

57823

C57BL/6JGpt-Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA

Strain Name: C57BL/6JGpt-Rosa26^{em1Cin(CAG-LSL-EGFP-3xHA-WPRE-polyA)}/Gpt

Strain Type: Knock-in

Strain Number: T057823

Background: C57BL/6JGpt

Description

This is a reporter tool strain for Cre-dependent EGFP fluorescent protein expression and lineage tracing. A loxp-stop-loxp cassette was placed inside CAG promoter and the coding sequence of EGFP, and the whole construct was knocked it into the mouse Rosa26 safe harbor site in mouse Chr6 by CRISPR/Cas9 technology. When Cre recombinase exists, stop cassette in the mouse genome will be removed due to loxp recombination, and EGFP will be expressed and emit dazzling green light. Note: according to our internal validation, this model is not suitable for tracing Cre expression in brain, spinal cord, white fat, thyroid gland, stomach and bone tissue.

Strategy

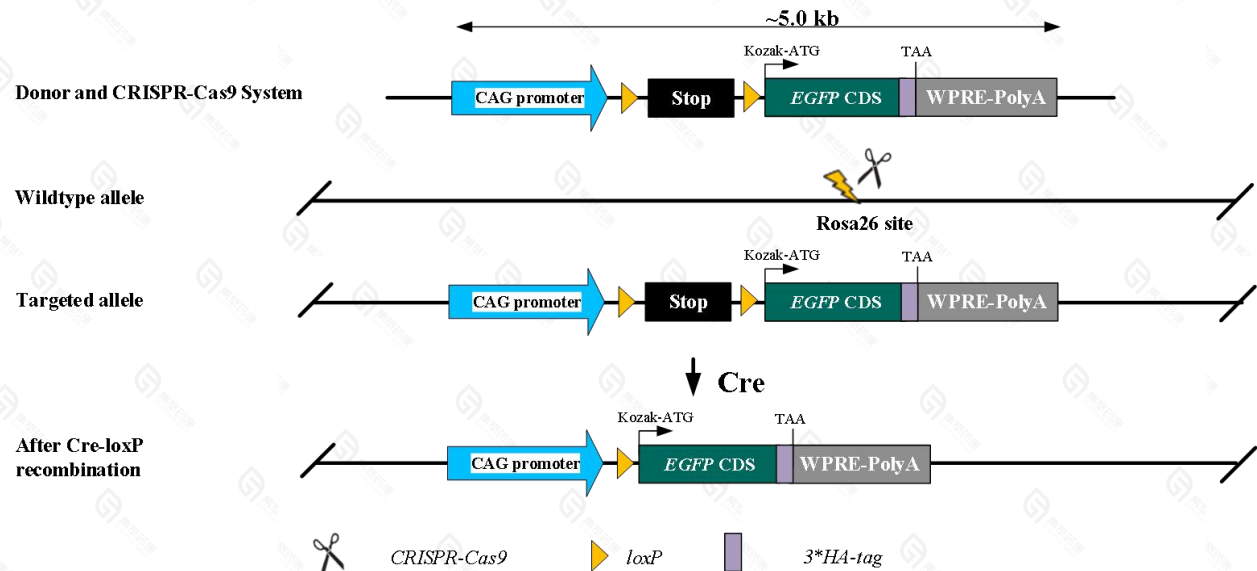


Fig.1 Schematic diagram of C57BL/6JGpt-CAG-LSL-EGFP-3xHA-WPRE-polyA model strategy.

Applications

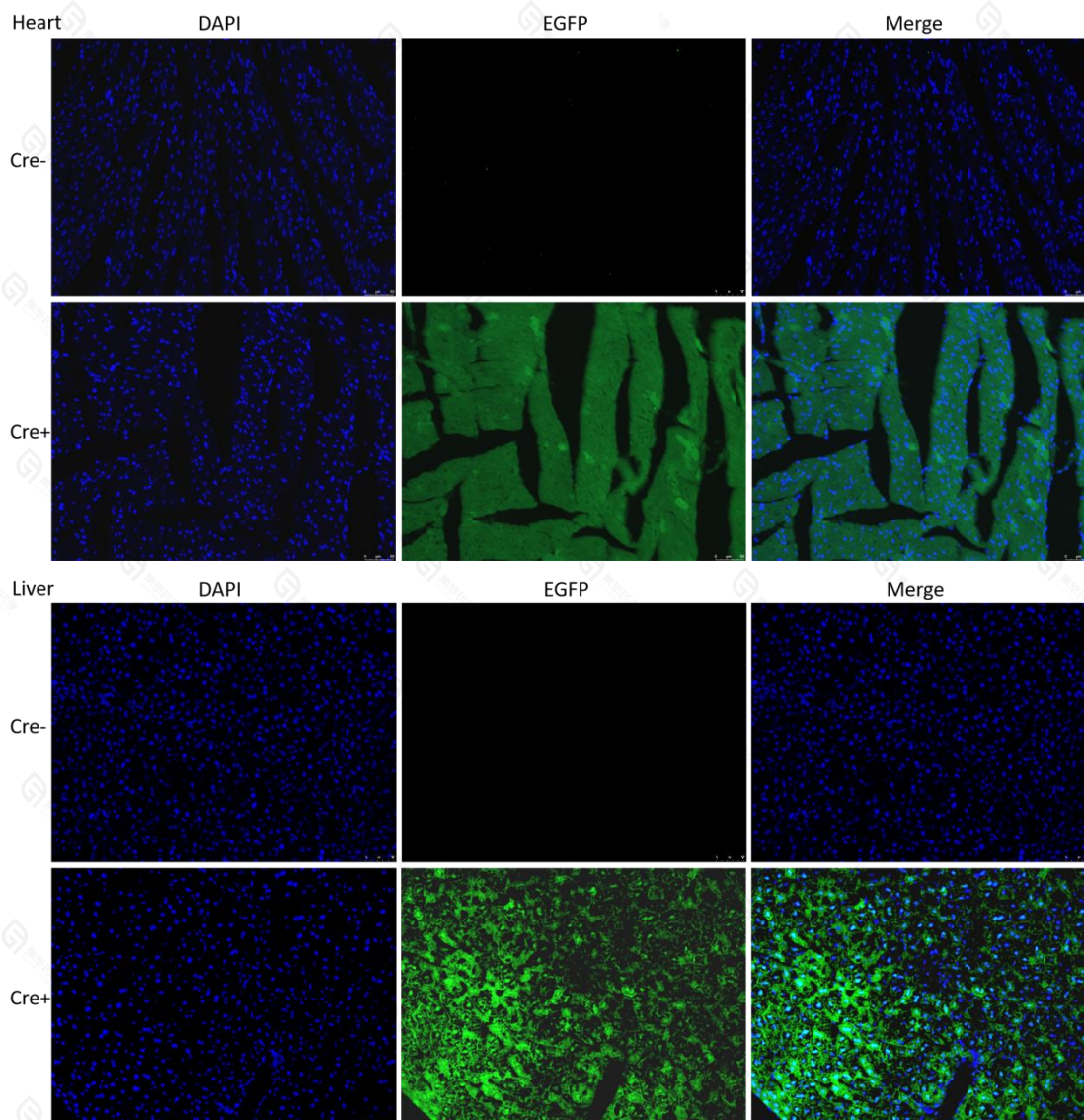
1. Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA mice is an ideal tool to trace the location of specific Cre expression and displaying Cell Fate [1].

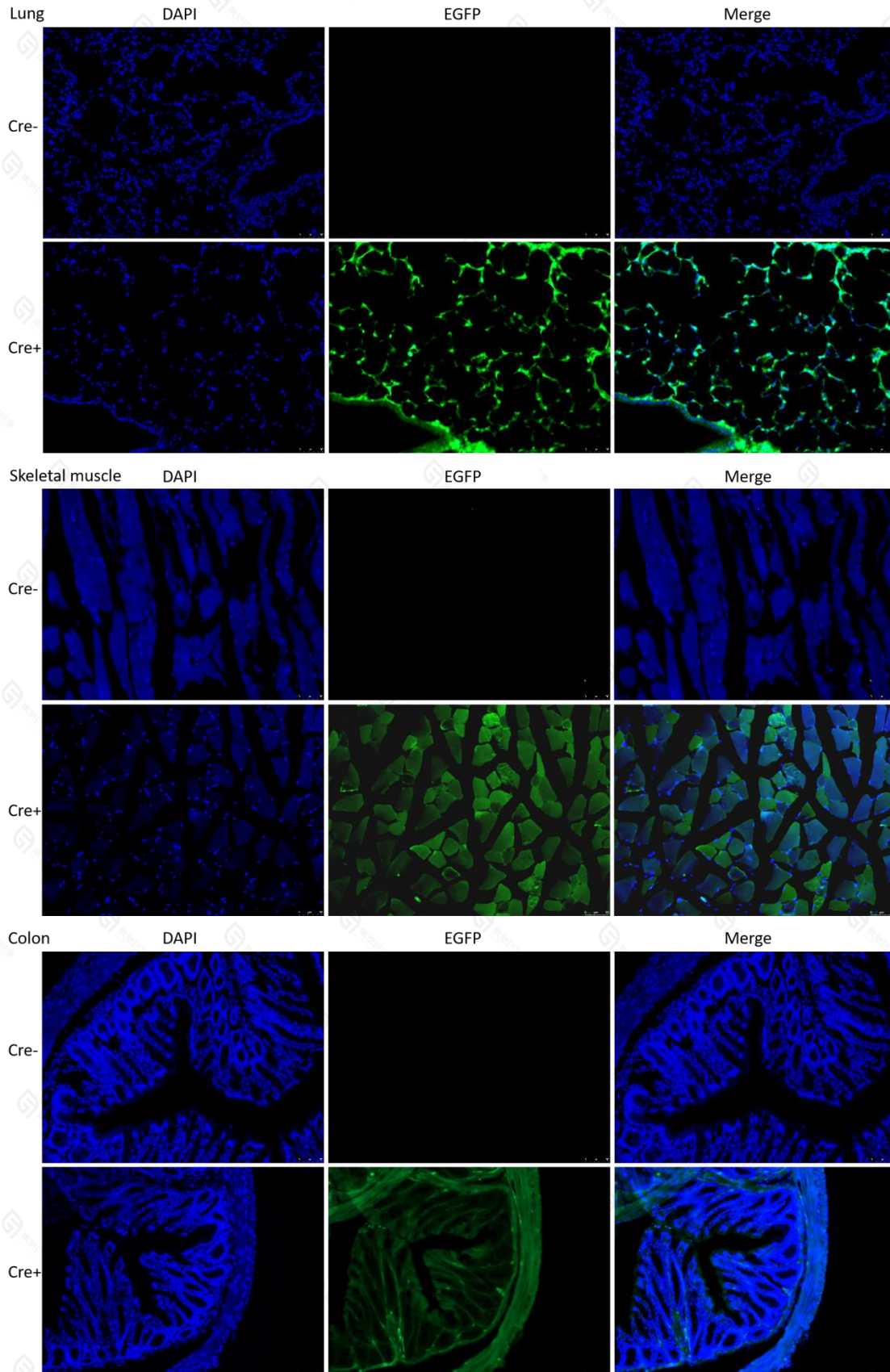
Data support

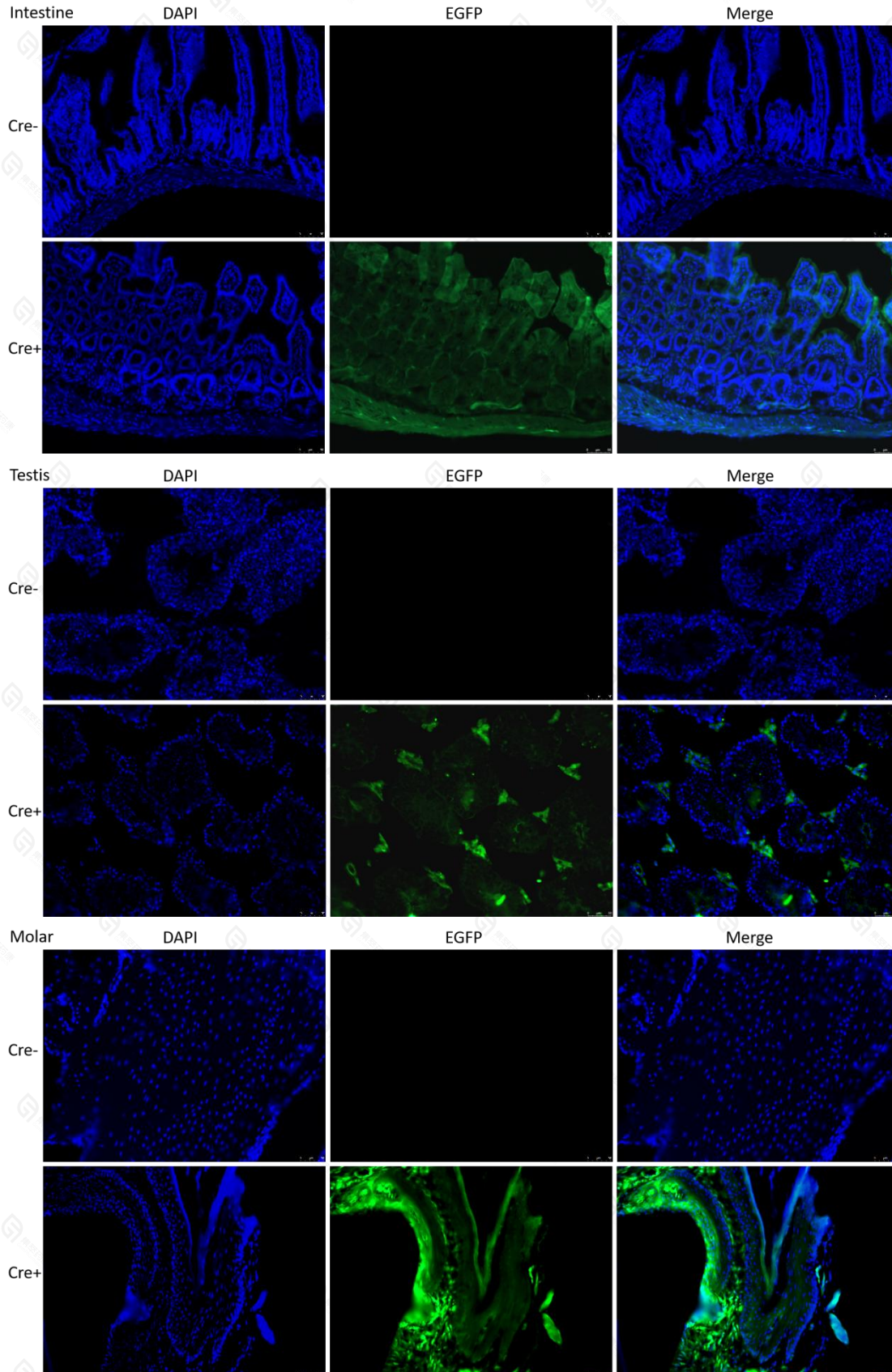
1. Validation methods & notes

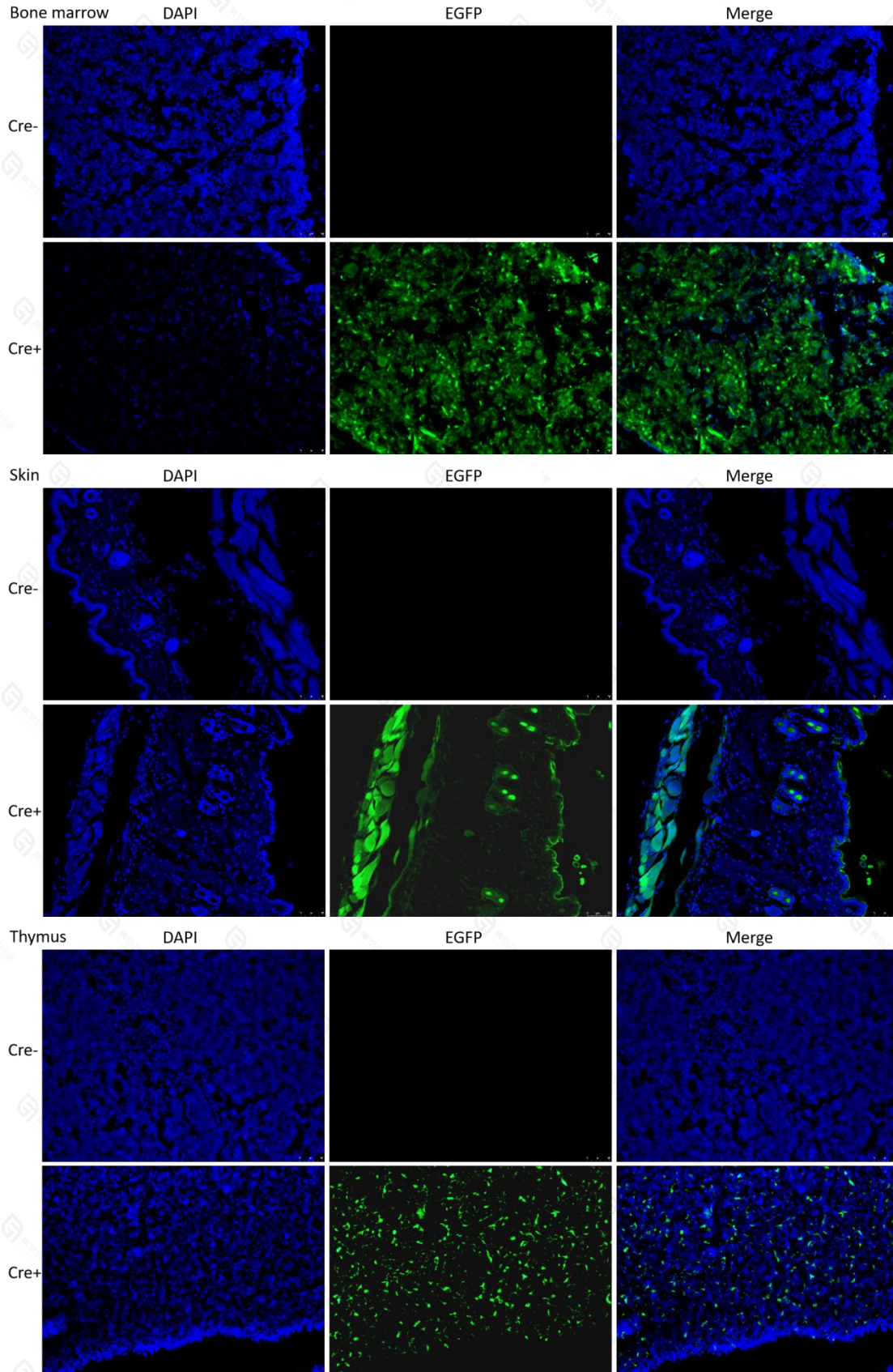
Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA mice was crossed with TgTn(pb-CAG-iCre) mice with ubiquitous expression, Cre-mediated recombination will lead to excision of the stop cassette and expression of EGFP. Fluorescence imaging of frozen sections were performed to exhibit expression of EGFP in various tissues and organs. Imaging of sections were performed under a 200x microscopy.

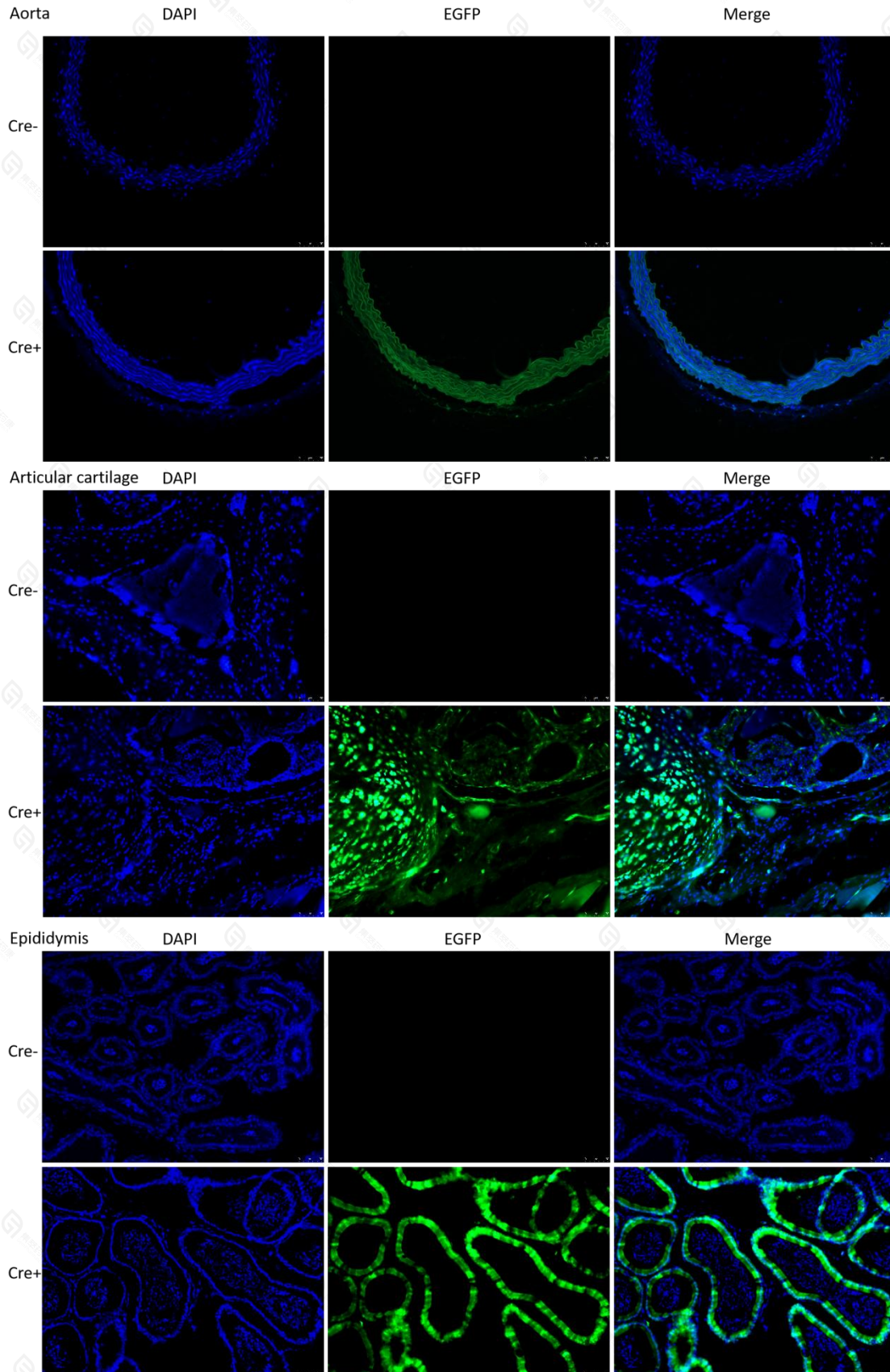
2. Images of tissues and organs with obvious EGFP expression

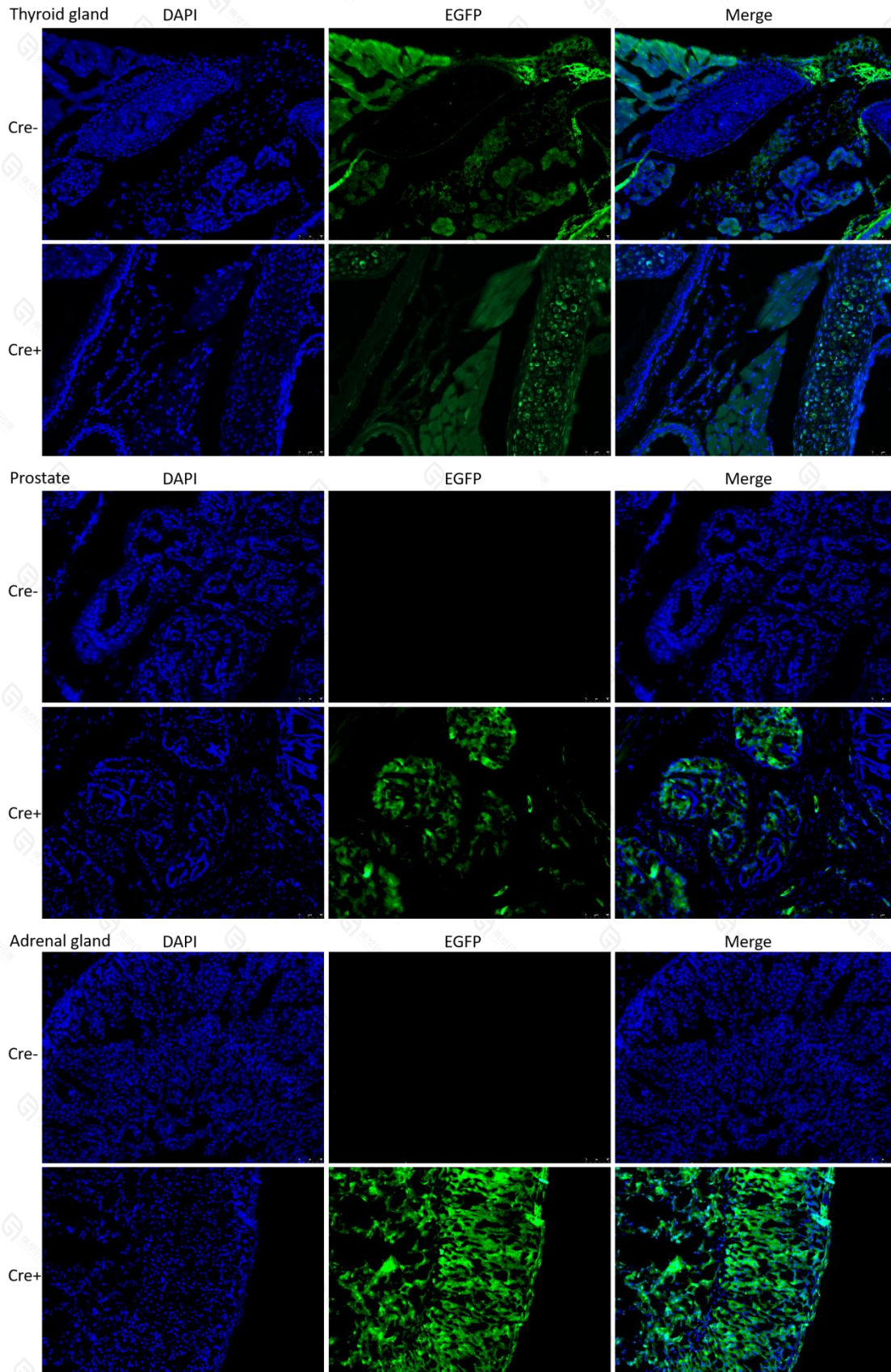


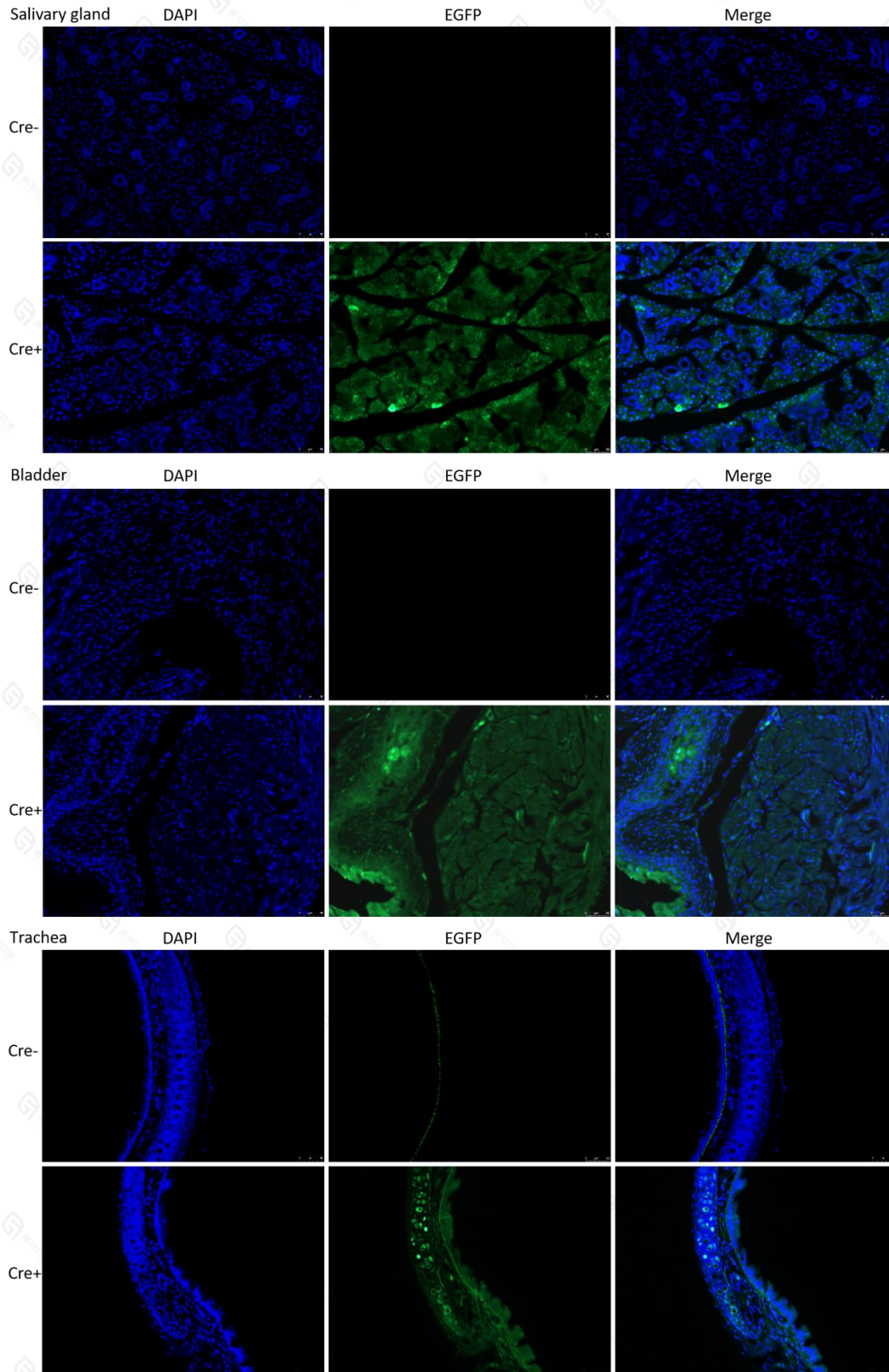


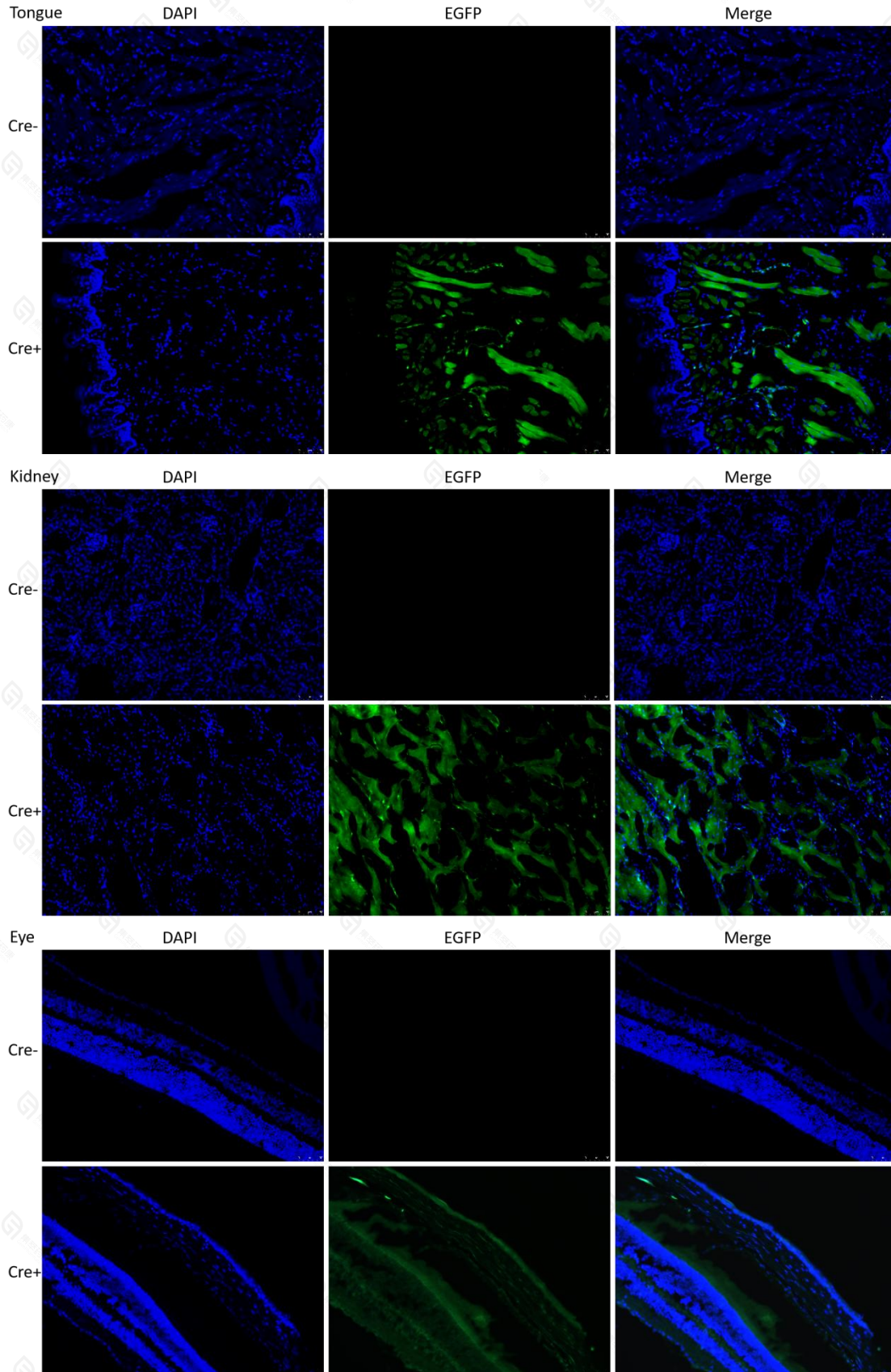


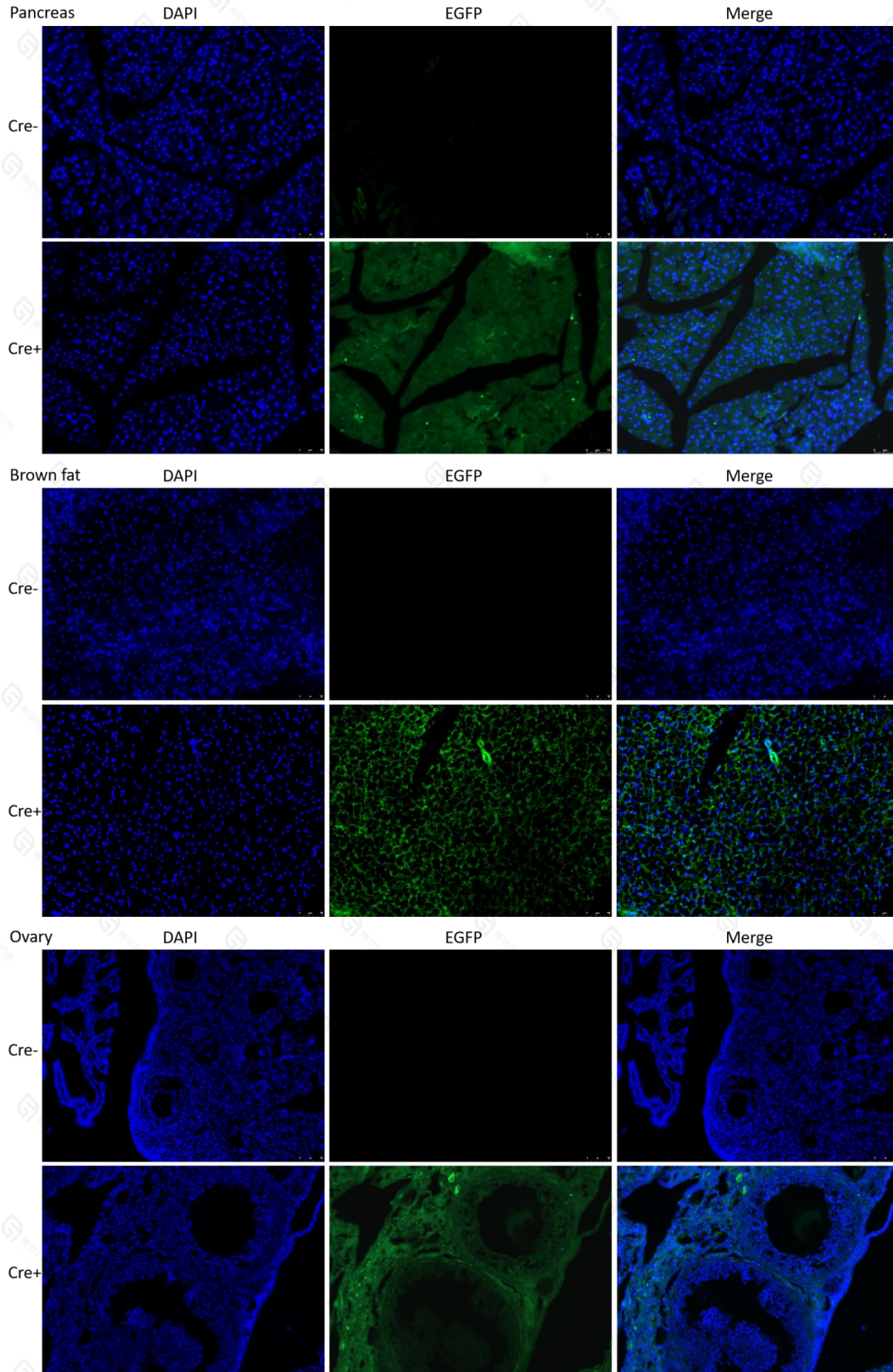












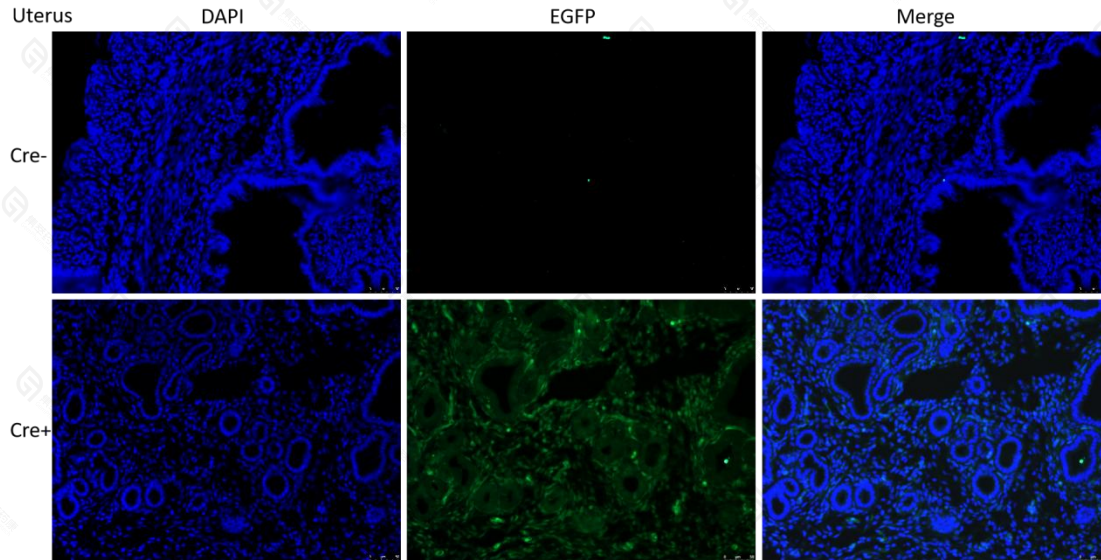
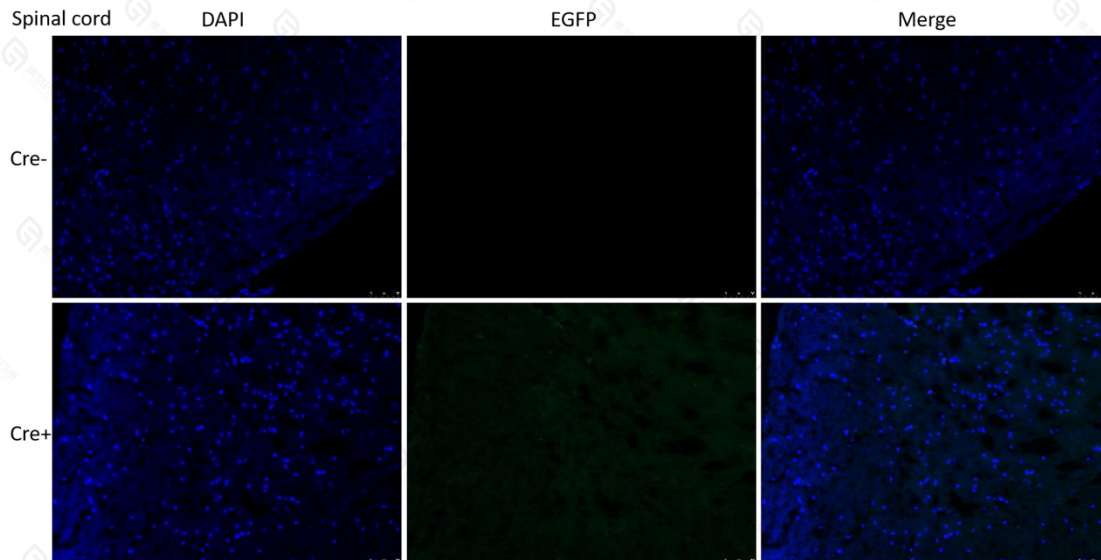


Fig 2. Fluorescence imaging of tissues and organs with obvious EGFP expression.

Organs were indicated in the left top of each subfigure group. Cre-: Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA single positive individuals; Cre+: TgTn(pb-CAG-iCre), Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA double positive individuals.

3. Images of tissues and organs with little or no EGFP expression



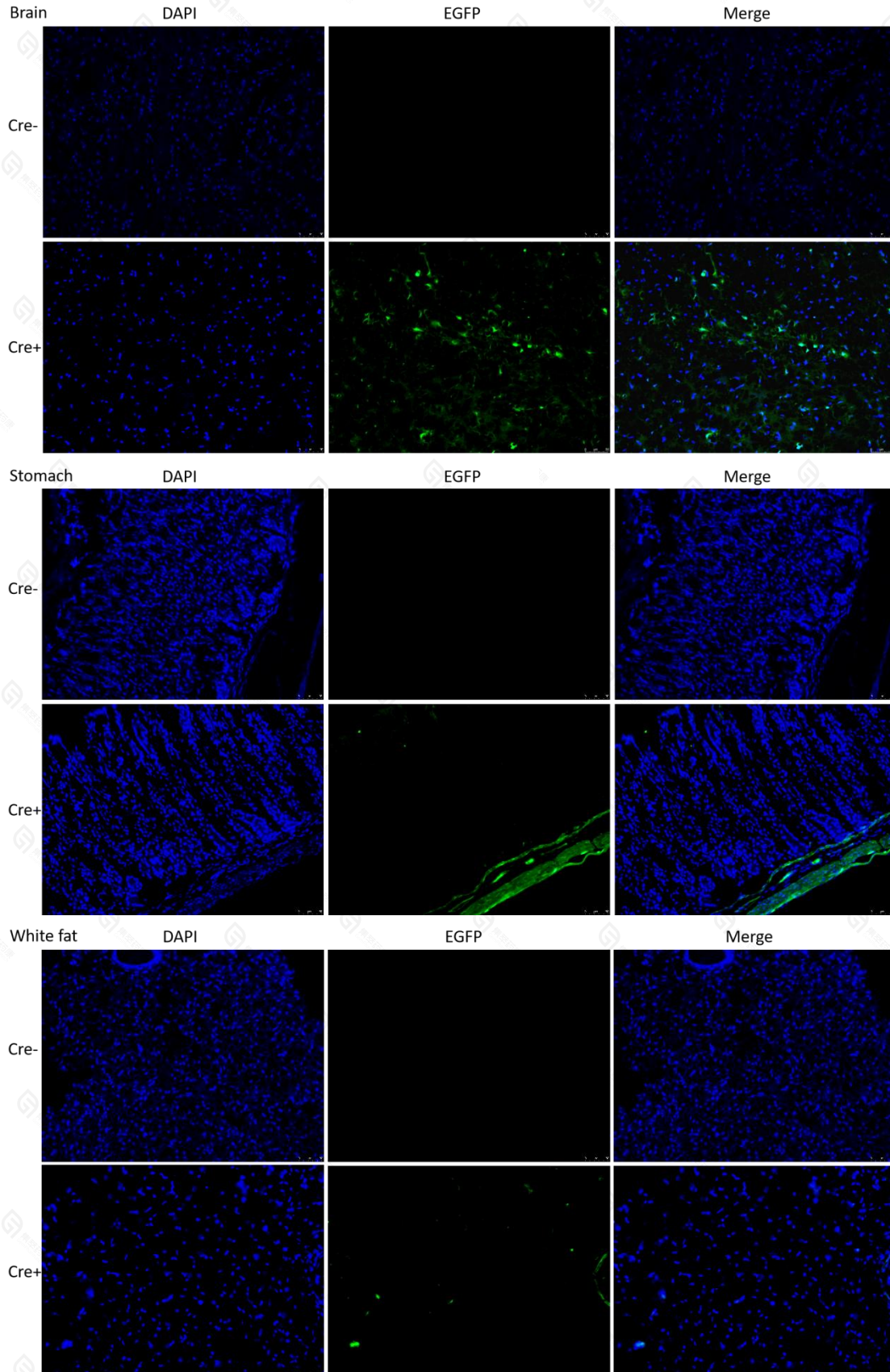


Fig 3. Fluorescence imaging of tissues and organs with little or no EGFP expression.

Organs were indicated in the left top of each subfigure group. Cre-: Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA single positive individuals; Cre+: TgTn(pb-CAG-iCre), Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA double positive individuals.

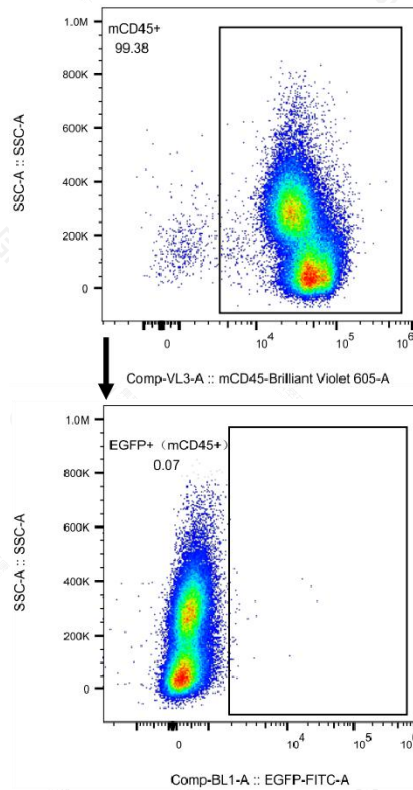
4. Gating Strategies for flow Cytometry

Cell population		Gating		
Leukocytes	mCD45+			
Total T cells	mCD45+	mCD3+mCD335-		
Natural killer cells	mCD45+	mCD3-mCD335+		
B lymphocytes	mCD45+	mCD3+mCD335-	mCD19+	
mCD4+ T cells	mCD45+	mCD3+mCD335-	mCD4+ mCD8-	
mCD8+ T cells	mCD45+	mCD3+mCD335-	mCD4- mCD8+	
Treg cells	mCD45+	mCD3+mCD335-	mCD4+mCD8-	mCD25+
Neutrophils	mCD45+	mCD11b+mLy6G+		
Monocytes	Not Neutrophils	mCD11b+mLy6C hi		
Eosinophils	Not Monocytes	mCD11b+SSC-H hi		
Macrophages	Not Eosinophils	mCD11b+mF4/80+		
Dendritic cells	Not Eosinophils	mCD11b+mCD11c+		
Platelets	mCD41+			

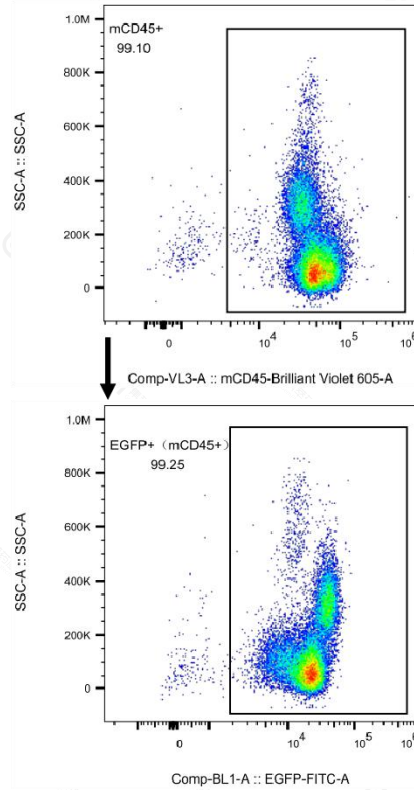
Table 1. Gating Strategies for flow Cytometry of Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA mice.

5. Flow cytometry analysis of cells with EGFP expression

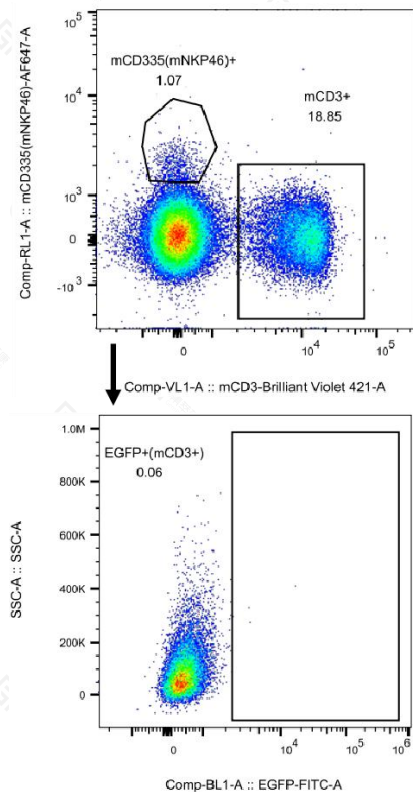
Blood: mCD45+ cells
Cre-



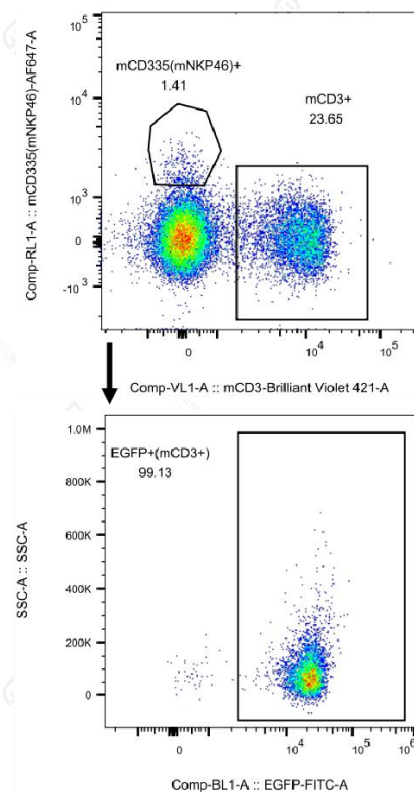
Cre+



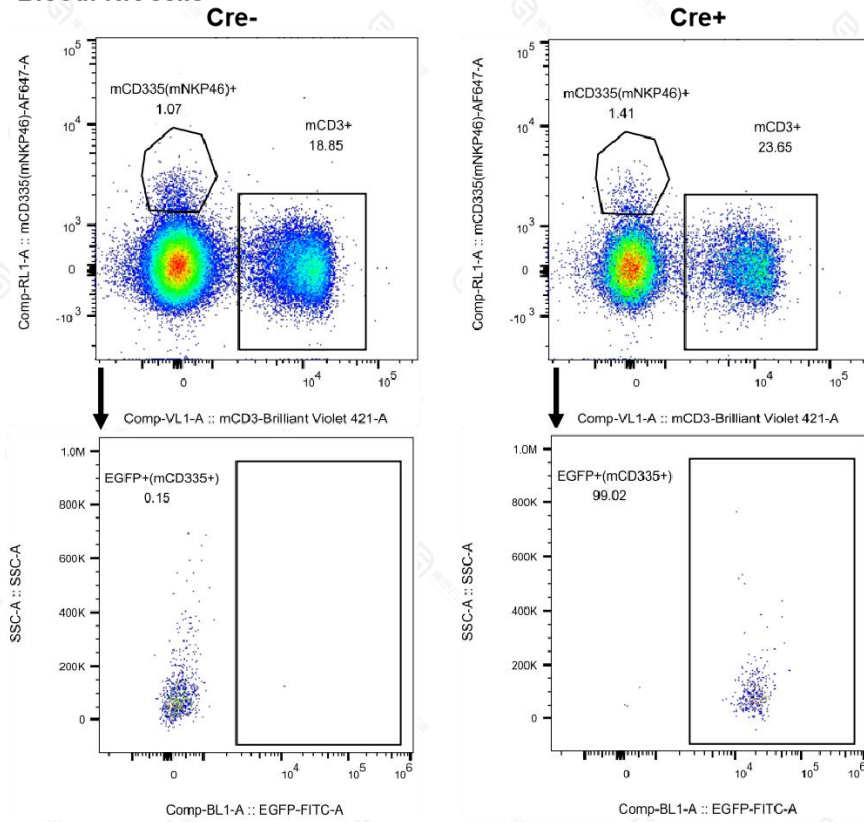
Blood: mCD3+ cells
Cre-



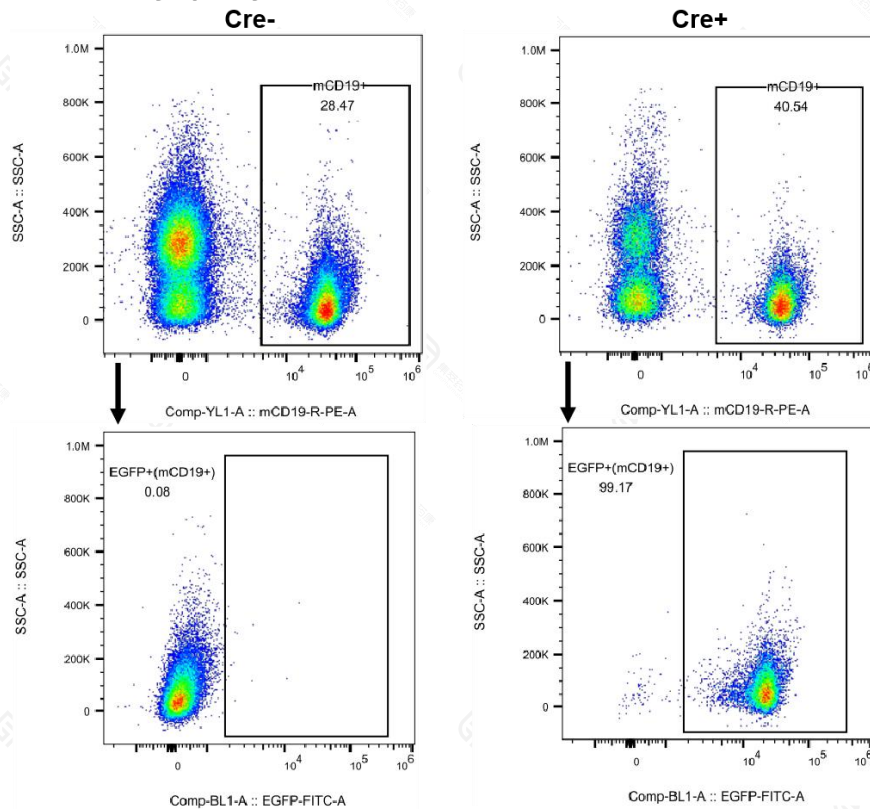
Cre+



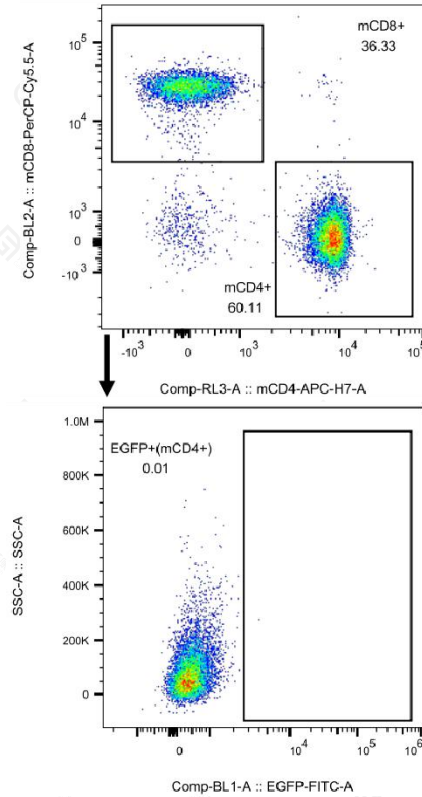
Blood: NK cells



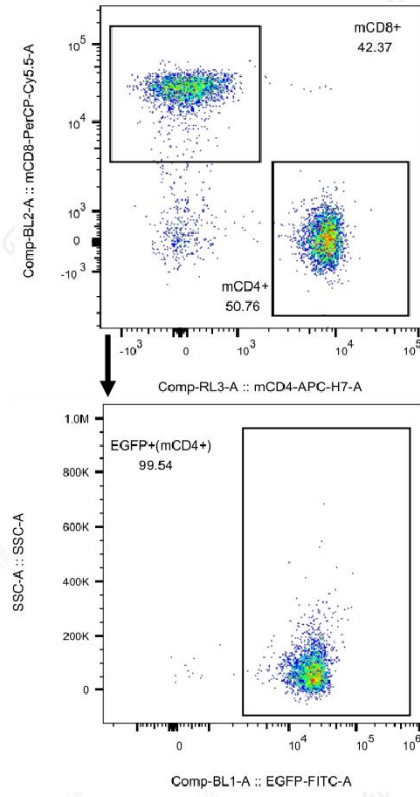
Blood: B lymphocytes



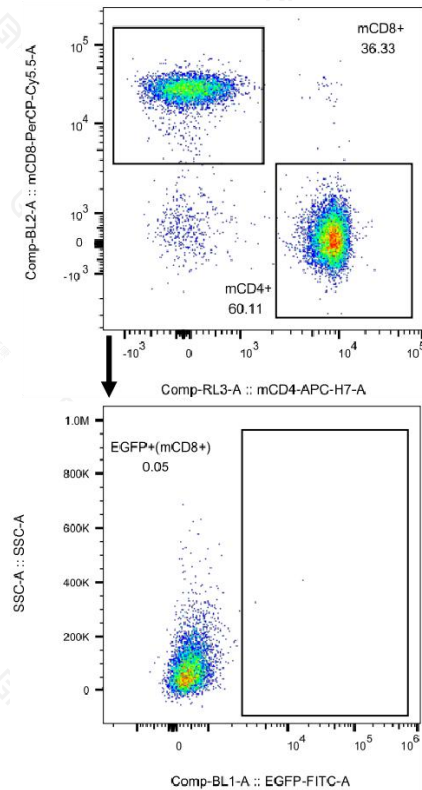
Blood: mCD4+ T cells
Cre-



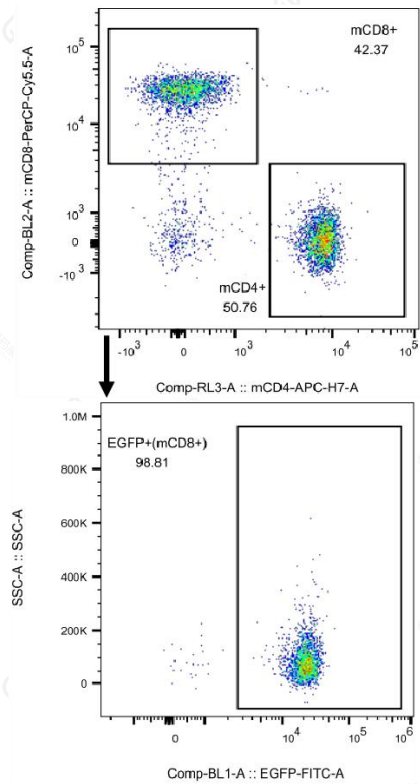
Cre+



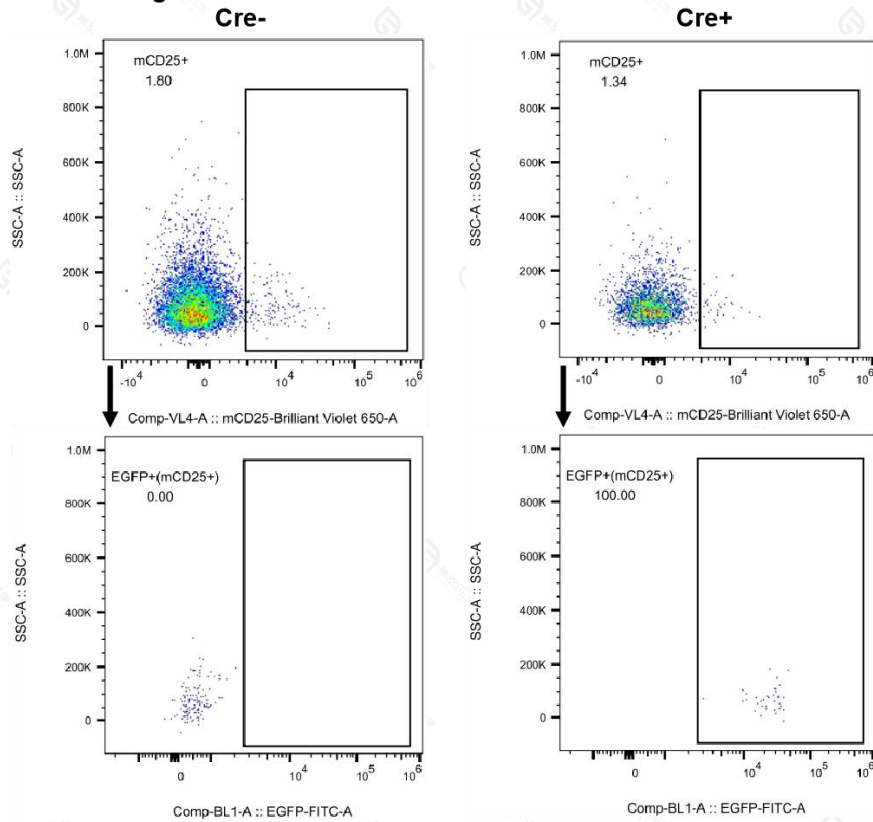
Blood: mCD8+ T cells
Cre-



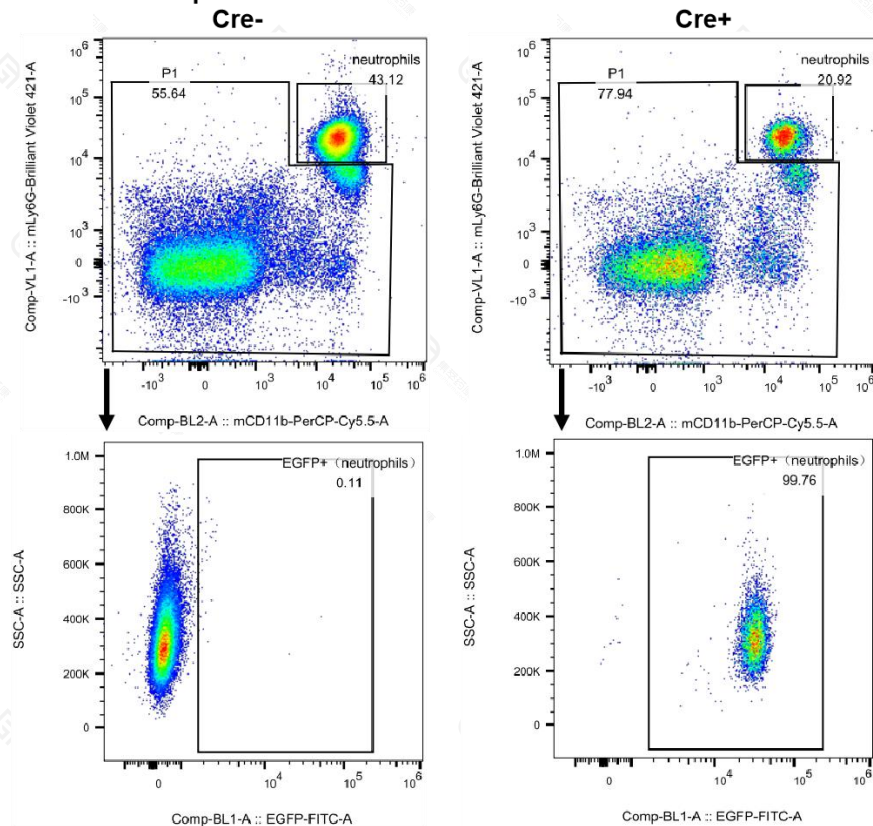
Cre+



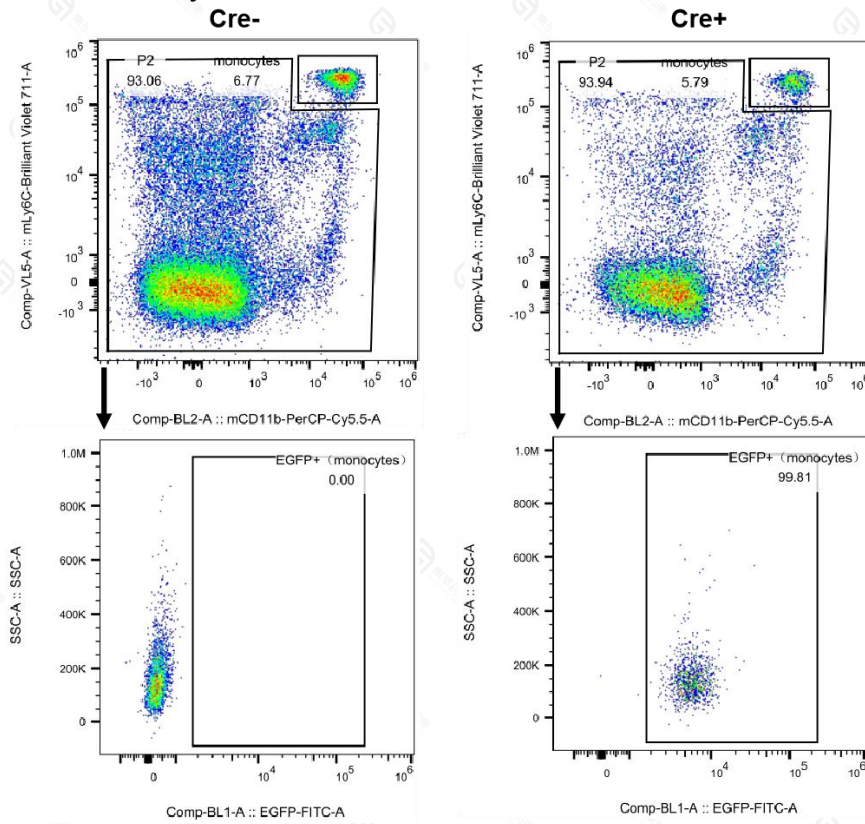
Blood: Treg cells



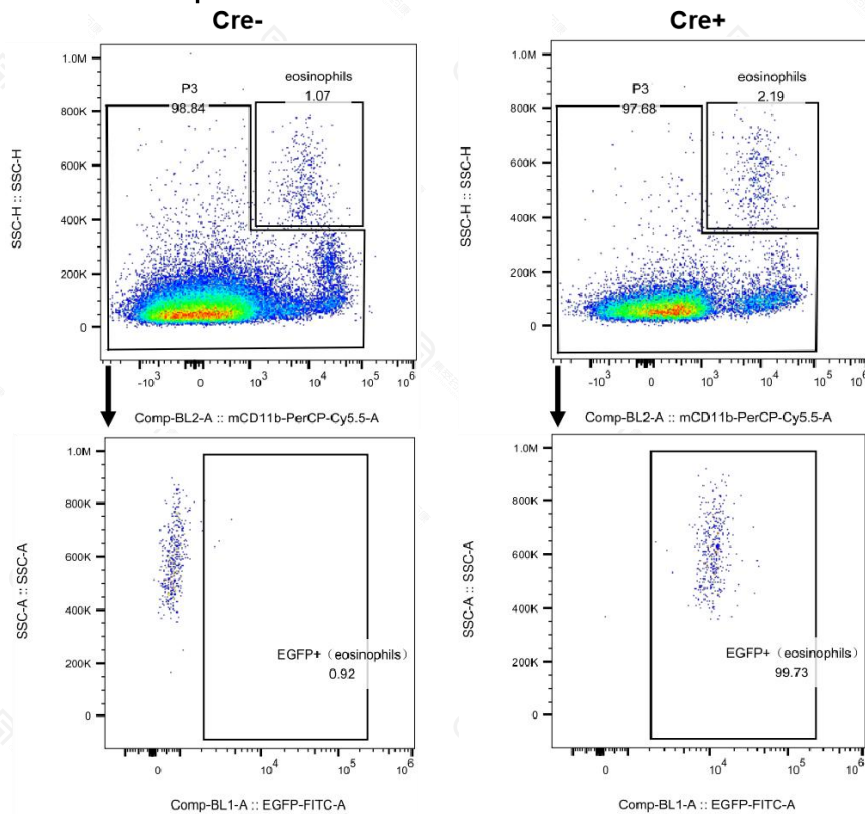
Blood: Neutrophils



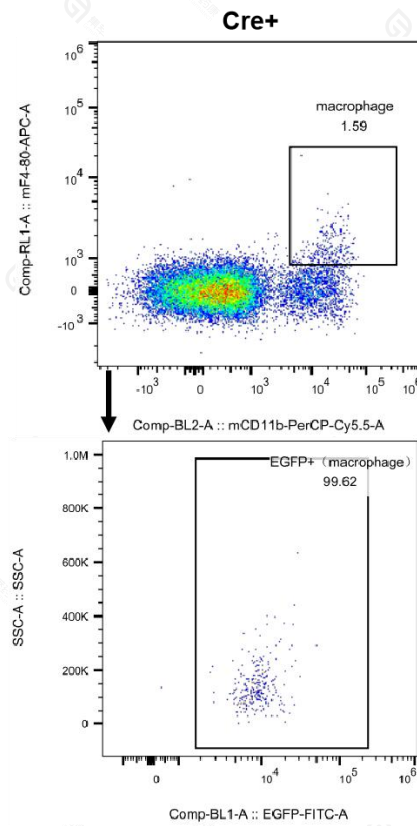
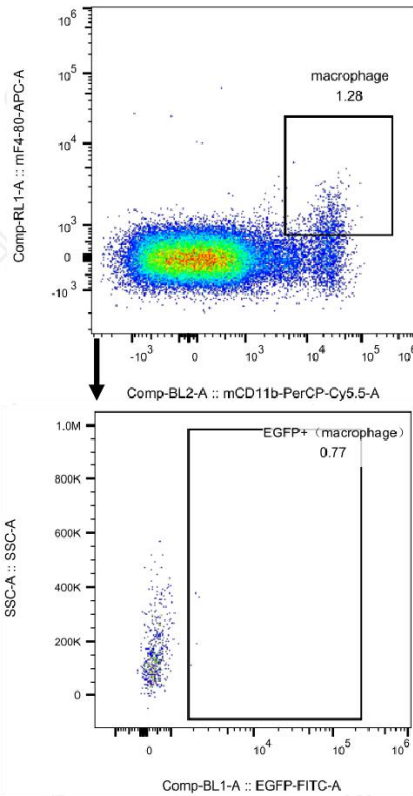
Blood: Monocytes



Blood: Eosinophils



Blood: Macrophages



Blood: Dendritic cells

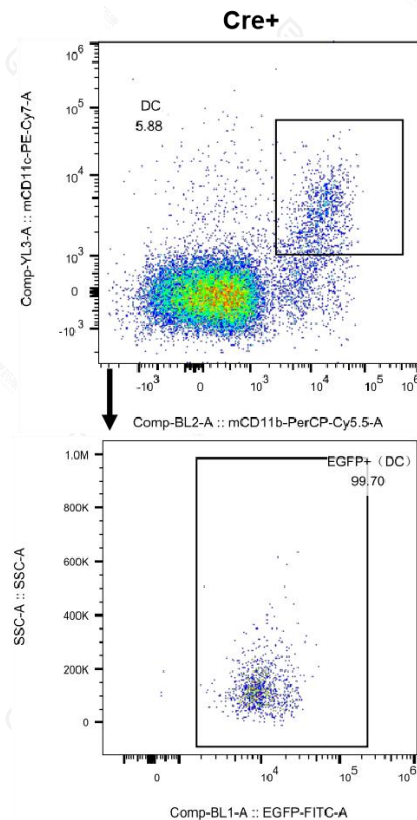
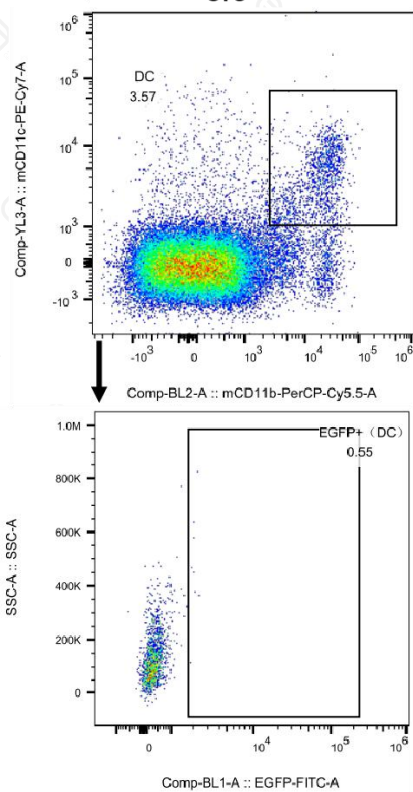


Fig 4. Flow cytometry analysis of cells with EGPF expression

Organs were indicated in the left top of each subfigure group. Cre-: Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA single positive individuals; Cre+: TgTn(pb-CAG-iCre), Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA double positive individuals. Whole blood cells were harvested from Cre- and Cre+ mice and analyzed for EGFP expression with flow cytometry.

5. Flow cytometry analysis of cells with little or no EGPF expression

Blood: Platelets

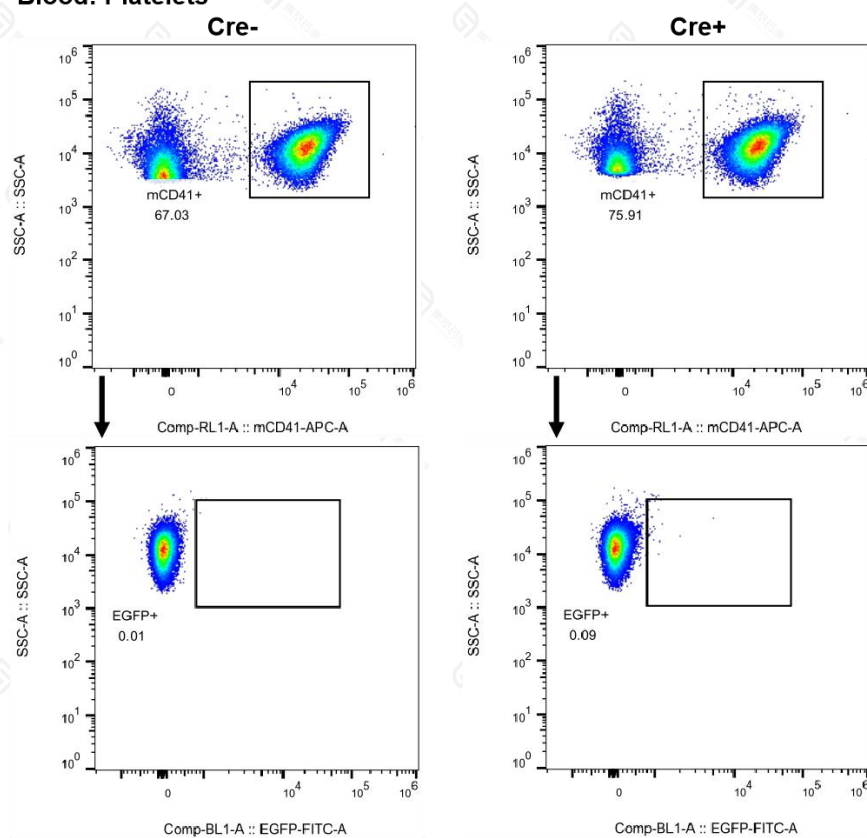


Fig 5. Flow cytometry analysis of cells with little or no EGPF expression

Cre-: Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA single positive individuals; Cre+: TgTn(pb-CAG-iCre), Rosa26-CAG-LSL-EGFP-3xHA-WPRE-polyA double positive individuals. Whole blood cells were harvested from Cre- and Cre+ mice and analyzed for EGFP 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.