B6-G/R

Strain Name： C57BL/6JGpt-*H11*$^{em1Cin(CAG\text{-}LoxP\text{-}ZsGreen\text{-}Stop\text{-}LoxP\text{-}tdTomato)}$/Gpt
Strain Type： Knock-in
Strain Number： T006163
Background： C57BL/6JGpt

Description

We combined the green fluorescent protein ZsGreen with the red fluorescent protein tdTomato, and knocked it into the mouse H11 safe harbor site at the same time. Under normal circumstances, the whole body of mice expresses ZsGreen, which can excite dazzling green light, which can illuminate the shape and structure of various cells and tissues. Therefore, the whole body of mice is green at birth, which is different. However, we buried loxP sites on both sides of ZsGreen. When Cre recombinase exists, ZsGreen in the mouse genome will be deleted, and the expression of tdTomato will be turned on, which can emit dazzling red light. Since Cre can be expressed tissue-specifically, mice turn on red light following the expression time and location of Cre. Note: This model is not suitable for tracing the expression location of Cre in brain and bone tissue, but articular cartilage was expressed normally.

Strategy

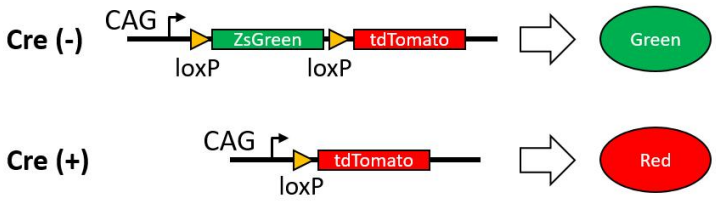


Fig.1 Schematic diagram of B6-G/R model strategy.

Applications

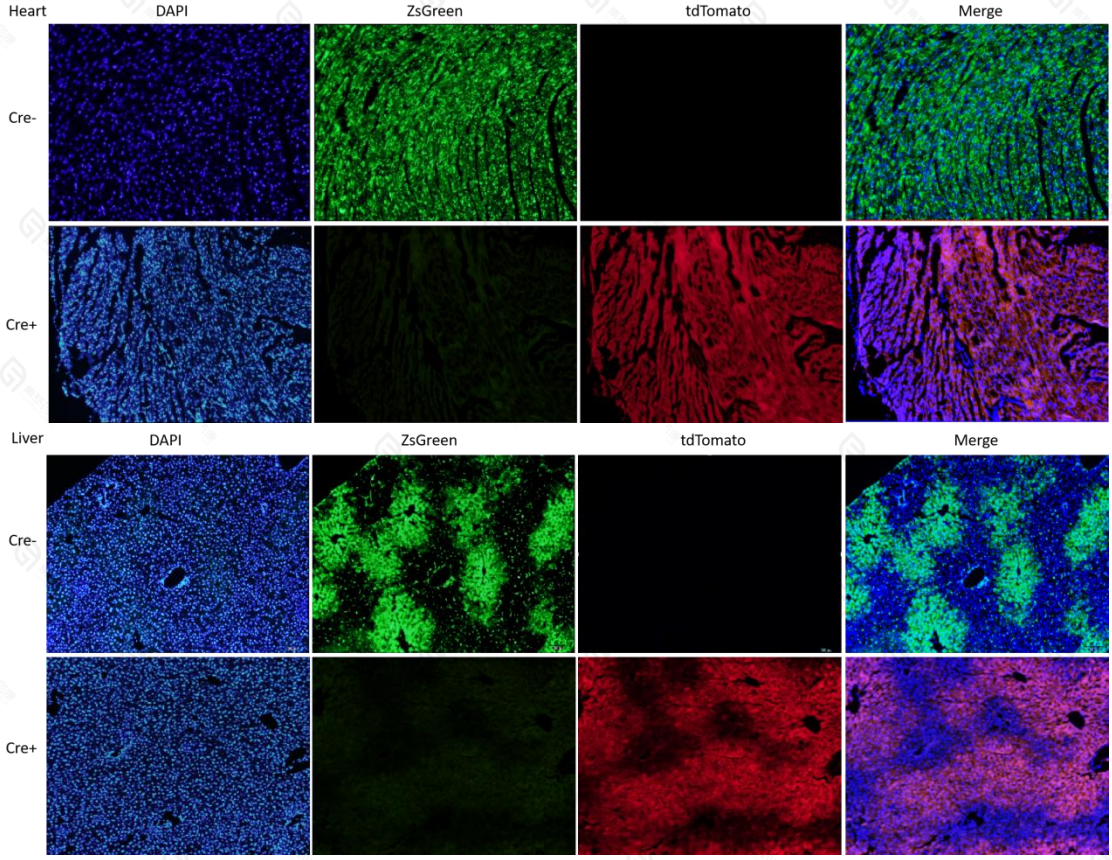
1. B6-G/R mice is an ideal tool to trace the location of specific Cre expression and displaying Cell Fate [1].

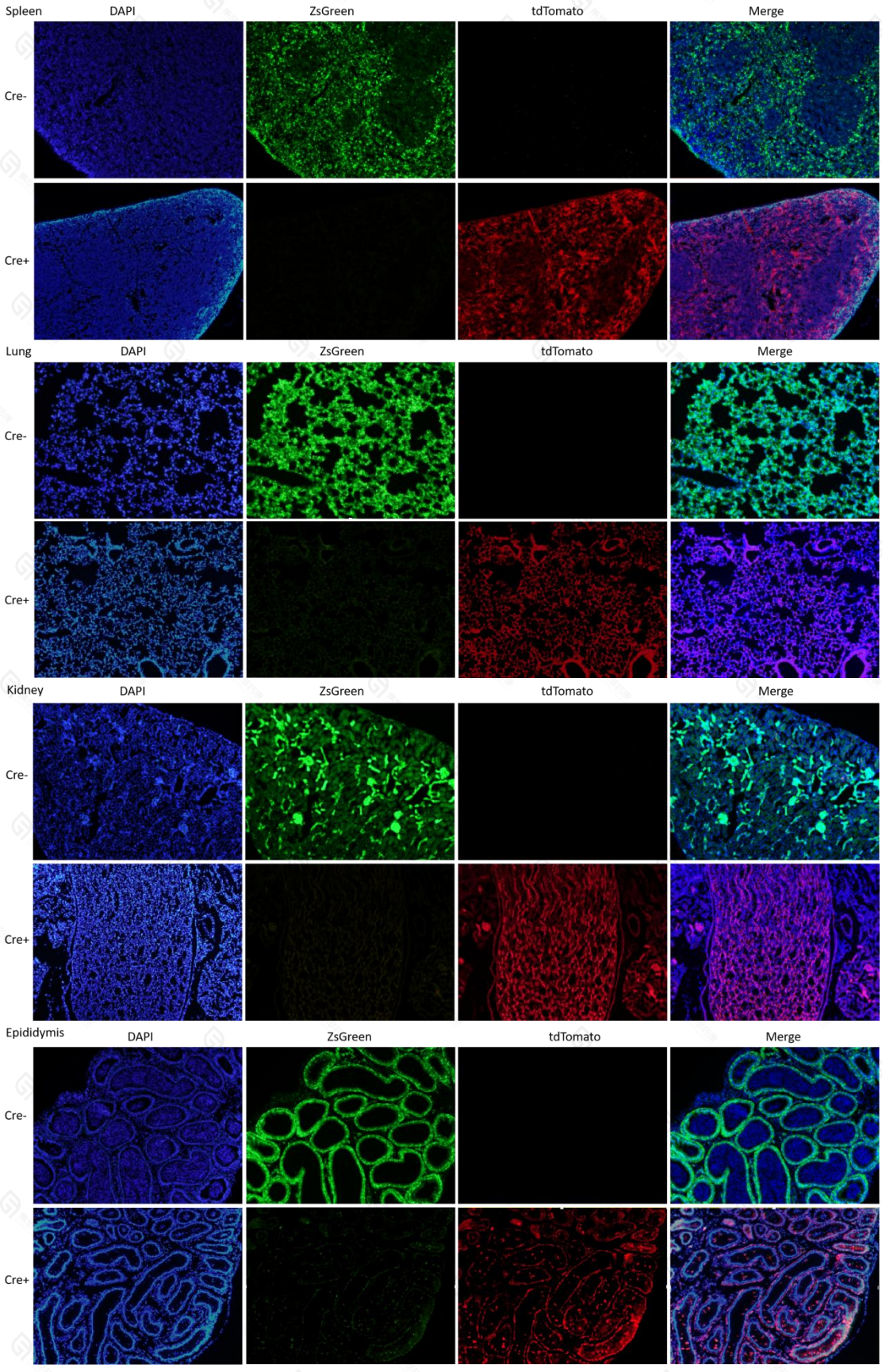
Data support

1. Validation methods & notes

TgTn(pb-CAG-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




Spleen
DAPI
ZsGreen
tdTomato
Merge
Cre-
Cre+
Lung
DAPI
ZsGreen
tdTomato
Merge
Cre-
Cre+
Kidney
DAPI
ZsGreen
tdTomato
Merge
Cre-
Cre+
Epididymis
DAPI
ZsGreen
tdTomato
Merge
Cre-
Cre+

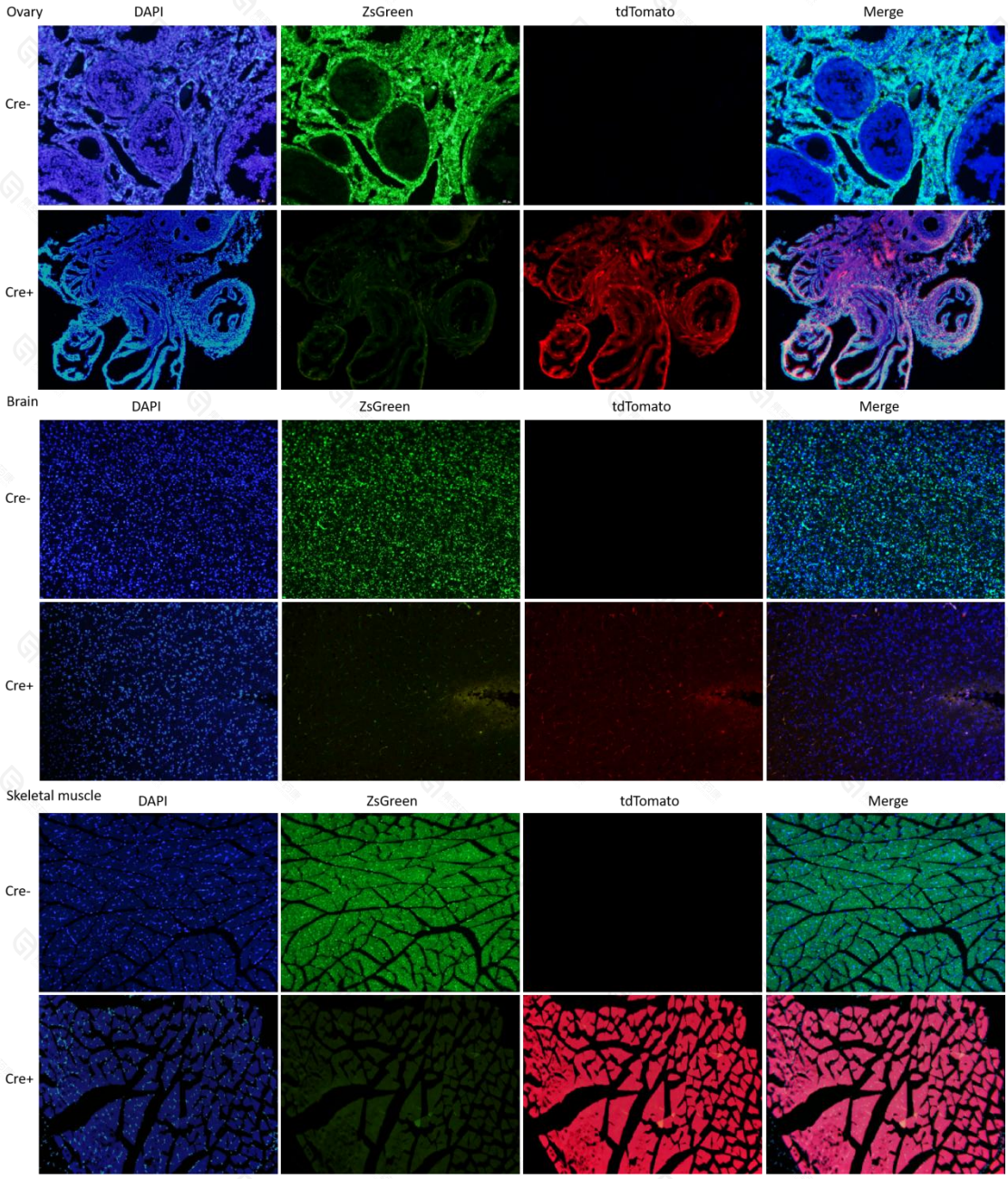


Fig 2. Fluorescence imaging of tissues and organs with obvious Cre activity.
Organ name was indicated in the left top of each subfigure group. Cre-: CAG-G/R single positive individuals; Cre+: TgTn(pb-CAG-iCre) , CAG-G/R double positive individuals.

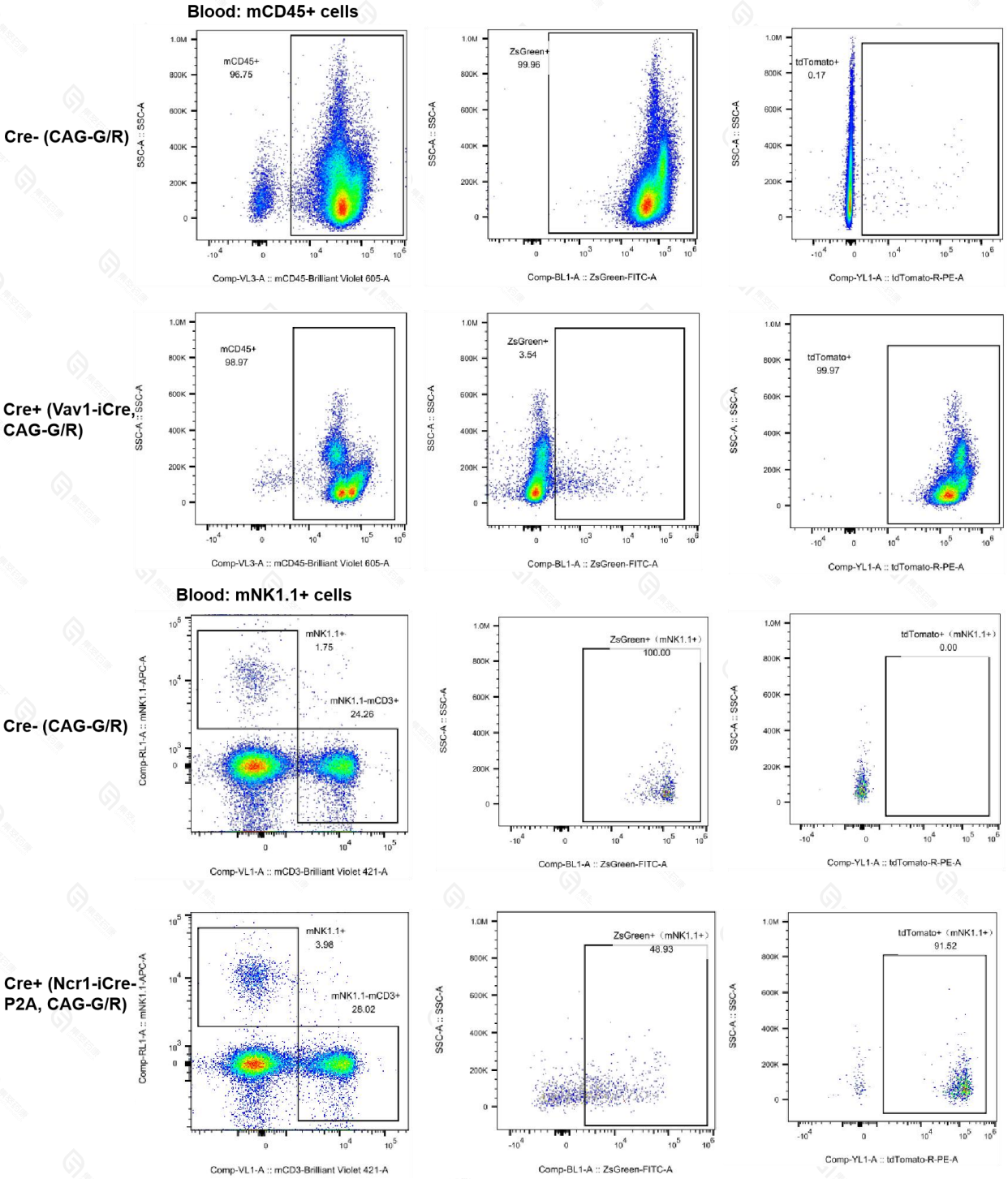
3. Gating Strategies for flow Cytometry

Cell population	Gating		
Leukocytes	mCD45+		
Natural killer cells	mCD45+	mCD3-mNK1.1+	
mCD4+ T cells	mCD45+	mCD3+mCD335-	mCD4+ mCD8-

mCD8+ T cells	mCD45+	mCD3+mCD335-	mCD4- mCD8+
Platelets	mCD41+		

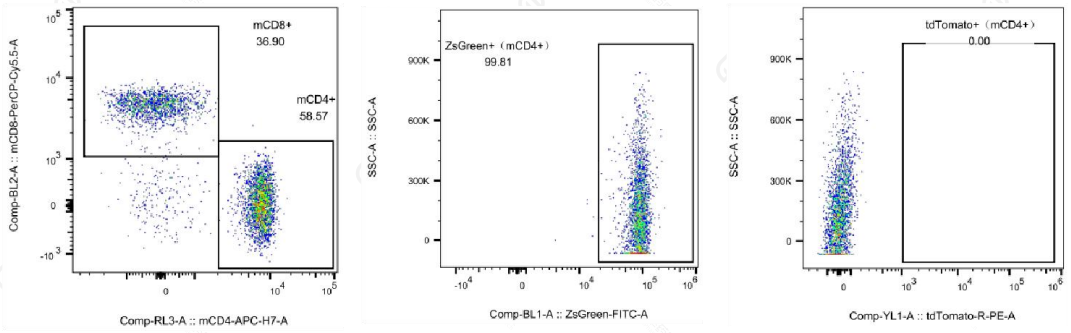
Table 1. Gating Strategies for flow Cytometry of B6-G/R mice.

4. Flow cytometry analysis of cells with Zsgreen and tdTomato expression

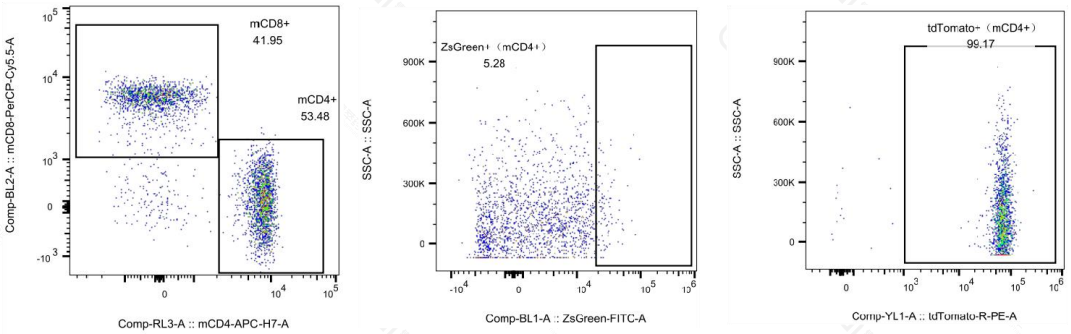


Blood: mCD4+ T cells

Cre- (CAG-G/R)

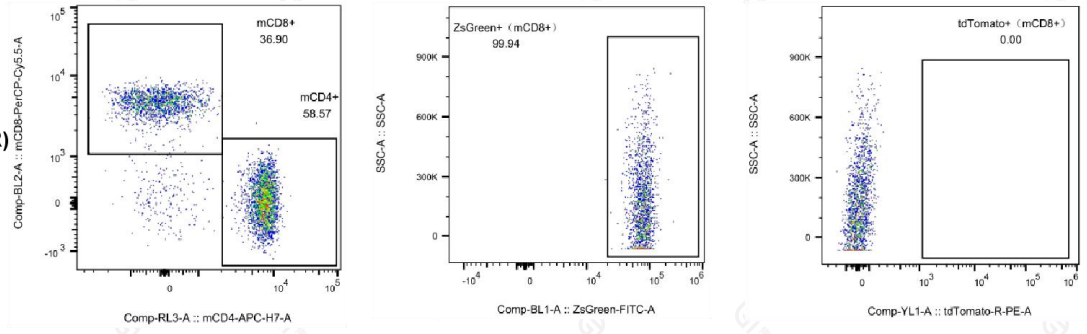


Cre+ (H11-Cd4-iCre, CAG-G/R)

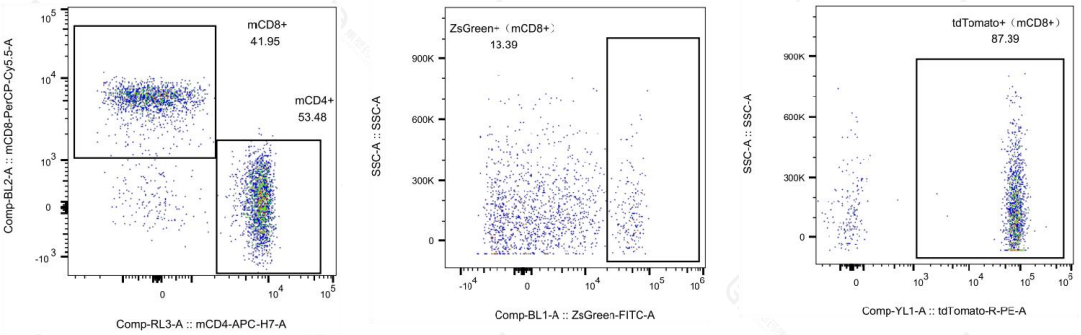


Blood: mCD8+ T cells

Cre- (CAG-G/R)



Cre+ (H11-Cd4-iCre, CAG-G/R)



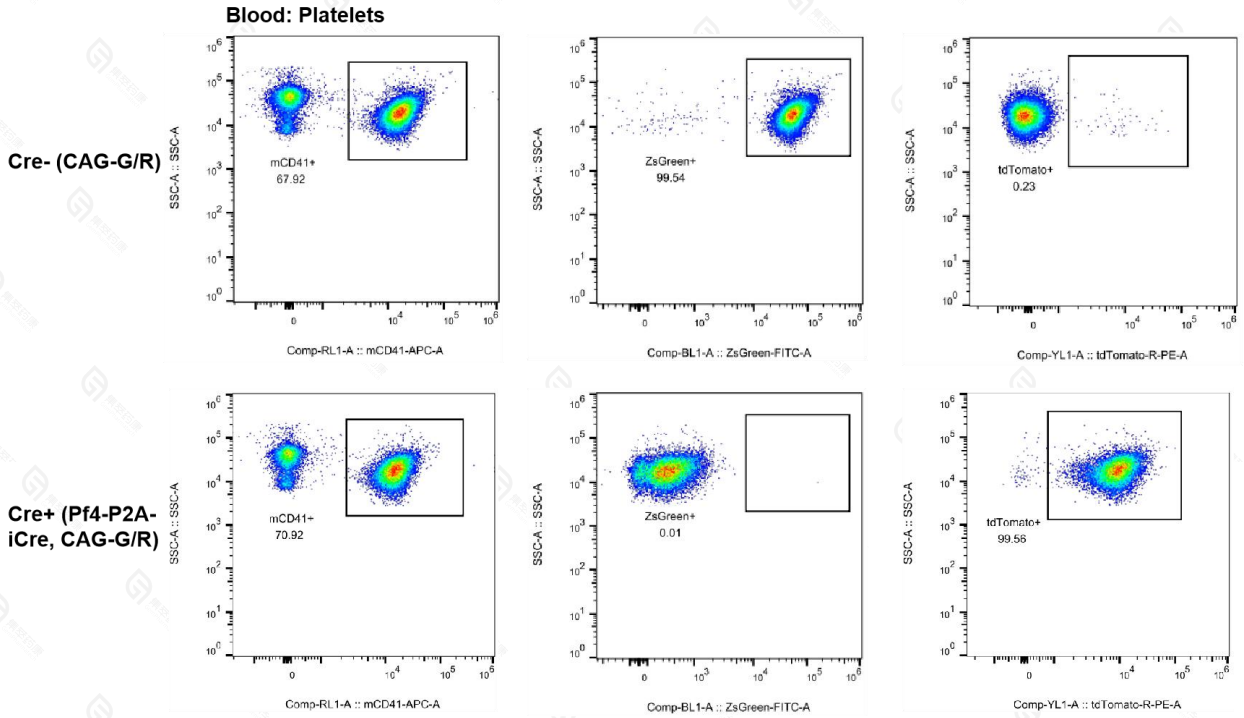


Fig 3. Flow cytometry analysis of cells with Zsgreen and tdTomato expression

Organs were was indicated in the left top of each subfigure group. Cre-: CAG-G/R single positive individuals. Whole blood cells were harvested and analyzed for Zsgreen and 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.