

## SUPPORTING INFORMATION

### Charge-converting nanoemulsions as promising retinal drug and gene delivery systems

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## Gating strategy for flow cytometry

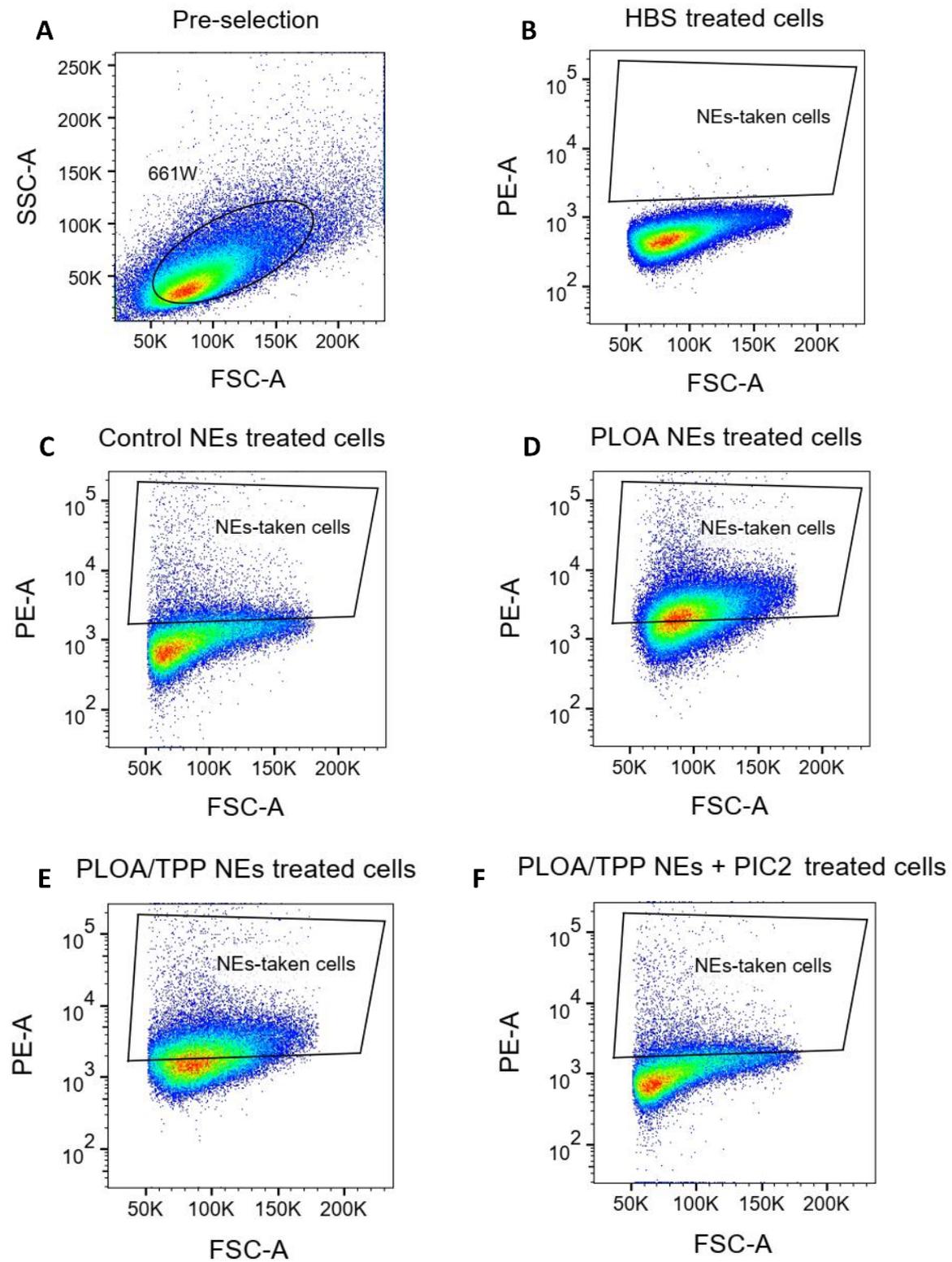


Figure S.1. Gating strategy for flow cytometry. Cellular uptake profile of Lumogen red loaded NEs. FSC-A: forward scatter-area. SSC-A: side scatter-area. PE-A: phycoerythrin-area channel

used as detection parameter for Lumogen red loaded NEs-positive cells. (A) Pre-selection of main cell populations based on FSC-A and SSC-A followed by doublet-elimination based on FSC-Width (not shown). (B) HBS-treated 661W cells served as control and for setting the gate for fluorescence-positive cells. (C) Control NEs-taken 661W cells show only a small percentage of fluorescence-positive cells within the gate. (D) PLOA NEs-taken 661W cells and (E) PLOA/TPP NEs-taken 661W cells show substantial fractions of fluorescence-positive cells within the gate. (F) PLOA/TPP NEs-taken 661W cells in samples incubated with phosphatase inhibitor cocktail 2 show a small number of fluorescence-positive cells within the gate.

## Calculation for transfection study on 661W photoreceptor-like cells

Each well was incubated with 250  $\mu\text{L}$  of either 0.05% nanoemulsion, pDNA:Lipofectamine<sup>®</sup> 2000 liposome solution, or pDNA solution.

Below is the calculation of the amount of pDNA in each 250  $\mu\text{L}$  solution of each type:

### 1. pGFP:DOTAP loaded NE formulation

Molar ratio DOTAP:pGFP ion pair = 2

MW (molecular weight): pGFP = 6100

DOTAP = 698.56

→ DOTAP-pGFP ion-pair  $\sim$  7497.12

Concentration of loaded DOTAP-pGFP in SEDDS preconcentrate = 0.2 %

Amount of pGFP in each 100 mg lipophilic preconcentrate = 0.163 mg

1  $\mu\text{L}$  lipophilic preconcentrate weighs = 0.97 mg

Each well incubated with 250  $\mu\text{L}$  of 0.05% nanoemulsion

This amount of NE contains = 0.125  $\mu\text{L}$  preconcentrate

→ equivalent to: 0.121 mg preconcentrate

→ equivalent to: 196.3 ng pGFP

### 2. pGFP:Lipofectamine<sup>®</sup> 2000 formulation

Concentration of Lipofectamine<sup>®</sup> 2000 reagent = 1  $\mu\text{g}/\mu\text{L}$

Concentration of pGFP solution = 4.30  $\mu\text{g}/\mu\text{L}$

0.3  $\mu\text{L}$  of pGFP solution contains = 1290 ng pGFP

Mix 0.75  $\mu\text{L}$  of Lipofectamine<sup>®</sup> 2000 stock solution and 0.3  $\mu\text{L}$  of pGFP solution in 100  $\mu\text{L}$  of buffer. Incubate for 5 min. Dilute pGFP:Lipofectamine<sup>®</sup> 2000 complex with buffer to the final volume of 1500  $\mu\text{L}$ .

Final Lipofectamine<sup>®</sup> 2000 concentration = 0.05 %

250  $\mu\text{L}$  pGFP:Lipofectamine<sup>®</sup> 2000 solution contains = 215 ng pGFP

### 3. pGFP solution alone

Concentration of pGFP solution = 4.30  $\mu\text{g}/\mu\text{L}$

0.3  $\mu\text{L}$  of 4.3  $\mu\text{g}/\mu\text{L}$  pGFP solution contains = 1.29  $\mu\text{g}$  pGFP

→ equivalent to = 1290 ng pGFP

Mix 0.3  $\mu\text{L}$  of pGFP solution with buffer to the final volume of 1500  $\mu\text{L}$ .

→ 250  $\mu\text{L}$  of pGFP diluted solution contains = 215 ng pGFP