SUPPLEMENTAL MATERIAL

Methods

Measurement of Infarct Volume. Following 24 hours of reperfusion, the mice were killed and transcardially perfused with heparinized saline (1U/mL). The brains were removed and post-fixed in 4% paraformaldehyde for 1 week. The cerebrum was then cut into 1 mm thick serial coronal sections. 10 μm paraffin embedded sections were stained with hematoxylin and eosin and the cerebral infarct volume was quantified using NIH image analysis software (Image J ver 1.43). Infarct volume was corrected for brain edema.

Immunohistochemistry. Microglia and macrophage activation and localization were determined after 24 hours of reperfusion with the microglia/macrophage selective antibody ionized calcium-binding adapter protein 1 (Iba1) (Abcam) at a 1:300 dilution using standard staining protocols on paraffin embedded sections. $Iba1^+$ cells were quantified and expressed as number of cells/field (40X objective). Two 40X fields were counted per anatomical region and averaged to obtain the number of $Iba1^+$ microglia and macrophages.

Quantitative real-time RT-PCR. mRNA expression was measured after 24 hours reperfusion. Total RNA was extracted from frozen whole cerebral hemispheres using TRIzol reagent and then purified with the RNeasy Mini Kit (Qiagen). Purified RNA (1μg) was reverse transcribed to cDNA using an Applied Biosystems kit. QRT-PCR was performed using a Bio-Rad iCycler. The relative mRNA expression was quantified using the comparative method and mRNA was normalized to β-actin.

Laser Doppler Flowmetry and Blood Gas. Cortical perfusion in the MCA territory was measured used laser Doppler flowmetry and was determined before and during occlusion of the

MCA. For measurement of pH, PO_2, and PCO_2, a catheter was implanted into the femoral artery and arterial blood was collected during pre-ischemic and ischemic periods.

Microglia Isolation and Culture. Isolation of cerebral microglia was performed as described elsewhere[1]. Briefly, 10-12 wk old mice FC and MyMRKO mice were euthanized and transcardially perfused with heparinized saline (1U/mL). The cerebrum was homogenized in ice cold PBS in a Tenbroeck homogenizer. The homogenate was then filtered through a 50 µm strainer and then resuspended in 70 % isotonic Percoll. A 0/40/70% Percoll gradient was set up and centrifuged at 1200 x g for 45 min at 20°C. The microglia containing fraction was then collected, resuspended in RPMI + 10% FBS and plated at a density of 2 x 10^5 cells/mL/well. Cells were washed with PBS (+ calcium chloride, + magnesium chloride) after 2 hours to remove non-adherent cells and then incubated for 24 hours at 37 °C, 5% CO_2.

Figure Legend

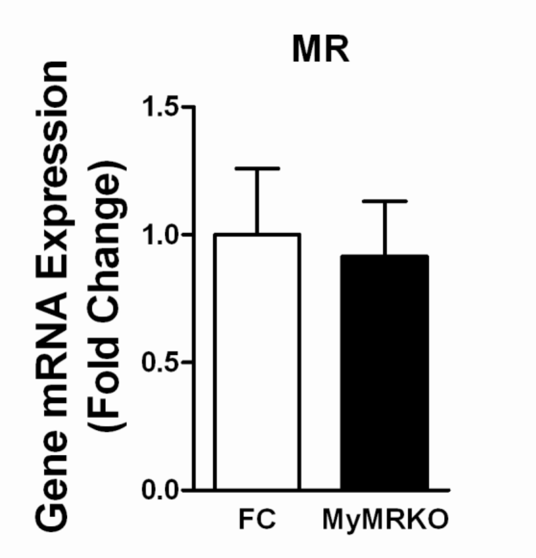
S2. Expression of MR in cultured microglia. Microglia were isolated from the cerebrum of FC and MyMRKO mice and cultured for 24 hours. No significant change in MR expression was detected between FC and MyMRKO mice. N = 5 per group.

Table S1. Cerebral blood flow and arterial blood gas measurements.

	CBF (%)	pH	P_{O2}, mm Hg	P_{CO2}, mm Hg
Pre-Ischemia				
FC	100	7.34 ± 0.04	19.3 ± 3.9	158.7 ± 17.4
MyMRKO	100	7.36 ± 0.06	17.6 ± 2.5	149.0 ± 14.8
Ischemia				
FC	46.1 ± 0.9	7.28 ± 0.06	20.8 ± 4.1	138.6 ± 17.0
MyMRKO	46.6 ± 3.8	7.39 ± 0.05	20.9 ± 4.6	140.7 ± 12.3

Values represent mean ± S.E. The ischemic cerebral blood flow (CBF) is represented as the percentage of the pre-ischemic, baseline CBF. There were no significant differences between FC and MyMRKO mice (N = 4 per group). FC = Floxed Control, MyMRKO = myeloid MR knockout.

Figure S2.



Supplemental References

1. Frank MG, Wieseler-Frank JL, Watkins LR, Maier SF. Rapid isolation of highly enriched and quiescent microglia from adult rat hippocampus: Immunophenotypic and functional characteristics. *J Neurosci Methods*. 2006;151:121-130