SUPPORTING INFORMATION

Supplemental Methods

S1.1. Marker enzyme assays

For lactate dehydrogenase activity, 1 µL of each sample was added to 100 µL NADH/Tris/NaCl (0.244 mM NADH, 81.3 mM Tris, 203.2 mM NaCl, pH 7.2) and equilibrated to 30 °C. At t_0, 20 µL of Tris/NaCl/Na-pyruvate (81.3 mM Tris, 203.2 mM NaCl, pH 7.2, 9.76 mM Na-pyruvate) was added to each sample and the absorbance at 339 nm was measured every 10 min for 1 h at 30 °C using a plate reader (Tecan Safire^2). The linear portion of the slope of the absorbance at 339 nm vs. time was compared to a purified lactate dehydrogenase enzyme standard.

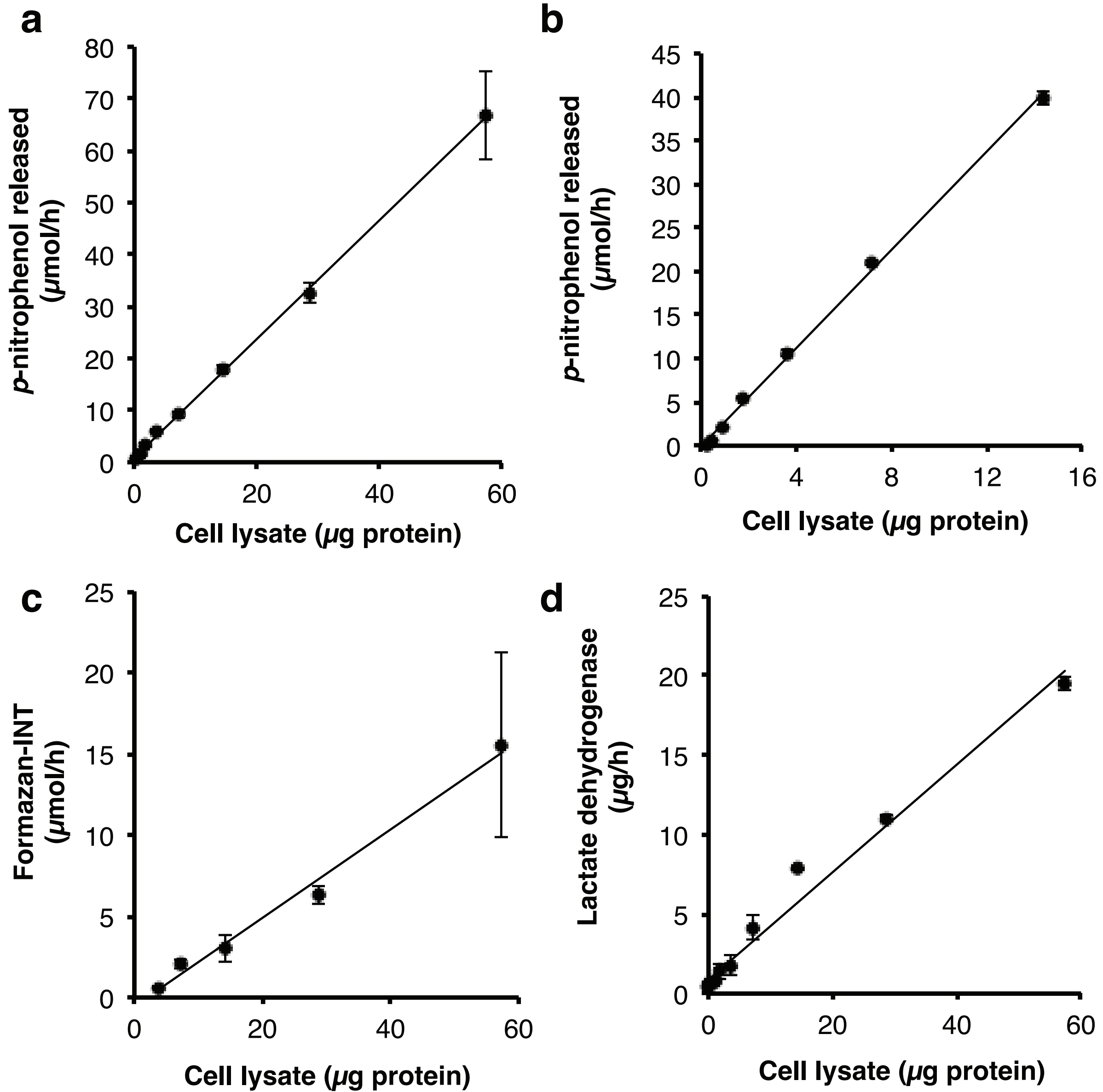
For hexosaminidase A activity, 10 µL of each sample was added to 70 µL of citrate-phosphate buffer containing substrate (100 mM citrate-phosphate buffer, pH 4.7, 7.5 mM *p*-nitrophenol-*N*-acetyl-ß-d-glucosaminide) and incubated for 1 h at 37 °C. To stop the reaction, 200 µL of borate buffer (200 mM borate buffer, pH 9.8) was added to each sample. For alkaline phosphatase activity, 20 µL of each sample was added to 140 µL borate buffer containing substrate (50 mM sodium borate, 1 M $MgCl_2$, pH 9.8, 16 mM *p*-nitrophenyl phosphate) and incubated for 1-4 h at 37 °C. The specific enzyme activity was calculated by measuring the absorbance at 405 nm using a plate reader and comparing the released *p*-nitrophenol to a standard of *p*-nitrophenol.

For succinate dehydrogenase activity, 20 µL of each sample was added to 140 µL of Tris-EDTA-succinate buffer containing substrate (20 mM Tris-HCl, pH 7.4, 100 µM EDTA, 200 mM sodium succinate, 203 mM NaCl, 2.5 mg/mL 2-*p*-iodonitrotetrazolium violet (INT)) and incubated for 1-4 h at 37 °C. The specific enzyme activity was calculating by measuring the absorbance at 492 nm using a plate reader and comparing the reduced INT to a standard of formazan-INT.

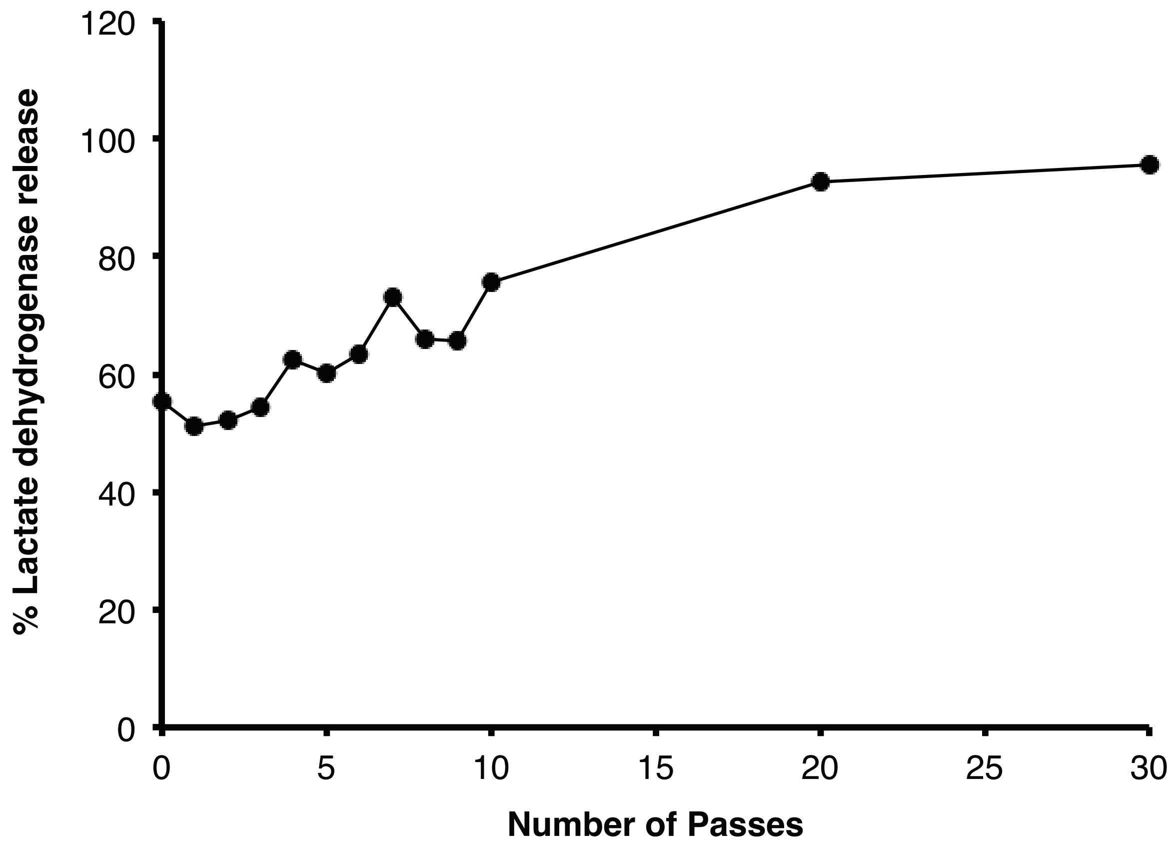
S1.2. Gel retardation assay

Polyplex condensation was determined using a gel retardation assay. An equivolume of polymer was added to 0.5 µg plasmid DNA at different charge ratios (N/P) and allowed to complex for 10 min at room temperature. Prior to loading into a 0.8% agarose gel containing TAE buffer (40 mM Tris-acetate, 1 mM EDTA), 10X BlueJuice loading buffer (Life Technologies, Carlsbad, CA) was added to the samples. The gel was then electrophoresed at 100 V for 30-45 min. Plasmid DNA was visualized using ethidium bromide staining via an UV transilluminator (laser-excited fluorescence gel scanner, Kodak, Rochester, NY).

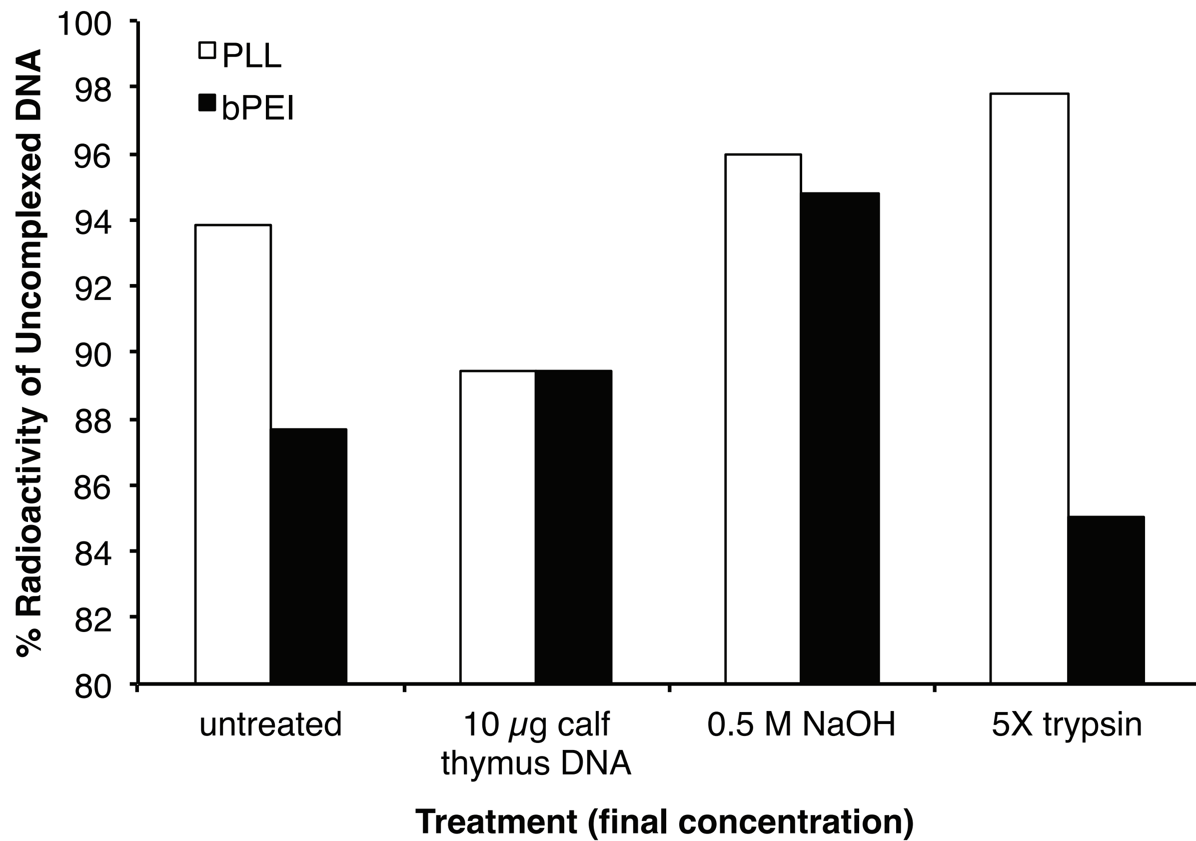
Supplemental Figures



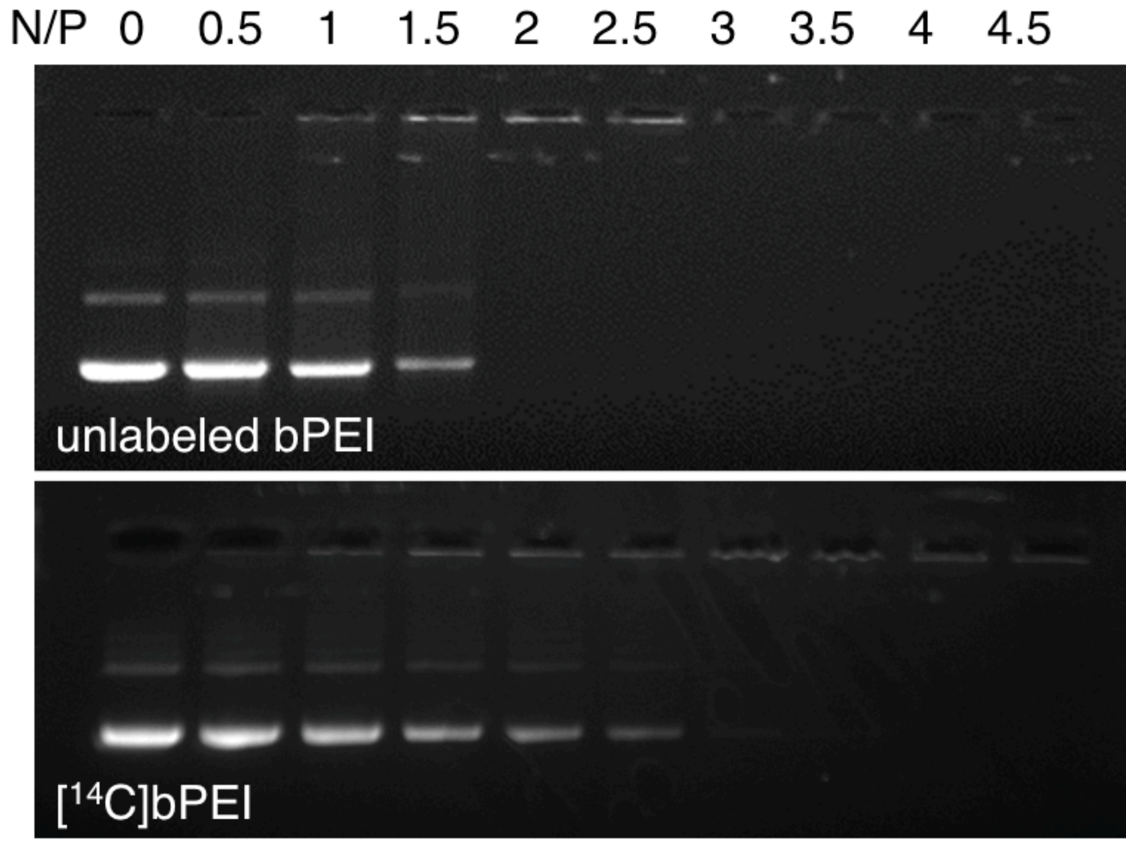
Supplemental Figure 1. Evaluation of marker enzyme assays. (a) HeLa cells (2×10^7) were lysed through six freeze-thaw cycles and the resulting cell lysate was used to evaluate marker enzyme assays for (b) alkaline phosphatase (plasma membrane), (c) hexosaminidase A (lysosomes), (d) succinate dehydrogenase (mitochondria), and (e) lactate dehydrogenase (cytosol). Data are presented as mean ± S.D., $n = 3$.



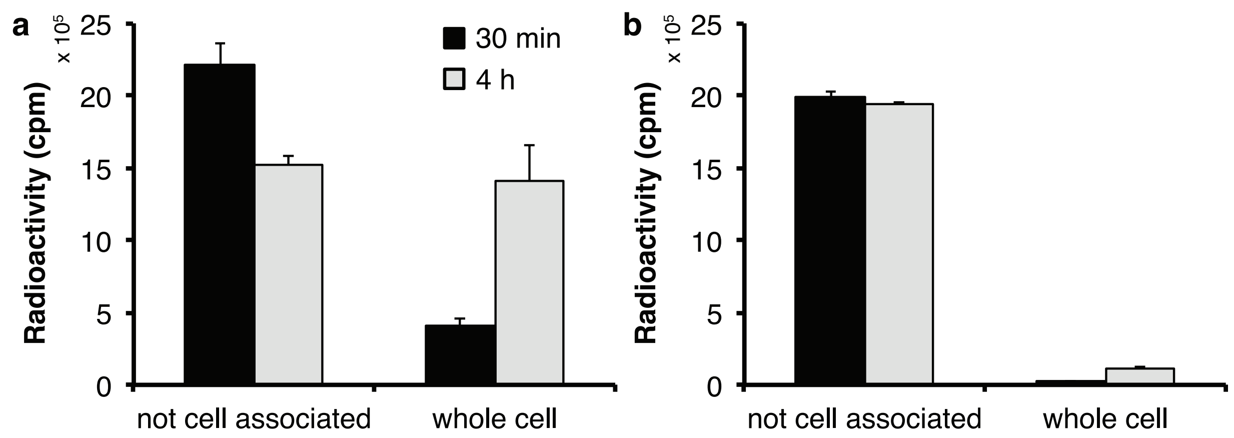
Supplemental Figure 2. Optimization of cell lysis with a needle and syringe. HeLa cells (2×10^7) were lysed through a 25-gauge needle and the supernatant was measured for lactate dehydrogenase release after a various number of passages.



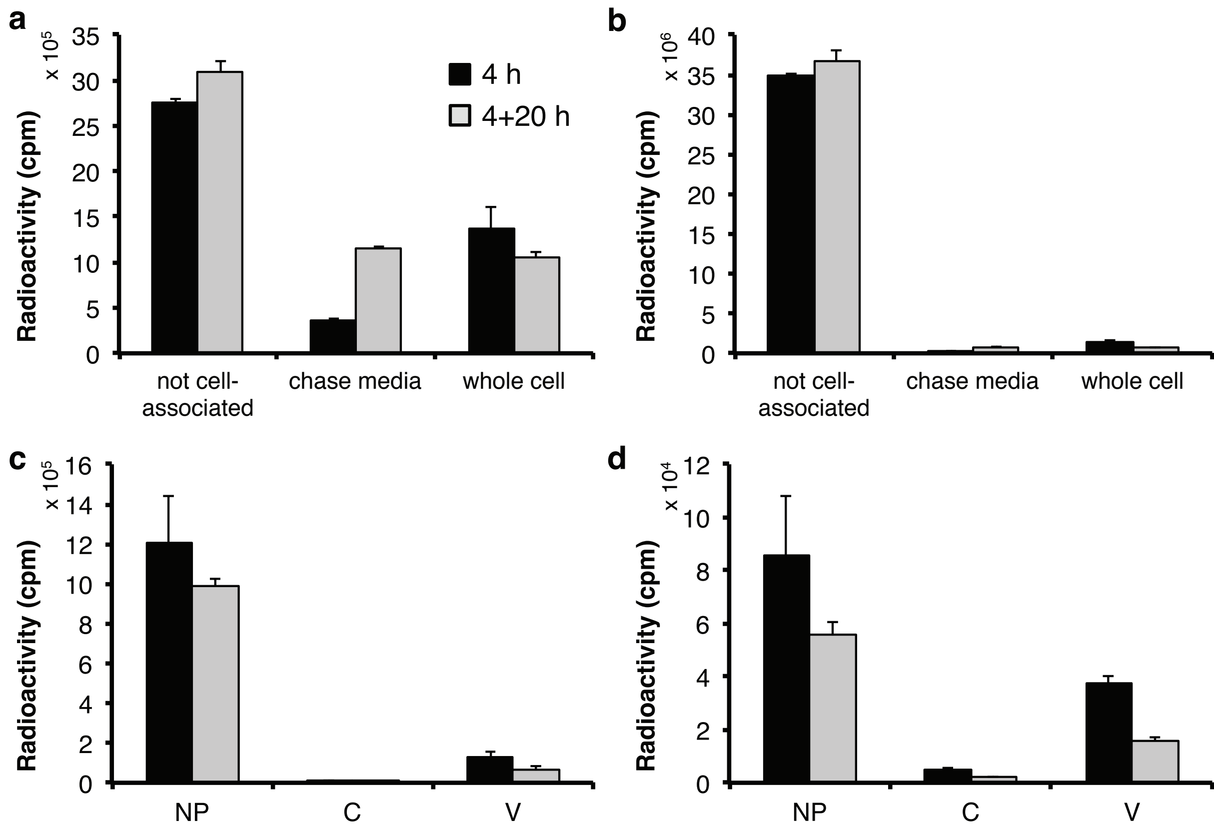
Supplemental Figure 3. Optimization of polyplex unpackaging for radioactivity measurements. Polyplexes were formed with 1 μg [^{3}H]DNA/unlabeled DNA mixture and unlabeled polymer, and subsequently treated with either 10 μg calf thymus DNA (to compete off radiolabeled DNA), 0.5 M NaOH (to deprotonate amines on the polymer), and 5X trypsin (to degrade polymer). Radioactivity counts are expressed as a percentage of the radioactivity count from uncomplexed DNA.



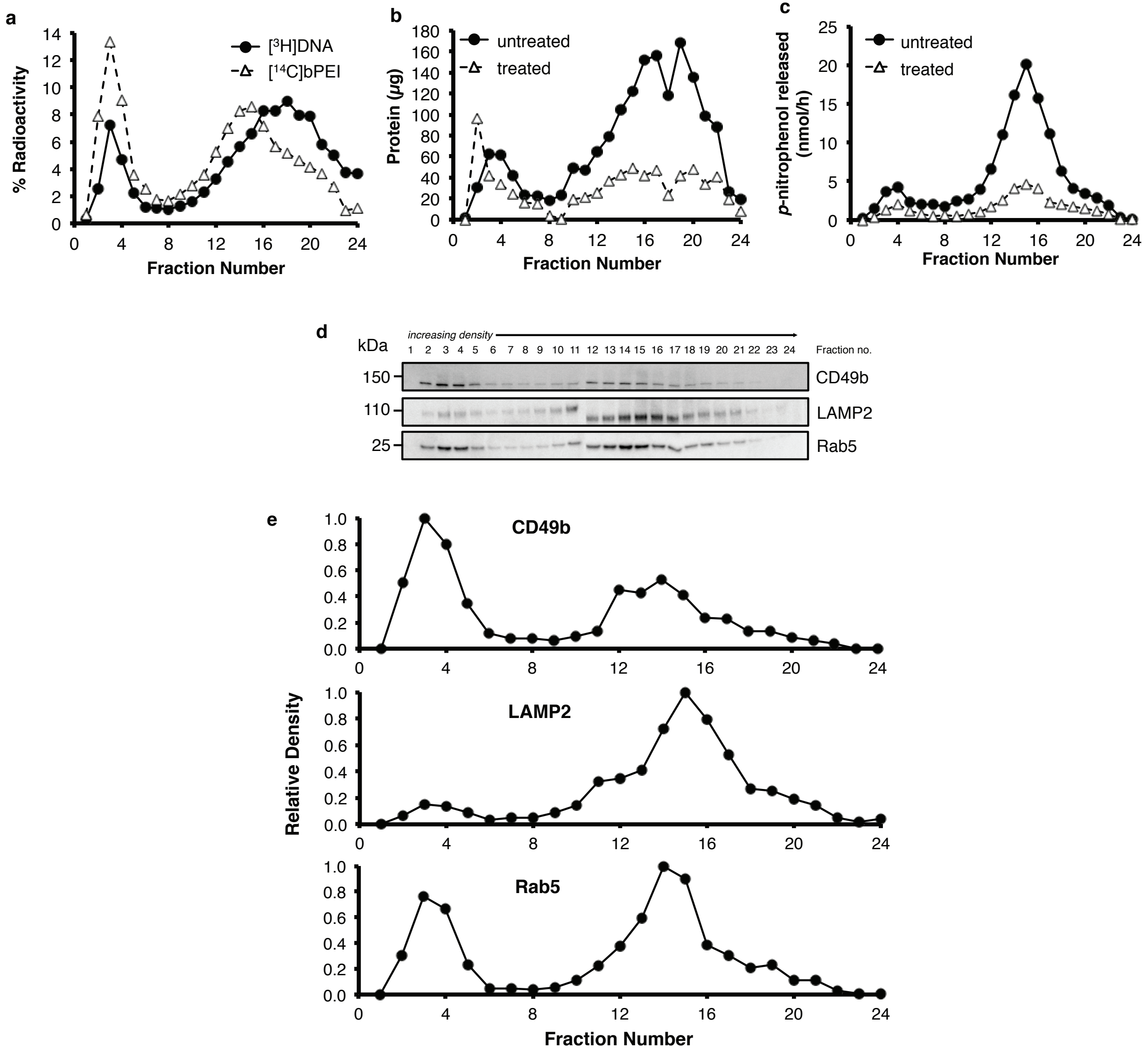
Supplemental Figure 4. Gel retardation assay of unlabeled and [^{14}C]-labeled bPEI. [^{14}C]-labeled and unlabeled bPEI were complexed with unlabeled plasmid DNA at different charge ratios (N/P) and loaded onto 0.8% agarose gels. Plasmid DNA was visualized using ethidium bromide.



Supplemental Figure 5. Extracellular and cellular distribution of [^{3}H]DNA/[^{14}C]bPEI polyplexes in treated cells fractionated using differential centrifugation. HeLa cells (10^7) were incubated with [^{3}H]DNA/[^{14}C]bPEI polyplexes for 1 h at 4 °C to allow for binding and then at 37 °C for 30 min (black bars) or 4 h (grey bars) to allow for internalization prior to fractionation. The radioactivity of (a) [^{3}H]DNA and (b) [^{14}C]bPEI measured in media and washes, which are "not cell associated", and whole cell fractions. Data are presented as mean ± S.D., n = 3.



Supplemental Figure 6. Distribution of [^{3}H]DNA/[^{14}C]bPEI polyplexes in treated cells fractionated using density-gradient centrifugation. HeLa cells (2×10^7) were pulsed with [^{3}H]DNA/[^{14}C]bPEI polyplexes for 4 h, and then prepared for fractionation. A 5-20% continuous iodixanol gradient was used to separate vesicular organelles.The radioactivity of (a) [^{3}H]DNA and (b) [^{14}C]bPEI measured in pulse media and washes, which are "not cell associated", chase media, and whole cell fractions. The radioactivity of (c) [^{3}H]DNA and (d) [^{14}C]bPEI measured in the nuclear, cytosolic, and vesicular fractions. Data are presented as mean ± average deviation (range divided by 2), $n = 2$.



Supplemental Figure 7. Distribution of [^{3}H]DNA/[^{14}C]bPEI polyplexes after 4 h pulse-20 h chase in cells fractionated using a 5-20% continuous iodixanol density gradient. HeLa cells (2 × 10^{7}) were pulsed with [^{3}H]DNA/[^{14}C]bPEI polyplexes for 4 h, chased in complete media for 20 h, and then prepared for fractionation. A 5-20% continuous iodixanol gradient was used to separate vesicular organelles. (a) The percent radioactivity measured in fractions from the 5-20% gradient. 100% radioactivity is equal to the sum of the radioactivity found in all 24 fractions collected from the 5-20% gradient. (b) Total protein was measured in untreated and treated gradient fractions. (c) Hexosaminidase A (lysosome) activity was also measured in untreated and treated gradient fractions. (d) An equivolume (250 μL) of gradient fractions from treated samples was precipitated, concentrated, and probed for *CD49b* (plasma membrane), *LAMP2* (lysosome), and *Rab5* (endosome). (e) The optical density of each band was measured using ImageJ. Each time point presented is representative of duplicate experiments.