



Figure 2. Comparison of polymers for transient transfection of cytosolic mCherry in HEK and CHO cells. (A) HEK cells were transfected with 2 μ g/mL mCherry-encoding DNA via PBAE or PEI nanoparticles ($n=5$). In HEK cells, PEI was used at a 2:1 polymer:DNA w/w ratio, whereas in CHO cells, PEI was used at a 3:1 polymer:DNA w/w ratio. All PBAEs were used at a 60:1 polymer:DNA w/w ratio in both cell lines. mCherry fluorescence was assessed via plate reader each day, and significance was calculated on day 5. (B) mCherry transfection efficiency was determined via flow cytometry 5 days following transient transfection of HEK cells with 4 μ g mCherry-encoding DNA via PBAE or PEI nanoparticles ($n=5$). (C) CHO cells were transfected with 4 μ g/mL mCherry-encoding DNA via PBAE or PEI nanoparticles ($n=5$). mCherry fluorescence was assessed via plate reader each day, and significance was calculated on day 5. (D) mCherry transfection efficiency determined by flow cytometry 5 days following transient transfection of CHO cells with 8 μ g mCherry-encoding DNA via PBAE and PEI nanoparticles ($n=5$). (E) Representative fluorescence microscopy images of HEK and CHO cells 5 days following transient transfection of mCherry-encoding DNA (4 μ g for HEK cells; 8 μ g for CHO cells) with 4-4-6 or PEI nanoparticles. Scale bars are 200 μ m. For all panels, error bars represent SD. Significance on day 5 was calculated using one-way ANOVA with Dunnett post-test, comparing all conditions to treatment with PEI. Increases relative to PEI are designated: ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.