

[Supplementary Information]
Nanoconjugation Potentiates Epidermal Growth Factor Induced Apoptosis

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Fig. S1 NP-EGF Stability.

SEM images of NP-EGF conjugates before (left) and after incubation (right) with A431 cells in serum containing medium confirm a high stability of nanoconjugated EGF against agglomeration. NP-EGF conjugates incubated with the cells were washed through centrifugation (3600rpm, 15min) and resuspended in 0.5x PBS. All particles were randomly deposited on a silicon substrate through incubation for 1h. Subsequently the silicon chips were washed with ddi water and dried before imaging in SEM.

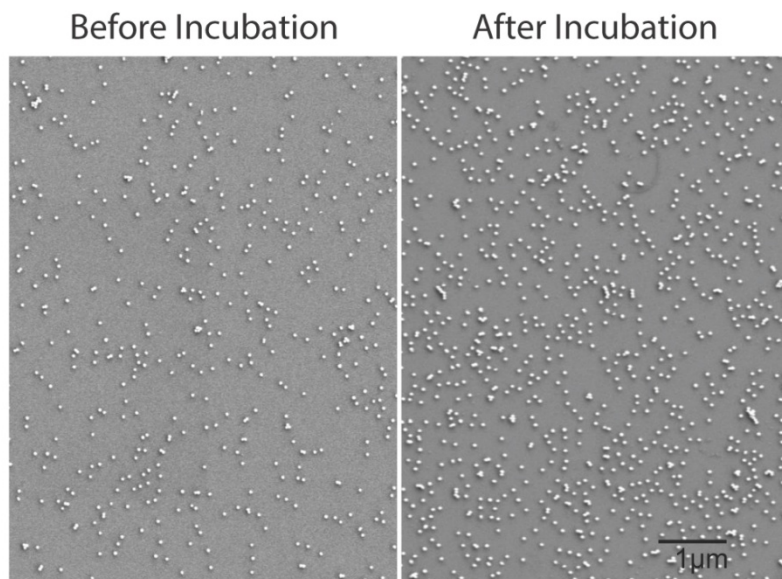


Fig. S2 Darkfield and Fluorescence Image of HeLa Cell Controls (No NP Uptake). a) Darkfield scattering image. The darkfield image was scaled to the same intensity as Fig. 4a. Due to the absence of NPs the scattering intensity is lower than in Fig. 4a. b) Lysosomes were stained with fluorescent LysoTracker. c) Cell nuclei were stained with Hoechst 33342. d) Overlay image of all three channels.

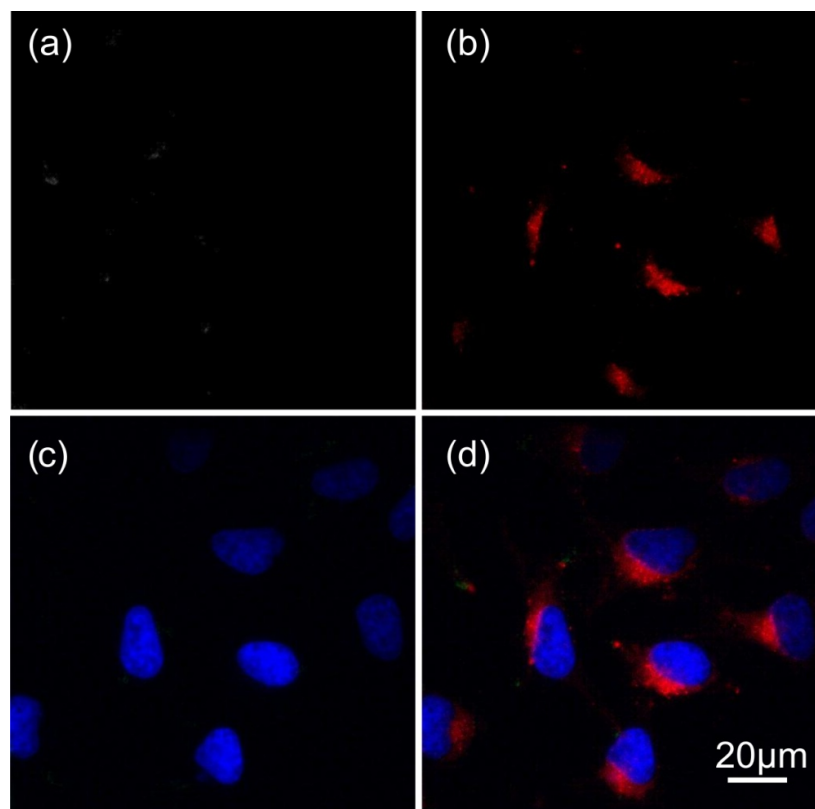


Fig. S3 Morphological Cell Changes.

Darkfield images of A431 cells cultured for 72h in serum containing DMEM (top row), in medium with 33nM EGF (middle row) and in medium containing P3 (bottom row). Rounding-up of cells (arrow) is observed in 33nM EGF and P3 treated cells but not in the controls. Rounding-up is a morphological indication of apoptosis.^[1,2]

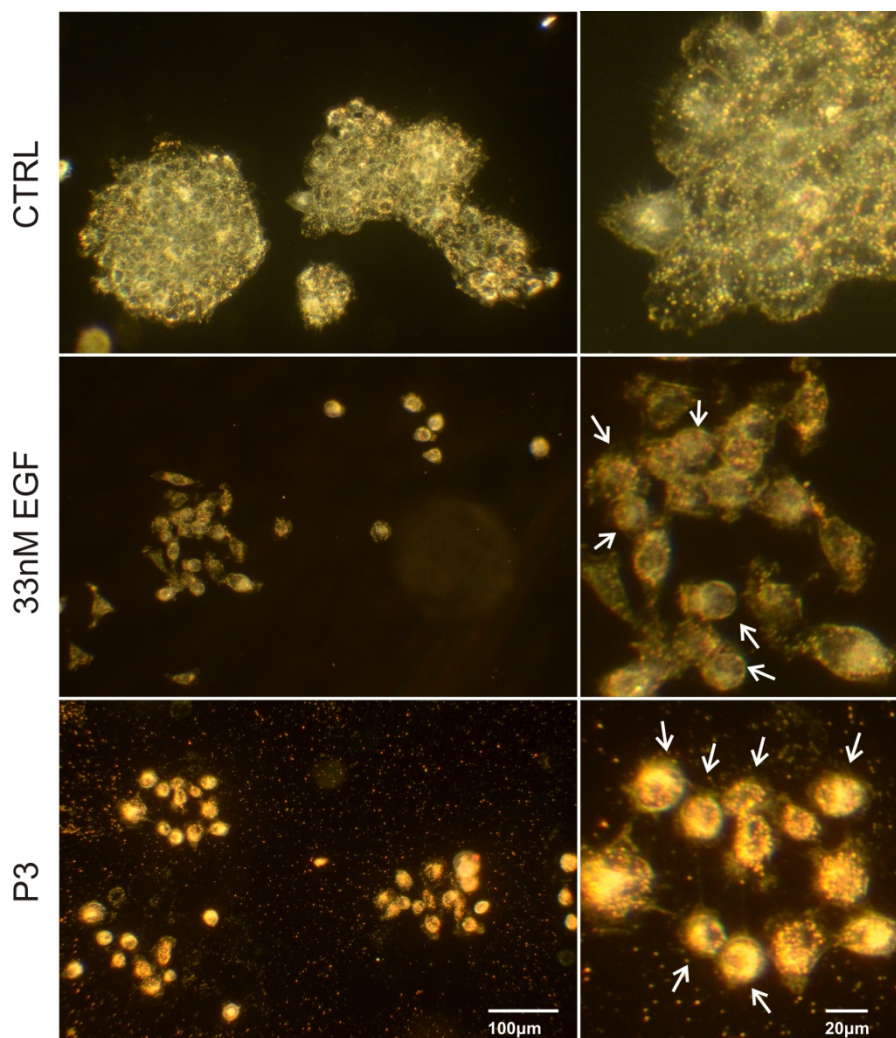


Fig. S4 Nucleus Condensation.

Nucleus size and morphology was evaluated after staining with Hoechst 33342 nuclear fluorescent stain. A431 cells exposed to 33nM EGF and P3 contain distinctly smaller nuclei. relative nucleus cross-section was determined as relative to the blank control. Nuclear condensation is another morphological indication of apoptosis. ^[1-3]

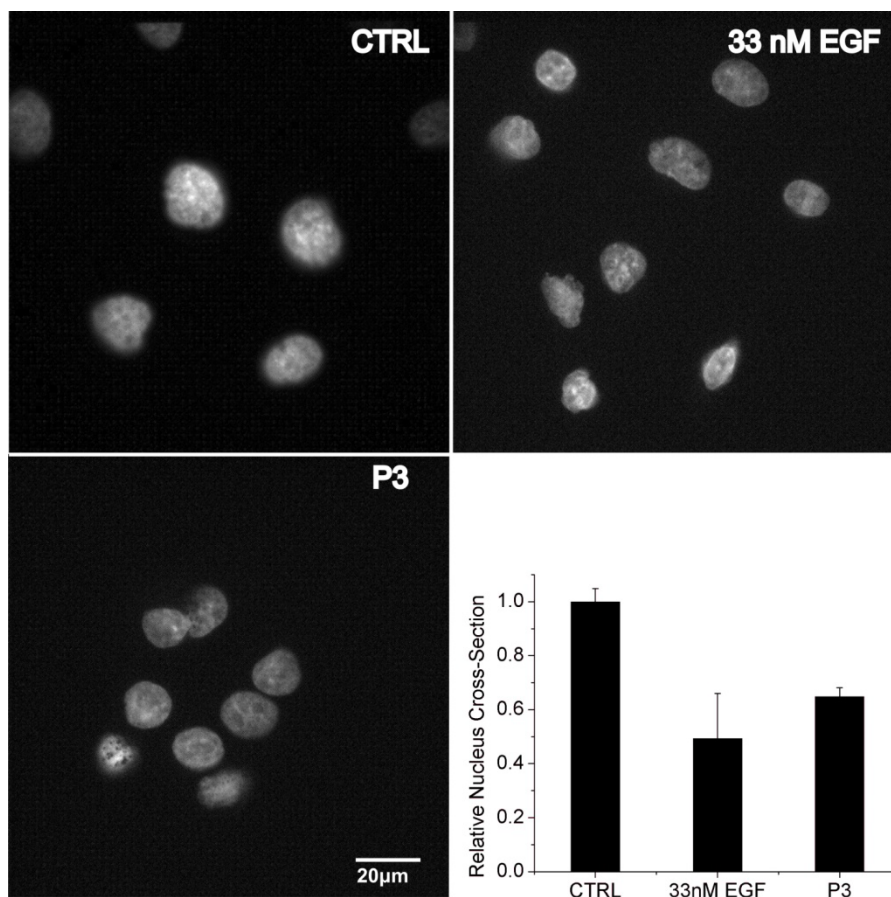
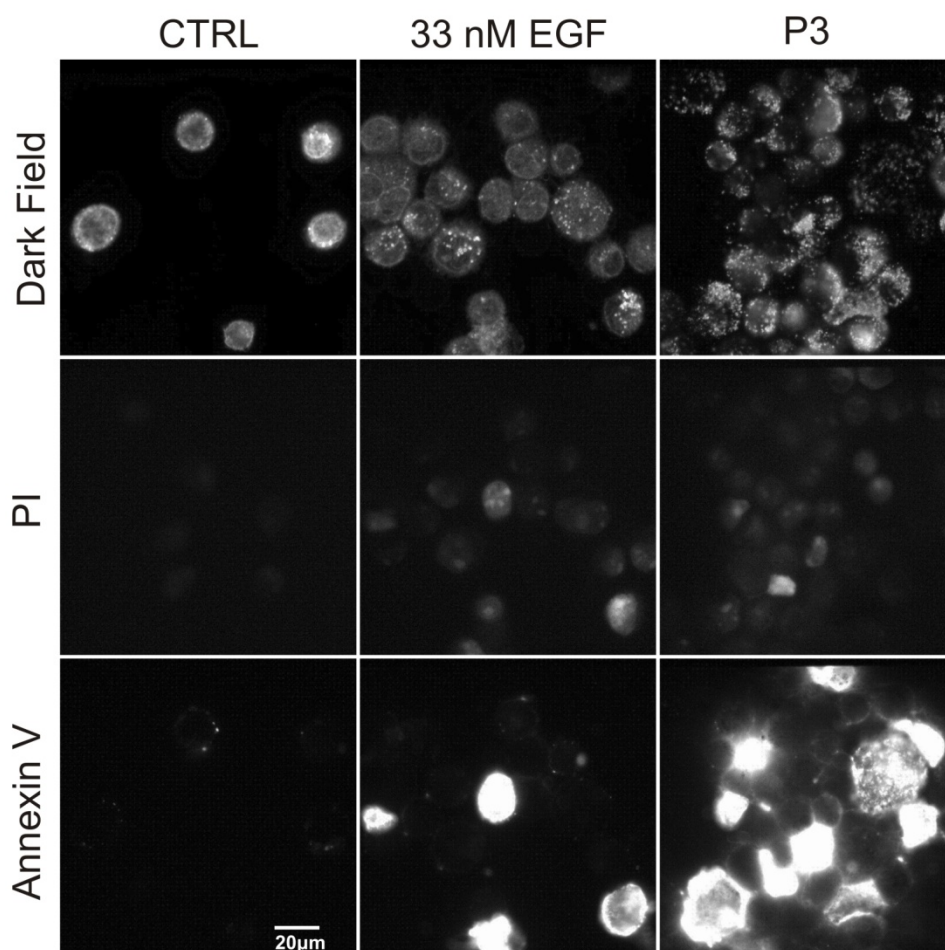


Fig. S5 Phosphatidylserine (PS) Translocation/Plasma Membrane Integrity Assay.

A431 control cells and cells exposed to EGF and NP-EGF for 72h were harvested and stained with fluorescent propidium iodide (PI) and Annexin V at room temperature for 25min and imaged after washing two times through centrifugation. DNA staining through PI is indicative of ruptured membranes in dead cells, whereas Annexin V staining indicates PS translocation into the outer membrane leaflet, which indicates apoptosis. The overall low PI intensity and strong Annexin V labeling intensity in cells treated with EGF or P3 is characteristic of apoptosis.



Reference

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2. A. Saraste, K. Pulkki, *Cardiovasc. Res.*, 2000, **45**, 528-537.
3. S. Toné, K. Sugimoto, K. Tanda, T. Suda, K. Uehira, H. Kanouchi, K. Samejima, Y. Minatogawa, W. C. Earnshaw, *Exp. Cell Res.*, 2007, **313**, 3635–3644