Supplementary materials

Methods

Cell culture and TF expression

U87MG cells and HUVECs were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO_2. The medium was changed every other day. Cell viability was assessed by 0.4% trypan blue exclusion before experiments.

For cell immunofluorescence staining, U87MG cells and HUVECs were seeded onto coverslips in a 12-well plate and allowed to 70% confluence. After washed twice with PBS, cells were fixed with 4% PFA for 20 min, followed by incubation with 5% BSA for 1h. Cells were then incubated with anti-TF antibody (1:200 dilution) at 4°C overnight, and washed thrice for 5 min each. After incubation with Cy3-conjugated secondary antibody for 2 h and counterstained with DAPI, cells were visualized by a fluorescent microscopy.

For quantitative RT-PCR analysis of TF mRNA, total RNA was extracted from U87MG cells and HUVECs using Trizol Reagent. RNA samples were subjected to quantitative reverse transcription polymerase chain reaction (RT-qPCR). Primers used for PCR amplification were as follows: TF (sense: 5'-GACGAGATTGTGAAGGATGTGA-3', anti-sense: 5'- ATTGTTGGCT GTCCGAGGT-3'), GAPDH (sense: 5'-ATCATCAGCAATGCCTCCT-3', anti-sense: 5'-CCATCACGCCACAGTTTC-3'). The amplification cycling conditions were as follows: 95℃ for 10 min, followed by 40 cycles at 95℃ for 10 s, 60℃ for 30 s and 72℃ for 20 s. The relative TF mRNA levels were calculated as 2^-△△Ct, where Ct represents the threshold cycle. GAPDH

were used as the internal reference.

Targeting ability assessment of EGF1-EGFP in vivo

The distribution of EGF1-EGFP in U87MG glioma *in vivo* was also assessed. On the 14th day after tumor inoculation, EGF1-EGFP was injected at a dose of 3 mg/kg body weight via tail vein. After 24 h, mice were sacrificed, brains were isolated, fixed in 4% PFA, and sectioned to a thickness of 20 μm. Nuclei was stained with DAPI. The distribution of EGF1-EGFP in U87MG glioma was visualized by a fluorescent microscopy.

Supporting Figures

ATGAATCACAAAGTGCATCATCATCATCATCATATGGATGGTGACCAGTGTGCCTCTTCTCC

ATGCCAGAATGGTGGCTCCTGCAAGGACCAGCTGCAGTCCTATATCTGCTTCTGCCTGCCT

GCCTTCGAGGGCCGTAACTGTGAGGGATCCATGGTGAGCAAGGGCGAGGAGCTGTTCAC

CGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCG

TGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGC

ACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGT

GCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCAT

GCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGAC

CCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCA

TCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCC

ACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCG

CCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCAAT

CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGA

GCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCACC

GGGATCACTCTCGGCATGGACGAGCTGTACAGTGAATTCAAGCTTGTCGACCTGCAGTCT

AGATAG

Figure S1 cDNA sequence of EGF1-EGFP fusion protein.

MNHKVHHHHHHM<u>DGDQCASSPCQNGGSCKDQLQSYICFCLPAFEGRNCE</u>GSMVSKGEELFT

GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSR

YPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNIL

GHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYL

STQSALSKDPNEKRDHMVLLEFVTATGITLGMDELYSEFKLVDLQSR

Figure S2 Amino acid sequence of EGF1-EGFP fusion protein. The EGF1 domain is underlined.

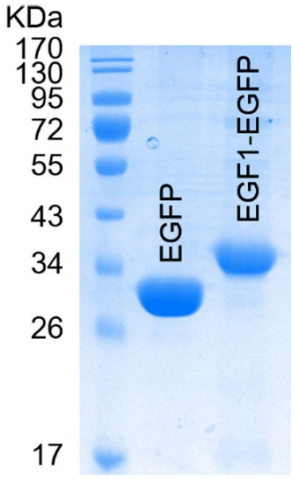

Figure S3 SDS-PAGE analysis of EGF1-EGFP fusion protein expressed in E. coli. BL21 (DE3). EGFP protein was set as control. The objective protein was obtained with high purity after purification.

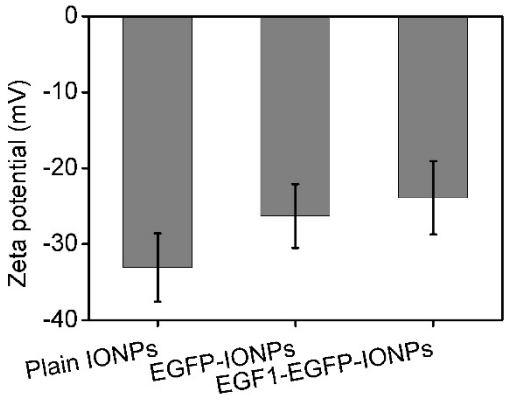

Figure S4 Zeta potential of NPs determined by DLS.

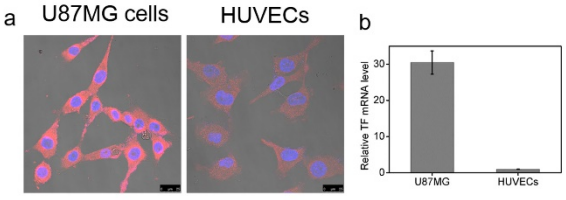

Figure S5 Immunofluorescence images (a) and quantitative RT-PCR (b) of TF expression on

U87MG cells and HUVECs. Scale bar, 20 μm.

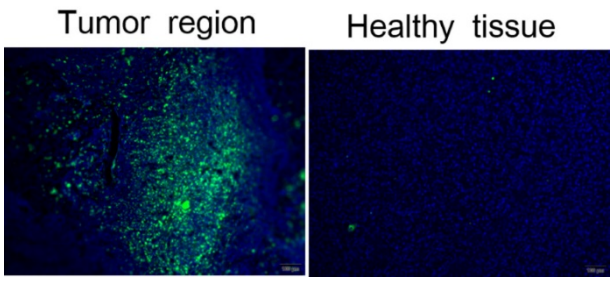

Figure S6 Accumulation of EGF1-EGFP in U87MG glioma *in vivo*. The green fluorescence from EGF1-EGFP was localized within the tumor region. Scale bar, 100 μm.