Fes Cas9-CKO Strategy

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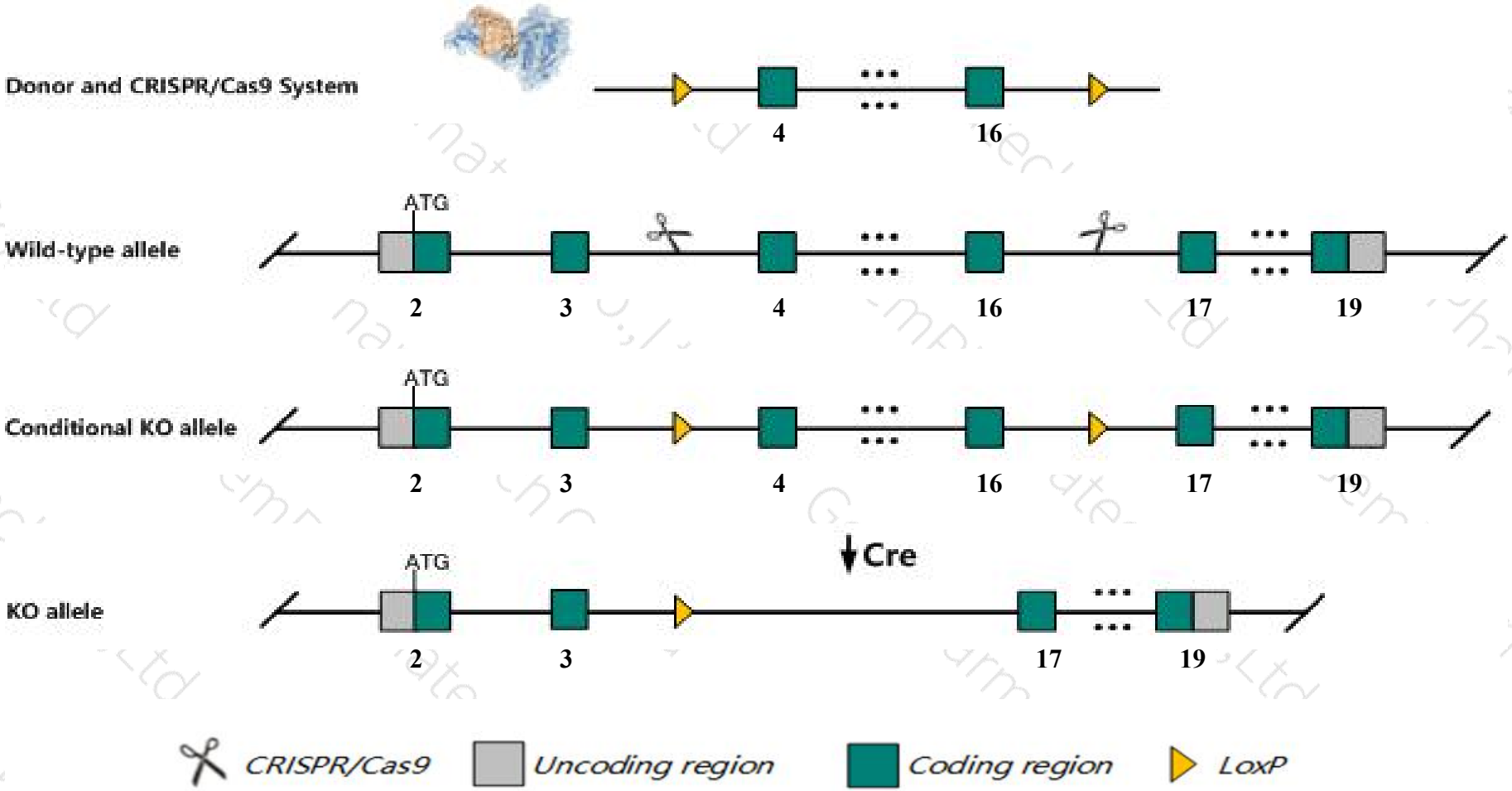
Project Overview

Project Name	***Fes***
Project type	**Cas9-CKO**
Strain background	**C57BL/6J**

Conditional Knockout strategy

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



Technical routes

- The *Fes* gene has 10 transcripts. According to the structure of *Fes* gene, exon4-exon16 of *Fes-201* (ENSMUST00000080932.7) transcript is recommended as the knockout region.The region contains 1658bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Fes* gene. The brief process is as follows:CRISPR/Cas9 system and Donor 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.

- 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,homozygotes for a null allele show partial in utero lethality, runting, altered hematopoietic homeostasis and macrophage function, skin lesions and susceptibility to bacterial infection. homozygotes for another null allele show enhanced lps sensitivity, altered hematopoiesis and larger litter size.
- The *Fes* gene is located on the Chr7. 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）

集萃药康 GemPharmatech

Fes feline sarcoma oncogene [Mus musculus (house mouse)]

Gene ID: 14159, updated on 13-Mar-2020

Summary

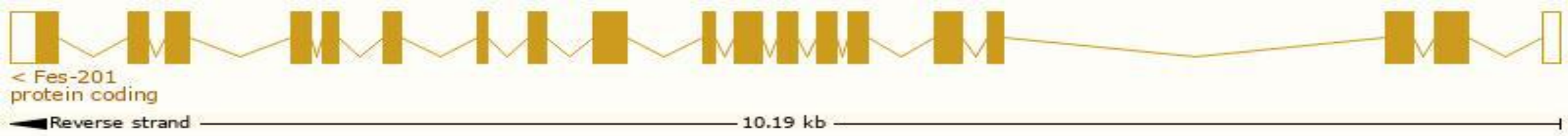
Official Symbol	Fes provided by MGI
Official Full Name	feline sarcoma oncogene provided byMGI
Primary source	MGI:MGI:95514
See related	Ensembl:ENSMUSG00000053158
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI586313, BB137047, FPS, c-fes
Expression	Ubiquitous expression in spleen adult (RPKM 20.4), lung adult (RPKM 18.5) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

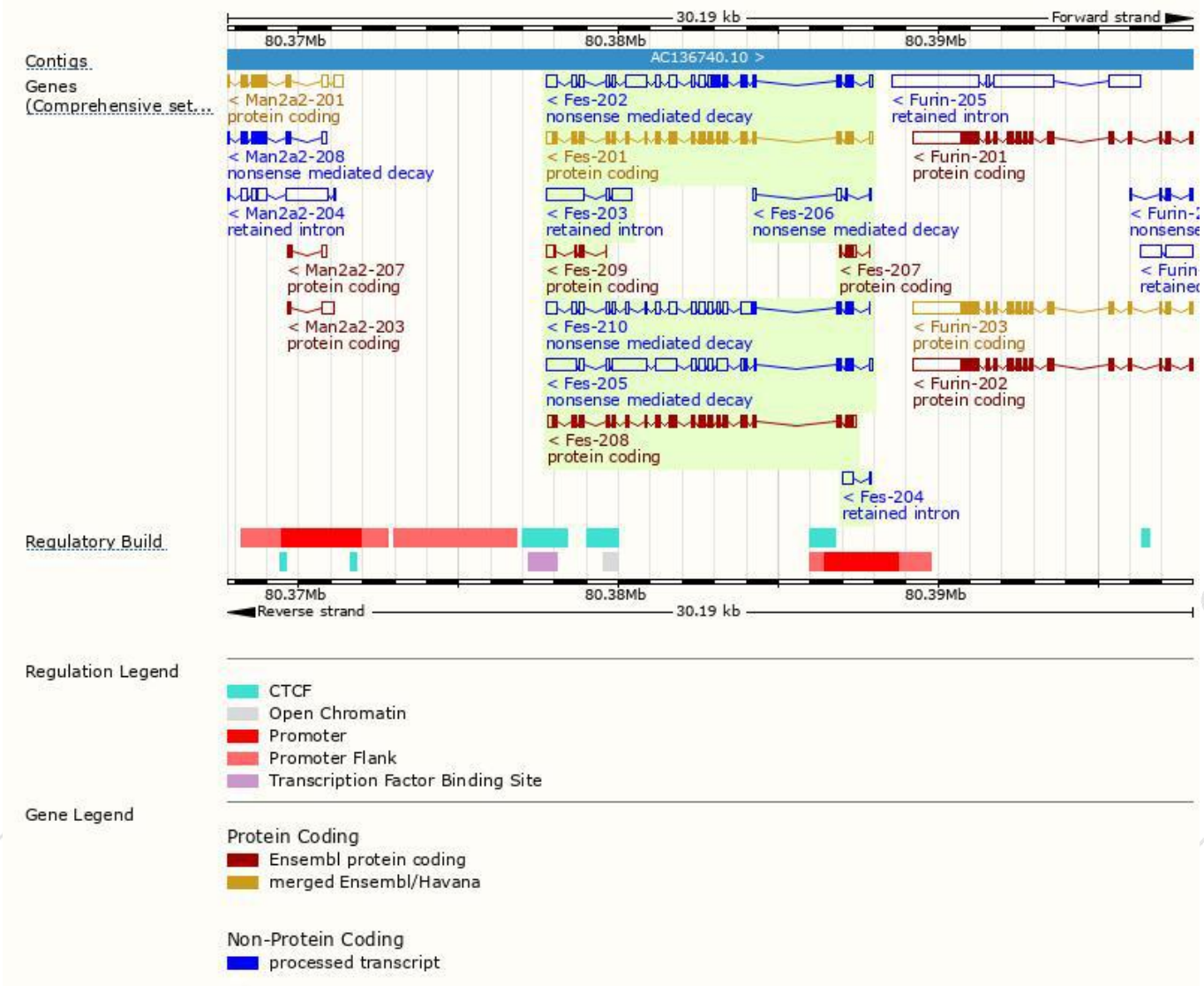
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fes-201	ENSMUST00000080932.7	2762	822aa	Protein coding	CCDS39999	P16879	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 P2
Fes-208	ENSMUST00000206728.1	2707	820aa	Protein coding	-	A0A0U1RPM2	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 ALT1
Fes-209	ENSMUST00000206735.1	472	75aa	Protein coding	-	A0A0U1RPN6	CDS 5' incomplete TSL:5
Fes-207	ENSMUST00000206698.1	443	95aa	Protein coding	-	A0A0U1RPD6	CDS 3' incomplete TSL:3
Fes-205	ENSMUST00000206479.1	4403	173aa	Nonsense mediated decay	-	A0A0U1RPB9	TSL:2
Fes-202	ENSMUST00000205617.1	3371	360aa	Nonsense mediated decay	-	A0A0U1RNL3	TSL:1
Fes-210	ENSMUST00000206744.1	2890	177aa	Nonsense mediated decay	-	A0A0U1RQ80	TSL:1
Fes-206	ENSMUST00000206539.1	362	41aa	Nonsense mediated decay	-	A0A0U1RPQ5	CDS 5' incomplete TSL:2
Fes-203	ENSMUST00000206002.1	1921	No protein	Retained intron	-	-	TSL:1
Fes-204	ENSMUST00000206271.1	363	No protein	Retained intron	-	-	TSL:2

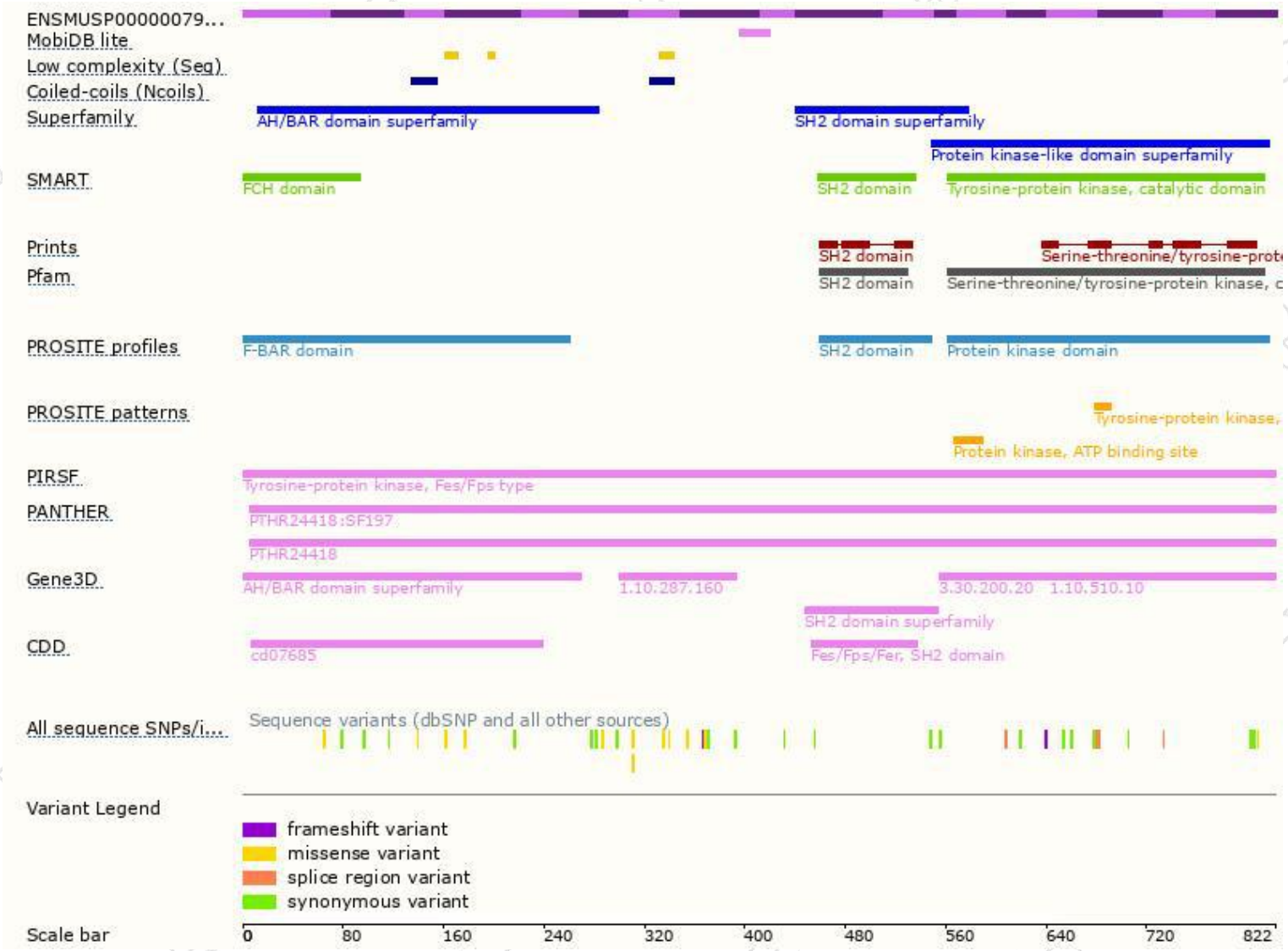
The strategy is based on the design of *Fes-201* transcript,the transcription is shown below:



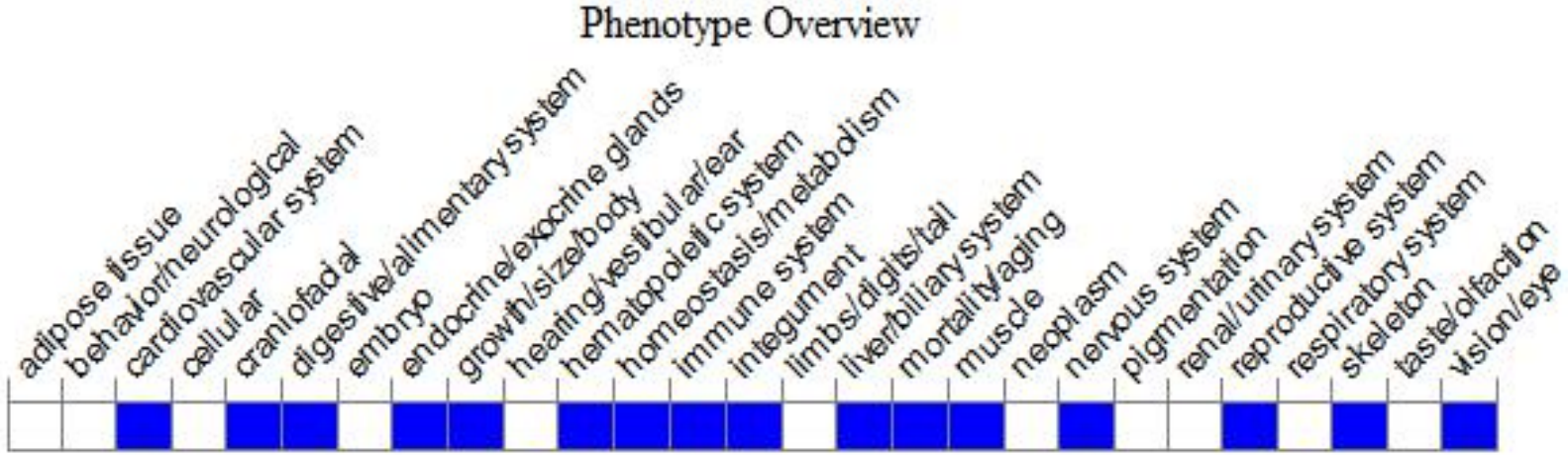
Genomic location distribution



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,homozygotes for a null allele show partial in utero lethality, runting, altered hematopoietic homeostasis and macrophage function, skin lesions and susceptibility to bacterial infection. Homozygotes for another null allele show enhanced LPS sensitivity, altered hematopoiesis and larger litter size.

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

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