Manuscript Title: Plasma exosomes can deliver exogenous siRNA to monocytes and lymphocytes

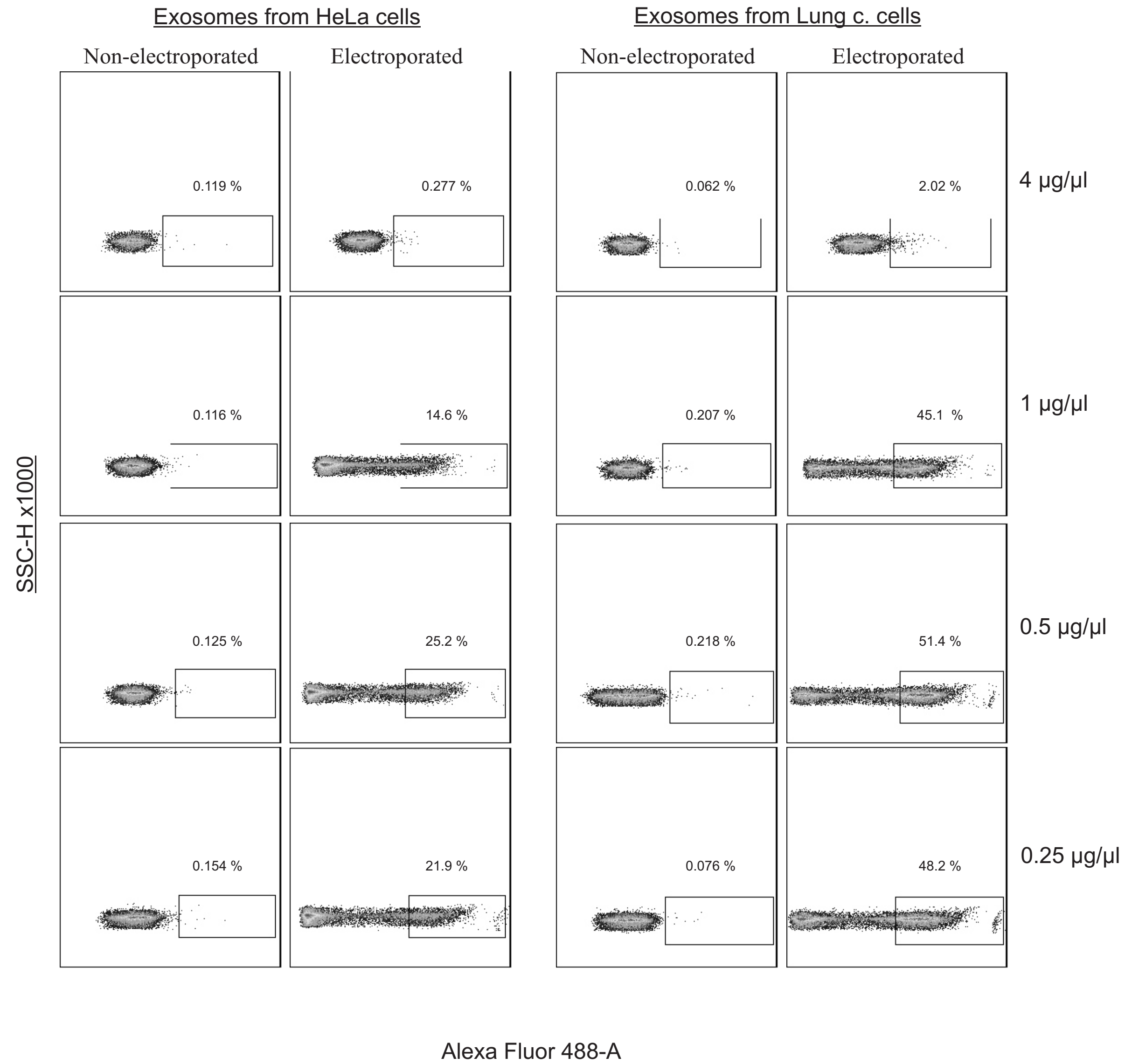
Authors: Jessica Wahlgren[1,3], Tanya De L. Karlson[1,3], Mikael Brisslert[1], Forugh Vaziri Sani[1], Esbjörn Telemo[1], Per Sunnerhagen[2], and Hadi Valadi[1§]

Supplementary Data

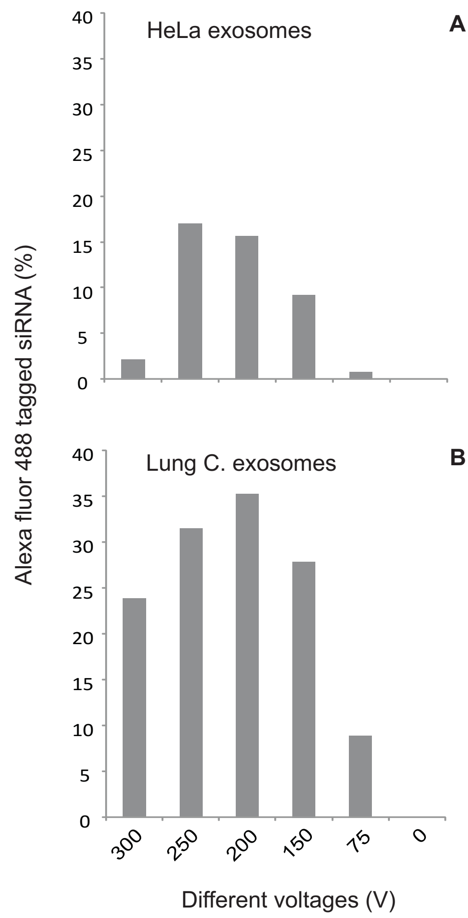
Supplementary Figure S1. Exosome concentration has an effect on electroporation efficiency. To determine the optimal exosome concentration, exosomes at four different concentrations (0.25–4 µg/µl) were mixed with Alexa Fluor 488-tagged siRNA (2 nmol/ml) and exposed to electroporation. Samples containing exosomes and siRNA but which were not exposed to the electric pulse were used as a negative control. The exosomes were purified using latex beads and the presence of siRNA in the exosomes was determined by FACS analysis.

Supplementary Figure S2. Different scenarios regarding the variation of voltages on electroporation efficiency.
The exosome vesicles were mixed with an Alexa Fluor 488-tagged siRNA against the transcript of MAPK1, incubated on ice for 10 min, and exposed to an electric field pulse. Samples containing exosomes and siRNA but which were not pulsed were used as a negative control. The influence of voltage (sample 1 = 300V and sample 5 = 75V) on the electroporation efficiency was monitored using FACS for the detection of the fluorescently-labeled siRNA. HeLa exosomes (**A**) and HTB-177 exosomes (**B**).

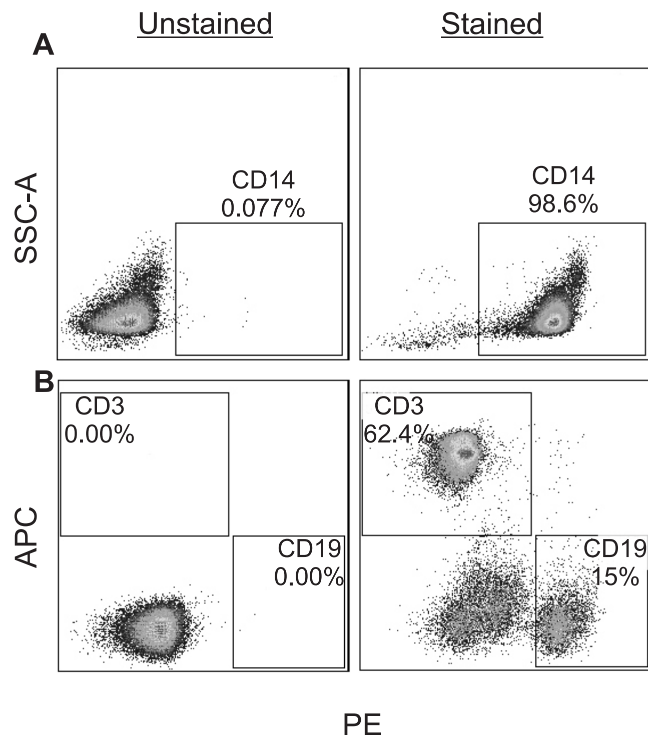
Supplementary Figure S3. Flow cytometry of recipient cells isolated from PBMCs using CD14 microbeads and LS column. The $CD14^+$ fraction *i.e.* the monocytes were stained with CD14-PE antibody or corresponding isotype yielding a purity of 98.6 % (A). The $CD14^-$ (CD14 minus) cells herein termed the lymphocytes were identified by staining with CD3-APC antibody, identifying t-cells as 62.4 % of the lymphocytes, and with CD19 antibody identifying the b-cells as 15 % of the lymphocytes Gates were set using fluorescence minus one (FMO) control (B).



Supplementary Figure S1 (Wahlgren J. *et al*)



Supplementary Figure S2 (Wahlgren J. *et a*l)



Supplementary Figure S3 (Wahlgren J. *et al*)