

***Mcam-CreERT2 cas9-ki* Mouse Model Strategy**

-CRISPR/Cas9 technology

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Project Overview

Project Name

Mcam-CreERT2

Project Type

cas9-ki

Background

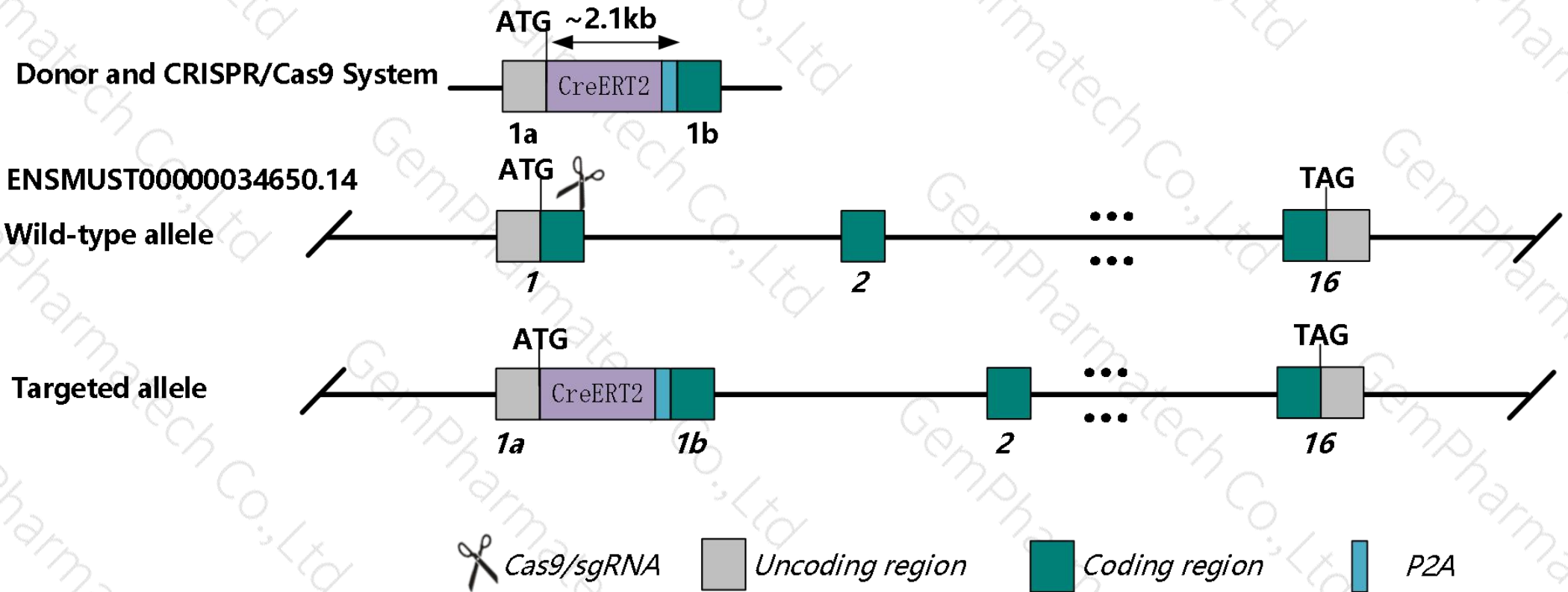
C57BL/6JGpt

Technical Description

- The mouse *Mcam* gene has 6 transcripts.
- According to the structure of *Mcam* gene, The element CreERT2-P2A will be inserted after the translation start codon of *Mcam*-201(ENSMUST00000034650.14), the length of inserted fragment is about 2.1kb.
- In this project, *Mcam* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR, sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

Strategy

This model uses CRISPR/Cas9 technology to edit the *Mcam* gene and the schematic diagram is as follow:



- According to the data of MGI, Mice homozygous for a conditional allele activated in endothelial cells exhibit impaired VEGF-induced angiogenesis in Matrigel..
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide
- Mouse *Mcam* gene is located on Chr9. Please take the loci in consideration when breeding this knockin mice with other gene modified (e.g., Tg, iCre) strains, if the other gene is also on Chr9, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 5'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

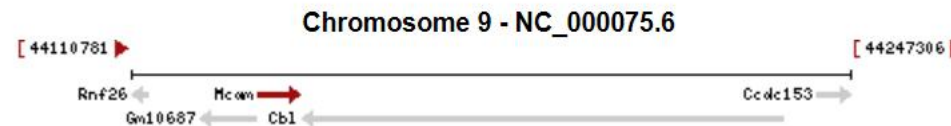
Gene name and location (NCBI)

Mcam melanoma cell adhesion molecule [*Mus musculus* (house mouse)]

Gene ID: 84004, updated on 25-Aug-2020

Summary

Official Symbol	Mcam provided by MGI
Official Full Name	melanoma cell adhesion molecule provided by MGI
Primary source	MGI:MGI:1933966
See related	Ensembl:ENSMUSG00000032135
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	s-e; s-gi; 1-gic; CD146; CD149; Muc18; s-endo; AV025631; 1-gicerin; s-gicerin
Expression	Broad expression in subcutaneous fat pad adult (RPKM 97.6), lung adult (RPKM 73.6) and 21 other tissues See more
Orthologs	human all

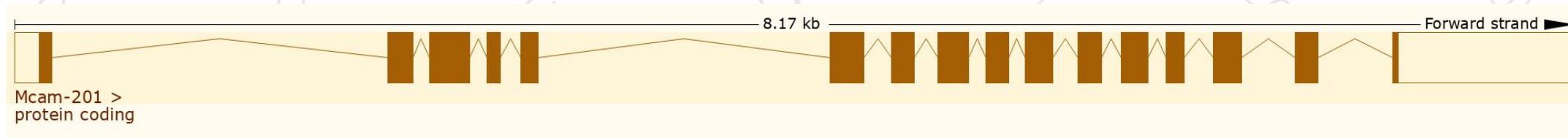


Transcript information (Ensembl)

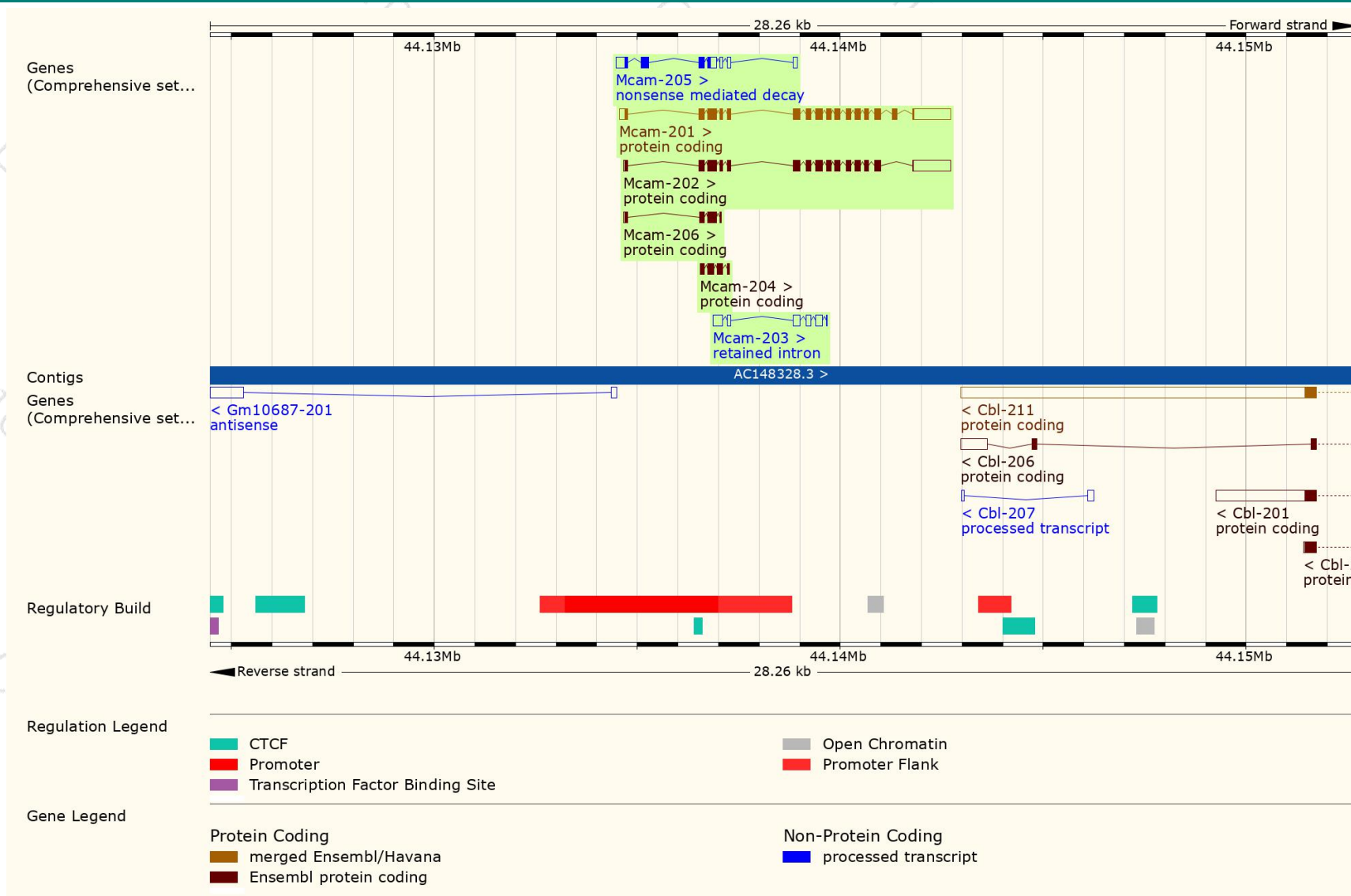
The gene has 6 transcripts, and all transcripts are shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲
Mcam-201	ENSMUST00000034650.14	2989	648aa	Protein coding	CCDS23097	Q8R2Y2	TSL:1 GENCODE basic APPRIS P2
Mcam-202	ENSMUST00000098852.2	2772	606aa	Protein coding	-	Q8R2Y2	TSL:1 GENCODE basic APPRIS ALT2
Mcam-203	ENSMUST00000132490.1	832	No protein	Retained intron	-	-	TSL:5
Mcam-204	ENSMUST00000147836.1	449	149aa	Protein coding	-	F7ATH5	CDS 5' and 3' incomplete TSL:2
Mcam-205	ENSMUST00000149241.7	1061	146aa	Nonsense mediated decay	-	D6RFP0	TSL:5
Mcam-206	ENSMUST00000216002.1	457	142aa	Protein coding	-	A0A1L1SQC5	CDS 3' incomplete TSL:2

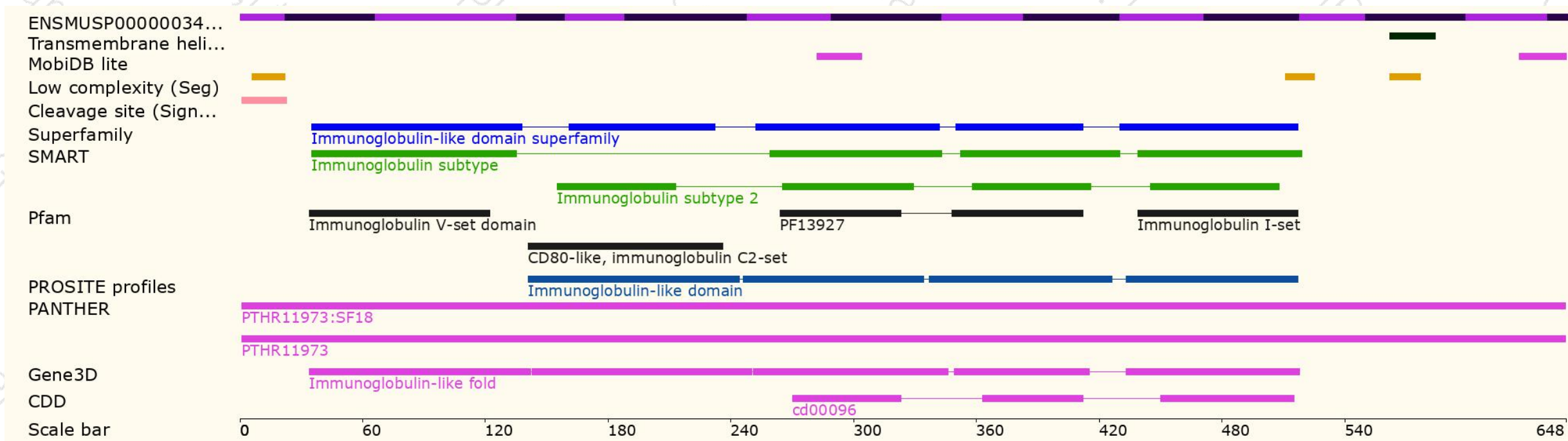
The strategy is based on the design of *Mcam-201* transcript, The transcription is shown below:



Genomic location distribution



Protein domain

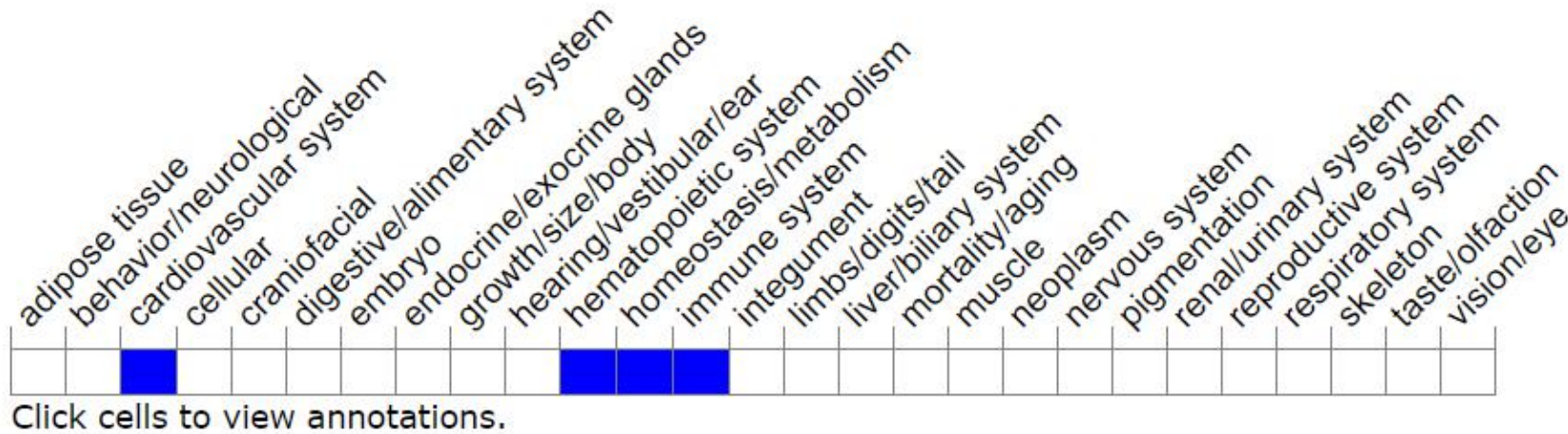


Mouse phenotype description(MGI)

URL link is as follows:

<http://www.informatics.jax.org/marker/MGI:1933966>

Phenotype Overview ?



Mice homozygous for a conditional allele activated in endothelial cells exhibit impaired VEGF-induced angiogenesis in Matrigel.

If you have any questions, please feel free to contact us.
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