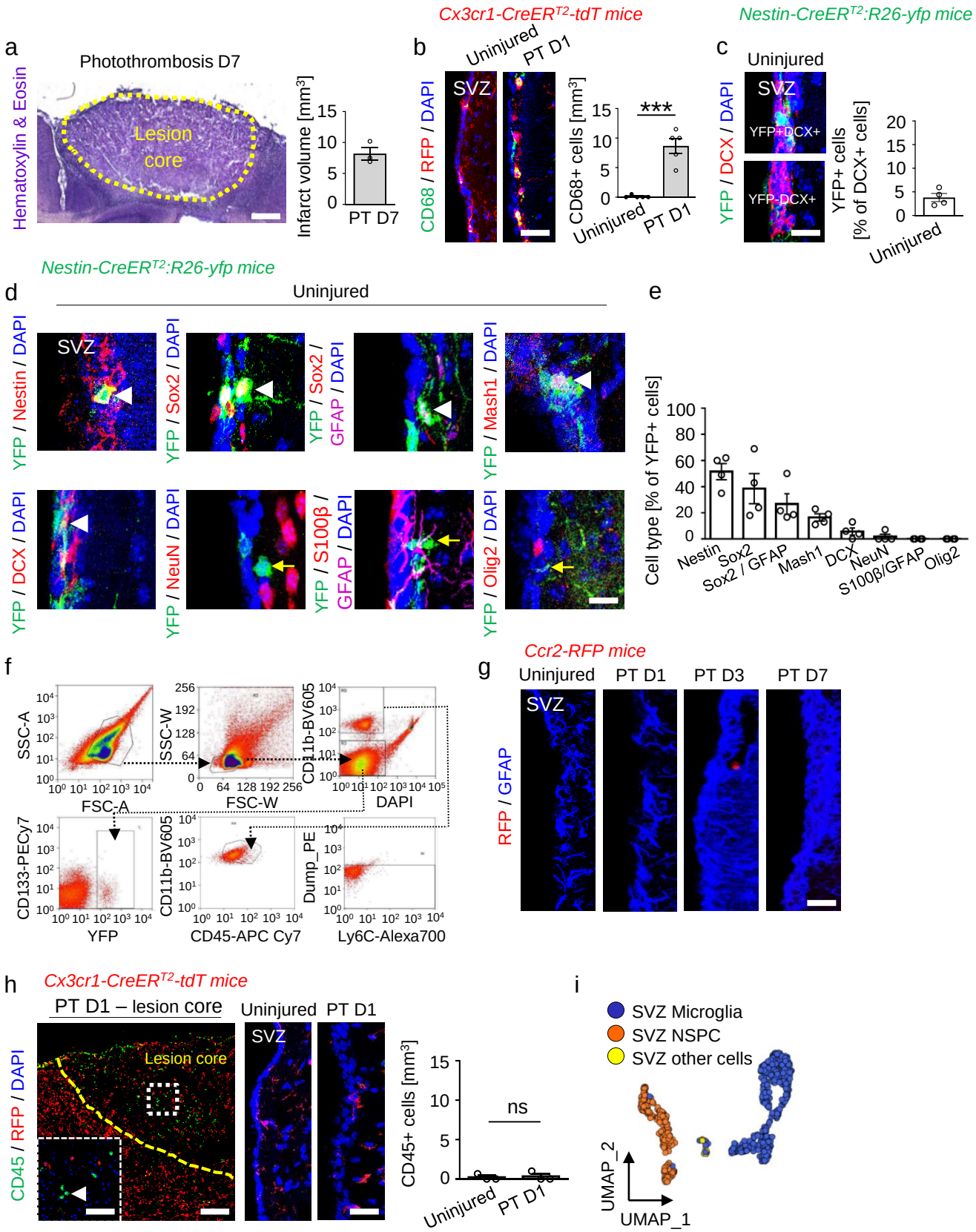
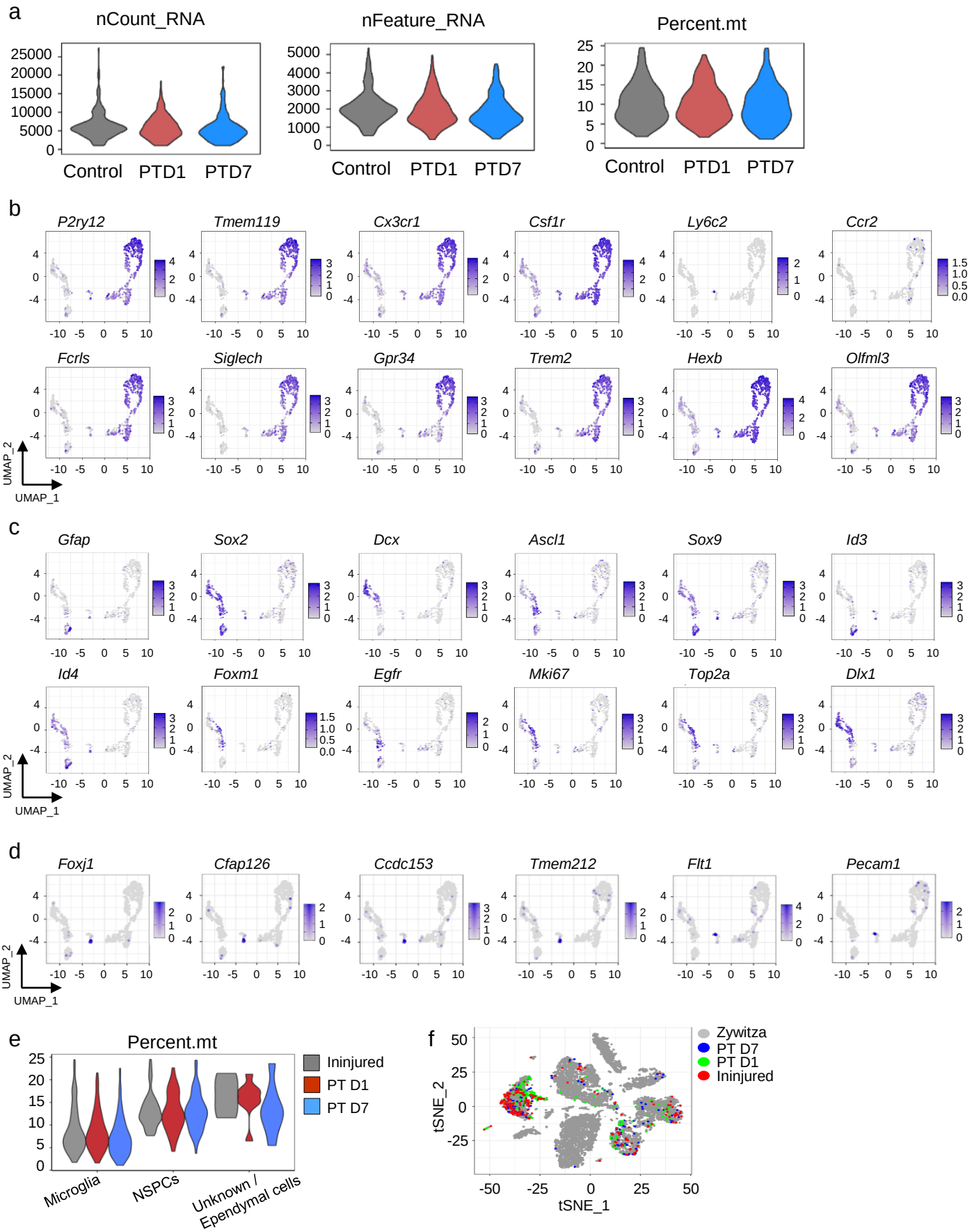
Interaction between subventricular zone microglia and neural stem cells impacts the neurogenic response in a mouse model of cortical ischemic stroke

Nath *et al.*

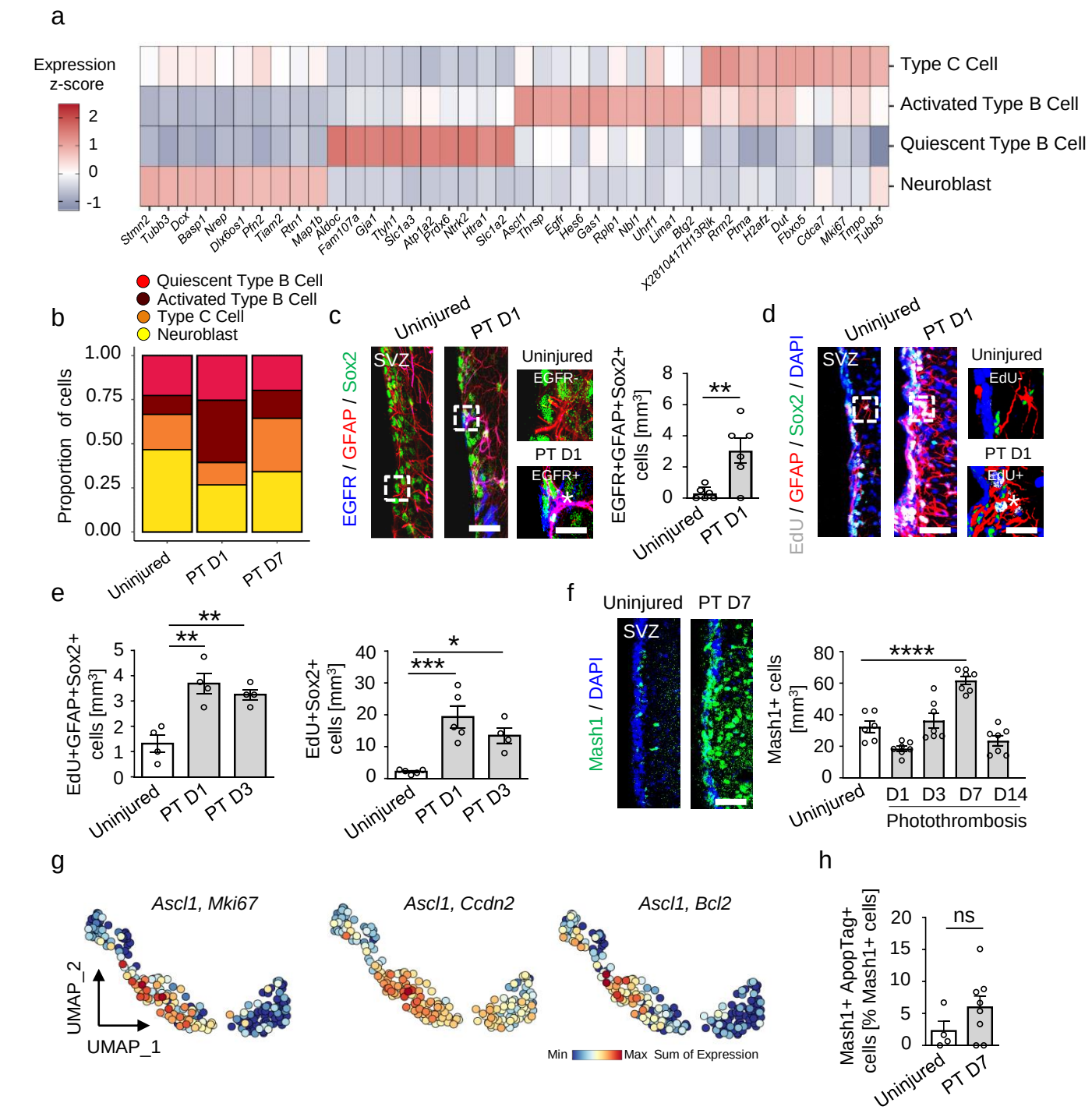
Supplementary Information


a
Photothrombosis D7
Hematoxylin & Eosin
Lesion core
Infarct volume [mm³]
PT D7
Cx3cr1-CreER^T2-tdT mice
b
Uninjured
PT D1
CD68 / RFP / DAPI
SVZ
CD68+ cells [mm³]
***
Uninjured
PT D1
Nestin-CreER^T2:R26-yfp mice
c
Uninjured
YFP / DCX / DAPI
SVZ
YFP+DCX+
YFP-DCX+
YFP+ cells [% of DCX+ cells]
Uninjured
Nestin-CreER^T2:R26-yfp mice
d
Uninjured
YFP / Nestin / DAPI
SVZ
YFP / Sox2 / DAPI
YFP / Sox2 / GFAP / DAPI
YFP / Mash1 / DAPI
YFP / DCX / DAPI
YFP / NeuN / DAPI
YFP / S100β / GFAP / DAPI
YFP / Olig2 / DAPI
e
Cell type [% of YFP+ cells]
Nestin
Sox2
Sox2 / GFAP
Mash1
DCX
NeuN
S100β/GFAP
Olig2
f
SSC-A
FSC-A
SSC-W
FSC-W
CD11b-BV605
DAPI
CD133-PECy7
YFP
CD11b-BV605
CD45-APC Cy7
Dump_PE
Ly6C-Alexa700
Ccr2-RFP mice
g
Uninjured
PT D1
PT D3
PT D7
SVZ
RFP / GFAP
h
Cx3cr1-CreER^T2-tdT mice
PT D1 – lesion core
Uninjured
PT D1
CD45 / RFP / DAPI
Lesion core
SVZ
CD45+ cells [mm³]
ns
Uninjured
PT D1
i
SVZ Microglia
SVZ NSPC
SVZ other cells
UMAP_2
UMAP_1

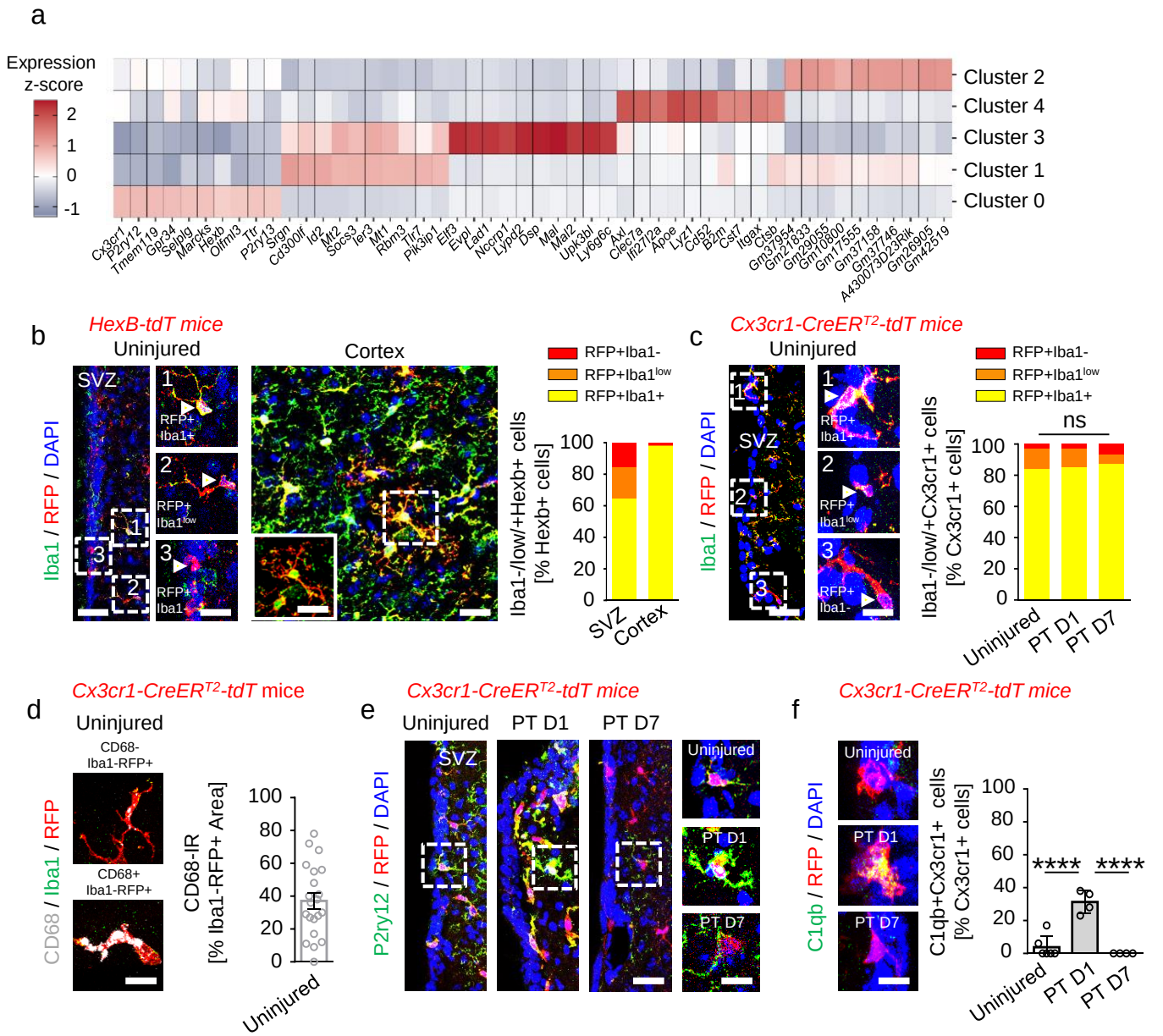
Supplementary Fig. 1. Cluster identification of the scRNA-Seq dataset. a, Hematoxylin and eosin staining of brain sections 7 days after PT. Dotted yellow line delineates lesion core. Scale, 240 µm. Quantification of the infarct volume of mice at day 7 after PT (n = 3 mice). **b**, Immunolabeling for CD68 and RFP in the SVZ 1 day after PT. Scale, 36 µm. Quantification of the number of CD68+ cells in the SVZ 1 day after PT (n = 5). **c**, Immunolabeling for YFP and DCX in the SVZ of uninjured *Nestin-CreER^{T2}:R26-yfp* mice 12 days after the last tamoxifen injection. Scale, 12 µm. Quantification of the percentage of YFP+DCX+ cells relative to all DCX+ cells (n = 4). **d**, Immunolabeling for YFP with Nestin, Sox2, GFAP, Mash1, DCX, NeuN, S100β, and Olig2 labeling in the SVZ of uninjured *Nestin-CreER^{T2}:R26-yfp* mice. Arrowheads indicate YFP+marker+ and arrows indicate YFP+marker− cells for the marker combinations. Scale, 8 µm. **e.** Quantification of percentage of cell types per total YFP+ cells in the SVZ (n = 4). **f**, FACS isolation of SVZ YFP+ NSPCs and CD11b+CD45^{low} microglia. **g**, Immunolabeling for RFP+ blood-derived circulating monocytes (red) in the SVZ at the indicated times after PT and in uninjured *Ccr2-RFP* reporter mice (n = 5 mice). Scale, 18 µm. **h**, Immunolabeling for CD45 and RFP in the lesion core (left) and in the SVZ 1 day after PT. Dashed box indicates magnified area showing CD45+ cells in the lesion core (arrowhead). Scales, 180 µm (lesion core), left; 90 µm, magnification; 30 µm, SVZ. Quantification of CD45+ cells in the SVZ in uninjured mice and 1 day after PT (n = 3 mice). **i**, UMAP representation of 658 individual NSPCs and microglia from the SVZ 1 and 7 days after PT and uninjured mice measured by scRNA-Seq. Each dot represents an individual cell. All plots show the mean ± SEM. ns, no significant; ***P<0.001, unpaired Student's *t*-tests (b, h). SVZ, subventricular zone. Source data are provided as a Source Data file.


a
nCount_RNA
nFeature_RNA
Percent.mt
Control
PTD1
PTD7
b
P2ry12
Tmem119
Cx3cr1
Csf1r
Ly6c2
Ccr2
Fcrls
Siglech
Gpr34
Trem2
Hexb
Olfml3
UMAP_2
UMAP_1
c
Gfap
Sox2
Dcx
Ascl1
Sox9
Id3
Id4
Foxm1
Egfr
Mki67
Top2a
Dlx1
d
Foxj1
Cfap126
Ccdc153
Tmem212
Flt1
Pecam1
e
Percent.mt
Ininjured
PT D1
PT D7
Microglia
NSPCs
Unknown / Ependymal cells
f
Zywitza
PT D7
PT D1
Ininjured
tSNE_2
tSNE_1

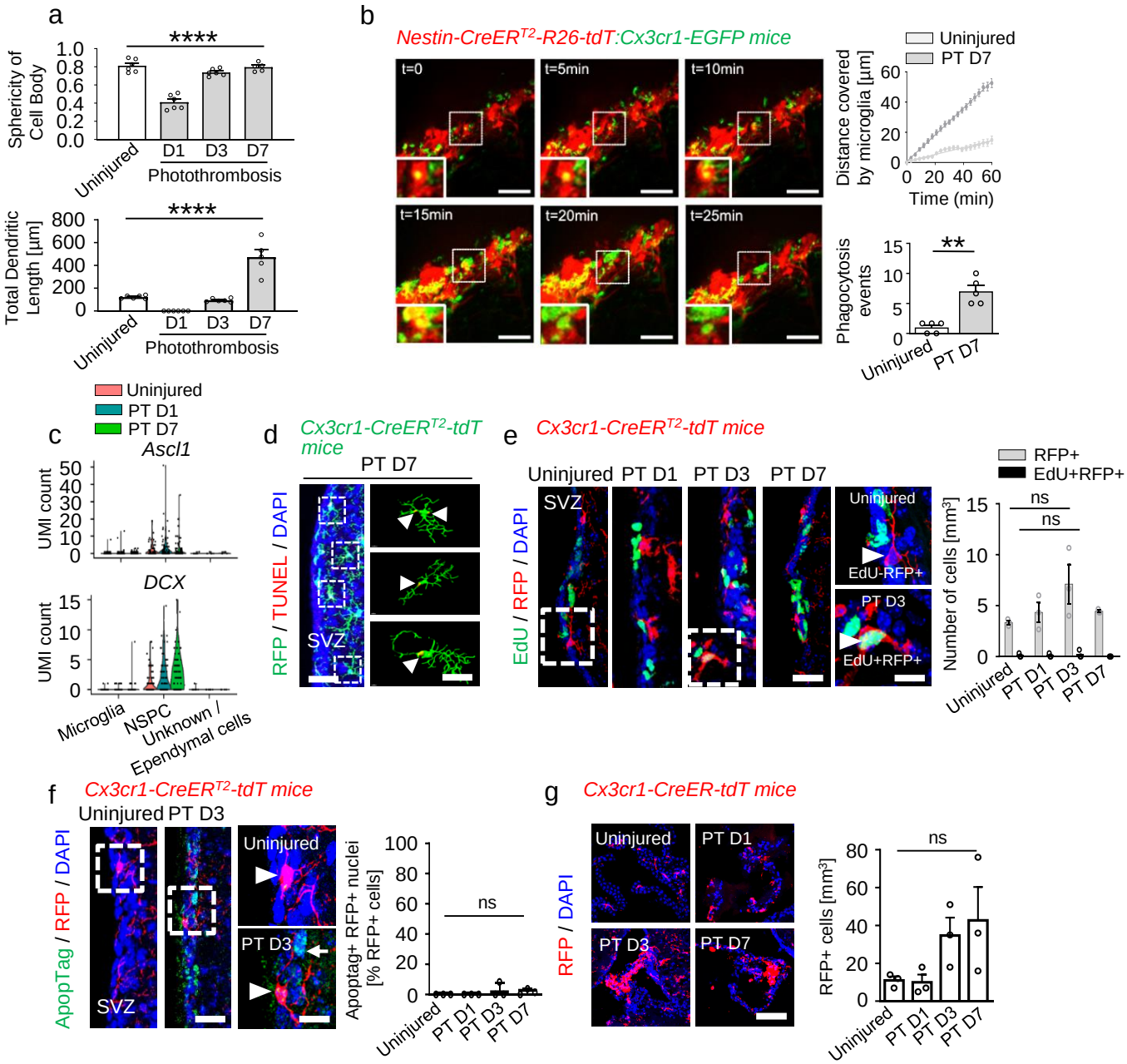
Supplementary Fig. 2. Quality-control metrics and cluster identification for the scRNA-Seq dataset. a, Violin plots depicting number of genes and unique features related to genes, and percentage of transcripts mapping to the mitochondrial genome 1 and 7 days after PT and in the uninjured control. **b**, UMAP plots colored for expression of canonical genes to verify the microglial cluster. **c**, UMAP plots colored for expression of canonical genes to verify the NSPC cluster. **d**, UMAP plots colored for expression of canonical genes to verify the mixed cell population cluster. **e**, Violin plots of mitochondrial genes enriched in different cell populations. **f**, t-SNE plot of microglia cells with the time point of origin, compared with Zywitza's study[11].


a
Expression z-score
2
1
0
-1
Type C Cell
Activated Type B Cell
Quiescent Type B Cell
Neuroblast
Stmn2 Tubb3 Dcx Basp1 Nrep Dlx6os1 Ptn2 Tiam2 Rtn1 Map1b Aldoc Fam107a Gja1 Tlyh1 Slc1a3 Atp1a2 Prdx6 Ntrk2 Htra1 Slc1a2 Ascl1 Thrsp Egfr Hes6 Gas1 Rplp1 Nbl1 Uhrf1 Lima1 Btg2 X2810417H13Rik Rrm2 Ptma H2afz Dut Fbxo5 Cdca7 Mki67 Tmpo Tubb5
b
Quiescent Type B Cell
Activated Type B Cell
Type C Cell
Neuroblast
Proportion of cells
1.00
0.75
0.50
0.25
0.00
Uninjured
PT D1
PT D7
c
Uninjured
PT D1
EGFR / GFAP / Sox2
SVZ
Uninjured
EGFR-
PT D1
EGFR+
EGFR+GFAP+Sox2+ cells [mm3]
**
Uninjured
PT D1
d
Uninjured
PT D1
EdU / GFAP / Sox2 / DAPI
SVZ
Uninjured
EdU-
PT D1
EdU+
e
EdU+GFAP+Sox2+ cells [mm3]
**
**
Uninjured
PT D1
PT D3
EdU+Sox2+ cells [mm3]
*
***
Uninjured
PT D1
PT D3
f
Uninjured
PT D7
Mash1 / DAPI
SVZ
Mash1+ cells [mm3]
****
Uninjured
D1
D3
D7
D14
Photothrombosis
g
Ascl1, Mki67
Ascl1, Ccdn2
Ascl1, Bcl2
UMAP_2
UMAP_1
Min
Max
Sum of Expression
h
Mash1+ ApoptTag+ cells [% Mash1+ cells]
ns
Uninjured
PT D7

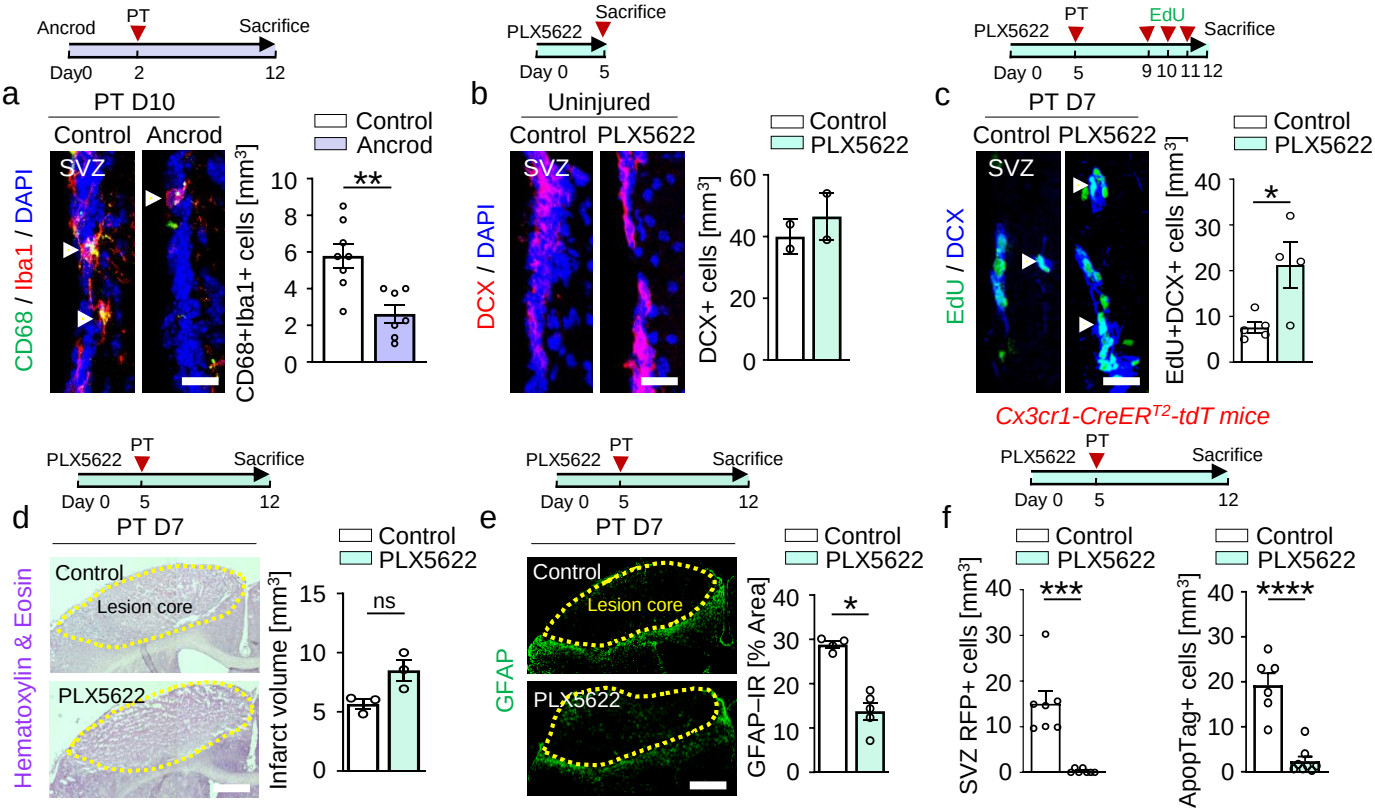
Supplementary Fig. 3. NSPC activation and apoptosis in the SVZ after cortical stroke. **a**, Heat map of the top-10 differentially expressed genes (DEGs) in NSPC clusters. **b**, Quantification of NSPC cluster proportions 1 and 7 days after PT and in uninjured mice. **c**, Immunolabeling for EGFR, GFAP, and Sox2 in uninjured mice and 1 day after PT. The white dashed boxes indicate magnification of EGFR−GFAP+Sox2+ quiescent type B (right, top) and EGFR+GFAP+Sox2+ activated type B cells (star) (right, bottom) in the SVZ in an uninjured mouse and 1 day after PT, respectively. Scales, 25 µm, left; 12 µm, magnification. Quantification of EGFR+GFAP+Sox2+ cells in the SVZ per area 1 day after PT and in uninjured mice (n = 6). **d**, Immunolabeling for EdU, GFAP, and Sox2 in the SVZ in uninjured mice and 1 day after PT. Dashed boxes indicate the magnification of an EdU−GFAP+Sox2+ quiescent type B cell (right, top) and an EdU+GFAP+Sox2+ activated type B cell (star) (right, bottom) in uninjured mice and 1 day after PT, respectively. Scale bars, 27 µm, left; 10 µm, magnification. **e**, Quantification of EdU+GFAP+Sox2+ (n = 4) and EdU+Sox2+ (n = 5 mice, uninjured, PT D1; n = 4, PT D3) cells in the SVZ per area 1 day and 3 days after PT and in uninjured mice. **f**, Immunolabeling for Mash1 (green) in the SVZ 7 days after PT, compared with uninjured mice. Scale, 27 µm. Quantification of Mash1+ cells in the SVZ per area 1, 3, 7, and 14 days after PT, and in uninjured mice (n = 6, uninjured; n = 7, PT D1-D14). **g**, *Ascl1*+ proliferative type C cells co-express *Mki67*, *Ccdn2*, and *Bcl2*, as in the projected expression of the UMAP after PT. **h**, Quantification of Mash1+ApopTag+ cell percentage in the SVZ 7 days after PT, compared with uninjured mice (n = 4, uninjured; n = 8, PT D7). All graphs show the mean ± SEM. *P<0.05, **P<0.01, ***P<0.01, ****P<0.0001, ns not significant, one-way ANOVAs with Bonferroni corrections for multiple comparisons (e, f) and unpaired Student's *t*-tests (c, h). SVZ, subventricular zone. Source data are provided as a Source Data file.


a
Expression z-score
2
1
0
-1
Cluster 2
Cluster 4
Cluster 3
Cluster 1
Cluster 0
Cx3cr1 P2ry12 Tmem119 Gpr34 Selplg Marcks Hexb Olfml3 Ttr P2ry13 Srgn Cd300lf Id2 Mt2 Socs3 Ier3 Mt1 Rbm3 Tlr7 Pik3ip1 Elf3 Evpl Lad1 Nccrp1 Lypd2 Dsp Mal Mal2 Upk3bl Ly6g6c Axl Clec7a Ifi27l2a Apoe Lyz1 Cd52 B2m Csf7 Itgax Clsb Gm37954 Gm21833 Gm29055 Gm10800 Gm17555 Gm37158 Gm37746 A430073D23Rik Gm26905 Gm42519
b
HexB-tdT mice
Uninjured
Cortex
SVZ
Iba1 / RFP / DAPI
RFP+ Iba1+
RFP+ Iba1low
RFP+ Iba1-
Iba1-/low/+Hexb+ cells [% Hexb+ cells]
RFP+Iba1-
RFP+Iba1low
RFP+Iba1+
SVZ
Cortex
c
Cx3cr1-CreERT2-tdT mice
Uninjured
Iba1 / RFP / DAPI
Iba1-/low/+Cx3cr1+ cells [% Cx3cr1+ cells]
ns
Uninjured
PT D1
PT D7
d
Cx3cr1-CreERT2-tdT mice
Uninjured
CD68- Iba1-RFP+
CD68+ Iba1-RFP+
CD68 / Iba1 / RFP
CD68-IR [% Iba1-RFP+ Area]
e
Cx3cr1-CreERT2-tdT mice
Uninjured
PT D1
PT D7
P2ry12 / RFP / DAPI
SVZ
f
Cx3cr1-CreERT2-tdT mice
C1qb / RFP / DAPI
C1qb+Cx3cr1+ cells [% Cx3cr1+ cells]
****
****

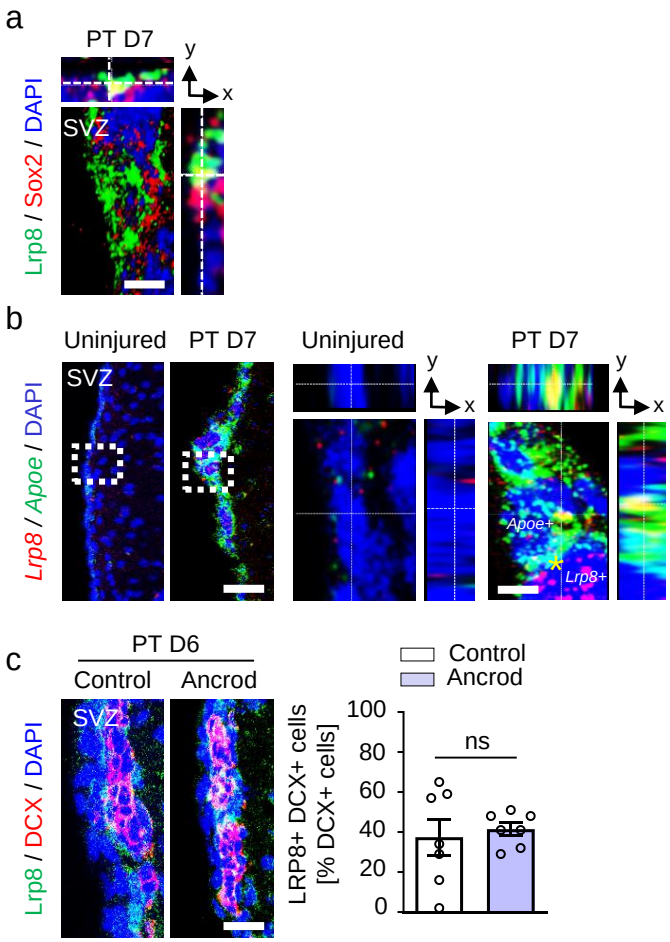
Supplementary Fig. 4. SVZ microglial molecular heterogeneity after PT. a, Heat map of the top-10 DEGs in microglial clusters. **b**, Immunolabeling for Iba1 and RFP in the SVZ (left) and cortex (right) of uninjured *HexB-tdTomato* mice. White boxes indicate magnifications of a RFP+Iba1+ cell (left, top), a RFP+Iba1^{low} cell (left, middle) and a RFP+Iba1− cell (left, bottom) in the SVZ, and a RFP+Iba1+ cell (right, bottom) in the cortex. Examples of each cell type are indicated by arrowheads. Scales, 20 µm, SVZ; 10 µm, magnifications of SVZ cells; 35 µm, cortex; 8 µm, magnification of the cortical cell. Quantification of the percentage of RFP+Iba1+, RFP+Iba1^{low} and RFP+Iba1− cells in the SVZ and cortex of uninjured mice (n = 4 mice). **c**, Immunolabeling for Iba1 and RFP in the SVZ of uninjured *Cx3cr1-CreER^{T2}:R26-tdTomato* mice. Boxes indicate magnifications of RFP+Iba1+ (right, top), RFP+Iba1^{low} cells (right, middle) and a RFP+Iba1− cell (right, bottom) in the SVZ. Examples of each type of cell are indicated by the white arrowheads. Scales, 33 µm, SVZ; 9 µm, magnifications. Quantification of the percentage of RFP+Iba1+, RFP+Iba1^{low}, and RFP+Iba1− cells in the SVZ of uninjured mice and mice 1 day and 7 days after PT (n = 3). **d**, Immunolabeling for CD68, Iba1, and RFP in the SVZ of uninjured *Cx3cr1-CreER^{T2}:R26-tdTomato* mice representing CD68+Iba1−RFP+ (bottom) and CD68−Iba1−RFP+ cells (top). Scale, 12 µm. Quantification of the CD68 immunoreactivity (IR) in Iba1-RFP+ cells in the SVZ of uninjured mice (n = 3 mice, a total of 21 cells were analyzed). **e**, Immunolabeling for P2ry12 and RFP in the SVZ of uninjured *Cx3cr1-CreER^{T2}:R26-tdTomato* mice and mice 1 and 7 days after PT. Boxes indicate magnification of representative cells showing P2ry12 expression at 1 and 7 days after PT and in uninjured mice (n = 3). Scales, 25 µm; 8 µm, magnifications. **f**, Immunolabeling for C1qb and RFP of uninjured *Cx3cr1-CreER^{T2}:R26-tdTomato* mice and mice 1 and 7 days after PT. Scale, 8 µm. Quantification of the percentage of C1qb^{+} cells of uninjured mice and mice 1 and 7 days after PT (n = 6, uninjured; n = 4, PT D1, PT D7). All graphs show the mean ± SEM. ****P<0.0001, ns not significant, one-way ANOVAs with Bonferroni corrections for multiple comparisons. SVZ, subventricular zone. Source data are provided as a Source Data file.


a
Sphericity of Cell Body
****
Uninjured
D1
D3
D7
Photothrombosis
Total Dendritic Length [µm]
****
b
Nestin-CreER^T2-R26-tdT:Cx3cr1-EGFP mice
t=0
t=5min
t=10min
t=15min
t=20min
t=25min
Distance covered by microglia [µm]
Uninjured
PT D7
Time (min)
Phagocytosis events
**
c
Uninjured
PT D1
PT D7
Ascl1
DCX
UMI count
Microglia
NSPC
Unknown / Ependymal cells
d
Cx3cr1-CreER^T2-tdT mice
PT D7
RFP / TUNEL / DAPI
SVZ
e
Cx3cr1-CreER^T2-tdT mice
Uninjured
PT D1
PT D3
PT D7
EdU / RFP / DAPI
EdU-RFP+
RFP+
EdU+RFP+
Number of cells [mm³]
ns
f
Cx3cr1-CreER^T2-tdT mice
Uninjured
PT D3
ApopTag / RFP / DAPI
Apoptag+ RFP+ nuclei [% RFP+ cells]
g
Cx3cr1-CreER-tdT mice
RFP / DAPI
RFP+ cells [mm³]

Supplementary Fig. 5. Activated SVZ microglia phagocytose dying neuroblasts after PT. a, IMARIS-based automatic quantification of cell morphology of GFP+ microglia 1, 3, and 7 days after PT (n = 6, uninjured, PT D1, PT D3; n = 5, PT D7). **b**, Time-lapse imaging montages revealed phagocytosis of tdT+ NSPCs by GFP+ microglia in the SVZ of *Nestin-CreER^{T2}-R26-tdTomato:Cx3cr1-EGFP* mice. Magnification of regions indicated shows phagocytosis of an NSPC by a microglial cell (bottom insets). Scale, 10 µm. Quantification of velocity dynamics of microglia and phagocytosis events 7 days after PT compared with uninjured control in SVZ acute slices (velocity: n = 2 (35 cells), uninjured; n = 3 (62 cells), 7 days after PT; phagocytosis events: n = 2, uninjured (5 slices); n = 3, 7 days after PT (5 slices). **c**, Analysis of NSPC RNA in microglia. Violin plots for the type C cell and neuroblast genes *Ascl1* and *Dcx* in SVZ microglia. **d**, Immunolabeling for RFP and TUNEL dying cells 7 days after PT. Dashed boxes indicate 3-D IMARIS reconstruction of the spatial localization of TUNEL+ dying cell fragments revealing microglia phagocytosis of dying cells (right, arrowheads) (n = 3). Scales, 30 µm, left; 5 µm, magnifications. **e**, Immunolabeling for EdU and RFP in the SVZ 1, 3, and 7 days after PT and uninjured mice. Dashed boxes indicate magnification of an EdU−RFP+ microglial cell (right, top, arrowhead) and an EdU+RFP+ proliferating microglial cell (right, bottom, arrowhead) in the SVZ in uninjured mice and 3 days after PT, respectively. Scales, 48 µm, left; 14 µm, magnifications. Quantification of RFP+ and EdU+RFP+ cells in the SVZ 1, 3, and 7 days after PT compared to uninjured mice (n = 3). **f**, Immunolabeling for ApopTag and RFP in the SVZ 3 days after PT and uninjured mice. Dashed boxes indicate magnification of ApopTag−RFP+ microglia (arrowheads). An ApopTag+ cell is indicated by an arrow. Scales, 24 µm, left; 12 µm, magnifications. Quantification of the percentage of ApopTag+RFP+ cells in the SVZ 1, 3, and 7 days after PT, compared with uninjured mice (n = 3 mice). **g**, Immunolabeling for RFP in the choroid plexus 1, 3, and 7 days after PT, compared with uninjured mice. Scale, 80 µm. Quantification of RFP+ cells in the choroid plexus in uninjured mice and at different times after PT (n = 3). All graphs show the mean ± SEM. **P<0.01, ****P<0.0001, ns, not significant, one-way ANOVAs with Bonferroni corrections for multiple comparisons (a, e-g), Mann-Whitney test (b). SVZ, subventricular zone. Source data are provided as a Source Data file.



Supplementary Fig. 6. Depletion of SVZ microglia increases the newborn neuroblast cell number after PT. a, Immunolabeling for CD68 and Iba1 in the SVZ of fibrinogen-depleted mice and control mice 10 days after PT. Arrowheads indicate CD68+Iba1+ cells. Scale, 16 µm. Quantification of CD68+Iba1+ cells in the SVZ of fibrinogen-depleted mice and control mice 10 days after PT (n = 8, control; n = 7, ancrod). **b**, Immunolabeling for DCX (red) in the SVZ of PLX5622-fed or control uninjured mice. Scale, 13 µm. Quantification of DCX+ cells in the SVZ in PLX5622-fed or control uninjured mice (n = 2). **c**, Immunolabeling for EdU and DCX in the SVZ of PLX5622-fed or control mice 7 days after PT. Arrowheads indicate EdU+DCX+ cells. Scale bar, 19 µm. Quantification of EdU+DCX+ cells in the SVZ in PLX5622-fed and control mice 7 days after PT (n = 5 mice, control; n = 4 mice, PLX5622). **d**, Hematoxylin and eosin staining of the brain cortex 7 days after PT in PLX5622-treated and control mice. Area delineated by dotted line corresponds to lesion core. Scale, 500 µm. Quantification of infarct volume (n = 3). **e**, Immunolabeling for GFAP in the cortex 7 days after PT in PLX5622-treated and control mice. Area delineated by the dotted yellow line corresponds to the lesion core. Scale bar, 600 µm. Quantification of GFAP-immunoreactivity in the glial border (n = 4, control; n = 5, PLX5622). **f**, Quantification of RFP+ microglia and ApopTag+ cells in the SVZ in PLX5622-fed mice 7 days after PT, compared with control group 7 days after PT (n = 7 mice for microglial quantification (left graph); n = 6, control; n = 8, PLX5622 for ApopTag quantification (right graph)). All graphs show the mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, ns, not significant, Mann-Whitney test (d-e), and unpaired Student's *t*-tests (a, c, f). SVZ, subventricular zone. Source data are provided as a Source Data file.



Supplementary Fig. 7. Lrp8 and ApoE expression in SVZ NSPCs and microglia after PT. a, Representative immunolabeling image showing Lrp8 (green) and Sox2 (red) in the SVZ 7 days after PT (n = 4 mice). Scale bar, 10 µm. **b**, *In situ* hybridization for *Lrp8* (red) and *Apoe* (green) 7 days after PT, compared with uninjured mice. White dashed boxes indicate the magnification of an *Lrp8*+ cell next to a *Apoe*+ cell (yellow asterisk) in the SVZ 7 days after PT, compared with *Lrp8*− and *Apoe*− cells in the SVZ of uninjured mice (n = 2 mice, uninjured; n = 4 mice, PT D7). Scale bars, 27 µm, left; 7 µm, right. **c**, Immunolabeling for Lrp8 (green) and DCX (red) in the SVZ in fibrinogen-depleted mice compared with control-treated mice 6 days after PT. Scale bar, 20 µm. Quantification of the percentage of Lrp8+DCX+ cells in fibrinogen-depleted mice compared with control-treated mice 6 days after PT (n = 7 mice). The plot shows the mean ± SEM. ns = not significant, unpaired Student's *t*-tests. SVZ, subventricular zone. Source data are provided as a Source Data file.