A Split-Cre system designed to detect simultaneous expression of two genes based on SpyTag/SpyCatcher conjugation and Split-GFP dimerization

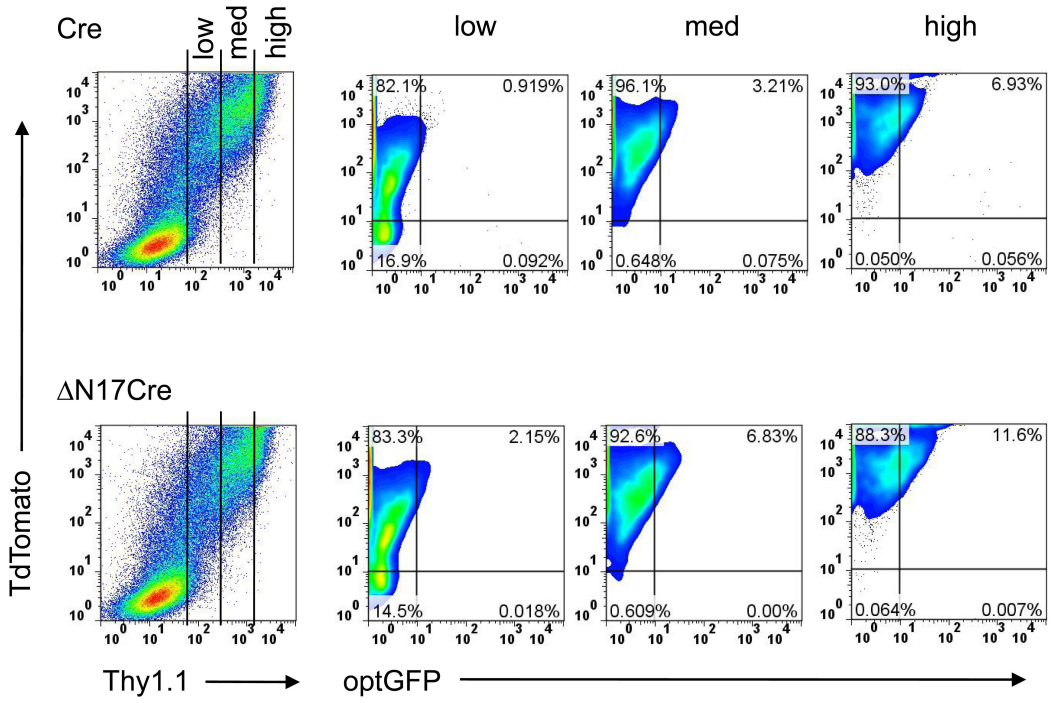
Xundong Wei[1,2,#], Jianhua Zhang[2,3,#], Jian Cui[4], Wei Xu[2,3], Xuyu Zhou[2,3,*], Jie Ma[1,2,*]

List of Supporting Material

Figure S1-S3

A

	Report vector	pEGFP-N1
1	CAG-LSL-TdTomato	Cre
2	pMSCV-IRES-Thy1.1	ΔN17Cre



B

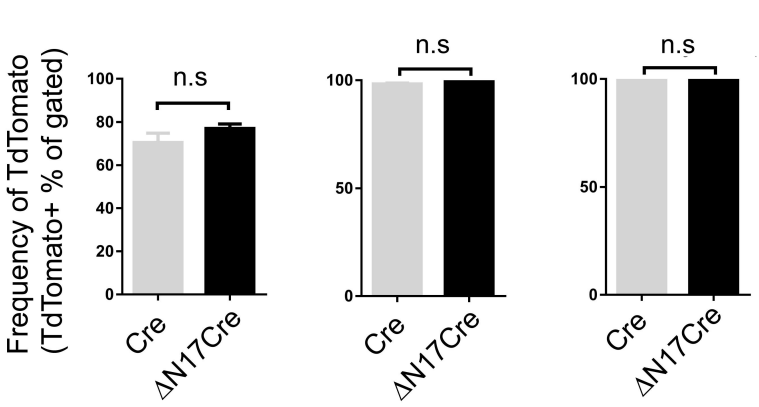


Figure S1. Analysis of the recombinase activity of ΔN17Cre

A, Analysis of recombinase activities by transient transfection. HEK293T cells were transiently transfected with the Cre or N17Cre constructs, CAG-loxp-STOP-loxp-TdTomato, and MSCV-Thy1.1. The expression of Thy1.1 on the cell surface was used as an indicator of intracellular expression of transfected genes. The

cells were harvested 20 h after transfection, and cells with different Cre expression levels as indicated by the Thy1.1 expression level were analyzed by flow cytometry.

B, The average frequencies of TdTomato^{+} cells among cells with different Cre expression levels (Thy1.1 low, medium, high) in **A** are shown.

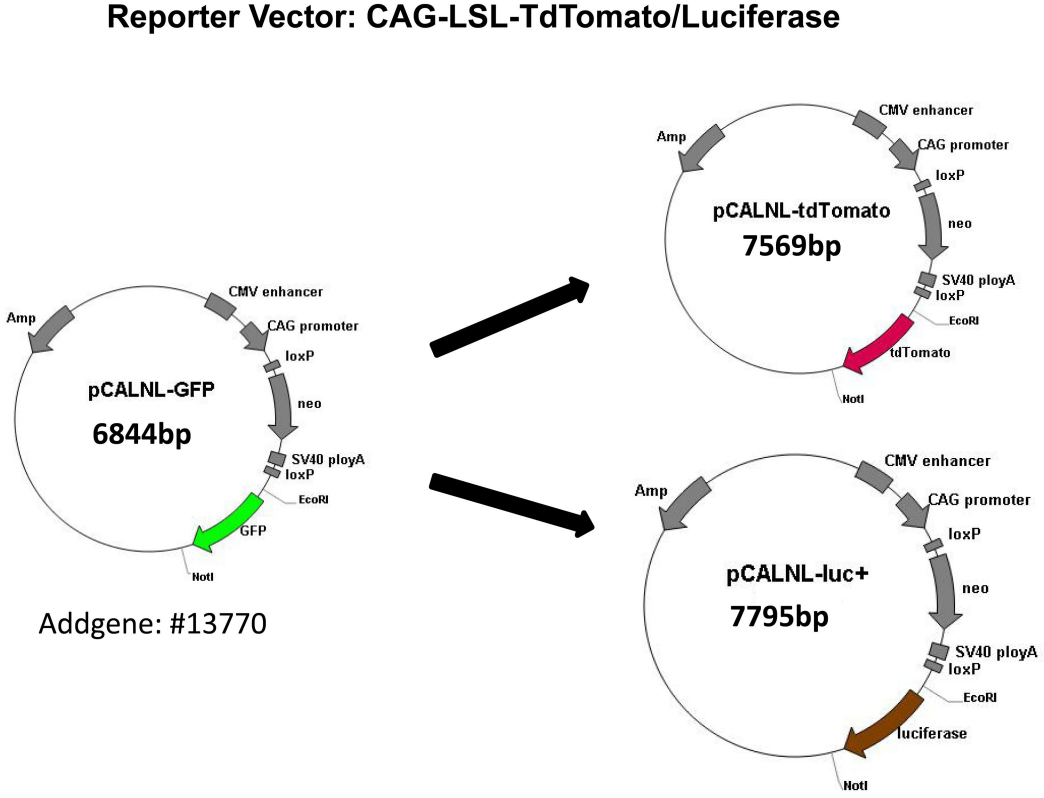


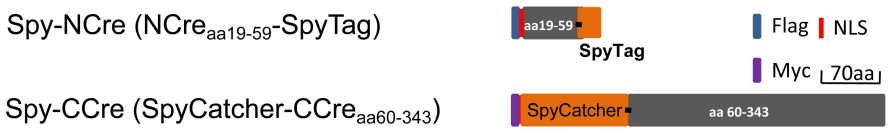
Figure S2. Cre recombinase-dependent expression of TdTomato or Luciferase

The genes encoding TdTomato or luciferase were cloned into the *Eco*R I/*Not* I sites of pCALNL-GFP (Addgene: #13770) after deletion of the GFP gene. TdTomato or Luciferase is only expressed in the presence of Cre due to the transcriptional stop between the *loxP* sites.

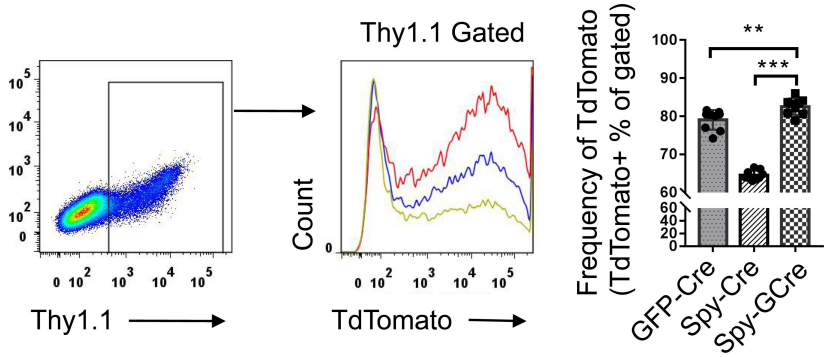
A

	Report vector	pMSCV-IRES-Thy1.1	pMSCV-IRES-Thy1.1	
1	CAG-LSL-TdTomato	NGFP-NCre	CGFP-CCre	GFP-Cre
2		Spy-NCre	Spy-CCre	Spy-Cre
3		Spy-GNCre	Spy-GCCre	Spy-GCre

B



C



D

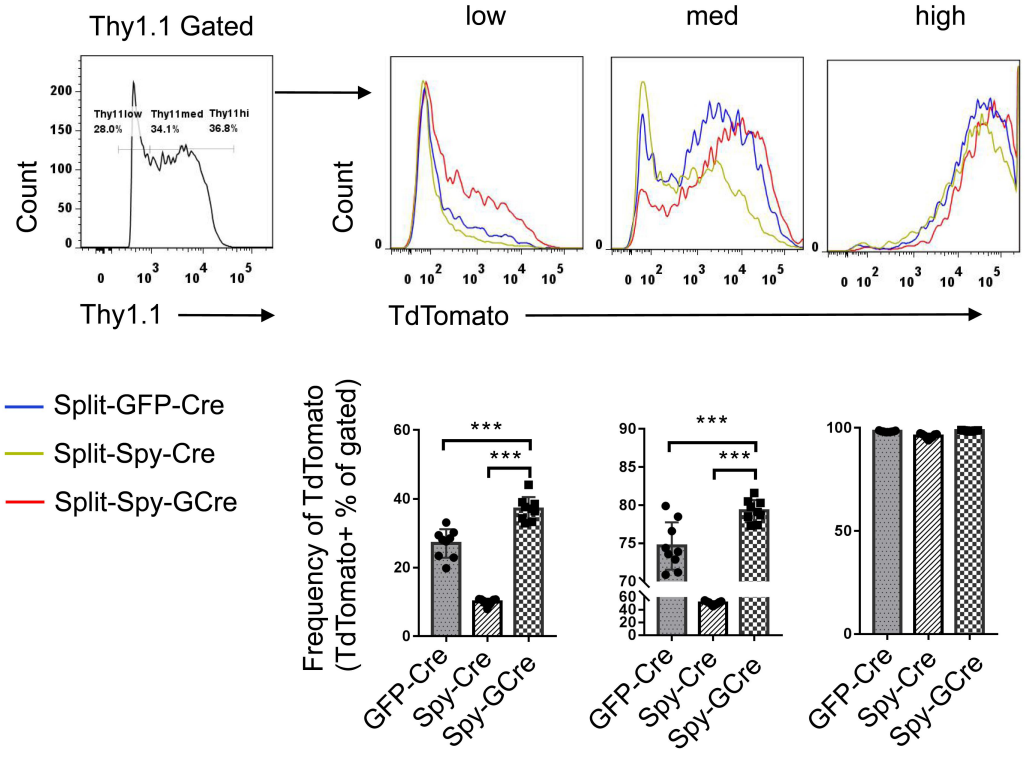


Figure S3. SpyTag/SpyCatcher-assisted complementation of NCre/CCre

A, List of plasmids used in this experiment.

B, Schematic representation of the structures of the Spy-NCre ($NCre_{aa19-59}$-SpyTag) and Spy-CCre (SpyCatcher-$CCre_{aa60-343}$) fusion proteins in this study. All genes were inserted into the MSCV-Thy1.1 expression reporter vector.

C, Comparison of the level of Cre recombinase activity following the transient transfection of different complementary pairs of Cre fragments. HEK293T cells were transiently transfected with the components of the Split-GFP-Cre, Split-Spy-Cre, or Split-Spy-GCre system. All constructs were first inserted into the MSCV-Thy1.1 expression reporter vector. The CAG-loxp-STOP-loxp-TdTomato reporter was used to visualize Cre reconstitution efficiency. The cells were harvested 20 h after transfection and analyzed by flow cytometry according to Thy1.1 expression. The average frequencies of $TdTomato^+$ cells among $Thy1.1^+$ cells are shown.

D, Cells with different Cre expression levels as indicated by the Thy1.1. expression level were analyzed by flow cytometry. The average frequencies of $TdTomato^+$ cells among cells with different Cre expression levels (Thy1.1 low, medium, high) are shown.

The data shown are typical results from 2 experiments. The small horizontal bars indicate the mean ± SEM. ns, not significant, $^{**}p < 0.01$, and $^{***}p < 0.001$ (ANOVA with Bonferroni post-test)