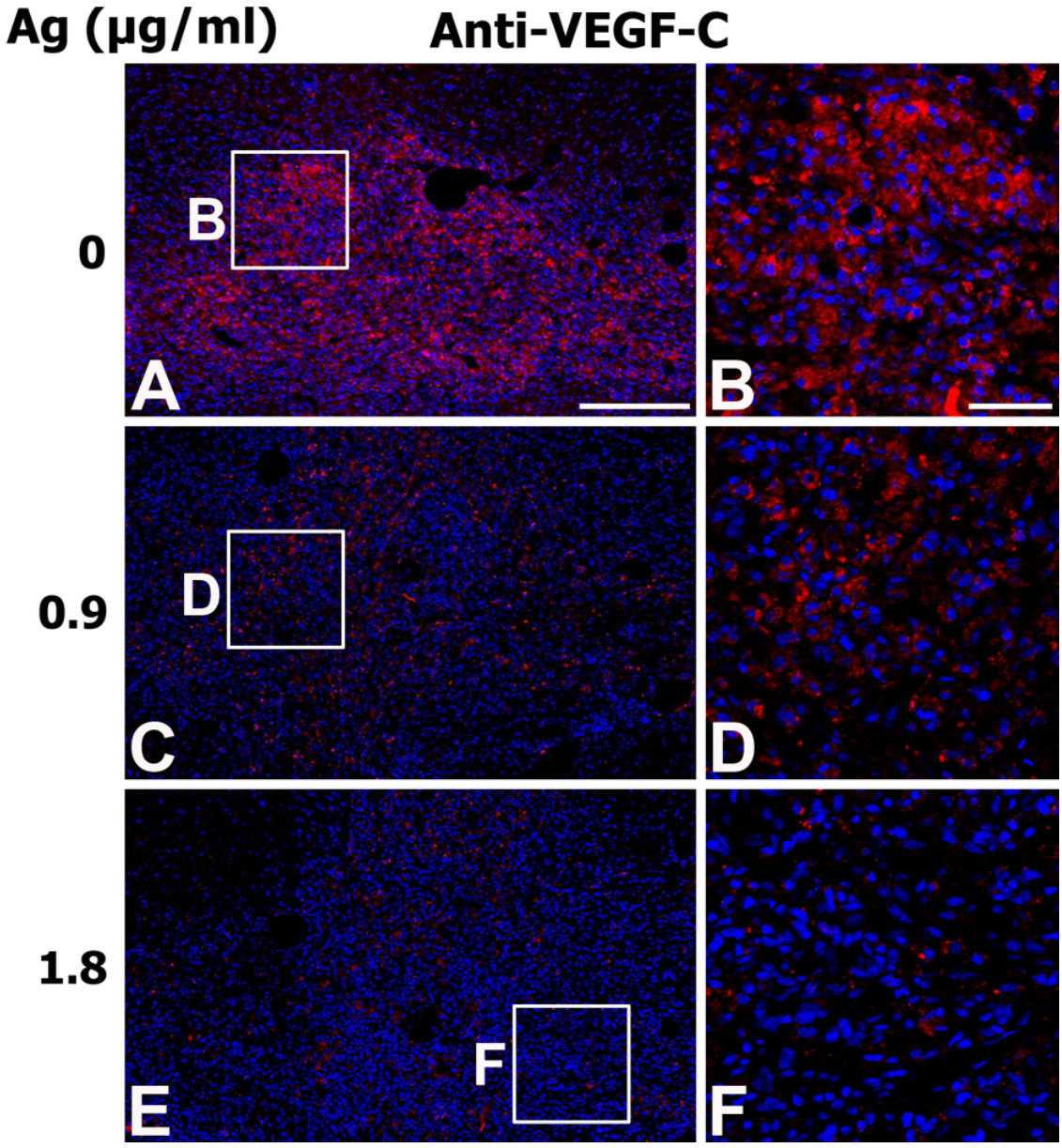
JHC—Journal of Histochemistry & Cytochemistry
DOI: 10.1369/0022155414564218
Authors: Shin et al.



Supplementary Figure 1. Antigen preadsorption blocks the VEGF-C immunoreactivity in a dose-dependent pattern. The primary antibody was preadsorbed overnight with several concentrations (0, 0.9, 1.8 μg/mL) of the peptide-antigens (Ag) for immunization, before being used for labeling serial tissue sections from the striatum of the MSC-grafted rats on day

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7 after transplantation. Note that the intensity of the VEGF-C immunoreactivity is inhibited in a dose-dependent pattern by preadsorption with its immunizing protein. (B, D, F) Higher magnifications of the boxed areas from A, C and E, respectively. Cell nuclei appear blue after DAPI staining. Scale bar = 200 μm for A, C, E; 50 μm for B, D, F.

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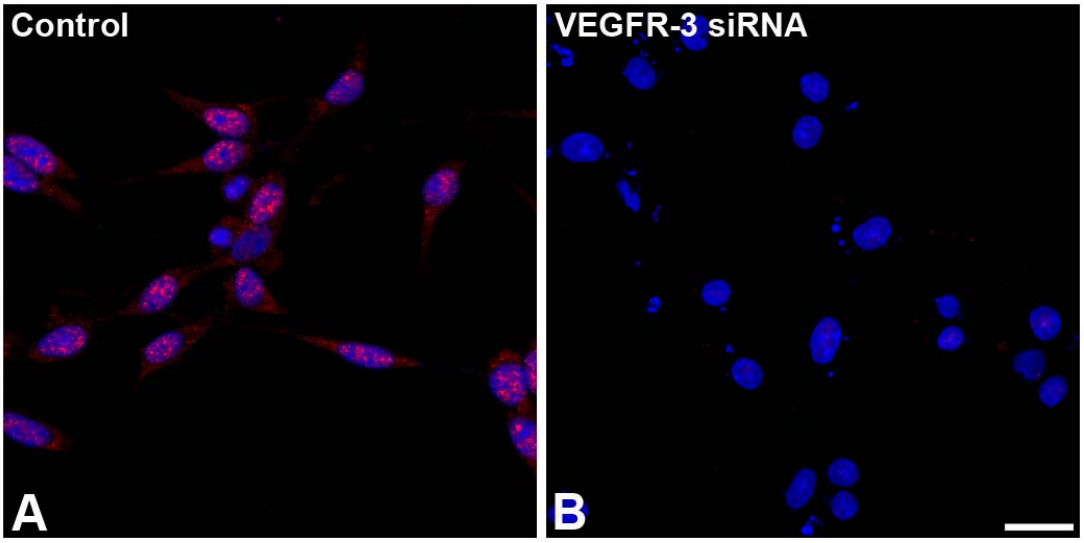

Supplementary Figure 2. The specificity for VEGFR-3 antibody is confirmed by the absence of VEGFR-3 immunoreactivity in VEGFR-3-silenced C6 glioma cells using small interfering RNA (siRNA) transfection. Transient transfection of VEGFR-3 siRNA into C6 cells is based on our previous study (Park et al. 2014). Target sequences of siRNA duplexes are as follows: sense (5′-GCAAACUUCGCUACACUAA (dTdT)-3′), and antisense (5′-GGUUGCCAGUUAUAGGUAU (dTdT)-3′). Transfected cells are fixed with absolute ethanol for 10 min and used in immunocytochemistry assays. Intense VEGFR-3 immunoreactivity is observed in C6 cells transfected with scrambled RNA (A), while VEGFR-3-silenced C6 cells displayed little or no immunoreactivity for VEGFR-3 (B). Cell nuclei become fluorescent blue after DAPI staining. Scale bar = 20 μm for A and B.

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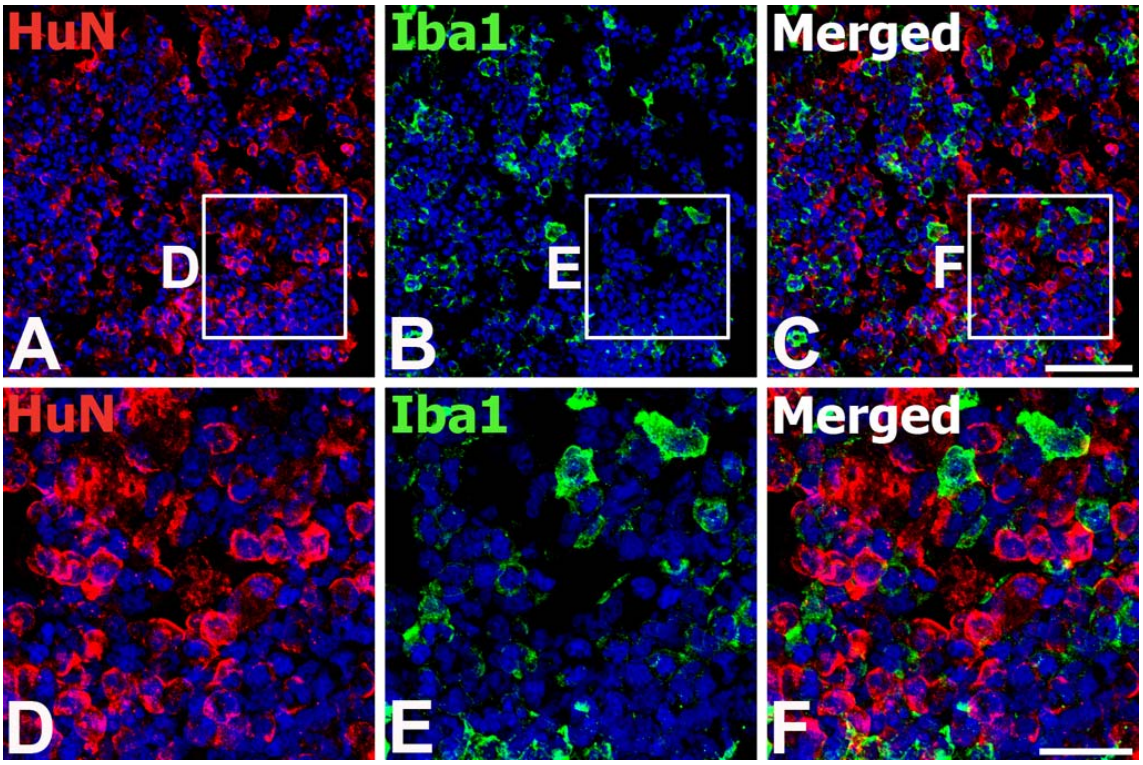

Supplementary Figure 3. Higher magnification views of the area shown in Fig. 2C. Double labeling with the human-specific marker HuN and Iba1 shows that, while HuN staining is observed in the grafted MSCs, it is rarely observed within the nuclei of Iba1-positive brain macrophages. (D–F) Higher magnifications of the boxed areas from A–C, respectively. Cell nuclei appear blue after DAPI staining. Scale bar = 50 μm for A–C; 20 μm for D–F.

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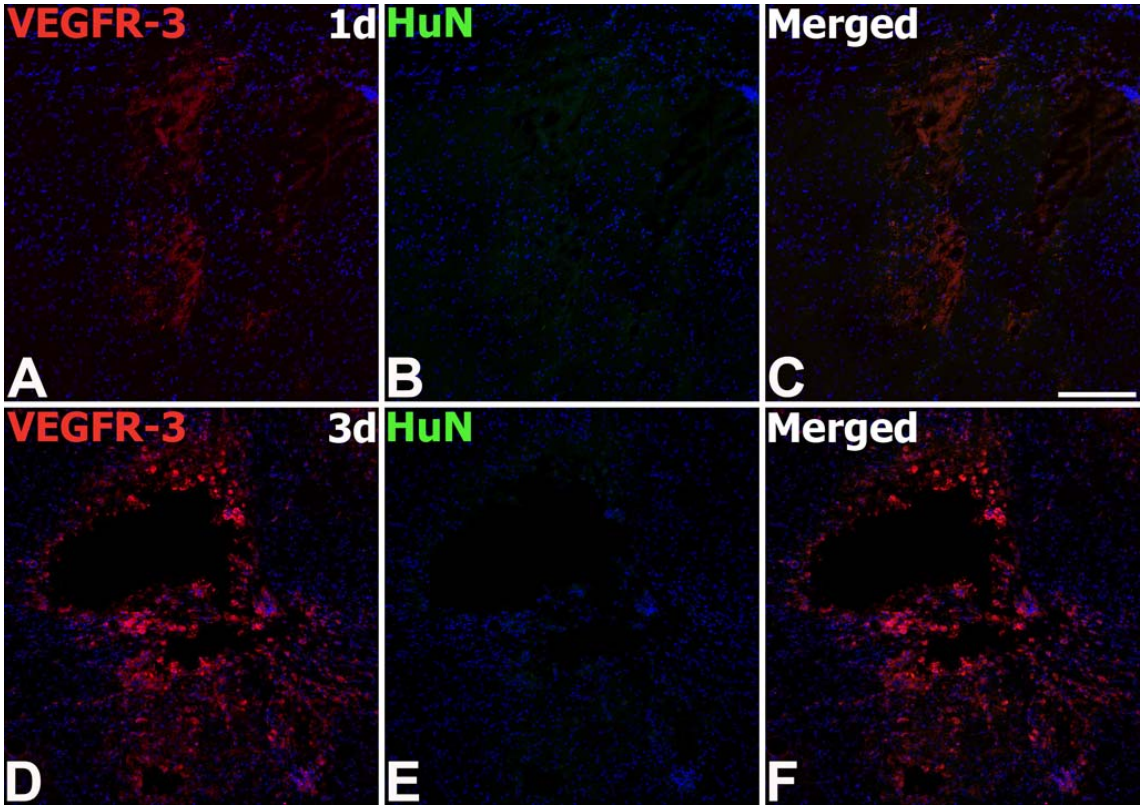

Supplementary Figure 4. Double labeling for HuN and VEGFR-3 at sham-injected sites on days 1 (A–C) and 3 (D–F) after saline injection. No specific staining for HuN is detected within the needle track in sham controls, where VEGFR-3 immunoreactivity is weak at 1 day, but increases by day 3 after saline injection. Cell nuclei appear blue after DAPI staining. Scale bars = 200 μm for A–F.