

# ***Cd28 Cas9-KO Strategy***

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# Project Overview

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**Project Name**

***Cd28***

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**Project type**

**Cas9-KO**

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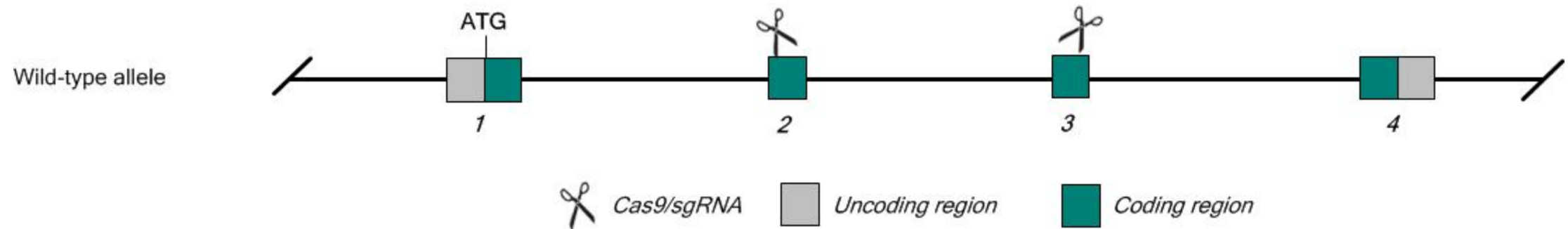
**Strain background**

**C57BL/6J**

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# Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cd28* gene. The schematic diagram is as follows:



# Technical routes

- The *Cd28* gene has 3 transcripts. According to the structure of *Cd28* gene, partial exon2 and partial exon3 of *Cd28-201* (ENSMUST00000027165.2) transcript is recommended as the knockout region. The region contains key coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cd28* 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.

- According to the existing MGI data, Homozygous mutation of this gene results in impairment of some T cell responses and decreased basal immunoglobulin levels. Mutant animals have reduced T helper cell activity and impaired T cell response to lectins, but cytotoxic T cells can still be induced.
- The *Cd28* gene is located on the Chr1. 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)

## Cd28 CD28 antigen [Mus musculus (house mouse)]

Gene ID: 12487, updated on 9-Apr-2019

### Summary



<b>Official Symbol</b>	Cd28 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	CD28 antigen provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:88327</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG000000026012</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Expression</b>	Biased expression in thymus adult (RPKM 17.9), spleen adult (RPKM 1.8) and 1 other tissue <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>



# Transcript information (Ensembl)

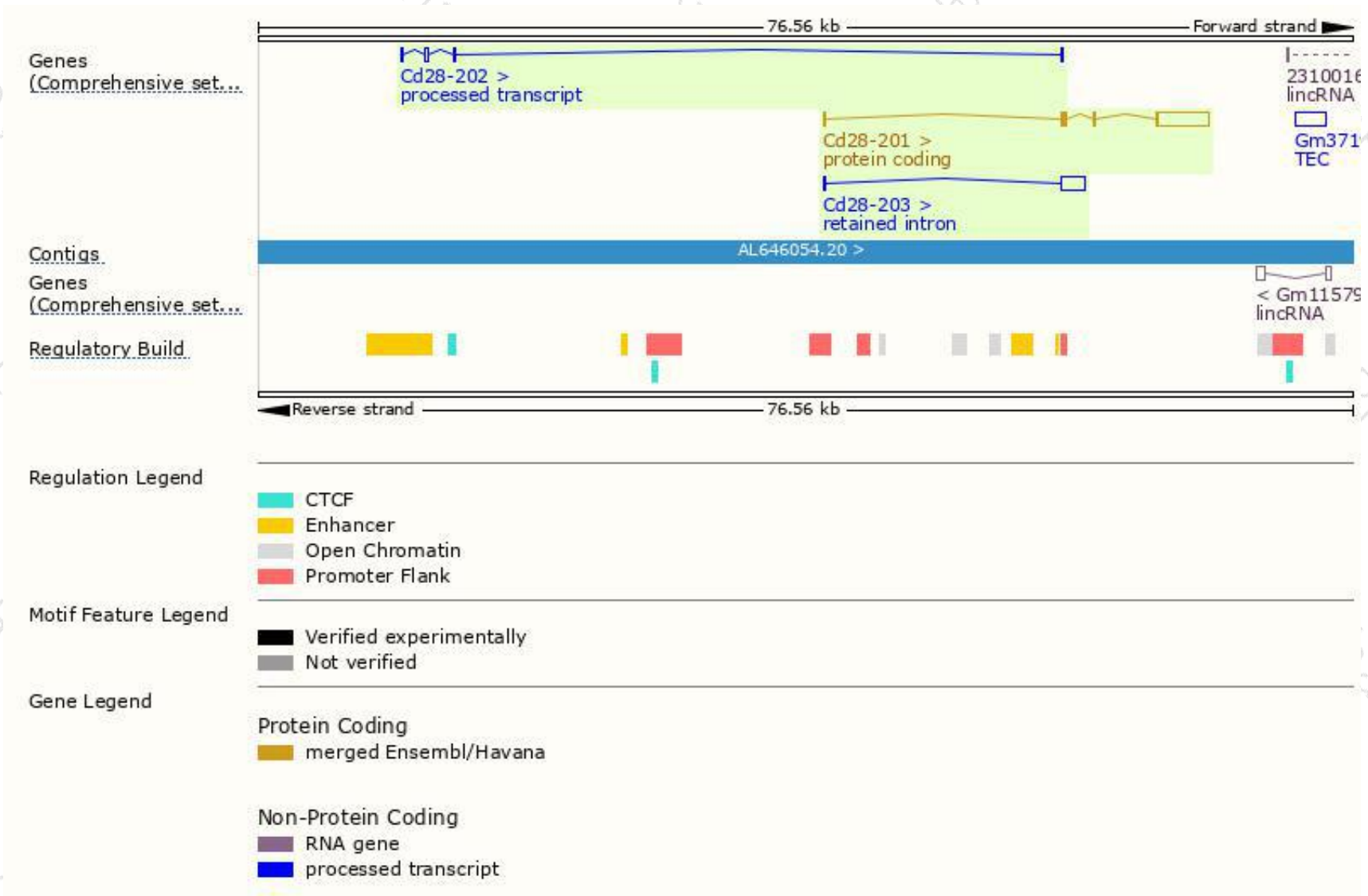
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cd28-201	<a href="#">ENSMUST00000027165.2</a>	4347	<a href="#">218aa</a>	Protein coding	<a href="#">CCDS14992</a>	<a href="#">P31041</a>	TSL:1 GENCODE basic APPRIS P1
Cd28-202	<a href="#">ENSMUST00000132833.1</a>	669	No protein	Processed transcript	-	-	TSL:5
Cd28-203	<a href="#">ENSMUST00000153207.1</a>	1775	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Cd28-201* transcript,The transcription is shown below

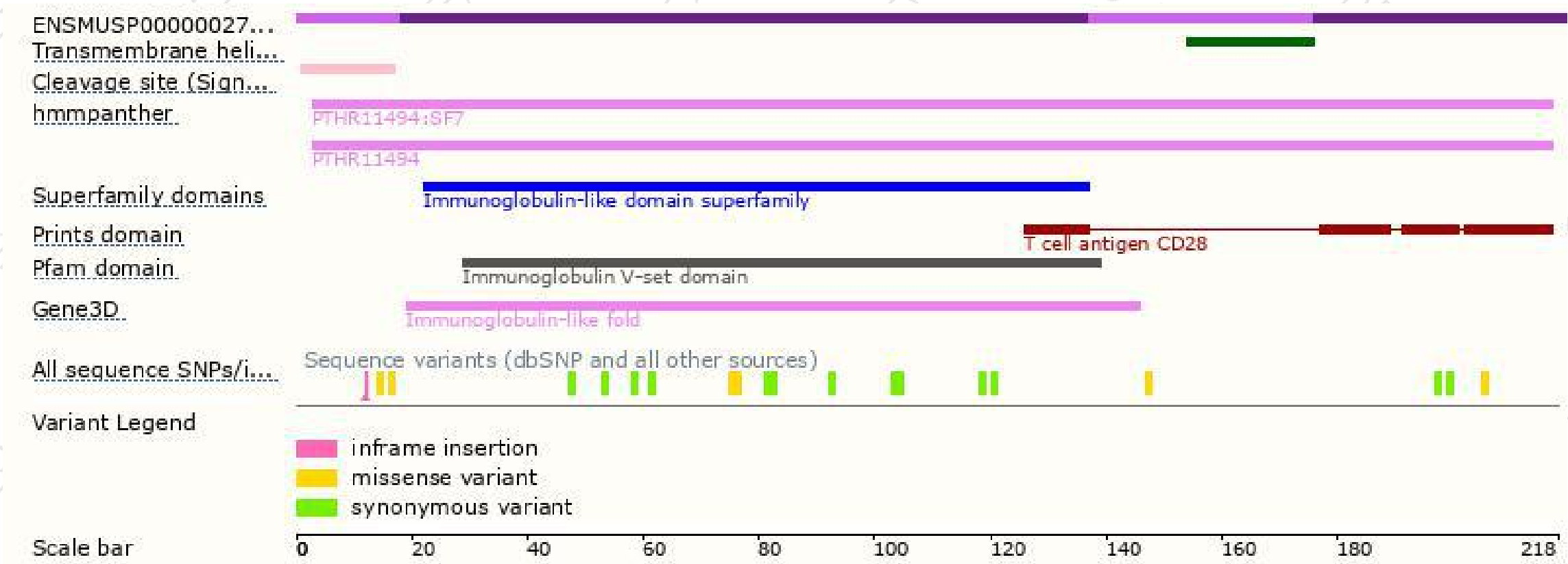


# Genomic location distribution

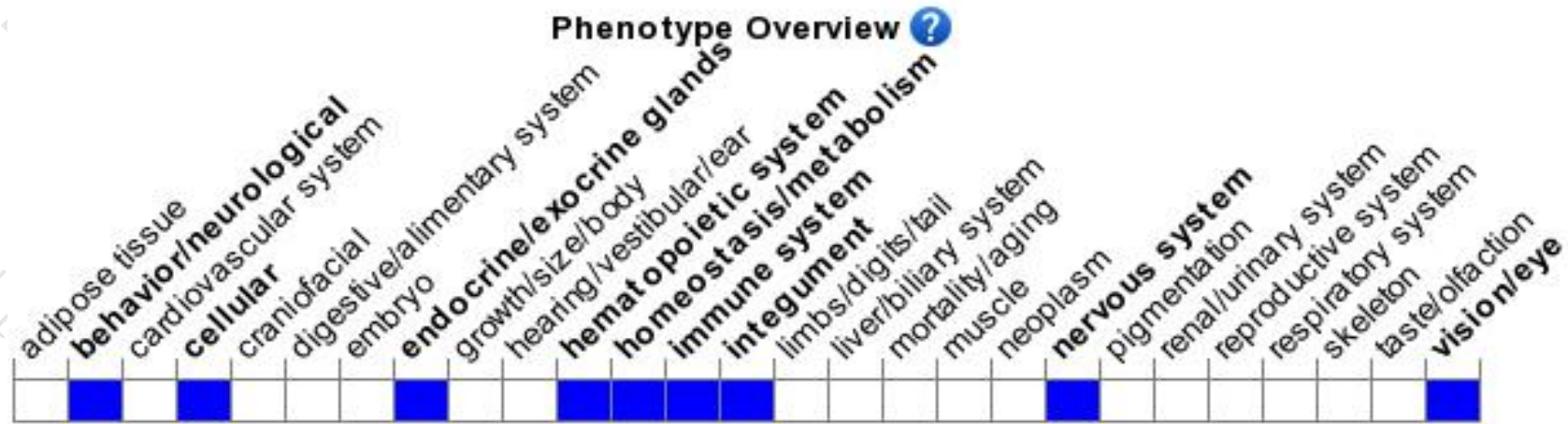




# Protein domain



# 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 mutation of this gene results in impairment of some T cell responses and decreased basal immunoglobulin levels. Mutant animals have reduced T helper cell activity and impaired T cell response to lectins, but cytotoxic T cells can still be induced.

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

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