

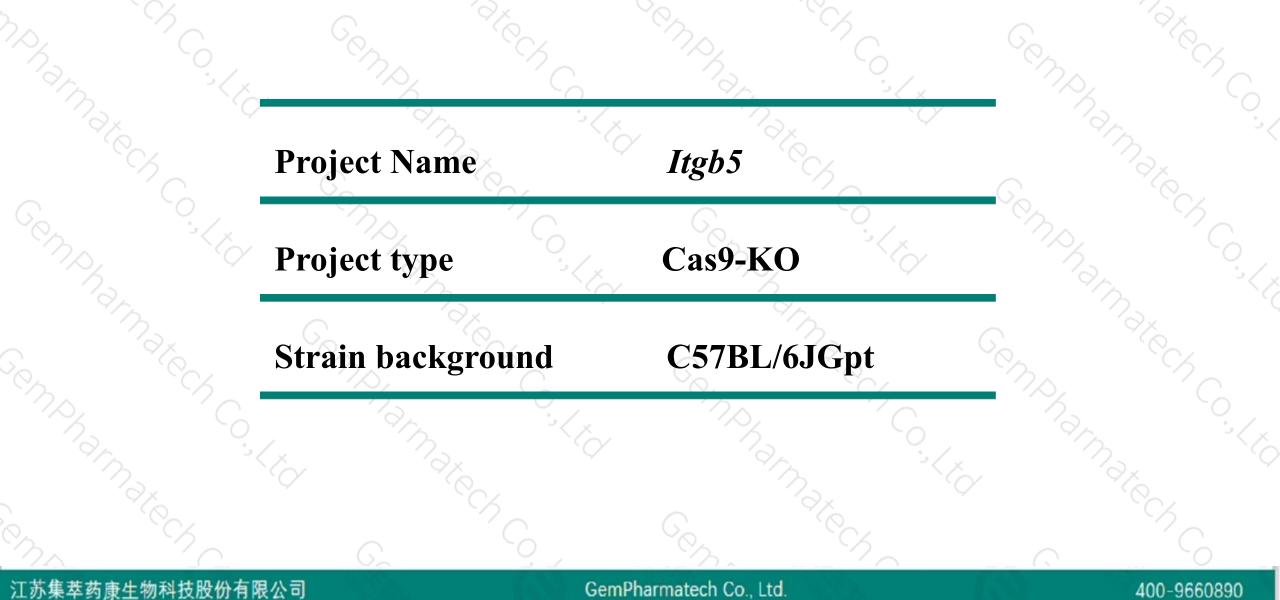
# Itgb5 Cas9-KO Strategy

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empharmatec.

### **Project Overview**

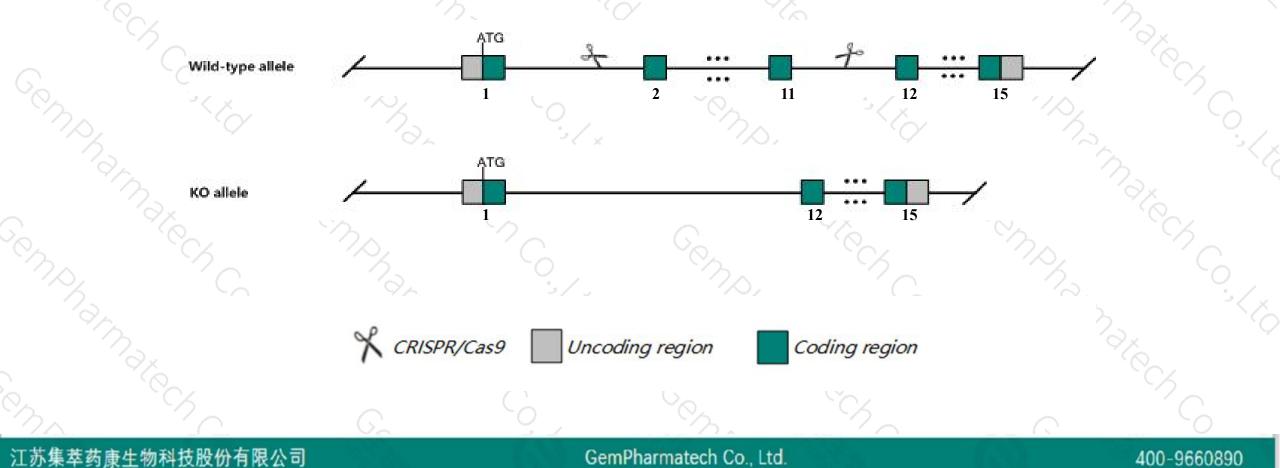




# **Knockout** strategy



This model will use CRISPR/Cas9 technology to edit the *Itgb5* gene. The schematic diagram is as follows:





- The *Itgb5* gene has 7 transcripts. According to the structure of *Itgb5* gene, exon2-exon11 of *Itgb5-201* (ENSMUST00000069345.5) transcript is recommended as the knockout region. The region contains 1846bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Itgb5* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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, Homozygotes for a targeted null mutation do not appear to differ from normal in respect to development, reproduction, adenovirus infection, or wound healing. Mutant keratinocytes do show reduced migration on, and adhesion to, vitronectin in vitro.
- ➤ Transcript *Itgb5*-206 may not be affected.
- The strategy will delete the start codon of transcrip *Itgb5*-206, which may form a new ATG and translate unknown proteins.
  The *Itgb5* gene is located on the Chr16. 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)**



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### Itgb5 integrin beta 5 [Mus musculus (house mouse)]

Gene ID: 16419, updated on 16-Feb-2019

#### Summary

Official SymbolItgb5 provided by MGIOfficial Full Nameintegrin beta 5 provided by MGIPrimary sourceMGI:MGI:96614See relatedEnsembl:ENSMUSG0000022817Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;<br/>Muroidea; Murinae; Mus; MusAlso knownasAA475909, Al874634, ESTM23, [b]-5, [b]5A, [b]5B, beta-5, beta5ExpressionUbiquitous expression in adrenal adult (RPKM 97.5), ovary adult (RPKM 81.3) and 28 other tissues<br/>See more

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# **Transcript information (Ensembl)**



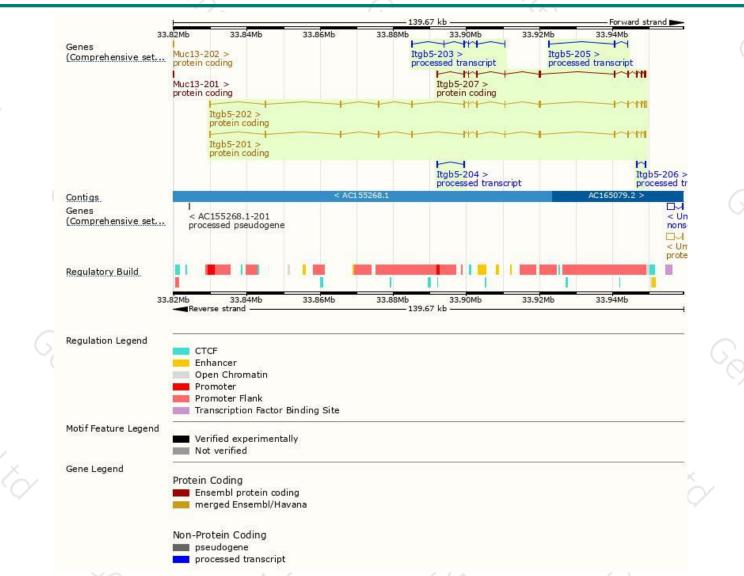
### The gene has 7 transcripts, all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ltgb5-202	ENSMUST00000115028.10	3282	<u>799aa</u>	Protein coding	CCDS49835	<u>Q6PE70</u>	TSL:1 GENCODE basic APPRIS P1
ltgb5-201	ENSMUST0000069345.5	3056	<u>816aa</u>	Protein coding	CCDS28135	<u>G5E8F8</u>	TSL:1 GENCODE basic
ltgb5-207	ENSMUST00000232262.1	2130	<u>486aa</u>	Protein coding	1943	A0A338P795	GENCODE basic
ltgb5-203	ENSMUST00000134262.7	631	No protein	Processed transcript	N <u>1</u> 5	<u>84</u>	TSL:2
ltgb5-205	ENSMUST00000151930.1	364	No protein	Processed transcript	1751	65	TSL:3
ltgb5-206	ENSMUST00000231409.1	297	No protein	Processed transcript	( <del>.</del>	. <del>.</del>	
ltgb5-204	ENSMUST00000148462.1	265	No protein	Processed transcript	(23)	84-	TSL:5

The strategy is based on the design of *Itgb5-201* transcript, The transcription is shown below

### **Genomic location distribution**



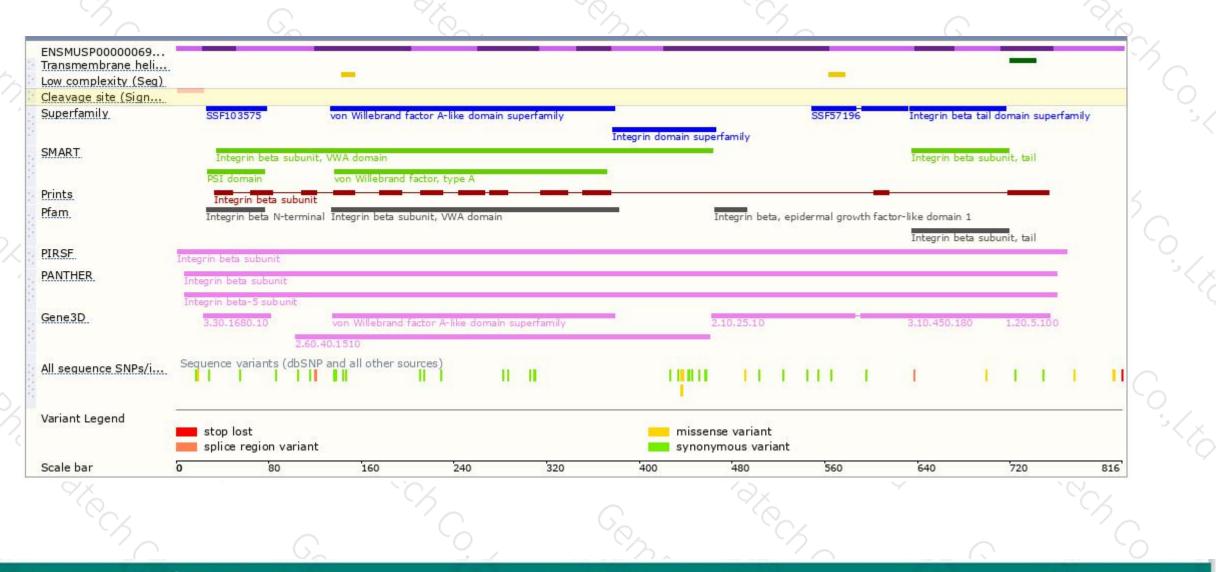


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### **Protein domain**



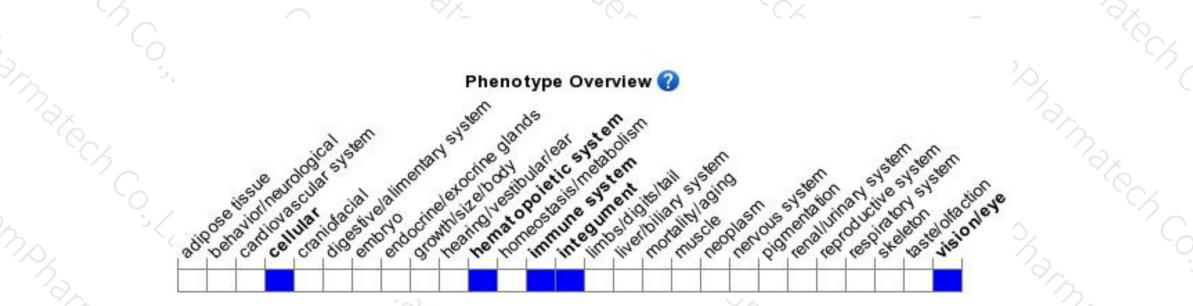


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### Mouse phenotype description(MGI)





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, Homozygotes for a targeted null mutation do not appear to differ from normal in respect to development, reproduction, adenovirus infection, or wound healing. Mutant keratinocytes do show reduced migration on, and adhesion to, vitronectin in vitro.

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



