

Supplementary Information

Title: Low Electric Treatment activates Rho GTPase via Heat Shock Protein 90 and Protein Kinase C for Intracellular Delivery of siRNA

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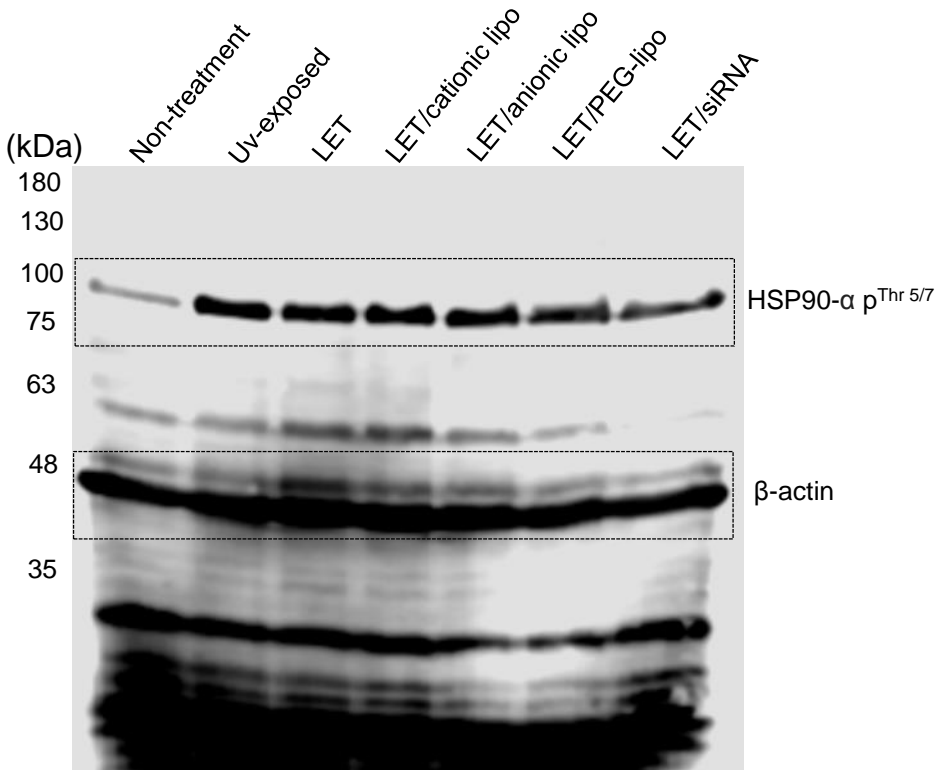
GO slim analysis of GO term-attached proteins among the top Top 15 phosphorylated proteins upregulated by LET

Names	Cellular component	Molecular function	Biological process
Hnrnpc	Cell; intracellular; organelle; cytoplasm; nucleus; cytosol; nucleoplasm; protein complex	RNA binding; mRNA binding	N.D
Pdap1	Extracellular space, extracellular region	RNA binding	N.D
Mtdh	Cell; intracellular; organelle; cytoplasm; nucleus; endoplasmic reticulum; nucleolus	RNA binding	N.D
Hmgbl	Cell; intracellular; organelle; cytoplasm; nucleus; plasma membrane; extracellular space; extracellular region	Lyase activity	N.D
Marcks	Cell; intracellular; cytoplasm	N.D	N.D
Eef1b2	Cell; intracellular; organelle; cytoplasm; endoplasmic reticulum;	N.D	N.D
Hsp90aa1	Cell; intracellular; organelle; cytoplasm; nucleus; cytosol; protein complex	RNA binding; unfolded protein binding; ATPase activity	N.D
Huwe1	Cell; intracellular; organelle; cytoplasm; nucleus; cytosol; nucleoplasm;	RNA binding; DNA binding	Cell differentiation

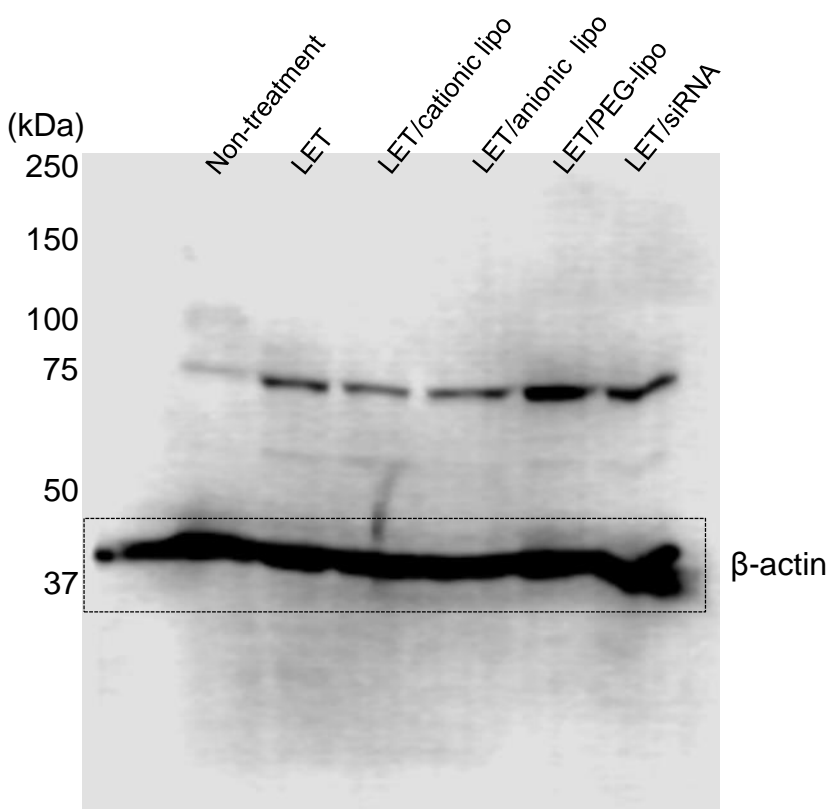
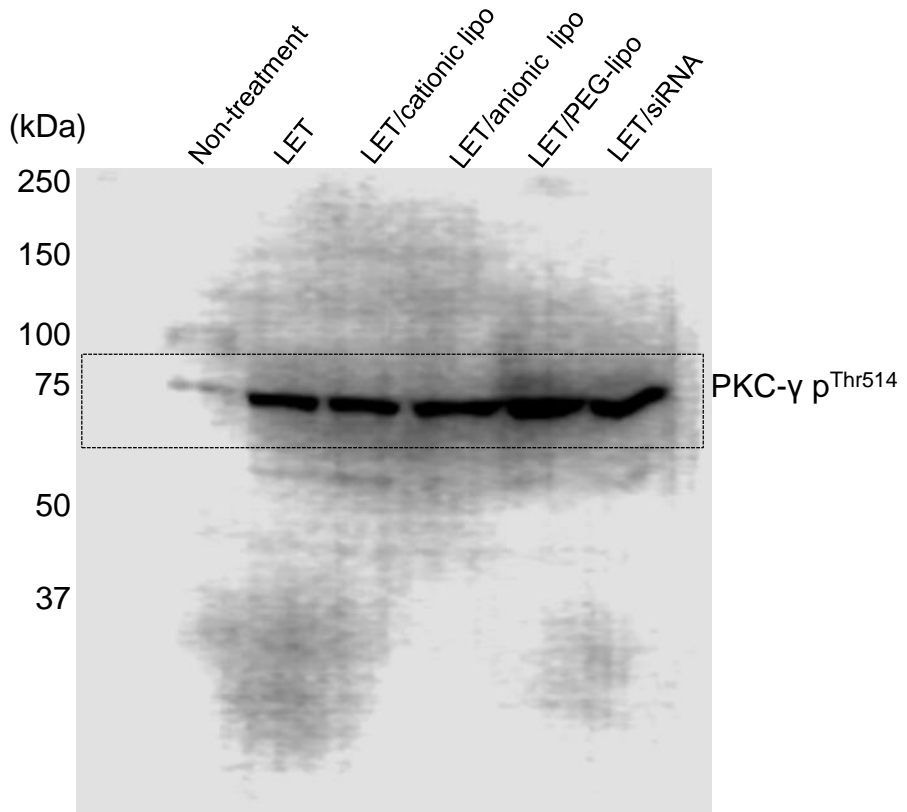
N.D.: Not determined

Physicochemical properties of liposomes

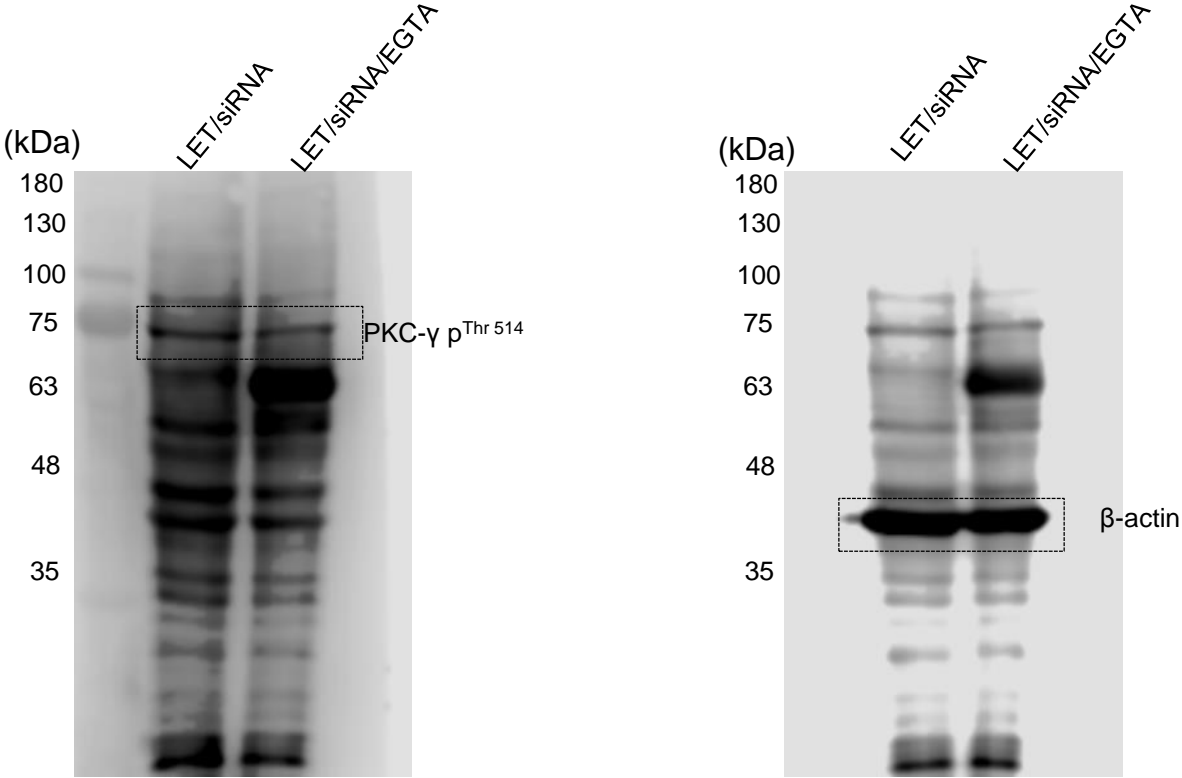
	Particle size (nm)	ζ -potential (mV)
Cationic lipo	96 \pm 8.1	10 \pm 1.9
Anionic lipo	97 \pm 14	-17 \pm 3.1
PEG-lipo	87 \pm 7.8	-1.8 \pm 0.47



Full-length blots regarding figure 2 (a)

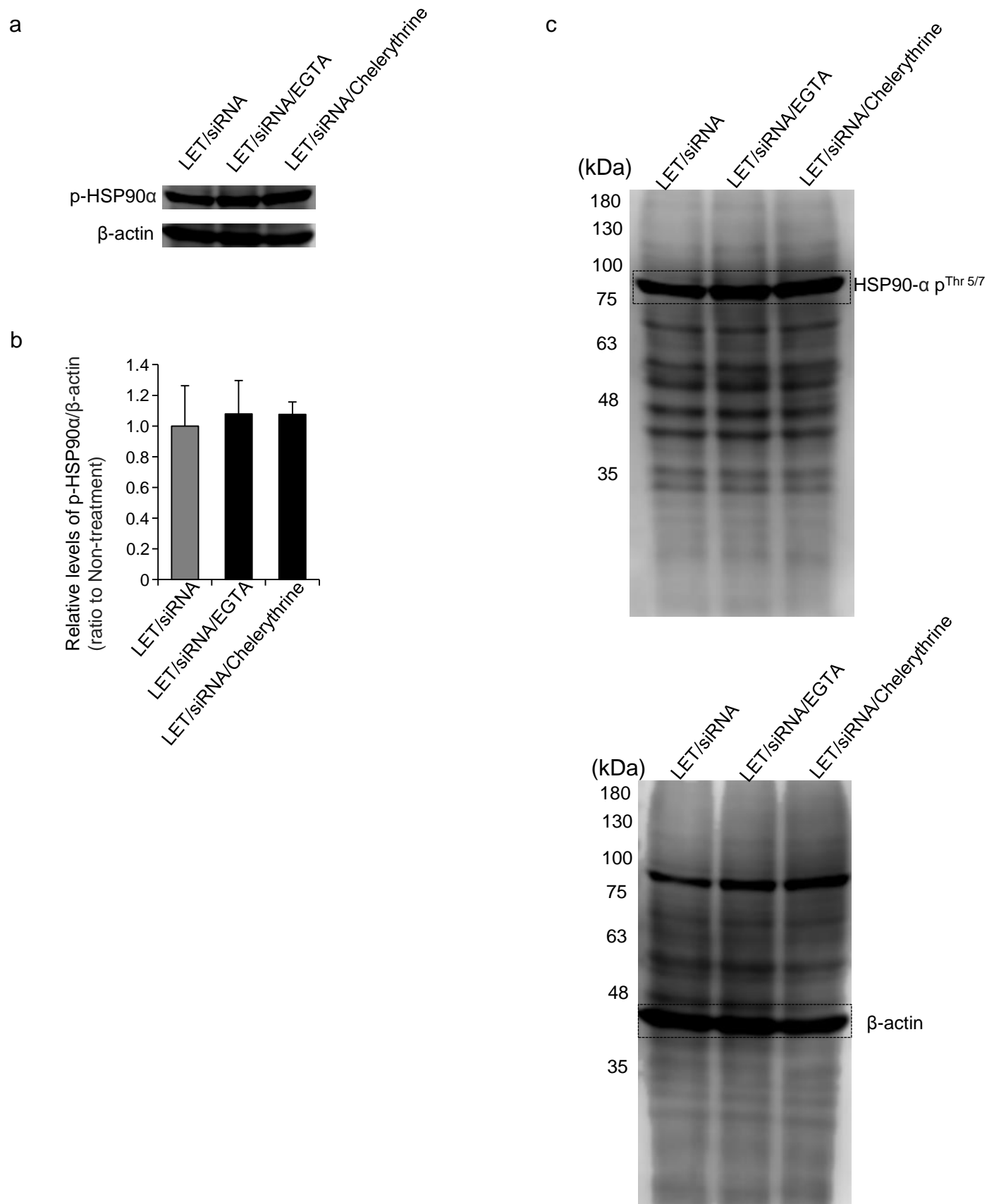


Full-length blots regarding figure 3 (a)



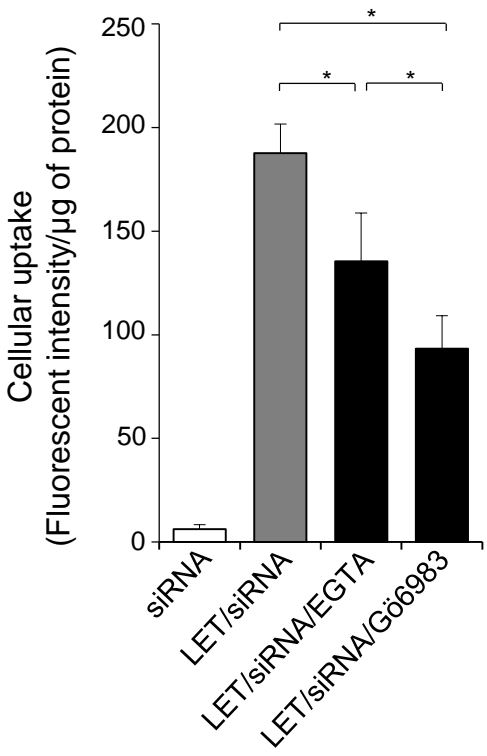
Full-length blots regarding figure 3 (d)

Supplementary Fig. S4



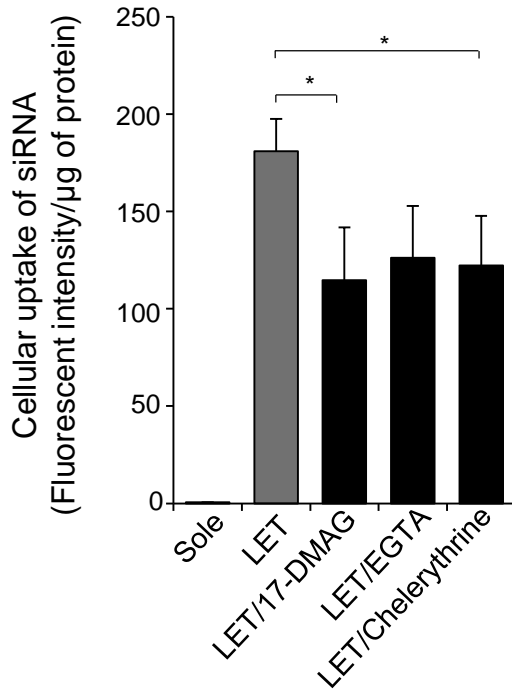
Effects of EGTA and chelerythrine on Hsp90α phosphorylation induced by LET with siRNA

Cells were pretreated with EGTA or chelerythrine for 30 min prior to the LET in presence of siRNA. Phospho-Hsp90α (Thr5/7) levels were determined by western blotting. β-actin was used as a loading control. (a) Typical images from at least 3 individual experiments. (b) Relative levels p-Hsp90α/β-actin. Data are shown as the ratio to non-treatment. Values represent the means of 3 individual experiments. Bars represent standard deviations. (c) Full-length blots regarding Fig. S4a



Effects of EGTA or PKC inhibitor Gö6983 on cellular uptake of siRNA

NIH3T3 cells were pretreated with EGTA or PKC inhibitor Gö6983 prior to LET in presence of rhodamine-labeled siRNA. After incubation for 45 min, cells were lysed, and fluorescence intensity in the lysate was measured. Values represent the means of 3 individual experiments. Bars represent standard deviations. * $p < 0.05$.



Effects of Hsp90 inhibitor, EGTA or PKC inhibitor on cellular uptake of siRNA in B16-F1 cells

B16-F1 cells were pretreated with 17-DMAG, EGTA or Chelerythrine prior to LET in presence of rhodamine-labeled siRNA. After incubation for 45 min, cells were lysed, and fluorescence intensity in the lysate was measured. Values represent the means of 3 individual experiments. Bars represent standard deviations. * $p < 0.05$.