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Supplemental Information

Involvement of a Rac1-Dependent Macropinocytosis Pathway in Plasmid DNA Delivery by Electrotransfection

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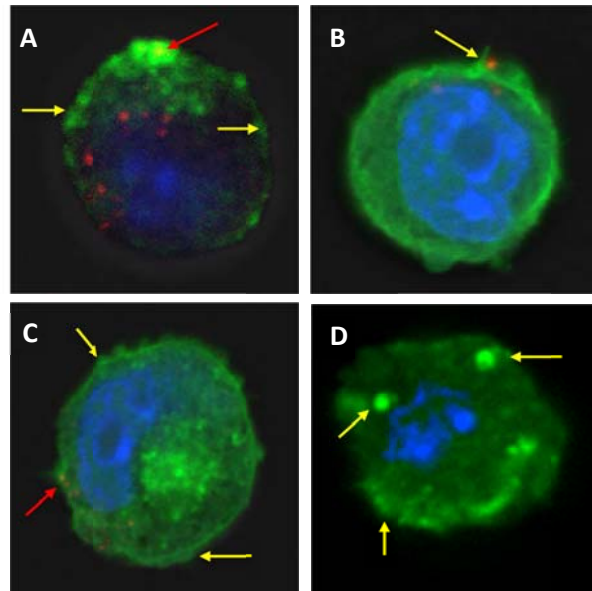


Figure S1. Representative images of pDNA uptake and actin remodeling in B16.F10 cells expressing Lifeact-GFP after electrotransfection with rhodamine-labeled DNA. Experimental conditions were the same as those for the image shown in Figure 1A. (A) A cell with punctate-like structures of actin. (B) A cell with filopodia-like protrusion around pDNA. (C) A cell with multiple membrane ruffling structures. (D) Actin remodeling in a cell pulsed without pDNA. Yellow arrows denote the actin structures described above. Red arrows denote the pDNA co-localized with actin.

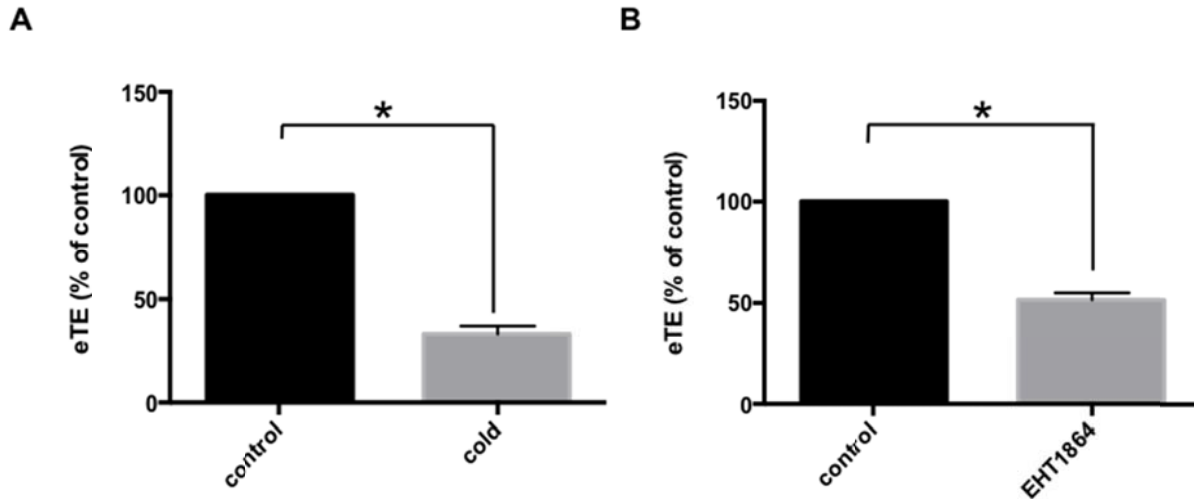


Figure S2. Effects of treatments with cold medium and Rac1 inhibitor on eTE. (A) eTE in COS7 cells treated with cold medium. After electrotransfection of pcDNA3.1(+) Luc2=tdT pDNA, the cells were divided into two groups. The control groups were incubated at 37°C for 10 min to allow uptake of pDNA. The cold treatment groups were incubated on ice for 10 min to minimize macropinocytosis. The eTE was quantified at 24 hr. (B) Effect of Rac1 inhibitor EHT1864 on eTE in COS7 cells. Cells were pretreated with 10 μ M EHT1864, or equivalent volume of DMSO as control for 1 hr prior to electrotransfection with the pcDNA3.1 (+) Luc2=tdT pDNA. After electrotransfection, cells were plated in 6-well plates and cultured for 24 hr before eTE measurement using luciferase assay. Luminescence readings (LU) of each sample were normalized by protein concentration to obtain relative luminescence units (RLU). RLU in treated group was further normalized by that in the control. *P < 0.05 (Mann-Whitney U test).