Eftud2 Cas9-CKO Strategy

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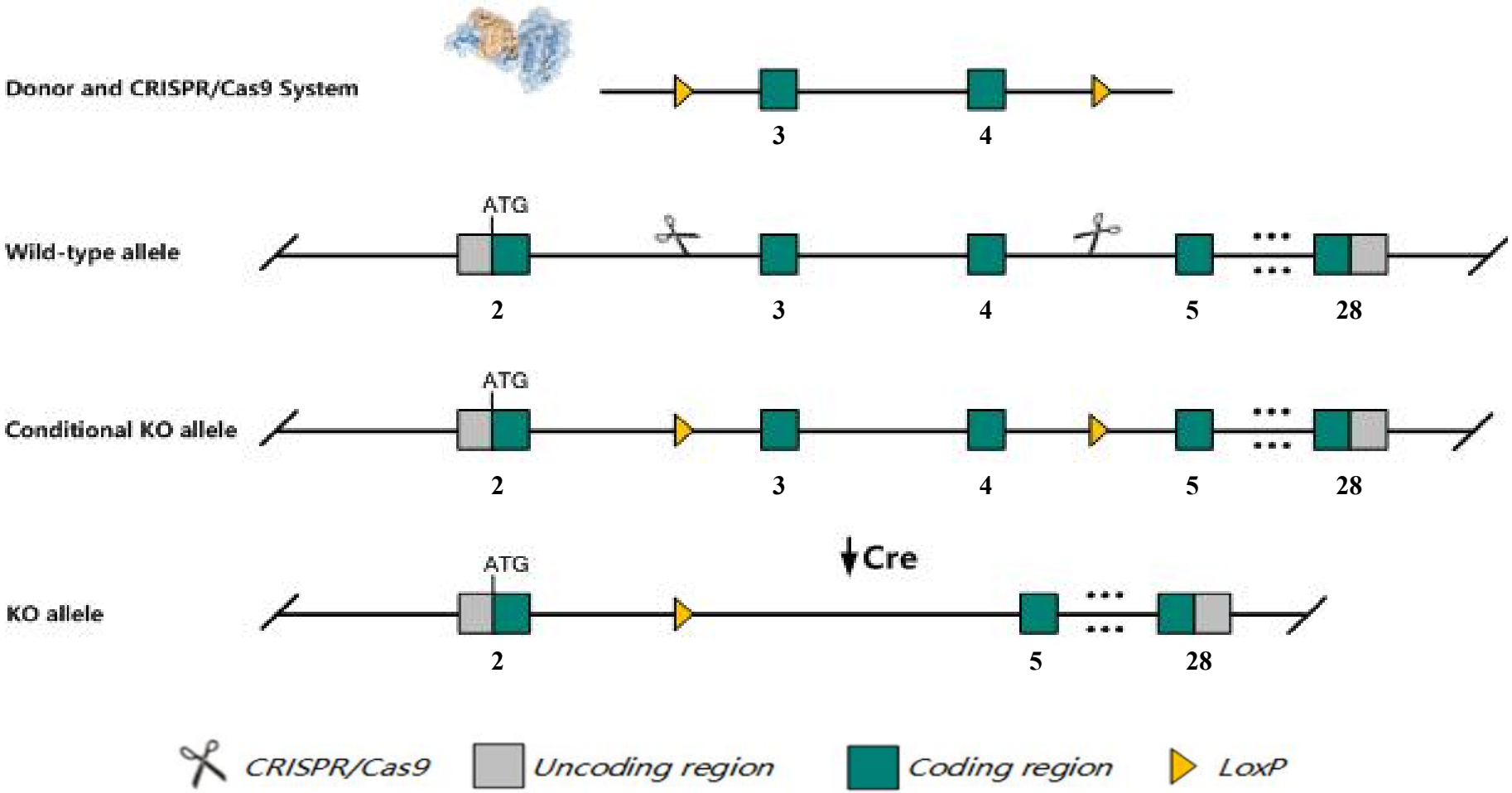
Project Overview

Project Name	***Eftud2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Eftud2* gene. The schematic diagram is as follows:



Technical routes

The *Eftud2* gene has 13 transcripts. According to the structure of *Eftud2* gene, exon3-exon4 of *Eftud2-201*(ENSMUST00000021306.13) transcript is recommended as the knockout region.The region contains 245bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Eftud2* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

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.

Notice

According to the existing MGI data,homozygous mice lacking exon 2 die before implantation while heterozygous mice show a transient developmental delay between E8.5-E9.5.

The *Eftud2* gene is located on the Chr11. 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 biological processes,all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information NCBI

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Eftud2 elongation factor Tu GTP binding domain containing 2 [Mus musculus (house mouse)]

Gene ID: 20624, updated on 13-Mar-2020

Summary

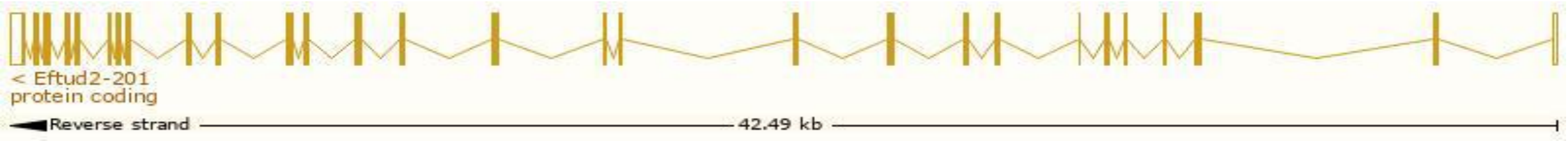
Official Symbol	Eftud2 provided by MGI
Official Full Name	elongation factor Tu GTP binding domain containing 2 provided by MGI
Primary source	MGI:MGI:1336880
See related	Ensembl:ENSMUSG00000020929
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	116kDa, Snrp116, U5-116kD
Expression	Ubiquitous expression in CNS E11.5 (RPKM 41.6), whole brain E14.5 (RPKM 30.4) and 28 other tissues See more
Orthologs	human all

Transcript information Ensembl

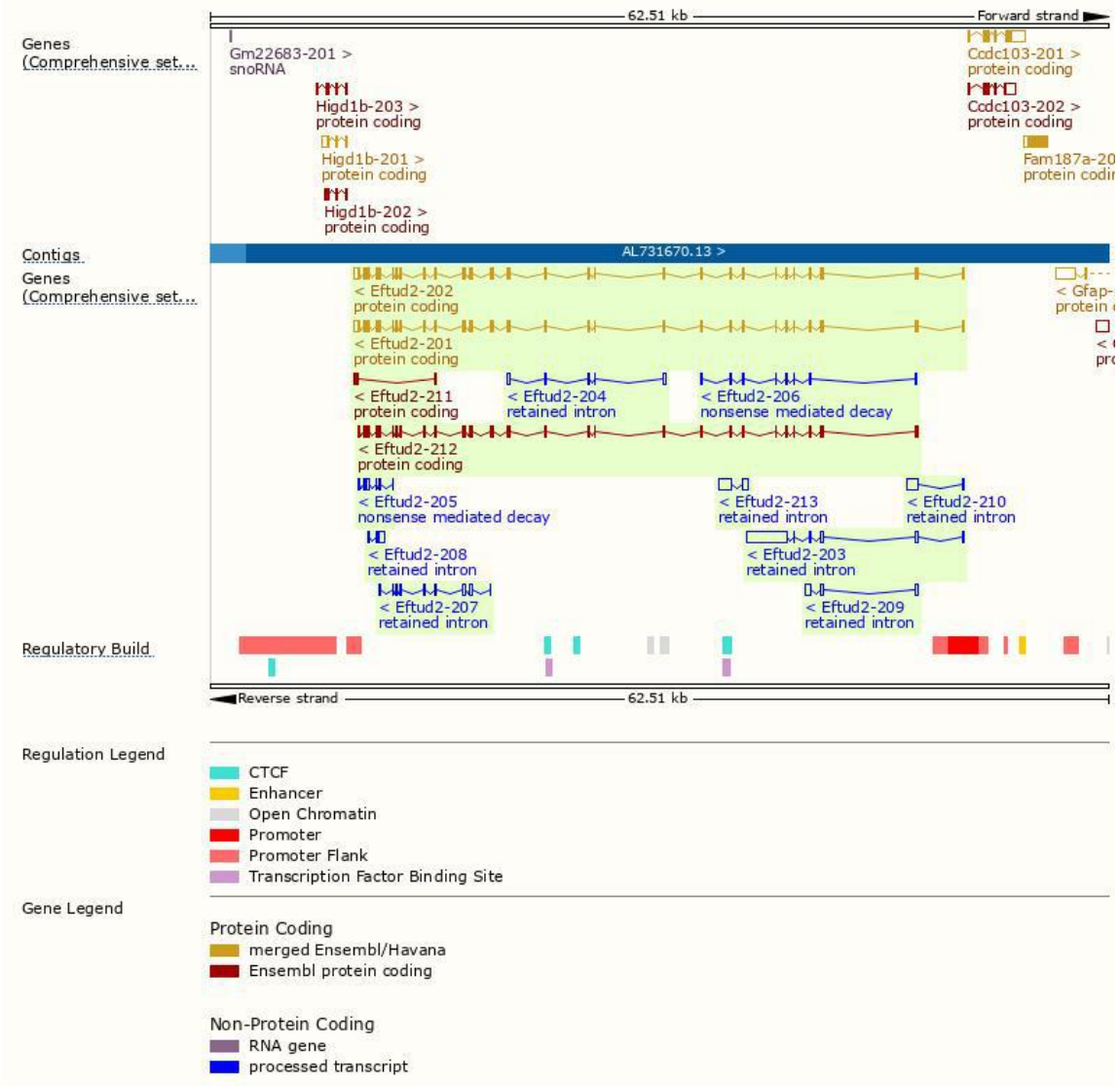
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Eftud2-202	ENSMUST00000107060.7	3339	971aa	Protein coding	CCDS48947	O08810 Q543F1	TSL:1 GENCODE basic
Eftud2-201	ENSMUST00000021306.13	3324	972aa	Protein coding	CCDS48948	A2AH85	TSL:5 GENCODE basic APPRIS P1
Eftud2-212	ENSMUST00000173679.7	2889	962aa	Protein coding	-	G3UZ34	TSL:5 GENCODE basic
Eftud2-211	ENSMUST00000172611.1	397	98aa	Protein coding	-	G3UZ25	CDS 5' incomplete TSL:3
Eftud2-206	ENSMUST00000138483.7	679	30aa	Nonsense mediated decay	-	G3V021	CDS 5' incomplete TSL:5
Eftud2-205	ENSMUST00000132543.1	567	85aa	Nonsense mediated decay	-	G3UXK8	CDS 5' incomplete TSL:3
Eftud2-203	ENSMUST00000123833.8	3288	No protein	Retained intron	-	-	TSL:1
Eftud2-213	ENSMUST00000173974.1	1326	No protein	Retained intron	-	-	TSL:5
Eftud2-207	ENSMUST00000141273.1	934	No protein	Retained intron	-	-	TSL:5
Eftud2-210	ENSMUST00000148209.1	796	No protein	Retained intron	-	-	TSL:2
Eftud2-208	ENSMUST00000143323.1	631	No protein	Retained intron	-	-	TSL:3
Eftud2-204	ENSMUST00000131678.1	629	No protein	Retained intron	-	-	TSL:2
Eftud2-209	ENSMUST00000147368.1	581	No protein	Retained intron	-	-	TSL:2

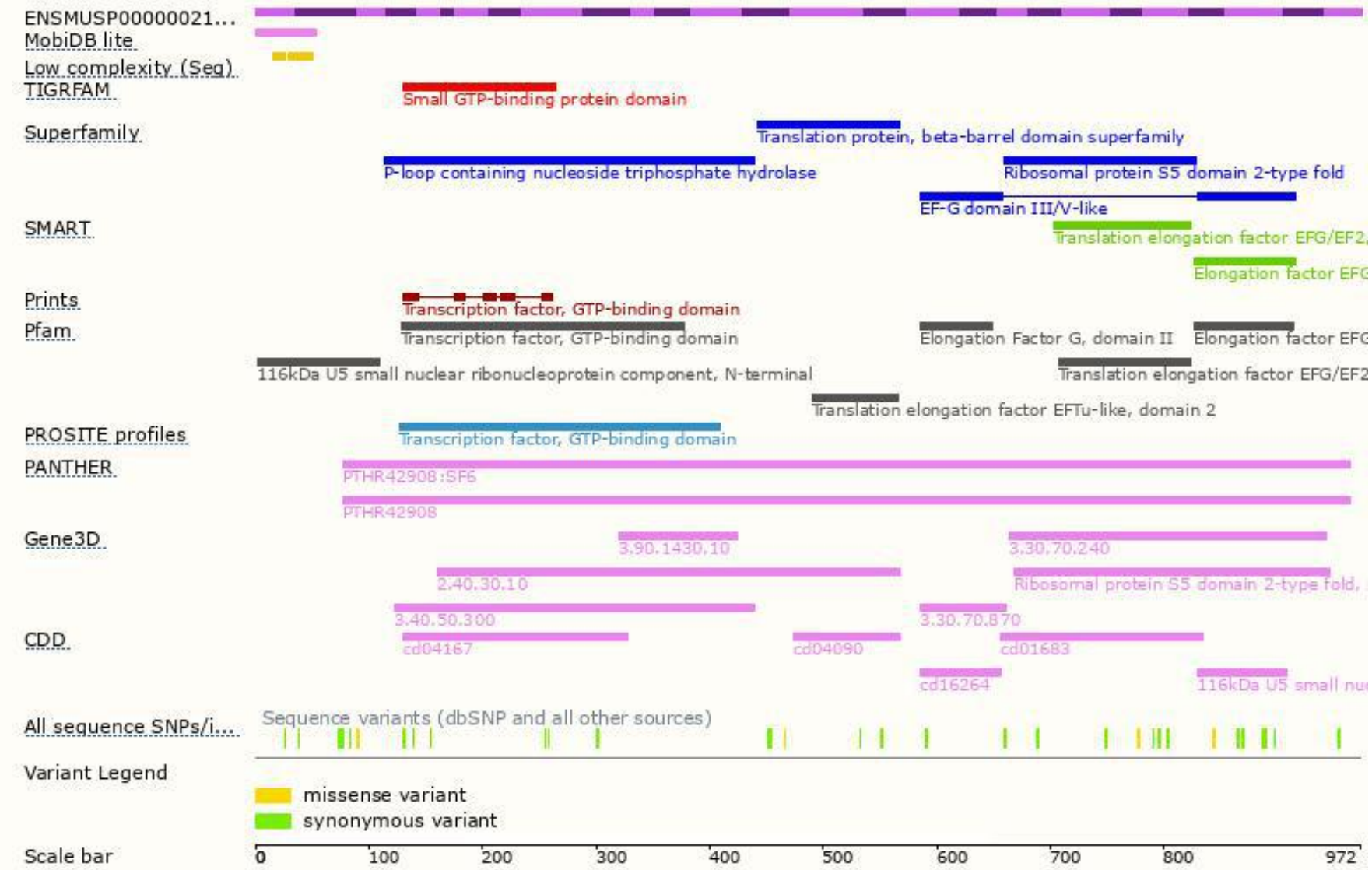
The strategy is based on the design of *Eftud2-201* transcript,the transcription is shown below:



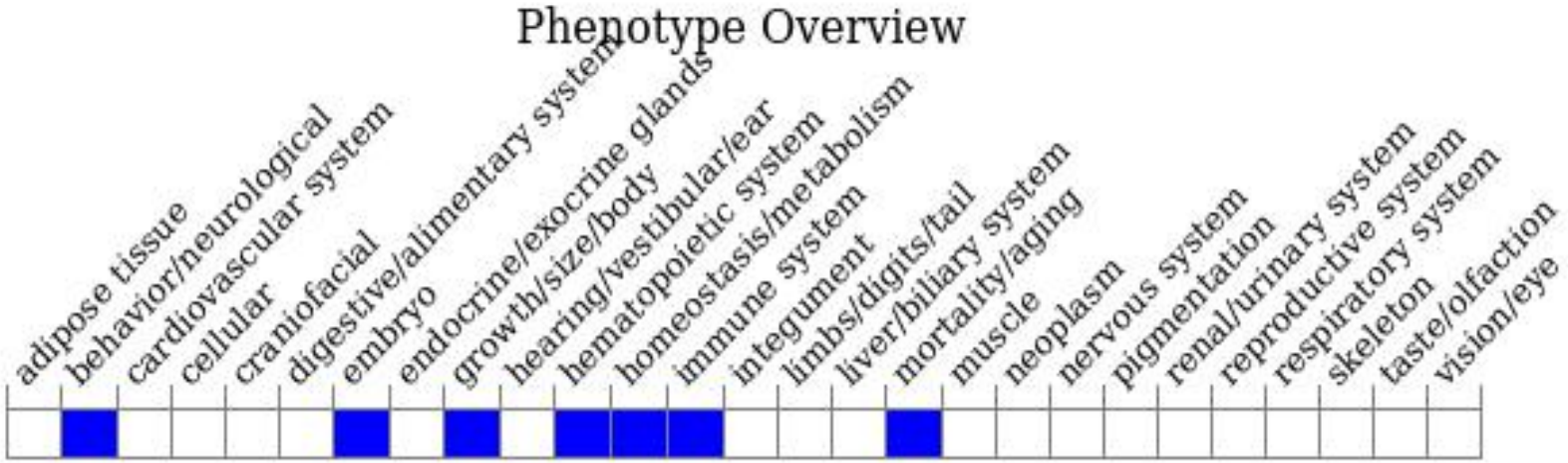
Genomic location distribution



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/).

According to the existing MGI data,homozygous mice lacking exon 2 die before implantation while heterozygous mice show a transient developmental delay between E8.5-E9.5.

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