Tsc2 Cas9-KO Strategy

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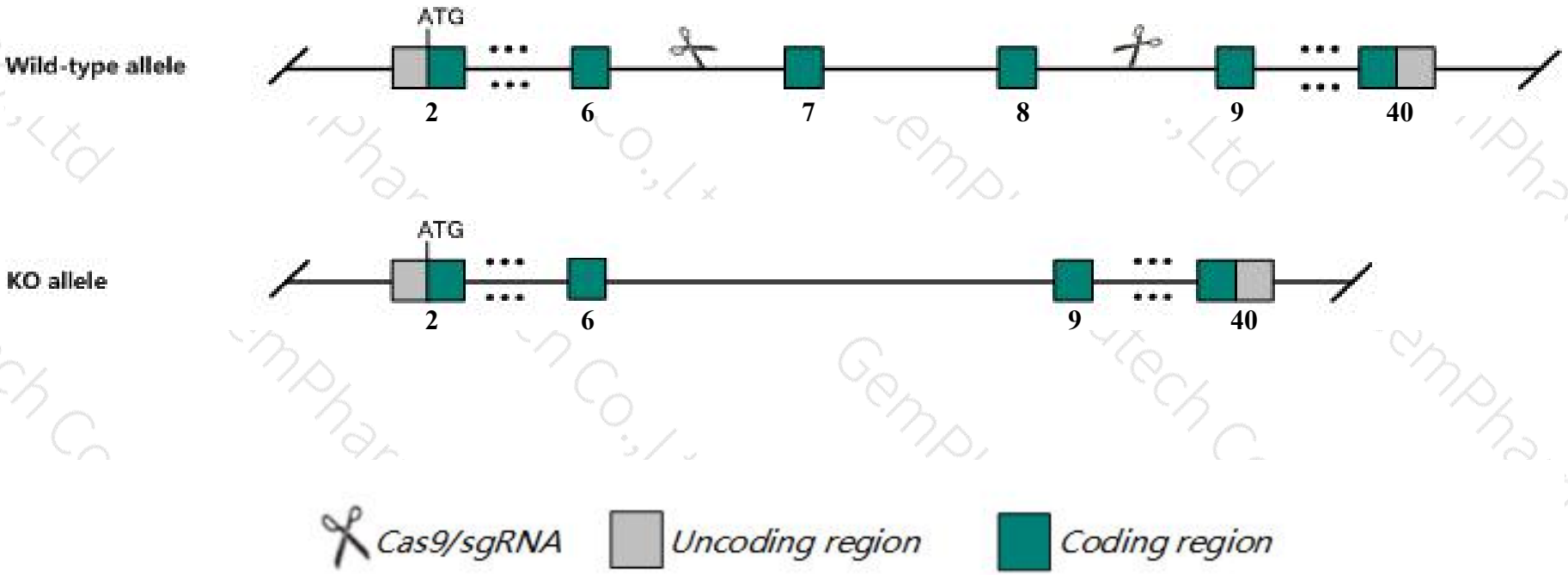
Design Date: 2019-10-18

Project Overview

Project Name	***Tsc2***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tsc2* gene. The schematic diagram is as follows:



Technical routes

- The *Tsc2* gene has 22 transcripts. According to the structure of *Tsc2* gene, exon7-exon8 of *Tsc2-201* (ENSMUST00000097373.1) transcript is recommended as the knockout region.The region contains 175bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Tsc2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

- According to the existing MGI data,Homozygous null mutants exhibit liver hypoplasia, open neural tube, thickened myocardium and die by embryonic day 9.5-12.5. Heterozygotes develop renal cystadenomas, liver hemangiomas (sometimes resulting in fatal bleeding) and lung adenomas.

- Transcript *Tsc2-213,219* may not be affected.

- The *Tsc2* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Tsc2 TSC complex subunit 2 [Mus musculus (house mouse)]

Gene ID: 22084, updated on 26-Mar-2019

Summary

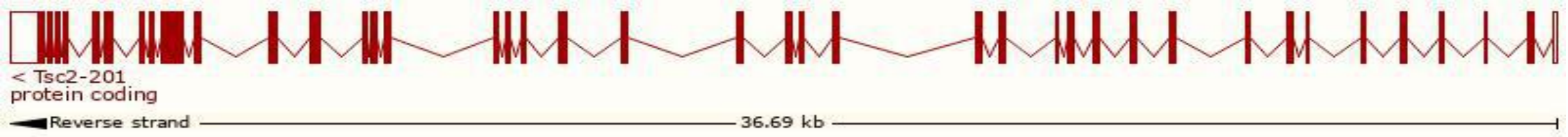
Official Symbol	Tsc2 provided by MGI
Official Full Name	TSC complex subunit 2 provided byMGI
Primary source	MGI:MGI:102548
See related	Ensembl:ENSMUSG00000002496
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Nafld, Tcs2
Expression	Ubiquitous expression in lung adult (RPKM 31.9), ovary adult (RPKM 27.3) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

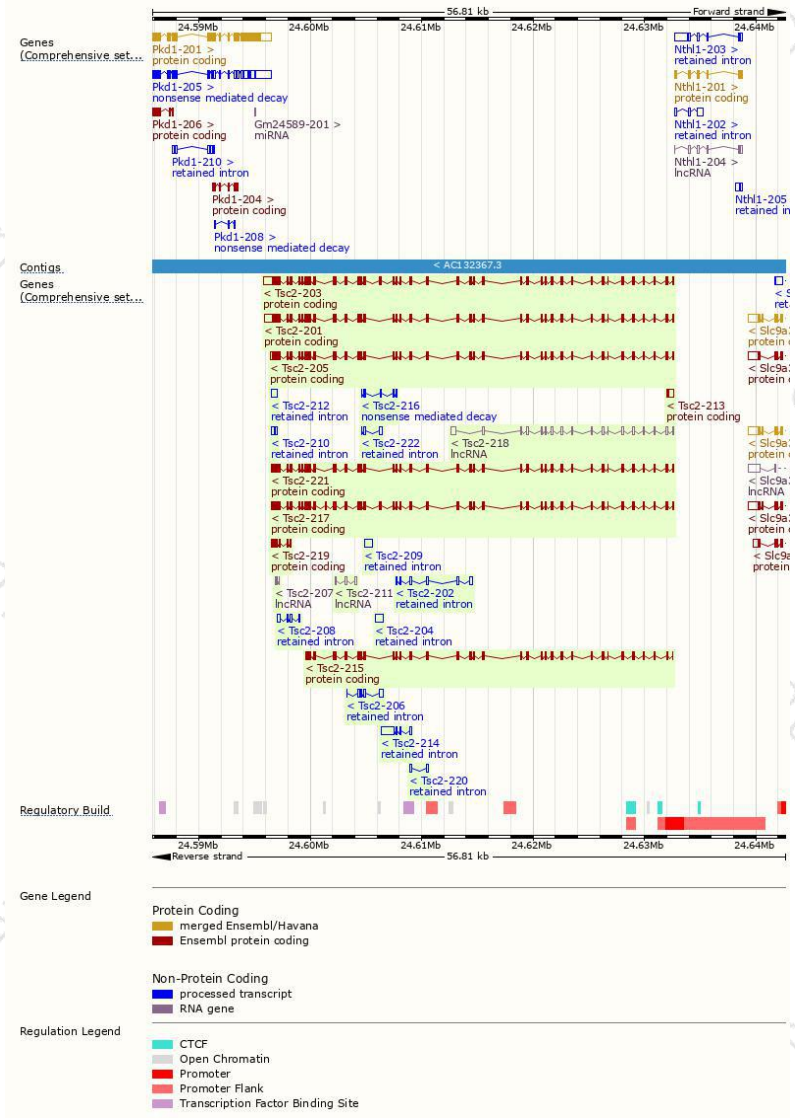
The gene has 22 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tsc2-201	ENSMUST00000097373.1	6018	1742aa	Protein coding	CCDS28486	Q7TT21	TSL:1 GENCODE basic APPRIS P2
Tsc2-205	ENSMUST00000226398.1	5459	1742aa	Protein coding	CCDS28486	Q7TT21	GENCODE basic APPRIS P2
Tsc2-203	ENSMUST00000226284.1	6266	1785aa	Protein coding	-	A0A2I3BPP1	GENCODE basic APPRIS ALT2
Tsc2-217	ENSMUST00000227745.1	5598	1808aa	Protein coding	-	Q3UHB2	GENCODE basic APPRIS ALT2
Tsc2-221	ENSMUST00000228412.1	5432	1741aa	Protein coding	-	A0A2I3BRT5	GENCODE basic APPRIS ALT2
Tsc2-215	ENSMUST00000227607.1	4269	1359aa	Protein coding	-	A0A2I3BPE9	CDS 3' incomplete
Tsc2-219	ENSMUST00000227804.1	798	237aa	Protein coding	-	Q3TQ10	CDS 5' incomplete
Tsc2-213	ENSMUST00000227509.1	432	7aa	Protein coding	-	A0A2I3BRA1	CDS 3' incomplete
Tsc2-216	ENSMUST00000227658.1	393	97aa	Nonsense mediated decay	-	A0A2I3BPR7	CDS 5' incomplete
Tsc2-214	ENSMUST00000227543.1	1545	No protein	Retained intron	-	-	
Tsc2-202	ENSMUST00000226242.1	859	No protein	Retained intron	-	-	
Tsc2-206	ENSMUST00000226428.1	782	No protein	Retained intron	-	-	
Tsc2-209	ENSMUST00000226985.1	699	No protein	Retained intron	-	-	
Tsc2-204	ENSMUST00000226309.1	677	No protein	Retained intron	-	-	
Tsc2-208	ENSMUST00000226691.1	623	No protein	Retained intron	-	-	
Tsc2-212	ENSMUST00000227432.1	570	No protein	Retained intron	-	-	
Tsc2-222	ENSMUST00000228729.1	461	No protein	Retained intron	-	-	
Tsc2-210	ENSMUST00000227094.1	454	No protein	Retained intron	-	-	
Tsc2-220	ENSMUST00000228220.1	376	No protein	Retained intron	-	-	
Tsc2-218	ENSMUST00000227754.1	2357	No protein	lncRNA	-	-	
Tsc2-211	ENSMUST00000227330.1	354	No protein	lncRNA	-	-	
Tsc2-207	ENSMUST00000226473.1	137	No protein	lncRNA	-	-	

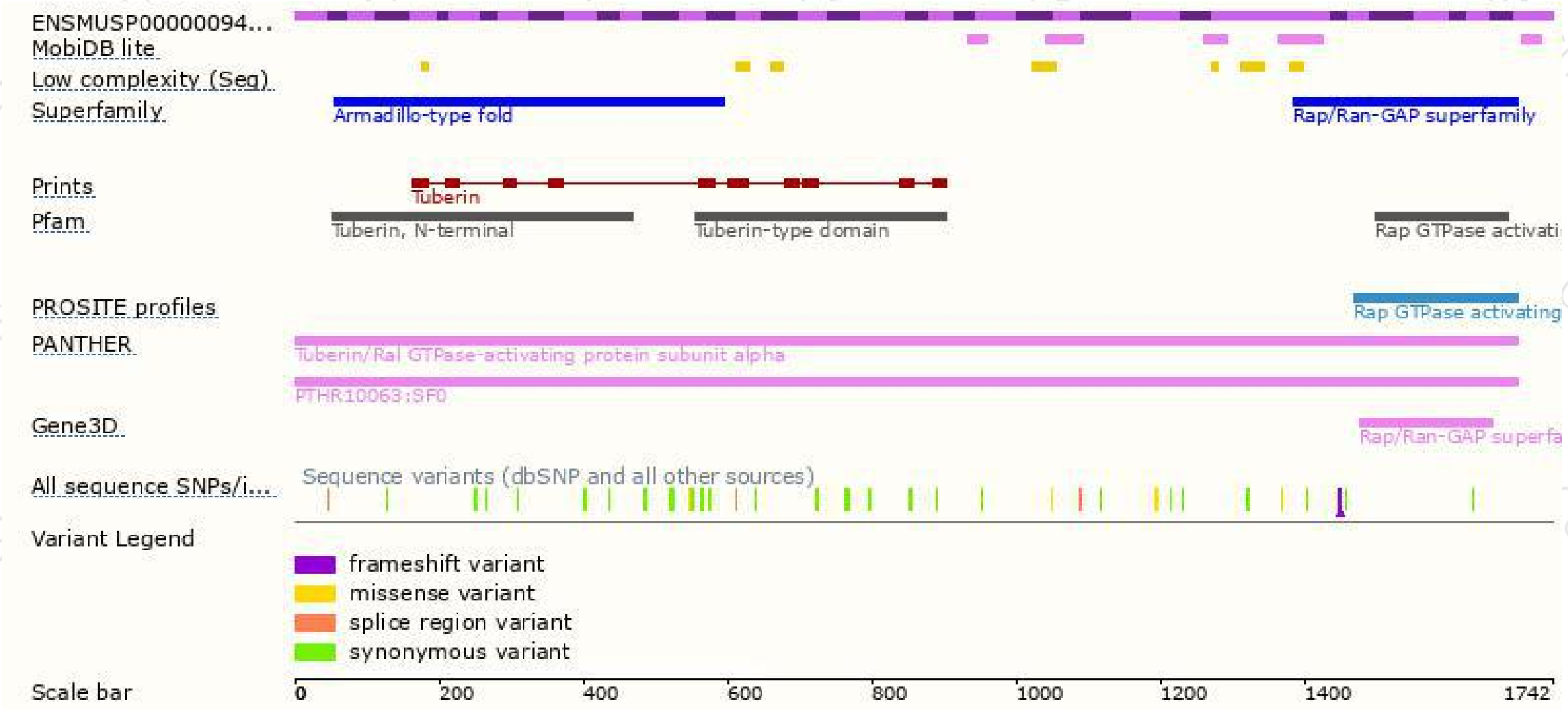
The strategy is based on the design of *Tsc2-201* transcript,The transcription is shown below



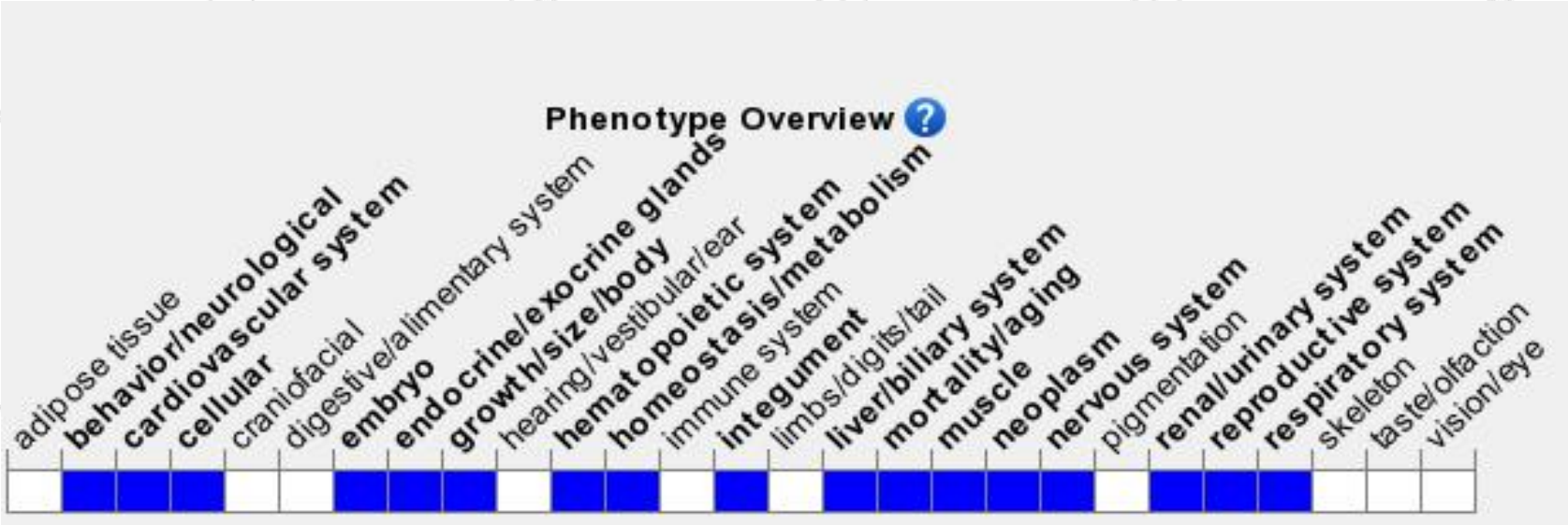
Genomic location distribution



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,Homozygous null mutants exhibit liver hypoplasia, open neural tube, thickened myocardium and die by embryonic day 9.5-12.5. Heterozygotes develop renal cystadenomas, liver hemangiomas (sometimes resulting in fatal bleeding) and lung adenomas.

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

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