Supplementary information

Microfluidic Co-Assembly of PEG/PEG-Lipid-Incorporated PBAE Nanoparticles for Enhanced Non-Viral Gene Delivery

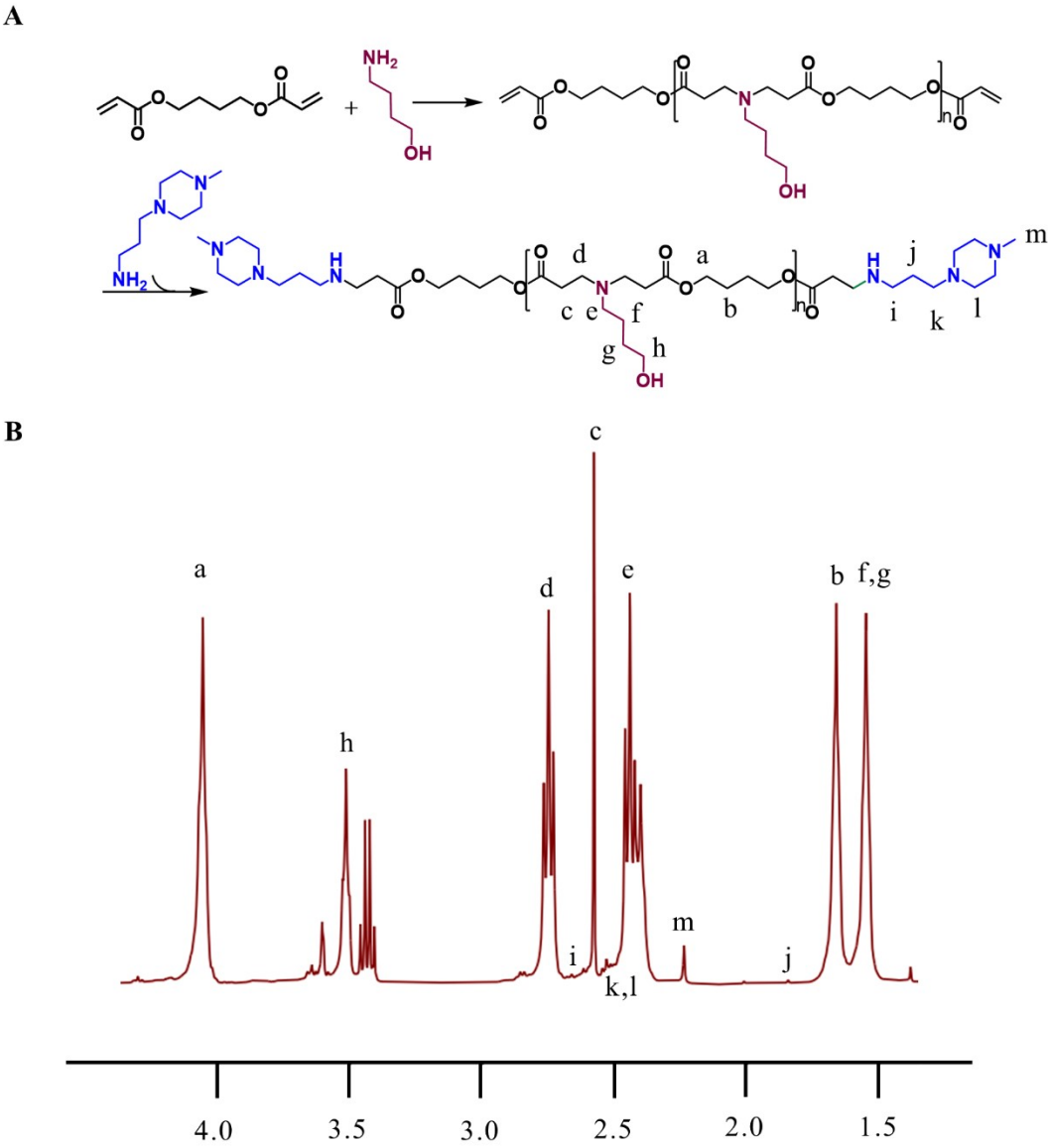

Fig. S1 (A) The base polymer PBAE-447 was synthesized via a two-step process. Briefly, diacrylate and amine undergo Michael addition to form acrylate-capped prepolymer, which then react with end-capping amine to yield amino-capped polymer PBAE-447. (B)^{1} H NMR spectrum of the 70° synthesized PBAE-447 polymer.

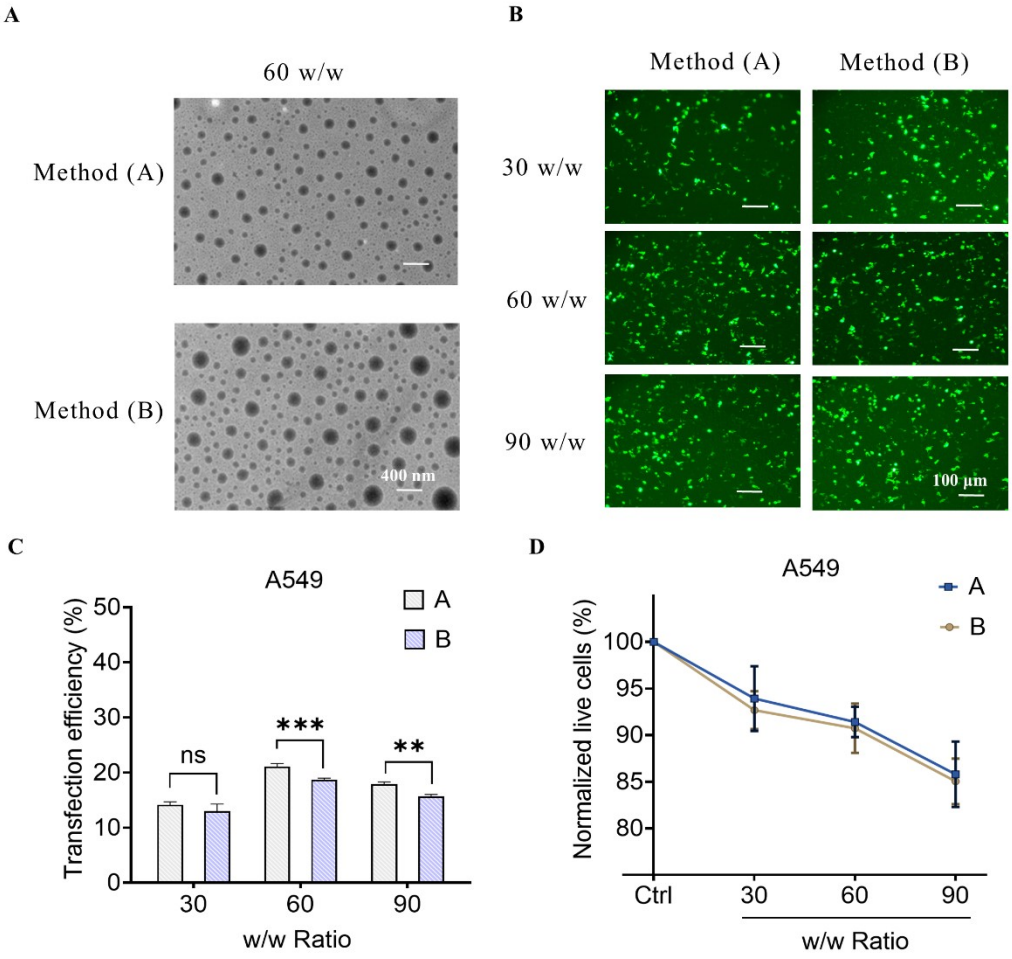

Fig. S2 (A) The TEM image of PBAE nanoparticles at a PBAE:DNA ratio of 60 (w/w) prepared via microfluidic mixing Methods A and B. (B-D) The efficiency of PBAE nanoparticles (1.5 µg DNA/well) at a PBAE:DNA ratios of 30, 60, and 90 (w/w) prepared via microfluidic mixing Methods A and B, using eGFP as a reporter gene in A549 cells. (B) Fluorescence microscopy images (20×) of eGFP expression. Scale bars represent 100 µm in all panels. (C) Flow cytometry data showing transfection efficiency [1]at 48 h post-treated of A549 cells. (D) Cytotoxicity of PBAE nanoparticles, quantified by normalizing metabolic activity to untreated A549 cells ("Ctrl" represents untreated A549 cells) (mean ± SD, n=3) (***$p < 0.001$; **$p < 0.01$).

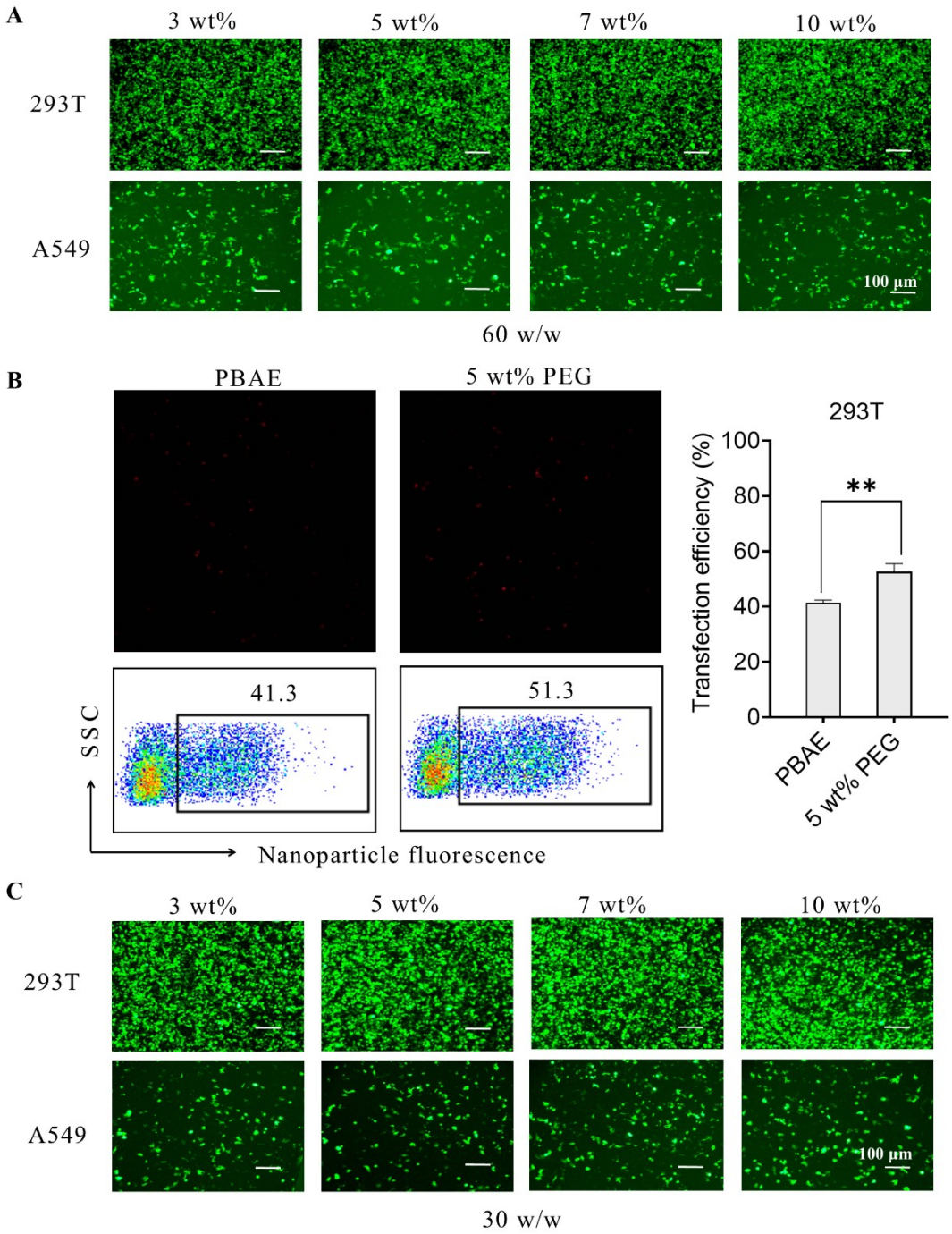

Fig. S3 (A) Fluorescence microscopy images (20×) of eGFP expression at 48 h post-treatment of 293T cells (1.0 μg DNA/well) and A549 cells (1.5 μg DNA/well) transfected with PBAE/PEG co-assembled nanoparticles at a PBAE:DNA ratio of 60 (w/w). Scale bars indicate 100 μm in all panels. (B) Qualitative confocal laser scanning microscopy (CLSM) visualization and subsequent quantitative flow cytometric analysis

of cellular association efficiency of Cy5-labeled PBAE-only nanoparticles and 5 wt% PEG-incorporated nanoparticles in 293T cells after 60 min incubation. (C) Fluorescence microscopy images (20×) of eGFP expression at 48 h post-treatment of 293T cells (1.0 μg DNA/well) and A549 cells (1.5 μg DNA/well) transfected with co-assembled nanoparticles at a PBAE:DNA ratio of 30 (w/w). Scale bars indicate 100 μm in all panels.

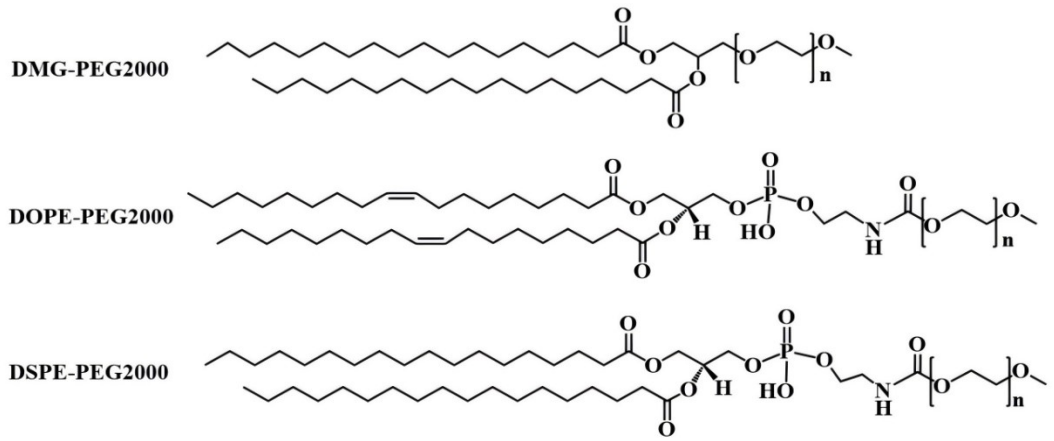

Fig. S4 Three representative PEG-lipid examples for PBAE/PEG-Lipid co-assembled nanoparticles formulations: DMG-PEG2000 (short saturated chains C14:0 and small monomethoxy headgroup), DOPE-PEG2000 (long unsaturated chain C18:1 and large phosphatidylethanolamine headgroup,), and DSPE-PEG2000 (long saturated chain C18:0 and large phosphatidylethanolamine headgroup).

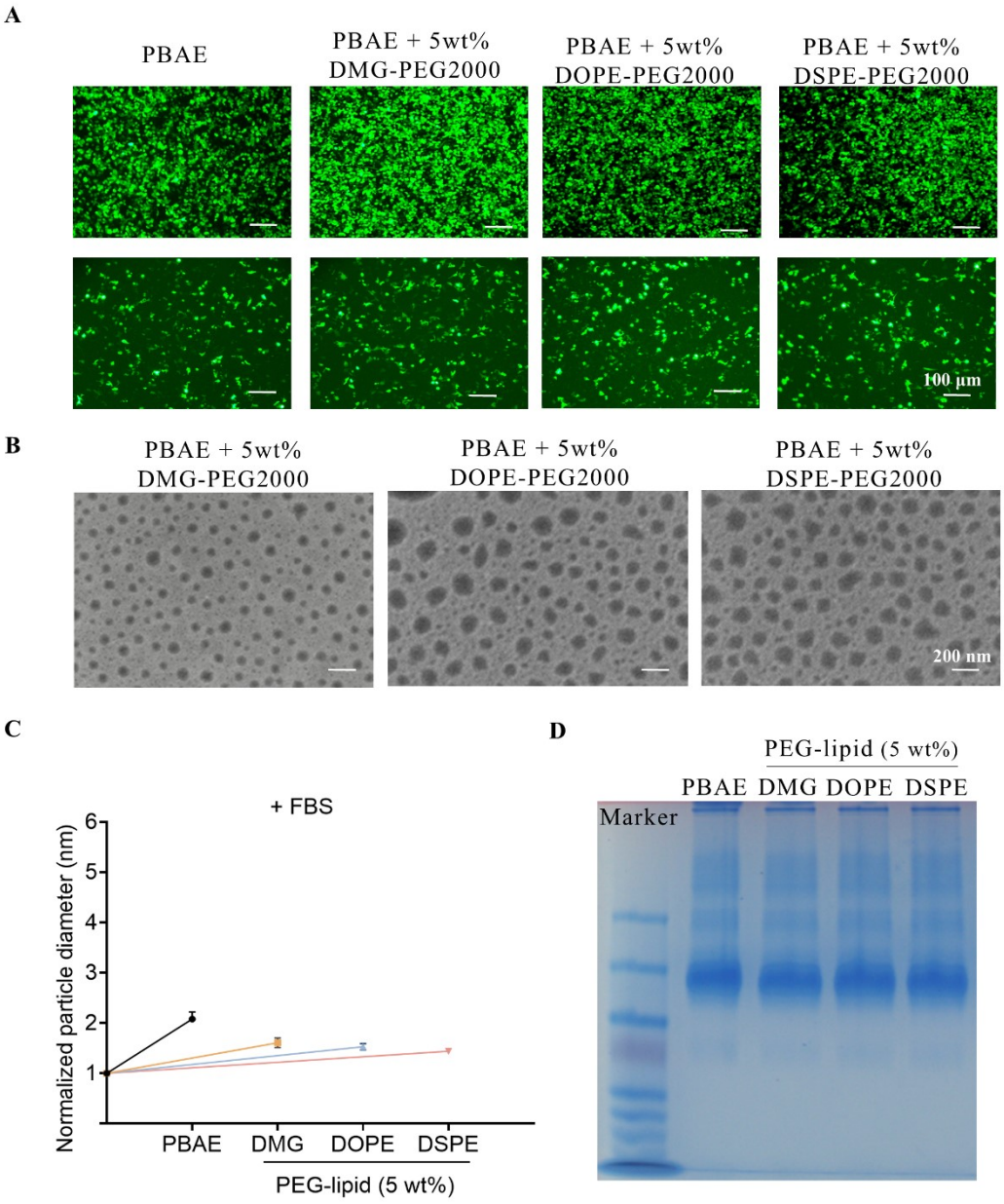

Fig. S5 (A) Fluorescence microscopy images (20×) of eGFP expression at 48 h post-treatment of 293T cells and A549 cells transfected with co-assembled nanoparticles containing 5 wt% PEG-lipid. Scale bars represent 100 μm in all panels. (B) The TEM image of co-assembled nanoparticles containing 5 wt% PEG-lipid (PBAE:DNA = 60 w/w) prepared via microfluidic mixing Method A. (C) Size variation of 5wt% PEG-lipid co-assembled nanoparticles in 50% v/v fetal bovine serum (FBS) at 37°C,

measured by dynamic light scattering (DLS). (D) Native-PAGE gel electrophoretic analysis of 5 wt% PEG-lipid co-assembled nanoparticles.

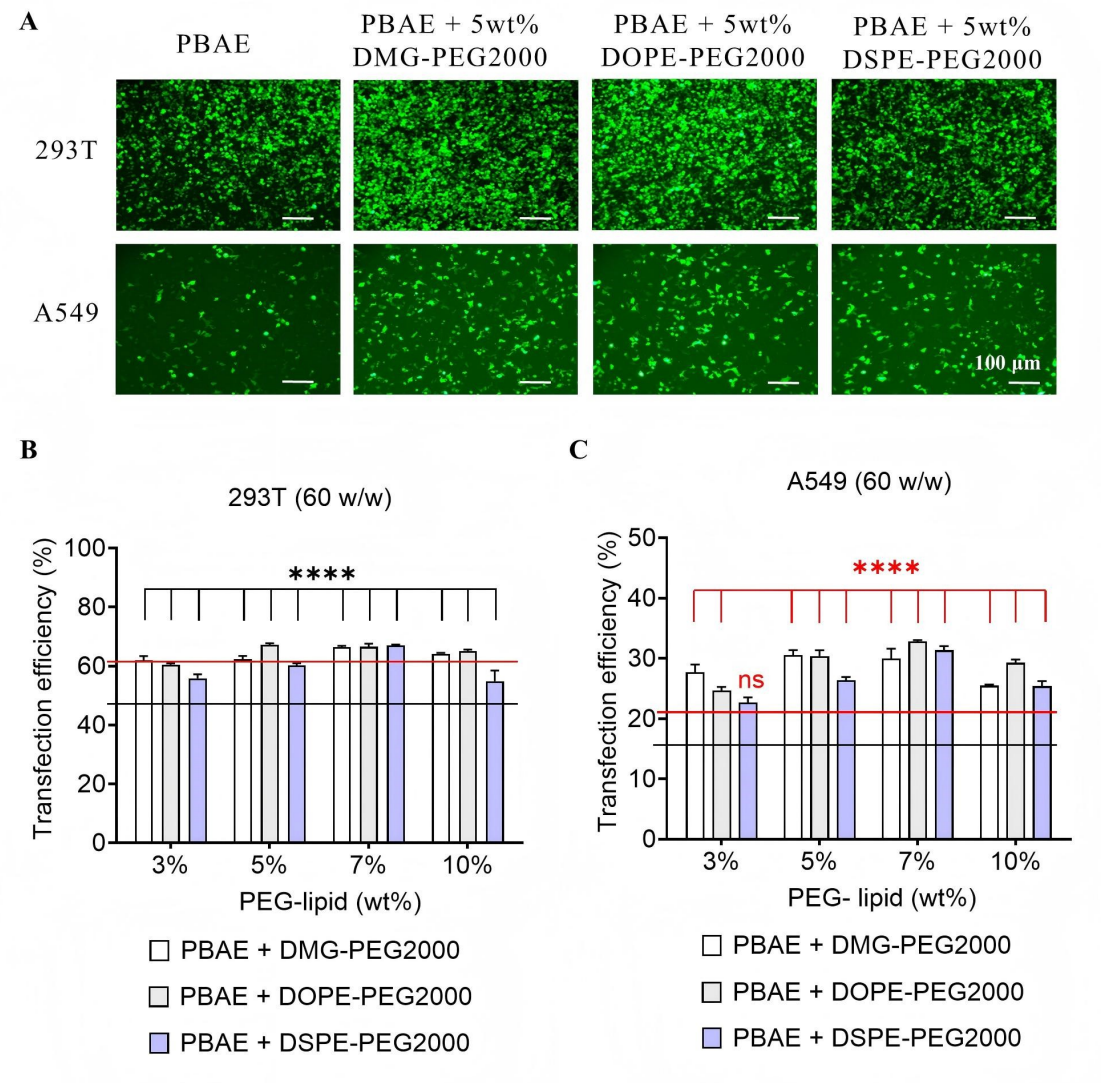

Fig. S6 The efficacy of PBAE/PEG-lipid co-assembled nanoparticles at a PBAE:DNA ratio of 60:1 (w/w) prepared using microfluidic mixing Method A with eGFP as a reporter gene in 293T cells (0.5 μg DNA/well) and A549 cells (1.0 μg DNA/well). (A) Fluorescence microscopy images (20×) of eGFP expression at 48 h post-treatment of 293T cells and A549 cells transfected with co-assembled nanoparticles containing 5

wt% PEG-lipid. Scale bars indicate 100 μm in all panels. (B) Flow cytometry data showing transfection efficacy at 48 h post-treatment of 293T cells with co-assembled nanoparticles (****$p < 0.0001$ compared to PBAE-only nanoparticles under the same condition). (C) Flow cytometry data showing transfection efficacy at 48 h post-treatment of A549 cells with co-assembled nanoparticles (mean ± SD, n=3) (****$p < 0.0001$ compared to PBAE-only nanoparticles under standard transfection conditions) (Red lines indicate the transfection efficiency of PBAE-only nanoparticles under standard conditions: PBAE:DNA=60:1 w/w, 293T cells: 1.0 μg DNA/well, A549 cells: 1.5 μg DNA/well ; Black lines indicate the transfection efficiency of PBAE-only nanoparticles at reduced DNA transfection doses: PBAE:DNA=60:1 w/w, 293T cells: 0.5 μg DNA/well, A549 cells: 1.0 μg DNA/well).

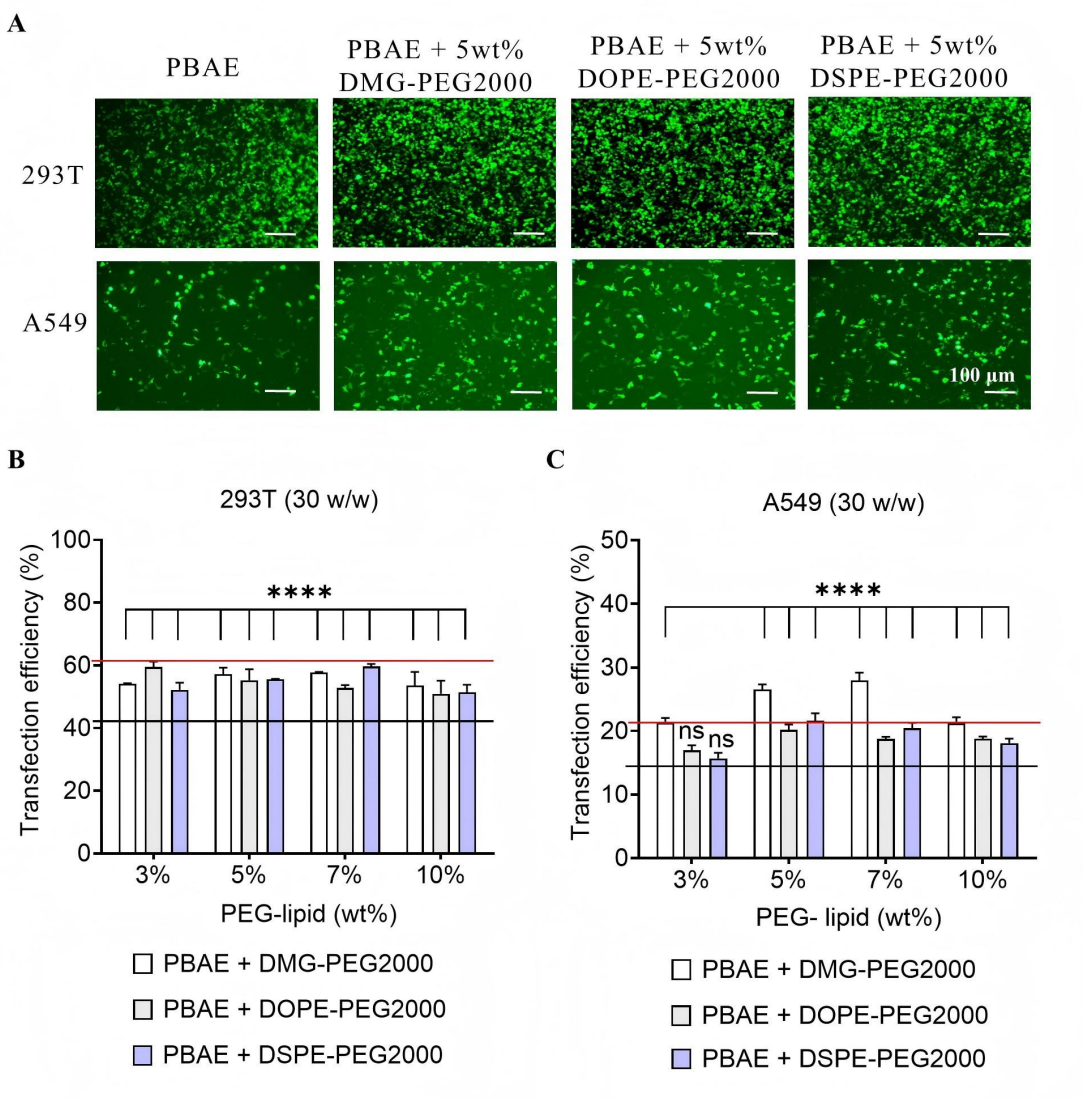

Fig. S7 The efficacy of PBAE/PEG-lipid co-assembled nanoparticles at a PBAE:DNA ratio of 30:1 (w/w) prepared using microfluidic mixing Methods A with eGFP as a reporter gene in 293T cells (1.0 μg DNA/well) and A549 cells (1.5 μg DNA/well). (A) Fluorescence microscopy images (20×) of eGFP expression at 48 h post-treatment of 293T cells and A549 cells transfected with co-assembled nanoparticles containing 5 wt% PEG-lipid. Scale bars indicate 100 μm in all panels. (B) Flow cytometry data showing transfection efficacy at 48 h post-treatment of 293T cells with co-assembled nanoparticles. (C) Flow cytometry data showing transfection efficacy at 48 h post-treatment of A549 cells with co-assembled nanoparticles (mean ± SD, n=3) (****p <

0.0001 compared to PBAE-only nanaparticles under the same condition) (Red lines indicate the transfection efficiency of PBAE-only nanoparticles under standard conditions: PBAE:DNA=60:1 w/w, 293T cells: 1.0 μg DNA/well, A549 cells: 1.5 μg DNA/well; Black lines indicate the transfection efficiency of PBAE-only nanoparticles at reduced mass ratio: PBAE:DNA=30:1 w/w, 293T cells: 1.0 μg DNA/well, A549 cells: 1.5 μg DNA/well).

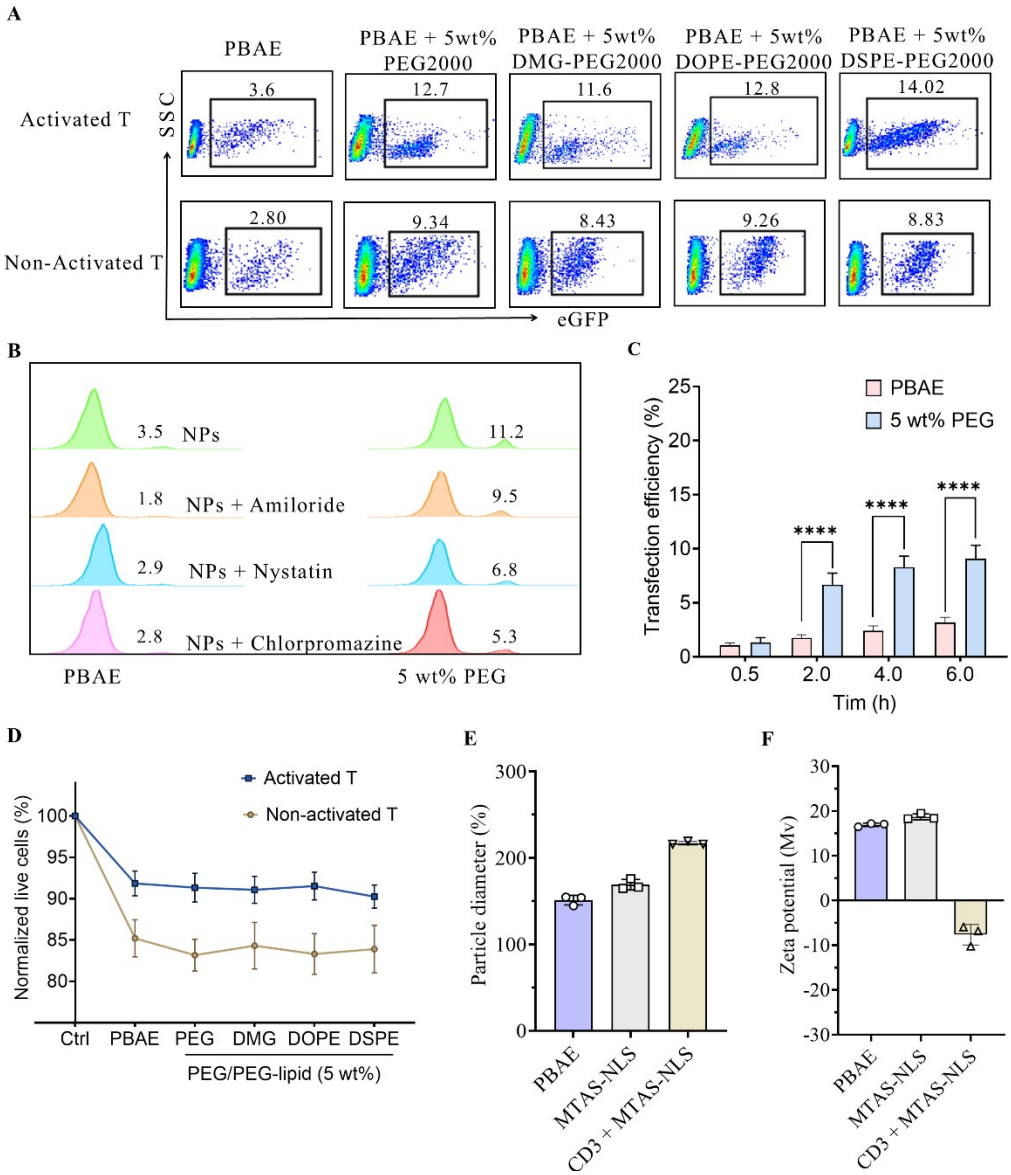

Fig. S8 (A) Representative flow cytometry plots of eGFP expression in activated and non-activated T cells transfected with nanoparticles at a PBAE:DNA ratio of 60:1 (w/w). (B) Representative flow cytometry plots for the uptake inhibition assays using three specific endocytic inhibitors (amiloride, nystatin, and chlorpromazine). (C) Transfection efficiency of PBAE-only nanoparticles and 5 wt% PEG-incorporated nanoparticles in non-activated T cells at different incubation time points (0.5h, 2 h, 4 h, and 6 h). (D) Cytotoxicity of nanoparticles, quantified by normalizing metabolic activity to untreated T cells. (E-F) Size and zeta potential of PBAE-only nanoparticles, MTAS-NLS peptide-conjugated PBAE nanoparticles, and PBAE nanoparticles co-conjugated with MTAS-NLS peptide and CD3 antibody (mean ± SD, n=3).

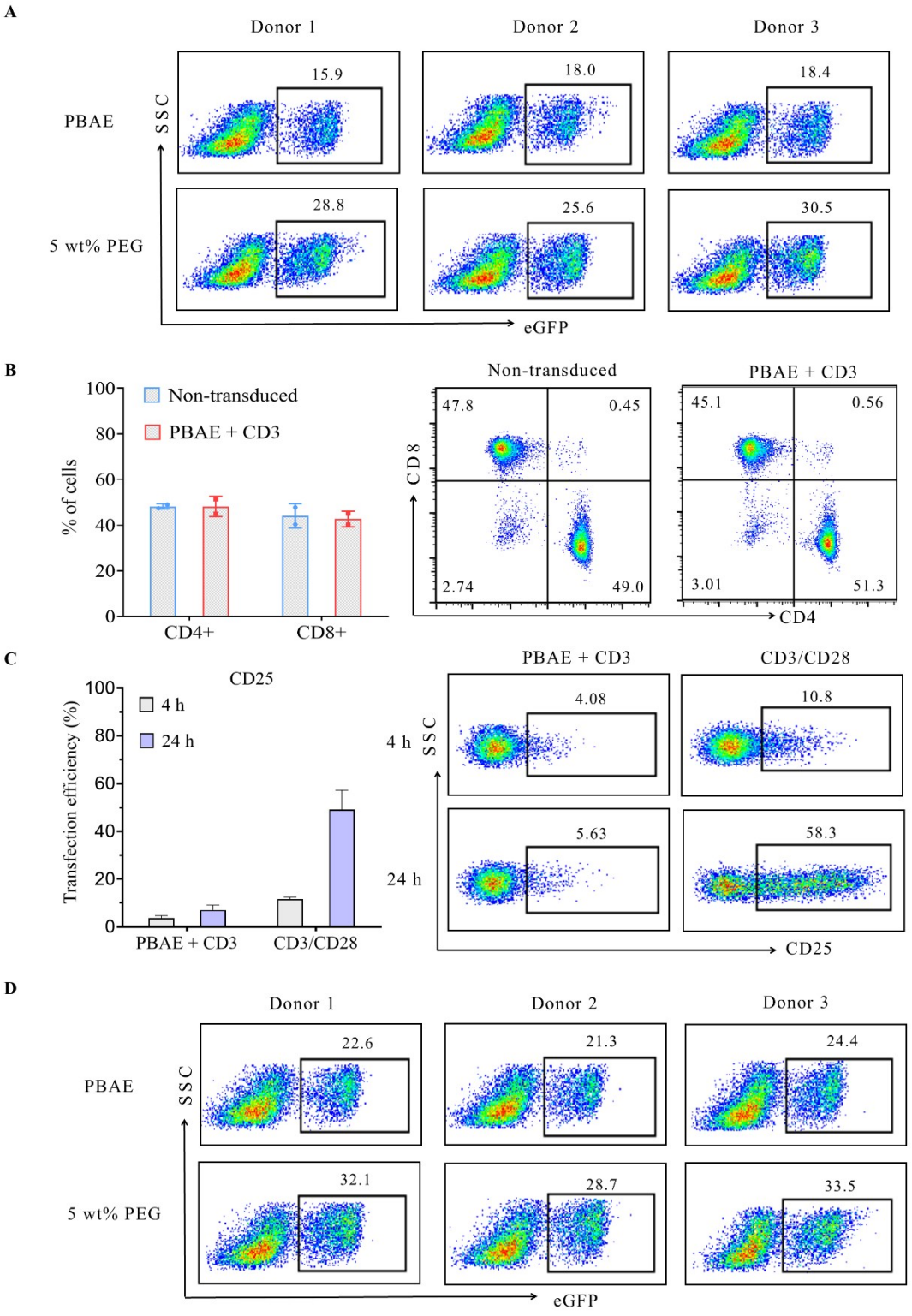

Fig. S9 (A) Flow cytometry analysis of eGFP expression in non-activated T cells from three different donors after transfection with MTAS-NLS peptide-modified nanoparticle. (B) Immunophenotypic levels of CD4 and CD8 on the surface of non-

activated primary T cells determined at 48 h post-nanoparticle transfection, including non-transduced T cells and T cells transduced with CD3 antibody-conjugated PBAE nanoparticles. (C) Immunophenotypic levels of CD25 on the surface of primary T cells under different stimulation conditions. T cells were treated with CD3 antibody-conjugated PBAE nanoparticles or CD3/CD28 activation beads for 4 h followed by washing, or continuously incubated for 24 h, and CD25 expression was analyzed by flow cytometry at 24 h. (D) Flow cytometry analysis of eGFP expression in non-activated T cells from three different donors after transfection with CD3 antibody and MTAS-NLS peptide-modified nanoparticle.

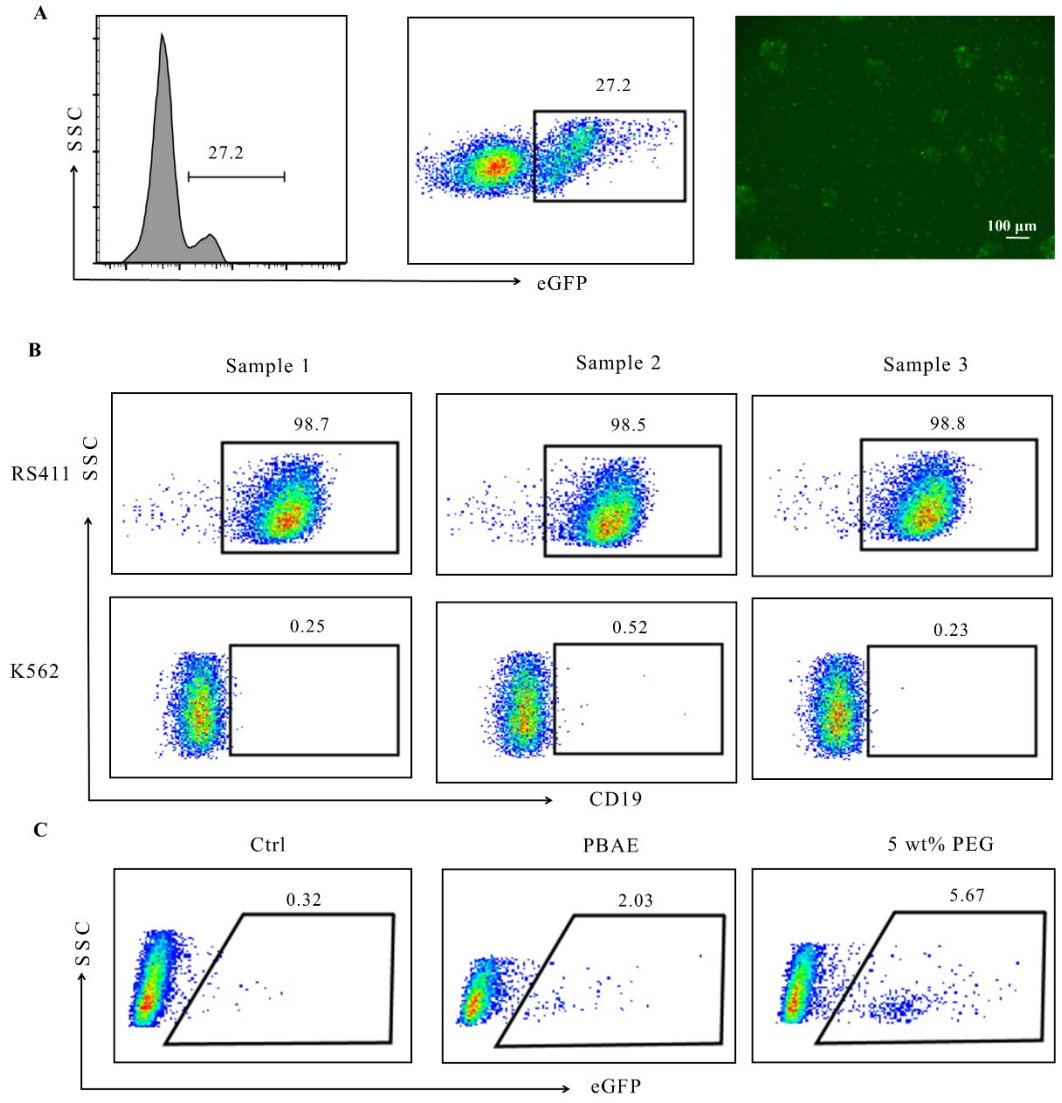

Fig. S10 (A) Representative flow cytometry plots and fluorescence images of eGFP expression in non-activated T cells at 48 h after transfection with MTAS-NLS peptide-modified nanoparticles incorporating 5 wt% PEG. (B) Flow cytometric analysis of CD19 antigen expression in RS411 and K562 cells. (C) Representative flow cytometric analysis of mice peripheral blood cells after injection of PBAE or 5 wt% DSPE-PEG2000 co-assembled nanoparticles both conjugated with MTAS-NLS peptide ("Ctrl" represents the untreated control group).