

## C57BL/6JGpt-Cx3cr1-iCre

**Strain Name:** C57BL/6JGpt-Cx3cr1<sup>em1Cin(iCre)</sup>/Gpt

**Strain Type:** Knock-in

**Strain Number:** T006768

**Background:** C57BL/6JGpt

### Description

This mouse strain expresses codon optimized iCre recombinase <sup>[1]</sup> under the control of the Mouse *Cx3cr1* promoter, the iCre replaced the the entire coding region of the *Cx3cr1* 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 monocytes and macrophages, as well as microglia. Recombinase activity was also detected in NK cells and CD8+ T cells.

### Strategy

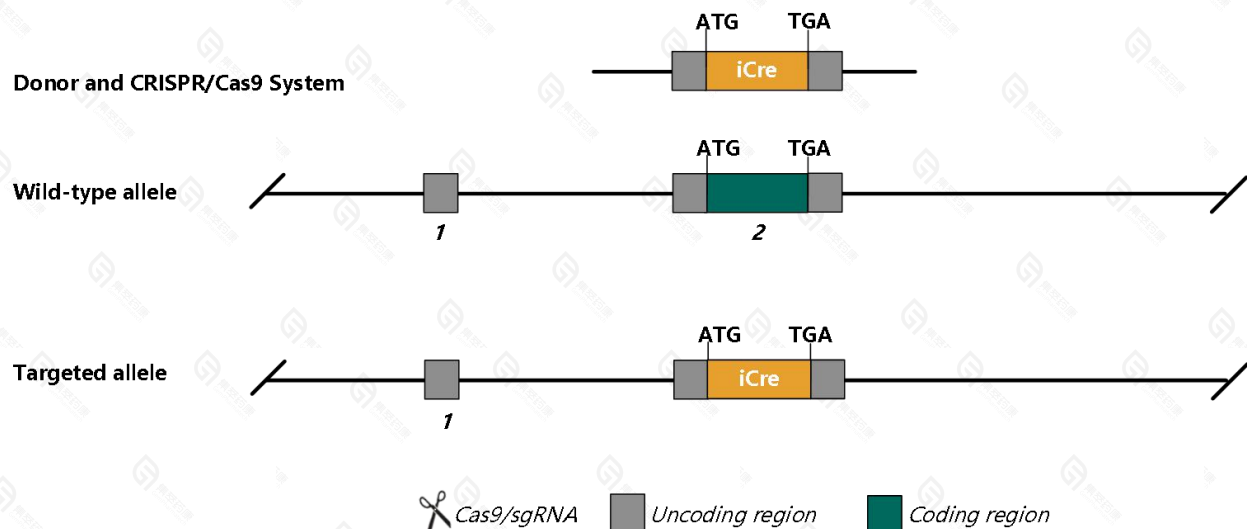


Fig.1 Schematic diagram of C57BL/6JGpt-Cx3cr1-iCre model strategy.

### Applications

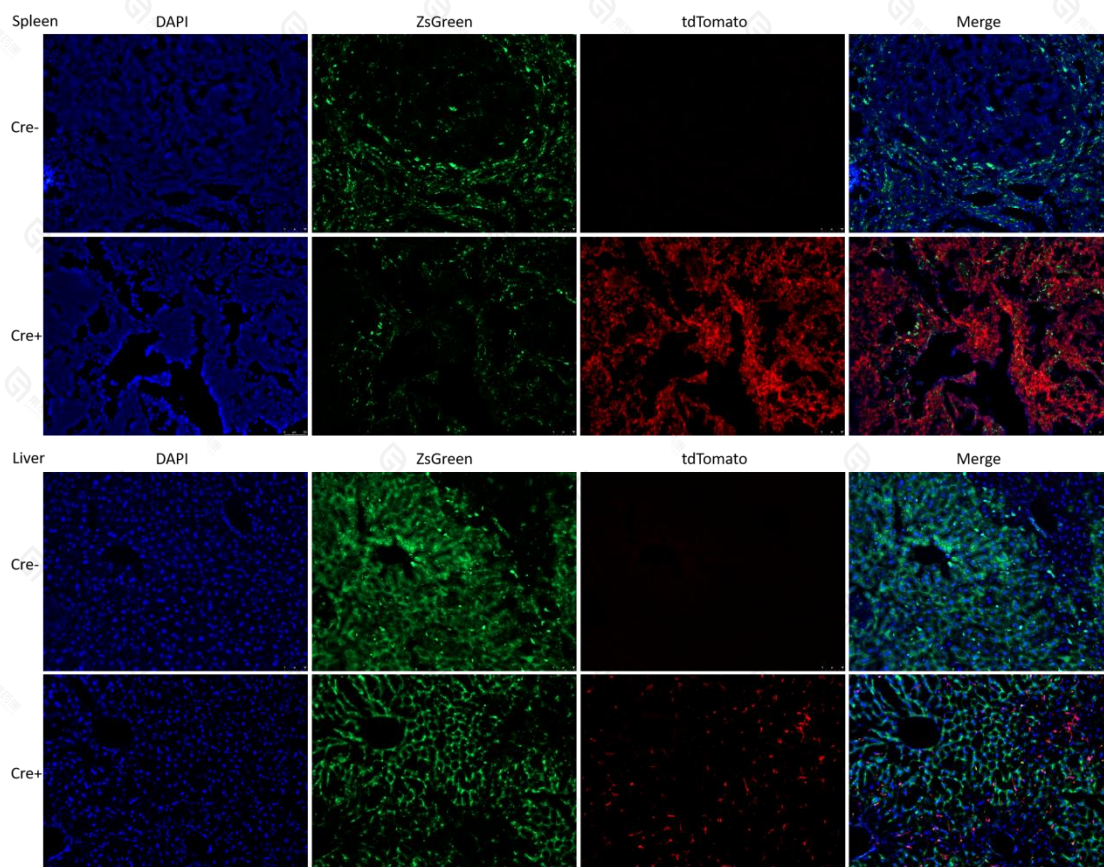
1. Cre tool mice for specific induction of loxP recombination in monocytes and macrophages, as well as microglia <sup>[2]</sup>.

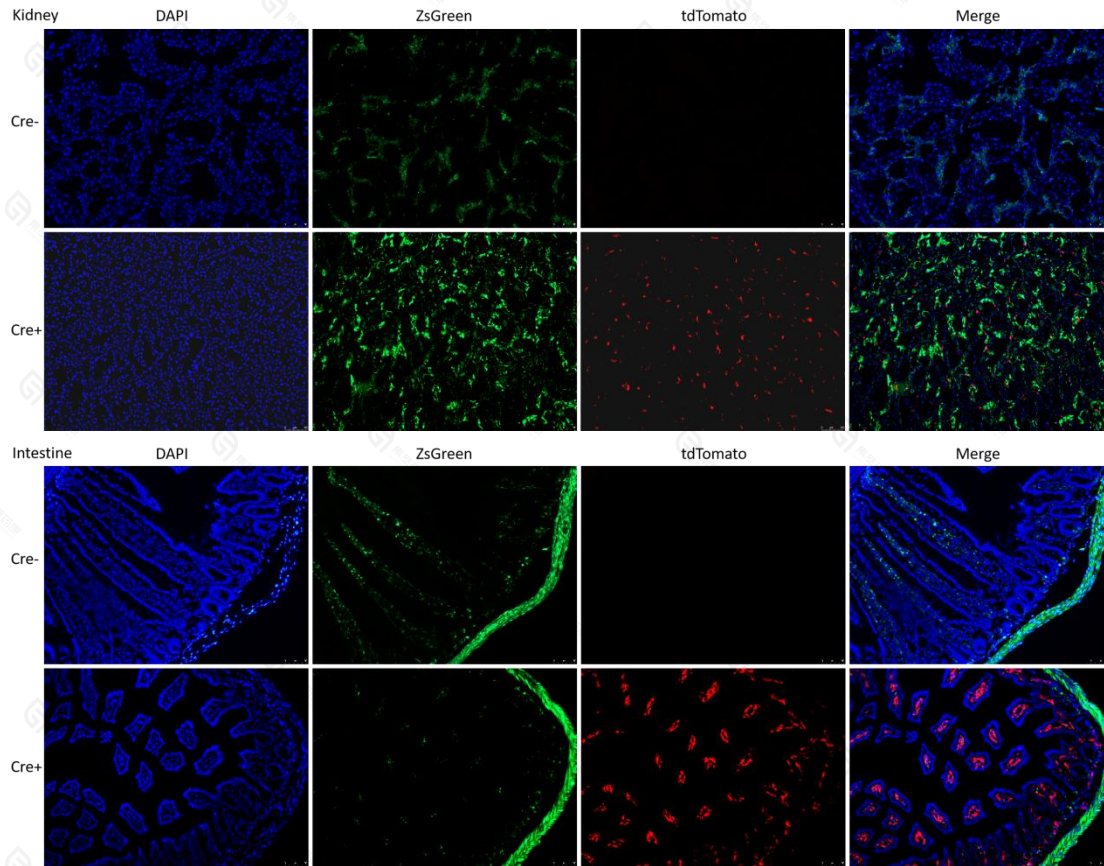
### Data support

#### 1. Validation methods & notes

Cx3cr1-iCre 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. 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

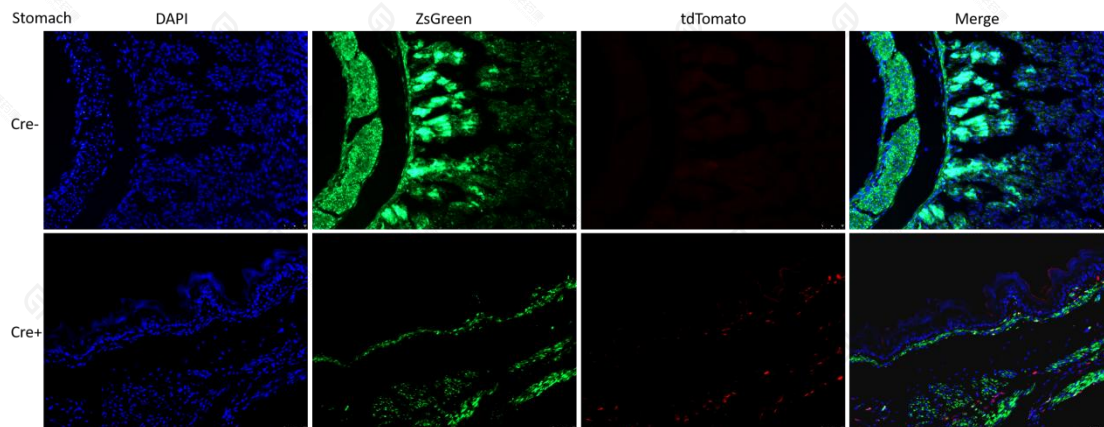




**Fig 2. Fluorescence imaging of tissues and organs with obvious Cre activity.**

Organ name was indicated in the left top of each subfigure group. Cre-: CAG-G/R single positive individuals; Cre+: Cx3cr1-iCre, CAG-G/R double positive individuals.

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



**Fig 3. Fluorescence imaging of tissues and organs with little or no Cre activity.**

Organ name was indicated in the left top of each subfigure group. Cre-: CAG-G/R single positive individuals; Cre+: Cx3cr1-iCre, CAG-G/R double positive individuals.



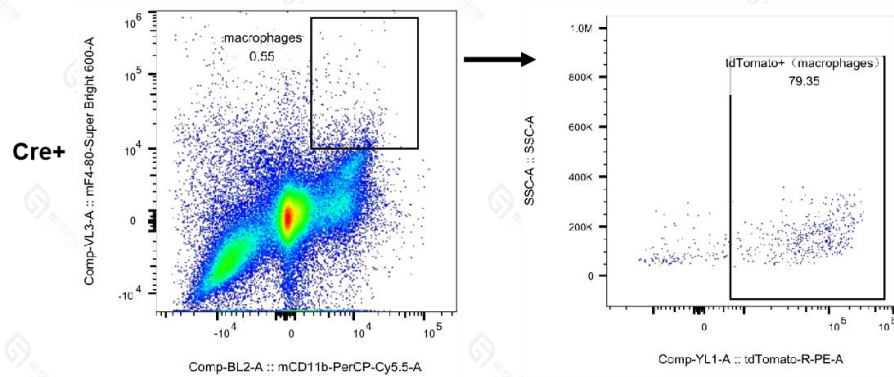
#### 4. Gating Strategies for flow Cytometry

Cell population		Gating		
Monocytes	mCD45+	Not (mCD11b+mLy6G+)	mCD11b+mLy6C hi	
Macrophages	Not Monocytes	Not (mCD11b+SSC-H hi)	mCD11b+mF4/80+	
CD8+ T cells	Not Monocytes	Not (mCD11b+SSC-H hi)	mCD3+mCD335-	mCD8+
NK cells	Not Monocytes	Not (mCD11b+SSC-H hi)	mCD3-mCD335+	

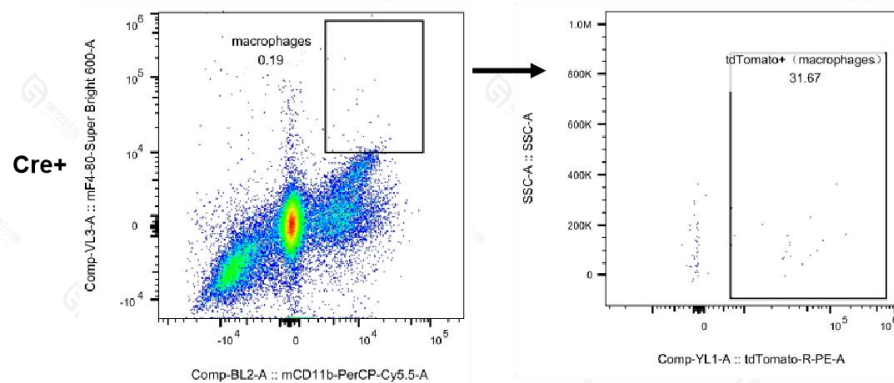
**Table 1. Gating Strategies for flow Cytometry of Cx3cr1-iCre mice.**

#### 5. Flow cytometry analysis of cells with Cre activity

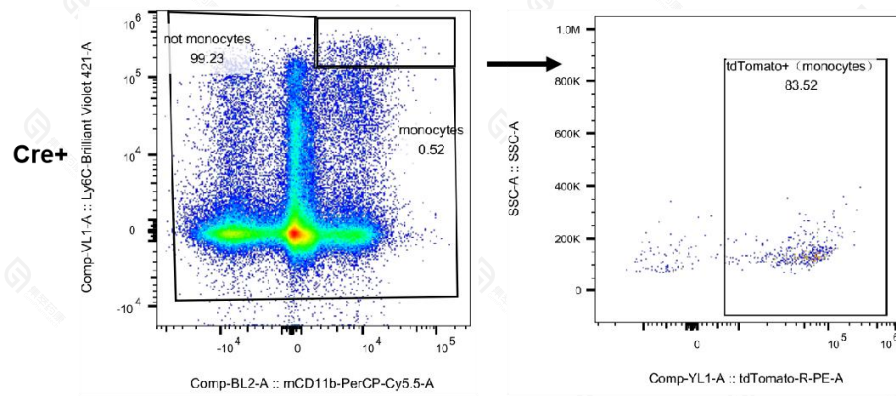
##### Spleen: Macrophages



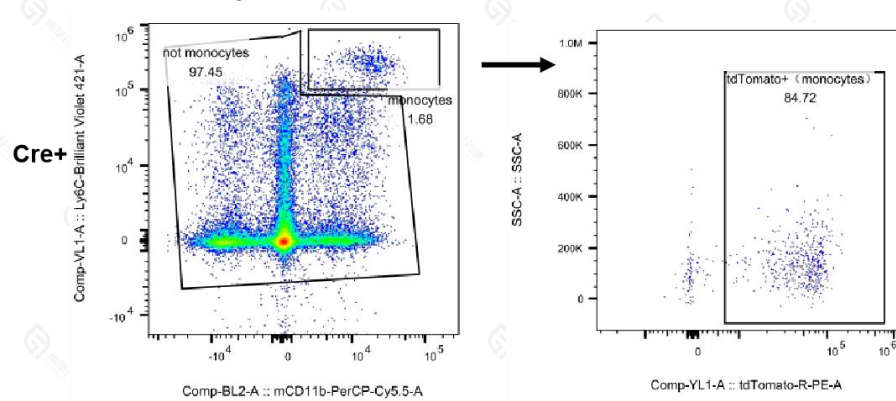
##### Blood: Macrophages



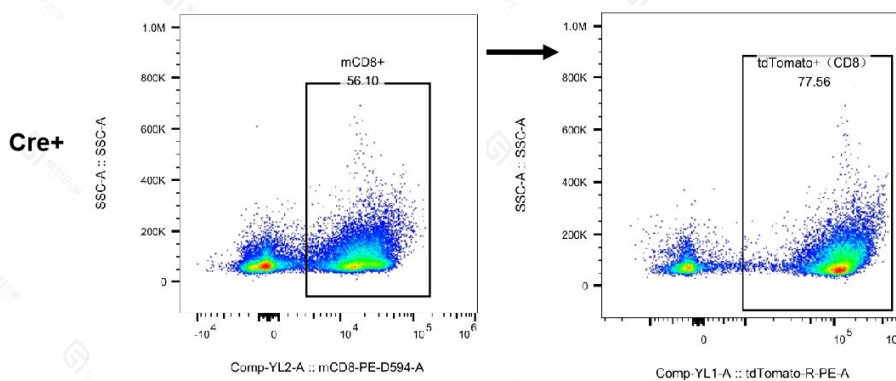
### Spleen: Monocytes

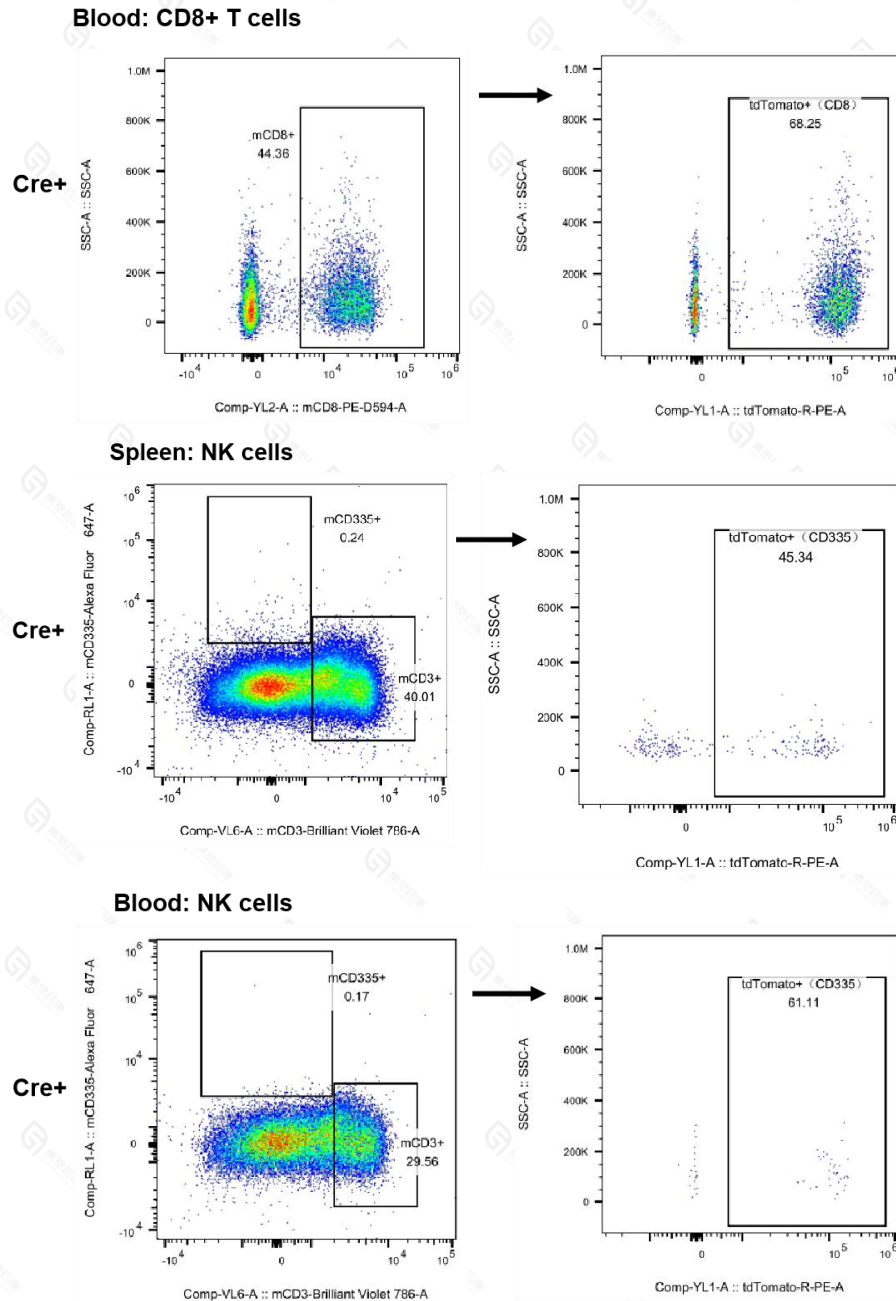


### Blood: Monocytes



### Spleen: CD8+ T cells





**Fig 5. Flow cytometry analysis of cells with Cre activity**

Organ name was indicated in the left top of each subfigure group. Cre+: Cx3cr1-iCre, CAG-G/R 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. Yona S, Kim KW, Wolf Y, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity*, 2013, 38(1): 79-91.