Cish Cas9-CKO Strategy

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Project Overview

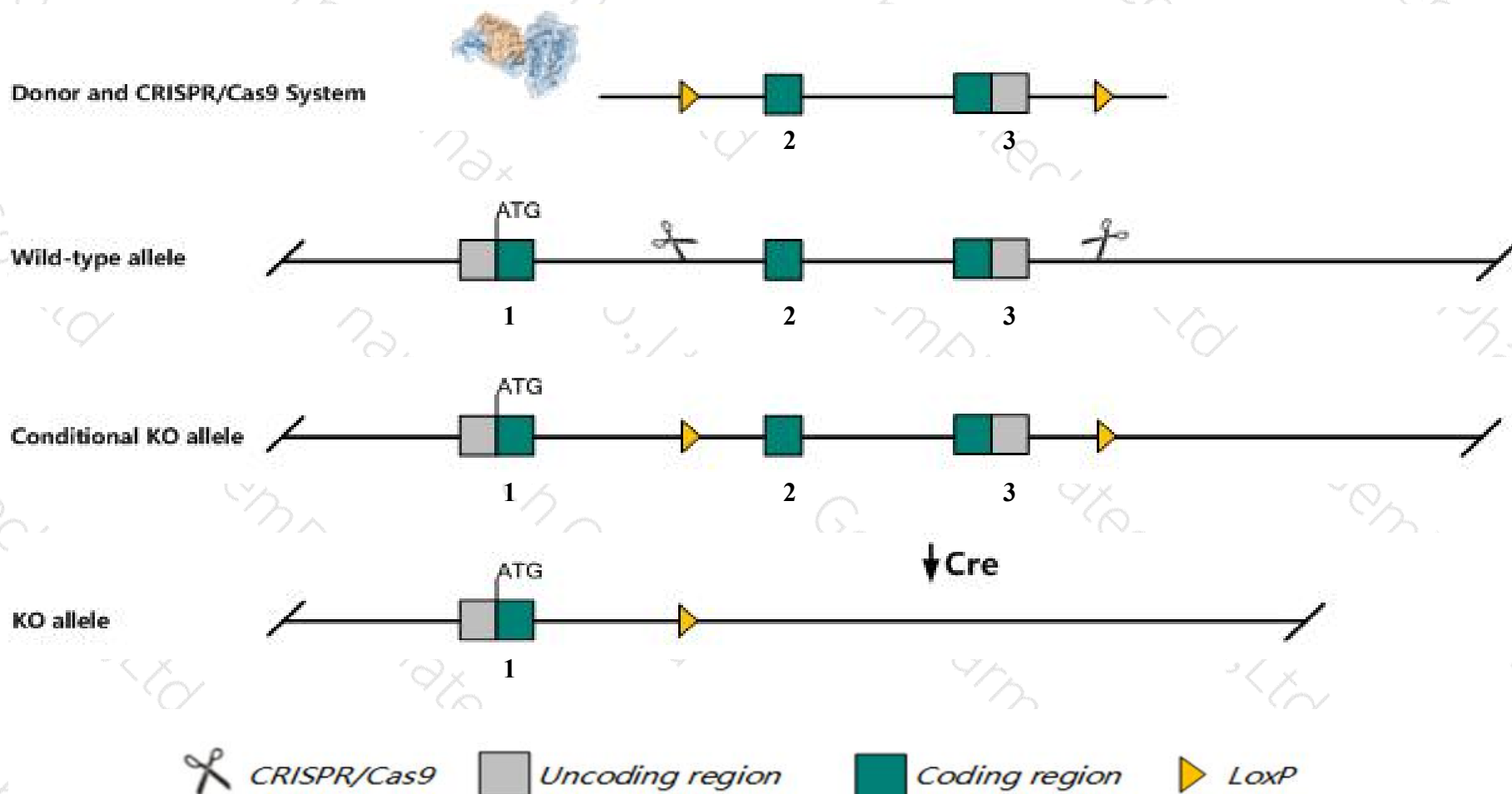|	
Project Name	*Cish*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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

Technical routes

➢ The *Cish* gene has 5 transcripts. According to the structure of *Cish* gene, exon2-exon3 of *Cish-201*(ENSMUST00000085102.5) transcript is recommended as the knockout region.The region contains Ens 754bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Cish* 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 a knock-out allele exhibit enhanced Th2 and Th9 differentiation and allergic airway inflammation.

➢ The *Cish* gene is located on the Chr9. 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.

➢ The deleted area contains GM17040-202, 5 'loxp will be placed on E1 of Cish-202(processed transcript).

Gene information （NCBI）

Cish cytokine inducible SH2-containing protein [Mus musculus (house mouse)]
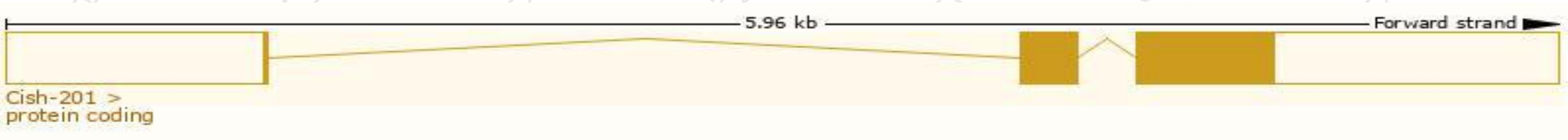
Gene ID: 12700, updated on 13-Mar-2020

▲ Summary

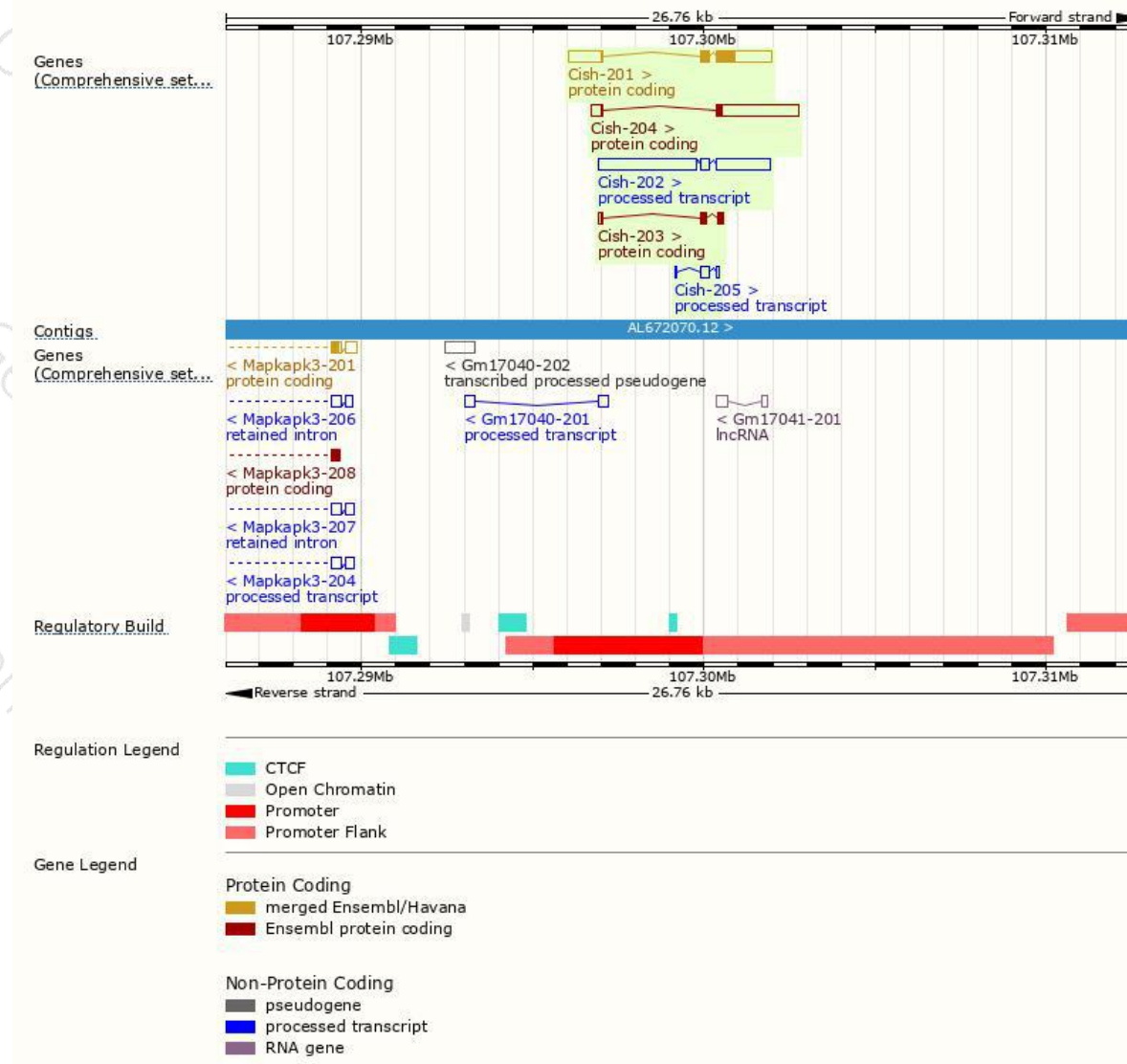|	
Official Symbol	Cish provided by MGI
Official Full Name	cytokine inducible SH2-containing protein provided by MGI
Primary source	MGI:MGI:103159
See related	Ensembl:ENSMUSG00000032578
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CIS-1, CIS1, Cis, F17, F23, SOCS
Expression	Broad expression in heart adult (RPKM 25.8), lung adult (RPKM 20.1) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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

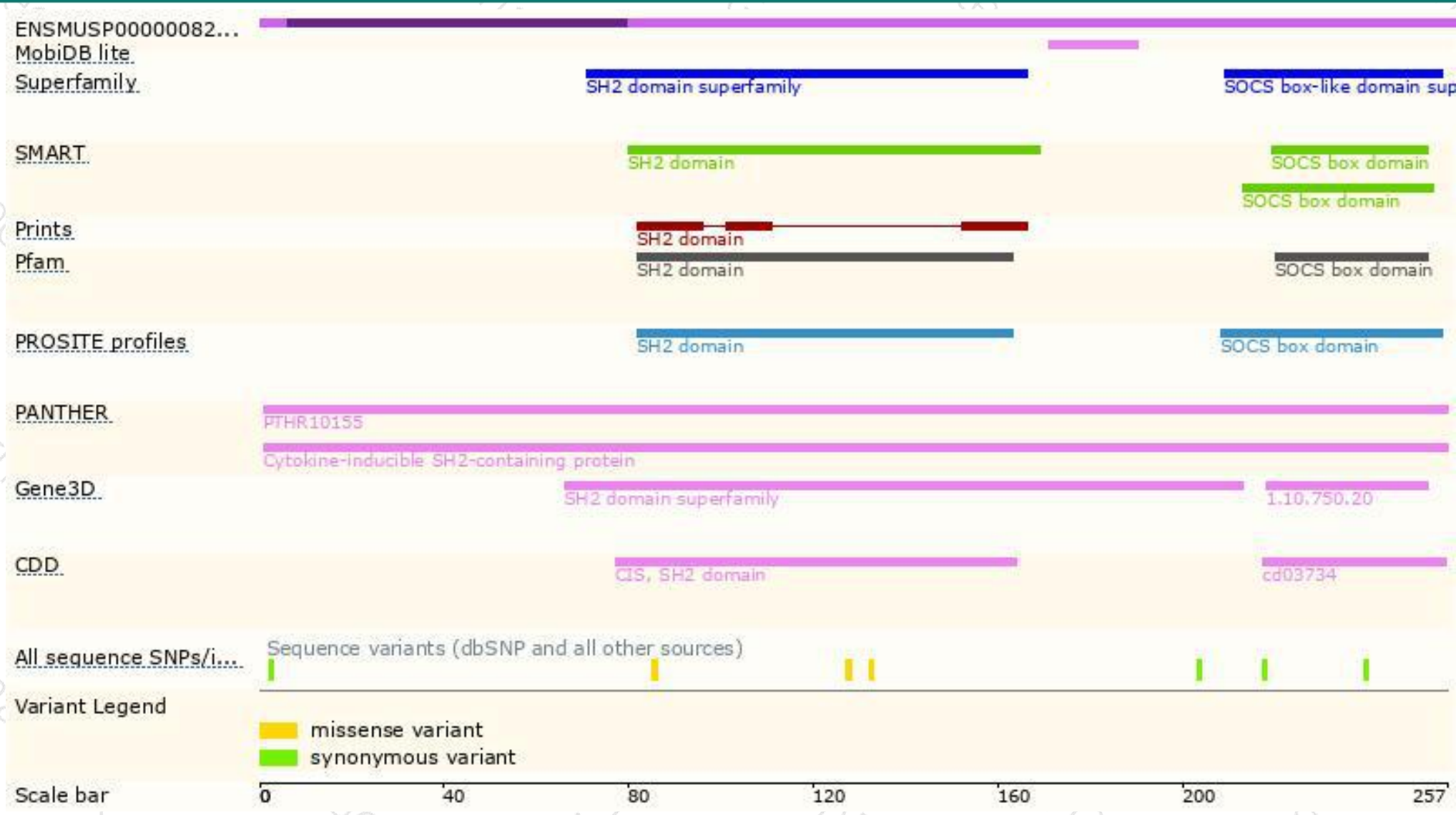| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cish-201	ENSMUST00000085102.5	2850	257aa	Protein coding	CCDS40761	Q62225	TSL:1 GENCODE basic APPRIS P1
Cish-204	ENSMUST00000168260.1	2742	62aa	Protein coding	-	E9Q273	TSL:1 GENCODE basic
Cish-203	ENSMUST00000167072.1	411	102aa	Protein coding	-	E9Q2F4	CDS 3' incomplete TSL:5
Cish-202	ENSMUST00000165664.1	4697	No protein	Processed transcript	-	-	TSL:5
Cish-205	ENSMUST00000171568.1	371	No protein	Processed transcript	-	-	TSL:3

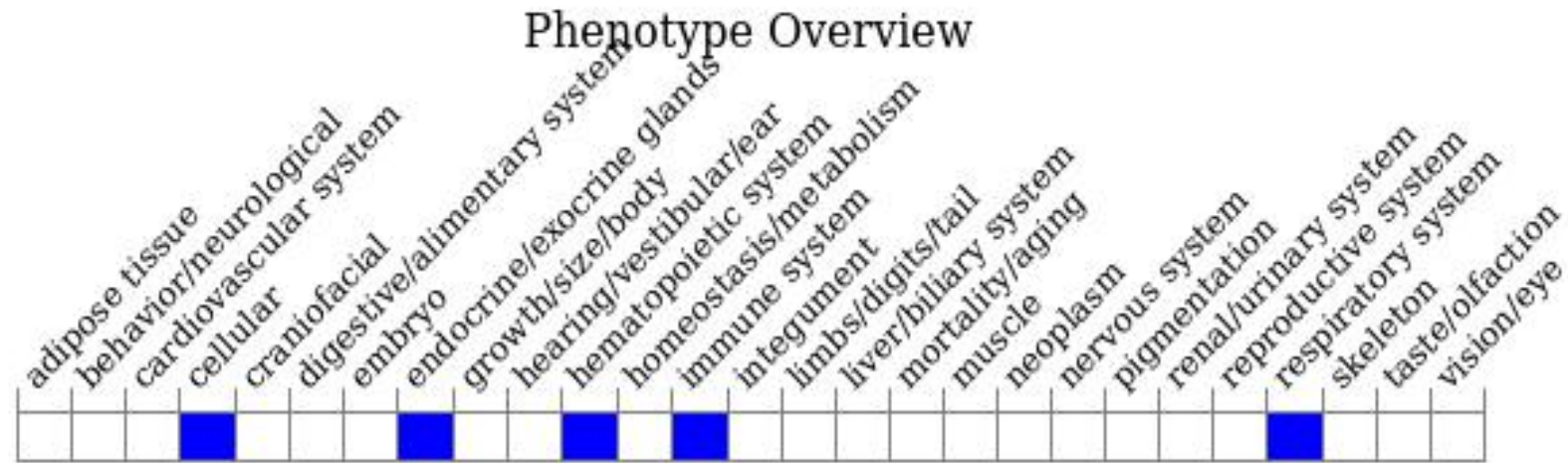The strategy is based on the design of *Cish-201* transcript,the transcription is shown below:

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 exhibit enhanced Th2 and Th9 differentiation and allergic airway inflammation.

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