Materials and methods

Apoptosis assays. For detecting apoptosis, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assays were performed using the *In Situ* Cell Death Detection kit (Roche Diagnostics), with cells grown on coverslips according to the manufacturer's instructions. At least five images per coverslip were analyzed with a Cell Observer microscope (Zeiss AG), and the percentage of TUNEL-positive cells was determined using an ImageJ macro [Damir Krunic, Light Microscopy Core Facility, German Cancer Research Center (Deutsches Krebsforschungszentrum; DKFZ)] in relation to the total cell count determined by DAPI (Cell Signaling Technology, Inc.) staining.

Electron microscopic examination. The ultrastructure of brain organoids was observed by electron microscopy. The tissue was placed in fixative and washed with PBS. It was then post-fixed in phosphate-buffered 1% osmium tetroxide. Following serial dehydration in increasing concentrations of alcohol, the tissue was embedded in epon resin. Ultrathin Sects. (70-80 nm) were cut using an ultramicrotome (Ultracut UCT, Leica Microsystems GmbH). The sections were subsequently stained with uranyl acetate and lead citrate. The ultrastructure of these specimens was examined using an electron microscope (JEM-1010, JEOL, Ltd.).

Co-culture of glioma cell line and cerebral organoids. For the co-culture experiments, individual organoids were transferred to a 24-well plate (one organoid per well). Excess medium was removed, and 1,000 cells were plated in each organoid-containing well (1,000 U87-luc, U373-luc in 50% DMEM and 50% brain organoid mature media per well). Plates were incubated at 37˚C for 24 h with no agitation. Each organoid was subsequently washed in PBS and transferred to a clean well with 2 ml organoid differentiation medium. Tumor-bearing organoids were maintained on an orbital shaker for up to 7 days at 37˚C.

Figure S1. TUNEL assay of U87 and U373 cells treated with TTFfields. The portion of TUNEL-positive cells in U87 and U373 spheres treated with 75 and 100% duty cycle of TTFfields was higher than that in cells treated with the 50% duty cycle of TTFfields. TUNEL-positive cells were absent in the control (scale bar, 200 μm). TTFfields, tumor-treating fields.

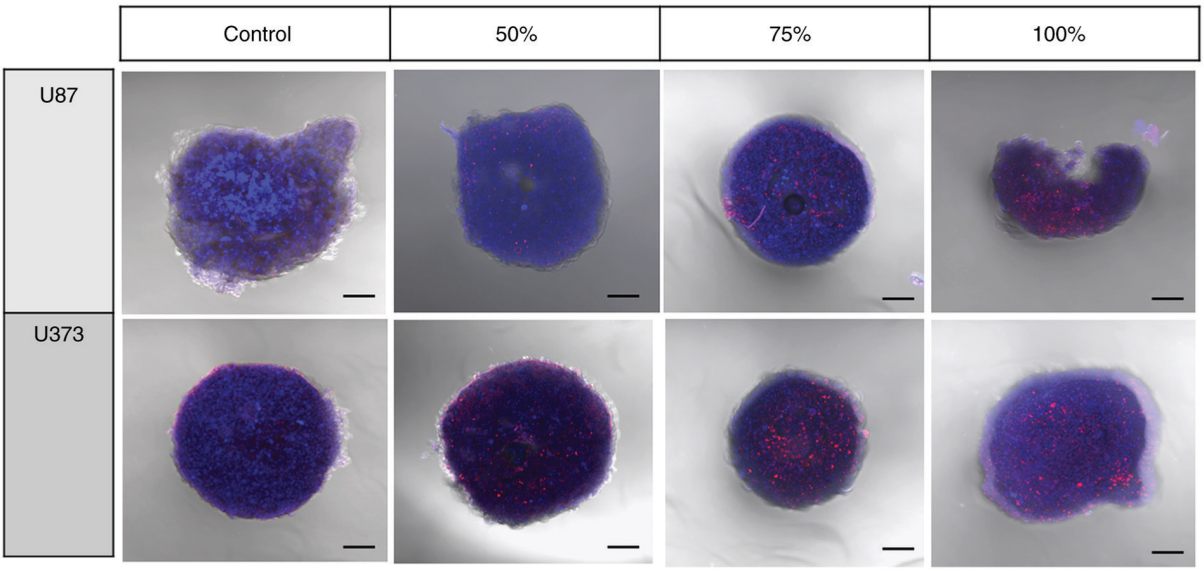

Figure S2. Electron microscopy of human brain organoids showed axon-like microtubules (arrowheads) and astrocytes (scale bar, 100 nm). N, nucleus; C, cytoplasm.

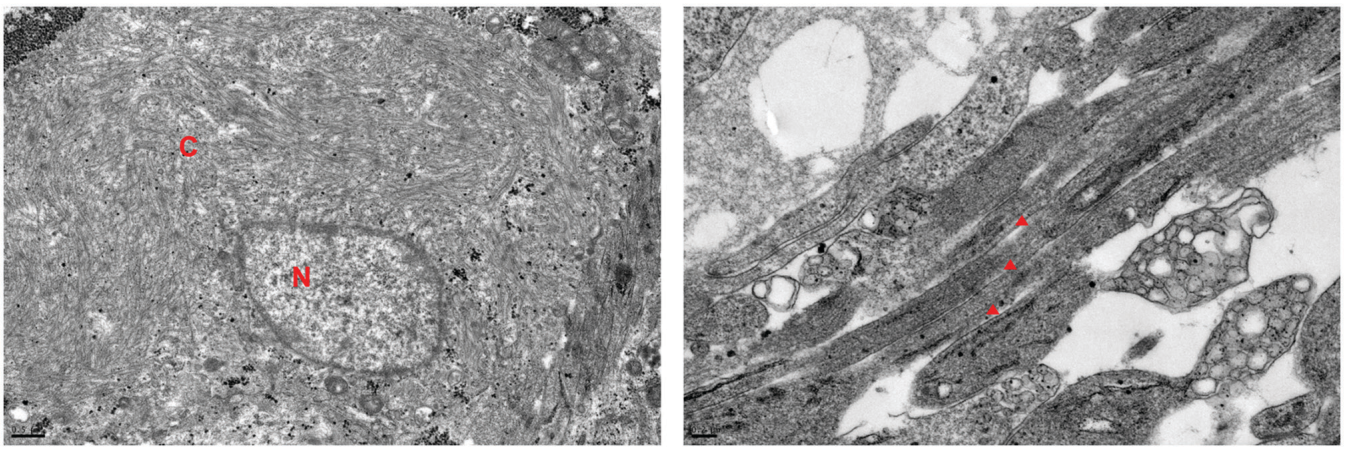

Figure S3. TUNEL assay of brain organoids treated with TTFfields. The portion of TUNEL-positive cells in brain organoids treated with 50 and 75% duty cycle of TTFfields was higher than that of those without TTF exposure. As brain organoids treated with 100% duty TTF were severely disorganized, the comparison of TUNEL-positive cells was not appropriate (scale bar, 200 μm). TTFfields, tumor-treating fields.

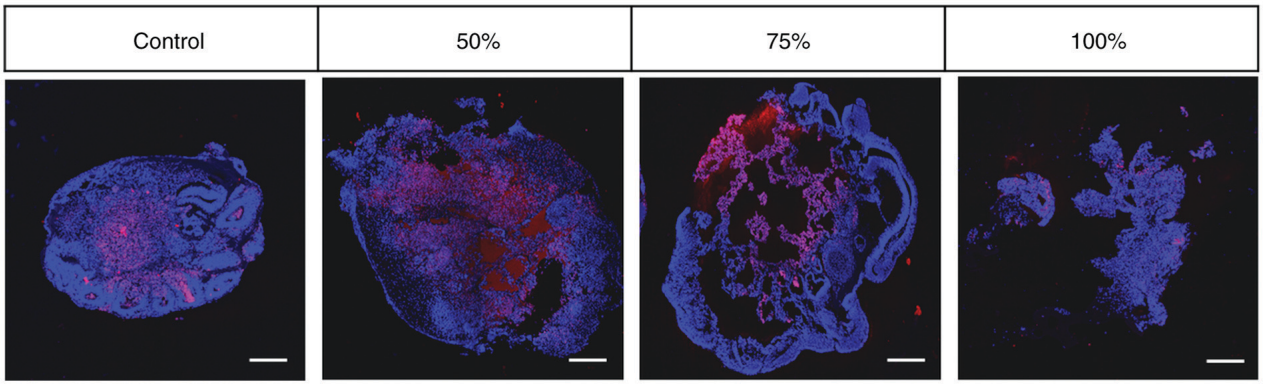

Figure S4. (A) H&E staining of normal kidney organoid and (B) confocal microscopy image of normal kidney organoid stained with DAPI (blue) and glial fibrillary acidic protein (green).

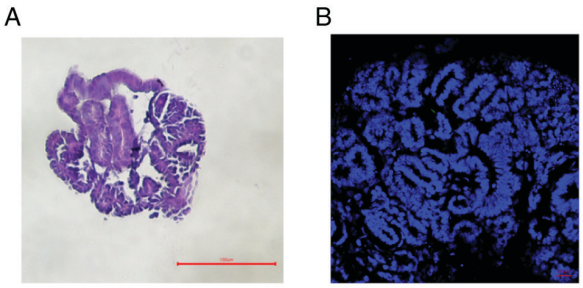

Figure S5. Co-culture of normal brain organoid and U87-luc. (A) An increase of bioluminescence was more prominent on day 7 (right panel), compared with that on day 2 (left panel). TUNEL assay of brain organoids treated with tumor-treating fields. (B) The portion of TUNEL-positive cells in the co-culture of normal brain organoid and U87 without TTF exposure and with TTF exposure is shown (scale bar, 100 μm).