

***H11-Ctsk-iCre- ρ loyA* Cas9-KI Strategy**

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Reviewer:

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Design Date:

2019-8-18

Project Overview

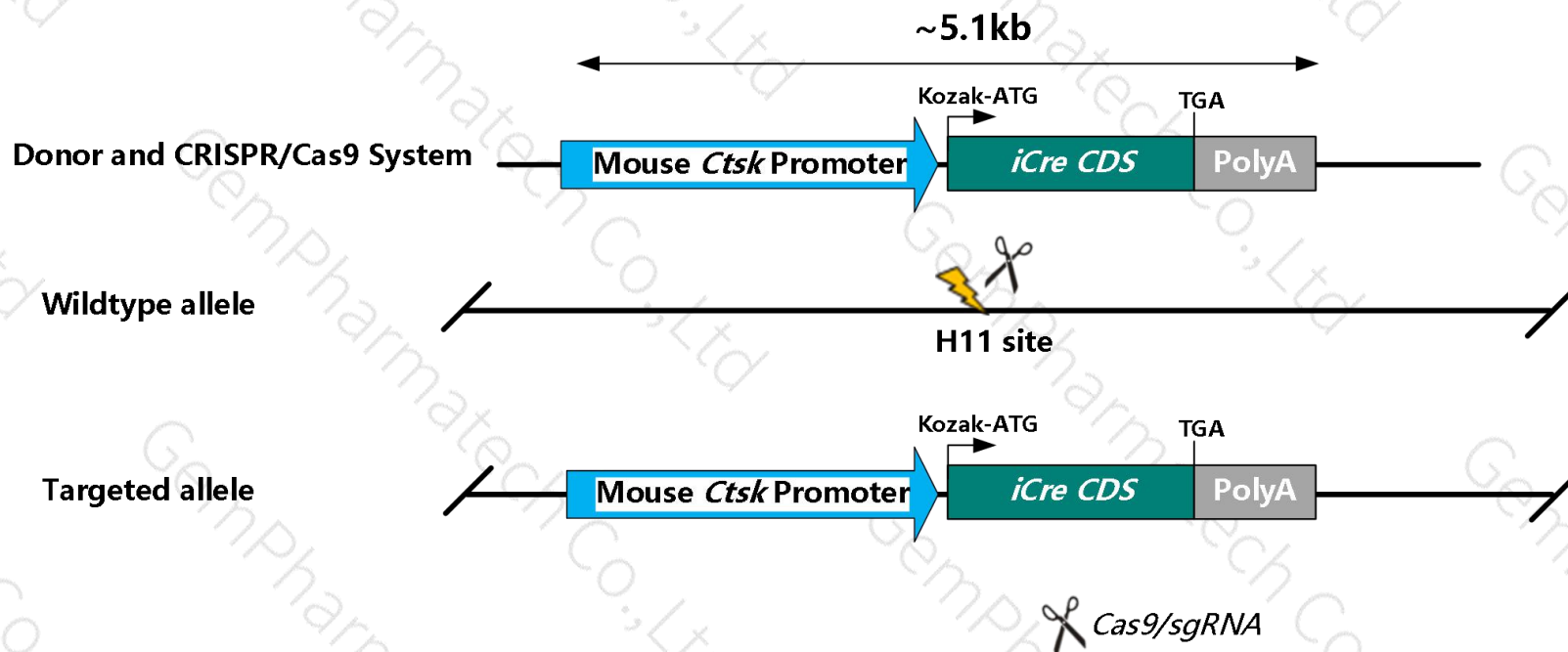
Project Name	<i>H11-Ctsk-iCre-<i>ployA</i></i>
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Project type	Cas9-KI (H11)
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Strain background	C57BL/6J
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Knockin strategy

The *Ctsk-iCre-ployA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.
- In this study, the *Ctsk-iCre-ployA* gene fragment was inserted into H11 site of mice by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and sgRNA were constructed in vitro, Cas9, donor and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6J mice by PCR, sequencing and southern blot, then the stable inheritance of F1 positive mice model was obtained.

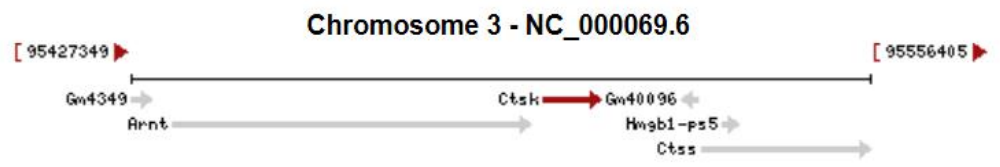
- H11 is located on Chr11. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., iCre) strains, if the other gene is also on Chr11, it may be extremely hard to get double gene positive homozygotes.
- According to the existing data, Cre-mediated recombination is expressed in osteoclasts and can be detected at E17.5 days of embryonic phase.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Ctsk cathepsin K [*Mus musculus* (house mouse)]

Gene ID: 13038, updated on 12-Aug-2019

Summary

Official Symbol	Ctsk provided by MGI
Official Full Name	cathepsin K provided by MGI
Primary source	MGI:MGI:107823
See related	Ensembl:ENSMUSG00000028111
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	catK; Ms10q; MMS10-Q; AI323530
Summary	This gene encodes a member of the cathepsin family of cysteine proteases that is highly expressed in osteoclasts and is involved in the degradation of collagen and other matrix proteins in bone. The encoded preproprotein undergoes proteolytic processing to generate a mature, functional enzyme. Mice lacking the encoded protein exhibit phenotypic features of pycnodysostosis such as increased bone density and bone deformity, which become progressively more pronounced with age. [provided by RefSeq, Jan 2016]
Expression	Biased expression in bladder adult (RPKM 27.4), limb E14.5 (RPKM 13.7) and 12 other tissues See more
Orthologs	human all



Transcript information (Ensembl)

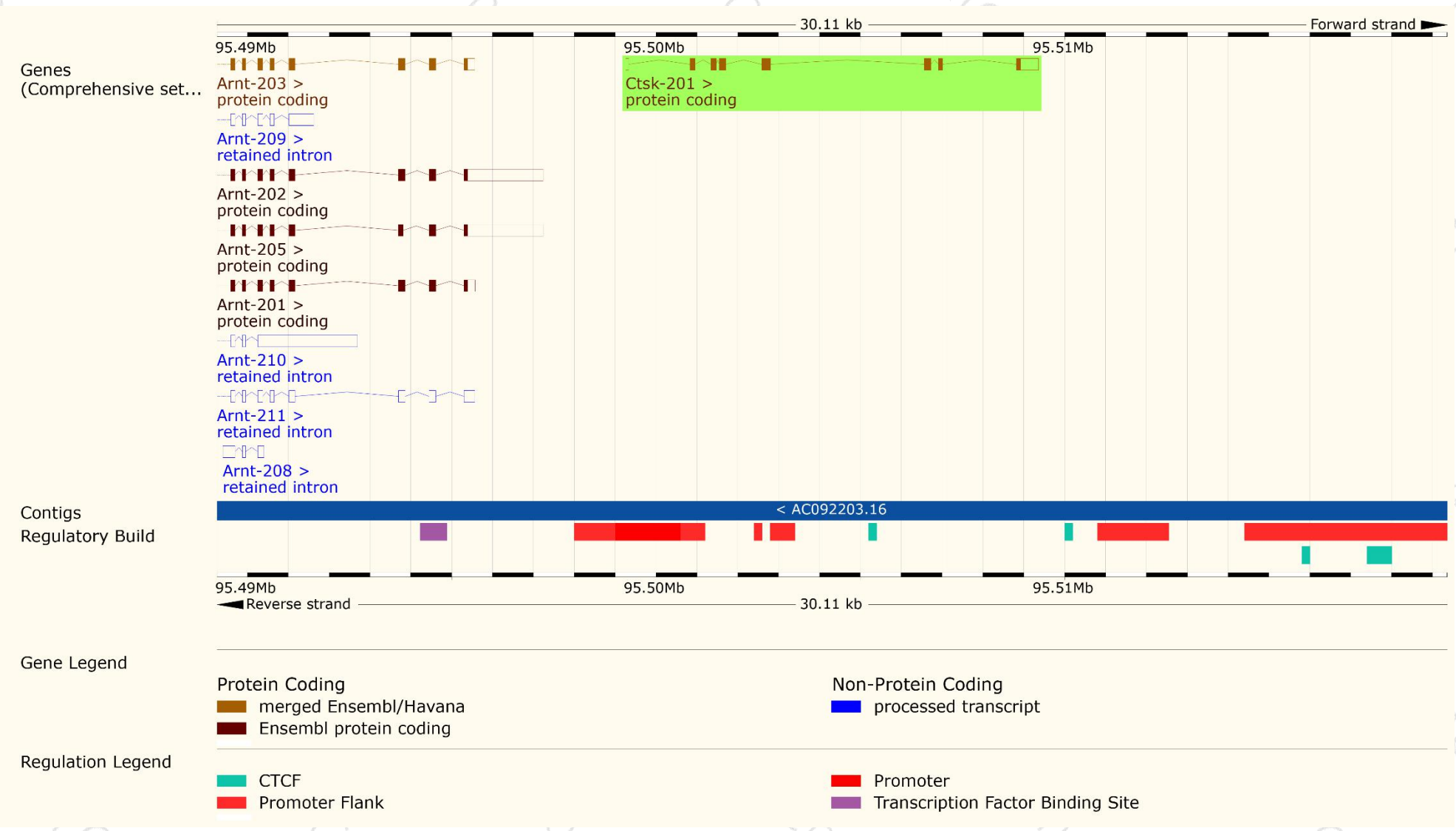
The gene has 1 transcript, and the transcript is shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Translation ID ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲
Ctsk-201	ENSMUST00000015664.4	1512	329aa	ENSMUSP00000015664.3	Protein coding	CCDS17615	P55097 Q545T0	TSL:1 Gencode basic APPRIS P1

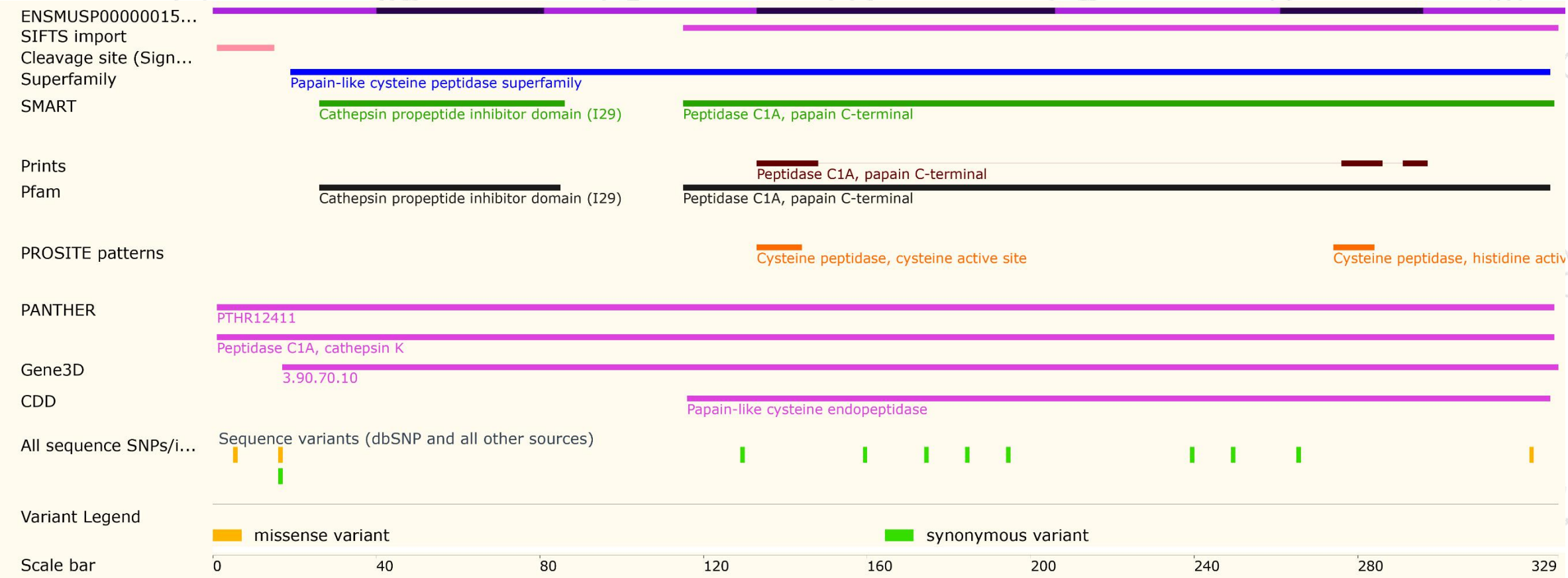
The strategy is based on the design of *Ctsk-201* transcript, The transcription is shown below



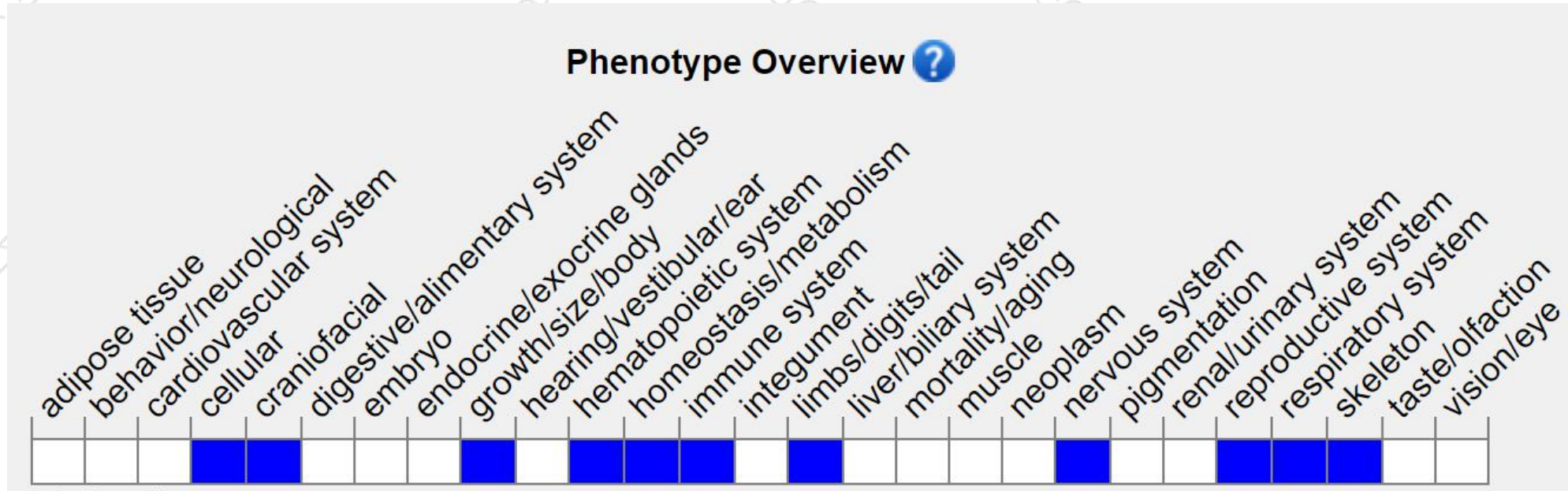
Genomic location distribution



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/marker/MGI:107823>) .

According to the existing MGI data, Mice homozygous for disruptions in this gene survive well and are fertile. They have osteopetrosis, reduced levels of thyroxine and increased pulmonary fibrosis.

iCre Sequence^[1] (1056bp)

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGCCACCTCTGATGAAGTCAGGAAGA
ACCTGATGGACATGTTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAGATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAA
CAACAGGAAATGGTTCCCTGCTGAACCTGAGGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTG
GGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATCAGAAAGGAGAATGTGG
ATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCAGATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAG
GAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGA
ATGCTGATCCACATTGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGTTACCAAGCTGGTGGAGAGATGGATCT
CTGTGTCTGGTGTGGCTGATGACCCCAACAACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCTGCCCCTTCTGCCACCTCCCAACTGTCCACCCG
GGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGTGCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGA
GTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCA
GAAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

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[1] Madison BB, Dunbar L, Qiao XT, Braunstein K, Braunstein E, Gumucio DL. *cis* Elements of the Villin Gene Control Expression in Restricted Domains of the Vertical (Crypt) and Horizontal (Duodenum, Cecum) Axes of the Intestine. *J Biol Chem*. 2002 Sep 6;277(36):33275-83. Epub 2002 Jun 13.

[2] Maria Arantzazu Sanchez-Fernandez, et al. Transgenic Mice for a Tamoxifen-Induced, Conditional Expression of the Cre Recombinase in Osteoclasts. *PLoS One*. 2012; 7(5): e37592.

To generate an inducible osteoclast specific Cre transgenic mouse line, we first cloned the mouse cathepsin K promoter (-3429 to +52 from the start codon). This fragment was inserted

A

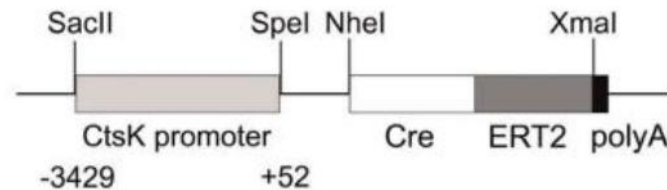


Figure 1. CtsKCreERT2 construct and *In vitro* functionality. A: Schematic diagram of transgenic CtsKCreERT2 expression construct used to generate transgenic mice, which includes the full sequence of the CtsK promoter, the CreERT2 fusion sequence, and the SV40 polyA signal. **B.** Schematic representation of the floxed neomycin construct

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