

Nanogel-based particles for treatment of systemic lupus erythematosus

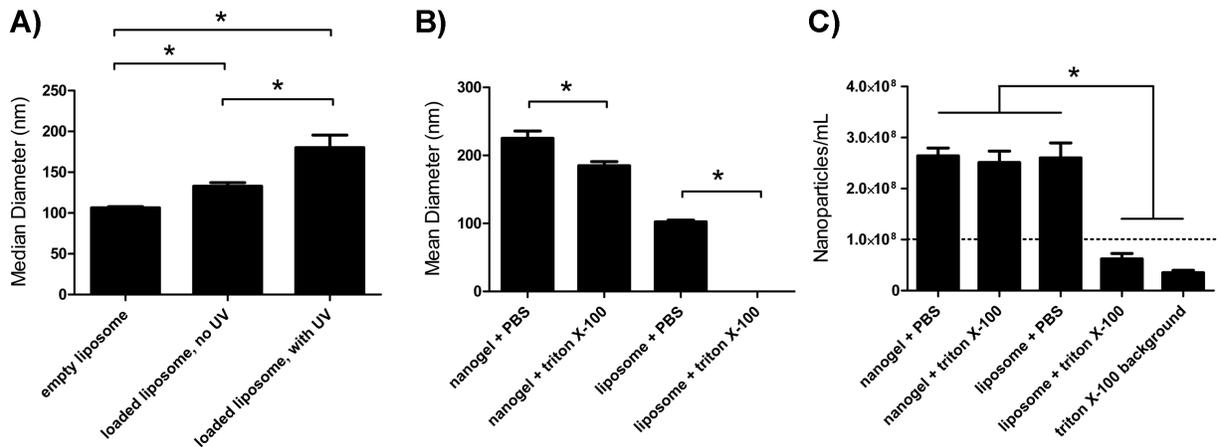
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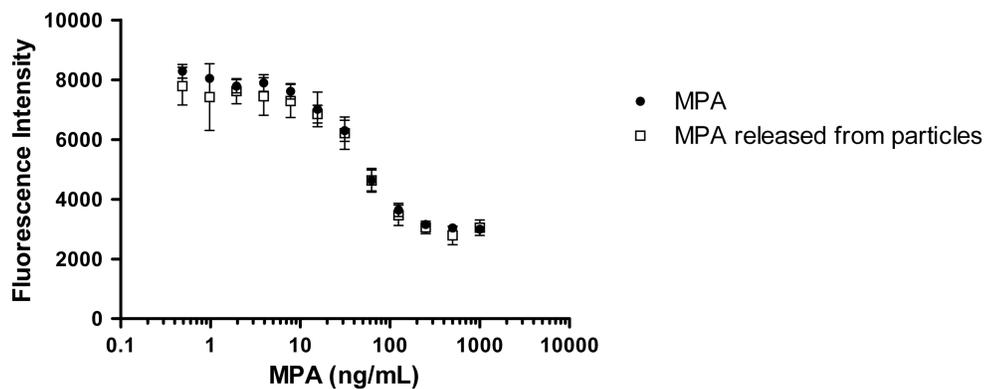
Supplementary Material

List of Supplementary Material

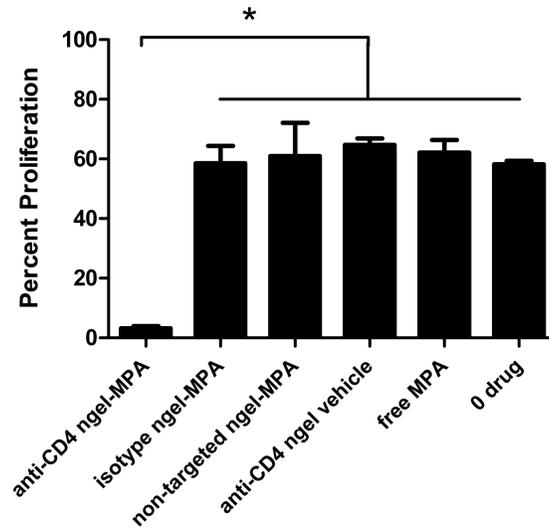
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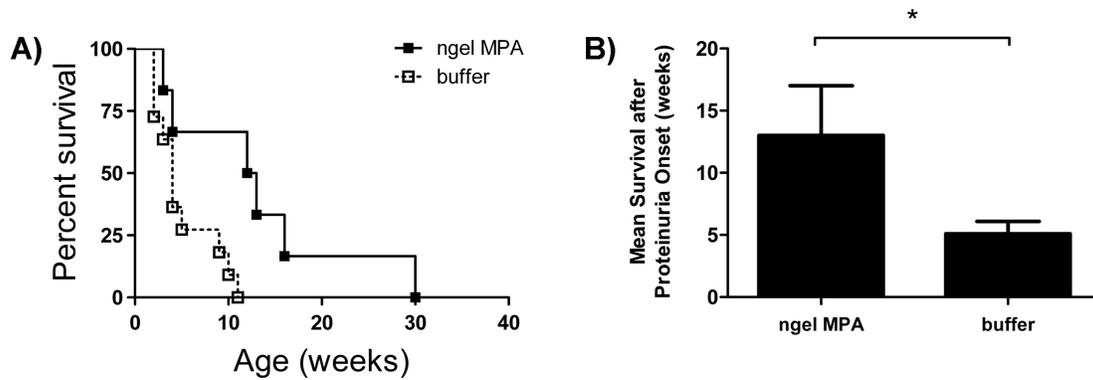
Supplementary Figure 1. Nanogels are characterized as a swollen, gel-like interior. **a)** The median diameter of liposomes swelled after being filled with drug and PEG-oligomers. UV photopolymerization of filled-particles increased the particle size even more. **b)** The formation of a stable interior was verified by exposing nanogels to triton X-100: liposomes ruptured upon exposure to this surfactant, whereas lipogels do not. PBS is phosphate buffered saline at pH 7.4. **c)** Single particle counting verified that triton X-100 ruptured and decreased the number of liposomes but not nanogels. Horizontal dashed line indicates the limit of detection of the Nanosight particle tracking instrument. In all subfigures, error bars represent standard error measurement, with at least 3 measurements per group. * $p < 0.05$ or less by 1-way ANOVA with Bonferroni multiple comparison test.



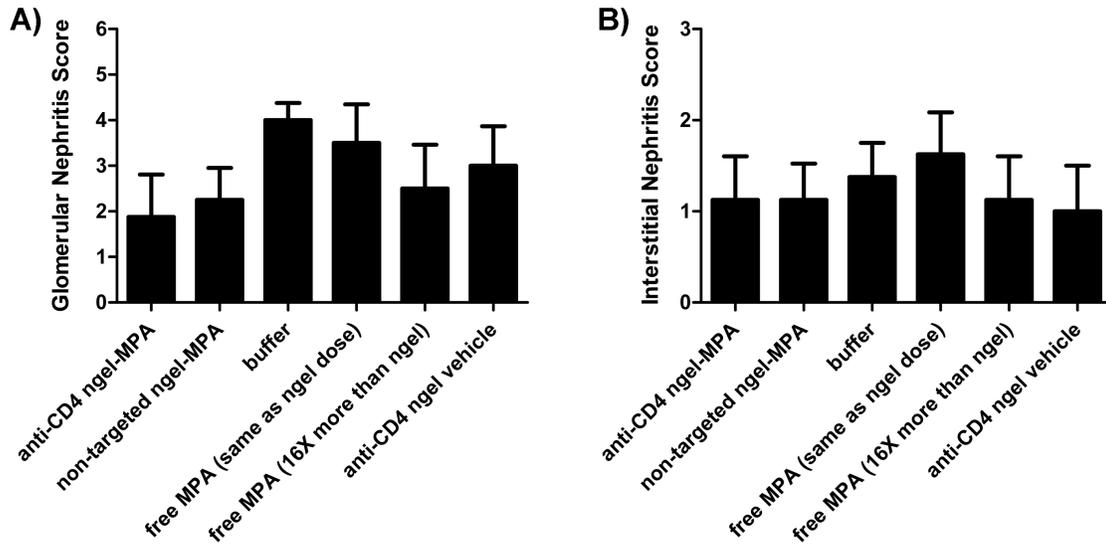
Supplementary Figure 2. MPA released from nanogels remain bioactive. Nanogels released MPA into PBS, and the supernatant (containing released drugs but no particles) was added to Jurkat cells for 2 days. Jurkat cell proliferation was quantified by cell titer blue assay (Promega), in which an increase of fluorescence correlates with increased proliferation. MPA released from nanogels had equivalent bioactivity as stock drug. Results are representative of two independent experiments.



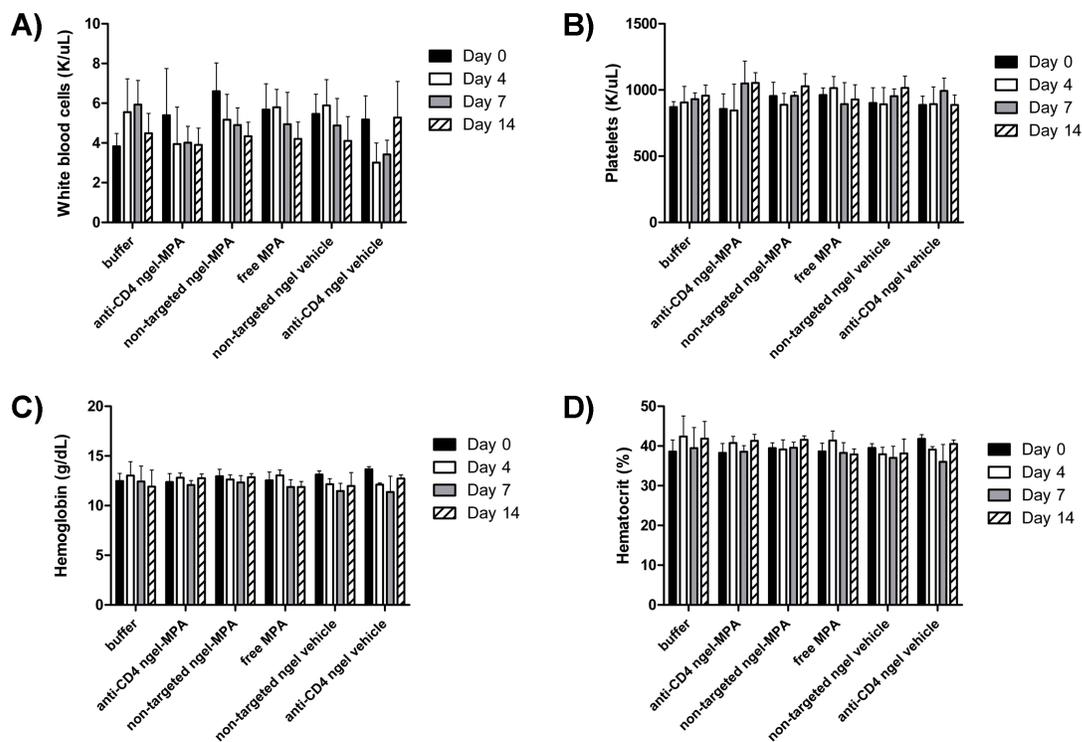
Supplementary Figure 3. CD4-targeted particles inhibit T cell proliferation. CD4 T cells were incubated with nanogels for 15 min at 37 °C, washed, and then cultured for 4 days with anti-CD3 and anti-CD28. Proliferation was measured with CFSE. Only T cells treated with targeted particles had inhibited proliferation, whereas non-targeted particles and free MPA did not because the wash step removed unbound drug. Results are based on triplicates of sample conditions, and are representative of three independent experiments. Error bars represent standard deviation. * $p < 0.0001$ by 1-way ANOVA with Bonferroni multiple comparison test.



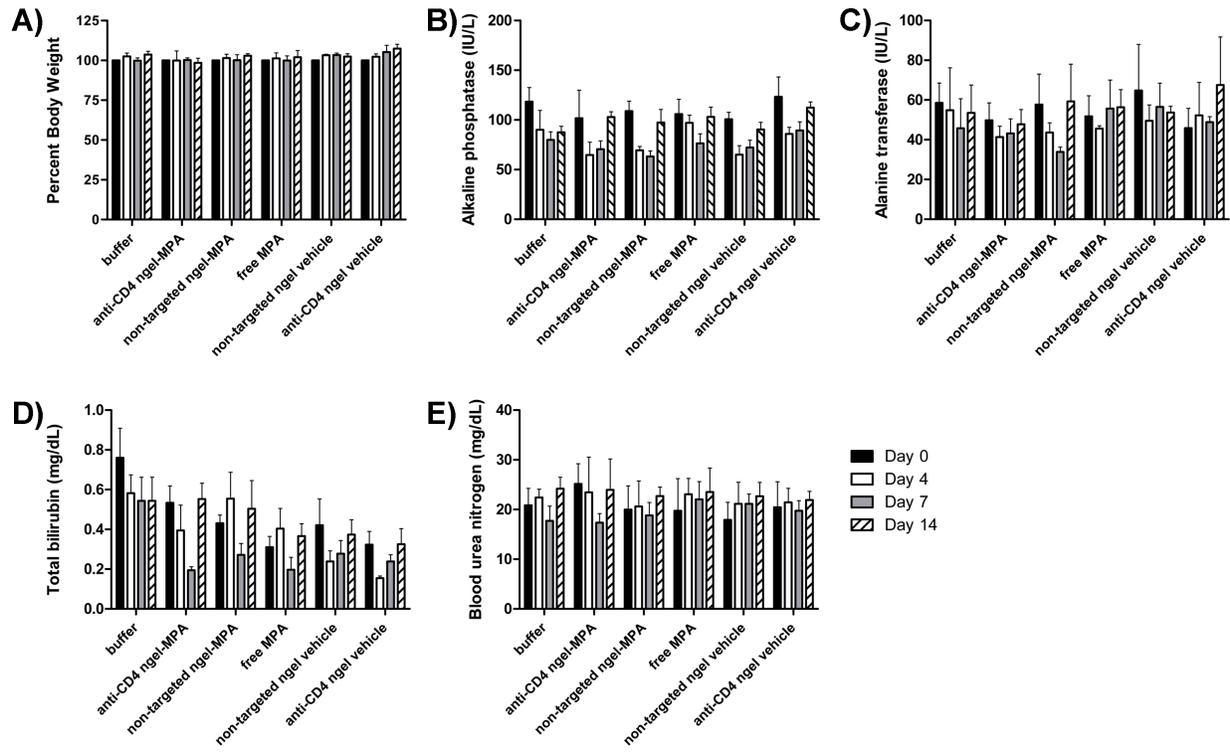
Supplementary Figure 4. Nanogel therapy initiated after proteinuria onset extends survival. NZB/W F1 mice that developed proteinuria (≥ 300 mg/dL protein on two consecutive days) were administered a weekly treatment of 0.625 mpk of MPA in particles. The average age (with standard deviation) of proteinuria onset was 37.2 ± 5.9 weeks (ngel-MPA) and 36.3 ± 5.2 weeks (buffer). Survival analysis is shown with **a)** Kaplan-Meier curves, and **b)** mean survival age. Error bars represent the standard error measurement. The sample size is $n = 6 - 11$ mice per group. * $p = 0.0198$ by log-rank (Mantel-Cox) method.



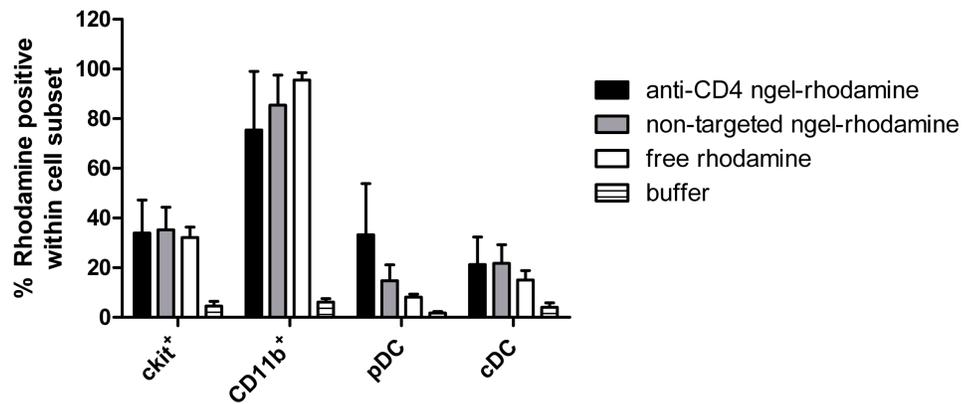
Supplementary Figure 5. Nanogel therapy prevents kidney damage. NZB/W F1 mice received prophylactic therapy, and hematoxylin and eosin stained kidney sections were prepared from 36-40-weeks-old NZB/W F1 mice and blindly scored for **a)** glomerular nephritis (0-6) and **b)** interstitial nephritis (0-3). Marked reductions in score were observed for glomerular inflammation but not intertubular infiltration in mice receiving particulate treatment. A score of “0” indicates no nephritis, with higher values indicating greater severity in inflammatory damage. The sample size is n = 8-16 mice per group, with error bars representing the standard error measurement.



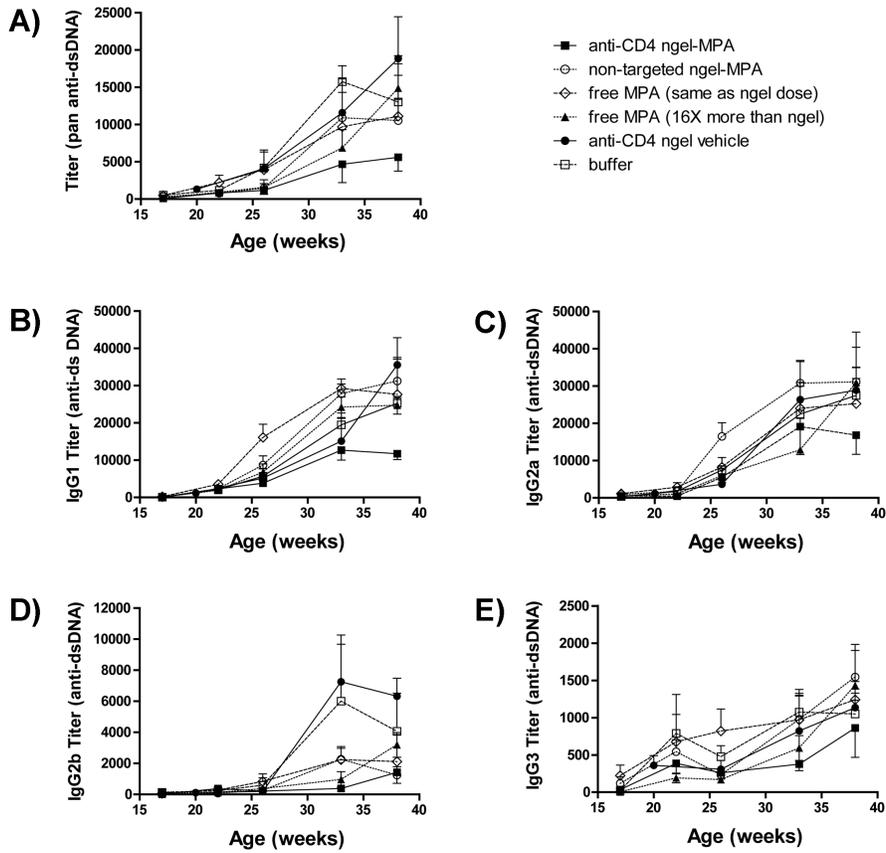
Supplementary Figure 6. Complete blood counts (CBC) are normal after nanogel administration. C57BL/6 mice received 4 consecutive, daily doses of particles, and EDTA-anti-coagulated blood was analyzed for hematoxicity. All CBC measurements were within the normal reference range for **a)** white blood cells (1.8 – 10.7 K/ μ L), **b)** platelets (592 - 2971 K/ μ L), **c)** hemoglobin (11.0 – 15.1 g/dL), and **d)** hematocrit (35.1 – 45.1 %). Mouse CBC reference ranges are from *Drew Scientific Hemavet 950 Reference Ranges* (2010). Error bars represent the standard deviation. The sample size is n = 5 mice per group.



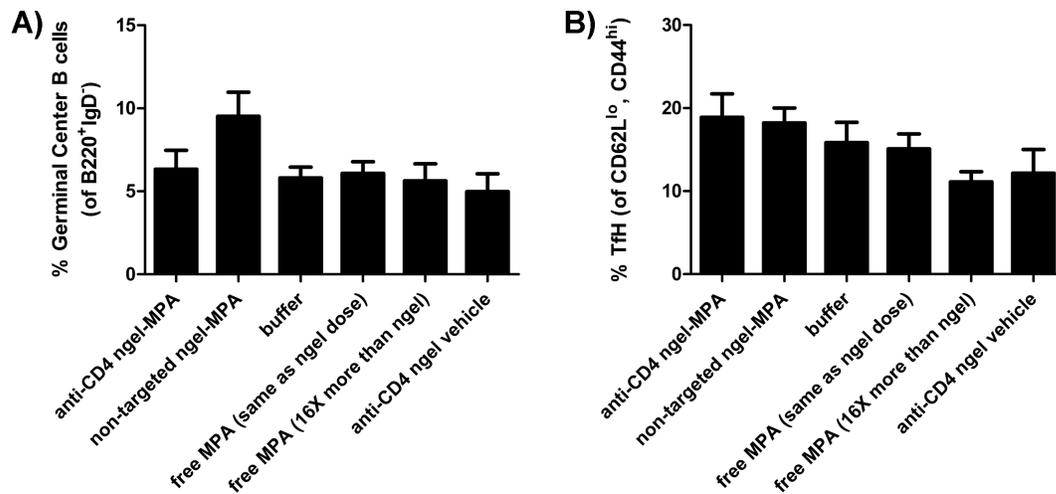
Supplementary Figure 7. Nanogels do not cause liver or renal toxicity. C57BL/6 mice received 4 consecutive, daily doses of particles. **a)** Body weight was normal. Serum clinical chemistries were within normal physiological range for **b)** alkaline phosphatase (62-209 IU/L), **c)** alanine transferase (28-152 IU/L), **d)** total bilirubin (0.1-0.9 mg/dL), and **e)** blood urea nitrogen (18-29 mg/dL). Mouse physiological reference ranges are from *IDEXX VetTest Operator's Reference Manual* (2007). Error bars represent the standard deviation. The sample size is n = 5 mice per group.



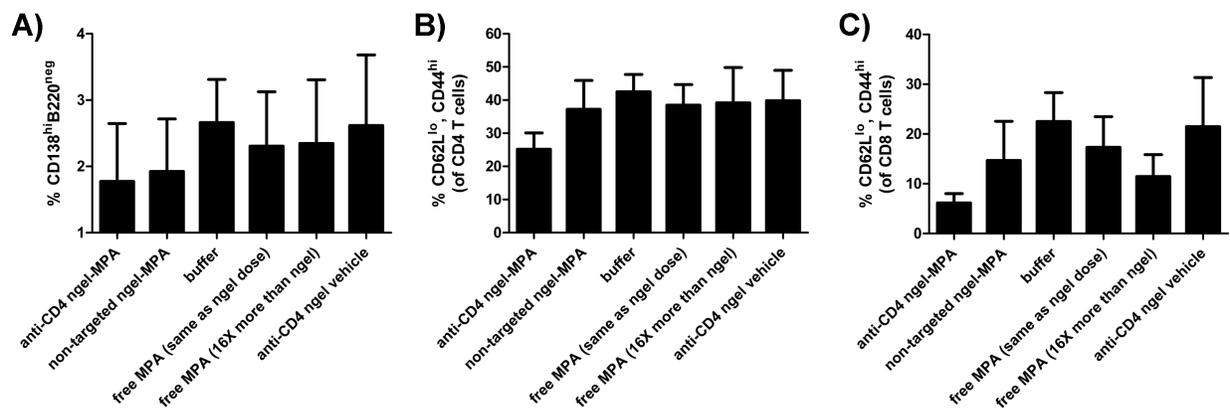
Supplementary Figure 8. Cells in the bone marrow associate with nanogels. C57BL/6 mice were vaccinated with sheep red blood cells to induce T and B cell activation, and 7 days later, the mice were given an intraperitoneal injection of free rhodamine or nanogels loaded with rhodamine. Approximately 2-3 hours later, bone marrow cells were harvested and analyzed by flow cytometry. Hematopoietic stem cells (ckit⁺), macrophages (CD11b⁺), pDCs (plasmacytoid dendritic cells), and cDCs (conventional dendritic cells) were analyzed. Error bars represent the standard error measurement. The sample size is n = 4 mice per group.



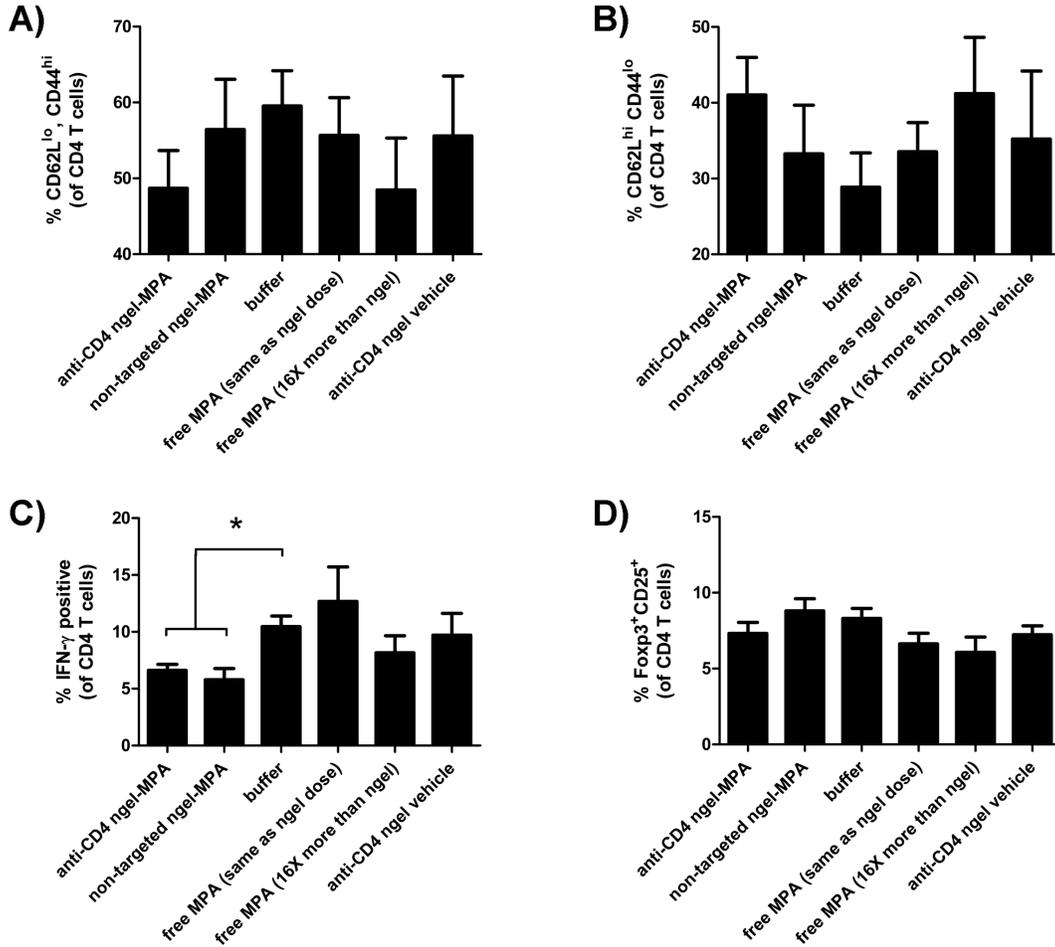
Supplementary Figure 9. Analysis of anti-ds DNA titers. NZB/W F1 mice received weekly treatments of nanogels beginning at 18-20 weeks of age. Anti-ds DNA titers were measured for **a)** total IgG, **b)** IgG1, **c)** IgG2a, **d)** IgG2b, and **e)** IgG3. Some moderate, but statistically insignificant (by ANOVA test) reductions of total IgG, IgG1, and IgG3 titers were observed with CD4-targeted MPA particles. The sample size is $n = 8$ animals per group. Error bars represent the standard error measurement.



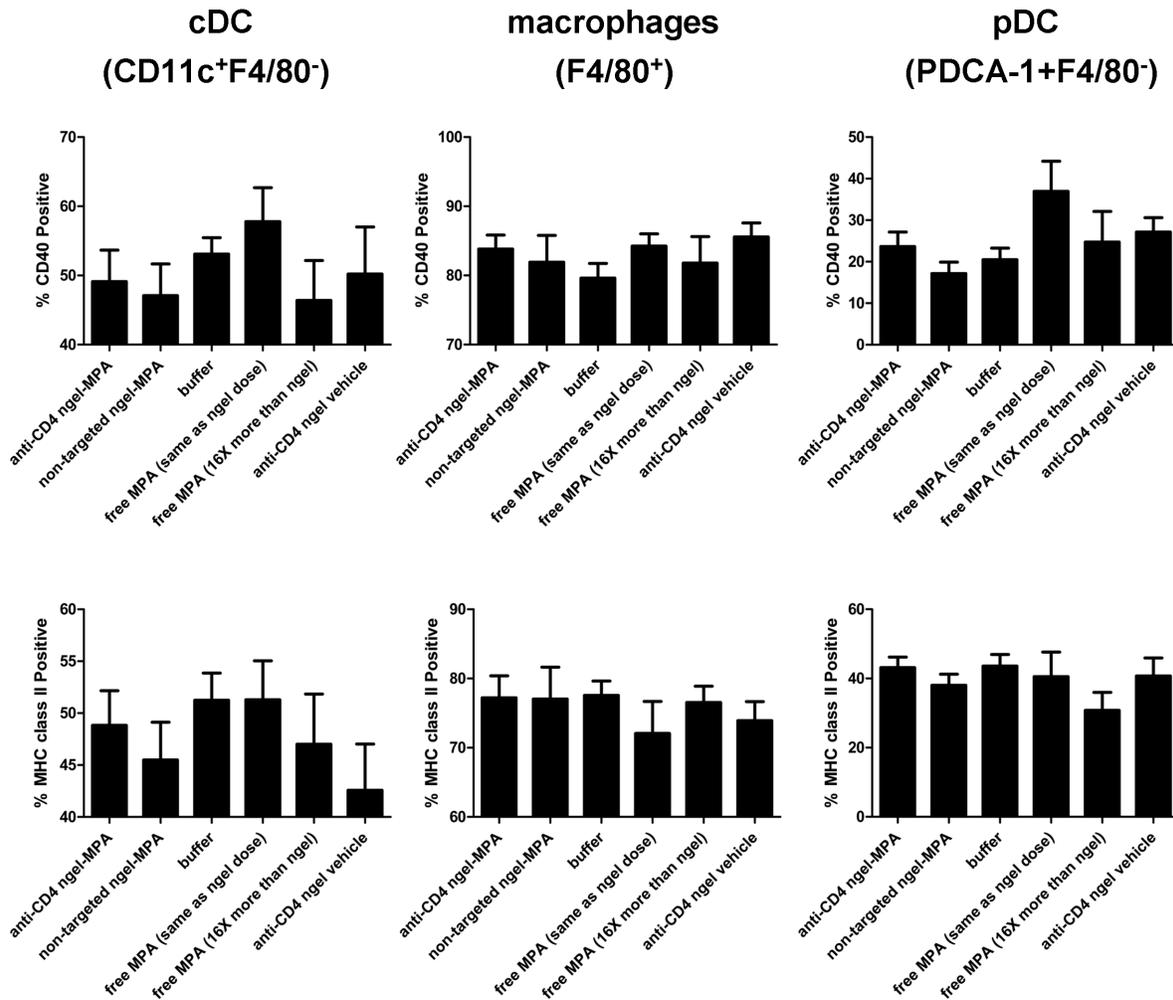
Supplementary Figure 10. T follicular helper cell and germinal center B cell percentages are unaffected by nanogel therapy. In 36-40-weeks-old NZB/W F1 mice that received prophylactic therapy, the percentage of splenic **a)** germinal center B cells and **b)** T follicular helper cells (defined as CXCR5⁺PD-1⁺ activated CD4 T cells) were comparable among all treatment groups. The sample size is n = 6 to 15 animals per group. Error bars represent the standard error measurement.



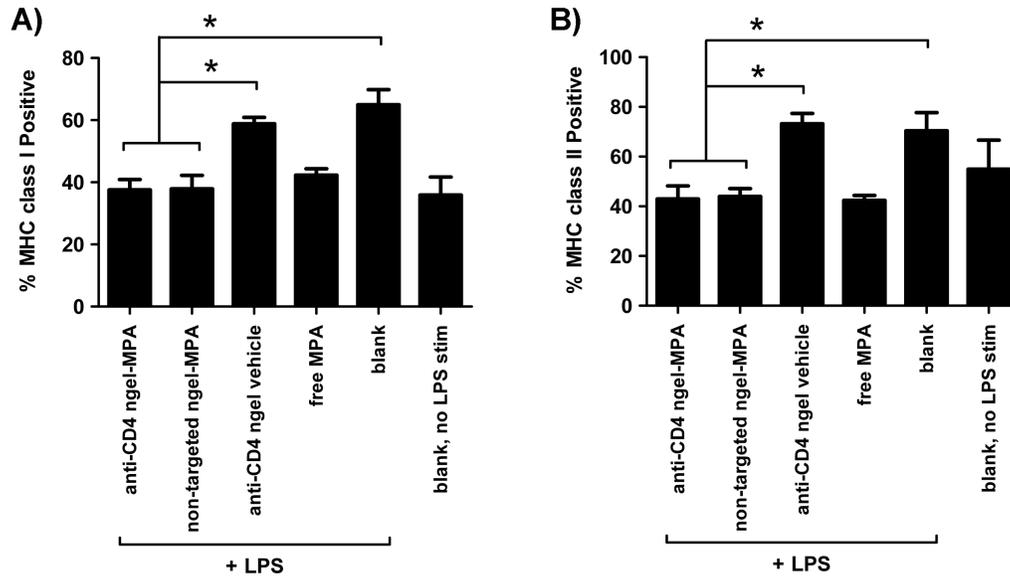
Supplementary Figure 11. Peripheral blood lymphocyte analysis in 36-40-week-old NZB/W F1 mice. Mice received particulate therapy beginning at 18-20 weeks of age. Particulate therapy resulted in partial reductions in the percentage of **a)** plasmablasts, **b)** activated CD4 T cells, and **c)** activated CD8 T cells. The sample size is n = 6 to 15 animals per group. Error bars represent the standard error measurement.



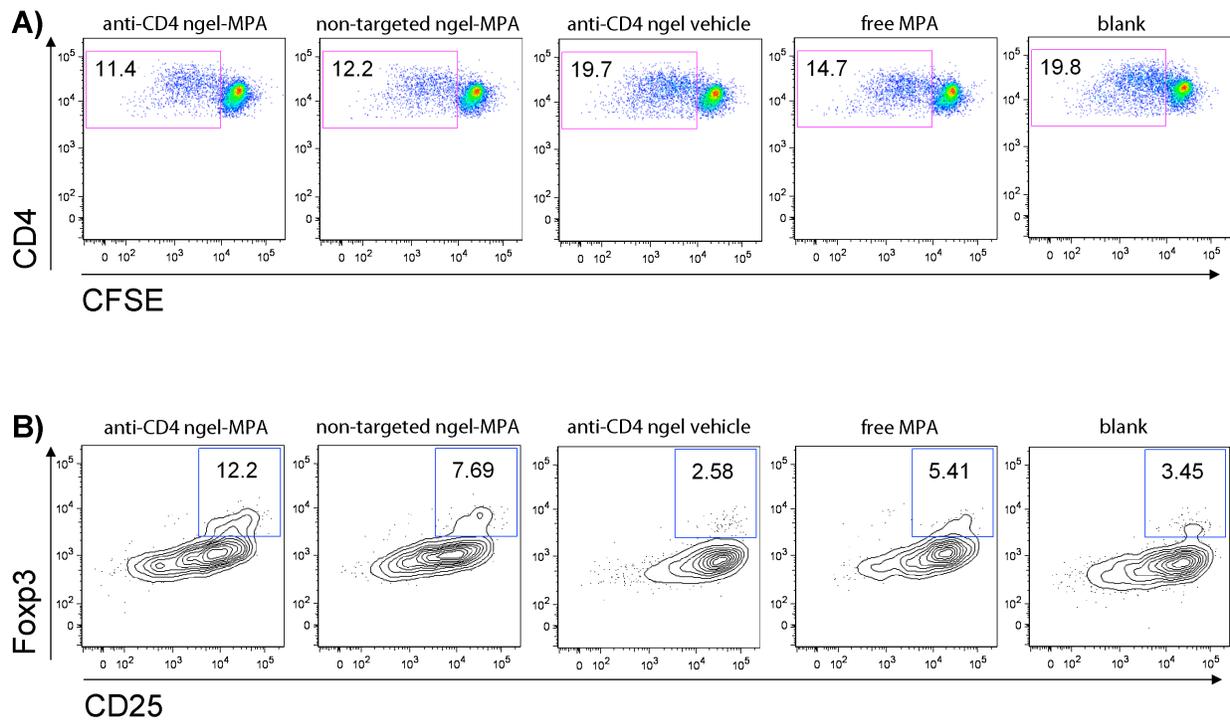
Supplementary Figure 12. Activation state of splenic CD4 T cells. Splenocytes were harvested from 36-40-weeks-old NZB/W F1 mice that received weekly therapy beginning at 18-20 weeks of age. Nanogel treatment resulted in **a)** decreased percentages of activated cells, and **b)** increased percentages of naïve cells. **c)** The percentage of IFN- γ ⁺ cells was reduced in mice that received particulate treatment, compared to all other groups. * $p < 0.05$ by two-tailed t-test. **d)** No differences were observed in CD4 T regulatory cells. The sample size is $n = 6$ to 15 animals per group. Error bars represent the standard error measurement.



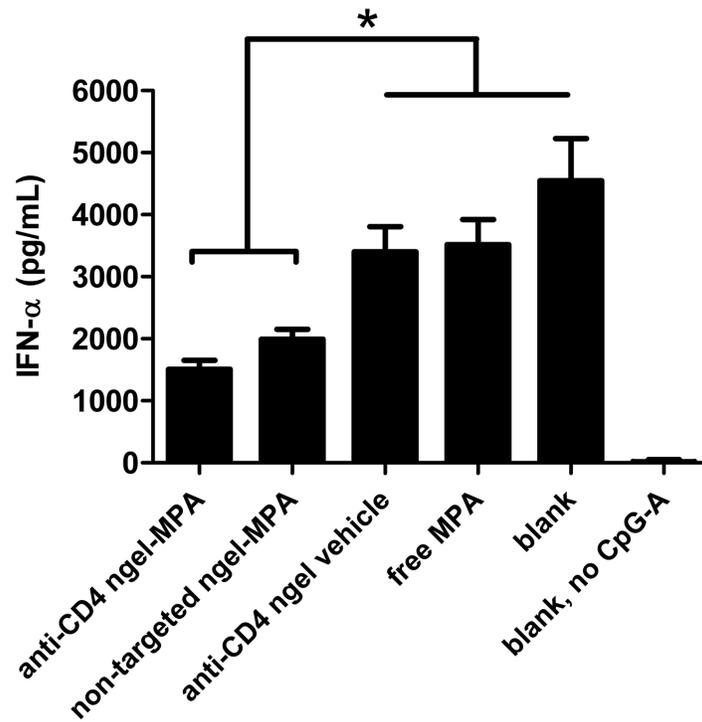
Supplementary Figure 13. CD40 and MHC class II expression on splenic innate antigen presenting cells. Cells were harvested from 36-40-weeks-old mice that received weekly particulate therapy beginning at 18-20 weeks of age. Conventional dendritic cells (cDCs), macrophages, and plasmacytoid dendritic cells (pDCs) were analyzed. The sample size is n = 6 to 15 animals per group. Error bars represent the standard error measurement.



Supplementary Figure 14. MHC class I and II expression on bone marrow derived dendritic cells (BMDCs). BMDCs were treated with immunosuppressants beginning on day 1 of culture, and then stimulated for 18 hr with lipopolysaccharide (LPS) on day 6. The surface expression of **a)** MHC class I and **b)** MHC class II was reduced with MPA treatment in vitro. Results are the average of 3 separate experiments, with error bars representing the standard error measurement. * $p < 0.05$ or less by 1-way ANOVA using Bonferroni post-test.



Supplementary Figure 15. MPA-treated bone marrow-derived dendritic cells (BMDCs) are less allostimulatory to CD4 T cells, and can induce some expansion to Treg cells. MPA-treated BMDCs from Balb/c mice were irradiated and then cultured with purified C57BL/6 CD4 T cells for four days. **a)** T cells had reduced proliferation, as measured by CFSE labeling. **b)** T cells also had increased differentiation to a Fxp3⁺CD25⁺ phenotype. Results are representative of two independent experiments.



Supplementary Figure 16. Nanogels attenuated IFN- α production from plasmacytoid dendritic cells. Bone marrow cells were incubated with 1 $\mu\text{g}/\text{mL}$ of MPA in nanogels for 1 hr at 37 $^{\circ}\text{C}$, washed, and then stimulated with CpG-A for 18 hr. Data are averaged triplicates in one representative experiment from three repeated trials. Error bars are the standard deviation. * $p < 0.05$ or less by 1-way ANOVA with Bonferroni multiple comparison post-test.