SUPPORTING INFORMATION

Piezoelectric Barium Titanate Nanostimulators for the Treatment of Glioblastoma Multiforme

Attilio Marino[1,†,], Enrico Almici[2,†], Simone Migliorin[2], Christos Tapeinos[1], Matteo Battaglini[1,3], Valentina Cappello[4], Marco Marchetti[5,6], Giuseppe de Vito[5,7], Riccardo Cicchi[5,7], Francesco Saverio Pavone[5,6,7], Gianni Ciofani[1,2,*]*

[1]Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Viale Rinaldo Piaggio 34, 56025 Pontedera, Italy

[2]Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Corso Duca degli Abruzzi 24, 10129 Torino, Italy

[3]Scuola Superiore Sant'Anna, The Biorobotics Insitute, Viale Rinaldo Piaggio 34, 56025 Pontedera, Italy

[4]Istituto Italiano di Tecnologia, Center for Nanotechnology Innovation, Piazza San Silvestro 12, 56127 Pisa, Italy

[5]European Laboratory for Nonlinear Spectroscopy (LENS), Via Nello Carrara 1, 50019 Sesto Fiorentino, Italy

[6]Università di Firenze, Department of Physics and Astronomy, Via Giovanni Sansone 1, 50019 Sesto Fiorentino, Italy

[7]National Institute of Optics, National Research Council (INO-CNR), Largo Enrico Fermi 6, 50125 Firenze, Italy

[†]These authors contributed equally to this work

[*]CORRESPONDING AUTHORS:

attilio.marino@iit.it; gianni.ciofani@iit.it

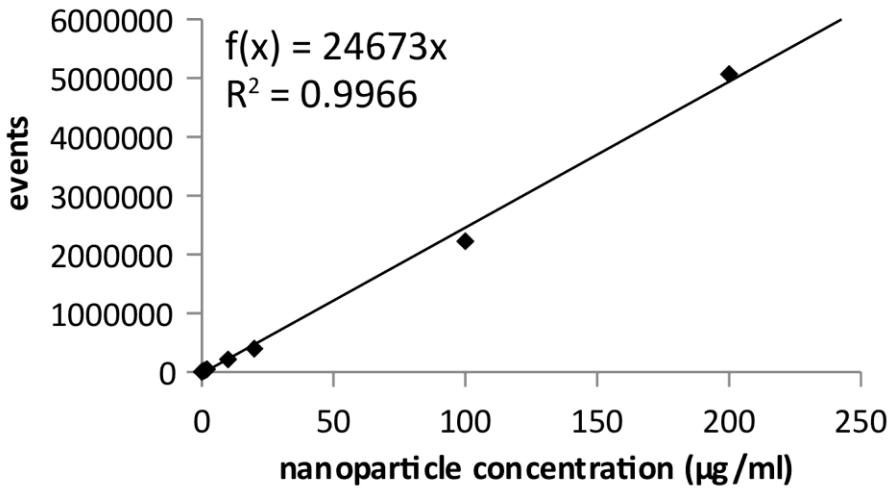

Figure S1. Calibration curve reporting the number of events recorded by flow cytometry as a function of nanoparticle concentration.

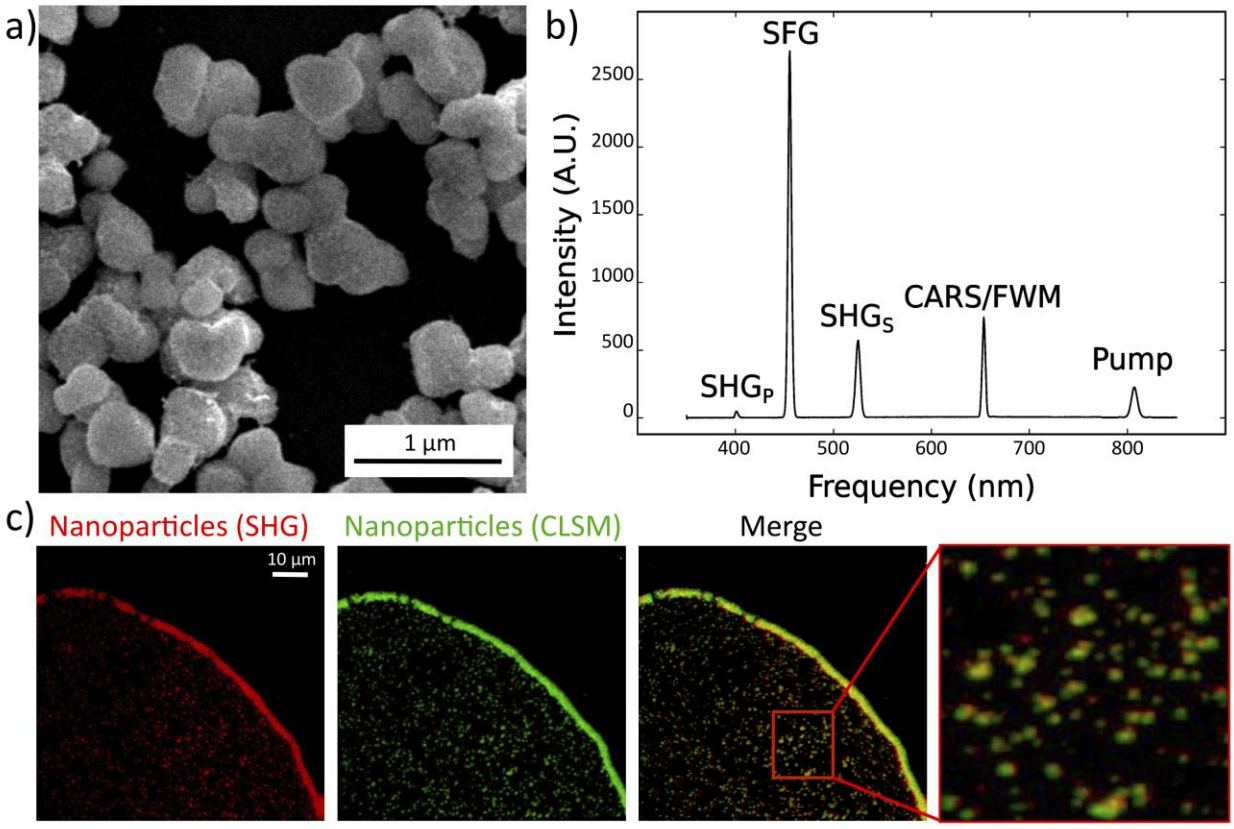

Figure S2. a) Scanning electron microscopy (SEM) imaging, b) emission spectrum (induced by pump-and-probe beam and Stokes beam illumination), and c) second harmonic generation (SHG, in red) and confocal laser scanning microscopy (CLSM, in green) imaging of the nanoparticles.

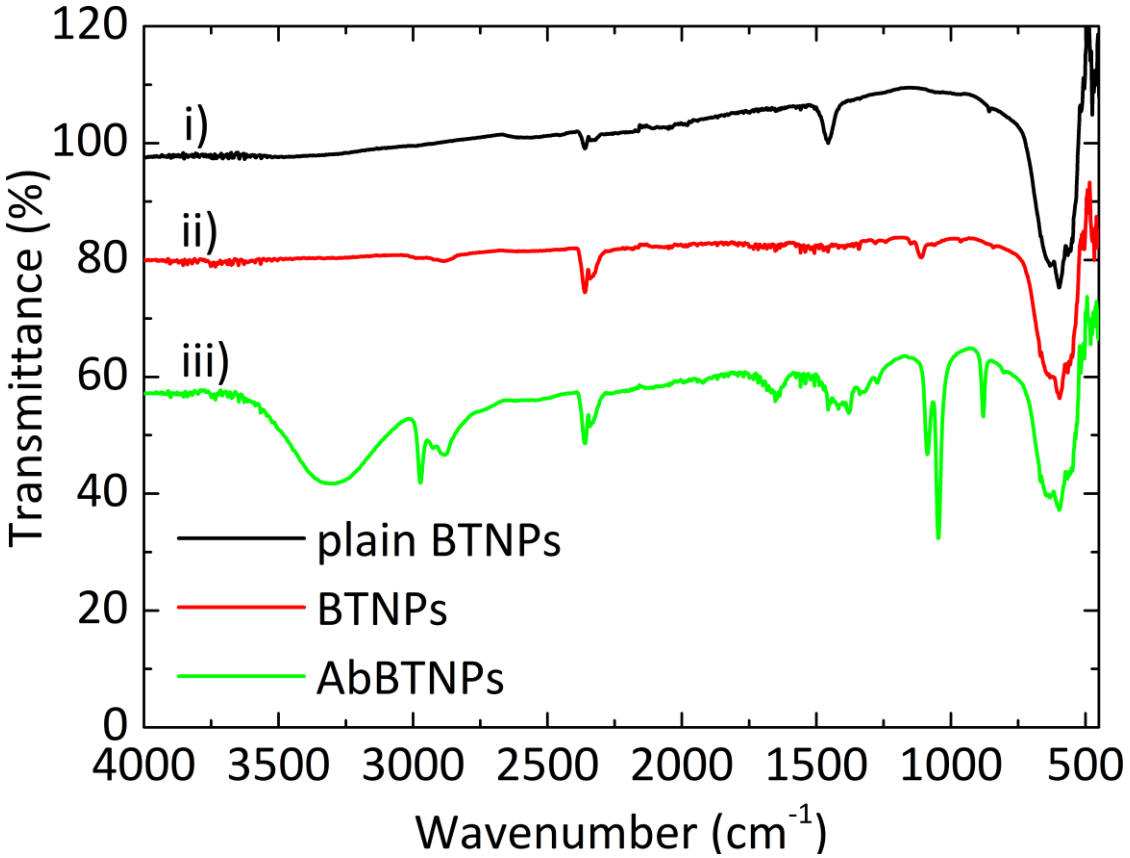

Figure S3. FT-IR spectra of *i*) non-functionalized plain BTNPs (plain BTNPs), *ii*) BTNPs coated with DSPE-PEG (BTNPs), and *iii*) of BTNPs coated with DSPE-PEG and functionalized with an antibody against the transferrin receptor (AbBTNPs).

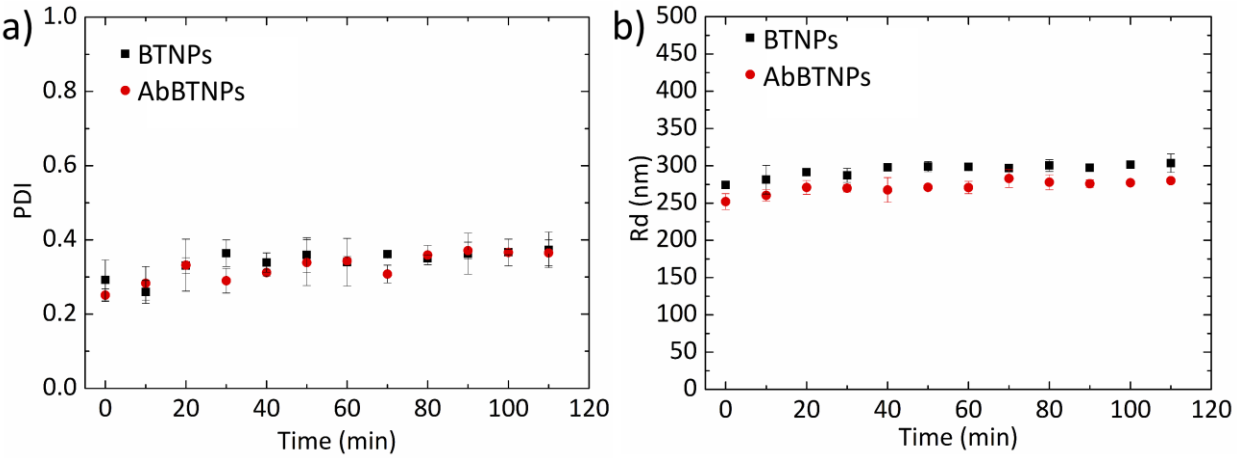

Figure S4 Nanoparticle stability. a) polydyspersity index (PDI) and b) hydrodynamic diameter (*Rd*) of BTNPs and AbBTNPs at different time points.

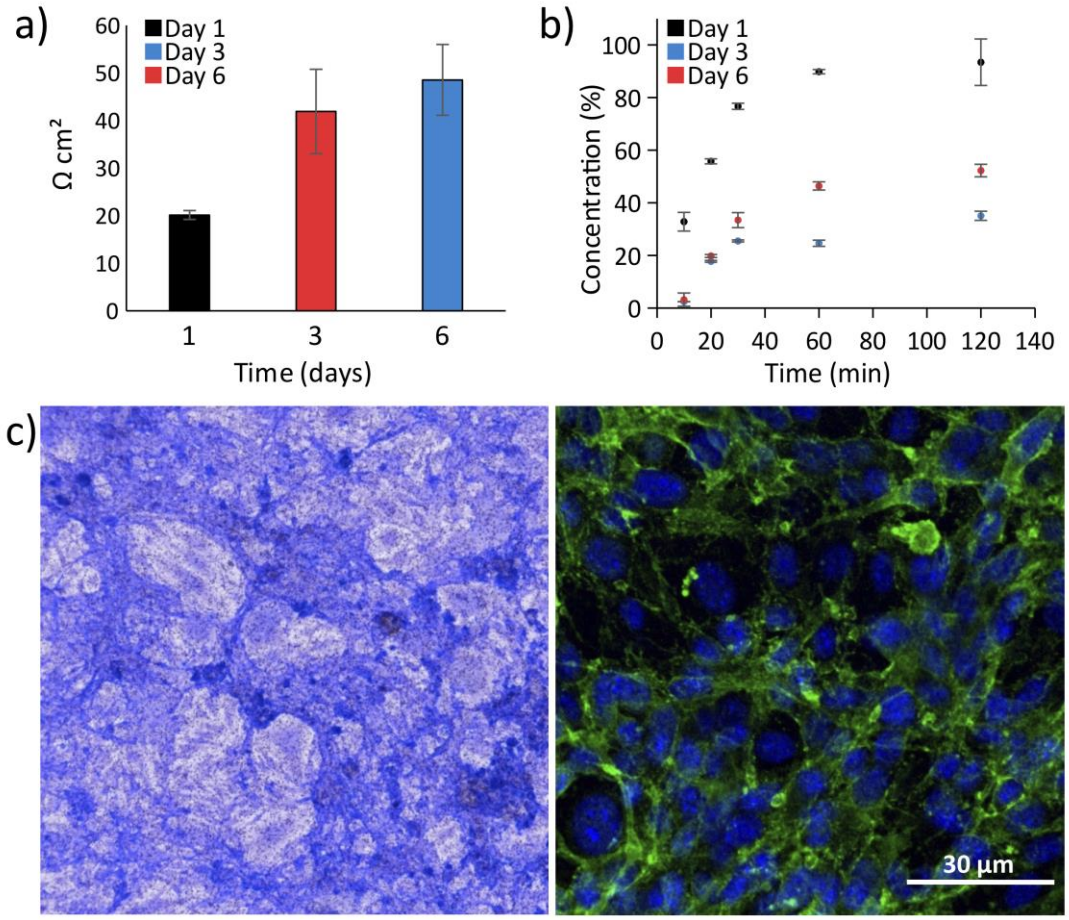

Figure S5. Characterization of the BBB model developed on 3 µm porous transwell. a) Transendothelial electric resistance (TEER) and b) permeability to FITC-conjugated dextran of the model at day 1, 3 and 6 of culture. c) Coomassie (left image) and immunofluorescence staining (right image; ZO-1 in green and nuclei in blue) of the BBB model after 3 days of maturation.

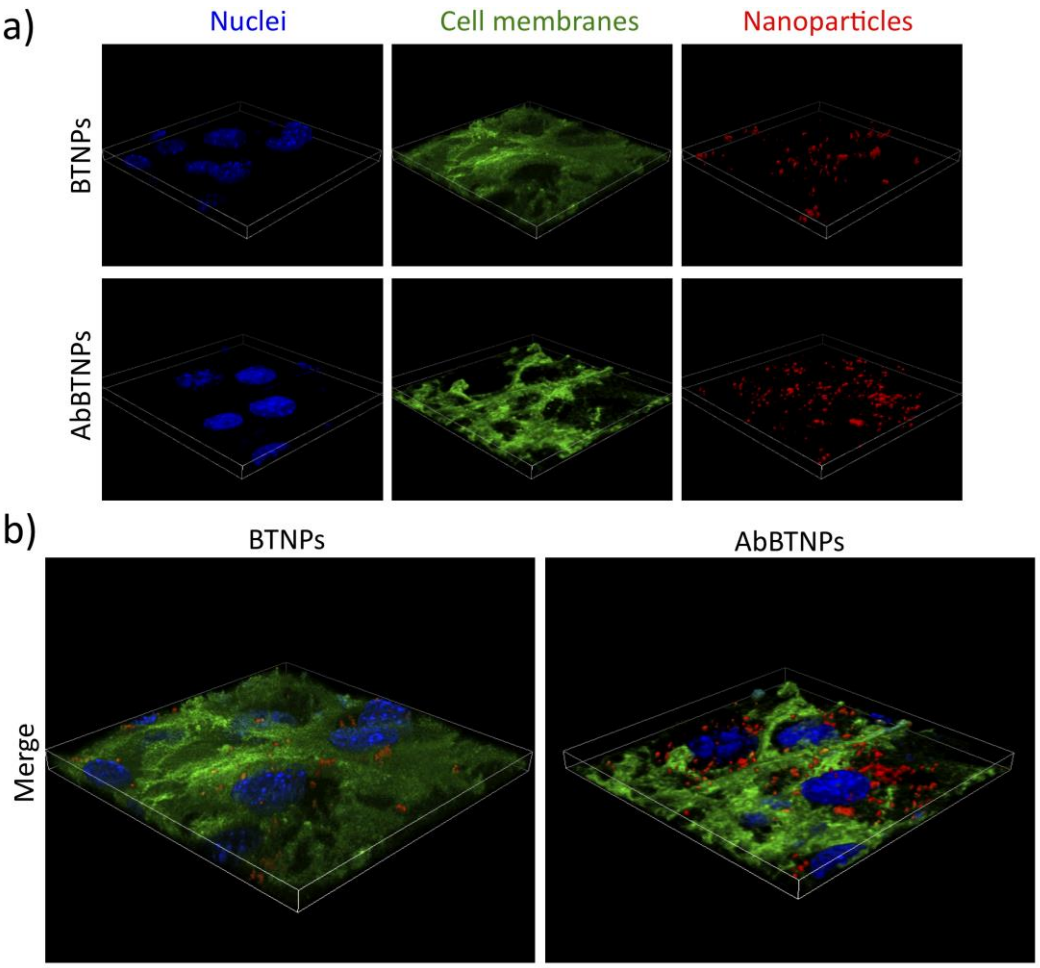

Figure S6. 3D reconstructions of confocal *Z*-stack acquisitions of nanoparticles (BTNPs / AbBTNPs in red) and of bEnd.3 plasma membranes (in green). Separated and merged signals are shown in a) and b), respectively.

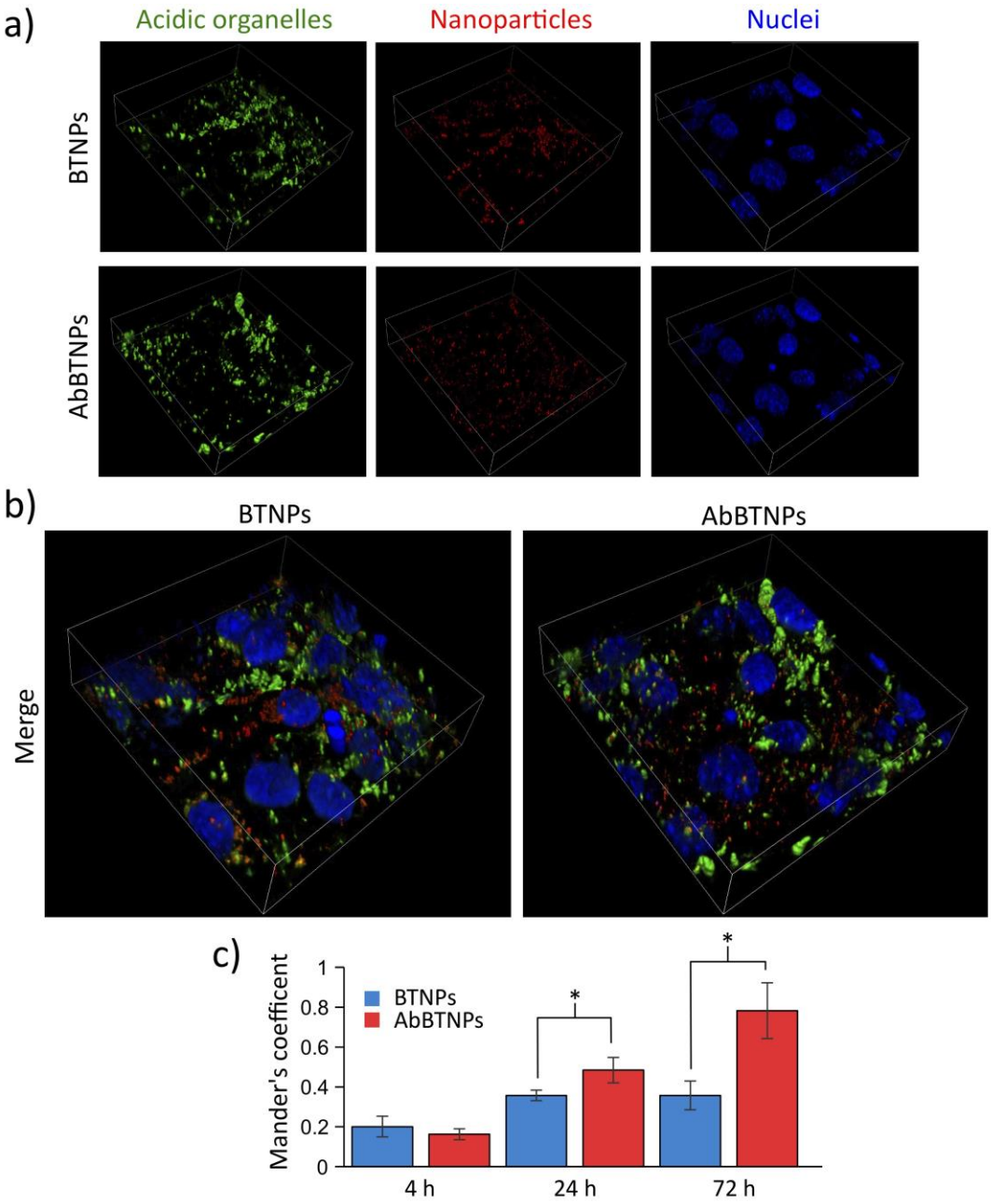

Figure S7. 3D reconstructions of confocal *Z*-stack acquisitions of nanoparticles (BTNPs / AbBTNPs, in red) and of bEnd.3 acidic organelles (in green). Separated and merged signals are respectively shown in a) and b). c) Co-localization analysis showing Mander's coefficients at different incubation points (4, 24 and 72 h).

Table S1. Stretching frequencies of the most relevant vibrations of plain and functionalized BTNPs.

Wavenumber (cm^{-1}) range	**Vibrations/Description**
530 - 600	Ti-O ($BaTiO_3$)
1450	C-O ($BaCO_3$)
1000 - 1100	C-O-C and C-O-H stretching
1600 - 1670	Amide I (C=O stretching)
1300 - 1460	Amide III
2280 - 2400	C-H stretching
2850 - 3000	C-H stretching
3320	Amide A (N-H stretching)

Video S1. Fluorescence time lapses of Ca^{2+} imaging performed following US stimulation, Pseudo-color intensity indicates F/F_0. Total duration of the time lapse = 40 min.

Video S2. Fluorescence time lapses of Ca^{2+} imaging performed following AbBTNPs+US stimulation. Pseudo-color intensity indicates F/F_0. Total duration of the time lapse = 40 min.