Intracellular mRNA Regulation with Self-Assembled Locked Nucleic Acid-Polymer Nanoparticles

Anthony M. Rush,¹ Dave A. Nelles,³,⁵ Angela P. Blum,¹ Sarah A. Barnhill,¹ Erick T. Tatro,⁴ Gene W. Yeo²,³ and Nathan C. Gianneschi,*¹,⁵

¹Department of Chemistry & Biochemistry, ²Stem Cell Program and Institute for Genomic Medicine, ³Department of Cellular and Molecular Medicine, ⁴Department of Psychiatry, ⁵Materials Science and Engineering

University of California, San Diego, La Jolla, CA 92093-0303, United States
Supporting Information

Contents

S1. Materials and Methods

S2. Polymer Synthesis
   S2.1 Synthesis of monomer 1
   S2.2 Synthesis of chain transfer agent 2
   S2.3 Synthesis of polymer $1_{18}-2$

S3. LNA Synthesis
   S3.1 HPLC purification of oligonucleotides
   S3.2 MALDI-TOF MS of oligonucleotides

S4. LNA-Polymer Amphiphile Synthesis and Micellar Nanoparticle Formation
   S4.1 LPA concentration determination
   S4.2 Transmission electron microscopy
   S4.3 Dynamic Light Scattering

S5. LPA Micelle Uptake Analyzed via Fluorescence-Activated Cell Sorting
   S5.1 Antisense versus nonsense LPA micelle uptake
   S5.2 LPA micelle versus ssLNA uptake
   S5.3 Cytotoxicity via propidium iodide assay
   S5.4 Pharmacological inhibition studies of LPA micelle uptake
   S5.5 LPA micelle uptake kinetics across multiple cell lines
   S5.6 LPA micelle uptake in human embryonic kidney cells

S6. LPA Micelle Internalization Analyzed via Live-Cell Laser Scanning Confocal Fluorescence Microscopy

S7. RNA Regulation with LPA Micelles Analyzed via RT-qPCR
   S7.1 Treatment
   S7.2 RNA harvest, reverse transcription, qPCR
S1. Materials and Methods

All reagents were purchased from commercial sources and used without further purification. \((\text{IMesH}_2)(\text{C}_5\text{H}_5\text{N})_2(\text{Cl})_2\text{Ru}=\text{CHPh}\) was prepared as described by Sanford et. al.\(^1\) DNA synthesis was carried out on an ABI 394 DNA/RNA synthesizer utilizing standard phosphoramidite chemistry. DNA synthesis reagents and custom phosphoramidites were purchased from Glen Research Corporation. CPG support columns and standard phosphoramidites were purchased from Azco Biotech Inc. LNA phosphoramidites were purchased from Exiqon Inc. DNA/RNA oligos that were not synthesized were purchased from Integrated DNA Technologies. All cell lines were purchased from ATCC (HeLa CCL-2, MCF7 HTB-22, A549 CCL-185, HT1080 CCL-121, HEK-293 CRL-1573). All deuterated solvents were purchased from Cambridge Isotope Laboratories Inc. \(^1\text{H}\) (400 MHz) and \(^{13}\text{C}\) (100 MHz) NMR spectra were recorded on a Varian Mercury Plus spectrometer. Chemical shifts (\(^1\text{H}\)) are reported in \(\delta\) (ppm) relative to the CDCl\(_3\) residual proton peak (7.27 ppm). \(^{13}\text{C}\) chemical shifts are reported in \(\delta\) (ppm) relative to the CDCl\(_3\) carbon peak (77.00 ppm). Mass spectra were obtained at the UCSD Chemistry and Biochemistry Molecular Mass Spectrometry Facility. Low-resolution mass spectra were obtained using a Thermo LCQdeca mass spectrometer and high-resolution mass spectra were obtained using an Agilent 6230 Accurate Mass time of flight mass spectrometer. Polymer molecular weight and dispersity were determined via size-exclusion chromatography (Phenomenex Phenogel 5u 10, 1K-75K, 300 x 7.80 mm in series with a Phenomex Phenogel 5u 10, 10K-1000K, 300 x 7.80 mm (mobile phase: 0.05 M LiBr in DMF)) using a Hitachi-Elite LaChrom L-2130 pump equipped with a DAWN HELEOS multi-angle light scattering (MALS) detector (Wyatt Technology) and a refractive index detector (Hitachi L-2490) normalized to a 30,000 g/mol polystyrene standard. Hydrodynamic diameter (\(D_h\)) of DPA nanoparticles was measured via DLS using a DynaPro NanoStar (Wyatt Technology). Concentrations of oligonucleotides and DPA nanoparticles were determined using a Thermo Scientific NanoDrop 2000c spectrophotometer. HPLC analysis and purification of oligonucleotides was accomplished utilizing a Phenomenex Clarity 5u Oligo-RP (150 x 4.60 mm) or Clarity 10u Oligo-WAX (150 x 4.60 mm) column and a Hitachi-Elite LaChrom L-2130 pump equipped with a UV-Vis detector (Hitachi-Elite LaChrom L-2420). Oligonucleotide molecular weights were determined by mass spectrometry performed on a Bruker Daltronics Biflex IV MALDI-TOF instrument using a combination of 2’, 4’, 6’-trihydroxyacetophenone monohydrate (THAP) and 3-hydroxypicolinic acid (3-HPA) as matrices and a three-point calibration standard composed of three purchased oligonucleotides. Denaturing polyacrylamide gel electrophoresis was performed using a Bio Rad Criterion Mini-PROTEAN Tetra cell and precast TBE-Urea gels. Size-exclusion FPLC was accomplished using a HiPrep 26/60
S5

Supporting Information

Sephacryl S-200 High Resolution-packed size-exclusion column (mobile phase: 50 mM Tris, pH 8.5) and an Äkta purifier (Pharmacia Biotech) equipped with a P-900 pump and a UV-900 UV-Vis multi-wavelength detector. TEM samples were deposited on carbon/formvar-coated copper grids (Ted Pella Inc.), stained with 1% w/w uranyl acetate, and imaged using a Technai G2 Sphera operating at an accelerating voltage of 200 kV.

S2. Polymer Synthesis

S2.1 Synthesis of (N-Benzyl)-5-norborne-exo-2,3-dicarboximide (1). To a stirred solution of N-benzylamine (2.85 g, 26.6 mmol) in dry toluene (125 mL) was added 5-norbornene-exo-2,3-dicarboxylic anhydride (4.10 g, 25.0 mmol) and triethylamine (3.83 mL, 27.5 mmol). The reaction was heated to reflux overnight under an atmosphere of N₂. The reaction was cooled to room temperature and washed with 10% HCl (3 x 50 mL) and brine (2 x 50 mL). The aqueous layers were combined and extracted with ethyl acetate (60 mL). The combined organic layers were dried with MgSO₄, filtered and concentrated to dryness yielding a pale yellow solid that was then recrystallized from ethyl acetate/hexanes to give 1 (4.98 g, 79%) as white crystals. ¹H NMR (CDCl₃): δ (ppm) 1.07 (d, 1H, CH₂, J=9.6 Hz), 1.42 (d, 1H, CH₂, J=9.6 Hz), 2.69 (s, 2H, 2 x CH₂), 3.26 (s, 2H, 2 x CH₂), 4.61 (s, 2H, CH₂), 6.28 (s, 2H, CH=CH₂), 7.25-7.40 (m, 5H, Ar). ¹³C NMR (CDCl₃): δ (ppm) 42.18, 42.28, 45.13, 47.62, 127.74, 128.48, 135.76, 137.76, 177.48. LRMS (CI), 253.99 [M+H]+. HRMS, theo: 254.1176 [M+H]+, found: 254.1175 [M+H]+.

S2.2 Synthesis of (Z)-4,4'-((but-2-ene-1,4-diylbis(oxy)) dibenzoic acid (2). To a stirred solution of ethyl 4-hydroxybenzoate (5.5 g, 33.1 mmol) in 100 mL dry DMF was added potassium carbonate (7.28 g, 52.7 mmol). To this stirred suspension was added cis-1,4-dichlorobutene (2.0 g, 16 mmol). The solution turned brown within minutes and the reaction was allowed to stir under an atmosphere of N₂ at 90 °C overnight. The mixture was then cooled to room temperature, filtered, and concentrated to dryness. The resulting solid was dissolved in ethyl acetate and washed three times with H₂O. The organic layer was dried over MgSO₄ and concentrated to dryness to yield solid white crystalline needles. This solid was recrystallized from ether to yield the pure diester (2.18 g, 35%). ¹H NMR (CDCl₃): δ (ppm) 1.38 (t, 6H, 2 x CH₃), 4.35 (q, 4H, 2 x CH₂), 4.74 (d, 4H, 2 x CH₂), 5.96 (t, 2H, CH=CH₂), 6.92 (d, 4H, 4 x ArH), 8.0 (d, 4H, 4 x ArH). The diester (2.18 g, 5.66 mmol) was dissolved in 95% ethanol and potassium hydroxide was added (12.0 g, 215 mmol). The reaction was heated to reflux for 5 hours, cooled to room temperature, diluted with an equal volume of H₂O and acidified with HCl to form a white precipitate. The precipitate was filtered off to yield 2 as an orange-tan solid (1.78 g, 100%). ¹H
Supporting Information

NMR (DMSO-d6, residual $^1$H = 2.50 ppm): $\delta$ (ppm) 3.38 (s broad, 2H, 2 x COOH), 4.80 (d, 4H, 2 x CH$_2$), 5.89 (t, 2H, CH=CH$_2$), 7.03 (d, 4H, 4 x ArH), 7.87 (d, 4H, 4 x ArH). $^{13}$C NMR (DMSO-d6, residual $^{13}$C = 39.51 ppm): $\delta$ (ppm) 64.11, 114.50, 123.18, 128.33, 131.34, 161.72, 166.98. LRMS, 327.03 [M-H], HRMS, theo: 327.0874 [M-H], obs: 327.0877 [M-H].

**S2.3 Synthesis of Polymer (1$_{18}$-2).** Monomer 1 (870 mg, 3.4 mmol) was dissolved in 5 mL CDCl$_3$ and cooled to −78 °C. Ruthenium catalyst (IMesh$_2$)(C$_5$H$_5$N)$_2$(Cl)$_2$Ru=CHPh (124 mg, 0.17 mmol) was added as a powder, followed by 1 mL additional CDCl$_3$ to solubilize the catalyst. The reaction was then allowed to warm to room temperature and stir under N$_2$ for 35 minutes (NMR confirms the absence of the original olefin resonance from monomer 1 at 6.28 ppm, and the presence of broad cis and trans polymer backbone olefin resonances at 5.45 and 5.71 ppm). At this point, 200 μL of the reaction mixture was removed and quenched with an excess of ethyl vinyl ether to provide a homopolymer for molecular weight determination (SEC-MALS: $M_w$ = 4575 g/mol, PDI = 1.001, Figure S2). Chain transfer agent 2 (111 mg, 0.34 mmol) was dissolved in 2.0 mL DMF-d7, added to the reaction mixture, and the mixture was allowed to stir at room temperature for 20 minutes. The ruthenium alkylidene proton resonance was monitored in order to track end-labeling of the polymer. Once the reaction was determined to be complete, excess ethyl vinyl ether was added to quench the ruthenium catalyst. The crude polymer was precipitated from cold methanol and further purified by column chromatography in order to eliminate any traces of unreacted chain transfer agent 2. The crude precipitated polymer was dry loaded onto a silica column, the column was washed with 200 mL CH$_2$Cl$_2$, and the polymer was eluted with 3% methanol in CH$_2$Cl$_2$ to yield a glassy yellow-brown solid as the pure polymer (905 mg, 97%, $r_f$ = 0.56). Alternatively, the polymer can be precipitated from cold basic methanol (0.1% triethylamine) three times in order to yield pure polymer free of excess chain transfer agent.

![Synthesis diagram](image)

Synthesis of carboxylic acid-terminated phenyl-norbornene polymer 1$_{18}$-2 via ROMP.
Polymer termination with termination agent 2 (T.A.) monitored via $^1$H NMR.

SEC-MALS chromatogram for polymer 1_{18}2 (LS = light scattering Rayleigh ratio, RI = refractive index difference, UV = UV absorbance at 280 nm, $M_w$ = polymer molecular weight).
Oligonucleotides were synthesized in house using automated phosphoramidite chemistry (ABI 394 DNA/RNA Synthesizer) with 4,5-Dicyanoimidazole (Glen Research cat. #30-3150-52) as the activator and 0.02 M iodine in tetrahydrofuran/pyridine/water (Glen Research cat. #40-4330-52) as the oxidizer. Standard 2-cyanoethyl protected phosphoramidites include dA (N-Bz), dG (N-dmf), (N-acetyl) dC, and T. LNA phosphoramidites were vacuum dried using a Schlenk line for 24 hours prior to dissolving at 0.07 M in anhydrous acetonitrile or 25% tetrahydrofuran in acetonitrile v/v (for LNA mC only). Coupling times of 35 s (dC and T), 100 s (dA and dG), and 120 s (LNA amidites) were used. Oxidation time for LNA phosphoramidites was increased to 45 s as compared to 15 s for standard phosphoramidites. Oligonucleotides were synthesized on a 1.0 μmol scale using columns packed with 1000 Å CPG beads (both AZCO universal support, cat. #19-8051-10, and Glen Research Universal Support III 40 μmol/g, cat. #G308237). No significant differences between the two support types were noticed. However, a pore size of at least 1000 Å was found to be critical for polymer conjugation on solid support. A 5’-amino modifer was incorporated into each synthetic oligonucleotide through use of 5’-amino modifer C12 phosphoramidite (Glen Research). FL-AS-ssLNA and CY5-AS-ssLNA 5’-amino termini were acetylated on solid support using the automated synthesizer as follows: the MMT group was removed by treatment with 3% trichloroacetic acid in CH₂Cl₂ for three minutes (until the yellow color due to the MMT⁺ cation was no longer visible in the eluting deblock solution) followed by a standard capping cycle to acetylate the free amine with acetic anhydride. Fluorescein and CY5 labels were incorporated into the oligonucleotides via use of Fluorescein dT and Cyanine 5 phosphoramidites (Glen Research) using coupling times of 10 minutes and 3 minutes respectively. Oligos were cleaved from solid support by treatment with ~150 μl 2.0 M ammonia in methanol for 1 hour at room temperature (recommended for universal support III chemistry) and further deprotected by addition of ~1.5 ml concentrated ammonium hydroxide at room temperature for 24 hours (to avoid degradation of CY5), purified by HPLC, and characterized by MALDI-TOF MS. Survivin RNA oligo was purchased from Integrated DNA Technologies (purified by HPLC, confirmed by ESI-MS). Detailed sequences are shown in Figure S4.

**S3.1 HPLC Purification of Oligonucleotides.** Synthetic LNA oligonucleotides were purified via reverse-phase HPLC using a binary gradient (buffer A: 10% methanol, 90% 50 mM triethyl ammonium acetate (TEAA) pH 7.1; buffer B: 100% methanol.) For FL-AS-ssLNA, weak anion-exchange (WAX) HPLC was also necessary to purify the oligonucleotide. A quaternary gradient was used for WAX HPLC analyses and purification (Solvent A: nanopure H₂O, solvent B: methanol, solvent C: 100 mM
Supporting Information
Tris(hydroxymethyl)aminomethane (Tris) pH 8.0, solvent D: 2 M NaCl. FL-AS-ssLNA was desalted post WAX HPLC purification using a Sep-Pak Plus C18 Environmental Cartridge.

**S3.2 MALDI-TOF MS of Oligonucleotides.** A MALDI target plate was spotted with 1 µL of matrix solution A for each sample to be analyzed and allowed 20 minutes to dry completely. Matrix solution A was prepared by dissolving 50 mg of 3-HPA in 1000 µL of HPLC grade acetonitrile/Nanopure H₂O (1:1 v/v). Subsequently, 454 µL of this solution was mixed with 45 µL of 100 mg/ml diammonium hydrogen citrate in Nanopure H₂O. Oligonucleotide samples were prepared for MALDI-TOF MS analysis using Zip-Tip C18 pipette tips. Oligos were loaded onto the C18 tips from concentrated stock solutions (ca. 50-100 µM), desalted (protocol: Sample Preparation of Oligonucleotides Prior to MALDI-TOF MS Using ZipTipC18 and ZipTip µ-C18 Pipette Tips), and eluted with matrix solution B. Briefly, desalting was achieved by washing/wetting the Zip-Tip with 50% acetonitrile, equilibrating with 0.1 M TEAA, loading the oligo, washing with 0.1 M TEAA, washing with H₂O, and finally eluting the bound oligo with ~ 3.0 µl of matrix solution B. Matrix solution B was prepared by dissolving 50 mg of THAP in 500 µL of HPLC grade acetonitrile. Dissolution was assisted by sonication and the resulting solution was centrifuged to pellet any remaining solid. Subsequently, 250 µL of this supernatant was mixed with 250 µL of 23 mg/mL diammonium hydrogen citrate in Nanopure H₂O. 1 µL of this solution was then spotted onto the MALDI plate on top of crystallized matrix A. The samples were allowed to dry for 15-30 minutes before analyzing via MALDI-TOF MS. External three-point calibration was achieved using a mixture of three oligonucleotides purchased from Integrated DNA Technologies. Aliquots of 25 pmol 5'-TCTTATACTTAAT-3' (3898.6 g/mol), 125 pmol 5'-CAGTGGACTGGACATGACTCT-3' (6077.0 g/mol), and 700 pmol 5-AAGATCGATCAGACTGGAC-3' (11644.6 g/mol) were all dissolved together in 100 µl nanopure H₂O and spotted (1 µl) on top of crystallized matrix A (1 µl spot) for MALDI-TOF MS analysis in order to create an external calibration reference.
Supporting Information

**5'-Acetyl-Antisense Fluorescein**

HPLC chromatograms and corresponding MALDI-TOF MS spectra for synthesized LNA sequences. HPLC was carried out using a linear binary gradient from 0-100% Buffer B in 60 minutes while recording UV absorbance at 260 nm (Buffer A: 10% methanol, 90% 50 mM triethyl ammonium acetate (TEAA) pH 7.1; Buffer B: 100% methanol).

**5'-Acetyl-Antisense CY5**

**5'-MMT-Nonsense Fluorescein**

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**S4. LNA-Polymer Amphiphile Synthesis and Micellar Nanoparticle Formation**

To a solution of polymer (1k-2) (50 mg, 10.9 μmol) dissolved in 150 μL DMF was added N,N-diisopropylethylamine (5 μL, 29.1 μmol) and HATU (3.42 mg, 9 μmol). The solution was vortexed for 10 minutes at room temperature in order to activate the polymer carboxylic acid terminus. At this point, 5’-amino modified LNA on CPG solid support (ca. 0.5-1.0 μmol, MMT deprotected by washing with 3% TCA in DCM for 3 minutes) was added. 100 μL DMF was added to wash CPG beads to the bottom of the microcentrifuge tube and immerse the beads in solution entirely. The mixture was allowed to vortex at room temperature for 2 hours. At this point, the CPG beads were centrifuged and washed with DMF three times followed by a final rinse with DCM. The beads were dried using a SpeedVac vacuum concentrator and subsequently a second coupling of 50 mg polymer for 2 hours was carried out under conditions identical to those described above. The CPG beads were then filtered away from the solution using an empty synthesis column and then washed with DMF (2 x 20 ml) and CHCl₃ (2 x 20 ml). The LNA-polymer amphiphile (LPA) was cleaved from solid support via treatment with concentrated ammonium hydroxide for 24 hours at room temperature. The CPG beads were away from the solution using glass wool and subsequently washed consecutively with H₂O (2.0 ml), DMSO (2.0 ml), Formamide (2.0 ml), H₂O (3.0 ml), and DMSO (1.0 ml). This solution was transferred to 3,500 MWCO...
S11

Supporting Information

snakeskin dialysis tubing (Thermo Scientific) and 2.0 mL H₂O, used to wash the filtrate container, was added. The resulting solution was dialyzed against 2.0 L of Nanopure H₂O overnight. This dialyzed solution was then concentrated to ~3.0 ml via rotary evaporation (water bath temperature set to 55 °C). The resulting crude LPA-nanoparticle/ssLNA mixture was analyzed by denaturing PAGE and agarose gel electrophoresis to confirm the presence of LPA conjugates and free ssLNA. It is important to note that low molecular weight ssLNA impurities (≤ 10,000 g/mol) remain present despite extensive attempts to dialyze away these species using 20,000 MWCO slide-a-lyzer dialysis cassettes or mini dialysis units (Thermo cat. #PI87736, #PI69590). Therefore, the crude mixture was purified via SEC FPLC (mobile phase: 50 mM Tris pH 8.5, flow rate: 1.8 ml/min, λ_{abs} = 260, 492, 646 nm). The LPA-nanoparticles elute at ca. 55 minutes while unconjugated ssLNA impurities elute at ca. 85 minutes. All crude LPA micelles were purified using HiPrep 26/60 Sephacryl S-200 High Resolution SEC media. The pure LPA fraction (~15 ml) was then concentrated to ~0.5-1.0 ml via rotary evaporation (water bath temperature set to 55 °C) and dialyzed (20,000 MWCO slide-a-lyzer mini dialysis unit, Thermo Cat. #PI69590) against 10 mM Tris pH 8.5 overnight. The pure LPA micelles in 10 mM Tris pH 8.5 were then filtered through a 13 mm 0.45 μm pore size PTFE syringe filter (GE Whatman cat. #6784-1304).

S4.1 LNA Concentration Determination. Nucleic acid concentrations were determined by UV absorbance at 260 nm using a NanoDrop 2000c spectrophotometer (pathlength = 1.0 mm). Extinction coefficients of 357,779 L/mol·cm for FL-AS-ssLNA/FL-NS-ssLNA and 328,979 L/mol·cm for CY5-AS-ssLNA were used. This coefficient was calculated as the extinction coefficient of the entire sequence without the dye modified base at 260 nm (318,979 L/mol·cm, OligoCalc) plus the extinction coefficients for each dye-labeled base at 260 nm (38,800 L/mol·cm for Fluorescein dT, and 10,000 L/mol·cm for CY5 phosphoramidite, Glen Research). LNA bases were treated as standard DNA bases in these calculations. For determining the concentration of LPA micelles an aggregation number of 200 LPAs/LPA micelle was assigned based on SLS/DLS measurements. Therefore, ssLNA concentration was divided by 200 to yield the approximate concentration of LPA micelle in each instance.

S4.2 Transmission Electron Microscopy. Copper grids (formvar/carbon-coated, 400 mesh copper, Ted Pella # 01754) were prepared by glow discharging the surface at 20 mA for 1.5 minutes followed by treatment with 3.5 μL 250 mM CaCl₂ in order to prepare the surface for DPA nanoparticle adhesion. The CaCl₂ solution was wicked away with filter paper, the grid allowed to dry for 1 minute, and 3.5 μL of LPA nanoparticle (ca. 50 μM DNA in 10 mM Tris pH 8.5) solution was deposited on the grid surface. This solution was allowed to sit for 10 seconds before being washed away with 20 drops of glass distilled H₂O and subsequent staining with 3 drops of 1% w/w uranyl acetate. The stain was allowed to sit for 10 seconds before wicking away with filter paper. All grid treatments and sample
Supporting Information

depositions were on the dark/shiny/glossy formvar-coated face of the grid (this side face up during glow discharge). Samples were then imaged via TEM.

S4.3 Dynamic Light Scattering. All samples were filtered through a 0.45 μm PTFE filter (Whatman #6784-1304) prior to DLS analysis using a DynaPro NanoStar (Wyatt Technology) and Dynamics version 7 software. A total of ten 4.0 s acquisitions were averaged for each reported measurement. Analyses are reported as both the reweighted intensity by mass % and the raw intensity (see Figures S1-3).

S5. LPA Micelle Uptake Analyzed via Fluorescence-Activated Cell Sorting

All FACS analyses were carried out using an Accuri C6 flow cytometer set to the default “3 blue 1 red” configuration with standard optics. Medium fluidics (35 μl/min, 16 μm core size) were used with no compensation set for any channel. All cells were grown in Dulbecco’s Modified Eagle Medium (DMEM + 4.5 g/L glucose, - L-Glutamine, - Sodium Pyruvate, Gibco Life Tech. #11960-044) with fetal bovine serum (FBS, Omega Scientific, Cat. # FB-02) added to 10%, and antibiotics (100 x Penicillin-Streptomycin, Corning Cellgro, #30-002-CI), non-essential amino acids (100x MEM-NEAA, Gibco Life Tech. #11140-050), sodium pyruvate (100x, Gibco Life Tech. #11360-070) and glutamine (Glutamax 100x, Gibco Life Tech. #35050-061) all added to a final concentration of 1x. All cells were treated similarly for all FACS analyses, unless otherwise noted. In each experiment cells were grown to 80-100% confluency before splitting and plating 15-24 hours prior to treatment and subsequent FACS analysis. All incubations were carried out in Opti-MEM reduced serum media (Gibco Life Tech. #31985-070). Cells were plated in plastic 24-well tissue culture plates (24-well, flat bottom, 1.93 cm² growth area, Genesee Scientific Olympus Plastics #25-107) at 20,000 cells/well with 500 μl DMEM (as used for growth) per well. After treatment, cells were washed three times with Dulbecco’s Phosphate Buffered Saline (DPBS, without Calcium or Magnesium, Corning Cellgro #21-031-CM) and trypsinized with 250 μl 0.25% trypsin (diluted from 10x stock with DPBS, 2.5% trypsin, Gibco Life Tech. #15090-046) for 10 minutes at 37 °C and 5% CO₂. Following trypsinization, 500 μl DMEM (as used for growth) was added and the cells were aspirated and dispensed three times before harvesting in 1.5 mL microcentrifuge tubes (CNT-1.5F, DNase/RNase free, Bio Pioneer Inc.). Cells were then pelleted by centrifugation at 300 g for five minutes. Subsequently, the media was aspirated and the pellet was resuspended in 100 μl cold DPBS and put on ice until FACS analysis (analysis carried out within one hour of resuspension). Cells were never fixed at any point for any experiment. Cells were vortexed gently immediately prior to FACS analysis.
Supporting Information

S5.1 Antisense versus nonsense LPA micelle uptake. HeLa cells were plated at 20,000 cells/well in a 24-well plastic tissue culture plate ca. 24 hours prior to incubation with 5 nM antisense fluorescein (AS-FL-LPA) or nonsense fluorescein (NS-FL-LPA) LPA micelles in 250 μl Opti-MEM for 2 hours at 37 °C and 5% CO₂. Cells were then washed three times with DPBS and harvested via trypsinization and subsequently pelleted and resuspended in cold DPBS. A total of 2500 event counts were collected and gated to include only events in the population that gave rise to significant scattering (>2M FSC). These cells comprised 32-45% of the total population analyzed for each experiment. Fluorescence for each event was recorded using an excitation wavelength of 488 nm and an emission filter of 533 ± 15 nm. Data is plotted as overlaid histograms displaying relative event count versus fluorescence per event.

S5.2 LPA micelle versus ssLNA uptake. HeLa cells were plated at 20,000 cells/well in a 24-well plastic tissue culture plate ca. 24 hours prior to incubation with 5 nM antisense LPA micelles or 1 μM ssLNA (equivalent concentration with respect to LNA and fluorophore) analogue in 250 μl Opti-MEM for 10 minutes or 2 hours at 37 °C and 5% CO₂. Cells were then washed three times with DPBS, harvested via trypsinization, and subsequently pelleted and resuspended in cold DPBS. Cells harvested at 10 minutes were put on ice until 2 hour treatment was finished, hence FACS analysis was carried out at the same time for both time points. No significant reduction in LPA micelle-cell association was noticed for cell samples stored on ice for less than 4 hours. A total of 2500 event counts were collected and gated to include only events in the population that gave rise to significant scattering (>2M FSC). Ungated histograms are also displayed for each experiment. These cells comprised 52-86% of the total population analyzed for each experiment. Fluorescence for each event was recorded using an excitation wavelength of 488 nm and an emission filter of 533 ± 15 nm (FL-1, fluorescein-labeled materials) or an excitation wavelength of 640 nm and an excitation filter of 675 ± 12.5 nm (FL-4, cyanine 5-labeled materials). Data is plotted as overlaid histograms displaying relative event count versus fluorescence per event.

S5.3 Cytotoxicity via propidium iodide assay. HeLa cells were plated at 20,000 cells/well in a 24-well plastic tissue culture plate ca. 24 hours prior to incubation with 5 nM antisense fluorescein LPA (AS-FL-LPA) micelles or 10 nM antisense fluorescein LPA (AS-FL-LPA) micelles in 250 μl Opti-MEM for 1 hour at 37 °C and 5% CO₂. Cells treated with 0.25% Triton X 100 in 250 μl DMEM were incubated for 5 minutes at room temperature. The shorter incubation time in this instance was necessary due to nearly complete cell lysis after 1 hour of incubation (thus precluding FACS analysis). After incubation, cells were then washed three times with DPBS, harvested via trypsinization, and subsequently centrifuged and resuspended in 100 μl of DPBS. To this cell suspension was added 10 μl of a 200 μg/ml solution of propidium iodide in DPBS. The cells were then incubated at room
Supporting Information

temperature for 5 minutes before putting on ice. Cells were incubated on ice for 15 minutes prior to FACS analysis. A total of 5000 event counts were collected for each sample. Gating for the population of cells with significant scattering signal in the regions associated with healthy HeLa cells excluded greater than 95% of the cell population treated with Triton-X 100. Therefore, histograms were constructed for ungated cell populations in order to assess the fluorescence due to propidium iodide for each event. Fluorescence for each event was recorded using an excitation wavelength of 488 nm and a 670 nm LP emission filter. Data is plotted as overlaid histograms displaying relative event count versus fluorescence per event.

S5.4 Pharmacological inhibition studies of LPA micelle uptake. HeLa cells were plated at 20,000 cells/well in a 24-well plastic tissue culture plate ca. 24 hours prior to incubation for 30 minutes with a given pharmacological inhibitor in 211 μl Opti-MEM at 37 °C and 5% CO₂ in order to compare results to previous literature.⁴ Following incubation with inhibitor, 39 μl of 32 nM antisense fluorescein LPA (AS-FL-LPA) micelle was added to each well to achieve a final concentration of 5 nM LPA micelle. The cells were then incubated for 1 hour at 37 °C and 5% CO₂. After incubation, cells were then washed three times with DPBS, harvested via trypsinization, and subsequently centrifuged and resuspended in 100 μl of DPBS. A total of 2500 event counts were collected for each sample. As methyl-β-cyclodextrin was the only inhibitor to show a significant effect on LPA micelle-cell association, this data was compared directly to the experiments not involving any inhibitor treatment in both gated and ungated FACS analyses. In the instance of gated populations, these cells comprised 25-59% of the total population analyzed for each experiment. Fluorescence for each event was recorded using an excitation wavelength of 488 nm and an emission filter of 533 ± 15 nm. Data is plotted as both as overlaid histograms displaying relative event count versus fluorescence per event and as bar graphs displaying average fluorescence per event (mean FL-1 A, gated and ungated) in each experiment (error bars are the result of running the experiment three times with three separate passages of the cells, see Figures S13 and S14). Experimental and stock concentrations of pharmacological inhibitors are shown in Table S5.

S5.5 LPA micelle uptake kinetics across multiple cell lines. HeLa, A549, HT1080, and MCF7 cells were plated at 20,000 cells/well in a 24-well plastic tissue culture plate ca. 24 hours prior to incubation with 5 nM antisense fluorescein LPA (AS-FL-LPA) micelles in 250 μl Opti-MEM for 10 minutes, 30 minutes, 1 hour, or 4 hours at 37 °C and 5% CO₂. The data for the four-hour incubations are not reported in the main text, as triplicate measurement were only carried out for the first three time points. At the end of each time point, the cells were washed three times with DPBS, harvested via trypsinization, and subsequently pelleted and resuspended in cold DPBS and put on ice. All cells were analyzed via FACS ca. 90 minutes after the start of the experiment. No significant reduction in LPA
Supporting Information
micelle-cell association was noticed for cell samples stored on ice for less than 4 hours. A total of 5000 event counts were collected and gated to include only events in the population that gave rise to significant scattering (>2M FSC). These cells comprised 44-88% of the total population analyzed for each experiment. Fluorescence for each event was recorded using an excitation wavelength of 488 nm and an emission filter of 533 ± 15 nm. Data is plotted as overlaid histograms displaying relative event count versus fluorescence per event.

S5.6 LPA micelle uptake in human embryonic kidney cells. HEK 293 cells were plated at 20,000 cells/well in a 24-well plastic tissue culture plate ca. 24 hours prior to incubation with 5 nM antisense fluorescein LPA (AS-FL-LPA) micelles in 250 μl Opti-MEM for 10 minutes or 2 hours. At the end of each time point, 250 μl of 0.25 % trypsin was added to each well and the cells were incubated for 10 minutes at 37 °C 5% CO₂. After three aspirate/dispose cycles, the cells were collected in a 1.5 ml microcentrifuge tube and centrifuged at 300 g for 5 minutes. The supernatant was aspirated and the cells resuspended in 1.0 ml DPBS. The cells were pelleted again at 300 g, the supernatant removed, and the pellet resuspended in 100 μl cold DPBS for FACS analysis. A total of 5000 event counts were collected and gated to include only events in the population that gave rise to significant scattering (>2M FSC). These cells comprised 17-26% of the total population analyzed for each experiment. Fluorescence for each event was recorded using an excitation wavelength of 488 nm and an emission filter of 533 ± 15 nm. Data is plotted as overlaid histograms displaying relative event count versus fluorescence per event.

S6. LPA Micelle Internalization Analyzed via Live-Cell Laser Scanning Confocal Fluorescence Microscopy.

HeLa cells were plated at 20,000 cells/well in a 24-well glass-bottom tissue culture plate (No. 1.5, γ-irradiated, MatTek Corporation cat. #P24G-1.5-10-F) ca. 24 hours prior to incubation with 10 nM antisense fluorescein (AS-FL-LPA) or 10 nM antisense cyanine 5 (AS-CY5-LPA) micelles in 250 μl Opti-MEM for 5 minutes or 2 hours at 37 °C and 5% CO₂. Glass bottom wells were coated with human fibronectin (BD Biosciences cat. #354008) and stored at 8 °C the evening before plating the cells. Fibronectin coating was achieved by first treating each well with 250 μl of 1 M HCl for 15 minutes. Following this treatment, the wells were washed three times with DPBS and two times with ultrapure H₂O. Fibronectin solution (250 μl, diluted to 50 μg/ml with DPBS) was added to each well and incubated for 1 hour before aspirating and washing three times with DPBS. At each time point, the Opti-MEM media was removed and replaced with warm DPBS. The cells were then imaged via confocal fluorescence microscopy in an incubation chamber at 37 °C and 5% CO₂. For figures S7 and S8A,
Supporting Information

Imaging was accomplished using a 20x air objective at 2x optical zoom. For single-slice images (main text Figure 2), scan size was set to 800 x 800 pixels with a scan speed of 10 µs/pixel. For Z-stack imaging, slice thickness was 1.25 µm with a scan size of 512 x 512 pixels and a scan speed of 10 µs/pixel. Cell imaging for fluorescein fluorescence was accomplished using a 488 nm laser and the following settings for all images acquired: 488 laser set to 30% power, HV=458, Gain=2, Offset=7. Cell imaging for cyanine 5 fluorescence was accomplished using a 635 nm laser and the following settings for all images acquired: 635 laser set to 50% power, HV=361, Gain=2, Offset=6. Identical contrast, brightness, and intensity settings were applied to each fluorescent channel for all images post acquisition. To image a high-resolution z-profile of cells treated with LPA nanoparticles with an added nuclear stain, a separate experiment was conducted. In this case, HeLa cells were plated exactly as described for the previous experiment. Treatment with 5 nM AS-CY5-LPA for 3.5 h was then carried out, followed by fixation with paraformaldehyde, permeabilization with Triton X-100, and staining according to standard procedures. Imaging was accomplished using a 60x oil objective at 2x optical zoom. Scan size was set to 640 x 640 pixels at a scan speed of 10 µs/pixel and a slice thickness of 0.5 µm. For cyanine 5 imaging, a 635 nm laser was used with the following settings: 3% laser power, HV = 484, gain = 4, offset = 6. For NucBlue imaging, a 405 nm laser was used with the following settings: 32% laser power, HV = 355, gain = 3, offset = 6.

S7. RNA Regulation with LPA Micelles Analyzed via RT-qPCR

S7.1 Treatment. HeLa cells were treated with 5 nM LPA micelle for three consecutive days as follows:

Day 0, morning: HeLas were grown in DMEM in a T75 flask to 60-80% confluency. Media was aspirated, cells were washed with PBS and dissociated with trypsin (TrypLE Express, Life Technologies #12605010) for 5 minutes. DMEM with FBS was added to inactivate trypsin and the cells were aspirated and dispensed gently to break up any aggregates. 20,000 cells were plated in each well of a 24-well tissue culture plate.

Day 1: Micelles were diluted in Opti-MEM in a 1.5mL microcentrifuge tube to 5 nM immediately before use. For each LPA experiment (3 wells for fluorescein antisense micelle, 3 wells for fluorescein nonsense micelle), 39 µL of LPA Micelle (concentration = 32 nM LPA micelle) was added to 211 µL of Opti-MEM. For the untreated control (6 wells), 39 µL molecular-grade water was added to 211 µl of Opti-MEM. The media was aspirated from the cells gently followed by the gentle addition of micelle suspension. Beginning at 3:00 pm, the cells were incubated with LPA micelles for four hours at 37 °C.
Supporting Information
and 5% CO₂. After four hours the media with LPA micelles was aspirated and replaced with DMEM growth media.

Day 2: Cells were incubated with 5 nM LPA micelle from 6:00-10:00 pm. After four hours the media with LPA micelles was aspirated and replaced with DMEM growth media.

Day 3: Cells were incubated with 5 nM LPA micelle from 6:00-10:00 pm. At 10:00 pm 400 μl DMEM was added to each well without aspiration of Opti-MEM micelle solution.

Day 4: Cellular RNA was harvested with RNAeasy Mini Kit (Qiagen cat. #74-104) at 5:00 pm.

S7.2 RNA harvest, reverse transcription, and qPCR. On Day 4, HeLa RNA was harvested from each experimental well using Qiagen’s RNAeasy mini kit following the associated protocol. The cells were lysed directly in each well using 350 μl RLT buffer and the lysate collected by vigorous aspirate and dispense cycles. The lysate was then homogenized using QIAshredder spin columns and RNA was harvested using RNeasy spin columns. Remaining DNA was digested using TURBO DNase and a TURBO DNA-free kit (Life Technologies #AM1907M) and following the associate protocol. Subsequently, the total RNA concentration was determined using a NanoDrop 2000c spectrophotometer. Reverse transcription of cellular RNA into cDNA was achieved using Superscript III reverse transcriptase and Superscript III First-Strand Synthesis System (Invitrogen #18080-051). Reverse transcription was carried for each experimental well (3 experimental wells for antisense micelle treatment, 3 experimental wells for nonsense micelle treatment, and 6 experimental wells for untreated HeLa cells) using oligo dT primers, 1 μg of RNA per reaction, and digesting RNA with RNase H after the reaction was complete. Quantification of GAPDH and survivin transcripts in 1 μl of resulting cDNA for each experiment was achieved by qPCR using Fast SYBR Green Master Mix (Life Technologies #4385612) and measuring threshold cycle in the presence of survivin primers³ relative to cycle threshold in the presence of glyceraldehyde 3-phosphate dehydrogenase primers (GAPDH, Primer Bank ID 83641890b1) for each experimental condition in triplicate. Data analysis was carried out using GraphPad Software t test calculator (http://www.graphpad.com/quickcalc/ttest1/?Format=SEM) by entering the mean, standard error of the mean (SEM), and number of repeats (N) and using the unpaired t test. Survivin forward: 5’-ATG GGT GCC CCG ACG TTG, Survivin reverse: 5’-AGA GGC CTC AAT CCA TGG, GAPDH forward: 5’-AAG GTG AAG GTC GGA GTC AAC, GAPDH reverse: 5’-GGG GTC ATT GAT GGC AAC AAT A. All primers were purchased from Integrated DNA Technologies with standard desalting used as the sole purification method.
**Figure S1.** Antisense fluorescein (AS FL) LPA nanoparticle characterization. Negative stain (1% w/w uranyl acetate) TEM micrographs are at 29,000x (A), 80,000x (B), and 150,000x (C) magnification. FPLC (D) was carried out using 50 mM Tris pH 8.5 at a flow rate of 1.8 mL/min while monitoring absorbance at 260 nm. DLS data is reported as the reweighted intensity by mass % (E), the raw intensity % (F), and the correlation function (G).
**Figure S2.** Antisense CY5 (AS CY5) LPA nanoparticle characterization. Negative stain (1% w/w uranyl acetate) TEM micrographs are at 29,000x (A), 80,000x (B), and 150,000x (C) magnification. FPLC (D) was carried out using 50 mM Tris pH 8.5 at a flow rate of 1.8 mL/min while monitoring absorbance at 260 nm. DLS data is reported as the reweighted intensity by mass % (E), the raw intensity % (F), and the correlation function (G).
Figure S3. Nonsense fluorescein (NS FL) LPA nanoparticle characterization. Negative stain (1% w/w uranyl acetate) TEM micrographs are at 29,000x (A), 80,000x (B), and 150,000x (C) magnification. FPLC (D) was carried out using 50 mM Tris pH 8.5 at a flow rate of 1.8 mL/min while monitoring absorbance at 260 nm. DLS data is reported as the reweighted intensity by mass % (E), the raw intensity % (F), and the correlation function (G).
**Figure S4.** LNA and RNA sequence information, modifications, and chemical structure. $T_r$ denotes fluorescein dT phosphoramidite and CY5 denotes cyanine 5 phosphoramidite. Underlined positions indicate LNA bases. LNA C is incorporated as the 5-methyl cytosine analogue as sold by Exiqon.
**Figure S5.** FACS data showing the difference in HeLa uptake between fluorescein-labeled LPA nanoparticles and the corresponding fluorescein-labeled ssLNA at both 10 minutes (top) and 2 hours (bottom). HeLa cells were incubated with 5 nM LPA micelle for the denoted time at 37 °C in 5% CO₂. FACS data displays 2500 event counts for each experimental condition. The cells contained within the gated area (pink polygon) are denoted as a percent of the total event count. The histogram data on the left is gated to each corresponding scatter plot and displays FL-1 Area as the x-axis ($\lambda_{ex} = 488$ nm, $\lambda_{em} = 533 \pm 15$ nm).
**Cy5 Antisense ssLNA vs LPA Micelle**

Figure S6. FACS data showing the difference in HeLa uptake between fluorescein-labeled LPA nanoparticles and the corresponding fluorescein-labeled ssLNA at both 10 minutes (top) and 2 hours (bottom). HeLa cells were incubated with 5 nM LPA micelle for the denoted time at 37 °C in 5% CO₂. FACS data displays 2500 event counts for each experimental condition. The cells contained within the gated area (pink polygon) are denoted as a percent of the total event count. The histogram data on the left is gated to each corresponding scatter plot and displays FL-4 Area as the x-axis (λ_ex = 640 nm, λ_em = 675 ± 12.5 nm).
Figure S7. Confocal fluorescence microscopy Z-stack montage for live HeLa cells incubated with FL-AS-LPA micelles for 2 hours ($\lambda_{ex} = 488$ nm). The distance between each image in the z-axis is 1.25 μm.
Figure S8A. Confocal fluorescence microscopy Z-stack montage for live HeLa cells incubated with CY5-AS-LPA micelles for 2 hours ($\lambda_{ex} = 635$ nm). The distance between each image in the z-axis is 1.25 μm.
Supporting Information

Figure S8B. Confocal fluorescence microscopy Z-stack imaging for fixed HeLa cells incubated with CY5-AS-LPA micelles for 3.5 hours ($\lambda_{ex}=635$ nm, colored red). Nuclei are stained with NucBlue ($\lambda_{ex}=405$ nm, colored blue). Z-spacing is 0.5 $\mu$m.
Table S1. RT-qPCR cycle threshold (CT) data for qPCR of survivin cDNA normalized to endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA.

| experiment   | CT (with Survivin Primers) | CT (with GAPDH Primers) |
|--------------|----------------------------|-------------------------|
|              | repeat 1 | repeat 2 | repeat 3 | repeat 1 | repeat 2 | repeat 3 |
| antisense1   | 22.80    | 22.82    | 22.85    | 17.07    | 16.73    | 17.10    |
| antisense2   | 24.36    | 24.36    | 24.51    | 18.01    | 17.80    | 17.84    |
| antisense3   | 22.39    | 22.44    | 22.48    | 16.95    | 16.79    | 16.87    |
| nonsense1    | 21.09    | 21.03    | 21.08    | 16.96    | 16.97    | 16.91    |
| nonsense2    | 20.91    | 20.86    | 20.84    | 16.88    | 16.75    | 16.80    |
| nonsense3    | 21.36    | 21.18    | 21.26    | 17.00    | 17.02    | 17.16    |
| untreated1   | 20.55    | 20.64    | 20.50    | 16.69    | 16.61    | 16.68    |
| untreated2   | 20.61    | 20.69    | 20.59    | 16.83    | 16.63    | 16.81    |
| untreated3   | 20.52    | 20.71    | 20.56    | 16.61    | 16.55    | 16.55    |
| untreated4   | 20.77    | 20.56    | 20.59    | 17.92    | 16.58    | 16.69    |
| untreated5   | 20.91    | 20.82    | 20.81    | 16.85    | 16.60    | 16.58    |
| untreated6   | 21.03    | 21.07    | 21.19    | 16.80    | 16.78    | 16.63    |

| Survivin-GAPDH | 2^(-survivin-GAPDH) |
|----------------|---------------------|
| repeat 1 | repeat 2 | repeat 3 | repeat 1 | repeat 2 | repeat 3 |
| antisense1   | 5.73    | 6.09    | 5.75    | 0.019    | 0.015    | 0.019    |
| antisense2   | 6.35    | 6.56    | 6.67    | 0.012    | 0.011    | 0.010    |
| antisense3   | 5.44    | 5.65    | 5.61    | 0.023    | 0.020    | 0.020    |
| nonsense1    | 4.13    | 4.06    | 4.17    | 0.057    | 0.060    | 0.056    |
| nonsense2    | 4.03    | 4.11    | 4.04    | 0.061    | 0.058    | 0.061    |
| nonsense3    | 4.36    | 4.16    | 4.10    | 0.049    | 0.056    | 0.058    |
| untreated1   | 3.86    | 4.03    | 3.82    | 0.069    | 0.061    | 0.071    |
| untreated2   | 3.78    | 4.06    | 3.78    | 0.073    | 0.060    | 0.073    |
| untreated3   | 3.91    | 4.16    | 4.01    | 0.067    | 0.056    | 0.062    |
| untreated4   | 2.85    | 3.98    | 3.90    | 0.139    | 0.063    | 0.067    |
| untreated5   | 4.06    | 4.22    | 4.23    | 0.060    | 0.054    | 0.053    |
| untreated6   | 4.23    | 4.29    | 4.56    | 0.053    | 0.051    | 0.042    |
**Supporting Information**

**Table S2.** RT-qPCR statistical analysis for antisense versus nonsense LPA micelle treatments.

|                | antisense | untreated |
|----------------|-----------|-----------|
| **Mean**       | 0.0164    | 0.0652    |
| **SD**         | 0.0047    | 0.0201    |
| **SEM**        | 0.0027    | 0.0082    |
| **N**          | 3         | 6         |
| two-tailed P value | 0.0051 |          |
| conclusion     | very statistically significant |

|                | nonsense | untreated |
|----------------|----------|-----------|
| **Mean**       | 0.0572   | 0.0652    |
| **SD**         | 0.0037   | 0.0201    |
| **SEM**        | 0.0021   | 0.0082    |
| **N**          | 3        | 6         |
| two-tailed P value | 0.5332 |          |
| conclusion     | not statistically significant |

*Figure S9.* FACS data showing HeLa uptake of both antisense and nonsense fluorescein-labeled LPA nanoparticles. HeLa cells were incubated with 5 nM LPA micelle for 2 hours at 37 °C in 5% CO2. FACS data displays 2500 event counts for each experimental condition. The cells contained within the gated area (pink polygon) are denoted as a percent of the total event count. The histogram data is gated to each corresponding scatter plot and displays FL-1 Area as the x-axis ($\lambda_{ex} = 488$ nm, $\lambda_{em} = 533 \pm 15$ nm).
**Figure S10.** FL-AS-LPA micelle uptake kinetics for four different cell lines as analyzed via FACS. Histograms were constructed from gated data (see Figure S16) on a total of 5000 event counts. The x-axis is plotted as FL-1 Area ($\lambda_{\text{ex}} = 488$ nm, $\lambda_{\text{em}} = 533 \pm 15$ nm).

**Table S3.** Tabulated data for LPA micelle uptake kinetics in four different cell lines.

| Cell Line | 10 min | 30 min | 1 hr | 4 hr | untreated |
|-----------|--------|--------|------|------|-----------|
| HeLa      | 7.50   | 6.10   | 5.76 | 5.39 | 0.80      |
| HT1080    | 11.5   | 14.5   | 14.6 | 11.4 | 0.83      |
| A549      | 5.02   | 6.72   | 6.59 | 6.81 | 0.67      |
| MCF7      | 20.6   | 17.2   | 16.3 | 13.6 | 1.26      |

*values reported as mean FL-1 area of gated population divided by $1 \times 10^4$
**Figure S11.** Representative FACS scatter plots showing percentage (5000 events total) of cells falling within the gated region for each experiment. Each gate was drawn to accommodate the population of events scattering significantly in the forward direction for the untreated cells.

**Figure S12.** FACS data showing HEK-293 uptake of antisense fluorescein-labeled LPA nanoparticles after 10 minute and 2 hour incubations. Histograms were constructed from gated and ungated data on a total of 5000 event counts and the x-axis is plotted as FL-1 Area ($\lambda_{ex} = 488$ nm, $\lambda_{em} = 533 \pm 15$ nm).
Figure S13. FACS data showing HeLa uptake of fluorescein antisense LPA (FL-AS-LPA) micelles after treatment with pharmacological inhibitors of various pathways of endocytosis. FACS histograms (left) display only untreated, LPA micelle-treated, and HeLa cells treated with methyl-β-cyclodextrin and LPA micelle. Treatment of HeLa cells with other known inhibitors of endocytosis did not show a significant difference in LPA micelle uptake as compared to incubation of HeLa cells with LPA micelles only. Untreated cells were not incubated with inhibitor or LPA micelle in order to serve as a reference for minimum cell-associated fluorescence. HeLa cells were incubated with inhibitor for 30 minutes at 37 °C in 5% CO₂ followed by 5 nM LPA micelle for 1 hour at 37 °C in 5% CO₂. FACS histograms are displayed as ungated (top) and gated (bottom) data (see Fig. S14) and are based on 2500 total event counts for each experimental condition with the x-axis plotted as FL-1 Area ($\lambda_{ex} = 488$ nm, $\lambda_{em} = 533 +/- 15$ nm).
Figure S14. FACS scattering analysis of HeLa cells after treatment with endocytosis inhibitors and/or fluorescein antisense LPA (FL-AS-LPA) micelles. Gates for relevant experiments are drawn as pink polygons and the percentage of the total cell population (2500 events total) within the gate is displayed.
Supporting Information

Table S4. Tabulated data for pharmacological inhibition of endocytosis effect on LPA micelle uptake in HeLa cells.

| Treatment | exp 1 | exp 2 | exp 3 | average | std. dev. |
|-----------|-------|-------|-------|---------|-----------|
| Micelle   | 15.4  | 16.7  | 14.7  | 15.6    | 1.02      |
| MBCD      | 8.10  | 8.57  | 6.89  | 7.85    | 0.87      |
| FCD       | 11.2  | 15.1  | 11.7  | 12.7    | 2.14      |
| POLY I    | 13.4  | 13.3  | 9.00  | 11.9    | 2.51      |
| NaN$_3$   | 14.9  | 16.3  | 12.5  | 14.6    | 1.94      |
| FPN III   | 18.2  | 3.32  | 12.8  | 11.4    | 7.53      |
| PLC       | 19.2  | 19.0  | 15.1  | 17.8    | 2.33      |
| Untreated | 7.97  | 7.60  | 7.31  | 7.63    | 0.33      |

*values reported as mean FL-1 area of ungated population divided by 1x10$^3$

Mean Ungated Fluorescein Fluorescence/Cell* via FACS

| Treatment | exp 1 | exp 2 | exp 3 | average | std. dev. |
|-----------|-------|-------|-------|---------|-----------|
| Micelle   | 22.4  | 18.4  | 16.8  | 19.2    | 2.87      |
| MBCD      | 13.2  | 13.6  | 11.6  | 12.8    | 1.05      |
| FCD       | 18.3  | 14.4  | 13.4  | 15.4    | 2.60      |
| POLY I    | 15.4  | 16.5  | 12.5  | 14.8    | 2.11      |
| NaN$_3$   | 20.0  | 17.5  | 14.5  | 17.3    | 2.72      |
| FPN III   | 23.0  | 21.3  | 16.3  | 20.2    | 3.50      |
| PLC       | 26.2  | 20.7  | 17.4  | 21.4    | 4.43      |
| Untreated | 10.0  | 10.4  | 10.1  | 10.2    | 0.22      |

*values reported as mean FL-1 area of gated population divided by 1x10$^3$

Mean Gated Fluorescein Fluorescence/Cell* via FACS

Table S5. Pharmacological inhibitor concentrations for HeLa treatment.

| Inhibitor       | Stock                   | Experimental | Volume (µl) in 250 µl |
|-----------------|-------------------------|--------------|----------------------|
| MβCD            | 250 mg/ml in H$_2$O    | 12.5 µg/ml   | 12.5                 |
| Filipin III     | 2 mg/ml in DMSO diluted to 0.28 mg/ml with DPBS | 2.5 µg/ml | 2.23 |
| Phospholipase C | 5 units/ml in DPBS      | 0.5 units/ml | 25.0                 |
| Sodium Azide    | 1 M in H$_2$O          | 50 mM        | 12.5                 |
| Fucoidan        | 0.83 mg/ml in H$_2$O:DPBS, 10:2 | 50 µg/ml   | 15.0                 |
| Polyinosinic acid | 1 mg/ml in DPBS       | 50 µg/ml     | 12.5                 |
Figure S15. FACS cytotoxicity analysis measured via fluorescence of propidium iodide incorporated into compromised HeLa cells. HeLa cells were incubated with 5 nM LPA micelle for 1 hour at 37 °C in 5% CO₂. FACS data displays 5000 event counts for each experimental condition. The cells contained within the gated area (pink polygon) are denoted as a percent of the total event count. The histogram data is not gated (compromised cells do not fall within the same scattering region) and displays FL-3 Area as the x-axis (λ<sub>ex</sub> = 488 nm, λ<sub>em</sub> filter = 670 nm LP).

Table S6. Tabulated data for LPA micelle toxicity studies by propidium iodide fluorescence.

|          | Untreated | 5 nM Micelle | 10 nM Micelle | Triton-X |
|----------|-----------|--------------|---------------|----------|
| repeat 1 | 1.07      | 1.05         | 1.53          | 57.2     |
|          | 1.33      | 1.17         | 1.13          | 59.4     |
| repeat 2 | 2.09      | 1.11         | 1.07          | 52.4     |
|          | 1.08      | 4.73         | 1.25          | 58.0     |
| repeat 3 | 1.07      | 2.89         | 1.94          | 49.7     |
|          | 0.87      | 1.86         | 1.67          | 58.5     |
| Average  | 1.25      | 2.14         | 1.43          | 55.9     |
| Std. Dev.| 0.44      | 1.45         | 0.34          | 3.89     |

*values reported as mean FL-3 area divided by 1.0 x 10<sup>5</sup>
Supporting Information

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