Supporting Information

Self-Assembly of Four Generations of RNA Dendrimers for Drug Shielding with Controllable Layer-by-Layer Release

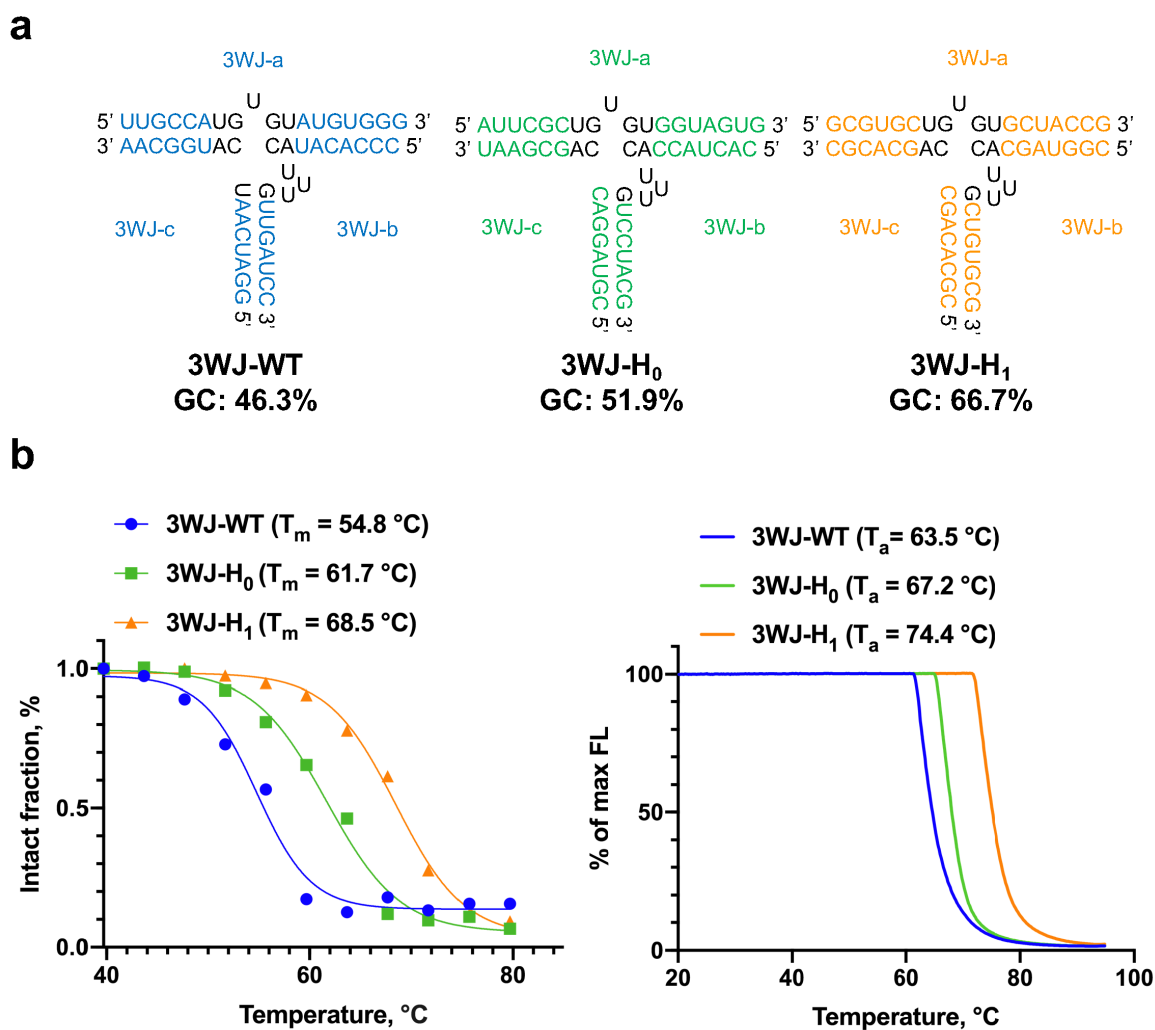*Xin Li[1,2], Dr. Mario Vieweger[1,2], Dr. Peixuan Guo[1,2,3,4,5]* ∗

Figure S1. Sequence design and thermostability of new 3WJs. a) Sequence design and GC content of 3WJs, 3WJ-WT, 3WJ-H_0 and 3WJ-H_1. b) Thermostability evaluation of 3WJs including melting and annealing curve.
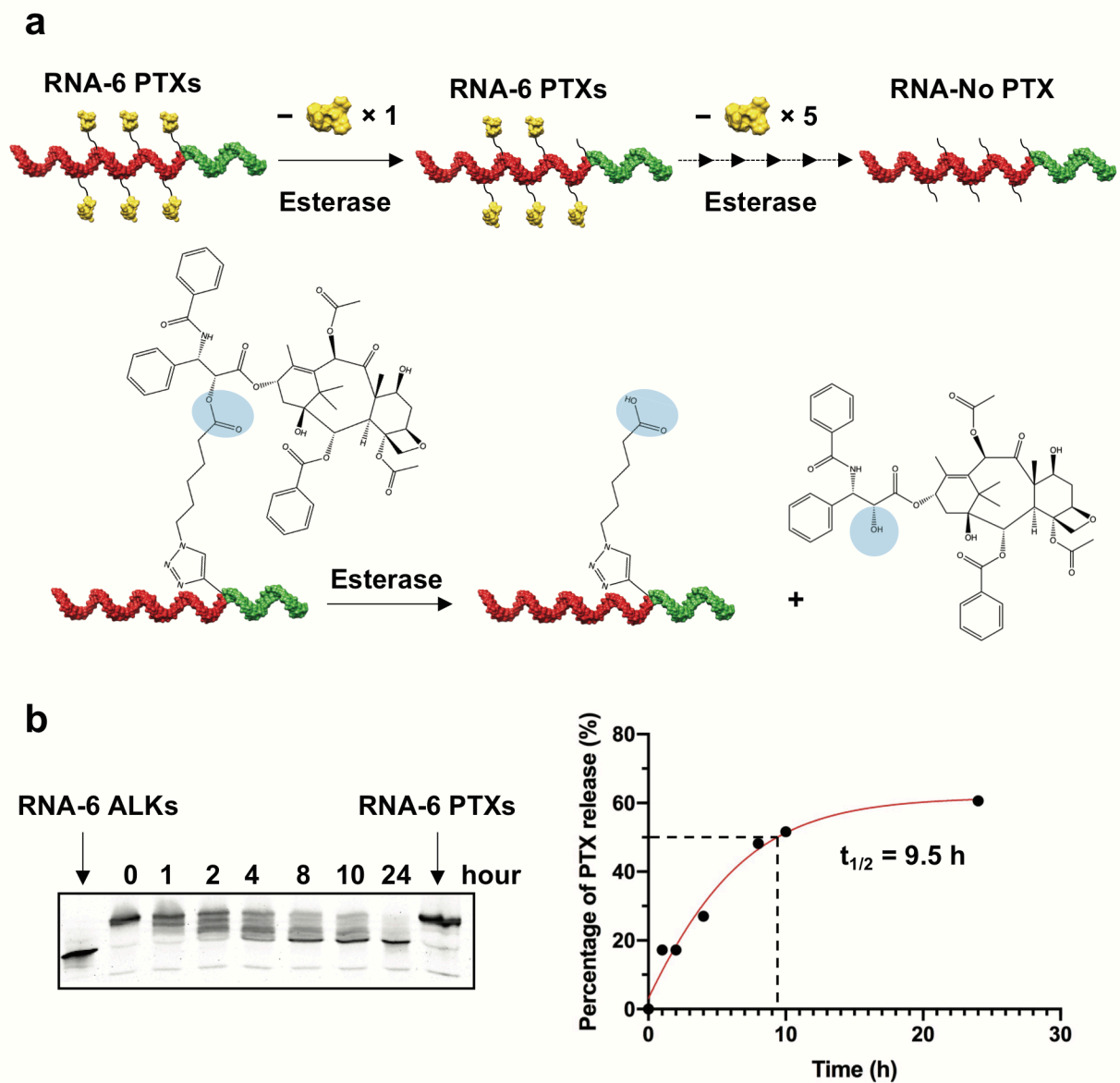
Figure S2. Paclitaxel release from RNA strands. a) Schematics of PTX release from RNA strands by esterase hydrolysis. b) Release profile of RNA-6 PTXs strands in 50% FBS for up to 24 h.
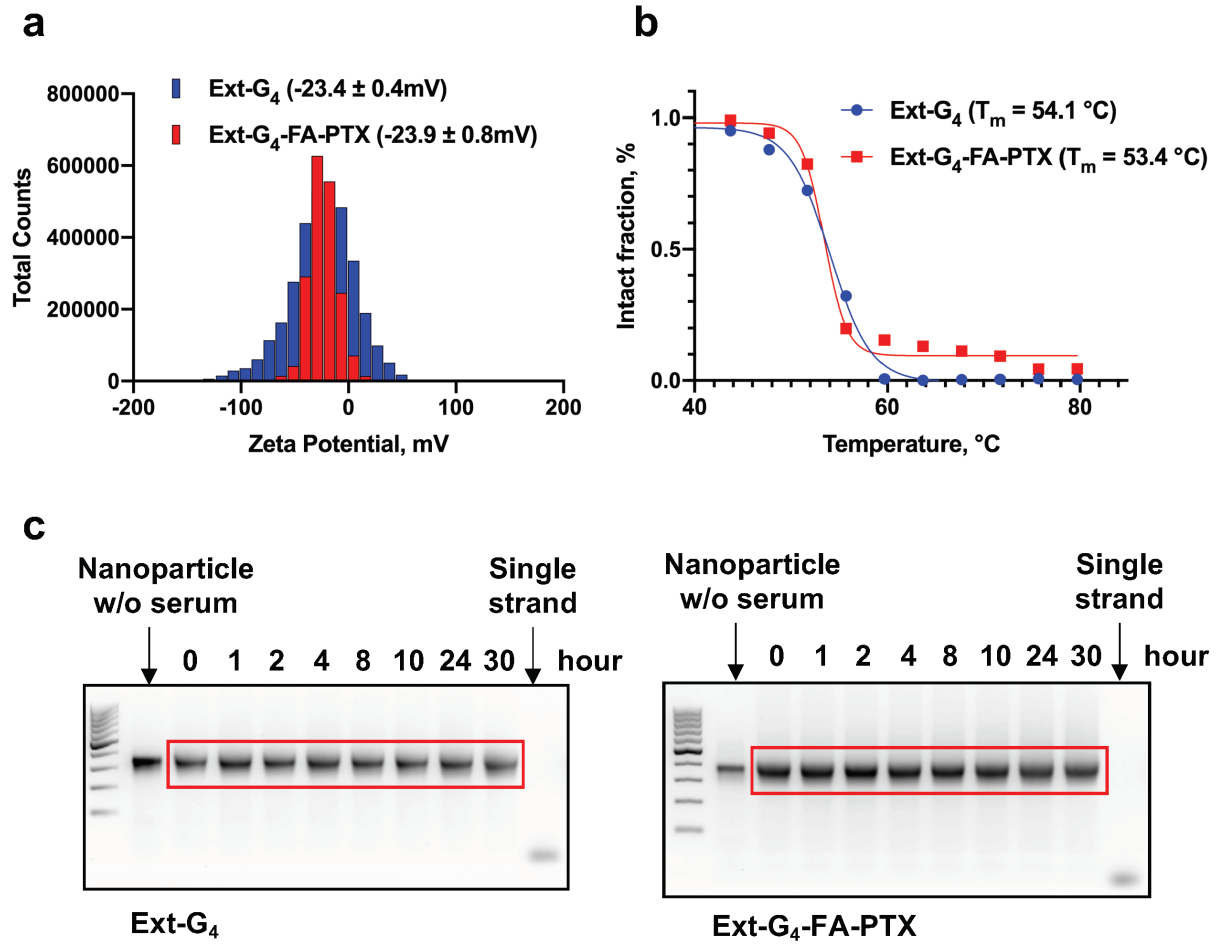
Figure S3. Characterizations of Ext-G$_4$-FA-PTX RNA dendrimers (a) Zeta potential measurement (mean $\pm$ SD), (b) Thermal stability assay and (c) Enzymatic stability assay of Ext-G$_4$ and Ext-G$_4$-FA-PTX RNA dendrimers.
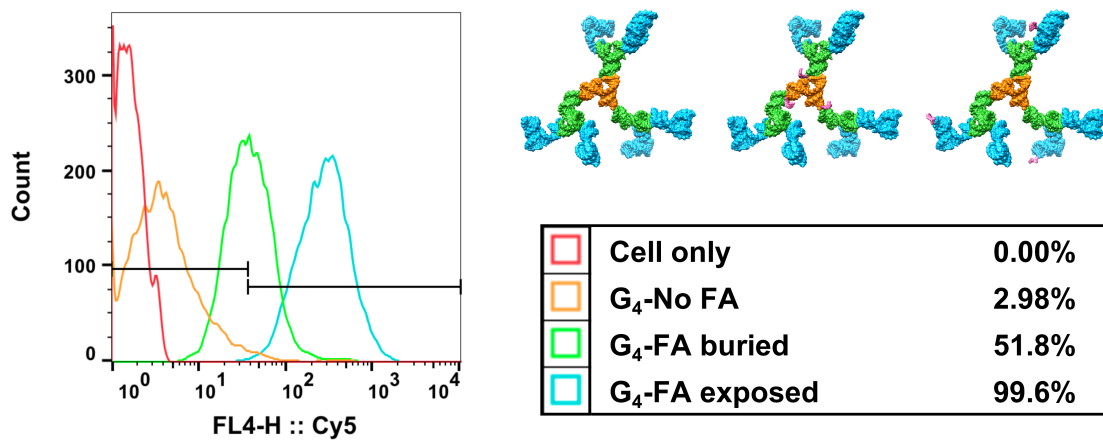
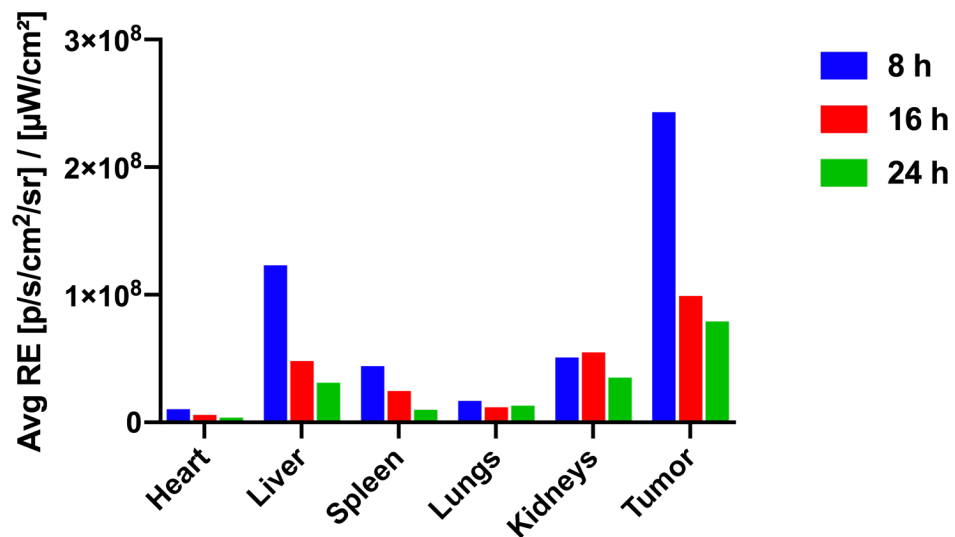**Figure S4.** Shielding effect of RNA dendrimers using folate as model cargo demonstrated by flow cytometry.

Figure S5. Quantitative analysis of biodistribution in tumors and vital organs derived from organ images in Figure 6 (Average Radiant Efficiency, [p/s/cm^2/sr] / [μW/cm^2]).

Table S1. Summary of sequences used in RNA dendrimer construction.

	RNA Strand	Sequence	Length (nt)	MW (g/mol)
1	H_0-a/WT-b	5'-AUUCGCUGUGUGGUAGUGCCCACAUACUUUGUUGAUCC-3'	38	12099
2	H_0-b/WT-b	5'-CACUACCACUUUGUCCUACGCCCACAUACUUUGUUGAUCC-3'	40	12566
3	H_0-c/WT-b	5'-CGUAGGACCAGCGAAUCCCACAUACUUUGUUGAUCC-3'	36	11470
4	H_1-a/ H_0-b	5'-GCGUGCUGUGUGCUACCGCACUACCACUUUGUCCUACG-3'	38	12074
5	H_1-b/ H_0-b	5'-CGGUAGCACUUUGCUGUGCGCACUACCACUUUGUCCUACG-3'	40	12711
6	H_1-c/ H_0-b	5'-CGCACAGCCAGCACGCCACUACCACUUUGUCCUACG-3'	36	11407
7	Ext-H_1-a/ H_0-b	5'-CCUAUUCAGGUGCGUGCUGUGUGCUACCGAUGUAAUUCAACACUACCACUUUGUCCUACG-3'	60	19108
8	Ext-H_1-b/ H_0-b	5'-UUGAAUUACAUCGGUAGCACUUUGCUGUGCGAGGCUGAACAGCACUACCACUUUGUCCUACG-3'	62	19841
9	Ext-H_1-c/ H_0-b	5'-CUGUUCAGCCUCGCACAGCCAGCACGCACCUGAAUAGGCACUACCACUUUGUCCUACG-3'	58	18471
10	WT-b/H_0-a	5'-CCCACAUACUUUGUUGAUCCAUUCGCUGUGUGGUAGUG-3'	38	12099
11	WT-b/H_0-c	5'-CCCACAUACUUUGUUGAUCCCGUAGGACCAGCGAAU-3'	36	11470
12	3WJ-WT-a	5'-UUGCCAUGUGUAUGUGGG-3'	18	5784
13	3WJ-WT-b	5'-CCCACAUACUUUGUUGAUCC-3'	20	6253
14	3WJ-WT-c	5'-GGAUCAAUCAUGGCAA-3'	16	5140
15	3WJ-H_0-a	5'-AUUCGCUGUGUGGUAGUG-3'	18	5784
16	3WJ-H_0-b	5'-CACUACCACUUUGUCCUACG-3'	20	6252
17	3WJ-H_0-c	5'-CGUAGGACCAGCGAAU-3'	16	5155
18	3WJ-H_1-a	5'-GCGUGCUGUGUGCUACCG-3'	18	5760

19	3WJ-H$_1$-b	5'-CGGUAGCACUUUGCUGUGCG-3'	20	6398
20	3WJ-H$_1$-c	5'-CGCACAGCCAGCACGC-3'	16	5093
21	Ext-H$_1$-a/H$_0$-b_ 6 ALKs	<u>5'</u>- <u>C</u>CUAU<u>U</u>CAGG<u>U</u>GCGUGCUGUGUGCUAC<u>C</u>G AUG<u>U</u>AAUU<u>C</u>AACACUACCACUUUGUCCUA <u>C</u>G-3'	60	19284
22	Ext-H$_1$-b/H$_0$-b_ 6 ALKs	<u>5'</u>- UUGAA<u>U</u>UACA<u>U</u>CGGUAGCACUUUGCUGUG <u>C</u>GAGG<u>C</u>UGAA<u>C</u>AGCACUACCACUUUGUCCU A<u>C</u>G-3'	62	20017
23	Ext-H$_1$-c/H$_0$-b_ 6 ALKs	<u>5'</u>- <u>C</u>UGUU<u>C</u>AGCC<u>U</u>CGCACAGCCAGCA<u>C</u>GCAC<u>C</u> UGAA<u>U</u>AGGCACUACCACUUUGUCCUACG-3'	58	18585

All C and U are 2'-fluoro modified. All <u>5'</u>, <u>C</u> and <u>U</u> are 2'-O-propargyl modified.

Table S2. Summary of RNA dendrimers characteristics.

	RNA nanoparticle	Strand Type	Strand Amount	Nucleotide Amount	Terminal Ends	Size (nm)	T_m (°C)	T_a (°C)	MW (g/mol)
1	3WJ-WT	3	3	54	3	N/A	54.8	63.5	17177
2	3WJ-H_0	3	3	54	3	N/A	61.7	67.2	17191
3	3WJ-H_1	3	3	54	3	N/A	68.5	74.4	17251
4	G_3-1	5	9	216	6	7.98	52.0	72.3	68908
5	G_3-2	5	9	216	6	8.39	58.3	79.4	69010
6	Ext-G_3	5	9	282	6	10.38	59.7	N/A	90238
7	G_4	7	21	540	12	13.12	52.6	76.5	172444
8	Ext-G_4	7	21	606	12	14.24	53.6	95.0	193672
9	Ext-G_4-FA-PTX	7	21	606	12	17.90	53.4	N/A	217780