Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Materials

mRNA-Carrying Lipid Nanoparticles that Induce Lysosomal Rupture Activate NLRP3 Inflammasome and Reduce mRNA Transfection Efficiency

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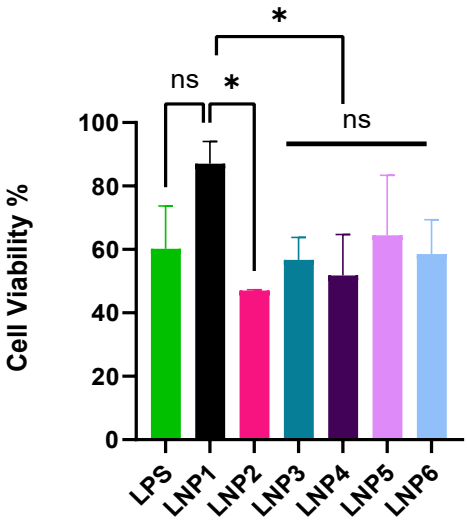
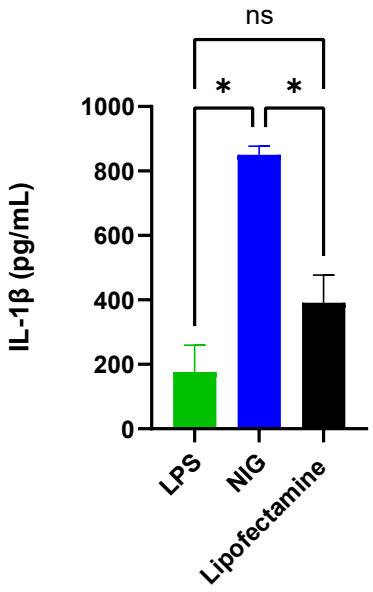
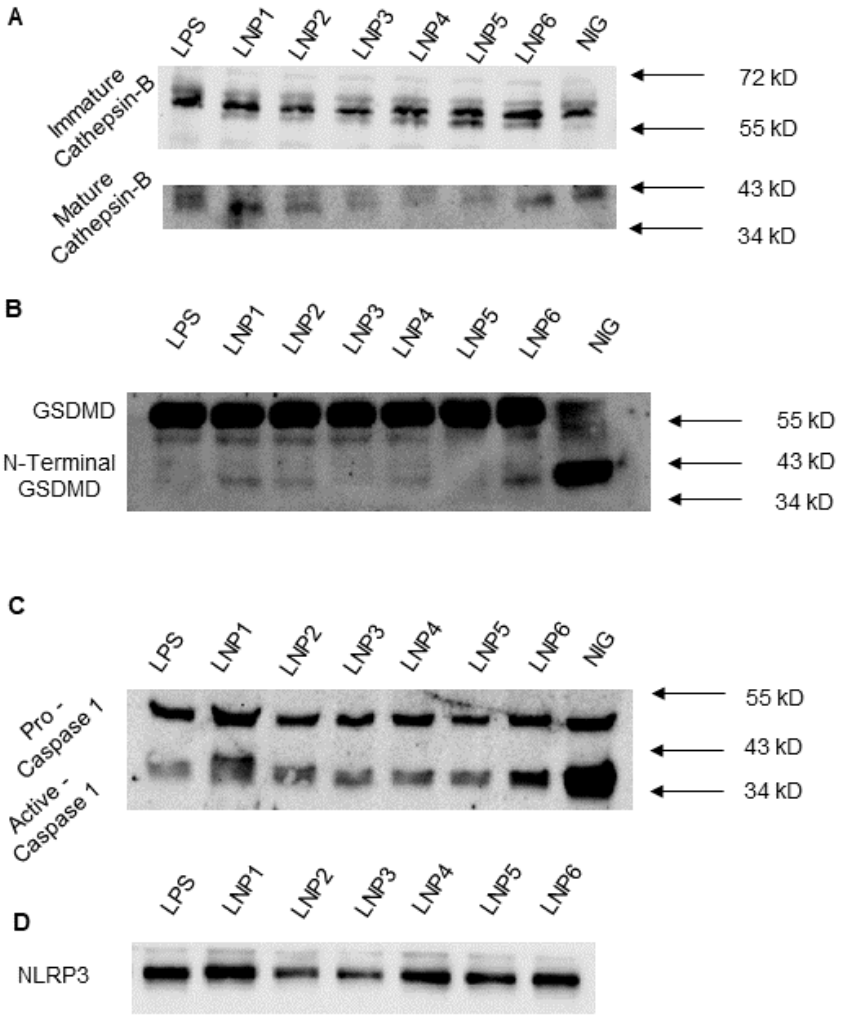


Supplementary Figure S1: Cell death assessed by MTS assay (one step MTT assay): Evaluation of cell death mediated by the panel of LNPs. Graph denotes the percent viability of LPS primed (4 hours) and 100µM LNPs treated (24 hours) iBMDMs using the one-step MTT assay, known as the MTS assay, to provide a correlation between pyroptosis measured in the previous assays. Data shown are mean ± S.E.M (n=3). Statistical significance was determined using two-way ANOVA analysis followed by Tukey's post-test. ns – not significant, *p < 0.05.



Supplementary Figure S2: Assessment of NLRP3 Inflammasome Activation by Lipofectamine MessengerMax Reagent by IL-1β ELISA IL-1β release in the supernatant of LPS primed iBMDMs incubated with 3.75µL/mL of Lipofectamine MessengerMAX reagent, the positive transfection control used in Figure 7. Data shown is mean ± S.E.M (n=3). Statistical significance was determined using one-way ANOVA analysis followed by Tukey's post test post-test. ns – not significant, *p < 0.05.



Supplementary Figure S3: Western Blot images for mature Cathepsin B, active Caspase-1 and active GSDMD protein. (A) Representative Western blot images run on lysate collected from LPS-primed and LNPs treated iBMDMs, showcasing the expression of immature cathepsin-B (top), and the lower-running mature cathepsin-B (bottom). **(B)** Additional representative Western blot image of both inactive (top) and active (bottom) GSDMD. The very high nigericin positive control is shown as to confirm the identity of the band between 43 and 34 kD as cleaved N-terminal GSDMD, which has a theoretical molecular weight of 37 kD. **(C)** Representative Western blot image showing the separation between pro-caspase-1 (top) and active-caspase-1 (bottom). The very high active-caspase-1 expression in the nigericin positive control treatment group is shown to confirm the identity of the lower band as active-caspase-1. **(D)** Additional Western blot showing the expression of NLRP3 in lysates collected from the LPS-primed LNP treated iBMDMs.