Unc13d Cas9-CKO Strategy

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Project Overview

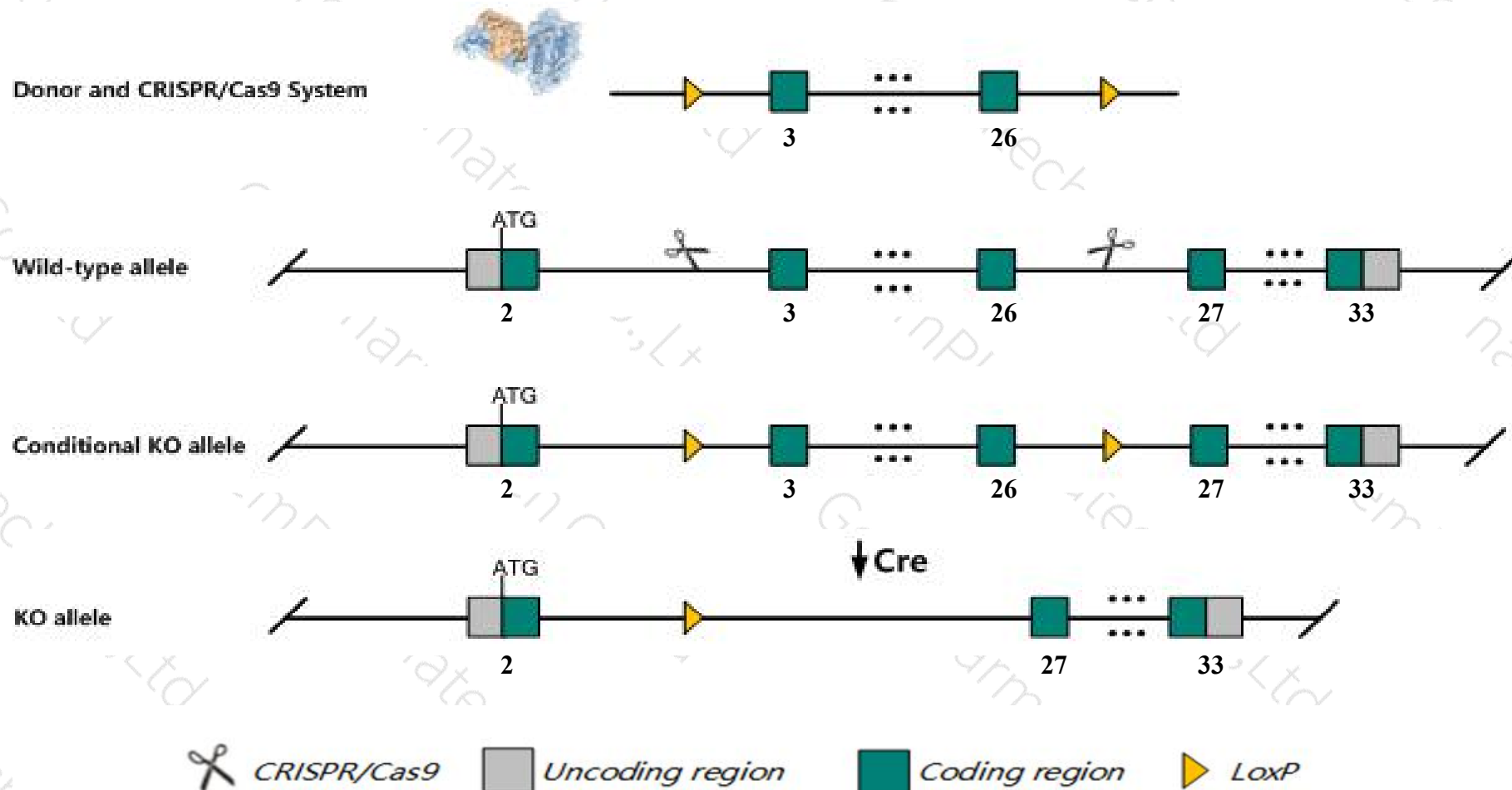|	
Project Name	*Unc13d*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Unc13d* gene. The schematic diagram is as follows:

Technical routes

➤ The *Unc13d* gene has 9 transcripts. According to the structure of *Unc13d* gene, exon3-exon26 of *Unc13d-201* (ENSMUST00000075036.8) transcript is recommended as the knockout region.The region contains Ens 2324bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Unc13d* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

➤ 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.

Notice

➢ According to the existing MGI data,Targeted deletion of this gene leads to defective hemostasis, abrogated thrombus formation and protection of homozygotes from ischemic stroke in the absence of intracranial bleeding. Homozygous ENU mutant mice are sensitive to infection by mouse cytomegalovirus.

➢ Transcript *Unc13d*-204 may be directly destroyed in this strategy.

➢ The *Unc13d* gene is located on the Chr11. 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）

Unc13d   unc-13 homolog D [*Mus musculus* (house mouse)]

Gene ID: 70450, updated on 10-Dec-2019

▲ Summary

Official Symbol	Unc13d provided by MGI
Official Full Name	unc-13 homolog D provided by MGI
Primary source	MGI:MGI:1917700
See related	Ensembl:ENSMUSG00000057948
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	Jinx; Munc13-4; mFLJ00067; 2610108D09Rik
Expression	Broad expression in thymus adult (RPKM 20.5), spleen adult (RPKM 18.5) and 15 other tissues See more
Orthologs	human all

▲ Genomic context

Location: 11; 11 E2

See Unc13d in Genome Data Viewer

Exon count: 35

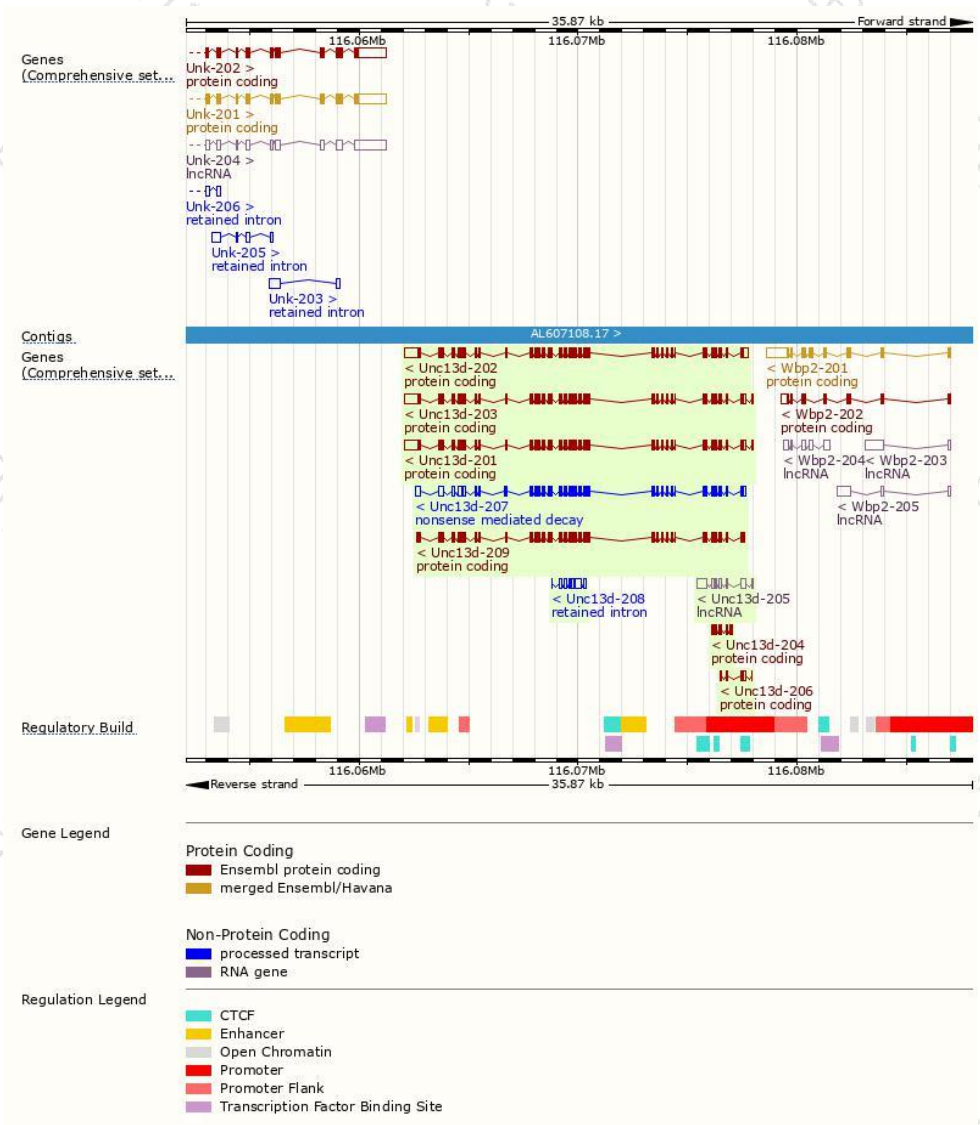| Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	11	NC_000077.6 (116062095..116078495, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	11	NC_000077.5 (115923410..115939275, complement)

Transcript information（Ensembl）

The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Unc13d-201	ENSMUST00000075036.8	3967	1083aa	Protein coding	CCDS25656	B2RUP2	TSL:5 GENCODE basic APPRIS P2
Unc13d-209	ENSMUST00000174822.7	3337	1083aa	Protein coding	CCDS25656	B2RUP2	TSL:1 GENCODE basic APPRIS P2
Unc13d-202	ENSMUST0000106450.7	4046	1085aa	Protein coding	-	B2RUP2	TSL:5 GENCODE basic APPRIS ALT2
Unc13d-203	ENSMUST0000106451.7	3973	1085aa	Protein coding	-	B2RUP2	TSL:5 GENCODE basic APPRIS ALT2
Unc13d-204	ENSMUST0000153408.7	416	117aa	Protein coding	-	A2A855	CDS 3' incomplete TSL:3
Unc13d-206	ENSMUST0000156545.1	353	76aa	Protein coding	-	A2A858	CDS 3' incomplete TSL:5
Unc13d-207	ENSMUST00000173345.7	3514	834aa	Nonsense mediated decay	-	A0A0R4J257	TSL:1
Unc13d-208	ENSMUST00000173943.1	766	No protein	Retained intron	-	-	TSL:5
Unc13d-205	ENSMUST0000155120.1	991	No protein	lncRNA	-	-	TSL:1

The strategy is based on the design of *Unc13d-201* transcript,The transcription is shown below

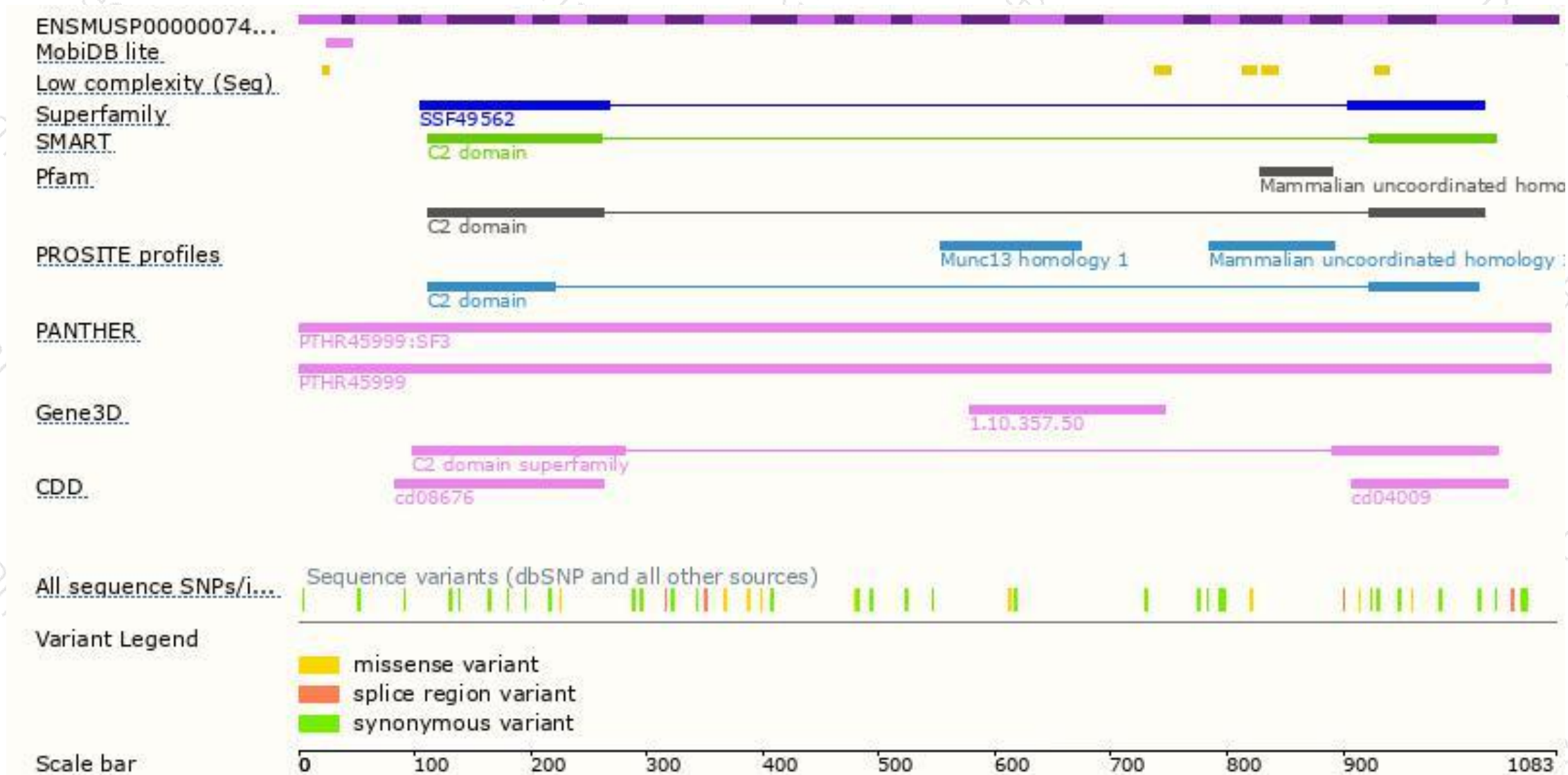< Unc13d-201
protein coding

◀ Reverse strand

15.87 kb

Genomic location distribution
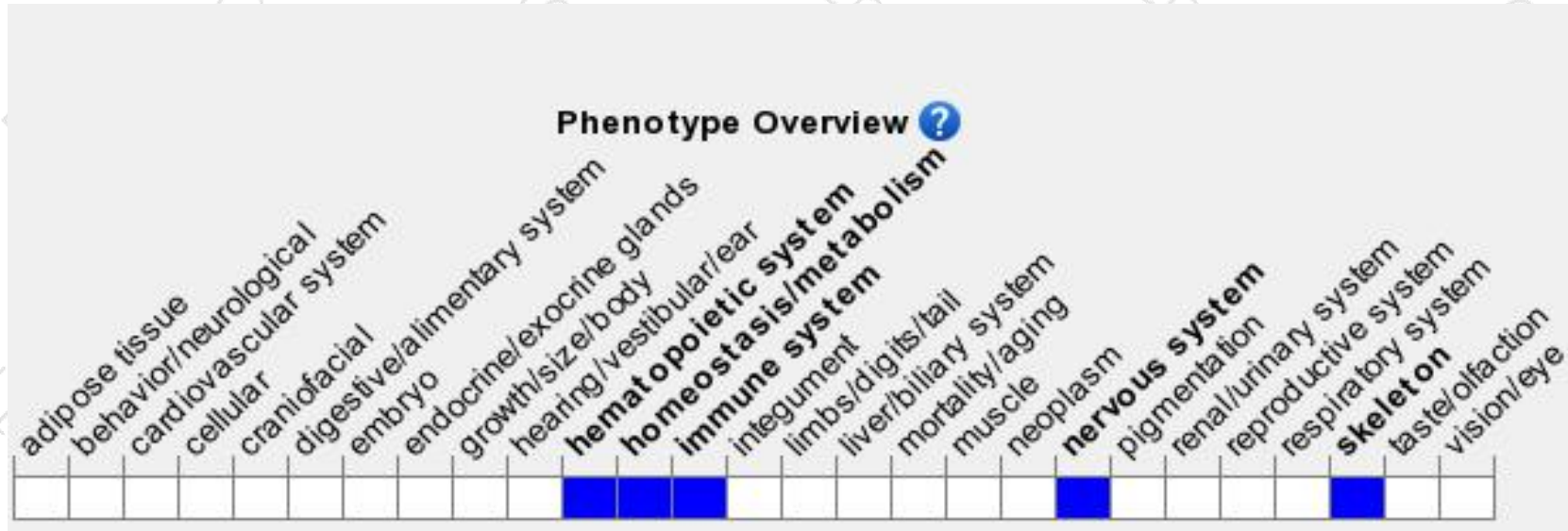
Protein domain

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,Targeted deletion of this gene leads to defective hemostasis, abrogated thrombus formation and protection of homozygotes from ischemic stroke in the absence of intracranial bleeding. Homozygous ENU mutant mice are sensitive to infection by mouse cytomegalovirus.

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