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Supplemental Information

Antisense Oligonucleotides Promote Exon Inclusion and Correct the Common c.-32-13T>G *GAA* Splicing Variant in Pompe Disease

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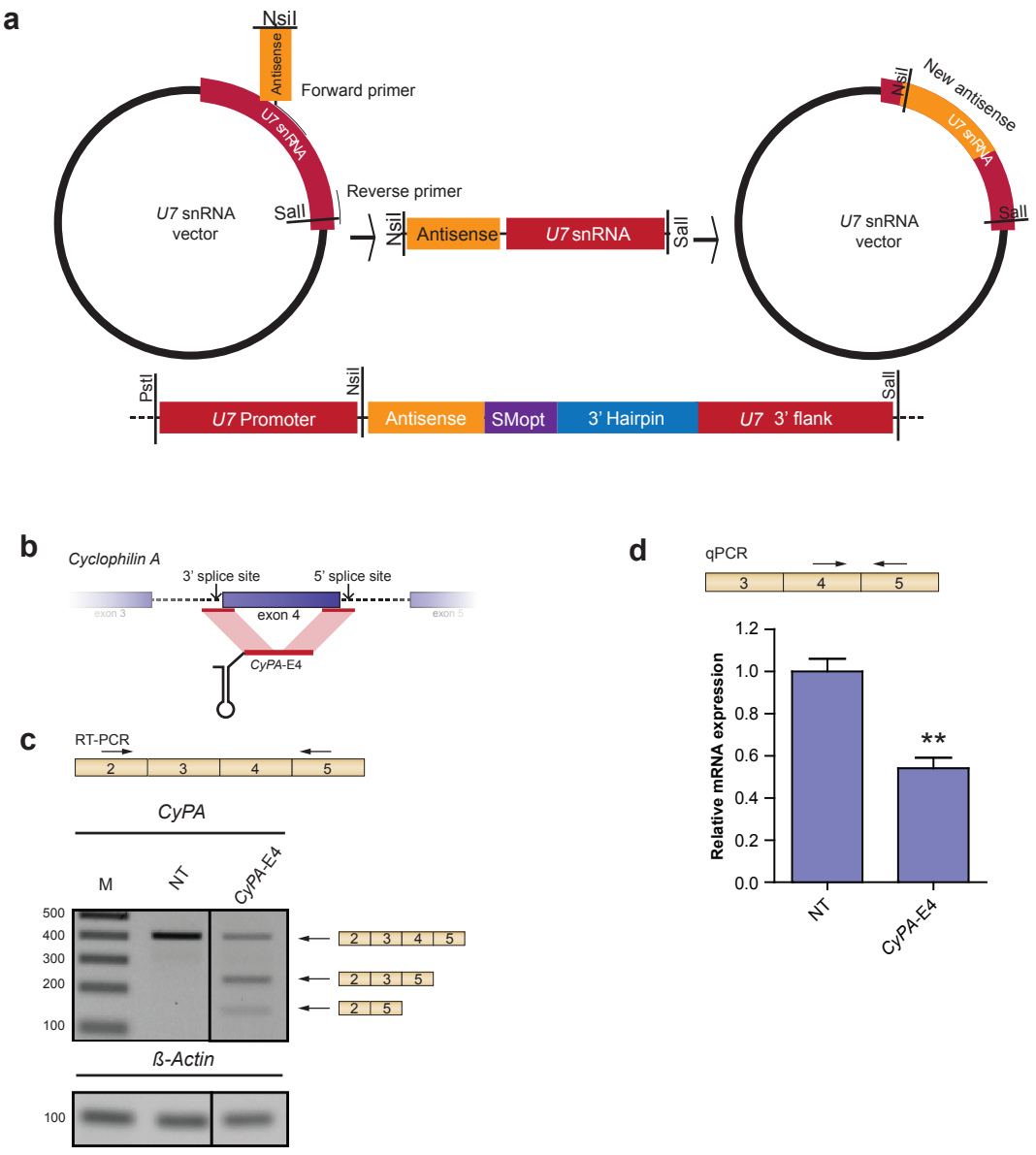


Figure S1. One step *U7* snRNA cloning system and validation. (a) One-step cloning strategy for rapid cloning of AONs in the lentiviral *U7* snRNA expression vector. A unique NsiI site was introduced in the *U7* snRNA. AON sequenc-es and the NsiI site were part of a forward primer in PCR, and a unique SalI site was included in the reverse PCR primer. **(b)** Cartoon of the region of the *Cyclophilin A* (*CypA*) gene that was targeted using a *U7* snRNA-expressed AON (*CyPA*-E4) as described previously by Liu et al.[1] **(c)** RT-PCR analysis of patient 1 fibroblasts in which the *CypA* pre-mRNA was targeted using *CyPA*-E4. As control, non-transduced cells were used (NT). The PCR strategy is shown above the gel. ß-Actin was used as loading control. **(d)** RT-qPCR analysis of the samples of **(c)**.

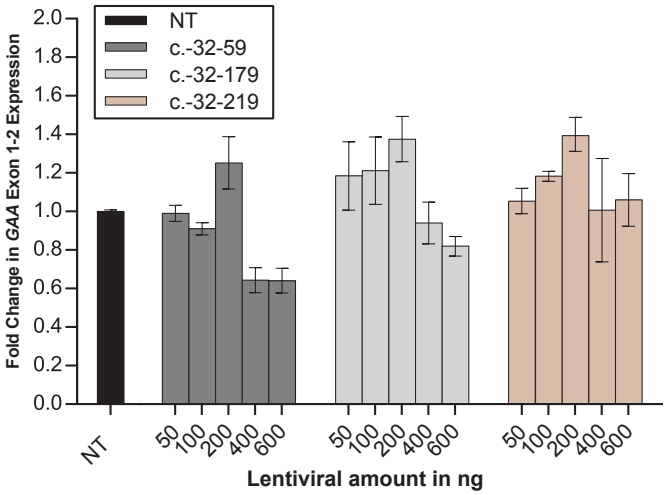


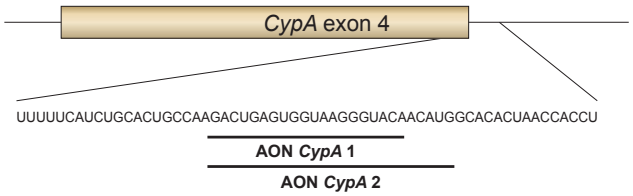
Figure S2. Testing of the optimal viral amount for detection of splicing modulation sequences. Patient 1 fibroblasts were infected with various lentiviruses at the amounts indicated. The optimum amount was determined to be 200 ng lentivirus per ml of medium. Data are means +/- SD of two biological replicates. Data points from 200 ng were taken from Figure 2B (N = 3). NT: non-transduced.

a

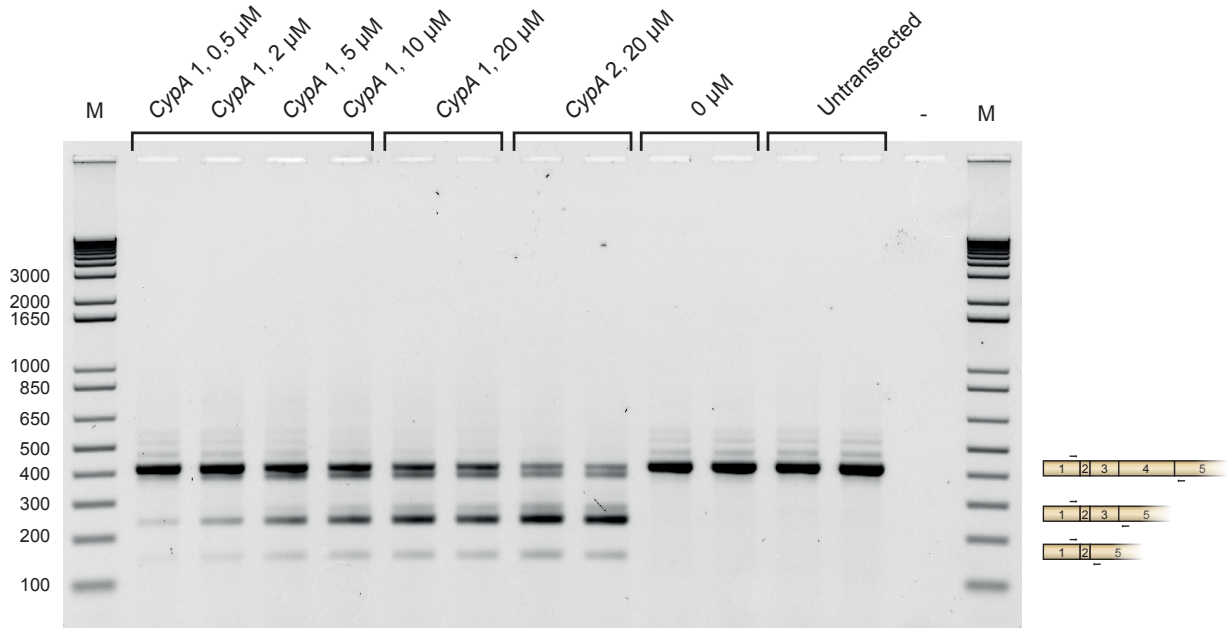
Name:	cDNA location	sequence 5' to 3'	nucleotides
CypA 1	*CypA* c.165_173+11	TGTACCCTTACCACTCAGTC	20
CypA 2	*CyPA* c.165_173+16	CATGTTGTACCCTTACCACTCAGTC	25
AON 1	*GAA* c.-32-219_-200	GAGTGCAGAGCACTTGCACA	20
AON 2	*GAA* c.-32-224_-200	GAGTGCAGAGCACTTGCACAGTCTG	25
AON 3	*GAA* c.-32-187_-167	CCAGAAGGAAGGGCGAGAAAA	21
AON 4	*GAA* c.-32-190_-166	GCCAGAAGGAAGGGCGAGAAAAGCT	25

reference sequence for cDNA annotation is NM_000152.3

b



c



d

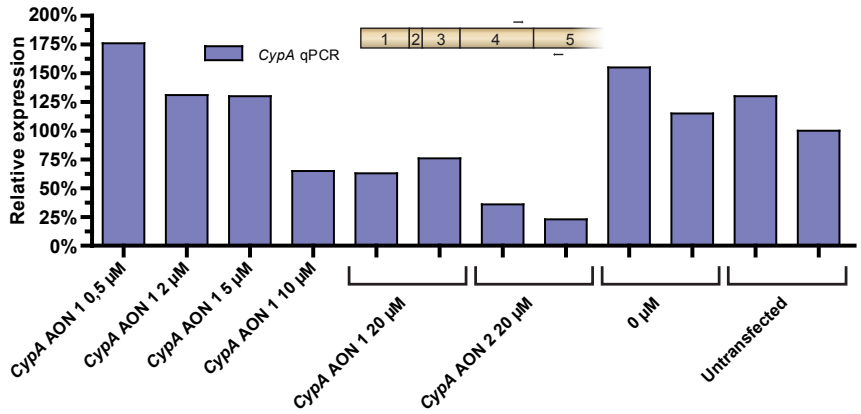


Figure S3. Validation of PMO-based AONs in primary fibroblasts. (a) Sequences of PMO-AONs used. **(b-d)** Test of PMO-based AONs on the positive control gene *CypA*. **(b)** Location of AONs designed to block the splice donor of *CypA* exon 4. **(c)** Fibroblasts from patient 1 were transfected with AONs at various concentrations as indicated, and *CyPA* mRNAs were analyzed by RT-PCR. Cartoons at the right side of the gel indicate spliced products. **(d)** RT-qPCR analysis of exon 4 skipping of the experiment in **(c)**. The cartoon highlights the primer locations. Data represent means of 3 technical replicates.

Table S1. Primers used for RT-qPCR, RT-PCR, cloning and sequencing

Primer target	Sequence (5'-3')	Used for
β-Actin fw	AACCGCGAGAAGATGACCC	qPCR/RT-PCR
β-Actin rv	GCCAGAGGCGTACAGGGATAG	qPCR/RT-PCR
GAA Exon 1-2 fw	AAACTGAGGCACGGAGCG	qPCR
GAA Exon 1-2 rv	GAGTGCAGCGGTTGCCAA	qPCR
GAA Cryptic Exon 2 fw	GGCACGGAGCGGGACA	qPCR
GAA Cryptic Exon 2 rv	CTGTTAGCTGGATCTTTGATCGTG	qPCR
GAA Full Skip Exon 2 fw	AGGCACGGAGCGGATCA	qPCR
GAA Full Skip Exon 2 rv	TCGGAGAACTCCACGCTGTA	qPCR
GAA Pseudo Exon fw	AAACTGAGGCACGGAGCG	qPCR
GAA Pseudo Exon rv	GCAGCTCTGAGACATCAACCG	qPCR
CypA Exon 2-5 fw	CACCGTGTTCTTCGACATTG	RT-PCR
CypA Exon 2-5 rv	CCATGGCCTCCACAATATTC	RT-PCR
CypA Exon 4-5 fw	GGACCCAACACAAATGGTTC	qPCR
CypA Exon 4-5 rv	GGCCTCCACAATATTCATGC	qPCR
Fw-*U7*snRNA-smOPT	GCTCTTTTAGAATTTTTGGAGCAGGTTTTCTGACTTCG	Cloning
Rv-*U7*snRNA-smOPT	CGAAGTCAGAAAACCTGCTCCAAAAATTCTAAAAGAGC	Cloning
Fw-*U7*snRNA-NsiI	CCTGGCTCGCTACAGATGCATAGGAGGACGGAGGACG	Cloning
Rv-*U7*snRNA-NsiI	CGTCCTCCGTCCTCCTATGCATCTGTAGCGAGCCAGG	Cloning
Fw-*U7*snRNA-PstI	GCGCCTGCAGTAACAACATAGGAGCTGTG	Cloning
Rv-*U7*snRNA-SalI	GCGCGTCGACCAGATACGCGTTTCCTAGGA	Cloning
M13 fw	GTAAAACGACGGGCCAG	Sequencing
M13 rv	CAGGAAACAGCTATGAC	Sequencing
GAA Exon1-3 fw	AGGTTCTCCTCGTCCGCCCGTTGTTCA	RT-PCR
GAA Exon1-3 rv	TCCAAGGGCACCTCGTAGCGCCTGTTA	RT-PCR

Supplemental References

1. Liu, S, Asparuhova, M, Brondani, V, Ziekau, I, Klimkait, T, and Schumperli, D (2004). Inhibition of HIV-1 multiplication by antisense U7 snRNAs and siRNAs targeting cyclophilin A. *Nucleic Acids Res* **32**: 3752-3759.