Supporting Information

Protein corona formation on Supramolecular Polymer Nanoparticles causes differential endosomal sorting resulting in an attenuated NLRP3 inflammasome activation

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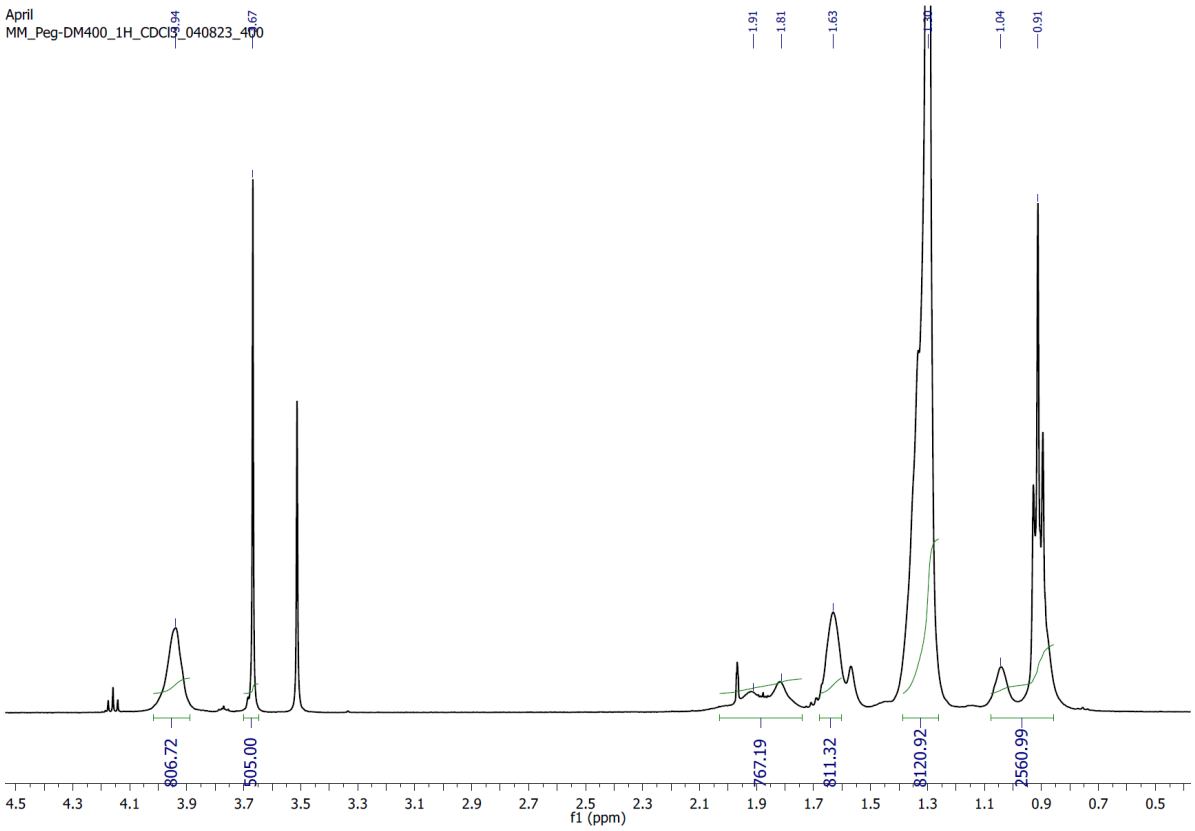


Figure S1 . NMR characterization of COOH-PEG$_{5000}$-b-DM$_{400}$. ^{1}H NMR plot of COOH-PEG$_{5000}$-b-DM$_{400}$ in $CDCl_3$. The spectra are plotted up to 4.50 ppm for simplicity.

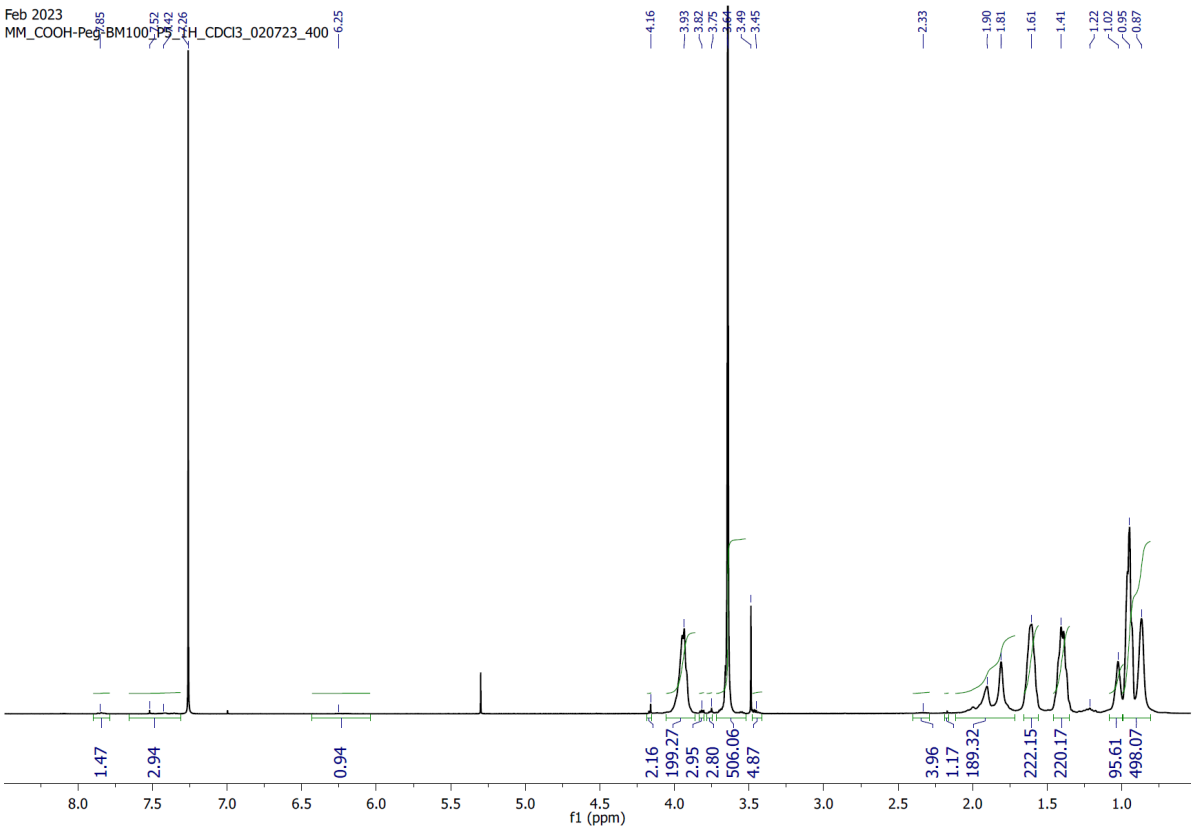


Figure S2. NMR characterization of COOH-PEG$_{5000}$-b-BM$_{100}$. ^{1}H NMR plot of COOH-PEG$_{5000}$-b-BM$_{100}$ in $CDCl_3$. The spectra are plotted up to 8.50 ppm for simplicity. The degree of polymerization (Xn) was determined by comparing the peak intensities from the macro chain transfer agent PEG-CTA to the hydrophobic backbone counterpart in the 1H-NMR analysis. The peak at 3.64 ppm, corresponding to the PEG protons was chosen as the reference peak and given the integration of 505. Accordingly, the peak integration for the ester protons in the methacrylate backbone at 3.90 ppm was calculated.

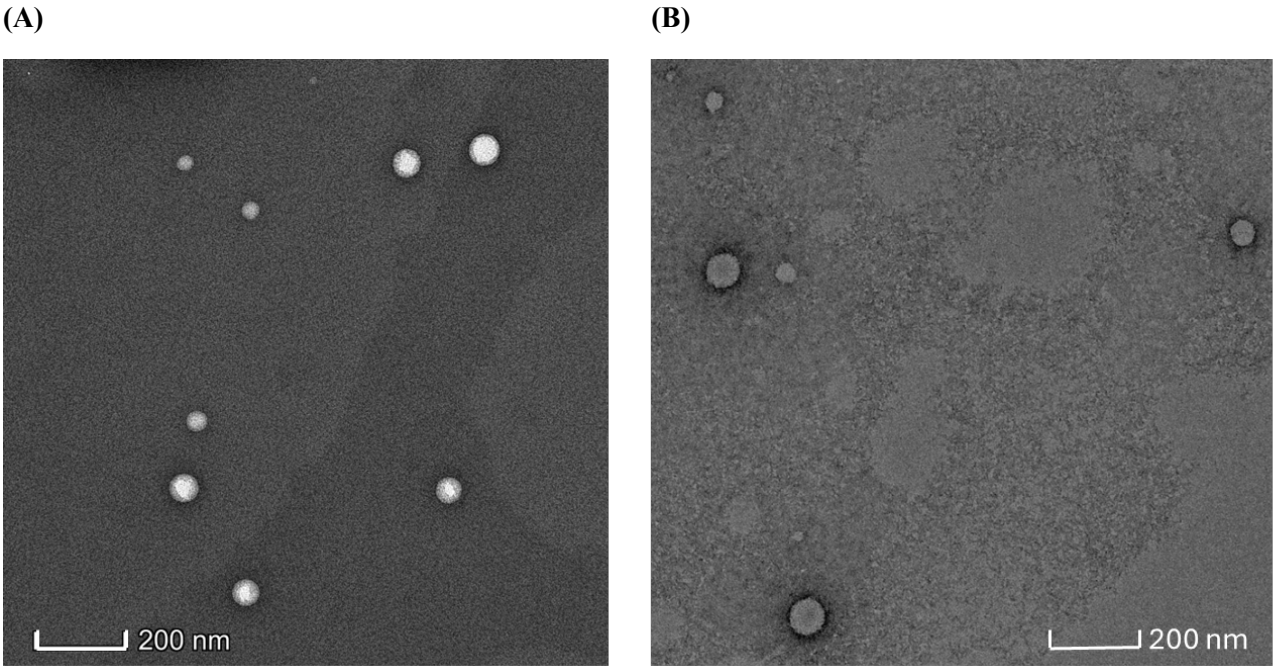
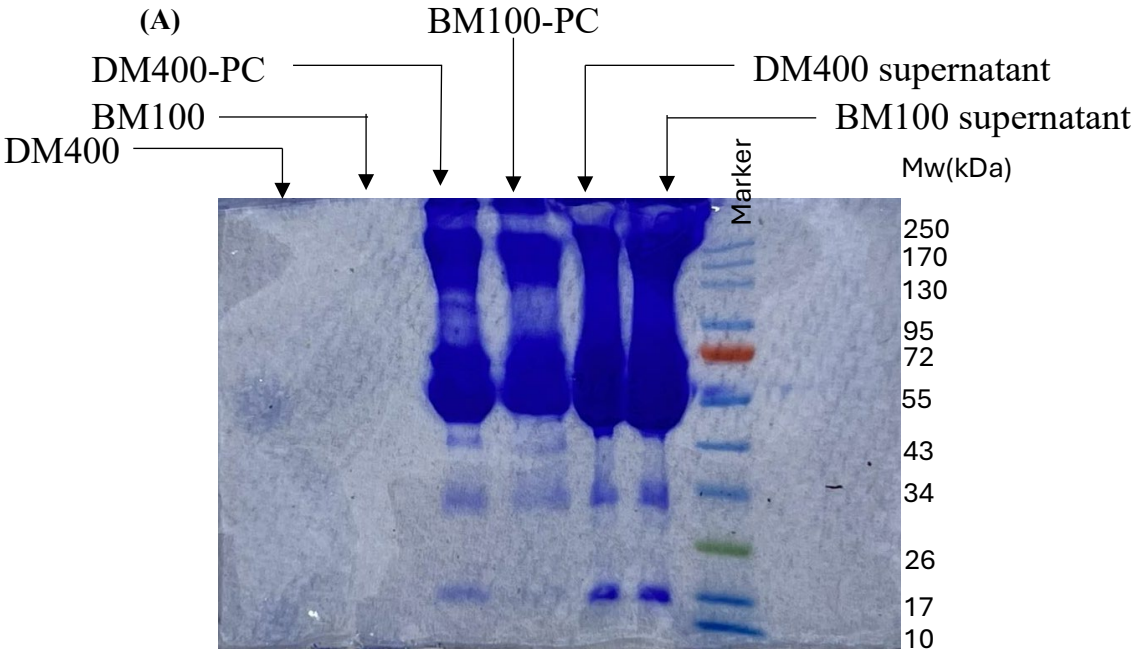


Figure S3. Cryo TEM images of **(A)** DM100 and **(B)** BM100 nanoparticles



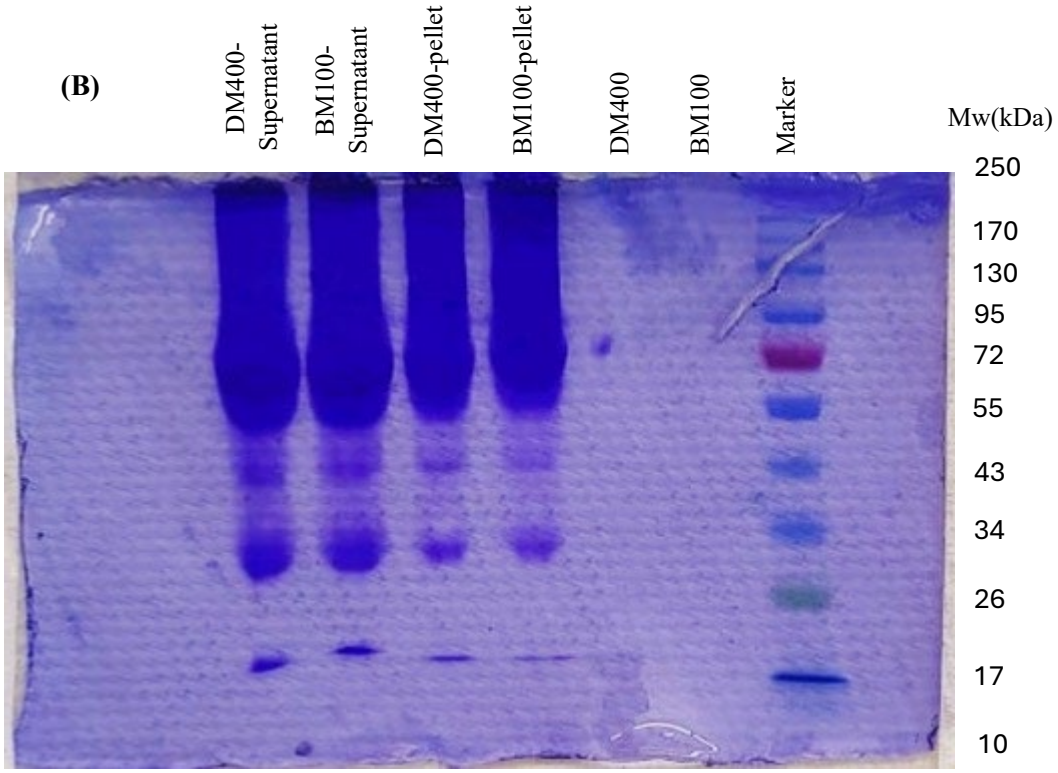


Figure S4. Representative fractions of **(A, B)** hard corona proteins and the proteins in supernatants for DM400 and BM100 using traditional SDS-PAGE method

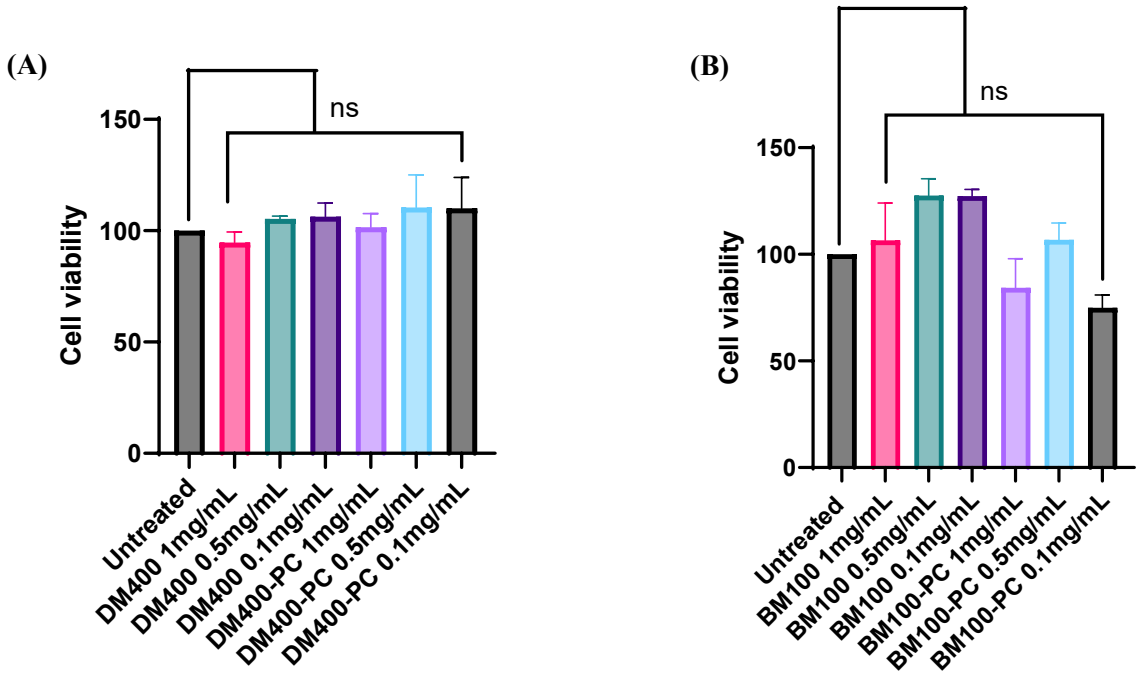


Figure S5. Representative images of cell viability accessed by MTT assay by treating the iBMDMs with **(A)** DM400 and **(B)** BM100 at three different concentrations of 1mg/mL, 0.5 mg/mL and 0.1 mg/mL for 24 hours. Data shown are ±S.E.M. ($n = 3$). Statistical analysis was performed with one-way ANOVA followed by Tukey post-test. ns: not significant, *$p < 0.05$.

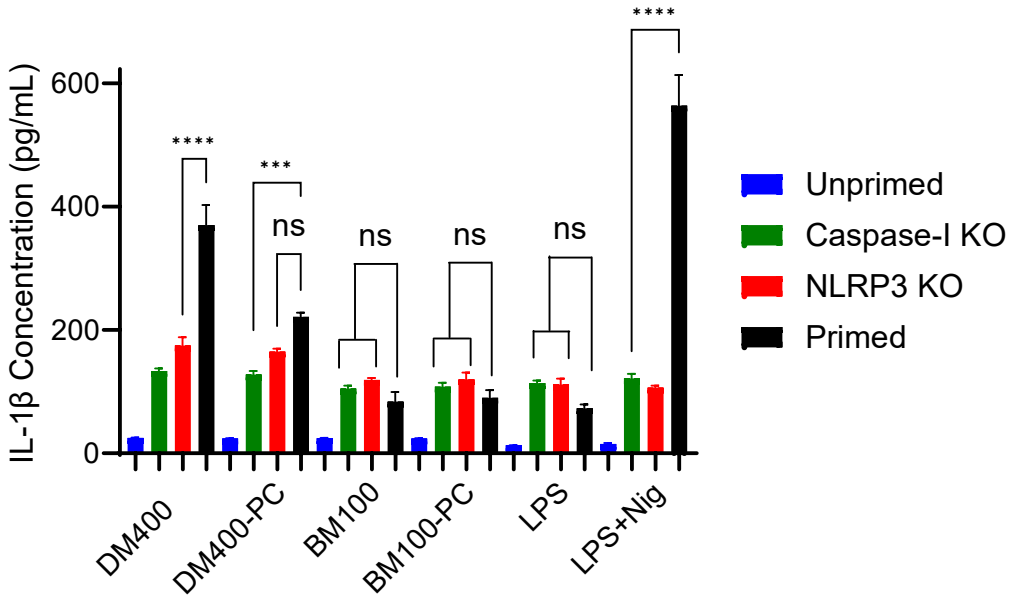


Figure S6. We confirmed the involvement of the NLRP3 inflammasome in IL-1β release by incubating SNPs and their protein corona at a concentration of 0.5 mg/mL with NLRP3 and Caspase-1 Knockout iBMDMs, as well as iBMDMs unprimed with LPS, showing minimal activation. Data shown is ±S.E.M (n = 3). Statistical analysis was performed with one-way ANOVA followed by Tukey post-test. ns – not significant, **$p < 0.01$, ****$p < 0.0001$.

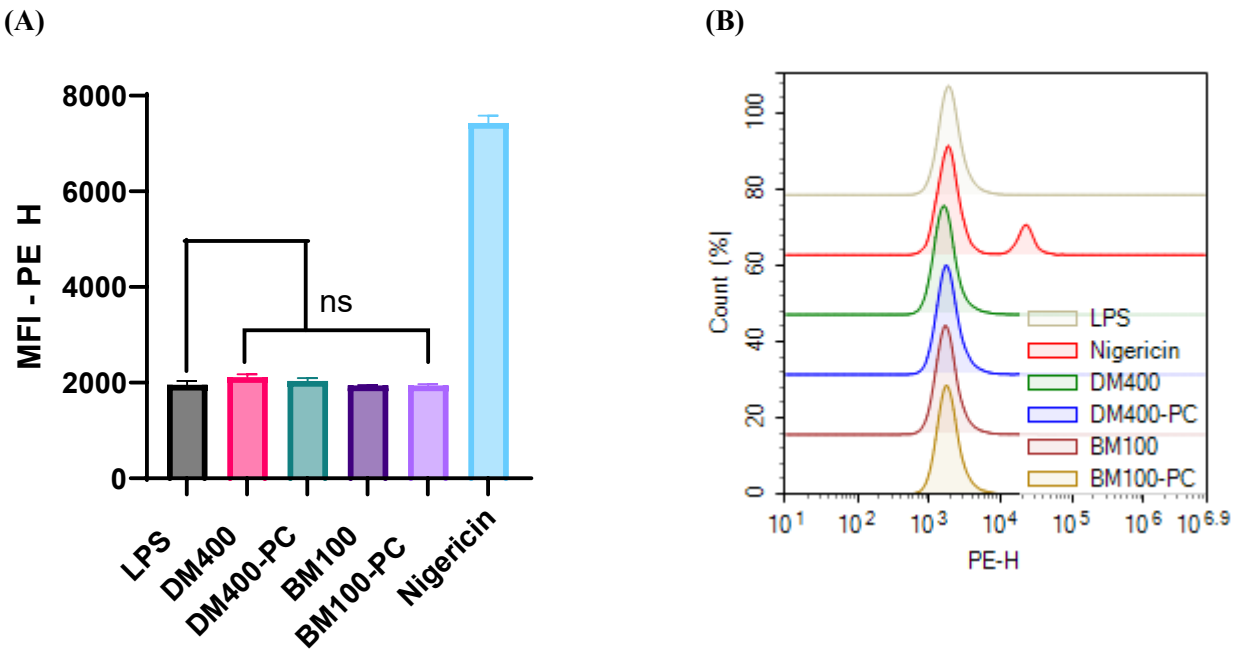


Figure S7. DM400 and BM100 show minimal mitochondrial ROS pathways. **(A)** Representative flow cytometry histogram showing MitoSOX fluorescent shifts in LPS-primed iBMDMs after 0.5 mg/mL of nanoparticle treatment for 4 hours. **(B)** Median fluorescence intensity as determined by flow cytometry. Data shown is ±S.E.M (n = 3). Statistical analysis was performed with one-way ANOVA followed by Tukey post-test. ns – not significant.

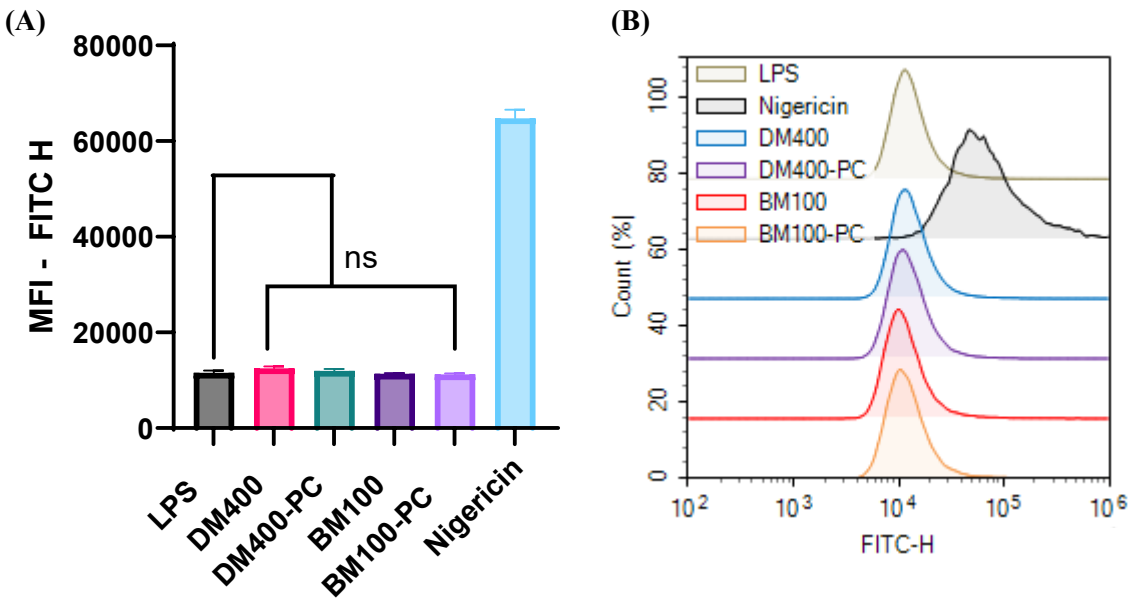


Figure S8. Calcium influx is not the primary method for NLRP3 inflammasome activation. (A) Representative flow cytometry histogram showing Fluo-4 fluorescent shifts in LPS primed iBMDMs after 0.5 mg/mL of nanoparticle treatment for 4 hours. **(B)** Median fluorescence intensity as determined by flow cytometry. Data shown is ±S.E.M (n = 3). Statistical analysis was performed with one-way ANOVA followed by Tukey post-test. ns – not significant.

Rab11

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
DM400 vs. DM400-PC	-57.73	-69.00 to -46.46	Yes	****	<0.0001	A-B		
DM400 vs. BM100	5.428	-6.917 to 17.77	No	ns	0.6568	A-C		
DM400 vs. BM100-PC	-55.07	-66.09 to -44.05	Yes	****	<0.0001	A-D		
DM400-PC vs. BM100	63.16	50.82 to 75.51	Yes	****	<0.0001	B-C		
DM400-PC vs. BM100-PC	2.666	-8.355 to 13.69	No	ns	0.9203	B-D		
BM100 vs. BM100-PC	-60.5	-72.61 to -48.38	Yes	****	<0.0001	C-D		

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
DM400 vs. DM400-PC	30	87.74	-57.73	4.29	21	21	19.03	76
DM400 vs. BM100	30	24.58	5.428	4.699	21	15	1.633	76
DM400 vs. BM100-PC	30	85.07	-55.07	4.196	21	23	18.56	76
DM400-PC vs. BM100	87.74	24.58	63.16	4.699	21	15	19.01	76
DM400-PC vs. BM100-PC	87.74	85.07	2.666	4.196	21	23	0.8985	76
BM100 vs. BM100-PC	24.58	85.07	-60.5	4.613	15	23	18.54	76

LAMP1

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
DM400 vs. DM400-PC	74.11	48.15 to 100.1	Yes	****	<0.0001	A-B		
DM400 vs. BM100	-9.936	-34.76 to 14.88	No	ns	0.7179	A-C		
DM400 vs. BM100-PC	76.03	50.49 to 101.6	Yes	****	<0.0001	A-D		
DM400-PC vs. BM100	-84.05	-110.0 to -58.09	Yes	****	<0.0001	B-C		
DM400-PC vs. BM100-PC	1.919	-24.73 to 28.57	No	ns	0.9976	B-D		
BM100 vs. BM100-PC	85.97	60.43 to 111.5	Yes	****	<0.0001	C-D		

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
DM400 vs. DM400-PC	94.26	20.15	74.11	9.852	19	16	10.64	67
DM400 vs. BM100	94.26	104.2	-9.936	9.42	19	19	1.492	67
DM400 vs. BM100-PC	94.26	18.23	76.03	9.694	19	17	11.09	67
DM400-PC vs. BM100	20.15	104.2	-84.05	9.852	16	19	12.06	67
DM400-PC vs. BM100-PC	20.15	18.23	1.919	10.11	16	17	0.2684	67
BM100 vs. BM100-PC	104.2	18.23	85.97	9.694	19	17	12.54	67

Figure S9. Source Data file for the summary of multiple comparisons using one-way ANOVA followed by Tukey-post test for the colocalization of the SNPs with and without protein corona with Rab11(recycling endosome) and LAMP1(lysosome).

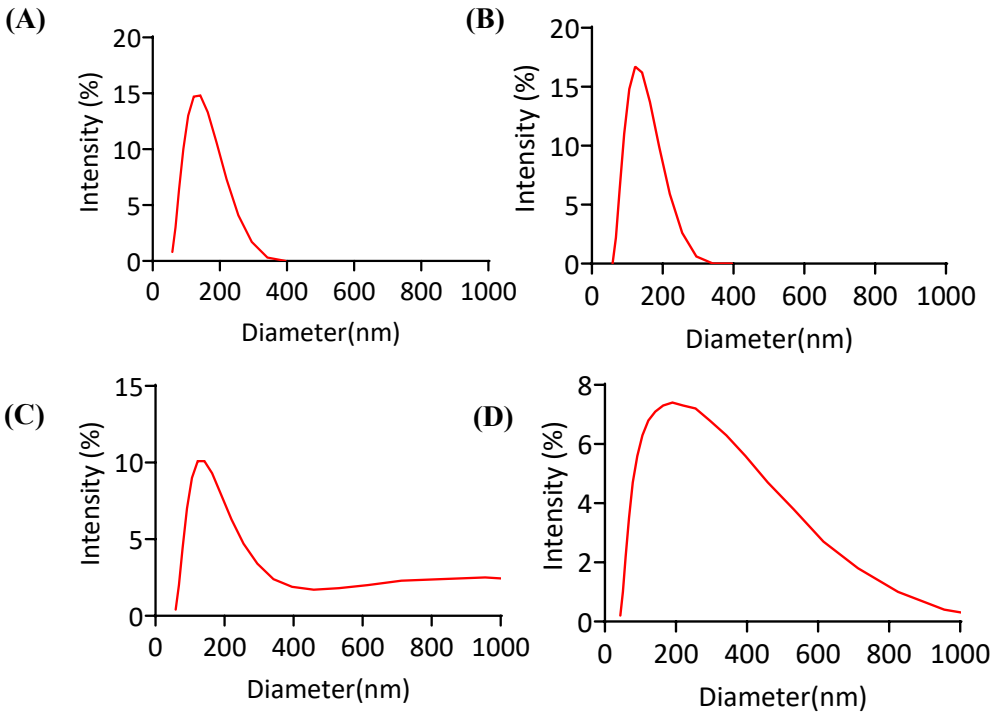


Figure S10. Size distribution of (**A**) DM400 (**B**) BM100 (**C**) DM400 protein corona pellet after ultracentrifugation and washing (**D**) BM100 protein corona pellet after ultracentrifugation and washing measured via dynamic light scattering (concentration = 0.01 mg/mL). Data is represented as mean ± SEM (n = 3).

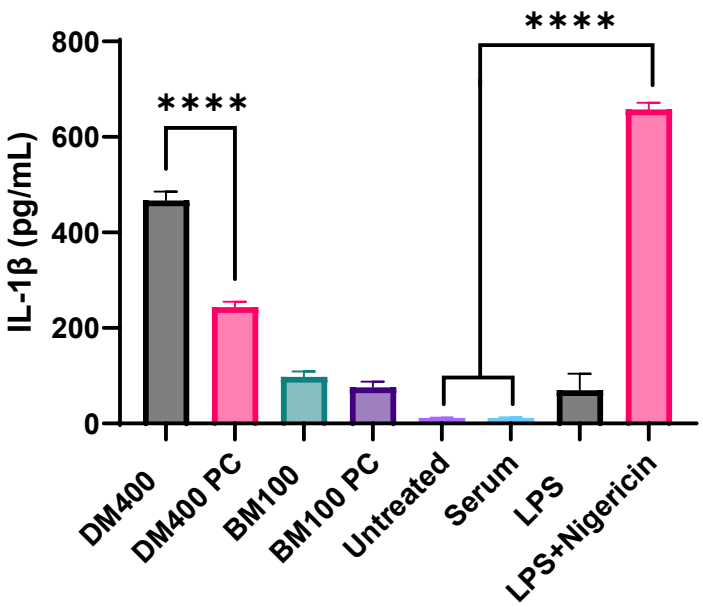


Figure S11 : IL-1β release in the supernatant of LPS primed iBMDMs incubated with DM400, BM100, their corresponding protein corona, LPS only treatment groups, serum proteins for 24 hours, Nigericin for 2 hours and untreated groups quantified by ELISA. Data shown are ±S.E.M. Statistical analysis was performed with one-way ANOVA followed by Tukey post-test. ns: not significant, *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

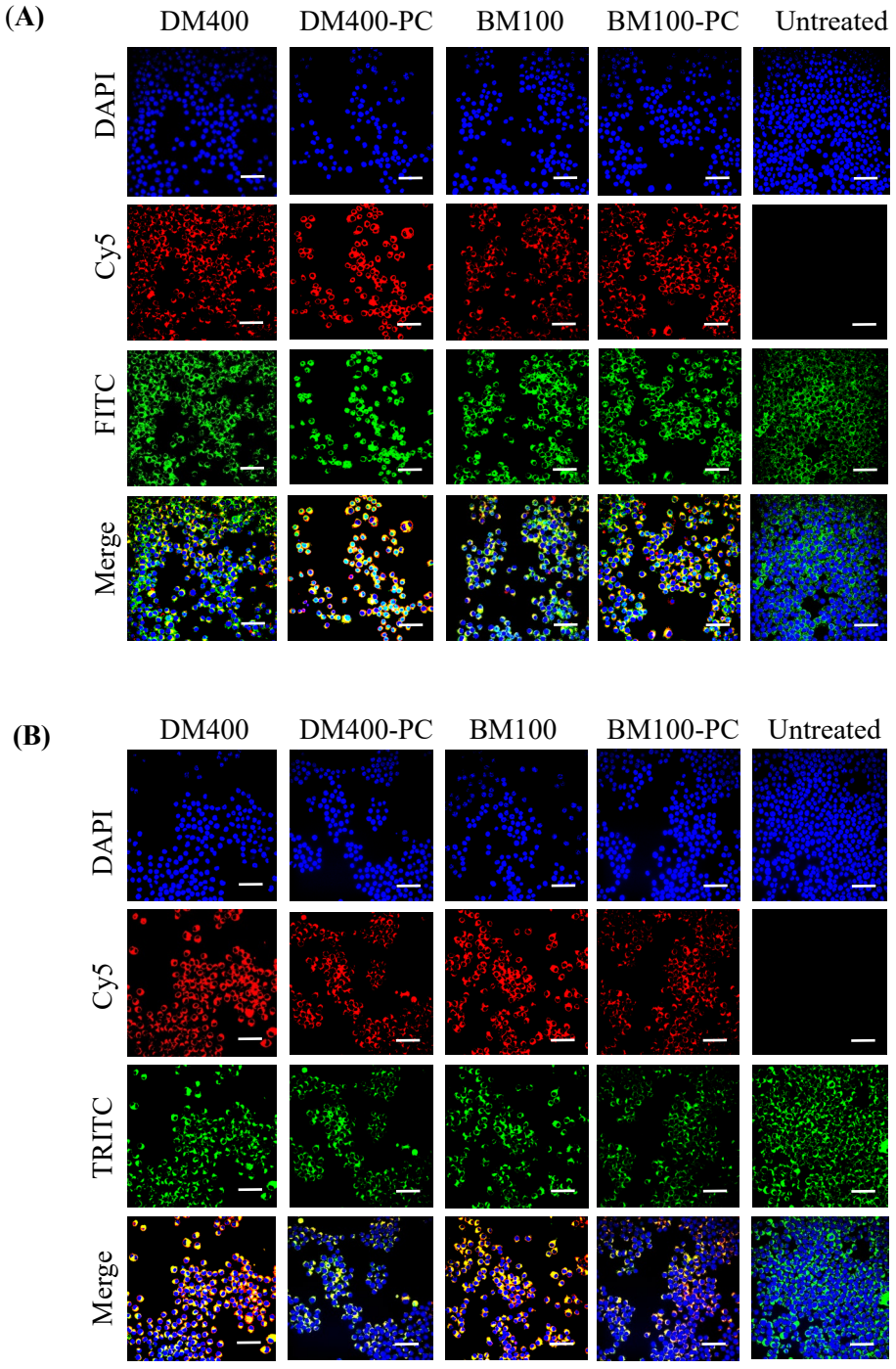


Figure S12 : Additional representative fluorescence microscopy imaging of iBMDMs treated with DiR-encapsulating SNPs for 4 h and stained with **(A)** Rab11 primary and secondary antibody, **(B)** LAMP1 primary and secondary antibody and NucBlue. Blue fluorescence correlates with stained nuclei by NucBlue, green fluorescence correlates with recycling endosomes (FITC) and late endosomes/lysosomes (TRITC), and red fluorescence indicates internalized DiR-SNPs. Scale bar : 50uM. Yellow color in the merged images represent colocalization of nanoparticles with recycling endosomes and late endosomes.

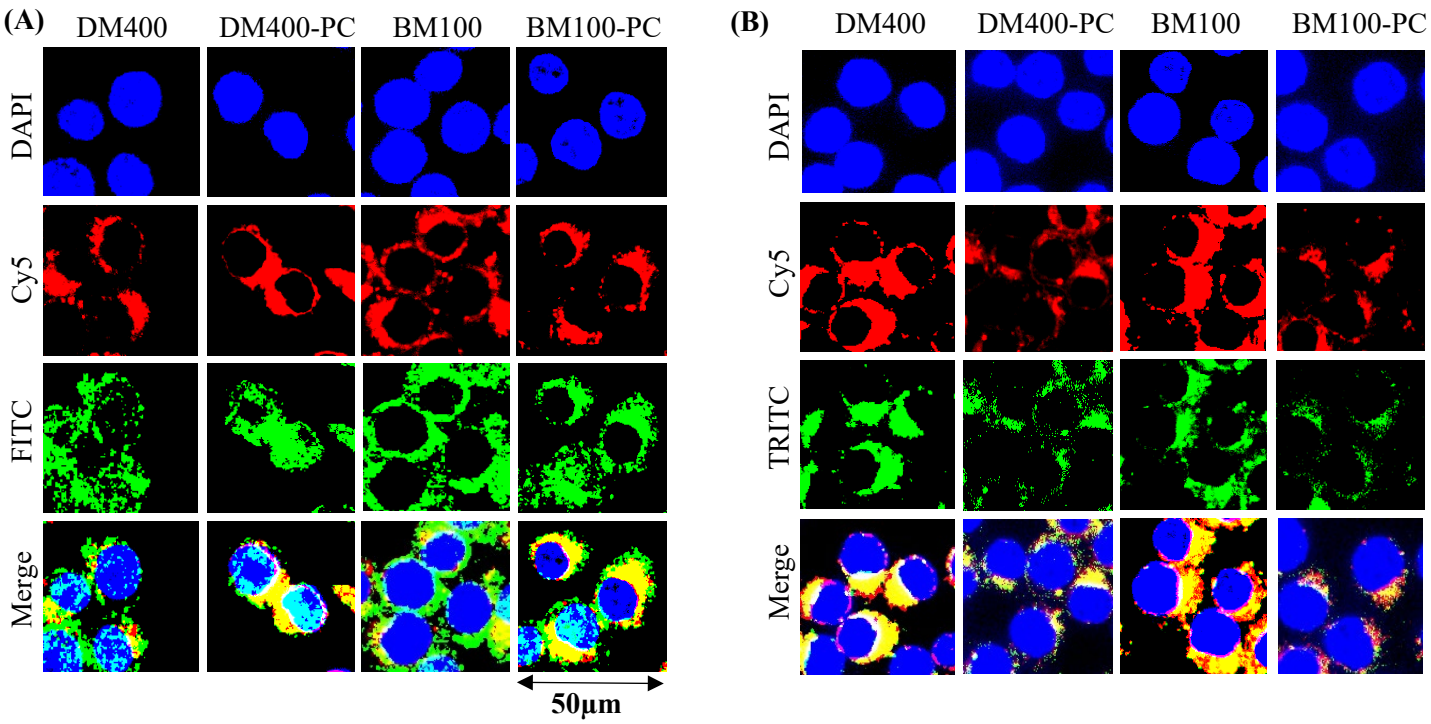


Figure S13 : Magnified representative fluorescence microscopy imaging of iBMDMs treated with DiR-encapsulating SNPs for 4 h and stained with **(A)** Rab11 primary and secondary antibody, **(B)** LAMP1 primary and secondary antibody and NucBlue. Blue fluorescence correlates with stained nuclei by NucBlue, green fluorescence correlates with recycling endosomes (FITC) and late endosomes/lysosomes (TRITC), and red fluorescence indicates internalized DiR-SNPs.