C57BL/6JGpt-Lyz2-iCre

Strain Name： C57BL/6JGpt-*Lyz2*$^{em1Cin(iCre)}$/Gpt
Strain Type： Knock-in
Strain Number： T003822
Background： C57BL/6JGpt

Description

This mouse strain expresses codon optimized iCre recombinase [1] under the control of the mouse *Lyz2* endogenous promoter, iCre was inserted downstream of the start codon of *Lyz2* gene by CRISPR/Cas9 technology. To ensure the tissue specificity, 3' enhancer of *Lyz2* was maintained and the endogenous *Lyz2* Poly A was used directly. Endogenous *Lyz2* is disrupted, and homozygous offspring appear *Lyz2* knockout phenotype. 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 myeloid cell lineage (monocytes, mature macrophages and granulocytes) and alveolar type II cells (ATII). Recombinase activity was detected in a proportion of cells in lung, spleen and liver.

Strategy

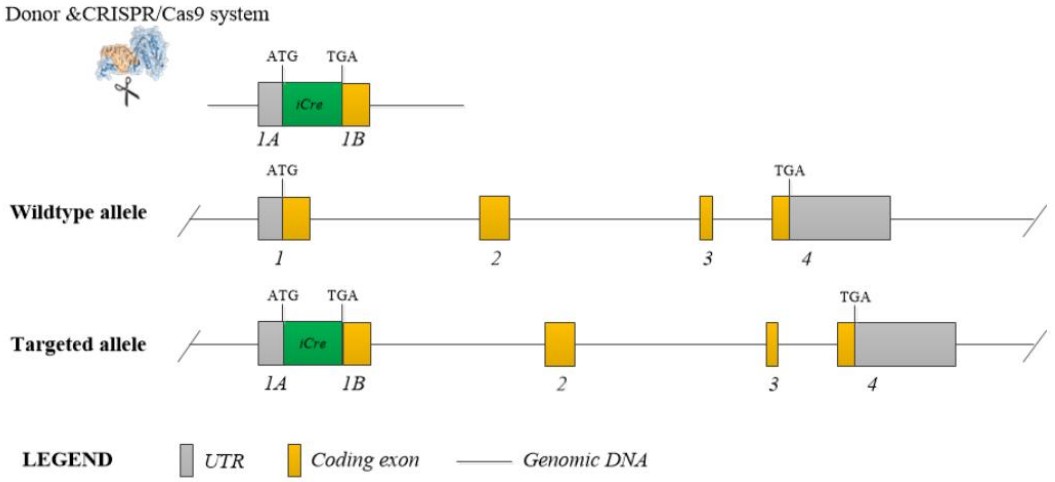


Fig.1 Schematic diagram of C57BL/6JGpt-Lyz2-iCre model strategy.

Applications

1. Cre tool mice for specific induction of loxP recombination in myeloid cell lineage (monocytes, mature macrophages and granulocytes) and alveolar type II cells (ATII) [2-3].

Data support

1. Validation methods & notes

Lyz2-iCre mice was crossed with Rosa26-loxp-tdTomato-loxp-GFP mice with ubiquitous reporter expression, Cre-mediated recombination will lead to excision of tdTomato and the stop cassette and expression of GFP, thus loss of red fluorescence and gain of green fluorescence will indicate Cre activity. Fluorescence imaging of frozen sections were performed to exhibit Cre activity in various tissues and organs. Imaging of sections were performed under a 200x microscopy.

Lyz2-iCre mice was crossed with H11-CAG-LSL-tdTomato mice with ubiquitous reporter expression, Cre-mediated recombination will lead to excision of stop cassette and expression of tdTomato, thus gain of red fluorescence will indicate Cre activity. Flow cytometry analysis of splenic cells and whole blood cells were performed to exhibit Cre activity.

Note: these results may only represent the activity of Cre in this strain at the identical stage. Recombinase activity may be different at other stages in your application.

2. Images of tissues and organs with obvious Cre activity

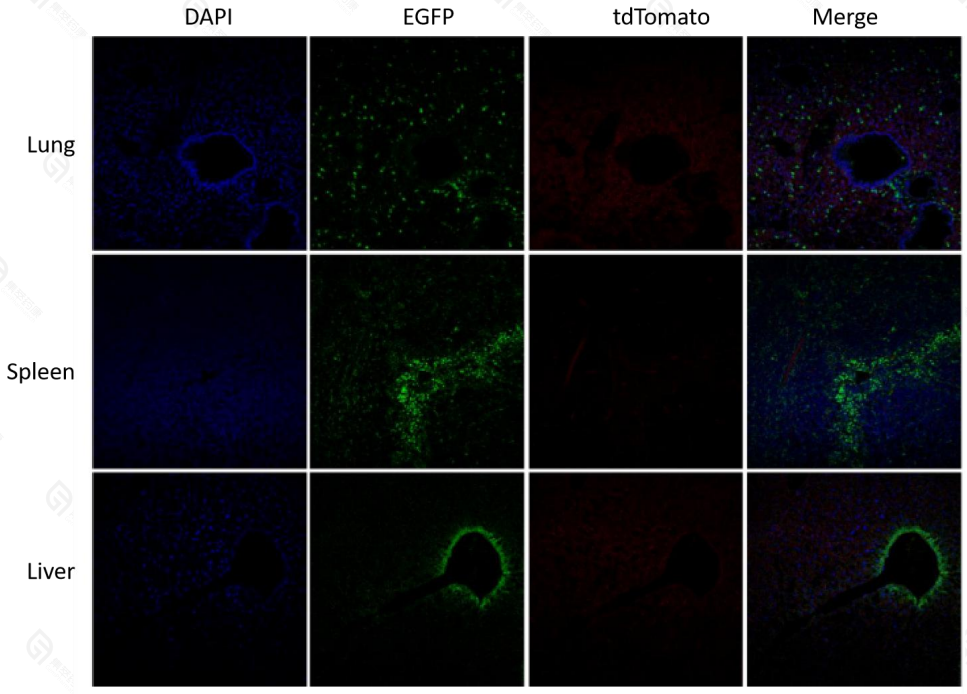


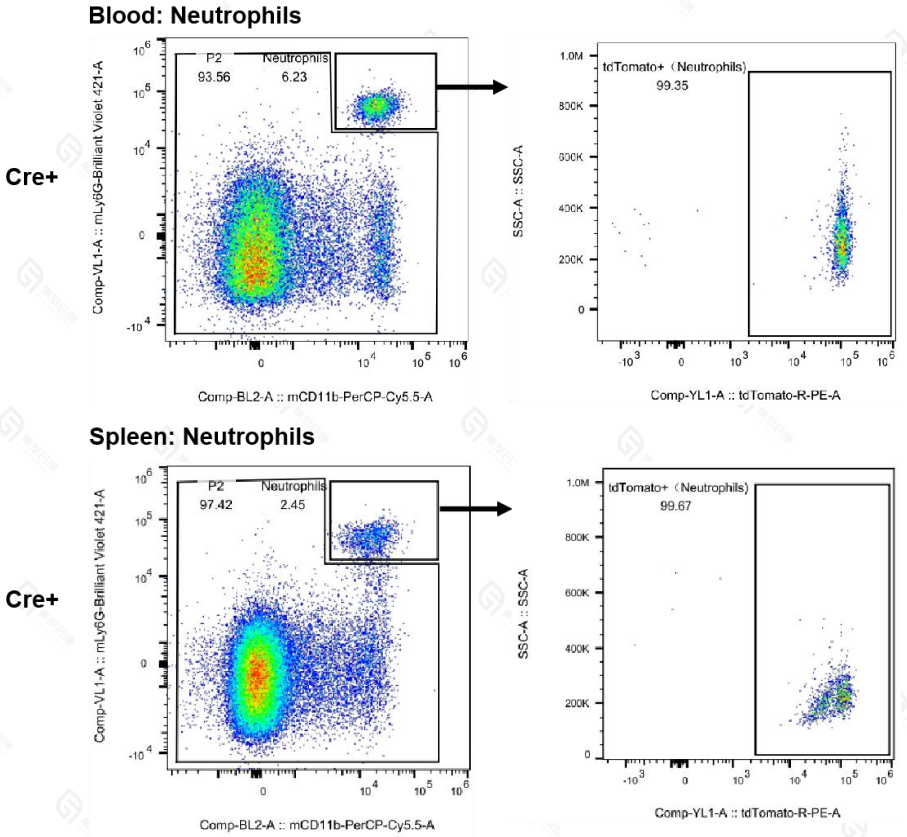
Fig 2. Fluorescence imaging of tissues and organs with obvious Cre activity.

2. Gating Strategies for flow Cytometry

Cell population	Gating	
Neutrophils	mCD45+	mCD11b+mLy6G+
Monocytes	Not Neutrophils	mCD11b+mLy6C hi
Eosinophils	Not Monocytes	mCD11b+SSC-H hi
Macrophages	Not Eosinophils	mCD11b+mF4/80+

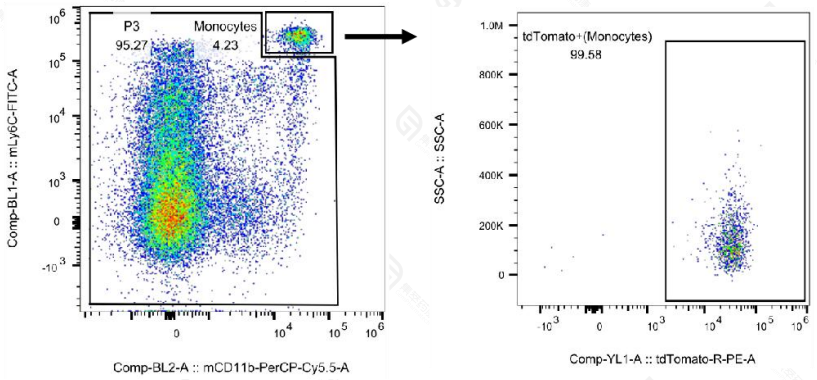
Table 1. Gating Strategies for flow Cytometry of Lyz2-iCre mice.

3. Flow cytometry analysis of cells with Cre activity



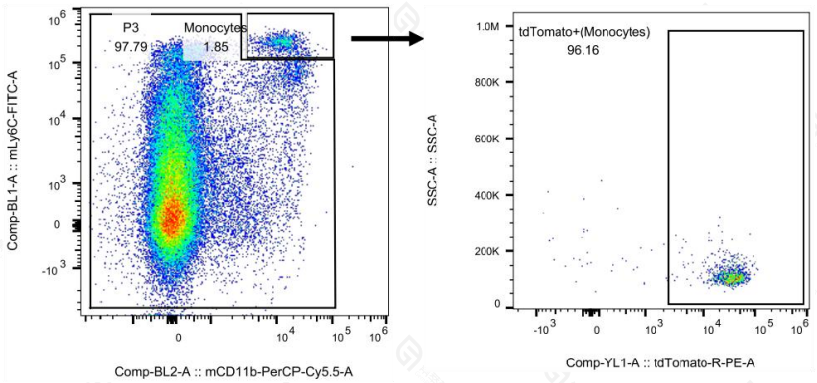
Blood: Monocytes

Cre+



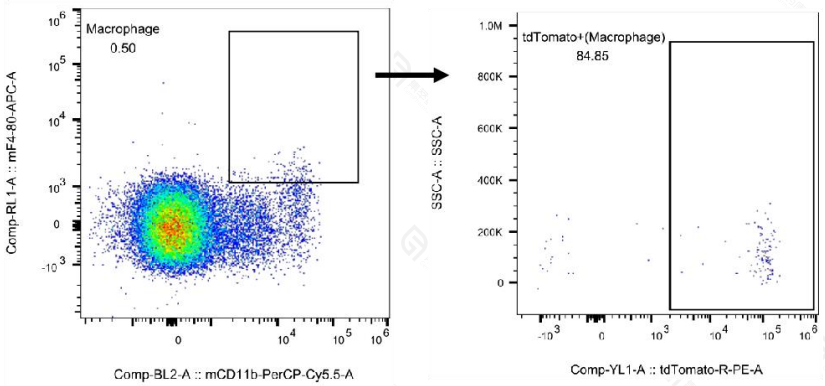
Spleen: Monocytes

Cre+



Blood: Macrophages

Cre+



Spleen: Macrophages

Cre+

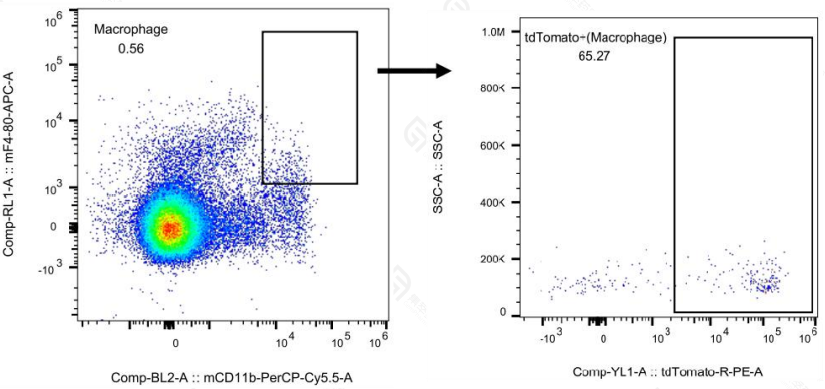


Fig 3. Flow cytometry analysis of cells with Cre activity

Organ name was indicated in the left top of each subfigure group. Cre+: Lyz2-iCre, H11-CAG-LSL-tdTomato double positive individuals. Splenocytes and whole blood cells were harvested from Cre+ mice and analyzed for tdTomato expression with flow cytometry.

4. Flow cytometry analysis of cells with little Cre activity

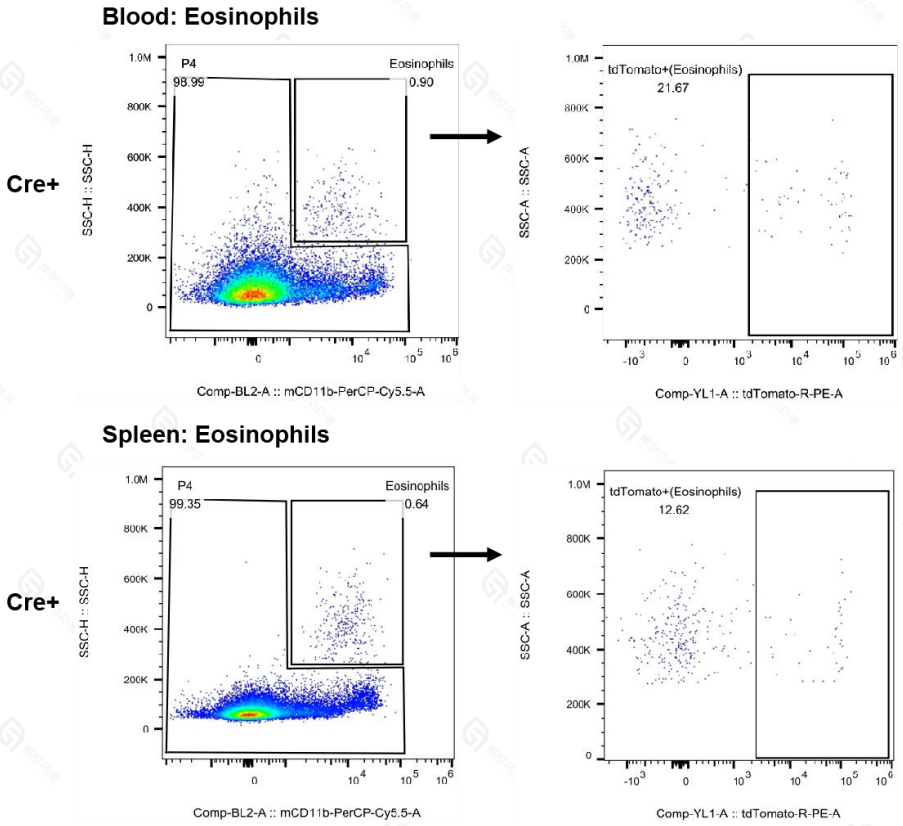


Fig 4. Flow cytometry analysis of cells with little Cre activity

Organ name was indicated in the left top of each subfigure group. Cre+: Lyz2-iCre, H11-CAG-LSL-tdTomato double positive individuals. Splenocytes and whole blood cells were harvested from Cre+ mice and analyzed for tdTomato expression with flow cytometry.

Reference

1.Shimshek D R, Kim J, Hübner M R, et al. "Codon-improved Cre recombinase (iCre) expression in the mouse." genesis 2002, 32(1): 19-26.

2.Clausen BE, Burkhardt C, Reith W, et al. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. Transgenic Res, 1999, 8(4): 265-77.

3.Singh G, Katyal SL, Brown WE, et al. Pulmonary lysozyme--a secretory protein of type II pneumocytes in the rat. Am Rev Respir Dis, 1988, 138(5): 1261-7.