Supporting Information

Self-assembly into nanoparticles is essential for receptor mediated uptake of therapeutic antisense oligonucleotides

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Figure S1.

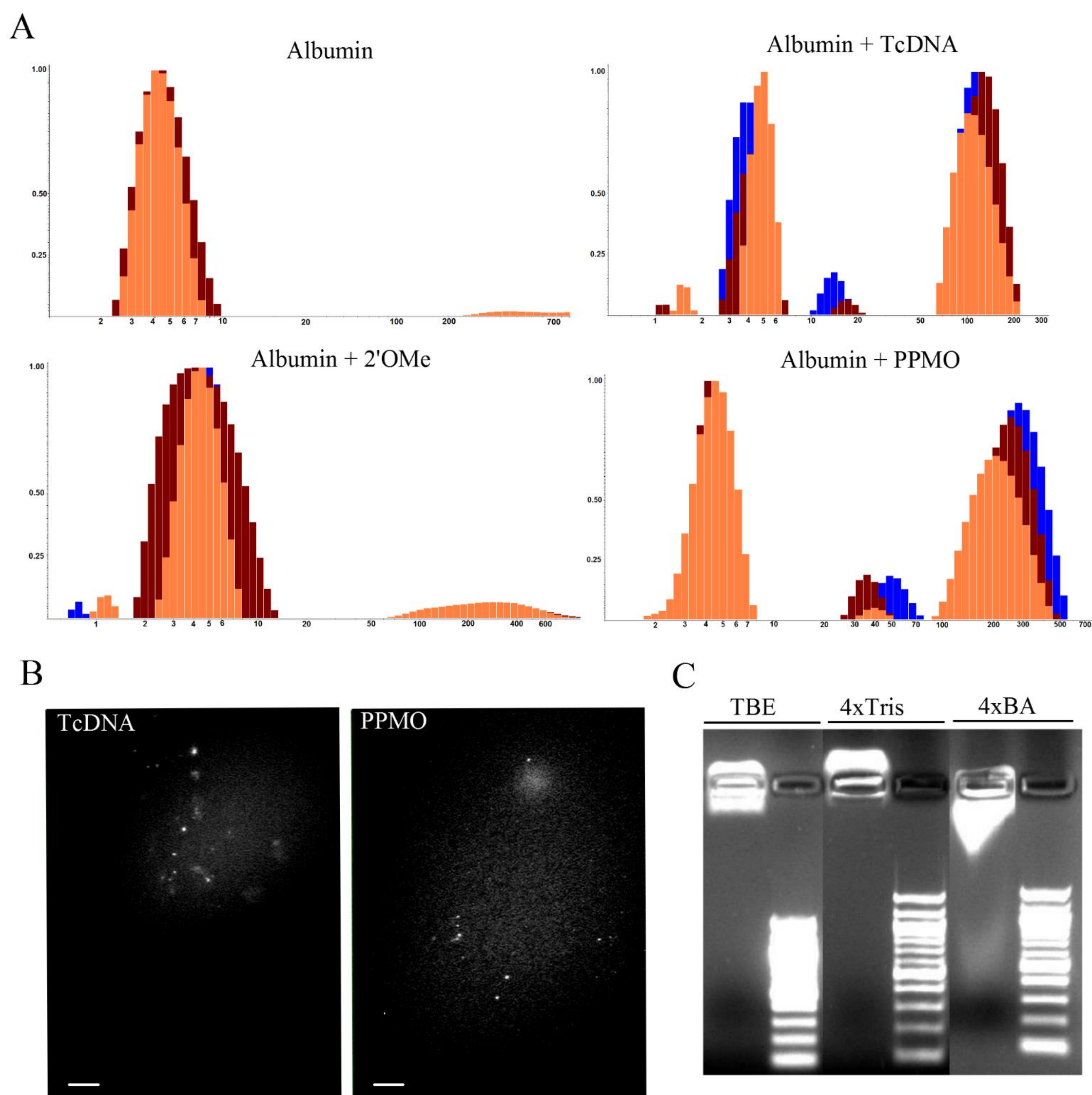

Figure S1. (**A**) DLS analysis of tcDNA, 2'OMe and PPMO in the presence of physiological concentrations of albumin (BSA). Each was measured 3 times (different colors) at final concentration of 500 µM for tcDNA and 2'OMe and 50 µM for PPMO after incubation with albumin solution in PBS at 37 ºC for 1h (**B**) Nanoparticles formed by FITC-labelled tcDNA and PPMO after incubation with full serum for 1h at 37° C and separation via a continuous sucrose gradient visualized by fluorescence microscopy, scale bar = 5 µm. (**C**) PPMO at the concentration of 50 µM was loaded on a 1.25% agarose gel with ethidium bromide and run in 3 different conditions, TBE, TBE with 4x Tris and TBE with 4x boric acid (BA).

Figure S2.

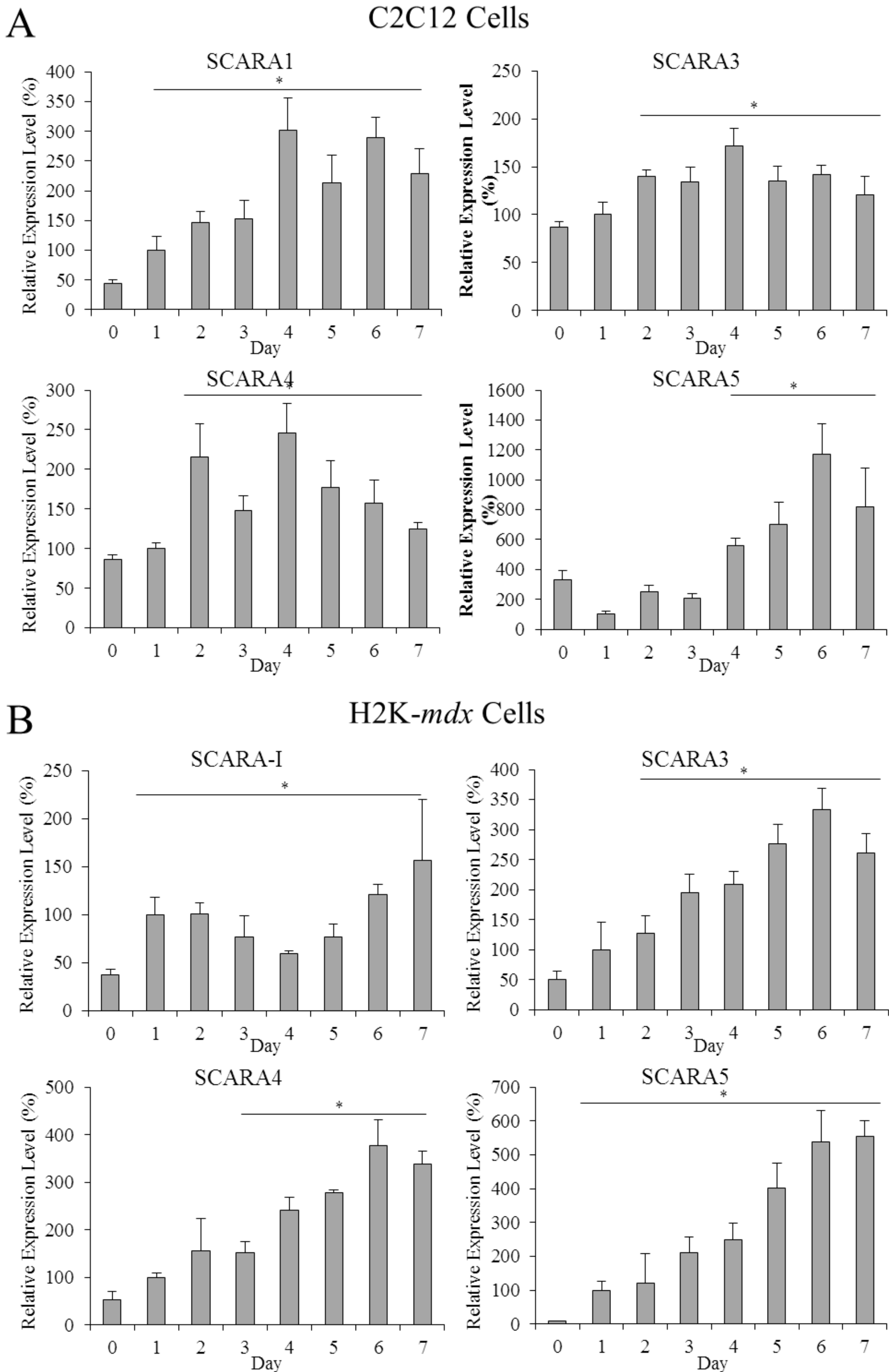

Figure S2. qPCR analysis of SCARAs in (**A**) C2C12 and (**B**) H2k *mdx* myoblast or myotube cells. Day 1 levels were set to 100% expression and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control to determine relative RNA expression levels throughout samples, n=3. *$P < 0.05$; one-way ANOVA, error bars represent mean ± SEM.

Figure S3.

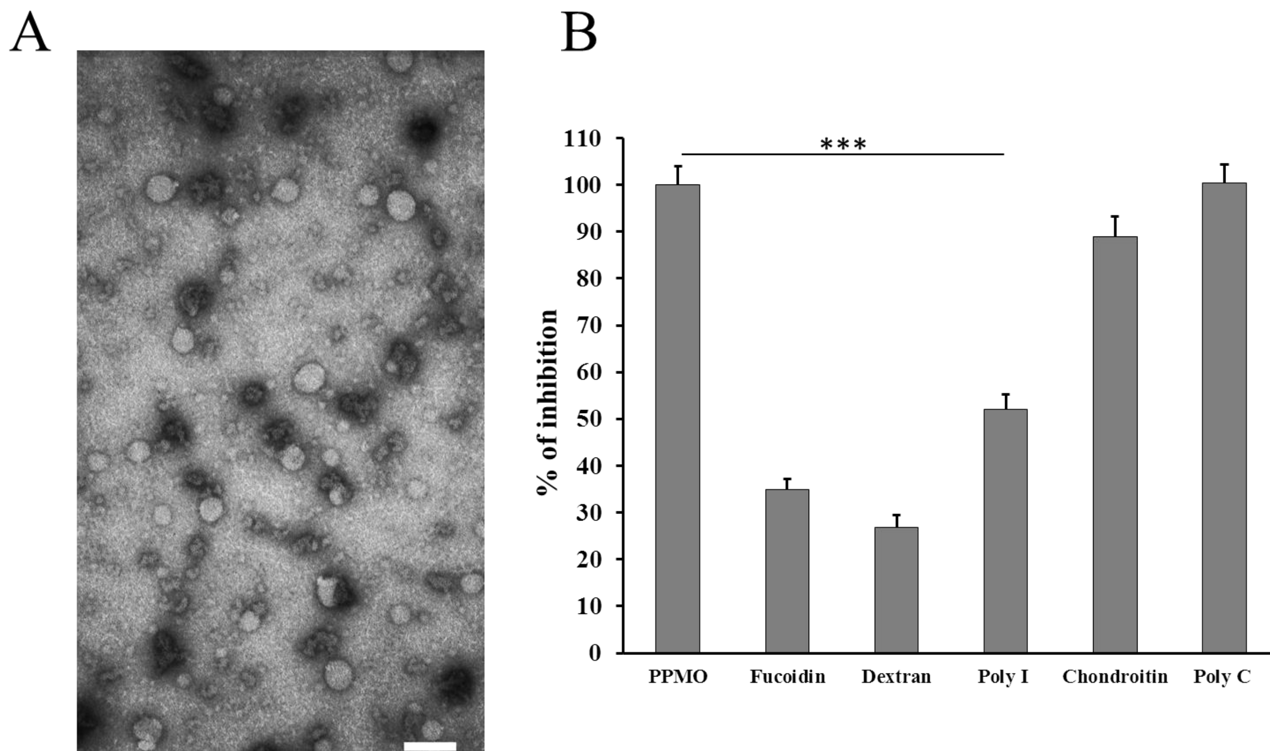

Figure S3. (**A**) Negatively stained TEM pictures of nanoparticles formed by Pip6a-PMO; Bar = 100 nm. (**B**) Analysis of Pip6a-PMO uptake in the presence of SR ligands. Differentiated C2C12 cells were pretreated with ligands or controls (5 μg/ml for fucoidin, dextran and chondroitin sulfate, 25 μg/ml for poly I and poly C) for 1 h and subsequently incubated for 4 h with fluorescein labelled Pip6a-PMO at 250 nm, then the cells were washed and internalization was assessed by fluorescence spectrophotometry. *** $P< 0.001$; Student's t-test, error bars represent mean ± SEM.

Figure S4.

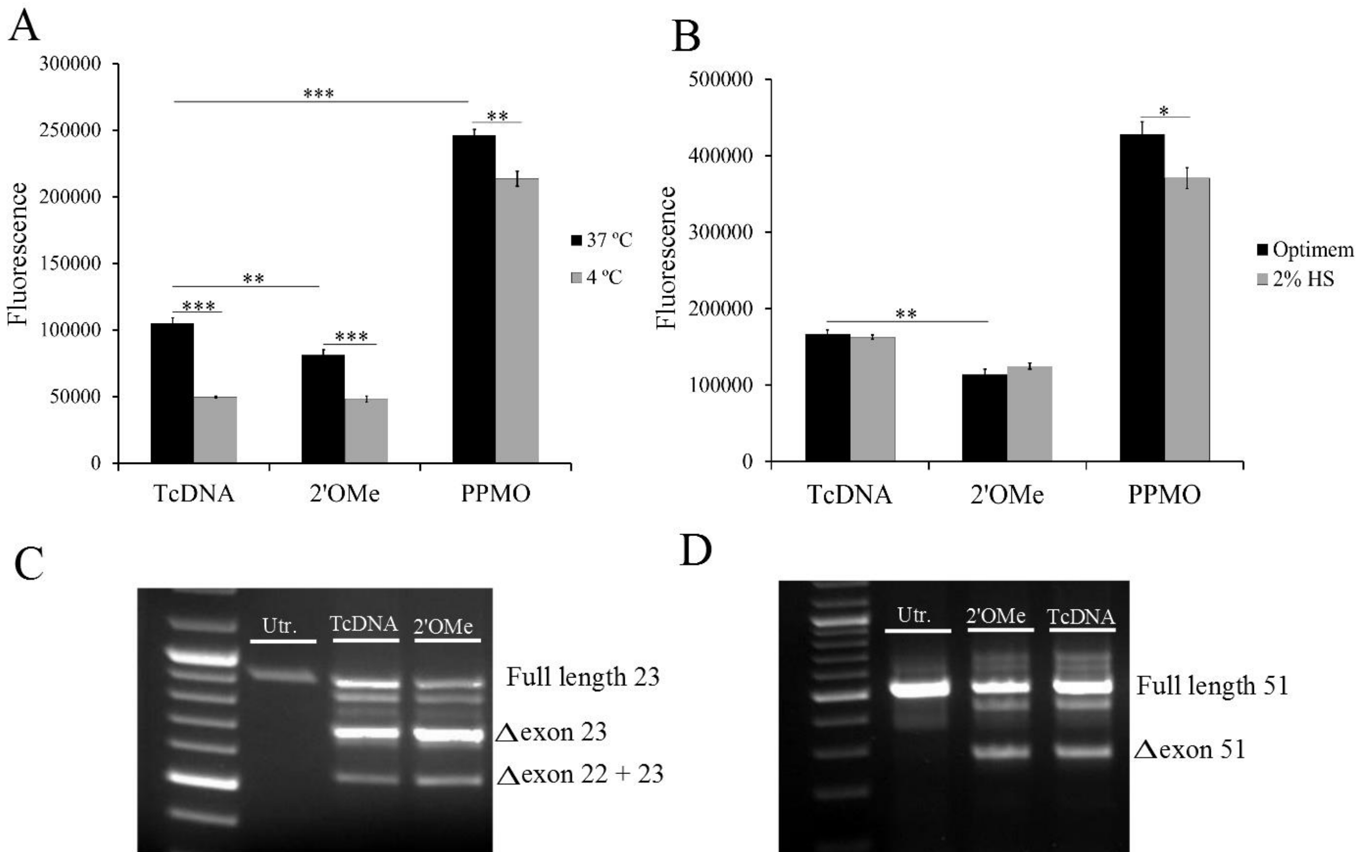

Figure S4. Differentiated C2C12 cells ($10x10^3/cm^2$) were treated with FITC-labelled ASOs at 2μM for 24h at (**A**) 37 ºC compared to 4 ºC , or (**B**) in Opti-MEM® compared to differentiation medium with 2% horse serum (2% HS), then the total fluorescence was quantified in each well using spectrophotometry . $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Student's t-test; error bars represent mean ± SEM. *Mdx* primary myoblasts seeded in 6 well-plate treated with (**C**) tcDNA or 2'OMe targeting exon 23 (final concentration 1.17 μM) or (**D**) tcDNA or 2'OMe targeting exon 51 (final concentration 265 nM) using lipofectamine 2000 reagent according to manufacturer's protocol. Sequences for the exon 51 skipping ASOs are 5'-UCAAGGAAGAUGGCAUUUCU-3' for 2'OMe, and 5'-TCAAGGAAGATGGCATTTCT-3' for tcDNA. The products of nested reverse transcription-PCR (RT-PCR) were examined by electrophoresis on a 2% agarose gel 48 h after transfection.