

Supporting information

Dye-loaded polymeric nanorods as bright fluorescent nano-platforms for efficient cellular uptake and bioimaging

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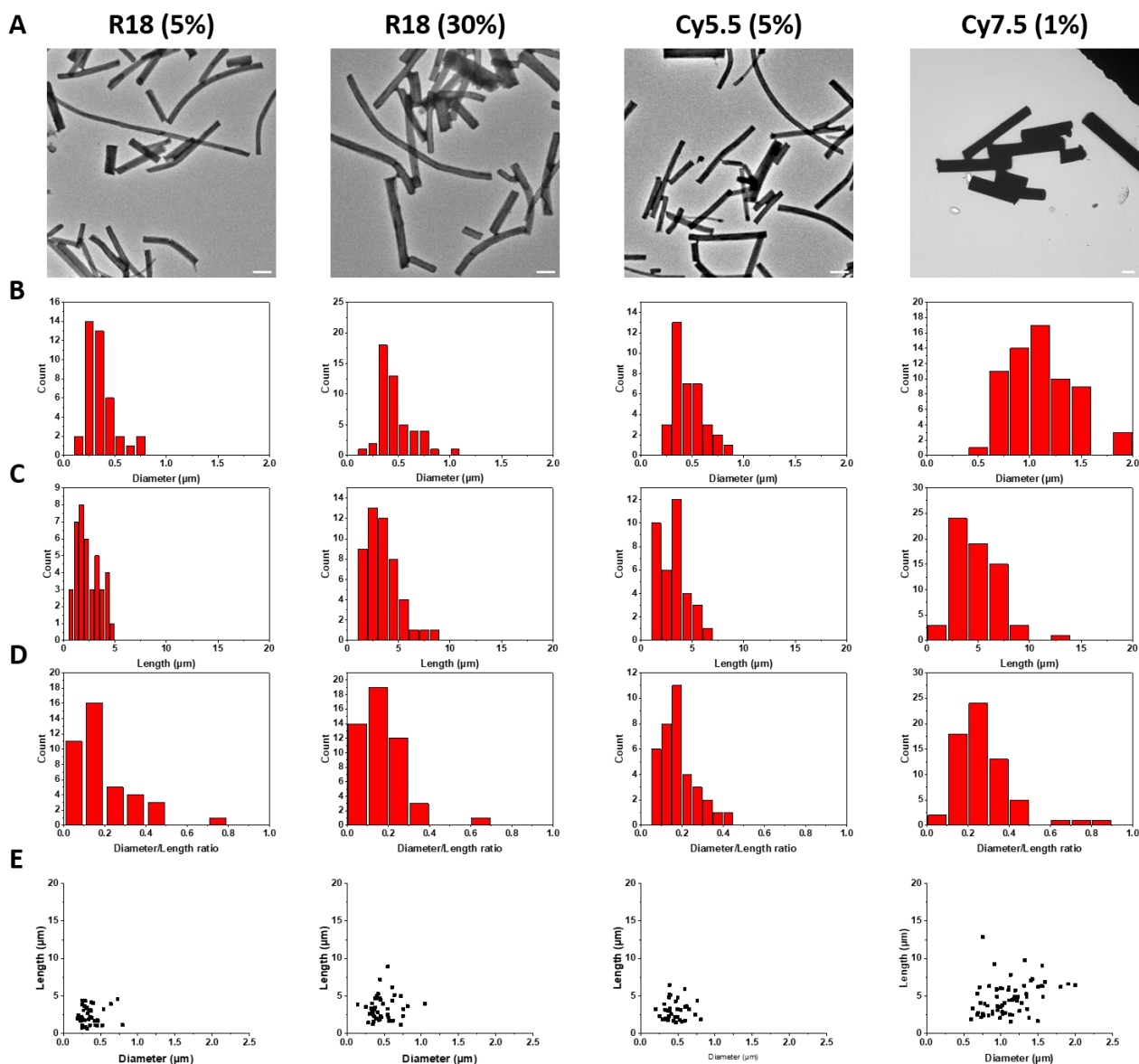


Figure S1. Diameter and length measurements of nanorods by transmitted electron microscopy (A) TEM micrographs of nanorods. Scale bar 1 μm. (B) Diameter distribution determined from TEM images. Calculated on 40 nanorods for each samples. (C) Lengths distribution determined from TEM images. Calculated on 40 nanorods for each samples. (D) Diameter to lengths ratio distribution determined from TEM images. Calculated on 40 nanorods for each samples. (E) Scatter plot of diameter versus lengths determined from TEM images. Calculated on 40 nanorods for each samples.

Table S1. Mean values and standard deviations of dimensions of nanorods measured on TEM micrographs.

	Diameter (μm)	Length (μm)	Ratio diameter/length
R18 5%	0.356 ± 0.137	2.37 ± 1.16	0.198 ± 0.143
R18 30%	0.466 ± 0.154	3.26 ± 1.54	0.175 ± 0.108
Cy5.5 5%	0.467 ± 0.143	3.13 ± 1.31	0.176 ± 0.091
Cy7.5 1%	1.114 ± 0.315	4.73 ± 2.20	0.279 ± 0.140

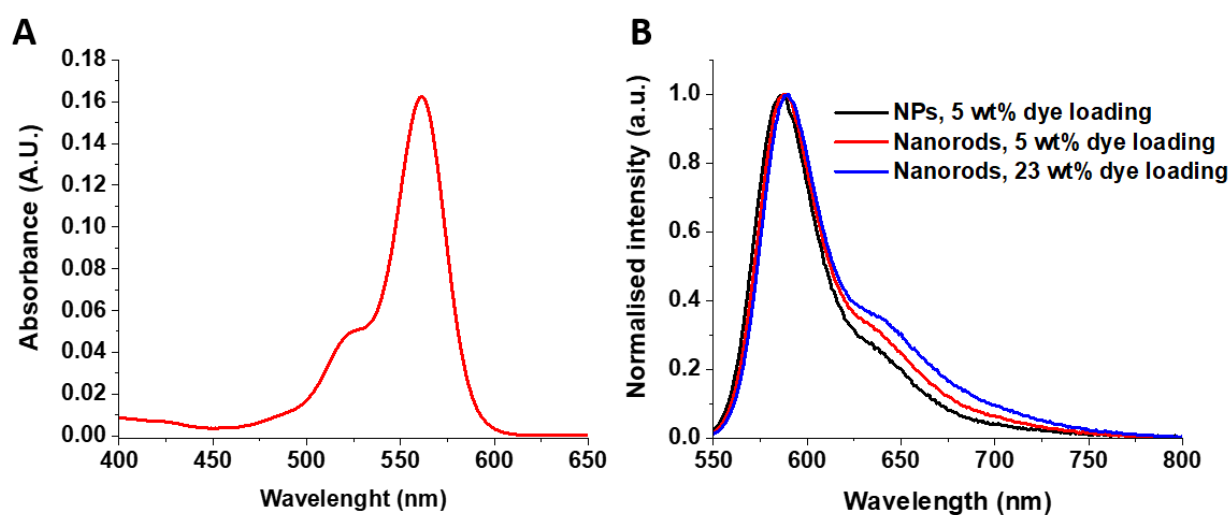


Figure S2. (A) Absorption and (B) normalized fluorescence spectra of PMMA-MA NPs loaded with 5 wt% of R18/F5-TPB. According to DLS, the hydrodynamic diameter of these NPs was 58 ± 9 nm and polydispersity was 0.121. (B) Comparisons of normalized fluorescence spectra of NPs with those of nanorods loaded with 5 wt% and 23 wt% of R18/F5-TPB. Excitation wavelength was 530 nm.

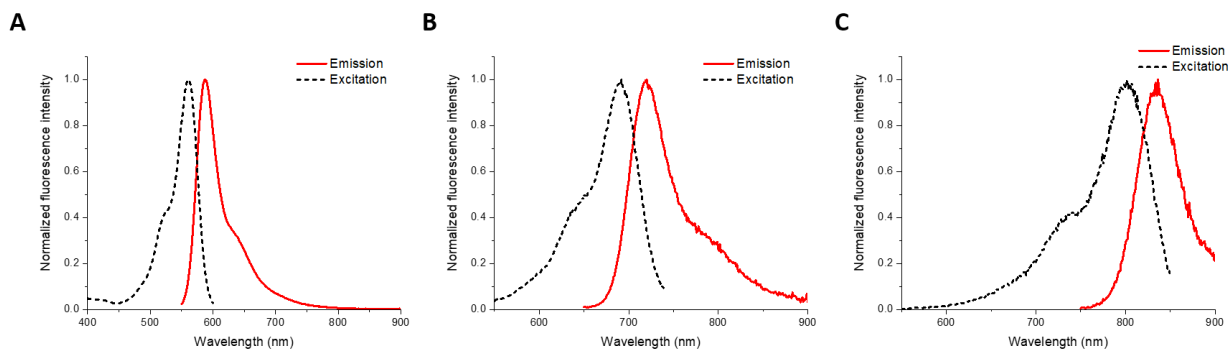


Figure S3. Normalized excitation (black dashed) and emission (red line) spectra of nanorods suspensions. (A) Nanorods loaded with 5 wt% of R18 5F-TPB. For excitation spectra the emission is recorded at 625 nm, for the emission spectra the excitation is set at 530 nm. (B) Nanorods loaded with 5 wt% of Cy5.5 5F-TPB. For excitation spectra the emission is recorded at 750 nm, for the emission spectra the excitation is set at 630 nm. (C) Nanorods loaded with 1 wt% of Cy7.5 TPB. For excitation spectra the emission is recorded at 860 nm, for the emission spectra the excitation is set at 730 nm.

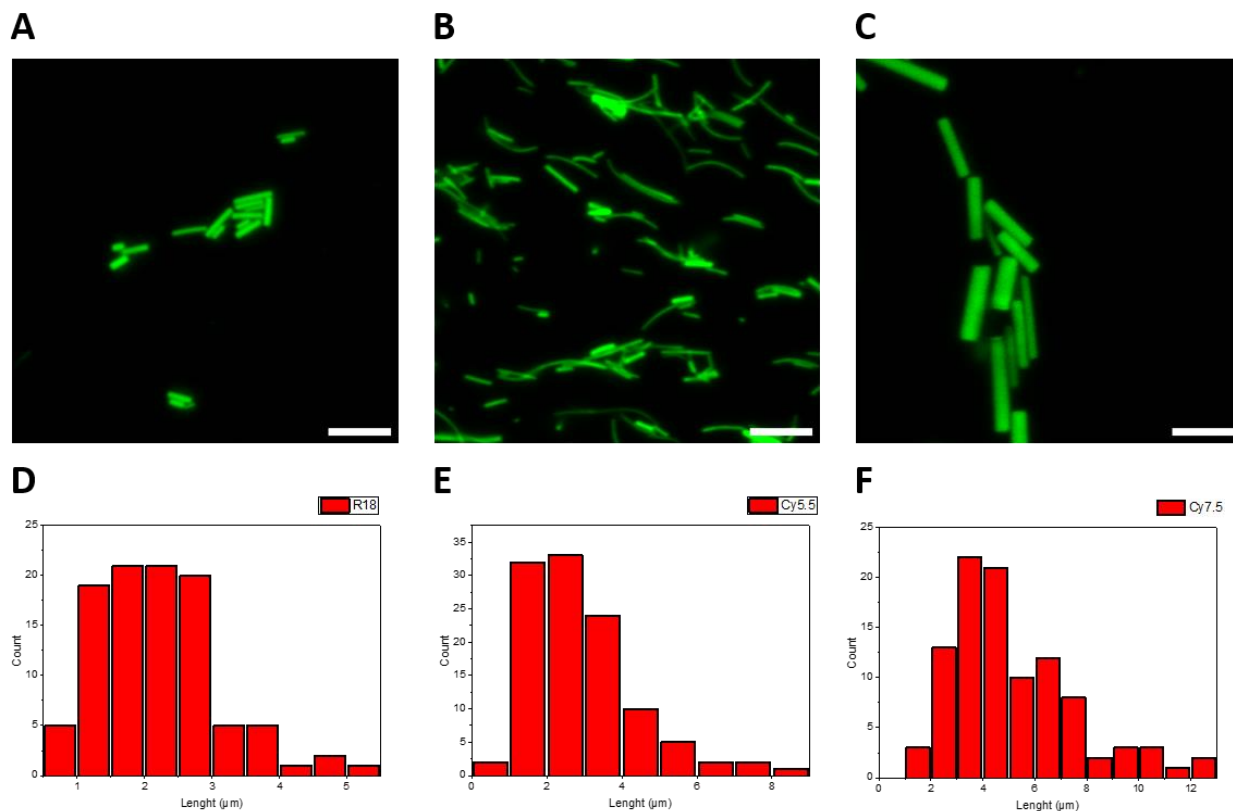


Figure S4. Confocal fluorescence microscopy images of fluorescent nanorods with (A) 5wt% R18 5-FTPB dye loading (B) 5 wt% Cy5.5 5F-TPB dye loading (C) 1 wt% Cy7.5 dye loading. Pinhole has been opened to obtain 5 μm optical section. Scale bar: 5 μm. (D-F) show length distribution in μm of corresponding nanorods observed (calculated on 100 nanorods).

Table S2. Mean values and standard deviations of nanorods lengths determined by confocal fluorescence microscope.

	R18/F5-TPB 5 wt%	Cy5.5/F5-TPB 5 wt%	Cy7.5/F5-TPB 1 wt%
Length (μm)	2.24 ± 0.92	2.92 ± 1.51	5.13 ± 2.38

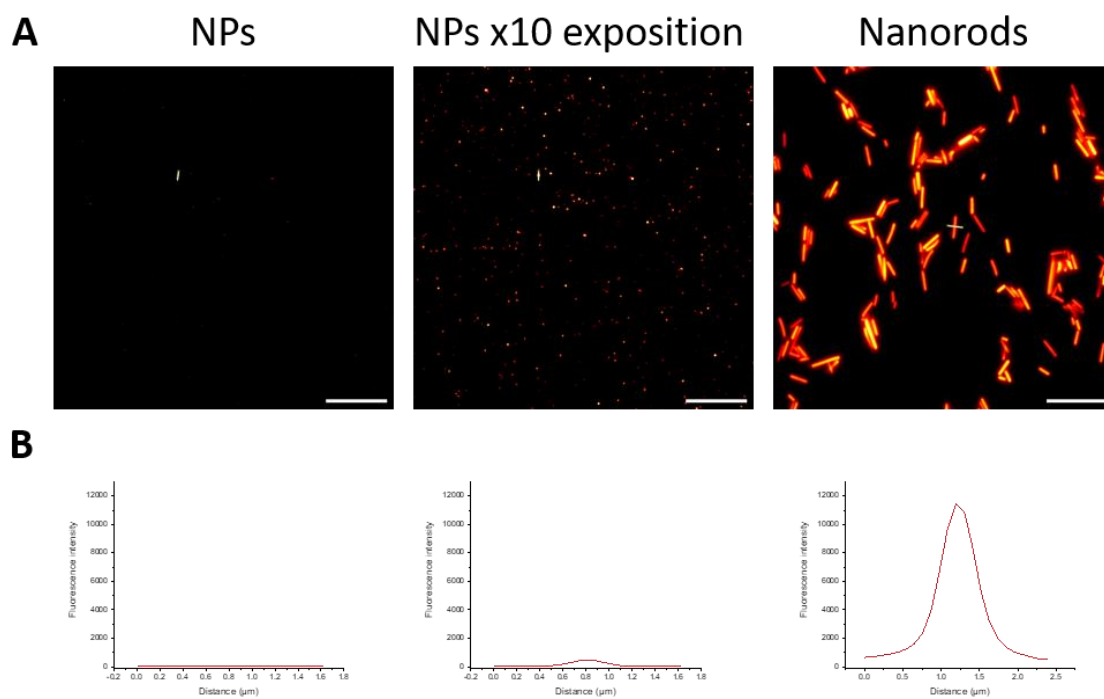


Figure S5. (A) Epifluorescence images of NPs with 10 ms and 100 ms exposition time and nanorods with 10 ms. Scale bar 10 μm . (B) Intensity profile along one NP or nanorod (white lines). Maximum intensities are respectively at 41, 458 and 11414. Average and standard deviation of maximal intensity calculated on 40 nano-objects provide values of 26.8 ± 0.9 , 167.4 ± 14.1 and 13311.5 ± 584.5 for NPs at 10 ms exposition time, NPs at 100 ms exposition time and nanorods at 10 ms exposition time.

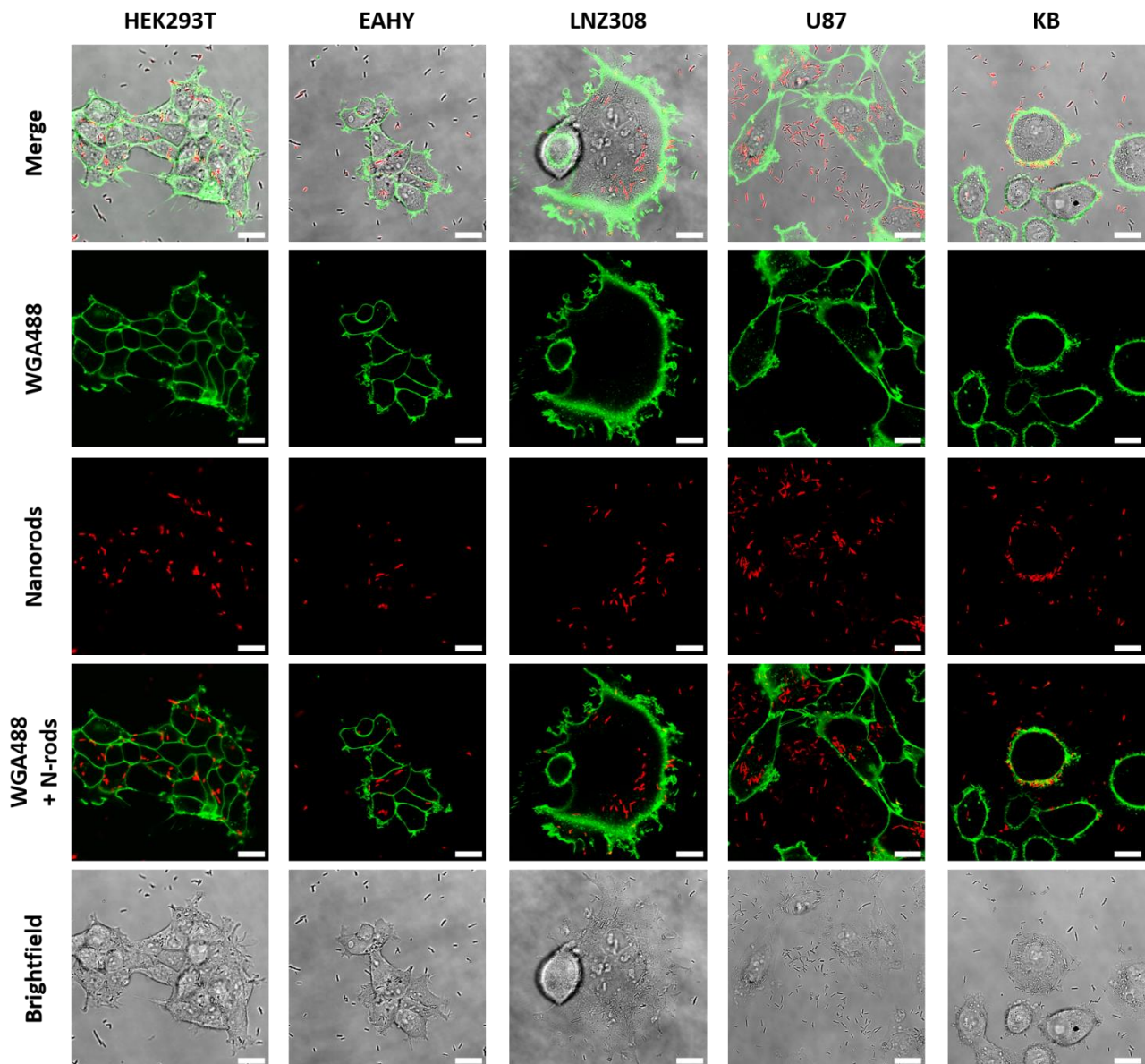


Figure S6. CLSM images of live cells (HEK293T, EAHY, LNZ308, KB and U87) after 24h incubation in presence of fluorescent nanorods (shown in red). Plasma membrane was stained with WGA-AF488 before imaging (shown in green). Scale bar: 20 μm .

**Pluronic treated
nanorods**

**Non-treated
nanorods**

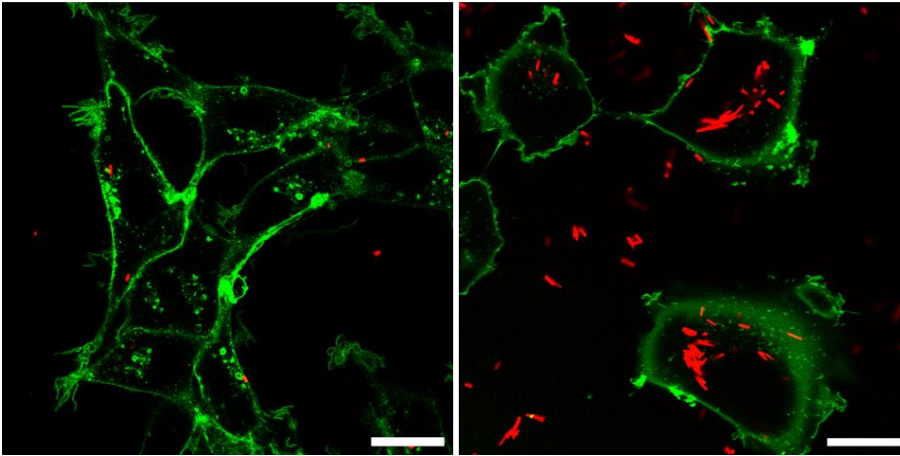


Figure S7. CLSM images of live U87 cells after 24h incubation in complete growth medium at 37°C with fluorescent nanorods (shown in red) pre-treated or not with Pluronic F127. Plasma membrane was stained with WGA-AF488 before imaging (shown in green). Scale bar: 20 μ m.

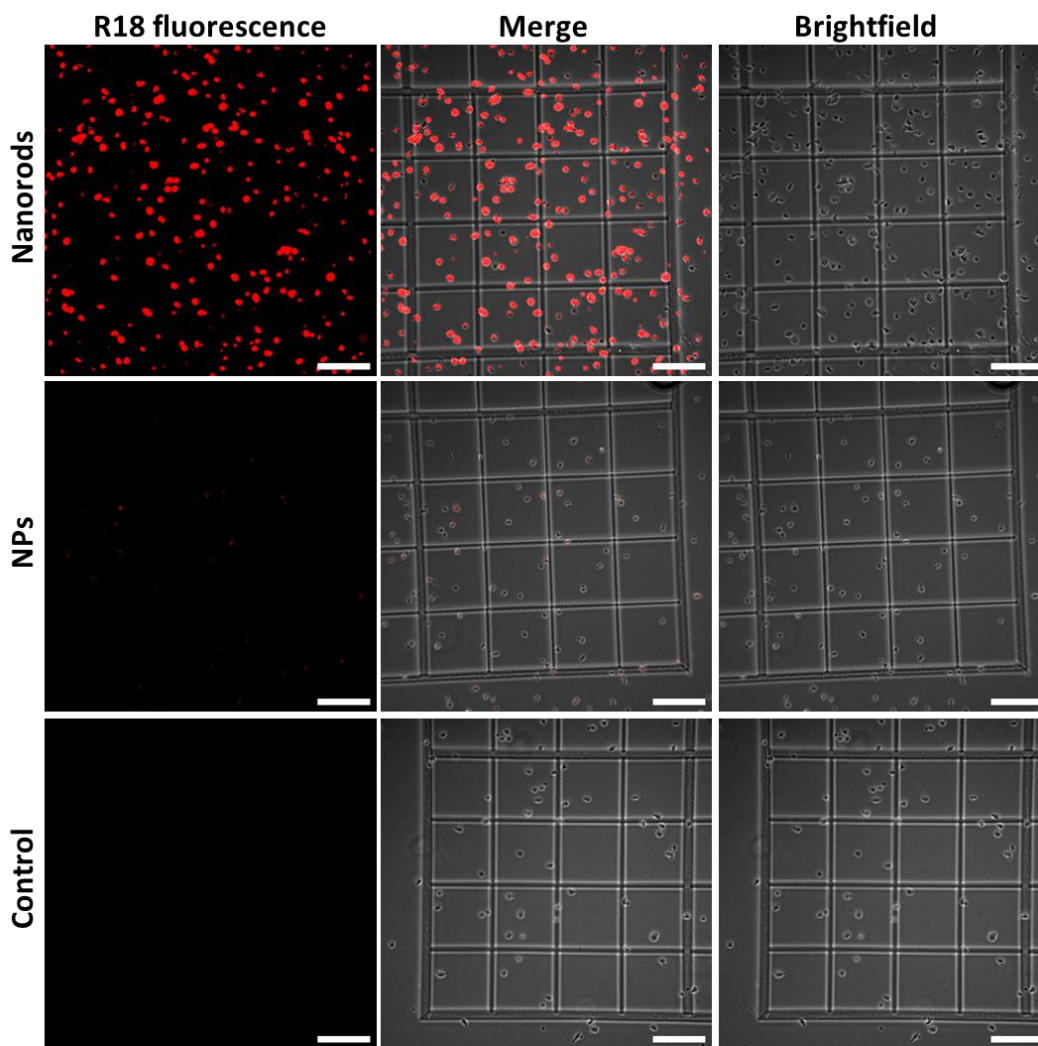


Figure S8. Examples of fixed U87 cells after 24h incubation with NPs or nanorods at 50 μ g/mL on counting slide before dissolving in 1,4-dioxane. Fluorescence channel is displayed at the same intensity for all samples. Scale bar: 200 μ m.

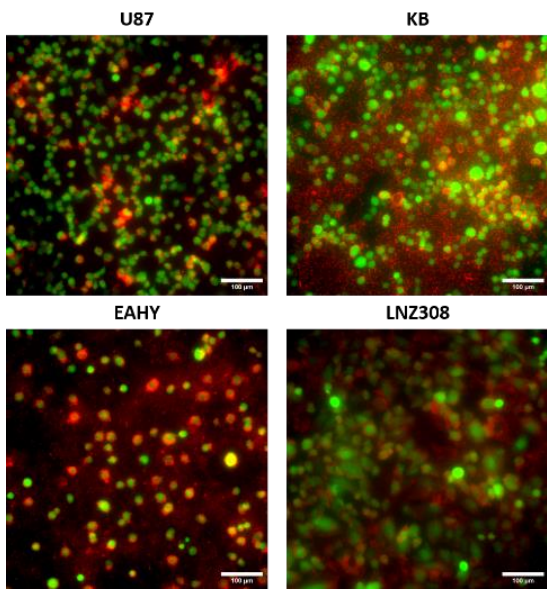


Figure S9. Different cell lines observed in epifluorescence microscopy after 24 h incubation with nanorods (red) and stained with fluorescein diacetate (green) 30 min before imaging. Scale bar: 100 μm.

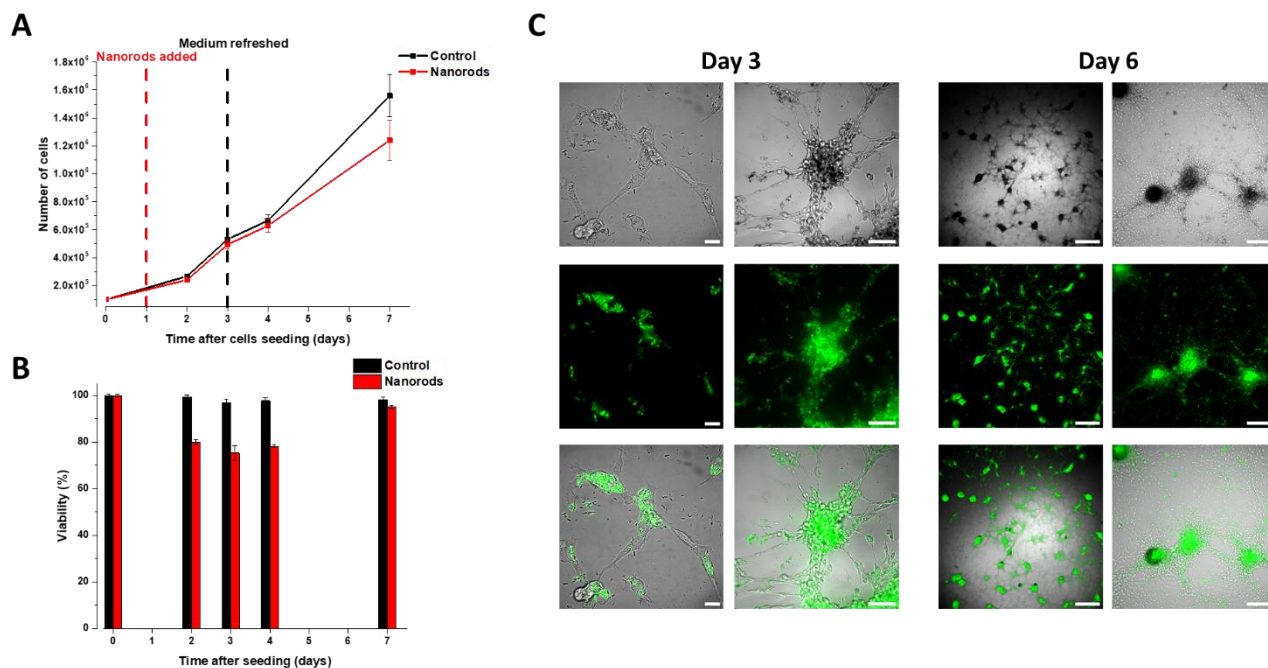


Figure S10. (A) Number of cells numbered over time of U87 control cells and U87 cells incubated with nanorods. Error bars are displayed for $n = 3$. (B) Viability percentage over time of corresponding cell populations determined by trypan blue counting. (C) Transmitted light microscopy images of U87 cells incubated with nanorods at day 3 and day 6. Nanorods fluorescence is displayed in green. Scale bars: 20 μm (day 3, left); 200 μm (day 3 right); 500 μm (day 6 left); 200 μm (day 6 right).

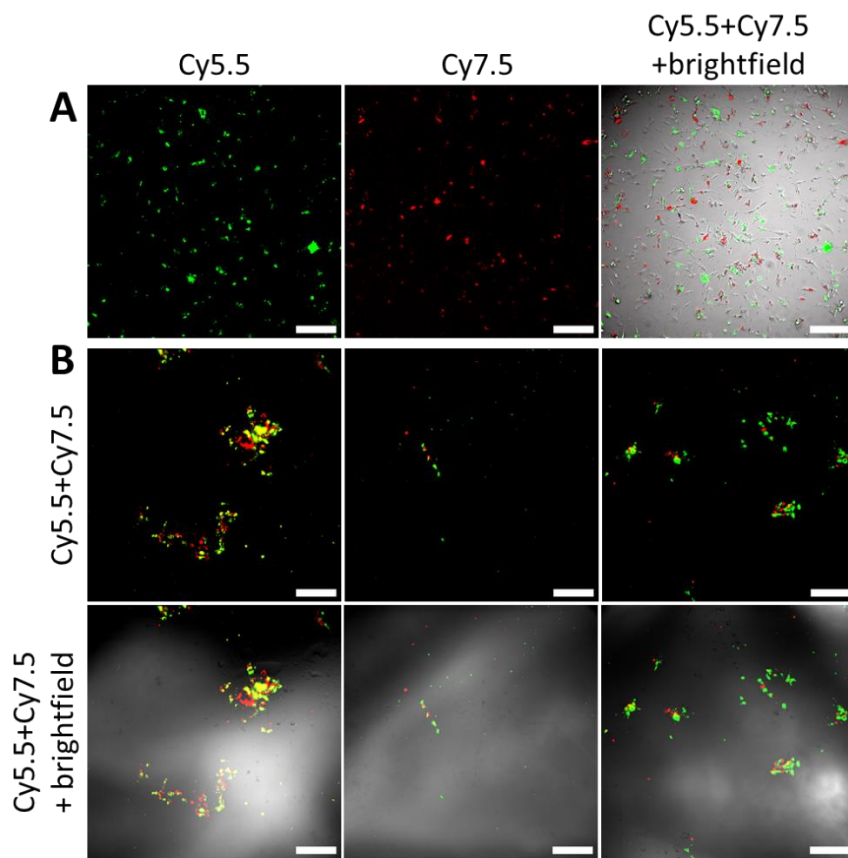


Figure S11. (A) Widefield images of a co-culture of two U87 cells batches incubated respectively with nanorods loaded with 5wt% Cy5.5/5F-TPB and nanorods loaded with 1wt% Cy7.5 TPB. (B) Similar co-cultured U87 cells microscopy images through chicken skin. Scale bar: 200 μm .