Supporting information

A Virus-Inspired RNA Mimicry Approach for Effective Cancer Immunotherapy

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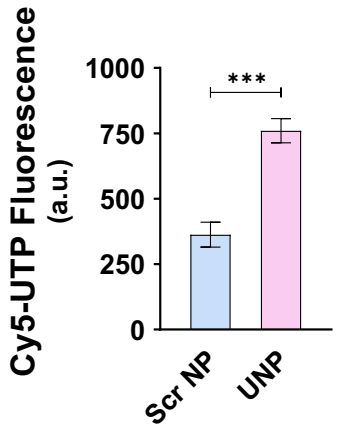

Fig. S1 Cy5-UTP fluorescence measured of scrambled NP and UNP after isolation. The data shown in this figure represent the mean ± SD of three replicate samples for each group. **p<0.001 determined by the Student's t-test. Scr NP stands for scrambled NP

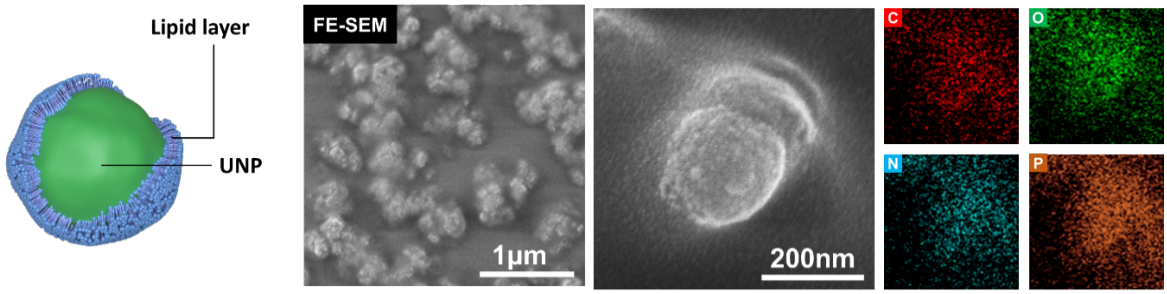

Fig. S2 SEM image and SEM-based EDX-mapping images of lipid coated UNP.

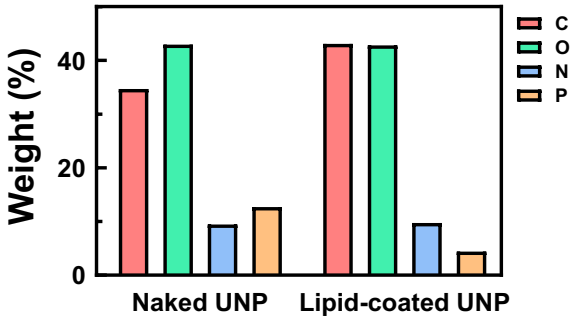

Fig. S3 SEM based EDX elemental results of differentiation in naked UNP and lipid-coated UNP.

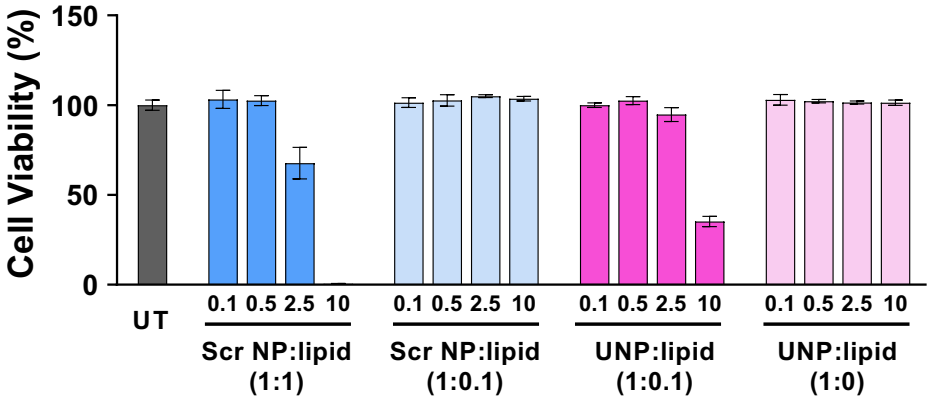

Fig. S4 Cell viability assay of HeLa cells cultured with varying concentrations of Scrambled NP and UNP, based on different lipid ratios (w/w). The data shown in this figure represent the mean ± SD of three replicate samples for each group. Scr NP stands for scrambled NP

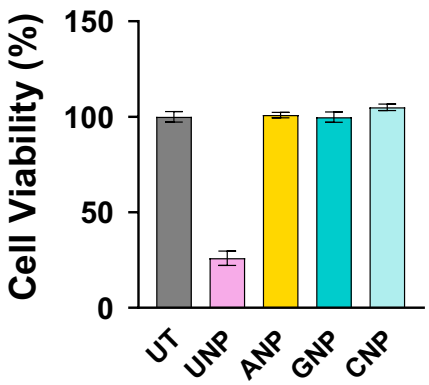

Fig. S5 Cell viability assay of HeLa cells cultured with lipid-coated U, A, G and C-rich nanoparticles at 10 μg/mL concentration. The data shown in this figure represent the mean ± SD of three replicate samples for each group. Scr NP stands for scrambled NP

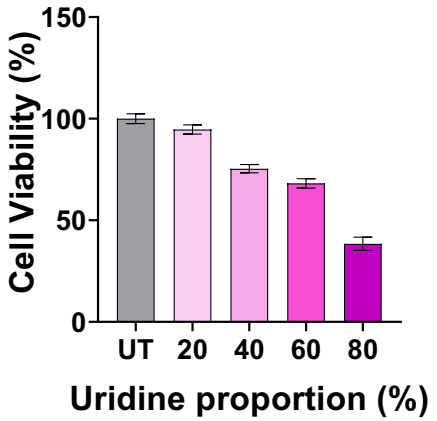

Figure S6. Cell viability assay of HeLa cells cultured with nanoparticles based on uridine proportion in RNA sequence at 10 μg/mL concentration. The data shown in this figure represent the mean ± SD of three replicate samples for each group. Scr NP stands for scrambled NP.

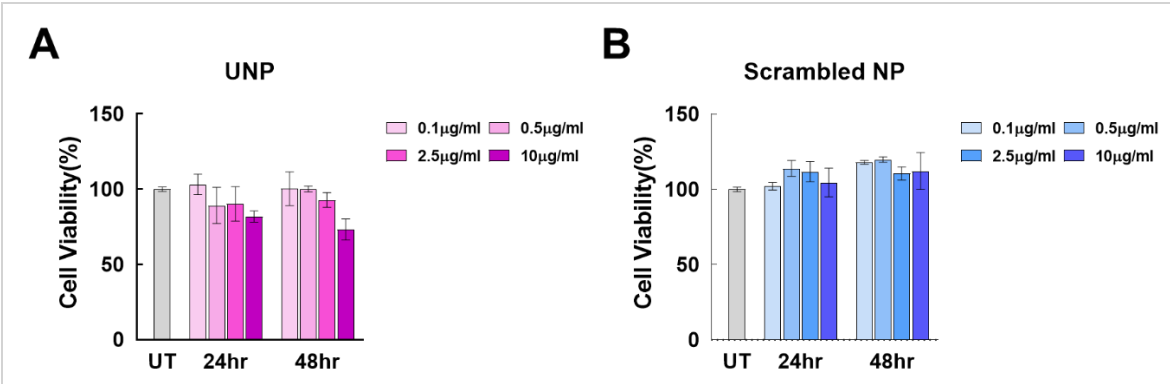

Figure S7. Cell viability assay of HDF cells cultured with (A) UNP and (B) scrambled NP with various concentrations for 24 h, 48 h.

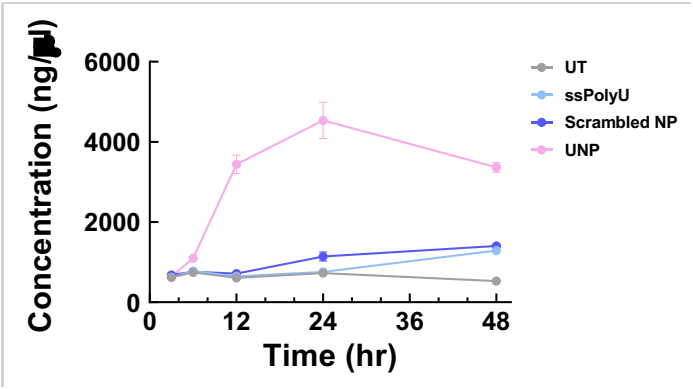

Figure S8. Time dependent IL-6 releases in HeLa cells, cultured with ssPolyU strand, scrambled NP and UNP for various times. IL-6 detection was confirmed by ELISA.

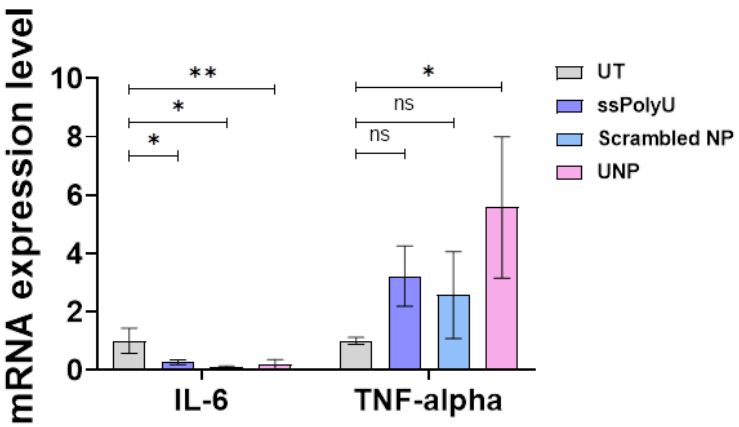

Figure S9. TNF-α and IL-6 mRNA expression of dendritic cells, cultured with cancer cell-conditioned media. TNF-α and IL-6 mRNA level were confirmed by RT-qPCR. The data shown in this figure represent the mean ± SD of three replicate samples for each group. *p<0.1, **p<0.01 determined by the Student's t-test. UT stands for untreated.

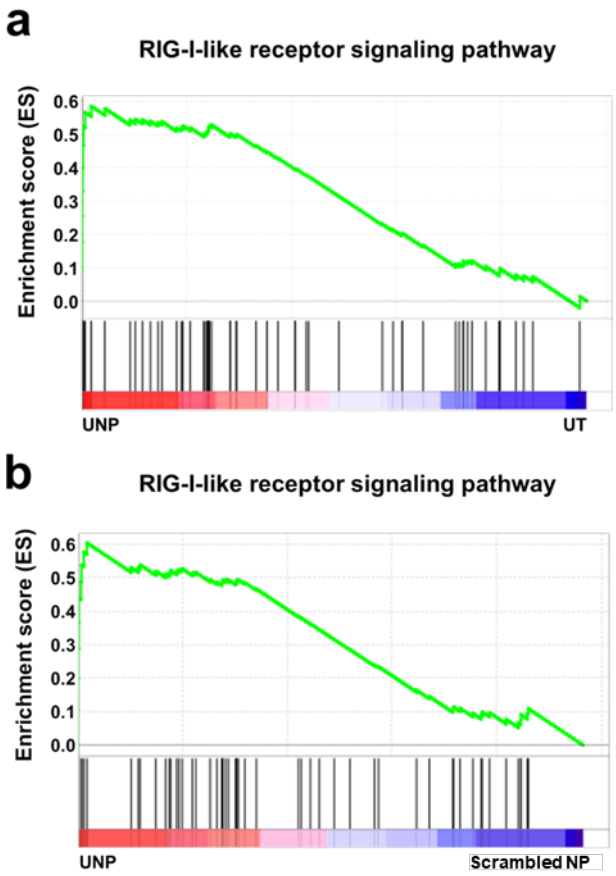

Fig. S10 Gene Set Enrichment Analysis (GSEA) of genes highly expressed in cells treated with UNP compared to (a) untreated cells and (b) cells treated with scrambled NP. The predefined gene set represents the RIG-I like receptor signaling pathway, sourced from the KEGG database.

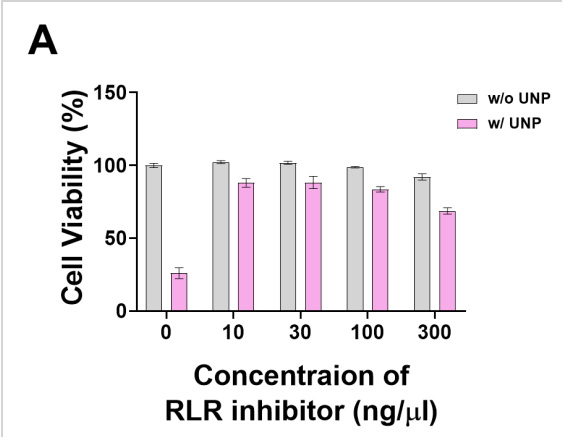

Fig. S11 Cell viability assay of HeLa cells cultured with UNP with 10 ng/μl concentrations for 48 h after treating RLR pathway inhibitor with various concentrations.

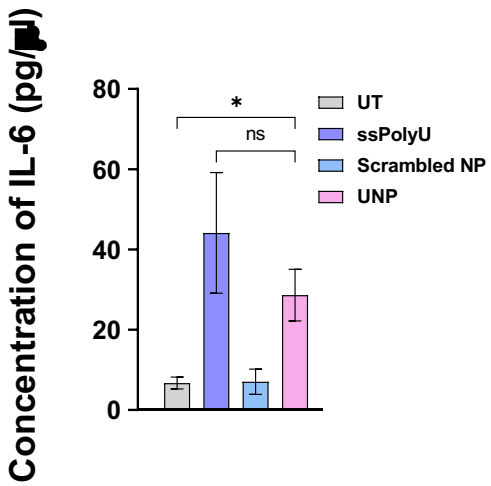

Fig. S12 In vivo cytokine secretion data, injected intravenously with ssPolyU strand, scrambled NP and UNP. IL-6 detection was confirmed by ELISA, 3 hours after injection. The data shown in this figure represent the mean ± SD of three replicate samples for each group. *p<0. 1 determined by the Student's t-test. UT stands for untreated.

DNA strands	Length (nt)	Sequence
T7 promoter	22	5’- TAA TAC GAC TCA CTA TAG GGA T - 3'
Linear DNA for U-rich NP	90	5’- ATA GTG AGT CGT ATT AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA ACA AAA AAA AAA AAA AAA AAA ATC CCT - 3'
Linear DNA for scrambled NP	92	5’- ATA GTG AGT CGT ATT AGG TCA CGA GGG TGG GCC AGG GCA CGG GCA GCT TGC CGG TGG TGC AGA TGA ACT TCA GGG TCA GCT TGC CGA TCC CT - 3'
Linear DNA for A-rich NP	90	5’- ATA GTG AGT CGT ATT ATT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT ATC CCT - 3'
Linear DNA for G-rich NP	90	5’- ATA GTG AGT CGT ATT ACC CCC CCC CCC CCC CCC CCC CCC CCC CCC CTC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCT CCC CCC ATC CCT - 3'
Linear DNA for C-rich NP	90	5’- ATA GTG AGT CGT ATT AGG GGG GGG GGG GGG GGG GGG TGG GGG GGG GGG GGG GGG GGG TGG GGG GGG GGG GGG GGG GGG TGG GGG ATC CCT - 3'
Linear DNA for 40% U-rich NP	90	5’- ATA GTG AGT CGT ATT AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA TTC GGT CCT TCG CTC TCG CTG GTG GTG GGT CGG ATC CCT - 3'
Linear DNA for 60% U-rich NP	90	5’- ATA GTG AGT CGT ATT AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAC AAA AAA CTC TCG CTG GTG GTG ATC CCT - 3'

Table S1. Oligonucleotide sequences for circularization of linear DNA and fabrication of various nanoparticles.