## Supplemental Material for

The Mechanism of Polyplexes Internalization into Cells: Testing the GM1/Caveolin-1-Mediated Lipid Raft Mediated Endocytosis Pathway

Rong Qi, ${ }^{1,2,3,5 \text { * }}$ Douglas G. Mullen, ${ }^{4,5}$ James R. Baker, Jr., ${ }^{5}$ Mark Banaszak Holl ${ }^{3,4,5^{*}}$

Peking University Institute of Cardiovascular Sciences, ${ }^{1}$ Peking University Health Science Center, Peking University, Beijing 100083, China

Key Laboratory of Molecular Cardiovascular Sciences, ${ }^{2}$ Ministry of Education, China Chemistry Department, ${ }^{3}$ Program in Macromolecular Science and Engineering, ${ }^{4}$ and the Michigan Nanoscience Institute for Medicine and Biological Science, ${ }^{5}$ University of Michigan, Ann Arbor Michigan 48109-1055

Fig. S1 CTB colocalization with polyplexes in Cos-7 cells. CTB was labeled by AF-555 (yellow), G5 and G7 were labeled by AF-488 (green), and plasmid luciferase (Luc.) was labeled by Cy5 (red). Cos-7 cells were first transfected by G5 or G7 polyplexes (N/P ratio at 10) for 3 h . The cells were rinsed and then incubated with $1 \mu \mathrm{~g} / \mathrm{mL}$ CTB for 1 h . Cell nuclei were stained by DAPI resulting in blue fluorescence in the images.

Fig. S2 Images of GFP expression 48h after transfection by G7 dendrimers at an N/P ratio of 10 on Cos-7, 293A, C6, HeLa, KB and HepG2 cells. Left: bright field images; Right: Fluorescent images.

Table S1 The percentage of the positive uptake cell populations for CTB, G5 polyplexes and G7 polyplexes in the six different cell lines before and after the treatment of cells with GM1.
(The six cell lines: C6, HepG2, HeLa, KB, Cos-7 and 293A)

Fig. S1


Fig. S2


Table S1

|  | CTB | $\begin{gathered} \text { GM1 } \\ + \text { CTB } \end{gathered}$ | G5pp | $\begin{gathered} \text { GM1 } \\ + \text { G5pp } \end{gathered}$ | G7pp | $\begin{gathered} \text { GM1 } \\ + \text { G7pp } \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| C6 | $\begin{gathered} 8 \% \\ \pm 1 \% \end{gathered}$ | $\begin{gathered} 100 \% \\ \pm 1 \% \end{gathered}$ | $\begin{gathered} 100 \% \\ \pm 1 \% \end{gathered}$ | $\begin{gathered} 100 \% \\ \pm 1 \% \end{gathered}$ | $\begin{gathered} 100 \% \\ \pm 1 \% \end{gathered}$ | $\begin{gathered} 100 \% \\ \pm 1 \% \end{gathered}$ |
| HepG2 | $\begin{aligned} & 12 \% \\ & \pm 3 \% \end{aligned}$ | $\begin{aligned} & 87 \% \\ & \pm 6 \% \end{aligned}$ | $\begin{aligned} & 55 \% \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & 59 \% \\ & \pm 1 \% \end{aligned}$ | $\begin{array}{r} 60 \% \\ \pm 1 \% \end{array}$ | $\begin{aligned} & \mathbf{6 5 \%} \\ & \pm 1 \% \end{aligned}$ |
| HeLa | $\begin{aligned} & 74 \% \\ & \pm 2 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 8 \%} \\ & \pm 3 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 9 \%} \% \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 4 \%} \\ & \pm 2 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 9 \%} \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 6 \%} \\ & \pm 2 \% \end{aligned}$ |
| KB | $\begin{aligned} & \mathbf{3 7 \%} \\ & \pm 7 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 6 \%} \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & 69 \% \\ & \pm 3 \% \end{aligned}$ | $\begin{aligned} & 72 \% \\ & \pm 2 \% \end{aligned}$ | $\begin{aligned} & \mathbf{7 2 \%} \\ & \pm 2 \% \end{aligned}$ | $\begin{aligned} & \mathbf{7 2 \%} \\ & \pm 1 \% \end{aligned}$ |
| Cos-7 | $\begin{gathered} 100 \% \\ \pm 1 \% \end{gathered}$ | $\begin{aligned} & 99 \% \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 8 \%} \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & 98 \% \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 1 \%} \\ & \pm 3 \% \end{aligned}$ | $\begin{aligned} & 87 \% \\ & \pm 2 \% \end{aligned}$ |
| 293A | $\begin{gathered} 100 \% \\ \pm 1 \% \end{gathered}$ | $\begin{aligned} & \mathbf{9 9 \%} \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 4 \%} \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 8 \%} \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & \mathbf{9 3 \%} \\ & \pm 1 \% \end{aligned}$ | $\begin{aligned} & 99 \% \\ & \pm 1 \% \end{aligned}$ |

