GemPharmatech™

Rnf220 Cas9-KO Strategy

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Reviewer: Daohua Xu

Design Date: 2023-7-25

Overview

Target Gene Name

- Rnf220

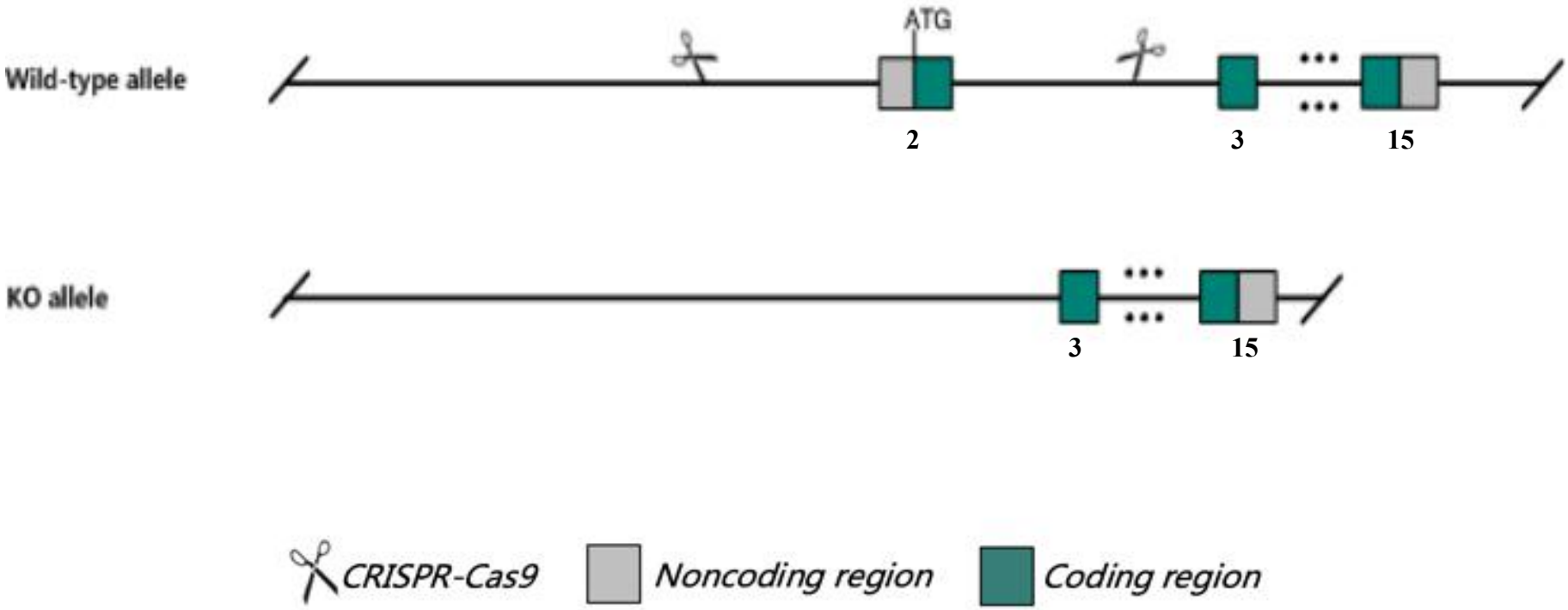
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Technical Information

- The *Rnf220* gene has 14 transcripts. According to the structure of *Rnf220* gene, exon2 of *Rnf220*-201 (ENSMUST00000030439.15) transcript is recommended as the knockout region. The region contains start codon ATG. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Rnf220* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

Gene Information

Rnf220 ring finger protein 220 [Mus musculus (house mouse)]

Gene ID: 66743, updated on 31-May-2023

Summary

Official Symbol	Rnf220 provided by MGI
Official Full Name	ring finger protein 220 provided by MGI
Primary source	MGI:MGI:1913993
See related	Ensembl:ENSMUSG00000028677
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	4732477A13, 4931406I20Rik, 5730503K05Rik
Summary	Enables ubiquitin protein ligase activity. Involved in protein autoubiquitination. Acts upstream of or within several processes, including nervous system development; positive regulation of DNA-binding transcription factor activity; and protein monoubiquitination. Located in cytoplasm. Is expressed in several structures, including central nervous system; dorsal root ganglion; genitourinary system; retina; and tooth. Orthologous to human RNF220 (ring finger protein 220). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in testis adult (RPKM 15.8), ovary adult (RPKM 14.8) and 28 other tissues See more
Orthologs	human all

Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

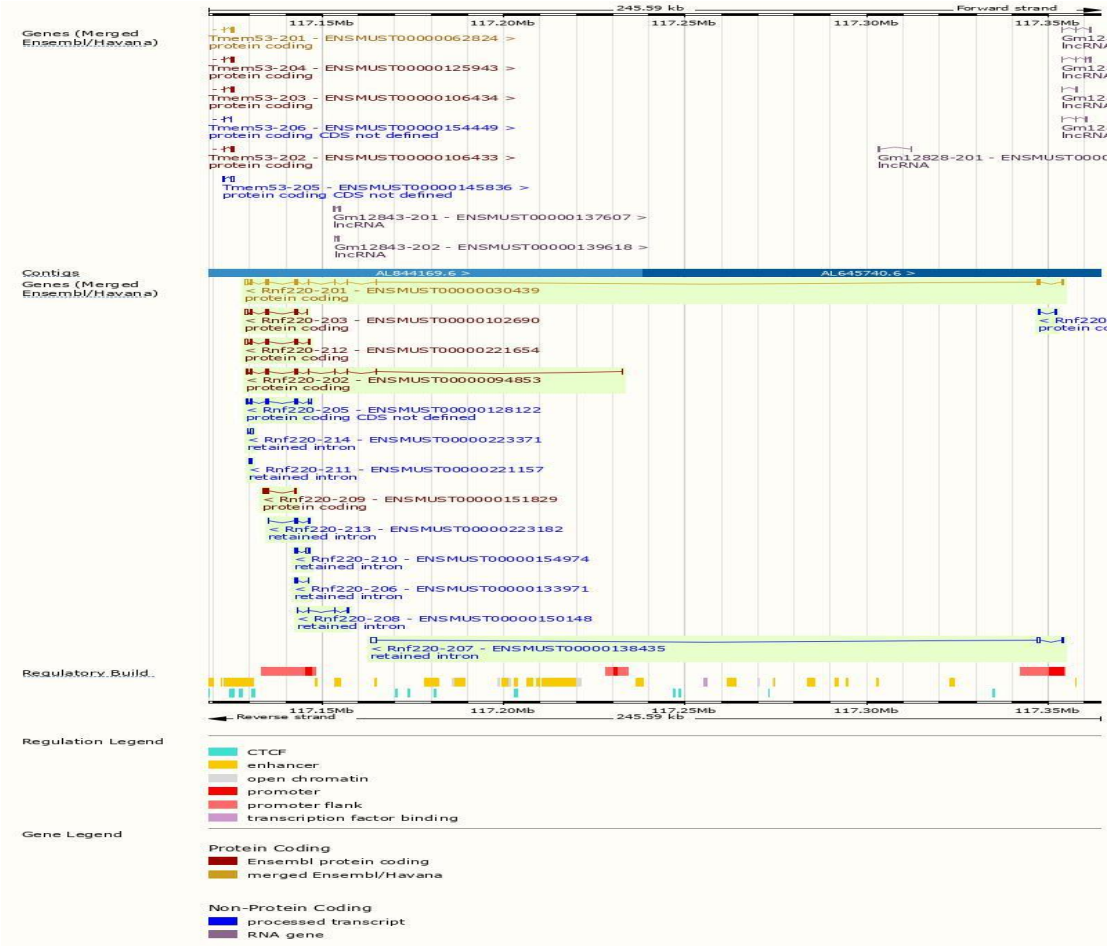
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rnf220-201	ENSMUST00000030439.15	3029	566aa	Protein coding	CCDS18534		A single transcript chosen for a gene which is the most conserved, most highly expressed, has the longest coding sequence and is represented in other key resources, such as NCBI and UniProt. This is defined in detail on http://www.ensembl.org/info/genome/genebuild/canonical.html Ensembl Canonical , The GENCODE set is the gene set for human and mouse. GENCODE basic , TSL:1 ,
Rnf220-212	ENSMUST00000221654.2	1949	308aa	Protein coding			The GENCODE set is the gene set for human and mouse. GENCODE basic , TSL:1 ,
Rnf220-203	ENSMUST00000102690.9	1842	245aa	Protein coding	CCDS80149		The GENCODE set is the gene set for human and mouse. GENCODE basic , TSL:1 ,
Rnf220-202	ENSMUST00000094853.9	1675	353aa	Protein coding	CCDS80150		The GENCODE set is the gene set for human and mouse. GENCODE basic , APPRIS P1 , TSL:1 ,
Rnf220-209	ENSMUST00000151829.2	761	188aa	Protein coding			TSL:3 , CDS 5' incomplete ,
Rnf220-205	ENSMUST00000128122.9	1330	No protein	Protein coding CDS not defined			TSL:5 ,
Rnf220-204	ENSMUST00000123222.2	458	No protein	Protein coding CDS not defined			TSL:3 ,
Rnf220-207	ENSMUST00000138435.2	2630	No protein	Retained intron			TSL:1 ,
Rnf220-210	ENSMUST00000154974.8	1492	No protein	Retained intron			TSL:1 ,
Rnf220-214	ENSMUST00000223371.2	728	No protein	Retained intron			TSL:2 ,
Rnf220-211	ENSMUST00000221157.2	720	No protein	Retained intron			TSL:1 ,
Rnf220-208	ENSMUST00000150148.2	713	No protein	Retained intron			TSL:3 ,
Rnf220-213	ENSMUST00000223182.2	534	No protein	Retained intron			TSL:5 ,
Rnf220-206	ENSMUST00000133971.3	343	No protein	Retained intron			TSL:1 ,

The strategy is based on the design of *Rnf220*-201 transcript, the transcription is shown below:

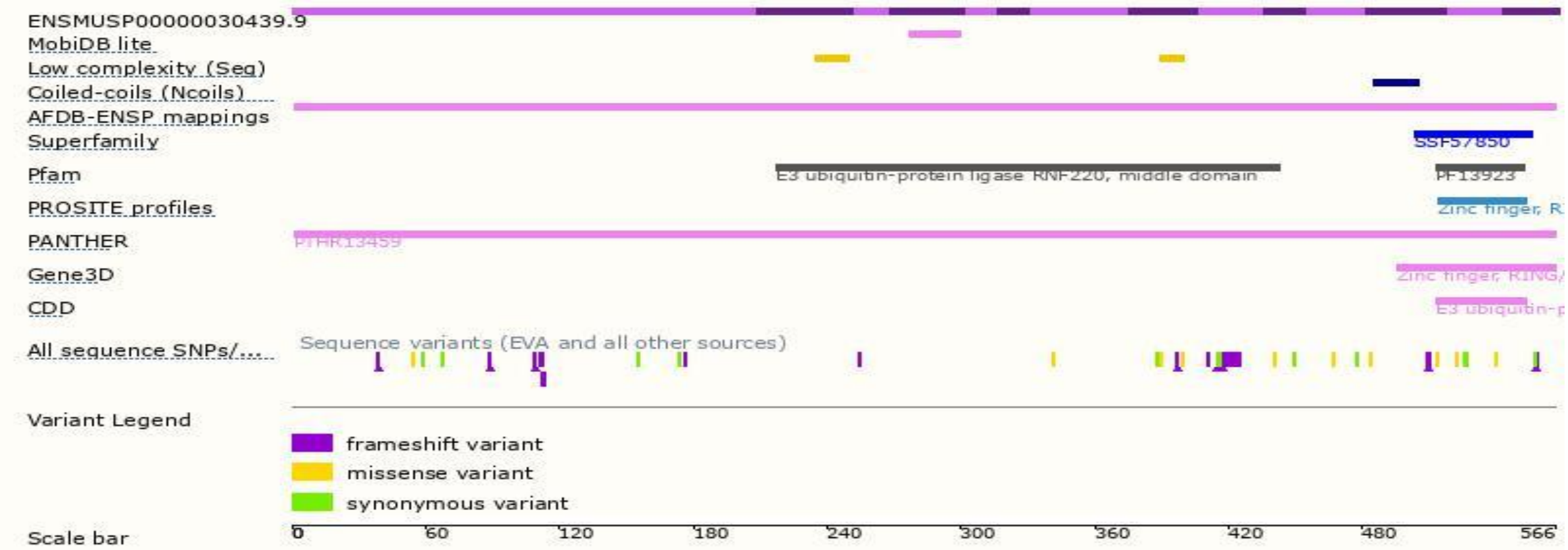
Source: https://www.ensembl.org

Genomic Information



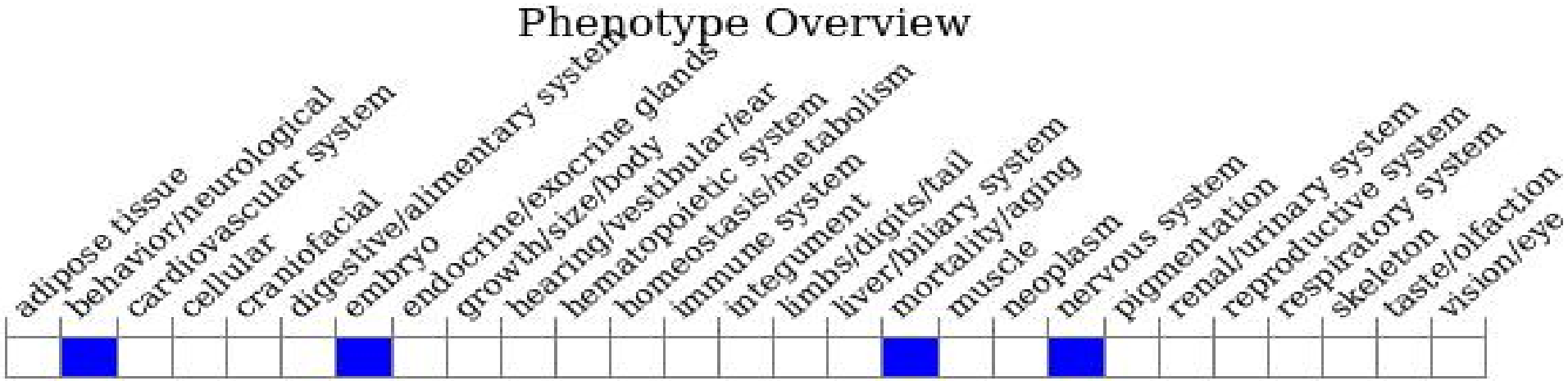
Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Homozygous knockout results in the reduction of the number of motor neurons and some types of spinal interneurons and the increase of other types of spinal interneurons in embryos.

Source: https://www.informatics.jax.org

Important Information

- The effect on transcript-202&203&209&212 is unknown.
- *Rnf220* is located on Chr4. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

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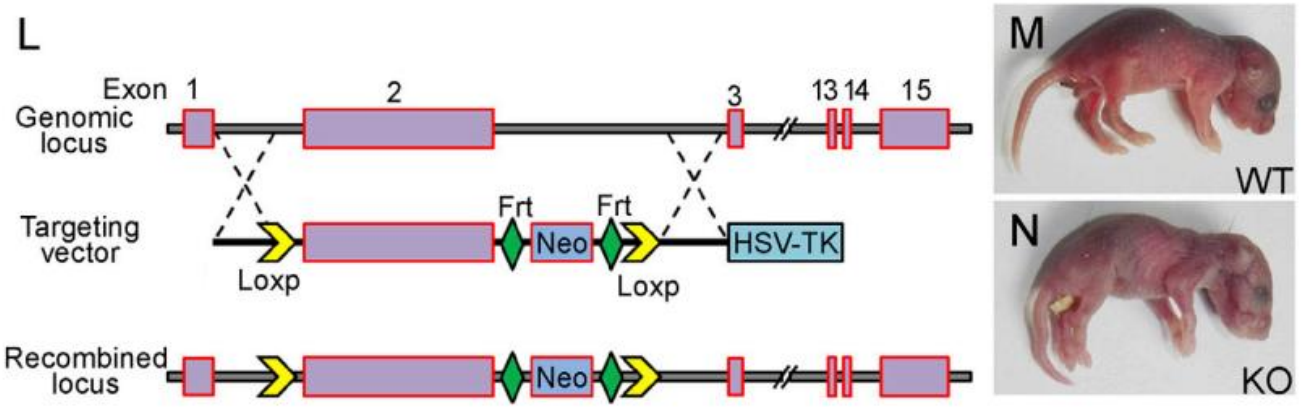
Reference Information

Ma P, et al., Fine-Tuning of Shh/Gli Signaling Gradient by Non-proteolytic Ubiquitination during Neural Patterning. Cell Rep. 2019 Jul 9;28(2):541-553.e4



(L) Diagram of the targeting construct and expected recombination events. Exon2 is floxed by two LoxP sites.
(M and N) Photographs showing control (M) and $RNF220^{-/-}$ (N) pups on the day of birth. The $RNF220^{-/-}$ pups die after birth. WT, wild-type; KO, knockout.

Animals, Staging and Genotyping

All mice were maintained and handled according to guidelines approved by the Animal Care and Use Committee of the Kunming Institute of Zoology, Chinese Academy of Sciences. All mice were maintained on a C57BL/6 background. Analysis was performed only after lines were crossed to C57BL/6 for at least three generations. The conditional RNF220 knockout allele, $RNF220^{fl}$ was generated by insertion of two loxP sites into introns flanking exon 2 through homologous recombination. To obtain RNF220 knockout embryos, Vasa-cre mice were used to generate germ cell RNF220 conditional knockout female mice ($RNF220^{fl/wt}$; Vasa-cre) first and then the female mice were used to mate with the male RNF220 floxed mice ($RNF220^{fl/fl}$).