

# Mfsd2b Cas9-KO Strategy

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## **Project Overview**



**Project Name** 

Mfsd2b

**Project type** 

Cas9-KO

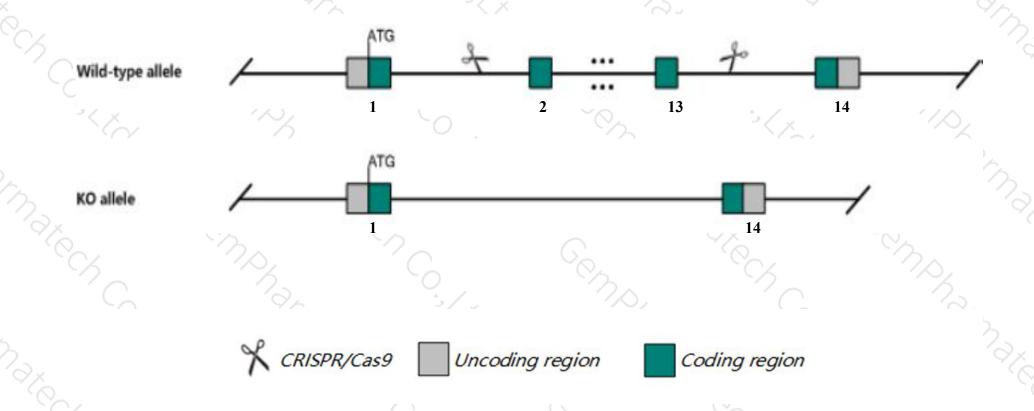
Strain background

C57BL/6JGpt

# **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: CRISPR/Cas9 system 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.

### **Notice**



- > 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology 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 tissuesSee more

Orthologs <u>human</u> all

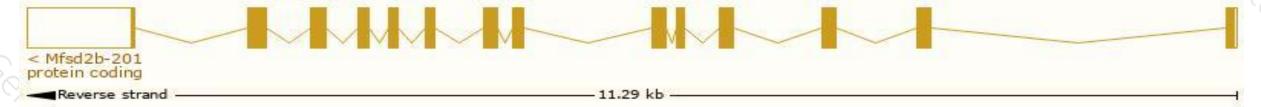
## Transcript information (Ensembl)



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

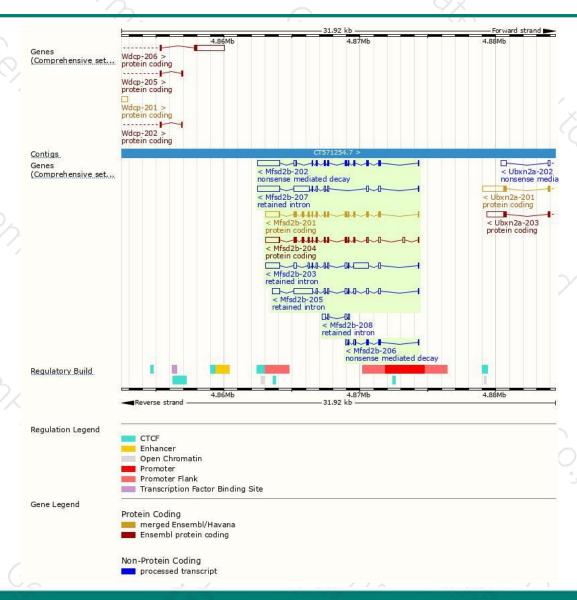
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Mfsd2b-201	ENSMUST00000045921.13	2490	<u>494aa</u>	Protein coding	CCDS36401	B8JK43	TSL:1 GENCODE basic APPRIS P	
Mfsd2b-204	ENSMUST00000137337.7	2641	<u>391aa</u>	Protein coding		B8JK42	TSL:2 GENCODE basic	
Mfsd2b-202	ENSMUST00000085790.11	2868	<u>347aa</u>	Nonsense mediated decay	u u	Q3T9M1	TSL:2	
Mfsd2b-206	ENSMUST00000147241.1	653	117aa	Nonsense mediated decay	-	D6RDD7	TSL:3	
Mfsd2b-207	ENSMUST00000150764.7	3486	No protein	Retained intron	ч	840	TSL:2	
Mfsd2b-203	ENSMUST00000125344.7	3173	No protein	Retained intron		10.70	TSL:2	
Mfsd2b-205	ENSMUST00000143446.7	2893	No protein	Retained intron		19#1	TSL:2	
Mfsd2b-208	ENSMUST00000153676.1	576	No protein	Retained intron	2	12	TSL:3	

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



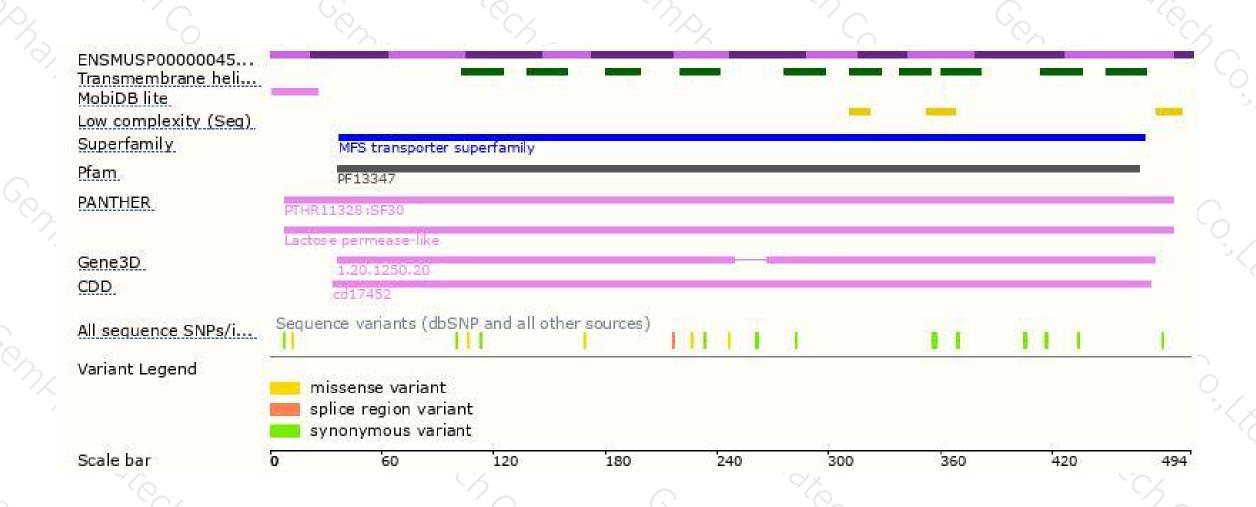
### Genomic location distribution





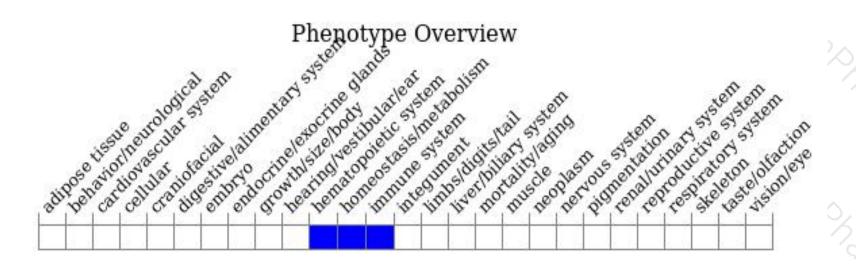
### Protein domain





### 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. Tel: 400-9660890





