Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

Cell-specific optoporation with near-infrared ultrafast laser and functionalized gold nanoparticles

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FIGURES

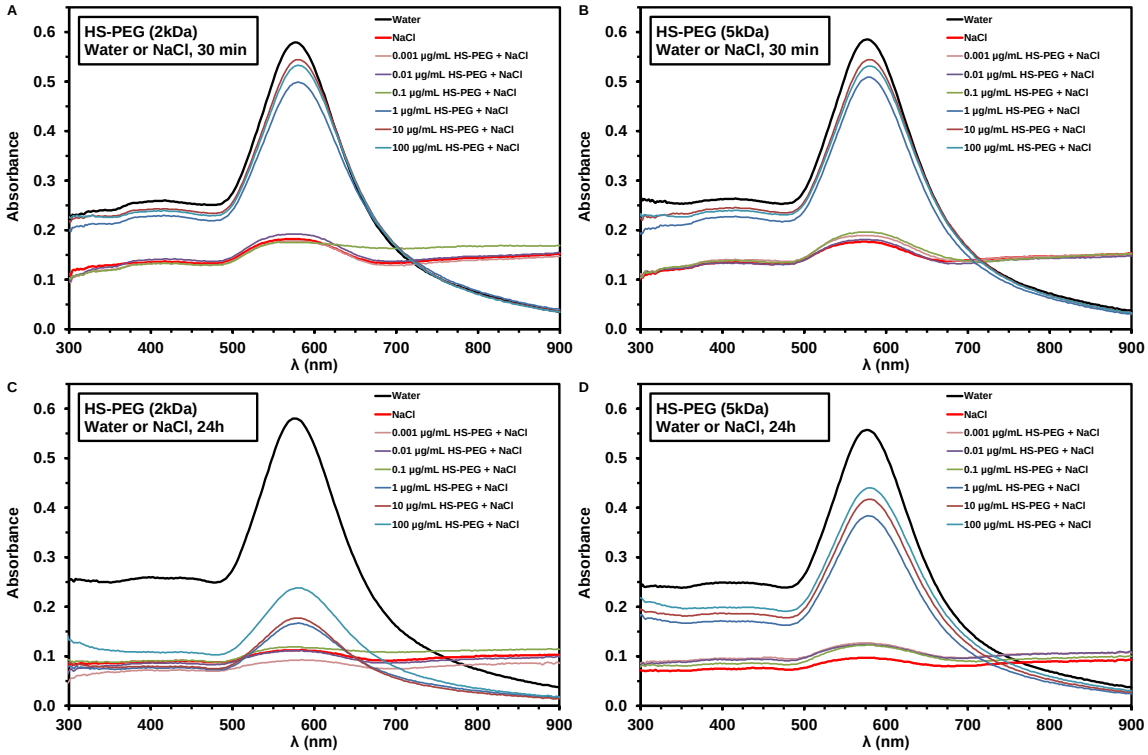

Fig. S1. UV-visible-NIR spectra of AuNPs functionalized with HS-PEG (2kDa) (A,C) or HS-PEG (5kDa) (B,D) incubated for 30 min (A,B) or 24 h (C,D) with water or 1% NaCl. Four other independent experiments gave similar results.

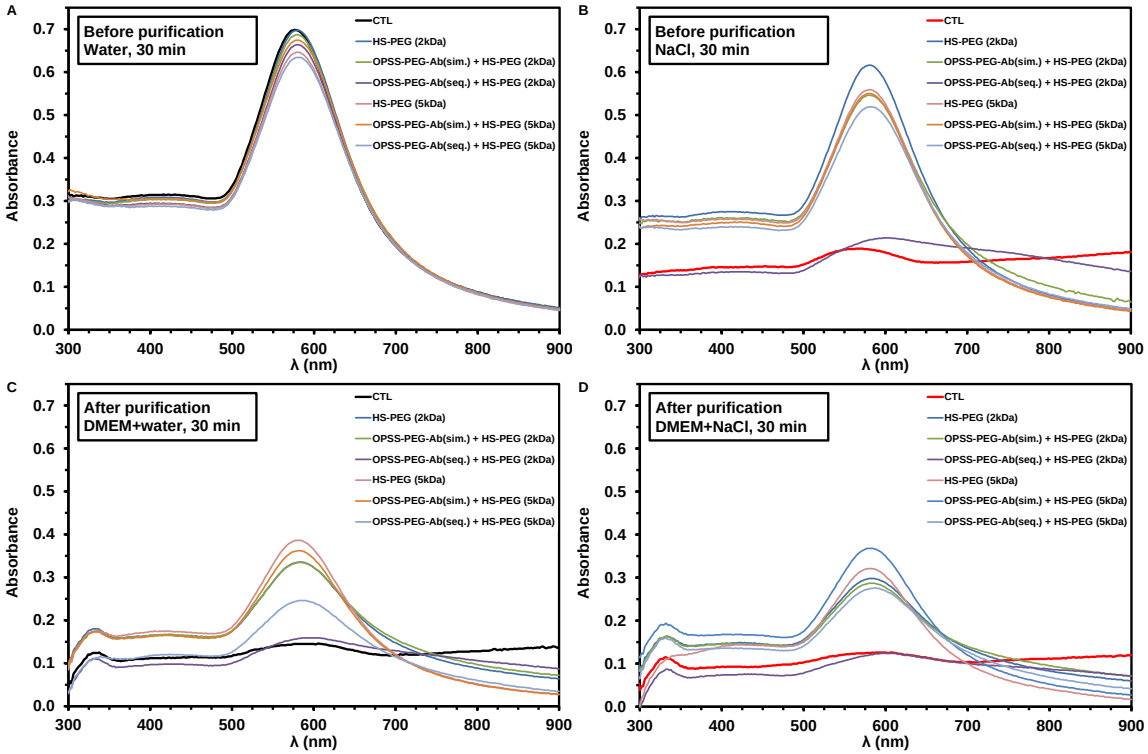

Fig. S2. UV-visible-NIR spectra of AuNPs functionalized with i) HS-PEG (2 or 5kDa), ii) OPSS-PEG-Ab (0.45%) simultaneously (sim.) with HS-PEG or iii) OPSS-PEG-Ab sequentially (seq.) followed by HS-PEG. Samples before purification (A,B) or after centrifugation and resuspension in phenol red-free DMEM (C,D) were incubated for 30 min in water (A,C) or 1% NaCl (B,D). Five other independent experiments gave similar results.

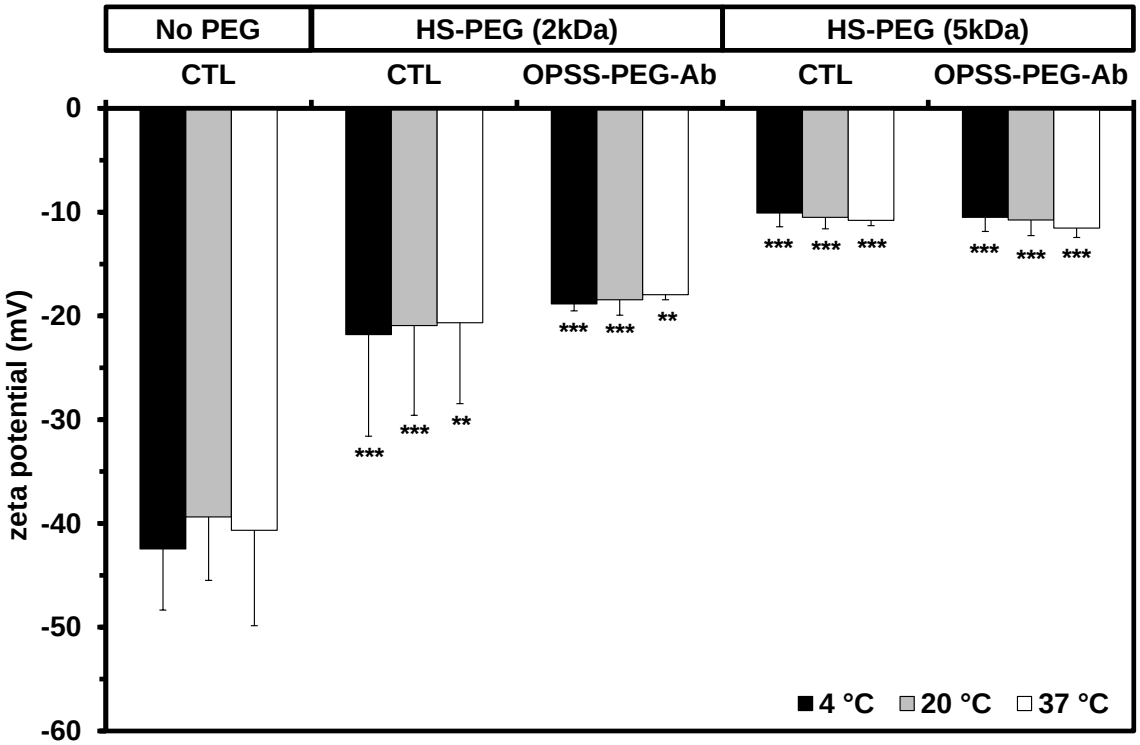

Fig. S3. Zeta potential of AuNPs functionalized with i) HS-PEG (2 or 5kDa) or ii) OPSS-PEG-Ab (0.45%) during 1 h sequentially followed by HS-PEG measured at 4, 20 and 37 °C. Results are expressed as means ± SD from three independent experiments. Statistically significant differences are indicated by **p < 0.01 and ***p < 0.001 in comparison to citrate-capped AuNPs for each temperature.

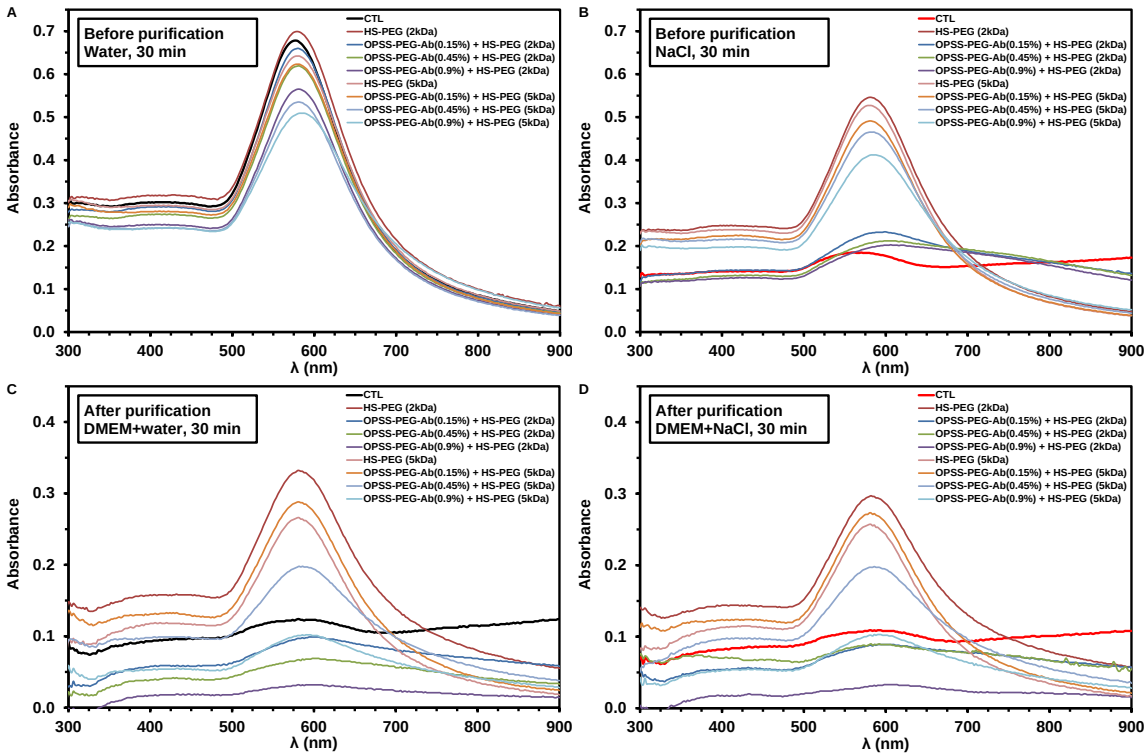

Fig. S4. UV-visible-NIR spectra of AuNPs functionalized with i) HS-PEG (2 or 5kDa) or ii) OPSS-PEG-Ab (0.15, 0.45 or 0.9%) during 1 h sequentially followed by HS-PEG. Samples before purification (A,B) or after centrifugation and resuspension in phenol red-free DMEM (C,D) were incubated for 30 min in water (A,C) or 1% NaCl (B,D). Two other independent experiments gave similar results.

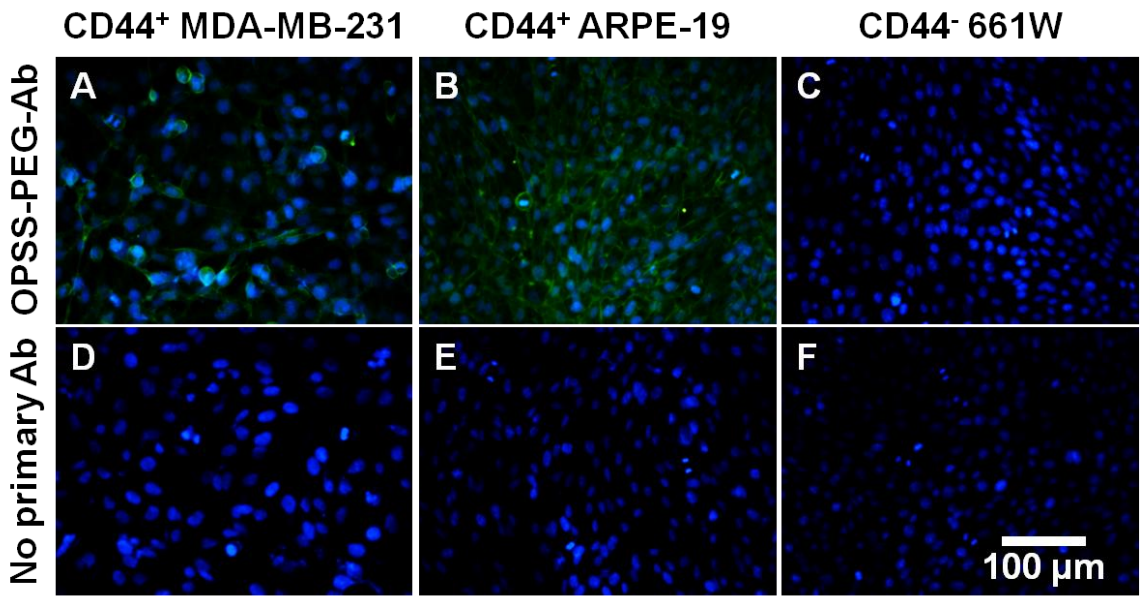

Fig. S5. Immunofluorescence of CD44 cell surface receptors in $CD44^+$ MDA-MB-231, $CD44^+$ ARPE-19 and $CD44^-$ 661W cells. OPSS-PEG-Ab (A-C) or negative controls without primary Abs (D-F) were detected with green-emitting Alexa Fluor 488 dye conjugated to goat anti-rat IgG Abs and cell nuclei were stained with DAPI (blue). Representative images were obtained from five independent experiments.

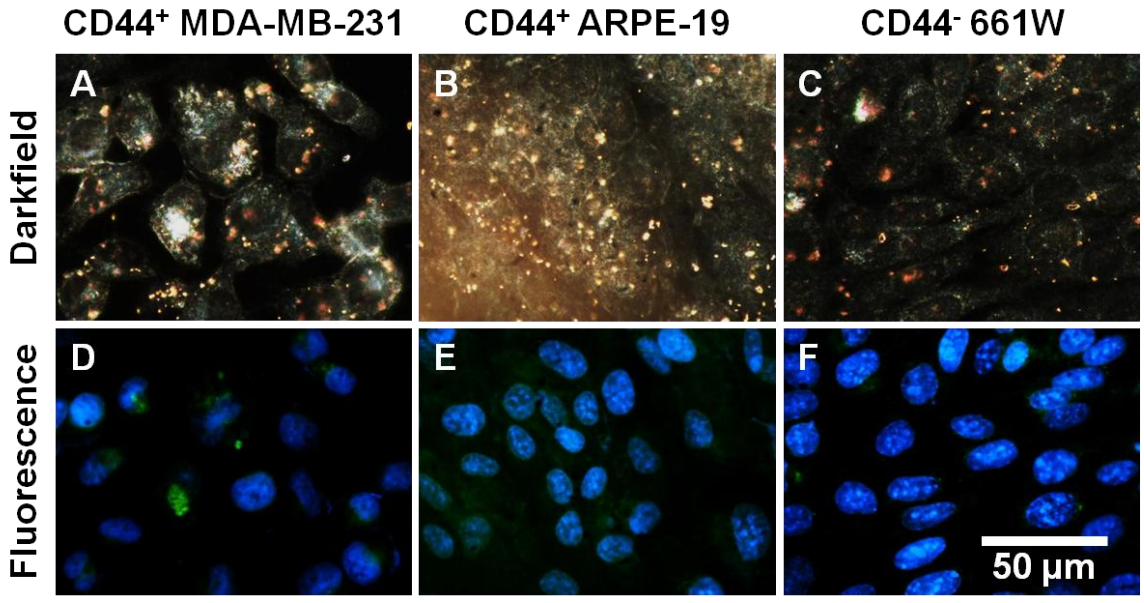

Fig. S6. Darkfield (A-C) and fluorescence (D-F) imaging of $CD44^+$ MDA-MB-231, $CD44^+$ ARPE-19 and $CD44^-$ 661W cells incubated for 3 h in DMEM/10% FBS/1% PS with 8 µg/mL purified citrate-capped AuNPs. Anti-CD44 Abs were detected with green-emitting Alexa Fluor 488 dye conjugated to goat anti-rat IgG Abs and cell nuclei were stained with DAPI (blue, D-F). Representative images were obtained from two independent experiments.

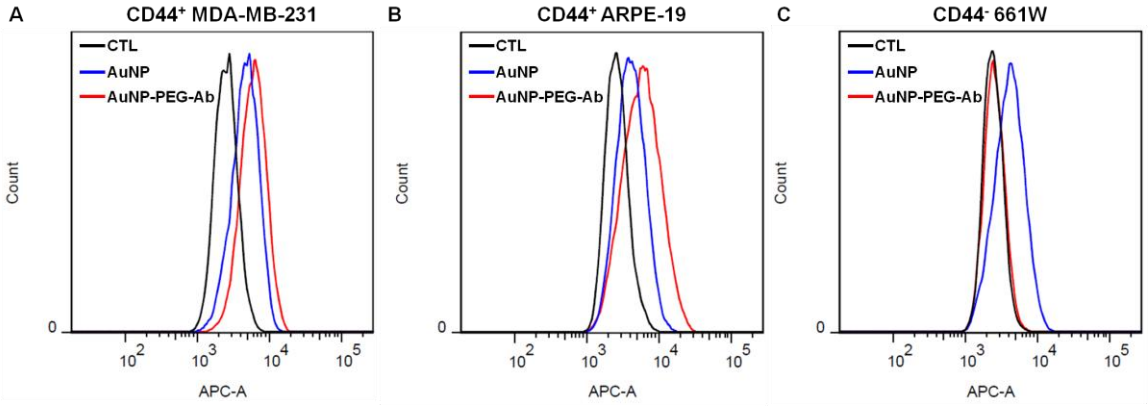

Fig. S7. Flow cytometry of CD44$^+$ MDA-MB-231 (A), CD44$^+$ ARPE-19 (B) and CD44$^-$ 661W (C) cells incubated for 3 h in DMEM/10% FBS/1% PS without AuNPs (CTL), with 8 µg/mL purified citrate-capped AuNPs or AuNPs functionalized with OPSS-PEG-Ab (0.45%) during 1 h sequentially followed by HS-PEG (5kDa). Forward scatter signal from a 633 nm laser without filter (APC-A) was recorded.

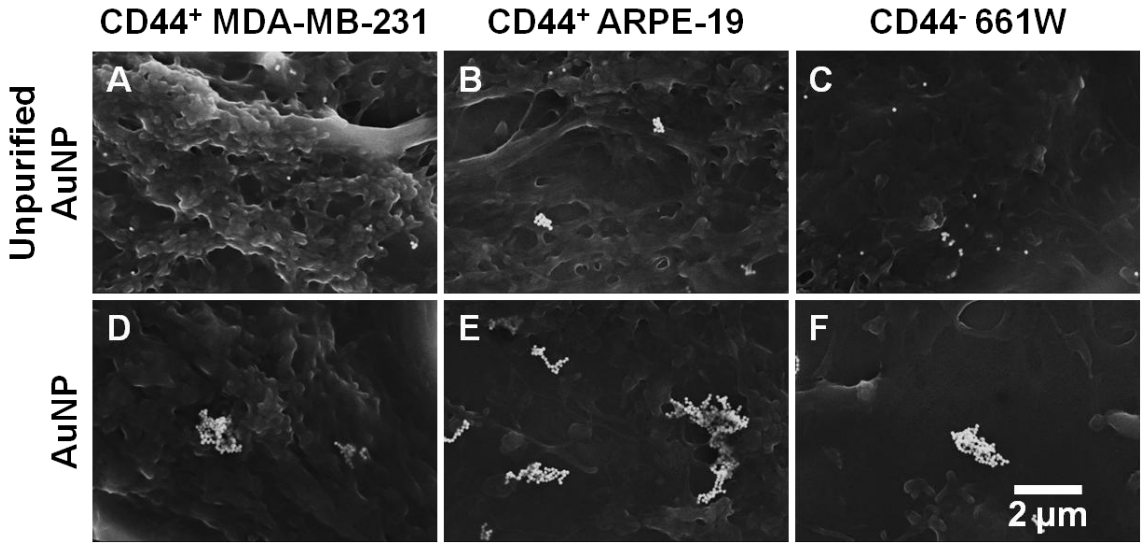

Fig. S8. SEM of CD44$^+$ MDA-MB-231, CD44$^+$ ARPE-19 and CD44$^-$ 661W cells incubated for 3 h in DMEM/10% FBS/1% PS with 8 µg/mL unpurified citrate-capped AuNPs (A-C) or purified citrate-capped AuNPs (D-F).

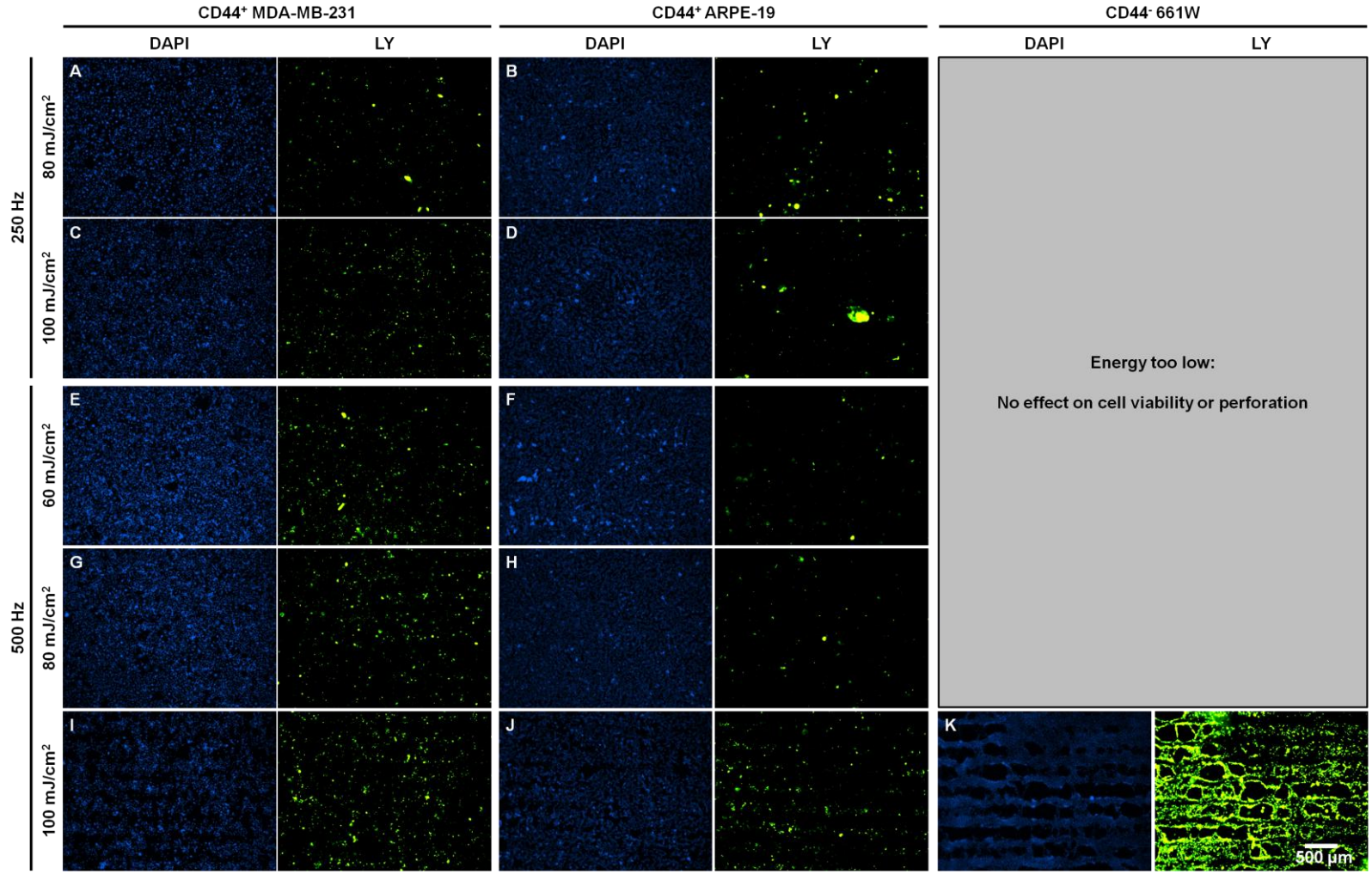

Fig. S9. Fluorescence imaging of laser-treated CD44$^+$ MDA-MB-231, CD44$^+$ ARPE-19 and CD44$^-$ 661W cells incubated for 3 h in DMEM/10% FBS/1% PS without AuNPs (A-K). LY (green) was added before laser treatment (60-100 mJ/cm^2, 250-500 Hz) and cell nuclei were stained with DAPI (blue). Representative images were obtained from two independent experiments.

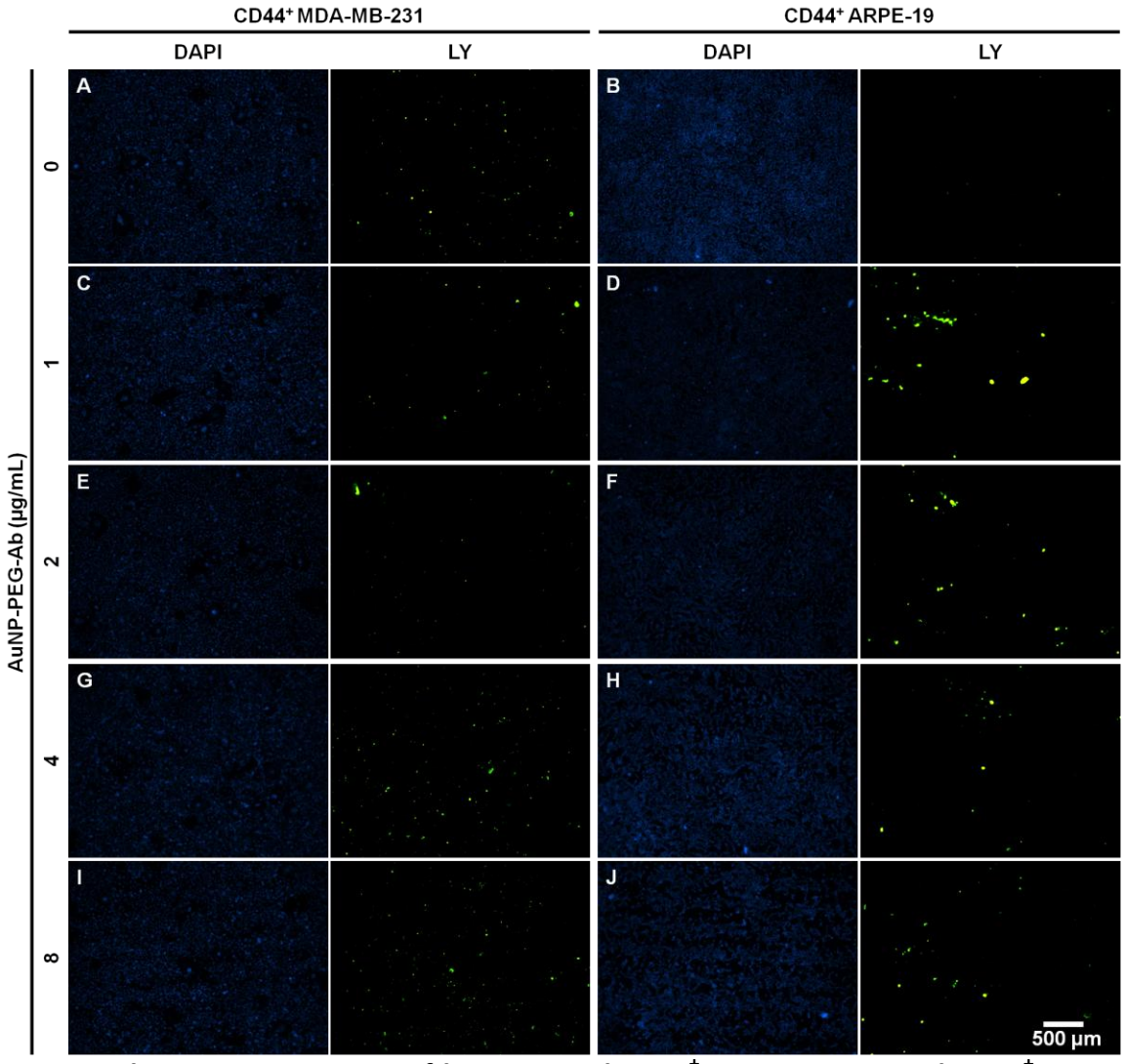

Fig. S10. Fluorescence imaging of laser-treated CD44$^+$ MDA-MB-231 and CD44$^+$ ARPE-19 cells incubated for 3 h in DMEM/10% FBS/1% PS without AuNPs (A,B) or with 1-8 μg/mL AuNPs functionalized with OPSS-PEG-Ab (0.45%) during 1 h sequentially followed by HS-PEG (5kDa) (C-J). LY (green) was added before laser treatment (60 mJ/cm^2, 500 Hz) and cell nuclei were stained with DAPI (blue). Representative images were obtained from three independent experiments.

TABLES

Table S1. Concentration of the reagents for the functionalization of AuNPs.

OPSS-PEG-Ab (%)	OPSS-PEG-Ab (nM)	OPSS-PEG-NHS (nM)	HS-PEG (μM)	HS-PEG/OPSS-PEG-Ab	OPSS-PEG-Ab/AuNP
0.15	0.84	0.75	5	5933	110
0.45	2.53	2.24	5	1978	329
0.9	5.05	4.48	5	989	658

Table S2. Increase in forward signal of light scattering from cells with citrate-capped AuNPs or functionalized AuNPs in comparison to cells without AuNPs.

	AuNP		AuNP-PEG-Ab	
Cells	Δ vs CTL (mean)	Δ vs CTL (median)	Δ vs CTL (mean)	Δ vs CTL (median)
$CD44^+$ MDA-MB-231	1831 ± 454	1691 ± 495	3583 ± 121	3270 ± 224
$CD44^+$ ARPE-19	2323 ± 1241	2089 ± 1185	4220 ± 1103	3372 ± 848
$CD44^-$ 661W	1665 ± 262	1385 ± 310	-28 ± 136	-34 ± 119

Table S3. Normalized forward signal of light scattering from cells with citrate-capped AuNPs or functionalized AuNPs in comparison to cells without AuNPs.

	AuNP		AuNP-PEG-Ab	
Cells	APC-A mean	APC-A median	APC-A mean	APC-A median
$CD44^+$ MDA-MB-231	1.98 ± 0.37	1.96 ± 0.38	2.62 ± 0.19	2.58 ± 0.15
$CD44^+$ ARPE-19	2.06 ± 0.70	2.03 ± 0.70	2.67 ± 0.36	2.45 ± 0.28
$CD44^-$ 661W	1.83 ± 0.12	1.73 ± 0.06	0.99 ± 0.05	0.98 ± 0.05

Table S4. Cell viability after 3 h incubation with citrate-capped AuNPs or functionalized AuNPs.

	Cell viability (%)	
Cells	AuNP	AuNP-PEG-Ab
$CD44^+$ MDA-MB-231	89 ± 18	89 ± 15
$CD44^+$ ARPE-19	75 ± 11	94 ± 6
$CD44^-$ 661W	93 ± 38	112 ± 27

Table S5. Cell size and number of unpurified citrate-capped AuNPs, purified citrate-capped AuNPs or functionalized AuNPs on cells.

Cells	Cell size (μm^2)	Unpurified AuNP/cell	Purified AuNP/cell	AuNP-PEG-Ab/cell
$CD44^+$ MDA-MB-231	736 ± 418	151 ± 108	901 ± 479	229 ± 263
$CD44^+$ ARPE-19	439 ± 188	260 ± 389	1633 ± 318	237 ± 67
$CD44^-$ 661W	448 ± 143	237 ± 265	378 ± 482	2 ± 3