

# Fap Cas9-CKO Strategy

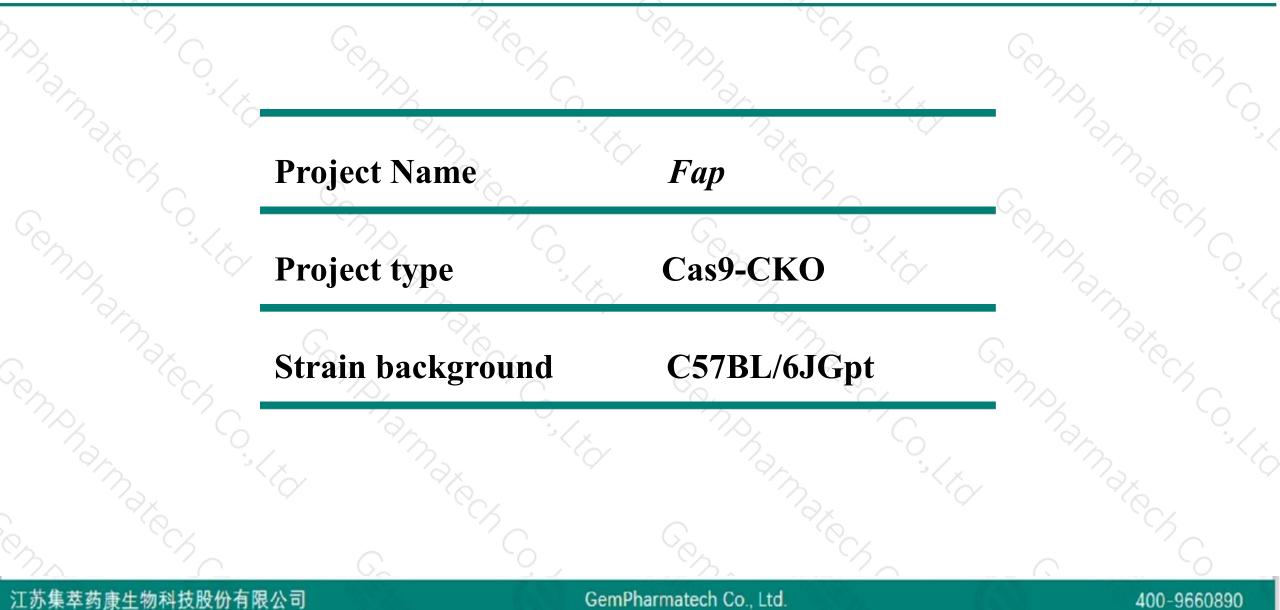
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**Reviewer: Lingyan Wu** 

**Design Date: 2020-7-2** 

## **Project Overview**



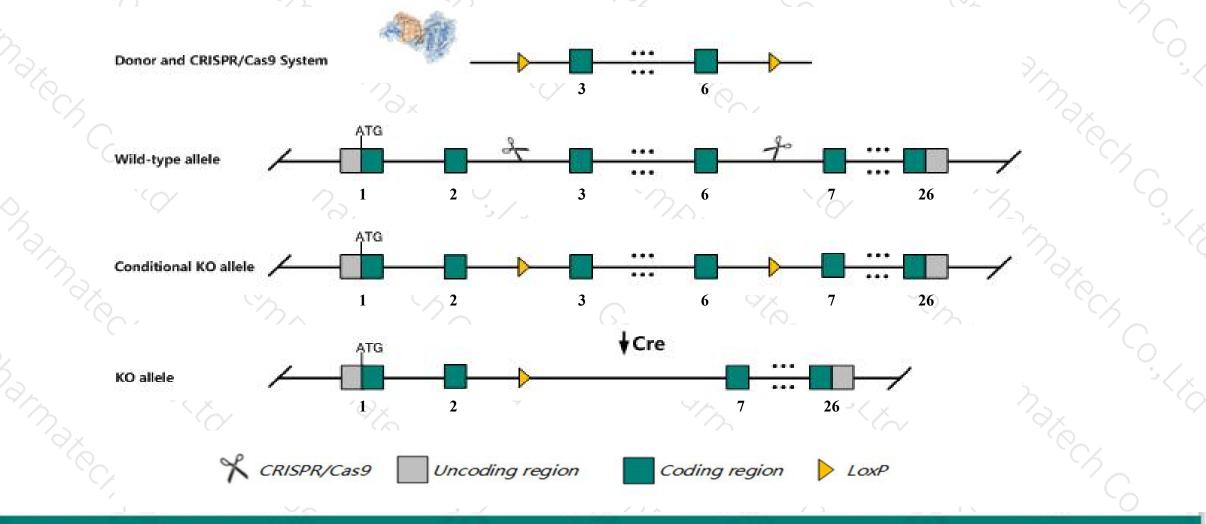


## **Conditional Knockout strategy**



400-9660890

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



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> The Fap gene has 9 transcripts. According to the structure of Fap gene, exon3-exon6 of Fap-202(ENSMUST00000102732.9) transcript is recommended as the

knockout region. The region contains 322bp coding sequence. Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify *Fap* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 targeted null mutations exhibit no discernable phenotype; mice are viable and fertile with no change in cancer susceptibility.
- The *Fap* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

## Gene information (NCBI)



2

#### Fap fibroblast activation protein [Mus musculus (house mouse)]

Gene ID: 14089, updated on 13-Mar-2020

#### Summary

Official Symbol Fap provided by MGI Official Full Name fibroblast activation protein provided by MGI Primary source MGI:MGI:109608 See related Ensembl:ENSMUSG0000000392 Gene type protein coding RefSeg 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 SIMP This gene belongs to the serine protease family. The encoded protein is an inducible cell-surface bound glycoprotein specifically expressed in Summary tumor-associated fibroblasts and pericytes of epithelial tumors and has protease and gelatinase activity. The protein plays a role in remodeling of the extracellular matrix (ECM) and may affect tumorigenesis and tissue repair. Alternately spliced transcript variants of this gene are described in the literature (PMID 9139873), but the full-length sequence of these variants is not available. [provided by RefSeq, Apr 2013] Biased expression in limb E14.5 (RPKM 7.4), mammary gland adult (RPKM 2.8) and 9 other tissuesSee more Expression Orthologs human all

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## **Transcript information (Ensembl)**



#### The gene has 9 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fap-202	ENSMUST00000102732.9	2751	<u>761aa</u>	Protein coding	CCDS16067	P97321	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 gene. APPRIS P
Fap-209	ENSMUST00000174448.7	2271	<u>756aa</u>	Protein coding	-	<u>P97321</u>	TSL:5 GENCODE basic
Fap-208	ENSMUST00000174234.7	2211	<u>736aa</u>	Protein coding	920	G3UXR2	TSL:5 GENCODE basic
Fap-201	ENSMUST0000000402.15	2187	<u>728aa</u>	Protein coding	1.2	<u>P97321</u>	TSL:5 GENCODE basic
Fap-207	ENSMUST00000173745.1	503	<u>63aa</u>	Protein coding	-	G3UZ14	CDS 3' incomplete TSL:3
Fap-203	ENSMUST00000128139.2	467	<u>47aa</u>	Nonsense mediated decay	-	G3UYG3	CDS 5' incomplete TSL:5
Fap-205	ENSMUST00000152085.8	1269	No protein	Processed transcript	-		TSL:5
Fap-206	ENSMUST00000172676.7	2867	No protein	Retained intron	12	120	TSL:1
Fap-204	ENSMUST00000136297.7	2343	No protein	Retained intron		-	TSL:1

The strategy is based on the design of *Fap-202* transcript, the transcription is shown below:

< Fap-202 protein coding

Reverse strand

- 73.13 kb -

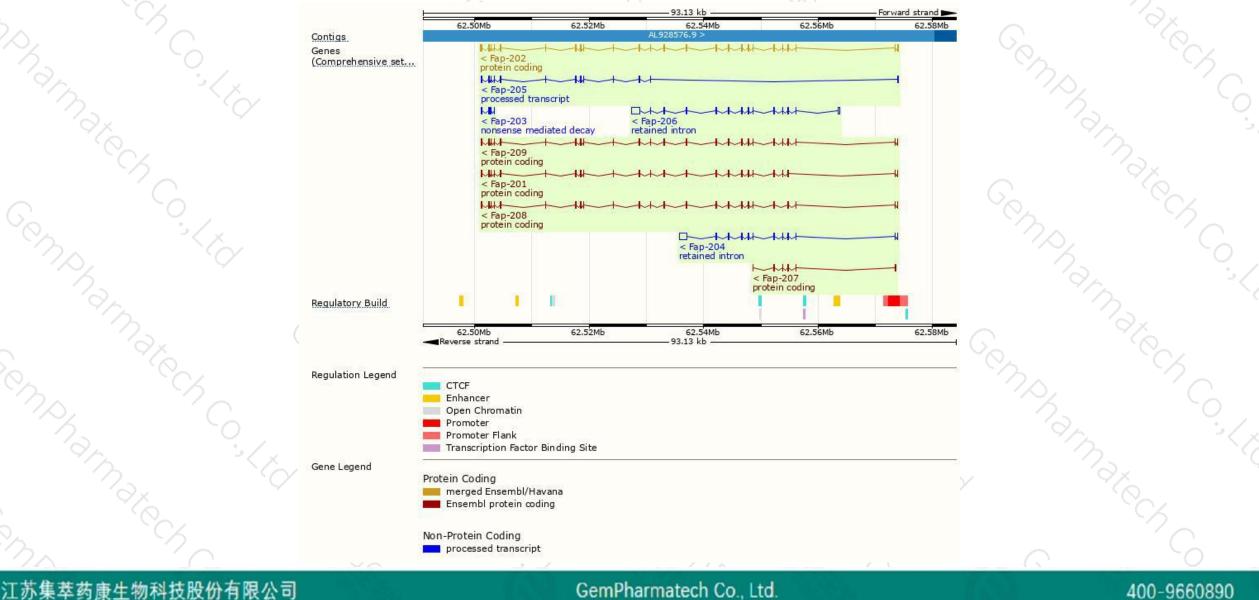
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### **Genomic location distribution**





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## **Protein domain**



5.97	ENSMUSP00000099 Transmembrane heli Superfamily	SSF82171				Alpha/Beta hyd	rolase fold	97. 200		
	Pfam.	Dipe	ptidylpeptidase IV, N-te	erminal domain		Peptida	se S9, prolyl o	ligopeptidase, c		
?/	PROSITE patterns PANTHER	Dipeptidyl peptidase F/	A.P:				Peptidase S9,	serine active si		
	Gene3D	PTHR11731 Dipleptidylpeptid	ase IV, N-terminal dom	iain superfamily		Alpha/Beta hydrolase fold				
32	All sequence SNPs/i Variant Legend	Sequence variants (	dbSNP and all other	sources)	ĩ					
	Scale bar	synonymous v o 80	ariant 160 240	320	400	480 560	640	761		

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



