

Ano1 Cas9-CKO Strategy

Designer:

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Project Overview

Project Name

Ano1

Project type

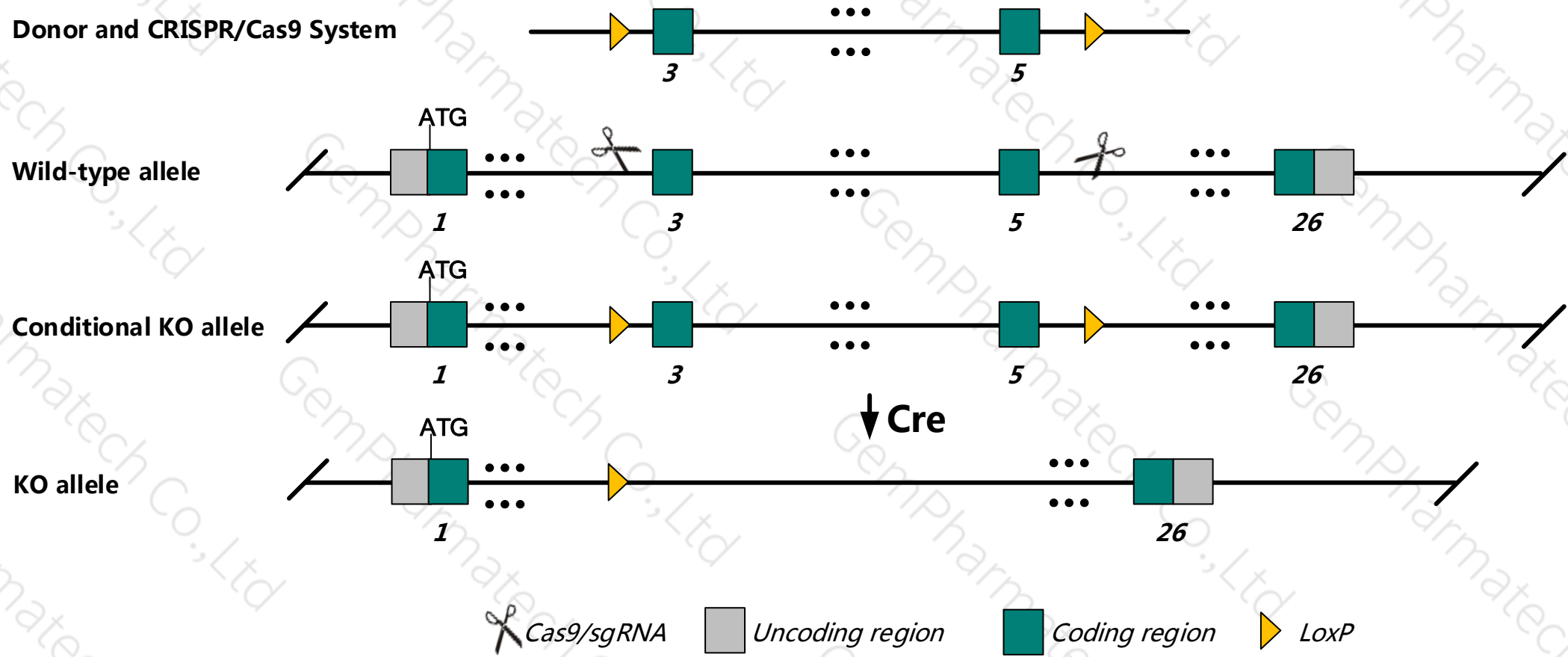
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ano1* gene. The schematic diagram is as follows:



- The *Ano1* gene has 10 transcripts. According to the structure of *Ano1* gene, exon3-exon5 of *Ano1*-203 transcript is recommended as the knockout region. The region contains 584bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ano1* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

- According to the existing MGI data , Mice homozygous for a knockout allele exhibit postnatal death associated with aerophagia, slow postnatal weight gain, cyanosis, and abnormal tracheal morphology. Mice homozygous for a different knock-out allele exhibit proteinuria and intracellular endosomal vesicles in PTE cells.
- The *Ano1* gene is located on the Chr7. 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Ano1 anoctamin 1, calcium activated chloride channel [

(house mouse)]

Gene ID: 101772, updated on 19-Mar-2019

Summary

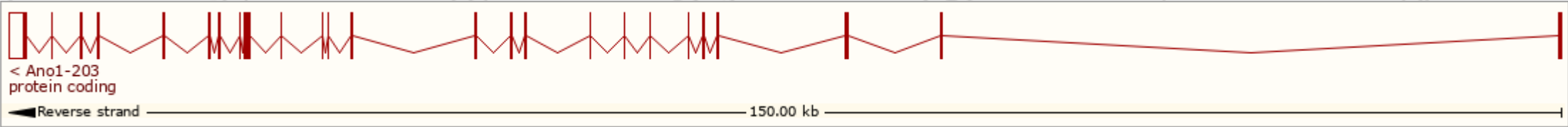
Official Symbol Ano1 provided by [MGI](#)
Official Full Name anoctamin 1, calcium activated chloride channel provided by [MGI](#)
Primary source [MGI:MGI:2142149](#)
See related [Ensembl:ENSMUSG00000031075](#)
Gene type protein coding
RefSeq status VALIDATED
Organism [Mus musculus](#)
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as Tmem16a
Expression Broad expression in colon adult (RPKM 33.8), genital fat pad adult (RPKM 27.4) and 19 other tissues [See more](#)
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

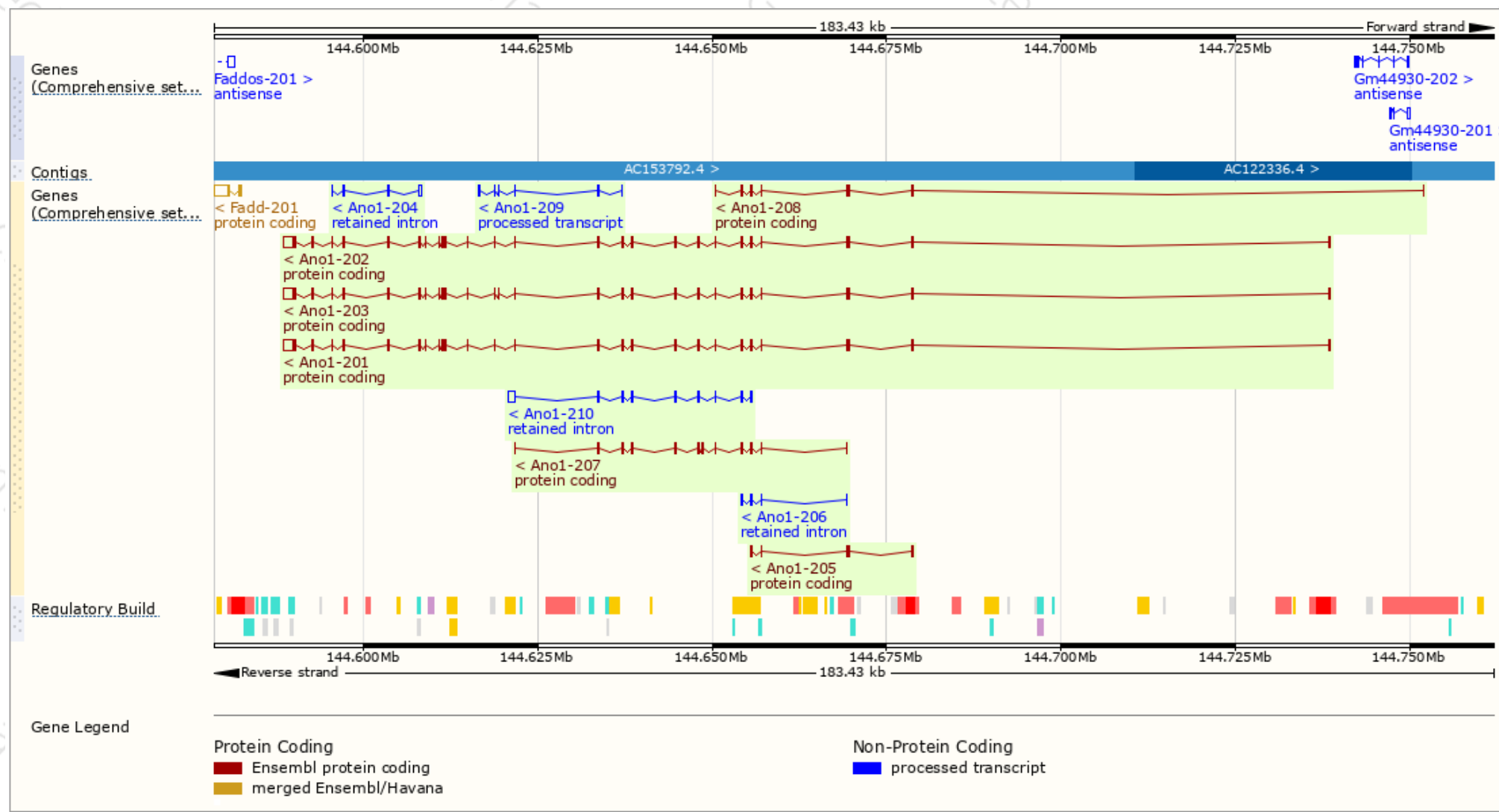
The gene has 10 transcripts, and all transcripts are shown below :

Show/hide columns (1 hidden)							Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Ano1-202	ENSMUST00000118556.8	4566	1014aa	Protein coding	CCDS57600	G8JL82	TSL:1	GENCODE basic APPRIS ALT2
Ano1-203	ENSMUST00000121758.7	4552	1017aa	Protein coding	CCDS40202	A0A0A0MQF2	TSL:1	GENCODE basic APPRIS P3
Ano1-201	ENSMUST00000033393.14	4585	956aa	Protein coding	-	Q8BHY3	TSL:5	GENCODE basic APPRIS ALT2
Ano1-207	ENSMUST00000152531.7	1004	335aa	Protein coding	-	F6R6E1	CDS 5' and 3' incomplete TSL:5	
Ano1-208	ENSMUST00000155175.7	926	290aa	Protein coding	-	D3Z035	CDS 3' incomplete TSL:5	
Ano1-205	ENSMUST00000131731.1	834	230aa	Protein coding	-	D3YYA4	CDS 3' incomplete TSL:2	
Ano1-209	ENSMUST00000208094.1	551	No protein	Processed transcript	-	-	TSL:5	
Ano1-210	ENSMUST00000208985.1	1620	No protein	Retained intron	-	-	TSL:2	
Ano1-204	ENSMUST00000131571.1	769	No protein	Retained intron	-	-	TSL:3	
Ano1-206	ENSMUST00000141632.1	450	No protein	Retained intron	-	-	TSL:3	

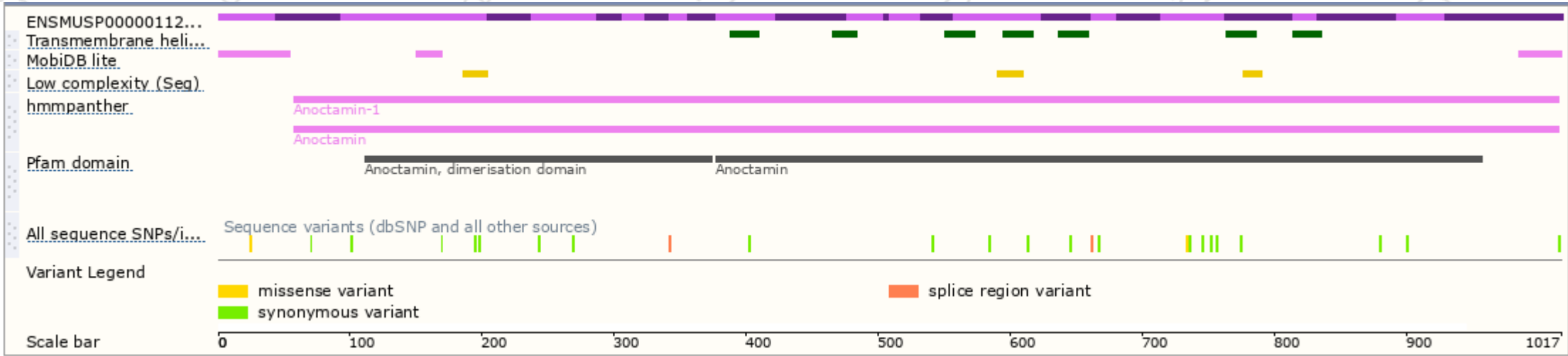
The strategy is based on the design of *Ano1-203* transcript, The transcription is shown below



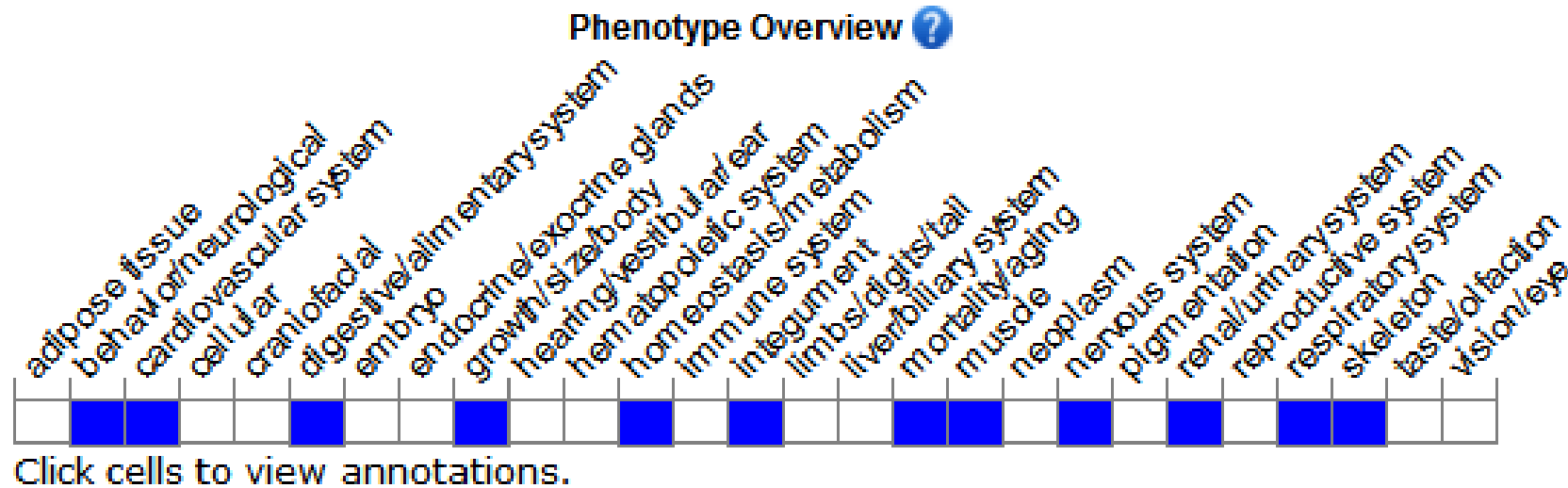
Genomic location distribution



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, Mice homozygous for a knockout allele exhibit postnatal death associated with aerophagia, slow postnatal weight gain, cyanosis, and abnormal tracheal morphology. Mice homozygous for a different knock-out allele exhibit proteinuria and intracellular endosomal vesicles in PTE cells.

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