224282.1 | 996 | 257aa | Protein coding | - | P11032 | GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2 |

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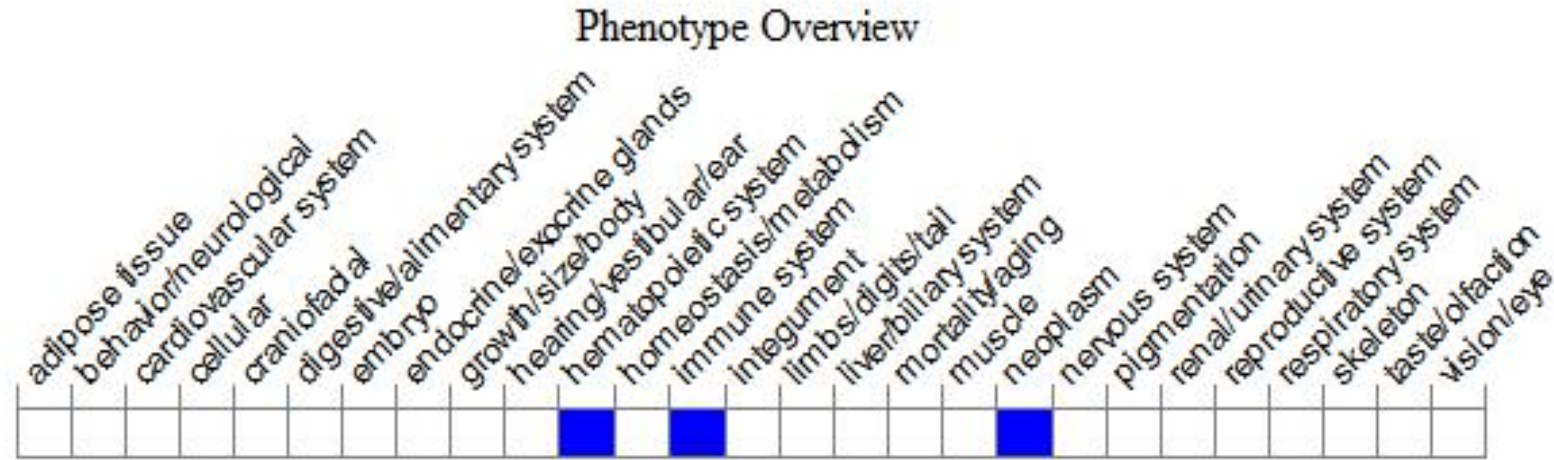


< Gzma-201
protein coding

Reverse strand

7.16 kb
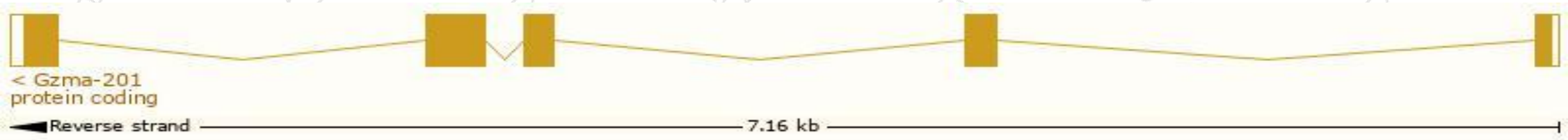
# Genomic location distribution
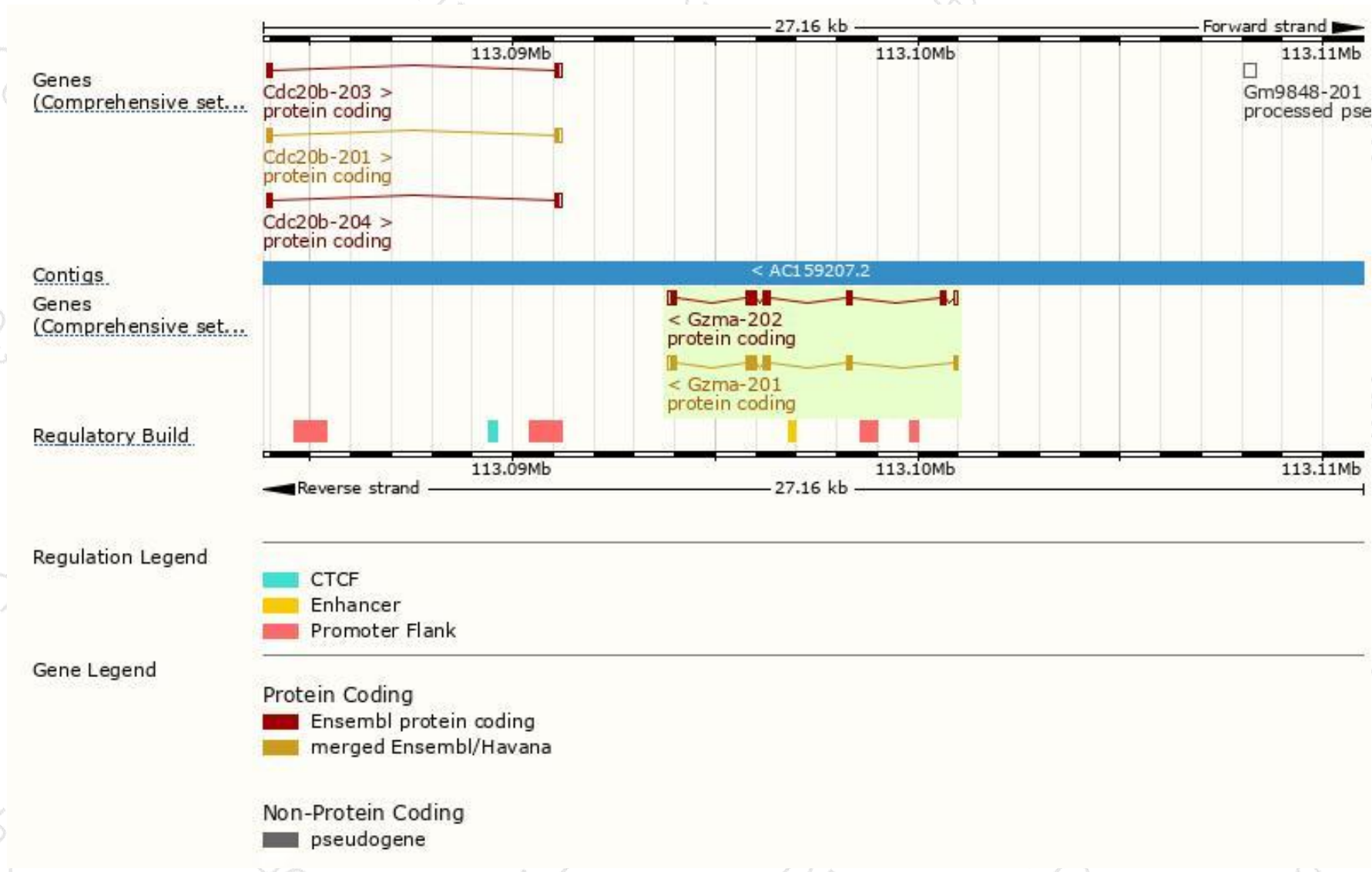
# Protein domain
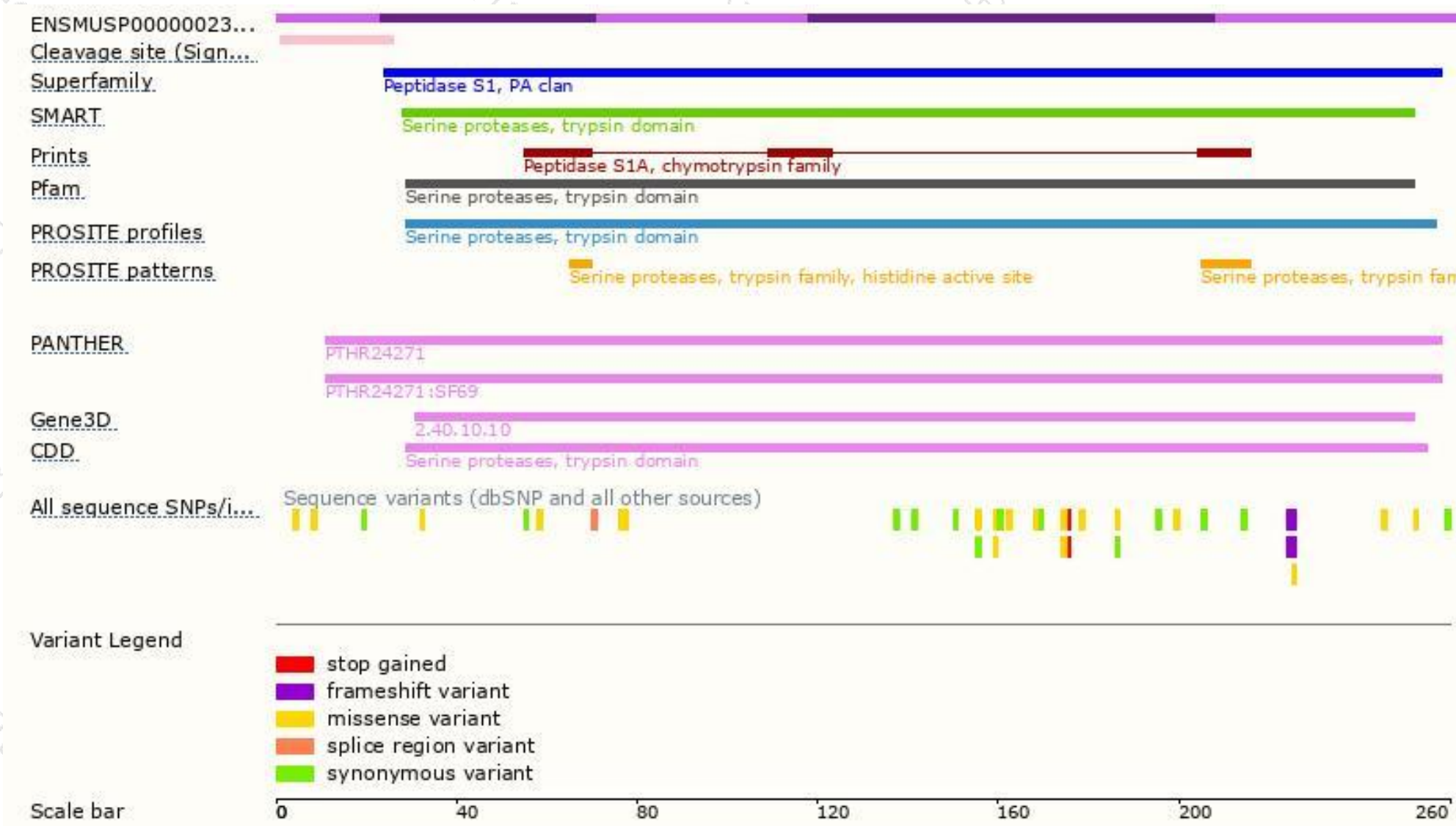
# Mouse phenotype description(MGI )

Phenotype Overview



*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 normal T/NK cell-mediated cytotoxicity, recovery from LCM virus or L. monocytogenes infection, and control of syngeneic tumor growth. Homozygotes for a different null allele show defective CTL cytolysis and increased tumor burden upon challenge with RMAS cells.