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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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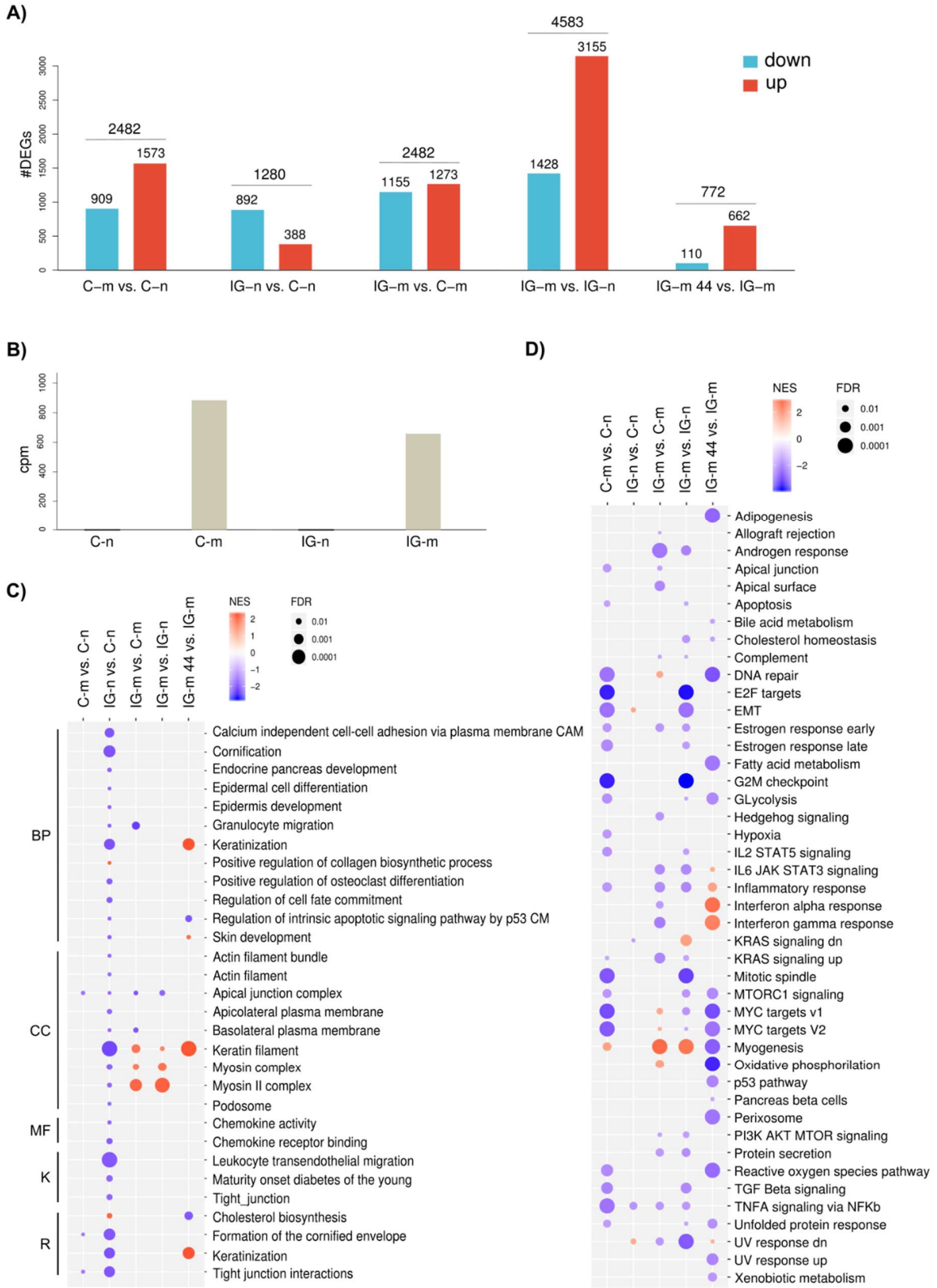
Supplemental Methods

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).


A)
#DEGs
down
up
2482
1573
909
1280
892
388
2482
1155
1273
4583
3155
1428
772
662
110
C–m vs. C–n
IG–n vs. C–n
IG–m vs. C–m
IG–m vs. IG–n
IG–m 44 vs. IG–m
B)
cpm
C-n
C-m
IG-n
IG-m
C)
C-m vs. C-n
IG-n vs. C-n
IG-m vs. C-m
IG-m vs. IG-n
IG-m 44 vs. IG-m
NES
FDR
0.01
0.001
0.0001
BP
CC
MF
K
R
Calcium independent cell-cell adhesion via plasma membrane CAM
Cornification
Endocrine pancreas development
Epidermal cell differentiation
Epidermis development
Granulocyte migration
Keratinization
Positive regulation of collagen biosynthetic process
Positive regulation of osteoclast differentiation
Regulation of cell fate commitment
Regulation of intrinsic apoptotic signaling pathway by p53 CM
Skin development
Actin filament bundle
Actin filament
Apical junction complex
Apicolateral plasma membrane
Basolateral plasma membrane
Keratin filament
Myosin complex
Myosin II complex
Podosome
Chemokine activity
Chemokine receptor binding
Leukocyte transendothelial migration
Maturity onset diabetes of the young
Tight_junction
Cholesterol biosynthesis
Formation of the cornified envelope
Keratinization
Tight junction interactions
D)
C-m vs. C-n
IG-n vs. C-n
IG-m vs. C-m
IG-m vs. IG-n
IG-m 44 vs. IG-m
NES
FDR
0.01
0.001
0.0001
Adipogenesis
Allograft rejection
Androgen response
Apical junction
Apical surface
Apoptosis
Bile acid metabolism
Cholesterol homeostasis
Complement
DNA repair
E2F targets
EMT
Estrogen response early
Estrogen response late
Fatty acid metabolism
G2M checkpoint
GLycolysis
Hedgehog signaling
Hypoxia
IL2 STAT5 signaling
IL6 JAK STAT3 signaling
Inflammatory response
Interferon alpha response
Interferon gamma response
KRAS signaling dn
KRAS signaling up
Mitotic spindle
MTORC1 signaling
MYC targets v1
MYC targets V2
Myogenesis
Oxidative phosphorilation
p53 pathway
Pancreas beta cells
Perixosome
PI3K AKT MTOR signaling
Protein secretion
Reactive oxygen species pathway
TGF Beta signaling
TNFA signaling via NFKb
Unfolded protein response
UV response dn
UV response up
Xenobiotic metabolism

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 MisgDB. 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-*m* vs. C-*n*		IG-*m* vs. IG-*n*	
		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 USCs as compared to control. The upregulation in DMD myogenic USCs (IG-*m*) as compared to control cells (C-*m*; FDR≤0.05 and Fold Change≥2) is reverted by the treatment of IG-m cells with AON44.

	IG-m.vs.C-m		IG-m 44.vs.IG-m	
geneSymbol	**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