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Supplemental Information

RNA-Seq Analysis of an Antisense Sequence Optimized for Exon Skipping in Duchenne Patients Reveals No Off-Target Effect

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Table S1: Raw reads data after mapping on the human genome.

Samples	Number of input reads	Average input read length	Uniquely mapped reads number	Uniquely mapped reads %
MT_NT_1	77195401	197	68279964	88.45%
MT_NT_2	74927455	196	70292542	93.81%
MT_NT_3	76346921	197	69890600	91.54%
MT_U7_1	77803902	196	70904842	91.13%
MT_U7_2	75010698	196	70165223	93.54%
MT_U7_3	84426161	196	79012958	93.59%
HEP_NT_1	59522053	196	53843790	90.46%
HEP_NT_2	73721154	195	67687077	91.81%
HEP_NT_3	92869178	196	82104643	88.41%
HEP_U7_1	77212823	195	67039545	86.82%
HEP_U7_2	76094381	196	66281471	87.10%
HEP_U7_3	78118817	195	71631495	91.70%

Table S2: Ratios obtained between *DMD* transcripts and U7-E53 RNA in human primary hepatocytes and myotubes. The RPKM values (reads per kilobase per million mapped reads) represent the number of uniquely mapped reads normalized by the transcript length.

Samples	RPKM values for *DMD* transcript	Mean RPKM for *DMD* transcript	RPKM values for U7-E53 RNA	Mean RPKM for U7-E53 RNA	Ratio *DMD* transcript/U7-E53 RNA
HEP_NT_1	2.9	2.6	0.0	0.0	
HEP_NT_2	2.6		0.0		
HEP_NT_3	1.9		0.0		
HEP_U7_1	3,5		1.9	1.3	**2.0**
HEP_U7_2	2.8		0.5		
HEP_U7_3	1.9		1.4		
MT_NT_1	28.4	28.3	0.1	0.1	
MT_NT_2	32.7		0.1		
MT_NT_3	32.2		0.0		
MT_U7_1	14.9		5.9	4.7	**6.0**
MT_U7_2	27.3		4.7		
MT_U7_3	34.4		3.5		

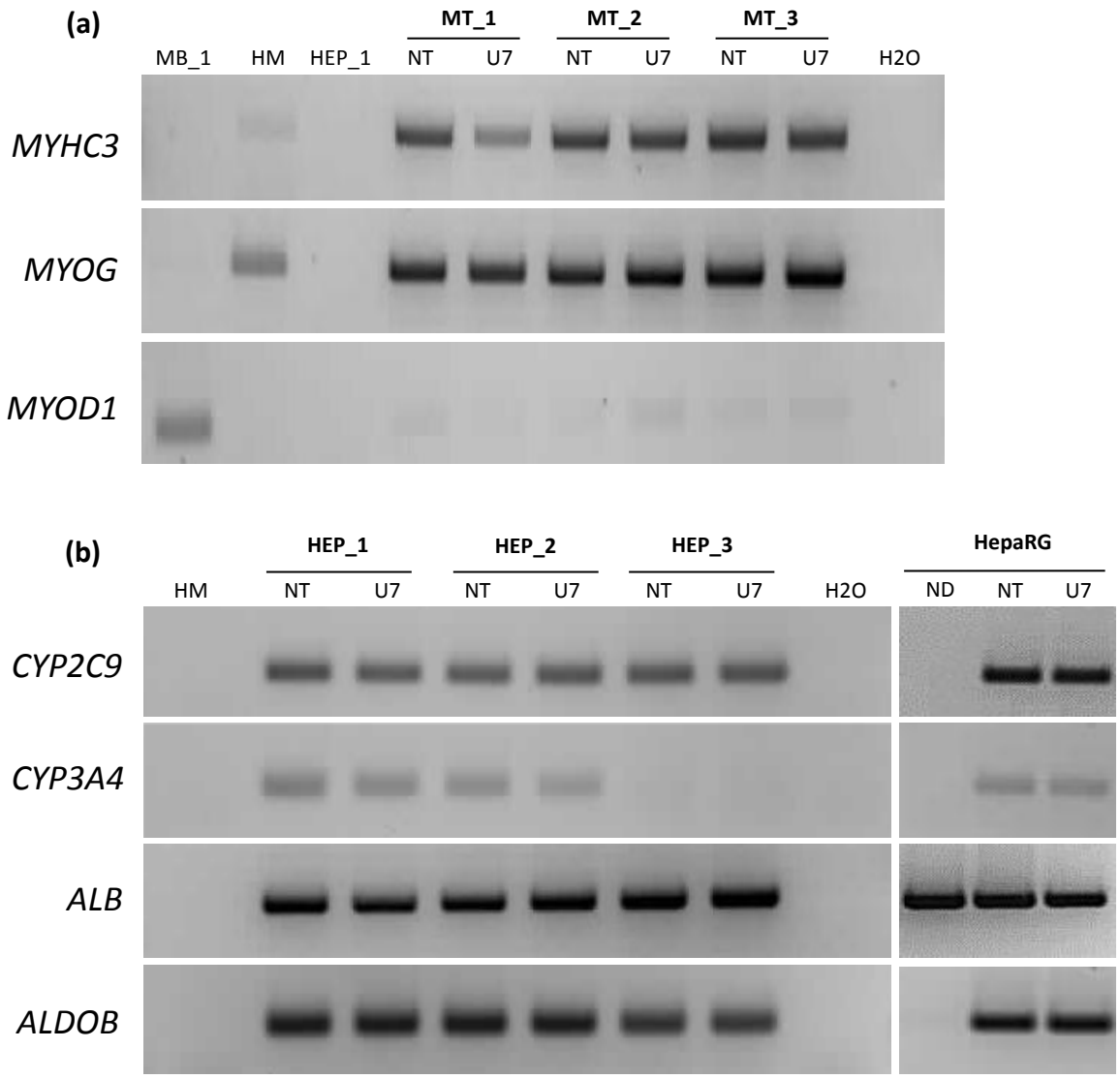

Figure S1: Confirmation of the muscular or hepatic transcriptomic pattern of human cellular models through RT-PCR on specific genes. **(a)** *MYHC3*, *MYOG*, and *MYOD1* expression analysis on one representative batch of human primary myoblasts (MB_1), human skeletal muscle (HM), human primary hepatocytes (HEP_1) and on 3 batches of different primary myotubes obtained from 3 different patients (MT_1, 2, and 3) non-transduced (NT) or 3 days after transduction with rAAV3b-U7-E53 (U7). **(b)** *CYP2C9* and *CYP3A4*, *ALB* and *ALDOB* expression analysis on human skeletal muscle (HM), on 3 batches of different primary hepatocytes obtained from 3 different patients (HEP_1, 2, and 3) non-transduced (NT) or transduced with rAAV3b-U7-E53 (U7) and on non-differentiated HepaRG (ND) and differentiated HepaRG non-transduced (NT) or transduced with rAAV3b-U7-E53 (U7). H2O: water.

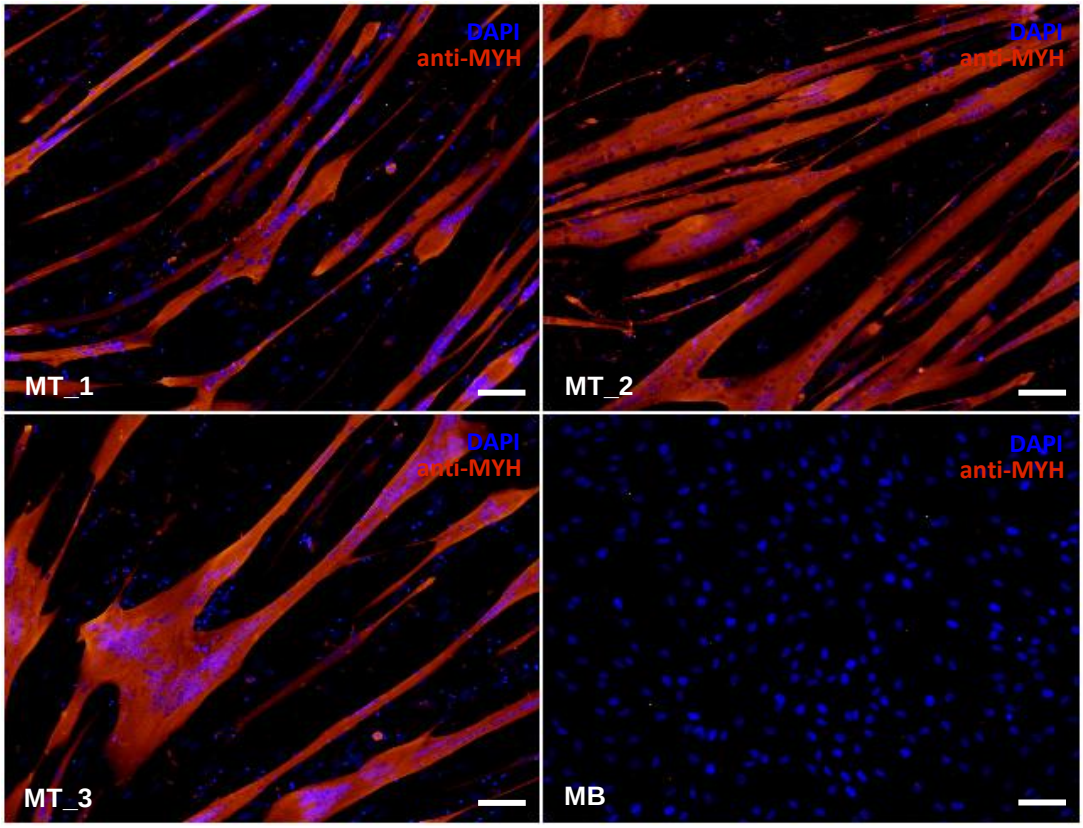

Figure S2: Analysis of the myosin heavy chain expression in human primary myotubes using immunostaining. Representative panels of 3 batches of different primary myotubes obtained from 3 different patients (MT_1, 2, and 3) and one representative batch of human primary myoblasts (MB) stained with an anti-myosin antibody (red) and DAPI nuclear staining (blue). Scale bar = 100 µm

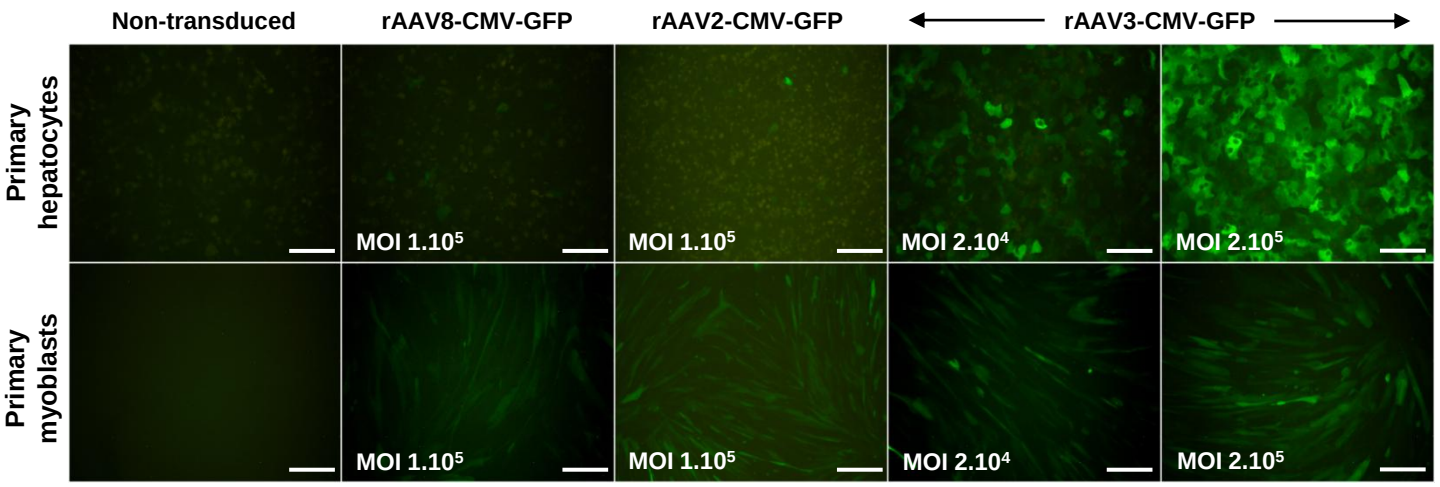

Figure S3: Representative examples of GFP expression after transduction of human primary hepatocytes and myoblasts with different serotypes of rAAV vectors encoding GFP under the control of the CMV promoter. Primary hepatocytes (upper) and myoblasts (lower) were transduced with rAAV8, rAAV2, or rAAV3b at different MOI. Cells were observed 3 days after transduction. Scale bar = 100 µm

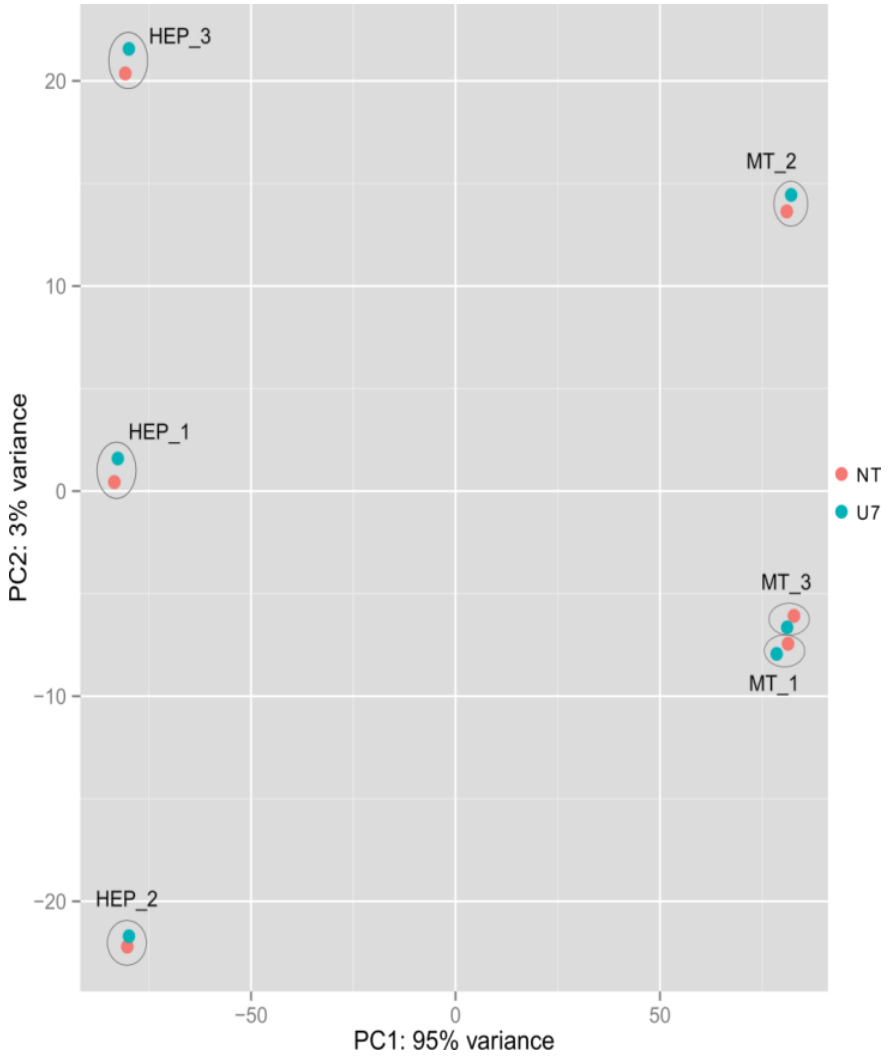

Figure S4: Graphic representation of principal component analyses (PCA) of raw expression data obtained from human primary myotubes and hepatocytes transduced or not with rAAV3b-U7-E53. Samples from the same patients are circled in gray.