SUPPLEMENTAL MATERIAL

Power analysis. For behavior experiments we need 8 animals per group to achieve > 80% power (88% calculated), considering the following parameters: $\alpha = 0.05$; effect size = 0.5; 5 repetitive measures; 4 groups, and a correlation between measures = 0.5. For OIS experiments we need 5 animals per group to achieve > 80% power (87% calculated), considering the following parameters: $\alpha=0.05$; effect size 1; 5 repetitive measures; 2 groups, and correlation between measures = 0.5. OIS parameters were determined from our prior work, in which we demonstrate both significant behavior and OIS effects[1]. It should be noted that our prior work examined the effects of stroke itself and not differences in stroke recovery; thus we used much more conservative effect sizes for the present analysis. For immunohistochemistry experiments, with an effect size of 0.37 and α of 0.05, we need 4 animals per group, and 6 measures per animal to achieve 80% power. For Western blot experiments, with an effect size of 0.48 and α (two-sided) of 0.05, we need 4 animals per group to achieve 80% power. Power calculations were performed with G Power Software (version 3.1.5).

Histology image analysis. Stained sections were examined and photographed using brightfield microscopy (5X magnification) with the same microscope settings (Zeiss, Oberkochen, Germany) and on the same day. Image analysis was conducted using ImageJ 1.40g[2]. Prior to any quantification, contrast in all the original images was automatically and uniformly adjusted. The contralateral hemicortex served as a control. Immunohistochemical images from animals with stroke were analyzed from the end of the scar to a lateral distance of 2100 μm, divided into 6 regions (350 μm width, height was thickness of cortex; **Figures 1C, 4, 5**). Sham treated animals were analyzed over identical cortical regions. Because post-stroke scarring causes changes in cortical thickness, all measures were normalized to the area of the region analyzed. For NeuN quantification the color images were binarized and a minimal threshold size of $7\mu m^2$ was established for all images. The percent area occupied by NeuN immunoreactivity in each individual area was measured using ImageJ (Analyze Particles Plugin). For GFAP quantification (**Figure 4**), the original color images were decomposed into their color channels (blue, green and red) and the green image was chosen for posterior analysis as it exhibited the greatest contrast. An arbitrary threshold of intensity of 100 was established for all images and the percent area occupied by GFAP inmunoreactivity in each area was measured automatically. For PECAM-1 quantification (**Figure 5**) the background for each color was subtracted and the resulted images were binarized. For vascular quantification, in order to evaluate tubelike structures, a minimal threshold of $10\mu m^2$ and maximum circularly of 0.5 was established for all images (ImageJ Analyze Particles Plugin). The percent of each area occupied by PECAM-1 immunoreactivity and the size of individual pixel cluster were measured using ImageJ and then averaged. For scar

quantification, the scar was manually traced in GFAP images and area measured using ImageJ. For all quantifications, the pixels were converted to μm (pixel size=0.738 μm).

References

1. Clarkson A, Lopez-Valdes H, Overman J, Charles A, Brennan K, Carmichael S. Multimodal examination of structural and functional remapping in the mouse photothrombotic stroke model. *J Cereb Blood Flow Metab*. 2013;33:716–23.

2. Rasband WS. ImageJ. http://imagej.nih.gov/ij/. 1997. Accessed 5/1/13.