Electronic Supplementary Material (ESI) for Materials Advances.

SUPPORTING INFORMATION

FOR

Capsid-like biodegradable poly-glycolic acid nanoparticles for a long-time release of nucleic acid molecules

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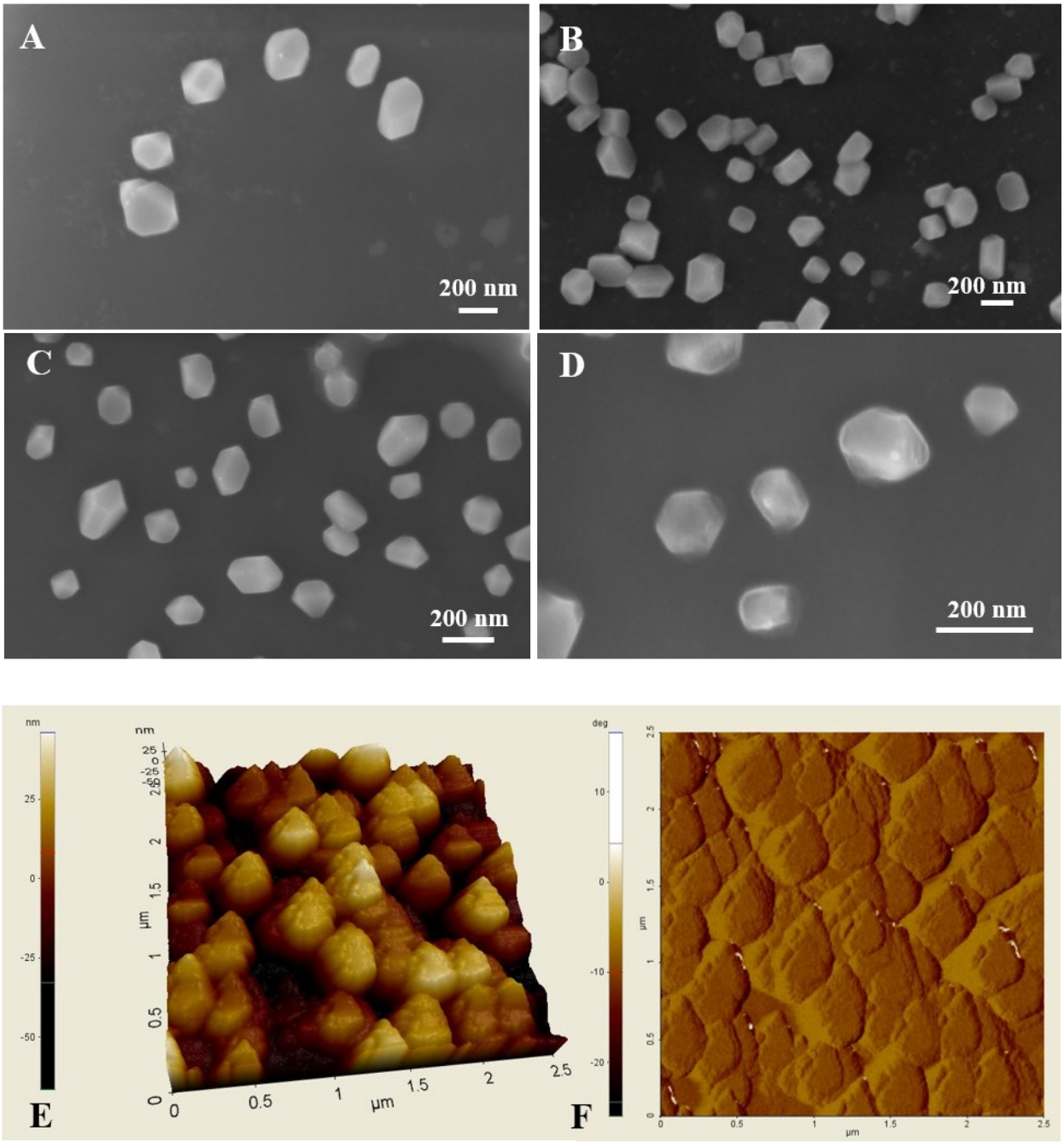

Figure S1. SEM images of empty PGA NPs (A), CH-DNA loaded PGA NPs (B), PEI-DNA loaded PGA NPs (C) and PRM-DNA loaded PGA NPs (*scale bar: 200 nm*). Representative 3D topographical (E) and phase (F) AFM images for PGA NPs (*scale bar: 2.5 µm*). Representative images of three independent experiments are shown.

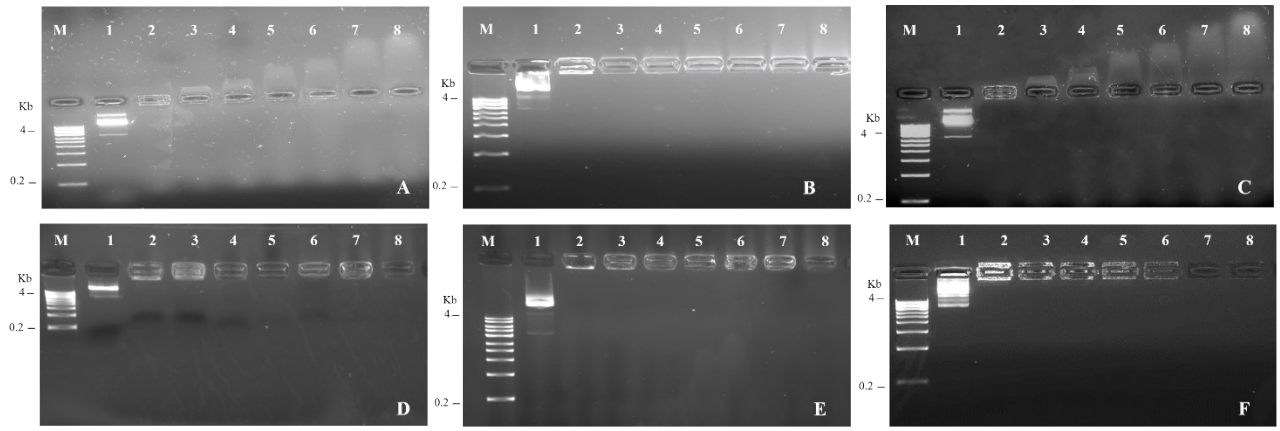

Figure S2. Agarose retardation assay of DNA complexed with polycation polymers (A,B,C) and DNA-loaded PGA NPs (D,E,F) with different DNA: polymer mass ratios. DNA refer to the pT7-EGFP plasmid. A) Lane M: DNA marker1 Kb; Lane 1: naked DNA; Lane 2-8: CH-DNA complexes at a DNA: polymer mass ratio of 1:1, 1:3, 1:5, 1:10, 1:20, 1:50 and 1:100 respectively. E) Lane M: DNA marker 1 Kb; Lane 1: naked DNA; Lane 2-8: PEI-DNA complexes at mass ratio of 1:1, 1:3, 1:5, 1:10, 1:20, 1:50 and 1:100 respectively. F) Lane M: DNA marker 1 Kb; Lane 1: naked DNA; Lane 2-8: PRM-DNA complexes at mass ratio of 1:1, 1:3, 1:5, 1:10, 1:20, 1:50 and 1:100 respectively. D) Lane M: DNA marker1 Kb; Lane 1: naked DNA; Lane 2-8: CH-DNA loaded PGA NPs at a DNA: polymer mass ratio of 1:1, 1:3, 1:5, 1:10, 1:20, 1:50 and 1:100 respectively. E) Lane M: DNA marker 1 Kb; Lane 1: naked DNA; Lane 2-8: PEI-DNA loaded PGA NPs at mass ratio of 1:1, 1:3, 1:5, 1:10, 1:20, 1:50 and 1:100 respectively. F) Lane M: DNA marker 1 Kb; Lane 1: naked DNA; Lane 2-8: PRM-DNA loaded PGA NPs at mass ratio of 1:1, 1:3, 1:5, 1:10, 1:20, 1:50 and 1:100 respectively. A representative of three independent experiments is shown.

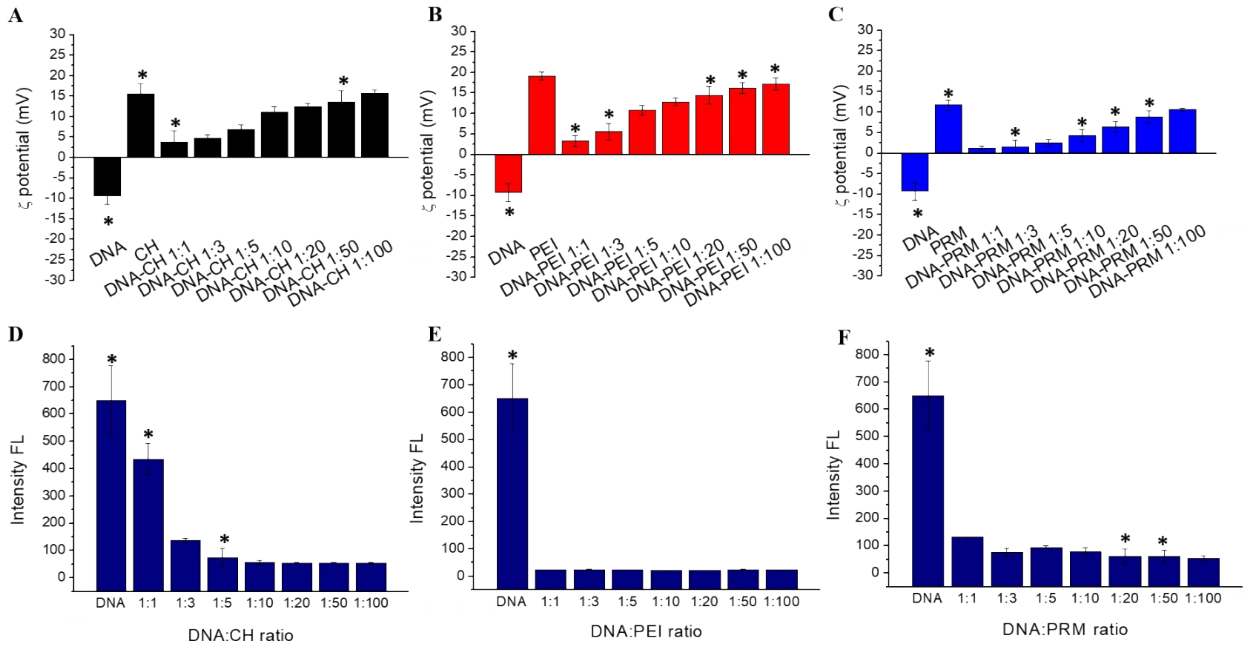

Figure S3. A, B, C) Zeta potential of naked DNA, CH, PEI and PRM, and of DNA-CH complexes (A), DNA-PEI complexes (B) and DNA-PRM complexes (C) with different mass ratio. SYBR safe displacement

assay of naked DNA and DNA-CH complexes (D), DNA-PEI complexes (E) and DNA-PRM complexes (F) with different mass ratio. DNA refers to pT7-EGFP plasmid. Means and sd of three distinct sets of data have been reported; * indicates P-values of <0.05 for t-Student test.

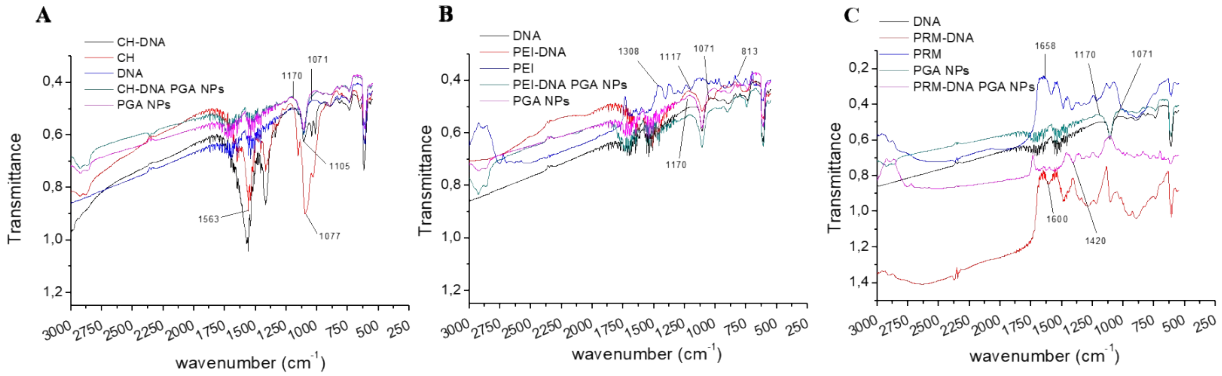

Figure S4. FT-IR analysis of CH-DNA loaded PGA NPs (A), PEI-DNA loaded PGA NPs (B) and PRM-DNA loaded PGA NPs (C).

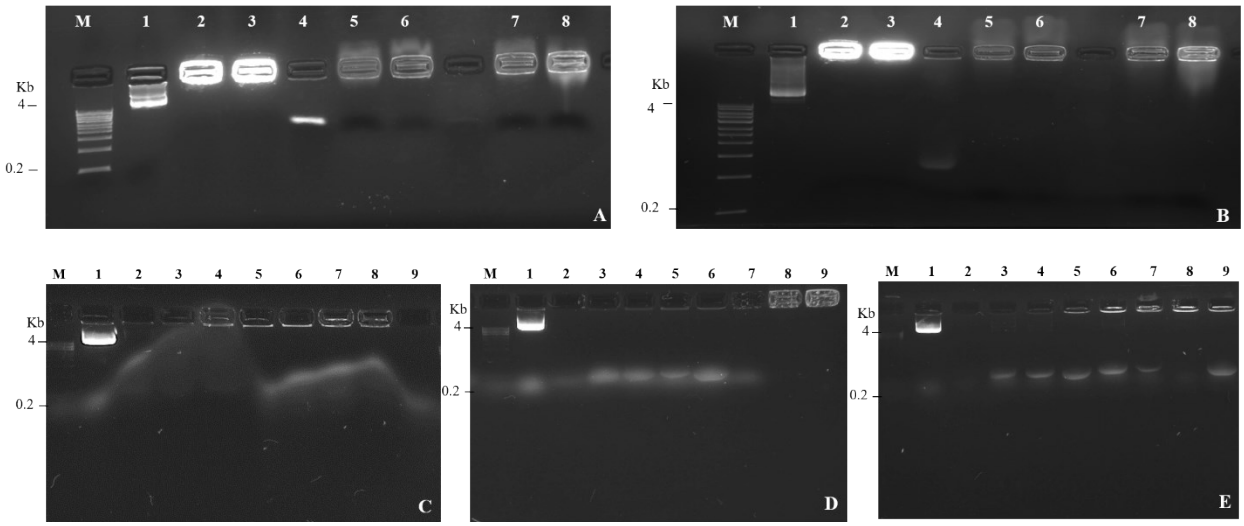

Figure S5. DNase protection assay of PGA NPs loaded with DNA complexed with CH, PEI and PRM after incubation with DNase I for 30 (A) and 60 minutes (B). Lane M: DNA marker 1 Kb; Lane 1: naked DNA; Lanes 2-3 for A and B, represent DNA-CH loaded PGA NPs at different ratio (1:50 and 1:100), after incubation with DNase I for 30 (A) and 60 minutes (B). Lane 4 for A and B represent naked DNA after incubation with DNase I for 30 (A) and 60 minutes (B). Lane 5-6 represent DNA-PEI loaded PGA NPs at different ratio (1:50 and 1:100), after incubation with DNase I for 60 (A) and 90 minutes (B). Lane 7-8 represent DNA-PRM loaded PGA NPs at different ratio (1:50 and 1:100), after incubation with DNase I for 30 (A) and 60 minutes (B).

DNase protection assay of PGA NPs loaded with DNA complexed with CH (C), PEI (D) and PRM (E) after incubation with DNase I for 90 minutes. Lane M: DNA marker 1 Kb; Lane 1: free DNA; Lane 2 for D and E, and lane 9 for C represent naked DNA after incubation with DNase I for 90 minutes. Lanes 2-8 for C and 3-9 for D and E represent, DNA-CH loaded PGA NPs, DNA-PEI loaded PGA NPs and DNA-PRM loaded PGA NPs at different ratio (1:1, 1:3, 1:5, 1:10, 1:20, 1:50, 1:100) respectively, after incubation with DNase I for 90 minutes. DNA refer to pT7-EGFP plasmid. Representative measurements of three independent experiments have been reported.

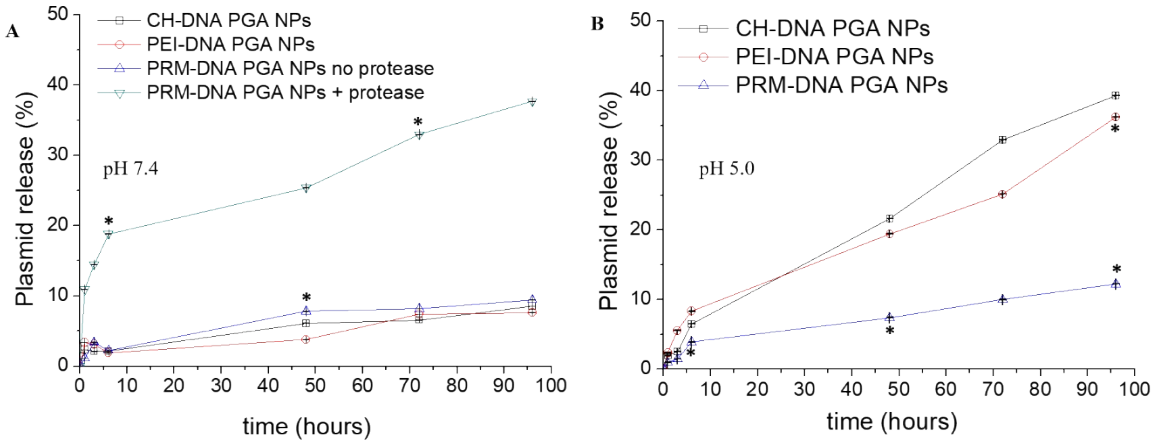

Figure S6. *In vitro* cumulative release (%) at 37°C and pH 7.4 (A) or pH 5.0 (B) of DNA from CH, PEI and PRM loaded PGA NPs over time. DNA refer to pT7-EGFP plasmid. Representative measurements of three distinct sets of data have been reported; * indicates P-values of <0.05 for t-Student test.

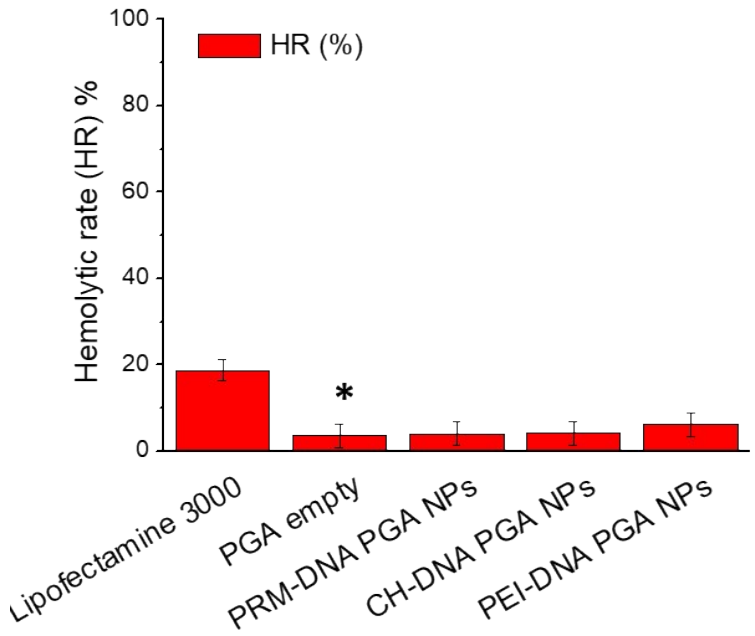

Figure S7. Hemolysis assay performing after 1 hours of incubation at 37°C with DNA-Lipofectamine 3000, empty PGA NPs and DNA-PRM, -CH and -PEI PGA NPs. DNA refer to pT7-EGFP plasmid. Mean ± sd of three independent experiments are shown; * indicates P-values of <0.05 for t-Student test.

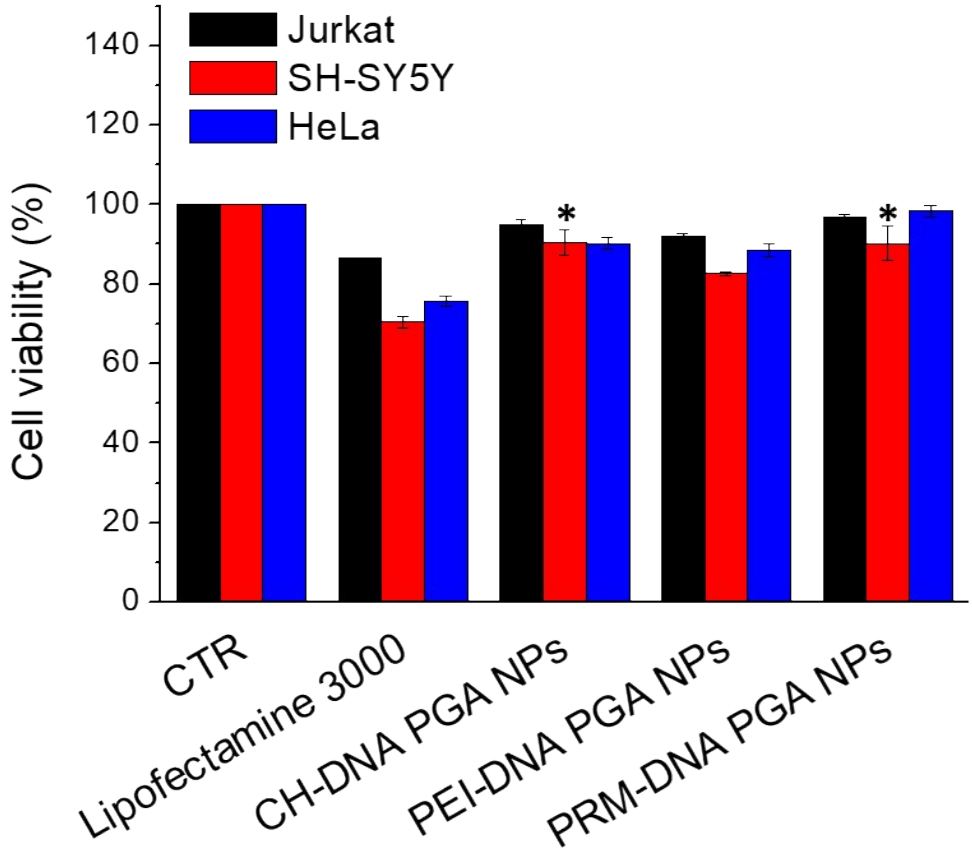

Figure S8. Cytotoxicity analysis of Jurkat, SH-SY5Y and HeLa cells incubated with DNA- lipofectamine 3000, DNA loaded CH-, PEI- and PRM- PGA NPs for 48 hours compared with untreated control cells (CTR). DNA refer to pT7-EGFP plasmid. Representative measurements of three independent experiments have been reported; * indicates P-values of <0.05 for t-Student test.

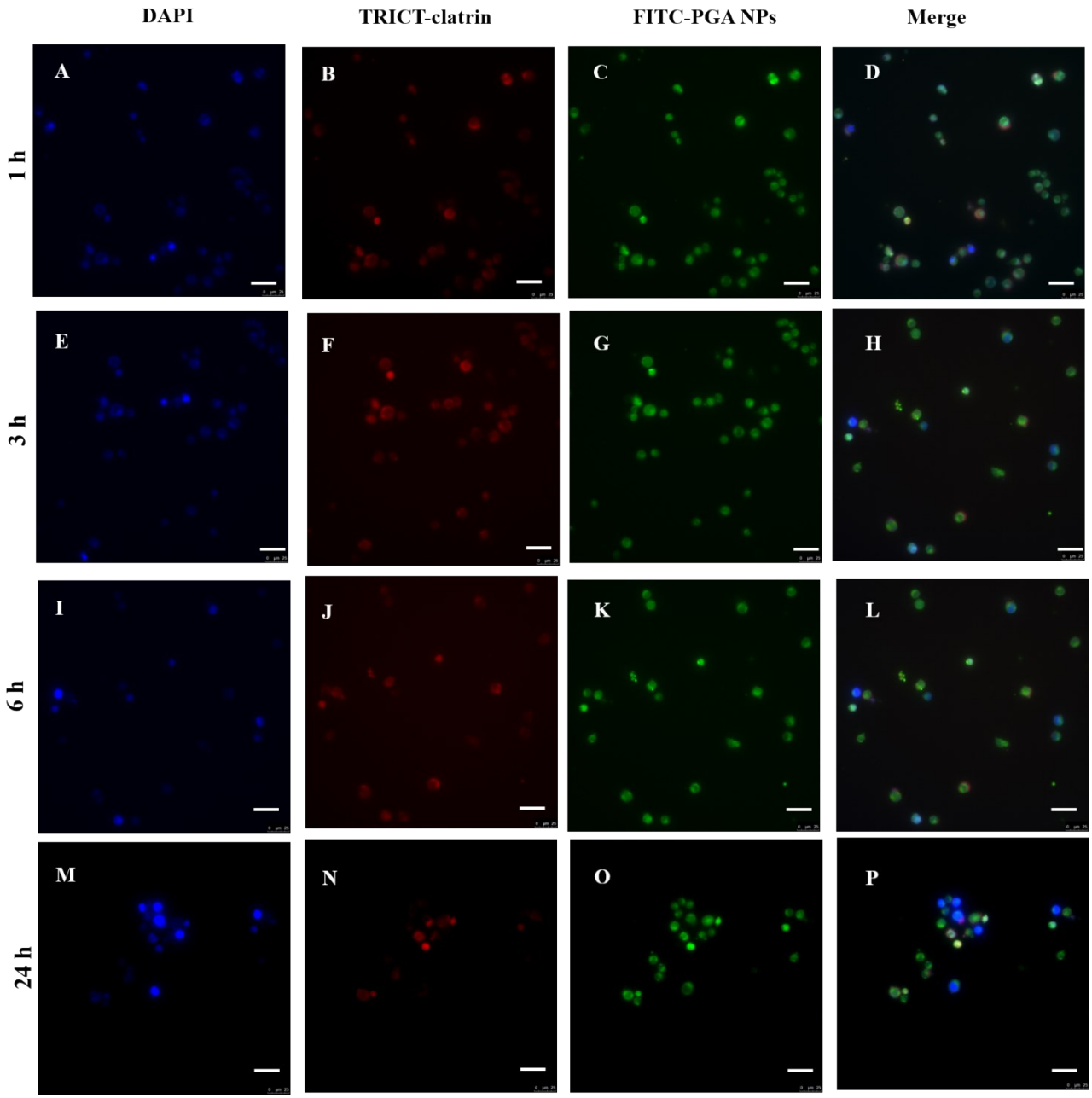

Figure S9. Qualitative colocalization analysis by confocal laser scanning microscopy between PGA NPs (FITC, Green) and clatrin (Red) over time for Jurkat cells. Cell nuclei were stained with DAPI (blue). Scale bars: 25 µm. A representative result of three independent experiments is shown.

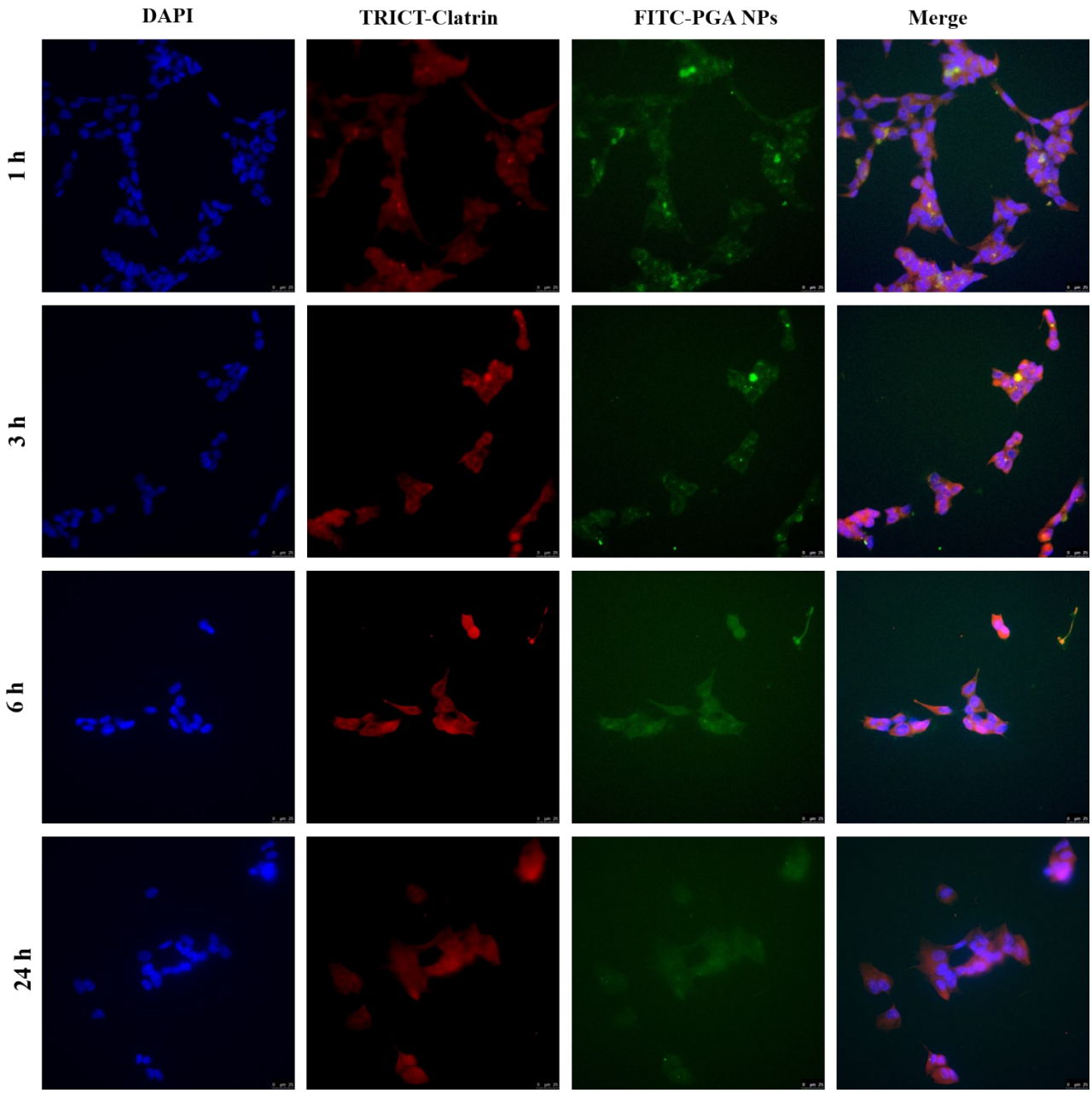

Figure S10. Qualitative colocalization analysis by confocal laser scanning microscopy between PGA NPs (FITC, Green) and clatrin (Red) over time for HeLa cells. Cell nuclei were stained with DAPI (blue). Scale bars: 25 µm. A representative result of three independent experiments is shown.

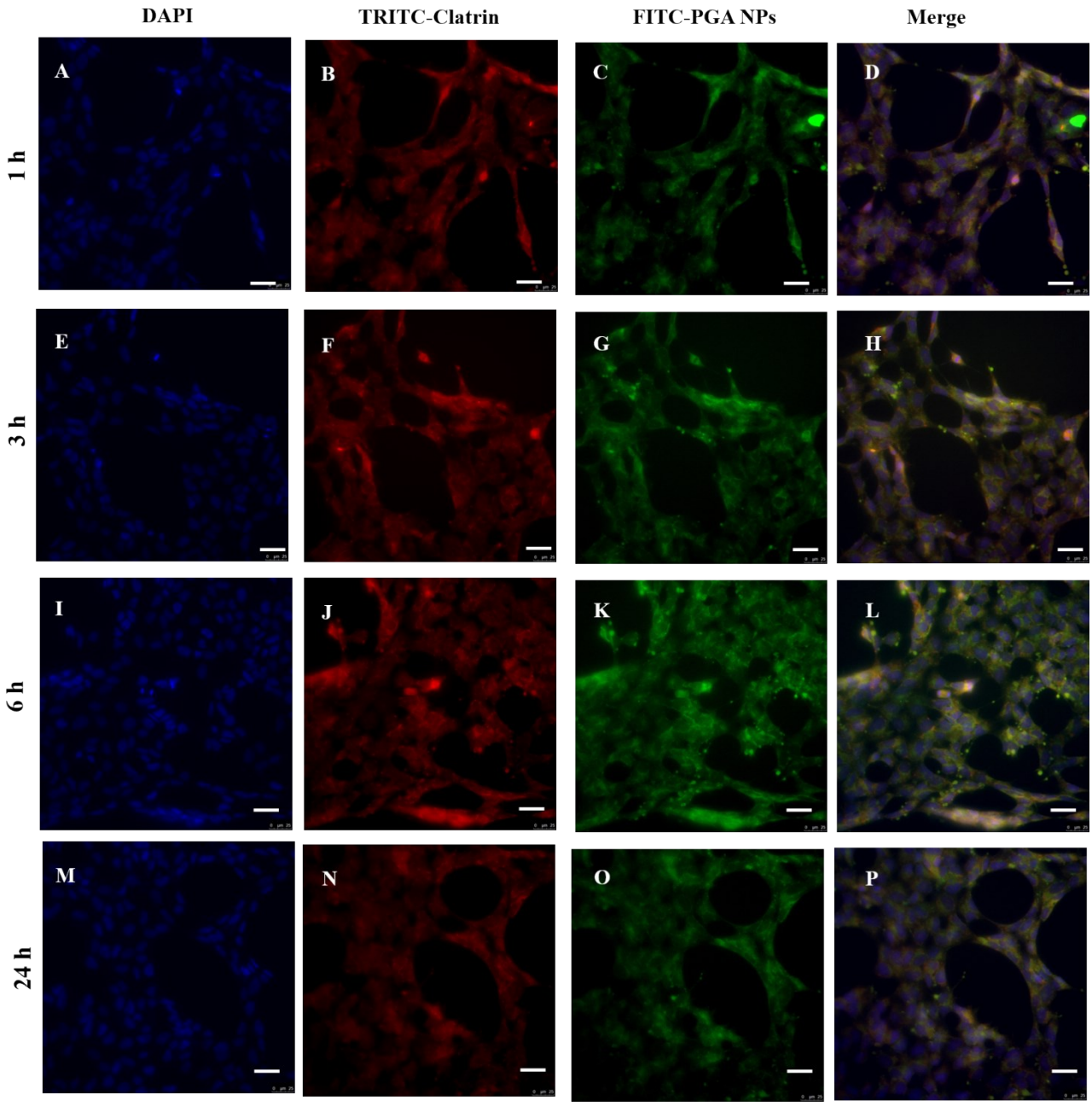

Figure S11. Qualitative colocalization analysis by confocal laser scanning microscopy between PGA NPs (FITC, Green) and clatrin (Red) over time for SH-SY5Y cells. Cell nuclei were stained with DAPI (blue). Scale bars: 25 µm. A representative result of three independent experiments is shown.

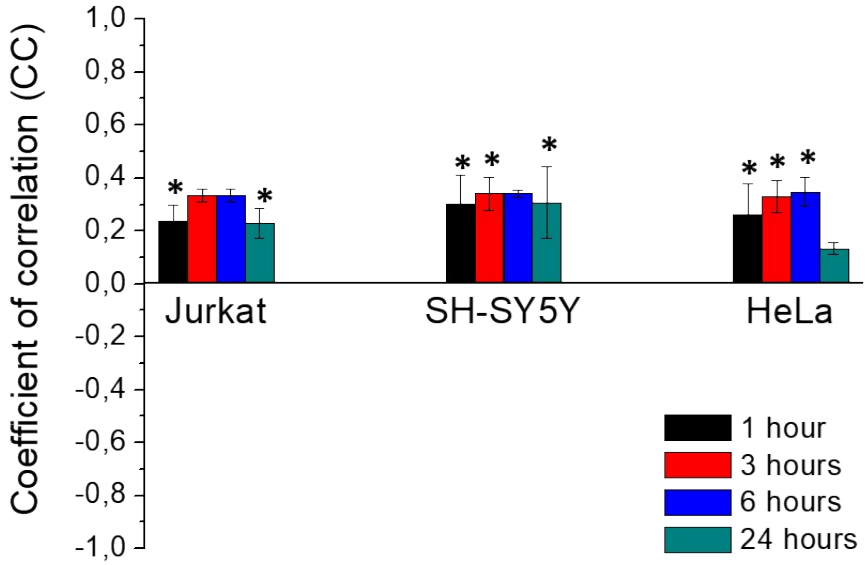

Figure S12. Quantitative colocalization analysis (expressed as coefficient of correlation, CC) between PGA NPs and caveolin over time for Jurkat, SH-SY5Y and HeLa cells. The coefficient of Correlation (CC) ranges from -1 to 1, where 1 means the perfect overlap, and 0 means random distribution. Ten different fields were randomly selected for each sample, and three distinct experiments were performed; * indicates P-values of <0.05 for t-Student test.

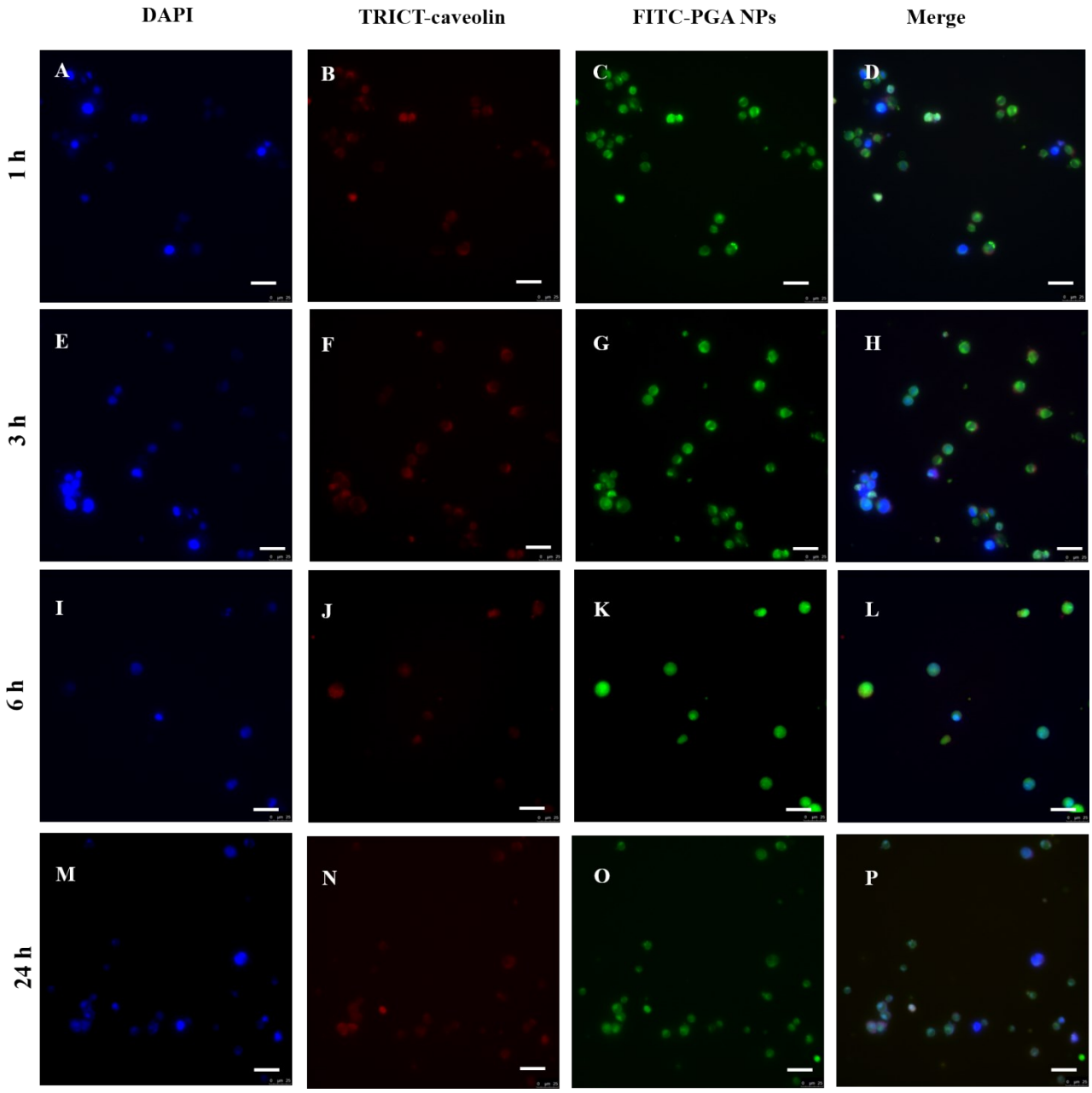

Figure S13. Qualitative colocalization analysis by confocal laser scanning microscopy between PGA NPs (FITC, Green) and caveolin (Red) over time for Jurkat cells. Cell nuclei were stained with DAPI (blue). Scale bars: 25 µm. A representative result of three independent experiments is shown.

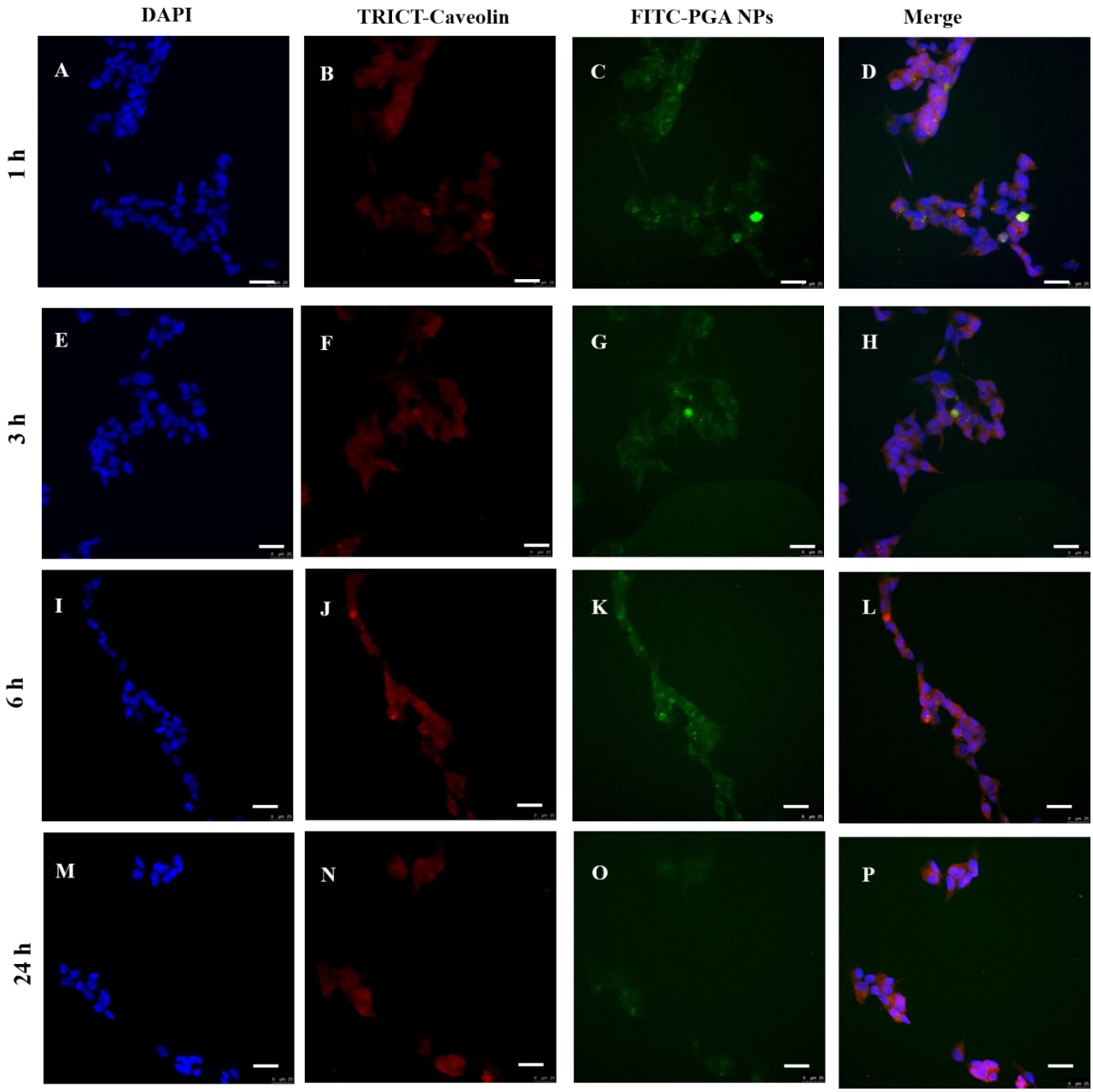

Figure S14. Qualitative colocalization analysis by confocal laser scanning microscopy between PGA NPs (FITC, Green) and caveolin (Red) over time for HeLa cells. Cell nuclei were stained with DAPI (blue). Scale bars: 25 µm. A representative result of three independent experiments is shown.

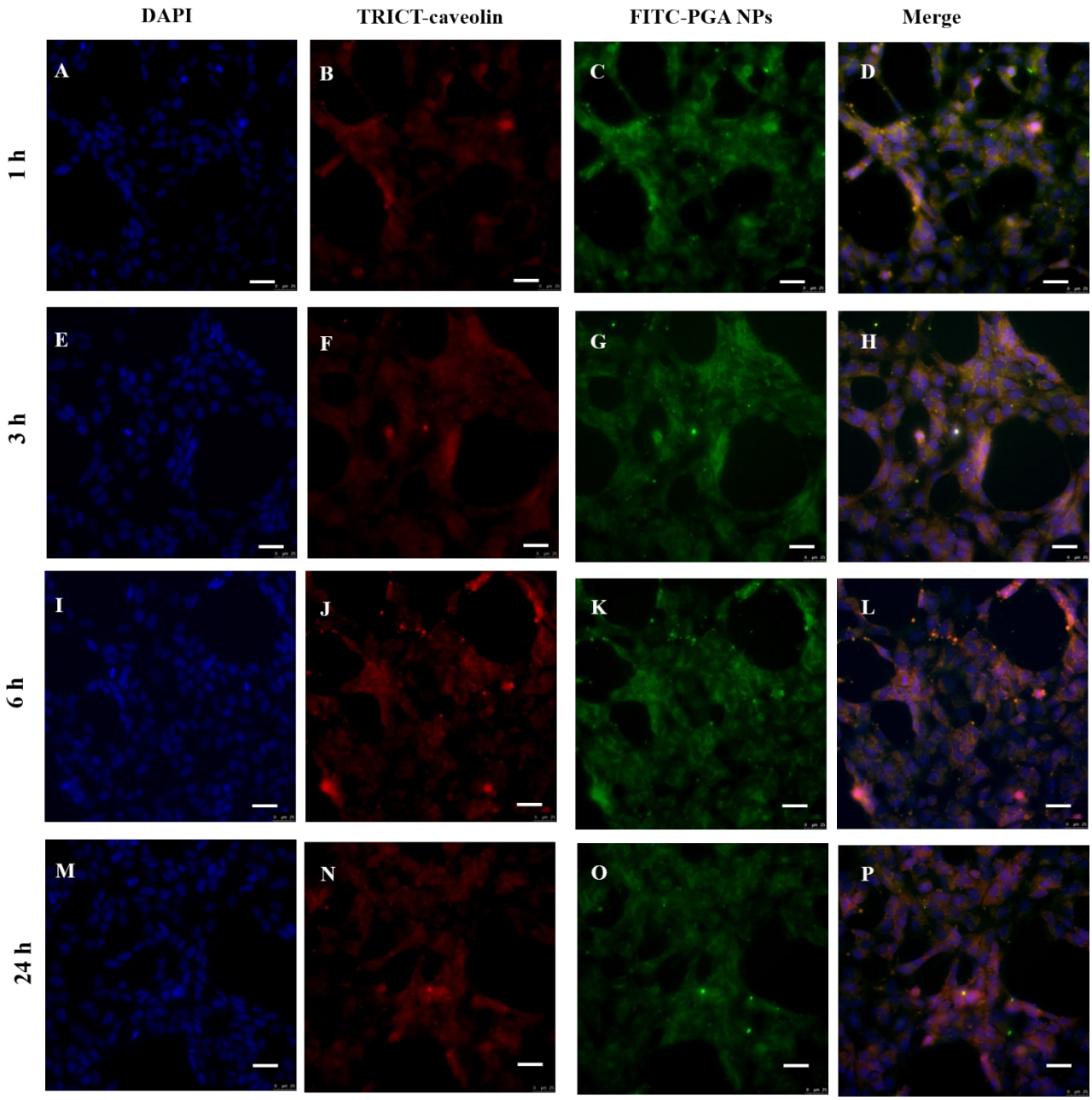

Figure S15. Qualitative colocalization analysis by confocal laser scanning microscopy between PGA NPs (FITC, Green) and caveolin (Red) over time for SH-SY5Y cells. Cell nuclei were stained with DAPI (blue). Scale bars: 25 µm. A representative result of three independent experiments is shown.

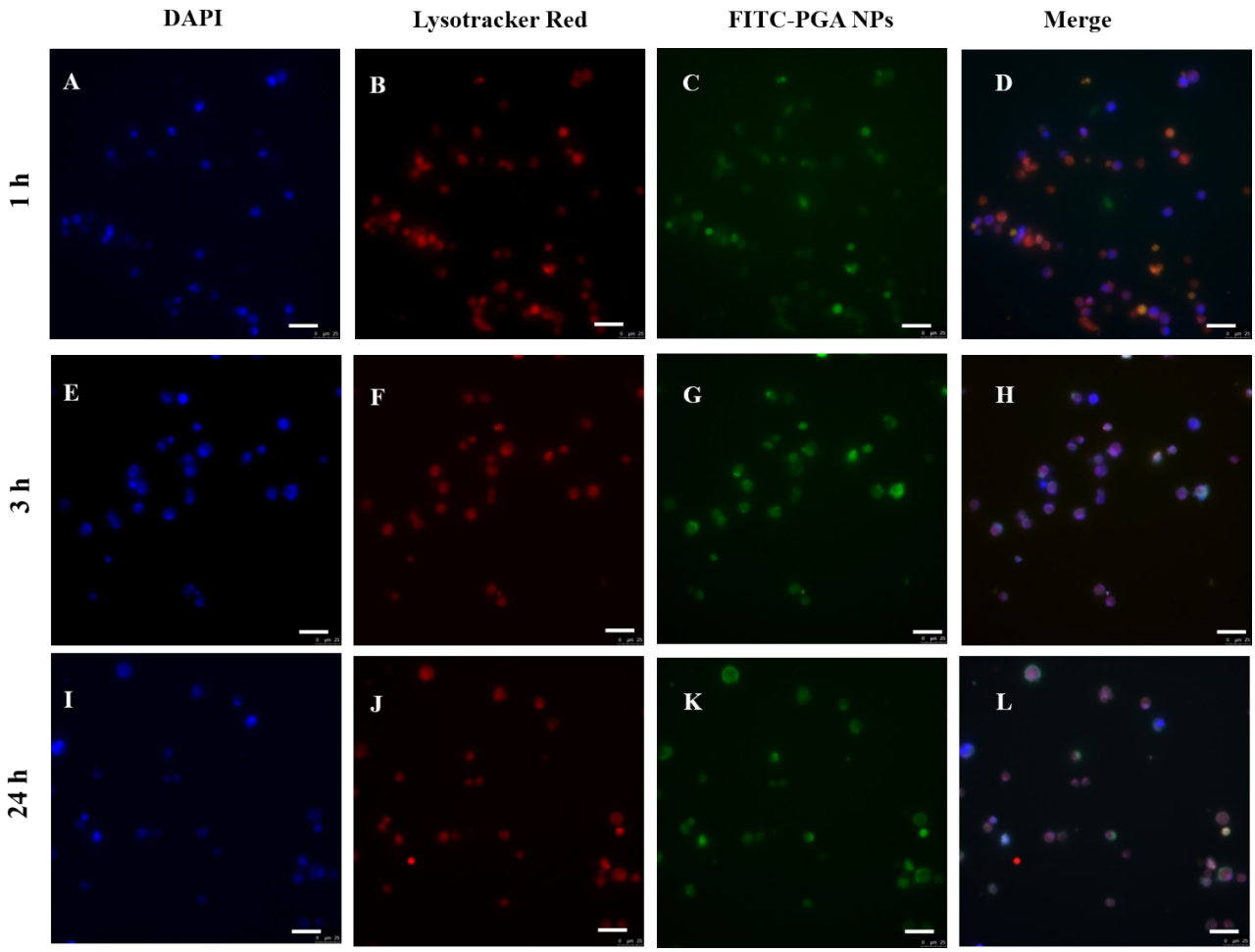

Figure S16. Qualitative colocalization analysis by confocal laser scanning microscopy between PGA NPs (FITC, Green) and Lysotracker (Red) over time for Jurkat cells. Cell nuclei were stained with DAPI (blue). Scale bars: 25 µm. A representative result of three independent experiments is shown.

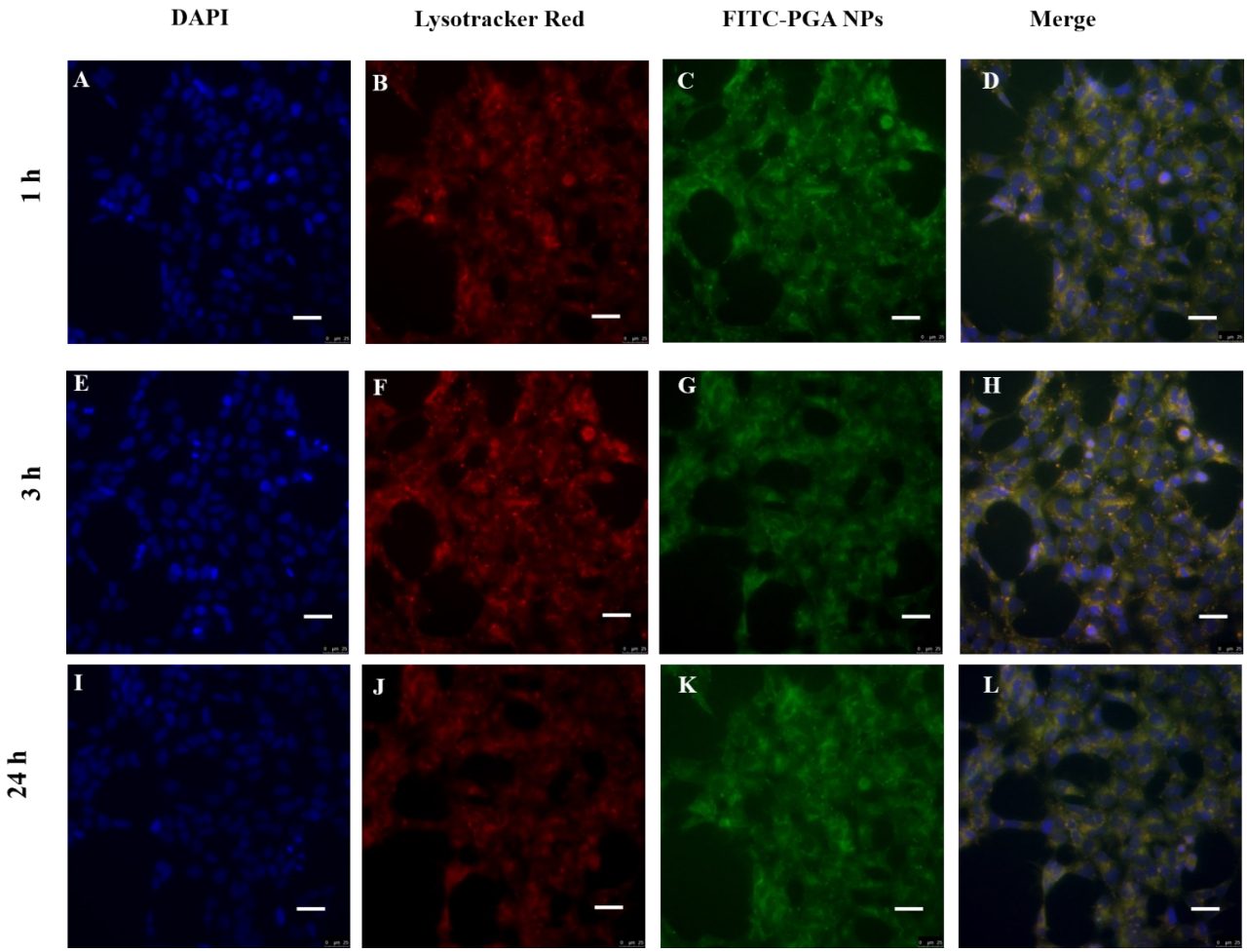

Figure S17. Qualitative colocalization analysis by confocal laser scanning microscopy between PGA NPs (FITC, Green) and Lysotracker (Red) over time for HeLa cells. Cell nuclei were stained with DAPI (blue). Scale bars: 25 µm. A representative result of three independent experiments is shown.

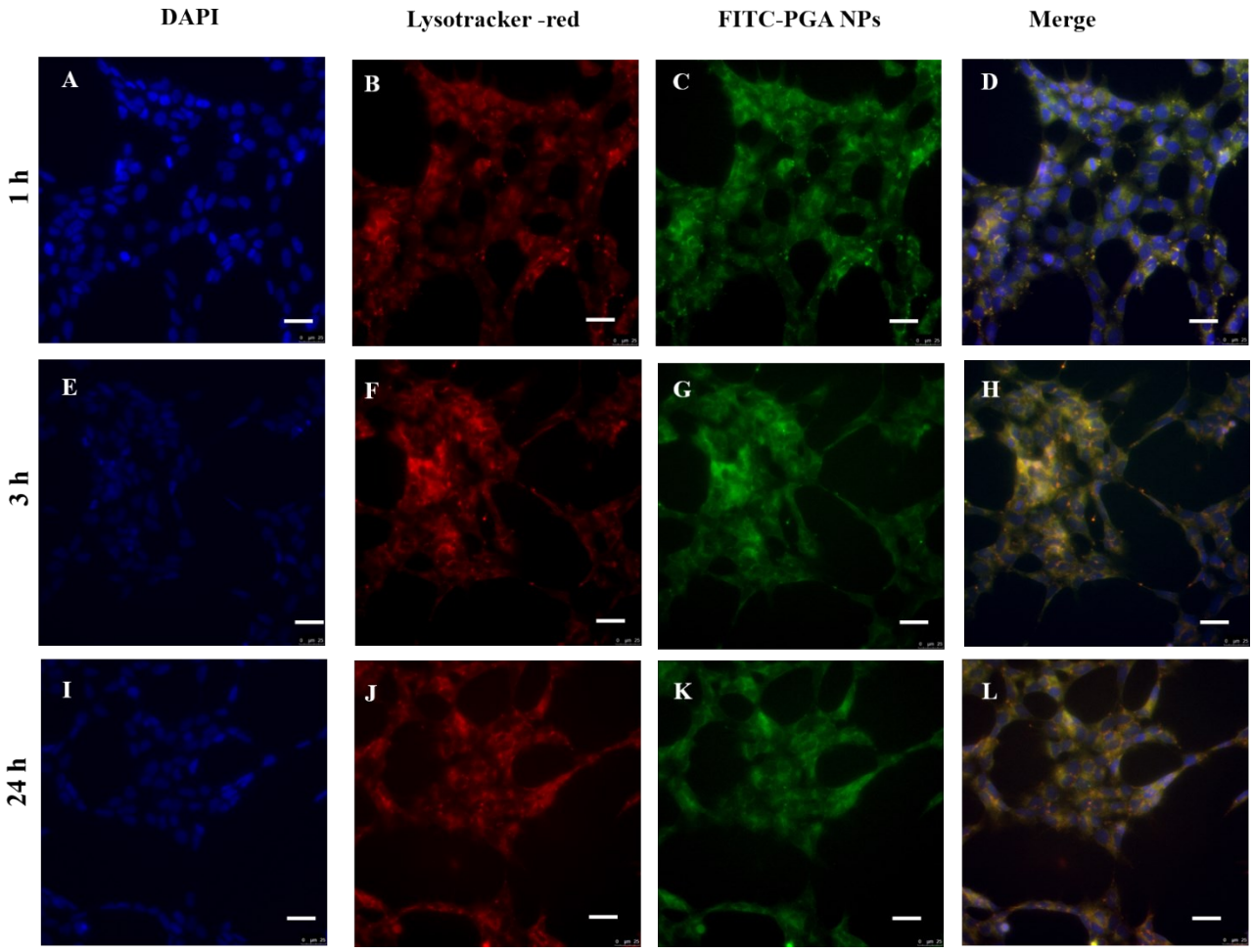

Figure S18. Qualitative colocalization analysis by confocal laser scanning microscopy between PGA NPs (FITC, Green) and Lysotracker (Red) over time for SH-SY5Y cells. Cell nuclei were stained with DAPI (blue). Scale bars: 25 µm. A representative result of three independent experiments is shown.

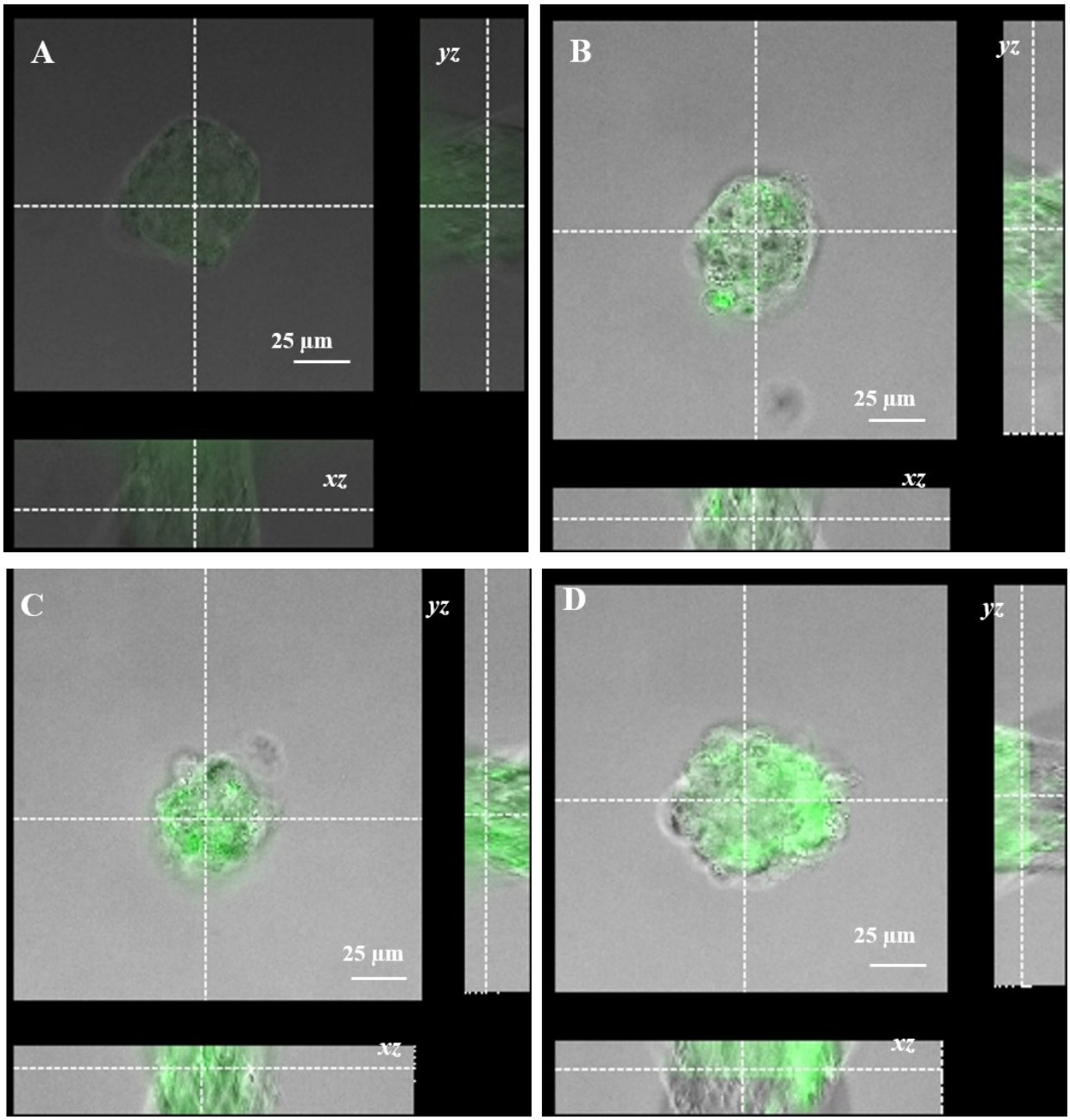

Figure S19. Qualitative transfection efficacy analyzed after 5 days in 3D human neuroblastoma (SH-SY5Y) spheroids treated with DNA-Lipofectamine 3000 (A), PEI-DNA loaded PGA NPs (B), PRM-DNA loaded PGA NPs (C) and CH-DNA loaded PGA NPs (D). z-stack optical sections (yz and xz), obtained through photoluminescence reconstruction in the z-direction with a z-resolution of 200 nm, confirm the spatial distribution of EGFP inside spheroids. Scale bars: 25 µm. DNA refer to pT7-EGFP plasmid A representative result of three independent experiments is shown.

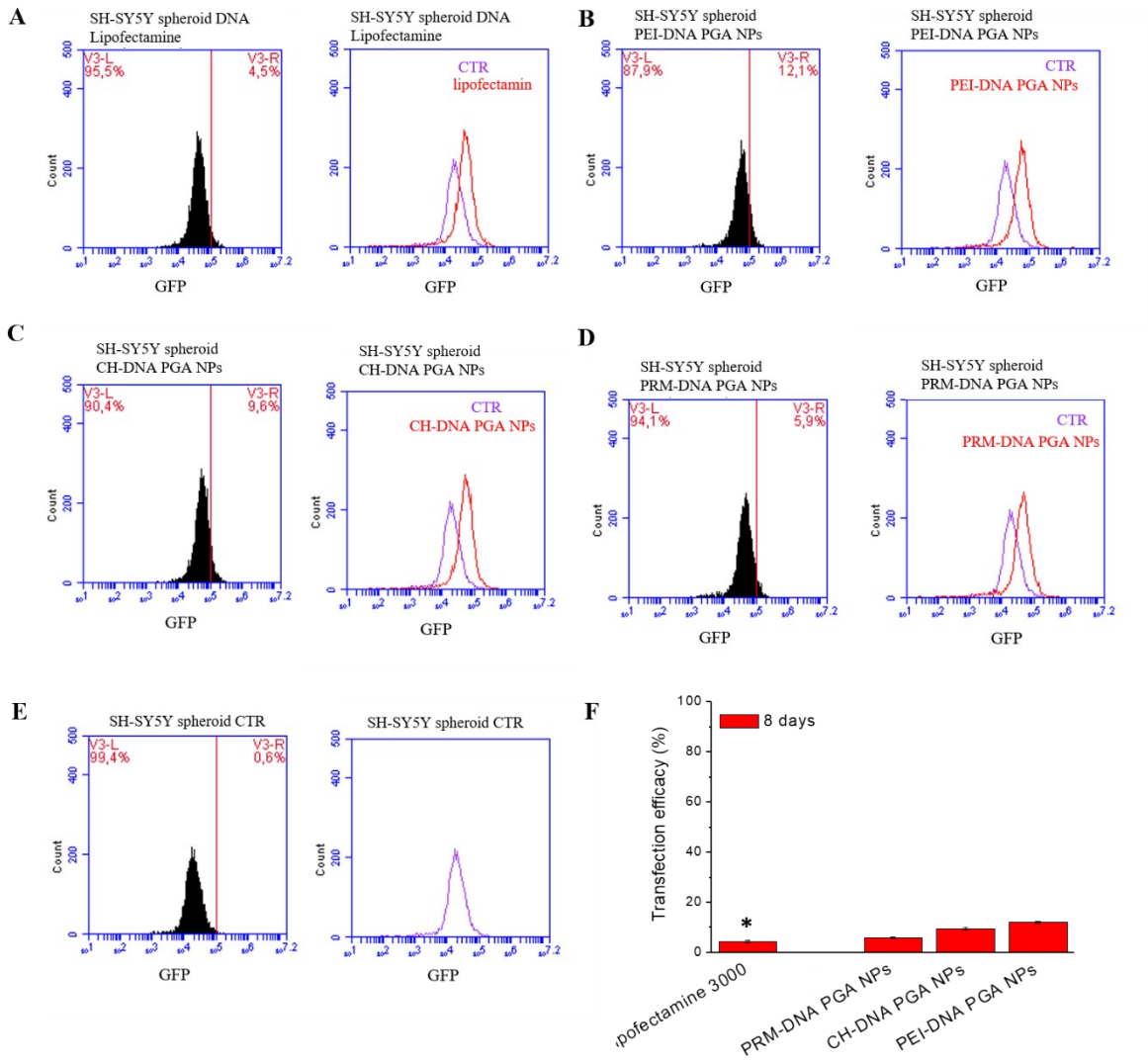

Figure S20. Transfection analysis determined by the EGFP content as assessed by cytofluorimetric analysis after 8 days in 3D human neuroblastoma (SH-SY5Y) spheroids treated with DNA-Lipofectamine 3000 (A), PEI-DNA loaded PGA NPs (B), CH-DNA loaded PGA NPs (C), PRM-DNA loaded PGA NPs (D), untreated spheroid as control (CTR, E). Quantitative transfection efficacy (%) determined by the EGFP content as assessed by cytofluorimetric analysis (F). DNA refer to pT7-EGFP plasmid. A representative result of three independent experiments is shown; * indicates P-values of <0.05 for t-Student test.