Rims2 Cas9-CKO Strategy

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Project Overview

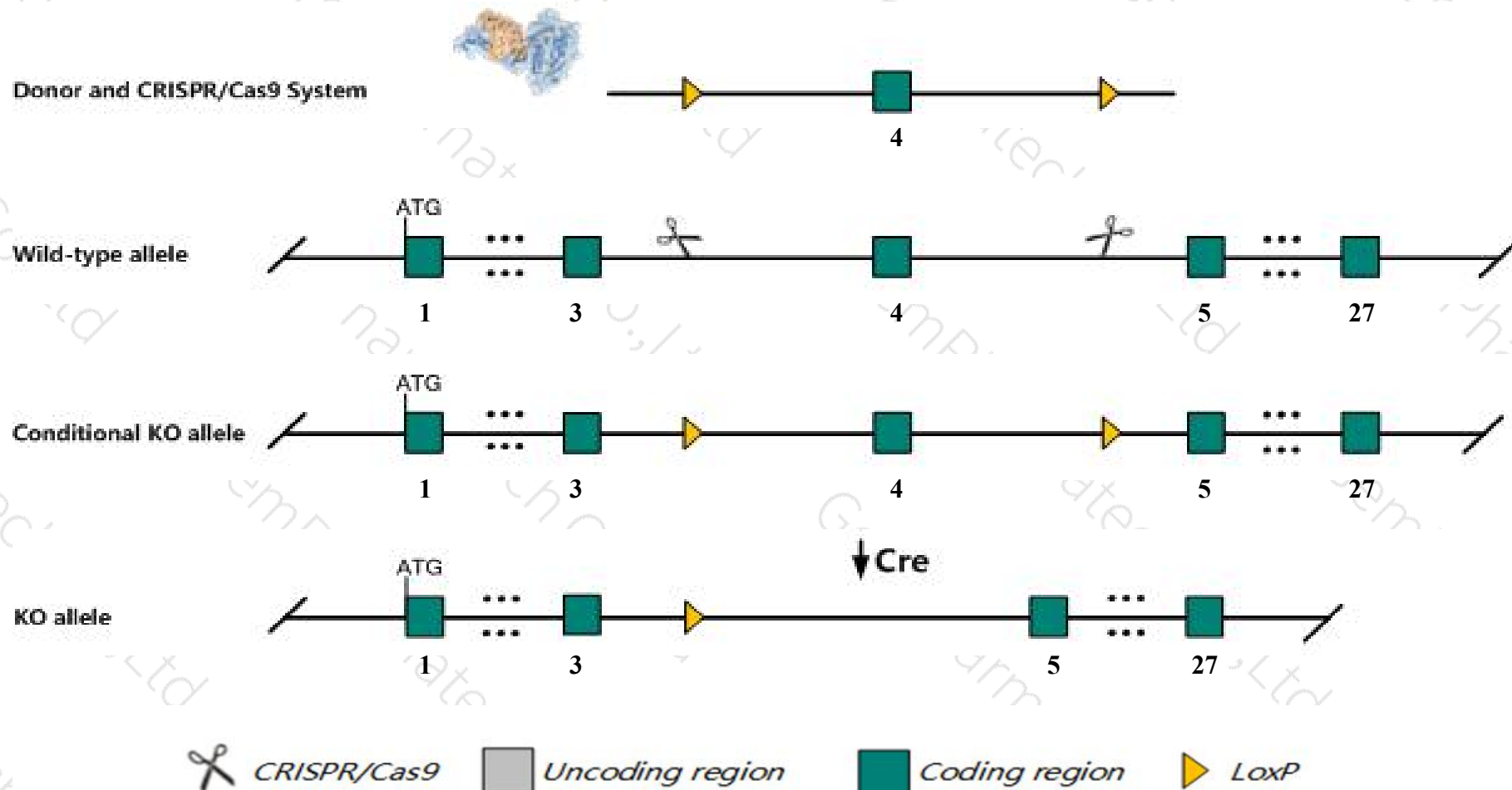|	
Project Name	*Rims2*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rims2* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

4

Wild-type allele

ATG

1   3   4   5   27

Conditional KO allele

ATG

1   3   4   5   27

Cre

KO allele

ATG

1   3   5   27

CRISPR/Cas9   Uncoding region   Coding region   LoxP

Technical routes

➢ The *Rims2* gene has 12 transcripts. According to the structure of *Rims2* gene, exon4 of *Rims2-201*(ENSMUST00000042917.9) transcript is recommended as the knockout region.The region contains Ens 926bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rims2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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,mice homozygous for a knock-out allele show reduced body size, aberrant insulin granule exocytosis, and impaired secretion of hormones associated with glucose homeostasis. Mice homozygous for another knock-out allele show a slightly reduced body size, abnormal maternal behavior and premature death.

➢ Transcript *Rims2*-204 may not be affected.

➢ The *Rims2* gene is located on the Chr15. 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）

Rims2　regulating synaptic membrane exocytosis 2 [*Mus musculus* (house mouse)]

Gene ID: 116838, updated on 26-Jun-2020

Summary

Official Symbol	Rims2 provided by MGI
Official Full Name	regulating synaptic membrane exocytosis 2 provided by MGI
Primary source	MGI:MGI:2152972
See related	Ensembl:ENSMUSG00000037386
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	RIM2; Serg2; Rab3ip2; Syt3-rs; AW048769; Rim2(+4A); mKIAA0751; Rim2(+40A); Rim2(+44A); 2810036I15Rik
Expression	Biased expression in cerebellum adult (RPKM 7.7), cortex adult (RPKM 5.5) and 7 other tissues See more
Orthologs	human all

# Transcript information（Ensembl）

The gene has 12 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rims2-202	ENSMUST00000082054.11	4800	1530aa	Protein coding	CCDS37067	Q9EQZ7	TSL:2 GENCODE basic
Rims2-201	ENSMUST00000042917.9	4719	1572aa	Protein coding	CCDS56982	D9HP81	TSL:1 GENCODE basic APPRIS P2
Rims2-205	ENSMUST00000227243.1	4760	1550aa	Protein coding	-	Q0VF51	GENCODE basic APPRIS ALT1
Rims2-206	ENSMUST00000227381.1	4581	1297aa	Protein coding	-	A0A2I3BRM1	CDS 5' incomplete
Rims2-204	ENSMUST00000226410.1	3685	285aa	Protein coding	-	Q9EQZ7	GENCODE basic
Rims2-211	ENSMUST00000228839.1	1379	352aa	Protein coding	-	A0A2I3BRN6	CDS 3' incomplete
Rims2-210	ENSMUST00000228460.1	1381	No protein	Processed transcript	-	-	
Rims2-212	ENSMUST00000228867.1	803	No protein	Processed transcript	-	-	
Rims2-203	ENSMUST00000226243.1	581	No protein	Processed transcript	-	-	
Rims2-207	ENSMUST00000227469.1	458	No protein	Processed transcript	-	-	
Rims2-209	ENSMUST00000228269.1	299	No protein	Processed transcript	-	-	
Rims2-208	ENSMUST00000227830.1	862	No protein	Retained intron	-	-	

The strategy is based on the design of *Rims2-201* transcript,the transcription is shown below:
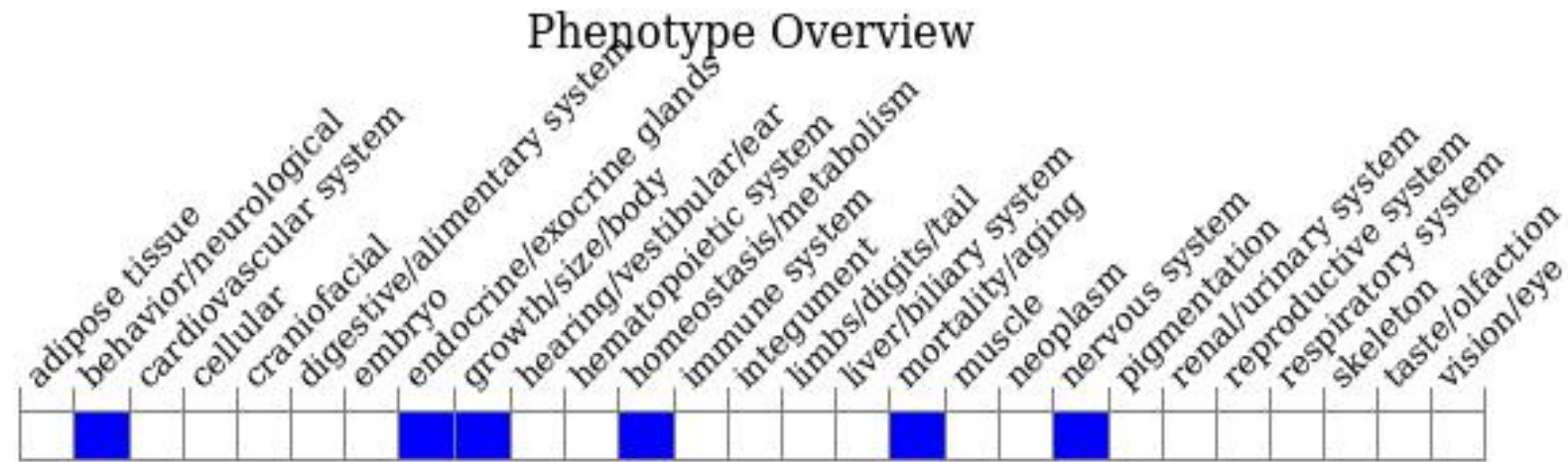

483.40 kb — Forward strand

Rims2-201 >
protein coding
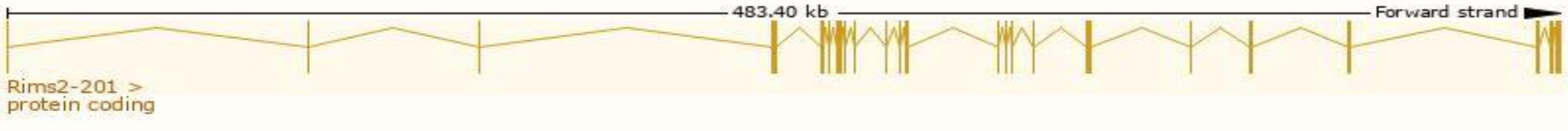
Genomic location distribution
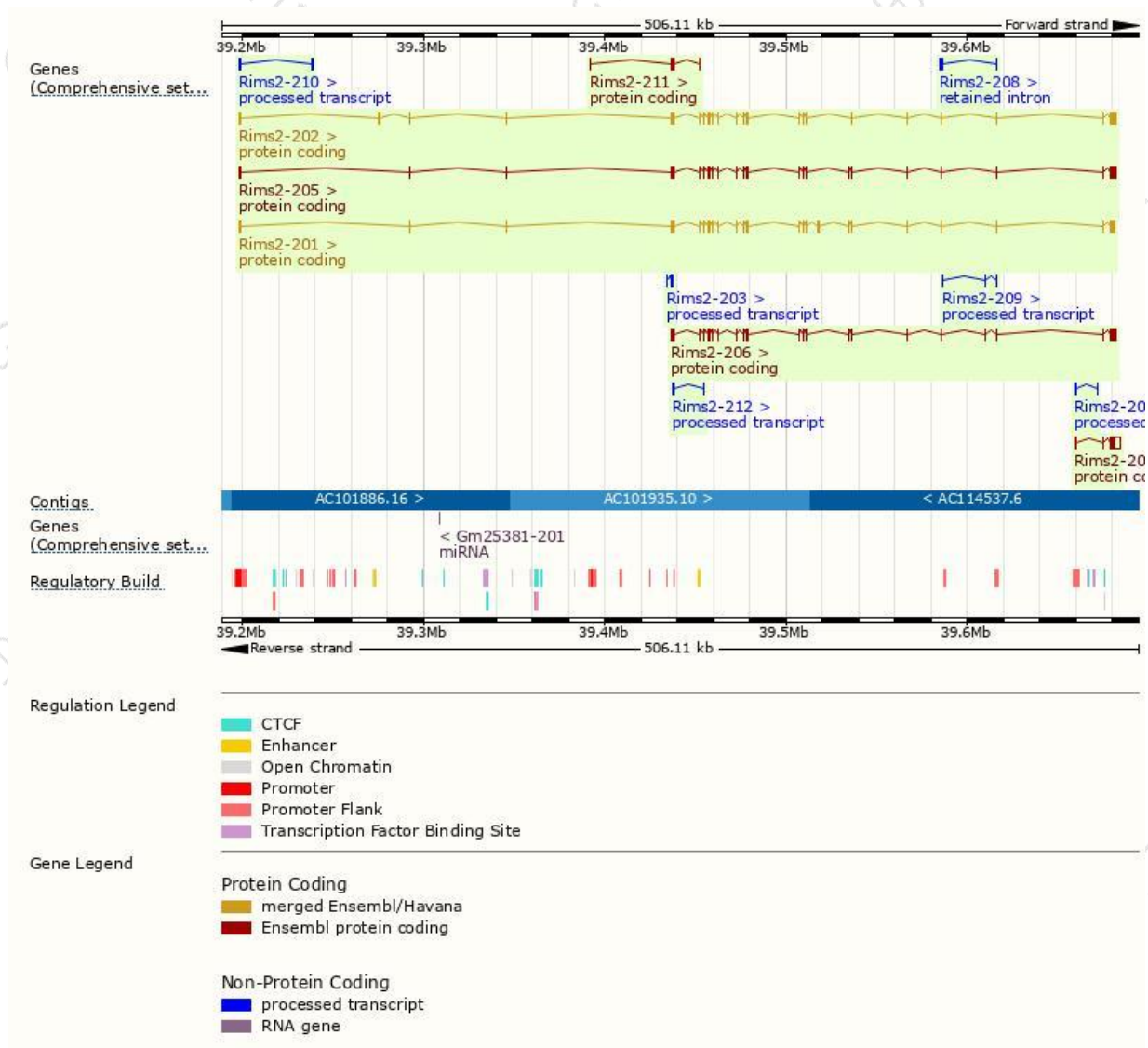
Protein domain
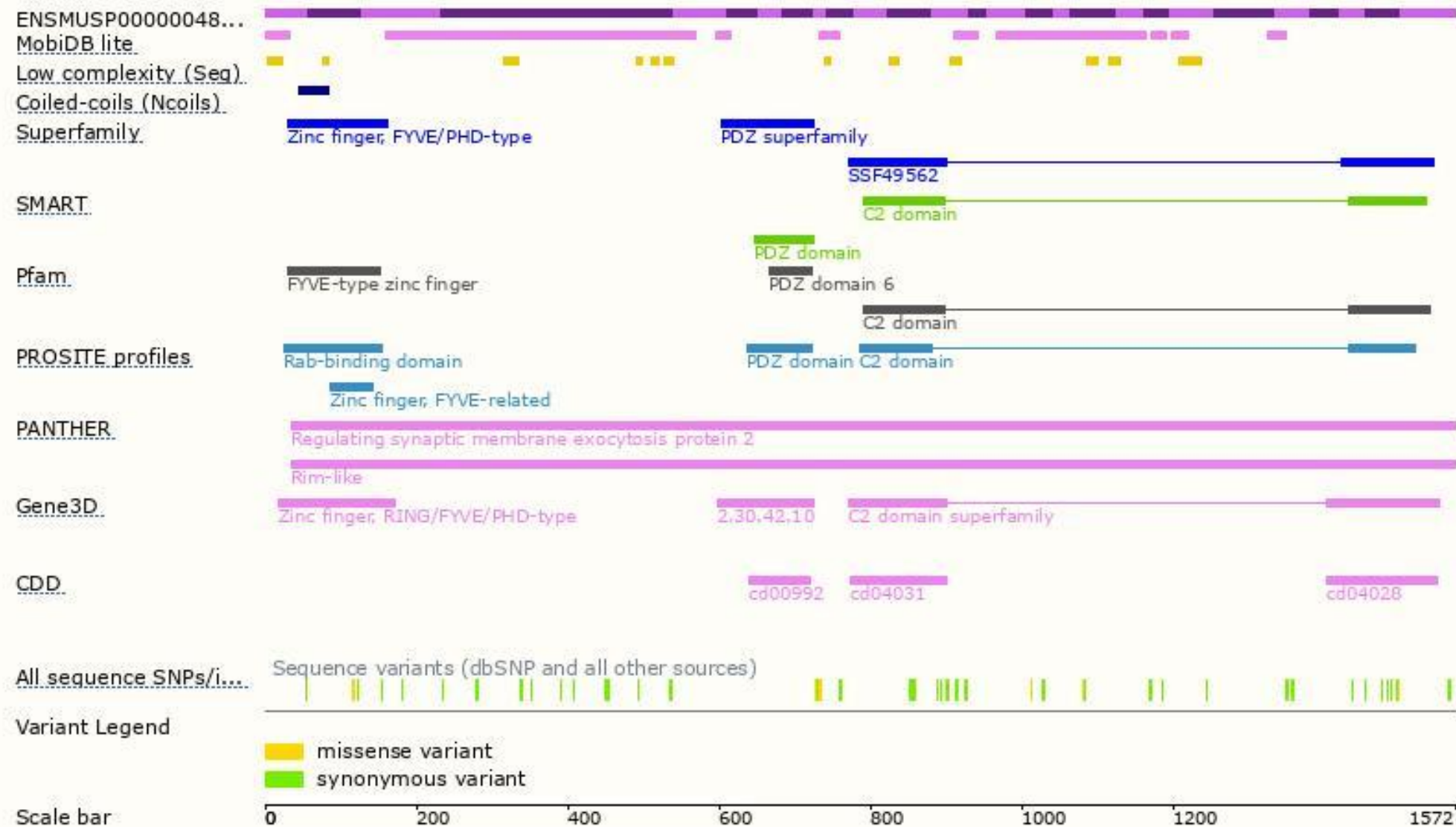
Mouse phenotype description(MGI)

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,mice homozygous for a knock-out allele show reduced body size, aberrant insulin granule exocytosis, and impaired secretion of hormones associated with glucose homeostasis. Mice homozygous for another knock-out allele show a slightly reduced body size, abnormal maternal behavior and premature death.

If you have any questions, you are welcome to inquire.
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