

## SUPPLEMENT

### **PEGylation of cationic, shell-crosslinked-knedel-like nanoparticles modulates inflammation and enhances cellular uptake in the lung**

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Supplement contents: Methods and Table: 1

#### **Cell culture and endocytosis inhibitor treatments**

*Cell culture.* MLE 12 (ATCC, Manassas, VA), a mouse cell line with features of alveolar type II cells was cultured in media recommended by ATCC.

*Endocytosis inhibitors.* Inhibitors were from Sigma-Aldrich. Phagocytosis was inhibited by latrunculin B (1  $\mu$ M), and macropinocytosis by cytochalasin D (10  $\mu$ M) and nocodazole (33  $\mu$ M). Methyl- $\beta$ -cyclodextran (MbCD, 100  $\mu$ M) was used to disrupt lipid raft-mediated endocytosis by sequestration of cholesterol. Monodansylcadaverine (MDC, 200  $\mu$ M) and chlorpromazine (CPM, 100  $\mu$ M) were used to inhibit clathrin-mediated endocytosis, and dynasore (10  $\mu$ M) to inhibit dynamin-mediated endocytosis. Cells ( $5 \times 10^4$ /well) were pretreated with inhibitors for 30 min, then incubated with nanoparticles (7.5 mg/mL) for 1 h, followed by flow cytometry. cSCK labeled with Alexa Fluor 488 were co-localized in cells using a FACSCalibur flow cytometer with CELLquest software (BD Biosciences).

**Supplementary Table 1: Effect of endocytosis inhibitors on cell uptake of cSCK**

Endocytosis inhibitor	Pathway affected and effect on endocytosis	Percent of nanoparticle cell uptake			
		non-PEG cSCK	cSCK-2PEG	cSCK-5PEG	cSCK-10PEG
Latrunculin B	Phagocytosis: Disrupts microfilaments	112.1 ± 5.2	110.9 ± 1.8	102.4 ± 5.8	101.1 ± 2.2
Nocodazole	Macro-pinocytosis: Depolymerizes microtubule	90.9 ± 19.2	88.6 ± 5.2	94.6 ± 4.6	91.0 ± 4.9
Cytochalasin D	Macro-pinocytosis: Inhibitor of F-actin polymerization	110.0 ± 12.5	110.6 ± 20.3	106.4 ± 7.6	101.3 ± 5.1
Monodansyl-cadaverine (MDC)	Clathrin-mediated pinocytosis: Inhibitor of fibrin stabilization	72.4 ± 7.1*	89.7 ± 22.1	103.1 ± 15.7	89.8 ± 3.3
Chlorpromazine (CPM)	Clathrin-mediated pinocytosis: Inhibitor of AP2 function	70.6 ± 7.9*	88.3 ± 10.1	98.4 ± 9.8	96.6 ± 5.8
Methyl- $\beta$ -cyclodextran (M $\beta$ CD)	Lipid raft - mediated pinocytosis: Cholesterol depletion	92.2 ± 8.7	100.5 ± 9.0	103.2 ± 3.3	99.2 ± 6.4
Dynasore	Dynamin-mediated pinocytosis: Dynamin 1 and 2 GTPase inhibitor	12.6 ± 5.4*	33.1 ± 5.6*	39.2 ± 9.0*	58.0 ± 13.4*

A significant difference in uptake compared to no inhibitor controls is indicated (ANOVA, \* $p < 0.05$ ).