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Supplementary Methods

Patient-derived GBM stem-like cells and cell lines. MGG6, MGG8 and MGG23 primary glioblastoma stem-like cells (GSCs) were derived from surgical specimens obtained from glioblastoma patients undergoing treatment at the Massachusetts General Hospital, in accordance with the appropriate Institutional Review Board approval and have been previously characterized [1, 2]. GSCs are cultured as neurospheres in serum-free DMEM-F12 medium supplemented with 2% B27 supplement (v/v) and 2μg/mL heparin (Life Technologies, Carlsbad, CA), 20 ng/mL human recombinant EGF (R&D Systems, Minneapolis, MN), and 20 ηg/mL human recombinant bFGF-2 (Peprotech, Rocky Hill, NJ). U87 glioma cell line was obtained from ATCC and maintained in DMEM/10% FBS with 100 U/mL penicillin/streptomycin. For bioluminescence imaging, cells were transduced with a lentivirus vector expressing firefly luciferase (Fluc) and mCherry fluorescent protein separated by an IRES under CMV promoter (Fluc-ImCherry).[3] For knockdown experiments, similar strategy was used to infect cells with lentivirus expressing specific shRNA (obtained from Sigma).

IL-6 and TNF-α knockdown. MGG8 or U87 cells were plated in six-well plates and infected with lentivirus vectors expressing shRNA against either TNF or IL-6 or pLKO.1-puro Non-Target shRNA Control (shRNA CTRL; MISSION® shRNA obtained from Sigma) in the presence of polybrene (10 μg/mL) for 12 hours. The infected cells were selected using a medium containing puromycin (0.5 μg/mL) and knockdown efficiency in both cell lines was quantified by qRT-PCR.

RNA isolation, cDNA synthesis and qRT-PCR. RNA was isolated using mini columns (Qiagen, Venlo, the Netherlands) following the instructions of the manufacturer. Five hundred microgram of total RNA was transcribed into cDNA using OneScript® cDNA synthesis kit (Applied Biological Materials). Primer pairs for studying expression of IL-6 and TNF-α were designed using Primer-BLAST tool (available at NCBI website) using NCBI Reference Sequence and

checked against the genebank to avoid cross reactivity with other known sequences as follows: IL-6 (for, 5'-TTCCAAAGATGTAGCCGCCC-3'; rev, 5'-ATTTGTGGTTGGGTCAGGGG-3') and TNF-α (for, 5'-CACCACTTCGAAACCTGGGA-3'; rev, 5'-AGGAAGGCCTAAGGTCCACT-3').

In vitro luciferase cell proliferation/viability analysis. *Gaussia* luciferase (Gluc) assay. Coelenterazine, the Gluc substrate, was obtained from NanoLight™ (Pinetop, AZ) and resuspended at 5 mg/mL in acidified methanol and further diluted in phosphate buffered saline (PBS) immediately before use. Cells were plated in a 96-well clear bottom, black plates at a density of 3000-5000 cells/well, and the media were refreshed 4 hours before measurements to avoid accumulation of the reporter. To monitor cell growth, aliquots of cell-free conditioned medium (10 µL) were transferred to a white 96-well plate. Gluc activity was determined using FlexStation3 microplate reader (Molecular Device, Sunnyvale, CA) under "Flex" mode after injecting 100 µL 40 µM of coelenterazine.

Firefly luciferase (Fluc) assay. D-Luciferin, the Fluc substrate, was prepared by dissolving luciferin powder (25 mg/mL; Gold Biotech, St. Louis, MO) in PBS**.** For monolayer cells, conditioned medium was removed and cells were washed with PBS. For GSCs neurospheres, cells were transferred to V-bottomed plate, pelleted at 800xg for 5 minutes, and washed with PBS. 100 µL of lysis buffer (Promega) was added to each well and plates were placed on an orbital shaker with gentle shaking for 15 minutes to ensure complete lysis. Then cell lysate was transferred into solid, white 96-well microplates and mixed then at a 1:1 (v/v) ratio with the D-luciferin solution. Luciferase activity was determined using FlexStation3 microplate reader.

OEC migration assay. The migration assay was performed in 6-well Transwell® inserts (Corning Life Sciences) with polycarbonate filters (8 µm pore size) pre-coated on the upper surface with laminin (40 µg/mL). OECs (10^4 cells) were plated in the upper chamber of the Transwell insert in DMEM-F12 complete medium (0.4 mL per well). The lower chamber of the

transwell insert was filled with either DMEM-F12 complete medium (as negative control) or with conditioned medium collected from GSCs/GBM cell cultures. Twenty-four hours later, OECs that migrated to the bottom surface of the Transwell membrane were stained with crystal violet and quantified by taking pictures from five randomly chosen fields using an inverted microscope (Nikon E400 light) attached to a SPOT CCD digital camera (Diagnostics Instruments). All experiments were performed at-least in triplicate.

Apoptosis detection in co-cultured cells. OCU cells were cultured in serum-containing medium overnight, then the medium was drained and the cell monolayer was washed with PBS. GSCs were then added on top of the OCU. 5-FC was added at the same time for the treatment group. Neurospheres were collected and dissociated with short incubation with Accutase 24 hours later. Single cell suspensions were washed twice with cold PBS and then resuspended in Binding Buffer at a concentration of ~10^6 cells/mL and stained with Annexin V-FITC and propidium iodide kit (BD biosciences, Billerica, MA), per manufacturer's instructions. Cells were then analyzed using BD LSR II flow cytometer and data were analyzed using Flowjo software (BD).

Caspase 3 and 7 activity in GSCs was measured using Caspase-Glo® 3/7 Assay kit (Promega, Madison, WI) per manufacture's protocol.

Bioluminescence Imaging. Mice are injected intraperitoneally with 150 µg of D-luciferin per gram body weight and transferred into the Xenogen IVIS 200 Imaging System (PerkinElmer, Waltham, MA). Imaging was acquired 10 min post-luciferin injection and the image intensity was quantitated using the Living Image software 3.0 from Xenogen Imaging Technologies (Perkin-Elmer, Waltham, MA) as we previously described [4].

Immunofluorescence. For OECs primary culture phenotypic characterization, cells were detached using TrypLE and placed on chamber slides (Nunc, Thermo Fisher Scientific) pre-treated with poly-L-lysine (20 μg/mL, Sigma-Aldrich), covered with laminin (40 μg/mL, Life Technologies). After adhering on coverslips, OECs were fixed with 4% paraformaldehyde (PFA). We first washed the glass slide chambers with 0.3%Triton in PBS and blocked with blocking solution containing 10% normal goat serum (NGS) and 2% Bovine serum albumin diluted in a Triton/PBS for 1 h. OECs were then incubated overnight with the following primary antibodies: anti-2′ 3′ cyclic nucleotide 3′-phosphodiesterase (anti-CNPase, monoclonal mouse IgG, 1:100, Sigma–Aldrich); anti-smooth muscle α-Actin (anti-SMA, monoclonal rabbit IgG, 1:100, Sigma–Aldrich); anti-S100b (anti-S100β, polyclonal rabbit IgG, 1:100, Abcam) and anti-glial fibrillary acidic protein (anti-GFAP, polyclonal rabbit IgG, 1:80, StemCell Technologies). Chamber slides were washed with Triton/PBS and incubated with appropriate species secondary antibodies conjugated to Alexa Fluor 488 or 633 (1:500, Life Technologies) for 2 hours at room temperature. After several washes with 0.9% NaCl, nuclei were stained with 6-diamidino-2-phenylindole (DAPI). The slides were mounted with anti-fade medium n-propyl gallate (Sigma-Aldrich) and analyzed by fluorescence microscopy.

For ex vivo histological evaluation, two animals from each group were perfused with 4% PFA under deep anesthesia. Tissues were harvested, embedded in Microm Neg-50 frozen section medium (Thermo, Waltham, MA) and stored at −80 °C. Brains were cryosectioned coronally (15 μm) using a Microm HM-500M CryoStat, mounted on slides. Tissues were stained with Hematoxylin-Eosin (H&E) per standard protocol. For immunostaining, different tissue sections were blocked with blocking solution for 1 hour, followed by an overnight incubation with anti-GFP (monoclonal rabbit IgG, 1:100, Cell Signaling), anti-CD133 (monoclonal mouse IgG conjugated with APC, non-diluted, Milteny Biotec) and anti-cleaved Caspase-3 (anti-Caspase-3, monoclonal rabbit IgG, 1:50, R&D Systems). Brain slices were washed with Triton/PBS and incubated with corresponding secondary antibody conjugated to Alexa Fluor 488 or 633

antibody respectively (1:500) for 2 hours at room temperature. After several washes with 0.9% NaCl, nuclei were stained with DAPI, brain sections were mounted with n-propyl gallate medium and analyzed by fluorescence microscopy.

References

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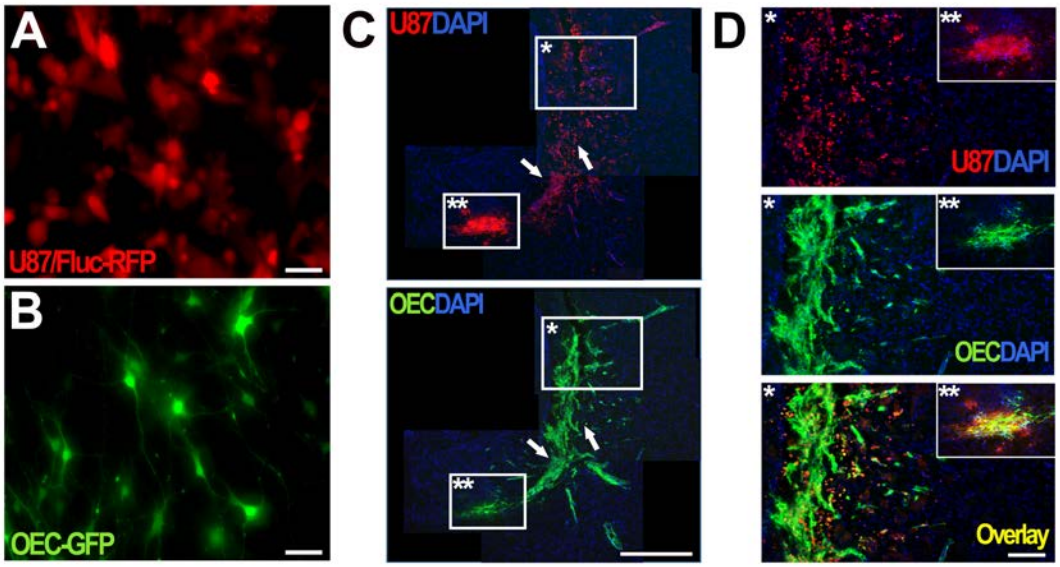
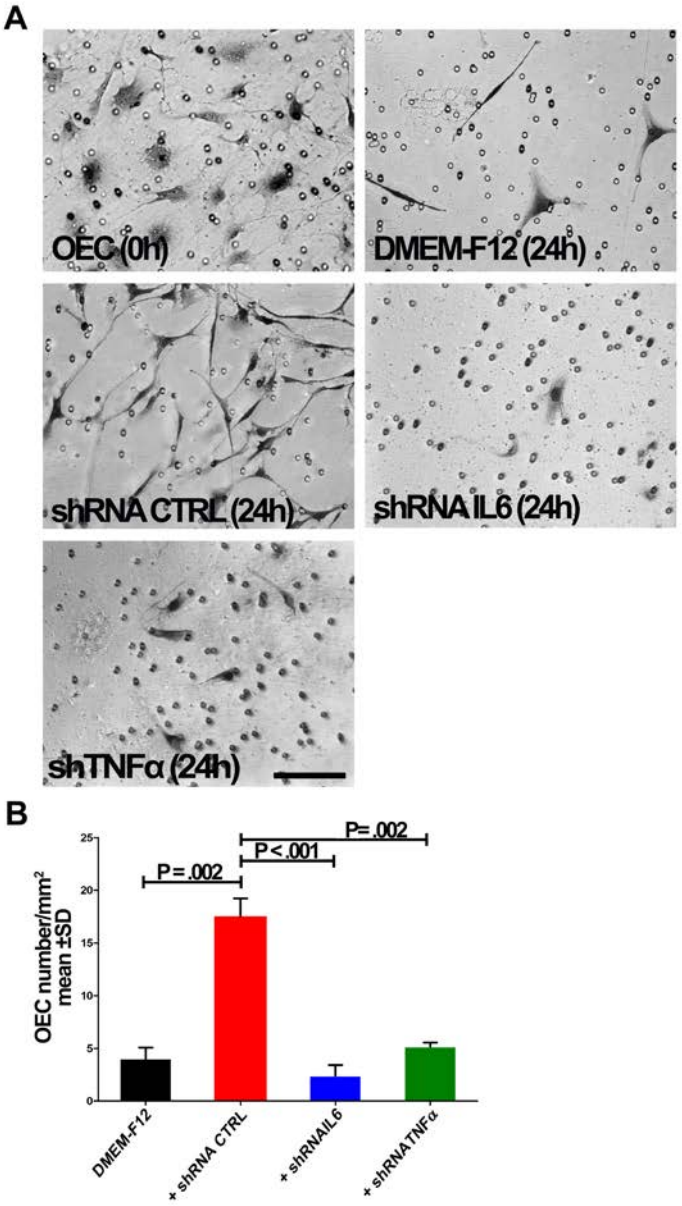
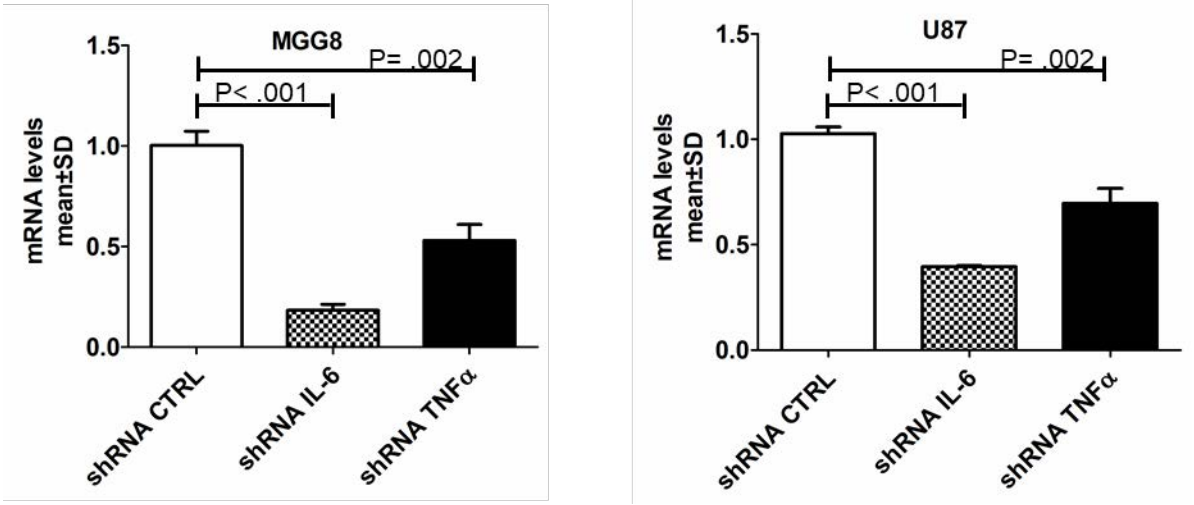


Supplementary figure 1. OEC targets U87 glioma tumors upon intranasal administration. (**A-D)** U87-Fluc-ImCherry cells (A) were injected into the midstriatum of nude mice brain. The needle was retracted in a fast manner on purpose to have single cells at the needle track. Three-week post-injection, mice received 10^5 OECs-GFP cells (in 10 µl PBS; B) via intranasal administration. One week later, mice were euthanized and the brains were removed, sectioned and analyzed for mCherry (U87 cells) and GFP (OEC) by immuno-fluorescence. OEC (GFP) migrated towards U87 cells (RFP). Arrows in (C) indicate OEC tracking tumor cells. Interestingly, OEC-GFP cells were found at the primary tumor site (**) and along the needle track targeting single cells (*). Higher magnification of these 2 sites are showing in (D). Scale bar, 150µm (C); 50µm (A-B and D).



Supplementary figure 2. OEC displays tropism to U87 glioma cells *in vitro*. Conditioned medium collected from U87 cells expressing shCTRL, shIL6, or shTNFα were placed in lower chambers and OECs in top chambers of a transwell. (**A)** 24 hrs later, lower chamber was analyzed by microscopy. (**B)** Quantitation of OECs in the lower chamber under different conditions. (mean ± SD, n = 3 independent experiments, P value from two-sided Student's t test is shown). Scale bar, 50 μm. Abbreviations: olfactory ensheathing cell (OEC); shRNA against tumor necrosis factor alpha (shRNA TNFα), shRNA against interleukin 6 (shRNA IL-6); shRNA Non-Target (empty vector) as control (shRNA CTRL).



Supplementary figure 3. Knockdown Efficiency of of IL-6 and TNF-α in tumor cells. MGG8 cells or U87 cells were infected with three different shRNA lentivirus constructs targeting human IL-6, TNF-α or empty vector as control (shRNA CTRL). After selection with puromycin, cells were lysed and RNA was isolated. Expression and quantification of both cytokines was analyzed by qRT-PCR and alpha-actin as housekeeping gene. Quantification showed a significant reduction of IL-6 (82% [0.18±0.03] and 61% [0.53±0.07] vs CTRL [1.02±0.07]) and TNF-α (47% [0.39±0.004] and 32%[0.69±0.07] vs CTRL [1.03±0.03]) levels in both MGG8 and U87 cells, respectively. (mean±SD, n = 3, P value from two-sided Student's t test is shown).