Tor1a Cas9-CKO Strategy

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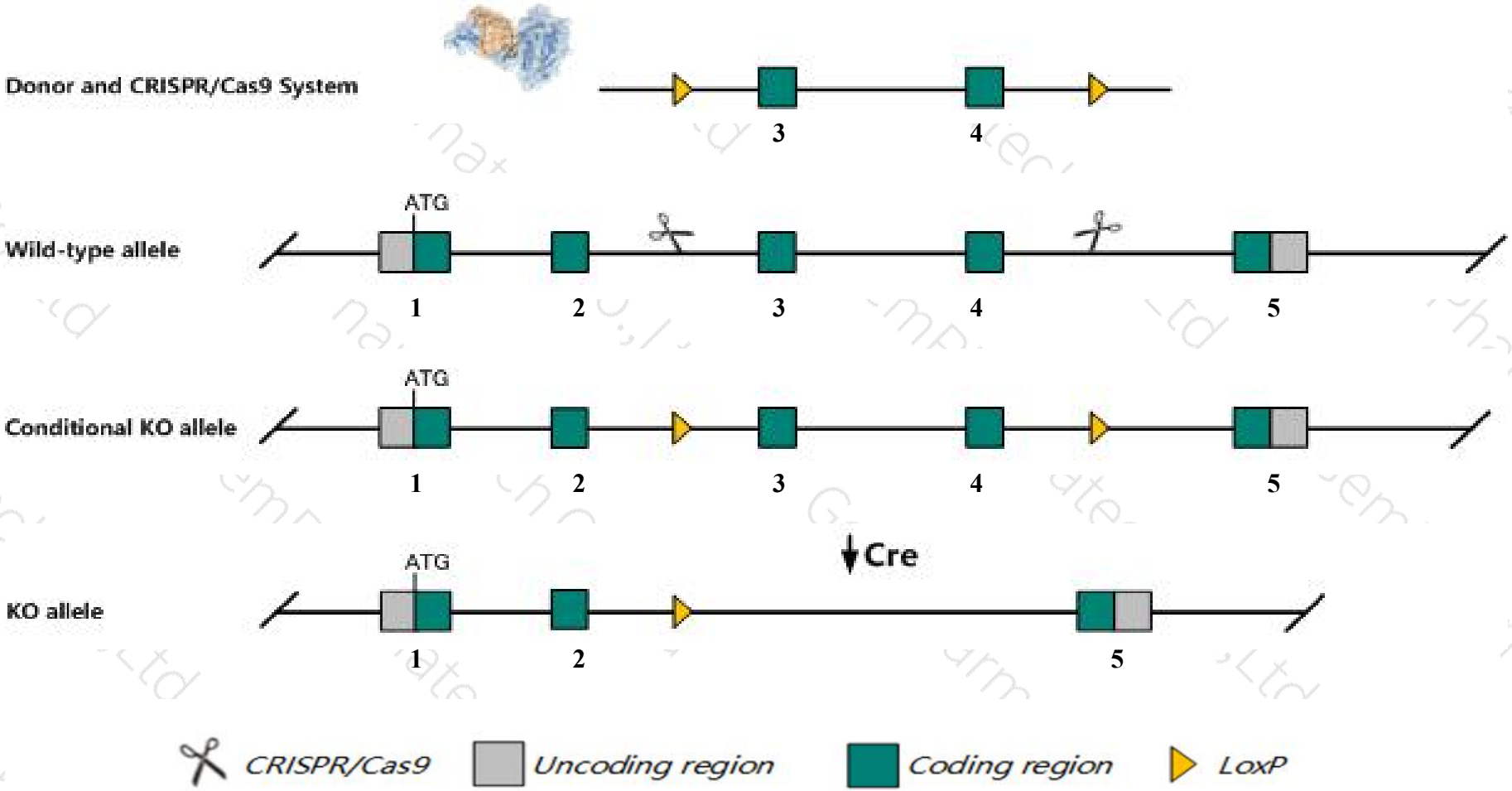
Project Overview

Project Name	***Tor1a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tor1a* gene. The schematic diagram is as follows:



Technical routes

- The *Tor1a* gene has 5 transcripts. According to the structure of *Tor1a* gene, exon3-exon4 of *Tor1a-201* (ENSMUST00000028200.8) transcript is recommended as the knockout region.The region contains 304bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Tor1a* 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.

Notice

➢ According to the existing MGI data,mice homozygous for disruptions in this gene die either embryonically or very soon after birth. heterozygous males display hyperactivity and coordination difficulties.

➢ Transcript *Tor1a*-204 may not be affected.

➢ The N-terminal of *Tor1a* gene will remain several amino acids ,it may remain the partial function of *Tor1a* gene.

➢ The *Tor1a* gene is located on the Chr2. 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）

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Tor1a torsin family 1, member A (torsin A) [Mus musculus (house mouse)]

Gene ID: 30931, updated on 13-Mar-2020

Summary

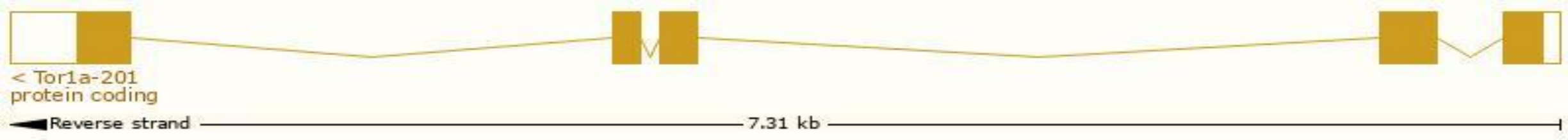
Official Symbol	Tor1a provided by MGI
Official Full Name	torsin family 1, member A (torsin A) provided by MGI
Primary source	MGI:MGI:1353568
See related	Ensembl:ENSMUSG00000026849
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	DQ2, Dyt1, torsinA
Expression	Ubiquitous expression in duodenum adult (RPKM 31.6), ovary adult (RPKM 22.9) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

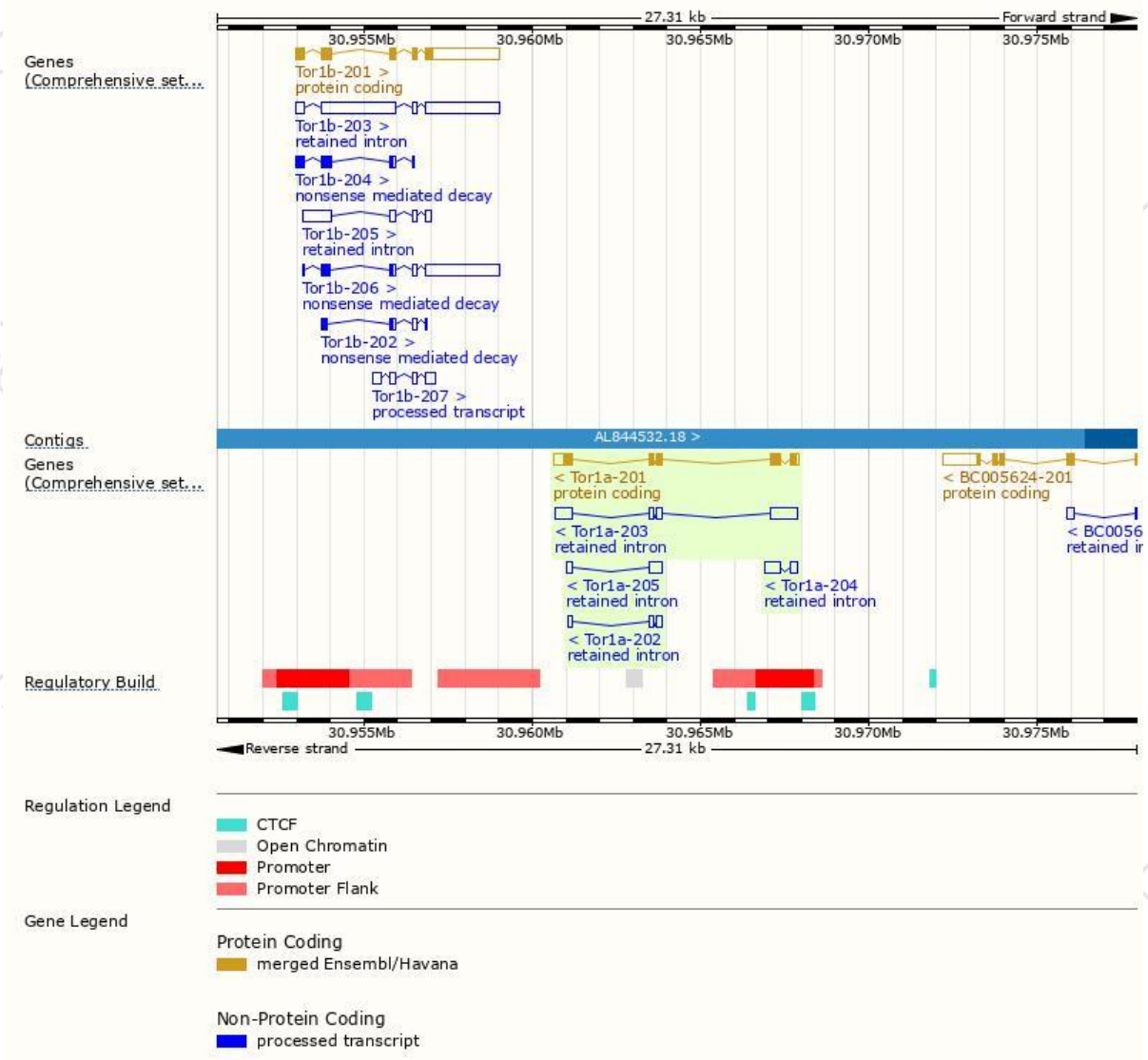
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tor1a-201	ENSMUST00000028200.8	1401	333aa	Protein coding	CCDS15891	Q3TV62 Q9ER39	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 P1
Tor1a-203	ENSMUST00000137694.7	1605	No protein	Retained intron	-	-	TSL:1
Tor1a-204	ENSMUST00000143199.1	660	No protein	Retained intron	-	-	TSL:2
Tor1a-205	ENSMUST00000144152.1	560	No protein	Retained intron	-	-	TSL:2
Tor1a-202	ENSMUST00000123762.1	455	No protein	Retained intron	-	-	TSL:2

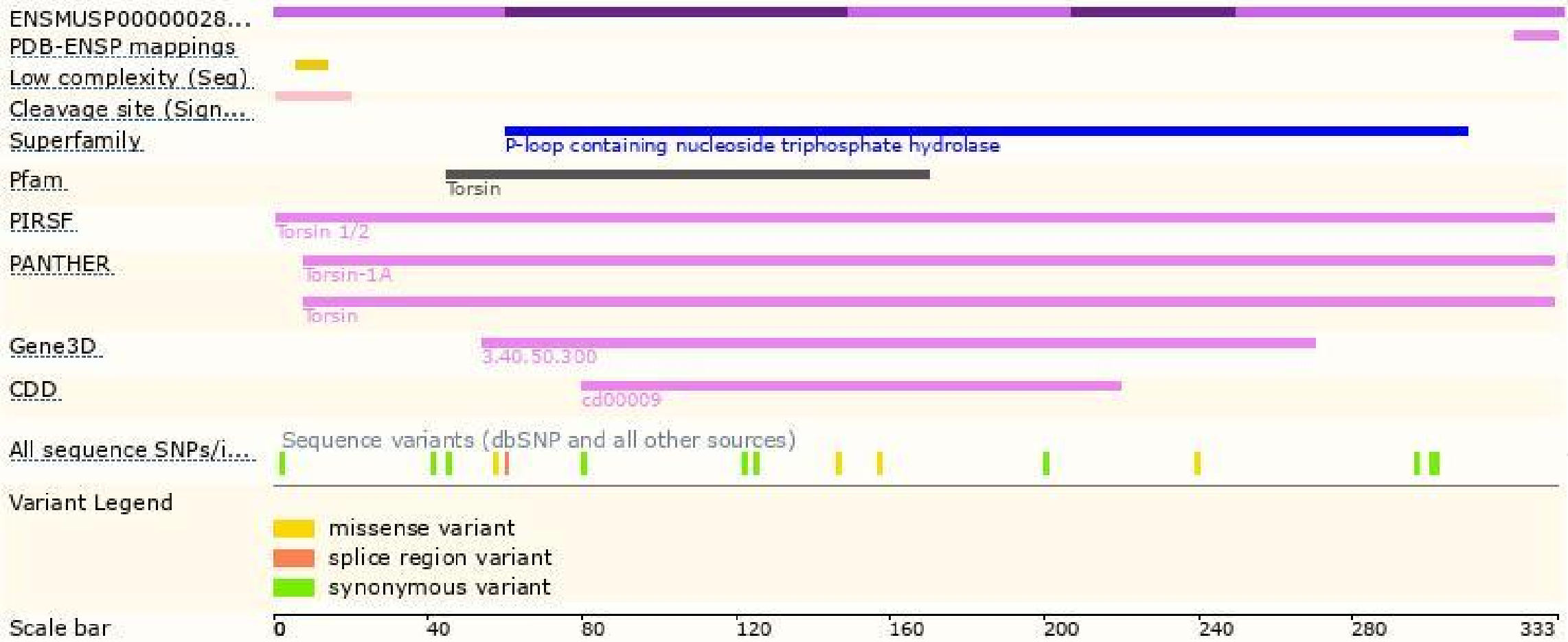
The strategy is based on the design of *Tor1a-201* transcript,the transcription is shown below:



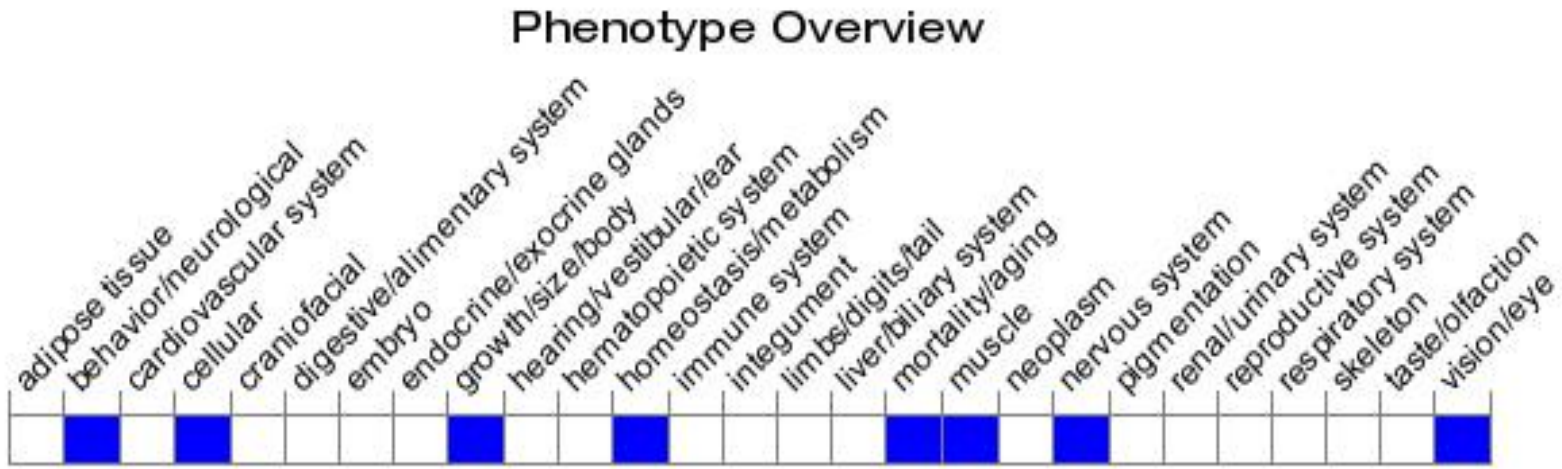
Genomic location distribution



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,mice homozygous for disruptions in this gene die either embryonically or very soon after birth. Heterozygous males display hyperactivity and coordination difficulties.

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

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