C57BL/6JGpt-Csf1r-P2A-CreERT2

Strain Name： C57BL/6JGpt-*Csf1r*$^{em1Cin(P2A-CreERT2)}$/Gpt
Strain Type： Knock-in
Strain Number： T006204
Background： C57BL/6JGpt

Description

This mouse strain expresses CreERT2 inducible recombinase [1] under the control of the mouse *Csf1r* endogenous promoter, CreERT2-P2A was inserted downstream of the start codon ATG of *Csf1r* 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 macrophages and dendritic cells after tamoxifen administration. Recombinase activity after tamoxifen induction was detected in a proportion of cells in liver, spleen, intestine, lung and kidney.

Strategy

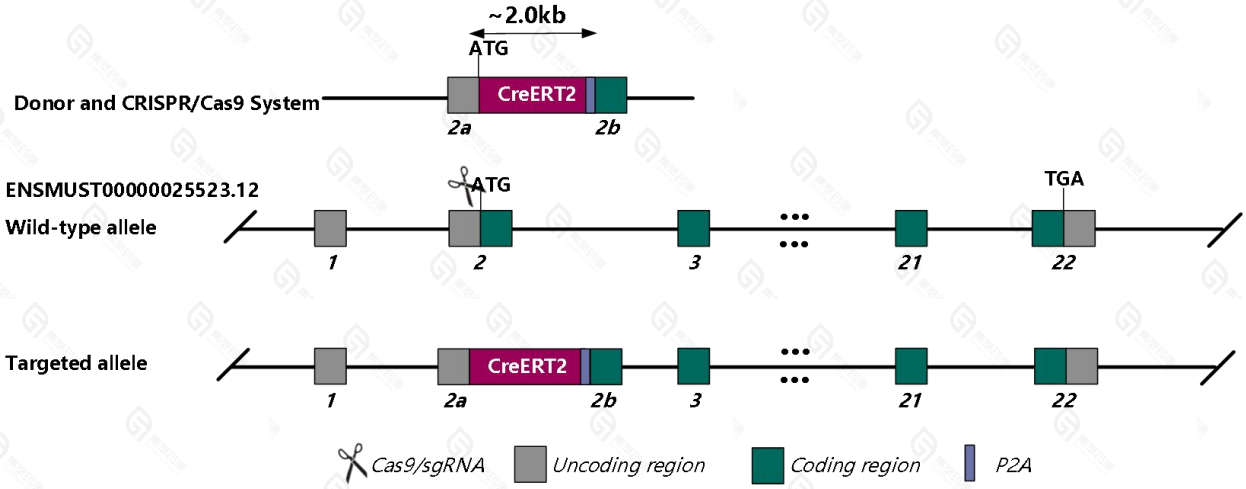


Fig.1 Schematic diagram of C57BL/6JGpt-Csf1r-P2A-CreERT2 model strategy.

Applications

1. Cre tool mice for specific, tamoxifen dependent induction of loxP recombination in macrophages and dendritic cells [2].

Data support

1. Validation methods & notes

Csf1r-P2A-CreERT2 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 loss of green fluorescence and gain of red 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. Flow cytometry analysis of splenic cells was performed to exhibit Cre activity. For tamoxifen administration, 0.25 mL of 5 mg/mL tamoxifen was treated through intraperitoneal injection daily from P57 to P63 (8.1 w~9.0 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.

2. Timeline of tamoxifen treatment and imaging

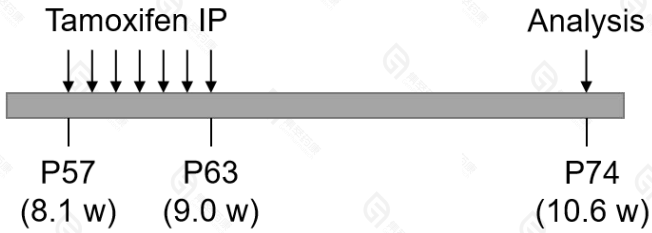
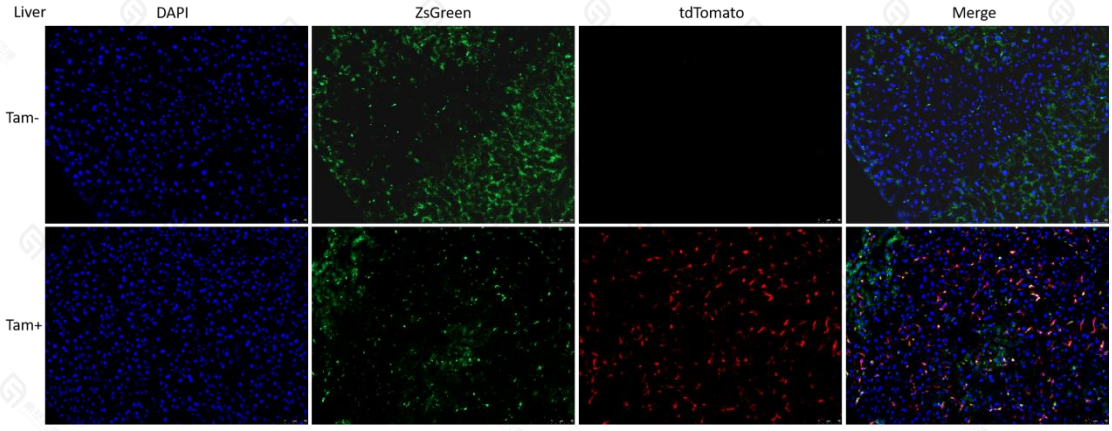
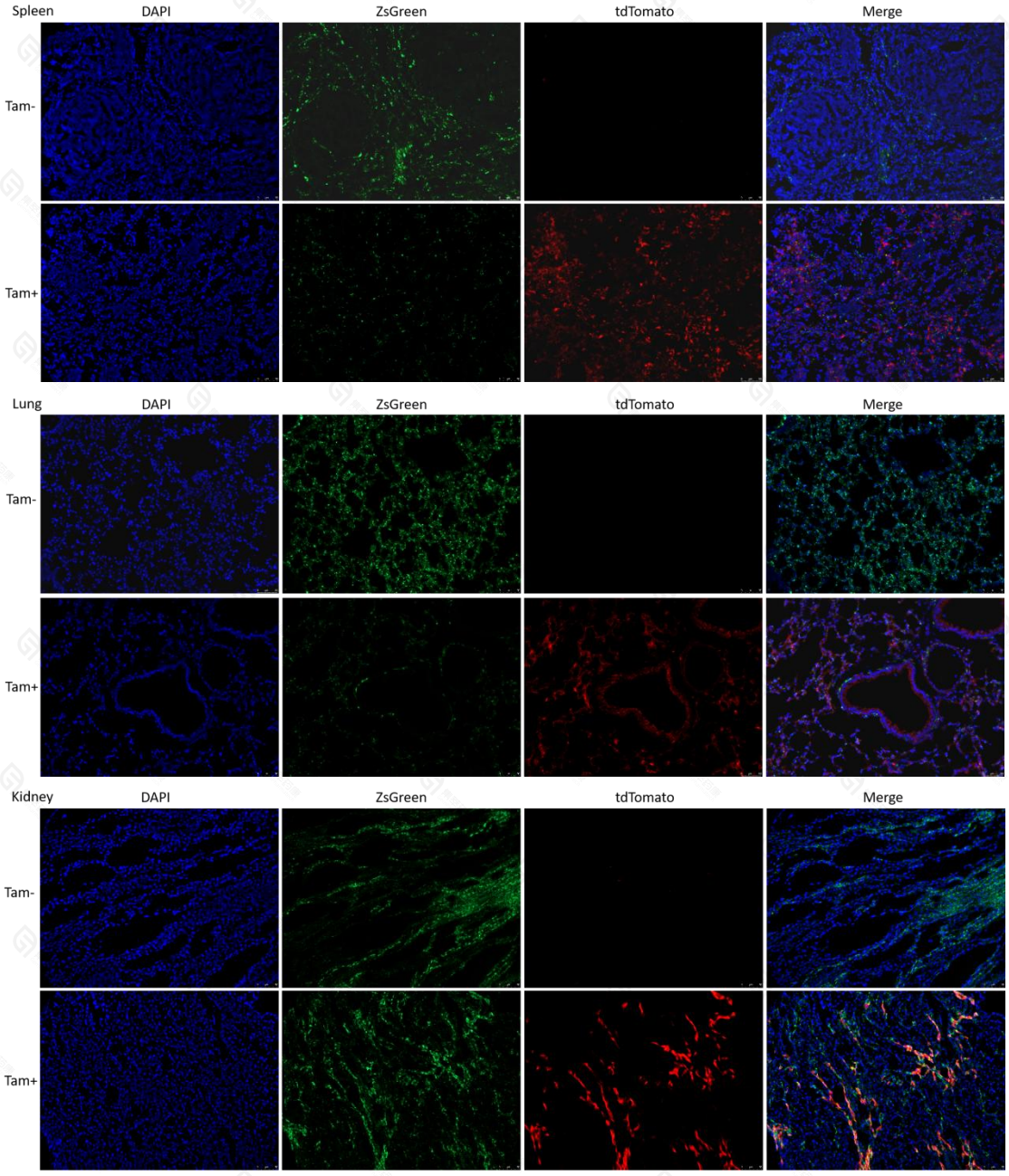


Fig 2. Timeline of tamoxifen treatment and experiment analysis of Csf1r-P2A-CreERT2 mice.

3. Images of tissues and organs with obvious Cre activity




Spleen
DAPI
ZsGreen
tdTomato
Merge
Tam-
Tam+
Lung
DAPI
ZsGreen
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Merge
Tam-
Tam+
Kidney
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ZsGreen
tdTomato
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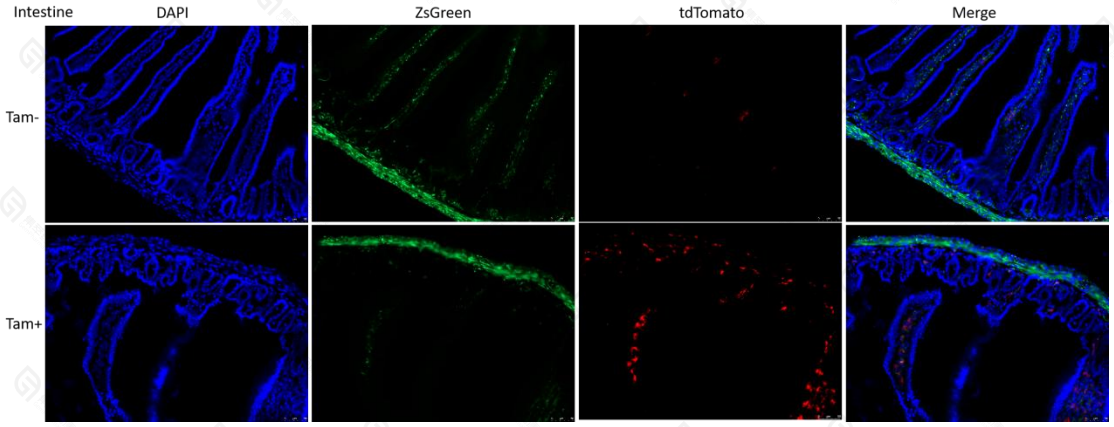
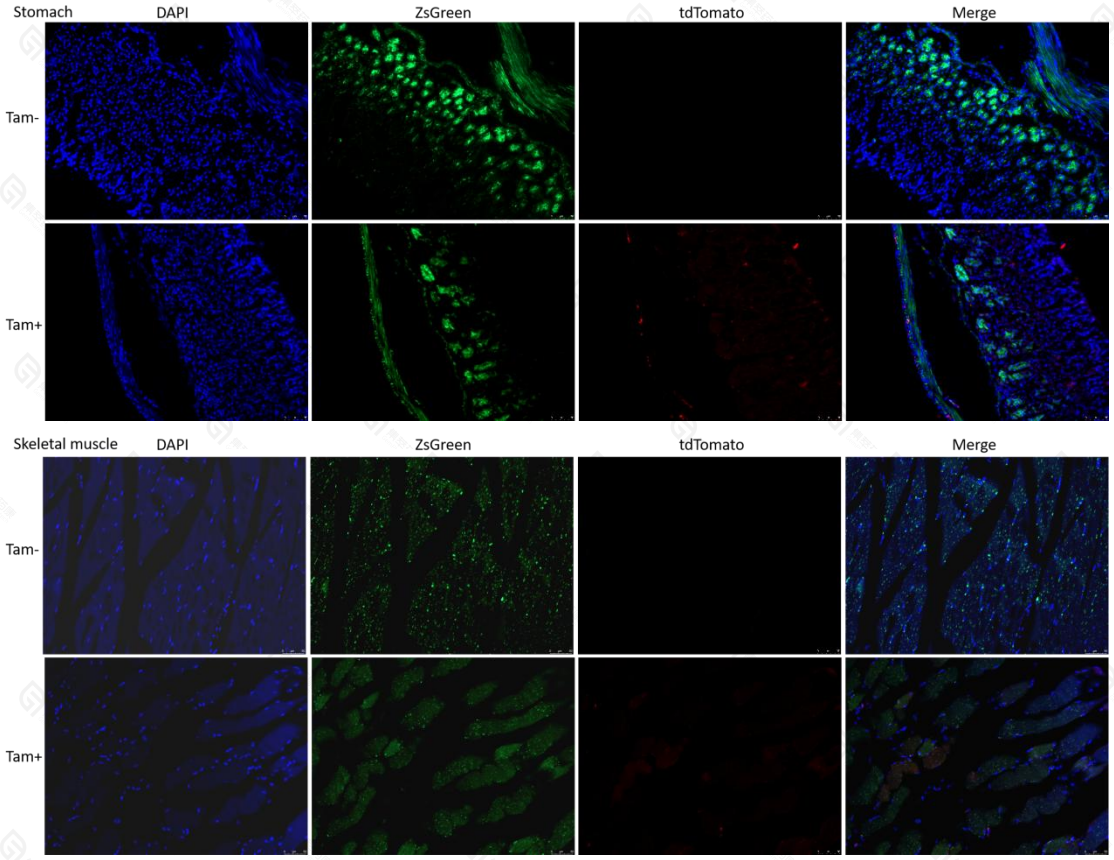


Fig 3. Fluorescence imaging of tissues and organs with obvious Cre activity.
Organ name was indicated in the left top of each subfigure group. Tam-: Csf1r-P2A-CreERT2, CAG-G/R double positive individuals without tamoxifen administration; Tam+: Csf1r-P2A-CreERT2, CAG-G/R double positive individuals with tamoxifen administration.

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



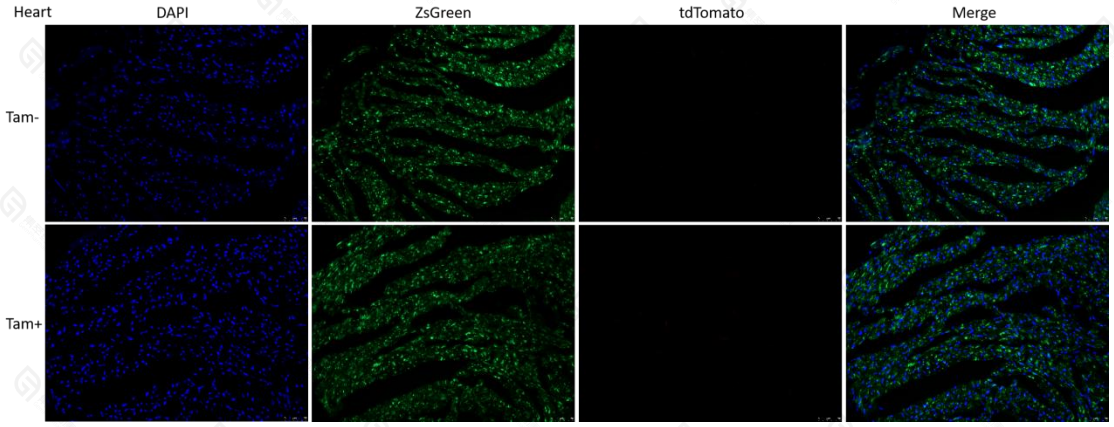


Fig 4. Fluorescence imaging of tissues and organs with little or no Cre activity.
Organ name was indicated in the left top of each subfigure group. Tam-: Csf1r-P2A-CreERT2, CAG-G/R double positive individuals without tamoxifen administration; Tam+: Csf1r-P2A-CreERT2, CAG-G/R double positive individuals with tamoxifen administration.

5. Gating Strategies for flow Cytometry

Cell population	Gating		
Monocytes	mCD45+	Not (mCD11b+mLy6G+)	mCD11b+mLy6C hi
Dendritic cells	Not Monocytes	mCD11c+	
Macrophages	Not Dendritic cells	mCD11b+mF4/80+	

Table 1. Gating Strategies for flow Cytometry of Csf1r-P2A-CreERT2 mice.

6. Flow cytometry analysis of cells with Cre activity

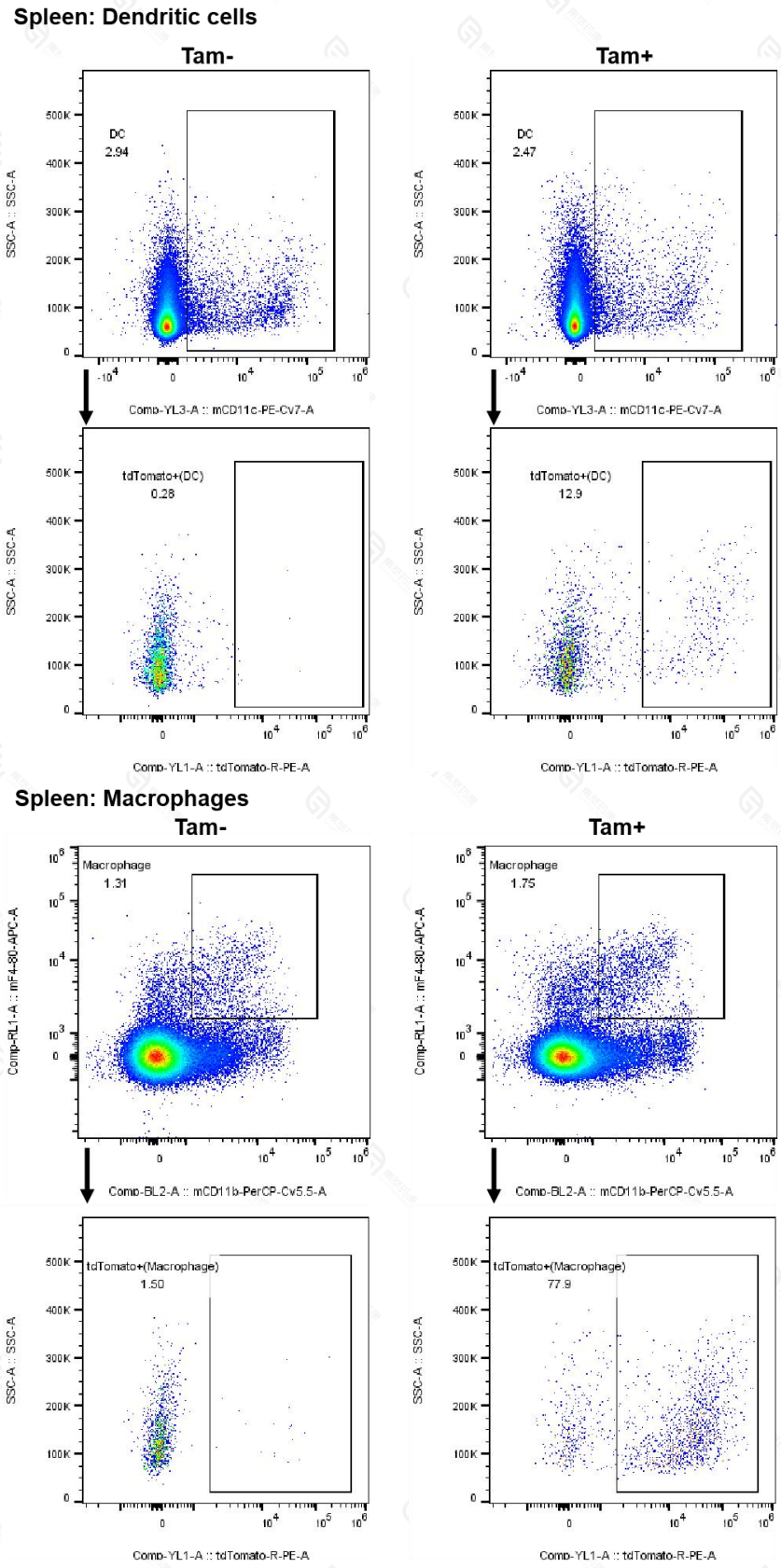


Fig 5. Flow cytometry analysis of cells with Cre activity

Organ name was indicated in the left top of each subfigure group. Tam-: Csf1r-P2A-CreERT2, CAG-G/R double positive individuals without tamoxifen administration; Tam+: Csf1r-P2A-CreERT2, CAG-G/R double positive individuals with tamoxifen administration. Splenocytes were harvested from Tam- and Tam+ mice and analyzed for tdTomato expression with flow cytometry.

7. Flow cytometry analysis of cells with little or no Cre activity

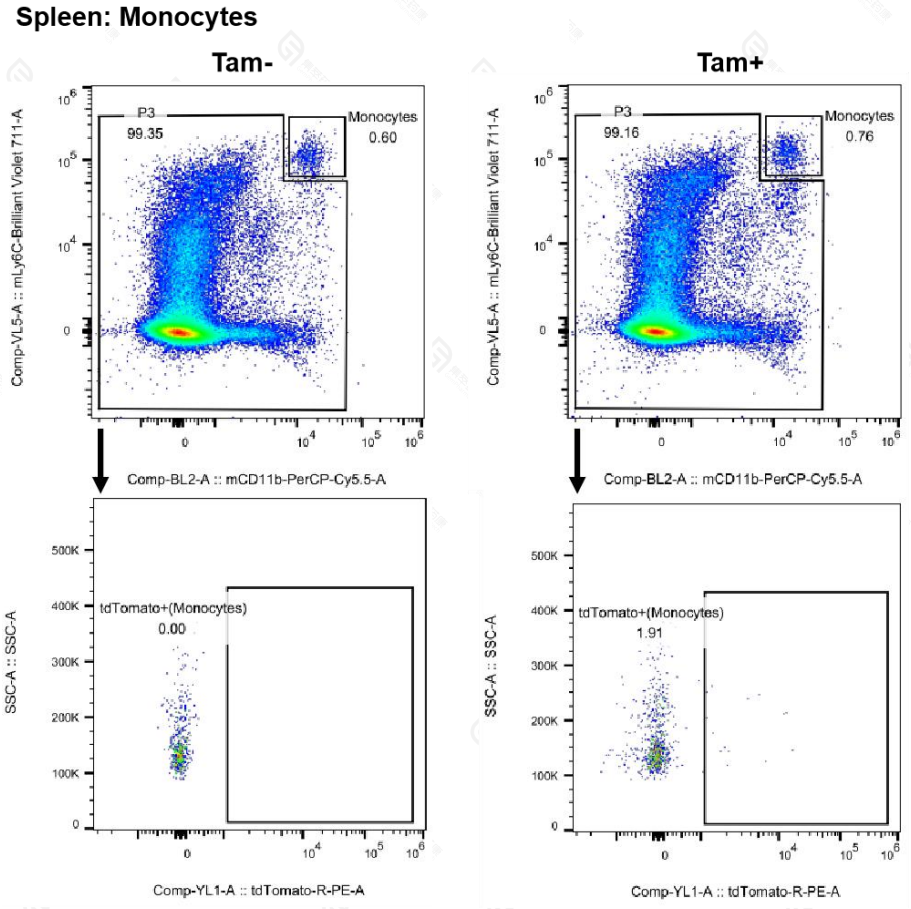


Fig 6. Flow cytometry analysis of cells little or no Cre activity

Organ name was indicated in the left top of each subfigure group. Tam-: Csf1r-P2A-CreERT2, CAG-G/R double positive individuals without tamoxifen administration; Tam+: Csf1r-P2A-CreERT2, CAG-G/R double positive individuals with tamoxifen administration. Splenocytes were harvested from Tam- and Tam+ mice and analyzed for tdTomato expression with flow cytometry.

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.Qian BZ, Li J, Zhang H, et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature. 2011, 475(7355): 222-5.