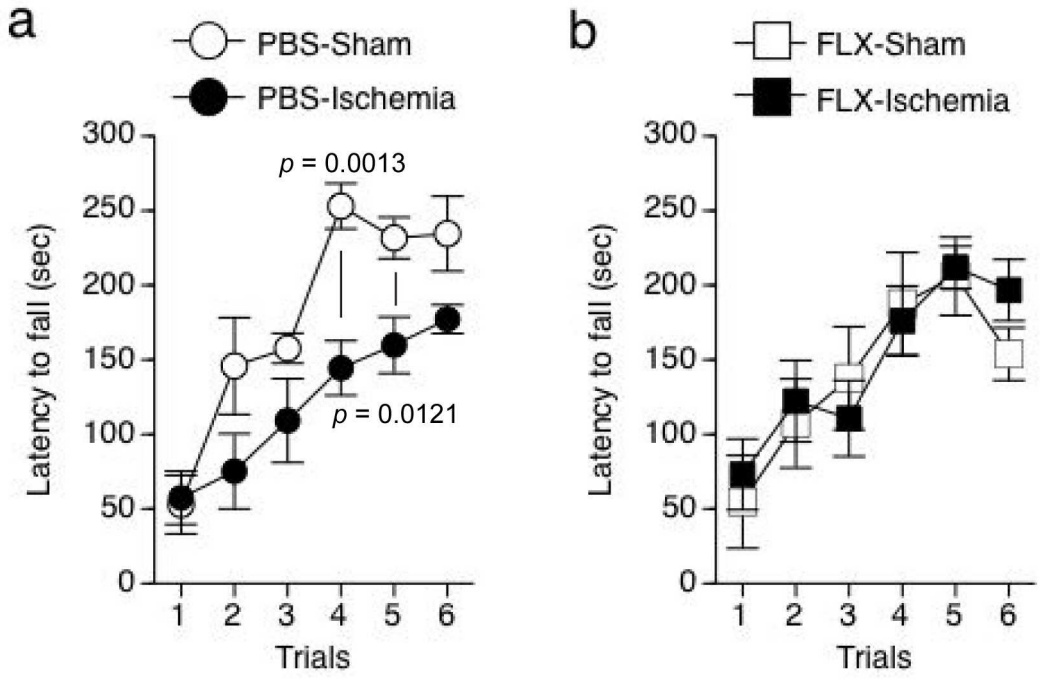
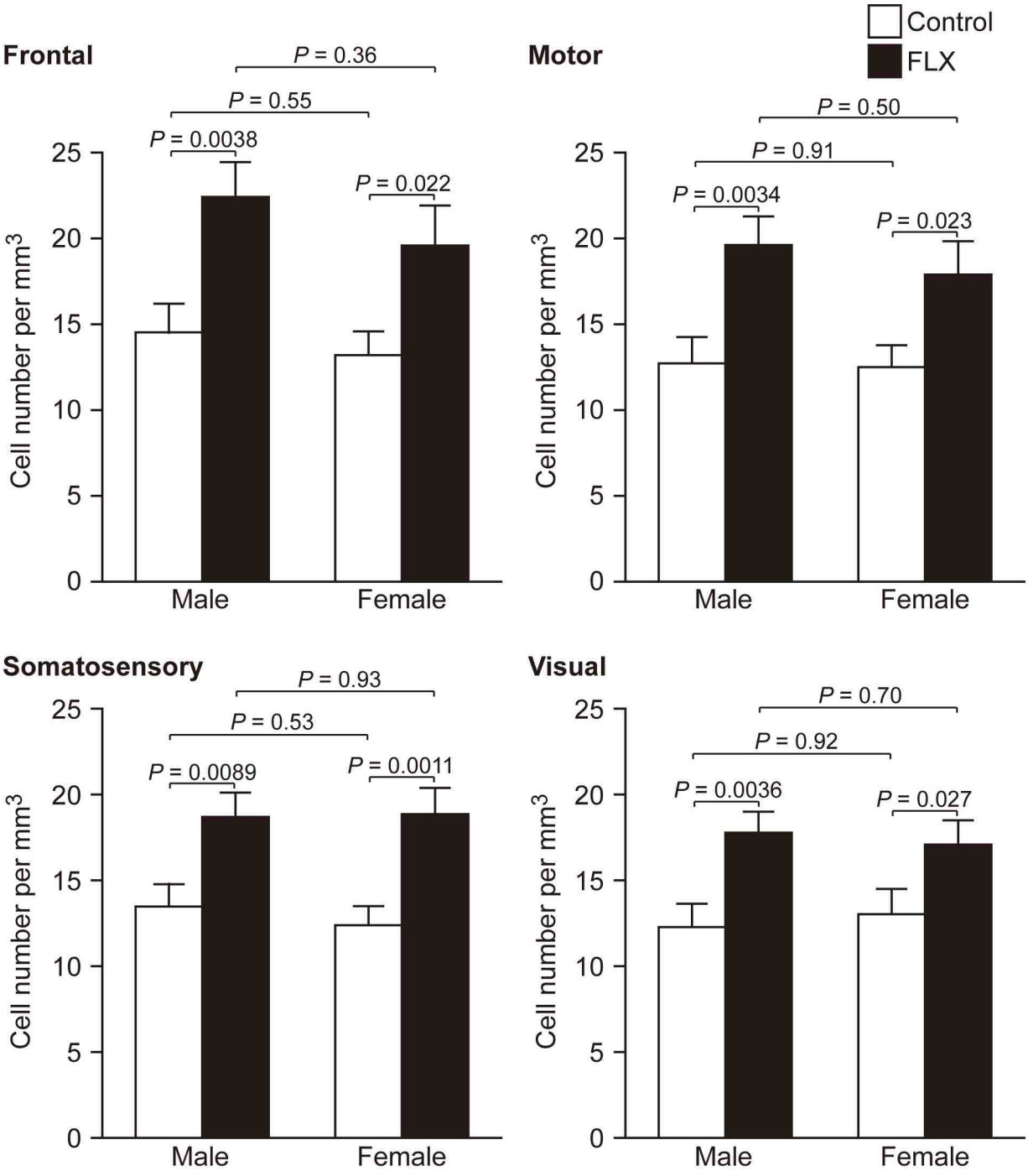
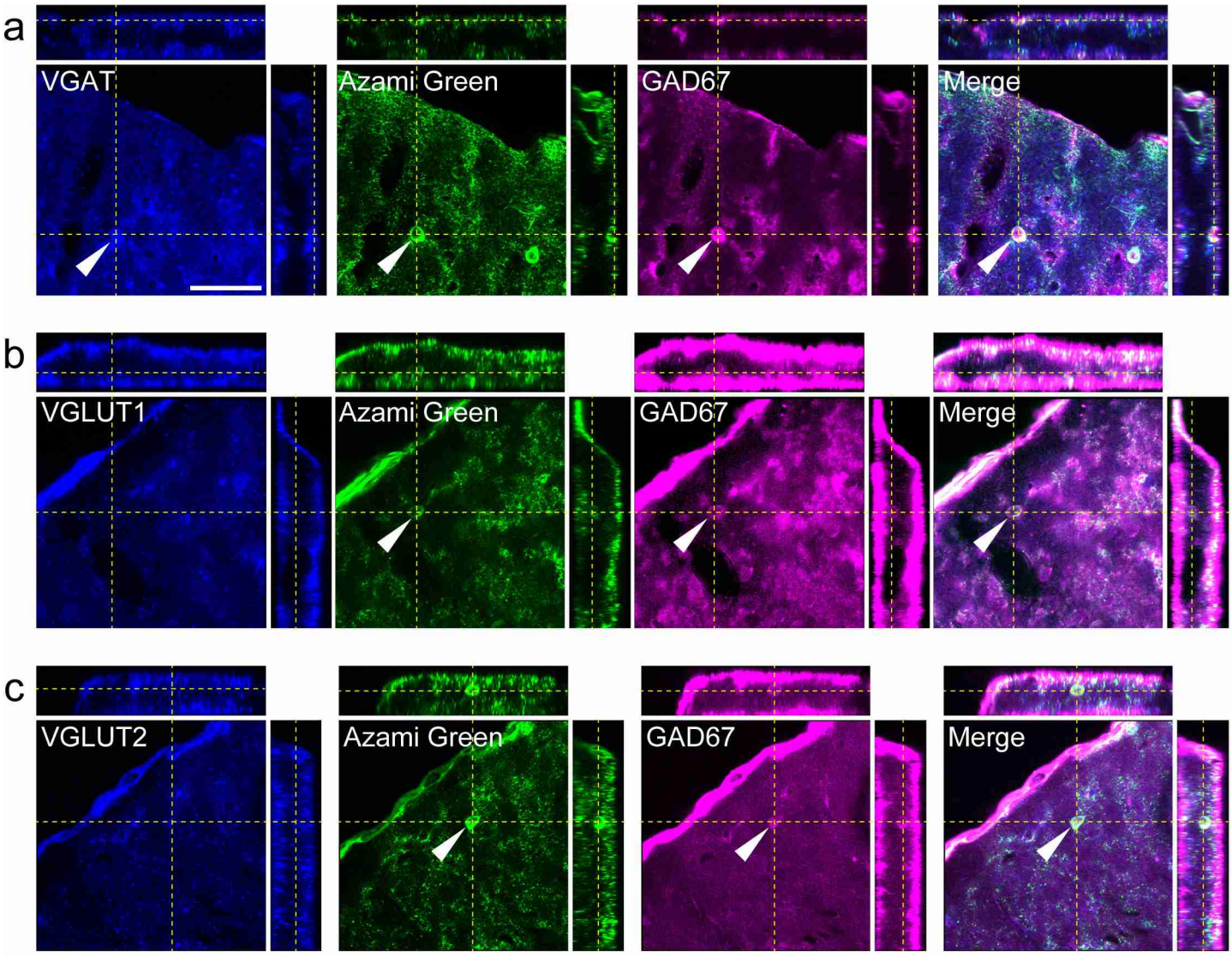


Supplementary Figure S1. Attenuating effect of FLX treatments on ischemia-induced decrease in latency to fall off rotarod.
An overall three-way ANOVA including all data showed that there was a tendency in a FLX × ischemia interaction ($F(1,22) = 2.996$, $p = 0.0975$), suggesting that the effect of ischemia is different between FLX-treated and PBS-treated groups. Therefore, we next conducted two-way ANOVA for PBS-treated group and FLX-treated group, separately. In the PBS-treated mice, ischemia treatment significantly reduced latency to fall off rotarod (Supplementary Figure S1a) ($F(1,9) = 6.239$, $p = 0.034$), and a TRIAL × ISCHEMIA interaction was highly significant ($F(5,45) = 3.6$, $p = 0.008$). Further analysis for each trial revealed that ischemia significantly decreased latency to fall in the trials #4 ($p = 0.0013$) and #5 ($p = 0.0121$). As shown in Supplementary Figure S1b, there was no significant main effect of ischemia on the motor performance in the FLX-treated mice ($F(1,13) = 0.068$, $p = 0.7979$), nor TRIAL × ISCHEMIA interaction ($F(5,65) = 0.84$, $p = 0.5265$), indicating that ischemia did not impair motor performance under FLX treatment. All data are shown as mean±SEM.

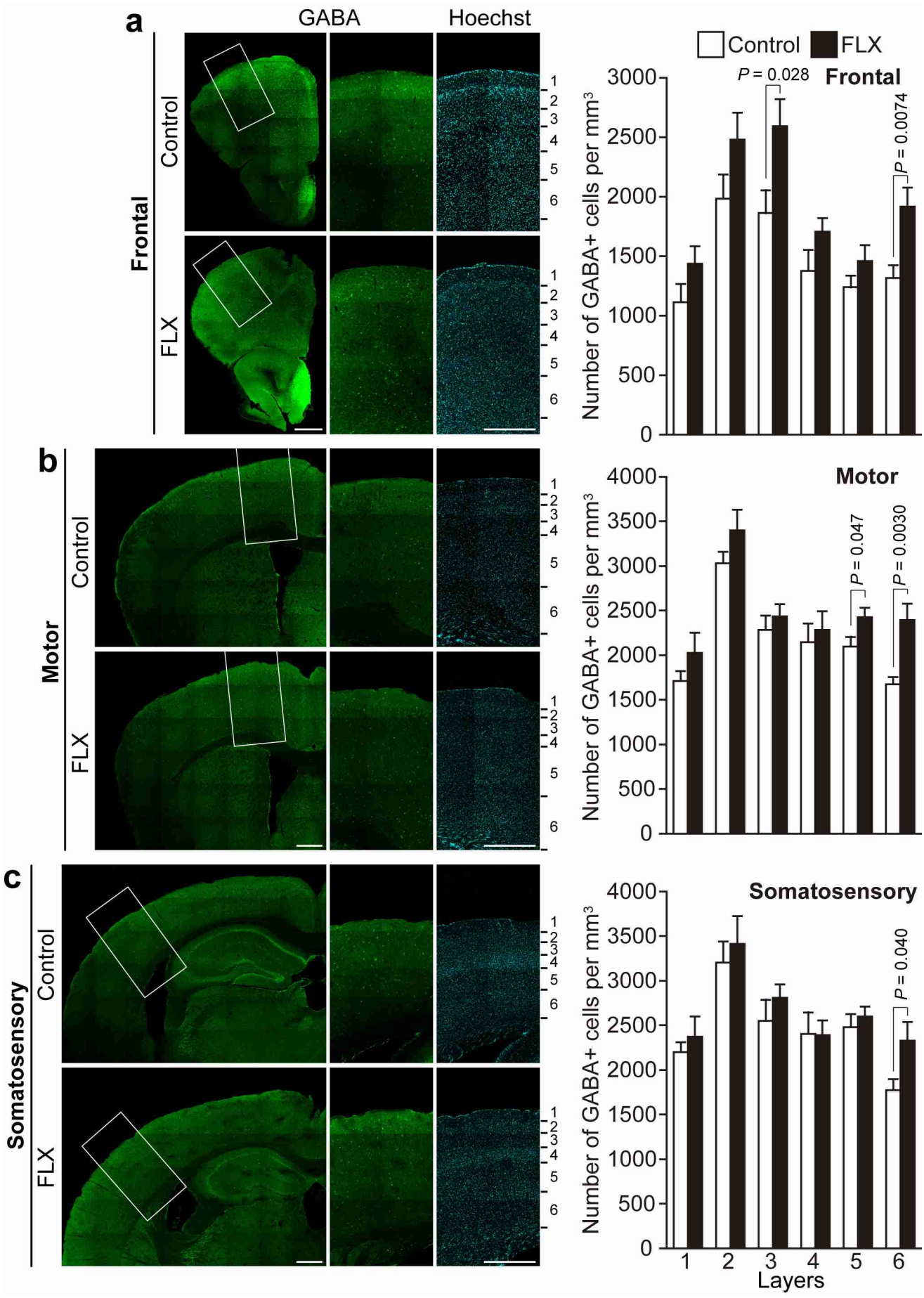


Supplementary Figure S2. No sex difference in the number of L1-INP cells in layer 1 of FLX-treated mice.

Both male and female groups were treated with FLX (15 mg/kg/day) for 3 weeks. FLX pellets were subcutaneously embedded into the dorsal interscapular region of mice. In all the cortical regions examined, no significant differences between the male and female groups were observed (two-way ANOVA, $n = 5$ each). All data are shown as mean ± SEM.

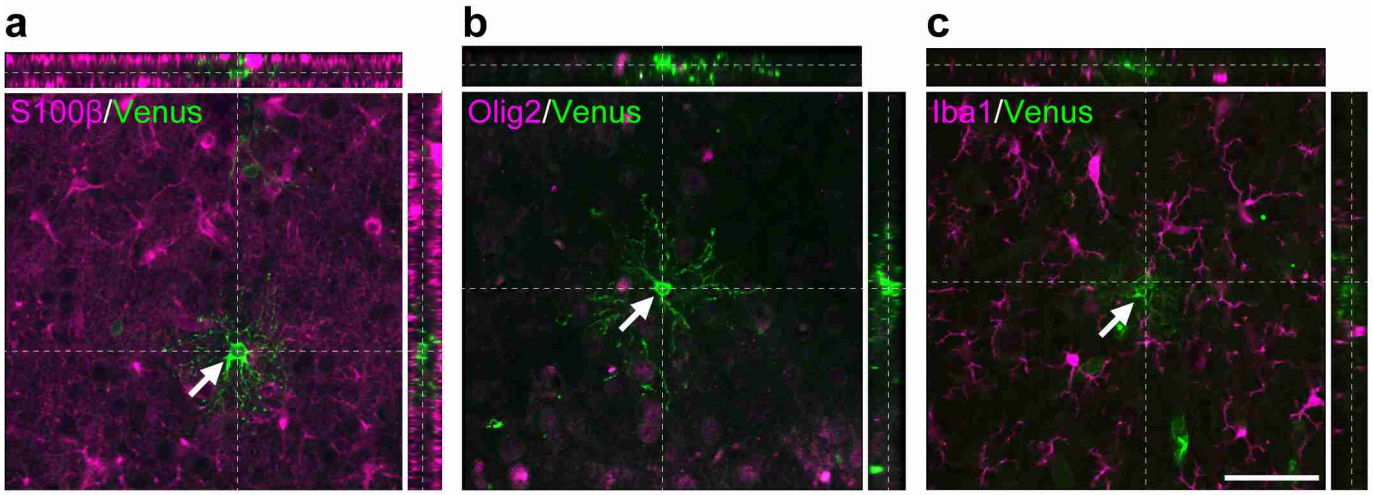


Supplementary Figure S3. Expression of vesicular GABA transporter in L1-INP cells. The expressions of vesicular glutamate 1 and 2 (VGLUT) and GABA transporters (VGAT) were examined by triple-labeling immunofluorescence microscopy. L1-INP cells expressed VGAT (a), but neither VGLUT1 (b) nor VGLUT2 (c). Arrowheads means L1-INP cells, which are labeled with Azami Green and GAD67. Scale bar, 50 μm.

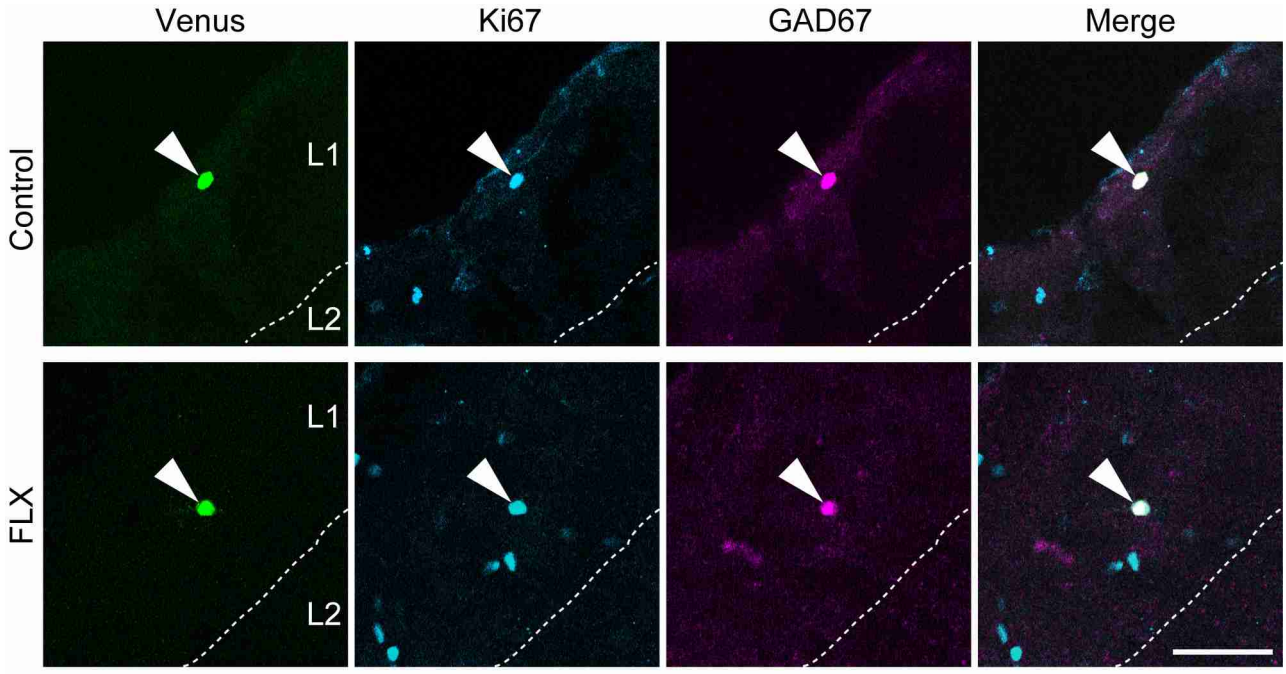

a
GABA
Hoechst
Frontal
Control
FLX
1
2
3
4
5
6
Control
FLX
Frontal
P = 0.028
P = 0.0074
Number of GABA+ cells per mm³
3000
2500
2000
1500
1000
500
0
b
Motor
Control
FLX
Motor
P = 0.047
P = 0.0030
4000
3500
3000
2500
2000
1500
1000
500
0
c
Somatosensory
Control
FLX
Somatosensory
P = 0.040
1
2
3
4
5
6
Layers

Supplementary Figure S4. Increases in the number of GABAergic interneurons in the cortex of FLX-treated mice.

(a-c) Mice were treated with FLX pellets (15 mg/kg/day) for 3 weeks. Brain sections were stained with anti-GABA (green signal). FLX treatments increased the number of GABAergic interneurons in layer 6 (Student's *t* test, $n = 8$ each). In addition, layer-specific increases in GABAergic interneurons were also found in layer 3 of the frontal cortex (a) and layer 5 of the motor cortex (b). Scale bars, 500 μm.



Supplementary Figure S5. No expression of glial cell markers in neurons derived from L1-INP cells. L1-INP cells were infected with a retrovirus vector, which expresses Venus reporter proteins under the neuron-specific promoter enhanced synapsin I. At 4 weeks after the FLX treatments (15 mg/kg/day), Venus+ neurons were found in layers 2–6, but they were not co-stained with glial markers, such as astrocyte marker S100β (a), oligodendrocyte lineage marker Olig2 (b), or microglia marker Iba1 (c). Scale bar, 50 μm.

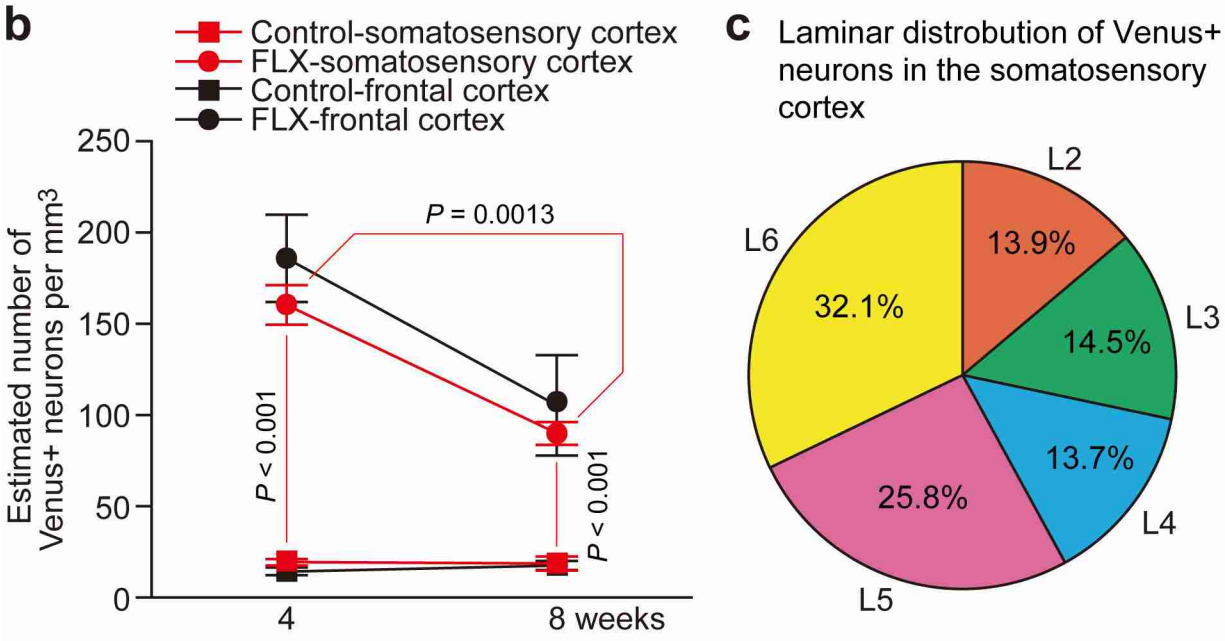


Supplementary Figure S6. Retrovirus-mediated Venus labeling of L1-INP cells.
L1-INP cells were labeled with a retrovirus vector, which expresses Venus proteins driven by the neuron-specific promoter enhanced synapsin I. Mice were treated with FLX (15 mg/kg/day) for 3 weeks. Arrowheads indicate the same cells in layer 1 of control (upper) and FLX-treated mice (lower). Scale bar, 50 μm.

a Number of layer 2–6 Venus+ cells and labeling efficiency *r* in the somatosensory cortex of each mouse

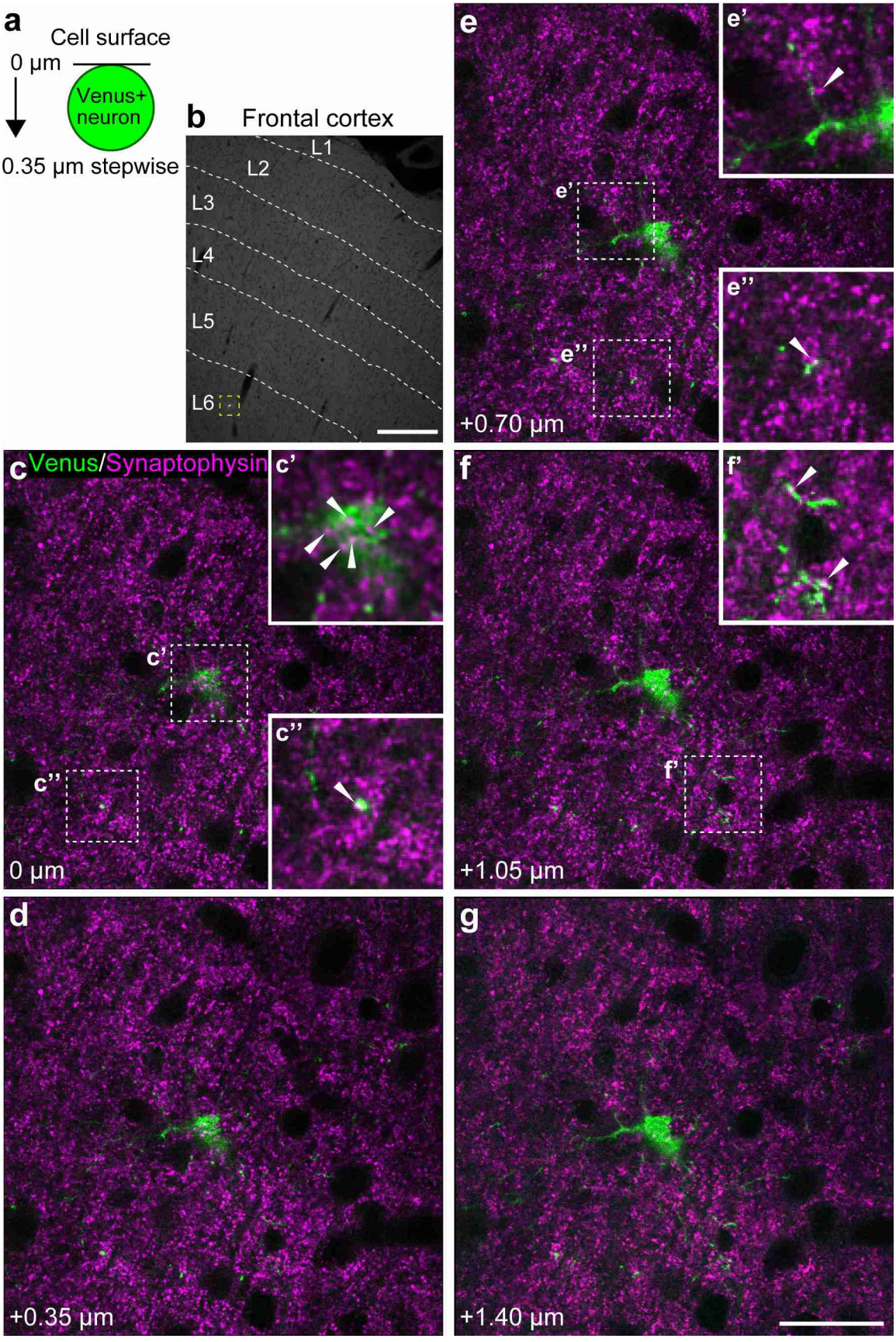
Weeks	4	8
Control mice	3.06, 0.159	2.26, 0.192
	1.90, 0.128	1.48, 0.113
	2.00, 0.086	2.93, 0.114
	2.51, 0.126	2.23, 0.077
FLX-treated mice (15 mg/kg/day)	16.7, 0.112	10.2, 0.137
	25.9, 0.138	12.7, 0.142
	19.3, 0.117	9.66, 0.092
	23.8, 0.172	12.1, 0.132

Each cell represents ([number of layer 2–6 Venus+ neurons per mm^3], [labeling efficiency *r*]) for each mouse.

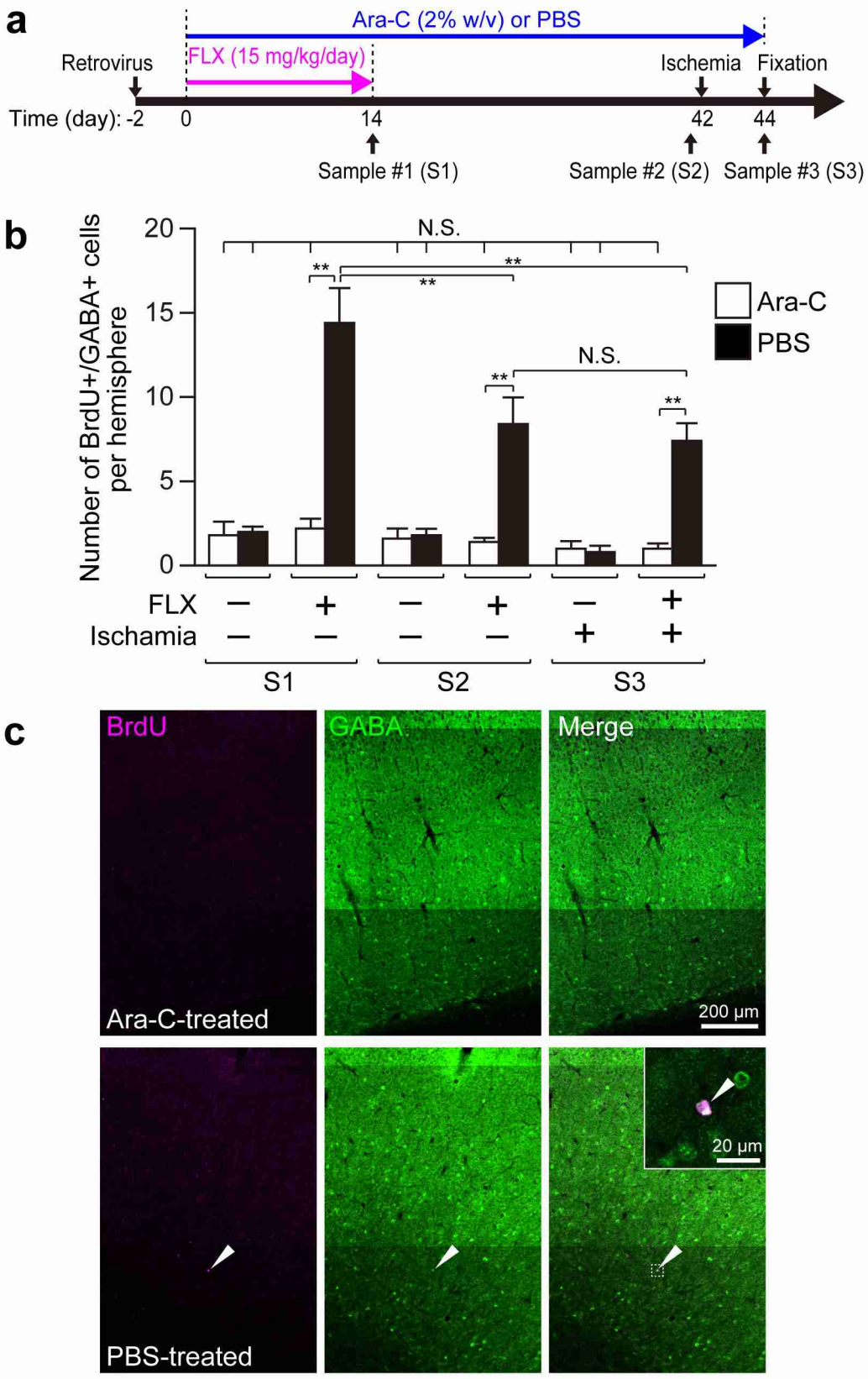


Supplementary Figure S7. Increase in the number of Venus+ neurons and their laminar distribution in the somatosensory cortex of FLX-treated mice.

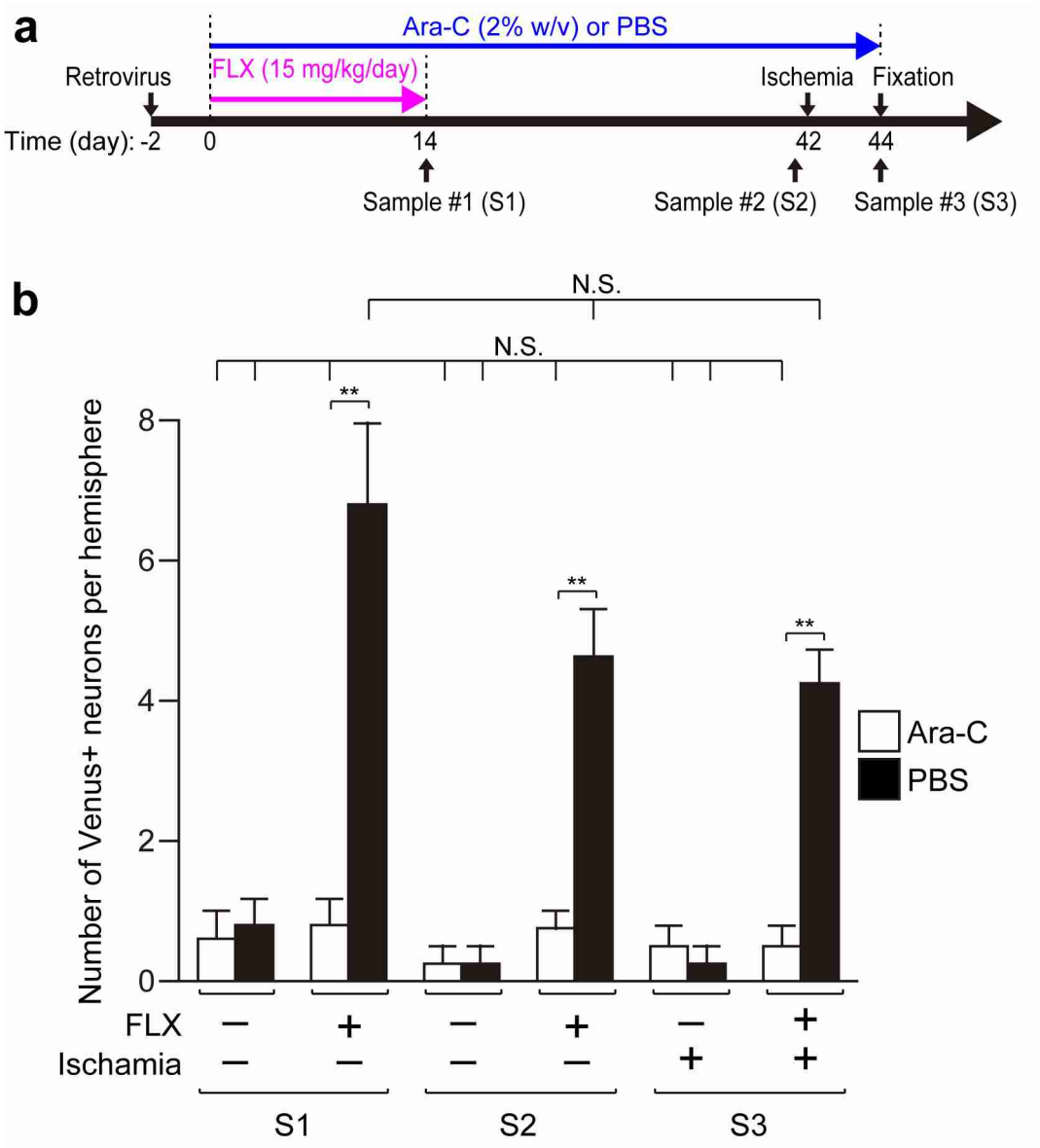
(a) The number of layer 2-6 Venus+ neurons and labeling efficiency *r* in the somatosensory cortex 4 and 8 weeks after the last FLX administration. (b) Time-course of the estimated number of layer 2–6 Venus+ cells in the frontal and somatosensory cortex of FLX-treated mice (two-way ANOVA followed by Scheffé's *post hoc* test, $n = 4$ each). Data from the somatosensory cortex is indicated by red square (control) and circle (FLX). Data from the frontal cortex (black square [control] and circle [FLX]) are identical to those in Fig. 3c. All data are shown as mean ± SEM. (c) Laminar distribution of Venus+ neurons in the somatosensory cortex of FLX-treated mice. Data are shown as mean of four mice. L, layer.


a
Cell surface
0 μm
Venus+
neuron
0.35 μm stepwise
b
Frontal cortex
L1
L2
L3
L4
L5
L6
e
e'
e''
+0.70 μm
c
Venus/Synaptophysin
c'
c''
0 μm
f
f'
+1.05 μm
d
+0.35 μm
g
+1.40 μm

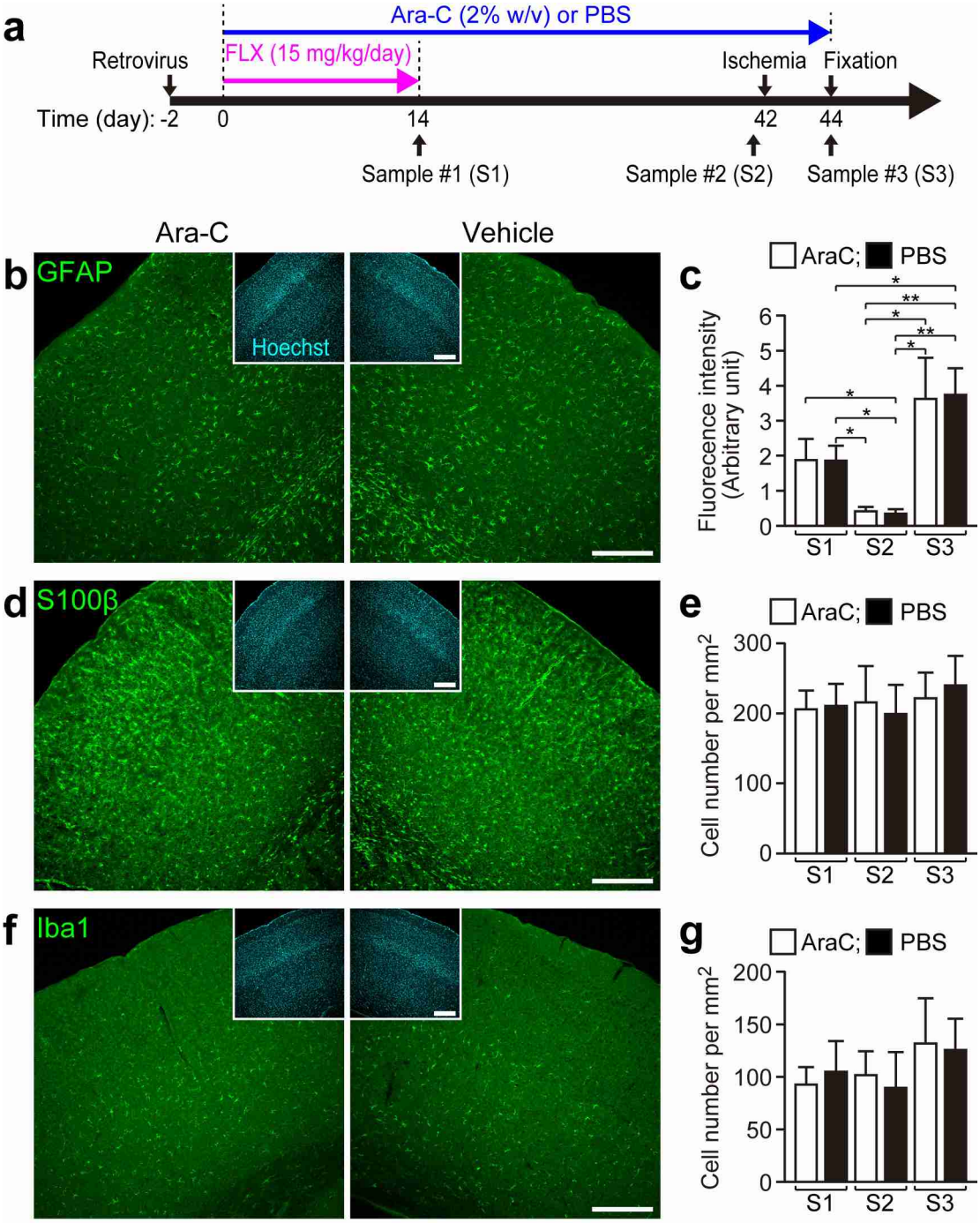
Supplementary Figure S8. Synaptic inputs to new neurons.
Synaptophysin was used as a presynaptic marker. (a) Schematic representation of the analysis. (b) Cell images were taken from layer 6 of the frontal cortex 4 weeks after 3 weeks-FLX treatments (15 mg/kg/day). (c-g) A z-series analysis revealed that the synaptophysin-labeled presynaptic structures (arrowheads) were in close apposition with a different focal plane of the Venus+ neuron. Scale bars, 300 μm (b), 30 μm (c-g).



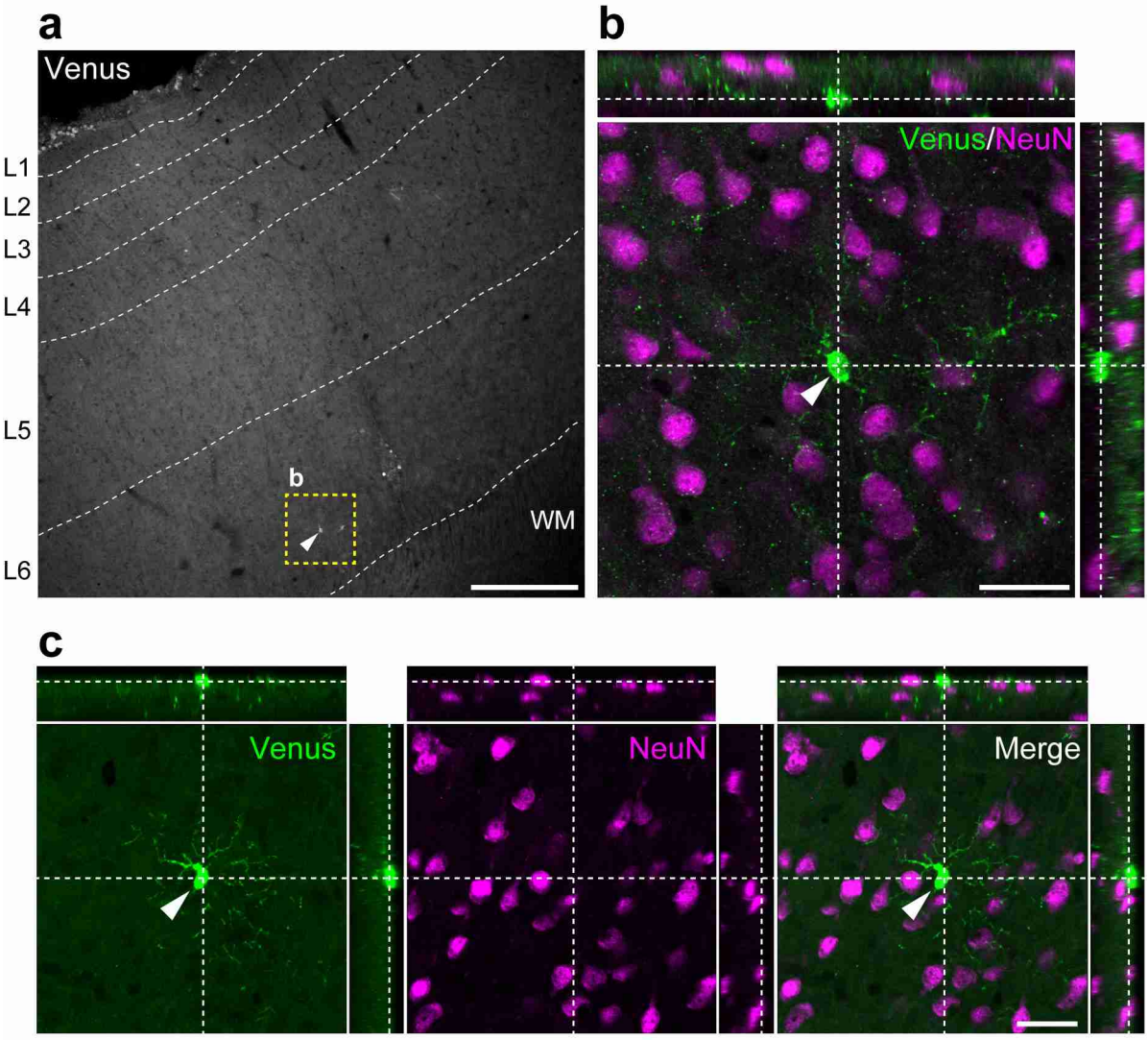
Supplementary Figure S9. Quantification of the number of BrdU+/GABA+ cells before and after ischemia. (a) Time course experiments. (b) Quantification of the number of BrdU+/GABA+ cells in the Ara-C- and PBS-treated hemispheres before and after ischemia. S1, sample #1; S2, sample #2; S3, sample #3, as shown in (a). All data are shown as mean ± SEM (**P < 0.01; two-way ANOVA followed by Scheffé's post hoc test, n = 4 each). N.S., not significant. (c) Representative images of S3, which were treated with FLX. Tissue sections were stained with anti-BrdU (magenta) and anti-GABA (green). Arrowhead indicates a BrdU+/GABA+ cell.



Supplementary Figure S10. No changes in the number of Venus+ neurons before and after ischemia. (a) Time course experiments. (b) Quantification of the number of Venus+ neurons in the Ara-C- and PBS-treated hemispheres before and after ischemia. S1, sample #1; S2, sample #2; S3, sample #3, as shown in (a). All data are shown as mean ± SEM (**$P < 0.01$; two-way ANOVA followed by Scheffé's post hoc test, $n = 4$ each). N.S., not significant.

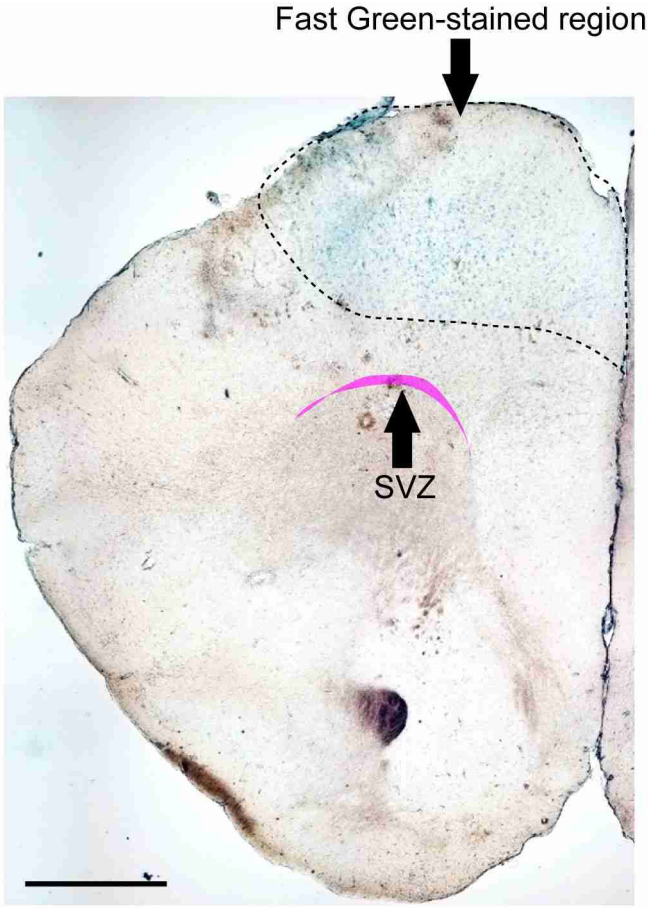


Supplementary Figure S11. Involvement of glial cells in neuroprotective effects. (a) Time course experiments. Glial activation was evaluated with the expressions of GFAP, astrocyte activation (b and c); S100β, cell number of astrocytes (d and e); and Iba1, microglia activation (f and g). S1, sample #1; S2, sample #2; S3, sample #3, as shown in a. All sections were counter-stained with Hoechst to assess the cytoarchitecture (inset). All data are shown as mean ± SEM (*$P < 0.05$, **$P < 0.01$; two-way ANOVA followed by Scheffé's post hoc test, $n = 4$ each). Scale bars, 250 μm.



Supplementary Figure S12. No expression of NeuN in new neurons derived from L1-INP cells.

(a) The cell images were taken from layer 6 of the frontal cortex 4 weeks after 3 weeks-FLX treatments (15 mg/kg/day). L, layer. WM, white matter. (b) NeuN signals (magenta) were hardly detected in Venus+ neurons by mouse monoclonal anti-NeuN antibody. (c) Sections were stained with rabbit polyclonal anti-NeuN antibody, but no venus-expressing neurons expressed NeuN. Scale bars, 250 μm (a), 30 μm (b, c).



Supplementary Figure S13. The regions stained with Fast Green.
A representative Fast Green-stained region was outlined by a dotted line. The SVZ was indicated by magenta. Scale bar, 1 mm.

Supplementary Table S1

Phenotypes of Venus+ neurons in the somatosensory cortex

	Labeling percentages of the markers expressed in Venus+ cells	
Periods after the FLX treatments	4 weeks	8 weeks
GABA	78 ± 6.1 (28/37)	81 ± 7.0 (19/24)
CR	45 ± 6.8 (17/38)	54 ± 8.1 (11/20)
NPY	7.9 ± 3.2 (4/33)	19 ± 5.9 (5/18)
SOM	4.6 ± 2.9 (2/36)	7.6 ± 4.0 (2/19)
CB	0 (0/33)	0 (0/15)
PV	0 (0/41)	0 (0/25)
S100β	0 (0/36)	0 (0/18)
Olig2	0 (0/30)	0 (0/21)
Iba1	0 (0/34)	0 (0/19)

Data are presented as means ± SEM from four mice at each time-point. The denominator and numerator mean the number of total Venus+ cells and that of marker+/Venus+ cell in the somatosensory cortex, respectively, in parentheses (one-way ANOVA and Scheffé's *post hoc* test, all $P > 0.05$). However, there was no significant difference between the values of each marker at 4 and 8 weeks. CR, calretinin. NPY, neuropeptide Y. SOM, Somatostatin. CB, calbindin. PV, parvalbumin. S100β, S100 calcium binding protein β. Olig2, Oligodendrocyte transcription factor 2. Iba1, ionized calcium binding adaptor molecule 1.

Supplementary Table S2

Number of layer 2–6 Venus+ cells and labeling efficiency *r* in the frontal cortex of each mouse

Weeks	1	2	4	8
Control mice	3.37, 0.186	2.18, 0.157	2.23, 0.108	1.75, 0.093
	2.56, 0.211	2.01, 0.150	1.43, 0.103	1.23, 0.119
	3.20, 0.154	2.73, 0.121	1.75, 0.153	3.20, 0.168
	2.59, 0.275	2.88, 0.184	2.26, 0.200	2.23, 0.103
FLX-treated mice	40.2, 0.146	30.9, 0.129	19.3, 0.167	13.1, 0.128
	47.2, 0.108	43.0, 0.107	31.3, 0.157	11.7, 0.193
	46.6, 0.182	32.3, 0.183	25.7, 0.126	13.4, 0.073
	35.5, 0.173	40.1, 0.145	27.4, 0.122	12.6, 0.169

Each cell represents ([number of layer 2–6 Venus+ neurons per mm^3], [labeling efficiency *r*]) for each mouse.