Supplementary Methods

Cell lines. IMR-90 and WI38 cell lines were obtained from ATCC and grown in complete EMEM supplemented with 10% fetal bovine serum, L-glutamine, pen/strep, sodium pyruvate, HEPES, and sodium bicarbonate. Both cell lines were authenticated by ATCC. The cell lines were initially grown, cryopreserved into multiple aliquots and used within 6 months of resuscitation.

High-throughput screening of PBAE polymer library. H446 cells were seeded at 15,000 cells/well in 96-well plates in 100 µL of complete RPMI one day prior to transfection. The nanoparticles were made as discussed in the *nanoparticle preparation* section, with luciferase (CMV-Luc) plasmid DNA at 30, 60, 75, 100, 125, or 150 w/w of PBAE. Twenty µL of the nanoparticle solution was then added per well to the complete RPMI. Untreated wells served as negative controls, while Lipofectamine 2000 and FuGene HD, leading commercially available non-viral reagents, were used as positive controls as per the manufacturer's instructions. After 4 hours, the transfection solution was removed and fresh media was added to the cells. Transfection efficacy was analyzed 2 days after transfection by measuring the luminescence using the BrightGlo luciferase assay as we have previously described using a BioTek Synergy 2 plate reader. Luminescence was averaged within groups of quadruplicates and normalized by the untreated group.

Transfection protocol. Adherent (DMS-114, WI-38, IMR-9) and suspension cells (H69, H82, H345, H1930) were transfected following the protocol described in the Material and Methods.

Fluorescence microscopy. GFP positive cells were analyzed using a Motic AE31 inverted microscope using a FITC 480nm filter. Cy5 labeled cells were visualized and analyzed using a Zeiss Axio Observer microscope fitted with a Cy5 specific filter (emission wavelength of 690nm).

Flow cytometry. GFP fluorescence was used to indicate successful transfection, propidium iodide (PI) was used for dead cell discrimination, and Cy5 fluorescence to indicate successful nanoparticle association using a FACSCalibur flow cytometer (BD Biosciences, Rockville, MD). Briefly, adherent cells were trypsinized, centrifuged and resuspended in 500 µL of fresh media

and analyzed by flow cytometry. A total of 10,000 cells were acquired per analysis. All analyses were run with triplicate samples. Data analysis was performed using BD CellQuest Pro software (BD Biosciences).