Supplementary Data

Supplementary Materials and Methods

Culturing of spheroid by hanging drop method

ΔGli36 glioma spheroids were formed based on a modified hanging drop method. About 5×10^3 cells in 10 μL were dispensed on the lid of cell culture dish and inverted to form the hanging drops. They were incubated for 48 h and then transferred by overlaying them, in presence of media, onto 0.75% agarose-coated wells.

Quantification of soluble TRAIL by ELISA

TRAIL was quantified using the human TRAIL/TNFSF10 Immunoassay Kit (R & D Systems) according to the manufacturer's protocol. Absorbance reading at 450 nm was obtained by TECAN plate reader.

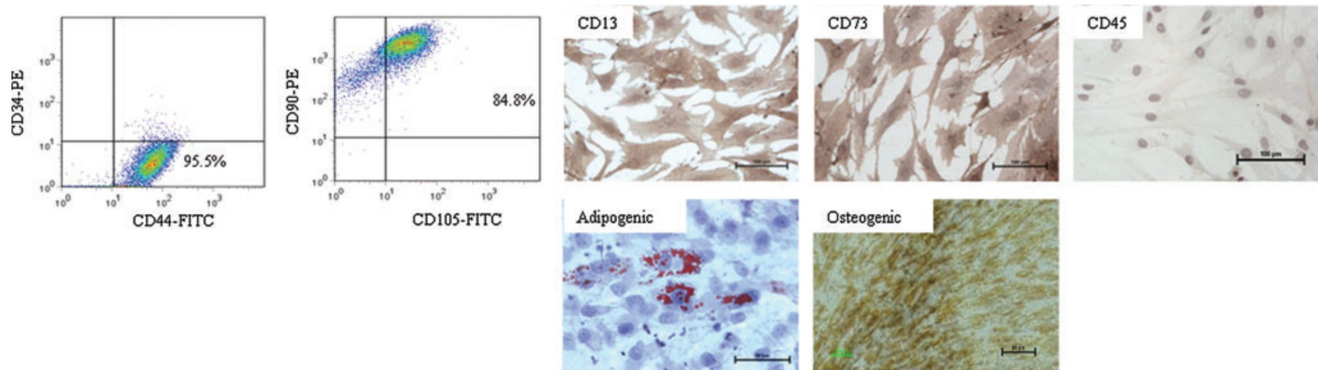
Real-time reverse transcriptase–polymerase chain reaction

Total RNA was isolated using TRIzol (Invitrogen) method according to the manufacturer's protocol. For first-strand cDNA synthesis, 2 μg of total RNA was generally used. cDNA was synthesized using random hexanucleotide primers and oligo-dT12–17 primers (Invitrogen), in the presence of superscript II reverse transcriptase (Invitrogen). The expression level of Cx43 and 18S was quantified using QuantiTech^{TM} SYBR Green PCR kit (Qiagen). The following primers were used to determine the Cx43 level: forward primer 5′-ATGAGCAGTCTGCCTTTCGT-3′ and reverse primer 5′-TCTGCTTCAAGTGCATGTCC-3′. All quantitative PCR reactions were conducted in duplicate. Standard curves for Cx43 and 18S were generated independently. The relative copy number of each sample was calculated according to the corresponding standard curve using RotorGene software version 6.0. Normalization was performed in each sample by dividing the copy number of the target genes to that of 18S. The relative expression levels were calculated by arbitrarily designating the lowest normalized value to 1.

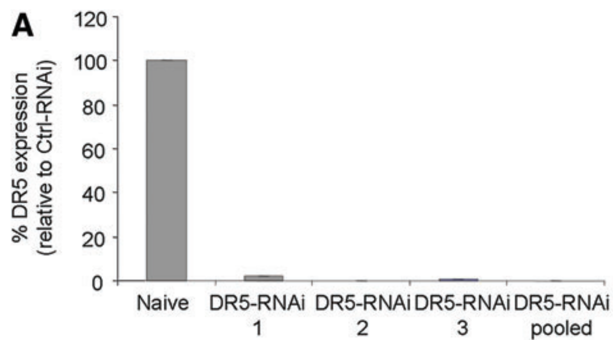
Immunohistochemistry and immunofluorescence staining

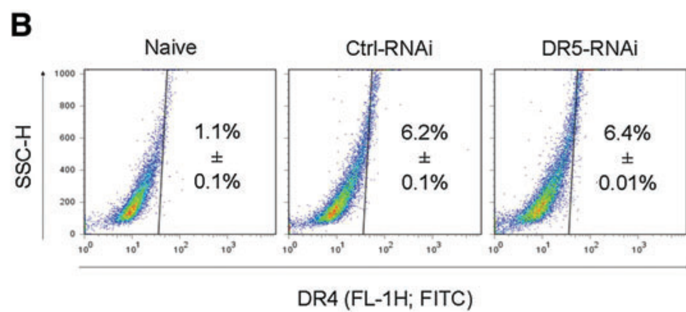
For immunochemistry staining, cells seeded on glass coverslips were fixed with 4% paraformaldehyde (PFA) and permeabilized with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 5 min each before washing with PBS. Cells were then incubated in blocking buffer (0.1% Tween-20 + 10% goat serum + PBS) for 1 h at room temperature followed by incubation with primary antibodies at room temperature. Cells were then stained with horseradish peroxidase-conjugated anti-mouse polymer followed by detection using 3,3′-Diaminobenzidine (EnVision + System; Dako). Coverslips were then mounted onto microscope slides and visualized using bright-field microscopy (Nikon). Images were captured at original magnification ×10.

For immunofluorescence staining, cells seeded on glass coverslips were fixed with 4% PFA or cold acetone for GFAP and Cx43 staining, respectively, followed by permeabilization with 0.1% Triton X-100 in PBS for 5 min each before washing with PBS. Following 1 h incubation in blocking buffer, cells were incubated in mouse anti-GFAP (BD Pharmingen) or rabbit anti-Cx43 (Sigma Aldrich) at room temperature for 1 h. Subsequently, cells were incubated in respective secondary antibodies at room temperature for 1 h and counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Images were obtained using a 63×/NA 1.4 Plan-Apochromat oil immersion objective mounted on the LSM 510 Meta Confocal Microscope system (Carl Zeiss).

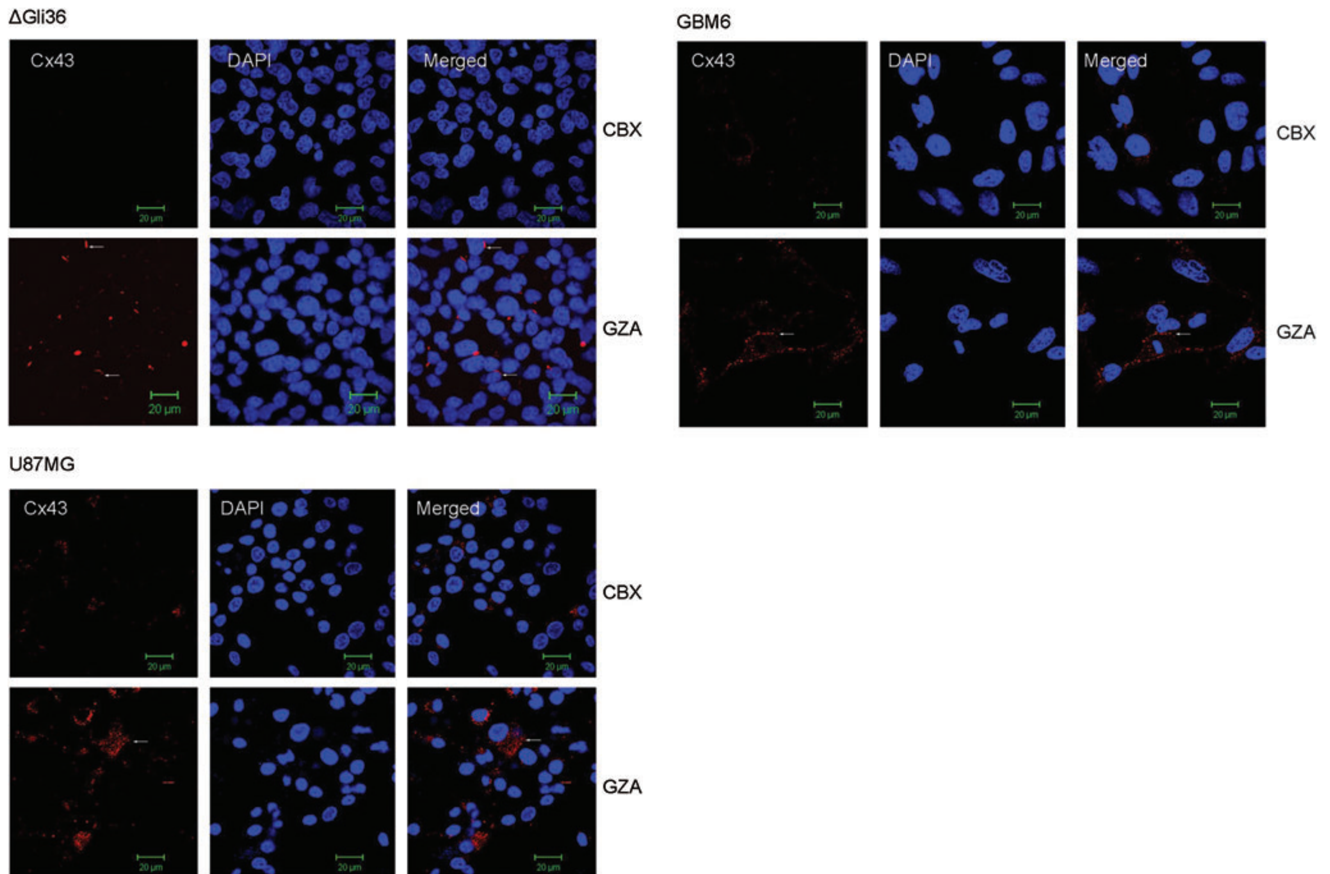


SUPPLEMENTARY FIG. S1. Characterization of bone marrow-derived mesenchymal stem cells (MSC). Expression of the characteristic surface markers that identify MSC was analyzed by FACS and immunohistochemistry staining. MSC were able to differentiate toward the adipogenic and osteogenic lineages.

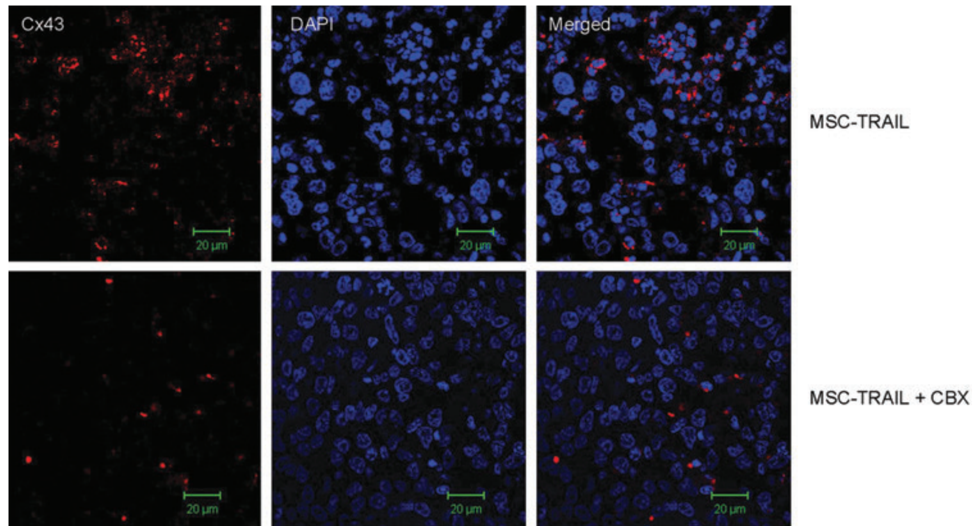




SUPPLEMENTARY FIG. S2. Transient knockdown of DR5 expression in ΔGli36 cells. Flow cytometry analysis was performed 72 h post-transfection to assess **(A)** the knockdown efficiency of different siRNA constructs targeting DR5 and **(B)** its effect of DR4 expression level.



SUPPLEMENTARY FIG. S3. CBX downregulates Cx43. The level of Cx43 protein in glioma cells, ΔGli36, and U87MG and primary glioma GBM6 were assessed by immunofluorescence staining (red) 72 h post-treatment. DAPI (*blue*) stained the nucleus. Confocal images represented were captured at original magnification ×630.



SUPPLEMENTARY FIG. S4. CBX downregulates Cx43 in tumor sections harvested from mice. The level of Cx43 on the sections was determined by immunofluorescence staining (*red*) and quantified. Confocal images represented were captured at original magnification $\times 630$.