SUPPLEMENTARY INFORMATION

Nanoconjugation Prolongs Endosomal Signaling of the Epidermal Growth Factor Receptor and Enhances Apoptosis

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SUPPLEMENTARY FIGURES

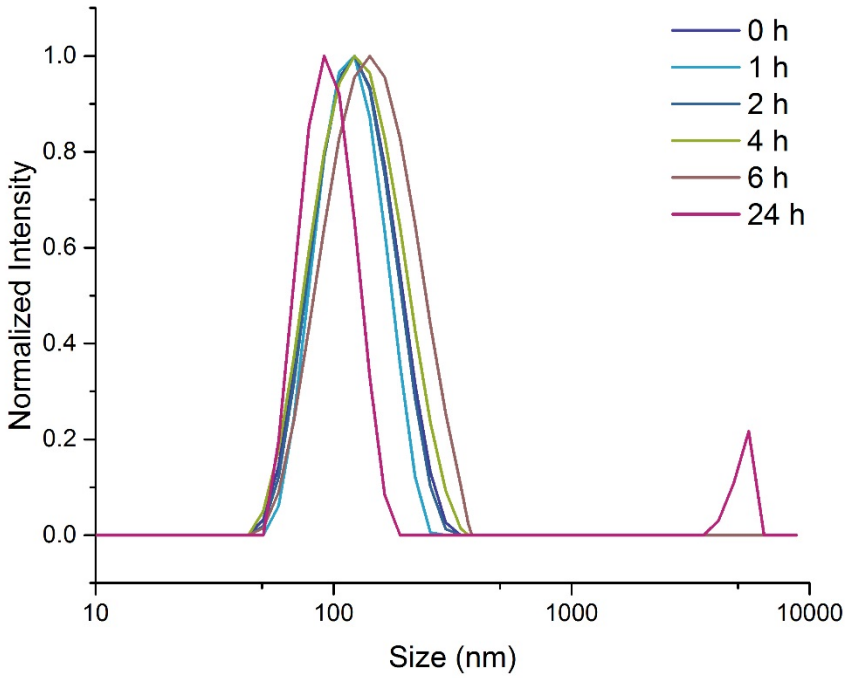

Figure S1. Stability of NP-EGF. NP-EGF was suspended in DMEM at 37 °C for durations as indicated before DLS data were collected. For incubation times as long as 4 h, the hydrodynamic diameter of NP-EGF did not exhibit any change. After 6 h, there was an obvious shift of the peak and after 24 h a distinct population of agglomerates was detected.

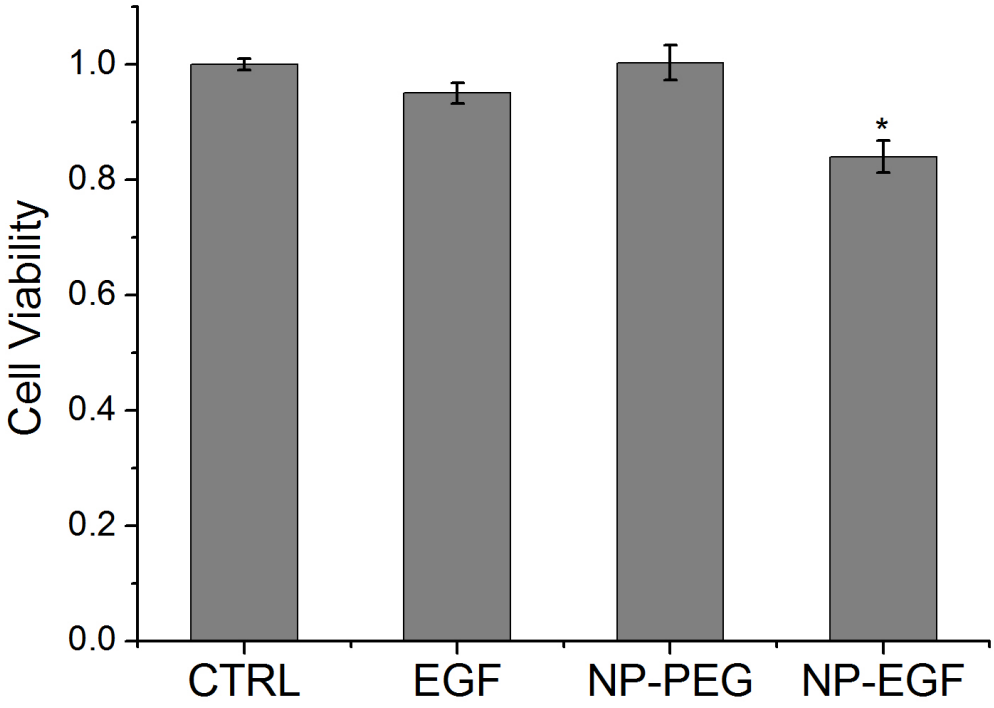

Figure S2. MTT cell viability assay. Cells were treated with 1) No treatment 2) EGF (33 nM) 3) NP-PEG (8 pM) and 4) NP-EGF (8 pM) for 4 h at 37 °C and chased in fresh growth medium for another 20 h. MTT assay was applied to measure the cell viability. Data were plotted as the ratio of the treated cells to the non-treated control group in DMEM and were collected from three independent experiments. Error bars represent the SEM and p values were calculated with one-way ANOVA with Tukey post-hoc test (* $p<0.05$).

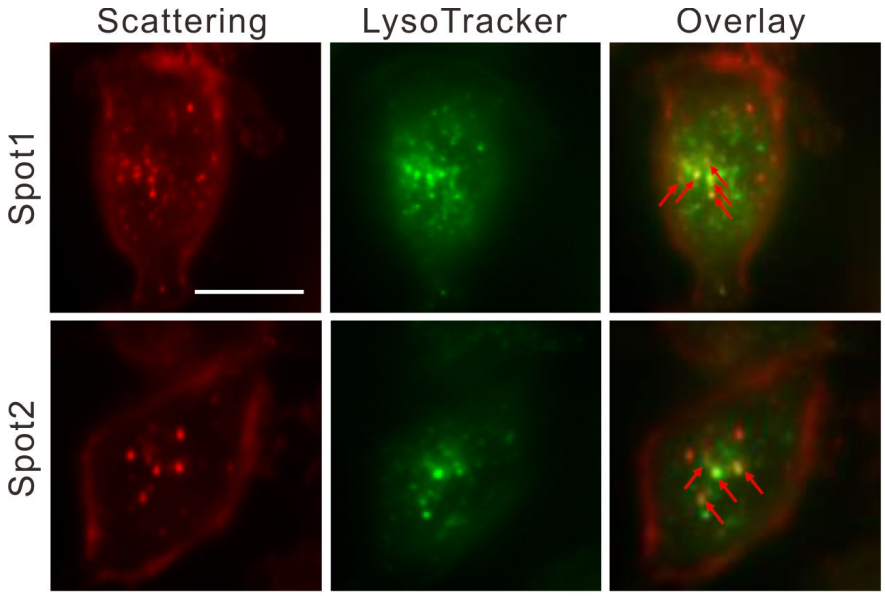

Figure S3. MW-NPs are collected in lysosomal compartments. Cells were treated with MW-NP (1 pM) for 30 min and were then stained with lysosome tracker deep red for 30 min. After that, the cells were fixed with 4% PFA. Left: darkfield scattering images of two MW-NP containing cells. Middle: fluorescence images of the lysotracker treated cells. Right: overlay of fluorescence and darkfield channels. The red arrows in the merged images indicate MW-NPs localized in lysosomal compartments.

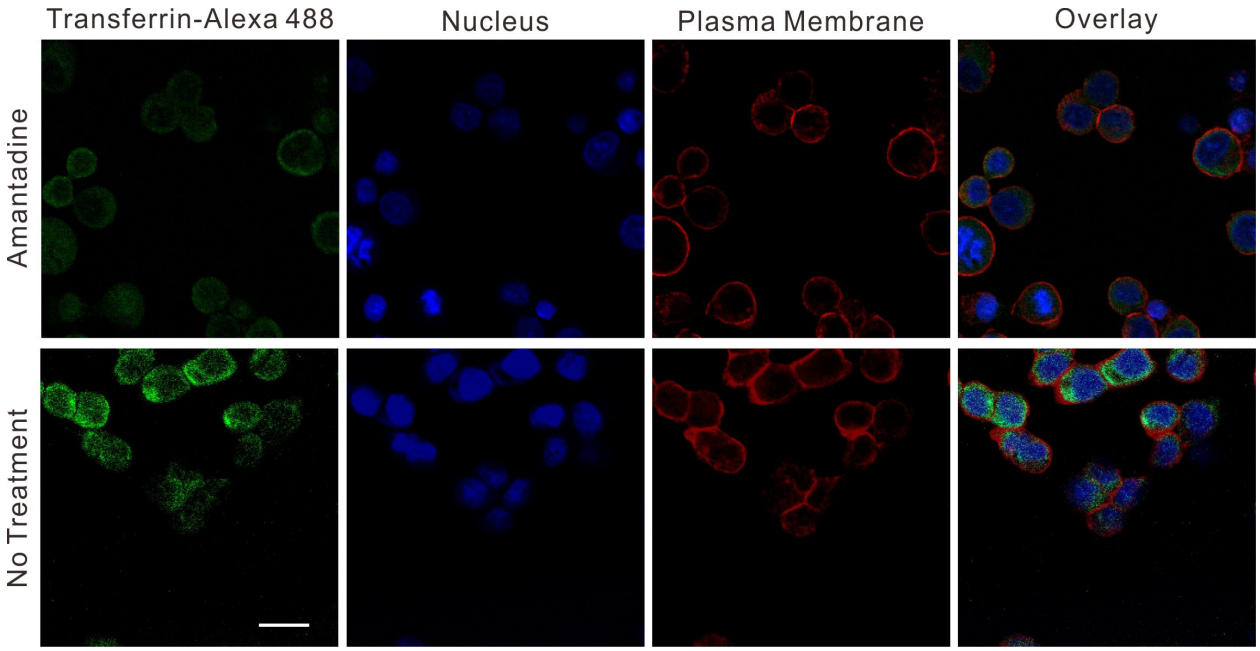

Figure S4. Inhibition of clathrin mediated endocytosis with amantadine. MDA-MB-468 cells were pretreated with amantadine (5 mM) for 30 min (top row). Then Transferrin Alexa 488 conjugate (5 μg/mL) was incubated with cells in the presence of amantadine for 15 min. The cells were washed twice with HBSS buffer and fixed with 4% PFA. After that, the cells were imaged in the confocal microscope. The bottom row shows the control without amantadine treatment. All other conditions are identical to the top row. Left to right: Confocal images of Transferrin (green), nucleus (blue), plasma membrane (red) and overlay images. Scale bar 10 μm.

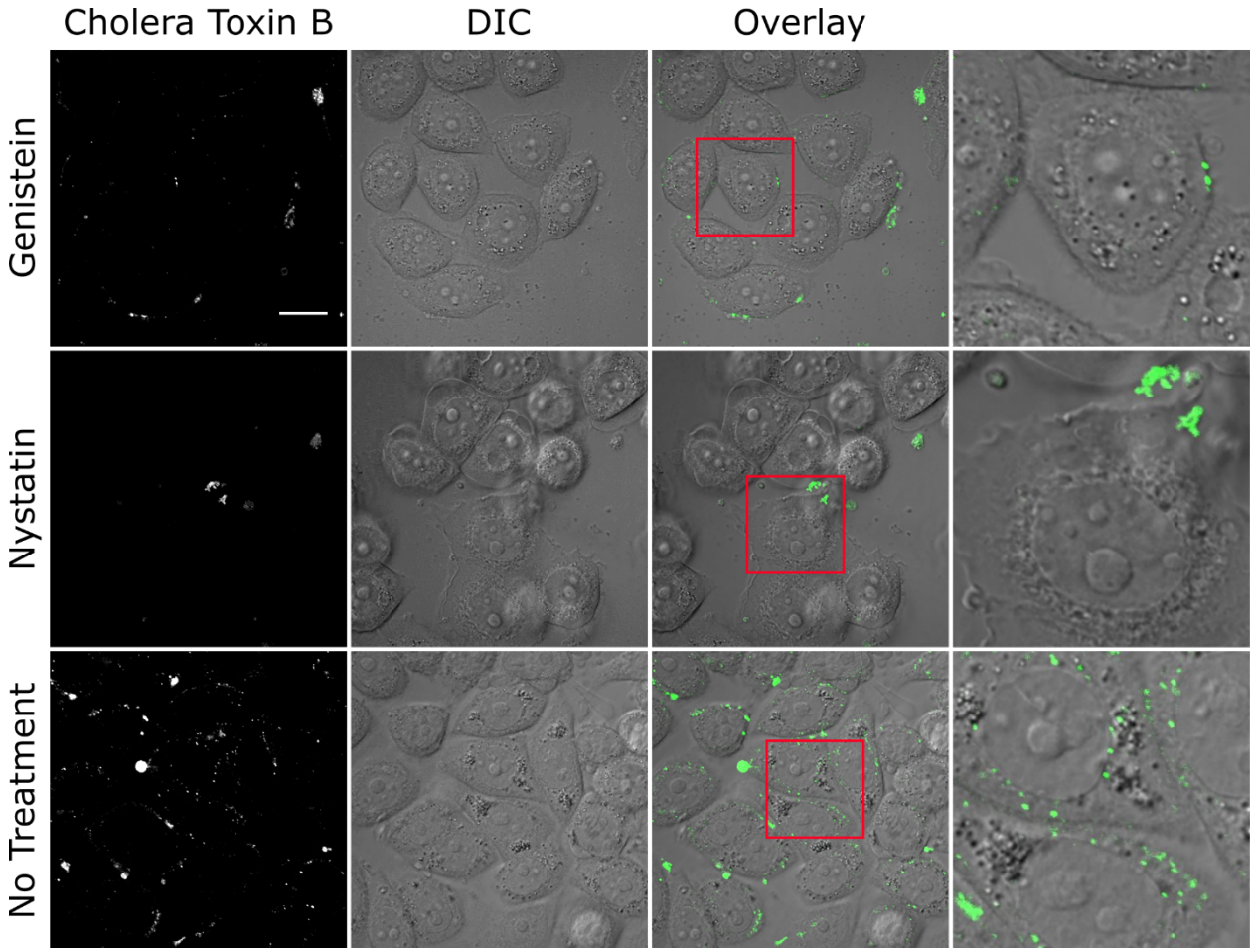

Figure S5. Inhibition of caveolae mediated endocytosis with genistein and nystatin. MDA-MB-468 cells were pretreated with genistein (200 μM) for 60 min or nystatin (50 μg/mL) for 15min. Then cholera toxin B Alexa 488 conjugate (CTB) (5 μg/mL) was incubated with cells for 20 min in the presence of inhibitors. Cells were washed twice with HBSS buffer and fixed with 4% PFA before they were imaged in the confocal microscope. Left to right: Confocal images of CTB, DIC images of cells, overlay images of CTB and cells, enlarged images of the red squared area. Genistein (top) and nystatin (middle) treated cells show a decrease in uptake of CTB compared to cells without inhibitor treatment which show a significantly higher uptake (bottom). Scale bar: 10 μm.

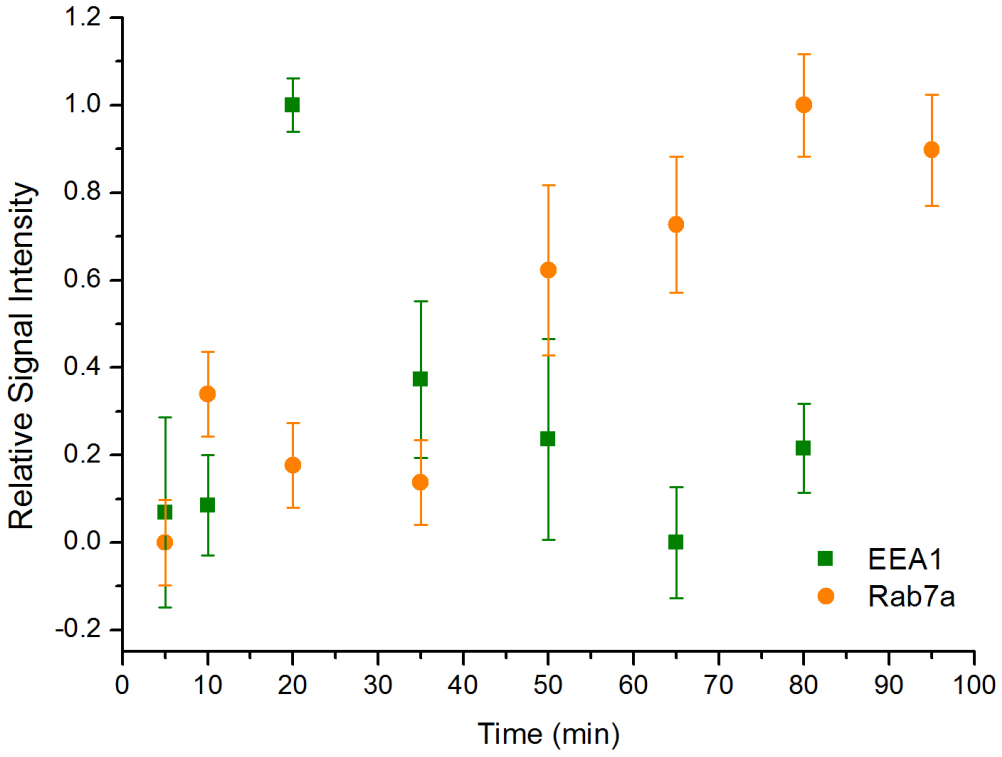

Figure S6. Relative signal intensity for early (olive) and late (orage) endosomes in endosome sorting experiments. Relative signal intensity was defined as $(I(t) - I_{min}) / (I_{max}-I_{min})$ where I(t) is the normalized ELISA intensity at time t, and I_{max} and I_{min} are the minimum and maximum intensities. Early time points were included to illustrate the accumulation after NP-EGF in EEA1 positive endosomes after transfer of the cells from 4°C to 37°C a t = 0. Data were collected from at least 6 independent experiments. Error bars represent the SEM.

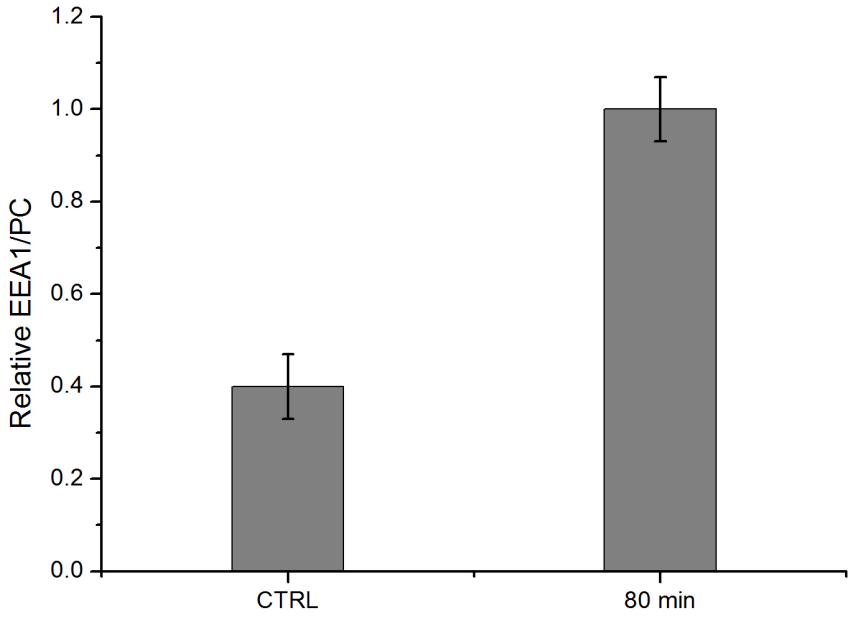

Figure S7. Comparison of EEA1 levels obtained for NP-EGF after t = 80 min (for experimental details see main text) and background (Au NPs mixed with cell lysate). The background sample was prepared by incubating 80 nm NP-EGF with non-treated cell lysate for 30 min at room temperature. After that, the particles were collected by centrifugation at 800 g for 10 min, and EEA1 levels were determined by ELISA. EEA1 levels are normalized by the measured PC levels to account for differences in the sample sizes. Data were collected from three independent experiments. Error bars represent the SEM.

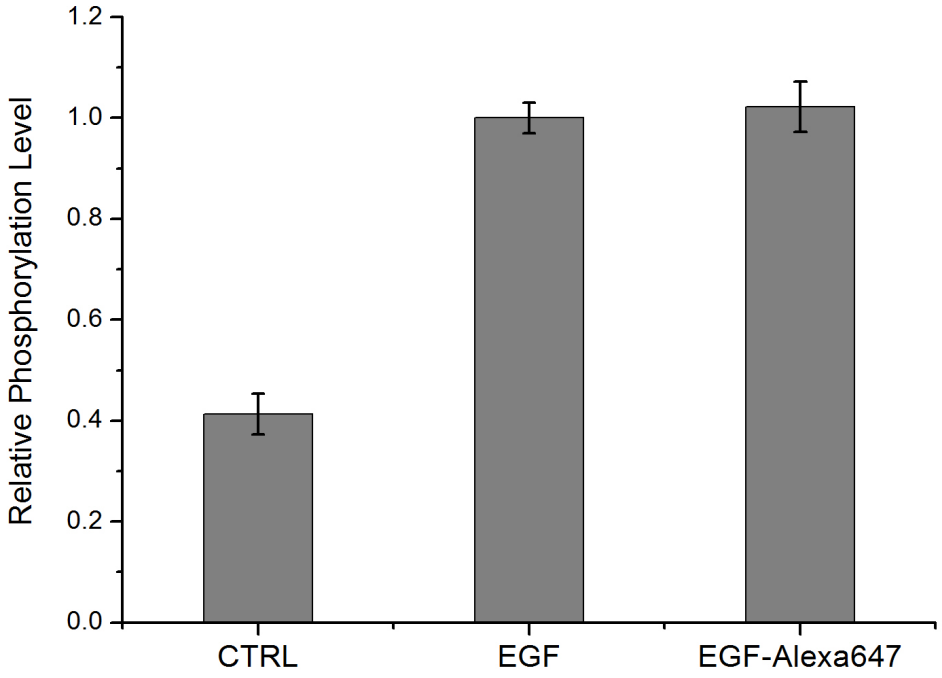

Figure S8. Validation of activity of fluorescently labeled EGF (EGF-Alexa647). MDA-MB-468 cells were incubated with EGF (16 nM) or EGF-Alexa647 (16 nM) for 10 min before cells were collected and lysed. pEGFR level was measured by ELISA and normalized to the total protein concentration (BCA). The phosphorylation levels induced by EGF and EGF-Alexa647 are nearly identical and much higher than for the no-treatment control. Conjugation of Alexa647 to EGF has no measurable effect on the receptor activation. Data were collected from three independent experiments. Error bars represent the SEM.

SUPPLEMENTARY MOVIE

Movie S1. Example of colocalized living cell movie of MDA-MB-468 cells expressing Rab5a-GFP (green) 4.5 h after exposure to 8 pM NP-EGF (red). The movie was acquired at 2 frames per second (fps) and is speeded up here to 15 fps. The field of view is 40 by 40 μm.