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Rnf20 Cas9-CKO Strategy

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Reviewer: Xueting Zhang

Design Date: 2023-9-6

Overview

Target Gene Name

- Rnf20

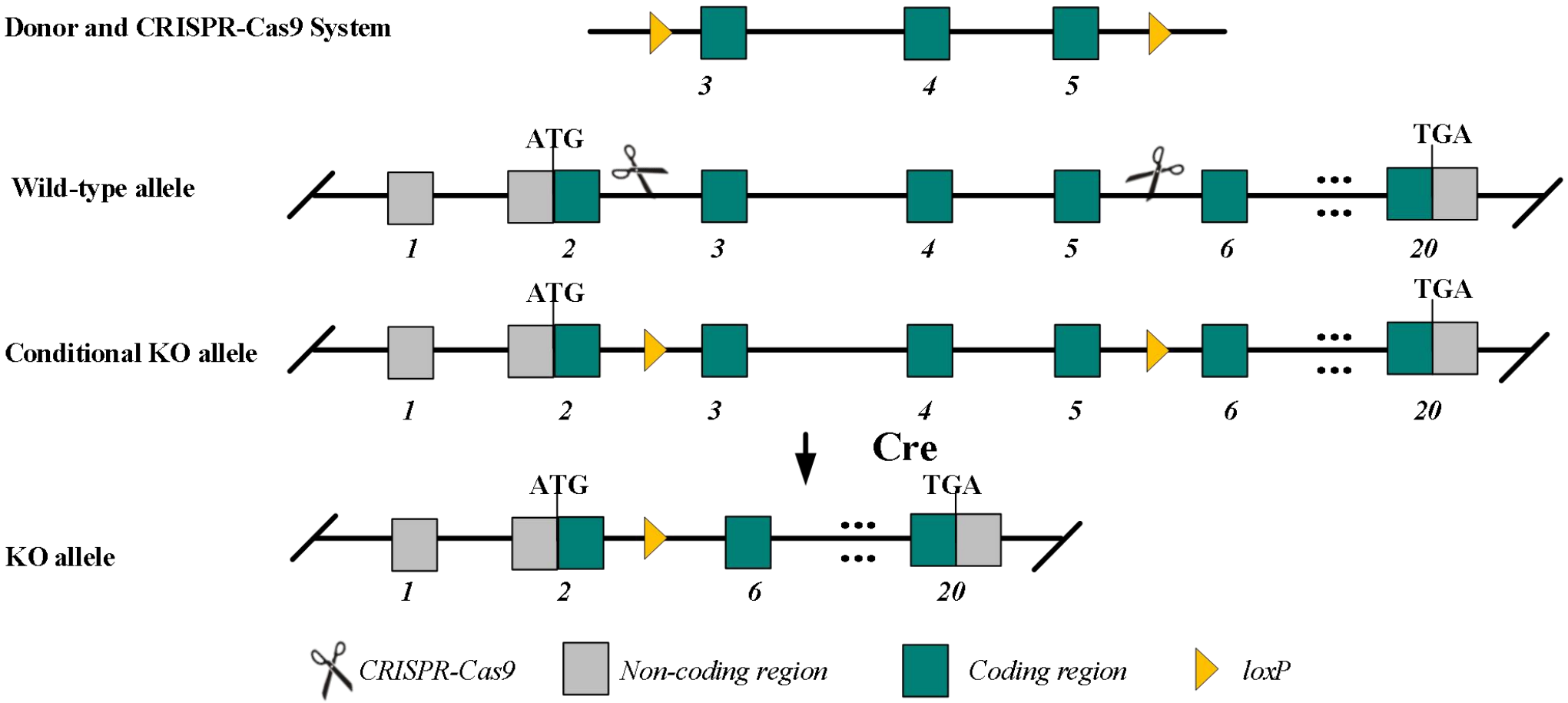
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Rnf20* gene.

Technical Information

- The *Rnf20* gene has 10 transcripts. According to the structure of *Rnf20* gene, exon 3-exon 5 of *Rnf20*-210 (ENSMUST00000167496.8) transcript is recommended as the knockout region. The region contains 499 bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Rnf20* 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 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.
- 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.

Transcript Information

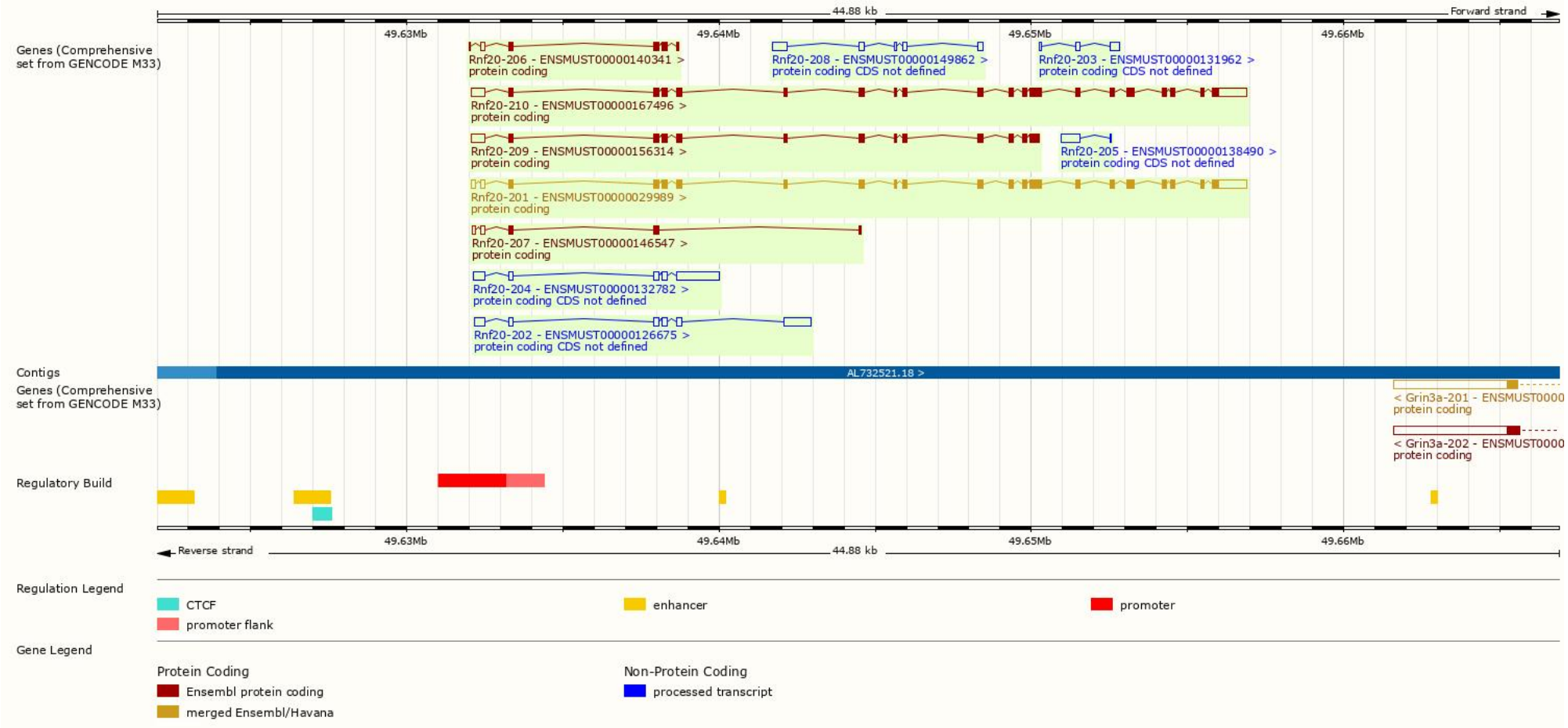
The gene has 10 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000167496.8	Rnf20-210	4312	973aa	Protein coding	CCDS18178	Q5DTM8-1	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000029989.11	Rnf20-201	4154	973aa	Protein coding	CCDS18178	Q5DTM8-1	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000156314.8	Rnf20-209	2300	606aa	Protein coding		A2AIR2	TSL:1 CDS 3' incomplete
ENSMUST00000140341.8	Rnf20-206	727	174aa	Protein coding		A2AIR0	TSL:2 CDS 3' incomplete
ENSMUST00000146547.2	Rnf20-207	648	117aa	Protein coding		A2AIR1	TSL:3 CDS 3' incomplete
ENSMUST00000132782.8	Rnf20-204	2238	No protein	Protein coding CDS not defined		-	TSL:1
ENSMUST00000126675.2	Rnf20-202	1904	No protein	Protein coding CDS not defined		-	TSL:1
ENSMUST00000149862.2	Rnf20-208	966	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000138490.2	Rnf20-205	610	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000131962.2	Rnf20-203	488	No protein	Protein coding CDS not defined		-	TSL:2

The strategy is based on the design of *Rnf20*-210 transcript, the transcription is shown below:

Source: https://www.ensembl.org

Genomic Information



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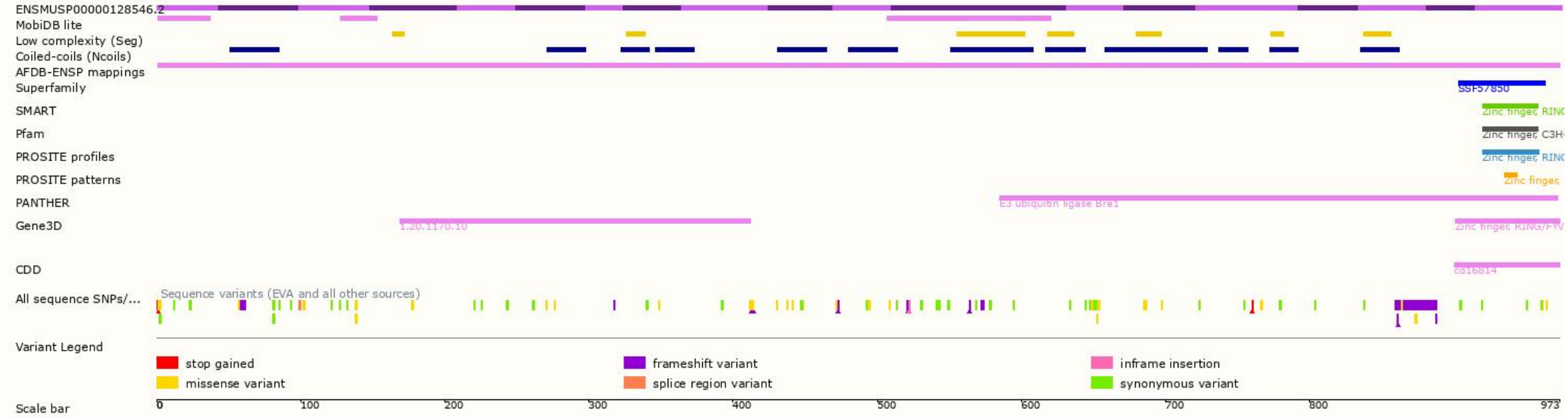
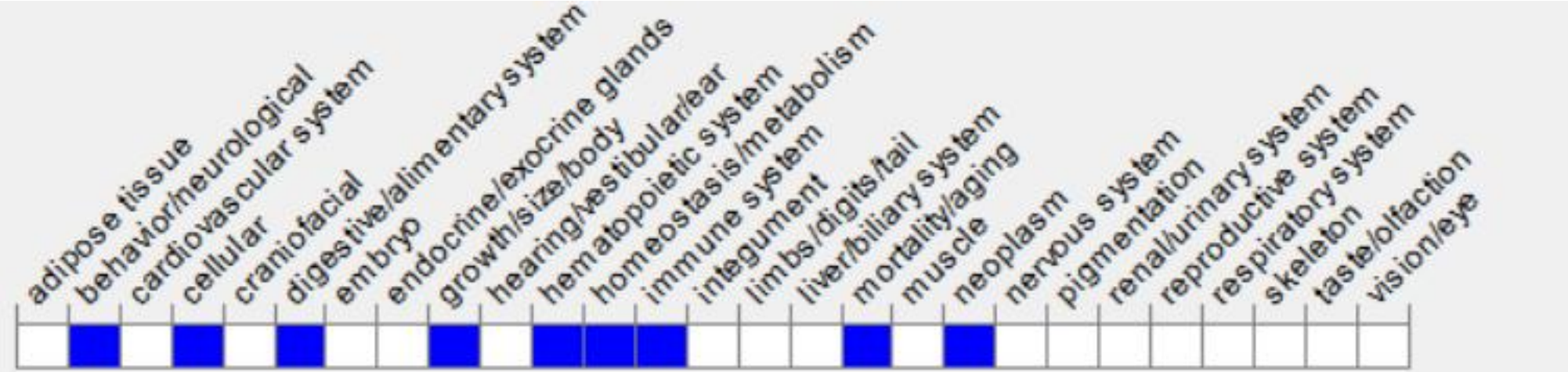
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



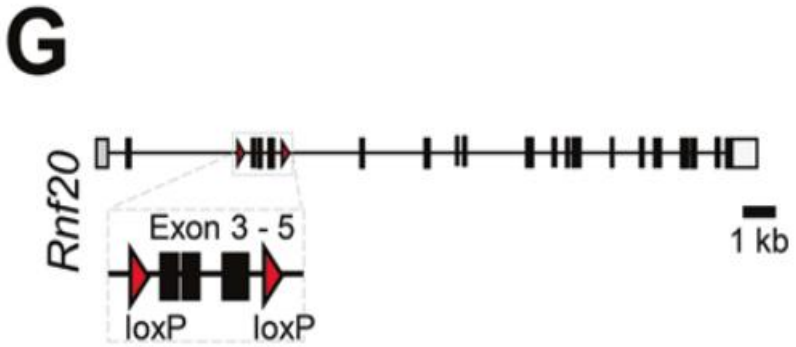
- Mice homozygous for a knock-out allele exhibit complete preimplantation embryonic lethality. Heterozygotes are predisposed to acute and chronic colonic inflammation and development of colorectal cancer.

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

Important Information

- According to the MGI information,Mice homozygous for a knock-out allele exhibit complete preimplantation embryonic lethality. Heterozygotes are predisposed to acute and chronic colonic inflammation and development of colorectal cancer.
- The effect of the knock-out region on the *Rnf20* -206 and *Rnf20* -207 transcripts is unknown.
- *Rnf20* 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Reference



The *Rnf20*$^{tm1c(EUCOMM)Wtsi}$ mouse line, referred to here as *Rnf20*^{flox}, containing loxP sites flanking exons 3–5 was generated from the mouse embryonic stem cell line G11 (EPD0701_3_G11) purchased from the European Conditional Mouse Mutagenesis Program (EUCOMM) by crossing "knockout first" allele (*Rnf20*$^{tm1a(EUCOMM)Wtsi}$) mice with a mouse line ubiquitously expressing the Flp

Kosinsky RL, Zerche M, Kutschat AP, et al. RNF20 and RNF40 regulate vitamin D receptor-dependent signaling in inflammatory bowel disease. Cell Death Differ. 2021;28(11):3161-3175. doi:10.1038/s41418-021-00808-w