Supplementary Information

Multifunctional temozolomide-loaded lipid superparamagnetic nanovectors: Dual targeting and disintegration of glioblastoma spheroids by synergic chemotherapy and hyperthermia treatment

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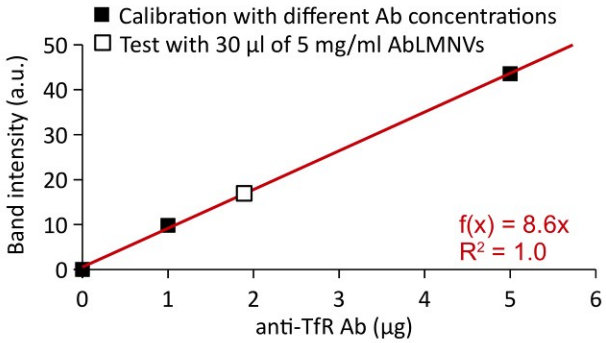

Figure S1. Linear relation between the intensity of the band attributed to the light chain (MW ~ 25 kDa) of the anti-TrR Ab and the amount of Ab loaded in the gel. The value depicted in white box represents the band intensity and the corresponding amount of Ab, detected in 30 µl of 5 mg/ml AbLMNVs.

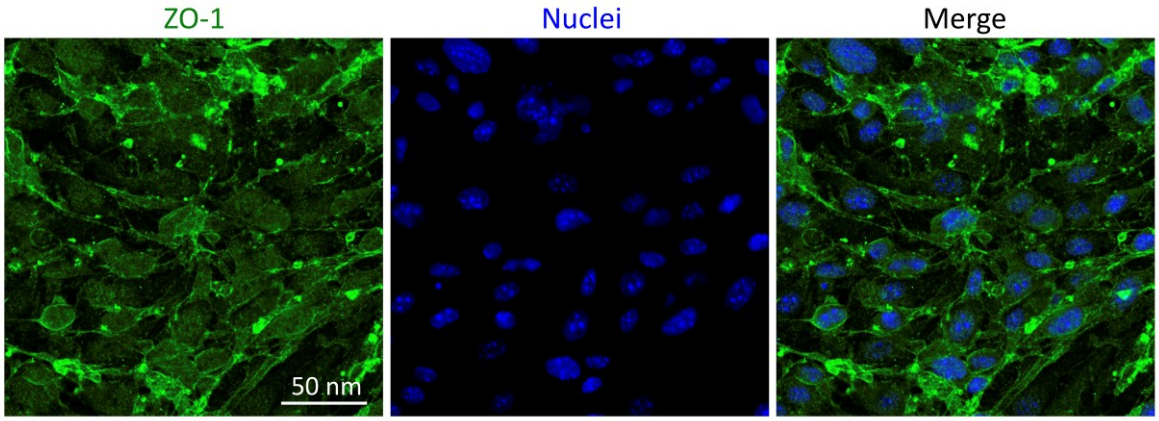

Figure S2. Confocal laser scanning microscopy imaging of ZO-1 (in green) and nuclei (in blue) showing the complete formation of an endothelial layer on the luminal side of the BBB model.

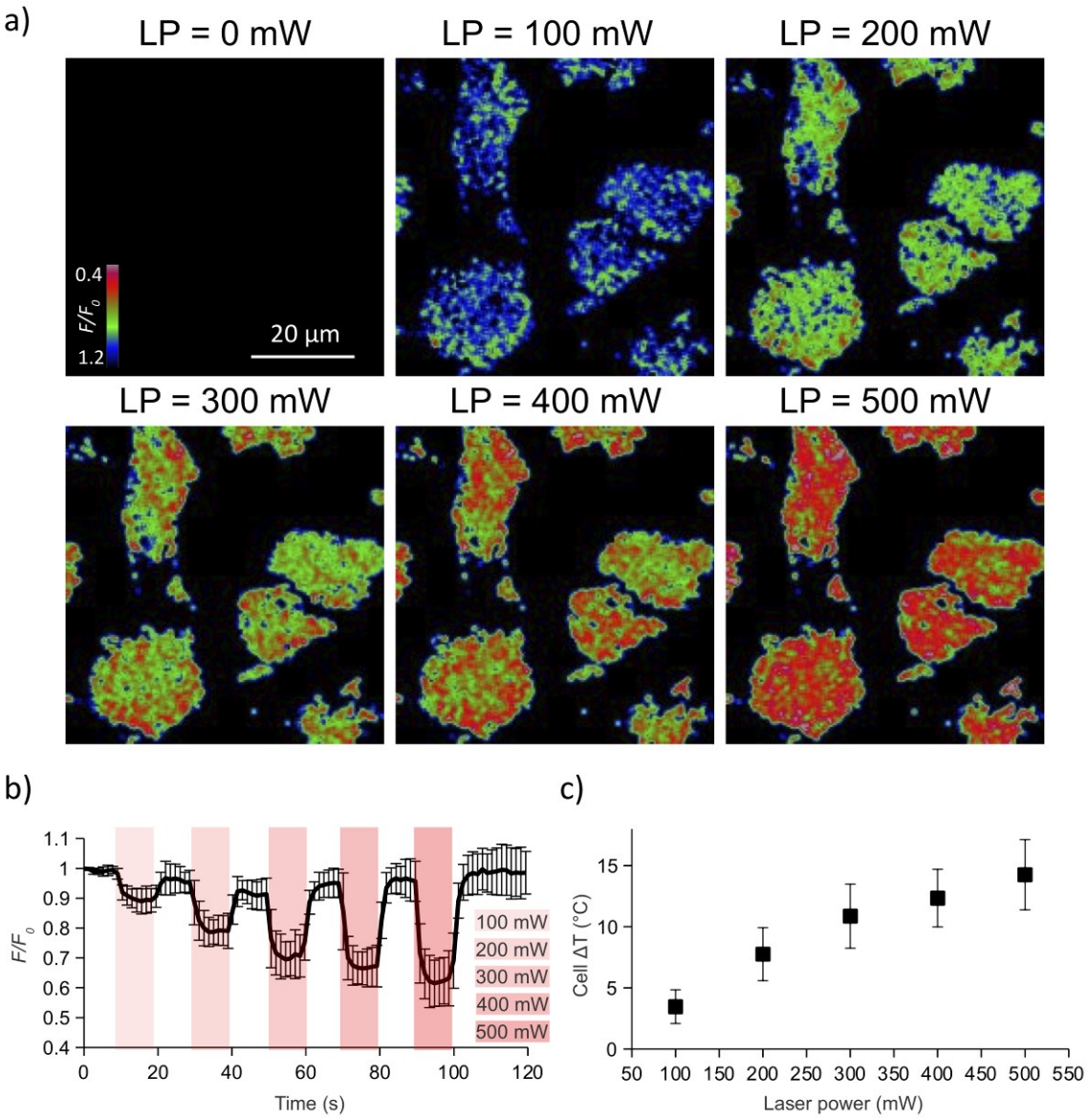

Figure S3. Temperature monitoring in GBM cells that underwent irradiation with infrared (IR) radiation at different laser powers (LP) by exploiting ER-thermo yellow fluorescent thermometer. a) Time-lapse imaging and b) fluorescence intensity (F/F_0) time course during irradiations with IR. c) Intracellular temperature increments in response to IR stimulations.

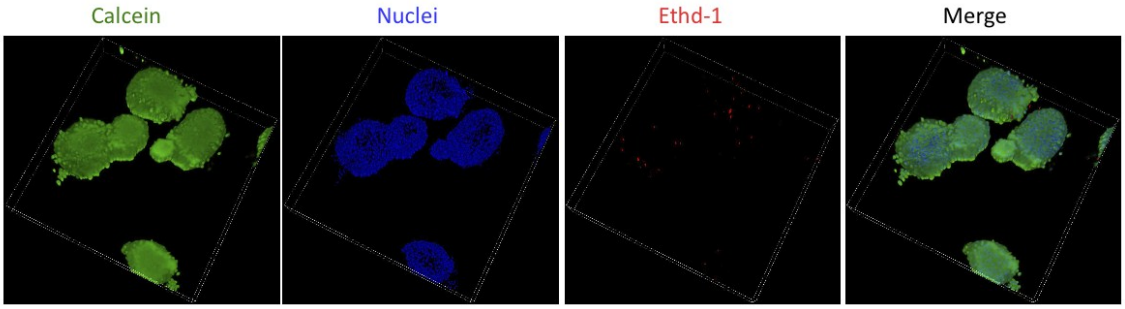

Figure S4. GBM spheroids before the magnetothermal treatment. Spheroids are stained with calcein (live cells in green), Hoechst (nuclei in blue), ethidium homodimer-1 (ethd-1; dead cells in red); scan volume is 1270 µm (*x* axis) × 1270 µm (*y* axis) × 185 µm (*z* axis).

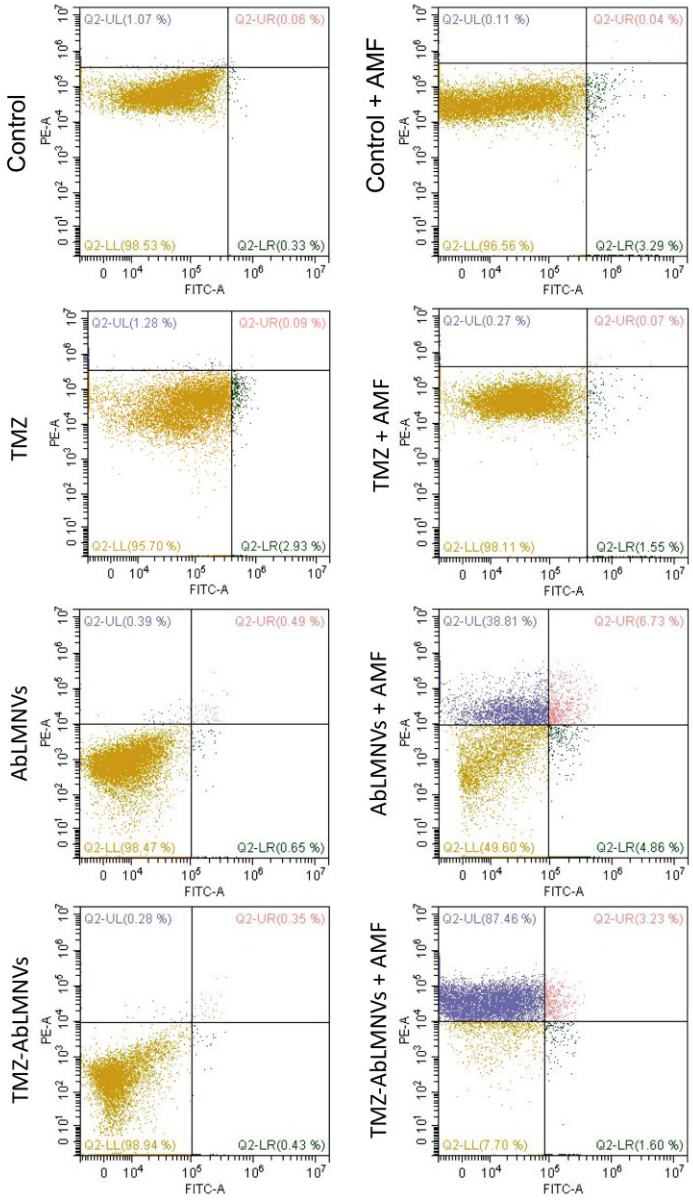

Figure S5. Representative scatter plots of fluorescence emission of cells dissociated from the spheroids and stained with PI / FITC-annexin V after magnetothermal and chemotherapy treatment.

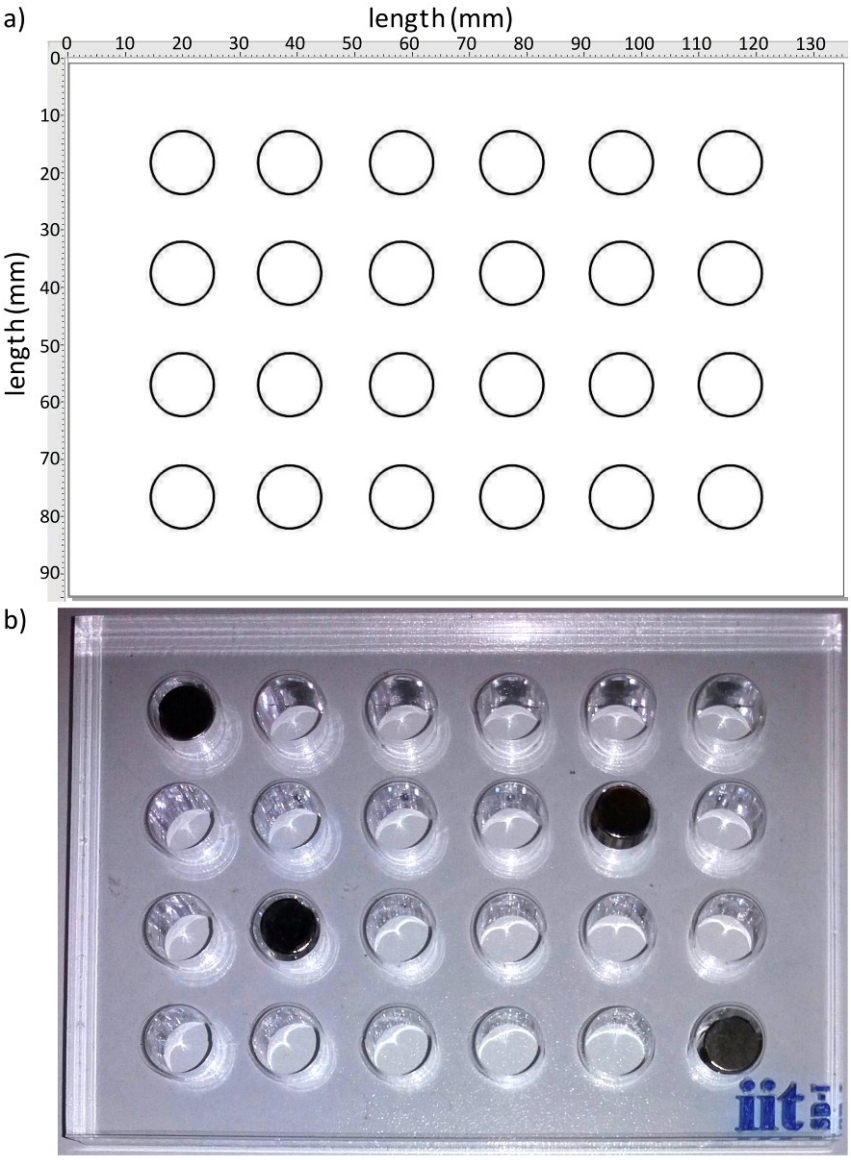

Figure S6. Design and fabrication of a custom-made multi-magnet support for multiwell plates.

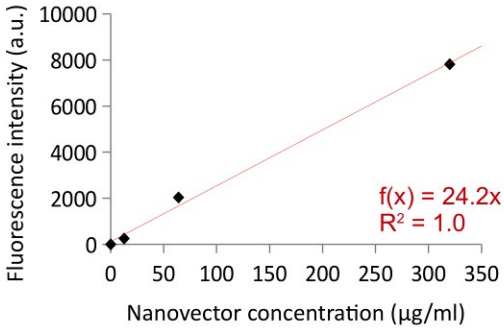

Figure S7. Calibration curve of the fluorescence intensity of nanovectors at different concentrations.

Video S1. Time-lapse fluorescence imaging of propidium iodide (PI) in AbLMNV-incubated GBM cells non-stimulated with AMF.

Video S2. Time-lapse fluorescence imaging of propidium iodide (PI) in GBM cells non-incubated with AbLMNVs that underwent AMF stimulation.

Video S3. Time-lapse fluorescence imaging of propidium iodide (PI) in GBM cells incubated with AbLMNVs and stimulated with AMF.

Video S4. Temperature time-lapse imaging of DiI-stained AbLMNVs in GBM spheroids during AFM stimulation.