Functional Delivery of siRNA in Mice using Dendriworms

Supporting Information

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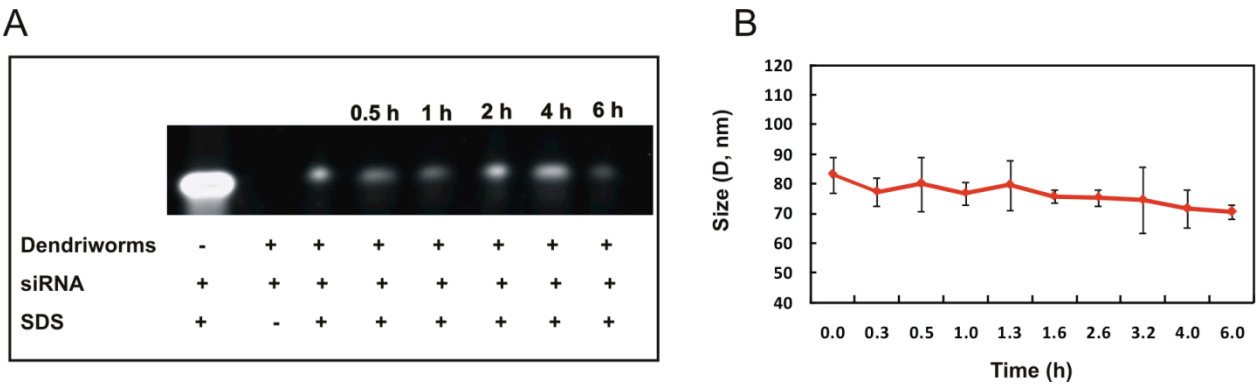

Figure S1: Stability of siRNA-Dendriworm nanoparticle in artificial cerebrospinal fluid (ACSF). (A) Gel electrophoresis indicating the stability of siRNA in ACSF. (B) DLS of the siRNA-Dendriworm complex in ACSF indicating colloidal stablility over time.

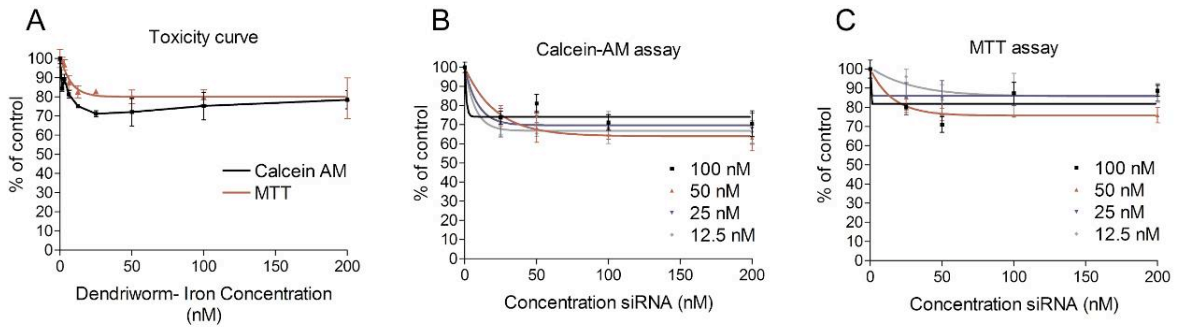

Figure S2. (A) Calcein AM and MTT toxicity assays with Dendriworms incubated with HeLa cells. Formulations were incubated using a protocol similar to that used for assessment of siRNA mediated gene silencing (4 hour incubation with nanoparticle containing solution followed by 20 hours of incubation in cell culture medium) and viability was assessed 24 hours later (B) Calcein AM toxicity assay with varying Dendriworm and siRNA concentrations following the same protocol as in A (C) MTT toxicity assay with varying Dendriworm and siRNA concentrations following the same protocol as in A. Toxicity was also assessed immediately after 4 hour incubation with the nanoparticle formulations (data not shown) and no formulation was found to reduce the viability below 50%.

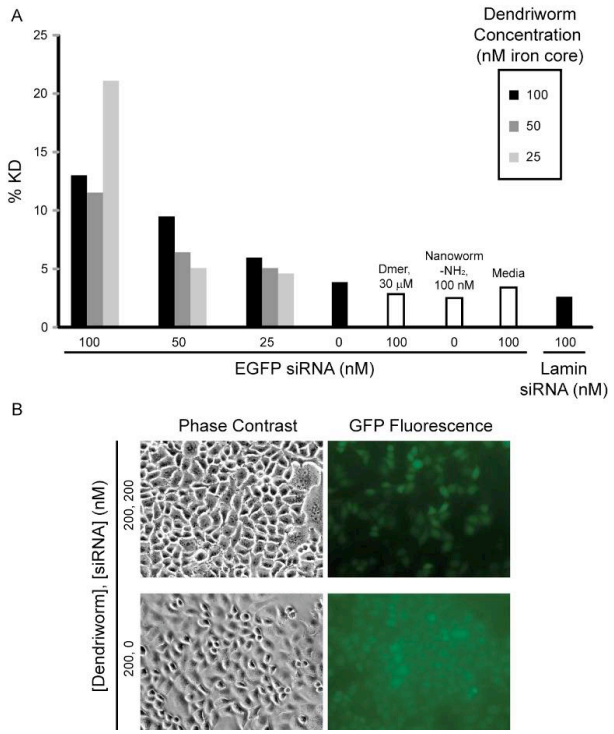

Figure S3. GFP knockdown in HeLa cells. HeLa-GFP cells were incubated with dendriworms at different siRNA and dendriworm concentrations (nM iron) and dose dependent GFP knockdown was observed. The knockdown was low but significant when compared with various controls (dendrimer only, nanoworm-NH2, Lamin siRNA treatment or no treatment). Lipofectamine 2000 produced as high as 70-80% knockdown in these experiments when 100 or 50 nM siRNA was used. Nanoworm concentration expressed as iron core concentration.

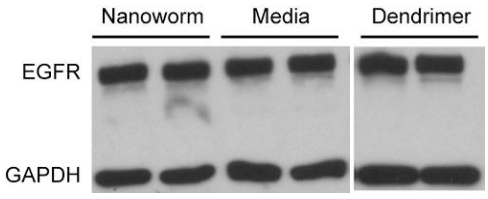

Figure S4. Controls for EGFR siRNA delivery in GBM-6 cells with dendriworms. No difference in EGFR/GAPDH ratio was seen for any of MION, dendrimer treatment or no treatment conditions.

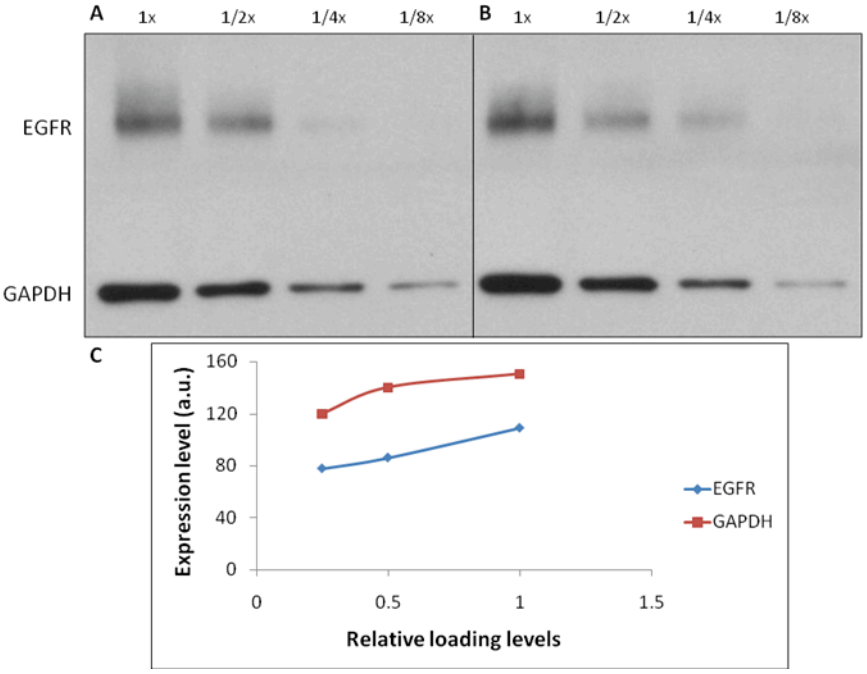

Figure S5. EGFR/GAPDH ratio is linear over the range of band intensities analyzed in the manuscript. A,B: Series dilutions of protein lysate obtained from GBM-6 cells were tested for EGFR and GAPDH protein levels via western blot. C: Quantification of band intensities in A and B reveals linear and proportional increas in EGFR and GAPDH levels as concentration increases.

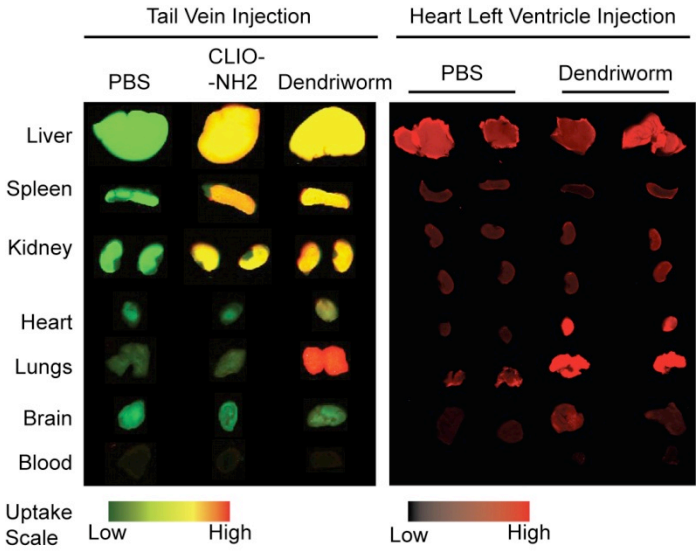

Figure S6. Biodistribution of dendriworms *in vivo* when injected sytemically. (Left): When dendriworms are injected in the tail vein of a Swiss Webster mouse, they accumulate in the lungs within 5 minutes while CLIO-NH_2 particles do not exhibit this behavior and end up in the liver, spleen and

kidneys (Right): dendriworms home to the lungs even when injected into the heart left ventricle of Swiss Webster mouse so that they arrive at the lungs the last. Heart left ventricle injections were done in duplicate, at the same time to eliminate potential error arising from accidental right ventricle injection. The gradient scales indicate least uptake on the left end and the maximum uptake at the right end.

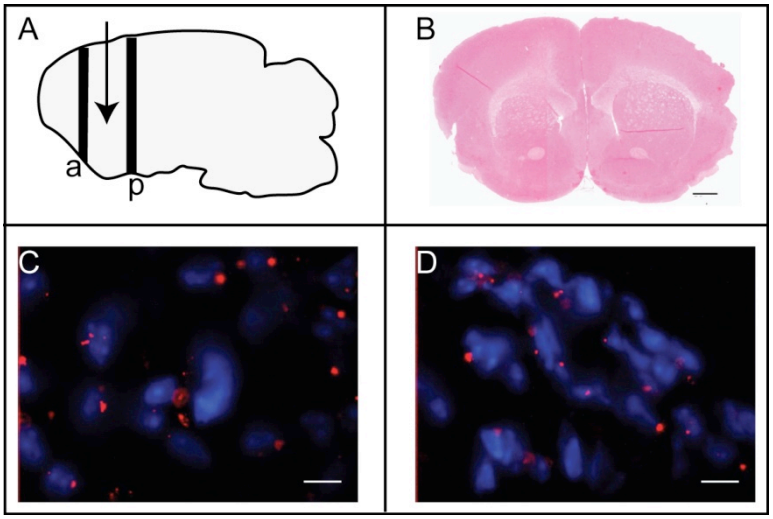

Figure S7. Dendriworms formulated with siRNA are well tolerated in the brain tissue. A) Sagittal schematic representation of a mouse brain indicating the site of injection (arrow). B) Coronal H&E stained paraffin-embedded section at the site of injection. Scale bar is 500 μm. The nanoparticles penetrate in both the anterior (a, C) and posterior (p, D) directions from the site of injection after 7 days of delivery. Scale bars in C and D are 10 μm long.

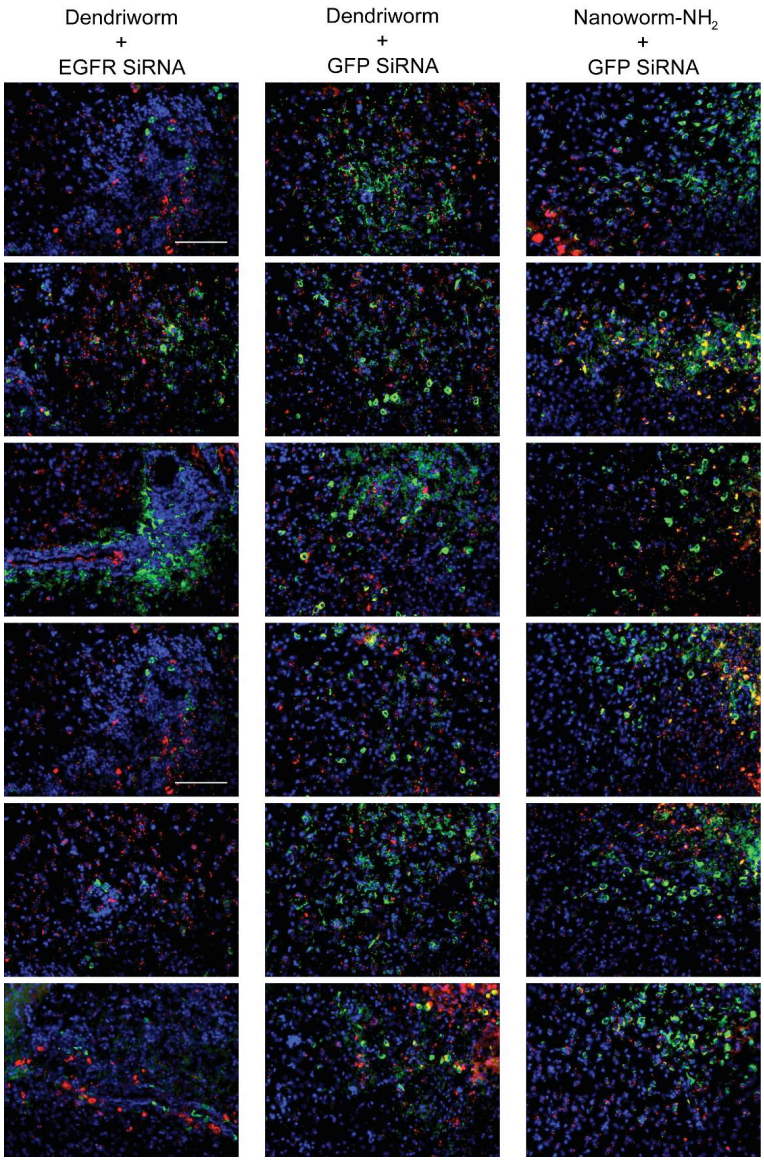

Figure S8. Histology data from all six sections imaged for various nanoparticle + siRNA formulations used in the *in vivo* studies

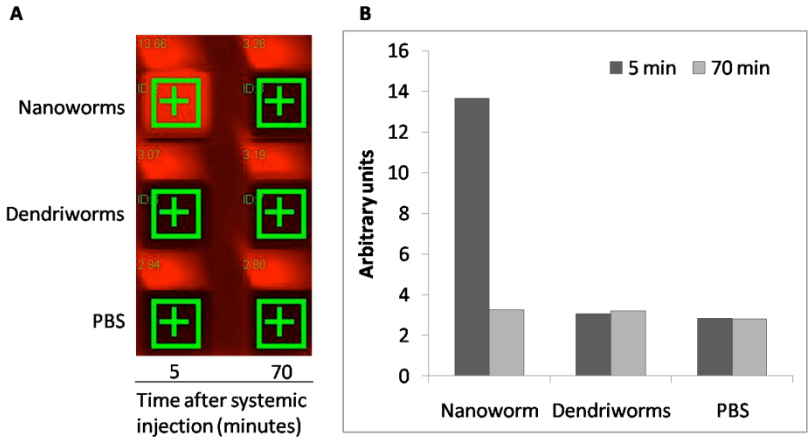

Figure S9. Circulation time of dendriworms and nanoworms. A: Nanoworms or dendriworms (12 ug iron) were injected into the tail vein of Swiss Webster mice and blood was drawn at 5 min or 70 min after injection. While nanoworms were present in blood at 5 min post injection, dendriworms were cleared in under 5 min. B: Quantification of data obtained via infrared imaging of the samples.