Supplementary Information

Efficient protection and transfection of siRNA by cationic shell-crosslinked knedel-like nanoparticles (cSCKs)

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Table S1. LC_{50} for $PAEA_{128}$-*b*-PS_{40} cSCKs, cM and Lipofectamine 2000 with control and lethal siRNAs.

Transfection agent	LC50 control siRNA (μg/mL)	Hill slope coefficient	LC50 lethal siRNA (μg/mL)	Hill slope coefficient
cSCK-pa_{100}	11.1 ± 0.1	6.3 ± 0.2	3.4 ± 0.1	5.2 ± 0.5
cSCK-$pa_{75}ta_{25}$	13.4 ± 0.6	3.4 ± 0.5	5.5 ± 0.2	5.4 ± 0.5
cSCK- $pa_{50}ta_{50}$	22.4 ± 1.5	1.8 ± 0.3	8.3 ± 0.6	2.6 ± 0.4
cSCK- $pa_{25}ta_{75}$	30.6 ± 1.3	2.6 ± 0.4	12.2 ± 0.7	2.7 ± 0.3
cM-ta_{100}	39.1 ± 1.9	1.9 ± 0.2	21.5 ± 1.0	3.1 ± 0.5
Lipofectamine 2000	11.1 ± 0.3	2.7 ± 0.2	4.1 ± 0.5	1.8 ± 0.4

Table S2. LC_{50} for ($PAEA_{160}$-b-PS_{30}) cSCKs, cM and Lipofectamine 2000 with control and lethal siRNAs in HeLa cells.

		LC50 control siRNA (μg/ml)	Hill slope coefficient	LC50 lethal siRNA (μg/ml)	Hill slope coefficient
HeLa	cSCK	16.9 ± 0.8	3.2 ± 0.8	3.9 ± 0.2	2.6 ± 0.3
293T	cSCK	23.6 ± 2.3	2.3 ± 0.2	7.5 ± 0.8	1.3 ± 0.2
	Lipofectamine 2000	10.6 ± 0.5	1.2 ± 0.1	3.2 ± 0.7	0.7 ± 0.1
HEK	cSCK	10.2 ± 0.3	3.3 ± 0.3	6.2 ± 0.2	2.4 ± 0.2
	Lipofectamine 2000	7.4 ± 0.3	3.1 ± 0.4	5.0 ± 0.3	2.2 ± 0.2
BEAS-2B	cSCK	13.6 ± 2.3	1.4 ± 0.4	7.2 ± 1.6	0.9 ± 0.2
	Lipofectamine 2000	6.0 ± 0.7	0.6 ± 0.1	1.2 ± 0.6	0.6 ± 0.2
MCF-10A	cSCK	13.8 ± 0.6	5.5 ± 1.1	12.1 ± 0.3	4.6 ± 0.5
	Lipofectamine 2000	5.7 ± 0.2	1.9 ± 0.1	3.7 ± 0.3	1.6 ± 0.2

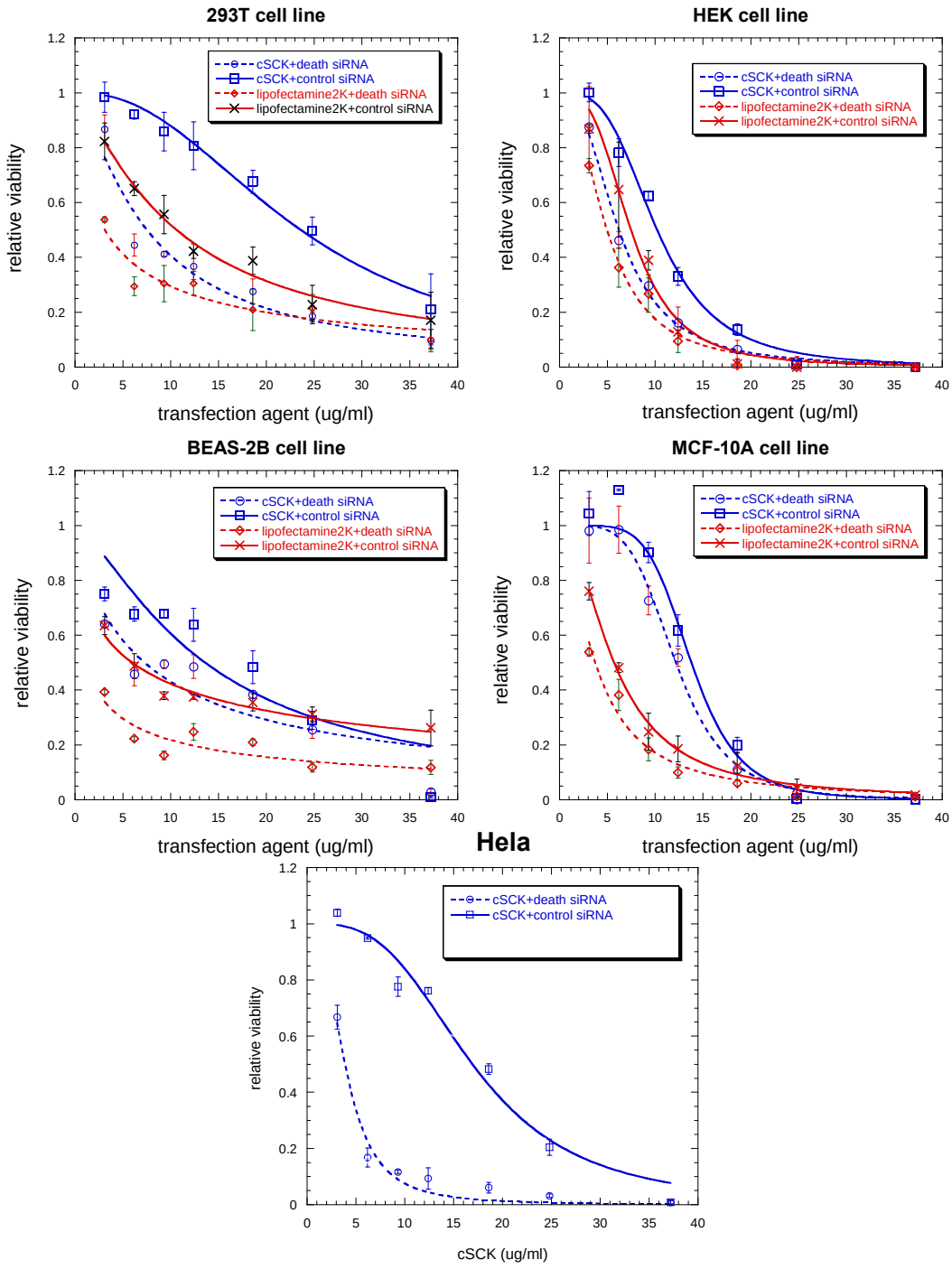


Figure S1. Cytotoxicity assay for siRNA silencing efficiency in different mammalian cell lines. Cells were transfected with 100 nM AllStars Hs Cell Death Control siRNA or ALLStars Negative Control siRNA in the presence of for ($PAEA_{160}$-b-PS_{30}) cSCK at different N/P ratios, or Lipofectamine 2000 at the same µg/mL as for cSCK-pa_{100}. After 48 h, the relative cell viability was quantified using CellTiter-Glo® Luminescent Cell Viability Assay. The concentration of the cSCKs for N/P=1 was: cSCK-pa_{100} 0.62 µg/mL.

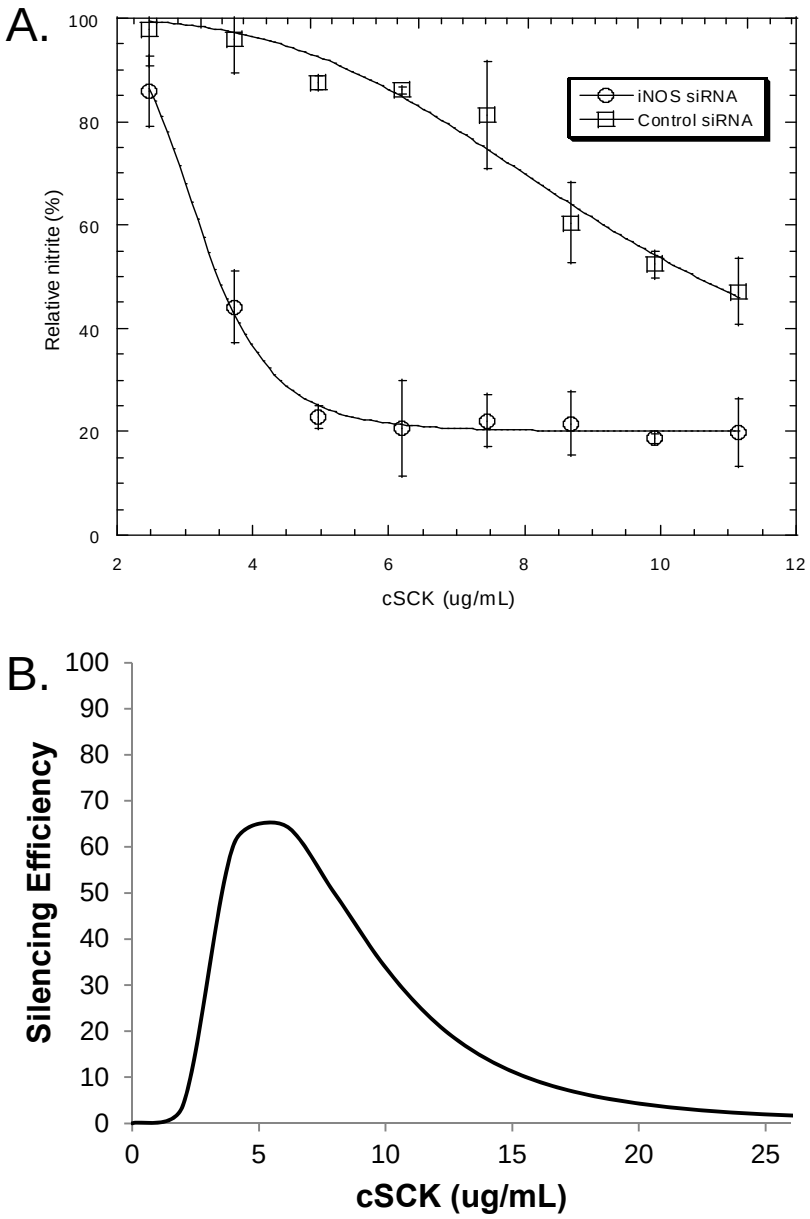


Figure S2. Silencing efficiency of siRNA mediated knockdown of iNOS mRNA by $PAEA_{128}$-*b*-PS_{40} cSCK-pa100 in RAW264.7 cells. A) Curve fits for the taken from Figure 6A with a modified Hill equation to take into account plateauing of the inhibition curve at 20% relative nitrite (80/(1+[cSCK]/IC50)^Hill) + 20), to give LC50 and Hill coefficients of 3.8 ± 0.4 and 2.1 ± 0.5 for knockdown by the iNOS siRNA, and 10.5 ± 0.3 and 3.2 ± 0.4 for non-specific knockdown, presumably cell death, by the control siRNA. B) Silencing efficiency plot, with 58% maximum efficiency at 6 ug/mL.

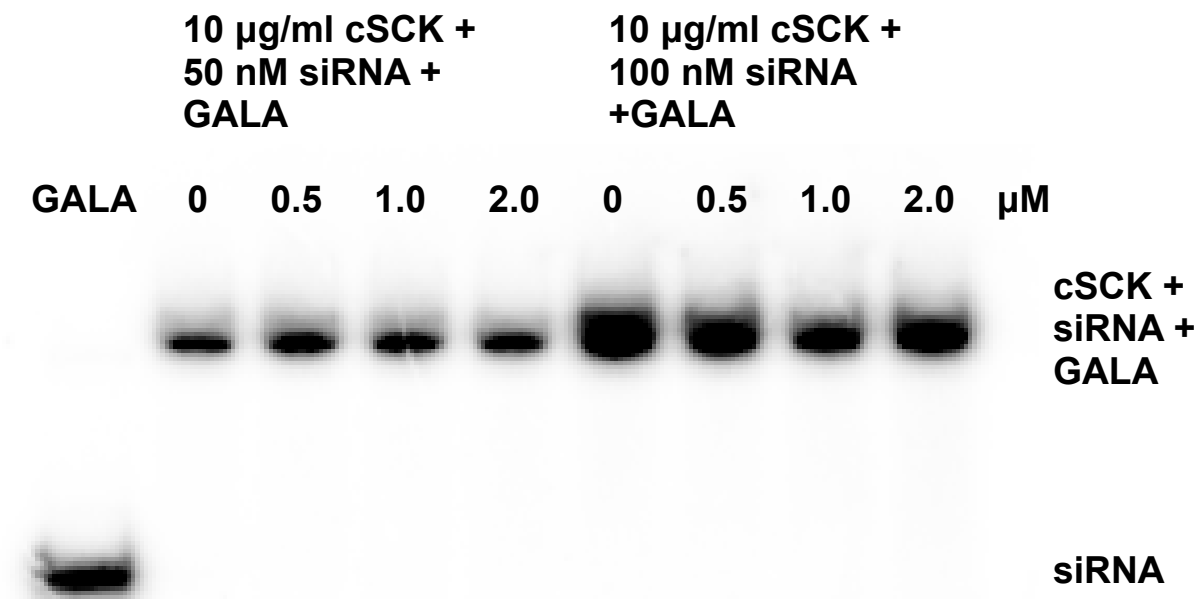
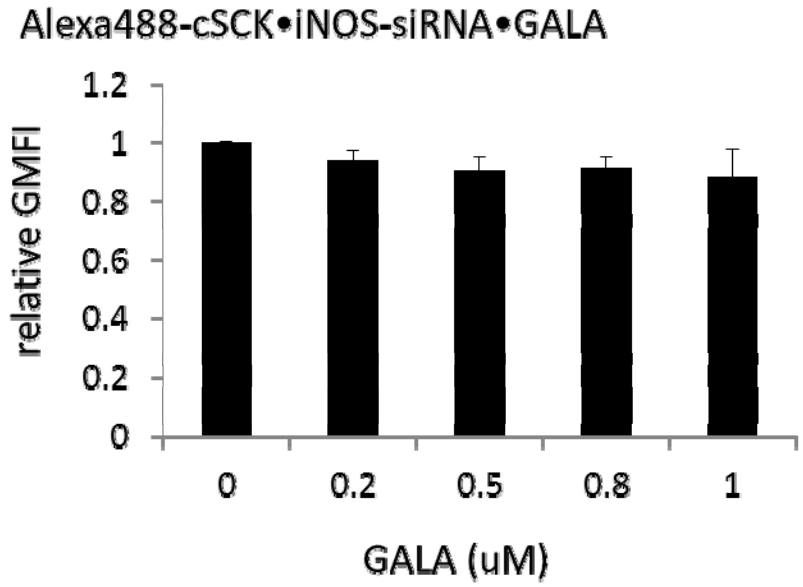


Figure S3. Electromobility shift assay of siRNA and GALA binding to $PAEA_{160}$-b-PS_{30} cSCK. ^{32}P-labeled iNOS siRNA (50 nM, 100 nM) and GALA (0, 0.5, 1.0, 2.0 µM) were incubated with cSCK (10 µg/ml) in OptiMEMI media for 30 min and electrophoresed on a 15% native polyamide gel.

A.



B.

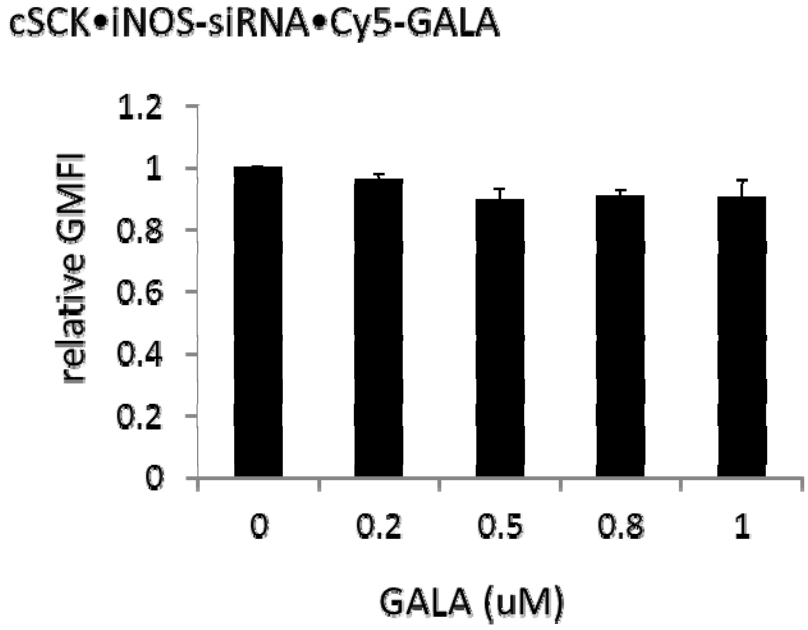


Figure S4. Cellular uptake of $PAEA_{160}$-b-PS_{30} cSCK(Alexa488)-siRNA(Cy5)-GALA nanocomplexes in RAW264.7 cell line by flow cytometry analysis. (A) Alexa488 channel (B) Cy5 channel.

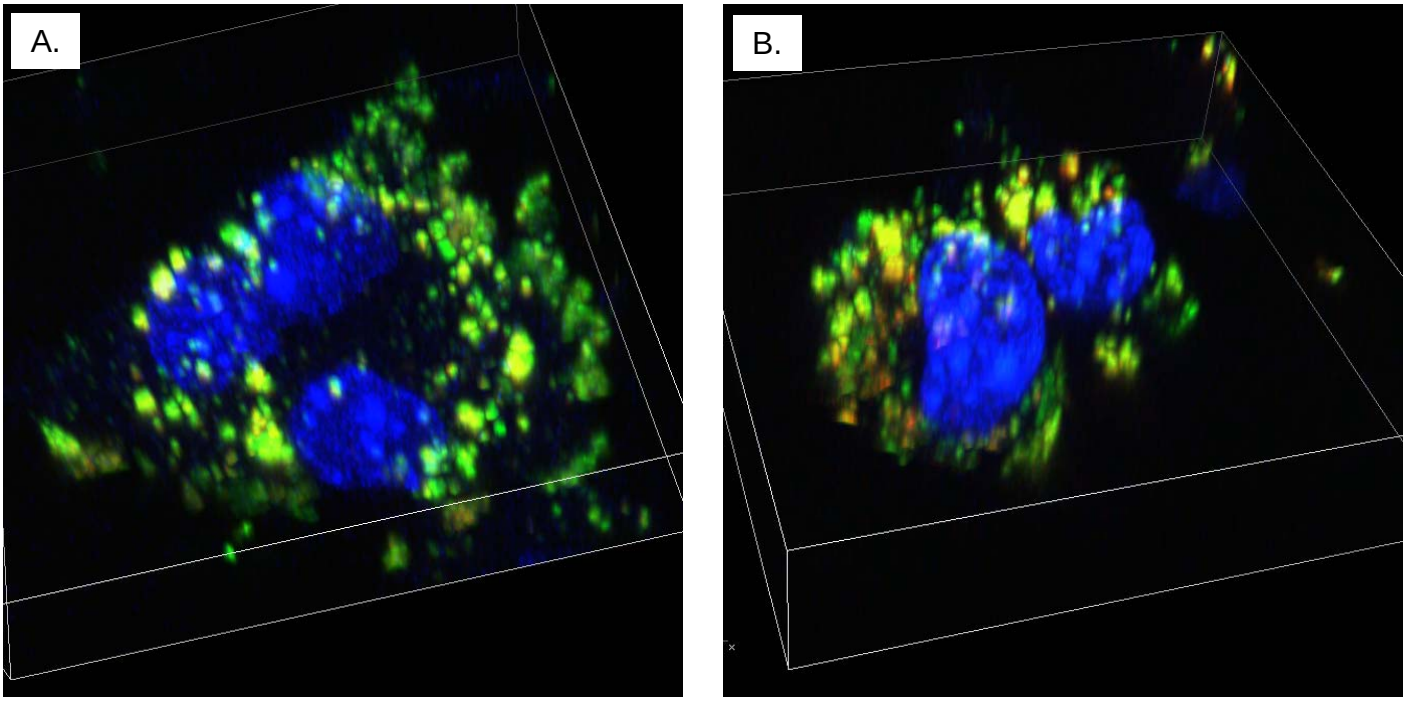


Figure S5. Representative confocal fluorescent microscopic image of intracellular localization of cSCK•siRNA•GALA nanocomplexes in RAW 264.7 cell line. cSCK-Alexafluor488 (green), siRNA-Cy5 (red), Nucleus (Blue). A complex of 10 μg/mL cSCK and 50 nM siRNA A) without GALA and B) with 0.2 μM GALA.

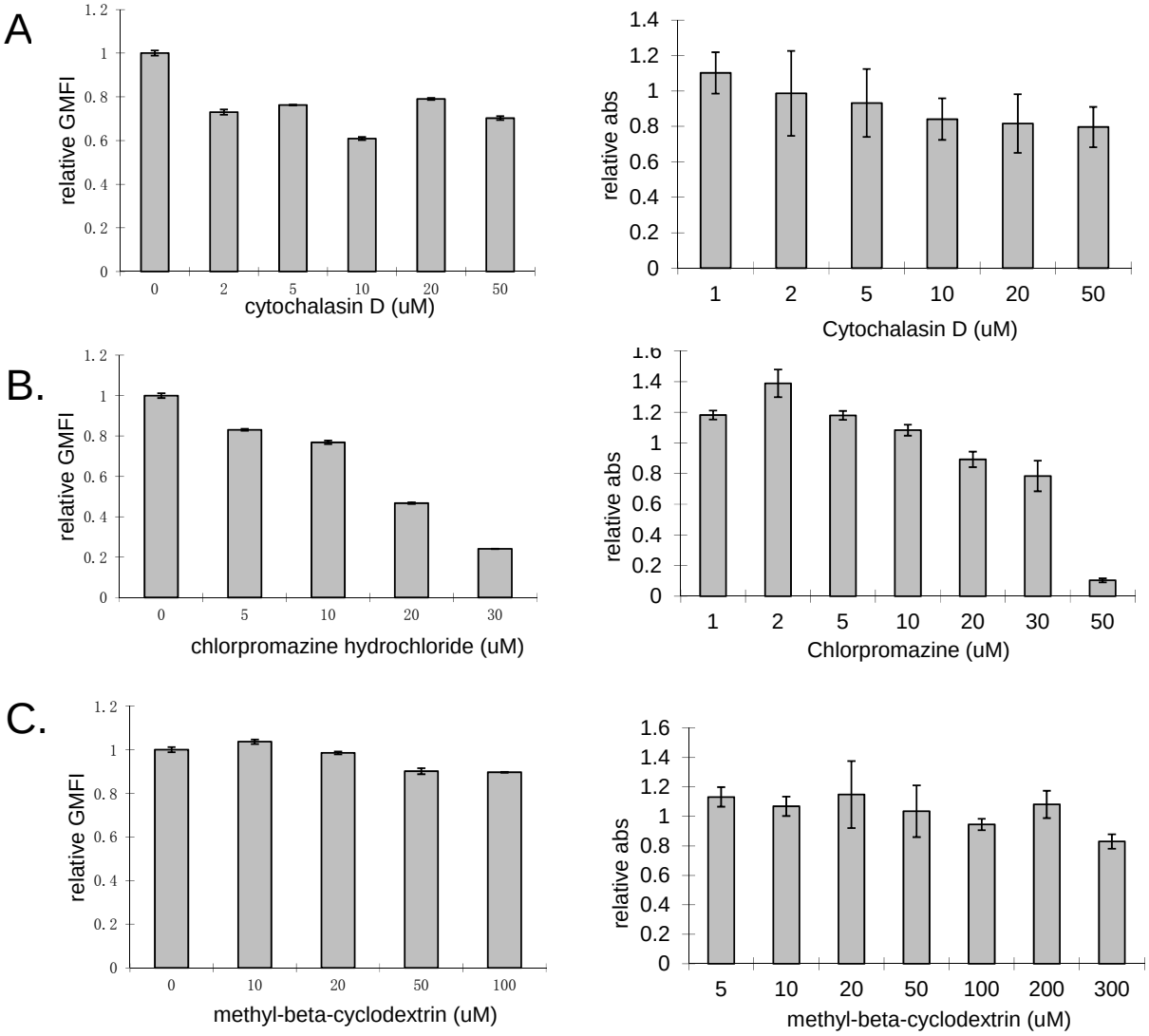


Figure S6. Inhibition of cSCK uptake and cytotoxicity of endocytosis inhibitors. RAW 264.7 cells were treated with the indicated inhibitors and incubated for 1 h after which cSCK uptake was monitored by flow cytometry and cell viability by the CellTiter 96 Non-Radioactive Cell proliferation Assay (MTT assay).

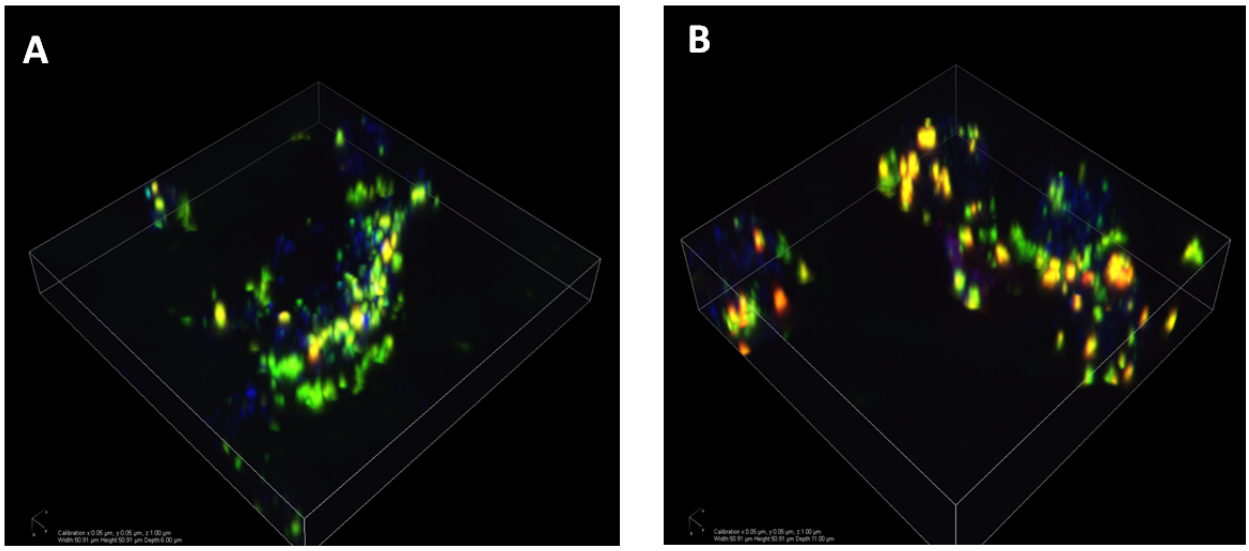


Figure S7. Representative confocal fluorescent microscopic image of intracellular localization of cSCK•siRNA•GALA nanocomplexes with Lysotracker-Blue in RAW 264.7 cell line. cSCK-Alexafluor488 (green), siRNA-Cy5 (red), Lysotracker-Blue (Blue). Lysotracker-Blue (50 nM) with complexes of 10 ug/mL cSCK and 50 nM siRNA without GALA (A) and with 0.2 uM GALA (B).

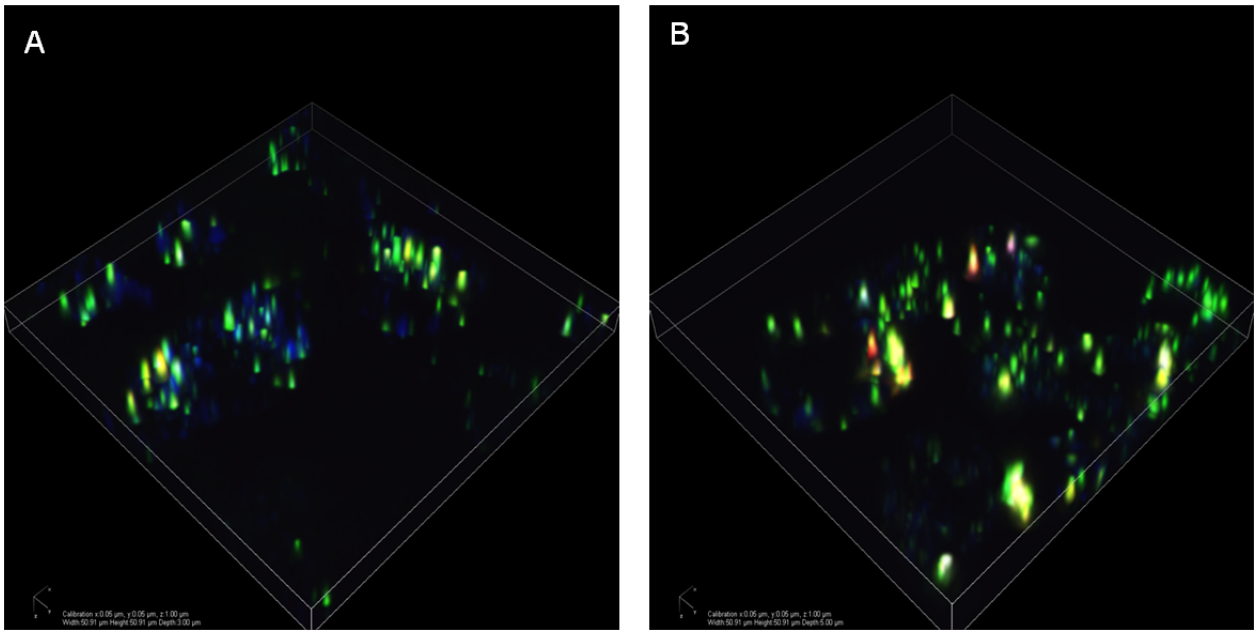


Figure S8. Representative confocal fluorescent microscopic image of intracellular localization of cSCK•siRNA•GALA nanocomplexes with Dextran in RAW 264.7 cell line. cSCK-Alexafluor488 (green), siRNA-Cy5 (red), Dextran-Cascade Blue (Blue). Dextran (100 ug/ml) with complexes of 10 ug/mL cSCK and 50 nM siRNA without GALA (A) and with 0.2 uM GALA (B).