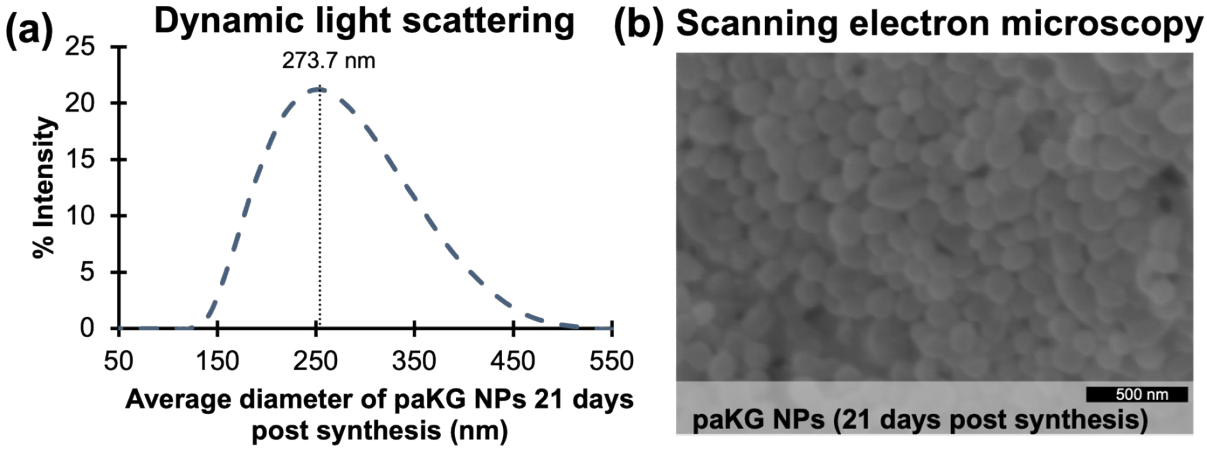
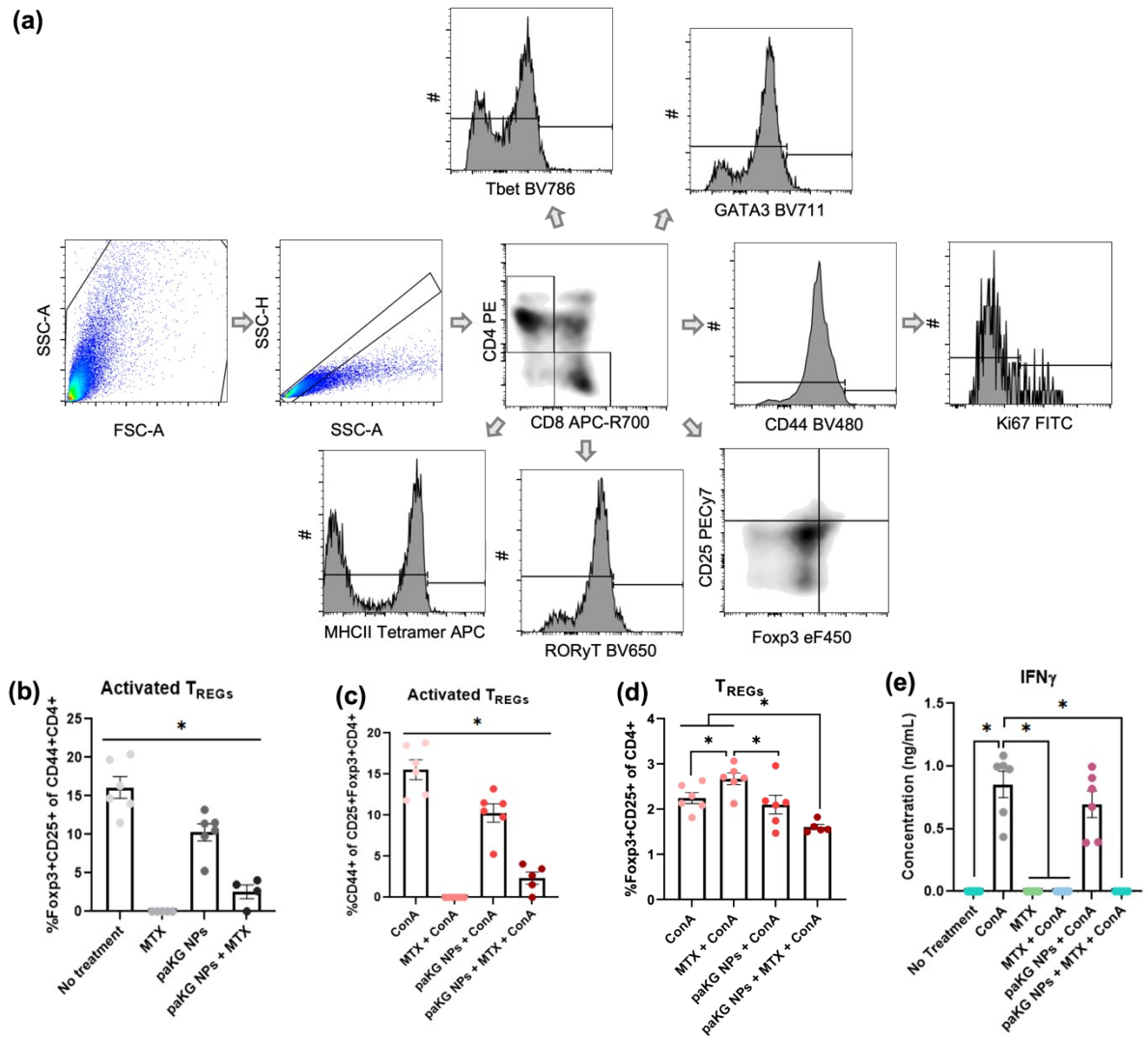
Appendix A. Supplementary material

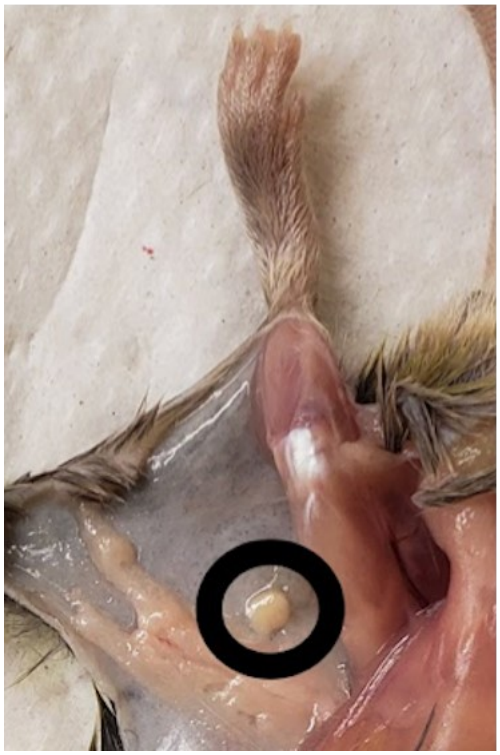


Supplementary Figure 1: Characterization of paKG NPs 21 days post synthesis. (a) Dynamic light scattering data demonstrates the average diameter of nanoparticles that were stored in -20°C for 21 days to be 273.7 ± 62.6 nm (n = 3; avg ± SEM). Measurement was executed in DI H2O. **(b)** Scanning electron microscope images of paKG NPs with a gold sputter coating of ~15 nm (nanoparticles were gold coated only for the purposes of imaging) continued to demonstrate a smooth and spherical surface morphology after being stored in -20°C for 21 days (scale bar = 500 nm; magnification = 100,000x).



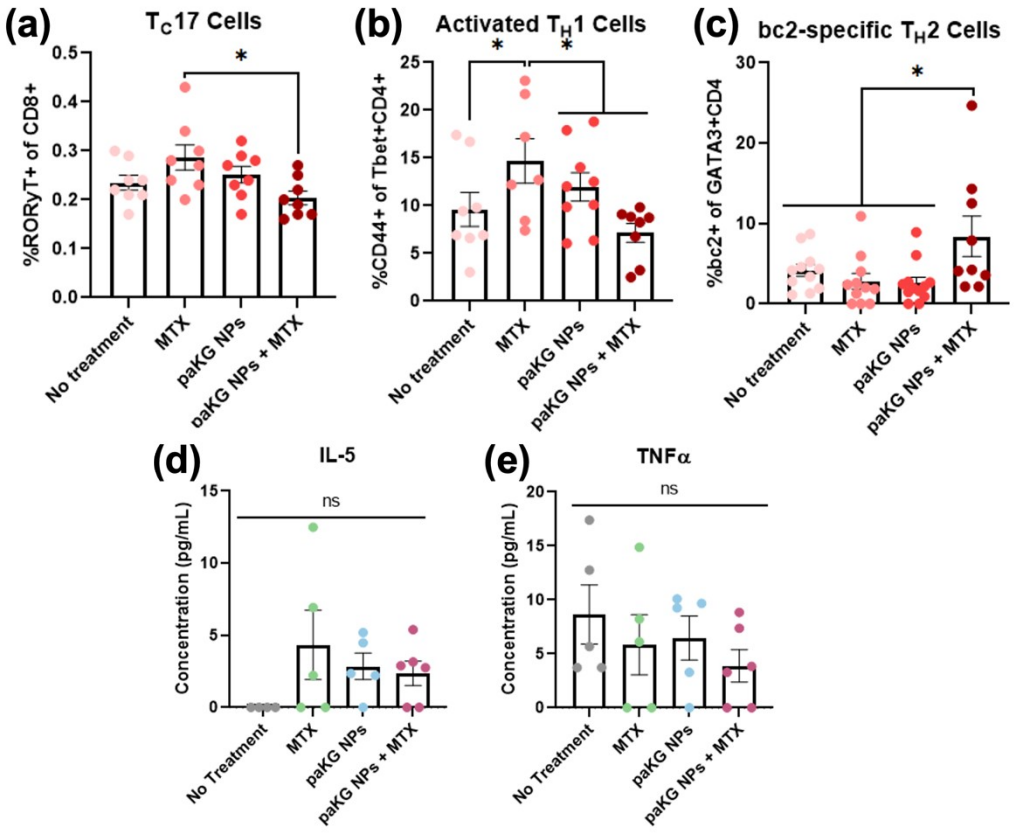
Supplementary Figure 2: T cell flow cytometry schematic and *in vitro* T cell responses to paKG NPs + MTX. (a) Representative schematic of T cell flow plot analysis. **(b-d)** Flow cytometry of *in vitro* splenic T_{REG} responses treated with a final well concentration of 1 mg/mL of methotrexate and/or 0.1 mg/mL of paKG nanoparticles and cultured in 37°C for 48-72 hrs in the absence or presence of ConA with a final well concentration of 2.5 µg/mL, (b) Activated T_{REGs} without ConA (n= 4-6; avg ± SEM; * = $p \leq 0.05$; two-tailed unpaired Student's t-test), (c) T_{REGs} with ConA (n= 4-6; avg ± SEM; * = $p \leq 0.05$; One-Way ANOVA, Brown-Forsythe and

Welch ANOVA tests, unpaired t with Welch's correction), (d) Activated T_{REGs} with ConA (n= 4-6; avg ± SEM; * = $p \leq 0.05$; two-tailed unpaired Student's t-test), **(e)** Extracellular IFNγ levels within *in vitro* supernatant of splenocyte cultures were quantified by ELISA (n= 4-9; avg ± SEM; * = $p \leq 0.05$ as compared to ConA; two-tailed unpaired Student's t-test)

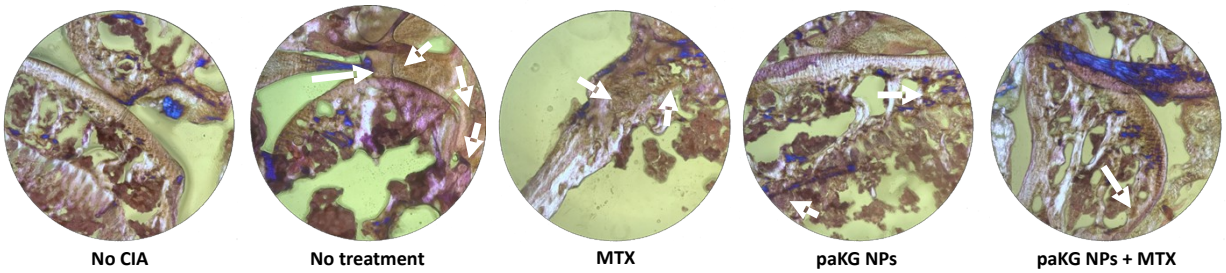


Supplementary Figure 3: Representative injection site image. The nanoparticle injection sites of mice were present when mice were euthanized on day 57 shown within a black circle.

Supplementary Figure 4: Representative paw scoring photos. The arthritic severity of mice was scored on a scale of 0-6 (see Table 1 for the points and scoring system that was utilized).



Supplementary Figure 5: paKG NPs + MTX alter T cell responses *in vivo* but do not increase blood serum IL-5 and TNFa cytokine levels. (a-c) Flow cytometry of *in vivo* T cell responses on day 57, (a) splenic T_C17 cells, (b) splenic activated T_H1 cells, (c) bc2-specific T_H2 cells in the cervical lymph nodes (n = 6 mice; n = 2 replicates; avg ± SEM; * = $p \leq 0.05$; Ordinary one-way ANOVA, uncorrected Fisher's LSD, with a single pooled variance). **(d,e)** Multiplex luminex analysis of blood serum cytokine levels, (d) IL-5, (e) TNFa (n = 4 - 6; avg ± SEM; * = $p \leq 0.05$; One-Way ANOVA, Brown-Forsythe and Welch ANOVA tests, unpaired t with Welch's correction, with individual variances computed for each comparison).



Supplementary Figure 6: paKG NPs + MTX reduce infiltration of immune cells.

Representative images of knee tissue with H&E staining is shown with white arrows showing cell infiltration. N = 3 biological replicates for no CIA, and N = 6 biological replicates for all other groups. Pink = Cytoplasm, Brown = drying artifact or blood, Blue = nuclei, and blue large splotches = artifact.