

Supplementary Information

# Immunostimulatory Silica Nanoparticle Boosts Innate Immunity in Brain Tumors

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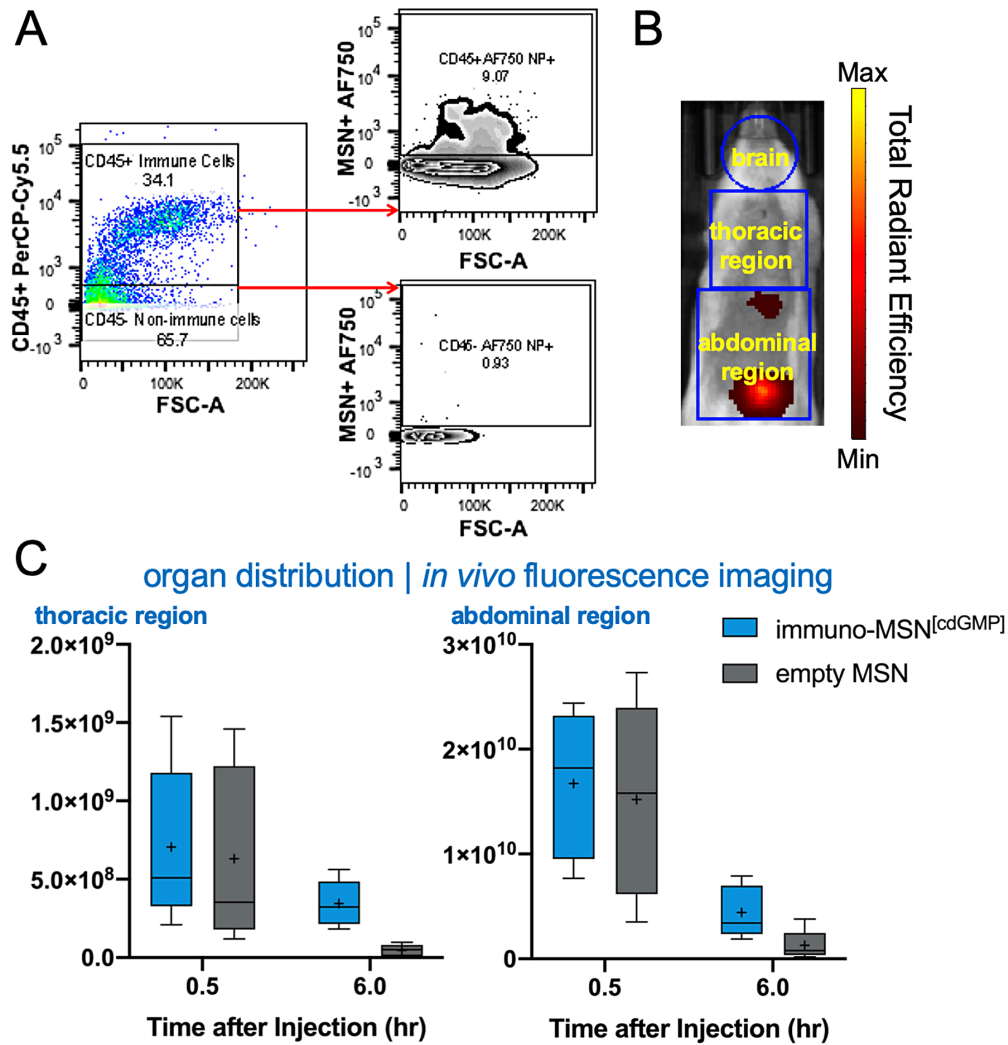
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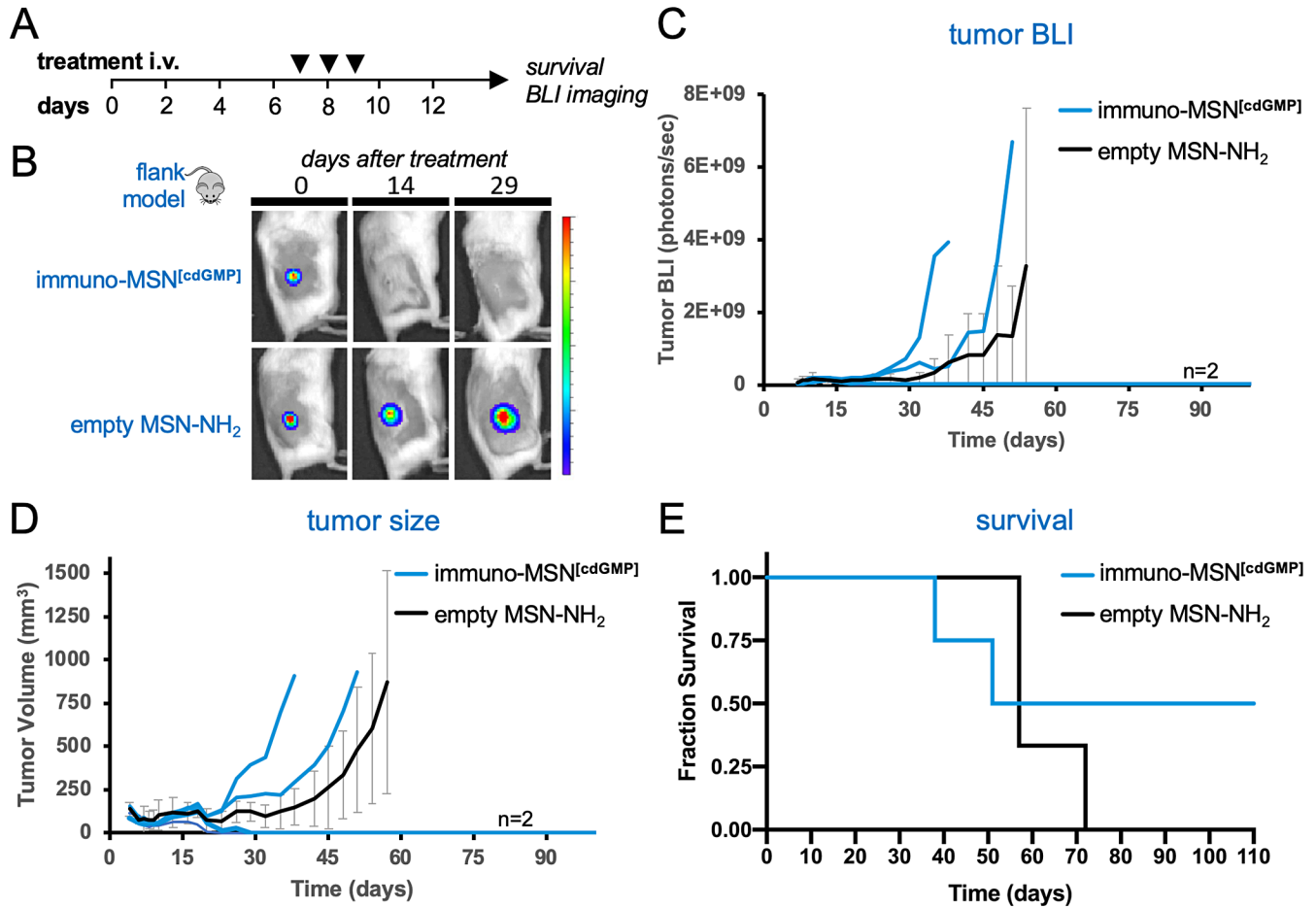
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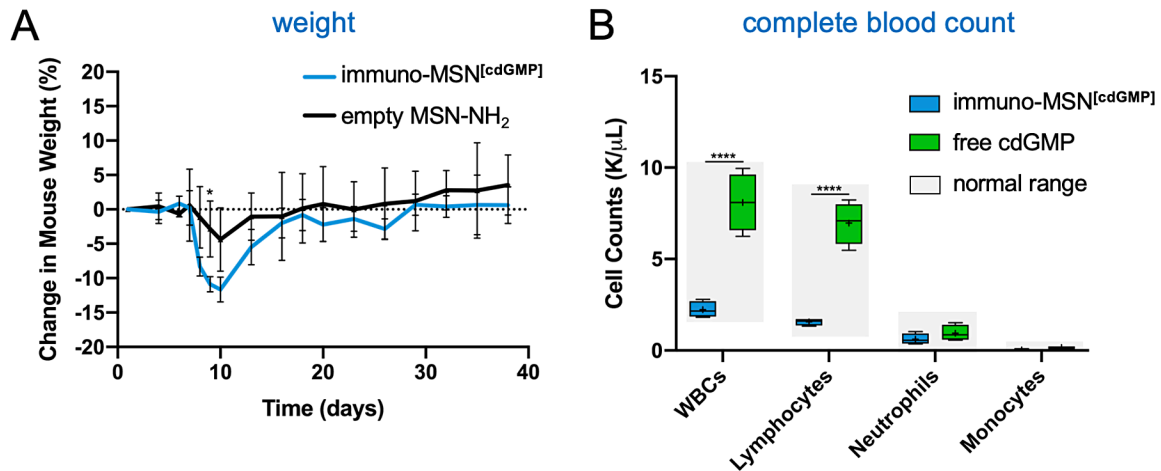
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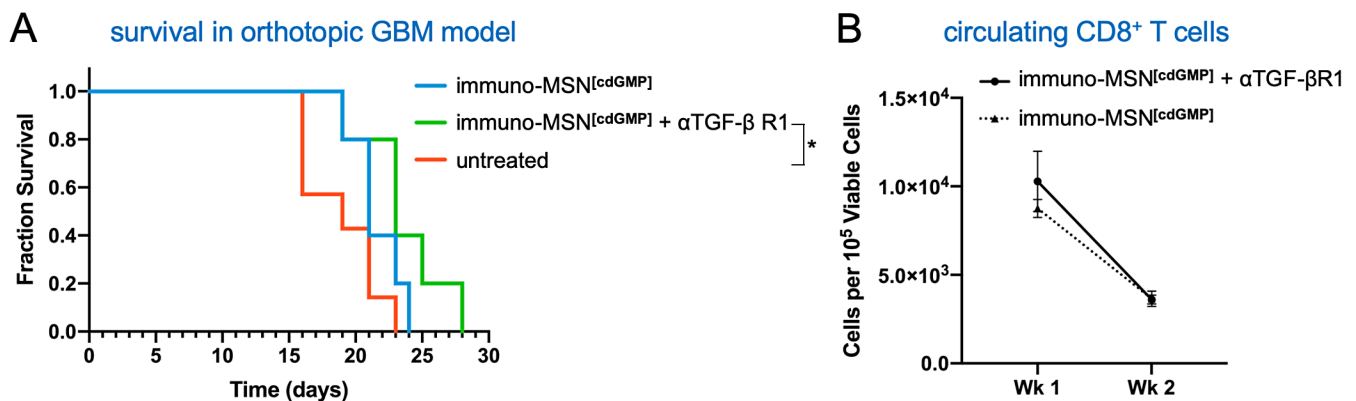
**Supplementary Figure S1.** (A) A representative flow cytometry dot plot shows the threshold gating for MSN<sup>+</sup> cells. (B) Live-animal spectrum imaging for fluorescently labeled MSN was performed longitudinally. A representative image shows the regions of interest (ROIs) used to measure signal 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 intravenously administered immuno-MSN particles delivering 30  $\mu\text{g}$  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<sub>2</sub> 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<sup>3</sup>) from physical caliper measurements ( $\text{volume} = 0.5 \times \text{length} \times \text{width}^2$ ). **(E)** Fractional survival of mice treated with immuno-MSN particles ( $n = 4$ ) compared to MSN-NH<sub>2</sub> 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<sub>2</sub> vehicle control ( $n = 3$ ). Mice treated with immuno-MSNs received 30  $\mu$ g 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  $\mu$ g of cdGMP loaded into MSNs or an equivalent amount of free cdGMP. Cell counts (K/ $\mu$ L) 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  $\mu$ g of cdGMP. 2.5 mg/kg of  $\alpha$ -TGF- $\beta$ R1 (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  $\alpha$ -TGF- $\beta$ R1) compared to untreated controls ( $n \geq 5$ ). 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<sup>+</sup> T cells in the blood circulation after the start of immuno-MSN treatment. Cell count data from flow cytometry analysis is represented as mean  $\pm$  standard error and was normalized to 10<sup>5</sup> viable cells (Wk 1 and Wk 2:  $n = 5$  for immuno-MSN and immuno-MSN +  $\alpha$ -TGF- $\beta$ R1). Statistical significance was conducted by two-way ANOVA with Sidak's post-test.