Supporting Information

Gene Regulation using Nanodiscs Modified with HIF-1-α Antisense Oligonucleotides

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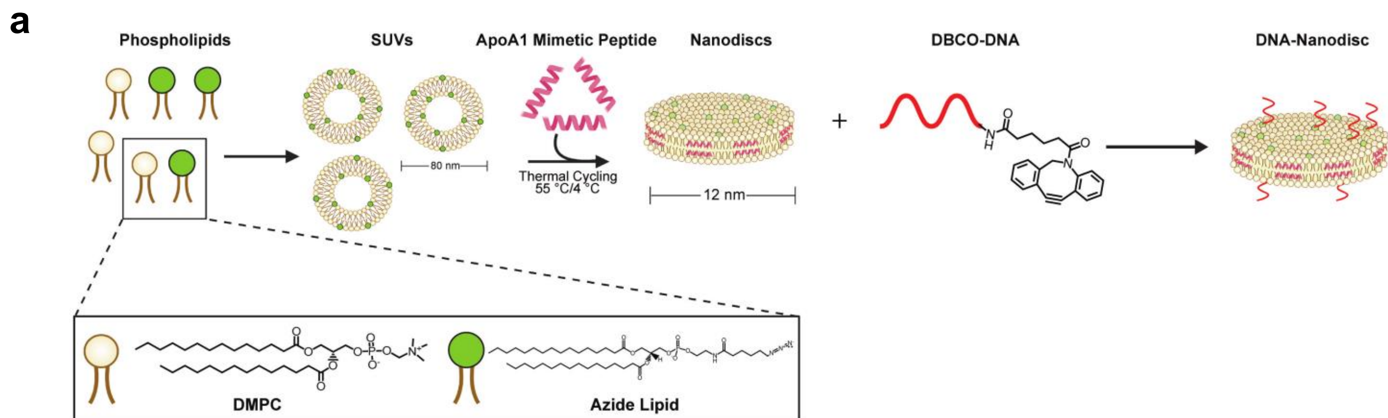
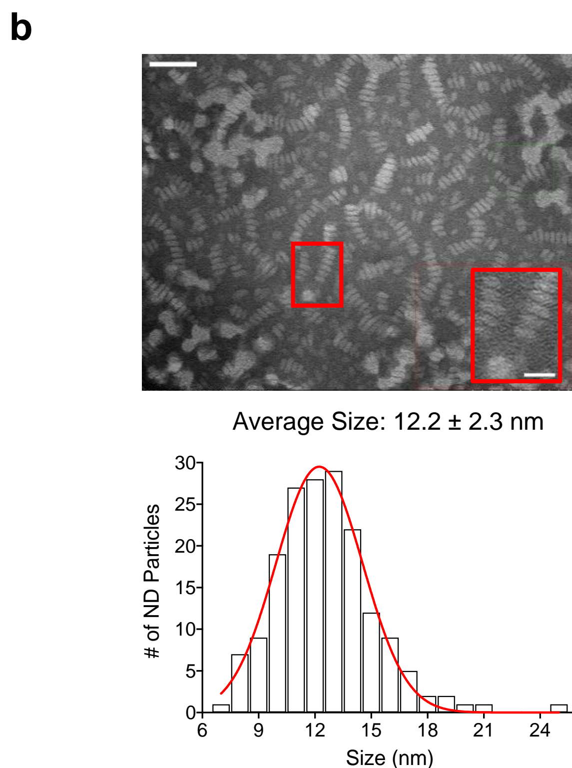




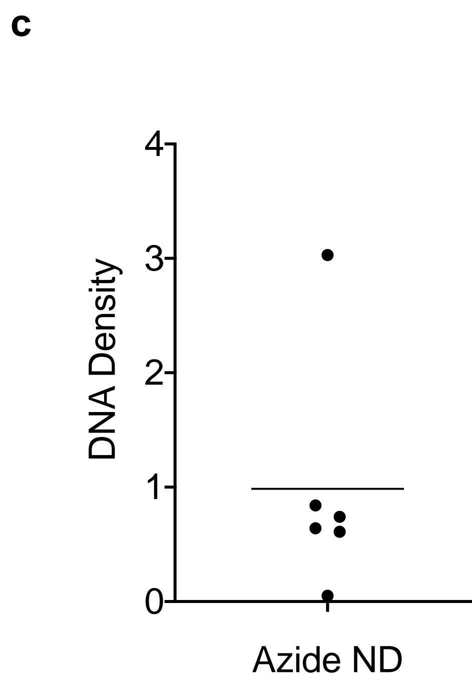


Figure S1: DNA density using the click chemistry approach to assemble NDs. **(a)** Scheme depicting that azide-NDs were prepared from SUVs comprised of 10% DPPE-Azide phospholipid (Avanti Polar Lipids) and 90% DMPC. DBCO DNA (modification obtained from Integrated DNA Technologies) was conjugated to the ND using copper-free click chemistry in KPi buffer. **(b)** TEM image (top) and binned sized analysis of nanodiscs (bottom) of n = 170 NDs revealing that NDs prepared with DPPE-azide yield an average size of 12.2 nm. Samples were prepared using a plasmon-etched 400-mesh copper grid and stained was performed using Nano-W™. Scale bar: 50 nm, inset: 30 nm. **(c)** Plot showing the low DNA density as measured using OliGreen™. This approach results in an average DNA density of < 1 DNA/ND suggesting this attachment chemistry was not favorable for dense DNA conjugation onto the ND surface.

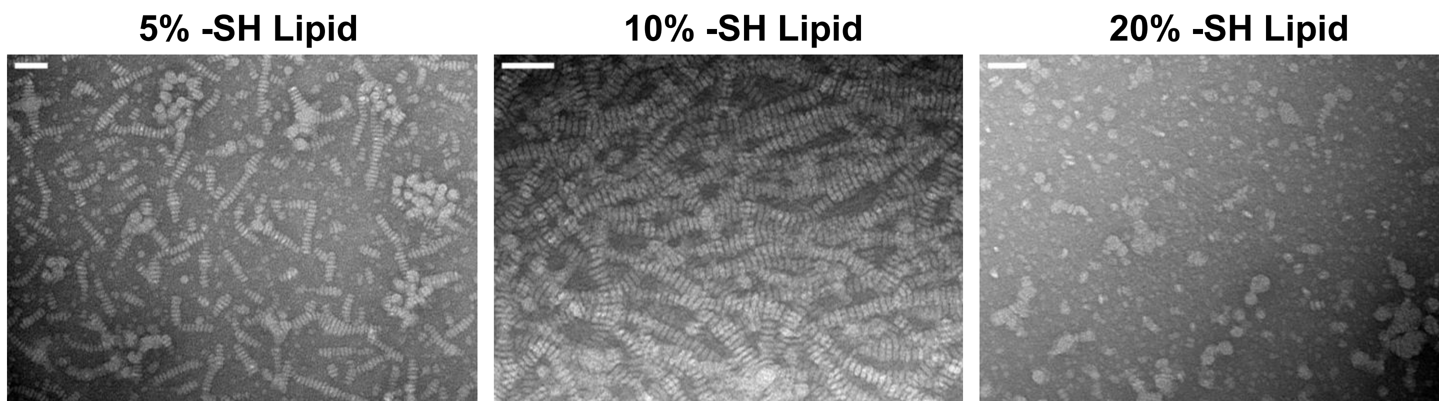


Figure S2: TEM panel of NDs incorporated with different ratios of thiol lipids. Thiol NDs incorporated with 5% and 10% thiol lipids formed monodisperse homogeneous populations, whereas the 20% thiol lipids were heterogeneous and aggregated, suggesting that a doping ratio of 10% represents the highest tolerated concentration that still assembles as a ND. Samples were prepared using a plasmon-etched 400-mesh copper grid and stained was performed using Nano-W™. Scale bar: 50 nm.

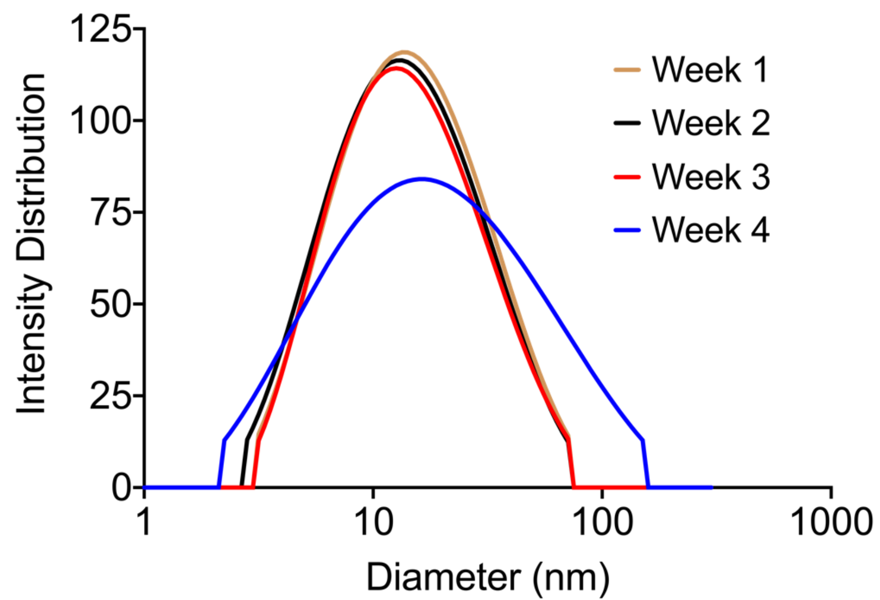


Figure S3: DLS stability data of 10% thiol DNA-NDs. DNA-NDs were stored at 4 °C and DLS measurements were taken each week. This graph suggests that DNA-NDs are stable for up to 3 weeks.

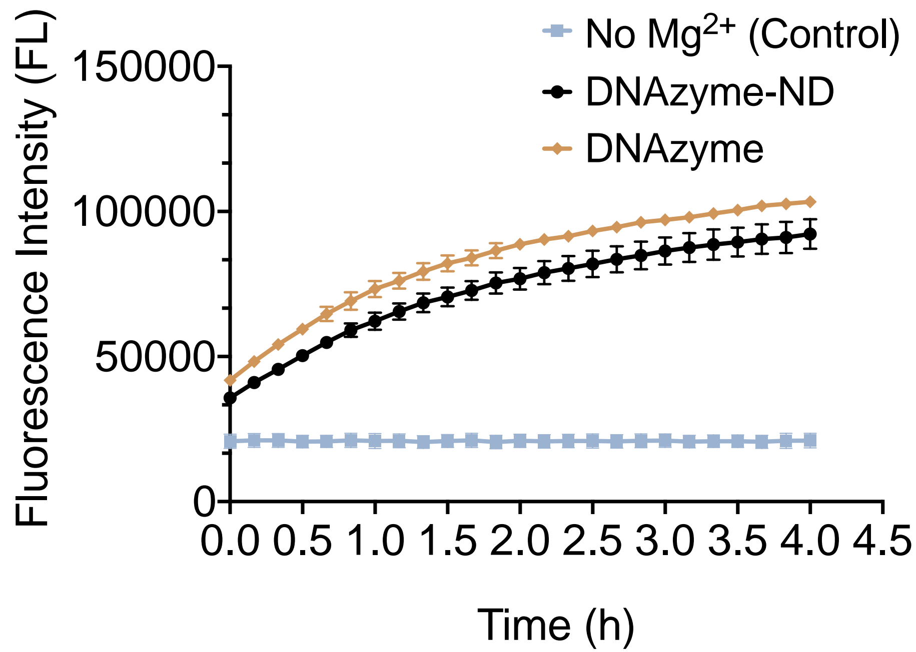


Figure S4: Catalytic activity of the DNAzyme-NDs. The mock RNA substrate used in this fluorescence kinetics assay was labeled with a FAM fluorophore on one terminus and a quencher on the opposite terminus. Graph shows a time-dependent increase in fluorescence as a result of cleavage of the mock RNA substrate by the soluble DNAzyme or the DNAzyme conjugated to the ND surface. DNAzyme incubated with the substrate in a magnesium-free buffer was added as a control. This plot shows that the DNA-ND is functional and active. Samples represent SEM of n = 3 independent replicates.

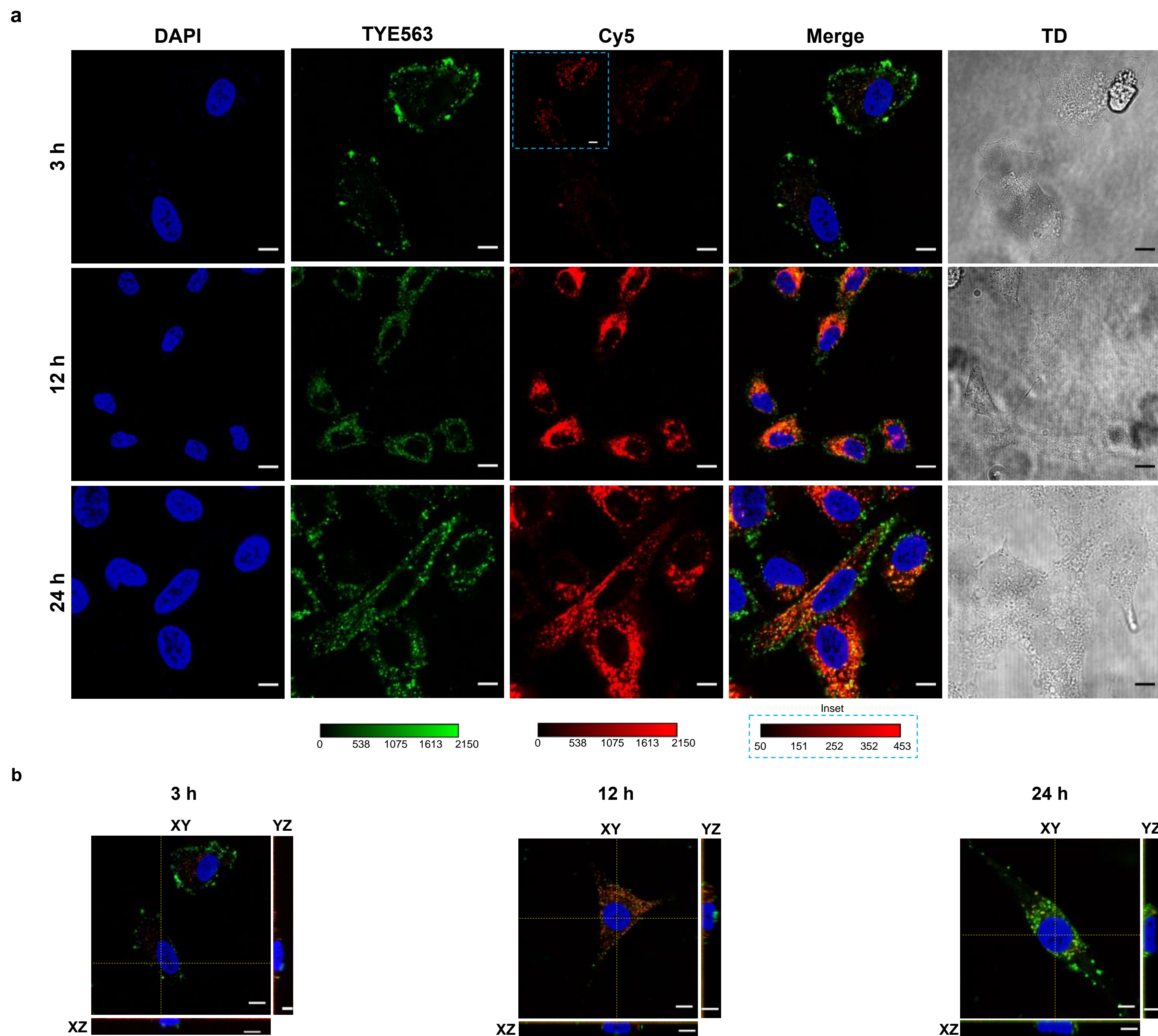


Figure S5: Confocal microscopy showing the time course for internalization of ASO-NDs into HeLa cells. **(a)** Panel shows representative images of cells treated with ASO-ND at 3 h (top), 12 h (middle), and 24 h (bottom). ASO-NDs initially localize near the cell membrane, and then at later time points there is increased localization in the cytoplasm. **(b)** Confocal Z-stack scans performed to determine the localization of ASO-ND conjugates internalized in HeLa cells at different 3 h, 12 h, and 24 h timepoints. Panel shows representative images taken at the middle layer of the cell. For each timepoint there is a planar and orthogonal projection, as derived from the indicated yellow line on XY field. We do not detect nuclear localization in these experiments. ASO was labeled with a TYE dye in the Cy3 channel and NDs were prepared using a Cy5 phospholipid. The DNA concentration was maintained at 100 nM in all groups. Cells were fixed and the nuclei were stained with DAPI (blue) prior to imaging. Scale bar: 5 µm.

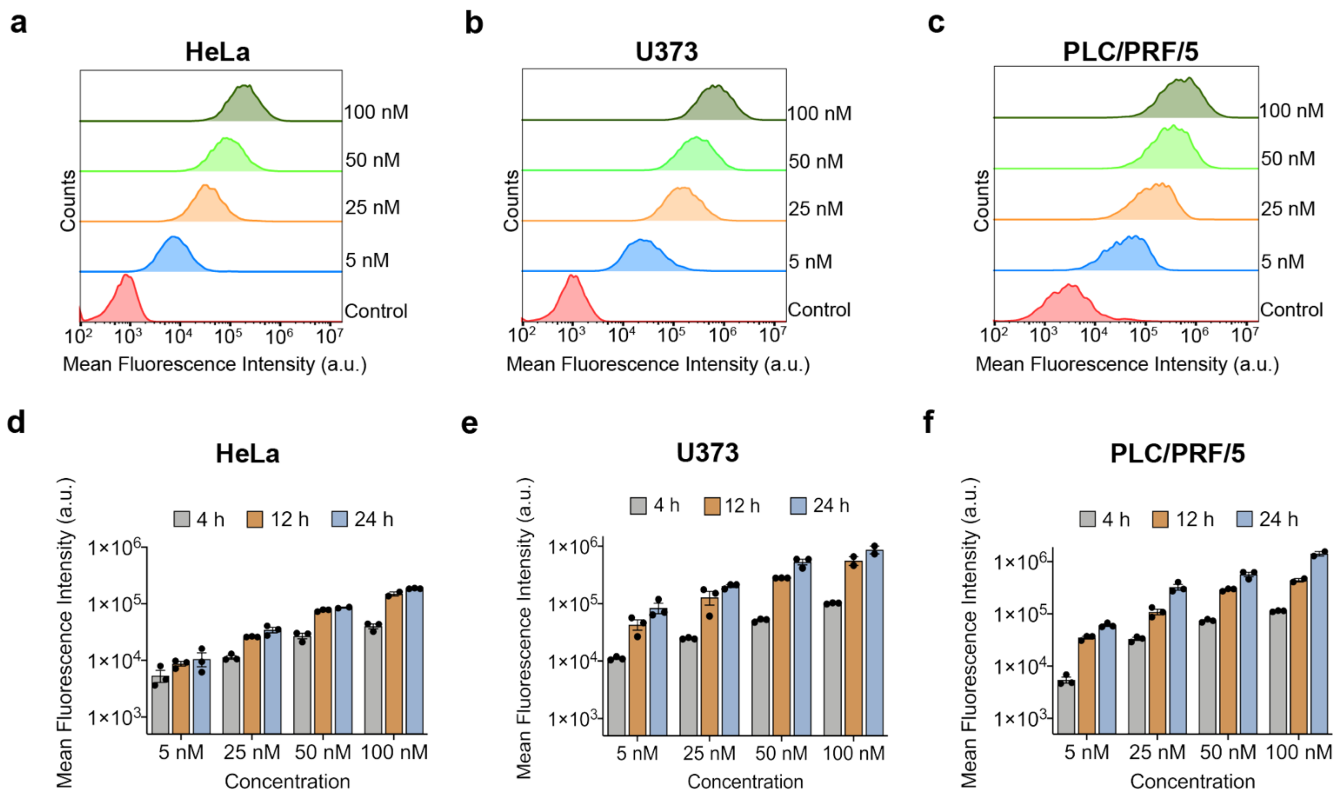


Figure S6: Quantification of ND uptake into HeLa, U373, and PLC/PRF/5 cells. Representative flow cytometry histograms of cells treated with ND tagged with 0.1% Cy5-lipids for 12 h for **(a)** HeLa, **(b)** U373, and **(c)** PLC/PRF/5 cells. Flow data represent Cy5 intensities for a minimum of 5000 cells. (**d**)-(**f**) show plots of the mean fluorescence intensity for cells treated with NDs as a function of time and ND concentration. There is an increase in uptake of the ND as a function of time at 4 h, 12 h, and 24 h for the three cell lines tested. HeLa cells generally showed the least ND uptake within this cohort of cells. Each data point represents one independent replicate. Error bars represent SEM of $n = 3$.

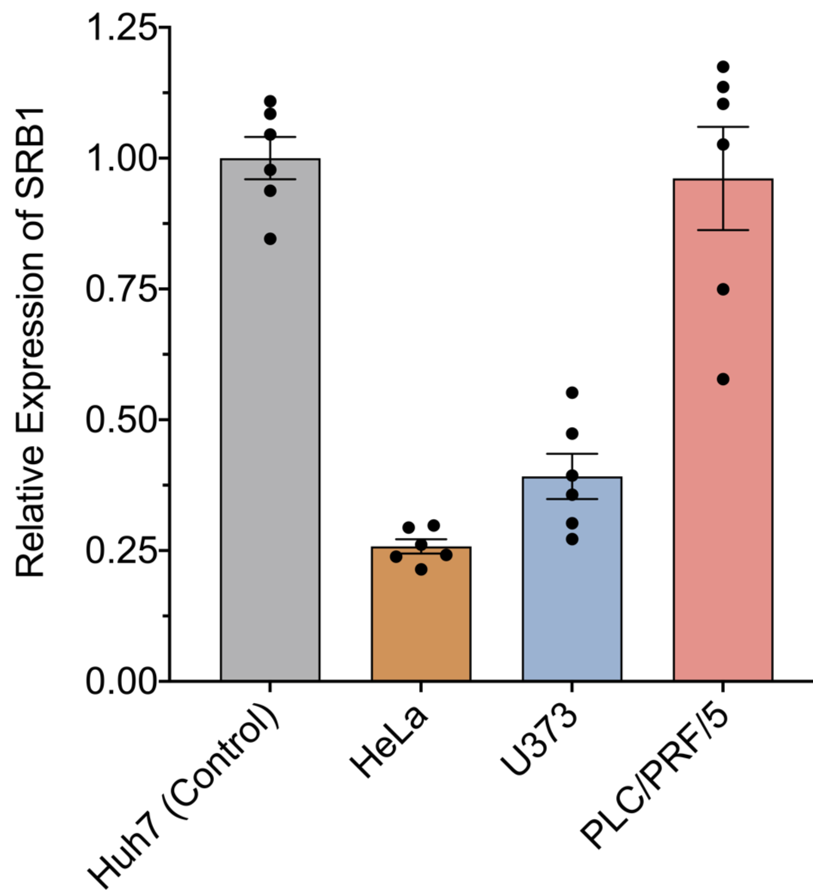


Figure S7: RT-qPCR experiment confirming the expression of SRB1 in HeLa, U373, and PLC/PRF/5 cells. We used 18S as the housekeeping gene. SRB1 expression is compared against wildtype Huh7 cells as a control because it is known to express high levels of SRB1. High SRB1 mRNA transcript levels were detected in PLC/PRF/5 cells, followed by U373 cells, followed by HeLa cells showing the lowest amount of expression. Each data point represents one independent replicate. Error bars represent SEM for $n = 6$ independent replicates.

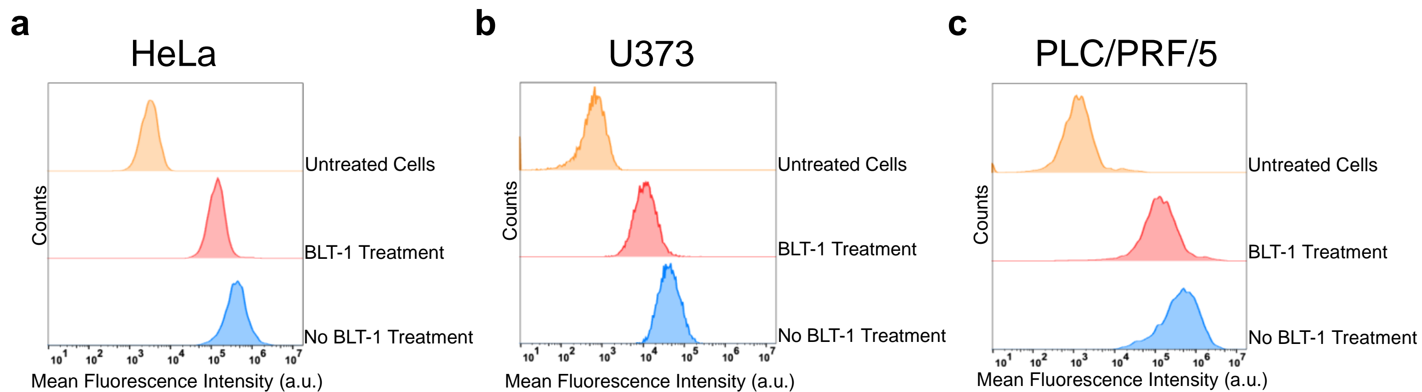
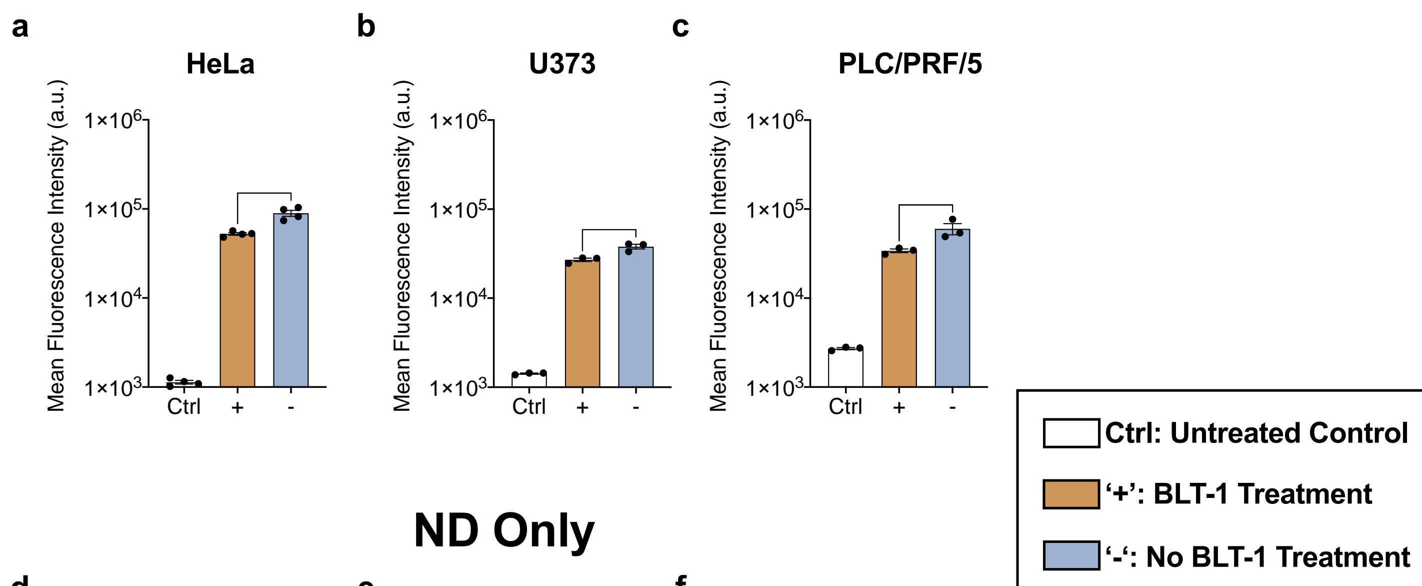


Figure S8: Testing the role of SRB1 in mediating ND uptake. Representative flow cytometry histograms measuring Cy5 intensity for cells pretreated with 50 µM BLT-1 for 1 h, and then incubated with the ND for 2 h for **(a)** HeLa, **(b)** U373, and **(c)** PLC/PRF/5 cells. The flow data reports mean intensities from a minimum of 5000 cells. Reduced Cy5 intensity for HeLa, U373, and PLC/PRF/5 cells after BLT-1 treatment indicates that SRB1 partially mediates the uptake of NDs into cells.



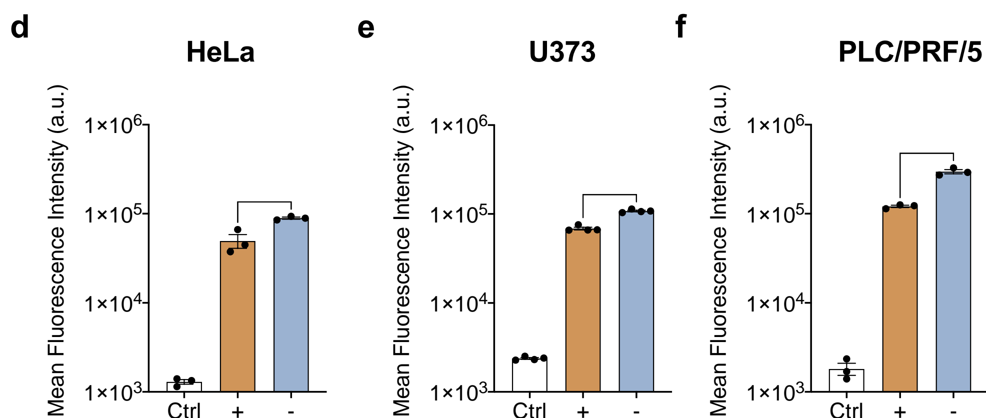


Figure S9: Quantitative Cy5 mean fluorescence intensity plots of the HeLa, U373, and PLC/PRF/5 cells treated with BLT-1. The indicated cell lines were pre-treated with 50 µm BLT-1 for 1 h prior to adding ASO-ND or ND for 2 h. The flow data reports mean intensities from 5000 cells. Bar graphs are comparing the uptake of ASO-ND **(a)** – **(c)** or ND **(d)** – **(f)** into cells after BLT-1 treatment. The raw data is a summary and representative flow cytometry histograms are shown in **Figure 5** and **Figure S8**. Each data point represents one independent replicate. Error bars represent SEM of a minimum of n = 3 and * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

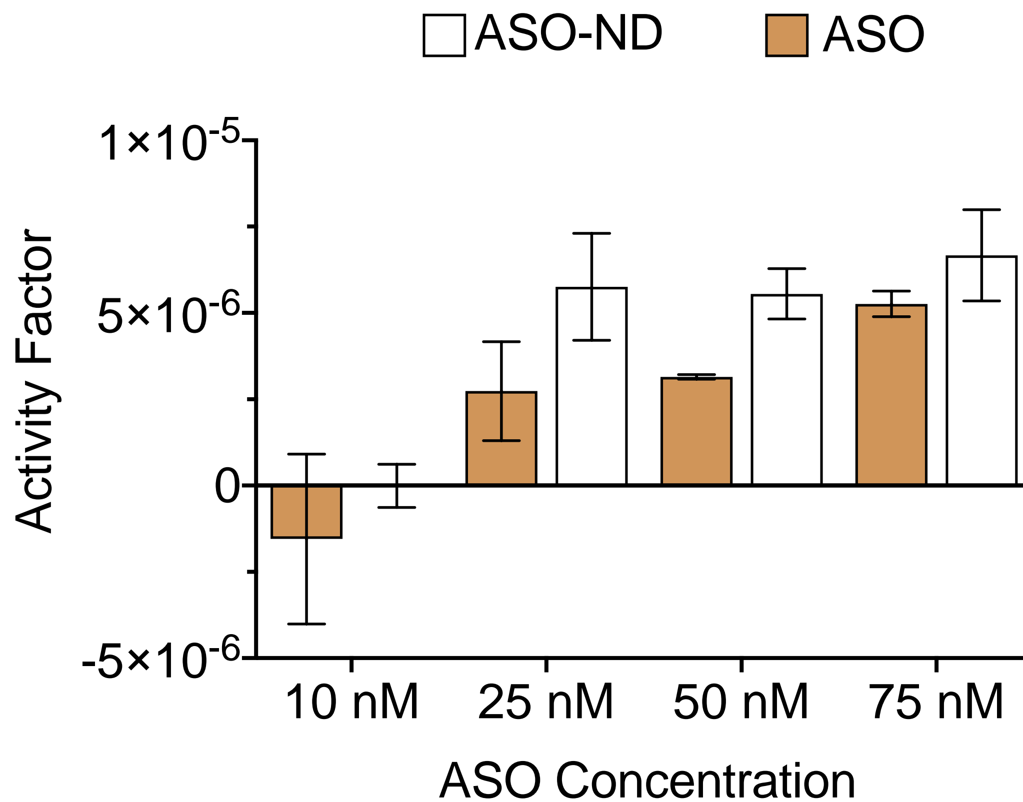


Figure S10: Plot of the activity factor comparing the activity of ASO-ND vs. naked ASOs on a per molecule basis. The ASOs and ASO-NDs were introduced to HeLa cells for 24 h without the use of a transfection reagent. Activity factor is determined by dividing the knockdown efficiency of the ASO-ND or ASO (**Figure 6c**) with the uptake value as determined by flow cytometry (**Figure 6b**). ASO-ND conjugates display enhanced activity compared to soluble HIF-1- α ASO. Error bars represent the SEM from n=3 replicates.

Table S1: Lipids used for preparing thiol-modified NDs.

Lipid	Catalog Number	Structure
DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine)	850345	
Thiol lipid (1,2-dipalmitoyl-sn-glycero-3-phosphothioethanol)	870160	
Cy5 lipid [1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(cyanine 5)]	810335	

Table S2: Oligonucleotide and primer sequences used for *in vitro* studies**.** Sequences consist of the catalytic DNAzyme, the HIF-1-α ASOs, scrambled ASO sequences, and primers used for RT-qPCR.

"+" = LNA Modification "*" = PS Modification "3AmMo" = 3' Amino Modifier "5TYE563" = 5' TYE™ 563 Fluorophore

"rX" = RNA base "56-FAM" = 5' 6-Fluorescein fluorophore "3IABkFQ" = 3' Iowa Black® Hole FQ Quencher

ID	Sequence (5' → 3')
Catalytic Dz	ATT CCT TAA AGG CTA GCT ACA ACG ATT CTT GGC TTT
Catalytic Dz-Amine	ATT CCT TAA AGG CTA GCT ACA ACG ATT CTT GGC TTT /3AmMO/
Catalytic Dz Substrate	/56-FAM/ GCC AAG AArG rUTT AAG GAA T /3IABkFQ/
HIF-1-α ASO	+T*+G*+G* C*A*A* G*C*A* T*C*C* +T*+G*+T* A
HIF-1-α ASO-Amine	+T*+G*+G* C*A*A* G*C*A* T*C*C* +T*+G*+T* A /3AmMO/
TYE563-HIF-1-α ASO-Amine	/5TYE563/ T*G*G* C*A* A* G*C*A* T*C*C* T*G*T* A /3AmMO/
Scrambled HIF-1-α ASO-Amine	+C*+G*+T* C*A*G* T*A*T* G*C*G* +A*+A*+T* C /3AmMO/
SRB1 Forward Primer	TCC TCA CTT CCT CAA CGC TG
SRB1 Reverse Primer	TCC CAG TTT GTC CAA TGC C
HIF-1-α Forward Primer	TAT GAG CCA GAA GAA CTT TTA GGC
HIF-1-α Reverse Primer	CAC CTC TTT TGG CAA GCA TCC TG
18S Forward Primer	AGG AAT TGA CGG AAG GGC ACC A
18S Reverse Primer	GTG CAG CCC CGG ACA TCT AAG

Table S3: ApoA1 mimetic peptide sequence.

Peptide ID	Sequence (N → C)
ApoA1 Mimetic	PVLDLFRELLNELLEALKQKLK