Supplementary Figures

Acute brain injuries trigger microglia as an additional source of the proteoglycan NG2

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Supplementary Figure S1. Assessment of infarct-affected area in the brain.

**a and b** Images showing the TTC staining of coronal brain sections at rostral (a) and caudal (b) level 24 h after tMCAO. c Proportion of ischemic areas (as indicated in a and b by dashed line, n = 5 mice). d and e Bar graphs quantify the percentage of microgliosis (as area) indicated by enhanced EGFP expression (d), and the relative fluorescent intensity (FI) of the ipsilateral brain normalized to the contralateral part of coronal sections (e) from mice in Figure 1f and 1g. f and g Data points of the density of tdT+ EGFP+ cells in striatum shown in Figure 1g were plotted with the corresponding data points in d and e. No obvious correlation between the density of tdT+ EGFP+ cells and the extent of microgliosis from each experimental time points could be detected.
Supplementary Figure S2. After SWI tdT+ EGFP+ cells were not derived from spontaneous recombination or pre-existing NG2-expressing cells.

a Overview of transgene structures of NG2\textsuperscript{tdT}xCXCR\textsuperscript{EGFP} mice used in SWI model. b NG2\textsuperscript{tdT}xCXCR\textsuperscript{EGFP} mice were used for SWI and analyzed at 3 or 7 dpi, without tamoxifen treatment (for c). In addition, NG2\textsuperscript{tdT}xCXCR\textsuperscript{EGFP} mice were also injected with tamoxifen 70 days prior to SWI and analyzed at 3 or 14 dpi (for d/e). c Micrographs showing only sporadic tdT expression at the lesion site and never in EGFP+ cells without tamoxifen induced recombination. d and e Confocal images showing EGFP+ (triangles), tdT+ glial cells (open arrowheads), and tdT+ pericytes (open triangles) at the lesion sites at 3 (d) or 14 (e) dpi. EGFP and tdT signals were never co-localized as shown by orthogonal views of the white boxes, indicating that tdT+ cells did not differentiate into EGFP+ cells. Scale bars = 20 μm.
Supplementary Figure S3. Microglial phagocytosis of dying tdT⁺ cells.

a Overview of transgene structures of NG2<sup>tdT⁻xCXCR<sup>EGFP</sup> mice used in SWI model. b After SWI, NG2<sup>tdT⁻xCXCR<sup>EGFP</sup> mice were injected with TAM from 3-5 dpi to induce Cre activity, and subsequently analyzed at 7, 14, or 28 dpi. c-e Confocal images showing EGFP⁺ (triangles) and tdT⁺ cells (open triangles) along the lesion site of NG2<sup>tdT⁻xCXCR<sup>EGFP</sup> mice at 7 (c), 14 (d), and 28 (e) dpi. Notably, some condensed tdT⁺ particles trapped in EGFP⁺ cells (open arrowheads) could be always observed (c-e), indicating dying tdT⁺ cells being phagocytosed by EGFP⁺ microglia. Please note, that no overlay of tdT and EGFP can be observed. Orthogonal views of selected phagocytosing EGFP⁺ cells (white cross) were shown at the right panel. Scale bars = 20 μm.