

# **Tiel Cas9-CKO Strategy**

Designer: Reviewer:

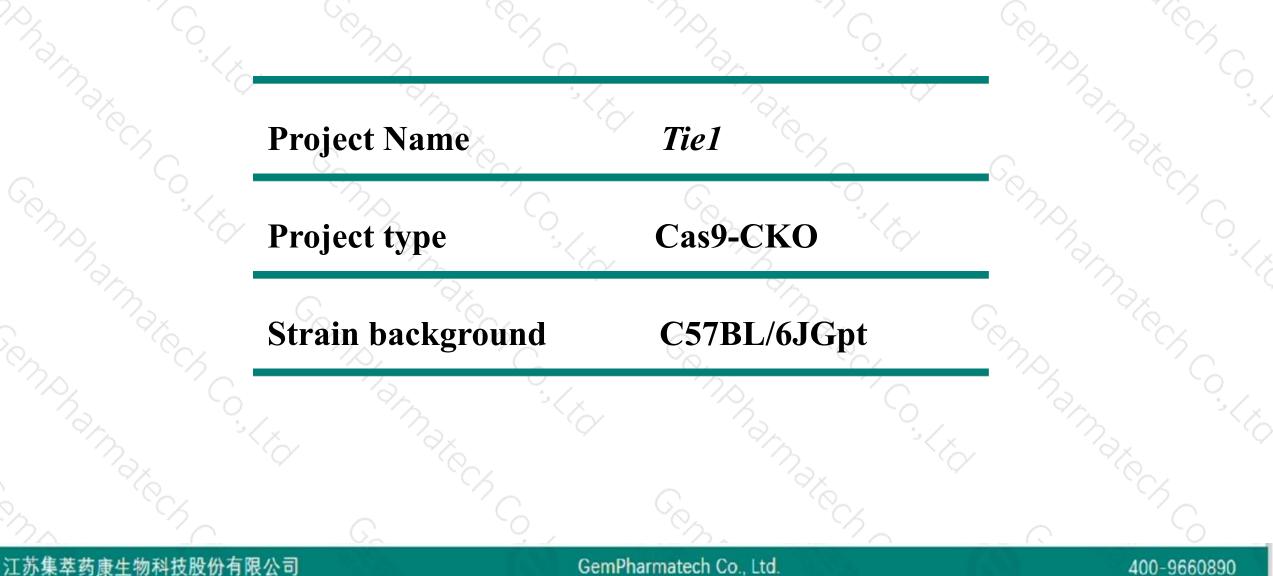
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**Design Date:** 

Daohua Xu Huimin Su 2019-9-5

## **Project Overview**





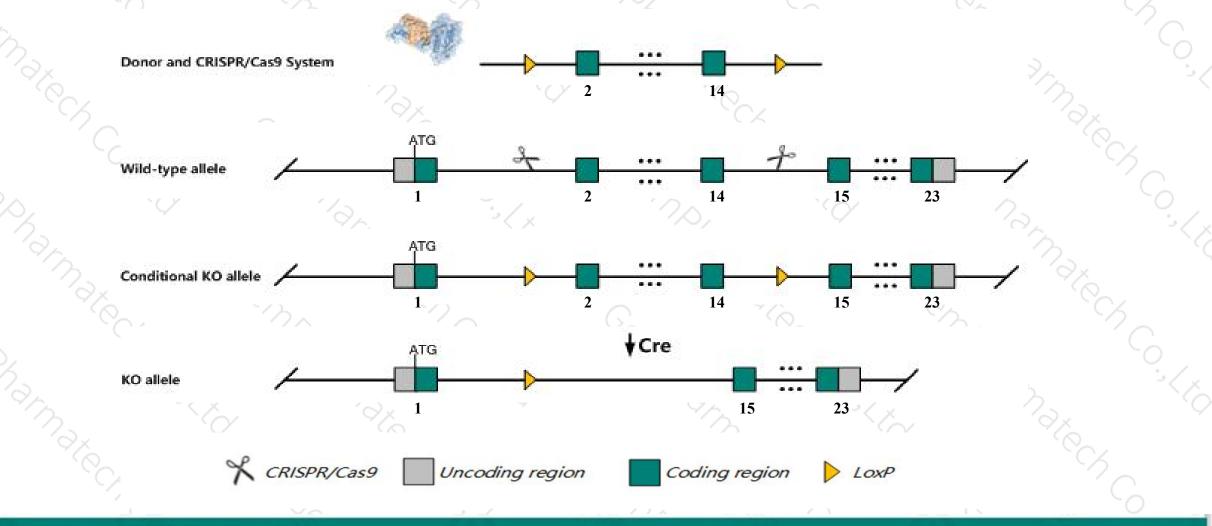
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### **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Tie1* gene. The schematic diagram is as follows:



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The *Tiel* gene has 3 transcripts. According to the structure of *Tiel* gene, exon2-exon14 of *Tiel-201* (ENSMUST00000047421.5) transcript is recommended as the knockout region. The region contains 2339bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Tie1* 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.



- According to the existing MGI data, Homozygous mutation of this gene results in embryonic or neonatal lethality, hemorrhages, edema, increased vascular branching, and abnormal vascular endothelial cell development. Mice homozygous for a hypomorphic allele exhibit dilated and disorganized lymphatic vessel, edema, and hemorrhage.
- The *Tiel* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

# **Gene information (NCBI)**



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### Tie1 tyrosine kinase with immunoglobulin-like and EGF-like domains 1 [Mus musculus (house mouse)]

Gene ID: 21846, updated on 12-Mar-2019

### Summary

Tie1 provided by MGI
tyrosine kinase with immunoglobulin-like and EGF-like domains 1 provided by MGI
MGI:MGI:99906
Ensembl:ENSMUSG00000033191
protein coding
VALIDATED
Mus musculus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Muridae; Murinae; Mus; Mus
D430008P04Rik, TIE, tie-1
Biased expression in lung adult (RPKM 126.3), adrenal adult (RPKM 37.3) and 14 other tissues See more
human all

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tie1-201	ENSMUST00000047421.5	4091	<u>1134aa</u>	Protein coding	CCDS18553	<u>Q06806</u>	TSL:1 GENCODE basic APPRIS P1
Tie1-203	ENSMUST00000184261.7	4234	<u>647aa</u>	Protein coding	-	Q6PAP2	TSL:2 GENCODE basic
Tie1-202	ENSMUST00000153286.1	2851	No protein	IncRNA	-	2	TSL:1

The strategy is based on the design of *Tie1-201* transcript, The transcription is shown below

< Tie1-201 protein coding

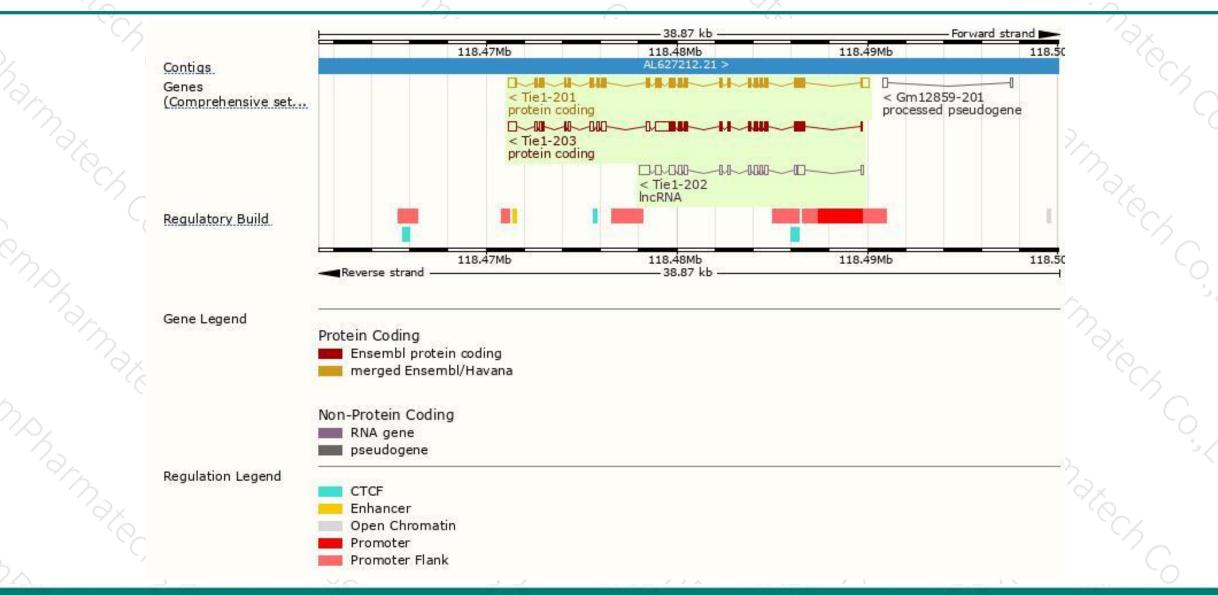
Reverse strand

— 18.87 kb -

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### **Genomic location distribution**



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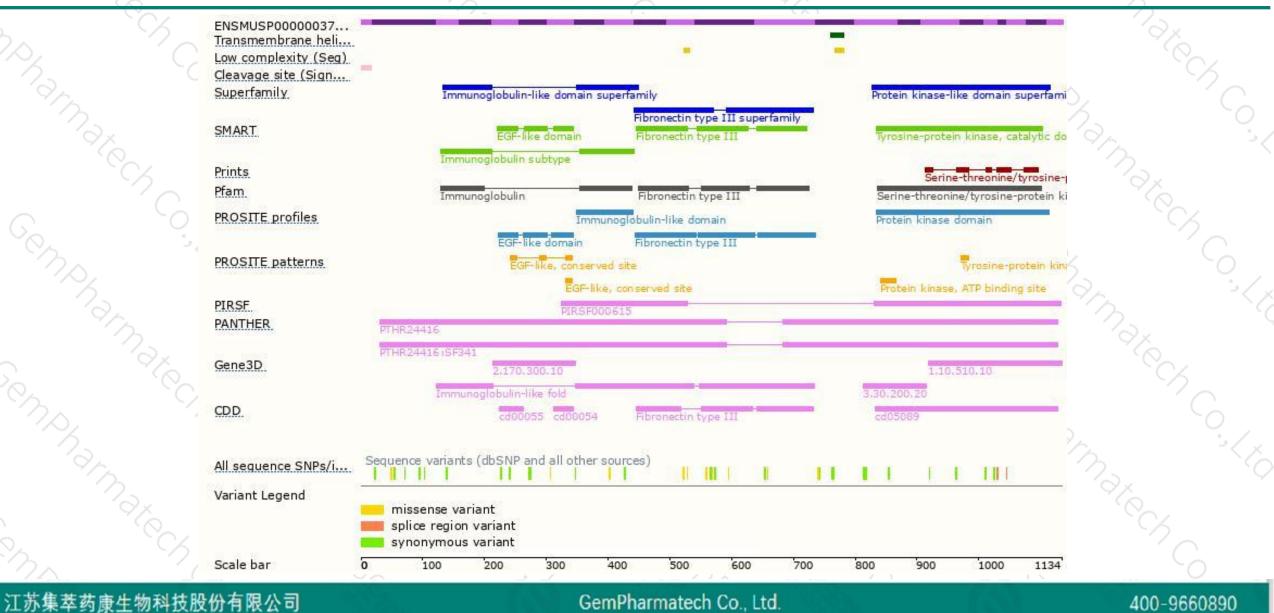
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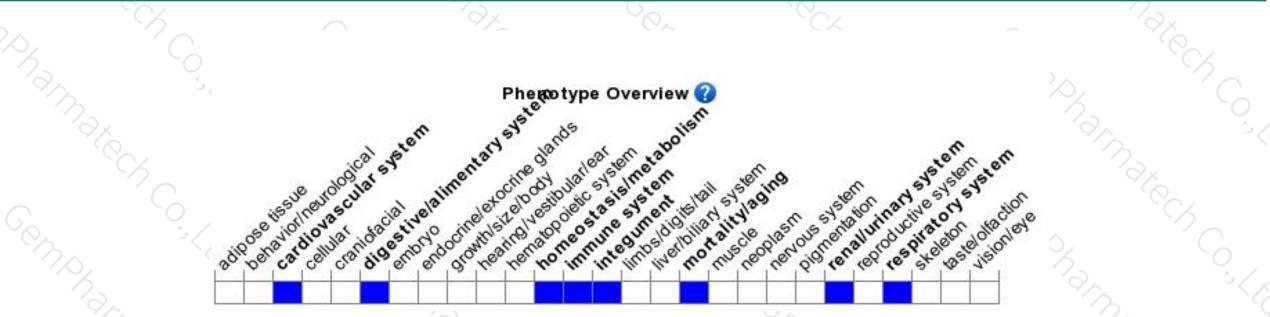
### **Protein domain**





### 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 embryonic or neonatal lethality, hemorrhages, edema, increased vascular branching, and abnormal vascular endothelial cell development. Mice homozygous f hypomorphic allele exhibit dilated and disorganized lymphatic vessel, edema, and hemorrhage.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



