Tgif1 Cas9-CKO Strategy

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Reviewer: **Ruirui Zhang**

Design Date: **2020/2/12**

Project Overview

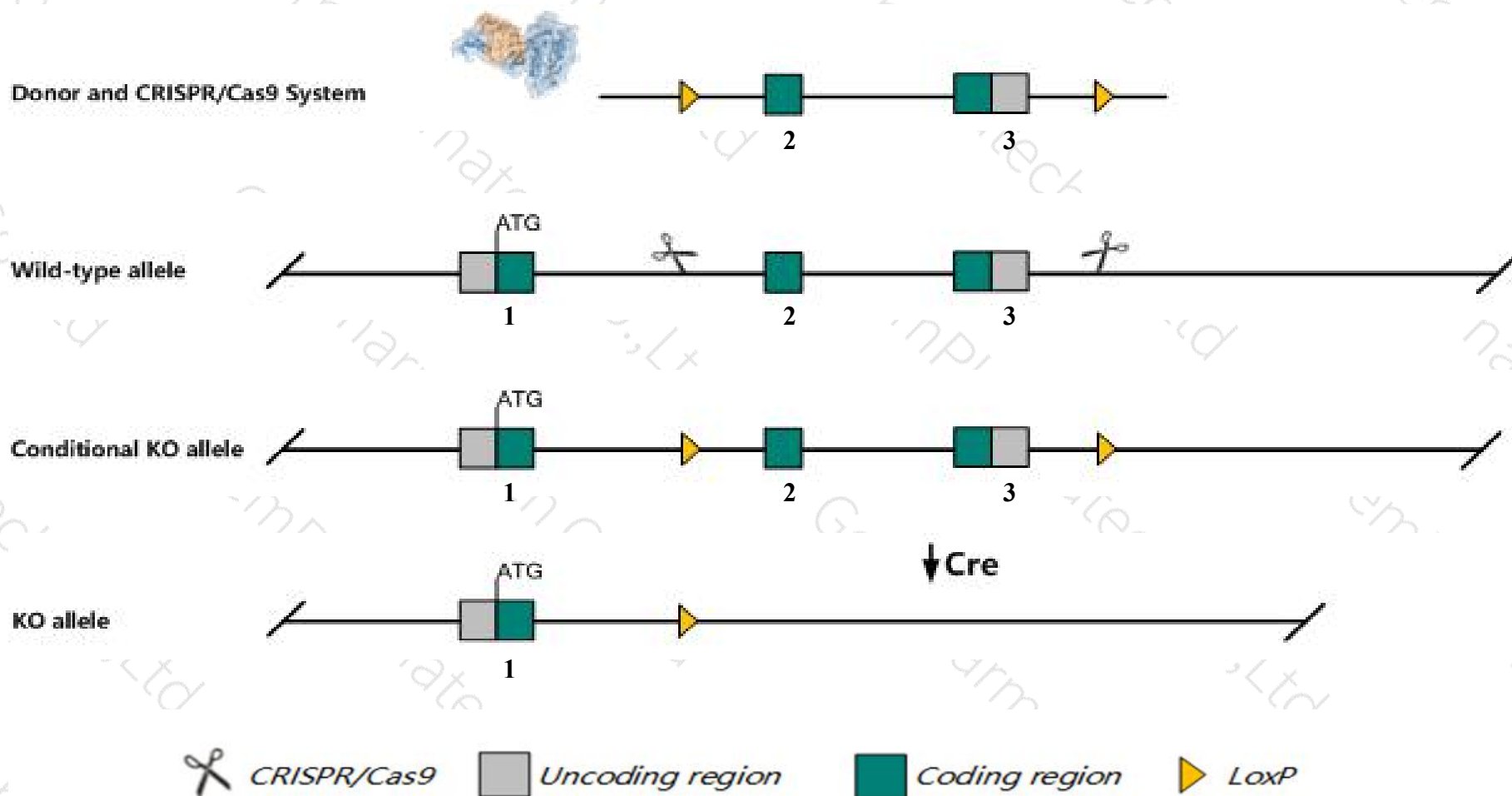|	
Project Name	*Tgif1*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tgif1* gene. The schematic diagram is as follows:

Technical routes

➤ The *Tgif1* gene has 12 transcripts. According to the structure of *Tgif1* gene, exon2-exon3 of *Tgif1-209* (ENSMUST00000166395.8) transcript is recommended as the knockout region.The region contains most of coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Tgif1* 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,Homozygous null mice display normal growth, behavior and fertility.

➢ The insertion of 5'loxp may affect the 5-terminal regulation of *Tgif1-207* transcript.

➢ The *Tgif1* 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.
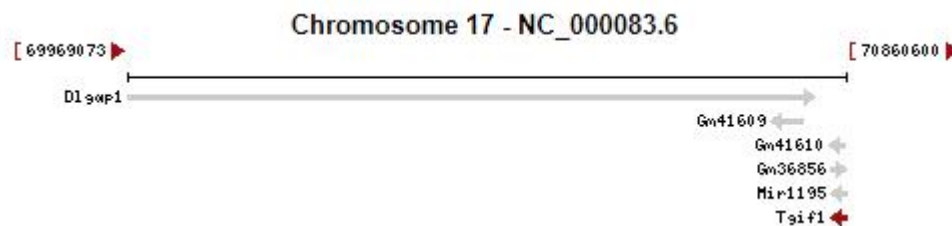
Gene information （NCBI）

Tgif1   TGFB-induced factor homeobox 1 [*Mus musculus* (house mouse)]

Gene ID: 21815, updated on 28-Oct-2019

Summary

|	
Official Symbol	Tgif1 provided by MGI
Official Full Name	TGFB-induced factor homeobox 1 provided by MGI
Primary source	MGI:MGI:1194497
See related	Ensembl:ENSMUSG00000047407
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	Tgif; AA959811; AI462167
Expression	Ubiquitous expression in ovary adult (RPKM 15.8), bladder adult (RPKM 9.9) and 24 other tissues See more
Orthologs	human all

Chromosome 17 - NC_000083.6

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Dlgap1

Gm41609

Gm41610

Gm36856

Mir1195

Tgif1

Transcript information（Ensembl）

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

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tgif1-209	ENSMUST00000166395.8	1714	305aa	Protein coding	CCDS50175	G3UWC5	TSL:1 GENCODE basic
Tgif1-201	ENSMUST00000059775.14	1631	272aa	Protein coding	CCDS37683	P70284	TSL:1 GENCODE basic APPRIS P1
Tgif1-210	ENSMUST00000172229.7	1446	252aa	Protein coding	CCDS50174	P70284 Q3TVD4	TSL:3 GENCODE basic
Tgif1-202	ENSMUST00000118283.7	1388	252aa	Protein coding	CCDS50174	P70284 Q3TVD4	TSL:1 GENCODE basic
Tgif1-211	ENSMUST00000186358.5	1455	84aa	Protein coding	-	A0A087WNP4	TSL:3 GENCODE basic
Tgif1-207	ENSMUST00000135007.2	1291	158aa	Protein coding	-	E0CYI0	CDS 3' incomplete TSL:2
Tgif1-204	ENSMUST00000127719.1	923	242aa	Protein coding	-	D3Z0Q5	CDS 3' incomplete TSL:3
Tgif1-206	ENSMUST00000134654.1	679	58aa	Protein coding	-	E0CXJ4	CDS 3' incomplete TSL:3
Tgif1-208	ENSMUST00000156484.1	613	57aa	Protein coding	-	F6TUU5	CDS 5' incomplete TSL:2
Tgif1-203	ENSMUST00000125329.1	772	No protein	Processed transcript	-	-	TSL:5
Tgif1-205	ENSMUST00000132825.1	522	No protein	Processed transcript	-	-	TSL:3
Tgif1-212	ENSMUST00000190687.1	3145	No protein	Retained intron	-	-	TSL:NA

The strategy is based on the design of *Tgif1-209* transcript,The transcription is shown below
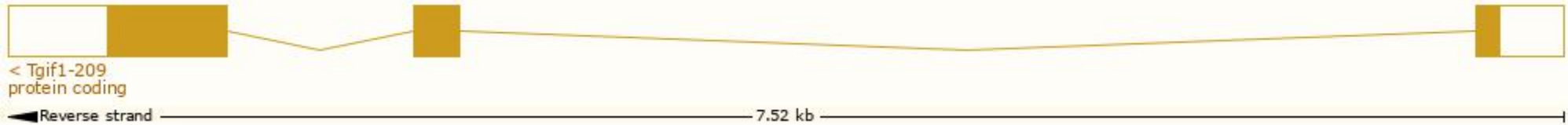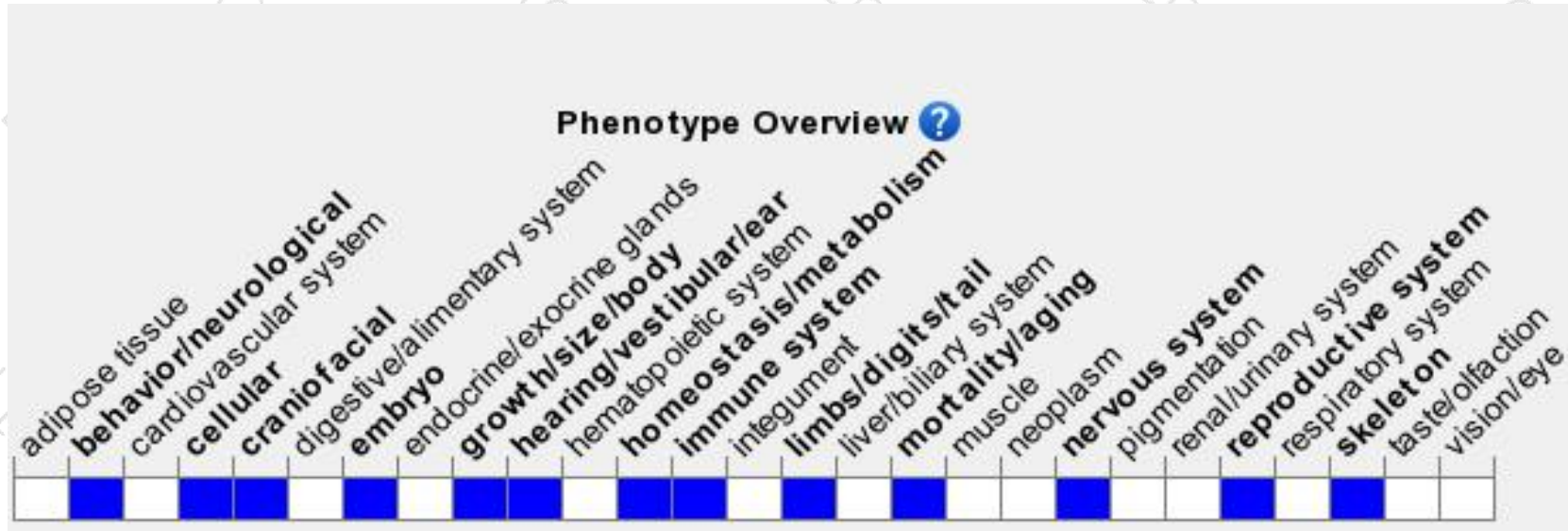
Genomic location distribution
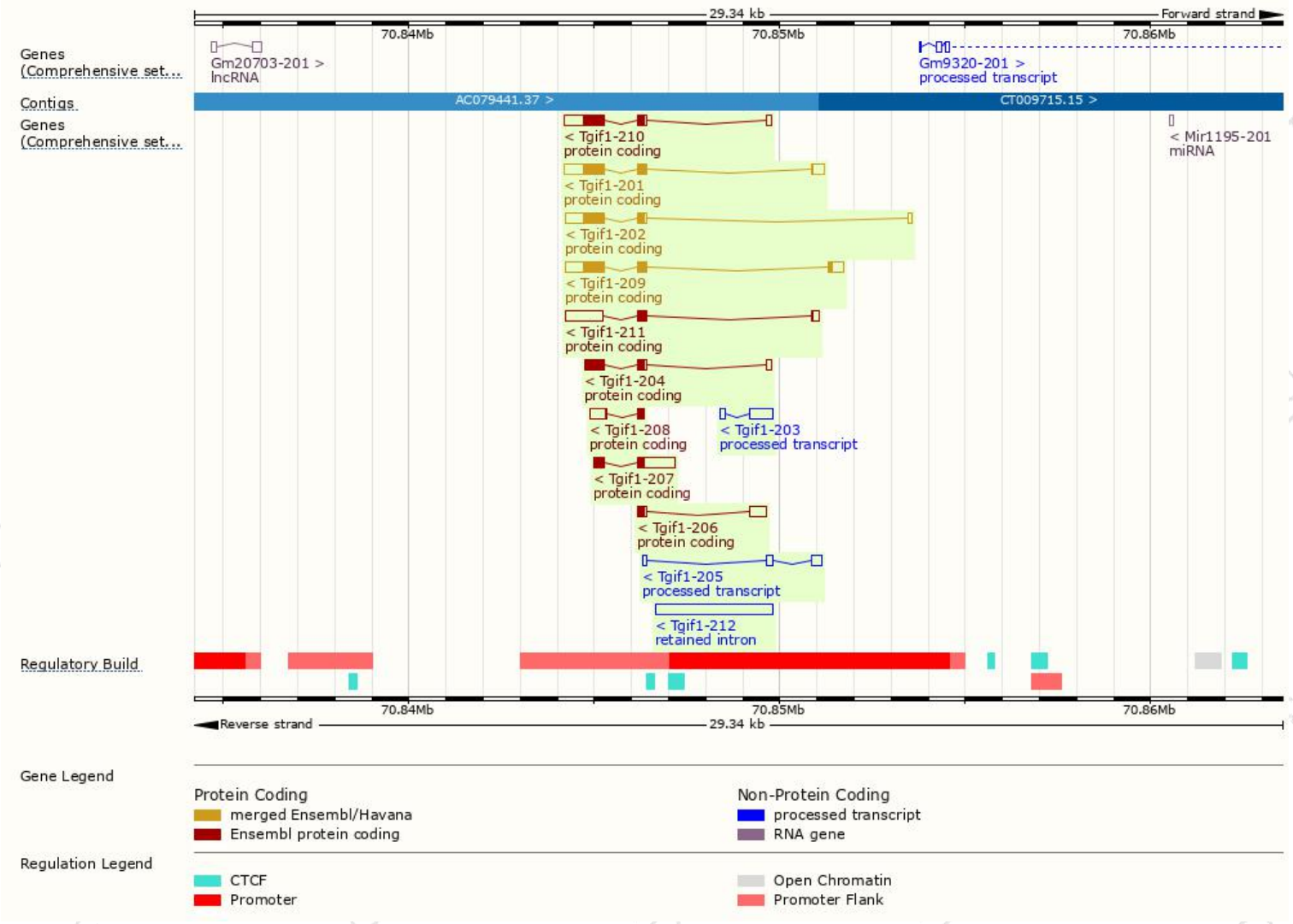
Protein domain
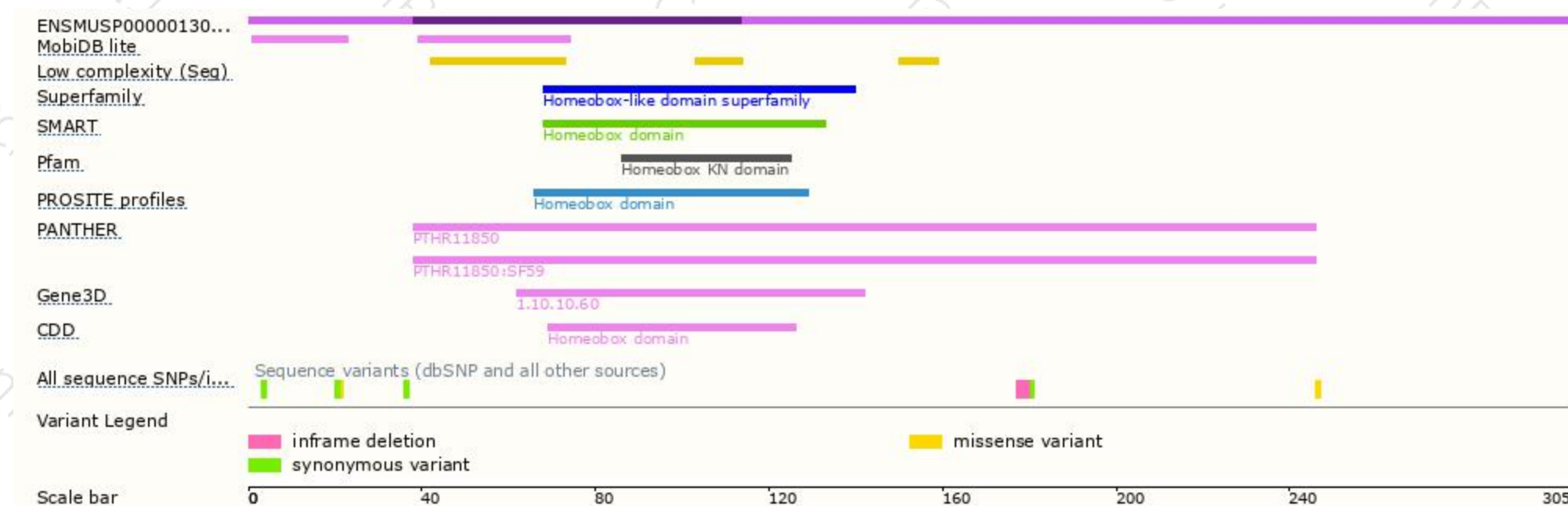
Mouse phenotype description(MGI)

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,Homozygous null mice display normal growth, behavior and fertility.

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