

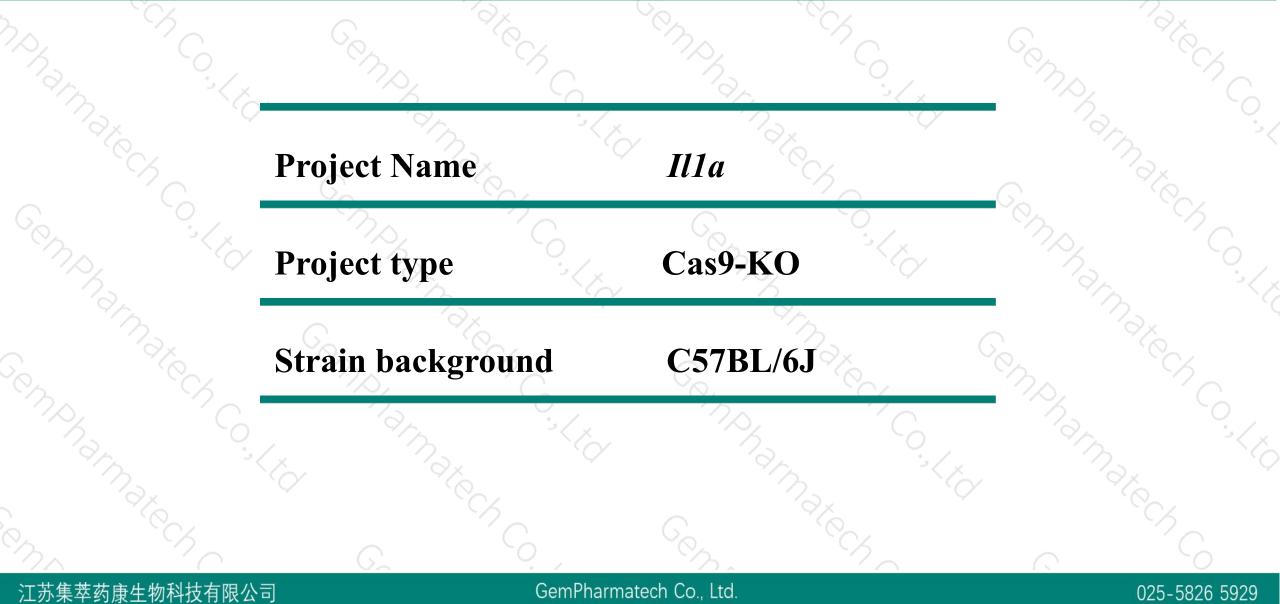
# Illa Cas9-KO Strategy

Cemphamatech, Comphannated Co Designer: Yanhua Shen

enphamatech,

### **Project Overview**

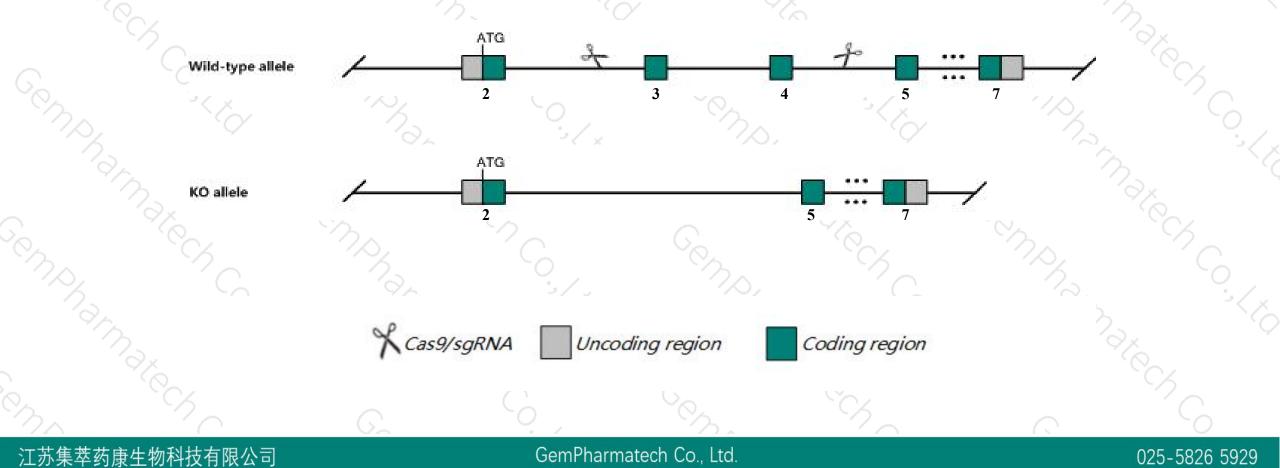




# **Knockout** strategy



This model will use CRISPR/Cas9 technology to edit the *Il1a* gene. The schematic diagram is as follows:





- The Illa gene has 1 transcript. According to the structure of Illa gene, exon3-exon4 of Illa-201 (ENSMUST0000028882.1) transcript is recommended as the knockout region. The region contains 278bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Il1a* 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.



025-5826 5929

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit abnormal tumor vascularization, decreased metastatic potential, and decreased interleukin-1 beta secretion.
- The Illa gene coincides with the Gm14023 gene, and the exon4 and exon5 of the Gm14023 gene are deleted at the same time, and it is uncertain whether the phenotype of the mouse is caused by knockout of which gene.
- The *Illa* gene is located on the Chr2. 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.

#### 江苏集萃药康生物科技有限公司

#### GemPharmatech Co., Ltd,

# **Gene information (NCBI)**



\$ ?

#### II1a interleukin 1 alpha [Mus musculus (house mouse)]

Gene ID: 16175, updated on 9-Apr-2019

#### Summary

Official Symbol	II1a provided by MGI
Official Full Name	interleukin 1 alpha provided by <u>MGI</u>
<b>Primary source</b>	MGI:MGI:96542
See related	Ensembl:ENSMUSG0000027399
Gene type	protein coding
<b>RefSeq status</b>	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	ll-1a
Expression	Broad expression in liver E18 (RPKM 1.2), liver E14 (RPKM 1.1) and 20 other tissues See more
Orthologs	human all

#### 江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.



## **Transcript information (Ensembl)**



025-5826 5929

The gene has 1 transcript, and the transcript is shown below:

Name 🍦	Transcript ID 👙	bp 🌢	Protein 🖕	Biotype 🖕	CCDS 🖕	UniProt 🝦	Flags 🖕		
II1a-201	ENSMUST0000028882.1	1974	<u>270aa</u>	Protein coding	<u>CCDS16725</u> &	<u>P01582</u> & <u>Q3U0Y6</u> &	TSL:1	GENCODE basic	APPRIS P1

The strategy is based on the design of *Il1a-201* transcript, The transcription is shown below

#### < Il1a-201 protein coding

Reverse strand

- 10.36 kb -

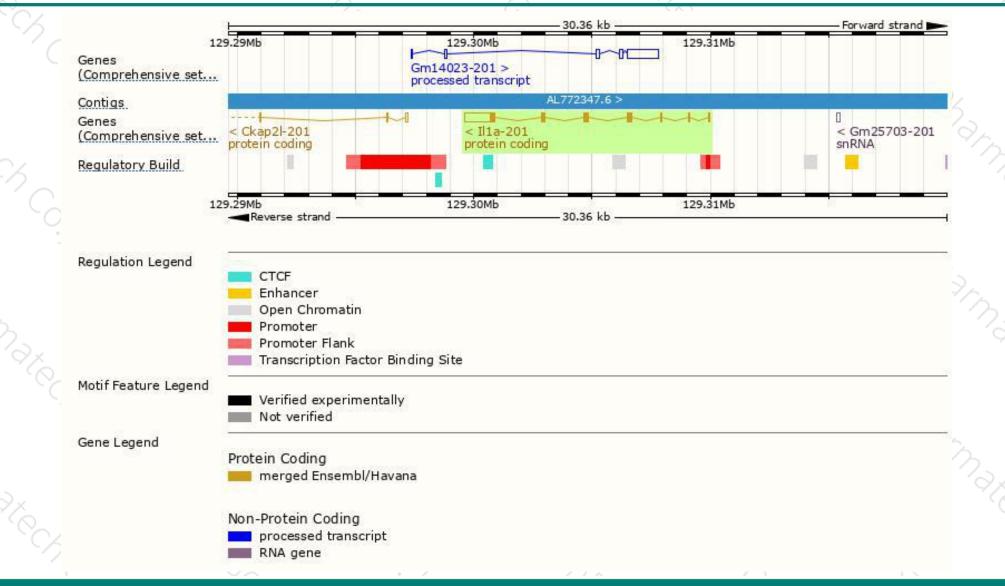
#### 江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

### **Genomic location distribution**



025-5826 5929

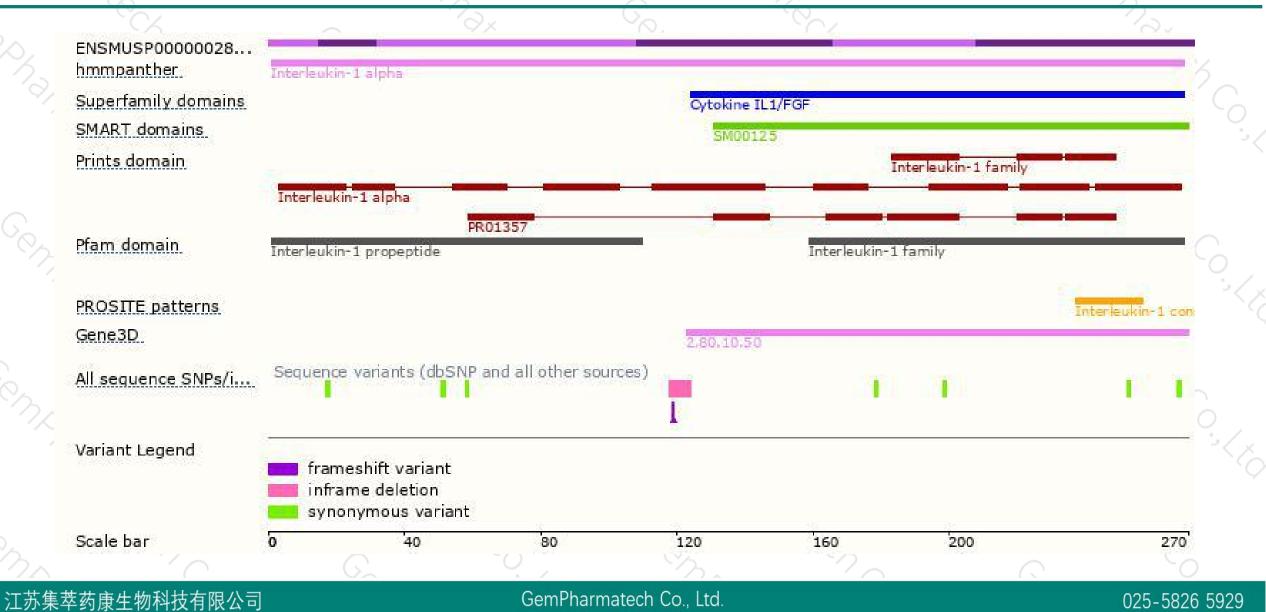


江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

### **Protein domain**

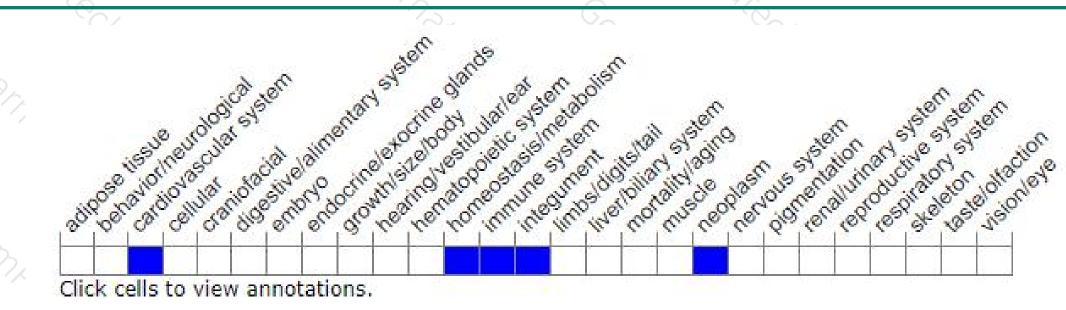




### Mouse phenotype description(MGI)



025-5826 5929



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 abnormal tumor vascularization, decreased metastatic potential, and decreased interleukin-1 beta secretion.



If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



