

1. Magnetic resonance imaging

In vivo MRI experiments were performed on a 4.7-T small-animal MR scanner (Agilent/Varian, Santa Clara, CA) equipped with a DirectDrive™ console. The scanner is built around a 33-cm, clear-bore magnet equipped with a 21-cm inner diameter (Oxford Instruments, Oxford, UK), actively shielded, Agilent/Magnex gradient coil (maximum gradient, 28 G/cm; rise time, approximately 200 μ s), and model A-240 amplifiers (300 V, 300 A; Oy International Electric Company, Helsinki, Finland).

MRI data were collected using an actively decoupled coil pair: a 9-cm inner diameter volume coil (transmit) and a 1.5-cm outer diameter surface coil (receive). Before each imaging experiment, mice were anesthetized with isoflurane/O₂ [2% (vol/vol)] and maintained on isoflurane/O₂ [1% (vol/vol)] throughout the experiment. Mice were restrained in a laboratory-constructed, three-point, Teflon head holder and were placed on a pad with circulating warm water to maintain body temperature at 37 \pm 1°C. Before being placed into the magnet, mice were injected intraperitoneally with 0.25 mL of MultiHance (gadobenate dimeglumine; Bracco Diagnostics Inc, Princeton, NJ) contrast agent, diluted 2:10 in sterile saline.

2. Primary antibodies

Immunohistochemical (IHC) stains used included:

rabbit anti-mouse VEGF antibody (Biorbyt, Cambridge, UK), diluted 1:300;

rabbit anti-mouse HIF-1 α antibody (Novus Biotechnology, Littleton, CO, USA), diluted 1:400;

rabbit anti-mouse CD68 antibody (Biorbyt, Cambridge, MA, USA), diluted 1:100;

rabbit anti-mouse CD3 antibody (Origene Technologies, Rockville, MD, USA), diluted 1:100;

rabbit anti-mouse Iba-1 antibody (Abcom Biotechnology, Cambridge, MA, USA), diluted 1:1500;

rabbit anti-mouse CXCR4 antibody (Thermo Scientific, Waltham, MA, USA), diluted 1:200;

rabbit anti-mouse CXCL12 antibody (Abcom Biotechnology), diluted 1:200;

rabbit anti-mouse TNF- α antibody (Abcom Biotechnology), diluted 1:100;

rabbit anti-mouse IL6 antibody (Bioss Antibodies, Woburn, MA, USA), diluted 1:100.