

Madcam1 Cas9-CKO Strategy

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Reviewer: Yao Yu

Design Date: 2023-12-18

Overview

Target Gene Name

• Madcam1

Project Type

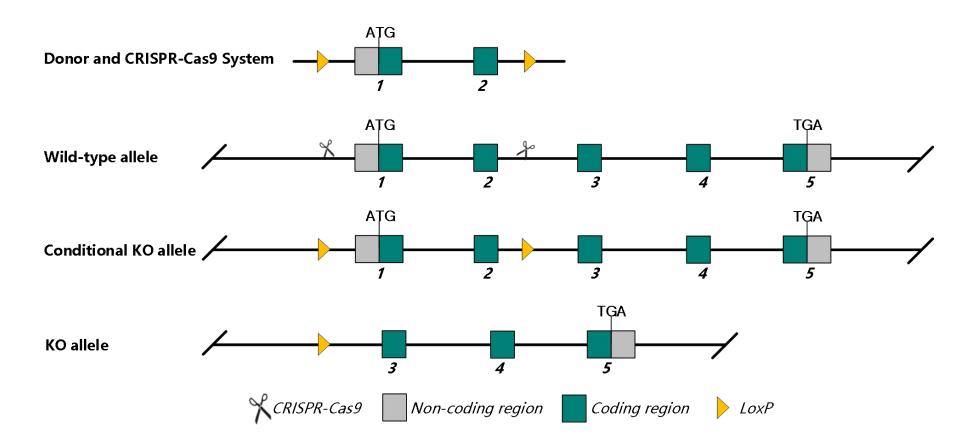
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Madcam1 gene.

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Technical Information

- The *Madcam1* gene has 2 transcripts. According to the structure of *Madcam1* gene, exon 1-2 of *Madcam1*-201 (ENSMUST0000020554.8) is recommended as the knockout region. The region contains the start codon ATG. Knocking out the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Madcam1* gene. The brief process is as follows: CRISPR-Cas9 system 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.



Gene Information

Madcam1 mucosal vascular addressin cell adhesion molecule 1 [Mus musculus (house mouse)]

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Gene ID: 17123, updated on 23-Nov-2023

Summary

Official Symbol	Madcam1 provided by MGI
Official Full Name	mucosal vascular addressin cell adhesion molecule 1 provided by MGI
Primary source	<u>MGI:MGI:103579</u>
See related	Ensembl:ENSMUSG0000020310 AllianceGenome:MGI:103579
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	MAdCAM-1
Summary	Predicted to enable integrin binding activity involved in cell-matrix adhesion. Acts upstream of or within keratinocyte differentiation and leukocyte migration. Predicted to be located in membrane. Predicted to be integral component of membrane. Is expressed in eyelid; hair follicle; and hemolymphoid system. Orthologous to human MADCAM1 (mucosal vascular addressin cell adhesion molecule 1). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Biased expression in spleen adult (RPKM 8.6), large intestine adult (RPKM 4.9) and 7 other tissues See more
Orthologs	human all
NEW	Try the new <u>Gene table</u> Try the new <u>Transcript table</u>
Genomic context	
Location: 10 C1; 10 39	.72 cM See Madcam1 in Genome Data Viewer
Exon count: 5	

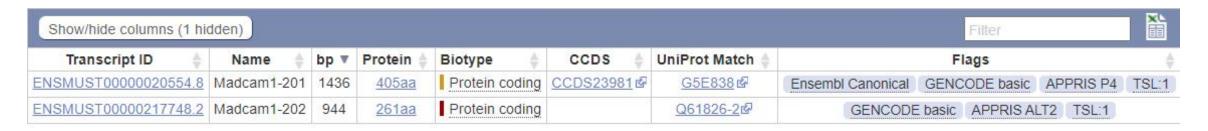
https://www.ncbi.nlm.nih.gov/gene/17123



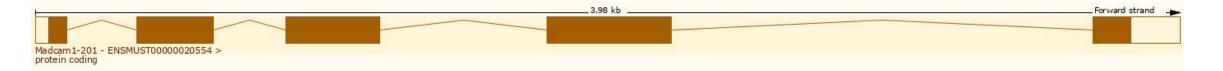
Transcript Information

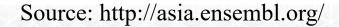
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The gene has 2 transcripts, all transcripts are shown below:

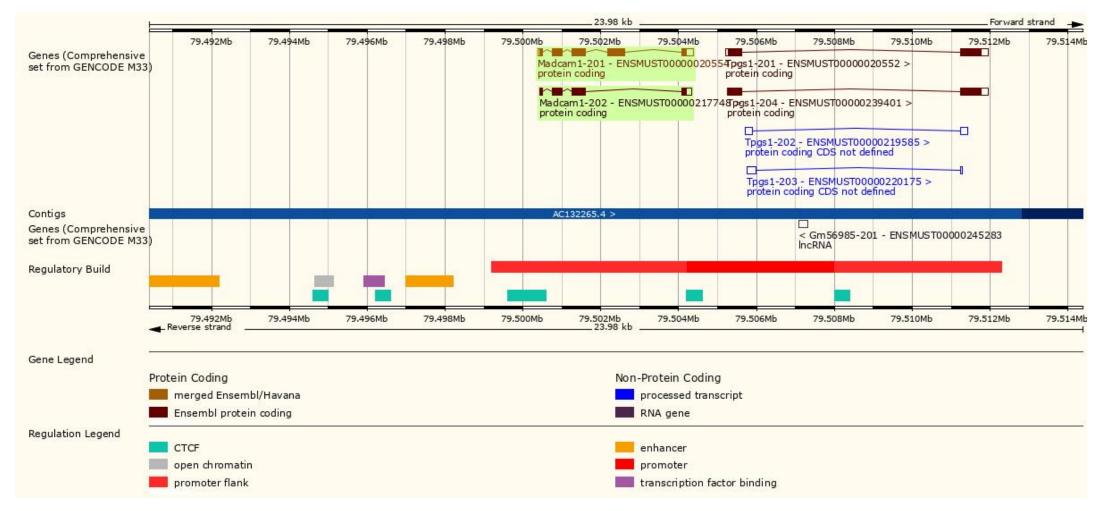


The strategy is based on the design of *Madcam1*-201 transcript, the transcription is shown below:





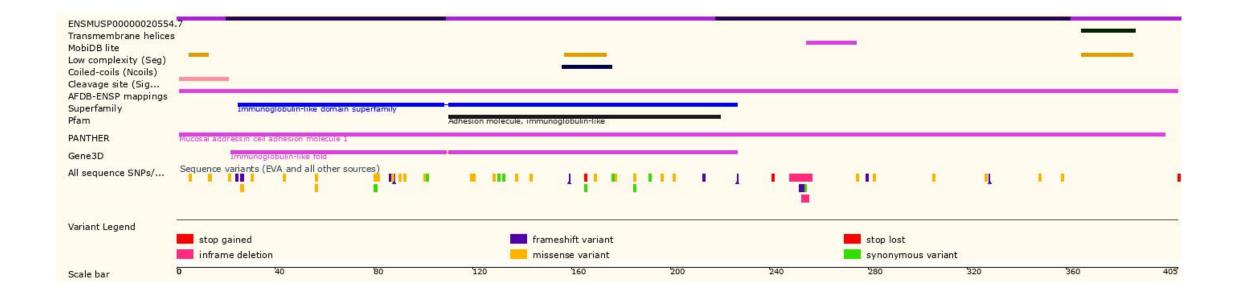
Genomic Information



Source: http://asia.ensembl.org/

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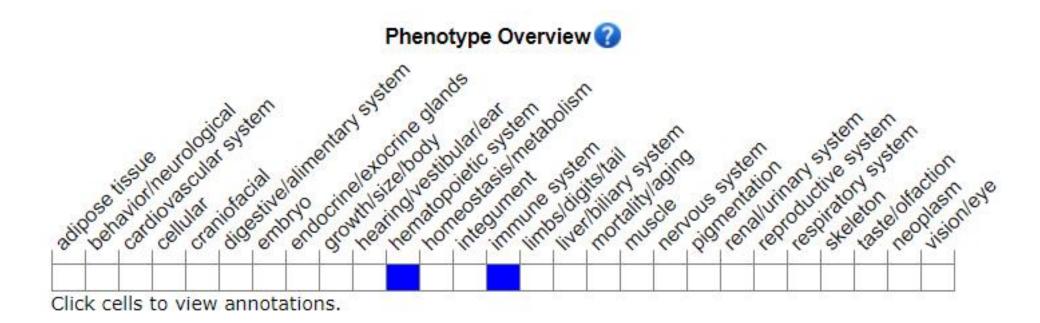
Protein Information



Source: https://www.ensembl.org

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Mouse Phenotype Information (MGI)



Mice homozygous for a knock-out allele exhibit small Peyer's patches and decreased homing of IgA-secreting plasma cells in the lamina propria.

Source: https://www.informatics.jax.org

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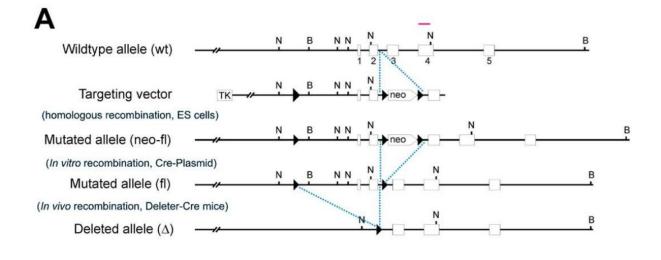
Important Information

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- The knockout region is about 4 kb away from the 5' of the *Tpgs1* gene, which may affect the regulation of this gene.
- The knockout region contains start codon, translation may recognize new start codon and form new unknown protein.
- The intron 2-3 of *Madcam1* is 252 bp, the loxp insertion may affect the regulation of this gene.
- *Madcam1* is located on Chr 10. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

Reference

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Deletion of MAdCAM-1 in Mice

MAdCAM-1-deficient mice ($MAdCAM^{\Delta}$) were generated by removal of the promoter region and exons 1 and 2 of the MAdCAM-1 gene (Figure 1A). Complete deletion of the MAdCAM-1 gene was confirmed by Southern blot analysis (Figure 1B), Northern blot/reversetranscription polymerase chain reaction (RT-PCR) analysis (Figure 1C, D), and immunofluorescence staining of spleen sections using an anti-MAdCAM-1 antibody (Figure 1E). MAdCAM-1-deficient mice were viable, fertile, and did

[1] Schippers A, Leuker C, Pabst O, Kochut A, Prochnow B, Gruber AD, Leung E, Krissansen GW, Wagner N, Müller W.
Mucosal addressin cell-adhesion molecule-1 controls plasma-cell migration and function in the small intestine of mice.
Gastroenterology. 2009 Sep;137(3):924-33. doi: 10.1053/j.gastro.2009.05.039. Epub 2009 May 18. PMID: 19450594.