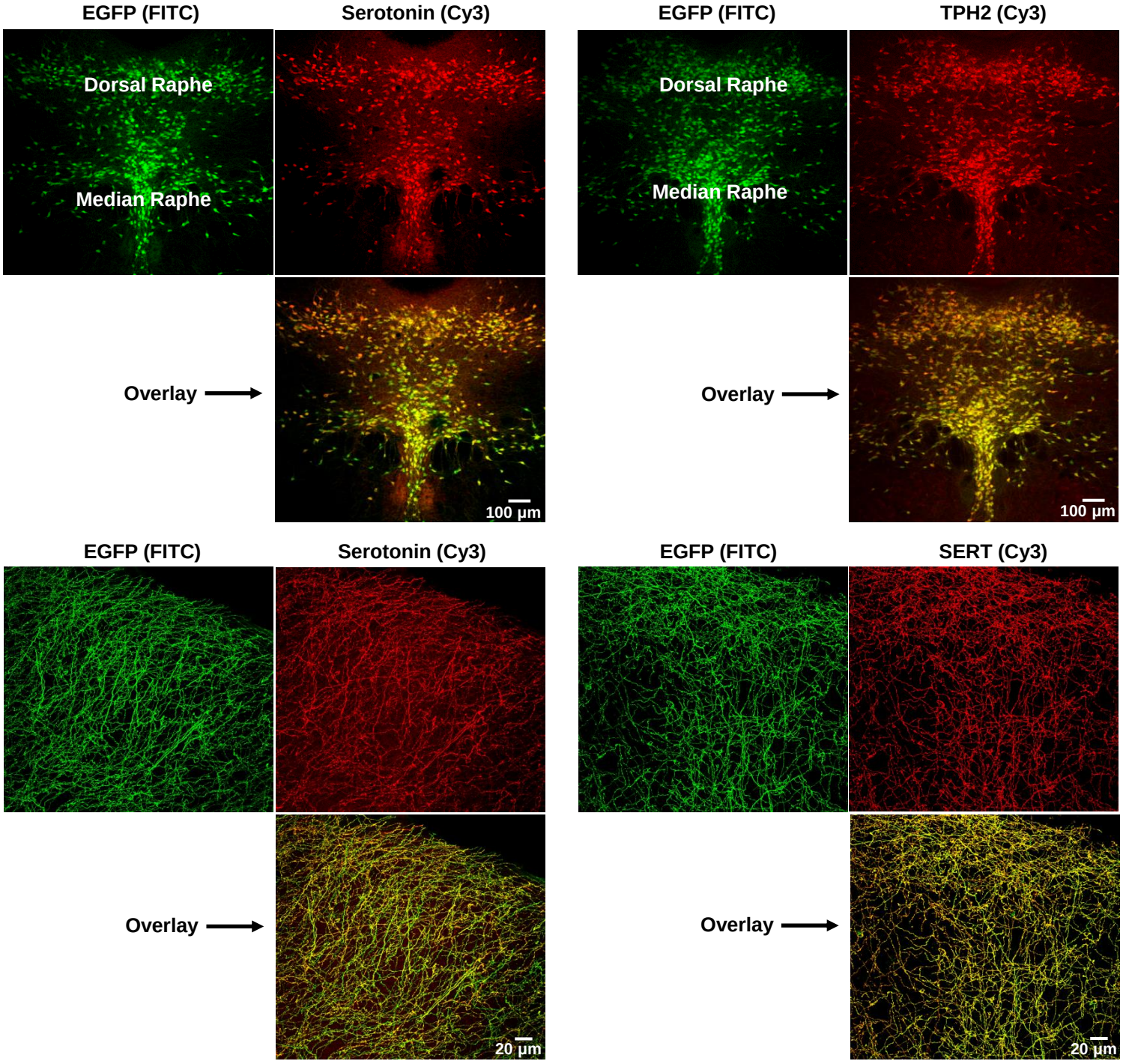
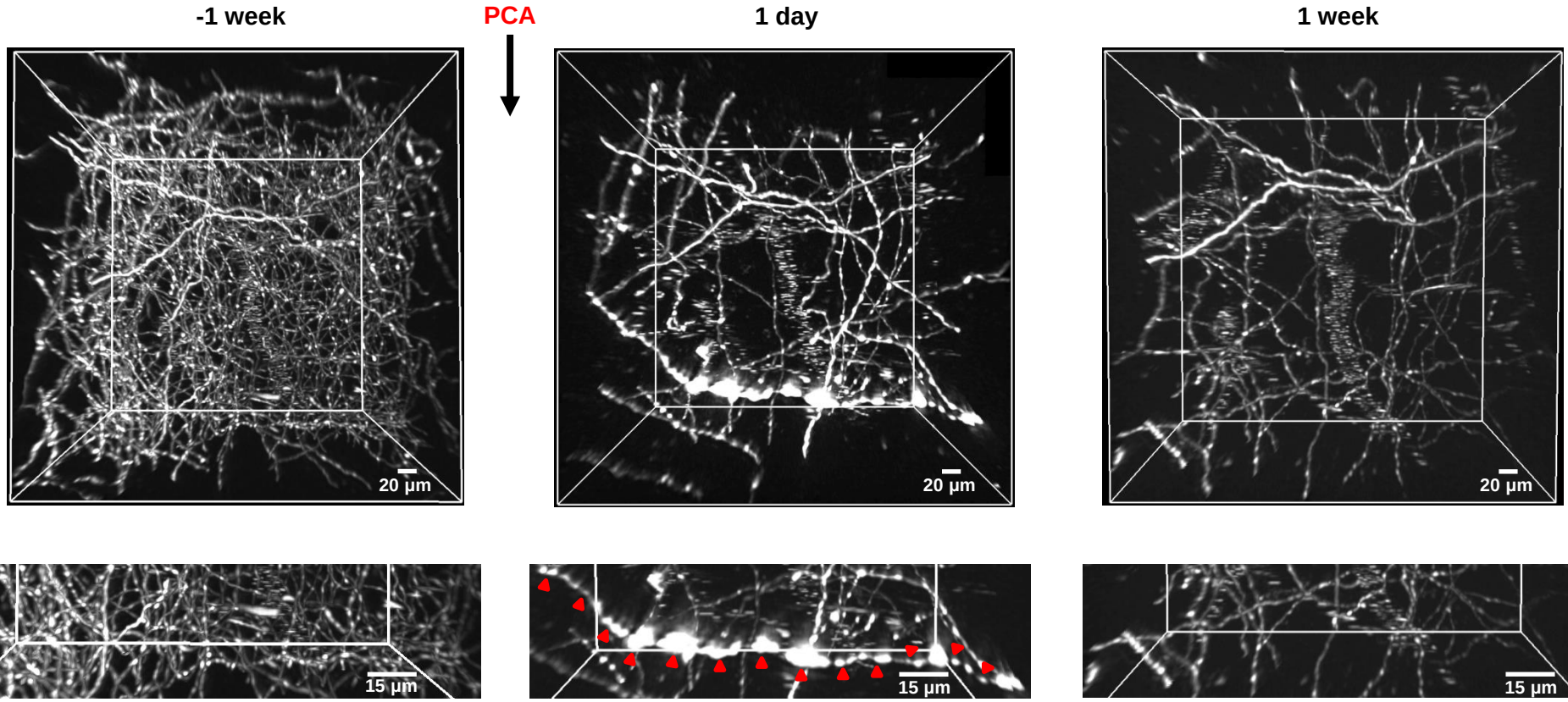
Figure S1


EGFP (FITC)
Serotonin (Cy3)
EGFP (FITC)
TPH2 (Cy3)
Dorsal Raphe
Median Raphe
Dorsal Raphe
Median Raphe
Overlay
Overlay
100 µm
100 µm
EGFP (FITC)
Serotonin (Cy3)
EGFP (FITC)
SERT (Cy3)
Overlay
Overlay
20 µm
20 µm

Supplemental Figure 1. EGFP positive neurons in serotonin transporter (Slc6a4)-EGFP mice express three different serotonin neuron markers, related to Figure 1.

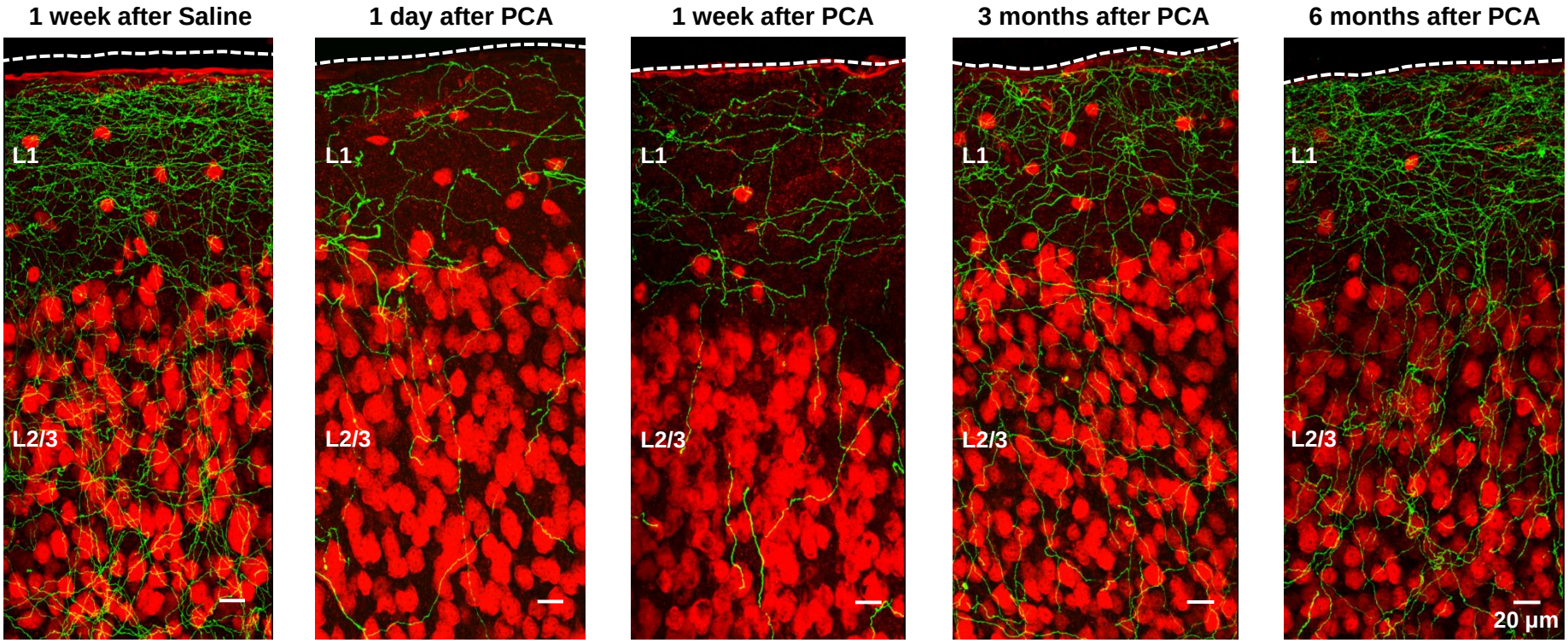
Top: Coronal sections through the raphe nuclei were subjected to dual-label immunohistochemistry. EGFP-positive cell bodies in the dorsal raphe showed a near complete overlap with immunofluorescence for serotonin or tryptophan hydroxylase 2 (TPH2). Bottom: Sagittal sections though the frontal cortex were subjected to dual-label immunohistochemistry. EGFP-positive axons showed near complete overlap with immunofluorescence for serotonin or serotonin transporter (SERT).

Figure S2



Supplemental Figure 2. In vivo images reveals swollen and fragmented serotonin axons in somatosensory cortex after PCA treatment, related to Figure 1

High resolution in vivo images show degenerating features of swollen and fragmented serotonin axons at 1 day after the end of PCA treatment. One particular swollen and fragmented axon, indicated with red arrows at 1 day after PCA treatment, is gone 1 week after PCA treatment.

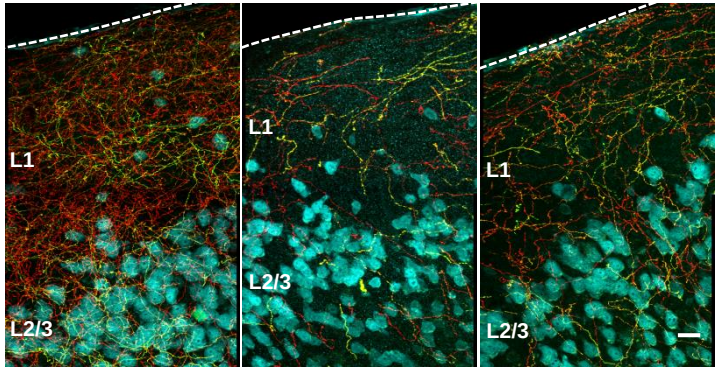

1 week after Saline
1 day after PCA
1 week after PCA
3 months after PCA
6 months after PCA
L1
L2/3
20 µm

Supplemental Figure 3. Exemplar images of somatosensory cortex show rapid loss and slow recovery of serotonin-EGFP axons following PCA treatment, related to Figure 2

Serotonin-EGFP axons are shown in green and NeuN immunoreactivity is shown in red in these sagittal sections. See Figure 2C for quantification of group data.

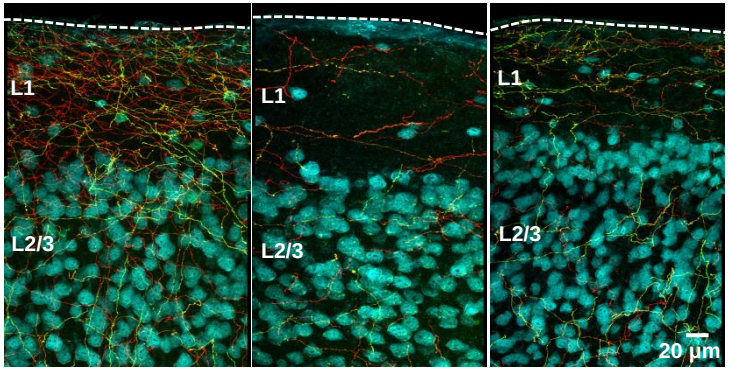
A

Frontal Cortex (6)

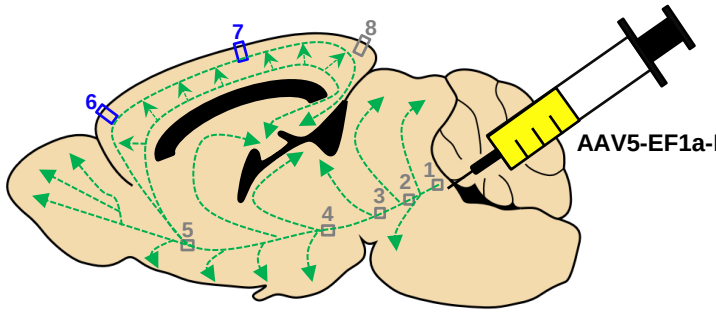


1 week after Saline | 1 week after PCA | 3 month after PCA

Somatosensory Cortex (7)

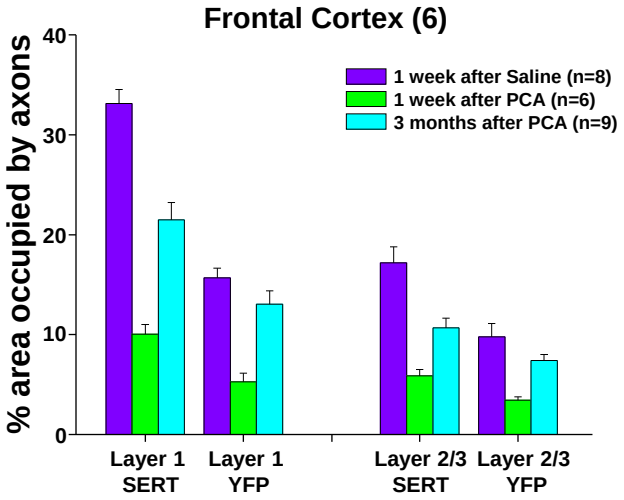


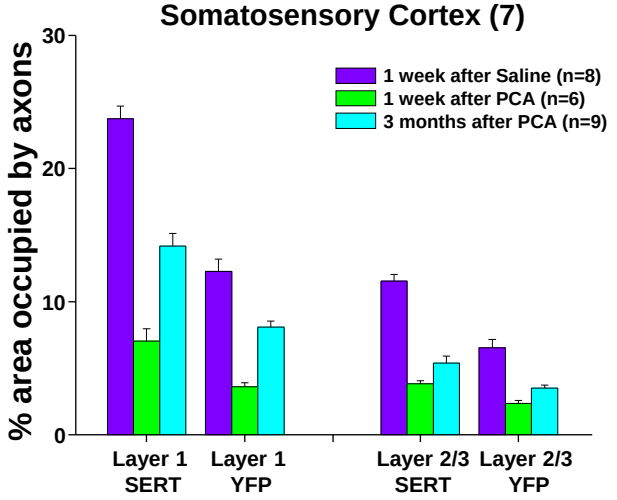
1 week after Saline | 1 week after PCA | 3 month after PCA



AAV5-EF1a-DIO-hChR2(H134R)-EYFP-WPRE-pA

B



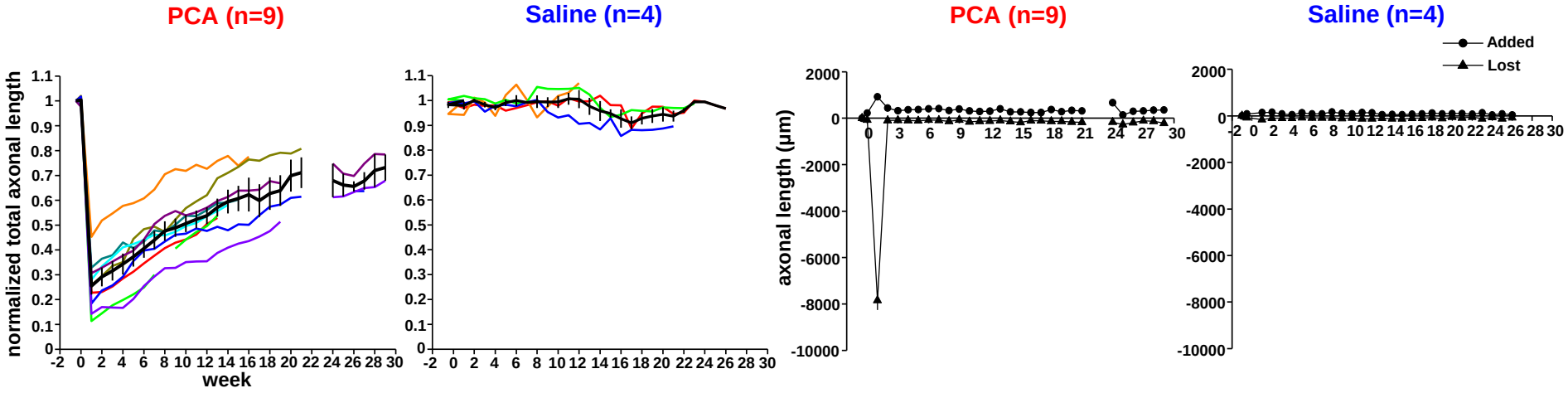


Supplemental Figure 4. PCA lesion evokes degeneration and subsequent recovery of serotonin axons in the neocortex in a mouse in which EYFP expression is no longer under the control of the promoter of the serotonin transporter. Related to Figure 2.

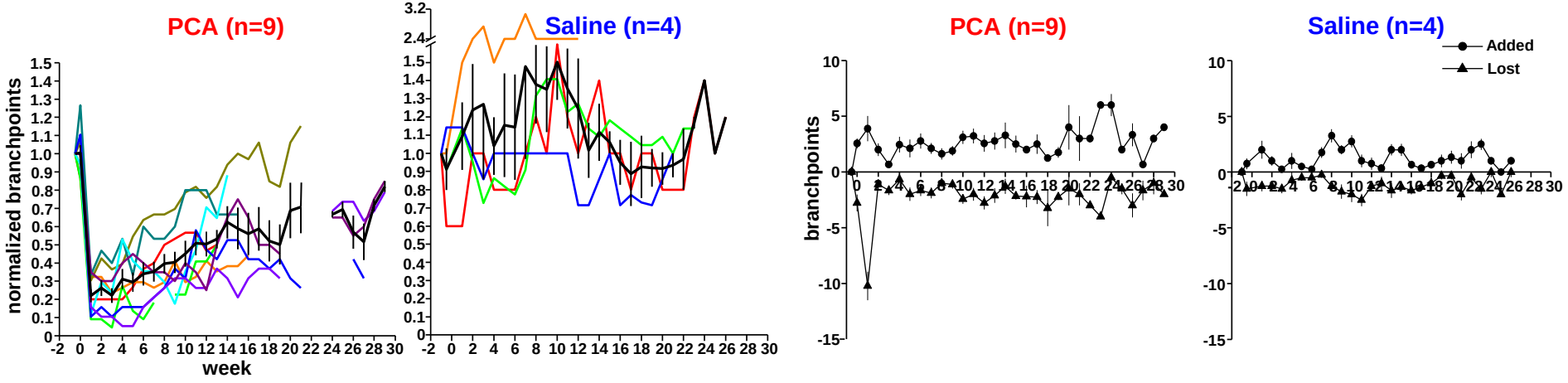
(**A**) Serotonin transporter-Cre mice were injected with the virus AAV5-EF1a-DIO-hChR2(H134R)-EYFP-WPRE-pA to produce irreversible EYFP surface labeling of serotonin neurons. Triple-label immunohistochemistry was performed on sagittal sections of frontal cortex and somatosensory cortex to reveal EYFP positive serotonin axons. These sections were also stained for NeuN (to mark a subset of neuronal nuclei, cyan) and serotonin transporter (to mark serotonin axons, red). The central panel shows a schematic representation of the serotonin neurons of the dorsal raphe and their C-shaped projection to the neocortex in the sagittal plane and the location of virus injection in the dorsal and median raphe. Images were taken from frontal cortex (location 6) and somatosensory cortex (location 7).

(**B**) % area occupied by serotonin transporter (SERT) and YFP immuno-positive axons are quantified in layer 1 and layer 2/3 in frontal and somatosensory cortex are calculated for a population of mice.

A Axonal Length



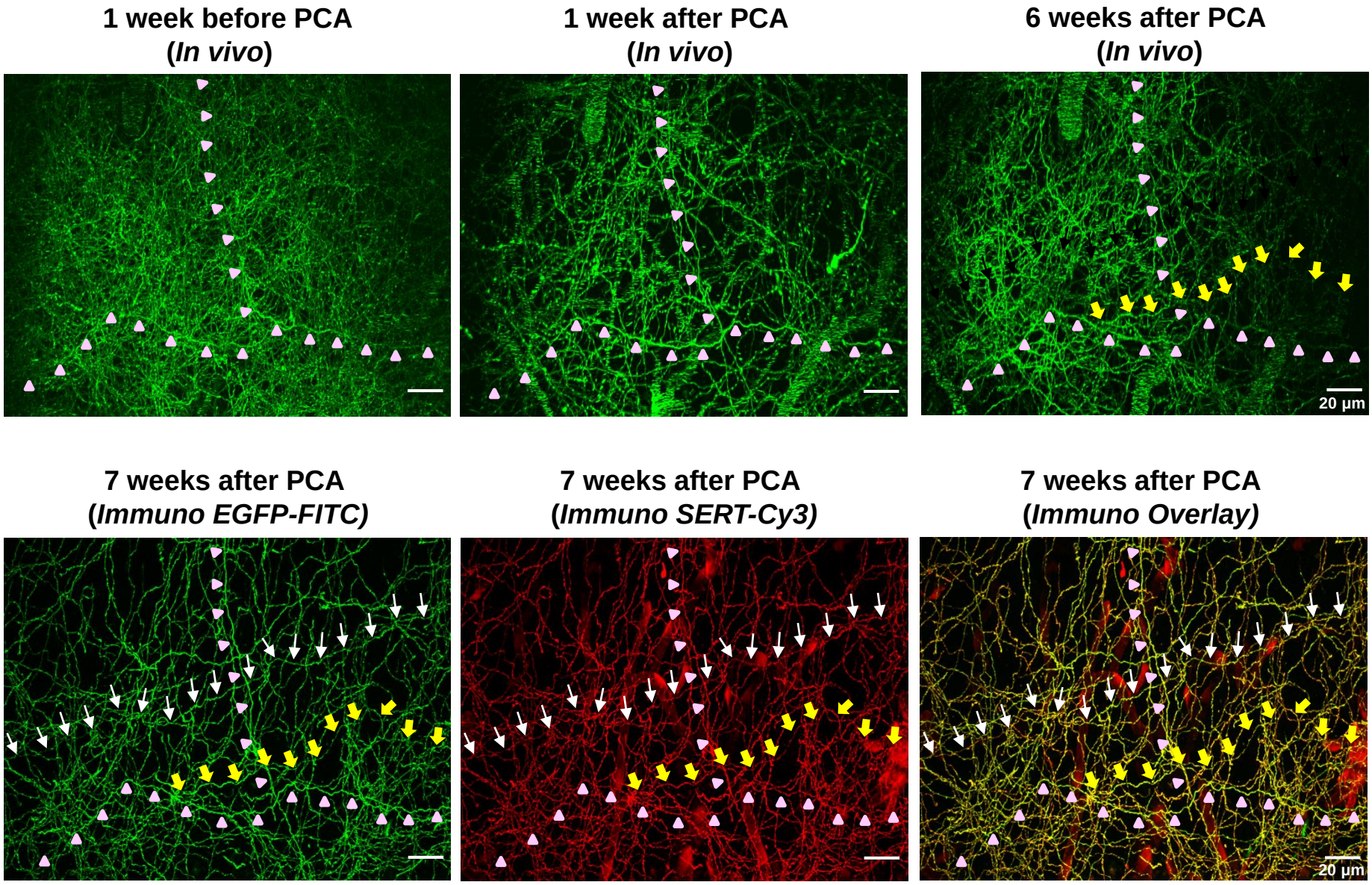
B Axonal Branchpoints



Supplemental Figure 5. Total axonal length and branch points are decreased by PCA treatment and slowly recovered over many weeks, related to Figure 3

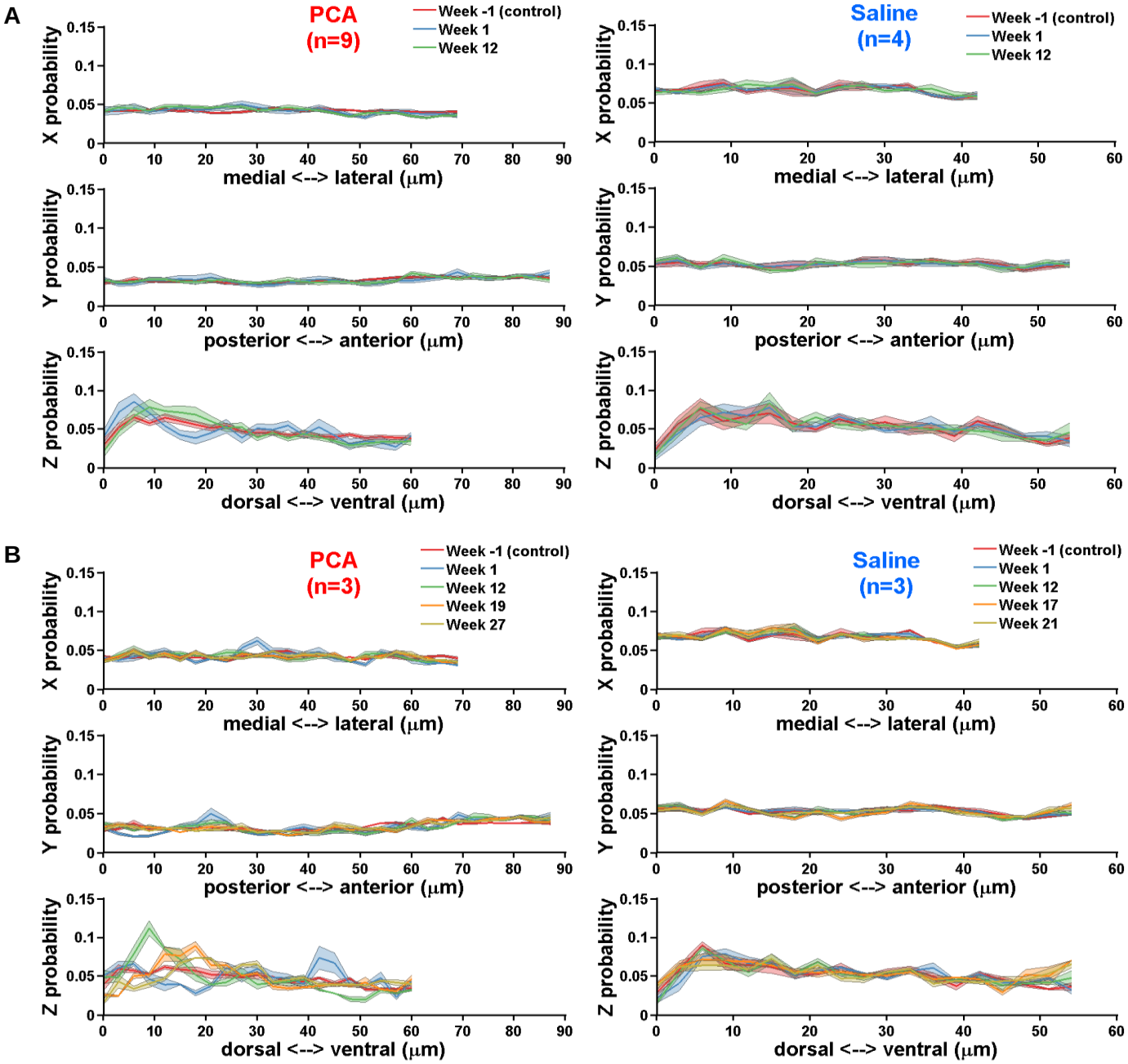
(**A**) Left: Normalized axonal lengths are plotted for PCA and saline treated mice. Each color represents a different mouse. Note that the maximum monitoring time varies among individual mice so that the effective n is reduced at later time points (see also figure 5D). The black line depicts the mean. Right: The mean absolute changes in axonal length from the previous time point are potted for PCA and saline-treated mice.

(**B**) The same style plots as in a are shown here for the number of branch points rather than axonal length.


1 week before PCA
(*In vivo*)
1 week after PCA
(*In vivo*)
6 weeks after PCA
(*In vivo*)
20 μm
7 weeks after PCA
(*Immuno EGFP-FITC*)
7 weeks after PCA
(*Immuno SERT-Cy3*)
7 weeks after PCA
(*Immuno Overlay*)
20 μm
: Survived axon
: Sprouted axon
: New axon

Supplemental Figure 6. New and sprouted axons appearing after PCA treatment are immunopositive for the serotonin transporter (SERT), related to Figure 4

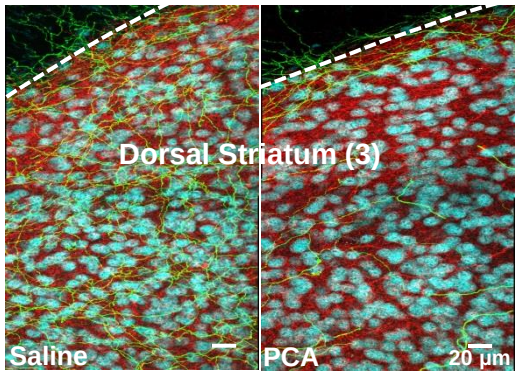
6 weeks after PCA treatment, surviving, sprouted and new axons were imaged *in vivo*. Then, at week 7, the mouse was euthanized and perfused. The same field of view seen in the vivo image was recovered in fixed tissue slices and was stained for serotonin transporter-EGFP (green) and the serotonin transporter protein (red). The overlay of these two color channels shows that survived, sprouted and new axons were all immunopositive for the serotonin transporter.

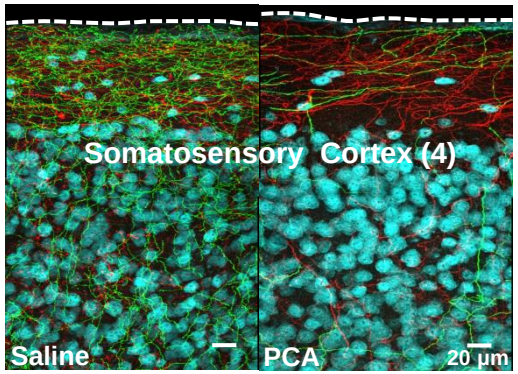

A
PCA
(n=9)
Week -1 (control)
Week 1
Week 12
Saline
(n=4)
Week -1 (control)
Week 1
Week 12
X probability
medial <--> lateral (μm)
Y probability
posterior <--> anterior (μm)
Z probability
dorsal <--> ventral (μm)
B
PCA
(n=3)
Week -1 (control)
Week 1
Week 12
Week 19
Week 27
Saline
(n=3)
Week -1 (control)
Week 1
Week 12
Week 17
Week 21
X probability
medial <--> lateral (μm)
Y probability
posterior <--> anterior (μm)
Z probability
dorsal <--> ventral (μm)

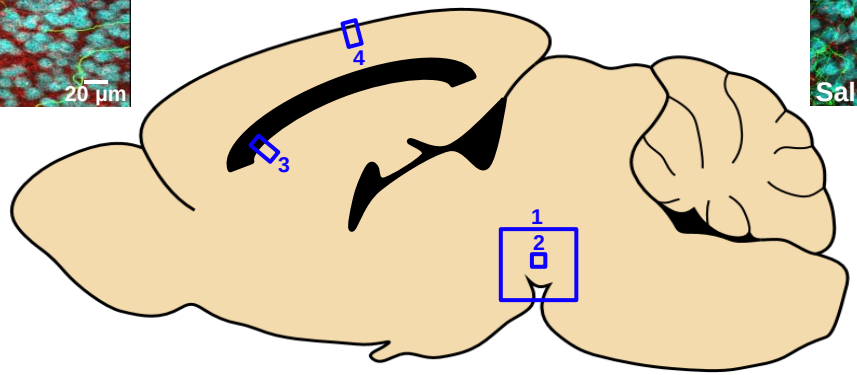
Supplemental Figure 7. The distribution of normalized axonal densities in different imaging planes does not significantly change following PCA lesion or subsequent recovery, related to Figure 5.

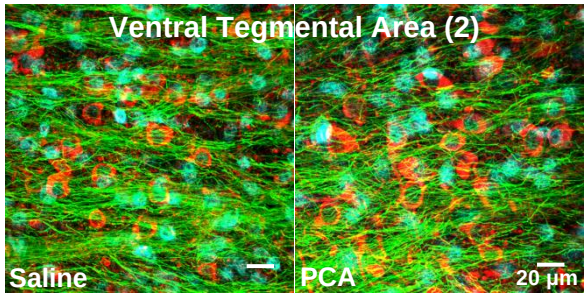
(**A**) The distribution of axons in three different imaging planes (medial-lateral, posterior-anterior and dorsal-ventral) for a number of weeks before and after PCA treatment (left column) and in saline treated animals (right-column). Following PCA treatment, new axons (week 1 and week 12) recapitulate the density seen in both control and in saline treated mice. Most notably, there is a slight increase in density of axons in the most superficial 5-10 µm of the dorsal-ventral plane while in other imaging planes the densities remain flat. N = 9 PCA and 4 saline mice. Analysis divided the planes into 3 µm thick slices. Data were normalized to a probability mass function by dividing by the total number of points in the image.

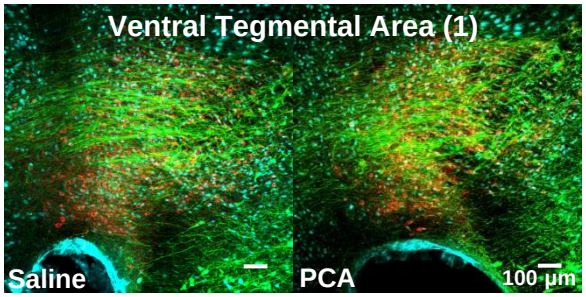
(**B**) The distribution of axons in three different imaging planes for subset of mice for which imaging remained possible for 27 weeks. N = 3 PCA and 3 saline mice.

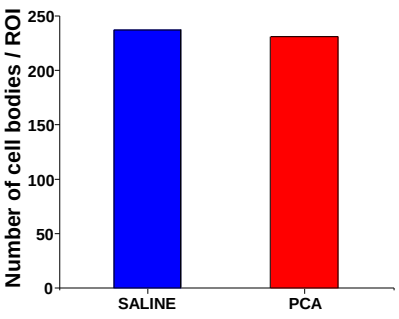

Dorsal Striatum (3)
Saline
PCA
20 µm

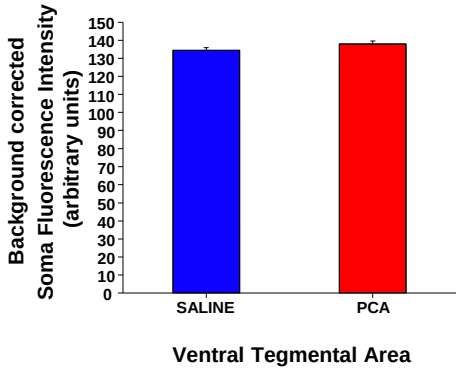

Somatosensory Cortex (4)
Saline
PCA
20 µm

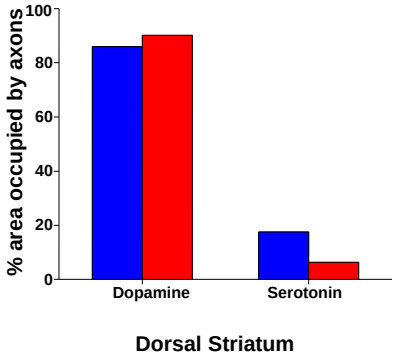

4
3
1
2

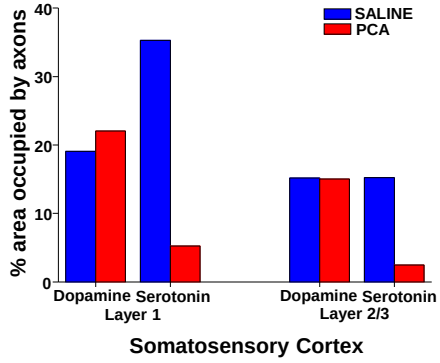

Ventral Tegmental Area (2)
Saline
PCA
20 µm


Ventral Tegmental Area (1)
Saline
PCA
100 µm


Number of cell bodies / ROI
250
200
150
100
50
0
SALINE
PCA

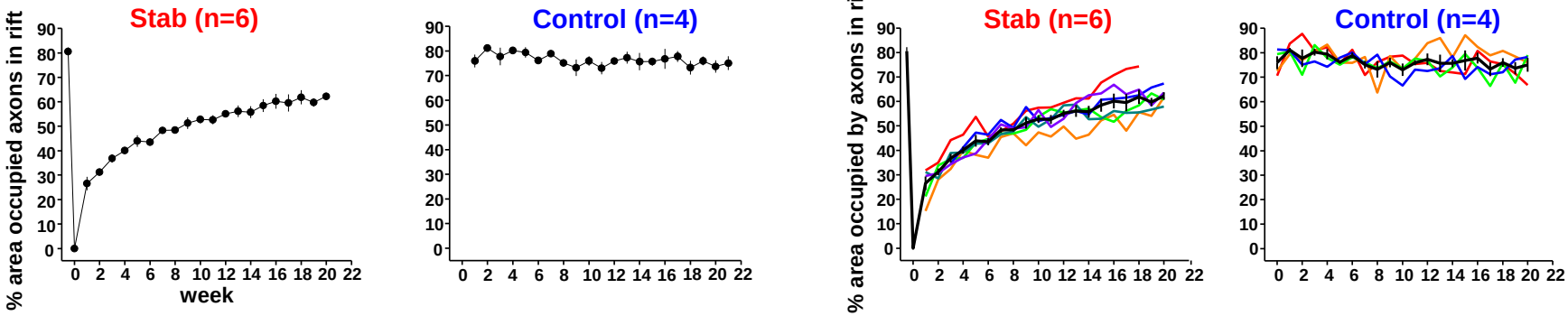

Background corrected Soma Fluorescence Intensity (arbitrary units)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
SALINE
PCA
Ventral Tegmental Area


% area occupied by axons
100
80
60
40
20
0
Dopamine
Serotonin
Dorsal Striatum

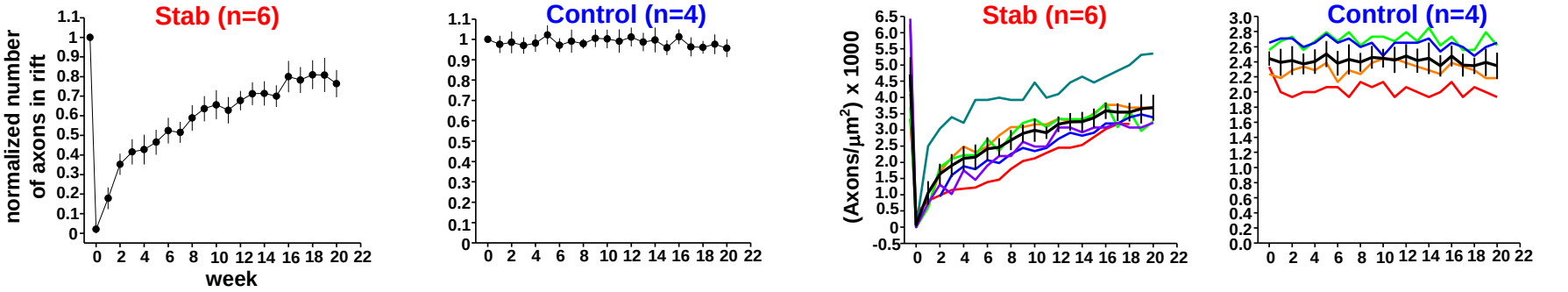

% area occupied by axons
40
30
20
10
0
SALINE
PCA
Dopamine
Serotonin
Layer 1
Dopamine
Serotonin
Layer 2/3
Somatosensory Cortex

Supplemental Figure 8. PCA treatment does not affect dopaminergic neurons, related to Figure 6.
Triple-label immunohistochemistry was performed on sagittal sections of serotonin transporter-EGFP mice. These sections were also stained for NeuN (to mark a subset of neuronal nuclei, cyan) and tyrosine hydroxylase (TH; to mark dopamine neurons, red). Dopamine neuron cell bodies in the ventral tegmental area (1 and 2; sections are ~0.48 – 0.60 mm lateral to the midline), dopamine axons in the dorsal striatum (2; sections are ~1.08 – 1.80 mm lateral to the midline) and somatosensory cortex (4; sections are ~1.08 – 1.80 mm lateral to the midline) were intact 1 week following PCA treatment. The number of cell bodies and background-corrected soma fluorescence intensity are quantified in the ventral tegmental area. The % area occupied axons is quantified for the dorsal striatum, and somatosensory cortex.

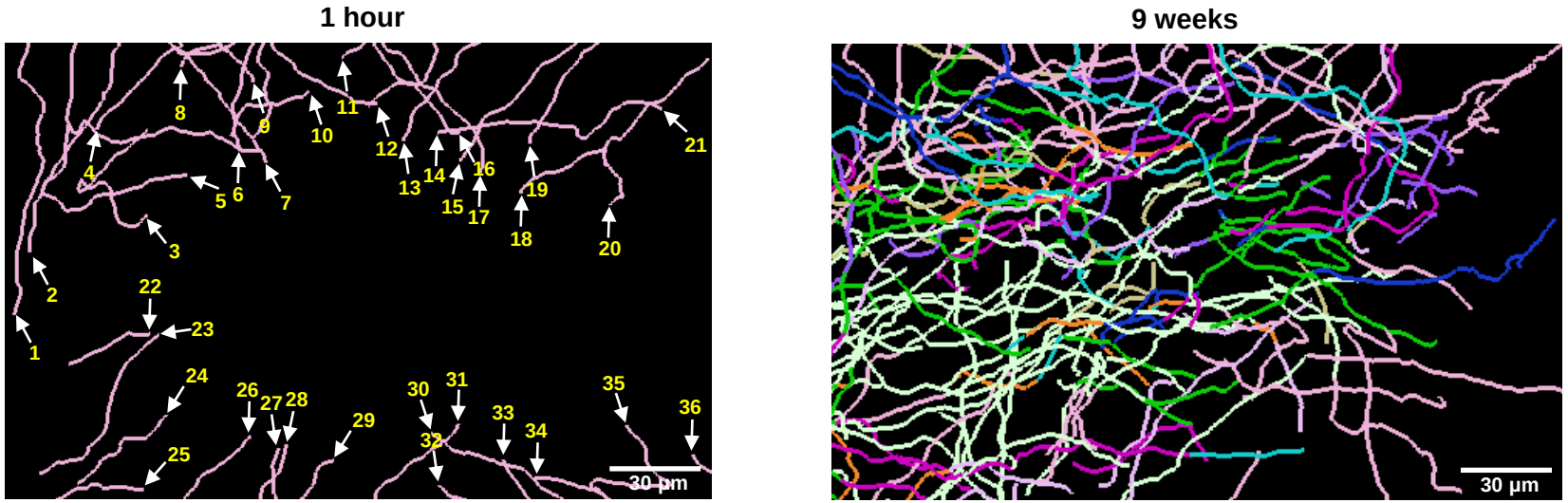
A % area occupied by axons



B Axon Numbers



C



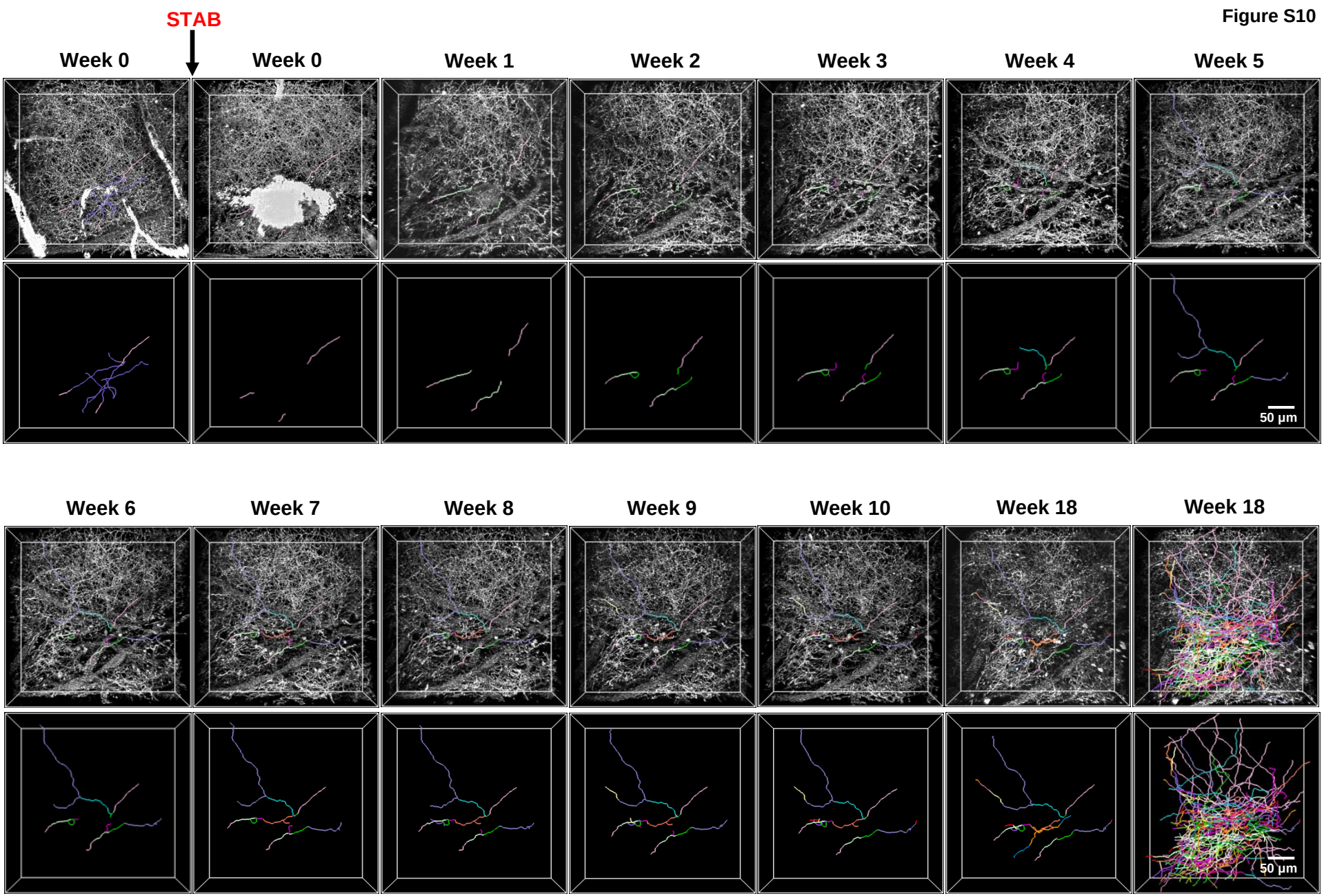
Supplemental Figure 9. Total axonal length and branch points are decreased by stab injury and slow recovery over many weeks, related to Figure 7

(**A**) Left: % area occupied by axons in rift are plotted for stab injured and control mice. Right: Each color represents a different mouse. The black line depicts the mean.

(**B**) Left: Normalized number of axons in rift are plotted for stab injured and control mice. Right: Each color represents a different mouse. The black line depicts the mean.

(**C**) Enlarged and tilted panels from Figure 7A show traced serotonin axons surrounding the stab rift 1 hour and 9 weeks after injury. The 36 individual severed ends of axons are indicated with white arrows and numbers.

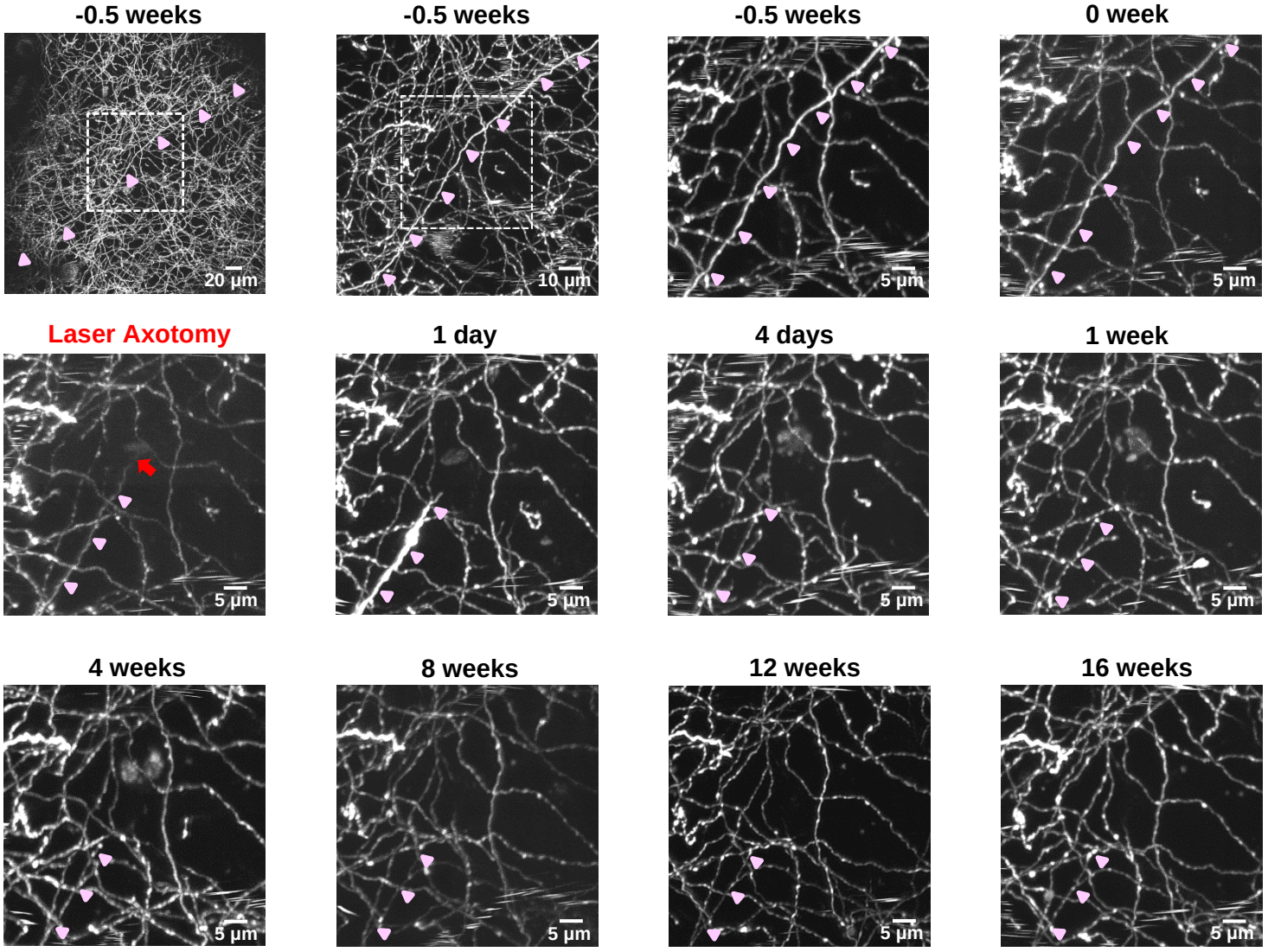
Figure S10


STAB
Week 0
Week 0
Week 1
Week 2
Week 3
Week 4
Week 5
50 µm
Week 6
Week 7
Week 8
Week 9
Week 10
Week 18
Week 18
50 µm

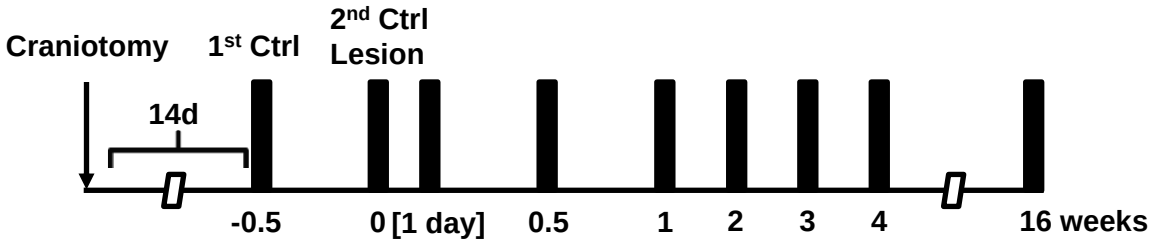
Supplemental Figure 10. By tracing only 3 of 36 severed axons, regrowth from severed ends following a stab injury may be clearly visualized, related to Figure 7.

The top panels show 3-D images of serotonin-EGFP, immediately before, immediately after and weekly for 10 weeks after stab injury in layer 1 of the somatosensory cortex. We also show images from the final time point, 18 weeks after the stab injury. 3 out of 36 axons present are traced and superimposed on the 3-D images. In the first pre-injury panel, the pink segments are the portions of the axons that survive the stab injury and the purple segments are the portions that will be destroyed. Subsequent new growth is coded using a different color for each post-injury week. The final panel shows all 36 labeled axons at week 18, each drawn in a different color. The bottom panels show the corresponding tracings alone. The 3 axons traced here correspond to axons 15, 22 and 29 in Supplemental Figure 9C.

A


-0.5 weeks
-0.5 weeks
-0.5 weeks
0 week
20 µm
10 µm
5 µm
5 µm
Laser Axotomy
1 day
4 days
1 week
5 µm
5 µm
5 µm
5 µm
4 weeks
8 weeks
12 weeks
16 weeks
5 µm
5 µm
5 µm
5 µm

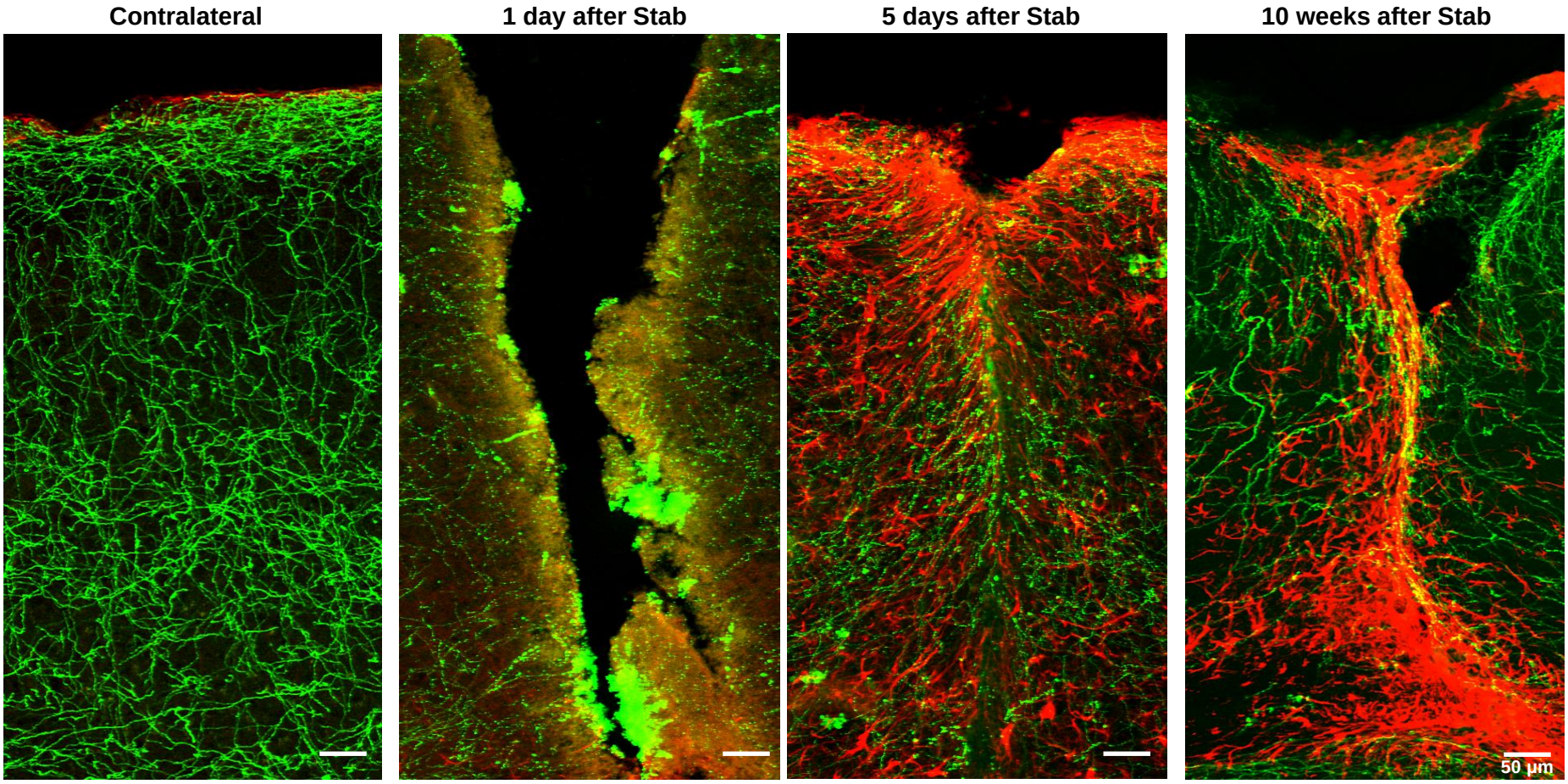
B


Craniotomy
1st Ctrl
2nd Ctrl
Lesion
14d
-0.5
0 [1 day]
0.5
1
2
3
4
16 weeks

Supplemental Figure 11. Serotonin axons fail to regenerate after discrete laser microlesion, related to Figure 8

(**A**) Exemplar maximal z-projection images show a stable serotonin-EGFP axon in layer 1 of somatosensory cortex that was targeted for laser microlesion, indicated by pink arrow heads. The red arrow indicates the position of IR laser beam. The surviving portion of the targeted axon was swollen 1 day after the lesion, but at 4 days after the lesion had returned to a superficially normal. The cut end of this axon was then entirely static measured over a 16 week monitoring period. The red arrow indicates the position of IR laser beam.

(**B**) Experimental time course for long-term imaging before and after laser axotomy.


Contralateral
1 day after Stab
5 days after Stab
10 weeks after Stab
50 µm

Supplemental Figure 12. Stab injury produces persistently activated astrocytes surrounding the stab rift, related to Figure 8. Exemplar sagittal sections showing serotonin-EGFP (green) and GFAP 9 (red) immunoreactivity. Note that at the 10 week time point, serotonin-EGFP axons run adjacent to activated astrocytes in the stab rift.

Supplemental Movie 1. A movie showing rotation of *in vivo* imaging volumes illustrates degeneration and recovery of serotonin axons following PCA treatment , related to Figure 3.

Before PCA (left), 1 week after PCA (middle), 28 week after PCA (right) treatment images are shown in the three dimensional animation. The volume had the dimensions X: 72.85 μm, Y:117.8 μm, Z: 74 μm.