# Cx3cl1 Cas9-CKO Strategy

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Reviewer: Shilei Zhu

Date: 2018/9/7

# **Project Overview**



**Project Name** 

Cx3cl1

**Project type** 

Cas9-CKO

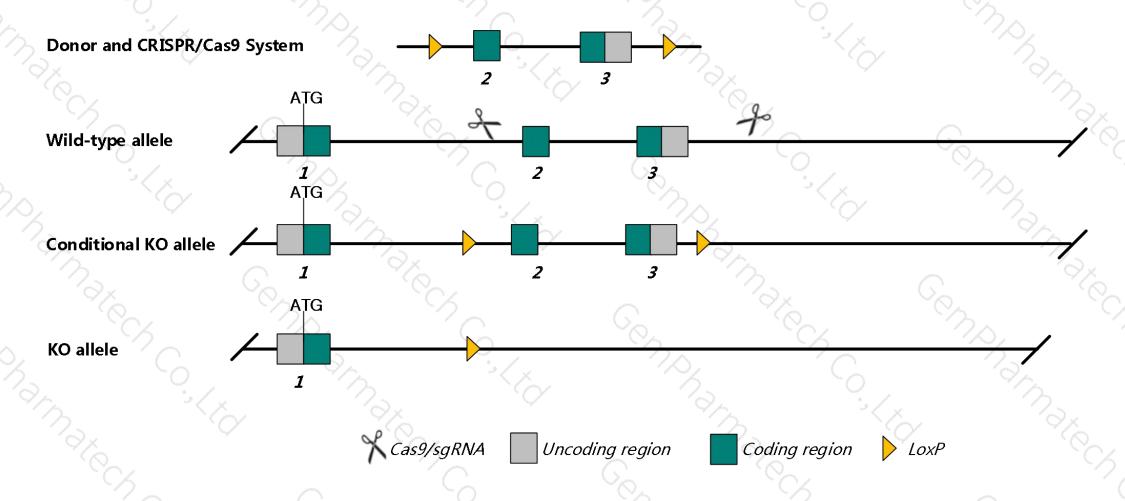
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Cx3cl1* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Cx3cl1* gene has 6 transcripts. According to the structure of *Cx3cl1* gene, exon2-3 of *Cx3cl1*-201 (ENSMUST00000034230.6) transcript is recommended as the knockout region. The region contains most 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 *Cx3cl1* 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 or cell types.

### **Notice**



- According to the existing MGI data, Mice homozygous for a knock-out allele show a specific reduction in Gr1(low) monocyte levels, and increased neuronal cell loss in a neurotoxin (MPTP)-induced model of Parkinson disease. Mice homozygous for a different knock-out allele are less susceptible to cerebral ischemia-reperfusion injury.
- ➤ The *Cx3cl1* 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Gene information (NCBI)



#### Cx3cl1 chemokine (C-X3-C motif) ligand 1 [ Mus musculus (house mouse) ]

Gene ID: 20312, updated on 13-Mar-2020

#### Summary

Official Symbol Cx3cl1 provided by MGI

Official Full Name chemokine (C-X3-C motif) ligand 1 provided by MGI

Primary source MGI:MGI:1097153

See related Ensembl: ENSMUSG00000031778

RefSeq status 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 CX3C; Cxc3; Scyd1; ABCD-3; AB030188; Al848747; D8Bwg0439e

Expression Biased expression in cortex adult (RPKM 170.7), frontal lobe adult (RPKM 132.2) and 14 other tissues See more

Orthologs human all

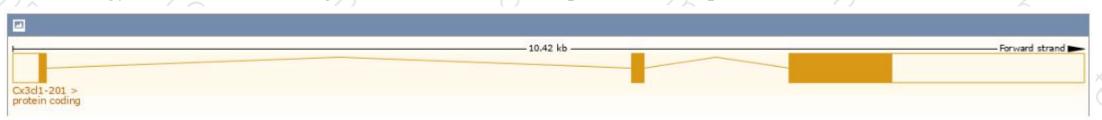
# Transcript information (Ensembl)



The gene has 6 transcripts, and all transcripts are shown below:

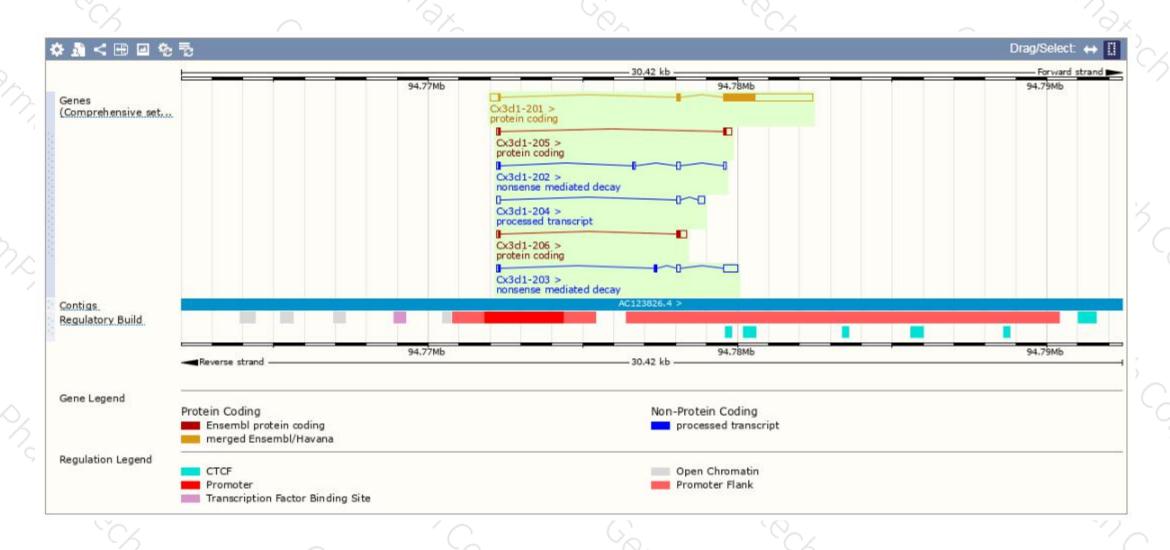
Name	Transcript ID	bp 🛊	Protein	Biotype	CCDS	UniProt	Flags
Cx3cl1-201	ENSMUST00000034230.6	3315	<u>395aa</u>	Protein coding	CCDS22548@	<u>O35188</u> ₽	TSL:1 GENCODE basic APPRIS P
Cx3cl1-206	ENSMUST00000211956.1	470	<u>64aa</u>	Protein coding	-	A0A1D5RMK8₽	TSL:2 GENCODE basic
Cx3cl1-205	ENSMUST00000211947.1	377	<u>45aa</u>	Protein coding		A0A1D5RLW9₽	TSL:5 GENCODE basic
Cx3cl1-203	ENSMUST00000150307.1	780	<u>57aa</u>	Nonsense mediated decay	:-	D6RCV0條	TSL:3
Cx3cl1-202	ENSMUST00000135970.1	432	<u>37aa</u>	Nonsense mediated decay	-	E0CXC3₽	TSL:3
Cx3cl1-204	ENSMUST00000151783.8	462	No protein	Processed transcript	2	- 24	TSL:3

The strategy is based on the design of *Cx3cl1*-201 transcript, The transcription is shown below



### Genomic location distribution





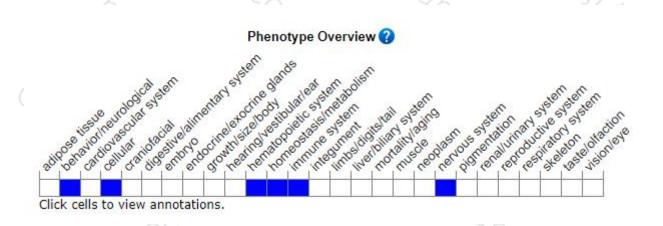
### Protein domain





### 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, Mice homozygous for a knock-out allele show a specific reduction in Gr1(low) monocyte levels, and increased neuronal cell loss in a neurotoxin (MPTP)-induced model of Parkinson disease.

Mice homozygous for a different knock-out allele are less susceptible to cerebral ischemia-reperfusion injury.

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





