Electronic Supplementary Material (ESI) for Nanoscale.

This supplementary information was **updated** on **28/05/2020.**

Near-infrared nanosecond-pulsed laser-activated high efficient intracellular delivery mediated by nano-corrugated mushroom-shaped gold-coated polystyrene nanoparticles

The previous supplementary information contained errors in Figures S9, S20 and S21, and in the captions of Figures S10, S11 and S17. Labels (b) and (c) in Figure S9 have been reversed. The second image in row three of Figure S20 has been labelled as (j). Incorrect fluence values in the leftmost column of Figure S21 have been removed, with the correct values stated in the caption.

The images that have been replaced, and their original captions, are copied below for future reference. The original captions of S10, S11 and S17 are also copied below.

Please contact Nanoscale@rsc.org with any inquiries, citing the DOI: doi.org/10.1039/D0NR01792B

Fig S10: Control experiment using nm-AuPNPs and pulse laser interaction of different days for CL1-0 cells. (a) Bright field image using nm-AuPNPs and laser interaction after one day (b) viability image using calcein AM (c) dead cell detection using EthD-1 (d) (a) Bright field image using nm-AuPNPs and laser interaction after two days (b) viability image using calcein AM (c) dead cell detection using EthD 1 (d) (a) Bright field image using nm-AuPNPs and laser interaction after three days (b) viability image using calcein AM (c) dead cell detection using EthD-1 (d)The laser exposure was 1 minutes at 10 Hz pulsing frequency with 40 mJ/cm^2 laser fluence at 945 nm.

Fig. S11: Time dependent delivery efficiency and viability at 945 nm for CL1-0 cells with nm-AuPNPs as a mediator. Due to increase of time, the delivery efficiency increases and it is maximum (delivery efficiency 94% and viability close to 100%) at 30 seconds and then the delivery efficiency and viability decreases with increase of time. The laser exposure was 30 seconds at 10 Hz pulsing frequency with 35 mJ/cm^2 laser fluence. Data show average ± standard deviation (n =3 replicate).

Fig. S17: PI dye delivery on non-exposure area of AGS cells and P-19 stem cells (a) bright field image on non-exposure area of AGS cells (b) corresponding cell nucleus image (using hoechst 33342) (c) non-exposure area indicating no PI dye delivery (d) cell viability test with merge image of PI dye and calcein AM shows all cells are viable in non-exposure area (e) bright field image on non-exposure area of P-19 cells (f) corresponding cell nucleus image (using hoechst 33342) (c) no PI dye delivery on non-exposure area (d) cell viability test with merge image of PI dye and calcein AM shows all cells are viable in non-exposure area. For each experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 35 mJ/cm^2 laser fluence at 945 nm.

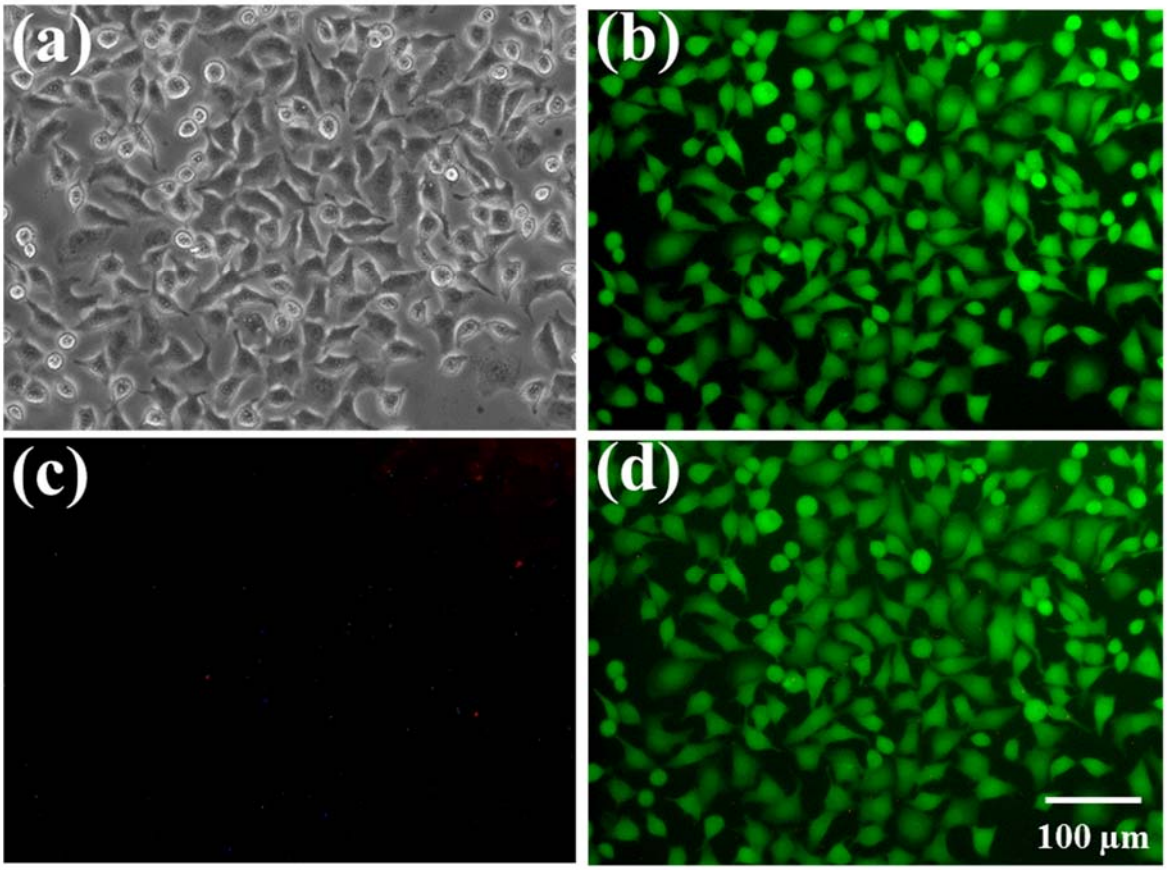

Fig. S9 PI dye delivery and cell viability of AGS cells without nm-AuPNPs attachment onto the cell surface after pulsed laser exposure (a) Bright field image on laser exposure area without nm-AuPNPs (b) no PI dye delivery without nm-AuPNPs (c) cell viability test using calcein AM shows all cells are viable after laser exposure (d) merge image of PI dye and calcein AM. The laser exposure was 30 seconds at 10 Hz pulsing frequency with 30 mJ/cm^2 laser fluence at 945 nm.

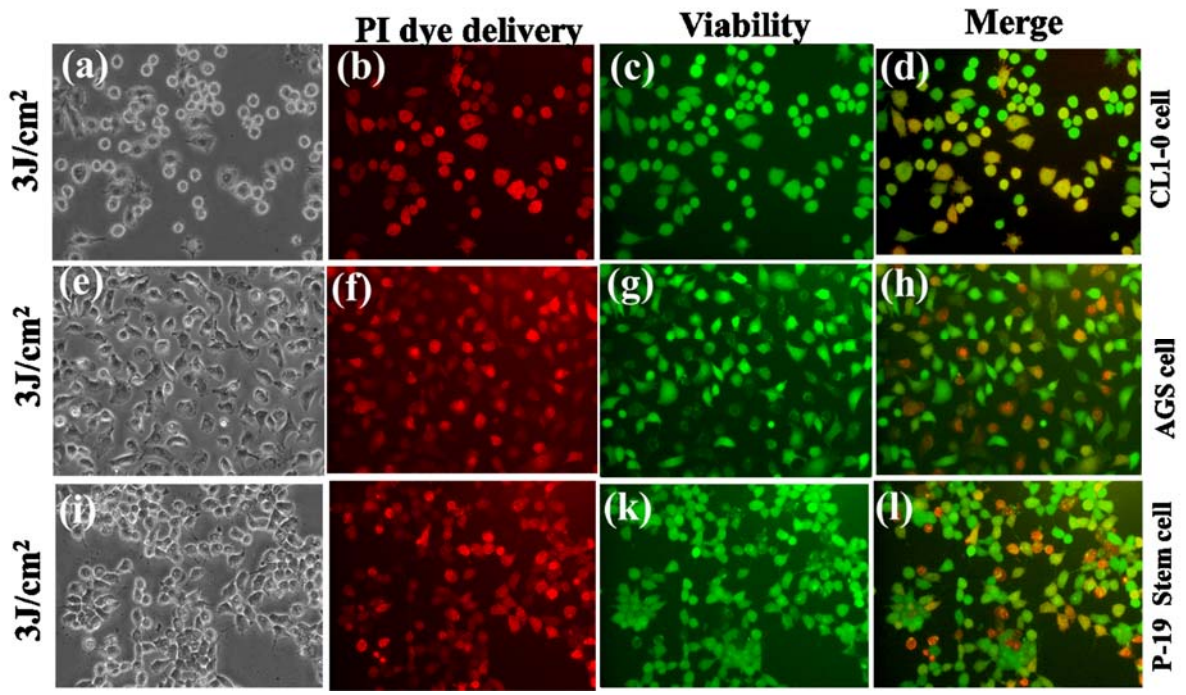

Fig. S20 Pulsed laser exposure PI dye delivery and cell viability of CL1-0, AGS and P-19 embryonic stem cell at 630 nm with spherical AuPNPs as a mediator (a) bright field image (b) red fluorescence indicating successful PI dye delivery into lung cancer (CL1-0) cells (c) green fluorescence indicating maximum cells viable using calcein AM (d) merge image of PI dye and calcein AM (e) bright field image of AGS cells (f) successful PI dye delivery (g) cell viability test using calcein AM (h) merge image of PI dye and calcein AM (i) bright field image of P-19 stem cells (j) successful PI dye delivery into P-19 embryonic stem cells (k) cell viability test using calcein AM (l) merge image of PI dye and calcein AM. For this experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 120 mJ/cm^2 laser fluence.

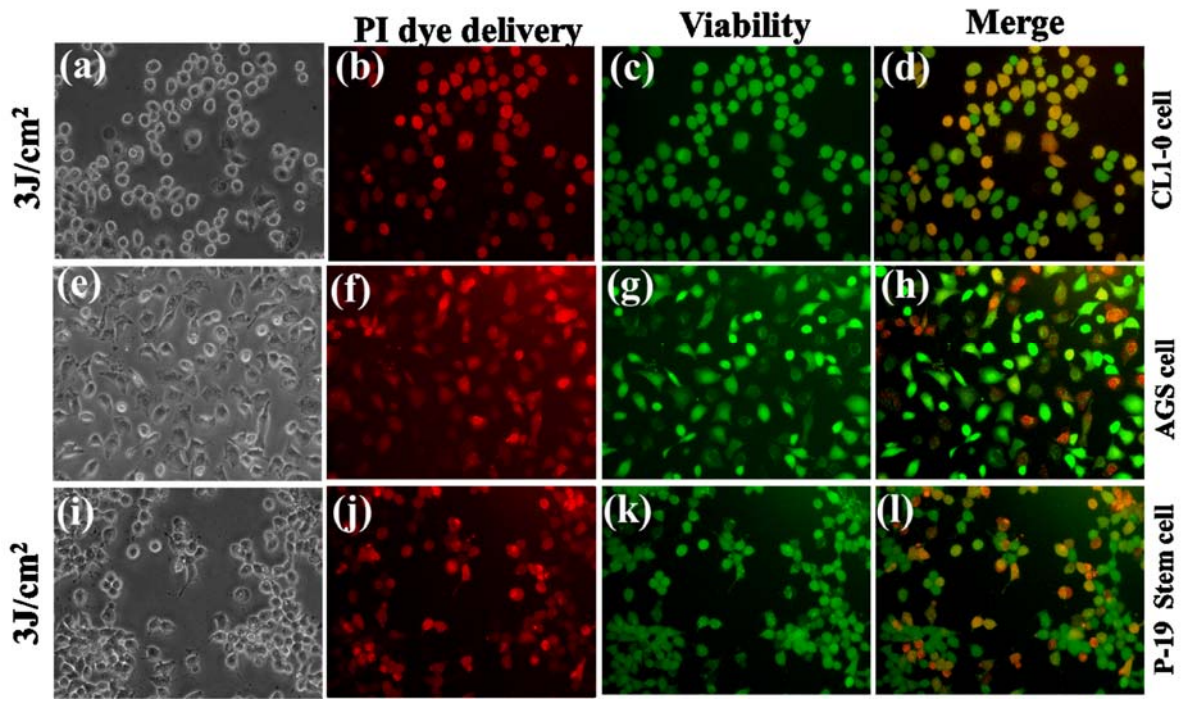

Fig. S21 Pulsed laser exposure PI dye delivery and cell viability of CL1-0, AGS and P-19 embryonic stem cell at 730 nm with spherical AuPNPs as a mediator (a) bright field image (b) red fluorescence indicating successful PI dye delivery into lung cancer (CL1-0) cells (c) green fluorescence indicating maximum cells viable using calcein AM (d) merge image of PI dye and calcein AM (e) bright field image of AGS cells (f) successful PI dye delivery (g) cell viability test using calcein AM (h) merge image of PI dye and calcein AM (i) bright field image of P-19 stem cells (j) successful PI dye delivery into P-19 embryonic stem cells (k) cell viability test using calcein AM (l) merge image of PI dye and calcein AM. For this experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 110 mJ/cm^2 laser fluence.

Supplementary information

Near infrared nanosecond-pulsed laser activated highly efficient intracellular delivery mediated by nano-corrugated mushroom-shaped gold coated polystyrene nanoparticles

Simulation:

We modeled electric field enhancement using electromagnetic waves, frequency domain (Radio Frequency Module) in COMSOL Multiphysics. A Gaussian electromagnetic wave was incident on a nanoparticle to model electric field enhancement using finite element method with frequency. For the simulation of temperature distribution, time dependent PDE were used in COMSOL Multiphysics.

Fabrication

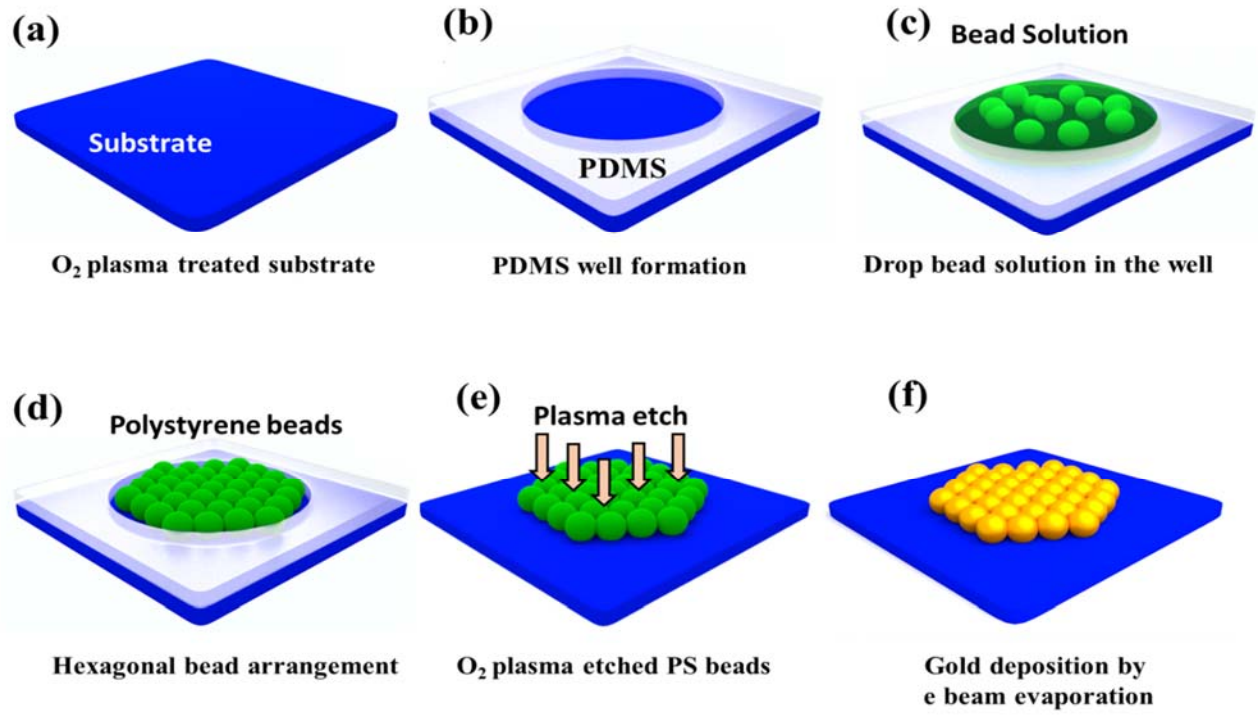

Fig. S1 nm-AuPNPs fabrication (a) O_2 plasma treatment on clean silicon substrate (b) PDMS well formation on silicon substrate (c) polystyrene beads (PS) solution into the PDMS well (d) hexagonal array formation of PS beads (e) O_2 plasma etched of PS beads using reactive ion etching (RIE) to form nanocorrugated mushroom shape beads (f) gold deposition using e-beam evaporation to form high-aspect-ratio based nm-AuPNPs. The details fabrication and its application is in reference [1-2]

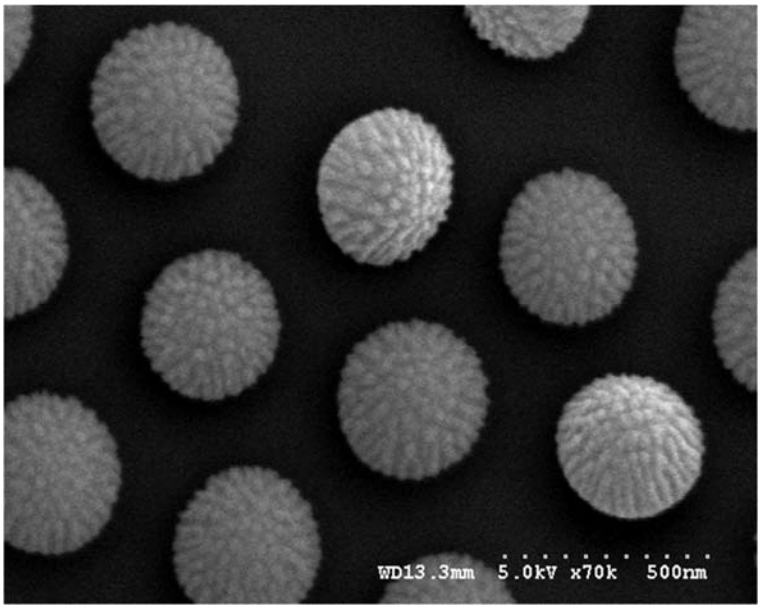

Fig. S2 Scanning electron microscopy (SEM) image of high-aspect-ratio nm-AuPNPs.

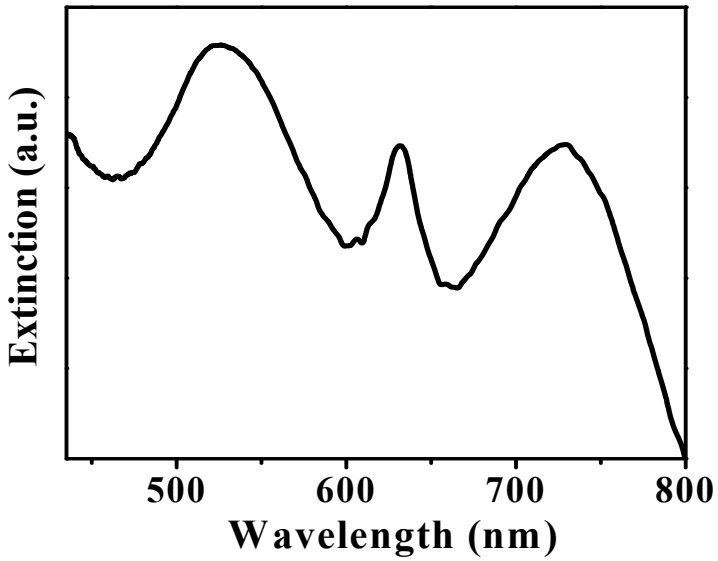

Fig. S3 UV extinction spectra of 300 nm spherical AuPNPs with higher peak at 522 nm, 630 nm and 730 nm wavelength.

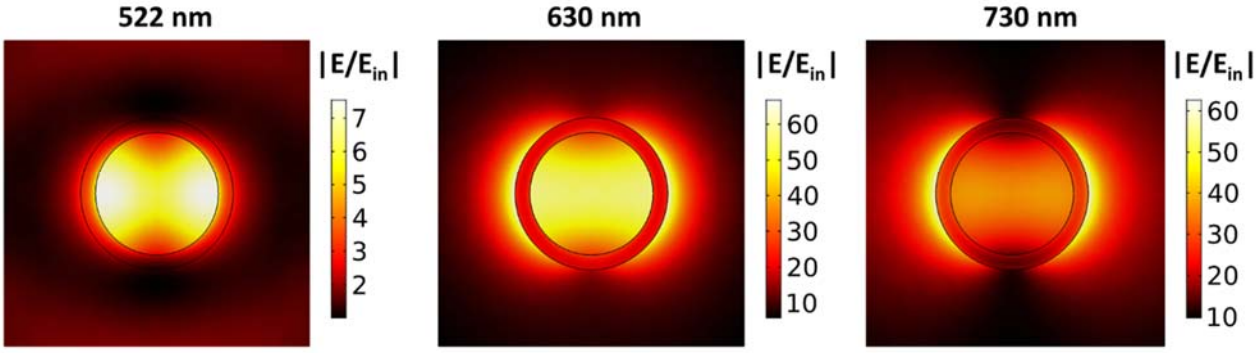

Fig. S4 Cross-sectional view of Electromagnetic field enhancement results at the middle of spherical core-shell AuPNPs at 522 nm, 630 nm and 730 nm.

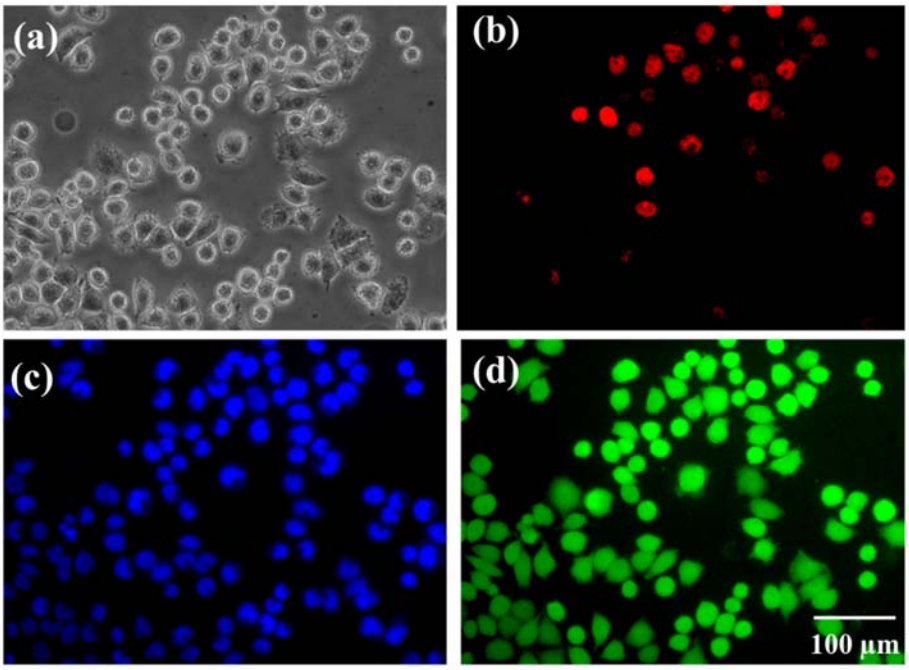

Fig. S5 PI dye delivery using spherical AuPNPs for CL1-0 cells (a) bright field image before laser exposure (b) PI dye delivery after laser exposure, where few cells successfully deliver PI dye into cells (c) cell nucleus stained using hoechst 33342 (d) cell viability test using calcein AM after PI

dye delivery where all cells are viable after exposure. For this experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 25 mJ/cm^2 laser fluence at 522 nm.

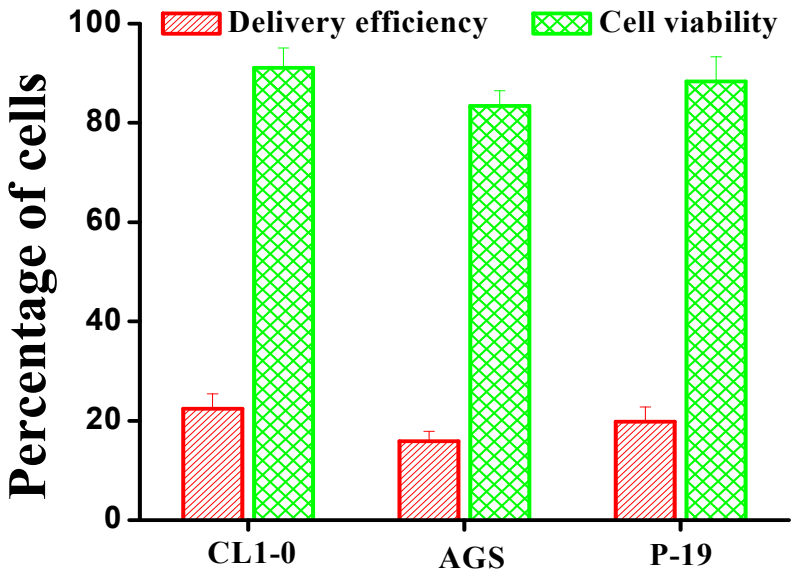

Fig. S6 Delivery efficiency and cell viability of different cells using spherical AuPNPs with 30 seconds laser exposure at 10 Hz pulsing frequency with 25 mJ/cm^2 laser fluence at 522 nm. Data show average ± standard deviation (n =3 replicate).

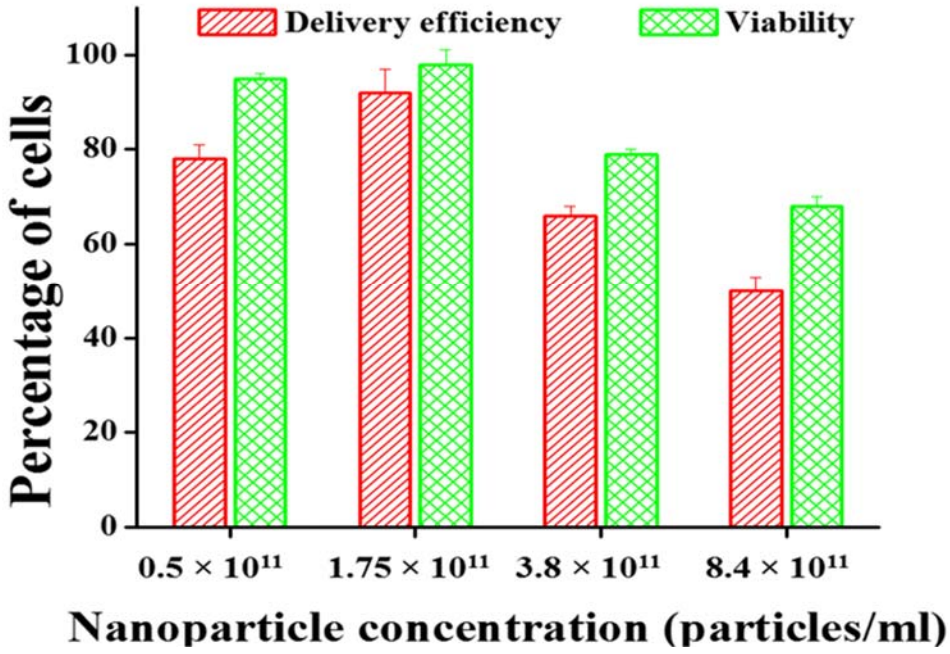

Fig. S7 Delivery efficiency and viability of CL1-0 cells with different nm-AuPNPs concentration at 945 nm. The maximum efficiency and viability were observed at 1.75×10^{11} particles/ml. For higher particles concentration, the delivery efficiency and viability reduces dramatically. The laser exposure was 30 seconds at 10 Hz pulsing frequency with 30 mJ/cm^2 laser fluence. Data show average ± standard deviation (n =3 replicate).

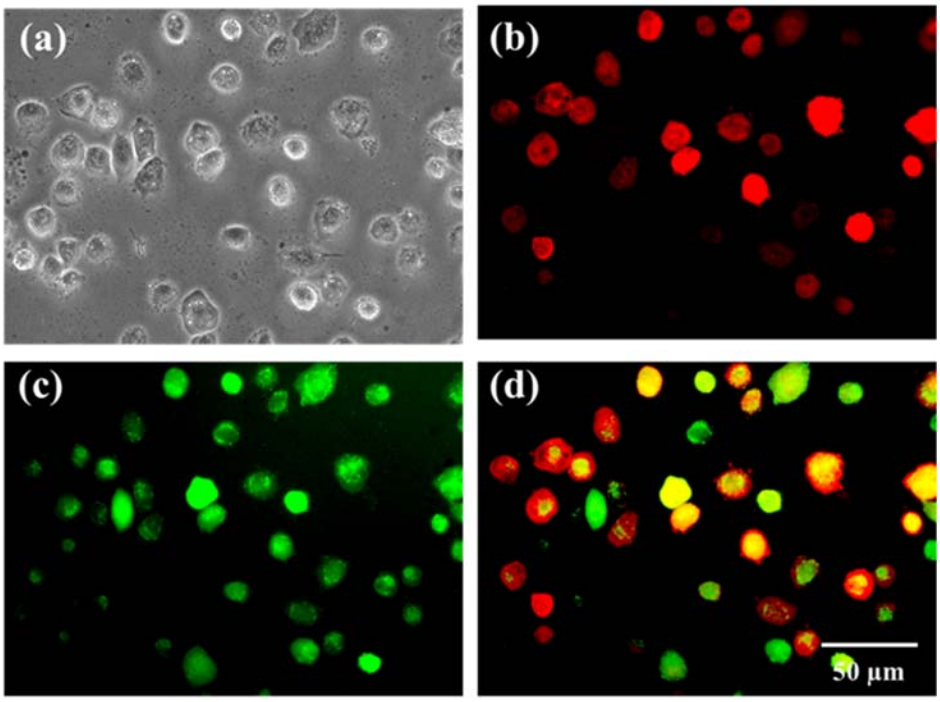

Fig. S8 PI dye delivery and viability with higher concentration of nm-AuPNPs (3.8×10^{11} particles/ml) for CL1-0 cells (a) bright field image after laser exposure area shows many debris on top of the cells as well as surrounding the cells (b) PI dye delivery (c) cell viability test using calcein AM (d) merge image of PI dye and calcein AM. The laser exposure was 30 seconds at 10 Hz pulsing frequency with 30 mJ/cm^2 laser fluence at 945 nm.

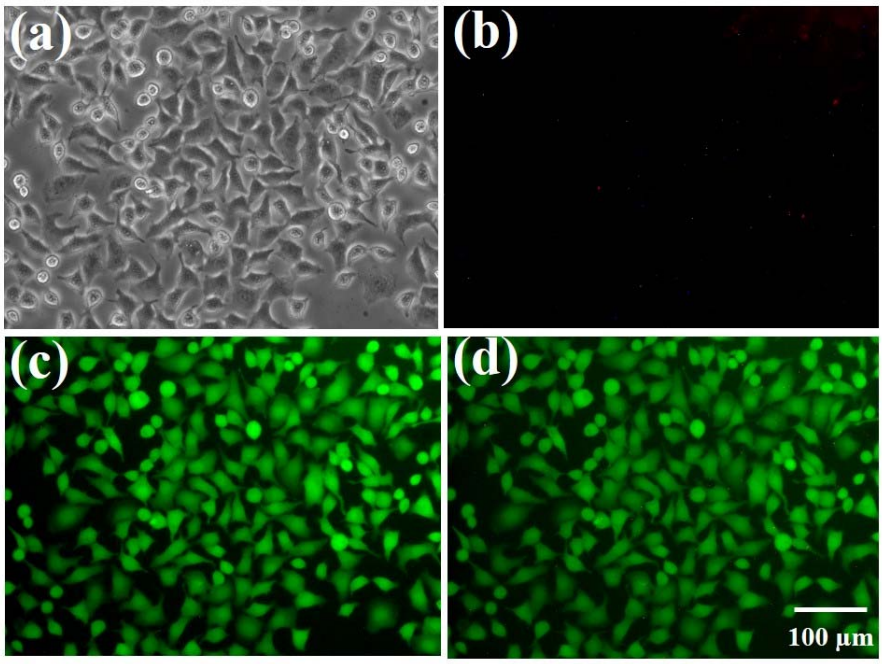

Fig. S9 PI dye delivery and cell viability of AGS cells without nm-AuPNPs attachment onto the cell surface after pulsed laser exposure (a) Bright field image on laser exposure area without nm-AuPNPs (b) no PI dye delivery without nm-AuPNPs (c) cell viability test using calcein AM shows all cells are viable after laser exposure (d) merge image of PI dye and calcein AM. The laser exposure was 30 seconds at 10 Hz pulsing frequency with 30 mJ/cm^2 laser fluence at 945 nm.

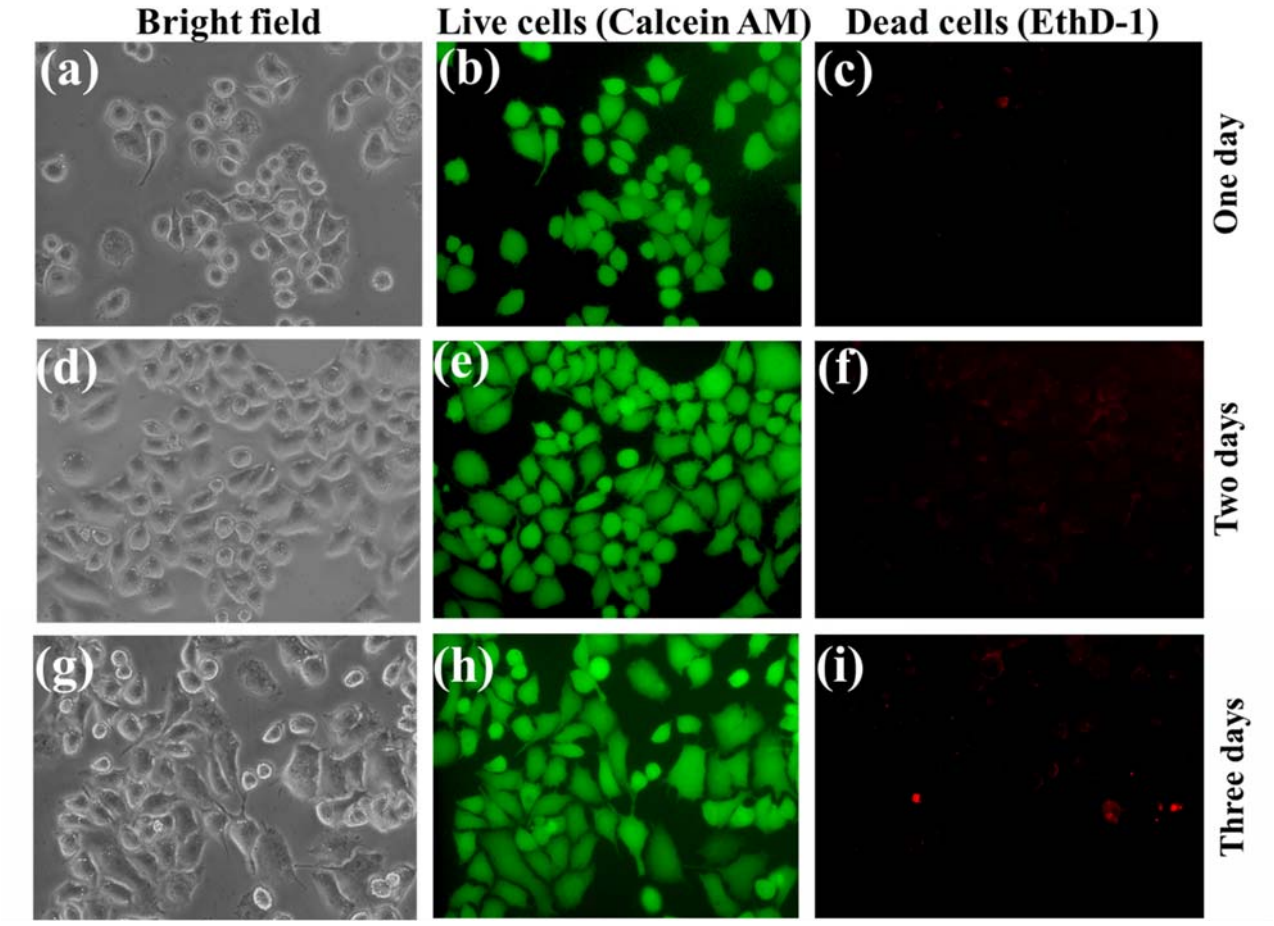

Fig S10: Control experiment using nm-AuPNPs and pulse laser interaction of different days for CL1-0 cells. (a) Bright field image using nm-AuPNPs and laser interaction after one day (b) viability image using calcein AM (c) dead cell detection using EthD-1 (d) Bright field image using nm-AuPNPs and laser interaction after two days (e) viability image using calcein AM (f) dead cell detection using EthD-1 (g) Bright field image using nm-AuPNPs and laser interaction after three days (h) viability image using calcein AM (i) dead cell detection using EthD-1. The laser exposure was 1 minutes at 10 Hz pulsing frequency with 40 mJ/cm^2 laser fluence at 945 nm.

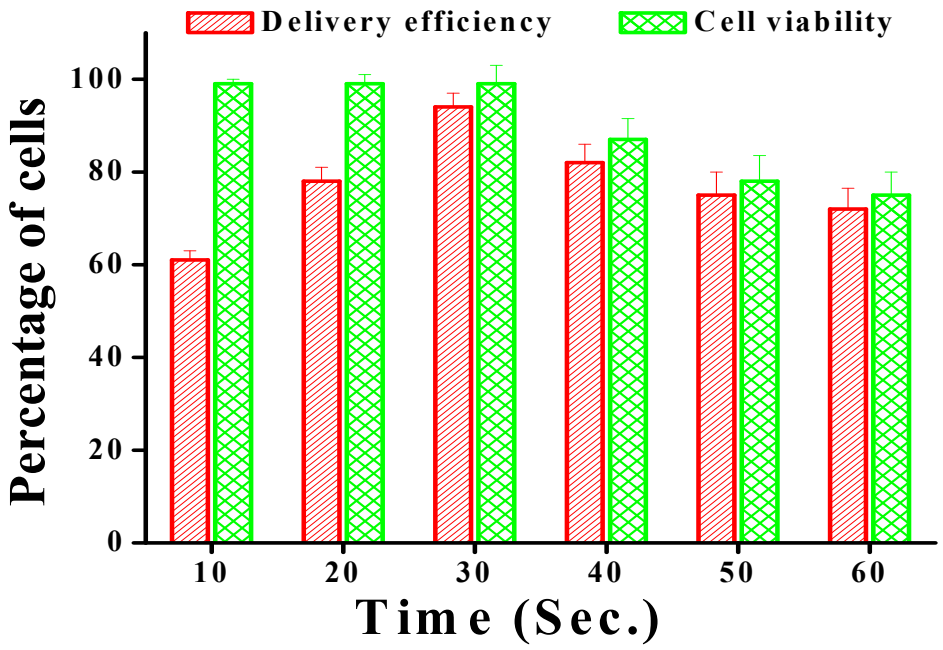

Fig. S11 Time dependent delivery efficiency and viability at 945 nm for CL1-0 cells with nm-AuPNPs as a mediator. Due to increase of time, the delivery efficiency increases and it is maximum (delivery efficiency 94% and viability close to 100%) at 30 seconds and then the delivery efficiency and viability decreases with increase of time. The laser fluence was 35 mJ/cm^2 with 10 Hz pulse repetition rate. Data show average ± standard deviation (n =3 replicate).

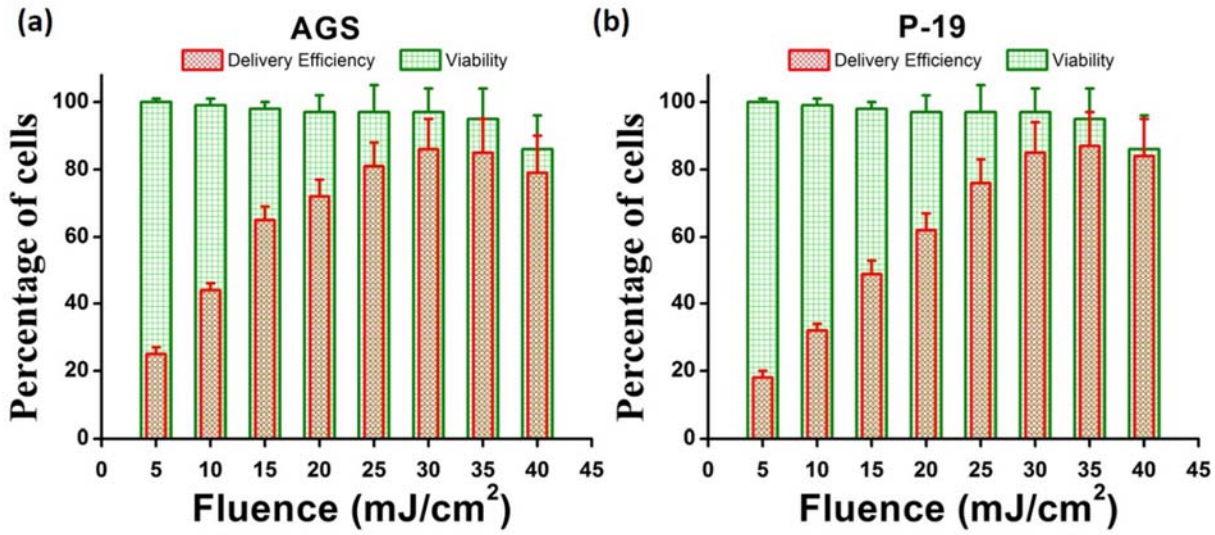

Fig. S12 Delivery efficiency and cell viability of AG S and P-19 stem cells with different laser energy at 945 nm for 30 seconds laser exposure.

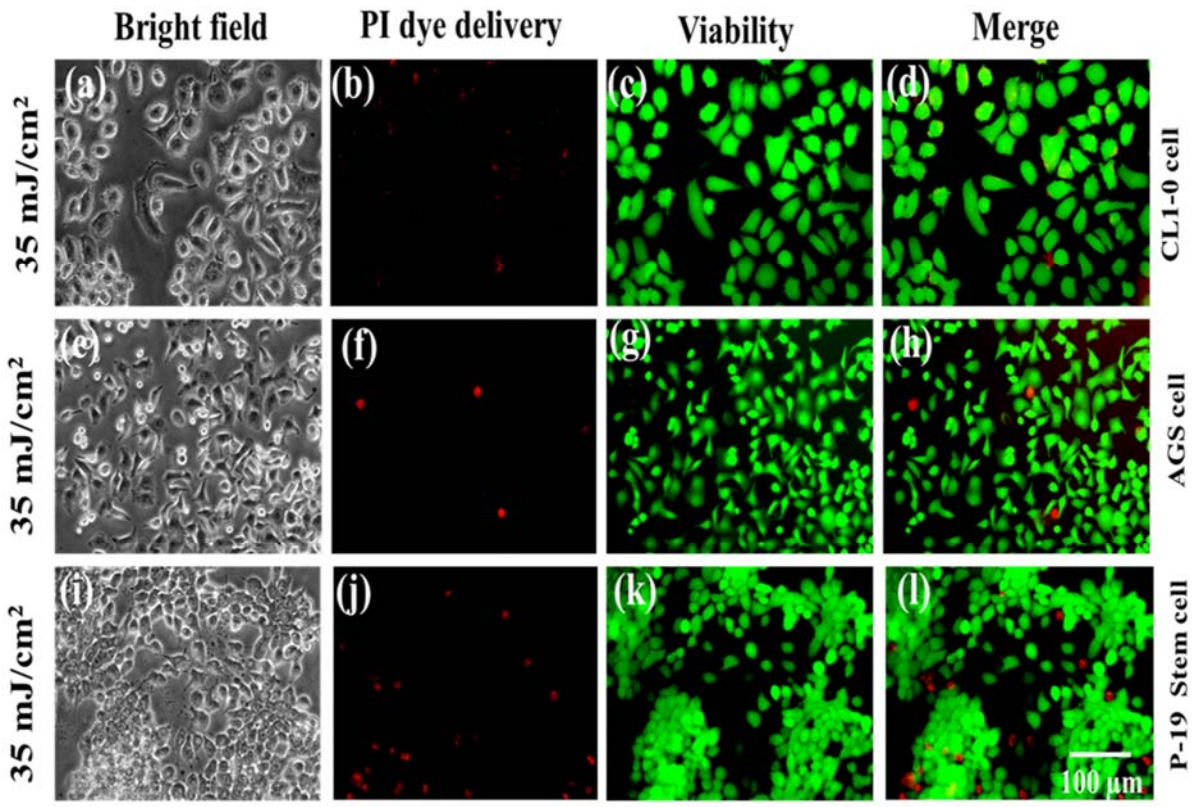

Fig. S13: Pulsed laser exposed PI dye delivery and cell viability of CL1-0, AGS and P-19 embryonic stem cell at 660 nm laser excitation （under nm-AuPNPs mediation) (a) bright field image of CL1-0 cells after laser exposure (b) red fluorescence indicating PI dye delivery and very less dye deliver into cells (c) green fluorescence indicating maximum cells viable by using calcein AM (d) merge image of PI dye and calcein AM (e) bright field image of AGS cells after laser exposure (f) corresponding PI dye delivery into cells (g) cell viability tested by using calcein AM (h) merge image of PI dye and calcein AM (i) bright field image of P-19 cells after laser exposure (j) PI dye delivery into P-19 embryonic stem cells (k) cell viability tested by using calcein AM (l) merge image of PI dye and calcein. For each experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 35 mJ/cm^2 laser fluence.

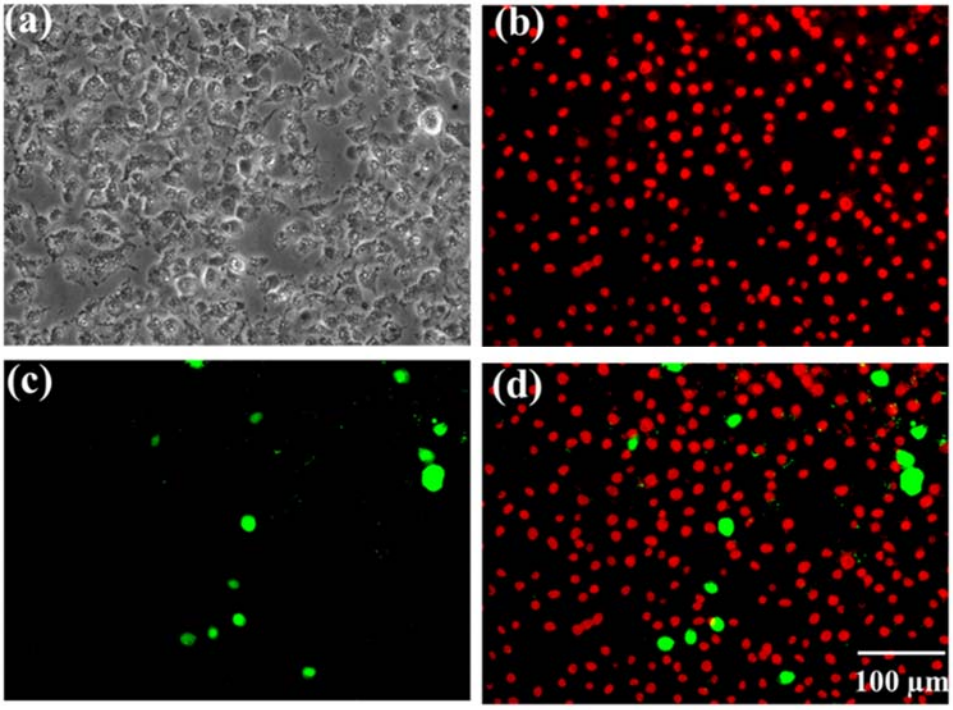

Fig. S14: PI dye delivery at 516 nm with nm-AuPNPs (a) bright field image shows maximum cells are dead after laser exposure (b) due to higher laser exposure, PI dye only stain all nucleus of dead cells (c) cells viability test using calcein AM shows only 3.7 % cells are live (d) merge image of PI dye and calcein AM stain. For this experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 50 mJ/cm^2 laser fluence.

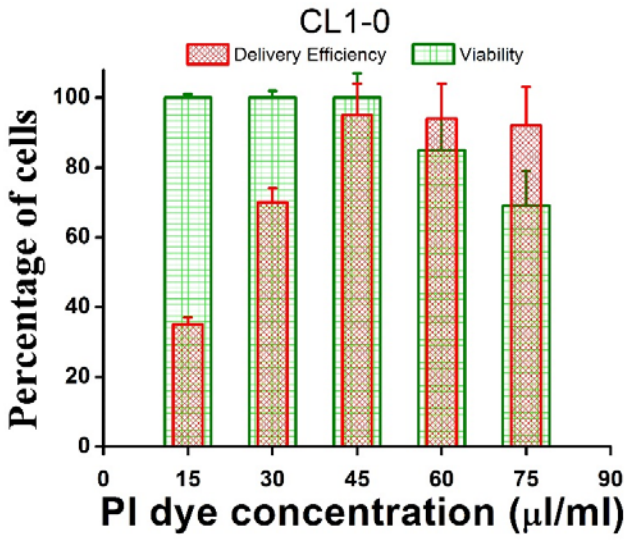

Fig. S15 PI dye concentration dependent delivery efficiency and cell viability of CL1-0 cells at 945 nm wavelength with 35 mJ/cm^2 laser fluence for 30 seconds.

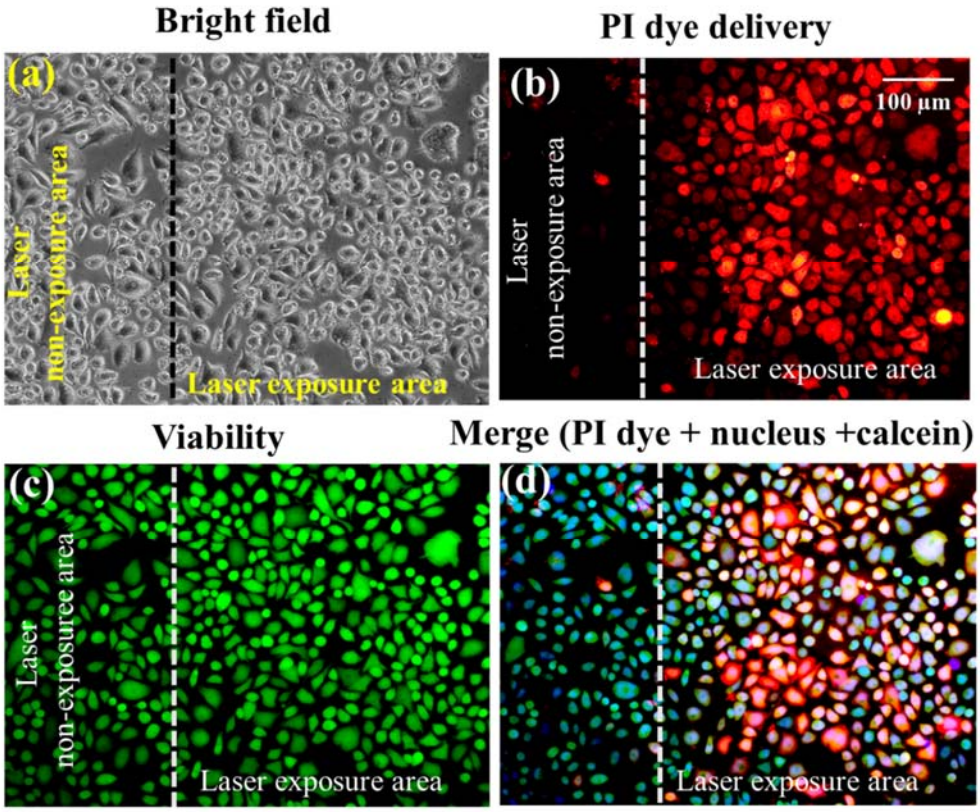

Fig. S16 PI dye delivery on laser exposure and non-exposure area of CL1-0 cells (a) bright field image shows laser exposure and non- exposure area separated with black dotted line (b) PI dye only delivered on laser exposure area (c) cell viability test using calcein AM after PI dye delivery where all cells are viable in exposure and non-exposure area (d) merge image of PI dye, cell nucleus (hoechst 33342) and calcein AM. For this experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 35 mJ/cm^2 laser fluence at 945 nm.

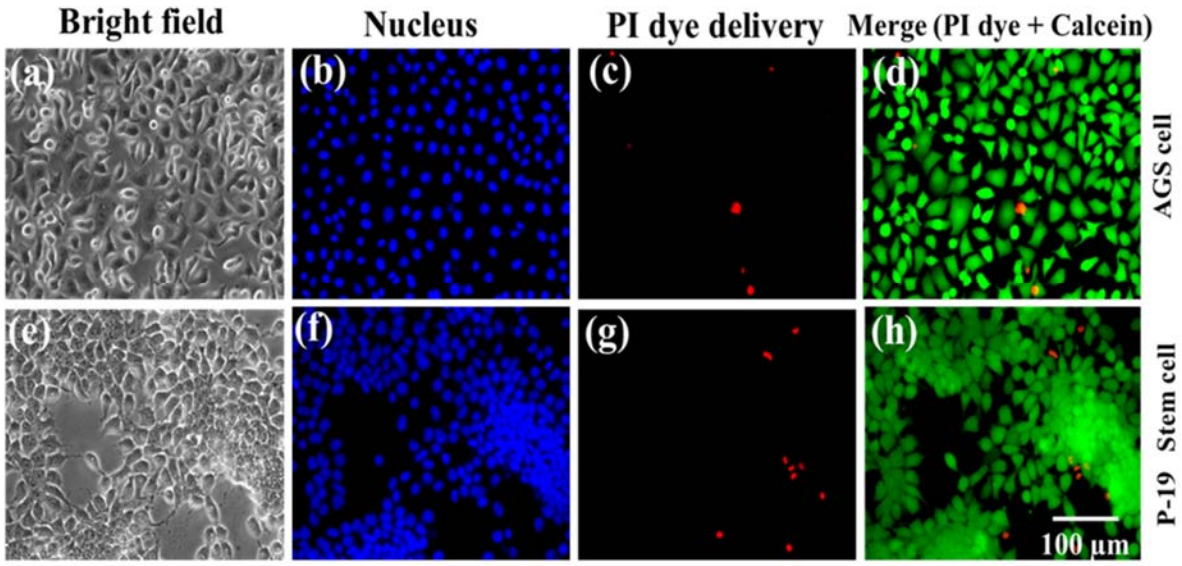

Fig. S17 PI dye delivery on non-exposure area of AGS cells and P-19 stem cells (a) bright field image on non-exposure area of AGS cells (b) corresponding cell nucleus image (using hoechst 33342) (c) non-exposure area indicating no PI dye delivery (d) cell viability test with merge image of PI dye and calcein AM shows all cells are viable in non-exposure area (e) bright field image on non-exposure area of P-19 cells (f) corresponding cell nucleus image (using hoechst 33342) (g) no PI dye delivery on non-exposure area (h) cell viability test with merge image of PI dye and calcein AM shows all cells are viable in non-exposure area. For each experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 35 mJ/cm^2 laser fluence at 945 nm.

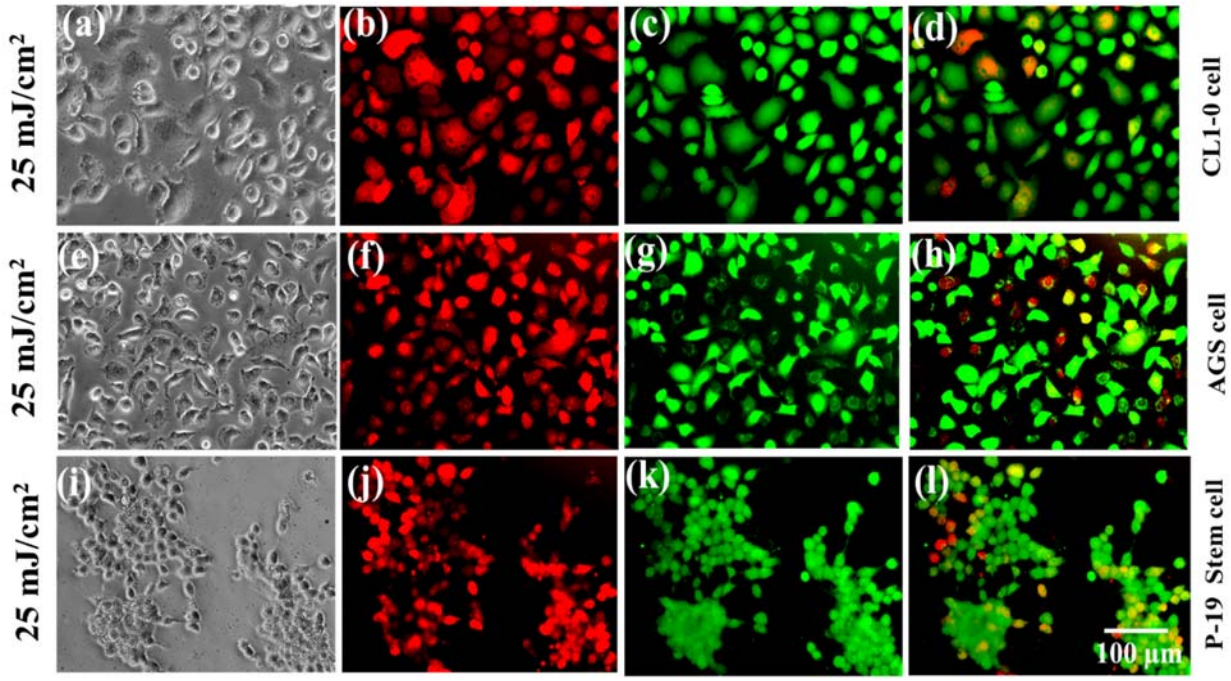

Fig. S18 Pulsed laser exposure PI dye delivery and cell viability of CL1-0, AGS and P-19 embryonic stem cell at 516 nm with nm-AuPNPs as a mediator (a) bright field image (b) red fluorescence indicating successful PI dye delivery into lung cancer (CL1-0) cells (c) green fluorescence indicating maximum cells viable using calcein AM (d) merge image of PI dye and calcein AM (e) bright field image of AGS cells (f) successful PI dye delivery (g) cell viability test using calcein AM (h) merge image of PI dye and calcein AM (i) bright field image of P-19 stem cells (j) successful PI dye delivery into P-19 embryonic stem cells (k) cell viability test using calcein AM (l) merge image of PI dye and calcein AM. For this experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 25 mJ/cm^2 laser fluence.

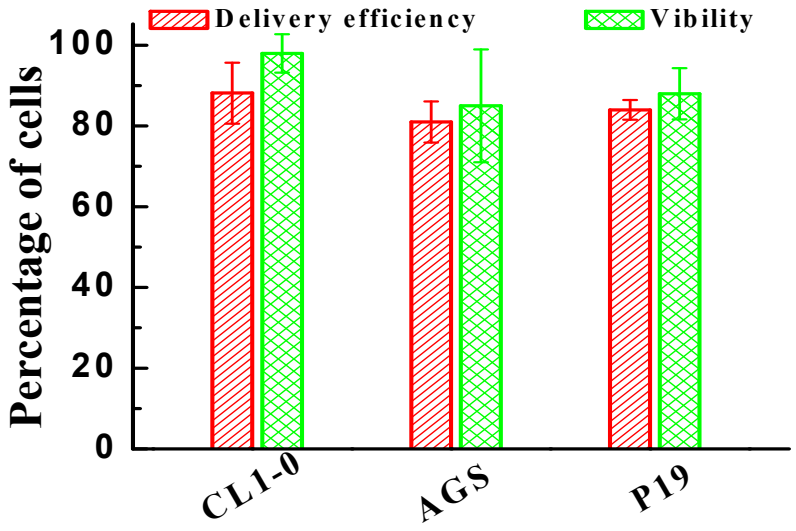

Fig. S19 Delivery efficiency and cell viability at 516 nm wavelength under nm-AuPNPs mediation. The maximum efficiency for CL1-0 cells were 88% with 94% cell viability. While for AGS and P-19 cells, the delivery efficiency were 81% and 84% with 85% and 88% cell viability. The laser exposure was 30 seconds at 10 Hz pulsing frequency with 25 mJ/cm^2 laser fluence. Data show average ± standard deviation (n =3 replicate).

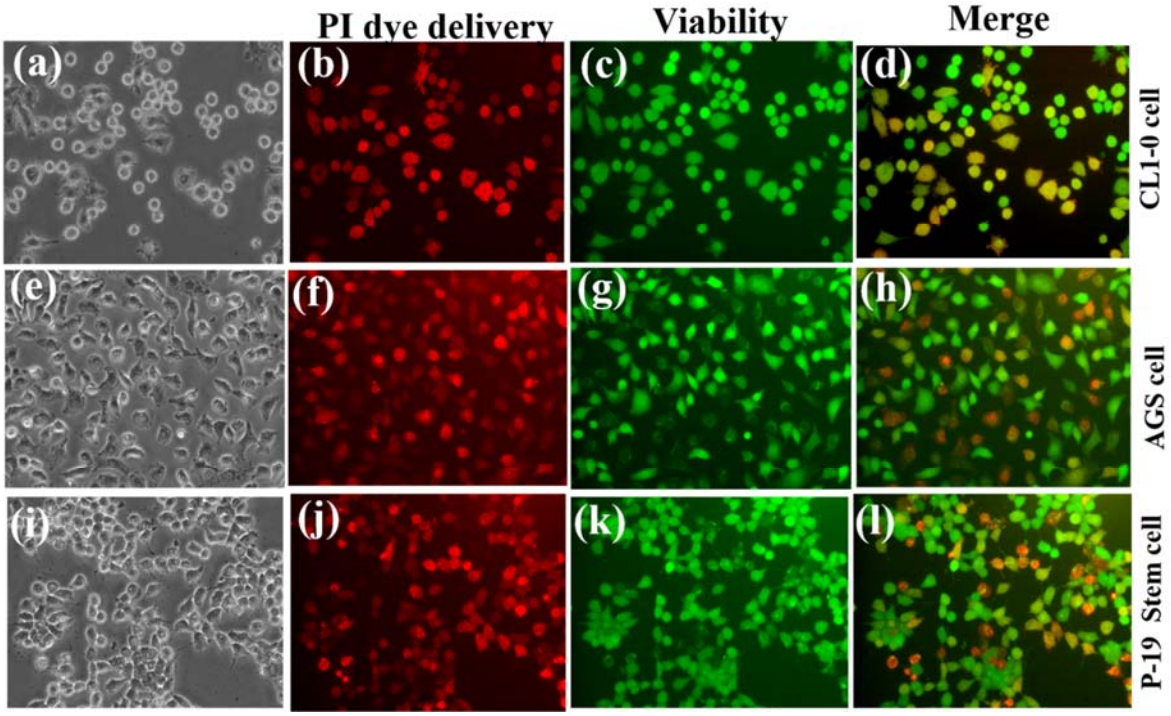

Fig. S20 Pulsed laser exposure PI dye delivery and cell viability of CL1-0, AGS and P-19 embryonic stem cell at 630 nm with spherical AuPNPs as a mediator (a) bright field image (b) red fluorescence indicating successful PI dye delivery into lung cancer (CL1-0) cells (c) green fluorescence indicating maximum cells viable using calcein AM (d) merge image of PI dye and calcein AM (e) bright field image of AGS cells (f) successful PI dye delivery (g) cell viability test using calcein AM (h) merge image of PI dye and calcein AM (i) bright field image of P-19 stem cells (j) successful PI dye delivery into P-19 embryonic stem cells (k) cell viability test using calcein AM (l) merge image of PI dye and calcein AM. For this experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 120 mJ/cm^2 laser fluence.

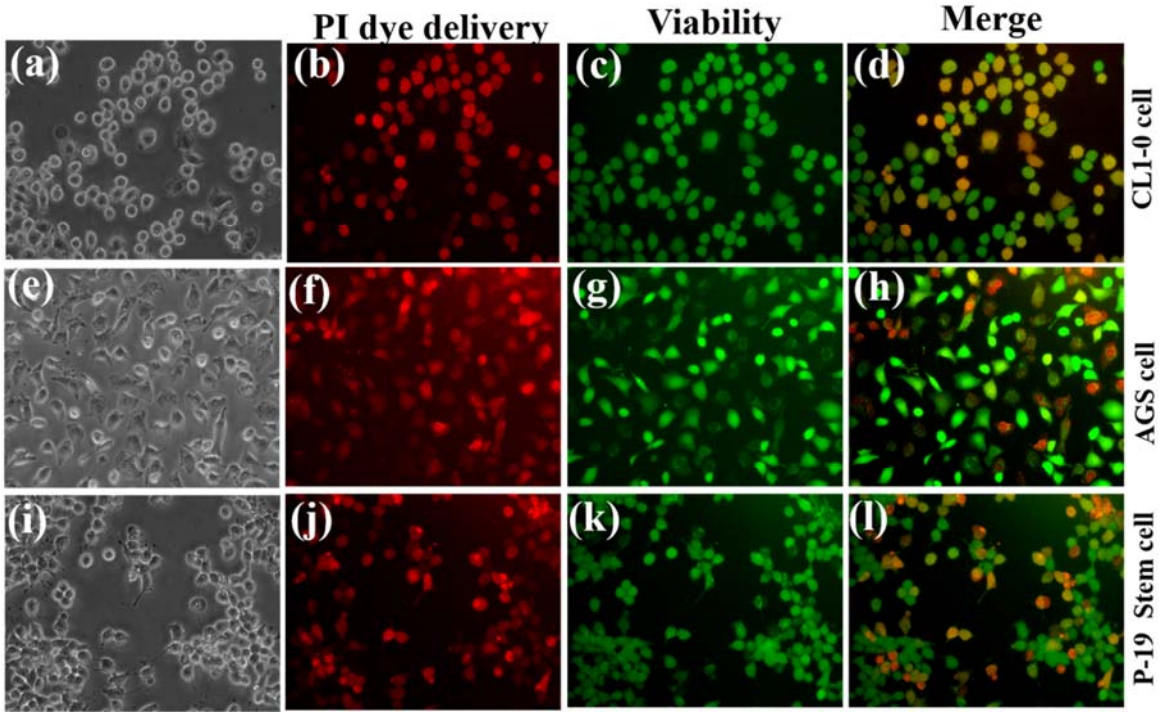

Fig. S21 Pulsed laser exposure PI dye delivery and cell viability of CL1-0, AGS and P-19 embryonic stem cell at 730 nm with spherical AuPNPs as a mediator (a) bright field image (b) red fluorescence indicating successful PI dye delivery into lung cancer (CL1-0) cells (c) green fluorescence indicating maximum cells viable using calcein AM (d) merge image of PI dye and calcein AM (e) bright field image of AGS cells (f) successful PI dye delivery (g) cell viability test using calcein AM (h) merge image of PI dye and calcein AM (i) bright field image of P-19 stem cells (j) successful PI dye delivery into P-19 embryonic stem cells (k) cell viability test using calcein AM (l) merge image of PI dye and calcein AM. For this experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 110 mJ/cm^2 laser fluence.

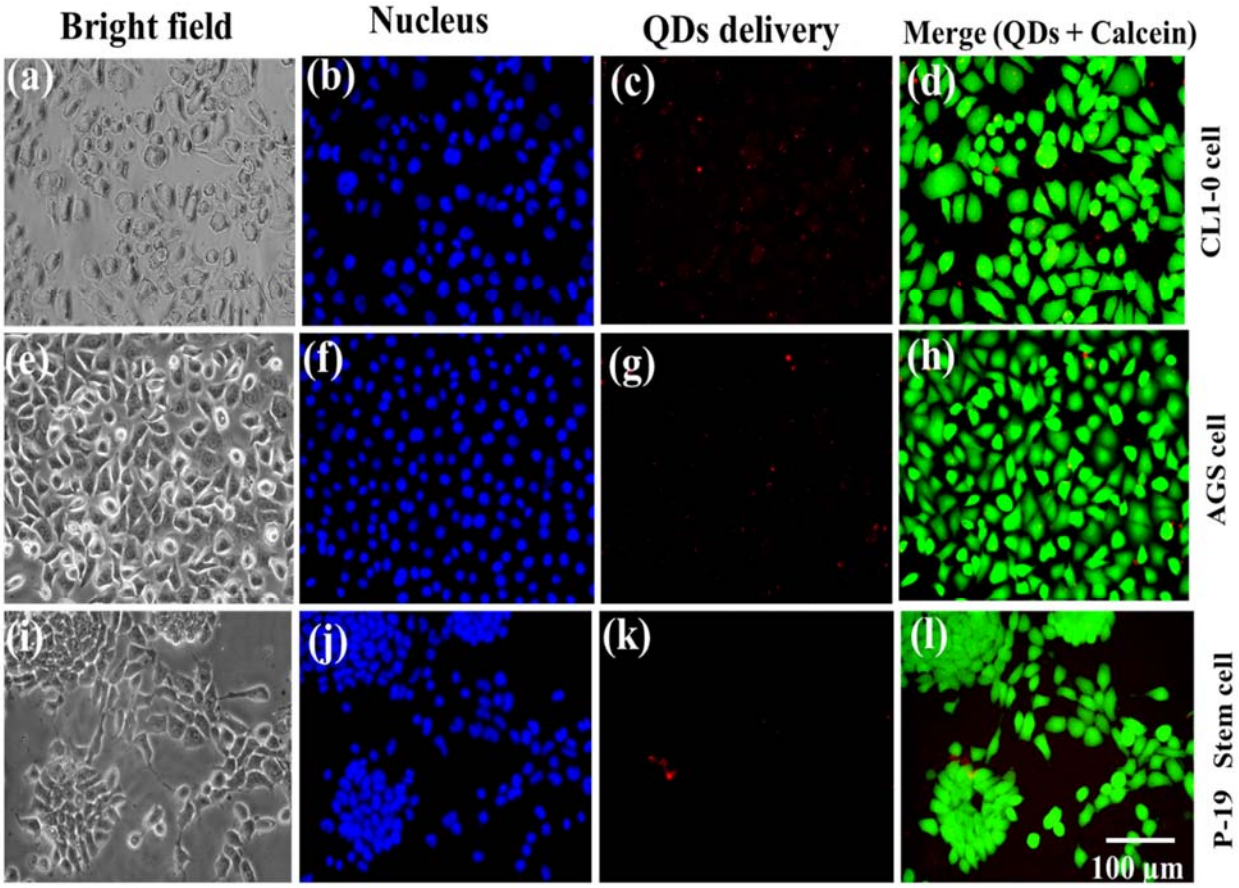

Fig. S22 QDs delivery on non-exposure area of CL1-0, AGS cells and P-19 stem cells (a) bright field image of non-exposure area of CL1-0 cells (b) corresponding cell nucleus image (using hoechst 33342) (c) non-exposure area indicating no QDs delivery (d) cell viability test with merge image of QDs and calcein AM shows, all cells are viable on non-exposure area (e-h) and (i=l), similar phenomena for AGS cells and P-19 cells. For each experiment, the laser exposure was 30 seconds at 10 HZ pulsing frequency with 35 mJ/cm^2 laser fluence at 945 nm.

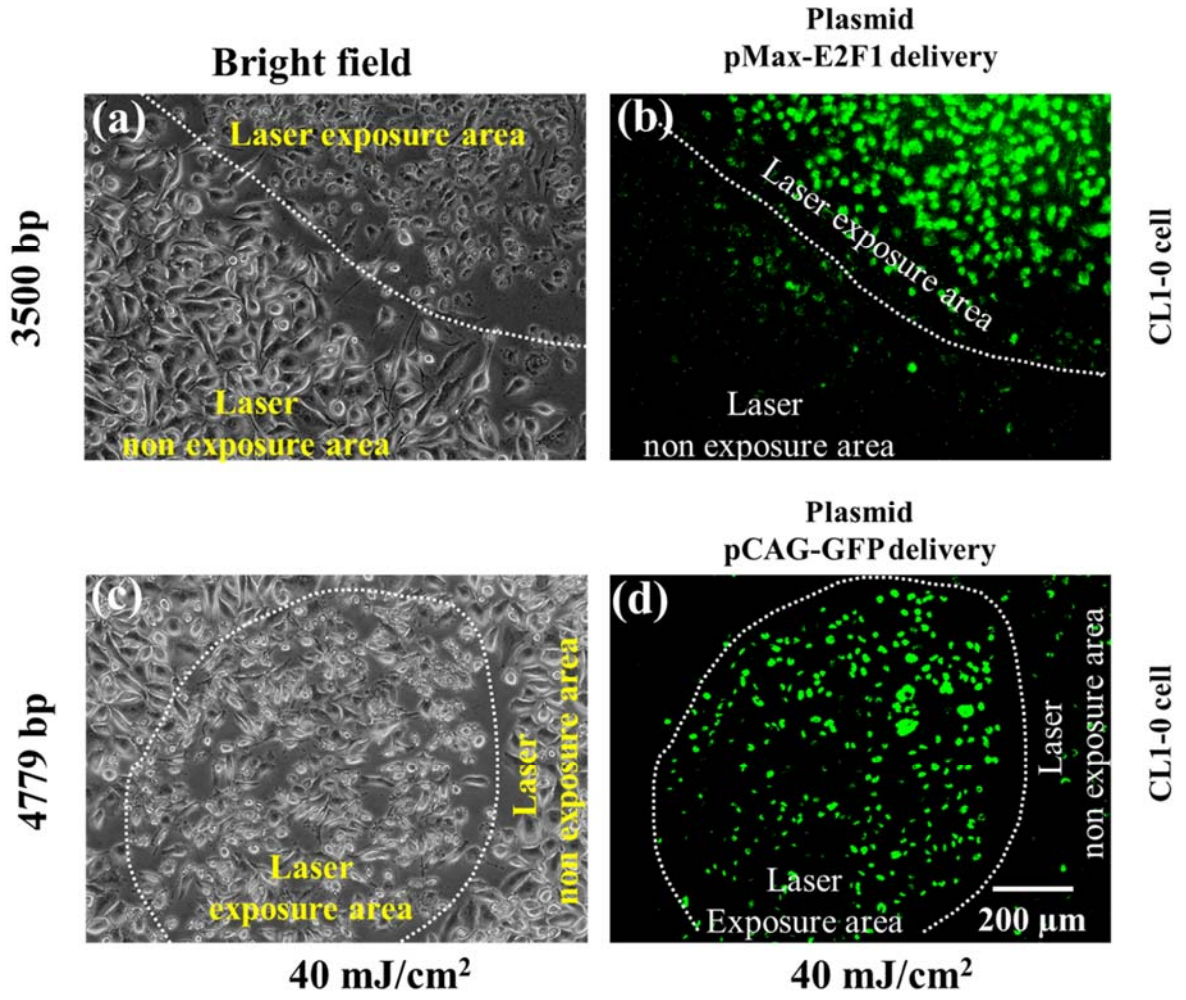

Fig. S23 Plasmid delivery on laser exposure and non-exposure area of CL1-0 cells (a) bright field image of laser exposure and non-exposure area separated with white dotted line (b) plasmid pMax-E_2F_1 delivery and protein expression on laser exposure area (c) bright field image of laser exposure (inside circle with white dotted line) and non-exposure area (outside circle area) (d) plasmid pCAG-GFP delivery and protein expression on laser exposure area (inside white circle area). For each experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 40 mJ/cm^2 laser fluence at 945 nm.

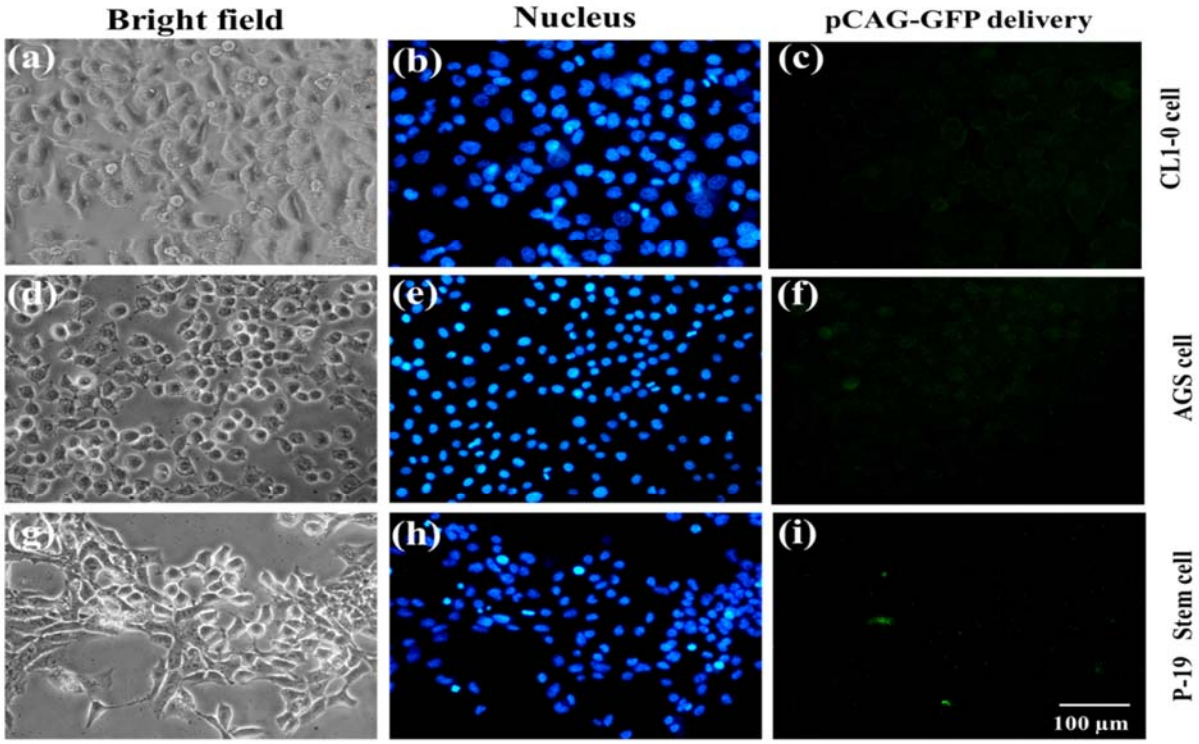

Fig. S24 Plasmid delivery (pCAG-GFP) on non-exposure area of different cell lines (a) bright field image on non-exposure area of CL1-0 cells (b) cell nucleus (c) plasmid delivery on non-exposure area after 24 hours indicating no protein expression (d) bright field image on non-exposure area of AGS cells (e) cell nucleus (f) no protein expression after 24 hours (g) bright field image on non-exposure area of P-19 cells (h) cell nucleus (i) no protein expression after 24 hours. For each experiment, the laser exposure was 30 seconds at 10 Hz pulsing frequency with 40 mJ/cm^2 laser fluence at 945 nm.

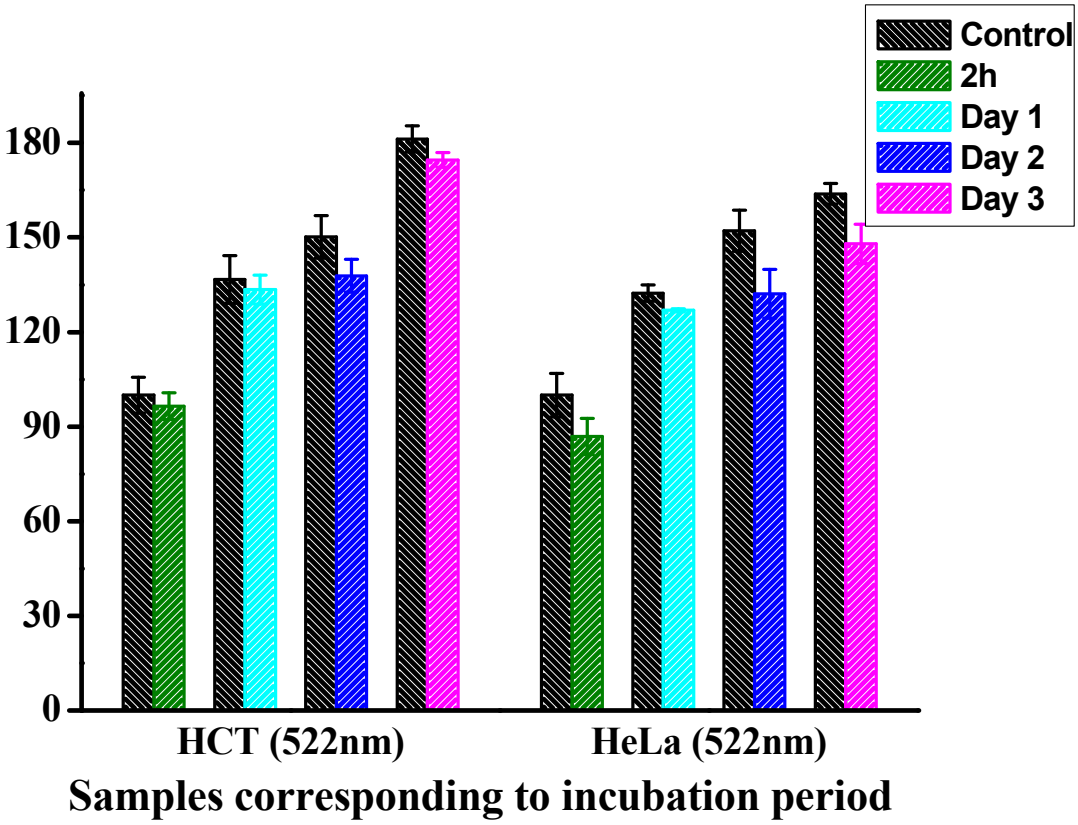

Fig. S25 Cell viability of HCT-8 and HeLa cells using MTT assay on different days after laser exposure at 516 nm wavelength corresponding to 25 mJ/cm^2 energy for the 30s each.

References:

[1] Hsieh, H-Y.; Huang, T-W.; Xiao, J-L.; Yang, C-S.; Chang, C-C.; Chu, C-C.; Lo, L-W.; Wang, S-H.; Wang, P-C.; Chieng, C-C.; Lee, C-H.; Tseng, F.G. Fabrication and Modification of Dual-Faced Nano-mushrooms for Tri-functional Cell Theranostics: SERS/fluorescence Signaling, Protein Targeting, and Drug Delivery. *J. Mater. Chem*. **2012**, 22, 20918.

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