

Mfsd2b Cas9-CKO Strategy

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Project Overview

Project Name

Mfsd2b

Project type

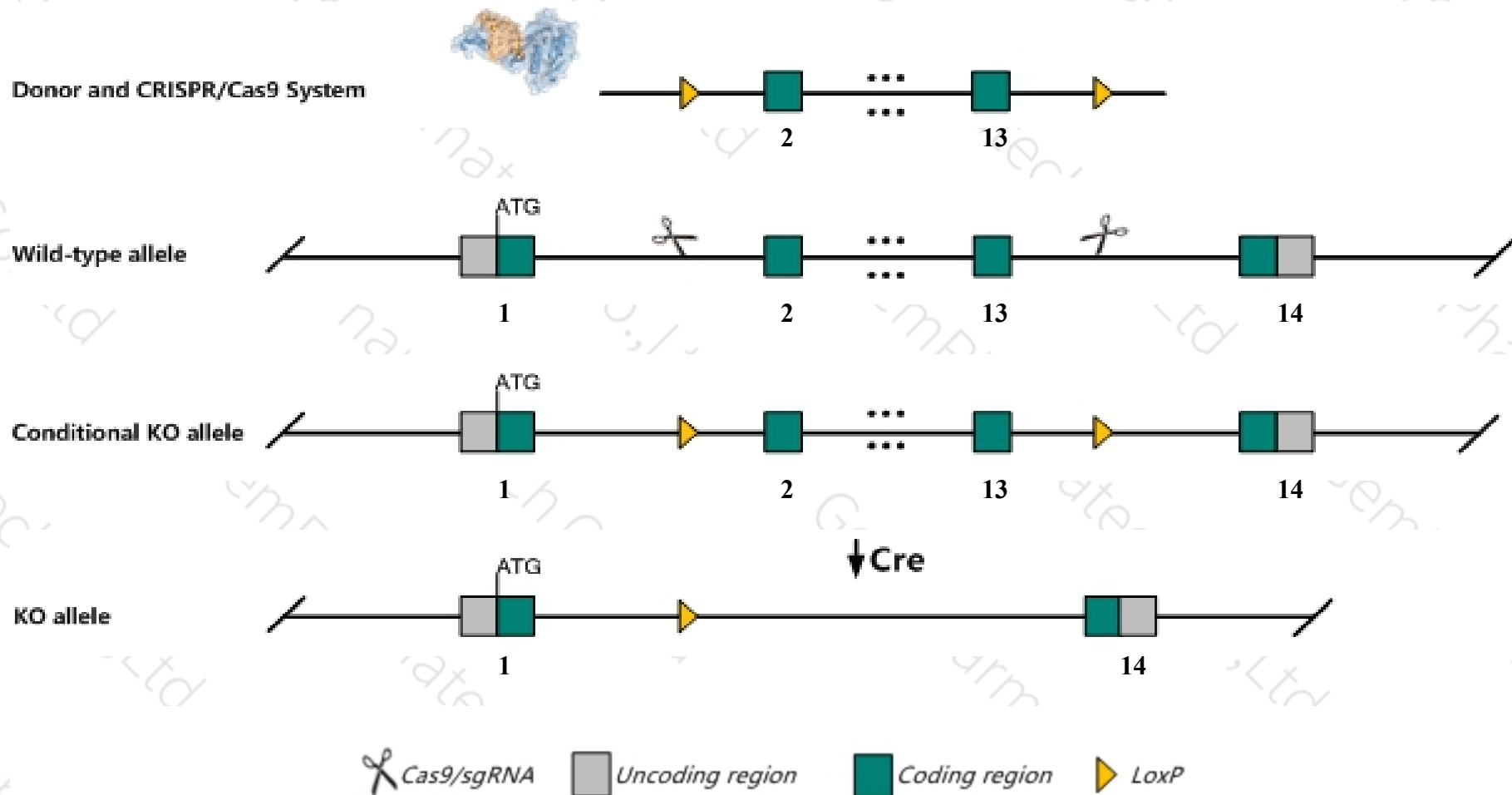
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mfsd2b* gene. The schematic diagram is as follows:



Technical routes

- The *Mfsd2b* gene has 8 transcripts. According to the structure of *Mfsd2b* gene, exon2-exon13 of *Mfsd2b*-201(ENSMUST00000045921.13) transcript is recommended as the knockout region. The region contains 1394bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mfsd2b* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was 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 KO reduces sphingosine-1-phosphate export from, and increases its accumulation in, red blood cells and platelets, and leads to reduced leukocyte, reticulocyte, and erythrocyte cell numbers.
- The *Mfsd2b* gene is located on the Chr12. 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)

Mfsd2b major facilitator superfamily domain containing 2B [Mus musculus (house mouse)]

Gene ID: 432628, updated on 13-Mar-2020

Summary



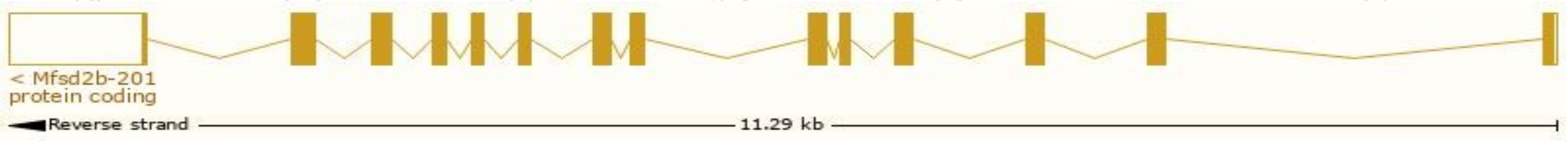
Official Symbol	Mfsd2b provided by MGI
Official Full Name	major facilitator superfamily domain containing 2B provided by MGI
Primary source	MGI:MGI:3583946
See related	Ensembl:ENSMUSG00000037336
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Gm1964
Expression	Biased expression in liver E14.5 (RPKM 21.0), liver E14 (RPKM 17.3) and 5 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

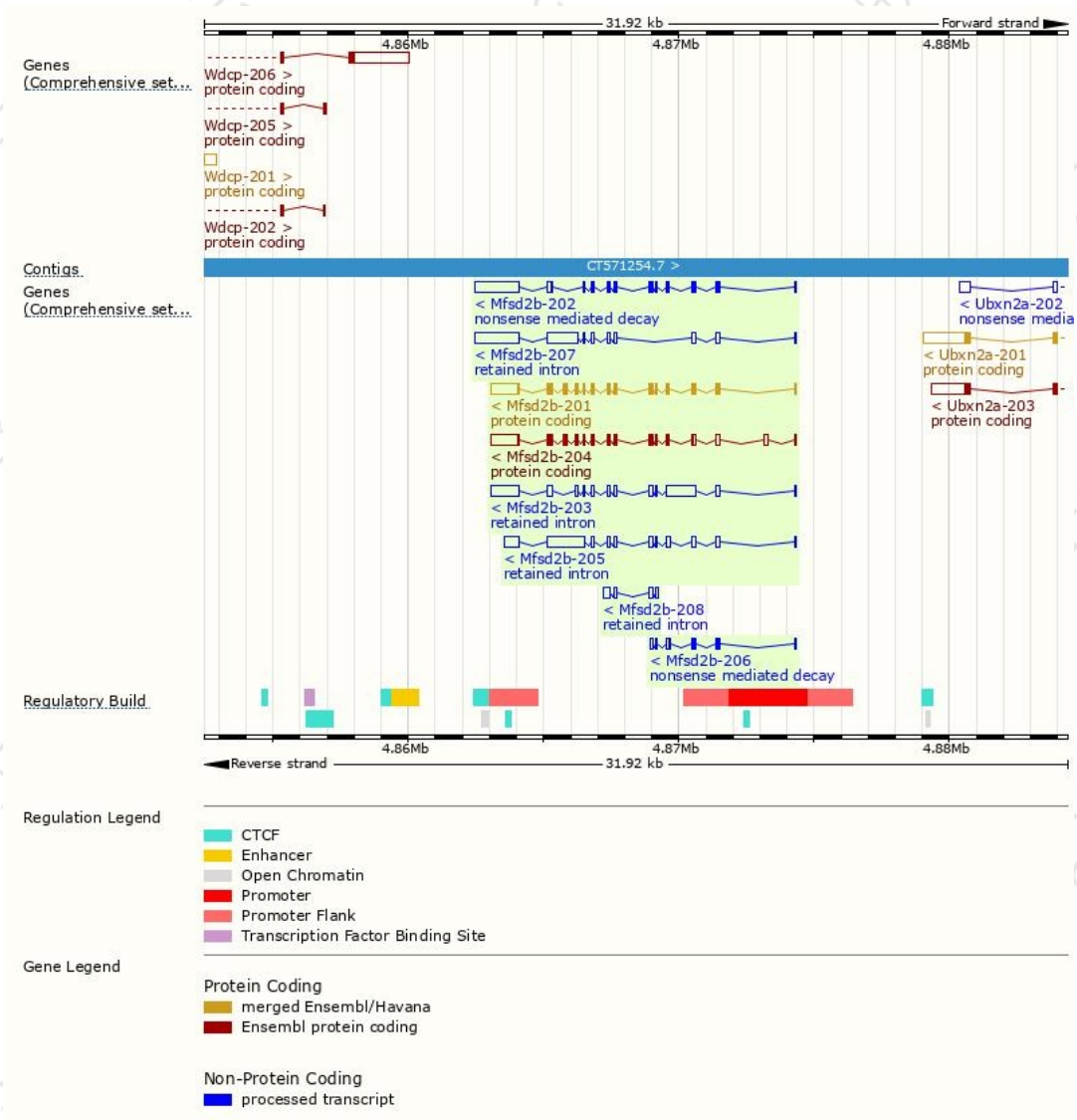
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mfsd2b-201	ENSMUST00000045921.13	2490	494aa	Protein coding	CCDS36401	B8JK43	TSL:1 GENCODE basic APPRIS P1
Mfsd2b-204	ENSMUST00000137337.7	2641	391aa	Protein coding	-	B8JK42	TSL:2 GENCODE basic
Mfsd2b-202	ENSMUST00000085790.11	2868	347aa	Nonsense mediated decay	-	Q3T9M1	TSL:2
Mfsd2b-206	ENSMUST00000147241.1	653	117aa	Nonsense mediated decay	-	D6RDD7	TSL:3
Mfsd2b-207	ENSMUST00000150764.7	3486	No protein	Retained intron	-	-	TSL:2
Mfsd2b-203	ENSMUST00000125344.7	3173	No protein	Retained intron	-	-	TSL:2
Mfsd2b-205	ENSMUST00000143446.7	2893	No protein	Retained intron	-	-	TSL:2
Mfsd2b-208	ENSMUST00000153676.1	576	No protein	Retained intron	-	-	TSL:3

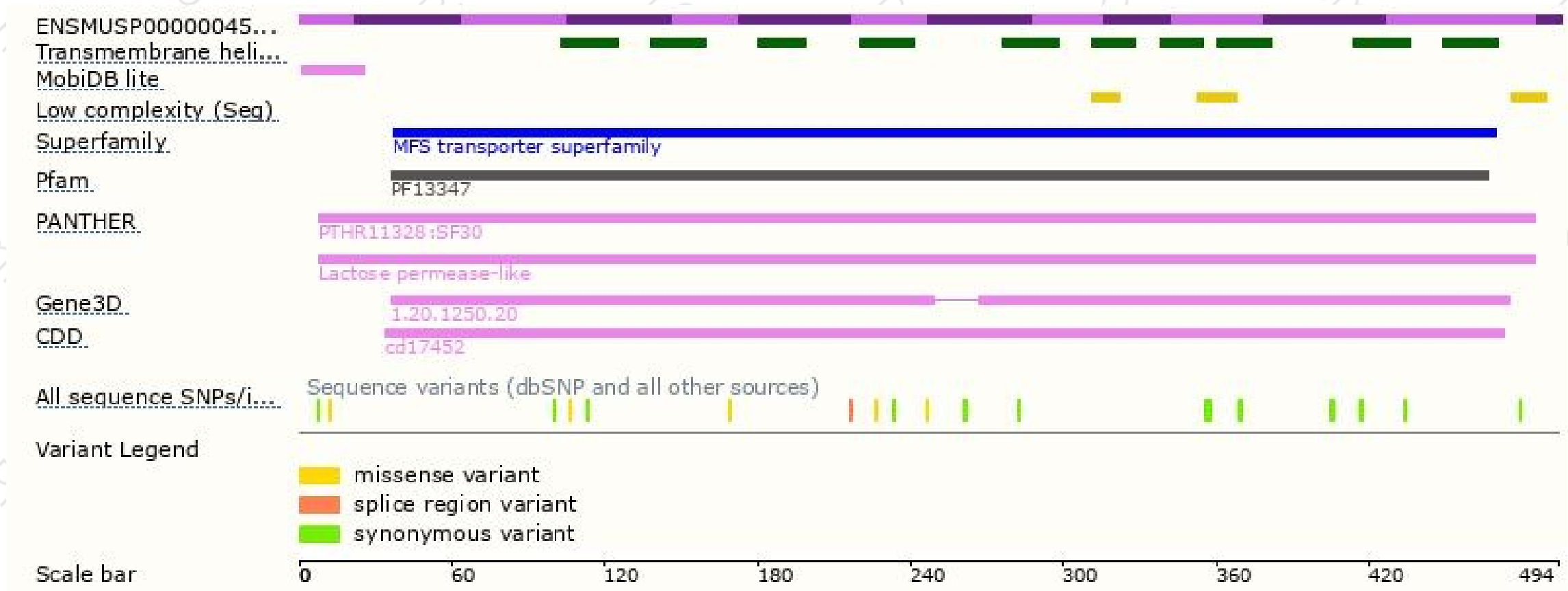
The strategy is based on the design of *Mfsd2b-201* transcript,the transcription is shown below:



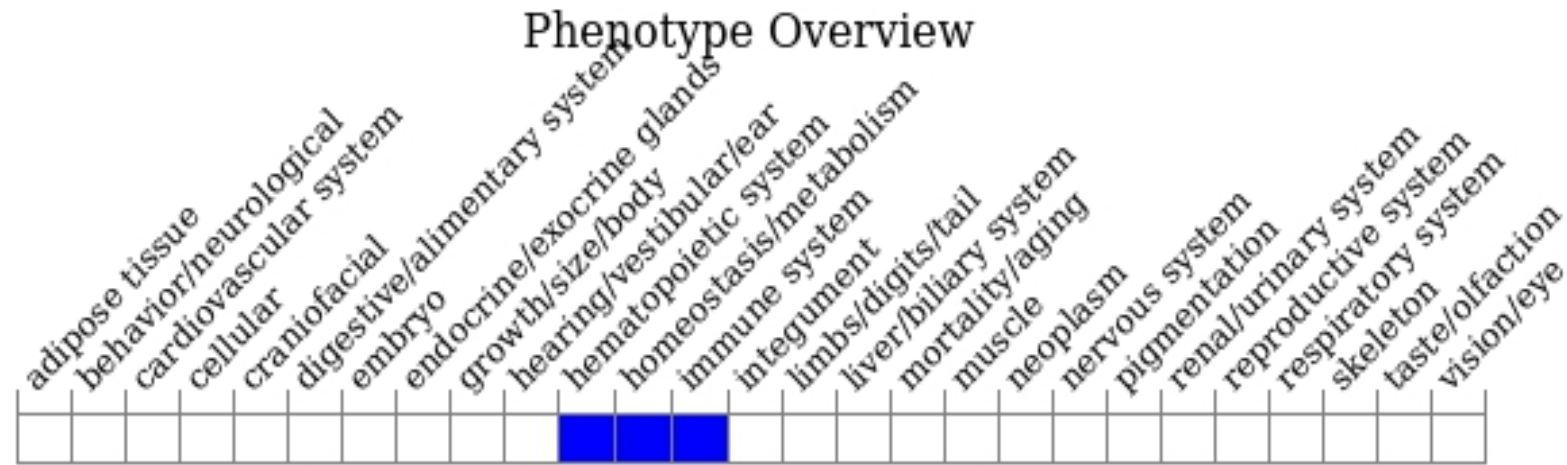
Genomic location distribution



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, homozygous KO reduces sphingosine-1-phosphate export from, and increases its accumulation in, red blood cells and platelets, and leads to reduced leukocyte, reticulocyte, and erythrocyte cell numbers.

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

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