

# ***Tnfrsf25 Cas9-KO Strategy***

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# Project Overview

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**Project Name**

***Tnfrsf25***

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**Project type**

**Cas9-KO**

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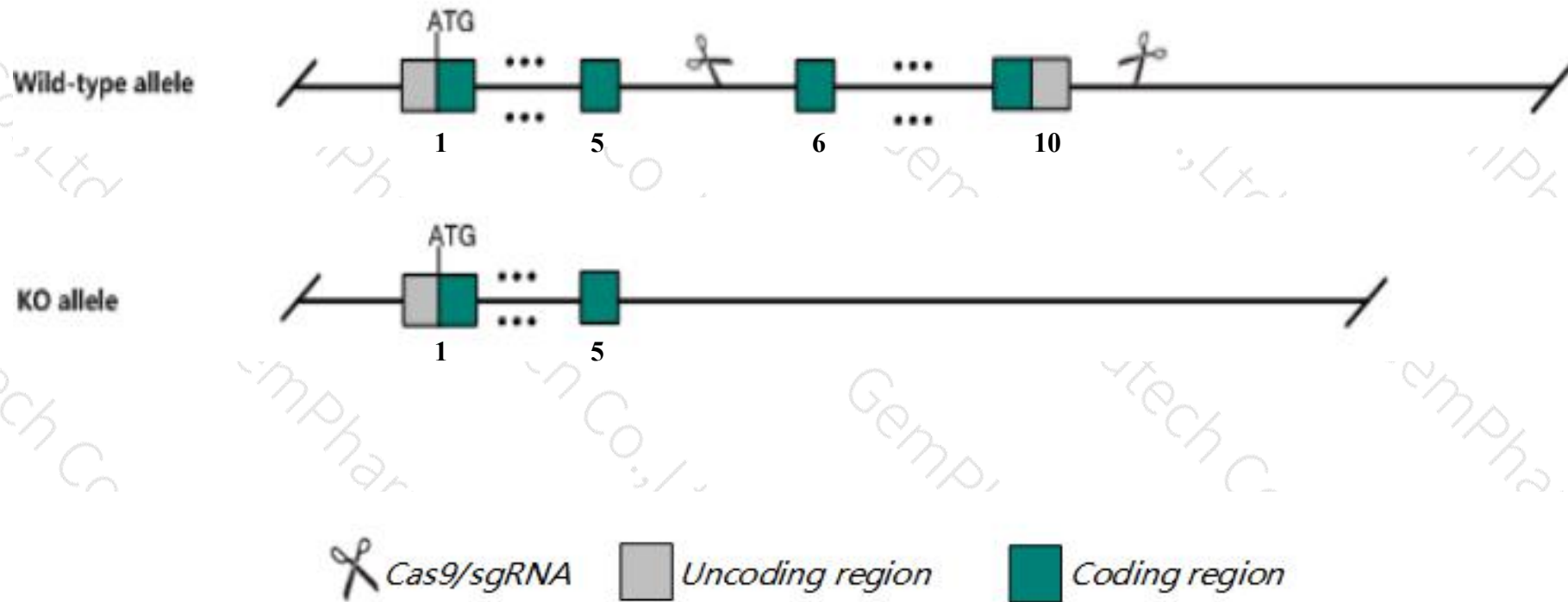
**Strain background**

**C57BL/6JGpt**

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# Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tnfrsf25* gene. The schematic diagram is as follows:



- The *Tnfrsf25* gene has 4 transcripts. According to the structure of *Tnfrsf25* gene, exon6-exon10 of *Tnfrsf25-201*(ENSMUST00000025706.9) transcript is recommended as the knockout region. The region contains 706bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tnfrsf25* gene. The brief process is as follows: sgRNA was transcribed in vitro. Cas9 and sgRNA 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.

- According to the existing MGI data, homozygous mutant mice show no developmental defects or impairments of early thymocyte development. Negative selection and anti-CD3-induced apoptosis, however, are significantly impaired.
- The KO region may affect the 3-terminal regulation of *Espn* gene.
- There are more amino acids left at the N end of the *Tnfrsf25* gene, which may retain some functions.
- The *Tnfrsf25* gene is located on the Chr4. 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)

## Tnfrsf25 tumor necrosis factor receptor superfamily, member 25 [Mus musculus (house mouse)]

Gene ID: 85030, updated on 13-Mar-2020

### Summary



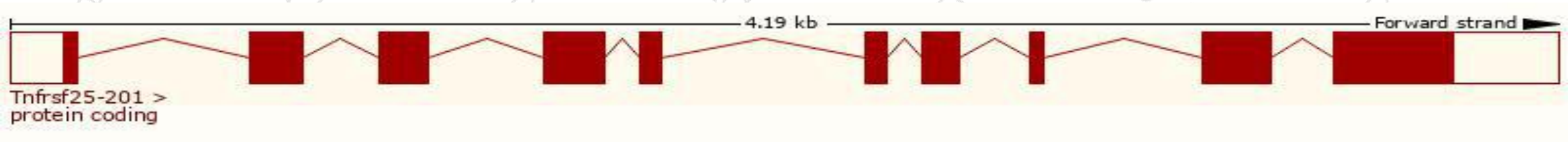
<b>Official Symbol</b>	Tnfrsf25 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	tumor necrosis factor receptor superfamily, member 25 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1934667</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000024793</a>
<b>Gene type</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>Also known as</b>	APO-3, DDR3, DR3, LARD, TR3, TRAMP, Tnfrsf12, WSL-1, WSL-LR, Wsl
<b>Expression</b>	Ubiquitous expression in mammary gland adult (RPKM 5.7), spleen adult (RPKM 5.0) and 25 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

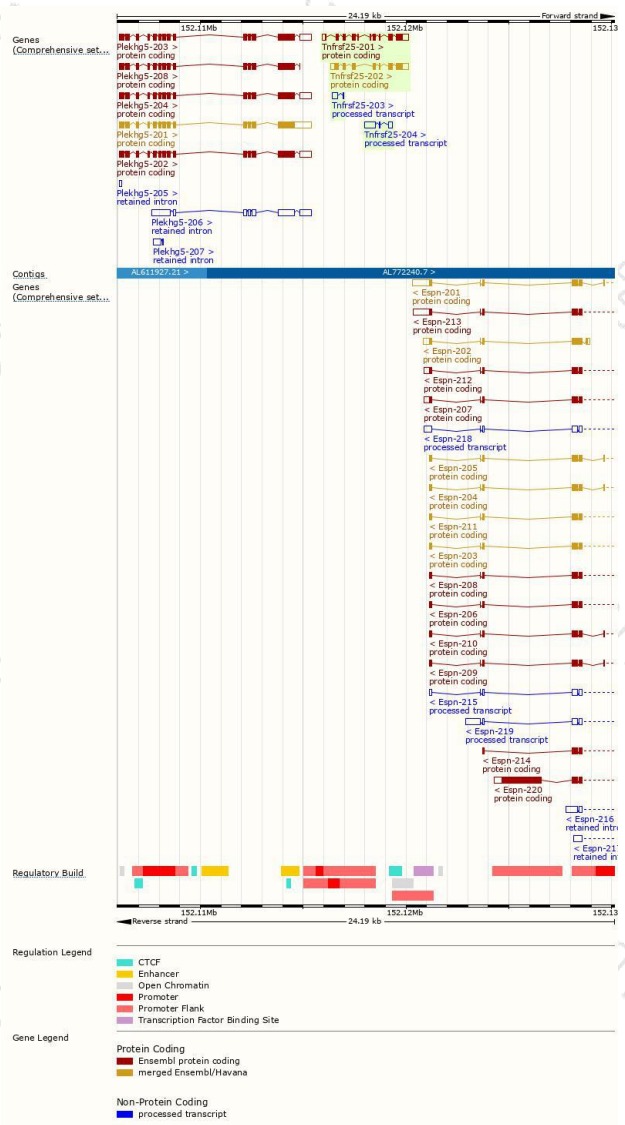
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tnfrsf25-201	<a href="#">ENSMUST00000025706.9</a>	1661	<a href="#">411aa</a>	Protein coding	<a href="#">CCDS71527</a>	<a href="#">B1AWN9</a>	TSL:1 GENCODE basic APPRIS ALT2
Tnfrsf25-202	<a href="#">ENSMUST00000035275.7</a>	1603	<a href="#">387aa</a>	Protein coding	<a href="#">CCDS38983</a>	<a href="#">Q8VD70</a>	TSL:1 GENCODE basic APPRIS P3
Tnfrsf25-204	<a href="#">ENSMUST00000153619.1</a>	731	No protein	Processed transcript	-	-	TSL:3
Tnfrsf25-203	<a href="#">ENSMUST00000127111.1</a>	346	No protein	Processed transcript	-	-	TSL:3

The strategy is based on the design of *Tnfrsf25-201* transcript,the transcription is shown below:



# Genomic location distribution





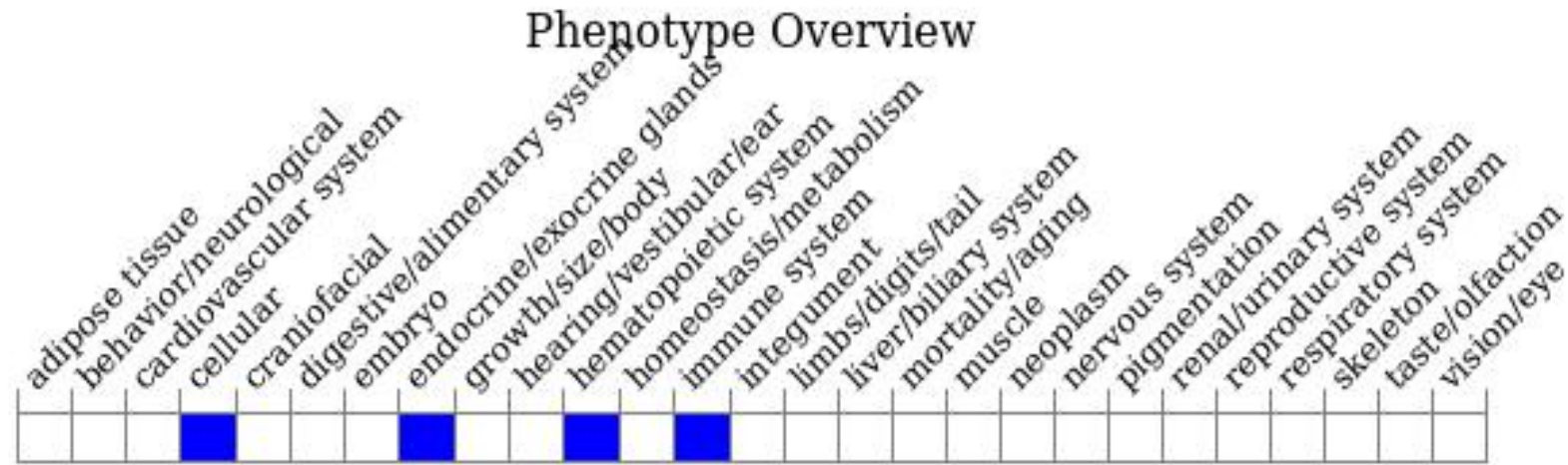
# Protein domain



集萃药康  
GemPharmatech



# 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 mutant mice show no developmental defects or impairments of early thymocyte development. Negative selection and anti-CD3-induced apoptosis, however, are significantly impaired.

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

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