C57BL/6JGpt-Clu-P2A-CreERT2-P2A-EGFP

Strain Name： C57BL/6JGpt-*Clu*$^{em1Cin(P2A\text{-}CreERT2\text{-}P2A\text{-}EGFP)}$/Gpt
Strain Type： Knock-in
Strain Number： T053653
Background： C57BL/6JGpt

Description

This mouse strain expresses CreERT2 inducible recombinase [1] under the control of the mouse endogenous *Clu* promoter, the construct was inserted into the targeted stop codon of the *Clu* gene by CRISPR/Cas9 technology. When crossed with a strain with loxP site flanked sequence in its genome, Cre-mediated recombination will result in excision of the DNA fragment between the two loxPs in *Clu*-positive cells or tissues after tamoxifen administration. Note: mild CreER leaky activity was also observed in some cells in liver without tamoxifen treatment.

Strategy

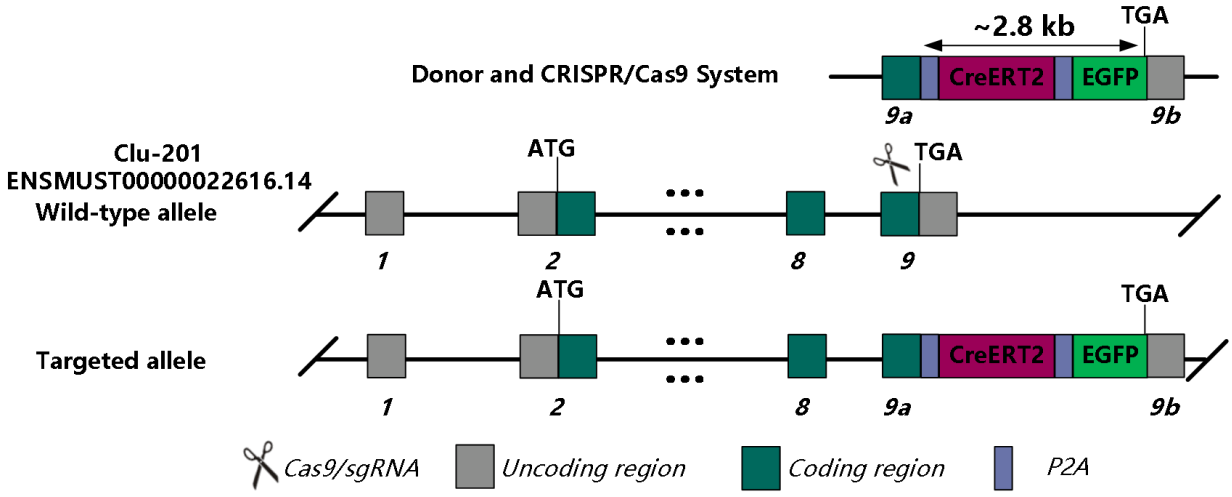


Fig.1 Schematic diagram of C57BL/6JGpt-Clu-P2A-CreERT2-P2A-EGFP model strategy.

Applications

1. Cre tool mice for specific, tamoxifen dependent induction of loxP recombination in *Clu*-positive cells or tissues [2].

Data support

1. Validation methods & notes

Clu-P2A-CreERT2-P2A-EGFP mice was crossed with CAG-loxp-ZsGreen-Stop-loxp-tdTomato mice with ubiquitous reporter expression (hereafter referred as CAG-G/R mice), Cre-mediated recombination will lead to excision of ZsGreen and the stop cassette and expression of tdTomato, thus gain of red fluorescence will indicate Cre activity. Fluorescence imaging of frozen sections was performed to exhibit Cre activity in various tissues and organs. Imaging sections were performed under a 200x microscopy. For tamoxifen administration, 0.25 mL of 5 mg/mL tamoxifen was treated through intraperitoneal injection daily from P60 to P66 (8.6 w~9.4 w).

Note: these results may only represent the activity of CreERT2 in this strain under this certain tamoxifen treatment condition at the identical stage. Recombinase activity may be different at other stages or under different tamoxifen induction conditions in your application.

Clu-P2A-CreERT2-P2A-EGFP mice exhibited high CreERT2 activity in the heart, liver, pancreas, and stomach, with little or no CreERT2 activity in the spleen, lung, duodenum, kidneys, brain, skeletal muscle and sperm after tamoxifen administration. It should be noted that other tissues remain untested, and the presence of CreERT2 in unexamined organs is not guaranteed.

2. Timeline of tamoxifen treatment and imaging

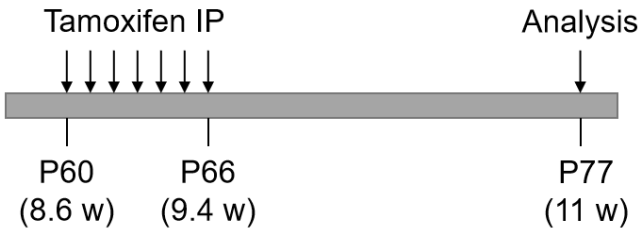
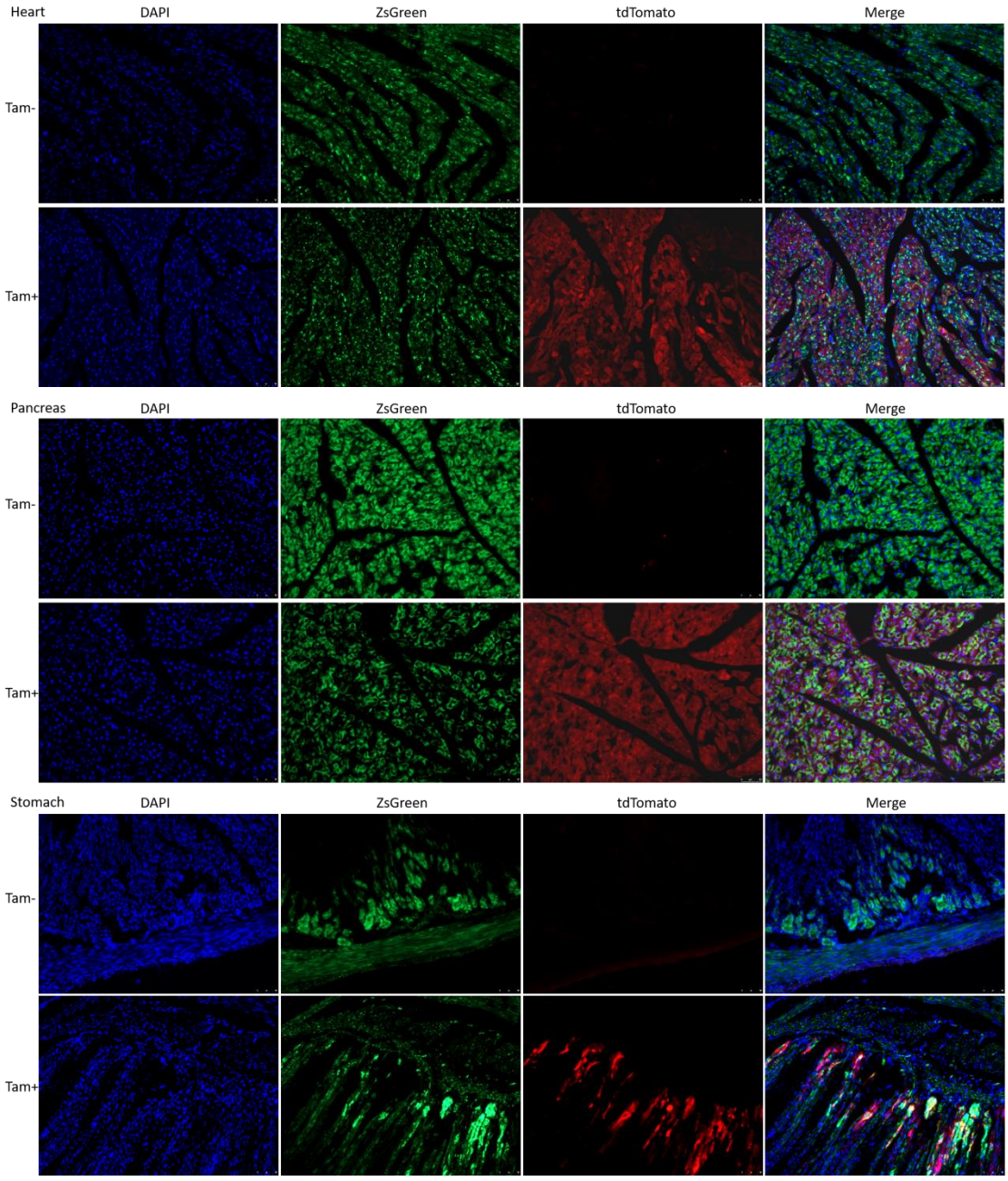


Fig 2. Timeline of tamoxifen treatment and experiment analysis of Clu-P2A-CreERT2-P2A-EGFP mice.

3. Images of tissues and organs with obvious Cre activity


Heart
DAPI
ZsGreen
tdTomato
Merge
Tam-
Tam+
Pancreas
DAPI
ZsGreen
tdTomato
Merge
Tam-
Tam+
Stomach
DAPI
ZsGreen
tdTomato
Merge
Tam-
Tam+

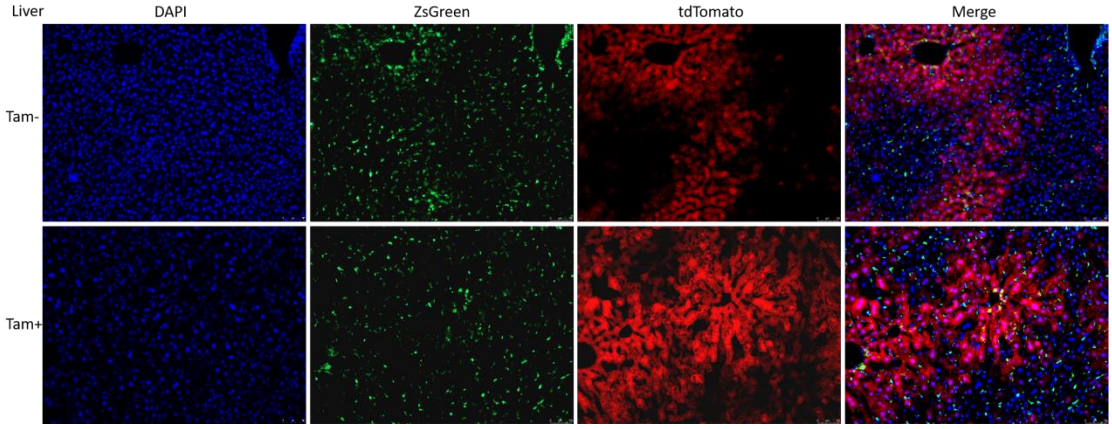


Fig 3. Fluorescence imaging of tissues and organs with obvious Cre activity.

Organ name was indicated in the left top of each subfigure group. Tam-: Clu-P2A-CreERT2-P2A-EGFP, CAG-G/R double positive individuals without tamoxifen administration; Tam+: Clu-P2A-CreERT2-P2A-EGFP, CAG-G/R double positive individuals with tamoxifen administration.

4. Images of tissues and organs with little or no Cre activity

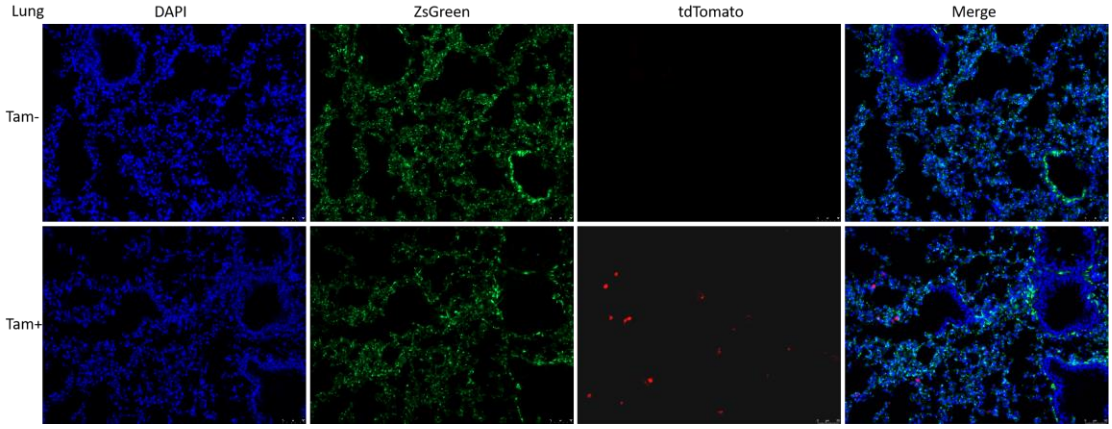


Fig 4. Fluorescence imaging of tissues and organs with little or no Cre activity.

Organ name was indicated in the left top of each subfigure group. Tam-: Clu-P2A-CreERT2-P2A-EGFP, CAG-G/R double positive individuals without tamoxifen administration; Tam+: Clu-P2A-CreERT2-P2A-EGFP, CAG-G/R double positive individuals with tamoxifen administration.

Reference

1. Feil R, Wagner J, Metzger D, et al. "Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains." Biochem Biophys Res Commun, 1997, 237(3): 752-757.

2. Ayyaz A, Kumar S, Sangiorgi B, et al. Single-cell transcriptomes of the regenerating intestine reveal a revival stem cell. Nature, 2019, 569(7754): 121-125.