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Supplementary Materials for

ssDNA nanotubes for selective targeting of glioblastoma and delivery of doxorubicin for enhanced survival

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Figs. S1 to S11
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Legends for movies S1 to S3

Other Supplementary Material for this manuscript includes the following:

Movies S1 to S3

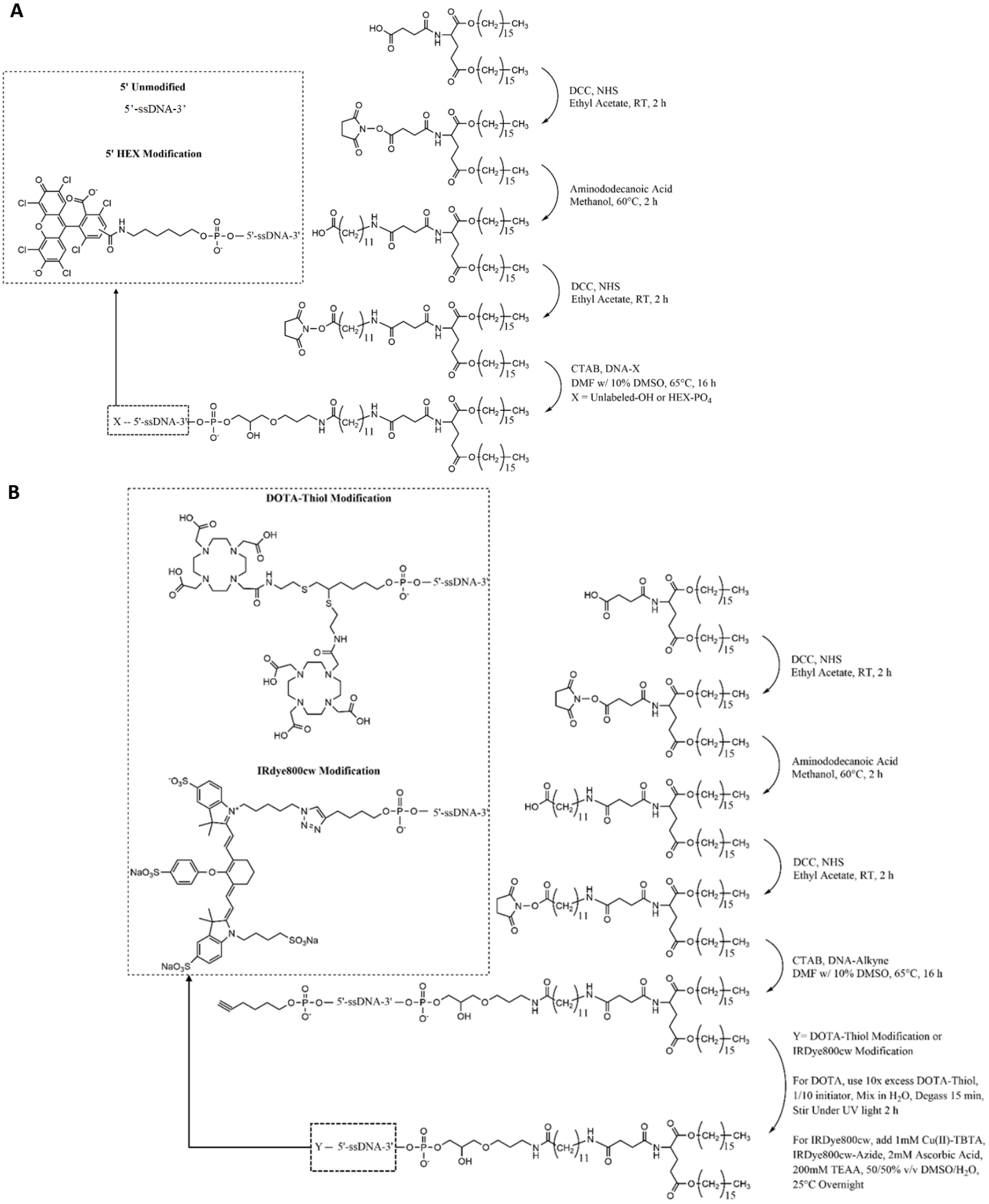

Figure S1. Synthesis schemes of ssDNA-amphiphiles. (**A**) Synthesis scheme of ssDNA-amphiphiles with or without a HEX fluorophore at the 5' end. (**B**) Synthesis scheme of ssDNA-amphiphiles with a DOTA or IRDye 800CW fluorophore. Inserts show the chemical structures of the different probes attached to the 5' end of the ssDNA.

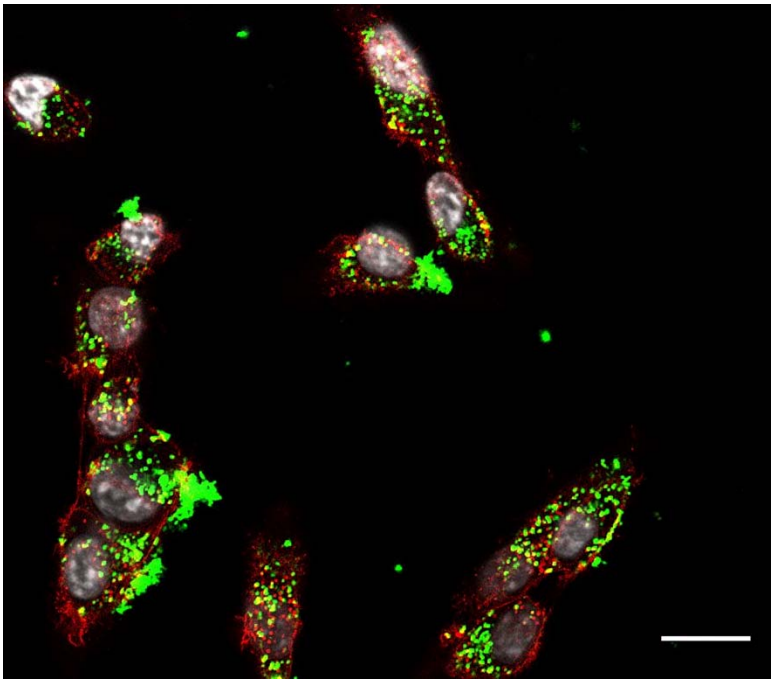

Figure S2. Nanotube internalization in GL261 cells after 3 h incubation. Confocal microscopy of HEX-labeled nanotubes (green) after incubation with GL261 GBM cells for 3 h at 37 °C. Nuclei are shown in gray and cell membranes in red. Scale bar is 20 µm.

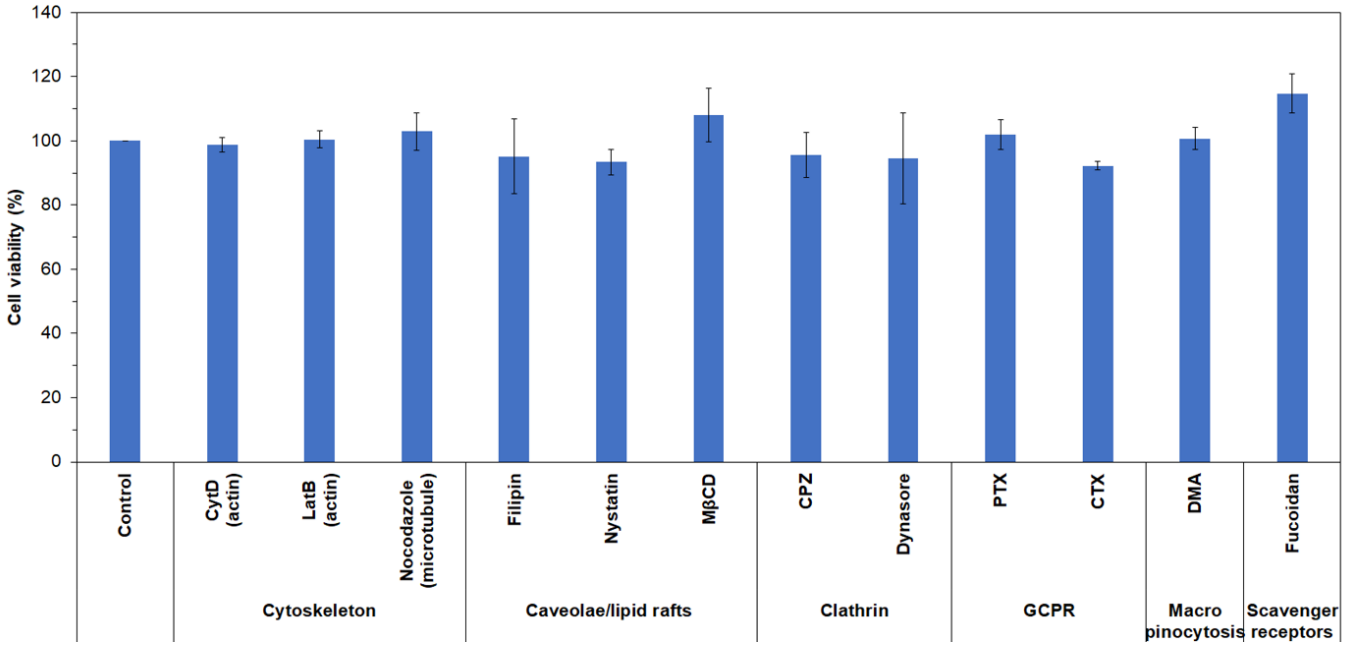

Figure S3. Cell viability after exposure to endocytosis inhibitors. Viability of GL261 cells treated with different endocytosis inhibitors at the same concentrations used for Fig. 2E. Data are shown as mean ± SD (n = 3). Statistical significance with that of the control was determined using a two-sided unpaired *t*-test; $P > 0.05$ for all inhibitors.

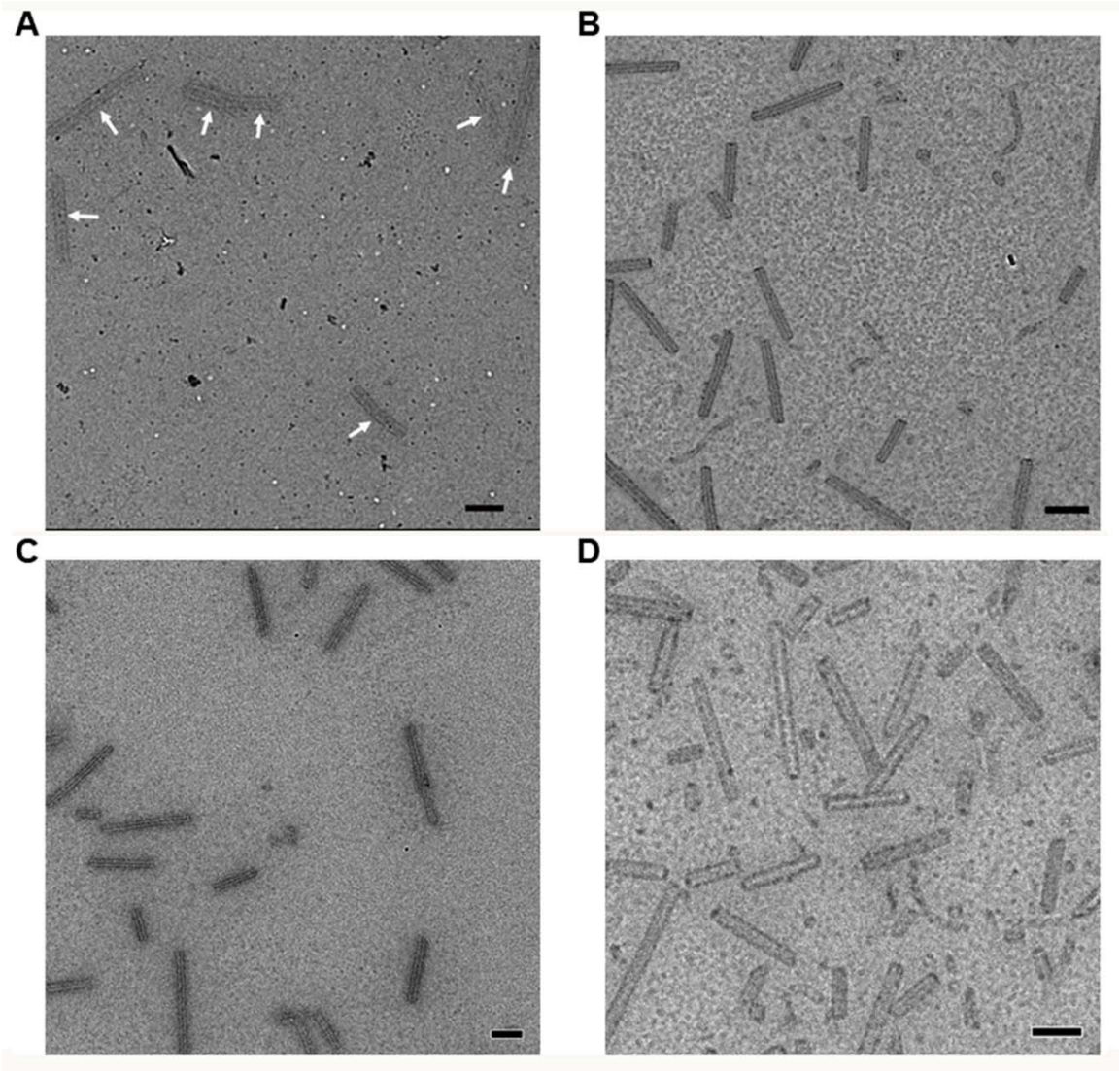

Figure S4. Stability of nanotubes in serum and nucleases. Room temperature TEM of nanotubes after exposure for 24 h at 37 °C to (**A**) 50% (v/v) FBS, (**B**) 5 U/mL of DNase I, (**C**) 5 U/mL of exonuclease III, and (**D**) 5 U/mL T5 exonuclease. The white arrows in **A** point to nanotubes, as the high protein concentration in the sample made visualization of the nanotubes difficult. All scale bars are 100 nm.

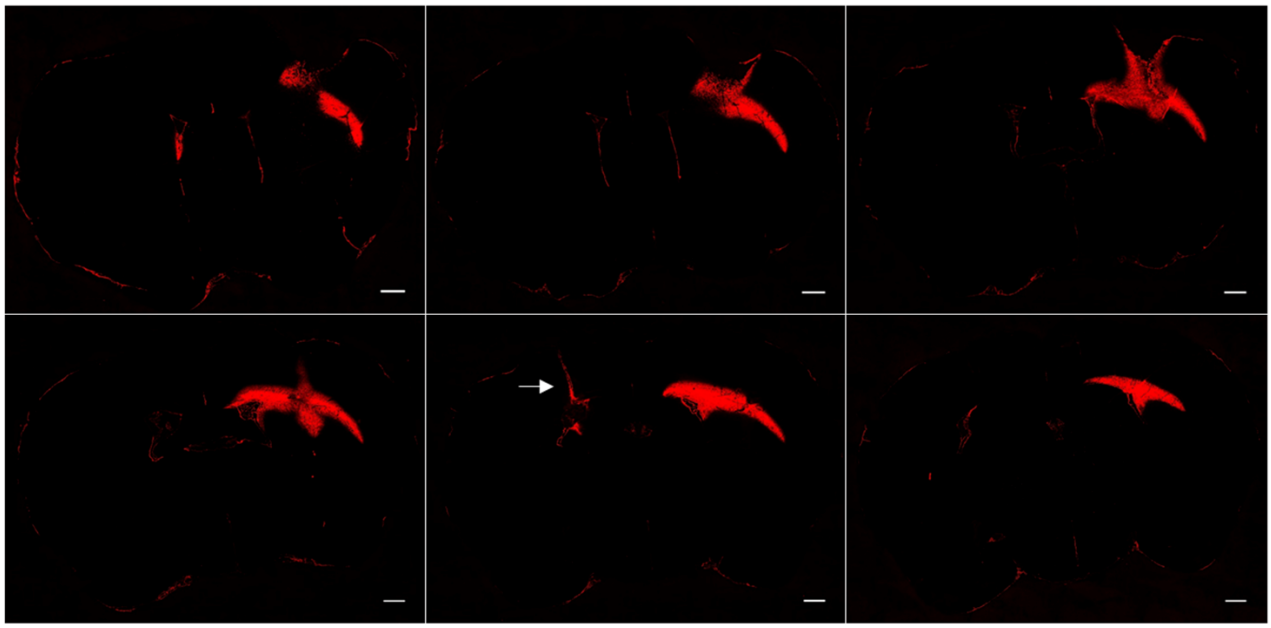

Figure S5. Nanotube retention by tumor hemisphere. Mouse 1 (Fig. 3B) bearing an orthotopic GL261 tumor on the right hemisphere received bilateral intracranial injections of NIR fluorescently-labeled nanotubes. Wide-field fluorescent microscopy images of brain tissue sections excised from mouse 1 show the nanotubes (red) only on the right, tumor-bearing hemisphere. The white arrow shows an area close to the left hemisphere injection point. Scale bars are 500 μm.

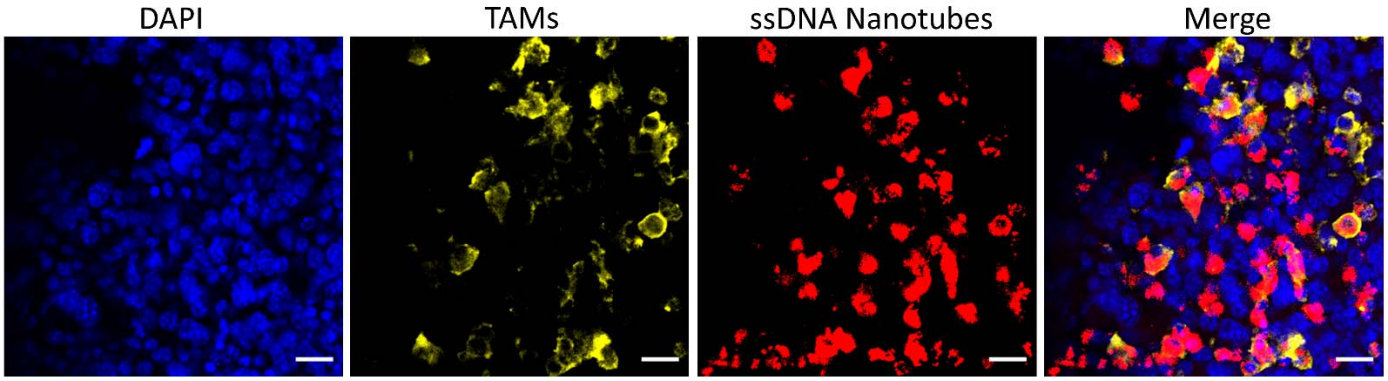

Figure S6. Nanotube colocalization with tumor associated macrophages (TAMs). Mice bearing orthotopic GL261 tumors received intracranial injections of HEX-labeled nanotubes. Mice were sacrificed 3 h post intracranial injection, and tumor tissues were removed and processed. Confocal microscopy images show colocalization of nanotubes (red) with TAMs (yellow). Nuclei were stained with DAPI (blue). Scale bars are 20 μm.

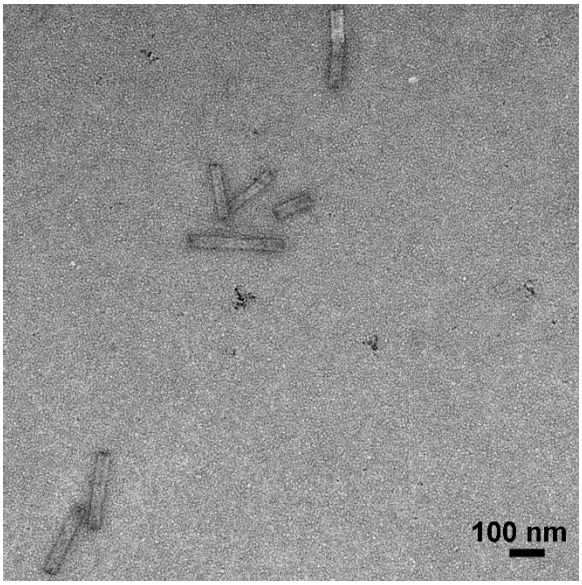

Figure S7. DOTA-labeled nanotubes. Room temperature TEM of nanotubes labeled with DOTA.

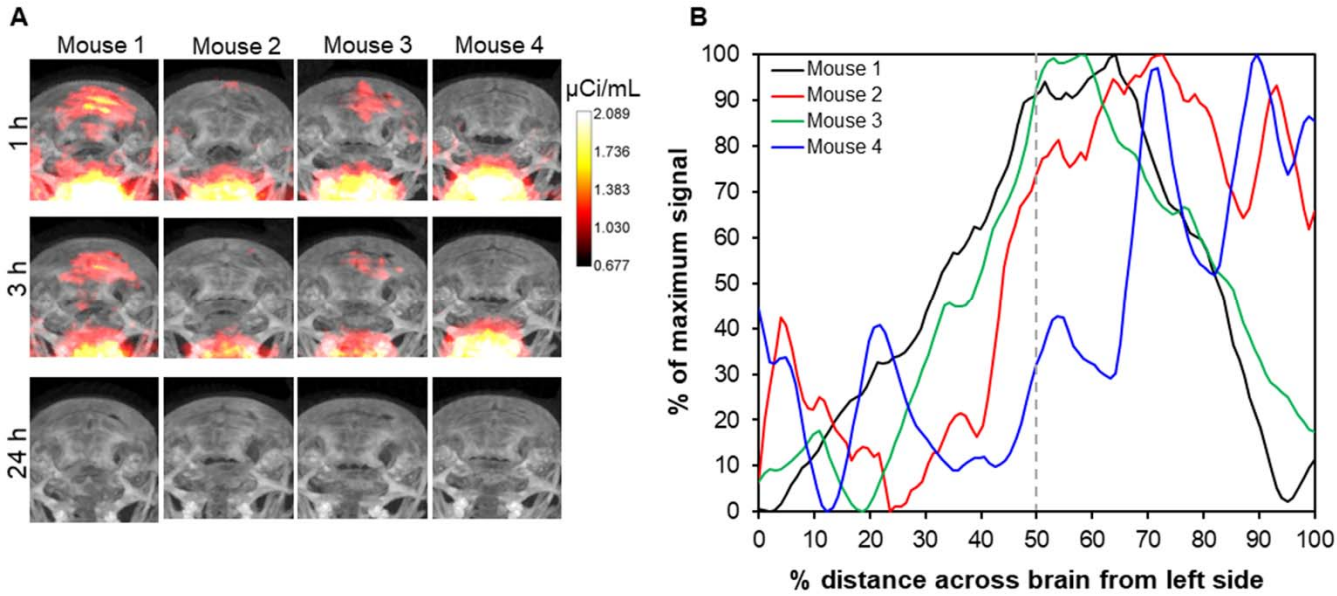

Figure S8. Nanotube accumulation in the brain after intravenous injection. (**A**) Tail-view maximum intensity projection of μPET/CT scans of mice heads at 1, 3 and 24 h after intravenous injection of ^{64}Cu-radiolabeled nanotubes to mice bearing GL261 orthotopic tumors. The intensity of the μPET signal was not adjusted for the half-life of ^{64}Cu. (**B**) Percent of maximum μPET brain signal as a function of distance from the left side of the brain from the head-view images in **A** at 1 h. Background signal was subtracted, and radiation intensity values were not adjusted for the half-life decay of ^{64}Cu.

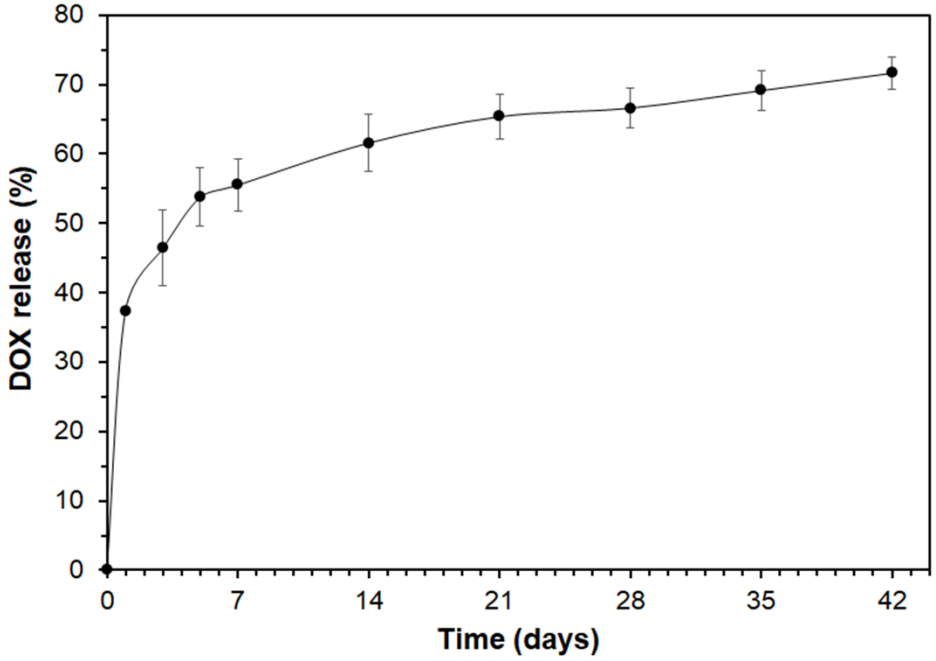

Figure S9. DOX release from nanotubes. Release profile of DOX from nanotubes in PBS at 37 ºC. Results are reported as mean ± SD (n =3).

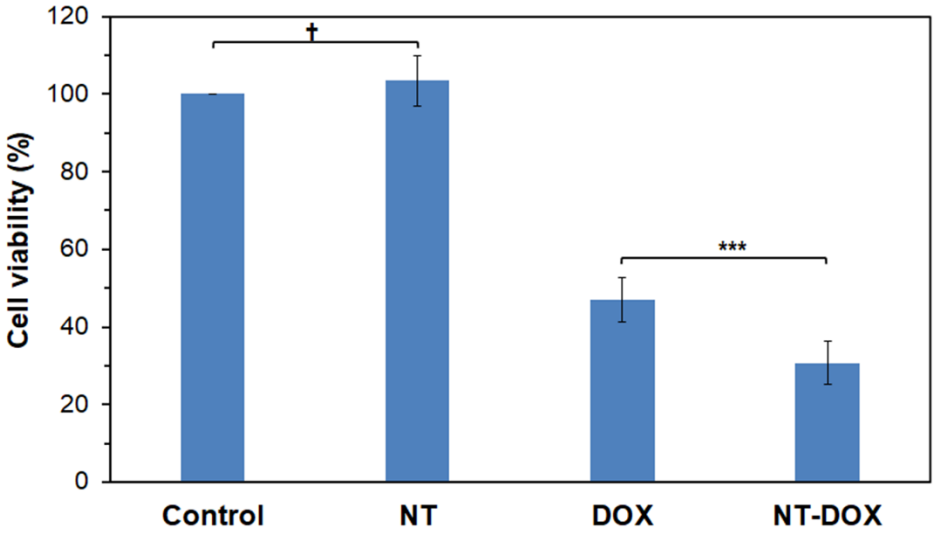

Figure S10. GL261 viability after exposure to different treatments. Viability of GL261 cells treated with ssDNA nanotubes (NT) at 5-6.4 µM of ssDNA-amphiphiles, free DOX at 5 µg/mL, or DOX intercalated in the ssDNA nanotubes (NT-DOX) at the same DOX and amphiphile concentrations. Cells were incubated with the different samples for 12 h at 37 ºC, washed, replenished with media and incubated for another 36 h at 37 ºC. Data are presented as mean ± SD (n = 6). Statistical analysis was performed by one-way ANOVA with Tukey's honest significant difference post-hoc test; † $P > 0.05$, *** $P < 0.005$, for all other groups $P < 0.00001$.

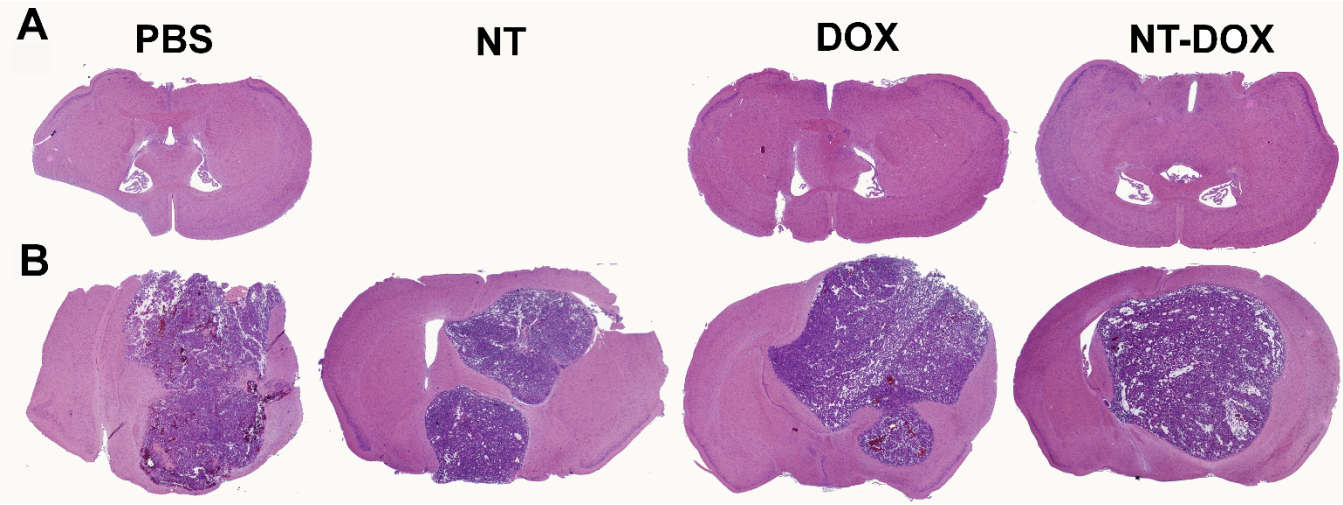

Figure S11. Hematoxylin and eosin (H&E) staining of brains. Representative images of H&E staining of brain tissues from mice that received different treatments (PBS, NT, DOX, NT-DOX) and either (**A**) survived at the end of the experiment (day 82 from day of surgery) or (**B**) died during the experiment. Images were taken with 1x objective lens.

Table S1. Masses of ssDNA-amphiphiles as determined by LC-MS.

ssDNA-amphiphiles	Expected mass (Da)	Measured mass (Da)
10nt ssDNA-amphiphile	4161.6	4159.3
HEX-10nt ssDNA-amphiphile	4905.7	4901.2
DOTA-10nt ssDNA-amphiphile	5245.8	5243.7

Movie S1. Confocal microscopy 3D movie showing internalization of nanotubes in GL261 GBM cells (Fig. 2A).

Movie S2. Confocal microscopy 3D movie showing lack of nanotube internalization in C8-D1A normal astrocytes (Fig. 2A).

Movie S3. Confocal microscopy 3D movie showing colocalization of nanotubes with early endosomes and acidic organelles in GL261 GBM cells (Fig. 2B).