Itgav Cas9-CKO Strategy

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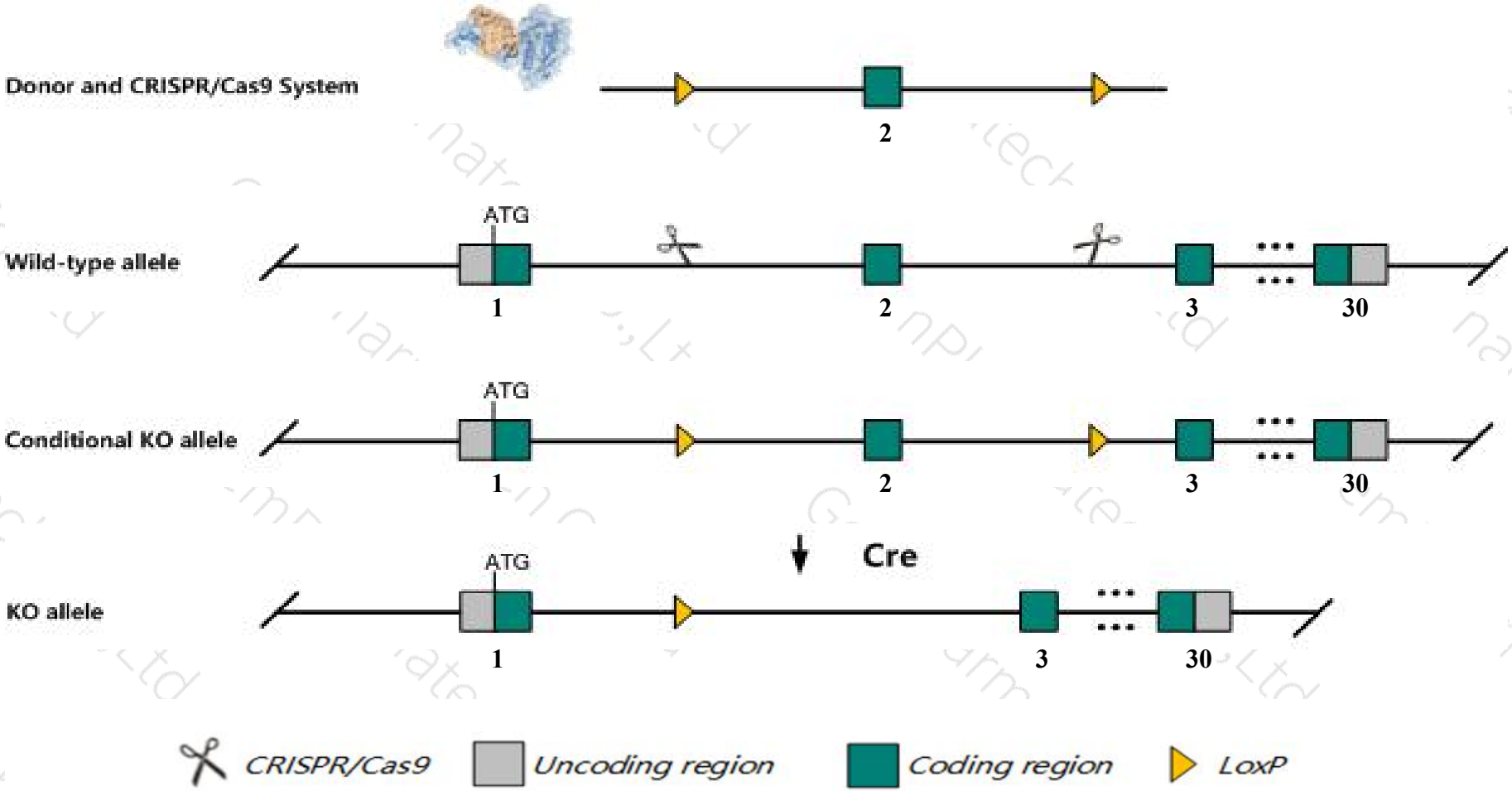
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Itgav* gene has 9 transcripts. According to the structure of *Itgav* gene, exon2 of *Itgav-201* (ENSMUST00000028499.10) transcript is recommended as the knockout region.The region contains 131bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Itgav* 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,Homozygotes for a targeted null mutation exhibit placental defects, intracerebral and intestinal hemorrhages, and cleft palate, resulting in death occurring as early as midgestation and as late as shortly after birth.

➢ Because the N-terminal of transcript 204&205 are incomplete,the impact of this strategy on them is unknown.

➢The distance between the floxed region and *Gm13684* is about 0.5kb, the deletion of floxed region may influence the 3'regulation of this gene.

➢ The *Itgav* gene is located on the Chr2. 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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Itgav integrin alpha V [Mus musculus (house mouse)]

Gene ID: 16410, updated on 25-Mar-2019

Summary

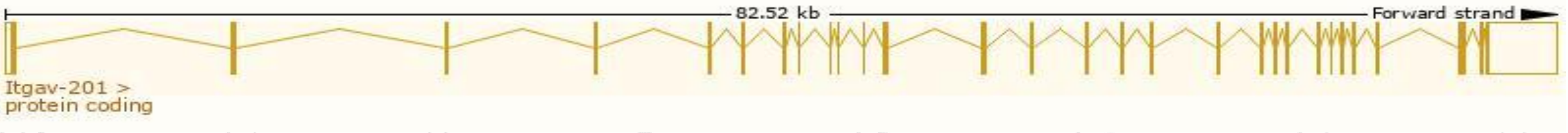
Official Symbol	Itgav provided by MGI
Official Full Name	integrin alpha V provided by MGI
Primary source	MGI:MGI:96608
See related	Ensembl:ENSMUSG00000027087
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110004F14Rik, 2610028E01Rik, CD51, D430040G12Rik
Summary	This gene encodes a protein that is a member of the integrin superfamily. Integrins are transmembrane receptors involved cell adhesion and signaling, and they are subdivided based on the heterodimer formation of alpha and beta chains. This protein has been shown to heterodimerize with beta 1, beta 3, beta 6 and beta 8. The heterodimer of alpha v and beta 3 forms the Vitronectin receptor. This protein interacts with several extracellular matrix proteins to mediate cell adhesion and may play a role in cell migration. In mouse, deficiency of this gene is associated with defects in vascular morphogenesis in the brain and early post-natal death. [provided by RefSeq, May 2013]
Expression	Ubiquitous expression in placenta adult (RPKM 13.0), ovary adult (RPKM 12.9) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

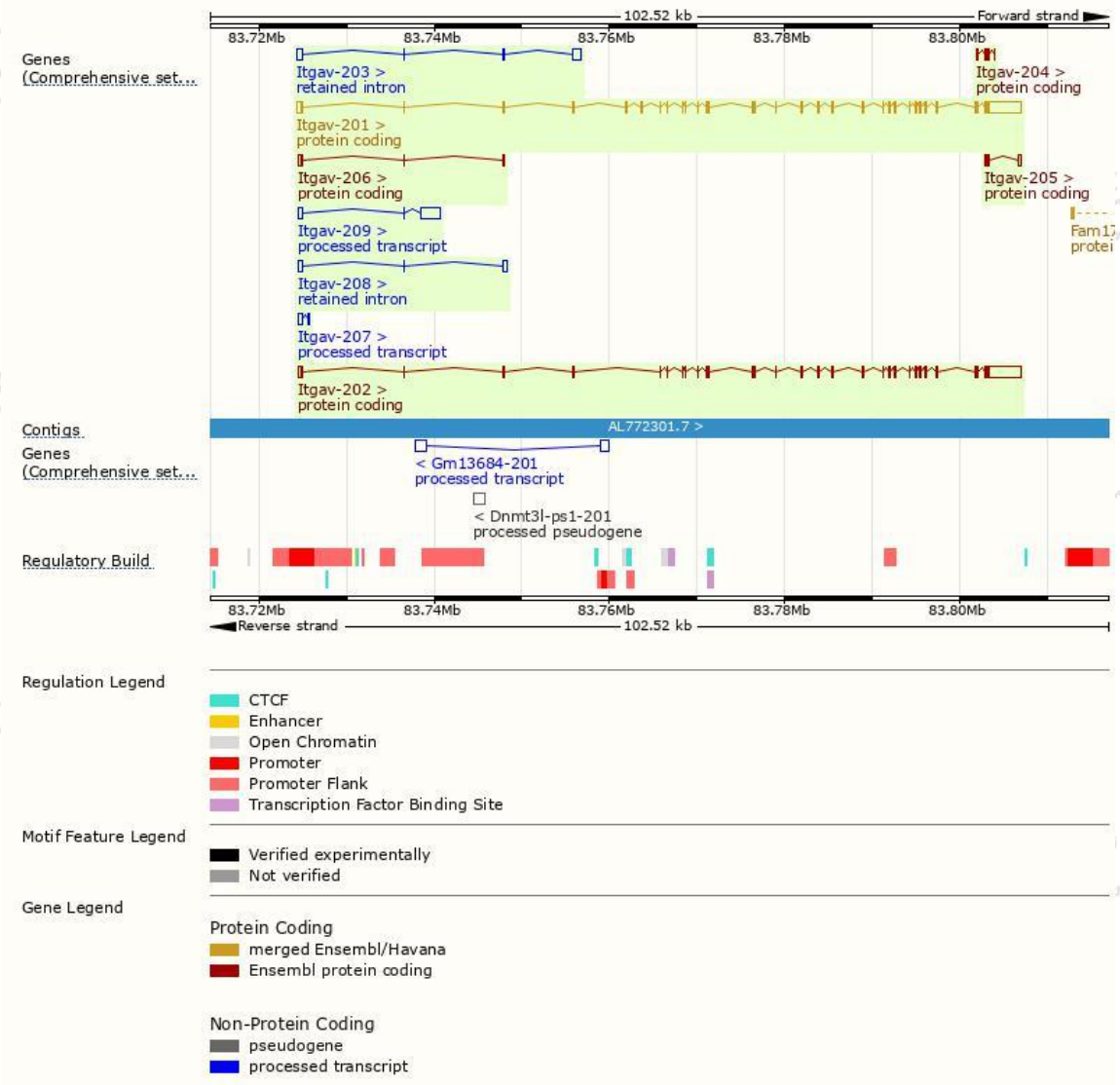
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Itgav-201	ENSMUST00000028499.10	7054	1044aa	Protein coding	CCDS16181	P43406	TSL:1 GENCODE basic APPRIS P2
Itgav-202	ENSMUST00000111740.8	6788	1008aa	Protein coding	-	A2AKI5	TSL:5 GENCODE basic APPRIS ALT2
Itgav-206	ENSMUST00000141725.2	688	135aa	Protein coding	-	B7ZCI9	CDS 3' incomplete TSL:1
Itgav-205	ENSMUST00000131192.1	463	59aa	Protein coding	-	F6RWM8	CDS 5' incomplete TSL:5
Itgav-204	ENSMUST00000125402.7	444	120aa	Protein coding	-	F6W6Q6	CDS 5' incomplete TSL:3
Itgav-209	ENSMUST00000155521.1	2777	No protein	Processed transcript	-	-	TSL:2
Itgav-207	ENSMUST00000148256.1	592	No protein	Processed transcript	-	-	TSL:1
Itgav-203	ENSMUST00000125360.7	1738	No protein	Retained intron	-	-	TSL:5
Itgav-208	ENSMUST00000151463.1	1105	No protein	Retained intron	-	-	TSL:2

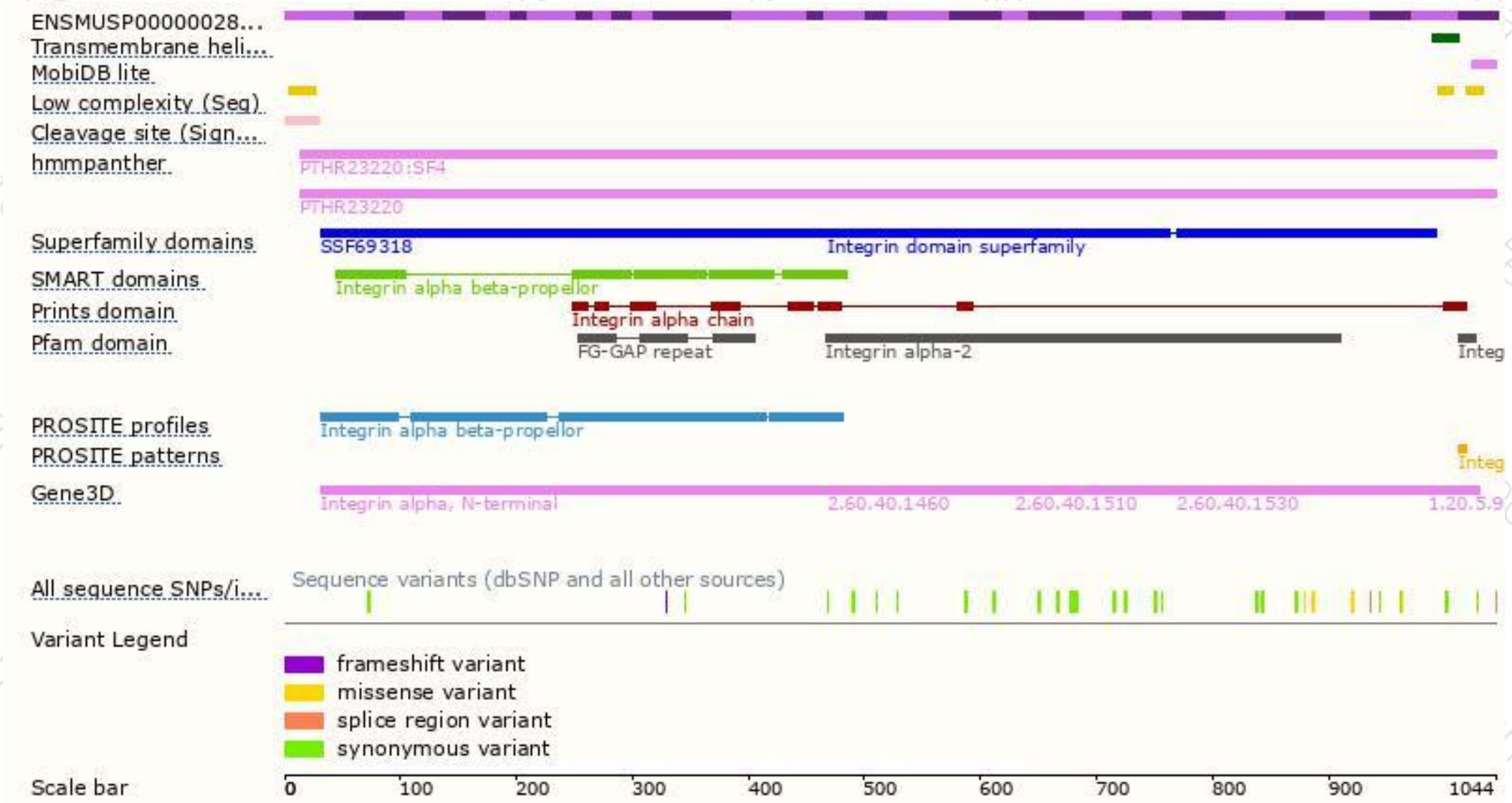
The strategy is based on the design of *Itgav-201* transcript,The transcription is shown below



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,Homozygotes for a targeted null mutation exhibit placental defects, intracerebral and intestinal hemorrhages, and cleft palate, resulting in death occurring as early as midgestation and as late as shortly after birth.

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

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