

Supplementary Materials: Investigations on Cellular Uptake Mechanisms and Immunogenicity Profile of Novel Bio-Hybrid Nanovesicles

Yi-Hsuan Ou, Jeremy Liang, Wei Heng Chng, Ram Pravin Kumar Muthuramalingam, Zi Xiu Ng, Choon Keong Lee, Yub Raj Neupane, Jia Ning Nicolette Yau, Sitong Zhang, Charles Kang Liang Lou, Chenyuan Huang ^{6,8}, Jiong-Wei Wang and Giorgia Pastorin

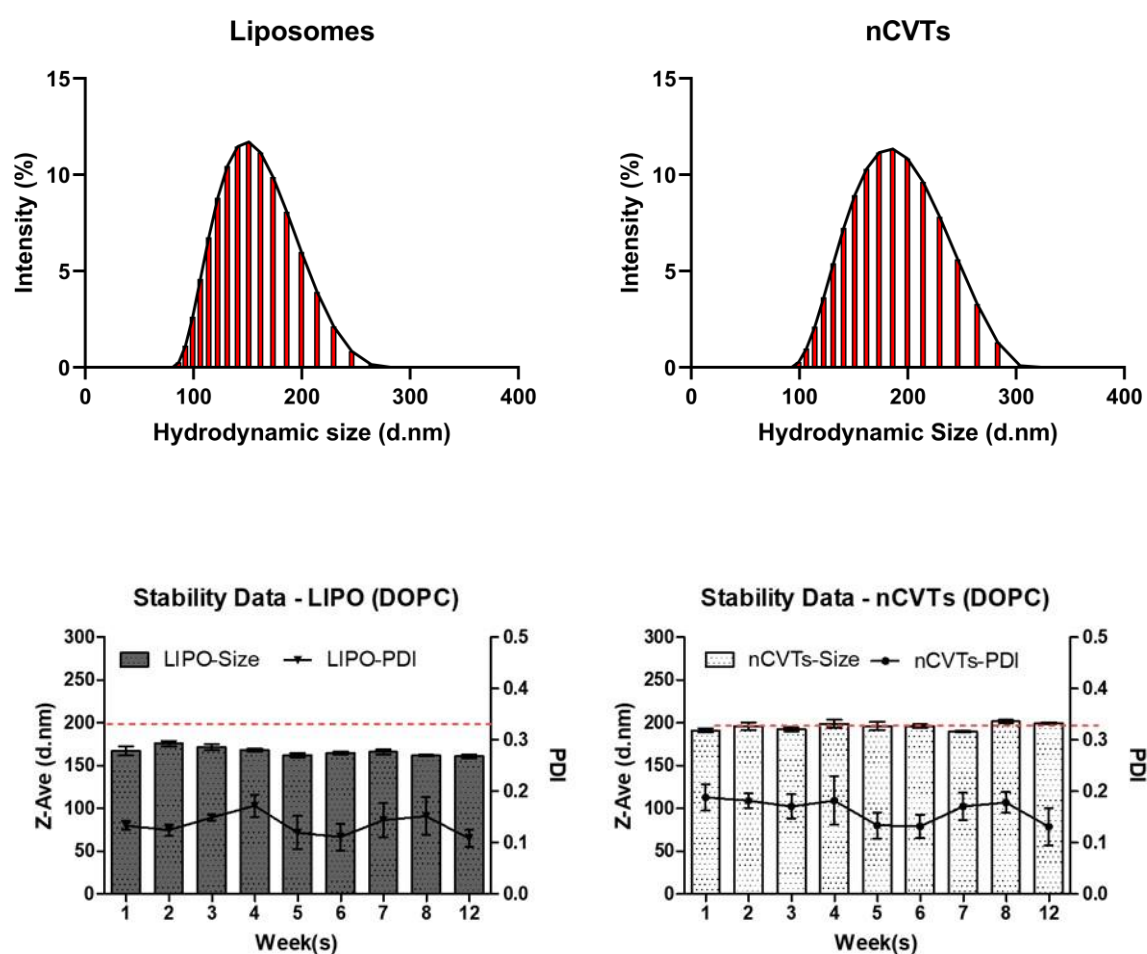


Figure S1: (top) Size distribution of liposomes and nCVTs by density. **(bottom)** The stability of DOPC:Cho (70:30 mol%) formulated nCVTs and Liposomes (LIPO) over a time course of 3 months under that storage condition of 4°C. No significant changes in size and PDI through the course for both liposomes and nCVTs, indicating a good stability (n = 3/group).

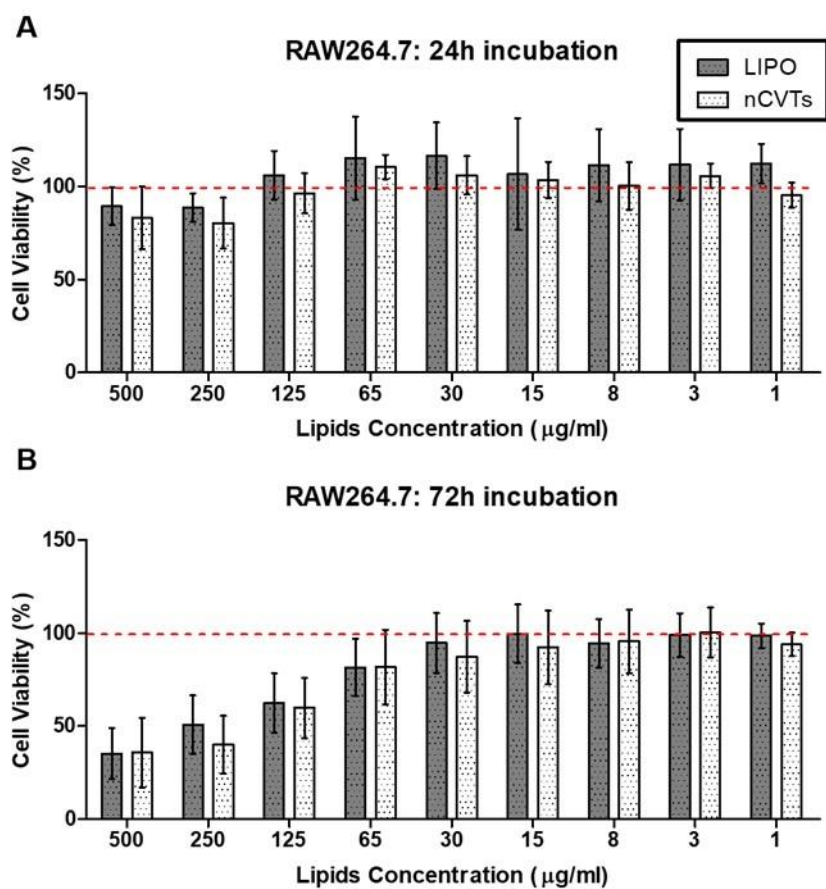


Figure S2A: Cytotoxic effect on immune cells after incubation with the respective amount of nCVTs and Liposomes (LIPO) for (A) 24 hours and (B) 72 hours (n = 3/group).

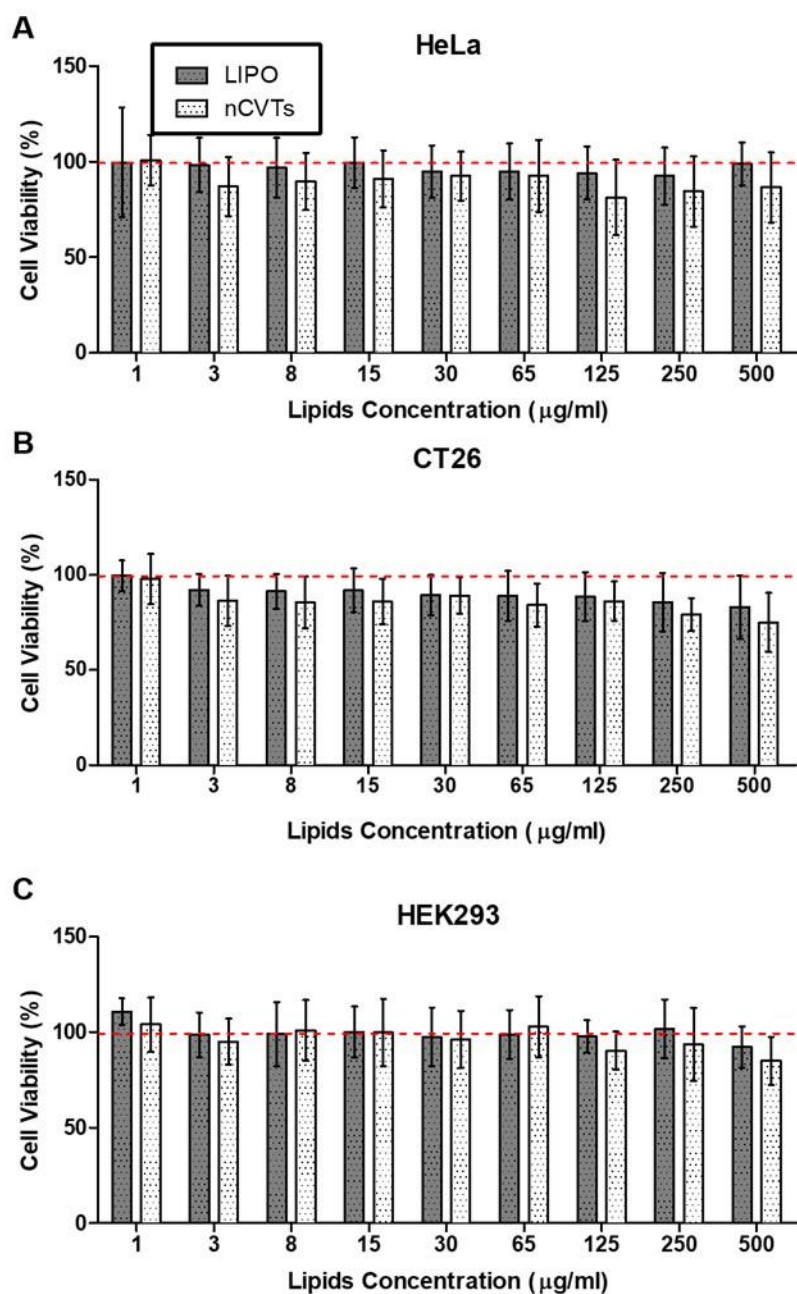


Figure S2B: Effect of Liposomes (LIPO) and nCVTs on cell viability (72 hours). (A) HeLa cells, (B) CT26 cells and (C) HEK293 cells (n = 5/group).

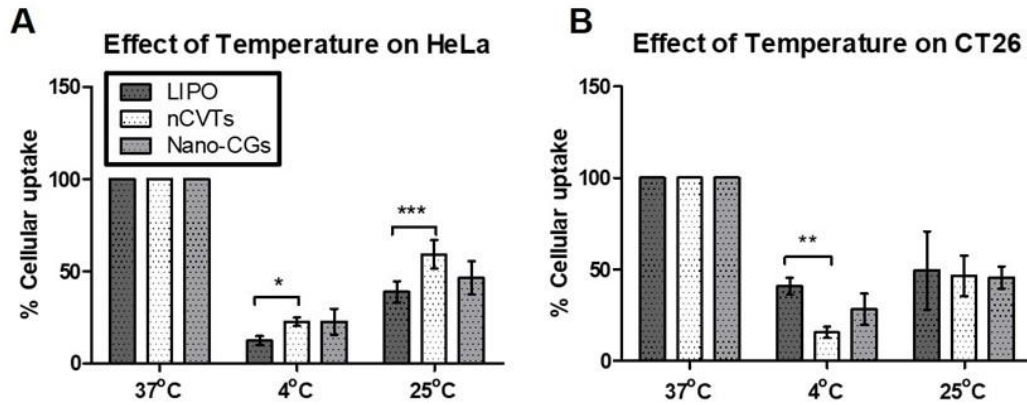


Figure S3: Effect of temperature on cellular uptake of different nanoparticles in (A) HeLa and (B) CT26. (n = 3/group) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S1. Endocytotic Inhibitors and Respective Concentrations. Clathrin-mediated endocytosis (CLME), Caveolae-mediated endocytosis (CVME), and Clathrin-/Caveolae-Independent Endocytosis (CCIE)).

Endocytic Inhibitors	Endocytic Pathway Blocked	Concentrations of Inhibitors tested
Amiloride [18]	Macropinocytosis	250 μ M, 1 mM, 2 mM
Dynasore [19]	Dynamin-dependent Endocytosis (CLME, CVME, CCIE)	8 μ M, 80 μ M, 160 μ M
Methyl- β Cyclodextrin (M β CD) [19]	Lipid raft-dependent Endocytosis (CVME, CCIE)	1 mM, 5 mM, 10 mM
Chlorpromazine [18,19]	CLME	1 μ g/ml, 10 μ g/ml, 20 μ g/ml (in HeLa) 2.5 μ g/ml, 5 μ g/ml, 10 μ g/ml (in CT26)
Genistein [18]	CVME	100 μ M, 200 μ M, 400 μ M (in HeLa) 400 μ M, 700 μ M, 1000 μ M (in CT26)

CCIE: Clathrin- & Caveolae- Independent Endocytosis.

CLME: Clathrin-mediated Endocytosis

CVME: Caveolae-mediated Endocytosis