

Supplemental information

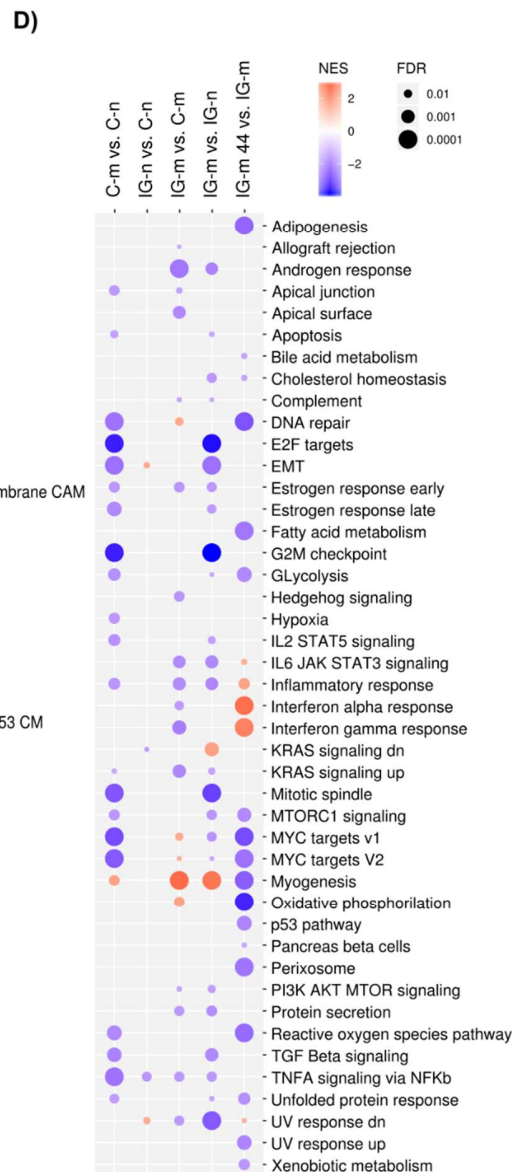
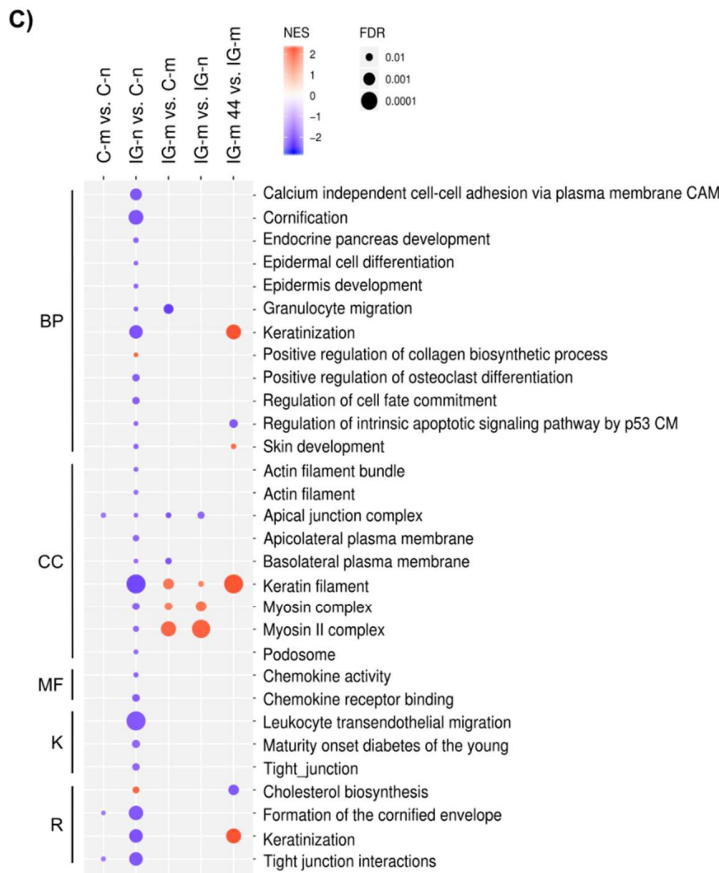
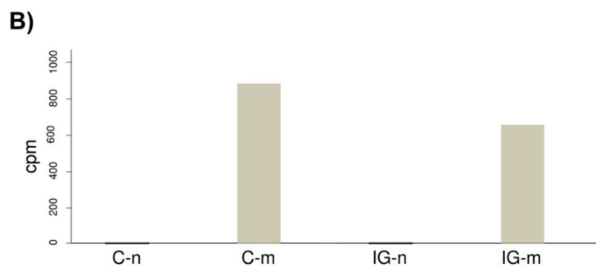
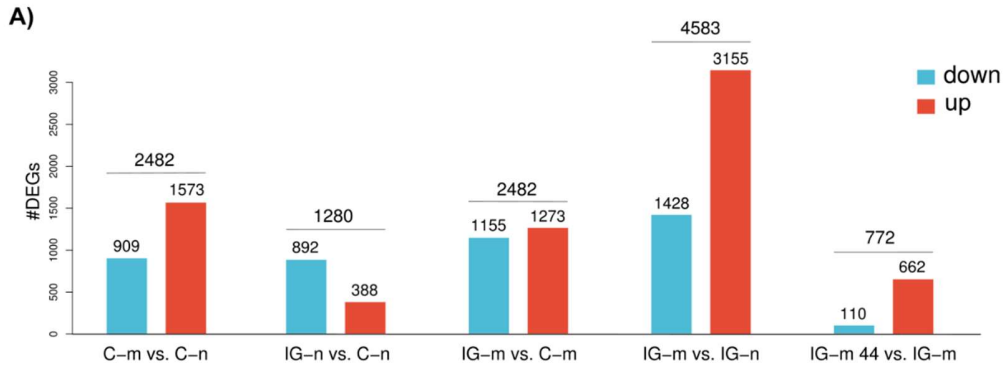
RNA-seq in DMD urinary stem cells recognized muscle-related transcription signatures and addressed the identification of atypical mutations by whole-genome sequencing

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Supplemental Methods

Celector® technology

The cell separation exploits the Non-Equilibrium, Earth Gravity Assisted Fractionation (NEEGA-DF) principles where cells are separated and eluted based only on their physical characteristics such as dimension, density, morphology, and membrane rigidity. In general, these characteristics influence the cell position across the capillary device, generated by the opposite forces acting on cells, gravity, and lift forces. Cells having different positions possess different velocity and therefore elute at different times, allowing the separation and collection of several subpopulations. A camera with a microscopic object placed at the outlet of the capillary channel and connected to imaging software records live images of eluting cells and plots the number of counted cells related to elution time in a fractogram (profile). Dimension inclusion/exclusion criteria are set by the operator to refine the counting procedure. A decontamination procedure of the fractionation system by flushing a cleaning solution of sterile, demineralized water is performed every day before starting. Subsequently, to block unspecific interaction sites on the plastic walls, a sterile coating solution is flushed at 1 ml/min. The system is then ready to be used after filling it with sterile mobile phase. All solutions are provided by Stem Sel s.r.l. (Italy).



Supplementary Fig. 1. A) Number of genes differentially expressed in native (*n*) and myogenic (*m*) USCs from DMD (IG) or controls (C) and following AON treatment (44). B) MYOD gene expression in native and MyoD-induced USCs. C) Enrichment analysis highlighted the downregulation of gene sets mainly involved in the inflammatory response and in skin-related circuits in IG-*n* compared to C-*n* USCs. The plot shows a manual selection of gene sets from different collections of the MsigDB database. BP: Gene ontology, biological processes; CC: Gene ontology, cellular component; MF: Gene ontology, molecular function; K: KEGG gene set; R: Reactome gene set. NES: normalized enrichment score. Red, positive NES, i.e., activation of the gene set in the first condition; Blue, negative NES, i.e., repression of the gene set in the first condition; FDR: p-value after false discovery rate correction. Only significantly ($FDR \leq 0.05$) enriched gene sets are shown. Abbreviations in the gene set labels: CAM: cell adhesion molecules; CM: class mediator. D) Enrichment analysis using the gene sets from the Hallmark collection of MsigDB. The MyoD treatment induced a significant deregulation of both inflammatory and myogenesis pathways in healthy donors and patients' native USCs. Both signaling pathways are reverted after AON transfection. Figure legend as in panel C.

Table S1. Total number of reads and number of reads for each main step of RNA-seq analysis for all analyzed samples.

Sample ID	raw fastq	trimmed	aligned: uniquely mapped	counted: assigned to coding regions
C-m	20,600,102	20,065,900	18,135,177	15,567,734
C-n	59,505,396	31,751,714	30,011,295	26,706,107
IG-n	32,956,669	22,756,422	21,872,447	19,532,133
IG-m 44	15,033,477	14,806,346	13,831,392	9,023,157
IG-m	31,431,592	19,914,094	16,951,806	14,547,339

Table S2. Genes of different myogenic pathways deregulated by *MYOD* gene expression in USCs from both control and DMD IG as compared to their native counterparts (up-regulated genes in red; down-regulated genes in green; FDR≤0.05).

Gene symbol	Function (gene description)	C- <i>m</i> vs. C- <i>n</i>		IG- <i>m</i> vs. IG- <i>n</i>	
		logFC	FDR	logFC	FDR
CDH15	cadherin 15	10.98	1.18E-12	12.65	3.66E-17
TNS4	tensin 4	-11.30	9.90E-08	-8.68	2.17E-04
Cell Cycle/DNA Replication					
GADD45G	Gadd45g	2.90	2.26E-02	7.10	1.49E-08
BRCA1	breast cancer 1	-3.99	8.46E-04	-7.02	1.11E-07
TACC3	Tacc3	-3.03	1.10E-02	-3.19	4.21E-03
CDK2	cyclin-dependent kinase 2	-3.50	3.42E-03	-3.22	4.35E-03
MKI67	Ki 67	-11.07	1.18E-12	-7.28	4.95E-09
BUB1B	Bub1b	-2.83	1.94E-02	-6.51	1.54E-07
Growth Factors/Ligands					
PDGFA	platelet derived growth factor a	2.62	3.14E-02	3.55	1.55E-03
TGFBI	transforming growth factor b	-2.60	3.19E-02	-5.04	1.03E-05
IGFBP5	insulin-like growth factor binding protein 5	3.02	1.10E-02	3.52	1.59E-03
Metabolism					
GPC1	glypican 1	2.53	3.74E-02	3.64	1.06E-03
Nuclear Regulatory Factors					
EYA1	eyes absent 1	5.24	7.40E-05	4.60	2.31E-03
MYOG	Myogenin	7.02	3.50E-02	12.61	2.72E-14
Receptors/Signaling					
RYR1	ryanodine receptor 1	6.52	2.92E-07	11.88	4.07E-15
RAPSN	receptor-associated prot	5.12	6.41E-05	8.24	1.45E-09
CHRNB1	cholinergic receptor nicotinic b1	3.54	2.60E-03	4.47	7.59E-05
RAB9B	Rab9	2.89	1.89E-02	2.84	1.94E-02
CHRNG	cholinergic receptor, nicotinic g	4.34	3.83E-03	8.44	2.08E-09
ITGA7	integrin alpha 7	6.43	2.83E-07	6.50	5.66E-08
VIPR2	vip receptor 2	5.26	3.73E-04	8.25	8.41E-04
EGFR	epidermal growth factor receptor	-2.45	4.55E-02	-5.77	1.07E-06
ADCY7	adenylate cyclase 7	-2.69	2.78E-02	-2.77	1.41E-02
Structural/Cytoskeletal					
TNNI1	troponin I, skeletal, slow 1	6.88	1.45E-05	11.05	2.64E-17
NES	nestin	9.48	6.44E-12	3.90	4.68E-04
MYBPH	myosin-binding protein H	7.91	6.00E-07	9.02	9.98E-12
TNNT2	troponin T2, cardiac	10.01	6.44E-12	10.24	7.00E-14
KIF4A	kinesin heavy chain member	-13.09	2.37E-10	-6.10	6.82E-07
LMNB2	lamin B2	-3.12	8.39E-03	-2.96	7.76E-03

Table S3. Genes belonging to the core enrichment genes of the Hallmark myogenesis gene set were deregulated in myogenic DMD USC_s as compared to control. The upregulation in DMD myogenic USC_s (IG-*m*) as compared to control cells (C-*m*; FDR \leq 0.05 and Fold Change \geq 2) is reverted by the treatment of IG-*m* cells with AON44.

geneSymbol	IG- <i>m</i> .vs.C- <i>m</i>		IG- <i>m</i> 44.vs.IG- <i>m</i>	
	logFC	FDR	logFC	FDR
ACHE	3.68	2.17E-03	-0.71	7.06E-01
ACTA1	5.14	1.93E-05	-2.47	7.48E-02
ACTC1	4.59	1.12E-04	-1.11	4.99E-01
ACTN2	4.87	5.22E-05	-0.70	7.06E-01
ACTN3	7.41	4.21E-08	-1.84	1.98E-01
AEBP1	3.03	1.20E-02	-0.86	6.25E-01
AK1	4.27	3.52E-04	-2.57	6.63E-02
APOD	9.14	1.20E-04	-1.83	2.57E-01
ATP2A1	4.06	6.10E-04	-1.15	4.81E-01
CACNG1	10.33	2.93E-06	-1.41	3.83E-01
CAV3	7.98	1.13E-08	-2.21	1.10E-01
CFD	3.34	2.30E-02	-0.92	6.75E-01
CKM	8.01	2.59E-09	-1.83	1.98E-01
COX6A2	3.53	3.36E-03	-2.59	6.62E-02
COX7A1	7.23	7.56E-08	-2.69	5.84E-02
CRYAB	2.74	2.41E-02	-1.70	2.44E-01
CSRP3	4.09	6.04E-04	-1.58	2.92E-01
DES	3.87	1.02E-03	-2.04	1.44E-01
EFS	9.87	1.24E-05	-0.54	8.08E-01
ENO3	4.76	6.66E-05	-2.25	1.05E-01
FOXO4	2.80	2.28E-02	-0.22	9.41E-01
FST	2.78	2.20E-02	-1.22	4.46E-01
GADD45B	2.82	2.39E-02	-0.49	8.16E-01
GJA5	5.34	4.42E-03	-1.08	5.81E-01
GPX3	3.43	3.95E-03	-1.27	4.23E-01
GSN	3.60	2.57E-03	-2.09	1.34E-01
HRC	9.52	3.99E-11	-1.61	2.75E-01
HSPB2	5.50	9.41E-06	-1.90	1.82E-01
ITGA7	2.52	3.91E-02	-0.80	6.56E-01
KCNH2	3.60	2.61E-03	-0.84	6.34E-01
LSP1	6.71	1.21E-05	-1.04	5.59E-01
MAPRE3	2.52	4.61E-02	-0.94	5.93E-01
MB	5.69	1.81E-05	-1.89	1.96E-01
MYBPH	5.95	1.50E-06	-1.32	3.92E-01
MYF6	10.70	9.09E-07	-1.43	3.81E-01
MYH2	11.30	1.39E-07	-0.29	9.10E-01
MYH3	9.23	4.41E-11	-0.99	5.60E-01
MYH7	7.74	2.94E-06	-0.91	6.16E-01
MYH8	17.08	1.02E-16	-0.40	8.57E-01
MYL1	6.61	1.78E-07	-1.58	2.86E-01
MYL3	3.89	1.06E-03	-2.84	4.53E-02

MYL4	6.55	2.21E-07	-2.12	1.27E-01
MYL6B	3.69	1.79E-03	-2.43	8.32E-02
MYL7	5.88	1.93E-05	-2.31	1.10E-01
MYLPF	11.28	2.00E-14	-2.48	7.45E-02
MYOG	8.86	3.69E-10	-0.94	5.83E-01
MYOM1	4.58	1.47E-04	-0.68	7.15E-01
MYOZ1	7.16	8.33E-08	-1.55	3.00E-01
PC	2.65	2.97E-02	-1.48	3.27E-01
PGAM2	4.03	8.17E-04	-3.34	1.86E-02
PTP4A3	3.22	7.21E-03	-1.85	1.97E-01
PYGM	7.70	3.58E-08	-1.54	3.07E-01
REEP1	3.62	2.27E-03	-0.39	8.64E-01
SGCA	5.81	2.67E-06	-1.80	2.10E-01
SGCG	7.13	3.13E-06	-0.59	7.75E-01
SLN	10.30	3.13E-06	-2.26	1.23E-01
SOD3	4.33	2.99E-04	-1.86	1.96E-01
SORBS3	2.65	3.07E-02	-1.07	5.23E-01
TAGLN	2.65	2.93E-02	-1.21	4.53E-01
TCAP	4.78	1.26E-04	-2.42	9.06E-02
TEAD4	3.72	1.63E-03	-1.68	2.50E-01
TNNC1	3.78	1.35E-03	-2.96	3.35E-02
TNNC2	7.85	5.04E-09	-3.55	1.02E-02
TNNI1	3.93	8.40E-04	-1.94	1.70E-01
TNNI2	10.02	9.07E-11	-3.27	1.95E-02
TNNT1	4.53	1.36E-04	-2.61	6.21E-02
TNNT2	4.19	3.91E-04	-1.85	1.95E-01
TNNT3	9.85	1.92E-11	-2.13	1.23E-01
TPM2	3.38	4.41E-03	-2.03	1.47E-01