

Figure S1. Effects of cationic liposomes on Luc activity in HeLa cells post-transfection. HeLa cells were transfected only with cationic liposomes (mock transfection) at the indicated charge ratios and incubated for 24 h. Each value represents the mean  $\pm$  standard deviation (n=3). FLuc, Firefly luciferase.

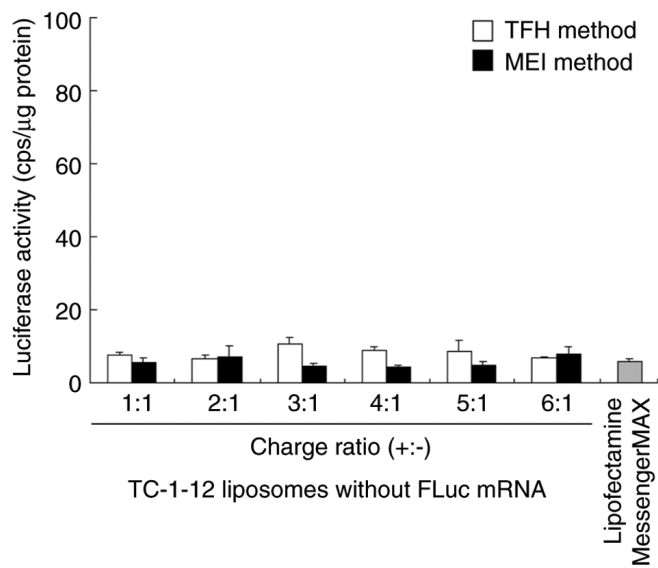


Figure S2. Representative histograms of flow cytometry after transfection with TC-1-12-based EGFP mRNA lipoplexes to HeLa cells. EGFP mRNA lipoplexes were prepared using the MEI and TFH methods at charge ratios of 2:1 to 5:1, transfected into HeLa cells, and incubated for 24 h. Lipofectamine MessengerMAX was used as a control. Percentage of EGFP-positive cells was determined using flow cytometry. TC-1-12, 11-((1,3-bis(dodecanoyloxy)-2-((dodecanoyloxy)methyl)propan-2-yl)amino)-*N,N,N*-trimethyl-11-oxoundecan-1-amium bromide; EGFP, enhanced green fluorescent protein; MEI, modified ethanol injection; TFH, thin-film hydration.

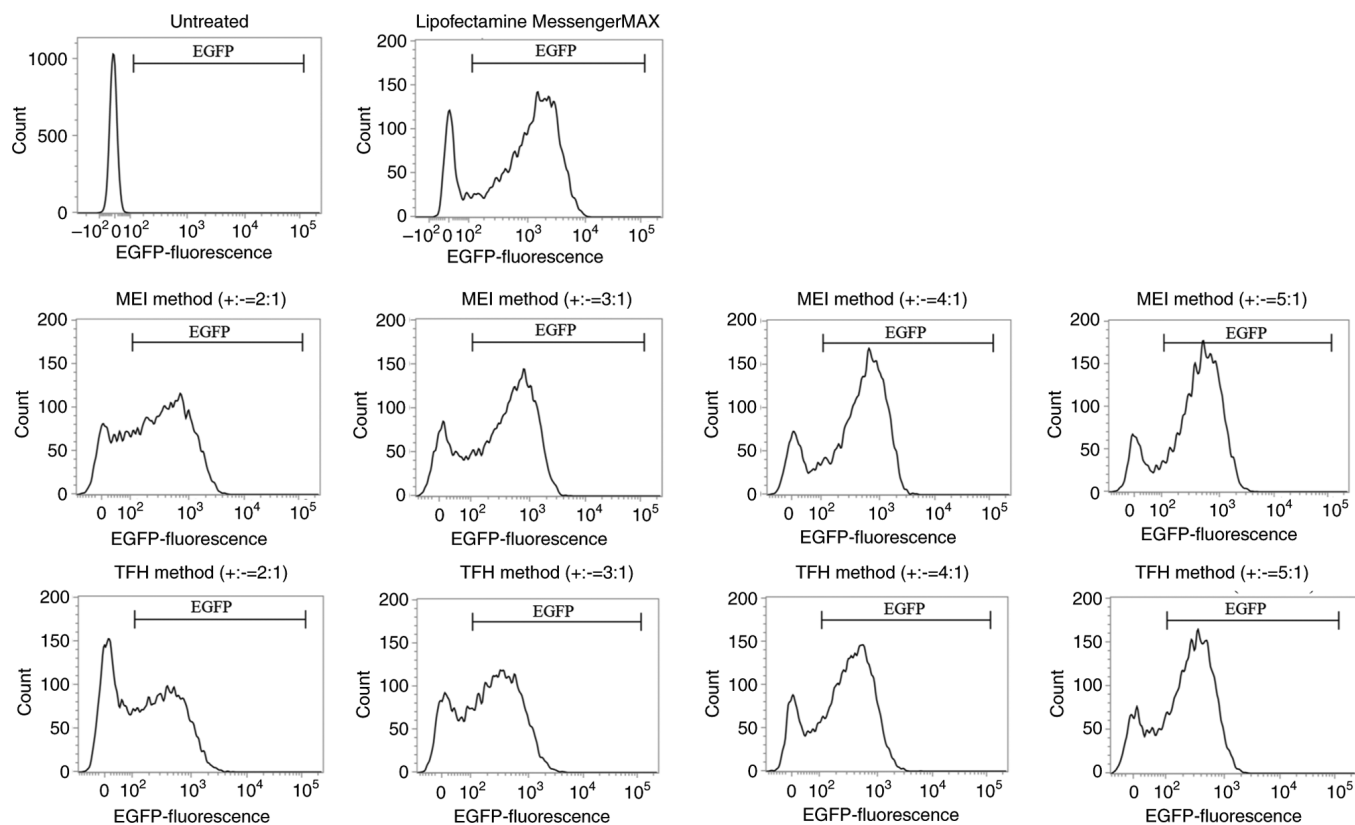


Figure S3. Representative histograms of flow cytometry after transfection with the TC-1-12-based Cy5-labeled mRNA lipoplexes to HeLa cells. Cy5-labeled mRNA lipoplexes were prepared at charge ratios of 3:1 and 4:1 using the MEI and TFH methods, respectively, and added to HeLa cells at 0.5  $\mu\text{g}/\text{ml}$  mRNA. As controls, Cy5-labeled mRNA solution (free mRNA) and Lipofectamine MessengerMAX mRNA lipoplexes were added to the HeLa cells. Fluorescence intensity in Cy5-labeled mRNA-transfected cells was measured using flow cytometry 3 h after incubation. MEI method. TC-1-12, 11-((1,3-bis(dodecanoyloxy)-2-((dodecanoyloxy)methyl)propan-2-yl)amino)-*N,N,N*-trimethyl-11-oxoundecan-1-amium bromide; Cy5, cyanine 5; MEI, modified ethanol injection; TFH, thin-film hydration.

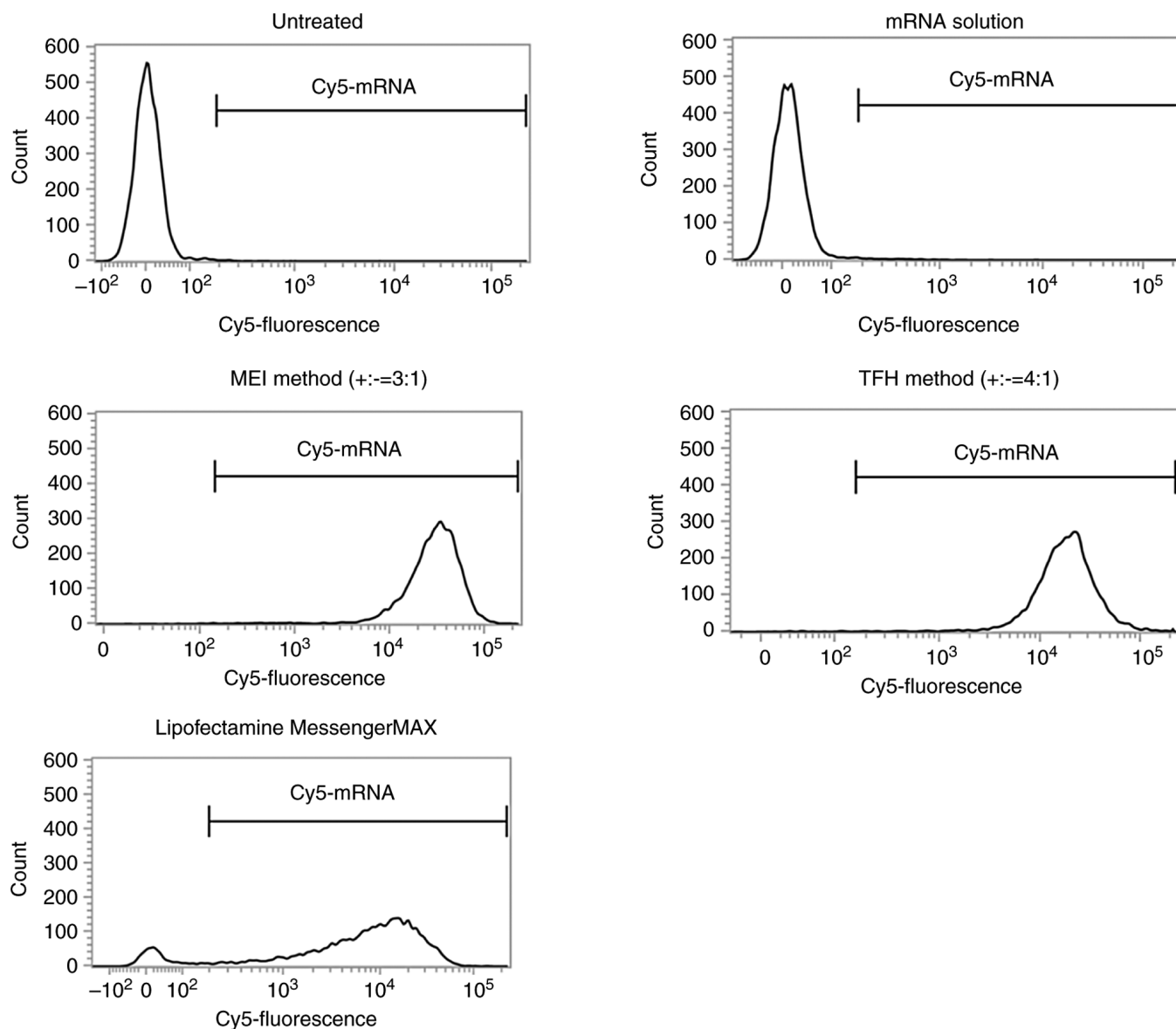


Figure S4. Cytotoxicity in HeLa, PC-3 and HepG2 cells after treatment with the culture medium containing ethanol. HeLa, HepG2, and PC-3 cells were incubated with the culture medium containing 0.156-5% (v/v) ethanol for 24 h. Each value is represented as the mean  $\pm$  standard deviation (n=5 for 2.5% ethanol in HeLa cells, n=8 for 0.313 and 0.156% ethanol in HeLa cells, n=7 for other groups in HeLa cells; n=3 for 5% ethanol in HepG2 cells and n=4 for other groups in HepG2 cell; n=5 for all groups in PC-3 cells). \*\*P<0.01 and \*\*\*P<0.001 vs. untreated cells (0% ethanol).

