

# **Gdf1** Cas9-CKO Strategy

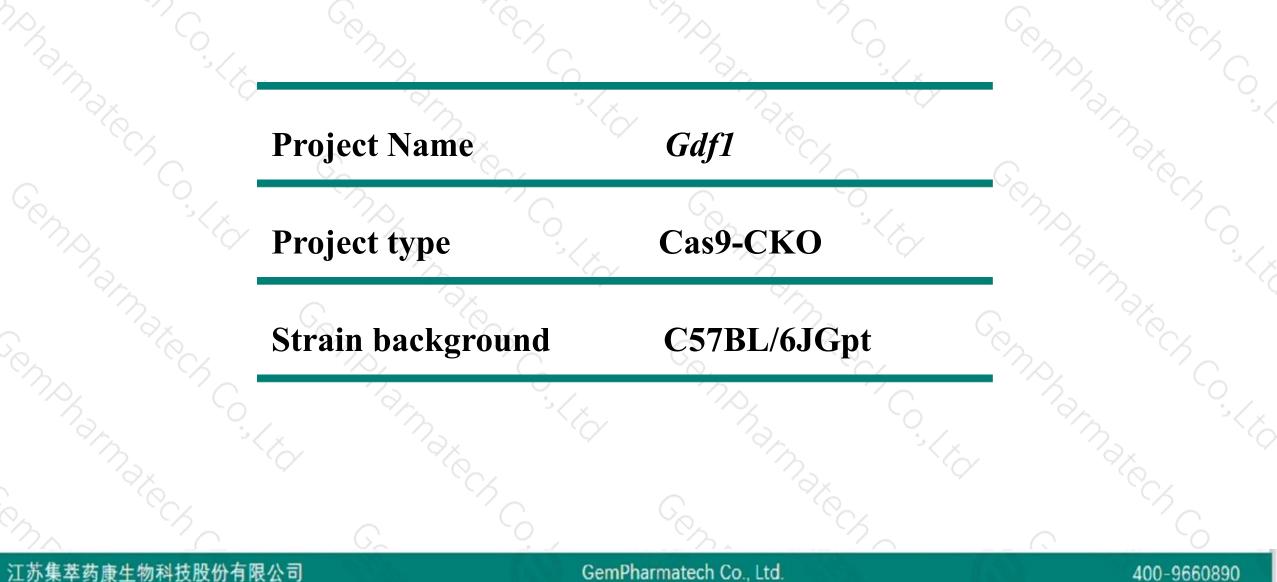
Designer: Daohua Xu

**Reviewer: Xueting Zhang** 

Design Date: 2020-11-18

## **Project Overview**





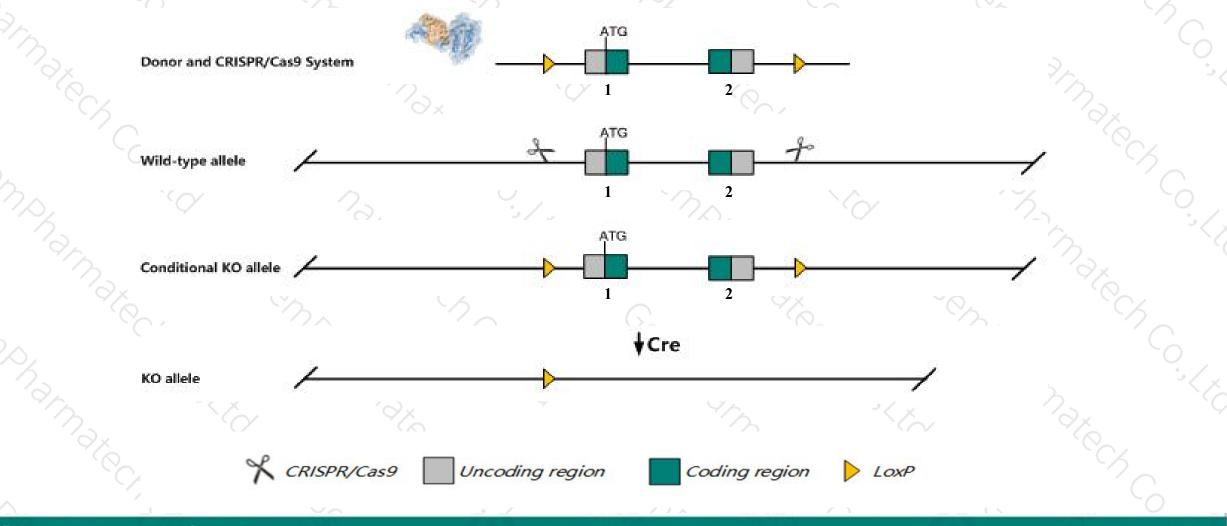
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## **Conditional Knockout strategy**



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Gdf1* gene. The schematic diagram is as follows:



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> The *Gdf1* gene has 1 transcript. According to the structure of *Gdf1* gene, exon1-exon2 of *Gdf1*-201(ENSMUST00000207684.1) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Gdf1* 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 null mice display partially penetrant late embryonic lethality, neonatal lethality, situs inversus, right pulmonary isomerism, and other left-right patterning defects, cardiac septal defects, annular pancreas, and abnormal spleen morphology.

> The KO region contains functional region of the *Upf1,Cers1* gene.Knockout the region may affect the function of *Upf1,Cers1* gene.

The *Gdf1* gene is located on the Chr8. 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)



400-9660890

#### Gdf1 growth differentiation factor 1 [Mus musculus (house mouse)]

Gene ID: 14559, updated on 13-Mar-2020

#### - Summary

<b>Official Symbol</b>	Gdf1 provided by MGI
Official Full Name	growth differentiation factor 1 provided by MGI
Primary source	MGI:MGI:95683
See related	Ensembl:ENSMUSG00000087408 Ensembl:ENSMUSG00000109523
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Al385651, Gdf-1
Summary	This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate each subunit of the disulfide-linked homodimer. This protein is involved in the establishment of left-right asymmetry in early embryogenesis and in neural development in later embryogenesis. This protein is translated from a monocistronic mRNA early in development, and from a bicistronic mRNA in later stages that also encodes ceramide synthase 1. [provided by RefSeq, Jul 2016]
Expression	Biased expression in frontal lobe adult (RPKM 27.2), cortex adult (RPKM 24.4) and 11 other tissues See more
Orthologs	human all

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## **Transcript information (Ensembl)**



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

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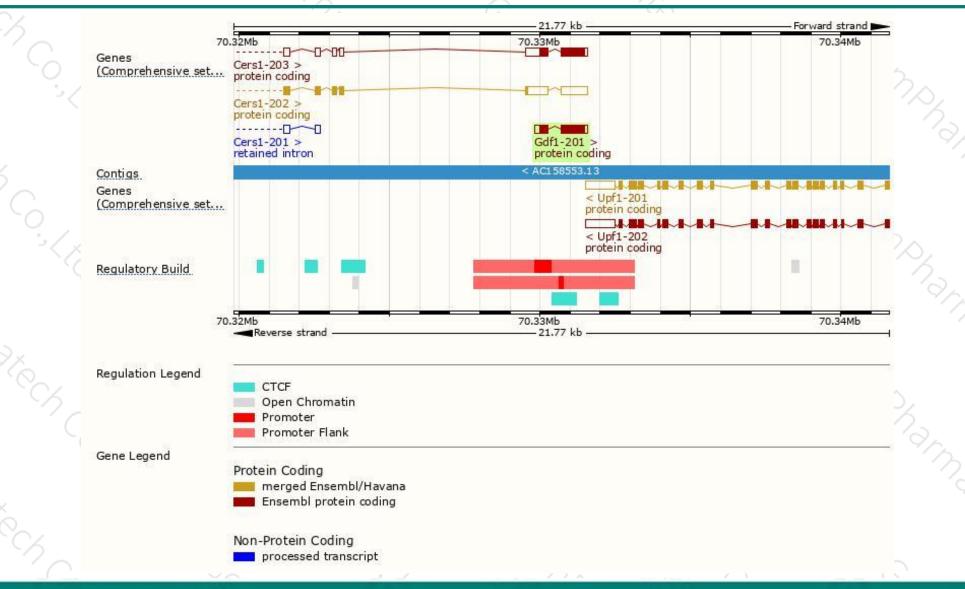
Gdf1-201 ENSMUST00000207684.1 1349 357aa Protein coding CCDS52571 P20863 TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a generatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a generatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a generatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a generatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a generatively spliced transcript based on a range of computational methods to identify the most functionally important transcript(s) of a generatively spliced transcript based on a range of computational methods to identify the most functionally important transcript(s) of a generatively spliced transcript based on a range of computational methods to identify the most functionally important transcript(s) of a generatively spliced transcript based on a range of computational methods to identify the most functionally important transcript(s) of a generatively spliced transcript based on a range of computational methods to identify the most functional methods to identi	
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The strategy is based on the design of *Gdf1-201* transcript, the transcription is shown below:

		— 1.77 kb —		— Forward strand 🗩
Gdf1-201 > protein coding				
2	62	$\sim 20$	10	 <u>`</u> 0

### **Genomic location distribution**





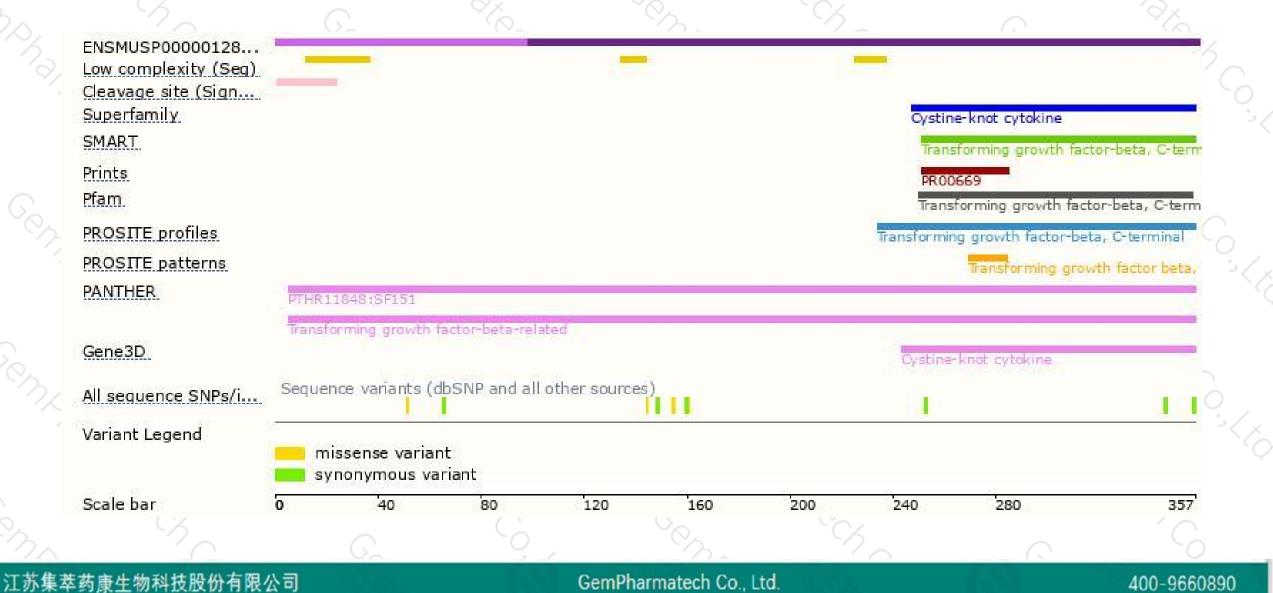
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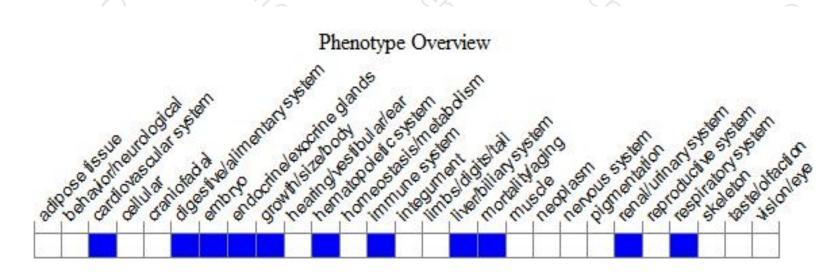
### **Protein domain**





## 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 null mice display partially penetrant late embryonic lethality, neonatal lethality, situs inversus, right pulmonary isomerism, and other left-right patterning defects, cardiac septal defects, annular pancreas, and abnormal spleen morphology.

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



