Rab32-C222S&C223S Mouse Model Strategy

-CRISPR/Cas9 technology

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Project Overview

Project Name	**Rab32-C222S&C223S**
Project type	**cas9-ki(LSL)**
Strain background	**C57BL/6JGpt**

Technical Description

- The mouse *Rab32* gene has 2 transcripts.
- This project produced *Rab32*-C222S&C223S point mutation on exon 3 of the transcript of *Rab32*-201(ENSMUST00000019974.4). The 222th and 223th amino acid will be mutated from C to S, and the corresponding nuclearinic acid will be mutated to AGC from the TGC.This model expresses wild-type *Rab32* gene before breed *Cre*, and after matching with tissue or cell-specific *Cre* tool mice, it will theoretically express the mutant type in the corresponding tissue or cell.
- In this project, *Rab32* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

Mutation Site

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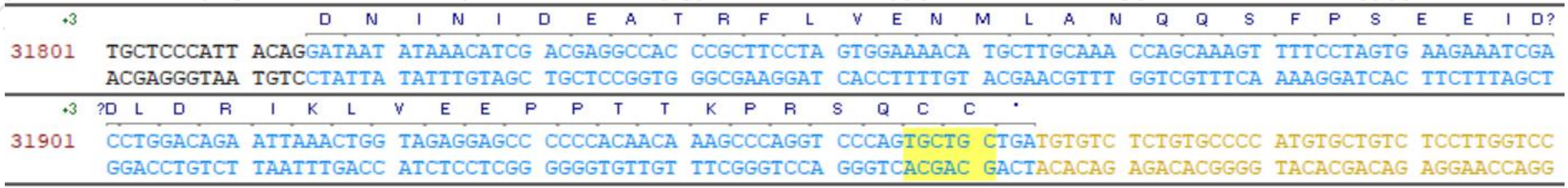
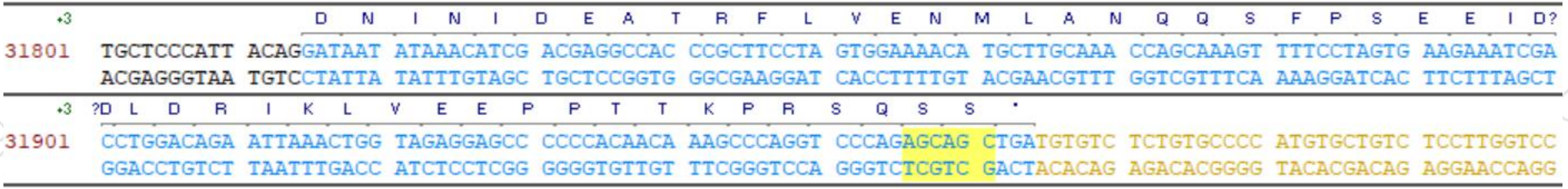
Before mutation



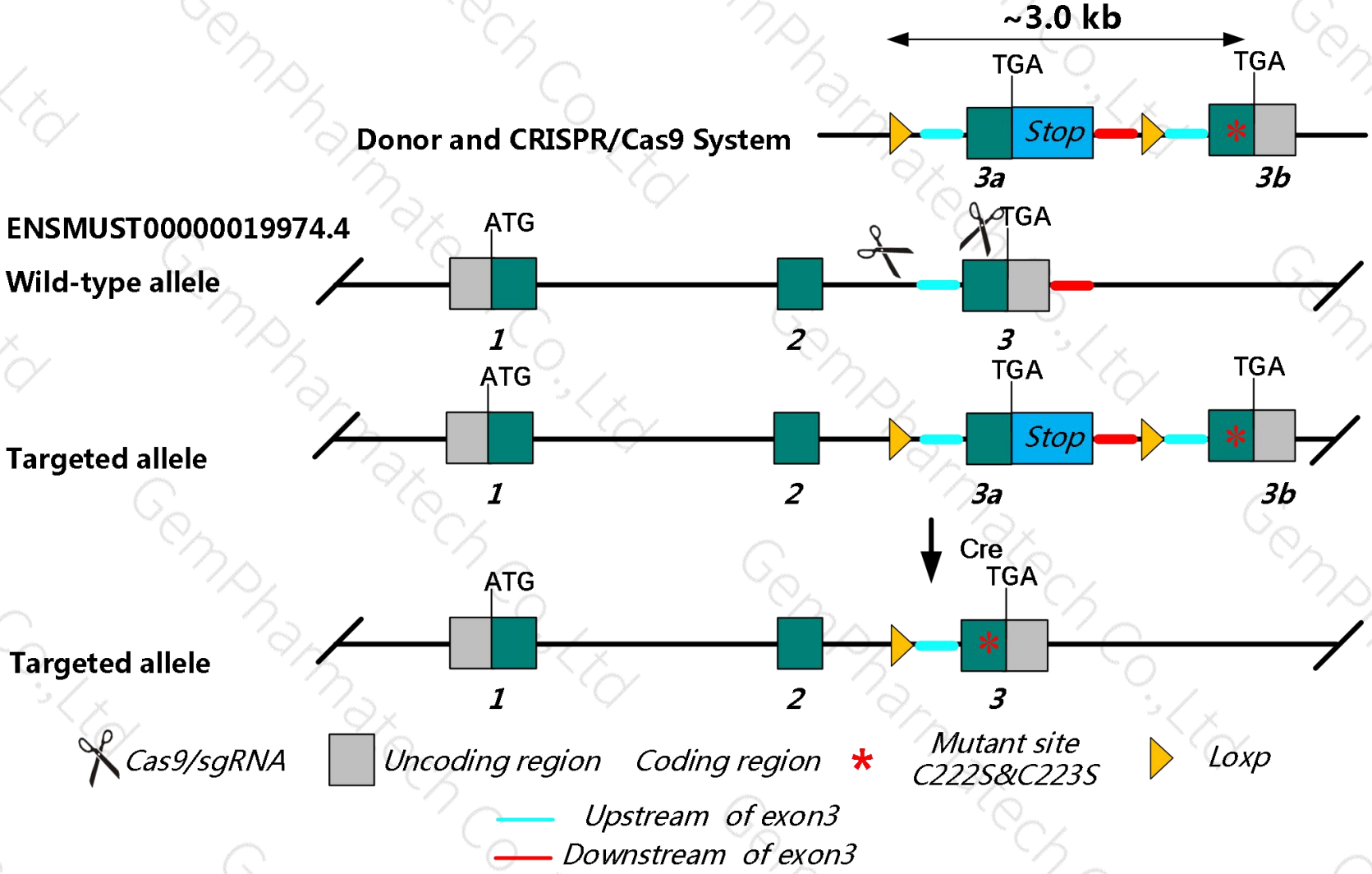
After mutation



The blue region is exon 3 of *Rab32-201,* and the yellow region represents the C222S&C223S mutation site.

Strategy

This model uses CRISPR/Cas9 technology to edit the *Rab32* gene and the schematic diagram is as follow:



Notice

- According to the existing MGI data, homozygous dendritic cell-specific conditional knockout results in increased pathogen load in liver and spleen after bacterial infection. It also increases susceptibility to, and morbidity and mortality of, DSS-induced colitis.
- One or two synonymous mutations of amino acids will be intronduced on exon3 of *Rab32*.
- Mouse *Rab32* gene is located on Chr10. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr10, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene name and location (NCBI)

Rab32 RAB32, member RAS oncogene family [*Mus musculus* (house mouse)]

Gene ID: 67844, updated on 17-Nov-2020

Summary

Official Symbol	Rab32 provided by MGI
Official Full Name	RAB32, member RAS oncogene family provided by MGI
Primary source	MGI:MGI:1915094
See related	Ensembl:ENSMUSG00000019832
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AU022057; 2810011A17Rik
Expression	Ubiquitous expression in subcutaneous fat pad adult (RPKM 13.2), liver adult (RPKM 10.4) and 22 other tissues See more
Orthologs	human all

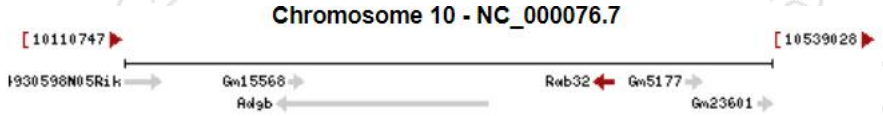
Genomic context

Location: 10; 10 A1

See Rab32 in Genome Data Viewer

Exon count: 3

Annotation release	Status	Assembly	Chr	Location
109	current	GRCm39 (GCF_000001635.27)	10	NC_000076.7 (10420783..10433951, complement)
108.20200622	previous assembly	GRCm38.p6 (GCF_000001635.26)	10	NC_000076.6 (10545039..10558207, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	10	NC_000076.5 (10264837..10278005, complement)

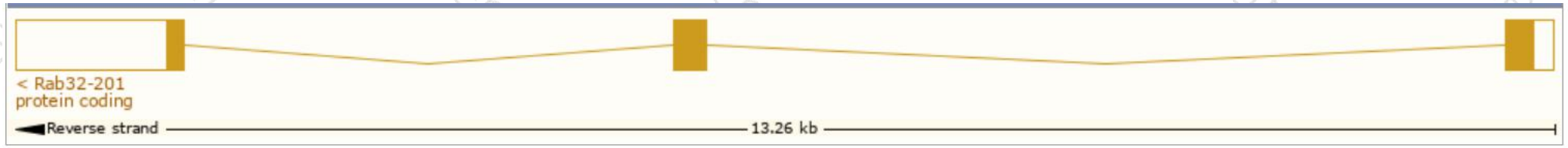


Transcript information (Ensembl)

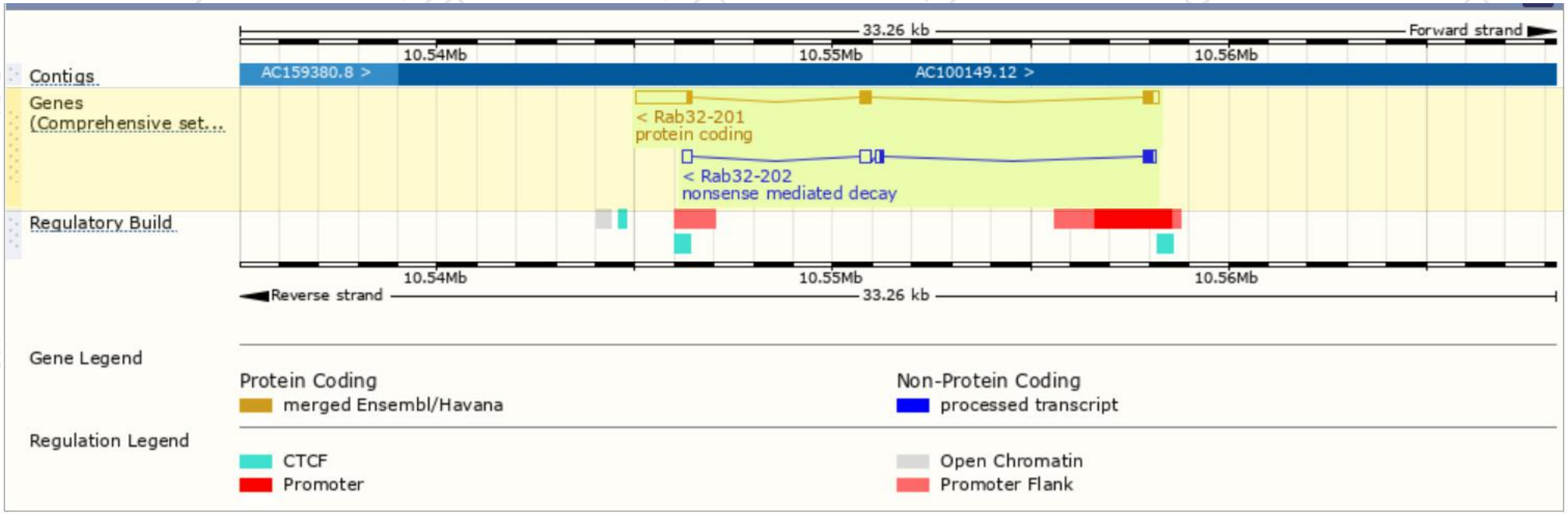
The gene has 2 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Rab32-202	ENSMUST00000220018.2	1062	100aa	Nonsense mediated decay	-	A0A1Y7VIZ0	TSL:5
Rab32-201	ENSMUST00000019974.4	2149	223aa	Protein coding	CCDS23695	Q0PD23 Q9CZE3	TSL:1 GENCODE basic APPRIS P1

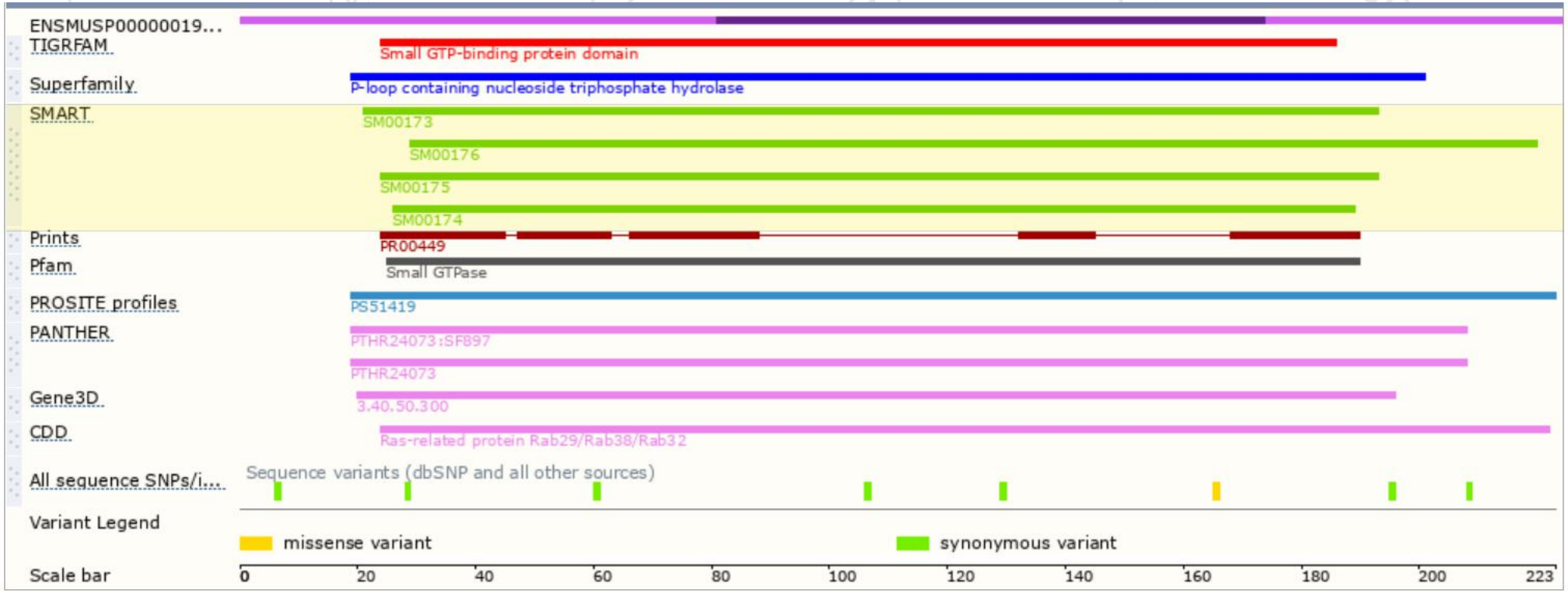
The strategy is based on the design of *Rab32-201* transcript, the transcription is shown below:



Genomic location distribution

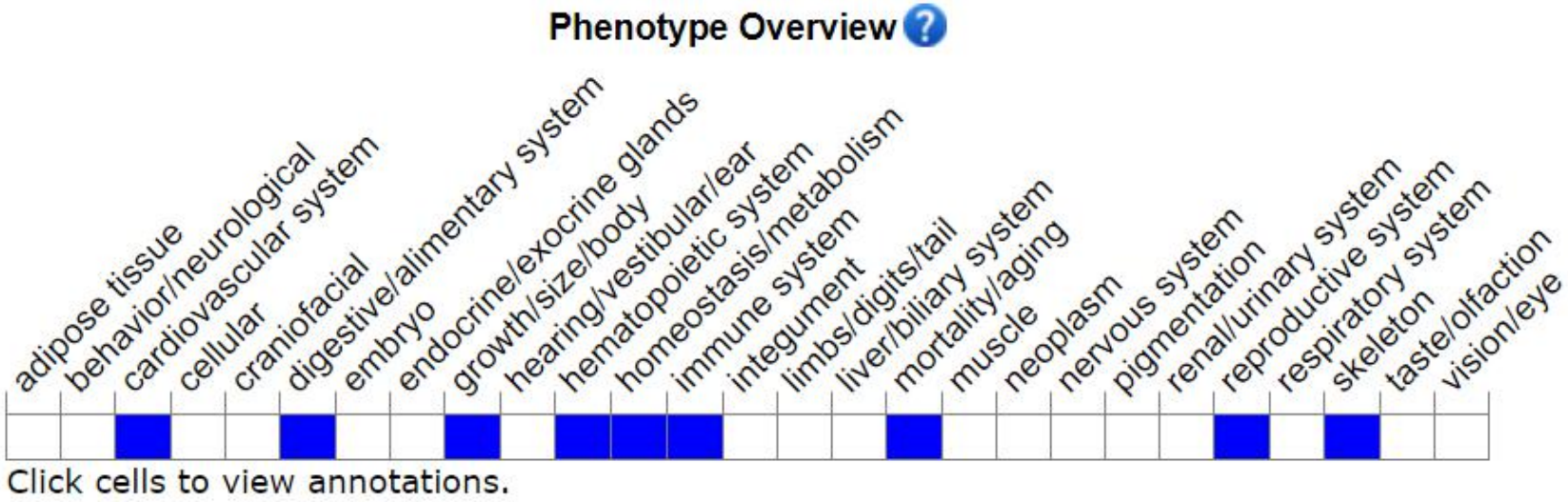


Protein domain



Mouse phenotype description(MGI)

URL link is as follows:
http://www.informatics.jax.org/marker/MGI:1915094



According to the existing MGI data, homozygous dendritic cell-specific conditional knockout results in increased pathogen load in liver and spleen after bacterial infection. It also increases susceptibility to, and morbidity and mortality of, DSS-induced colitis.

If you have any questions, please feel free to contact us.
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