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Supplemental Information

Functional Delivery of Lipid-Conjugated siRNA by Extracellular Vesicles

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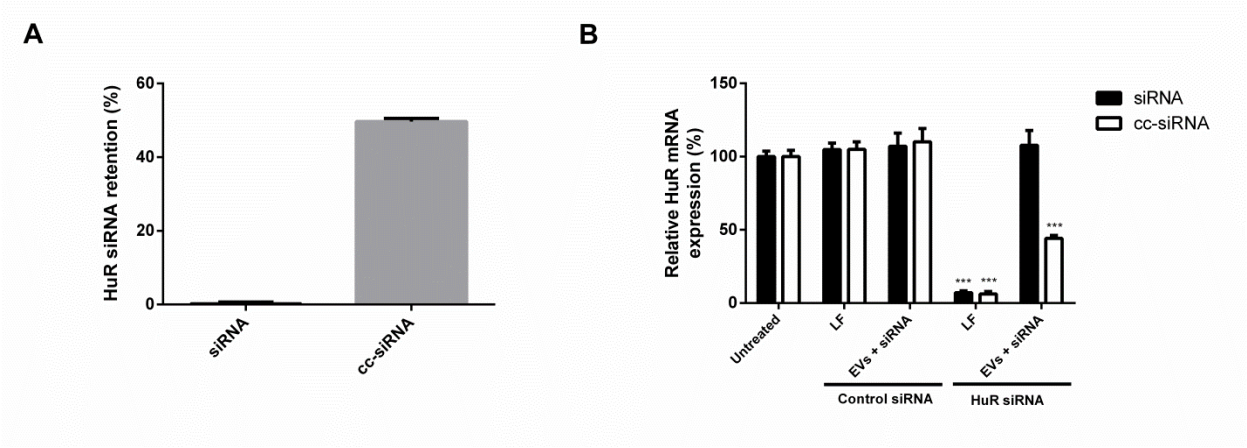

Figure S1. Cholesterol conjugation is critical for successful siRNA loading into EVs and subsequent HuR silencing in HEK cells. A) siRNA retention in pellet following mixing of EVs derived from Neuro2A cells with unconjugated siRNA or cc-siRNA. EVs were mixed with fluorescent siRNA at a ratio of 1:15 at 37 °C for 1 hour in 100 µl PBS. B) HEK293 cells were treated with cc-siRNA loaded Neuro2a EVs at a final concentration of 600 nM cc-siRNA or with an equal number of EVs loaded with unconjugated siRNA. EVs were loaded by incubating EVs with siRNA or cc-siRNA using a ratio of EVs:siRNA of 1:15 at 37 °C for 1 hour in 100 µl PBS. *HuR* expression relative to *GAPDH* and *ACTB* were measured 48 hours post-treatment with EVs loaded with a control or *HuR* cc-siRNA or transfection with control siRNA or *HuR* cc-siRNA. Values represent mean + SEM. n = 3. Statistical differences were calculated by one-way ANOVA followed by Tukey's post hoc analysis. Statistical differences indicated are compared with negative control. ***p<0.001, **p<0.01, *p<0.05.

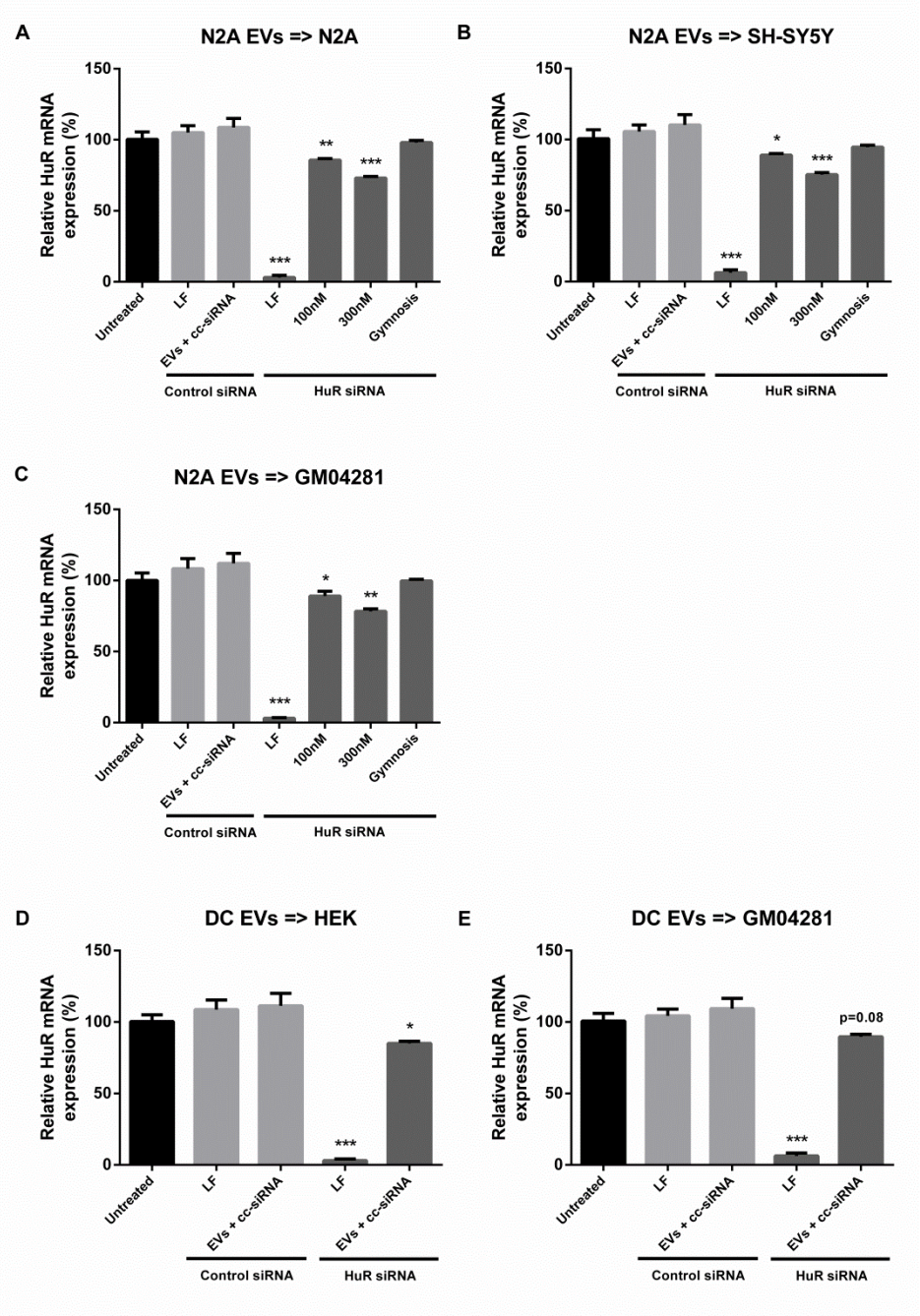

Figure S2. HuR silencing in various cell types treated with cholesterol conjugated siRNA-loaded extracellular vesicles at optimised conditions. A) Neuro2a (N2A) cells, SH-SY5Y cells, GM04281 cells, or HEK293 (HEK) cells were treated with cc-siRNA loaded Neuro2a EVs (A-C) at final concentrations of 100 and 300 nM cc-siRNA or with cc-siRNA loaded dendritic cell (DC) EVs (D,E) at a final concentration of 100 nM cc-siRNA. EVs were loaded by incubating EVs with cc-siRNA using a ratio of EVs:cc-siRNA of 1:15 at 37 °C for 1 hour in 100 µl. *HuR* expression relative to *GAPDH* and *ACTB* were measured 48 hours post-treatment with EVs loaded with a control or *HuR* cc-siRNA or transfection with control siRNA or *HuR* cc-siRNA. The effect of gymnosis was evaluated through the inclusion of cells treated with cc-siRNA in the absence of EVs or a transfection reagent. Values represent mean + SEM. n = 3. Statistical differences were calculated by one-way ANOVA followed by Tukey's post hoc analysis. Statistical differences indicated are compared with negative control. ***p<0.001, **p<0.01, *p<0.05.

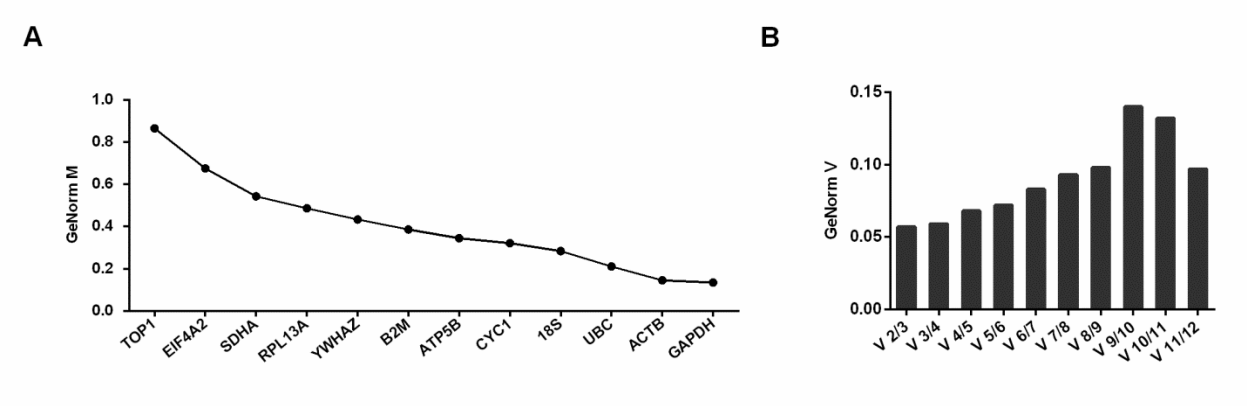

Figure S3: Expression stability (M) analysis and the optimal reference gene number determination with geNorm. The 12 gene geNorm Perfect Probe Plus Mouse and Human Kits (PrimerDesign) were used to identify the most stably expressed and therefore appropriate reference genes for analysis. Using this kit, the expression of 12 candidate reference genes identified from over 30,000 microarray experiments were compared in a representative set of experimental samples. For *in vitro* assessment for HEK293 cells for HTT silencing, samples included untreated cells (n=2), and cells treated with a mid-range dose of each of the therapeutic nucleic acids (n=2). The expression of each of the 12 candidate reference genes was assessed by qPCR using 25 ng of cDNA. **A)** Data was analysed using qbase+ software (Biogazelle) which ranked the candidate reference genes by their gene expression stability (geNorm M). In this study the optimal reference genes, those with highest stability (lowest geNorm M), were GAPDH and ACTB. **B)** Pairwise variation was used to calculate the optimal number of reference genes for normalisation (GeNorm V). All V (n/n+1) values, including V2/3, were below 0.15 in each pool, which indicated that combination of the geometric mean of the two genes was optimal for normalization.