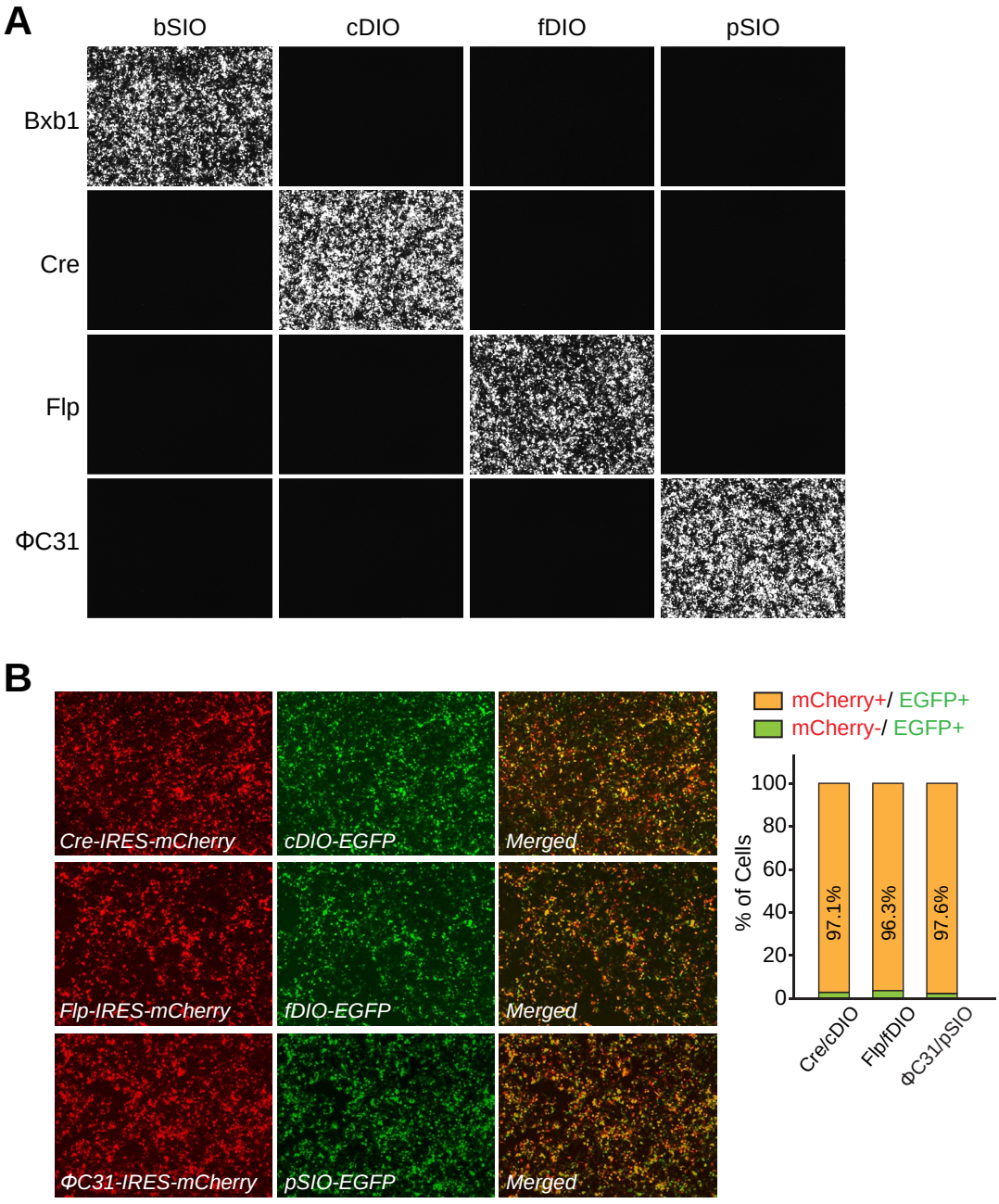
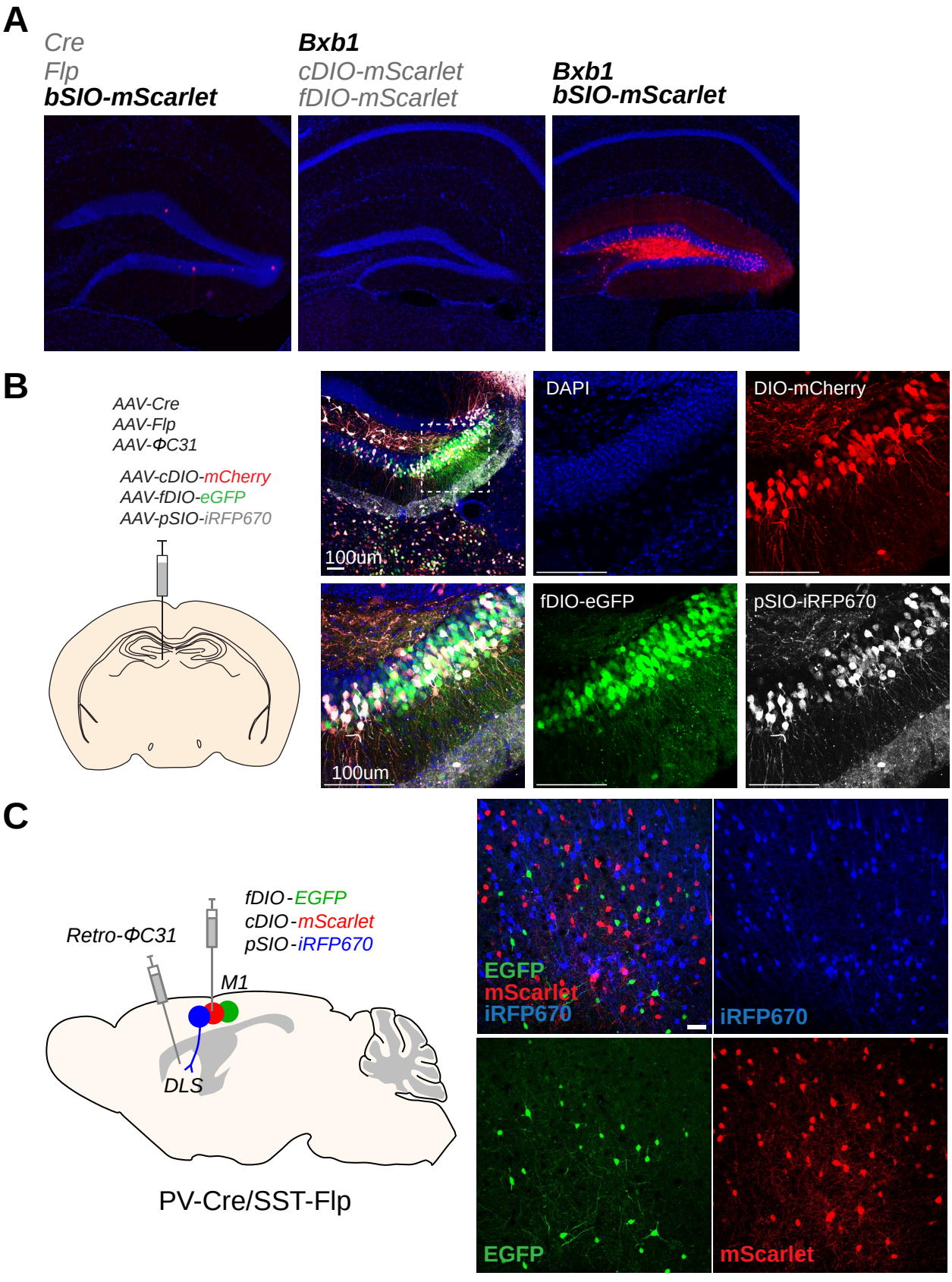


Supplementary Figure. 1

Supplementary Fig. 1. The efficiency and cross-reactivity for the Cre, Flp, ΦC31, and Bxb1 recombinase systems in HEK293T cells, related to Figure 1D.

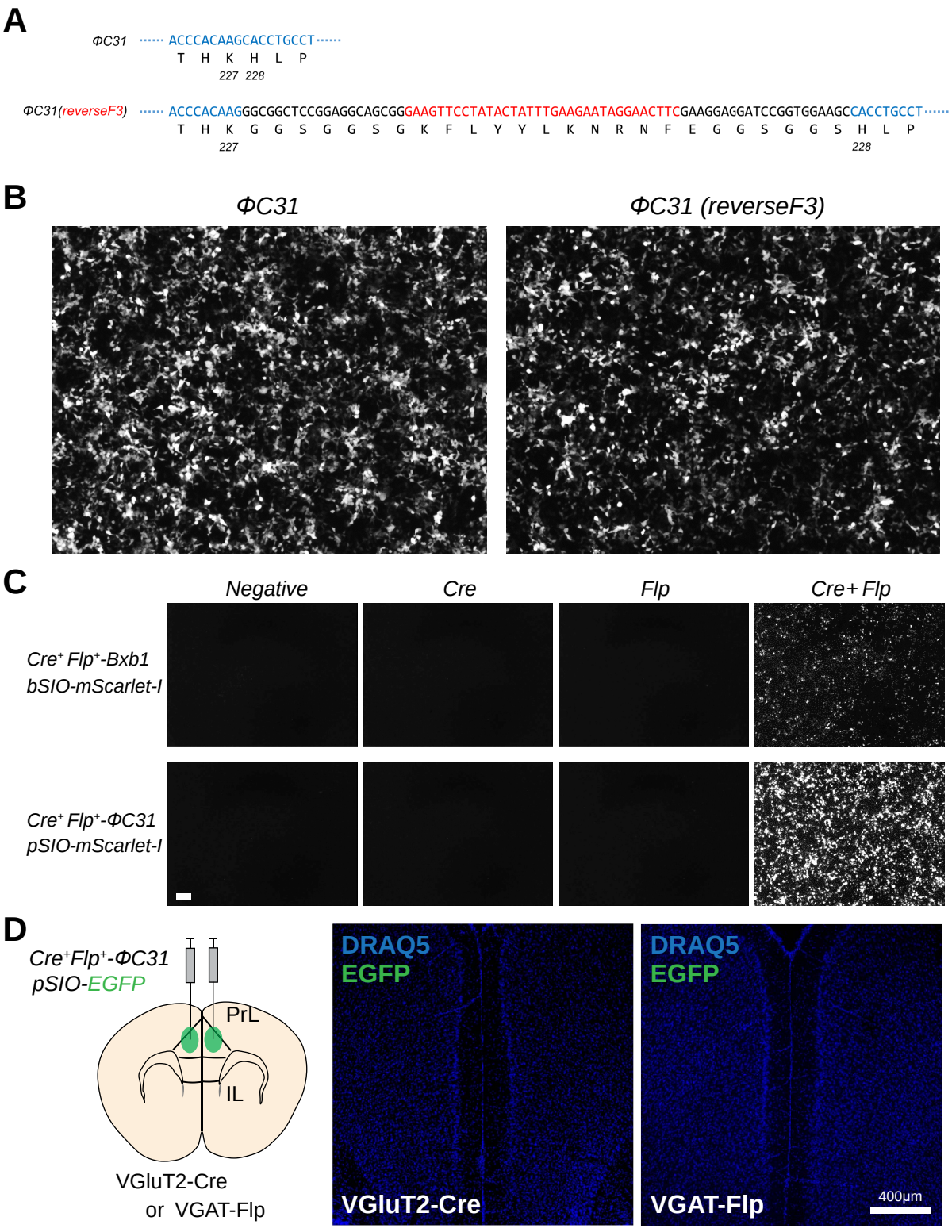
A, Specificity and efficiency of transgenes expression by bSIO, cDIO, fDIO, and pSIO vectors. Either one of the transgenes (mScarlet-I) expressing plasmids and either one of the recombinases expressing plasmid were co-transfected in HEK293T cells. **B,** By co-transfecting a plasmid encoding recombinase and mCherry (Cre-IRES-mCherry, Flp-IRES-mCherry, or ΦC31-IRES-mCherry) along with a plasmid producing EGFP in a recombinase-dependent manner (cDIO-EGFP, fDIO-EGFP, or pSIO-EGFP), we were able to examine the specificity and effectiveness for each particular recombinase-reporter pair. The most of cells that express EGFP also express mCherry for all Cre-, Flp-, and ΦC31 pair, indicating that the ΦC31-pSIO system exhibits specific recombinase-dependent expression similar to that of Cre and Flp recombinase.



Supplementary Figure. 2

Supplementary Fig. 2. The efficiency and cross-reactivity for the Cre, Flp, ΦC31, and Bxb1 recombinase systems *in vivo*, related to Figure 1E-G.

A, Specificity and efficiency of transgenes expression by Bxb1/bSIO system in vivo. Combinations of AAVs as indicated were co-injected in the DG. Leaky expression from AAV-bSIO-mScarlet-I alone was observed. **B,** The injection of Cre-cDIO, Flp-fDIO, ΦC31-pSIO pairs into hippocampal areas. A similar amount of viral particle for each pair was injected. The result shows that a similar number of neurons are labeled with different colors, suggesting that recombinase combinations do not have significant competing or interfering effects when used together *in vivo*. **C**, Usage of cDIO, fDIO, and pSIO in order to express three different transgenes in three different populations defined by promoter activities or anatomical connectivity. AAV-EF1α-fDIO-EGFP, AAV-EF1α-cDIO-mScarlet-I, and AAV-EF1α-pSIO-iRFP670 were co-injected into M1, and retroAAV-ΦC31 were injected into DLS of PV-Cre/SST-Flp transgenic mice.

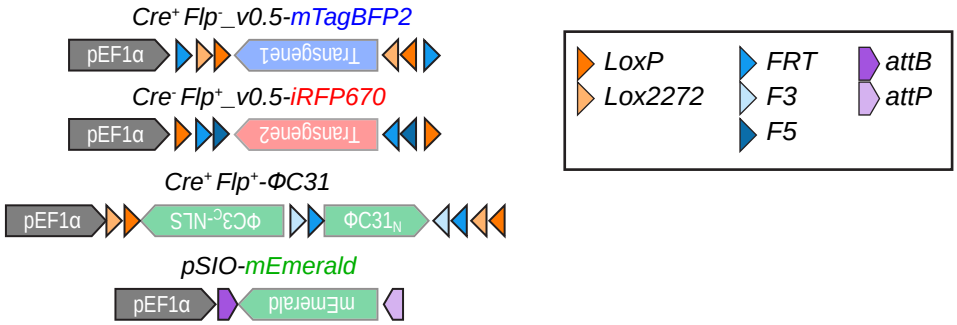


Supplementary Figure. 3

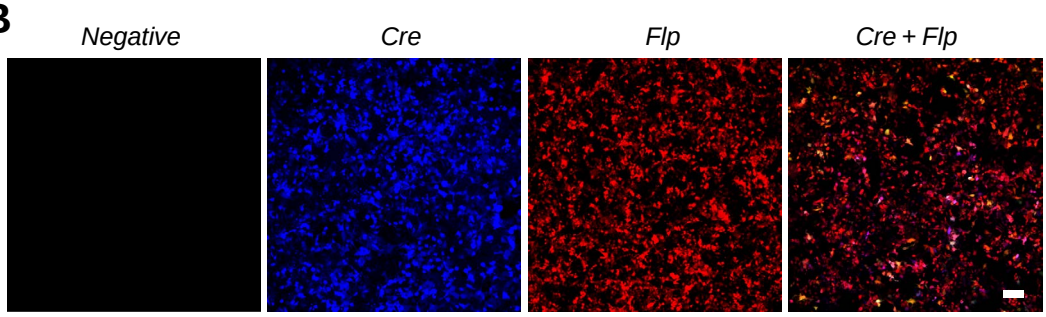
Supplementary Fig. 3. The efficiency and specificity of ΦC31-dependent intersectional expression in cells expressing both Cre and Flp recombinase, related to Figure 5.

A, Insertion site of the FRT variant (F3) in the ΦC31. The numbers indicate the resides in intact ΦC31.
B, Comparison of the efficiency of the intact ΦC31 and ΦC31 with FRT variant insertion. Either one of the recombinases expressing vector were co-transfected in HEK293T cells together with pSIO-Scarlett-I plasmid. **C,** Specificity and efficiency of $Cre^{+}Flp^{+}$-ΦC31 and $Cre^{+}Flp^{+}$-Bxb1 in HEK293T cells. Upper panels: $Cre^{+}Flp^{+}$-Bxb1, bSIO-mScarlet-I, and Cre and/or Flp expressing plasmids were cotransfected. Lower panels: $Cre^{+}Flp^{+}$-ΦC31, pSIO-mScarlet-I, and Cre and/or Flp expressing plasmids were co-transfected. **D.** The injection of Cre+Flp+-ΦC31, pSIO-EGFP into the prelimbic cortex (PrL) of VGluT1-Cre or VGAT-Flp (left). Four weeks after the injection, we did not observe any EGFP-positive neurons in the injected area, suggesting very low level of leaky expression with Cre+Flp+-ΦC31 and pSIO combination.

A



B



C

Cre

	Palindromic armL	Spacer	Palindromic armR
LoxP	ATAACTTCGTATA	GCATACAT	TATACGAAGTTAT
Lox2272	ATAACTTCGTATA	GGATACTT	TATACGAAGTTAT
M3	ATAACTTCGTATA	TAATACCA	TATACGAAGTTAT
M7	ATAACTTCGTATA	AGATAGAA	TATACGAAGTTAT
FAS	ATAACTTCGTATA	TACCTTTC	TATACGAAGTTAT

Flp

	Palindromic armL	Spacer	Palindromic armR
FRT	GAAGTTCCTATTC	TCTAGAAA	GTATAGGAACTTC
F5	GAAGTTCCTATTC	TTCAAAAG	GTATAGGAACTTC
F2	GAAGTTCCTATTC	TCTACTTA	GTATAGGAACTTC
F3	GAAGTTCCTATTC	TTCAAATA	GTATAGGAACTTC
F10	GAAGTTCCTATTC	ACTAGAAT	GTATAGGAACTTC
F15	GAAGTTCCTATTC	TTATAGGA	GTATAGGAACTTC

Supplementary Figure. 4

Supplementary Fig. 4. Schematic diagram of the composition of Cre+/Flp+, Cre+/Flp-, and Cre-/Flp+ vectors, related to Figure 6.

A, Design of $Cre^{+}Flp^{-}$, $Cre^{-}Flp^{+}$, and $Cre^{+}Flp^{+}$ vectors with overlapping target sites among the vectors. **B,** Expression of three transgenes in cells expressing a different combination of recombinases. $Cre^{+}Flp^{-}$_v0.5-mTagBFP, $Cre^{-}Flp^{+}$_v0.5-iRFP670, $Cre^{+}Flp^{+}$-ΦC31, pSIO-mEmerald, and Cre and/or Flp-expressing plasmids were co-transfected in HEK293T cells. The iRFP670 signal is pseudocolored in red. Scale bar = 50 μm. **C,** The sequence information for the different recognition sites for Cre- and Flp recombinases used in this work.