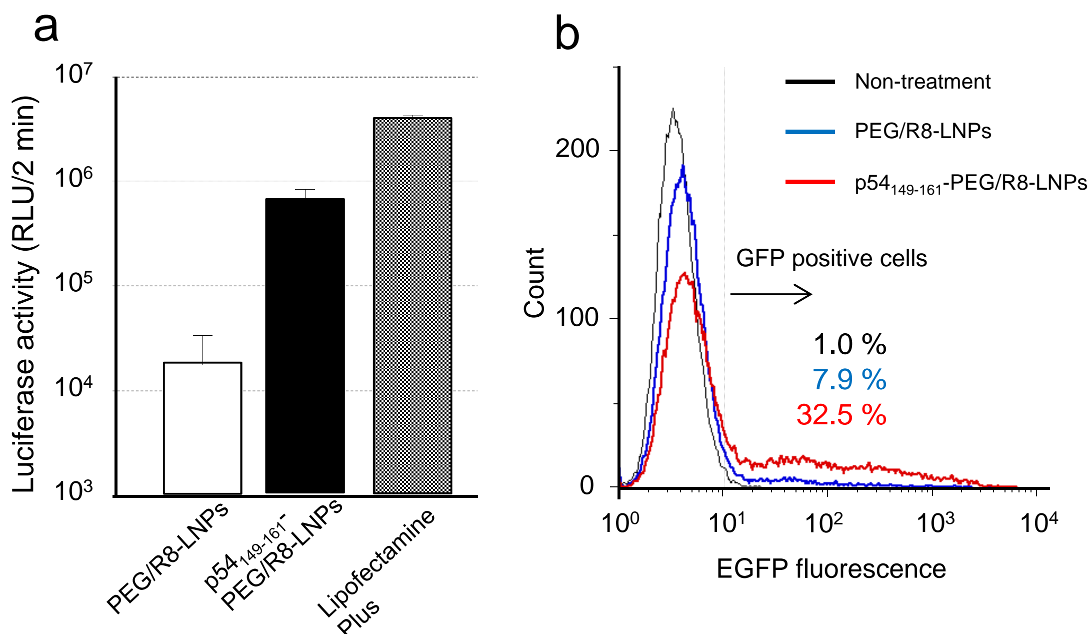
Supplemental Figure S3



Supplemental Figure S3:

Transfection activities of PEG/R8-NLPs and $p54_{149\text{-}161}$-PEG/R8-NLPs

8 x 10^4 cells were seeded on 35-mm culture dishes in 2 mL of culture medium 1 day before transfection. For the transfection, a 2 ml aliquot of PEG/R8-NLPs or $p54_{149\text{-}161}$-PEG/R8-NLPs including 1.6 μg DNA encoding (**a**) pcDNA3.1-GL3 or (**b**) pEGFP-N1 (Clontech, Palo Alto, CA, USA) was incubated with the cells in serum- and antibiotics-free DMEM for 3h. Transfection with the Lipofectamine Plus reagent was demonstrated according to the manufacture's instructions. The following procedures to evaluate the gene expression efficacies of luciferase (**a**) and EGFP (**b**) were evaluated as follows;

a. The medium was then replaced with fresh phenol red-free medium containing 10% serum and 100mM $_D$-luciferin. The dishes were set in a luminometer incorporated in a small CO_2 incubator (ATTO Co. Ltd.), and the bio luminescence was monitored at 8 h (2min collection time).

b. At 24 h post-transfection, The cells were then trypsinized and collected by centrifugation at 3000 rpm at 4°C for 5 min. Cells were washed in ice-cold PBS two times by repeating the centrifugation and suspension steps. Finally, cells were suspended in 500 μL of FACS buffer (ice-cold PBS solution containing 0.1% sodium azide and 0.5% BSA). The cell suspension was filtered through a nylon mesh (45 μm mesh size) to remove cell aggregates and dust, and the cells were then analyzed using a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA).

Histograms indicated the resultant fluorescence intensity of EGFP when transfected by PEG/R8-NLPs (blue) and $p54_{149\text{-}161}$-PEG/R8-NLPs PEG-LPs (red) analyzed 30,000 cells by FACS. Autofluorescence derived from the non-transfected (control) cells were represented by black lines.