Supplementary Information

SUPPLEMENTARY FIGURES

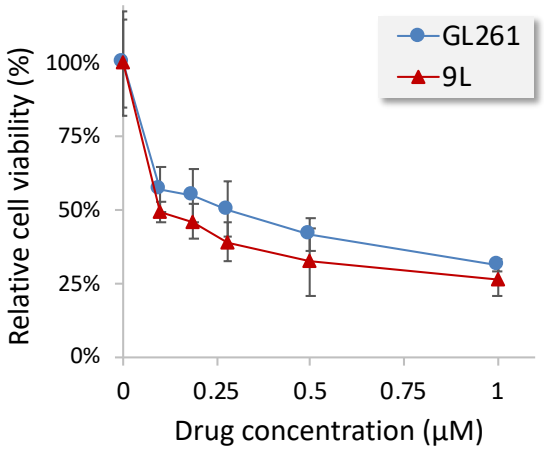

Figure S1. Cytotoxicity of DOX on glioma cells. Cell viability was used as an indication of the cytotoxic effects of DOX on GL261 and 9L glioma cells. The cytotoxicity tests were performed at a drug concentration varying from 0-1 µM of DOX (n=5 per condition). The cells were seeded at a density of 10^5 cells/well in a 96-well plate 48 h before incubation with drugs. Cells were incubated with the treatment for 48 h and were incubated for 48 h at 37°C and 5% CO_2 in a humidified environment. The treated cells were then washed three times with fresh medium and the number of viable cells was determined using a formazan-based cell counting assay (CCK-8). Untreated cells were served as live controls for normalization of the data.

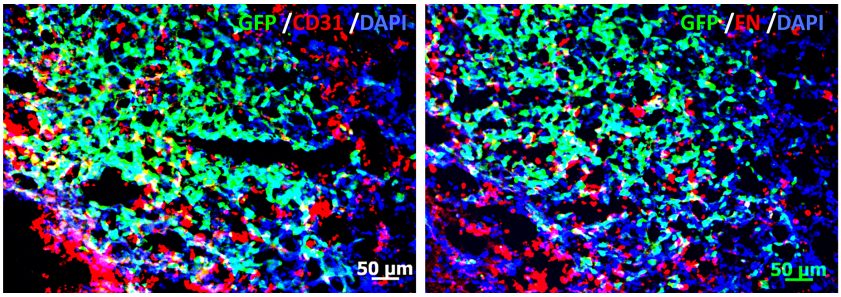

Figure S2. Histological analysis. Histological analysis of the topology of fibronectin in the GL261 model in mice (10x magnification; GFP: glioma cells; DAPI: cell nuclei; CD31: endothelial cells; FN: fibronectin).

SUPPLEMENTARY METHODS

Synthesis of Fe@MSN nanoparticles

Iron oxide cores were synthesized by the coprecipitation method. A typical synthesis procedure is as follow: 0.6757 g of $FeCl_3.6H_2O$ and 0.2478 g of $FeCl_2.4H_2O$ were dissolved in 5 mL of deoxygenated water. To this solution, 2.5 mL of 0.4 M HCl was added under vigorous stirring. This iron precursor solution was added to a solution of 25 mL of 0.5 M NaOH, which was preheated to 80 °C under a constant flow of argon. The reaction mixture was then stirred for another 15 minutes at 80 ^{0}C under argon and the black precipitate was separated by using a powerful magnet. The nanoparticles were then washed several times with Milli-Q water until stable ferrofluid was obtained. To prevent the nanoparticles from agglomeration 170 mg of citric acid (in 10 mL of deionized water) was introduced

and allowed to react at 80 ^{o}C for 1.5 h. The pH of the reaction mixture was adjusted to 5.2 using concentrated ammonia solution prior to heating. The reaction was protected under argon in order to avoid any undesired side-reactions. Finally, uncoated nanoparticles and aggregates were removed by repeated centrifugation. Excess citric acid was removed by centrifugation with Amicon Ultra-15 centrifugal filters.

To prepare the final Fe@MSN nanoparticles, we generated the mesoporous silica shell around the iron oxide core. We used a base-catalyzed sol–gel process with a slight modification. Specifically, 50 mg of the iron oxide nanoparticles was first dispersed in 25 mL 80% ethanol by ultra-sonication, to which 1 g of cetyltrimethylammonium bromide (CTAB) solution (in 5 mL of di water) was added and the resulting solution was stirred vigorously for 30 minutes. Then the mixture was heated at 60 °C for another 20 minutes to evaporate ethanol. The resulting Fe_3O_4/CTAB solution was added to a mixture of 45 mL of water and 0.3 mL of 2M NaOH solution and the mixture was heated up to 70°C under stirring. Then, 0.5 mL of tetraethylorthosilicate (TEOS) was added to the reaction solution under vigorous stirring. After stirring for another 10 min, 3.3 mg of silane-PEG-NH_2 was added and stirred for 24 h at room temperature. After that, 54 µL of Trihydroxysilylpropylmethylphosphonate was added and the solution was stirred for 4 h. The unreacted species were removed by washing the nanoparticles 3 times with ethanol. Finally, CTAB was extracted by refluxing the nanoparticles at 60 °C for 3 hrs. with acidic ethanol (pH ~ 1.4). Finally, aggregates were removed by repeated centrifugation.

Loading of drugs into Fe@MSN nanoparticles

For DOX loading, 10 mg of phosphonate-functionalized Fe@MSN nanoparticles were suspended in 2 mL of PBS at pH of 7.4. A solution of DOX.HCl (5 mg in 1 mL water) was added to the Fe@MSN suspension. The mixture was kept under mild stirring for 12 h. The DOX-loaded Fe@MSN nanoparticles were collected by centrifugation. The nanoparticles were washed with PBS several times to remove unbound DOX molecules.

To evaluate drug loading capacity, the residual drug was measured after the loading procedure. The washing solutions were collected and the residual drug content was measured by UV-Vis absorption spectroscopy at λ=480 nm.

Infrared analyses for DOX were obtained using a Thermo Nexus 870 FTIR spectrometer with an attenuated total reflection (ATR) accessory. Spectra over the 4000−500 cm^{-1} range were obtained by the co-addition of 64 scans with a resolution of 4 cm^{-1}.

Functionalization with CREKA peptide

The CREKA peptide was conjugated onto Fe@MSN via maleimide chemistry. First, amine-functionalized Fe@MSN in PBS were vortexed with 10 molar excess of sulfo-SMCC (sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) for 15 minutes. Next, 5 molar excess (relative to the number of amines on Fe@MSN) of CREKA was added and allowed to react for 2 hours. The product was dialyzed against PBS using a 10,000 Da MW cut-off membrane to remove unbound CREKA molecules. Bio-Rad DC protein assay was used to quantify the number of peptides per Fe@MSN particle. Briefly, 200 µL of Bio-Rad dye solution (1 part of commercially available Bio-Rad was diluted with 2 parts of distilled water and filtered through a whatman filter) was added to 800 µL of 10 mg/mL Fe@MSN and vortexed. The absorbance of the sample was obtained at 595 nm after incubating the sample for 15 minutes. The absorbance value was compared to a standard curve, which was obtained by measuring the absorbance of known concentrations of CREKA with Bio-Rad dye solution.