Supplemental Figure for

PTBP1-Mediated Inhibition of circSCMH1 Biogenesis Impairs Brain Recovery after Ischemic Stroke

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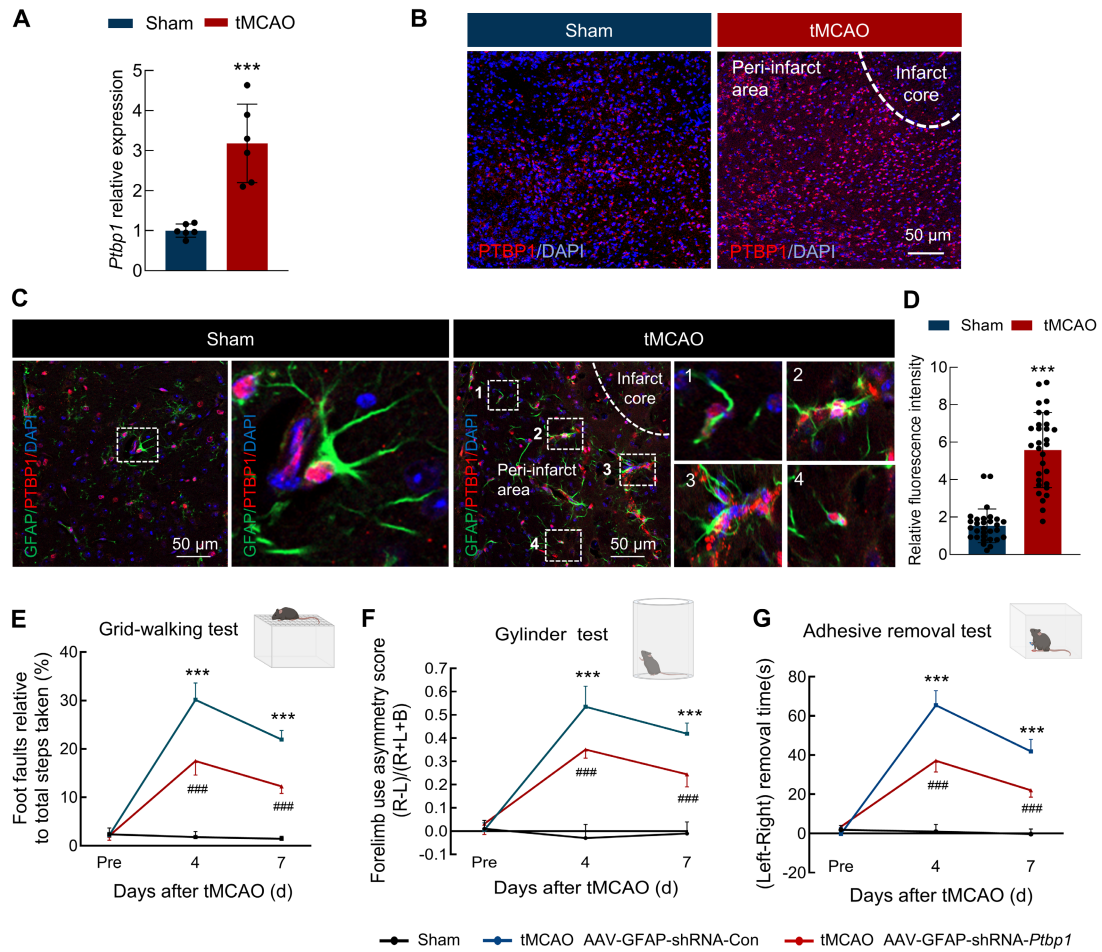
Supplemental Figure 1. PTBP1 functions as an RNA-binding protein for circSCMH1 and significantly increases in the infarct area in tMCAO stroke model. (A) The expression of *Ptbp1* in the peri-infarct area of tMCAO mice compared with that in sham mice by real-time PCR. n=6 samples/group. $^{***}P < 0.001$ versus Sham group. **(B)** The immunofluorescence staining of PTBP1 in the peri-infarct area of tMCAO mice compared with that in sham mice. Scale bar: 50 μm. **(C-D)** The immunofluorescence staining of PTBP1 in astrocytes on the peri-infarct area of tMCAO mice compared with that in sham mice. $^{***}P < 0.001$ versus sham group. **(E-G)** AAV-GFAP-shRNA-*Ptbp1* improved behavioral recovery at different time points after tMCAO mouse stroke model, as measured by the grid walking test **(E)**, cylinder test **(F)**, and adhesive removal test **(G)**. n = 12/group. All data are presented as the mean ± SD. $^{***}P < 0.001$ versus Sham group, $^{###}P < 0.001$ versus tMCAO AAV-GFAP-shRNA-Con group.
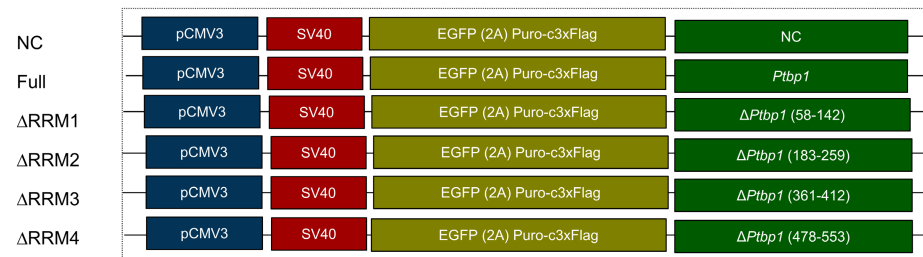
Supplemental Figure 2. Schematic diagram of *Ptbp1* plasmid construction.
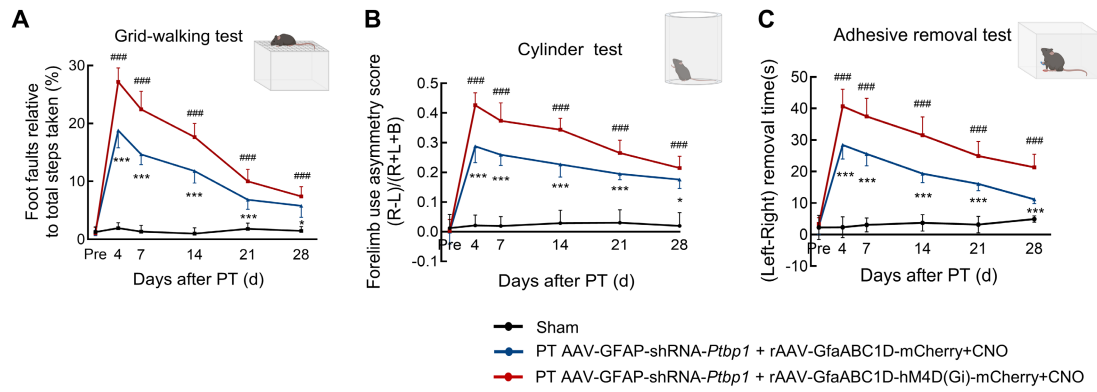
Supplemental Figure 3. Loss of function in astrocytes markedly diminished the therapeutic efficacy of AAV-GFAP-shRNA-*Ptbp1*. (A-C) AAV-GFAP-shRNA-*Ptbp1* improved behavioral recovery at different time points after PT mouse stroke model, as measured by the grid walking test **(A)**, cylinder test **(B)**, and adhesive removal test **(C)**. n = 12/group. All data are presented as the mean ± SD. *P <0.05, ***P <0.001 versus Sham group, #P<0.05, ##P<0.01, ###P<0.001 versus PT AAV-GFAP-shRNA-*Ptbp1*+ rAAV-GfaABC1D-mCherry+CNO.
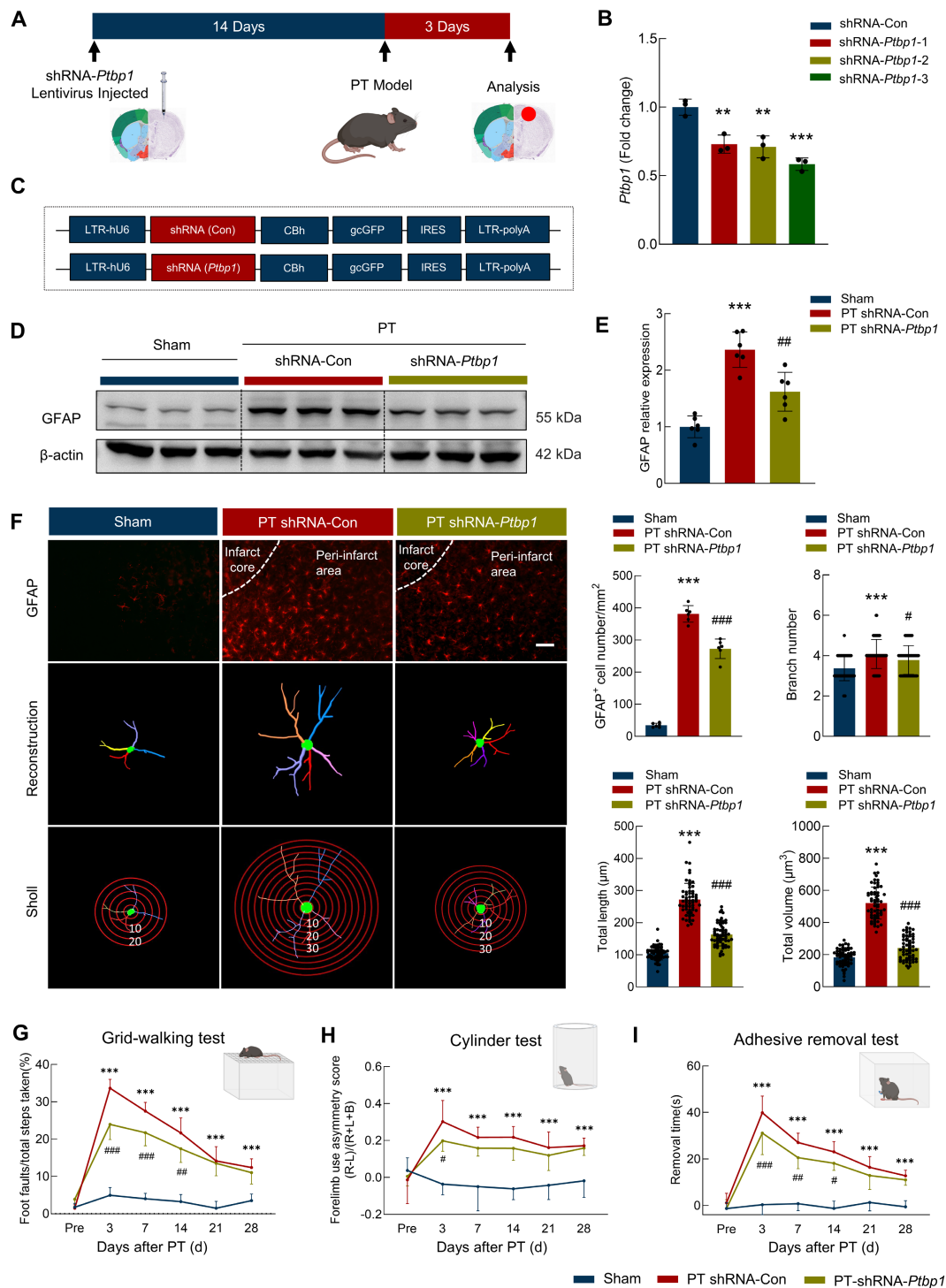
Supplemental Figure 4. Downregulating PTBP1 significantly inhibits the activation of astrocytes and promotes the function recovery after ischemic stroke. (A) Timeline of the experimental procedure in the PT mouse model. **(B)** Expression of *Ptbp1* transduced with shRNA-*Ptbp1*-1/2/3. ******P <0.01, *******P <0.001 versus shRNA-Con group. **(C)** Schematic of the lentivirus encoding *Ptbp1*. **(D-E)** Western blot analysis of GFAP expression after PT stroke model. Three representative immunoblots from 6 mice/group are presented. ***P<0.001 versus Sham group. $^{##}$P<0.01 versus PT+shRNA-Con group. **(F)** Effect of PTBP1 on the astrocyte activation. Representative images of astrocyte immunostaining for GFAP in the peri-infarct area, followed by

3D reconstruction and Sholl analysis, branch number, total length, and total volume. 60 cells/group. Scale bar: 50 μm $^{***}P$ <0.001 versus Sham group. $^{#}P$ <0.05, $^{###}P$ <0.001 versus PT+shRNA-Con group. **(G-I)** lentivirus-shRNA-*Ptbp1* improved behavioral recovery at different time points after stroke, as measured by the grid walking test **(G)**, cylinder test **(H)**, and adhesive removal test **(I)**. n = 12/group. All data are presented as the mean±SD. n=6 mice/group, $^{***}P$ <0.001 versus Sham group.

$^{#}P < 0.05$, $^{##}P < 0.01$, $^{###}P < 0.001$ versus PT shRNA-Con group.
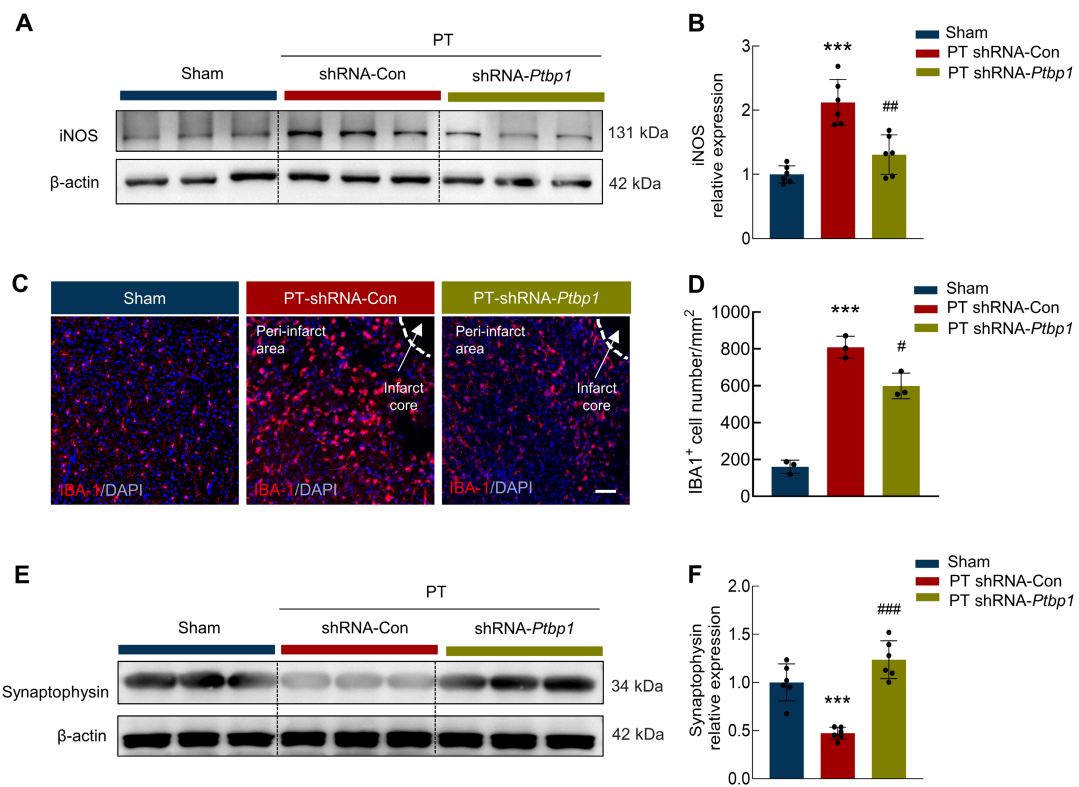
Supplemental Figure 5. Downregulating PTBP1 reduces neuroinflammation and promotes the neuroplasticity. (A-B) Western blot analysis of iNOS expression after PT stroke model. Three representative immunoblots from 6 mice/group are presented. $^{***}P$ <0.001 versus Sham group. $^{##}P$ <0.01 versus PT+shRNA-Con group. **(C-D)** Immunohistochemistry using antibodies against IBA-1. Scale bar: 50 μm. $^{***}P$ <0.001 versus Sham group. $^{#}P$<0.05 versus PT+shRNA-Con group. **(E-F)** Western blot analysis of synaptophysin expression after PT stroke model. $^{***}P$ <0.001 versus Sham group. $^{###}P$ <0.001 versus PT+shRNA-Con group. Three representative immunoblots from 6 mice/group are presented.