Supporting Information for

ORIGINAL ARTICLE

Synchronous conjugation of i-motif DNA and therapeutic siRNA on the vertexes of tetrahedral DNA nanocages for efficient gene silence

Xiu Han[a,b,†], Xiang Xu[a,†], Ziheng Wu[c], Zhenghong Wu[a,*], Xiaole Qi[a,*]

[a]*Key Laboratory of Modern Chinese Medicines, China Pharmaceutical University, Nanjing 210009, China*

[b]*Medical School of Nanjing University, Nanjing University, Nanjing 210093, China*

[c]*Parkville Campus, Monash University, VIC 3052, Australia*

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*Corresponding authors. Tel.: +86 15062208341 (Zhenghong Wu); +86 15996229832 (Xiaole Qi).

E-mail addresses: zhenghongwu66@cpu.edu.cn (Zhenghong Wu), qixiaole523@cpu.edu.cn (Xiaole Qi).

†These authors made equal contributions to this work.

Oligonucleotides

The tetrahedrons were designed from Turberfield and co-workers which had 20 bp on each side along with an overhang (double strands) for siRNA hybridization and a linker (single strand) for X hybridization[1,2]. The sequences for Td backbones (A, B, C, D) were showed in Table S2. Each edge of the tetrahedron was identified by the same color. The sequences (5′-TTTTTTT-3′ and 5′-AAAAAAA-3′) were used as an overhang. Different numbers of T were used as linkers for X hybridization. The strategy for *a*X-Td@*b*siRNA formation was displayed in Table S1.

Table S1 DNA strands for aX-Td@bsiRNA formation.

aX-Td@bsiRNA	Strand	Component of strands (5′–3′)
X-Td@3siRNA	A_1	A-linker-Y
	B_1	B-overhang
	C_2	overhang-C
	D_1	D-overhang
	Y′	Y′
	$Antisense_1$	Antisense-overhang
	Sense	Sense
2X-Td@2siRNA	A_1	A-linker-Y
	B_1	B-overhang
	C_1	Y-linker-C
	D_1	D-overhang
	Y′	Y′
	$Antisense_1$	Antisense-overhang
	Sense	Sense
3X-Td@siRNA	A_1	A-linker-Y
	B_2	B-linker-Y
	C_1	Y-linker-C
	D_1	D-overhang
	Y′	Y′
	$Antisense_1$	Antisense-overhang
	sense	sense

Table S2 DNA sequences.

Strand	Sequences (5′–3′)
A	CGTATCACCAGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAG
B	CGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCG
C	CGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCGGGTGATAAA
D	CTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGACCCTCGCATG
A_1	CGTATCACCAGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGTTTCCCAATCCCAATCCC AATCCC
B_1	CGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGTTTTTTT
B_2	CGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGTTTCCCAATCCCAATCCC AATCCC
C_1	CCCAATCCCAATCCCAATCCCTTTTTTCGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG GGTGATAAA
C_2	TTTTTTTCGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCGGGTGATAAA
D_1	CTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGACCCTCGCATGTTTTTTT
Antisense	AUUUCUCAUGGGCAGCUCCdTdT[3]
$Antisense_1$	AUUUCUCAUGGGCAGCUCCdTdTAAAAAAA
Sense	GGAGCUGCCCAUGAGAAAUdTdT[3]
Y	CCCAATCCCAATCCCAATCCC[4]
Y′	GGGATTGGGATTGGGATTGGG[4]

Table S3 Sizes and zeta potentials of aX-Td@bsiRNA.

Sample	pH	Size (nm)	PDI	Zeta potential (mV)
Td	7.4	19.62 ± 2.36	0.97 ± 0.05	–7.25 ± 0.07
	5.5	20.69 ± 5.00	0.94 ± 0.07	–7.27 ± 0.24
X-Td@3siRNA	7.4	26.46 ± 3.50	0.75 ± 0.03	–19.37 ± 0.84
	5.5	24.64 ± 1.82	0.72 ± 0.02	–10.63 ± 0.92
2X-Td@2siRNA	7.4	27.35 ± 2.88	0.44 ± 0.02	–19.50 ± 1.22
	5.5	23.44 ± 1.73	0.54 ± 0.15	–8.71 ± 0.15
3X-Td@siRNA	7.4	26.16 ± 8.46	0.72 ± 0.16	–10.63 ± 1.16
	5.5	26.59 ± 1.43	0.27 ± 0.01	–9.33 ± 1.11

Data are presented as mean±SD, *n*=3.

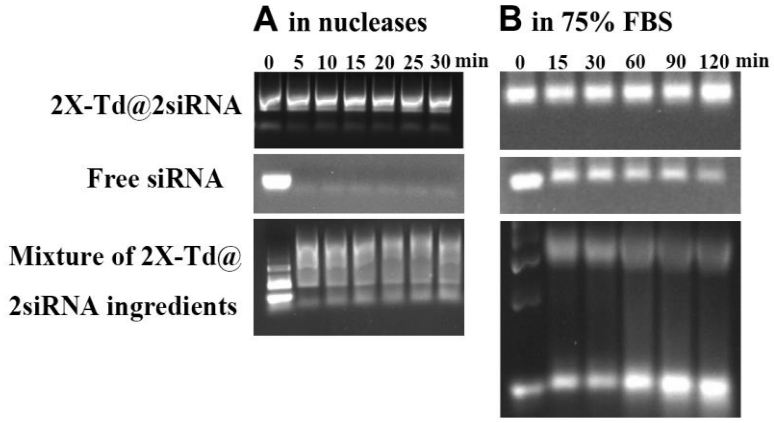

Figure S1 Stability of 2X-Td@2siRNA, free siRNA and mixture of 2X-Td@2siRNA strands in nucleases (100 U DNase and 5 mg/mL RNase) and 75% FBS incubating for different time at 37 °C.

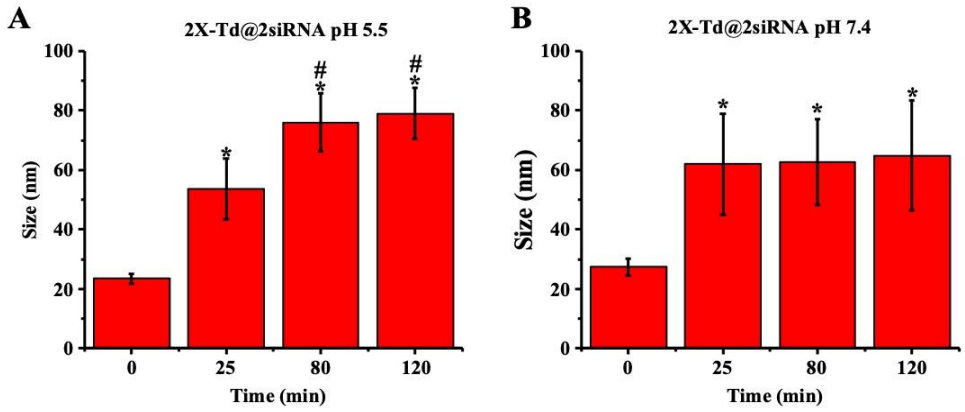

Figure S2 The particle size changes of 2X-Td@2siRNA (A) in pH 5.5 PBS. The significance of the differences ($^{*}P < 0.05$ *versus* 0 min, $^{\#}P < 0.05$ *versus* 25 min) was evaluated by two-tailed Student's t-test. (B) in pH 7.4 PBS. The significance of the differences ($^{*}P < 0.05$ *versus* 0 min) was evaluated by two-tailed Student's *t*-test.

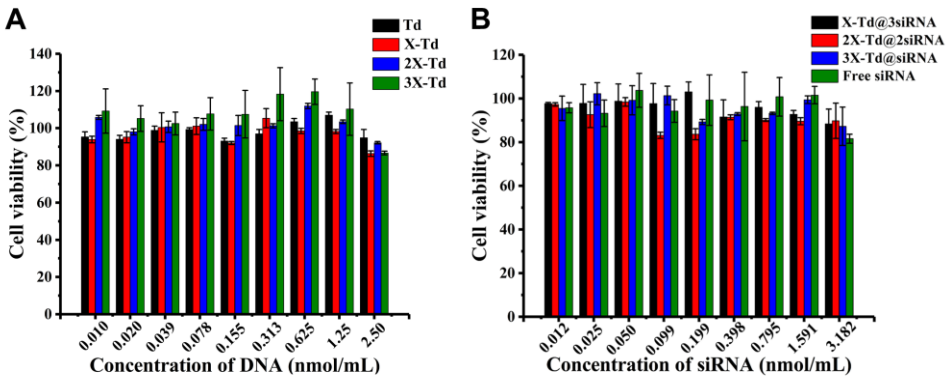

Figure S3 Cell viability of A549 cells treated with A) empty cages (aX-Td), and B) siRNA loaded DNA nanocages (aX-Td@bsiRNA) with increasing concentrations after 48 h.

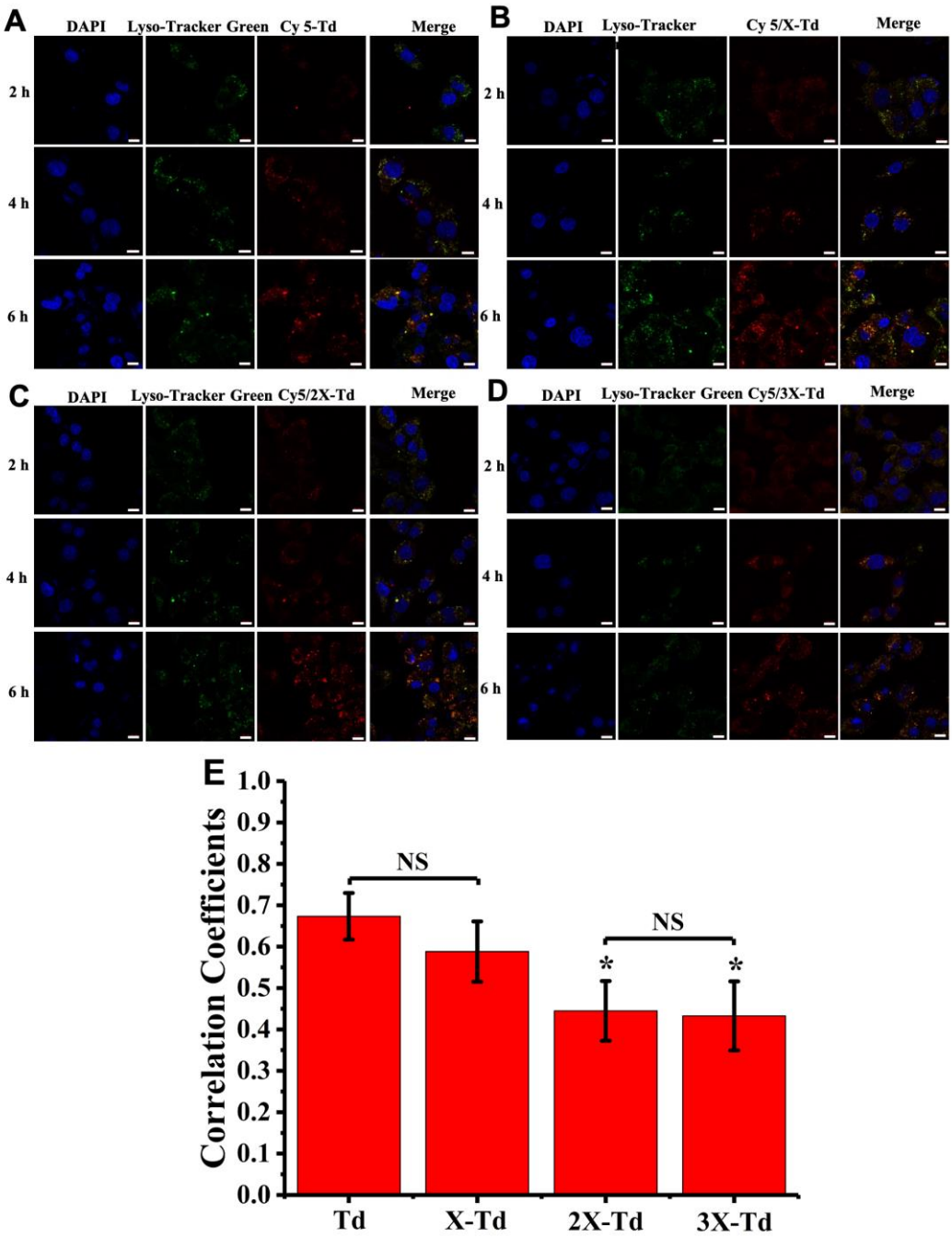

Figure S4 Endosomal escape behavior of Td (A), X-Td (B), 2X-Td (C) and 3X-Td (D) in A549 cells at different times analyzed by confocal laser scanning microscopy. The scale bar is 10 μm. (E) The Pearson correlation coefficients between Cy 5 and endosomes at 6 h were calculated by Image J software. The significance of the differences (*$P < 0.05$ *versus* Td group) was evaluated by two-tailed Student's *t*-test.

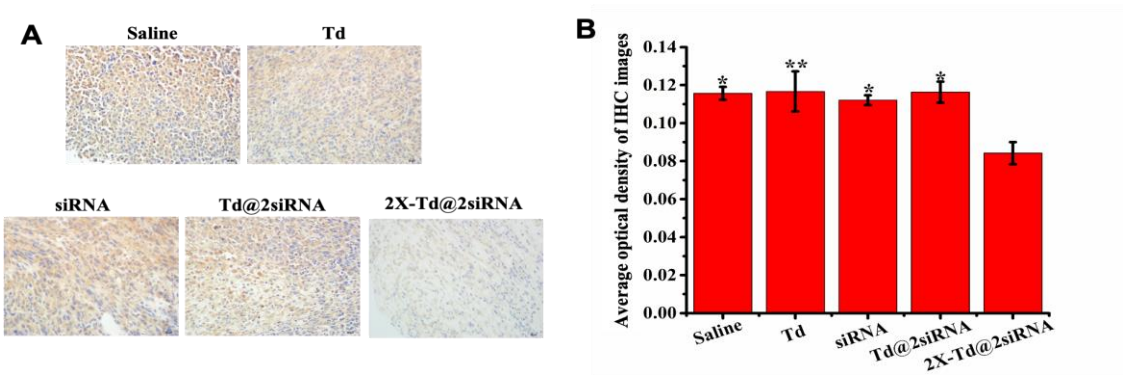

Figure S5 EGFR protein expression quantification (A) Immunohistochemistry (IHC) images of the tumors collected from different groups of mice after 12 days injection treatments (400 ×). (B) Average optical density of IHC images quantified by Image J. The significance of the differences ($^{**}P < 0.01$, $^{*}P < 0.05$ *versus* 2X-Td@2siRNA group) was evaluated by two-tailed Student's *t*-test ($n = 3$)

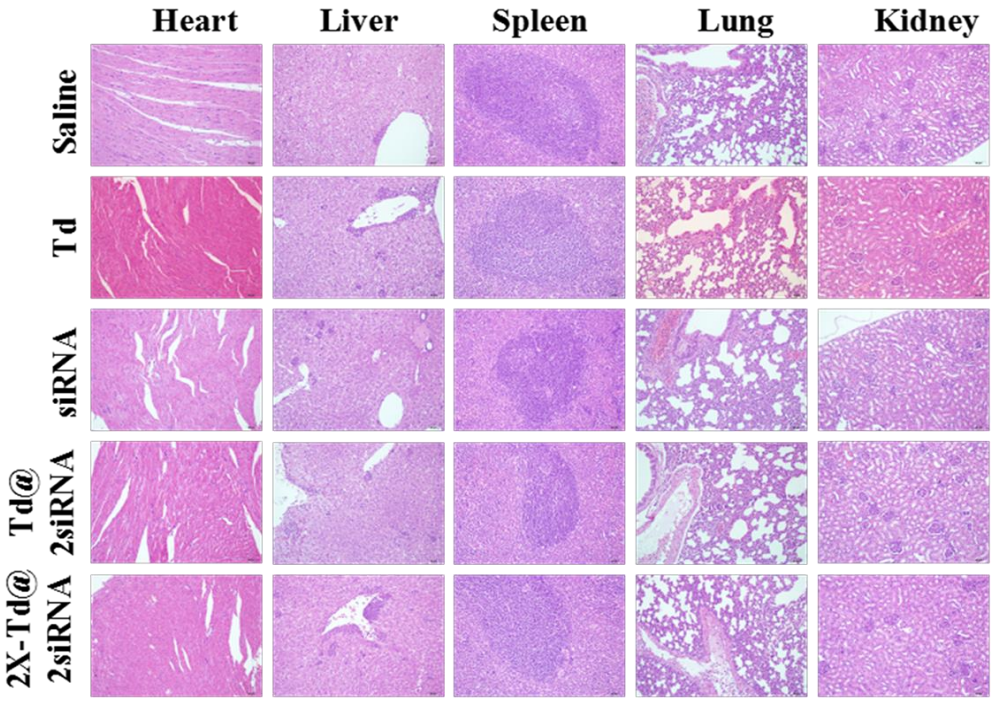

Figure S6 H&E staining of main organs collected from different groups of mice after 12 days injection treatments (200 ×).

References

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