Supplementary Information

for

Optimizing TDP-43 silencing with siRNA-loaded polymeric nanovectors in neuronal cells for therapeutic applications: balancing knockdown and function

Annamaria Russo[a], Gabriele Maiorano[a], Barbara Cortese[b], Stefania D'Amone[a], Alessandra Invidia[c], Angelo Quattrini[d], Alessandro Romano[d,e]*, Giuseppe Gigli[a,f] and Ilaria E. Palamà[a]*

[a]Nanotechnology Institute, CNR-NANOTEC, Monteroni street - 73100 Lecce, Italy;
[b]Nanotechnology Institute, CNR-NANOTEC, c/o La Sapienza University, P. le A. Moro- 00185 Rome, Italy
[c]Department of Mathematics and Physics, University of Salento, Monteroni Street, 73100 Lecce, Italy
[d]IRCCS San Raffaele Scientific Institute, Neuropathology Unit, Division of Neuroscience, Institute of Experimental Neurology, Milan, 20132 Italy
[e]Department of Life Sciences, Health and Health Professions, Link Campus University del Casale di San Pio V street, 44 , I-00165 Rome, Italy
*[f]Department of Experimental Medicine, University of Salento, c/o Campus Ecotekne, Monteroni street, 73100 Lecce, Italy.**
Correspondence: romano.alessandro@hsr.it , ilaria.palama@nanotec.cnr.it

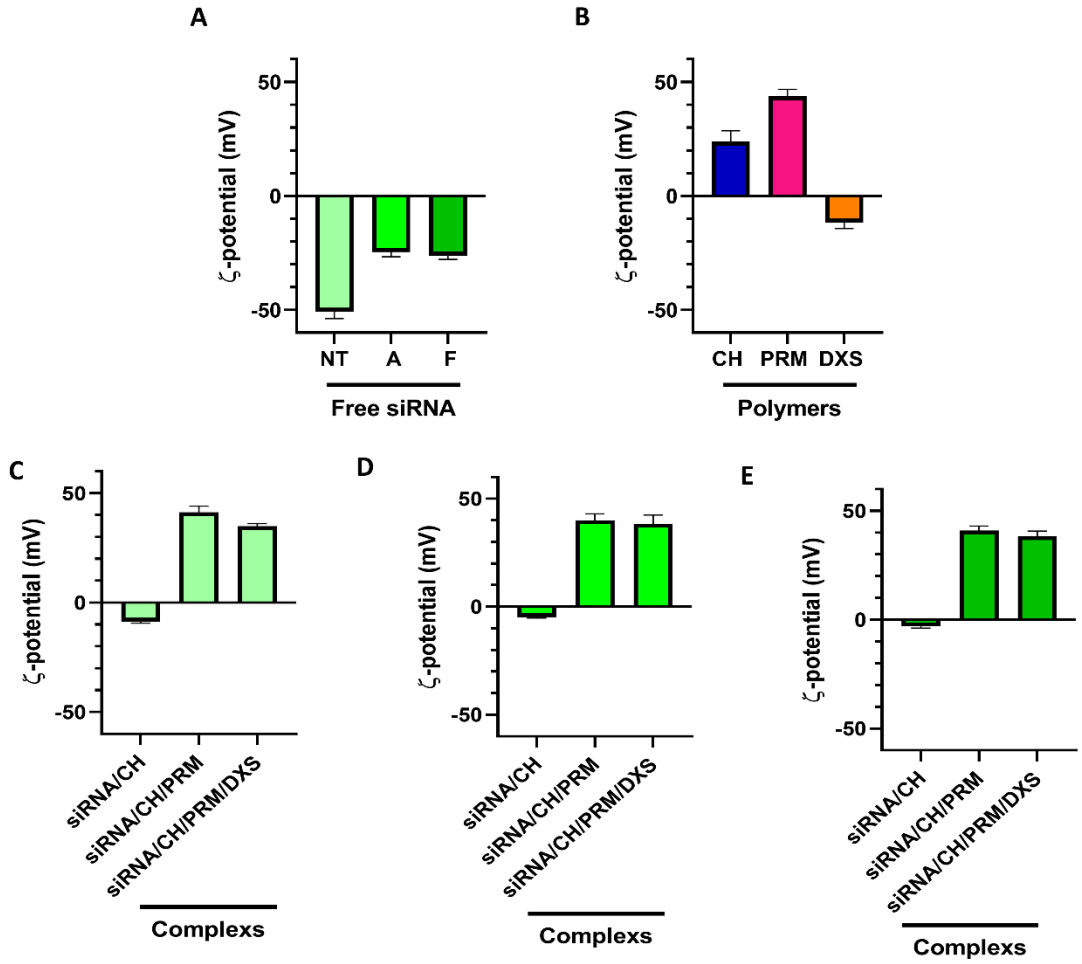

Figure S1. Histograms of ζ-potential measurements. (A) Free siRNA (NT, A, F); (B) polymers (chitosan, CH; protamine, PRM; and dextran, DXS); (C-E) complexs (siRNA/CH; siRNA/CH/PRM; and siRNA/CH/PRM/DXS) with siRNA variants. (C) siRNA-NT, (D) siRNA-A, and (E) siRNA-F.

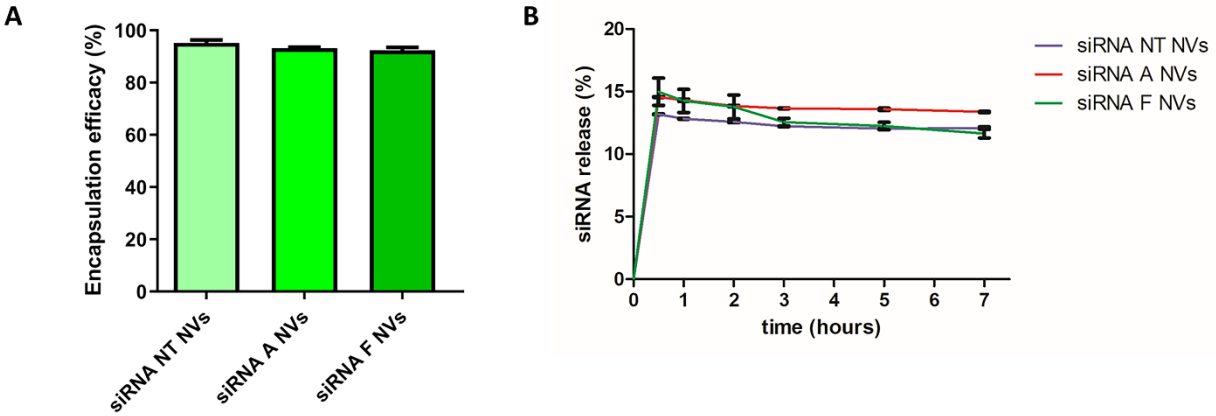

Figure S2. Encapsulation efficacy of different siRNA loaded NVs (A) and their release (%, B) when subject to acid conditions (pH 4.5) for a time window of up to 7 hours. Representative measurements of three distinct sets of data have been reported. Data are shown as mean ± SEMs and analyzed by one-way ANOVA (no statistical difference).

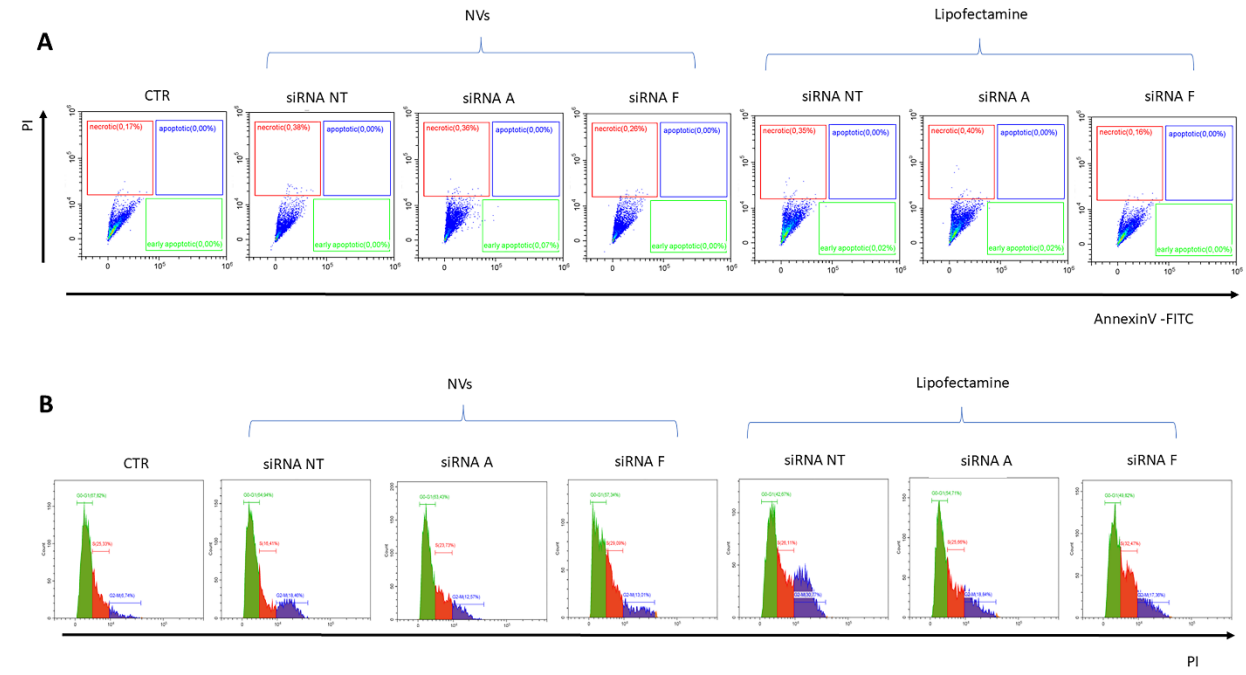

Figure S3. Analysis of cell apoptosis (A) and cell cycle (B) of neuroblastoma cells after treatments with siRNA-loaded NVs or delivered with lipofectamine.

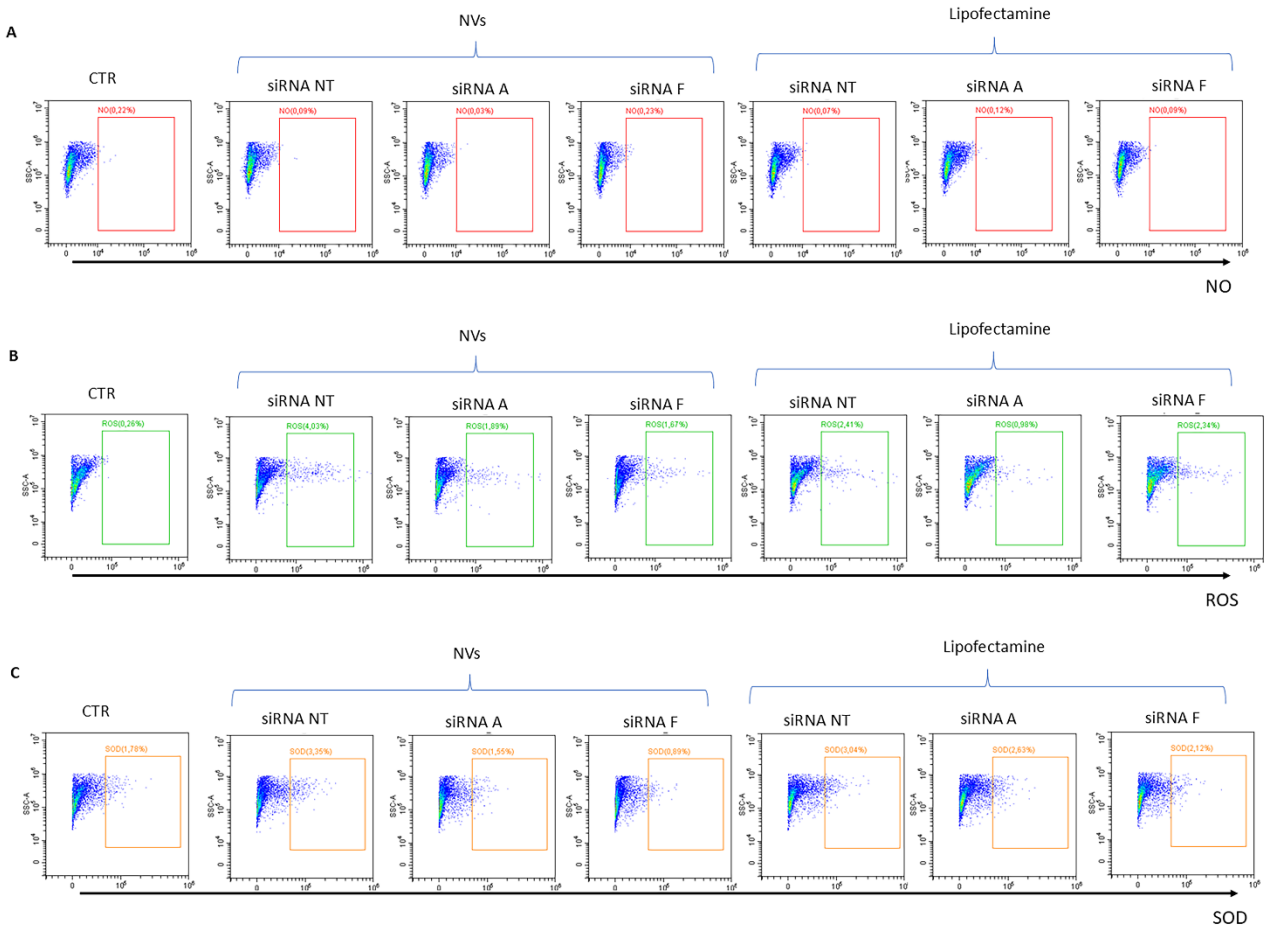

Figure S4. Cytofluorimetric analysis of the production of NO (A), ROS (B) and SOD activity inhibition (C) in neuroblastoma cells after treatment with siRNA-loaded NVs or delivered with lipofectamine.

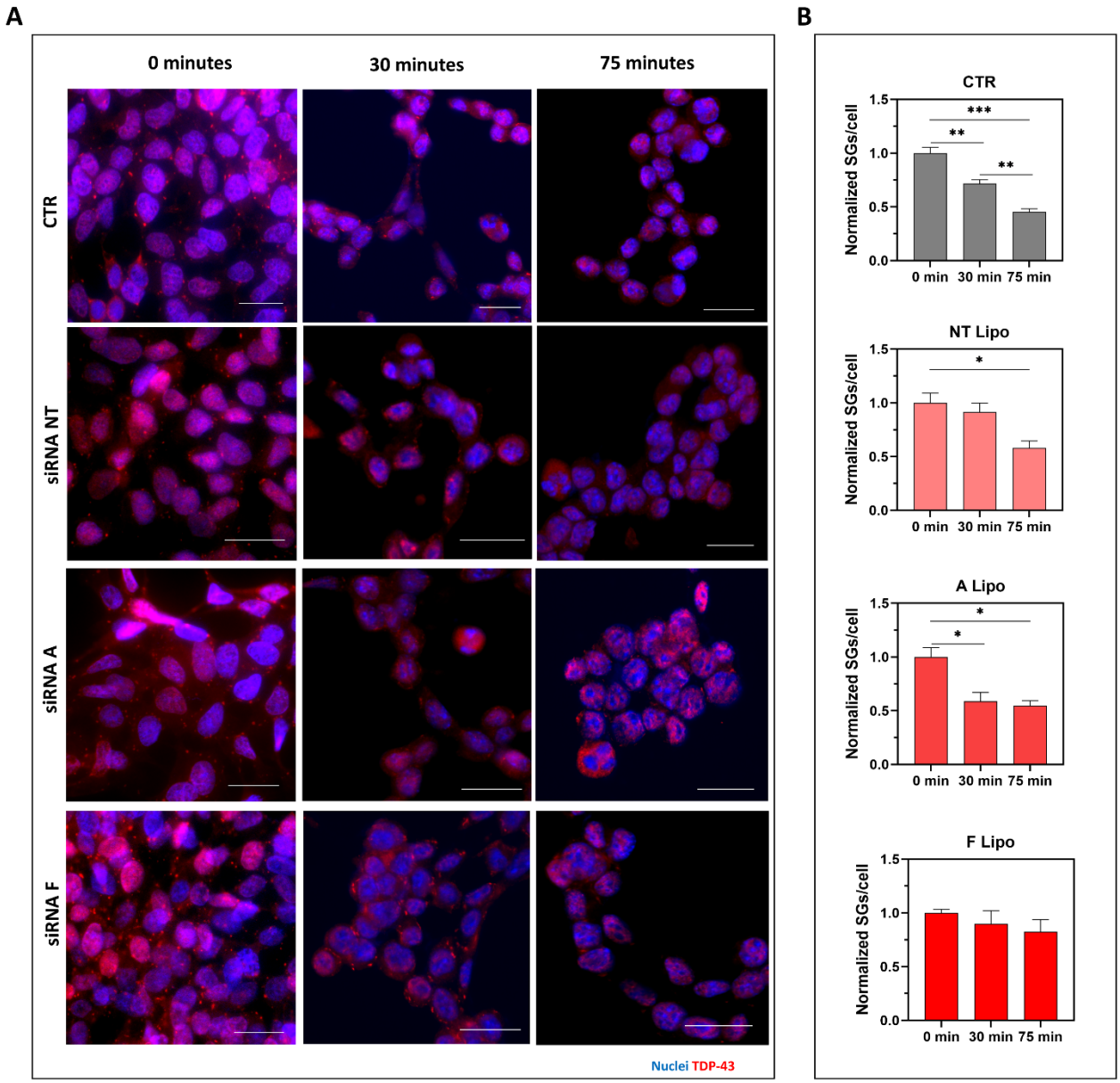

Figure S5. Fluorescent images of localization of SG in neuroblastoma cells (A) after treatments with siRNA-loaded lipofectamine for 48 hours and stressed with SA for 45 minutes and after recovery of 30 minutes or 75 minutes. Nuclei were stained with DAPI (blue), TDP-43 labeled in red. *Scale bars: 25 µm.* Number of TDP-43 positive SGs per cells (B), after treatment with siRNA NT (NT Lipo), siRNA A (A Lipo), or siRNA F (F Lipo) loaded lipofectamine for 48 hours and SA stressed for 45 minutes, untreated cells are used control (CTR). Data are shown as mean ± SEMs and analyzed by one-way ANOVA followed by Tukey's Multiple Comparison tests (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).