Figure S1. Brain endothelial cell viability is not affected by incubation with glioma stem-like cells and semaphorin 3A

(A) Human brain endothelial microvascular endothelial cells (ECs) were starved for 6h (ctrl) and treated for 1h with staurosporine (stauro, 1 µM, Sigma), glioma stem-like cell conditioned medium (GSC-CM#4), and semaphorin 3A (S3A, 100 ng/ml). Nuclei were observed with DAPI staining.

(B) ECs were cultured at confluence on collagen and further cultured with GSC (#4) for 18h in serum-free medium. Cells were stained for the stemness marker Sox2 (red) and for nucleus with DAPI (blue).

(C) ECs were seeded on collagen-coated porous membranes for permeability assays and treated with serum-free medium for 6h (ctrl) then with GSC-CM#4 for 1h. Cells were fixed and processed for DAPI staining. Representative images were obtained using confocal microscopy and processed with Image J. Scale bars: 10 µm.

(D) Cell proliferation was measured by MTT (Sigma, as described in Galan-Moya et al., 2011) in hCMEC/D3 starved for 6h (ctrl) and then exposed to S3A (50 ng/ml), GSC-CM#4 and staurosporine (1 µM) for 24h. Alternatively, cells were left in serum and FGF-containing medium (pos). Data were normalized to ctrl and graph represents the average ± sem from 3 independent experiments.

(E) Cells were treated as in (D) and their viability was measured by Trypan Blue. Briefly, cells were trypsinized, centrifuged, resuspended in starving medium and trypan blue (0.4%, Sigma) was added. Percentage of cell viability was calculated as the ratio between viable cells (unstained) over total number of cells.
Figure S2. VE-cadherin internalization in human cerebral microvascular endothelial cells
(A) Cartoon depicting the VE-cadherin internalization assay protocol.
(B-C) Internalization assays as described above. hCMEC/D3 were co-stained for VE-cadherin (iVEC, green), Early Endosomal Antigen 1 (EEA1, BD, red) and adaptin α (AP2, BD, red). Scale bars: 10 µm.
Figure S3. GSC-induced endothelial repulsion is barely affected by inhibitors of actomyosin contractility and collagenase, but is impaired in the absence of NRP1 expression

(A) Three day-old human brain endothelial microvascular endothelial cells (ECs) were starved 6h and pre-incubated 30 min with DMSO (ctrl), inhibitors of collagenase (10 µM, 4-Aminobenzoyl-Gly-Pro-D-Leu-D-Ala hydroxamic acid, Sigma) and of actomyosin contractility (10 µM, Blebbistatin, Sigma). Afterwards doses were reduced by a 10 fold to minimize toxicity upon prolonged incubation with GSC#4 for 18h in serum-free medium.

(B) Sic and NRP1<sup>si3</sup>-siRNA transfected ECs were processed for repulsion assays in the presence of GSC#4.
Figure S4. Glioma stem-like cells are negative for TubulinβIII in coculture experiments
Three day-old human brain microvascular endothelial cell monolayers were exposed to glioma stem-like cells (GSC#4) for 18h in serum-free medium. Cells were fixed and processed for immunostaining for VE-cadherin (VEC, red) and TubulinβIII (Millipore, green). Nuclei were counterstained with DAPI. Representative images were obtained using confocal microscopy and analysed with Image J. Scale bars: 10 µm.
Figure S5. VE-cadherin is required to maintain brain endothelial cell integrity
(A) Human brain microvascular endothelial cells (ECs) were transfected with 50 nM of non-silencing sequence (sic) and three independent VE-cadherin (VEC)-targeting siRNA sequences (HSS101680, HSS101681, HSS101682, Invitrogen). Three days later, total cell lysates were analyzed for VEC and Tubulin expression by western-blot.
(B-C) Similarly transfected cells were processed for permeability assays (B) and reshaping assays in the presence of GSC#4 (C).
Figure S6. Controls for co-immunoprecipitation experiments
(A) Three day-old human brain microvascular endothelial cell monolayers were starved for 6 hours and total cell lysates (input) were processed for immunoprecipitations (IP) using pre-immune polyclonal (IgG) and anti-VE-cadherin (VEC) antibodies. Membranes were blotted against VEC and PP2A.
(B) Similarly cultured cells were treated with semaphorin 3A (S3A, +, 100 ng/ml, 5 min) or left in serum-free medium (-). Total cell lysates (input) were processed for IP using pre-immune monoclonal (IgG) and anti-PP2A antibodies. Membranes were blotted against VEC, Set, Src and PP2A.