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14. ABSTRACT

The purpose of this project is to investigate the effect of dystrophin loss on 3D genome organization and gene expression output of skeletal myofibers, and to determine whether recovery of expression of a short form of dystrophin (micro-dystrophin) currently used in clinical trial with Duchenne Muscular Dystrophy (DMD) boys can restore in full or partly the original 3D nuclear landscape and gene expression. The goal of this research is the identification of pathological alterations in chromatin interactions than impair the expression of genes implicated in the pathogenesis of DMD.

We have generated datasets of RNAseq, ATACseq and promoter capture HiC (pcHiC) from cultures of DMD muscles (or wild type controls), before or after micro-dystrophin re-expression. During the analysis of these samples, we have discovered that some samples did not reach the standard quality control check and therefore we have prepared and added more experimental replicates. We have completed the RNAseq and ATACseq analysis, while pcHiC is still undergoing. The analysis so far is showing that the absence of dystrophin causes profound changes in chromatin accessibility at regulatory elements of genes implicated in many features of DMD pathogenesis and disease progression, leading to deregulation of their expression. We found that re-expression of a micro-dystrophin (µDys5) without the nitric oxide (NO) binding domain could not rescue a large majority of these alterations, while re-expression of a micro-dystrophin (µDys5) that retains the nitric oxide (NO) binding domain could partly recover some of these alterations and restored some gene expression pattern to the levels of wild type myotubes. As for the samples from mdx mice, we have also encountered problems in sample preparation, mostly related to the isolation of the required amount and purity of RNA from myonuclei; we therefore had to prepare new mice treated or not to micro-dystrophin re-expression, which will be ready for sample collection by December 2023.

15. SUBJECT TERMS

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1. **INTRODUCTION:**
   The purpose of this project is to investigate the effect of dystrophin loss on 3D genome organization and gene expression output of skeletal myofibers, and to determine whether recovery of expression of a short form of dystrophin (micro-dystrophin) currently used in clinical trial with boys affected by Duchenne Muscular Dystrophy (DMD) can restore in full or partly the original 3D nuclear landscape and gene expression.
   The ultimate scope of this research is the identification of pathological alterations in chromatin interactions than impair the expression of genes implicated in the pathogenesis of DMD.

2. **KEYWORDS:** Duchenne Muscular Dystrophy (DMD); Dystrophin; Skeletal muscle; Myonuclei; Chromatin; Gene expression; Enhancers; Promoters; Topologically Associating Domains (TADs); Nucleosomes
3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*

   - **What were the major goals of the project?**

   The major goals of this project were (as stated in the SOW):

   Perform FACS-mediated isolation of myonuclei from TA muscles of mdx and wild type mice (months: 1-6)

   Perform parallel pcHiC, RNAseq and ATACseq on isolated myonuclei (sample library preparation and deep sequencing)
   (months: 1-10)

   Produce and test AAVs for viral delivery of µDys5 to mdx mice
   (months: 1-6)

   AAV-mediated delivery of µDys5 to mdx mice
   (months: 1-6)

   Perform FACS-mediated isolation of myonuclei from TA muscles of mdx after AAV delivery of µDys5 or control vector
   (months: 6-18)

   Perform parallel pcHiC, RNAseq and ATACseq on isolated myonuclei (sample library preparation and deep sequencing)
   (months: 6-18)

   Prepare cultures of DMD patient (or control)-derived myotubes
   (months: 1-6)

   Perform parallel pcHiC, RNAseq and ATACseq on myonuclei isolated from cultured myotubes (sample library preparation and deep sequencing)
   (months: 1-10)

   Produce and test Lentiviruses for viral delivery of µDys5 to DMD cultured myotubes
   (months: 1-6)

   Lentiviral-mediated delivery of µDys5 to DMD cultured myotubes
   (months: 1-6)

   Perform parallel pcHiC, RNAseq and ATACseq on myonuclei isolated from cultured myotubes (sample library preparation and deep sequencing)
   (months: 6-18)

- **What was accomplished under these goals?**

The major activities of the second year of funding were:

We have continued the production and test of viral preps for *in vivo* (AAV-µDys5) and *in vitro* (lentiviral- µDys5) delivery of µDys5

We have conclusively set optimal and reproducible experimental conditions for preparation of cultures of DMD patient (or control)-derived myotubes. In particular, we have solved the problem of the contamination of the residual amount of hiPSCs that did not convert into skeletal muscles, by exposing our cultures to ArabinosideC (AraC), which eliminates all mitotic cell types.

We have collected and sequenced samples for the first replicates of parallel RNAseq, ATACseq and HiChIP on myonuclei isolated from cultured WT and DMD myotubes in which µDys5 (without the Nitric oxide binding domain) was delivered by infection with lentiviruses. As an independent point (no planned in the original version of this project, but still included in the analysis) we have included samples in which a µDys5 mutant that retains the ability to bind nitric oxide was re-expressed by genome editing

We have collected the material for the first replicates of the experiment of parallel pcHiC, RNAseq and ATACseq on myonuclei isolated by FACS from HSAcre;MDX; Rosa26-Lsl-H2B-GFP mice at two time points – 4 weeks and 4 months of life – with or without AAV-mediated delivery of µDys5 or control vector. Note that because of the poor yield and quality of the RNA extracted from the myonuclei in the previous experiment, we had to prepare more mice for additional replicates.

The specific objectives of the second year of funding were:

Produce and test Lentiviruses for viral delivery of µDys5 to DMD cultured myotubes (Chamberlain lab)

Produce and test AAVs for viral delivery of microdystrophin to mdx mice (Chamberlain lab)

Collect myotubes generated from hiPSCs wild type or in which dystrophin deficiency is caused by CRISPR-mediated mutation in exons 8-9 (Δ8-9) or rescued CRSPR-mediated skipping of mutated exons (Δ6-9) and process them for parallel, RNAseq, ATACseq and pcHiC (Puri lab)

Analysis of RNAseq, ATACseq and pcHiC from of myotubes generated from hiPSCs wild type or in which dystrophin deficiency is caused by CRISPR-mediated mutation in exons 8-9 (Δ8-9) or rescued by lentiviral-mediated delivery of µDys5 (without NO-binding domain) or CRSPR-mediated skipping of mutated exons (Δ6-9), which produces a µDys5 retaining the NO binding domain (Puri lab)

Collect myonuclei isolated by FACS from HSAcre;MDX; Rosa26-Lsl-H2B-GFP mice at two time points – 4 weeks and 4 months of life – with or without AAV-mediated delivery of µDys5 or control vector and process them for parallel pcHiC, RNAseq and ATACseq (Puri lab)

The significant results of the first year of funding were:

Chamberlain lab progress

Production of AAVs for viral delivery of µDys5 ***(from Dr Chamberlain, Washington University sub-award)***

Dr Chamberlain has continued generating and testing viral preps for of pAAV-CK8e-µDys5-pA for in vivo delivery and viral prep for lentiviral delivery of HSAµDys5

Specific Aim 1: Analysis of the epigenetic landscape in *mdx* mice.
Major Task 2.

Subtask 1: *Produce and test AAVs for viral delivery of microdystrophin to mdx mice*

1) *Major activities:* The major activity at the University of Washington was to generate AAV vectors expressing micro-dystrophin (µDys) proteins under control of the CK8e muscle promoter/enhancer. Plasmids for vector preps were prepared and used to generate vectors.
2) *Specific objectives:* For this task we needed to generate more vector preps for additional studies. As before, we generated plasmids for the AAV (pAAV-CK8e-µDys5-pA, and also for pAAV-CK8e-H3µDys-pA) and the helper plasmids, which encodes the capsid and rep proteins needed to make AAV (pDG6). Preps were done using GigaPrep kits (Thermofisher). Production of AAV was done in HEK293 using calcium phosphate mediated co-transfecting of the pAAV and pHelper plasmids. Vector was purified from culture lysates and supernatants, and purified via HPLC, heparin affinity columns, sucrose gradients and dialysis. Initial yields were a bit low so we are completing a larger set of preps.
3) *Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative):* The vectors performed as expected, but initial yields were less than planned, so additional preps were needed. Titer is determined in 2 ways, by Q-PCR and by Southern analysis, with final yields and titers determined by an average of the 2 methods.
4) *Other achievements.* The final purified AAV vector was tested for full (genome-containing) and empty-no genome) particles. For this we have begun using a newer analytic tool, a Stunner spectrophotometer (unchained labs). This enables rapid determination of 'empty/full' rations, aggregation and impurities in the preps.

Specific Aim 2: Analysis of the epigenetic landscape in patient derived skeletal muscles
Major Task 2.

Subtask 1: *Produce and test Lentiviruses for viral delivery of µDys5 to DMD cultured myotubes*

1) *Major activities:* The major activity here was to generate a lentiviral vector (Lv) expressing the µDys protein under control of a muscle-specific promoter (Lv-HSA-µDys-eGFP; Kimura, Mol Th 2010; 18:206). The initial preps have all been used and we completed a second set of preps and are finishing a third. Plasmids needed to grow the virus were prepared and used to generate vector. The helper plasmids used were originally obtained from Luigi Naldini, with minor modifications to accommodate muscle-specific applications.
2) *Specific objectives:* For this task we use 4 plasmids for the Lv (pRRL-cPPT-HSA-µDy-PRE-SIN) and the helper plasmids (pMDLg/pRRE; pRSV-REV; and pCMV-VSVG – Fig. J3). This system generates replication-incompetent lentiviral vectors. Vector was generated by co-transfection of all 4 plasmids into HEK293 cells. Vector was harvested from the supernatant and purified via ultracentrifugation and dialysis.
3) *Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative):* As before, the vector grew well, as determined from 2 independent preps. Prep size has been increased to a 10 roller bottle range, and was concentrated to ~1 ml.
4) *Other achievements.* We have cloned several new versions of micro- and mini-dystrophin in Lv vectors, and while not used here, they will be available for follow-up studies.

What opportunities for training and professional development has the project provided? As noted last year, vector production for this project has not yielded specific opportunities for training and professional development. However, the Chamberlain lab in general offers hand-on training for AAV and lentiviral production for individuals wishing to use these methods in their own lab.

How were the results disseminated to communities of interest?
The main focus in the Chamberlain lab has been on vector production and quality control. Data on these items is provide d to Dr. Puri, and will be included in submitted manuscripts.

What do you plan to do during the next reporting period to accomplish the goals?
As this is the final reporting period there will not be formal follow-up. However, the reagents continue to be developed and will be available for follow-up studies.

What was the impact on technology transfer?
No new technology transfer has resulted from our AAV and Lv preps under this project.

Puri lab progress

Analysis of RNAseq, ATACseq and pcHiC from cultured WT and DMD myotubes with or without re-expression of micro-dys
New experimental replicates from control iPSC-derived myotubes (WT), iPSC-derived dystrophin deficient myotubes (DMDΔ8-9) or iPSC-derived dystrophin deficient myotubes expressing lentiviral delivered micro-dys (DMDΔ8-9μDys) or CRISPR-restored micro-dys (DMDΔ6-9) have been prepared and subjected to deep sequencing for parallel RNAseq, ATACseq and pc-Hi-C – see explanation in the section "Changes in approach and reasons for change") analysis.

Principal Component Analysis (PCA) of RNAseq datasets shows that while all experimental duplicates are well clustered, the DMD myotubes are clearly separated by the PC1 from the control WT myotubes, regardless the re-expression micro-dystrophin; however, DMDΔ6/9 myotubes, which re-expressed micro-dys that retains the NO binding domain clustered away from DMDΔ8-9 myotubes and DMDΔ8-9μDys myotubes (which re-expressed micro-dys that lacks the NO binding domain) along with the PC2 which accounts for a substantial variance (24%) (Fig. 1A). Indeed, heatmap of the RNAseq shows patterns of up and downregulation of gene expression in DMD myotubes with respect to the WT control myotubes, with a subset of genes that are upregulated in DMDΔ6/9 myotubes whose expression is recovered in DMDΔ6-9 myotubes, but not DMDΔ8-9μDys myotubes (Fig. 1B)

RNAseq analysis

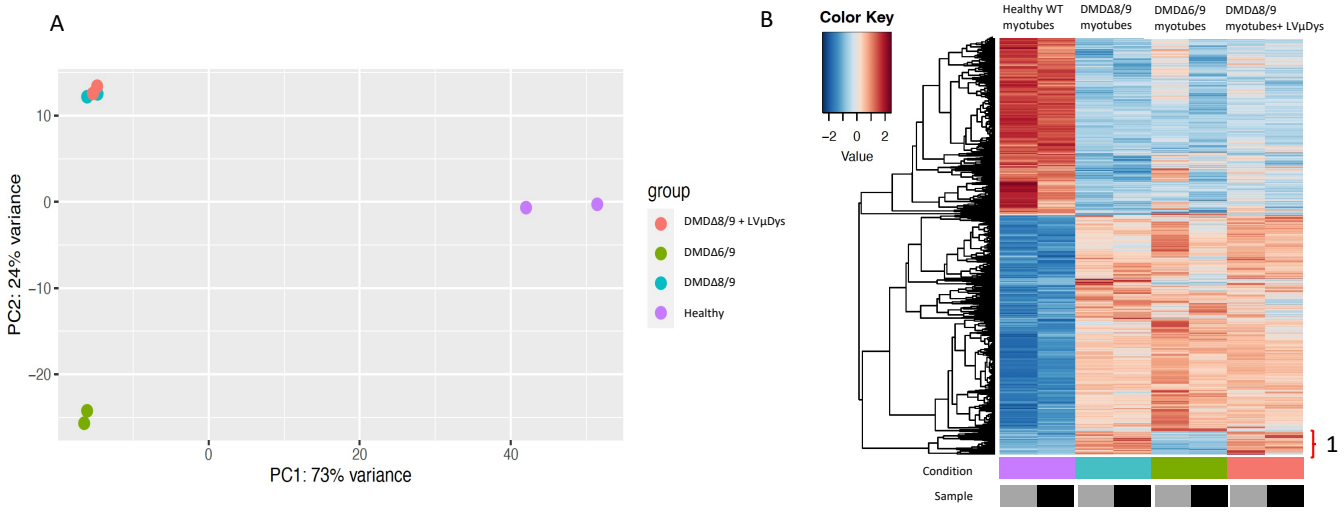


Figure 1: A) Principal Component Analysis (PCA) and **B)** heatmap of the RNAseq from the samples indicated in the list.

Figure 2 shows IGV tracks that demonstrate the expression of controls genes not altered in WT vs DMD myotubes (myogenin and MYHC3) (Fig. 2 A-C) as well as of DMD, which shows absence of reads in all exons in dystrophin deficient myotubes (DMDΔ8-9) and the predicted patterns of partial recovery in DMDΔ8-9μDys) or DMDΔ 6-9 myotubes

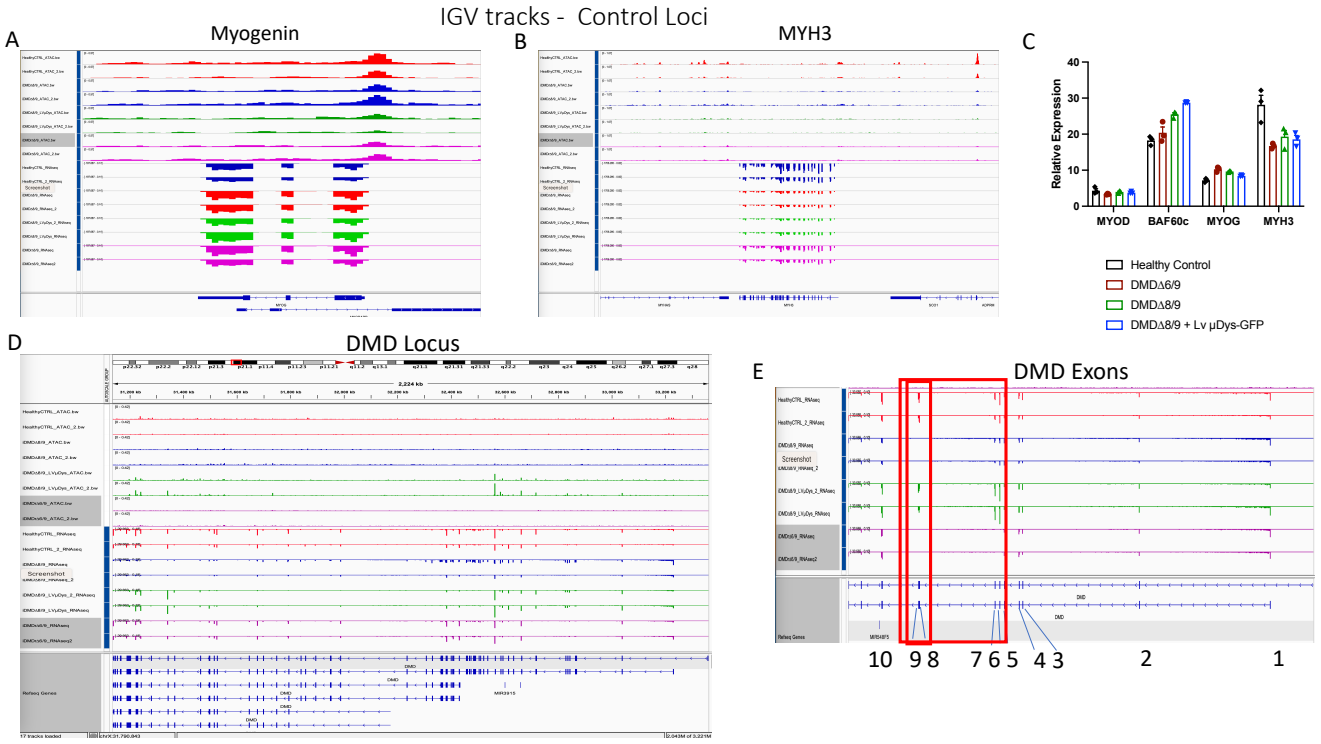


Figure 2: A and B) IGV tracks of controls genes that are not altered in WT vs DMD myotubes, and **C)** as also shown by qPCR analysis. **D-E)** IGV tracks of DMD expression showing the predicted patterns of expression in DMD samples without or with restoration of micro-Dys mutants

Gene ontology analysis revealed that the genes differentially expressed (DE) in DMD myotubes are related to biological processes implicated in the pathogenesis of DMD, including Calcium uptake and handling, remodelling of extracellular matrix, proteolysis, contraction, sarcomere organization, DNA repair and metabolism (Figs 3 and 4).

GO Analysis of DMDΔ8/9 vs WT of downregulated genes

Term GO BIOLOGICAL PROCESS	P-value	Adjusted P-value
DNA Metabolic Process (GO:0006259)	1.58E-25	7.76E-22
DNA-templated DNA Replication (GO:0006261)	6.49E-19	1.60E-15
DNA Repair (GO:0006281)	1.11E-17	1.81E-14
Positive Regulation Of DNA-templated Transcription (GO:0045893)	4.47E-15	5.50E-12
DNA Replication (GO:0006260)	7.02E-15	6.90E-12
DNA Damage Response (GO:0006974)	1.04E-13	8.53E-11
Regulation Of Cell Migration (GO:0030334)	1.45E-13	9.28E-11
Mitotic Sister Chromatid Segregation (GO:0000070)	1.51E-13	9.28E-11
Regulation Of Cell Population Proliferation (GO:0042127)	2.06E-11	1.13E-08
Regulation Of Apoptotic Process (GO:0042981)	2.53E-11	1.24E-08

Term GO Cellular Component	P-value	Adjusted P-value
Nucleus (GO:0005634)	2.96E-29	1.27E-26
Intracellular Membrane-Bounded Organelle (GO:0043231)	1.26E-26	2.70E-24
Focal Adhesion (GO:0005925)	4.10E-19	5.85E-17
Cell-Substrate Junction (GO:0030055)	3.36E-18	3.59E-16
Endoplasmic Reticulum Lumen (GO:0005788)	1.51E-13	1.29E-11
Collagen-Containing Extracellular Matrix (GO:0062023)	8.70E-12	6.21E-10
Spindle (GO:0005819)	1.24E-11	7.56E-10
Intracellular Non-Membrane-Bounded Organelle (GO:0043232)	3.96E-10	2.08E-08
Cell-Cell Junction (GO:0005911)	4.37E-10	2.08E-08
Nuclear Chromosome (GO:0000228)	3.04E-09	1.30E-07

Term GO Molecular Function	P-value	Adjusted P-value
Cadherin Binding (GO:0045296)	1.03E-12	1.05E-09
DNA Binding (GO:0003677)	9.37E-11	4.76E-08
Protein Serine/Threonine Kinase Activity (GO:0004674)	6.55E-10	2.22E-07
Kinase Binding (GO:0019900)	8.77E-09	2.23E-06
Protein Kinase Binding (GO:0019901)	1.26E-08	2.56E-06
Single-Stranded DNA Helicase Activity (GO:0017116)	2.93E-08	4.95E-06
RNA Binding (GO:0003723)	5.02E-08	7.28E-06
Ubiquitin-Like Protein Ligase Binding (GO:0044389)	1.55E-07	1.97E-05
Damaged DNA Binding (GO:0003684)	4.69E-07	5.30E-05
Ubiquitin Protein Ligase Binding (GO:0031625)	5.40E-07	5.48E-05

Figure 3: Gene ontology analysis of genes downregulated in DMD myotubes

GO Analysis of DMDΔ8/9 vs WT of upregulated genes

Term GO BIOLOGICAL PROCESS	P-value	Adjusted P-value
Striated Muscle Contraction (GO:0006941)	1.59E-14	7.63E-11
Myofibril Assembly (GO:0030239)	3.09E-13	7.43E-10
Muscle Contraction (GO:0006936)	1.04E-11	1.67E-08
Heart Contraction (GO:0060047)	1.60E-10	1.92E-07
Actomyosin Structure Organization (GO:0031032)	2.76E-10	2.65E-07
Sarcomere Organization (GO:0045214)	1.22E-09	9.74E-07
Cardiac Muscle Contraction (GO:0060048)	5.76E-09	3.96E-06
Regulation Of Heart Contraction (GO:0008016)	1.32E-07	7.95E-05
Muscle Organ Development (GO:0007517)	9.17E-07	4.90E-04
Regulation Of Calcium Ion Transmembrane Transport (GO:1903169)	1.52E-06	7.29E-04

Term GO Cellular Component	P-value	Adjusted P-value
Sarcoplasmic Reticulum (GO:0016529)	5.22E-10	2.19E-07
Sarcolemma (GO:0042383)	1.71E-09	3.59E-07
Calcium Channel Complex (GO:0034704)	4.09E-09	5.71E-07
Sarcoplasmic Reticulum Membrane (GO:0003017)	1.65E-08	1.72E-06
Cytoskeleton (GO:0005856)	2.61E-06	2.19E-04
Voltage-Gated Calcium Channel Complex (GO:0005891)	1.53E-05	0.00106997
Cell-Cell Contact Zone (GO:0044291)	2.47E-05	0.001478
Actin Cytoskeleton (GO:0015629)	2.88E-05	0.00151048
Junctional Sarcoplasmic Reticulum Membrane (GO:0014701)	7.63E-05	0.00355349
Neuron Projection (GO:0043005)	9.51E-05	0.0039859

Term GO Molecular Function	P-value	Adjusted P-value
Actin Binding (GO:0003779)	6.99E-08	6.93E-05
Voltage-Gated Calcium Channel Activity (GO:0005245)	3.35E-07	1.66E-04
Alpha-Actinin Binding (GO:0051393)	2.50E-06	6.20E-04
High Voltage-Gated Calcium Channel Activity (GO:0008331)	2.50E-06	6.20E-04
Actinin Binding (GO:0042805)	1.27E-05	0.0025224 7
Voltage-Gated Monoatomic Cation Channel Activity (GO:0022843)	1.95E-05	0.0032299 6
Calcium Channel Activity (GO:0005262)	4.01E-04	0.0567807 3
Calmodulin-Dependent Protein Kinase Activity (GO:0004683)	5.16E-04	0.0640394 2
Microtubule Binding (GO:0008017)	6.89E-04	0.0759556 1
Ligand-Gated Calcium Channel Activity (GO:0099604)	8.04E-04	0.0797721 8

Figure 4: Gene ontology analysis of genes upregulated in DMD myotubes

Figure 5 shows the IGV track of plastin-2 (LCP1) a gene upregulated in DMD myotubes, as also previously reported by Guiraud et al (Scientific Reports 2017) and whose expression was recovered only by the restoration of micro-Dys which retains the NO binding domain.

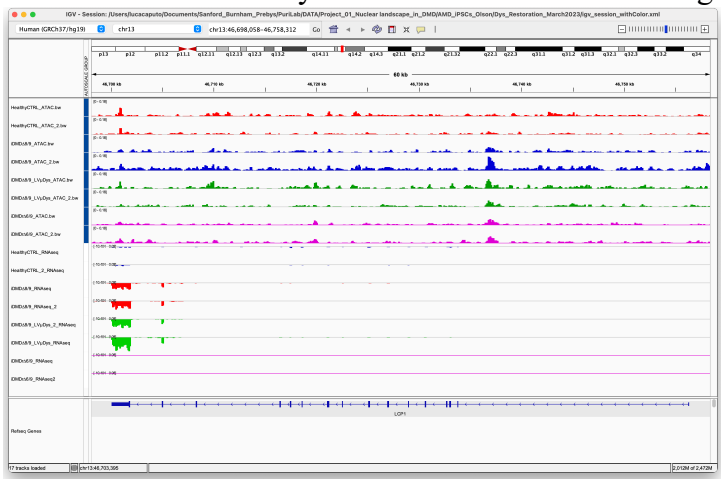


Figure 5: IGV tracks of plastin-2 (LCP1)

Figure 6 shows the IGV track of the expression of an intronic transcript of uncertain nature and function from the HDAC9 gene that was induced in DMD myotubes and whose expression was recovered by the restoration of micro-Dys which retains the NO binding domain.

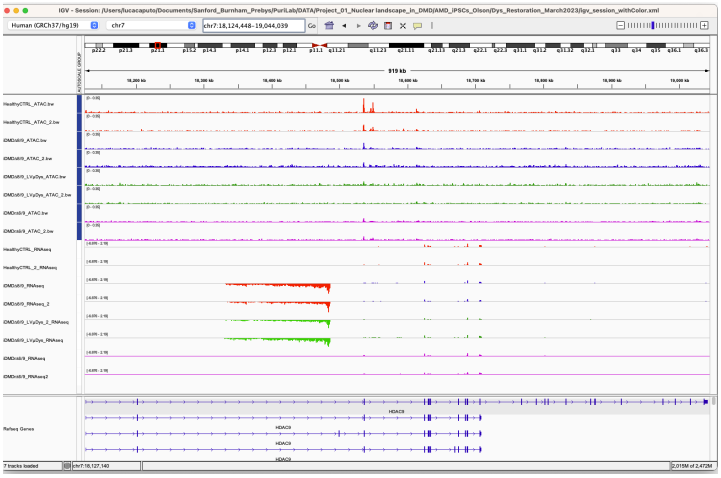


Figure 6: IGV tracks of the HDAC9 locus

Parallel ATACseq analysis revealed coherent patterns of differential chromatin accessibility between WT and DMD myotubes. PCA shows again that DMDΔ8-9 myotubes were separated by the PC1 from the control WT myotubes, regardless the re-expression micro-dystrophin (Fig. 7A). Interestingly, DMDΔ6/9 myotubes, which re-expressed micro-dys that retains the NO binding domain, and DMDΔ8-9μDys myotubes, which re-expressed micro-dys that lacks the NO binding domain, both clustered in close proximity of DMDΔ8-9 along with the PC2, which account for a small percentage of variance (14%) (Fig. 7A). This suggests that while chromatin alterations in DMD parallel the patterns of differential gene expression detected by RNAseq, the correction of specific patterns of DE genes by micro-dystrophin might operate through a mechanism different than recovering the patterns of chromatin accessibility. Of note, the genomic distribution of alterations in chromatin accessibility indicates that a large amount of differential peaks of chromatin accessibility was mostly located at gene promoters in DMDΔ8-9, a pattern that was partly restored in DMDΔ8-9μDys, but not DMDΔ6/9 myotubes (Fig. 7B and C), again suggesting that micro-dystrophins might individually recover altered patterns of gene expression and chromatin accessibility, likely by different mechanisms.

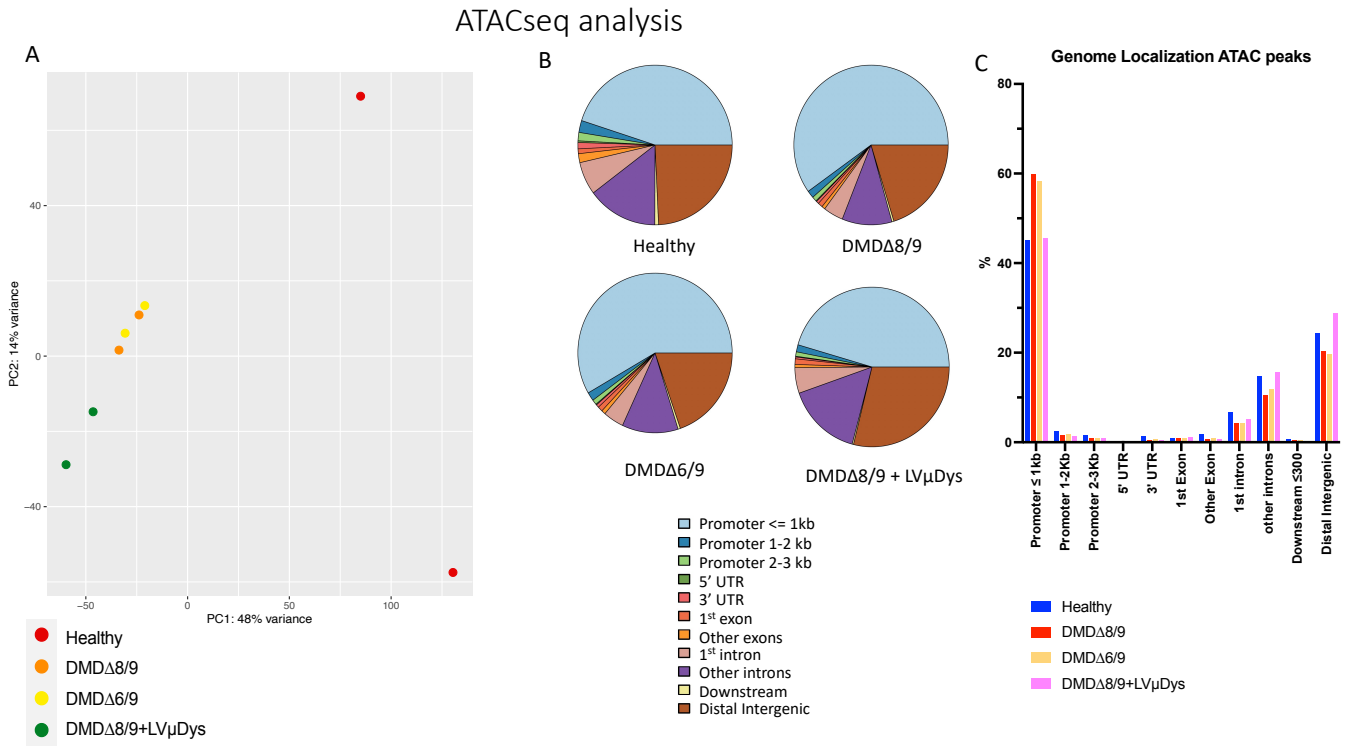


Figure 7: A) Principal Component Analysis (PCA) and **B)** genomic distribution and **C)** localization of differential peaks of chromatin accessibility of the samples indicated in the list.

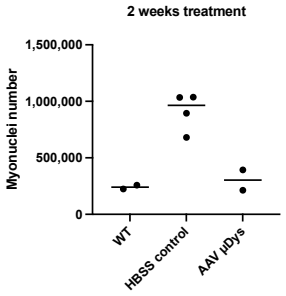
While in the year 1 we have performed an initial analysis of HiChIP H3K27Ac, the large amount of differential peaks of chromatin accessibility at promoter level prompted an interest to perform a promoter capture HiC (pc-HiC), as initially planned in the original proposal. We have therefore subjected the sample replicates to a parallel pcHiC whose analysis is current undergoing.

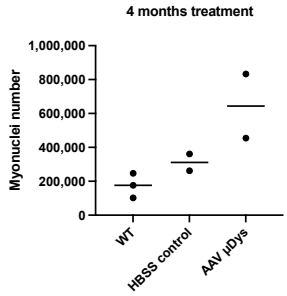
Collection of myonuclei isolated by FACS from HSAcre;MDX; Rosa26-Lsl-H2B-GFP mice

We have collected myonuclei from HSAcre;MDX; Rosa26-Lsl-H2B-GFP mice at 4 weeks and 4 months of life, with or without AAV-mediated delivery of μDys5 or control vector, and we are currently processing them for parallel pcHiC, RNAseq and ATACseq. A representative representation of the samples collected from this experiment is illustrated below

Statistic Post-Sorting: Number of GFP nuclei for each animal

	WT mice					mdx mice									
	80 days old		200 days old			2 weeks treatment						4 months treatment			
						HBSS control				AAV Dys5+NOS		HBSS control		AAV Dys5+NOS	
Animal ID	227	229	223	220	221	184	185	186	188	164	238	149	158	154	157
HSA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H2B	+/+	+/+	+/+	+/+	+/+	+/+	+/-	+/-	+/-	+/+	+/-	+/+	+/-	+/-	+/+
Age at injection (days)						66	66	66	66	66	66	82	76	76	76
Age at sac (days)	81	81	201	205	205	81	81	81	81	81	81	202	196	196	196
Days of treatment						15	15	15	15	15	15	120	120	120	120
Total GFP+ Myonuclei	224802	257566	246728	101716	176210	681116	1037365	1034128	894176	213000	393067	261882	361211	832737	454206
Weight (g)	50,5	44,29	57,21	54,9	50,6	32,19	29,5	32,75	33,57		28,81	41,2	30,2	34,3	33,7





- **What opportunities for training and professional development has the project provided?**

The Sanford Burnham Prebys Medical Discovery Institute (SBP) Office of Education, Training & International Services (OETIS) oversees and coordinates an annual individual development planning (IDP) process for all postdocs at the Institute. The focus of the IDP process at SBP is the career goal of the postdoc; identification of what skills, knowledge, and accomplishments will be necessary for the postdoc to obtain a desired independent position following training; and identification of training and professional development opportunities that are available for the postdoc to obtain the necessary skills and knowledge. The SBP Office of Education, Training & International Services provides guidance and advising to both postdocs and PIs throughout the postdoc's training with respect to developing IDPs and preparing for a successful transition to independence post-training. The SBP Office of Education, Training & International Services also maintains webpages containing comprehensive resources on career path identification, career planning, and creating an IDP that can be utilized in conjunction with the formal annual IDP process.

The SBP IDP process includes two components:

1) First-Year IDP (effective in 2014). Within the first 3 months of beginning postdoctoral training at SBP, all postdocs receive and fill out an initial "planning and expectations" document to discuss with their PI. This document serves as the foundation for their postdoctoral IDP and is designed to facilitate discussion between the PI and new postdoc regarding goals and expectations for the first year of training, as well as stimulate initial discussions about long-term career goals and training plans.

2) Postdoctoral IDP (effective January 2013). At the end of the first year of training SBP postdocs receive notification that it is time to update their IDP, and they receive the information they included in their first-year planning and expectations document in the form of a full IDP that they can update with their accomplishments over the past year and their goals for the coming year, mid-term future, and long-term future. Each subsequent year of their postdoctoral training, postdocs will receive notification and the previous year's IDP form to update and expand. The IDP forms are designed to build upon each previous year as well as provide a solid foundation from which a postdoc can easily build his or her CV/resume.

The SBP Office of Education, Training & International Services also maintains webpages containing comprehensive resources on career path identification, career planning, and creating an IDP that can be utilized in conjunction with the formal annual IDP process.

Though the most recent review has not been conducted for Drs. Chiara Nicoletti and Luca Caputo, Dr. Puri plans to ensure that all IDPs are updated over the coming year to be consistent with SBP IDP process for Postdocs.

- **How were the results disseminated to communities of interest?**
  - Nothing to report
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - We plan to ask for a no cost extension in which we aim at completing the datasets analysis of RNAseq, ATACseq and pc-Hi-C samples that have been already generated from iPSC-derived myotube cultures (and are under generation from mdx mice. Once we will have available standard "quality-grade" datasets from all samples, we will proceed with the final integrative analysis (Aim 3).

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - Nothing to report
   - **What was the impact on other disciplines?**
     - Nothing to report
   - **What was the impact on technology transfer?**
     - Nothing to report
   - **What was the impact on society beyond science and technology?**
     - Nothing to report.
5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**
     - None
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
     - Because of lack low quality control standards in some replicates of the experiments performed in year 1, we have prepared completely new sample replicates that have been analyzed as described in "significant results" section.
   - **Changes that had a significant impact on expenditures**
     - Failed experiments did not have any significant impact on originally budgeted DoD expenditures, as they have been covered by using personal discretionary fundings from the institute.
   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents** None
   - **Significant changes in use or care of human subjects** None
   - **Significant changes in use or care of vertebrate animals.** None
   - **Significant changes in use of biohazards and/or select agents** None
6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**
     Nothing to report
     - **Journal publications.**
       Nothing to report
     - **Books or other non-periodical, one-time publications.**
       Nothing to report
     - **Other publications, conference papers, and presentations.**
       Nothing to report
   - **Website(s) or other Internet site(s)**
     Nothing to report
   - **Technologies or techniques**
     Nothing to report
   - **Inventions, patent applications, and/or licenses**
     Nothing to report

- **Other Products**
  Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

Name:	Lorenzo Puri
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-4964-0095
Nearest person month worked:	0.96 calendar months
Contribution to Project:	Planning and supervision of the experiments. Data dissemination.
Funding Support:	N/A

Name:	Chiara Nicoletti
Project Role:	Postdoctoral Associate
Researcher Identifier (e.g. ORCID ID):	0000-0002-0872-6506
Nearest person month worked:	0.72 calendar months
Contribution to Project:	Analysis of RNAseq, ATACseq and HiC generated from mice and cultured cells
Funding Support:	N/A

Name:	Luca Caputo
Project Role:	Postdoctoral Associate
Researcher Identifier (e.g. ORCID ID):	0000-0002-1697-9968
Nearest person month worked:	0.20 calendar months
Contribution to Project:	Setting experimental protocols. Generation of RNAseq, ATACseq and HiC datasets from mice and cultured cells
Funding Support:	N/A

Name:	Alessandra Sacco
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-1669-0221
Nearest person month worked:	0.23 calendar months
Contribution to Project:	Counseling on FACS-mediated isolation of myonuclei
Funding Support:	N/A

Name:	Jimmy Massenet
Project Role:	Postdoctoral Associate
Researcher Identifier (e.g. ORCID ID):	0000-0001-9699-5580
Nearest person month worked:	4.10 calendar months
Contribution to Project:	Generation of RNAseq, ATACseq and HiC datasets from mice and cultured cells
Funding Support:	N/A

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** The following are changes to Dr.Puri's Active Other Support:
  Current Grants

NEW

Title: Interdependency of fibroadipogenic progenitors and extracellular matrix that drive skeletal muscle fibrosis

Major Goals: The goal of this proposal is to establish the mechanisms of interaction between fibroadipogenic progenitors and the extracellular matrix that drive progressive fibrosis in Duchenne muscular dystrophy.

Specific Aims:
1. Determine fibroadipogenic progenitor sensitivity to fibrotic extracellular matrix architecture and mechanical properties.
2. Determine the regulation of myogenesis by fibrotic fibroadipogenic progenitor derived extracellular matrix.
3. Determine the effect of microdystrophin gene therapy on fibrosis and fibroadipogenic progenitor phenotypes in vivo.

Project Number: R01 AR079545
Name of PD/PI: Puri, Lorenzo
Source of Support: University of California, Davis/NIH/NIAMS
Proposed Performance Period: 04/2022-03/2027
Total Proposed Amount:

Time Commitment per Budget Period:

YEAR (YYYY)	Person Months (##.##)
1. 2023	0.30 calendar months
2. 2024	0.30 calendar months
3. 2025	0.30 calendar months
4. 2026	0.30 calendar months
5. 2027	0.30 calendar months

Grants Management Officer: Susan Toy, Phone: Email: Susan.Toy@nih.gov
Overlap: None

NEW

Title: Blocking Fibroadipogenic Progenitor Response to Fibrotic Stiffness to Enhance Efficacy of Microdystrophin Gene Therapy

Major Goals: The goals of this project blocking FAPs production of fibrosis and limitation of dystrophin gene therapy is particularly aligned well with the goals of my lab, while the focus on mechanical stiffness signaling is distinct.

Specific Aims:
1. Determine the relationship between fibrosis and the efficacy of

micro-dystrophin treatment
2. Determine anti-fibrotic properties of inhibiting FAP response to stiffness
3. Enhance efficacy of micro-dystrophin gene therapy by inhibiting FAP response to stiffness

Project Number: W81XWH-22-1-1058
Name of PD/PI: Puri, Pier Lorenzo
Source of Support: University of California, Davis/DoD
Proposed Performance Period: 07/2022 - 06/2024
Total Awarded Amount:
Time Commitment per Budget Period:

YEAR	Person Months
1. 2023	0.34 calendar months
2. 2024	0.34 calendar months

Grants Management Officer: Asha Phillips, asha.k.phillips.civ@health.mil None
Overlap:

- **What other organizations were involved as partners?**
  - **Organization Name: University of Washington**
  - **Location of Organization:** USA
  - **Partner's contribution to the projec**t *(identify one or more)*
    - **Financial support;**
    - **In-kind support** *(e.g., partner makes software, computers, equipment, etc., available to project staff)*;
    - **Facilities** *(e.g., project staff use the partner's facilities for project activities)*;
    - **Collaboration** *(e.g., partner's staff work with project staff on the project)*;
    - **Personnel exchanges** *(e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*; and
    - **Other.**

Nothing to Report

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** *N/A*
   - **QUAD CHARTS:** *N/A*
9. **APPENDICES:** Nothing to Report