SUPPLEMENTAL MATERIAL

Supplemental Methods

Western Blotting Analysis

MSCs cultured in 100-mm dishes were detached by scraping and sonicated for 35 seconds in ice-cold lysis buffer. Lysates were measured for protein concentrations as previously described.[1] Equal amounts of protein from each sample were loaded into a 4-12% Nupage Bis-Tris gel (Invitrogen, Eugene, OR), and subjected to electrophoresis. After separation, proteins were transferred to a nitrocellulose membrane (Invitrogen), blocked with Odyssey blocking buffer (LI-COR, Lincoln, NE) for one hour at room temperature, and incubated overnight at 4°C with a primary antibody against CXCR4 (1:500; Abcam, Cambridge, MA), acetylated histone-H3 on Lys9 and Lys14 (1:2000; Upstate Biotechnology, Lake Placid, NY), MMP-9 (1:1000; Millipore, Billerica, MA), GSK-3β (1:2000; BD, Franklin Lakes, NJ), phospho-GSK-3β^{Ser9} (1:1000; Cell Signaling, Beverly, MA), or GAPDH (1:4000; Sigma-Aldrich, St. Louis, MO), in 0.1% Tween 20/Odyssey blocking buffer and then with goat anti-rabbit 800 and goat anti-mouse 680 secondary antibodies (1:10000; LI-COR) at room temperature for two hours. After the final washes, the membranes were scanned and the signals of reactive bands were quantified using the Odyssey Infrared Imager (LI-COR).

Immunohistochemistry

Animals were anesthetized with a lethal dose of isoflurane and fixed by transcardial perfusion with saline, followed by 4% formaldehyde. The brains were submerged in 30% sucrose, and then frozen at -80°C. A series of 30 µm-thick sections were cryo-cut, acidified with 1 mol/L HCl for 10 minutes on ice and then 2 mol/L HCl for 30 minutes at 37°C, blocked with 5% normal goat/rabbit serum (Sigma-Aldrich) for one hour at room temperature, and then incubated overnight at 4°C with the following primary antibodies: anti-BrdU (1:130; Abcam), anti-panendothelium (RECA1 for endothelium; 1:200; Abcam), anti-CD54 (for MSCs; 1:100; Millipore), anti-fibronectin (for MSCs; 1:1500; Millipore), anti-glial fibrillary acidic protein (GFAP for astroctyes; 1:200; Millipore), anti-NeuN (for neurons; 1:100; Millipore),anti-ionized calcium binding adaptor molecule 1 (Ibal for microglial cells; 1:200; Abcam), anti-osteocalcin (for osteoblasts; 1:200; Abcam), and anti-Ki67 (for cell proliferation; 1:200; Abcam). Samples were then washed and incubated for two hours at room temperature with the appropriate fluorescence dye-conjugated secondary antibodies (1:200; Invitrogen and Abcam). Nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI) included in the mounting medium. Fluorescent labeling was examined with a fluorescence microscope (BX61 Olympus, Center Valley, PA) or a LSM510 laser-scanning confocal microscope (Carl Zeiss, Göttingen, Germany).

Under fluorescence microscopy, the penumbra has higher background noise, moderately

damaged structure, distortive microvessels, and higher cell density, compared with normal brain. We analyzed the MSC homing efficacy and vessel density at the junction of infarcted and non-infarcted brain tissues with an area of approximately 1 mm^2 within the boxed areas shown in Figures 2 and 5. Five coronal sections at the penumbra cortex and striatum were evaluated for each rat. The microvessel density was analyzed using ImageJ (Free download at http://rsbweb.nih.gov/ij/) and the results were shown as the ratio of microvessels versus whole picture in pixels.

Behavioral Testing

For the rotarod test, rats were placed on an accelerating rotarod cylinder (Rotor-rod, San Diego Instuments, San Diego, CA), in which the speed was slowly increased from 4 to 40 rpm within four minutes. The time that animals remained on the rotarod was measured. The animals were trained for three consecutive days before MCAO. Each test consisted of three independent measurements, and the longest time spent on the device was recorded.

The Neurological Severity Score test was modified based on a previous report.[2] Twelve different tests for motor, sensation, and reflex abnormalities were performed by a blinded investigator. Six tests of motor performance (flexion of forelimb or hind limb, head movement 10^o to the vertical axis, inability to walk straight, circling towards the paralytic side, falling down

to the paralytic side, and immobility) were included to evaluate hemiplegia in the extremities and trunk. In sensation tests, visual and tactile placement and a proprioceptive test were adapted to evaluate sensory abnormalities of the body. In reflex tests, pinna, corneal, and startle reflex were evaluated. A score of 0 (normal) or 1 (abnormal) was given to each test.

Body asymmetry was quantitatively analyzed using the elevated body swing test as previously described.[3] Briefly, rats were examined for lateral movements/turning when their bodies were suspended by the tail 200 mm above the testing table. The number of initial head or upper body turns was counted in 20 consecutive trials.

To validate these functional tests, we analyzed the above studies in 21 rats before and one day after MCAO. The correlation coefficients between infarct volume and changes in rotarod test, Neurological Severity Score, body asymmetry, and body weight were -0.72, 0.84, 0.75, and -0.9, respectively.

Magnetic Resonance Imaging (MRI)

Infarct volume on Days 1, 8, and 15 after MCAO was evaluated using MRI. Each group comprised five rats.

All MRI experiments were performed on a 7-T (Bruker Avance, Billerica, MA.), 21 cm horizontal scanner. The rats were anesthetized with 1.5% isofluorane and placed in a steriotaxic

holder, which was mounted in a transmit (72 mm) / receive (25 mm) radio frequency coil ensemble. Body core temperature was maintained at 37°C using a circulating water heater and monitored by means of a rectal temperature probe.

Three mutually perpendicular slices were acquired through the brain as scout images. A contiguous set of 1 mm thick T_2 weighted axial slices, encompassing the ischemic region (15 slices), was acquired using a fast spin-echo sequence to delineate anatomical details (Field-of-view [FOV] = 32 mm, in-plane resolution of 125 μm, echo time [TE] = 12 ms, repetition time [TR] = 2500 ms, echo train length = 8 and number of averages [NA] = 8). Subsequently, diffusion-weighted images (in three orthogonal directions), based on Echo Planer Imaging (EPI) sequence (diffusion gradient duration = 4 ms, b values, = 1000 mT/m, TE/TR = 25.4/4500 ms, delay between diffusion gradients = 15 ms, in-plane resolution of 250 μm, NA =10) were acquired at the same slice positions as the T_2. These images were used to calculate the extent of the lesions. Infarct volume was then calculated using ImageJ software.

Hematoxylin and Eosin Staining

Hematoxylin and eosin (H&E) staining was performed to detect the brain infarct area. Briefly, brain slices were washed in PBS and stained in hematoxylin solution (Sigma-Aldrich) for four minutes followed by washing in running tap water. After differentiation in 1% acid

alcohol, slices were counterstained in eosin-phloxine B solution (Sigma-Aldrich) for one minute followed by washing, dehydrating and clearing. Stained slices were scanned with Epson scanner and infarct area was calculated using ImageJ software.

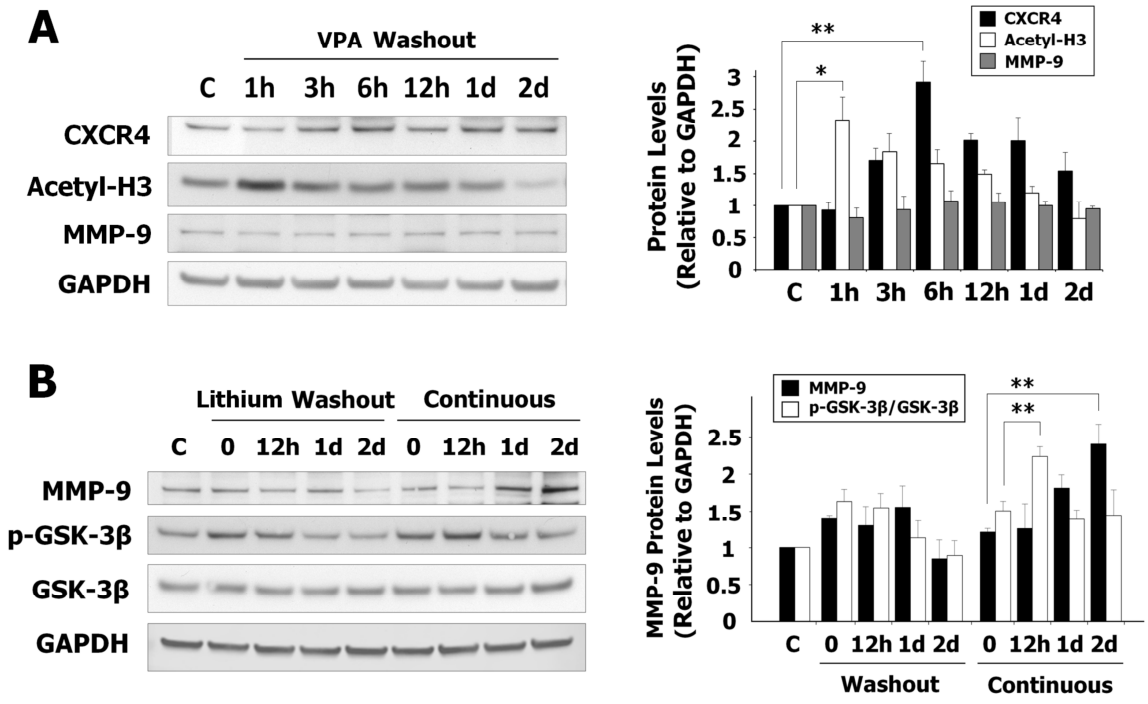
References

1. Tsai LK, Leng Y, Wang Z, Leeds P, Chuang DM. The mood stabilizers valproic acid and lithium enhance mesenchymal stem cell migration via distinct mechanisms. *Neuropsychopharmacology*. 2010;35:2225-2237.

2. Li Y, Chopp M, Chen J, Wang L, Gautam SC, Xu YX, Zhang Z. Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. *J Cereb Blood Flow Metab*. 2000;20:1311-1319.

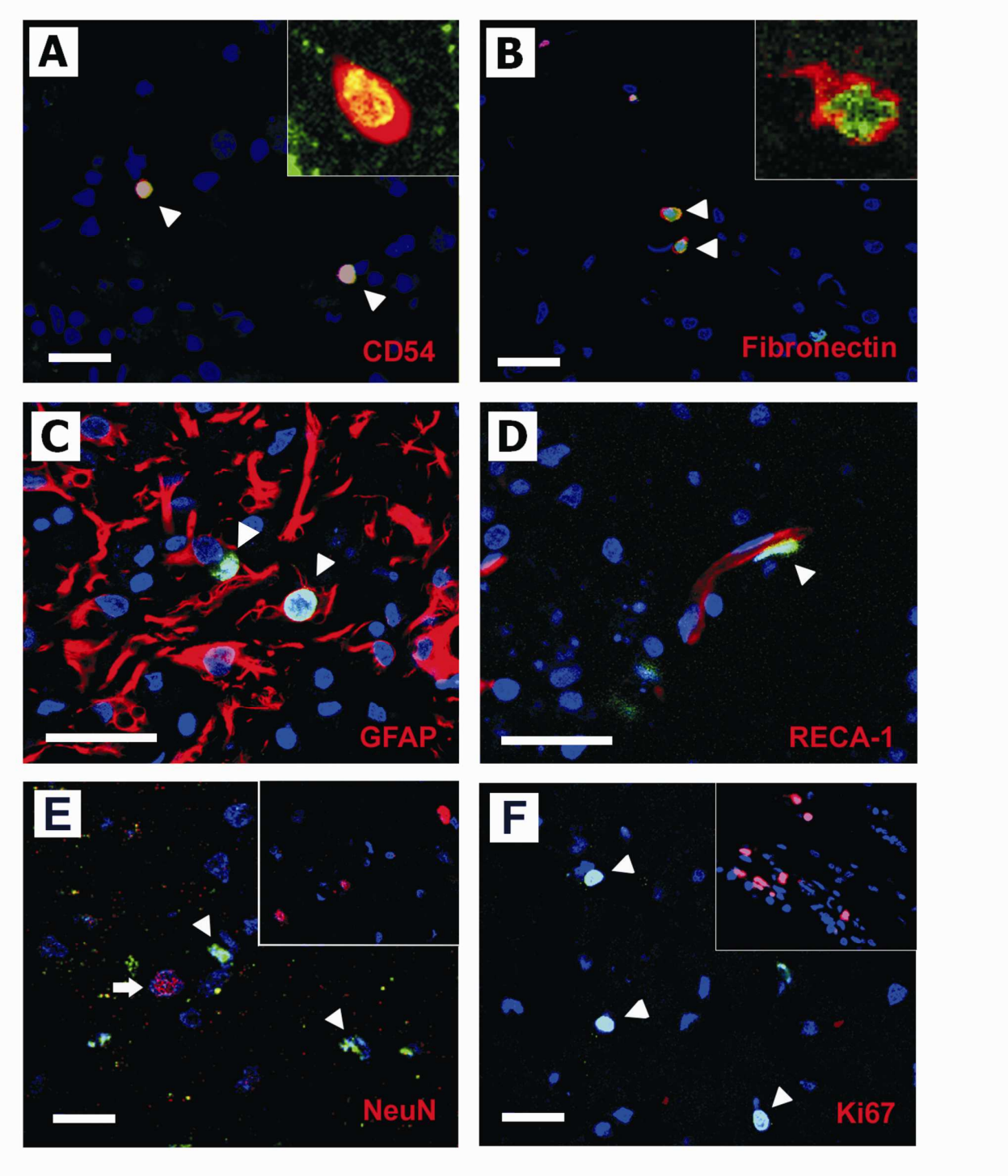
3. Chang CF, Lin SZ, Chiang YH, Morales M, Chou J, Lein P, Chen HL, Hoffer BJ, Wang Y. Intravenous administration of bone morphogenetic protein-7 after ischemia improves motor function in stroke rats. *Stroke*. 2003;34:558-564.

Supplemental Figures

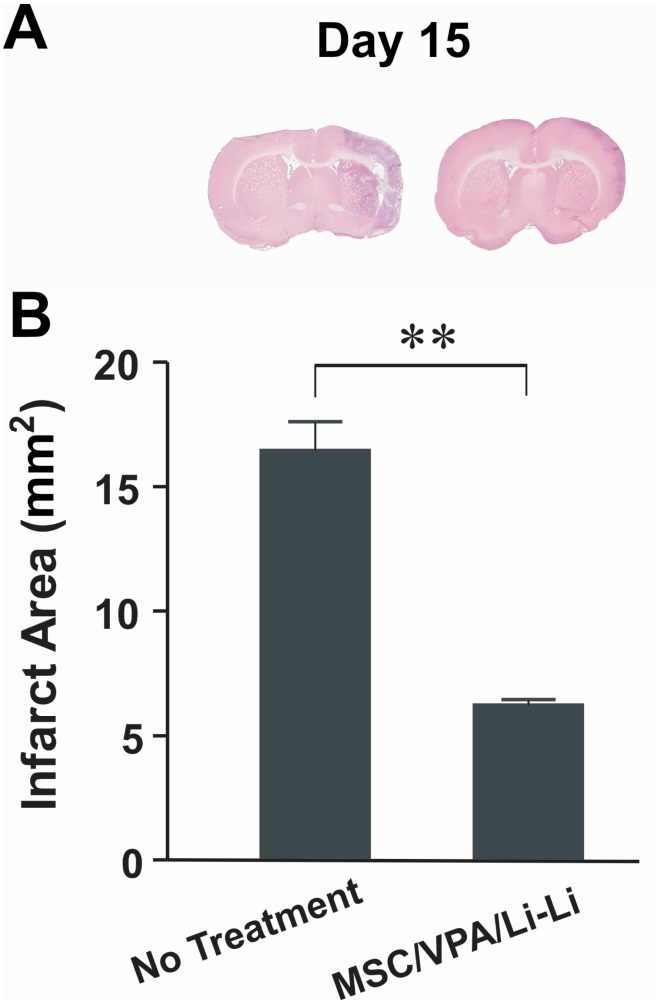


Supplemental Figure S1. Treatment of MSCs with VPA and lithium causes increases in CXCR4 and MMP-9, respectively. (A) MSCs were treated with 2.5 mmol/L sodium VPA for three hours and then cultured with fresh medium; cells were harvested at different time points after VPA washout. Levels of acetylated histone-H3 and CXCR4 protein peaked at one and six hours after VPA washout, respectively, as determined by Western blotting. (B) When MSCs were treated with 2.5 mmol/L lithium for 24 hours followed by drug washout, MMP-9 levels did not significantly increase. However, when MSCs were treated with 2.5 mmol/L lithium for 24 hours followed by continuous treatment with 1 mmol/L lithium, levels of MMP-9 gradually increased,

following an elevation of phosphorylated GSK-3β levels. *p < 0.05; **p < 0.01.



Supplemental Figure S2. VPA and/or lithium priming does not affect the differentiation and proliferation of MSCs in the infarcted brain. (A and B) Most of BrdU-labeled MSCs (green) expressed MSC markers CD54 (red) and fibronectin (red). Enlarged colocalization is shown in the upper right insert. (C and D) A few BrdU-labeled MSCs (green) expressed the astrocyte marker GFAP (red) and endothelial cell marker RECA-1 (red). (E) No BrdU-labeled MSCs (green) colocalized with the neuronal marker NeuN (red). NeuN staining in the infarcted cortex is shown in the upper right insert as the positive control. (F) No BrdU-labeled MSCs (green) colocalized with a proliferation marker Ki67 (red). Ki67 staining in the infarcted cortex is shown in the upper right insert as the positive control. Blue, DAPI; arrow head, BrdU-positive cell; arrow, NeuN staining; Bar = 50 μm.



Supplemental Figure S3. Transplantation of MSCs primed with VPA and lithium reduces infarct area in MCAO rats. H&E staining was used to measure brain infarct area on Day 15 after MCAO. MCAO rats that received transplanted VPA- and lithium-primed MSCs had significantly smaller infarct area than rats in the untreated MCAO group. (A) Representative brain slices. (B) Quantified results. N = 4-6 per group; $^{**}p < 0.01$.