Cell

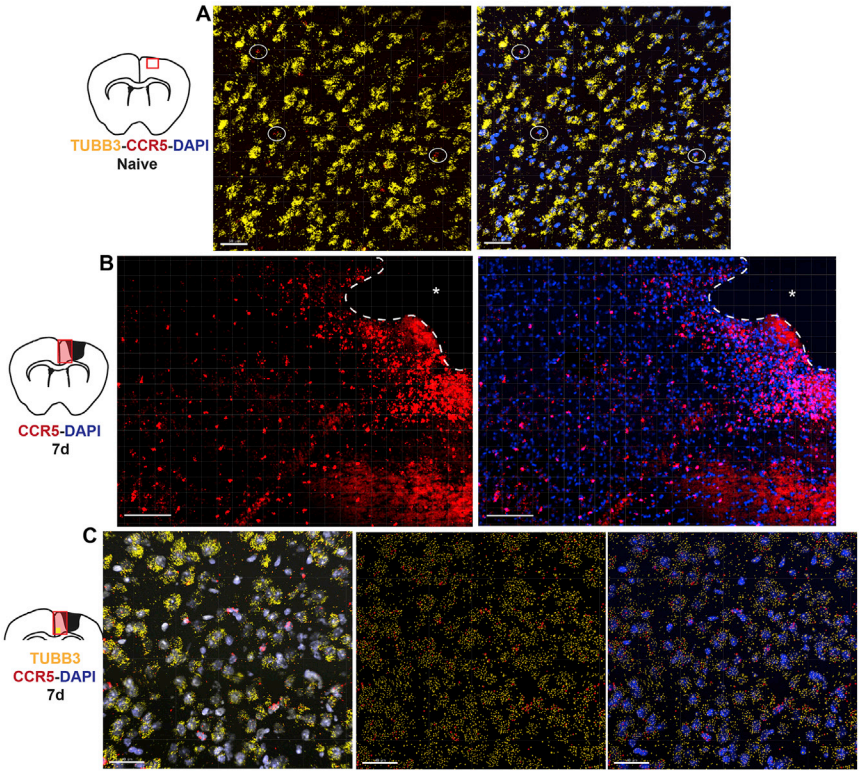


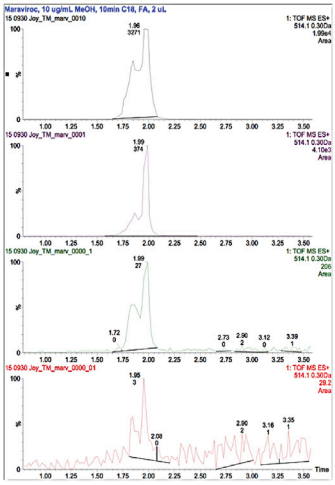
Figure S1. CCR5 Transcripts Are Expressed in Cortical Neurons 7 Days after Stroke, Related to Figure 1

(A) Projection images from larger field of view show absence of CCR5 co-localization with TUBB3+ve neurons. Circles represent CCR5 transcripts that localize to DAPI+ve nuclei but absent in TUBB3+ve cells. Scale bar-50μm.

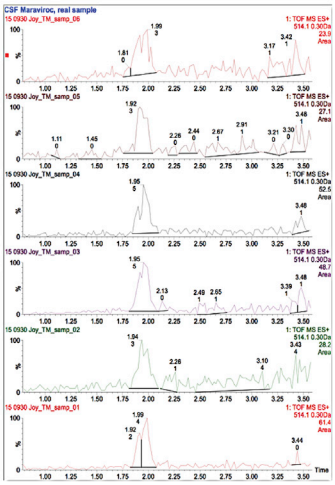
(B) CCR5 expression at 7 days post-stroke. Infarct border marked with dotted line. Asterisk denotes stroke site.

(C) Left, projection image from region of interest within field of view shown in (B). TUBB3+ve neurons express CCR5 at 7 days after stroke. Middle and right -images processed with spot detection feature for improved visualization of CCR5 transcript localization.

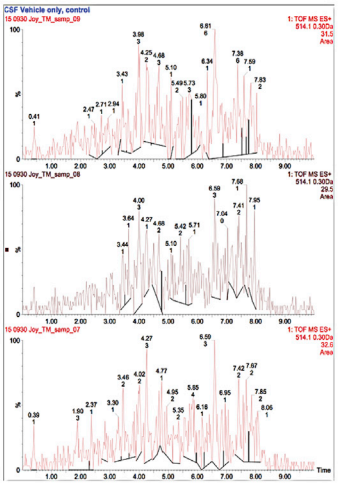
A



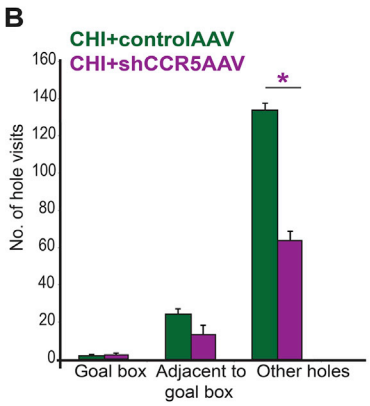
Chromatogram of CSF samples spiked with 10,1,0.1 and 0.01 µg/ml of Maraviroc.



Chromatogram of all CSF samples from treated animals.



Negative controls from animals with vehicle injections and stroke showing the absence of Maraviroc.



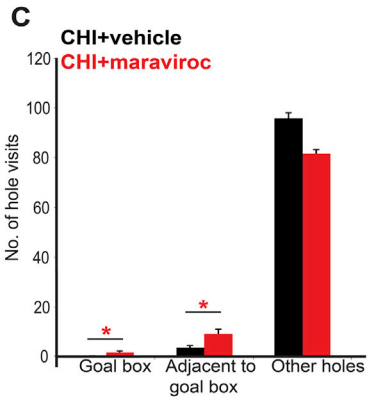


Figure S2. Detection of CCR5 Knockdown and Effect on Cognitive Improvement, Related to Figure 2

(A) Detection of maraviroc in mouse CSF with UPLC. Chromatogram of CSF, spiked with known concentrations of maraviroc (left panel), peaks at 1.95-1.99 min. CSF samples from stroke-induced mice treated with 100mg/kg of maraviroc shows similar peak (middle panel) and is absent in control CSF from animals treated with vehicle (right panel). Mean concentration of maraviroc detected was 13.8+ 5.4 ng/mL. n = 6. The mean minimum concentration for maraviroc given as 300 mg twice/day for HIV patients ranges from 33.6–60 ng/mL (Pfizer Selzentry maraviroc package insert. New York, NY: 2010).

(B,C) CCR5 knockdown improves performance on Barnes maze. Animals with CCR5 knockdown and CHI show better performance on the Barnes maze (which has 1 goal box, two adjacent, located on either side of the goal hole, and 17 "other" holes.

(B) shCCR5 AAV decreases the number of errors in hole visits to the other holes (N = 7/group; *$p < 0.01$ compared to control virus + CHI.

(C) Treatment with maraviroc following CHI increases the number of successful hole visits at the goal box as well as at adjacent holes compared to vehicle treatment *$p < 0.05$; n = 6 for vehicle and 7 for maraviroc. Data are mean ± SEM.

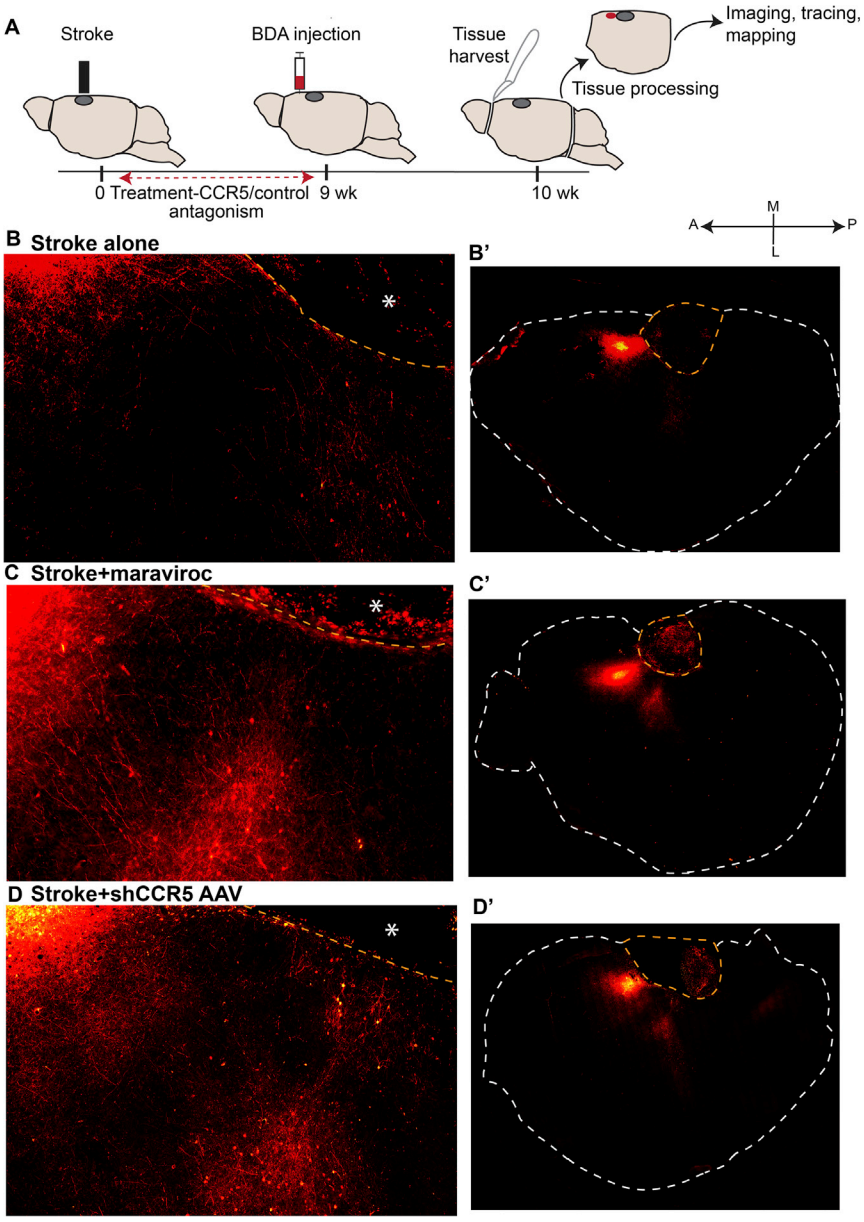


Figure S3. BDA Labeling of Axonal Projections across Groups, Related to Figure 5

(A) Schematic on experimental outline.

(B-D) Representative images of BDA-labeled fibers in cortical sections from stroke (B,B'), stroke+maraviroc (C,C') and stroke+shCCR5 AAV (D,D'). Images on the left are magnified from images from tissue sections on right. Infarct area marked by yellow dotted line. Asterisk denotes stroke site.

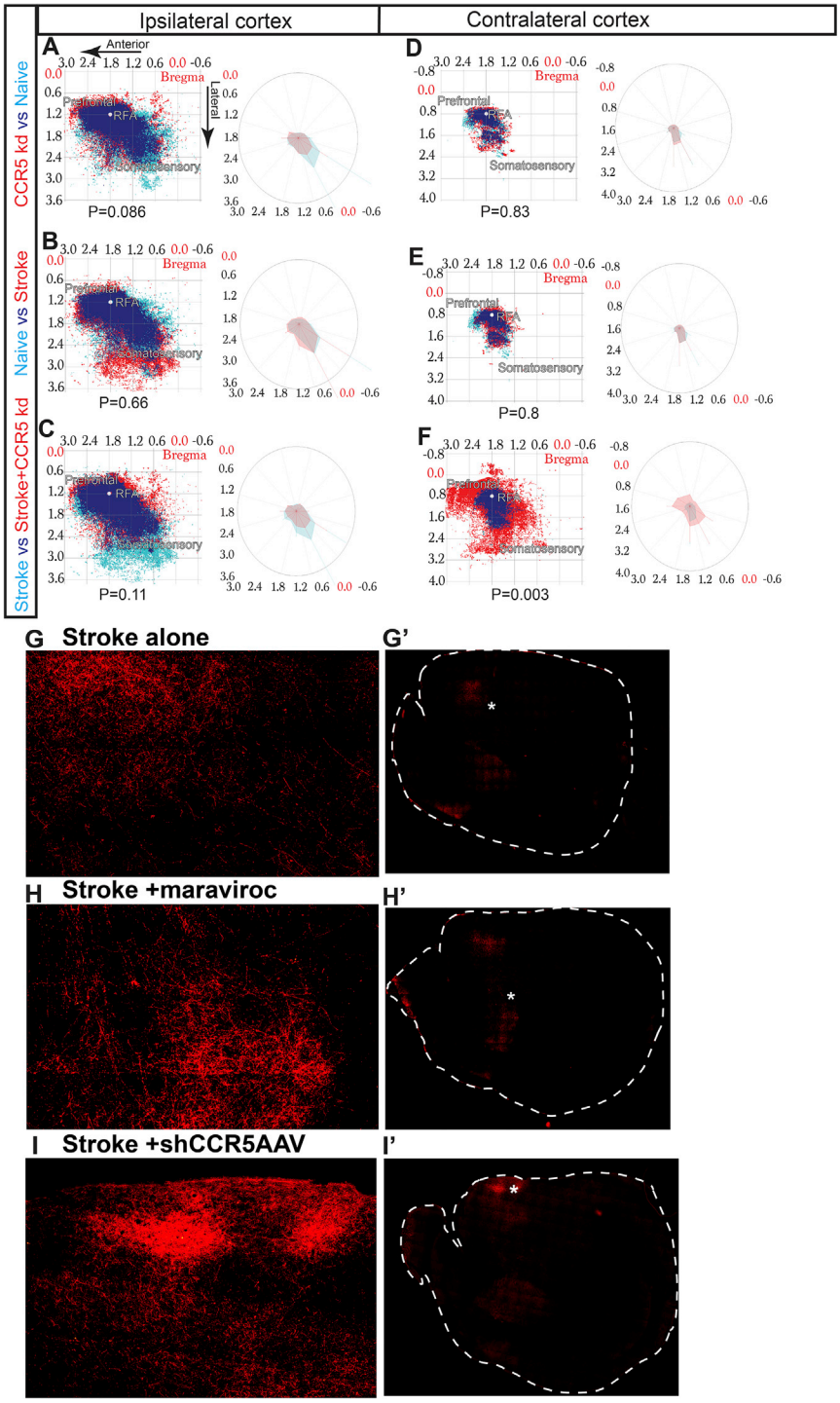


Figure S4. Axonal Projections from Stroke and Non-stroke Groups, Related to Figure 5

(A,D) CCR5 knockdown alone does not induce axonal sprouting in the uninjured brain in ipsilateral ($p = 0.086$) and contralateral ($p = 0.83$) cortices when compared to naive animals.

(B,E)) Following stroke, more axons are seen in the somatosensory area of the ipsilateral cortex but this sprouting response does not statistically differ from naive ($p = 0.66$). In the contralateral hemisphere (E), pattern of sprouting induced by stroke does not differ from naive groups ($p = 0.8$).

(legend continued on next page)

(C,F) Following stroke and CCR5 knockdown, more axons are seen in peri-infarct cortex, but overall pattern of sprouting did not differ when compared to stroke alone, ($p = 0.11$). However, in contralateral cortex following stroke and CCR5 knockdown (f), vast numbers of ipsilateral pre-motor projections were seen in contralateral pre-motor, pre-frontal and somatosensory cortices when compared to animals with stroke alone ($p = 0.003$); $n = 5$ animals per group.
(G-l) Representative images of BDA-labeled fibers in contralateral cortex in whole tissue sections (right side) and fields of view on left from areas marked by asterisk.

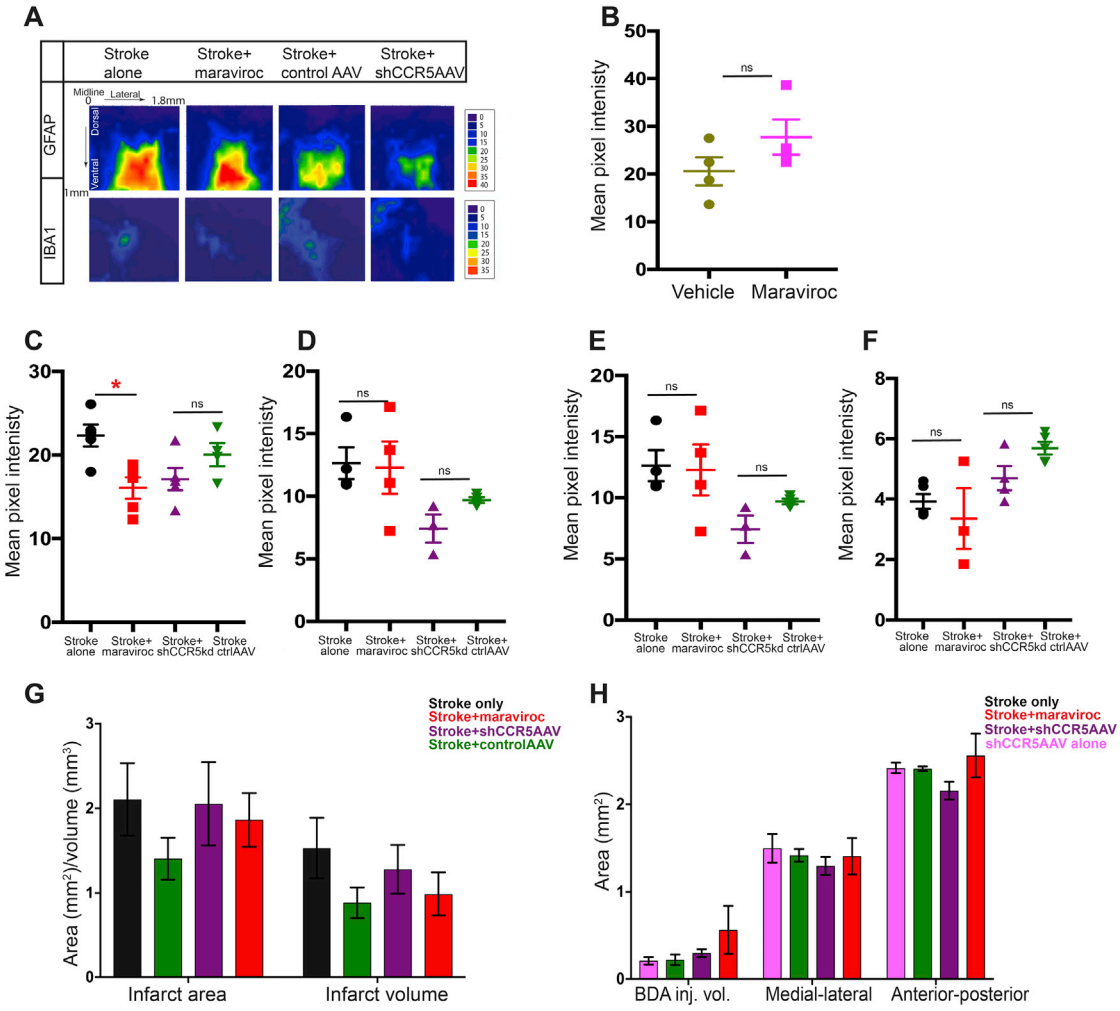


Figure S5. CCR5 Knockdown Does Not Alter Infarct Volume or Glial Reactivity at 2 Months post Stroke, Related to Figure 6

(A) At 2 months post-stroke GFAP immunoreactivity does not significantly differ between stroke and stroke+ maraviroc ($p = 0.95$) or stroke+control AAV and stroke+shCCR5 AAV ($p = 0.066$). Similarly, IBA-1 reactivity does not significantly differ between stroke and stroke+ maraviroc ($p = 0.50$) or stroke+control AAV and stroke+ shCCR5 AAV ($p = 0.159$). For GFAP n = 3 stroke+shCCR5 AAV, n = 4 for all other groups; for IBA-1, n = 3 for stroke+shCCR5AAV/maraviroc; n = 5 stroke alone, n = 4 stroke+control AAV.

(B) IBA-1 immunoreactivity is similar between groups with maraviroc treatment (uninjured) and vehicle (uninjured); n = 4 animal'/group. Data are mean ± SEM.

(C, D) Data on average pixel intensities for GFAP immunoreactivity (non-spatial) show significant decline in with maraviroc treatment at 7 days after stroke ($p = 0.01$) compared with stroke alone (C). Treatment at 2 months (D) and treatment with shCCR5AAV at 7 days and 2 months did not statistically differ across groups.; n = 4-5 animals/group.

(E-F) Mean pixel intensities for IBA-1 immunoreactivity do not statistically differ across groups at 7 days (E) and 2 months (F) post-stroke.

(G) Treatment with maraviroc or CCR5 AAV does not affect stroke area or stroke volume when compared to stroke alone or stroke+control AAV; n = 5 animals per group.

(H) BDA injection location and size based on area of injection site, anterior-posterior and medial-lateral co-ordinates did not significantly vary across animals from groups with CCR5 AAV alone, stroke + control AAV, stroke+ CCR5 AAV and stroke alone; n = 5 stroke+shCCR5 AAV; n = 4 for all other groups.

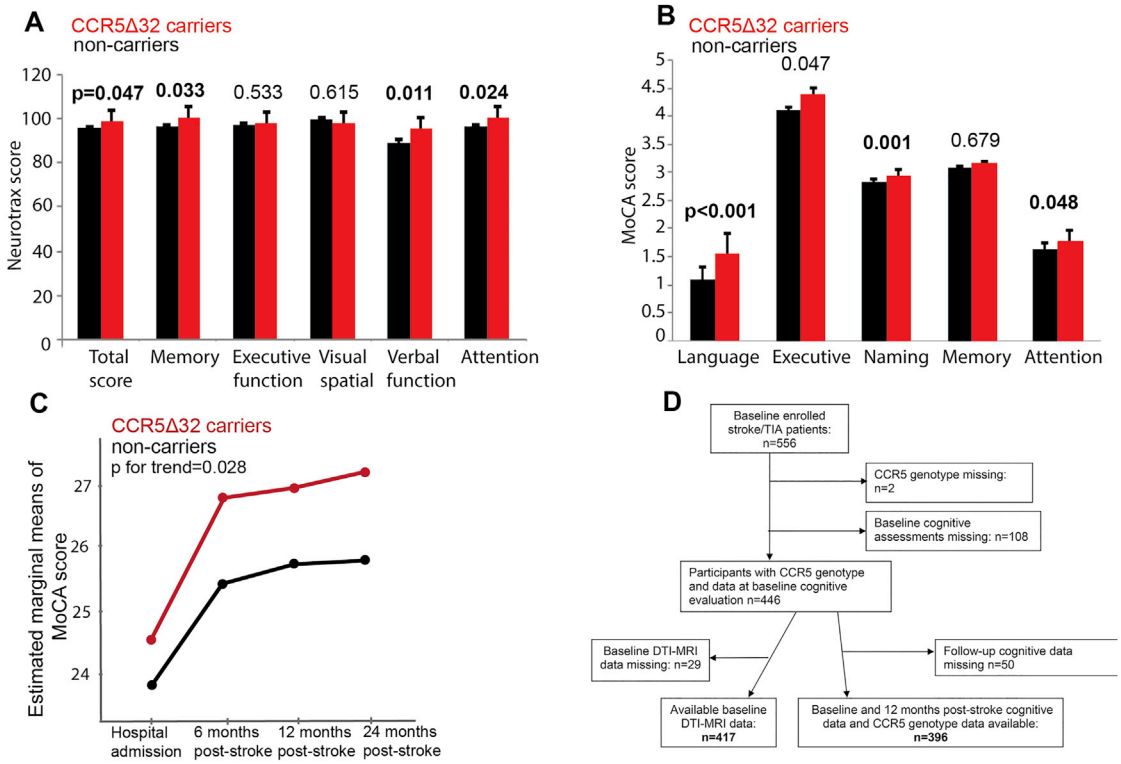


Figure S6. CCR5Δ32 Carriers Show Better Cognitive Performance, Related to Table 1

Cognitive scores at 1 year following stroke assessed by:

(A) Neurotrax for total score, memory, executive function, visual spatial, verbal function, attention.

(B) MoCA assessments for language, executive function, naming and attention. Significant p values in bold. n = 396.

(C) General linear model analysis of repeated-measures of MoCA scores from stroke patients comparing CCR5-Δ32 carriers to non-carriers.

(D) Flow chart of patients included in this study.