Supplementary Figure legends:

Supplementary Figure 1:

Post-stroke neurogenesis and Ascl1creERT2::tdTomato line in stroke

(A) Low magnification confocal micrograph of whole brain coronal section stained with DCX and GFAP 14 days after stroke. The peri-infarct region is outlined by the dashed line. Scale bar: 500 μm. **(B)** High magnification confocal micrographs of DCX+ and Ki67+ cells in the peri-infarct cortex 14 days after stroke. **(C)** In the absence of CreERT2 expression, no tdTomato expression was induced by Tamoxifen (left). In the presence of CreERT2 expression, Tamoxifen administration for 5 days induced a robust and specific expression of tdTomato in the SVZ and rostral migratory stream (RMS). **(D)** High magnification confocal micrographs of tdTomato+ cells in the SVZ and RMS. tdTomato+ cells co-express the progenitor marker Ki67 and neuroblast marker DCX. **(E)** Confocal micrographs of tdTomato+ and GFP+ cells in the SVZ, 21 days after virus injection. Scale bar: 20 um (B) Confocal micrographs of tdTomato+ and GFP+ cells in the OB, 21 days after virus injection. Scale bar: 5 μm. **(G,H)** Expression of Ascl1creERT2:: tdTomato cells at 3 days and 10 days after stroke. Tamoxifen was administered once per day for 5 consecutive days, starting from 2 days before stroke till 3 days after stroke. Confocal micrographs of tdTomato+ cells at 3 days **(G)** and 10 days **(H)** after stroke. Expression of tdTomato+ cells in the white matter (WM) and peri-infarct is shown in higher magnification (regions highlighted in box). Scale bar: 100μm in lower magnification, 20μm in higher magnification.

Supplementary Figure 2:

Viral labeling of SVZ progenitors using the lentivirus EF1a-FLEXEGFP in the Ascl1creERT2:: tdTomato animals

(A-D) Cre-dependent lentivirus expressing the EF1a-FLEXEGFP was injected into the lateral ventricle of Ascl1-CreERT2 mice. 7 days after injection, Tamoxifen was administered once per day for 5 consecutive days starting from 2 days before stroke till 3 days after stroke. **(A)** Cre-induced tdTomato from the germline and GFP from the lentivirus, 14 days after stroke in the SVZ or in the peri-infarct cortex. Scale bar: 10μm. **(B-C)** Low magnification images of SVZ-derived cells migrating from the SVZ to the peri-infarct cortex at 3 days **(B)** and 10 days **(C)** after stroke. Expression of EGFP+ cells in the white matter (WM) and peri-infarct is shown in higher magnification on the right of each row (regions highlighted in box). Scale bar: 100μm in lower magnification, 20μm in higher magnification. **(D)** Confocal micrograph of the viral injection site in the CTX, with higher magnification micrographs showing the injection regions highlighted with the boxes. Scale: 100μm in lower magnification, 50μm in higher magnification.

Supplementary Figure 3:

Neuronal marker expression of Ascl1creERT2:: tdTomato cells in the peri-infarct at 2 months after stroke

Ascl1creERT2:: tdTomato+ neurons expressed both the inhibitory neuronal marker GABA **(A)** and excitatory neuronal marker CAMKIIα **(B)** in the peri-infarct at 2 months after stroke. tdTomato+ cells co-labeled with either GABA or CAMKIIα are highlighted by white arrowheads. Scale bar: 10μm

Supplementary Figure 4:

Immediate early gene expression in the DREADD modulated cells

(A) Confocal micrographs of tdTomato+ and Zif268+ cells in the peri-infarct cortex of animals receiving CAMKII-Gq tdTomato injection in the peri-infarct region, with either saline or CNO treatment. Open arrowheads: TdTomato+Zif268- cells in (A) and EGFP+pERK- cells in (C)Scale bar: 5 μm **(B)** Percentage of tdTomato+ Zif268+ among all tdTomato+ cells in the peri-infarct, compared between the saline and CNO treatment group. **(C)** Confocal micrographs of EGFP+ and pERK+ cells in the peri-infarct cortex of animals receiving GFAP-Gi GFP injection in the peri-infarct region, with either saline or CNO treatment. Scale bar: 5 μm **(D)** Percentage of EGFP+ pERK+ among all EGFP+ cells in the peri-infarct, compared between the saline and CNO treatment group. Asterisk: p value < 0.05

Supplementary Figure 5:

Pre- and post-synaptic marker expression of Ascl1creERT2:: tdTomato cells in the peri-infarct at 2 months after stroke

(A) Confocal micrographs showing expression of presynaptic marker vGlut1 and post-synaptic marker Homer1 in the Ascl1creERT2:: tdTomato cells. Synaptic marker expression in the region highlighted with a box is shown with higher magnification. Scale bar: lower magnification: 10 μm, higher magnification: 5 μm. **(B)** BDA was injected into the pre-motor cortex 7 days before the Ascl1creERT2:: tdTomato animals were sacrificed at 2 months after stroke. Confocal micrographs showing expression of BDA with the post-synaptic marker Homer1 in the Ascl1creERT2:: tdTomato cells. Synaptic marker expression in the region highlighted with a box is shown with higher magnification. Scale bar: lower magnification: 10 μm, higher magnification: 5 μm.

Supplementary Figure 6:

Schematic of approach rabies-based and cre-dependent monosynaptic tracing in the Ascl1CreERT2:: tdTomato transgenic animals targeted neurons specifically derived from SVZ

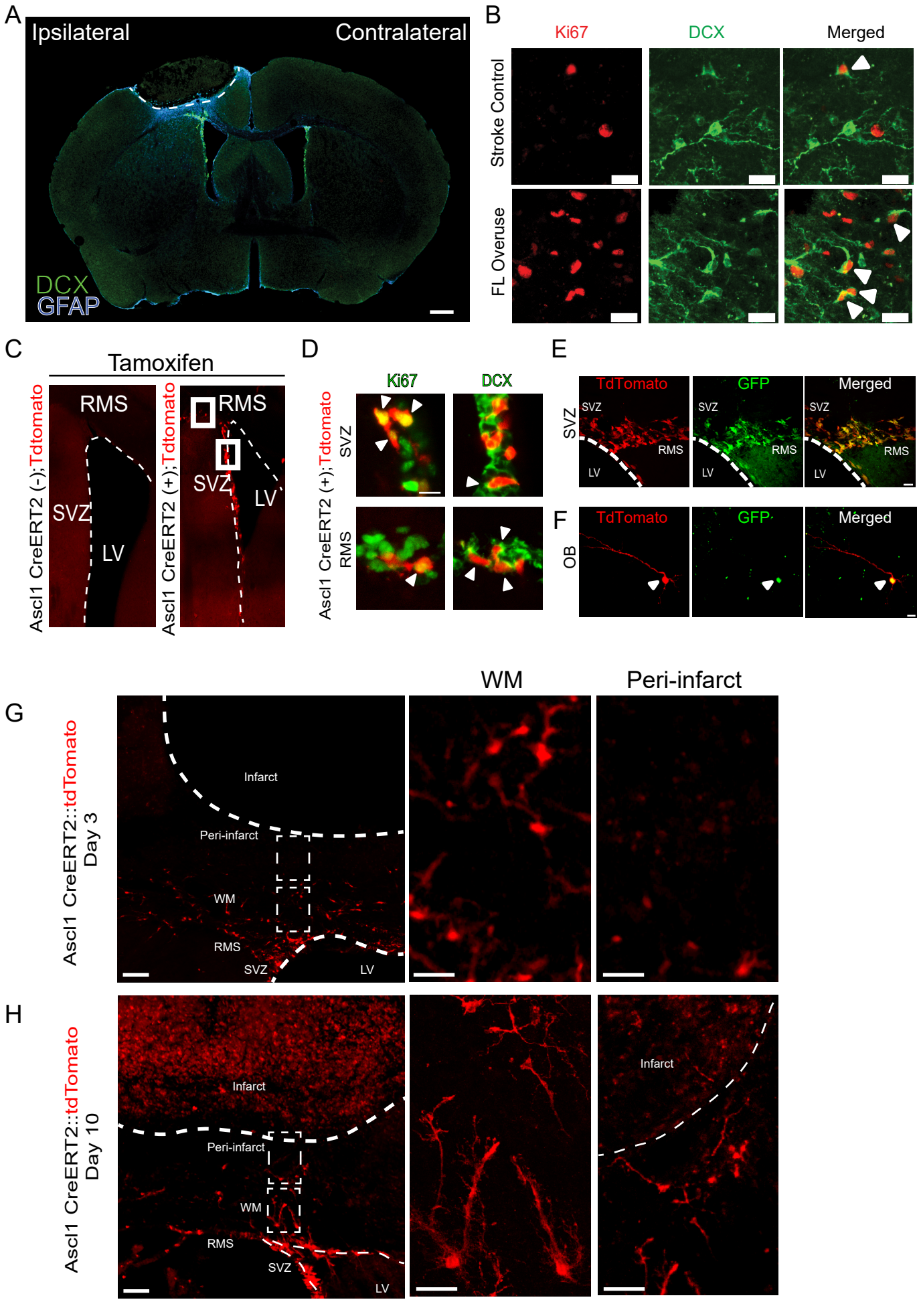
(A) Schematic demonstration of the helper plasmid EF1-α - FLEX BTG. AAV2 expressing the helper EF1-α - FLEX BTG was injected into the lateral ventricle of Ascl1 CreERT2 (+);tdTomatoanimals 7 days before stroke. Animals were allowed to survive 2 months after stroke and the rabies BFP was injected into the peri-infarct region 4 days before sacrifice. (B) Schematic demonstration of the rabies virus-based monosynaptic tracing from the SVZ-derived Ascl1 CreERT2 (+);tdTomato cells. (C) Schematic demonstration of the Syn-FLEX-EYFP and Syn-FLEX-TeNT-EYFP plasmids. AAV2 expressing either the Syn-FLEX-EYFP or Syn-FLEX-TeNT-EYFP was co-injected with the AAV2 CMV-cre into the lateral ventricle of Ascl1 CreERT2 (+);tdTomato animals, 7 days before stroke. Behavioral tasks including grid walking and pasta handling were performed during the 2 month of post-stroke survival period. **(D)** Images of EYFP+ cells in the periinfarct regions 2 months after stroke. **(E)** Images (quantified with Imaris) of VAMP2 located within the EYFP+ cell surface. Scale bar: 10 μm

Supplementary Figure 7:

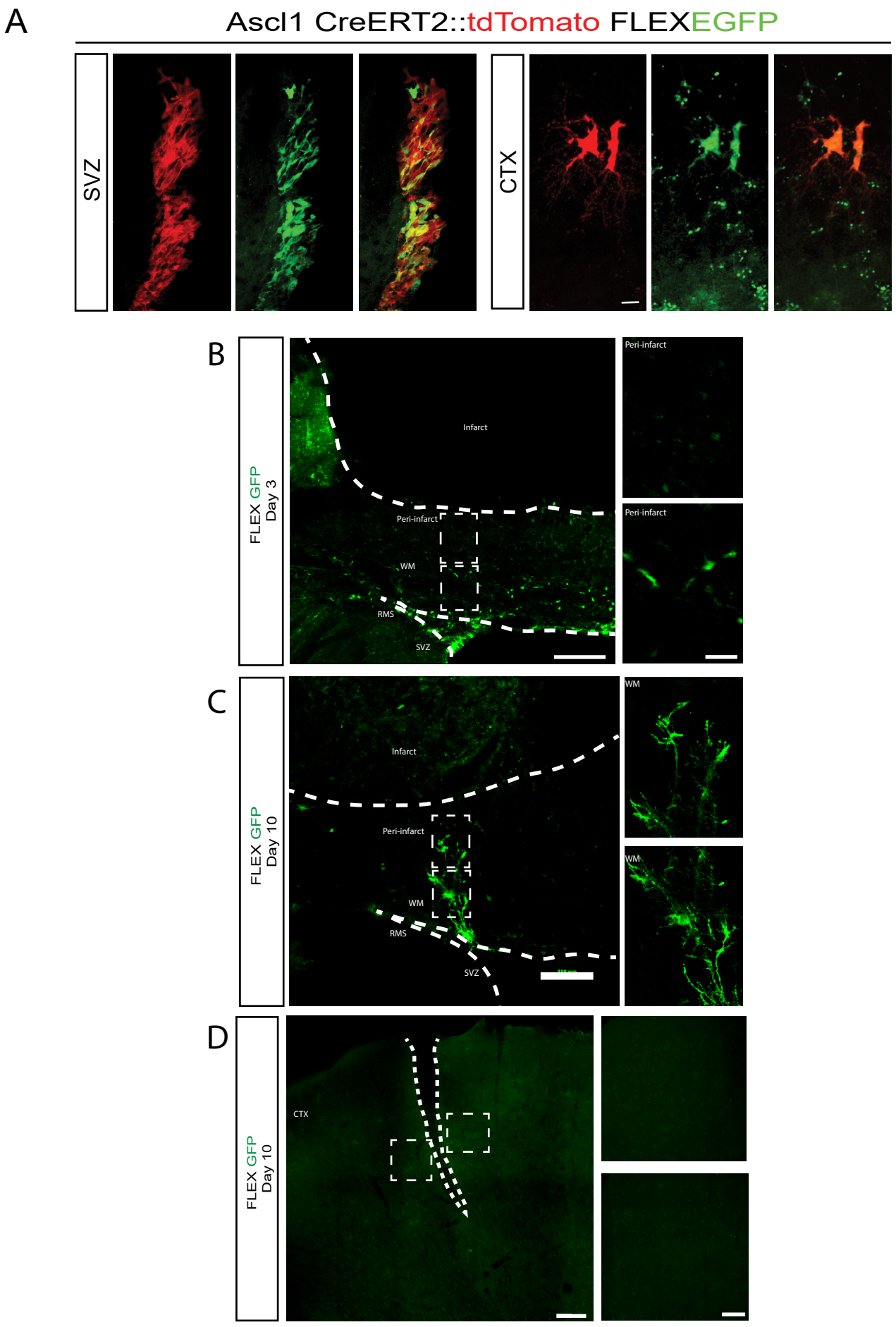
Monosynaptic connections formed between SVZ-derived neurons

(A) In the rabies-based and cre-dependent monosynaptic tracing experiment, BFP+GFP- (monosynaptic connected cells) cells were found to be tdTomato+ (open arrowheads) or tdTomato- (filled arrowheads). **(B)** Quantification showing percentage of BFP+GFP-tdTomato+ cells among BFP+GFP- cells, in stroke control and FL overuse group. $p>0.05$**.** (**C**) Sparse BFP+ cells were found in the contralateral cortex. Scale bar: 10 μm.

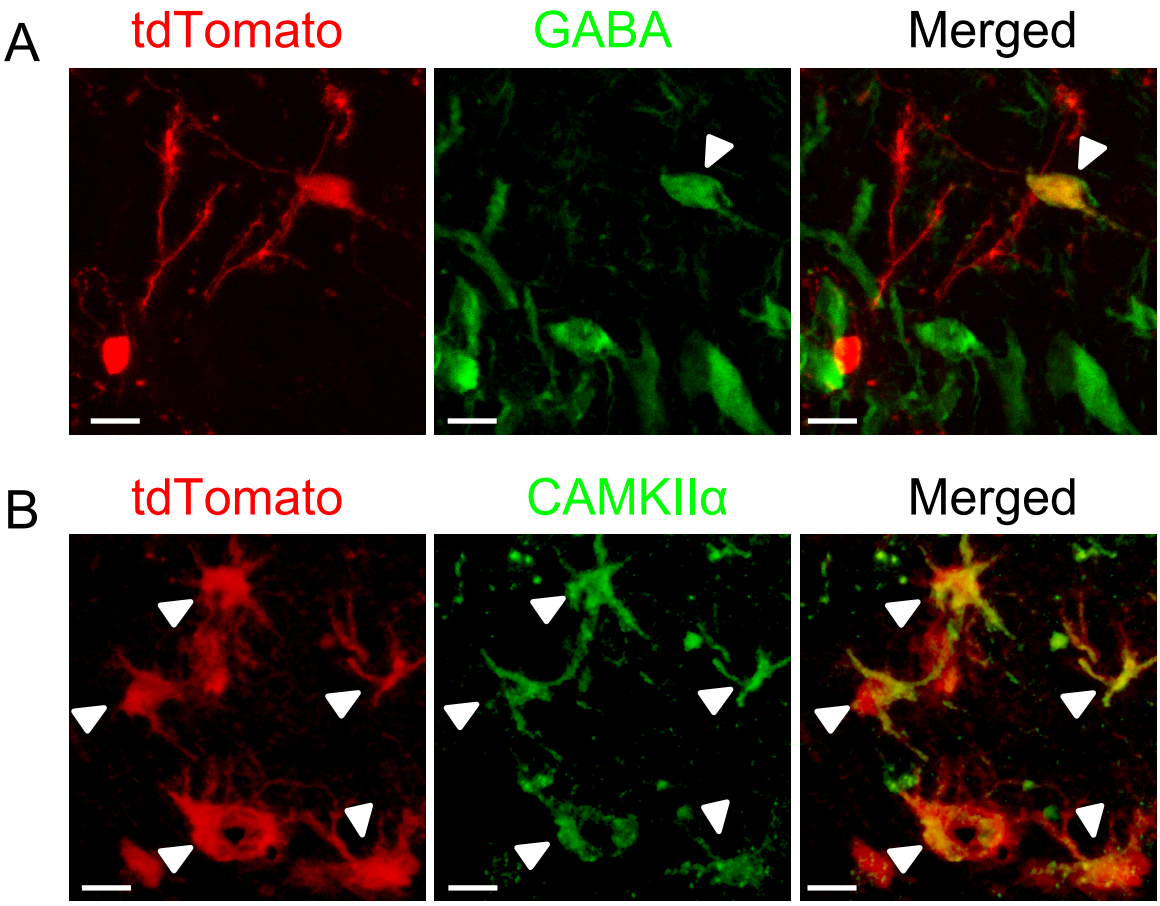
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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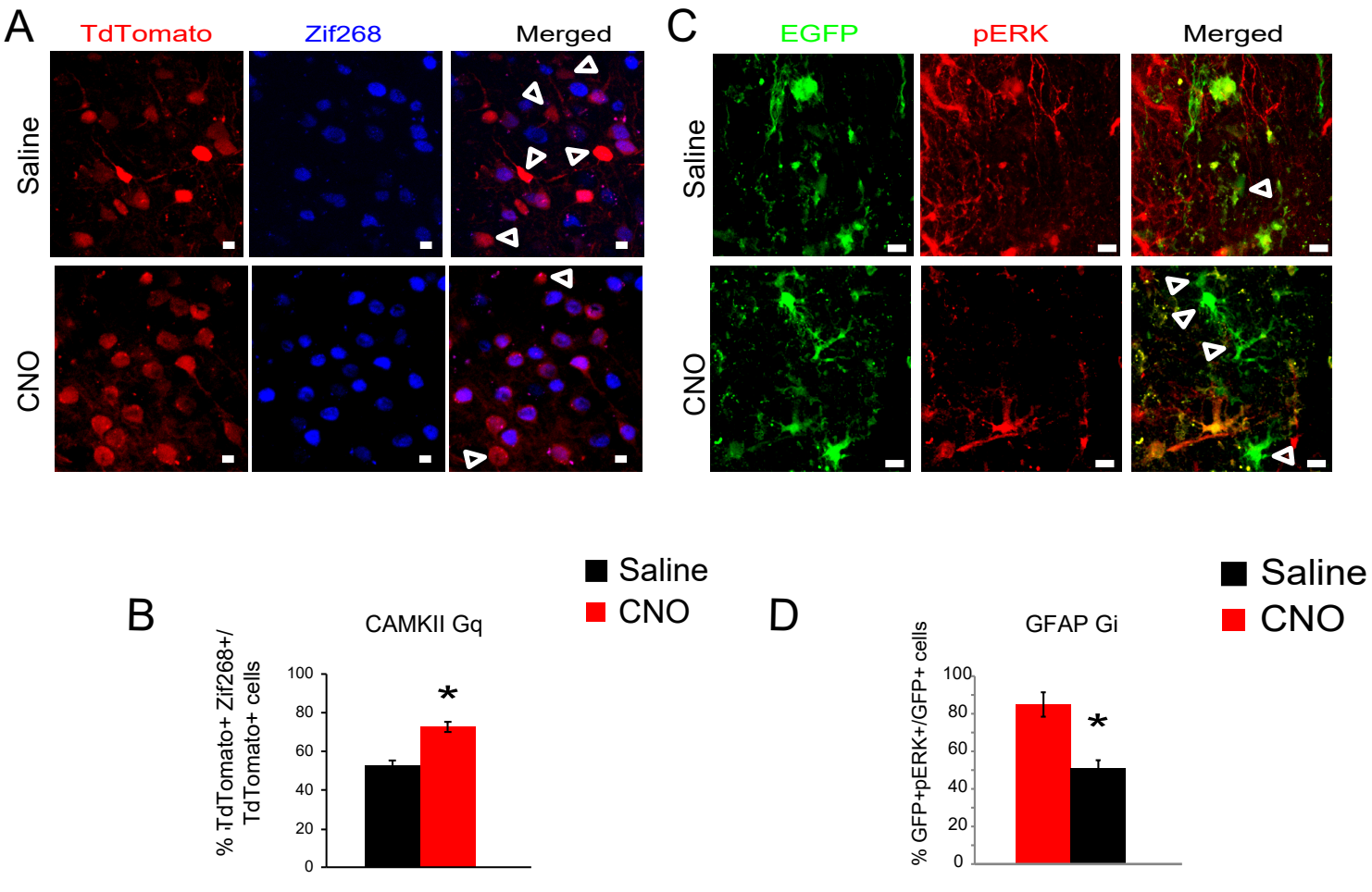

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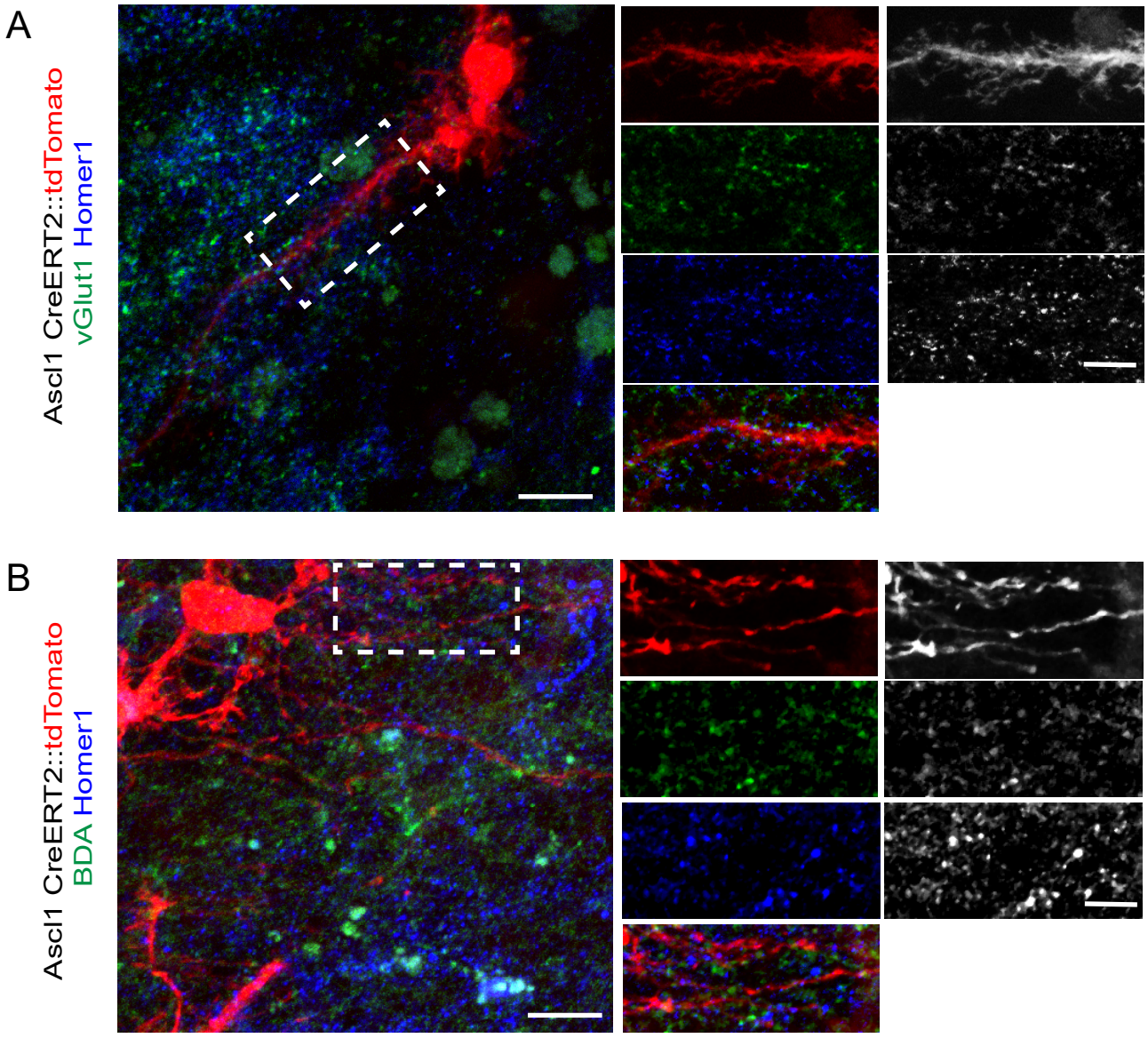
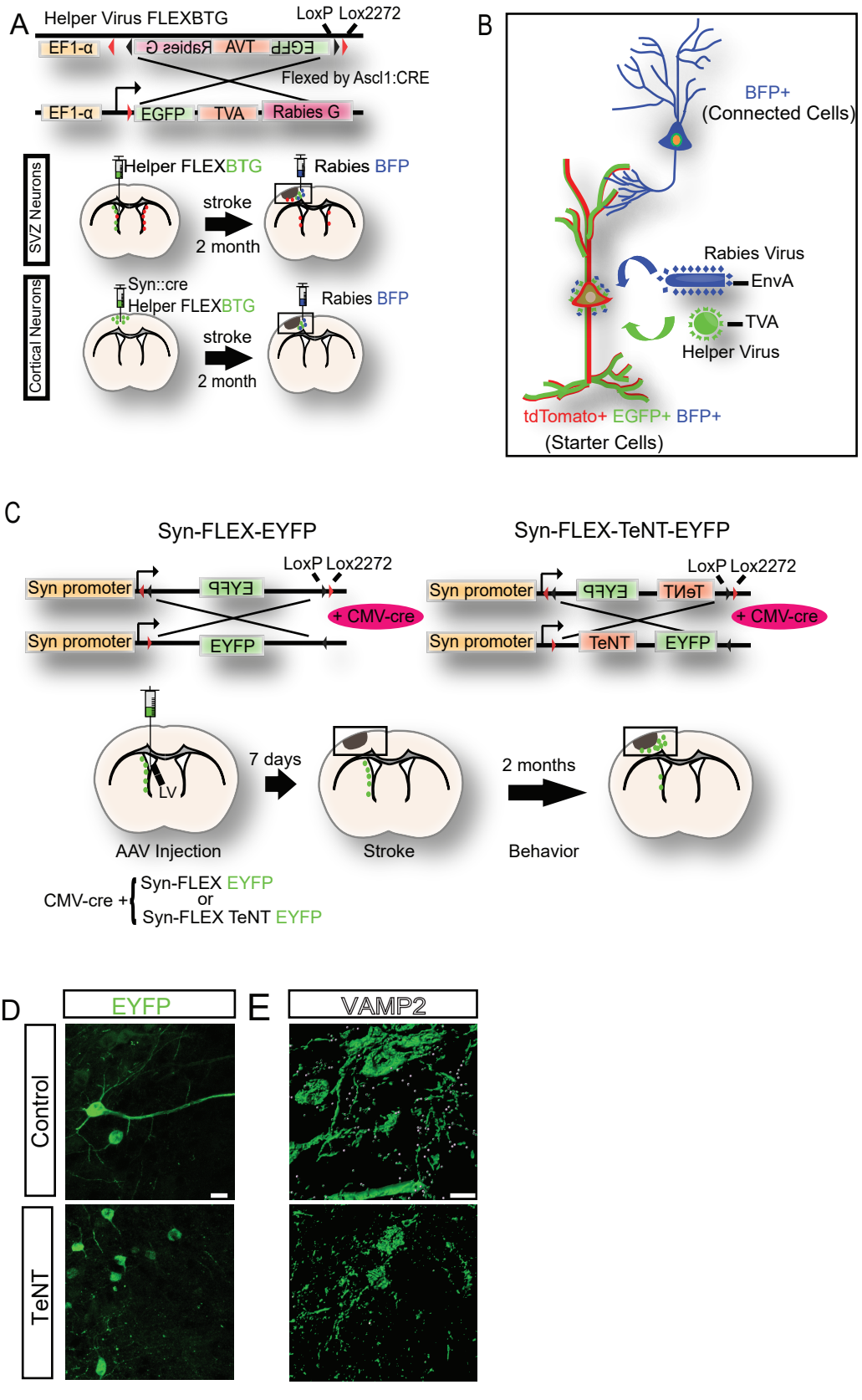


Supplementary Figure 6



Supplementary Figure 7