Osman, N, Curley, P, Box, H, Liptrott, N, Sexton, DW and Saleem, I

In vitro evaluation of physicochemical-dependent effects of polymeric nanoparticles on their cellular uptake and co-localization using pulmonary calu-3 cell lines

Supplementary Data:

Figure. S.1. flow cytometry gating strategy:

Flow Cytometry Settings and Gating Strategy: BD Accuri C6 FC was set to fast flow rate (66 µl/min), and 3 blue-1 red lasers. Dot-plot FSC-A vs SSC-A was used to gate around the cell population and exclude the debris and particles (Figure S.1. A, P1 gate). Dot-plot FSC-A vs FSC-H was used to gate around the singlet cells (Figure S.1. B, P2 gate). The acquisition limits were set based on the gating to 5000 events/P2 in the singlets gate with 50 µL sample volume as a secondary limit. MFI was collected in FL-3 (M1 for NR, 550/647 nm on red channel) (Figure S.1. C). The results were expressed as a percentage of NP treated cells under no inhibition at 37 ˚C.

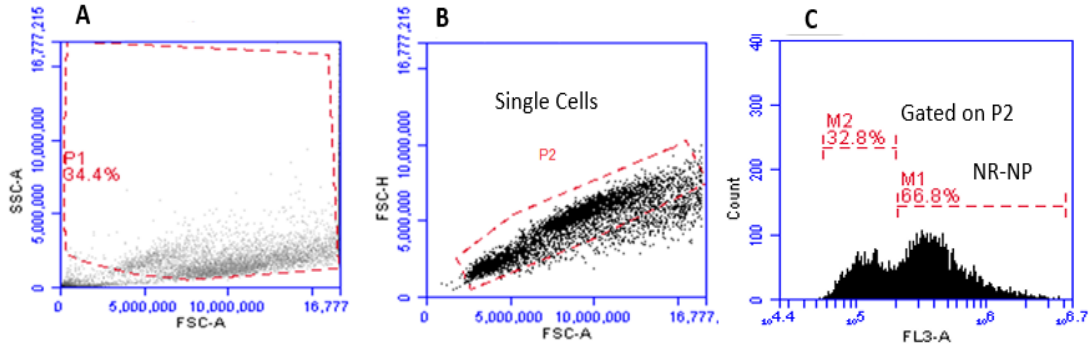

Figure. S.1. flow cytometry gating strategy: (A) FCS-A vs SCC-A: gating around the cells/P1 gate, (B)FSC-A vs FSC-H: gating around single cells/P2 gate, and (C) FL-3 histogram: gating around the negative (M2) and positive (M1) populations.

Figure. S.2. TEER values

TEER values of Calu-3 cells grown under ALI increased over time, reaching a maximum of ~1000 ohms.cm^2 at day 15 in Figure. S.2. This indicates that the cells had formed polarized monolayers with tight junctions as previously reported (27, 61).

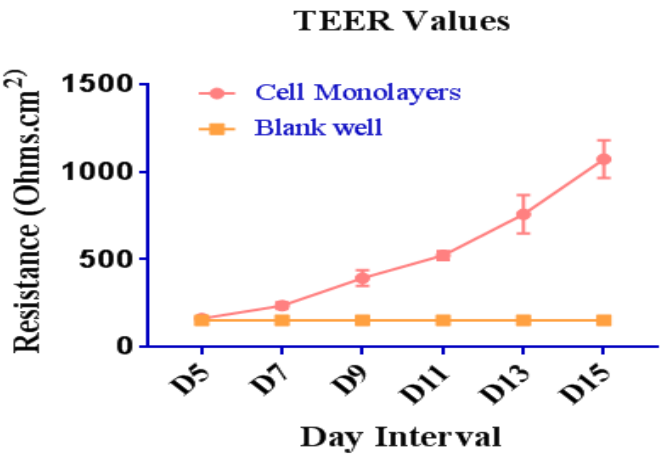

Figure. S.2. TEER values measured over time for Calu-3 cell lines grown under ALI.

Figure. S.3. Confocal microscopy

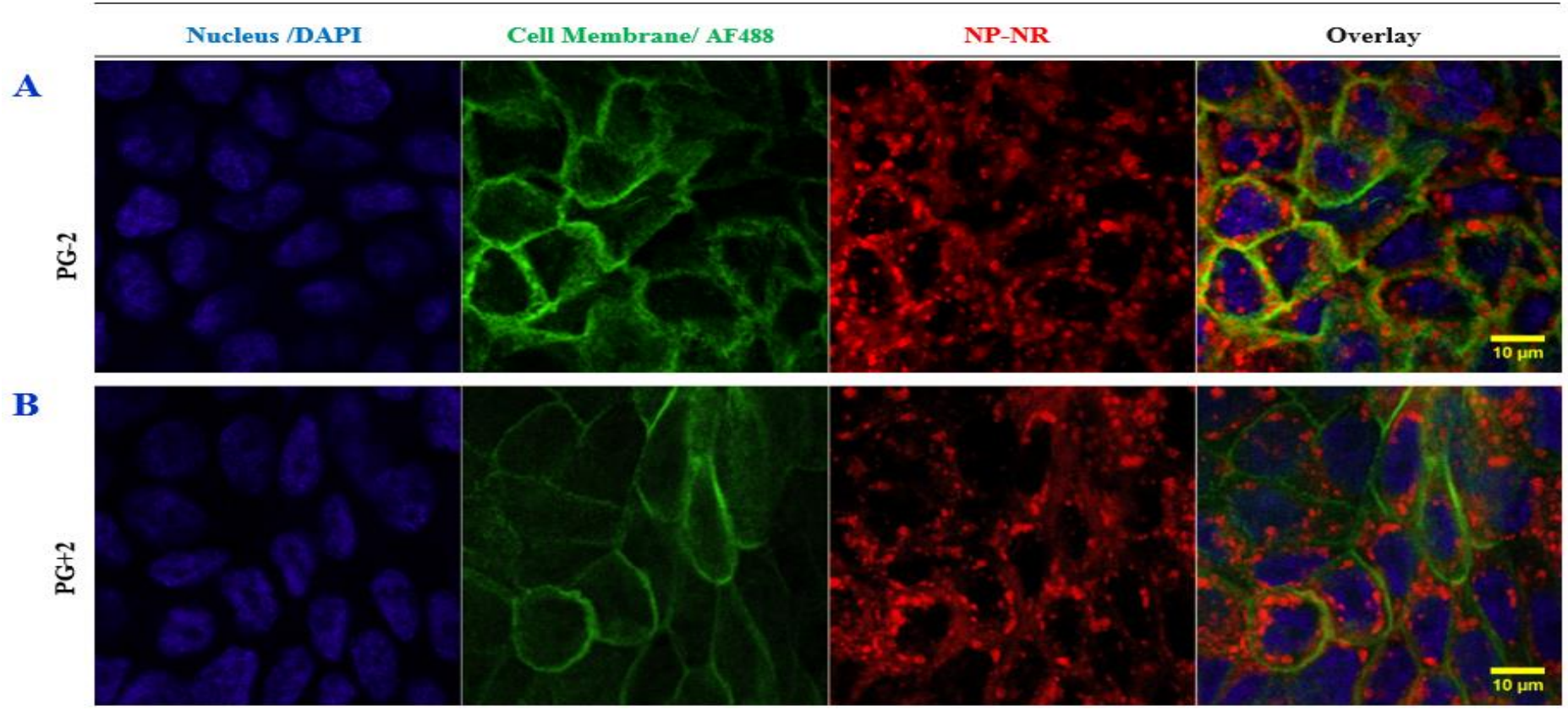

Figure. S.3. Confocal microscopy images of Calu-3 cells where nuclei labelled by DAPI and cell membranes labelled by AF488 after incubation for 1 hour with (0.5 mg/mL) NP-NRs (A) PG-2, (B) PG+2.

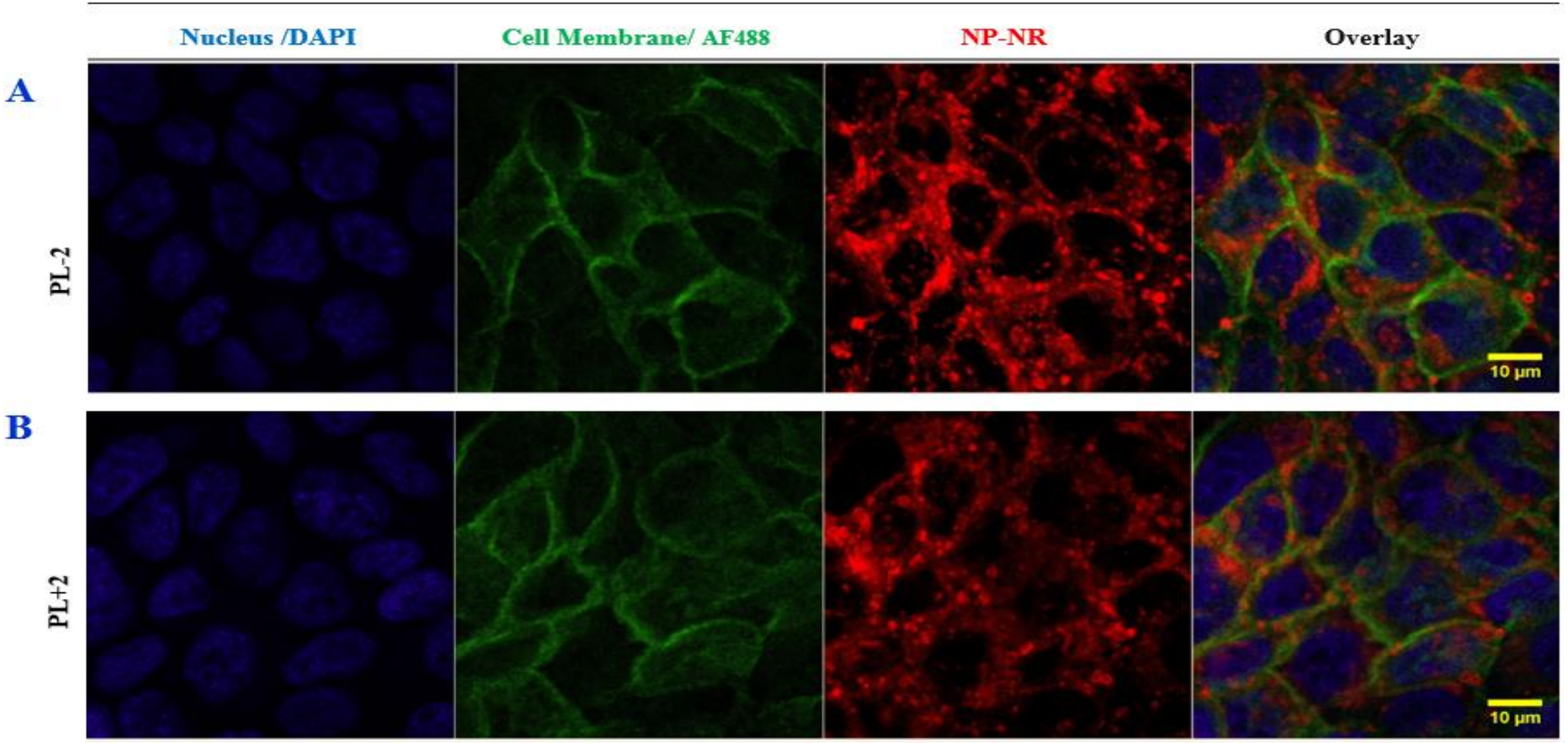

Figure. S.3. Confocal microscopy images of Calu-3 cells where nuclei labelled by DAPI and cell membranes labelled by AF488 after incubation for 1 hour with (0.5 mg/mL) NP-NRs (A) PL-2, (B) PL+2.

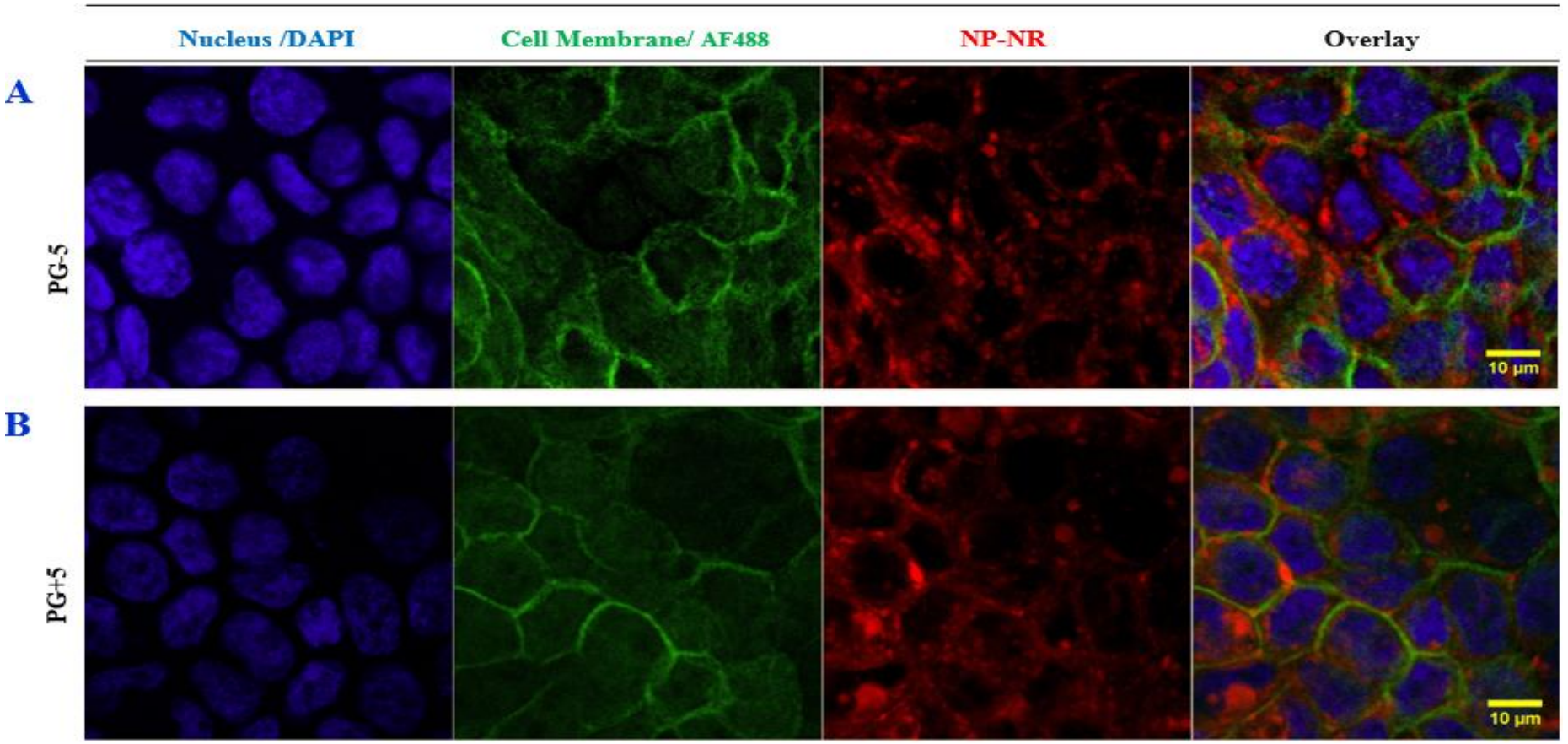

Figure. S.3. Confocal microscopy images of Calu-3 cells where nuclei labelled by DAPI and cell membranes labelled by AF488 after incubation for 1 hour with (0.5 mg/mL) NP-NRs (A) PG-5, (B) PG+5.

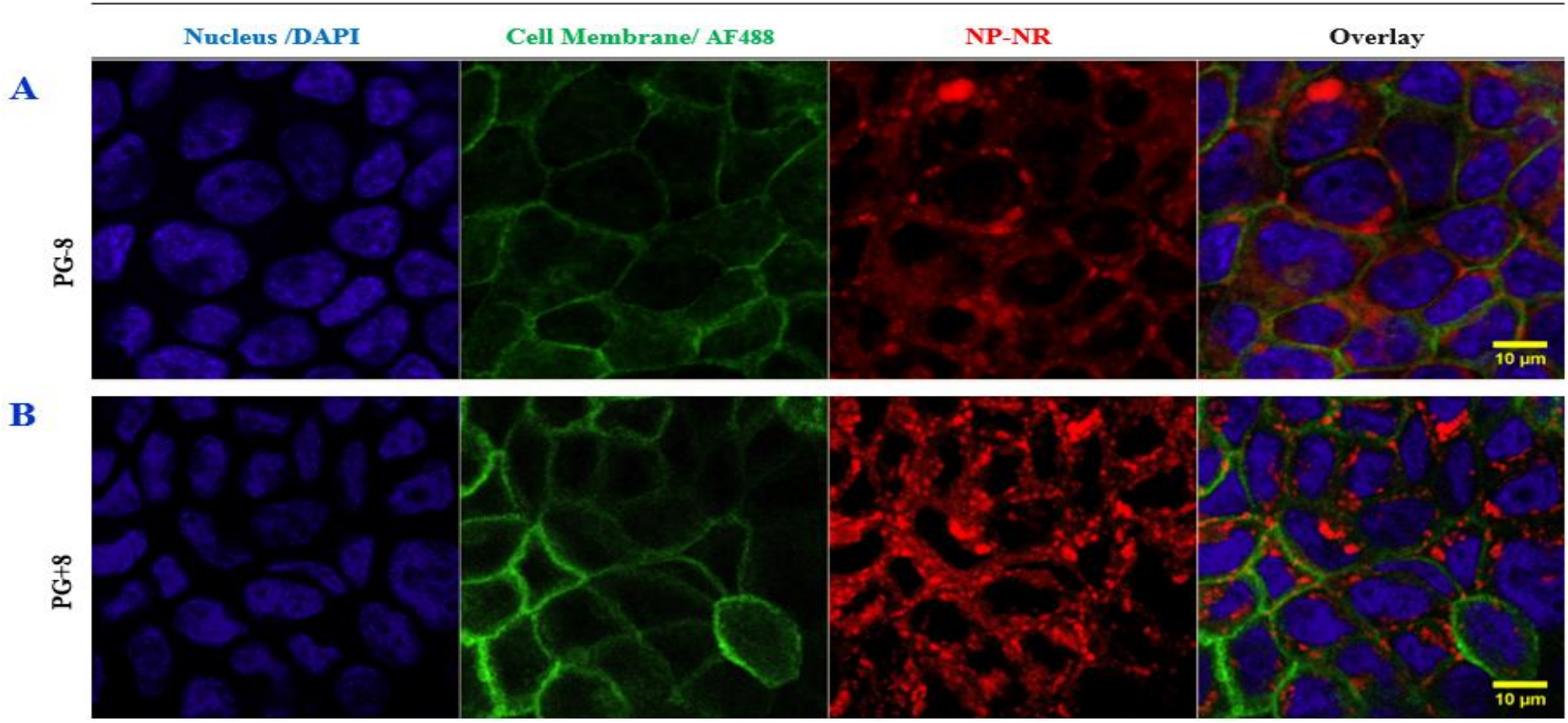

Figure. S.3. Confocal microscopy images of Calu-3 cells where nuclei labelled by DAPI and cell membranes labelled by AF488 after incubation for 1 hour with (0.5 mg/mL) NP-NRs (A) PG-8, (B) PG+8.

Figure. S.4. Confocal microscopy

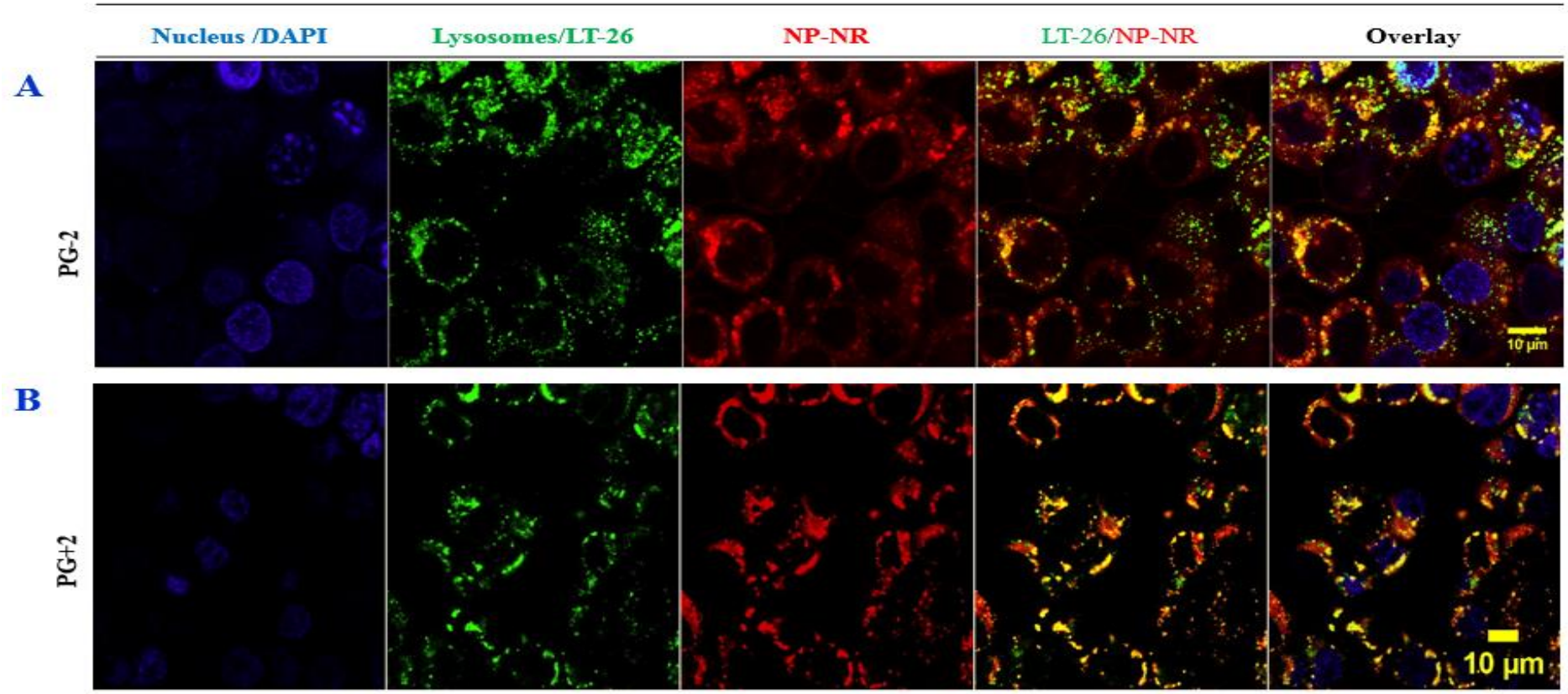

Figure. S.4 Confocal microscopy images of Calu-3 cells where nuclei labelled by DAPI and lysosomes labelled by LT-26 after incubation for 1 hour with (0.5 mg/mL) NP-NRs (A) PG-2, (B) PG+2.

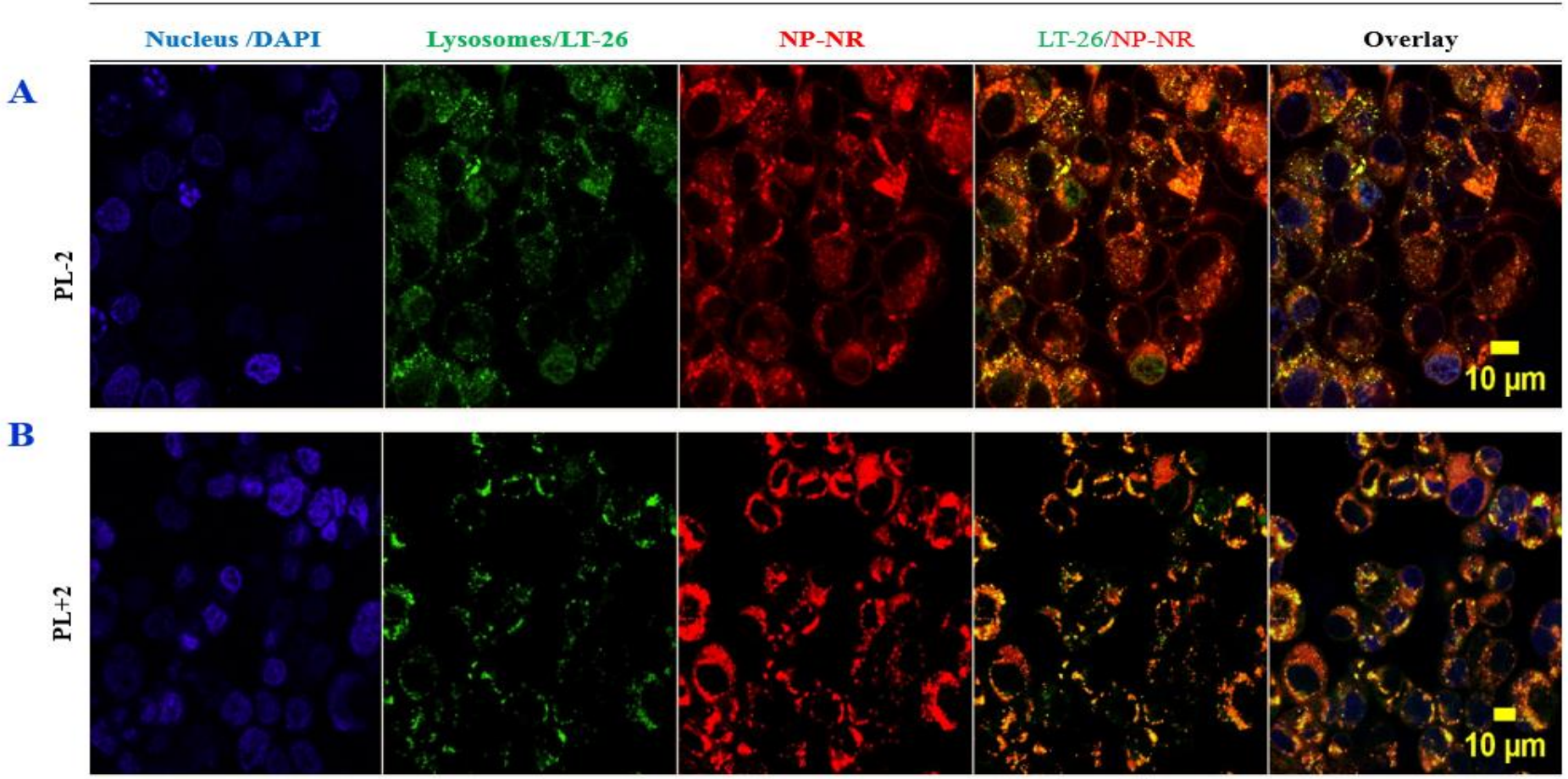

Figure. S.4. Confocal microscopy images of Calu-3 cells where nuclei labelled by DAPI and lysosomes labelled by LT-26 after incubation for 1 hour with (0.5 mg/mL) NP-NRs (A) PL-2, (B) PL+2.

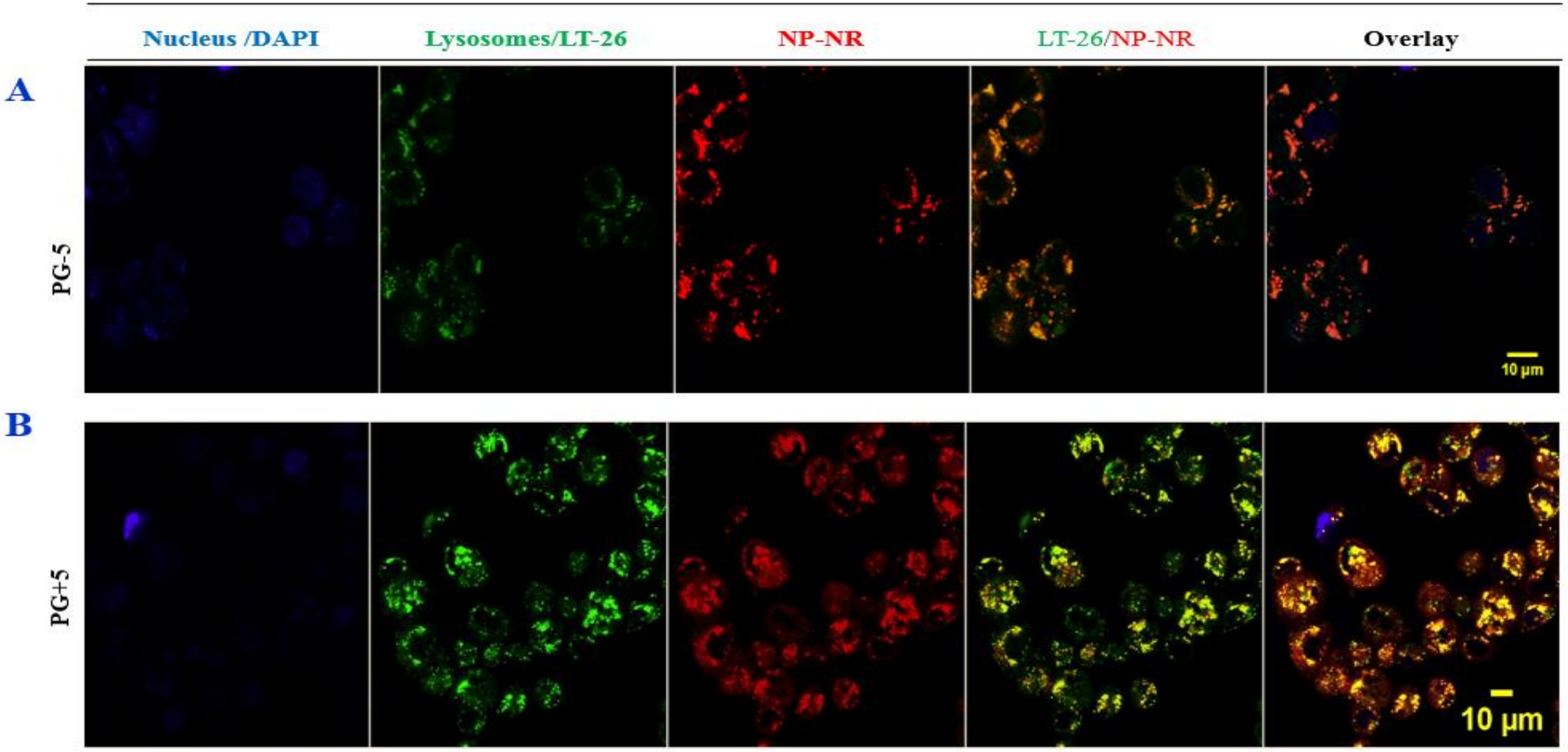

Figure. S.4. Confocal microscopy images of Calu-3 cells where nuclei labelled by DAPI and lysosomes labelled by LT-26 after incubation for 1 hour with (0.5 mg/mL) NP-NRs (A) PG-5, (B) PG+5.

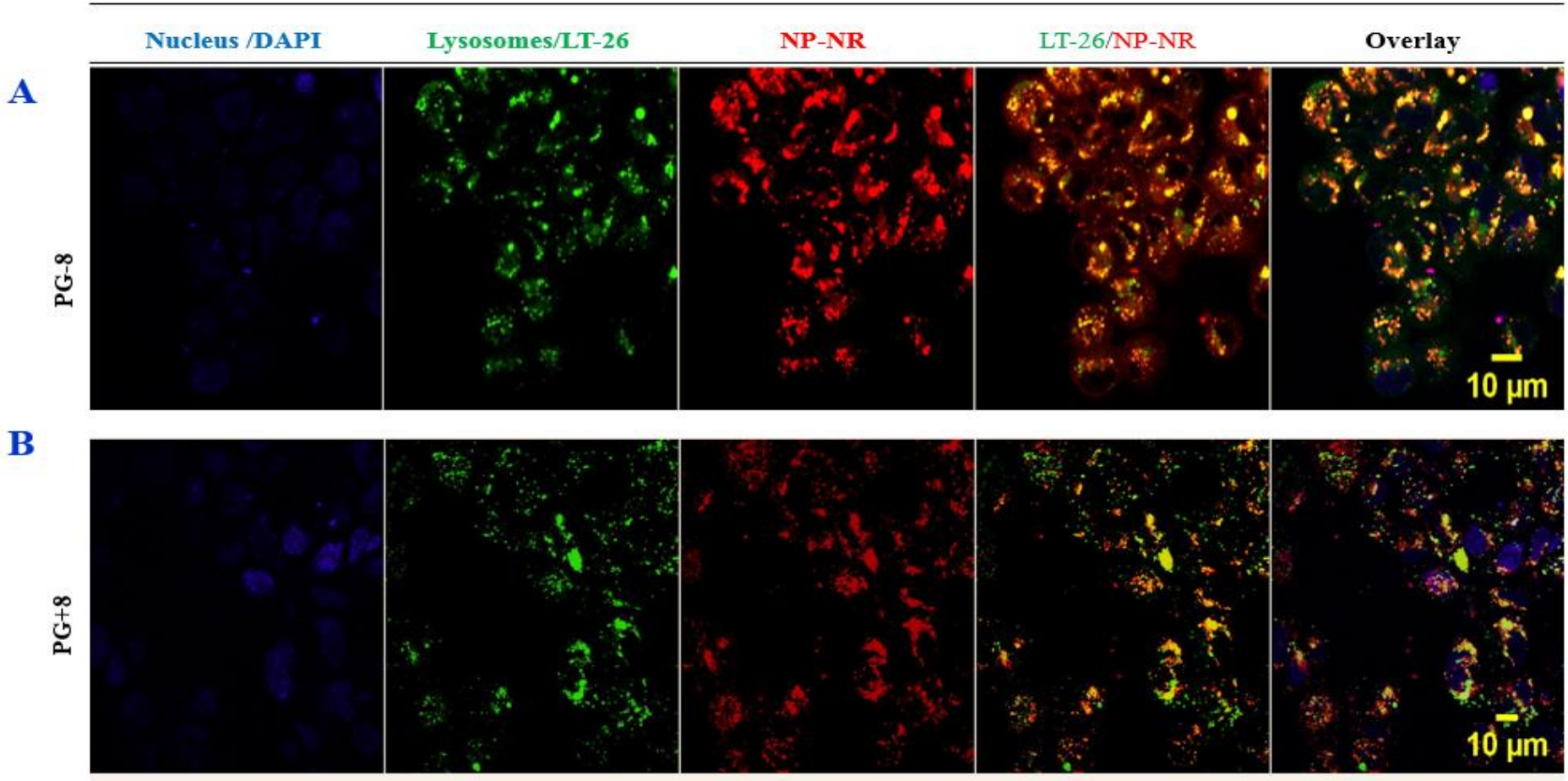

Figure. S.4. Confocal microscopy images of Calu-3 cells where nuclei labelled by DAPI and lysosomes labelled by LT-26 after incubation for 1 hour with (0.5 mg/mL) NP-NRs (A) PG-8, (B) PG+8.