Supporting Information for

Multifunctional Porous Microspheres Encapsulating Oncolytic Bacterial Spores and Its Potential for Cancer Immunotherapy

Experimental methods

***In vitro* colony formation assay:** The *in vitro* survival of CT26 cells in response to *C. novyi*-NT supernatant was assessed using a colony formation assay. Initially, 500 CT26 cells per well were seeded into a 6-well plate and incubated for 12 hours. Subsequently, the *C. novyi*-NT supernatant was added and the cells were further incubated for 7 days. Colonies were then fixed and stained with a mixture of 6% glutaraldehyde and 0.5% crystal violet. Finally, the colonies were counted, and representative images were taken.

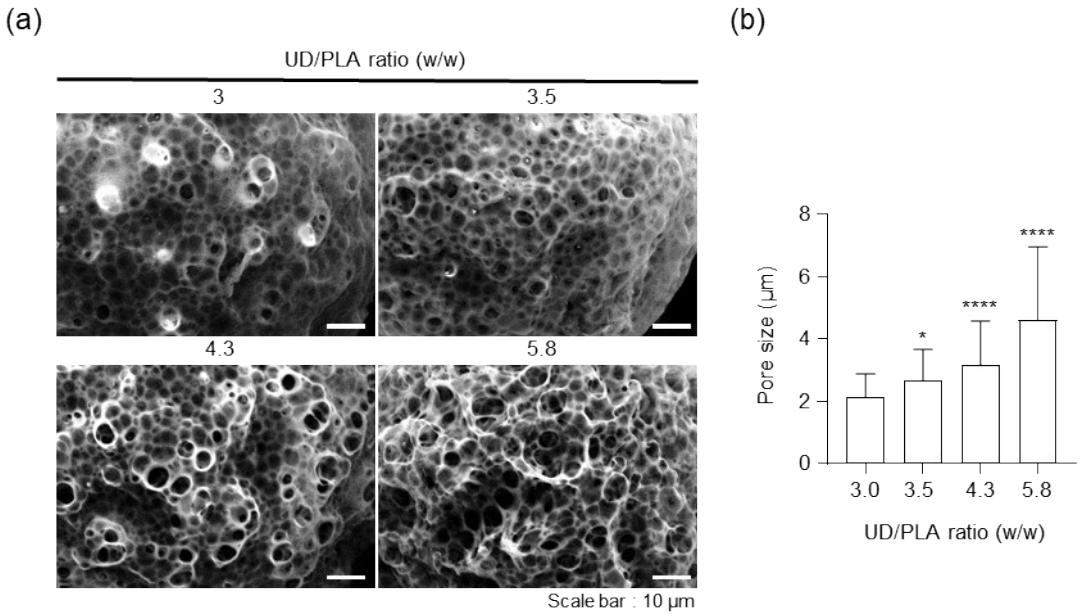

Figure S1. Pore size analysis of microspheres prepared at various UD/PLA ratios (w/w). (a) SEM images of the microsphere surface pores prepared at various UD/PLA ratios. (b) Bar graph analysis of the pore size obtained from the SEM images. Data are presented as the mean ± SD (n = 100, *$p < 0.05$, ****$p < 0.0001$; ns, not significant).

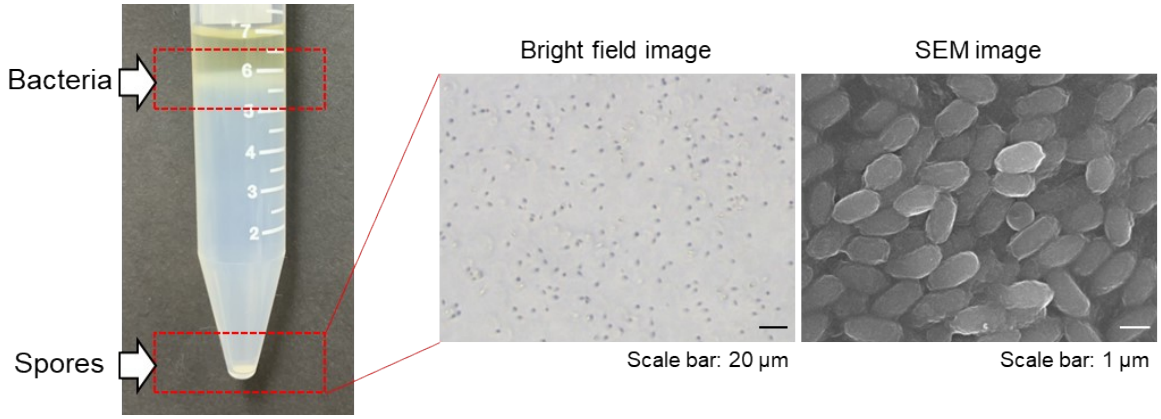

Figure S2. *C. novyi*-NT spores isolated by Percoll density gradient separation. A density gradient seperation was performed in 55% and 70% Percoll in PBS solution with centrifugation (3000 rpm, 15 min, accel 0, and decel 0). After centrifugation, the isolated *C. novyi*-NT spores were separated. Bright field and SEM images were taken.

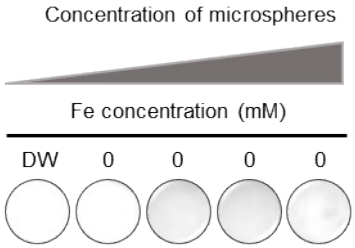

Figure S3. ***In vitro*** **T2-weighted MR phantom images of porous PLA microspheres without magnetic nanoparticles at various concentrations.**

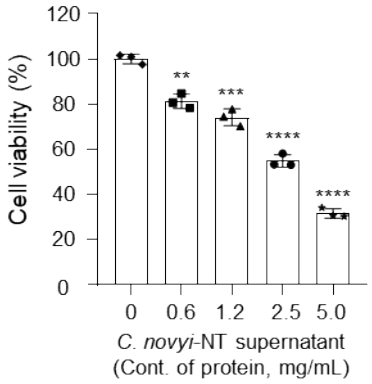

Figure S4. *In vitro* cell viability of RAW 264.7 cells after treatment with culture supernatants (at various protein concentrations) of MPMs encapsulated with *C. novyi*-NT spores. Data are presented as the mean ± SD (n = 3, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$).

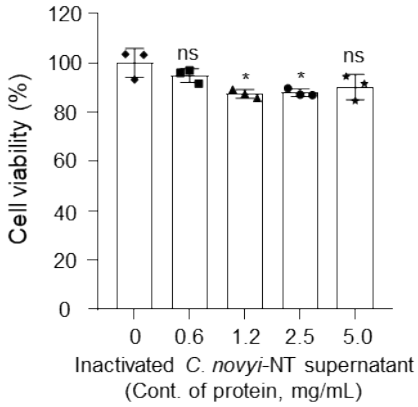

Figure S5. *In vitro* cell viability of CT26 cells after treatment with heat-inactivated culture supernatants (at various protein concentrations) of MPMs encapsulated with *C. novyi*-NT spores. Data are presented as the mean ± SD (n = 3, **p* < 0.05; ns, not significant).

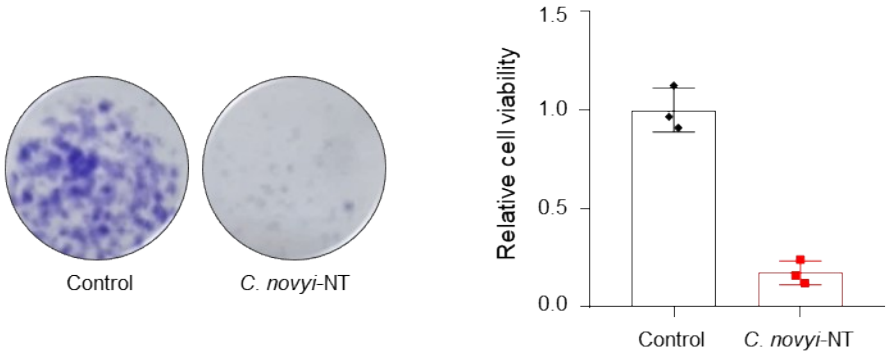

Figure S6. ***In vitro*** **colony formation assay of CT26 cells after treatment with culture supernatants of MPMs encapsulated with *C. novyi*-NT spores.**