

**Supporting Information**

**Cell Internalization Kinetics and Surface Charge Accessibility of Surface-Modified PAMAM Dendrimers**

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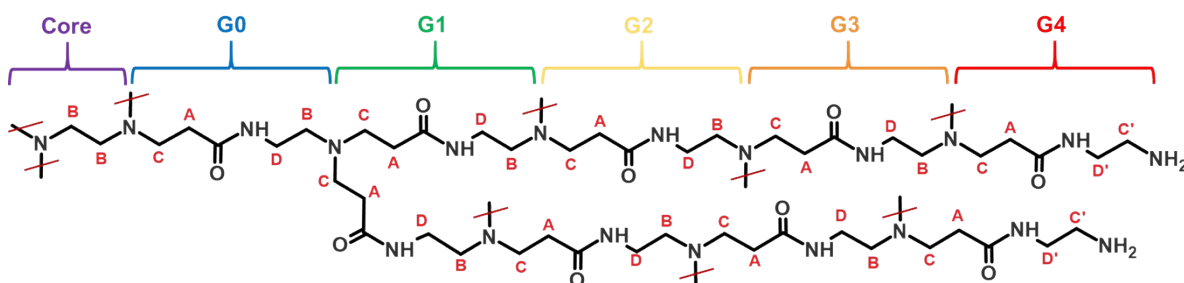
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## 1. NMR characterization of modified PAMAM dendrimers

Table S1 reports the  $^1\text{H-NMR}$  chemical shifts and signal integrals of native PAMAM dendrimer of the fourth-generation (G4). The signal corresponding to  $\alpha$ -methylene groups (A) is not significantly affected by the surface functionalization of the dendrimer. Therefore, it was used to estimate the functionalization degree of substituted counterparts containing acetyl (ACE), folic acid (FA), and polyethyleneglycol (PEG) groups.

**Table S1.** Chemical shifts  $\delta$  (ppm) and signal integrals corresponding to the  $^1\text{H-NMR}$  spectrum of the fourth-generation PAMAM dendrimer (G4). The signal marked with \* was used for the determination of the functionalization degree.



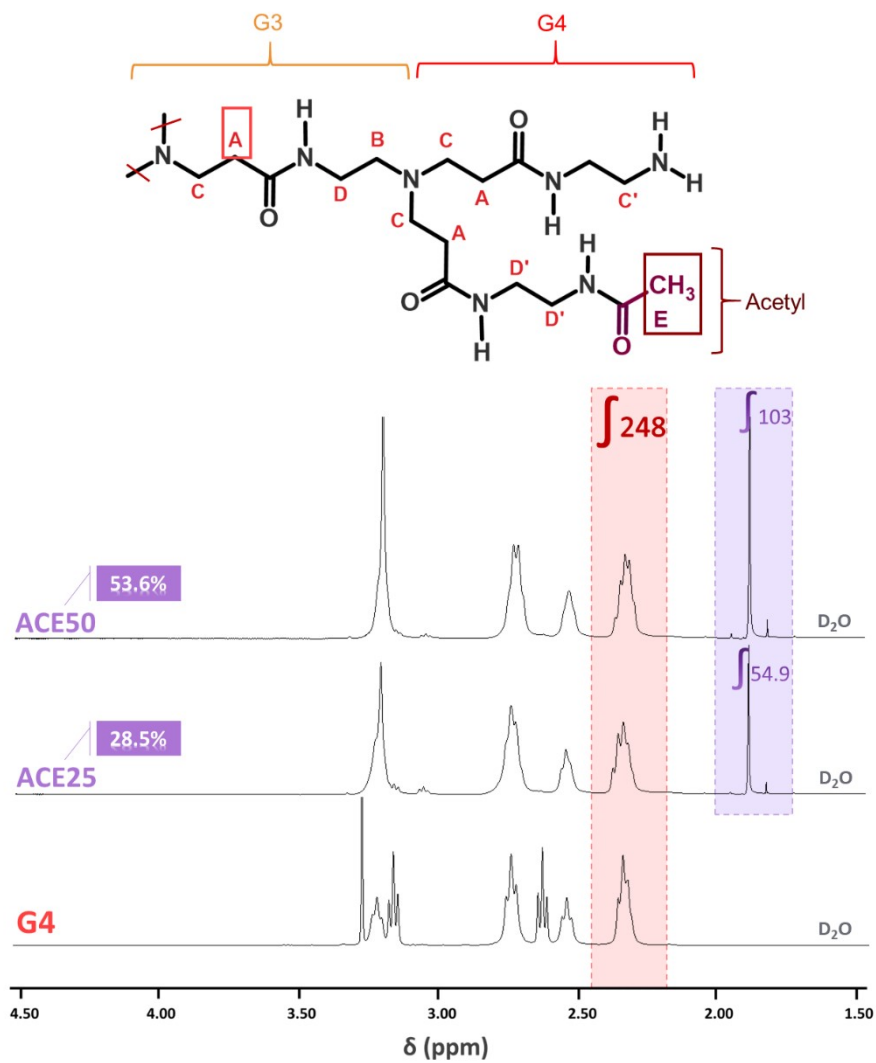
Hydrogen type	$\delta$ (ppm)	Integral	Number of hydrogens
<b>A*</b>	<b>2.35</b>	<b>248</b>	<b>248</b>
B	2.54	123	120
C'	2.63	131	128
C	2.74	242	248
D'	3.16	131	128
D	3.21	127	124

## Acetylated dendrimers

Table S2 reports the chemical shifts and signal integrals corresponding to the  $^1\text{H-NMR}$  spectra of acetylated PAMAM dendrimers with nominal substitution degrees of 25% (ACE25) and 50% (ACE50). The ratio between the signals corresponding to the methyl group of the ACE moiety (E) and the  $\alpha$ -methylene groups (A) of the PAMAM dendrimer was used to estimate the functionalization degree in each system, resulting in acetylation degrees of 28.5% for ACE25 and 53.6% for ACE50 (Figure S1). After acetylation, the signals C and C' of PAMAM merged into a single signal, just like D and D'.

**Table S2.** Chemical shifts  $\delta$  (ppm) and signal integrals corresponding to the  $^1\text{H-NMR}$  spectrum of acetylated PAMAM dendrimers of the fourth generation with nominal substitution degrees of 25% (ACE25) and 50% (ACE50).

Hydrogen type	System					
	ACE25			ACE50		
	$\delta$ (ppm)	Integral	Number of hydrogens	$\delta$ (ppm)	Integral	Number of hydrogens
E*	1.88	54.9	54	1.89	103	103
A*	2.34	248	248	2.33	248	248
B	2.54	120	120	2.54	123	120
C+C'	2.74	305	340	2.74	285	307
D+D'	3.21	269	292	3.21	314	325



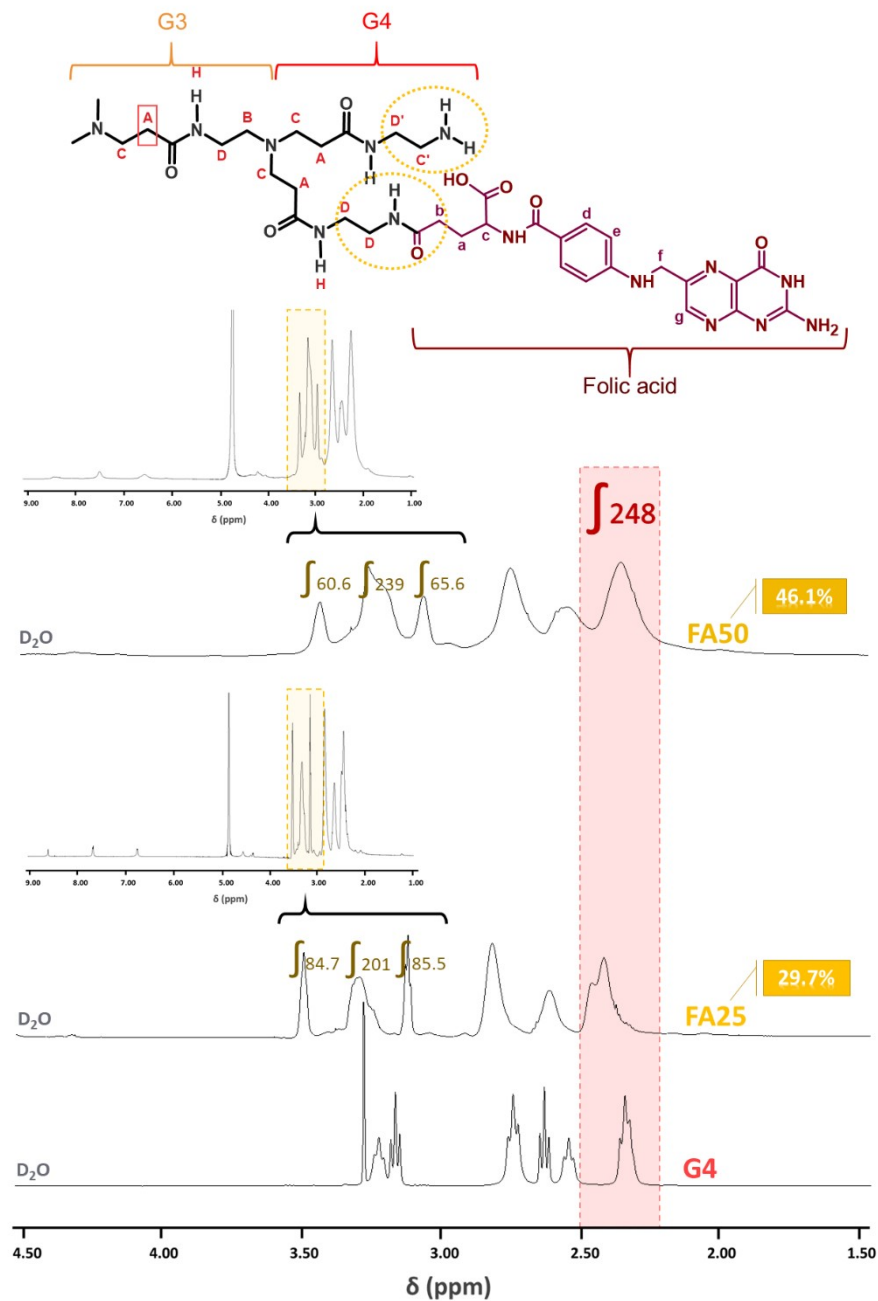
**Figure S1.**  $^1\text{H}$  NMR spectra of native PAMAM of the fourth generation (G4) and acetylated dendrimers with nominal substitution degrees of 25% (ACE25) and 50% (ACE50).

## Folate-conjugated dendrimers

The determination of the substitution degree of PAMAM dendrimers conjugated with folic acid (FA) was carried out indirectly. The characteristic signals of FA undergo a strong distortion after conjugation to PAMAM, making integration difficult. Each conjugated FA causes a decrease in signals C' and D' while increasing signal D. The summary of the integrals is compiled in Table S3 and Figure S2. On average, the calculation for each signal yielded a functionalization of 29.7% for FA25 and 46.1% for FA50

**Table S3.** Chemical shifts  $\delta$  (ppm) and signal integrals corresponding to the  $^1\text{H-NMR}$  spectrum of PAMAM dendrimers of the fourth generation conjugated with folic acid with nominal substitution degrees of 25% (FA25) and 50% (FA50).

Hydrogen type	System					
	FA25			FA50		
	$\delta$ (ppm)	Integral	Number of hydrogens	$\delta$ (ppm)	Integral	Number of hydrogens
a	1.74	-	38	1.91	-	60
b	1.93	-	38	1.97	-	60
A*	2.41	248	248	2.34	248	248
B	2.60	122	120	2.53	129	120
C	2.81	225	248	2.73	213	248
C' - % *	3.11	85.5	90	3.04	65.6	68
D + % *	3.29	201	200	3.23	239	244
D' - % *	3.48	84.7	90	3.40	60.6	68
c	3.61	-	19	3.55	-	30
f	4.52	-	38	4.27	-	60
e	6.63	-	38	6.63	-	60
d	7.54	-	38	7.54	-	60
H	7.99	-	124	8.00	-	124
g	8.48	-	19	8.47	-	30



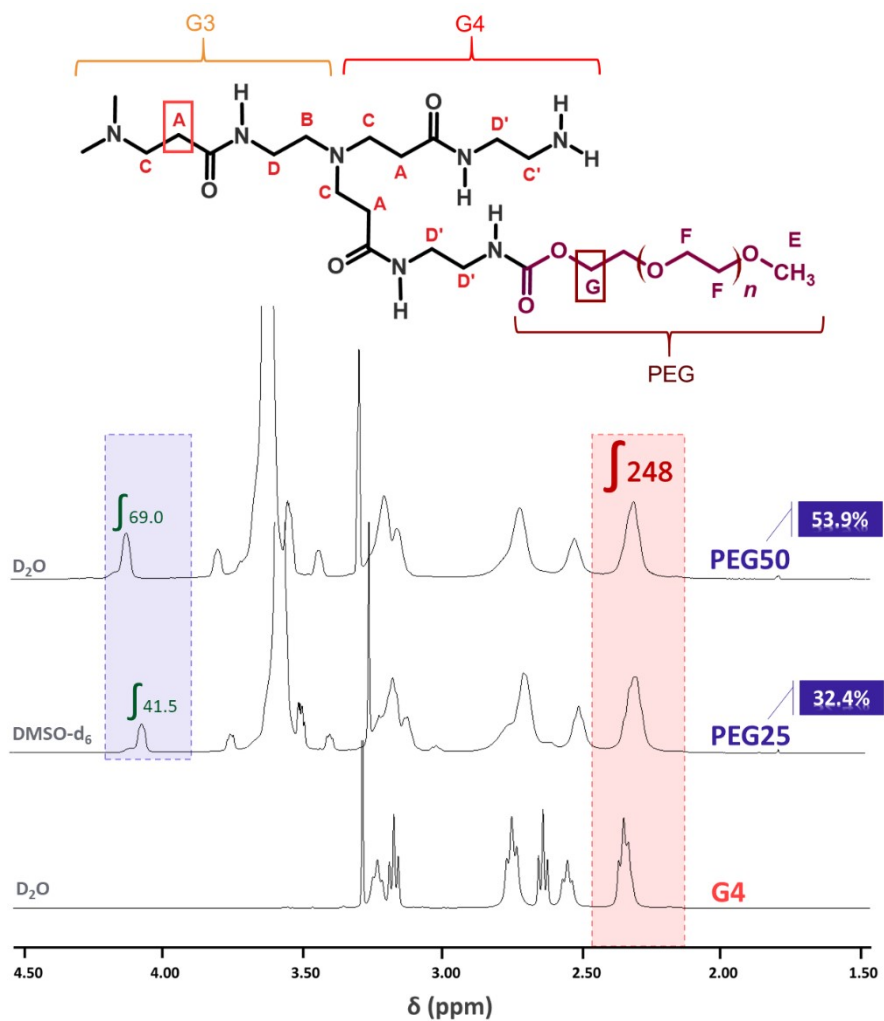
**Figure S2.**  $^1\text{H}$  NMR spectra of native PAMAM of the fourth generation (G4) and dendrimers conjugated with folic acid with nominal substitution degrees of 25% (FA25) and 50% (FA50).

## PEGylated dendrimers

Table S4 summarizes the chemical shifts  $\delta$  (ppm) and signal integrals corresponding to the  $^1\text{H}$ -NMR spectrum of PAMAM dendrimers of the fourth generation conjugated with PEG chains (2 kDa) with nominal substitution degrees of 25% (PEG25) and 50% (PEG50). The degree of functionalization was determined by comparing signal A of PAMAM with signal G of PEG chains, which corresponds to the hydrogen atoms of the methylene group closest to the PEG amide bond. The functionalization percentages were found to be 32.4% for PEG25 and 53.9% for PEG50 (Figure S3).

**Table S4.** Chemical shifts  $\delta$  (ppm) and signal integrals corresponding to the  $^1\text{H}$ -NMR spectrum of PAMAM dendrimers of the fourth generation conjugated with PEG chains (2 kDa) with nominal substitution degrees of 25% (PEG25) and 50% (PEG50)

Hydrogen type	System					
	PEG25			PEG50		
	$\delta$ (ppm)	Integral	Number of hydrogens	$\delta$ (ppm)	Integral	Number of hydrogens
A*	2.27	248	248	2.33	248	248
B	2.48	120	120	2.54	123	120
C+C'	2.68	331	334	2.73	282	306
D+D*'	3.15	290	294	3.21	345	322
E	3.23	80.3	41	3.30	129	69

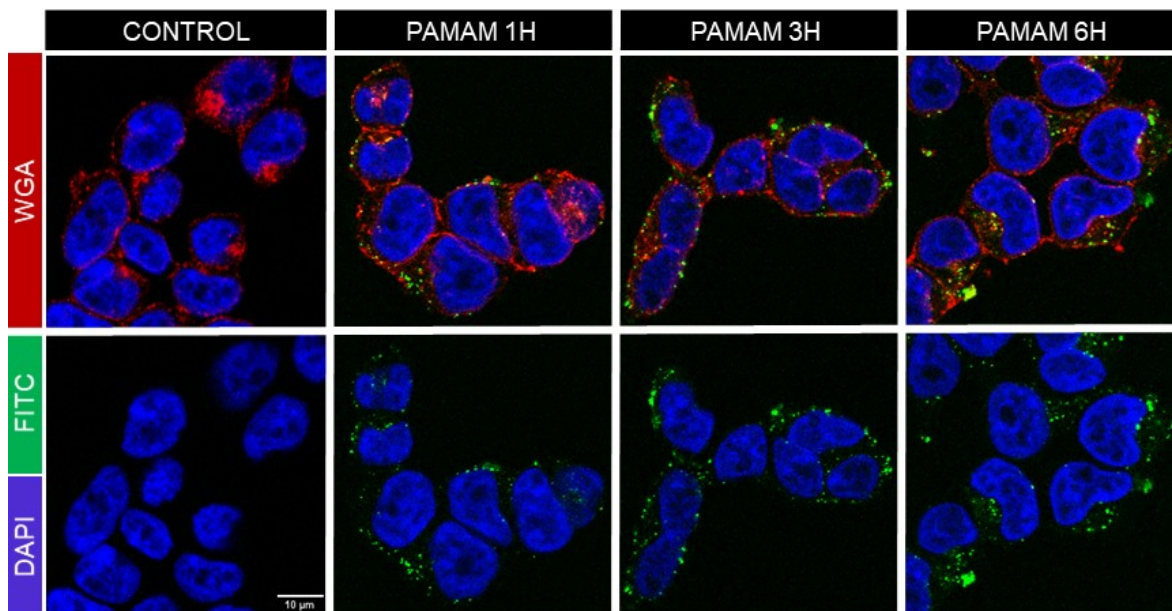


**Figure S3.**  $^1\text{H}$  NMR spectra of native PAMAM of the fourth generation (G4) and dendrimers conjugated with 2 kDa PEG chains with nominal substitution degrees of 25% (PEG25) and 50% (PEG50).

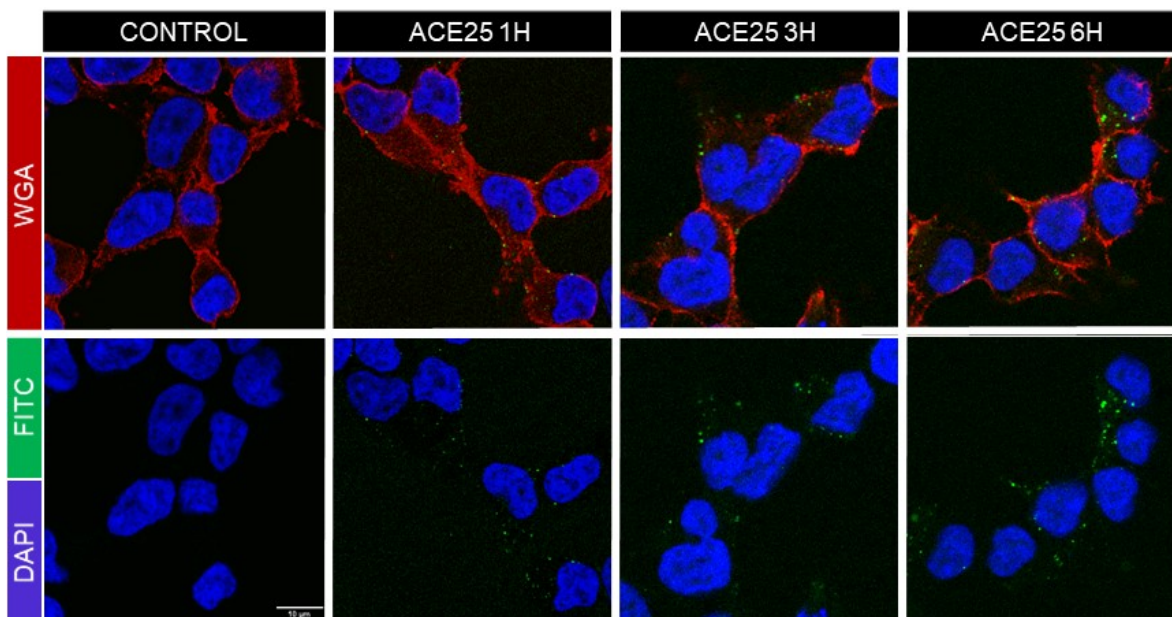


## 2. Cell internalization kinetics – Confocal captures at 1, 3, and 6 h

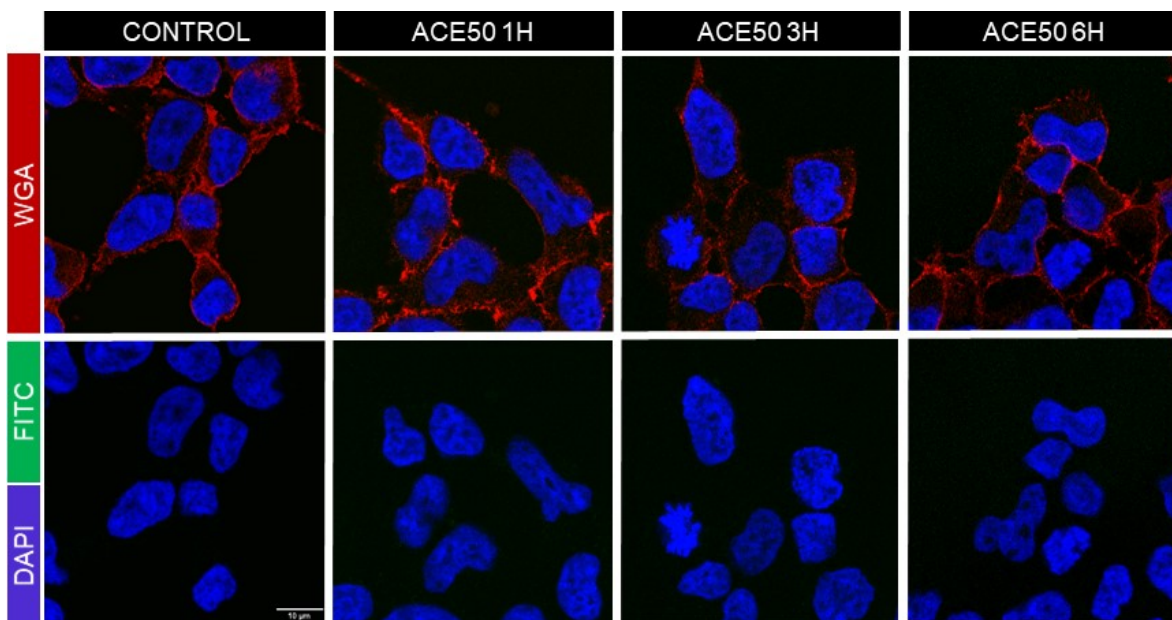
Figures S4-S10 display the confocal images used to quantify the extent of HEK cells internalization of native (G4) and surface-modified PAMAM dendrimers of the fourth generation with acetyl (ACE), folic acid (FA) and polyethylene glycol (PEG) groups, with nominal substitution degrees of 25% and 50%, at 1, 3, and 6 hours of incubation. Dendrimers were labeled with a green probe (FITC), the nucleus appear in blue (DAPI), and the plasma membrane in red (WGA-Alexa fluor 647) for better cell visualization.



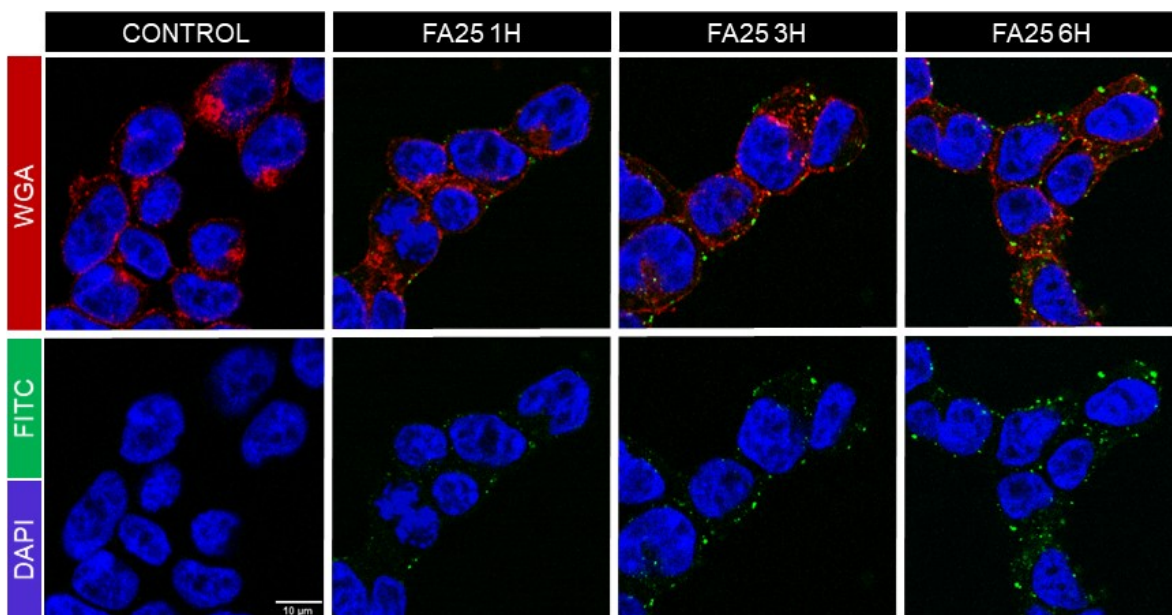
**Figure S4.** Confocal images for HEK cells incubated for 1 h, 3 h and 6 h with 0.5 mM solutions of native PAMAM (G4).



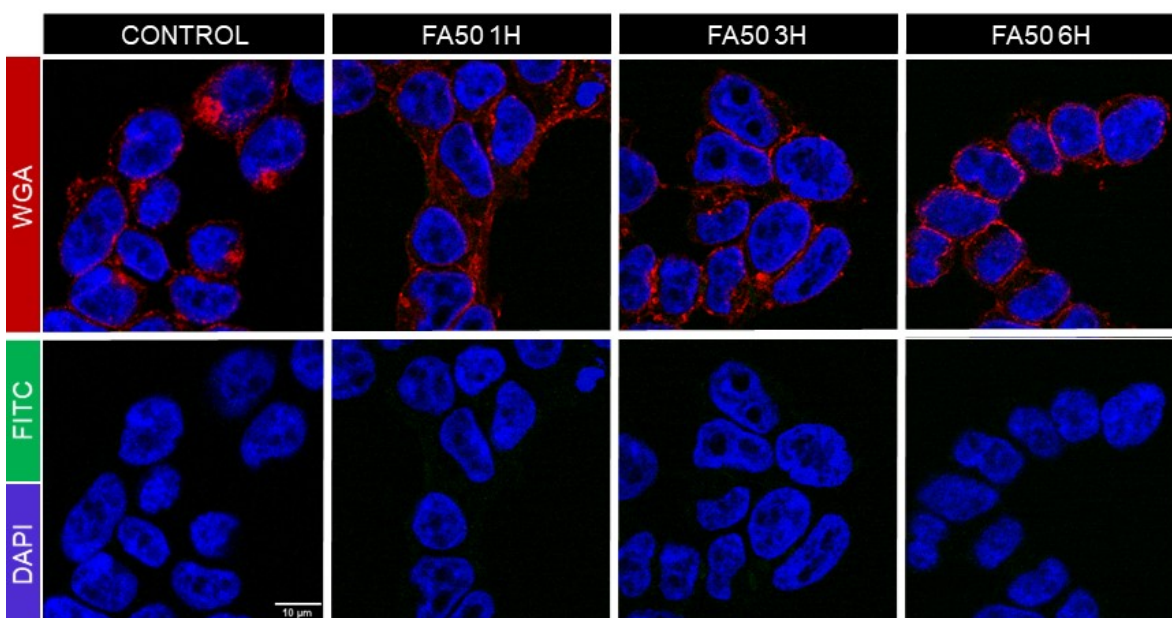
**Figure S5.** Confocal images for HEK cells incubated for 1 h, 3 h and 6 h with 0.5 mM solutions of the acetylated PAMAM dendrimer with a nominal substitution degree of 25% (ACE25).



**Figure S6.** Confocal images for HEK cells incubated for 1 h, 3 h and 6 h with 0.5 mM solutions of the acetylated PAMAM dendrimer with a nominal substitution degree of 50% (ACE50).

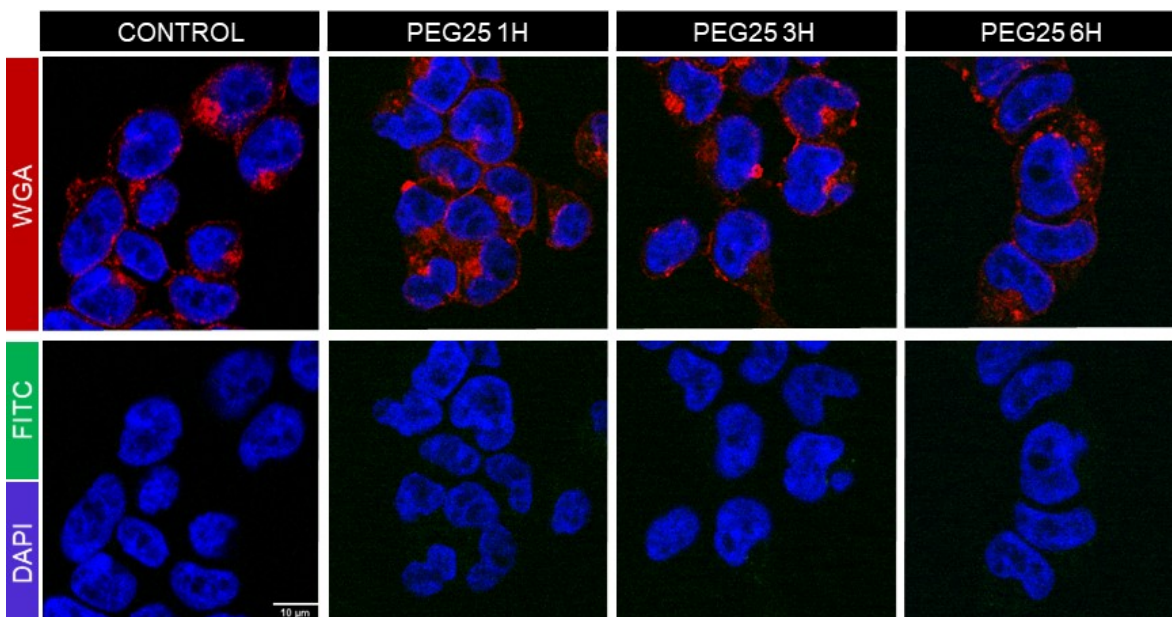


**Figure S7.** Confocal images for HEK cells incubated for 1 h, 3 h and 6 h with 0.5 mM solutions of the PAMAM dendrimer conjugated with folic acid with a nominal substitution degree of 25% (FA25).

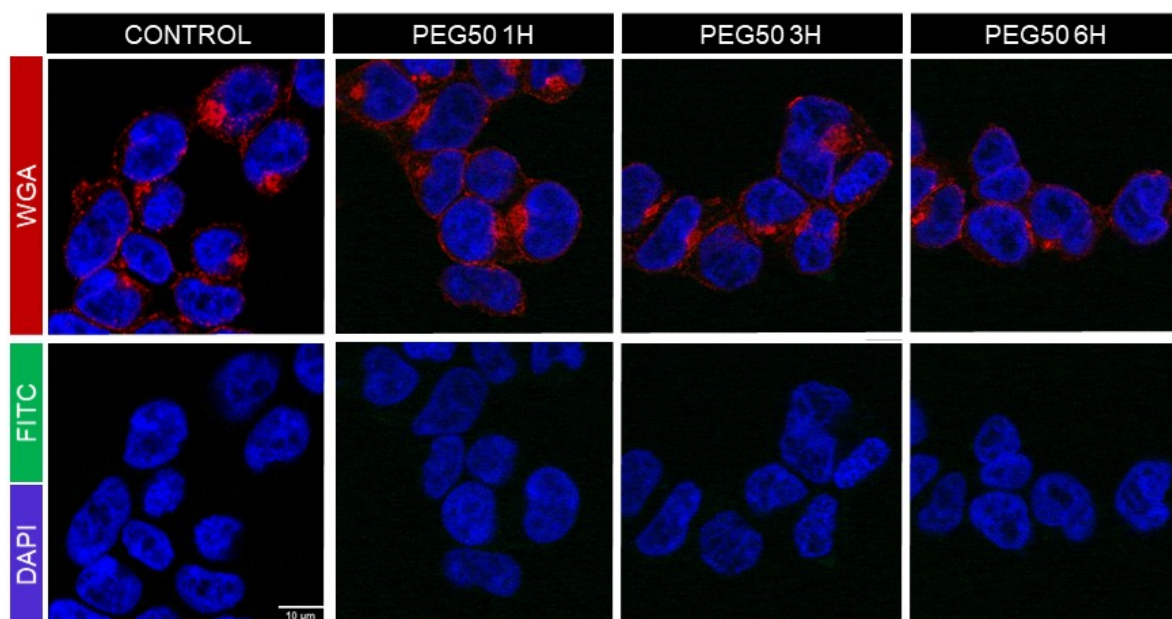


**Figure S8.** Confocal images for HEK cells incubated for 1 h, 3 h and 6 h with 0.5 mM solutions of the PAMAM dendrimer conjugated with folic acid with a nominal substitution degree of 50% (FA50).





**Figure S9.** Confocal images for HEK cells incubated for 1 h, 3 h and 6 h with 0.5 mM solutions of the PEGylated PAMAM dendrimer with a nominal substitution degree of 25% (PEG25).



**Figure S10.** Confocal images for HEK cells incubated for 1 h, 3 h and 6 h with 0.5 mM solutions of the PEGylated PAMAM dendrimer with a nominal substitution degree of 50% (PEG50).