

Spp1-CreERT2-IRES-EGFP Mouse Model Strategy

-CRISPR/Cas9 technology

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Project Overview

Project Name

Spp1-CreERT2-IRES-EGFP

Project Type

cas9-ki

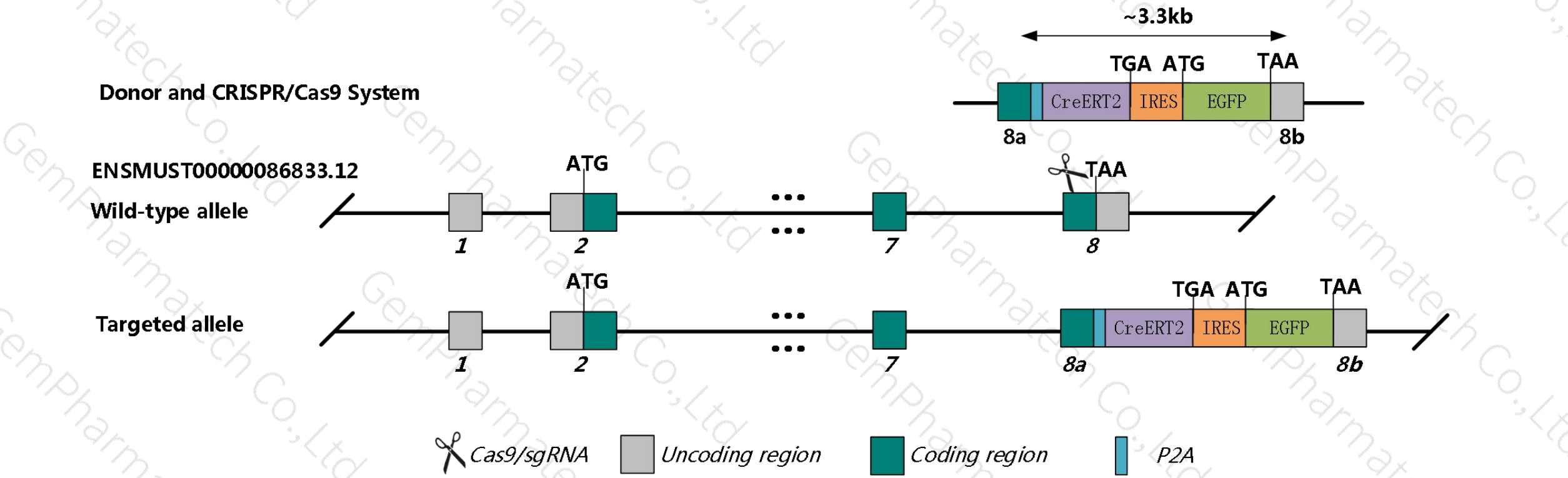
Background

C57BL/6JGpt

Technical Description

- The mouse *Spp1* gene has 7 transcripts.
- According to the structure of *Spp1* gene, The element CreERT2-IRES-EGFP will be inserted at the translation stop codon of Spp1-202(ENSMUST00000086833.12), the length of inserted fragment is about 3.3kb.
- In this project, *Spp1* 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.

This model uses CRISPR/Cas9 technology to edit the *Spp1* gene and the schematic diagram is as follow:



- According to the data of MGI , Two alleles determine natural resistance/susceptibility to the lethal effects of the Gilliam strain of Rickettsia tsutsugamushi. Mice homozygous for a knock-out allele exhibit abnormal osteoclast physiology, macrophage recruitment, wound healing, response to injury, and inflammatory response.
- It is necessary to introduce 1-2 synonymous mutation in exon8.
- The effect of this strategy on Spp1-203206207 transcript is unknown due to its incomplete 3-terminal.
- zsGreen and CreERT2 are linkerd with EGFP , and these two genes will be transcribed together and then be translated two protein separately. Otherwise, IRES may produce lower amounts of the downstream protein in relation to the upstream protein.
- Mouse *Spp1* gene is located on Chr5. 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 Chr12, 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 3'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.

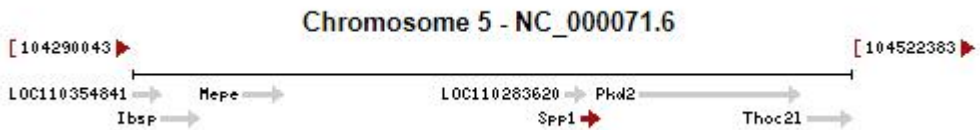
Gene name and location (NCBI)

Spp1 secreted phosphoprotein 1 [*Mus musculus* (house mouse)]

Gene ID: 20750, updated on 24-May-2020

Summary

Official Symbol	Spp1 provided by MGI
Official Full Name	secreted phosphoprotein 1 provided by MGI
Primary source	MGI:MGI:98389
See related	Ensembl:ENSMUSG00000029304
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	OP; 2AR; Bsp; Eta; Opn; Ric; BNSP; BSPI; Opnl; Apl-1; ETA-1; Spp-1
Expression	Biased expression in kidney adult (RPKM 580.4), liver E18 (RPKM 36.2) and 1 other tissue See more
Orthologs	human all

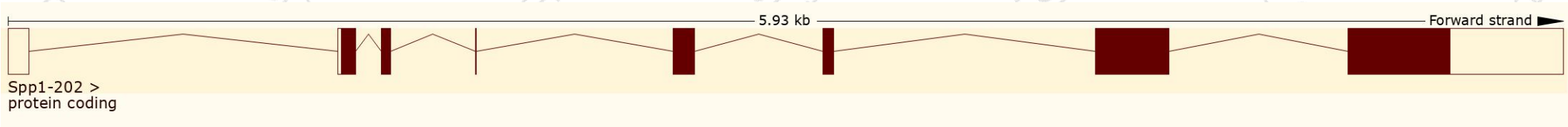


Transcript information (Ensembl)

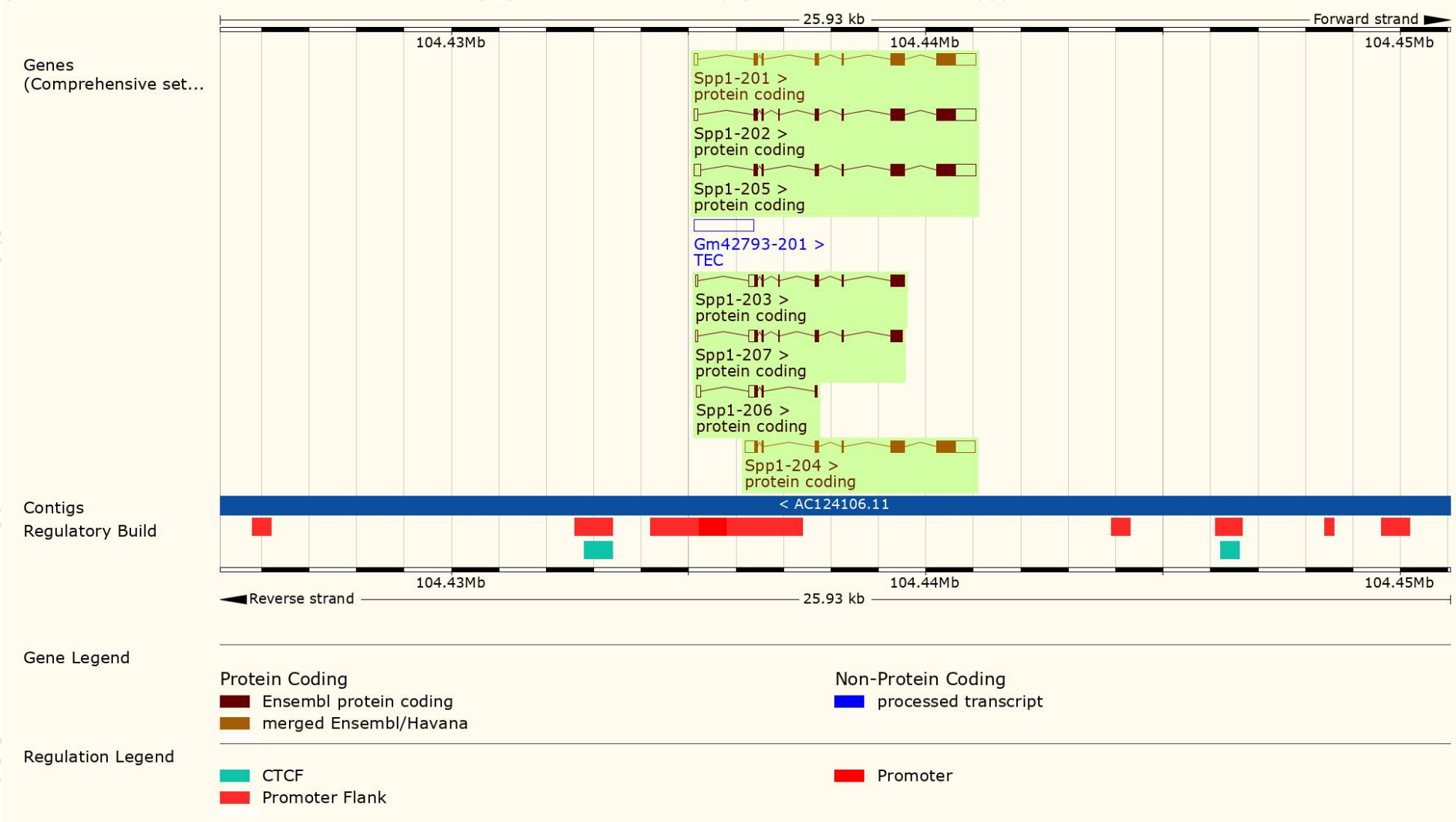
The gene has 7 transcripts, and all transcripts are shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲
Spp1-201	ENSMUST00000031243.14	1410	294aa	Protein coding	CCDS19486	P10923 Q547B5	TSL:1 GENCODE basic APPRIS P3
Spp1-202	ENSMUST00000086833.12	1411	295aa	Protein coding	CCDS57367	F8WIP8	TSL:5 GENCODE basic APPRIS ALT2
Spp1-203	ENSMUST00000112746.7	669	164aa	Protein coding	-	D3Z4N2	CDS 3' incomplete TSL:5
Spp1-204	ENSMUST00000112747.1	1507	294aa	Protein coding	CCDS19486	P10923 Q547B5	TSL:1 GENCODE basic APPRIS P3
Spp1-205	ENSMUST00000112748.7	1456	294aa	Protein coding	CCDS19486	P10923 Q547B5	TSL:1 GENCODE basic APPRIS P3
Spp1-206	ENSMUST00000132457.7	354	48aa	Protein coding	-	D3Z116	CDS 3' incomplete TSL:3
Spp1-207	ENSMUST00000145084.7	634	152aa	Protein coding	-	D3Z513	CDS 3' incomplete TSL:5

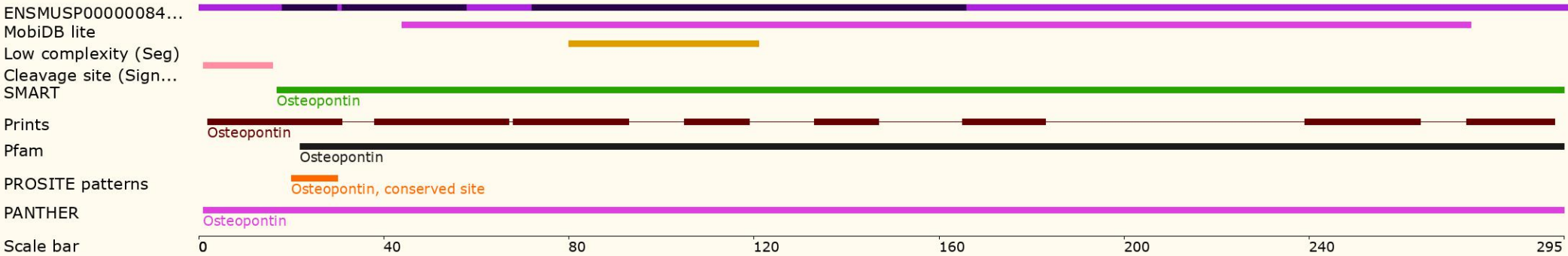
The strategy is based on the design of *Spp1-202* transcript,The transcription is shown below:



Genomic location distribution

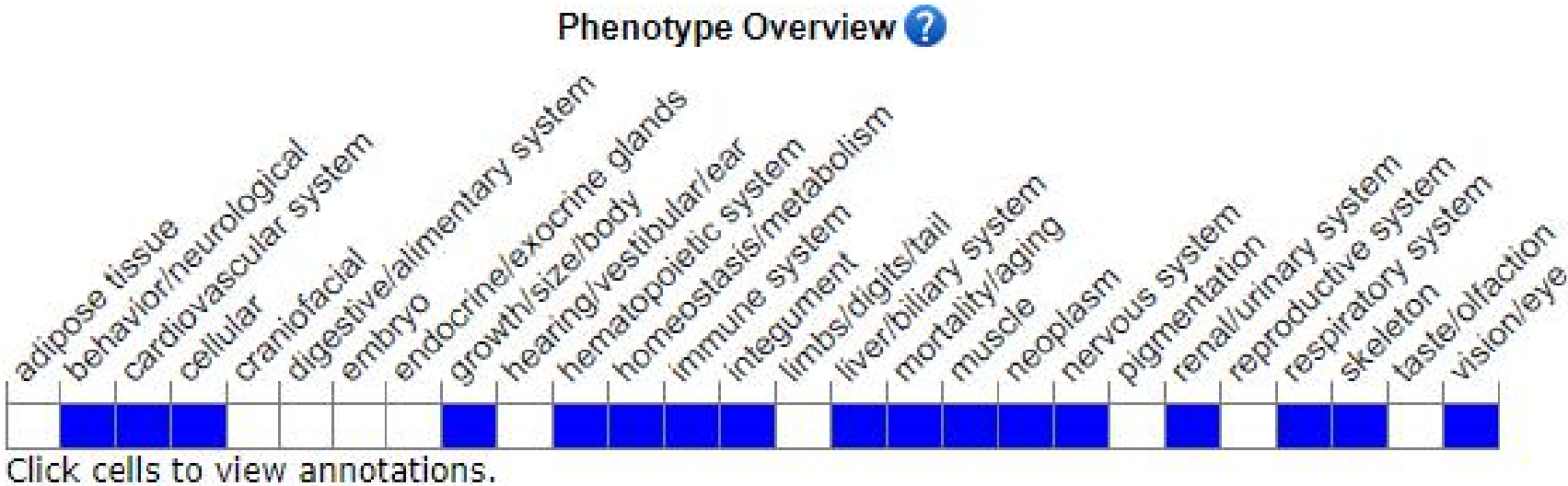


Protein domain



Mouse phenotype description(MGI)

URL link is as follows:
<http://www.informatics.jax.org/marker/MGI:98389>



Two alleles determine natural resistance/susceptibility to the lethal effects of the Gilliam strain of *Rickettsia tsutsugamushi*. Mice homozygous for a knock-out allele exhibit abnormal osteoclast physiology, macrophage recruitment, wound healing, response to injury, and inflammatory response.

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