Sftpc-IRES-iCre Cas9-KI Strategy

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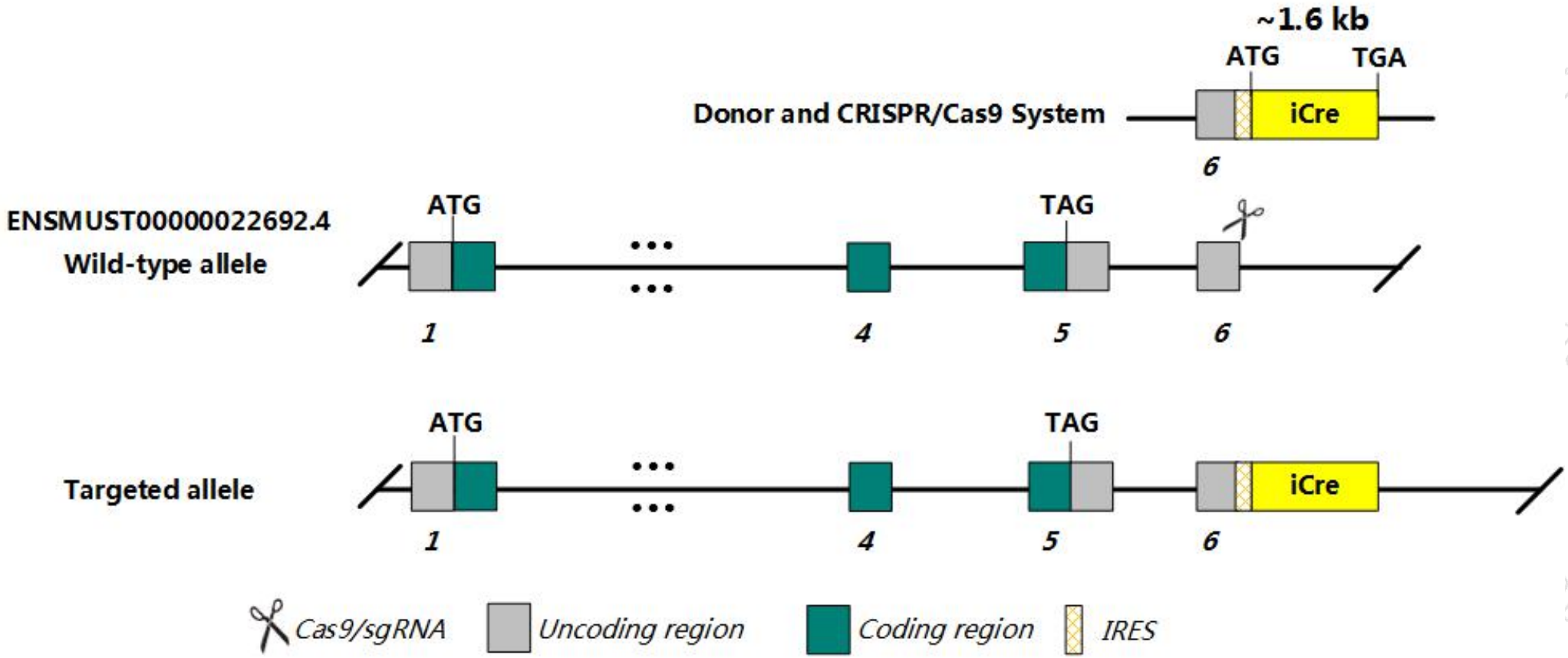
Project Overview

Project Name	***Sftpc-IRES-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

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



Technical routes

- The *Sftpc* gene has 2 transcripts.According to the structure of *Sftpc* gene, *Sftpc-201*(ENSMUST00000022692.4) is selected for presentation of the recommended strategy.

- *Sftpc-201* gene has 6 exons, with the ATG start codon in exon1 and TAG stop codon in exon5.

- We make *Sftpc-IRES-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage in exon6 of Sftpc gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in IRES-iCre after exon6 of Sftpc gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, mice homozygous for disruptions in this gene display respiratory abnormalities similar to emphysema.
- The IRES-linked *Sftpc* gene and the iCre gene are expressed by the same promoter driver. The transcription levels are consistent but the translation is independent. Often the latter gene translates at a lower level than the former.
- Insertion of iCre may affect the regulation of the 3' end of the *Sftpc* gene.
- There will be 2 to 4 base mutations in exon6 of *Sftpc* gene in this strategy.
- The distance between the site of insertion and *Lgi3*/*Bmp1* gene is about 9.9kb/0.8kb, insertion of IRES-iCre may influence the 5'regulation of these two genes.
- The *Sftpc* gene is located on the Chr14. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

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Sftpc surfactant associated protein C [*Mus musculus* (house mouse)]

Gene ID: 20389, updated on 16-Jul-2019

Summary

Official Symbol	Sftpc provided by MGI
Official Full Name	surfactant associated protein C provided by MGI
Primary source	MGI:MGI:109517
See related	Ensembl:ENSMUSG00000022097
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	SP5; SPC; SP-C; Sftp2; Bricd6; Sftp-2; pro-SpC
Expression	Restricted expression toward lung adult (RPKM 8709.5) See more
Orthologs	human all

Genomic context

Location: 14 D2; 14 36.32 cM

See Sftpc in Genome Data Viewer

Exon count: 6

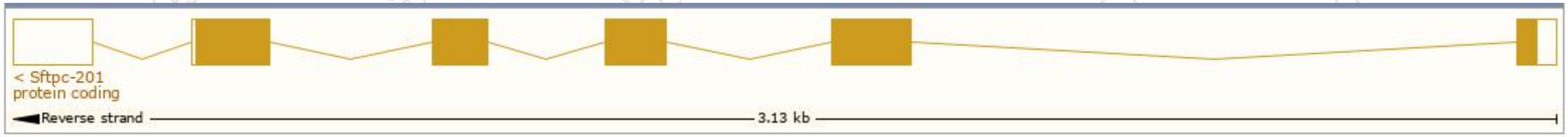
Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	14	NC_000080.6 (70520941..70524081, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	14	NC_000080.5 (70920748..70923888, complement)

Transcript information （Ensembl）

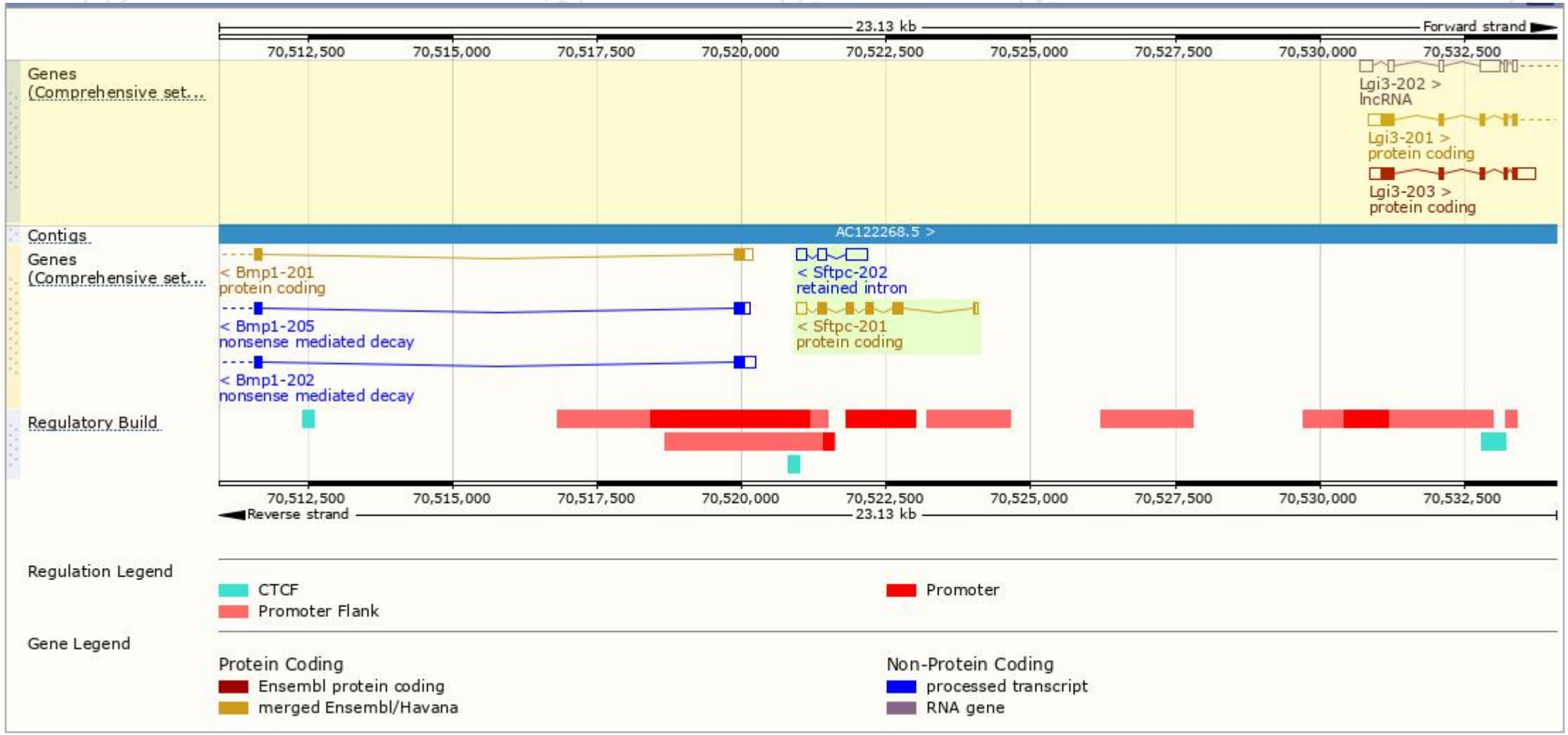
The gene has 2 transcripts, and all transcripts are shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sftpc-201	ENSMUST00000022692.4	792	193aa	Protein coding	CCDS27254	Q6P8P8	TSL:1 GENCODE basic APPRIS P1
Sftpc-202	ENSMUST00000227152.1	695	No protein	Retained intron	-	-	-

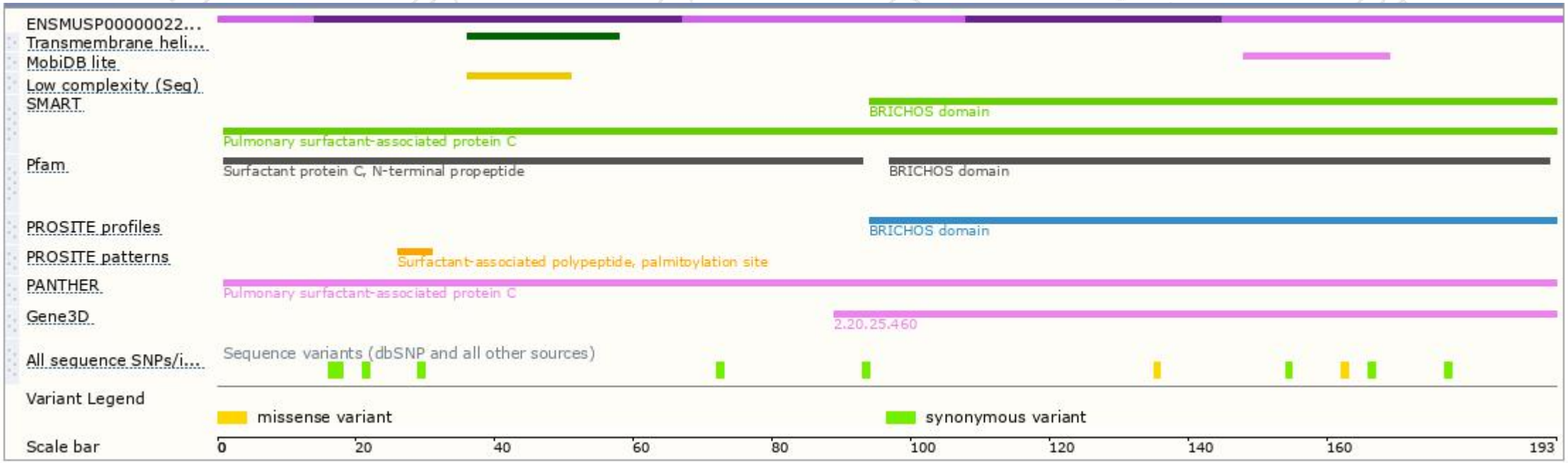
The strategy is based on the design of *Sftpc-201* transcript,The transcription is shown below



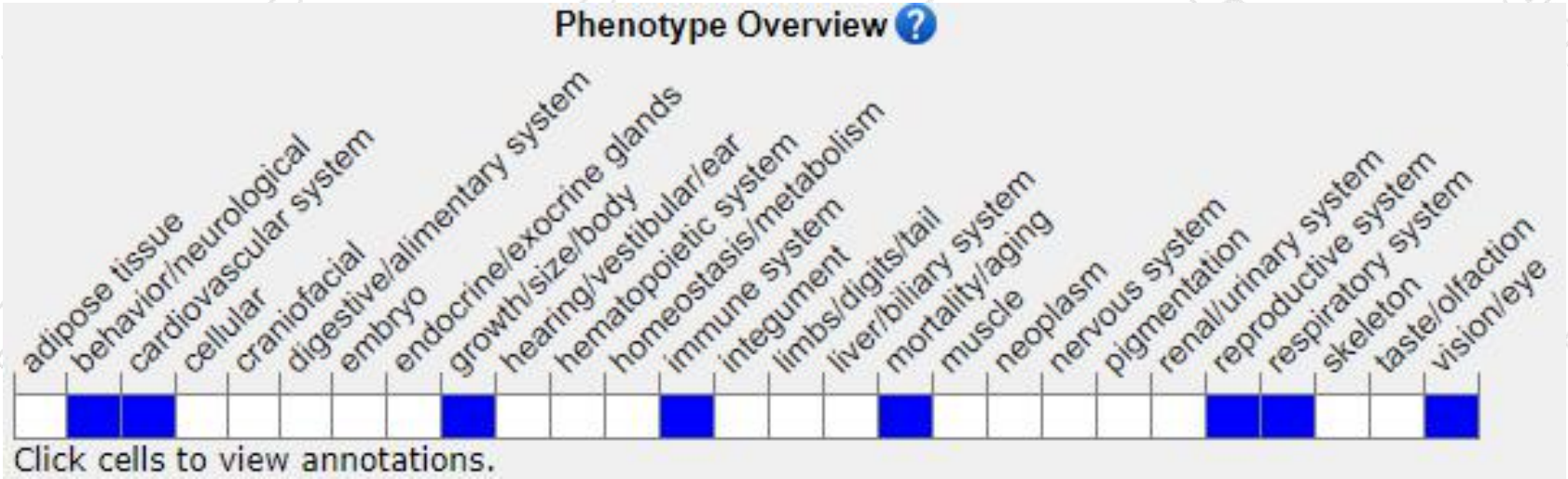
Genomic location distribution



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:109517) .

Mice homozygous for disruptions in this gene display respiratory abnormalities similar to emphysema.

Targeted Progress (from Jax)

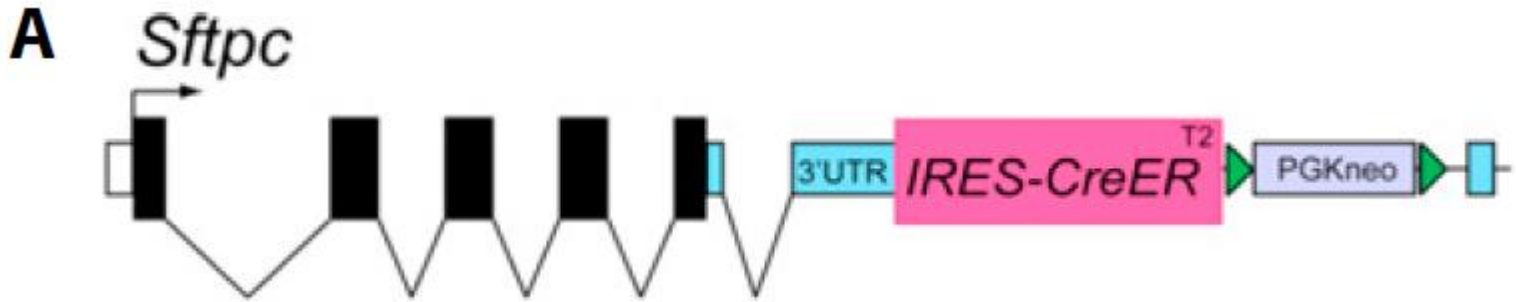


Fig. S4. (*A*) Schematic of *Sftpc-CreER* construct. The coding sequence and 3′ UTR of *Sftpc* were retrieved from a BAC by recombineering into a vector upstream of a DT cassette for negative selection in ES cells. An *IRES-CreERT2* cassette and a PGKneo cassette flanked with FRT sites were recombined into the 3′ UTR. (*B*

Materials and Methods

Mice. The *CC10-CreER* and *Rosa26R-CAG-farnesylated GFP* (*Rosa26R-fGFP*) mouse lines have been described previously (7). To generate *Sftpctm1(cre/ERT)Blh* (*Sftpc-CreER*) mice, the coding sequence and 3′ UTR of *Sftpc* were retrieved from a BAC by recombineering into a vector upstream of a diphtheria toxin (DT) cassette for negative selection in ES cells. An *IRES-CreERT2* cassette and a *PGKneo* cassette flanked with Flp recognition target (FRT) sites were recombined into the 3′ UTR (Fig. S4). The construct was linearized and electroporated into 129S6/SvEvTac ES cells. Ten correctly targeted clones were identified by Southern blot and PCR, and ES cells from three clones were injected into C57BL/6 blastocysts. Mice heterozygous for *Sftpc-CreER*

http://www.informatics.jax.org/allele/MGI:5305340

https://www.jax.org/strain/028054

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

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