



H2-Oa Cas9-CKO Strategy

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Reviewer:

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Design Date:

2020-3-13

Project Overview

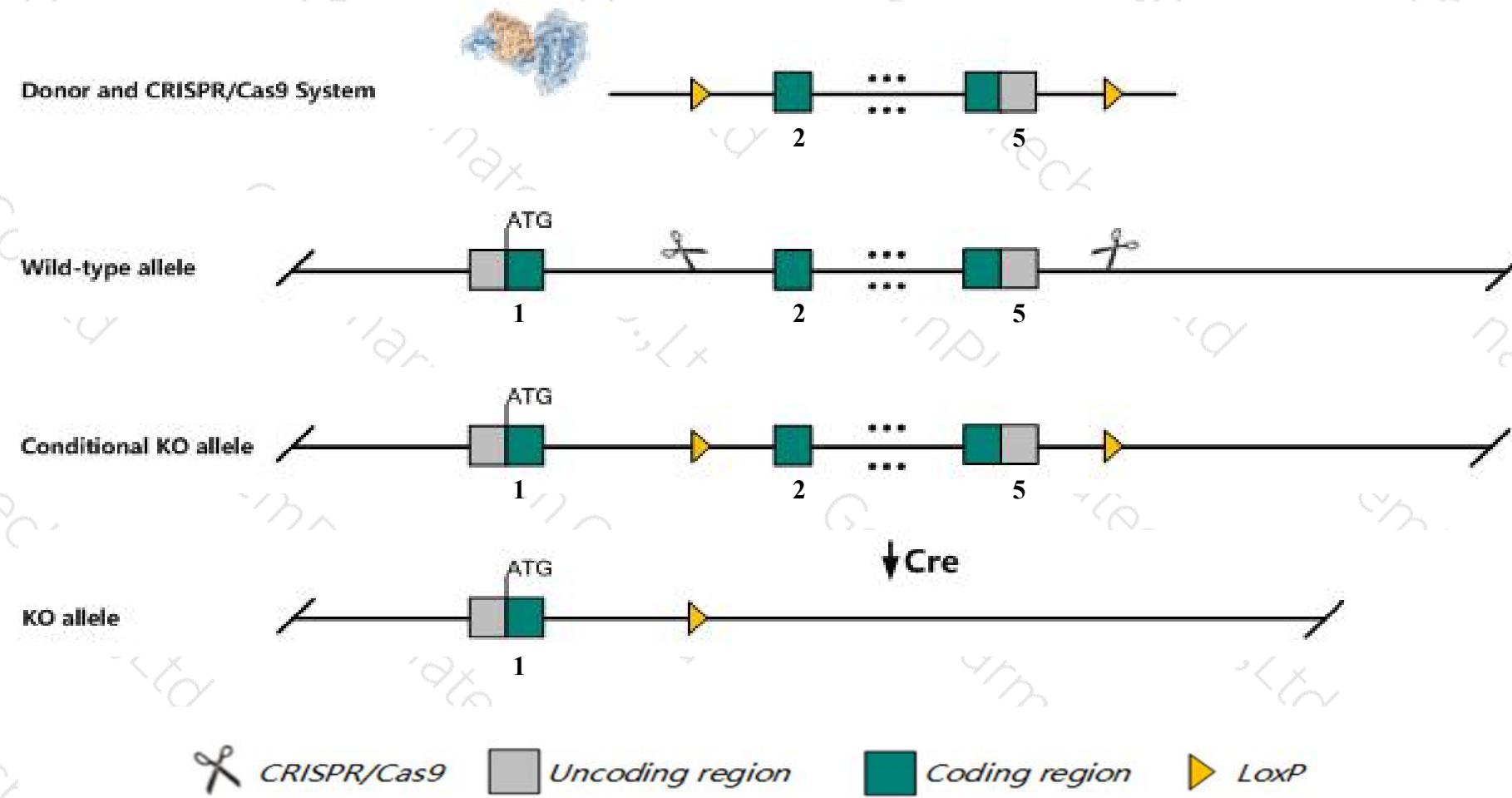
Project Name**H2-Oa**

Project type**Cas9-CKO**

Strain background**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *H2-Oa* gene. The schematic diagram is as follows:



Technical routes

- The *H2-Oa* gene has 7 transcripts. According to the structure of *H2-Oa* gene, exon2-exon5 of *H2-Oa-201* (ENSMUST00000025192.7) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *H2-Oa* 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.



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Notice

- According to the existing MGI data, Homozygous inactivation of this gene results in abnormal antigen presentation via MHC class II. Mice homozygous for a knock-out allele show enhanced selection of CD4+ single positive thymocytes. Mice homozygous for a different knock-out allele show increased serum IgG1 levels.
- The flox region overlap with part of the BC051537 gene, which may affect the regulation of this gene.
- The *H2-Oa* 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)

H2-Oa histocompatibility 2, O region alpha locus [*Mus musculus* (house mouse)]

Gene ID: 15001, updated on 5-Nov-2019



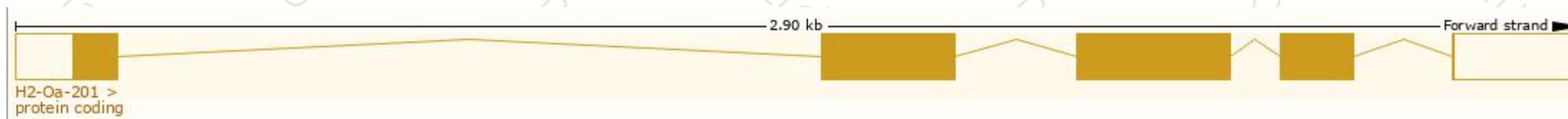
Official Symbol	H2-Oa provided by MGI
Official Full Name	histocompatibility 2, O region alpha locus provided by MGI
Primary source	MGI :MGI:95924
See related	Ensembl:ENSMUSG00000024334
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; <i>Mus</i> ; <i>Mus</i>
Also known as	H-2Oa
Expression	Biased expression in spleen adult (RPKM 116.7), thymus adult (RPKM 40.6) and 3 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

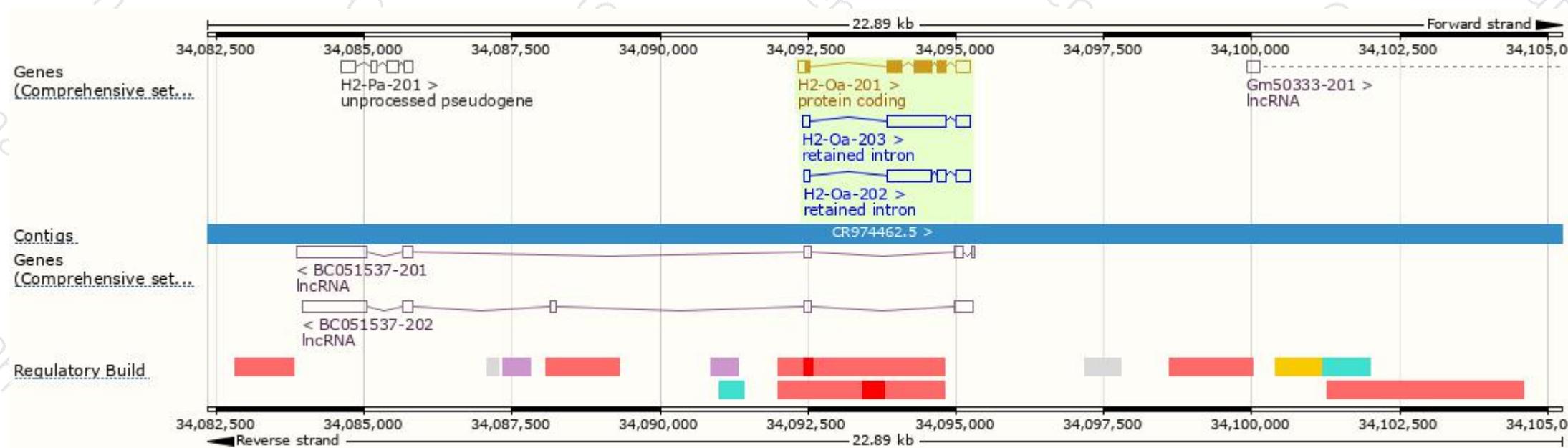
The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
H2-Oa-201	ENSMUST00000025192.7	1088	250aa	Protein coding	CCDS37577	Q9QWV1	TSL:1 GENE CODE basic APPRIS P1
H2-Oa-203	ENSMUST00000236684.1	1340	No protein	Retained intron	-	-	-
H2-Oa-202	ENSMUST00000174670.1	1231	No protein	Retained intron	-	-	TSL:1

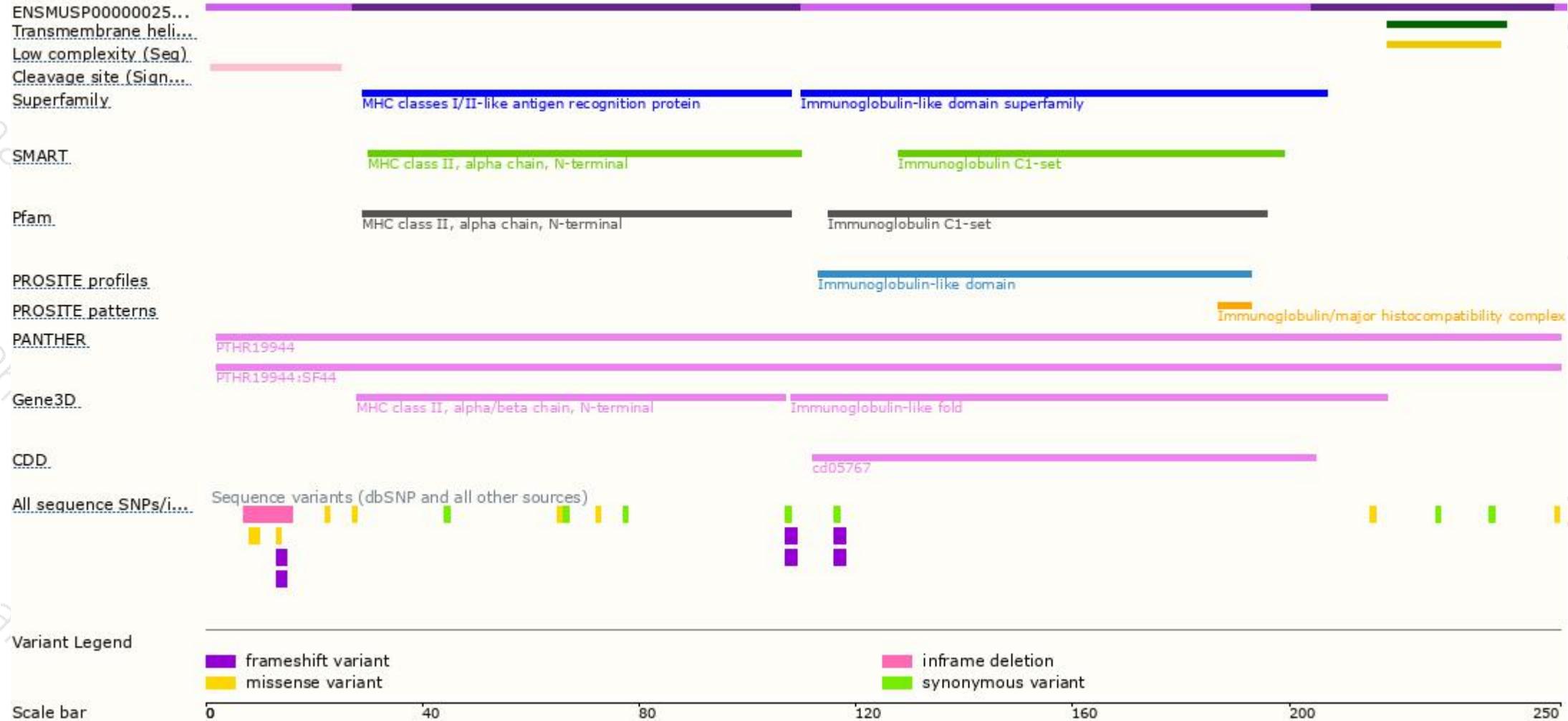
The strategy is based on the design of *H2-Oa-201* transcript, The transcription is shown below



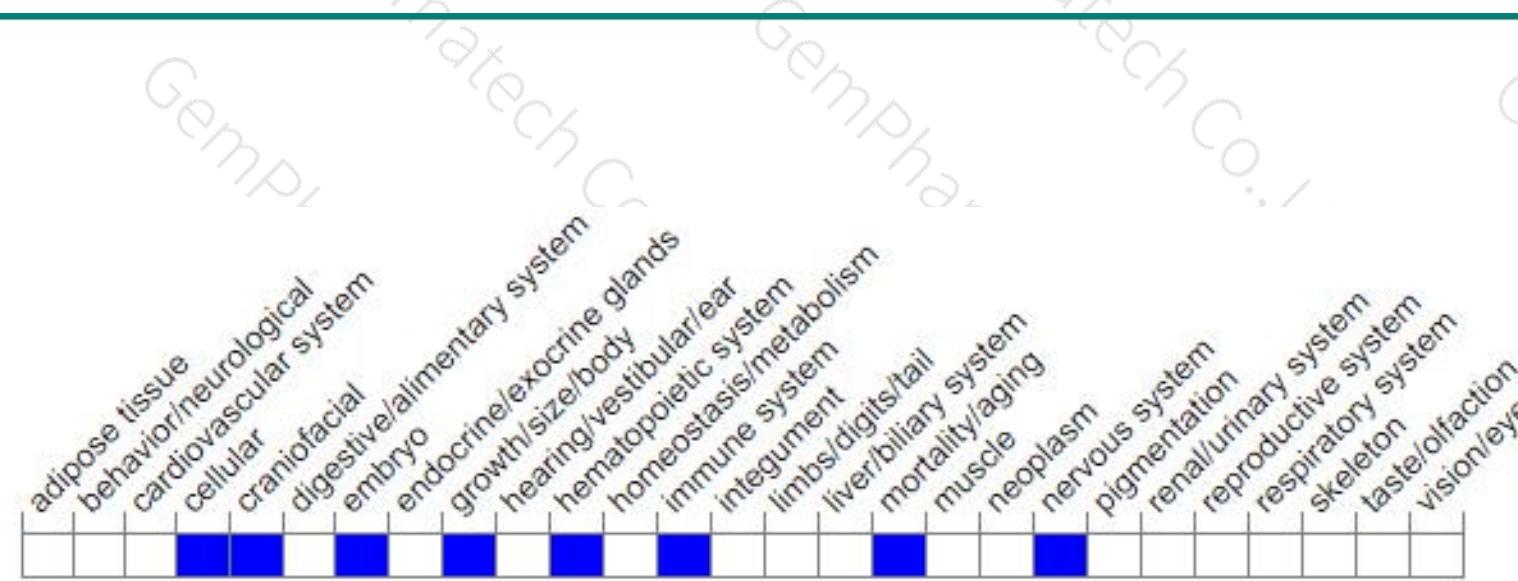
Genomic location distribution



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 inactivation of this gene results in abnormal antigen presentation via MHC class II. Mice homozygous for a knock-out allele show enhanced selection of CD4+ single positive thymocytes. Mice homozygous for a different knock-out allele show increased serum IgG1 levels.



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

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