Cx3cr1-iCre Cas9-KI Strategy

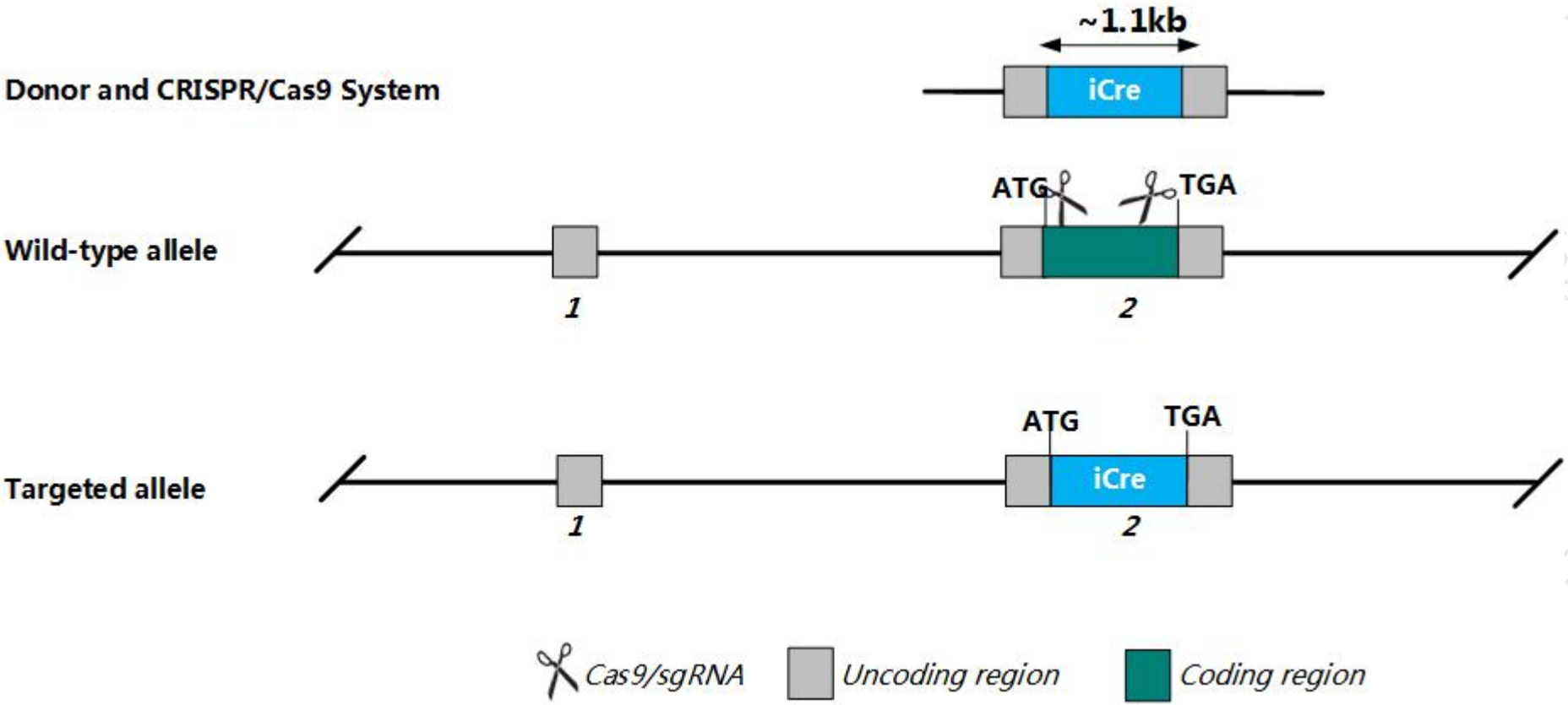
Designer:	**Xiaojing Li**
Design Date:	**2019-8-16**
Reviewer	**JiaYu**

Project Overview

Project Name	***Cx3cr1-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Cx3cr1* gene. The schematic diagram is as follows:



Technical routes

- The *Cx3cr1* gene has 3 transcripts.According to the structure of *Cx3cr1* gene, *Cx3cr1-201*(ENSMUST00000064165.4) is selected for presentation of the recommended strategy.

- *Cx3cr1-201* gene has 2 exons, with the ATG start codon in exon2 and TGA stop codon in exon2.

- We make *Cx3cr1-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage at exon 2 near the start codon ATG and exon 2 near the stop codon TGA, and create a DSB(double-strand break). Such breaks will be repaired, and result in *iCre* inserted into the targeted gene replacing exon2 coding region by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, Age related retinal degeneration with abnormal subretinal microglial cell accumulation in one homozygous null mice. Other null mice shows impaired monocyte recruitment after vascular injury, kidney ischemia and reperfusion, and bacterial infection of the instestine.
- Expression of Cx3cr1-Cre may be mainly expressed in the mononuclear phagocyte system.
- Insertion of iCre directly destroys the expression of target genes.
- The Cx3cr1 gene is located on the Chr9. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information （NCBI)

集萃药康 GemPharmatech

Cx3cr1 chemokine (C-X3-C motif) receptor 1 [*Mus musculus* (house mouse)]

Gene ID: 13051, updated on 13-Aug-2019

Summary

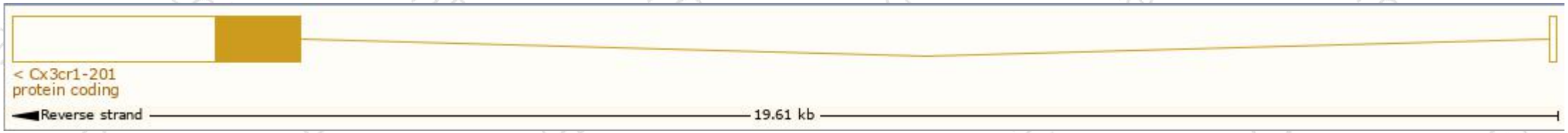
Official Symbol	Cx3cr1 provided by MGI
Official Full Name	chemokine (C-X3-C motif) receptor 1 provided by MGI
Primary source	MGI:MGI:1333815
See related	Ensembl:ENSMUSG00000052336
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Ubiquitous expression in cortex adult (RPKM 8.3), frontal lobe adult (RPKM 7.0) and 27 other tissues See more
Orthologs	human all

Transcript information （Ensembl）

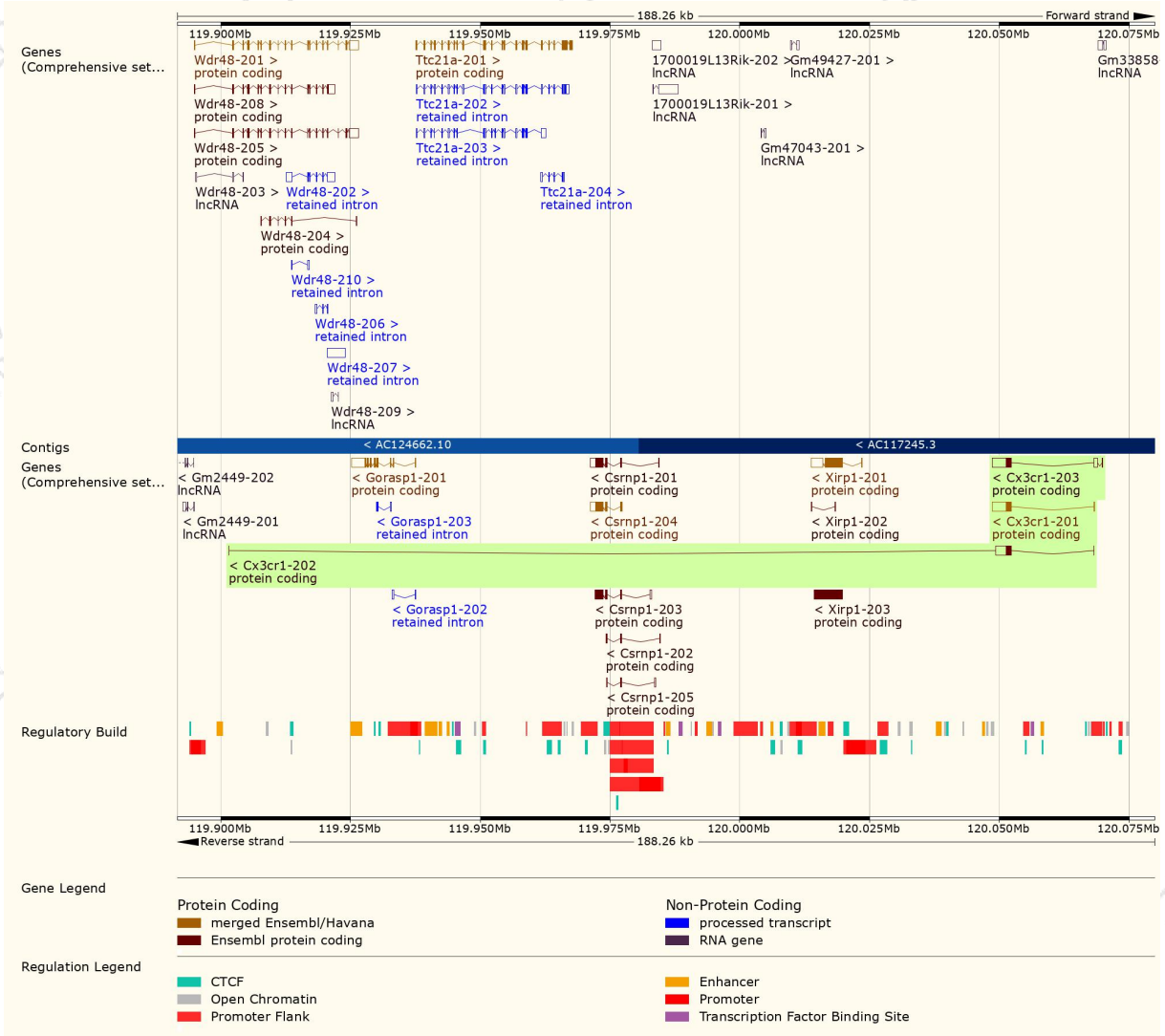
The gene has 3 transcripts, and all transcripts are shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cx3cr1-201	ENSMUST00000064165.4	3753	354aa	Protein coding	CCDS40806	Q543X3 Q9Z0D9	TSL:1 GENCODE basic APPRIS P1
Cx3cr1-202	ENSMUST00000177637.1	3156	354aa	Protein coding	CCDS40806	Q543X3 Q9Z0D9	TSL:5 GENCODE basic APPRIS P1
Cx3cr1-203	ENSMUST00000215016.1	4493	354aa	Protein coding	CCDS40806	Q543X3 Q9Z0D9	TSL:5 GENCODE basic APPRIS P1

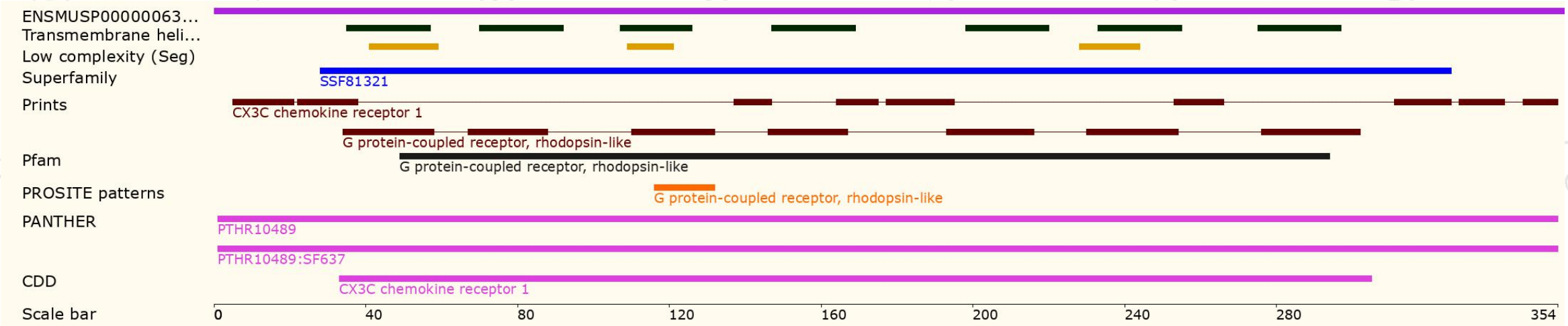
The strategy is based on the design of *Cx3cr1-201* transcript,The transcription is shown below



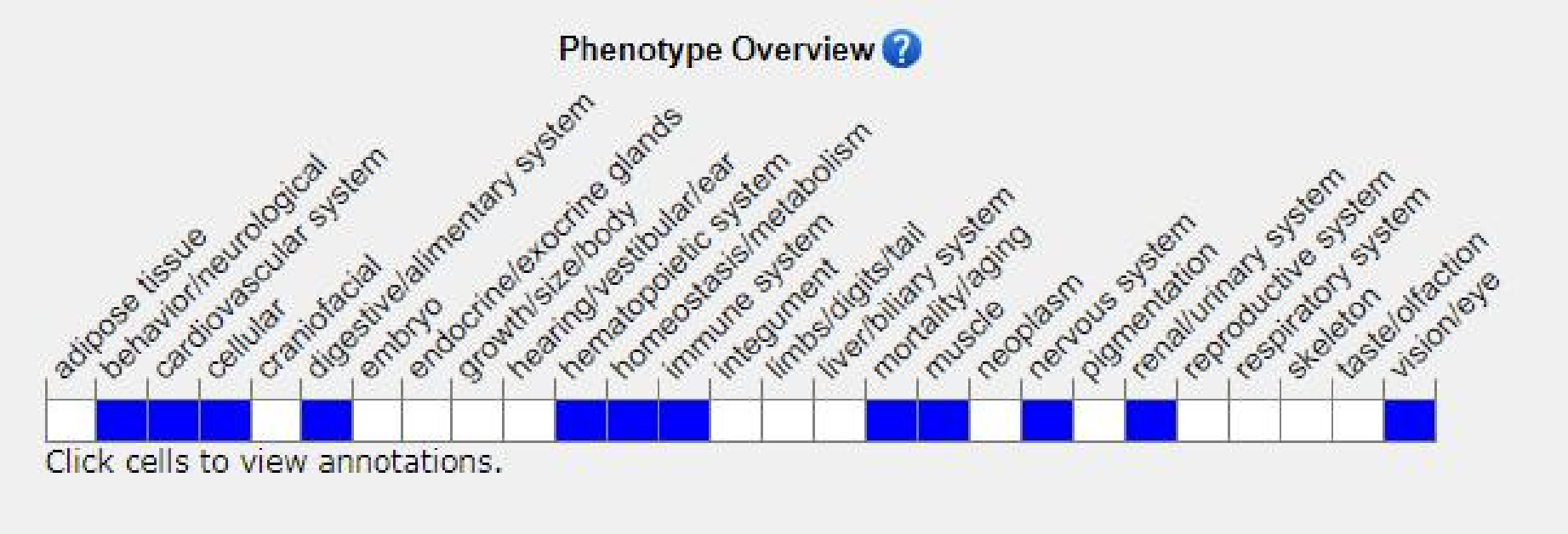
Genomic location distribution



Protein domain



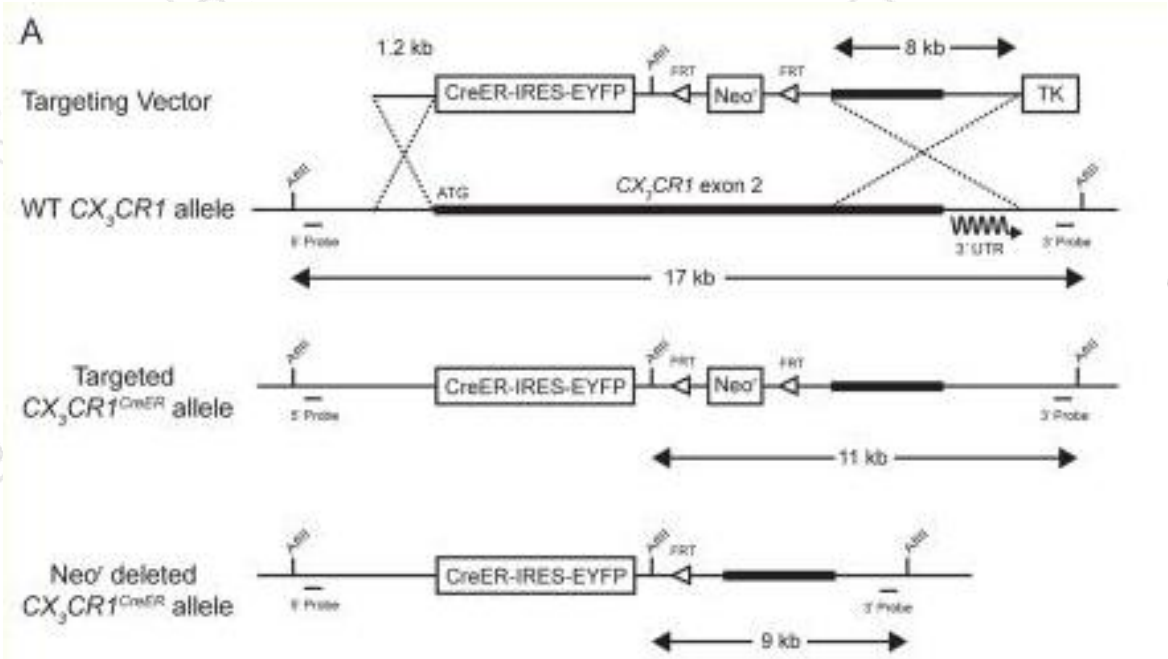
Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/marker/MGI:1333815) .

Age related retinal degeneration with abnormal subretinal microglial cell accumulation in one homozygous null mice. Other null mice shows impaired monocyte recruitment after vascular injury, kidney ischemia and reperfusion, and bacterial infection of the instestine.

Existing Model Reporting 1



Generation of CX_3CR1^{CreER} mice to manipulate gene expression in microglia

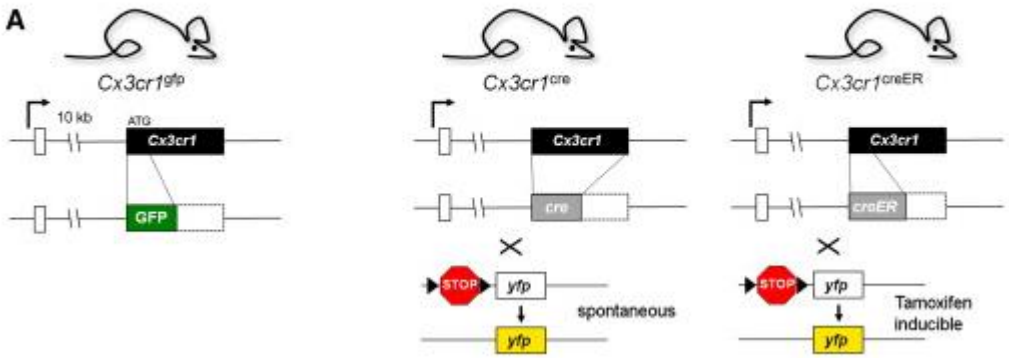
In order to manipulate microglial function, we generated CX_3CR1^{CreER} mice expressing tamoxifen-inducible Cre recombinase (CreER) in microglia under the control of the endogenous *CX3CR1* promoter (Fig. 1A). The gene encoding CreER was followed by an IRES-EYFP element and the insertion site was chosen according to previous studies in which the *CX3CR1* coding region was replaced with EGFP (Jung et al., 2000). Correct targeting of the *CX3CR1* locus and subsequent FLP recombinase mediated excision of

Molecular Note

A targeting vector was designed to replace exon 2 of the chemokine (C-X3-C) receptor 1 (Cx3cr1) gene with a cre/ERT2 (cre recombinase fused to an estrogen receptor ligand binding domain) coding sequence, followed by an internal ribosome entry site (IRES) and an enhanced yellow fluorescent protein (EYFP). A frt-flanked neomycin resistance cassette, in reverse orientation to the gene, wasalso inserted downstream of the EYFP sequence. Flp-mediated recombination excised the neomycin resistance sequence.

[1] Parkhurst CN; Yang G; Ninan I; Savas JN; Yates JR 3rd; Lafaille JJ; Hempstead BL; Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell; 2013; 155 (7) 1596-609;

Existing Model Reporting 2



Allele Symbol: *Cx3cr1*$^{tm1.1(cre)Jung}$ MGI

Allele Name	targeted mutation 1.1, Steffen Jung
Allele Type	Targeted (Recombinase-expressing)
Allele Synonym(s)	Cx3cr1^{cre}
Gene Symbol and Name	*Cx3cr1* MGI, chemokine (C-X3-C motif) receptor 1
Gene Synonym(s)	CCRL1; CMKBRL1; CMKDR1; GPR13; GPRV28; Rbs11; V28
Promoter	*Cx3cr1*, chemokine (C-X3-C motif) receptor 1, mouse, laboratory
Expressed Gene	*cre*, cre recombinase, bacteriophage P1
Site of Expression	Cre recombinase is expressed in the mononuclear phagocyte system.
Strain of Origin	C57BL/6N
Chromosome	9
Molecular Note	A cre recombinase cassette with a floxed neo was introduced into a BAC by RedE/T recombineering, replacing the coding exon, prior to homologous recombination to target the gene in ES cells. Cre-mediated recombination removed the selection cassette.

Dual Origins of Resident Macrophages

To exploit the pronounced activity of the CX_3CR1 promoter for the study of the mononuclear phagocyte system, we manipulated the murine CX_3CR1 loci to harbor Cre recombinase genes. Specifically, we replaced the *Cx3cr1* gene with genes encoding either *cre* recombinase or a *cre* recombinase fusion to a mutant estrogen ligand-binding domain that requires the presence of the estrogen antagonist tamoxifen for activity (*Cre*ERT2) (Metzger et al., 1995), yielding *Cx3cr1*^{cre} and *Cx3cr1*^{creER} mice (Figure 1A).

[2] Yona S; Kim KW; Wolf Y; Mildner A; Varol D; Breker M; Strauss-Ayali D; Viukov S; Guilliams M; Misharin A; Hume DA; Perlman H; Malissen B; Zelzer E; Jung S. 2013. Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis. Immunity 38(1):79-91. ;

If you have any questions, you are welcome to inquire.

Tel: 025-5864 1534

集萃药康生物科技

GemPharmatech Co.,Ltd