Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Material

A comparison of acyl-moieties for noncovalent functionalization of PLGA and PEG-PLGA nanoparticles with a cell-penetrating peptide

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Running head: C16-hLF enhances PLGA and PEG-PLGA nanoparticle uptake.

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Supplemental Materials & Methods

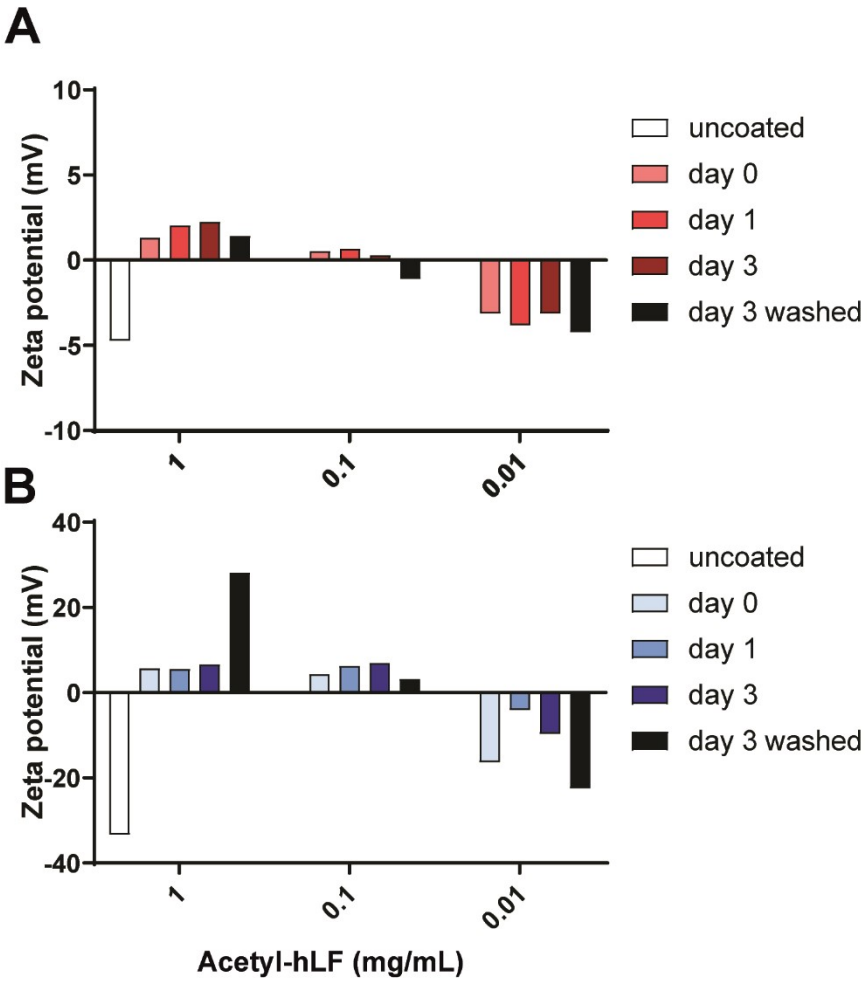
Quantification of pH, zeta potential and hLF adsorbed onto the nanoparticle surface

FA-PLGA nanoparticles were prepared as described in section 2.2.1. Samples were resuspended at 5 mg/mL in 1 mL of Milli-Q water and 50 mM Hepes (Sigma Aldrich, Taufkirchen, Germany) at pH 8. Next, 450 μL of each sample was diluted to 2.5 mg/mL with corresponded solution (Milli-Q water and Hepes) and incubated with 50 μL of 1, 0.1, and 0.01 mg/mL acetyl-hLF. Incubation with peptides was done overnight at RT. To remove the unbound peptides after the coating, the particles were centrifuged at 16,000 *g* and redispersed in the same buffer in which they were before.

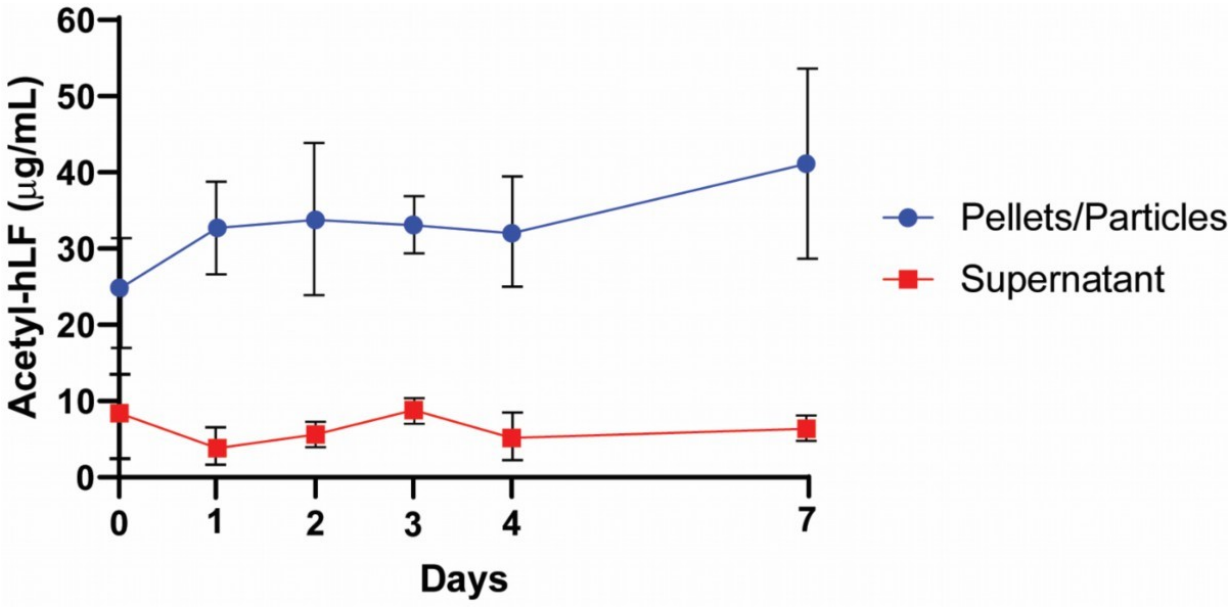
Measurements were conducted on days 0, 1, 2, 3, 4 and 7. For a measurement 100 µL were taken from the particle suspension and diluted 1:10 in Milli-Q water. From this resulting diluted particle suspension pH and zeta potential (measured with a Malvern Zetasizer ZS, Malvern Instruments, Worcestershire, United Kingdom) were measured. To determine particle-bound and free peptide, 200 µL of the suspension were centrifuged (24,000 *g*, 20-25 min) and kept for a BCA assay. The pellet was washed again with MQ water and then redispersed in 200 µL MQ water. The BCA assay was conducted with a QuantiPro BCA Assay Kit (Sigma Aldrich, Taufkirchen, Germany). The BCA solution was mixed according to the manual, then samples (either 150 µL particle suspension, or 150 µL supernatant) were mixed with 150 µL BCA solution in a 96-well plate and incubated for 2 hours at 37 ° C. After incubation, sample intensities were measured with a TECAN Reader Infinite 200 PRO (Tecan Group, Männedorf, Switzerland) at a wavelength of 562 nm. Furthermore, intensities for pure Hepes and

uncoated nanoparticle solution were determined and subtracted from the respective sample to correct for background.

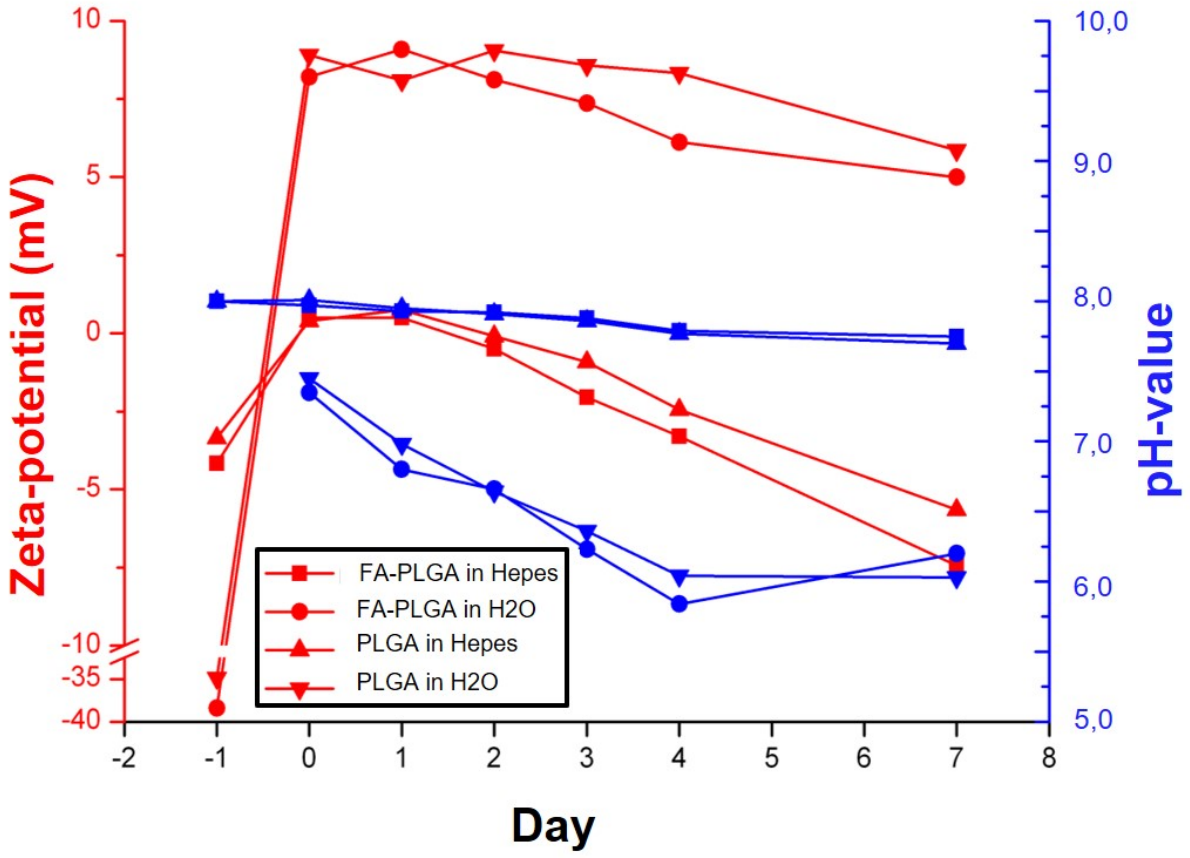
Supplemental Figures



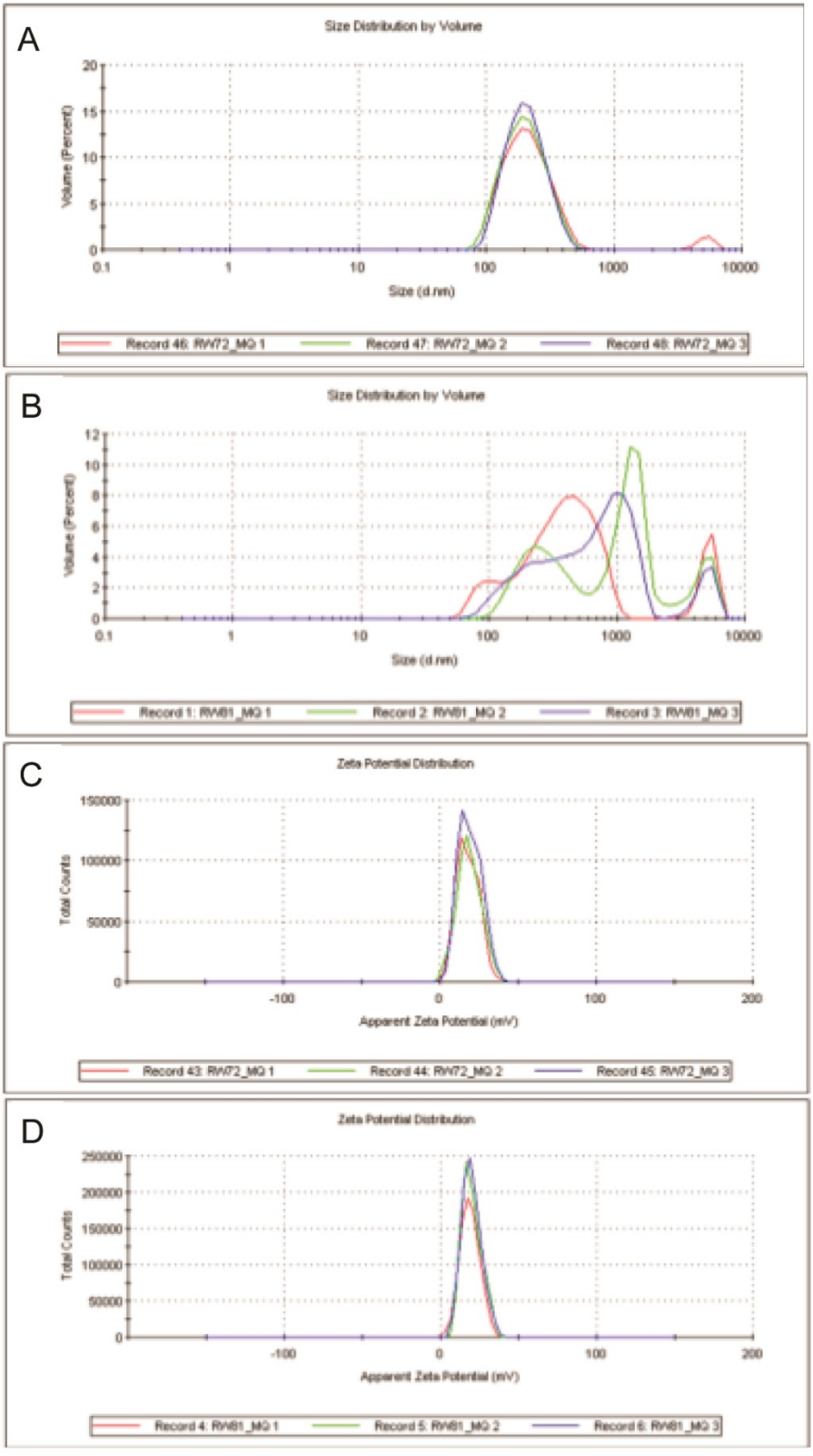
Supplemental Figure 1. **Time- and concentration dependence of zeta potentials.** PLGA (Resomer RG 503 H) nanoparticles were prepared in water but resuspended in (A) water or (B) HEPES. Suspensions of particles were incubated with the indicated concentrations of acetyl peptide (acetyl-hLF) for 1h at RT and zeta potentials of the particles were measured on the indicated days. To remove loosely bound peptide, on day 3, parts of the particle suspension were washed and the zeta potentials were measured again. There is no explanation for the strong increase of zeta potential for particles at 1 mg/mL peptide after washing. This experiment was only performed once.



Supplemental Figure 2. **Amounts of acetyl-hLF peptide adsorbed to the surface of PLGA particles and released into the supernatant.** The amount of peptide in the supernatant and on the particles was determined by a BCA assay. Values of day 0 were taken right after the coating process. N=3, error bars describe standard deviations.

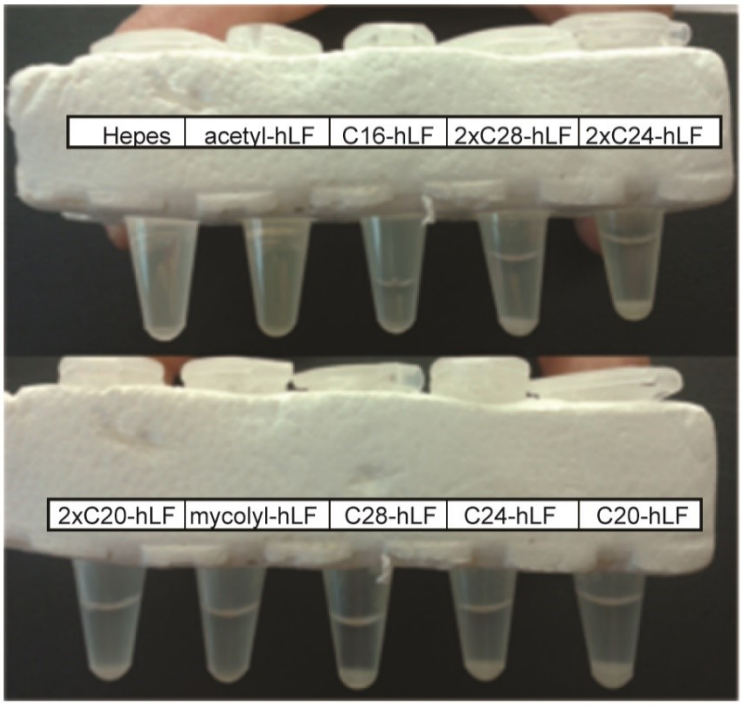


Supplemental Figure 3. **Zeta potentials and pH values of acetyl-hLF-coated PLGA particles over time.** Measurements were performed in comparison over 8 days in 1:10 diluted in different solutions. Day -1 shows the values prior to nanoparticle coating, day 0 the values directly after coating. Storing the particles in buffered solution ensured a stable pH and thereby prevented acidification through degradation of PLGA.

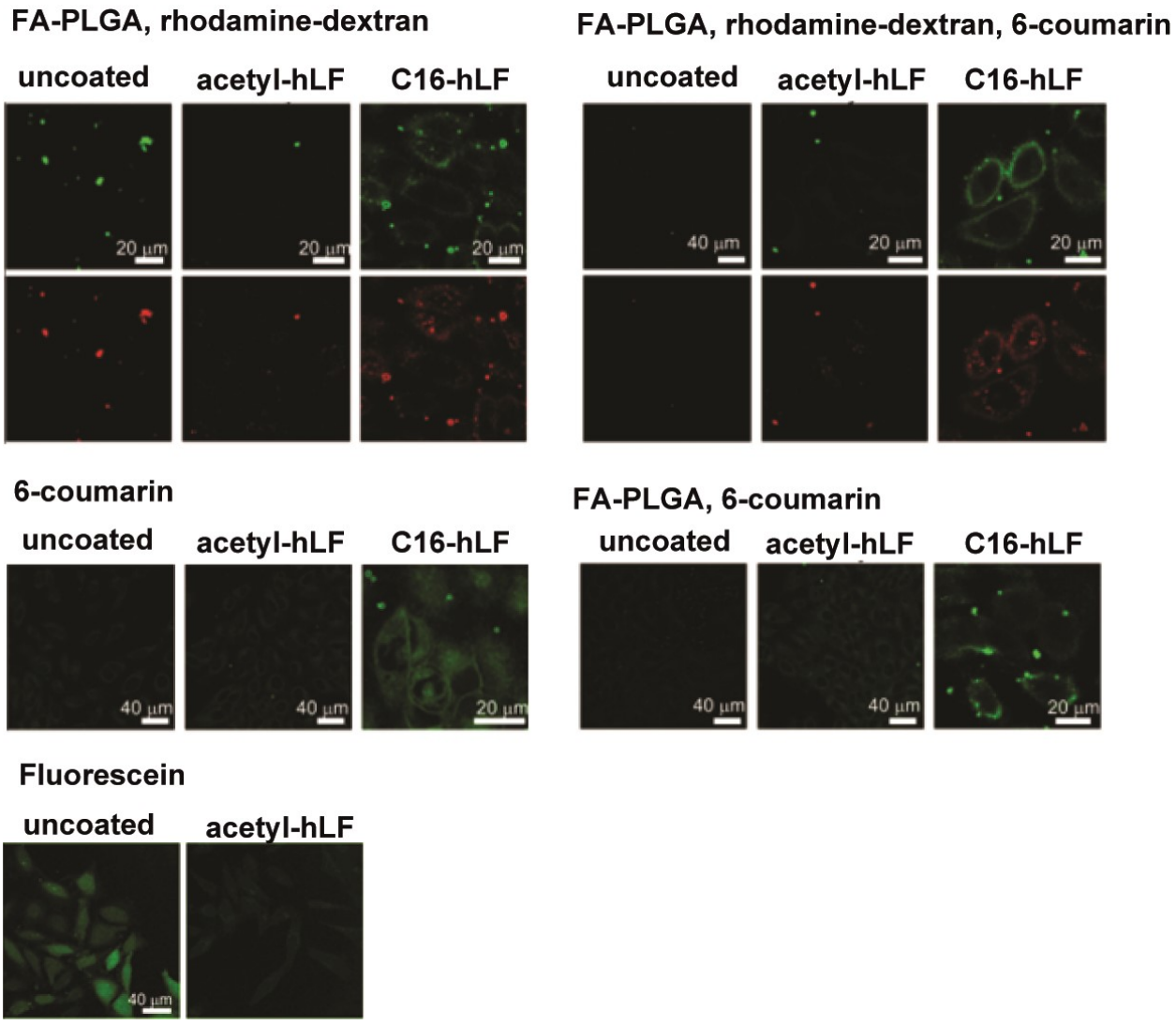


Supplemental Figure 4. **PLGA nanoparticle size and surface net charge after functionalization with acylated peptides.** Size distribution and zeta-potential of

PLGA nanoparticles functionalized with (A and C) C16-hLF, and (B and D) 2xC20-hLF.



Supplemental Figure 5. **Aggregation of defrosted acyl-hLF peptides**.



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88 *Supplemental Figure 6.* **Comparison of different fluorescent compounds for**
89 **particle labelling.** HeLa cells were incubated for 2 h with 0.4 mg/mL particles. After a
90 comparison of the different combinations of fluorophores, we selected rhodamine-
91 dextran, as the ideal fluorophore to be encapsulated into PLGA particles without
92 significant leakage. Green: FA-PLGA, 6-coumarin, and fluorescein; Red: rhodamine-
93 dextran

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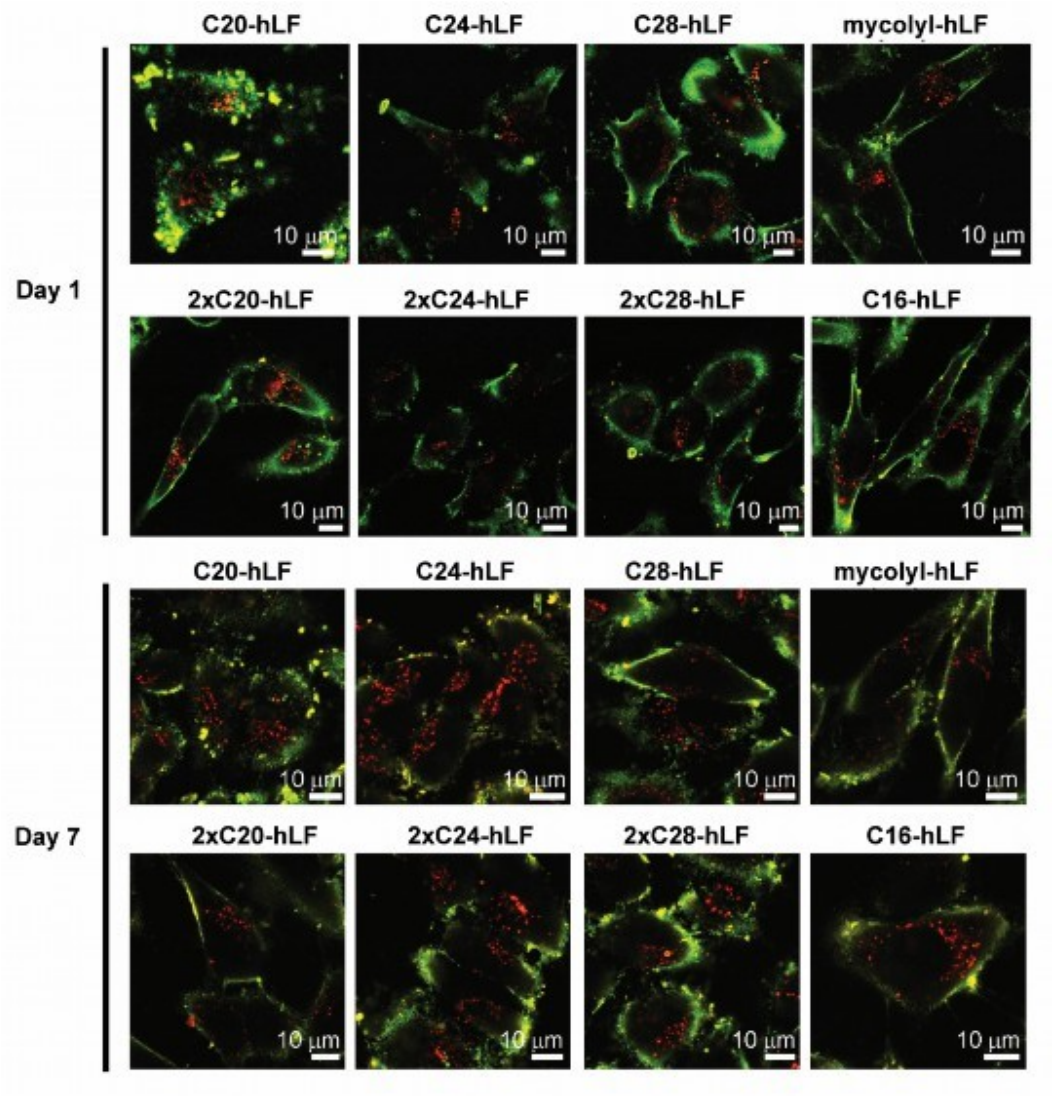
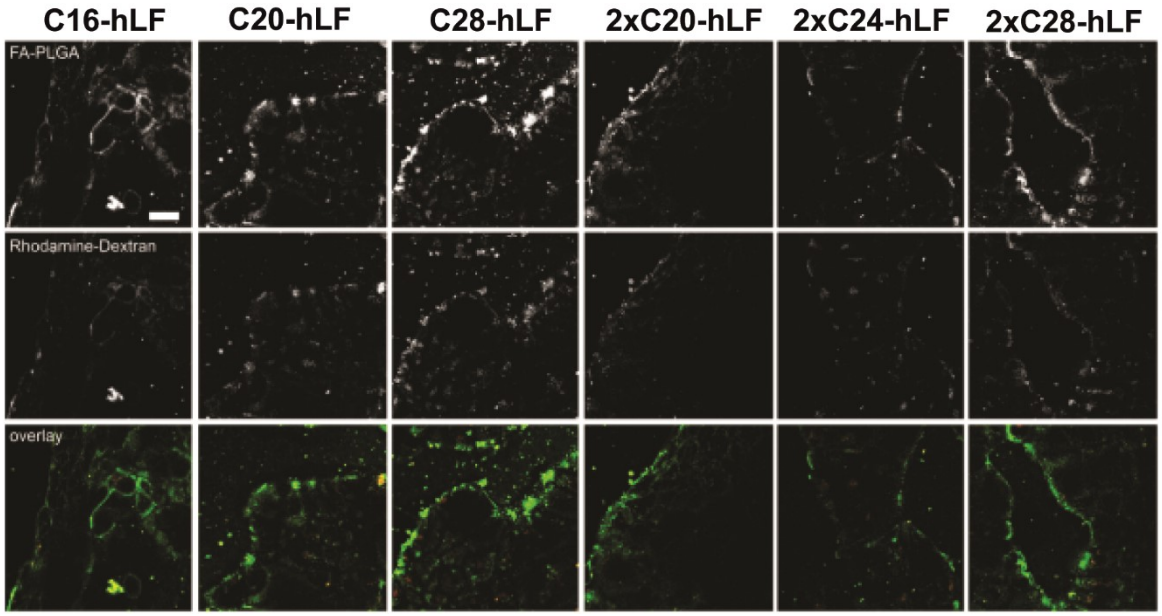
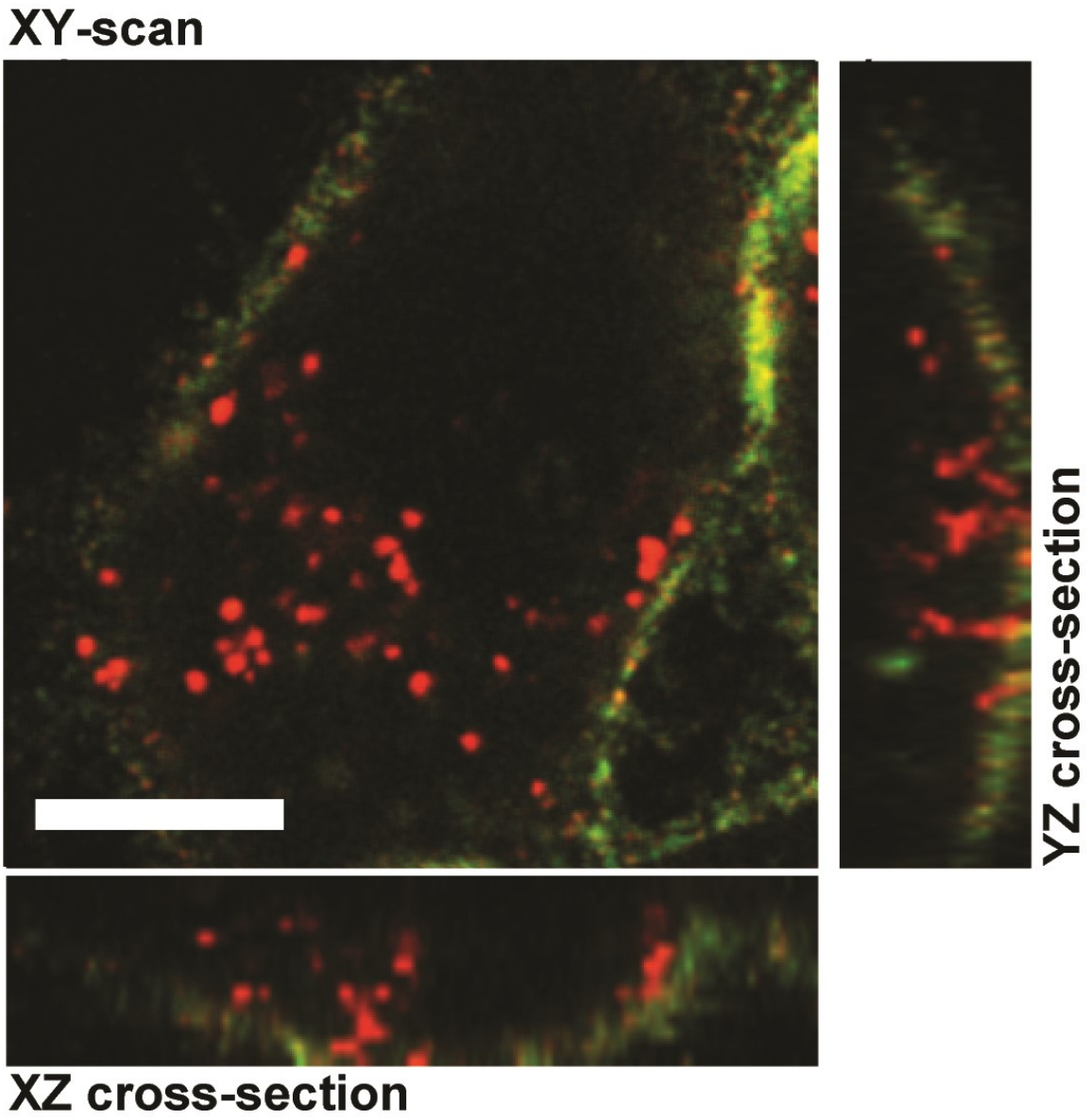


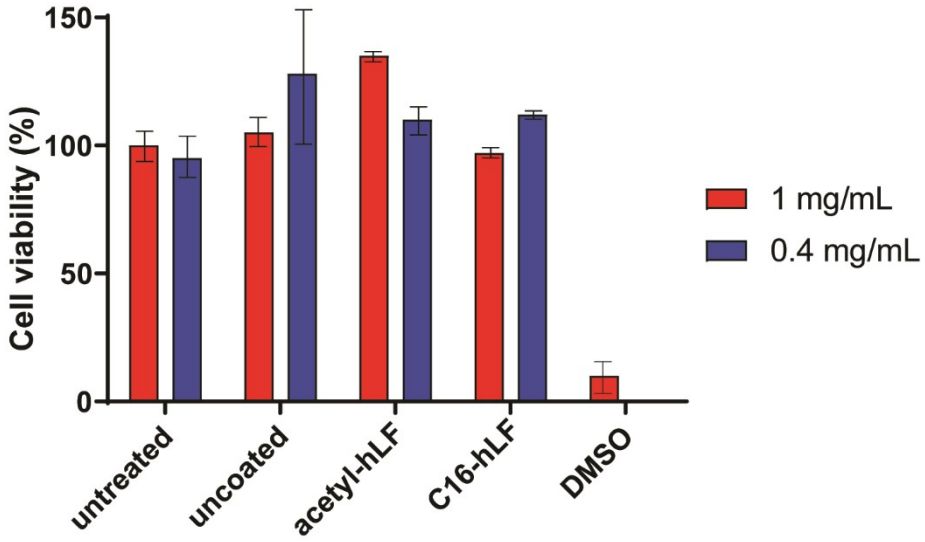
Supplemental Figure 7. **Uptake of FA-PLGA rhodamine-dextran particles into HeLa cells.** Particles were coated with the indicated peptides followed by incubation for the indicated number of days and incubated for 2 h with HeLa cells. Green: FA-PLGA; Red: Rhodamine-dextran.



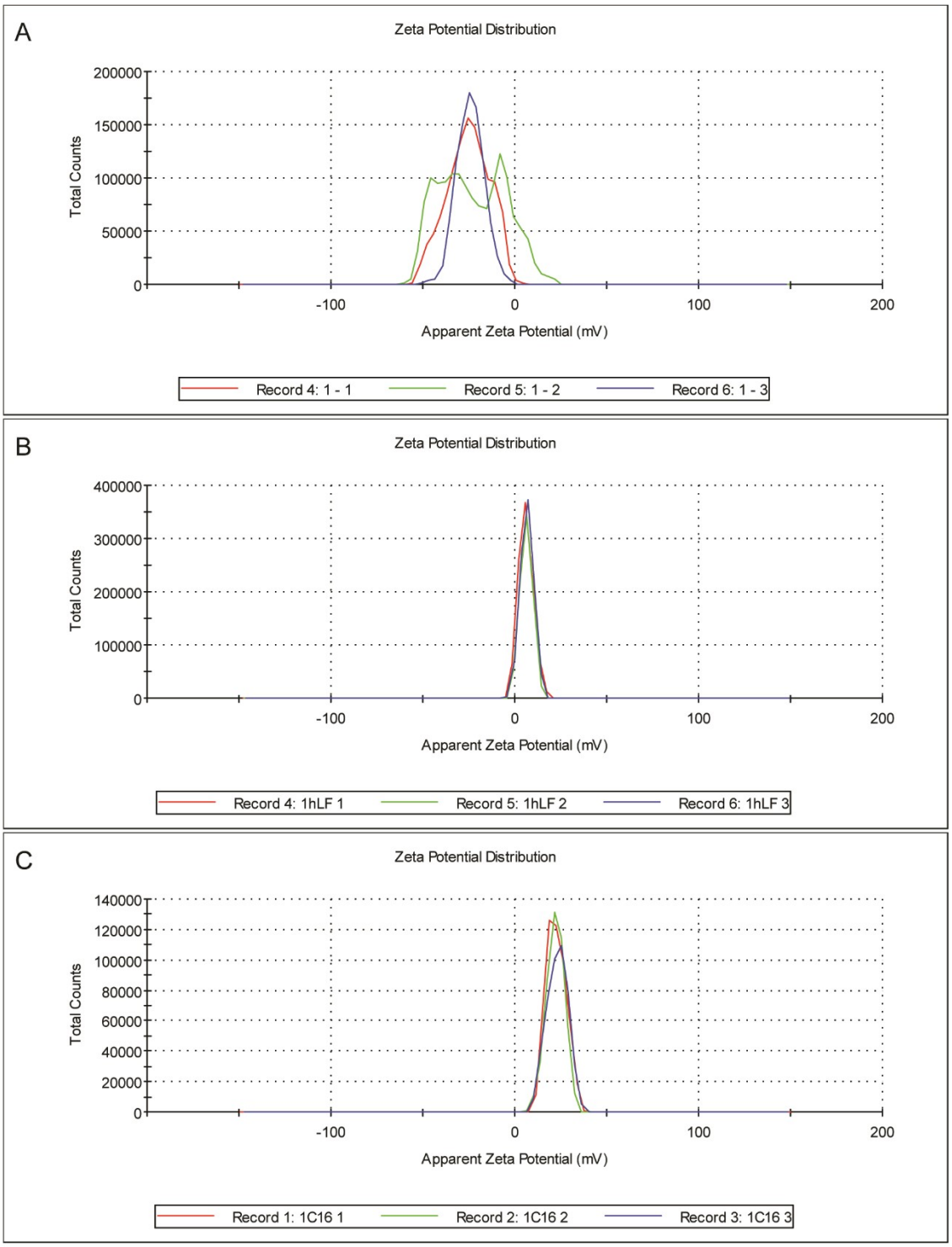
Supplemental Figure 8. **Uptake of FA-PLGA rhodamine-dextran particles in Caco-2 cells.** Particles were coated with the indicated peptides and incubated for 2 h with Caco-2 cells. Scale bar denotes 40 µm.



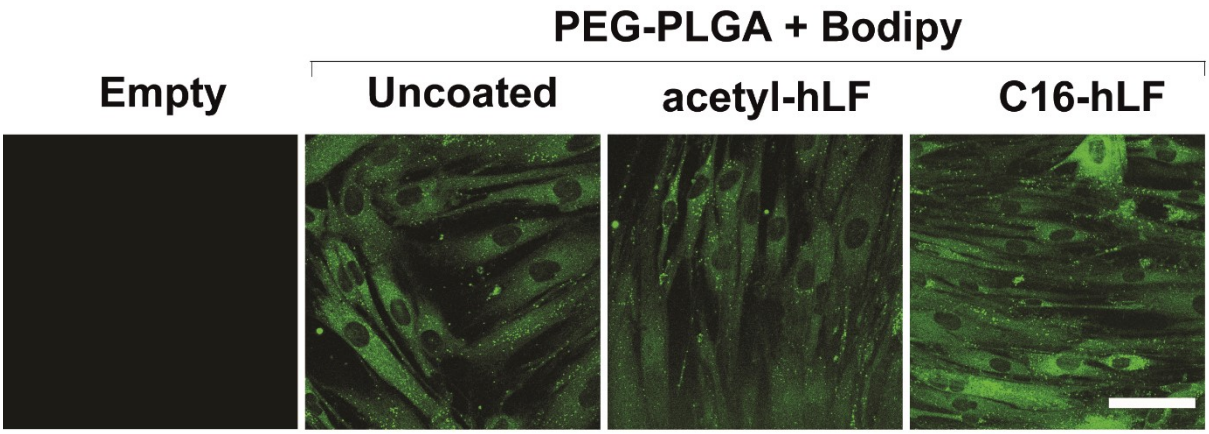
Supplemental Figure 9. **Intracellular distribution of rhodamine-dextran.** Hela cells incubated with 0.4 mg/mL FA-PLGA PLGA rhodamine-dextran particles coated with C16-hLF peptide for 2 hours.



Supplemental Figure 10. **Toxicity of PLGA particles coated with acylated hLF peptides.** HeLa cells were incubated for 24h in RPMI + 10% FCS. Then, the Resazurin assay was performed to determine cell viability. Bars represent one single experiment. Standard deviations were calculated from two replicates in one experiment.



Supplemental Figure 11. **Surface net charge of PEG-PLGA nanoparticles.** Zeta-potential measurement of uncoated (A) PEG-PLGA, and PEG-PLGA functionalized with (B) acetyl-hlF or (C) C16-hLF.



Supplemental Figure 12. **Periodontal ligament cells incubated with Bodipy-loaded PEG-PLGA particles.** C16-hLF coated particles outperformed acetyl-hLF and uncoated PEG-PLGA nanoparticles. Scale bar represents 50 μm. Green: BODIPY.