Supplementary Figure 1 Verification of species specific primers

Ethidium bromide stained gels for verification of species specific primers. Species specificity was tested by using cDNA derived from mouse brain (m) and from the human glioma cell lines SF126 (h) and SF767 (h’) used for the xenotransplantation experiments. The respective primer specificity and identity of the cDNA used is denoted. Absence of competitive products was achieved for ephrinB1, EphB2, and EphB4. For ephrinB2 and EphB3, PCR-products of different species could be discriminated by product size.

Supplementary Figure 2 In-situ Hybridization: sense transcripts

In situ hybridization with sense transcripts (D’-G’, corresponding to Figure 1) served as control. Related in-situ hybridizations of normal brain (D’ and E’) and corresponding hybridizations of high-grade glioma (F’, G’) for ephrinB2 and EphB4 using DIG-labeled sense cRNA-probes. NE = necrosis. Counterstain with methyl green. Bar scale as indicated.

Supplementary Figure 3 Retroviral vectors

(A and B). Scheme of design of retroviral vectors containing cDNA either encoding EphB4wt (A) or EphB4dn (B), lacking the cytoplasmic signaling domain. Primer localization for PCR analysis given as arrows.
Supplementary Materials and Methods

RT-PCR was used to evaluate the expression of ephrinA1, ephrinB1, ephrinB2, ephrinB3, EphA2, EphA5, EphB2, EphB3, and EphB4 mRNA. In order to discriminate between tumor-derived (human origin) and host-derived (mouse origin) expression Ephs and ephrins in glioma xenografts, primers that were specific for human and mouse ephrinB1, ephrinB2, EphB2, EphB3, and EphB4 were designed using the octamer frequency disparity method (Chenal et al., 1996; Griffais et al., 1991). Evaluation of mouse Tie2, mouse Ang-1, mouse Ang-2, and mouse GAPDH expression on xenografted tumors was also assessed by means of RT-PCR. Dilution of cDNA samples and numbers of PCR-cycles have been evaluated to assure non-saturating PCR conditions. The RT-PCR experiments were performed in triplicates. Total RNA was isolated from tumor tissue using the RNAEasy-Kit (Qiagen; Hilden, Germany) and subjected to reverse transcription using poly-dT-Primers. Single-stranded cDNA was used for PCR amplification, according to standard protocols. PCR for the presence of the neomycin-cassette in transfected cells or xenografted tumors was performed according to standard protocols. Xenografted tumors were checked for cross-contamination with EphB4wt or EphB4dn expressing viruses by using primers localized in the SV40 promoter of the pLXSN vector and the extracellular domain of EphB4 (see supplementary Figure 2A and 2B for primer localization). PCR products were separated on 1.5% agarose gels and stained with ethidium bromide.

The primer sequences were as follows (Target, Sequence 5’-3’, Annealing Temperature (°C), MgCl2):
mouse ephrinB1 fw: AGC AGG GCC GCC CTA CGA GT, rv: ACA CAG CCG GCC CCG ACG GC, 55°, 1.5mM; mouse ephrinB2 fw: AGA GCT GTG GGA GAG TCC GT, rv: CCA CCA CCA CCA CCG AAC TC, 55°C, 1.5mM; mouse EphB2 fw: GGA CCT GGG TGG CCG CGT CA, rv: TAA ACA AGA TCC TCG CGA CC, 55°C, 1.5mM; mouse EphB3 fw: TGT TGG CCC ACA CAC GCT AC, rv: TCG ATC TCC TTG GCG AAC TC, 55°C, 1.5mM; mouse EphB4 fw: GGA CCT GCC GGG TGG CCG CGT CA, rv: TAA ACA AGA TCC CGG GCC CCG CCG ACG GC, 55°, 1.5mM; mouse ephrinA1 fw: AGA GCT GTG GGA GAG TCC GT, rv: CCA CCA CCA CCA CCG AAC TC, 55°C, 1.5mM; mouse EphB3 fw: TGT TGG CCC ACA CAC GCT AC, rv: TCG ATC TCC TTG GCG AAC TC, 55°C, 1.5mM; mouse EphB4 fw: ATT GGT GCA GGT GAG TTC GG, rv: CAG GGG CGG GCC ATC GGA TG, 64°C, 1.5mM; human ephrinA1 fw: CTG GCCAGG CCC CGC GCT AT, rv: CAA CCT CAA GCA GCG GTC TT, 55°C, 1.5mM; human ephrinB1 fw: CAG AGC AGG AAA TAC GCT TT, rv: CAC CGA CAG CCG CGA ACA AT, 55°C, 1.5mM; human ephrinB2 fw: TCC CAA TGC TCA GCG CTT AA, rv: TAC TTC CTA GTC TAC
GGT TC, 55°C, 1.5mM; human ephrinB3 fw: CCG CTC GCA CCA CGA TTA CT, rv: GCC CGC CGT CTC CGC CAA CA, 55°C, 1.5mM; human EphA2 fw: GGG GTG AAG AGC CCC GTA TG, rv: GTGTGC AAG GCA TCG ACG CT, 55°C, 1.5mM; human EphA5 fw: GTA GAG GAA GGC TAT CGT CT, rv: GAA AAT CTC TGT ATA CCG GC, 55°C, 2.0mM; human EphB2 fw: GCC ATT GAG CAG GAC TAT CG, rv: GAT CAT CTT GTC TAG CGT GT, 66°C, 1.5mM; human EphB3 fw: CTC TGC CGC CTC GTT ATG CG, rv: GCT TCC TGA GGC AGA CGA TA, 55°C, 1.5mM; human EphB4 fw: CCT TCC TGC GGC TAA ACAG AC, rv: GTT GAC TAG GAT GTT GCC AG, 66°C, 1.5mM; hGAPDH fw: GGT GAA GGT CGG AGT CAA CGG A, rv: GAG GGA TCT CGC TCC TGG AAG A, 66°C, 1.5mM; hALD fw: AGC TGT CTG ACA TCG CTC ACC G, rv: CAC ATA CTG GCA GCG CTT CAA G, 66°C, 2.0mM; mouse Ang-1 fw: CCA CCA TGC TTG AGA TAG GAA CC, rv: CTG TGA GTA GGC TCG GTT CCC, 64°C, 1.5mM; mouse Ang-2 fw: GTG GTG CAG AAC CAG ACA GCT G, rv: CAC TTC CTG GTT GGC TGA TGC, 64°C, 2.0mM; mouse Tie2 fw: CAA CAG CGT CTA TCG GAC TCC, rv: GAA AAG GCT GGG TTG CTT GAT C, 64°C, 2.0mM; mouse GAPDH fw: CTC ACT CAA GAT TGT CAG CAA TGT G, rv: GAG GGA GAT GCT CAG TGT TGG, 64°C, 1.5mM; Neo fw: ACG AAG AGC ATC AGG GGG CTC; rv: GGG TAG CCA ACG CTA TGT CC, 64°C, 2.0mM; SV 40, fw: GAG AGA AGC AGA ATA TTC GG, EphB4 ED rv: CGG AGT TAG GGG CGG GAC TA, 64°C, 1.5mM).
Supplementary Figure 1 (Erber et al.)

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m: mouse brain  
h: SF126 cells  
h': SF767 cells
Supplementary Figure 2 (Erber et al.)

**ephrinB2**

- **D′**
  - normal brain:
  - sense
  - Scale: 100µm

- **F′**
  - high grade glioma:
  - sense
  - Scale: 200µm

**EphB4**

- **E′**
  - normal brain:
  - sense
  - Scale: 100µm

- **G′**
  - high grade glioma:
  - sense
  - Scale: 200µm

- **NE**
Supplementary Figure 3 (Erber et al.)

A

pLXSN EphB4 wt
8837 bp

EphB4 wt

5' LTR

psi+

EcoRI

XbaI/HpaI

3' LTR

Neo

P SV40e

B

pLXSN Ephb4 DN
7678 bp

EphB4 dn

5' LTR

psi+

EcoRI

XbaI/HpaI

3' LTR

Neo

P SV40e