Supplementary Information

Magnetosomes extracted from Magnetospirillum gryphiswaldense as theranostic agents in an experimental model of glioblastoma

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Information S1: *Cancer cells culture*

U87MG cell line (purchased by ATCC Manassas, VA), was cultured in Eagle's Minimum Essential Medium (EMEM) with 10% of Fetal Bovine Serum (FBS), 1% of a mix of penicillin/streptomycin 1:1 and 1% of L-glutamine 200 mM, in 25 cm^2 plates and incubated at 37°C in humidified air with 5% CO_2. Medium and L-glutamine were purchased by Sigma-Aldrich (Italy),

while serum and antibiotic mix were acquired by GIBCO Life Technologies (USA). When at confluence, cells were treated with trypsin-EDTA 1% (GIBCO Life Technologies, USA), harvested and centrifuged at 1200 rpm for 5 min. The supernatant was discarded and cells pellet was resuspended in 1 ml of complete medium, placed in 75 cm^2 plates and incubated at 37°C and 5% of CO_2 until 80% confluence was detectable.

Information S2: *MNs-uptake in cancer cells*

Batches of $2X10^5$ cells were seeded in specific glasses multiwell for optical microscopy (Falcon BD, Italy) with 1 ml of growth medium. Cells were incubated at 37°C in humidified air with 5% CO_2 for 24 h. Different concentrations of MNs (1 mg/ml, 0.5 mg/ml, 0.2 mg/ml) were added to the wells and incubated for 6 h, 12 h and 24 h. At the selected time points, the growth medium was discarded and cells were washed with 1 ml of 1X sterile phosphate buffer saline (PBS, GIBCO, Life Technologies, USA). Cells were fixed with 1 ml of 4% buffered formalin (Bioptica, Italy) for 30 min at room temperature. Once formalin was discarded, samples were double stained with Prussian Blue and Nuclear Fast Red (Bioptica, Italy).

Information S3: *Transmission Electron Microscopy (TEM) of cells incubated with MNs*

Whole mount bacteria of different samples of MNs, extracted and purified, were fixed with glutharaldehyde 2% in Sorensen buffer pH 7.4 for 2 h, post-fixed in 1% osmium tetroxide in aqueous solution for 2 h, dehydrated in graded concentrations of acetone. At the end of dehydrating process, samples were positioned in a multi-well grid for electron microscopy and observed using Philips Morgagni TEM and equipped with a Megaview II camera for digital image acquisition.

U87MG cancer cells, plated on a 2.4 cm culture glass, were positioned on the bottom of 3.5 cm Petri dishes and incubated at 37°C in humidified air with 5% of CO_2 in 3 ml of growth medium. After 24h, the growth medium was then discarded and replaced with fresh medium added with MNs at different concentrations: 1 mg/ml, 0.5 mg/ml, 0.2 mg/ml. Cells were incubated as previously described for 6 h, 12 h and 24 h. At each time point, the growth medium was discarded and cells were washed with 1 ml of 1X sterile phosphate buffer saline (PBS, GIBCO, Life Technologies,

USA).Cells were fixed with glutaraldehyde 2% in Sorensen buffer pH 7.4 for 2 h, then post-fixed in 1% osmium tetroxide (OsO_4) in aqueous solution for 2 h, and finally dehydrated in graded concentrations of acetone. At the end of dehydrating process, glasses were stained with lead citrate.

Information S4: *Magnetic Resonance Imaging*

MRI was performed using a Bruker tomograph operating at 4.7 T, equipped with an actively shielded gradient insert (Bruker, Germany) having a maximum gradient strength of 40 G/cm. T_2 and T_2* weighted images were acquired with the following parameters: T_2 w RARE 3D sequence with TR=1200 ms, TE_{eff}=47.5 ms, FOV=5x2.5x2.5 cm^3, NEX=1, MTX=256/128/32, Slice Thickness=0.78 mm; T_2*weighted FLASH gradient echo sequence with TR=400 ms, TE=4.4ms, flip angle=10°, FOV=5x2.5 cm, NEX=2, MTX=256/128, NSLICES=8, Slice Thickness=2 mm.

Information S5: *Histology*

At the end of the last imaging session, tumors were excised, washed with PBS 0.1 M and fixed with 10% buffered formalin for 4 h. Afterwards, samples were dehydrated with increasing concentration gradient of alcohol from 70% to 100% and then with xylene for final processing. Tissues were embedded in paraffin and sections of 5 µm were obtained and dried at 37°C for 24 h. Section were then stained with Prussian blue and Nuclear Fast Red (Bioptica, Italy).