Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Nanoparticle-mediated co-delivery of inflammasome inhibitors provide protection against sepsis

Dipika Nandi [1 2], Maharshi Debnath [1], James Forster III [1], Ankit Pandey [2], Ashish Kulkarni [1 2 3 4]*

[1]Department of Chemical Engineering, University of Massachusetts Amherst, MA, USA

[2]Department of Veterinary and Animal Sciences, University of Massachusetts Amherst, MA, USA

[3]Department of Biomedical Engineering, University of Massachusetts Amherst, MA, USA

[4]Center for Bioactive Delivery, Institute for Applied Life Sciences, University of Massachusetts, Amherst, MA, USA

*Corresponding Author: akulkarni@engin.umass.edu

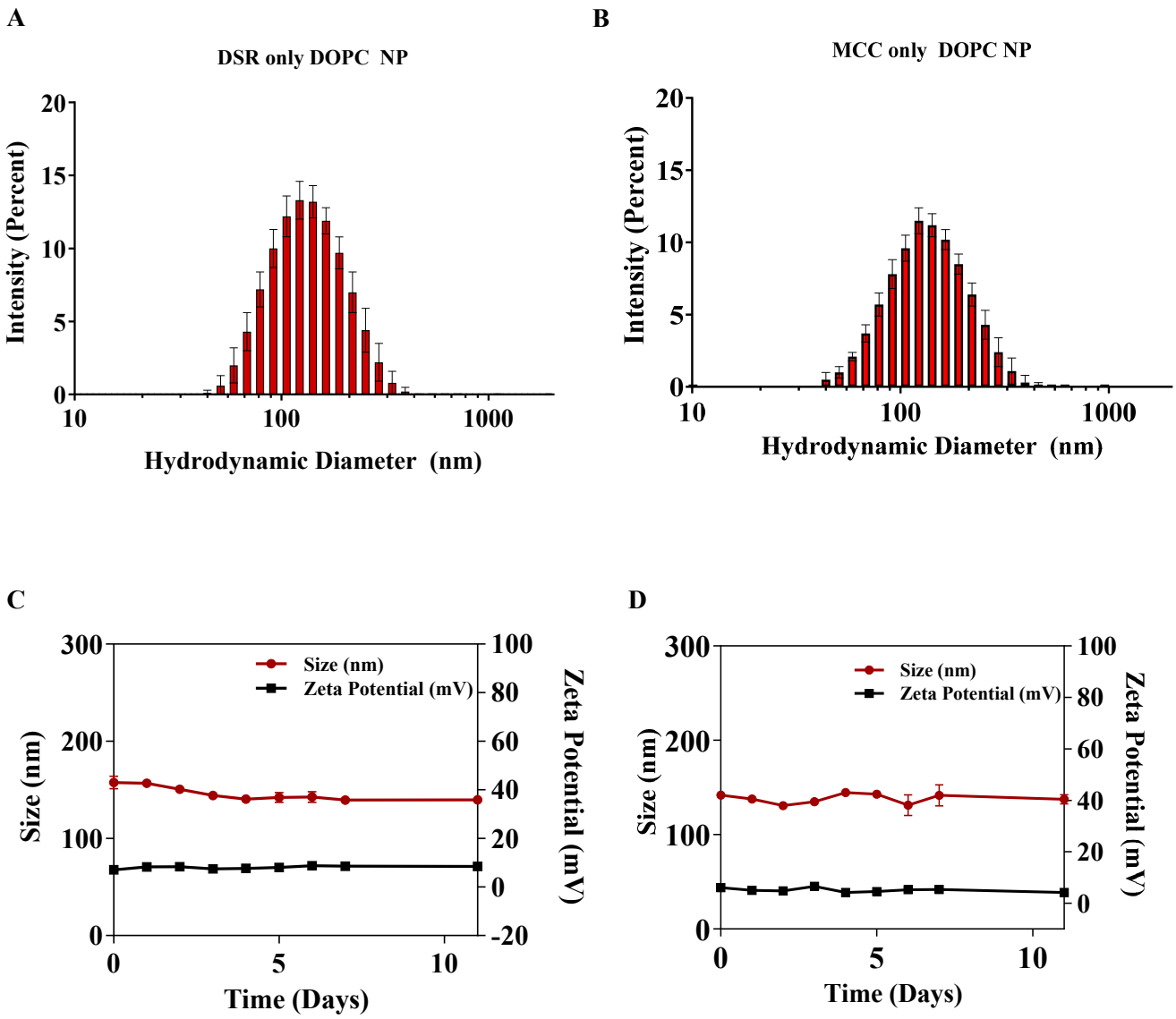

Figure-S1. Single drug nanoparticle characterization. (**A**) Graph plots the hydrodynamic diameter of DSR single drug nanoparticle. Data shown as mean ± S.D. (n=3). (**B**) Graph plots the hydrodynamic diameter of MCC-950 single drug nanoparticle. Data shown as mean ± S.D. (n=3). (**C**) PBS Stability plot shows size and zeta potential of DSR Np in PBS over a period of 11 days. Data shown is mean ± S.E.M. (n=3). (**D**) PBS Stability plot shows size and zeta potential of MCC Np in PBS over a period of 11 days. Data shown is mean ± S.E.M. (n=3).

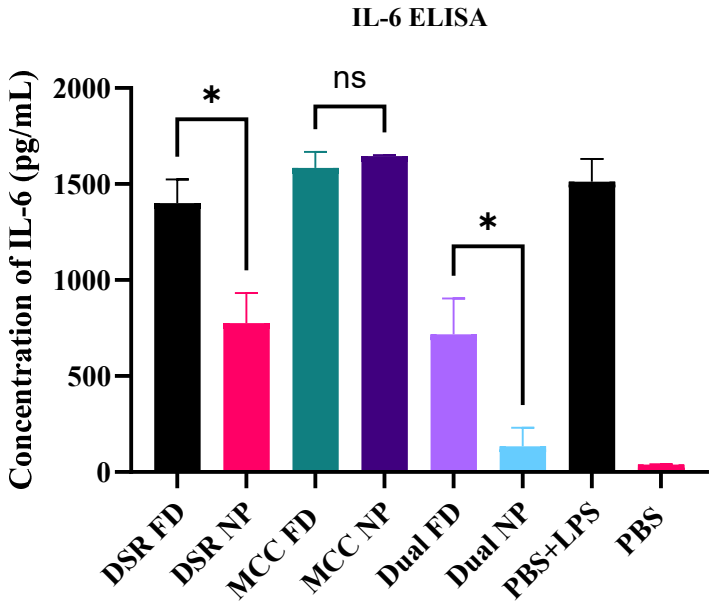

Figure-S2. IL-6 concentrations of serum samples obtained from the mice injected with single or dual drug nanoparticle. Graph plots serum IL-6 cytokine levels in the different treatment groups. Data shown is mean ± S.E.M. (n=3). Statistical analysis was performed by ordinary one-way ANOVA and Dunnett's multiple comparisons test. *p < 0.05, **p<0.01, ***p<0.001

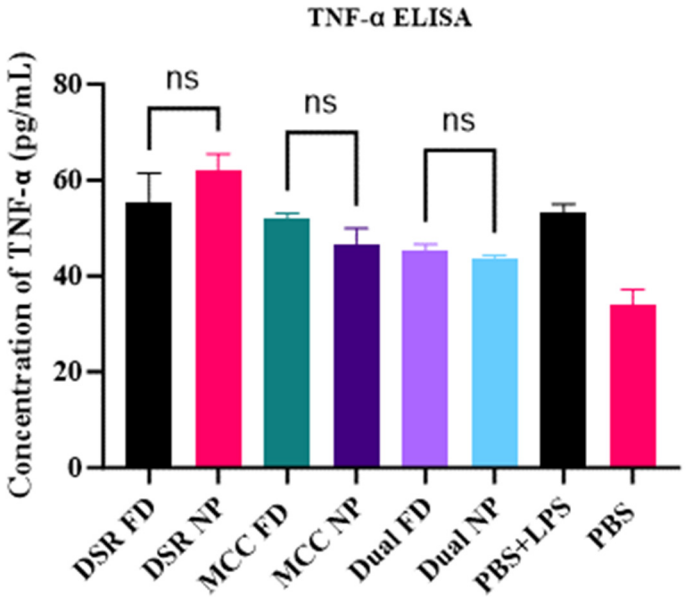

Figure-S3. Serum TNF-α levels after single and dual drug nanoparticle treatment in sepsis mice model
Graph plots serum TNF-α levels in the different treatment groups. Data shown is mean ± S.E.M. (n=3). Statistical analysis was performed by ordinary one-way ANOVA and Dunnett's multiple comparisons test. *p < 0.05, **p<0.01, ***p<0.001

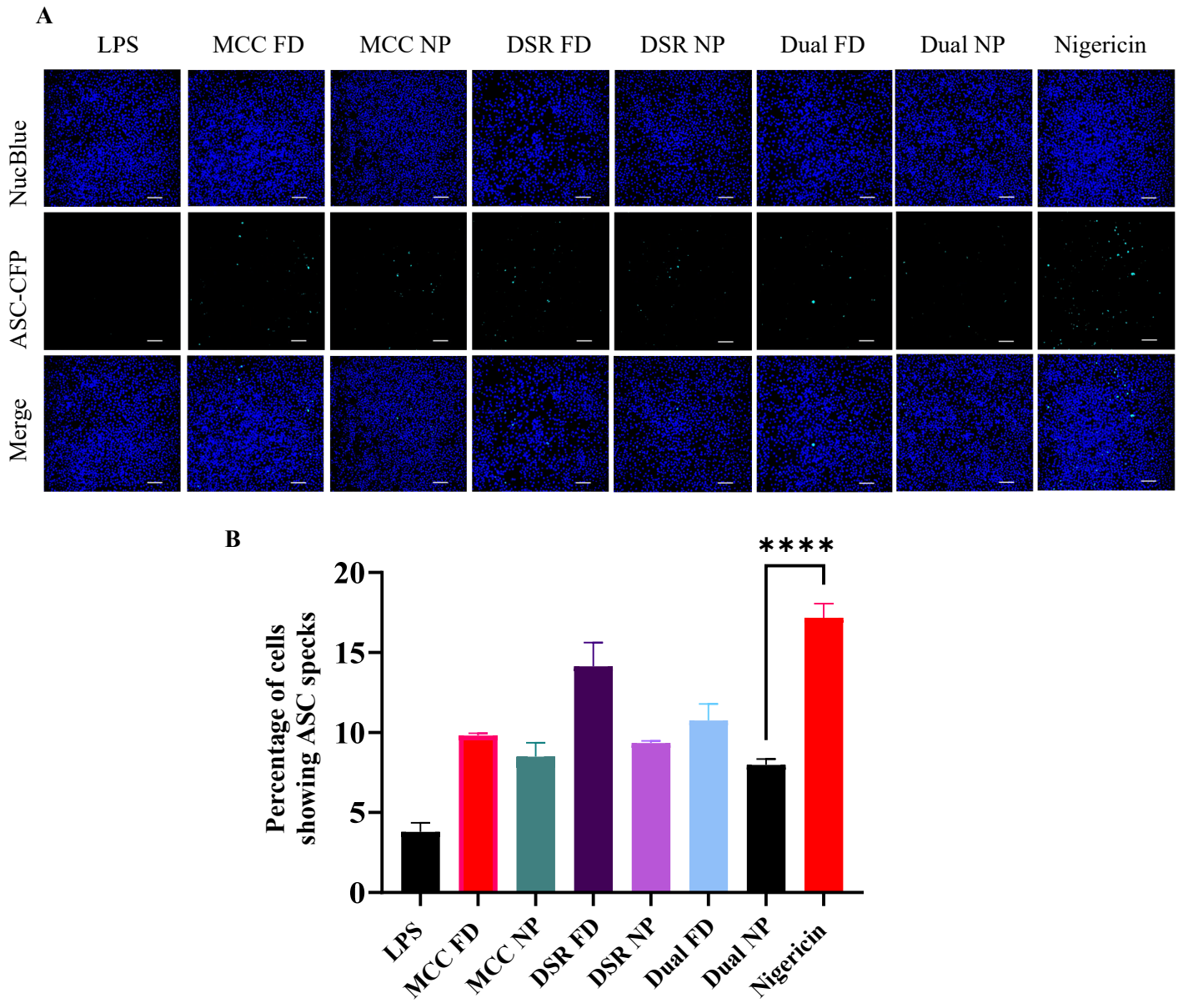

Figure S4. ASC specks formation in iBMDMs treated with indicated groups. (**A**) Representative fluorescence microscopy imaging of LPS-primed and NP/FD treated ASC-CFP expressing iBMDMs stained with NucBlue. Blue fluorescence correlates with stained nuclei by NucBlue. Cyan fluorescence correlates with the expression of ASC, where small dots in the sample indicate the formation of ASC specks in the inflammasome complex. Scale bar- 100 µm. (**B**) Quantification of ASC specks after free drug and NP incubation normalized by the total number of live cells counted by the NucBlue signal. Data shown are ±S.E.M. ($n = 3$). Statistical analysis was performed with one-way ANOVA followed by Tukey post-test. *$p < 0.05$.

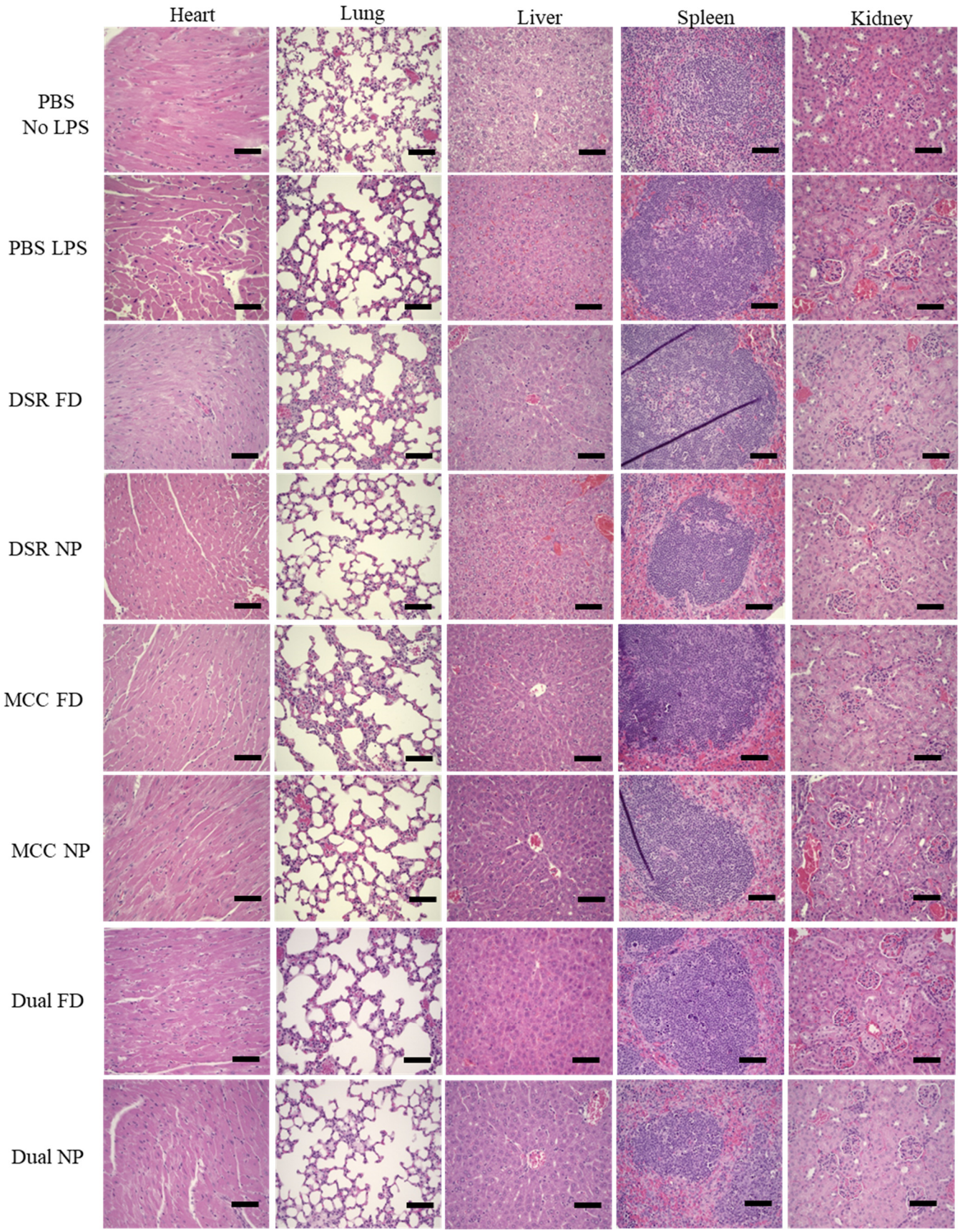

Figure-S5. Biosafety analysis of indicated treatment groups in different organs via H&E. Hematoxylin and Eosin (H&E) stains from heart, lung, liver, spleen, and kidney sections of sepsis induced mice treated with different nanoparticles and free drug. Control groups include wild type mice and sepsis induced mice injected with PBS, labelled as PBS No LPS and PBS LPS respectively. Scale bar - 100μm.

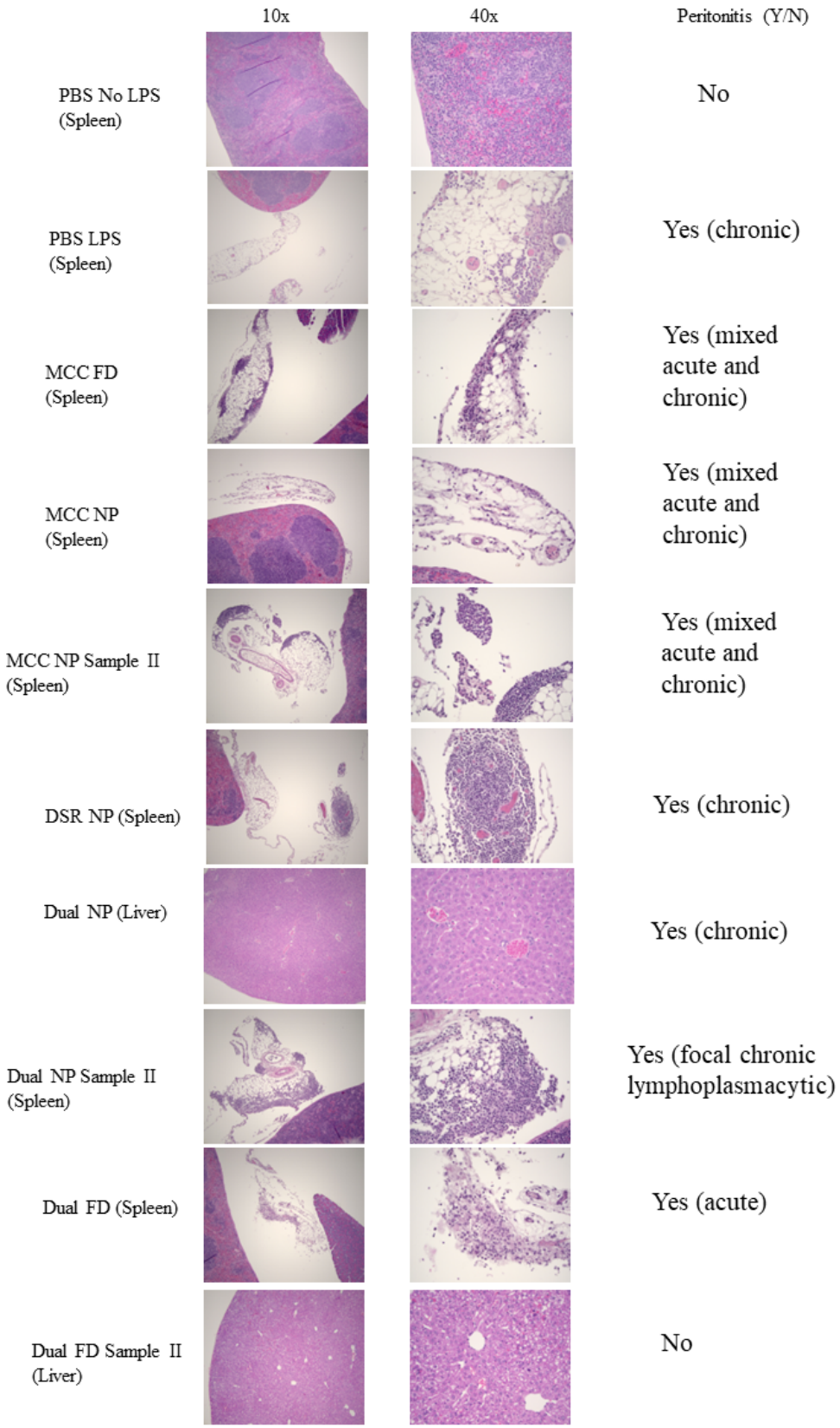

Figure-S6. H&E staining of peritoneal linings taken from excised spleen or liver tissue from sepsis induced mice treated with various treatment groups.

Hematoxylin and Eosin (H&E) stains from the splenic or liver peritoneal lining sections of sepsis induced mice treated with different nanoparticles and free drugs. Both 10x and 40x magnification images are shown, as well as the pathological assessment of the sample.

Spleen and Kidney Peritonitis Assessment

A

Slide label	Peritonitis (Y/N)
PBS No LPS	N
PBS LPS	Y (chronic)
DSR FD	Y (chronic)
DSR NP	Y (chronic)
MCC FD	Y (mixed acute and chronic)
MCC NP	Y (mixed acute and chronic)
MCC NP Sample II	Y (mixed acute and chronic)
Dual FD	Y (acute)
Dual FD Sample II	N
Dual NP	N
Dual NP Sample II	Y(chronic lymphoplasmacytic)

Heart, Lung, and Liver Peritonitis Assessment

B

Slide label	Peritonitis (Y/N)
PBS No LPS	N
PBS LPS	N
DSR FD	N
DSR NP	N
MCC FD	N
MCC NP	N
MCC NP Sample II	N
Dual FD	Y (focal acute and chronic)
Dual FD Sample II	N
Dual NP	Y (chronic)
Dual NP Sample II	Y(focal chronic lymphoplasmacytic

Figure-S7. Assessing the presence of peritonitis from the histology stains of septic mice. Hematoxylin and Eosin (H&E) staining was conducted on sepsis induced and treated mouse samples sectioned from the peritoneal lining of (A) the spleen and kidney, the principal organs for assessing peritonitis, as well as from (B) the heart, lung, and liver.