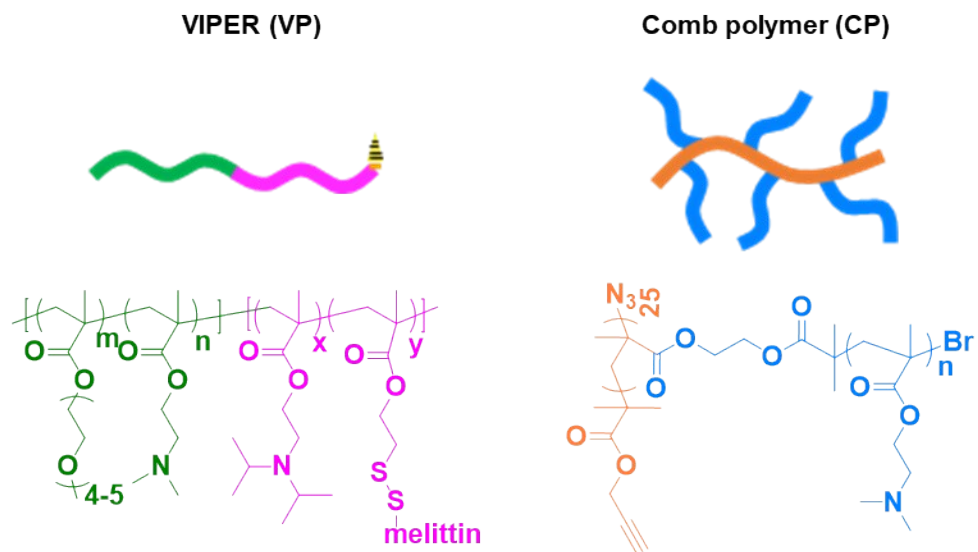


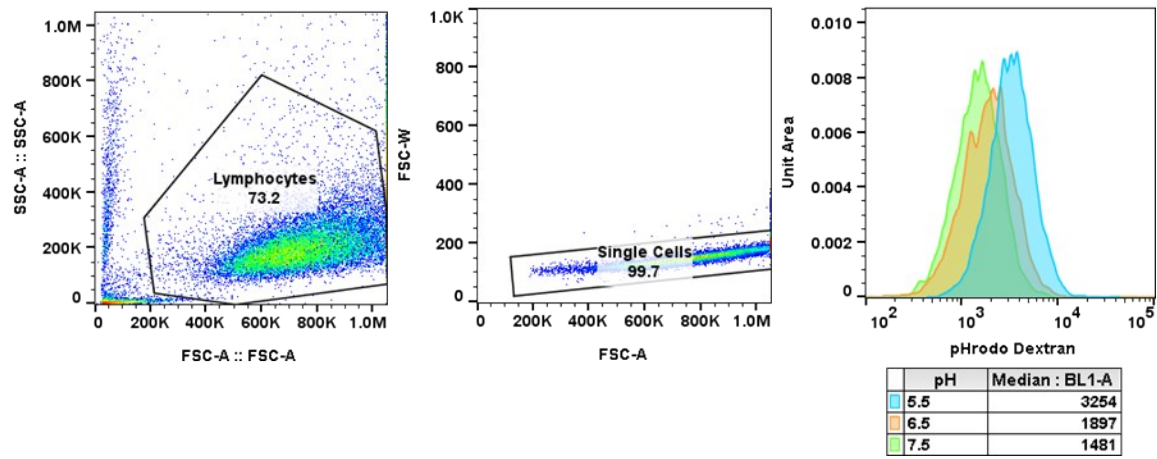
## **Supplementary Information**

### **Identifying key barriers in cationic polymer gene delivery to human T cells**

Brynn R. Olden<sup>a</sup>, Emmeline Cheng<sup>a</sup>, Yilong Cheng<sup>a,b</sup>, Suzie H. Pun<sup>\*a</sup>

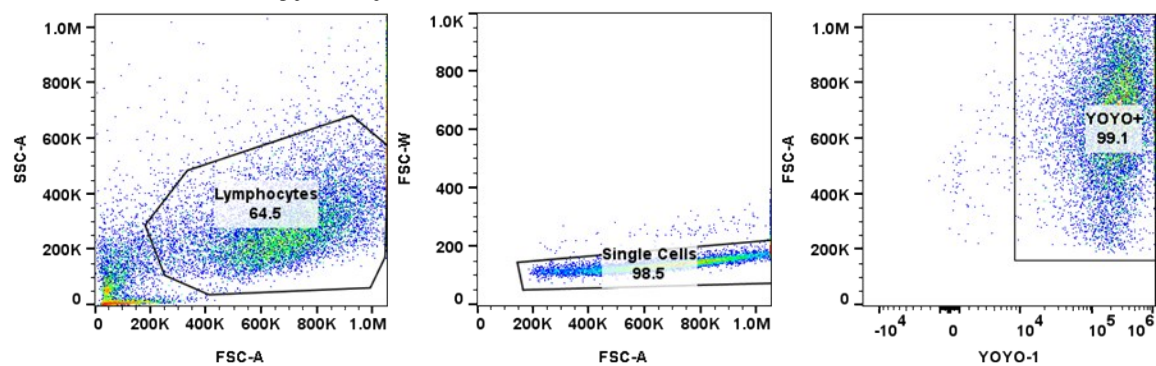


**Fig. S1** Schematic and chemical structures of virus-inspired polymer for endosomal release (VIPER) and comb polymer (Comb).

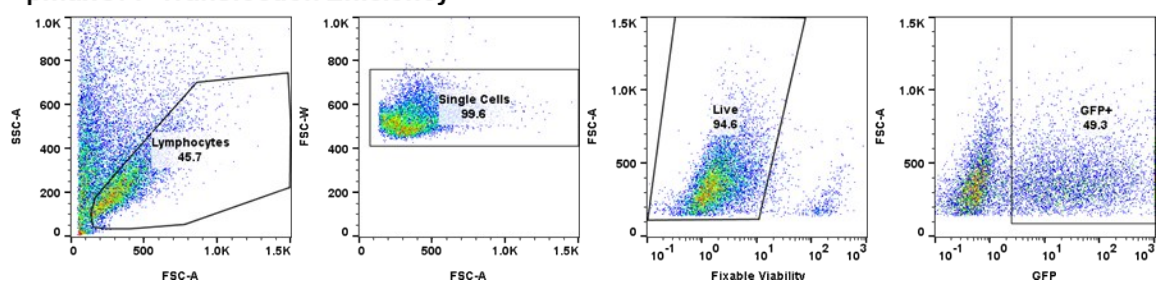


**Fig. S2** Gating strategies for pHrodo dextran uptake studies.

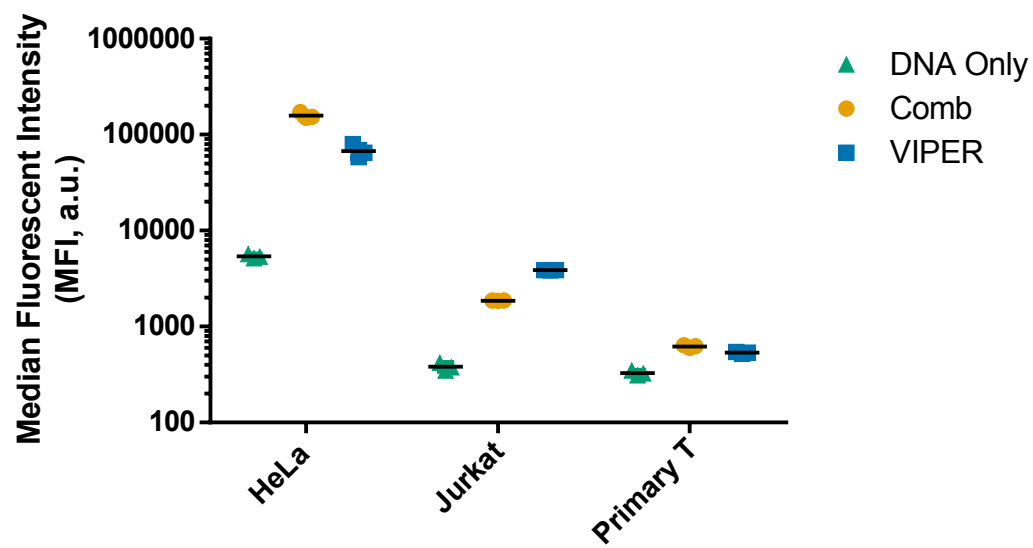
### YOYO-1 Labeled Polyplex Uptake



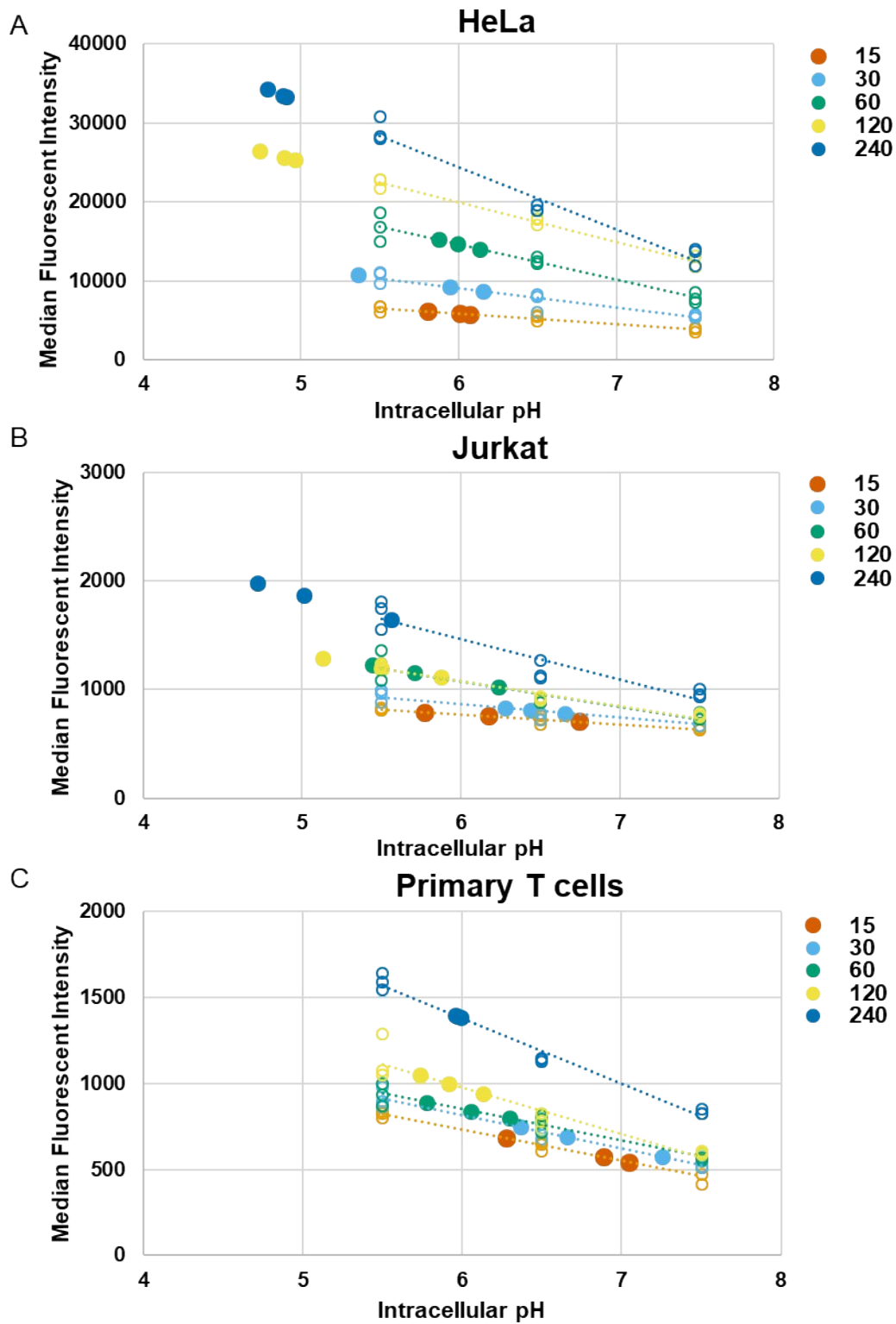
### pmaxGFP Transfection Efficiency



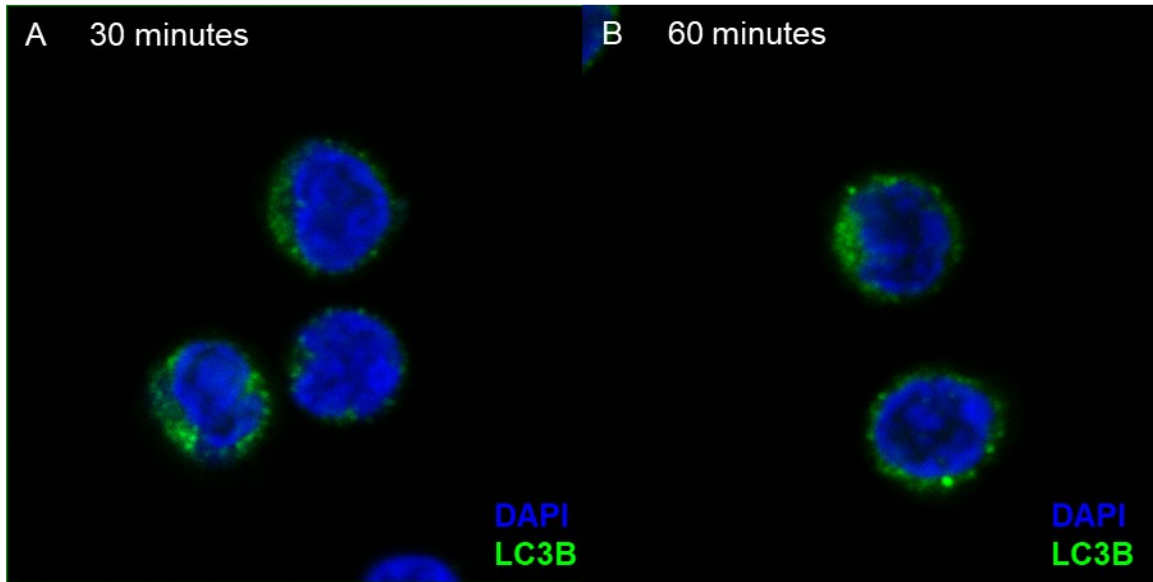
**Fig. S3** Gating strategies for uptake and transfection studies.



**Fig. S4** Uptake of free DNA, Comb, and VIPER polyplexes in HeLa, Jurkat, and primary T cells. Uptake is expressed as median fluorescent intensity (MFI) of all cells treated with YOYO-1 labeled DNA in indicated formulations.



**Fig. S5** Calibration curves (open circles with dotted trendline) used for experimental intracellular pH calculations (filled circles) for (A) HeLa, (B) Jurkat, and (C) primary human T cells using pHrodo labeled 10 kDa MW dextran.



**Fig. S6** Confocal microscopy images of autophagy in primary human T cells. T cells transfected with VIPER polyplexes for (A) 30 minutes or (B) 60 minutes stained with LC3B antibody that labels autophagosomes.