

# **Tigar Cas9-CKO Strategy**

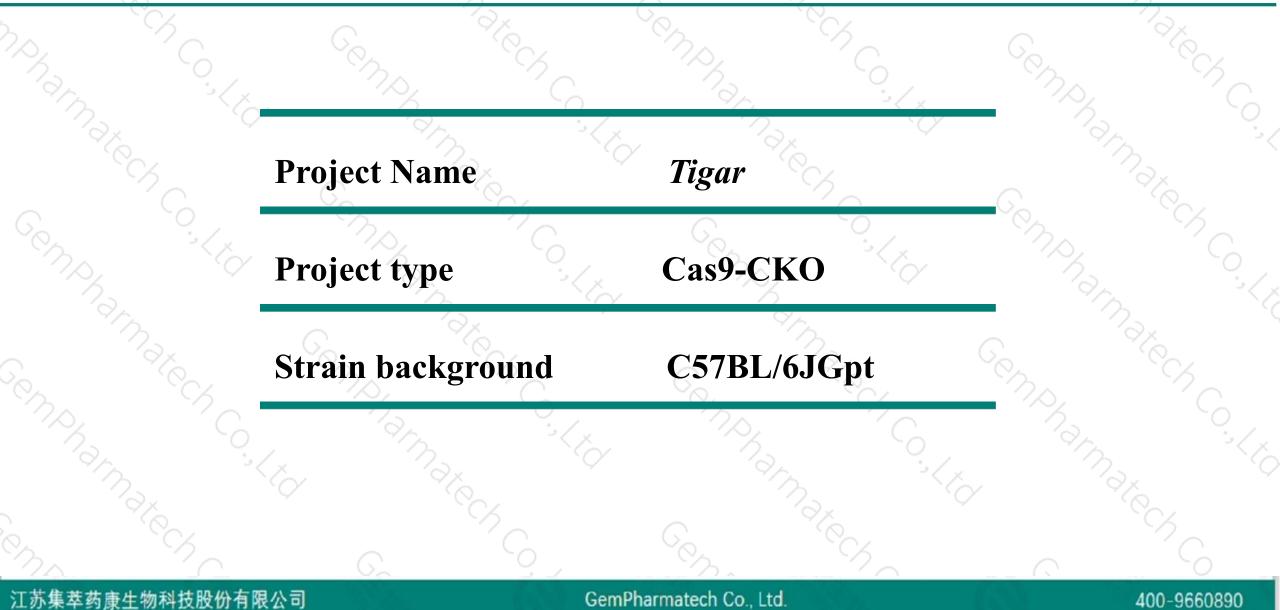
Designer: Design Date:

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Daohua Xu 2019-8-5

## **Project Overview**



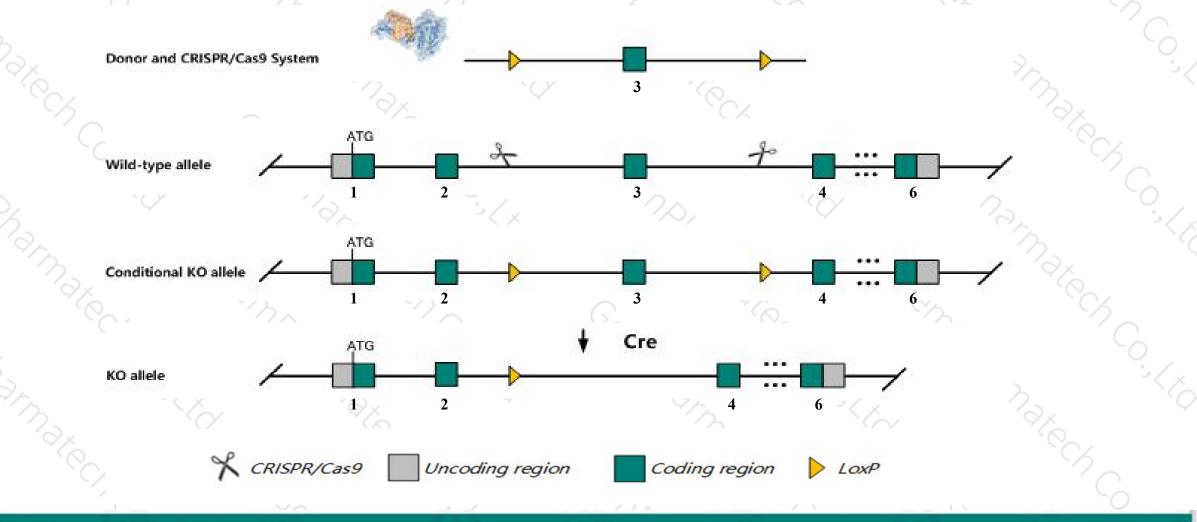


## **Conditional Knockout strategy**



400-9660890

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



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The *Tigar* gene has 2 transcripts. According to the structure of *Tigar* gene, exon3 of *Tigar-201* (ENSMUST00000039913.8) transcript is recommended as the knockout region. The region contains 122bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Tigar* 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, Mice homozygous for a knock-out allele exhibit improved response to myocardial infarction associated with increased autophagy, mitophagy, levels of reactive oxygen species production and decreased mitochondria DNA damage. Mice homozygous for a different allele exhibit impaired crypt regeneration.
- The *Tigar* gene is located on the Chr6. 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.

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## **Gene information (NCBI)**



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#### Tigar Trp53 induced glycolysis repulatory phosphatase [Mus musculus (house mouse)]

Gene ID: 319801, updated on 5-Feb-2019

#### Summary

Official Symbol	Tigar provided by MGI
Official Full Name	Trp53 induced glycolysis repulatory phosphatase provided by MGI
<b>Primary source</b>	MGI:MGI:2442752
See related	Ensembl:ENSMUSG0000038028
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	9630033F20Rik, AA793651, Al595337, C79710, C85509
Expression	Ubiquitous expression in heart adult (RPKM 8.1), cerebellum adult (RPKM 5.2) and 28 other tissues See more
Orthologs	human all

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The gene has 2 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tigar-201	ENSMUST0000039913.8	3651	<u>269aa</u>	Protein coding	CCDS20563	B2RWB7 Q8BZA9	TSL:1 GENCODE basic APPRIS P1
Tigar-202	ENSMUST00000200988.1	855	<u>49aa</u>	Nonsense mediated decay		A0A0J9YV11	TSL:5

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

< Tigar-201 protein coding

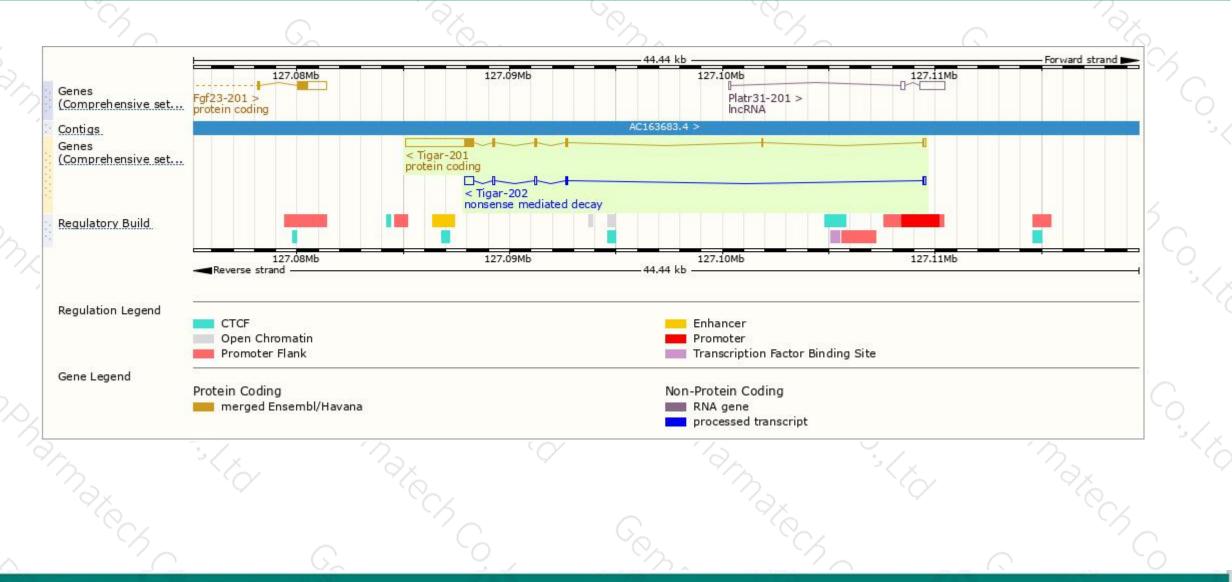
Reverse strand -

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24.43 kb

### **Genomic location distribution**



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## **Protein domain**



ENSMUSP00000048... Conserved Domains hmmpanther

Superfamily domains SMART domains Pfam domain

PROSITE patterns PIRSF domain Gene3D

All sequence SNPs/i...

Variant Legend

Scale bar

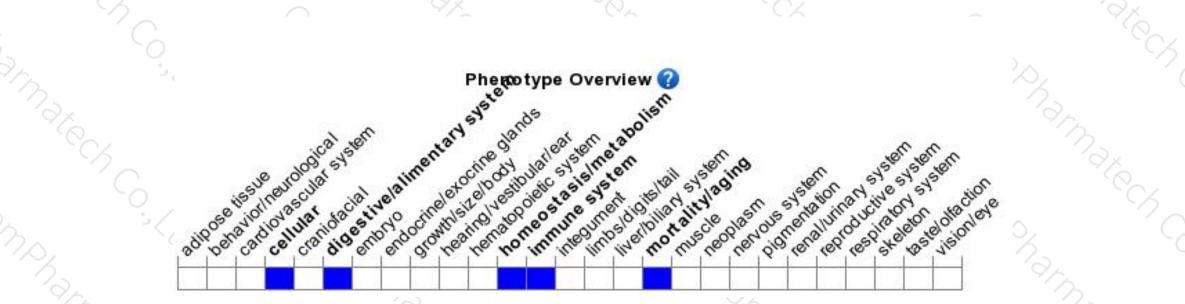
PTHR2302	19					-
PTHR2302	29:9F41			144.5		
Histidine	phosphatase supe	rfamily				
Histidine	phosphatase super	family, clade-1				
Histidin	e phosphatase supe	rfamily, clade-1				
Phosph	oglycerate/bisphos	phoglycerate mutase	a, active site			
PIRSFOOD					-	
ALC: NOT THE OWNER OF THE OWNER	phosphatase superf	amily				
Sequence	variants (dbSNP	and all other sour	ces)	1.15	1	111
	ense variant nymous variant					
	40	80	120	160	200	26
0				(		

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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, Mice homozygous for a knock-out allele exhibit improved response to myocardial infarction associated with increased autophagy, mitophagy, levels of reactive oxygen species production and decreased mitochondria DNA damage. Mice homozygous for a different allele exhibit impaired crypt regeneration.

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



