

Supporting Information

Intracellular Delivery of Therapeutic Antisense Oligonucleotides Targeting mRNA Coding Mitochondrial Proteins by Cell-Penetrating Peptides

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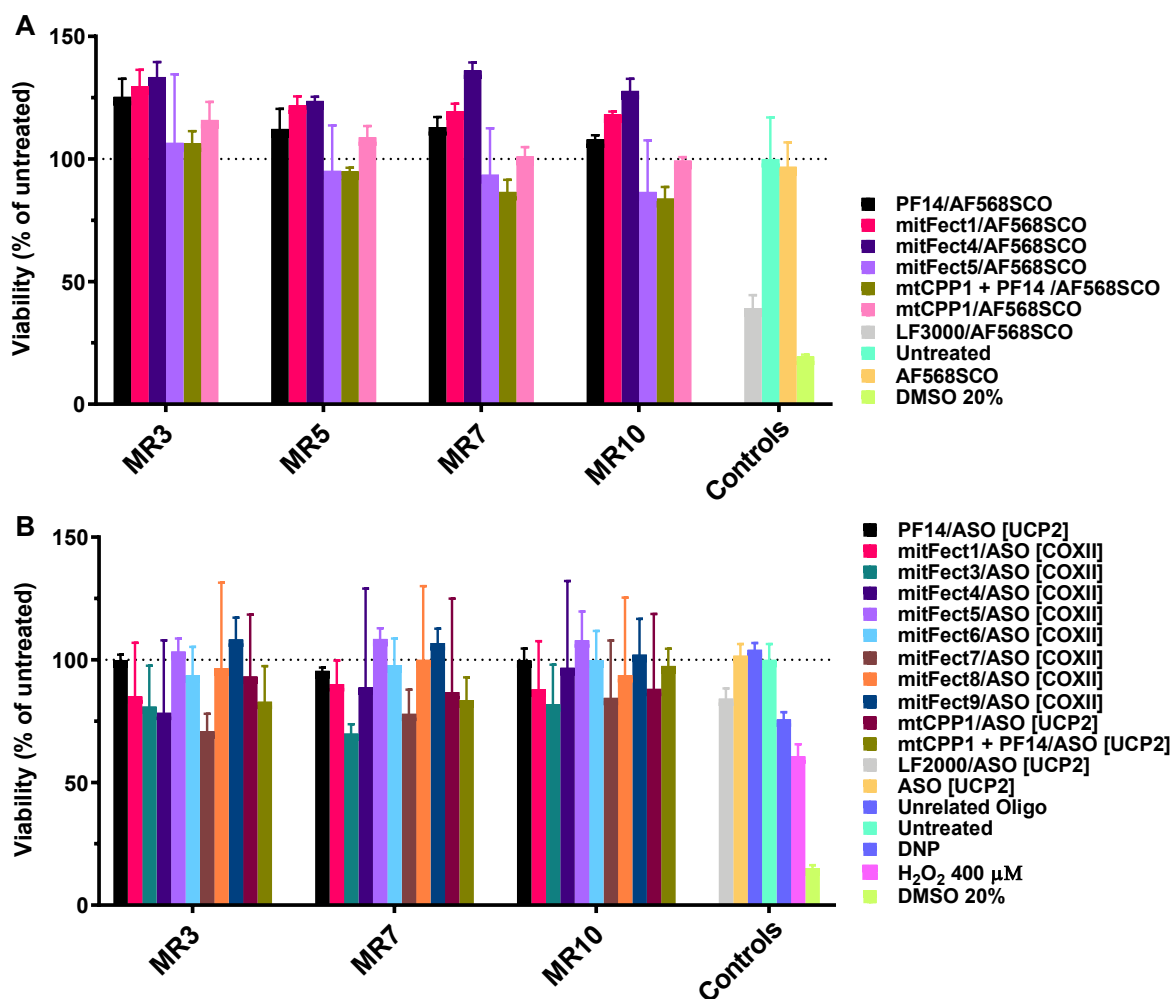


Figure S1. Effect of treatment with AF568SCO (A) or ASO [UCP2] (B) at different molar ratio on HeLa pLuc705 cells viability. HeLa pLuc705 cells were treated with peptide/oligonucleotides for 24 hr. DMSO 20% was chosen as positive controls. Treatments with any of the complexes have shown no toxicity. Data shown represent a mean of a minimum of two individual experiments performed in triplicates (n=1); SD for each data point is shown. The cell viability was quantified by conventional WST-1 assay.

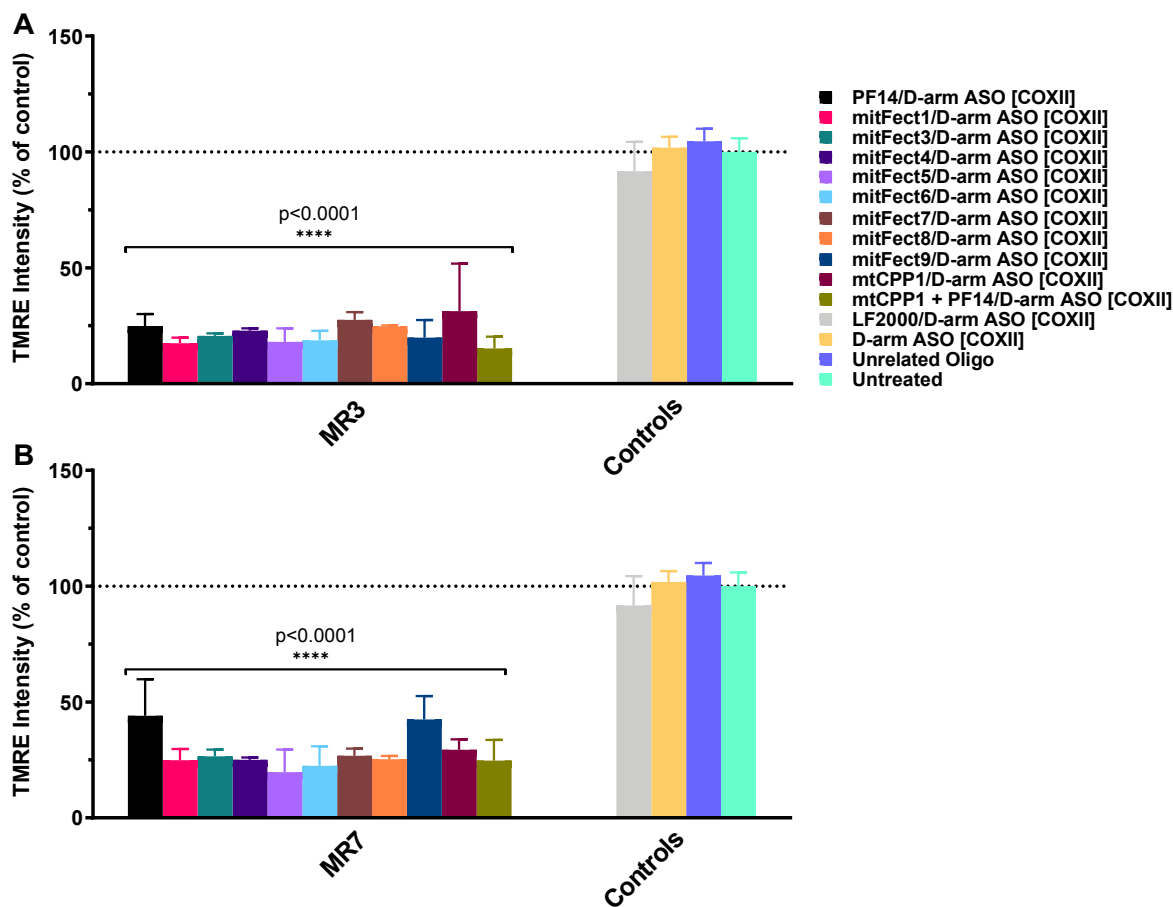


Figure S2. Effect of D-arm ASO [COXII] on mitochondrial membrane potential and intracellular ROS production in HeLa pLuc705 cells. The oligonucleotide treatment was performed as described in the materials and methods section. HeLa pLuc705 were treated for 6 h at MR3 (A) and MR7 (B) with 100 nM D-arm ASO [COXII], or LF2000, D-arm ASO [COXII] alone, or unrelated oligonucleotide, as indicated in the Materials and methods. The mitochondrial membrane potential was evaluated by TMRE uptake. Results are normalized and expressed as percentage of results obtained from untreated cells. The values represent the mean of one experiment performed in triplicate (mean \pm SD, n=1).

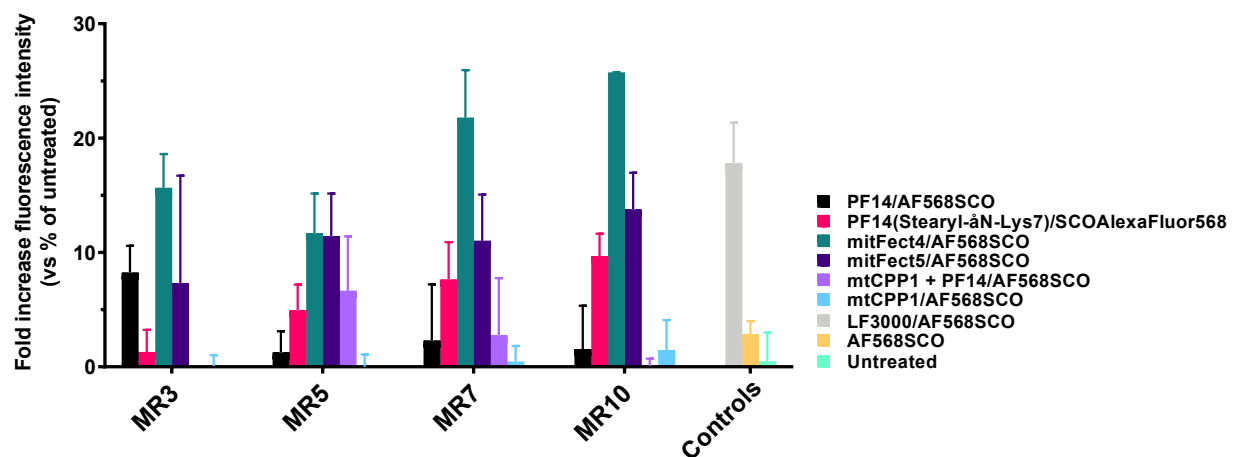


Figure S3. Uptake of SCOAlexaFluor568 in HeLa pLuc705. HeLa pLuc705 cells (7×10^3) were seeded 24 h prior to experiments into 96-well plates. Cells were treated with peptide/AF568-labeled SCO nanocomplexes at MR3, 5, 7, and 10 and incubated for 24 h. Cells were washed with twice with fresh complete media and fluorescence was measured at 578/603 nm. The values represent the mean of one experiment performed in triplicate (mean \pm SD, n=1).

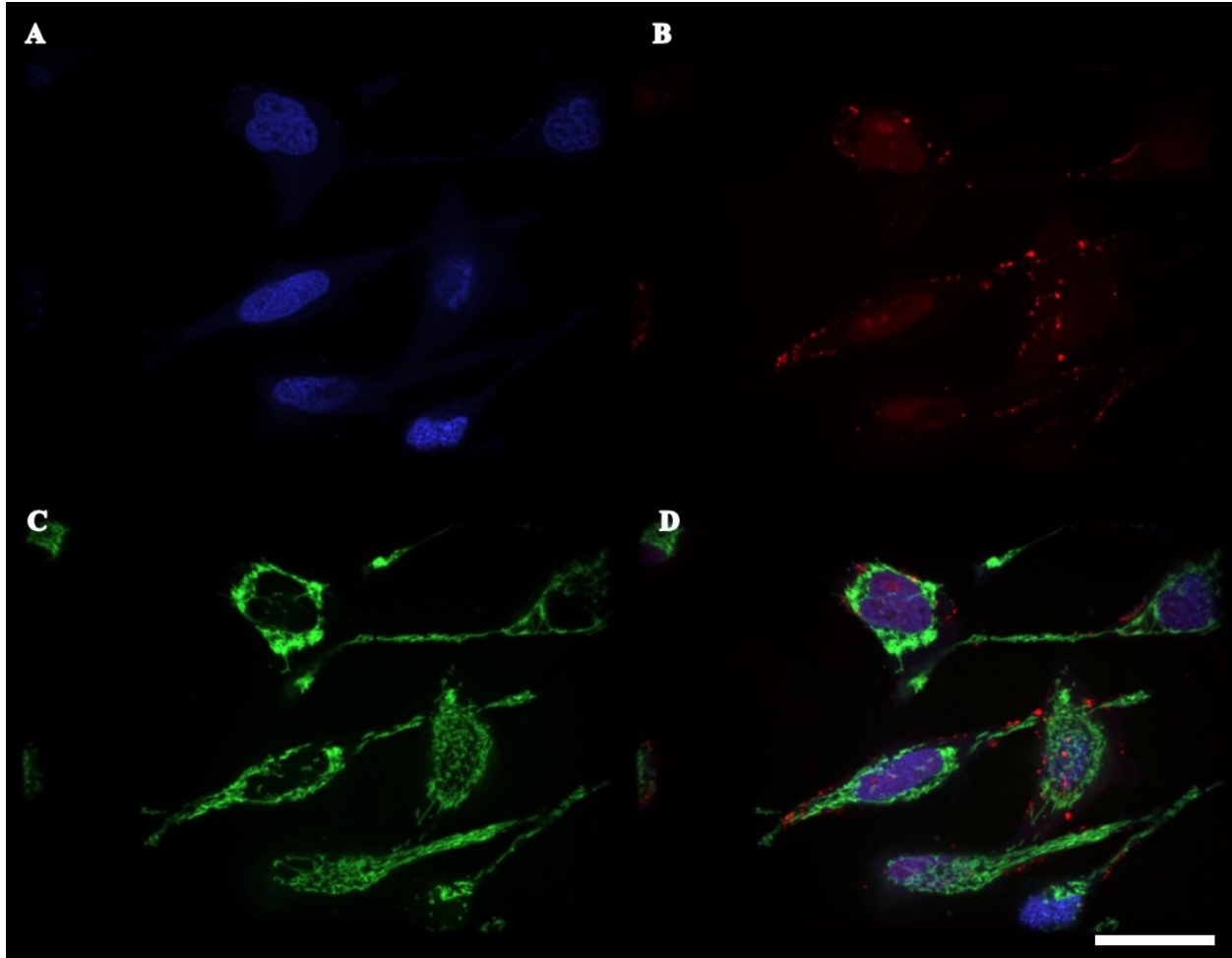


Figure S4. Localization of mitFect3/SCO complexes. HeLa pLuc705 cells were incubated with complexes of 100 nM AF568SCO (red, B) and 1 μ M mitFect3 for 1 h at 37 $^{\circ}$ C. Mitochondria were stained using MitoTracker Green (green, C) and nuclei using Draq5 (blue, A). Overlay picture is presented in section D. Scale bar = 10 μ m.

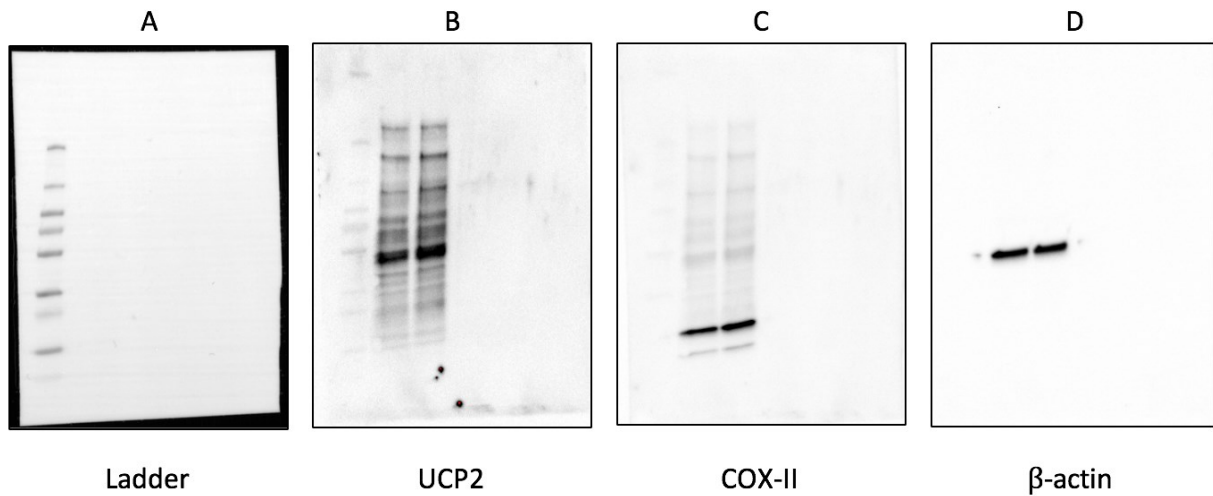


Figure S5. Complete pictures of the western blot membranes. Each blot comes from the same gel and the immunostaining was performed in the following order: B, C and D. The membrane was stripped from antibody between each staining.

Table S1. Complete statistical data analysis for Fig. 1. Ordinary two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test of PF14/SCO vs. each other complex formulation at MR3, 5, and 10.

Dunnett's multiple comparisons test	Mean Diff.	95% CI of diff.	Summary	Adjusted P Value
MR3				
PF14/SCO vs. mitFect1/SCO	12038	3878 to 20197	***	0.001
PF14/SCO vs. mitFect3/SCO	14303	6143 to 22463	****	<0.0001
PF14/SCO vs. mitFect4/SCO	24647	16487 to 32807	****	<0.0001
PF14/SCO vs. mitFect5/SCO	26594	18434 to 34754	****	<0.0001
PF14/SCO vs. mitFect6/SCO	26178	18019 to 34338	****	<0.0001
PF14/SCO vs. mitFect7/SCO	23589	15429 to 31749	****	<0.0001
PF14/SCO vs. mitFect8/SCO	22355	14196 to 30515	****	<0.0001
PF14/SCO vs. mitFect9/SCO	26797	18637 to 34956	****	<0.0001
PF14/SCO vs. mtCPP1/SCO	27050	18891 to 35210	****	<0.0001
PF14/SCO vs. mtCPP1 + PF14/SCO	23636	15476 to 31796	****	<0.0001
Dunnett's multiple comparisons test	Mean Diff.	95% CI of diff.	Summary	Adjusted P Value
MR5				
PF14/SCO vs. mitFect1/SCO	-21527	-29686 to -13367	****	<0.0001
PF14/SCO vs. mitFect3/SCO	-10065	-18224 to -1905	**	0.0083
PF14/SCO vs. mitFect4/SCO	34614	26454 to 42774	****	<0.0001
PF14/SCO vs. mitFect5/SCO	42106	33946 to 50266	****	<0.0001
PF14/SCO vs. mitFect6/SCO	40834	32675 to 48994	****	<0.0001
PF14/SCO vs. mitFect7/SCO	33961	25802 to 42121	****	<0.0001
PF14/SCO vs. mitFect8/SCO	28863	20703 to 37023	****	<0.0001
PF14/SCO vs. mitFect9/SCO	41963	33803 to 50122	****	<0.0001
PF14/SCO vs. mtCPP1/SCO	42508	34348 to 50667	****	<0.0001
PF14/SCO vs. mtCPP1 + PF14/SCO	-20364	-28523 to -12204	****	<0.0001
Dunnett's multiple comparisons test	Mean Diff.	95% CI of diff.	Summary	Adjusted P Value
MR10				
PF14/SCO vs. mitFect1/SCO	-20426	-28586 to -12266	****	<0.0001
PF14/SCO vs. mitFect3/SCO	15845	7685 to 24004	****	<0.0001
PF14/SCO vs. mitFect4/SCO	58378	50218 to 66537	****	<0.0001
PF14/SCO vs. mitFect5/SCO	66328	58169 to 74488	****	<0.0001
PF14/SCO vs. mitFect6/SCO	65148	56988 to 73307	****	<0.0001
PF14/SCO vs. mitFect7/SCO	54462	46303 to 62622	****	<0.0001
PF14/SCO vs. mitFect8/SCO	54918	46759 to 63078	****	<0.0001
PF14/SCO vs. mitFect9/SCO	66889	58729 to 75049	****	<0.0001
PF14/SCO vs. mtCPP1/SCO	66223	58063 to 74382	****	<0.0001
PF14/SCO vs. mtCPP1 + PF14/SCO	25211	17052 to 33371	****	<0.0001

Table S2a. Complete statistical data analysis for Fig. 2a. Ordinary two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test of untreated vs. each complex formulation at MR3, 7, and 10.

Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR3				
Untreated vs. PF14/ASO [UCP2]	-6.124	-48.07 to 35.82	ns	0.9994
Untreated vs. mitFect1/ASO [UCP2]	-28.68	-70.62 to 13.26	ns	0.3731
Untreated vs. mitFect3/ASO [UCP2]	-82.4	-124.3 to -40.46	****	<0.0001
Untreated vs. mitFect4/ASO [UCP2]	-108.6	-150.5 to -66.63	****	<0.0001
Untreated vs. mitFect5/ASO [UCP2]	-21.16	-63.10 to 20.78	ns	0.758
Untreated vs. mitFect6/ASO [UCP2]	-43.97	-85.92 to -2.033	*	0.0342
Untreated vs. mitFect7/ASO [UCP2]	-101.8	-143.8 to -59.87	****	<0.0001
Untreated vs. mitFect8/ASO [UCP2]	-126.6	-168.5 to -84.65	****	<0.0001
Untreated vs. mitFect9/ASO [UCP2]	-39.76	-81.70 to 2.179	ns	0.0737
Untreated vs. mtCPP1/ASO [UCP2]	-51.9	-93.84 to -9.957	**	0.0065
Untreated vs. mtCPP1 + PF14/ASO [UCP2]	-44.23	-86.18 to -2.294	*	0.0324
Untreated vs. LF2000/ASO [UCP2]	-22.2	-64.14 to 19.74	ns	0.7036
Untreated vs. ASO [UCP2]	-34.43	-76.37 to 7.513	ns	0.1736
Untreated vs. Unrelated Oligo	-17.6	-59.54 to 24.34	ns	0.9103
Untreated vs. DNP 150 μ M	-18.71	-60.65 to 23.23	ns	0.8702
Untreated vs. H ₂ O ₂ 400 μ M	-25.84	-67.78 to 16.10	ns	0.509
Untreated vs. H ₂ O ₂ 800 μ M	-38.55	-80.49 to 3.394	ns	0.0907
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR7				
Untreated vs. PF14/ASO [UCP2]	-32.59	-74.53 to 9.347	ns	0.2259
Untreated vs. mitFect1/ASO [UCP2]	-60.88	-102.8 to -18.94	***	0.0008
Untreated vs. mitFect3/ASO [UCP2]	-120.8	-162.7 to -78.86	****	<0.0001
Untreated vs. mitFect4/ASO [UCP2]	-168.3	-210.2 to -126.4	****	<0.0001
Untreated vs. mitFect5/ASO [UCP2]	-35.13	-77.07 to 6.809	ns	0.1562
Untreated vs. mitFect6/ASO [UCP2]	-48.31	-90.25 to -6.367	*	0.0143
Untreated vs. mitFect7/ASO [UCP2]	-82.89	-124.8 to -40.95	****	<0.0001
Untreated vs. mitFect8/ASO [UCP2]	-94.92	-136.9 to -52.98	****	<0.0001
Untreated vs. mitFect9/ASO [UCP2]	-21.78	-63.72 to 20.16	ns	0.7258
Untreated vs. mtCPP1/ASO [UCP2]	-106.2	-148.1 to -64.22	****	<0.0001
Untreated vs. mtCPP1 + PF14/ASO [UCP2]	-52.86	-94.80 to -10.92	**	0.0052
Untreated vs. LF2000/ASO [UCP2]	-22.2	-64.14 to 19.74	ns	0.7036
Untreated vs. ASO [UCP2]	-34.43	-76.37 to 7.513	ns	0.1736
Untreated vs. Unrelated Oligo	-17.6	-59.54 to 24.34	ns	0.9103
Untreated vs. DNP 150 μ M	-18.71	-60.65 to 23.23	ns	0.8702

Untreated vs. H ₂ O ₂ 400 μM	-25.84	-67.78 to 16.10	ns	0.509
Untreated vs. H ₂ O ₂ 800 μM	-38.55	-80.49 to 3.394	ns	0.0907
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR10				
Untreated vs. PF14/ASO [UCP2]	-68.14	-110.1 to -26.15	***	0.0001
Untreated vs. mitFect1/ASO [UCP2]	-96.46	-138.4 to -54.47	****	<0.0001
Untreated vs. mitFect3/ASO [UCP2]	-140.4	-182.4 to -98.43	****	<0.0001
Untreated vs. mitFect4/ASO [UCP2]	-105.8	-152.8 to -58.88	****	<0.0001
Untreated vs. mitFect5/ASO [UCP2]	-28.98	-70.96 to 13.01	ns	0.3633
Untreated vs. mitFect6/ASO [UCP2]	-59.56	-101.5 to -17.57	**	0.0011
Untreated vs. mitFect7/ASO [UCP2]	-110.2	-152.2 to -68.26	****	<0.0001
Untreated vs. mitFect8/ASO [UCP2]	-97.39	-139.4 to -55.40	****	<0.0001
Untreated vs. mitFect9/ASO [UCP2]	-31.4	-73.39 to 10.58	ns	0.2676
Untreated vs. mtCPP1/ASO [UCP2]	-109.1	-151.1 to -67.12	****	<0.0001
Untreated vs. mtCPP1 + PF14/ASO [UCP2]	-55.27	-97.25 to -13.29	**	0.003
Untreated vs. LF2000/ASO [UCP2]	-22.2	-64.19 to 19.78	ns	0.7077
Untreated vs. ASO [UCP2]	-34.43	-76.41 to 7.556	ns	0.1752
Untreated vs. Unrelated Oligo	-17.6	-59.58 to 24.39	ns	0.9126
Untreated vs. DNP 150 μM	-18.71	-60.69 to 23.28	ns	0.8732
Untreated vs. H ₂ O ₂ 400 μM	-25.84	-67.82 to 16.15	ns	0.5132
Untreated vs. H ₂ O ₂ 800 μM	-38.55	-80.53 to 3.437	ns	0.0915

Table S2b. Complete statistical data analysis for Fig. 2b. Ordinary two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test of untreated vs. each complex formulation at MR3, 7, and 10.

Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR3				
Untreated vs. PF14/ASO [UCP2]	-20648	-82662 to 41365	ns	0.9854
Untreated vs. mitFect1/ASO [UCP2]	-15446	-77460 to 46568	ns	0.9958
Untreated vs. mitFect3/ASO [UCP2]	-57705	-119719 to 4308	ns	0.0836
Untreated vs. mitFect4/ASO [UCP2]	-28705	-90719 to 33308	ns	0.8406
Untreated vs. mitFect5/ASO [UCP2]	-86551	-148564 to -24537	**	0.0014
Untreated vs. mitFect6/ASO [UCP2]	-161043	-223057 to -99029	****	<0.0001
Untreated vs. mitFect7/ASO [UCP2]	-213681	-275694 to -151667	****	<0.0001
Untreated vs. mitFect8/ASO [UCP2]	-167412	-229426 to -105398	****	<0.0001
Untreated vs. mitFect9/ASO [UCP2]	-128684	-190698 to -66670	****	<0.0001
Untreated vs. mtCPP1/ASO [UCP2]	-161599	-223613 to -99585	****	<0.0001
Untreated vs. mtCPP1 + PF14/ASO [UCP2]	50202	-11812 to 112215	ns	0.1861

Untreated vs. LF2000/ASO [UCP2]	46285	-15728 to 108299	ns	0.2686
Untreated vs. ASO [UCP2]	8809	-53205 to 70822	ns	0.9994
Untreated vs. Unrelated Oligo	7574	-54440 to 69588	ns	0.9995
Untreated vs. DNP 150 μ M	9464	-52550 to 71477	ns	0.9994
Untreated vs. H ₂ O ₂ 400 μ M	24464	-37549 to 86478	ns	0.9406
Untreated vs. DMSO 20%	109765	47752 to 171779	****	<0.0001
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR7				
Untreated vs. PF14/ASO [UCP2]	-32333	-101737 to 37072	ns	0.8378
Untreated vs. mitFect1/ASO [UCP2]	-95494	-157571 to -33417	***	0.0003
Untreated vs. mitFect3/ASO [UCP2]	-187566	-249644 to -125489	****	<0.0001
Untreated vs. mitFect4/ASO [UCP2]	-151652	-213729 to -89575	****	<0.0001
Untreated vs. mitFect5/ASO [UCP2]	-127785	-189863 to -65708	****	<0.0001
Untreated vs. mitFect6/ASO [UCP2]	-161134	-223212 to -99057	****	<0.0001
Untreated vs. mitFect7/ASO [UCP2]	-212318	-274395 to -150240	****	<0.0001
Untreated vs. mitFect8/ASO [UCP2]	-172691	-234768 to -110614	****	<0.0001
Untreated vs. mitFect9/ASO [UCP2]	-49142	-111219 to 12935	ns	0.2082
Untreated vs. mtCPP1/ASO [UCP2]	-186313	-248390 to -124236	****	<0.0001
Untreated vs. mtCPP1 + PF14/ASO [UCP2]	-86682	-148760 to -24605	**	0.0013
Untreated vs. LF2000/ASO [UCP2]	46285	-15792 to 108363	ns	0.271
Untreated vs. ASO [UCP2]	8809	-53269 to 70886	ns	0.9994
Untreated vs. Unrelated Oligo	7574	-54503 to 69651	ns	0.9995
Untreated vs. DNP 150 μ M	9464	-52614 to 71541	ns	0.9994
Untreated vs. H ₂ O ₂ 400 μ M	24464	-37613 to 86542	ns	0.9424
Untreated vs. DMSO 20%	109765	47688 to 171843	****	<0.0001
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR10				
Untreated vs. PF14/ASO [UCP2]	-44880	-114284 to 24525	ns	0.4475
Untreated vs. mitFect1/ASO [UCP2]	-181422	-243500 to -119345	****	<0.0001
Untreated vs. mitFect3/ASO [UCP2]	-208855	-270932 to -146777	****	<0.0001
Untreated vs. mitFect4/ASO [UCP2]	-159915	-221993 to -97838	****	<0.0001
Untreated vs. mitFect5/ASO [UCP2]	-95383	-157461 to -33306	***	0.0003
Untreated vs. mitFect6/ASO [UCP2]	-158735	-220812 to -96658	****	<0.0001
Untreated vs. mitFect7/ASO [UCP2]	-236400	-298477 to -174323	****	<0.0001
Untreated vs. mitFect8/ASO [UCP2]	-177261	-239338 to -115184	****	<0.0001
Untreated vs. mitFect9/ASO [UCP2]	-96518	-158595 to -34441	***	0.0003
Untreated vs. mtCPP1/ASO [UCP2]	-116071	-178149 to -53994	****	<0.0001
Untreated vs. mtCPP1 + PF14/ASO [UCP2]	-150884	-212962 to -88807	****	<0.0001

Untreated vs. LF2000/ASO [UCP2]	46285	-15792 to 108363	ns	0.271
Untreated vs. ASO [UCP2]	8809	-53269 to 70886	ns	0.9994
Untreated vs. Unrelated Oligo	7574	-54503 to 69651	ns	0.9995
Untreated vs. DNP 150 μ M	9464	-52614 to 71541	ns	0.9994
Untreated vs. H ₂ O ₂ 400 μ M	24464	-37613 to 86542	ns	0.9424
Untreated vs. DMSO 20%	109765	47688 to 171843	****	<0.0001

Table S2c. Complete statistical data analysis for Fig. 2c. Ordinary two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test of untreated vs. each complex formulation at MR3, 7, and 10.

Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Summary	Adjusted P Value
MR3				
Untreated vs. PF14/ASO [UCP 2]	-0.6348	-15.24 to 13.97	ns	0.9998
Untreated vs. mitFect1/ASO [UCP2]	-5.803	-20.40 to 8.797	ns	0.8827
Untreated vs. mitFect3/ASO [UCP2]	-23.72	-38.32 to -9.117	***	0.0005
Untreated vs. mitFect4/ASO [UCP2]	-24.24	-38.84 to -9.640	***	0.0004
Untreated vs. mitFect5/ASO [UCP2]	-4.15	-18.75 to 10.45	ns	0.9875
Untreated vs. mitFect6/ASO [UCP2]	-21.55	-36.15 to -6.948	**	0.0015
Untreated vs. mitFect7/ASO [UCP2]	-30.5	-45.10 to -15.90	****	<0.0001
Untreated vs. mitFect8/ASO [UCP2]	-36.96	-51.56 to -22.36	****	<0.0001
Untreated vs. mitFect9/ASO [UCP2]	-7.444	-22.04 to 7.157	ns	0.6657
Untreated vs. mtCPP1/ASO [UCP 2]	-22.33	-36.93 to -7.732	***	0.001
Untreated vs. mtCPP1 + PF14/ASO [UCP 2]	-16.7	-31.30 to -2.099	*	0.0183
Untreated vs. LF2000/ASO [UCP 2]	3.409	-11.19 to 18.01	ns	0.9958
Untreated vs. ASO [UCP 2]	3.602	-11.00 to 18.20	ns	0.9925
Untreated vs. Unrelated Oligo	-1.354	-15.95 to 13.25	ns	0.9996
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Summary	Adjusted P Value
MR7				
Untreated vs. PF14/ASO [UCP 2]	-0.6348	-15.24 to 13.97	ns	0.9998
Untreated vs. mitFect1/ASO [UCP2]	-5.803	-20.40 to 8.797	ns	0.8827
Untreated vs. mitFect3/ASO [UCP2]	-23.72	-38.32 to -9.117	***	0.0005
Untreated vs. mitFect4/ASO [UCP2]	-24.24	-38.84 to -9.640	***	0.0004
Untreated vs. mitFect5/ASO [UCP2]	-4.15	-18.75 to 10.45	ns	0.9875
Untreated vs. mitFect6/ASO [UCP2]	-21.55	-36.15 to -6.948	**	0.0015
Untreated vs. mitFect7/ASO [UCP2]	-30.5	-45.10 to -15.90	****	<0.0001
Untreated vs. mitFect8/ASO [UCP2]	-36.96	-51.56 to -22.36	****	<0.0001
Untreated vs. mitFect9/ASO [UCP2]	-7.444	-22.04 to 7.157	ns	0.6657
Untreated vs. mtCPP1/ASO [UCP 2]	-22.33	-36.93 to -7.732	***	0.001
Untreated vs. mtCPP1 + PF14/ASO [UCP 2]	-16.7	-31.30 to -2.099	*	0.0183
Untreated vs. LF2000/ASO [UCP 2]	3.409	-11.19 to 18.01	ns	0.9958
Untreated vs. ASO [UCP 2]	3.602	-11.00 to 18.20	ns	0.9925

Untreated vs. Unrelated Oligo	-1.354	-15.95 to 13.25	ns	0.9996
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Summary	Adjusted P Value
MR10				
Untreated vs. PF14/ASO [UCP 2]	-0.6348	-15.24 to 13.97	ns	0.9998
Untreated vs. mitFect1/ASO [UCP2]	-5.803	-20.40 to 8.797	ns	0.8827
Untreated vs. mitFect3/ASO [UCP2]	-23.72	-38.32 to -9.117	***	0.0005
Untreated vs. mitFect4/ASO [UCP2]	-24.24	-38.84 to -9.640	***	0.0004
Untreated vs. mitFect5/ASO [UCP2]	-4.15	-18.75 to 10.45	ns	0.9875
Untreated vs. mitFect6/ASO [UCP2]	-21.55	-36.15 to -6.948	**	0.0015
Untreated vs. mitFect7/ASO [UCP2]	-30.5	-45.10 to -15.90	****	<0.0001
Untreated vs. mitFect8/ASO [UCP2]	-36.96	-51.56 to -22.36	****	<0.0001
Untreated vs. mitFect9/ASO [UCP2]	-7.444	-22.04 to 7.157	ns	0.6657
Untreated vs. mtCPP1/ASO [UCP 2]	-22.33	-36.93 to -7.732	***	0.001
Untreated vs. mtCPP1 +PF14/ASO [UCP 2]	-16.7	-31.30 to -2.099	*	0.0183
Untreated vs. LF2000/ASO [UCP 2]	3.409	-11.19 to 18.01	ns	0.9958
Untreated vs. ASO [UCP 2]	3.602	-11.00 to 18.20	ns	0.9925
Untreated vs. Unrelated Oligo	-1.354	-15.95 to 13.25	ns	0.9996

Table S3a. Complete statistical data analysis for Fig. 3a. Ordinary two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test of untreated vs. each complex formulation at MR3, 7, and 10.

Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR3				
Untreated vs. PF14/Darm ASO [UCP2]	-23.2	-79.36 to 32.96	ns	0.919
Untreated vs. mitFect1/Darm ASO [UCP2]	-55.15	-111.3 to 1.009	ns	0.0573
Untreated vs. mitFect3/Darm ASO [UCP2]	-98.89	-155.1 to -42.73	****	<0.0001
Untreated vs. mitFect4/Darm ASO [UCP2]	-135.3	-191.5 to -79.16	****	<0.0001
Untreated vs. mitFect5/Darm ASO [UCP2]	-67.79	-124.0 to -11.63	**	0.0086
Untreated vs. mitFect6/Darm ASO [UCP2]	-90.87	-147.0 to -34.71	***	0.0001
Untreated vs. mitFect7/Darm ASO [UCP2]	-144.6	-200.8 to -88.48	****	<0.0001
Untreated vs. mitFect8/Darm ASO [UCP2]	-174.6	-230.7 to -118.4	****	<0.0001
Untreated vs. mitFect9/Darm ASO [UCP2]	-14.77	-70.93 to 41.39	ns	0.9954
Untreated vs. mtCPP1/Darm ASO [UCP2]	-22.59	-78.75 to 33.57	ns	0.9322
Untreated vs. mtCPP1 + PF14/Darm ASO [UCP2]	-24.01	-80.18 to 32.15	ns	0.8991
Untreated vs. LF2000/Darm ASO [UCP2]	-52.23	-108.4 to 3.931	ns	0.0839
Untreated vs. Darm ASO [UCP2]	-71.28	-127.4 to -15.11	**	0.0048
Untreated vs. Unrelated Oligo	1.436	-54.73 to 57.60	ns	>0.9999
Untreated vs. DNP 150 μ M	-68.6	-124.8 to -12.43	**	0.0076
Untreated vs. H ₂ O ₂ 400 μ M	-78.13	-134.3 to -21.97	**	0.0014
Untreated vs. H ₂ O ₂ 800 μ M	-50.91	-107.1 to 5.250	ns	0.0991
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR7				
Untreated vs. PF14/Darm ASO [UCP2]	-49.4	-105.6 to 6.761	ns	0.119
Untreated vs. mitFect1/Darm ASO [UCP2]	-91.75	-147.9 to -35.59	***	0.0001
Untreated vs. mitFect3/Darm ASO [UCP2]	-147.2	-203.4 to -91.05	****	<0.0001
Untreated vs. mitFect4/Darm ASO [UCP2]	-201	-257.1 to -144.8	****	<0.0001
Untreated vs. mitFect5/Darm ASO [UCP2]	-71.23	-127.4 to -15.07	**	0.0048
Untreated vs. mitFect6/Darm ASO [UCP2]	-113	-169.2 to -56.84	****	<0.0001
Untreated vs. mitFect7/Darm ASO [UCP2]	-184.9	-241.1 to -128.8	****	<0.0001
Untreated vs. mitFect8/Darm ASO [UCP2]	-186.3	-242.5 to -130.2	****	<0.0001
Untreated vs. mitFect9/Darm ASO [UCP2]	2.833	-53.33 to 58.99	ns	0.9998
Untreated vs. mtCPP1/Darm ASO [UCP2]	-48.85	-105.0 to 7.309	ns	0.1269
Untreated vs. mtCPP1 + PF14/Darm ASO [UCP2]	-38.65	-94.81 to 17.51	ns	0.3653
Untreated vs. LF2000/Darm ASO [UCP2]	-52.23	-108.4 to 3.931	ns	0.0839
Untreated vs. Darm ASO [UCP2]	-71.28	-127.4 to -15.11	**	0.0048
Untreated vs. Unrelated Oligo	1.436	-54.73 to 57.60	ns	>0.9999
Untreated vs. DNP 150 μ M	-68.6	-124.8 to -12.43	**	0.0076

Untreated vs. H ₂ O ₂ 400 μM	-78.13	-134.3 to -21.97	**	0.0014
Untreated vs. H ₂ O ₂ 800 μM	-50.91	-107.1 to 5.250	ns	0.0991
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR10				
Untreated vs. PF14/Darm ASO [UCP2]	-130	-186.1 to -73.81	****	<0.0001
Untreated vs. mitFect1/Darm ASO [UCP2]	-146.3	-202.4 to -90.12	****	<0.0001
Untreated vs. mitFect3/Darm ASO [UCP2]	-191.9	-248.1 to -135.8	****	<0.0001
Untreated vs. mitFect4/Darm ASO [UCP2]	-203.6	-259.8 to -147.5	****	<0.0001
Untreated vs. mitFect5/Darm ASO [UCP2]	-90.28	-146.4 to -34.12	***	0.0001
Untreated vs. mitFect6/Darm ASO [UCP2]	-152.7	-208.9 to -96.55	****	<0.0001
Untreated vs. mitFect7/Darm ASO [UCP2]	-236.9	-293.0 to -180.7	****	<0.0001
Untreated vs. mitFect8/Darm ASO [UCP2]	-201.8	-257.9 to -145.6	****	<0.0001
Untreated vs. mitFect9/Darm ASO [UCP2]	-9.55	-65.71 to 46.61	ns	0.9993
Untreated vs. mtCPP1/Darm ASO [UCP2]	-92.48	-148.6 to -36.32	****	<0.0001
Untreated vs. mtCPP1 + PF14/Darm ASO [UCP2]	-100.8	-156.9 to -44.62	****	<0.0001
Untreated vs. LF2000/Darm ASO [UCP2]	-52.23	-108.4 to 3.931	ns	0.0839
Untreated vs. Darm ASO [UCP2]	-71.28	-127.4 to -15.11	**	0.0048
Untreated vs. Unrelated Oligo	1.436	-54.73 to 57.60	ns	>0.9999
Untreated vs. DNP 150 μM	-68.6	-124.8 to -12.43	**	0.0076
Untreated vs. H ₂ O ₂ 400 μM	-78.13	-134.3 to -21.97	**	0.0014
Untreated vs. H ₂ O ₂ 800 μM	-50.91	-107.1 to 5.250	ns	0.0991

Table S3b. Complete statistical data analysis for Fig. 3b. Ordinary two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test of untreated vs. each complex formulation at MR3, 7, and 10.

Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR3				
Untreated vs. PF14/Darm ASO [UCP2]	-122.7	-235.9 to -9.537	*	0.0255
Untreated vs. mitFect1/Darm ASO [UCP2]	-276.5	-389.7 to -163.3	****	<0.0001
Untreated vs. mitFect3/Darm ASO [UCP2]	-380.5	-493.7 to -267.3	****	<0.0001
Untreated vs. mitFect4/Darm ASO [UCP2]	-471.4	-584.6 to -358.2	****	<0.0001
Untreated vs. mitFect5/Darm ASO [UCP2]	-125.2	-238.4 to -12.03	*	0.0212
Untreated vs. mitFect6/Darm ASO [UCP2]	-137.2	-250.4 to -24.00	**	0.0083
Untreated vs. mitFect7/Darm ASO [UCP2]	-187.5	-300.7 to -74.26	****	<0.0001
Untreated vs. mitFect8/Darm ASO [UCP2]	-306.2	-419.4 to -193.0	****	<0.0001
Untreated vs. mitFect9/Darm ASO [UCP2]	-61.11	-174.3 to 52.08	ns	0.6799
Untreated vs. mtCPP1/Darm ASO [UCP2]	-81.59	-194.8 to 31.60	ns	0.3086
Untreated vs. mtCPP1 + PF14/Darm ASO [UCP2]	-155.8	-269.0 to -42.57	**	0.0017

Untreated vs. LF2000/Darm ASO [UCP2]	-69.52	-182.7 to 43.67	ns	0.5137
Untreated vs. Darm ASO [UCP2]	-150.5	-263.7 to -37.35	**	0.0027
Untreated vs. Unrelated Oligo	-28.82	-142.0 to 84.37	ns	0.9957
Untreated vs. DNP 150 μ M	-164.9	-278.1 to -51.70	***	0.0007
Untreated vs. H ₂ O ₂ 400 μ M	-181.9	-295.1 to -68.73	***	0.0001
Untreated vs. H ₂ O ₂ 800 μ M	-46.79	-160.0 to 66.40	ns	0.9184
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR7				
Untreated vs. PF14/Darm ASO [UCP2]	-145.8	-258.9 to -32.56	**	0.004
Untreated vs. mitFect1/Darm ASO [UCP2]	-448.9	-562.1 to -335.7	****	<0.0001
Untreated vs. mitFect3/Darm ASO [UCP2]	-426.7	-539.9 to -313.5	****	<0.0001
Untreated vs. mitFect4/Darm ASO [UCP2]	-620	-733.2 to -506.8	****	<0.0001
Untreated vs. mitFect5/Darm ASO [UCP2]	-123.7	-236.9 to -10.54	*	0.0237
Untreated vs. mitFect6/Darm ASO [UCP2]	-160.6	-273.8 to -47.38	**	0.0011
Untreated vs. mitFect7/Darm ASO [UCP2]	-232.6	-345.8 to -119.4	****	<0.0001
Untreated vs. mitFect8/Darm ASO [UCP2]	-230.5	-343.7 to -117.3	****	<0.0001
Untreated vs. mitFect9/Darm ASO [UCP2]	-32.49	-145.7 to 80.71	ns	0.9917
Untreated vs. mtCPP1/Darm ASO [UCP2]	-130.5	-243.7 to -17.33	*	0.0141
Untreated vs. mtCPP1 + PF14/Darm ASO [UCP2]	-87.02	-200.2 to 26.18	ns	0.237
Untreated vs. LF2000/Darm ASO [UCP2]	-69.52	-182.7 to 43.67	ns	0.5137
Untreated vs. Darm ASO [UCP2]	-150.5	-263.7 to -37.35	**	0.0027
Untreated vs. Unrelated Oligo	-28.82	-142.0 to 84.37	ns	0.9957
Untreated vs. DNP 150 μ M	-164.9	-278.1 to -51.70	***	0.0007
Untreated vs. H ₂ O ₂ 400 μ M	-181.9	-295.1 to -68.73	***	0.0001
Untreated vs. H ₂ O ₂ 800 μ M	-46.79	-160.0 to 66.40	ns	0.9184
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR10				
Untreated vs. PF14/Darm ASO [UCP2]	-207	-320.3 to -93.67	****	<0.0001
Untreated vs. mitFect1/Darm ASO [UCP2]	-454.4	-567.7 to -341.1	****	<0.0001
Untreated vs. mitFect3/Darm ASO [UCP2]	-550.1	-663.4 to -436.8	****	<0.0001
Untreated vs. mitFect4/Darm ASO [UCP2]	-739.3	-866.0 to -612.6	****	<0.0001
Untreated vs. mitFect5/Darm ASO [UCP2]	-141.5	-254.8 to -28.15	**	0.0058
Untreated vs. mitFect6/Darm ASO [UCP2]	-207.4	-320.7 to -94.11	****	<0.0001
Untreated vs. mitFect7/Darm ASO [UCP2]	-280.3	-393.6 to -166.9	****	<0.0001
Untreated vs. mitFect8/Darm ASO [UCP2]	-217.6	-330.9 to -104.3	****	<0.0001
Untreated vs. mitFect9/Darm ASO [UCP2]	-62.84	-176.2 to 50.47	ns	0.6499
Untreated vs. mtCPP1/Darm ASO [UCP2]	-147.7	-261.0 to -34.40	**	0.0034
Untreated vs. mtCPP1 + PF14/Darm ASO [UCP2]	-342.9	-456.2 to -229.6	****	<0.0001

Untreated vs. LF2000/Darm ASO [UCP2]	-69.52	-182.8 to 43.79	ns	0.5178
Untreated vs. Darm ASO [UCP2]	-150.5	-263.9 to -37.23	**	0.0027
Untreated vs. Unrelated Oligo	-28.82	-142.1 to 84.49	ns	0.9957
Untreated vs. DNP 150 μ M	-164.9	-278.2 to -51.59	***	0.0007
Untreated vs. H ₂ O ₂ 400 μ M	-181.9	-295.2 to -68.62	***	0.0001
Untreated vs. H ₂ O ₂ 800 μ M	-46.79	-160.1 to 66.52	ns	0.9207

Table S3c. Complete statistical data analysis for Fig. 3c. Ordinary two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test of untreated vs. each complex formulation at MR3, 7, and 10.

Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR3				
Untreated vs. PF14/Darm ASO [UCP2]	-50.62	-80.91 to -20.33	****	<0.0001
Untreated vs. mitFect1/Darm ASO [UCP2]	-64.14	-94.43 to -33.86	****	<0.0001
Untreated vs. mitFect3/Darm ASO [UCP2]	-51.45	-81.74 to -21.17	****	<0.0001
Untreated vs. mitFect4/Darm ASO [UCP2]	-53.97	-84.26 to -23.69	****	<0.0001
Untreated vs. mitFect5/Darm ASO [UCP2]	-59.64	-89.92 to -29.35	****	<0.0001
Untreated vs. mitFect6/Darm ASO [UCP2]	-90.12	-120.4 to -59.84	****	<0.0001
Untreated vs. mitFect7/Darm ASO [UCP2]	-100.4	-130.7 to -70.12	****	<0.0001
Untreated vs. mitFect8/Darm ASO [UCP2]	-90.6	-120.9 to -60.32	****	<0.0001
Untreated vs. mitFect9/Darm ASO [UCP2]	-0.3495	-30.63 to 29.94	ns	>0.9999
Untreated vs. mtCPP1/Darm ASO [UCP2]	-46.3	-76.59 to -16.02	***	0.0003
Untreated vs. mtCPP1 + PF14/Darm ASO [UCP2]	-31.79	-62.08 to -1.508	*	0.0337
Untreated vs. LF2000/Darm ASO [UCP2]	-40.51	-70.79 to -10.22	**	0.0025
Untreated vs. Darm ASO [UCP2]	-92.03	-125.9 to -58.17	****	<0.0001
Untreated vs. Unrelated Oligo	-22.22	-52.50 to 8.067	ns	0.2893
Untreated vs. DNP 150 μ M	-84.91	-115.2 to -54.63	****	<0.0001
Untreated vs. H ₂ O ₂ 400 μ M	-99.35	-129.6 to -69.06	****	<0.0001
Untreated vs. H ₂ O ₂ 800 μ M	-21.23	-51.51 to 9.057	ns	0.344
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR7				
Untreated vs. PF14/Darm ASO [UCP2]	-34.88	-65.17 to -4.597	*	0.0142
Untreated vs. mitFect1/Darm ASO [UCP2]	-86.58	-116.9 to -56.30	****	<0.0001
Untreated vs. mitFect3/Darm ASO [UCP2]	-100.9	-131.2 to -70.59	****	<0.0001
Untreated vs. mitFect4/Darm ASO [UCP2]	-82.54	-112.8 to -52.25	****	<0.0001
Untreated vs. mitFect5/Darm ASO [UCP2]	-43.79	-74.08 to -13.51	***	0.0008
Untreated vs. mitFect6/Darm ASO [UCP2]	-71.24	-101.5 to -40.95	****	<0.0001
Untreated vs. mitFect7/Darm ASO [UCP2]	-92.84	-123.1 to -62.55	****	<0.0001
Untreated vs. mitFect8/Darm ASO [UCP2]	-72.22	-102.5 to -41.94	****	<0.0001

Untreated vs. mitFect9/Darm ASO [UCP2]	19.72	-10.57 to 50.01	ns	0.4382
Untreated vs. mtCPP1/Darm ASO [UCP2]	-75.32	-105.6 to -45.04	****	<0.0001
Untreated vs. mtCPP1 + PF14/Darm ASO [UCP2]	-27.08	-57.37 to 3.205	ns	0.108
Untreated vs. LF2000/Darm ASO [UCP2]	-40.51	-70.79 to -10.22	**	0.0025
Untreated vs. Darm ASO [UCP2]	-92.03	-125.9 to -58.17	****	<0.0001
Untreated vs. Unrelated Oligo	-22.22	-52.50 to 8.067	ns	0.2893
Untreated vs. DNP 150 μ M	-84.91	-115.2 to -54.63	****	<0.0001
Untreated vs. H ₂ O ₂ 400 μ M	-99.35	-129.6 to -69.06	****	<0.0001
Untreated vs. H ₂ O ₂ 800 μ M	-21.23	-51.51 to 9.057	ns	0.344
Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95% CI of diff.	Summary	Adjusted P Value
MR10				
Untreated vs. PF14/Darm ASO [UCP2]	-31.67	-61.96 to -1.386	*	0.0349
Untreated vs. mitFect1/Darm ASO [UCP2]	-87.1	-117.4 to -56.82	****	<0.0001
Untreated vs. mitFect3/Darm ASO [UCP2]	-77.75	-108.0 to -47.47	****	<0.0001
Untreated vs. mitFect4/Darm ASO [UCP2]	-85.46	-115.7 to -55.18	****	<0.0001
Untreated vs. mitFect5/Darm ASO [UCP2]	-40.46	-70.74 to -10.17	**	0.0025
Untreated vs. mitFect6/Darm ASO [UCP2]	-58.45	-88.74 to -28.17	****	<0.0001
Untreated vs. mitFect7/Darm ASO [UCP2]	-82.31	-112.6 to -52.03	****	<0.0001
Untreated vs. mitFect8/Darm ASO [UCP2]	-41.43	-71.71 to -11.14	**	0.0018
Untreated vs. mitFect9/Darm ASO [UCP2]	-15.51	-45.79 to 14.78	ns	0.7443
Untreated vs. mtCPP1/Darm ASO [UCP2]	-48.3	-78.59 to -18.02	***	0.0002
Untreated vs. mtCPP1 + PF14/Darm ASO [UCP2]	-48.52	-78.80 to -18.23	***	0.0001
Untreated vs. LF2000/Darm ASO [UCP2]	-40.51	-70.79 to -10.22	**	0.0025
Untreated vs. Darm ASO [UCP2]	-92.03	-125.9 to -58.17	****	<0.0001
Untreated vs. Unrelated Oligo	-22.22	-52.50 to 8.067	ns	0.2893
Untreated vs. DNP 150 μ M	-84.91	-115.2 to -54.63	****	<0.0001
Untreated vs. H ₂ O ₂ 400 μ M	-99.35	-129.6 to -69.06	****	<0.0001
Untreated vs. H ₂ O ₂ 800 μ M	-21.23	-51.51 to 9.057	ns	0.344

Table S4a. Complete statistical data analysis for Fig. 4a. Ordinary one-way analysis of variance (ANOVA) with Holm-Sidak's multiple comparisons test of untreated vs. each complex formulation at MR3.

Holm-Sidak's multiple comparisons test	Mean Diff.	Summary	Adjusted P Value
Untreated vs. PF14/D-arm ASO [COX II]	-25.3	****	<0.0001
Untreated vs. mitFect1/D-arm ASO [COXII]	76.55	****	<0.0001
Untreated vs. mitFect3/D-arm ASO [COXII]	73.28	****	<0.0001
Untreated vs. mitFect4/D-arm ASO [COXII]	76.67	****	<0.0001
Untreated vs. mitFect5/D-arm ASO [COXII]	80.07	****	<0.0001
Untreated vs. mitFect6/D-arm ASO [COXII]	79.43	****	<0.0001
Untreated vs. mitFect7/D-arm ASO [COXII]	71.59	****	<0.0001
Untreated vs. mitFect8/D-arm ASO [COXII]	75.36	****	<0.0001
Untreated vs. mitFect9/D-arm ASO [COXII]	55.33	****	<0.0001
Untreated vs. mtCPP1/D-arm ASO [COX II]	58.61	****	<0.0001
Untreated vs. mtCPP1 + PF14/D-arm ASO [COX II]	78.01	****	<0.0001
Untreated vs. LF2000/D-arm ASO [COX II]	8.342	ns	0.2807
Untreated vs. D-arm ASO [COX II]	-1.831	ns	0.7151
Untreated vs. Unrelated Oligo	-4.578	ns	0.5959

Table S4b. Complete statistical data analysis for Fig. 4b. Ordinary one-way analysis of variance (ANOVA) with Holm-Sidak's multiple comparisons test of untreated vs. each complex formulation at MR3.

Dunnett's multiple comparisons test	Mean Diff.	95% CI of diff.	Summary	Adjusted P Value
Untreated vs. PF14/D-arm ASO [COXII]	-4.855	-31.13 to 21.42	ns	0.9992
Untreated vs. mitFect1/D-arm ASO [COXII]	15.81	-10.46 to 42.08	ns	0.4756
Untreated vs. mitFect3/D-arm ASO [COXII]	21.04	-5.229 to 47.31	ns	0.175
Untreated vs. mitFect4/D-arm ASO [COXII]	15.41	-10.86 to 41.68	ns	0.5063
Untreated vs. mitFect5/D-arm ASO [COXII]	14.18	-12.09 to 40.46	ns	0.6052
Untreated vs. mitFect6/D-arm ASO [COXII]	12.48	-13.79 to 38.75	ns	0.7441
Untreated vs. mitFect7/D-arm ASO [COXII]	-30.64	-56.91 to -4.367	*	0.0153
Untreated vs. mitFect8/D-arm ASO [COXII]	-11.92	-38.19 to 14.35	ns	0.7875
Untreated vs. mitFect9/D-arm ASO [COXII]	20.86	-5.411 to 47.13	ns	0.1821
Untreated vs. mtCPP1/D-arm ASO [COXII]	-4.517	-30.79 to 21.75	ns	0.9993
Untreated vs. mtCPP1 + PF14/D-arm ASO	36.53	10.26 to 62.80	**	0.0027
Untreated vs. LF2000/D-arm ASO [COXII]	8.342	-17.93 to 34.61	ns	0.9713
Untreated vs. D-arm ASO [COXII]	-1.831	-28.10 to 24.44	ns	0.9997
Untreated vs. Unrelated Oligo	-4.578	-30.85 to 21.69	ns	0.9993

Table S5. Primers used for the RT-qPCR assay.

Primers	Biorad Unique assay ID	Sequence
UCP2	qHsaCID0013753	GCGCTACTGTACTGGCCAGGGCAGAGTTCATGTATCTCGTCTTG ACCACGTCTACAGGGGAGGCGATGACAGTGGTGCAGAAGCCTGC CCCAAAGGCAGAAGTGAAGTGGCAAGGGAGGTCATCTGTCATGA GGTTGGCTTTCAGGAGGGCATCCTTGATGAGGTCATAGGTCACCA GCTCAGCACAGTTGACAAATGGCATTACGAGCAACA
COX II	qHsaCED0048349	AAACCGTCTGAACTATCCTGCCCCGCATCATCCTAGTCCTCATCG CCCTCCCATCCCTACGCATCCTTTACATAACAGACGAGGTCAACG ATCCCTCCCTTACCATCAAATCAATTGGCCACCAATGGTAC
UBC	qHsaCED0023867	AGCTGCTTTCGGCAAAGATCAACCTCTGCTGGTCAGGAGGAATG CCTTCCTTGTCTGGATCTTTGCTTTGACGTTCTCGATGGTGTAC TGGGCTCGACCTCAAGGGTGATGGTCTTGCCAGTGAGTGTCTTCA CGAAGATTTGCAT
GAPDH	qHsaCED0038674	GTATGACAACGAATTTGGCTACAGCAACAGGGTGGTGGACCTCAT GGCCACATGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCC CCAGCAAGAGCACAAAGAGGAAGAGAGACCCTCACTGCTGGGG AGTCCCTGCCACAC