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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 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 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.
We have successfully established a experimental pipelines that allows the isolation of myonuclei from a mouse model of DMD (mdx mice) as well as from DMD skeletal muscles derived from iPSCs.
We have generated datasets of RNAseq, ATACseq and HiChIP from cultures of DMD muscles (or wild type controls), before or after micro-dystrophin re-expression, that have been partly analyzed, as well as prepared samples from mdx mice that have been sent out for sequencing.

15. SUBJECT TERMS None listed.

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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 major goals of the first year 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)

Accomplishment under these goals

The major activities of the first year of funding were:

We have successfully standard parameters for FACS-mediated isolation of myonuclei from HSAcre;MDX; Rosa26-Lsl-H2B-GFP mice

We have collected the material for the first replicate experiment of parallel pcHiC, RNAseq and ATACseq on myonuclei isolated from HSAcre;MDX; Rosa26-Lsl-H2B-GFP mice at two time points – 4 weeks and 4 months of life.

We have produced AAVs for viral delivery of μDys5 and successfully tested the delivery to 4 week-old HSAcre;MDX; Rosa26-Lsl-H2B-GFP mice

We have successfully set optimal and reproducible experimental conditions for preparation of cultures of DMD patient (or control)-derived myotubes

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.

We have produced Lentiviruses for viral delivery of μDys5 and successfully tested the delivery to DMD cultured myotubes

The specific objectives of the first year of funding were:

Set reproducible protocols of myonuclei isolation from mdx mice and from cultures of myotubes derived from DMD hiPSCs

Generate samples for the first replicates of parallel, RNAseq, ATACseq and pcHiC/HiChIP analysis

Produce and test viral preps for in vivo and in vitro delivery of μDys5

The significant results of the first year of funding were:

Production of AAVs for viral delivery of µDys5 ***(from Dr Chamberlain, Washington University sub-award)*** We generated plasmids for the AAV (pAAV-CK8e-µDys5-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. The vector grew moderately well, but was not one of our higher titer clones. Yields were 8.4E11 vector genomes per roller bottle, which came to a final purified titer of 1.6E13 vg/ml. Titer was determined in 2 ways, by Q-PCR and by Southern analysis, with final yields and titers determined by an average of the 2 methods. As the yield was lower than ideal, we re-cloned the µDys cDNA to eliminate extraneous sequences, such as polylinkers and spacer DNA segments. The AAV vector was then re-prepped, yielding titers nearly 10X higher than with the initial pAAV (range of 5-10X higher on 3 preps). The final purified AAV vector was tested for full (genome-containing) and empty-no genome) particles by CsCl gradients. As shown in Figure 1A, the newer preps gave >80% empty capsids, in line with previous preps. Testing the prep in mdx mice gave robust expression of the µDys protein, also in line with previous preps (Figure 1B).

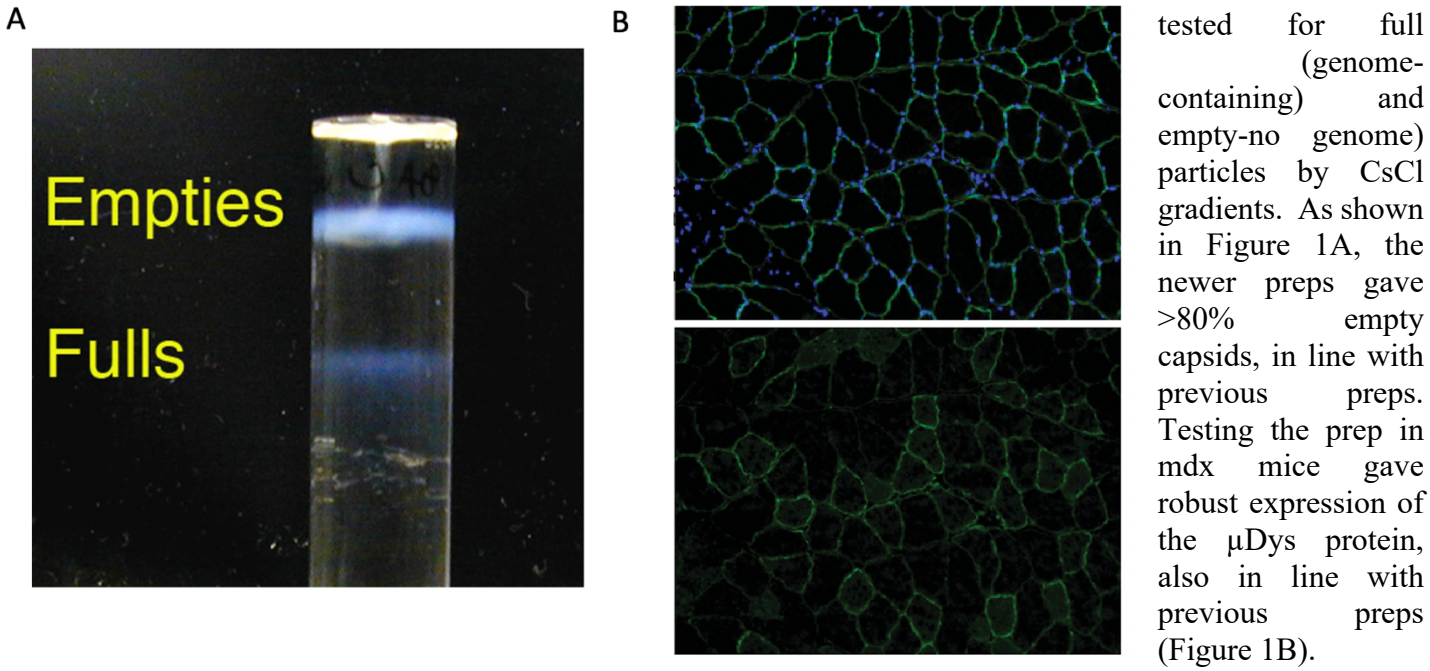
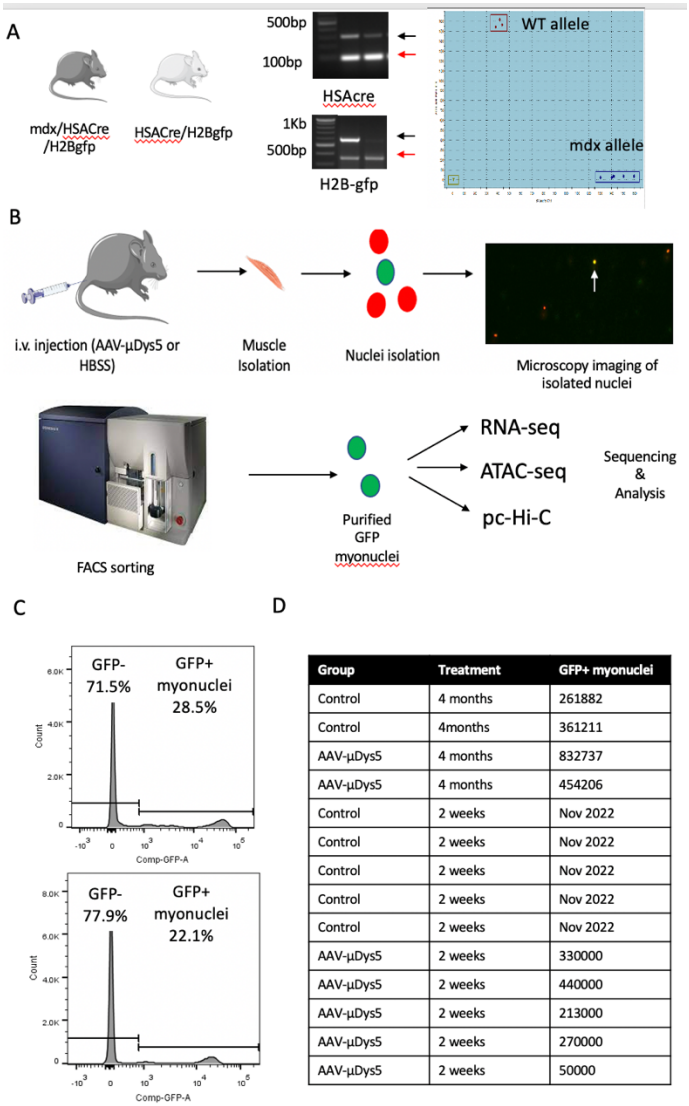


Figure 1 Legend: A) *CsCl gradient to separate empty (top band) from full (lower band) particles from a pAAV6-CK8e-µDys5 preparation. B) Representative images following intramuscular injection of 1E11 vg of AAV6-CK83-µDys5 into dystrophic mdx mouse TA muscles. Top: µDys immunostaining. Bottom: nNOS activity, showing re-expression of nNOS following µDys expression in the muscles. Blue staining is DAPI to visualize nuclei.*

FACS-mediated isolation of myonuclei from HSAcre;MDX; Rosa26-Lsl-H2B-GFP mice and collection of the material for the first replicates of parallel pcHiC, RNAseq and ATACseq.

We have set an optimal strategy for the isolation of myonuclei from skeletal muscles of mdx mice, by using the HSAcre;MDX; Rosa26-Lsl-H2B-GFP mouse models (and wild type control - HSAcre; H2B-GFP mice), in which the expression of GFP allows purification of myonuclei by FACS (Fig. 2A). Figure 2B shows the workflow of myonuclei nuclei isolation from a cohort of mdx mice with all the experimental points planned in the project with the FACS-sorted GFP-positive nuclei that are used for parallel RNAseq, ATACseq and pc-Hi-C. Figure 2C shows a representative image for typical amount of sorted myonuclei from mdx mice (with or without injection of micro-dys). In Fig. 2D it is shown the table dysplaying the number of GFP-positive myonuclei that were collected from mdx mice at all the experimental points planed in the Aim 1. The numbers are all within the expected range that allows to proceed with the parallel RNAseq, ATACseq and pc-Hi-C, except for mdx mice treated with AAV-microDys for 2 weeks, for which we are going to collect another couple of mice to increase the number of available myonuclei. All the other samples have been sent out for deep-sequencing.

Control mice will be sacrified on November 2022 for isolation of GFP-positive myonuclei, which will also be send out for sequencing (parallel RNAseq, ATACseq and pc-Hi-C).

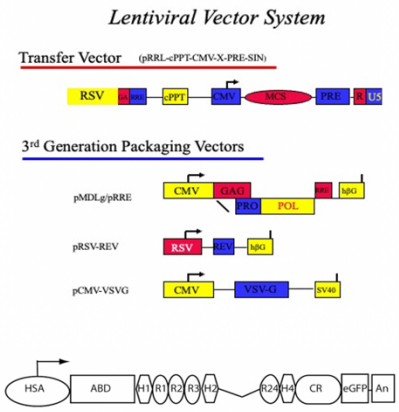


Group	Treatment	GFP+ myonuclei
Control	4 months	261882
Control	4months	361211
AAV-µDys5	4 months	832737
AAV-µDys5	4 months	454206
Control	2 weeks	Nov 2022
Control	2 weeks	Nov 2022
Control	2 weeks	Nov 2022
Control	2 weeks	Nov 2022
Control	2 weeks	Nov 2022
AAV-µDys5	2 weeks	330000
AAV-µDys5	2 weeks	440000
AAV-µDys5	2 weeks	213000
AAV-µDys5	2 weeks	270000
AAV-µDys5	2 weeks	50000

Figure 2 Legend: **A)** *Scheme of genotyping of mdx:HSACre:H2BGFP animals: HSACre WT band 324 bp (black arrow) Mutant band 100bp (red arrow); H2BGFP WT band 600 bp (black arrow) Mutant band 324bp (red arrow); mdx genotyping with TaqMan array;* ***B)*** *Workflow of the isolation procedure for purification of GFP-positive myonuclei from experimental mdx:HSACre:H2BGFP mice - 4 week-old mdx mice njected with AAV- mDys or (control AAV empty solution) and sacrificed either 2 weeks or 4 months after injection. In the microscopy imaging of isolated nuclei (visualized under the fluorescence microscopy) the arrow indicates a GFP-positive nucleus, at the expected frequency of 1:3. GFP-positive nuclei are proceesed and sent out for deep sequencing to generate datasets of RNAseq, ATACseq and pc-Hi-C analysis.* ***C)*** *Representative FACS plot of isolated GFP+ myonuclei in Control treated (top) and AAV-mDys injected (bottom) mdx mice (2 weeks and 4 month old).* ***D)*** *Summary table for isolated GFP+ myonuclei in different conditions - all mdx:HSA-Cre:H2BGFP animals (either control or AAV-mDys injected)*

Production of Lentiviruses for viral delivery of µDys5 and successfully tested the delivery to DMD cultured myotubes *(from Dr Chamberlain, Washington University sub-award)*

We generated a lentiviral vector (Lv) expressing the µDys protein under control of a muscle-specific promoter. The plasmids used were originally obtained from Dr L. Naldini, with minor modifications to accommodate muscle-specific use. For this task we generated 4 plasmids for the Lv (pRRL-cPPT-HSA-µDy-PRE-SIN) and the



helper plasmids (pMDLg/pRRE; pRSV-REV; and pCMV-VSVG – Fig. 3). 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. The vector grew well, as determined from 2 independent preps. Prep size ranged from 5-10 roller bottles, and was concentrated to ~1 ml. The final purified Lv was tested for µDys expression in iPSCs-derived myotubes (see Figs 5 and 11).

Figure 3 Legend: *Plasmids used to generate LV vectors expression µDystrophin. A four plasmid co-transfection system used to generate lentiviral vectors. The Transfer plasmid carries the gene of interest, in this case the HSA promoter and the µDys cDNA (not CMV as shown in the figure).*

Setting optimal and reproducible experimental conditions for preparation of cultures of DMD patient (or control)-derived myotubes

We have experienced initial and unpredicted troubles with obtaining 100% purity of cultures of iPSC-derived skeletal myotubes. While analyzing our first experiments of RNAseq, ATACseq and pc-Hi-C generated from cultures during the initial 6 months of grant activity, we noticed unexpected patterns of samples alignment and clustering, due to the of presence of non-muscle cells (Fig, 4 – see DAPI positive/MyoD negative cells) which likely reflects contamination of cells undergoing differentiation possibly into various types of lineages.

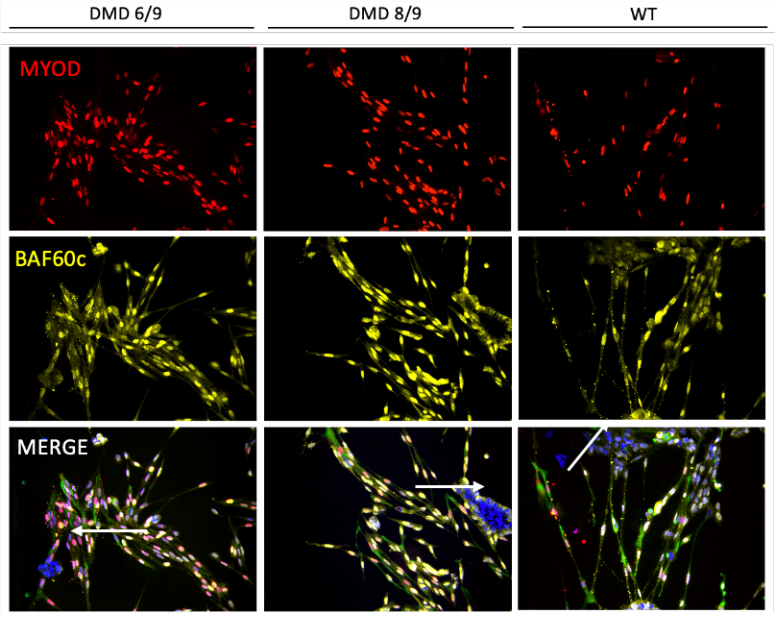
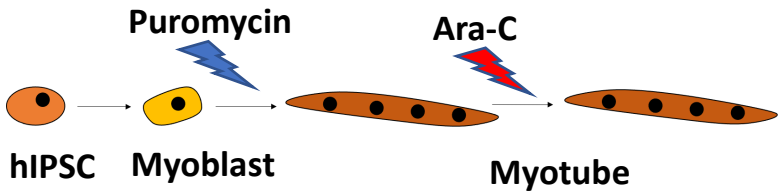


Figure 4 Legend: *Immunofluorescence staining of MYOD (upper panels) and BAF60C (middle panels) and counterstaining with DAPI (bottom panels) in hiPSC-derived cells from iPSC-derived dystrophin deficient myotubes (DMD 8/9) or micro-dys restored counterpart (DMD 6/9) or wild type control iPSC-derived myotubes.*

We have resolved this issue by using a protocol of sequential treatment of myotubes with Puromycin (to further select Puromycin-resistant, MYOD/BAF60C-expressing myotubes) and Ara-C (to selectively eliminate contaminating cycling cells interspersed between the myotubes) 5 days prior cell collection for analysis – see scheme below.



The resulting cultures of myotubes show no or negligible evidence of contamination (see Fig. 5)

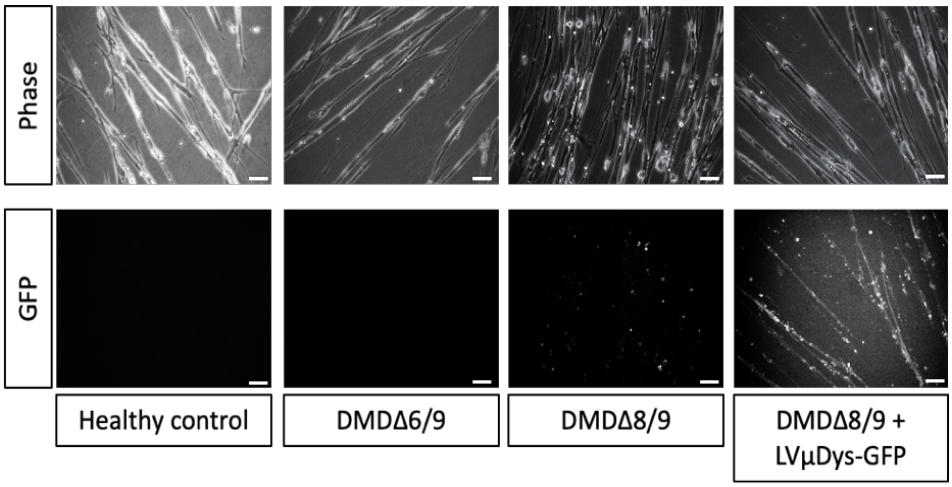


Figure 5 Legend: *Representative images of cultures of iPSC-derived myotubes 5 days after the sequential exposure to Puromycin (6ng/ml, 48 hours) and AraC (5μM, 48hours) - Scale bar 150μm; CNote that GFP visualize the expression of μDys in myotubes infected with Lentivirus expressing μDys-GFP under HSA promoter– Scale bar 100 μm)*

We confirmed by qPCR that myotubes from each experimental samples expressed comparable levels of MYOD and BAF60C, as well as the two markers of early and late skeletal muscle differentiation, myogenin and MYH3, respectively (Fig. 6).

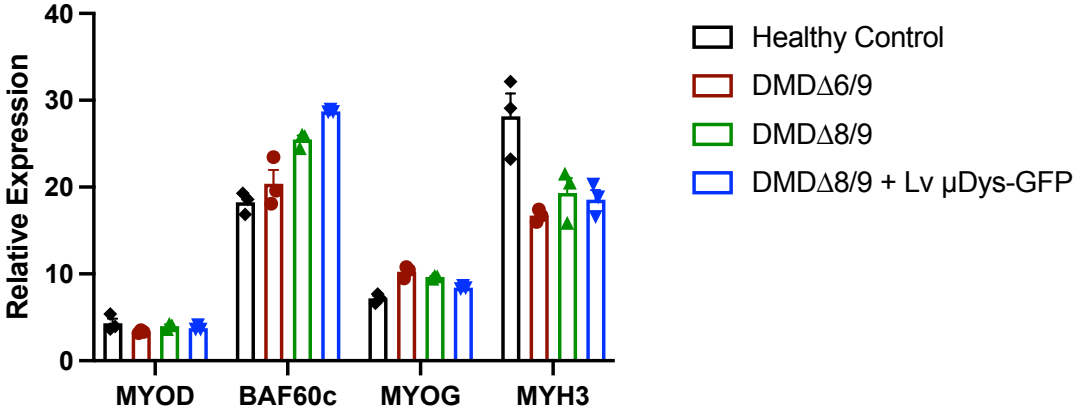


Figure 6 Legend: *qPCR expression analysis for exogenous MYOD and BAFC60c and myogenic markers MYOG and MYH3 at d21 of differentiation protocol (n=3, data are shown as MEAN + SEM)*

First replicates of parallel pcHiC, RNAseq and ATACseq on myonuclei isolated from cultured WT and DMD myotubes.

Experimental replicates from control iPSC-derived myotubes (WT), iPSC-derived dystrophin deficient myotubes (8-9) or iPSC-derived dystrophin deficient myotubes (8-9) expressing lentiviral delivered micro-dys (8-9μDys) or CRISPR-restored micro-dys (6-9) were subjected to deep sequencing for parallel RNAseq, ATACseq and HiChIP H3K27Ac (instead of pc-Hi-C – see explanation in the section "Changes in approach and reasons for

change") analysis. Figure 7 shows the list of the samples and the general quality statistics relative to the sequencing of each samples for the 3 different analysis.

FASTQC
General Statistics

Sample Name	% Dups	% GC	M Seqs
WT-ATAC-seq_rep1_R1	19.5%	50%	164.5
WT-RNA-seq_rep1_R1	88.4%	72%	39.3
WT-ATAC-seq_rep1_R2	24.9%	50%	164.5
WT-RNA-seq_rep1_R2	84.6%	74%	39.3
WT-ATAC-seq_rep2_R1	58.9%	51%	99.0
WT-RNA-seq_rep2_R1	72.1%	50%	88.2
WT-ATAC-seq_rep2_R2	54.0%	51%	99.0
WT-RNA-seq_rep2_R2	64.6%	51%	88.2
WT-ATAC-seq_rep3_R1	43.7%	48%	234.1
WT-RNA-seq_rep3_R1	76.6%	53%	77.3
WT-ATAC-seq_rep3_R2	46.6%	47%	234.1
WT-RNA-seq_rep3_R2	70.4%	52%	77.3
WT-RNA-seq_rep4_R1	55.4%	49%	73.7
WT-RNA-seq_rep4_R2	47.5%	50%	73.7
WT-HiChIP_H3K27ac_rep1_R1	40.6%	44%	320.3
WT-HiChIP_H3K27ac_rep1_R2	39.6%	44%	320.3
6-9-ATAC-seq_rep2_R1	61.2%	50%	91.6
6-9-ATAC-seq_rep2_R2	57.1%	50%	91.6
6-9-ATAC-seq_rep3_R1	49.6%	48%	245.7
6-9-ATAC-seq_rep3_R2	51.5%	48%	245.7
6_9-ATAC-seq_rep1_R1	32.2%	48%	278.0
6_9-RNA-seq_rep1_R1	56.0%	52%	20.3
6_9-ATAC-seq_rep1_R2	31.6%	48%	278.0
6_9-RNA-seq_rep1_R2	52.1%	52%	20.3
6_9-RNA-seq_rep2_R1	67.5%	53%	38.1
6_9-RNA-seq_rep2_R2	63.4%	52%	38.1
6_9-RNA-seq_rep3_R1	59.8%	48%	131.0
6_9-RNA-seq_rep3_R2	49.9%	49%	131.0
6_9-HiChIP_H3K27ac_R1	59.1%	44%	327.9
6_9-HiChIP_H3K27ac_R2	57.8%	44%	327.9
8_9-ATAC-seq_rep1_R1	26.4%	49%	161.8
8_9-RNA-seq_rep1_R1	64.2%	55%	25.9
8_9+μDys-ATAC-seq_rep1_R1	21.3%	50%	150.5
8_9+μDys-RNA-seq_rep1_R1	52.3%	48%	40.2
8_9-ATAC-seq_rep1_R2	26.4%	49%	161.8
8_9-RNA-seq_rep1_R2	61.1%	56%	25.9
8_9+μDys-ATAC-seq_rep1_R2	23.8%	50%	150.5
8_9+μDys-RNA-seq_rep1_R2	48.0%	49%	40.2
8_9+μDys-ATAC-seq_rep2_R1	55.8%	49%	157.1
8_9-ATAC-seq_rep2_R1	69.8%	50%	200.7
8_9+μDys-RNA-seq_rep2_R1	72.6%	53%	61.3
8_9-RNA-seq_rep2_R1	80.8%	52%	352.9
8_9+μDys-ATAC-seq_rep2_R2	52.3%	49%	157.1
8_9-ATAC-seq_rep2_R2	63.5%	50%	200.7
8_9+μDys-RNA-seq_rep2_R2	66.7%	53%	61.3
8_9-RNA-seq_rep2_R2	81.1%	51%	352.9
8_9+μDys-ATAC-seq_rep3_R1	48.6%	48%	291.0
8_9-ATAC-seq_rep3_R1	39.1%	49%	290.0
8_9+μDys-RNA-seq_rep3_R1	53.8%	51%	61.8
8_9-RNA-seq_rep3_R1	52.2%	48%	43.9
8_9+μDys-ATAC-seq_rep3_R2	46.6%	48%	291.0
8_9-ATAC-seq_rep3_R2	37.6%	48%	290.0
8_9+μDys-RNA-seq_rep3_R2	47.0%	52%	61.8
8_9-RNA-seq_rep3_R2	43.6%	49%	43.9
8_9-HiChIP_H3K27ac_rep1_R1	56.1%	44%	362.1
8_9+μDys-HiChIP_H3K27ac_rep1_R1	58.9%	44%	306.4
8_9-HiChIP_H3K27ac_rep1_R2	55.4%	44%	362.1
8_9+μDys-HiChIP_H3K27ac_rep1_R2	58.2%	44%	306.4

Figure 7 Legend: *Quality controls for ATAC-seq, HiChIP H3K27ac and RNA-seq from human myotubes with DMD wild type (WT), dystrophic (8_9), and restored by CRISPR/Cas9 (6_9) or through microdystrophyn (DM). The first column on this table represents the proportion of PCR duplicates in each sample, the second focuses on the GC content of the sequences (the human genome should have a 42% GC content), the third informs us on the proportion of failed quality tests per sample and the last column containes the million reads sequenced per sample.*

RNAseq datasets from the samples listed in Fig. 7 were subjected to alignment and gene count analysis, which shows that all the samples were within the range of accepted standard criteria for analysis, expect for one of the 4 replicates of control iPSC derived myotubes (WT) (Fig. 8), which was therefore eliminated and excluded from subsequent analysis.
Thus, we carry over for further analysis 3 replicates for each sample.

Sample Name	% Aligned	M Aligned
WT-RNA-seq_rep1	19.9%	7.0
WT-RNA-seq_rep2	82.2%	68.6
WT-RNA-seq_rep3	80.3%	49.0
WT-RNA-seq_rep4	81.1%	59.7
6_9-RNA-seq_rep1	65.2%	13.2
6_9-RNA-seq_rep2	80.7%	24.2
6_9-RNA-seq_rep3	81.0%	105.3
8_9-RNA-seq_rep1	54.0%	13.9
8_9+μDys-RNA-seq_rep1	83.2%	33.3
8_9+μDys-RNA-seq_rep2	80.0%	37.3
8_9-RNA-seq_rep2	82.1%	238.0
8_9+μDys-RNA-seq_rep3	74.4%	45.9
8_9-RNA-seq_rep3	79.0%	34.5

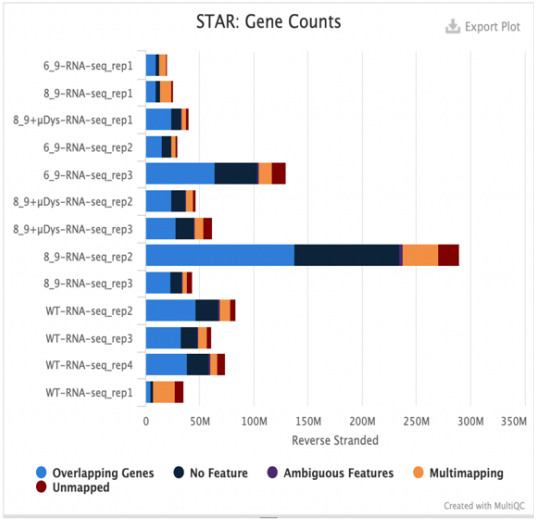


Figure 8 Legend: A*nalysis of sample alignment and gene counts*

We have started an initial RNAseq analysis by performing a Principal Component Analysis (PCA) of two biological replicates derived from cultures of two distinct experiments. While PC1 separates different samples batches, PC2 could clearly separate WT myotubes from dystrophin deficient DMD 8-9 myotubes. Interesting, re-expression of a version of micro-dys that contains the binding domain for nNOS (DMD 6-9) could partly restore a pattern of gene expression closer to that of WT myotubes. Conversely, expression of a version of micro-dys that does not contain the binding domain for nNOS (8-9 µDys) could not significantly change the gene expression as compared to DMD 8-9 myotubes (Fig. 9).

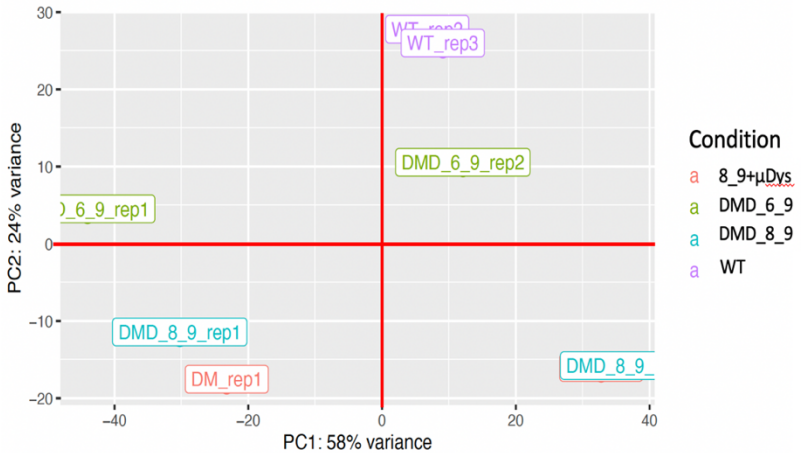


Figure 9 Legend: *Principal Component Analysis of RNAseq datasets from duplicates of experimental samples of WT myotubes, dystrophin-deficient DMD 8-9 myotubes or DMD 8-9 infected with lentiviral expressed µDys (8-9 µDys) or myotubes re-expressing µDys from CRISPR-based genome editing (DMD 6-9)*

Consistently, UpSetR plot of differentially expressed genes (DEG) shows that DMD 8-9 and 8-9 µDys myotubes exhibit most different patterns of gene expression than WT myotubes, as compared to DMD 6-9 (Fig. 10)

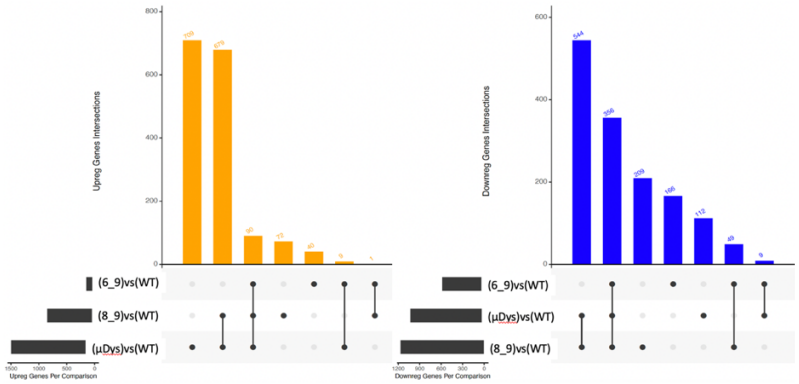


Figure 10 Legend : *UpSetR plots of DEGs, UP in yellow and DOWN in blue for the three differential comparisons. UP reg genes almost lost with 6_9, DOWN reg genes still present*

Of note, we used RNAseq to verify that correct isoforms of micro-dys were indeed expressed in mDys 8-9 myotubes and DMD 6-9 myotubes. Figure 11 shows that indeed the micro-dystrophin expression pattern was consistent with the predicted sequences of re-expressed micro-dys in the different cell types. Note that we have also included a samples of iPSC-derived myoblasts infected with lentiviral micro-dys, to show the similar pattern, but lower expression, of micro-dys as compared to µDys 8-9 delivered to myotubes

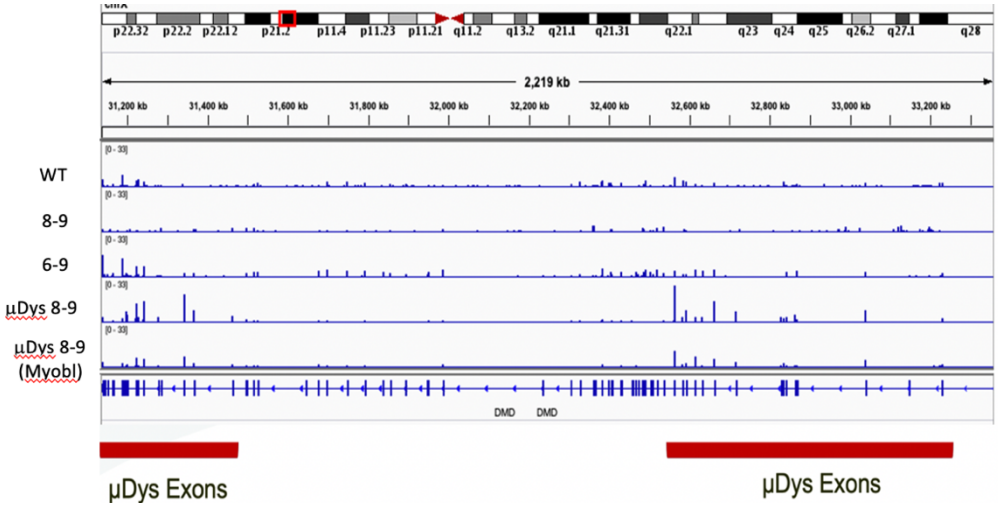


Figure 11 Legend: *RNAseq tracks of dystrophin locus from all experimental samples.*

We have also started an initial analysis of HiChIP H3K27Ac. The heatmap shown in Fig. 12 illustrates the HiChIP signal enrichment at ChIP peaks for each sample (Fig. 12)

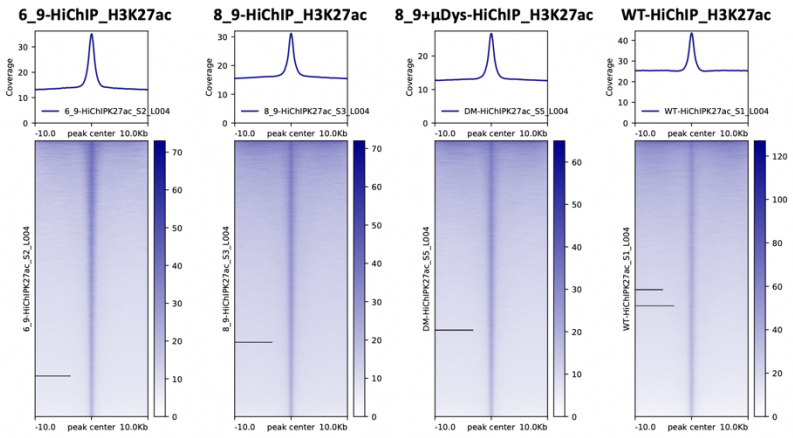


Figure 12 Legend: *HiChIP signal enrichment at ChIP peaks for each condition.*

Analysis of these samples is currently ongoing and will be completed by the end of the second year. This analysis will include integration of datasets at multiple levels by bio-informatic analysis. Initial inspection of parallel RNAseq and HiChIP H3K27ac tracks, as illustrated by a representative locus in Fig. 13, demonstrate how HiChIP signal defines H3K27ac-anchored loops formed between promoters and enhancers of differentially expressed myosins at the MYH human locus.

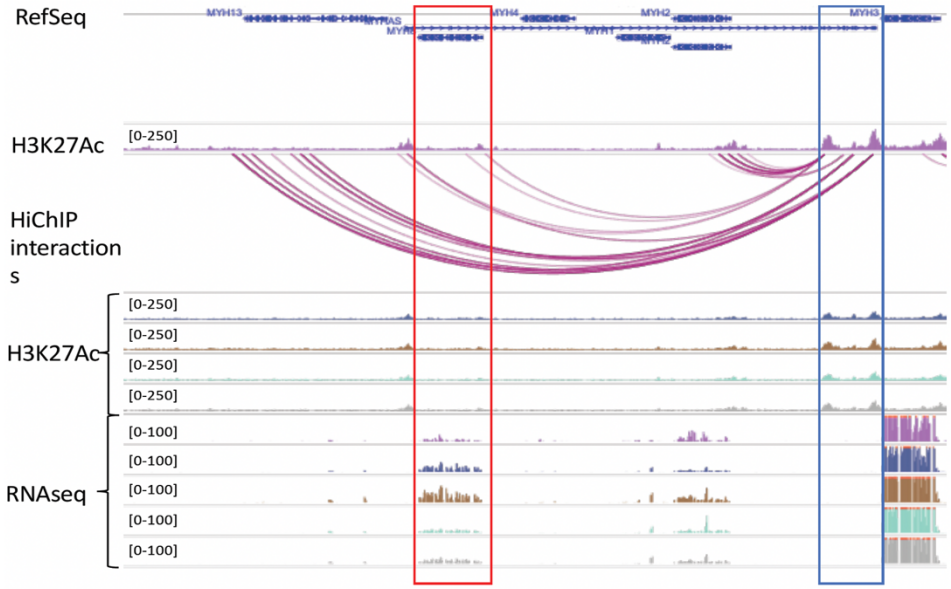


Figure 13 Legend: *Epigenome Browser screenshot of MYH locus on chr17. From top to bottom: RefSeq gene track; H3K27ac signal in CTRL myotubes; 3D genome interactions identified by HiChIP in CTRL myotubes; H3K27ac signal in DMDrΔ6/9, DMDΔ8/9, DMDΔ8/9- µDysMb and DMDΔ8/9-µDysMt; RNA-seq tracks for CTRL myotubes, DMDrΔ6/9, DMDΔ8/9, DMDΔ8/9-µDysMb and DMDΔ8/9-µDysMt . MYH8 is highlighted with red box; the locus controlling enhancer is highlighted with blue box. MYH8 expression, upregulated in DMDΔ8/9 compared to CTRL myotubes, is efficiently downregulated by LV-µDys restoration, but not by expression of DMDrΔ6/9*

4) other achievements.
Nothing to report

- **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?**

Results from the first year of funding were presented at the conferences listed below:

Abstract Presented at the Virtual Blitz at the Frontiers in Myogenesis Conference: Skeletal muscle: development, regeneration, and disease.
Herradura, Costa Rica; November 15th-19th, 2021.

Title of the abstract presentation: Alterations of regulatory chromatin interactions leading to aberrant patterns of gene expression in Duchenne Muscular Dystrophy.

Abstract presented at the 2021 Interuniversity Institute of Myology (IIM) Meeting: Pathogenesis and Therapies of Neuromuscular Diseases.
Online, October 22nd-24th, 2021.
Title of the abstract presentation: Alterations of regulatory chromatin interactions leading to aberrant patterns of gene expression in Duchenne Muscular Dystrophy.

- **What do you plan to do during the next reporting period to accomplish the goals?**

We plan to complete the sequencing and datasets analysis of RNAseq, ATACseq and pc-Hi-C samples that have been already generated (or are under generation – see Fig. 2D) from mdx mice during the first year of activity (Aim 1). As for the analysis of iPSC-derived myotube cultures, we plan to continue analyzing the first datasets of RNAseq, ATACseq and HiChIP H3K27Ac generated during the first year of activity; in case of samples that will not align to standard quality controls, we plan to generate additional samples from new cultures (Aim 2). Once we will have available standard "quality-grade" datasets from all samples, we will proceed with the final integrative analysis (Aim 3).

2. **IMPACT:**
   - **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

3. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**

For experiments in vitro on iPSC-derived myotubes we have decided to perform HiChIP H3K27Ac instead of pcHiC, for two major reasons. One was a delay in obtaining a "quality grade" full array of human promoters. However, the major reason why we prefer to generate HiChIP H3K27ac datasets was suggested by our parallel studies performed in IMR90-derived muscle cells that show an enrichment of H3K27Ac at the large majority of chromatin interactions occurring between promoters and enhancers in myotubes. Thus, HiChIP H3K27ac would capture most of the promoter-enhancer interactions. We note that, unlike mouse myotubes, for which high resolution

3D chromatin interaction have not been mapped yet, the 3D genome structure of human myotubes has been defined by recent (Dall'Agnese et al. 2018) as well as current studies from our lab. Therefore, we propose to use HiChIP H3K27ac analysis in human iPSC-derived myotubes, while confirming that pc-Hi-C will be used to analyze the 3D chromatin interactions in mouse myotubes.

Actual or anticipated problems or delays and actions or plans to resolve them

As described in the "significant results" section, we have experienced problems of lack of purity of HiPSC-derived muscle cultures, with contamination by non-muscle cells that prevented accurate statistics applied to the bio-informatic analysis. Thus, during the first 8 months of activity we have lost several Arima kits of pc-Hi-C with contaminated cultures. We note that this financial loss has been covered by discretional personal institute fundings, in order to avoid loss of budgeted DoD funds. We have now solved these problems and we have prepared new pure cultures of ipSC-derived myotubes for parallel RNAseq, ATACseq and HiChIP (in place of pcHiC – see above for the explanation). Results are shown in the "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**
- **Significant changes in use or care of human subjects**
- **Significant changes in use or care of vertebrate animals.**
- **Significant changes in use of biohazards and/or select agents**

None

4. **PRODUCTS:**

- **Publications, conference papers, and presentations**

  - **Journal publications.**

    Nothing to report

    Books or other non-periodical, one-time publications.

    Nothing to report

  - **Other publications, conference papers, and presentations.**
    Abstract Presented at the Virtual Blitz at the Frontiers in Myogenesis Conference: Skeletal muscle: development, regeneration, and disease.
    Herradura, Costa Rica; November 15th-19th, 2021.
    Title of the abstract presentation: Alterations of regulatory chromatin interactions leading to aberrant patterns of gene expression in Duchenne Muscular Dystrophy.

Abstract presented at the 2021 Interuniversity Institute of Myology (IIM) Meeting: Pathogenesis and Therapies of Neuromuscular Diseases.
Online, October 22nd-24th, 2021.
Title of the abstract presentation: Alterations of regulatory chromatin interactions leading to aberrant patterns of gene expression in Duchenne Muscular Dystrophy.

- **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

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

Name:	Pier Lorenzo Puri
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-4964-0095
Nearest person month worked:	0.96
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:	2.96
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:	2.11
Contribution to Project:	Setting experimental protocols. 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:

* Title:	A Multidisciplinary Stem Cell Training Program at Sanford Burnham Prebys Institute, A Critical Component of the La Jolla Mesa Educational Network
Major Goals:	Sanford Burnham Prebys (SBP) proposes a "CIRM Scholar" Research Training Program, which will serve as one of the hubs in a robust synergistic network of training programs on the La Jolla Mesa (that includes Scripps & UCSD) designed to train PhD candidates, postdoctoral scientists, & clinical fellows in basic & translational stem cell biology. SBP's extant stem cell program is vibrant & multidisciplinary (comprised of biologists, chemists, engineers & clinicians with extensive stem cell expertise) & has a particular focus on using stem cells to model diseases for drug discovery
Project Number:	EDUC4-12813
Name of PD/PI:	Snyder, Evan
*Source of Support:	California Institute for Regenerative Medicine
*Primary Place of Performance:	Sanford Burnham Prebys Medical Discovery Institute
Project Period:	12/2021 – 11/2026
*Total Award Amount:	

*Person Months Per Award Period:

Year	Person Months
1. 2021	0.60 calendar months
2. 2022	0.60 calendar months
3. 2023	0.60 calendar months
4. 2024	0.60 calendar months
5. 2025	0.60 calendar months

* Title:	Role of Acute Phase Response Protein SAA-1 in Pancreatic Cancer-Induced Cachexia
Major Goals:	The goal of this study is to define SAA1's mechanism of action in both MuSC and myofibers, and evaluate SAA1 as a potential therapeutic target to counteract pancreatic cancer-induced cachexia.
Project Number:	CCSG 2022
Name of PD/PI:	Sacco, Alessandra; Puri, Pier Lorenzo
*Source of Support:	SBP
*Primary Place of Performance:	Sanford Burnham Prebys Medical Discovery Institute
Project Period:	05/2022 – 05/2023

*Total Award Amount:
*Person Months Per Award Period:

Year	Person Months
1. 2022	0.12 calendar months

* Title: Pathogenic alterations of the 3D epigenetic landscape in dystrophin-deficient skeletal muscles and reversal by dystrophin re-expression

Major Goals: To analyze the effect of dystrophin deficiency and restoration of 3D genome organization of skeletal muscle

Project Number: R01 AR056712
Name of PD/PI: Puri, Pier Lorenzo
*Source of Support: NIH/NIAMS
*Primary Place of Performance: Sanford Burnham Prebys Medical Discovery Institute
Project Period: 07/2022 -05/2027
*Total Award Amount:
*Person Months Per Award Period:

Year	Person Months
1. 2023	3.0 calendar months
2. 2024	3.0 calendar months
3. 2025	3.0 calendar months
4. 2026	3.0 calendar months
5. 2027	3.0 calendar months

- **What other organizations were involved as partners?**
  - **Organization Name: University of Washington**
  - **Location of Organization:** *(if foreign location list country)*
  - **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

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