Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Supplementary Information

Immunostimulatory Silica Nanoparticle Boosts Innate Immunity in Brain Tumors

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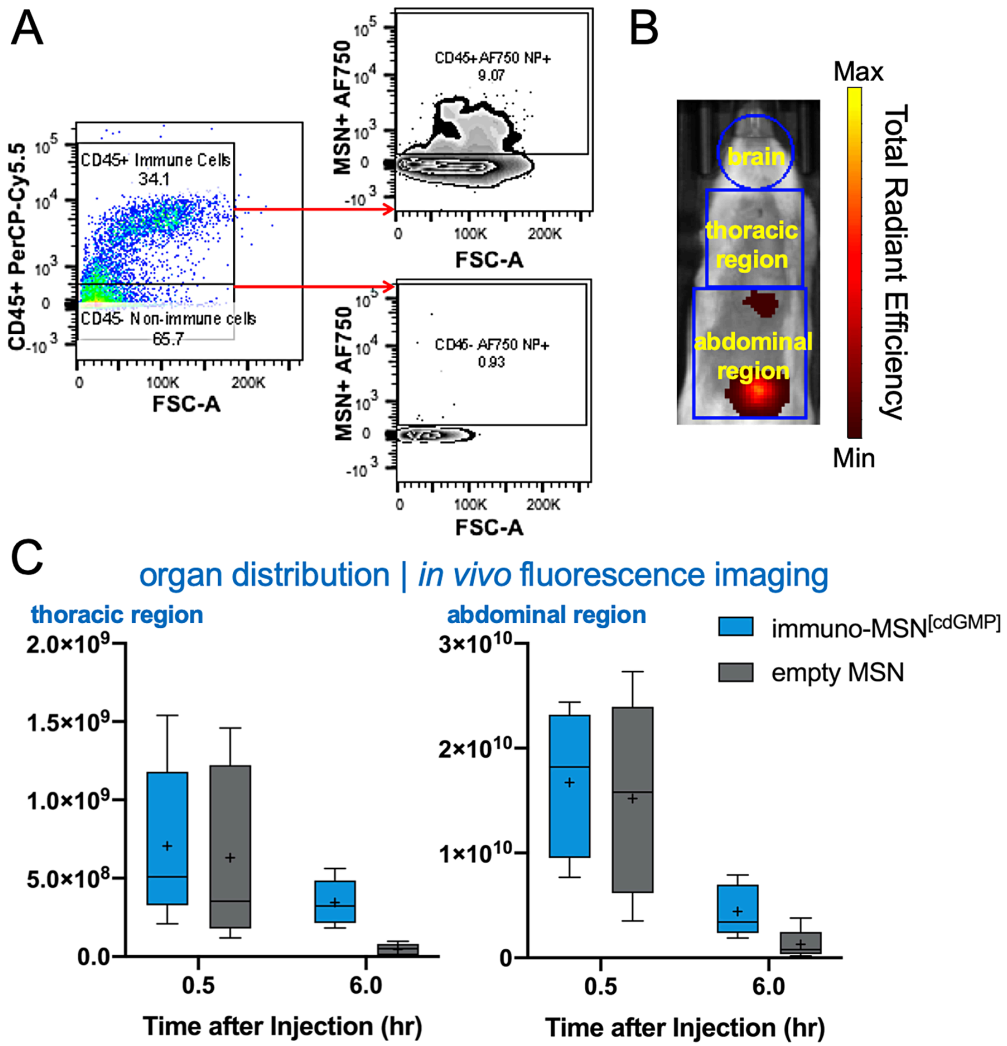
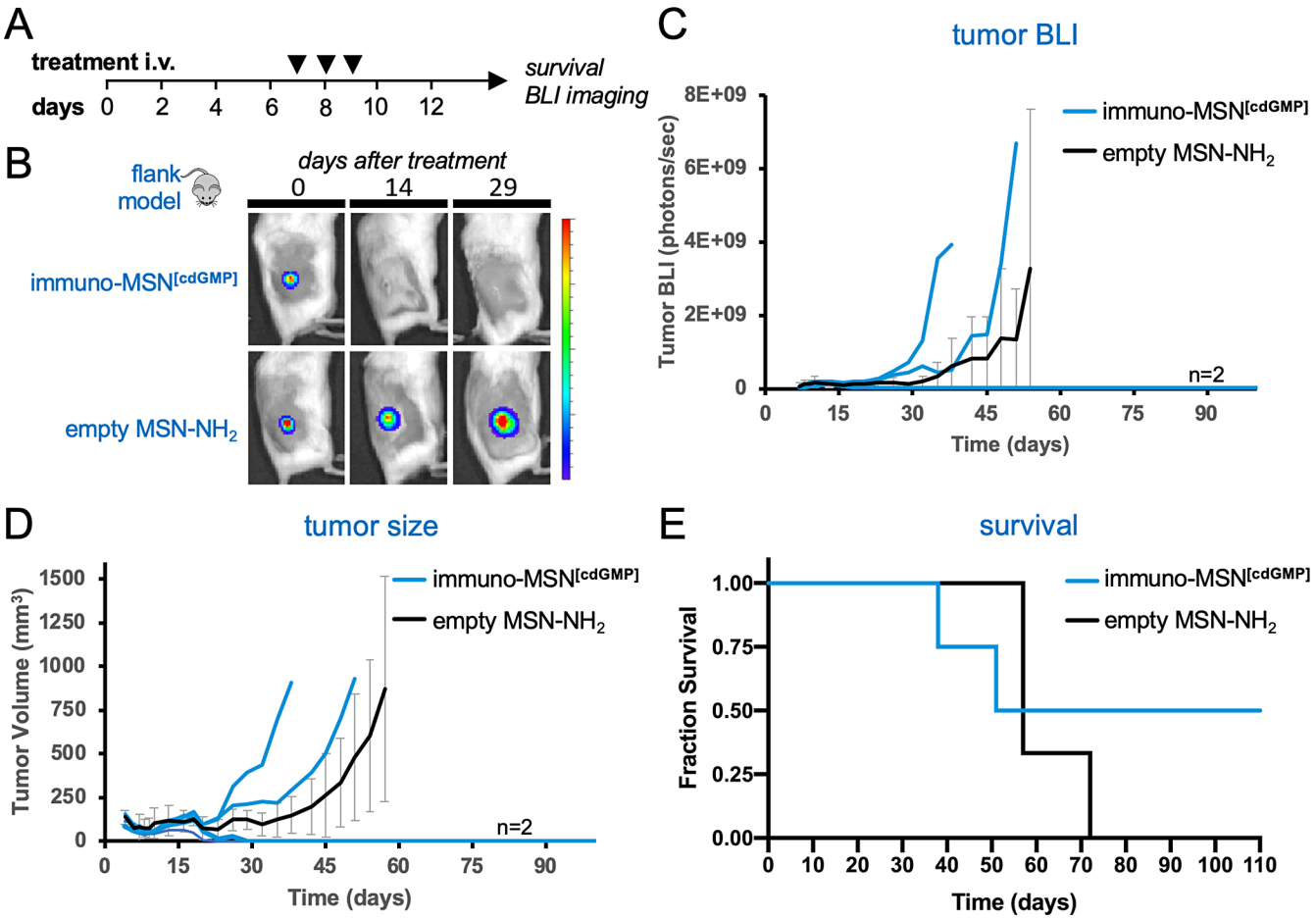
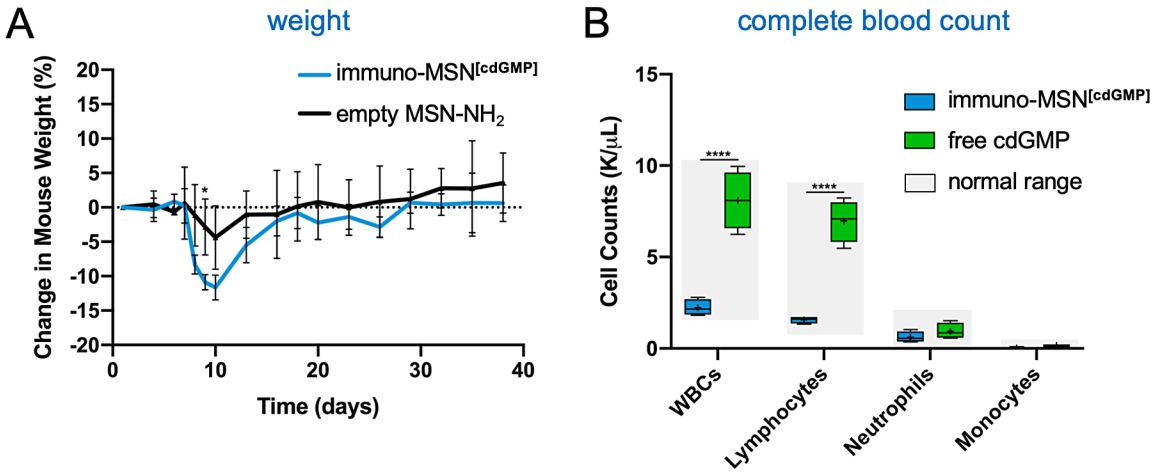


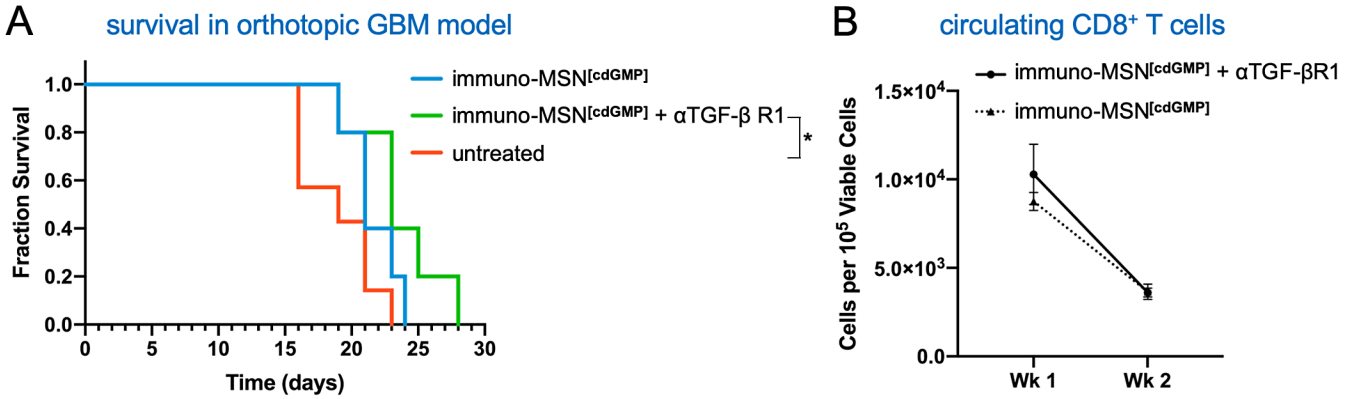
Supplementary Figure S1. (A) A representative flow cytometry dot plot shows the threshold gating for MSN^{+} cells. **(B)** Live-animal spectrum imaging for fluorescently labeled MSN was performed longitudinally. A representative image shows the regions of interest (ROIs) used to measure signal in the brain, thoracic region and abdominal region. **(C)** The fluorescent immuno-MSN and the fluorescent MSN (empty, no cdGMP cargo) were compared following the administration schedule showed in Fig. 4A.



Supplementary Figure S2. Treatment with immuno-MSN particles can induce complete tumor regression in a flank GBM model. **(A)** Treatment regimen of intravascularly administered immuno-MSN particles delivering 30 μg of cdGMP per dose. **(B)** Representative BLI images from live-animal Spectrum imaging of responding mice treated with immuno-MSN particles compared to treatment with the MSN-NH_2 vehicle control. Units of radiance is in photons/s. Flank GBM tumor progression measured by **(C)** total GL261 tumor cell bioluminescence signal (photons/s) and **(D)** tumor volume (mm^3) from physical caliper measurements (*volume = 0.5 × length × width²*). **(E)** Fractional survival of mice treated with immuno-MSN particles (n = 4) compared to MSN-NH_2 vehicle controls.



Supplementary Figure S3. (A) Change in mouse weight (%) of mice bearing flank GBM tumors and injected i.v. with immuno-MSN particles (n = 4) or the MSN-NH_2 vehicle control (n = 3). Mice treated with immuno-MSNs received 30 μg of cdGMP on days 7, 8, and 9 post-inoculation of GL261 cells. Statistical significance was conducted by two-way ANOVA with Sidak's post-test (*P<0.05). **(B)** Short-term safety study of healthy C57BL/6 immunocompetent mice injected i.v. with either 10 μg of cdGMP loaded into MSNs or an equivalent amount of free cdGMP. Cell counts (K/μL) were collected from a sample size of n = 4 and measured on a HemaVet 950. Statistical significance in the box and whisker plots (5-95 percentile, "+" mean) was conducted by two-way ANOVA with Sidak's post-test (****P<0.0001).



Supplementary Figure S4. Long-term efficacy study of orthotopic GBM mice injected i.v. with immuno-MSN particles on days 7, 8, and 14 post-inoculation of GL261 cells. Immuno-MSN particles were administered in doses of 10 μg of cdGMP. 2.5 mg/kg of α-TGF-βR1 (Galunisertib) was administered by intraperitoneal injection for five consecutive days per week beginning on day 3 after tumor inoculation. **(A)** Fractional survival of mice treated with immuno-MSN particles (with or without α-TGF-βR1) compared to untreated controls (n ≥ 5). Statistical significance was conducted by Log-rank (Mantel-Cox) test (*P<0.05). **(B)** Flow cytometry analysis from Wk 1 and Wk 2 blood draws measuring levels of $CD8^+$ T cells in the blood circulation after the start of immuno-MSN treatment. Cell count data from flow cytometry analysis is represented as mean ± standard error and was normalized to 10^5 viable cells (Wk 1 and Wk 2: n = 5 for immuno-MSN and immuno-MSN + α-TGF-βR1). Statistical significance was conducted by two-way ANOVA with Sidak's post-test.