Rosa26-CAG-LSL-tdTomato

Strain Name： B6/JGpt-*Rosa26*$^{tm1(CAG-LSL-tdTomato)}$/Gpt
Strain Type： Knock-in
Strain Number： T002249
Background： C57BL/6JGpt

Description

Genetically modified mice (knockout, knock-in, mutation, eterologous gene expression, etc)have become important models for gene function and disease research. With the advent of new technologies such as ZFN, TALEN and CRISPR, the technical difficulty of gene editing has been reduced. so the cost and time of making genetically modified mice models. especially the emergence of the CRISPR system, makes it easy to modify multiple sites at the same time.

Mouse Rosa26 gene (Friedrich and Soriano, 1991) has been proved to be able to stably express the foreign gene integrated into this site, and it has been widely used to make various knock-in and Gene Trap mice. We inserted the expression element driven by the CAG promoter (Niwa et al., 1991) into the Rosa26 site, and placed the STOP element with loxP on both sides between CAG and to prevent expression. In addition, although this strain also fused the red fluorescent label tdTomato after to facilitate the detection of expression, according to the internal test results and technical support feedback, **this model cannot be used as fluorescent reporter mice alone, especially for labeling of cardiomyocytes, skeletal muscles and CD45+ immune cells. Notably, the protein is barely expressed in cardiac muscle, skeletal muscle and CD45+ immune cells.**

When Cre recombinases are present, is used after recombination, combined with sgRNA delivered by transgenic or viral vectors to achieve gene knockout in specific tissues and organs.

Strategy

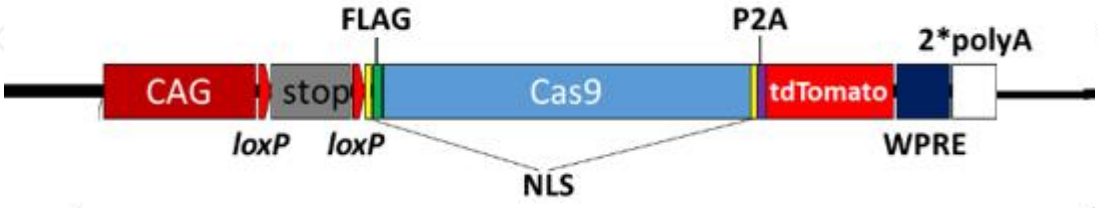

Fig1. Rosa26-CAG-LSL-tdTomato model strategy

Application

1. CRISPR/ gene editing
2. Functional studies of single or multiple genes after knockout/knockout in vivo
3. Conditional gene editing

Data Support

1. MEF electrotransfection experiment

Group	Transfection plasmid		Transfection result		
	pCAG-Cre-GFP	U6-GPR50-S51	Green fluorescence	Red fluorescence	DNA cleavage
1A	2ug	4ug	Y	Y	Y
1B	1ug	2ug	Y	Y	Y
1C	3ug	6ug	Y	Y	Y
2	2ug	0	Y	Y	N
3	0	4ug	N	N	N

Table. 1 Groups and results of MEF electrotransfection experiments

The results show that both group 1A and 1C can observe green fluorescence and red fluorescence, and the T7E1 test shows that cuts the sgRNA target obviously; Group 2 can observe green fluorescence and red fluorescence, but sgRNA target cleavage conducted by is not detected; In group 3, no green or red fluorescence was detected, and no cleavage of the sgRNA target conducted by was detected.

18h, 24h, 48h and 66h were chosen to observe the fluorescence, and found that the green fluorescence was strongest at 18h, and gradually weakened with time, and it was almost impossible to observe at 66h. The red fluorescence cannot be observed at 18h, and it only starts to appear at 24h, it becomes strongest at 48h, while weakened at 66h. The changes in green and red fluorescence were consistent with the expression order of Cre and . When the plasmid enters the cell through transfection, Cre is expressed and the green fluorescence can be observed. Cre cuts off the loxP-STOP-loxP at the position of Rosa26 and turns on the expression of to see the red fluorescence. Therefore, the red fluorescence should appear later than the green fluorescence. The T7E1 cleavage test showed that cleavage did not occur in both groups 2 and 3, which proves that cleavage did not occur in the absence of Cre or

sgRNA. The expression detection results were consistent with the Rosa26-LSL-EGFP mice made by Dr. Zhang's laboratory of the BROAD Institute.

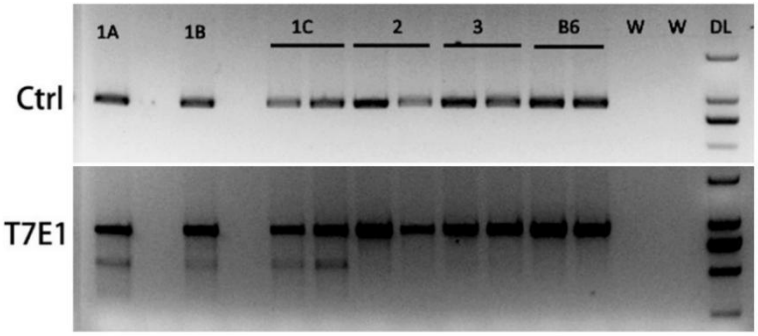

Fig.2 T7E1 identification (B6: C57BL/6JGpt genomic DNA, W: blank control, DL: DL2000 DNA Marker)

T7E1 restriction enzyme digestion results showed that the three groups of DNA 1A, 1B and 1C co-transfected with Cre and sgRNA plasmids all had cleavage of the sgRNA target, while the Cre plasmid (group 2) or the sgRNA plasmid (The two lines of group 3) were the same as B6 (negative control), and cleavage was not detected.

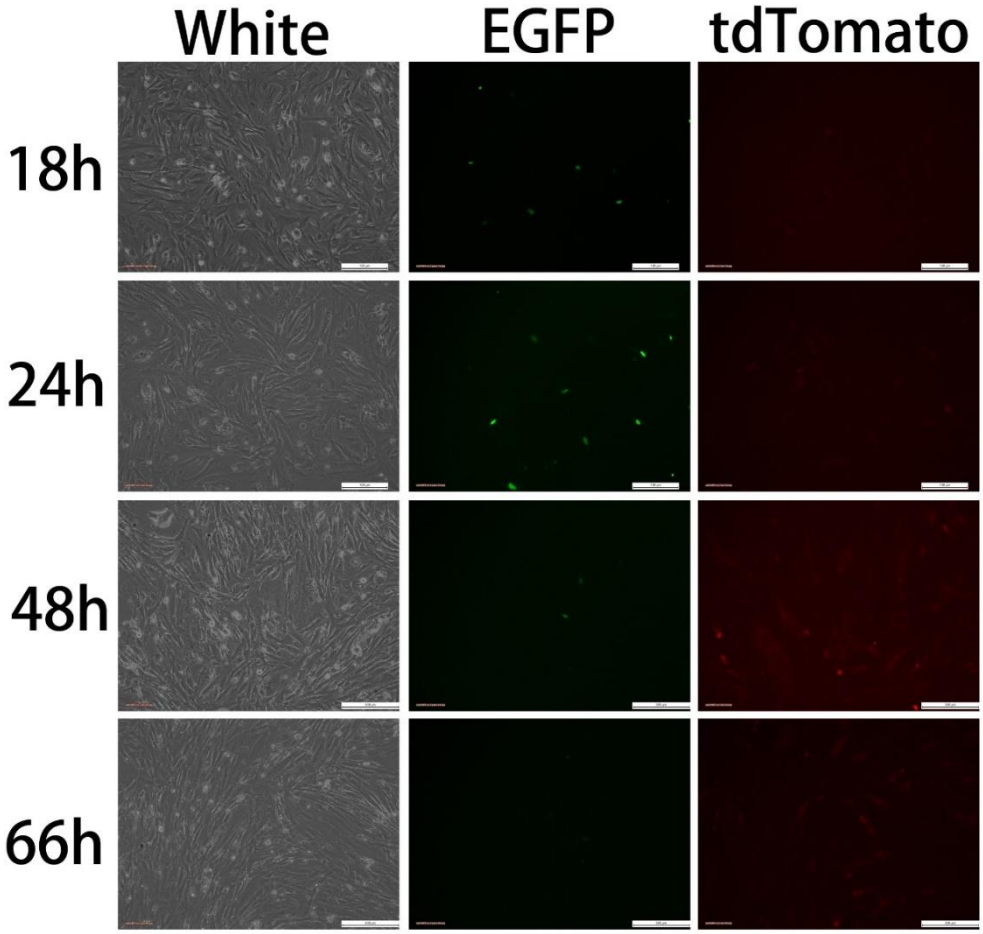

Fig.3 Group2 Transfected with pCAG-Cre:GFP

The transfected plasmid is pCAG-Cre:GFP (2ug), green fluorescence can be observed after Cre-GFP expression, while tdTomato requires Cre/loxP reaction to remove the STOP element before expression can be turned on, and red fluorescence appears. Therefore, the time of appearance of red fluorescence should be later than the time of appearance of green fluorescence. The experimental results were as expectations. Green fluorescence appeared at 18h. As time passed, green fluorescence gradually disappeared and was no longer visible at 66h; red fluorescence began to appear at 24h, fluorescence increased at 48h, and red fluorescence decreased at 66h.

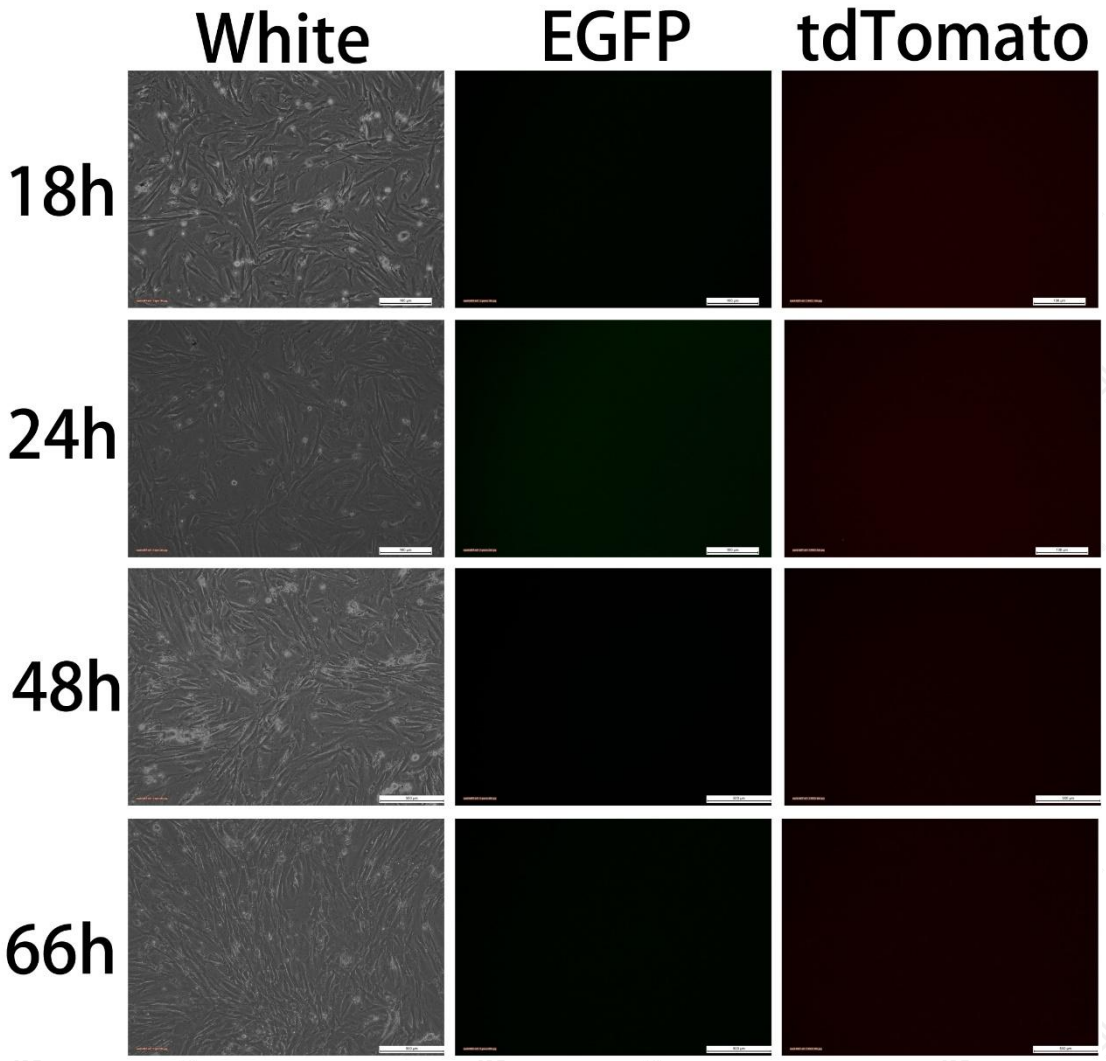

Fig.4 Group3 Transfected with U6-GPR50-S51

Transfected plasmid U6-GPR50-S51 (4ug),as there is no Cre expression, no green fluorescence is observed, loxP-STOP-loxP cannot be removed, expression cannot be turned on, and red fluorescence cannot be observed. The experimental results were in line with expectations, and no fluorescence was observed.

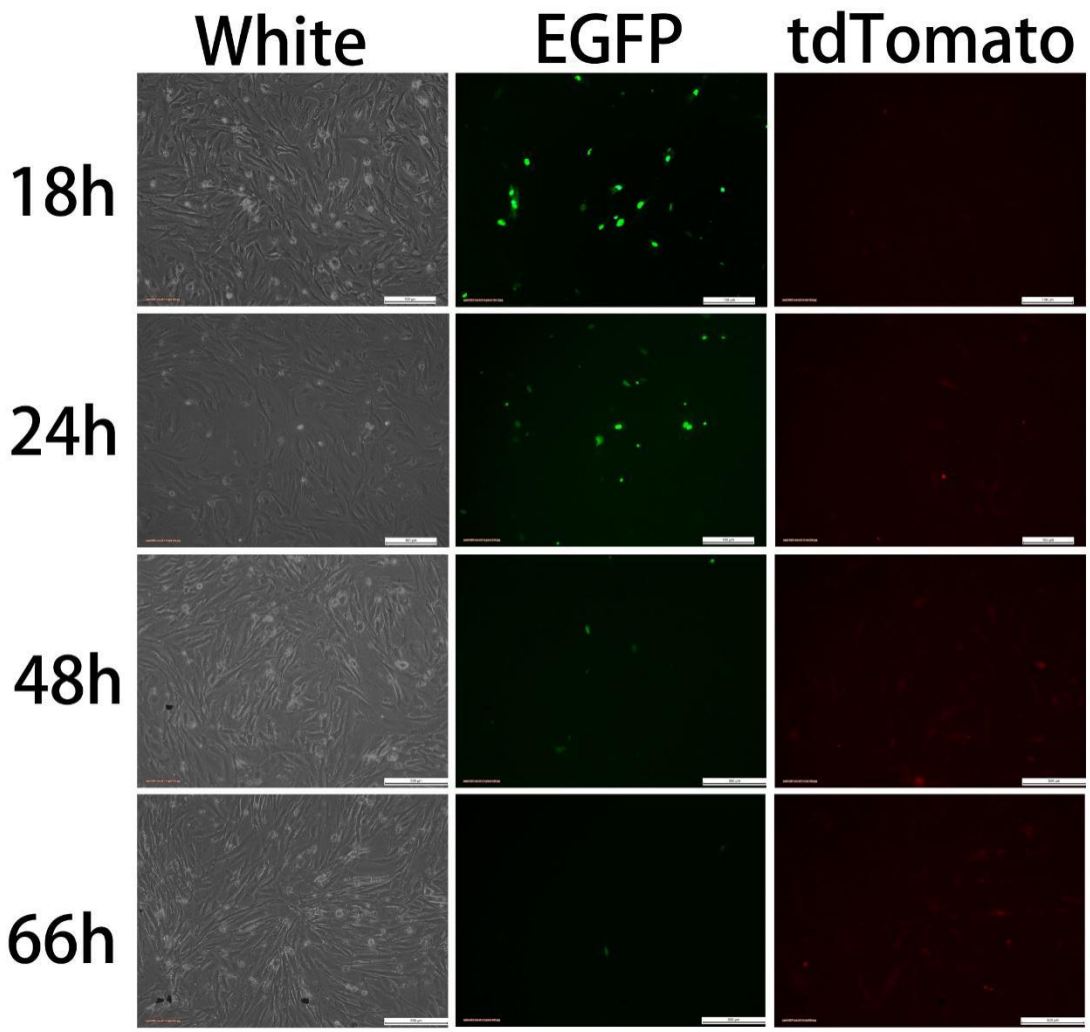

Fig.5 Group1A Transfected with pCAG-Cre:GFP(2ug)+U6-GPR50-S51(4ug)

The transfected plasmid is pCAG-Cre:GFP (2ug) + U6-GPR50-S51 (4ug), green fluorescence can be observed after Cre-GFP expression, while tdTomato requires the Cre/loxP reaction to remove the STOP element before the expression can be turned on, Red fluorescence appears, so the time of red fluorescence should be later than the time of green fluorescence. The experimental results were in line with expectations. Green fluorescence appeared at 18h. As time passed, green fluorescence gradually disappeared and was no longer visible at 66h; red fluorescence began to appear at 24h, fluorescence increased at 48h, and red fluorescence decreased at 66h.

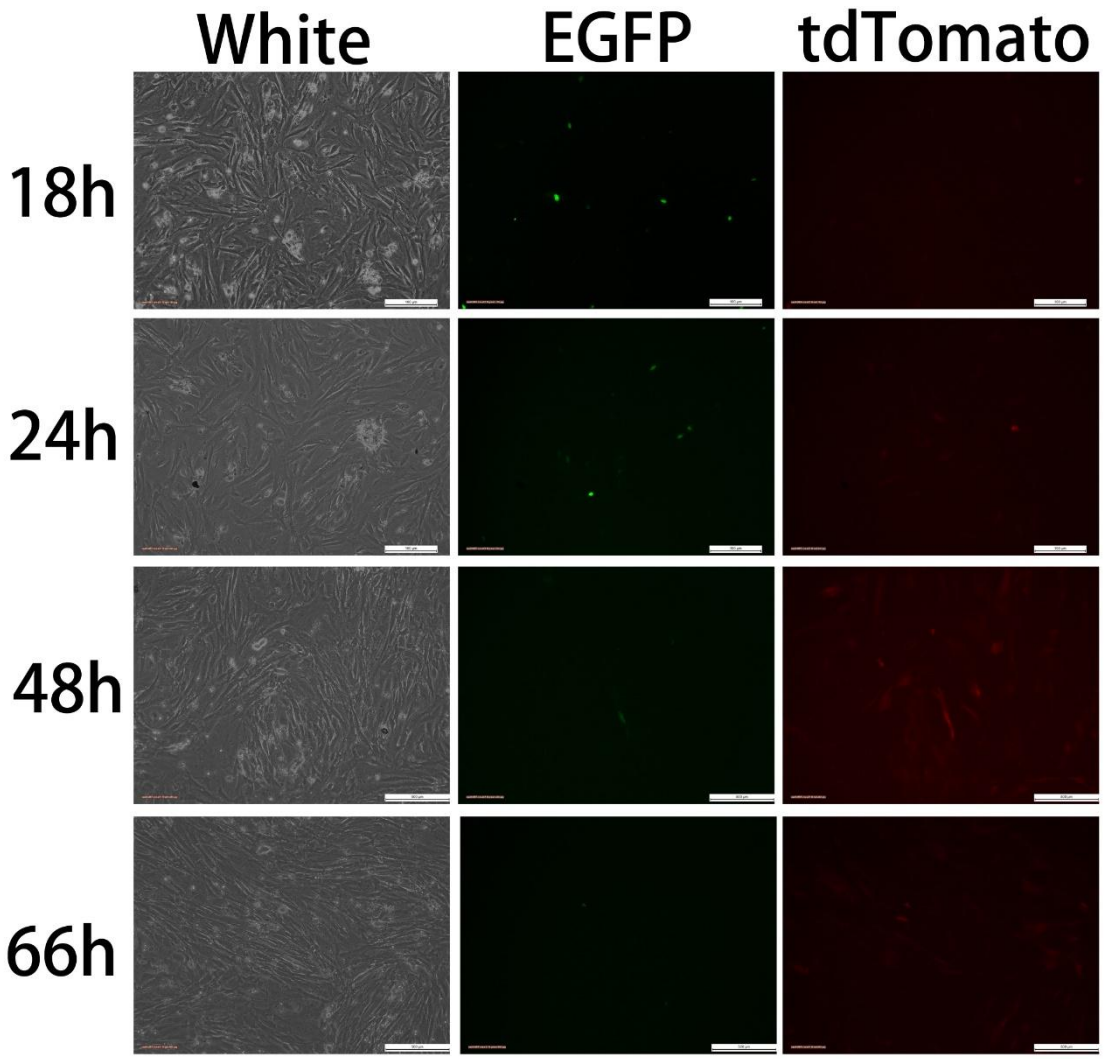

Fig.6 Group1B Transfected with pCAG-Cre:GFP(1ug)+U6-GPR50-S51(2ug)

The transfected plasmid is pCAG-Cre:GFP (1ug) + U6-GPR50-S51 (2ug), green fluorescence can be observed after Cre-GFP expression, while tdTomato requires the Cre/loxP reaction to remove the STOP element before the expression can be turned on, Red fluorescence appears, so the time of red fluorescence should be later than the time of green fluorescence. The experimental results were in line with expectations. Green fluorescence appeared at 18h. As time passed, green fluorescence gradually disappeared and was no longer visible at 66h; red fluorescence began to appear at 24h, fluorescence increased at 48h, and red fluorescence decreased at 66h.

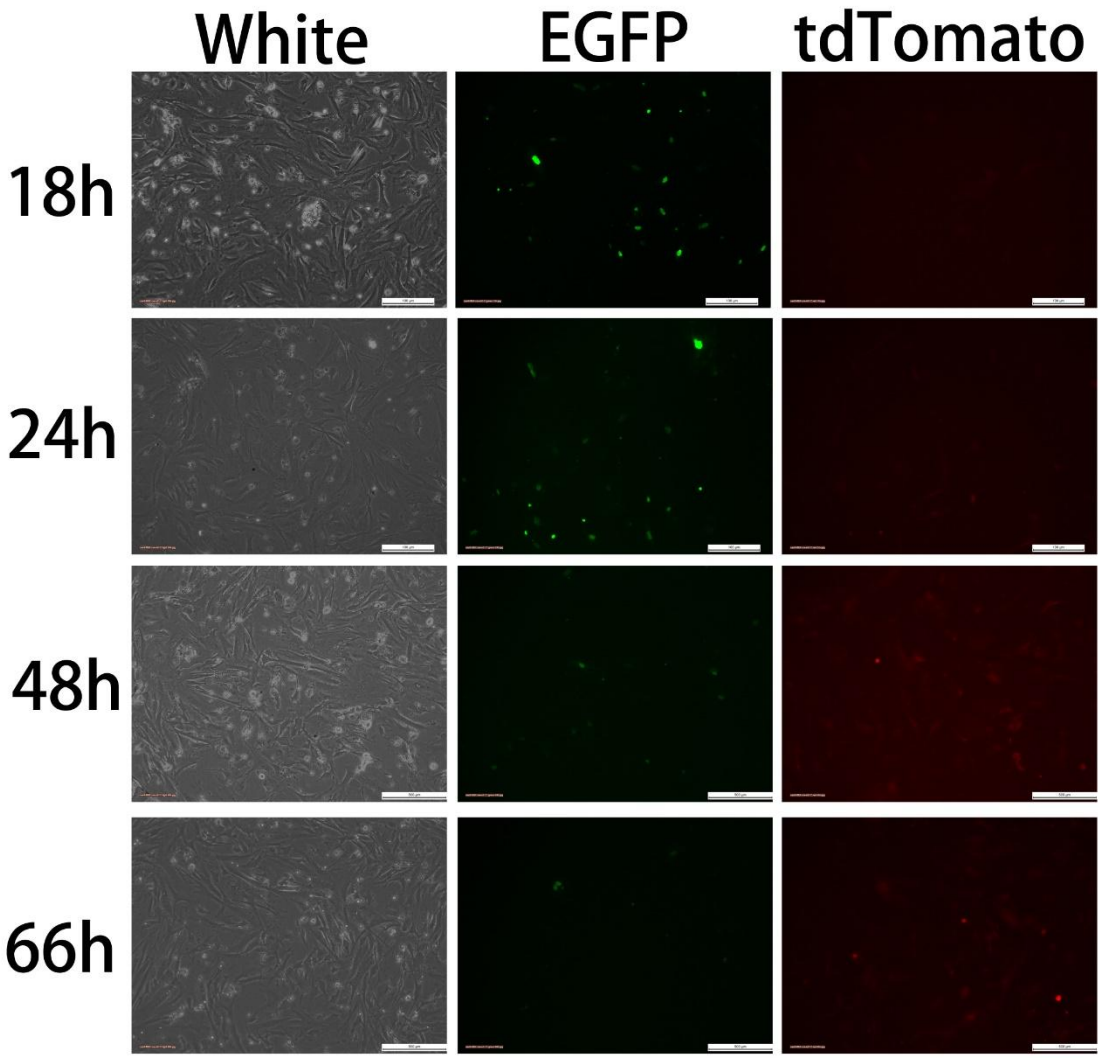

Fig.7 Group1C Transfected with pCAG-Cre:GFP(3ug)+U6-GPR50-S51(6ug)

The transfected plasmid is pCAG-Cre:GFP (3ug) + U6-GPR50-S51 (6ug), green fluorescence can be observed after Cre-GFP expression, while tdTomato requires the Cre/loxP reaction to remove the STOP element before the expression can be turned on, Red fluorescence appears, so the time of red fluorescence should be later than the time of green fluorescence. The experimental results were in line with expectations. Green fluorescence appeared at 18h. As time passed, green fluorescence gradually disappeared and was no longer visible at 66h; red fluorescence began to appear at 24h, fluorescence increased at 48h, and red fluorescence decreased at 66h.

Conclusion: MEF cells isolated from rosa26-LSL-tdTomato mice were transfected with Cre expression plasmid in vitro. This model can turn on the expression of and tdTomato after Cre/loxP removal of the STOP element; when co-transfecting Cre expression plasmid and guide RNA When expressing the plasmid, red fluorescence can be observed and the cleavage of the target gene can be detected. According to the

experimental results, it can be determined that the model is successfully prepared. However, due to the low transfection efficiency, the fluorescence signal observed in this experiment was weak.

2. Expression of validation

Based on the T002249 B6-CAG-LSL-tdTomato mouse model, mated with the systemic expression of Cre recombinase to remove the STOP element and open the expression of and tdTomato, remove the expression of Cre by backcrossing, established of B6-CAG-tdTomato strain.

1）Systemic expression of tdTomato assay

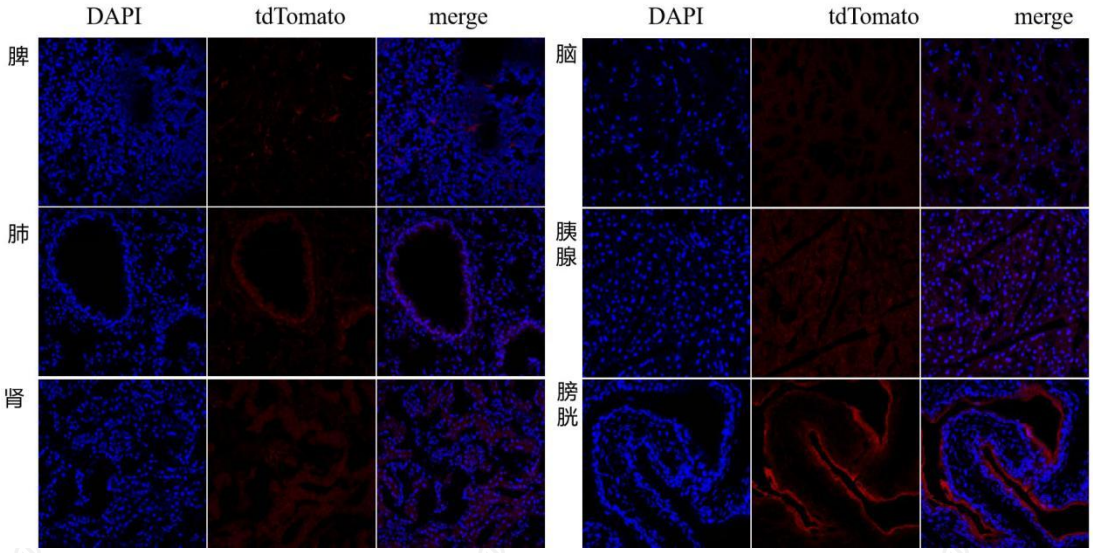

Fig1. Detection of red fluorescent protein expression in B6-CAG-tdTomato.

The tissues of the liver, spleen, lung, kidney, brain, pancreas, stomach, bladder and intestine were all expressed red fluorescent protein with different intensities in B6-CAG-tdTomatomice. The results indicate that B6-CAG-tdTomato mice can express tdTomato protein.

2） protein expression assay

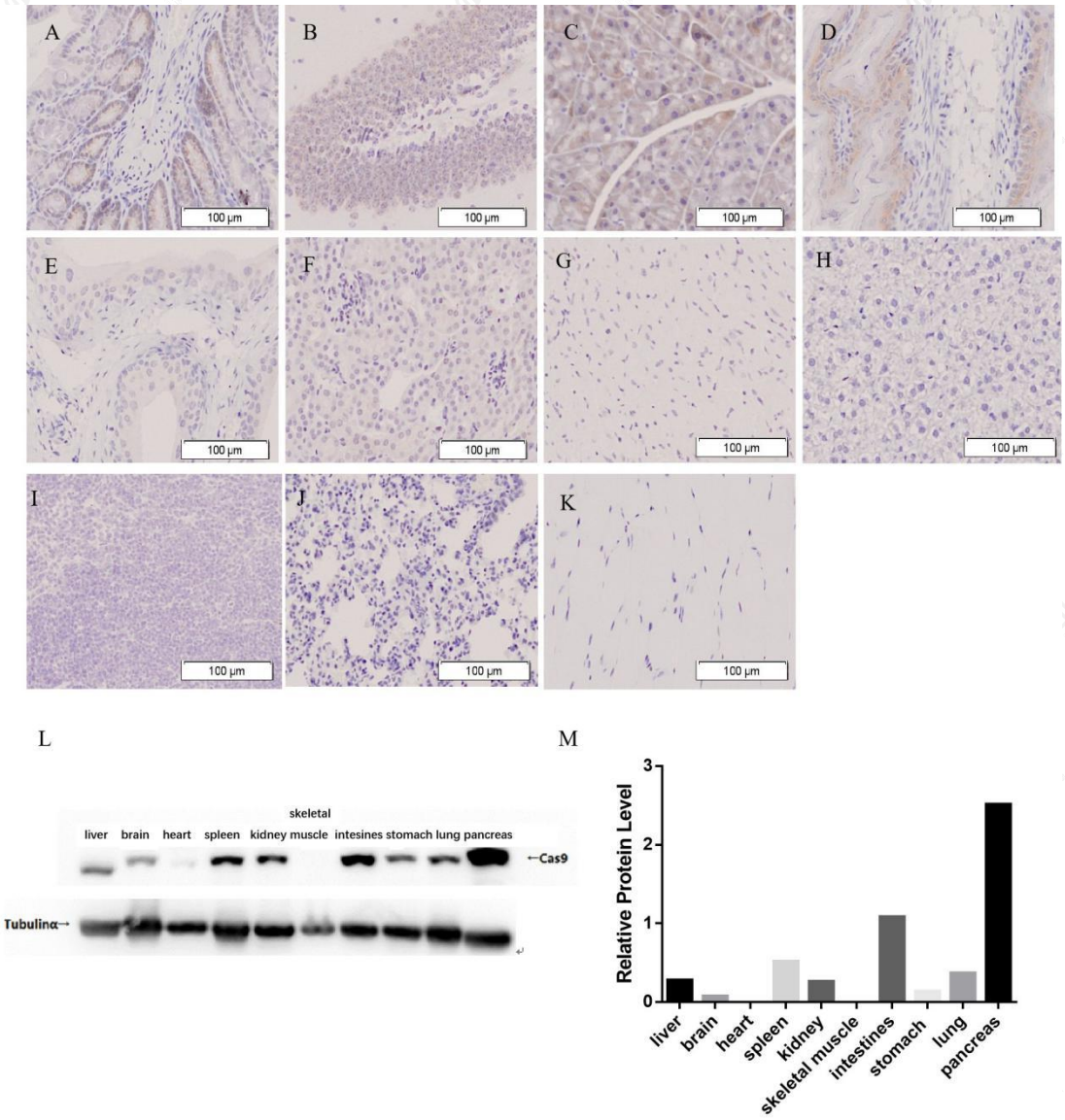

Fig2. Detection of protein expression in B6-CAG-tdTomato.

To investigate the expression of protein in different tissues of B6-CAG-tdTomato mice, we used immunohistochemical(A-K) and western(L-M) methods to detect the expression of protein. The results showed that protein was expressed in liver, spleen, lung, kidney, brain, pancreas and stomach. The main expression regions of brain are the hippocampus and the cortex, that it was hardly expressed in the heart and muscle. The results indicated that protein can be expressed in the main tissues of B6-CAG-tdTomato mice.

Reference

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