

Figure S1. Representative histograms and gating areas for the flow cytometry analysis of EGFP positive cells. EGFP mRNA lipoplexes containing DC-1-16, DC-6-14 or TC-1-12 were reverse transfected to HeLa cells. The percentage of EGFP positive cells was measured 24 h after the transfection. Control: Group subjected to reverse transfection with non-lyophilized mRNA lipoplexes. EGFP, enhanced green fluorescent protein; DC-1-16, *N*-hexadecyl-*N,N*-dimethylhexadecan-1-aminium bromide; DC-6-14, 2-(bis(2-(tetradecanoyloxy)ethyl)amino)-*N,N,N*-trimethyl-2-oxoethan-1-aminium chloride; TC-1-12, 11-((1,3-bis(dodecanoyloxy)-2-((dodecanoyloxy)methyl) propan-2-yl) amino)-*N,N,N*-trimethyl-11-oxoundecan-1-aminium bromide.

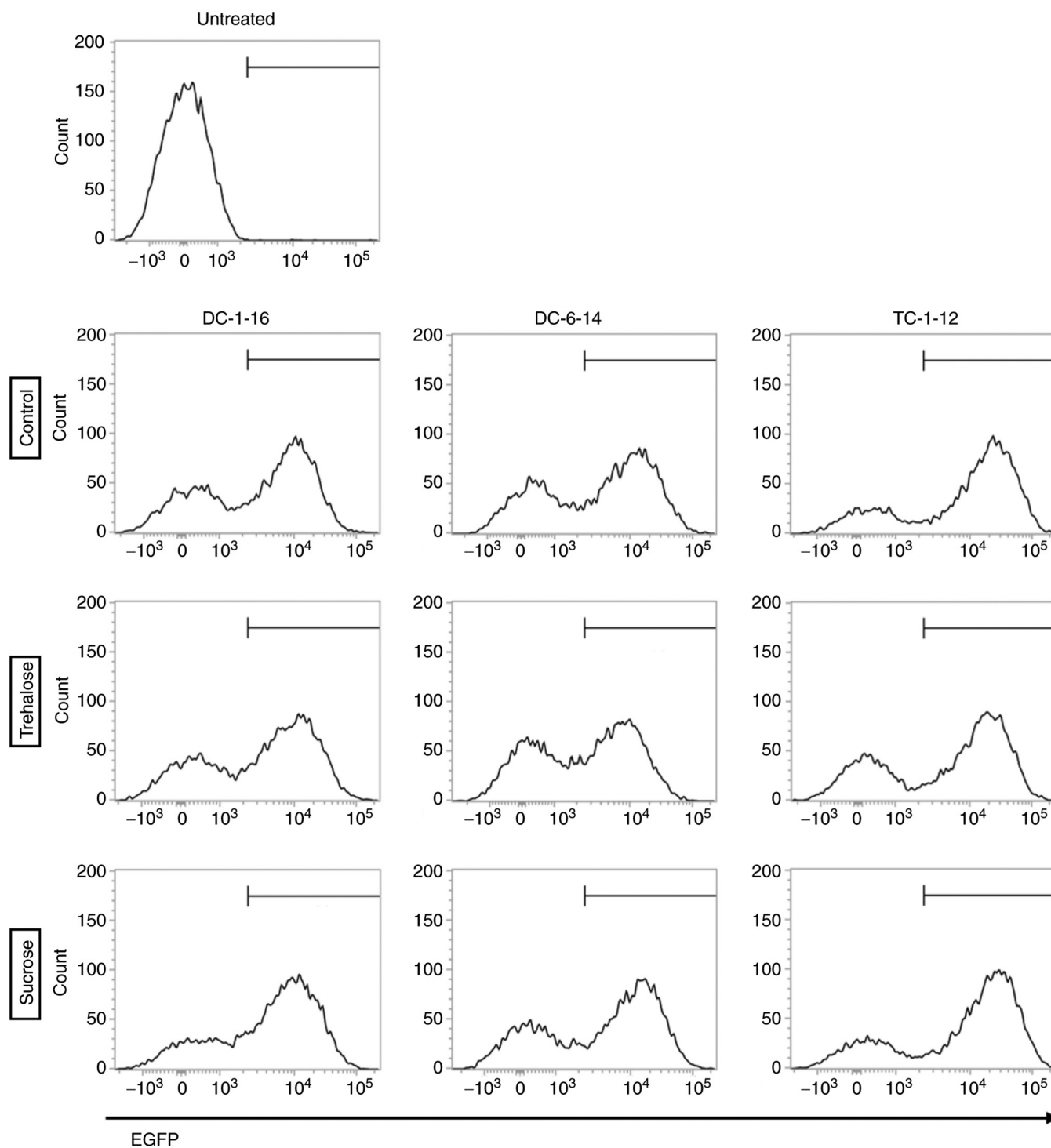


Figure S2. Representative histograms and gating areas for the flow cytometry analysis of Cy5 positive cells. Cy5-mRNA lipoplexes containing DC-1-16, DC-6-14 or TC-1-12 were reverse transfected to HeLa cells. The fluorescence intensity was measured 3 h after the transfection. Control: Group subjected to reverse transfection with non-lyophilized mRNA lipoplexes. DC-1-16, *N*-hexadecyl-*N,N*-dimethylhexadecan-1-aminium bromide; DC-6-14, 2-(bis(2-(tetradecanoyloxy)ethyl) amino)-*N,N,N*-trimethyl-2-oxoethan-1-aminium chloride; TC-1-12, 11-((1,3-bis(dodecanoyloxy)-2-((dodecanoyloxy)methyl) propan-2-yl) amino)-*N,N,N*-trimethyl-11-oxoundecan-1-aminium bromide.

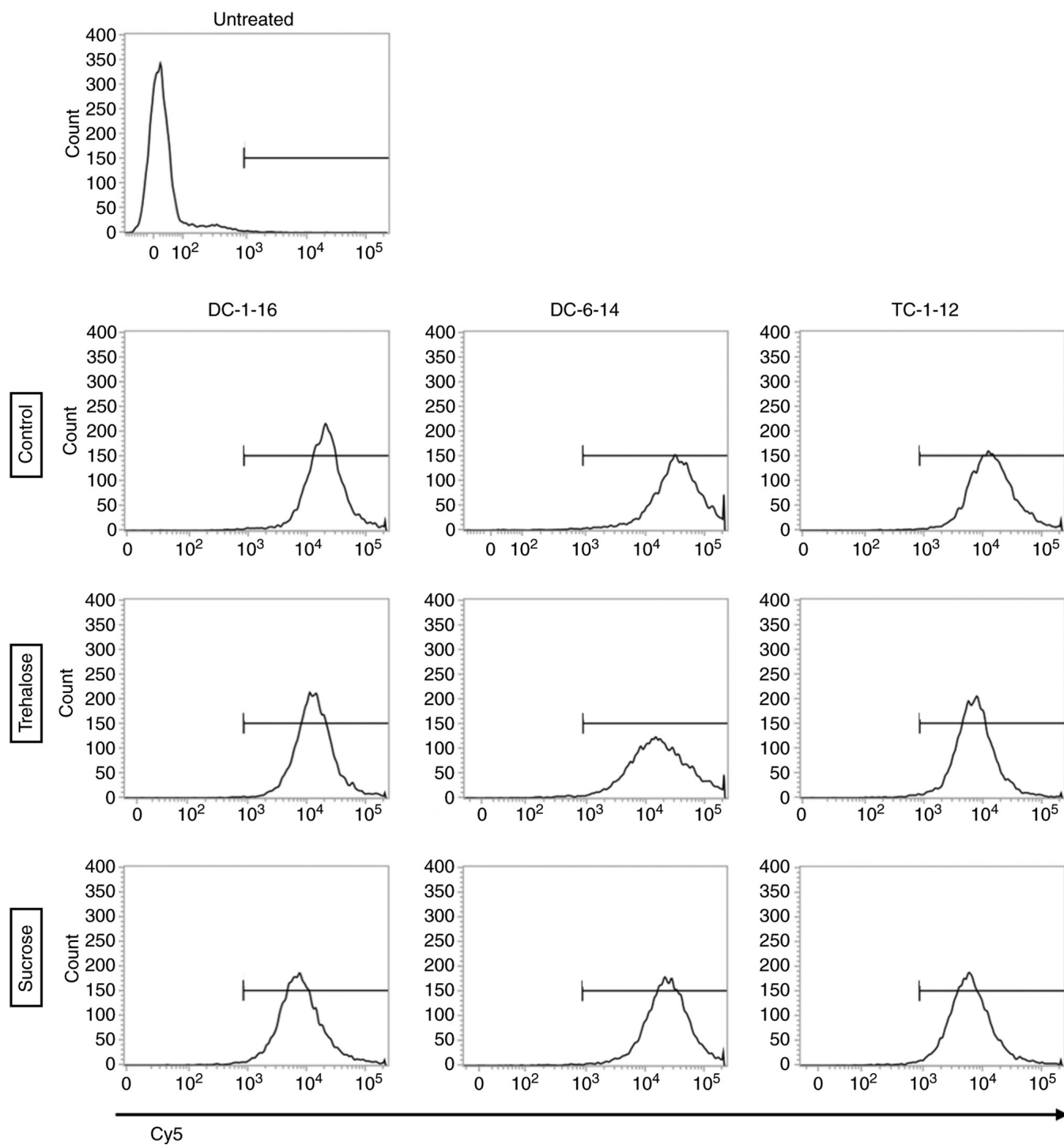


Figure S3. Localization of mRNAs and lysosomes in HeLa cells after reverse transfection with lyophilized mRNA lipoplexes. Cy5-mRNA lipoplexes containing DC-1-16, DC-6-14 or TC-1-12 were reverse transfected to HeLa cells. Lysosomes were stained by LysoTracker 3 h after the transfection. Blue indicates cell nuclei stained with Hoechst 33342, green indicates lysosomes labeled with LysoTracker Red, and red indicates the localization of Cy5-mRNA. Scale bar, 20 μ m. DC-1-16, *N*-hexadecyl-*N,N*-dimethylhexadecan-1-aminium bromide; DC-6-14, 2-(bis(2-(tetradecanoyloxy)ethyl) amino)-*N,N,N*-trimethyl-2-oxoethan-1-aminium chloride; TC-1-12, 11-((1,3-bis(dodecanoyloxy)-2-((dodecanoyloxy)methyl) propan-2-yl) amino)-*N,N,N*-trimethyl-11-oxoundecan-1-aminium bromide.

